ATTEMPTS TO LABEL CHOLESTEROL IN RING B: B-NOR-*i*-CHOLESTAN-7-ONE AND B-NOR-3(β)-BROMOCHOLESTAN-7-ONE: RING A C^{14}-LABELLED CHOLESTEROL.

A Thesis

by

William Werner Zorbach

Submitted to the Faculty of
Graduate Studies and Research
in partial fulfilment of the
requirements for the degree of
Doctor of Philosophy.

McGill University
Montreal, Canada.
April 1951

ACKNOWLEDGEMENTS

Grateful acknowledgement is expressed to my research director, Dr. R.D.H. Heard, for his supervision during this course of study.

I wish to thank Dr. Richard B. Turner of Harvard University for generously suggesting the sequence studied in Section II, Part E, herein, and also Dr. E. Schwenk of the Worcester Foundation for Experimental Biology for kindly furnishing us with information which satisfactorily brought the synthesis of cholesterol-4-C^{14} in Section III to fruition.

I wish, especially, to express sincere gratitude to Dr. R. Norman Jones of the National Research Council of Canada for preparing the infra-red curves of the four compounds in Section II, Part D; and for his valuable suggestions in interpreting the finer aspects of the spectra.

Finally, thanks are offered to my wife for her assistance in sundry matters, both technical and clerical, which were necessary for a proper consummation of this work.

TABLE OF CONTENTS

SECTION I

SECTION II

PART A

PART B

3(β)-acetoxycholestan-6-one as a Synthetic Goal.

PART C

Attempted Preparation of B-nor-cholestan-3(β)-ol-7-one.

PART D

PART E

PART F

SECTION III

Ring A C^{14}-Labelled Cholesterol

Chronological Listing of Compounds

I - Cholesterol

Ia - 3-benzyloxy-Δ^5-cholestene

I* - Cholesterol-4-C^{14}

II - 6-nitro-Δ^5-cholestene-3-nitrate

III - 3(β)-acetoxycholestan-6-one

IV - 3(β)-acetoxycholestan-6(β)-ol

V - 3(β)-acetoxy-Δ^5-cholestene

VI - 3(β),6(β)-diacetoxycholestane

VII - Cholestan-3(β),6(β)-diol

VIII - 3(β)-acetoxy-7-bromocholestan-6-one

IX - 3(β)-acetoxycholestan-6,7-dione

X - Cholestan-3(β)-ol-6,7-dione

XI - B-nor-cholestan-3(β),7-diol-7-carboxylate^x (referred to in text as seco-6-cholestan-3(β),7-diol-7-carboxylate).

XII - B-nor-cholestan-3(β)-ol-7-one^x (referred to in text as seco-6-cholestan-3(β)-ol-7-one).

XIII - 7-cyano-B-nor-cholestan-3(β),7-diol^x (referred to as 7-cyano-seco-6-cholestan-3(β),7-diol in text).

XIV - B-nor-cholestan-3(β),7-diol-7-methylamine^x (referred to in text as seco-6-cholestan-3(β),7-diol-7-methyleneamine).

XV - Cholestan-3(β)-ol-6-one

XVI - 3(α)-chlorocholestan-6-one

---

x This superscript refers to compounds which have not been prepared

XVII - 3(α)-chloro-6,7-secocholestan-6,7-dioic acid (referred to in text as 3(α)-chlorocholestan-6,7-dioic acid)

XVIII - 6,7-secocholestan-3(β)-ol-6,7dioic acid (referred to in text as cholestan-3(β)-ol-6:7-dioic acid)

XIX - Barium 6,7-secocholestan-3(β)-ol-6,7-dioate (referred to in text as Barium Cholestan-3(β)-ol-6:7-dioate)

XX - Unsaturated pyroketone (uncharacterized)

XXI - Alternate XXXI, B-nor-i-cholestan-7-one (referred to in text as seco-6-i-cholestan-7-one)

XXII - 7-cyano-B-nor-i-cholestan-7-ol[x] (referred to in text as 7-cyano-seco-6-i-cholestan-7-ol)

XXIII - B-nor-i-cholestan-7-ol-7-methylamine[x] (referred to in text as seco-6-i-cholestan-7-ol-7-methyleneamine)

XXIV - i-cholestan-6-one

XXV - 3(β)-chloro-Δ^5-cholestene

XXVI - 3(β)-chloro-6-nitro-Δ^5-cholestene

XXVII - 3(β)-chlorocholestan-6-one

XXVIII - 3(β)-bromocholestan-6-one

XXIX - 6,7-seco-i-cholestan-6,7-dioic acid (referred to in text as i-cholestan-6:7-dioic acid)

XXX - Lead 6,7-seco-i-cholestan-6,7-dioate (referred to in text Lead i-cholestan-6:7-dicarboxylate)

XXXI - B-nor-i-cholestan-7-one (referred to in text as seco-6-i-cholestan-7-one)

XXXII - 3(β)-bromo-B-nor-cholestan-7-one (referred to in text as seco-6-3(β)-bromocholestan-7-one)

XXXIIIa - 6,7-secocholestan-3(β)-ol-6,7-dioic acid-6→3-lactone (referred to in text as cholestan-3(β)-ol-6:7-dioic acid-6→3-lactone)

XXXIIIb - 6,7-secocholestan-3(β)-ol-6,7-dioic acid-6→3-lactone methyl ester (referred to in text as cholestan-3(β)-ol-6:7-dioic acid-6→3-lactone methyl ester)

XXXIV - 6,7-secocholestan-3(β)-ol-6,7-dioic acid-6→3-lactone acid chloride(referred to in text as cholestan-3(β)-ol-6:7-dioic acid-6→3-lactone acid chloride)

XXXV - 6,7-secocholestan-3(β)-ol-6-oic acid-6→3-lactone-7-diazomethyl-7-one[x] (referred to in text as cholestan-3(β)-ol-6:7-dioic acid-6→3-lactone-7-diazomethyl-7-one)

XXXVI - Methyl 6,7-secocholestan-3(β)-ol-6-oic acid-6→3-lactone-7-carboxylate[x] (referred to in text as Methyl Cholestan-3(β)-ol-6-oic acid-6→3-lactone-7-carboxylate)

XXXVII - 6,7-secocholestan-3(β)-ol-6-oic acid-7-carboxylic acid [x] (referred to in text as cholestan-3(β)-ol-6-oic acid-7-carboxylate)

XXXVIII - 6,7-secocholestan-3(β)-ol-6-oic acid-7-carboxylic acid dimethyl ester[x] (referred to in text as Dimethyl Cholestan-3(β)-ol-6-oic acid-7-carboxylate)

XXXIX - Cholestan-3(β),5,6(α)-triol

XXXIXa - 3-benzyloxycholestan-5,6(α)-diol

XL - 5,6-secocholestan-3(β)-ol-5-one-6-al[x](referred to in text as 6-aldehydo-cholestan-3(β)-ol-5-one)

XLI - 5,6-secocholestan-3(β)-ol-5-one-6-oic acid methyl ester [x] (referred to in text as Methyl Cholestan-3(β)-ol-5-one-7-carboxylate)

XLII - 6,7-secocholestan-3(β),5-diol-6-oic acid-7-carboxylic acid[x] (referred to in text as cholestan-3(β),5-diol-6-oic acid -7-carboxylate)

XLIII - 3(β)-acetoxy-6,7-seco-Δ^4-cholesten-6-oic acid-7-carboxylic acid dimethyl ester[x] (referred to in text as Dimethyl 3(β)-acetoxy-Δ^4-cholesten-5,7-dicarboxylate)

XLIV - 3(β)-acetoxy-Δ^4-cholesten-6-one[x]

XLV - Δ^4-cholesten-3-one

XLV* - Δ^4-cholesten-3-one-4-C^{14}

XLVI - 3-acetoxy-$\Delta^{3,5}$-cholestadiene

XLVI* - 3-acetoxy-$\Delta^{3,5}$-cholestadiene-4-C^{14}

XLVII - 3,5-secocholestan-5-one-3-oic acid (referred to in text as cholestan-5-one-3-oic acid)

XLVIII - 3,5-seco-Δ^5-cholesten-5-ol-3-oic acid-3,5-lactone (referred to in text as cholestan-5-one-3-oic acid enol lactone)

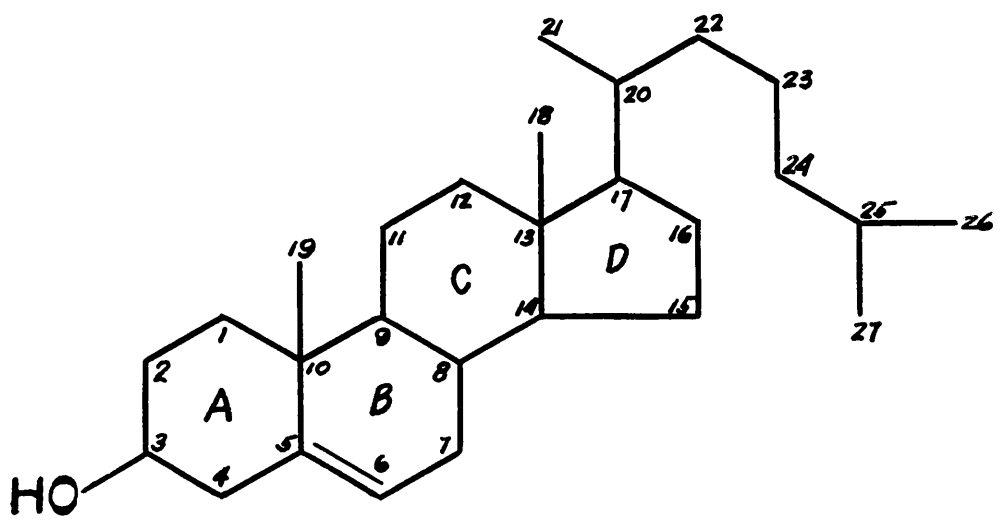
SECTION I

HISTORICAL INTRODUCTION

SECTION I

HISTORICAL INTRODUCTION

Cholesterol is a waxy, crystalline substance of definite molecular weight, having the empirical formula $C_{27}H_{46}O$. Its melting point is 147.5 - 148.5°C. The established system of designating the rings by letter and the numbering of individual carbon atoms accompany the structure at this time.

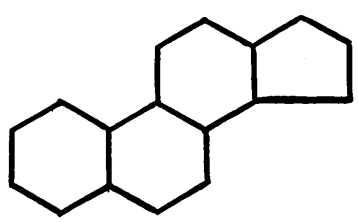


Cholesterol

Hereafter, the location of various positions by number may be made by reference to this formula. The parent hydrocarbon, cholestane, may be derived by completely saturating cholesterol with hydrogen. This provides us with a basis of naming members of this series. Thus, cholesterol would be completely identified by Δ^5-cholesten-3(β)-ol, where the Δ signifies a double bond and the superscript its position in the molecule. The symbols α and β refer to the orientation of substituents attached to the nucleus. Those bearing the symbol β are visualized as projecting above the plane of the paper and are shown by a solid line for the bond. α-oriented substituents are regarded as lying below the plane of the paper and are indicated

by a dotted line for the bond.

Cholesterol belongs to a class of organic compounds called sterols, which are crystalline alcohols(Gr. stereos, solid) and which are isolated from the unsaponifiable residues of lipids derived from animals and plants[1]. In recent years, this classification has been extended to include a great many other compounds, all having the same characteristic ring sys-



perhydro-1,2-cyclopentenophenanthrene

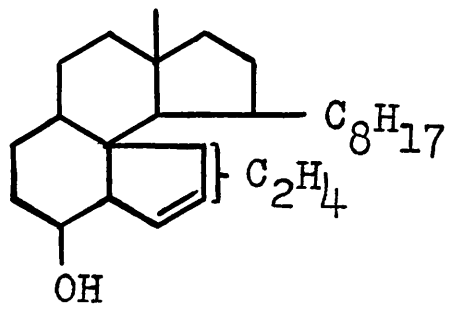
tem, but differing in the side chain; and which include, in addition to cholesterol and the bile acids, androgens, estrogens, progestational hormones, adrenal cortical hormones, cardiac glycosides and sapogenins. This larger and more general classification has been given the name steroid. Today, cholesterol and related substances, although still bearing the name sterols, constitute but one category in the steroid class of compounds. They may also be correctly referred to as steroid alcohols, since all have at least one secondary alcoholic group.

Cholesterol was first isolated as a morbid product in gallstones by Valliseri in 1733[2]. It is the principal animal sterol and is found in all tissues in amounts ranging from a

few hundredths of a per cent to ca. 5 per cent[3]. The correct empirical formula was established by Reinitzer in 1888 as $C_{27}H_{46}O$. Other miscellaneous experiments up to the year 1903 demonstrated the presence of a double bond and a secondary hydroxyl group, but not their respective positions in the molecule[4].

In the second phase of the study, an attempt was made to gain some insight into the structure of the complicated molecule[5]. This was accomplished through an examination of various products of oxidation. A brilliant and arduous series of investigations was initiated by Windaus in 1903 and by Wieland in 1912. For a time, these workers followed parallel but independent paths, although the method of attack in each case was by degradation. Resolution of the problem by synthesis of substances related to the complicated natural products was impossible, chiefly because at that time there was no characteristic property to serve as a guide. Furthermore, since the compounds themselves and any degradation products thereof were colorless, the often valuable technique of spectroscopy could indicate nothing due to the absence of characteristic absorption spectra.

Success was eventually achieved in 1927 and in the following year Wieland and Windaus reviewed in the Nobel Prize addresses the results of their investigations on the sterols and bile acids[6]. The formula for cholesterol suggested at this time barely reflects the real progress made, since it was unduly distorted by certain errors which had crept into



Cholesterol in 1927

the train of evidence. A few years later, in 1932, Rosenheim and King called attention to a neglected piece of evidence, - the formation of chrysene as a product of the selenium dehydrogenation of cholesterol and cholic acid. On the basis of this fact and of the x-ray measurments of ergosterol and of calciferol by Bernal, it was suggested that the ring nucleus of the sterols was perhydrochrysene. Study of the evidence in the light of this suggestion led Rosenheim and King, as well as Wieland and Dane, to modify the perhydrochrysene to a perhydrocyclopentenophenanthrene nucleus. This new structure for the sterols and the bile acids was immediately compatible with the vast amount of experimental material which had been accumulated. Finally, in 1939-40, Bachmann and co-workers carried out a total synthesis of one of the sex hormones. Thus, by degradation and by synthesis, the structure of the nucleus had been established.

The complete elucidation of the structure of cholesterol paved the way for quantitative metabolic experiments. Indeed, the first experiments of this sort date back only to around 1931[7]. As usual, two individual sets of problems evolve in

studying the metabolism of substances of biological importance. One of these, from an anabolic viewpoint, is to provide information relating to the in vivo synthesis of the substance under consideration. The other type of experiments mentioned relate to the disposition of the substance after it has either been synthesized in the body or ingested as such. The interest in this laboratory has been with the latter in the case of cholesterol and, more specifically at the inception of this work, with the suspected transformation of this substance into androgens, estrogens and adrenal cortical hormones within the body. Even before convincing metabolic experiments were performed, the strong structural relationship between cholesterol and the steroid hormones justified speculation regarding its transformation in the body to these regulators. That cholesterol labelled in the nucleus with tracer carbon would be of inestimable value in resolving this problem was a foregone conclusion.

To elucidate regarding the desirability of obtaining cholesterol labelled with isotopic carbon for tracer purposes, a brief account will be made of the use of isotopes, both stable and radioactive, in biological investigations[8]. The development of the cyclotron, whereby non-radioactive atoms may be bombarded by high-speed subatomic particles(protons, neutrons, deuterons, etc.) to produce artificially radioactive modifications of a number of elements, stimulated interest in the use of radioactive isotopes, and considerable work has been done with material from this source. Further, due to the availability of a great number of radioactive isotopes as the result

of controlled nuclear fission, the application of radioactive isotopes to biological studies promises to become far greater.

Radioactive isotopes are measured in terms of the radiation which they emit on decomposition to form other elements, with the accompanying emission of α- or β-particles, and sometimes γ-rays. From a practical point of view, the β-ray emission is usually the most important. Most investigators employ for β-ray measurement a Geiger-Müller counter. This depends on the ability of a single β-particle to produce a separate current impulse in a suitable ionization tube, which impulse can be amplified and made to activate a counting device. Theoretically, each ionizing particle can be counted; in practice, the counter is scaled to count a predetermined fraction of the total pulses. The activity of the material (and, hence, the isotope content) may, therefore, be expressed in counts per unit time, or in millicuries or microcuries, where 1 millicurie = 1000 microcuries = 3.7×10^7 counts per minute.

Radioactive isotopes do, however, differ with respect to availability, half-life and type of radiation emitted. Availability now appears to be largely a matter of technical development. The type of radiation emitted (kind and intensity) influences the sensitivity and method of measurement. Regarding half-life, those isotopes with a very short one or those with a very long half-life are of less value in biological investigations than those with a half-life of the

order of weeks or months. A short half-life means that too great a proportion of the original material will have lost its activity (and original chemical nature) during the time required for transport after preparation to the place of use and for manipulative details of the experiment. A very long half-life means that a high proportion of the isotope must be incorporated into a compound if accurate radioactivity measurements are to be made.

Most of the common elements as ordinarily encountered consist of stable isotopes. Over 200 stable isotopes of the various elements have been recognized as existing, but only a very few have been obtained in concentrated form. To obtain the separate isotopes from a mixture, or fractions relatively enriched with respect to one isotope, advantage is taken of properties such as diffusion or reaction velocity which may vary with mass differences. The first of these to become available in amounts sufficient for purposes of biological investigations was deuterium, obtained by Urey and Washburn in 1932, by the fractional electrolysis of water. Indeed, it was the availability of deuterium which suggested to Schoenheimer and Rittenberg the possibility of its use in metabolic studies, a concept which may be said to have initiated the present phase of application of isotopes to biological problems.

In tagging a molecule, or part of a molecule, stable isotopes have the advantage over the radioactive type in

that the time of preparation of a compound incorporating the isotope, or the duration of the experiment, are of no importance. There is no question concerning the possible effect of radiation on the experiment, and in some instances the isotope may be recovered at the end and used again. Disadvantages relative to stable isotopes include the increased difficulty of measurement, which is usually accomplished by mass spectrography, a rather laborious technique. Higher concentrations of the isotope are required of the same experiment employing a radioactive isotope, since measurements of radioactivity are far more sensitive than measurements of mass. Finally, there are relatively few stable isotopes available for metabolic work of this type.

There are five isotopes of carbon. Of these, as shown in Table 1, C^{12} is ordinary carbon which is encountered in nature, and always carries 1.1 per cent of stable C^{13}. C^{10}

Table 1

Symbol	Nucleus		Planetary Electrons	Relative Abundance (Stable)	Half-life (Radio-active)
	Protons	Neutrons			
C^{10}	6	4	6	...	8.8 sec.
C^{11}	6	5	6	...	21.0 min.
C^{12}	6	6	6	98.9	...
C^{13}	6	7	6	1.1	...
C^{14}	6	8	6	...	6100 yrs.

has such a short half-life that we may eliminate it immediately in consideration of its use in biological studies. C^{13}, the only stable isotope besides natural carbon, has been obtained in fairly concentrated form from natural carbon by suitable methods. It has found considerable use in tracer studies; but, as just indicated, the methods for its measurement are difficult. C^{11} has also a short half-life, but despite this, a number of satisfactory metabolic experiments have been performed employing it. It was, however, necessary for the workers to conduct the syntheses and subsequent experiments with a maximum of speed and efficiency, lest so much radioactivity be lost as to render the counting impossible. Also, enormous intensities are available due to its short half-life, and even after several half-lives have elapsed, measurements may be made by extrapolating the counting back to some arbitrary zero time.

C^{14}, on the other hand, has a very long half-life. The spectrum is simple, the radiation being almost entirely confined to β-rays (electrons). Although this radiation is by no means as energetic as that of C^{11}, accurate counting may be accomplished by means of a suitable ionization tube. Offsetting any disadvantage due to the "softness" of the radiation, the extremely long half-life of C^{14} makes it amenable to any type of investigation employing tracer carbon, regardless of the time consumed in the synthesis of the compound to be studied and the ensuing biological experiments. We thus have no correction to make for delay, as in the case

of C^{11}. Finally, the nature and low intensity of the radiation presents relatively much less a hazard to the worker, compared to that of C^{11}.

SECTION II

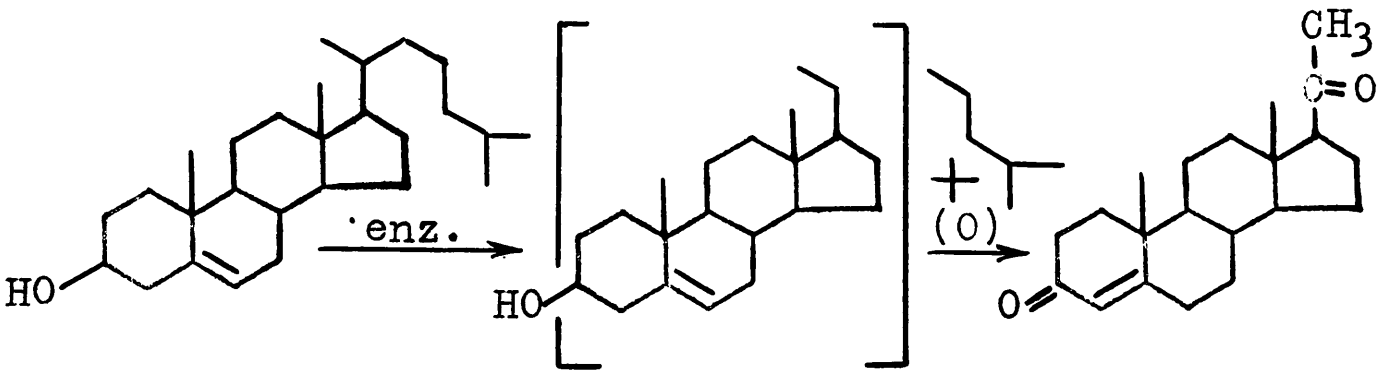
ATTEMPTS TO LABEL CHOLESTEROL IN RING B

PART A

Introductory Remarks

1) Nucleus C^{14} Labelled Cholesterol

The desirability for developing a synthetic scheme designed to permit the inclusion of radioactive C^{14} in the nucleus of cholesterol rather than in the side chain stems from the interest, in this laboratory, regarding the suspected conversion of cholesterol in the body to steroid hormones, as has been discussed previously (p. 5). Speculating further along the lines that if cholesterol were the precursor of these hormones, then its transformation in that direction would most certainly not take place by degradation and resynthesis of the nucleus; but rather by enzymatic degradation of the side chain down to a point where the residual side chain carried just as many carbon atoms as the hormone being synthesized, the nucleus remaining intact save for certain functional groups attached thereto. For example, if progesterone were being formed, according to this



Cholesterol — Hypothetical Intermediate — Progesterone

hypothesis, a 6-carbon chain would be removed from cholesterol, leaving two carbon atoms still attached. Then, in some unknown manner, the intermediate would be oxygenated to yield progesterone. This method of synthesis by degradation is by no means a certainty, and it is entirely possible that the entire side chain is removed in one step and the required groupings joined to the nucleus afterward. This would invalidate the usefulness of cholesterol labelled in the side chain with respect to the biological transformations just postulated, since the label would disappear during this time.

The value of cholesterol labelled in the nucleus for other types of biological investigations has not been overlooked. It is of major interest to ascertain in what manner cholesterol is destroyed or disposed with in the body. Obviously, its transformation into steroid hormones can account for only a small amount. Further, it has been demonstrated that mice can destroy several times the normal body content of this substance[9]. Some, however, is converted in small amounts to cholestanol, and in relatively larger amounts to coprostanol and epicoprostanol, all of which appear in the feces. This does not imply that all the cholesterol is accounted for, nor whether cholestanol, coprostanol, and epicoprostanol are not further degraded, while only that fraction escaping degradation appears in the feces.

Cholesterol thus labelled should produce unequivocal proof of its transformation to the three reduction products mentioned, as well as indicate whether the nucleus is broken down into smaller fragments in some manner not yet determined.

2) Choice of Ring B for the Site of the Label

In July of 1948, when this work was proposed, only two methods for preparing ring-C^{14} labelled steroids in the cholestane series had evolved[10,11], and which will be discussed fully in Section III. The compound in each case was Δ^4-cholesten-3-one (XLV), labelled in ring A. It was felt that if this compound, already labelled in ring A by described methods, could be conveniently converted to cholesterol (I), then the problem at hand would be relatively straightforward. An examination of the literature, however, disclosed only one method, by competent Swiss workers, for the transformation of Δ^4-cholesten-3-one (XLV) to cholesterol (I)[12]. This was accomplished in seven steps and with low overall yield. This fact weighed heavily against the possibility of obtaining radioactive cholesterol via this intermediate.

The possibility of devising some method for cleaving ring A of cholesterol (I), introducing a new carbon atom and cyclizing back without affecting the Δ^5 unsaturation also presented itself. Theoretically

it was possible to accomplish this, but to preserve the oxygen function at position 3, all schemes envisaged, which must of necessity terminate in a cyclization procedure, would lead to carbonyl group at this position, a highly unfavorable circumstance, since Δ^5-3-ketones are most unstable, the double bond shifting spontaneously to $C_4:C_5$. This phenomenon is well-known, for the direct oxidation of cholesterol (I) to Δ^5-cholesten-3-one is impossible, - the formation of an α,β-unsaturated ketone being so strongly favored that Δ^4-cholesten-3-one (XLV) always results[13]. It is, therefore, evident that the mere presence of a ketonic group at position 3 in a Δ^5-cholestene molecule is sufficient to shift the double bond to position 4. The difficulty of proceeding from here to cholesterol (I) has already been mentioned (p. 13).

This fact and other minor considerations not favoring the approach to a synthetic scheme making possible the incorporation of C^{14} into ring A of cholesterol (I), attention was therefore diverted to ring B. The results of these investigations appear in the following Parts of the Section.

PART B

3(β)-acetoxycholestan-6-one as a Synthetic Goal

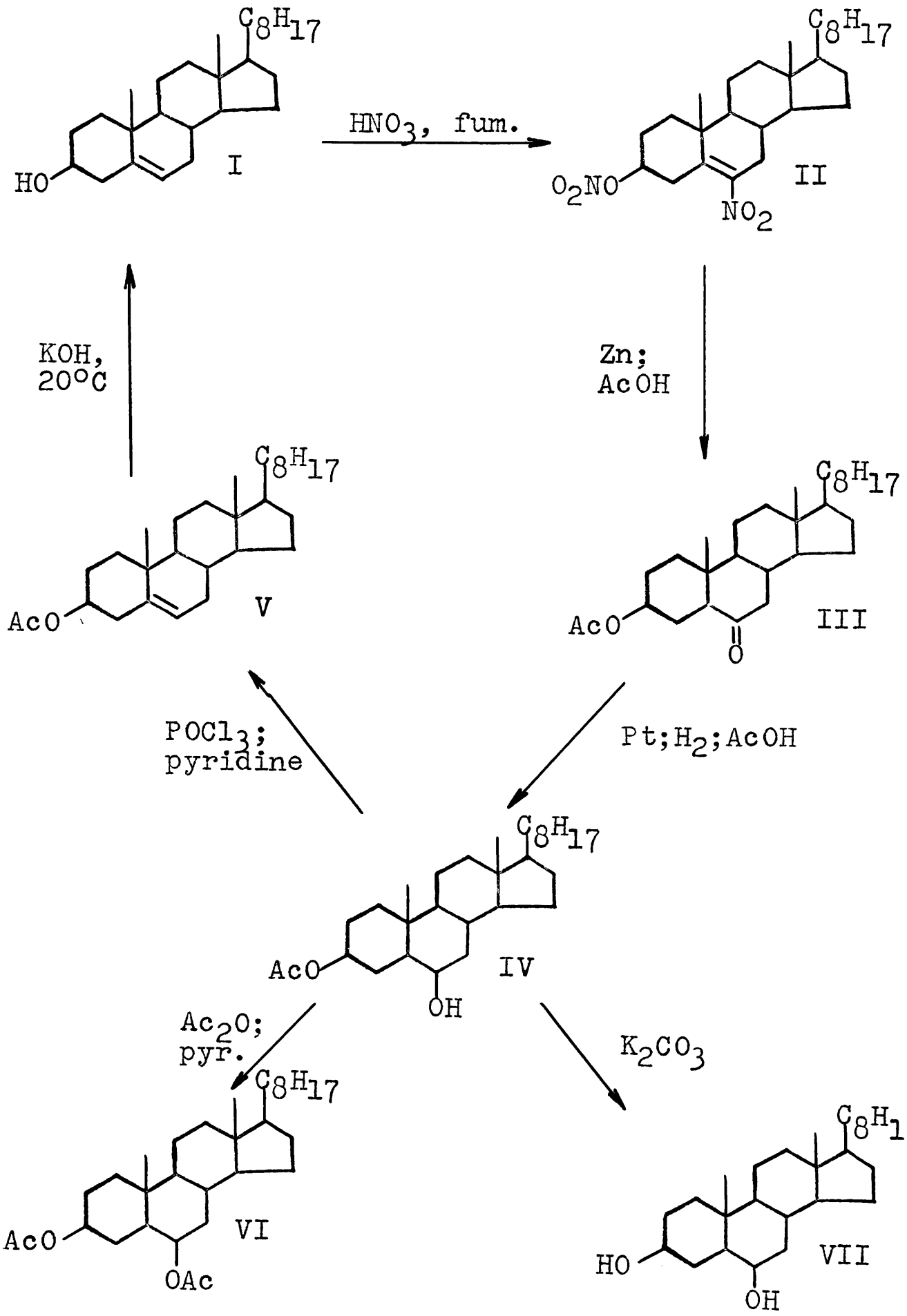
1. Theoretical

At the outset, several schemes were envisaged whereby a carbon atom in ring B could be replaced with another from a new source (hence, ultimately, a C^{14} atom), with subsequent restoration of the 6-membered ring either by cyclization procedures or by methods of ring expansion. It was felt, however, that even with the accomplishment of such a scheme or schemes, the resultant intermediate might be converted to cholesterol only with difficulty, thus rendering the method useless. In other words, whatever plan was adopted, it must allow for an economical conversion to cholesterol, once C^{14} was introduced into the molecule.

On this basis, it was decided to resolve the latter part of the problem first. This necessitated searching for a common pathway through which all proposed procedures could lead to cholesterol. Careful study disclosed that if the compound 3(β)-acetoxycholestan-6-one(III) were chosen as a common intermediate, cholesterol should be obtained, theoretically, in a minimum of steps and with a satisfactory overall yield.

The conversion of 3(β)-acetoxycholestan-6-one(III) to 3(β)-acetoxycholestan-6(β)-ol(IV) was accomplished by catalytic hydrogenation[14], while the remainder of

Chart 1


C8H17
HO
I
HNO3, fum.
O2NO
NO2
II
KOH,
20°C
Zn;
AcOH
AcO
V
AcO
O
III
POCl3;
pyridine
Pt;H2;AcOH
AcO
OH
IV
Ac2O;
pyr.
K2CO3
AcO
OAc
VI
HO
OH
VII

the transformation was effected by dehydrohalogenation[12]. This latter treatment, in effect a two-step reaction, the first of which replaces the 6-hydroxyl with halogen, while the second removes elements of hydrochloric acid to leave a double bond at C_5:C_6, yielded 3(β)-acetoxy-Δ^5-cholestene(V), which on mild saponification was converted to cholesterol(I).

In order to study this conversion, it was necessary to obtain a quantity of III sufficient for experimental purposes. The literature revealed a satisfactory method starting with cholesterol(I), which was nitrated to form 6-nitro-Δ^5-cholestene-3-nitrate(II). This, in turn, was reduced by the action of zinc in acetic acid to yield 3(β)-acetoxycholestan-6-one(III).

2. Discussion

a) 6-nitro-Δ^5-cholestene-3-nitrate(II)

Workers prior to 1938 experienced considerable difficulty in preparing this compound, obtaining low and variable yields. This was due, presumably to the employment of room temperatures for conducting the nitration. At such temperatures, oxidation largely occurs, with little or no nitration. The Heilbron work in 1938[15] solved this problem by adding a mixture of fuming nitric acids to a stirred suspension of cholesterol(I) at ca. -15°C. At the

end of the prescribed reaction time, a fine slurry of 6-nitro-Δ^5-cholestene-3-nitrate(II) separated which, upon recrystallization, melted at 128°C.

b) 3(β)-acetoxycholestan-6-one(III)

The preparation of this compound from 6-nitro-Δ^5-cholestene-3-nitrate(II) is also given by the same reference in (a) above, but is very time consuming (12 hours refluxing) and with a rather laborious work-up. Dodson and Riegel[16] have modified this procedure in such a manner that the entire reduction takes place in only four hours. By greatly diluting the reaction mixture with water at the termination of the reaction, they are able to filter off the reduction product and crystallize directly. The yields obtainable in this way based on the nitro compound(II) are good, usually around 60 per cent. The pure compound melts at 128-129°C.

c) 3(β)-acetoxycholestan-6(β)-ol(IV).

The reduction of 1150 mg. 3(β)-acetoxycholestan-6-one(III) was accomplished by the method of Plattner and Lang[14] employing pre-reduced platinum oxide and hydrogen, except that acetic acid was substituted for ethanol as the hydrogen-

ation solvent. This was done to allow the mixture, at the end of the experiment, to be filtered from the platinum before crystallization took place, which is commonly observed when ethanol is employed. During the hydrogenation which was carried out in this laboratory, the theoretical amount of hydrogen was absorbed.

The product so obtained was recrystallized twice from methanol and melted at 157-158°C., yield 1010 mg., 88 per cent of theory. Both Plattner and Lang[14] and Reich and Lardon[12] publish for 3(β)-acetoxycholestan-6(β)-ol(IV) a melting point of 141-142°C., with an $[\alpha]_D^{20} = -6$. The lack of agreement between the published melting points and that as determined herein prompted a further literature search, where it was found that a Japanese worker, Misao Chuman, obtained IV by the catalytic hydrogenation of cholesterol β-oxide in acetic acid[17]. The melting point reported by this worker was 155-156°C., which agrees with our value. Chuman also states that the 3(β)-acetoxycholestan-6(β)-ol(IV) thus obtained was the same as that prepared from 3(β)-acetoxycholestan-6-one(III). He further saponified IV to cholestan-3(β),6(β)-diol(VII), m.p. 188-189°C., which agrees with the value of

191°C. given by Plattner and Lang[14] for the same compound.

The value of $[\alpha]_D^{20} = -6.0$ for the specific rotation as determined in this laboratory is, on the other hand, in excellent agreement with that of the Swiss workers. As a final verification, both saponification[18] and acetylation of IV were attempted with the expectation that cholestan-3(β),6(β)-diol(VII) and 3(β),6(β)-diacetoxycholestane(VI) would be formed, respectively, both of which by melting points could be correlated with compounds already prepared by Plattner and Lang[14]. As anticipated, two compounds were obtained, both differing from the starting material, 3(β)-acetoxycholestan-6(β)-ol(IV). The melting points were carefully determined and are submitted in Table 2, where the melting points from all sources for compounds IV, VI and VII are listed for comparison. It

Table 2

	Plattner	Chuman	Determined
IV	141-142°C.	155-156°C.	157-158°C.
VI	136-137°C.	...	136-138°C.
VII	189-190°C.	188-189°C.	191-192°C.

seems certain, therefore, that compound IV is indeed 3(β)-acetoxycholestan-6(β)-ol and that the melting point as reported here is correct. It is difficult to overlook the fact that the choice of solvent for carrying out the hydrogenation was responsible for the discrepancy noted. Chuman and this laboratory, both employing acetic acid, obtained melts of 155-156°C. and 157-158°C., respectively. The Swiss workers, on the other hand, using ethanol, recorded 141-142°C. for the same compound. It is possible that in the latter case, drying was not sufficient to remove either water or solvent associated with the crystals; but the agreement established here with the value of the specific rotation as obtained by these workers indicates, to the contrary, that their compound was pure. Hence, no satisfactory explanation is possible.

d) Conversion of 3(β)-acetoxycholestan-6(β)-ol(IV) to Cholesterol(I).

In a preliminary run, 120 mg. IV were dehydrohalogenated according to Reich and Lardon[12]. The resulting material was worked up and crystallized without regard to yield, solely for purpose of identification. This material melted at 113-114°C. and gave no depression when admixed with

an authentic sample of 3(β)-acetoxy-Δ^5-cholestene(V). A positive test with tetranitromethane was obtained, denoting a double bond. On this basis, the compound was identified as cholesterol acetate(V).

A second similar run was accomplished, starting with 200 mg. IV. In order to obtain an optimum yield of cholesterol(I), the cholesterol acetate(V) resulting from the dehydrohalogenation was not purified, but saponified directly employing potassium hydroxide at room temperature. Crystallization of the saponified material gave 140 mg. of substance, melting at 146-148°C., which did not depress the melting point of an authentic sample of cholesterol(I). Overall yield of I based on 3(β)-acetoxycholestan-6-one(III), 71 per cent of theory. This was further purified and the specific rotation determined. This gave an $[\alpha]_D^{20} = -38$, which agrees well with the published value $[\alpha]_D^{20} = -39$[19].

3. Experimental

a) Preparation of 6-nitro-Δ^5-cholestene-3-nitrate(II).

To 20.0 gm. of cholesterol(I) of the commercial variety in a 500 ml. Erlenmeyer flask were added 80 ml. glacial acetic acid, and the

mixture stirred with a mechanical stirrer until all the cholesterol(I) was in suspension. From a mixture of 80 ml. nitric acid, d. 1.50, plus 50 ml. nitric acid, d. 1.515, 30 drops were subtracted and added to this suspension. The flask was then surrounded with an ice-salt bath (at -10 to -15°C.) and the remainder of the nitric acid added over a period of one hour with constant stirring. After the acid had been added, the reaction mixture was allowed to stir for an additional half-hour, after which time the separating solid was filtered off as rapidly as possible through a sintered-glass funnel by suction. Suction was maintained for one hour to promote drying, whereupon the solid was transferred to a flask and dissolved in a minimum quantity of boiling acetic acid. The 6-nitro-Δ^5-cholestene-3-nitrate(II) crystallized in fine, hard needles and weighed 15.4 gm., m.p. 125-127°C. Yield, 62.6 per cent of theoretical.

b) Preparation of 3(β)-acetoxycholestan-6-one(III).

To a warm, stirred solution of 15.0 gm. 6-nitro-Δ^5-cholestene-3-nitrate(II) in 300 ml. glacial acetic acid, 30 ml. water were added. Stirring was continued while 30 gm. zinc dust were added carefully and in small portions over a period of one hour.

After 0.5 hours, however, when the initial exothermic reaction subsided, a small flame was applied to bring the temperature slowly up to 110°C. This temperature was maintained for an additional 3.5 hours, at the end of which time the reaction was considered complete. Solvent losses from the heating were overcome by adding 50 ml. of a 1:10 mixture of water and acetic acid at the end of each hour. Next, the reaction mixture was filtered hot, thus removing any unreacted zinc, followed by a wash of 25 ml. of cold glacial acetic acid. The hot filtrate was added to an equal volume of cold water, the entire contents cooled in an ice-bath, then set aside in the refrigerator overnight. On the following day, the semi-crystalline 3(β)-acetoxycholestan-6-one(III) was filtered on a Büchner funnel, washed well with water, taken up in hot methanol and allowed to crystallize slowly overnight. The crystalline material was filtered on the following day and amounted to 8.5 gm., 60.5 per cent of theory, m.p. 124-127°C. A further crystallization brought the melt to 127-128°C.

c) Hydrogenation of 3(β)-acetoxycholestan-6-one(III).

To 200 mg. platinum oxide in a 200 ml. round bottom flask were added 10 ml. pure acetic acid.

The flask was attached to a hydrogenation apparatus, hydrogen admitted at a pressure of approximately 1.1 atmospheres and the flask gently shaken for 0.5 hours, at the end of which time the platinum oxide was considered completely reduced to colloidal platinum. The flask was disconnected and 1150 mg. 3(β)-acetoxycholestan-6-one(III) in 40 ml. acetic acid added. The flask was then returned to the hydrogenation rack as before, hydrogen admitted at the same pressure, and shaking resumed. At the end of 2.5 hours 50 ml. hydrogen (one mole equivalent) were absorbed. Shaking was continued for an additional 0.5 hours, during which time no further take-up of hydrogen was observed. The flask was disconnected and the contents filtered by suction through a Whatman No. 50 paper to remove the catalyst. The filtrate was removed quantitatively to a clean round bottom flask and taken down to dryness in vacuo at 60°C. The residue was taken up with methanol and allowed to crystallize slowly at room temperature. The material was filtered and crystallized once more from methanol. This resulted in 1010 mg. 3(β)-acetoxycholestan-6(β)-ol(IV), m.p. 157-158°C., $[\alpha]_D^{20} = -6.0$ (c2.0 Chf). Yield, 88.0 per cent of theory.

d) <u>3(β),6(β)-diacetoxycholestane(VI)</u>.

In a small flask containing 200 mg. 3(β)-acetoxycholestan-6(β)-ol(IV) was added a mixture of 0.4 ml. pyridine and 0.2 ml. acetic anhydride. The flask and contents were heated for one hour at 80°C., then water was carefully added to precipitate the acetylated material. After cooling the crystals were filtered, washed well with water and sucked dry. Two crystallizations from methanol gave VI, m.p. 136-138°C.

e) <u>Cholestan-3(β),6(β)-diol(VII)</u>.

In a 100 ml. round bottom flask containing 100 mg. 3(β)-acetoxycholestan-6(β)-ol(IV) in 10 ml. methanol was added 0.6 ml. water containing 32 mg. potassium carbonate. The flask was stoppered and the entire contents shaken gently for 16 hours. The solution was then transferred quantitatively to a small flask, warmed on the steam-bath and water added dropwise to a point of slight turbidity. The flask was then removed and the material allowed to crystallize. The material obtained from this crystallization melted at 191-192°C., and was identified as cholestan-3(β),6(β)-diol(VII).

f) 3(β)-acetoxy-Δ^5-cholestene(V).

To 120 mg. 3(β)-acetoxycholestan-6(β)-ol(IV) in a small flask were added 1.0 ml. pure pyridine and 0.4 ml. pure phosphorus oxychloride. The flask was tightly stoppered and allowed to stand at room temperature for 20 hours. After this time, the flask was unstoppered and water carefully added in a dropwise manner. The entire contents were then removed quantitatively, by means of alternate washings with ether and water, to a 125 ml. separatory funnel. More ether was added, the funnel shaken, and the contents allowed to separate. The lower layer was discarded, the ether extract washed twice with dilute hydrochloric acid, once with 5 per cent aqueous potassium carbonate, and finally with water. The separating ether layer was transferred to a flask and dried for one hour over anhydrous magnesium sulfate. This was then filtered and evaporated, the residue taken up and crystallized from methanol-ether. The crystalline material thus obtained melted at 112-114°C., and was identified as 3(β)-acetoxy-Δ^5-cholestene(V).

g) Cholesterol(I).

In an identical manner as described in (f) above, 200 mg. 3(β)-acetoxycholestan-6(β)-ol(IV) were treated with 10.0 ml. pure pyridine and 4.0

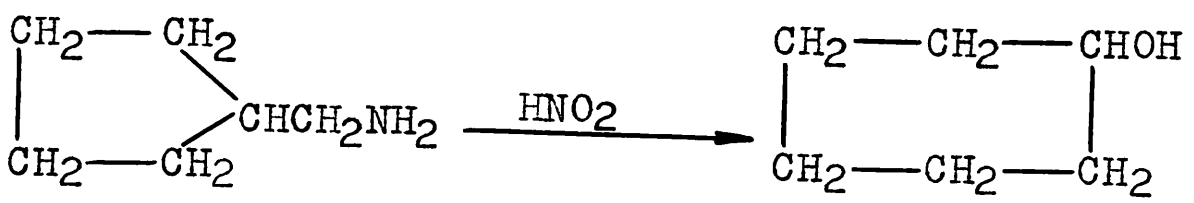
ml. pure phosphorus oxychloride. Instead of crystallizing, however, the crude cholesterol acetate(V) thus obtained was dissolved in a mixture of 50 ml. methanol and 25 ml. ether. To this was added a solution of 250 mg. potassium hydroxide in 3 ml. water, and the flask allowed to stand at room temperature for 16 hours. The solution was then concentrated on the steam-bath to about 25 ml. and allowed to cool. At the end of one hour, the separating material was filtered and washed well with water. Crystallization from methanol gave 140 mg. of material melting at 146-148°C., and giving no depression when mixed with an authentic sample of cholesterol(I). $[\alpha]_D^{20} = -38.$ (c 1.5, Chf). Yield based on 3(β)-acetoxycholestan-6(β)-ol(IV), 81.0 per cent of theory.

PART C

Attempted Preparation of Seco-6-cholestan-3(β)-ol-7-one(XII).

1. Theoretical.

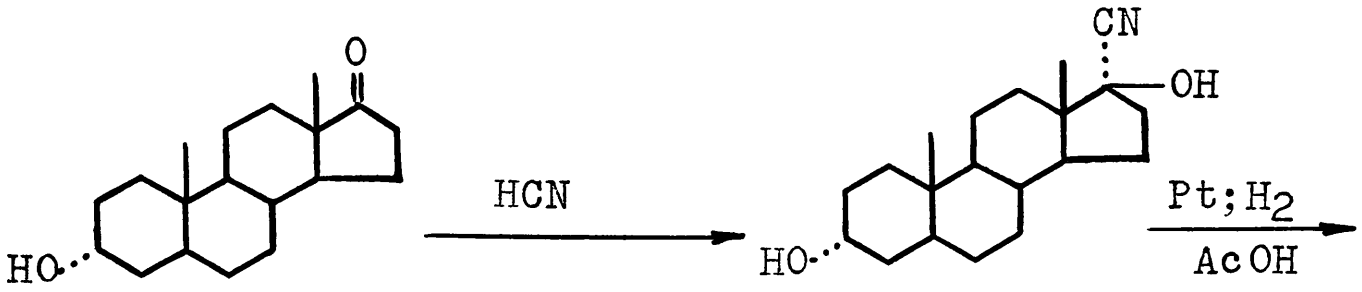
The plan for incorporating C^{14} into ring B adopted herein devolved about the success in obtaining the compound seco-6-cholestan-3(β)-ol-7-one(XII), not hitherto described. Such compounds can, theoretically, be made to undergo a sequence of reactions whereby a ring containing a ketonic function can be enlarged to give the next higher homolog. This is called the Tiffeneau ring-enlargement reaction[20], and is merely an extension of the Demjanow rearrangement[21], which involves the action of nitrous acid on cycloalkylmethylamines, an example of which is the formation of cyclohexanol from cyclopentylmethylamine:



cyclopentyl-methylamine

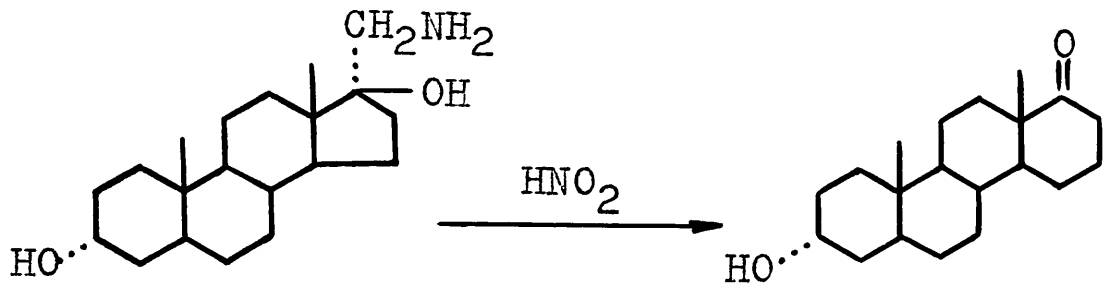
cyclohexanol

The extension of this rearrangement given by Tiffeneau provides for the transformation of an alicyclic ketone to the next higher homolog which is also ketonic. The application to steroids was first made in 1940 by Goldberg and Monnier[22] for the preparation of D-homoandrosterone which will serve as an example of the



androsterone

androsterone cyanohydrin



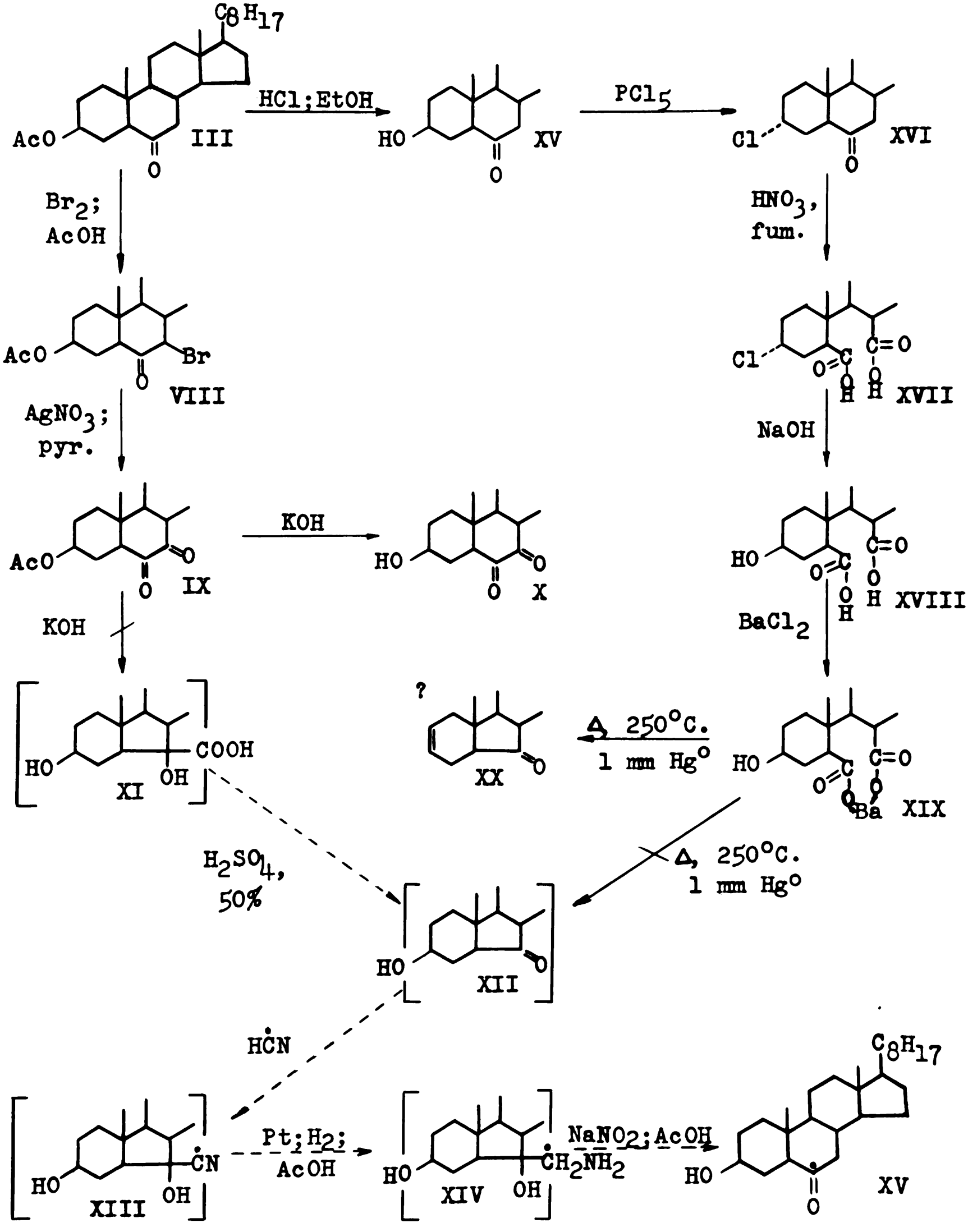
androstan-3(α),17(β)-diol-17(α)-methyleneamine

D-homoandrosterone

reaction, as well as indicate the feasibility of its application to the expansion of ring B of seco-6-cholestan-3(β)-ol-7-one(XII). It will be noted, however, that in this case, where the hydroxy-methyl-eneamine underwent the Demjanow rearrangement, the result was a cyclic ketone and not a secondary alcohol.

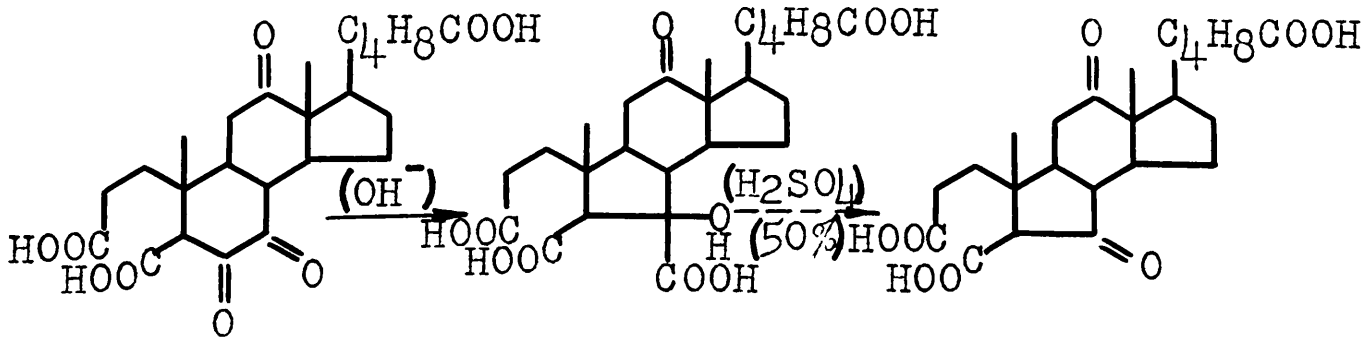
Provided that XII could be prepared and successfully subjected to the Tiffeneau reaction (by anology with the preparation of D-homoandrosterone), the compound anticipated would be cholestan-3(β)-ol-6-one(XV) which, on acetylation, yields 3(β)-acetoxycholestan-6-one(III). The desirability for obtaining this compound

Chart 2


C8H17
AcO
III
O
HCl;EtOH
HO
XV
O
PCl5
Cl
XVI
O
Br2;
AcOH
HNO3,
fum.
AcO
Br
O
VIII
Cl
C=O
O=C
O
H
XVII
AgNO3;
pyr.
NaOH
AcO
O
O
IX
KOH
HO
O
O
X
HO
C=O
O=C
O
H
XVIII
KOH
BaCl2
HO
COOH
OH
XI
?
XX
Δ 250°C.
1 mm Hg°
HO
C=O
O=C
O
Ba
XIX
H2SO4,
50%
Δ, 250°C.
1 mm Hg°
HO
O
XII
HCN
HO
CN
OH
XIII
Pt;H2;
AcOH
HO
CH2NH2
OH
XIV
NaNO2;AcOH
HO
O
XV
C8H17

was discussed in Part B. The key reaction in this proposed transformation is the addition of hydrogen cyanide to the carbonyl group of XII. If $HC^{14}N$ is employed for this addition, then the method allows for the incorporation of C^{14} into ring B, this same carbon atom eventually locating itself at position 6 of cholestan-3(β)-ol-6-one(XV) (Chart 2, XII ⟶ XV).

Two methods for the preparation of seco-6-cholestan-3(β)-ol-7-one(XII) were theoretically possible. The first involves the benzil-benzilic acid rearrangement as applied to cyclic diketones, the net effect being to contract the ring to form a cyclic monoketone with one carbon atom less[23]. The application of this reaction to steroids was made pursuant to the structure investigation in the bile acid series by Lassar-Cohn in 1899[24], the immediate product being cilianic acid, an α-hydroxy acid, which could lose carbon monoxide and water to yield a ketone. The contraction in this experiment was



cilianic acid

accomplished by warming with 12 per cent aqueous sodium hydroxide. Carbon monoxide and water may be split off, either by heating or by using some mild dehydrating agent, such as 50 per cent sulfuric acid. It was noted, however, that this rearrangement was undertaken where ring A of the steroid nucleus was open. As proposed herein (IX→XI), ring A is kept intact, hence ring B is doubly annulated with the result that such a reaction might be hindered. Despite this, the rearrangement of 3(β)-acetoxycholestan-6,7-dione(IX) to seco-6-cholestan-3(β),7-diol-7-carboxylate(XI) was attempted. Satisfactory procedures for the preparation of 3(β)-acetoxycholestan-6,7-dione(IX) were found in the literature[24,25]. 3(β)-acetoxycholestan-6-one(III) was monobrominated in the 7 position to yield 3(β)-acetoxy-7-bromocholestan-6-one(VIII) which, in turn, was oxidized to IX.

The second and alternate proposal for procuring seco-6-cholestan-3(β)-ol-7-one(XII) involved the pyrolysis of the barium salt of cholestan-3(β)-ol-6:7-dioic acid(XIX). This procedure stems from the work of Stange[26] who, taking cognizance of certain evidence leading to a revision of the Wieland-Windaus formulae for cholesterol and the bile acids (p. 4), pyrolyzed the barium salt of cholestan-6:7-dioic acid and obtained seco-6-cholestan-7-one, thus furnishing for the first

time unequivocal chemical proof that ring B is 6-membered.

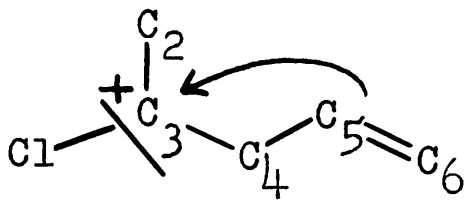
The preparation of the barium salt of cholestan-3(β)-ol-6:7-dioic acid(XIX) is outlined in Chart 2, III → XV → XIX. 3(β)-acetoxycholestan-6-one(III) was saponified to free the 3(β)-hydroxyl, yielding cholestan-3(β)-ol-6-one(XV). This, in turn, was chlorinated, with inversion at C_3, to give 3(α)-chlorocholestan-6-one(XVI). In this manner, a function was preserved at C_3 which would easily resist the vigorous oxidative attack in the following reaction employed to cleave ring B with the concomitant formation of 3(α)-chlorocholestan-6:7-dioic acid(XVII). Saponification of this compound resulted in the replacement of the 3(α)-chloro with a 3(β)-hydroxy group, again with inversion at C_3, to yield cholestan-3(β)-ol-6:7-dioic acid(XVIII).

This seems, at first glance, a circuitous route whereby 3 (α)-chlorocholestan-6-one(XVI) is obtained. One might be prone to suggest that if, starting with cholesterol(I) (which was, indeed, necessary to obtain 3(β)-acetoxycholestan-6-one(III)), 3(α)-chloro-Δ^5-cholestene were prepared, it would be necessary only to nitrate and reduce in a manner entirely similar to that as disclosed in Chart 1, I → III. That such a sequence could be realized is more than theory, since 3(β)-chloro-Δ^5-cholestene(XXV) has been converted to 3(β)-

chlorocholestan-6-one(XXVII) in this manner[27]. It is, however, impossible to invert the configuration at C_3 in cholesterol(I) by replacement reactions, even though these reactions may be operating via an S_N1 mechanism (nucleophilic substitution reaction of the first order). Despite whether phosphorus pentachloride, thionyl chloride, or thionyl chloride plus pyridine is employed to replace the C_3-hydroxyl, retention of configuration is observed in each case, the resulting chloro group being attached in the β-position.

This anomaly has been carefully studied by Shoppee[28], who points out that the presence of the double bond at C_5:C_6 is responsible, due to the polarizability of the electrons at C_5. Considering the chlorination of cholesterol(I) by phosphorus pentachloride then, one obtains the following picture: Under the influence of the reagent the ionization of the C_3-hydroxyl is favored, and as this ionization passes over its energy barrier, the polarizable electrons of the C_5:C_6 double bond interact with the carbon cation formed at C_3 before the reagent can attack at this point. This interaction is sufficient to overcome both the energetic and geometric factors which normally operate to favor the production of a linear transition state. Instead, the formation of

a pyramidal transition state obtains, with the result of retention of configuration after attack by the reagent and completion of reaction. This concept is not



valid, however, for intramolecular mechanisms, in which cases a carbon cation is not possible, but chemical evidence strongly suggests that the S_N1 is the operating mechanism herein.

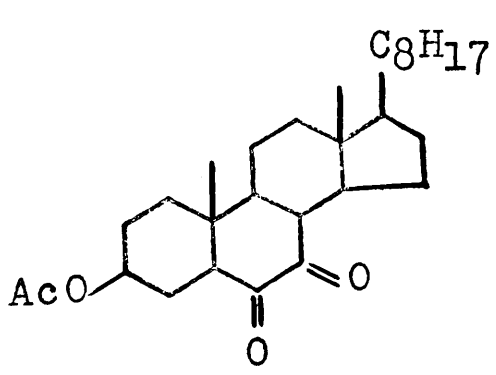
In the saturated series, an example of which is cholestan-3(β)-ol-6-one(XV), the electrons at C_5 have no longer any potential influence on the disposition of the carbon cation formed at C_3 during attack by a nucleophilic reagent. This allows for the production of a linear transition state which requires an S_N2 mechanism, hence such replacement reactions(halogenation, hydroxylation, acetoxylation, etc.) proceed in a perfectly normal manner, inversion of configuration being observed in the case of the chlorination of cholestan-3(β)-ol-6-one(XV) and of the saponification of 3(α)-chlorocholestan-6:7-dioic acid(XVII).

2. Discussion.

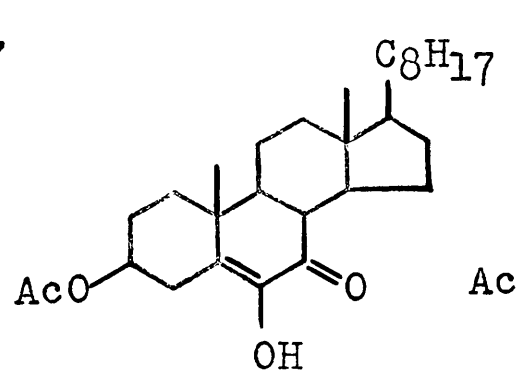
a) The Benzil-Benzilic Acid Rearrangement.

The preparation of 3(β)-acetoxycholestan-

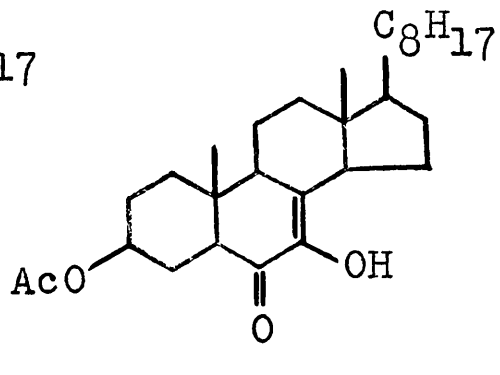
6,7-dione IX, was accomplished by previously described procedures. 3(β)-acetoxycholestan-6-one(III), the preparation of which has been described in detail (pp. 22-23), was treated with bromine in acetic acid and thus monobrominated at position 7 to yield 3(β)-acetoxy-7-bromocholestan -6-one(VIII)[24], m.p. 144-145°C. This, in turn, was treated with silver nitrate in pyridine, replacing the bromine at C_7 with a carbonyl group, affording 3(β)-acetoxycholestan-6,7-dione(IX)[25], m.p. 156-157°C. The formula as depicted in Chart 2 is for purpose of simplification only. As it is now well-known, IX exists as a mono-enol either as IXa or IXb[29], since the structure contains one active hydrogen. Unfortunately the absorption does not distinguish between



IX



IXa



IXb

the structures IXa and IXb, since the two unsaturated systems are identical in chromophoric power. From theoretical considerations, the exaltation of the absorption maximum due to the enolic hydroxyl group amounts to a shift of 31 mμ.

Repeated attempts to effect rearrangement of 3(β)-acetoxycholestan-6,7-dione(IX) failed. These attempts were carried out under a variety of conditions, employing 10,20,30,40 and 50 gm. potassium hydroxide per 100 ml. of 1:5 water-methanol, with refluxing times varying from 2 to 20 hours. In each case, saponified starting material was obtained and identified as cholestan-3(β)-ol-6,7-dione(XV)[30], m.p. 152-153°C. A typical trial will be discussed under Experimental.

It was apparent, therefore, that the double annulation of ring B prevented its contraction by the benzil-benzilic acid rearrangement. In this case, attention was diverted to the alternate method proposed for securing seco-6-cholestan-3(β)-ol-7-one(XII). This is discussed in the following.

b) Preparation and Pyrolysis of the Barium Salt of Cholestan-3(β)-ol-6:7-dioic Acid(XIX).

The preparation of XIX also requires as starting material 3(β)-acetoxycholestan-6-one(III). In this sequence, however, III was first saponified with hydrochloric acid in ethanol to yield cholestan-3(β)-ol-6-one(XV)[31], m.p. 141-142°C. The hydroxy-ketone XV was chlorinated at position 3 by grinding the steroid and phosphorus pentachloride in the dry state, thus preser-

ving the ketonic function at C_6 which would have otherwise been attacked[32]. This treatment resulted in 3(α)-chlorocholestan-6-one(XVI), melting at 180-181°C. which, in turn, was oxidized with fuming nitric acid at 60-70°C. to yield 3(α)-chlorocholestan-6:7-dioic acid(XVII)[33], separating easily from the chilled reaction mixture and which, upon recrystallization, melted at 241-243°C. Saponification of the chloro-diacid XVII gave cholestan-3(β)-ol-6:7-dioic acid(XVIII)[34], melting at 236-239°C., and which was precipitated quantitatively with barium chloride to yield the barium salt(XIX)[26].

The pyrolysis of the barium salt(XIX)[26] yielded a clear, light yellow oil which, upon recrystallization, gave crystals(XX) melting at 110-111°C. The presence of a carbonyl group was established by the formation of an oxime[26], m.p. 157-158°C. Since one of the chief concerns here was the preservation of the 3-hydroxyl group, attempts were made to precipitate the material with digitonin[35]. No digitonide was formed, which indicated that the hydroxyl group was split off. Attempts to acetylate XX were in vain also, starting material being returned in each case. On the basis that the 3(β)-hydroxyl was no longer present, it should follow that a point of unsaturation had been introduced. A positive

test with tetranitromethane confirmed these suspicions and, although the position of the double bond cannot be deduced from this evidence, it is suggested that this position might reasonably be at C_2:C_3. The characterization of compound XX was not further pursued since, obviously, it would be of no value in the proposed transformation to cholesterol(I).

3. Experimental.

a) Bromination of 3(β)-acetoxycholestan-6-one(III).

In a solution of 25 ml. acetic acid and 130 ml. ether were dissolved 11.0 gm. 3(β)-acetoxycholestan-6-one(III). 40 ml. of a solution of bromine in acetic acid (containing 5.0 gm. bromine per 100 gm. solution) were added drop-wise over a period of 75 minutes at 35°C. The resulting mixture was refluxed for 2.0 hours, the ether boiled off on a steam-bath and 5 ml. water added. The material separating on cooling was filtered and recrystallized from acetic acid. In this way 8.1 gm. 3(β)-acetoxy-7-bromocholestan-6-one(VIII) were obtained, melting at 143-145°C. Yield, 62.3 per cent of theory.

b) 3(β)-acetoxycholestan-6,7-dione(IX).

To 5.0 gm. 3(β)-acetoxy-7-bromocholestan-

6-one(VIII) contained in 100 ml. pyridine were added 10 gm. silver nitrate, and the mixture heated under reflux for 5.5 hours, or until such time when the evolution of nitrogen dioxide ceased. The reaction mixture was transferred quantitatively to a one liter separatory funnel, 500 ml. ether added, and the pyridine extracted with small portions of 5 M sulfuric acid. The ether layer was then washed with 5 per cent potassium carbonate, separated and dried over anhydrous magnesium sulfate. The dried ether solution was filtered, evaporated <u>in vacuo</u>, and the resulting residue taken up in 200 ml. ethanol. 5 gm. Norit were added and the mixture placed under reflux for 0.5 hours. The hot solution was then filtered rapidly and the resulting filtrate boiled down on a steam-bath until a faint turbidity was noted. The material separating upon cooling was filtered and recrystallized twice from methanol, giving 2.2 gm. 3(β)-acetoxycholestan-6,7-dione (IX), melting at 156-157°C. Yield, 49.3 per cent.

c) <u>Attempted Rearrangement of 3(β)-acetoxycholestan-6,7-dione(IX) to Seco-6-cholestan-3(β),7-diol-7-carboxylate(XI)</u>.

To a solution containing 5.0 gm. potassium

hydroxide, 5.0 ml. water and 20.0 ml. ethanol were added 200 mg. 3(β)-acetoxycholestan-6,7-dione(IX), and the mixture placed under reflux for 2.0 hours. At the end of this time, the solution was cooled and neutralized to a litmus end-point with 12 M hydrochloric acid, then reduced to two-thirds of its original volume in vacuo. The separating solid was collected on a small funnel, washed with water and dried. One crystallization from methanol gave 185 mg. material, melting at 151-153°C., depressing the melt when mixed with an authentic sample of 3(β)-acetoxycholestan-6,7-dione(IX), and was identified as cholestan-3(β)-ol-6,7-dione(X).

d) Cholestan-3(β)-ol-6-one(XV).

A mixture of 700 ml. ethanol and 85 ml. 12 M hydrochloric acid containing 25,0 gm. 3(β)-acetoxycholestan-6-one(III) was heated under reflux for 1.5 hours. Upon allowing the contents to cool, a voluminous precipitate of crystalline material separated, which was filtered by suction and washed with a small quantity of cold methanol. More material was obtained by concentrating the filtrate from this crystallization and was combined with the first crop, all of which was recrystallized once

more from ethanol. In this manner 20.7 gm. cholestan-3(β)-ol-6-one(XV) were obtained, melting at 140-142°C. Yield 91.5 per cent.

e) 3(α)-chlorocholestan-6-one(XVI).

In a mortar were placed 20.0 gm. dry cholestan-3(β)-ol-6-one(XV) which was ground thoroughly with 15.0 gm. phosphorus pentachloride for 0.5 hours. The resulting paste was covered with a minumum amount of water and heated on the steam-bath for 15 minutes. The material was transferred quantitatively to a one liter separatory funnel by means of water and ether. The volume of the ether in the funnel was brought up to approximately 600 ml., 200 ml. water added, and the contents shaken thoroughly. The two phases were allowed to separate overnight whereupon the aqueous layer was drawn off and discarded. The ether layer was then washed with two 200 ml. portions of 2.5 per cent aqueous potassium carbonate, separated, and dried over anhydrous magnesium sulfate. The dried ether extract was filtered through a Whatman No. 50 filter, evaporated *in vacuo* to dryness and crystallized twice from acetone-benzene. 12.0 gm. 3(α)-chlorocholestan-6-one(XVI) were thus obtained, melting at 179-181°C. Yield, 57.4 per cent.

f) 3(α)-chlorocholestan-6:7-dioic acid(XVII).

In a flask containing 10.0 gm. 3(α)-chlorocholestan-6-one(XVI) was added a cold mixture of 100 ml. fuming nitric, d. 1.50, and 100 ml. glacial acetic acid. The flask and the contents were placed in a water-bath and maintained at 60-70°C. for 25 minutes. The flask was then transferred to an ice-bath and cooled with swirling. The fine, crystalline material which separated was filtered through a sintered-glass funnel by suction, washed well with water and recrystallized twice from acetic acid. This gave 6.0 gm. 3(α)-chlorocholestan-6:7-dioic acid(XVII), melting at 239-242°C. Yield, 53.0 per cent.

g) Cholestan-3(β)-ol-6:7-dioic acid(XVIII).

In 200 ml. of 10 per cent aqueous potassium hydroxide were dissolved 5.0 gm. 3(α)-chlorocholestan-6:7-dioic acid(XVII). The flask and contents were placed directly on the steam-bath and saponified for 1.0 hours. After cooling, the material was precipitated with 5 M sulfuric acid, yielding 4.5 gm. crude cholestan-3(β)-ol-6:7-dioic acid(XVIII) after filtration. 1.0 gm. of this crude, dry material was taken up in 20 ml. acetone and placed on the steam-bath.

Benzene was added drop-wise until the solution became cloudy, whereupon gentle boiling was continued until crystalline material began to separate. The flask was then removed and allowed to cool, the crystals filtered by suction and dried, m.p. 236-239°C. Yield of crude XVIII, 93.7 per cent.

h) Barium Salt of Cholestan-3(β)-ol-6:7-dioic Acid XIX.

Without further purification, 3.0 gm. crude cholestan-3(β)-ol-6:7-dioic acid(XVIII) were covered with 150 ml. of 40 per cent methanol and titrated with 5 per cent aqueous potassium hydroxide to a phenolphthalein end-point. 2.76 gm. barium chloride (two mole-equivalents) in a small volume of water were added rapidly to this, and the resulting precipitate filtered by suction through a Whatman No. 50 filter. The precipitate was then washed in succession with methanol and water, removed to a watch-glass and dried in a vacuum oven at 65°C. under 5 mm. mercury for 3.0 hours.

i) Thermal Decomposition of the Barium Salt XIX.

The pyrolysis was conducted in the apparatus as shown in Fig. 1. 1.3 gm. dry barium cholestan-

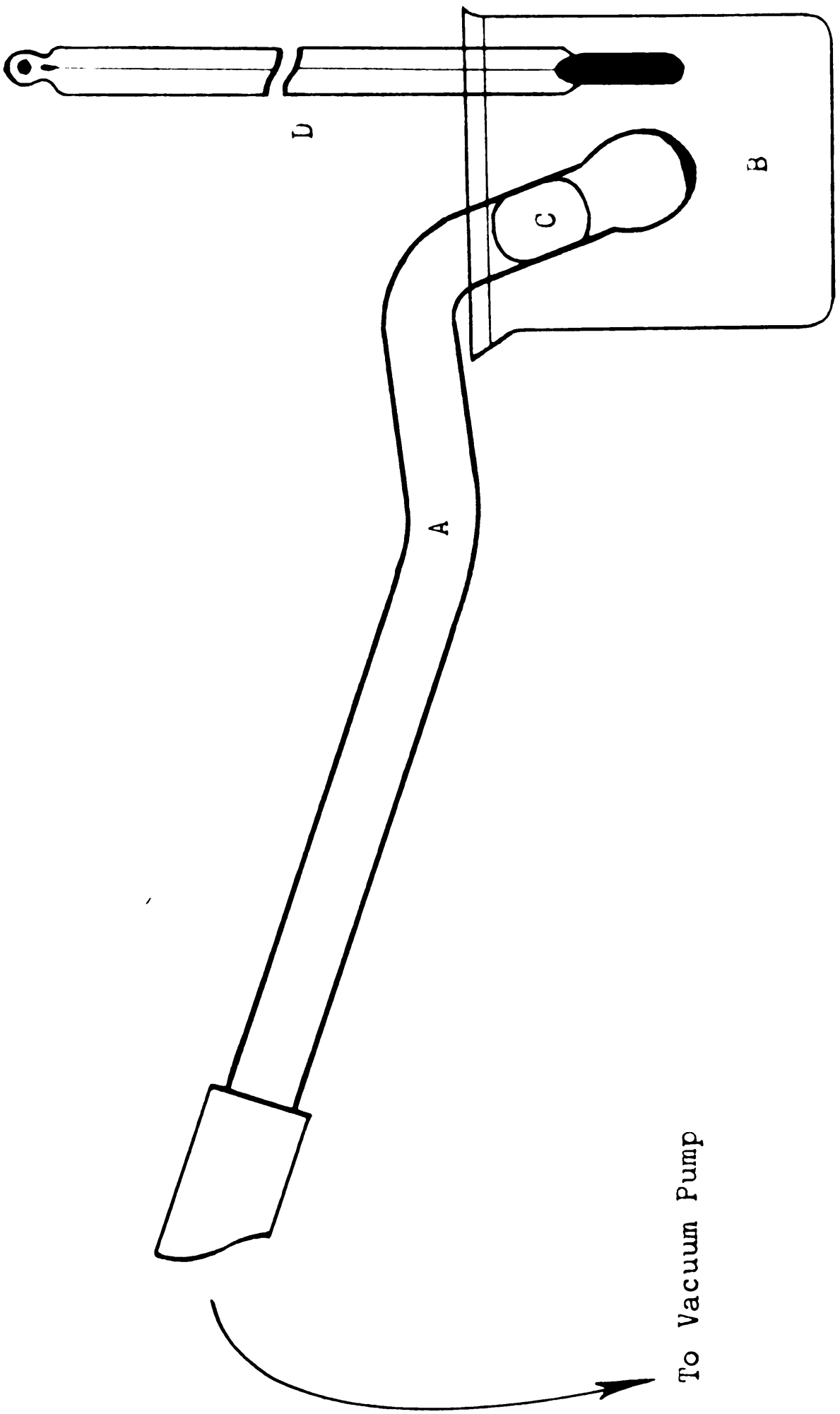


Fig. 1 - Apparatus for Thermal Decomposition of Barium or Lead Salts of Cholestane-6:7-dioic acid Derivatives.

3(β)-ol-6:7-dicarboxylate(XIX) were placed in the bottom of a 10 mm. pyrex bent sealing-tube (A) and protected by a glass-wool plug (C). The tube was then connected to a vacuum pump and subjected to a reduced pressure of ca. 1 mm. mercury. The lower end of the tube was placed in a 100 ml. beaker (B) into which a thermometer (D), calibrated from 0-400°C., was inserted. The beaker, thus arranged, was filled completely with a coarsely ground mixture of 50-50 sodium nitrite-sodium nitrate. A flame was employed and when the temperature had risen to 240-245°C., the nitrite-nitrate bath was completely molten. The temperature was raised another 5 degrees, and the bath maintained at 250°C. for 3.0 hours, at the end of which time the decomposition was considered terminated. The tube was now removed from the bath, disconnected from the pump and allowed to cool. After washing and drying the outside, the tube was broken just above the glass-wool plug, and the oil which distilled and collected in the upper part was washed into a small Erlenmeyer flask with successive rinses of ether. The solution thus obtained was evaporated on a steam-bath and the oily residue crystallized from a small amount of methanol. A further crystal-

lization gave 225 mg. XX, melting at 110-111°C.

j) Preparation of the Oxime of Compound XX.

In a 30 ml. flask fitted with a condenser were placed 50 mg. XX, 50 mg. hydroxylamine hydrochloride and 50 mg. anhydrous sodium acetate. 15 ml. methanol and 5 drops of water were added to the flask, and the contents placed under reflux for 3.0 hours. After cooling, the separating material was filtered and recrystallized from methanol, affording the oxime of XX, melting at 156-157°C.

Part D

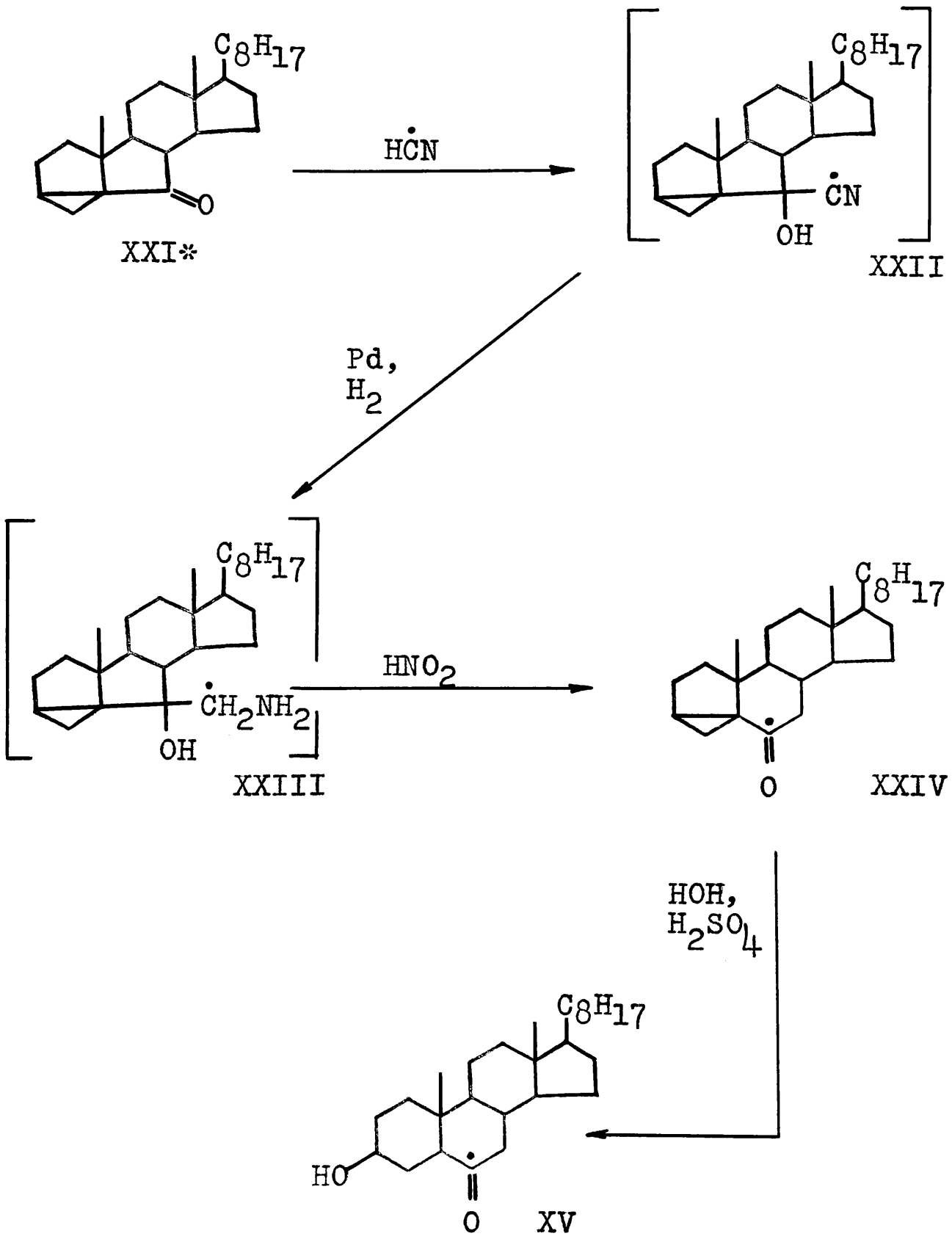
Seco-6-i-cholestan-7-one(XXXI) and Seco-6-3(β)-bromo-cholestan-7-one(XXXII).

1. Theoretical.

To reiterate, the application of the Tiffeneau reaction[20] in Part C was precluded by the loss of the 3(β)-hydroxyl during the thermal decomposition of the barium salt of cholestan-3(β)-ol-6:7-dioic acid(XIX). Since, theoretically, this reaction might still be employed as envisaged, a search was made for some suitable derivative of cholestan-6:7-dioic acid which, on pyrolysis of the barium salt, would maintain the integrity of the 3(β)-hydroxyl, either directly or indirectly. A survey of numerous possibilities was made, with the result that i-cholestan-6:7-dioic acid(XXIX) was chosen as a successor to cholestan-3(β)-ol-6:7-dioic acid(XVIII), employed in Part C.

Two factors of paramount importance were considered concerning this choice. The first of these was the possible effect of thermal treatment on the cyclopropane structure in ring A, although it seemed quite certain that pyrolysis of a divalent metallic salt of i-cholestan-6:7-dioic acid(XXIX) should give

Chart 3

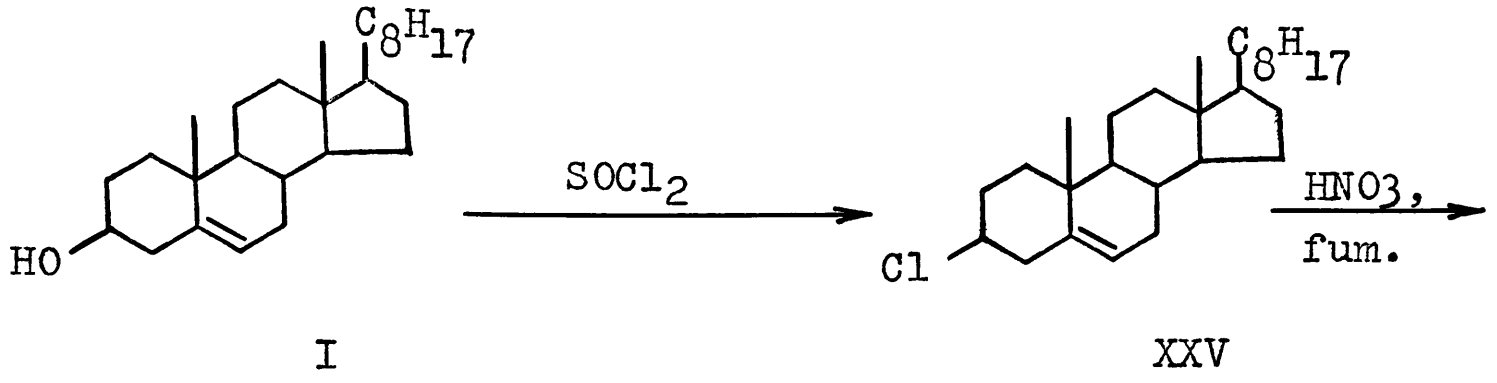


* Chart 4, XXXI

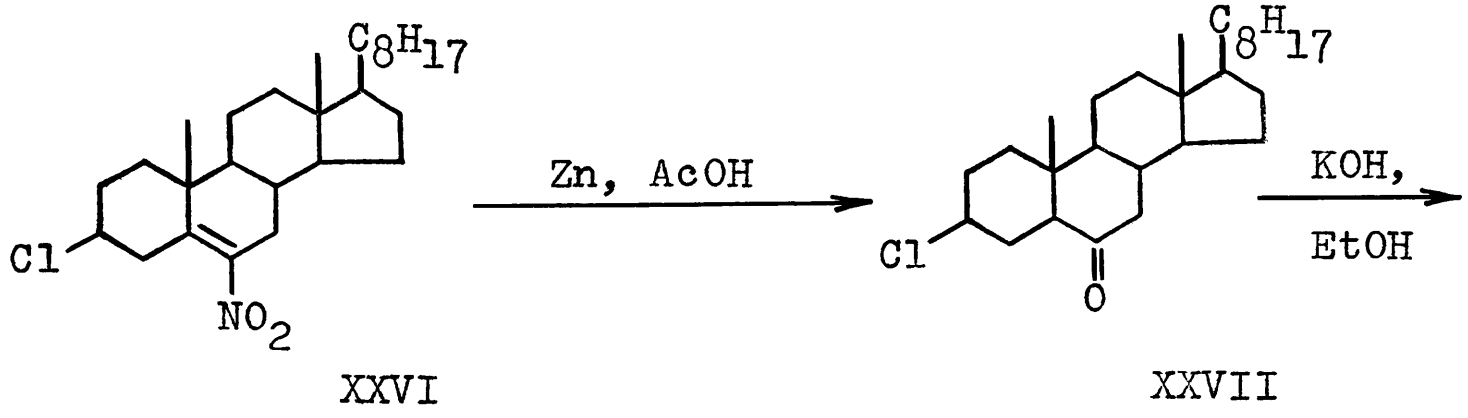
rise, without disturbing this system, to seco-6-i-cholestan-7-one(XXXI), not previously described. The second factor was the restoration of the 3(β)-hydroxyl group after the nitrous acid treatment in the Tiffeneau reaction as proposed in Chart 3. The compound anticipated by this rearrangement would be i-cholestan-6-one(XXIV) which, fortunately, undergoes easy conversion to cholestan-3(β)-ol-6-one(XV) by hydrolytic measures[36]. Acetylation of XV, then, would yield 3(β)-acetoxycholestan-6-one (III) which had already been established as a synthetic goal in Part B.

The preparation of i-cholestan-6:7-dioic acid(XXIX) may be prepared as shown in Chart 4 starting with cholesterol(I), which is chlorinated at C_3 to yield 3(β)-chloro-Δ^5-cholestene(XXV)[37]. This is nitrated to give 3(β)-chloro-6-nitro-Δ^5-cholestene(XXVI)[15]. Reduction of XXVI gives 3(β)-chlorocholestan-6-one(XXVII)[16] which, by alkaline treatment, loses elements of hydrochloric acid at C_3 and C_5 to form a bridge linkage between these two carbon atoms and which provides, in quantitative yield, i-cholestan-6-one(XXIV)[33]. This, in turn, undergoes mild oxidation cleaving ring B between C_6:C_7, with the formation of i-cholestan-6:7-dioic acid(XXIX)[36]. It was proposed, therefore,

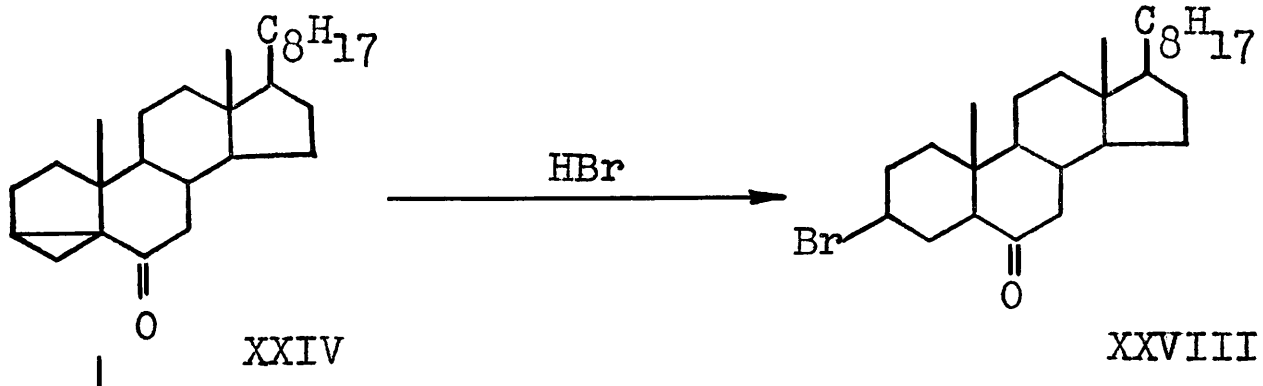
Chart 4



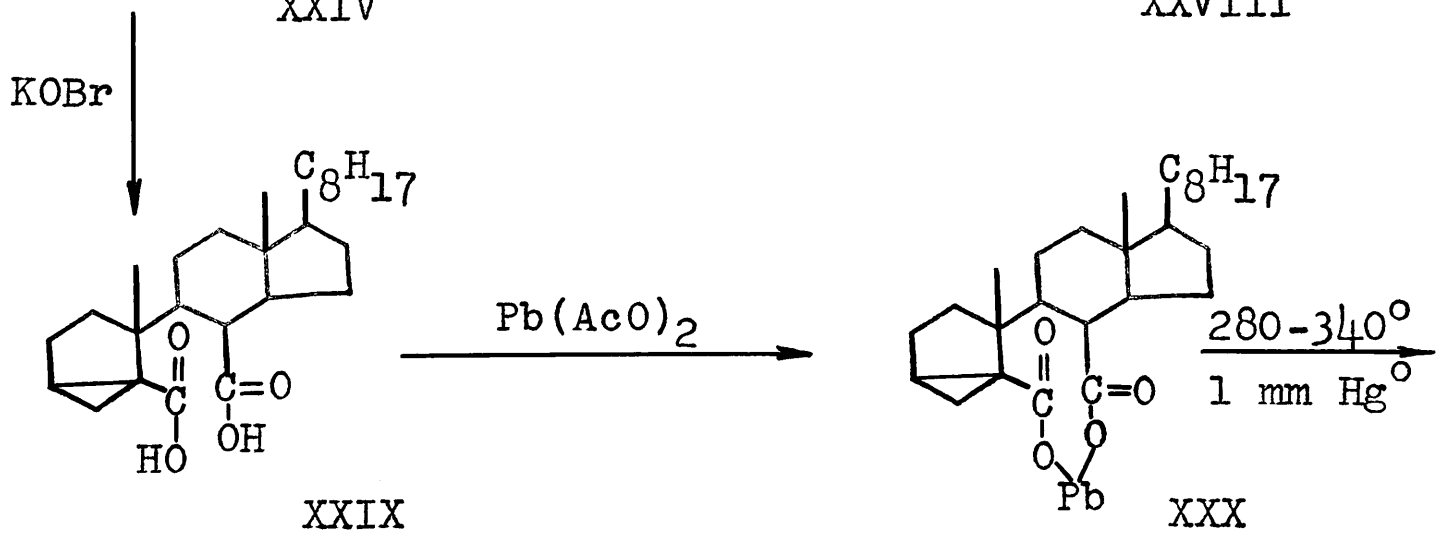
I XXV



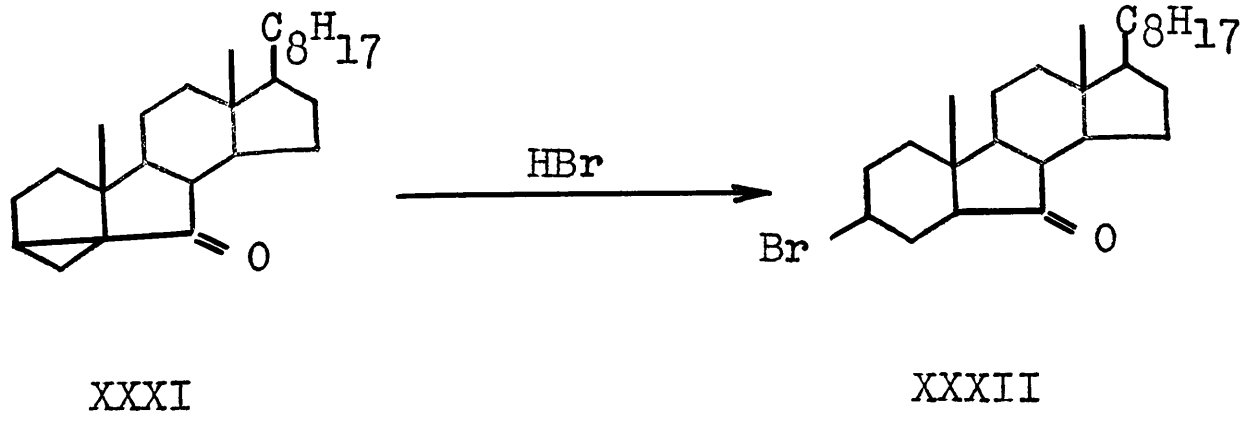
XXVI XXVII



XXIV XXVIII



XXIX XXX



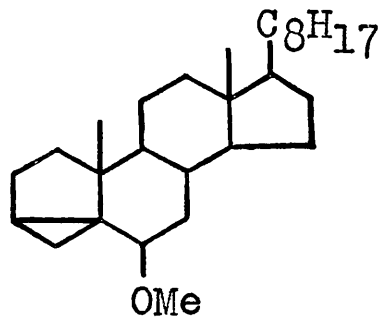
XXXI XXXII

to prepare the barium salt of this di-acid and pyrolyze in a manner entirely similar to that described on pp.43-44 herein. As mentioned above, the compound expected by this treatment would be seco-6-i-cholestan-7-one(XXXI).

2. Discussion.

a) i-cholestan-6-one(XXIV).

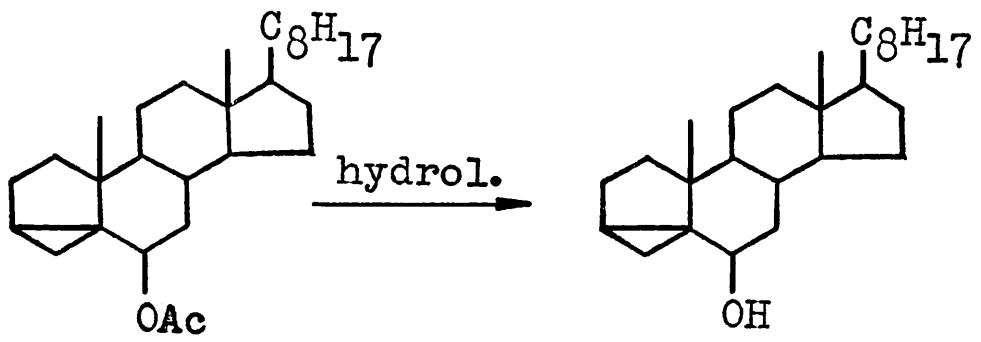
The preparation of i-cholestan-6:7-dioic acid(XXIX) was investigated first. Since entry into the i-cholestane series was provided by the compound i-cholestan-6-one(XXIV), attention was primarily centered at this point. One route to this compound is provided in a paper by Wallis, et al.[38], who showed that the abnormal cholesterol methyl ether of Stoll was, indeed, i-cholesterol



i-cholesterol methyl ether

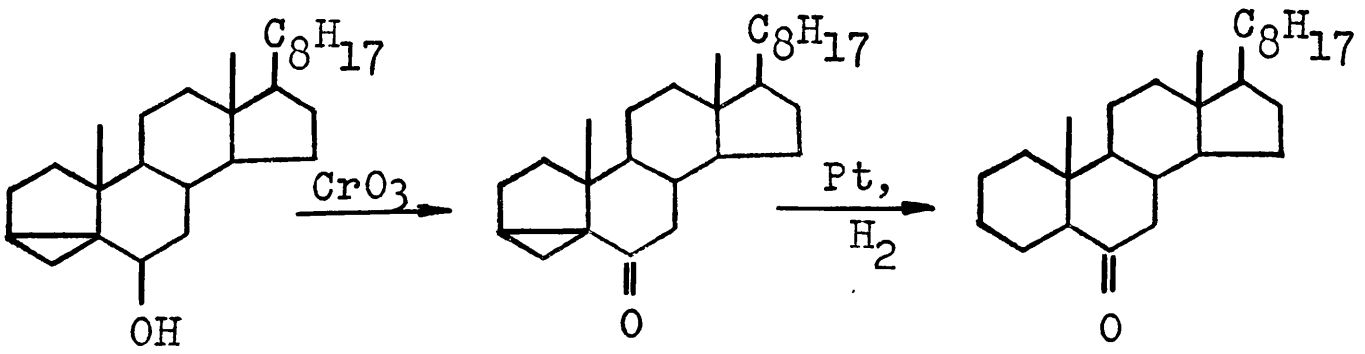
methyl ether. By reacting cholesterol p-toluene-sulfonate with potassium acetate in acetic anhydride, they obtained an abnormal, dextrorotatary acetate

which, on hydrolysis, gave an isomeric cholesterol that did not precipitate with digitonin. These



i-cholesterol acetate i-cholesterol

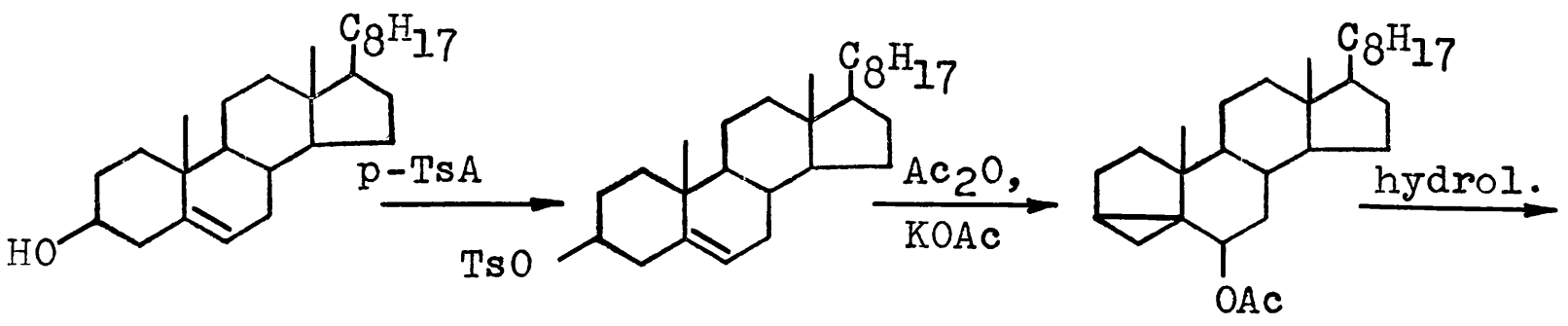
workers inferred the cyclopropane structure in ring A and designated this new isomer i-cholesterol. Later work confirmed this inference[36,39,40]. In many respects, the bridge linkage at C_3-C_5 is remarkably unreactive, particularly with reference to reactions applicable to double bonds. It can, however, be hydrogenated in the presence of Adams catalyst, as demonstrated by Heilbron, et al.[41], who carried out the chromic acid oxidation of i-cholesterol to the corresponding ketone i-cholestanone (XXIV) which, in turn, was converted by hydrogenation into cholestan-6-one, thus establishing



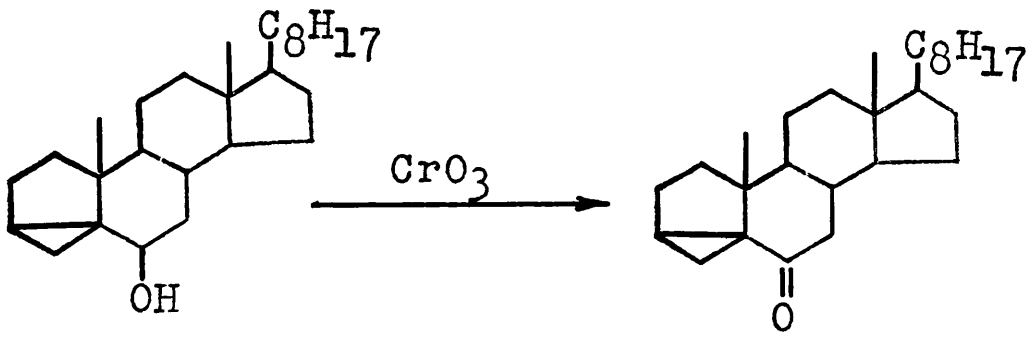
i-cholesterol (XXIV) cholestan-6-one

the position of the hydroxyl group. Heilbron[41] found that the i-cholestan-6-one(XXIV) thus prepared was identical with an unidentified product prepared nearly twenty years earlier by Windaus and Dalmer[27] and which they called "heterocholestenone", believing that this compound was unsaturated at C_4:C_5. In a later paper, Windaus and Staden prepared i-cholestan-6-one(XXIV) in almost quantitative yield by treating 3(β)-chlorocholestan-6-one(XXVII) with alcoholic potassium hydroxide (Chart 4, XXVII → XXIV), although at that time, as just pointed out, the structure was not known[33].

Thus, two routes were open for the preparation of i-cholestan-6-one(XXIV). Although Heilbron[42] greatly improved the method of Wallis[38] for the



I — cholesterol-para-toluenesulfonate — i-cholesterol acetate



i-cholesterol — XXIV

preparation of i-cholesterol, the work-up proved to be fairly involved, entailing the use of the expensive digitonin and did not, therefore, lend itself to large-scale preparations, as would be the case herein. Furthermore, an additional oxidative step converting i-cholesterol to i-cholestan-6-one (XXIV) was still necessary.

On the other hand, i-cholestan-6-one(XXIV) may be prepared in almost quantitative yield from 3(β)-chlorocholestan-6-one(XXVII) by the method of Windaus and Staden[33]. The 3(β)-chlorocholestan-6-one(XXVII) is conveniently prepared from cholesterol(I) in three steps as outlined in Chart 4, I $\longrightarrow$ XXVII. The yields are good, reagents inexpensive and the reactions lend themselves well to large-scale syntheses since, in all steps involved, crystalline products separate easily from the reaction mixtures. On this basis, it was decided to employ the latter synthetic sequence for obtaining i-cholestan-6-one(XXIV).

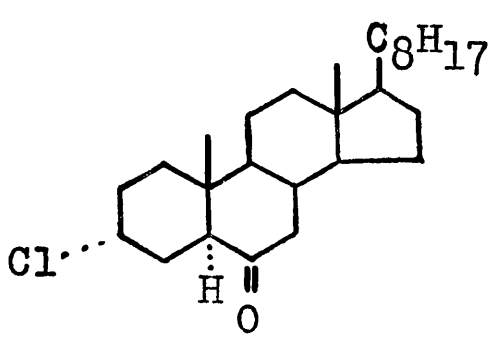
Cholesterol(I) was chlorinated at C_3 without inversion, by the use of thionyl chloride to yield 3(β)-chloro-Δ^5-cholestene(XXV), m.p. 96°C. This was nitrated with fuming nitric acid to give 3(β)-chloro-6-nitro-Δ^5-cholestene(XXVI) m.p. 154-155°C. which, in turn, was reduced by the action of zinc

and acetic acid to give 3(β)-chlorocholestan-6-one (XXVII), m.p. 129-130°C.[27], these two latter reactions being conducted in an identical manner to the conversion of cholesterol(I) to 3(β)-acetoxycholestan-6-one(III) as described on pp. 21-23 herein[15,16].

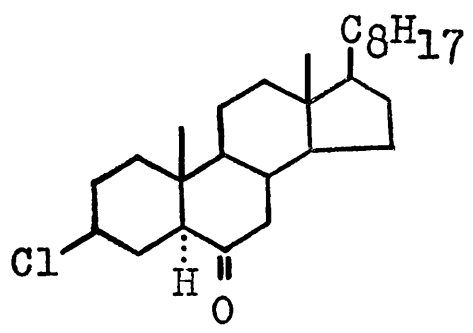
The 3(β)-chlorocholestan-6-one(XXVII) thus prepared was treated with ethanolic potassium hydroxide at 50°C., whereupon a fine precipitate of potassium chloride appeared almost immediately and in three to five minutes the reaction was complete[33]. On cooling, crystalline i-cholestan-6-one(XXIV) was obtained and melted at 97°C. This is, indeed, a remarkable reaction, both from the standpoint of the velocity with which it proceeds and of the establishment of the bridge linkage between C_3 and C_5.

The reaction has been studied in detail by Dodson and Riegel[16], who demonstrated that it is a stereospecific one, since 3(α)-chlorocholestan-6-one (XVI) subjected to identical conditions is recovered unchanged at the end of the experiment[33]. Now, in the cholestane series it has been established that rings A and B are fused in the trans-configuration, with C_1 and C_9 regarded as projecting below the plane of the paper, while C_4 and C_6 project above. This circumstance necessitates a downward projection of the C_5 hydrogen in order to satisfy the requirements of

the tetrahedral character of carbon. Regarding, then, the two isomeric 3-chlorocholestan-6-ones, XVI and XXVII, it is seen that in the case of 3(α)-chlorocholestan-6-one(XVI), the C_3-chlorine and C_5-hydrogen are <u>cis</u>, whereas in 3(β)-chlorocholestan-



XVI



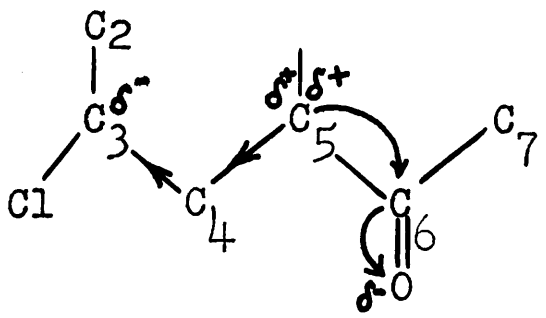
XXVII

6-one(XXVII), the C_3-chlorine and C_5-hydrogen are <u>trans</u>. Observations of Michael[43], leading to the concept of <u>trans</u> elimination, show that it is easier to remove elements of HX from a <u>trans</u> olefinic derivative than the <u>cis</u> isomer. On this basis, it appears that the hydrogen at C_5 and the chlorine at C_3 in 3(β)-chlorocholestan-6-one(XXVII) are ideally suited for elimination.

In the elimination of elements of hydrochloric acid from 3(β)-chlorocholestan-6-one(XXVII) it is highly probable, according to Dodson and Riegel[16], that the attack of the hydroxyl ion first results in the ionization of the C_5-hydrogen, removing it as a proton. The pair of unshared electrons remaining at

C_5 are then visualized as attacking the back side of C_3, eliminating the chlorine atom as an ion with inversion at C_3. What has not been demonstrated clearly, however, is that the extreme readiness of the C_5-hydrogen to ionize is a property characteristic, apparently, only to molecules with certain specific groupings and which are disposed in such a way as to favor this ionization. The prime requisite appears to be the presence of a ketonic function at position 6 in conjugation with the bridge structure and some grouping at C_3 which may be removed as an ion, notably halogen atoms. For example, in the literature there is no evidence of similar trans-elimination reactions as applied to 3(β)-chlorocholestan-6(β)-ol or to 3(β)-chlorocholestan-7-one.

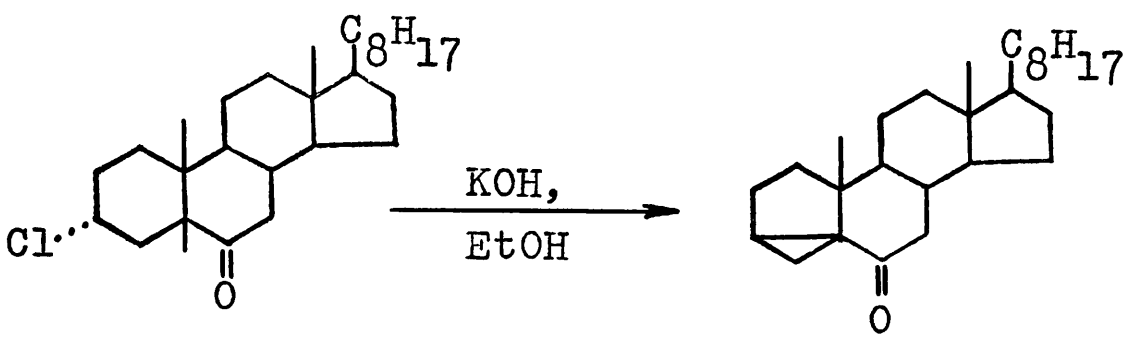
For a suggested explanation of this phenomenon one might consider the electronic theories as set forth by Ingold and Robinson and which are summarized by Johnson in Gilman's Treatise[44]. Now, considing the case of 3(β)-chlorocholestan-6-one(XXVII), it is seen that the ketone group located at C_6 is adjacent to C_5. These systems are known to involve a dynamic



shift of electrons away from the carbon atom carrying the ketonic function and it is entirely possible that such a displacement may also effect the C_5-carbon atom, thus reducing the electron density. Moreover, the presence of a strongly electronegative group at C_3 such as the chlorine atom would be responsible for an inductive effect with the result that a permanent electron displacement would obtain, both at C_4 and C_5. This would further reduce the electron density at C_5 and, hence, increase the ease of ionization of the C_5-hydrogen. Explanation of this elimination reaction on a basis of enolization seems unlikely, since it has been demonstrated that i-cholestan-6-one (XXIV) may be formed by the vacuum distillation of 3(β)-chlorocholestan-6-one(XXVII)[33].

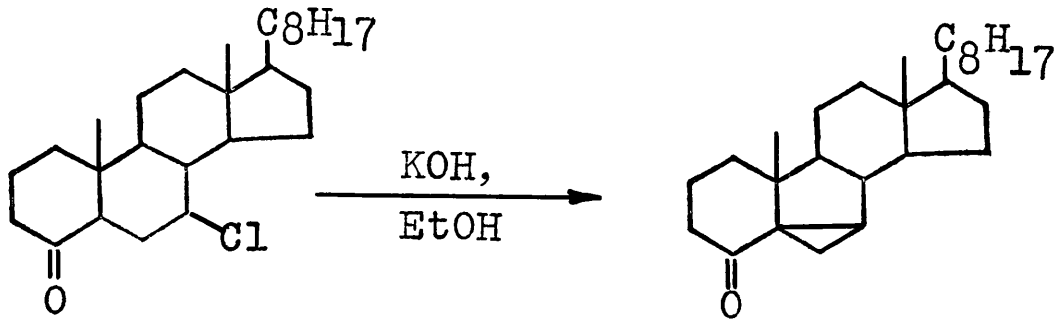
On this basis, it is further suggested that such a reaction need not necessarily be restricted to the 3(β)-chloro-6-keto derivatives of cholestane. If one considers, for example, the coprostane series where the A/B ring fusion is cis and, hence, where the C_5-hydrogen is oriented α, it will be noted that in the compound 3(α)-chlorocoprostan-6-one(not described), the C_3-chlorine and the C_5-hydrogen are trans, this relationship being entirely analogous to that displayed by these two same groups in 3(β)-chlorocholestan-6-one(XXVII). It should follow, then, that alcoholic

alkaline treatment of 3(α)-chlorocoprostan-6-one



3(α)-chlorocoprostan-6-one i-coprostan-6-one

should result in a new i-steroid, i-coprostan-6-one. Since in i-cholestan-6-one(XXIV) the methylene group at position 4 projects upward, i-coprostan-6-one would differ by a downward projection of this same group. Moreover, if this concept is valid, the reaction may be envisaged for other derivatives of cholestane, notably 7(β)-chlorocholestan-4-one which also satisfies



7(β)-chlorocholestan-4-one "B"-i-cholestan-4-one

the requirements as demonstrated above. Proof of these suggested variations will be forthcoming when the proper derivatives can be prepared.

That the elimination of elements of hydrochloric acid from 3(β)-chlorocholestan-6-one(XXVII) is stereospecific is further evidenced by the extreme ease with which the C_3-C_5 bridge of i-cholestan-6-one(XXIV) adds elements of HA, where HA is a powerful proton donor[45]. In all cases, A^- is added at C_3 in the β-position. Thus, for example, i-cholestan-6-one(XXIV) adds hydrobromic acid at room temperature and water(in the presence of hydrogen ions furnished by dilute sulfuric acid) under conditions of refluxing to give almost quantitative yields of 3(β)-bromocholestan-6-one(XXXII) and cholestan-3(β)-ol-6-one(XV), respectively[36]. This property of the cyclopropane system in i-cholestan-6-one(XXIV) may then be taken advantage of in exposing its presence in molecules possessing a ketone in conjugation with the system. A specific application of this will be demonstrated subsequently.

It was of interest to determine, at this point, if normal replacement of the C_3-chlorine in 3(β)-chlorocholestan-6-one(XXVII) could occur under conditions other than hydroxylation, where the formation of the i-steroid always takes precedence. Pursuant to this, acetoxylation at C_3 in 3(β)-chlorocholestan-6-one(XXVII) was attempted according to the directions of Shoppee[28], employing fused potassium acetate in acetic acid. Under these conditions, if acetoxylation

Fig. 2

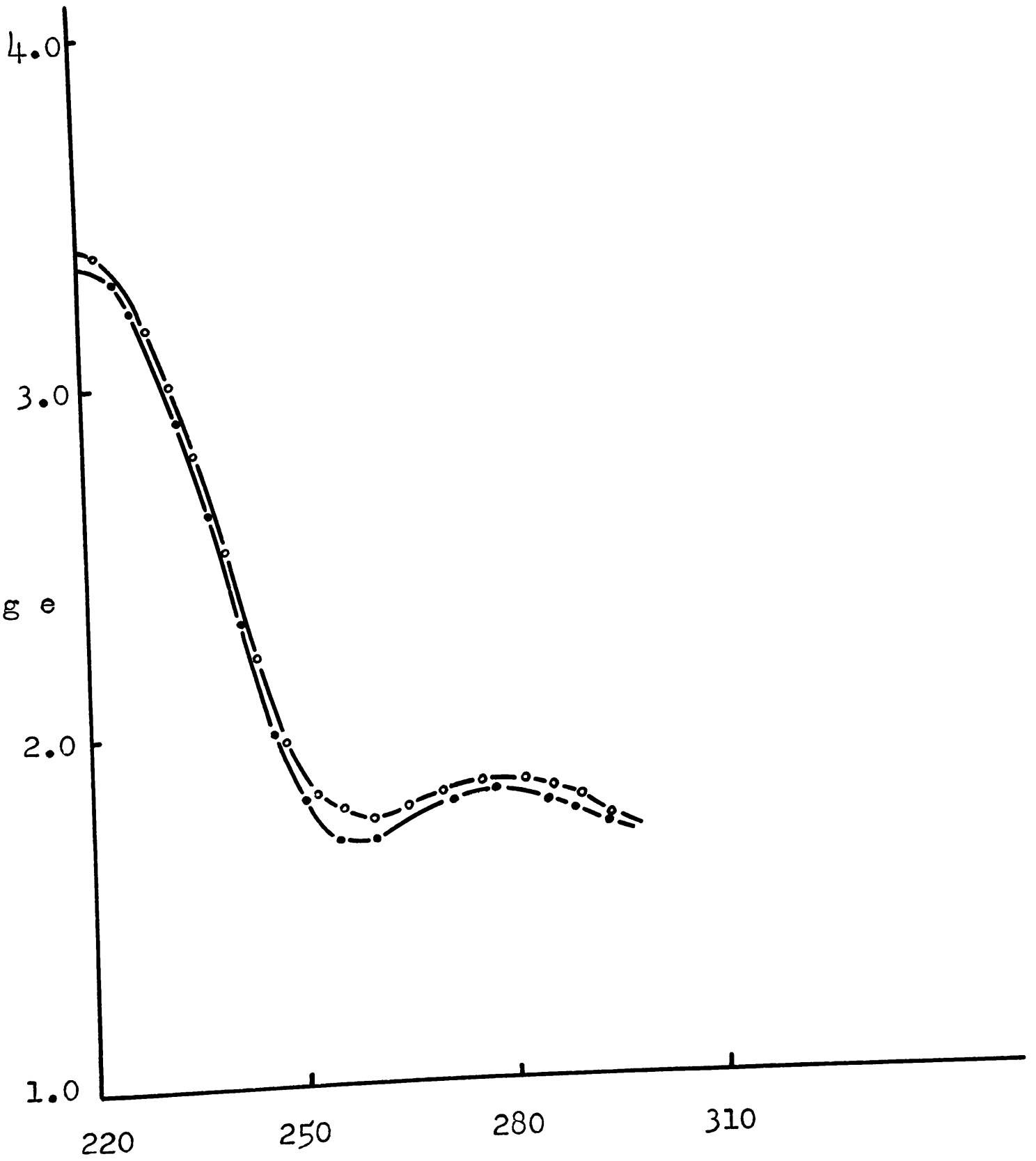


Fig. 2 - Ultra-violet absorption spectra.

open circles (o), i-cholestan-6-one (XXIV);
closed circles (•), product from attempted acetolysis of 3{β}-chlorocholestan-6-one (XXVII).

proceeded normally, the compound expected would be 3(α)-acetoxycholestan-6-one, m.p. 107-108°C.[16]. On working up the material, however, crystals were obtained which melted at 97°C. and which did not depress the melt of an authentic sample of i-cholestan-6-one (XXIV). A test with tetranitromethane was negative, and by treatment of a small quantity in methanol with 12 M hydrochloric acid[36], crystals were obtained which were identified as 3(β)-chlorocholestan-6-one(XXVII). As a final verification, the ultra-violet absorption spectra of the unknown compound and of an authentic sample of i-cholestan-6-one(XXIV) were determined simultaneously and curves for both are shown in Fig. 2[46]. The agreement obtained therein established beyond doubt that the compound resulting from the attempted acetoxylation of 3(β)-chlorocholestan-6-one(XXVII), is indeed, i-cholestan-6-one(XXIV). It is apparent, then, that in a similar manner, the high concentration of acetate ions provided by the potassium acetate provoked ionization of the C_5-hydrogen before replacement at C_3 could occur, with the result that the i-steroid XXIV was formed.

b) Preparation and Pyrolysis of Lead-i-cholestan-6:7-dicarboxylate(XXX).

Returning, now, to the main body of the work in

this Part, the i-cholestan-6-one(XXIV) prepared was subjected to a mild oxidative procedure, employing alkaline hypobromite. This treatment effected scission of ring B, between C_6:C_7, affording i-cholestan-6:7-dioic acid(XXIX) in good yield[36]. This was precipitated quantitatively with barium chloride to furnish the barium salt, which was pyrolyzed according to Stange[26]. The pyrolysis proceeded with difficulty, and only by elevating the temperature to 380°C., could sufficient oil be caused to distil over for purpose of identification. During this time, considerable decomposition took place. The oil which was recovered, however, was treated with a variety of solvents, but crystallization was not realized.

Attention was, therefore, directed toward other metals which could combine divalently with i-cholestan-6:7-dioic acid(XXIX). It was felt that a metal with a larger atomic volume than barium might provide a solution to the difficulty encountered in the case of the thermal decomposition of the barium salt. Attempts to precipitate the diacid XXIX with thorium nitrate and uranyl acetate failed, but methanolic lead acetate gave an immediate, quantitative precipitate of lead i-cholestan-6:7-dicarboxylate(XXX).

The thermal decomposition of the lead salt XXX proceeded in a far superior manner to that of the barium

salt. A clear, yellow oil began to distil at ca. 270°C., and by gradually elevating the temperature over a period of 3 hours to 350°C., complete decomposition of the lead salt was accomplished with a considerable amount of amber oil distilling over. Attempts to crystallize this material failed, consequently the separation of the crystalline components was carried out by chromatography. Crystalline material appeared early and, even under the most exacting conditions of elution, was always attended by traces of slightly aromatic, amber oil (likely of a hydrocarbon nature) which separated at the outset. Crystallization was effected in a small volume of methanol but since the accompanying oil was relatively insoluble in the solvent purification was difficult, and only by repeated recrystallization was a small amount of material obtained which gave a sharp melt of 97-98°C., and which was suitable for carbon and hydrogen analysis.

An attempt was made to form an oxime on the basis that the compound from the column was seco-6-i-cholestan-7-one(XXXI). Usually, a 3 hour reflux period is sufficient for the formation of steroid oximes, but in this case, only ill-defined crystalline material was obtained with a wide melting point range, even after two recrystallizations. When the compound was,

however, refluxed vigorously for 5 hours, a splendid array of felted needles separated which, on one recrystallization from methanol, gave crystals melting at 176-177°C. Thus, the presence of a ketonic function was established. Carbon and hydrogen analysis gave results which agreed with those calculated for seco-6-i-cholestan-7-one(XXXI) and as a final verification, addition of elements of hydrobromic acid to the C_3-C_5 bridge bond was attempted under the prescribed conditions[36]. This treatment resulted in a new compound, crystallizing in long, hard needles melting at 92-93°C., and giving a strong Beilstein test for halogen. This evidence supported strongly the suspected structure of the original compound formed during the thermal decomposition of the lead salt of i-cholestan-6:7-dioic acid(XXIX). The compound was, then, identified as seco-6-i-cholestan-7-one (XXXI). It was pointed out earlier on p. 57 that the addition of halogen acids to the C_3-C_5 bridge in steroids possessing a ketone conjugated with this system is a stereospecific reaction, the anion attaching itself at C_3 in the β-position[45]. It follows, then, that the addition of hydrobromic acid to seco-6-i-cholestan-7-one(XXXI) yields seco-6-3(β)-bromocholestan-7-one(XXXII), not hitherto described (Chart 4, XXXI ⟶ XXXII). The oxime of this compound was also prepared

and melted at 170-171°C.

As the result of the successful preparation of seco-6-*i*-cholestan-7-one(XXXI), the investigation of the Tiffeneau reaction[20] was renewed. The key step would be the addition of hydrogen cyanide to this compound, and an attempt to do so by the method of Butenandt and Thomé[47] failed. Owing to the low yields of seco-6-*i*-cholestan-7-one(XXXI), the addition of hydrogen cyanide to the 7-ketone could not be exhaustively studied, hence five separate sets of pyrolyses were conducted to obtain additional XXXI, starting each time with cholesterol(I). A typical sequence will be discussed in the experimental portion herein.

Several other attempts to effect a cyanohydrin addition by the Butenandt method failed, even though refluxing times were increased. Starting material was returned in each case. Repeated trials, by treating the ketone in aqueous ethanol with potassium cyanide at pH 10, also failed. A final method remained, that of Kuwada[48] who employs potassium cyanide in the presence of a large excess of hydrochloric acid. Although it was realized that the hydrochloric acid would add to the C_3-C_5 bridge of the seco-6-*i*-cholestan-7-one(XXXI), attempts to add hydrogen cyanide were made without regard to this circumstance, merely to determine

whether the 7-ketone would react under any conditions. Failure was encountered again and, as in previous cases, starting material was always returned. Butenandt and Thomé[47], refluxing androsterone with acetic acid and potassium cyanide in ethanol, effected addition of hydrogen cyanide to the 17-ketone in 15 minutes. It is evident, therefore, that there is a vast difference between the reactivities of ring D and ring B ketones. The additional reaction time necessary for the formation of the oxime of XXXI as discussed on p. 60 was also suggestive of the unreactive character of the 7-ketone. One reason for the failures of the addition of hydrogen cyanide may be due to the reversibility of the reaction as shown in (a), while the success of oxime formation even with unreactive ketones, for example, depends upon an irreversible loss of water(b).

a) $R_2{=}C{=}O + HCN \rightleftharpoons R_2{=}C\begin{matrix}OH\\CN\end{matrix}$

b) $R_2{=}C{=}O + NH_2OH \rightleftharpoons R_2{=}C\begin{matrix}OH\\NHOH\end{matrix} \xrightarrow{-H_2O} R_2{=}C{=}NOH$

In view of these considerations, the Tiffeneau reaction was abandoned.

c) <u>The Infra-red Spectra of Seco-6-i-cholestan-7-one (XXXI) and Seco-6-3(β)-bromocholestan-7-one(XXXII).</u>

Since seco-6-<u>i</u>-cholestan-7-one(XXXI) and seco-6-3(β)-bromocholestan-7-one(XXXII) were new compounds, it was of interest to determine the absorption spectra. Ultra-violet spectra determinations were not considered, for saturated steroid ketones do not display characteristic absorption in this region. Characteristic curves have been prepared for <u>i</u>-steroid ketones (p.58a), but these proved to be not as instructive as ultra-violet absorption curves for true α,β-unsaturated ketones which display bands of strong absorption, even though the pseudo unsaturation of the cyclopropane ring may be in "conjugation" with the ketone. Consequently, attention was directed to infra-red absorption measurements, which have proved to be a much more specific and valuable tool for the elucidation of the structure of an organic molecule. A brief account of the nature of infra-red absorption is given in the following.

In a large molecule such as a steroid, very complex interactions can occur between the vibrational motions of one atom and those of its neighbors. As a result of such interactions, new oscillations arise which are associated with groups of atoms or with the entire molecule. Such vibrations will also give rise to absorption bands in the spectrum but not in such a

way that all the various absorption bands observed can be related specifically to individual bonds or atoms in the molecule. Any change, therefore, in the relative positions of the atoms in the molecule will disturb the delicately adjusted vibrational system and alter the infra-red absorption spectrum. Owing to this sensitivity to small structural changes, the infra-red spectrum is, perhaps, the most specific of all the physical properties of a molecule.

This specificity does not apply to all of the absorption bands, however. Considering the vibratory motions of the atoms, which may be separated into longitudinal stretching and transverse bending, it usually requires more energy to change the length of a bond than to deflect the bond angle, hence absorption bands associated with stretching motions tend to occur at higher frequencies, while the bending motions occur at the lower frequencies.

Certain of these stretching motions do not interact to any considerable extent with the other vibrations of the molecule and can be clearly recognized at the higher frequency end of the spectrum, notably the stretching vibrations of the O-H, C-H, C=O and C=C bonds. Thus, the recognition of the structure groupings by their characteristic absorption bands may be employed in the elucidation of the

structure of steroid molecules[49].

It was of further interest, in this connection, to compare the infra-red absorption spectra of seco-6-i-cholestan-7-one(XXXI) and seco-6-3(β)-bromocholestan-7-one(XXXII) with their normal analogues, i-cholestan-6-one(XXIV) and 3(β)-bromocholestan-6-one(XXVIII), respectively. This proposal was warranted by the fact that infra-red spectra had not yet been determined for compounds in the i-series, hence i-cholestan-6-one(XXIV) would serve as a reference compound. As mentioned above, ultraviolet spectra are not too instructive, since the "conjugation" of the cyclopropane structure with the 6-ketone does not give rise to a strong absorption maximum as in the case of α,β-unsaturated ketones[50].

Of the four compounds to be studied spectroscopically, XXIV, XXXI and XXXII were available. It was, therefore, necessary to prepare the 3(β)-bromocholestan-6-one(XXVIII) which was conveniently accomplished according to the directions of Wallis, employing aqueous hydrobromic acid in methanol at room temperature[36] (Chart 4, XXIV⟶XXVIII). Two recrystallizations gave XXVIII, melting at 122-123°C.

The resulting infra-red absorption curves of

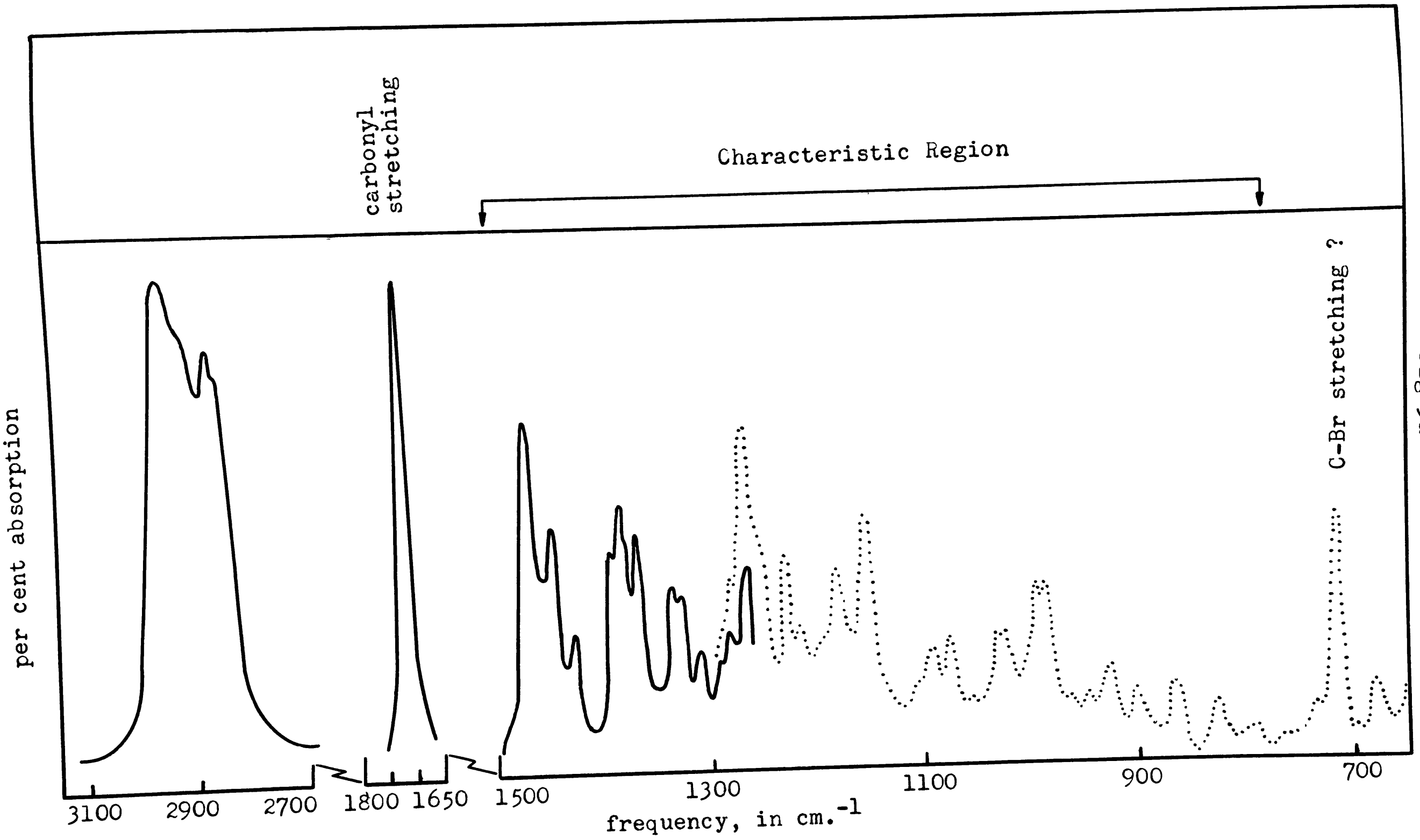


Fig 3a

Fig. 3a - Infra-red spectrum of 3(β)-bromocholestan-6-one (XXVIII).

———CaF_2 prism, CCl_4 solution, ·····NaCl prism, CS_2 solution.

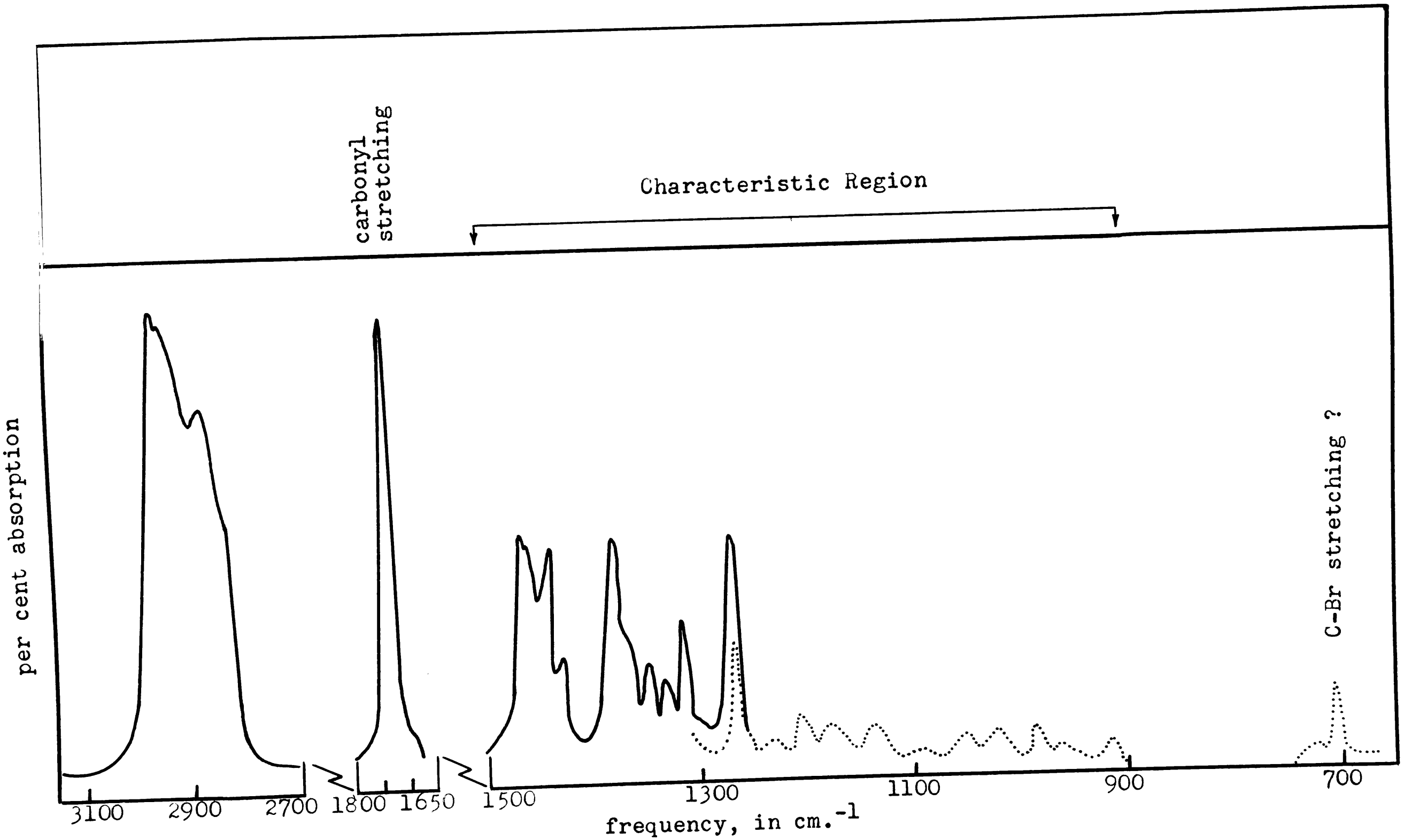


Fig. 3b - Infra-red spectrum of seco-6-3(β)-bromocholestan-7-one (XXXII).

——CaF_2 prism, CCl_4 solution, ...NaCl prism, CS_2 solution.

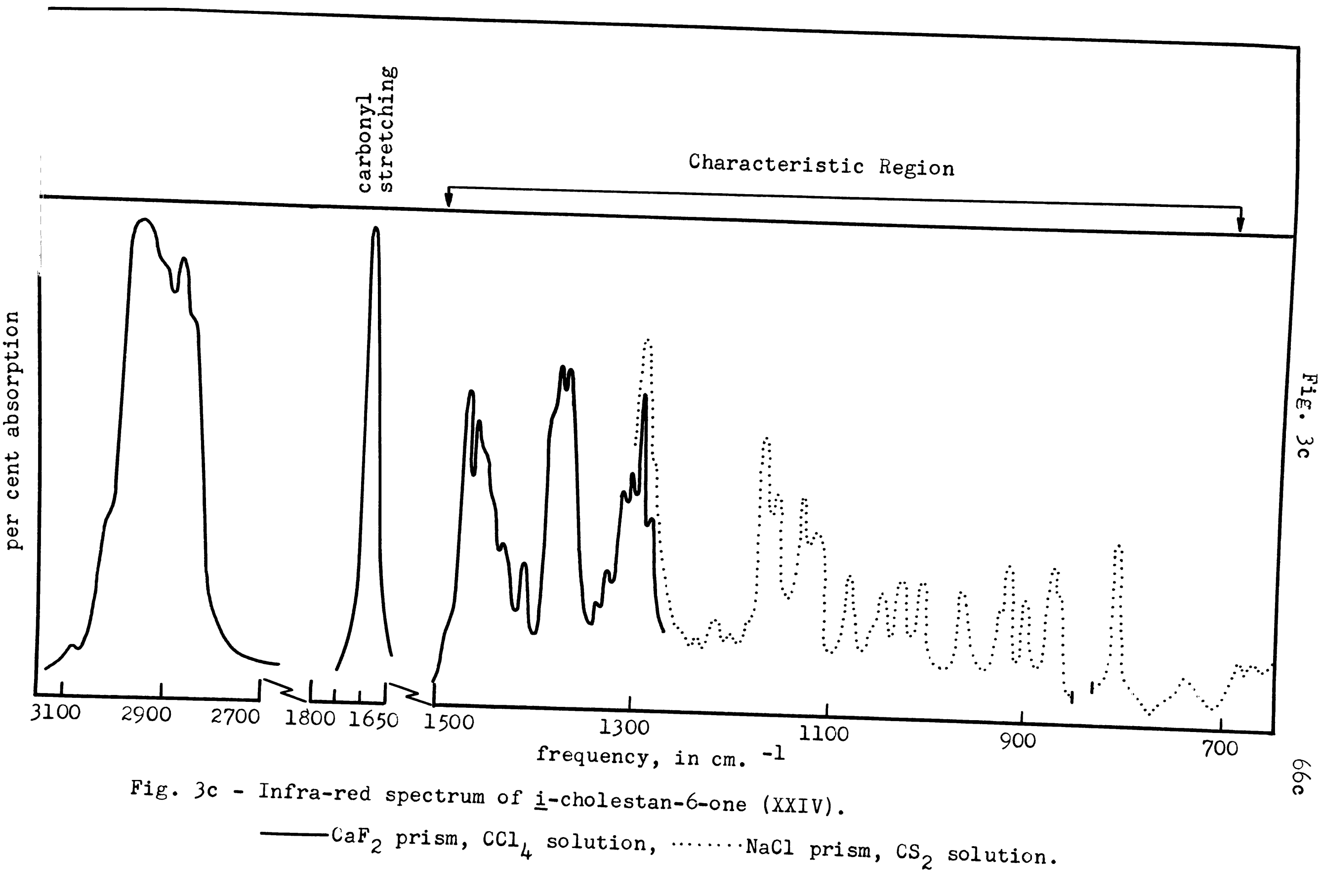


Fig. 3c

Fig. 3c - Infra-red spectrum of i-cholestan-6-one (XXIV).

———CaF_2 prism, CCl_4 solution, ·····NaCl prism, CS_2 solution.

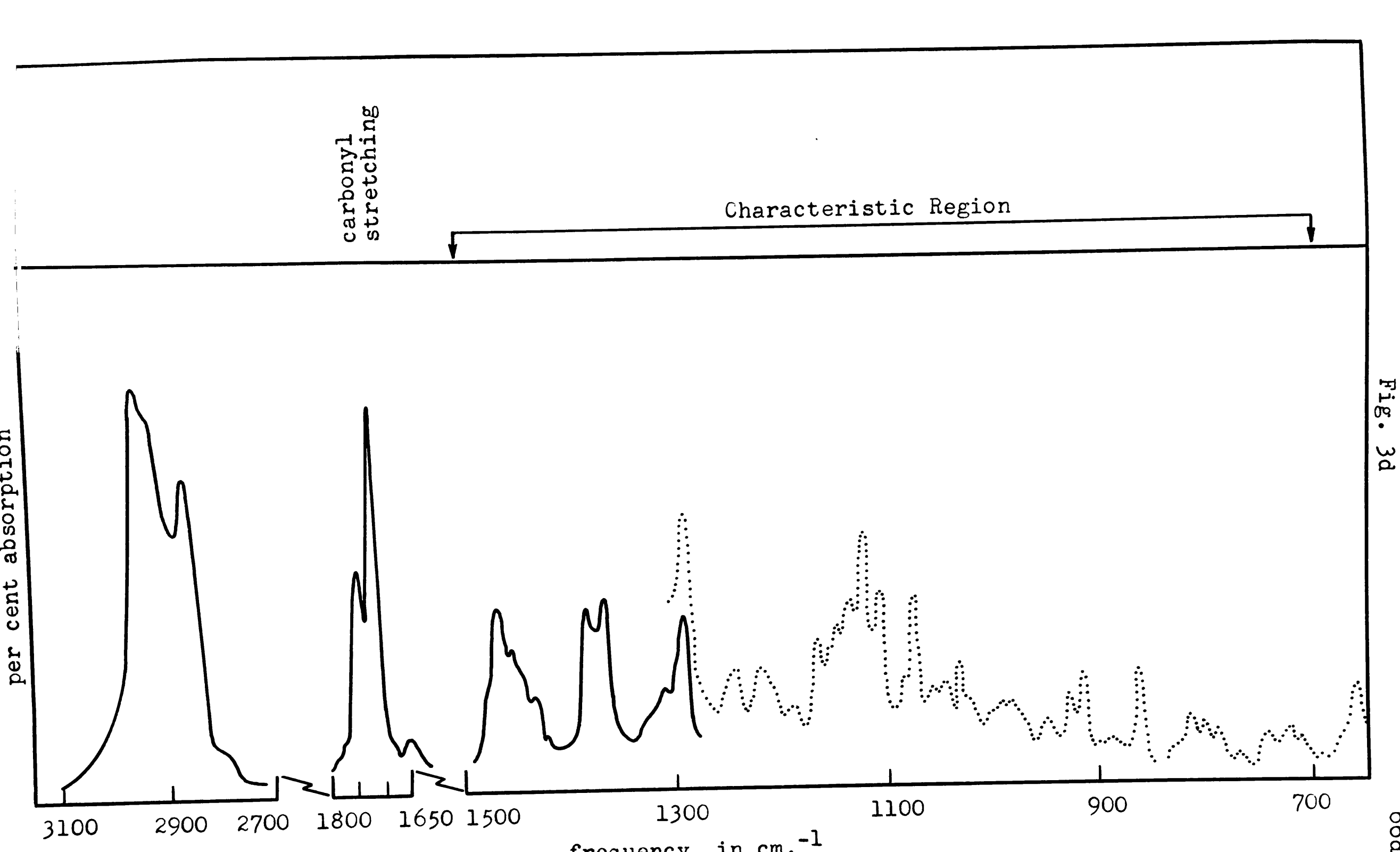


Fig. 3d - Infra-red spectrum of seco-6-i-cholestan-7-one (XXXI).

——— CaF_2 prism, CCl_4 solution
..... NaCl prism, CS_2 solution

the four compounds were scaled down and copied in order to be accomodated herein, and are so shown in Figs. 3a to 3d, inclusive. Before interpretation of these curves is outlined, some general remarks on steroid ketones are proffered. The normal position of the 6-ketone band is 1715-1713 cm.$^{-1}$ in the allocholane series, which includes cholesterol(I), and where the A/B ring fusion is <u>trans</u>. In the cholane series, however, where the A/B ring fusion is <u>cis</u>, the position of this band is at 1708-1719 cm.$^{-1}$. All other saturated 6-membered ring ketones have bands between 1706 and 1719 cm.$^{-1}$, while typical values for α,β-unsaturated ketones are 1684-1680 cm.$^{-1}$ for Δ^{1}-3-ketones and $\Delta^{9:11}$-12-ketones, 1677 cm.$^{-1}$ for Δ^{5}-7-ketones and, finally, 1677-1674 cm.$^{-1}$ for Δ^{4}-3-ketones[49]. Utilizing this information as a basis, the infra-red curves for compounds XXIV, XXVIII, XXXI and XXXII may be interpreted as follows:

i) <u>3(β)-bromocholestan-6-one(XXXVIII) - Fig. 3a</u>.

This compound displays a carbonyl maximum at 1715 cm.$^{-1}$, which is the normal position for a 6-ketone in the allocholane series, hence the γ-bromine atom at C_3 has no effect. It is suggested that the weak absorption band at 714 cm.$^{-1}$ may be due to the C-Br stretching.

ii) Seco-6-3(β)-bromocholestan-7-one(XXXII) - Fig. 3b.

The carbonyl maximum at 1735 cm.$^{-1}$ appears to be in agreement with the bands displayed by other 5-membered ring ketones. Again, as in the above case, the γ-bromine atom has no effect on the position of this band. The weak absorption at 705 cm.$^{-1}$ is probably due to the C-Br stretching.

iii) i-cholestan-6-one(XXIV) - Fig. 3c.

The two points of weak absorption over 3000 cm.$^{-1}$, at 3014 and 3078 cm.$^{-1}$, respectively, may be noted in slight inflections at the extreme left of the curve in this figure. These may be due to the cyclopropane structure in ring A, since they are suggestive of a -C=C-H system.

The frequency of the 1689 cm.$^{-1}$ band fits in excellently with the general theoretical concept of the "double bond" character of the cyclopropane ring. Thus, the effect of the structure in "conjugation" with the 6-ketone is to depress the carbonyl frequency from its normal position of 1715-1713 cm.$^{-1}$ in the saturated series, but since this structure is not a true unsaturation, it

is to be expected that the shift to lower wave numbers would not be as marked as with true α,β-unsaturated ketones.

iv) Seco-6-i-cholestan-7-one(XXXI) - Fig. 3d.

This compound shows a strong carbonyl maximum at 1722 cm.$^{-1}$ and a weak carbonyl maximum at 1746 cm.$^{-1}$. Since the carbonyl band for saturated seco-6-cholestane derivatives has been established at 1735 cm.$^{-1}$ in (ii) above, it is apparent that the introduction of the cyclopropane ring in "conjugation" with the 7-ketone depresses this band from 1735 cm.$^{-1}$ to 1722 cm.$^{-1}$.

It is suggested, however, that the weak absorption at 1746 cm.$^{-1}$ may be due to an impurity, since it has already been mentioned that this compound was purified only with difficulty (p. 60). This band might be due to a ketone in a 5-membered ring without the complication of a cyclopropane ring.

3. Experimental.

a) 3(β)-chloro-Δ^5-cholestene(XXV).

To 100.0 gm. commercial cholesterol(I) in a 500 ml. round bottom flask were added

100.0 gm. purified thionyl chloride. A vigorous reaction took place immediately, whereupon the flask and contents were placed in a fume-cupboard and allowed to stand for 24 hours at room temperature. On the following day the excess thionyl chloride was removed *in vacuo* at 60°C., the resulting semi-crystalline residue taken up in hot acetone and transferred quantitatively to a one liter Erlenmeyer flask. The separating crystalline material was filtered and the filtrate concentrated to one-half its original volume. More material thus obtained was combined with the first crop, all of which was recrystallized once more from acetone. This gave 85.3 gm. 3(β)-chloro-Δ^5-cholestene(XXV), melting at 94-96°C. Yield, 81.3 per cent.

b) 3(β)-chloro-6-nitro-Δ^5-cholestene(XXVI).

The treatment of 80.0 gm. 3(β)-chloro-Δ^5-cholestene(XXV) with 320 ml. glacial acetic acid, 320 ml. nitric acid, d. 1.50, and 200 ml. nitric acid, d. 1.515, was carried out in an identical manner as that for the nitration of cholesterol(I), described on pp. 21-22 herein. The crystalline material separating from the reaction mixture was crystallized twice from

acetic acid, resulting in 63.8 gm. 3(β)-chloro-6-nitro-Δ^5-cholestene(XXVI), m.p. 152-155°C. Yield, 71.9 per cent.

c) 3(β)-chlorocholestan-6-one(XXVII).

The details for the reduction of 60.0 gm. 3(β)-chloro-6-nitro-Δ^5-cholestene(XXVI) with 120 gm. zinc dust in 1200 ml. glacial acetic acid containing 120 ml. water are given previously for the preparation of 3(β)-acetoxy-cholestan-6-one(III) on pp. 22-23. The precipitated ketone was recrystallized twice from ethanol, furnishing 37.2 gm. 3(β)-chlorocholestan-6-one(XXVII), melting at 128-129°C. Yield, 66.3 per cent.

d) i-cholestan-6-one(XXIV).

To 600 ml. ethanol in a one liter flask at 50°C. were added 30.0 gm. 3(β)-chlorocholestan-6-one(XXVII), with this temperature maintained until all the substance was in solution. At this point, 120 ml. aqueous 20 per cent potassium hydroxide were added rapidly with stirring, whereupon a separation of fine crystals of potassium chloride took place almost immediately. In three to five minutes, the reaction appeared completed and the flask

was removed to cool. At the end of two to three hours the voluminous, fine crystalline precipitate was filtered by suction and the filtrate concentrated to ca. one-half its original volume. This afforded an additional quantity of material which was combined with the first crop, all of which was recrystallized from methanol. In this manner 23.4 gm. i-cholestan-6-one(XXIV) were obtained, melting at 93-96°C. Yield, 85.5 per cent of theory.

e) Attempted Acetolysis of 3(β)-chlorocholestan-6-one(XXVII).

In a solution of 40 ml. glacial acetic acid containing 20 gm. fused potassium acetate per 100 gm. solution were added 5.0 gm. 3(β)-chlorocholestan-6-one(XXVII). The flask and contents were then transferred to a "Glascol" heating mantle and maintained at 100-105°C. for 72 hours under exclusion of moisture. At the end of this time, the acetic acid was removed in vacuo and the residue transferred quantitatively to a one liter separatory funnel by means of alternate washings of water and ether. The volume of the ether was brought up to about 500 ml. and washed three times with

water. The ether layer was then dried over anhydrous magnesium sulfate, filtered and evaporated *in vacuo*. The brown, oily residue resisted crystallization under ordinary conditions, but when covered with 25 ml. acetic acid and allowed to stand at room temperature for two days, some oily globules which contained some crystalline material separated. By shaking this solution vigorously and allowing to stand for a few hours, more crystalline material appeared; and by sporadically shaking the flask in this manner over a period of two days, crystallization was virtually complete. The material was then filtered by suction, washed with a small quantity of cold methanol and recrystallized twice from this solvent. The amount thus recovered was 3.8 gm., m.p. 96-97°C., giving no depression when admixed with an authentic sample of *i*-cholestan-6-one(XXIV) and was, therefore, identified as such. Yield, 83.4 per cent. For the ultra-violet curves of this and the genuine sample of *i*-cholestan-6-one(XXIV), the concentration employed in each case was 0.025 per cent.

To substantiate the cyclopropane structure, 100 mg. of the acetolysis product in 10 ml.

methanol were treated with 5 drops of 12 M hydrochloric acid at room temperature. On the following day, the separated crystalline material was filtered and recrystallized twice from methanol. The pure compound melted at 128-129°C., gave a positive Beilstein test and was identified as 3(β)-chlorocholestan-6-one(XXVII), not depressing the melt of an authentic sample.

f) i-cholestan-6:7-dioic acid(XXIX).

To a solution of 400 ml. of 10 per cent potassium hydroxide in a 2 liter flask were added 10 gm. bromine and the mixture shaken until all was in solution. 10.0 gm. i-cholestan-6-one(XXIV) were added and the mixture warmed to 50°C. This was followed by the addition of 1000 ml. pyridine whereupon the flask and contents were tightly stoppered and shaken thoroughly for 24 hours. The reaction mixture was then cooled in an ice-bath and 18 M sulfuric acid added drop-wise with stirring until the odor of pyridine was no longer discernable. The semi-crystalline product which separated was filtered on a Buchner funnel, washed with water and transferred to a separatory funnel. 700 ml. ether were added and the product extracted with small portions of 1 per cent potassium

hydroxide. The material was then precipitated with dilute sulfuric acid, filtered, dried and recrystallized twice from aqueous methanol. This resulted in 7.4 gm. i-cholestan-6:7-dioic acid (XXIX), melting at 231-232°C. Yield, 66.0 per cent of theory.

g) Lead Salt of i-cholestan-6:7-dioic acid(XXX).

To 50 ml. methanol were added 7.0 gm. i-cholestan-6:7-dioic acid(XXIX). 5.25 gm.(excess) lead diacetate in a minimum quantity of warm methanol were added rapidly to this, whereupon a quantitative precipitate of lead i-cholestan-6:7-dicarboxylate(XXX) was instantly obtained. This was filtered by suction on a No. 50 Whatman paper, washed with small successive portions of cold methanol and dried in a small oven at 50°C. under a reduced pressure of 5 mm. of mercury. The dry salt amounted to 10.1 gm., which was equivalent to 99.0 per cent of the theoretical yield, and displayed no characteristic melting point.

h) Thermal Decomposition of the Lead Salt of i-cholestan-6:7-dioic acid(XXX).

This was accomplished in the same manner as described on pp. 43-44. 5.0 gm. of the lead salt XXX were divided equally among 6 bent sealing-tubes

identical to that shown in Fig. 2. Instead of heating each individually, however, the 6 tubes were connected to a central evacuating tube of 10 mm. pyrex glass by affixing each, with rubber tube connections, to one of 6 glass nipples joined thereon. The bent sealing-tubes, thus arranged, were subjected to a reduced pressure of ca. 1 mm. of mercury and heated simultaneously in a 250 ml. nitrite-nitrate bath at 280°C. The temperature was then gradually elevated over a period of 3.0 hours until it had reached 340°C., at which point the decomposition of the material in the tubes was considered complete. The vacuum was released, the 6 tubes disconnected from the central evacuating tube and allowed to cool. After cooling, the tubes were washed on the outside with water and dried, then broken by means of a file just above the glass-wool plugs and the oil which had distilled over into the upper part of the tubes washed with ether into a 125 ml. Erlenmeyer flask. The ether solution thus obtained was evaporated and the residue freed from solvent under reduced pressure provided by a water-pump. In this manner 1.8 gm. oil was collected. An additional 5.0 gm. of the lead salt(XXX) was pyrolyzed in an identical manner, resulting in 1.9 gm. oil. The two yields were combined, giving a total of

3.7 gm. oil, all of which was dissolved in a minimum amount of n-pentane and placed on a column of 70 gm. "Alcoa" F-20 grade activated alumina.

Table 3

Fraction	Eluent	Wt. in mg.	Nature
1	Benzene 10: Hexane 90	205	amber, aromatic oil
2-12	Benzene 10: Hexane 90	1557	crystals, plus oil
13-16	Benzene 15: Hexane 85	173	crystals, plus oil
17-19	Benzene 20: Hexane 80	107	crystals, plus oil
20-33	.....	....	oil

Fractions 2-19, amounting to 1837 mg., were consolidated and recrystallized from methanol to give 1561 mg. seco-6-i-cholestan-7-one(XXXI), melting at 89-94°C., yield 24.7 per cent. Five further recrystallizations from methanol gave 326 mg. XXXI, m.p. 97-98°C.

		Carbon	Hydrogen
Calculated for $C_{26}H_{42}O$:		84.26	11.42
Found:	1)	84.20	11.42
	2)	84.26	11.39

i) Oxime of seco-6-i-cholestan-7-one(XXXI).

In a 60 ml. round bottom flask fitted with a reflux condenser were added 100 mg. seco-6-i-cholestan-7-one(XXXI), 100 mg. anhydrous sodium acetate and 100 mg. hydroxylamine hydrochloride. 15 ml. methanol and 3 ml. water were added, respectively, and the mixture placed under vigorous reflux for 5.0 hours. On cooling, a fine array of felted needles separated which, on recrystallizing from methanol, melted at 176-177°C.

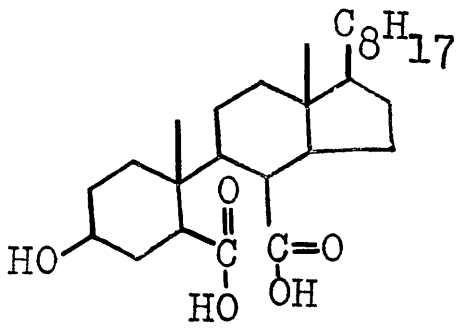
j) Seco-6-3(β)-bromocholestan-7-one(XXXII).

To a solution of 200 mg. seco-6-i-cholestan-7-one(XXXI) in 20 ml. acetic acid were added 2 ml. of 48 per cent aqueous hydrobromic acid, and the resulting mixture allowed to stand overnight at room temperature. The separating crystalline material thus obtained was filtered and recrystallized twice from methanol. For proper crystallization of this compound, 1-2 weeks were necessary under conditions of refrigeration. The pure compound, crystallizing in long hard needles, melted at 92-93°C., gave a strong positive Beilstein test for halogen and was identified as seco-6-3(β)-bromocholestan-7-one(XXXII). The oxime of this compound was prepared according to the directions in (i) above, and melted at 170-171°C.

k) Attempted Addition of Hydrogen Cyanide to Seco-6-i-cholestan-7-one(XXXI).

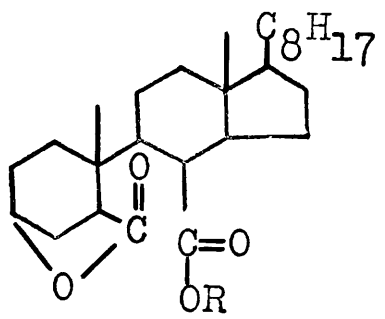
To 1.0 gm. XXXI in 30 ml. pure ethanol were added 2.0 gm. potassium cyanide and 2.0 ml. acetic acid. The mixture was boiled gently under reflux for 0.5 hours and, after cooling, the reaction mixture was poured into water. The precipitated material was filtered by suction, washed well with water and dried, giving impure starting material, melting at 86-93°C.

Chart 5



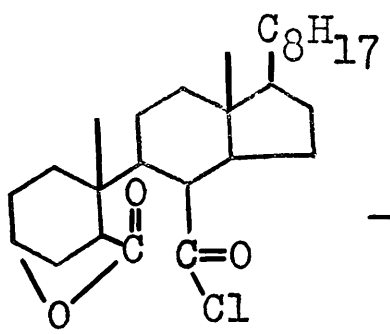
XVIII

Ac_2O



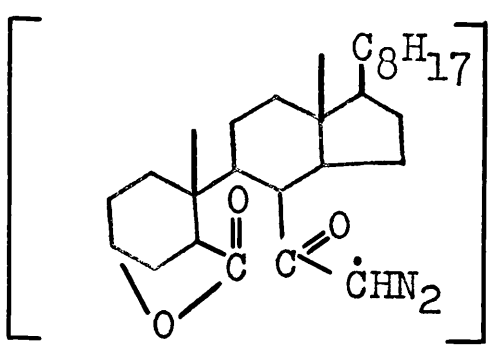
XXXIIIa R = H
XXXIIIb R = CH_3

$SOCl_2$



XXXIV

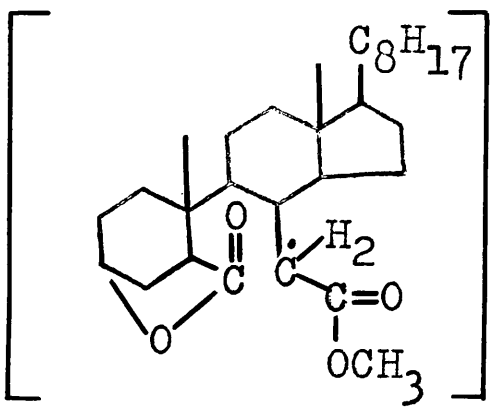
$\dot{C}H_2N_2$



XXXV

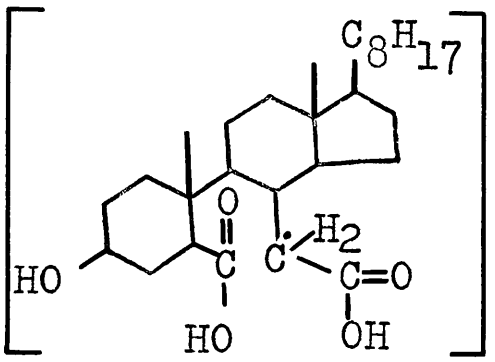
Ag

MeOH



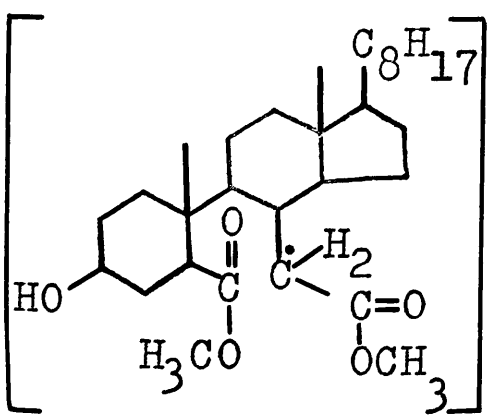
XXXVI

KOH



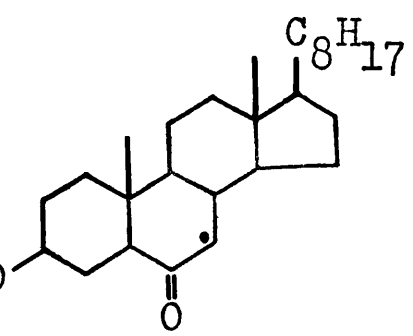
XXXVII

CH_2N_2



XXXVIII

1. NaOMe
2. AcOH



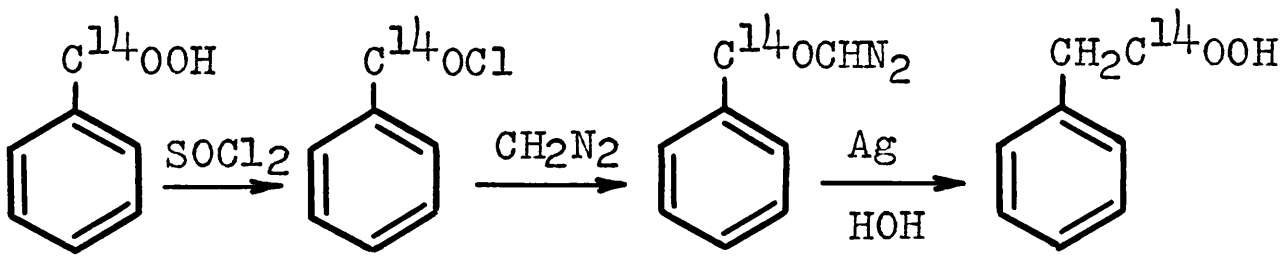
XV

transformed, by means of a unique reaction called the Wolff rearrangement,[52] into an ester containing one more carbon atom than the original acid.

This synthesis has been employed to excellent advantage as part of both the Bachmann total synthesis of equilinin[53] and the Miescher total synthesis of estrone[54]. In view of this application to the field of steroids, it was proposed to convert cholestan-3(β)-ol-6:7-dioic acid-6⟶3-lactone(XXXIIIa) to the homolactone acid XXXVII and cyclize to cholestan-3(β)-ol-6-one(XV) as outlined in Chart 5, XVIII ⟶XV. Although, in reaction (c) above, one may substitute water for the alcohol and thus bring about a direct transformation of the diazoketone to the homo acid (XXXVII)[51], the fact that steroids are insoluble in water would necessitate the employment of an alcoholic solvent, notably methanol.

The application of the Arndt-Eistert synthesis for the specific purpose of introducing C^{14} into ring B as envisaged in Chart 5 would, from a cursory examination of the reactions therein, be of relatively little value since there is an equal probability that the carbon atom lost in the cyclization procedure (XXXVIIIa ⟶XV) may come either from C_6 or from the new one in the extension of C_7. This consideration is, fortunately,

voided by the fact that the Wolff rearrangement of the diazoketone XXXV would involve an interchange in the positions of the carbonyl carbon atom and the newly affixed carbon atom furnished by the diazomethane. By referring to Chart 5, one may trace the carbon atom of the diazomethane (indicated with a solid circle), observing that this same atom eventually locates itself at position 7 (XXXIV⟶XV). That the rearrangement proceeds in this manner was clearly demonstrated in quantitative experiments by Hugget, Arnold and Taylor[55]; and more recently by Calvin, et al.[56], who converted C^{14} carboxyl-labelled benzoic acid, by means of the Arndt-Eistert synthesis, to phenylacetic acid with 100 per cent of the labelled carbon appearing in the carboxyl group of the latter.



benzoic acid — benzoyl chloride — diazoacetophenone — phenylacetic acid

It is, therefore, evident that the carbon atom furnished by the diazomethane and the labelled carbon atom of the benzoic acid were interchanged. Final conclusive proof of this exchange was produced in this

laboratory by Thompson[57], who applied the Arndt-Eistert synthesis as described by Anner and Miescher in the total synthesis of estrone[54]. The acid chloride of Marrianolic half-ester was converted to the corresponding diazoketone using diazomethane-C^{14}. This was subjected to the Wolff rearrangement, furnishing the homodiester of Marrianolic acid which, upon saponification and cyclization, gave estrone-16-C^{14}.

Regarding the synthesis as outlined herein, steps were taken to obtain cholestan-3(β)-ol-6:7-dioic acid-6⟶3-lactone(XXXIIIa) from cholestan-3(β)-ol-6:7-dioic acid(XVIII)[30]. The accomplishment of this, then, would permit investigation of the Arndt-Eistert synthesis.

2. Discussion.

Cholesterol(I) was converted to 3(β)-acetoxy-cholestan-6-one(III) in the manner described on pp. 21-23. Details for the transformation of III to cholestan-3(β)-ol-6:7-dioic acid(XVIII) are also given herein on pp. 40-43. The treatment of cholestan-3(β)-ol-6:7-dioic acid(XVIII) with hot acetic anhydride gave an oil which crystallized from hot hexane, furnishing crystalline material melting at 214-215°C. The presence of a carboxyl group was established by

treatment of a small quantity of the substance with ethereal diazomethane, the color of which was rapidly discharged with a vigorous evolution of gas. Determination of the neutral equivalent, and carbon and hydrogen analysis gave good agreement with the values calculated for the structure of cholestan-3(β)-ol-6:7-dioic acid-6⟶3-lactone(XXXIIIa)[30].

With the establishment of the structure of XXXIIIa its transformation to the acid chloride XXXIV was next considered. Although a variety of chlorinating agents were available, it was felt that by choosing some agent which promoted chlorination under mild conditions, destruction of the molecule might be minimized. Wilds and Shunk[58], employing oxalyl chloride at 0°C., converted the sodium salt of 3-keto-Δ^4-etiocholenic acid to the corresponding acid chloride in excellent yield with a minimum of side effects. Yates[59], of this laboratory, repeated this work with consistent success, noting an immediate, vigorous evolution of bubbles upon addition of the oxalyl chloride. The reaction was usually complete in 20-30 minutes and yields obtained in this manner were virtually quantitative. It was, therefore, decided to employ this method for the conversion of the lactone acid XXXIIIa to its acid chloride XXXIV.

In sharp contrast to the reactivity of the sodium

salt of 3-keto-Δ^4-etiocholenic acid, addition of oxalyl chloride to the sodium salt of XXXIIIa elicited no observable effects and, even after the reaction mixture was brought to room temperature, evolution of bubbles was sluggish and sporadic. Despite this, the reaction mixture was allowed to remain at room temperature for one hour, a condition far in excess of the requirements for the formation of the steroid acid chlorides by this method[58,59]. At the end of this time, the reaction was assumed to be appreciably complete, and the resulting material worked up. This yielded an oil which was impossible to crystallize, hence it was decided to treat this, without further purification, with an ethereal solution of diazomethane[60], conveniently prepared from nitroso-methylurea[61]. This treatment again resulted in an oil which resisted crystallization. The material was then chromatographed with the expectation of obtaining some crystalline diazoketone XXXV. The only crystalline material thus obtained melted at 104-105°C., and was identified as the methyl ester of cholestan-3(β)-ol-6:7-dioic acid-6⟶3-lactone(XXXIIIb)[30]. It is evident, then, that the methyl ester XXXIIIb was formed by the interaction of the diazomethane with the unchanged starting acid XXXIIIa[62].

From this, it was apparent that the lactone acid

XXXIIIa displays considerably less reactivity toward oxalyl chloride than other steroid acids; however, it was felt that with the proper choice of chlorinating agents this obstacle might be circumvented. Now, it is known that phosphorus pentahalides are the most intense of all halogenating agents[63]. Pursuant to this, a qualitative test was made with 25 mg. of the lactone acid XXXIIIa and a similar quantity of phosphorus pentachloride. The result was an exceedingly sluggish reaction, which was somewhat enhanced by heating on a steam-bath. The inevitable conclusion was that an extremely unreactive carboxyl group was being dealt with. Provided that the acid chloride XXXIV was formed by this treatment, it was reasonable to expect that it would be more resistant to hydrolysis than ordinary acid chlorides. This fact was borne out by washing an ethereal extract of the reaction product with aqueous potassium bicarbonate, with the result that non-acidic, oily material was returned, giving a strong, positive Beilstein test for halogen.

Thionyl chloride was employed as a final measure to effect chlorination of the lactone acid XXXIIIa. This was desirable, particularly from a standpoint of working up since the by-products formed in the reaction are hydrochloric acid and sulfur dioxide, two gases which may be easily removed from the reaction mixture.

Further, thionyl chloride itself is quite volatile and is easily removed. Treatment of 500 mg. XXXIIIa with an excess of thionyl chloride produced a slow evolution of bubbles. By warming on the steam-bath, however, this evolution became quite brisk, the escaping gas being identified as hydrochloric acid. In 15-20 minutes, the reaction subsided and the material worked up. The resulting oil resisted attempts to crystallize and gave a positive Beilstein test. Treatment of a small quantity with methanol under conditions of refluxing yielded the methyl ester XXXIIIb, confirming the formation of the acid chloride XXXIV.

Treatment of this oil with diazomethane did not produce the usual rapid liberation of nitrogen, indicating greatly reduced reactivity. In view of this, the acid chloride was allowed to remain in contact with the diazomethane for 24 hours at room temperature. On working this up, a non-crystallizable product was obtained which, without further characterization, was subjected to the conditions prescribed by the Wolff rearrangement, employing colloidal silver in methanol[52,54]. For a third time, an intractable oil was obtained, which in this case was chromatographed. The sole crystalline product procured in this manner melted at 103-105°C., and was identified as the normal methyl ester XXXIIIb of the lactone acid XXXIIIa. It may be deduced, therefore, that the acid chloride of XXXIIIa was, indeed,

formed but failed to react with the diazomethane, since the following treatment with hot methanol effected esterification to yield the methyl ester XXXIIIb.

To conclusively demonstrate the inability of the acid chloride XXXIV to react with diazomethane, several experiments were conducted under a variety of conditions. All attempts to effect the condensation failed, hence studies in this direction were discontinued.

3. Experimental.

a) Preparation of Cholestan-3(β)-ol-6:7-dioic acid-6⟶3-lactone(XXXIIIa).

In a flask containing 4.5 gm. crude cholestan-3(β)-ol-6:7-dioic acid(XVIII) (pp. 42-43) were added 100 ml. acetic anhydride and the mixture warmed for one hour at 90-95°C. The acetic anhydride was removed *in vacuo* and the oily residue transferred to a one liter separatory funnel by means of ether. The volume of the ether was brought up to *ca.* 500 ml. and the ethereal extract washed twice with 200 ml. portions of 1 M sodium carbonate. The ether phase was then separated, dried over anhydrous magnesium sulfate and filtered. The solvent was removed under reduced pressure and the

resulting oil taken up in a little ether. Pentane was added under conditions of gentle boiling, but crystallization was complicated by the formation of a gel. Rubbing the gel with a few drops of acetone was of no avail; therefore the solvent was removed and the material taken up in hot hexane. By boiling down to a small volume and removing from the steam-bath, some crystalline material appeared while still hot; and before the formation of a gel could take place, the flask was returned to the bath and heated again just to the boiling point, whereupon more material separated. By repeating this process, a quantity of material was obtained which, by filtering on a small Büchner funnel and washing with a small volume of cold pentane, gave 2.7 gm. cholestan-3(β)-ol-6:7-dioic acid-6⟶3-lactone(XXXIIIa), melting at 211-214°C. Yield, 62.5 per cent. A further crystallization from ether:pentane gave pure XXXIIIa, m.p. 214-215°C.

Titration of 100 mg. of XXXIIIa in 10 ml. pure acetone with 0.01 M sodium hydroxide gave a neutral equivalent of 438. Calculated for $C_{27}H_{44}O_4$: 432. Carbon and Hydrogen analyses were performed, furnishing the following results:

		Carbon	Hydrogen
Calculated for $C_{27}H_{44}O_4$:		74.96	10.25
Found:	1)	74.84	10.14
	2)	74.88	10.12

b) Diazomethane.

To a mixture of 200 ml. ether and 60 ml. 50 per cent potassium hydroxide at -5°C. were added, with constant stirring and in small portions, 20 gm. nitrosomethylurea. After complete dissolution, the flask was removed from the ice-salt bath and allowed to warm to 15°C. The flask was then fitted with a condenser and adapter, the end of which dipped just below a small quantity of ether in the receiver flask; and distillation carried out by heating in a water-bath at 55°C. When the ether came over colorless, distillation was discontinued. The resulting ethereal solution, containing about 5.5 gm. diazomethane, was dried over solid potassium hydroxide and stored in a refrigerator.

c) Cholestan-3(β)-ol-6:7-dioic acid-6⟶3-lactone Methyl Ester(XXXIIIb).

In a small flask containing 100 mg. XXXIIIa were added 10 ml. (excess) of an ethereal solution of diazomethane. After the cessation of evolution of gas, the mixture was allowed to stand for 0.5 hours, whereupon the ether and unreacted diazomethane were removed *in vacuo*. The oily residue was taken up in 5 ml. pentane and set aside in the refrigerator for one week. At the end of this time, sufficient material separated to permit filtration.

A further similar crystallization gave the methyl ester XXXIIIb, melting at 104-105°C.

d) Attempt to Form the Acid Chloride XXXIV of Cholestan-3(β)-ol-6:7-dioic acid-6⟶3-lactone(XXXIIIa) Using Oxalyl Chloride.

To 500 mg. of the lactone acid XXXIIIa in a round bottom flask were added 11.6 ml. (one mole-equivalent) of 0.01 methanolic sodium hydroxide. The methanol was removed and the resulting sodium salt dried for 10 hours at 100°C. under a reduced pressure of <1.0 mm. mercury. The dry salt was then lyophilized with 5 ml. anhydrous benzene and placed in an ice-bath at 0°C. 5 drops of pyridine were added to the mixture, followed by the rapid addition of 5.0 ml. redistilled oxalyl chloride. The flask and contents were maintained at this temperature for an additional 15 minutes and then removed to warm at room temperature. During this time, the evolution of bubbles was barely discernable; consequently the flask was allowed to remain at this temperature for one hour. The benzene and unreacted oxalyl chloride were removed under reduced pressure and the residue taken down three times with dry ether. The resulting oil was then dissolved in a small amount of ether and filtered to remove inorganic material. Attempts to crystallize the oily material from ether, benzene and pentane failed.

e) Attempt to Form the Diazoketone XXXV.

Assuming a reasonable conversion of the lactone acid XXXIIIa to the corresponding acid chloride XXXIV in (d) above, the material thus obtained was dissolved in 25 ml. ether and the resulting solution added dropwise, with constant stirring, to 50 ml. (excess) of ethereal diazomethane at -10°C., whereupon a vigorous evolution of gas was noted. The mixture was maintained at this temperature for 30 minutes and then removed to warm at room temperature. At the end of one hour, solvent and excess diazomethane were distilled off and the oily residue dried at room temperature under a reduced pressure of 1.0 mm. mercury. All attempts to effect crystallization failed, hence the material was dissolved in a small quantity of pentane and placed on a column of 10 gm. "Alcoa" F-20 grade activated alumina. The sole crystalline product, eluted by 25 ml. portions of benzene:pentane (20:80, 40:60, 60:40, 80:20) and benzene alone, appeared in fractions 4-20. Consolidation of these fractions gave 291 mg. of the methyl ester XXXIIIb which, when recrystallized from pentane, melted at 103-105°C. and did not give a depression in the melt of an authentic sample, prepared in (c) above.

f) Acid Chloride of the Lactone Acid(XXXIIIa).

In a small round bottom flask containing 500 mg. XXXIIIa were added 5.0 ml. purified thionyl chloride. The flask was fitted with a small condenser and the contents placed under gentle reflux for 0.5 hours. During this time, hydrochloric acid was slowly evolved. The thionyl chloride was then removed in vacuo and the residue taken up with 200 ml. ether. This was transferred to a 500 ml. separatory funnel and washed rapidly but thoroughly with two 100 ml. portions of potassium bicarbonate. The ether layer was separated, dried over anhydrous magnesium sulfate and filtered. On removal of the solvent, a colorless oil was obtained which gave a strong, positive Beilstein test for halogen and resisted efforts to effect crystallization.

To 5 ml. pure methanol were added about 50 mg. of this oil and the mixture placed under reflux for one hour. Removal of the solvent and crystallization from pentane gave crystals, melting at 103-105°C., which were identified as the methyl ester XXXIIIb. This evidence strongly supported the identification of the chlorination product above as XXXIV, the acid chloride of cholestan-3(β)-ol-6:7-dioic acid-6⟶3-lactone(XXXIIIa).

g) Repeated Attempt to Form the Diazoketone XXXV from the Acid Chloride XXXIV.

The remainder of the non-crystalline acid chloride XXXIV was taken up in dry ether and treated in an identical manner to that described in (e) above, except that the reaction mixture was allowed to remain at room temperature for 24 hours. On working up, an oily residue was obtained which could not be crystallized. The material was, therefore, utilized as such in the following.

h) Attempted Wolff Rearrangement.

The oil obtained in (g) above was transferred to a 100 ml. round bottom flask, covered with 50 ml. pure methanol and placed in a water-bath at 55°C. To this was added, with constant stirring, 0.5 gm. freshly prepared silver oxide in small portions over a period of 0.5 hours. At the end of this time the flask was fitted with a condenser and the contents placed under reflux for 5.0 hours. After cooling, the bulk of the metallic silver was removed by filtering on a No. 50 Whatman paper, and the methanol removed *in vacuo*. The residue was taken up in ether and filtered once more, thus removing last traces of silver. The ether was then removed, the material taken up in benzene and placed on a column of 15 gm. "Alcoa" F-20

grade activated alumina. The only crystalline product from the column was eluted by 25 ml. portions of benzene: pentane, (20:80, 40:60, 60:40, 80:20) and benzene alone; and appeared in fractions 4-27. Consolidation of these fractions gave 193 mg. substance, melting at 103-105°C., which was identified as the methyl ester XXXIIIb of the starting material XXXIIIa.

PART F

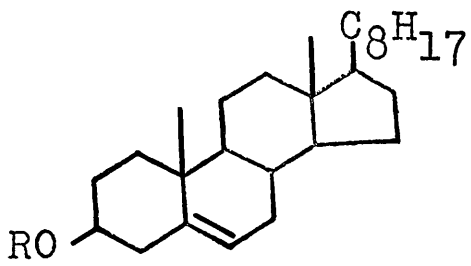
Proposed Synthetic Route via Cholestan-3(β),5,6(α)-triol(XXXIX).

1. Theoretical.

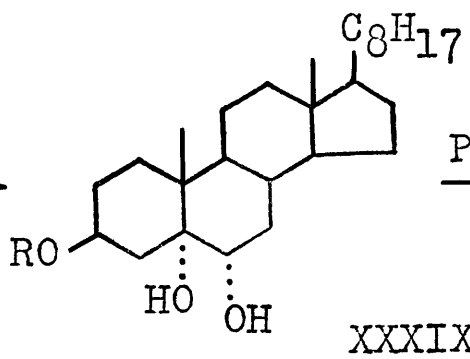
The final method proposed for a possible means of obtaining ring B C^{14}-labelled cholesterol(I), depended on the successful preparation of 6-aldehydo-cholestan-3(β)-ol-5-one(XL). As noted in Chart 6, XL would be converted to the methyl ester XLI of the corresponding 6-acid. Addition of hydrogen cyanide (which could be radioactive as shown by the solid circle above the carbon atom) to the 5-ketone of XLI and subsequent alkaline hydrolysis would give the diol-homodiacid XLII. Methylation of the carboxyl groups with diazomethane, acetylation of the 3-hydroxyl and dehydrohalogenation at C_5 could yield the 3(β)-acetoxy-Δ^4-homodiacid XLIII, which by a Dieckmann cyclization procedure employing sodium methylate, followed by decarboxylation with acetic acid would then give 3(β)-acetoxy-Δ^4-cholestene-6-one(XLIV). This in turn, could be reduced with hydrogen in the presence of platinum to furnish 3(β)-acetoxycholestan-6(β)-ol(IV) which is easily converted to cholesterol(I) as shown in Part B.

Attention was first directed toward the preparation

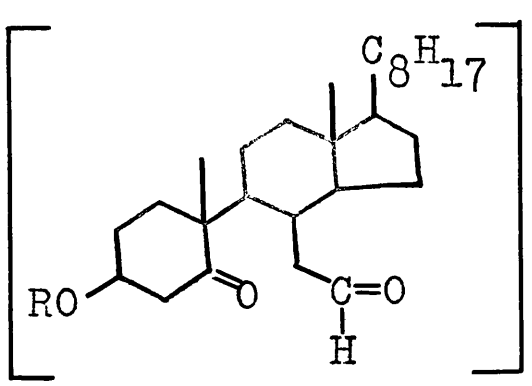
Chart 6



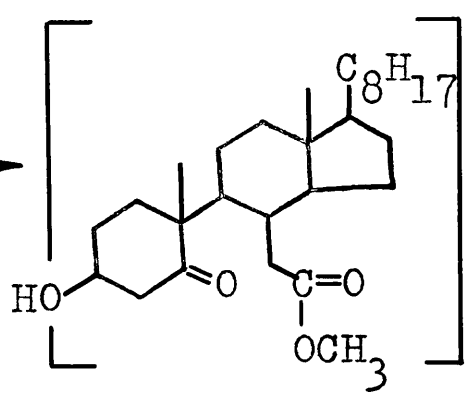
I R=H
Ia R=Ø–CH_2



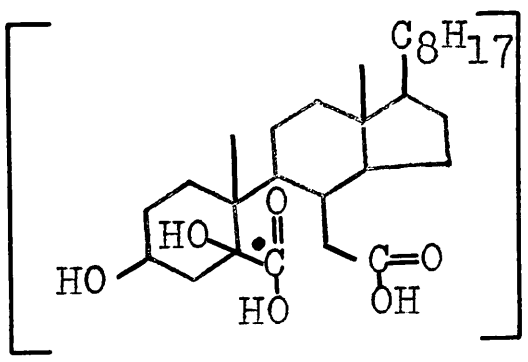
XXXIX R=H
XXXIXa R=Ø–CH_2



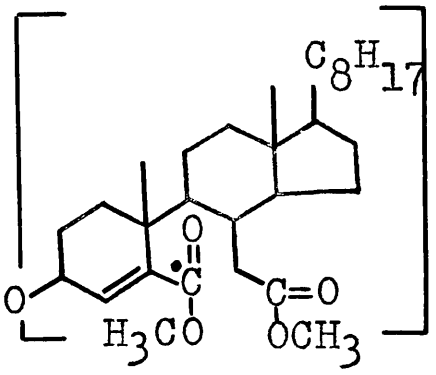
XL R=H
XLa R=Ø–CH_2



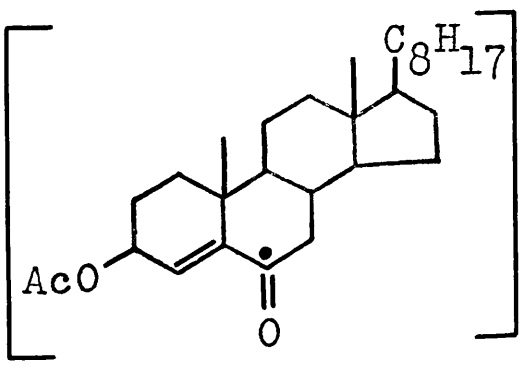
XLI



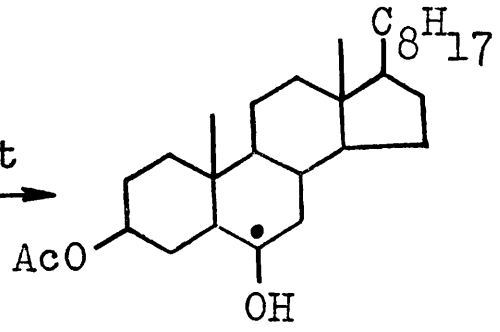
XLII



XLIII

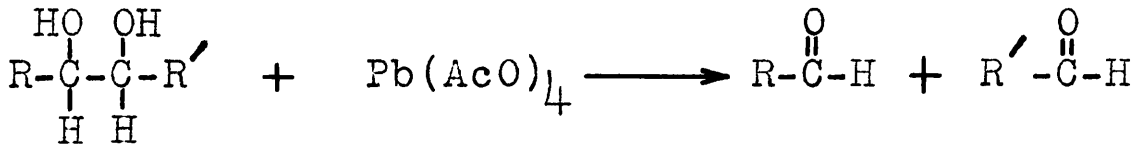


XLIV



IV

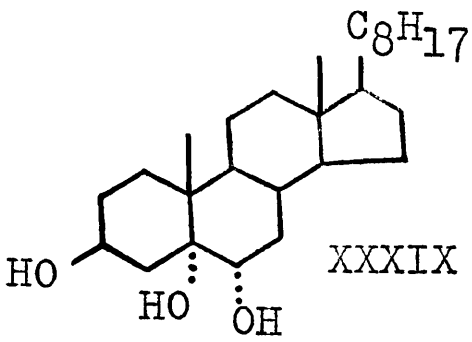
of the keto-aldehyde compound XL. It was felt that oxidative cleavage of a cholestan-3(β),5,6-triol should accomplish this, since in ring B a 1,2- glycol is present. Oxidative cleavage of such glycols is carried out in a general manner by the use of either periodic acid[64] or lead tetraacetate[65] which, in the case of aliphatic 1,2-glycols, ruptures the C-C bond between the two alcoholic groups with the concomitant production of two carbonyl fragments. It should fol-



low, therefore, that similar treatment of a cholestan-3(β),5,6-triol should result in cleavage of the C_5-C_6 bond with the formation of the 6-aldehydo-5-keto derivative XL. The choice of lead tetraacetate rather than periodic acid was due to the insolubility of periodic acid in organic solvents, whereas oxidative cleavage with lead tetraacetate may be accomplished conveniently in acetic acid.

Since cholestan-3(β),5,6-triol exists as two isomers, it was necessary to determine which of these would be more suitable for the proposed cleavage. The two isomers differ only by the orientation of the hydroxyl at C_6, the position of the C_5 being fixed as α in the cholestane series. These are commonly referred to

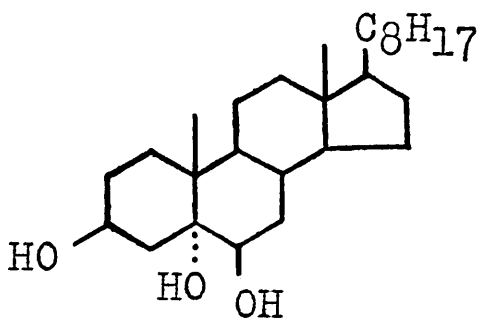
as the "cis" and "trans" triols where the 6-hydroxyl is present in the α and β positions, respectively.



XXXIX

"cis"-triol

m.p. 236°C.



"trans"-triol

m.p. 239°C.

The "trans"-triol may be conveniently prepared in excellent yield by the performic oxidation of cholesterol(I)[66], while the "cis"-isomer XXXIX requires osmium tetroxide for "glycolysis" of the double bond of I. In a study by Criegee, however, it was shown that the effect of lead tetraacetate on the "trans"-triol was almost negligible compared to that of the "cis"-isomer XXXIX. By evaluating rate constants for these two reactions, he was able to establish the orientation of the 6-hydroxyl in each of the two isomers[67]. With this information, cholestan-3(β),5,6(α)-triol(XXXIX) was chosen as a preparative precursor to the 6-aldehydo-5-keto derivative XL.

2. Discussion.

Pure cholesterol(I) was "glycolyzed" according to Prelog and Tagman[68], employing osmium tetroxide and

pyridine in absolute ether. This furnished the expected "cis"-triol XXXIX, melting at 234-236°C., which was then subjected to the action of an acetic acid solution of lead tetraacetate[69], the preparation of which was accomplished by treating red lead oxide with acetic anhydride in acetic acid[70]. The titer of the solution of this reagent was determined according to Dimroth and Schweitzer[71] by treating an aqueous solution of potassium iodide and sodium acetate with a known volume of the reagent, followed by titration of the liberated iodine with standard thiosulfate.

The reaction between the cholestan-3(β),5,6(α)-triol(XXXIX) and the lead tetraacetate was followed by 15 minute interval determinations of the reagent present by the Dimroth method[71]. At the end of one hour, a theoretical uptake of lead tetraacetate was observed, after which time the titer remained constant. On working up, no crystalline material was obtained, but a strong, positive test with ammoniacal silver oxide suggested the presence of a reducing group; and it was provisionally assumed that this might be the aldehyde function at the C_6 carbon atom of XL (Chart 6).

The oil thus obtained was placed on a column of alumina and chromatographed. Elution was carried out in a complete a manner as possible, returning nearly

all the material from the column but no crystalline components were forthcoming, even after allowing the separate fractions to stand for one week. It was noted, however, that some of the fractions which appeared late during the chromatography contained a clear gum of a hard and brittle consistency. This material gave a strong, positive test with ammoniacal silver oxide. Using this as a guide, these fractions were consolidated and treated with ammoniacal silver oxide in methanol[72]. On working up the product and extracting from ether with aqueous potassium hydroxide, a quantity of non-reducing acidic material was obtained. Methylation of this component gave an oil which was placed on a column of alumina and chromatographed. Complete elution failed to yield any crystalline fractions.

It was felt, at this point, that the introduction of some substituent at C_3 in cholesterol(I) might resolve the difficulty encountered in obtaining crystalline intermediates beyond the triol XXXIX stage. In order to accomplish "glycolysis" of the double bond by means of osmium tetroxide, it was necessary to choose a group which would not alter the reactivity of the double bond toward this reagent. On this basis, it was decided to prepare an ether of cholesterol(I) which should, theoretically, meet these requirements. Further,

it was expected that the presence of such a group at position 3 would not hinder the subsequent cleavage of the 5,6-diol by lead tetraacetate, since Heilbron showed that oxidative cleavage of 3-methoxyergostadiene-5,6(α)-diol under these conditions proceeded in the same manner as with the unsubstituted dienetriol[69]. In particular, the benzyl ether was chosen, for it was felt that this would more satisfactorily furnish crystalline derivatives.

The preparation of the benzyl ether Ia, m.p. 117-118°C., was accomplished by directions given by Heilbron[73], who treated potassium cholesterolate with benzyl chloride. This in turn, was subjected to the action of osmium tetroxide[68] which gave hard needles, melting at 183-184°C. Carbon and hydrogen analyses gave results which agreed with values calculated for 3-benzyloxycholestan-5,6(α)-diol(XXXIXa). Lead tetraacetate oxidation of XXXIXa gave an intractable oil which was reducing toward ammoniacal silver oxide, but could not be obtained in crystalline form, even by chromatography. At this time, a suitable method for the preparation of radioactive cholesterol(I) appeared; consequently, these studies were discontinued. Investigation of the newly published method appears in Section III.

3. Experimental.

a) Cholestan-3(β),5,6(α)-triol(XXXIX).

To a solution of 1.0 gm. osmium tetroxide in 33 ml. dry, absolute ether was added a solution of 1.53 gm. pure cholesterol(I) in 165 ml. absolute ether, followed by 0.8 ml. pure pyridine. The mixture was allowed to stand at room temperature, under exclusion of light, for 6 days. At the end of this time, the ether was removed under reduced pressure at 30°C. and a solution of 4.1 gm. mannitol in 75 ml. 0.1 N potassium hydroxide added. The flask was tightly stoppered and shaken gently for 24 hours to bring about the decomposition of the osmic ester. The white, crystalline material which separated was filtered, washed well with water and dried in a small vacuum oven at 100°C. under a reduced pressure of ca. 5 mm. mercury. This was then taken up in hot ethyl acetate, but crystallization was superseded by the formation of a gel. Finally, the solution was allowed to cool thoroughly and the solid mass of gel rubbed with a few drops of chloroform. On the following day, a small amount of crystalline material appeared at the site of rubbing and after 5 days, crystallization was complete. The resulting material was filtered, washed with small portions of cold

ethyl acetate and dried. In this manner 1.3 gm. cholestan-3(β),5,6(α)-triol(XXXIX) was obtained, melting at 229-235°C. Yield, 78.0 per cent. A further recrystallization gave XXXIX, m.p. 234-236°C.

b) Lead Tetraacetate.

i) Preparation of the Crystalline Material.

To a mixture of 108 ml. glacial acetic acid and 36 ml. acetic anhydride were added 60 gm. red lead oxide (minium) in small portions with constant, vigorous stirring, over a period of 0.5 hours with the temperature maintained below 65°C. When all the lead oxide was added, the flask was transferred to a water-bath at 60°C., and stirring continued until only traces of the red lead remained. The flask was then stoppered and set aside in the refrigerator overnight. On the following day, the crystalline lead tetraacetate was filtered and washed with two small portions of cold acetic acid. Suction was maintained until a faint yellow coloration appeared on the surface of the crystalline material, whereupon the entire cake was immediately removed to a desiccator containing 100 gm. solid potassium hydroxide. The desiccator was rapidly evacuated by means of an efficient water pump and

thus maintained for two hours, at the end of which time the stop-cock of the desiccator was closed and the pump disconnected. The lead tetraacetate was stored in this manner and to avoid decomposition, was weighed only when required.

ii) Preparation and Standardization of the Acetic Acid Solution.

To 600 ml. glacial acetic acid were added roughly 5 gm. of the above material and the resulting solution placed in a flask equipped for distillation. The solution was then distilled with vigorous ebullition until only about 75-100 ml. of liquid remained in the flask. An additional 14.0 gm. of the freshly prepared lead tetraacetate was weighed out and rapidly transferred to the acetic acid thus purified. This was bottled, shaken thoroughly for 15 minutes and allowed to stand for two days after which time all the material had dissolved. The resulting solution was then titrated in the following manner:

To a solution of 6.0 gm. anhydrous sodium acetate and 1.0 gm. potassium iodide in 20 ml. water were added exactly 2.0 ml. of the lead tetraacetate solution. The iodine liberated was titrated

against 0.0101 M sodium thiosulfate, using one per cent aqueous β-amylose as an indicator. This required 23.8 ml. which, by calculating from $Pb(AcO)_4^{=} \equiv I_2 \equiv 2S_2O_3^{=}$, furnished a titer of 26.7 mg. lead tetraacetate per ml. of solution.

c) Oxidative Cleavage of Cholestan-3(β),5,6(α)-triol (XXXIX).

In a flask containing 1.0 gm. XXXIX (2.38×10^{-3} moles) were added 50.0 ml. (1.25 mole-equivalents) of the lead tetraacetate solution prepared above. From this solution, 2.0 ml. samples were withdrawn every 15 minutes and the lead tetraacetate present determined by titration. At the end of one hour, the theoretical amount of lead tetraacetate was consumed, the titer remaining constant after this time. After allowing the mixture to stand for an additional hour, 1.0 ml. glycerol was added, whereupon the flask and contents were set aside overnight at room temperature. On the following day, the bulk of the acetic acid was removed under reduced pressure at 60°C. When the volume was brought down to ca. 10 ml., evaporation was discontinued and the solution transferred quantitatively to a 500 ml. separatory funnel by means of ether and water. 200 ml. ether were added and the solution washed with two 100 ml.

portions of one per cent aqueous potassium hydroxide. This treatment extracted only a negligible quantity of acidic material, hence the aqueous washes were discarded. The separated ether extract was dried over anhydrous magnesium sulfate, filtered and evaporated, furnishing 953 mg. of a clear oil which could not be brought to crystallize.

This was then placed on a column of 20 gm. "Alcoa" F-20 grade alumina and chromatographed. By elution, proceeding from pentane to pure methanol, 875 mg. material was recovered; however, no crystalline fractions were obtained. Noting that some of the late appearing fractions contained a hard, brittle gum, tests were made with ammoniacal silver oxide which indicated reducing material. Fractions 42-57, eluted with 25 ml. portions of chloroform:ether (20:80, 30:70, 50:50, 60:40, 80:20) and chloroform alone, were arbitrarily selected and consolidated, affording 343 mg. material which was employed as such in the following reaction.

d) Silver Oxide Oxidation of the Reducing Oil in the Lead Tetraacetate Cleavage of the Triol XXXIX.

The oil from (c) was dissolved in 100 ml. methanol and to this was added a solution of ammoniacal silver oxide (prepared by treating fresh

silver oxide with just sufficient 10 per cent aqueous ammonium hydroxide to effect solution). The mixture was allowed to stand at room temperature for one hour, during which time the mixture became very black with reduced silver. The solution was then transferred to a 250 ml. centrifuge bottle and centrifuged for 15 minutes. This was filtered through a Whatman No. 50 paper, the filtrate reduced in volume to about 25 ml. and taken up with ether. The ether was extracted with successive small portions of 2 per cent potassium hydroxide, separated, dried and filtered, yielding 115 mg. neutral material. The alkaline extracts were combined, neutralized with dilute sulfuric acid and extracted with ether. This afforded 172 mg. acidic non-crystalline material which was methylated with diazomethane and chromatographed. Complete elution from the column failed to give any crystalline substance.

e) 3-Benzyloxy-Δ^5-cholestene(Ia).

To 35 ml. benzene containing 5.0 gm. pure cholesterol(I) was added a suspension of 1.2 gm. potassium in 30 ml. benzene (prepared by vigorously stirring the metal in the benzene at its boiling point, and allowing to cool while still stirring). The mixture was then warmed to 50°C., with stirring,

and maintained under these conditions for 2.0 hours. Last traces of potassium metal disappeared when the solution was gently refluxed for 0.5 hours. Through the upright condenser attached were added 30 ml. benzyl chloride and the contents of the flask placed under reflux for 10 hours. At the end of this time, the reaction mixture was diluted with an equal volume of ether, transferred to a 500 ml. separatory funnel and washed thoroughly with water. The ether-benzene phase was separated, concentrated to about 25 ml., and 75 ml. ethanol added. The separating material was filtered, crystallized once from ethyl acetate and once from acetone. In this manner, 4.6 gm. of 3-benzyloxy-Δ^5-cholestene(Ia) were obtained, melting at 117-118°C. Yield, 81.3 per cent.

f) 3-benzyloxycholestan-5,6(α)-diol(XXXIXa).

To a solution of 1.89 gm. of the benzyl ether Ia in 34 ml. absolute ether was added a solution of 1.0 gm. osmium tetroxide in 165 ml. absolute ether, followed by 0.8 ml. pyridine. The brown osmic ester began to separate in about two hours and at the end of five days, under exclusion of light, the reaction was considered complete. Decomposition of the ester with 75 ml. 0.1 M sodium hydroxide and 4.1 gm mannitol did not proceed as in (a) above and it was subsequently

perceived that this was due merely to the inability of the aqueous solution to wet the ester. The addition of 0.25 gm. Aerosol O.T. caused an instantaneous wetting and, by shaking for 10 hours, the decomposition was complete. The material separating was filtered, washed well with water and dried. Two recrystallizations from ethyl acetate gave 1.43 gm. 3-benzyloxycholestan-5,6(α)-diol(Ia), melting at 183-184°C.

		Carbon	Hydrogen
Calculated for $C_{34}H_{54}O_3$:		79.94	10.66
Found:	1)	80.16	10.85
	2)	80.25	10.80

g) Oxidative Cleavage of the Benzyl Ether-Diol XXXIXa.

To a flask containing 1.0 gm. XXXIXa (1.95×10^{-3} moles) were added 41.0 ml. (1.25 mole equivalents) of a lead tetraacetate solution containing 26.7 mg. salt per ml. The resulting solution was treated in an identical manner to that as described in (c) above. On working up the material, 960 mg. oil were obtained. This was placed on a column of 18 gm. "Alcoa" F-20 grade activated alumina and chromatographed. Elution failed to furnish any crystalline components.

SECTION III

RING A C^{14}-LABELLED CHOLESTEROL

SECTION III

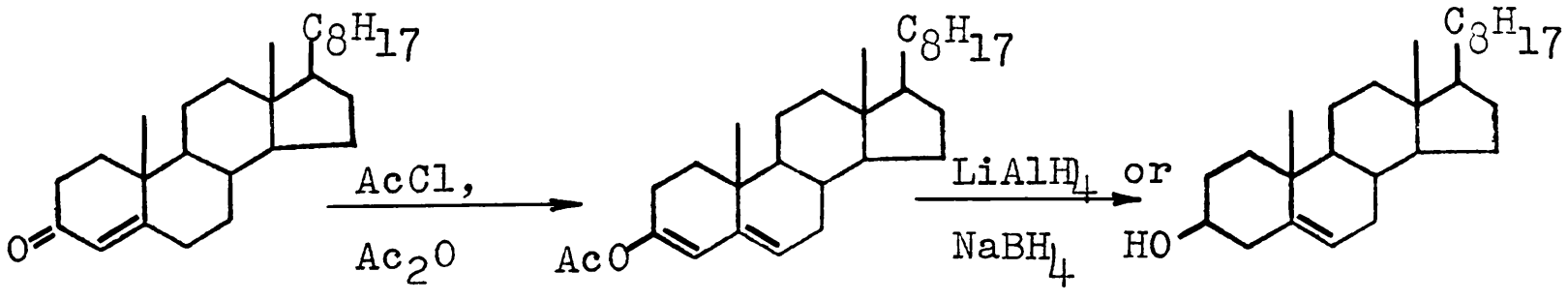
Ring A C^{14}-Labelled Cholesterol(I).

1. Introduction.

a) Reduction of 3-acetoxy-$\Delta^{3,5}$-cholestadiene(XLVI).

Before the problem of devising some means for labelling cholesterol(I) in ring B could be resolved, a satisfactory method for the preparation of ring A labelled cholesterol(I) appeared. Due to the urgent requirement of this laboratory for the labelled compound, studies in the preceding section were brought to a close and the new method investigated.

This method, developed by Dauben and Eastham[74], takes advantage of the accessibility to ring A labelled Δ^4-cholesten-3-one(XLV)[75], m.p. 80-81°C. This compound was converted, by means of acetyl chloride and acetic anhydride, to 3-acetoxy-$\Delta^{3,5}$-cholestadiene(XLVI)[76], m.p. 80-81°C. which, by the action of lithium aluminum



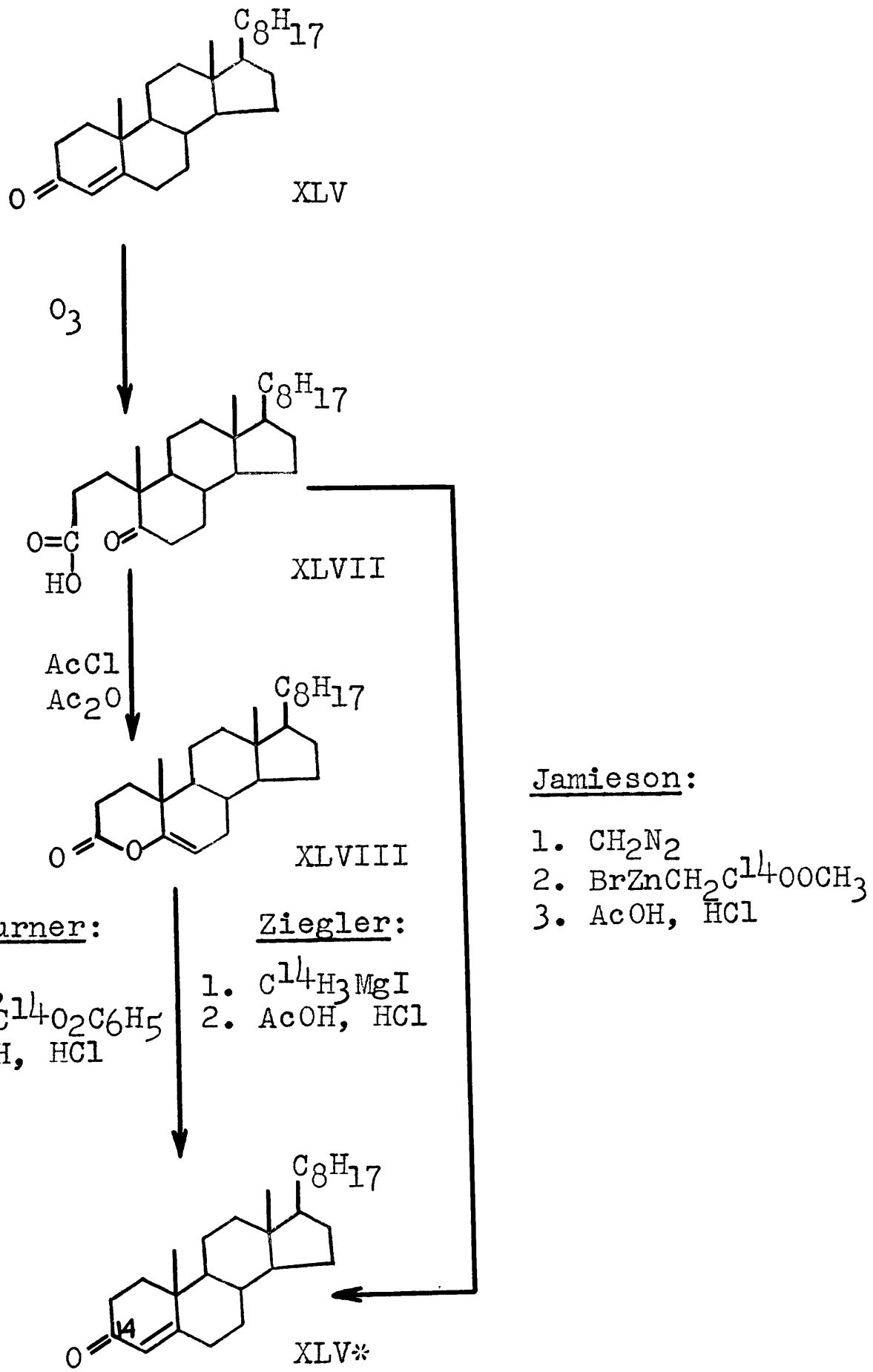
XLV XLVI I

hydride, afforded cholesterol(I) in 34 per cent yield. The method as published, unfortunately, was presented as a "Communication to the Editor" in the Journal of American Chemical Society, hence experimental details were not furnished. A reference given therein provided a satisfactory means for working up the reaction product[77] and, on this basis, an experiment was carried out in order to evaluate the method.

The reaction product of this experiment was first treated with very dilute ethanolic hydrochloric acid, which converted the α and β-Δ^4-stenols to cholestadiene. This was then followed by precipitation with digitonin[35]. Filtration of the digitonide removed the α epimers where-upon the dried precipitate was decomposed in pyridine, thus regenerating the original digitonin and freeing the steroid moieties. The crystalline steroid residue thus obtained was chromatographed, furnishing cholesterol(I) in only 17 per cent yield. A small amount of epicholestanol, m.p. 182°C. was also removed from the column, which indicated that ethereal extraction of the digitonide prior to decomposition was not complete[77].

At this point, the suggestion was made that if sodium borohydride were substituted for lithium aluminum hydride, yields of cholesterol(I) upward to 65 per cent could be obtained[78]. A trial reduction of a methanolic

Chart 7


C_8H_{17}
O
XLV
O_3
C_8H_{17}
O=C
HO
O
XLVII
AcCl
Ac_2O
C_8H_{17}
O
O
XLVIII
Turner:
1. NaH, $CH_3C^{14}O_2C_6H_5$
2. AcOH, HCl
Ziegler:
1. $C^{14}H_3MgI$
2. AcOH, HCl
Jamieson:
1. CH_2N_2
2. $BrZnCH_2C^{14}OOCH_3$
3. AcOH, HCl
C_8H_{17}
O
14
XLV*

solution of 1.0 gm. 3-acetoxy-$\Delta^{3,5}$-cholestadiene(XLVI) at room temperature gave cholesterol(I) in 55 per cent yield without resorting to chromatography.

b) Radioactive Δ^{4}-cholesten-3-one(XLV*).

It was mentioned earlier that until quite recently, only two methods were available for the preparation of radioactive Δ^{4}-cholesten-3-one(XLV*). The first of these, by Turner[75], involves the keto-acid XLVII, prepared by the ozonolysis of Δ^{4}-cholesten-3-one(XLV), as shown in Chart 7. This was dehydrated to yield the enol lactone XLVIII, which was then treated with C^{14}-carboxyl labelled phenylacetate. Cyclization gave Δ^{4}-cholesten-3-one-4-C^{14}(XLV*). The second method, by Jamieson, converts the keto-acid XLVII, by means of diazomethane, to the corresponding methyl ester which, in turn, is reacted with zinc and C^{14}-carboxyl labelled bromomethylacetate. Cyclization gives, in this case also, Δ^{4}-cholesten-3-one-4-C^{14}(XLV*)[10,79]. The yield afforded by these two methods were 45 and 30 per cent, respectively.

Observations of Belleau[80] regarding the condensation

---

* This symbol is used in conjunction with compound numerals to indicate the C^{14}-labelled molecule.

of Grignard reagents with enol lactones led to an improved method for obtaining Δ^4-cholesten-3-one(XLV). Ziegler, treating the enol lactone(XLVIII) with methyl magnesium iodide and cyclizing, obtained Δ^4-cholesten-3-one(XLV) in 70 per cent yield[79] (Chart7). This synthesis was then applied, employing methyl magnesium iodide-C^{14}, to give Δ^4-cholesten-3-one-4-C^{14}(XLV*) in 45 per cent yield. The total amount of XLV* thus obtained was 526 mg. and had a specific activity of 6.12×10^8 counts per minute per millimole.

c) Cholesterol-4-C^{14}(I*).

A second similar reduction was carried out, starting with 1.0 gm. Δ^4-cholesten-3-one-4-C^{14}(XLV*), prepared by Ziegler according to the directions of Jamieson[10], and counting ca. 2500 per minute per mg. This was converted to the enol acetate XLVI* which, when recrystallized twice from methanol, amounted to 725 mg. Reduction of this with sodium borohydride gave 404 mg. cholesterol-4-C^{14}(I*), melting at 147-148°C. Yield, based on the enol acetate XLVI*, 65 per cent.

A final experiment was accomplished wherein 510 mg. Δ^4-cholesten-3-one-4-C^{14}(XLV*), m.p. 74-76°C., prepared by Ziegler of this laboratory and having a specific activity of 6.12×10^8 counts per minute per millimole, was converted to the enol acetate XLVI*. This was thoroughly

evacuated to remove reagents and, without purification by crystallization, reduced with sodium borohydride. On working up the material, 135 mg. cholesterol-4-C^{14} (I*) was obtained, melting at 144-148°C. Yield based on XLV*, 27 per cent. By concentrating the mother liquors an additional quantity of material was obtained, melting at 139-145°C. The specific activity of the first crop, determined by means of a windowless Q-gas counter, was found to be 6.0×10^8 counts per minute per millimole.

Since the advent of the Dauben method for preparing radioactive cholesterol(I), two other precedures have been published. The first of these is designed to prepare cholesterol-26-C^{14}, and is accomplished by the addition of methyl magnesium iodide to 25-ketonorcholesterol [81]. The second method, taking advantage of Δ^4-cholesten-3-one(XLV) already labelled in ring A, converts this compound to the enol acetate XLVI which, by the action of potassium amide in liquid ammonia, is transformed into Δ^5-cholesten-3-one. This, in turn, is reduced by lithium aluminum hydride to yield cholesterol (I)[82]. Obviously, since this scheme involves an additional step not required by the Dauben method, its investigation was not considered.

2) Experimental.

a) Reduction of 3-acetoxy-$\Delta^{3,5}$-cholestadiene(XLVI) with Lithium Aluminum Hydride.

To a flask containing 80 ml. absolute ether was added 1.0 gm. of the enol acetate XLVI. The flask was then cooled to -10°C. and gentle stirring accomplished by means of a magnetic stirrer. A dropping funnel containing a solution of 500 mg. lithium aluminum hydride in 200 ml. absolute ether was affixed, and this solution added to the contents of the flask over a period of 0.5 hours. Stirring was maintained for an additional 15 minutes and 5 ml. acetone added to decompose the excess reagent. The reaction mixture was then allowed to warm to room temperature, whereupon this was transferred to a 500 ml. separatory funnel and shaken with three 75 ml. portions of 3 M sodium hydroxide. The ether was dried over anhydrous magnesium sulfate, filtered and evaporated *in vacuo*. This material was taken up in 250 ml. 95 per cent ethanol, 36 drops of 12 M hydrochloric acid added and the solution gently refluxed for 4.0 hours. The alcoholic solution was evaporated to about 25 ml., and the crystalline material separating washed well with water and dried. The material thus obtained was dissolved in 50 ml. 90 per cent ethanol

and 1.0 gm. digitonin in 100 ml. of 90 per cent ethanol added.

The flask was then set aside in the refrigerator overnight. The digitonide thus formed was filtered and washed with small, successive portions of cold 90 per cent ethanol, dried and decomposed in 30 ml. pure pyridine. To this was added 200 ml. ether, the mixture transferred to a 250 ml. centrifuge bottle and centrifuged for 15 minutes. The ether solution was then decanted and filtered. This process was repeated with two 100 ml. portions of ether, the filtrates combined and solvent removed under reduced pressure. This treatment afforded 350 mg. crystalline product, melting in the range 100-125°C. Consequently, the material was taken up in a little ether and placed on 10 gm. "Alcoa" F-20 grade alumina and chromatographed.

Fractions 13-30, consisting of 171 mg. material, were consolidated and recrystallized from methanol, affording cholesterol(I), melting at 147-148°C. and not depressing the melt of an authentic sample. A test with tetranitromethane was positive. Yield of total material in fractions 13-30, 17.1 per cent.

Table 4

Fraction	Eluent	Wt. in mg.	Nature
1-4	Benzene	2	oil
5-8	Ether 5: Benzene 45	37	epicholestanol m.p. 181-182°C.
9-10	Ether 10: Benzene 40	12	"
11-12	Ether 15: Benzene 35	7	"
13-14	Ether 20: Benzene 30	20	crystals, m.p. 145-148°C.
15-20	Ether 25: Benzene 25	85	crystals, m.p. 146-148°C.
21-26	Ether 30: Benzene 20	61	crystals,m.p. 147-148°C.
27-28	Ether 40: Benzene 10	2	crystals, m.p. 146-148°C.
29-30	Ether	3	crystals, m.p. 144-147°C.
31 et seq.			oil

b) Conversion of Δ^4-cholesten-3-one-4-C^{14}(XLV*) to Cholesterol-4-C^{14}(I*) Employing Sodium Borohydride.

To a small flask containing 510 mg. Δ^4-cholesten-3-one-4-C^{14}(XLV*), m.p. 74-76°C., specific activity 6.12×10^8 counts per minute per millimole, was added a mixture of 5.0 ml. acetyl chloride and 5.0 ml. acetic anhydride. This mixture was heated at 105°C. for 1.5

hours and the reagents removed *in vacuo* at 100°C. The resulting iridescent oil was taken down three times with ether and maintained under a reduced pressure of <1.0 mm. mercury for 3.0 hours. The material thus dried was dissolved in a mixture of 5 ml. ether and 35 ml. pure methanol, and 300 mg. sodium borohydride added. Stirring, by means of a magnetic stirrer, was initiated and continued for 2.0 hours, after which time the flask and contents were set aside at room temperature for an additional 24 hours. The reaction mixture was then taken up with 200 ml. ether and washed with two 100 ml. portions of water. The ether layer was separated and evaporated *in vacuo*, and the resulting residue dissolved in 175 ml. ethanol to which 25 drops of 12 M hydrochloric acid had been added. This was gently refluxed for 3.0 hours, after which time water was added to a point of great turbidity. On allowing the flask to remain at refrigerator temperature overnight, crystalline material separated, which was filtered, washed well with water and dried. The product so obtained was taken up in 75 ml. 95 per cent ethanol and to this was added a solution of 1.5 gm. digitonin in 25 ml. 95 per cent ethanol, whereupon the digitonide separated almost immediately. This, again, was refrigerated overnight and on the following day filtered, washed with a little

cold 90 per cent ethanol and dried. This was transferred to a flask, extracted with two 100 ml. portions of ether and filtered once more. The digitonide thus purified was decomposed in a 250 ml. centrifuge bottle with 30 ml. pure pyridine, and 200 ml. ether added. The regenerated digitonin was thrown out of suspension by centrifugation for 15 minutes and the ethereal solution of the product filtered. By repeating this process twice more, combining filtrates and evaporating under reduced pressure, 268 mg. crude material were obtained, melting at 125-140°C. One recrystallization from methanol gave cholesterol-4-C^{14}(I*), melting at 145-148°C. An additional quantity of material was secured by concentrating the mother liquors and melted at 139-145°C. The specific activity of the first crop was determined by means of a windowless Q-gas counter, and was found to be 6.0×10^8 counts per minute per millimole. A small sample of this material was admixed with an authentic sample of cholesterol(I) and gave no depression in the melt.

SUMMARY AND CONCLUSION

The formula, nature and occurrence of cholesterol(I) have been sketched. The researches of Wieland and Windaus were delineated to indicate some of the difficulties encountered in resolving problems relating to the elucidation of its structure. The suspected transformation of cholesterol(I) to the steroid hormones of the body provided the impetus, in this laboratory, to obtain the molecule labelled with tracer carbon. A brief account of isotopes, both stable and radioactive, has been given and the relative merits of these as "tags" in molecules of biological importance discussed.

In Section II, the desirability for nucleus, rather than side chain, labelled cholesterol(I) was discussed. 3(β)-acetoxycholestan-6-one(III) was chosen as a common pathway in the proposed chemical studies due to its facile conversion to cholesterol(I); hence, the attack was made at ring B. Several synthetic schemes which would allow for the incorporation of C^{14} into this ring were investigated, The first of these involved the Tiffeneau reaction as applied to seco-6-cholestan-3(β)-ol-7-one(XII). The failure to prepare this compound was due to the loss of the 3(β)-hydroxyl. This difficulty was overcome in a second procedure by preparing XXXI, the i-analogue of XII, thereby maintaining

indirectly the integrity of this function. The extremely unreactive character of the carbonyl group of XXXI, however, made addition of hydrogen cyanide impossible. A third method was to extend, with rearrangement, the chain at C_7 of the lactone acid XXXIIIa by the Arndt-Eistert synthesis and cyclize, but the acid chloride XXXIV failed completely to react with diazomethane. Lastly, an attempt was made to attach a carbon atom at C_5 of the keto-aldehyde compound XL. This sequence failed also due to the impossibility of preparing crystalline intermediates prior to the proposed addition of hydrogen cyanide. These difficulties may be ascribed to the anomalous and unreactive character of ring B, a fact well-known to workers in this field.

Incident to these investigations, the trans-elimination of elements of hydrohalide from 3(β)-halo-6-keto derivatives of cholestane was studied in detail, as well as the reverse reaction. The infra-red spectra of seco-6-i-cholestan-7-one(XXXI) and seco-6-3(β)-bromocholestan-7-one(XXXII), along with their normal, 6-membered ring B analogues, have been determined, interpreted and compared. Thus, it was shown that the cyclopropane structure in ring A "conjugated" with the 6-ketone of i-cholestan-6-one(XXIV) depresses the carbonyl frequency in a manner similar to that of α,β-unsaturated ketones, but to a lesser extent; whereas the effect of contracting ring B was to exalt the carbonyl frequency from its normal position in the saturated series. The bands of weak

absorption at the extreme end of the low frequency region displayed by the two bromo derivatives, XXVIII and XXXII, have tentatively been assigned to the C-Br stretching.

In the final section, a method for converting Δ^4-cholesten-3-one(XLV), by the lithium aluminum hydride reduction of its enol acetate XLVI, to cholesterol(I) was evaluated. A variation of this procedure, employing sodium borohydride, was carried out and found to be superior with respect to yield. The latter synthesis was then followed through, starting with Δ^4-cholesten-3-one(XLV*) of high specific activity. In this manner, a quantity of cholesterol(I*) with a sufficiently high concentration of C^{14} to permit tracer studies was obtained. This material had a specific activity of 6.0×10^8 counts per minute per millimole.

CLAIMS TO ORIGINAL RESEARCH

1) It was found that the benzil-benzilic acid rearrangement may not be applied to steroid ring B diketones where rings A and C are intact, since the energy requirements imposed by the double annulation cannot, apparently, be surmounted.

2) It was shown that acetoxylation of 3(β)-chlorocholestan-6-one(XXVII), by means of potassium acetate in acetic acid, is impossible owing to the precedence of a trans-elimination reaction provoked by the high concentration of acetate ions and that, under these conditions, the formation of i-cholestan-6-one(XXIV) is favored. Thus, an alternate method for the preparation of this compound has been revealed.

3) The difficulty encountered in pyrolyzing barium i-cholestan-6:7-dioate was overcome by treating a methanolic solution of i-cholestan-6:7-dioic acid(XXIX) with a solution of normal lead acetate in methanol. The pyrolysis of the lead salt thus obtained proceeded with much greater facility, affording the expected product.

4) B-nor-i-cholestan-7-one(XXXI), a new compound, has been prepared by the pyrolysis of lead i-cholestan-6:7-dioate(XXX). Advantage was taken of the trans-addition of elements of hydrohalide to the C_3-C_5 bridge of such compounds to furnish evidence of the cyclopropane structure

in ring A. Addition of hydrobromic acid to XXXI gave the derivative B-nor-3(β)-bromocholestan-7-one(XXXII), not previously described.

5) 3(β)-bromocholestan-6-one(XXVIII), B-nor-3(β)-bromocholestan-7-one(XXXII), i-cholestan-6-one(XXIV) and B-nor-i-cholestan-7-one(XXXI) have been submitted for infra-red spectral analysis. Such studies of steroids with a contracted ring B and i-steroids with the cyclopropane structure in "conjugation" with a carbonyl group have been accomplished for the first time.

6) The acid chloride XXXIV of cholestan-3(β)-ol-6:7-dioic acid-6⟶3-lactone(XXXIIIa) has been prepared; but, owing to the impossibility of obtaining it in crystalline form, could not be fully characterized. It proved to be exceedingly inert.

7) 2 3-benzyloxycholestan-5,6(α)-diol(XXXIXa), not hitherto described, has been prepared by the osmium tetroxide hydroxylation of 3-benzyloxy-Δ^5-cholestene(Ia), which places the 6-hydroxyl in the α position. Further support of the structure was afforded by the reducing property of the lead tetraacetate cleavage product, although the latter could not be obtained in crystalline form.

8) Δ^4-cholesten-3-one(XLV*) of high specific activity and labelled at position 4 with C^{14} was transformed into its enol acetate XLVI* which, in turn, was converted into

cholesterol-4-C^{14} by the action of sodium borohydride. The cholesterol-4-C^{14} thus obtained had a specific activity of 6.0 counts per minute per millimole.

BIBLIOGRAPHY

1. Fieser and Fieser, Natural Products Related to Phenanthrene, 3d Ed., Rheinhold, New York (1949), p. 92.
2. Sobotka, Chemistry of the Sterids, Williams and Wilkins, Baltimore (1938), p. 5.
3. Gilman, Organic Chemistry, An Advanced Treatise, 2d Ed., Wiley, New York (1943), p. 1392.
4. Fieser and Fieser, Natural Products Related to Phenanthrene, 3d Ed., Rheinhold, New York (1949), pp. 120-121.
5. ibid. , pp. 121-122.
6. Gilman, Organic Chemistry, An Advanced Treatise, 2d Ed., Wiley, New York (1943), pp. 1348-9.
7. Fieser and Fieser, Natural Products Related to Phenanthrene, 3d Ed., Rheinhold, New York (1949), p. 474.
8. Hawk, Oser and Summerson, Practical Physiological Chemistry, 12th Ed., Blakiston, Toronto, (1947), pp. 987-995.
9. Fieser and Fieser, Natural Products Related to Phenanthrene, 3d Ed., Rheinhold, New York, (1949), pp. 476-7.
10. Jamieson, Ph. D. Thesis, McGill University (1947).
11. Turner, J. Am. Chem. Soc., 69: 726 (1947).
12. Reich and Lardon, Helv. Chim. Acta, 29: 676 (1946).

13. Fieser and Fieser, Natural Products Related to Phenanthrene, 3d Ed., Rheinhold, New York (1949), pp. 97-8.

14. Plattner and Lang, Helv. Chim. Acta, 27: 1877(1944).

15. Heilbron, et al., J. Chem. Soc., 1938: 102-7.

16. Dodson and Riegel, J. Org. Chem., 13: 432 (1948).

17. Chuman, J. Chem. Soc. Japan, 64: 1369-74 (1943).

18. Barnett, Ryman and Smith, J. Chem. Soc., 1946: 524-6.

19. Fieser and Fieser, Natural Products Related to Phenanthrene, 3d Ed., Rheinhold, New York, (1949), p. 95.

20. Tiffeneau, Weill and Tchoubar, Compt. rend., 205: 54 (1937).

21. Gilman, Organic Chemistry, An Advanced Treatise, 2d Ed., Wiley, New York,(1943), pp. 96-7.

22. Goldberg and Monnier, Helv. Chim. Acta, 23: 376, 840 (1940).

23. Lassar-Cohn, Ber., 32: 683 (1899).

24. Heilbron, et al., J. Chem. Soc., 1937: p. 803.

25. ibid. , p. 805.

26. Stange, Z. physiol. Chem., 218: 74 (1933).

27. Windaus and Dalmer, Ber., 52: 162 (1919).

28. Shoppee, J. Chem. Soc., 1946: 1147-51.

29. Fieser and Fieser, Natural Products Related to Phenanthrene, 3d Ed., Rheinhold, New York (1949), pp. 268-9.

30. Shoppee, J. Chem. Soc., 1948: 1032 et seq.

31. Gilman and Blatt, ed., Organic Syntheses, Coll. Vol. I, 2d Ed., Wiley, New York (1941), p. 452 (c).

32. Ruzicka, et al., Helv. Chim. Acta, 17: 1392 (1934).

33. Windaus and Staden, Ber., 54: 1065 (1921).

34. Windaus, Ber., 37: 3705 (1904).

35. Fieser and Fieser, Natural Products Related to Phenanthrene, 3d Ed., Rheinhold, New York (1949), pp. 102-4.

36. Ladenburg, Chakravorty and Wallis, J. Am. Chem. Soc., 61: 3483-7 (1939).

37. Diels and Abderhalden, Ber., 44: 2848 (1911).

38. Wallis, Fernholz and Gephart, J. Am. Chem. Soc., 59: 137 (1937).

39. Ford and Wallis, J. Am. Chem. Soc., 59: 1415 (1937).

40. Ford, Chakravorty and Wallis, J. Am. Chem. Soc., 60: 413 (1938).

41. Heilbron, Hodges and Spring, J. Chem. Soc., 1938: 759.

42. Benyon, Heilbron and Spring, J. Chem. Soc., 1937: 1459.

43. Michael, J. prakt. Chem., 52: 289 (1895).

44. Gilman, Organic Chemistry, An Advanced Treatise, 2d Ed., Wiley, New York (1943), 1840-62.

45. King, J. Am. Chem. Soc., 70: 2685-6 (1948).

46. Klotz, J. Am. Chem. Soc., 66: 88-91 (1944).

47. Butenandt and Thome, Ber., 71: 1487 (1938).

48. Kuwada and Miyasaka, J. Pharm. Soc. Japan, 58: 540-8 (1938).

49. Jones and Dobriner, Vitamins and Hormones, Thimann ed., Vol. VII, Academic Press, New York (1949), pp. 293-363.

50. Fieser and Fieser, Natural Products Related to Phenanthrene, 3d Ed., Rheinhold, New York, (1949), pp. 184-198.

51. Bachmann and Struve, in Organic Reactions, Vol. I, Wiley, New York (1942), pp. 38-62.

52. Wolff, Ann., 394: 25 (1912).

53. Bachmann, Cole and Wilds, J. Am. Chem. Soc., 61: 974 (1939).

54. Anner and Miescher, Helv. Chim. Acta, 31: 2173-83 (1948).

55. Hugget, Arnold and Taylor, J. Am. Chem. Soc., 64: 3043 (1942).

56. Calvin, et al., J. Am. Chem. Soc., 72: 122 (1950).

57. Thompson, Ph.D. Thesis (to be presented), McGill Univ.

58. Wilds and Shunk, J. Am. Chem. Soc., 70: 2427 (1948).

59. Yates, Ph.D. Thesis (to be presented), McGill Univ.

60. Blatt, ed., Organic Syntheses, Coll. Vol. II, Wiley, New York (1943), pp. 165-7.

61. ibid. , pp. 461-2.

62. Weygand, Organic Preparations, Interscience, New York (1945), pp. 177-80.

63. ibid., p. 103.

64. Malaprade, Compt. rend., 186: 382 (1928).

65. Criegee, Ann., 481: 268 (1930).

66. Fieser and Rajagopalan, J. Am. Chem. Soc., 71: 3940 (1949).

67. Criegee, Ber., 65: 1770 (1932).

68. Prelog and Tagman, Helv. Chim. Acta, 27: 1870 (1944).

69. Heilbron, J. Chem. Soc., 1933: 302.

70. Booth, ed., Inorganic Syntheses, Vol. I, McGraw-Hill, New York (1939), p. 47.

71. Dimroth and Schweitzer, Ber., 56: 1375 (1923).

72. McElvain, The Characterization of Organic Compounds, McMillan, New York (1946), p. 133.

73. Heilbron, et al., J. Chem. Soc., 1932: 270.

74. Dauben and Eastham, J. Am. Chem. Soc., 72: 2305 (1950).

75. Turner, J. Am. Chem. Soc., 72: 579 (1950).

76. Westphal, Ber., 70: 2128 (1937).

77. McKennis and Gaffney, J. Biol. Chem., 175: 217 (1948).

78. Schwenk, Worcester Foundation for Experimental Biology, private communication to R.D.H. Heard.

79. Ziegler, Ph.D. Thesis (to be presented), McGill Univ.

80. Belleau, Ph.D. Thesis, McGill Univ. (1950).

81. Ryer, Gebert and Murrill, J. Am. Chem. Soc., 72: 4247 (1950).

82. Birch, J. Chem. Soc., 1950: 2325-6.