STEREOCHEMICAL ASPECTS OF ^{13}C-^{1}H COUPLING

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STEREOCHEMICAL ASPECTS OF ^{13}C-^{1}H COUPLING AND RELATED STUDIES

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A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfilment of the requirements for the degree of Doctor of Philosophy

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August 1974

ACKNOWLEDGEMENTS

The author wishes to acknowledge above all the interest, expertise, and invaluable assistance of Professor A.S. Perlin in connection with every aspect of the present work.

Special appreciation is extended to Dr. N. Cyr for performing the theoretical calculations herein, to Dr. S.S. Bhattacharjee for his many suggestions concerning the phase of this research dealing with the reduction of aldonolactones and to Dr. R.G.S. Ritchie for his useful comments. Deep felt thanks go to Dr. H.J. Koch for numerous valuable discussions in diverse areas of chemistry.

Thanks are also due to Mr. R. Simoneau for running NMR spectra, to Dr. A. Gray at the Ontario Research Foundation for recording the 220 MHz spectra, to Dr. J. Saunders at Sherbrooke University and Dr. I.C.P. Smith at N.R.C. in Ottawa for kindly recording some Fourier Transform ^{13}C spectra, to Dr. O. Mamer at Royal Victoria Hospital for recording mass spectra, to L. Jones for her experimental help and to Mrs. C. Ewan for typing the manuscript. The financial support of the National Research Council of Canada and the Pulp and Paper Research Institute of Canada is gratefully acknowledged.

Finally, I express my sincere appreciation to my wife, Alice, for putting up graciously with the turmoil accompanying the writing of this thesis and for an excellent job of proofreading.

ABSTRACT

Various factors determining the magnitude of internuclear coupling between directly bonded, geminal and vicinal ^{13}C and ^{1}H nuclei have been examined with ^{13}C labelled carbohydrates. Compounds synthesized for this purpose include derivatives of D-glucose-1- and -6-^{13}C, L-idose-6-^{13}C, D-mannose-1-^{13}C, and related lactones. Directly bonded ^{13}C-^{1}H coupling shows a dependence on the disposition of adjacent substituents and electron lone pairs relative to the ^{13}C-^{1}H bond. Orientation effects also characterize geminal ^{13}C-^{1}H coupling, the magnitude as well as the sign of which is determined by the orientation of substituents on the ^{13}C nucleus relative to the coupling proton. Vicinal ^{13}C-^{1}H coupling both through C-C and C-O bonds shows a general dihedral angle dependence similar to that for protons [i.e., dihedral angles of 60-100° are associated with smaller coupling (0-3 Hz) than angles of 140-180° (4.5-5.5 Hz)] although the coupling can be modulated by secondary effects: e.g., the observed spacing is decreased by the presence of an oxygen substituent on the ^{1}H bearing carbon in the plane of the coupling pathway, and is increased when adjacent lone pairs approach this pathway. In conjunction with the synthesis of ^{13}C-enriched sugars, two high yield new procedures have been developed for the reduction of aldonolactones to aldoses, employing either diborane or lithium aluminum hydride-aluminum chloride.

Résumé

Nous avons examiné les différents facteurs déterminant la grandeur des couplages internucléaires entre les noyaux ^{13}C et ^{1}H directement liés, géminés et vicinaux à l'aide d'une série d'hydrates de carbone marqués au ^{13}C. Nous avons donc synthétisé à cet effet les dérivés du ^{13}C-1 et ^{13}C-6-D-glucose, du ^{13}C-6-L-idose, du ^{13}C-1-D-mannose et les lactones correspondantes. La valeur du couplage ^{13}C-^{1}H de noyaux directement liés dépend de l'orientation des substituants adjacents et de celle des doublets de l'atome d'oxygène par rapport à la liaison ^{13}C-^{1}H. La constante de couplage géminée ^{13}C-^{1}H est aussi fonction de l'orientation des substituants portés par le noyau ^{13}C, par rapport au proton couplé à ce noyau, cette orientation déterminant l'amplitude et le signe de la constante de couplage. Les couplages vicinaux à travers les liaisons C-C et C-O montrent toujours une dépendance vis à vis de l'angle dièdre, similaire à celle observée pour le proton [par exemple les angles dièdres de 60 - 100° sont associés à de plus petites constantes de couplages (0-3Hz) que les angles de 140° - 180° (4.5-5Hz)] même si les couplages peuvent être affectés par des effets secondaires: l'écart observé est diminué par la présence d'un atome d'oxygène sur le carbone portant le proton ^{1}H et se trouvant dans le plan du couplage, il est augmenté lorsqu'un doublet libre est au voisinage de ce plan. Nous avons mis au point deux nouvelles méthodes de réduction des aldonolactones en aldoses, en utilisant soit le diborane soit le mélange hydrure de lithium et aluminium - chlorure d'aluminium.

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[illegible]-pyranose [illegible]

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[illegible]-isopropylidene-[illegible]-acetyl-[illegible]-glucofuranose-[illegible]

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[illegible]-isopropylidene-[illegible]-mannofuranose-[illegible]

[illegible]-isopropylidene-[illegible]-acetyl-[illegible]-mannofuranose-[illegible]

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LIST OF ABBREVIATIONS

Ac	acetyl, CH_3CO-
DHP	dihydropyran
DMF	N,N-dimethylformamide
DMSO	dimethyl sulfoxide
Ft	Fourier transform
g.l.c.	gas-liquid chromatography
Hz	Hertz (c.p.s.)
J	coupling constant (Hz)
k	force constant
MHz	megahertz
m.p.	melting point
mmole	millimole
NMR	nuclear magnetic resonance
Ph	phenyl
pmr	proton magnetic resonance
p.p.m.	parts per million (chemical shift, δ)
r.f.	radio frequency
R.T.	room temperature
THF	tetrahydrofuran
THP	tetrahydropyranyl
t.l.c.	thin layer chromatography
TMS	tetramethylsilane
$\sim$	approximately
$<$	less than

CHAPTER 1

INTRODUCTION

1.1 Introductory remarks

Perhaps no other single instrumental method has had as profound an influence on organic chemistry as nuclear magnetic resonance spectroscopy. The hydrogen nucleus, or proton, has proven to be particularly amenable to study by NMR techniques and owes its importance to the fact that it occurs in most organic compounds. Since all such compounds contain carbon, the emergence of ^{13}C magnetic resonance spectroscopy during the last decade as an area of rapidly growing interest has been an inevitable development (1). Early work in the field was hindered by the fact that the natural abundance of the ^{13}C nucleus is only 1.13%, but recent advances in instrumental methods have been able to compensate for this drawback to a great extent. The advent of Fourier transform (Ft) Spectroscopy has now further reduced the experimental complexity of measuring ^{13}C spectra and has made these spectra available in a relatively short experimental time. Most of the work to date has been concerned with ^{13}C chemical shifts and this parameter is taking an important place in the organic chemist's repertoire. Chemical shift data have been used to advantage in many chemical problems dealing with structure determination, conformational analysis and bond polarization effects (1). Massive amounts of ^{13}C chemical shift data have now been compiled (1) and are being analyzed with the hope of gaining further insight into the fundamental nature of molecules by comparing the experimental values to theoretically calculated chemical shifts (2).

1.2 Some general comments on ^{13}C-^{1}H coupling

Coupling constants between ^{13}C and protons have received relatively little attention due mostly to the experimental difficulties involved in measuring this parameter. The ^{13}C nucleus, like the proton, has a spin of 1/2 and consequently couples to other magnetic nuclei; however, because of its

low natural abundance (1.13%) the lines in the proton magnetic spectrum caused by coupling of a proton to a ^{13}C nucleus (referred to as the ^{13}C satellites) are much less intense than the signals which do not exhibit such coupling. Figure 1 shows schematically the vinyl proton spectrum of a 1,2-disubstituted propene. The very intense central peak is due to the most common isotopic species, that is, those molecules which do not contain any ^{13}C nuclei. This central peak is flanked by three pairs of satellites, (A,B,C), which are the result of molecules containing one ^{13}C nucleus. Because of the low natural abundance of the ^{13}C isotope, the probability that a single molecule contains two ^{13}C nuclei is negligible.

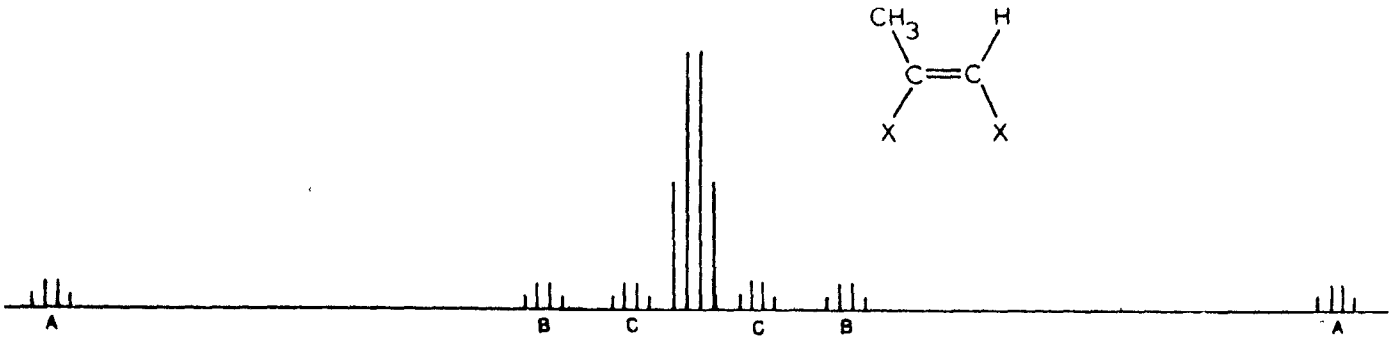


FIG. 1 Representation of ^{13}C-^{1}H coupling in a disubstituted propene (X contains no hydrogen); vinyl proton spectrum.

The intensity of each of the satellite signals in the figure is about ∿0.5% of the intensity of the central peak. The A lines can be attributed to those molecules having a ^{13}C nucleus in the 1-position, while the B and C signals are caused by molecules containing a ^{13}C nucleus in either the 2- or the 3-position of the substituted propene. The distance between the signals

constituting each pair, measured in cycles, then gives directly the relevant proton - ^{13}C coupling constant. When the ^{13}C and proton nuclei are separated by one bond the coupling is referred to as "directly bonded" and is denoted by $^{1}J_{C-H}$; when two or three bonds intervene between these nuclei the coupling is termed "geminal" or "vicinal" and is denoted by $^{2}J_{C-H}$ and $^{3}J_{C-H}$ respectively. The latter two couplings as well as any that may be observed over distances greater than those bonds are collectively referred to as "long-range coupling".

The A lines in Figure 1 which are the result of coupling between the vinyl proton and the directly bonded ^{13}C nucleus (i.e. ^{13}C in the 1-position) represent the largest type of ^{13}C-^{1}H coupling and hence these lines are well removed from the central peak and can usually be detected by greatly increasing the spectrum amplitude. The inner B and C satellites on the other hand, arising from geminal and vicinal ^{13}C-^{1}H coupling are usually masked by the much more intense central peak. (As will become evident in later chapters, the magnitude of these couplings is not proportional to the number of intervening bonds, so that in many instances vicinal coupling can be greater than geminal coupling). It is evident then that even for such a simple system as discussed above, the derivation of geminal and vicinal ^{13}C-^{1}H couplings from the proton spectrum presents great difficulties. Furthermore, it is not always the case even for simple molecules that the satellite pattern is readily analyzable. While the experimental satellite spectrum of acetylene is simple (Fig. 2), and readily affords $^{1}J_{C-H}$ and $^{2}J_{C-H}$ (3), that of ethylene is highly complex (Fig. 3).

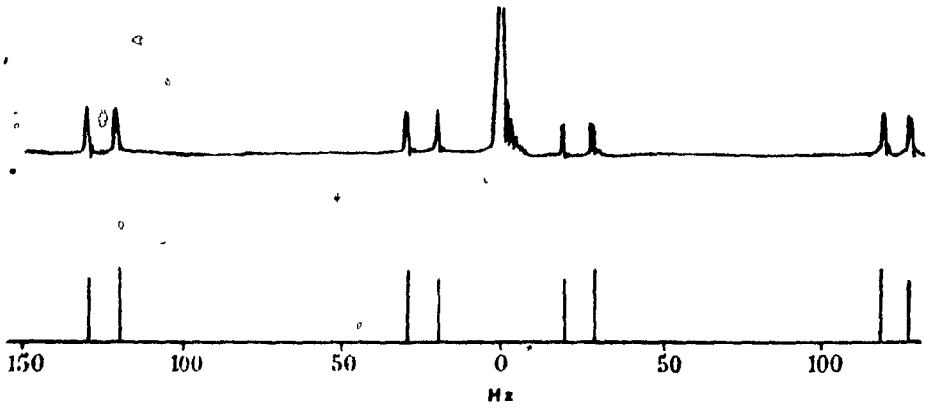


FIG. 2 Observed pmr spectrum of an approximately 1:1 mixture of H-^{13}C≡C-H and H-C≡C-H (3).

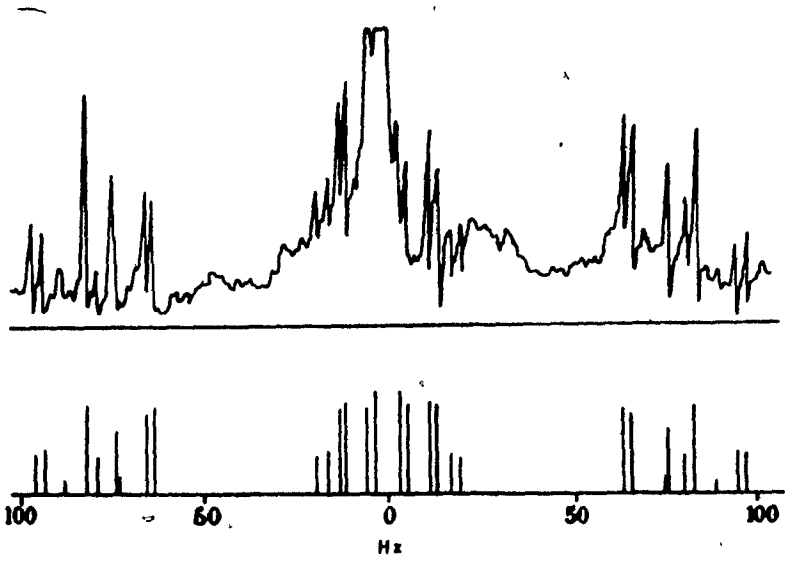


FIG. 3 Observed and computer calculated pmr spectrum of an approximately 1:1 mixture of $H_2C=^{13}CH_2$ and $H_2C=CH_2$ (3).

The substitution of a ^{13}C nucleus for one of the ^{12}C atoms in ethylene results in a system of interacting nuclei best described as A_2XA_2' which of course gives rise to a complex splitting pattern. The desired coupling however can still be derived from this pattern by comparison with the computer calculated spectrum.

As is evident from the preceding, directly bonded ^{13}C-^{1}H coupling constants can be obtained from the proton spectrum with relative ease for suitable compounds, whereas the two and three bond couplings present a much more difficult problem. It must be pointed out, however, that the qualifying word "suitable" in the above statement is not trivial, since even for slightly more complex compounds the directly bonded satellites are often obscured by absorptions due to other protons. In view of these difficulties it is understandable that ^{13}C-^{1}H coupling constants have received considerably less attention than the related ^{1}H-^{1}H coupling constants. Nevertheless, substantial information on the subject is available, especially in the area of the directly bonded couplings (1).

The reason for interest in this field of ^{13}C NMR is two-fold. Firstly, it is now widely accepted that the magnitude of coupling between magnetic nuclei reflects the electronic situation in the bonds involved and as a consequence these couplings can potentially furnish information about the nature of chemical bonding.

Secondly, it is known that coupling constants are greatly affected by factors such as the relative geometry of the coupling nuclei, and the electronegativity of substituents along the coupling pathway (4) (4a). Since both the nature of carbon-hydrogen bonds and the relative orientation of the nuclei are of fundamental interest to chemists, a close examination of relevant ^{13}C-^{1}H coupling constants should provide useful information both from a theoretical and an applied viewpoint.

1.3 The pmr spectrum of D-glucose-U-^{13}C

The present investigation was prompted by an analysis of the ^{13}C-^{1}H coupling constants measured from the proton spectrum of an anomeric mixture of D-glucose, 50% ^{13}C enriched in all positions (5). As can be seen in Figure 4, the β anomeric proton signal is split into a doublet ($^{1}J_{C-H}$ = 160 Hz) by $^{13}C_1$ but shows no further coupling to any other ^{13}C nucleus. The α anomeric absorption on the other hand is split into a doublet by $^{13}C_1$ ($^{1}J_{C-H}$ = 169 Hz) and is further coupled to at least one other ^{13}C nucleus to an extent of about 6-7 Hz. These results suggested that ^{13}C-^{1}H coupling is dependent on stereochemistry and conversely that this parameter may potentially serve as a stereochemical probe.

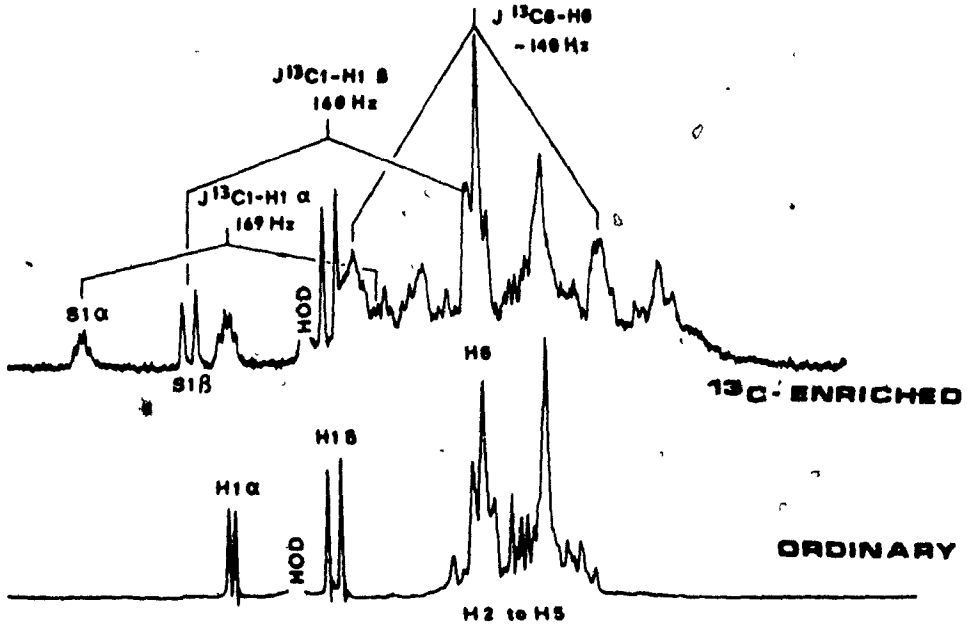
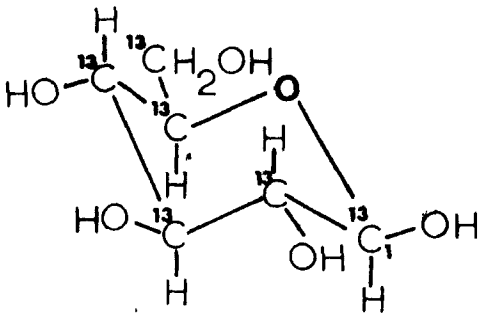
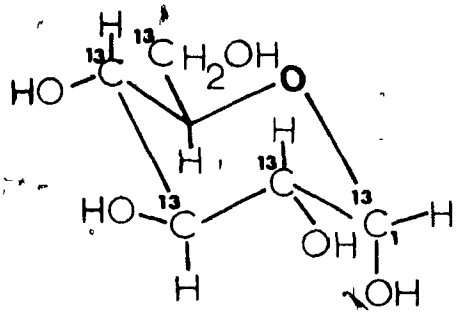


FIG. 4 Pmr spectrum of α,β-D-glucose-U-^{13}C (50% enriched) in D_2O (5).

The most evident rationale for the observed splitting pattern involves a dihedral angle dependence of $^{3}J_{C-H}$. The C_1-H_1 bond of the β anomer subtends a dihedral angle of 60° with respect to the C_2-C_3 and O_5-C_5 bonds whereas the C_1-H_1 bond of the α anomer maintains a 180° relationship with respect to the same bonds as can be seen below.



β-D-Glucopyranose-U-^{13}C



α-D-Glucopyranose-U-^{13}C

If the behaviour of vicinal ^{13}C-^{1}H coupling parallels that of the corresponding three bond proton-proton coupling (4) one would indeed expect a small coupling for a 60° dihedral angle relationship and a considerably larger coupling when the relevant dihedral angle is 180°. This is the case for H_1 with respect to $^{13}C_3$ and $^{13}C_5$ in the β and α anomers respectively.

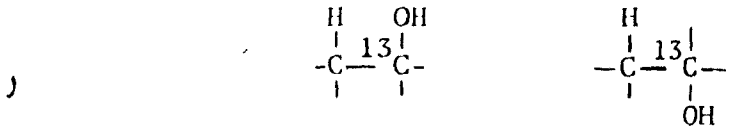
Examination of the above spectrum also reveals the striking observation that whereas H_1 of the α anomer may be coupled to $^{13}C_2$, H_1 of the β anomer assuredly shows no such coupling. The preceding implies not only that three bond ^{13}C-^{1}H couplings may on occasion be larger than two bond couplings but also that at least under certain conditions such two bond or geminal couplings may be nonexistent ($^{2}J_{C-H}$ = 0 Hz).

1.4 Measurement of ^{13}C-^{1}H coupling constants (J_{C-H})

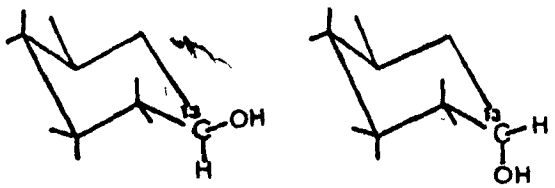
In order to further investigate the possible dihedral angle dependence of vicinal ^{13}C-^{1}H couplings as well as the seemingly anomalous nature of geminal ^{13}C-^{1}H couplings, and to evaluate their potential as an aid in stereochemical problems, it was decided to investigate several series of compounds in which the relevant coupling constants could be measured while dihedral angle

and other likely coupling determinants were systematically varied.

In order to examine the various factors that can affect the magnitude of J_{C-H}, ideally, the most meaningful results would be obtained if each variable were isolated and studied independently. For example; to investigate how $^{2}J_{C-H}$ varies with the relative orientation of the coupling proton and a substituent on the ^{13}C nucleus (as is done in Chapter 3), it would be optimal to consider isolated cases as below:



In practice, one can at best examine such effects when the partial structure is incorporated into a ring :



a **b**

It is recognized, however, that in going from a to b, in addition to changing the relative orientations of the C-H and C-OH bonds, one may be introducing other effects such as the 1,3-diaxial interaction present in b but not in a, which may also have an impact on the observed $^{13}C-^{1}H$ coupling. As a further example, introduction of an OH group in place of H to study electronegativity effects may alter the state of hybridization, bond angles and bond lengths in addition to providing an electronegative substituent. Hence, when a structure is altered to examine one effect, it cannot be assumed that all else remains constant. Nevertheless, valuable information pertaining to $^{13}C-^{1}H$ coupling can be derived from a systematic analysis of the factors likely to

have an influence on this parameter.

In general, there are four basic ways in which ^{13}C-^{1}H coupling constants can be obtained experimentally:

A) From the natural abundance proton spectrum -

This method has already been described (Figure 1) and its limitations have been noted. At best, in appropriate compounds, directly bonded ^{13}C-^{1}H coupling constants can be determined.

B) From the natural abundance ^{13}C spectrum -

The proton coupled ^{13}C spectrum can furnish information about ^{13}C-^{1}H couplings but the problems of resolution and signal assignment are usually present. With the advent of Fourier transform ^{13}C NMR the resolution difficulties have been alleviated but the assignment of specific absorptions is still problematic. Even if the carbon signals can be correctly identified and the proton couplings observed, the uncertainty in assigning the correct coupling pathway to the observed splitting remains. Figure 5 illustrates the ^{13}C Fourier transform spectrum of 1,6-anhydro-β-D-galactopyranose. In this case the anomeric carbon signal is readily identified by its downfield position and the absorption is seen to be split by proton coupling. However, it is not known which splitting is a result of coupling to H_2, which to H_3 etc. This problem can potentially be solved by specific proton decoupling, a technique which is within the capabilities of the most advanced Fourier transform spectrometers. The exact resonant frequency of the proton absorption in question is determined and the relevant ^{13}C absorption is then observed while the proton absorption is irradiated with a second radio-frequency in order to attain the desired heteronuclear decoupling. The collapse of a coupling on account of the irradiation then enables this coupling to be assigned with a high degree of certainty. Under

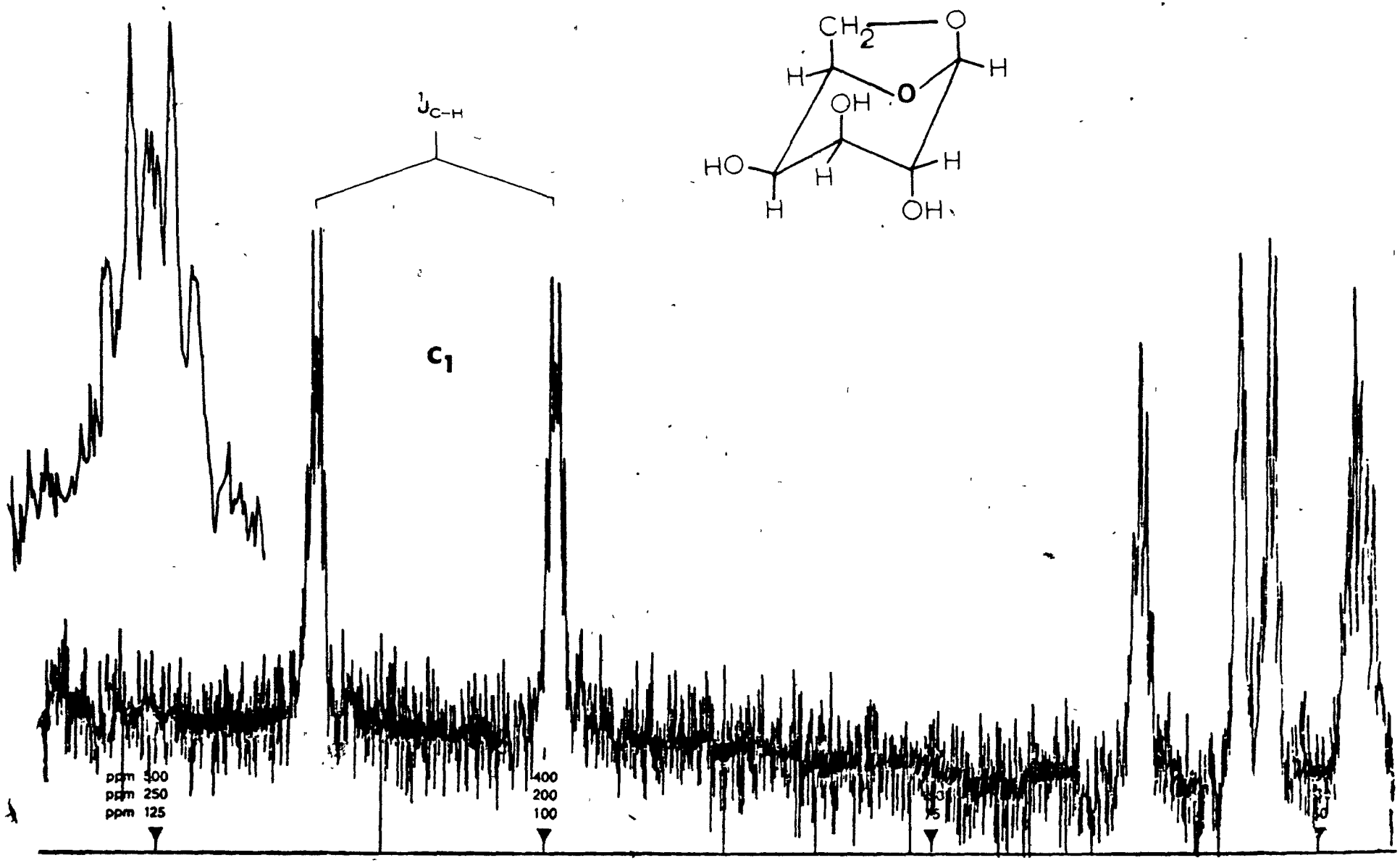


FIG. 5 Partial Ft ^{13}C proton coupled spectrum (22.6 MHz) of 1,6-anhydro-β-D-galactopyranose in D_2O.

ideal conditions, and with the proper instrumentation, conceivably almost all ^{13}C-^{1}H couplings can be systematically assigned in this manner.

The problem of coupling assignment can also be tackled by specific deuteration. If the ^{13}C Ft NMR spectrum of 1,2:5,6-di-O-isopropylidene-α-D-glucofuranose is examined (Figure 6A), the downfield anomeric carbon signal is readily assigned and is seen to be split by two ^{13}C-^{1}H couplings. Again, however, assignment of these splittings to specific coupling pathways is not possible. This ambiguity can be alleviated by comparing the spectrum of the above compound to that of its 3-deutero and 4-deutero derivatives, (Figures 6B and 6C, respectively). There is no observable difference in the splitting pattern of C_1 on deuteration at position four indicating that there is no coupling between $^{13}C_1$ and H_4. Introduction of deuterium at position three however results in the disappearance of one of the $^{13}C_1$ splittings, which can then be assigned to $^{3}J_{C_1\text{-}H_3}$. The remaining coupling then must be due to $^{2}J_{C_1\text{-}H_2}$. Although this method can be useful in certain cases it is, of course, limited by the practicality of the synthesis of necessary deuterated compounds.

C) From the proton spectrum of ^{13}C enriched compounds -

This technique essentially increases the size of the ^{13}C satellites and thus alleviates the isotopic abundance problem.

D) From the ^{13}C spectrum of ^{13}C enriched compounds -

Using this technique the enriched carbon absorption is readily assigned on the basis of its intensity but the uncertainty involving the assignment of the observed couplings to specific protons still remains.

For the purposes of the present study, all things considered,* the synthesis of ^{13}C enriched compounds appeared to be the most promising approach

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*An Ft spectrometer was not available until most of this study had been completed.

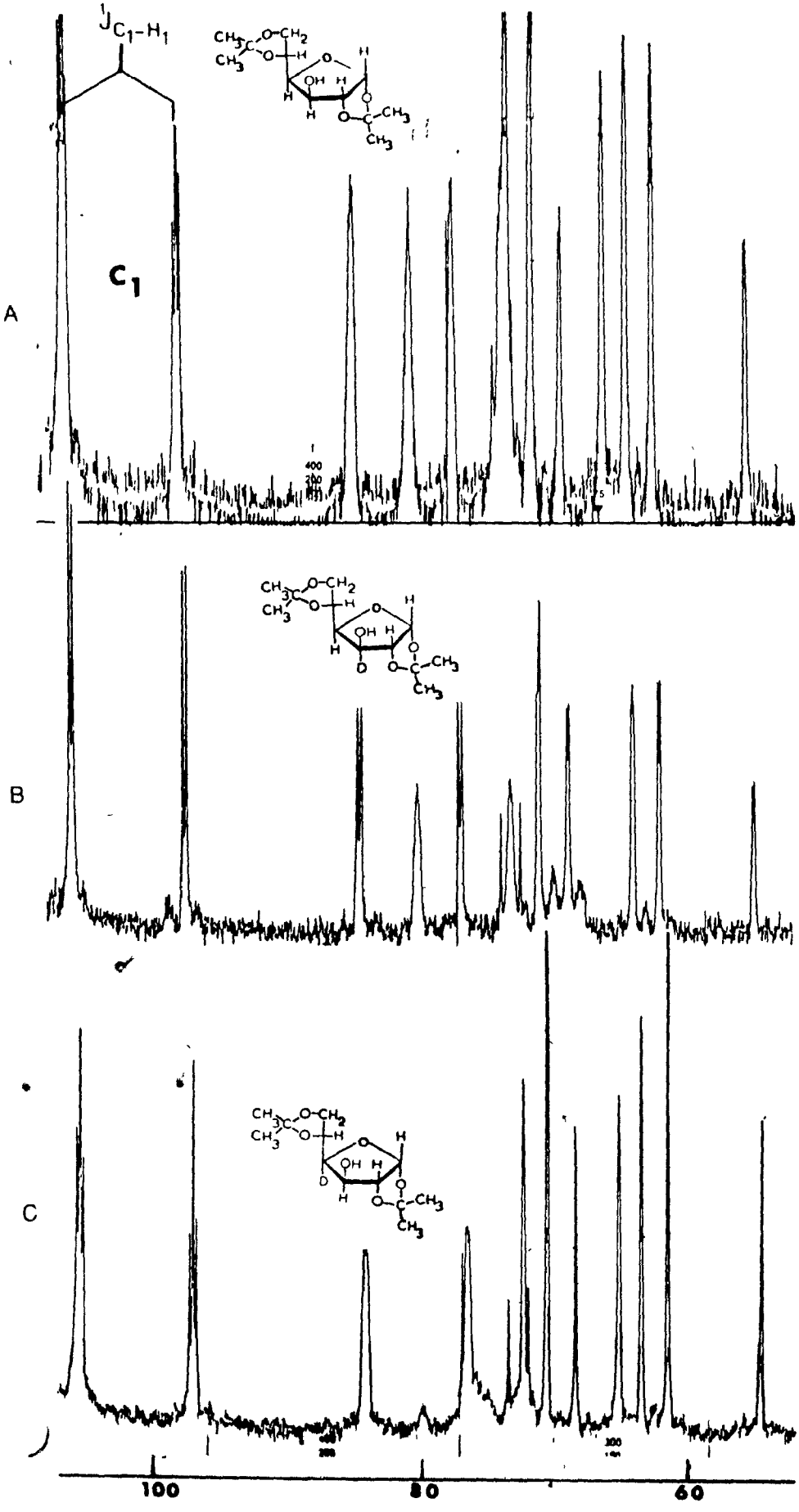


FIG. 6 Partial Ft ^{13}C spectrum (22.6 MHz) of A) 1,2:5,6-Di-O-isopropylidene α-D-glucofuranose; B) 1,2:5,6-Di-O-isopropylidene-α-D-glucofuranose-3-d; C) 1,2:5,6-Di-O-isopropylidene-α-D-glucofuranose-4-d. (Kindly provided by G.R.S. Ritchie)

for the determination of ^{13}C-^{1}H coupling constants in complex systems. This method can be illustrated by comparing the 220 MHz spectrum of 1,2,-O-isopropylidene-α-D-glucofuranurono-6,3-lactone to that of its 6-^{13}C analog, one of the compounds synthesized for the purposes of this investigation (Figure 7). It is important to recognize the fact that proper assignment of the ^{13}C-^{1}H couplings in this case hinges on the correct assignment of the proton absorptions. For example, once the H_5 and H_4 signals in the spectrum of the above compound are assigned (a relatively routine procedure on the basis of proton-proton coupling constants) the $^{13}C_6$-C_5-C_4-H_4 coupling is easily measured. Nevertheless, as emphasized in later chapters, this method has several drawbacks. The synthesis of ^{13}C enriched compounds is expensive and necessitates a small scale operation which can present experimental difficulties. Also if, as in the present study, the compounds of interest are relatively complex, it is of paramount importance that the proton signals which are expected to exhibit ^{13}C coupling be visible and identifiable in the spectrum. The possibility also exists that the satellites due to the directly bonded coupling can obscure signals showing long range couplings.

It appeared, however, that a careful selection of compounds to be investigated would circumvent or minimize the potential difficulties mentioned above and would in turn provide heretofore unavailable data concerning stereochemical aspects of ^{13}C-^{1}H coupling. These couplings, it was hoped, would then serve as a complement to proton-proton coupling constants in solving problems of a stereochemical nature.

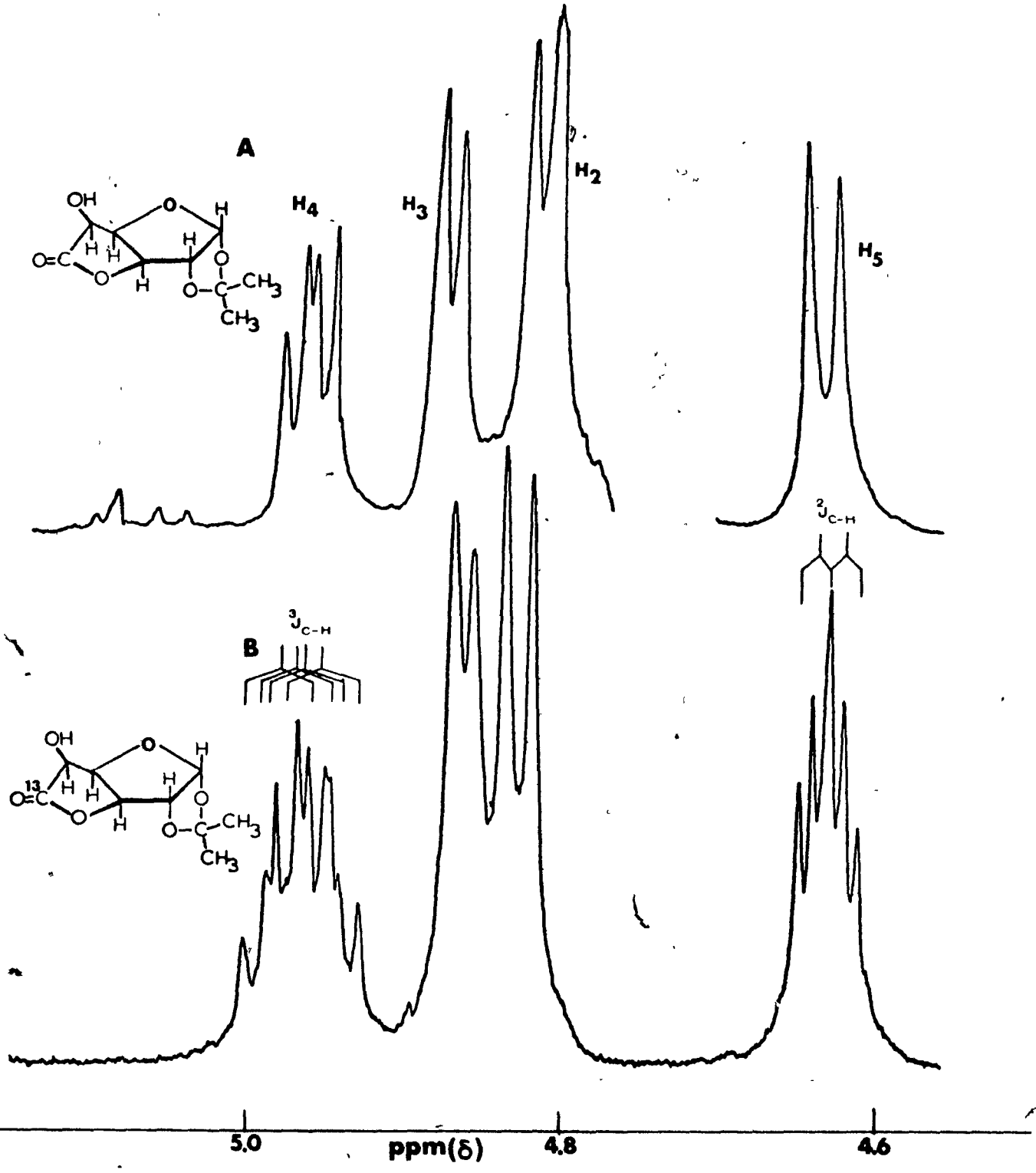


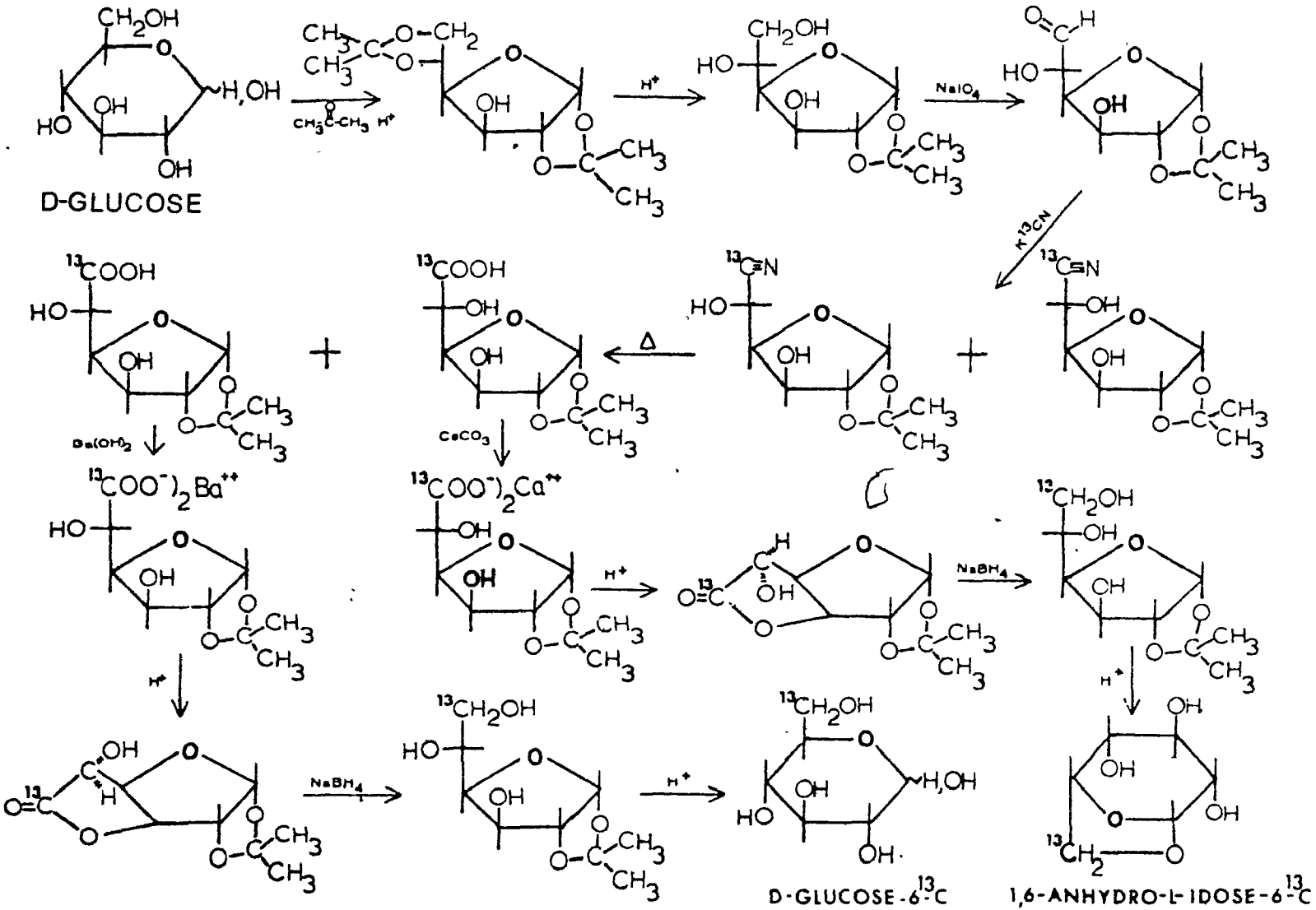
FIG. 7 Partial pmr spectrum (220 MHz) of A) 1,2-O-Isopropylidene-α-D-glucofuranurono-6,3-lactone and B) 1,2,-O-Isopropylidene-α-D-glucofuranurono-6,3-lactone-6-^{13}C (60%) in $CDCl_3$.

1.5 Synthesis of ^{13}C-enriched sugars

The choice of ^{13}C-enrichment as the basis of the present study was expedited by the availability of extensive information on the synthesis of ^{14}C-labelled sugars (6)(6a). This latter situation promised direct access to the required compounds containing ^{13}C although, in practice, a significant modification of procedures and some innovation proved to be advisable, as described below. Two reaction schemes provided for the synthesis of a basic group of ^{13}C-enriched sugars, which in turn could be converted into sundry derivatives or other sugars, as necessary for the examination of specific structural features.

One of these schemes led to enriched compounds in which the ^{13}C bears one oxygen substituent, and resulted in the synthesis of D-glucose-6-^{13}C and L-idose-6-^{13}C as outlined in Scheme 1. This scheme involves the preparation of 1,2:5,6-di-O-isopropylidene-α-D-glucofuranose from glucose, the hydrolysis of the 5,6-O-isopropylidene function and cleavage of the 5,6-diol by means of sodium periodate to yield 5-aldo-1,2-O-isopropylidene-D-xylo-pentofuranose. The 5-aldehydo function now allows introduction of carbon-13 (as K^{13}CN) by means of the cyanohydrin reaction. Due to the high cost of the enriched cyanide all subsequent steps were carried out on a small scale and necessitated numerous trial runs on non-enriched material.

The addition of cyanide to the 5-aldehydo function results in the formation of epimeric cyanohydrins having the D-gluco and L-ido configurations, the proportions of which can be altered by the use of suitable buffers. In slightly acid solution the reaction proceeds slowly (20-25 days are necessary for completion), but the yield of the D-gluco epimer is high (60%); this yield decreases with an increase in alkalinity. The desired degree of



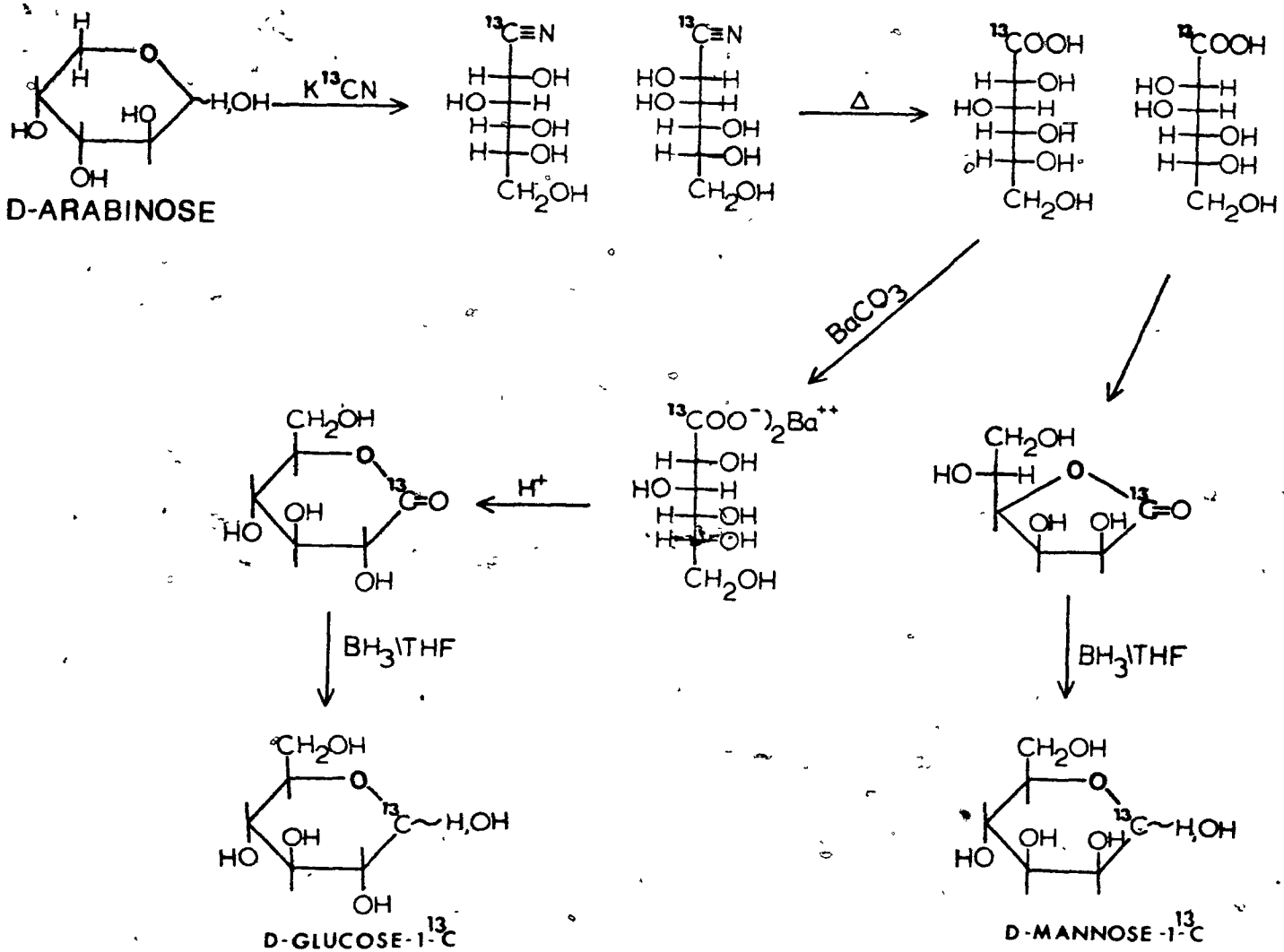
SCHEME 1

acidity was maintained by the addition of proportionate amounts of sodium hydroxide and acetic acid to the reaction mixture. The resulting cyanohydrins were hydrolyzed *in situ* to their corresponding uronic acids; this is best carried out under alkaline conditions, the pH being adjusted by the addition of sodium carbonate. At this stage separation of the epimers is possible due to the differences in solubility behaviour of the barium and calcium salts of the two uronic acids: the barium salt of 1,2-O-isopropylidene-α-D-glucuronic acid crystallizes well whereas the corresponding salt of the L-*ido* epimer does not. Therefore, passing the hydrolyzate through a cation exchange column followed by neutralization of the effluent with barium hydroxide affords barium 1,2-O-isopropylidene-α-D-glucuronate as a crystalline material. Care must be exercised during the ion exchange process to prevent partial hydrolysis of the isopropylidene groups. This process is therefore best carried out at low temperatures. After crystallization of barium 1,2-O-isopropylidene-α-D-glucuronate, the L-*ido* epimer can be isolated from the mother liquor as the calcium salt. This procedure again involves the removal of positive ions from the mother liquor by means of cation exchange resin and neutralizing the effluent with calcium carbonate. Calcium 1,2-O-isopropylidene-β-L-iduronate can then be crystallized from the resulting solution.

For the production of D-glucose-6-^{13}C and L-idose-6-^{13}C, the crystalline salts are converted to the 6,3-urono lactones which are then reduced. Lactonization is best carried out by passing a solution of these crystalline salts through a cold cation exchange column for conversion to the uronic acids, followed by lyophilization and subsequent heating of the residues with toluene to arrive at the desired 6,3-urono lactones. These lactones can then be reduced to the corresponding mono-O-isopropylidene derivatives. The standard technique

has employed $LiAlH_4$ as the reducing agent (6) but it was found that the reaction could be carried out more easily, and higher yields could be obtained, using $NaBH_4$. The relatively lower yield of the $LiAlH_4$ procedure is due to difficulties encountered in the isolation of the product from the reaction mixture, a difficulty which does not arise when $NaBH_4$ is used. In this latter procedure the lactone is taken up in methanol, sodium borohydride is added followed by neutralization with cation exchange resin. The solution is recovered and evaporated several times with methanol to remove borate as methyl borate, leaving as a residue the mono-O-isopropylidene derivative in almost quantitative yield. Hydrolysis of 1,2-O-isopropylidene-α-D-glucofuranose-6-^{13}C then yields D-glucose-6-^{13}C directly. L-Idose however cannot be isolated from the analogous hydrolyzate of 1,2-O-isopropylidene-β-L-idofuranose due to the preponderant formation of 1,6-anhydro-α-L-idose. This latter compound is favoured since the 2,3 and 4 hydroxyl groups are all equatorial as opposed to an all axial arrangement in the free sugar. The resulting 6-^{13}C labelled compounds and their various derivatives proved useful in the investigations of J_{C-H} in structures where the ^{13}C atom formed part of a ring, as well as in studies of conformationally mobile systems.

The second reaction scheme (Scheme 2) pertains to the synthesis of carbohydrates enriched with ^{13}C in the one position. This scheme again involves increasing the chain length of an aldose by the cyanohydrin reaction. Since the desired end products were glucose-1-^{13}C and mannose-1-^{13}C derivatives, D-arabinose was used as the starting material. As was the case with the previously described scheme, the proportion of epimers produced upon cyanide addition to the aldose depends upon the reaction conditions (6a). It was found that the best yields were obtained when the reaction was carried out under mildly basic conditions, which were maintained by the addition of calculated


D-ARABINOSE
K13CN
Δ
BaCO3
H+
BH3\THF
BH3\THF
D-GLUCOSE-1-13C
D-MANNOSE-1-13C

SCHEME - 2

amounts of NaOH and $NaHCO_3$ to the reaction mixture. The reaction was essentially complete within 48 hours after which time hydrolysis to the glyconic acids was carried out by heating the solution gently in a stream of air in order to drive off the ammonia produced. Separation of the epimers was again based on the different solubility patterns of the barium salts of the two glyconic acids. The barium salt of gluconic acid crystallizes quite readily as the trihydrate whereas the corresponding salt of mannonic acid does not crystallize. Consequently, prior removal of cations from the reaction mixture by means of ion exchange resin followed by neutralization with barium hydroxide afforded barium D-gluconate trihydrate as a crystalline material.

Reduction of aldonic acids to the corresponding aldoses takes place through the lactone and not through the free acid. D-Gluconic acid is known to form two lactones; the five-membered ring gamma (γ) lactone and the six-membered ring delta (δ) lactone. The product obtained depends upon temperature, solvent, concentration and the presence of seed crystals. Crystallization of the delta lactone can be obtained in near quantitative yields by a slow concentration of a methyl cellosolve solution of gluconic acid. D-Mannono-1,4-lactone can be isolated directly from the mother liquor of the barium gluconate preparation by passing this solution over a cation exchange column to remove positive ions, and evaporating the effluent. Crystallization of the lactone, induced by seeding, then proceeds readily.

The reduction of the aldonolactone to the aldose is traditionally the most difficult step of the reaction sequence due to the possibility of over-reduction to the alditol. Consequently this reduction was examined in detail in order to maximize the yields, and is discussed extensively in Chapter 5. This investigation resulted in the development of two novel techniques for the

reduction of aldonolactones to aldoses: reduction by borane and reduction by means of a mixed hydride ($LiAlH_4$-$AlCl_3$) reagent. Both of these techniques afforded higher yields than reduction with sodium amalgam or with sodium borohydride under acidic conditions, the classical methods for conversion of aldonolactones to aldoses.

Scheme 2, then, leads to compounds in which the stereochemistry about $^{13}C_1$ is known and can be varied. The ^{13}C-^1H coupling constants derived from these compounds played an essential role in elucidating the stereochemical nature of this type of internuclear coupling.

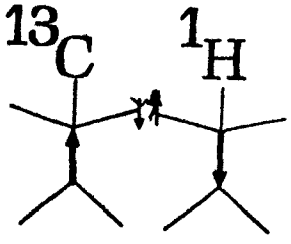
1.6 A comment on the literature dealing with ^{13}C-^1H coupling

At the beginning of this study, and during most of its experimental phase, there was only a single article (5) on ^{13}C-^1H coupling in carbohydrates. However, substantial data were available for other classes of compounds - alkanes, alkenes, and derivatives* - and various characteristics of J_{C-H} had been intensively explored. Although structural analogies are not always easily drawn between such molecules and the sugars, an attempt has been made to assess the current findings in this broader context, and hence also to present a comprehensive treatment of the overall literature on ^{13}C-^1H coupling.

* Most of these are relatively simple molecules and, as neat liquids, could be examined adequately well with the instrumentation then available.

CHAPTER 2

DIRECTLY BONDED ^{13}C-^{1}H COUPLING



2.1 Introductory remarks

As mentioned previously, experimental measurement of directly bonded $^{13}C-^{1}H$ coupling constants ($^{1}J_{C-H}$) is relatively facile in comparison with the analogous measurements for geminal ($^{2}J_{C-H}$) and vicinal ($^{3}J_{C-H}$) couplings between the ^{13}C and ^{1}H nuclei. The first compilation of such data was achieved by Lauterbur (7) who noted that there was considerable variation in this parameter ranging from 120 Hz for the methyl carbon-hydrogen coupling in tetramethylsilane to 208 Hz for the corresponding coupling in bromoform, and that in general $^{1}J_{C-H}$ increases with decreasing magnetic shielding of the proton. It thus appeared likely that some relationship between $^{13}C-^{1}H$ coupling constants and bond polarity or hybridization exists. Research in this area has focused on the preceding concept with investigators attempting to correlate the observed couplings with various parameters associated with the carbon-hydrogen bond. Such research has a dual goal: to investigate the potential of $^{1}J_{C-H}$ as an index of any of these parameters and to use the measured couplings to gain information about the nature of chemical bonding by comparing the experimental values to theoretically calculated ones. Correspondingly, for an in-depth analysis of directly bonded $^{13}C-^{1}H$ couplings it is most fitting to begin with a discussion of the results that have heretofore been accumulated concerning $^{1}J_{C-H}$ as an index of hybridization.

2.2 $^{1}J_{C-H}$ as an index of hybridization

The findings of Lauterbur reported above were not totally unexpected since Karplus and Grant had already suggested in 1959 (8) that electron coupled nuclear spin interactions when interpreted in terms of the theory of localized electon pairs, could conceivably serve as a measure of the extent of orbital hybridization and bond polarization. Consequently, Muller and Pritchard

examined the $^{1}J_{C-H}$ parameter theoretically (9) using Ramsay's formulation of the expression to calculate coupling constants (10). Ignoring terms other than the Fermi contact interaction (11), the investigators formulated the expression:

$$^{1}J_{C-H} = 2 \times 10^{3} a^{2} b^{2}$$

where a and b are the coefficients of the hydrogen 1s and carbon 2s atomic orbitals, respectively, used to describe the two-center molecular orbital encompassing the nuclei in question. Inherent in the above expression is the notion that the percent "s" character of the carbon orbital involved in the bond under consideration is proportional to the directly bonded $^{13}C-^{1}H$ coupling constant.

This relationship can be demonstrated in a general manner by considering acetylene, ethylene and ethane which show couplings of 248.7, 156.2 and 125.0 Hz, respectively (3)(12), parallelling a decrease in "s" character (Fig. 1).

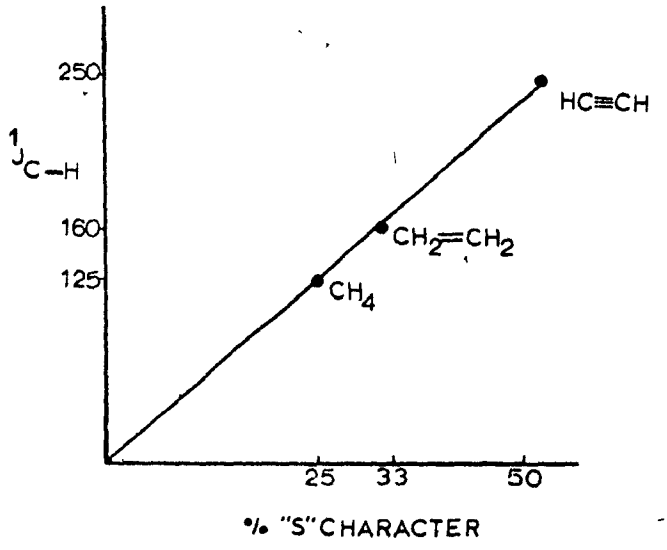


FIG. 1 Plot of $^{1}J_{C-H}$ versus % "s" character in methane, ethylene and acetylene.

It can also be noted that carbonium ions such as $(CH_3)_2\overset{+}{C}H\ SbF_5Cl^-$ (13) produce directly bonded couplings (~168 Hz) which are consistent with sp^2 hybridization (i.e. 33% "s" character) for the central carbon. Such a relationship is reasonable if the coupling is indeed adequately described by the Fermi contact mechanism. This mechanism predicts that the magnitude of the interaction between a ^{13}C nucleus and an adjacent proton depends on the probability of finding the bonding electrons at the two nuclei in question (11). Since an electron in a pure "p" orbital has zero probability of being found at the nucleus, whereas "s" electrons have a finite probability, it would seem plausible that the coupling should depend at least to some extent on the state of hybridization of the carbon atom.

Further theoretical support for the "$^1J_{C-H}$ - percent "s" character" relationship was provided by Muller (14) as well as by Juan and Gutowsky (15)(16) who used valence bond theory to show that at least for hydrocarbons $^1J_{C-H}$ was explicitly dependent upon the "s" character of the carbon atom and was relatively insensitive to the degree of polarization of the carbon-hydrogen bond. Other calculations, using only the contact contribution according to the molecular orbital theory of Pople and Santry (17) and energies generated according to extended Hückel theory (18) also showed a correlation between percent "s" character and the measured $^1J_{C-H}$. Reasonable correlations were also obtained using the Linear Combination of Atomic Orbitals - Self Consistent Field Molecular Orbital (LCAO-SCF) theory (19)(20). Randic et al. (21) employed the "maximum overlap method" to develop the expression:

$$^1J_{C-H} = \frac{a^2_C \cdot a^2_H}{1 + S^2_{C-H}}$$

where a^2 is the "s" character of the bonding orbitals involved in the.

carbon-hydrogen bond and S is the overlap integral. The "maximum overlap method" of calculation involves a search for the optimum hybrid coefficients to make a suitably weighted sum of bond overlaps maximal. It can be shown that when variations in bond overlap are neglected, the above expression reduces to:

$$^{1}J_{C\text{-}H} = 500\ a^{2}$$

The proportionality of directly bonded $^{13}C\text{-}^{1}H$ coupling to percent "s" character is evident in the above relationship, which in turn concurs with the previous proposals of Muller and Pritchard (9) and Juan and Gutowsky (15)(16).

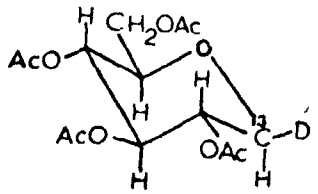
It has also been suggested, using Hückel M.O. theory, that the directly bonded $^{13}C\text{-}^{1}H$ coupling is proportional to the square of the bond order of the C-H bond (22)(23). Bond order can be described as:

$$p = 2 \sum_{i}^{occ} a^{i}_{1s_H}\ b^{i}_{2s_C}$$

where a_{s_H} and b_{s_C} are coefficients of the hydrogen 1s and carbon 2s orbitals in the i^{th} molecular orbital.

In virtually all of the theoretical calculations mentioned above, it has been tacitly assumed that the Fermi contact term is the sole contributor to the coupling. Indeed, this assumption has been questioned by some (24). For example, it is known that a C-D bond is shorter than a C-H bond by about .005Å (25). Therefore, the carbon orbitals associated with the C-H bonds in CH_2DX should have less "s" character than in CH_3X. However it is found that $^{1}J_{C\text{-}H}$ for CH_2DBr is about 4Hz larger than the corresponding coupling in CH_3Br (24). A possible explanation is that the Fermi term is not the only contributor to the coupling. By contrast, Murray (26) has shown that deuteration does not have a large effect on $^{1}J_{C\text{-}H}$: e.g., the methyl carbon of toluene shows a coupling

of 126 Hz in comparison to 125.4 Hz for the partially deuterated analog. This latter result is corroborated by findings in the present study. The $^{13}C_1$-H_1 coupling in 1,5-anhydro-D-glucitol-1-^{13}C-1-d was measured and was not found to deviate significantly from the ^{13}C-^{1}H coupling in methanol, both couplings being 141 Hz.

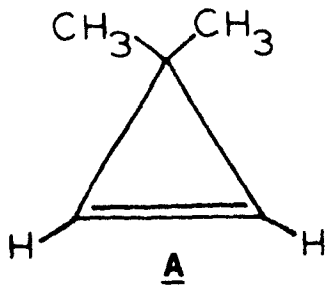


1,5-Anhydro-D-glucitol-1-^{13}C-1-d

Nevertheless, the concept of coupling between ^{13}C and ^{1}H nuclei being determined only by the Fermi contact term must not be accepted as dogma and the possible contributions from other coupling mechanisms must be recognized.

2.3 Some applications of the hybridization model

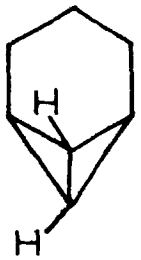
The possible calculation of hybridization from $^{1}J_{C-H}$ intrigued many chemists since it provided a method of investigating the nature of bonding in many unusual systems. Cyclopropenes, were studied for example (27), and it was found that for 3,3-dimethylcyclopropene (A) the measured value of 220 Hz corresponds to 44% "s" character in the exocyclic carbon orbital.



A

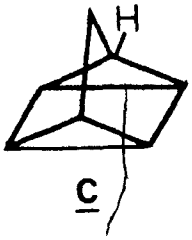
Therefore, cyclopropenes appear to be more closely related to acetylenes than olefins. This is in fact evidenced by the relatively high acidity of the

vinyl protons in this compound (28). A study of tricyclo [4.1.0.0.^{2,7}] heptane (B) reveals a coupling of 200 Hz for the protons indicated below (29).



B

This coupling corresponds to about 40% "s" character for the exocyclic carbon orbital. Again, this remarkably high degree of "s" character leads one to expect unusual acidity; indeed, this hypothesis is verified since the compound reacts readily with n-butyl lithium. In compound C, $^{1}J_{C-H}$ for the coupling between $^{13}C_1$ and H_1 is 179 Hz (30).



C

Again, it is evident that the bridgehead carbon atoms have extraordinarily high "s" character. A systematic examination of the variation of $^{1}J_{C-H}$ with ring size (31) reveals that the coupling varies directly with the C-C-C interatomic angle, and consequently when dealing only with hydrocarbons this angle can be derived from the measured coupling. Agreement is good, especially if interorbital instead of interatomic bond angles are used (32). A similar situation persists with cyclic olefinic systems (33), the olefinic $^{1}J_{C-H}$ being inversely proportional to the C-C-C interatomic angle. This

relationship is maintained for heterocycles if the heteroatom is kept constant (34), although the curve is displaced depending on the nature of the heteroatom.

In general then, when dealing with hydrocarbons a correlation between $^1J_{C-H}$ and angular distortion is evident (34)(35). The more strained the molecule under consideration, the larger the observed coupling (see Section 2.10).

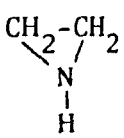
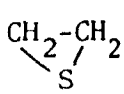
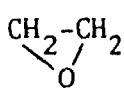
The structure of Grignard reagents has been the subject of speculation for a long time and consequently prompted examination by NMR. The directly bonded coupling constant in CH_3MgI was found to be 108 Hz (36) which corresponds to a hybridization of $s^{.8}p^{1.2}$ thereby giving a rough measure of the degree of ionization of the C-Mg bond. If ionization were complete, the coupling should not be influenced by the nature of the metal atom. However CH_3Li shows a coupling of 98 Hz (37) indicating a bonding character different than in Grignard reagents.

2.4 Electronegativity effects

Calculation of percent "s" character from $^1J_{C-H}$ is an obvious extension of the relationship discussed above. It must be recognized, however, that whereas this relationship is followed for large changes in $^1J_{C-H}$ as in going from an sp^3 to an sp^2 hybridized carbon atom, it does not necessarily apply for the smaller changes in $^1J_{C-H}$ brought about by electronegative substitution. The directly bonded $^{13}C-^1H$ coupling constant is known to vary with the electronegativity of the substituent (7)(38)(39) as is evident in Table 1.

TABLE 1

Variation of $^{1}J_{C-H}$ with electronegative substitution

Compound	$^{1}J_{C-H}$ (Hz)	Reference
CH_4	125	38
CH_3NH_2	133	38
CH_3SH	138	16
CH_3CN	138	40
CH_3OH	141	38
CH_3NO_2	147	41
CH_3Cl	150	38
$CH_2(OCH_3)_2$	162	42
CH_2Cl_2	178	38
$CHCl_3$	209	38
	169	43
	171	43
	176	43

Since, as previously mentioned, these differences cannot be fully rationalized in terms of bond polarity (14)(15), the hybridization approach must be examined again. It is generally recognized (44) that replacement of X in the structure H-^{13}C-X by a more electronegative group causes the carbon atom to rehybridize in such a manner as to increase the "s" character of the ^{13}C-H bond. In other words, this replacement increases the effective electronegativity of the carbon towards the hydrogen atom and since electronegativity

of an atomic orbital is expected to increase with "s" content, the carbon atom is rehybridized in such a manner as to increase the "s" content of its orbital with H. This rehybridization is accompanied by a commensurate shortening of the $^{13}C-^{1}H$ bond and has led to the development of expressions relating bond lengths and electronegativity to $^{1}J_{C-H}$. Examination of the available experimental data, for example, has led to the following empirical relationships (45):

$$r(C-H) = 1.1597 - 4.17 \times 10^{-4}\ {}^{1}J_{C-H}$$

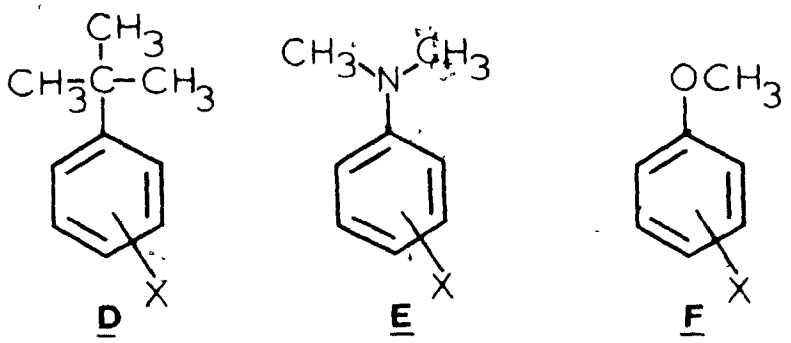
and $$^{1}J_{C-H} = 22.6\ Ex + 40.1\ r(C-X) + 5.5$$

where r(C-H) and r(C-X) represent bond lengths and Ex is the electronegativity of the substituent X.

These relationships seemed promising since they afforded data for bond angles, bond lengths and electronegativities from directly bonded coupling constants which, at least for simple compounds, are readily measurable. Nevertheless, great care must be taken in the application of such relationships. For example, if an attempt is made to predict bond angles in this manner it must be recognized that interorbital angles are not necessarily equal to interatomic angles, that is, the possibility of bent bonds must be appreciated. Using the above equation for methyl halides, the percent "s" character is calculated to be 30%, which gives an interorbital angle of 102° for the H-C-X bonds instead of the experimental value of 107°. Furthermore, as will become apparent later in this chapter, electronegativity effects are not the sole determinants of $^{1}J_{C-H}$; lone pair effects, steric effects and substituent orientation effects can all play vital roles. In the light of these aspects, all results relating to bond lengths and electronegativities derived from directly bonded

carbon-hydrogen coupling are best recognized as approximations.

Variations in $^1J_{C-H}$ due to electronegative substitution have also been examined in aromatic systems (46). These systems are particularly amenable to such a study since the transmission of electronic effects can be pronounced. Examination of various ring substituted toluenes shows that the methyl carbon-hydrogen coupling varies with the electronegativity of the substituent. Intuitively, the substituent should modify the electronic structure of the molecule and since $^1J_{C-H}$ also should be dependent upon this factor, a relationship can be expected. The impact of electronegative substitution on the directly bonded carbon-hydrogen coupling is further demonstrated by plots of the Hammet σ constants versus $^1J_{C-H}$ of the methyl group for compounds D, E, and F (46).



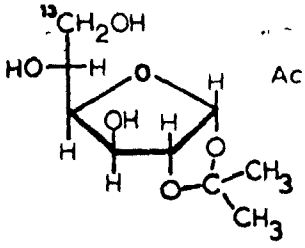
D E F

The above mentioned plots indicate a direct proportionality between σ and $^1J_{C-H}$ for the meta and para derivatives. If one regards $^1J_{C-H}$ as a function of "s" character and effective nuclear charge, (see Section 2.6) one must conclude that the coupling constant is a function of the electronegativity of X since both of these parameters are related to the electronegativity of X. For electron releasing groups (σ negative), the effective electronegativity of the whole ring is reduced and J is decreased. The slopes of the plots should accordingly be a measure of the efficiency of transmission of electronic effects. In the particular case above these slopes are in the order E > F > D resulting probably from overlap of the nitrogen or oxygen lone pairs with the

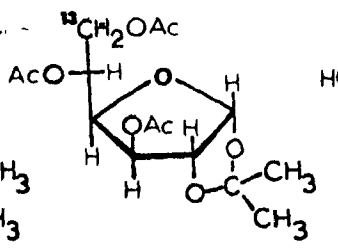
aromatic system.

Since the aforegoing trends are usually discussed in terms of the variation in "s" character it is desirable to have an a priori calculation of this parameter to compare with values derived from coupling data. Such an approach has been taken by Goldstein and Hobgood (47) who calculated "s" character using the method of maximum orbital overlap, and found good agreement with the corresponding data provided by coupling constants. Essentially the calculation of maximum overlap shows that replacement of H by, for example, halogen tends to favor the $C_{2p\sigma}$ orbital in the C-X bond and that H exhibits a marked preferance for the C_{2s} orbital. It is noteworthy that the calculations indicate that the halogen orbitals are not purely p orbitals but that they also have some "s" character (48). Inclusion of these "s" characteristics of the halogen orbitals does not change the overall agreement.

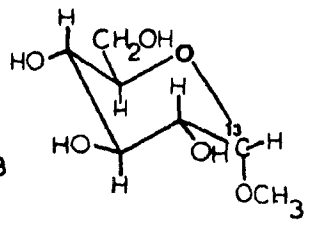
The overall variation of $^1J_{C-H}$ with electronegativity of substituents is reproduced in the present study encompassing carbohydrates. As is obvious from the examples cited below, $^1J_{C-H}$ increases with increasing electronegativities of substituents;



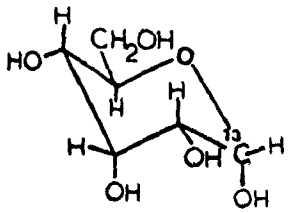
$^1J_{C-H}$ = 143 Hz



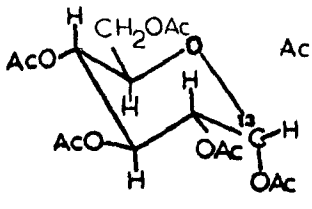
$^1J_{C-H}$ = 149 Hz



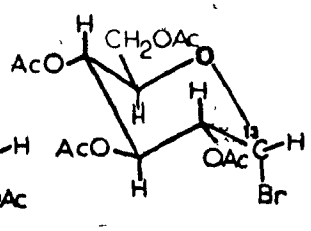
$^1J_{C-H}$ = 168 Hz



$^1J_{C-H}$ = 169 Hz



$^1J_{C-H}$ = 177 Hz



$^1J_{C-H}$ = 185 Hz

This general increase however is modulated by various stereochemical aspects of the molecules involved, as will become evident in later sections.

2.5 Additivity relationships

The variation of $^1J_{C-H}$ with electronegative substitution prompted attemps to find additivity relationships for directly bonded carbon-hydrogen coupling constants. Malinowski (38) examined substituent additivity effects in compounds of the type CHXYZ and formulated the relation:

$$^1J_{C-H} = S_X + S_Y + S_Z$$

where the S's are coupling components for various substituents determined empirically. This relationship leads to reasonable agreement with experiment as is evident from the representative examples in Table 2.

TABLE 2 Additivity relationships for $^1J_{C-H}$ (38)

Substituent	S (Hz)
H	41.7
Cl	68.6
Br	68.6
I	67.6
OH	59.6
C_6H_5	42.6
CH_3	42.6
$C(CH_3)_3$	38.6

.... continued

Table 2 (cont'd)

Compound	$^1J_{C-H}$ (calc.) Hz	$^1J_{(C-H)}$ (exp.) Hz	Δ
$C_6H_5\underline{C}H_2C_6H_5$	127	127	0
$\underline{C}H_2Br_2$	179	178	+1
$CH_3\underline{C}H_2I$	152	149	+3
$\underline{C}H_2I_2$	177	173	+4
$\underline{C}HCl_3$	206	209	-3
$(CH_3)_3C\underline{C}H_2OH$	139	132	+7

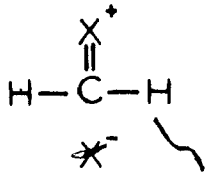
To illustrate, the value of 178 Hz for dibromomethane is arrived at by summing the substituent effects of hydrogen, bromine and bromine, i.e., 41.7 + 68.6 + 68.6 respectively. In general, the substituent effects depend on the first atom of the substituent and decrease in the order X > O > N > C. A similar relationship was arrived at by Juan and Gutowsky (16) accompanied by a theoretical justification. Other relationships were subsequently found for aldehydes (49)(50) and heteroaromatics (51). An increase in coupling with increase in substituent electronegativity is also seen with aromatic compounds (52) but no additivity relation is evident.

It was soon realized, however, that the Malinowski type relationships do not hold equally well for all compounds; considerable deviation occurs when one or more of the substituents attached is highly electronegative, as can be seen in Table 3 (40)(42).

TABLE 3 - Deviations from additivity relationships for $^1J_{C-H}$

Compound	$^1J_{C-H}$ (exp.) Hz	$^1J_{C-H}$ (calc.) Hz	Δ
CH_2F_2	185	173	+12
CHF_3	238	197	+41
$CHFCl_2$	220	203	+17
$CH_2(OCH_3)_2$	162	155	+ 7
$CH(OCH_3)_3$	186	170	+16

The possibility arises that the lone pairs on the substituent in question play a role in determining the extent of coupling (41). This can be visualized in valence bond terms as contributions to the ground state of the molecule of structures such as below:



This structure leads to an increase in "s" character of the carbon and consequently a larger coupling than predicted is observed. Accordingly, Douglas (53) and Malinowski himself (54) modified the additivity relationship to include possible pairwise interactions:

$$^1J_{C-H} = 125 + N_X f(X) + N_{X-Y}\, g(X,Y)$$

where N_X = number of X substituents

$f(X)$ = direct substituent effect

N_{X-Y} = number of interactions

$g(X,Y)$ = interaction effect

Both the direct and the pairwise interaction effects were obtained empirically and lead to good agreement with experiment. The pairwise interaction concept has

also received theoretical support (41)(55). Table 4 lists these effects as well as the results obtained for the compounds of Table 3 by taking pairwise interactions into account.

TABLL 4a - Direct and pairwise interaction effects for the calculation of $^1J_{C-H}$ in disubstituted methanes

Direct effect		Interaction effect		
X	f(X) Hz	X	Y	g(X,Y) Hz
F	24	F	F	13
Cl	25	F	Cl	9
OCH_3	15	OCH_3	OCH_3	6

TABLE 4b - Calculation of $^1J_{C-H}$ by additivity relationships taking pairwise substituent interactions into account

Compound	$^1J_{C-H}$ (calc.) Hz	$^1J_{C-H}$ (exp.) Hz	Δ
$\underline{C}H_2F_2$	186	185	+1
$\underline{C}HF_3$	236	238	-2
$\underline{C}HFCl_2$	220	220	0
$\underline{C}H_2(OCH_3)_2$	161	162	-1
$\underline{C}H(OCH_3)_3$	188	186	+2

The contrast in the Δ values between Tables 3 and 4b affirms the notion that factors other than substituent electronegativities can influence the extent of coupling between the carbon-13 and hydrogen nuclei even in simple substituted methanes. Other relationships taking pairwise interactions into account also have been derived (56), but those for alkenes are not promising (57). It has been suggested that π electron contributions may account for the differences in this case.

2.6 Effective nuclear charge

Although it is generally accepted that correlations exist between hybridization and $^1J_{C-H}$, there has been controversy as to whether or not hybridization is the main factor determining the extent of observed coupling. Some of the problems associated with ascribing electronegative substituent effects to rehybridization have already been described. One knows, for example, that although $^1J_{C-H}$ in methyl halides is about 25 Hz larger than in methane, the bond angles do not show the necessary rehybridization. Similarly some sp^2 systems such as fluoroformaldehyde would require an angle of 108° whereas 122° is observed experimentally (50). Attempts to account for this deviation using delocalized MO theory (58) proved to be unsuccessful.

In view of such anomalies, Grant and Litchman proposed (59) that the determining factor was in fact the so-called "effective nuclear charge" and formulated the relationship:

$$^1J_{C-H}(K) = \left[\frac{\Delta_{CH_4}}{\Delta_K}\right]\left[\frac{N_K}{N_{CH_4}}\right]^2\left[\frac{\alpha_K}{\alpha_{CH_4}}\right]^2\left[\frac{Z_K}{Z_{CH_4}}\right]^3 \cdot {}^1J_{CH_4}$$

where Δ is the average excitation energy (which should not vary greatly for C-H bonds)

N is the bond normalization constant

α^2 is the parameter which is directly proportional to the "s" character

Z is the effective nuclear charge

This equation relates the coupling constant in the K^{th} bond of a substituted methane to that in methane itself. The effective nuclear charge is essentially determined by the screening effect of the electrons. If the electrons are withdrawn, the hydrogen "sees" a larger charge at the nucleus. Grant and

Litchman calculated $(Z_K/Z_{CH_4})^3$ from bond dipole moments (Table 5).

TABLE 5 - Effective nuclear charge in substituted methanes (59)

Compound	Effective nuclear charge on carbon
CH_4	1
CH_3F	1.213
CH_2F_2	1.454
CHF_3	1.725

If the excitation energies and N are assumed to be constant, it is evident that variations in the effective nuclear charge can account for variations in $^1J_{C-H}$ and that it is not necessary to invoke large changes in hybridization. The Malinowski additivity parameters may also be explained by additive incremental changes in $(Z_K)^3$ for a substituted carbon atom.

Lunazzi and Taddei have also shown that $^1J_{C-H}$ is proportional to the effective nuclear charge both of proton and carbon (60) and suggest that these can be derived from the coupling constant. Nevertheless, the assumption of constant excitation energy, which is vital to the "effective nuclear charge" argument, has been criticized (61)(62). In fact, it has been demonstrated that ΔE is not necessarily constant; the larger the number of substituent atoms, the smaller the excitation energy (Table 6) and consequently the larger the coupling.

TABLE 6 - Excitation energies in substituted methanes (63)

Compound	Excitation energy relative to methane (E_X/E_{CH_4})
CH_4	1
CH_3F	0.78
CH_2F_2	0.54
CHF_3	0.45

On the other hand, there exists theoretical support for the effective nuclear charge concept (63)(64) as well as the following experimental evidence (65). If the coupling constant represents in some manner the character of the valence electrons from a hybridization and energetic viewpoint, it should correlate with the force constant "k" for the bond, which is also a measure of such interactions (66)(67). The force constant can be derived from infrared data. Such a correlation has in fact been demonstrated at least for CH_3F, CH_3Cl, CH_3Br, CH_3I and for the methylene groups in cyclohexane, cyclopentane, cyclobutane, propane and ethylene (68), as well as for $(CH_3)_4X$ where X = C, Si, Sn and Pb (69). The orbital contributions to the force constant have been examined theoretically (65) and it was found that a variation in fractional "s" character of the carbon orbital to hydrogen does not, of itself, lead to a significant variation in "k". The force constant, however, does increase with increasing ionic character of the C-H bond. The good correlation between k and $^1J_{C-H}$ is therefore a form of experimental support for Grant and Litchman's treatment.

A correlation of $^1J_{C-H}$ with group electronegativities (70)(71) (e.g. CH_nCl, $CHCl_2$, CCl_3 groups) in substituted methanes (72) has been demonstrated. Since the calculation of group electronegativities does not necessitate changes in the hybridization of various atoms it is again

evident that it is possible to account for the variations in $^1J_{C-H}$ without invoking corresponding changes in the hybridization of the carbon atom.

Also, the fact that $^1J_{C-H}$ in $CHCl_3$ increases with an increase in the extent of hydrogen bonding (73)(74), i.e., with increasing bond length, would not favor the hybridization approach. Similarly, $^1J_{C-H}$ in CH_3Cl is 148.6 Hz for the neat liquid and 147.5 Hz for the vapour (75), again implying that the coupling is larger in the associated state where the bond is longer and is consequently believed to possess less "s" character.

It is important to note, however, that the effective nuclear charge and hybridization effects are not completely separable since orbital electronegativity (70) is dependent on the "s" character of a bond. In other words, changes in hybridization can also bring about changes in Z. If a halomethane is considered, increase in "s" character in the hydrogen bonding orbital of the carbon will result in a decrease in "s" character and electronegativity of the bonding orbital towards halogen, thereby increasing the positive charge on the carbon.

It is obvious that at present no conclusive theoretical interpretation of $^1J_{C-H}$ is available. It seems that the "s" character approach is suitable for simple hydrocarbons, but for substituted compounds (76) the computed $^1J_{C-H}$ is substantially more sensitive to substituent variation than the "s" character approach would indicate. It may therefore be possible to observe large substituent effects without large variations in "s" character. Although both "s" character and "effective nuclear charge" approaches to the interpretation of the directly bonded coupling constant can be useful it must again be emphasized that neither of these factors <u>alone</u> determines the extent of observed coupling.

The various factors dealt with later in this Chapter must always be taken into account as well.

2.7 Stereochemical aspects of directly bonded ^{13}C-^{1}H coupling

Directly bonded ^{13}C-^{1}H coupling constants were readily measured from the proton spectra of many of the enriched carbohydrates and these values are listed in Table 7. As an illustrative example of how these couplings were obtained, Figure 2 depicts the 220 MHz pmr spectra of 1,2-O-isopropylidene-3,5,6-tri-O-orthoformyl-α-D-glucofuranose and that of its 6-^{13}C analog. For this compound the directly bonded couplings are easily measured since the relevant ^{13}C satellites are clearly identifiable in the lower spectrum. Such is not always the case, especially for the 6-^{13}C derivatives; often one of the ^{13}C satellites is masked by other proton absorptions thereby preventing the measurement of the separation of the satellites. Even in these instances it is sometimes possible to obtain the coupling constant from the spectrum by measuring the distance between one satellite and the central absorption due to non-enriched material and equating this distance to $1/2\ ^{1}J_{C-H}$. In this procedure it must be recognized that the central absorption is not necessarily exactly half-way between the satellites due to a possible isotope shift (77)(78)(79). In other words the chemical shift of a proton appended to a ^{13}C nucleus is slightly displaced from the shift of this proton when attached to ^{12}C. All such isotope effects were measured to be less than 1 Hz in the present study and consequently are not a source of significant error.

Examination of the values for $^{1}J_{C-H}$ in Table 7 along with the observations of Bock et al. (80) indicates that there is a difference between the observed coupling at the anomeric centre in the α, as compared with the β series. In all cases the α, or equatorial proton, exhibits a coupling with

TABLE 7 - Directly bonded ^{13}C-^{1}H coupling in enriched carbohydrates

Compound	$^{1}J_{C-H}$ ($\pm\frac{1}{2}$ Hz)
1,2-O-Isopropylidene-α-D-glucofuranose-6-^{13}C	143, 143
1,2-O-Isopropylidene-3,5,6-tri-O-acetyl-α-D-glucofuranose-6-^{13}C	148, 148
1,2-O-Isopropylidene-α-D-glucofuranose-1-^{13}C	184
1,2-O-Isopropylidene-3,5,6-tri-O-orthoformyl-α-D-glucofuranose-6-^{13}C	148, 154
1,2-O-Isopropylidene-3,5,6-tri-O-orthoformyl-α-D-glucofuranose-1-^{13}C	184
1,2-O-Isopropylidene-α-D-glucofuranose-6-^{13}C periodate complex	148, 153
1,2:5,6-Di-O-isopropylidene-α-D-glucofuranose-6-^{13}C	148, 149
1,2:5,6-Di-O-isopropylidene-α-D-glucofuranose-1-^{13}C	183
1,2:5,6-Di-O-isopropylidene-3-O-acetyl-α-D-glucofuranose-1-^{13}C	183
1,2:5,6-Di-O-isopropylidene-α-D-ribohexofuranose-3-ulose-1-^{13}C	180.5
1,2:5,6-Di-O-isopropylidene-α-D-ribohexofuranose-3-ulose (monohydrate)-1-^{13}C	186
1,2:5,6-Di-O-isopropylidene-α-D-allofuranose-1-^{13}C	183
1,2-O-Isopropylidene-β-L-idofuranose-6-^{13}C	143, 143
1,2-O-Isopropylidene-3,5,6-tri-O-acetyl-β-L-idofuranose-6-^{13}C	148, 148
2,3:5,6-Di-O-isopropylidene-α-D-mannofuranose-1-^{13}C	174.5
2,3:5,6-Di-O-isopropylidene-1-O-acetyl-α-D-mannofuranose-1-^{13}C	181

Compound	$^{1}J_{C-H}$ (Hz)
α-D-Glucofuranurono-6,3-lactone-1-^{13}C	170
β-D-glucofuranurono-6,3-lactone-1-^{13}C	175
Methyl-α-D-glucopyranoside-6-^{13}C	141, 142
Methyl-α-D-glucopyranoside-1-^{13}C	168.5
α,β-D-Glucopyranose-6-^{13}C	143, 143
α-D-Glucopyranose-1-^{13}C	169
β-D-Glucopyranose-1-^{13}C	161
α-D-Mannopyranose-1-^{13}C	169
β-D-Mannopyranose-1-^{13}C	160
1,2,3,4,6-Penta-O-acetyl-α-D-glucopyranose-6-^{13}C	148, 150
1,2,3,4,6-Penta-O-acetyl-β-D-glucopyranose-6-^{13}C	148, 150
1,2,3,4,6-Penta-O-acetyl-α-D-glucopyranose-1-^{13}C	177
1,2,3,4,6-Penta-O-acetyl-β-D-glucopyranose-1-^{13}C	166.5
1,2,3,4,6-Penta-O-acetyl-α-D-mannopyranose-1-^{13}C	177
1,2,3,4,6-Penta-O-acetyl-β-D-mannopyranose-1-^{13}C	162
β-D-Allose-1-^{13}C	163
1,2,3,4,6-Penta-O-acetyl-β-D-allopyranose-1-^{13}C	168

Compound	$^{1}J_{C-H}$ (Hz)
1,5-Anhydro-D-glucitol-1-^{13}C-1-d	141
2,3,4,6-Tetra-O-acetyl-1,5-anhydro-D-glucitol-1-^{13}C-1-d	142
1,6-Anhydro-α-L-idopyranose-6-^{13}C	149.5, 154
2,3,4,6-Tetra-O-acetyl-α-D-glucopyranosyl bromide-1-^{13}C	184
2,3,4,6-Tetra-O-acetyl-α-D-mannopyranosyl bromide-1-^{13}C	184
3,4,6-Tri-O-acetyl-β-D-mannopyranose-1,2(methyl orthoacetate)-1-^{13}C	176
Methyl 4,6-O-benzylidene-α-D-glucopyranoside-1-^{13}C	168
Methyl 4,6-O-benzylidene-α-D-glucopyranoside-6-^{13}C	141.5, 151

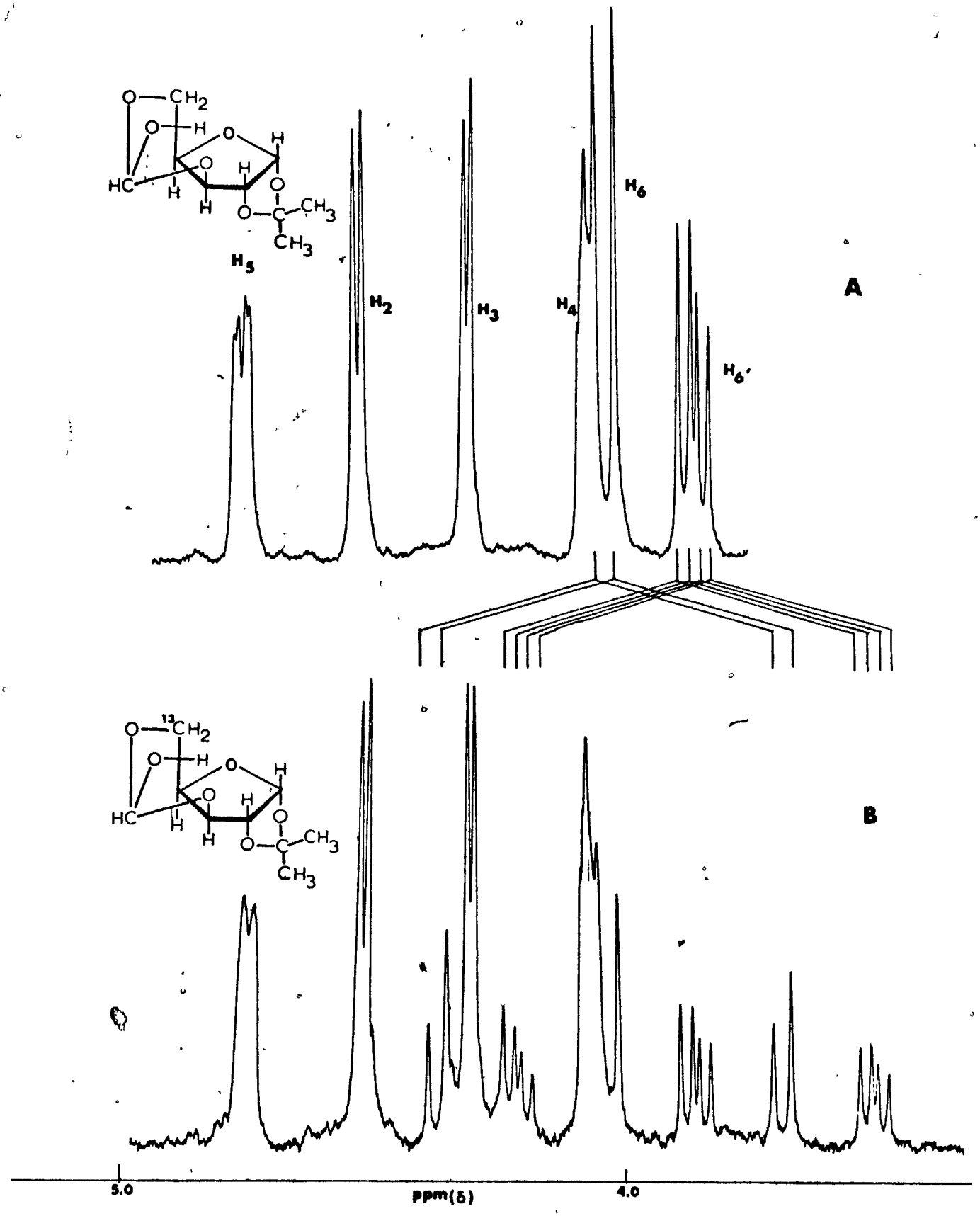
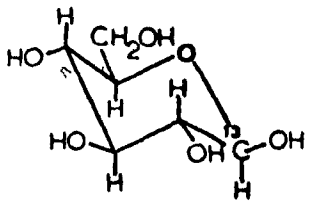
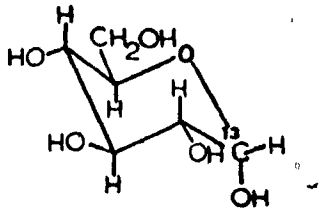


FIG. 2 Partial pmr spectrum (100 MHz) of A) 1,2-O-Isopropylidene-3,5,6-tri-O-orthoformyl-α-D-glucofuranose and B) 1,2-O-Isopropylidene-3,5,6-tri-O-orthoformyl-α-D-glucofuranose-6-^{13}C (60%) in $CDCl_3$.

$^{13}C_1$ roughly 8-10 Hz greater than the β, or axial proton. For example, comparing α and β-D-glucose 1-^{13}C, the α coupling was found to be 169 Hz while the β coupling was measured to be 161 Hz.



$^1J_{C-H}$ = 161 Hz



$^1J_{C-H}$ = 169 Hz

According to any of the aforementioned additivity relationships, these values should be equal since the central carbon atom bears equivalent substituents. In other words, if C_1 with its four substituents is isolated from the sugar ring, the resulting situations for the α and β forms are equivalent. It is evident then that the nature of such coupling is more complex than previously believed. However, the very fact that the two values differ can be potentially useful even aside from the obvious empirical value of differentiating between anomers.

Most of the theoretical treatments performed to date, as well as the various empirical additivity relationships, do not take into account the respective orientation of the atoms in question. It now becomes obvious that the orientation of the substituents with respect to the C-H bond being investigated, as well as the orientation of these substituents with respect to the rest of the molecule may effect the observed ^{13}C-^1H coupling constant significantly.

The best acyclic model for the systems under investigation here, and for which the directly bonded coupling is measurable, is dimethoxy methane, $^{13}CH_2(OCH_3)_2$, which exhibits a coupling of 162 Hz (51). This coupling is much closer in value to those observed for the β sugar series than for the α. The most relevant comparison can be made with the anomeric methyl-D-glucopyranosides-1-^{13}C which show couplings of 159 Hz and 168 Hz for the β and α anomers respectively. It seems, therefore, that it is the coupling observed in the α anomer which is extraordinarily large and consequently necessitates a rationale.

Since internuclear coupling is essentially transmitted through bonding electrons (10), any condition that leads to a perturbation of the electronic environment around the ^{13}C and ^{1}H nuclei experiencing coupling can conceivably affect the magnitude of $^{1}J_{C-H}$. The structural features that can give rise to such perturbations and consequently may have an impact on $^{1}J_{C-H}$ can be examined individually. While a 1,3 diaxial hydrogen-hydrogen interaction normally is not expected to have any destabilizing effects associated with it, the interaction of OH_1 with H_3 in the α anomer is certainly destabilizing and can result in changes in the electronic environment at C_1. It can also be noted that in the β anomer the C_2-C_3 and ring O_5-C_5 bonds are oriented gauche with respect to the C_1-H_1 bond whereas a trans situation exists in the α anomer. A similar possible variable is the orientation of the C_2-O_2 bond relative to the C_1-H_1 bond. This orientation is the same for the glucose anomers discussed above, but is different for anomers possessing the manno configuration. Another factor which can conceivably influence ^{13}C-^{1}H directly bonded coupling in the 1-^{13}C sugars is the orientation of the non-bonded electron pair lobes on the ring oxygen and on OH_1 with respect to the ^{13}C-^{1}H

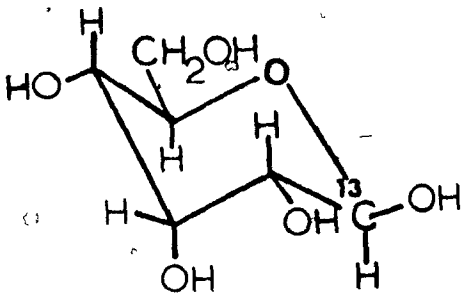
bonds in the α and β anomers.

2.8 Steric interaction effects on $^{1}J_{C-H}$

Three basic types of steric interactions which can influence internuclear $^{13}C-^{1}H$ coupling are conceivable. The first two involve 1,3 diaxial type interactions; either the coupling proton, or one of the substituents appended to the coupling ^{13}C nucleus, can interact with other atoms in the molecule. The third type of steric interaction involves the destabilization associated with a decreased dihedral angle between a C-H bond and a neighbouring bond and is usually referred to as "torsional" strain (81).

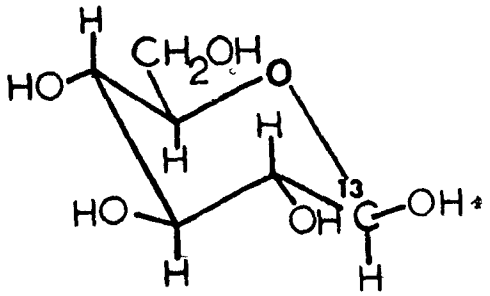
1,3 Diaxial interaction effects

In order to investigate the first of the above possibilities, the coupling between $^{13}C_1$ and H_1 of β-D-allopyranose-1-^{13}C (for the synthesis of this compound see p. 102) was compared to the corresponding coupling in β-D-glucopyranose-1-^{13}C:



β-D-Allopyranose-1-^{13}C

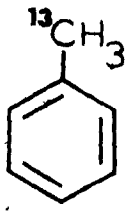
$^{1}J_{C-H}$ = 163 Hz



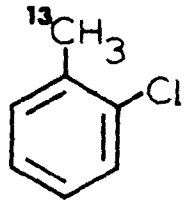
β-D-Glucopyranose-1-^{13}C

$^{1}J_{C-H}$ = 161 Hz

The allose and glucose molecules differ in their configuration about C_3: the C_3 hydroxyl substituent is axial in allose and equatorial in glucose thereby setting up a significant 1,3 diaxial interaction between H_1 and OH_3 in the allose moiety. This interaction is seen to result in an increase in coupling between $^{13}C_1$ and H_1 of 2 Hz. The increase may also have an electronic component, as a secondary factor may be involved here: that of situating an electronegative atom in proximity to the hydrogen undergoing coupling. Such "through space" effects are possible although the data available are limited: toluene and o-chlorotoluene show couplings for the methyl groups of 125.5 and 127.6 Hz respectively (82).



$^1J_{C-H}$ = 125.5 Hz

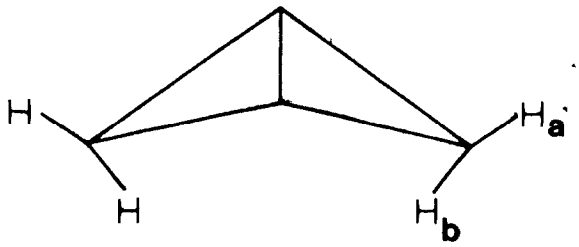


$^1J_{C-H}$ = 127.6 Hz

The distance between the methyl protons and the chlorine atom in the above compound is about 2.5Å, the same as the distance separating the protons and the chlorine atom in $CHCl_3$. Evidently then, placing a halogen in close proximity to the protons does not provide major changes in $^1J_{C-H}$ unless, of course, the halogen is chemically bound to the ^{13}C nucleus, but nevertheless changes of about 2 Hz can be realized. Similarly, placing a methoxyl group in the ortho position of toluene results in a change of less than 2 Hz for the methyl ^{13}C-^1H coupling (46). In all probability even a part of these increases is due to inductive effects transmitted through the bonding network, although in general such γ substitution effects are small. Furthermore the

distance between the 1,3 diaxial substituent (i.e. H_1 and OH_3) in β-D-allose is greater than the distance giving rise to the "through space" effects described above, so that it can be deduced that the steric interaction introduced by inversion of configuration at C_3 of β-D-glucose likely accounts for the observed difference of 2 Hz in $^1J_{C-H}$.

This concept is further substantiated by an examination of the bicyclopropane system (83), perhaps the most extreme example available for proton-proton steric interaction:



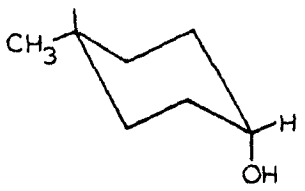
$^1J_{C-H_a}$ = 153 Hz

$^1J_{C-H_b}$ = 169 Hz

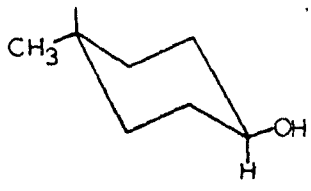
In this compound the endo protons are expected to experience a particularly strong steric compression, which is reflected in the observed coupling of 169 Hz in comparison to 153 Hz for the exo protons.

The second type of steric interaction which may be considered a possible influencing force on directly bonded coupling, is a situation where one of the substituents on the ^{13}C nucleus to which the proton is appended interacts sterically with another part of the molecule. This is the case for α-D-glucose and its derivatives; the axial substituent on C_1 maintains a presumably unfavourable 1,3 diaxial relationship with H_3. The resulting perturbation may then affect C_1 and consequently the $^{13}C_1$-H_1 coupling. This possibility was investigated by synthesizing a cis-trans mixture of 4-methylcyclohexanol and examining the $^{13}C_1$-H_1 couplings for the isomers. The cis-trans mixture is readily prepared by reduction of 4-methylcyclohexanone, and the couplings can be measured from the ^{13}C-Fourier transform spectrum of

the mixture. The C_1 absorption in this case is readily assigned since it is the only carbon which bears an electronegative substituent and consequently has a downfield chemical shift. The axial hydrogen was found to give rise to a coupling of 140 Hz whereas the equatorial hydrogen coupled to the extent of 143 Hz.*



$^1J_{C-H}$ = 143 Hz



$^1J_{C-H}$ = 140 Hz

It appears that the entire difference (∿8-10 Hz) between the $^{13}C_1$-H_1 couplings of the carbohydrate anomers cannot be explained by this type of steric interaction. Intuitively, such steric effects are expected to have an impact on $^1J_{C-H}$ due to the perturbation of the electronic environment of $^{13}C_1$ caused by the 1,3 diaxial interaction. On consideration of relevant ^{13}C chemical shift values it is evident that such a perturbation can indeed result from the interaction between H_3 or H_5 and OH_1. In the carbohydrates, $^{13}C_1$ of the α anomer is consistently more shielded than is $^{13}C_1$ of the β anomer (85)(86). This shielding has been attributed to a repolarization of the C_1-H_1 bond on going from the β to the α derivative, a hypothesis that receives support from the observed deshielding of H_1 in the α anomer

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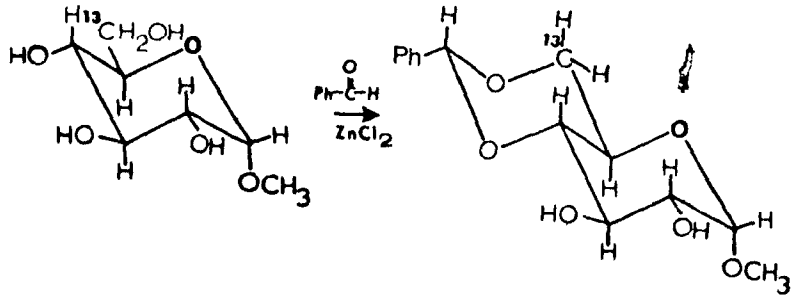
* Although the cis isomer can flip to the alternate chair form, the chair with CH_3 equatorial and OH axial, predominates to an extent of 85% (84).

relative to the β (87). Parallel effects have been noted in cyclohexane derivatives (88)(89)(90)(91), an increase in ^{13}C shielding being associated with steric crowding. Since it is known that ^{13}C chemical shifts can be related to the electron densities at the carbon in question (92)(93) and that internuclear ^{13}C-^{1}H coupling is also dependent on the electronic environment of the ^{13}C nucleus (10), one would expect that such coupling should be affected by the type of steric crowding just mentioned above. It still seems imperative, however, to examine a system where such steric interactions are absent and determine whether or not a difference in ^{13}C coupling to the equatorial and axial protons is nevertheless observed. A model is necessary then in which no substituent capable of giving rise to 1,3 diaxial type interactions is present on the carbon experiencing hydrogen coupling; ideally the axial and equatorial protons in question should be bonded to the same ^{13}C nucleus. Such a situation can be realized in methyl 4,6-O-benzylidene-α-D-glucopyranoside-6-^{13}C which was accordingly synthesized in the following manner:



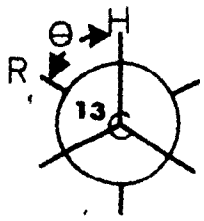
There is no 1,3 diaxial interaction involving the C_6 protons in the above compound. The H_6 and H_6' absorptions in the proton magnetic resonance spectrum can be easily assigned since the axial proton couples

strongly with H_5 (dihedral angle = 180°) whereas the equatorial proton couples to a smaller extent (dihedral angle = 60°). The measured $^{13}C_6$-H_6 and $^{13}C_6$-$H_{6'}$ couplings are striking; the coupling of $^{13}C_6$ to the equatorial proton is 151 Hz, compared with 141 Hz to the axial proton. The difference between these couplings is in the same range as that observed for the anomeric sugars at C_1. This then implies that the observed difference in $^{13}C_1$-$^1H_1$ coupling in the sugar anomers and in the methylcyclohexanols is not a function of the orientation of OH_1. If as previously mentioned, one assumes that ^{13}C chemical shifts are dependent on electron density, one now is led to the curious conclusion that the directly bonded ^{13}C-^1H coupling constants do not reflect electron density differences at the ^{13}C nucleus. Thus either the applicability of the effective nuclear charge concept as discussed earlier in this chapter, or the validity of attributing the shielding of a sterically hindered carbon to changes in electron density, becomes questionable.

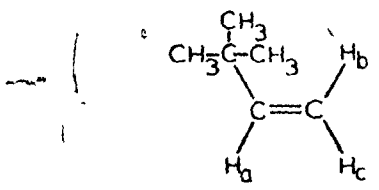
The 3 Hz difference between the ^{13}C-H_e and ^{13}C-H_a coupling in cis and trans 4-methylcyclohexanol must then be attributed to factors other than a 1,3 diaxial interaction. This deduction leads naturally into a discussion of the third possible type of steric effect, that associated with torsional strain.

Torsional strain effects

If the projection below is considered, it is anticipated that $^1J_{C-H}$ varies with θ since the approach of the ^{13}C-R bond towards the ^{13}C-^1H bond is expected to have a perturbing effect on the electron distribution in the ^{13}C-^1H bond.

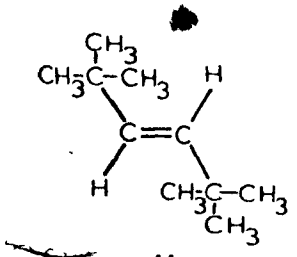


Inherent to the cyclohexane system is the gauche interaction of the axial C-H bond and the trans relationship of the equatorial C-H bond with adjacent carbon-carbon bonds. It is then conceivable that a gauche interaction between the ^{13}C-H bond in question and a neighbouring carbon-carbon bond has a different impact on the electronic nature of the ^{13}C-H bond than does a trans interaction, leading to differences in the observed values of $^{1}J_{C-H}$. Pertinent information along these lines can be derived from a study of certain substituted ethylenes. In ethylene itself the observed $^{13}C-^{1}H$ coupling is 156.4 Hz (42). When a tertiary butyl group is introduced as in G the $^{13}C-H_a$ and $^{13}C-H_b$ couplings are reduced to 150.7 and 153.3 Hz, respectively, whereas the $^{13}C-H_c$ coupling is essentially unaffected (94).



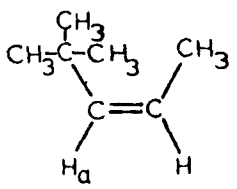
G

Introduction of a second tertiary butyl group as in H leads to a further decrease in $^{1}J_{C-H}$ to 147.6 Hz.

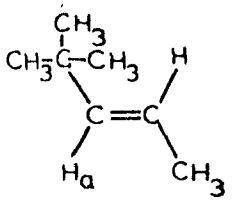


H

Similar effects are noted when a methyl substituent is introduced as in I and J.



I

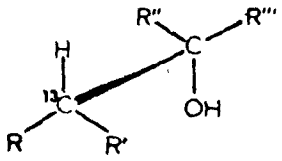


J

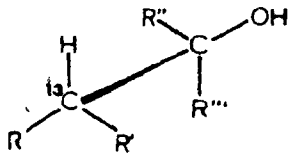
The coupling of the ^{13}C nucleus to the appended proton (H_a) is 2.5 Hz larger when the methyl substituent is trans to the ^{13}C-H bond than when a cis situation is maintained. The preceding results imply that directly bonded ^{13}C-^{1}H coupling is decreased by steric interaction between the ^{13}C-^{1}H bond and an adjacent carbon-carbon bond. It must be noted however that a steric interaction in the above compounds between the ^{13}C-H bond and the carbon-carbon bond, which in this case lie in the same plane, is expected to be larger than the gauche type of interaction in cyclohexane rings. Consequently, large differences are not expected when comparing the directly bonded ^{13}C-^{1}H coupling in systems which differ only by relative orientation (i.e. anti or gauche) of a neighbouring carbon-carbon bond with respect to the ^{13}C-^{1}H bond.

Steric effects involving the orientation of an adjacent C-O bond

In the previous section it was suggested that the relative orientation of an adjacent carbon-carbon bond with respect to a C-H bond has an impact on the $^{13}C-^{1}H$ coupling in this latter bond. Correspondingly, one would intuitively anticipate a difference in $^{1}J_{C-H}$ for rotamers K and L.



K

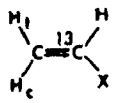


L

As mentioned before, the extent of coupling between nuclei is determined by the electronic character of the bonds through which the coupling is transmitted (10). This electronic character on the other hand can be expected to be influenced by the orientation of neighbouring substituents with respect to the bond involved in $^{13}C-^{1}H$ coupling. A gauche interaction of a polar C-O bond with a C-H bond is expected to perturb the C-H bond in a manner different from the corresponding anti interaction.

Halogen substituted ethylenes do in fact exhibit such a substituent orientation dependence of $^{1}J_{C-H}$ as indicated in Table 8 (95)(96)(97).

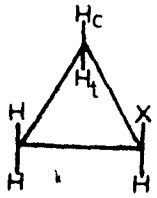
TABLE 8 - $^{1}J_{C-H}$ in substituted ethylenes



X	$^{1}J_{C-H_c}$ (Hz)	$^{1}J_{C-H_t}$ (Hz)
F	159.2	162.2
Cl	162.6	160.9
Br	163.6	160.3
I	164.1	159.2

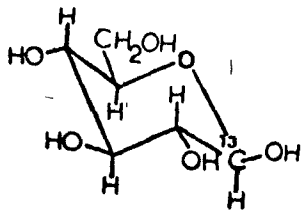
It is apparent from the above data that at least for the ethylene derivatives considered, the coupling of ^{13}C to the proton trans to the halogen decreases as the size of the halogen atom increases whereas an opposite and more pronounced trend is noted for the coupling between ^{13}C and the proton cis to the halogen. This trend is likely caused by overlap of halogen unshared electrons with the ^{13}C-H bond.

Similar effects are noted in other systems as well. In cyclopropyl halides, below, the ^{13}C-H_c coupling is consistently 2 Hz larger than the ^{13}C-H_t coupling (98)(99).



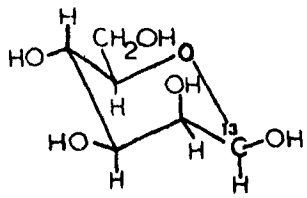
Again, a likely rationale involves the interaction of the halogen lone pairs with the C-H bond, an interaction which is expected to be dependent on the spatial separation of the nuclei in question.

A starting point for examining this aspect of ^{13}C-^{1}H internuclear coupling in carbohydrates is a consideration of the couplings between $^{13}C_1$ and H_1 in β-D-glucose-1-^{13}C and β-D-mannose-1-^{13}C. In this case the C_2-O_2 bond is oriented gauche with respect to the $^{13}C_1$-H_1 bond in β-D-glucose whereas an anti relationship is maintained in β-D-mannose:



β-D-Glucose-1-^{13}C

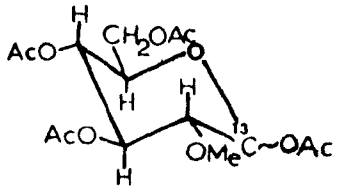
$^{1}J_{C-H}$ = 161 Hz



β-D-Mannose-1-^{13}C

$^{1}J_{C-H}$ = 160 Hz

The measured couplings between $^{13}C_1$ and H_1 in the cases above, however, were within experimental error ($^1J_{C-H}$ = 160 Hz). As this seemed to be a curious result, the corresponding acetates were also examined spectroscopically. In general, acetylation of an hydroxyl appended to the ^{13}C nucleus leads to an increase in $^1J_{C-H}$ of 5 Hz, as exemplified by the comparison of methanol (141 Hz) with methyl acetate (146 Hz) (100). This increase in all likelihood can be attributed to the electronegative character of the acetate function. Such increments in coupling can be expected for the carbohydrate series as well, but since peracetylation leads to the difficulty that the acetyl group at C_2 also can withdraw electrons, it is perhaps more profitable then to examine 1,3,4,6-tetra-O-acetyl-2-O-methyl-D-glucopyranose:



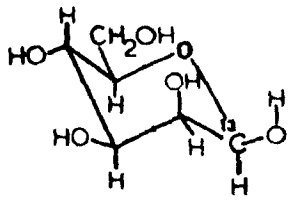
The ^{13}C-^1H couplings in the above compound are measured to be 175 and 165 Hz, for the α and β anomers, respectively (80). Comparison of these values with the corresponding ones in α and β-D-glucose-1-^{13}C (170 and 161 Hz) again shows an increase of 5 Hz upon acetylation at C_1. When D-glucose-1-^{13}C is peracetylated the $^{13}C_1$-H_1 coupling in the α anomer becomes 177 Hz whereas that in the β is measured to be 167 Hz; the extra increase of 2 Hz is therefore attributed to the acetate group at the 2 position of the pyranose ring.

When, on the other hand, 1,2,3,4,6-penta-O-acetyl-D-mannopyranose-1-^{13}C is examined, it is found that whereas the α anomer shows the same increase of 7 Hz for $^1J_{C-H}$ on going from the free sugar to the pentacetate as does glucose,

(Table 7), the β anomer shows the strikingly small increase of 2 Hz. It is clear that in the pentacetates the difference in $^{1}J_{C_1-H_1}$ which had been anticipated for β-D-mannopyranose and β-D-glucopyranose becomes noticeable; a coupling of 162 Hz is measured for mannose pentacetate as compared with 167 Hz for the glucose isomer. No difference is expected, and none is observed, in the couplings between $^{13}C_1$ and H_1 of the α isomers since the $C_2'-O_2$ bond assumes a gauche relationship with the C_1-H_1 bond in both compounds. Seemingly, the relative orientation of the oxygen substituent at C_2 has no impact upon the $^{13}C_1-H_1$ coupling in the free sugars but an orientation effect is exerted in the acetates. The consequent deduction, then, is that electronegative substitution at the carbon once removed from the carbon involved in the coupling can, in certain cases, influence the directly bonded coupling; the specific effect seems to be a decrease in observed coupling when this substituent is oriented trans with respect to the ^{13}C-H bond. Apparently this effect is intensified as the electronegativity of the C_2 substituent is increased.

The problem of why no effect is noted for the free sugars still remains. Both in the unsubstituted sugars and in the acetates there is free rotation about the C_1-O_1 bond. As will become evident in the next section, the extent of coupling between ^{13}C and ^{1}H nuclei can be affected by the relative orientation of the ^{13}C-H bond with respect to unshared electron pairs on neighbouring substituents. Consequently, $^{1}J_{C_1-H_1}$ in the free sugars and in the acetates is also a function of the rotamer populations about the C_1-O_1 bond. It is then conceivable that the effect exerted on $^{1}J_{C_1-H_1}$ by a neighbouring gauche C-O bond relative to a trans C-O bond, as in β-D-mannopyranose, is counteracted by different rotamer populations at C_1. Experimental evidence for these rotamer populations is difficult to obtain. Some indication

though can be had from the H -C-O-H proton-proton coupling which can be measured in DMSO (101). For α-D-glucopyranose and α-D-mannopyranose the couplings are identical suggesting similar rotamer populations, whereas for the β anomers, which are of interest here, there is a difference in this coupling of 1.5 Hz (8 Hz is observed in β-D-mannose, 6.5 Hz in β-D-glucose). Assuming that the 8 Hz reasonably indicates a trans situation whereas 6.5 Hz is less conclusive, the rotamer below should predominate for β-D-mannopyranose.



In this conformation the O_1 free electron pairs are close to the C_1-H_1 bond, thereby creating a stereochemical situation which is expected to lead to increased coupling (Sect. 2.9). This rotamer may be less predominant in β-D-glucopyranose. Hence, in β-D-mannopyranose the effect of a trans oxygen may be partly counteracted by the state of the rotamer population about the C_1-O_1 bond. Upon acetylation however, this rotamer is destabilized due to the unfavourable steric 1,3 diaxial type of interaction for the larger acetate groups. Consequently the electron lone pair effect no longer compensates for the trans oxygen effect, and a difference in coupling between $^{13}C_1$ and H_1 for 1,2,3,4,6-penta-O-acetyl-β-D-glucopyranose-1-^{13}C and the manno epimer now becomes observable.

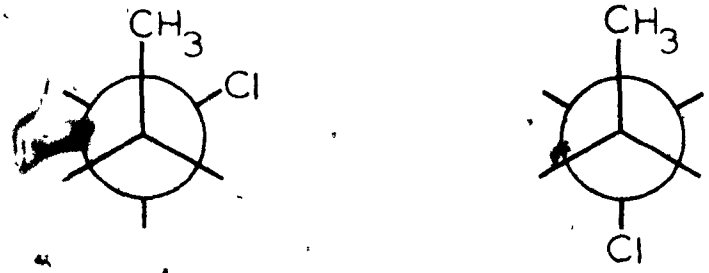
Validity of classical steric effects

The "steric effect" or "steric crowding" terminology was widely used in the previous sections; however, the specific type of mechanism giving rise to the observed effects on $^1J_{C-H}$ is not clear

since it is probable now that the classical bulk or size approach to steric effects is neither quantitative nor adequate. It is known, for example, that in 1-fluoro-1-propene the cis form is more stable than the trans (102).

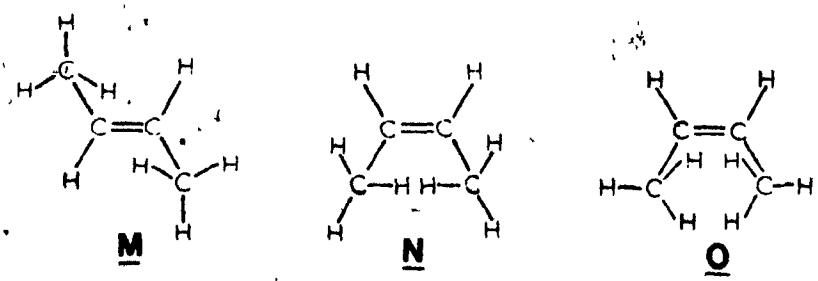
more stable form

In the same vein, the gauche conformation of chloropropane is more stable than the anti form (103).



more stable form

Taking these results into account, it does not seem irrational that adjacent C-O and C-C bonds have seemingly opposite effects on $^{1}J_{C-H}$. Some unexpected steric effects are also found for methyl-methyl interactions. Pople has calculated that for the compounds below M is more stable than N, but is less stable than O (104).



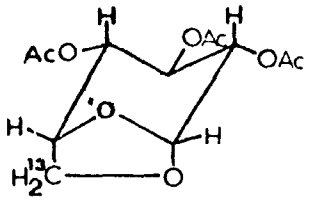
M N O

Experimental evidence is in accord with these findings (105)(106). Intuitively, one would quickly rule out O as a favored rotamer because of the assumed sterically unfavourable interactions between the methyl hydrogens. Pople's calculations show, however, that three separate and distinct terms must be considered when attempting to determine stability. These are respectively the attraction of electrons for nuclei (Vne), interelectronic repulsion (Vee) and internuclear repulsion (Vnn) (107)(108). In general, Vnn + Vee is larger than Vne, and this leads to a situation that can be described by "classical" steric effects. This, however, is not exclusively the case; in certain conformations (as in O above) the protons in question assume such a relationship that the electrons of one are rather strongly attracted to the nucleus of the other thereby making Vne > Vee + Vnn and stabilizing that conformation.

It is evident then that steric effects do not follow the simple patterns which are commonly accepted and relied upon, and caution must be exercised whenever any conclusion is drawn on the basis of "unfavourable" steric effects.

Application of steric effect concepts

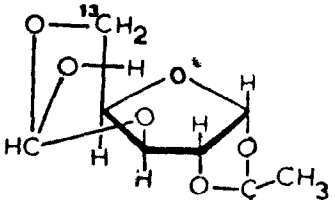
In conjunction with the investigation of $^{1}J_{C-H}$ and steric effects a study of 2,3,4-tri-O-acetyl-1,6-anhydro-α-L-idopyranose-6-^{13}C (P), 1,2-O-isopropylidene-3,5,6-tri-O-orthoformyl-α-D-glucofuranose-6-^{13}C (Q) and the periodate complex of 1,2-O-isopropylidene-α-D-glucofuranose-6-^{13}C (R) is valuable because of the inherent rigidity of these compounds.



P

$^1J_{^{13}C\text{-}H_6}$ = 154 Hz

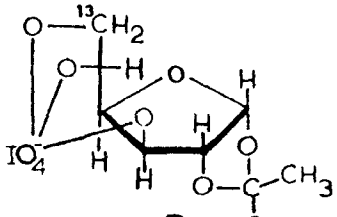
$^1J_{^{13}C\text{-}H_6'}$ = 149 Hz



Q

$^1J_{^{13}C\text{-}H_6}$ = 154 Hz

$^1J_{^{13}C\text{-}H_6'}$ = 148 Hz

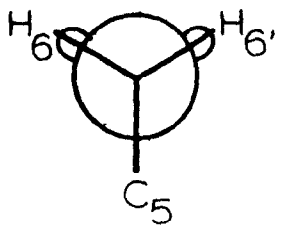


R

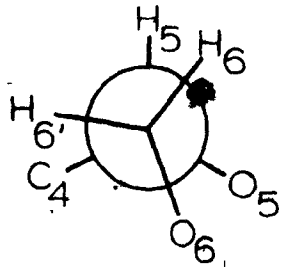
$^1J_{^{13}C\text{-}H_6}$ = 153 Hz

$^1J_{^{13}C\text{-}H_6'}$ = 148 Hz

As noted above, there is again a difference in the couplings between the ^{13}C nucleus and its two appended protons. The two observed couplings can be assigned to the specific protons since the relevant absorptions in the proton magnetic resonance spectrum are readily identified. One of the C_6 protons is coupled to H_5, the other experiences no such coupling. Examination of molecular models provides an explanation: one of the C_6-H_6 bonds is nearly eclipsed by the C_5-H_5 bond whereas the other maintains a dihedral angle of 90° with respect to the C_5-H_5 bond. In each case the coupling of the ^{13}C nucleus to the endo proton is smaller than that to the exo proton. In these instances the observed difference cannot be attributed to the lone pair effects of the appended oxygen (lone pair effects are discussed in the next section) since both C_6-H_6 bonds are eclipsed by a lone pair as illustrated below in projection along the C_6-O_6 bond:



This type of situation is expected to increase both couplings equally and consequently the absolute values of these couplings deserve some attention. The carbon undergoing coupling is incorporated into a five-membered ring and can thus be likened to C_1 of tetrahydrofuran. The coupling observed in this latter compound is 144.6 Hz (43). Even if an allowance of 2 Hz is made for a β oxygen substituent, it is seen that the couplings in P, Q and R are in excess of this value with, in each case, one of the couplings being decidely so. The small increase in $^1J_{C-H}$ relative to tetrahydrofuran for one of the C_6-H_6 couplings, in comparison to the other, can now be attributed to the relative orientation of the C_5 substituents with respect to the C_6-H_6 bonds. The projection along the $^{13}C_6$-C_5 bond for the above compounds is is depicted below:

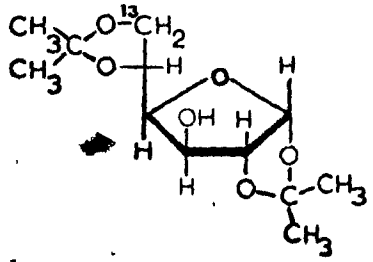


In all cases $^1J_{C-H_6'}$ is less than $^1J_{C-H_6}$. The distinguishing features between the C_6-H_6 and C_6-H_6' bonds are their relative orientation with respect to the C_5-O_5 and C_5-C_4 bonds. The C_6-H_6' bond maintains a

dihedral angle of about 150° with the C_5-O_5 bond and an angle of 30° with the C_5-C_4 bond. For the C_6-H_6 bond these angles are 90° and 150°, respectively. As discussed above, a trans electronegative substituent tends to decrease $^1J_{C-H}$ but, as is apparent from a comparison with β-D-glucose-1-^{13}C, this effect is minimal when a C-OH bond is involved. The C_5-C_4 bond, however, practically eclipses the C_6-H_6' bond whereas it is essentially anti to the C_6-H_6 bond. As noted in the previous section, such an eclipsing type of interaction is expected to result in decreased $^1J_{C-H}$. This notion is now seen to be in agreement with the experimental results.

To summarize the preceding argument, it can be noted that whereas both ^{13}C-H_6 couplings are increased by virtue of these bonds being eclipsed by oxygen lone pairs, the effect is partially counteracted for $^1J_{C-H_6'}$ by the interaction of the C_6-H_6' bond with the C_5-C_4 bond and probably to a smaller extent by the anti orientation of the C_5-O_5 bond.

It is of interest to compare the above systems with a situation that is similar, except for the absence of such fixed steric relationships. A suitable model is found in the 5,6-O-isopropylidene ring of 1,2:5,6-di-O-isopropylidene-α-D-glucofuranose-6-^{13}C:



$^1J_{C_6-H_6'}$ = 149 Hz

$^1J_{C_6-H_6}$ = 148 Hz

In the above compound the ^{13}C nucleus is incorporated into a non-rigid five-membered ring so that the C_5-O_5 and C_5-C_4 bonds do not maintain a fixed orientation* with respect to the C_6-H_6 or C_6-$H_{6'}$ bond. Correspondingly, the difference observed (~1 Hz) between $^1J_{C_6-H_6}$ and $^1J_{C_6-H_{6'}}$ is minimal.

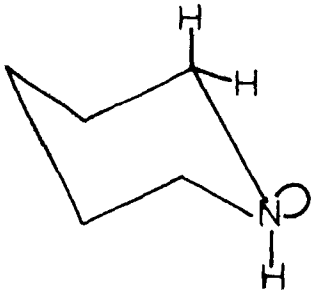
The preceding discussions lead to the overall conclusion that directly bonded ^{13}C-^1H couplings are affected by the relative orientation of neighbouring substituents relative to the ^{13}C-H bond. Although these interactions are probably more of asteric than electronic nature, the presence or absence of unfavourable steric interactions must be interpreted with great care. Also, whereas the gauche interaction of an axial ^{13}C-H bond with adjacent carbon-carbon bonds in cyclohexane type systems (as in trans 4-methylcyclohexanol) can account for a small decrease in $^1J_{C-H}$ relative to a situation where this interaction is absent (see Section 2.8), the observed differences in coupling between $^{13}C_1$ and H_1 of α-aldohexoses relative to the β-anomers (~10 Hz) necessitate another explanation.

2.9 Lone pair effects on $^1J_{C-H}$

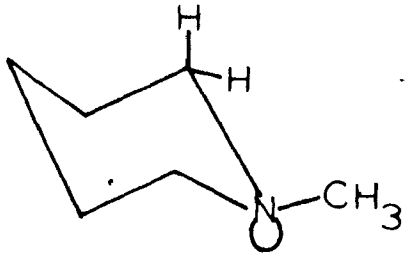
As seen in the previous section, steric interactions cannot account for all of the 10 Hz difference between the $^{13}C_1$-H_1 couplings of the sugar anomers and consequently it is necessary to examine other aspects of the rigid pyranose ring. An obvious difference between the anomeric protons is the proximity of the lone pair electrons of the ring oxygen to the equatorial or α proton. It must be emphasized at the beginning of this discussion that the actual size and directional character of non-bonded lone pairs is a controversial topic. Wolfe et al. (109) have stated that the common conception

*Although a favoured conformation for this ring may be described (p. 139).

of these lone pairs as "rabbit ears" is not justified; the electron density actually is concentrated much closer to the nucleus. However, evidence is available from some systems that the actual size of a lone pair is somewhere in between that of a hydrogen and a methyl group. For a compound such as N-methylpiperidine, which possesses an axial lone pair, the α axial proton absorption in the proton magnetic resonance spectrum is shifted upfield as compared with cases where there is no trans lone pair (110). The corresponding shift is much smaller for piperidine implying that in this compound the lone pair is not anti-coplanar with the axial proton.



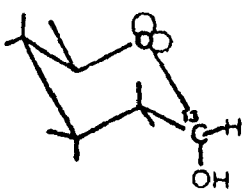
Piperidine



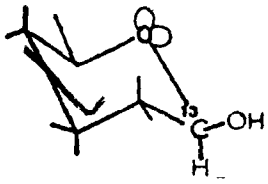
N-Methylpiperidine

Since it is commonly accepted that bulky substituents prefer an equatorial orientation, the preceding observations suggest that a lone pair is larger than a hydrogen atom but smaller than a methyl group. Furthermore, these results indicate that chemical shift can be influenced by the orientation of a lone pair on an adjacent atom. Chemical shift is determined by the electronic environment of the nucleus under observation and this factor has also been seen to influence $^{1}J_{C-H}$. It seems reasonable therefore that the directly bonded coupling constant should also be a function of the

orientation of the free electron pair lobes on an adjacent atom if such are present. Even though the size and shape of such lobes are not established beyond doubt it is clear that for α and β-D-glucose the equatorial C-H bond is nearer the concentration of electron density of the oxygen unshared electrons than is the axial C-H bond:



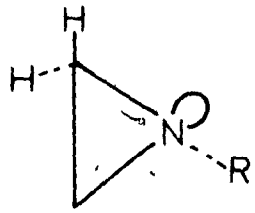
α-D-Glucose



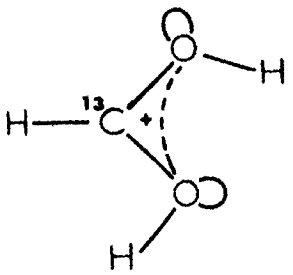
β-D-Glucose

Since the equatorial proton exhibits the greater coupling, the inference has to be that proximity of lone pairs increases the absolute value of directly bonded ^{13}C-^{1}H coupling.

With this hypothesis it is then of interest to examine other organic molecules where lone pair effects are expected to be significant. In the aziridine derivative below, the ^{13}C-^{1}H coupling syn to the lone pair is 171 Hz as compared with 161 Hz for the trans C-H bond (111).

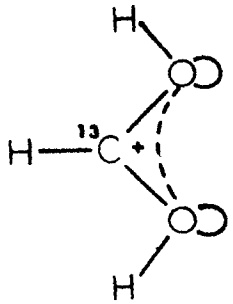


An analogous situation is found for the carbonium ions S and T (112) as well as other similar positive ions (113).



S

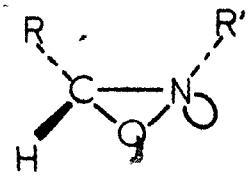
$^1J_{C-H}$ = 244.8 Hz



T

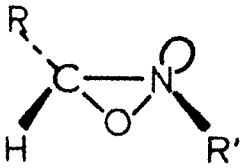
$^1J_{C-H}$ = 235.8 Hz

The above species can be differentiated spectroscopically by making use of the fact that the hydroxyl protons couple to the central proton differently in the two carbonium ions. The directly-bonded ^{13}C-^1H coupling in S is 9 Hz larger than the corresponding coupling in T, and this has been attributed to the proximity of the oxygen lone pair to the C-H bond in question. Again, in the oxazirines below, the values for $^1J_{C-H}$ are always about 6 Hz larger when the proton is syn with respect to the lone pair (114).



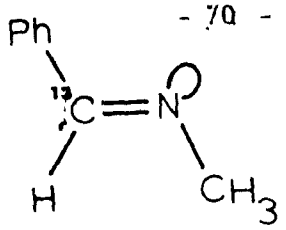
R = p-Nitrophenyl
R' = Methyl

$^1J_{C-H}$ = 185 Hz



$^1J_{C-H}$ = 179.5 Hz

It is also of interest to examine systems where the lone pair is oriented trans with respect to the carbon-hydrogen bond involved in the coupling. This type of situation can be realized in the unsaturated system below:

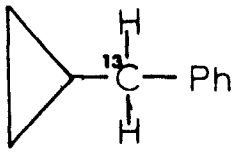


$^{1}J_{C-H}$ = 154 Hz

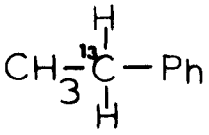
The $^{13}C-^{1}H$ coupling between the ethylenic carbon and the proton in this compound is 154 Hz (115). This is an interesting result because the corresponding coupling in ethylene is 156.4 Hz (42). It has already been shown that electronegative substitution increases the directly bonded coupling significantly and that such effects are even more pronounced in unsaturated than saturated systems. The smaller than expected value for the above compound in which one of the carbons of ethylene is replaced by the more electronegative nitrogen atom then is likely due to the trans lone pair of the nitrogen atom. It seems then that it is possible for the electron pair effect to be so large as to overcome the expected electronegativity effects.

On a theoretical basis, increasing the electron density between the coupling nuclei should lead to more extensive coupling since such nuclear-nuclear coupling is transmitted through electrons (10). This increase in electron density can be envisaged as the overlap of the adjacent lone pair with the antibonding molecular orbital of the C-H bond. Theoretical calculations have been carried out in this vein (116), and were found to be in agreement with experimental results. It was noted that the effect of a lone pair is negative when the lone pair orbital and the C-H bond are trans to each other and positive when the relative orientation is cis.

These concepts can be used to advantage in the examination of some conformationally mobile systems. The $^{13}C-^{1}H$ couplings in the substituted methanes below are equal(117):

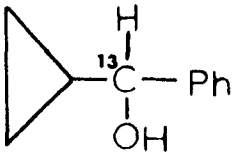


$^1J_{C-H}$ = 127 Hz

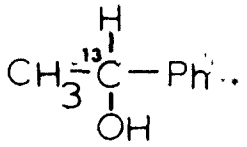


$^1J_{C-H}$ = 127 Hz

However, when one of the central hydrogens is substituted by a hydroxyl group, the equivalence of the couplings is destroyed (117):

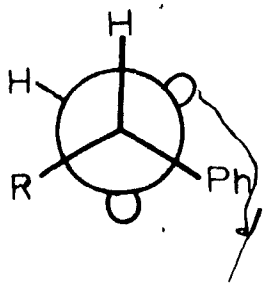


$^1J_{C-H}$ = 146



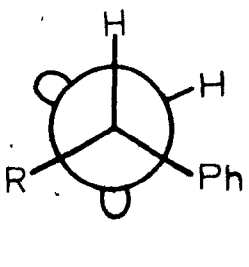
$^1J_{C-H}$ = 142.5 Hz

The difference in observed coupling between the latter two compounds must consequently be some function of the oxygen substituent. The most readily available explanation is that the unshared electron pairs of the oxygen assume different orientations with respect to the ^{13}C-H bond. This difference in orientation can be rationalized if it is assumed that the size of the non-bonded electron pair is somewhat greater than that of a bonding pair. Three possible orientations about the C-O bond can be proposed:



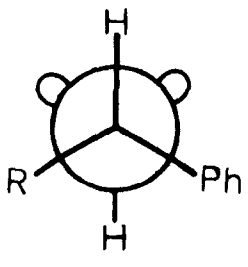
a; R = CH_3

a'; R = cyclopropyl



b; R = CH_3

b'; R = cyclopropyl

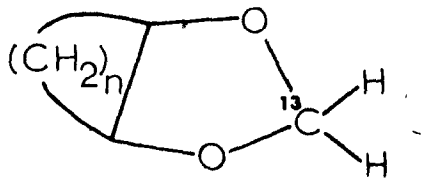


c; R = CH_3

c'; R = cyclopropyl

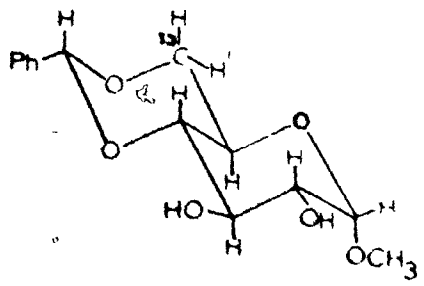
The most unfavourable orientation results when one of the unshared lobes is gauche with respect to both bulky substituents (R,Ph), as in a and b, and this orientation should become even less favoured energetically when the size of R is increased as in a' and b'. A possible inference then is that whereas a, b and c may be energetically similar, c' is favoured over a' and b'. Since in c' the ^{13}C-H bond has two oxygen lone pairs oriented gauche with respect to it as opposed to one gauche and one trans for a' and b' it is reasonable to expect increased coupling for the cyclopropyl substituted compound in comparison with the methyl substituted analog, as is observed (117).

Another interesting situation is encountered in the dioxolane derivative below:



It is found that the directly bonded ^{13}C-^{1}H coupling for the proton cis to the attached alkyl ring in all cases is about 1.5 Hz larger than for the trans proton (118). Due to steric interactions, the cis proton in the above series probably assumes a pseudo equatorial orientation thereby experiencing more gauche lone pair interactions with the oxygen unshared electron pairs than does the trans proton.

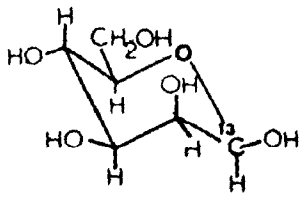
It would be valuable in this connection to have some approximate empirical values for the electron lone pair effects discussed above. To determine such values it is of course necessary to investigate systems where all other variable are essentially minimized. A suitable model seems to be methyl 4,6-O-benzylidene-α-D-glucopyranoside-6-^{13}C.



$^{1}J_{C-H}$ = 141.5 Hz $^{1}J_{C-H'}$ = 151 Hz

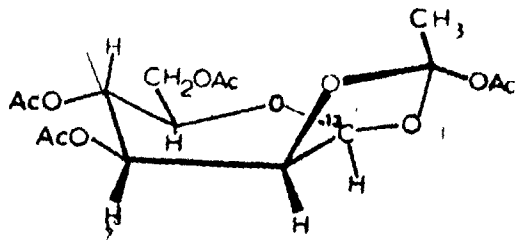
In this compound, as previously mentioned, there are no 1,3 diaxial type steric interactions involving $^{13}C_6$. Furthermore the C_5-O_5 bond bears the same relationship with respect to both C_6-H_6 bonds. Even if about 3 or 4 Hz are attributed to the effect of a gauche carbon-carbon bond with respect to the ^{13}C-^{1}H bond, lone pair effects have to be invoked to explain the remainder of the difference, i.e., 7 Hz between $^{1}J_{C-H_6}$ and $^{1}J_{C-H_6'}$. Since the equatorial proton experiences two gauche lone pair effects whereas the axial proton maintains a gauche relationship with one lone pair and a trans with the other, the observed difference in coupling may be attributed to the net result of the difference between a trans and a gauche electron pair effect.

In order to further examine these proposed effects of lone pairs it was deemed necessary to investigate a model where a conformationally free oxygen can be subsequently fixed and the coupling between the adjacent ^{13}C and ^{1}H nuclei can be measured and compared for the two forms. This situation can be realized in β-D-mannose-1-^{13}C and 3,4,6-tri-O-acetyl-β-D-mannose 1,2-(methyl orthoacetate)-1-^{13}C:



β-D-Mannose-1-^{13}C

$^{1}J_{C-H}$ = 160 Hz

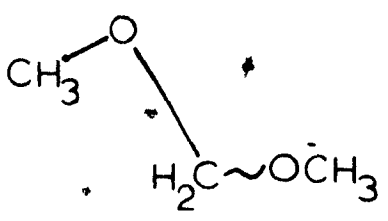


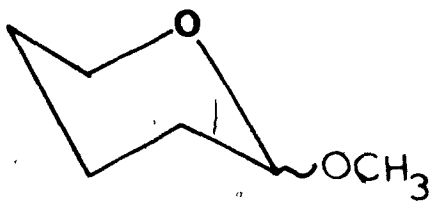
3,4,6-Tri-O-acetyl-β-D-mannose-1,2-(methyl orthoacetate)-1-^{13}C

$^{1}J_{C-H}$ = 176 Hz

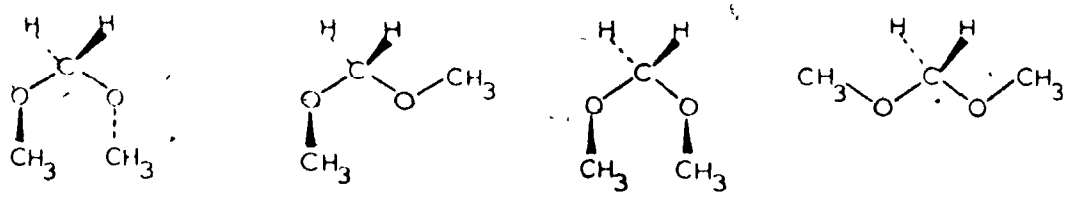
In the free sugar, although there may be a preferred conformer about the C_1-O_1 bond, the O_1 lone pairs do not maintain a fixed orientation relative to the C_1-H_1 bond. However, when O_2 and O_1 are incorporated in a ring in the orthoester derivative the lone pairs on O_1 of necessity become fixed relative to the C_1-H_1 bond. Molecular models indicate that the C_1-H_1 bond is oriented in between the two lone pairs; in fact this bond is close to being eclipsed by one of these lobes. This is exactly the type of situation for which increased coupling has been predicted. Experimental results show that the $^{13}C_1$-H_1 coupling in the ortho-ester is 16 Hz greater than the corresponding coupling in β-D-mannose. Not all of this increase can be attributed to the lone pair effect since, as will become evident in Section 2.10, incorporation of the coupling carbon into a five-membered ring leads to an increase in $^1J_{C-H}$. This latter increase, however, is in no case greater than 6-7 Hz. The effect of the orthoacetate substituent is also expected to be very small since it is known that γ electronegative substituents affect $^1J_{C-H}$ minimally (27). One can assume then that the rest of the difference in coupling between the above compounds is accounted for by the proximity of the oxygen lone pairs to the C_1-H_1 bond.

It is of interest in this connection to study a simple system which approximates the situation about C_1 of the sugars. A suitable model in this case is found in dimethoxymethane:



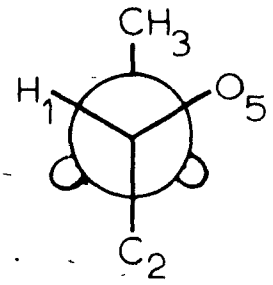


There are four possible conformations of this compound consistent with a staggered arrangement of substituents about the C-O bonds:



a b c d

Ab initio molecular orbital calculations (119) carried out on methanediol indicate that a is the preferred conformation. By analogy the suggestion has been made (119) that the favoured conformation about the C_1-O_1 bond of methyl-α-glycosides must then be the following:



This now means that the conformation of the C_5-O_5-C_1-O_1-CH_3 fragment of the sugar moiety is equivalent to a. In turn, the unshared electron pair effects experienced by the central C-H bonds in a are the same as those experienced by the C_1-H_1 bond in the glycoside.

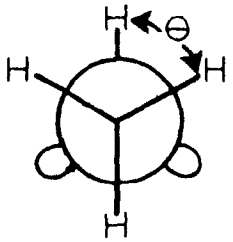
The only notable difference between the two systems, in fact, is a carbon substituent (i.e. C_2) in the sugar compared with a hydrogen in dimethoxymethanol. The observed coupling between $^{13}C_1$ and H_1 in methyl-α-D-glucopyranoside-1-^{13}C is 168 Hz, or, corrected for the β-OH substituent effect, 166 Hz. The measured coupling in dimethoxymethane is 162 Hz. There are no factors apparent in these systems which can account for this difference, other than

those introduced by differences in conformation. Therefore it is possible that the conformations are not strictly equivalent. Consequently the validity of using simple models to approximate conformations in more complex systems must be questioned.

A theoretical investigation of the lone pair effect was also carried out in conjunction with this study.* The Pople-Santry method was employed (17) and terms other than the Fermi contact term were ignored. The results for methanol, listed in Table 9, are in agreement with experiment: the directly bonded coupling increases as electron lone pairs approach the $^{13}C-^{1}H$ bond. In such calculations importance can be attached only to the trend and not to the absolute values.

TABLE 9 - Calculated $^{1}J_{C-H}$ in methanol

θ	$J_{calc.}$ (Hz)
0	162.3
30	163.0
60	165.0
90	167.4
120	169.5
150	170.8
180	171.3



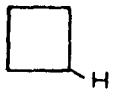
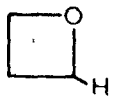
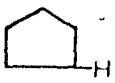
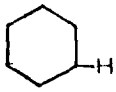
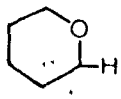
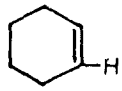
2.10 Effect of ring size on $^{1}J_{C-H}$

It is well known that directly bonded $^{13}C-^{1}H$ coupling is influenced by strain in ring systems. As is evident in Table 10, $^{1}J_{C-H}$ increases with decreasing ring size (27) (43) (120).

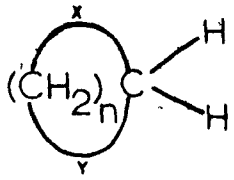
---

*The theoretical calculations were performed by Dr. N. Cyr.

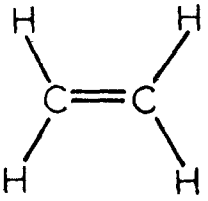
TABLE 10 - Variation of $^1J_{C-H}$ with ring size (27)(43)(120)

	$^1J_{C-H}$ (Hz)		$^1J_{C-H}$ (Hz)		$^1J_{C-H}$ (Hz)
	161.0		175.7		220.0
	134.6		148.0		170.0
	128.5		144.6		161.0
	127.0		139.4		158.0

The sugars in general manifest this phenomenon; all other factors being equivalent, larger couplings are associated with the C-H bonds of furanoses than of pyranoses. These observations can be rationalized by considering a and b below:



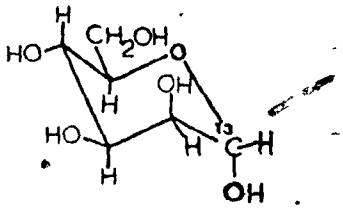
a



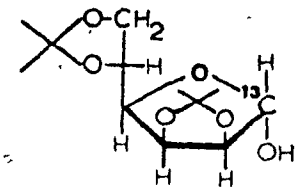
b

In a, as n decreases, the bonds X and Y move closer to each other, and the carbon atom approaches sp^2 hybridization which it ultimately achieves in b where n equals one. Since $^1J_{C-H}$ increases with an increase in "s" character of the carbon it is reasonable to anticipate a variation of this parameter with ring size.

It has already been shown that $^1J_{C-H}$ depends on several factors which can be additive or compensatory. It is important for purposes of comparison to consider models where variables other than ring size are held as constant as possible. The anomeric regions of α-D-mannopyranose-1-^{13}C and 2,3:5,6-di-O-isopropylidene-α-D-mannofuranose-1-^{13}C seemed to fulfill this condition and were consequently considered in this aspect of the study of $^1J_{C-H}$.



$^1J_{C-H}$ = 170 Hz

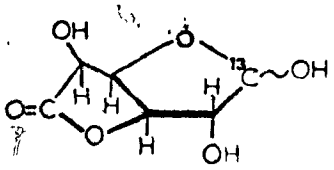


$^1J_{C-H}$ = 175 Hz

Although the conformation of five-membered rings is not readily assigned, in the proton magnetic resonance spectrum of the above furanose no coupling between H_1 and H_2 is observed. The dihedral angle between the C_1-H_1 and C_2-H_2 bonds then can be assumed to be close to 90° thereby fixing the conformation of the ring. The relative orientations of the ring oxygen lone pairs with respect to the C_1-H_1 bond are now seen to be similar to that in the pyranose. The orientation of the C_2-O_2 bond with respect to the C_1-H_1 bond is also roughly the same in the pyranose and furanose. The observed difference in coupling of 5 Hz is comparable to the difference between the C_1-H_1 couplings of tetrahydrofuran and tetrahydropyran and can be directly attributed to ring strain.

When a similar comparison is made for compounds possessing the α gluco configuration a difference such as is observed for the manno compounds is not

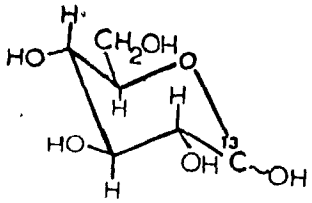
evident:



U

$^1J_{C-H_\alpha}$ = 170 Hz

$^1J_{C-H_\beta}$ = 175 Hz



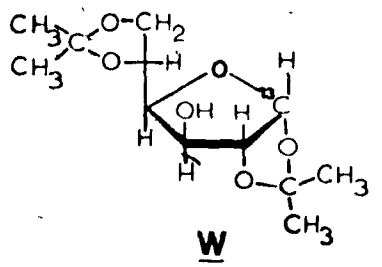
V

$^1J_{C-H_\alpha}$ = 169 Hz

$^1J_{C-H_\beta}$ = 161 Hz

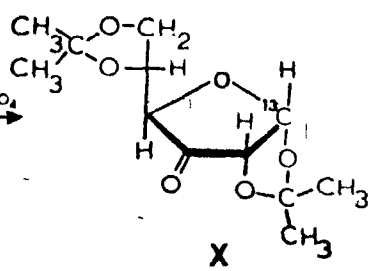
The conformation of the furanose ring can again be determined with some degree of certainty by utilizing the fact that the absence of coupling between H_1 and H_2 in the α anomer fixes the dihedral angle between the C_1-H_1 and C_2-H_2 bonds at 90°. In the manno compounds above it was noted that the C_2-O_2 bond was gauche with respect to the C_1-H_1 bond in the furanose as well as the pyranose. For the compounds possessing the α gluco configuration this is no longer true. In the furanose now the C_2-O_2 bond is almost trans with respect to the C_1-H_1 bond, a situation that should lead to decreased coupling. This effect then likely cancels the increase due to ring strain. Considering the β anomers, however, the C_2-O_2 bond of the furanose maintains a small dihedral angle with the C_1-H_1 bond and consequently not only is the expected increase observed, but it is amplified.

$^1J_{C-H}$ can, then, in certain cases serve as a measure of ring strain. It is noteworthy in this regard that when W below is oxidized to X, a decrease of 2.5 Hz is observed in $^1J_{C-H}$.



W

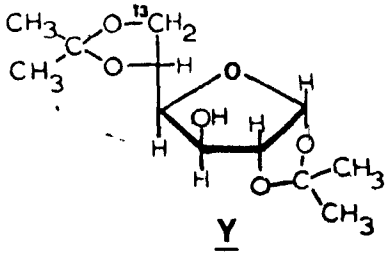
$^1J_{C-H}$ = 183 Hz



X

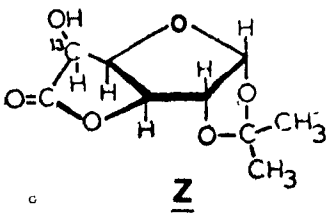
$^1J_{C-H}$ = 180.5 Hz

It is expected that in X ring strain is partially relieved by the incorporation of an sp^2 carbon in the furanose ring. A similar effect is manifested in Y relative to Z.



Y

$^1J_{C-H}$ = 149 Hz



Z

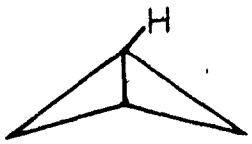
$^1J_{C-H}$ = 144 Hz

When C_1 of a furanose is incorporated into a second ring as in W, $^1J_{C-H}$ increases by about 13 Hz. The question of ring strain additivity has to be considered in this case; i.e. whether incorporation of the coupling ^{13}C nucleus into two fused rings results in twice the increase in coupling as is observed on incorporation into a single ring. This does seem to be the case when cyclopropanes are examined (35):

CH_4

$^1J_{C-H}$ = 125 Hz

$^1J_{C-H}$ = 161 Hz



$^1J_{C-H}$ = 202 Hz

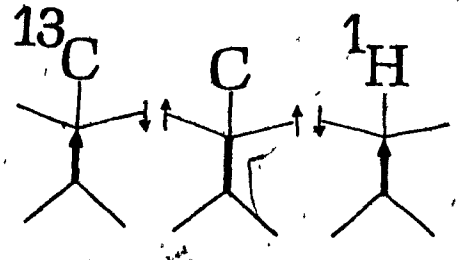
It has already been stated that incorporation of the ^{13}C nucleus into a five-membered ring results in an increase of about 5 Hz in the coupling to its appended proton, whereas the observed difference between the α anomer of II and W is certainly greater than twice this amount. Some of this increment becomes evident on the examination of molecular models. Incorporating O_1 into an isopropylidene ring fixes the orientation of the unshared electron pair lobes of O_1 relative to the C_1-H_1 bond in such a manner that the C_1-H_1 bond experiences two gauche lone pair interactions. This type of arrangement, as discussed in Section 2.9, leads to increased ^{13}C-^1H coupling.

2.11 Conclusion

The results of the present investigation suggest that directly bonded ^{13}C-^1H coupling constants vary according to structural features of molecules in a manner that cannot be described by the classical hybridization approach. The latter model, along with the Malinowski type of additivity relationship (38) is now seen as applicable only for very large changes in $^1J_{C-H}$, and the effective nuclear charge concept of Grant and Litchman (59) also seems to offer too limited a view. In fact $^1J_{C-H}$ varies in a rather complex manner. The coupling increases with electronegative substitution at the ^{13}C nucleus, with the proximity of lone pairs to the ^{13}C-^1H bond and with strain when the ^{13}C is incorporated into a ring. Gauche type steric effects as well as adjacent trans electronegative substituents cause decreased coupling. These couplings, as demonstrated, can be used to advantage in conformational analysis and will no doubt receive further attention to exploit their potential. It must be emphasized, however, that in order to obtain meaningful results it is imperative to take all variables into account. As the present investigation implies, previously calculated hybridization, bond angle and bond length data based on $^1J_{C-H}$ are best regarded as approximations.

CHAPTER 3

GEMINAL ^{13}C-^{1}H COUPLING



3.1 Introductory remarks

Internuclear coupling between the ^{13}C nucleus and a proton through two bonds, termed geminal coupling and denoted by $^{2}J_{C-H}$, was also extensively investigated in this general study of ^{13}C-^{1}H coupling constants, and the results are reported in Table 1. As with directly bonded coupling, emphasis was placed on stereochemical factors associated with this parameter.

The determination of ^{13}C-^{1}H geminal coupling interactions presents substantial experimental difficulties and consequently the literature data on this topic are limited (121)(122). These couplings are much smaller in magnitude than the previously discussed directly bonded couplings, ranging in the present investigation from 0 to 6 Hz in absolute value. For this reason their measurement from natural abundance spectra is at present frequently impossible except in the simplest cases (see p. 4), since the relevant signals are obscured by the much larger signals of those protons not coupled to a ^{13}C nucleus. Figure 1 depicts the theoretical and actual natural abundance proton magnetic resonance spectrum of the aldehydic proton in acetaldehyde, an optimal case in this regard due to the large coupling (26.6 Hz) between the methyl carbon and the aldehydic proton (123).

FIG. 1 Natural abundance pmr spectrum of the aldehydic proton in acetaldehyde.

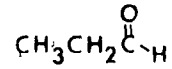
TABLE 1 - Geminal $^{13}C-^{1}H$ coupling in ^{13}C enriched carbohydrates

Compound	Coupling pathway	$^{2}J_{C-H}$ ($\pm\frac{1}{2}$ Hz)
1,2-O-Isopropylidene-α-D-glucofuranose-6-^{13}C	$C_6-C_5-H_5$	1
1,2-O-Isopropylidene-3,5,6-tri-O-acetyl-α-D-glucofuranose-6-^{13}C	$C_6-C_5-H_5$	1
1,2-O-Isopropylidene-3,5,6-tri-O-acetyl-α-D-glucofuranose-6-^{13}C-6-d_2	$C_6-C_5-H_5$	< 1
1,2-O-Isopropylidene-α-D-glucofuranose-1-^{13}C	$C_1-C_2-H_2$	5.5
1,2-O-Isopropylidene-3,5,6-tri-O-acetyl-α-D-glucofuranose-1-^{13}C	$C_1-C_2-H_2$	5.5
1,2-O-Isopropylidene-3,5,6-tri-O-orthoformyl-α-D-glucofuranose-6-^{13}C	$C_6-C_5-H_5$	< 1
1,2-O-Isopropylidene-3,5,6-tri-O-orthoformyl-α-D-glucofuranose-6-^{13}C-6-d_2	$C_6-C_5-H_5$	<1
1,2-O-Isopropylidene-3,5,6-tri-O-orthoformyl-α-D-glucofuranose-1-^{13}C	$C_1-C_2-H_2$	4.8
1,2:5,6-Di-O-isopropylidene-α-D-glucofuranose-6-^{13}C	$C_6-C_5-H_5$	< 1
1,2:5,6-Di-O-isopropylidene-α-D-glucofuranose-1-^{13}C	$C_1-C_2-H_2$	+5.5
1,2:5,6-Di-O-isopropylidene-3-O-acetyl-α-D-glucofuranose-1-^{13}C	$C_1-C_2-H_2$	5.5
1,2:5,6-Di-O-isopropylidene-α-D-ribohexofuranose-3-ulose-1-^{13}C	$C_1-C_2-H_2$	5.5
1,2:5,6-Di-O-isopropylidene-α-D-allofuranose-1-^{13}C	$C_1-C_2-H_2$	5

Compound	Coupling pathway	$^2J_{C-H}$ (Hz)
1,2-O-Isopropylidene-β-L-idofuranose-6-^{13}C	C_6-C_5-H_5	< 1
1,2-O-Isopropylidene-β-L-idofuranose-6-^{13}C-6-d_2	C_6-C_5-H_5	1.5
1,2-O-Isopropylidene-α-D-glucofuranurono-6,3-lactone-6-^{13}C	C_6-C_5-H_5	4.1
1,2-O-Isopropylidene-5-O-acetyl-α-D-glucofuranurono-6,3-lactone-6-^{13}C	C_6-C_5-H_5	5.5
1,2-O-Isopropylidene-β-L-idofuranurono-6,3-lactone-6-^{13}C	C_6-C_5-H_5	< 1
1,2-O-Isopropylidene-5-O-acetyl-β-L-idofuranurono-6,3-lactone-6-^{13}C	C_6-C_5-H_5	2.2
D-Mannono-1,4-lactone-1-^{13}C	C_1-C_2-H_2	4.8
2,3:5,6-Di-O-isopropylidene-α-D-mannofuranose-1-^{13}C	C_1-C_2-H_2	1.5
2,3:5,6-Di-O-isopropylidene-1-O-acetyl-α-D-mannofuranose-1-^{13}C	C_1-C_2-H_2	1.5
α-D-Glucopyranose-1-^{13}C	C_1-C_2-H_2	< 1
β-D-Glucopyranose-1-^{13}C	C_1-C_2-H_2	5.7
β-D-Allopyranose-1-^{13}C	C_1-C_2-H_2	-6.0
1,2,3,4,6-Penta-O-acetyl-β-D-allopyranose-1-^{13}C	C_1-C_2-H_2	-5.5
1,2,3,4,6-Penta-O-acetyl-α-D-glucopyranose-1-^{13}C	C_1-C_2-H_2	< 1
1,2,3,4,6-Penta-O-acetyl-β-D-glucopyranose-1-^{13}C	C_1-C_2-H_2	5

Compound	Coupling pathway	$^2J_{C-H}$ (Hz)
1,2,3,4,6-Penta-O-acetyl-β-D-mannopyranose-1-^{13}C	C_1-C_2-H_2	1.5
1,2,3,4,6-Penta-O-acetyl-α-D-glucopyranose-6-^{13}C	C_6-C_5-H_5	< 1
1,2,3,4,6-Penta-O-acetyl-β-D-glucopyranose-6-^{13}C	C_6-C_5-H_5	< 1
2,3,4,6-Tetra-O-acetyl-α-D-glucopyranosyl bromide-1-^{13}C	C_1-C_2-H_2	1.5
2,3,4,6-Tetra-O-acetyl-α-D-mannopyranosyl bromide-1-^{13}C	C_1-C_2-H_2	1.5
2,3,4,6-Tetra-O-acetyl-1,5-anhydro-D-glucitol-1-^{13}C	C_1-C_2-H_2	3.5
3,4,6-Tri-O-acetyl-β-D-mannopyranose-(1,2-methyl orthoacetate)-1-^{13}C	C_1-C_2-H_2	< 1
Methyl-α-D-glucopyranoside-1-^{13}C	C_1-C_2-H_2	< 1
Methyl 4,6-O-benzylidene-α-D-glucopyranoside-1-^{13}C	C_1-C_2-H_2	< 1
Methyl 4,6-O-benzylidene-α-D-glucopyranoside-6-^{13}C	C_6-C_5-H_5	< 1
1,6-Anhydro-α-L-idopyranose-6-^{13}C	C_6-C_5-H_5	1.5
2,3,4-Tri-O-acetyl-1,6-anhydro-α-L-idopyranose-6-^{13}C	C_6-C_5-H_5	1.5

The coupling in this case can be measured from the separation of the satellites which are the direct result of geminal ^{13}C-^{1}H coupling between $^{13}C_2$ and H_1. It is evident, however, that if such geminal coupling is of smaller magnitude than in the example above, the satellites of interest are likely to be obliterated by the intense central absorption. Furthermore, in any slightly more complex system, for example one containing three carbon atoms such as propanal,



even if the satellites are observable, additional problems may be encountered. Along with geminal ^{13}C-^{1}H coupling, there now exists the possibility of vicinal (three bond) coupling between $^{13}C_3$ and H_1 and consequently there is an uncertainty in which coupling is really being observed.

Fourier transform instrumentation capable of specific proton decoupling was not available during the period of research described here. Hence a rather high degree of ^{13}C enrichment was essential in our investigation of the relatively complex systems which appeared suited for the examination of various stereochemical determinants of $^{2}J_{C-H}$. Aside from the difficulties involved in the synthesis of ^{13}C enriched compounds, potential problems can arise in specific proton signal assignment. This may be illustrated by referring to two examples. Figure 2 shows an optimum case, that of 1,2-O-isopropylidene-3,5,6-tri-O-orthoformyl-α-D-glucofuranose, natural abundance and 1-^{13}C (80%). In this case the complete spectrum can be analyzed by making use of chemical shift principles, proton-proton coupling data and proton decoupling. In the 1-^{13}C labelling experiment, then, the coupling between $^{13}C_1$ and H_2 can be directly measured. Since the enrichment is about 80% the H_2 doublet of the non-enriched compound is still present in the spectrum and in some cases may still

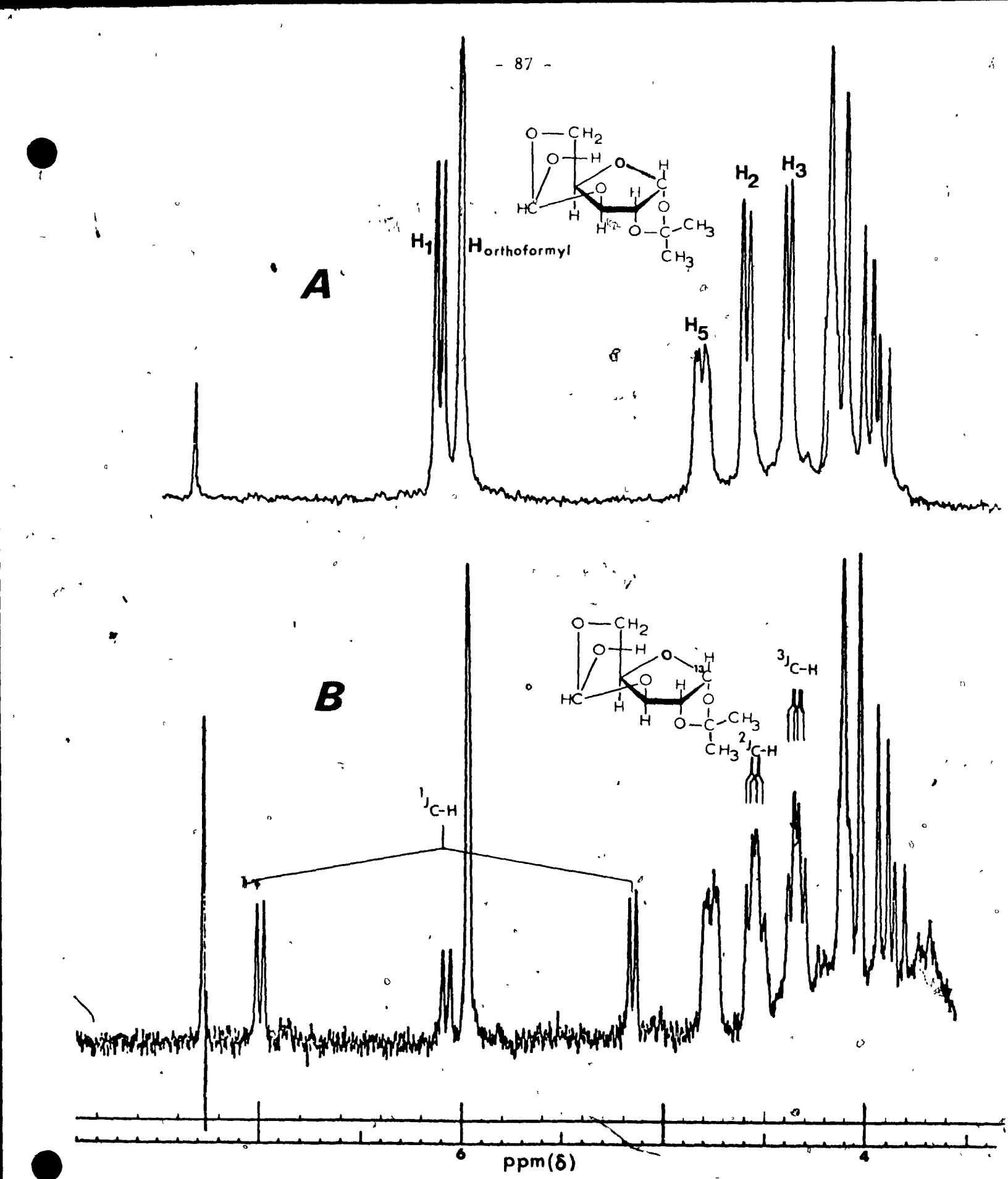
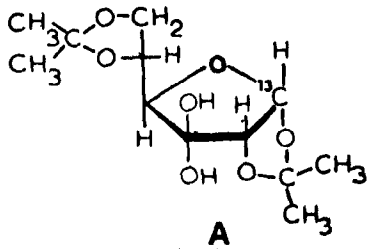


FIG. 2 Partial pmr spectrum (100 MHz) of A) 1,2-O-isopropylidene-3,5,6-tri-O-orthoformyl-α-D-glucofuranose and B) 1,2-O-isopropylidene-3,5,6-tri-O-orthoformyl-α-D-glucofuranose-1-^{13}C (80% enriched) in $CDCl_3$.

interfere with the necessary determination. For this reason a high degree of enrichement i.e. 80-90% is particularly beneficial in obtaining accurate values for the coupling.

In contrast to the above compound, the spectrum of 1,2:5,6-di-O-isopropylidene-α-D-ribohexofuranose-3-ulose (monohydrate)-1-^{13}C (A) (Fig. 3) is



A

so complex due to the proximity of the chemical shifts of H_2, H_4, H_5, H_6 and $H_{6'}$ that even at 220 MHz the H_2 signal cannot be identified and consequently the coupling to $^{13}C_1$ cannot be measured. In the light of these considerations it is obvious that even with enriched compounds one is limited in the type of systems that can be examined spectroscopically.

Primarily then, due to the aforementioned difficulties the ^{13}C-^{1}H geminal couplings that have been measured heretofore relate mostly to rather simple systems; there is a dire lack of data for conformationally rigid systems in which various stereochemical effects can systematically be evaluated. It should be mentioned at this point that the spectra encountered in this study can in general be described as AMX as far as the vicinal proton, geminal proton and the ^{13}C nucleus are concerned, so that the observed spacings should be close in value to the actual coupling constant.

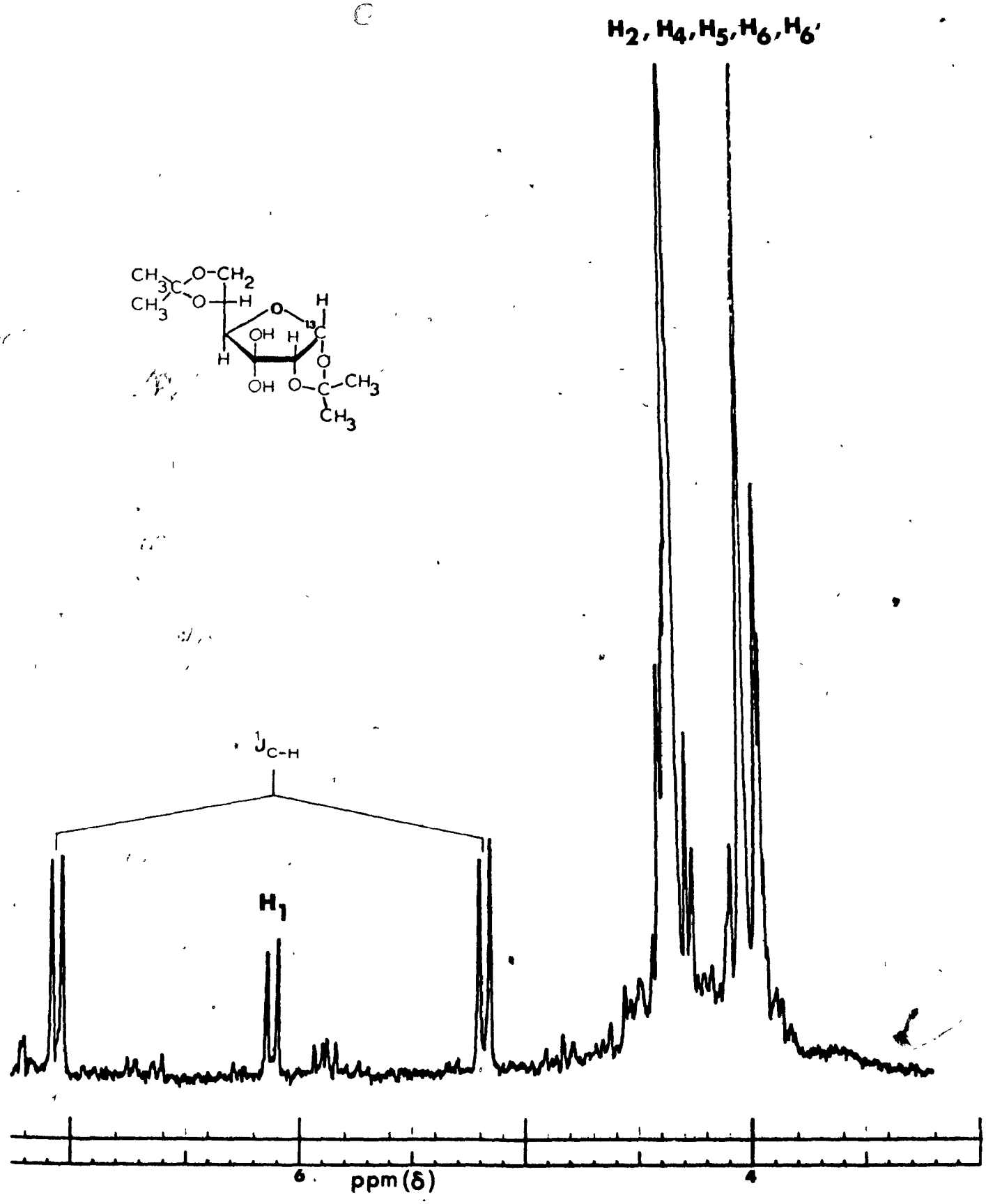
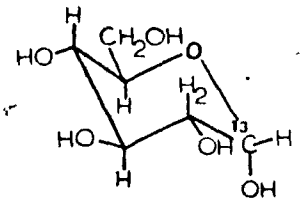


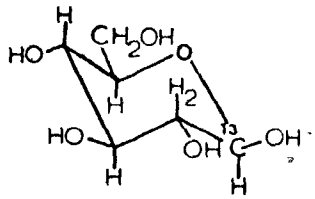
FIG. 3 Partial pmr spectrum (100 MHz) of 1,2:5,6-di-O-isopropylidene-α-D-ribohexofuranose-3-ulose (monohydrate)-1-^{13}C (80%) in $CDCl_3$.

3.2 Observation of relative bond orientation effects on $^{2}J_{C-H}$

As with the directly bonded couplings, a striking observation was made for the case of α and β-D-glucopyranose-1-^{13}C:

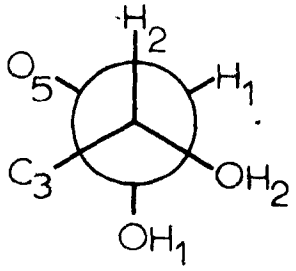


$^{2}J_{C-H}$ < 1 Hz

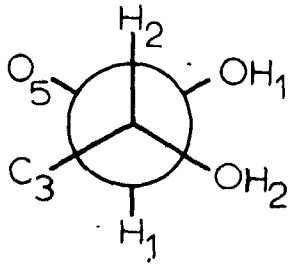


$^{2}J_{C-H}$ = 5.7 Hz

The β anomer exhibits a rather large geminal coupling of 5.7 Hz between $^{13}C_1$ and H_2 whereas no coupling is observable for the α anomer. The coupling pathway appears to have the same geometry in these two instances; the only obvious difference between the anomers is the orientation of the anomeric hydroxyl group with respect to the C_2-H_2 bond:

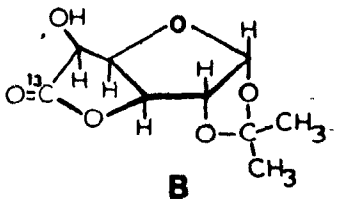


α-D-Glucose



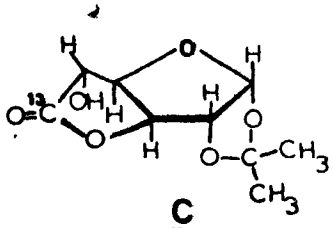
β-D-Glucose

Similar effects can be noted as well when the ^{13}C is sp^2 hybridized as in the case of 1,2-O-isopropylidene-α-D-glucofuranurono-6,3-lactone-6-^{13}C (B) and its L-ido epimer (C):



B

$^{2}J_{C-H}$ = 4.1 Hz

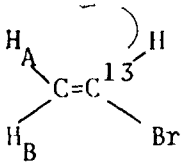


C

$^{2}J_{C-H}$ < 1 Hz

Based on these observations, the potential use of these couplings became apparent: if the factors determining the above differences were identified such ^{13}C-^{1}H couplings could prove to be a valuable tool in structural analysis.

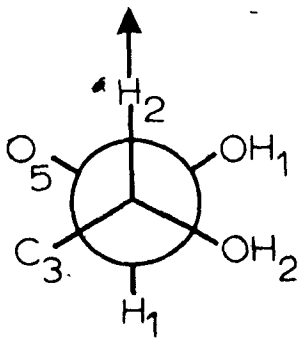
The present findings bear a resemblance to those described by Lynden-Bell (95) for bromo-ethylene:



$$^{2}J_{C\text{-}H_A} = +7.5 \text{ Hz}$$

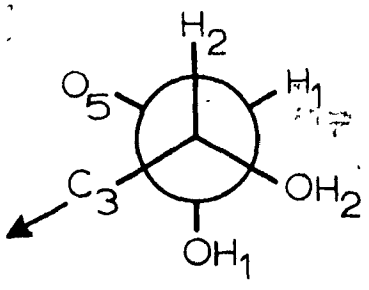
$$^{2}J_{C\text{-}H_B} = -8.5 \text{ Hz}$$

That is, the proton cis to the bromine atom shows a coupling of -8.5 Hz in contrast to the +7.5 Hz for the trans proton, a marked difference in steric dependence. Since in the sugars mentioned above there are two oxygen substituents on the ^{13}C nucleus, one way to describe their orientation with respect to the coupling path could be to consider a "resultant" by treating the C_1-O_1 bonds as vectors. For the glucose anomers these resultants can be represented by the following:



$^{2}J_{C\text{-}H}$ = 5.7 Hz

β-D-Glucose



$^{2}J_{C\text{-}H}$ < 1 Hz

α-D-Glucose

Using this terminology the relative positions of the oxygens with

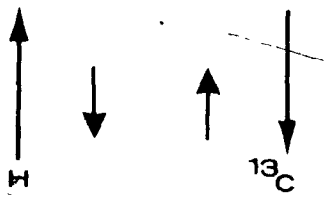
respect to the C_2-H_2 bond can be described by referring to the dihedral angle between the "resultant" and the C_2-H_2 bond. Accordingly, coupling decreases in absolute value as the dihedral angle is increased.

At this stage it becomes imperative to introduce a concept which was not considered in dealing with directly bonded couplings: the sign of coupling constants. It has already been indicated for bromoethylene that geminal ^{13}C-^{1}H coupling may have a positive or negative sign, depending on the inherent stereochemistry. In order then, to make meaningful correlations between geminal coupling and structure and to enable the application of these couplings in practical structural analysis, it became essential that the sign of such couplings be determined. It would then subsequently become clear whether the observed coupling was actually increasing or decreasing relative to the orientation of the substituents on the ^{13}C nucleus.

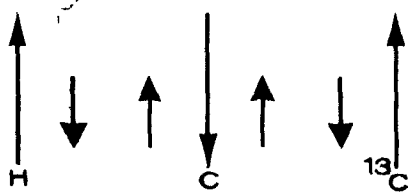
3.3 Signs of coupling constants

The great majority of the ^{13}C-^{1}H geminal coupling values available in the literature are absolute values owing to the fact that the experimental determination of signs is often difficult. Internuclear coupling is caused by the interaction of nuclear spins, as transmitted through electrons (10). In general, if the energy of interaction between two nuclei is more favourable when the spins are antiparallel than for the parallel arrangement, then the coupling constant J is positive. When the parallel arrangement is energetically favoured, the coupling constant is said to be negative. As an example, consider the ^{13}C-H bond, and let the high and low energy states of nuclei or electrons be denoted by α and β respectively. At any moment, then, there is a high probability of finding one electron in the vicinity of the ^{13}C nucleus and the other in the vicinity of the proton. Since nuclear and electron spins tend to pair,

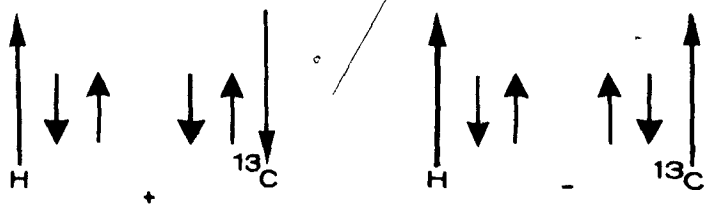
if the hydrogen nucleus is in the α or high energy state, the electron close to it will most frequently have a β spin. By the Pauli exclusion principle the second electron in the covalent bond will then be in the α spin state and consequently the β spin state of the ^{13}C nucleus will be favoured. This type of transmission of the energetic state of coupling nuclei leads to positive spin-spin coupling and can be represented schematically as below:



where the large arrows represent spins of nuclei whereas the small arrows refer to electron spin. It follows then that if coupling is transmitted by this mechanism (i.e. Fermi mechanism), directly bonded couplings should be positive. Sometimes, a similar argument is advanced to show that geminal spin-spin couplings are negative making use of the schematic below which leads to a parallel, or less favoured arrangement of nuclear spins.

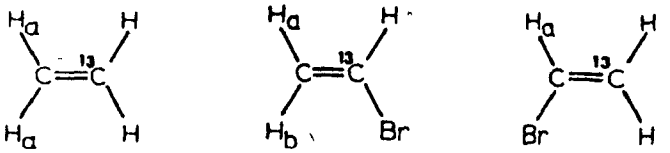


Such an argument is fallacious because in such systems 99% of the time the intervening carbon atom is the ^{12}C isotope and consequently has no spin! The above diagram accordingly becomes meaningless. It has already been indicated that $^{2}J_{C-H}$ can be of either sign; therefore depending on how spin information is transmitted from the electrons in one bond to the electrons in the other, according to the stereochemistry of the orbitals involved, both of the following coupling situations are possible:



positive coupling negative coupling

The signs of these couplings have mathematical significance, so that one can talk about positive and negative contributions to coupling. For example, consideration of the data below (124), leads to the notion that a Br substituent trans to the coupling proton makes a contribution of +9.9 Hz, a cis substituent contributes -6.1 Hz whereas a Br atom bonded to the intervening carbon contributes +8.2 Hz.



$^2J_{C-H} = -2.4$ Hz $^2J_{C-H_a} = +7.5$ Hz $^2J_{C-H_a} = +5.8$ Hz

$^2J_{C-H_b} = -8.5$ Hz

Based on this idea the geminal $^{13}C-^1H$ coupling in cis-dibromoethylene would be expected to equal -2.4 + 9.9 + 8.2, or 15.7 Hz. The experimental value for this compound is 14.7 Hz. It is evident then that signs of coupling constants have real algebraic significance and consequently it becomes important to investigate these effects in the sugar series.

Theory of sign determination

Three basic methods are available for sign determination of coupling constants. It must be emphasized that absolute sign determination is in general not possible; sign determinations are carried out relative to the one bond $^{13}C-H$ coupling which is widely accepted to be positive, based on studies

carried out with liquid crystals (125).

For some strongly coupled spectra it is possible to carry out sign determinations by the matching of ^{13}C satellite spectra with computed spectra. This method has been used by Goldstein and coworkers (126) with aromatic systems.

A second method of sign determination involves the investigation of solvent effects on the geminal coupling constant (127). It is known for example, that the geminal proton-proton coupling in styrene oxide is positive while in styrene sulfide it is negative. These couplings are seen to vary with solvent in the following manner:

	Styrene sulfide	Styrene oxide
Cyclohexane	-1.15 Hz	6.00 Hz
$CHCl_3$	-1.50 Hz	5.55 Hz
DMSO	-1.55 Hz	5.31 Hz

Both sets of coupling constants decrease algebraically with increasing dielectric constant of the solvent; styrene sulfide increasing in absolute value. Therefore, by observing the behaviour of |J| in different solvents one can determine the sign of the coupling. (The rationale here involves the shift of electrons out of, or into, certain carbon orbitals depending on the orientation of the dipole moment of the solute molecule with respect to the H-C-H plane (127)). Theoretically then, it should also be possible to determine the sign of geminal $^{13}C-^{1}H$ coupling by studying whether the coupling increases or decreases as the dielectric constant of the solvent is increased.

To investigate this possibility in the sugar series it was necessary to select compounds in which the desired pmr signals were sharp and clearly visible. 1,2:5,6-Di-O-isopropylidene-3-O-acetyl-α-D-glucofuranose-1-^{13}C (Fig. 4);

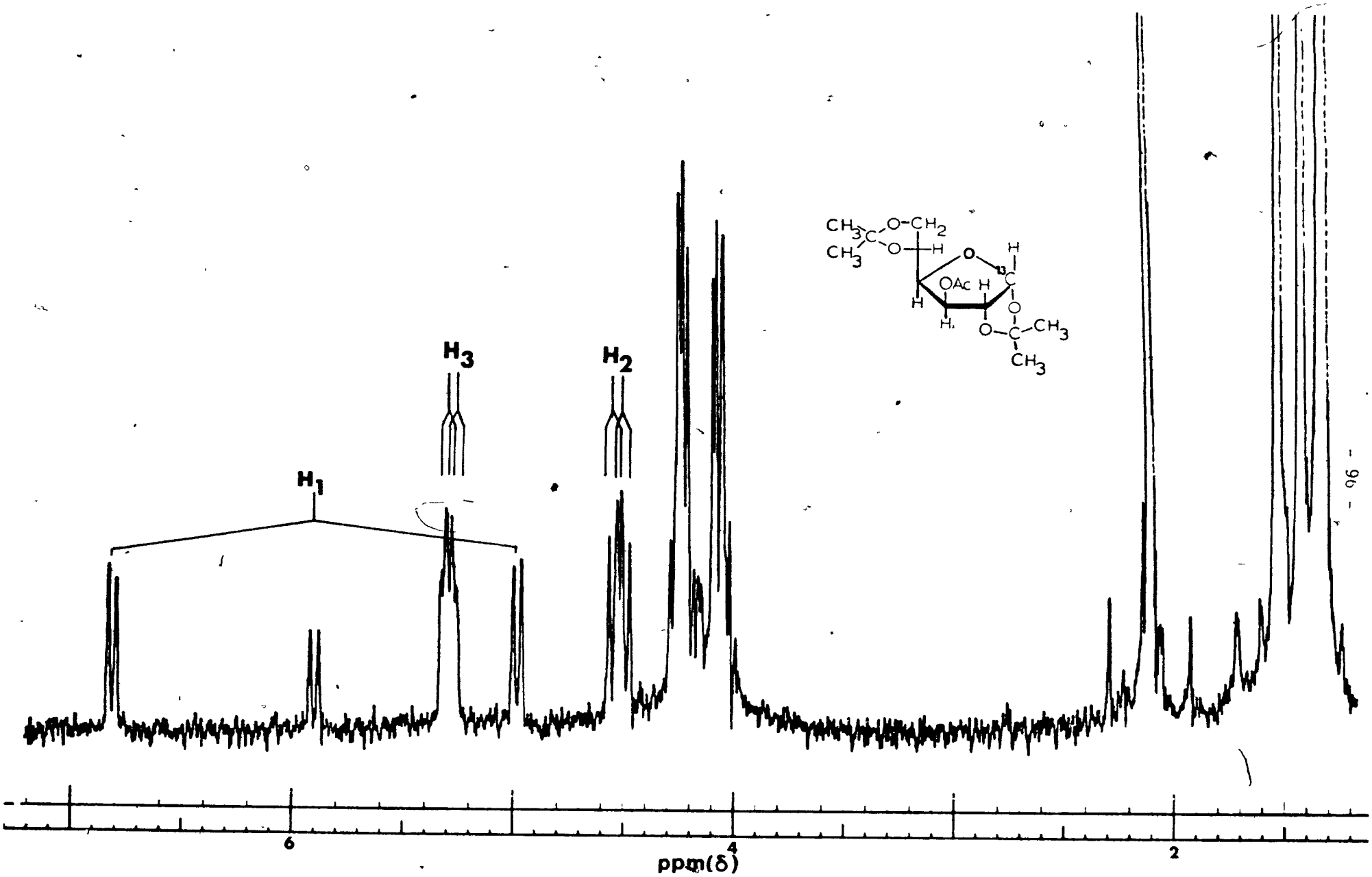


FIG. 4 Partial pmr spectrum (100 MHz) of 1,2:5,6-di-O-isopropylidene-3-O-acetyl-α-D-glucofuranose-1-^{13}C (80%) in $CDCl_3$.

and 1,2-O-isopropylidene-3,5,6-tri-O-acetyl-α-D-glucofuranose-6-^{13}C-6-d_2 (Fig. 5) were found to be suitable since the relevant proton absorptions were easily identified and the compounds can be dissolved in a variety of solvents.

The above compounds also represent the two general types of cases examined in this investigation: the coupling carbon bearing two oxygens or one oxygen, respectively. The solvents employed were CCl_4, CS_2, DMSO, acetonitrile, pyridine, acetone, hexafluoroacetone, benzene and chloroform along with various combinations of the solvents. It is evident that these solvents represent a wide range of dielectric constants, however, within the accuracy of the measurements (.5 Hz) no significant variation in $^2J_{C-H}$ was noted in either of the two cases. It must be concluded then that this method is not suitable for sign determination in the type of systems encountered in the present investigation. Correspondingly, it can also be concluded that the geminal couplings which are being discussed are essentially independent of solvent.

The third method, and the one found applicable here, involves double resonance techniques (128). The ideal case for such investigations is an AMX spin system. Consider a situation such as H_2-C-^{13}C-H_1 where X is ^{13}C. The prerequisites for sign determination include coupling of ^{13}C to H_1 and H_2 as well as proton-proton coupling between H_1 and H_2. Symbolically the spectrum can then be represented as in Fig. 6.

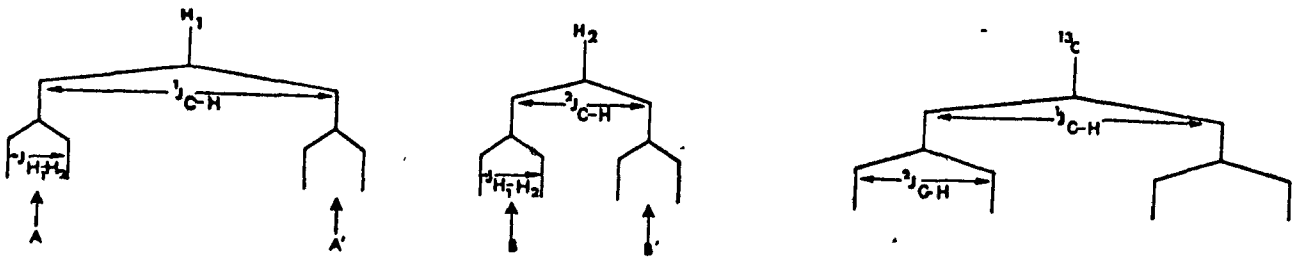


FIG. 6 Schematic NMR spectrum of H_2-C-^{13}C-H_1.

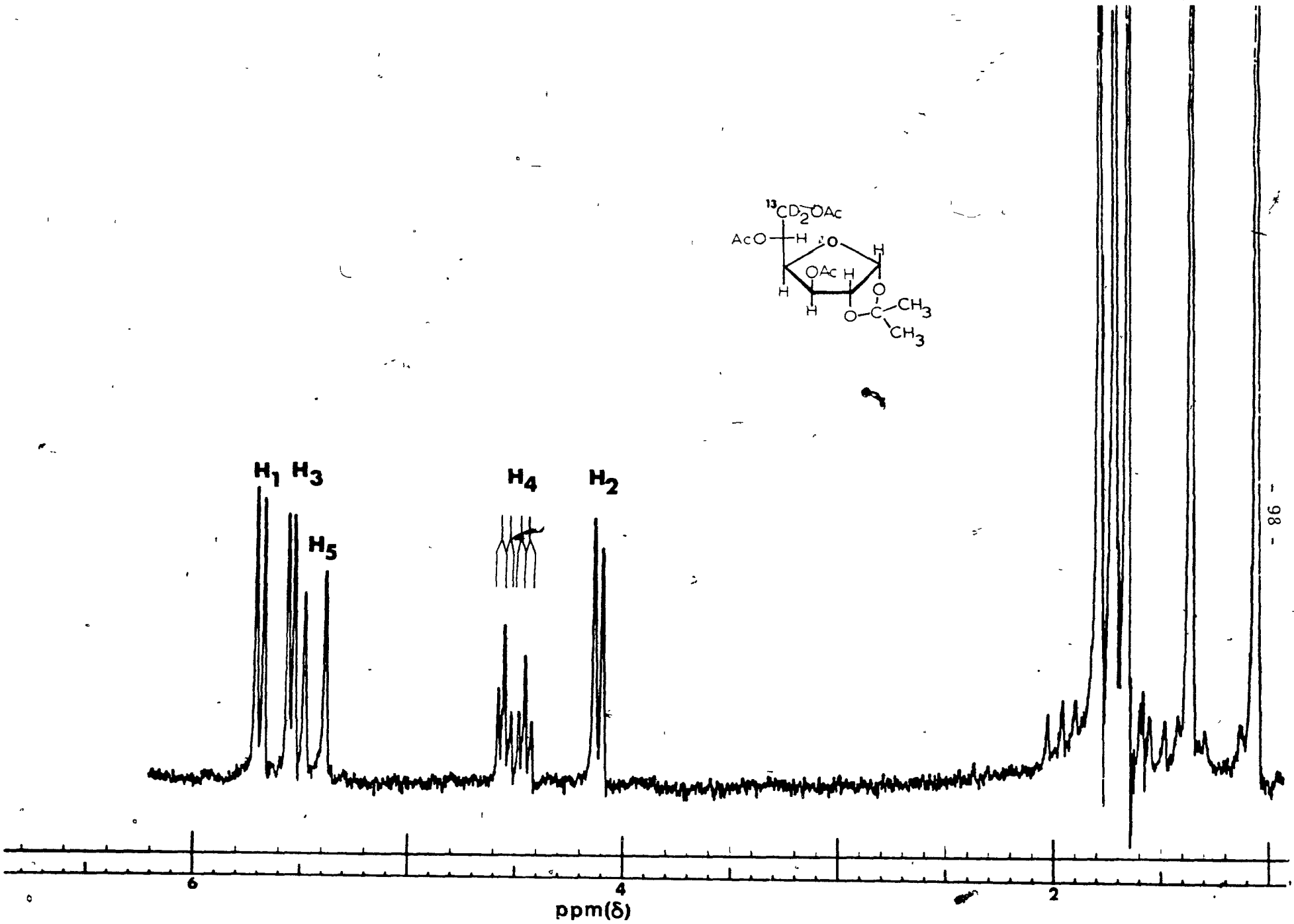


FIG. 5 Partial pmr spectrum (100 MHz) of 1,2-O-isopropylidene-3,5,6-tri-O-acetyl-α-D-glucofuranose-6-^{13}C-6-d_2 in $CDCl_3$.

In this case the chemical shifts of the H_1 and H_2 absorptions can be described by the following relationships:

$$\nu(A) = \nu_{H_1} + J_{H_1H_2} \cdot m_{H_2} + {}^1J_{C-H} \cdot m_{^{13}C}$$

$$\nu(B) = \nu_{H_2} + J_{H_1H_2} \cdot m_{H_1} + {}^2J_{C-H} \cdot m_{^{13}C}$$

where ν is the chemical shift in Hz and m assumes values of 1/2 or -1/2. For example, ${}^1J_{C-H}$ being positive, the chemical shifts of the low frequency H_1 lines would be described as:

$$\nu(A_1) = \nu_{H_1} - 1/2\ {}^1J_{C-H} - 1/2\ J_{H_1H_2}$$

and $$\nu(A_2) = \nu_{H_1} - 1/2\ {}^1J_{C-H} + 1/2\ J_{H_1H_2}$$

These absorptions are then said to be in the same ^{13}C state (i.e. - 1/2). Turning now to H_2, if ${}^2J_{C-H}$ is positive the low frequency H_2 lines can be described as:

$$\nu(B_1) = \nu_{H_2} - 1/2\ {}^2J_{C-H} - 1/2\ J_{H_1H_2}$$

and $$\nu(B_2) = \nu_{H_2} - 1/2\ {}^2J_{C-H} + 1/2\ J_{H_1H_2}$$

Again, both of these are seen to be in the -1/2 ^{13}C state. On the other hand, if ${}^2J_{C-H}$ is negative, the description of the low frequency H_2 lines becomes:

$$\nu(B_1) = \nu_{H_2} + 1/2\ {}^2J_{C-H} - 1/2\ J_{H_1H_2}$$

and $$\nu(B_2) = \nu_{H_2} + 1/2\ {}^2J_{C-H} + 1/2\ J_{H_1H_2}$$

These signals are now in the + 1/2 ^{13}C state. It is evident that if ${}^1J_{C-H}$ and ${}^2J_{C-H}$ are both positive, the low frequency pair of H_1 lines as well as the low frequency pair of H_2 lines arise from the -1/2 ^{13}C state. If the signs are opposite, then the -1/2 ^{13}C state is responsible for the low frequency pair

of one doublet of doublets and the high frequency pair of the other. Hence by introducing a second r.f. source with a frequency corresponding to that of one of the doublets, one can obtain selective decoupling within only half the molecules, i.e., those with a specific ^{13}C state. By observing which doublet collapses due to this irradiation, the relative signs of the coupling can be determined. If irradiation at A causes collapse at B in Fig. 6, the signs are the same; if this irradiation causes collapse at B', the signs are then opposite. Since $^{1}J_{C-H}$ is taken as positive (125), the sign of $^{2}J_{C-H}$ can be determined.

As a practical example, acetaldehyde-2-^{13}C can be considered. This is an AM_3X spin system with a schematic spectrum as represented in Fig. 7.

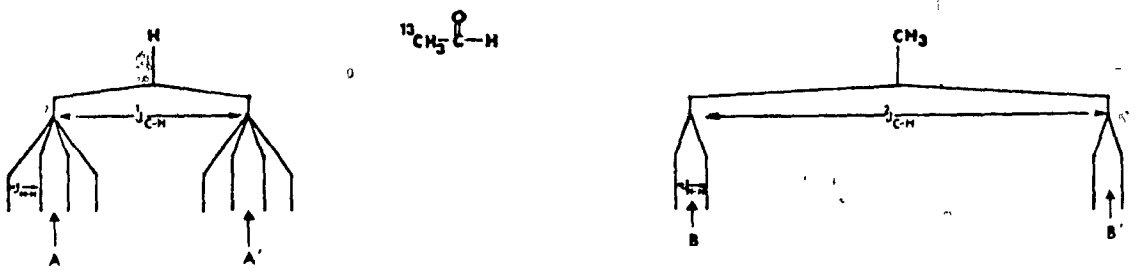


FIG. 7 Schematic spectrum of acetaldehyde-2-^{13}C.

The prerequisites for sign determination are all met. The sets of protons coupled to the ^{13}C nucleus are coupled to each other and all signals are clearly visible. In this instance irradiation at point A with a second r.f. oscillation causes the low field satellite B of the methyl proton to collapse, whereas irradiation at point A' has the same effect on B', indicating that $^{2}J_{C-H}$ and $^{1}J_{C-H}$ are of like sign (129). It should be noted that complete decoupling is often not possible, nor is it necessary. The same information can be obtained by the application of a weak r.f. field (spin tickling) and noting which satellite is perturbed as a result of this irradiation. It can also be mentioned

parenthetically at this point that it is possible in certain instances to determine the couplings and signs even when the transitions are obscured by the center peak. Each of the A transitions shares a common energy level with two of the B transitions and therefore when a second r.f. field is adjusted so as to coincide with one of the A transitions, one of the B transitions is split into a sharp doublet (regressive transition) but the other into a broad doublet (progressive transition) (130). In this manner the A lines can be detected and the signs can be established.

Experimental sign determination and orientation effects

When β-D-glucopyranose-1-^{13}C (which shows a geminal coupling of 5.7 Hz) is subjected to such a study several problems become immediately apparent. The spin system is no longer a simple AMX case since H_2 is coupled not only to H_1 but to H_3 as well. The pmr spectrum of this compound is represented symbolically in Fig. 8.

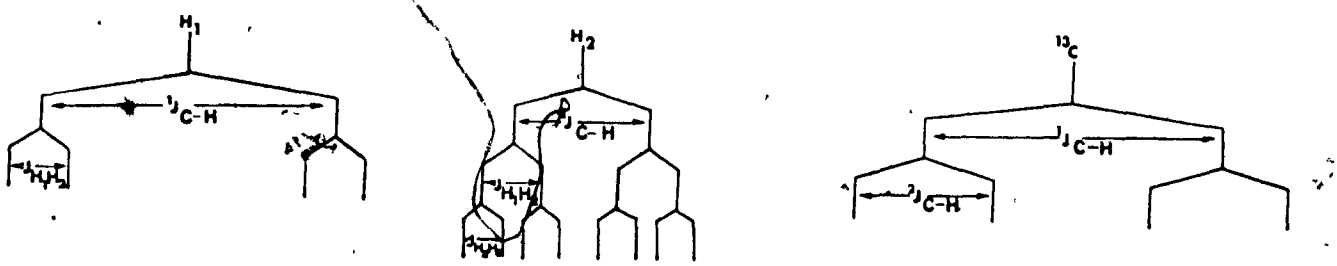


FIG. 8 Schematic partial NMR spectrum of β-D-glucopyranose-1-^{13}C.

This circumstance should not prevent relative sign determination. Instead of observing a collapse of a doublet, one would observe perturbation of the appropriate quartet, so that in principle the experiment is still feasible. In practice, however, due to the equality of $J_{H_1H_2}$ and $J_{H_2H_3}$ and the chance equality of $^2J_{C-H}$ to one half of these proton-proton couplings, the H_2 absorption is such as to prevent the experiment from being carried out. The

problem is evident in Fig. 9 which illustrates the H_2 absorption at 220 MHz of β-D-glucopyranose and β-D-glucopyranose-1-^{13}C.

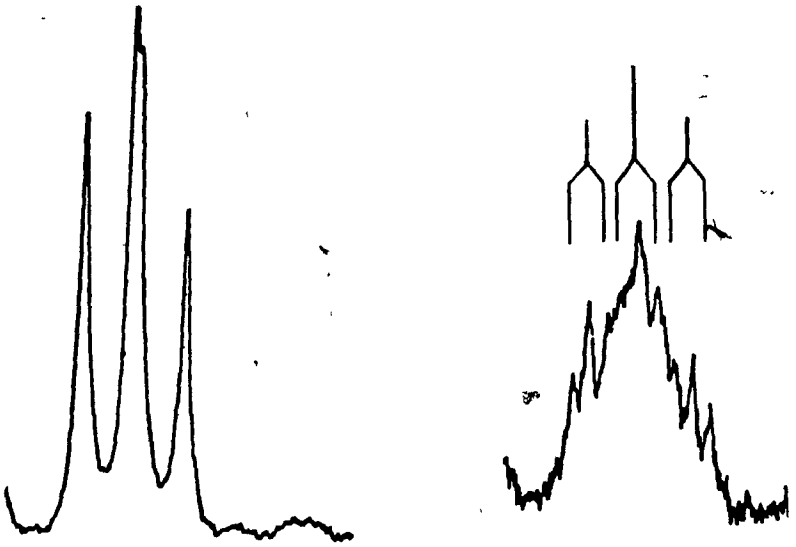


FIG. 9 H_2 absorption (220 MHz, 500 Hz sweep width) of β-D-glucopyranose and β-D-glucopyranose-1-^{13}C (60%).

Although $^2J_{C-H}$ can be derived from this signal, the individual absorptions necessary for sign determination cannot be clearly seen.

As the theory seemed promising, a search was carried out for a more suitable compound. β-D-Allopyranose-1-^{13}C proved to be amenable to such a study of the sign of ^{13}C-^{1}H geminal coupling; its synthesis is outlined in Fig. 10.

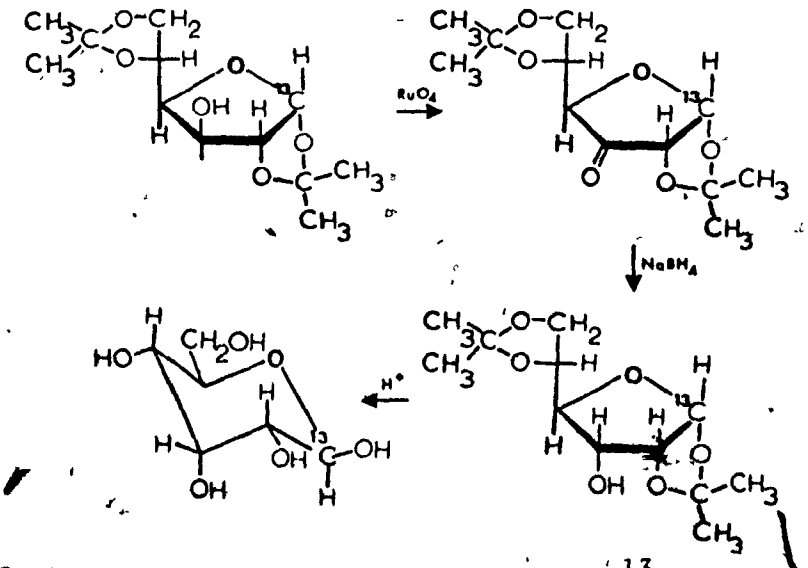
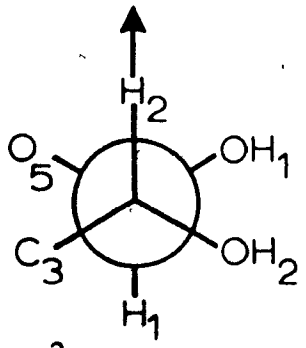


FIG. 10 Synthesis of β-D-allopyranose-1-^{13}C.

D-Allose differs from D-glucose in its configuration about C_3, a difference that does not influence the nature of the experiment but the very difference which makes it possible. Since $J_{H_1H_2}$ is no longer equal to $J_{H_2H_3}$ (the dihedral angles involved are close to 180° and 60° respectively), the ^{13}C coupled H_2 signal is now seen as an octet in Fig. 11.

Experimentally, it proved best to proceed by observation of the low frequency H_1 satellite while the two halves of the H_3 octet were subjected to the second r.f. field. As can be seen in Fig. 12, irradiation of the upfield half resulted in the collapse of the low field H_1 satellite while irradiation of the low field half left this signal unaffected. This result was confirmed by irradiation the low field H_1 satellite and observing the perturbation of the upfield half of the H_2 absorption.

The above result shows that $^1J_{C-H}$ and $^2J_{C-H}$ are of opposite sign; consequently $^2J_{C-H}$ for the stereochemical arrangement below is negative:



$^2J_{C-H}$ = -5.7 Hz

It now becomes possible to make the statement that as the dihedral angle between the resultant, (as introduced on p. 91) and the proton involved in the coupling decreases, the actual value of $^2J_{C-H}$ becomes more negative. Another way of stating this observation is that an oxygen trans to the H atom makes a positive contribution to the coupling, since no coupling to H_2 is observed for the steric situation encountered in α-D-glucopyranose-1-^{13}C:

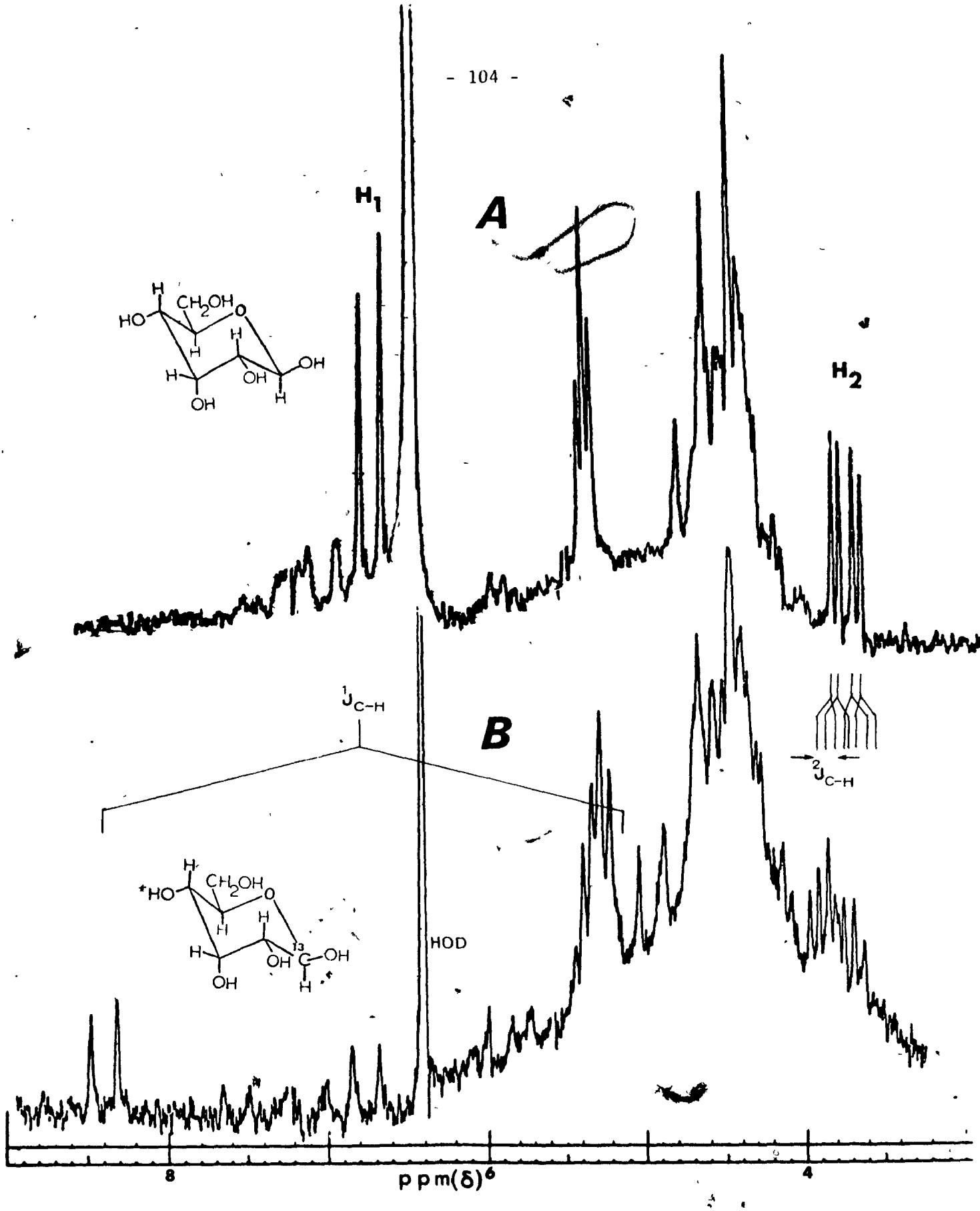


FIG. 11 Partial pmr spectrum (100 MHz) of A) β-D-Allose and B) β-D-Allose-1-^{13}C (80%) in D_2O.

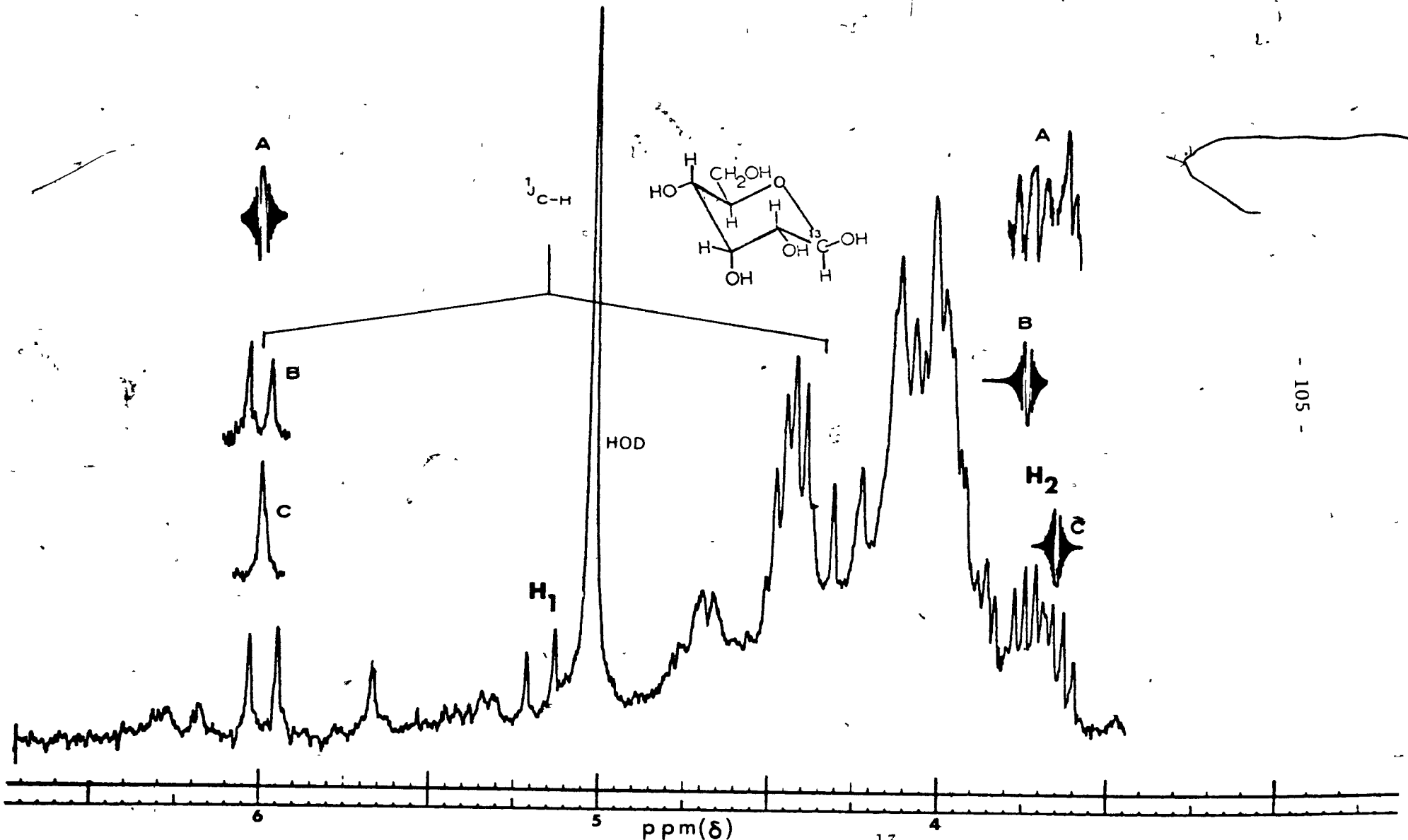
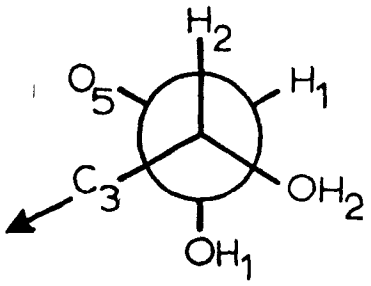
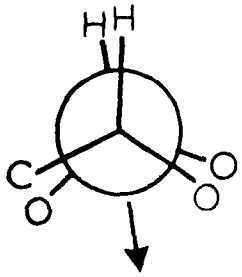


FIG. 12 Relative sign determination in β-D-Allose-1-^{13}C (80%) by double resonance.

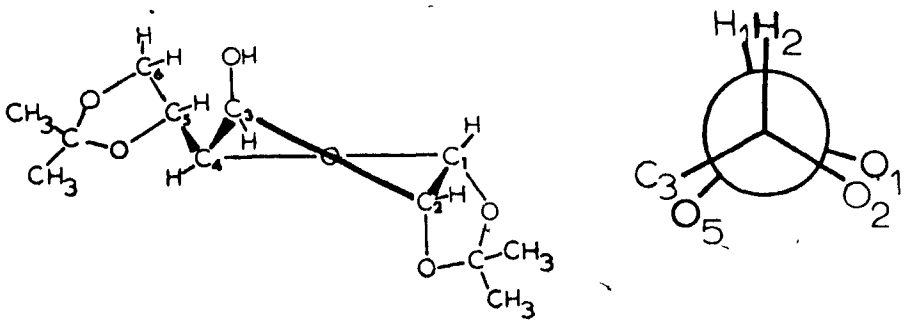


In order to make full use of such relationships in structural analysis it became necessary to investigate the effect exerted on $^{2}J_{C-H}$ when the dihedral angle maintained by the resultant and the relevant C-H bond is increased beyond the angle for which no coupling is observed, i.e., when the resultant and the C-H bond approach an anti relationship. The partial structure which would give rise to the desired situation can be represented by a projection along the C-^{13}C bond:



It is difficult to realize such a conformation in a six-membered ring system, but careful scrutiny led to the selection of 1,2:5,6-di-O-isopropylidene-α-D-glucofuranose-1-^{13}C as a suitable model.

The conformation of this molecule can be reasonably well established from proton-proton coupling data (131)(132). The fact that no coupling is observed between H_2 and H_3 fixes the orientation of the C_2-H_2 and C_3-H_3 bonds at ~90°; consequently the conformation of the molecule is fixed as represented by the projections below:



The "resultant" is now seen to be virtually trans to the proton in question, i.e., H_2. In selecting such a molecule with the desired orientations it was, of course, essential that the proton spectrum be suitable for sign determination. The spectrum of the above compound along with its 1-^{13}C (80%) analog is given in Fig. 13.

The H_2 signal is clearly identifiable as a doublet in the non-enriched spectrum. Since there is no coupling to H_3, we have an ideal AMX case with X being ^{13}C (Fig. 14).

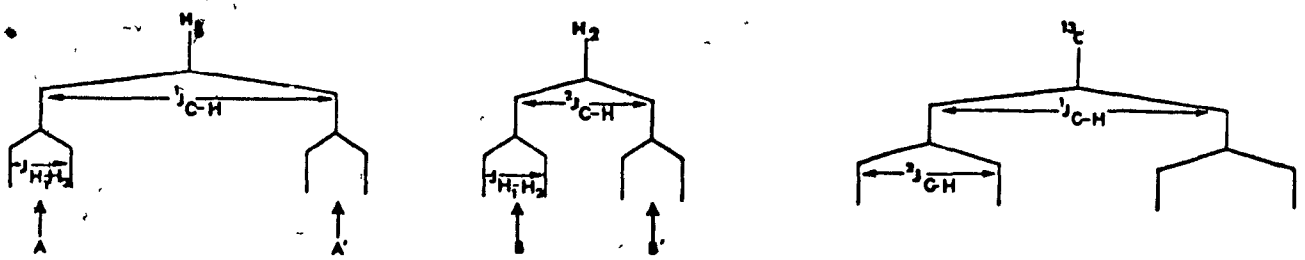


FIG. 14 Schematic partial NMR spectrum of 1,2:5,6-di-O-isopropylidene-α-D-glucofuranose-1-^{13}C.

This time irradiation at point B caused collapse of the A doublet whereas irradiation at B' exerted a similar effect on A'. The results were confirmed by irradiating at A and observing the appropriate decoupling at B as shown in Fig. 15.

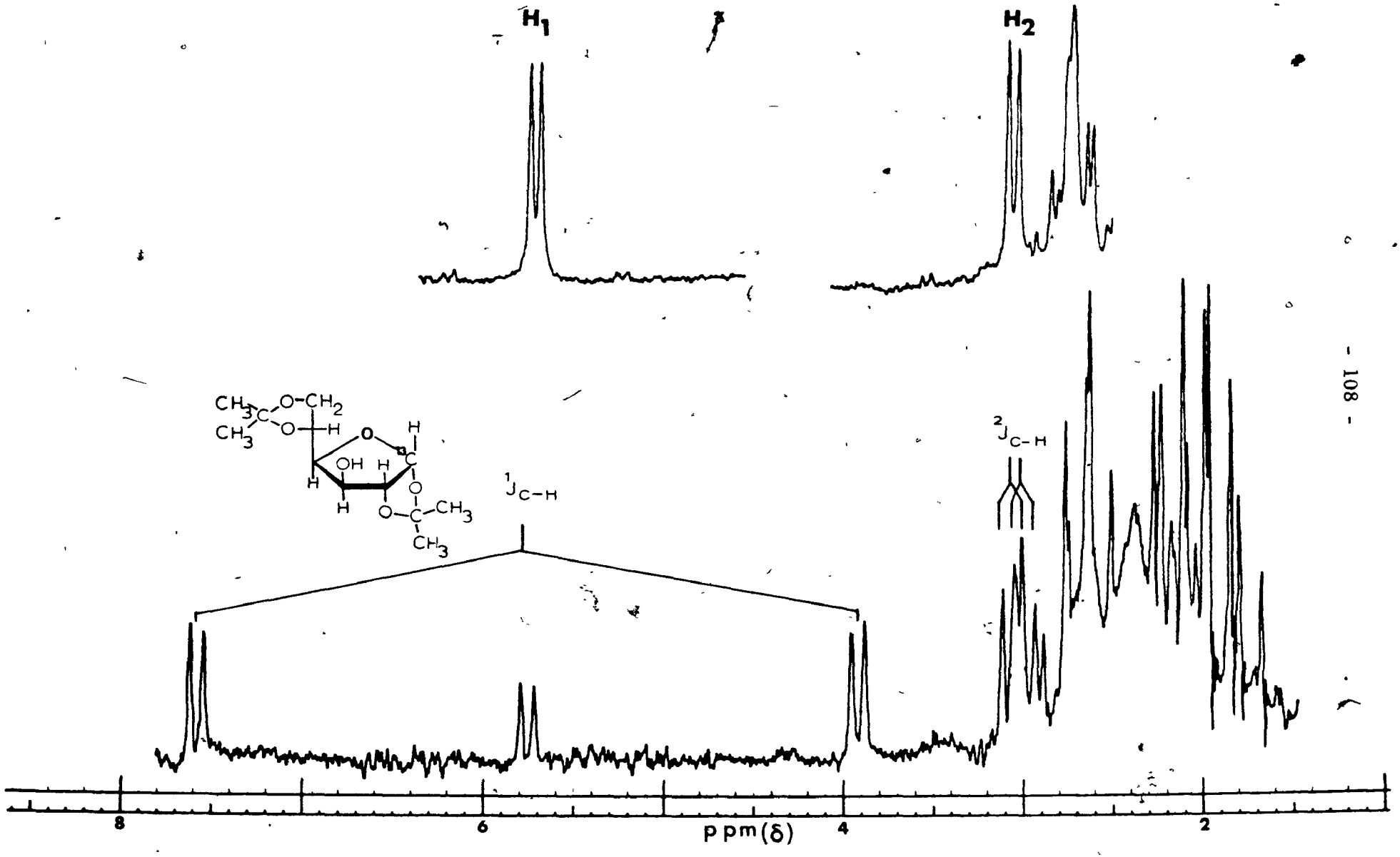


FIG. 13 Partial pmr spectrum (100 MHz) of A) 1,2:5,6-di-O-isopropylidene-α-D-glucofuranose and B) 1,2:5,6-di-O-isopropylidene-α-D-glucofuranose-1-^{13}C (80%) in $CDCl_3$.

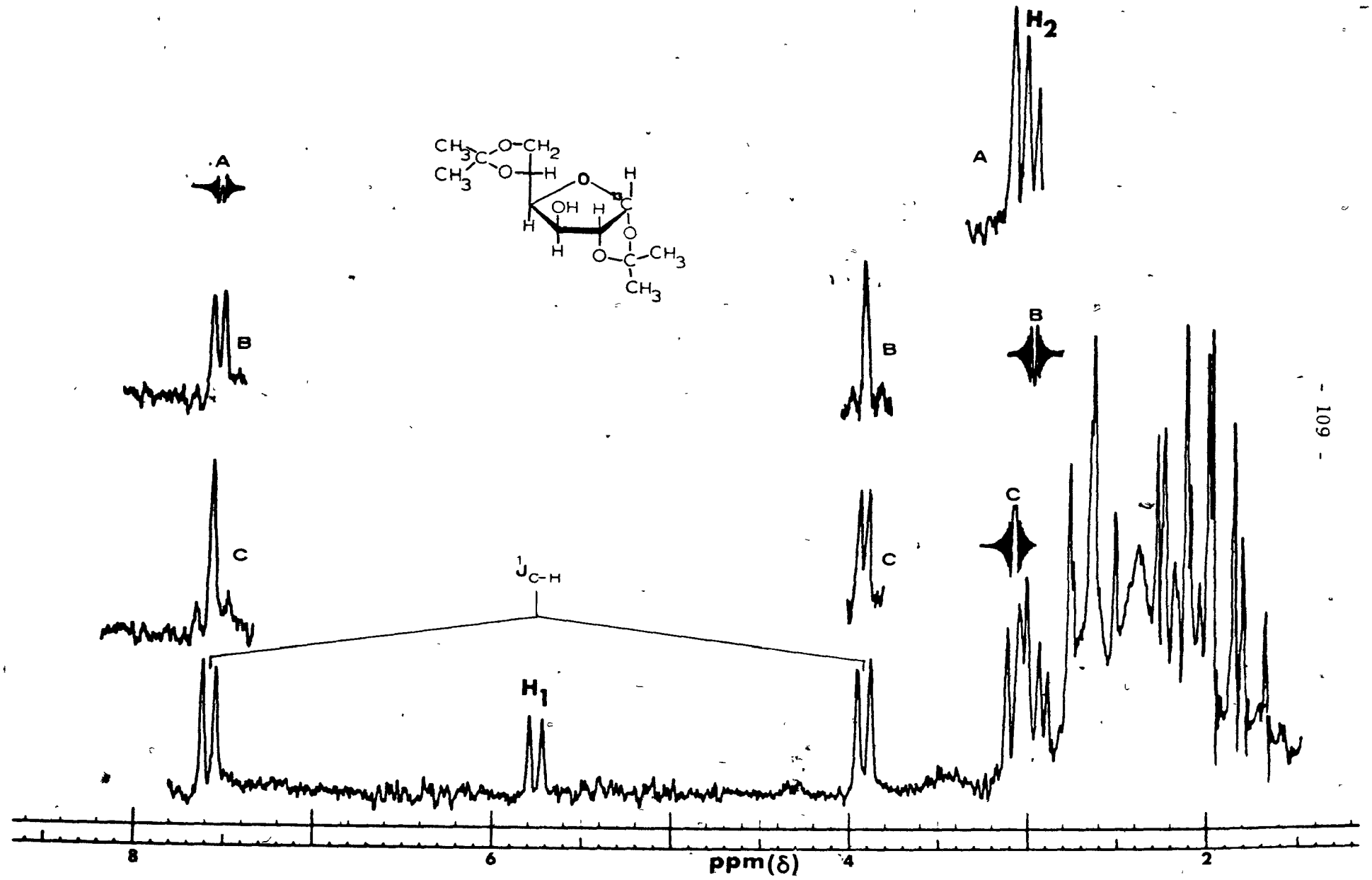
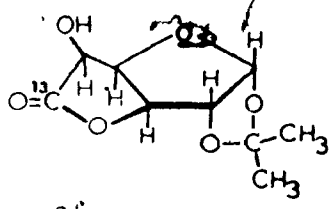


FIG. 15 Relative sign determination in 1,2:5,6-di-O-isopropylidene-α-D-glucofuranose-1-^{13}C (80%) by double resonance.

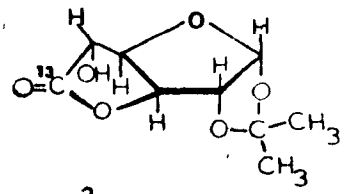
This experimental result demonstrates that $^{2}J_{C\text{-}H}$ is positive (+5.5 Hz) for the particular stereochemical situation examined.

The primary overall conclusion then is that geminal ^{13}C-H coupling is influenced by the orientation of the substituents on the ^{13}C atom with respect to the C-H bond involved in the coupling. At least for the case when the ^{13}C atom bears two oxygen substituents the coupling is negative when the dihedral angle between the oxygens and the proton is small; passes through zero and becomes positive as the oxygens move further away. A trans oxygen substituent then makes a positive contribution to the coupling, whereas a gauche one makes a negative contribution.

It now becomes possible to examine the previously-mentioned 1,2-O-isopropylidene-α-D-glucofuranurono-6,3-lactone-6-^{13}C and its L-ido epimer:

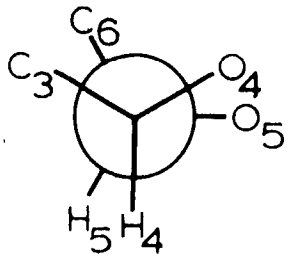


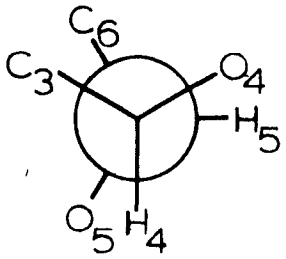
$^{2}J_{C\text{-}H}$ = 4.1 Hz



$^{2}J_{C\text{-}H}$ < 1 Hz

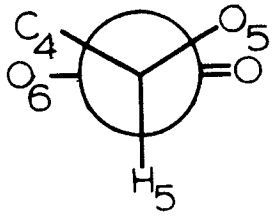
In these instances the ^{13}C in question is sp^2 hybridized. Although the inherent rigidity of a six-membered ring is now absent, the use of proton-proton couplings gives a fairly good estimate of the preponderant conformations. As in the case of 1,2:5,6-di-O-isopropylidene-α-D-glucofuranose there is no coupling between H_2 and H_3 indicating a 90° dihedral angle. Furthermore, the H_4-H_5 coupling is large in the case of the gluco isomer and is zero for the ido isomer. This is consistent with the following preponderant conformations in the region of C_4-C_5 bond:

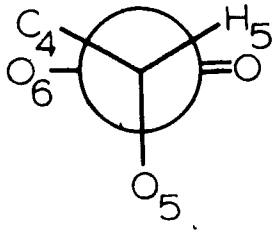




D-Gluco L-Ido

The relative orientations about the C_5-C_6 bond can then be represented as below:

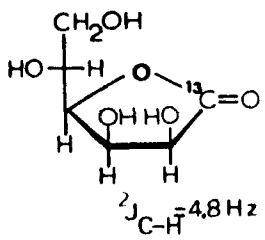




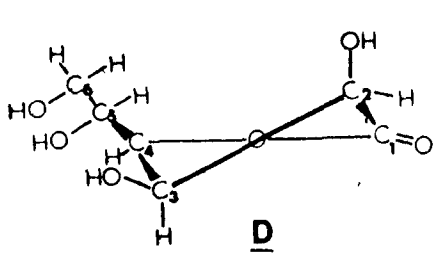
D-Gluco L-Ido

Once again it is obvious that there is a considerable difference in the orientation of H_5 with respect to the two C_6 oxygen substituents. Since we are dealing here with an sp^2 hybridized ^{13}C and a double bonded oxygen it is not possible to equate this type of arrangement to the cases already described (i.e. β-D-allose and 1,2:5,6-di-O-isopropylidene α-D-glucofuranose-1-^{13}C). It is clear, nevertheless, that the general trend seems to be the same; the presence of the trans oxygen results in ~0 Hz coupling. Although it is not possible to carry out sign determinations for these compounds since the ^{13}C atom bears no hydrogen, the results are consistent with a -4.1 Hz coupling for the gluco isomer and <1 Hz for the compound possessing the ido configuration; i.e., the trans oxygen makes a positive contribution to the coupling.

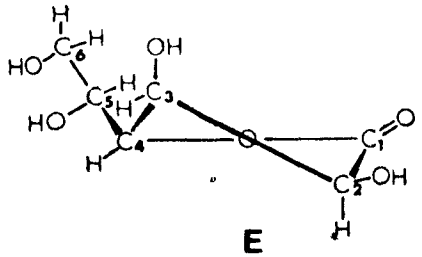
As a potential application of this method, if D-mannono-1,4-lactone,



is examined, it is evident that two major conformations, D and E, are possible depending on the puckering of the five-membered ring:

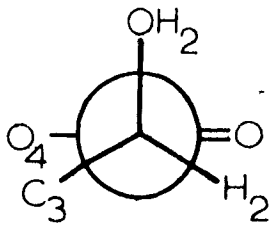


D

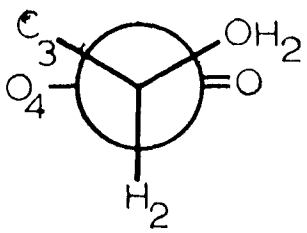


E

In projection along the C_2-$^{13}C_1$ bond these can be represented by:



D



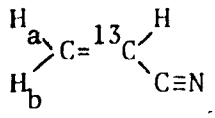
E

D is then seen to be analogous to the situation in 1,2-O-isopropylidene-β-L-idofuranurono-6,3-lactone as already described, whereas E corresponds to that in 1,2-O-isopropylidene-α-D-glucofuranurono-6,3-lactone. Measurement of the ^{13}C-H_2 coupling constant should then help to determine the preponderant conformation of this compound in solution. D-Mannono-1,4-lactone-1-^{13}C was available from D-mannonic acid-1-^{13}C which in turn was obtained from the cyanohydrin reaction of D-arabinose with enriched KCN. The measured ^{13}C-H_2 coupling of 4.8 Hz then indicates that the favoured conformation is E, and implies that the preponderant conformation is the same as in glucofuranuronolactone - a result that is to be

expected on the basis of chemical intuition.

3.4 Orientation effects when the ^{13}C atom bears only one substituent

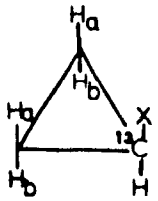
When it became evident that the orientation of the electronegative substituents about C_1 relative to H_2 in the enriched sugars can influence the extent of coupling between C_1 and H_2, the possibility arose that even if the carbon had only one such substituent, its orientation could in fact affect the coupling. As already mentioned on p. 94 such possibilities do exist in halogen substituted ethylenes (95)(124)(133)(134)(135) and the effect is also observable when the substituent is of a less electronegative nature as below (136)(137)(138):



$^2J_{C\text{-}H_a}$ = +0.29

$^2J_{C\text{-}H_b}$ = -4.42

The only apparent relevant example in the literature for single bonded systems is a substituted cyclopropane (139):



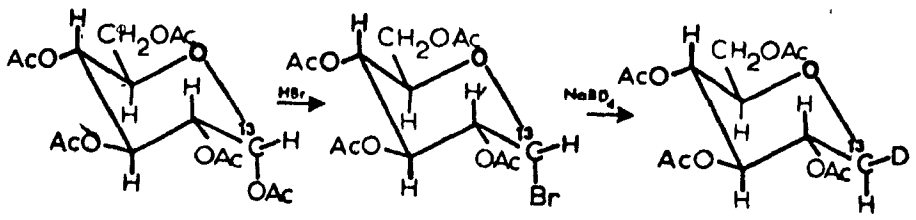
X	$^2J_{C\text{-}H_a}$ (Hz)	$^2J_{C\text{-}H_b}$ (Hz)
Br	-5.35	-1.55
NH_2	-4.0	-1.0
COCl	-3.2	-2.2

There is an ambiguity, however, in this type of compound since the couplings can be regarded either as of a two bond geminal or of a three bond vicinal nature. Nevertheless, the results are consistent with the hypothesis already proposed that

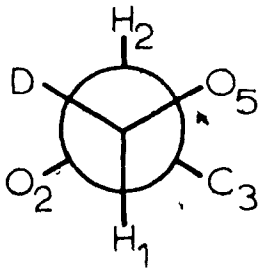
a trans electronegative substituent increases the actual value of the coupling, i.e. making it less negative if it already is negative.

The investigation of this possible angular dependence proved to be difficult on several accounts. Primarily, the ^{13}C-H geminal couplings were found to be in general considerably smaller in magnitude when the coupling carbon had only one oxygen substituent instead of two. Secondly, the selection and synthesis of molecules in which the dihedral angle between the substituent and the proton in question takes on different values presented problems. To make such a study truly representative it was necessary to examine a system having a gauche oxygen, one having a trans oxygen, and one having an oxygen substituent at roughly 90° relative to the coupling proton. Variables other than the oxygen orientation must be kept as constant as possible and of course these systems must essentially be conformationally static. A further complication is the fact that, of necessity, in the sugar series we are dealing mostly with 6-^{13}C labelled sugars and are observing the coupling to H_5. In general, H_5 is a complex signal (usually an octet) resulting from coupling to H_6, $H_{6'}$, and H_4. Consequently, this absorption often cannot be clearly identified in the 100 MHz or even the 220 MHz pmr spectrum; this factor as well had to be taken into account in the selection of illustrative compounds.

As a model for the 60° angular relationship, 2,3,4,6-tetra-O-acetyl-1,5-anhydro-D-glucitol-1-^{13}C-1-d proved to be suitable and was synthesized in the manner outlined below:

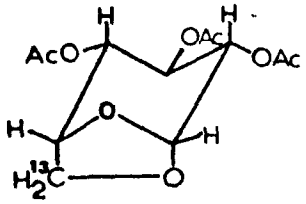


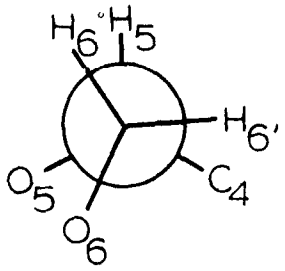
Introduction of deuterium instead of hydrogen was necessary in order to simplify the H_2 signal. This compound in projection along the C_1-C_2 bond can be represented by the following:



The measured coupling between $^{13}C_1$ and H_2 for the above case is 3-3.5 Hz.

A near trans arrangement between H_5 and O_6 is provided by 2,3,4-tri-O-acetyl-1,6-anhydro-α-L-idopyranose-6-^{13}C:





This compound can be synthesized by acid hydrolysis of 1,2:5,6-di-O-isopropylidene-β-L-idofuranose-6-^{13}C followed by removal of water under high vacuum. The partial spectra (H_2 and H_5 absorptions) of the enriched and non-enriched material are reproduced in Fig. 16. The "envelope" appearance of the H_5 signal for the 6-C^{13} labelled compound is not caused by poor resolution (as can be seen by comparing the H_2 signals) but is due to each line of the H_5 triplet being split into a narrow doublet by $^{13}C_6$.

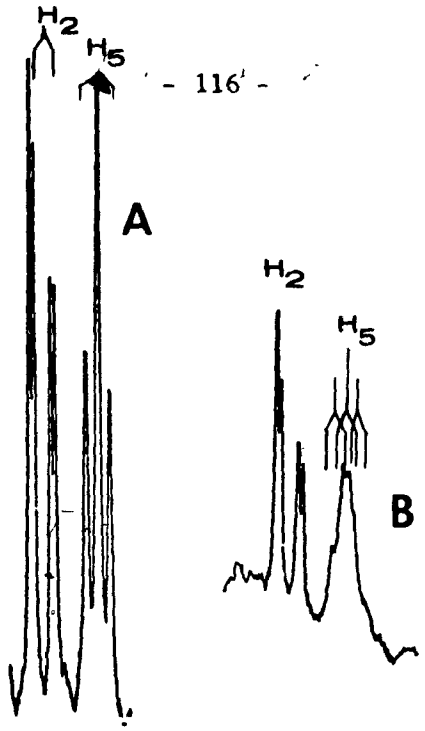
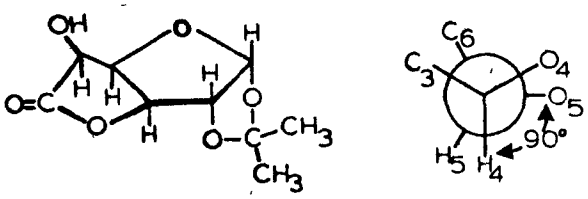


FIG. 16 Partial pmr spectrum (100 MHz) of A) 2,3,4-tri-O-acetyl-1,6-anhydro-α-L-idopyranose and B) 2,3,4-tri-O-acetyl-1,6-anhydro-α-L-idopyranose-6-^{13}C (60%) in $CDCl_3$.

The observed splitting in this case is ∿0.5 Hz; it is then evident that $^2J_{C-H}$ does show a dependence on the relative orientation of the coupling proton and the ^{13}C substituent. The possibility still remains that a maximum effect is exerted for a 90° angular relationship. The selection of a compound in which this arrangement can be realized poses a problem. It is not possible to incorporate the 6 position of any of the sugars into a rigid system where H_5 and O_6 subtend a dihedral angle of circa 90°; consequently an alternate approach had to be employed.

As previously mentioned, it is possible to obtain ^{13}C-^1H geminal coupling constants from Fourier transform ^{13}C spectra in certain cases where signal assignments can be made and where no ambiguity exists in determining which particular proton gives rise to a certain ^{13}C-^1H split. On examining molecular models it became apparent that in 1,2-O-isopropylidene-α-D-gluco-furanurono-6,3-lactone the C_5-O_5 and C_4-H_4 bonds subtend an angle of ∿90°, which is the desired relationship:



Synthesis of this molecule ^{13}C enriched at C_5 would involve the elaborate process of first synthesizing D-xylose-5-^{13}C by the cyanohydrin reaction then building up to glucose, with the appropriate separation of isomers. Nevertheless, the non-enriched compound should be suitable for Ft study. The absence of protons on C_6 and the fact that the dihedral angle between the C_4-C_5 and C_3-H_3 bonds is about 90° (as will be shown in the next chapter such a relationship results in no vicinal ^{13}C-^{1}H coupling) ensures that the only coupling to C_5 arises from interaction with H_4. If the C_5 signal in the Ft ^{13}C spectrum then can be properly resolved and assigned, the relevant spacing in turn can be measured from the proton coupled ^{13}C spectrum.

The natural abundance Ft ^{13}C proton decoupled spectrum of this molecule has the appearance shown in Fig. 17.

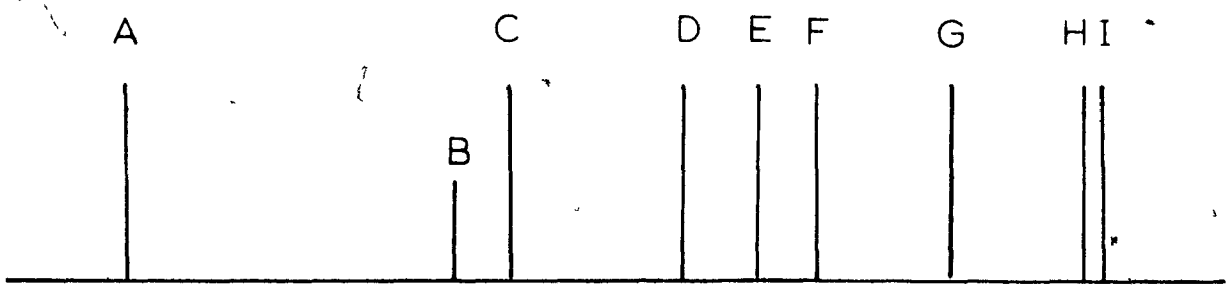


FIG. 17 Schematic proton decoupled ^{13}C Ft spectrum of 1,2-O-isopropylidene-α-D-glucofuranurono-6,3-lactone.

Some assignments can be made on the basis of chemical shifts and intensities. Accordingly, the lowest field signal, A, is assigned to the carbonyl carbon, B

to the central carbon of the isopropylidene group (lack of nuclear Overhauser effect) and C to C_1 of the furanose ring. The H and I absorptions are assigned to the isopropylidene methyls. The D, E, F and G absorptions representing C_2, C_3, C_4 and C_5 however cannot be sorted out. It seemed possible to make signal assignments in this case by making use of the 6-^{13}C enriched analog which was available by lactonizing 1,2-O-isopropylidene-D-glucuronic acid-6-^{13}C. The ^{13}C spectrum of this compound was expected to exhibit ^{13}C-^{13}C coupling between the enriched $^{13}C_6$ and C_5 thereby enabling the identification of the C_5 signal. Normally such coupling cannot be detected in the spectrum due to the low probability of finding two ^{13}C nuclei adjacent to each other. The proton decoupled 6-^{13}C enriched spectrum had the appearance shown in Fig. 18.

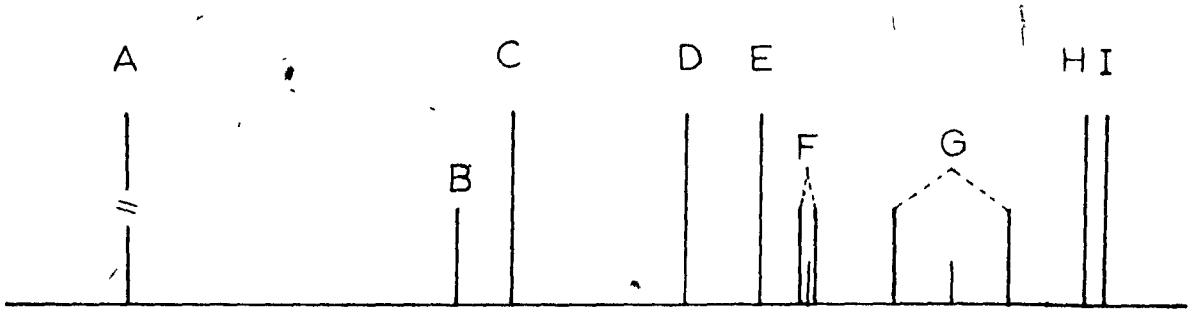


FIG. 18 Schematic proton decoupled ^{13}C Ft spectrum of 1,2-O-isopropylidene-α-D-glucofuranurono-6,3-lactone-6-^{13}C.

The carbonyl signal, of course, is now immense in comparison with the other signals due to enrichment. It is clearly obvious that G has become a doublet ($J_{^{13}C-^{13}C}$ = 55 Hz) and therefore it is the absorption due to C_5. (Some long range ^{13}C-^{13}C coupling between C_6 and F is also noted: F is then likely the C_4 or C_3 absorption).

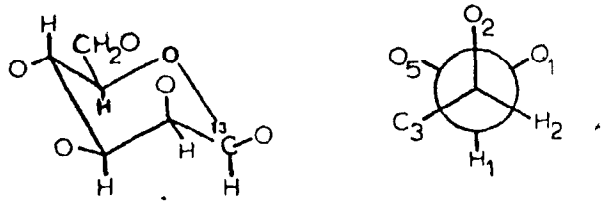
With the signal for C_5 assigned, the proton coupled ^{13}C spectrum was examined and it was found that coupling between C_5 and H_4 was less than 2 Hz.

The overall suggestion, then, is that in the type of compounds investigated in this study, the absolute value of the geminal coupling increases as the dihedral angle between the coupling proton and the electronegative substituent appended to the ^{13}C nucleus decreases.

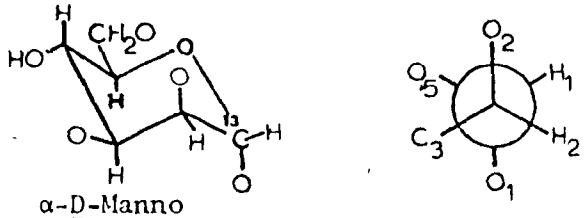
3.5 Possible steric interaction effects

The striking difference in geminal coupling ($^{13}C_1$-H_2) between α and β-D-glucopyranose-1-^{13}C has already been discussed and has been attributed to a difference in relative orientation between H_2 and the C_1 substituents. The possibility has to be considered, however, that the change is actually brought about by differing degrees of steric interaction in the anomers. For example, it is conceivable that the apparent absence of coupling in α-D-glucopyranose-1-^{13}C can be caused by a perturbation of the electron distribution about C_1 brought about by repulsion between axial H_3 and OH_1. The directly bonded ^{13}C-^1H couplings as discussed in the previous chapter suggest an increase with 1,3 diaxial interactions. Since the geminal ^{13}C-^1H coupling in β-D-glucopyranose-1-^{13}C, by analogy to β-D-allopyranose-1-^{13}C, is taken to be negative, a positive contribution due to a 1,3 diaxial interaction may cause the coupling to take on a value of close to zero.

In this connection it is useful to compare compounds which experience different degrees of steric interaction while maintaining essentially constant relative orientations. Information can be provided along these lines by compounds in the D-mannose-1-^{13}C series. Since H_2 is now equatorial, orientation of the C_1 substituents relative to H_2 is equivalent in the α and β anomers, i.e., one gauche and one trans relationship is maintained:



β-D-Manno



α-D-Manno

The steric interactions inherent to these compounds, however, are different. The β anomer has a 1,2 gauche oxygen-oxygen interaction whereas the α anomer has a 1,3-diaxial interaction. If steric effects are important in determining geminal ^{13}C-^{1}H coupling, the observed couplings for the above anomers should be different.

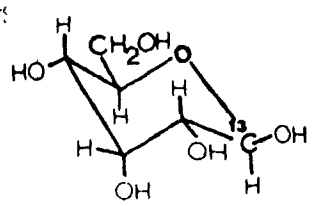
D-Mannose-1-^{13}C became available by the reduction of D-mannono-1,4-lactone-1-^{13}C, which itself had been synthesized from D-arabinose (p. 19). Unfortunately, it was not possible to adequately resolve the H_2 resonances of mannose itself in aqueous solution, in which it exists as a mixture of α and β anomers and it thus became necessary to resort to derivatives of the sugar.

To represent the α series, 2,3,4,6-tetra-O-acetyl-α-D-mannopyranosyl bromide-1-^{13}C was synthesized by the reaction of 1,2,3,4,6-penta-O-acetyl-D-mannopyranose-1-^{13}C with HBr. The β series was in turn represented by 1,2,3,4,6-penta-O-acetyl-β-D-mannopyranose-1-^{13}C (the H_2 resonance in the α anomer is obscured by other signals). The fact that neither a bromine nor an O-acetyl substituent introduces a significant change in geminal coupling in comparison

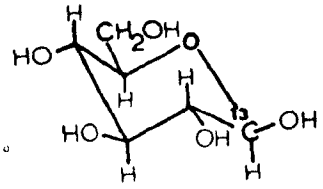
with an OH group was confirmed in the glucose series: i.e., the geminal $^{13}C_1$-H_2 couplings of the α and β pentacetates differ very little from those of the free sugar (5 Hz as compared with 5.7 Hz) and 2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl bromide-1-^{13}C shows a coupling of ∿1.5 Hz in comparison with < 1 Hz for α-D-glucopyranose-1-^{13}C.

The measured $^{13}C_1$-H_2 couplings both in the α and β mannose derivatives were found to be 1-1.5 Hz. This is comparable to the $^{13}C_1$-H_2 coupling in α-D-glucopyranose-1-^{13}C, a compound in which the orientation of H_2 relative to O_1 and O_5 is the same as in both mannose isomers. This result then implies that the relative orientations of these atoms, and not steric effects, are the main factors determining the extent of ^{13}C-^{1}H geminal coupling.

In this connection it is also significant that the coupling between $^{13}C_1$ and H_2 in β-D-allopyranose-1-^{13}C (6 Hz) is practically equivalent to that observed in β-D-glucopyranose-1-^{13}C (5.7 Hz). In the former compound the hydroxyl substituent on C_3 is axial whereas it is equatorial in the latter:



$^2J_{C-H}$ = 6 Hz



$^2J_{C-H}$ = 5.7 Hz

If geminal ^{13}C-^{1}H couplings reflect steric effects this should certainly be evident in the β-D-allose compound due to the strong 1,3 diaxial interaction between OH_3 and H_1. The equivalence of the above two couplings reinforces the hypothesis that steric interactions are not major determinants of the extent of ^{13}C-^{1}H geminal coupling.

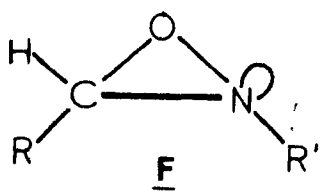
3.6 Lone pair effects

It has already been demonstrated that adjacent unshared electron pairs play a role in determining the extent of directly bonded $^{13}C-^{1}H$ couplings. Even intuitively then, similar effects can be expected for geminal $^{13}C-^{1}H$ coupling, and, this hypothesis is reinforced after the examination of other geminal systems. It is fruitful in this context to compare geminal $^{2}J_{C-H}$ to other types of geminal coupling; such comparisons were, of course, not possible for the directly bonded $^{13}C-^{1}H$ couplings.

Lone pair effects in geminal systems can be divided into two main categories: a) where the lone pair is located on a coupling atom, and b) where the lone pair is located on an adjacent atom. For the case of $^{13}C-^{1}H$ geminal coupling our concern is exclusively with case "b" since it is not possible to have a neutral carbon atom with a lone pair. Nevertheless it is important to examine the effect of lone pairs on geminal coupling in similar systems in order to establish their magnitude, as well as possible orientation effects.

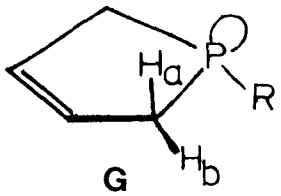
Geminal systems in which one of the coupling atoms bears a lone pair

An investigation of oxaziridines - ^{15}N (F) (114)(140) revealed that the $^{15}N-C-^{1}H$ coupling is 5 Hz in absolute value when the coupling proton is *cis* to the lone pair and is zero when it is *trans* thereby showing a steric dependence.



F

Similar effects are exhibited in $^{31}P\text{-}C\text{-}^{1}H$ coupling. In G, it was found that $^{2}J_{P\text{-}H_a} = \pm 25$ Hz whereas $^{2}J_{P\text{-}H_b} = \pm 6$ Hz (141).



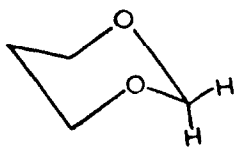
G

It has been shown, in fact (141)(142), that the $^{31}P\text{-}C\text{-}^{1}H$ coupling varies with the dihedral angle between the C-H bond and the lone pair, becoming zero at 80° and 180°.

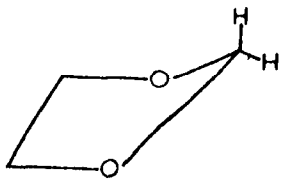
Geminal systems in which the lone pair is situated on a neighboring atom

a) Proton-proton geminal coupling

The proton-proton geminal coupling in 1,3-dioxane (H) is - 6 Hz (143) whereas the analogous coupling in dioxolane systems (I) is about 0±2 Hz (144)



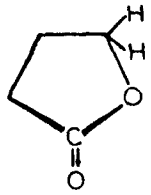
H



I

Such differences are not observed when the ring size is further varied (145). In I each of the C-H bonds appears to experience partial eclipsing by an oxygen electron pair whereas this is not so in the case of dioxane. The primary interpretation then is similar to that proposed for directly bonded $^{13}C\text{-}^{1}H$ couplings: proximity of a lone pair to a bond involved in the coupling increases the absolute value of this coupling. In theoretical terms the effect of the lone pair can be envisaged as electron transfer from the oxygen 2p orbitals into the CH_2 system. Experimentally, it seems that each time an α oxygen atom has

one of its free electron pair p orbitals parallel to the C-H bond of one of the protons involved in the coupling, it contributes an increment of 1.8 Hz (146)(147). For example in the lactone (147):

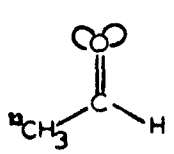


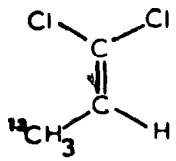
the observed value of -8.5 Hz for the geminal H-H coupling can be rationalized using this concept. In methane the geminal coupling is -12.5 Hz, a freely rotating oxygen would increase this by 1 Hz, and two lone pair contributions of 1.8 Hz would result in a final theoretical value of -7.8 Hz.

It can be concluded then that the relative orientation of a lone pair with respect to the coupling pathway is reflected in the magnitude of the observed geminal internuclear coupling, whether the lone pair is on one of the coupling atoms or on an adjacent atom.

b) ^{13}C-proton geminal coupling

When the type of geminal coupling examined occurs between a ^{13}C nucleus and a proton one has to be concerned only with lone pairs present on adjacent atoms. A remarkable observation suggesting lone pair effects on $^2J_{C-H}$ is noted when the geminal $^{13}C-^1H$ coupling in acetaldehyde (+26.6 Hz) (123) is compared to the coupling in 1,1-dichloropropene (+3.2 Hz):





$^2J_{C-H}$ = +26.6 Hz $^2J_{C-H}$ = +3.2 Hz

As noted in Section 3.8, it is very improbable that substituent effects on the intervening carbon should cause such large differences in coupling. However in the acetaldehyde molecule the lone pairs on the carbonyl oxygen lie in the plane of the coupling pathway and consequently each bond involved in the coupling is eclipsed by a lone pair. It is conceivable then that the large value of the coupling in acetaldehyde is the result of electron donation from the oxygen lone pairs into the coupling system.

It seems again appropriate to begin an examination of lone pair effects on $^{2}J_{C-H}$ in carbohydrates by a study of α and β-D-glucopyranose-1-^{13}C. The lone pair effect was invoked here to explain at least part of the difference observed between the directly bonded ^{13}C-^{1}H coupling of the respective anomers, since the relative orientations of the ring oxygen lone pairs with respect to the C_1-$H_{1\alpha}$ and C_1-$H_{1\beta}$ bonds are different. For geminal coupling, however, the C_2-C_1 bond is involved rather than the C_1-H_1 bond. In this instance, for both anomers the relative orientation of the ring oxygen lone pairs is equivalent and consequently is not expected to give rise to a difference in coupling. The possibility of some difference in coupling arising from differences in rotamer populations about the C_1-O_1 bond however cannot be ruled out.

The question of through space lone pair effects over longer distances can be raised at this point; for example, whether or not the fact that H_2 in D-glucose is in a 1,3 diaxial relationship with one of the ring oxygen lone pairs affects the value of the coupling. To examine this possibility it is fruitful to compare epimers having the manno and gluco configurations. In order to keep the relative orientations of the C_1 substituents with respect to H_2 constant, the α-gluco and β-manno configurations must be compared.

α-D-Gluco — β-D-Manno

No significant difference between these two couplings is observed (0.5 and 1.5 Hz respectively) and it thus seems that lone pair effects are not transmitted over this distance.

In order to investigate the role that the orientation of the lone pairs of a substituent bonded to the carbon intermediate to the coupling path (i.e., C_2) plays, it was necessary to synthesize and examine molecules in which factors other than this orientation could be kept relatively constant. 2,3:5,6-Di-O-isopropylidene-α-D-mannofuranose-1-^{13}C (J) and 3,4,6-tri-O-acetyl-β-D-mannose 1,2-(methyl orthoacetate)-1-^{13}C (K) were selected as suitable model compounds for this purpose and were synthesized as outlined in Fig. 19.

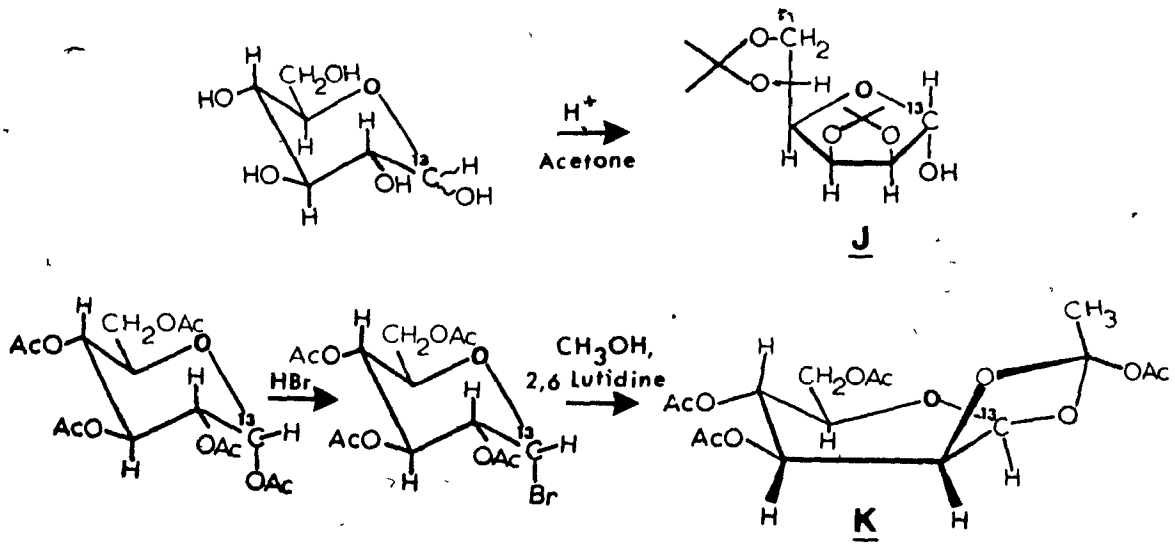
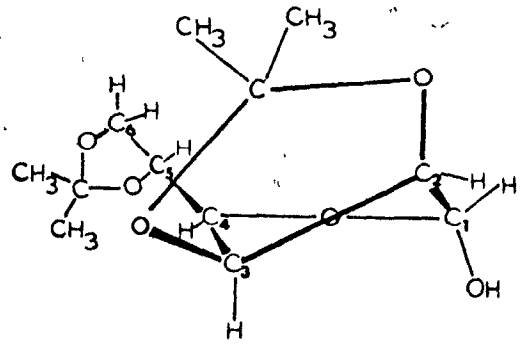
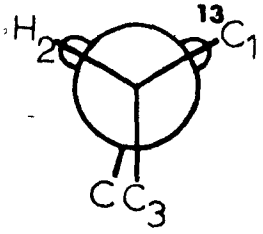


FIG. 19 Synthesis of 2,3:5,6-di-O-isopropylidene-α-D-mannofuranose-1-^{13}C and 3,4,6-tri-O-acetyl-β-D-mannose 1,2-(methyl orthoacetate)-1-^{13}C.

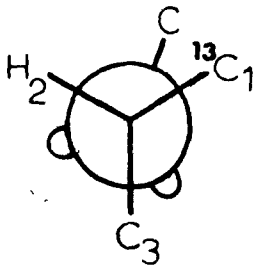
The coupling examined in each instance involves the $^{13}C_1$-C_2-H_2 pathway. The conformations of these two compounds can be assumed with some confidence. The orthoester is a rigid system and poses little conformational uncertainty; the O-isopropylidene compound shows no coupling between H_1 and H_2, establishing the dihedral angle between the C_1-H_1 and C_2-H_2 bonds as roughly 90° and consequently fixing the stereochemistry of the ring as below:



For both compounds, molecular models indicate that the orientation of the two C_1 substituent oxygens with respect to H_2 is very similar: each one has roughly one trans and one gauche interaction. However, the orientation of the O_2 lone pairs is considerably different as illustrated by a projection along the C_2-O_2 bond:



J



K

In the O-isopropylidene compound (J) the lone pairs coincide with the coupling pathway whereas in the orthoester (K) the lone pairs are displaced from the pathway. If the proximity of lone pairs to bonds involved in the coupling does, in fact, influence the magnitude of coupling, such effects should certainly

be noted here. A small effect is observed, a coupling of ~1.5 Hz is measured for 2,3:5,6-di-O-isopropylidene-α-D-mannofuranose whereas the corresponding coupling is <1 Hz for the orthoester. Hence, lone pair effects in these types of systems are unlikely to account for large differences in coupling. The small magnitude of the coupling can be attributed to the orientations of the C_1 substituents with respect to the C_2-H_2 bond, consistent with the previous discussions relating coupling to these orientations.

3.7 Hybridization effects

Hybridization of the coupling ^{13}C

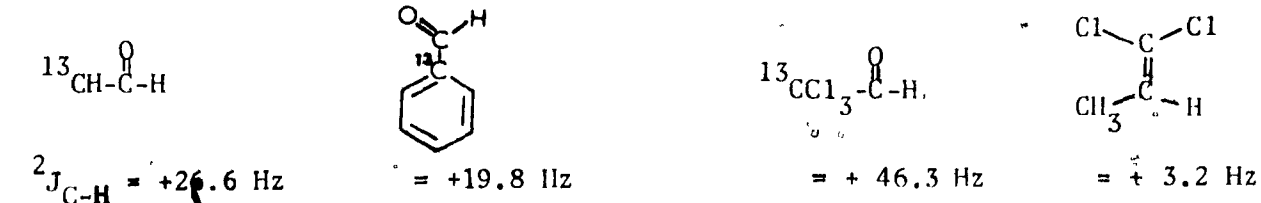
When simple conformationally mobile systems are examined it is found that the absolute value of geminal ^{13}C-^{1}H coupling increases with "s" character of the coupling carbon atom:

	$^2J_{C-H}$	Ref.
CH_3-$^{13}C\equiv C$-H	10.6 Hz	(148)
CH_3-CH_2-$^{13}C(=O)$-CH_2-CH_3	5.7 Hz	(61) (149)
CH_3-CH_2-$CD(OH)$-CH_2-CH_3	4.0 Hz	(61) (149)

The above trend, however, cannot be used practically. As indicated before, the spectroscopic investigation of α and β-D-glucopyranose-1-^{13}C, and 1,2-O-isopropylidene-β-L-idofuranurono-6,3-lactone-6-^{13}C and its D-gluco epimer showed that large differences in couplings can occur even when there is apparently no major change in hybridization. Again, variations due to hybridization can be masked by the relative orientation effects. It does not seem valid then to derive hybridization data from observed ^{13}C-C-^{1}H couplings.

Hybridization of the intervening carbon

Very few data are available for cases when the intervening carbon is not sp^3 hybridized (123)(150):



$^{2}J_{C-H}$ = +26.6 Hz = +19.8 Hz = + 46.3 Hz = + 3.2 Hz

Consideration of the above examples suggests an increase in the absolute value of $^{2}J_{C-H}$ when the "s" character of the intervening atom is increased. However, noting the small value for the ethylene derivative, it becomes questionable whether the large couplings are due to the change in hybridization at the intervening carbon or to the lone pairs on the carbonyl oxygen as discussed on p. 124.

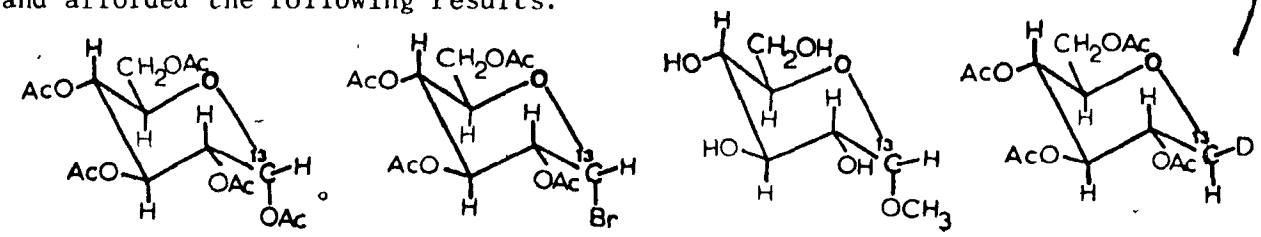
3.8 Electronegative substitution

In freely rotating systems geminal ^{13}C-H coupling generally increases in absolute value with electronegative substitution on the coupling carbon regardless of the hybridization of this carbon. These trends are evident in the following (12) (123) (150)(151):

	$^{2}J_{C-H}$		$^{2}J_{C-H}$		$^{2}J_{C-H}$
$^{13}CH_3-CH_3$	-4.8 Hz	$^{13}CH_3-C(=O)-H$	+26.6 Hz	$CH_3-^{13}C(=O)-CH_3$	5.9 Hz
$Cl_2{}^{13}CH-CH_3$	5.1 Hz	$^{13}CHCl_2-C(=O)-H$	+35.8 Hz	$HO-^{13}C(=O)-CH_3$	6.8 Hz
$Cl_3{}^{13}C-CH_3$	5.9 Hz	$^{13}CCl_3-C(=O)-H$	+46.3 Hz		

However, we have already seen that geminal ^{13}C-H couplings can be quite different even when the substituents are the same; in fact it is the orientation of these substituents with respect to the coupling proton that seems

to be the primary determinant force. It became necessary then to synthesize a series of compounds in which orientations could be kept essentially constant while the substituents were varied in order to analyze substituent effects. A group of related D-glucose derivatives was prepared as requisite model compounds, and afforded the following results:



$^2J_{C-H}$	< 1 Hz	1.5 Hz	< 1 Hz	3.5 Hz

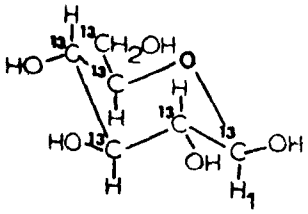
It is evident that the introduction of a second electronegative group in each case causes a decrease in the absolute value of the coupling, probably making a positive contribution to a negative coupling. The nature of the electronegative group does not seem to play a large role. As pointed out above, if this second electronegative group is introduced gauche with respect to H_2 the observed coupling is ~6 Hz, and consequently it is again evident that the orientation and not the nature of the electronegative group has a major effect.

Since the introduction of electronegative substituents on the coupling carbon atom influences the extent of coupling, the possibility arises that their introduction along the coupling pathway can also influence the magnitude of coupling. There is some experimental evidence to indicate a decrease in the absolute value of geminal $^{13}C-^1H$ coupling when an electronegative substituent is introduced along the coupling pathway (151):

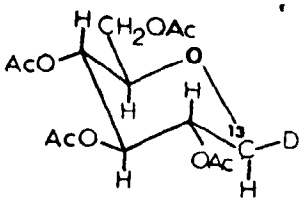
	$\lvert J \rvert$
$^{13}CH_3-CH_3$	4.8 Hz
$^{13}CH_3-CH_2Cl$	2.6 Hz
$^{13}CH_3-CHCl_2$	< 1.2 Hz

Because it is well established that the coupling in ethane is negative (12), an electronegative substituent along the coupling path makes a positive contribution to the coupling.

This question was investigated briefly in the sugar series as well. It was not feasible to make use of either C_1 labelled or C_6 labelled carbohydrates for this purpose due to the difficulty of introducing a second electronegative group on the adjacent carbon. Instead, spectral analysis of a mixture of α and β glucose uniformly enriched in ^{13}C (50%) seemed appropriate. The coupling between $^{13}C_2$ and H_1 of the β anomer is less than one cycle. This coupling can be compared to the $^{13}C_1$-H_2 coupling of 2,3,4,6-tetra-O-acetyl-1,5-anhydro-D-glucitol-1-^{13}C-1-d:



$^2J_{C_2-H_1} < 1$ Hz



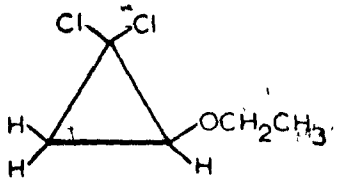
$^2J_{C_1-H_2} = 3.5$ Hz

The major difference between these two compounds is the presence of a second oxygen substituent on the carbon intervening between ^{13}C and ^1H. The orientation of the oxygen on the coupling carbon with respect to the coupling proton is the same in both cases. This observation then is consistent with the hypothesis that introduction of electronegative substituents along the coupling pathway decreases the absolute magnitude of geminal ^{13}C-H coupling.

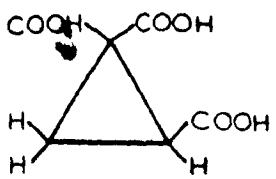
3.9 Effect of the C-C-H angle on ^{13}C-^1H geminal coupling

Early work on ^1H-C-^1H couplings (152) indicated that this type of internuclear coupling shows a dependence on the H-C-H bond angle. These data now are questionable since the originally assigned signs of the coupling were not correct. Experimental "evidence" for such dependence on the angle subtended by the

coupling atoms in general takes the form of the following argument. If the two substituted cyclopropenes below are considered,



L

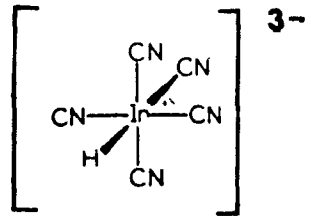


M

whereas $^{2}J_{H-H}$ in L is 8.1 Hz, with $^{3}J_{H-H}$ trans being 5.3 Hz, in M $^{2}J_{H-H}$ is 4.8 Hz and $^{3}J_{H-H}$ trans is 6.5 Hz (153). The increase in $^{3}J_{H-H}$ trans in M relative to L implies that the dihedral angle is increased (4) and consequently that the H-C-H angle is increased, leading to a smaller $^{2}J_{H-H}$. Such arguments fail to take into account the variation of $^{3}J_{H-H}$ with substituents along the coupling network (154) - a concept to be fully discussed in the next chapter. In general then, it is not at all clear how $^{2}J_{H-H}$ varies with angle or whether it varies at all; consequently no clue towards such possible variation in $^{2}J_{C-H}$ is to be had from $^{2}J_{H-H}$.

For $^{13}C-C-^{1}H$ coupling there is no reason to believe that in any of the cases we have examined the $^{13}C-C-^{1}H$ angle deviates greatly from the normal tetrahedral angle. For α and β-D-glucopyranose-1-^{13}C there may be slight angular differences perhaps introduced by a flattening of the ring of α-D-glucopyranose-1-^{13}C due to the 1,3 diaxial interaction between H_3 and OH_1. However, the observed difference in coupling between the anomers of ~5 Hz is certainly too large to be due to these small possible angular differences.

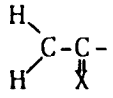
Some indication for angular dependence of $^{2}J_{C-H}$ comes from an examination of the spectra of some metal hydride complexes. For example in $[HIr(CN)_5]^{3-}$ K_3 (155):



cis $^{2}J_{C\text{-}Ir\text{-}H}$ was found to be 5.7 Hz and trans 37.2 Hz. (The identity of cis and trans couplings can be readily determined by noting the intensities of the relevant signals). In the analogous rhodium complex the cis coupling is 5.7 Hz and the trans 56.2 Hz whereas in H Mn $(CO)_5$ the values are reversed: the trans coupling is 7 Hz and the cis is 14 Hz. The signs here are not known, consequently it is possible that the cis coupling is actually -14 Hz so that the larger angle shows the larger coupling.

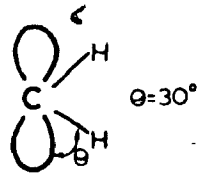
3.10 Neighbouring π bond effect

Since it has been shown that neighbouring unshared electron pairs can influence $^{13}C\text{-}H$ couplings it was of interest to consider whether or not an adjacent π bond can have similar effects. The lone pair effect has been attributed to a donation of electrons into the coupling system, a phenomenon which is theoretically also possible when the carbon atom next to a coupling system is involved in π bonding. In fact, it has been shown (156) that when a π bond is present on a carbon neighbouring a geminal H-H situation as in:

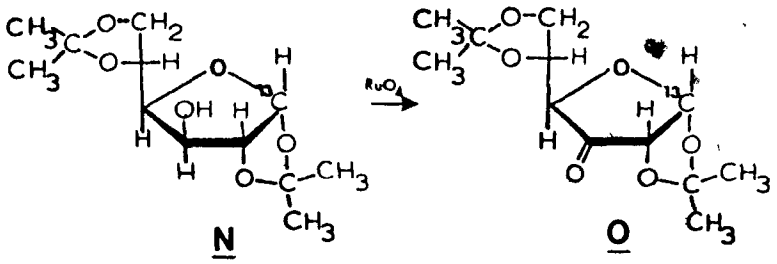


the magnitude of $^{2}J_{H\text{-}H}$ depends on the orientation of the π bond with respect to the protons in question. Experimentally and theoretically (157) the π bond makes the greatest contribution to the coupling when both protons are as close to

the π electrons of the double bond as possible. This situation arises when each proton subtends an angle of 30°, with respect to the π bond, and can be graphically represented by the projection of the methylene group on the π bond:

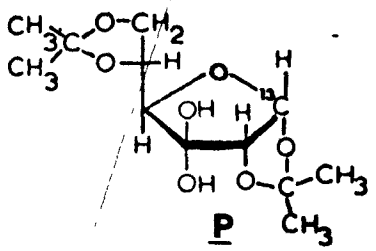


No literature data on geminal ^{13}C-H coupling with adjacent π bonds are available. Hence, it was desirable to examine a molecule having a π bond adjacent to the coupling system for comparison with a situation in which this π bond is absent. Once again the suitability of a compound for such study was governed by the limiting factor that the relevant protons must be identifiable in the pmr spectrum. The ketone formed on oxidation of 1,2:5,6-di-O-isopropylidene-α-D-glucofuranose-1-^{13}C proved suitable in this regard:



N O

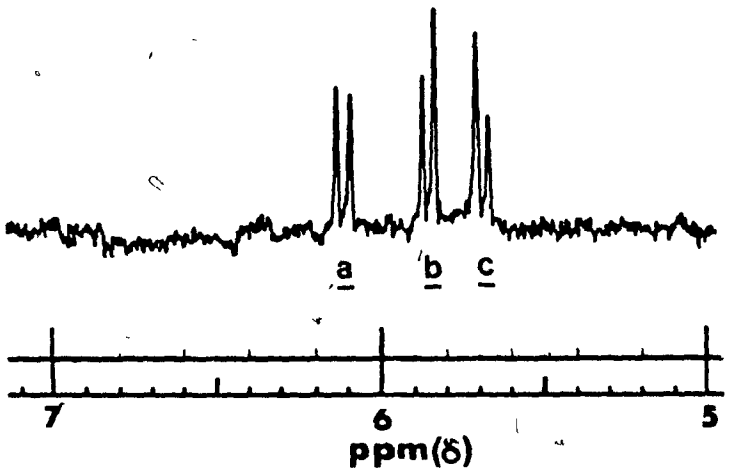
Oxidation of N introduces a double bond adjacent to the geminal $^{13}C_1$-C_2-H_2 system. The final spectrum however is complicated by the fact that along with the desired product O, there is extensive formation of P, the monohydrate of the ketone.



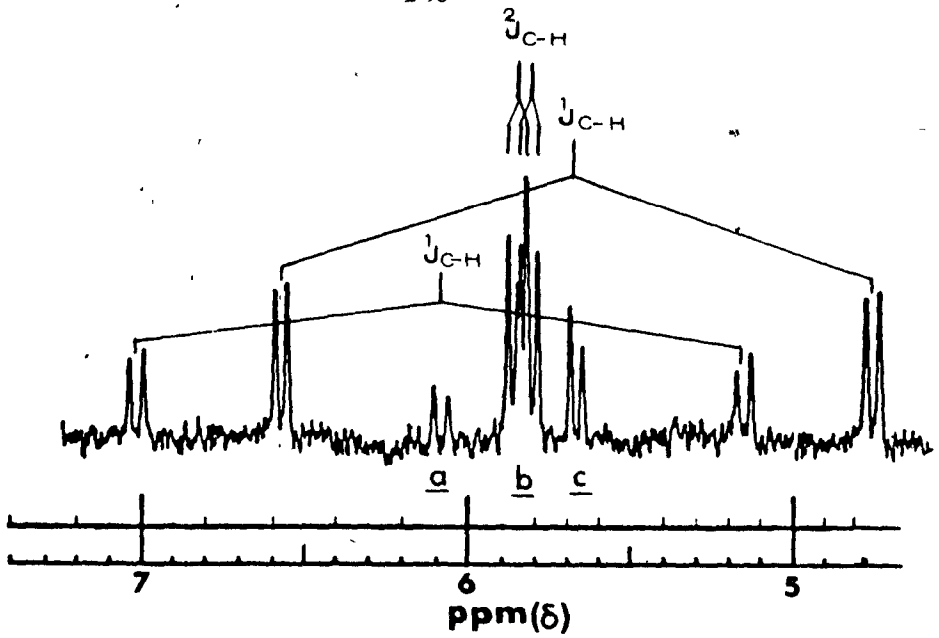
P

On the scale of the experiment (~50 mg) it was not possible to separate the ketone from the hydrate. However, the relevant proton signals in the mixture were identified, providing an interesting example of a spectroscopic problem solved by ^{13}C enrichment.

The anomeric region of the spectrum of the mixed product (non-enriched) had the following appearance:

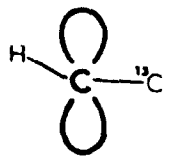


At first, the occurrence of three doublets with the same proton-proton coupling constant (or perhaps one doublet and an AB quartet) seemed somewhat anomalous. Since no starting material was present, all of the signals must have been due to either the ketone or the hydrate. On ^{13}C enrichment at the one position, the answer to the problem became obvious. The anomeric region of the pmr spectrum now had the following appearance:



Two large directly bonded ^{13}C-H couplings are noted thereby identifying the H_1 signals of the ketone and the hydrate (a and c). The lower field doublet (a) was then identified as that of H_1 of the hydrate by synthesis of the hydrate on a large scale and comparing its spectrum to the spectrum of the mixture. Furthermore, the third doublet (b) was also split by ^{13}C to an extent of 5.5 Hz. This signal must then be due to H_2 of the ketone. The fact that b is not H_2 of the hydrate was evident because the ratio of ketone to hydrate varied from experiment to experiment but doublets b and c always remained equal in intensity. The results were confirmed by the addition of H_2O which caused an increase in the size of the signals due to the hydrate in relation to that of the ketone. Subsequent lyophilization had the opposite effect.

This experiment therefore yielded the value of 5.5 Hz for the coupling between $^{13}C_1$ and H_2 of the ketone, which is about equivalent to the coupling before oxidation. Apparently then, a double bond on the carbon adjacent to a ^{13}C-C-^{1}H system oriented in projection as below;

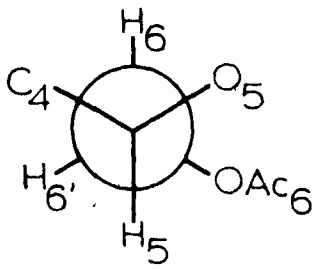


does not exert a significant effect on the extent of ^{13}C-H geminal coupling.

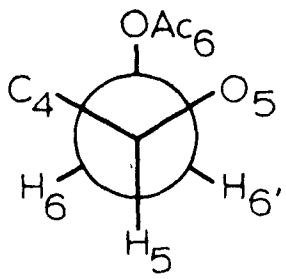
3.11 ^{13}C-^{1}H geminal coupling in rotamers

Rotational isomerism of carbinol groups exocyclic to five or six membered sugar rings has been studied extensively by pmr spectroscopy. However, the interpretation of vicinal H-H coupling within such systems is not straightforward because of time averaging due to the rapid interconversion of rotamers. It has been of interest, therefore, to examine the geminal coupling of rotamers of this class as an independent source of information for comparison with the H-H coupling data.

Two preferred rotamers about the 5,6-bond have been advanced for penta-O-acetyl-α or β-D-glucose (158)(159):

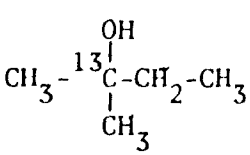


Q



R

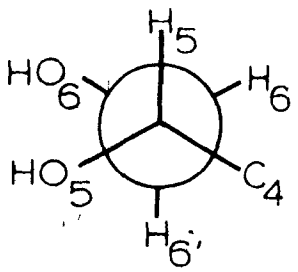
In one of these rotamers H_5 is oriented trans with respect to the acetate group on $^{13}C_6$ whereas in the other rotamer these substituents subtend a gauche relationship. On examining the 6-^{13}C enriched analogs of these compounds no geminal ^{13}C-H coupling was observed between $^{13}C_6$ and H_5. This is in sharp contrast to a freely rotating system such as 2-methyl-2-butanol-2-^{13}C (160),



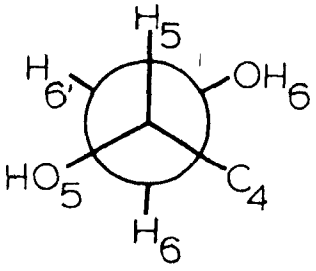
in which a coupling of 4 Hz is observed. Part of the difference is likely due to the presence of a second oxygen along the coupling pathway, which is expected

to decrease the coupling (see 3.8). The remainder can be rationalized in terms of rotamer populations. It has already been suggested that a trans oxygen leads to smaller observed coupling (most likely a positive contribution to an originally negative coupling) than a gauche oxygen (see 3.4). The fact that in the above system no geminal coupling is observed appears to be in accord with a preference for R rather than Q.

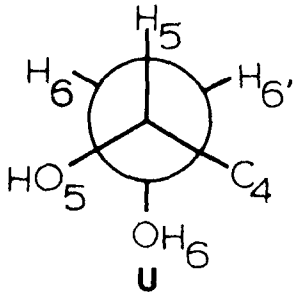
In the system of 1,2-O-isopropylidene-α-D-glucofuranose-6-^{13}C three staggered rotamers about the 5,6 bond are possible:



S



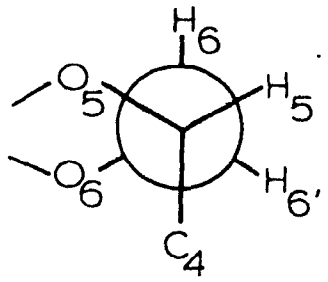
T



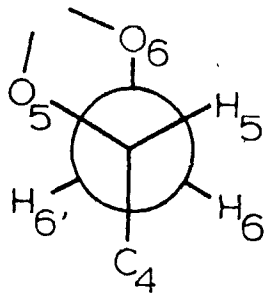
U

The fact that the H_5-H_6 and H_5-H_6' couplings are different (2.7 Hz and 6.0 Hz) suggests that rotamers S and T predominate. This kind of geometric arrangement is expected to give rise to a $^{13}C_6$-H_5 geminal coupling of ∿3 Hz (60° dihedral angle between H_5 and OH_6). The rotamer U has an oxygen trans to H_5 and this kind of stereochemistry leads to zero geminal ^{13}C-^{1}H coupling. The observed result of 2 Hz is then consistent with major contributions from S and T and a minor contribution from U to the rotamer population.

In this connection it is useful to consider the di-O-isopropylidene-6-^{13}C derivative because in this compound C_5 and C_6 must be constrained in a gauche relationship as in V and W:



V



W

Since spin-spin couplings between the 5- and 6-protons are almost equal (5.5 and 6.2 Hz) V appears to be more favored than W. No coupling between $^{13}C_6$ and H_5 is detectable in the spectrum of the labelled compound and again this is consistent with the dominance of V which has O_6 oriented trans with respect to H_5.

3.12 Theoretical considerations

It has been demonstrated that geminal ^{13}C-H coupling is orientation dependent, and consequently it is of interest to examine whether or not this dependence can be theoretically justified.

The contact contribution for two bond coupling can be expressed by the following (161):

$$^2K = 1/4\ A_{^{13}C} \cdot \gamma_{^{13}C\text{-}C\text{-}H} \cdot A_H/^3\Delta E$$

where K = reduced coupling constant

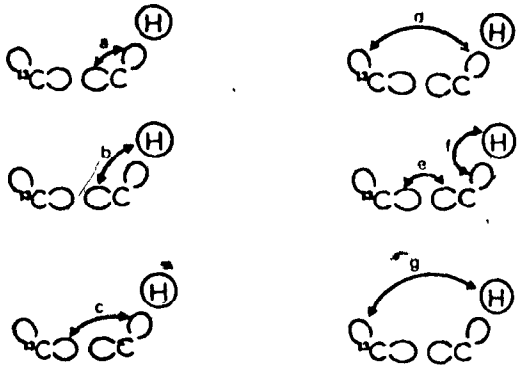
$A_{^{13}C}$ is the interaction corresponding to the transfer of spin information from the ^{13}C nucleus to the ^{13}C bonding orbital in the ^{13}C-C bond

$\gamma_{^{13}C\text{-}C\text{-}H}$ is the interaction which transmits electron spin information from the ^{13}C bonding orbital in the ^{13}C-C bond to the H bonding orbital in the C-H bond

A_H is the interaction which transmits information from the H bonding orbital in the C-H bond to the H nucleus

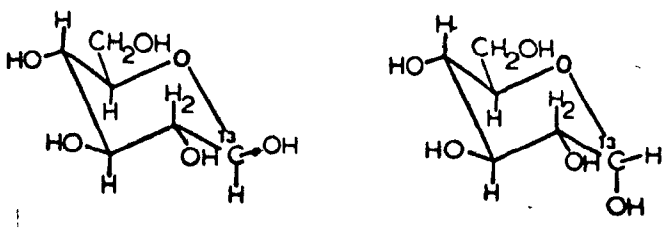
$^3\Delta E$ is the mean triplet excitation energy

The hybridization of the coupled atom affects $A_{^{13}C}$, whereas the stereochemical effects noted are expected to be involved in the $\gamma_{^{13}C\text{-}C\text{-}H}$ term. This term is a function of integrals which are sensitive to bond angles and the nature of the substituents on the coupled nuclei and the intervening nucleus, as well as to the stereochemistry of the rest of the molecule. If one considers a $^{13}C\text{-}C\text{-}H$ system, the integrals corresponding to interactions involved in the calculation can be represented by the following:



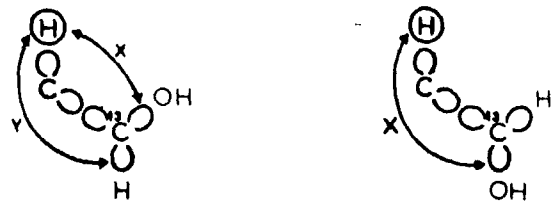
It is evident from the above that, theoretically, the relative orientations of the ^{13}C substituents and the proton in question are important in determining the extent of coupling (interactions of type g).

For the anomeric 1-^{13}C glucopyranoses,



all resonance integrals are the same except for those, indicated below, which

are angular dependent:



$A_{^{13}C}$ and A_H are the same for both cases, (positive), therefore the sign of γ is expected to determine the sign of the coupling.

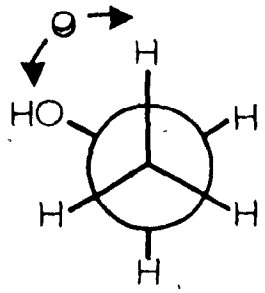
Calculations do show, in fact (161), that the signs of these types of integrals depend upon the dihedral angle and the "s" character of the carbon orbitals involved. Such an integral is large when the carbon orbitals involved in the integral are mostly p, and decreases with s participation. In the case cited above the substituent, (OH), is electronegative; therefore the ^{13}C orbital involved in the C-O bond is such as to put more electron density close to the oxygen, i.e., more p orbital participation in the C-O bond. This will make the X type of integral large and Y type small, (the C-H bond has more s character than when only carbon and hydrogen atoms are involved) and consequently these determine the coupling.

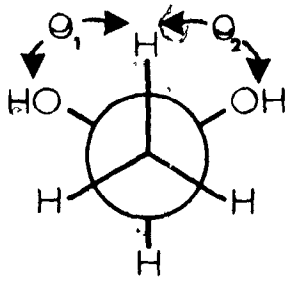
Calculations also indicate that integrals of this type become increasingly more negative with increasing dihedral angle (161). The inference to be drawn, then, is that X is large and negative for the α anomer and is much smaller or even positive for the gauche interaction in the β anomer. In the actual calculation of γ the above integrals are preceded by a negative sign so that the contributions become negative for the β anomer and positive for the α anomer. This is consistent with the proposed hypothesis that a trans

electronegative group makes a positive contribution to the coupling, as evidenced in the geminal ^{13}C-H couplings of the carbohydrates previously discussed.

Calculations have been carried out on ethanol and ethane-1,1-diol using the Pople and Santry equation (17) with orbital coefficients and energies calculated using the CNDO/2 method. The results are shown in Table 2.*

TABLE 2 - Calculated $^{2}J_{C-H}$ in ethanol and ethane-1,1-diol





θ	$^{2}J_{^{13}C-H}$ (calc.) Hz	θ_1	θ_2	$^{2}J_{^{13}C-H}$ (calc.) Hz
0	-3.2	60	60	-3.0
30	-3.0	30	90	-2.8
60	-2.2	0	120	-1.9
90	0.05	30	150	0
120	+3.9	60	180	+2.6
150	+7.2	90	150	+4.9
180	+9.3	120	120	+6.0

Although in this type of calculations no weight can be placed on the actual numerical values, the trends observed can be informative. For ethanol the positive contribution of a <u>trans</u> hydroxyl and the negative one of a <u>gauche</u> substituent is well demonstrated. The observed results in the

* See footnote p. 76.

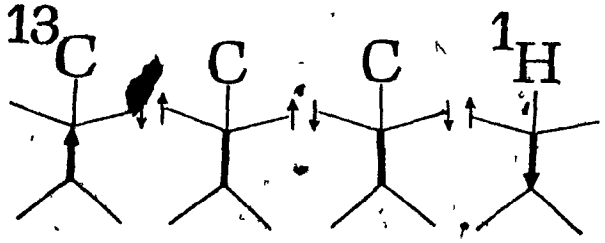
1-^{13}C enriched carbohydrates are also reproduced in the gem ethanediol system. The coupling is negative when the hydroxyls are oriented 60° with respect to the proton and becomes positive when the dihedral angles become 120°. This corresponds well, therefore, to what is observed with β-D-allose-1-^{13}C and 1,2:5,6-di-O-isopropylidene-α-D-glucofuranose-1-^{13}C which show couplings of -6.0 and +5.5 Hz respectively.

3.13 Conclusion

Coupling between ^{13}C and a geminal proton shows a striking dependence on the orientation of the coupling proton and the ^{13}C substituents. Values of this coupling ranging from -6.0 to +5.5 Hz have been measured in carbohydrate systems differing essentially only in relative orientations. The magnitude of this type of variation is such as to potentially obscure differences due to lone pair, hybridization and electronegativity effects. Conceivably, then, ^{13}C-^{1}H geminal coupling holds promise as a tool in conformational analysis for the determination of unknown substituent orientations.

CHAPTER 4

VICINAL ^{13}C-^{1}H COUPLING

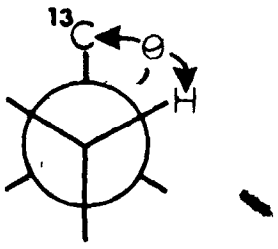


4.1 Introductory remarks

Coupling between the ^{13}C nucleus and a proton over three bonds is termed vicinal coupling and is denoted by $^{3}J_{C-H}$. These couplings have also been investigated in this general study of $^{13}C-^{1}H$ coupling constants. Again, because of the experimental difficulties involved in the measurement of this parameter, very few data are available and consequently a study of $^{3}J_{C-H}$ held appeal even if only as a supplement to vicinal proton-proton coupling in application to stereochemical problems. The latter parameter, mainly because of its dependence on dihedral angle (4) has proven to be of immense value in organic structural analysis. A "Karplus type" relationship between vicinal ^{13}C and ^{1}H would not only complement the proton-proton parameter but conceivably could also serve to solve structural problems in the absence of pertinent proton-proton data. The carbohydrate series again seemed especially suitable for realizing a variety of the types of dihedral angles generally encountered in organic compounds.

4.2 Dihedral angle dependence of $^{3}J_{C-H}$

The dihedral angle referred to in the present context can be denoted by Θ in the projection below:

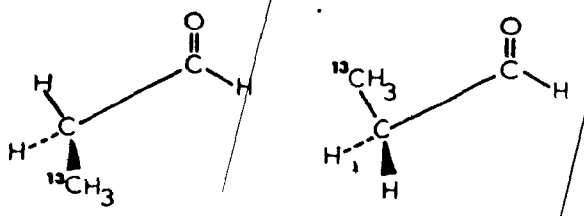


However, any discussion of dihedral angle effects in molecules has to be preceded by a statement of caution, or at best reservation. Although chemists (particularly of the organic variety) are accustomed to derive dihedral angles

from molecular models, the actual angles subtended by the particular bonds may be substantially different, especially for compounds in solution. Consequently, reference below to dihedral angle will always imply that the angle in question is only an approximation. The Karplus curve for proton-proton coupling, despite its unquestioned usefulness has limitations that need to be recognized (162), and does not necessarily afford an accurate measure of bond angles. Thus, dihedral angle is not the only determinant of vicinal proton-proton coupling; substitution, hybridization and bond length effects also have to be considered. It is best then to use the Karplus relationship in a general manner, for example, to differentiate between dihedral angles of 60, 180 or 90°. In a similar vein, the overall purpose of the present investigation has been to establish whether or not any such relationship exists for ^{13}C-^{1}H coupling and to study how such a relationship may be applied to practical structural problems.

During the course of the present study, a dependence of $^{3}J_{C-H}$ on dihedral angle has been reported (163)(163a) for a group of ^{13}C-enriched compounds related to uridine. The data in this instance deal with the systems ^{13}C-O-C-H and ^{13}C-N-C-H in which the carbon is sp^2 hybridized. Also concurrently, dihedral angle dependence has been demonstrated for $^{3}J_{P-C-C-H}$ (164), $^{3}J_{H-C-C-F}$ (165), $^{3}J_{^{14}N-C-C-H}$ (166), $^{3}J_{^{13}C-C-C-F}$ (167), $^{3}J_{P-O-C-^{13}C}$ (168), $^{3}J_{H-N-C-H}$ (169), and $^{3}J_{P-C-C-^{13}C}$ (170) so that intuitively one would expect $^{3}J_{C-H}$ to follow an analogous trend. On the other hand, $^{3}J_{F-C-C-F}$ (171) exhibits a much more complex relationship so that a Karplus type of behaviour cannot be assumed a priori.

The first experimental suggestion for dihedral angle dependence of $^{3}J_{C-H}$ came from Karabatsos et al. (172) who examined propanal-3-^{13}C. The relative populations of the rotamers A and B can be calculated as a function of temperature (173).



A B

It has been shown that ΔH° (A→B) = -800 cal/mole; consequently if trans $^{3}J_{C-H}$ is larger than gauche, an increase in temperature should decrease the coupling between $^{13}C_3$ and H_1 in the above compound. Such a decrease was indeed noted over the temperature range of -35° to +45°, $^{3}J_{C-H}$ decreasing from 2.65 to 2.30 Hz. On this basis Karabatsos estimated that $^{3}J_{C-H}$ trans = 3.5 Hz and gauche = 0.2 Hz. Although these data are indicative, one must be careful not to place too much significance on an observed change of 0.35 Hz in coupling.

The general reaction schemes employed in this study have already been outlined in chapter 1. The compounds used in the investigation of vicinal coupling and their measured $^{13}C-^{1}H$ coupling constants are listed in Table 1 and the angular dependence based on these data is depicted in Fig. 1. It is obvious that the relationship is not straightforward, as demonstrated by the variation in $^{3}J_{C-H}$ in compounds possessing nominally the same dihedral angle. The dotted line represents the best fit curve through these points but the significant deviations from the line raise questions concerning the approximation of the relationship by means of a Karplus type curve. It is to be emphasized, however, that most of these deviations can be questioned in terms of other possible factors coming into play as will be shown below. The procedure adopted for discussing these effects and the overall relationship, is to examine separately

TABLE 1 - Vicinal ^{13}C-H coupling in ^{13}C enriched carbohydrates

	Compound	Coupling pathway	Dihedral angle	$^3J_{C-H}$ ($\pm\frac{1}{2}$ Hz)
1.	1,2-O-Isopropylidene-α-D-glucofuranose-6-^{13}C	$C_5-C_5-C_4-H_4$		2.8
2.	1,2-O-Isopropylidene-α-D-glucofuranose-6-^{13}C-6-d_2	$C_6-C_5-C_4-H_4$		2.5
3.	1,2-O-Isopropylidene-3,5,6-tri-O-acetyl-α-D-glucofuranose-6-^{13}C	$C_6-C_5-C_4-H_4$		3
4.	1,2-O-Isopropylidene-3,5,6-tri-O-acetyl-α-D-glucofuranose-6-^{13}C-6-d_2	$C_6-C_5-C_4-H_4$		3
5.	1,2-O-Isopropylidene-α-D-glucofuranose-1-^{13}C	$C_1-C_2-C_3-H_3$	140°	5
		$C_1-O_4-C_4-H_4$	110°	< 1
6.	1,2-O-Isopropylidene-3,5,6-tri-O-orthoformyl-α-D-glucofuranose-6-^{13}C	$C_6-C_5-C_4-H_4$	70°	< 1
		C_6-O_6-C-H	160°	4.6
7.	1,2-O-Isopropylidene-3,5,6-tri-O-orthoformyl-α-D-glucofuranose-1-^{13}C	$C_1-C_2-C_3-H_3$	140°	5.2
		$C_1-O_4-C_4-H_4$	110°	< 1
8.	1,2-O-Isopropylidene-α-D-glucofuranose-6-^{13}C (periodate complex)	$C_6-C_5-C_4-H_4$	70°	< 1
9.	1,2-O-Isopropylidene-β-L-idofuranose-6-^{13}C	$C_6-C_5-C_4-H_4$		< 1
10.	1,2-O-Isopropylidene-3,5,6-tri-O-acetyl-β-L-idofuranose-6-^{13}C	$C_6-C_5-C_4-H_4$		1
11.	1,2-O-Isopropylidene-β-L-idofuranose-6-^{13}C-6-d_2	$C_6-C_5-C_4-H_4$		< 1
12.	1,2-O-Isopropylidene-α-D-glucofuranurono-6,3-lactone-6-^{13}C	$C_6-C_5-C_4-H_4$	140°	9
		$C_6-O-C_3-H_3$	100°	< 1

Compound	Coupling pathway	Dihedral angle	$^3J_{C-H}$ (Hz)
13. 1,2-O-Isopropylidene-β-L-idofuranurono-6,3-lactone-6-^{13}C	$C_6-C_5-C_4-H_4$	140°	6.6
	$C_6-O-C_3-H_3$	100°	< 1
14. 1,2-O-Isopropylidene-5-O-acetyl-α-D-glucofuranurono-6,3-lactone-6-^{13}C	$C_6-C_5-C_4-H_4$	140	8.5
	$C_6-O-C_3-H_3$	100	1.3
15. 1,2-O-Isopropylidene-5-O-acetyl-β-L-idofuranurono-6,3-lactone-6-^{13}C	$C_6-C_5-C_4-H_4$	140	6.7
	$C_6-O-C_3-H_3$	100	< 1
16. D-Mannono-1,4-lactone-1-^{13}C	$C_1-C_2-C_3-H_3$	140°	9
	$C_1-O_4-C_4-H_4$	110°	< 1
17. 1,2:5,6-Di-O-isopropylidene-α-D-glucofuranose-6-^{13}C	$C_6-C_5-C_4-H_4$		3
18. 1,2:5,6-Di-O-isopropylidene-α-D-glucofuranose-1-^{13}C	$C_1-C_2-C_3-H_3$	140°	5.5
	$C_1-O_4-C_4-H_4$	110°	< 1
19. 1,2:5,6-Di-O-isopropylidene-3-O-acetyl-α-D-glucofuranose-1-^{13}C	$C_1-C_2-C_3-H_3$	140°	4.5
20. 1,2:5,6-Di-O-isopropylidene-α-D-allofuranose-1-^{13}C	$C_1-C_2-C_3-H_3$	90°	0
	$C_1-O_4-C_4-H_4$	110°	< 1
21. 1,2:5,6-Di-O-isopropylidene-3-O-acetyl-α-D-allofuranose-1-^{13}C	$C_1-C_2-C_3-H_3$	90°	0
	$C_1-O_4-C_4-H_4$	110°	< 1
22. 2,3:5,6-Di-O-isopropylidene-α-D-mannofuranose-1-^{13}C	$C_1-C_2-C_3-H_3$	100°	3.5

	Compound	Coupling pathway	Dihedral angle	$^3J_{C-H}$ (Hz)
23.	2,3:5,6-Di-O-isopropylidene-1-O-acetyl-α-D-mannofuranose-1-^{13}C	C_1-C_2-C_3-H_3	100°	3.5
24.	2,3,4,6-Tetra-O-acetyl-1,5-anhydro-D-glucitol-1-^{13}C-1-d	C_1-C_2-C_3-H_3	60°	< 1
		C_1-O_5-C_5-H_5	60°	1.5
25.	Methyl-α-D-glucopyranoside-1-^{13}C	C_1-C_2-C_3-H_4	60°	< 1
		C_1-O_5-C_5-H_5	60°	2
		C_1-O_1-C-H		4
26.	Methyl-α-D-glucopyranoside-6-^{13}C	C_6-C_5-C_4-H_4	60°	3.3
27.	1,2,3,4,6-Penta-O-acetyl-α-D-glucopyranose-6-^{13}C	C_6-C_5-C_4-H_4	60°	2.8
28.	1,2,3,4,6-Penta-O-acetyl-β-D-glucopyranose-6-^{13}C	C_6-C_5-C_4-H_4	60°	2.4
29.	1,2,3,4,6-Penta-O-acetyl-α-D-glucopyranose-1-^{13}C	C_1-C_2-C_3-H_3	60°	< 1
		C_1-O_5-C_5-H_5	60°	2.5
30.	1,2,3,4,6-Penta-O-acetyl-β-D-glucopyranose-1-^{13}C	C_1-C_2-C_3-H_3	60°	< 1
		C_1-O_5-C_5-H_5	60°	2.5
31.	2,3,4,6-Tetra-O-acetyl-α-D-glucopyranosyl bromide-1-^{13}C	C_1-C_2-C_3-H_3	60°	< 1
32.	2,3,4,6-Tetra-O-acetyl-α-D-mannopyranosyl bromide-1-^{13}C	C_1-C_2-C_3-H_3	60°	< 1
33.	2,3,4,6-Tetra-O-acetyl-α-D-glucopyranosyl bromide-6-^{13}C	C_6-C_5-C_4-H_4	60°	3

Compound	Coupling pathway	Dihedral angle	$^3J_{C-H}$ (Hz)
34. 3,4,6-Tri-O-acetyl-β-D-mannopyranose 1,2-(methyl orthoacetate)-1-^{13}C	$C_1-C_2-C_3-H_3$	60°	< 1
	$C_1-O_5-C_5-H_5$	60°	2.5
35. β-D-Glucopyranose-U-^{13}C	$C_3-C_2-C_1-H_1$	60°	< 1
36. α-D-Glucopyranose-U-^{13}C	$C_3-C_2-C_1-H_1$	180°	5-6
37. β-D-Allopyranose-1-^{13}C	$C_1-C_2-C_3-H_3$	180°	5.5
38. 1,2,3,4,6-Penta-O-acetyl-β-D-allopyranose-1-^{13}C	$C_1-C_2-C_3-H_3$	180°	5.5
39. α,β-D-Glucopyranose-6-^{13}C-6-d_2	$C_6-C_5-C_4-H_4$	60°	3.3
40. β-D-Glucopyranose-1-^{13}C	$C_1-C_2-C_3-H_4$	60°	< 1
41. β-D-mannopyranose-1-^{13}C	$C_1-O_5-C_5-H_5$	60°	2
42. 1,2,3,4,6-Penta-O-acetyl-β-D-mannopyranose-1-^{13}C	$C_1-O_5-C_5-H_5$	60°	2
43. 2,3,4-Tri-O-acetyl-1,6-anhydro-α-L-ido-pyranose-6-^{13}C	$C_6-C_5-C_4-H_4$	160°	5.8
	$C_6-O_6-C_1-H_1$	160°	5
44. Methyl 4,6-O-benzylidene-α-D-glucopyranoside-1-^{13}C	$C_1-C_2-C_3-H_3$	60°	< 1
	$C_1-O_5-C_5-H_5$	60°	2
	C_1-O_1-C-H		4
45. Methyl 4,6-O-benzylidene-α-D-glucopyranoside-6-^{13}C	$C_6-C_5-C_4-H_4$	60°	2.5
	$C_6-O-C-H$	60°	1.5

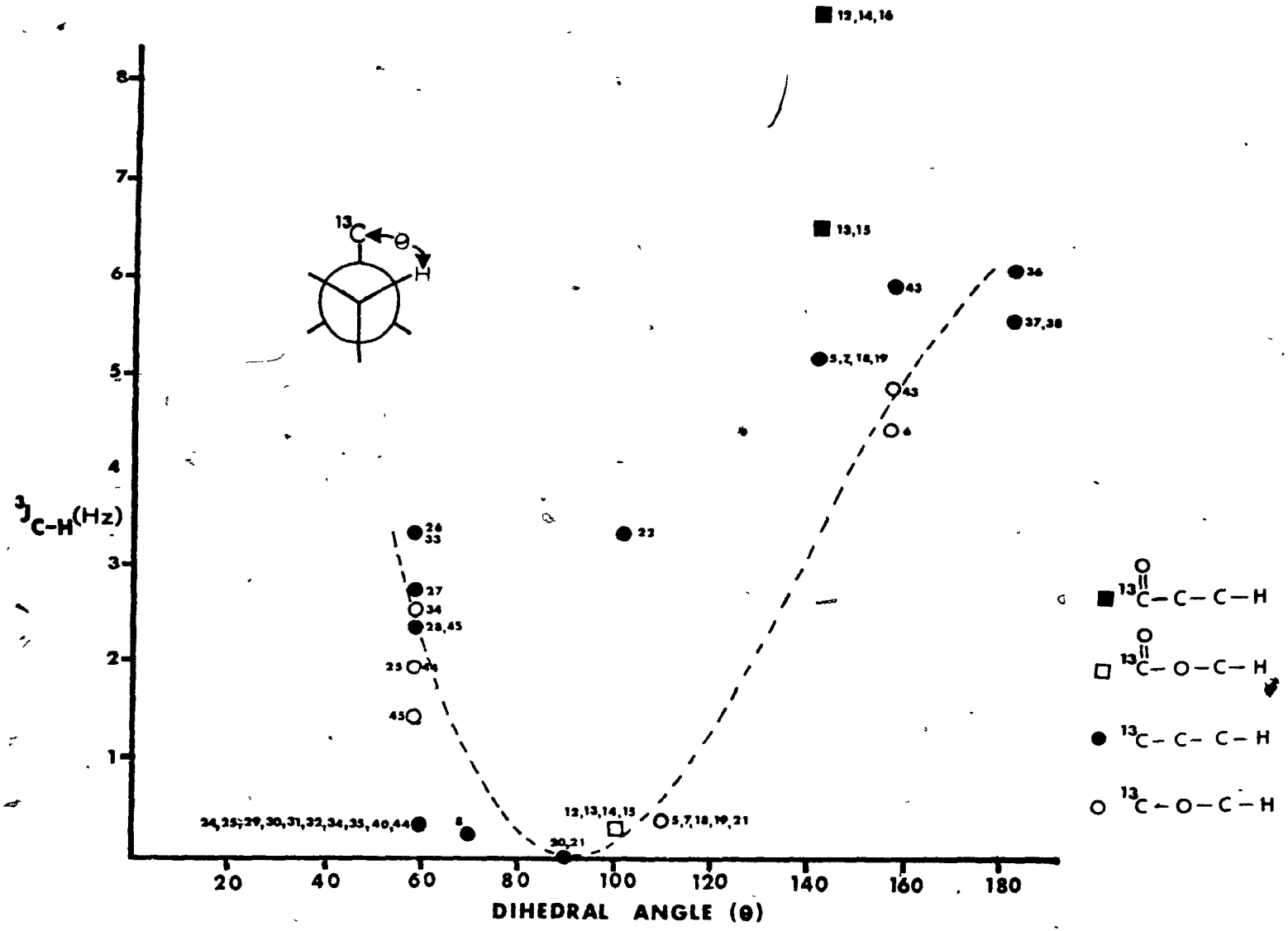


FIG. 1 Dihedral angle dependence of $^{3}J_{C-H}$.

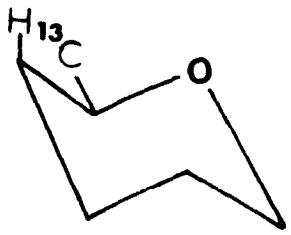
the groups of compounds giving rise to a particular dihedral angle.

60° Dihedral angle

Compounds well suited to the investigation of a nominal 60° (±5°) dihedral angle are the pyranose sugars enriched in the 6 or 1 position. The observed vicinal ^{13}C-^{1}H couplings range from 3.3 Hz for methyl-α-D-glucopyranoside-6-^{13}C to < 1 Hz for the α-D-glucopyranose-1-^{13}C derivatives. Measurement of these couplings presented some problems especially in the case of the 6-^{13}C labelled compounds where the ^{13}C satellites of H_6 and $H_{6'}$ sometimes obscured the vicinal H_4 absorption. In these instances it was necessary to resort to analogs deuterated at position 6, as is illustrated in Fig. 2 for D-glucopyranose-6-^{13}C-6-d_2. The deuteration is carried out by reducing 1,2-O-isopropylidene-α-D-glucofuranurono-6,3-lactone-6-^{13}C (Scheme 1, p. 16) with $NaBD_4$ instead of $NaBH_4$.

Although within the group of compounds discussed here substantial conformational differences may exist, the range of such variation surely cannot be as large as that observed among the coupling constants. The 60° angles are in general furnished by three distinct systems:

a) A pyranose ring enriched in the 6-position - the relevant coupling being $^{J}{}_{^{13}C_6\text{-}C_5\text{-}C_4\text{-}H_4}$:



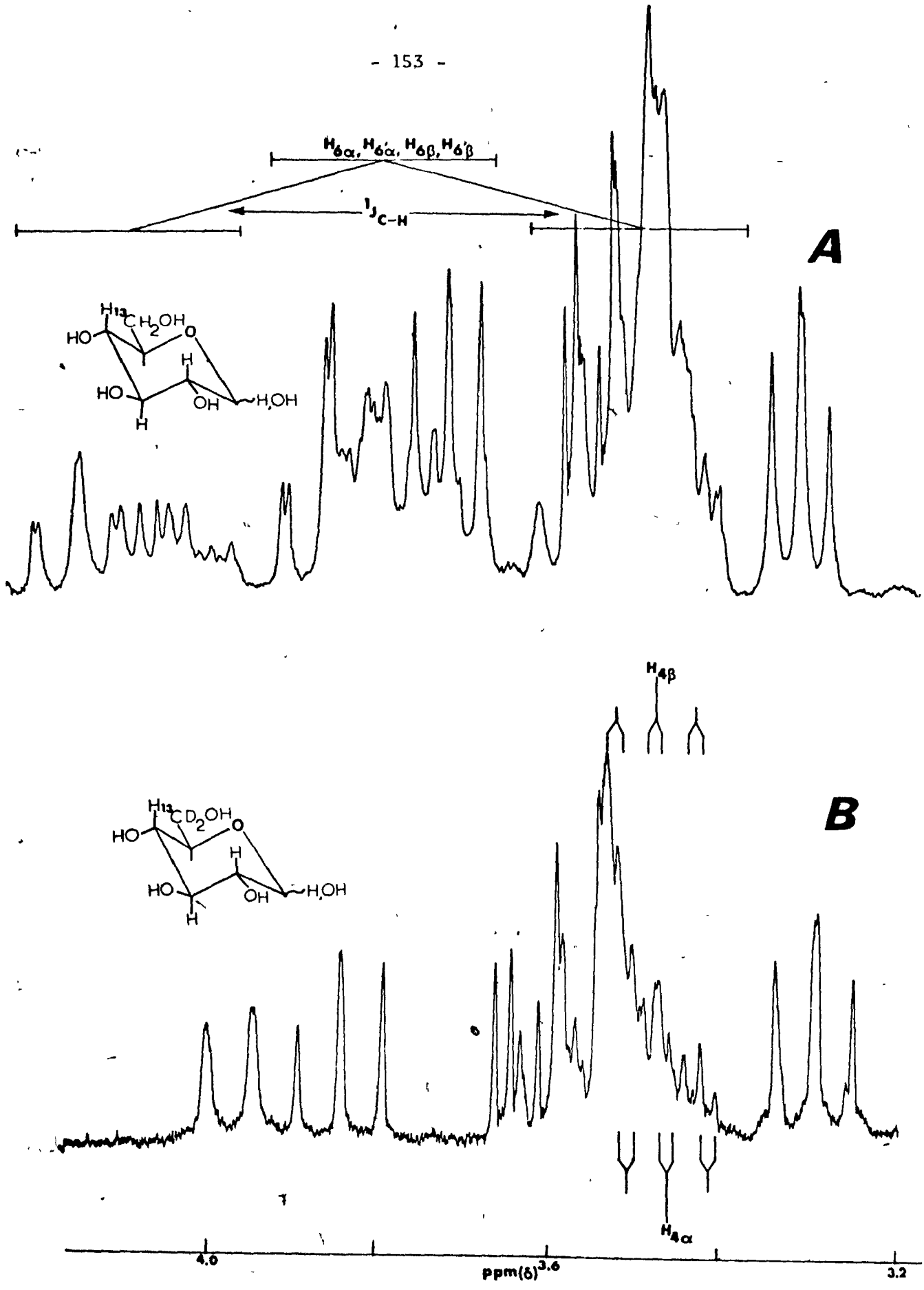
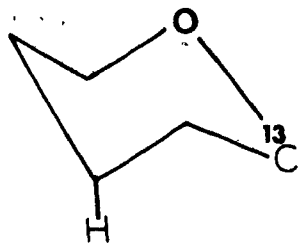
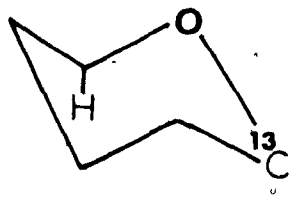


FIG. 2 Partial pmr spectrum (100 MHz) of A) α,β-D-Glucose-6-^{13}C (60%) and B) α,β-D-Glucose-6-^{13}C (80%)-6-d_2 in D_2O.

b) A pyranose ring enriched in the 1-position - the relevant coupling being $J_{^{13}C_1\text{-}C_2\text{-}C_3\text{-}H_3}$:

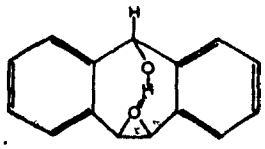


c) A pyranose ring enriched in the 1-position - the relevant coupling being $J_{^{13}C_1\text{-}O\text{-}C_5\text{-}H_5}$:

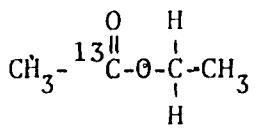


The above systems were selected not only because they furnish dihedral angles of 60° but also because they allow the examination of variations due to factors other than dihedral angle.

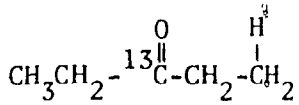
The first question encountered in the analysis of these results is whether coupling through a heteroatom is significantly different from coupling through carbon. It is difficult to obtain any information along these lines from proton-proton data because in general the orientation of the bonds in an H-C-O-H type of systems is not well known. Some indication can be had from hydrogen bonded systems (174) such as the following:



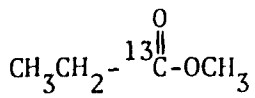
where the H-C-O-H coupling is observed to be 12 Hz, roughly what is expected for a trans geometry of the C-H and O-H bonds. More relevant information is available from the comparison of some conformationally mobile systems in which the coupling pathways between ^{13}C and a proton are similar except for the presence of a heteroatom along the path (150):



$J_{^{13}C\text{-}O\text{-}C\text{-}H} = 3.1$ Hz



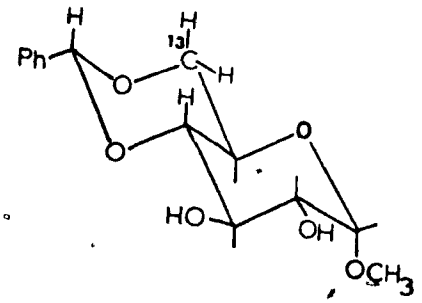
$J_{^{13}C\text{-}C\text{-}C\text{-}H} = 4.7$ Hz



$J_{^{13}C\text{-}C\text{-}C\text{-}H} = 5.3$ Hz

$J_{^{13}C\text{-}O\text{-}C\text{-}H} = 4.0$ Hz

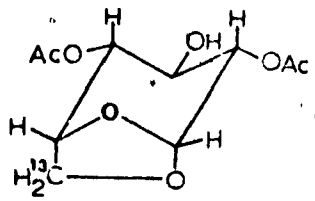
These data imply that coupling through oxygen is about 1 Hz less than through carbon. In the sugar series, the coupling through oxygen in $^{13}C_1$ enriched pyranoses can be related to the coupling through carbon in the $^{13}C_6$ enriched compounds; the coupling pathways being similar. In the former case the coupling between $^{13}C_1$ and H_5 is in general around 2 Hz whereas the coupling between $^{13}C_6$ and H_4 is on the average 3 Hz (Table 1). This is consistent with the hypothesis that coupling through oxygen is slightly smaller than through carbon. To further investigate this matter it was desirable to examine a compound in which the coupling pathways both originated from the same ^{13}C so that any hybridization effects could be minimized. Methyl 4,6-O-benzylidene-α-D-glucopyranoside-6-^{13}C is suited for this purpose:



$^{3}J_{C\text{-}C\text{-}C\text{-}H}$ = 2.5-3.0 Hz | $^{3}J_{C\text{-}O\text{-}C\text{-}H}$ = 1.5-2.0 Hz

The couplings under investigation here are $^{3}J_{C_6\text{-}C\text{-}C\text{-}H_4}$ and $^{3}J_{C_6\text{-}O_6\text{-}C\text{-}H}$. At 220 MHz the relevant signals were observable and the coupling through carbon was measured to be 2.5-3.0 Hz whereas that through oxygen was 1.5-2.0 Hz, again consistent with the hypothesis that couplings through oxygen are slightly smaller than the corresponding couplings through carbon.

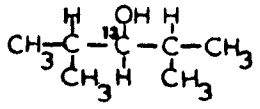
A second example investigated in this regard was 2,3,4-tri-O-acetyl-1,6-anhydro-α-L-idopyranose-6-^{13}C which was synthesized as discussed on p. 115.



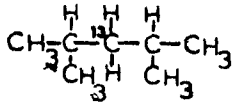
In this molecule both of the $^{13}C_6\text{-}C_5\text{-}C_4\text{-}H_4$ and $^{13}C_6\text{-}O\text{-}C_1\text{-}H_1$ couplings involve a dihedral angle of about 160°, but in one instance the coupling is through oxygen and in the other it is through carbon. The observed couplings are 5.8 Hz through the carbon pathway and 5 Hz through the oxygen. It seems reasonably well established then that there are no major differences in $^{13}C\text{-}^{1}H$ vicinal coupling whether through oxygen or through carbon. The consistently small difference of 1 Hz between pathways of type "a" and type "c" (p. 152) and then

likely due to the presence of the heteroatom in path "c".

Differences between couplings through path "a" and path "b" are more striking: all examples of the latter type that have been examined give no detectable coupling (i.e., < 1 Hz) as opposed to ∿2-3 Hz for path "a". The most evident distinction between these two pathways is the presence of two oxygen substituents on the enriched carbon in the "b" series as opposed to only one in the "a" series. However, the simple introduction of electronegative substituents does not seem to be the main factor judging from the equality of $^{3}J_{C-H}$ (4.5 Hz) in the two compounds below (149):

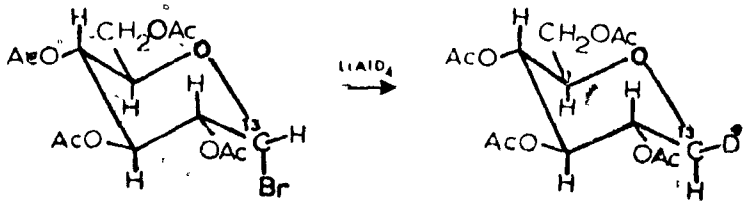


$^{3}J_{C-H}$ = 4.5 Hz



$^{3}J_{C-H}$ = 4.5 Hz

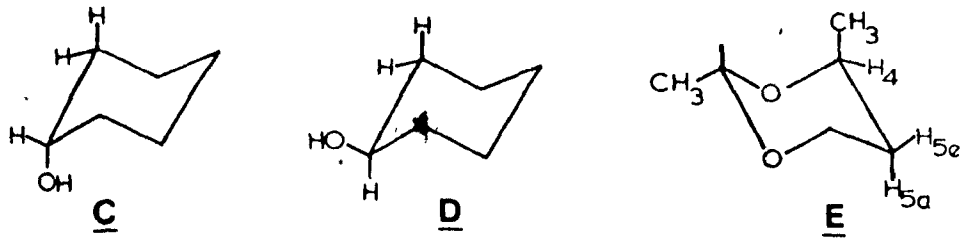
Similarly removal of one of the oxygen atoms in the C_1 labelled series, which can be accomplished in a highly stereoselective manner by the reaction:



showed that coupling to H_3 was still less than 1 Hz. Hence, the extra oxygen is not the determining factor. (The coupling through oxygen to H_5 also remained essentially unchanged, at 1.5-2.0 Hz). It seems established then that the 60° angle can furnish a relatively wide range of ^{13}C-H vicinal coupling constants.

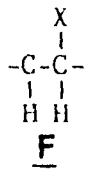
Since substitution effects on the enriched carbon do not account for the observed differences, the rest of the coupling pathway must be examined critically. The major difference between pathways "a" and "b" then becomes the orientation of the oxygen substituent on the vicinal proton-bearing carbon with respect to the coupling pathway. In the 6-^{13}C series the C_4-O_4 bond is gauche with respect to the $^{13}C_6$-C_5 bond whereas in the 1-^{13}C series the C_3-O_3 bond is antiperiplanar with respect to the $^{13}C_1$-C_2 bond:

Now, for related proton-proton vicinal couplings substituent orientation effects have been noted. For example, in the partial structures below the axial-equatorial proton-proton vicinal coupling is 5.5 Hz smaller for C than for D (175). Similarly in E, the coupling between H_4 and axial H_5 is 7.0 Hz whereas the coupling between H_4 and equatorial H_5 is 3.1 Hz.



The conclusion to be drawn from the above data is that the presence of an electronegative substituent bearing a trans orientation to part of the coupling pathway reduces the absolute value of the coupling. It is well established that proton-proton, proton-fluorine and fluorine-fluorine, vicinal couplings are

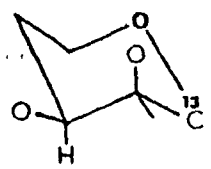
decreased by electronegative substitution at the pathway carbon atoms (97) (176) (177) (179). That is, in F the more electronegative X is, the smaller the coupling. A physical explanation may be that in an antiperiplanar situation



F

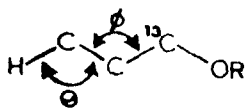
the substituent can withdraw electrons from the path more effectively; that is, its electronegativity is orientation dependent. A decrease of electron density along the coupling path should be reflected in a decrease in coupling since the coupling is transmitted through electrons (10). In fact, a study of electron densities in nucleosides (180) suggests that there is a correlation between unusually small ^{13}C-^{1}H vicinal couplings and low electron density in one of the coupling pathway bonds. Pertaining to the ^{13}C-C-C-^{1}H type of coupling then, the presence of an electronegative substituent parallel to part of the coupling pathway should lead to a decrease in coupling. Coupling pathways of type "c" do not have any such trans substituents associated with them and consequently in the pyranoses, coupling between $^{13}C_1$ and H_5 (despite being through oxygen) is consistently 1-2 Hz larger than coupling to axial H_3 even though the dihedral angles are essentially the same.

It was also of interest to investigate the effect of a second oxygen substituent antiparallel to the coupling pathway. This situation can be realized in the mannopyranose-1-^{13}C series:

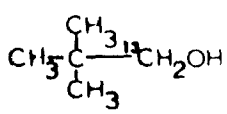


The C_3-O_3 bond remains antiperiplanar to the $^{13}C_1$-C_2 bond but, in addition, the C_2-O_2 bond now is antiperiplanar with respect to the C_3-H_3 bond. The observed couplings, however, are also about 1 Hz, i.e., comparable to the glucose series. This result, although seemingly anomalous, was not unexpected. In general, vicinal ^{13}C-^{1}H couplings are positive (see section 7.6). If the coupling for a 60° angle in the absence of an antiperiplanar electronegative substituent is about 2.5-3 Hz as derived from the 6-^{13}C series, then the presence of the antiperiplanar substituent must make a negative contribution of about 2 Hz, making the observable coupling about 1 Hz; this is the coupling noted in the glucose-1-^{13}C series. A second antiperiplanar substituent may decrease the coupling by another two cycles resulting in a final coupling of about -1 to -1.5 Hz, but the coupling detectable in the spectrum, of course, remains very small. Such possible shifts from positive to negative coupling are not unusual; even the Karplus curve for proton-proton vicinal coupling takes on negative values in the 90° dihedral angle region (4).

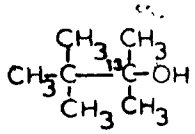
One further point concerning the small coupling observed for the 1-^{13}C enriched species bears discussion. This is the possible role of steric perturbation effects: if in the system,



θ and Ø deviate from the tetrahedral angle, the coupling may in fact reflect this deviation. If 2,2-dimethylpropanol-1-^{13}C and 2,3,3-trimethylbutan-2-ol-2-^{13}C are compared,



$^{3}J_{C-H}$ = 4.5 Hz



$^{3}J_{C-H}$ = 3.6 Hz

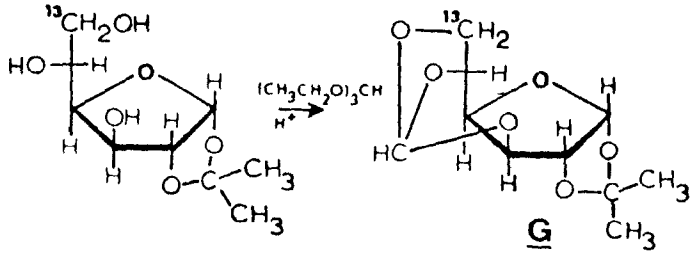
the observed couplings are 4.5 and 3.6 Hz respectively (181). The angles θ and ϕ are expected to be different in these two compounds due to steric interactions, yet there is no large difference in coupling. It can also be noted that if steric interactions were the reason for the small $^{13}C_1$-C_2-C_3-H_3 coupling in the 1-^{13}C sugars, the $^{13}C_1$-O-C_5-H_5 coupling should be similarly affected, which is not observed.

It has been clearly demonstrated that geminal ^{13}C-H coupling is very much dependent on the orientation of the substituents on the ^{13}C nucleus with respect to the coupling proton. However, this does not seem to be the case with vicinal coupling. For example, in 1,2,3,4,6-penta-O-acetyl-α-D-glucopyranose-1-^{13}C the $^{13}C_1$-H_3 and $^{13}C_1$-H_5 couplings are equivalent to the corresponding couplings in the β anomer. The proton likely is too far removed from C_1 substituents to experience the effect.

To summarize the results obtained from the various 60° dihedral angle situations examined, it can be noted that the extent of coupling through oxygen is virtually the same (generally 1 Hz smaller) as coupling through carbon and that an electronegative substituent antiperiplanar to the coupling pathway leads to a decrease in observed coupling.

Dihedral angles between 60° and 110°

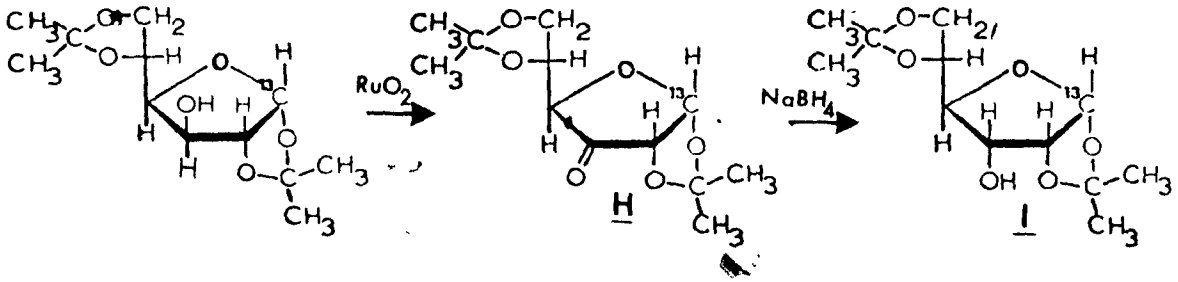
Dihedral angles between 60° and 90° are relatively difficult to realize. A dihedral angle somewhat larger than 60°, of nominally 70° is subtended by the C_5-C_6 and C_4-H_4 bonds of 1,2-O-isopropylidene-3,5,6-tri-O-orthoformyl-α-D-glucofuranose-6-^{13}C (G). This is a rather rigid structure, especially when the absence of coupling between H_2 and H_3 is taken into account, thereby fixing this dihedral angle between the C_2-H_2 and C_3-H_3 bonds at about 90°. The compound is synthesized from 1,2-O-isopropylidene-α-D-glucofuranose-6-^{13}C:



G

There is very little coupling observable between the ^{13}C nucleus and H_4 but, again, this is not unexpected since the C_5-O_5 bond and the ring O-C_4 bond are antiperiplanar to the C_4-H_4 and C_5-C_6 bonds respectively.

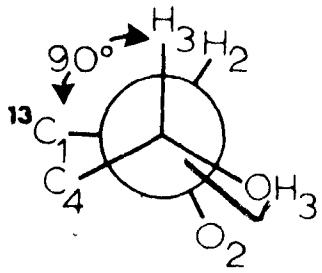
It is not really feasible to incorporate a 90° angle into a pyranose system; therefore it became necessary to examine furanose compounds for a potential 90° dihedral angle between the ^{13}C and ^{1}H nuclei. Conformational analysis of 1,2:5,6-di-O-isopropylidene-α-D-allofuranose-1-^{13}C (I) indicates that the C_1-C_2 and C_3-H_3 bonds subtend an angle of 90°. This compound is synthesized from its glucose epimer by inversion at C_3 as already described on p.102.



H I

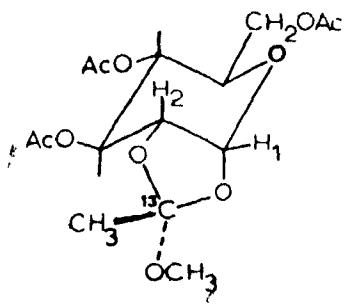
Once again, proton-proton coupling constants are used as a reference in the determination of the furanose ring conformation. The vicinal proton-proton coupling constants are in accord with a conformation similar to the one already established (131) for 1,2:5,6-di-O-isopropylidene-α-D-glucofuranose, ($^{3}J_{H_3-H_4}$, almost trans, equals 8.5 Hz; $^{3}J_{H_2-H_3}$, about 30°, equals 5 Hz). It should be noted that this conformation can be assumed with some confidence because if the furanose were in the alternate twist conformation, the dihedral

angle between the C_3-H_3 and C_4-H_4 bonds would be about 90° and consequently no coupling would be expected whereas in fact 8.5 Hz is measured. Consequently, in projection along the C_3-C_2 bond the conformation can be depicted as below:



The pmr spectrum of this compound presented interpretation problems since the H_3 absorption could not be unambiguously assigned. In order to fully analyze the spectrum it became necessary to synthesize 1,2:5,6-di-O-isopropylidene-α-D-allofuranose-3- and -4d. The former compound can be obtained by reducing the intermediate 3-hexulose (II) with $NaBD_4$ instead of $NaBH_4$, while the latter is synthesized by treating the ketone with D_2O and base to promote deuterium exchange in the α position. Comparison of the proton spectra of these compounds to the spectrum of the ^{13}C enriched derivative clearly indicates no additional coupling to H_3 introduced by the presence of $^{13}C_1$.

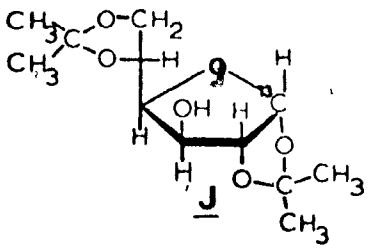
Lemieux et al. (163) have also examined the 1,2-(methyl orthoacetate) derivative of D-glucose, labelled with ^{13}C as shown below:

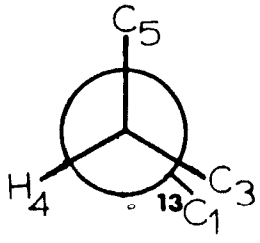


These workers observed no coupling to either H_1 or H_2 and explained this phenomenon by stating that the dihedral angles involved are 90°. Examination of molecular models reveals that it is not possible to have both of these dihedral angles simultaneously equal to 90°.

However, if the dihedral angle between the ^{13}C-O_2 and C_2-H_2 bonds is set equal to 90°, the ^{13}C-O_1 and C_1-H_1 bonds subtend an angle of about 120° but the C_1-O_5 bond now is practically <u>trans</u> to the ^{13}C-O_1 bond - a situation which results in decreased coupling (see p. 158). The rationale then is that one coupling is zero because of the 90° dihedral angle situation whereas the other coupling, although having a dihedral angle of ~120°, is decreased due to the <u>anti</u> orientation of the ring oxygen with respect to the coupling pathway.

Dihedral angles of 110° were obtained in the 1-^{13}C labelled furanose series. For example, as already mentioned, the conformation of 1,2:5,6-di-<u>O</u>-isopropylidene α-<u>D</u>-glucofuranose (<u>J</u>) is known with reasonable accuracy (131), since the C_2-H_2 and C_3-H_3 bonds subtend an angle of 90° ($^3J_{H_2H_3} = 0$). Consequently, the C_1-O_4 bond and the C_4-H_4 bond maintain a dihedral angle of about 110°, a situation which in projection along the C_4-O_4 bond can be depicted as below:



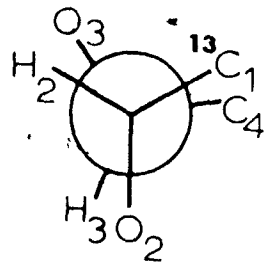


All couplings measured through the $^{13}C_1$-O-C_4-H_4 pathway were consistently < 1 Hz.

4.2 <u>Dihedral angles between 140° and 160°</u>

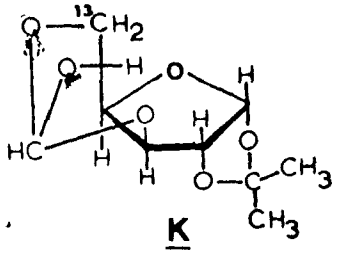
1,2:5,6 di-<u>O</u>-isopropylidene-α-<u>D</u>-glucofuranose-1-^{13}C as well as the

mono-O-isopropylidene derivative furnished dihedral angles of about 140°. The coupling pathway examined here is the $^{13}C_1$-C_2-C_3-H_3 route, or in projection along the C_2-C_3 bond:

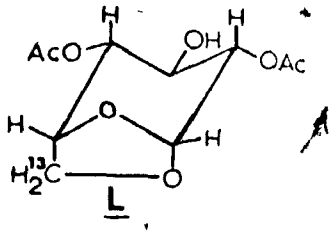


The observed couplings in these cases were 5-5.5 Hz.

Two different compounds supplied dihedral angles of 160°. In 1,2-O-isopropylidene-3,5,6-tri-O-orthoformyl-α-D-glucofuranose-6-^{13}C (K), the $^{13}C_6$-O_6 and C-H_{formyl} bonds of the $^{13}C_6$-O-C-H pathway subtend an angle of 160° whereas as already mentioned on p. 156, 2,3,4-tri-O-acetyl-1,6-anhydro-α-L-idopyranose-6-^{13}C (L) provides two routes with dihedral angles of 160°, i.e., the $^{13}C_6$-O-C_1-H_1 and $^{13}C_6$-C_5-C_4-H_4 pathways.



K



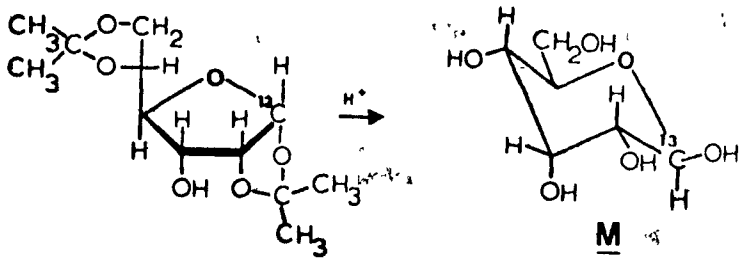
L

The observed couplings through the oxygen were 4.6 Hz and 5 Hz in K and L, respectively; whereas coupling through carbon in L was 5.8 Hz.

Dihedral angle of 180°

From the spectrum of α and β-D-glucopyranose-U-^{13}C (p. 6), it is evident that H_1 of the α anomer exhibits ^{13}C coupling but H_1 of the β form does not. The observed coupling (6-7 Hz) of the α proton has been attributed to vicinal

coupling to $^{13}C_3$ or $^{13}C_5$. In each of these situations the dihedral angle is 180°. In this system, however, it is impossible to exclude the possibility that the observed coupling arises, in fact, from geminal coupling to $^{13}C_2$. It became imperative therefore to examine a situation in which no such ambiguity could arise. In D-allopyranose, the C_3-H_3 and C_1-C_2 bonds subtend a dihedral angle of 180°. α-D-Allopyranose-1-^{13}C (M) can be obtained by acid hydrolysis of 1,2:5,6-di-O-isopropylidene-α-D-allofuranose-1-^{13}C which, in turn, is available by inversion at C_3 of the glucose epimer as outlined on p. 102. Only the β anomer forms upon hydrolysis and consequently the 100 MHz pmr spectrum of the product is not excessively complex. The α anomer is destabilized in this case on account of the unfavourable 1,3-diaxial interaction between OH_1 and OH_3.



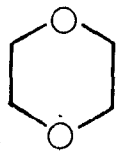
The measured coupling in this experiment was 5.5 Hz consistent with the splitting of $H_{1\alpha}$ observed in the D-glucose-U-^{13}C spectrum.

It is evident from the preceding that a general dihedral angle dependence of $^3J_{C-H}$ is followed: trans arrangements of ^{13}C and ^1H nuclei are associated with larger couplings than gauche arrangements. However, as has been indicated, some factors other than dihedral angle also must be taken into account

and consequently it becomes necessary to examine the impact of other variables such as lone pair and hybridization effects on this parameter.

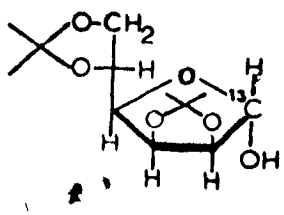
4.3 Lone pair effects

Lone pair effects have already been invoked to explain some phenomena related to directly bonded and geminal $^{13}C-^{1}H$ couplings, hence the possibility that they also play a role in vicinal couplings bears discussion. If proton-proton vicinal coupling of 1,4-dioxane is considered (146);



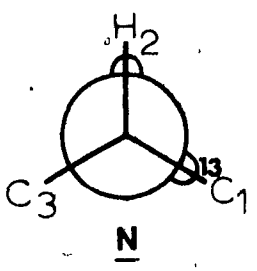
the sum of the vicinal couplings calculated according to the Karplus relationship (4) is 7 Hz, which becomes 4.8 Hz when corrected for the electronegativity effects of the oxygens. This is in sharp contrast to the observed coupling of 9.2 Hz. However, if overlap of an oxygen lone pair with a bond of the coupling pathway causes an increase in coupling the result becomes more rational. In this case the mean number of overlaps is two, consequently suggesting an increase in vicinal proton-proton coupling of about 2 Hz per lone pair overlap.

It is difficult to synthesize vicinal systems in which the orientations of the oxygen substituents can be controlled. One possibility for such study however is 2,3:5,6-di-O-isopropylidene-α-D-mannofuranose-1-^{13}C:

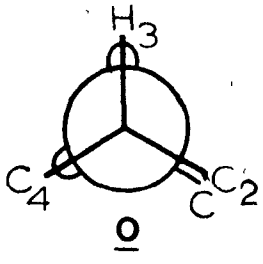


Again the conformation of this molecule is reasonably well established

from proton-proton coupling data (see p. 127). The absence of coupling between H_1 and H_2 fixes the relative orientation of the C_1-H_1 and C_2-H_2 bonds at 90° and this in turn establishes the conformation of the furanose ring. The 2,3-O-isopropylidene function fixes the orientation of the oxygens relative to the coupling pathway. The oxygen orientations are such that one of the lone pairs on O_3 eclipses the C_3-H_3 bond whereas a lone pair on O_2 eclipses the C_2-C_1 bond. This situation can be illustrated by projections along the C_2-O_2 (N) and C_3-O_3 (O) bonds:



N



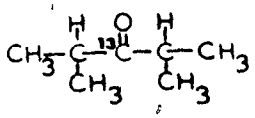
O

The lone pair effect, if such exists, is anticipated to be considerable in this case since two bonds (i.e., C_2-$^{13}C_1$ and C_3-H_3 bonds) of the coupling pathway are eclipsed by lone pairs. The dihedral angle between $^{13}C_1$ and H_3 is roughly 110°, a situation which from all previous experience should result in zero coupling. The experimentally measured coupling however is 3.5 Hz, a result which seems explicable only by attributing the increase to lone pair orientations.

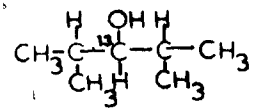
It is also noteworthy that in 1,2:5,6-di-O-isopropylidene-α-D-glucofuranose-1-^{13}C the observed coupling of 5.5 Hz for a dihedral angle of 140° seems large in comparison to the values observed for 160°. In this compound one of the lone pairs of the C_2 oxygen substituent eclipses the C_2-C_3 bond, a situation which should lead to increased coupling.

4.4 Hybridization and substituent effects

In conformationally mobile compounds a slight increase is noted when going from sp^3 to sp^2 carbon (149).

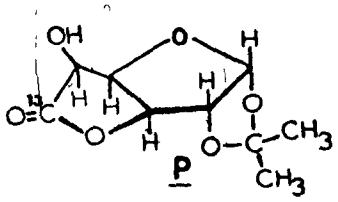


$^3J_{C-H}$ = 5.1 Hz

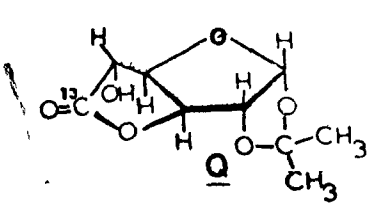


$^3J_{C-H}$ = 4.5 Hz

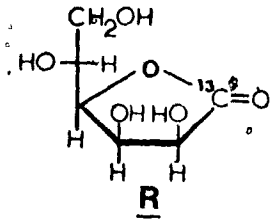
It was desirable to examine this relationship in systems of known geometry. The compounds employed in this study were 1,2-O-isopropylidene-α-D-glucofuranurono-6,3-lactone-6-^{13}C (P), 1,2-O-isopropylidene-β-L-idofuranurono-6,3-lactone-6-^{13}C (Q) and D-mannono-1,4-lactone-1-^{13}C (R).



P



Q



R

Fig. 3 illustrates the spectrum of R and is typical of those encountered in the above systems. The couplings from $^{13}C_6$ to H_4 in the uronolactones are 9 Hz and 6.6 Hz for the D-gluco and L-ido epimers, respectively, although the dihedral angle for this pathway of about 140° is associated above with smaller $^3J_{C-H}$ values. It seems clear that, other factors being equal, an increase in "s" character of the ^{13}C nucleus results in increased coupling. D-Mannono-1,4-lactone-1-^{13}C also exhibits a coupling of 9 Hz between $^{13}C_1$ and H_3; as discussed on p. 112, the stereochemical situation in this compound is analogous to that in the glucurone derivative.

The difference in coupling between the D-gluco and L-ido epimers is interesting. It is likely due to the different orientation of the C_5-O_5

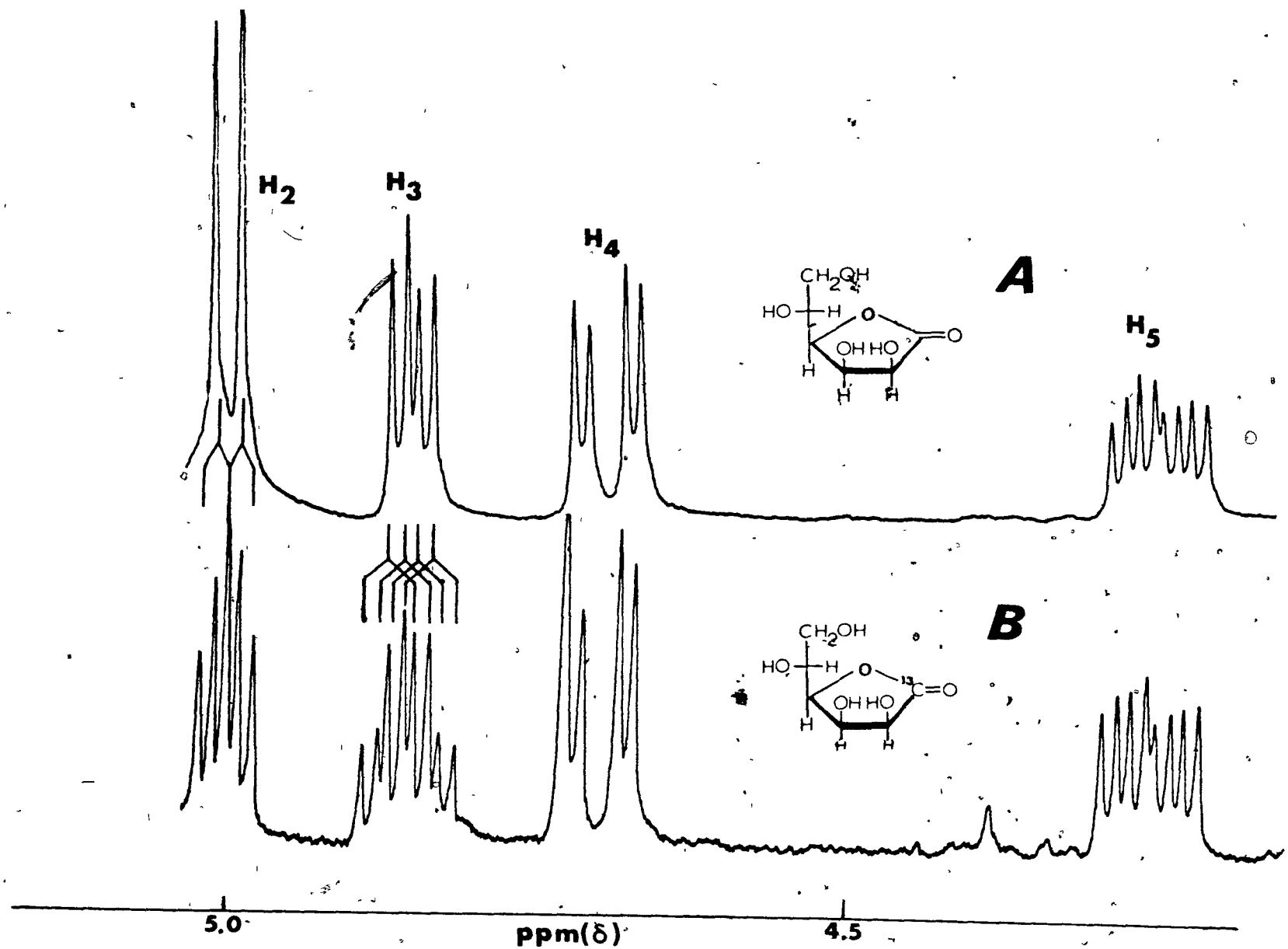
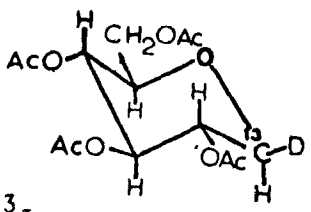


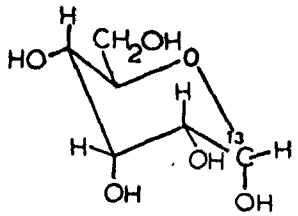
FIG. 3 Partial pmr spectrum (220 MHz) of A) D-Mannono-1,4-lactone and B) D-Mannono-1,4-lactone-1-^{13}C (60%) in D_2O.

bond with respect to the coupling pathway in the two compounds. In the L-ido derivative this bond nearly eclipses the C_4-H_4 bond whereas in the D-gluco derivative a 90° relationship is maintained. This orientation effect then is not unlike those observed in ^{13}C-^{1}H geminal couplings. All of the couplings through the lactone oxygen in the above compounds were found to be zero but the dihedral angles in these cases are roughly 110°, an angular region where little coupling is to be expected. In general, though, as has been shown by Lemieux et al. (163) ^{13}C-^{1}H vicinal couplings where the ^{13}C atom is sp^2 hybridized vary with dihedral angle in a Karplus manner.

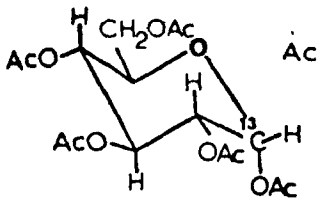
Substituent effects on vicinal ^{13}C-^{1}H coupling when the substituent is bonded to the coupling carbon are minimal. When the series below was examined, no variation in $^{3}J_{C_1\text{-}H_3}$ was noted, all couplings being less than 1 Hz.



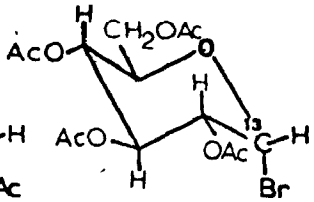
$^{3}J_{C\text{-}H}$ < 1 Hz



< 1 Hz

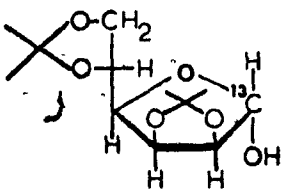


< 1 Hz

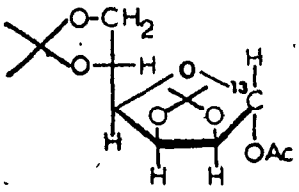


< 1 Hz

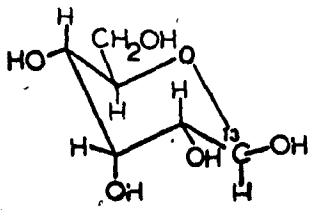
Even when large couplings were examined no substituent effects were observed. This is apparent on consideration of the data below:



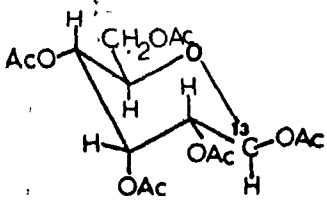
$^{3}J_{C\text{-}H_3}$ = 3.5 Hz



$^{3}J_{C\text{-}H_3}$ = 3.5 Hz



$^{3}J_{C\text{-}H_3}$ = 5.5 Hz



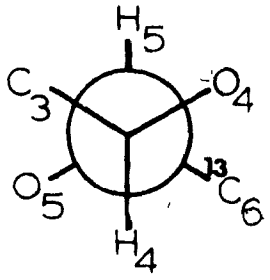
$^{3}J_{C\text{-}H_3}$ = 5.5 Hz

This lack of variation in coupling has already been noted by Karabatsos et al. (181) who consequently drew the conclusion that vicinal coupling is not governed by the Fermi contact term.

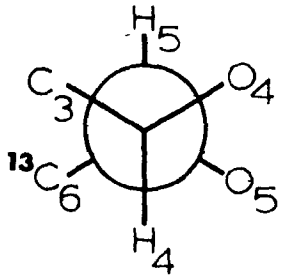
4.5 ^{13}C-^{1}H vicinal coupling in rotamers

As with geminal ^{13}C-^{1}H coupling it was of interest to examine what information could be obtained about rotamer populations in freely rotating carbinol groups with the aid of $^{3}J_{C-H}$.

The pmr spectra of 1,2-O-isopropylidene-α-D-glucofuranose-6-^{13}C, and of its L-ido epimer, show that H_4 and H_5 are strongly coupled. For each of these compounds the large value of $J_{H_4-H_5}$ observed (7.5-8 Hz) is consistent with a preponderance of either of two rotamers: in one, H_4 and H_5 are eclipsed and in the other antiperiplanar. On conformational grounds, obviously the latter arrangement is more likely. The preponderant rotamers for the D-gluco and L-ido derivatives then become the following:



D-Gluco



L-Ido

The experimentally observed $^{13}C_6$-H_4 couplings of 2.4 Hz and 0 Hz for the D-gluco (Fig. 4) and L-ido epimers respectively, agree with the dihedral angle of nominally 60° relating these two nuclei. The difference of 2.4 Hz can be attributed to the fact that in the L-ido epimer the ring oxygen is trans to the C_5-C_6 bond. This particular type of arrangement has already been associated with a decrease in

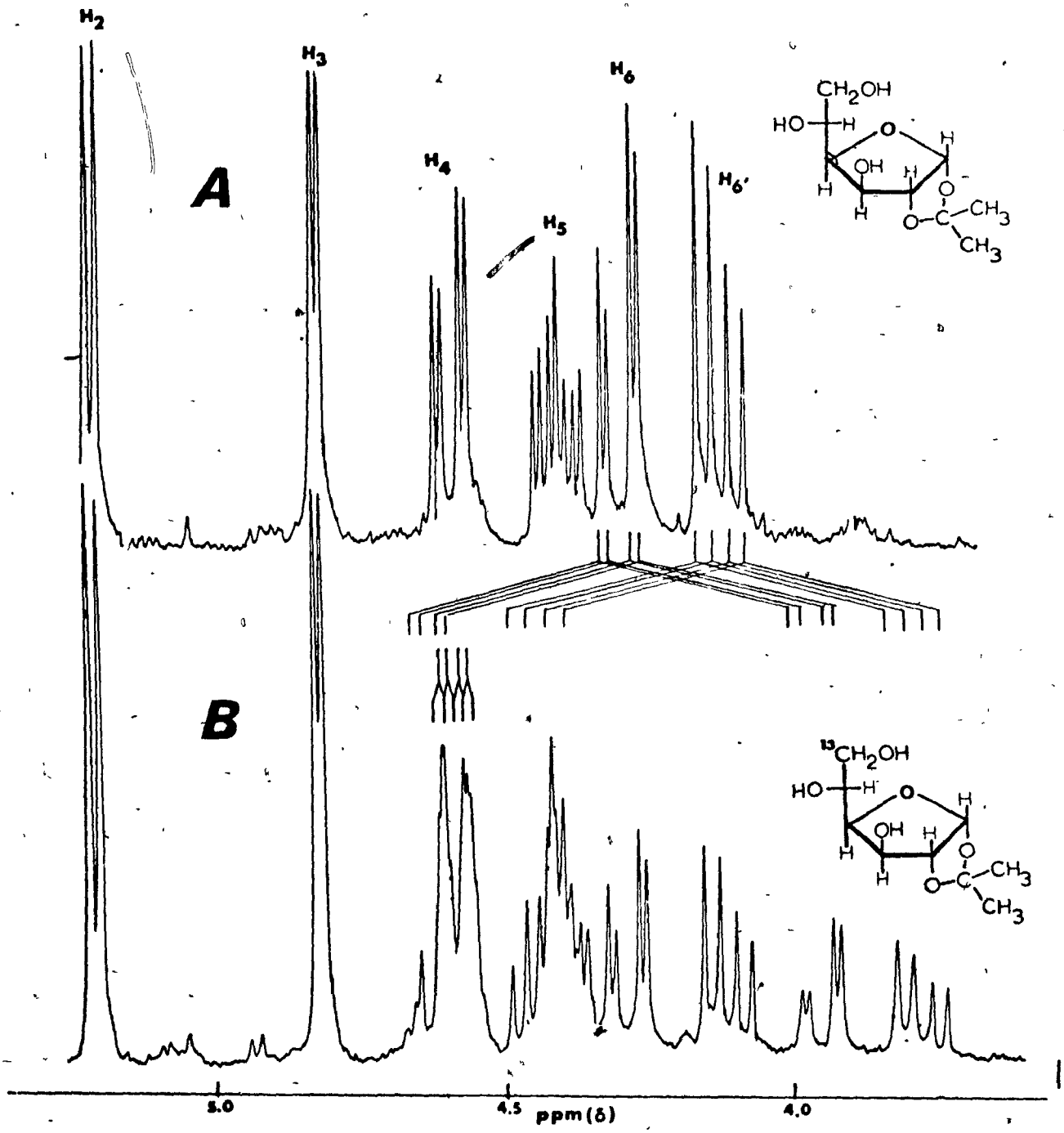
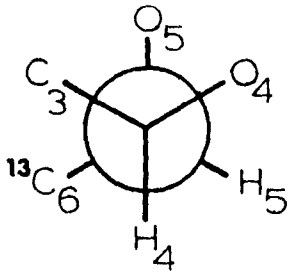


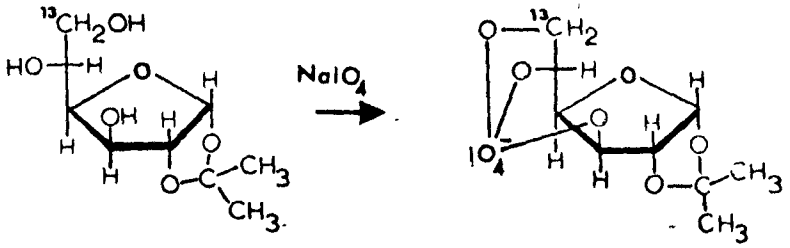
FIG. 4 Partial pmr spectrum (220 MHz) of A) 1,2-O-Isopropylidene-α-D-glucofuranose and B) 1,2-O-Isopropylidene-α-D-glucofuranose-6-^{13}C (60%) in $CDCl_3$.

observed coupling. In the gluco epimer, on the other hand, the same bonds subtend a gauche relationship. If the rotamer population about the C_5-C_6 bond for the gluco epimer could be influenced so as to favour the rotamer S,



S

the coupling should decrease since there are now two C-O bonds antiperiplanar to the coupling pathway. This situation can be realized by complexing the mono-O-isopropylidene derivative with periodate (183):

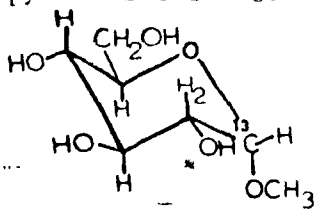


When periodate is added to a D_2O solution of 1,2-O-isopropylidene-α-D-glucofuranose-6-^{13}C in the NMR sample tube, the spectrum changes to that of the complex and the coupling between $^{13}C_6$ and H_4 disappears. (This result is analogous to that obtained with 1,2-O-isopropylidene-3,5,6-tri-O-orthoformyl-α-D-glucofuranose-6-^{13}C.) This observed phenomenon again reinforces the concept that an antiperiplanar electronegative substituent leads to decreased ^{13}C-H coupling.

In the 1,2:5,6-di-O-isopropylidene derivative of glucose the coupling between $^{13}C_6$ and H_4 is essentially equivalent to that in the mono-O-isopropylidene-

compound indicating that there is no major change in the rotamer population about the C_4-C_5 bond when OH_5 and OH_6 are constrained in a gauche relationship.

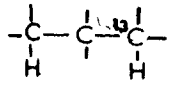
It was also of interest to examine the coupling between $^{13}C_1$ and the methyl protons in methyl-α-D-glucopyranoside-1-^{13}C:



The observed coupling of 4 Hz in this case is readily rationalized if the gauche coupling in absence of a trans oxygen is assumed to be ~2.5-3 Hz and the trans coupling is taken to have an average value of 5.5-6 Hz. $J_{Average}$ then equals $(2J_g + J_t)/3$ which is about 4 cycles, in agreement with the observed value. A similar result has been noted by Lemieux et al. who measured the coupling between H_1 and the glycosidic carbon (163a).

4.6 Signs of vicinal ^{13}C-^1H coupling constants

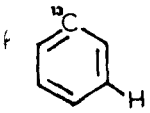
Very few sign determinations of vicinal proton -^{13}C coupling constants have been carried out. This stems mostly from the lack of suitable compounds. To carry out a sign determination by means of the spin decoupling method, the only method which can provide conclusive results, it is necessary for the vicinal proton to be coupled not only to the ^{13}C nucleus but also to the proton bonded to the ^{13}C. Since the directly bonded ^{13}C-^1H coupling constant is known to be positive, the sign of the vicinal coupling can then be obtained. The system necessary for such an experiment can be represented as:



where the protons must couple appreciably to each other. Such situations are very

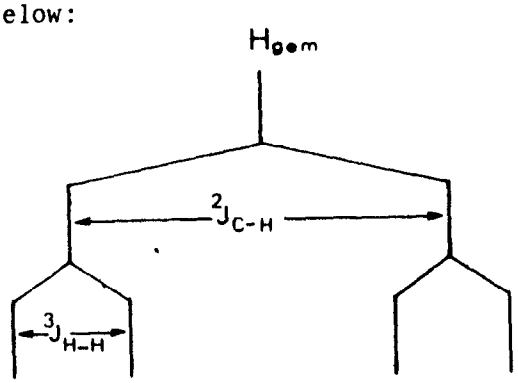
difficult to realize, consequently the sign determinations made heretofore have involved comparisons of ^{13}C satellite spectra with computed spectra. Again, this method is limited to rather simple compounds. All results obtained in this area indicate the the ^{13}C-^{1}H vicinal coupling is positive (Table 2).

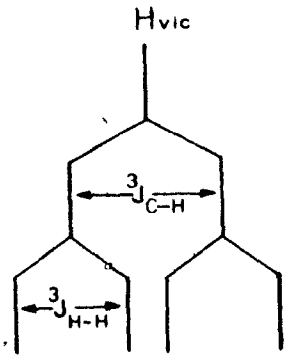
TABLE 2 - Signs of vicinal ^{13}C-^{1}H coupling constants

	$^{3}J_{C-H}$ (Hz)	Ref.
$H-{}^{13}C\equiv C-CH_3$	+4.8	183
$H-C\equiv C-{}^{13}CH_3$	+3.6	183
$CH_3-C(CH_3)(CH_3)-{}^{13}CH_3$	+4.4	184
	+7.4	185
$^{13}CH_3-C(=O)-CH_3$	+2.2	186

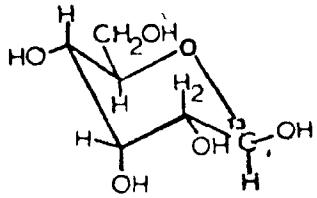
In the present study no compounds were encountered in which the vicinal proton and the directly bonded protons were coupled, consequently the sign of the vicinal coupling could not be directly determined.

Accordingly, an alternate approach was designed to determine the sign of the vicinal ^{13}C-^{1}H coupling relative to ^{13}C-^{1}H geminal coupling. The pre-requisites now become coupling of the vicinal and geminal protons to the ^{13}C nucleus and to each other. Symbolically this arrangement can be represented as below:

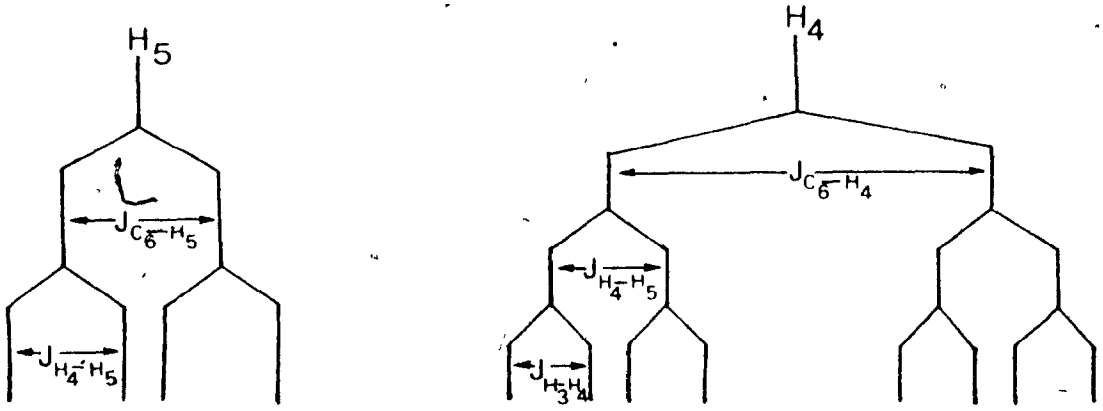




Accordingly, if irradiation of one of the doublets of either quartet leads to collapse of one of the doublets of the other quartet the relative values of $^{3}J_{C-H}$ and $^{2}J_{C-H}$ are obtained. Theoretically then, a compound such as β-D-allopyranose-1-^{13}C would be suitable for such an experiment:

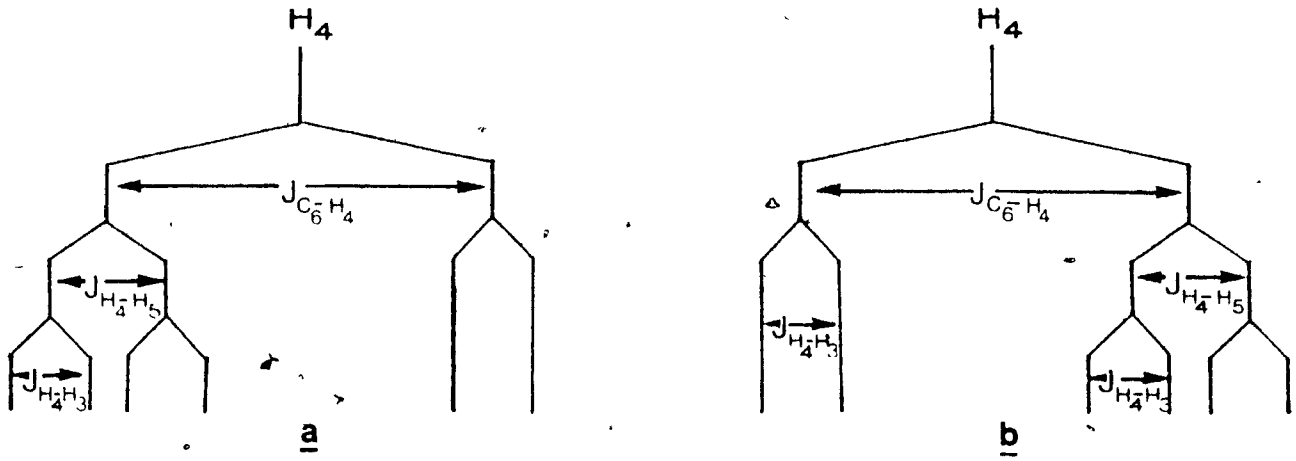


Both H_3 and H_2 are coupled to ^{13}C-1 and the sign of the geminal coupling between ^{13}C-1 and H_2 has already been determined to be negative. However, in this compound H_2 and H_3, aside from being coupled to each other, are also coupled to H_1 and H_4 respectively; this leads to a degree of complexity in the H_2 and H_3 absorptions as to preclude such an experiment. It seemed necessary, therefore, to examine a compound in which at least one of the extra couplings was absent. 1,2-O-Isopropylidene-5-O-acetyl-α-D-glucofuranurono-6,3-lactone-6-^{13}C was suitable for this purpose. Due to the absence of hydrogens on C_6, the H_5 signal in the non-enriched compound is a simple doublet. The proton spectrum of the 6-^{13}C compound can be represented schematically by the following:



Relative sign determination now involves irradiating one of the

doublets of the H_5 signal and observing the collapse of the upper or lower half of the H_4 signal. Upon irradiation the H_4 signal then should take on the appearance of either (a) or (b):



The experimental sign determination (Fig. 5) indicates that the vicinal and geminal couplings are of opposite sign: irradiation of the low field H_5 doublet results in collapse of the upfield half of the H_4 signal, whereas irradiation of the upfield H_5 doublet causes collapse of the low field half of the H_4 signal. Since the geminal coupling in this case is most likely negative (Chapter 3), the vicinal coupling must be positive. In any case, it is clearly demonstrated that the couplings are of opposite sign.

4.7 Theoretical considerations

Wasylishen and Schaefer (187) carried out calculations according to the INDO formulation using propane as a model. These workers found a Karplus type of relationship but suggested that there is an insensitivity of vicinal coupling to hybridization state as well as to substituent effects. Later examination of fluoropropane (188) indicated that the coupling decreases when a fluorine atom is present along the pathway as in T, but this decrease is modulated by the orientation of the substituent.

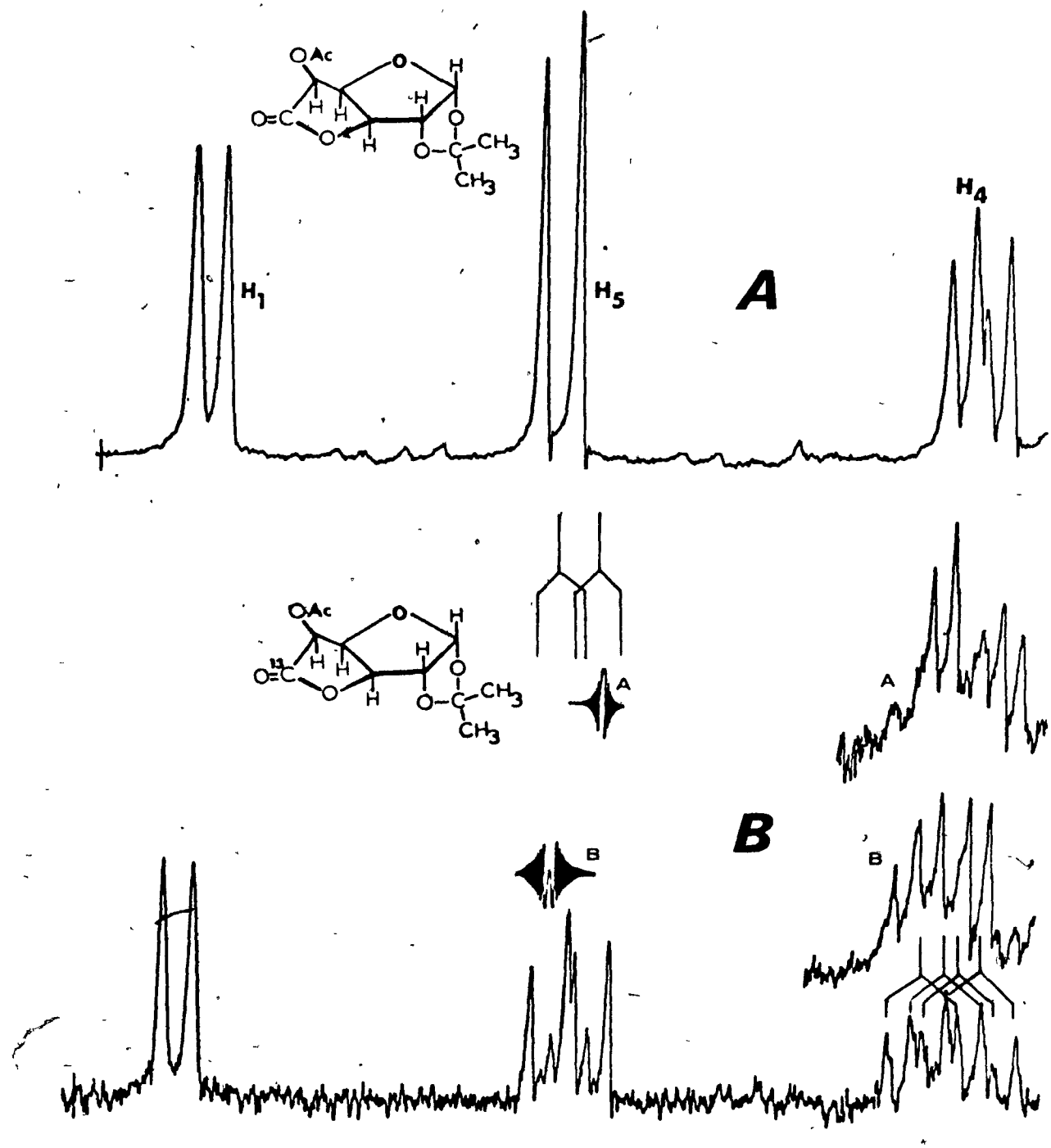
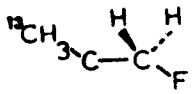
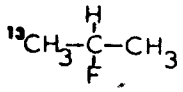


FIG. 5 Relative sign determination by double resonance in 1,2-O-isopropylidene-5-O-acetyl-α-D-glucofuranurono-6,3-lactone-6-^{13}C. Partial pmr spectrum (100 MHz) of A) the natural abundance compound and B) the 6-^{13}C (80%) derivative in $CDCl_3$.



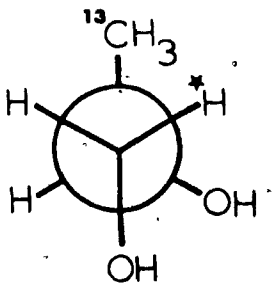
T

The effect of fluorine varies with the dihedral angle between the fluorine and ^{13}C nuclei exerting a maximum effect at $\theta = 180^{b}$ and a minimum at $\theta = 90°$. Similar relationships were noted in 2-fluoropropane-1-^{13}C:

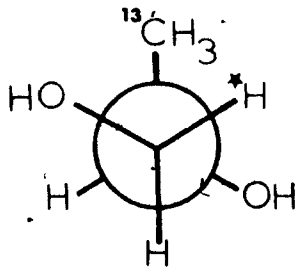


In general then these calculations support the experimental data previously discussed.

Calculations have also been carried out using the CNDO/2 method, and oxygen orientation effects were examined. 1,2-Dihydroxypropane was used as a model compound. Again the results obtained from the calculations are consistent with the experimental data - if constant dihedral angle is maintained, an <u>antiperiplanar</u> oxygen leads to decreased coupling (189):



smaller coupling



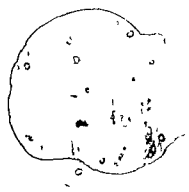
larger coupling

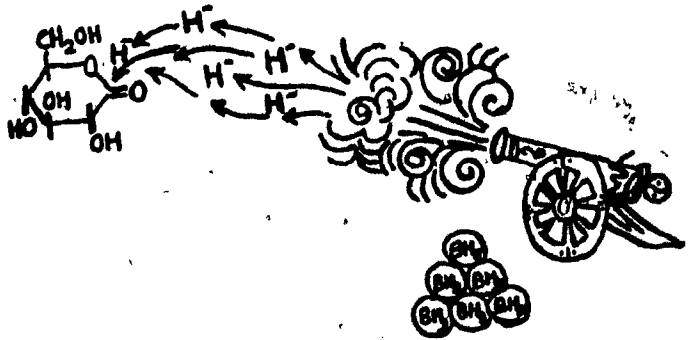
4.8 Conclusion

It has been demonstrated that vicinal $^{13}C-^{1}H$ coupling does not conform to a straightforward dihedral angle relationship. Substituents, substituent orientations, hybridization changes and lone pair effects can all significantly modulate the coupling expected on a dihedral angle basis. Consequently, no validity can be attached to calculations of dihedral angles from $^{3}J_{C-H}$ which do not take these factors into account.

CHAPTER 5

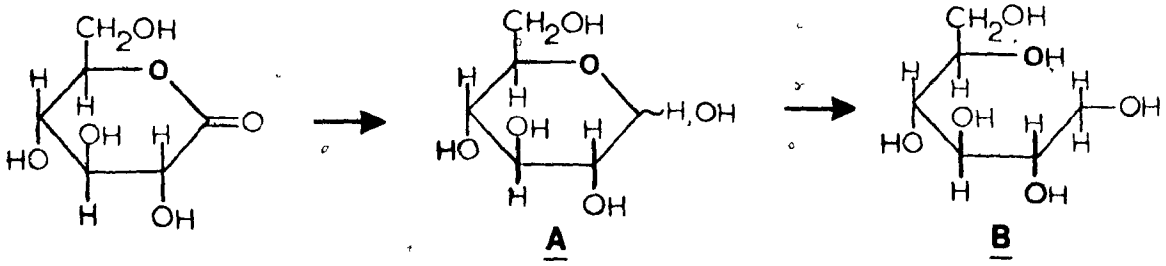
REDUCTION OF ALDONOLACTONES





5.1 Introductory remarks

The preparation of carbohydrates enriched with ^{13}C (or ^{14}C) in the one position presents some problems. The main difficulty in the synthesis arises from the last step of the Fischer-Kiliani sequence, namely the reduction of an aldonolactone to an aldose (A). This reaction must be carried out in such a way as to prevent over-reduction to the sugar alcohol (B). The reaction is illustrated below for D-glucono-1,5-lactone.



The classical reagent for reduction to the aldose is sodium-amalgam (190)(191); however, it has sometimes proven difficult to obtain good yields using this technique. Preliminary experiments showed that the sodium amalgam method was not suitable on a small scale. The maximum yields obtained were 60% for D-glucose and 30% for D-mannose. More recently it was found that esterified aldono-1,4-lactones (192-195) as well as some free aldonolactones (196) could be reduced to the corresponding aldose with bis-3-methyl-2-butylborane (disiamylborane) (197)(198). The highest yield achieved for the reduction of D-glucono-1,5-lactone to D-glucose using this method however is again only 60% (196). While lower yields can be tolerated when radioisotope (^{14}C) enrichment is desired due to the possibility of co-crystallizing the first product with non-enriched material, high yields in ^{13}C incorporation experiments are very important, especially for spectroscopic studies which are favored by as high degree of enrichment as possible.

Since one is already limited by the degree of enrichment of the starting material, $K^{13}CN$, any further dilution should be avoided. Wolfrom has described the reduction of aldonolactones using buffered sodium borohydride (199) but this reaction was found to be difficult to control and also gave poor yields.

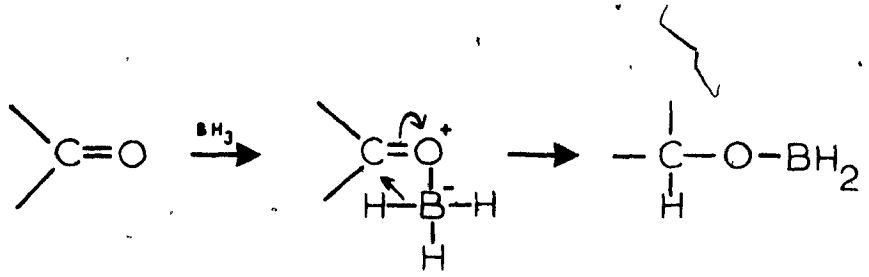
In the light of these results a search was carried out for a reduction method which would give higher yields of the sugars in question. As a result, two novel reduction techniques for the reduction of aldonolactones to aldoses were developed: reduction via BH_3 and reduction via a $LiAlH_4$-$AlCl_3$ mixed hydride reagent. Both methods afforded higher yields of the reducing sugar than had been previously attainable.

5.2 Reduction of aldonolactones by means of BH_3

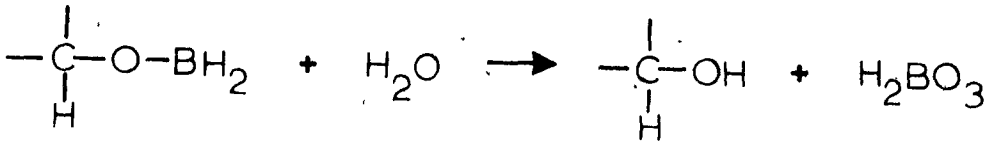
Diborane, which in its chemical action can be considered as BH_3, is a well known reducing agent, reducing aldehydes, ketones and carboxylic acids rapidly and esters more slowly (200-202). The reduction of lactones by diborane has received very little attention although Dias and Pettit (203) have reported a mixture of products when certain steroid lactones were treated with diborane. Diborane is readily prepared by the addition of a solution of sodium borohydride in diglyme to boron trifluoride etherate (204):

$$3NaBH_4 + 4BF_3 \longrightarrow 3NaBF_4 + 2B_2H_6$$

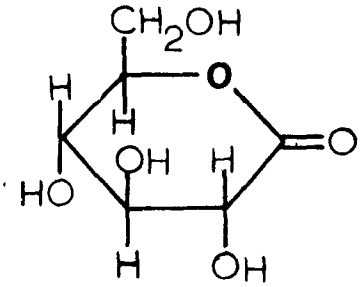
This diborane solution can be heated under an inert atmosphere to distil off diborane into a receiver containing tetrahydrofuran. A 1M solution of diborane in THF is also available commercially. Diborane is a Lewis acid and reduces carbonyl funotions by first complexing with the oxygen atom and then transferring a hydride, perhaps by a concerted mechanism analogous to the hydroboration of alkenes:



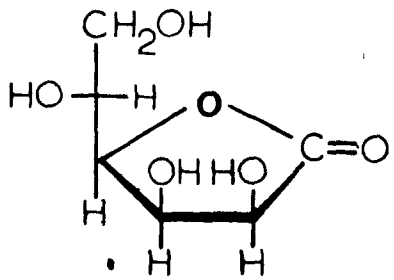
Treatment of the product with water generates the alcohol and boric acid:



It was hoped that if the reduction of lactones with borane could be controlled so that only the carbonyl group were reduced, a clean method for converting lactones to hemiacetals would be available. The lactones of major concern for the present purposes were D-glucono-1,5-lactone and D-mannono-1,4-lactone, the epimeric species formed upon the addition of cyanide to arabinose, since the corresponding ^{13}C-enriched hexoses and derivatives of them have constituted the main compounds examined in this investigation.



D-Glucono-1,5-lactone



D-Mannono-1,4-lactone

A ten fold excess of a 1M solution of diborane in THF was used to compare the reduction of these lactones under different conditions. The results of this study are reported in Table 1. Yields were obtained by g.l.c. analysis of the TMS or acetate derivatives of the products.

TABLE 1 - Reduction of aldonolactones by 1M BH_3

D-Glucono-1,5-lactone

Reaction time	Temp.	Aldose	Alditol	Lactone
15 min	R.T.	60%	2%	38%
60 min	"	88%	5%	7%
90 min	"	90%	8%	2%
3.5 hrs	"	90%	8%	2%
40 hrs	"	86%	13%	1%
84 hrs	"	81%	19%	-
3 hrs	reflux	60%	40%	-
3 hrs	-10°	40%	-	60%
18 hrs	-10°	48%	2%	50%
D-Mannono-1,4-lactone				
15 min	R.T.	52%	23%	25%
60 min	"	75%	18%	7%
3.5 hrs	"	65%	30%	5%
40 hrs	"	60%	38%	2%
84 hrs	"	57%	43%	-
3 hrs	reflux	35%	65%	-
3 hrs	-10°	35%	-	65%
18 hrs	-10	40%	10%	50%

The above results indicate that most probably there are two reactions: first, the lactone is reduced to the sugar, (or to a derivative which on hydrolysis yields the sugar) in a fast reaction, followed by the much slower conversion of the sugar into the sugar alcohol.

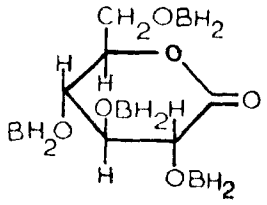
$$\text{Lactone} \xrightarrow{\text{fast}} \text{sugar} \xrightarrow{\text{slow}} \text{sugar alcohol}$$

This is emphasized by the very low rate of formation of alditol at -10°. It was found that the best and most reproducible results were obtained when the reaction was carried out at room temperature for 2 to 3 hours. Under these conditions the isolated yields were 85-90% for glucose and 65-70% for mannose. Both of these yields are higher than those obtained by any other procedure.

The reaction itself is much cleaner and is easier to carry out than amalgam reduction or reduction using buffered sodium borohydride. Finely powdered lactone is suspended in tetrahydrofuran and an excess of diborane, (about 10 fold) is added, followed by vigorous stirring. A rapid evolution of hydrogen gas is observed on the addition of the borane reagent; this is of course due to the reaction of borane with the hydroxyl groups of the lactone:

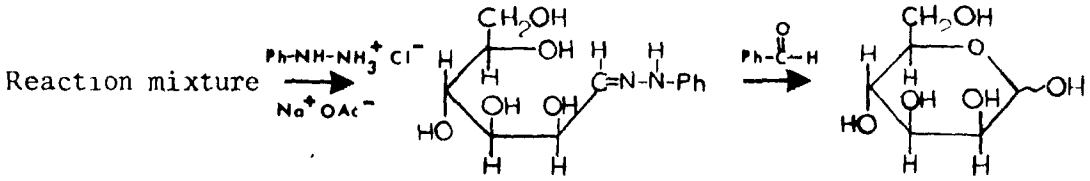
$$-\overset{|}{\underset{|}{C}}\text{-OH} + BH_3 \longrightarrow -\overset{|}{\underset{|}{C}}\text{-O-}BH_2 + H_2$$

After a short period the reaction mixture becomes gelatinous, presumably due to the formation of species such as the following:



It is also conceivable that dimeric or even trimeric borate esters can form, joining together two or three lactone units. At the end of the reduction period water is added to decompose the gel and the reaction mixture is concentrated several times with methanol to remove boric acid as volatile methyl borate. If the reaction is carried out on a scale of one or more grams the desired product can be fractionally crystallized at this point. On a smaller scale it was found that crystallization led to the loss of too much product and alternative

approaches were examined. For the reduction of D-glucono-1,5-lactone, the most efficient isolation technique was found to be acetylation of the reaction mixture followed by chromatography on silica gel. Mannose was best isolated from the reaction mixture through its phenylhydrazone derivative (evidence now favours an acyclic form for this compound (205)) from which mannose is readily regenerated:



Mechanism of reduction

A possible mechanism for the reduction of D-glucono-1,5-lactone (C) is outlined in Fig. 1. D-Glucose (F) is obtained by hydrolysis of the intermediate E. This intermediate however can conceivably undergo ring opening by three different pathways: via carbonium ion (a), via concerted mechanism (b), or via type J complex formation (c). All of these routes lead to the alditol as the final product. The corresponding scheme for D-mannono-1,4-lactone is given in Fig. 2. Note that in this case borates of type N can also be expected to form by analogy to 2,3:5,6-di-O-isopropylidene-α-D-mannofuranose. Hydrolysis of intermediate O or P affords D-mannose but these species can also undergo ring opening by routes analogous to a, b, and c of Fig. 1. The implication of the experimental results is that a furanose ring is more likely to undergo ring opening than is a pyranose ring. It appeared possible, however, that the configuration at C_2 and not ring size is the determining factor. In order to clear up this point the reduction of D-glucono-1,4-lactone was examined.*

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* A gift of D-glucono-1,4-lactone from Professor H.S. Isbell is gratefully acknowledged.

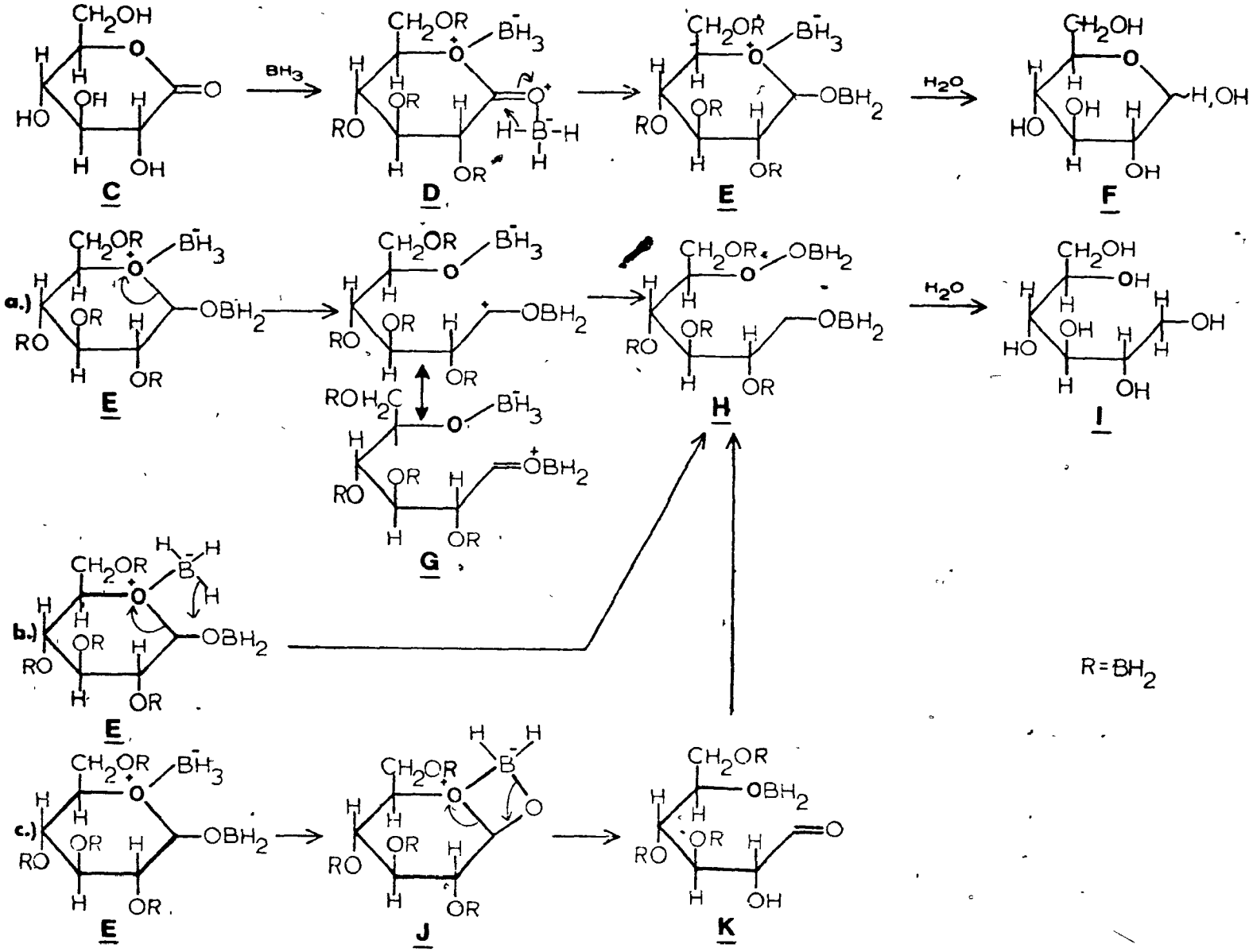


FIG. 1 Reduction of D-glucono-1,5-lactone with diborane.

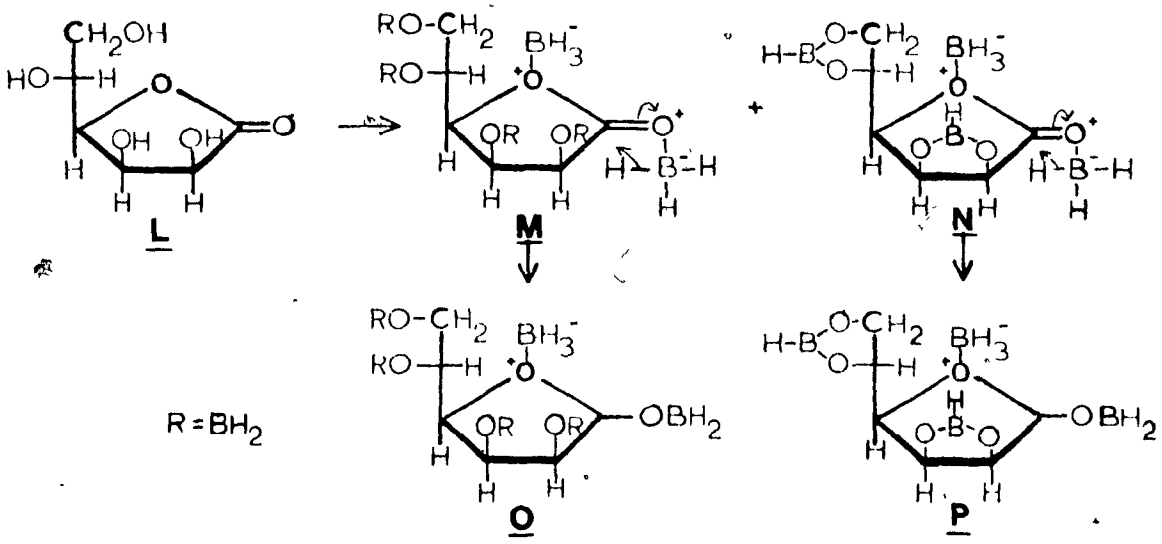


FIG. 2 Reduction of D-mannono-1,4-lactone with diborane.

The reduction of this compound now afforded yields of aldose (60%) and alditol (40%), very similar to those obtained from D-mannono-1,4-lactone. D-Galactono-1,4-lactone also gave almost equivalent yields. It seems established, then, that the difference in yield of aldose obtained by BH_3 reduction of D-glucono-1,5-lactone and D-mannono-1,4-lactone can be accounted for by the different ring sizes of these lactones.

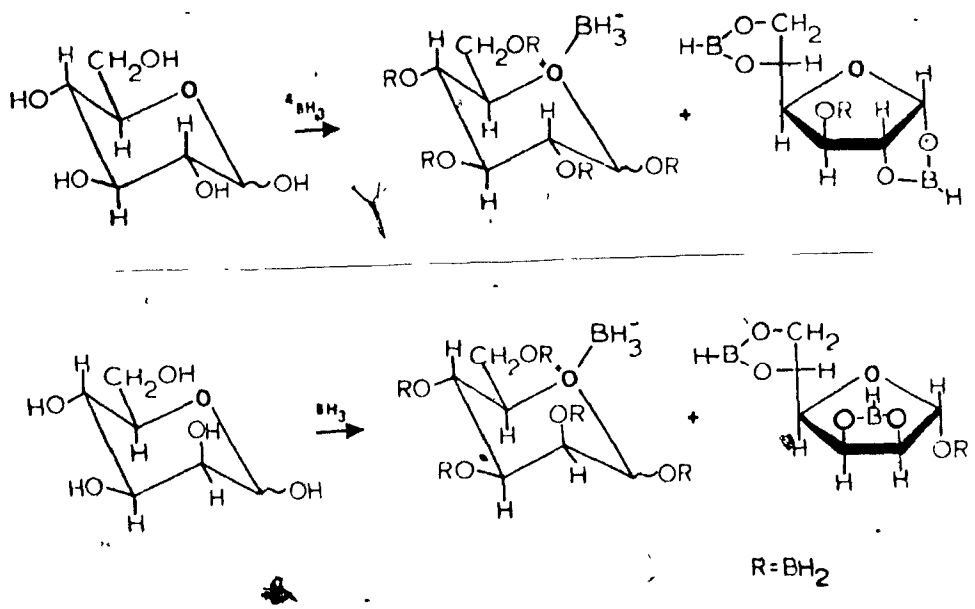
In order to gain further insight into the reaction of BH_3 with carbohydrates and to attempt to determine what factors promote ring opening, a series of reducing sugars was studied under experimental conditions equivalent to those used for the lactones (10 fold excess BH_3 for 3 hrs at R.T.).

These results are reported in Table 2.

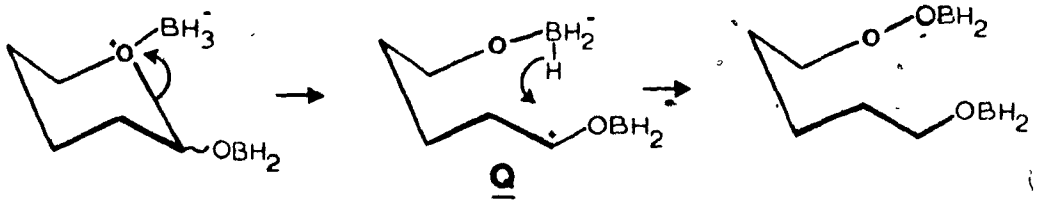
TABLE 2 - Reduction of aldoses by diborane

	% Aldose remaining	% Alditol
D-Glucose	95	5
β-D-Glucose	95	5
D-Mannose	60	40
D-Galactose	95	5
D-Erythrose	45	55
L- Fructose	75	25
D-6-Deoxyglucose	75	25
D-Lyxose	40	60
D-Xylose	30	70
D-Ribose	5	95
D-Arabinose	10	90
D-2-Deoxyarabinohexose	0	100
D-2-Deoxyribose	0	100

D-Glucose and D-mannose are again reduced to different extents but this time it was not possible to determine whether this difference is due to a difference in ring size. The major intermediate species that are expected to form on reaction of D-mannopyranose and D-glucopyranose with BH_3 are indicated below.



It was not possible to determine the relative ratios of these types of species in such a complex system and hence the differences in the rate of reduction of D-mannose and D-glucose cannot be attributed to any one specific factor such as ring size. More interesting, however, is the observation that when the C_2 oxygen substituent is removed, as in 2-deoxyarabinohexose, reduction to the alditol is 100%. There are two possible explanations that may be suggested in this regard. If ring opening proceeds by a carbonium ion mechanism,



Q

removal of an electronegative substituent adjacent to the prospective carbonium

ion Q, should favour the reaction. The second alternative is that reaction proceeds through the open chain or aldehydo form of the various sugars, and those sugars with higher aldehydo content in solution are more readily reduced. This latter rationale seems favourable since 2-deoxysugars and ribose are expected to have the highest (even though very small) aldehydo content, followed by pentoses and then hexoses (206). Since it is known that aldehydes are rapidly reduced (200-202) the overall trend in Table 2 becomes reasonable.

5.3 Reduction of aldonolactones by means of $LiAlH_4$-$AlCl_3$

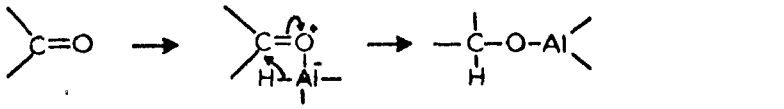
Lithium aluminum hydride has been extensively used in organic and inorganic synthesis (207). This compound is known to reduce lactones readily to diols and therefore is not suitable for the reduction of aldonolactones to aldoses. It is possible however to modify the reducing properties of lithium aluminum hydride by the addition of a measured amount of aluminum chloride (208-211). The nature of the reducing species in this "mixed hydride" reaction depends on the molar proportion of $AlCl_3$ and $LiAlH_4$ (212,213) according to the following relationships.

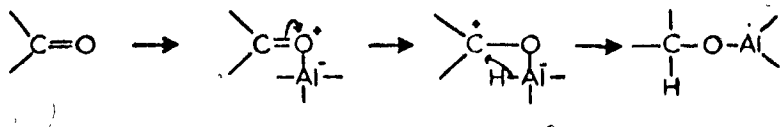
$$AlCl_3 + 3LiAlH_4 \longrightarrow 4AlH_3 + 3LiCl$$

$$AlCl_3 + LiAlH_4 \longrightarrow 2AlH_2Cl + LiCl$$

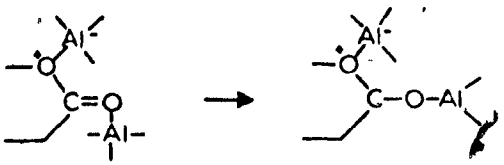
$$3AlCl_3 + LiAlH_4 \longrightarrow 4AlHCl_2 + LiCl$$

The aluminum hydrides produced in any of the above reactions are analogous to the previously discussed diborane, and reduction of lactones by them can be expected to proceed in a manner similar to the borane reductions. All three hydrides are Lewis acids and perform their function by first complexing with an available oxygen atom. The actual reduction can then proceed either by a four center or a carbonium ion mechanism as illustrated below:

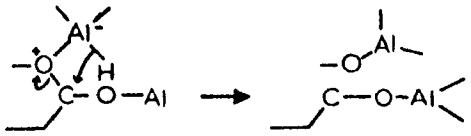




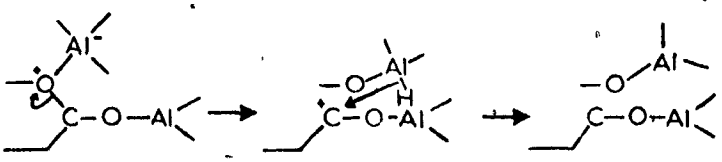
For lactones, the reaction is not expected to stop at the hemiacetal stage since it has been shown that acetals, ketals and orthoesters can be cleaved by the mixed hydride reagent (214-217). Mechanistically this type of cleavage is expected to be very similar to cleavage by diborane, as discussed previously. Once again, both four center and carbonium ion mechanisms can be envisaged:



a.)



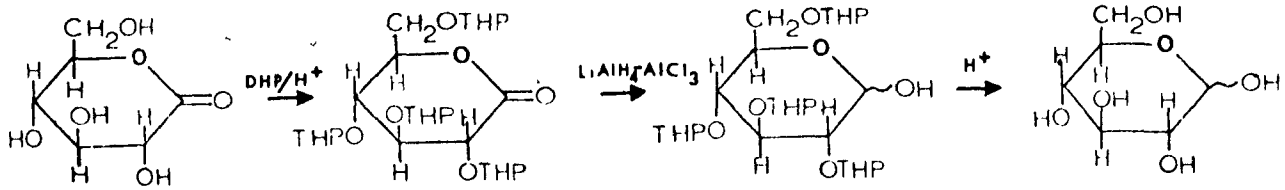
b.)



However, because the rate of these cleavage reactions is considerably slower than the rate of reduction of carbonyl functions (214), it was hoped that

under the right reaction conditions it would be possible to reduce aldonolactones to aldoses in good yield.

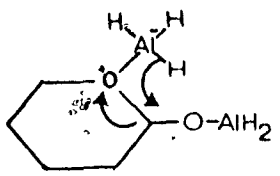
At first the reaction was attempted in a manner analogous to the borane reduction. The lactone was suspended in ether or THF, and this was followed by the addition of a mixture of $AlCl_3$ and $LiAlH_4$ in THF. Using this procedure the reaction rate was extremely slow due to the relative insolubility of the lactone in the solvent (a gel, characteristic of the borane reduction, does not form in this case). To circumvent this problem dihydropyran was used to block the free hydroxyl groups of the lactone: the resulting tetrahydropyranyl derivatives are hydrogenolyzed very slowly under the reaction conditions, but are readily removed by mild acid hydrolysis following reduction of the carbonyl function. Thus, for D-glucono-1,5-lactone:



Results obtained in these experiments were similar to those for the borane reduction; the reduction of D-glucono-1,5-lactone normally provided yields of glucose ranging from 80 to 90% whereas the reduction of D-mannono-1,4-lactone afforded yields of 60 to 70%. As before, the best method of isolation of mannose from the reaction mixture proved to be via the phenylhydrazone. Although reduction by means of the mixed hydride is experimentally somewhat complex, the yields obtained are better than by the standard reduction techniques, other than the previously discussed diborane reduction.

Both for D-glucono-1,5-lactone and D-mannono-1,4-lactone the best ratios of aldose to alditol were obtained when the molar ratio of $LiAlH_4$ to $AlCl_3$ was

1:3 and the poorest yields were obtained when a molar ratio of 3:1 was employed. In the former case the reducing species is $AlHCl_2$ whereas in the latter the preponderant species is AlH_3. Both of these readily reduce the carbonyl function, but the experiments indicate that ring opening is more extensive when the reducing species is AlH_3. If ring opening proceeds by a four center mechanism as in:

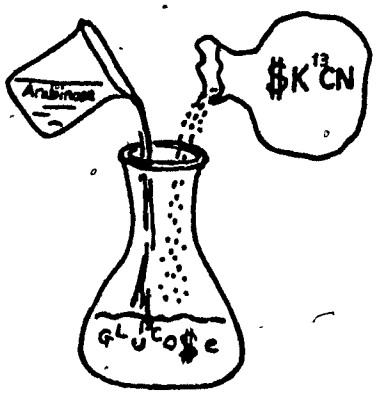


it is likely that the reason more diol is formed when AlH_3 is the reducing species can be traced to the fact that three hydrogens are available for the reaction as compared to one for $AlHCl_2$.

In summary, it can be stated that both of the reduction techniques developed for the conversion of aldonolactones to aldose sugars surpass or equal the existing methods in efficiency, and also for the diborane reduction in simplicity, and hence they can facilitate the synthesis of 1-^{13}C, (or 1-^{14}C) enriched carbohydrates by widening the classical bottleneck of the Fischer-Kiliani sugar synthesis, namely, the reduction of the aldonolactone to the aldose.

CHAPTER 6

EXPERIMENTAL



PART I

GENERAL METHODS

The natural abundance analogs of the compounds synthesized during the present research are known compounds. However, the synthetic methods used in many cases have had to be modified because in working with the ^{13}C-enriched species small scale experiments were mandatory. Such conditions were dictated by the high cost of the starting material, $K^{13}CN$ (Merck, Sharpe and Dohme, Montreal, Canada), which also necessitated numerous "dry runs" using non-enriched material before undertaking any synthesis involving ^{13}C enriched compounds.

Many of the compounds encountered here which are reported to be easily synthesized on a large scale proved to be far less accessible on a milligram scale, and when yields were critical. Generally, they have been isolated by crystallization, whereas in the current experiments it was usually necessary to employ column chromatography in order to obtain good yields. Identification of the ^{13}C enriched compounds was made by melting point, mass spectroscopy and most relevant to this study, by NMR spectroscopy. In each case, the spectrum of the enriched derivative was compared to the spectrum of the natural abundance analog. As an example of compound identification by mass spectroscopy, Figs. 1, 2 and 3 (Appendix) show the spectra of 1,2-O-isopropylidene-3,5,6-tri-O-acetyl-α-D-glucofuranose and its 1-^{13}C and 6-^{13}C derivatives, respectively. These spectra are seen to be identical except for the one m/e unit shift of some peaks due to the presence of the ^{13}C isotope. Such isotopic substitution can, in fact, be used to advantage in the analysis of fragmentation patterns and is discussed briefly in the Appendix.

Spectroscopy

Pmr spectra at 100 MHz were recorded with a Varian HA-100 spectrometer. Spectra at 220 MHz were recorded at the Canadian 220 MHz NMR Centre in Sheridan Park, Ontario. ^{13}C spectra were recorded with a Bruker WH90 spectrometer operating at 22.6 MHz.

Mass spectra were recorded with an LKB gas chromatograph mass spectrometer operating at 70 eV.

Chromatography

Thin layer chromatography plates were prepared using MN silica gel G as adsorbent. Benzene-ether (1:1, v/v) and benzene-methanol (9:1, v/v) were the solvents commonly used. Visualization was by spraying with sulfuric acid (5% solution v/v), and heating the sprayed plate at 120° in an oven.

Analytical paper chromatography was carried out by the descending technique on Whatman No. 1 paper using butanol-pyridine-acetic acid (10:3:3, v/v/v) as the solvent system. Spots were located with the aid of ammoniacal silver nitrate and alcoholic sodium hydroxide solutions (218).

Column chromatographic separations were carried out on columns packed with MN silica gel (grain size 0.08 mm) which had been previously washed with the solvent system being used.

Gas liquid chromatography was conducted with a Hewlett-Packard F & M 402 gas chromatograph using a silicone gum column (4% U.C.W.) on chromosorb W. Carbohydrates were analyzed either through their acetates (prepared with pyridine and acetic anhydride) or through their trimethylsilyl derivatives (prepared with pyridine, hexamethyldisilazane, and trimethylchlorosilane (219)).

Melting points

Melting points were determined with a Fisher-Johns hot plate apparatus and are uncorrected.

Acetylation

Small scale acetylations were carried out at 60° with pyridine-acetic anhydride. The compound to be acetylated (usually ~50 mg) was warmed with twenty drops of pyridine and ten drops of acetic anhydride for 1 hour. The solution was then transferred by means of a dropping pipette to a 10 ml separatory funnel containing 4 ml of ice-water. Extraction was carried out with chloroform (3 x 5 ml) and the combined extracts were washed successively with an ice-cold 3% sodium bicarbonate solution and ice-water before being dried over anhydrous sodium sulfate. The solution was concentrated and traces of pyridine were removed under high vacuum.

Evaporations

All evaporations were carried out under reduced pressure at 40°C.

PART II

PREPARATION OF 6 - ^{13}C CARBOHYDRATES

1,2:5,6-Di-O-isopropylidene-α-D-glucofuranose (1)

D-Glucose (200 g) was treated with anhydrous acetone in the presence of concentrated sulfuric acid according to known methods (220); yield, 173 g (60%); m.p. 108-110° (lit. 110° (220)).

1,2-O-Isopropylidene-α-D-glucofuranose (2)

Compound 1 (150 g) was dissolved in water and the pH of the solution was adjusted to 2 by the addition of HCl. After 4 hours at 40° the solution was neutralized with NaOH, filtered and concentrated to a crystalline material which was then recrystallized from ethanol (221); yield, 101 g (80%); m.p. 159-160° (lit. 161-162.5° (220)).

1,2-O-Isopropylidene-α-D-xylo-dialdopentofuranose (3)

To a stirred solution of 25 g of sodium metaperiodate in 250 ml of water was added 25 g of 2 in small portions, followed after 1 hr, by ethylene glycol to decompose excess periodate. The solution was concentrated and the residue was extracted with $CHCl_3$. The extract was then transferred onto a column of silica gel, and elution with ether resulted in dimeric 3 as the major fraction (yield, 50%); recrystallized from chloroform-petroleum ether, m.p. 171-172° (lit. 178-180° (221)).

Barium 1,2-O-isopropylidene-α-D-glucuronate-6-^{13}C (4)

This compound was prepared essentially according to the procedure of Isbell et al. (6). Twenty-five ml of an aqueous solution containing 1.1 g (5.0 mmole) of 3 was introduced into a 250 ml flask. To this was added a solution of 0.36 g (5.5 mmole) of $K^{13}CN$ followed by the addition of 0.2 g (5.0 mmole) of NaOH. Finally 20 ml of 1.0N acetic acid were added in

order to make the solution slightly acid. The resulting solution, now yellow, was stirred until it was homogenous and was then stored tightly stoppered for twenty days. (Trial experiments indicated that shorter reaction times resulted in lower yields and that heating the solution produced decomposition of the product.) After the 20 day reaction period the now enriched cyanohydrin was hydrolyzed in situ. (If this hydrolysis was not carried out carefully, the reaction mixture turned dark and there was much decomposition. Accordingly, partial hydrolysis was first carried out at low alkalinity and low temperature, then the pH and temperature were increased to complete the reaction.) The hydrolysis was carried out by adding 0.25 g of Na_2SO_3 to the reaction mixture and heating at 70° for two hours followed by the addition of 1.0 g of Na_2CO_3 and refluxing for two hours. The solution was then cooled with ice and passed through a column of cation exchange resin (Amberlite IR-120 (H)) at 10°, the effluent and washings being collected in a flask containing a well stirred solution of $Ba(OH)_2 \cdot 8H_2O$ (8.0 g). Excess of barium hydroxide was then neutralized with gaseous carbon dioxide and the $BaCO_3$ was removed by filtration. The filtrate was concentrated to 50 ml, again filtered, slowly concentrated further under a stream of air until crystals of barium 1,2-O-isopropylidene-α-D-glucuronate-6-^{13}C began to form and then stored at 10° for 24 hours (yield, 0.6 g). On concentration, the mother liquor yielded a further 0.1 g, and recrystallization from water-methanol (3:1) gave a final yield of 0.62 g of the ^{13}C enriched salt.

Calcium 1,2-O-isopropylidene-β-L-idofuranuronate-6-^{13}C (5)

To the mother liquor from the preparation of 4,700 ml of ethanol was added to precipitate out barium acetate, the filtrate was concentrated, cooled with ice, and was passed through a column of cation exchange resin (Amberlite

IR-120 (H)) into a flask containing 0.3 g of $BaCO_3$. The effluent was lyophilized, and addition of methanol to an aqueous solution of the resulting syrup afforded 0.15 g more of 4. After filtration, the mother liquor was again passed through a cation exchange column with cooling into a flask containing 0.15 g of $CaCO_3$. Concentration of the solution in a stream of air yielded crystals of 5, which were recrystallized from methanol-water (5:1); yield, 0.25 g.

1,2-O-Isopropylidene-α-D-glucofuranurono-6,3-lactone-6-^{13}C (6)

A solution of 0.5 g of 4 was passed through a column of cold cation exchange resin and the effluent was lyophilized to produce syrupy 1,2-O-isopropylidene-α-D-glucuronic acid-6-^{13}C. This residue was dissolved in acetone, toluene (50 ml) was added and the solution was slowly evaporated to produce crystals of 6; yield, 0.23 g (80%); m.p. 115-120° (lit. 119-120° (222)).

1,2-O-Isopropylidene-β-L-idofuranurono-6,3-lactone-6-^{13}C (7)

1,2-O-Isopropylidene-β-L-iduronic acid (100 mg) was lactonized in a manner analogous to the gluco epimer above; yield, 80 mg (87%), m.p. 133-136° (lit. 137-138° (223)).

1,2-O-Isopropylidene-5-O-acetyl-α-D-glucofuranurono-6,3-lactone-6-^{13}C (8) and 1,2-O-Isopropylidene-5-O-acetyl-β-L-idofuranurono-6,3-lactone-6-^{13}C (9)

A solution consisting of 25 mg of 6 or 7 in pyridine (0.5 ml) and acetic anhydride (0.5 ml) was heated at 60° for 1 hour, and then transferred to a seperatory funnel containing 4 ml of H_2O and 4 ml of $CHCl_3$. After partitioning, the $CHCl_3$ layer was washed with ice-water and the aqueous layer was further extracted with $CHCl_3$. The combined $CHCl_3$ extracts were washed with ice-cold sodium bicarbonate (3%) solution and dried over anhydrous Na_2SO_4. Concentration of the $CHCl_3$ solution then produced syrupy 8 or 9; yield, 20 mg

(70%). These compounds were used to study solvent effects on $^{2}J_{C-H}$ as reported in Chapter 2.

1,2-O-Isopropylidene-α-D-glucofuranose-6-^{13}C (10)

1,2-O-Isopropylidene-α-D-glucofuranurono-6,3-lactone-6-^{13}C (0.25 g) was dissolved in methanol (5 ml). With constant stirring, $NaBH_4$ (100 mg) was added in small portions and stirring was continued for an additional 30 minutes, excess of cation exchange resin (Amberlite IR-120 (H)) was then introduced, the resin was filtered off, and the filtrate was concentrated. Methanol was added to the residue and then distilled off, this procedure being repeated several times to remove boric acid as methyl borate. The product (10) crystallized and was recrystallized from hot ethyl acetate; yield, 230 mg (93%); m.p. 159-160°, (lit. 161-162.5° (221)).

1,2-O-Isopropylidene-β-L-idofuranose-6-^{13}C (11)

This compound was prepared from 8 (50 mg) by a method analogous to the preparation of the gluco epimer above; yield, 42 mg (85%); m.p. 110-112° (lit. 113-114° (223)).

1,2-O-Isopropylidene-α-D-glucofuranose-6-^{13}C-6-d_2 (12)

This compound was prepared by the same procedure as used for the preparation of 10, except that $NaBD_4$ instead of $NaBH_4$ was used for the reduction.

1,2-O-Isopropylidene-3,5,6-tri-O-acetyl-α-D-glucofuranose-6-^{13}C-6-d_2 (13)

Acetylation of 12 (25 mg) was carried out according to the procedure described under "General Methods"; yield, 30 mg (80%).

1,2-O-Isopropylidene-3,5,6-tri-O-orthoformyl-α-D-glucofuranose-6-^{13}C (14)

1,2-O-Isopropylidene-α-D-glucofuranose-6-^{13}C (100 mg) (10) was dissolved by heating in 1 ml of triethyl orthoformate. To this solution were then added 2 drops of a 1% solution of HCl in anhydrous methanol (prepared by adding acetyl

chloride to anhydrous methanol) and the solution was allowed to cool. After a short time crystals of 14 formed and were recovered by filtration; yield, 80 mg (78%); m.p. 198-201° (lit. 200-201° (224)).

1,2-O-Isopropylidene-α-D-glucofuranose-6-^{13}C periodate complex (15)

This complex was prepared by the procedure of Perlin and von Rudloff (182). 1,2-O-Isopropylidene-α-D-glucofuranose-6-^{13}C (20 mg) was dissolved in D_2O (0.5 ml) and 20 mg of anhydrous potassium carbonate and 20 mg of sodium metaperiodate were added. The pmr spectrum of the resulting complex was immediately recorded.

1,2:5,6-Di-O-isopropylidene-α-D-glucofuranose-6-^{13}C (16)

1,2-O-Isopropylidene-α-D-glucofuranose-6-^{13}C (100 mg) was suspended in anhydrous acetone (50 ml), conc. sulfuric acid (20 drops) was introduced, the resulting solution was stirred for 5 hours under anhydrous conditions, and neutralized with anhydrous Na_2CO_3. The precipitated Na_2SO_4 was filtered off, thoroughly washed with acetone, the filtrate was concentrated to about 1 ml and then transferred to a column (30 cm x 1.5 cm) of silica gel. Using benzene:ether (1:1) as the eluting solvent, 16 emerged from the column as the first major fraction; yield, 72 mg (60%); m.p. 106-108° (lit. 110° (220)). Using acetone as the next eluant, about 30 mg of 1,2-O-isopropylidene-α-D-glucofuranose-6-^{13}C were recovered from the column.

D-Glucose-6-^{13}C (17)

1,2-O-Isopropylidene-α-D-glucofuranose-6-^{13}C (100 mg) was dissolved in 5 ml H_2O. Cation exchange resin (Amberlite IR-120 (H), 500 mg) was then added and the solution was stirred at 90° for 1 hour. The ion exchange resin was then filtered off, thoroughly washed with water, and the filtrate was concentrated to give syrupy D-glucose-6-^{13}C; yield, 80 mg (97%).

1,6-Anhydro-α-L-idopyranose-6-^{13}C (18)

1,2-O-Isopropylidene-β-L-idofuranose-6-^{13}C (100 mg) was treated with cation exchange resin as above for the preparation of D-glucose-6-^{13}C, leading to preponderant formation of the 1,6-anhydro derivative (18). Conversion of the syrupy residue to 18 was promoted by subjecting it to a high vacuum. Yield of the final syrupy product was 70 mg (95%).

2,3,4-Tri-O-acetyl-1,6-anhydro-α-L-idopyranose-6-^{13}C (19)

Compound 18 (70 mg) was acetylated as described under "General Methods" to give syrupy 19; yield, 80 mg (92%).

1,2,3,4,6-Penta-O-acetyl-β-D-glucopyranose-6-^{13}C (20)

Acetylation of D-glucose with sodium acetate catalyst yields the peracetylated β-anomer (225). D-Glucose-6-^{13}C (100 mg) was suspended in acetic anhydride (2 ml) containing NaOAc (100 mg), the mixture was stirred at 90° for 18 hours, after which most of the acetic anhydride was evaporated off under reduced pressure. The residue was partitioned between $CHCl_3$ and H_2O and the aqueous layer was extracted with further portions of $CHCl_3$. The chloroform layer was washed with ice-cold sodium bicarbonate solution (3%), then ice-water and dried over anhydrous Na_2SO_4. The pmr spectrum of the first syrupy product showed the presence of only the β-anomer. Crystallization from 95% ethanol gave 20; yield, 150 mg (68%); m.p. 127-130°, (lit. 132° (225)).

1,2,3,4-6-Penta-O-acetyl-α-D-glucopyranose-6-^{13}C (21)

Conversion of 20 to the α-anomer can be effected by treatment with perchloric acid (226, 227). To acetic anhydride (0.35 ml) in a 1 ml flask at 40°, perchloric acid (0.005 ml) was added followed by the slow addition of 1,2,3,4,6-penta-O-acetyl-β-D-glucopyranose-6-^{13}C (100 mg). The solution was stirred for 30 minutes, diluted with ice-water (5 ml), extracted with

chloroform, and the chloroform layer was washed with ice-cold sodium bicarbonate (3%), then ice-water, dried over anhydrous Na_2SO_4 and concentrated. The residue was crystallized from 95% ethanol; yield, 80 mg (80%); m.p. 107-110° (lit. 112-113° (226)).

Methyl-α-D-glucopyranoside-6-^{13}C (22)

A solution of D-glucose-6-^{13}C (100 mg) in anhydrous methanolic hydrogen chloride (3 ml, 1%) was refluxed under anhydrous conditions for 1 hour. Neutralization was carried out with lead carbonate, the solution was filtered, the filtrate was concentrated, and the residue was crystallized from warm ethanol, affording 22; yield, 55 mg (51%); m.p. 164-167° (lit. 167-169° (228)).

Methyl 4,6-O-benzylidene-α-D-glucopyranoside-6-^{13}C (23)

The procedure employed was a modified form of the method of Freudenberg et al. (229). To methyl α-D-glucopyranoside-6-^{13}C (100 mg), benzaldehyde (2 ml) (which had been purified by washing with aqueous sodium carbonate to remove benzoic acid and subsequent distillation under reduced pressure) was added followed by 400 mg of $ZnCl_2$. The resulting mixture was shaken vigorously at room temperature for 4 hours, and poured into 5 ml of ice-water, which was then quickly extracted with several portions of chloroform. The chloroform extract was washed with ice-cold sodium bicarbonate (3%), then ice-water, dried over anhydrous Na_2SO_4, and concentrated, the residue being transferred to a column of silica gel. Using toluene-ether (1:1) as eluant, 23 was obtained in pure form; yield, 55 mg (36%); m.p. 158-161° (lit. 161-163° (229)).

PART III

PREPARATION OF 1-^{13}C CARBOHYDRATES

Barium-D-gluconate-1-^{13}C (24)

The procedure used is a modified form of the method of Isbell et al. (6a) developed for ^{14}C enrichment. To a solution of K^{13}CN (0.26 g) and NaOH (0.16 g) in water (20 ml), D-arabinose (0.60 g) dissolved in sodium carbonate (0.2 M, 20 ml) was added and the solution was stirred at room temperature for 48 hours. Hydrolysis of the resulting cyanohydrin was carried out in situ by heating at 60° in a current of air for twelve hours, and the residual solution was passed through a column of cation exchange resin (Amberlite-IR 120 (H)). Neutralization of the effluent was effected with barium hydroxide with stirring for 1 hour, excess of barium was then removed with gaseous carbon dioxide, the solution was concentrated to 3 ml, and methanol was added to the point of turbidity. Crystallization of barium gluconate-1-^{13}C proceeded over a period of 7 days at 10°; the resulting crystals were washed with cold water and methanol; yield, 300 mg.

D-Glucono-1,5-lactone-1-^{13}C (25)

Barium D-gluconate-1-^{13}C (500 mg) was passed through a column of cation exchange resin (Amberlite IR-120 (H)) and the effluent and washings were lyophilized. The residue was dissolved in methyl cellosolve, and the solvent was slowly evaporated in the presence of seed crystals of D-glucono-1,5-lactone leaving a partially crystalline mass, which upon storage in a desiccator for 7 days completely crystallized. A solution of the crystals in hot methyl cellosolve was decolorized with charcoal, and on cooling afforded D-glucono-1,5-lactone-1-^{13}C in a total yield of 75%.

D-Mannono-1,4-lactone-1-^{13}C (26)

The mother liquor from the preparation of barium D-gluconate-1-^{13}C

was passed through a column of cation exchange resin (Amberlite-IR 120 (H)), the effluent and washings were concentrated and the residue, moistened with isopropanol and seeded with D-mannono-1,4-lactone, was stored in a dessicator. Crystallization of the lactone proceeded for 14 days and recrystallization from isopropanol afforded 26; yield, 200 mg; m.p. 143-147° (lit. 148° (230)).

D-Glucose-1-^{13}C (27)

a) Reduction of D-glucono-1,5-lactone-1-^{13}C with $LiAlH_4$-$AlCl_3$ (231)

D-Glucono-1,5-lactone-1-^{13}C (100 mg) was dissolved in dry N,N-dimethylformamide (2 ml), 3,4-dihydropyran (2 ml) and p-toluensulfonic acid (10 mg) in N,N-dimethylformamide (1 ml), were then added, and the reaction mixture was stirred for 1 hour at room temperature. Ice water (5 ml) was introduced followed by thorough extraction with chloroform, and the chloroform extract was washed with ice water, and dried over anhydrous sodium sulfate and evaporated to a syrup. The latter was dissolved in anhydrous ether (10 ml). Meanwhile, $LiAlH_4$ (23 mg) and $AlCl_3$ (240 mg) were each suspended in ether (10 ml) in separate flasks, the $AlCl_3$ suspension was then poured into the $LiAlH_4$ suspension and the combined mixture was quickly transferred to the ether solution of the lactone. Following a 1 hour period of heating under reflux with efficient stirring, the reaction mixture was carefully decomposed with cold water and the resulting gelatinous mixture was extracted with chloroform. (It was found necessary to use especially large volumes of chloroform for this extraction in order to minimize emulsification.) The combined extracts were washed with water and were concentrated, water (30 ml) and cation exchange resin (Amberlite-IR 120 (H), 1 g) were then added to the syrupy product, and the mixture was heated on a steam bath for 1 hour. A second extraction with chloroform was carried out after removal of the ion exchange resin, and the aqueous

solution was concentrated to give a syrupy product, which by g.l.c. analysis was 95% glucose. The reaction product was acetylated as described above and, following column chromatography on silica gel using benzene-ether (6:4) as eluant, yielded 1,2,3,4,6-penta-O-acetyl-α,β-D-glucopyranose-1-^{13}C. De-O-acetylation was carried out with 0.1M sodium methoxide in methanol, cation exchange resin (Amberlite-IR (H)) was added, the solution filtered, and the filtrate concentrated to pure D-glucose-1-^{13}C; yield, 90 mg (90%). (It should be added that when the reaction is carried out on a larger scale purification through the acetate is not necessary; D-glucose can be crystallized from the syrupy reaction product using methanol-isopropanol (6:1) as solvent.)

b) Reduction of D-glucono-1,5-lactone-1-^{13}C by BH_3 in tetrahydrofuran

The procedure described here is typical of all reductions carried out by the use of BH_3 in THF. D-Glucono-1,5-lactone (100 mg) was suspended in THF (8 ml) and a 1 M solution of BH_3 in THF (4 ml), (available from Alfa Products, Beverly, Mass.) was introduced, anhydrous conditions being maintained. (The addition of BH_3 to the reaction mixture had to be carried out cautiously as hydrogen gas was evolved.) The reaction mixture was stirred at room temperature for 3 hours (after 15 minutes it becomes gelatinous), although experiments indicated that the stirring may not be essential. Water was then slowly introduced (again care had to be exercised on account of the vigorous effervescence which occurred). After the addition of water, the solution, which was now homogeneous, was concentrated at reduced pressure, and the residue was treated several times with methanol in order to remove boric acid as volatile methyl borate. The syrupy product was seen to be 95% D-glucose-1-^{13}C by g.l.c. analysis; where necessary, it was further purified through the acetate derivative as described above.

D-Mannose-1-^{13}C (28)

a) Reduction of D-mannono-1,4-lactone-1-^{13}C by $LiAlH_4$-$AlCl_3$

The procedure was analogous to the one described above for D-glucono-1,5-lactone. G.l.c. analysis of the syrupy product showed the presence of about 30% D-mannitol along with D-mannose. This product was dissolved in a minimum amount of water. A suspension of phenylhydrazine hydrochloride (100 mg) and sodium acetate (80 mg) in water (2 ml) was filtered through a cotton plug into the sugar-polyol solution, the reaction mixture was heated at 90° for 30 minutes and was then stored at 3°C for 12 hours. The hydrazone (232, 233) was recovered by filtration, washed with water, ethanol and ether; yield, 100 mg (this would imply about 65-70% of mannose in the reaction mixture, which is commensurate with g.l.c. analysis and also indicates that formation of the phenylhydrazone is practically quantitative) m.p. 196-200° (lit. 199-200° (232)).

D-Mannose phenylhydrazone-1-^{13}C (100 mg) was suspended in benzaldehyde (2 ml) and water (1 ml), the mixture was heated with stirring on the steam bath for 15 minutes, diluted with water and then extracted with chloroform. Following passage through a column of decolorizing charcoal and celite, the chloroform extract was concentrated to a colorless syrup which by g.l.c. analysis was essentially pure D-mannose-1-^{13}C; yield, 60-65 mg, corresponding to an isolated yield of about 65% D-mannose-1-^{13}C from the reduction of D-mannono-1,4-lactone-1-^{13}C.

b) Reduction of D-mannono-1,4-lactone-1-^{13}C by BH_3 in tetrahydrofuran

The method followed was analogous to that described above for D-glucono-1,5-lactone-1-^{13}C. Isolation of D-mannose-1-^{13}C was again through its phenylhydrazone derivative. Overall isolated yields of D-mannose-1-^{13}C based on D-mannono-1,4-lactone-1-^{13}C ranged from 60 to 70%.

1,2,3,4,6-Penta-O-acetyl-β-glucopyranose-1-^{13}C (29).

Prepared by treating D-glucose-1-^{13}C (50 mg) with acetic anhydride and sodium acetate as described for the preparation of 20; yield, 105 mg (71%); m.p. 128-130° (lit. 132° (225)).

1,2,3,4,6-Penta-O-acetyl-α-D-glucopyranose-1-^{13}C (30)

1,2,3,4,6-Penta-O-acetyl-β-D-glucopyranose-1-^{13}C (100 mg) was converted into 30 by use of perchloric acid as described for the preparation of 21; yield, 60 mg (60%); m.p. 106-108°(lit. 112-113° (226)).

1,2,3,4,6-Penta-O-acetyl-α-D-mannopyranose-1-^{13}C (31) and
1,2,3,4,6-Penta-O-acetyl-β-D-mannopyranose-1-^{13}C (32)

D-Mannose-1-^{13}C (50 mg) when treated with acetic anhydride and pyridine as described above, yields both anomeric acetates which could not be separated by chromatography nor by fractional crystallization; yield, 110 mg (72%).

2,3,4,6-Tetra-O-acetyl-α-D-glucopyranosyl bromide-1-^{13}C (33)

This compound was prepared by known procedures (234). 1,2,3,4,6-Penta-O-acetyl-β-D-glucopyranose-1-^{13}C (50 mg) was treated with a solution of HBr in acetic acid (2 ml) at 0°. Thin layer chromatographic analysis of the reaction mixture showed that after 1 hour the reaction was essentially complete. The solution was then poured into ice water (4 ml) and rapidly extracted with chloroform, the extract was washed with bicarbonate (3%) solution, then water, dried over anhydrous Na_2SO_4, passed through a column composed of layers of celite, decolorizing charcoal and silica gel, and concentrated. An almost colorless syrup of 33 was obtained; yield, 35 mg (65%).

1,5-Anhydro-D-glucitol-1-^{13}C-1-d (34)

To a stirred solution of 33 (35 mg) in anhydrous ether (2 ml), $LiAlD_4$ (20 ml) was added, and 1 hour later the reaction was arrested by the addition of first acetone, and then water. The etheral layer was decanted off, the aqueous

mixture centrifuged and the supernatant liquid was neutralized with Amberlite-IR 120 (H$^+$) resin and was concentrated to syrupy 34 ; yield, 20 mg (72%).

2,3,4,6-Tetra-O-acetyl-α-D-mannopyranosyl bromide-1-^{13}C (35)

This compound was prepared from the mixture of 31 and 32 (100 mg) in a manner analogous to the preparation of 33 from 29; yield, 50 mg (51%).

3,4,6-Tri-O-acetyl-β-D-mannose 1,2-(methyl orthoacetate)-1-^{13}C (36)

This compound was prepared by the method of Mazurek and Perlin (235). To a solution of 2,3,4,6-Tetra-O-acetyl-α-D-mannopyranosyl bromide-1-^{13}C (50 mg) in chloroform (2 ml) was added 2,6-lutidine (0.3 ml) in absolute methanol (2 ml). After 18 hours at room temperature, chloroform (5 ml) was introduced and the solution was washed with ice-cold sodium bicarbonate (3%), the latter being extracted with further portions of chloroform. The combined extracts were washed with ice-water, dried over anhydrous sodium sulfate, passed through a column consisting of layers of decolorizing charcoal and silica gel, and concentrated to give syrupy 36; yield, 20 mg (53%).

Methyl-α-D-glucopyranoside-1-^{13}C (37)

Prepared from D-glucose-1-^{13}C (100 mg) by the method used to prepare 22 from D-glucose-6-^{13}C ; yield, 60 mg (55%); m.p. 163-166° (lit. 167-169° (228)).

Methyl 4,6-O-benzylidene-α-D-glucopyranoside-1-^{13}C (38)

Prepared from 37 (60 mg) by the method used to prepare 23 from 22; yield, 40 mg (34%); m.p. 158-160° (lit. 161-163° (229)).

1,2:5,6-Di-O-isopropylidene-α-D-glucofuranose-1-^{13}C (39)

A suspension of syrupy D-glucose-1-^{13}C (100 mg) in anhydrous acetone (50 ml) containing concentrated sulfuric acid was shaken vigorously for 18 hours. Thin layer chromatographic analysis of the reaction mixture after that time showed that the major fraction was 39. Neutralization was carried out

with anhydrous Na_2CO_3, the precipitated Na_2SO_4 was filtered off and washed with acetone, the filtrate was concentrated to about 1 ml and transferred to a column of silica gel. Using benzene-ether (1:1) as the eluting solvent, 39 emerged from the column as the major fraction, yield, 70 mg (60%); m.p. 106-107° (lit. 110° (220)).

1,2:5,6-Di-O-isopropylidene-3-O-acetyl-α-D-glucofuranose-1-^{13}C (40)

Compound 39 (25 mg) was acetylated as described under "General Methods"; yield, 25 mg (83%).

1,2:5,6-Di-O-isopropylidene-α-D-ribohexofuranose-3-ulose-1-^{13}C (41) and

1,2:5,6-Di-O-isopropylidene-α-D-ribohexofuranose-3-ulose-1-^{13}C (monohydrate) (42)

These compounds were prepared according to the procedure of Jones et al. (236). 1,2:5,6-Di-O-isopropylidene-α-D-glucofuranose-1-^{13}C (100 mg) was dissolved in ethanol-free chloroform (3 ml), water (2 ml), potassium periodate (90 mg), potassium carbonate (10 mg) and ruthenium dioxide (20 mg) were introduced, and the reaction mixture was stirred for 3 hours. An excess of isopropanol was then added and the reaction mixture was extracted several times with chloroform (RuO_2 remains in the aqueous layer). The combined extracts were washed with water, dried over anhydrous Na_2SO_4 and concentrated to a syrup which by pmr was shown to be a mixture of 41 and 42; yield, 90 mg (90%).

1,2:5,6-Di-O-isopropylidene-α-D-allofuranose-1-^{13}C (43)

An excess of sodium borohydride was added to a cold methanolic solution of 41 and 42 (90 mg), the reaction mixture was stirred at 0° for 2 hours, then diluted with water, and extracted with ethyl acetate. The combined extracts were dried over anhydrous Na_2SO_4 and concentrated to a syrup; yield, 80 mg. (88%). Crystallization of 43 was effected from benzene-hexane (4:1); yield, 60 mg (67%); m.p. 74-77° (lit. 77-78° (237)).

1,2:5,6-Di-O-isopropylidene-3-O-acetyl-α-D-allofuranose-1-^{13}C (44)

Acetylation of 43 (20 mg) was carried out as described under "General Methods"; yield, 20 mg (83%).

β-D-Allopyranose-1-^{13}C (45)

1,2:5,6-Di-O-isopropylidene-α-D-allofuranose-1-^{13}C (60 mg) was dissolved in water (10 ml), cation exchange resin (Amberlite-IR 120 (H), 500 mg) was added, the mixture was stirred at 90° for 3 hours, then filtered, and the filtrate was concentrated to a syrup; yield, 40 mg (95%).

1,2,3,4,6-Penta-O-acetyl-β-D-allopyranose-1-^{13}C (46)

Compound 45 (20 mg) was acetylated as described above yielding a syrupy product, 46; yield, 41 mg (70%).

1,2-O-Isopropylidene-α-D-glucofuranose-1-^{13}C (47)

A solution of 1,2:5,6-di-O-isopropylidene-α-D-glucofuranose-1-^{13}C (100 mg) in dilute HCl (4 ml, pH2) was heated at 40° for 4 hours, then neutralized with anion exchange resin (Rexyn 203(OH)). The resin was filtered off, the filtrate was concentrated, and the residue was crystallized from ethyl acetate, affording 47; yield, 60 mg (71%); m.p. 157-159° (lit. 161-162.5° (221)).

1,2-O-Isopropylidene-3,5,6-tri-O-acetyl-α-D-glucofuranose-1-^{13}C (48)

Compound 47 (20 mg) was acetylated as described above; yield, 25 mg (80%).

1,2-O-Isopropylidene-3,5,6-tri-O-orthoformyl-α-D-glucofuranose-1-^{13}C (49)

Compound 47 (40 mg) was treated with triethyl orthoformate as described for the preparation of 14 from 10; yield, 30 mg (71%); m.p. 197-200° (lit. 200-201° (224)).

2,3:5,6-di-O-isopropylidene-α-D-mannofuranose-1-^{13}C (50)

Syrupy D-mannose-1-^{13}C (100 mg), anhydrous acetone (50 ml) and conc. sulfuric acid (20 drops) were shaken together for 18 hours; t.l.c. analysis

of the reaction mixture then showed the presence of only one major component (50). After neutralization with anhydrous Na_2CO_3 and removal of the precipitated Na_2SO_4 the solution was concentrated to a thin syrup which was transferred to a column of silica gel. Elution with toluene-ether (6:2) yielded crystalline (50), yield, 65 mg, (50%); m.p. 116-119° (lit. 122-123° (238)).

2,3:5,6-Di-O-isopropylidene-1-O-acetyl-α-D-mannofuranose-1-^{13}C (51)

Compound 50 (20 mg) was acetylated as described above to give syrupy 51; yield, 20 mg (83%).

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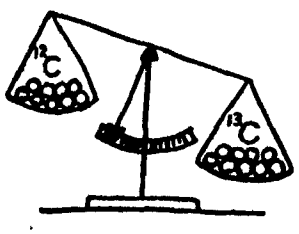
CLAIMS TO ORIGINAL RESEARCH

1. D-Glucose-1-^{13}C, D-mannose-1-^{13}C, D-glucose-6-^{13}C and L-idose-6-^{13}C, as well as derivatives of these compounds, have been synthesized. The proton magnetic resonance spectra of these compounds have been analyzed to yield values for internuclear coupling between the ^{13}C nuclei and directly bonded, geminal and vicinal protons.
2. Directly bonded coupling between ^{13}C and ^{1}H has been shown not to follow a straightforward relationship with percent "s" character. Various factors giving rise to differences in $^{1}J_{C-H}$ have been examined and it has been shown that steric, as well as adjacent lone pair effects, can have a substantial impact on the observed coupling.
3. An orientation dependence of $^{2}J_{C-H}$ has been demonstrated. The magnitude of $^{2}J_{C-H}$ is governed by the orientation of the coupling proton relative to the substituents on the coupling ^{13}C nucleus. Lone pair, hybridization, steric and substituent effects on $^{2}J_{C-H}$ have been investigated.
4. The signs of geminal coupling in β-D-allopyranose-1-^{13}C and in 1,2:5,6-di-O-isopropylidene-α-D-glucofuranose-1-^{13}C have been determined. Coupling between $^{13}C_1$ and H_2 in the former compound is -6.0 Hz, and in the latter +5.5 Hz. This difference delineates the importance of signs of coupling constants and demonstrates that orientation effects may give rise to large changes in $^{2}J_{C-H}$.
5. It has been shown that $^{3}J_{C-H}$ does not follow a straightforward dihedral angle dependence. The absolute value of the coupling between the ^{13}C nucleus and a proton separated by three bonds is decreased by electronegative substitution at the proton-bearing carbon when this substituent is <u>antiperiplanar</u> to a bond constituting part of the coupling pathway.

Problems inherent in deriving dihedral angles from $^{3}J_{C-H}$ have been emphasized. The roles of hybridization, lone pairs, substituent and steric effects as determinants of $^{3}J_{C-H}$ have been examined.

6. Two novel techniques for reducing aldonolactones to aldoses have been developed. D-Glucono-1,5-lactone and D-mannono-1,4-lactone have been reduced to D-glucose and D-mannose, respectively, in high yield, either with a 1 molar solution of BH_3 in THF or with a $LiAlH_4$-$AlCl_3$ mixed hydride reagent. Mechanistic aspects of these reductions have been discussed.
7. The usefulness of ^{13}C-labelling for elucidating mass spectral fragmentation patterns of carbohydrate derivatives has been demonstrated.

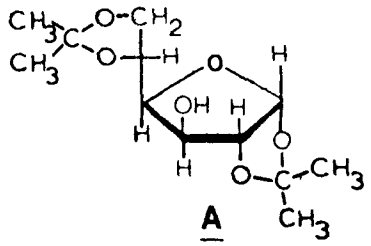
APPENDIX



MASS SPECTRA OF SOME ^{13}C ENRICHED CARBOHYDRATES

It was of interest to examine the mass spectra of some of the ^{13}C enriched carbohydrates made available in this study from the point of view of checking various features of fragmentation patterns. Deuterium is frequently used as an isotopic marker in this context (239-241) but ^{13}C may be more reliable because it is less likely to be involved in intramolecular migration.

O-Isopropylidene derivatives illustrate the potential of this approach. The mass spectrum (Fig. 4) of 1,2:5,6-di-O-isopropylidene-α-D-glucofuranose (A)

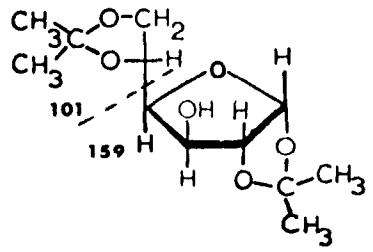


A

has been interpreted by Biemann and De Jongh (241). The major peaks are found at m/e 245, 187, 159, 131, 129, 127, 101, 59 and 43. The peak at m/e 245 represents the loss of a methyl group, whereas that at m/e 187 is due to the further loss of an O-isopropylidene group, as acetone. The peak at m/e 127 corresponds to a loss of CH_3 + CH_3COCH_3 + CH_3COOH, whereas those at m/e 59 and 43 are attributed to fragments from the O-isopropylidene moiety corresponding to C_3H_7O and C_2H_3O respectively.

Figures 5 and 6, which illustrate the mass spectra of 1,2:5,6-di-O-isopropylidene-α-D-glucofuranose-1-^{13}C (80% enriched) and -6-^{13}C (60% enriched), respectively, confirm the above assignments: both in the 1-^{13}C and 6-^{13}C derivatives the peaks at m/e 245, 187 and 127 now shift to 246, 188 and 128 (commensurate with the degree of enrichment) whereas the peaks at m/e 43 and 59

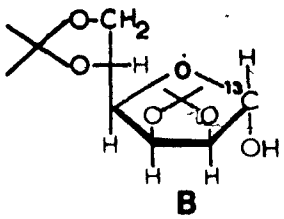
are unaffected. Cleavage of the C_4-C_5 bond is thought (241) to give rise to the peaks at m/e 101 and 159:



Accordingly, in the spectrum of the 6-^{13}C compound the corresponding peak is at m/e 102 whereas the m/e 159 peak remains; in contrast, the spectrum of the 1-^{13}C compound shows a shift from m/e 159 to 160 whereas the 101 peak is unaffected.

Peaks of m/e 129 and 131 probably represent a fragmentation by cleavage of the C_1-O_5 and C_3-C_4 bonds with either loss, or addition, of a hydrogen depending on the pathway (241). With the aid of Figures 5 and 6 these peaks can now be assigned. In the spectrum of the 6-^{13}C compound (Fig. 5) the peak at m/e 129 remains unaffected whereas there is now a peak at m/e 132 rather than at 131. In Figure 6, the opposite is noted, i.e., the m/e 129 peak is replaced by one at m/e 130, and the m/e 131 peak is unaffected. This means that the m/e 129 peak can now be assigned with confidence to the fragment containing C_1-C_2-C_3 and the m/e 131 peak to the fragment containing C_4-C_5-C_6.

Figures 7 and 8 represent the mass spectrum of 2,3:5,6-di-O-isopropylidene-α-D-mannofuranose (B) and is 1-^{13}C analog, respectively.



B

The fragmentation pattern is very similar to that observed for

1,2:5,6-di-O-isopropylidene-α-D-glucofuranose (Figs. 4-6) except for the peak at m/e 129, which is considerably more intense relative to the m/e 131 peak than in the glucose derivative. The former peak is again replaced by a peak at m/e 130 in the 1-^{13}C compound indicating that it is due to the fragment incorporating C_1, C_2 and C_3 as above. The ratio of the intensities of the peaks at m/e 128 and 131 implies that the C_1-C_2-C_3 fragment in the mannose derivative, bearing the isopropylidene function on C_2 and C_3, is more stable than the corresponding fragment of the glucose isomer in which this function is bonded to C_1 and C_2.

In general then, it is evident that incorporation of the ^{13}C nucleus into compounds can facilitate fragmentation assignments in mass spectroscopy and in turn lead to greater understanding of the fundamental nature of molecules.

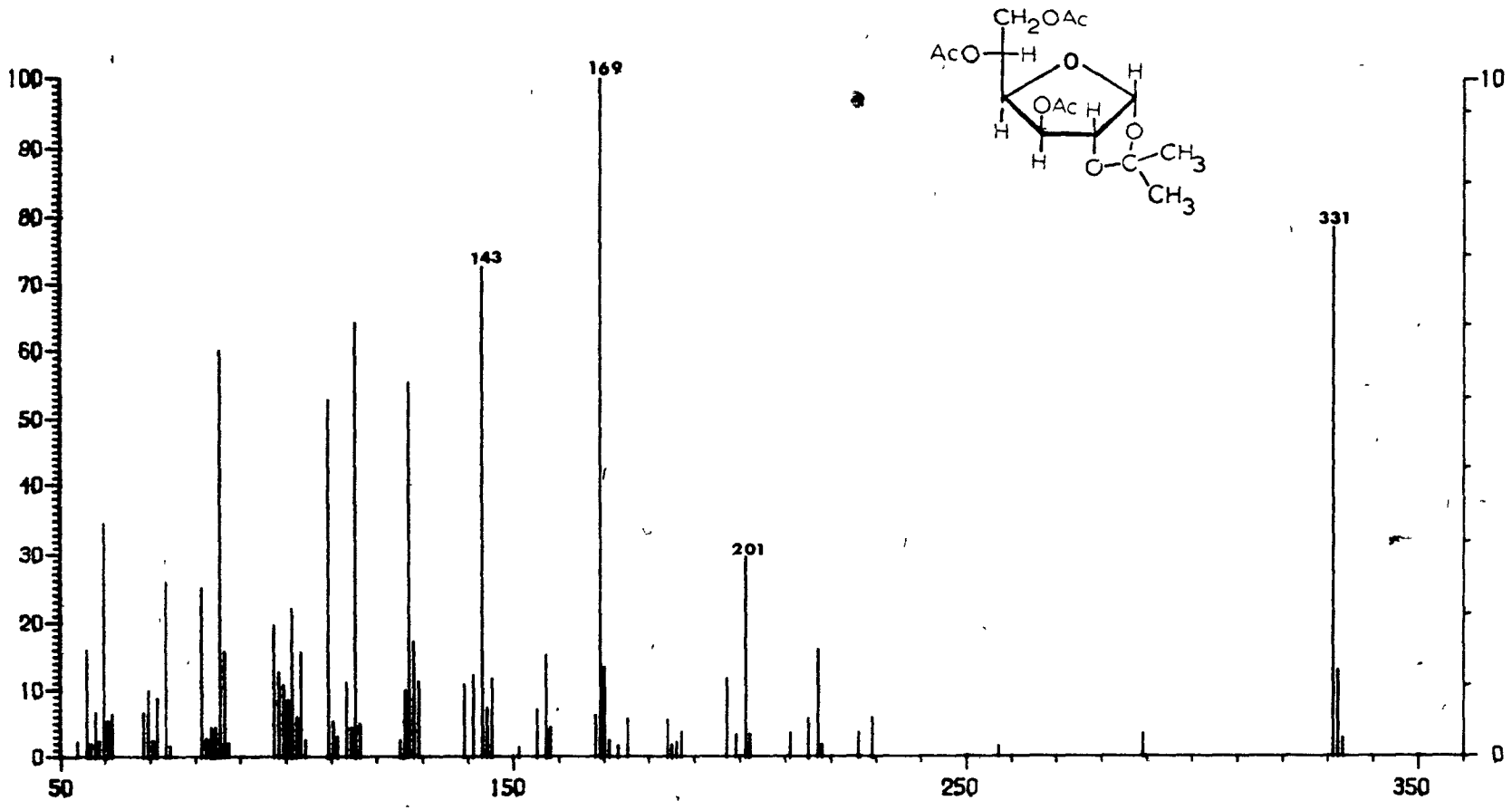


FIG. 1 Mass spectrum of 1,2-O-isopropylidene-3,5,6-tri-O-acetyl-α-D-glucofuranose.

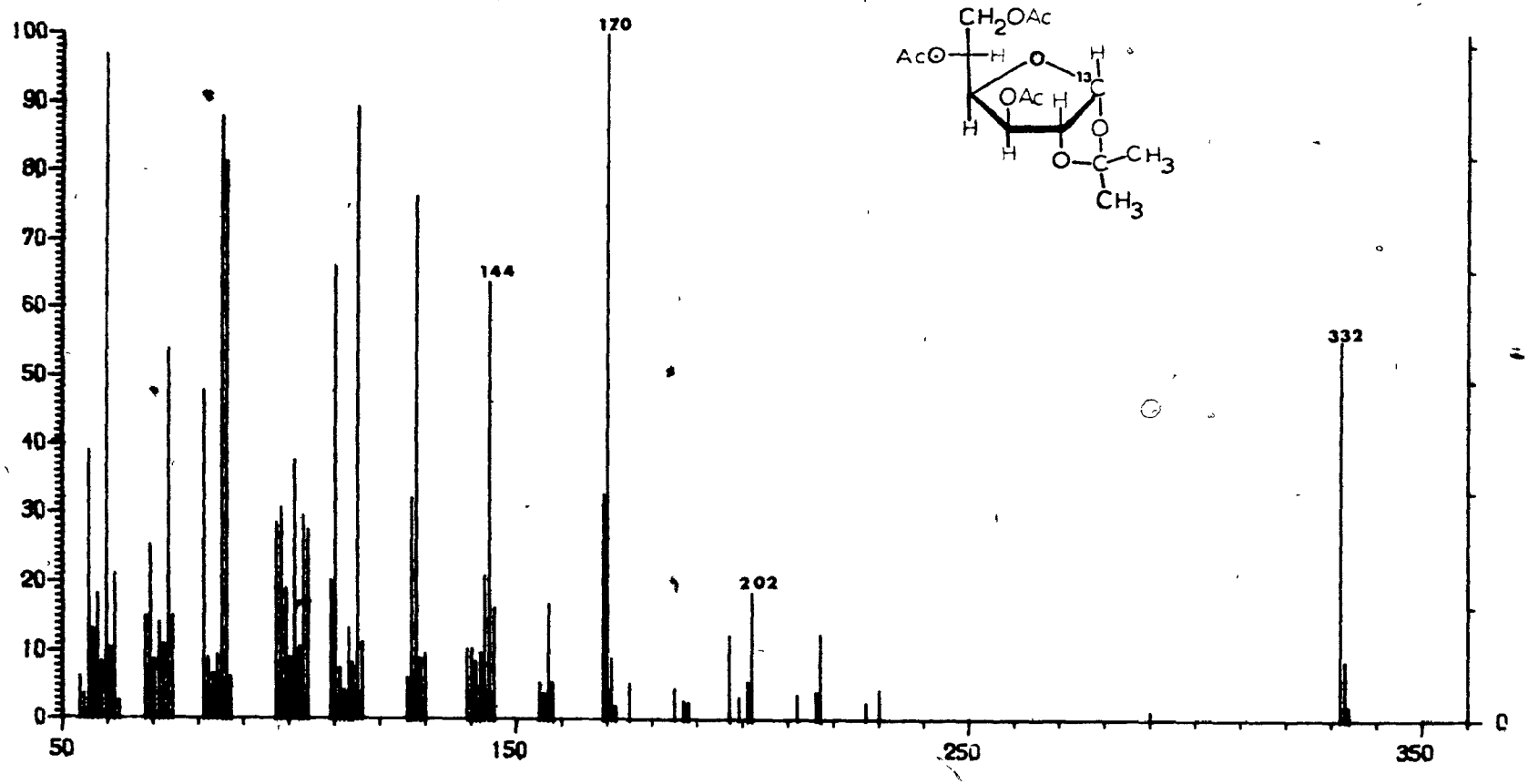


FIG. 2 Mass spectrum of 1,2-O-isopropylidene-3,5,6-tri-O-acetyl-α-D-glucofuranose-1-^{13}C (80%).

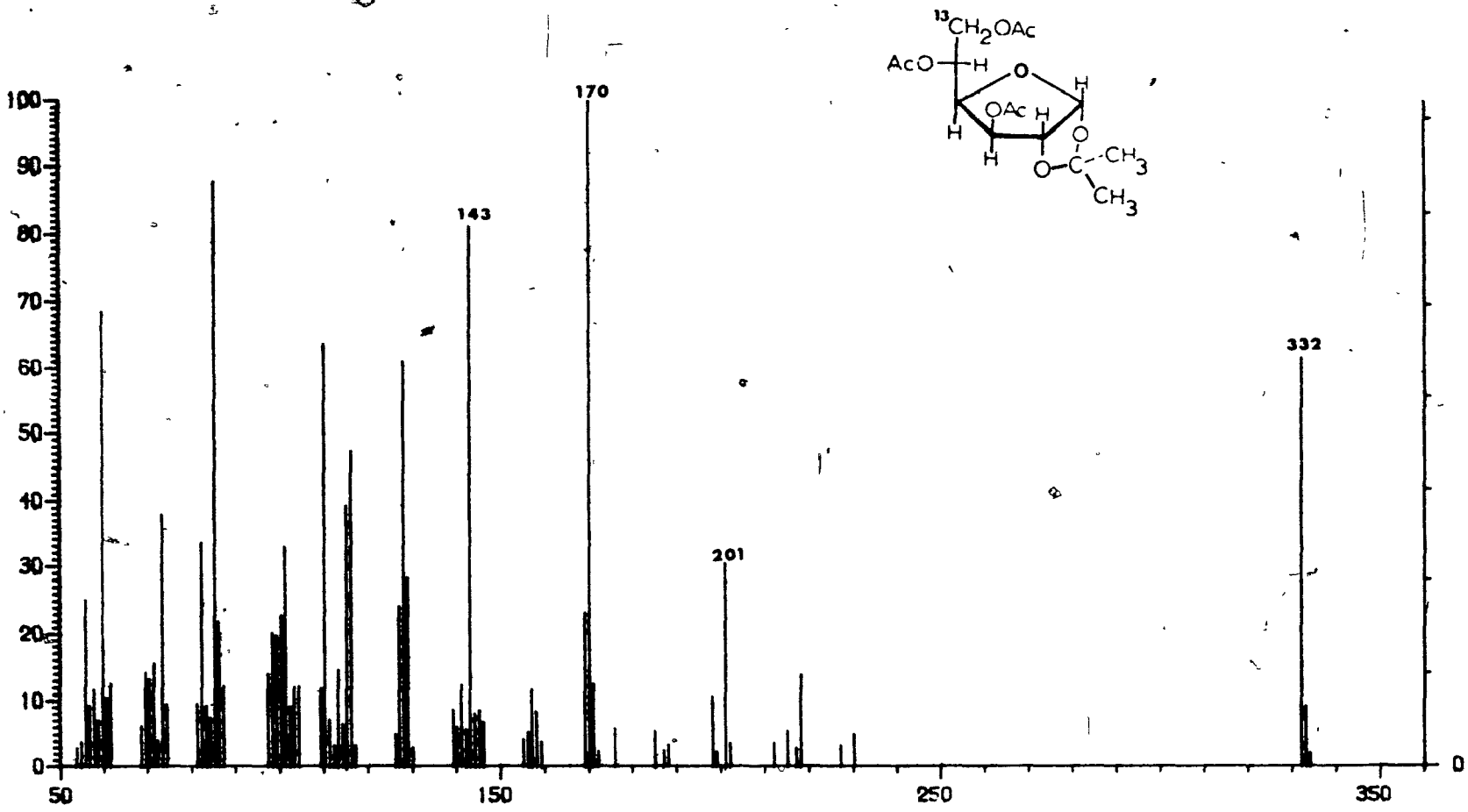


FIG. 3 Mass spectrum of 1,2-O-isopropylidene-3,5,6,-tri-O-acetyl-α-D-glucofuranose-6-^{13}C (80%).

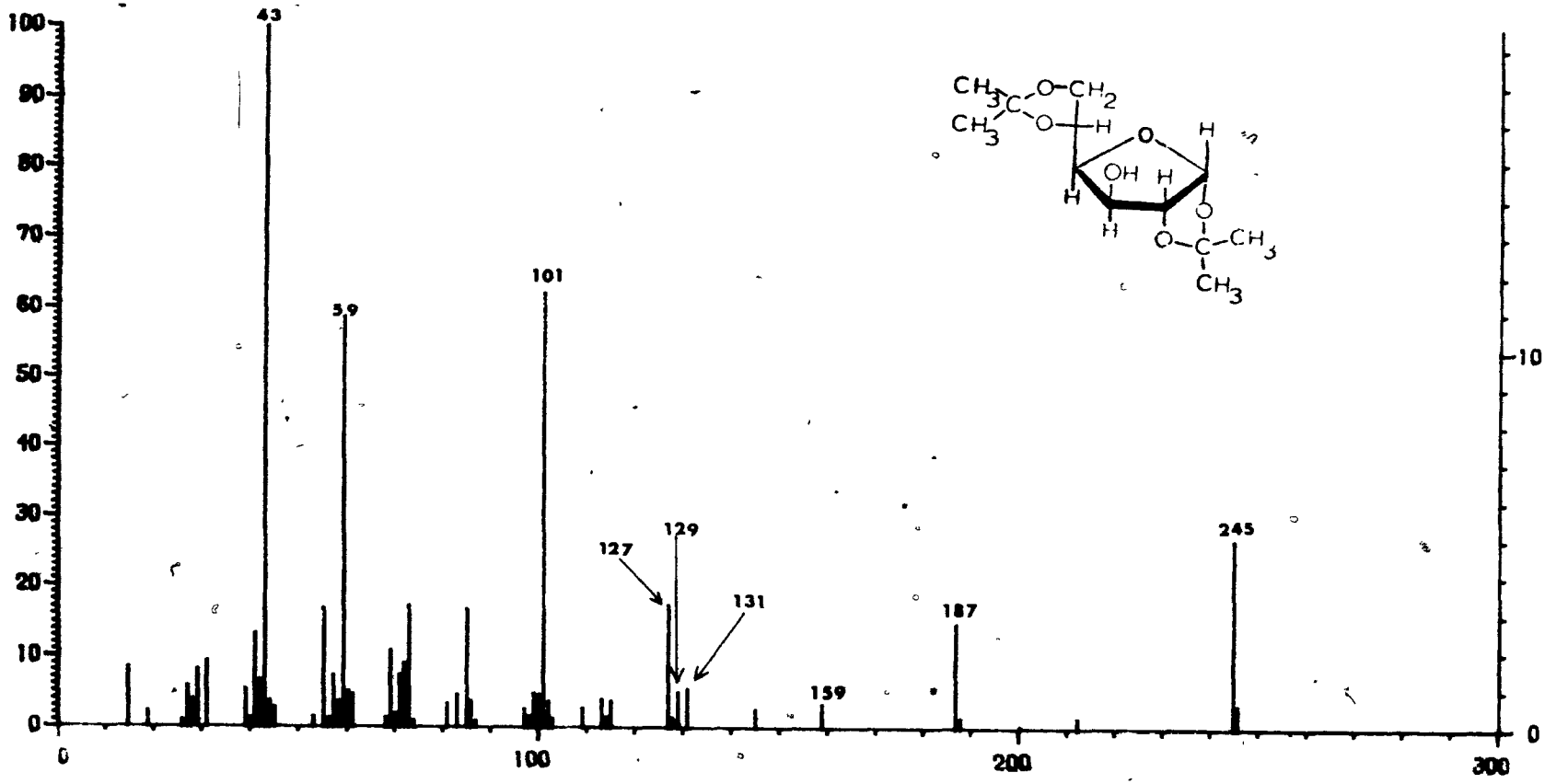


FIG. 4 Mass spectrum of 1,2:5,6-di-O-isopropylidene-α-D-glucofuranose.

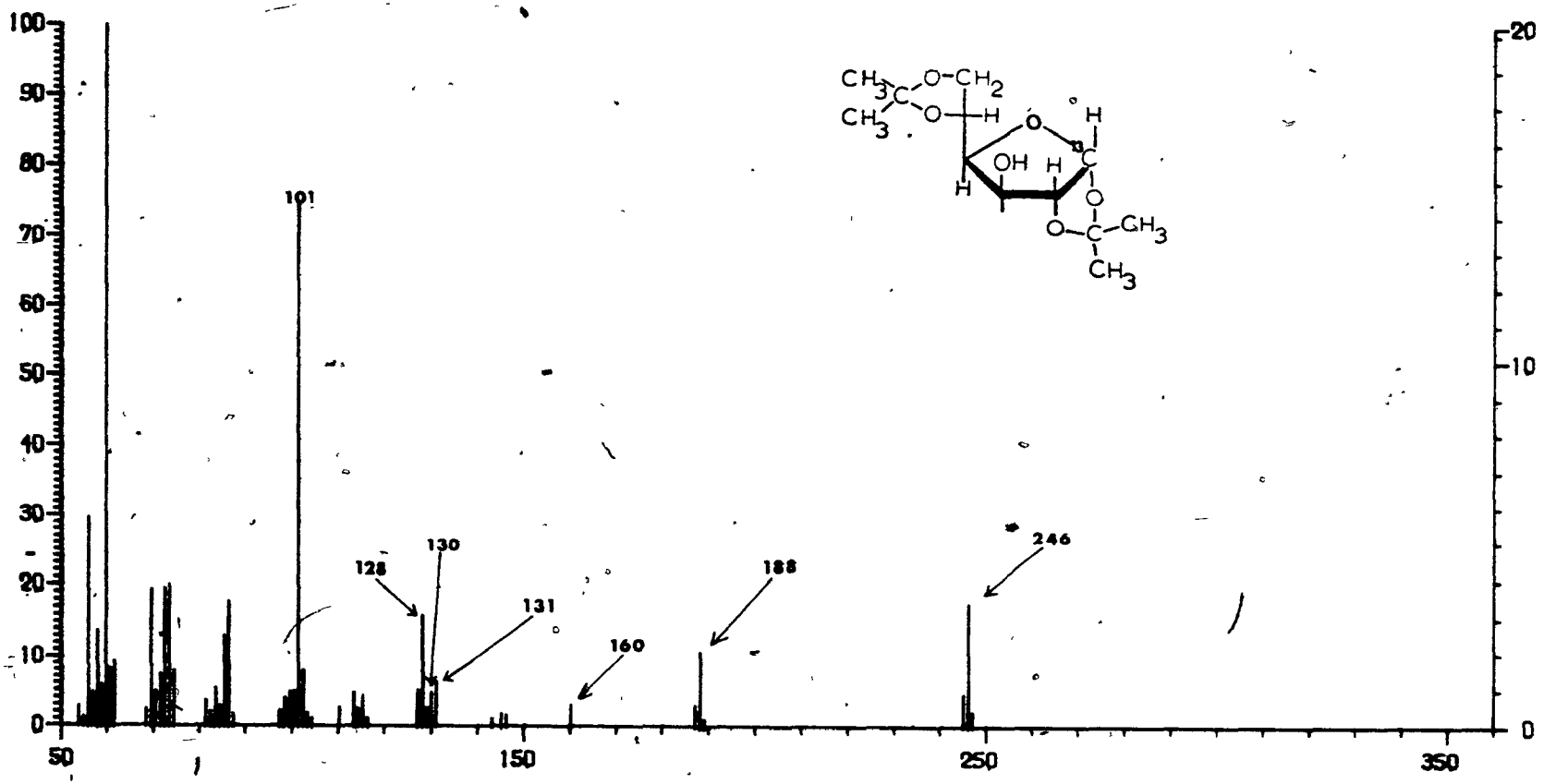


FIG. 5 Mass spectrum of 1,2:5,6-di-O-isopropylidene-α-D-glucofuranose-1-^{13}C (80%).

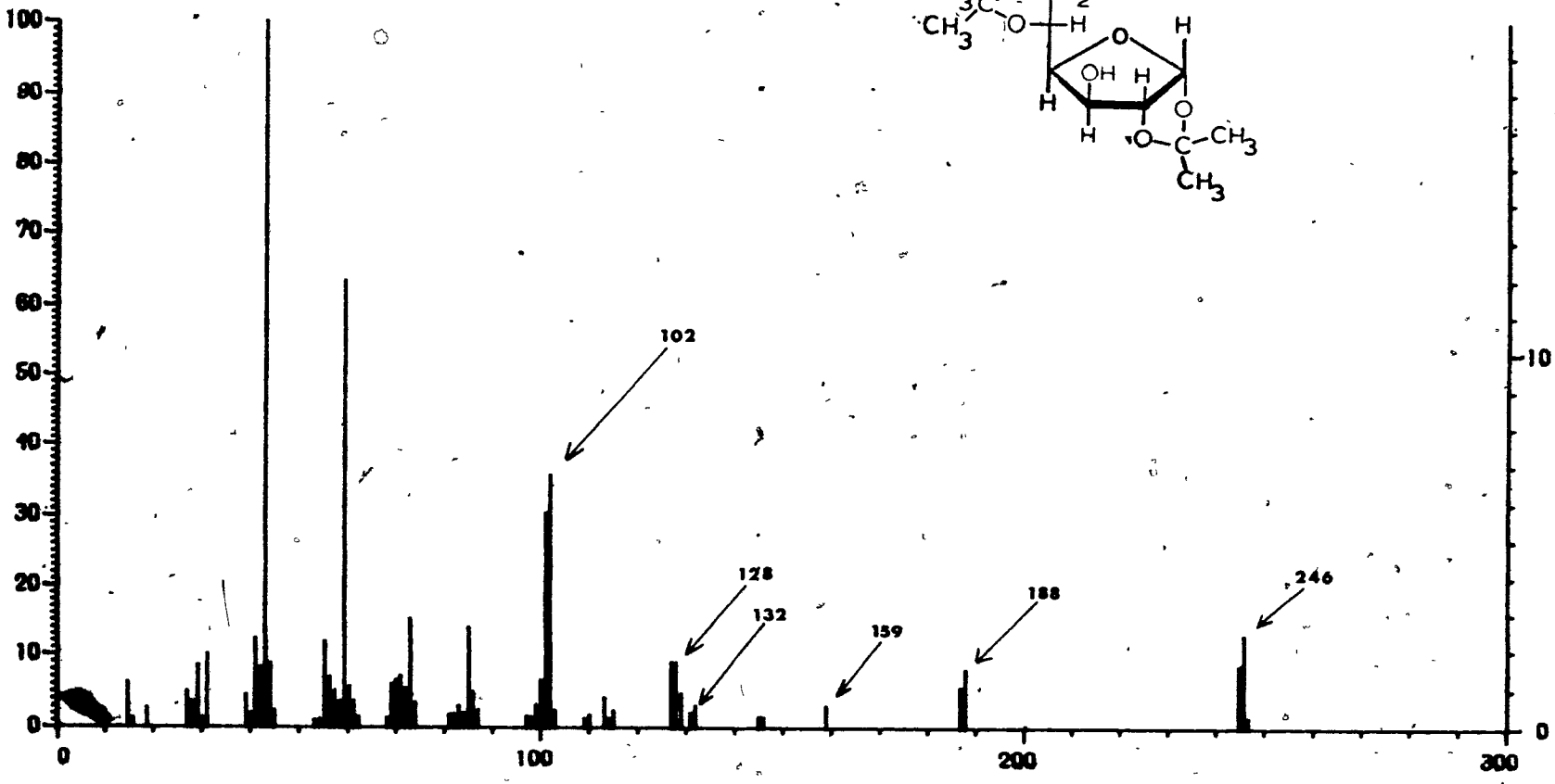


FIG. 6 Mass spectrum of 1,2:5,6-di-O-isopropylidene-α-D-glucofuranose-6-^{13}C (60%).

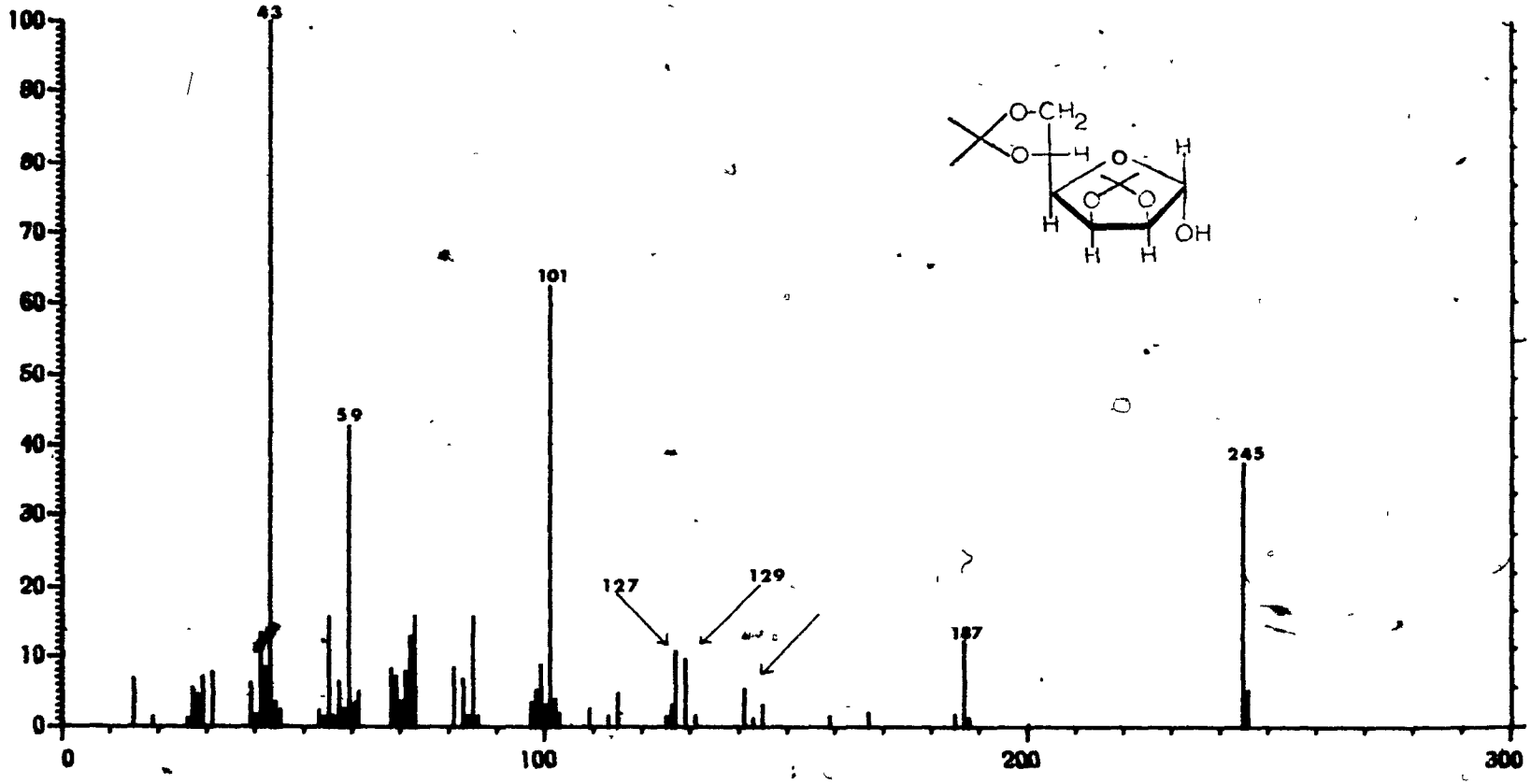


FIG. 7 Mass spectrum of 2,3:5,6-di-O-isopropylidene-α-D-mannofuranose.

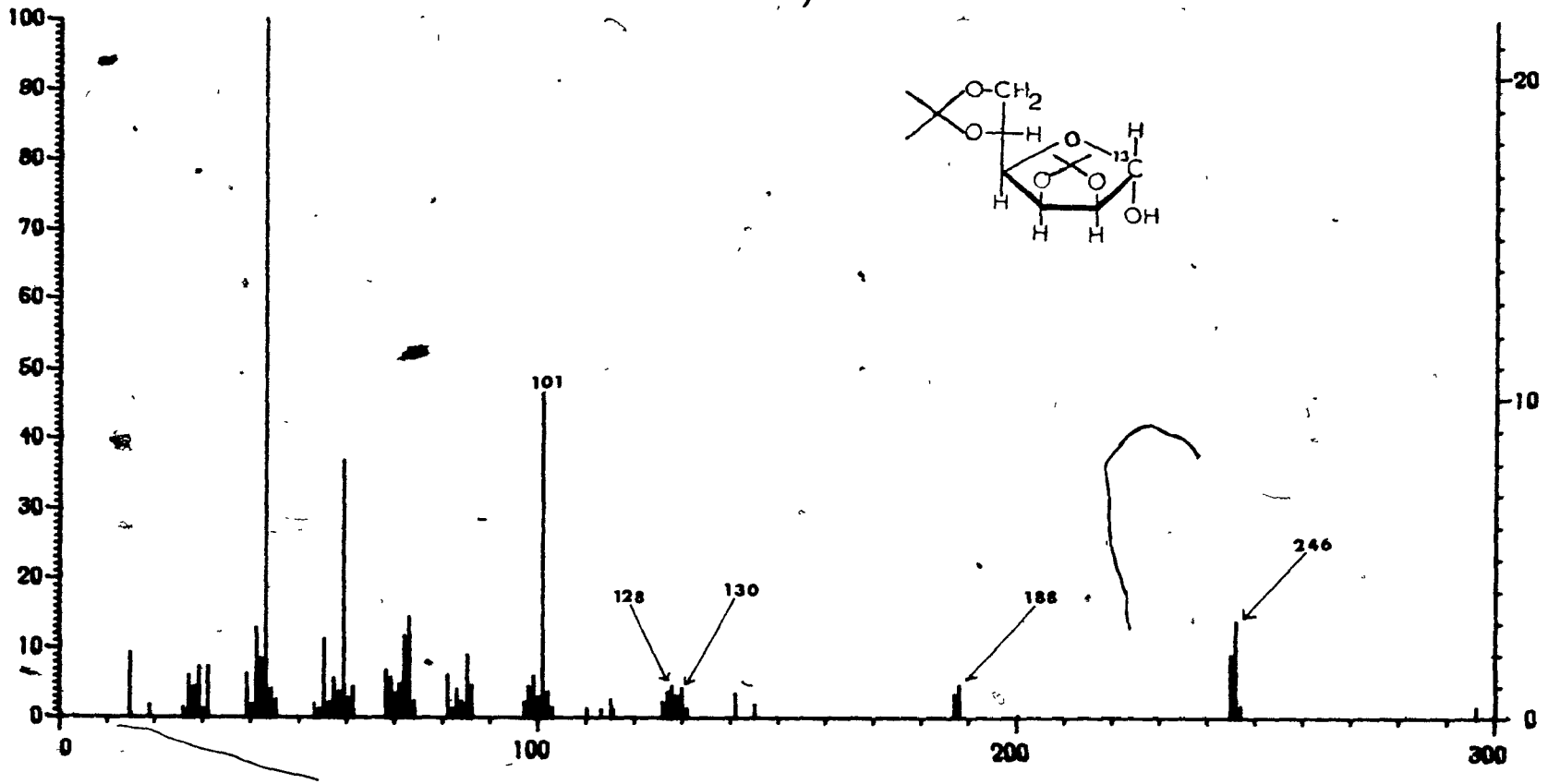


FIG. 8 Mass spectrum of ~~2,~~3:5,6-di-O-isopropylidene-α-D-mannofuranose-1-^{13}C (60%).