^{13}C MAGNETIC RESONANCE STUDIES OF CELLULOSE DERIVATIVES AND DISACCHARIDES

by

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^{13}CMR STUDIES OF CELLULOSE DERIVATIVES AND DISACCHARIDES

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RESUME

Cette étude a visé à évaluer les possibilités d'application de la r.m.n. du carbone-13 à la chimie de la cellulose. D'utiles renseignements, comme des estimations du degré de substitution et de la répartition des substituents ont pu être obtenus pour plusieurs dérivés de la cellulose.

Dans le cas des disaccharides et de leurs dérivés, il a été constaté que la conformation de la liaison glycosidique se reflète dans les couplages $^{3}J_{C-H}$ à cette liaison. Il est apparu que les molécules étudiées prennent une forme semblable à l'état solide et en solution et que les liaisons hydrogène entre unités n'ont que peu d'effet sur leur conformation.

Les limitations des mesures faites par r.m.n. sont dues au peu de solubilité des polymères examinés. Dans le cas des disaccharides l'interprétation des couplages vicinaux en termes de conformation est considérée comme valide surtout dans le cas de mesures comparatives obtenues sur des composés voisins.

La possibilité d'utiliser une route plus directe que celles utilisées habituellement pour la synthèse des 1,6-anhydro-disaccharides a aussi été étudiée.

ABSTRACT

This study was undertaken to evaluate the possibilities of c.m.r. spectroscopy in applications related to the chemistry of cellulose. It has been possible to obtain useful data for several cellulose derivatives, including direct estimations of their degree of substitution, as well as of the distribution of the substituents.

In the investigation of disaccharides and derivatives, the conformation of the glycosidic linkage was seen to be reflected in the $^{3}J_{C-H}$ across the bond, thus suggesting that the conformations are similar in the solid state and in solution. Inter-residue hydrogen bonding was also seen to have little effect on conformation.

The limits of measurements on the c.m.r. spectra have been found to originate in the low solubility of the polymers examined. As for disaccharides, the interpretation of vicinal couplings in terms of conformation was considered to be most valid when based on comparative measurements of line widths.

An attempt to devise an improved synthetic route to 1,6-anhydro disaccharides is described.

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∿	approximately
Me	CH_3-
Et	CH_3-CO-
A_c	CH_3-CO-
B_z	C_6H_5-CH_2-
Cell.	cellulose
CMC	O-carboxymethyl-cellulose
HEC	O-hydroxyethyl-cellulose
TMS	tetramethylsilane
n.m.r.	nuclear magnetic resonance
p.m.r.	proton magnetic resonance
^{13}C m.r. or c.m.r.	carbon-13 magnetic resonance
(P.) F T	(pulsed) Fourier transform
F.I.D.	free induction decay
R.F.	radio frequency
Hz	Hertz
MHz	Megahertz
C.A.T.	computer averaged transient
p.p.m. or δ	parts per million
J	coupling constant (Hz)
m.p.	melting point
t.l.c.	thin layer chromatography
g.l.c.	gas liquid chromatography
D.S.	degree of substitution
M.S.	molecular substitution
L.W.	line width (Hz) = width at half-height

CHAPTER I

INTRODUCTION

I.1. Cellulose and n.m.r. spectroscopy

Much progress has been achieved in the last few years in the study of biopolymers by nuclear magnetic resonance. This has been due to a large extent to improvements in instrumentation, and with them the introduction of ^{13}C magnetic resonance data.

Complex molecules like biopolymers, whether they are of animal origin (proteins, nucleic acids, mucopolysaccharides), found in the plant world (polysaccharides), or man made (cellulose derivatives) can be best approached through their monomeric components, and their various combinations (oligomers). This applies also to n.m.r. spectroscopy, because the spectra of model compounds greatly facilitate the characterization of the polymers themselves.

But a significant advantage of n.m.r., compared with classical chemical methods, is that it allows for direct observation of the polymer itself without, or sometimes with a little, degradation.

Owing to the recent development of this field of study, the literature available on ^{13}C m.r. spectroscopy of carbohydrates is not yet as extensive as that on proton magnetic resonance but at least two review articles (1,2) cover most publications until 1974. A more general approach to c.m.r. spectroscopy can be found in two recent textbooks (3,4).

The concern of the work presented here is the application of ^{13}C m.r. spectroscopy to cellulose derivatives, as well as to some relevant model compounds.

I.2. The ^{13}C m.r. experiment

Despite its low natural abundance (1.1%) the ^{13}C atom, which possesses a spin of $\frac{1}{2}$, can now be easily observed by n.m.r. spectroscopy. This first became possible with the use of the computer averaged transient (C.A.T.) technique, and, more recently and more conveniently, by means of the Fourier transform (FT) technique.

The latter involves irradiation of the sample by a series of short R.F. pulses which excite all the spins of the atoms considered (in the case of the ^{13}C atom the frequency applied is usually around 22-25 MHz). The resulting free induction decay (F.I.D.) signals are accumulated by a computer, which then performs the Fourier transformation of the signal, from the time domain to the conventional frequency domain. The time needed to obtain spectra depends mainly on the desired signal to noise ratio, which improves with $(W/L)^{\frac{1}{2}}$, where W is the spectrum width and L the line width (5).

The advantages of ^{13}C m.r. spectroscopy are manifold:

a) the ^{13}C chemical shifts cover a wide range of 200 p.p.m. (20 times that of protons), mostly downfield from the now widely accepted reference signal of T.M.S.

b) the ^{13}C signal assignments are facilitated by broad band complete proton decoupling (strong irradiation at the appropriate ^{1}H frequency) which makes the ^{13}C carbons appear as singlets. Indeed, their low natural abundance renders the coupling of two ^{13}C atoms (in the same molecule) highly improbable.

c) an interesting side effect of proton decoupling is the nuclear Overhauser enhancement (N.O.E.) (6). The increased population of the high energy spin states of the irradiated protons enhances the corresponding ^{13}C

signals through dipole-dipole interaction with geminal and vicinal protons.

d) off-resonance partial decoupling allows the advantage of the N.O.E. effect to be retained, while the ^{13}C-^{1}H couplings are seen, thereby helping in the signal assignments.

The lock signal, which stabilizes the field/frequency ratio, is usually provided by the ^{19}F signal of hexafluorobenzene, or, the ^{2}H signal of heavy water or deuteriobenzene. These can be used as solvents (internal lock) or in coaxial capillaries in the sample tube (external lock). T.M.S. is generally used as the reference signal, rather than CS_2, which was utilized earlier, and can be added to the sample tube. The $^{13}CS_2$ shift is -192 to -193 p.p.m. from T.M.S., depending on the solvent.

In such spectra, obtained by the FT technique in a relatively short time, the resolution is of the order of .1 p.p.m. and is limited mainly by the strength and stability of the lock signal.

Although integrated spectra are commonly obtained (7), they are usually not as satisfactory as those of protons. The relaxation times (T_1) of the various carbons are highly dependent on the position of these carbons in the molecule and the proximity of 1, 2, or 3 protons, as well as particular experimental conditions, and deeply influence the areas under each carbon signal (6). For consistent figures, therefore, it is best to restrict the peak area measurements to one class of carbon (primary, secondary or tertiary) in order to minimize the errors to less than approximately 5% (6).

I.3. ^{13}C n.m.r. of carbohydrates

I.3.1. Chemical shift data

The first studies of ^{13}C magnetic resonance in the field of carbohydrates, which date back to 1970 (8-11), have outlined the main features

of the spectra of these compounds:

a: between 55 and 62 p.p.m. one finds the carbons of primary alcohol groups.

b: between 70 to 80 p.p.m., appear the ring carbons, with a slightly higher shift for pyranose rings with equatorial substituents.

c: the anomeric carbons are found between 85 and 100 p.p.m., those bearing an equatorial hydroxyl being at lower field.

O-Alkyl substituents, whether they are on the anomeric carbon (11) or on the other ring carbons (12) have a deshielding effect ranging from 7 to 10 p.p.m.

Interestingly, this effect is slightly more pronounced with axial substituents than with equatorial ones (10), and is probably due to the increased steric crowding in the former case. On the other hand, neighbouring carbons are shielded by about 1 p.p.m., but this is true only for equatorial O-alkyl substituents (10). Anomeric O-phenyl or O-p-nitrophenyl groups have a lesser deshielding effect of about 6 p.p.m. (13). This can be explained by the fact that the electron density on the substituted carbons will be greater on the O-phenyl derivatives.

Carbonyl groups resonate at very low fields: from 170 p.p.m. for aldehydes to 190 p.p.m. for carboxylic acids (3,4).

O-Acetylation of ring hydroxyl groups has only a small effect (up to 1 p.p.m.) on the shift of the corresponding ring carbon. In general an upfield shift is associated with equatorial O-acetyl groups, and a downfield shift with axial ones (14).

What has been said so far applies as well to di- and oligosaccharides.

In the case of di-aldohexopyranoses, for example, although all 24 signals corresponding to the two anomeric forms are seen in D_2O, the overall spectrum can be easily sorted out: thus, the spectrum of cellobiose is very close to the superposition of those of methyl-β-D-glucopyranoside and that of D-glucose. Relative to D-glucose, C-4' (anomeric unit) and C-1 (non-reducing unit) appear to be deshielded by about 9 p.p.m. and 6 p.p.m. respectively (15). Comparable data have been obtained with maltose, lactose and their methyl glycosides.

It appears, therefore, that ^{13}C m.r. spectroscopy offers a very convenient means for establishing the position of the glycosidic linkage and its configuration. Indeed, such information has already been obtained or confirmed for numerous disaccharides (16,17) and their per-O-methylated derivatives (17a).

On the basis of results obtained with disaccharides, it can be expected that polysaccharides will be amenable to analysis by ^{13}C m.r. spectroscopy. In most cases, indeed, it should be possible to estimate the sequences of glycosidic linkages, their configuration and the amount of branching (18).

Despite the difficulties encountered in dealing with polymers, ^{13}C.m.r. spectroscopy is a particularly interesting technique for studying polysaccharides. Although their low solubility and the high viscosity of their solution (which makes for a weak internal lock signal) are problems, these drawbacks can be substantially overcome. Furthermore, the FT technique makes the acquisition of ^{13}C.m.r. spectra of polysaccharides a practical procedure in terms of the experimental time required.

I.3.2. ^{13}C-^{1}H couplings

As already mentioned, $J_{^{13}C-^{1}H}$ is the only type of coupling encountered in natural abundance ^{13}C m.r. spectra. One has to consider direct (^{1}J), geminal (^{2}J) or vicinal (^{3}J) couplings (i.e., across 1,2 or 3 bonds). This makes the appearance of proton coupled ^{13}C m.r. spectra generally complicated, and the problems inherent in selective decoupling make them difficult to examine. Still, since the directly bonded couplings dominate, one can assign doublets to CH groups, triplets to CH_2 groups and quartets to CH_3 groups.

The spacings due to ^{1}J are generally large, from 140 to 180 Hz (9,19). They have been found to be larger for anomeric centers, and even more so when the proton involved is axial.

Vicinal and geminal couplings are generally much smaller: 0-6 Hz in carbohydrates. ^{2}J is mainly affected by the orientation of substituents on carbon. If they are anti with respect to the proton considered, ^{2}J is close to zero. The gauche conformation, on the other hand, is generally associated with a large J value (9,19). However, some exceptions have been encountered, which prevent a generalization of these rules. For example, the presence of electronegative substituents on carbon reduces the extent of coupling.

The factors controlling ^{3}J have been also found to be mainly geometrical. From data accumulated for a large variety of carbohydrates a relationship similar to that originally found by Karplus (20) for vicinal J_{H-H} couplings has been derived. But, in the case of ^{13}C-X-X-^{1}H couplings (where X can be carbon or oxygen) other factors must be taken into account, which show that ^{3}J like ^{2}J is affected by electronegative substituents, as well as by the degree of hybridization of carbons on the coupling pathway (19). In broad terms, however,

and even though the angle values are not known with accuracy (in solution at least), large couplings (up to 6 Hz) can be associated with anti-conformations, while gauche ones give rise to little or no coupling.

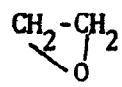
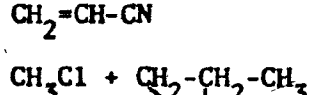
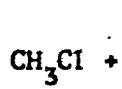
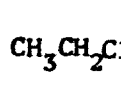
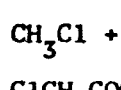
Vicinal proton couplings which also conform to the Karplus type of relationship, are much more extensively documented. Therefore, when the examination of $^{3}J_{C-H}$ and $^{3}J_{H-H}$ values lead to a similar conformational assignment, as has been found in several cases (19,21), this conformation is all the more probable.

It is on these grounds that the examination of a number of disaccharides and derivatives has been undertaken (cf Chapter 3). More precisely, the conformation of the glycosidic linkage has been examined through coupling constant measurements from their proton coupled ^{13}C m.r. spectra.

CHAPTER II

^{13}C.M.R. SPECTROSCOPY OF CELLULOSE DERIVATIVES

TABLE 1: Commercial cellulose ethers (22d)

Cellulose ether	Reagent	Solubility	D.S. range
Sodium carboxymethyl cellulose	$Cl\text{-}CH_2COOH$ (or -Na)	water	0.5-1.2
Methyl cellulose	$CH_3\text{-}Cl$	-	1.5-2.4
Ethyl cellulose	$CH_3\text{-}CH_2\text{-}Cl$	organic solvents	2.3-2.6
Hydroxyethyl cellulose		water	1.3-3
Cyanoethyl cellulose	$CH_2{=}CH\text{-}CN$	organic solvents	2.0
Hydroxypropyl-methyl cellulose	CH_3Cl + 	water	1.5-2
Hydroxyethyl-methyl cellulose	CH_3Cl + 	-	1.5-2
Ethyl-hydroxyethyl cellulose	CH_3CH_2Cl + 	-	1.4-1.6
Ethyl-methyl cellulose	$CH_3Cl + CH_3CH_2Cl$	-	1-1.3
Carboxymethyl-hydroxyethyl cellulose	$ClCH_2COOH$ (or -Na)	-	0.3-0.4
	+ 		0.3-0.7
Benzyl cellulose[a]	$C_6H_5CH_2Cl$		1.5-2

[a] manufactured in Europe only

II.1 Introduction. The cellulose ethers.

Etherification of the hydroxyl groups of cellulose yields a wide range of products whose properties and end uses can be varied to infinity. Most commercial cellulose ethers are synthesized by reacting alkali cellulose (cellulose treated with sodium hydroxide) with an etherifying agent (cf. Table 1).

Usually, as the degree of substitution (D.S.) increases, the solubility properties vary widely. As an example, methyl cellulose is soluble in water if D.S. < 2, and in organic solvents if D.S. > 2. Factors such as the distribution of substituents, temperature, pH, and the formation of gels greatly affect the solubility (22). Interestingly, the fact that methyl cellulose is less soluble in hot water than in cold water is explained by the fact that the methyl groups lessen the possibility of formation of hydrogen bonds between the hydroxyl groups and the water molecules, and the hydration complexes are then loosened by heat and molecular aggregates are formed.

In general, the colloidal properties of the water solutions of cellulose ethers make them useful to the food, pharmaceutical and plastics industries.

II.2 Necessity of the use of model compounds.

Although the basic chemistry of the formation of cellulose ethers is known, these compounds have mainly been characterized by chemical means such as the Zeisel method (alkoxyl group analysis) in the case of methyl and ethyl cellulose, carboxyl analysis (in the case of carboxymethyl cellulose) or other modifications of the Zeisel method in the case of benzyl and hydroxyethyl cellulose (22a). More commonly, viscosity measurements

provide a good means for routine testing of the commercial products.

By and large, however, no quantitative information is easily obtained about the distribution of the substituents on each anhydroglucose unit, and one has to go through hydrolysis and chromatographic examination of the hydrolyzates to obtain such data.

Several characteristics of ^{13}C m.r. spectra seem promising in this respect.

a) the wide range of ^{13}C chemical shifts generally makes for a better separation of signals than in p.m.r. spectra, and the identification of peaks is therefore easier.

b) the possibility of using the "proton decoupling" technique also facilitates the separation of signals by reducing the complexity of the spectra.

c) ^{13}C m.r. chemical shifts are very sensitive to stereochemical factors, and minor changes in structure and conformation can be detected.

d) the sensitivity of the instrument is mainly dependent on time and solutions of low concentration can be examined by simply increasing the number of F.I.D. signals accumulated.

It is with these ideas in mind that the examination of some cellulose ethers by ^{13}C m.r. spectroscopy was undertaken.

II.3 Carboxymethyl cellulose

This cellulose derivative was thought to be a priori the most amenable to ^{13}C m.r. analysis, because of its good solubility in water, which makes high concentrations relatively easy to attain. Furthermore, the low D.S. of the commercial product renders its structure somewhat close to that of the parent cellulose, in that most of its monomer units are nonsubstituted or monosubstituted anhydro glucoses.

In this study, the synthesis of mono-O-carboxymethyl-D-glucoses as model compounds was performed and their spectra recorded. The assignments of peaks of the parent polymer carbon atoms could then be made.

II.3.1 Synthesis of model compounds

The scheme followed for these syntheses was adapted from that of Timell (23) and is shown on Fig. 1. To make the 2-derivative, commercial D-glucose was treated with acetone in sulfuric acid to give 1,2-mono-O-isopropylidene-α-D glucofuranose (2) which was subsequently made to react with benzyl chloride in the presence of potassium hydroxide. The benzylated compound (3) was then heated with ion exchange resin to remove the isopropylidene group. Reaction of the product (4) with methanolic hydrochloric acid afforded the methyl glucoside (5). The sodio derivative, obtained from (5) by addition of sodium to its ethereal solution, was reacted with methyl bromoacetate to give the corresponding carboxymethyl ether at position 2 (6) which was isolated by column chromatography of the reaction product. De-benzylation was then effected by hydrogenolysis, and acid hydrolysis led to the anomeric mixture of α and β 2-O-carboxymethyl-D-glucoses (7). Their sodium salts, dissolved in D_2O, were used to obtain the ^{13}C m.r. spectrum.

The synthesis of the 3-derivative (Fig. 2) was carried out in the following way: 1,2:5,6-di-O-isopropylidene-α-D-glucofuranose (2) obtained by treatment of D-glucose with acetone in sulfuric acid, was treated with a sodium dispersion to make its sodio derivative. The latter was heated with methyl bromoacetate and yielded the 3-O-carboxymethyl derivative (9). Hydrolysis led to the corresponding mixture of α and β-anomers of the free 3-O-carboxymethyl-D-glucoses (10).

The 4-step synthesis of 6-O-carboxymethyl-D-glucose (Fig. 3)

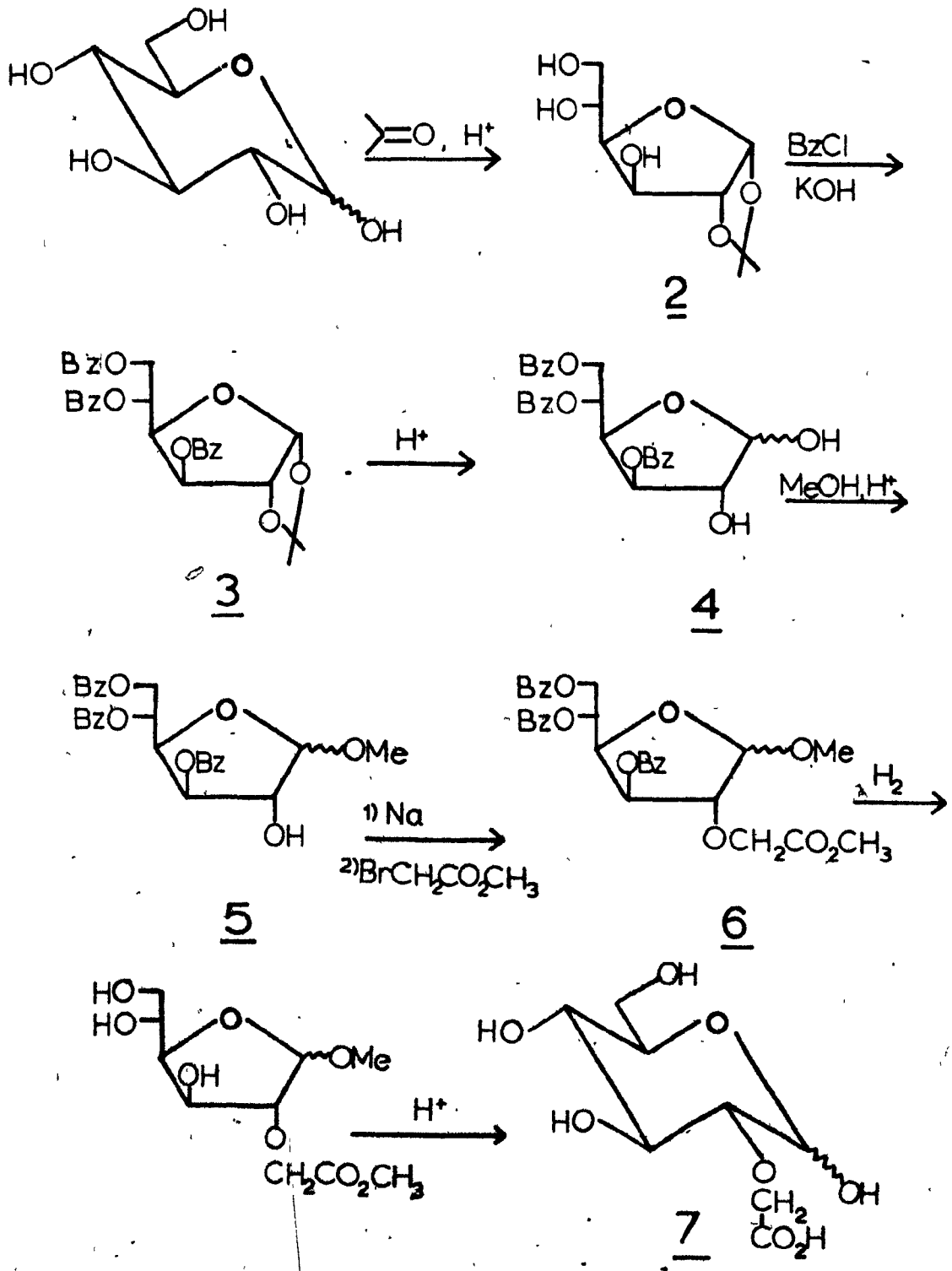


FIG. 1 Synthetic scheme for 2-O-carboxymethyl-D-glucose

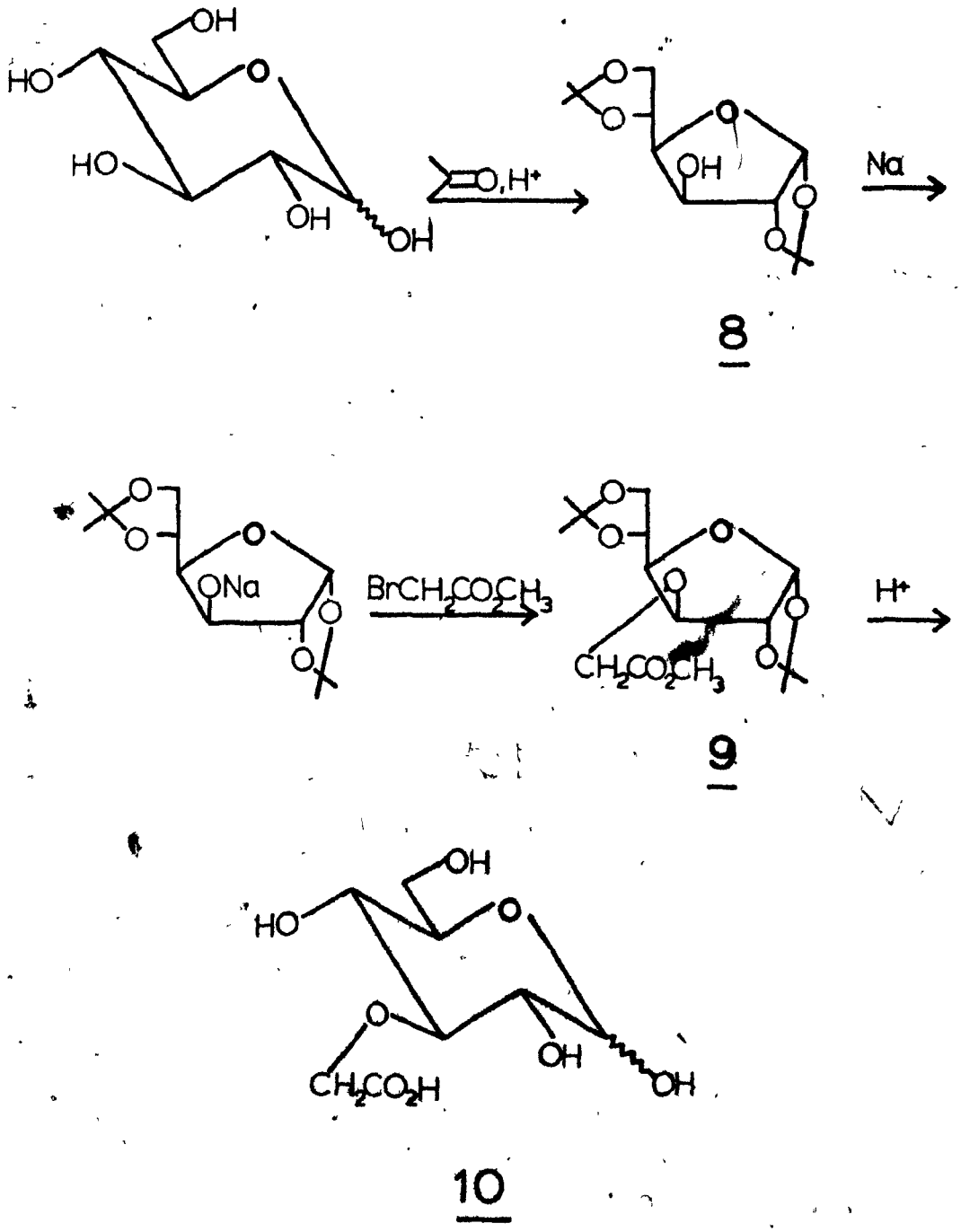


FIG. 2 Synthetic scheme for 3-O-carboxymethyl-D-glucose (10)

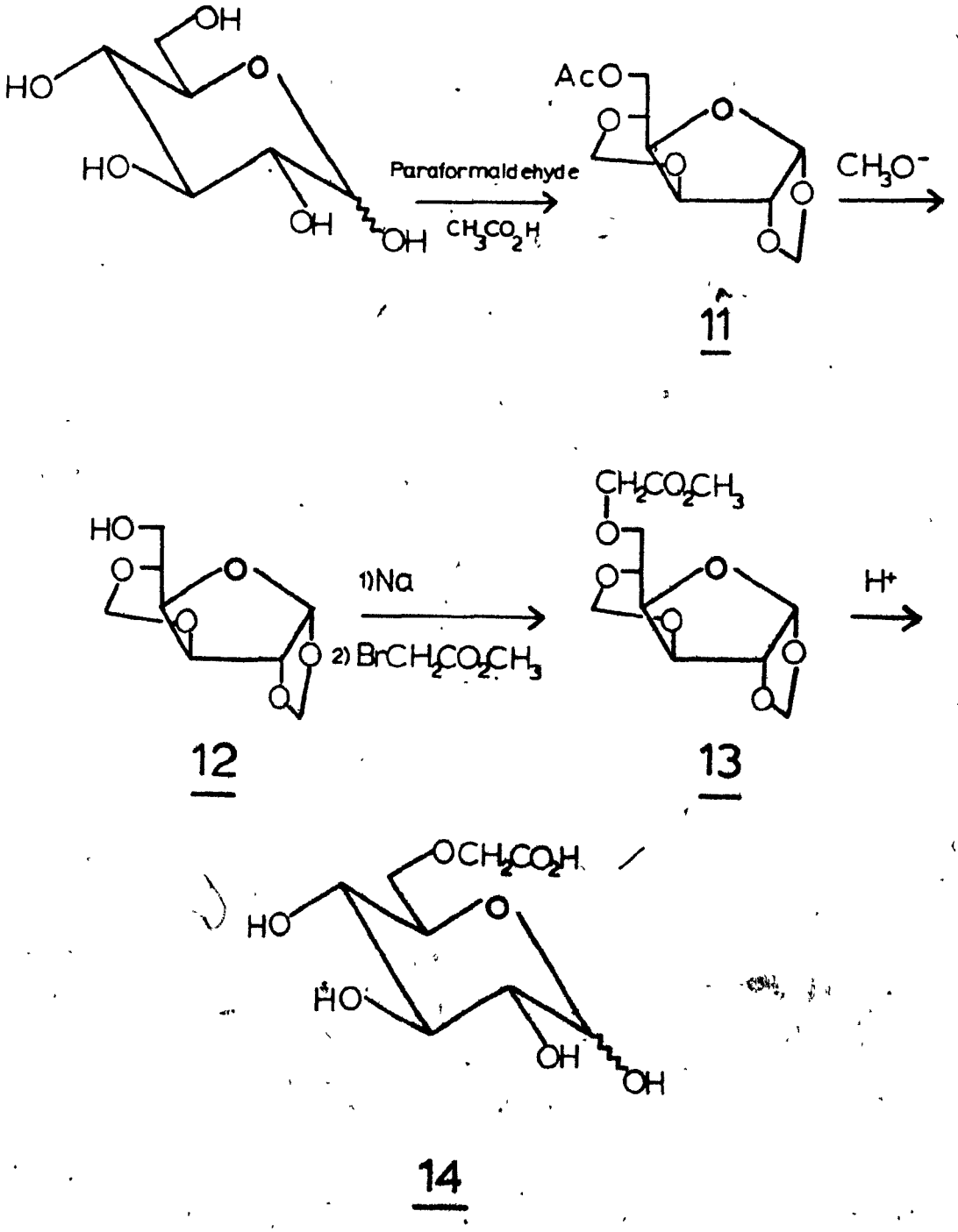


FIG. 3 Synthetic scheme for 6-O-carboxymethyl-D-glucose (14)

consisted of the following: treatment of D-glucose with paraformaldehyde led to the 6-O-acetyl derivative of di-O-methylene-α-D-glucofuranose (11) which was deacetylated to (12). Treatment of (12) with sodium and methyl bromoacetate, followed by acid hydrolysis, yielded the free 6-O-carboxymethyl-D-glucose (mixture of α and β anomers)(14).

II.3.2 ^{13}C m.r. spectra of model compounds

The ^{13}C m.r. spectra of compounds (8, 10, 14) are represented by Fig. 4 along with that of D-glucose (24). Examination of the differences between the spectra of D-glucose and its monosubstituted derivatives allows for a tentative assignment of the peaks.

The characteristic features usually seen in ^{13}C m.r. spectra of carbohydrates are retained here, i.e., primary hydroxyl carbons resonate around 60 p.p.m., ring carbons between 70 and 90 p.p.m. and anomeric carbons between 90 and 100 p.p.m. The carbonyl carbons have a large chemical shift of 180 p.p.m., and are relatively more difficult to detect because of their longer relaxation times.

For the 2-O-carboxymethyl derivative, all 12 resonances of the carbohydrate part of the two anomers are seen, apart from those of the two C-4 which give rise to a single peak, superimposed also upon that of the primary carbon of the carboxymethyl side chain at 70.8 p.p.m. The β-C-4, 5 and 6 almost retain the chemical shift they had in D-glucose, whereas C-2 shows a deshielding of 9 p.p.m. Conversely, the neighbouring C-3 is shifted upfield by 1.2 p.p.m., and C-1 is affected in the same way but by only 0.4 p.p.m. In the α anomer the corresponding effects are as follows: C-2 is shifted downfield by 8.2 p.p.m., while C-3 is shifted upfield by 1.4 p.p.m. and C-1 by 2 p.p.m.

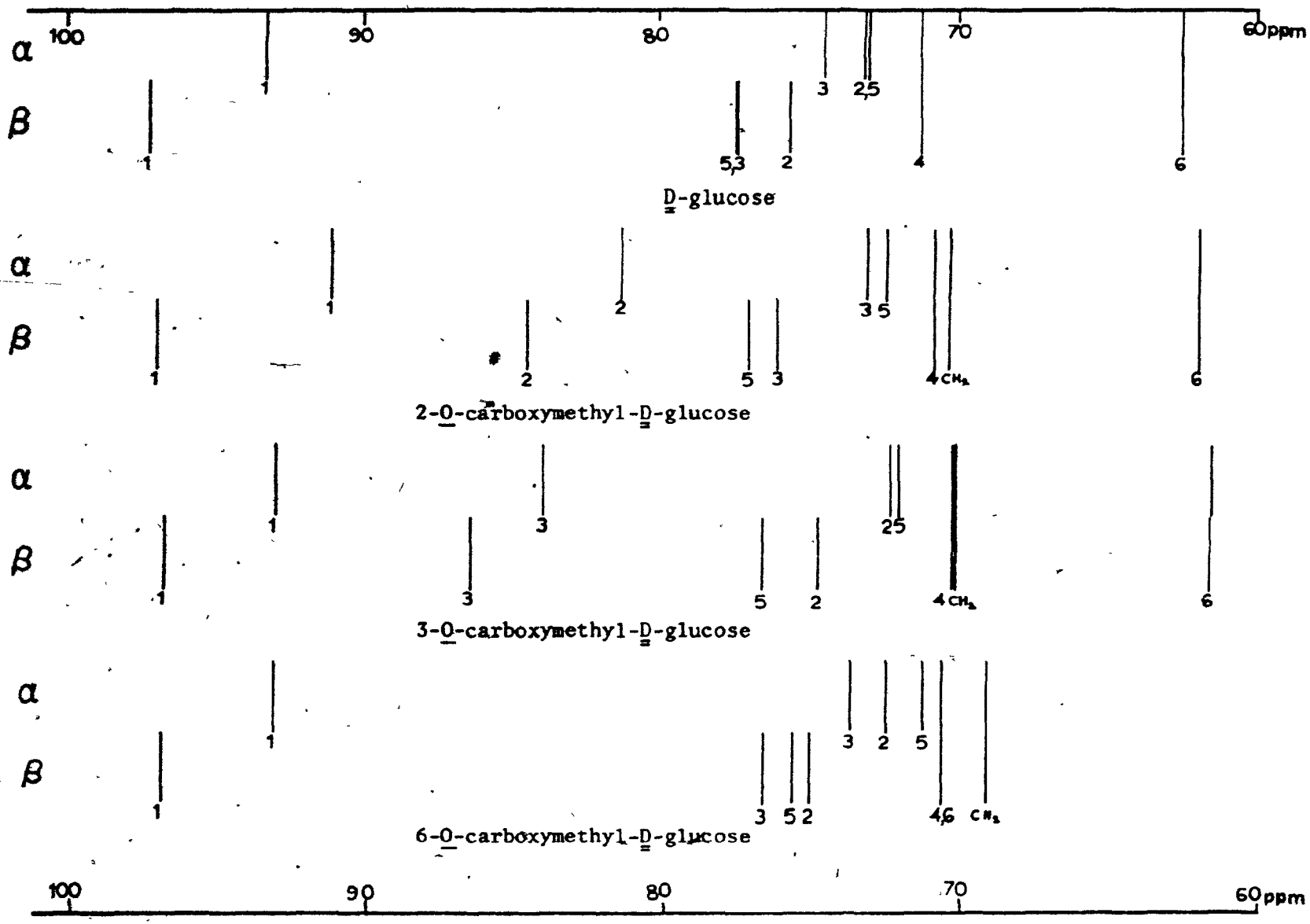


FIG. 4 ^{13}C n.m.r. spectra of the mono-O-carboxymethyl-D-glucoses

TABLE 2

^{13}C Chemical shifts of mono-O-carboxymethyl-D-glucoses.

	C-1	C-2	C-3	C-4	C-5	C-6	CH_2**
α-D-Glucose	93.3	73.1	74.4	71.2	72.9	62.4	
β-D-Glucose	97.1	75.6	77.3	71.2	77.3	62.4	
2-O-Carboxymethyl-α-D-glucose	91.1	81.3	73.0	70.8	72.4	61.3*	70.8
2-O-Carboxymethyl-β-D-glucose	96.7	84.5	76.1	70.8	77.0	61.9*	
3-O-Carboxymethyl-α-D-glucose	92.9	72.3	83.9	70.2*	72.0	61.5*	70.1
3-O-Carboxymethyl-β-D-glucose	96.7	74.7	86.4	70.4*	76.6	61.6*	
6-O-Carboxymethyl-α-D-glucose	92.9	72.3	73.5	70.1	71.1	70.5	68.9
6-O-Carboxymethyl-β-D-glucose	96.8	74.9	76.5	70.1	75.5	70.5	

*shifts for α and β anomers may be reversed

**shifts for all carboxyl carbons, at ~185 p.p.m.

Two explanations may be put forward to account for the fact that C-1 is much less shielded by 2-substitution in the β anomer than in its α analog. In the very similar case of 2-O-methyl glucoses it has been suggested that hydrogen bonding is stronger with the α anomer* (16), despite the fact that the distance between 1-OH and 2-O-Me is approximately the same in both anomers (Fig. 5).

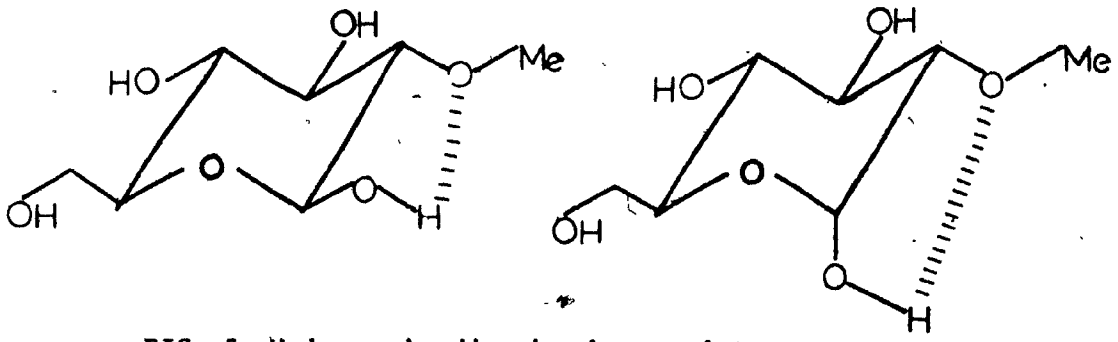


FIG. 5 Hydrogen bonding in the α and β anomers of 2-O-methyl-D-glucose

An alternative possibility is that the steric effect and the magnetic anisotropy of the O-Me group are more important. Indeed, for the cyclic polyalcohol, myo-inositol, it has been found that C-2 (axial) is shifted by 3.2 p.p.m. upfield and C-6 (equatorial) is shifted by 0.5 p.p.m. downfield upon methylation at C-1 (30) (Fig. 6).

Assignments are readily made for the 3-O-carboxymethyl-D-glucose: C-3 is shifted downfield by 9.5 and 9.1 p.p.m. in the α and β anomers respectively, while C-2 and C-4 are shifted upfield by about 0.9 p.p.m. for both anomers.

As for the 6-O-carboxymethyl-D-glucose, the downfield shift of C-6 amounts to 8.7 p.p.m. for both anomers, and C-5 has an upfield shift of 1.8 p.p.m. But, in this latter case, it must be emphasized that the

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*An early evidence of this provided by the infrared spectra of the cis- and trans-1,2-cyclohexanediols which show that trans-groups bend together less easily than their cis-analogs (25).

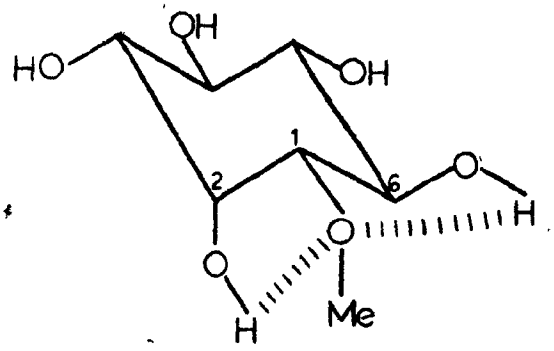

FIG. 6 Intramolecular hydrogen bonding in 1-O-methyl-myo-inositol

proximity of the signals due to C-6 and C-4 makes the assignment difficult and therefore subject to question. This is true even though a closely related pattern has previously been reported for the mono-O-methyl-D-glucoses (25) (vide infra p. 37).

On the basis of these data on the 2, 3 and 6-O-carboxymethyl-D-glucoses, it can be expected that the interpretation of the ^{13}C m.r. spectrum of carboxymethyl cellulose will be facilitated.

An interesting preliminary step, however, is the examination of the ^{13}C m.r. spectrum of the hydrolyzate of carboxymethyl cellulose. Accordingly, a sample of carboxymethyl cellulose of medium viscosity (D.S = 0.7) was hydrolyzed with 5% sulfuric acid in an autoclave. A paper chromatogram of the neutralized and concentrated hydrolyzate was obtained in solvent A (23) and six different O-carboxymethyl glucoses were separated (Fig. 7), although the spots corresponding to the disubstituted glucoses were hardly detectable. Accordingly, their presence is neglected in the following discussion.

R_F relative to glucose	1	2.7	4	1.3	4.1	5.1	5.7
position substituted on glucose	-	2	3	6	2,6	3,6	2,3

FIG. 7 R_F values of CMC hydrolyzate

Consequently, a ^{13}C m.r. spectrum of this hydrolyzate was obtained and is shown on Fig. 8. The various features of the mono-O-carboxymethyl glucoses are clearly distinguishable in this spectrum along with those of glucose itself. Although not all of the assignments can be made with confidence, especially for the nonsubstituted ring carbons of the monosubstituted glucoses, it is interesting to note that, at least for the 2- and 3-O-carboxymethyl-D-glucoses, the substituted ring carbons have a chemical shift such that they are well recognizable among the other signals. Indeed, they lie approximately half way between the anomeric carbons and the ring carbons. The substituted C-6, however, cannot be easily identified because the 9 p.p.m. downfield shift, noticed with the pure compound, brings its signal into the region occupied by those of the ring carbons. The strong signal at 70.3 p.p.m. corresponds to an overlap of the resonances of the primary carbon of the carboxymethyl side chain with that of C-4 of glucose.

Another useful feature of this spectrum is that the relative amount of 2- and 3-substitution can be derived from the integral of the peaks corresponding to the ring carbons C-2 and C-3. In the case studied here (D.S.= 0.75) the figure is approximately 2.2, which is in agreement with the molar ratio of 2.1 obtained by column fractionation of the same CMC by Croon and Purves (26).

It is known that the 3-position of the anhydro-glucose unit

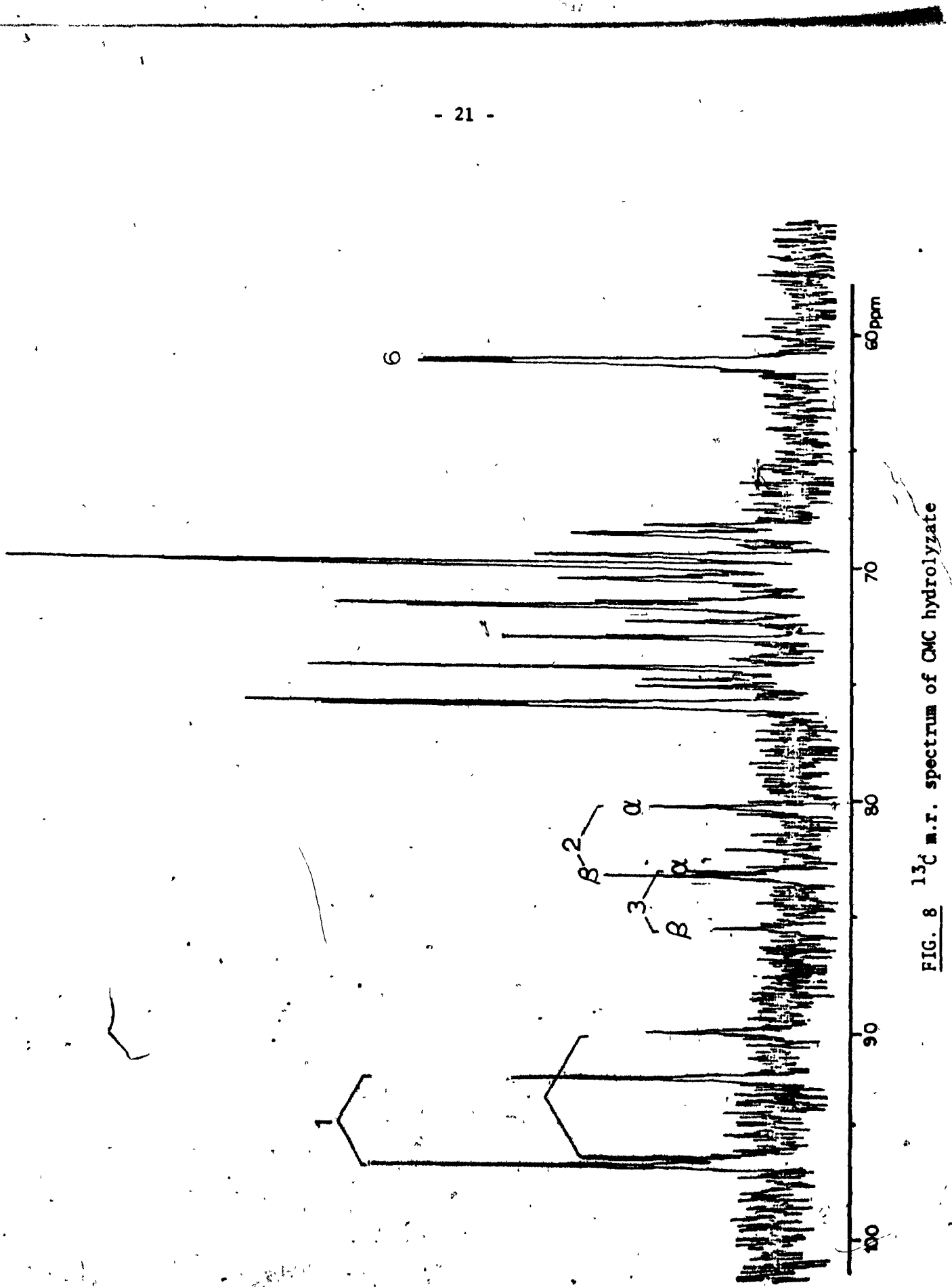

FIG. 8 ^{13}C n.m.r. spectrum of CMC hydrolyzate

is the least reactive, and it has been suggested (27) that this is due to hydrogen bonding between the C-3 hydroxyl and the ring oxygen on the neighbouring glucose residue as shown by Fig. 9. Recent crystallographic data show that this is indeed true, at least in the solid state, for the model compounds methyl-β—cellobioside (28) and cellobiose (29).

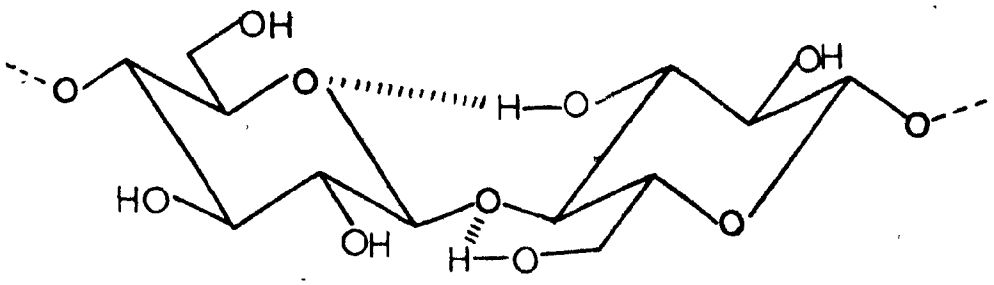

FIG. 9 Inter-residue hydrogen bonding in the cellulose chain

II.3.3 ^{13}C m.r. spectrum of CMC

Based on the interpretation of the ^{13}C m.r. spectrum of the hydrolysate of CMC it is now possible to turn to the examination of the polymer itself. However, the commercial compound is not directly amenable to a spectroscopic examination because of several factors:

a) its tendency to gelation (especially in neutral or weakly acidic media, where the sodium carboxymethyl cellulose is only partially in the salt form) due to electrostatic repulsion between the carboxylic groups.

b) the degree of polymerization (D.P.) can be as high as 1000 although it is usually in the range of 200 to 500 (22d);

c) substitution is not always ideally uniform along the chain, due to the varying crystallinity of the parent cellulose (31);

d) the presence of inorganic ions in the commercial material tends to

facilitate the formation of precipitates (22d).

A generally low solubility is the consequence of these properties, and this is true whatever the D.S. may be. In fact, the range of D.S. in which the commercial sodium carboxymethyl cellulose is made varies from 0.5 to 1.2, and therefore, it can be expected that some degradation by, say, an enzyme, would facilitate the examination of CMC by ^{13}C m.r. spectroscopy.

Previous studies (31,32) have given a substantial insight into the mechanism of enzyme degradation of CMC. It has been found that unsubstituted residues are the main focal points of attack by cellulase, and therefore a low D.S. is required if one is interested, as in this case, in a pattern as close as possible to randomness. It seemed reasonable, therefore, to take advantage of the enzyme to depolymerize the CMC to a small extent and thereby increase its solubility (or decrease the viscosity of the solution) and improve the resolution of the spectra. The enzyme utilized here was of the Streptomyces type (33), and, in order to further simplify the spectrum by eliminating the possibility of interference by monomers, the nondialyzable portion of the hydrolyzate was examined.

Several features are noteworthy in the sample spectrum shown in Fig. 10 (D.S.= 0.7).

1) the general aspect is basically that of a $\beta(1\rightarrow4)$ glucan, because of the low D.S. of the sample. By analogy with the spectrum of the complete hydrolyzate, one finds, in order of increasing chemical shift:

C-6 at 61.2 p.p.m.

C-2, C-3, C-4, C-5 between 70 and 76 p.p.m.

C-1 at 105 p.p.m.

C-2 to C-6 exhibit the same chemical shift as in glucose, whereas

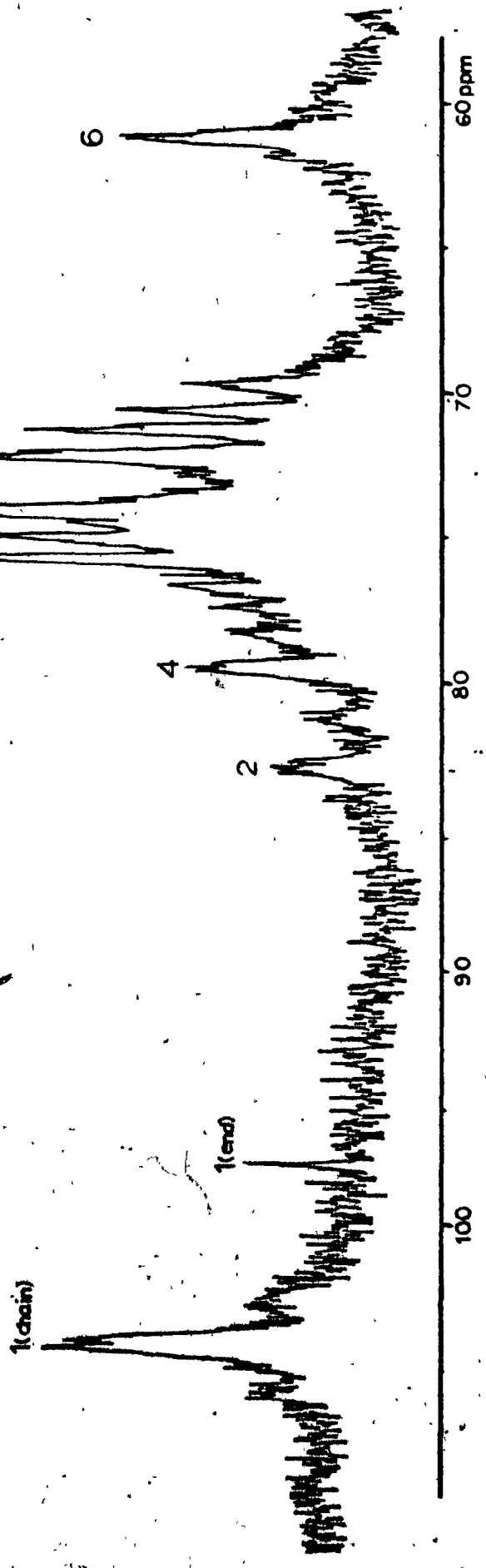

FIG. 10 ^{13}C n.r. spectrum of CMC (D.S. = 0.7)

C-1, linked through a β-glycosidic linkage to another anhydroglucose unit, is shifted downfield by about 6.5 p.p.m. from C-1 of β-D-glucose, which brings it to approximately the same value (103.7 p.p.m.) as for C-1 in cellobiose (103.5 p.p.m. (15))*. Similarly, C-4, also linked to another anhydroglucose, is shifted by about 5 p.p.m. from its position in β-D-glucose and comes to the same position (80.3 p.p.m.) that C-4' assumes in β-cellobiose.

2) The carbons bearing an O-carboxymethyl group are moved downfield by the same amount as in the monosubstituted glucoses. Thus, C-2 appears at 84.5 p.p.m. and C-6 at 69.8 p.p.m. C-3, unfortunately is not seen in the polymer presumably because of the lesser substitution at this position, the sample studied here having a low D.S. But its position can be estimated to be close to 86.4 p.p.m. Indeed the absence of the C-3 signal cannot be due to the upfield shift by the neighbouring glycosidic bond on C-4, whose effect is indeed very small, as seen on the fully methylated cellulose (vide infra, p.42).

3) The carboxyl group has a relatively long relaxation time and the intensity of its signal is smaller than those of the carbohydrate part of the polymer. The poorer resolution attainable with the polymer combined with a small difference in chemical shifts also prevents the identification of each type of carboxylic group (linked to C-2, C-3 or C-6) (see Footnote, Table 2).

4) The large number of relatively well resolved peaks in the 70-76 p.p.m. region is due to the presence of the methylene groups (at around 70 p.p.m.) in addition to the anhydroglucose ring carbons and their

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*converted relative to internal TMS, using δ_{CS_2} = 193.7 p.p.m. (4)

monosubstituted analogs. Monosubstitution of a ring carbon, as seen earlier, induces small upfield shifts on neighbouring carbons .

5) The small but well resolved peaks at 97 p.p.m. and 93 p.p.m. can be accounted for by the assumption that the extent of depolymerization was sufficient to allow the anomeric ends of the CMC chain to be seen. Theoretically, the combined integration of these 2 peaks, divided by that of the peak at 104 p.p.m. (C-1 glycosidic) should provide a good estimation of the D.P. of the sample, provided that it is not too polydisperse. In the case examined here however the extent of depolymerization was small and the D.P. is probably still higher than 100; therefore such a measurement cannot be made accurately. Spectra of samples of varying D.S. have been obtained in the same way, and their examination leads to the following conclusions:

A sample of low D.S. (0.5) is slightly more easily degradable, and therefore its spectrum is better resolved. However, the general appearance of the spectrum is the same, and in particular, no significant change is detectable in the signals attributed to substituted C-2 and C-6. On the other hand, if the D.S. is higher than 0.7, the signals of the substituted C-2 and C-6 are improved, but resolution is decreased due mainly to the higher D.P. of the depolymerized sample (the action of cellulase being hampered by the presence of more carboxymethyl groups).

In conclusion, despite the difficulties inherent to the observation of a polymer whose average molecular weight is well above 20000, it has been possible to obtain in a reasonable time (16 hrs) and analyze a ^{13}C m.r. natural abundance spectrum by using the F.T. technique. Its features show a fair agreement with earlier observations made by more laborious and time consuming methods.

II.4 O-Hydroxyethyl cellulose

The O-hydroxyethyl derivative of cellulose (HEC) is structurally a very close relative of the CMC previously studied. But its properties are different in several respects.

a) It is not a polyelectrolyte and therefore has less tendency to precipitate or to form gels, is less sensitive to salt effects and to oxidation (22d).

b) The way in which it is manufactured has an influence on its substitution pattern. Several molecules of the ethylene oxide which is made to react with alkali cellulose, can add successively to a given position on the cellulose backbone, leading to pendant side chains of appreciable length, especially in samples of high D.S. This leads to a polymer related less to cellulose and more to poly(ethylene oxide). Therefore, and although polymers having a D.S. of up to 3 have a commercial interest, this study will be limited to a sample of low D.S. The concept of molecular substitution (M.S.) has been introduced to take the length of the poly(ethylene oxide) side chains into account, and is defined as the average number of ($O-CH_2-CH_2$) units linked to each anhydroglucose of the cellulose.

c) An interesting feature (35) is that all glucose units have been found to be equally reactive towards ethylene oxide; the randomness of the substitution can therefore be expected to be higher than for CMC. The relative rate constants are 3:1:10:20 for reactions, respectively, at C-2, C-3, C-6 and the new hydroxyl introduced by hydroxyethylation (36).

The ^{13}C n.m.r. spectrum of HEC should therefore account for these characteristic properties. Beforehand, however, model compounds, namely two of the three possible mono-O-hydroxyethyl glucoses have been examined.

II.4.1 Model compounds

Following an approach similar to that used in the CMC study, the model compounds 3- and 6-O-hydroxyethyl-D-glucoses (10a,14a) were first synthesized from their carboxymethyl parents in a two step synthesis (34) (Fig. 11) involving reduction of the carboxymethyl group followed by hydrolysis. Their ^{13}C.m.r. spectra are illustrated in Fig. 12. In addition to the already known characteristics of glucose, they display a readily identifiable chemical shift pattern (Table 3):

a) Substitution on a given carbon induces an increase in its chemical shift of 8.9 and 8.6 p.p.m. (for C-3 α and β, respectively), and of 8.2 p.p.m. (for C-6 α or β). This means that the effect of the O-carboxymethyl and O-hydroxyethyl substituents on the ^{13}C m.r. chemical shifts of glucose is virtually the same.

b) The methylene groups show well defined chemical shifts, independent of their position of substitution: C-7 at 75.5 p.p.m. and C-8 at 62.9 p.p.m.

II.4.2 ^{13}C m.r. spectrum of O-hydroxyethyl cellulose

The same procedure used with CMC was applied here, i.e., the non-dialyzable portion of the enzyme hydrolyzate was examined and its ^{13}C m.r. spectrum is shown on Fig. 13.

The most striking feature is that the intensity of the methylene signals of the side chains is much higher than should be expected from a sample of D.S. = 0.8. This means that in several cases more than one hydroxyethyl group is attached to the same position. Since, as seen with the monomer 3-O-hydroxyethyl-D-glucose, the intensity of the signal due to C-1 and that of the methylene group is the same* (they have therefore comparable T_1

*In fact, T_1 may vary significantly in the polymer, (especially that of C-1) and therefore the validity of this kind of measurement can be questioned.

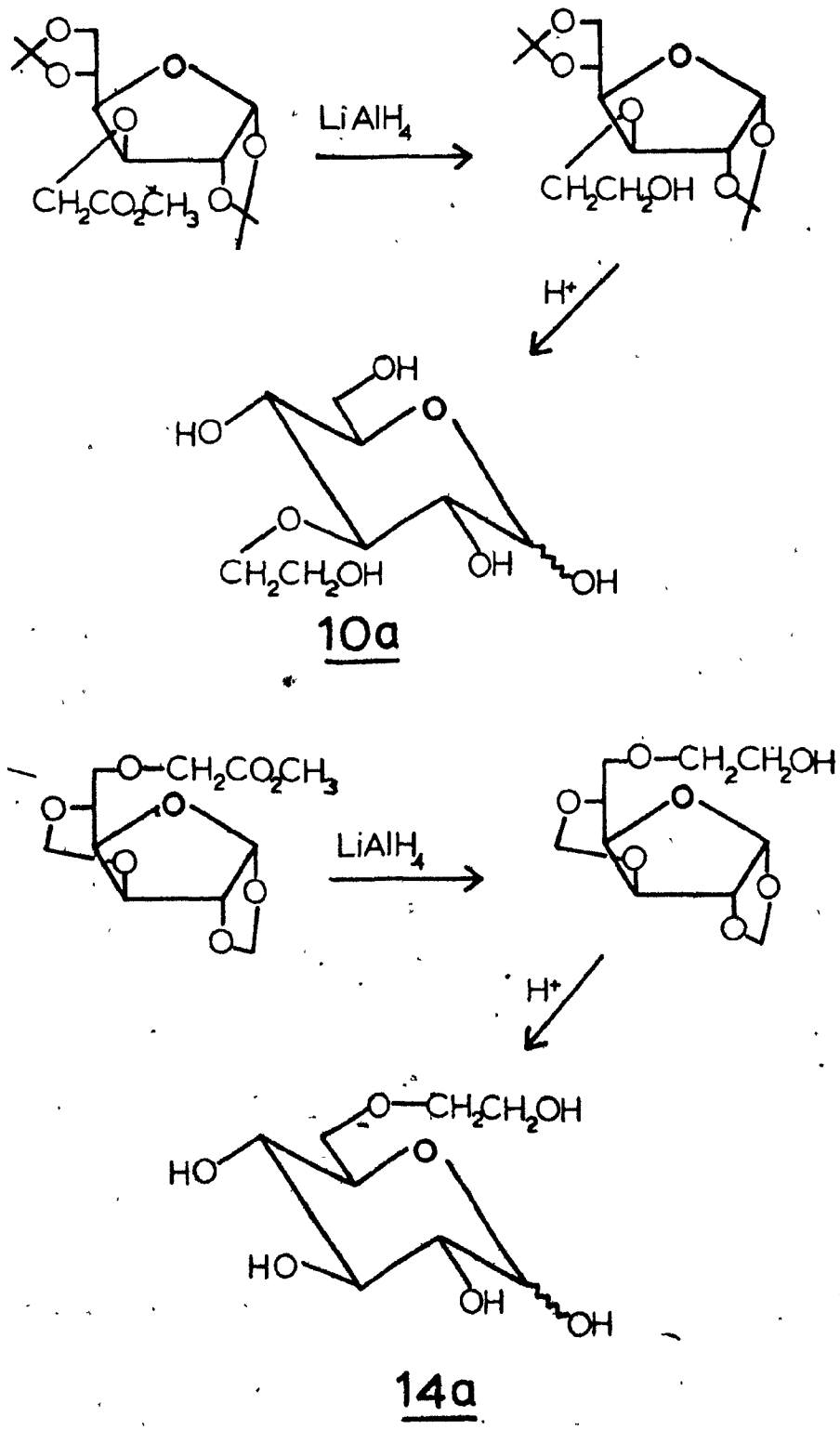

FIG. 11 Synthetic scheme for the 3-and 6-O-hydroxyethyl-D-glucoses (10a and 14a)

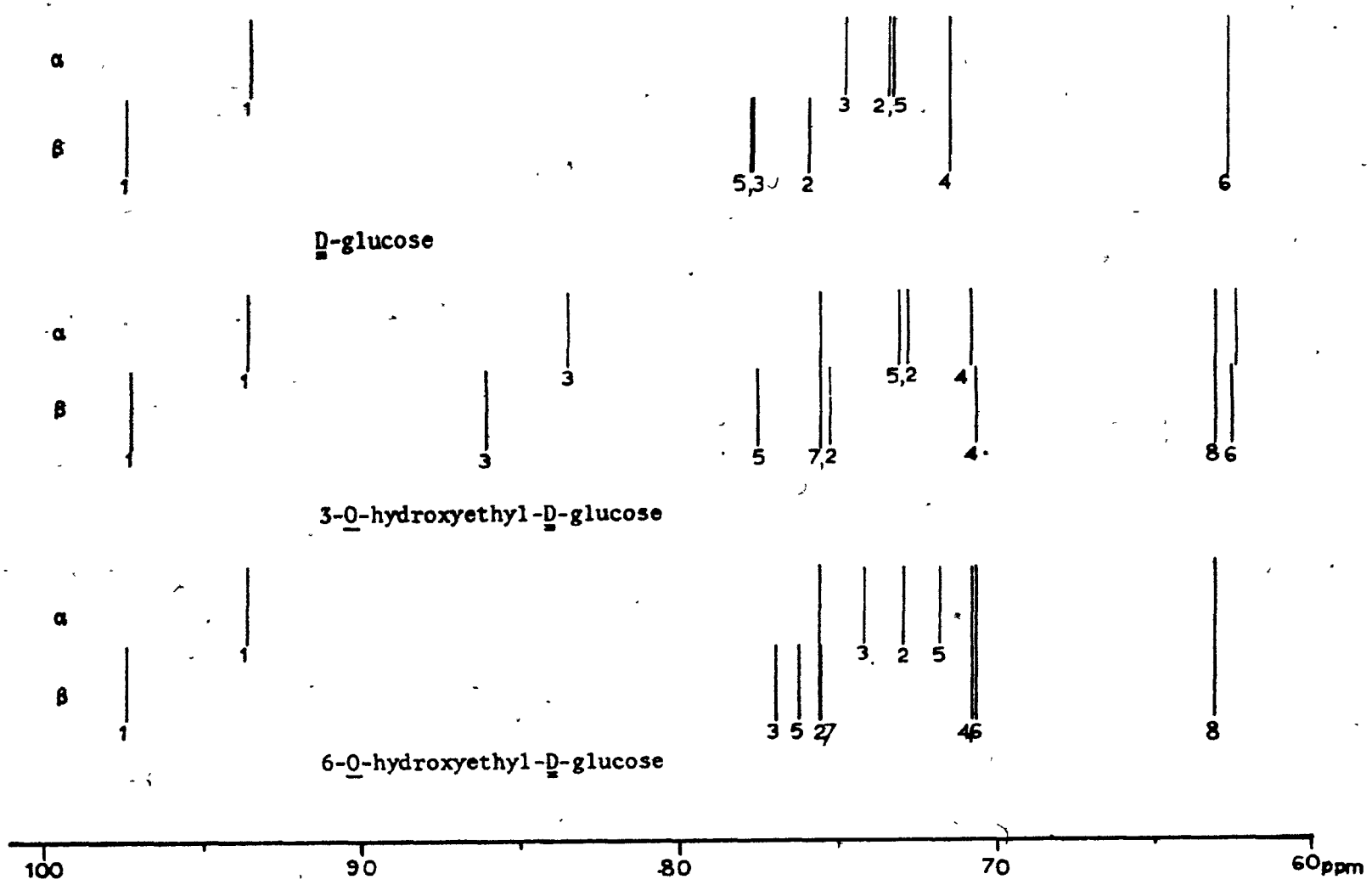

FIG. 12 ^{13}C m.r. spectra of mono-O-hydroxyethyl-D-glucoses

TABLE 3

^{13}C Chemical shifts of mono-O-hydroxyethyl-D-glucoses

	C-1	C-2	C-3	C-4	C-5	C-6	C-7	C-8
3-O-Hydroxyethyl-α-D-glucose	93.4	72.6	83.3	70.6*	72.8	62.2*	75.3	62.8
3-O-Hydroxyethyl-β-D-gluclose	97.2	75.1	85.9	70.4*	77.3	62.3*	75.3	62.8
6 O-Hydroxyethyl-α-D-glucose	93.3	72.6	73.8	70.4	71.5	70.5	75.5	62.9
6-O-Hydroxyethyl-β-D-glucose	97.2	75.2	76.7	70.4	75.9	70.5	75.5	62.9

*shifts for α and β anomers may be reversed

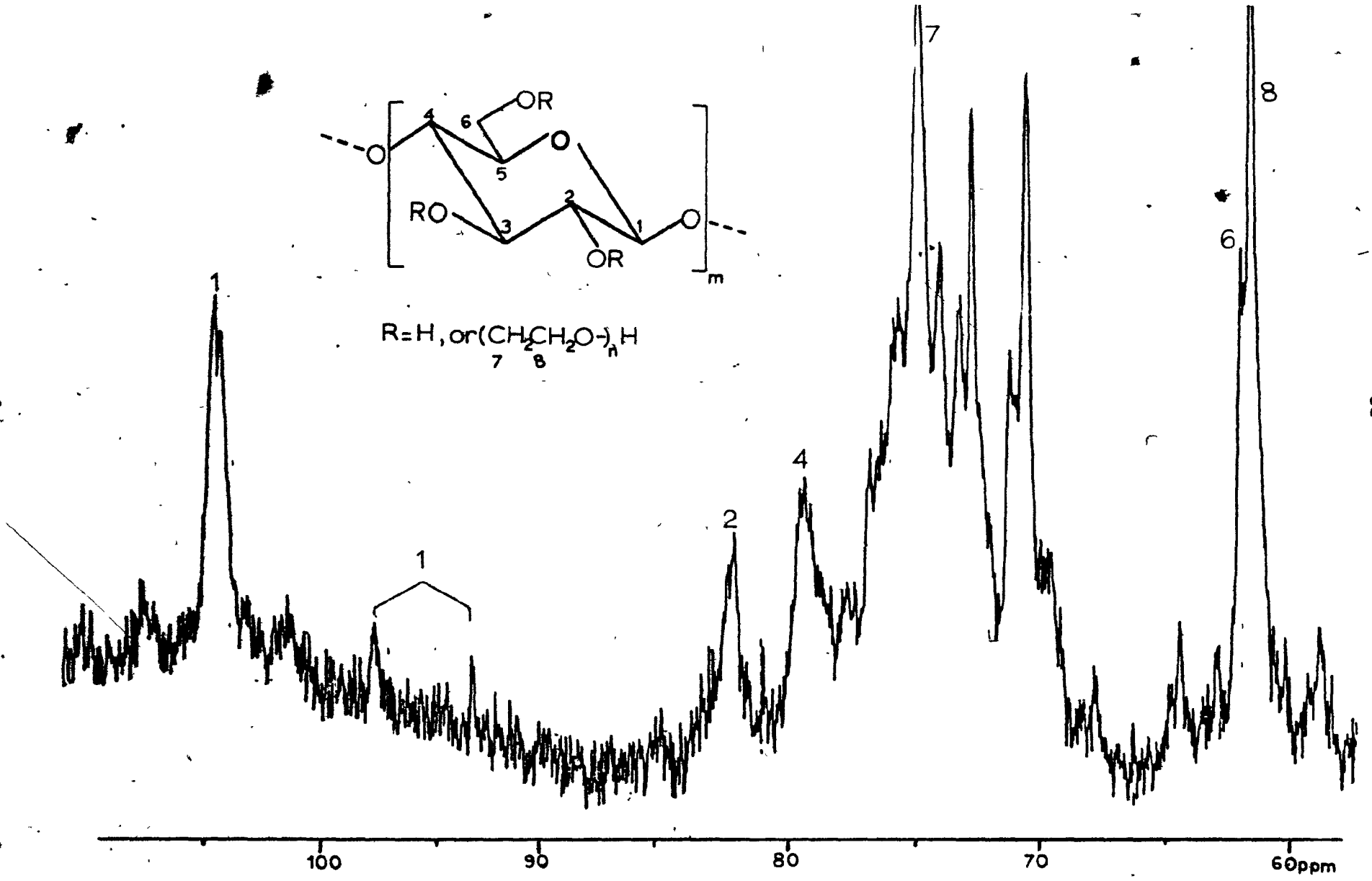

FIG. 13 ^{13}C.m.r. spectrum of -O-hydroxyethyl cellulose

relaxation times), it is possible to measure the avarage length of these side chains by taking the ratio:

$$\frac{\text{intensity of C-7 signal}}{\text{intensity of C-1 signal}}$$

In our case this value can be estimated to be around 2.5, meaning that, on the average 2.5 ethylene oxide molecules reacted with each glucose unit. It has been found, (37) that the D.S. of an HEC sample increases significantly when it is hydrolyzed by cellulase, because the enzyme attacks predominantly at the nonsubstituted positions, and the low molecular weight fragments produced are eliminated by dialysis. Thus, a sample having a D.S. of 0.8 is found to give a hydrolyzate having a D.S. of 1.1 to 1.2. Furthermore, a D.S. in this range is thought (38) to be associated with an M.S. of about 2.5, and this corresponds to the estimation based here on ^{13}C m.r. The ratio M.S./D.S. gives the average length of the ethylene oxide chains, and is here found to be 2.5:1.1 = 2.3.

^{13}C m.r. has thus made possible a confirmation of earlier calculations based on certainly more laborious methods. This is only a preliminary and approximate result, since it is based on one sample, and also, because the integral measurements in ^{13}C m.r. spectroscopy must be interpreted cautiously. But a more thorough quantitative approach along with a standardization of the procedure might be rewarding, and this technique might provide a simple way of characterizing O-hydroxyethyl celluloses.

II.5 Methyl cellulose

Methyl cellulose shares with hydroxyethyl cellulose some of its properties, including its water solubility, and its viscosity properties. But it differs in several respects.

a) Its water solutions gel on heating, or on addition of salts.

b) The range of D.S. of the commercial material varies from 1.5 to 2, and the solutions obtained in cold water are of low concentration, and their viscosity increases rapidly with concentration.

c) Nearly completely substituted methyl cellulose (D.S. > 2,5) is soluble in nonpolar organic solvents.

An approach similar to that used previously for CMC was used here, i.e., ^{13}C chemical shift data for the monosubstituted monomers were compared with those found for a partially degraded polymer, and tentative assignments could be made.

^{13}C chemical shift data for the 2-, 3-, and 6-O-methyl-D-glucoses were taken directly from the literature (16) and are presented in Fig. 14, along with those relative to the methyl glycosides and per-O-methyl derivatives of D-glucose (39) and cellobiose (40).

It is clear that the introduction of the O-methyl group at one given position on glucose induces an increase in the chemical shift of the substituted carbon of 8-10 p.p.m., while its neighbours experience a slight decrease in their chemical shift (0.5 to 1 p.p.m.). The methoxyl carbons have a chemical shift almost invariable with the position of the substitution (60-61 p.p.m.) and appear to be very close to C-6 of glucose.

These data have been used as a comparative basis for the interpretation of spectra of methyl cellulose of varying D.S., thus covering the whole possible range up to 3. The sample of low D.S was degraded by enzyme, (as described for CMC) while dilute aqueous HCl was used for the sample of medium D.S., and HCl in dichloroethylene/$CDCl_3$ was used for the sample of high D.S.

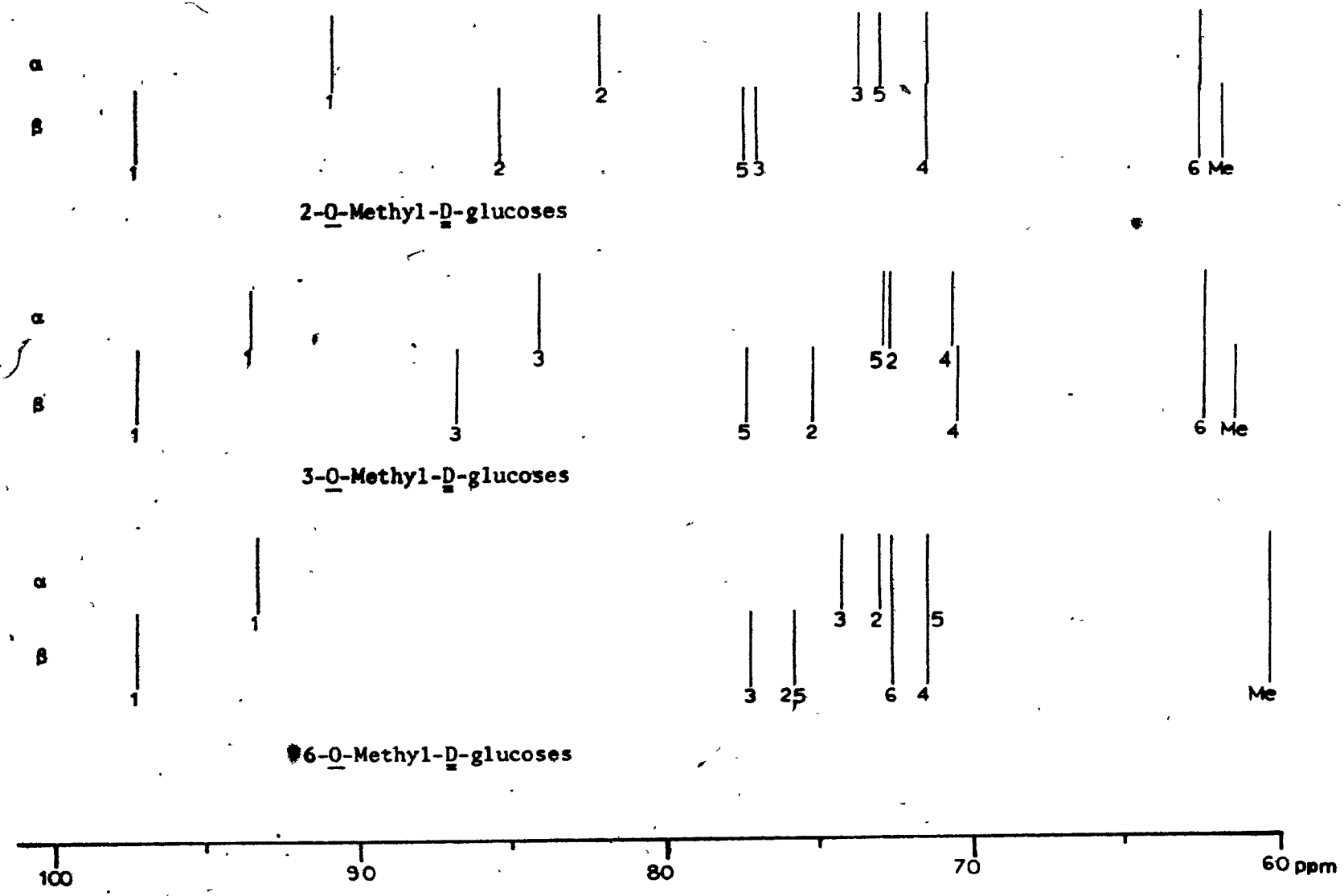

FIG. 14 ^{13}C.m.r. spectra of methylated glucoses and cellobiose

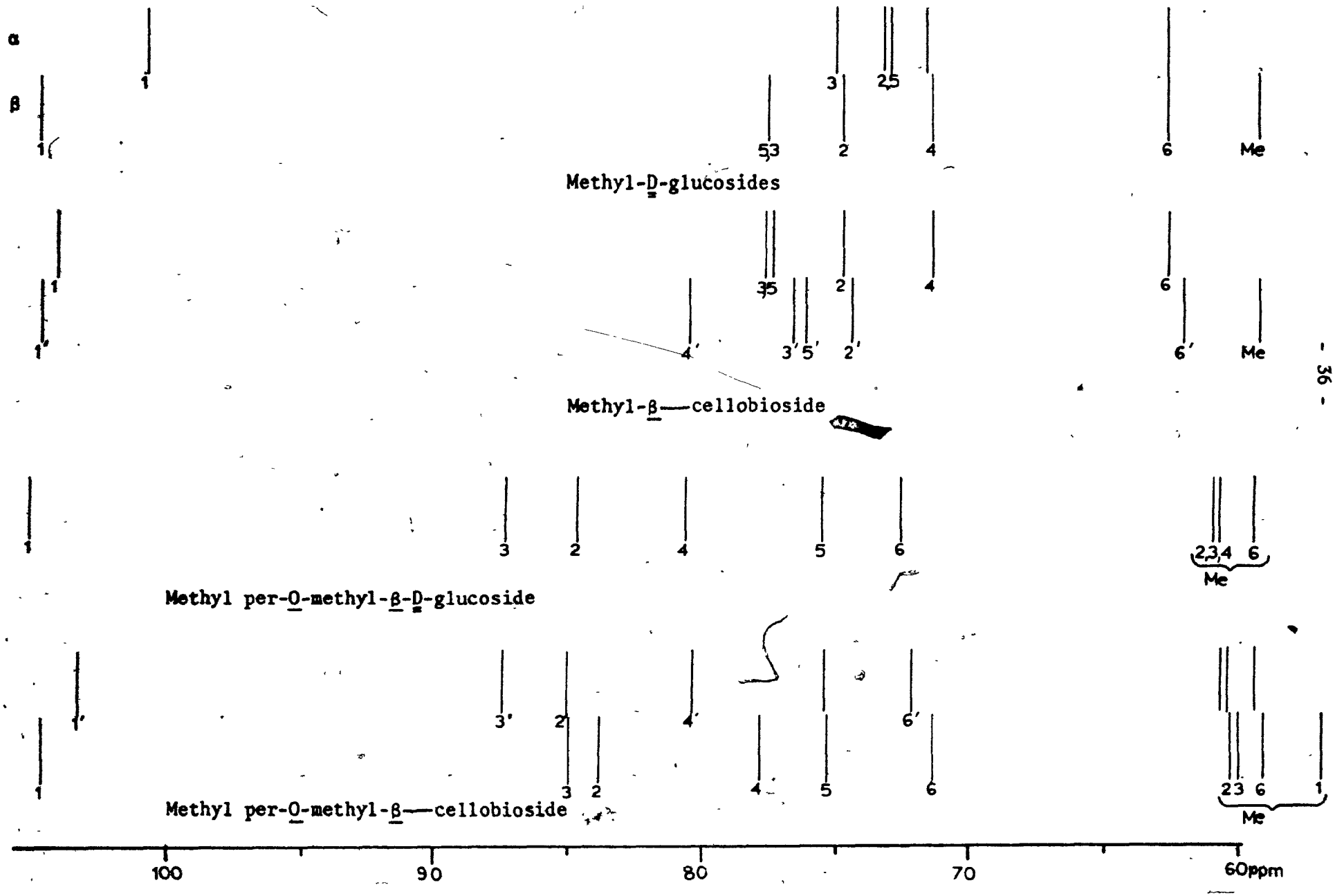

FIG. 14 2nd part

TABLE 4

^{13}C Chemical shifts of methylated glucoses and cellobiose

	C-1	C-2	C-3	C-4	C-5	C-6	O-Me
Methyl-α-D-glucoside	100.5	73.1	74.8	71.4	72.8	62.3	56.8
Methyl-β-D-glucoside	104.5	74.6	77.3	71.2	77.3	62.4	58.8
2-O-Methyl-α-D-glucose	90.7	81.9	73.5	71.3	72.8	62.4	59.3
2-O-Methyl-β-D-glucose	97.1	85.2	76.8	71.3	77.3	62.4	61.7
3-O-Methyl-α-D-glucose	93.4	72.6	84.1	70.6	72.8	62.3	61.3
3-O-Methyl-β-D-glucose	97.2	75.1	86.7	70.4	77.3	62.3	61.3
6-O-Methyl-α-D-glucose	93.3	73.0	74.3	71.4	71.4	72.6	60.3
6-O-Methyl-β-D-glucose	97.3	75.8	77.2	71.4	75.8	72.6	60.3
Methyl per-O-methyl-β-D-glucoside*	105.0	84.6	87.2	80.5	75.4	72.4	57.0; 60.5; 60.8; 60.6; 59.3
Methyl per-O-methyl-β-cellobioside**	104.9	83.9	85.0	77.9	75.4	71.5	56.9; 60.4; 60.1 - ; 59.1
C'	103.4	85.1	87.5	80.3	75.5	72.3	- ; 60.7; 60.7; 60.4; 59.4
Methyl β-cellobioside**	103.9	74.6	77.5	71.2	77.2	62.4	
C'	104.5	74.2	76.4	80.3	75.9	61.8	58.9

*ref. 39

**ref. 40

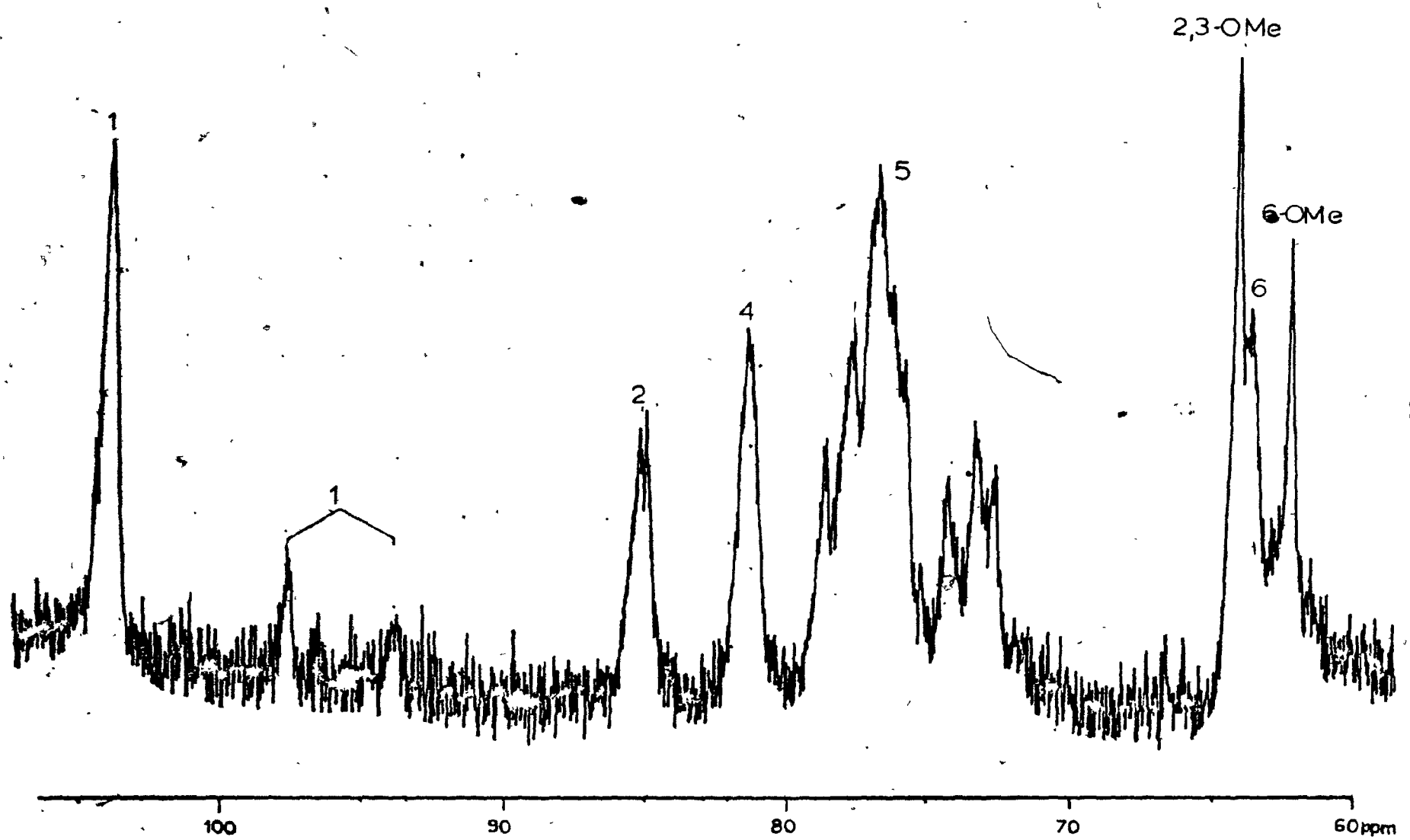

FIG. 15 ^{13}C m.r. spectrum of methyl cellulose (low D.S.)

The ^{13}C m.r. spectra of the degraded polymers are shown in Figs. 15, 16, 17.

1) Methyl cellulose of low D.S.

The sample used here had a D.S. of 0.7 originally, but it was found to have increased to a value of 1.1 (methoxyl content 19.5%) following enzymic degradation. It is a known fact that the cellulase attacks the cellulose chains mainly where it bears no substituents, and that at least two consecutive unsubstituted anhydroglucose units are required. If more than two such units are found along the chains, several glycosidic linkages will be broken, resulting in loss of free glucose or cellobiose, after dialysis, and thus increasing the overall D.S. of the material to be examined.

The spectrum of this sample retains characteristics of the anhydroglucose unit already found in CMC. In addition the methoxyl signals can be identified by reference to those of the corresponding free sugars. Thus, 2-O-Me appears at 61.6 p.p.m., 3-O-Me at 61.2 and 6-O-Me at 60.2 p.p.m. The relative intensity of these signals (at least that of the methoxyls at position 2 and 6) gives a measurement of the relative degree of subsitution at each of these positions. In our case, it is found to be approximately 1.5, which is very close to the relative reactivities proposed by Croon (27). C-2:C-6 = 3.5:2 in the case of methylation with methylsulphate. In fact, this small difference can be due to several factors.

a) Integration values are not always dependable in ^{13}C m.r. spectroscopy, since they are influenced by relaxation properties of carbon atoms. It may be that, in this case, the 6-O-methyl group has a shorter relaxation time than its C-2 analog, reflecting a higher degree of freedom expected for the C-6 methoxyl and therefore gives rise to a relatively higher peak.

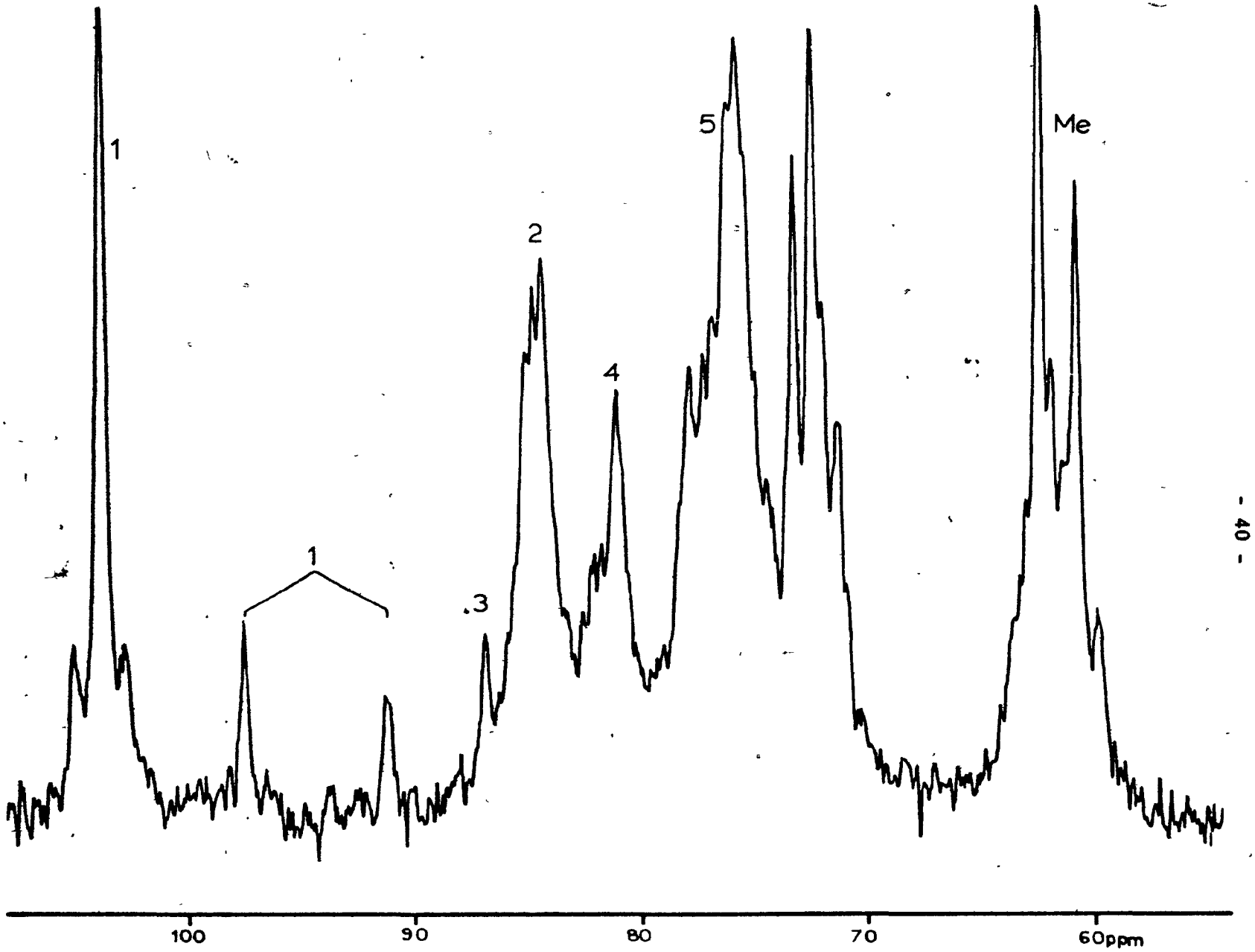

FIG. 16 ^{13}C.m.r. spectrum of methyl cellulose (medium D.S.)

b) The possibility of disubstitution on a single unit has been neglected here due to the low D.S. of the sample.

c) There may be some degree of overlap of the C-2 methoxyl signal, with the C-3 methoxy and with C-6 (nonsubstituted), which would contribute for its signal appearing stronger.

The ring carbons give rise to a complex pattern, due to overlap of nonsubstituted and partially substituted units. The stronger signal (at 75.5 p.p.m.) belongs to C-5, while C-4 is seen as a peak at 80.3 p.p.m. No satisfactory explanation was found to explain why the C-4 signal is weaker than the C-1 signal. Perhaps at this D.S. the solution properties are such that their relaxation behaviour differs by more than would be expected.

The substituted C-2 and C-3 are seen overlapping as a broad peak at 84.3 p.p.m., that is approximately at the same place as in the monomer. At 93.3 and 97.1 p.p.m the signals corresponding to the free reducing ends ($\underline{\alpha}$ and $\underline{\beta}$) of the chain are found. Their overall intensity is approximately 15% of that of the C-1 signal, seen at 103.9 p.p.m., which means that the average D.P. of the degraded polymer is around 115:15, or 7.

2) If one turns now to the examination of a sample of higher D.S. (Fig. 16), i.e. in the range of 1.5 to 2 corresponding to the commercial material, one finds that the same basic features are present. In addition, it is to be noted that the three types of methoxyl signals are now clearly resolved, the 3-$\underline{O}$-Me signal appearing at 61.3 p.p.m. Although the proximity of the 2-$\underline{O}$-Me signal does not permit a good quantitative measurement, its intensity is certainly much smaller than that of the other methoxyl signals.

In the ring carbon region, two peaks emerge with a strong integration: the one at 76 p.p.m. clearly belongs to C-5, since it will be significantly affected only by substitution at the neighbouring C-6 carbon. The C-4 signal

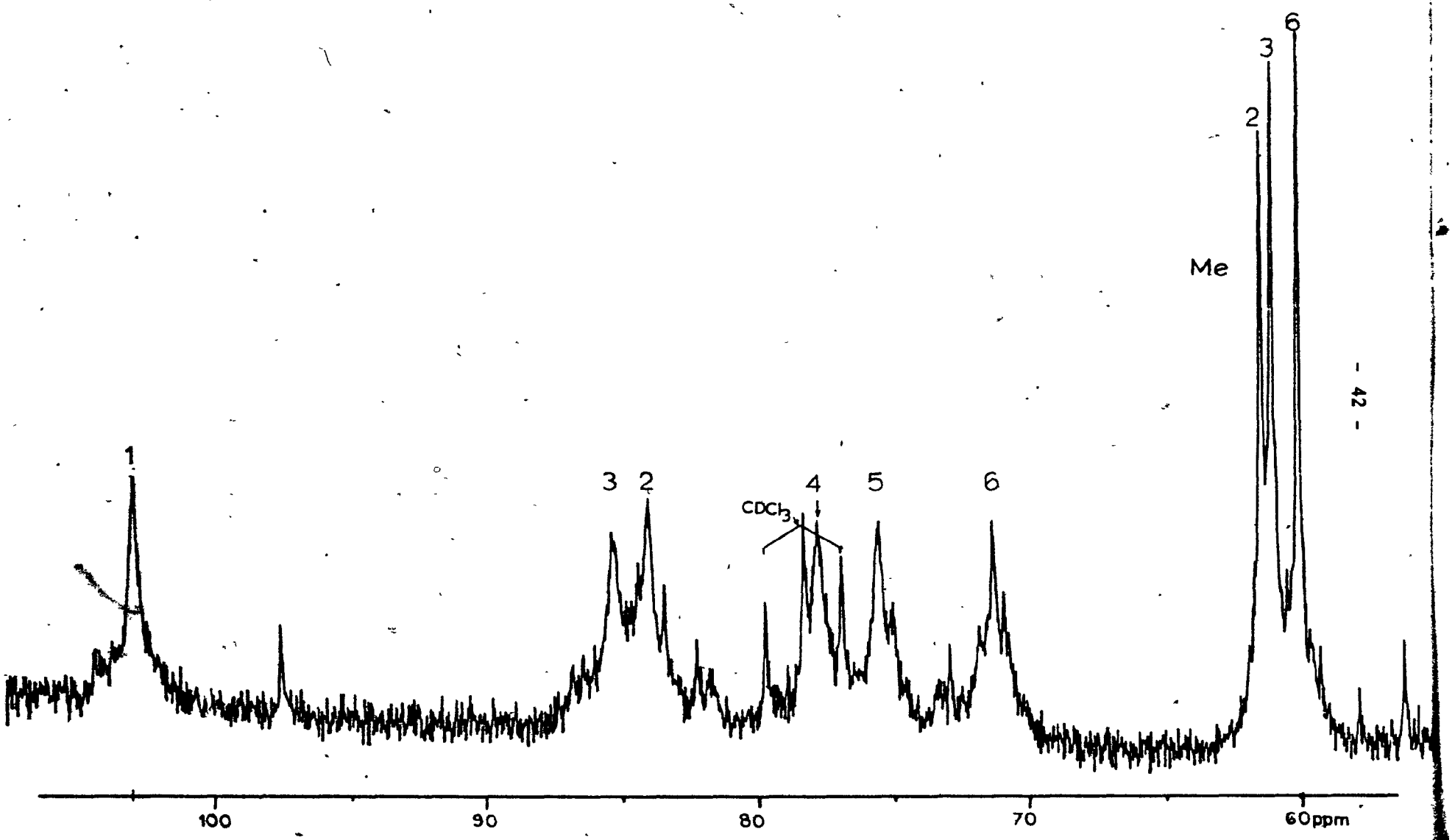

FIG. 17 ^{13}C m.r. spectrum of methyl cellulose (high D.S.)

at 81,3 p.p.m. is now a multiplet as a result of an increase in substitution at the neighbouring C-3, while the C-2 and C-3 (substituted) signals still overlap around 84 p.p.m.

3) As for the permethylated cellulose, whose building blocks are now 2,3,6-tri-O-methyl-β-D-anhydroglucose units, its spectrum (Fig. 17) as expected, is much simpler, and the six signals corresponding to each methylated glucose carbon are seen as strong peaks: 2 and 3 are now well separated. The minor peaks are due in part to the small number of residual unsubstituted positions (the D.S. is not expected to be higher than 2.8), along with the reducing ends of the chains (which, as seen from the intensity of their C-1 signals, are not to be neglected). Finally, the 3 methoxyl signals have practically the same intensity, showing that substitution is very close to completion.

II.6 Ethyl cellulose

A sample of ethyl cellulose (commercial material, D.S. = 1,5 to 2) was subjected to the same analysis as the methyl cellulose, and its ^{13}C m.r. spectrum obtained (Fig. 18). In addition to what has already been said for the methyl cellulose of similar D.S., one can mention the strong methylene signal at 79 p.p.m. corresponding to the CH_2 of the ethyl group. Its sharpness shows that its chemical shift is independent of the position of substitution, whereas the terminal methyl group is more sensitive to that factor.

II.7 Other derivatives of cellulose

^{13}C m.r. analysis could certainly be applied to other cellulose derivatives including esters. As a typical example, commercial cellulose acetate was partially acetolyzed in acetic anhydride. The ^{13}C m.r. spectrum

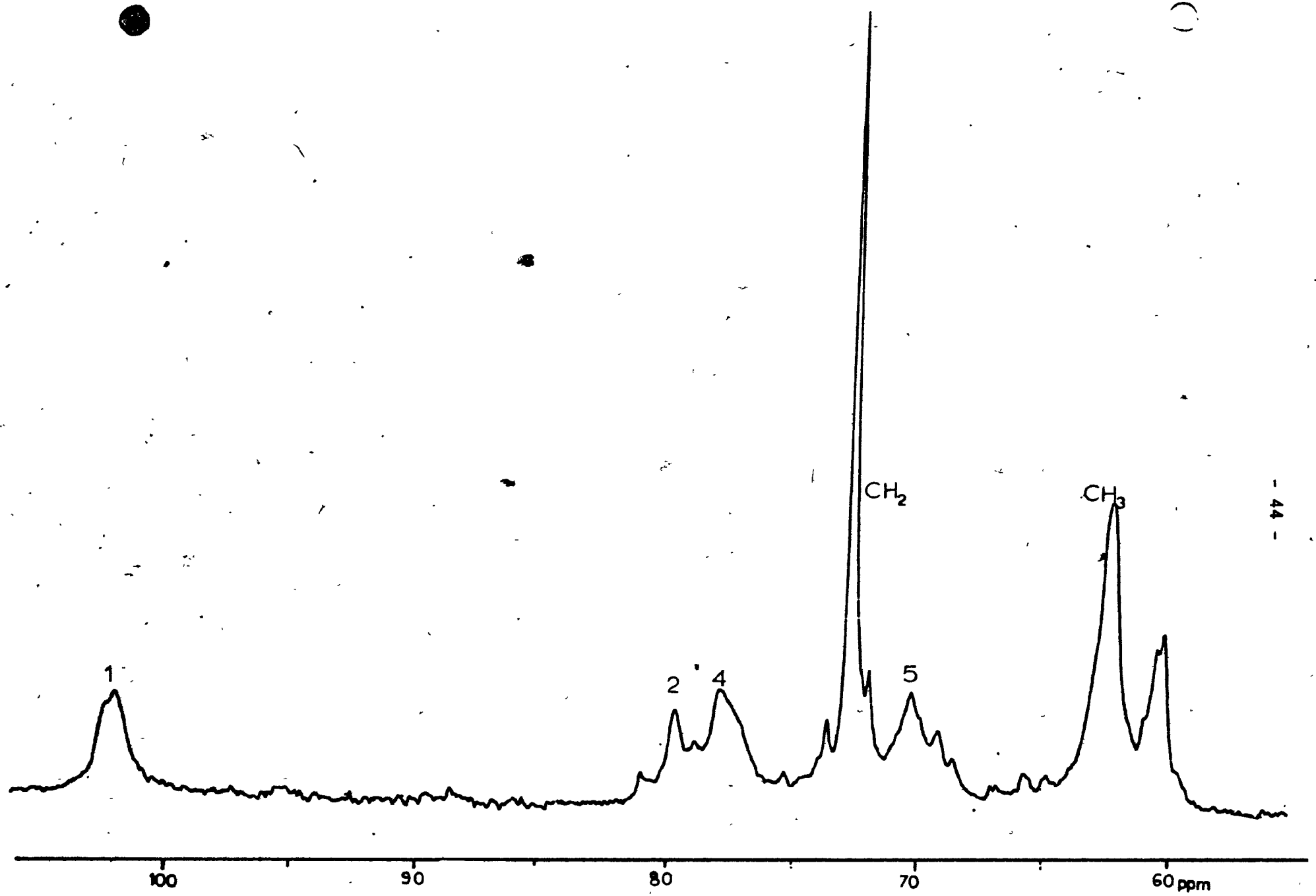

FIG. 18 ^{13}C m.r. spectrum of ethyl cellulose (D.S. = 1.5-2)

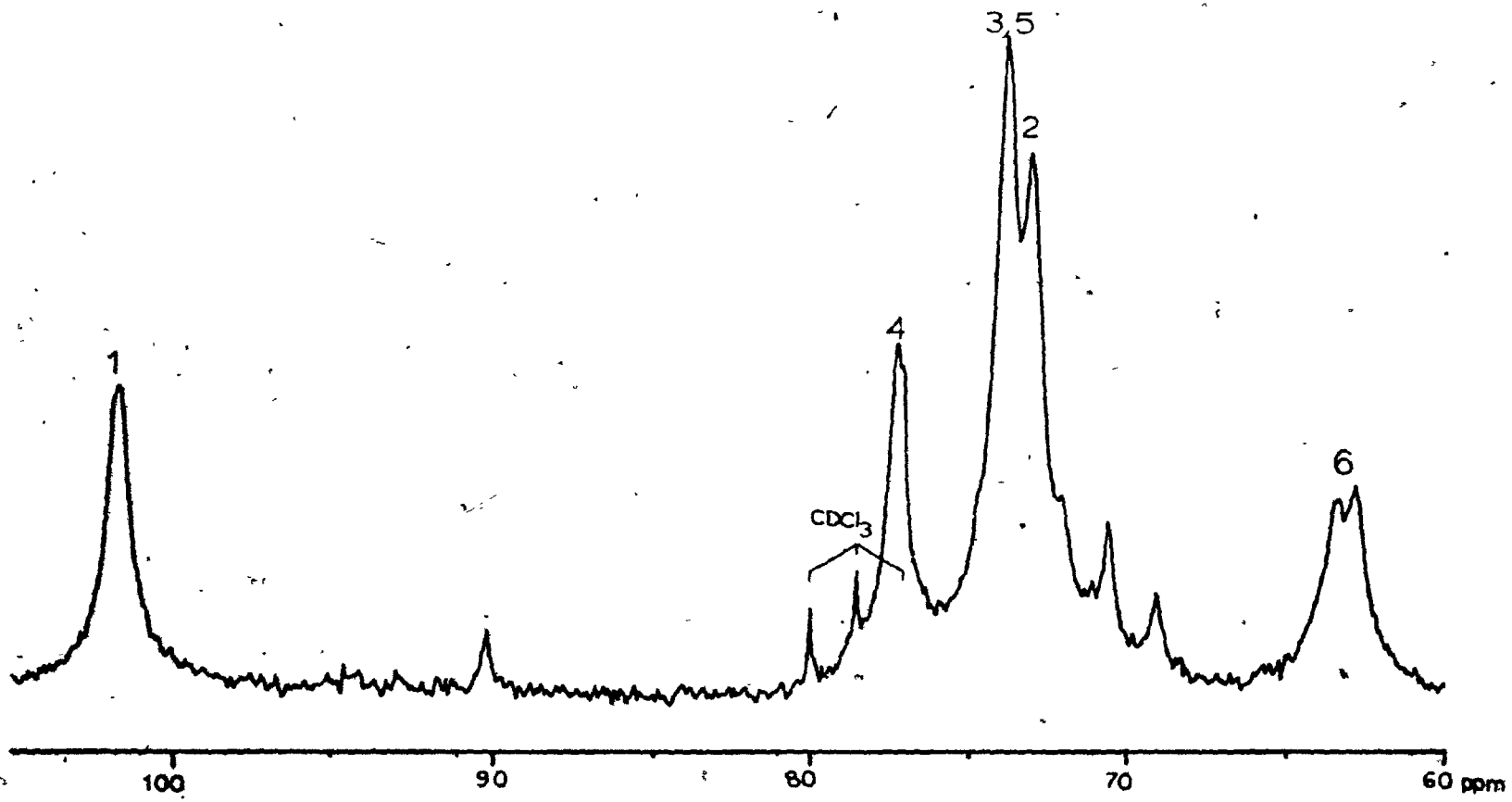

FIG. 19 ^{13}C m.r. spectrum of cellulose acetate

of the resulting, more readily soluble, material was then taken with deuteriochloroform as solvent (Fig. 19). It is seen that the material may be considered as comprised of 2,3,6-tri-O-acetyl-β-anhydroglucose building blocks, since the six signals of the glucose residues appear as strong peaks. They can be identified readily by reference to the corresponding penta-O-acetyl-D-glucoses and octa-O-acetyl-β-cellobiose (Fig. 20) and with the aid of the assignments proposed by Roberts (15) on a nondegraded material. The extent of degradation in the case studied here seems to be very small, since the anomeric end gives rise to a very small signal. Interestingly, the three carbonyl signals are clearly resolved, whereas the three methoxyl signals are not completely separated. This is probably due to the carbonyl groups being closer to the sugar part of the polymer.

II.8 Conclusion

It has therefore been possible to analyze the ^{13}C m.r. spectra of polymers very similar to commercial cellulose ethers, by carrying out a mild degradation of these derivatives with acid or enzyme. Although the pattern of degradation is different by these two means, it seems possible to get reasonable quantitative estimations of both the D.S. and the D.P. of these polymers, as well as, to a considerable extent, to confirm the distribution of substituents on the cellulose backbone.

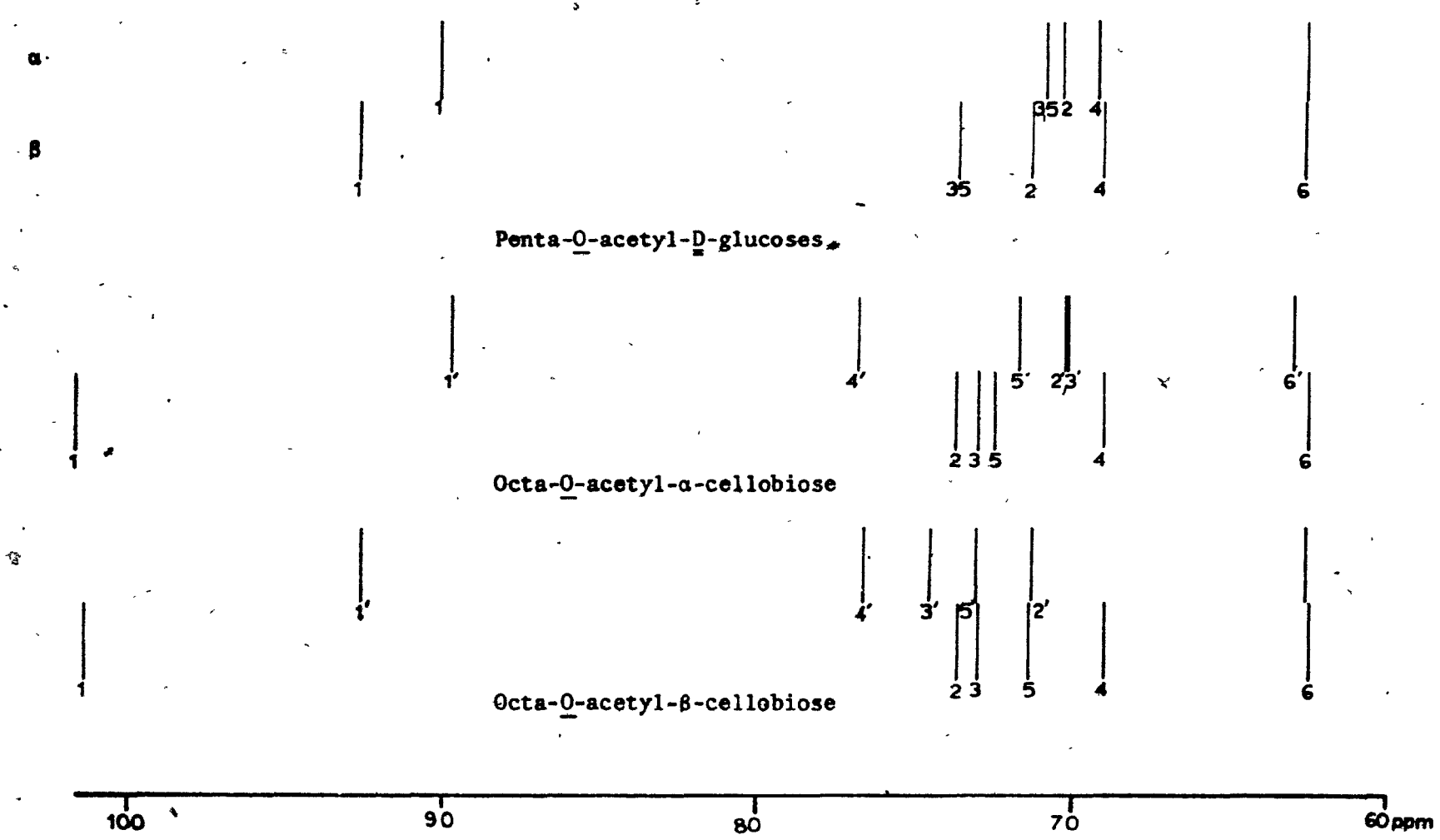

FIG. 20 ^{13}C n.m.r. spectra of the per-O-acetyl derivatives of glucose and cellobiose

CHAPTER III

CONFORMATIONAL ANALYSIS OF THE GLYCOSIDIC LINKAGE BY ^{13}C.M.R. SPECTROSCOPY

III.1 Introduction

While examining possible applications of proton decoupled c.m.r. spectroscopy in polysaccharide studies, it was also thought to be of interest to investigate some features of ^{13}C-^{1}H coupling. Since, in c.m.r. spectra, the C-1 and C-4 signals of cellulose are easily identified, the conformation of the glycosidic linkage was thought to be accessible, at least in principle, through the measurement of the $^{3}J_{C-H}$ coupling constants as shown in Fig. 21. The following section deals with model studies in this direction, in which the ^{1}H coupled c.m.r. spectra of a number of di-saccharides and their derivatives are examined.

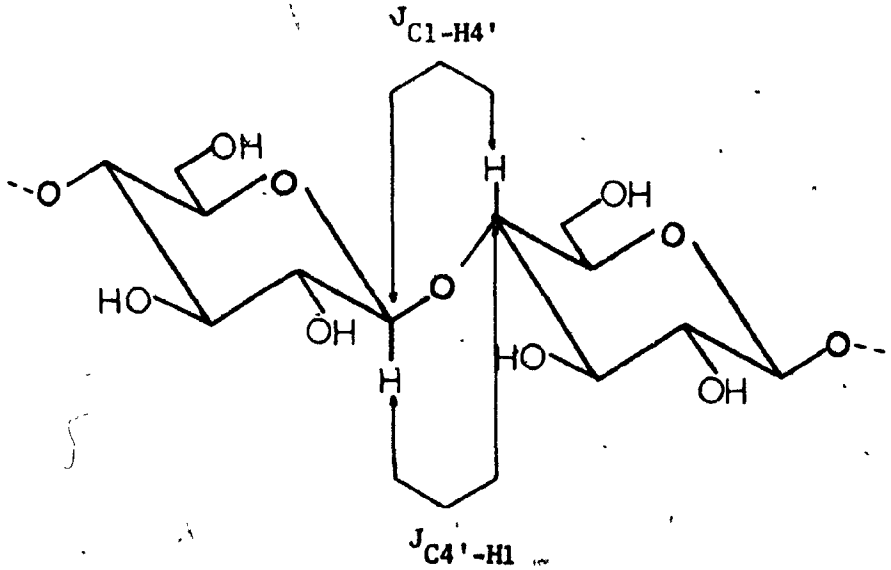

FIG. 21 Measurable coupling constants in a β(1→4) glycosidic linkage

III.2 Different approaches to the conformation of the glycosidic chain in cellulose

Recent studies by Norman (40), based on X-ray crystallographic studies combined with thermodynamical calculations, have suggested a -50° "screw dyad" conformation for the anhydrocellobiose unit, which can be considered to be the best model for cellulose. This represents a compromise

between the two extreme possible conformations described as the -90° and 0° "screw dyads", as seen in Fig. 22.

The X-ray crystallographic data on cellobiose itself do not confirm the proposed structure entirely but refine it by adding an "S twist" to the glycosidic linkage (Fig. 23).

On the basis of data obtained by n.m.r. spectroscopy (19) which suggest an orientational dependence of the Karplus type for the three bond coupling constants, an investigation of the possibility of using that dependence to predict dihedral angles was undertaken.

Previous investigations on nucleosides and glycosides (41) and cyclitols (42) have shown that this approach could be reasonably extended to other derivatives, including di- and polysaccharides. However, since the present state of instrumentation does not allow for such measurements to be made on polymers*, this study will be confined to disaccharides and some of their derivatives (Fig. 24).

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*In fact, the precision of measurements of small coupling constants is limited essentially by the following factors:

a) the resolution of the instrument (1 channel in an 8K memory is generally narrower than 1 Hz)

b) the instability of the lock signal over long periods of time. This factor varies with the solvent used, the temperature and the concentration. It can be evaluated by the line width of signals which are known to be singlets. This latter factor is generally the limiting one.

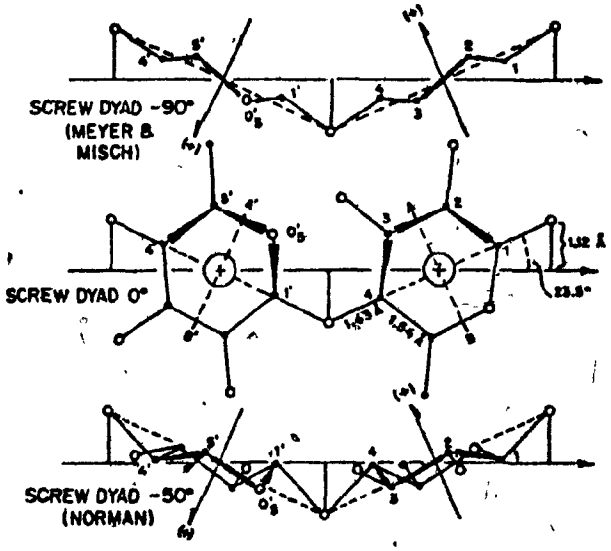


FIG. 22 The "screw dyad" conformations of the anhydrocellobiose unit in cellulose (40).

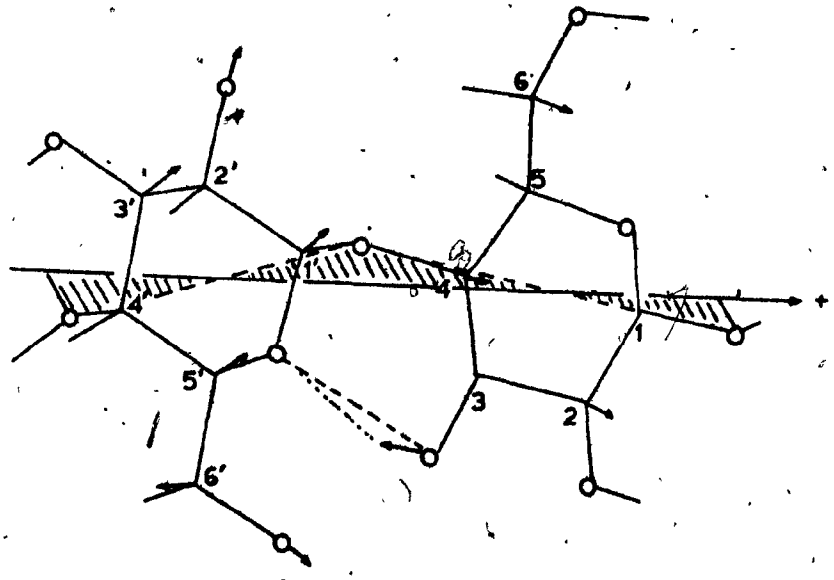
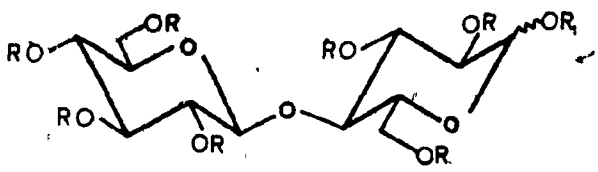
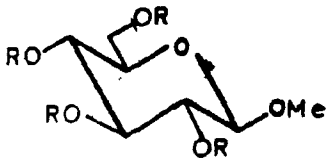


FIG. 23 Projection of the cellobiose molecule (40). Arrowheads indicate upward directions of the O-H and C-H bonds

FIG. 24 Disaccharide molecules

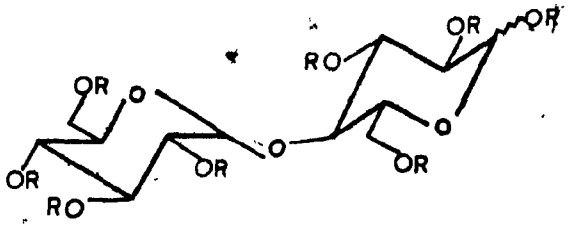


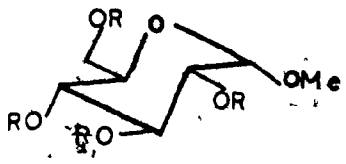


R_1 and R = H Cellobiose (15), methyl-β-D-glucopyranoside (18)

R_1 and R = Ac Octa-O-acetyl cellobiose (25), methyl-tetra-O-acetyl-β-D-glucopyranoside (30)

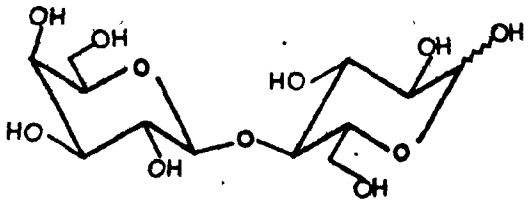
R_1 = H, R = Ac Hepta-O-acetyl cellobiose (27)

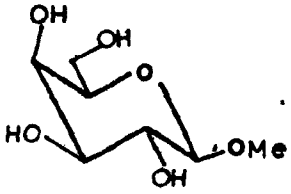




R = H Maltose (17)
R = Ac Octa-O-acetyl maltose (28)

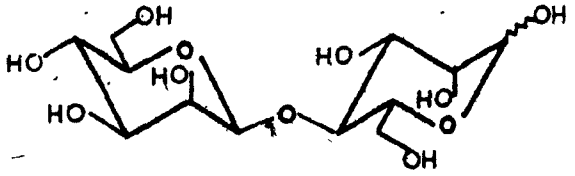
Methyl-α-D-glucopyranoside (16)
Methyl tetra-O-acetyl-α-D-glucopyranoside (31)

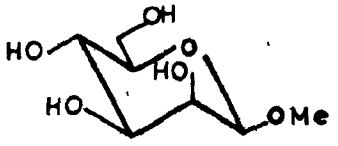




Lactose (20)

Methyl-β-D-galactopyranoside (21)





Mannobiose (22)

Methyl-β-D-mannopyranoside (23)

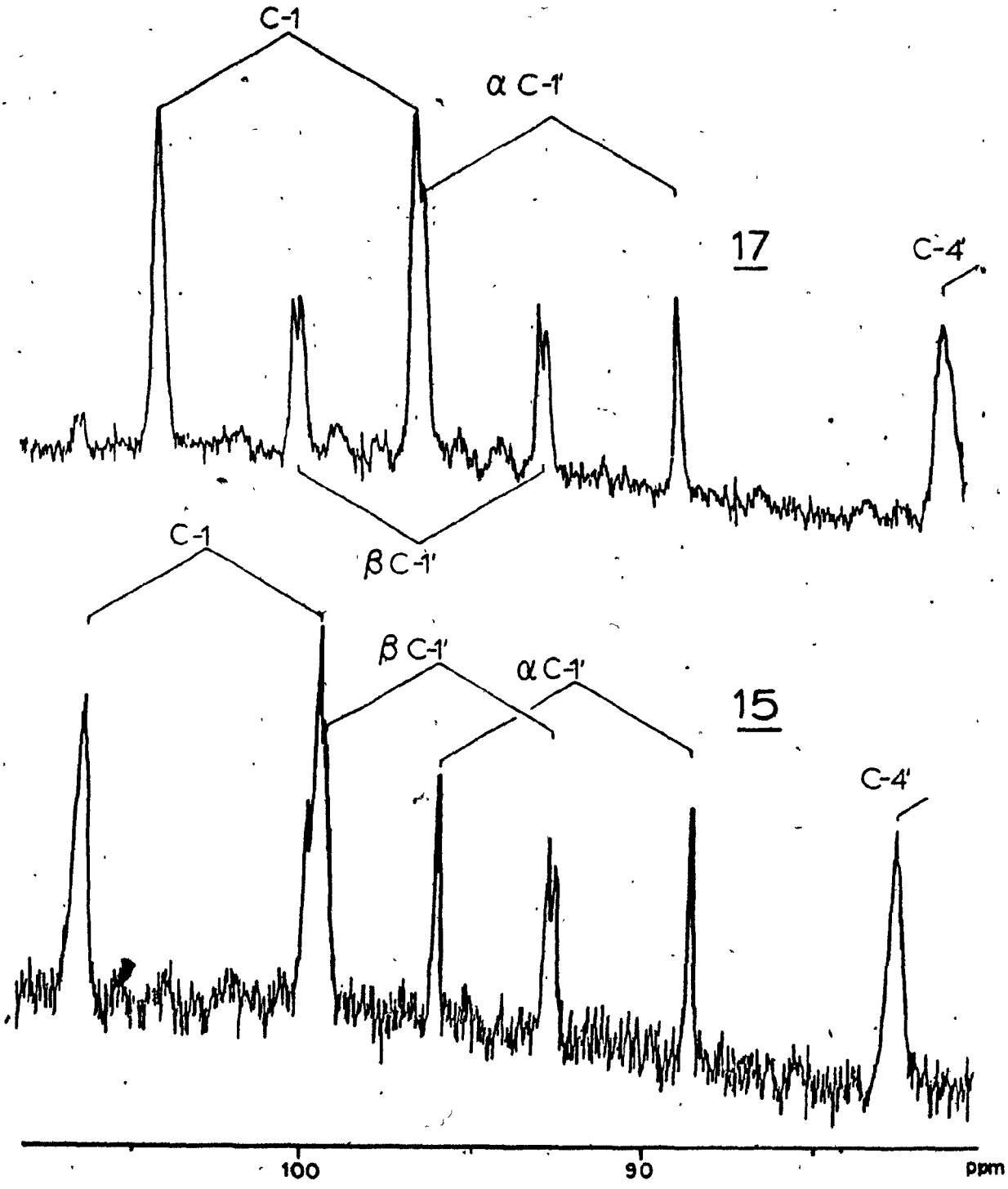
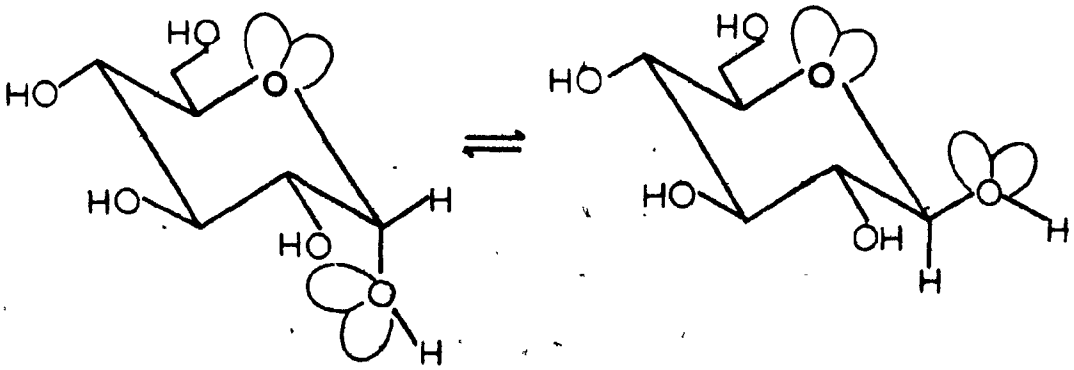


FIG. 25 ^{1}H-coupled, natural abundance ^{13}C n.m.r. spectra of cellobiose (15) and maltose (17) (low-field regions)

III.3 Reducing disaccharides (Fig. 24)*

Cellobiose (15) is a good model of the building blocks of cellulose, since it contains a β(1→4) glycosidic linkage between two glucose units. The anomeric part of its ^{1}H-coupled ^{13}C m.r. spectrum is shown in Fig. 25. Comparison with the corresponding decoupled spectrum shows that each carbon has given rise to a doublet due to coupling with the directly bonded hydrogen. The $^{1}J_{C-H}$ can therefore be easily measured: the α anomer has the larger coupling (170 Hz) while that of the β is only 161 Hz (Table 5). This may be accounted for by an increased electron density around the hydrogen atom due to lone pairs of electrons on the ring and anomeric oxygens as seen in Fig. 26.



$^{1}J_{C1-H1}$ = 170 Hz $^{1}J_{C1-H1}$ = 161 Hz

FIG. 26 The α and β configurations of glucose

In addition, the shape of these doublets show evidence of other couplings with hydrogens on neighbouring carbons. The line width (L.W.) of the carbon signals (width at half-height in Hz) can be expected to give

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*Some of the results presented here have already appeared in the literature (43).

TABLE 5

^{13}C.m.r. parameters for disaccharides and related glycosides

Compound		C-1 α	C-1 β	C-1' α	C-1' β	C-4 α/β	Coupling (^{3}J) C1-H4'	Coupling (^{3}J) C4'-H1
Cellobiose (15)	$^1J^\delta$	-	103.5	92.8	96.7	79.8	< 1	1.5
	^1J		160	170	161.3	147.5		
	L.W.		10	3	10	11.5		
Maltose (17)	$^1J^\delta$	100.9		93.2	97.2	78.9/78.6		
	^1J	175	-	175	165	138	4^6	3
	L.W.	8		4	10	12		
Methyl-D-gluco-pyranosides (16), (18)	$^1J^\delta$	100.5	104.5	-		71.4/71.2	-	
	^1J	169.9	160			145.2/142.5		
	L.W.	10^1	12.5^2			9/10	-	
D-Glucose (20)	$^1J^\delta$	93.3	97.1	-		71.2	-	
	^1J	169.6	161.9					
	L.W.	6	10^3			-		
Sucrose (19)	$^1J^\delta$	93.0		-		104.6	-	
	^1J	169.2	-			-		
	L.W.	3.7						
Lactose (20)	$^1J^\delta$	-	103.8	92.8	96.7	79.4/79.3	1	2-3
	^1J		162.5	169.5	161.5	145		
	L.W.		13	7.5	12	12.5		
Methyl-β-D-galactopyranoside (21)	$^1J^\delta$	-	105.2	-		70.1	-	
	^1J		160.5			147		
	L.W.		14^4			7		
Mannobiose (22)	$^1J^\delta$	-	102.3	96.0	95.9	-	1-2	0
	^1J		156	165	155	150		
	L.W.		8	3.7	7	10-12		
β-D-Mannose (24)	$^1J^\delta$	-	94.5	-		68.5	-	
	^1J		160.			143		
	L.W.		6			7		
Methyl-β-D-manno-pyranoside (23)	$^1J^\delta$	-	102.2	-		68	-	
	^1J		159			145		
	L.W.		12^2			12		

TABLE 5 (cont'd)

1 of which $J_{C1\text{-}H(Me)} \geq 4$ Hz

2 of which $J_{C1\text{-}H(Me)} \geq 4.3$ Hz

3 of which $J_{C1\text{-}H2} = 4$ Hz

4 of which $J_{C1\text{-}H(Me)} \geq 5$ Hz

5 this C-4' signal appears as a doublet J = 3.3 Hz

6 depending on model compound used, values of 2-5 Hz were obtained

the sum of all these coupling constants. An estimate of a particular coupling constant should therefore be accessible, for example, through a comparison with signals for corresponding carbons in glycosides and disaccharides.

As an example, the difference in the line widths of the C-1' and C-1 signals in cellobiose (15) (Fig. 25) can be attributed to coupling between C-1 and H-4' across the glycosidic linkage; similarly, comparison of the C-1 signals of β-D-glucose and cellobiose allows for an estimation of the same $^{3}J_{C1-H4'}$; in both cases, actually, it is found not to be measurable, and is probably smaller than 1 Hz. As for the C-4' signal, comparison with the corresponding signal of C-4 in D-glucose is not possible because of overlap problems, and the C-4 signal of methyl-β-D-glucopyranoside (18) is chosen instead; the difference is found to be around 1.5 Hz (Table 5).

Conversely, in maltose (17) the differences for the C-1 signals can be estimated to be about 2 to 5 Hz, depending on the model used: probably the best comparison is offered by the C-1' signal of maltose, because it can be taken from the same compound, but α-D-glucose can also be used. Sucrose (19) (Fig. 27) appears to be another good model for comparison

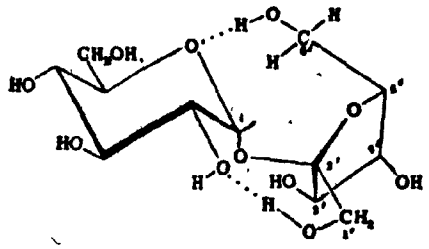


FIG. 27 The molecule of sucrose (19) (in the crystalline state)

with maltose, because of its α-D-glucopyranosyl residue, and because C-2' bears no hydrogen to couple with C-1 across the glycosidic linkage: indeed,

C-1 gives rise to a sharp signal (L.W. = 3.7-3.8 Hz), which, compared with the width of the C-1 signal of maltose, leads to a more precise value of 4-5 Hz for $J_{C1-H4'}$. This can now be interpreted by means of the Karplus-like relationship proposed for these compounds (19). Because of the wide scatter that has been observed, only rough approximations of average dihedral angles can be ventured at present. For example, little or no coupling can be associated with a dihedral angle of 90±30°, while for large couplings (3-6 Hz) angle values of 0-30° or 150-180° are possible alternatives. However, angle values higher than 90° have not been considered in this study, for the following reasons:

a) simple steric considerations based on the examination of molecular models show that they are very unfavourable.

b) a close correspondence with values obtained on crystals can only be obtained if a small ϕ or ψ angle value is considered. A gross dissimilarity between the solid and dissolved states would certainly be less attractive than the possibility suggested.

The fact that in maltose, the ^3J value is larger than in cellobiose can then be advanced as evidence that the "staggering" is more pronounced in the case of the β glycosidic linkage (larger ϕ and ψ values). This is consistent with computed energy diagrams (44) as well as the results of X-ray studies on cellobiose (45,46), maltose (47) and methyl β- maltoside (48). Accordingly, the J values suggest that these disaccharides assume similar conformations in the solid state and in aqueous solution.

Investigations of the p.m.r. spectra of a number of disaccharides in methyl sulphoxide (49,50), support the idea of inter-residue hydrogen bonding (OH-3→O-5' in cellobiose, OH-3'→OH-2 in maltose, for example) which, again

is suggestive of a similar conformation in this solvent and in the crystalline state. Also, the examination of the thermal expansibility of cellobiose and maltose in solution suggests that the β-glycosidic bond is less flexible than its α counterpart, the latter favouring an "hydrophobic folding" in the molecule of maltose (51). Of interest in this context is the ^{1}H-coupled c.m.r. spectrum of α-cyclodextrin (a cyclic trimer of maltose) obtained in this laboratory (Fig. 28). The observed splitting, attributable to $J_{C1-H4'}$ (3.5 Hz) corresponds closely to that found in maltose. The advantage of α-cyclodextrin as a model is that the molecule is rigid enough to limit bond angle fluctuations, and the $^{3}J_{C-H}$ coupling constant is readily measurable as a spacing, rather than a line broadening.

As for lactose (20) (Fig. 24), the comparison of the line widths of its C-1 and C-1' signals, and of its C-4' signal with that of methyl-β-D-glucoside (16) allows for an estimation of $J_{C1-H4'}$ of 1-1.5 Hz, whereas $J_{C-4',H-1}$ is around 2 Hz. This is not surprising, since the glycosidic linkage in lactose has basically the same environment as that in cellobiose, and O-1 and O-2 are in an eq,eq arrangement.

In mannobiose (22), whose β(1→4) glycosidic linkage is a model for the polysaccharide β(1→4) mannan, the couplings across the C1-O4'-C4' bond are both small (2 Hz and OHz, respectively, for $J_{C1-H4'}$ and $J_{C4'-H1}$) when estimated by comparison with the C-1 signal of β-D-mannose (24) and the C-4 signal of methyl-β-D-mannopyranoside (23). Extension of this to mannan then leads to a conformation of the cellulose type for these polysaccharides: the chain is extended, and assumes a ribbon-like shape. Indeed, the X-ray crystallographic patterns of ivory nut mannan (52) and sea-weed mannan (53) have been found to be similar to those of cellulose. More recent data, based on theoretical

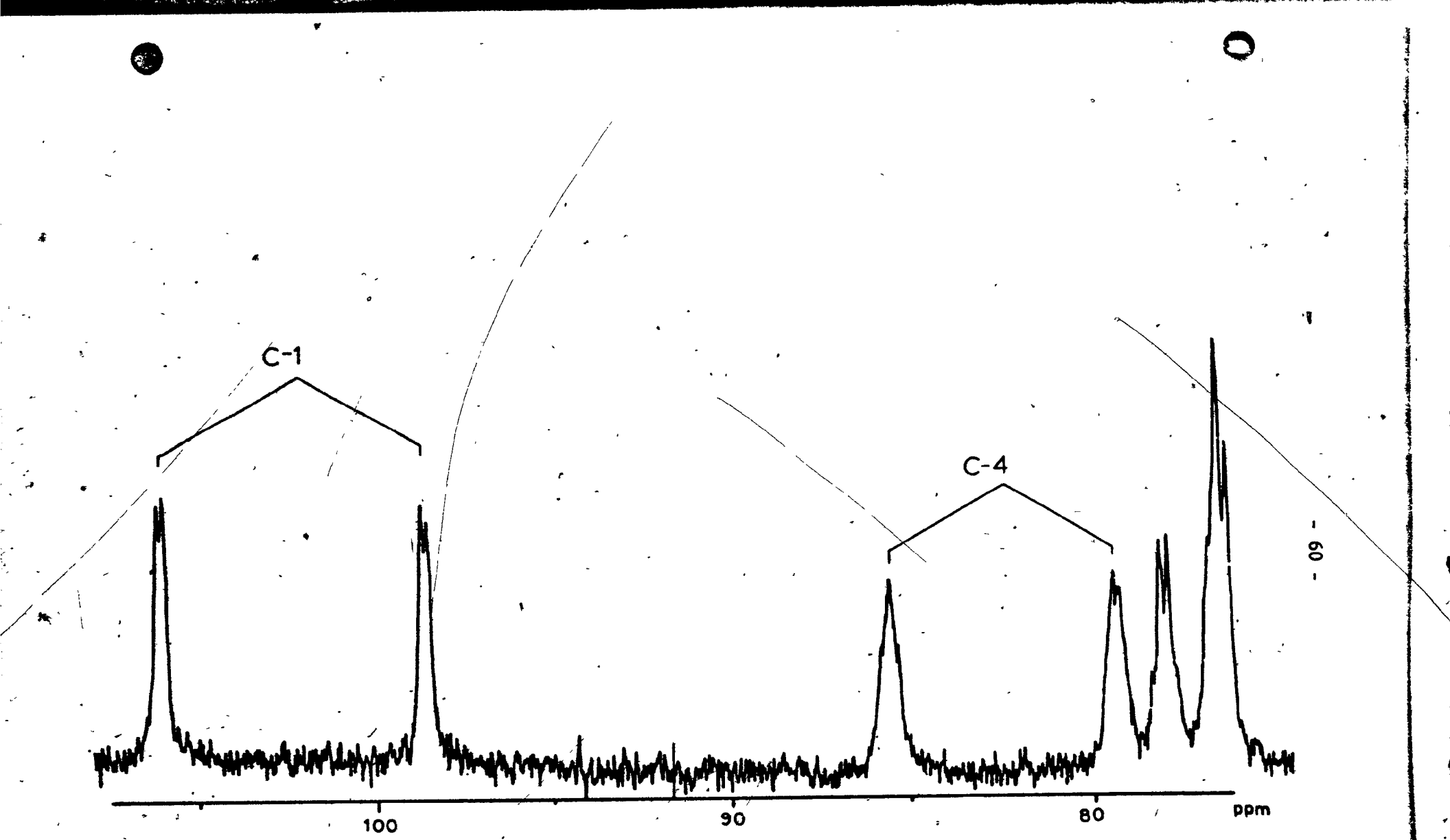


FIG. 28 Low field portion of the ^{1}H-coupled ^{13}C n.m.r. spectrum of α-cyclodextrin

calculations (54,55) have also shown a great similarity between β(1→4) glucan and β(1→4) mannan: the main difference lies in the different configuration at C-2, and some of the steric strain between O-2' and C-6 is relieved in mannan where OH-2 is ax. instead of eq, as in a glucan (54); the allowed area in the (ϕ,ψ) plane is therefore larger for mannan.

III.4 Disaccharide acetates

In acetate derivatives, the shape of the individual units is thought to be similar to that of the free sugars owing to the small size of the acetate groups (56). Moreover, no hydrogen bonding can intervene nor are there likely to be strong interactions with the nonpolar solvent used, which would influence the conformation of the disaccharide. The results of line width measurements on ^{13}C.m.r. proton coupled spectra of cellobiose and maltose derivatives are shown in Table 6. The same order of magnitude as that measured on the free sugars is found for the $^{3}J_{C-H}$ values in the acetate derivatives. Whatever the configuration of the reducing end may be, the staggering is less pronounced in derivatives of maltose (3-5 Hz) than in those of cellobiose and lactose (1-2 Hz). This finding can be proposed as evidence that inter-residue hydrogen bonding is not a predominant factor determining the conformation of the glycosidic linkage. In fact, this is what has been implied by the results of energy calculations mentioned earlier (22c), in which the same conclusion about conformation was obtained with and without consideration of hydrogen bonds.

III.5 Glycosides as disaccharide reference compounds

It is obvious, by looking at the preceding data, that the precision in the measurements of small coupling constants is low. This is in part due to the instability of the lock signal over long periods of time (16 hrs

TABLE 6

^{13}C.m.r. data on some disaccharide acetates

Compound		C1 α	C1 β	C1' α	C1' β	C4, α/β	Coupling (^{3}J) C-1,H-4'
1',2',3',6',2,3,4,6-Octa-O-acetyl-α-cellobiose (25)	^{1}J		161	178		144	2
	L.W.		12	4		11	
1',2',3',6',2,3,4,6-Octa-O-acetyl-β-cellobiose (26)	^{1}J		161		168	136	1
	L.W.		11		12	13	
2',3',6',2,3,4,6-Hepta-O-acetyl-α-cellobiose (27)	^{1}J		161	172		136	2
	L.W.		12	7.8		12	
1',2',3',6',2,3,4,6-Octa-O-acetyl-β-maltose (28)	^{1}J	174			170	164	4
	L.W.	6[1]			12.5	8	
2',3',6',2,3,4,6-Hepta-O-acetyl-β-maltose (29)	^{1}J	176		172		-	5-6
	L.W.	7-8		6		-	
Methyl-2,3,4,6-tetra-O-acetyl-α-D-gluco-pyranoside (30)	^{1}J	172		-			
	L.W.	6[2]					
Methyl-2,3,4,6-tetra-O-acetyl-β-D-gluco-pyranoside (31)	^{1}J		160			152	
	L.W.		14.5[3]			10	

(1) doublet J = 4 Hz

(2) of which $J_{C-1,H(Me)}$ = 4 Hz

(3) of which $J_{C-1,H(Me)}$ = 4.5 Hz

on the average) which causes line broadening, and hence could not be avoided with the instrumentation available. However, it should be possible to clarify the source of magnitude of long range, inter-residue, couplings by choosing suitable derivatives as reference compounds.

In the phenyl glycoside series, for example, the anomeric C-1 is not expected to be coupled with protons on the phenyl group. The C-1' signals of such derivatives, therefore, should provide a reasonably accurate basis for comparison with the line widths of C-1 signals in the proton coupled spectra of the disaccharide glycosides; the difference would be due to coupling across the glycosidic linkage ($^3J_{C1-H4'}$). The other coupling $^3J_{C4'-H1}$ is only accessible through comparison with the corresponding C-4 signal of, e.g., methyl β-D-glucoside (16), since the observed line width for C-4 of phenyl β-D-glucoside (35) is not comparable, due to a solvent change (methyl sulphoxide instead of water). Application of this reasoning is shown in Table 7; the phenyl derivatives of cellobiose (32) and lactose (34) are seen to exhibit a conformation similar to that of the corresponding free sugars, and $J_{C1-H4'}$ is found to be close to 2 Hz.

As for acetates of these derivatives, the comparisons were made between the C-1 and C-1' signals for the cellobiose (34) and lactose (36) derivatives, whereas the C-1 signal of the maltose derivative (35) had to be compared to the corresponding C-1 of methyl-α-D-glucoside (18). The β-glycosidic linkage is again clearly seen to be more twisted than the α one.

A similar observation could not be made for the C-4 signals, but this may be due to the poor quality of the model used (namely phenyl 2,3,4,6-tetra-O-acetyl-β-D-glucoside (39)). Indeed, this compound bears an acetate group at the 4 position, which may significantly influence the couplings at C-4 (as in methyl α-D-glucopyranoside (18), where $^1J_{C6-H6}$ is 141 Hz

TABLE 7

^{13}C m.r. data on phenyl glycosides of disaccharides

Compound		C-1 α	C-1 β	C-1'(β)	C4 α/β	Coupling (^3J) H1-C4'	Coupling (^3J) H4'-C1'
Phenyl-β-cellobioside (30)	δ		103.9	101.5	80		
	^1J		160	161	120	~1.5	0
	L.W.		10	8.5	10		
Phenyl-β-maltoside (31)	δ	100.9[1]		100.9[1]	77.8		
	^1J					-	-
	L.W.						
Phenyl-β-lactoside (32)	δ		94.8	87.6	81.8		
	^1J		160	160	146	3	2
	L.W.		12.5	9.5	12		
Phenyl-β-D-glucoside (33)	δ		101.8		71.2		
	^1J		158.8		143.6	-	-
	L.W.		9.5[2]		12[2]		

.... cont'd

TABLE 7 cont'd -D-Acetyl derivatives of phenyl glycosides of disaccharides

Compound		C1 α	C1 β	C-1'(β)	C1 α/β	Coupling H1-C4'	Coupling (^3J) H4'-C1'
Phenyl-β-cellobioside (34)	δ		101.7	99.9	77.5		
	^1J		159	162	144	3	4
	L.W.		13	10	12		
Phenyl-β-maltoside (35)	δ	104.1		99.3	79.3		
	^1J	164		174	156	6	2
	L.W..	12		9	10		
Phenyl-β-lactoside (36)	δ		102	99.9	77.3		
	^1J		157	160	146	3	4
	L.W.		13	10	12		
Phenyl-β-D-glucoside (37)	δ		100.2		69.5		
	^1J		160	-	150		
	L.W.		10		8		

(1) not measurable due to overlap of C-1 and C-4' signals.

(2) run in D.M.S.O.-d_6: Pine widths are to be interpreted cautiously.

whereas for the α-pentaacetate it is 148 Hz.

III.6 ^{13}C m.r. spectroscopy of 1,6-anhydromaltose and its heptaacetate

Anhydro-sugars are particularly interesting for conformational analysis because of the rigidity imparted to their molecule by the anhydro bridge. As a consequence, their conformation can be expected to be well defined and the relationship between dihedral angles and coupling constants to be more easily defined.

For the present purpose, a disaccharide possessing a 1,6-anhydro structure could provide a "reducing-end" unit having a greatly different shape than the C1 chair residues of the disaccharides already studied, and thus could introduce an additional variable for examination. The compound selected in this context is 1,6-anhydromaltose (42).

III.6.1 Peak assignments

The two model compounds used here, namely, methyl-α-D-glucoside (18) and levoglucosan (38) (Fig. 29) have already been examined by ^{13}C m.r. spectroscopy (16,57), and the chemical shifts are given in Table 8. Indeed, as far as chemical shifts are concerned, the non-reducing part of the maltosan is expected to be very similar to methyl-α-D-glucoside, while levoglucosan is a good model for the anhydro part of the molecule of maltosan. The only expected differences should arise from C-1 and C-4', the former because the effect of a methyl substituent is not quite equivalent to that of a glucose unit and the latter because substitution at C-4 induces a downfield shift of about 5 p.p.m. (an axial hydroxyl is less affected by substitution than an equatorial one*). These considerations apply well here (Table 8),

*As an example, the C-4 signal of methyl-β-D-galactoside (21) moved 9.8 p.p.m. downfield upon methylation (ax-O-Me) while that of methyl-β-D-glucoside (16) moved 6.1 p.p.m. upon methylation (eq. O-Me) (12).

TABLE 8

^{13}C Chemical shifts of some anhydro-sugars and model compounds

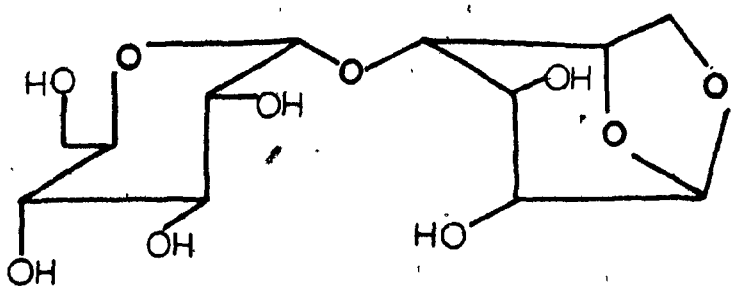
Compound	No.	Carbon number 1	2	3	4	5	6	1'	2'	3'	4'	5'	6'
Methyl-α-D-glucoside[1]	18	99.5	72.2	73.9	70.5	71.9	61.4						
Levoglucosan[2]	(40)							102.1	70.8	73.3	71.6	76.9	65.8
Maltosan	(42)	98.6	72.3	73.7[3]	70.5[4]	70.5[4]	61.5	102.0	70.5[4]	73.25[3]	76.2	76.5	66.1
Methyl-tetra-O-acetyl-α-D-glucoside	(30)	97.6	71.0	71.6	69.5	68.1	62.8						
Tri-O-acetyl-β-D-glucosan	(41)							99.5	70.1	69.9	71.0	79.0	65.5
Hexa-O-acetyl-β-maltosan	(43)	97.7	70.9	71.1	68.9	68.5	62.5	99.5	70.2	69.2	77.5	74.8	65.4

(1) from ref. 25, corrected to the int. TMS reference

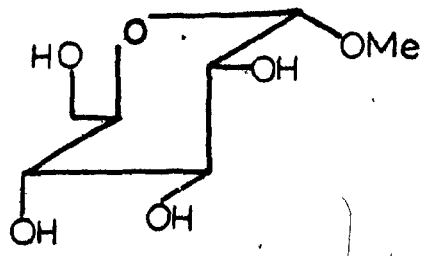
(2) from ref. 57

(3) α and β chemical shifts are reversible

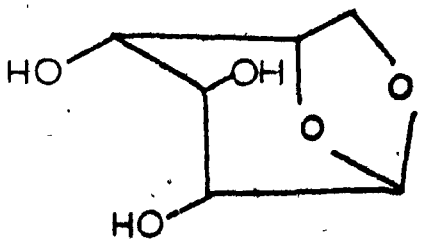
(4) not resolved



1',6'-Anhydro-maltose (42)
(maltosan)



Methyl-α-D-gluco-
pyranoside (18)



1,6-Anhydro-β-D-glucopyranose (40)
(levoglucosan)

FIG. 29

and the only remaining ambiguity is due to the overlap of signals corresponding to C-4, C-5 and C-2',

The same principles apply to the acetate derivative of maltosan, whose ^{13}C spectrum can be described as being close to the superposition of those of the acetate derivatives of glucosan (41) and methyl-α-D-glucoside (30), with the exceptions again being C-1 and C-4'. The correlation found is within the limits of 1 p.p.m., which is reasonable, given the relatively narrow range of chemical shifts examined.

III.6.2 Coupling across the glycosidic linkage

The C-1 signal of maltosan (Fig. 30), when proton coupled, appears

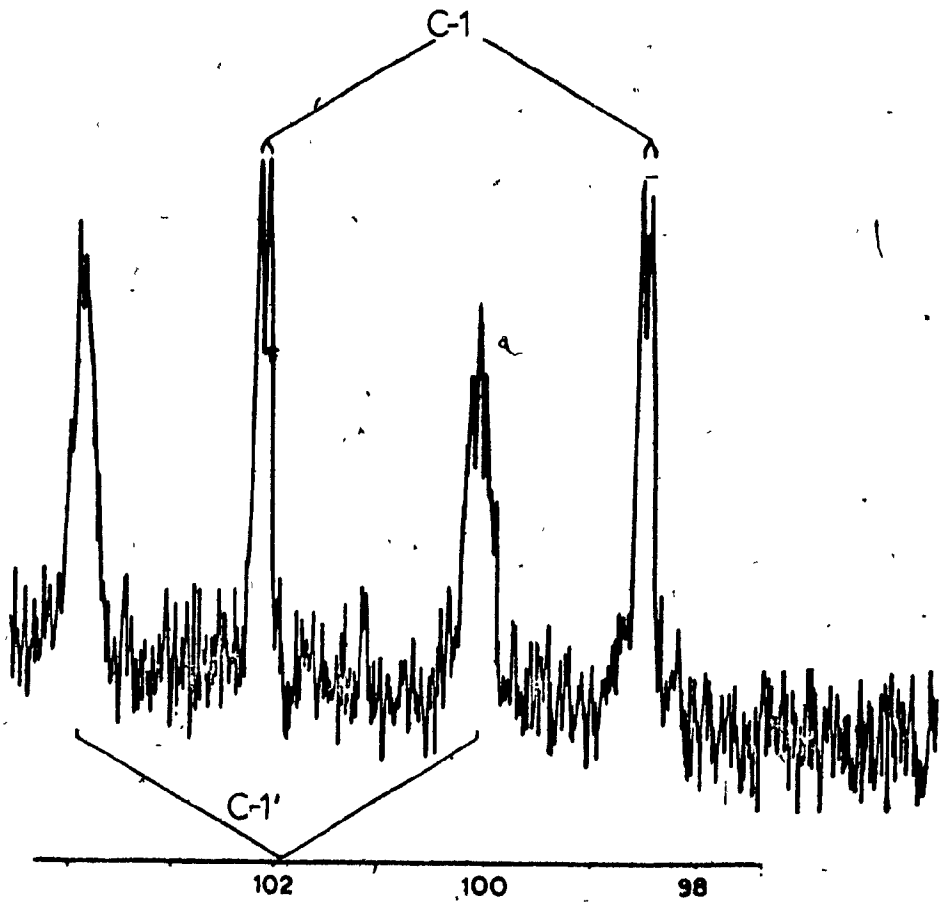


FIG. 30 Low-field portion of the ^{1}H-coupled ^{13}C m.r. spectrum of maltosan

as two doublets separated by 168 Hz corresponding to the directly bonded coupling $^{1}J_{C1-H1}$. The other measurable coupling constant is found to be 4.5. Hz. In theory, this can arise from coupling with H-2, H-3, H-5, or with H-4' across the glycosidic linkage. However, the first three possibilities can be eliminated with a fair degree of certainty, because, in the model compound methyl α-D-glucoside (Table 9) where the configuration is similar, they have all been found to be smaller than 2 Hz (21). Therefore, 4.5 Hz is attributed to the coupling constant $^{3}J_{C1-H4'}$, and if one applies the Karplus type of relationship mentioned earlier, this leads to a ψ angle of the order of 40° or 160°. The latter value can be excluded on the basis of simple steric considerations (with the help of molecular models).

Unfortunately, the other coupling constant $^{3}J_{C4'-H1}$ cannot be evaluated due to overlap between C-5 and C-4' signals. But, since steric hindrance seems to be the major contributing factor to disaccharide conformation, the examination of models suggests a small value for the ψ angle.

It is interesting to note that a very similar conformation seems to prevail in the acetate derivative of maltosan (Table 9 and Fig. 31). Again, the ψ value would be around 40°, while ϕ is not measurable. This tends to prove that the conformation of 43 in solution does not depend to a large extent on the possibility of inter-residue hydrogen-bonding. It may have a stabilizing effect, when formed, but most probably is a secondary factor, rather than the primary one determining the conformation of the inter-residue linkage.

TABLE 9

^{13}C m.r. data on maltosan and glucosan, and their acetate derivatives

Compound			C1	C1'	C4 or C4'	Coupling(^3J) C1-H4'
maltosan	(42)	δ	98.6	102	76.2	
		^1J	168	174	∿150	
		L.W.	8[1]	10	(2)	4.5
levoglucosan	(40)	δ	102.1		71.6	
		^1J	175.5		145	-
		L.W.	12.6		7.5	
hexa-O-acetyl-maltosan	(43)	δ	97.7	99.5	77.5	
		^1J	171	178	148	
		L.W.	9[1]	10	14-15	
tri-O-acetyl levoglucosan	(41)	δ	99.5		71	
		^1J	177		∿150	
		L.W.	12		(2)	

(1) appears as a doublet (J≈4.5 Hz)

(2) not measurable

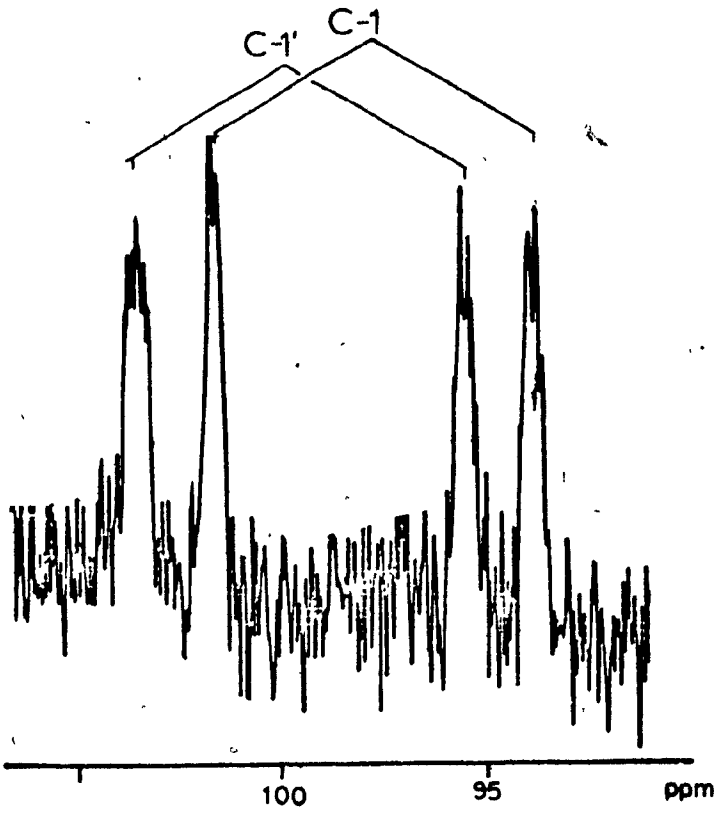


FIG. 31 Low-field portion of the ^{1}H-coupled ^{13}C m.r. spectrum of hexa-O-acetyl maltosan

III.6.3 An attempt to develop a new synthesis of 1,6-anhydro-sugars

1) Introduction

In conjunction with the preparation of disaccharides possessing a 1,6-anhydrohexose residue (previous section), it was of interest to examine the possibility of devising an improved route to anhydrides of this general class.

1,6-Anhydro-β-D-glucose (levoglucosan) has been known for many years as a product of the treatment of glycosides with a base (58). It is obtained nowadays routinely either as a product of the pyrolysis of starch (Pictet Method, 59) or by alkaline hydrolysis of phenyl β-D-glucoside (60)(Fig. 32). The

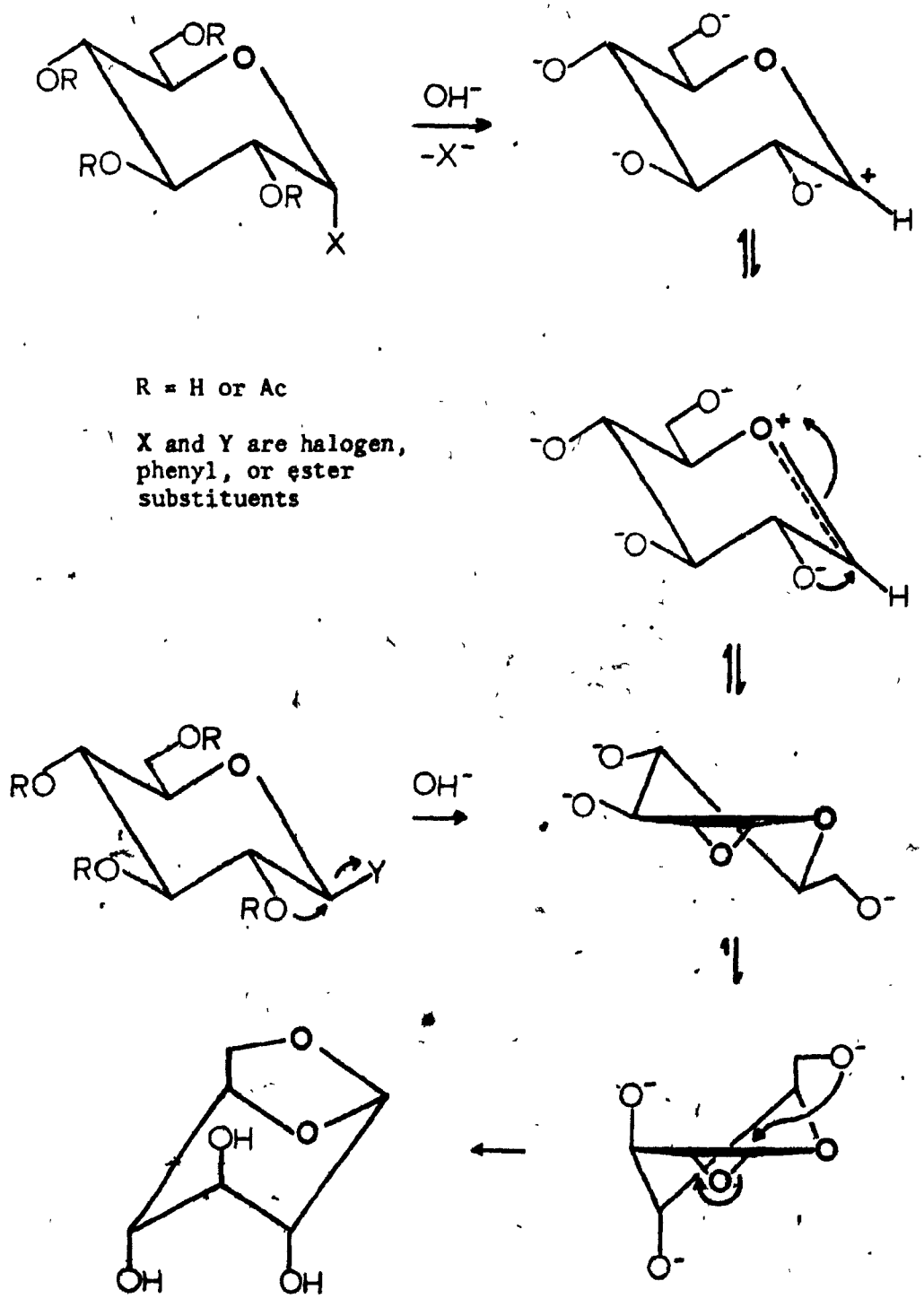


FIG. 32 Mechanism of formation of 1,6-anhydro-β-D-glucopyranose

Karrer method (61) using tetra-O-acetyl β-D-glucopyranosyl trimethylammonium bromide treated with base is another alternative. But these reactions suffer some drawbacks. The first gives a low yield; the second and the third methods involve more steps, and the levoglucosan can only be isolated through acetylation.

Most reactions leading to levoglucosan involve the base catalyzed nucleophilic displacement of a suitable substituent ~~on C-1~~ (Fig. 32).

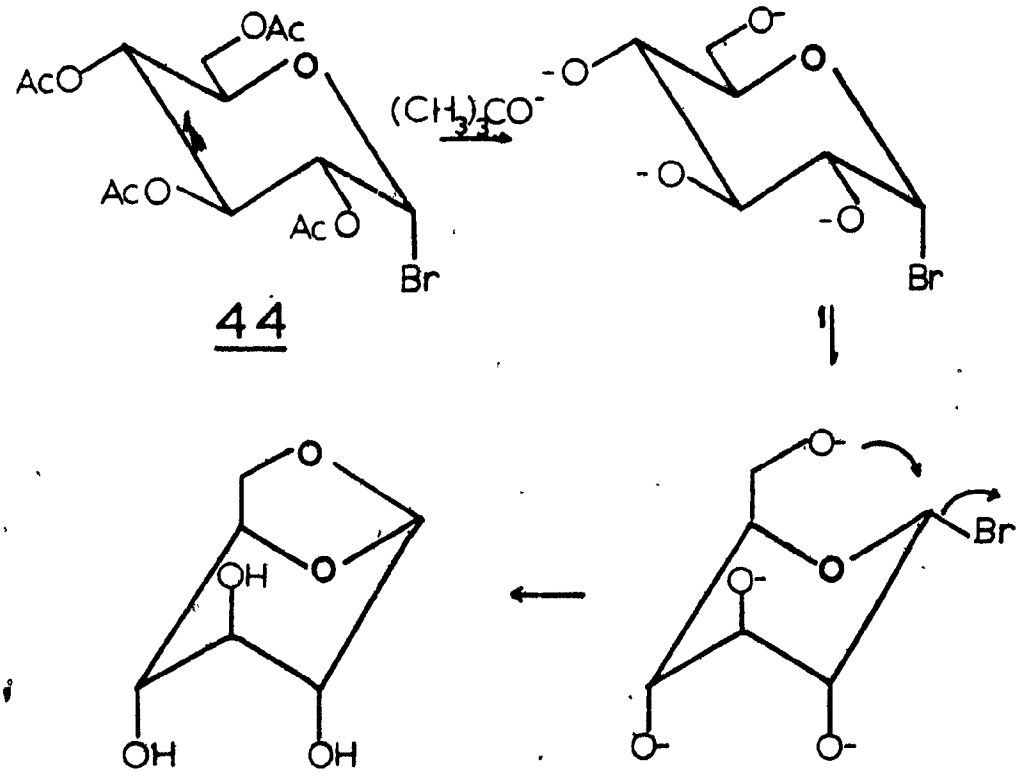


FIG. 33 Levoglucosan from acetobromoglucose

Thus, α- and β-D-glucosyl fluorides (62) as well as β-D-glucosyl azide (63) and phenyl-β-D-glucoside (64) yield levoglucosan when treated with

base, as do 2,3,4,6-tetra-O-acetyl-α-D-glucosyl nitrate (65), and 2,3,4-tri-O-acetyl-α-D-glucosyl bromide (66). In the case of 2,3,4,6-tetra-O-acetyl-D-glucosyl mesitoate, levoglucosan is obtained only with the β-anomer (67).

All these reactions possess several characteristics:

a) The substituent at position 1 must be a good leaving group, thanks to its size, or to its electronegativity.

b) The 6-hydroxyl group must be ionized rapidly in a strongly alkaline medium to the alkoxide ion, so that it can act as a nucleophile on C-1.

c) If the substituent at C-1 is a strongly electronegative group, the α- or β-anomer can be used as starting materials. Indeed, the intermediate carbonium ion is stabilized by resonance (involving the lone pairs on O-5) or by the participation of the neighbouring C-2 hydroxyl (Fig. 32) as demonstrated by the non-reactivity of the 2-O-methyl derivative (68) towards alkali.

For a better compliance with these requirements, tetra-O-acetyl-α-D-glucopyranosyl bromide (44), a readily available derivative of glucose and, in fact, an intermediate in some of the syntheses cited above was thought to be a suitable starting material. The base used here was t-BuOK (potassium tert-butoxide). Its advantage is that it is a very strong base whose nucleophilicity is poor enough not to compete with that of the primary alkoxide ion at C-6. In the reaction described, it is used both as a transesterification agent (in de-O-acetylation), and as a base to ionize the hydroxyl groups.

2) The reaction (Fig. 33) with acetobromoglucose

When a solution of t-BuOK was added to a solution of acetobromoglucose (44), the rapidly darkening colour of the reaction medium showed that at least part of the sugar moiety was destroyed. When up to two equivalents of t-BuOK were added, and after several hours, 1,6-anhydro-β-D-

glucose (40) could be detected by g.l.c. as by far the major product of the reaction, the other being glucose or its t-butyl-glycoside. An attempt to isolate 40 through acetylation was unsuccessful, which suggested that the quantitative yield was low. This may be due to several reasons:

a) the rate of deacetylation by the t-BuO$^-$ ion is too slow, and in any case slower than the rate of nucleophilic attack of this same t-BuO$^-$ ion on C-1.

b) the bromide ion is too good a leaving group, due to its size (apparently despite the stabilizing "anomeric effect" when this group is in the α position).

c) the strongly basic solution can attack the glucose ring as soon as C-1 is in the carbonium ion form, thus destroying it through elimination reactions.

3) Reaction with acetochloroglucose (Fig. 34)

In an attempt to overcome, at least partially, these difficulties, another starting material, namely, 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl chloride (45) was tried. The same procedure as with the α bromo derivative was applied, and a similar apparent result was obtained (dark colour, good relative yield of 1,6-anhydro-β-D-glucose), but acetylation of the reaction mixture allowed for the isolation of crystalline 2,3,4-tri-O-acetyl-1,6-anhydro-β-D-glucose (41) in a yield of 15%.

4) Conclusion

Although this must be considered as a preliminary result, it does suggest that the tertiary butoxide is not an ideal medium for such a reaction. However, the absence of glucose or t-butyl glucoside among the products of this reaction indicates that the direct nucleophilic attack of t-BuO$^-$ on C-1

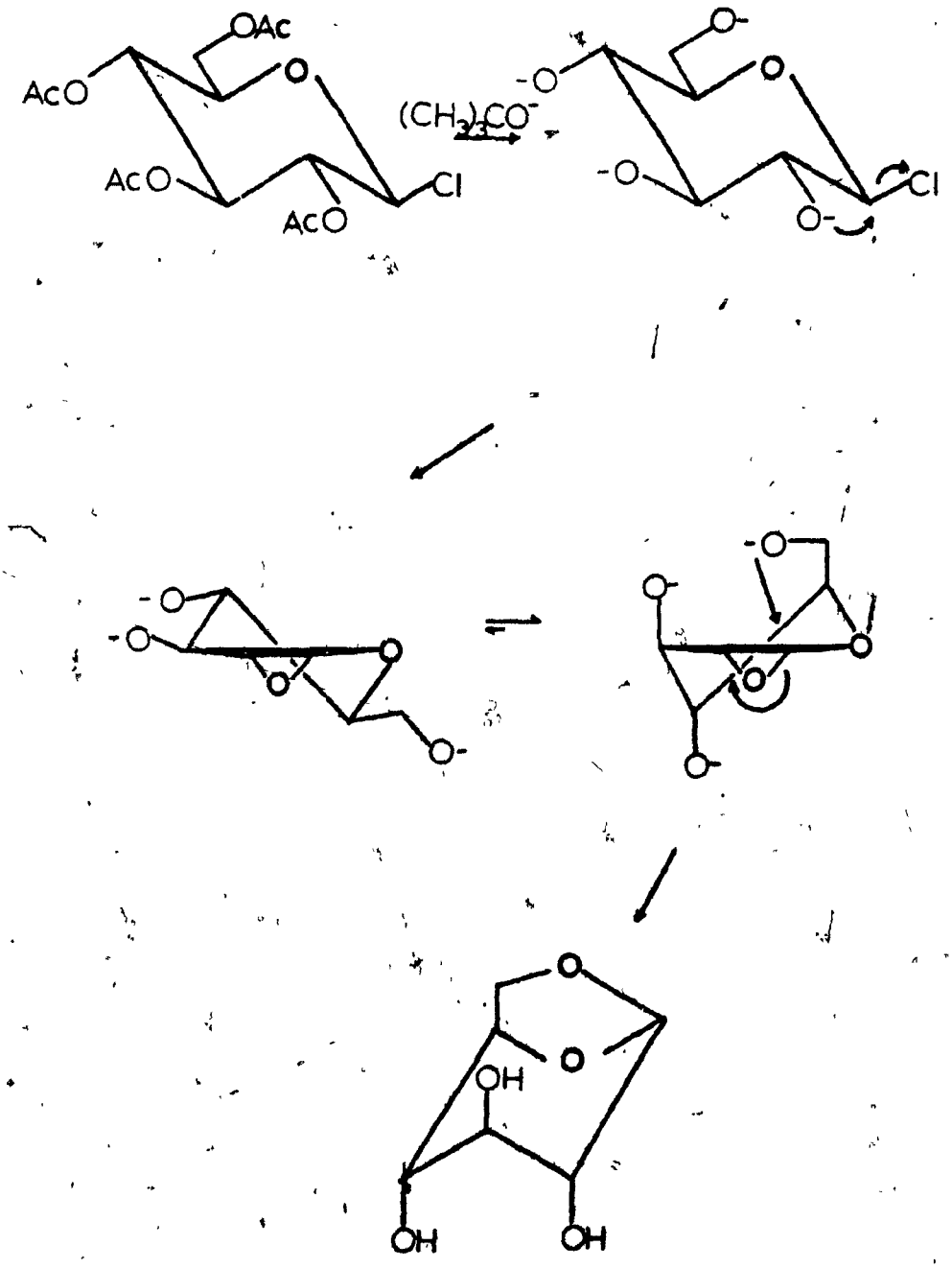


FIG. 34 Levoglucosan from acetochloroglucose

from the α side of the molecule is quite unlikely, due probably to steric hindrance.

The competition between levoglucosan formation and degradation might be more favorable in a less strongly basic medium such as sodium isopropoxide (measurements based on kinetic studies (69) have shown that basicity increases with alkylation of the alkoxide) as suggested by some results obtained in this laboratory (70), although glycoside formation might become more prominent with a less hindered alkoxide.

CHAPTER IV

EXPERIMENTAL

IV.1 General procedures

P.m.r. spectra were recorded at room temperature, using TMS as an internal lock signal, with a Varian HA-100 spectrometer (field sweep mode).

^{13}C m.r. spectra were recorded with a Bruker W H.-90 spectrometer operating at 22.63 MHz. Spectral accumulations and Fourier transformation were accomplished using its 8K memory, B.NC13 computer. Proton coupled spectra were recorded using the "gated decoupling" technique. Unless otherwise specified, the parameters were as follows:

sample temperature: 30°C.

concentration: 100 to 200 mg./ml.

pulse width: 24 μs.

The chemical shifts given are in the δ scale (TMS internal standard). The accuracy of the measurements is considered to be .05 p.p.m. When TMS was not present in the sample $CDCl_3$ (center line at δ = 140.1 p.p.m.) or methanol (singlet at 49.6 p.p.m.) was used as the reference.

Melting points were determined using a Fischer-Johns hot plate apparatus, and are uncorrected.

Evaporations were carried out at a pressure of about 20 mm Hg at a temperature of 40°C or less.

Gas chromatography was conducted with a Hewlett-Packard F and M 402 chromatograph using a 4 feet chromosorb (NAW, DMCS) column (4% U.C.W.) coated with silicone gum (SE 30).

Paper chromatograms were prepared with Whatman no.1 paper, using the descending technique. The solvents commonly used were:

A: Ethylacetate:98% formic acid:water = 12:1:12. (v/v) (top layer)

B: Ethylacetate:acetic acid:water: = 9:2:2 (v/v)

C: n-Butanol:ethanol:water = 5:1:4 (v/v)

D: Methyethyl ketone saturated with water (top layer)

Visualization was effected by spraying with aniline oxalate (71) and heating 5 min. at 80°C.

Thin layer chromatography plates were prepared using silica gel G (Macherey-Nagel) as adsorbent. Benzene-acetone (1:1, v/v), benzene-methanol (9:1, v/v) or ethyl acetate-ethanol (3:2, v/v) were commonly used. For developing, spraying with concentrated H_2SO_4 was followed by heating the plate with a flameless drier.

IV.2. Preparation of cellulose derivative solutions for spectroscopy

The following procedures were used to render the various derivatives suitable for examination by ^{13}C m.r. spectroscopy.

A) Enzyme degradation of carboxymethylcellulose (31)

One gm. of carboxymethylcellulose (Hercules Powder Co., Wilmington, Delaware) was triturated in 10 ml. of a solution of 30 mg. of cellulase (Streptomyces sp.QMB814) in 0.1M sodium acetate buffer (pH=5.5). When homogeneous, the mixture was kept at 40°C in a water bath for 12 hrs; then the enzyme was denaturated by heating the solution at 100°C for 15 min. The solution was then dialyzed against running tap water for 2 days and against distilled water for 8 hrs. After freeze-drying of the solution 0.2 g of the material was dissolved in 1-2 ml. of water, giving a viscous solution. To obtain a strong lock signal, a coaxial tube containing C_6D_6 was added to the sample tube.

A similar procedure was used for preparation of the hydroxyethyl-cellulose sample.

B) Complete acid degradation of carboxymethyl cellulose

Three gms. of carboxymethylcellulose powder (Hercules Powder Co.) were mixed mechanically with 20 cc. of 72% H_2SO_4 and kept at 40°C for 1 hour. After dilution to 1.5 l, the mixture was autoclaved for 1 hr. (2 atm.) (32), then neutralized with $BaCO_3$ and the suspension was filtered through Celite. The effluent was treated with Amberlite IR 120 (H^+) resin, concentrated, and and finally freeze-dried. A concentrated solution in D_2O was used for ^{13}C m.r. examination.

A similar method was used for the degradation of methylcelluloses.

C) Partial hydrolysis of methyl celluloses

a) For a low D.S. (< 1) sample, enzymatic degradation by a procedure similar to that used for CMC was employed.

b) Medium D.S. (1-2): the sample (1 g.) was triturated in hydrochloric acid (10 ml., 1 N) and the mixture kept 3-5 hrs. on a steam bath. The sample was then freeze-dried and redissolved in D_2O. The C.m.r. spectrum was obtained with a coaxial tube containing C_6D_6 which served as an external lock signal.

c) High D.S. (> 2.5): One gram of the sample was dissolved in dichloroethylene (10 ml.) and 1.5 ml. of conc. hydrochloric acid was added. The resulting viscous solution was kept under reflux for 1-2 hr., and the solution then concentrated to dryness. The c.m.r. spectrum was recorded using $CDCl_3$ or else $CHCl_3$, with C_6D_6 being then used as in b) to provide an external lock.

D) Partial hydrolysis of ethyl cellulose

One gram of ethyl cellulose (Fisher Scientific Co.) was dissolved in acetone-water (90:10, 10 ml.) which was 1 N with respect to HCl and heated under reflux for 1 hr. Water (100 ml.) was added, and the mixture was freeze-dried. A solution of 200 mg. in 1 ml. of D_2O was used for examination by

c.m.r. spectroscopy.

E) Partial acetolysis of cellulose acetate (72b)

Cellulose acetate (D.S. = 2.3, approximately) (5 g.) was stirred vigorously in acetic anhydride (100 ml.) until dissolved, concentrated sulfuric acid (10 ml.) was then introduced and stirring was continued for 5 hrs. at 40°C. The mixture was then carefully poured into ice-water (500 ml.), the precipitate was filtered off and thoroughly washed with water until the washings were neutral. The precipitate was dried in vacuum over sodium hydroxide. It exhibited no OH absorption band by infrared spectroscopy.

IV.3 Permethylation of methyl cellulose (73)

Methyl cellulose (Fisher Scientific, high viscosity grade) (4 g.) was dried at 50° under vacuum overnight, then suspended in dry methyl sulphoxide (over 4Å molecular sieves). A stream of dry nitrogen was introduced, and after 4 hours, a suspension of NaH in oil (1 g. 57%) was slowly added with stirring. The mixture was kept at room temperature for 12 hrs., then methyl iodide (10 ml.) was added in two portions at an interval of 2 hours. After a further reaction time of 2 hours, the excess CH_3I was evaporated off under vacuum, and the reaction was performed a second time in the same way.

The resulting mixture was then poured into ice-water (500 ml.) with stirring, the white precipitate was collected, washed with water and ethanol, dried, and redissolved in chloroform. Precipitation was effected with light petroleum ether, and the precipitate was vacuum dried. At this stage, the methyl cellulose did not show any OH absorption band in the infrared region, and was therefore considered to be fully methylated.

IV.4 Syntheses of mono-O-carboxymethyl glucoses (23)

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

D-Glucose (200 g.) was treated with anhydrous acetone in the presence of concentrated sulfuric acid (72a); yield: (1) 100 g., m.p. 108-110° (lit. 110°; (73b)), (2) 34 g., m.p. = 160° (lit. 160 (72a)).

1,2-O-Isopropylidene 3,5,6-tri-O-benzyl-α-D-glucofuranose (3)

Benzylation was carried out according to the procedure described by Fletcher (72a). Ten grams of 2 were finely ground in a Waring blendor and suspended in 150 ml. of freshly-distilled benzyl chloride contained in a 3-necked flask and protected against moisture. Powdered KOH (30 g.) was added, the mixture was stirred vigorously at 100°C for 1 hr., after which time another 30 g. of KOH was added. Heating was contined for another 4 hours, then water (300 ml.) was added to the cooled vessel to dissolve the mass of salts formed, and the resulting solution was extracted with ether. The extract was dried over anhydrous sodium sulfate and concentrated to give a light yellow syrup of 1,2-O-isopropylidene – 3,5,6-tri-O-benzyl-α-D-glucofuranose (3); yield, 16 g. (74%).

3,5,6-Tri-O-benzyl-D-glucofuranose (4)

A solution of 3 (7 g.) in sulfuric acid (1%, 125 ml.) was heated under reflux for 3 hrs. Neutralization was effected with a solution of sodium bicarbonate, and extraction into chloroform then gave a solution which was dried over anhydrous sodium sulfate, decolorized with charcoal (Darco G 60) and concentrated, yielding a clear syrup (5.6 g.) of 3,5,6-tri-O-benzyl-D-glucofuranose (4),

Methyl 3,5,6-tri-O-benzyl-D-glucofuranoside (5)

A solution of 4 in 1 N methanolic HCl (100 ml.) was heated under reflux

for 4 hrs. After neutralization with barium carbonate and filtration through a Celite bed a clear solution was obtained. Concentration, and drying of the residue over phosphorus pentoxide yielded a light yellow syrup (4.5 g, 88%) of 5.

Methyl-3,5,6-tri-O-benzyl-2-O-carboxymethyl-D-glucofuranoside methyl ester (6)

The method devised by Timell (23) was used with little modification: 4 g. of product 5, when treated with a dispersion of sodium followed by methyl bromoacetate, yielded a reddish syrup (6), which was passed through a column of silicic acid (Silica Gel), using toluene/ethanol 100/1, (v/v) as the eluent. The fractions containing 6 were combined (the process was followed by t.l.chromatography in toluene/ethanol:5/1, v/v), and on concentration yielded a red syrup (1.4 g., 30%).

Methyl 2-O-carboxymethyl-α/β-D-glucofuranoside methyl ester (7)

The crude preparation of 6 was dissolved in methanol, palladium black (10% on charcoal) (0.5 g.) was added, and hydrogen gas was introduced at atmospheric pressure, while the suspension was stirred vigorously. After 48 hrs., when hydrogen consumption had ceased, the suspension was filtered and concentrated yielding syrupy 7 (0.9 g., 94%).

2-O-Carboxymethyl-α,β-D-glucopyranose (8)

A solution of 7 in water (30 ml.) containing Amberlite IR-120 (H^+) was heated for 4 hrs., then treated with Darco G 60, and on concentration yielded a small amount of material which was freeze-dried for easier handling. Paper chromatography in solvent A (23) suggested that its composition was approximately 70% of 8, the rest being glucose. This was confirmed by the ^{13}C n.m.r. spectrum of 8 which exhibited 2 minor anomeric signals corresponding to the α and β anomers of D-glucose in addition to those of 8.

3-O-Carboxymethyl-1,2:5,6-di-O-isopropylidene-D-glucofuranose methyl ester (9)

1,2:5,6-Di-O-isopropylidene-D-glucofuranose (1) (10 g.; thoroughly dried) was dissolved in absolute ethyl ether (150 ml.) and a sodium hydride dispersion (2 g., 57% in oil) was added under dry nitrogen. Stirring was continued for 24 hrs., after which methylbromoacetate (10 ml.) was added, and the reaction was continued for 72 hrs. The mixture was then extracted with ether, the extract was concentrated, and diisopropyl ether added; crystallization occurred after 12 hours in the cold (5°C). The crystals (5 g., 48%) were washed with diisopropyl ether; m.p. 103° (lit. 103–104.5° (23)).

3-O-Carboxymethyl-D-glucopyranose (10)

The di-O-isopropylidene derivative (9) (2 g.) in water (30 ml.) was heated with Amberlite IR-120 (H^+) ion exchange resin under reflux for 8 hrs. Filtration and treatment with Darco G 60 decolorizing carbon yielded a colorless syrup (10) which was freeze-dried. The c.m.r. spectrum (Table 2) showed that the product had a high degree of purity, since no spurious signals of appreciable intensity were detected.

6-O-Acetyl 1,2:3,5-di-O-methylene-α-D-glucofuranose (11)

D-Glucose (100 g.), treated with paraformaldehyde in acetic acid as described by Jones et al. (74) yielded 15 g. (13%) of (11); m.p. 101-102° (lit. 104°; (74)).

1,2:3,5-Di-O-methylene-α-D-glucofuranose (12)

De-O-acetylation of 11 (12 g.) was effected with methanol (20 ml.) containing sodium methoxide (1 g.). After 1 hr. at room temperature, the solution was cooled in an ice bath and an excess of Amberlite IR-120 (H^+) was added. Filtration yielded a solution which was concentrated to a clear

syrup (12) and dried in vacuo. Yield: 9 g. (90%).

1,2:3,5-Di-O-methylene-6-O-carboxymethyl-α-D-glucofuranose methyl ester (13)

Compound 12 (5 g.) was dissolved in dry dioxane (50 ml.) and a dispersion of sodium (50% in oil, 10 ml.) was added under dry nitrogen. Stirring was continued for 16 hrs., and methyl bromoacetate (25 ml.) was added slowly to the suspension. After 12 hrs., the red brown mixture was extracted with ether, and the ether layer was washed with water (3 x 100 ml.). Concentration and drying yielded a thick red syrup which was treated once more with sodium and methyl bromoacetate. Thin layer chromatography of the material obtained after the second treatment indicated a yield of at least 50%. Thus it was purified by passage through a column of silicic acid, using as the eluant chloroform containing increasing amounts of acetone (5 to 40%, v/v). Addition of ethanol to the concentrated eluent induced crystallization of 13 in the form of long white needles (1 g., 20%) which melted at 46° (lit. 46.5-47.5° (23)).

6-O-Carboxymethyl-D-glucopyranose (14)

Hydrolysis of 13 (0.5 g.) was effected by treatment with an excess of Amberlite IR-120 (H^+) in boiling water for 8 hrs. The resulting solution was filtered, decolorized with Darco G 60 and freeze-dried (yield, 200 mg., 70%). The purity and identity of 14 were checked by c.m.r. spectroscopy (Table 2).

IV.4 Synthesis of mono-O-hydroxyethyl-D-glucoses

3-O-Hydroxyethyl-1,2:5,6-di-O-isopropylidene-D-glucofuranose

The procedure devised by Timell (36) was used here. The methyl ester of the O-carboxymethyl derivative 9, (1.0 g.) was reduced with lithium aluminum hydride, yielding 0.7 g. of compound 15 (90%).

3-O-Hydroxyethyl-α,β-D-glucopyranose (10a)

Product 15 (0.7 g.) was hydrolysed with Amberlite 1R-120 (H^+) resin in boiling water as indicated above under 14, yielding a syrup which was freeze-dried for easier handling. The c.m.r. spectrum of the product (Table 2) was consistent with structure 10a.

6-O-Hydroxyethyl-α,β-D-glucopyranose (14a)

Reduction of 13, (500 mg.) was carried out with lithium aluminum hydride (34), and the material obtained (17) was hydrolyzed with Amberlite 1R-120 (H^+) resin and freeze-dried (yield 200 mg., 45%). The c.m.r. spectrum of the product (Table 3) was consistent with structure 14a.

IV. 5 Preparation of disaccharide derivatives and model compounds

Cellobiose (Eastman), maltose and lactose (Anachemia) were commercial products and were used without further purification.

α-Cellobiose octaacetate was obtained by acetolysis of cellulose acetate as described in ref. 73c, whereas the β anomer was prepared from commercial cellobiose using acetic anhydride and sodium acetate (72b).

The phenyl glycosides were prepared via their acetate derivative, de-O-acetylation being effected with sodium methoxide in methanol (72b).

Other compounds whose spectra are described here were present in this laboratory and were used as found (or after recrystallization, if the melting point was not judged satisfactory).

IV. 6 Attempt to synthesize 1,6-anhydro-β-D-glucopyranose (38)

2,3,4,6-Tetra-O-acetyl-α-D-glucosyl bromide (42)

Acetobromoglucose (42) was synthesized from D-glucose using a mixture of acetic anhydride, bromine, and red phosphorus (73b). The product,

after recrystallization from ethyl ether, melted at 86-88° (lit. 88-89° (73b)).

1,6-Anhydro-β-D-glucopyranose (38)

Acetobromoglucose (1.0 g., 2.4 millimoles) was dissolved in dry benzene (2 ml.) in a 100 ml. flask under dry nitrogen. A solution of potassium t-butoxide (0.42 M, 15 ml., prepared by dissolving potassium metal in dry t-butanol) in t-butanol, was slowly added to the stirred solution. The mixture turned to a dark brown colour within a few minutes, but 1,6-anhydro-β-D-glucose (38) was detected by g.l.c. only after several hours. After 24 hrs., 38 was the main product in the mixture. Centrifugation left a yellow solution which was neutralized with Amberlite IR-120 (H^+) ion exchange resin, and partially decolorized with Darco G 60 carbon black. Evaporation yielded a dark syrup which was found by g.l.c., to consist mainly of 38.

2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyl chloride (43)

The title compound was prepared in one step from penta-O-acetyl-β-D-glucose following a known method (72a) using aluminum chloride in chloroform; m.p. 95° (lit. 99-100° (72a)).

1,6-Anhydro-β-D-glucopyranose (38)

The same basic procedure as above for 38 was followed here. However, a larger amount of syrup was recovered, when the reaction was performed on a 10 g. scale, and acetylation of the syrup with acetic anhydride and sodium acetate (72a) gave another brownish syrup. Crystallization from ethanol afforded 0.4 g. (~10%) of 2,3,4-tri-O-acetyl-1,6-anhydro-β-glucopyranose; m.p. 106-107° (lit. 108-109°, (72a)).

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CLAIMS TO ORIGINAL RESEARCH

1) Several cellulose derivatives have been examined by ^{13}C.m.r. spectroscopy and such characteristics as their degree of substitution and the distribution of substituents were found to be directly accessible in a semi-quantitative way. Results were consistent with those obtained by other techniques.

2) The orientational dependence of $^{3}J_{C-H}$ couplings across the glycosidic linkage was used to study the conformation of α and β(1→4) linked disaccharides and related compounds by ^{13}C.m.r. spectroscopy. The preferred conformation was found to be similar in the solid state and in solution, and to be fairly independent of inter-residue hydrogen bonding. Limits of the technique used have been discussed.

3) The possibility of using a more direct route than in current procedures for the synthesis of 1,6-anhydro-sugars was investigated. 1,6-Anhydro-β-D-glucopyranose was afforded when 2,3,4,6-tetra-O-acetyl-glucopyranosyl bromide or chloride was treated with potassium t-butoxide, along with some degradation products. Mechanistic aspects of these reactions have been discussed.