DIAZOMETHANE-C^{14}; RING B
DI-SUBSTITUTED CHOLESTANOLS

A Thesis

by

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TABLE OF CONTENTS

Page

PART I

PART II

PART I.

GENERAL INTRODUCTION

With the termination of the last World War, pile-produced radioactive isotopes have become generally available. As a result, the tracer technique has become an important tool in all branches of scientific research. This technique had early application in biochemical research and its usefulness is borne out by the large number of publications using radioactive tracers, which appear every month in scientific journals. Because of the very important role that is played by carbon in our physical and biological world, the outstanding importance of a radioactive isotope of carbon is readily seen. It affords a tool by which, for example, one may trace through all its metabolic ramifications, the utilization of organic materials by biological systems.

The most useful of the five known isotopes of carbon is C^{14}. Because of its long half-life, about 5300 years, one does not have to consider decay corrections while using it. Furthermore, C^{14} emits only a soft beta particle whose maximum energy is 0.15 Mev. This weak radiation minimizes health hazards when C^{14} is used but it gives rise to the only objectionable property of the isotope. Experimentally, a beta particle with maximum energy of 0.15 Mev. requires specialized techniques for accurate determination of its activity.

Our objective was the preparation of steroid hormones containing C^{14} in the ring and side chain. Since C^{14} was only available from Oak Ridge in the form of $BaC^{14}O_3$, when this work was begun, methods had to be worked out to convert the $BaC^{14}O_3$ into some intermediate organic compound which could be incorporated into the ring or side chain of the desired steroid hormones. The work of Jamieson (1), in 1947, and the later work of Yates (2) and Thompson (3) showed that diazomethane-C^{14} is such an intermediate. Diazomethane-C^{14} was used by Yates and Thompson in Arndt-Eistert extension to synthesize progesterone-21-C^{14}, desoxycorticosterone acetate-21-C^{14}, and estrone-16-C^{14}. Therefore, it is the worker's intention to describe, in this part of the thesis, a micro method for the synthesis of diazomethane-C^{14} of very high specific activity which was used to synthesize the above-mentioned steroid hormones.

HISTORICAL INTRODUCTION

The introduction of isotopic carbon into a molecule necessitates, in most cases, the formation of a new carbon to carbon bond. A considerable number of reactions that are standard practice in synthetic organic chemistry can be, and have been, used for this purpose. The applicability of a standard synthetic procedure to isotopic synthesis depends on a number of factors. First, it is necessary to choose a reaction for the introduction of the isotope that is most compatible with the starting material available. Second, the reaction chosen must proceed in high yield and good purity to the desired end-product, and in this case must also be adaptable on a micro scale. It is also imperative that organic intermediates used to label steroid hormones be produced in the highest specific activity possible. To this end, many standard reactions in organic chemistry have been modified in order to increase the yield and decrease the scale on which the necessary manipulations must be carried out. When known reactions could not be adapted, completely new methods have been devised to conserve the labeling isotope.

In 1948, Hershberg et al. (4) published for the first time an isotopic synthesis of diazomethane using C^{13} as a label. These authors reduced $NaC^{13}N$* by the electrolytic chromous chloride method of Traube et.al. (5) to methylamine-C^{13}. The amine was

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* Carbon-13 is available in the form of $NaC^{13}N$, obtained by chemical exchange or thermal diffusion methods for isotope concentration $HC^{12}N(g) + C^{13}N^- \rightleftharpoons CH^{13}N + C^{12}N^-$

converted to N-nitrosomethyl-C^{13}-urea which was decomposed with alkali to yield diazomethane-C^{13}. No yield was reported for the reduction of $NaC^{13}N$, but the over-all yield was given as 30%, based on the cyanide used. The synthesis was carried out on a two-gram scale. Our objection to the use of the above procedure was that the electrolytic reduction of sodium cyanide could not be readily adapted to a hundred milligram scale without a great lowering in the over-all yield of diazomethane. Still another dissuading factor was the difficulty in manipulating the electrolytic reduction on a micro scale.

It became evident to us at this time that $NaC^{14}N$ was a very necessary intermediate for the synthesis of diazomethane-C^{14}. This writer's attempt to synthesize $NaC^{14}N$ from $BaC^{14}O_3$, by the sodium azide fusion method of Adamson (6) proved to be unsuccessful. The difficulties encountered with Adamson's procedure in this laboratory were finally overcome by Henneberry and Baker (7). These authors improved Adamson's method to the stage where they can now obtain a constant yield of 82% of $NaC^{14}N$ from $BaC^{14}O_3$. However, Belleau (8), working in this laboratory, was successful in synthesizing $NaC^{14}N$ from $BaC^{14}O_3$ by a novel and simplified method. The readily available triphenylmethylsodium was carbonated in good yield to give triphenylacetic acid-1-C^{14}. The corresponding acid chloride and amide were obtained practically quantitatively as shown below. Dehydration of the amide gave the nitrile in very high yield. The nitrile readily undergoes hydrogenolysis to yield cyanide and triphenylmethane. The over-all yield of pure cyanide from CO_2 was 70%.

$$(C_6H_5)_3CNa \xrightarrow[90\%]{C^{14}O_2} (C_6H_5)3C\ C^{14}OOH \xrightarrow[95\%]{NH_4OH}$$

$$(C_6H_5)_3C\ C^{14}ONH_2 \xrightarrow[97\%]{P_2O_5} (C_6H_5)_3C\ C^{14}N \xrightarrow[90\%]{Na\ EtOH}$$

$$(C_6H_5)_3CH + NaC^{14}N$$

With the ready availability of $NaC^{14}N$, our attention now turned to the problem of converting the latter to diazomethane-C^{14}. The simplest standard method of synthesizing diazomethane is through methylamine hydrochloride to N-nitrosomethylurea and alkali decomposition of the latter to yield diazomethane. Therefore, a review was made of the literature to find a method of reducing $NaC^{14}N$ to monomethylamine hydrochloride-C^{14} that could best be adapted to our needs.

It has long been considered an established fact that hydrocyanic acid may be hydrogeneted to methylamine by the use of platinum catalysts. The early work in this field was of a qualitative nature to determine the physical form of the catalyst , and conditions of temperature and pressure that would reduce hydrocyanic acid. The earliest work reported is that of Dubus (9) who reduced gaseous hydrocyanic acid with hydrogen to methylamine in the presence of platinum black using the temperature range of 100 to 110° C. The rapid decrease in activity of the catalyst was attributed by him to the formation of a double cyanide of methylamine and platinum, which he claimed to have isolated. Linnemann (10) reduced hydrocyanic acid vapors at 300° C in the presence of platinum sponge. Reduction proceeded smoothly and the contact mass was apparently not attacked, but it soon lost much of its activity. Unlike Dubus, he found no double salt produced. The role of hydrocyanic acid as a catalyst poison was first shown by Denham (11), who found that platinized platinum reduces hydrocyanic acid to methylamine in solution. Later, Riedel (12), and independently Sieverts and Peters (13) , carried out the hydrogenetion of hydrocyanic acid in the presence of colloidal platinum. They too found methylamine as the product of reduction. These early workers did not have a good method for separating amine mixtures and as a result, quantitative data concerning the products of hydrogenetion is lacking. Barrett and Titley (14) made a comprehensive study of the reduction of hydrocyanic vapor using platinized asbestos as catalyst. The most favourable temperature range for the reduction was found to be 120-250° C, and a mixture of amines as well as ammonia resulted. A scheme was presented to explain the formation of mono-, di-, and trimethylamines and ammonia. It was also shown that when the ratio of hydrocyanic acid to hydrogen was maintained at 1 to 15, an 80% yield of bases was obtained, of which 75% was monomethylamine. Here, for the first time, was presented a method of separating the three amines and identifying them.

Hydrocyanic acid may also be reduced in the presence of base-metal catalyst, especially finely divided nickel, but the products are largely ammonia and methane, with comparatively small quantities of monomethylamine. Examples of this mode of reduction is the work of Sabatier and Senderens (15) and that of Dreyfuss (16). The first workers reduced hydrocyanic vapors in the presence of a nickel catalyst at 250° C, and identified ammonia, mono-, di-, and trimethylamine in the products of reduction. The latter reduced gaseous hydrocyanic acid in the presence of a large excess of hydrogen in the temperature range of 200-350° C, using copper, zinc and nickel cyanide catalyst deposited on pumice. He claimed to have obtained methylamine with small amounts of di- and trimethylamine and ammonia.

Although all the above methods for the catalytic reduction of hydrocyanic acid definitely showed that methylamine is one of the end products, none could be adapted to isotopic synthesis. The objections were as follows: Our initial idea was to reduce 100 mg. of $NaC^{14}N$, on this scale separation of the mixture of amines that would result from the hydrogenation, would greatly decrease the yield. High temperature vapor phase reduction on such a small scale presented many experimental difficulties which one should try to avoid in handling expensive materials such as isotopic compounds. Consequently, these methods of hydrogenetion were not tried.

There was, however, ample evidence in the literature pointing to PtO_2 as the desired catalyst for the reduction of hydrocynic acid. Voorhees and Adams (17) first prepared PtO_2 and showed it to be a very potent catalyst for the reduction of a wide variety of nitriles to their corresponding amines. Because of the great activity displayed by this catalyst, hydrogenation could be carried out at room temperature and atmospheric pressure. The low activity displayed by the catalyst preparations used by early workers was the governing factor that compelled them to employ vapor phase, high temperature hydrogenetion. Using PtO_2 as a catalyst, we therefore set out to find suitable experimental conditions for the hydrogenetion of $HC^{14}N$ at room temperature and atmospheric pressure that would give high yields of monomethylamine hydrochloride-C^{14}.

Very recently, however, Crompton and Woodruff (18) published an article containing notes on the synthesis of isotope labeled compounds, in which appeared an additional method for the synthesis of diazomethane-C^{14}, and a new method for the synthesis of methylamine hydrochloride-C^{14} as well as our own method for diazomethane-C^{14}. Skraba and Jones (19) reduced $NaC^{14}N$ in dilute acid solution using PtO_2 as a catalyst. The methylamine-hydrochloride-C^{14} produced was heated with potassium cyanate to give methyl-C^{14}-urea, which was nitrosylated to yield N-nitrosomethyl-C^{14}-urea. The nitrosomethylurea was decomposed with alkali to give diazomethane-C^{14}. The over-all yield claimed was 54% from sodium cyanide. Fields (20) reported the application of the Schmidt reaction on acetic acid-2-C^{14} to give methylamine hydrochloride-C^{14} in 80 to 90% yield. The best results were obtained with hydrazoic acid in chloroform and 100% sulfuric acid.

Another very recent synthesis of methylamine hydrochloride-C^{14} was reported by Gal et al. (21). These authors started with acetic acid-1, 2-C^{14}, biologically prepared by allowing Clostridium aceticum to catalyse the following reaction:

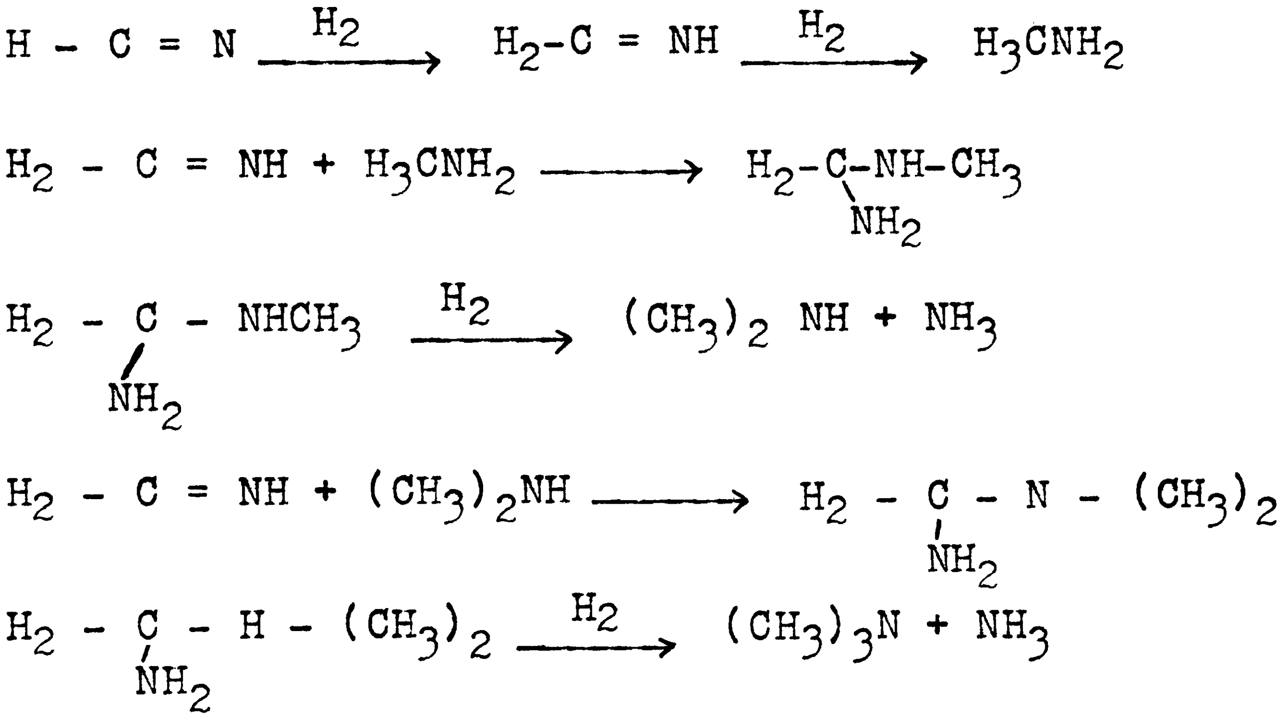
$$4H_2 + 2C^{14}O_2 \longrightarrow C^{14}H_3C^{14}OOH + 2H_2O$$

The doubly labeled acetic acid was converted to the corresponding acid chloride, which on treatment with sodium azide gave methyl isocyanate-C^{14}. Hydrolytic decarboxylation of the isocyanate produced methylamine hydrochloride-C^{14} in 72-76% yield, based on the acetyl chloride used.

The method of Fields for the synthesis of methylamine hydrochloride-C^{14} as outlined above uses acetic acid-2-C^{14} as starting material, which in turn is made from $C^{14}H_3OH$. The present methods for the synthesis of labeled methanol are very difficult to carry out and as a result this method for making methylamine hydrochloride-C^{14} will not be generally applicable. The method reported by Gal et al. for the synthesis of methylamine hydrochloride-C^{14} can be carried out with acetic acid-2-C14 but again it depends on the availability of $C^{14}H_3OH$.

DISCUSSION

As a rule, primary and secondary amines form simultaneously when cyanide and nitriles are catalytically reduced in the presence of nickel, platinum or other catalysts. Sometimes, tertiary amines and ammonia are also formed. The explanation of this fact is outlined as follows:



$$H - C = N \xrightarrow{H_2} H_2\text{-}C = NH \xrightarrow{H_2} H_3CNH_2$$

$$H_2 - C = NH + H_3CNH_2 \longrightarrow H_2\text{-}\underset{NH_2}{C}\text{-}NH\text{-}CH_3$$

$$H_2 - \underset{NH_2}{C} - NHCH_3 \xrightarrow{H_2} (CH_3)_2\ NH + NH_3$$

$$H_2 - C = NH + (CH_3)_2NH \longrightarrow H_2 - \underset{NH_2}{C} - N - (CH_3)_2$$

$$H_2 - \underset{NH_2}{C} - H - (CH_3)_2 \xrightarrow{H_2} (CH_3)_3N + NH_3$$

In the first stage of the reduction aldamines are formed which react with the primary amine in solution to form an aminodiamine. Reduction of the latter gives the secondary amine and ammonia. The formation of trimethylamine may be similarly explained by assuming the formation of aminomethyldimethylamine from formaldimine and dimethylamine and its subsequent reduction. It was shown by Escourrou (22) that under reduced pressure the hydrogenation may proceed only to the aldimine stage.

It is possible in certain cases to increase the yield of primary amines by the use of various solvents. Thus, if the reduction is carried out in acetic anhydride, the primary base is acetylated as fast as it is formed and a nearly quantitative yield of the acetylated base may be obtained. Corothers and Jones (23) reduced para and ortho tolunitrile with platinum oxide and acetic anhydride as solvent, and were able to isolate the primary amines in 88 and 94% yield respectively, as the N-substituted acylamides. These authors also showed that with platinum oxide the yield of primary amines may be increased by using acetic acid as the hydrogenation medium. Thus,benzonitrile yields 21% of primary amine when the reduction is carried out in alcohol, and 62% as primary amine when the reduction is carried out in acetic acid. Another method that can be used to increase the yield of primary amines is to carry out the reduction in a medium containing hydrochloric acid. This serves to trap the primary amine as the hydrochloride as soon as it is formed, and thus prevents its condensation with aldimine to yield secondary amines. Hartung (24) reduced benzonitrile with palladium-Norite catalyst, using absolute alcohol containing one equivalent of hydrochloric acid as a solvent, and obtained a high yield of the corresponding primary amine. Rosemund and Pfankuch (25), using palladium-barium sulfate catalyst, reduced phenylacetonitrile in acetic acid containing hydrochloric acid, to the corresponding primary amine in 73% yield. The primary amines were isolated as the hydrochlorides. Carothers and Jones (23) claimed that the speed of reduction increases rapidly with rise in temperature and have shown this to be true with a number of nitriles.

All the above mentioned methods for increasing the yield of primary amines, as applied to nitriles, were tried with hydrocyanic acid to see whether a high yield of monomethylamine could be obtained. The results of these preliminary experiments are summarized in Table I. The hydrogenation apparatus and a detailed account of the procedure used will be described in the experimental section. From experiment 1, Table I, it is readily seen that glacial acetic acid alone is not a very good solvent for the hydrogenation of cyanide. Contrary to the results of Hartung (24) using benzonitrile, alcohol and hydrochloric acid did not show a very large uptake of hydrogen during eight hours of hydrogenation (expt.2, Table I). Using acetic anhydride or acetic anhydride saturated with gaseous hydrochloric acid as a solvent, did not produce the N-substituted acylamide as was to be expected from the above-mentioned work of Carothers and Jones (expts.3 and 4, Table I). By far the best solvent was found to be glacial acetic acid containing a little in excess of 2 millimoles of hydrochloric acid per millimole of potassium cyanide used. When this solvent was used at room temperature and atmospheric pressure, the uptake of hydrogen was almost quantitative (expt.5, Table I). In this experiment the platinum oxide, glacial acetic acid and the hydrochloric acid were added together and the catalyst was reduced to platinum, prior to the addition of potassium cyanide. It was found, however, that some hydrochloric acid reacts with platinum oxide to give a yellow coloured solution, presumably containing platinum chloride complexes.

TABLE I

EFFECT OF SOLVENT AND TEMPERATURE ON THE HYDROGENATION OF CYANIDE AT ATMOSPHERIC PRESSURE *

Expt. No.	Compound	Solvent	Temp. °C.	Hydrogen Uptake % theoretical	Time in Hours
1	KCN	5 cc. of glacial acetic acid	25	65	6
2	KCN	5 cc. of 95% ETOH + 0.27 cc.conc.HCl	25	65	8
3	KCN	5 cc. of acetic anhydride	25	53	6
4	KCN	5 cc. of acetic anhydride sat'd with HCl (g)	25	44	5
5	KCN	5 cc. of acetic acid + 0.27 cc. conc.HCl	25	94	7½
6	KCN	5 cc. of acetic acid + 0.27 c.c. conc. HCl.	60	100	6½
7	KC N	5 cc. of acetic acid + 0.27 cc. conc.HCl	25	101	6½
8	NaCN	5 cc. of acetic acid + 0.35 cc. conc.HCl	25	100	7½

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* Hydrogenation was carried out on 100 mg. of compound using 40 mg. of PtO_2 as catalyst.

The physical state of the catalyst was not mossy but colloidal. Therefore, in experiment 7, Table I, the procedure was modified and the hydrochloric acid was added after the platinum oxide had all been reduced and just before the addition of potassium cyanide. This change produced a much more active catalyst and lowered the time required for quantitative uptake of hydrogen by one hour. Temperature had no appreciable effect on the course of the hydrogenation (expts. 6 and 7, Table I) since the time required for a quantitative uptake of hydrogen was the same at 60° C as at 25° C. Substituting sodium cyanide for potassium cyanide had no effect on the course of the hydrogenation (expt. 8, Table I). Thus, a method was developed for the conversion of sodium cyanide to methylamine hydrochloride in at least 85% yield.

In our experience with the hydrogenation of cyanide, we found that the best results are obtained when we use highly active platinum oxide as a catalyst. The commercial preparations of platinum oxide which we first used were found to be of low activity and a method was worked out to regenerate these into a more active form. The low activity platinum oxide was converted to chloroplatinic acid according to the procedure described by Baldeschwieler and Mikeska (26). Addition of ammonium chloride to the latter gave a precipitate of ammonium chloroplatinate which was isolated in pure form. A high yield of platinum oxide was obtained from the ammonium chloroplatinate by the procedure of Bruce (27). This freshly prepared platinum oxide was found to be very active for the reduction of cyanide to methylamine.

Storage in a vacuum dessicator preserved the catalyst in a very active form for at least six months,

The hydrogen used was made with a Kipp generator using a 50% solution of reagent grade hydrochloric acid and reagent grade zinc. The gas was scrubbed through a solution of 20% NaOH, 20% $AgNO_3$ and saturated $KMnO_4$. Electrolytic tank hydrogen scrubbed as above can also be used.

The synthesis of diazomethane from methylamine hydrochloride was simplified because of the availability of standard methods for this conversion. The method chosen was that described by Arndt (26) which was also used by Herschberg et al. (4) to synthesize diazomethane-C^{13}. The procedure is outlined as follows:

$$CH_3NH_2.HCl + H_2NCONH_2 \rightarrow CH_3NHCONH_2 + NH_4Cl$$
$$CH_3NHCONH_2 + HNO_2 \rightarrow CH_3N(NO)CONH_2 + H_2O$$
$$CH_3N(NO)CONH_2 + KOH \rightarrow CH_2N_2 + KCNO + 2H_2O$$

Methylamine hydrochloride condenses with urea to form N-methylurea, which is not isolated. Nitrosylation of the latter with nitrous acid in the cold gives N-nitrosomethylurea which comes out of solution as a white fluffy precipitate. Decomposition of the nitrosomethylurea with alkali in ether solution gives diazomethane, which is stable in ether in the cold. The problem that now arose was whether it was necessary to isolate methylamine hydrochloride beyond a qualitative isolation for identification purposes, before condensation with urea to form N-methylurea. Herschbert et al. showed that such an isolation was not necessary. These authors used crude methylamine hydrochloride from which they obtained pure N-nitrosomethylurea using the above procedure. Therefore, methylamine hydrochloride was not isolated, but a n-butanol extraction was carried out to separate it from KCl and to identify it.

$$KCN + HCl \rightarrow HCN + KCl$$

$$HCN + Pt + 2H_2 + HCl \rightarrow CH_3NH_2 \cdot HCl + Pt$$

In an inactive experiment, identical with experiment 8, Table I, the glacial acetic acid and excess HCl were removed under vacuo on the steam bath, when the hydrogenation was complete. The residue was extracted with hot n-butanol, and concentration of the butanol yielded 35% of methylamine hydrochloride. The melting point and admixture melting point was correct. This only served to identify the amine.

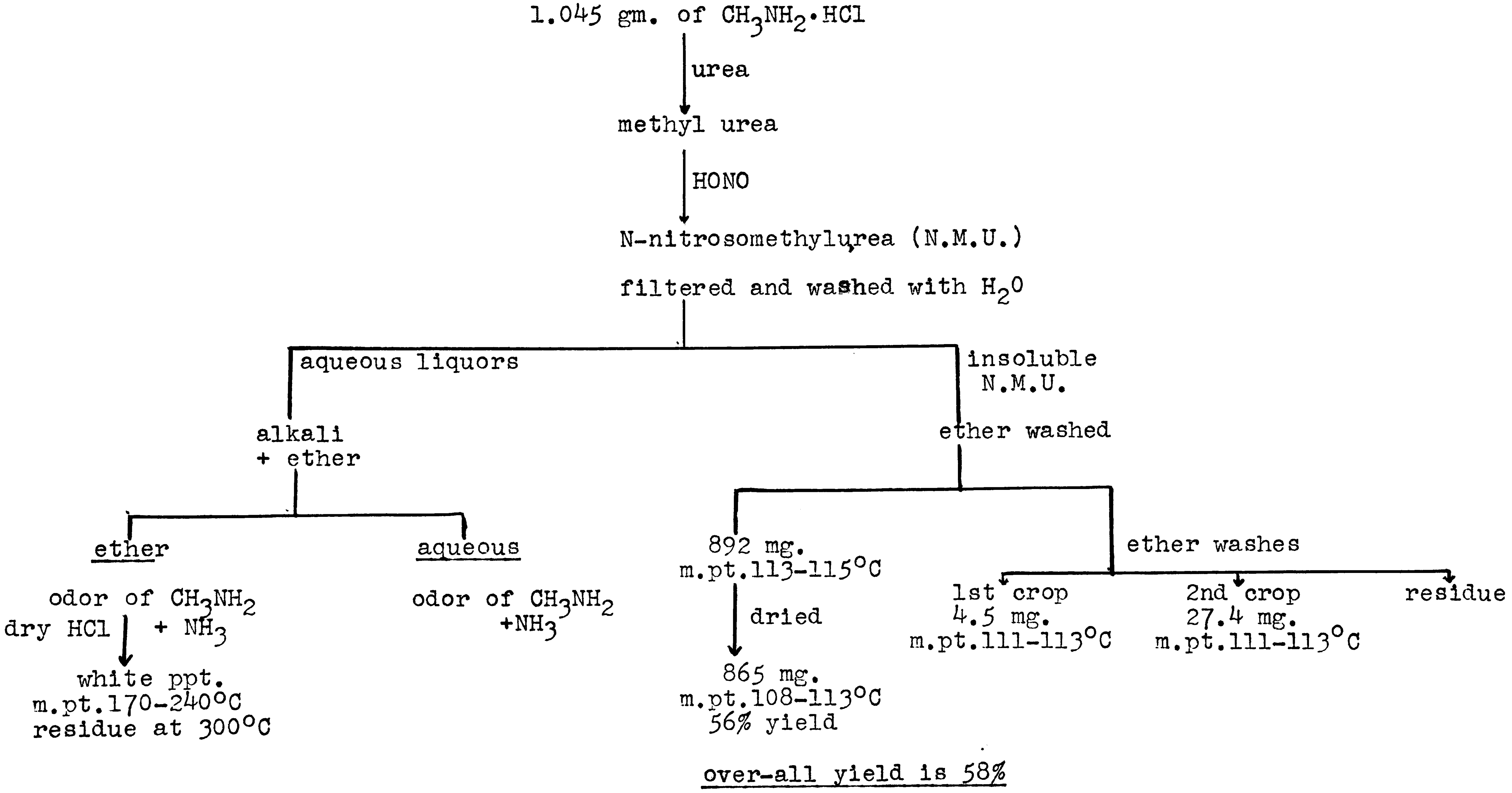
The possibility next considered was whether diazomethane could be obtained directly from sodium cyanide without isolating the intermediate nitrosomethylurea. To this end, an experiment identical with experiment 8, Table I, was carried out. The methylamine hydrochloride obtained was converted directly to N-nitrosomethylurea, and alkali decomposition of the latter in ether solution yielded diazomethane. The ether and the diazomethane was distilled into a flask kept in a salt-ice bath, which contained ether and a weighed quantity of benzoic acid. After all the diazomethane had been consumed, (the yellow colored solution became clear), the excess benzoic acid was titrated with standard alkali. This procedure gave an accurate estimation of the yield of diazomethane. The over-all yield of diazomethane from sodium cyanide in such an experiment was 35%. Since isolation of intermediates causes a loss of labeling isotope, this direct route to diazomethane was favoured by us because it would conserve the isotope.

To test the above procedure, a small radioactive run was carried out starting with 100 mg. of $NaC^{14}N$ containing 0.1 millicurries of C^{14}. The diazomethane-C^{14} obtained as above was diluted five-fold with inactive diazomethane, and the mixture reacted with the acid chloride of the monomethyl ester of Marrianolic acid, this being the method of introducing diazomethane-C^{14} into ring D of estrone (3). Unfortunately, no estrone-16-C^{14} was obtained by this procedure, and this direct route to diazomethane was abandoned. It is very probable that some volatile impurity, formed at the N-nitroso-methylurea stage, distills over with the diazomethane and interferes with the Arndt-Eistert reaction.

With the failure of the above-mentioned experiment, it became necesssary to obtain pure diazomethane-C^{14}. The only way to get pure diazomethane was to isolate and purify N-nitrosomethylurea. The difficulty with this procedure is that from 100 milligrams of sodium cyanide, the maximum yield of N-nitrosomethylurea is 126 milligrams (60%) of light, foaming precipitate. To isolate such a small quantity would entail too great a mechanical loss. In order to carry out Arndt-Eistert reactions on a workable scale, the diazomethane-C^{14} was diluted five-fold with inactive diazomethane. Instead of carrying out the dilution at the diazomethane stage, it was now decided to do it at the methylurea stage. When this was done in an inactive run and the mixture nitrosylated, the precipitate obtained was bulky enough to manipulate with ease. A radioactive run was then carried out starting with 100 milligrams of $NaC^{14}N$ (0.6 millicurries) containing 0.75 millimoles excess NaOH. The $NaC^{14}N$ was reduced to methylamine hydrochloride-C^{14}, which in turn was converted to N-nitrosomethyl-C^{14}-urea as described above. Diazomethane-C^{14}, prepared from the latter, was used to make estrone-16-C^{14} having a count of 66,000 disintegrations/mg/minute. In order to determine the optimum conditions of yield and purity of N-nitrosomethylurea, a series of experiments was designed, the results of which are summarized in Table II. N-nitrosomethylurea was filtered and washed with two 1 milliliter portions of water followed by 5 milliliters of ether. The ether and aqueous washes were both analysed for N-nitrosomethylurea as shown in Table II. It is readily seen from the data in the table that washing the precipitate with ether lowers the yield of N-nitrosomethylurea by 2%. The aqueous washes did not contain any N-nitrosomethylurea since there was no visible evolution of diazomethane when they were treated with ether and alkali in the cold, and the ether distilled into a flask kept in an ice-salt bath. The ether and aqueous residues from the above treatment both had an odor characteristic of methylamine

T A B L E II.

EFFECT OF ETHER AND WATER WASHINGS ON THE YIELD AND PURITY OF N-NITROSOMETHYLUREA



and ammonia. When the ether fraction was evaporated to dryness and treated with hydrochloric acid, a white precipitate settled out which melted at 170-240° C with a residue remaining at 300° C. This indicated the presence of methylamine hydrochloride and an inorganic salt, possibly ammonium chloride. Thus, it appears very possible that we were unable to make satisfactory use of diazomethane-C^{14}, obtained directly from $NaC^{14}N$ without isolation of intermediates, because of the interference of unreacted methylamine-C^{14} and ammonia with the Arndt-Eistert reaction. It was also found that the melting point of N-nitrosomethylurea dropped ten degrees if it was stored in a vacuum desiccator, in the cold, longer than two days, although it is stable for a much longer period of time when stored in large bulk. In radioactive runs, it was noticed that the residue left after distilling off the diazomethane - C^{14} was highly radioactive but no attempt was made to determine its exact nature other than the experiments reported above. When the scale of reduction of $NaC^{14}N$ was increased from 100 mg. to 200 mg. a new hydrogenation unit had to be constructed. The reasons for this are as follows:

In the preparation of $NaC^{14}N$ (6) from $BaC^{14}O_3$, the $HC^{14}N$ is distilled into a flask containing two millimoles excess sodium hydroxide. Water is removed under reduced pressure on the steam bath, leaving a mixture of $NaC^{14}N$ and NaOH spread on the bottom of the flask. In small scale, hydrogenation of $NaC^{14}N$ (100 mg.), it was necessary to transfer this mixture to a small cup (Figure 1). This transfer resulted in a small mechanical loss of isotope because it was impossible to scrape all the solids out of the flask. When the scale of the hydrogenation was increased to 200 milligrams of $NaC^{14}N$, a new apparatus was designed to enable us to reduce the cyanide in the same flask in which it had been taken to dryness. This new apparatus will be described in Figure 1.

When the reduction of sodium cyanide was carried out on a 200 milligram scale, it became possible to isolate directly N-nitrosomethylurea prepared from it. A number of inactive experiments were also carried out to obtain the yield of all the intermediate steps between sodium cyanide and diazomethane. It was found that the yield of diazomethane from sodium cyanide and methylamine hydrochloride respectively was 40 and 47%. From these, the calculated yield of sodium cyanide to methylamine hydrochloride is at least 85%. In another inactive run, methylamine hydrochloride was converted to N-nitrosomethylurea in 60% yield. Similarly, the yield of N-methylurea to N-nitrosomethylurea was found to be 85%.

Radioactive assay of all the compounds dealt with here proved to be a difficult task. Since the specific activity of all the compounds made was very high, "infinite thin" plates ($1.0\ \mu g/cm^2$) were made, for which no "self-absorption" corrections had to be made. Diazomethane-C^{14} itself cannot be assayed since it is volatile at room temperature. The only compound that could be counted was N-nitrosomethyl-C^{14}-urea, because it was isolated in a pure state. However, when the latter was plated from a methanol solution on a copper disc (1.0 $\mu g./cm^2$) it decomposed almost completely with apparent loss of the methyl carbon. When the assay was made with an end-window G.M. tube (1.8 mg. per cm^2), a large rise in background was noted, probably due to contamination from volatile decomposition product(s). We therefore decided to count diazomethane-C^{14} in the form of a methyl ester.

Diazomethane reacts with acids to form methyl esters. A search was therefore made for an acid that would rapidly react with diazomethane to produce a stable ester. The first methyl ester tried was that of benzoic acid. This methyl ester forms very readily when an excess of benzoic acid and diazomethane are brought into contact in ether. Because the methyl ester of benzoic acid is an oil at room temperature, it could not be separated from the unreacted benzoic acid on the micro scale used. Since an accurate radio-assay can only be carried out on a pure compound, benzoic acid methyl ester could not be used. Of the large number of acids considered, the one that was found most suitable to our need was 3-keto-Δ^4-etiocholenic acid. This acid reacts with diazomethane to form a methyl ester melting at 132-133° C. Its separation from unreacted etiocholenic acid was accomplished by chromatography on alumina.

In a radioactive experiment, the count of diazomethane-

C^{14} obtained by counting an "infinite thin" plate of the methyl-C^{14} ester of 3-keto-Δ^{4}-etiocholenic acid agreed within 10% with estrone-16-C^{14}, progesterone-21-C^{14} and DCA-21-C^{14} derived from it. The 10% discrepancy can be attributed to a 4% error in plating, a 4% error due to "self scattering" from infinite thin plates and a 2% error in counting.

EXPERIMENTAL.

All melting points were taken with the Fisher-Jones melting point apparatus and are therefore uncorrected. Radio-assay of C^{14} compounds was made with a thin window (1.8 mg./cm^2) G.M. tube and the results reported as counts/mM/min., unless otherwise stated. The radioactive compounds were plated from methanol solutions on copper discs as "infinitely thin" plates (1 µg./cm^2). The observed counts were corrected for background and the resolving time of the counter. The statistical error in counting was 2%.

PREPARATION OF PtO_2.

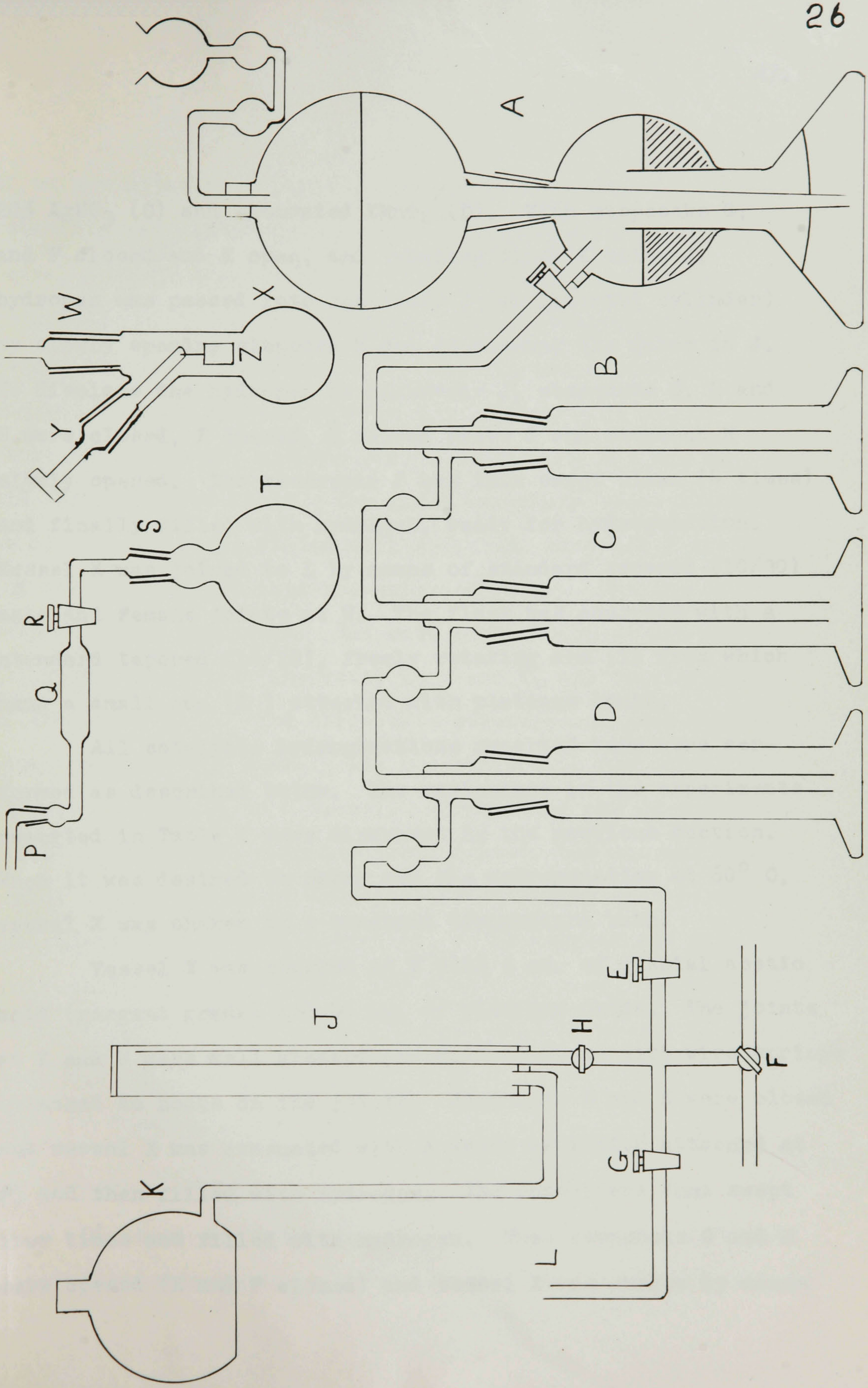
Ten g. of low activity platinum oxide was dissolved in aqua regia and any insoluble material was filtered off. The filtrate was evaporated to dryness three times with excess HCl. The residue was dissolved in dil. HCl and an excess of solid ammonium chloride was added to precipitate ammonium chloroplatinate. To this solution was added 95% ethyl alcohol (about 1/3 the total volume of the solution) and, after standing for one hour, the precipitate was filtered and dried at 100° C. The yield of ammonium chloroplatinate was 4.7 g.

To 4.7 g. of ammonium chloroplatinate was added 47 g. of sodium nitrate and the two were well mixed. The mixture was gradually heated to the fusion point; a temperature of 350-370° C. was attained in 10 minutes and 400° C. at the end of 15 minutes. At the end of 20 minutes, the temperature was at 500-550° C. The mixture was held at 500° C for 30 minutes and then allowed to cool to room temperature, and 50 ml. of water added. There was no splattering during the heating period. The nitrates took a long time to dissolve, and when solution was complete the platinum oxide was filtered on a Buchner funnel using a hard filter paper (Whatman No.50). The oxide was washed free of nitrate ions and dried overnight *in vacuo* at 60° C. The yield of platinum oxide was 2.37 g.

CATALYTIC HYDROGENATION

In Figure 1 is shown the catalytic hydrogenation apparatus. For small scale (100 mg.) hydrogenation of cyanide, the unit having a small cup suspended in it was used. When it was desired to hydrogenate larger amounts of cyanide (200 mg. or more), the larger S-shaped unit was used. Both units were attached to the main hydrogenation apparatus by means of flexible plastic tubing at junction L. Hydrogenation using the small unit will first be described. Hydrogen, generated from a Kipp generator (A), using reagent grade zinc and 50% reagent grade HCl, was slowly scrubbed through 20% KOH (B) ,

FIGURE 1.



20% $AgNO_3$ (C) and saturated $KMnO_4$ (D). With stopcocks G, and F closed and E open, and leveling flask K below J, hydrogen was passed into reservoir J (an inverted cylinder) by slowly opening stopcock H and displacing the water in J. To displace the hydrogen in reservoir J, stopcocks G, H and E were closed, F opened, K raised above J and stopcock H slowly opened. The reservoir J was thus swept clean (4 times) and finally filled with hydrogen, ready for hydrogenation. Vessel X was joined to L by means of standard tapered (10/30) male and female joints at W. The flask was equipped with a standard tapered (14/20), freely rotating arm (Y) from which hung a small cup (Z) attached with platinum hooks.

All catalytic hydrogenations reported here were performed as described below. The variations in the experiments reported in Table I were discussed in the previous section. When it was desired to carry out the hydrogenation at 60° C, vessel X was shaken in a constant temperature bath.

Vessel X was charged at Y with 5 cc. of glacial acetic acid (reagent grade) and 40 mg. of platinum oxide. The joints at Y and W were well greased and held in place with wire springs attached to hooks on the joints. Stopcocks H and E were closed and vessel X was evacuated with a water aspirator attached at F, and then filled with hydrogen. The vessel was thus swept four times and filled with hydrogen. Then stopcocks G and H were opened (E and F closed) and vessel X was shaken by means of an arm attached to it and leading to an eccentric. The eccentric was attached to a motor whose speed was regulated. Vessel X was shaken until all the platinum oxide had been reduced to platinum (10 minutes). Then, stopcock H was closed and the vessel opened by removing the arm at Y. To the acetic acid and platinum in X was added 0.27 ml. of conc. HCl (3.24 mM) and the cup Z, containing 105 mg. of 95% KCN suspended in it. The flask was again swept (4 times) as described above, filled with hydrogen, stopcock H opened and the cup dropped into the solution below. Shaking of vessel X then commenced. The rate of hydrogen uptake was followed by leveling the water in J and K, and reading the water height in J. The barometric pressure and room temperature were noted. Shaking was continued till the uptake of hydrogen ceased (6 to 8 hours). Towards the end of the hydrogenation, potassium chloride precipitated, but was not detrimental to the uptake of hydrogen. When sodium cyanide was used instead of potassium cyanide, an adjustment was made in the numbers of millimoles of HCl that were used. In a trial run with only 5 ml. of glacial acetic acid in vessel X, the apparatus only showed a leakage of 2 cc. of hydrogen in seven hours of shaking.

THE ISOLATION OF METHYLAMINE HYDROCHLORIDE

One hundred milligrams of sodium cyanide were reduced to methylamine hydrochloride as reported above. At the end of seven hours of shaking, the theoretical amount of hydrogen was consumed. The platinum was filtered off, using gravity filtration, and washed with dilute HCl. The filtrate was evaporated to dryness _in vacuo_ at 100° C. The residue was brought into solution with 40 ml. of hot n-butanol and the solution decanted from the insoluble sodium chloride. The solution was concentrated to a small volume (3 ml.), refrigerated and the resulting crystals filtered and vacuum dried. The methylamine hydrochloride thus obtained weighed 45 mg. (33% based on the sodium cyanide used) and had a melting point and admixture melting point of 222-224° C. The true melting point is 226° C.

DIAZOMETHANE

One hundred milligrams of sodium cyanide (2.04 mM) were again hydrogenated to methylamine hydrochloride as described above. The platinum was filtered off and the filtrate concentrated to dryness _in vacuo_ at 100° C. To the crude methylamine hydrochloride, in a 50 ml. standard tapered flask, was added 0.52 ml. of water and 400 mg. (6.6 mM) of urea (reagent grade). The mixture was refluxed gently for 2 3/4 hours and vigorously for fifteen minutes, and then allowed to cool to room temperature. To this solution was added 108 mg. of sodium nitrite (1.56 mM) and the whole immersed in an ice-salt bath. A mixture of 0.054 ml. of conc. sulfuric acid

(0.97 mM) in 600 mg. of ice was then added with constant stirring (20 minutes). When all the sulfuric acid had been added, a foaming precipitate of N-nitrosomethylurea settled out. The precipitate was not filtered but used in this crude form to synthesize diazomethane.

The flask containing the nitrosomethylurea was kept in an ice-salt bath and connected to a condenser the end of which was immersed in ether (20 ml.) contained in a receiving flask. The receiving flask was also kept in an ice-salt bath. To the nitrosomethylurea was added 10 ml. of ether and 2 ml. of 30% KOH. The alkali decomposed the nitrosomethylurea and a yellow coloured ethereal solution resulted. The decomposition was allowed to proceed fifteen minutes in the cold after which the ice-salt bath was replaced by a water bath at 50° C. and the ether solution brought to the boiling point with occasional shaking. The ether was distilled until it came over colorless and as an extra precaution another 10 ml. of ether was added and distilled into the receiving flask. To the combined ether solution was added a weighed amount of benzoic acid. The acid reacted with the diazomethane leaving a colourless ether solution in the receiving flask. Ten ml. of 95% alcohol was added to the ether and the latter was removed under *vacuo* at room temperature. The excess benzoic acid in alcohol was titrated with standard alkali. The calculated yield of diazomethane was 30 mg. or 35% based on the sodium cyanide used.

However, in another inactive experiment, similar to the one reported above, but starting with 200 mg. of sodium cyanide, the yield of diazomethane was 70 mg. or 40% based on the cyanide used.

DIAZOMETHANE-C^{14}

A hundred mg. of $NaC^{14}N$ (2.04 mM, 0.1 mc) containing 0.3 mM excess NaOH were hydrogenated as described above except that more HCl was added to neutralize the excess alkali. The resulting crude methylamine hydrochloride-C^{14} was converted to methyl-C^{14}-urea and the latter nitrosylated to N-nitrosomethyl-C^{14}-urea, as previously described. Alkali decomposition of the nitrosomethylurea gave diazomethane-C^{14} which was diluted five-fold by distilling it into an ether solution containing approximately 120 mg. of diazomethane. The total diazomethane was used in an Arndt-Eistert reaction with the acid chloride of the monomethyl ester of Marrianolic acid (3), which is one of the intermediate steps in the synthesis of oestrone-16-C^{14}. Unfortunately, this procedure did not produce the expected oestrone, probably due to impurities carried along with the diazomethane-C^{14} as was discussed in the previous section.

N-NITROSOMETHYL-C^{14}-UREA

A new route to diazomethane-C^{14} was therefore devised. Instead of making a five-fold dilution at the diazomethane stage, the dilution was accomplished by adding carrier methyl-urea. A second radioactive run was carried out starting with 100 mg. of $NaC^{14}N$ (2.04 mM, 0.6 mc) containing 0.75 mM excess NaOH. The $NaC^{14}N$ was reduced to methylamine hydrochloride-C^{14} using 40 mg. of platinum oxide, 5 ml. of glacial acetic acid and 0.41 ml. (4.92 mM) of conc. HCl. The hydrogenation procedure followed was the same as described above. Methyl-C^{14}-urea was formed by refluxing the crude amine in 0.6 ml. of water with 400 mg. (6.6 mM) of urea. To the methyl-C^{14}-urea in solution was added 910 mg. of methylurea in 3.9 ml. of water, 990 mg. (14.5 mM) of sodium nitrite and the whole immersed in an ice-salt bath. Nitrosylation of the methylurea was carried out by adding to the above solution a mixture of 0.9 ml. (16.2 mM) of conc. sulfuric acid in 5.4 g. of ice with constant stirring, over a period of 20 minutes. N-nitrosomethyl-C^{14}-urea settled out as a foamy precipitate. It was filtered, washed with cold water (2x0.5 ml.) and vacuum-dried overnight in the cold. The yield of dry, light yellow N-nitrosomethyl-C^{14}-urea was 1.26 g. It softened at 117°C and melted at 119-121° C. The true melting point is 123-124° C.

The N-nitrosomethyl-C^{14}-urea was decomposed with alkali, as described above, to give diazomethane-C^{14}. Oestrone-16-C^{14} produced from the latter assayed at 66,000 counts/mg./min.

THE RATE OF HYDROGENATION OF $NaC^{14}N$

In order to see whether $NaC^{14}N$ and $NaC^{12}N$ display any difference in their hydrogenation rates using platinum oxide as catalyst, the hydrogen uptake as a function of time was carefully followed in a small radioactive run. The room temperature and barometric pressure was also noted. One hundred and three ml. (0.4 mc) of $NaC^{14}N$ containing 0.45 mM excess NaOH were reduced as previously described. A quantitative uptake of hydrogen was obtained in 6 hours and 10 minutes of shaking. The data from this run was compared with the data obtained from a small inactive run starting with 100 mg. of NaCN. The time taken for a quantitative uptake of hydrogen is a direct function of the speed with which the hydrogenation vessel is shaken. The results of these two experiments are graphically presented in Figure 2. From these rate curves, it is readily seen that the catalytic hydrogenation of inactive and radioactive NaCN do not differ from one another except in the time taken for a quantitative uptake of hydrogen.

LARGE SCALE REDUCTION OF $NaC^{14}N$

A suitable hydrogenation unit was constructed for the reduction of more than 100 mg. of $NaC^{14}N$. This unit can also be used for small scale reductions in which case it would eliminate the operation of placing the cyanide-sodium hydroxide

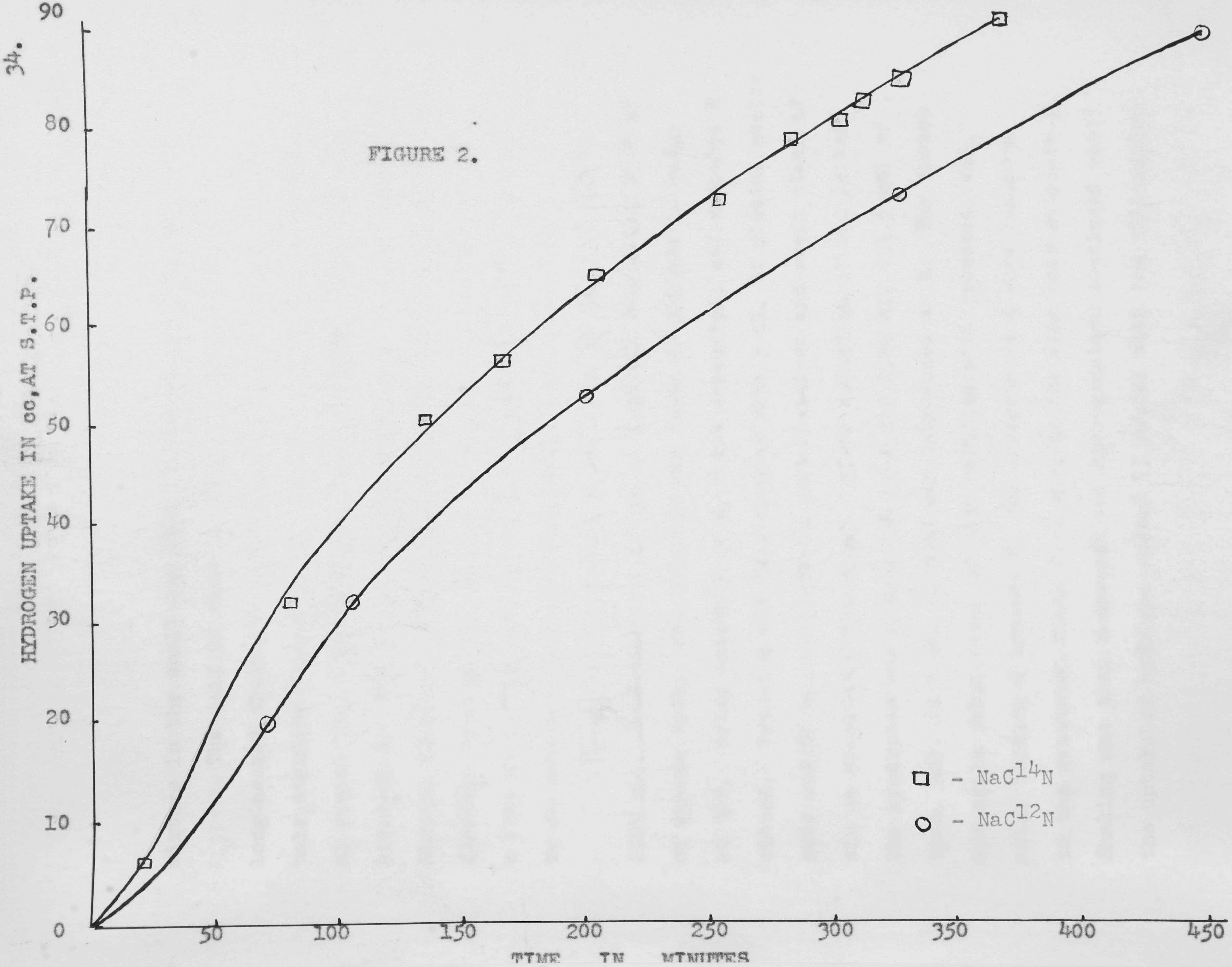

HYDROGEN UPTAKE IN cc,AT S.T.P.
0
10
20
30
40
50
60
70
80
90
50
100
150
200
250
300
350
400
450
TIME IN MINUTES
□ - NaC^{14}N
○ - NaC^{12}N

FIGURE 2.

mixture in the small cup (Z).

The unit is shown in Figure 1 and consists of a long-necked flask (T) connected by means of a male and female standard tapered joint (S,24/40) to a 30 cc. cylindrical vessel (Q). The latter can be closed off from T by the stopcock R. The whole unit is attached to the main apparatus, through standard tapered joints (14/35) at P, by means of flexible plastic tubing at L. The unit is clamped at S to a free revolving bar and shaken by means of an arm leading to an eccentric and attached to the long neck of T.

To flask T was added a solution (50 ml.) of $NaC^{14}N$ (205 mg., approximately 8.4 mc in 4.18 mM) containing 2.24 mM of excess NaOH. The solution was taken to dryness in vacuo at 30°. After assembly to Q, T was evacuated, and stopcock E closed. Vessel Q was then charged with 5 ml. of glacial acetic acid and 85 mg. of platinum oxide catalyst and swept clean of air as previously described. After reduction of the latter, the apparatus was dismantled at P and 0.96 ml. (11.5 mM) of conc. HCl. in 5 ml. of acid was introduced to Q. The system was again swept clean of air, refilled with hydrogen, and, after tilting Q towards T, the contents of Q were released to the evacuated flask T on opening the wide bore stopcock E. Shaking was then commenced and hydrogenation continued until the uptake of hydrogen ceased (7 hours) with the utilization of the theoretical two millimoles of hydrogen (210 ml.). After removal of the catalyst by filtration (gravity) and washing with dilute HCl, the filtrate was taken to dryness *in vacuo* at 40° C to give a residue of methylamine hydrochloride-C^{14} and sodium chloride. The residue dissolved in water (1 ml.) was refluxed for 3 hours with 711 mg. (11.85 mM) of urea. The solution was cooled to room temperature and an aqueous solution (5.6 ml.) of carrier methylurea (790 mg.; 10.7 mM), and sodium nitrite (1.3 g.), was added. The mixture was then cooled in an ice-salt bath and to it was added dropwise conc. sulfuric acid (0.6 ml. in 7.1 g. of ice-water mixture) with constant stirring (20 min.). The precipitated nitrosomethyl-C^{14}-urea was filtered, washed with cold water (1 cc.) and vacuum-dried. The product weighed 1.04 g. and had a melting point and admixture melting point of 120-123°C. The yield was 55% calculated from cyanide and ascertained gravimetrically from trial runs starting with NaCN without addition of methylurea.

N-nitrosomethyl-C^{14}-urea had a count of 9.97×10^{6} counts/mM/min. as against 1.39×10^{8} counts/mM/min. shown by diazomethane-C^{14} generated from it. This constitutes a 93% loss in activity which is probably due to decomposition of the product when plated as an "infinitely thin" plate.

The N-nitrosomethyl-C^{14}-urea (1.04 g.) was decomposed with alkali (3 ml. of 30% KOH) as previously described and the liberated diazomethane-C^{14} (332.0 mg. , 7.90 mM) was distilled into ether (50 ml.).

For determination of specific activity an aliquot (0.5 ml.) of the ether solution was pipetted into a solution of 50 mg. of 3-keto- Δ^4-etiocholenic acid in chloroform (5 cc.) and the mixture allowed to react in the cold overnight. The solvents were removed in vacuo at room temperature and the resulting oil was extracted with ether and separated into an acid and neutral fraction. The neutral fraction was dissolved in hexane-benzene and absorbed on alumina (prepared in hexane). The eluted fraction hexane-benzene (1:1) contained the C^{14}-methyl ester of 3-keto- Δ^4-etiocholenic acid. Its melting point and admixture melting point was 131-132° C. The C^{14}-methyl ester assayed at 4.2×10^5 counts/mg./min. The calculated count of diazomethane-C^{14} was 1.39×10^8 counts/mM/min. which compared favourably with oestrone-16-C^{14} (1.36×10^8 counts/mM/min.), progesterone-21-C^{14} (1.40×10^8 counts/mM/min.) and D.C.A. - 21-C^{14} (1.47×10^8 counts/mM/min.), all derived from it.

METHYLAMINE HYDROCHLORIDE TO N-NITROSOMETHYLUREA

To 1 g. (9.9 mM) of methylamine hydrochloride in 4 ml. of water was added 3 g. (50 mM) of urea and the mixture refluxed for 3 hours. To the solution of methylurea at room temperature was added 1.08 g. (14.8 mM) of 95% sodium nitrite and the whole cooled in an ice-salt bath. A mixture of 0.54 ml.

of conc. sulfuric acid in 6 g. of ice was added to this with constant stirring (20 minutes). The precipitated N-nitrosomethylurea was filtered on a Buchner funnel and washed with cold water (1 ml.). The vacuum-dried product had a melting point and admixture melting point of 122-123° C. The yield of pure compound was 622 mg. or 61% of theoretical.

METHYLAMINE HYDROCHLORIDE TO DIAZOMETHANE

N-nitrosomethylurea was prepared from 1 g. of methylamine hydrochloride as described above. Here, however, the product was not isolated but was decomposed with alkali to yield diazomethane. The yield of diazomethane, estimated by back titration of excess standard benzoic acid solution, was 200 mg. or 48% of theoretical.

METHYLUREA TO N-NITROSOMETHYLUREA

A solution of 1.01 g. of methylurea (Eastman Kodak) in 4.4 ml. of water was nitrosylated as previously described. The N-nitrosomethylurea formed was filtered, water washed and dried in vacuo. The product had a melting point and admixture melting point of 123-124° C. The yield was 573 mg. or 85% of theoretical.

SODIUM CYANIDE TO METHYLAMINE HYDROCHLORIDE

Having obtained the yields of the intermediate steps from sodium cyanide to diazomethane it is now possible to calculate the yield of sodium cyanide to methylamine hydrochloride without isolation of the latter. The yield of sodium cyanide to diazomethane is 40% as ascertained in an inactive experiment without isolation of intermediates. The yields of methylamine hydrochloride to diazomethane is 48% (above). Therefore, the calculated yield of sodium cyanide to methylamine hydrochloride is at least 85%.

N-NITROSOMETHYLUREA TO DIAZOMETHANE

One g. of N-nitrosomethylurea in 20 cc. of ether was decomposed with alkali (3 ml. of 30% KOH) as previously described. The diazomethane produced was distilled into an ether solution containing excess benzoic acid. Titration of the excess benzoic acid with standard alkali gave a calculated yield of 318 mg. of diazomethane or 78% of theoretical.

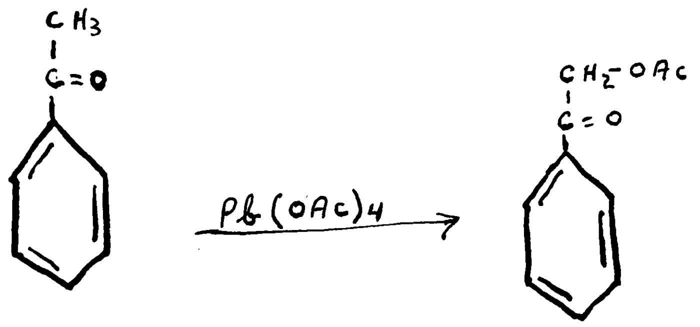
PART II.

RING B DI-SUBSTITUTED CHOLESTANOLS.

INTRODUCTION.

The object of the present investigation was to elucidate the spacial configuration of the acetoxy group, substituted,in ring B of the cholestane series, by the action of lead tetraacetate on 6-ketocholestanyl acetate (XXV). The initial work in this laboratory was carried out by Wasson (29) and Sainte-Marie (30), who made a study of the action of lead tetraacetate on the pregnane side chain, In 1947, Prado (31), subjected coprostanone to the action of this reagent, in order to explore in a more general manner the effect of lead tetraacetate on saturated, ring A oxygen-containing steroids.

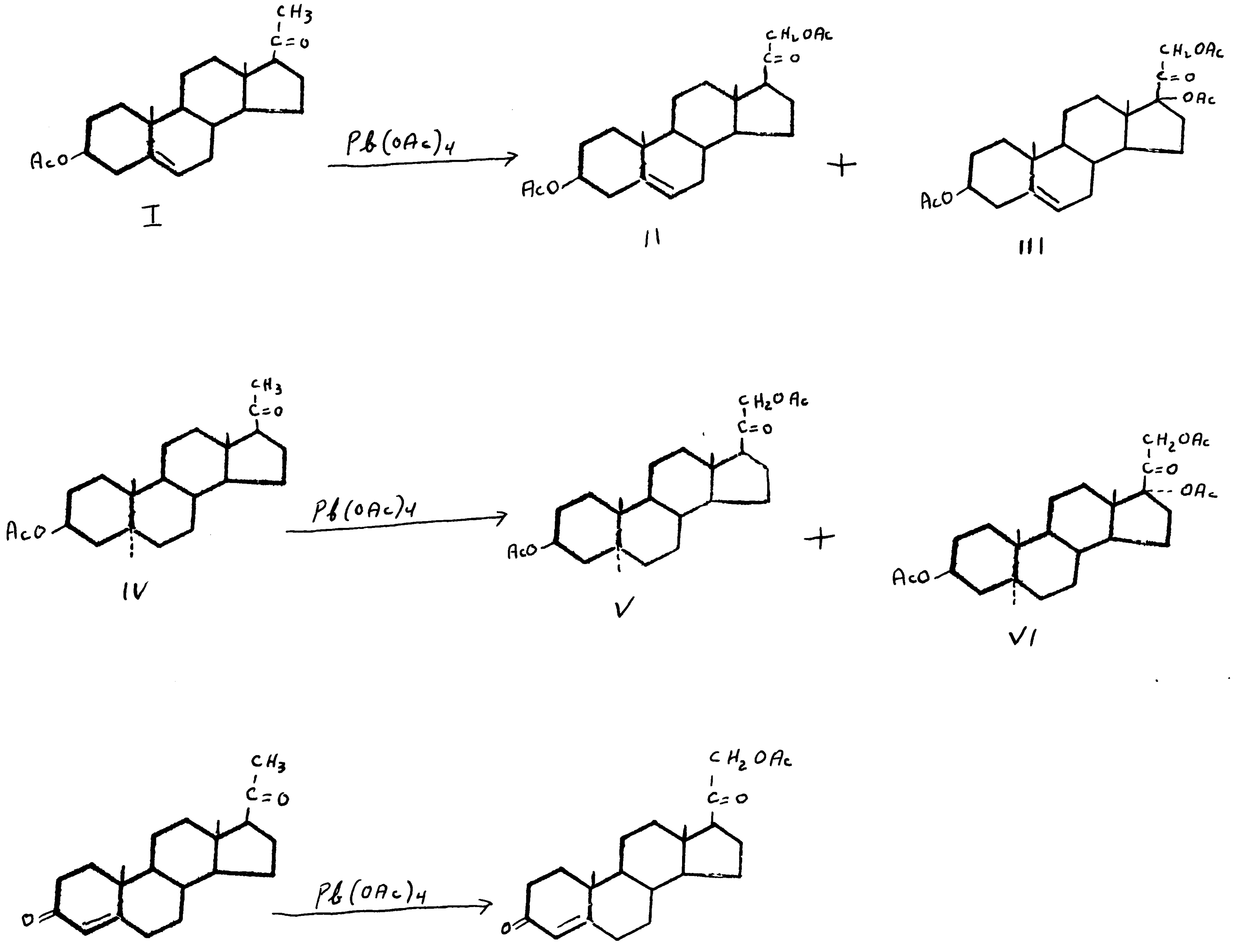
The observations that have prompted steroid chemists to use lead tetraacetate as an acetoxylating agent were first made by Dimroth and Schweizer (32). These authors observed that compounds possessing activated hydrogen atoms adjacent to a carbonyl group, could be oxidized by lead tetraacetate to give the corresponding acetoxy compound. Thus they showed that acetophenone could be oxidized by lead tetraacetate to yield the benzoylmethyl ester of acetic acid.



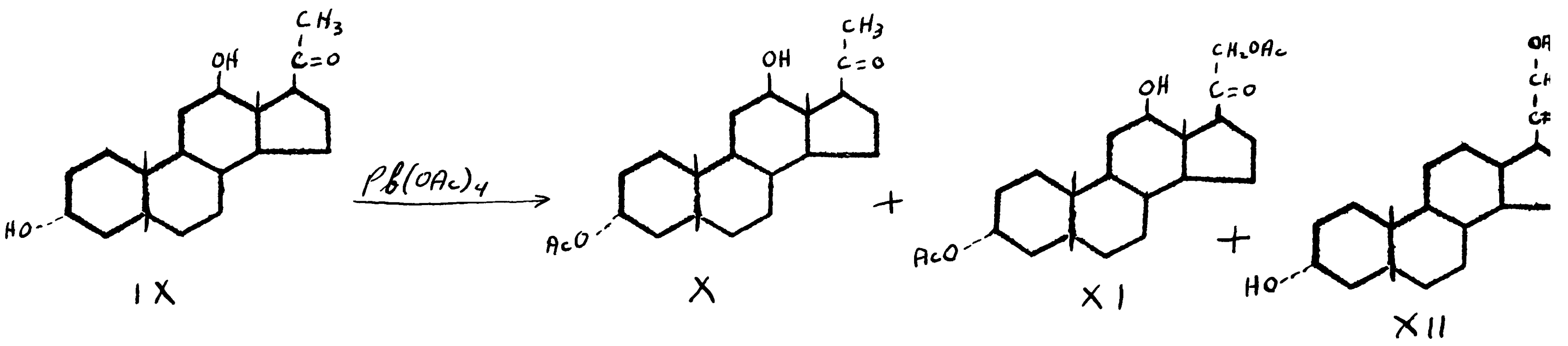
The first application of the lead tetraacetate oxidation to steroids was reported by Erhart, Ruschig and Aumueller (33) in 1939. These authors oxidized progesterone (VII) and pregnenolone acetate (I) in acetic acid from which they claim to have isolated high yields (80-100%) of desoxycorticosterone acetate (VIII) and 21-acetoxy-pregnenolone 3-acetate (II), respectively.

At the same time, Reichstein and Montigel (34) reported the results of lead tetraacetate oxidation of pregnenolone acetate (I), allopregnane-3β-ol-20-one acetate (IV) and progesterone (VII). The only variation in the method of oxidation, reported by these authors, from the method used by Erhart et. al., was the use of acetic anhydride as a catalyst along with the acetic acid usually used as solvent. The products isolated from the above compounds were as follows. Pregnenolone acetate (I) gave 21-acetoxy-pregnolone 3-acetate (II) and a small amount of a second compound formulated as 17β, 21-diacetoxy-pregnenolone 3-acetate (III). Allopregnane-3β-ol-20-one acetate (IV) gave allopregnane-3β, 21-diol-20-one diacetate (V) as the main reaction product and a small amount of a second compound formulated as allopregnane-3β, 17α, 21-triol-20-one triacetate (VI). Contrary to the claim of Erhart et.al. (33), progesterone (VII) yielded desoxycorticosterone acetate (VIII) in only 3% yield. The low yield of (VIII) may have been due to oxidative attack in ring A as will be shown later in this section.

CHART.1



Lead tetraacetate has found satisfactory use in the the oxidation of the C_{11} oxygenated steroid hormones of the pregnane series to give intermediates suitable for the partial synthesis of corticosterone and 11-dehydrocorticosterone (35). This reagent was also used by Sainte-Marie (30) who oxidized pregnane-3α, 12β-diol-20-one (IX) and isolated from the reaction pregnane-3α, 12-diol-20-one 3-acetate (X), pregnane-3α, 12β, 21-triol-20-one-3, 21-diacetate (XI) and pregnane-3α, 12β, 21-triol-20-one-21 acetate (XII), plus unreacted starting material (IX).



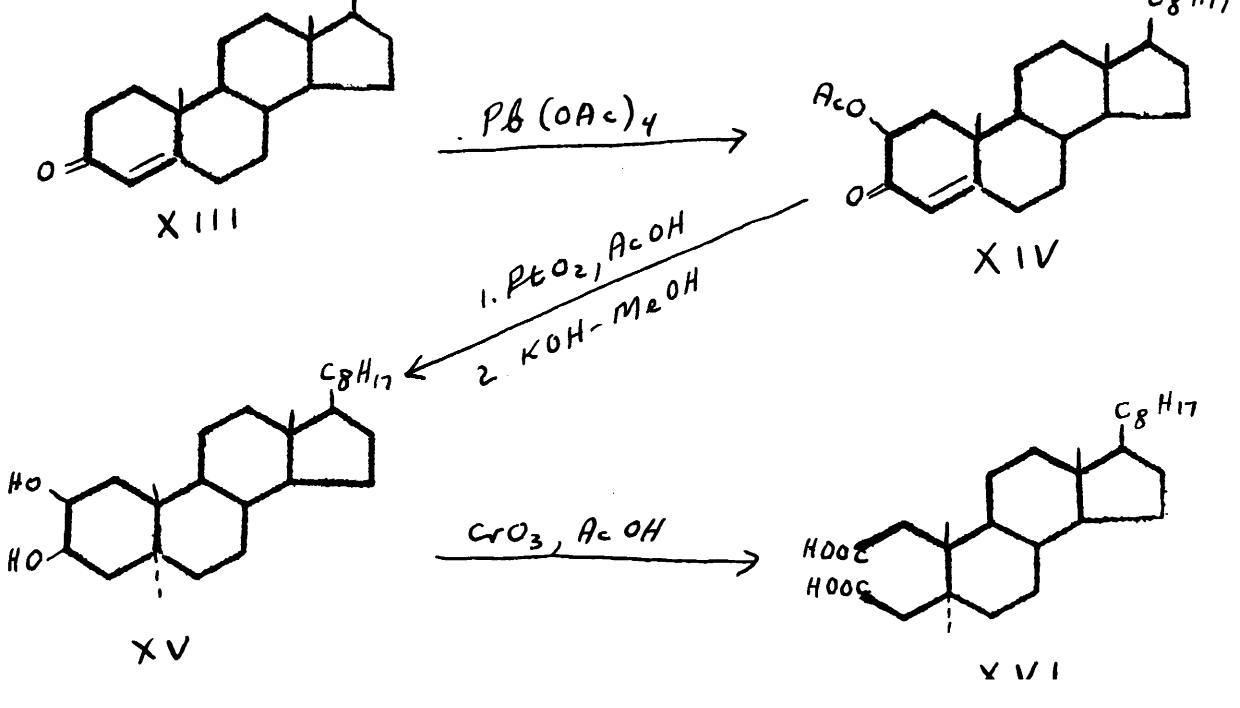
IX X XI XII

Recently, Giral (36) confirmed Reichstein's observations (34) by oxidizing pregnenolone with lead tetraacetate and isolating Δ^5- pregnene-3, 21-diol-20-one diacetate (II), showing that the C_3-hydroxyl group was acetylated during the process of acetoxylating the C_{21}-methyl group. This author also oxidized progesterone (VII) and pregnenolone benzoate. Progesterone did not yield any desoxycorticosterone acetate while pregnenolone benzoate gave a product which Giral formulated as Δ^5-pregnane-3β, 21-diol-20-one-3-benzoate-21-acetate.

In a subsequent publication, Mencera (37) repeated Giral's work on pregnenolone benzoate. He isolated a compound in 39% yield having physical characteristics quite different from those possessed by the product reported by Giral. This compound was formulated as the Δ^5-pregnene-3β , 21-diol-20-one-3-benzoate-21-acetate and shown to be identical with the product obtained by benzolation of Δ^5-pregnene-3β , 21-diol-20-one-21-acetate. Mencera did not propose a plausible structure for the product isolated by Giral.

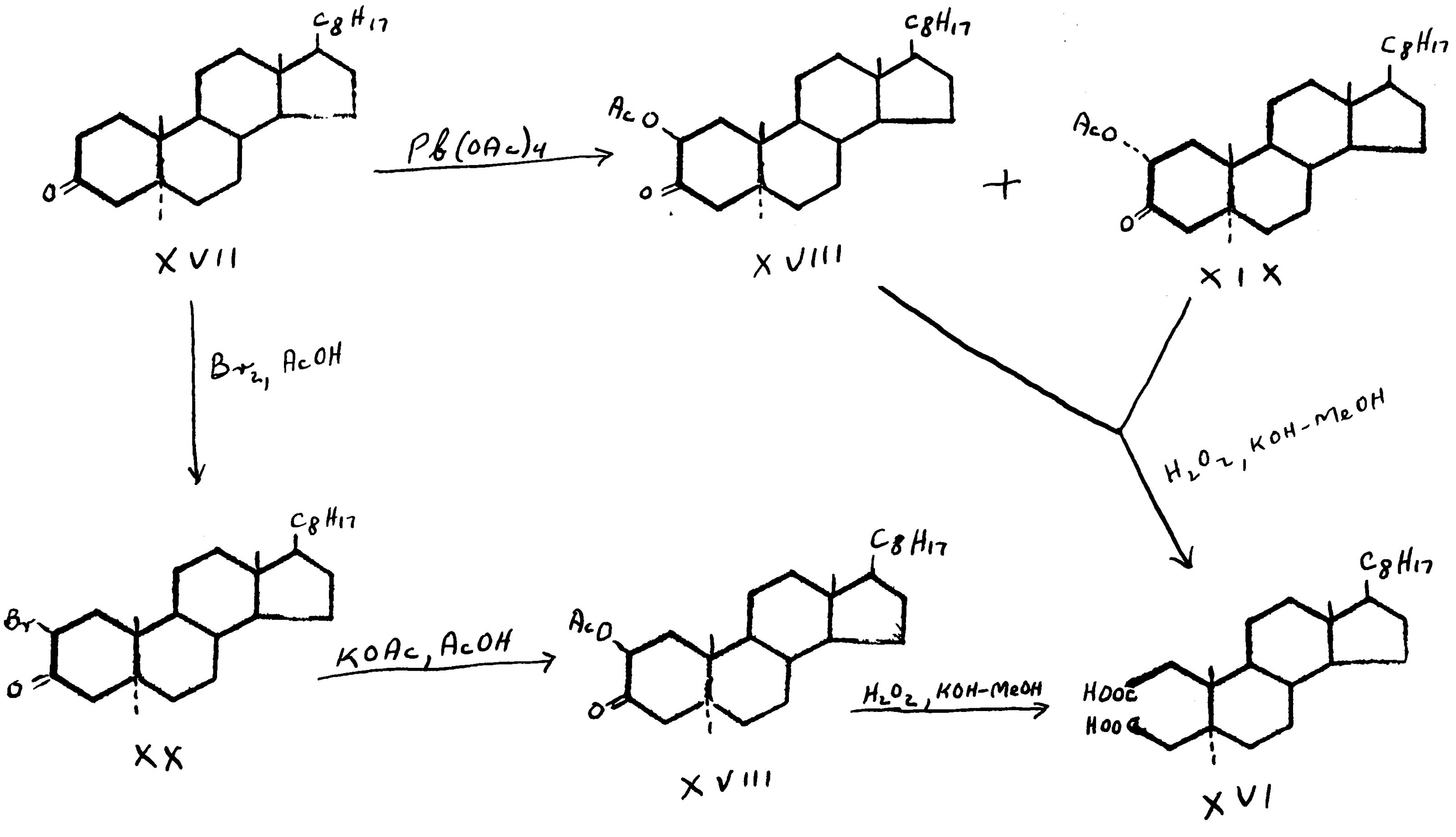
In 1944 Seebeck and Reichstein (38) oxidized cholestenone (XIII) with lead tetraacetate and reported the isolation of 2-acetoxycholestenone (XIV) plus unreacted starting material. To prove the structure of the acetoxy-ketone (XIV), these authors converted it to the 2, 3-dihydroxy-cholestane (XV) by reduction with platinum oxide in acetic acid followed by hydrolysis of the ester linkage. The diol (XV) was then oxidized with chromic acid to the well-known dibasic acid (XVI), thus furnishing good evidence for the structure of the 2-acetoxycholestenone (XIV).

CHAR. II



In the same year Wasson (29), oxidized cholestanone (XVII) with lead tetraacetate and was able to isolate two epimeric compounds which he formulated as 2-acetoxycholestanone (XVIII) and (XIX). Both epimers gave the well-known 2,3-diacid (XVI) on oxidation with alkaline hydrogen peroxide. One of the epimers (XVIII) was shown to be identical with 2-acetoxycholestanone (XVIII) prepared by the acetoxylation of 2-bromocholestanone (XX) which in turn was obtained by the bromination of cholestanone (XVII). The 2-acetoxycholestanone (XVIII) prepared in this manner also yielded 2,3-secocholestane-2,3-dioic acid (XVI) when oxidized with alkaline peroxide.

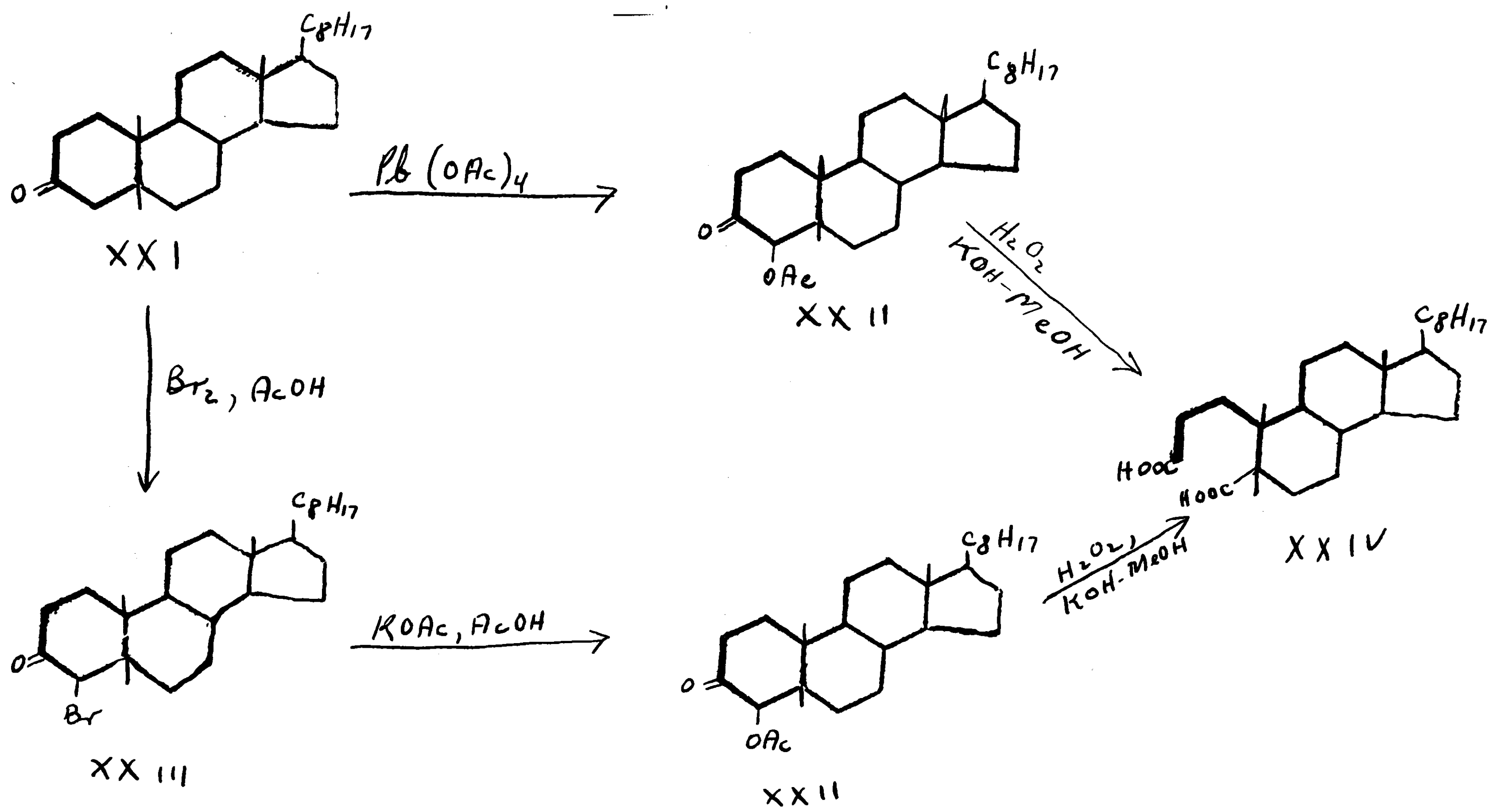
CHART III



In 1948, Prado (31) investigated the action of lead tetraacetate on coprostanone (XI). From the reaction products, on chromatographic separation, this investigator isolated a compound which was formulated as 4-acetoxy-coprostanone (XXII). The acetoxy-ketone (XXII) on oxidation with alkaline hydrogen peroxide gave a dibasic acid, identical with 3,4-**secoprostane 3,4-dioic** acid (XXIV). Since two C4 acetoxy epimers can theoretically exist and since only one of the six crystalline compounds eluted from the chromatogram was identified, it is quite possible that two epimers were actually formed in the lead tetraacetate oxidation of coprostanone (XXI).

Bromination of coprostanone gave 4-bromo-coprostanone (XXIII). Acetoxylation of the 4-bromide yielded a compound identified by Marker et. al. (39) as 4-acetoxy-coprostanone (XXII). This acetoxy-ketone (XXII) was identical with the 4-acetoxy-coprostanone (XXII) isolated by Prado from the lead tetraacetate oxidation of coprostanone (XXI), and also yielded 3,**4-secoprostane-3,4-dioic** acid (XXIV) on treatment with alkaline peroxide. The above evidence served to characterize the 4-acetoxy-coprostanone (XXII) prepared by Prado.

CHART IV



Shortly after the mode of action of lead tetraacetate on saturated and unsaturated 3-ketoneshad been elucidated, an investigation was begun to test the susceptibility of a saturated ketone in ring B to this type of oxidation. To this end Wasson (29) carried out a lead tetraacetate oxidation on 6-ketocholestanyl acetate (XXV). On chromatographic separation of the reaction products, this worker isolated unreacted starting material (XXV) and two compounds which he formulated as 7α-acetoxy-6-ketocholestanyl acetate (XXVI) and 7β-acetoxy-6-ketocholestanyl acetate (XXVII). Saponification of the lower melting more levorotatory epimer (XXVI), gave a product which was formulated as 3,7-dihydroxy-6-ketocholestane (XXVIII).

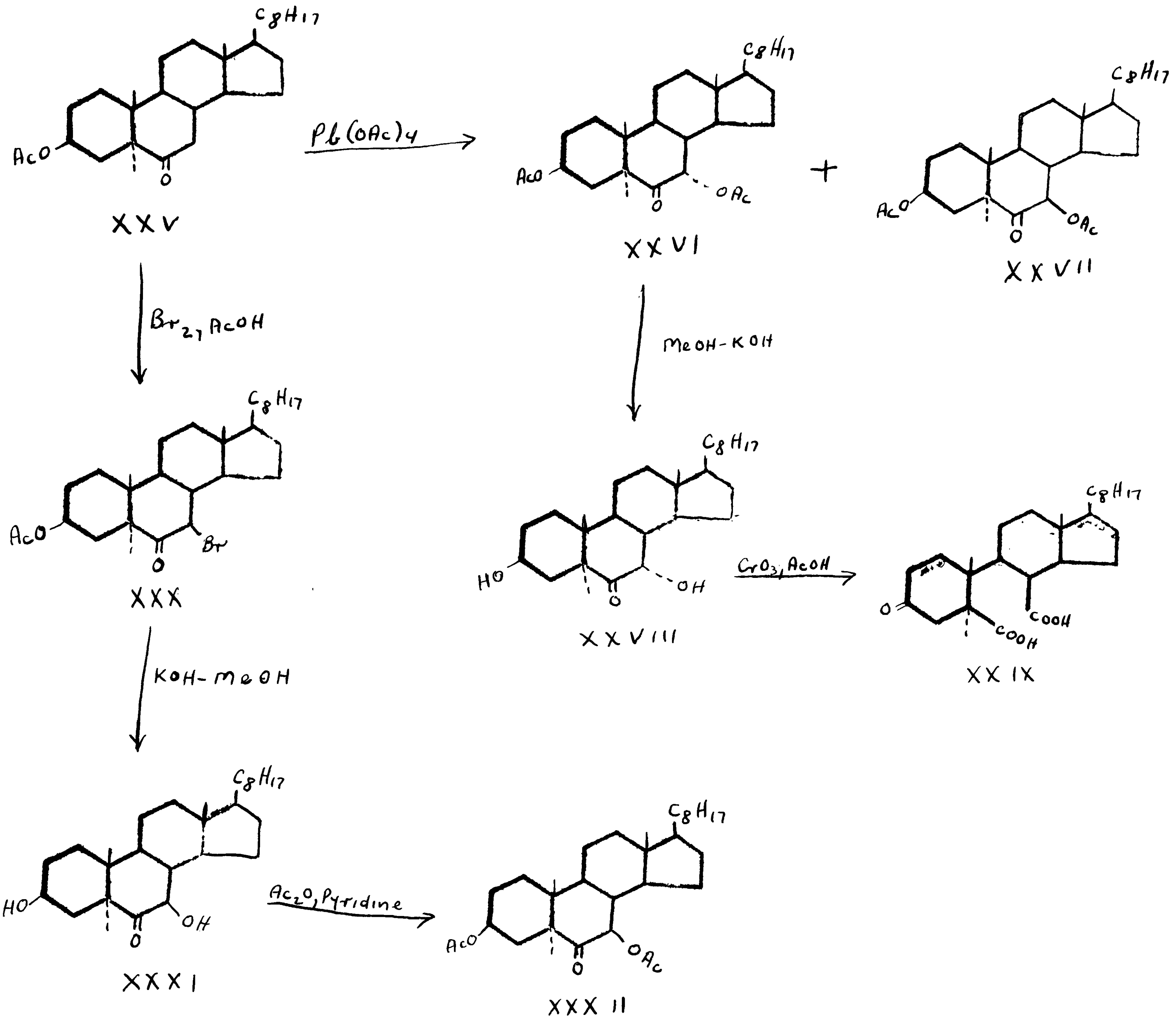
Chromic acid oxidation of this compound gave a dibasic acid which seemed to be identical with 6,7-secocholestane-3-one-

6,7-dioic acid (XXIX), first described by Windaus and Stein (40). Wasson then brominated 6-ketocholestanyl acetate (XXV) described by Heilbron (41) in 1937. Alkaline hydrolysis of the 7-bromide (XXX) gave a product which Heilbron formulated as 3,7-dihydroxy-6-ketocholestane (XXXI). This compound was not identical with the 3,7-dihydroxy-6-ketocholestane (XXVIII) obtained by the saponification of(XXVI), but it may have been its epimer. The ease with which the low melting epimer (XXVI) was saponified indicated that neither acetoxy group is at the C_5 position. Acetylation of the 3,7-dihydroxy-6-ketocholestane (XXXI), formulated by Heilbron, gave a product which was not identical with either of the two epimeric 3,7-diacetoxy-6-ketocholestanes (XXIX) and (XXX), formulated by Wasson.

Saponification of the higher melting more dextrorotatory compound, formulated by Wasson as 7β-acetoxy-6-ketocholestonyl acetate (XXVII), gave an oil which resisted crystallization and could not be separated in crystalline form by chromatography on alumina. This indicated that more than one compound was formed during the saponification, and that the compounds formed must have very similar polar properties.

Treatment of the 7-bromide (XXXI) with collidine did not give the expected Δ^7-cholestane-3-ol-6-one acetate. The products isolated were, unreacted starting material and collidine-hydrobromide.

CHART V



It was at this stage that the investigation of this problem was resumed in 1950. The results obtained by this author will be reported in this part of the thesis.

DISCUSSION.

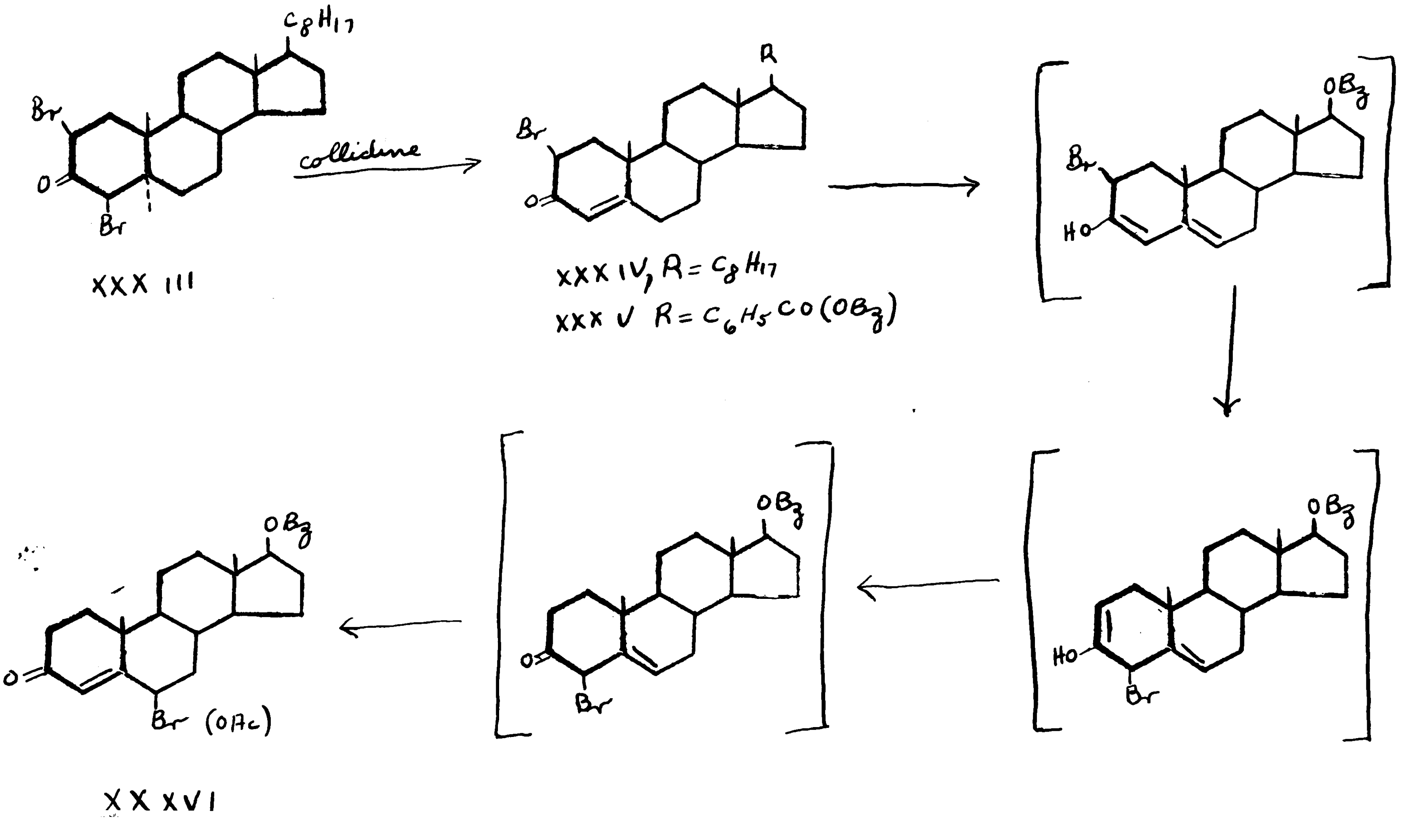
Our present knowledge of the action of lead tetraacetate on oxygen-containing steroids can be subdivided into three main groups; acetoxylation at the side chain in the pregnane and allopregnane series, acetoxylation adjacent to a 3-ketone in ring A in the cholestane and coprostane series and acetoxylation adjacent to an α, β-unsaturated ketone in ring A in the cholestane series.

An example of the first group is the oxidation of allopregnane-3β-ol-20-one-acetate (IV, Chart I) with lead tetraacetate (34). The products of the reaction are allopregnane-3β,21-diol-20-one- diacetate (V, 52%) and a small amount of allopregnane-3β,17α,21-triol-20-one triacetate (VI, 2%). Here, acetoxylation takes place mainly at the C_{21} position and substitution at the C_{17} position is only a side reaction. However, it is of interest to note that a tertiary hydrogen atom can be substituted by an acetoxy group in the five-membered ring C. Acetoxylation at the C_{17} position was not observed in the lead tetraacetate oxidation of either pregnane-3α,12β-diol-20-one (IX) or pregnenolone benzoate (30, 36).

Ring A saturated ketones in the cholestane and coprostane series exhibit a behaviour, on treatment with lead tetraacetate, analogous to that shown on treatment with bromine. Butenandt (42) observed that bromination of coprostanone (XXI, Chart IV) resulted in the substitution of a bromine atom at the C_4 position and that bromination of cholestanone (XVII, Chart III) fixed a bromine atom at the C_2 position. In an analogous manner, oxidation of cholestanone (XVII) and coprostanone (XXI) with lead tetraacetate resulted in the substitution of an acetoxy group in the C_2 and C_4 positions respectively. However, the analogy between bromination and acetoxylation is not fully realized because acetoxylation has been shown to produce epimers at the C_2 position in cholestanone (XVII) and this is probably also true for the C_4 position of coprostanone (XXI).

Lead tetraacetate oxidation of an α, β -unsaturated ketone in ring A (e.g. cholestenone, XIII) results in substitution of an acetoxy group in the C_2 position without the formation of two epimeric compounds (38). No analogy can be drawn here with bromination reactions because to our knowledge cholestenone on bromination does not give 2-bromocholestenone but rather 2,2-dibromocholestenone or 2,4-dibromocholestenone depending on whether potassium acetate is present or absent during the reaction. Therefore, since cholestenone behaves quite differently from cholestanone to the action of bromine, it is not surprising that in a parallel situation two epermeric acetoxy compounds are not formed at the C_2 position on oxidation with lead tetraacetate.

By analogy with the action of bromine on α , β -unsaturated ketones in ring A of the A:B trans-steroids, cholestenone might be expected to yield a mixture of 2-acetoxy-cholestenone and 6-acetoxy-cholestenone. Djerassi (43) prepared 2-bromo-cholestenone (XXXIV) by treatment of 2,4-dibromo-cholestanone (XXXIII) with collidine. Inhoffen and Zuehlsdorff (44) have shown that such a compound undergoes an allylic rearrangement in the presence of hydrogen bromide which results in the migration of the bromine atom from the 2- to the 6-position. These authors showed that 2-bromo-testosterone benzoate (XXXV) in the presence of hydrobromic acid at room temperature, yielded 6-bromo-testosterone benzoate (XXXVI). The mechanism postulated was as follows.



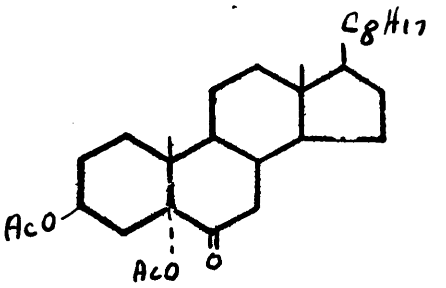
Seebeck and Reichstein (38) oxidized 4 g., (13 mM) of cholestenone with 8 g., (18 mM) of lead tetraacetate in 45 ml. of acetic acid and 5 ml. of acetic anhydride for 24 hours at 70°. Under these conditions it is quite possible that the concentration of lead tetraacetate was not large enough to yield two epimeric 2-acetoxy-cholestenones or ,6-diacetoxy-cholestenone (XXXVI), both of which are theoretically possible. Wasson (29) has shown that the optimum condition for the oxidation of steroids with lead tetraacetate is attained when the reaction is carried out at 70-75° with 1.66 moles of reagent per mole of steroid. Seebeck and Reichstein only used 1.4 moles of lead tetraacetate per mole of steroid. These authors claim that acetic anhydride is a catalyst in the oxidation, while Dimroth and Schweizer (32) found that acetic anhydride reacts with lead tetraacetate to give lead acetate and O-acetylglycolic anhydride.

$$Pb(OAc)_4 + Ac_2O \longrightarrow Pb(OAc)_2 + (CH_3COOCH_2CO)_2O$$

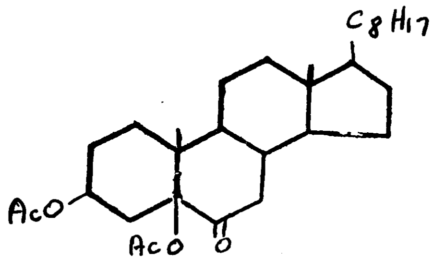
The above considerations may account for the 12% yield of 2-acetoxy-cholestenone obtained by Seebeck and Reichstein, as well as the non-appearance of the expected epimeric 2-acetoxy-cholestenone (XIV) and ,6-diacetoxy-cholestenone (XXXVI).

From the lead tetraacetate oxidation of 6-keto-cholestanylacetate (XXV), Wasson (29) isolated unreacted starting material plus two compounds both having the constitutional formula $C_{31}H_{50}O_5$. He designated these compounds as 7α-acetoxy-6-ketocholestanyl acetate (XXVI) m.p. 134-135°, $[\alpha]_D^{25}$ -45.2°($CHCl_3$) and 7β-acetoxy-6-ketocholestanyl acetate (XXVII), m.p. 195-196°, $[\alpha]_D^{25}$ -25.5°($CHCl_3$). Both compounds were non-reducing with ammoniacal silver diamine, negative to tetranitromethane and negative to the phosphomolybdic acid reducing test of Heard and Sobel (45). Both compounds were isolated by chromatography on alumina and occurred in the ratio of approximately 2:1, with the lower melting more levorotatory compound predominating. This fact eliminates the possibility that the oxidation of 6-keto-cholestanyl acetate (XXV) with lead tetraacetate proceeds in one direction to yield a very large amount of one compound with the formation of a second compound in small amounts being attributed to a possible side reaction, as was the case in the formation of allopregnane-3β, 17α,21-triol-20-one triacetate (VI) from allopregnane-3β-ol-20-one acetate (IV) with this reagent.

Substitution of the tertiary C_5 hydrogen atom with an acetoxy group was ruled out because a 3β, 5α-diacetoxy-6-keto-cholestane (XXXVII) having the constitutional formula $C_{31}H_{50}O_5$ had already been reported in the literature by Schenck (46). The 3β, 5α-diacetoxy-6-ketocholestane (XXXVII) has a m.p. 169-170°, $[\alpha]_D^{20}$ -15.4°($CHCl_3$) and therefore does not correspond to either of the two compounds (XXVI and XXVII) isolated by Wasson.(29). Acetoxylation at the C_5 position with inversion of configuration to give the unprepared hitherfore 3β, 5β-diacetoxy-6-ketocholestane (XXXVIII) is not very likely since the C_{10} methyl group would in all probability hinder the formation of such a compound.



XXXVII



XXXVIII

Having eliminated the possibility of acetoxylation at the C_5 position there remains only one theoretically possible centre at which substitution of an acetoxy group for a hydrogen atom can take place, and that is at the C_7 position. Therefore Wasson was justified in formulating the two compounds having the constitutional formula $C_{31}H_{50}O_5$ as C_7 epimers.

By an examination of the compounds known to be epimeric at the C_7 position, a tentative assignment of configuration can be given to the two compounds (XXVI) and (XXVII) isolated by Wasson. Plattner and Heusser (47) have recently characterized the two C_7 epimers of 7-hydroxycholesterol, first reported by Windaus et. al.(48) and Heilbron and co-workers (41). The epimer melting at 157°, $[\alpha]_D^{22}$ -88°($CHCl_3$), stanol, $[\alpha]_D^{22}$ +8.1°

($CHCl_3$) was characterized as 7α-hydroxycholesterol and the epimer melting at 178°, $[\alpha]_D^{22}$ +7.2°($CHCl_3$), stanol, $[\alpha]_D^{22}$ +5.3° ($CHCl_3$) was assigned the configuration of 7β-hydroxycholesterol. The lower melting more levorotatory epimer has a 7α- orientation and the higher melting more dextrorotatory epimer has a 7β- orientation. On this basis the two compounds isolated by Wasson having the constitutional formula $C_{31}H_{50}O_5$ were designated as 7α-acetoxy-6-ketocholestanyl acetate (XXVI) and 7β-acetoxy-6-ketocholestanyl acetate (XXVII). The only troublesome factor in the naming of these two compounds is that the 7β-acetoxy-ketone (XXVII) has a m.p. 195-196° which appears to be too high for a diacetate. The C_6 ketone should not contribute more than 5-10° to the rotation of the above-mentioned compounds.

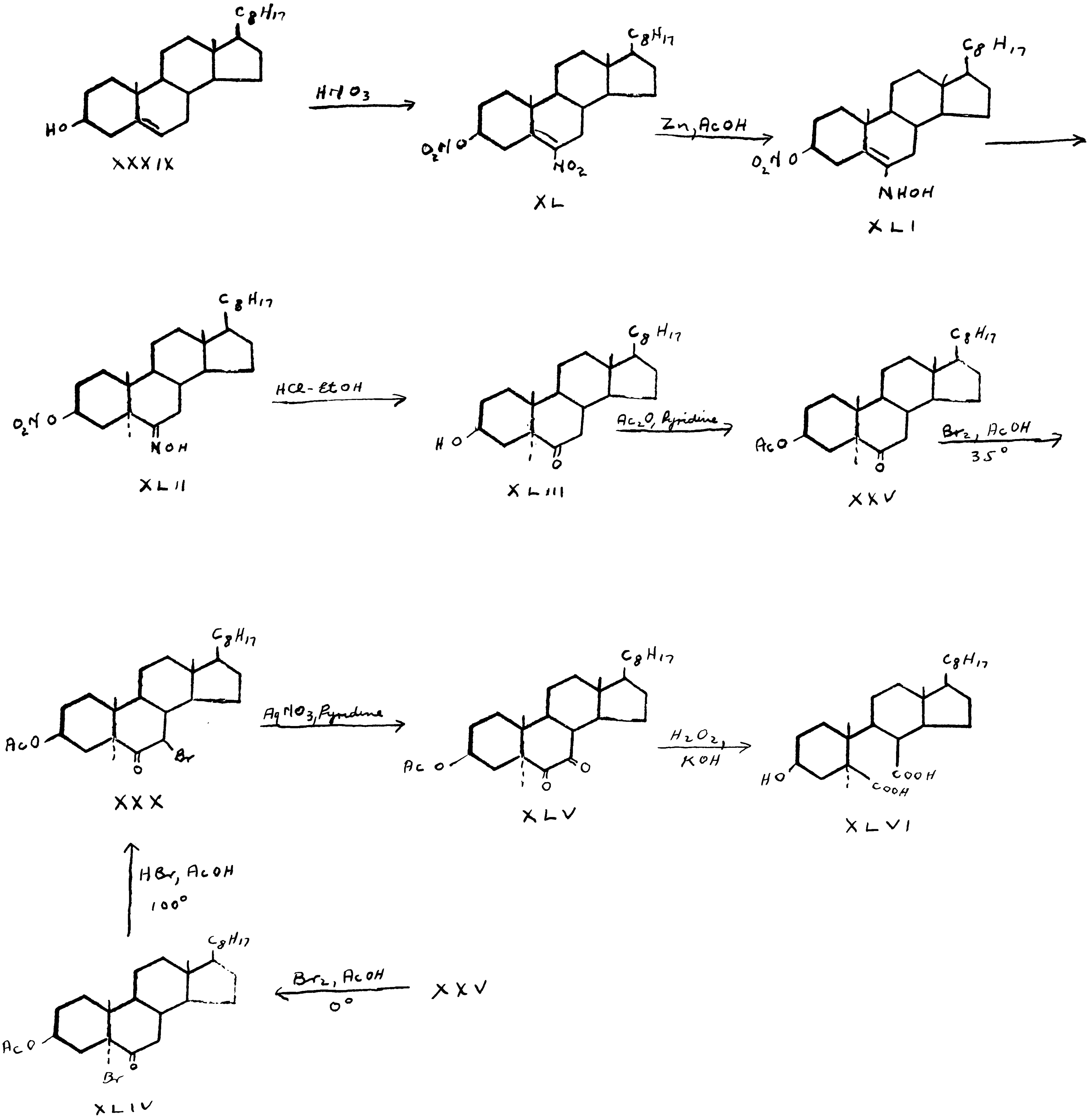
The key starting compouhds in this investigation, 6-ketocholestanyl acetate (XXV) and 7-bromo-6-ketocholestanyl acetate (XXX) were prepared as described by Heilbron et. al. (41). Cholesterol(XXXIX) was treated with fuming nitric acid to yield 6-nitrocholesteryl nitrate (XL). The nitration of cholesterol as described by Heilbron et. al. (41), yielded the desired product in 0-80% yield depending on the experimental conditions employed. It was found that consistently high yields could be obtained when the temperature, during the addition of the acid, was maintained at -10 to -15° and when the nitric acid used was made up exactly as prescribed by Heilbron, with the aid of a hydrometer.

The nitro ester (XL) was reduced by refluxing with zinc and acetic acid to give the corresponding imide (XLI), which rearranged to form an oxime (XLII). Hydrolysis of the oxime (XLII) gave 6-ketocholestanol (XLIII) in 80-90% yield, which on acetylation gave 6-ketocholestanyl acetate (XXV) in 90% yield. Bromination of the latter in acetic acid at 35° yielded 7-bromo-6-ketocholestanyl acetate (XXX) in approximately 50% yield.

Heilbron prepared 5-bromo-6-keto-cholestanyl acetate (XLIV) and showed that the bromine atom under the influence of hydrobromic acid at 100° could migrate to the C_7 position giving 7-bromo-6-ketocholestanyl acetate (XXX). In view of this, the bromination of 6-ketocholestanyl acetate was carried out with great care and only that material melting at 143-146°, $[\alpha]_D^{22}$ +41° ± 1(CHCl$_3$) was used in subsequent reactions. Proof of the structure of the 7-bromide (XXX) was afforded by oxidizing it at a high temperature with silver nitrate and pyridine to yield the corresponding α,β -diketone (XLV). The latter, on ring cleavage with alkaline peroxide yielded 6,7-seco-cholestanol-3β-6,7-dioic acid (XLVI), first prepared by Windaus and Stein (40).

Heilbron (41) stated that the bromine atom in 7-bromo-6-ketocholestanyl acetate (XXX) was trans to the hydrogen atom at C_8. This would make the 7-bromine atom α-oriented since

CHART VI


C8H17
HO
XXXIX
HNO3
O2NO
NO2
XL
Zn, AcOH
NHOH
XLI
NOH
XLII
HCl-EtOH
HO
O
XLIII
Ac2O, Pyridine
AcO
XXV
Br2, AcOH
35°
Br
XXX
AgNO3, Pyridine
XLV
H2O2, KOH
COOH
COOH
XLVI
HBr, AcOH
100°
Br2, AcOH
0°
XXV
XLIV

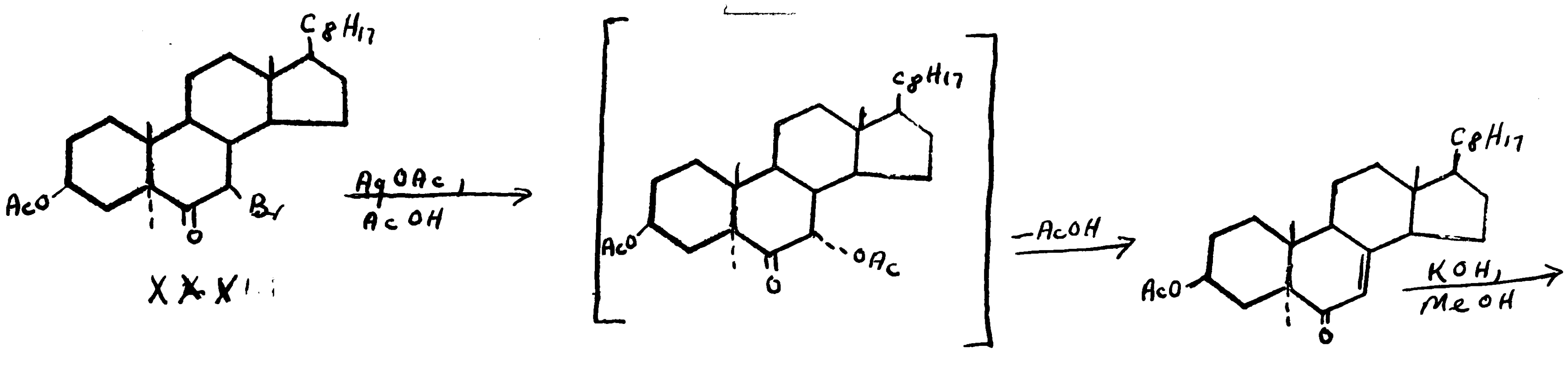
the C_8 hydrogen atom is β -oriented by convention. However, there is ample evidence that the former is not the case. Wasson (29) reacted the 7-bromide (XXX) with collidine and did not obtain the expected Δ^7-6-ketocholestanyl acetate (XLVII) that would have been produced by a trans dehydrohalogenation. Heilbron (41) found the C_7 bromine atom in 7-bromo-6-ketocholestanyl acetate (XXX) to be very unreactive. Under forcing conditions with silver nitrate and pyridine the above-mentioned author obtained a compound which he characterized as a α ,β -diketone (XLV). This compound was cleaved by Shoppee (49) with alkaline peroxide to give the known 6, 7-dicarboxylic acid, thus confirming its assigned structure.

Mattox and Kendall (50) have devised a method for the elimination of hydrogen bromide from 4-bromo-3-ketoallosteroids to give the corresponding α , β -unsaturated ketosteroids in excellent yields. When this method was applied to 7-bromo-6-ketocholestanyl acetate (XXX) the product obtained was unreacted starting material.

All the above-mentioned evidence adequately proves that the C_7 bromine atom in 7-bromo-6-ketocholestanyl acetate (XXX) is not trans with respect to the C_8 hydrogen atom and therefore must be considered as β -oriented.

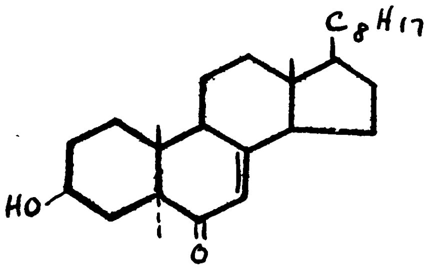
Lieberman, Hariton and Fukushima (51) treated sodium cholestanol-3α sulfate with silver acetate under reflux and isolated from the reaction mixture a small amount of neocholestene. These authors stated that displacement reactions with silver acetate should lead to inversion of configuration. In view of this, 7-bromo-6-ketocholestanyl acetate (XXX) was refluxed with silver acetate in acetic acid in the hope of obtaining a mixture of the two possible acetoxy derivatives epimeric at the C_7 position. This, however, was not realized because the neutral compound obtained had a constitutional formula $C_{29}H_{46}O_3$, was Beilstein negative, tetranitromethane positive and had a m.p. 169-170°. These facts indicated that the product isolated might be Δ^7-6-ketocholestenyl acetate (XLVII).

Saponification of this compound yielded a product having a constitutional formula $C_{27}H_{44}O_2$ (XLVIII), indicating that the compound with the formula $C_{29}H_{46}O_3$ had a saponifiable acetate group. A plausible mechanism that could explain the formation of a compound such as Δ^7-6-ketocholestenol acetate (XLVII) from 7-bromo-6-ketocholestanyl acetate (XXX) is as follows. Silver acetate displaces the C_7 bromine atom with an acetoxy group at the same time causing inversion of configuration. This is in agreement with the views held by Lieberman et. al. (51). Splitting out of acetic acid by trans deacetylation then gives the proposed Δ^7-6-ketocholestenyl acetate (XLVII).

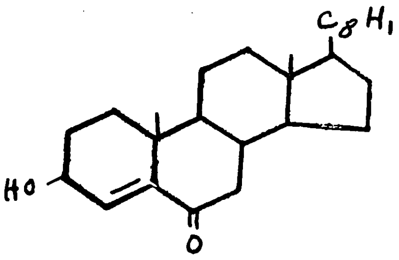


XXXVII

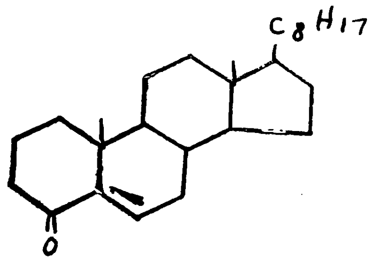
XLVII



XLVIII



XLIX

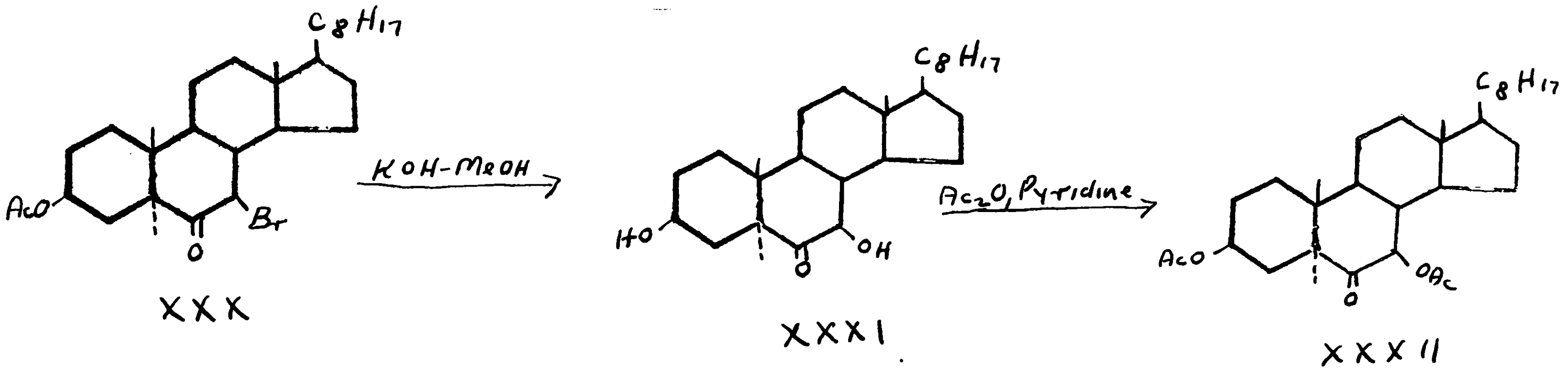


L

The only disturbing factor in the mechanism outlined above is that the compound $C_{29}H_{46}O_3$ postulated as XLVII exhibited a low molecular extinction coefficient at its absorption maximum in the ultraviolet region λ_{max}^{alc} 235 mμ , ϵ = 2,660. Such values are exhibited by α , β -unsaturated ketones, conjugated between ring A and B. Thus Δ^4-cholestene-3β -ol-6-one (XLIX) gives a value of λ_{max}^{alc} 239 mμ, ϵ = 6310 and Δ^5-androstene-17β -ol-4-one (L) shows a value of λ_{max}^{alc} 240 mμ, =3160 (52). The molar extinction coefficient at maximum absorption for an α ,β -unsaturated ketone in ring

B is about 12,000 as exhibited by 7-ketocholesterylacetate, λ_{max}^{alc} 234 mμ, ϵ =12,050 (53). Until more complete data on the compound $C_{29}H_{46}O_3$ is available, the low value of the molar extinction coefficient at maximum absorption cannot be explained.

Saponification of 7-bromo-6-ketocholestanyl acetate (XXX) yielded a compound formulated by Heilbron et.al. (41) as 3,7-dihydroxy-6-ketocholestane (XXXI) m.p. 179°, $[\alpha]_D^{22}$ + +31.4°($CHCl_3$). In our hands, this compound, prepared as described by Heilbron et.al. had a m.p. of 168-174°, $[\alpha]_D^{22}$ + +28.7°($CHCl_3$). The yield of the dihydroxy-ketone (XXXI) was usually 30-50%. The saponification of the 7-bromide (XXX) was carried out by refluxing with methanolic potassium hydroxide for two hours. This was thought to be too drastic a treatment and the saponification was subsequently carried out at room temperature for 48 hours. The latter treatment yielded a compound m.p. 181-182°, $[\alpha]_D^{22}$ + 31.9°($CHCl_3$), which was assumed to be identical with 3,7-dihydroxy-6-ketocholestane (XXXI) reported by Heilbron. The yield realized in this step was very low, the main product being an amber-coloured oil which resisted crystallization. The compounds obtained by these different methods of saponification showed no depression in the admixture melting point. The material melting at 168-174° gave a slightly positive test with ammoniacal silver diamine.



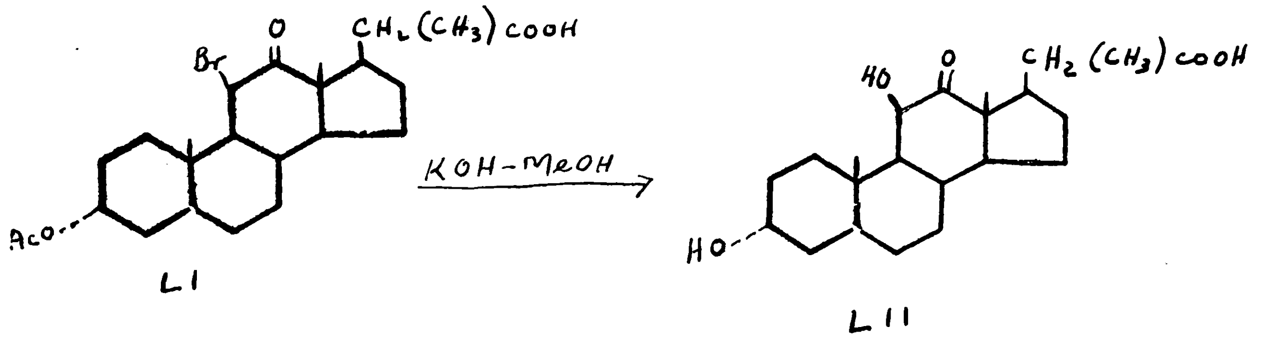
Our attention was centered on 3,7-dihydroxy-6-ketocholestane (XXXI) because on acetylation it should have yielded a compound identical with one of the two epimers of 7-acetoxy-6-ketocholestanyl acetate (XXVI) and (XXVII). Wasson (29), however, found that acetylation of the diolone (XXXI) produced two compounds crystallizing from methanol and melting at 129-133° and 127-130°. Neither of these compounds was identical to the two compounds designated as C_7 epimers of 7-acetoxy-6-ketocholestanyl acetate that were isolated by chromatography from the product resulting from the lead tetraacetate oxidation of 6-ketocholestanyl acetate (XXV).

This author's experience with the acetylation of 3,7-dihydroxy-6-ketocholestane (XXXI), formulated by Heilbron et.al., confirmed the results reported by Wasson (29). The product obtained had a m.p.123-127° which could be raised to 127-132° after numerous recrystallizations from methanol. These results indicate that the 3,7-dihydroxy-6-ketocholestane reported by Heilbron et.al. may well be a mixture of two compounds. To test this supposition, the dihydroxy-ketone (XXXI) was acetylated and the product obtained was adsorbed on a column of activated alumina. On eluting the alumina, a small amount of a crystalline compound m.p. 157-158° was obtained. This compound gave a negative test with ammoniacal silver diamine but has not as yet been further identified.

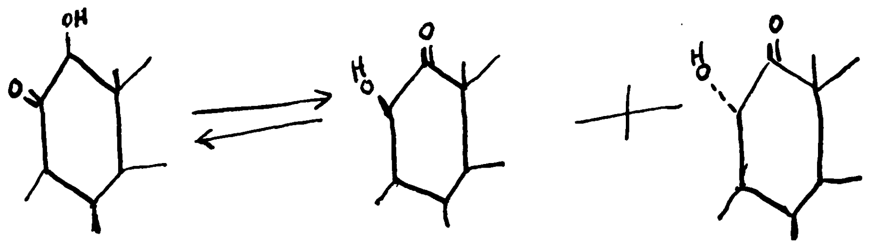
Acetylation of 3,7-dihydroxy-6-keto-cholestane (XXXI) at 100° gave a compound melting at 67-69°. This compound did not reduce ammoniacal silver diamine but no further data on it is available at present.

All the above-mentioned evidence seems to indicate that the product formed on saponification of 7-bromo-6-keto-cholestanyl acetate (XXX) with methanolic potassium hydroxide is not a single compound, as reported by Heilbron (41). The saponification of the 7-bromide (XXX) can cause inversion of configuration at the C_7 position, in which case two epimeric compounds should be produced. This has not yet been fully substantiated by this author.

There is, however, good evidence in the literature to substantiate such a postulate. Reigel and Moffett (54) saponified 3-acetoxy-11-bromo-12-ketobisnorcholanic acid (LI) and were able to isolate from the reaction two compounds that were designated as the C_{11} hydroxy epimers of 3,11-dihydroxy-12-ketobisnorcholanic acid (LII).



After the bromine atom in the 7-bromide (XXX) has been displaced by a hydroxyl group, a ketol results which complicates the picture still further. In addition to having inversion of configuration, we can also expect an exchange between the hydroxy group and the carbonyl group. This has been postulated by Gallagher and Long (55) for an analogous case in ring C in the cholic acid series. These authors postulated an equilibrium between an 11-keto-12-hydroxy acid and an 11-hydroxy-12-keto acid plus the corresponding C_{11} hydroxy epimer, as follows.



If an analogous situation exists in ring B in the cholestane series, then saponification of 7-bromo-6-ketocholestanyl acetate (XXX) could yield four compounds epimeric at the C_6 and C_7 positions. These possibilities may explain why the product of acetylation of the compound formulated by Heilbron as 3,7-dihydroxy-6-ketocholestane (XXXI) cannot be identified with either of the products from the lead tetraacetate oxidation of 6-ketocholestanyl acetate (XXV).

Chromatographic separation of the products from the lead tetraacetate oxidation of 6-ketocholestanyl acetate (XXV), as reported by Wasson (20), yielded two compounds melting at 134-135° (XXVI) and 195-196° (XXVII). Many attempts were made to obtain these two compounds by the procedure described by Wasson, but invariably only the high melting compound (m.p. 196-196°) was obtained. As a result, an investigation was begun to determine the factors responsible for the non-appearance of the low melting compound.

The lead tetraacetate oxidation of 6-ketocholestanyl acetate (XXV) was conducted at approximately 70° in pure acetic acid, using 1.66 moles of reagent per mole of steroid. The rate of reaction was followed by iodometric titration of the unreacted lead tetraacetate, withdrawn periodically from the reaction flask. When the rate of utilization of lead tetraacetate was equal to its rate of decomposition at 70°

(when 1.35 moles of reagent per mole of steroid had been used), the reaction was stopped, the product extracted with ether and concentrated. The resulting amber-coloured oil was dissolved in benzene and petroleum ether added to induce turbidity. The mixture was adsorbed on activated alumina and eluted as described in Table I. This table represents the chromatogram reported by Wasson (29).

TABLE I.

Separation of Products from Lead Tetraacetate Oxidation.

Fraction		Ratio	Weight eluted mg.	Nature on Crystallization from Methanol
1	Petroleum ether		7.7	crystalline
2-4	Petroleum ether-benzene	20:1	755.3	crystalline
5-14	Petroleum ether-benzene	20:1	539.1	crystalline
15-17	Petroleum ether-benzene	10:1	106.8	crystalline
18-24	Petroleum ether-benzene	5:1	354.8	crystalline
25-28	Petroleum ether-benzene	2:1	298.8	crystalline
29-33	Petroleum ether-benzene	1:1	279.0	oily
34-39	Benzene		376.6	crystalline
40-41	Benzene-ether	20:1	59.6	crystalline
42-44	Benzene-ether	4:1	296.0	crystalline
45-46	Benzene-ether	2:1	72.2	oily
47	Benzene -ether	1:1	20.2	oily
48-49	Ether		19.7	oily
50-51	Chloroform		5.7	oily

Fractions 1-4 contained unreacted starting material m.p.127-128° (763 mg.).

The crystalline compounds eluted in fractions 5-28 (1.58 g.), proved to be the low melting compound, m.p.134-135°, designated by Wasson as 7α-acetoxy-6-ketocholestanyl acetate XXVI).

The product eluted in fractions 29-33 (279 mg.) failed to crystallize and was discarded.

Fractions 34-44 (732 mg.) yielded a compound melting at 195-196°, which was designated as 7β-acetoxy-6-ketocholestane (XXVII).

In this author's experience, such a clean cut separation of products from the chromatogram was not realized. In a number of lead tetraacetate oxidations of 6-ketocholestanyl acetate (XXV), oils were eluted in the same region where Wasson obtained the compound with m.p. 134-135° (XXVI).

At first these oils were combined and re-chromatographed, but no crystalline material was eluted. In another oxidation, these eluted oils were saponified and the products obtained were fractionally crystallized. Such a procedure yielded two distinct compounds melting at 183-185° and 202-203°. The first compound did not depress the admixture melting point of 3 ,7-dihydroxy-6-ketocholestane (XXX) m.p.180-182°) formulated by Heilbron. The second compound thus obtained did not depress the admixture melting point of the saponified derivative (m.p.202-203) from the compound m.p. 134-135°, obtained by Wasson and designated by him as 7α-hydroxy-6-ketocholestanyl acetate (XXVIII). These results indicate that alumina is not the best medium to use in the separation of compounds containing three oxygen atoms, as was usually the case in the compounds encountered in this investigation. The highly oxygenated compounds are too strongly adsorbed and the alumina becomes alkaline during the course of eluting, causing hydrolysis of the compounds adsorbed on it. This would explain the appearance of the compound with a m.p. 202-203°.

The non-appearance of the compound with m.p.183-185 in Wasson's chromatography scheme (Table I), is probably due to the fact that the oily fractions 29-33 (279 mg.) were not examined. It is highly probable that this compound is in an oily fraction since it was obtained by saponification of the combined oily fractions appearing in the corresponding region of the chromatogram. It was further observed that the oils obtained from chromatography (Table V.)in the region just below the compound with m.p. 195-196 (XXVII) were very reducing and an investigation of these will be made shortly.

All the work reported on the compound with m.p. 134-135° was carried out with material supplied by Dr. Wasson. As a result little data was obtained concerning this compound and the available data on it is incomplete owing to the initial inexperience of this author in handling small amounts of steroid material.

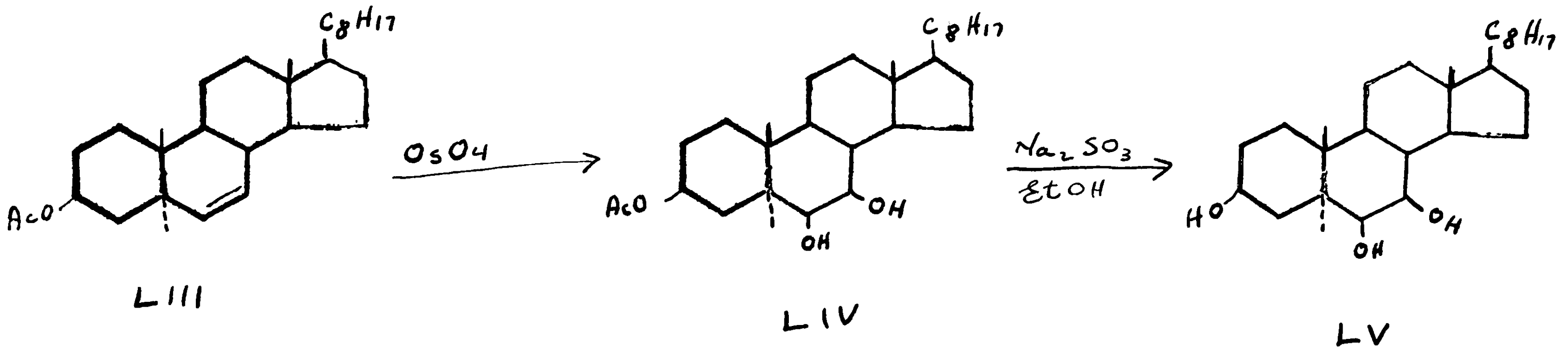
Room temperature and atmospheric pressure hydrogenation with platinum oxide in acetic acid, of the compound with m.p. 134-135°, designated by Wasson as 7α-acetoxy-6-ketocholestanyl acetate (XXVI), gave a compound which on saponification and crystallization from ethyl acetate yielded needles melting at 190-192°. This compound was not further itendified owing to the lack of sufficient starting material.

Hydrogenation of the compound designated as 7β-acetoxy-6-ketocholestanyl acetate (XXVII) under the above-mentioned conditions yielded unreacted starting material. This compound formed an oxime with difficulty and as a result, hydrogenation was again carried out at four atmospheres pressure, but again only unreacted starting material was isolated. No explanation can be offered for these results.

Acetylation of 7β-acetoxy-6-ketocholestanyl acetate (XXVII) yielded unreacted starting material. This disproved the presence of a free hydroxy group as was at first suspected because of the high melting point (195-196°) exhibited by this compound.

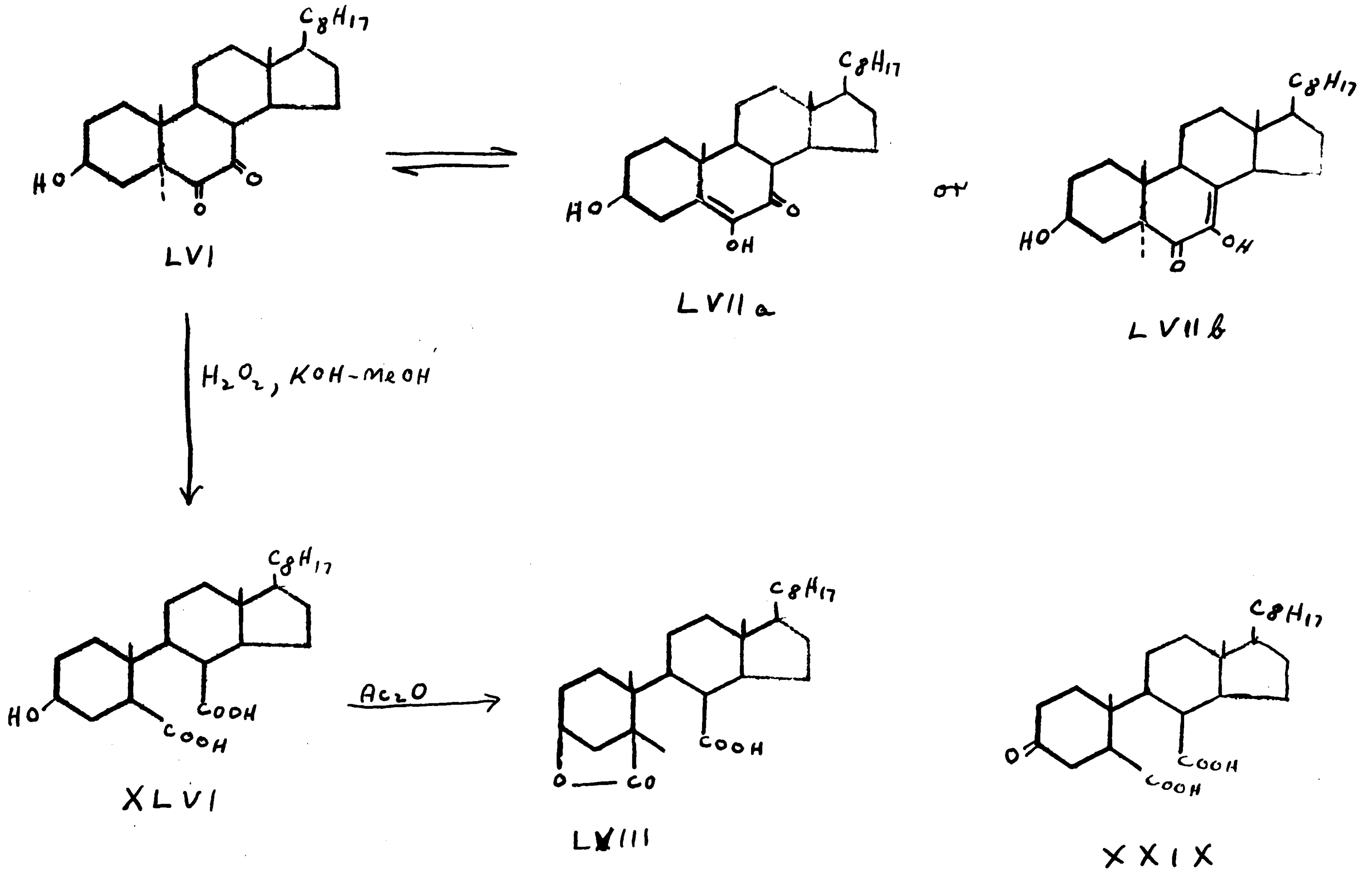
The aim of the above-described investigation was to prepare a 3,6,7-triol from the diacetoxy-ketones (XXVI) and (XXVII), in the hope that one of the triols formed would be identical with a 3,6,7-triol prepared by Wintersteiner and Moore (53).

These authors treated Δ^6-cholestenyl-3-acetate (LIII) with osmium tetroxide and isolated a product (LIV) which on saponification was characterized as cholestane-3β, 6β, 7β-triol (LV).



A triol formed from the compound formulated 7α-acetoxy-6-ketocholestanyl acetate (XXVI) was not expected to be identical with cholestane-3β, 6β, 7β-triol (LV). However, a triol formed from 7- -acetoxy-6-ketocholestanyl acetate (XXVII) should be identical with cholestane-3β, 6β, 7β-triol (LV). Efforts to establish this identity were frustrated by the (22b) failure of the 7β-acetoxy (XXVII) epimer to hydrogenate with platinum oxide. It should, however, be noted that the compound isolated from the hydrogenation of 7α-acetoxy-6-ketocholestanyl acetate (XXVI) is not identical with cholestane-3β, 6β, 7β-triol (LV) and therefore could be cholestane-3β, 6β, 7α-triol.

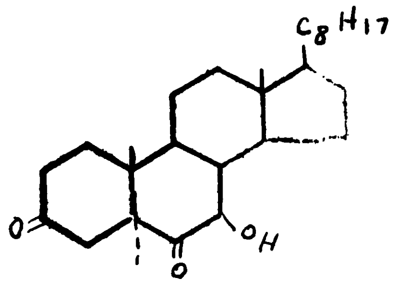
It is a well-known fact that a ketol occupying the 2,3-position in ring A can undergo oxidative cleavage with alkaline peroxide to yield the corresponding 2,3-dicarboxylic acid (56). The same applies to α, β -diketones in ring A (57) and in ring B (41) in the cholestane series. Heilbron et.al. (41) reported the preparation of 3β -hydroxy cholestane-6, 7-dione (LVI). The dione was characterized as a mono-enol (LVIIa and LVIIb) because it contained only one active hydrogen atom. Oxidation of the 3β -hydroxy-diketone (LVI) with alkaline peroxide was reported by Shoppee (49), to give β,7-seco - [illegible]l-cholestanol-3β-6,7-dioic acid (XLVI), a compound already identified by Windaus and Stein (40). The dibasic acid (XLVI) was further characterized by lactonizing it to the known lactonic acid (LVIII).



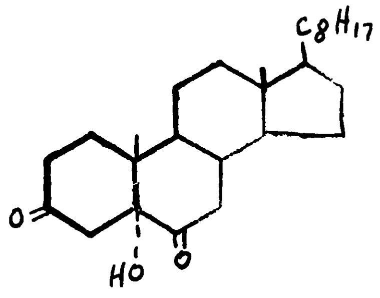
All the compounds encountered in this investigation that were suspected of having substituents at the C_6 and C_7 positions and that could be converted to a ketol or a diketone were oxidized with alkaline peroxide in the hope of obtaining β,7-secocholestanol-3β-6,7-dioic acid (XLVI). Another alternative that was employed was to attempt to oxidize the hydrolyzed derivatives of all compounds suspected of having a potential ketol group at the C_6 and C_7 positions to the 6,7-secholestane-one-3-6,7-dioicacid (XXIX), first described by Windaus and Stein (40). We thus had two known reference compounds to which any compound oxygenated at C_6 and C_7 encountered in this investigation could be converted. Such a conversion would automatically characterize the unknown compound as being a 6,7-disubstituted ketol derivative.

At first glance it seemed that a partial identification of the two compounds (XXVI) and (XXVII), isolated by Wasson from the lead tetraacetate oxidation of 6-ketocholestanyl acetate (XXV), could be afforded by cleaving them with alkaline peroxide to the known hydroxy-diacid (XLVI). This, however, was not fully realized because the above-mentioned compounds resisted cleavage with alkaline peroxide. Oxidation of the compound formulated by Wasson (29) as 7α-hydroxy-6-ketocholestanol ~~acetate~~ (XXVIII)with alkaline peroxide, yielded neutral unreacted starting material and a small amount of acidic material. The acid fraction failed to crystallize. Peroxide cleavage of the above compound could not be further investigated because of its scarcity. Chromic acid oxidation of (XXVI) has been reported by Wasson to give a compound which seems to be identical with the keto-diacid (XXIX) described by Windaus and Stein (40).

The higher melting more dextrorotatory compound (XXVII) resulting from the lead tetraacetate oxidation of 6-ketocholestanyl acetate (XXV) was saponified to yield an oily material. This oil was highly reducing when tested with ammoniacal silver diamine. This fact indicated the presence of a C_6-C_7 ketol group and the oil was therefore oxidized with chromic acid at 9°. The product obtained was neutral, had a m.p.121-122° and a constitutional formula $C_{27}H_{44}O_3$. This compound gave a positive Zimmerman test, and reduced ammoniacal silver diamine. It did not give an olive-green colour when treated with alcoholic ferric chloride and exhibited an absorption maximum in the ultraviolet at λ_{max}^{alc} 290 mμ, $\epsilon = 41$. The absorption characteristics usually exhibited by an α,β-diketone (41) is λ_{max}^{alc} 275 mμ, $\epsilon = 10.000$. These facts led us to believe that the compound isolated having the constitutional formula $C_{27}H_{44}O_3$ was 7-hydroxycholestane-3,6-dione (LIX). Further evidence for this structure will be obtained by forming the oxime derivative as well as by acetylation followed by hydrolysis. The above-described data can also fit 5α-hydroxycholestane-3,6-dione (LX). Fortunately, such a compound has been prepared by Marker and Rohrmann (58) and melts at 248-251°.



LIX



LX

Chromic acid oxidation of the compound described by Heilbron et.al. (41) as 3,7-dihydroxy-6-ketocholestane (XXXI) yielded a large acid fraction and a small neutral residue. Both fractions, however, failed to crystallize. The predominance of an acidic material indicated that cleavage of the C_6-C_7 bond had taken place.

Alkaline peroxide cleavage of 3,7-dihydroxy-6-ketocholestane (XXXI) formulated by Heilbron et.al.,gave a large acidic fraction with a negligible amount of neutral material. The acidic fraction on azeotropic distillation with dioxone and benzene still failed to crystallize. Gentle heating of the acidic fraction with acetic anhydride yielded a neutral product that was probably the 3-lactonic acid (LVIII), but the product could not be crystallized.

The 3-hydroxy-6,7-diacid (XLVI) and the 3-lactonic acid (LVIII) derived from it are both difficult to crystallize without the aid of a seed from an authentic sample (53). When authentic samples of the above-described compounds are prepared, crystallization of the oily products should give the desired compounds.

Thus, evidence has been presented to support the postulate, originally made by Wasson (41), that acetoxylation of 6-ketocholestanyl acetate (XXV), as a result of lead tetraacetate oxidation, occurs at the C_7 position. The spatial configuration of the designated 7α- and 7β-acetoxy-6-ketocholestanyl acetate (XXVI and XXCII) has not been fully elucidated but indirect evidence as to the correctness of the assigned structures has been presented. An investigation into this problem is being pursued further.

EXPERIMENTAL DETAIL

All melting points (m.p.) were recorded with the Fisher-Johns apparatus and were not corrected.

The acetic acid used was obtained in pure form by distillation over CrO_3 and the fraction with b.p.114-117° was used.

The optical rotations reported in this section were determined using chloroform solutions and a Goerz polarimeter (ℓ = 1).

Ultraviolet spectra were measured with a Beckman spectrophotometer (Model DU) using 95% ethanol solutions.

Chromatography was carried out on Alcoa alumina (Aluminum Company of Canada) prepared and activated by the method described by Shoppee (59). All solvents used were purified and dried over sodium sulfate.

1. 6-Nitrocholesteryl nitrate (XL) from Cholesterol(XXXIX). To a vigorously stirred slurry of cholesterol (50 g) in 200 ml. of acetic acid, at room temperature, was added 80 drops of a mixture of concentrated nitric acid (200 ml., d = 1.50) and fuming nitric acid (125 ml., d = 1.52).* The cholesterol slurry was then immersed in an ice:salt bath and the remaining nitric

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* The nitric acid was made up to the required strength with the aid of a hydrometer, by adding red fuming nitric acid (d = 1.61) to concentrated nitric acid (d = 1.50).

acid mixture added dropwise. When the temperature of the reaction mixture reached -10 to -12° the rate of addition of acid was increased. At the end of 1½ hours a precipitate settled out. Stirring was discontinued and the precipitate was quickly collected on a coarse sintered glass funnel with the aid of vacuum. The product thus obtained was air dried overnight and crystallized from acetic acid, from which 6-nitrocholesteryl nitrate (XL) separated in needles melting at 128-129°. The yield of pure product was usually 29-45 g. (48-72%).

2. 6-ketocholestanol (XLIII) from 6-nitrocholesteryl nitrate (XL). 6-nitrocholesteryl nitrate (41 g.) was dissolved in 600 ml. of acetic acid and to this solution were added 75 g. of zinc dust and 115 ml. of water. The mixture was allowed to stand at room temperature for one hour until the initial vigorous reaction had subsided. It was then heated on the steam-bath for two hours and under reflux for an additional ten hours. The solution was then decanted and the residual zinc washed with two 50 ml.-portions of acetic acid. After cooling the acetic acid solution, the oxime (XLII) corresponding to 6-ketocholestanol (XLIII) precipitated. This product was not purified but was converted directly to 6-ketocholestanol.

The isolated nitro ester (XLII) was hydrolyzed by heating under reflux with a mixture of hydrochloric acid (170 ml.)

and alcohol (600 ml.) for $1\frac{1}{2}$ hours. On cooling, the product precipitated and was filtered. From alcohol, 6-ketocholestanol (XLIII) separated as needles m.p.142-143°, $[\alpha]_D^{22}$ $-5.2 \pm 1°$($CHCl_3$). Concentration of the mother-liquors yielded an additional amount of product. The over-all yield of pure 6-ketocholestanol was 30-31 g. (88-91%).

3. 6-Ketocholestanyl acetate (XXV) from 6-ketocholestanol (XLIII). To a solution of 6-ketocholestanol (30 g.) in 60 ml. of pyridine was added 30 ml. of acetic anhydride and the solution heated on the steam-bath for one hour. Water was added, the solution cooled and the precipitated material collected by filtration. Crystallization gave 25 g. of 6-ketocholestanyl acetate m.p.128-129° $[\alpha]_D^{22}$ $-15.4 \pm 1°$($CHCl_3$). Concentration of the mother-liquors furnished an additional 4 g. of product. The over-all yield of pure product was 89%.

4. 7-Bromo-6-ketocholestanyl acetate (XXX) from 6-Ketocholestanyl acetate (XXV). To a solution of 6-ketocholestanyl acetate (8 g.) in 16 ml. of acetic acid and 80 ml. of ether, was added dropwise a solution of 4.8 g. of bromine in 60 ml. of acetic acid. The addition required $1\frac{1}{2}$ hours. The mixture was then gently refluxed for two hours, the ether removed by vacuum distillation and the solution allowed to stand at room temperature overnight. After heating the solution on the steam-bath, 10 ml. of water was added. On cooling this solution, sand-like crystals separated and were filtered. Two crystallizations from aqueous acetic acid yielded lustrous plates of 7-bromo-6-keto-

cholestanyl acetate m.p. 145-146°, $[\alpha]_D^{22}+40.2\pm 1°(CHCl_3)$, additional product was obtained by concentrating the mother-liquors. The yield of pure product was 5 g. (37%).

5.(a) 3,7-Dihydroxy-6-ketocholestane (XXI) from 7-bromo-6-ketocholestanyl acetate (XXX). A solution of 7-bromo-6-ketocholestanyl acetate (4 g.) in 122 ml. of 10% methyl-alcoholic potassium hydroxide was gently refluxed for two hours. The solution was diluted to a large volume with water, acidified and ether extracted (4x150 ml.). The ether extracts were washed neutral with water and dried over sodium sulfate. Evaporation of the ether under reduced pressure yielded an oily product which was dissolved in a minimum amount of methanol and the methanol solution decanted from the residual resins. The decanted solution was charcoaled twice and crystallized from methanol in the cold. From methanol,asbestos-like crystals separated, m.p.168-170°. Two further crystallizations from methanol yielded 3,7-dihydroxy-6-ketocholestane, m.p.170-174° $[\alpha]_D^{22}+ 27.7\pm 1°(CHCl_3)$. When this compound was dried in vacuo at 60° for ten hours no loss of water or elevation of the m.p. was observed. The over-all yield of product obtained was 2.3 g. (67%).

(b) 7-bromo-6-ketocholestanyl acetate (500 mg.) was dissolved in 16 ml. of 10% methyl-alcoholic potassium hydroxide with gentle heating on the steam bath. The solution was allowed to react at room temperature for three days and was then diluted with 5% HCl and ether extracted (5x50 cc.). The ether extracts were washed neutral with water, dried over sodium sulfate and the ether evaporated, leaving 344 mg. of oily residue. Crystallization from methanol in the cold yielded lustrous plates of 3,7-dihydroxy-6-ketocholestane m.p.180-182°, $[\alpha]_D^{22} + 28.6 \pm 1^\circ (CHCl_3)$. The over-all yield of pure product was 80 mg. (19%) with the main bulk of material remaining in an oily state. The admixture m.p. of the two above-described products was 172-176°.

6 (a) <u>Acetylation of 3,7-dihydroxy-6-ketocholestane (XXXI)</u>. To a solution of 3,7-dihydroxy-6-ketocholestane (325 mg., m.p.170-174°) in 6.5 ml. of pyridine was added 6.5 ml. of acetic anhydride. The mixture was allowed to react at room temperature for seventeen hours and was then diluted with 170 ml. of 10% hydrochloric acid. The resulting oil was ether extracted, the ether washed with cold sodium carbonate (5%) and then to neutrality with water. After drying the ether solution over sodium sulfate, the ether was evaporated leaving 310 mg. of light yellow residue. Crystallization from methanol yielded a white product m.p.127-129°. Two additional crystallizations from acetone-methanol raised the m.p. to 127-132°. The yield of pure product was 130 mg. (23%). The admixture m.p. of this compound with an authentic sample of 7 α -acetoxy-6-ketocholestanyl acetate (XXVI) m.p. 134-135°, supplied by Dr. Wasson, was 100-109°.

(b) To a solution of 3,7-dihydroxy-6-ketocholestane (500 mg., m.p. 170-174°) in 10.0 ml. of pyridine was added 10.0 ml. of acetic anhydride and the mixture allowed to react at room temperature for 24 hours. The solution was then acidified with 150 ml. of 10% hydrochloric acid and ether extracted. The ether extracts were first washed with cold 5% sodium carbonate (3x25 ml.), then to neutrality with water, and dried over sodium sulfate. Evaporation of the ether yielded 490 mg. of a light-yellow oil. The oils were dissolved in benzene, and petroleum ether was added to induce turbidity. The solution was adsorbed on a column of activated alumina and eluted as described in Table II.

TABLE II.

Separation of Products from Acetylation of 3,7-Dihydroxy-6-ketocholestane (XXXI).

Fraction	Solvent	Ratio	Weight eluted mg.	Nature on Crystallization from Methanol
1-2	Petroleum ether		0	
2-7	Petroleum ether-benzene	20:1	0	
8-12	Petroleum ether-benzene	10:1	0	
13-18	Petroleum ether-benzene	5:1	0	
19-22	Petroleum ether-benzene	2:1	5	oily
23-29	Petroleum ether-benzene	1:1	88	oily
30-33	Benzene		73	oily
34-36	Benzene-ether	10:1	10	oily
37-38	Benzene-ether	5:1	3	oily
39-44	Benzene-ether	2:1	80	crystalline
45-48	Benzene-ether	1:1	17	crystalline
49-51	Ether		0	
52-53	Ether-chloroform	1:1	21	oily
54-55	Chloroform		8	oily

Fractions 39-48 (97 mg.) yielded pure crystals from methanol, m.p. 157-158°. This compound was not reducing with ammoniacal silver diamine and was otherwise not investigated. An investigation is now being conducted to characterize this compound. All the other fractions obtained from the chromatogram failed to crystallize.

(c) To 1.68 g. of 3,7-dihydroxy-6-ketocholestane in 1.68 ml. of pyridine was added 1.4 ml. of acetic anhydride and the mixture heated at 95° for one hour. The solvents were removed invacuo and the residue was dissolved in ether. The ether solution was washed with dilute hydrochloric acid, cold 5% sodium carbonate and dried over sodium sulfate. Evaporation of the ether yielded 1.53 g. of residue which was dissolved in methanol and the solution cooled. From methanol micro needles separated and were collected by filtration. Two more crystallizations from methanol yielded 1.51 g. of pure product m.p.67-69°. This compound did not reduce ammoniacal silver diamine and a further identification of it is now being conducted.

7. Silver Acetate Treatment on 7-bromo-6-ketocholestanyl acetate (XXX). To a solution of 7-Bromo-6-ketocholestanyl acetate (3.08 g.) in 200 ml. of pure acetic acid was added 3.1 g. of silver acetate. The suspension was refluxed for 10 hours in the dark, diluted with one litre of water and ether extracted (3x200 ml.). The ether extracts were washed with 5% sodium bicarbonate, to neutrality with water and dried over sodium sulfate. Evaporation

of the ether yielded 2.4 g. of light-yellow oily residue. Acidification of the alkaline washes did not produce any turbidity.

The oils were dissolved in methanol from which a crude product precipitated m.p.162°. Crystallization of this material from acetone-methanol gave long needles m.p.169-170°. The overall yield of pure product was 1.8 g. (56%). For analysis, a pure sample was dried in vacuo for eight hours at 60°.

$C_{29}H_{46}O_3$	requires	C-78.69%	H - 10.46%
	found	C-78.51%	H-10.37%
		78.42%	H-10.22%

The ultraviolet characteristics exhibited by this compound were λ_{max}^{alc} 235 mμ, ϵ = 2,660. This compound was tentatively designated as Δ^7-6-ketocholestenyl acetate (XLVII).

8. Saponification of " Δ^7-6-ketocholestenyl Acetate" (XLVII). Δ^7-6-ketocholestenyl acetate (172 mg.) was dissolved in 17.5 ml. of 5% methanolic potassium hydroxide and the mixture allowed to react at room temperature for 48 hours. The solution was then acidified with 10% HCl and ether extracted. The ether extracts were washed neutral with small portions of water, dried over sodium sulfate, and the ether was distilled in vacuo. The residual oil (165 mg.) was dissolved in methanol but failed to crystallize. From acetone-methanol needles separated and were collected by filtration. A second crystallization from acetone-methanol yielded needles m.p. 243-244°. The overall yield after concentrating the mother liquors was 121 mg. (76%). For analysis, a pure sample was dried in vacuo for

10 hours at 60°.

$C_{27}H_{44}O_2$ requires C-80.94% H-11.07%

found C-79.03% H-10.48%
79.08% 10.55%

This compound was tentatively designated as Δ^7-6-ketocholestene (XLVIII).

9. *Attempted Preparation of Δ^7-6-ketocholestenyl acetate (XLVII) from 7-Bromo-6-ketochlestanyl acetate (XXX).* To a solution of 7-bromo-6-ketocholestanyl acetate (2.42 g.) in pure acetic acid (184 ml.) at 60°, were added 1.07 g. of 2,4- di - nitrophenylhydrazine and 45 mg. of sodium acetate. The solution was kept at 60° in a nitrogen atmosphere for forty minutes. A dark red colour developed but 2,4-dinitrophenylhydrazone failed to precipitate. The solution was then concentrated under reduced pressure and 124 ml. of chloroform, 31 ml. of pyruvic acid and 15 ml. of water were added. To this solution kept at 65° in a nitrogen atmosphere was added 15 ml. of pyruvic acid at hourly intervals. At the end of four hours the solution was diluted with ether and the ether thoroughly washed with sodium carbonate to remove the red hydrazone of pyruvic acid. The ether solution was then washed neutral with water, dried over sodium sulfate and evaporated to dryness. Crystallization from acetone yielded 1.8 g. (90%) of lustrous plates m.p. 145-146°. This compound was Beilstein positive and on admixture with 7-bromo-6-ketocholestanyl acetate gave a m.p. 145-146°.

10. Lead Tetraacetate Oxidation of 6-ketocholestanyl acetate (XXV). Lead tetraacetate, prepared by the method described by Fieser (60), was dissolved in acetic acid (distilled over lead tetraacetate) and the concentration of the reagent determined iodometrically.

$$Pb(OAc)_4 \quad 2KI \rightleftarrows Pb(OAc)_2 \quad 2KOAc \quad I_2$$

$$I_2 \quad 2Na_2S_2O_3 \rightleftarrows 2NaI \quad Na_2S_4O_6$$

The concentration of the standard solution was 21.56 mg. of $Pb(OAc)_4$ per ml. of solution.

(a) To 230 ml. of this solution (11.21 mM $Pb(OAc)_4$) in a 500 cc. standard tapered flask was added 3.00 g. (6.75 mM) of 6-ketocholestanyl acetate. The flask was stoppered and immersed in a constant temperature bath at 70-74°. At regular time intervals 2 ml. of the reaction mixture was withdrawn and the lead tetraacetate content of the sample was determined by iodometric titration. Thus the rate of lead tetraacetate oxidation was determined and the results are presented in Table III.

TABLE III.

The Rate of Lead Tetraacetate Oxidation of 6-ketocholestanyl acetate (XXV).

Time (hours)	$Pb(OAc)_4$ used (mM)
0	0
3.37	16
6.11	43
6.80	48
7.93	74

1.16 moles of $Pb(OAc)_4$ per mole of steroid were used.

At the end of 74 hours the solution was diluted with two litres of water and ether extracted (3x400 ml.). The ether extracts were washed with water, 5% sodium bicarbonate (3x150 ml.) and with additional portions of 5% sodium bicarbonate to remove any acidic material present. The combined alkali washes were acidified but no turbidity was observed. The ether solution was washed neutral with water, dried over sodium sulfate and evaporated to dryness leaving 3.38 g. of amber-coloured oils. The oils were dissolved in a minimum volume of benzene, and petroleum ether was added to induce turbidity. This solution was adsorbed on activated alumina (90 g.) and eluted as shown in Table IV.

TABLE IV.

Separation of Products from Lead Tetraacetate Oxidation.

Fractions	Solvent	Ratio	Weight eluted mg.	Nature on Crystallization from Methanol
1	Petroleum Ether		47	oily
2-9	Petroleum Ether-Benzene	20:1	95	crystalline
10-14	Petroleum Ether-Benzene	6.6:1	94	crystalline
15-22	Petroleum Ether-Benzene	5:1	125	crystalline
23-27	Petroleum Ether-Benzene	3:1	141	crystalline
28-29	Petroleum-Ether-Benzene	1:1	169	crystalline
30-34	Petroleum Ether-Benzene	1:1	232	oily
35-37	Benzene		250	oily
38-41	Benzene		166	crystalline
41-44	Benzene-Ether	20:1	78	crystalline
45-46	Benzene-Ether	10:1	39	crystalline
47-49	Benzene-Ether	5:1	202	crystalline
50-56	Benzene-Ether	5:1	346	oily
57-61	Benzene-Ether	3:1	193	oily
62-65	Benzene-Ether	1:1	151	oily
66-67	Ether		16	oily

The fractions eluted were analysed as follows:

Fractions 2-29 (624 mg.) crystallized from methanol to yield needles melting at 128-129°. This compound did not depress the admixture m.p. of 6-ketocholestanyl acetate (m.p.128-129°).

Fractions 30-37 (482 mg.) resisted crystallization and were rechromatographed on activated alumina, but no crystalline material was eluted.

Fractions 38-49 (485 mg.) crystallized from methanol and melted at 194-196°. This compound did not depress the admixture m.p. of an authentic sample of 7β-acetoxy-6-ketocholestanyl acetate (m.p.195-196°), supplied by Dr. Wasson.

(b) In another experiment, 6-ketocholestanyl acetate was again oxidized with lead tetraacetate (1.61 moles per mole of steroid) at 65°. After 96 hours, 1.17 moles of reagent had reacted per mole of steroid. The reaction products were isolated as previously described and the resulting oils (3.02 g.) chromatographed on activated alumina. The adsorbed material was eluted as shown in Table V.

TABLE V.

Separation of Products from Lead Tetraacetate Oxidation.

Fraction	Solvent	Ratio	Weight eluted mg.	Nature on Crystallization from Methanol
1	Petroleum Ether		269	crystals
2-9	Petroleum Ether-Benzene	20:1	818	crystalline
10-14	Petroleum Ether-Benzene	20:1	436	oily
15-20	Petroleum Ether-Benzene	10:1	434	oily
21-23	Petroleum Ether-Benzene	5:1	109	crystalline
24-26	Petroleum Ether-Benzene	2:1	73	crystalline
27-30	Petroleum Ether-Benzene	1:1	53	crystalline
31-36	Benzene		52	oily
37-38	Benzene-Ether	20:1	3	oily
39-42	Benzene-Ether	4:1	18	oily
43-45	Benzene-Ether	2:1	38	oily
46-48	Benzene-Ether	1:1	23	oily
49-50	Ether		14	oily

The fractions eluted were analysed as follows:

Fractions 1-9 (1.09 g.) crystallized from methanol to yield a compound melting at 127-129°. This compound did not depress the admixture m.p. with 6-ketocholestanyl acetate. The admixture m.p. with an authentic sample of 7α-acetoxy-6-ketocholestanyl acetate (XXVI), supplied by Dr. Wasson, was 100-110°.

Fractions 10-20 could not be induced to crystallize and as a result were saponified. The oily material (700 mg.) was dissolved in 30 ml. of 5% methanolic potassium hydroxide and the mixture allowed to react at room temperature for 60 hours. The solution was then diluted with 300 ml. of 5% hydrochloric acid and ether extracted (4x50 ml.). The ether extracts were washed neutral with water, dried over sodium sulfate and the ether distilled off under reduced pressure. The residue (616 mg.) dissolved in methanol was fractionally crystallized and yielded two compounds. The more insoluble compound had a m.p.183-185° and on admixture with 3, 7-dihydroxy-6-ketocholestane (m.p.180-182°), formulated by Heilbron (41), melted at 182-184°. The more soluble compound melted at 201-203° and on admixture with 3β, 7α-dihydroxy-6-ketocholestane (m.p.202-203°), supplied by Dr. Wasson, gave a m.p. 200-203°. The relative abundance of these two compounds has not been determined.

Fractions 21-30 crystallized from methanol to yield a compound melting at 194-197°. On admixture of this compound with an authentic sample of 7β-6-ketocholestanyl acetate (m.p.195-196°) the m.p. obtained was 195-196°.

11. <u>Chromic Acid Oxidation of the Saponified Product from 7β-acetoxy-6-ketocholestanyl acetate (XXVII)</u>. To 150 mg. of 7β-acetoxy-6-ketocholestanyl acetate was added 18 ml. of 5% methanolic potassium hydroxide. The mixture was allowed to react at room temperature for 3 days and was then acidified with 10% hydrochloric acid and ether extracted (3x50 cc.).

The ether extracts were washed neutral with water, dried over sodium sulfate and the ether removed under reduced pressure. The oily residue (128 mg.) was very reducing with ammoniacal silver diamine.

To these oils in 10 ml. of acetic acid at 17° was added 100 mg. of chromic anhydride dissolved in 10 ml. of 90% acetic acid. The mixture was allowed to react at 17° for one hour and was then set aside for 12 hours at 5°. After diluting with 200 ml. of water, methanol was added to decompose the excess chromic acid and the solution was ether extracted (4x50 ml.). The ether extracts were washed with 5% hydrochloric acid, 5% sodium bicarbonate and finally to neutrality with water. The ether solution was dried over sodium sulfate and evaporated to dryness leaving a residue (126 mg.) which crystallized spontaneously. The alkali extracts were acidified and ether extracted. The ether solution was washed neutral with water, dried over sodium sulfate and evaporated to dryness. The oily residue (18 mg.) failed to crystallize.

The neutral product crystallized from methanol to yield pure crystals m.p. 120-121. This compound gave a positive Zimmerman reaction, but did not give an olive green colour with alcoholic ferric chloride. The characteristics exhibited by this compound in the ultraviolet were λ_{max}^{alc} 290mμ, $\epsilon = 41$. On the basis of these results the compound was designated as 7-hydroxycholestane-3, 6-dione (LIX).

$C_{27}H_{44}O_3$	requires	C-77.83%	H-10.64%
	found	C-77.60%	H-10.32%
		77.63%	-10.37%

12. Hydrogenation of 7α-Acetoxy-6-ketocholestanyl acetate (XXVI). To a solution of 7α-acetoxy-6-ketocholestanyl acetate (100 mg.) in 3.5 ml. of acetic acid was added 40 mg. of platinum oxide. The diacetoxy-ketone was exhaustively hydrogenated at room temperature and atmospheric pressure. The platinum oxide was removed by filtration and the acetic acid evaporated under reduced pressure. The residue crystallized spontaneously but was not purified. To the residue dissolved in 1.3 ml. of ethanol was added 260 mg. of sodium sulfite. The mixture was refluxed for 25 minutes and immediately decanted away from the residual white precipitate. Evaporation of the ethanol gave a residue weighing 90 mg. From ethyl acetate the residue yielded needles m.p.190-192°. Derivatives of this compound are now being prepared.

13. Hydrogenation of 7β-Acetoxy-6-ketocholestanyl acetate (XXVII). To a solution of 7β-acetoxy-6-ketocholestanyl acetate (100 mg.) in 3.5 ml. of acetic acid was added 40 mg. of platinum oxide and the diacetoxy-ketone was exhaustively hydrogenated. On isolation of the reduction product as described above, pure crystals were obtained m.p. 194-195°. When these crystals were mix-melted with starting material (m.p.195-196°)

the resulting m.p. was 194-196°.

However, 7β-acetoxy-6-ketocholestanyl acetate does form an oxime, m.p.185-189°.

$C_{31}H_{51}O_5N$	requires	N-2.71%
	found	N-2.96%

As a result of this the hydrogenation of 7β-acetoxy-6-ketocholestanyl acetate was repeated at 4 atmospheres pressure and room temperature, but again unreacted starting material was recovered.

14. Hydrogen Peroxide Oxidation of 3,7-Dihydroxy-6-ketocholestane (XXXI) and Lactone Formation.

(a) To a solution of 3,7-dihydroxy-6-ketocholestane (100 mg.) in 12.5 ml. of hot ethanol were added 0.3 ml. of hydrogen peroxide and 0.63 ml. of 2N KOH. The mixture was warmed on the steam bath for ten minutes and a rapid evolution of oxygen was observed. An additional 0.3 ml. of hydrogen peroxide was added and the solution was set aside at room temperature for 20 hous. After removal of the ethanol under reduced pressure water was added and the residue was ether extracted. The ether extracts were washed neutral with water, dried over sodium sulfate and the ether removed under reduced pressure leaving a neutral residue weighing 4 mg. The alkaline solution was acidified, after removal of the residual ether under reduced pressure and the precipitate formed was ether extracted. The ether solution was washed neutral with water, dried over sodium sulfate and taken to dryness under reduced pressure. The residue (94 mg.) was dissolved in pure dioxane (distilled over sodium) and evaporated to dryness with the addition of consecutive small volumes of dry benzene. The residue from the aziotropic distillation was dissolved in acetone-pentane but it failed to crystallize. An attempt was therefore made to convert these oils to the corresponding 3-lactonic acid (LVIII).

(b) The oily residue was dissolved in 10 ml. of acetic anhydride and the solution heated gently on the steam bath for a half hour. The acetic anhydride was removed under reduced pressure and the resulting residue was dissolved in ether. The ether solution was washed with sodium carbonate (4x20 ml. of 10%) and then to neutrality with water. After drying the ether solution over sodium sulfate it was taken down to dryness under reduced pressure leaving 63 mg. of clear oily residue. The alkaline washes were acidified and ether extracted. The ether solution was washed neutral with water, dried over sodium sulfate and taken down to dryness under reduced pressure. The residual oil (10 mg.) was dissolved in acetone-pentane but failed to crystallize. The neutral product was dissolved in ether-pentane, but it too failed to crystallize.

15. Chromic Acid Oxidation of 3,7-Dihydroxy-6-keto-cholestane (XXXI). Two hundred milligrams of 3,7-dihydroxy-6-

ketocholestane was oxidized with chromic anhydride as described in experiment 11. From the oxidation products were isolated 167 mg. of acidic material and 67 mg. of neutrals. The acids were dissolved in ether-petroleum ether but resisted crystallization. The neutral product could not be induced to crystallize from methanol.

16. *Hydrogen peroxide oxidation of 7α-Hydroxy-6-ketocholestanol (XXVIII).* One hundred milligrams of 7α-hydroxy-6-ketocholestanol was oxidized with hydrogen peroxide as described in experiment 14 (a). From the reaction products were isolated a large neutral fraction (80 mg.) and a small amount of acid material (8 mg.). The neutral fraction crystallized from methanol to yield needles melting at 196-199°. A second crystallization raised the m.p. to 202-204°. The neutral compound on admixture with 7α -hydroxy-6-ketocholestane showed no depression in m.p. The acidic product failed to crystallize from acetone-pentane.

SUMMARY.

Part I.

1. A method has been developed for the synthesis of methylamine hydrochloride-C^{14} (85% yield) and diazomethane-C^{14} (41% yield) starting from $NaC^{14}N$.

2. The optimum conditions for good purity and high yield of the above-mentioned compounds have been worked out.

3. A method for the radioactive assay of diazomethane-C^{14} has been developed.

4. A review of the literature was presented to ascertain the most suitable procedure for the synthesis of diazomethane-C^{14} that would be most compatible with the starting material ($NaC^{14}N$) available to us.

Part II.

1. A review of the action of lead tetraacetate on steroids was presented.

2. An analogy between acetoxylation and bromination reactions was postulated.

3. Evidence has been presented to show that the 3,7-dihydroxy-6-ketocholestane (XXXI), reported by Heilbron et.al. (41), was not correctly formulated.

4. Evidence has been presented to substantiate the original claim made by Wasson (29), that acetoxylation through the action of lead acetate on 6-ketocholestanyl acetate (XXV),

takes place at the C_7 carbon atom to yield C_6-C_7 di-substituted cholestane derivatives.

5. The action of silver acetate in displacement reactions with a bromine atom, leading to possible inversion of configuration, has been discussed.

6. Indirect evidence was presented that seems to confirm the assigned spacial configuration of the products resulting from the lead tetraacetate oxidation of 6-ketocholestanyl acetate.

BIBLIOGRAPHY

1. R. Jamieson, Ph.D. Thesis, McGill University (1947).
2. C. Yates, Ph.D. Thesis, McGill University (1951).
3. L. Thompson, Ph.D. Thesis, McGill University (1951).
4. E.B. Hershberg, E. Schwenk and E. Stahl, Arch.Biochem., 19, 300 (1948).
5. W. Traube, E. Burmeister and R. Stahn, Z.Anorg.Chem.147, 50 (1925)
6. A.W. Adamson, J.Am.Chem.Soc.,69, 2564 (1947).
7. G.O. Henneberry and B.E. Baker, Can.J.of Research,28, 345 (1950).
8. B. Belleau and R.O.H. Heard, J.Am.Chem.Soc., 72, 4268 (1950).
9. J. Dubus, Ann., 128, 200 (1863).
10. E. Linnemann, Ann., 145, 38 (1868).
11. H.G. Denham, Z.physik.Chem., 72, 678 (1910).
12. J.D. Riedel, German Patent 264, 528.
13. A. Sieverts and E. Peters, Z.physik.Chem., 91, 226 (1916).
14. S. Barratt and A.F. Titley, J.Chem.Soc.,115, 902 (1919).
15. P. Sabatier and J.B. Senderens, Compt.rend.,140, 483 (1905).
16. H. Dreyfuss, Chem.Zentr., 1934, 124.
17. V. Voorhees and R. Adams, J.Am.Chem.Soc., 44, 1397 (1922).
18. C.E. Crompton and N.H. Woodruff, Nucleonics, 7, No.3, 49 (1950).
19. W.J. Skraba and A.R. Jones, Nucleonics 7, No.3,53 (1950).
20. M.Fields, Nucleonics, 7, No.3, 55 (1950).
21. E.M. Gal, R.E. Spenger and D.M.Greenberg, J.Org.Chem.,15 1261, (1950).

22. R. Escourrou, Bull.Soc.Chem., (4) 45, 735 (1929).
23. W.H. Carothers and J.A. Jones, J.Am.Chem.Soc., 47, 3051 (1925).
24. W.H. Hartung, J.Am.Chem.Soc., 50, 3370 (1928).
25. K.W. Rosenmund and E. Pfannkuch , Ber., 56, 2258 (1923).
26. B.L. Baldeschwieler and L.A. Mikaska, J.Am.Chem.Soc., 57, 977 (1935).
27. W.F. Bruce, J.Am.Chem.Soc., 58, 687 (1936).
28. F. Arndt, Org.Synthesis, Coll.Vol.II, pages 165 and 461 (1943).
29. B.K. Wasson, unpublished data.
30. D. Sainte-Marie, M.Sc.Thesis, McGill University, April 1944.
31. E. Prado, M.Sc. Thesis, McGill University, April 1948.
32. O. Dimroth and R. Schweizer, Ber., 56, 1375 (1923).
33. G. Erhart, H. Ruschig and W. Aumueller, Z.Angew.Chem., 52, 363 (1939).
34. T. Reichstein and C. Montigel, Helv.Chim.Acta, 22, 1212 (1939.
35. J. von Euw, A. Lardon and T. Reichstein, Helv.Chim.Acta, 27, 1287 (1944).
36. F. Giral, J.Am.Chem.Soc., 72, 1913 (1950).
37. O. Mancera, J.Am.Chem.Soc., 72, 5752 (1950).
38. E. Seebeck and T. Reichstein, Helv.Chim.Acta., 27, 948 (1944).
39. R.E. Marker, E.L. Wittle, L. Plambeck, Jr., E. Rohrmann, J. Krueger and P.R. Veshofer, J.Am.Chem.Soc.,61, 3317 (1939).
40. A. Windaus and G. Stein, Ber., 37, 3699 (1904).
41. I.M. Heilbron, H. Jackson, E.R.H. Jones and F.S. Spring, J.Chem.Soc., 102, 1938.
    I.M. Heilbron, E.R.H. Jones and F.S. Spring, J.Chem.Soc.,801,1937
42. A. Butenandt and A. Wolff, Ber., 68, 2091 (1935).
43. C. Djerassi, J.Am.Chem.Soc., 71, 1003 (1949).

44. H. Inhoffen and G. Zuhlsdorff, Ber., 76, 233 (1943).

45. R.D.H. Heard and H. Sobel, J.Biol.Chem., 165, 687 (1946).

46. Fr. Schenck, Z,physiol.Chem., 243, 119 (1936).

47. A. Plattner and H. Heusser, Helv.Chim.Acta., 27, 748 (1944).

48. A. Windaus, H.Lettre and Fr. Schenck, Ann., 52, 98 (1935).

49. C.W. Shoppee, J.Chim.Soc., 1032, 1948.

50. V.R. Mattox and E.C. Kendall, J.Am.Chem.Soc., 70, 882 (1948).

51. S. Lieberman, L. Hariton and D. Fukushima, J.Am.Chem.Soc., 70, 1427 (1948).

52. L. Fieser and M. Fieser, Natural Products Related to Phenanthrene, Reinhold Publishing Corp., 3rd edition, p.191, 1949.

53. O. Wintersteiner and M. Moore, J.Am.Chem.Soc., 72, 1923 (1950).

54. B. Riegel and B. Moffett, J.Biol.Chem., 162, 585 (1946).

55. T. Gallagher and W.P. Long, J.Biol.Chem., 162, 539 (1946).

56. L. Ruzicka, P. Plattner and H. Heusser, Helv.Chim.Acta., 21 866 (1938).

57. L. Fieser, M. Fieser and S. Rajagopalan, J.Org.Chem., 13, 800 (1948).

58. R.E. Marker and E. Rohrman, J.Am.Chem.Soc., 62, 516 (1940).

59. C.W. Shoppee and G. Summers, J.Chem.Soc., 687, 1950.

60. L. Fieser, Experiments in Organic Chemistry, Heath and Co., p.436, 1941.