STUDIES IN RING D OXYGENATED STEROIDS

A Thesis

by

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TABLE OF CONTENTS

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SECTION I

GENERAL INTRODUCTION

GENERAL INTRODUCTION

The catabolism of steroid hormones and compounds, though not perhaps as worthy of attention as their anabolism or manner of biological synthesis, is a subject which has interested investigators for many years. It embraces the problem of the manner in which such a structure as the steroid nucleus might be completely dismembered in the living cell as well as the possibility that such a structure may undergo metabolic alterations rendering it more or less active with respect to a particular physiological property. It must be admitted that only in the latter aspect of these investigations has much detailed knowledge been gained - particularly in the study of the steroids of the adrenal cortex.

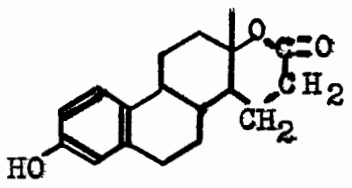
Most natural steroids have the common structural features of a saturated five membered hydrocarbon ring D attached to a phenanthrene nucleus, which may be completely saturated - or only partly so. In most cases ring D is also oxygenated to a greater or lesser degree, especially in the case of the androgenic and estrogenic sex hormones, and may also have a side chain attached to C_{17}. In considering the possibility of metabolic breakdown of steroids in the living cell, especially in the light of the long established biological fact that metabolism is essentially an oxidative process and the chemical rule that the points most susceptible to oxidative attack in a chemical structure are the carbon

atoms with attached oxygen functions, it is natural that attention would be focused on rings A and D as the logical structural sites where catabolism of an estrogen or an androgen might begin. Many investigators have sought out organs in the body where such catabolic processes might take place and the liver has often been implicated in this - particularly in the "inactivation" of the estrogens (1). Attempts have also been made to study the effect of pure enzyme preparations - as for example tyrosinase (2) - on the degradation of steroids.

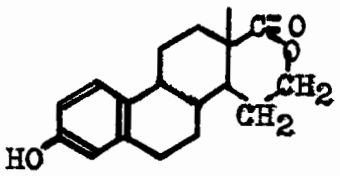
Most studies, however, on the catabolism of steroid hormones have focused on the fractionation of urinary steroid constituents in an attempt to isolate degradation products of the active steroid compounds. In this respect, for example, it now seems to be well established that the active male hormone testosterone and the estrogenic hormone β-estradiol appear in urine as transformation products possessing a 17 keto group where the steroid nucleus itself is still intact. In fact no steroid compound possessing a structure representing a degree of catabolism more advanced than this - i.e. where the steroid nucleus is actually ruptured - has ever been isolated from these urines and identified. There is no doubt of course that the steroid nucleus is catabolized in the body as was shown by the expiration of radioactive carbon dioxide by animals treated with C^{14} labelled steroids (3). It is true of course that one need not necessarily expect to isolate an intermediate in the breakdown of a steroid in the body, especially if one remembers that in the case of fatty acids (analagous in molecular size to steroids) the only structure with sufficient chemical permanency to merit the term "intermediate" is Acetyl CoA or "Active Two

Carbon Fragment", acetoacetic acid being rather a "pathological intermediate".

Pursuing an idea of Heard and Hoffman(5), Westerfeld (4) studied the effect of aqueous alkaline hydrogen peroxide on estrone expecting that, being a phenol, it would undergo oxidation at ring A and yield a dihydroxy phenol. However, he isolated instead a lactone which he represented as I, where the oxidative attack was directed not on ring A but on the 17-keto group of ring D.



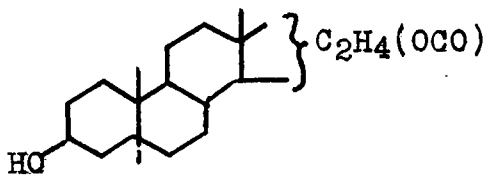
I



II

Assignment of this structure was based on the observation that while the compound was phenolic in character it was no longer Zimmerman positive and the hydroxy acid obtained by saponification was readily esterified by alcohol, which would not be so if the alternative structure II, representing attack on the C_{16-17} bond rather than C_{13-17}, obtained. The claim by Smith (6) and Westerfeld (4) that Westerfeld's lactone had slight estrogenic activity ($\frac{1}{4}$ that of estrone on the spayed mouse) and also markedly stimulated the pituitary of mature male rats to an extent that could not be accounted for in terms of this slight estrogenic activity aroused considerable interest in this topic and led many workers to study the peroxidation of steroid ketones.

CHART I



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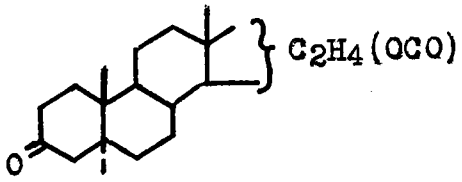

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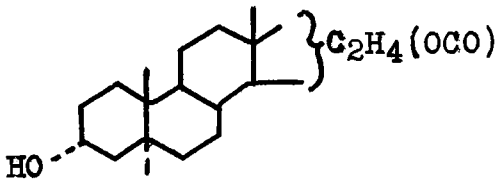

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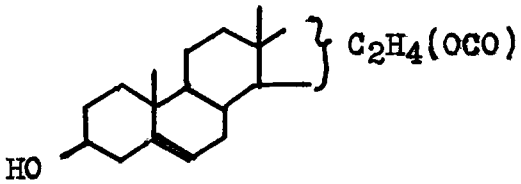

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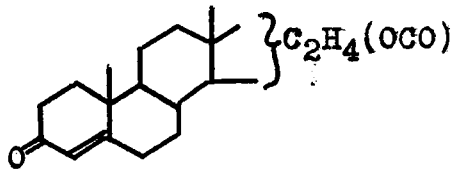

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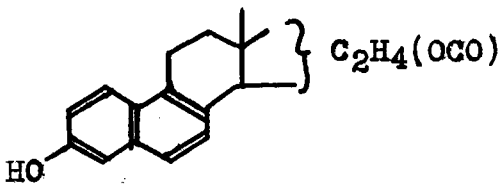
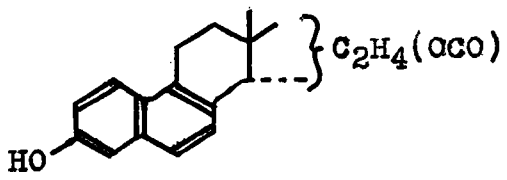


VIII



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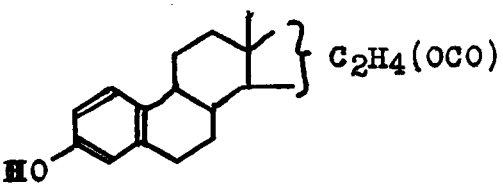


VIII$_a$

Jacobsen (7) improved the reaction on estrone and isolated a compound VIIIa which he termed "estrololactone" which on saponification yielded "estrolic acid". However, while his estrololactone was similar in chemical properties to Westerfeld's lactone, it had no estrogenic activity and inhibited the production of pituitary hormones in rats. Despite this, Keller and Weiss (8) and Jacques et al. (9) showed that in fact Westerfeld's lactone and the estrololactone of Jacobsen were identical.

Jacobsen (7), Jacobsen et al. (10) and Picha (11) extended this reaction, which involved essentially the use of peracetic acid, to the preparation of other steroid lactones with a view to studying their physiological activity and obtained the compounds (See Chart 1):

Isoandrololactone III (10_a) ..... from epiandrosterone

3-Keto Isoandrololactone IV (10_a) .... from Isoandrololactone

Andrololactone V (10_a) ..... from Androsterone

Dehydroisoandrololactone VI (10_a)...from Dehydroepiandrosterone

Testololactone VII (10_a) .....from VI

Bisdehydroestrololactone VIII (10_b)..from Equilenin.

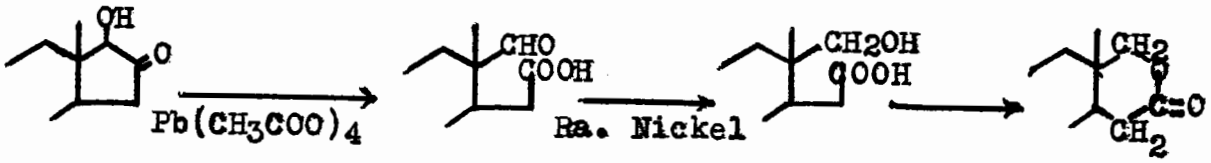
isoBisdehydroestrololactone IX (11)..from isoEquilenin.

It was claimed by Jacobsen (10_a) that while these compounds had no estrogenic or androgenic activity they markedly affected the pituitary, and the isoandrololactone, for example, stimulated pituitary growth and formation of gonadotrophins in male and female rats.

Despite the number of such lactones prepared by means

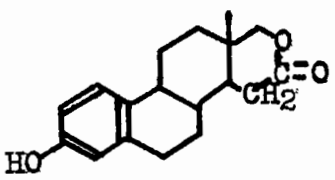
of this reaction, the exact structure of the lactone ring could not be formulated with certainty. As mentioned previously, Westerfeld had chosen structure I representing opening of ring D between C_{13} and C_{17}. Keller and Weiss however (8), in studying the action of X-rays on estrone in solution, isolated a lactone identical both with Westerfeld's lactone and Jacobsen's Estrololactone. Because such radiations are known to act on double bonds by virtue of the H and (OH) radicals they produce in water (12) and since in the case of estrone a double bond of a hypothetical intermediate in the reaction is only possible between carbons 16 and 17, Keller and Weiss formulated the structure of their lactone (and hence that of Westerfeld and Jacobsen) as in II.

This uncertainty in the structure of these ring D lactones prompted Von Seeman and Grant (13), Hershberg, Schwenk and Stahl (14), and Huffman et al. (15) to prepare ring D lactones of unequivocal structure. By the action of lead tetraacetate the latter authors were able to convert 16,17 ketols into aldehyde acids, which by reduction with Raney nickel yielded hydroxy acids. These hydroxy acids lactonized readily.

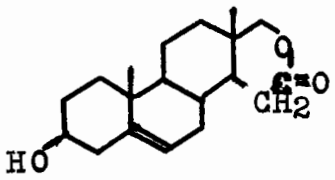


Ketol Aldehyde-Acid Hydroxy-Acid Lactone

CHART II

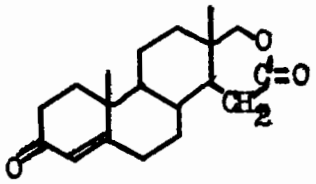


X

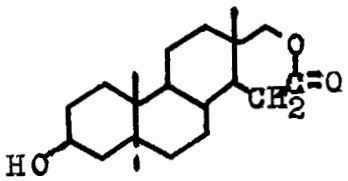


XI

(a)



XII



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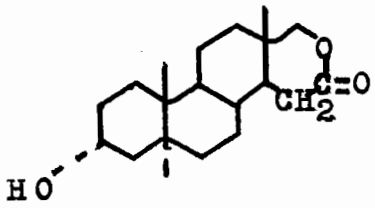

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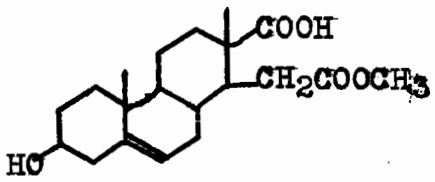

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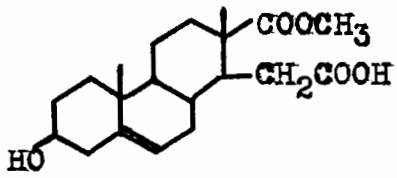
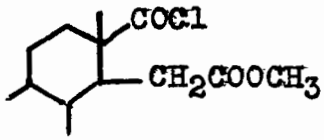
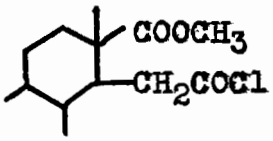
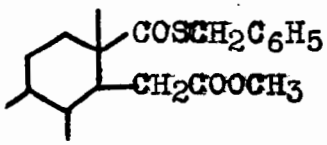


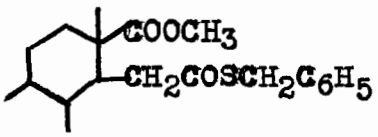
XVI

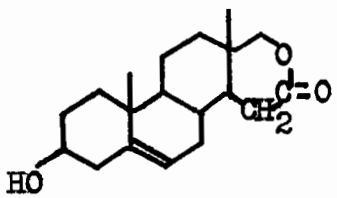




(b)

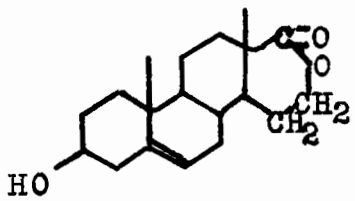






XV_a

" " lactone of Von Seeman and Grant

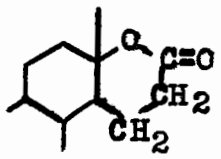


XVI_a

" " lactone of Von Seeman and Grant

In this manner, starting from estrone, dehydroepiandrosterone etc., they prepared the lactones X, XI, XII, XIII and XIV. (See Chart IIa). Starting from the monomethyl esters XV and XVI of 3β-acetoxy Δ^5-etiobilienic acid, Von Seeman and Grant (13) prepared two lactones of unequivocal structure which they referred to as the α and β lactones XV_a and XVI_a (ChartIIb).

Lactone XVI_b was prepared by Hershberg et al.(14) by another route. Perhaps the principal interest of these synthetic lactones is that by means of them it is possible to assign a structure with a high degree of certainty to the lactones of Westerfeld and Jacobsen. As the "beta" lactone of Von Seeman and Grant appears to be identical in chemical and physical properties with the lactone XVI_a of Hershberg et al., there is little doubt that the structure assigned is correct. But, whereas the "alpha" lactone of the former authors agrees well in melting point with the lactone XI of Huffman, neither the "alpha" nor "beta" lactone of Von Seeman and Grant is similar to the lactone VI prepared by Jacobsen from dehydroepiandrosterone. This must mean that of the two alternate structures postulated for Westerfeld's oxidation product of estrone and the series of compounds obtained by Jacobsen by the action of peracetic acid on a variety of steroid ketones, the one involving the rupture of the C_{13-17} bond of ring D must be the correct one - as shown in XVII.



XVII

More direct evidence in support of this has been supplied by Jacques et al. (9), although studies on infra red spectra by Jones and Dobriner (16) would still seem to favour the alternate structure.

Natural Lactones.

It must be remembered that the above compounds are the products of chemical reactions. The reaction is a peroxidation - involving simply hydrogen peroxide in the case of Westerfeld's compound - and as such, serves as a model for a plausible biochemical reaction on steroid ketones, since the possibility of oxidation by hydrogen peroxide exists in the organism involving perhaps catalysis by a peroxidase.

The systematic study of urinary steroid constituents by a great number of workers over a period of about 25 years has resulted in the isolation of only a very few compounds in which the ring structure of the steroid nucleus has actually been disrupted. Heard (17) and Jacobs and Laquer (18) isolated from the "neutral nonhydroxy ketonic fraction" of a mare pregnancy urine extract a compound of empirical formula $C_{19}H_{26}O_3$ of which one of the oxygens was ketonic and the other

seemed to be involved in a lactone ring, since saponification yielded a hydroxy acid. While the compound of Jacobs and Laquer approximated that of Heard in some of its properties there were marked discrepancies in certain respects - sufficient for Hoffman (19) to question the identity of the two compounds. The isolation of a third compound was achieved by Marker et al. (20) but, although these authors claimed that it was similar to the compound of Heard their data did not support this.

Heard's Lactone. This occurred in urine to the extent of about 1 mg. per 3 gallons of urine. It was subjected to as much chemical examination as the quantity available permitted and the results were published by Heard and Hoffman. As these chemical studies - and the structure assigned as a consequence of them - are pertinent to the work in this thesis they will be summarized in what follows as briefly as possible.

The compound of empirical formula $C_{19}H_{26}O_3$ gave a golden yellow colour with the Liebermann-Burchard Reagent, while a violet colour with the Zimmerman indicated the presence of a carbonyl group. The appropriate tests indicated that it was saturated and contained no free hydroxyl groups. Derivatives of the ketone such as semicarbazone and oxime could be readily prepared.

Investigation of the Structure.

Saponification of the Lactone. Refluxing the lactone in 2 N ethanolic potassium hydroxide yielded, after acidification, an acid whose empirical formula, $C_{19}H_{28}O_4.\frac{1}{2}H_2O$, indicated the up-

take of 1½ molecules of water. Titration showed that this was a monocarboxylic acid; the carbonyl function was still intact as a monosemicarbazone and monoxime of the acid could be prepared. The carboxylic group of the acid reacted with a cold ethereal solution of diazomethane to form a monomethyl ester.

The Hydroxy Acid.

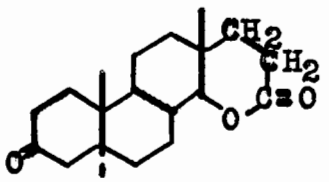
(a) Acetylation of the methyl ester of the acid obtained above could not be achieved at room temperature, but by heating in acetic anhydride and pyridine at 100°C. an acetate was formed. This together with the failure to undergo substitution by chlorine indicated the presence of a hydroxyl which was secondary, or which, if primary, was sterically hindered. Failure to isolate any acidic product after chromic acid oxidation further strengthened this viewpoint.

(b) Re-Lactonisation of the hydroxy acid itself could be readily effected by heating in dry acetic anhydride or by passing anhydrous hydrochloric acid through a solution in methanol at -4°C.

(c) Hydrogenation of the hydroxy acid in neutral solution with Adams Platinum Oxide effected reduction of the carbonyl and yielded a compound precipitable by digitonin. When done in glacial acetic acid the resultant compound was not precipitable by digitonin.

Structure Assigned to the Lactone.

In assigning a structure to the lactone on the basis of the above data, Hoffman - making the justified assumption that the compound was steroidal - chose the C_{19} androstane nucleus as basis. A δ-lactone structure (to which the above properties correspond) can only be fitted into ring D (rings A, B and C would involve ϵ-lactones) and the only position for a secondary hydroxyl is C_{14}. Hence the lactone was represented as arising from a hypothetical 15-keto steroid and the carbonyl group of the lactone itself was placed at C_3 in conformity with both this general rule for natural steroids and the behaviour of the hydroxy acid towards catalytic hydrogenation.



Heard's Lactone

This compound is the most completely characterized of the relatively few steroidal oxidation products of this type that have been isolated from urine. The identity of the lactone of Jacobs and Laquer (18) is obscure, for while it is similar to Heard's lactone in many respects, certain features - as for example the fact that acidification of the sodium salt of the corresponding hydroxy acid precipitates the lactone and not the free hydroxy acid - tend to show that they at least are not

identical. This property of the lactone of Jacobs and Laquer is reminiscent of the behaviour of the acids of the synthetic lactones of Von Seeman and Grant and of Huffman and may perhaps suggest that in Laquer's compound the lactone bridge is between C's 16 and 17.

The hypothetical derivation of Heard's lactone from a 15-keto steroid had at the time of its isolation some further justification based on the isolation by Heard and McKay (21) of an androstanol -3β - one, where the ketone was tentitatively placed at carbon 15. Recent synthetic work by Huffman et al. (22) has now placed this ketone grouping at C_{16}. However, despite this, the assigned structure of the lactone best fits its chemical behaviour. Furthermore, the lack of identity with any of the published synthetic lactones - particularly the 3-keto isoandrololactone of Jacobsen IV - would seem to eliminate carbons 16 and 17 as the site of the ketone parent to the lactone. In addition, the infra-red spectra determined recently on both the lactone itself and the hydroxy acid methyl ester confirm both the presence of the 3-keto grouping and the fact that the lactone ring is six membered, and is generally in agreement with the structure given. It is quite possible that the lactone of Laquer is an oxidation product of the androstanolone of Heard and McKay.

As all the available evidence thus points to this unusual structure for Heard's lactone, we became interested in this problem in the hope of proving it more conclusively by direct chemical means. As emphasized already, these lactones

represent the only known compounds that could be considered as intermediates in the complete catabolism of a steroid structure. Furthermore, the possibility that a natural steroid might possess an oxygen function at C_{15}, as implied in the structure of Heard's lactone, seemed worth while investigating both from the chemical and biological viewpoint, since in all the animal kingdom no such structure has ever been encountered.

While some more of Heard's lactone became available to us by isolation from a "neutral nonhydroxy ketonic oil fraction" the quantity was insufficient to attempt any degradative studies. Hence experiments were started with a view to devising a synthetic route to some compound of well-defined structure which would yield ultimately a compound identical with the natural lactone.

SECTION II

ATTEMPTED SYNTHESIS OF HEARD'S LACTONE

PART A

INTRODUCTORY REMARKS

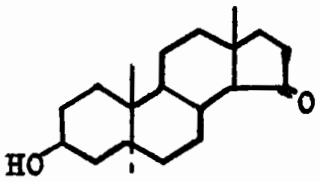
Consideration of the possible approaches towards the partial synthesis of a compound of the structure assigned to Heard's lactone (17,18) necessitates the manipulation of ring D of an androstane compound in such a manner as to introduce directly on C_{15} either the lactone grouping or some other function, such as a ketone, which would be ultimately convertible to a lactone, while at the same time maintaining on C_3 of ring A a ketone grouping - or potentially ketonic grouping - suitably protected throughout the chemical alterations on ring D.

Possible starting compounds were limited to C_{19} (i.e. without a side chain), partly because of the fact that even among natural steroids of plant origin there are very few compounds possessing a function on C_{15} with potentialities sufficiently attractive as to make the attempt to remove the side chain seem worth while, and partly because it was considered best to start from a compound - available in quantity - with the minimum of structural features capable of introducing difficulties into the synthesis.

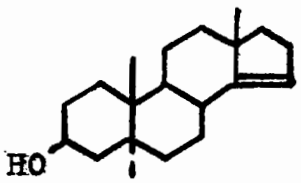
Within these limitations the problem resolves itself into two main approaches:

(A) the possibility of synthesising androstanol-3β-one-15 XVIII

(B) the possibility of synthesising Δ^{14} androstenol-3β XIX



XVIII

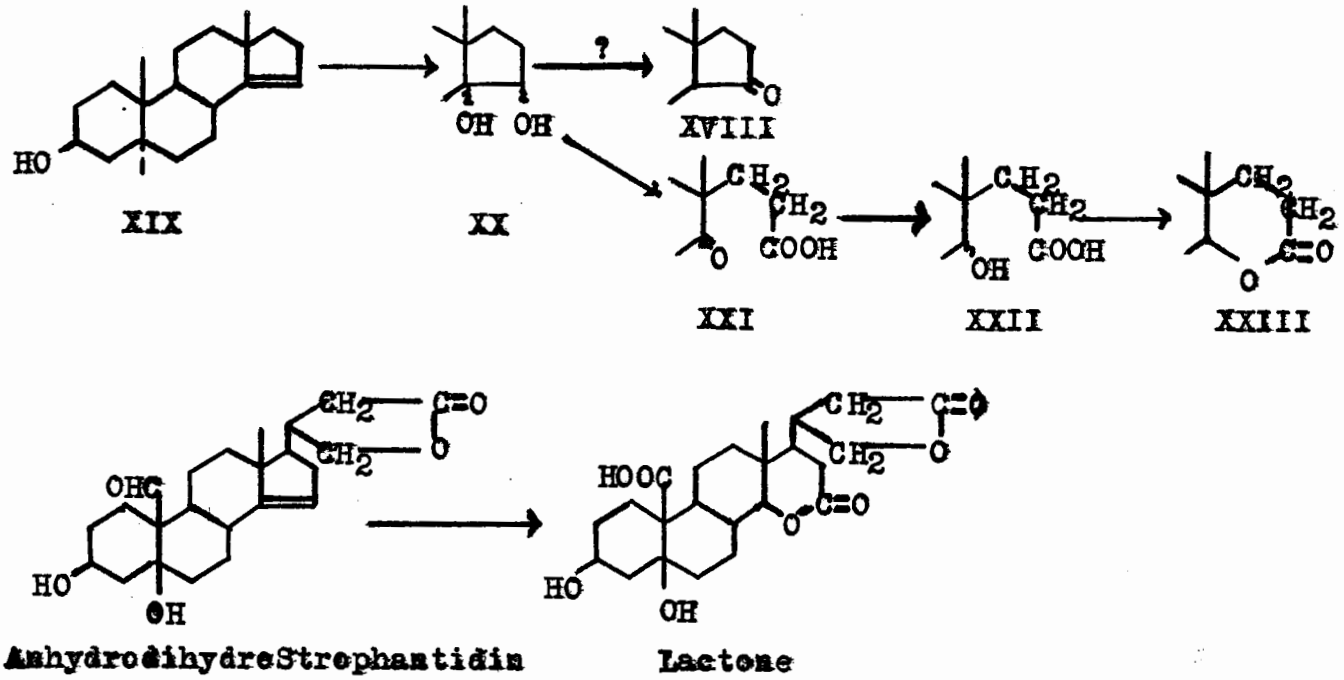


XIX

Both of these compounds would represent convenient precursors to the lactone. The conversion of ketones to lactones, as would be required in (A), is well illustrated in the literature (10,11) involving the use of peracetic acid, perbenzoic acid etc. In this particular instance there is also the possibility of obtaining two lactones and it would be of interest to see if the same selectivity would obtain in the peroxidation of a 15-ketone as was found in the reaction on 17-ketosteroids. Moreover, the hydroxyl at C_3 presents a grouping suitable for ultimate conversion to a ketone while at the same time offering the possibility - through both the acid-stable alkali-labile acetate and alkali-stable acid-labile tetrahydropyranyl (23) or triphenylmethyl ethers - of convenient protection throughout the reactions on ring D.

The androstenol XIX, though in some respects the less attractive of the two alternatives, is open to a number of considerations in speculating on a mode of conversion to the lactone. There is, for example, a plausible route involving glycollisation of XIX to the diol XX, oxidation of XX to the keto acid XXI, reduction of the keto acid to the hydroxy acid XXII and lactonisation to XXIII. The obvious

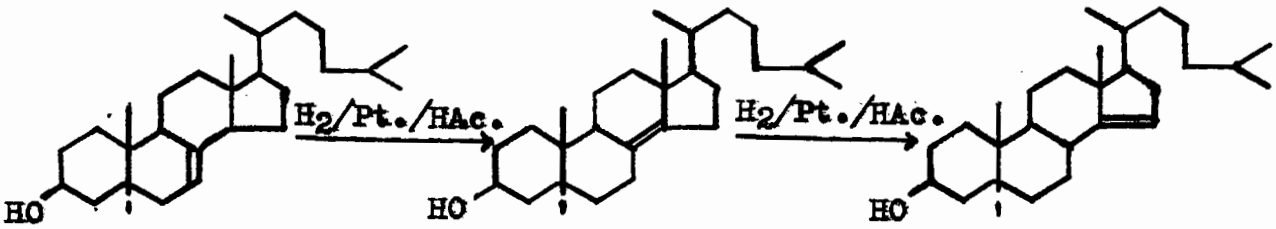
advantage in such a sequence is that it would lead directly to one lactone only.



In fact such a series of reactions has been used by Jacobs and Elderfield (24) in the conversion of anhydro dihydrostrophantidin to an analogous ring D lactone and, as such, offers a good chemical precedent for the series of reactions outlined above. There is also the possibility that the glycol XX might dehydrate to the 15-ketone XVIII.

At the outset of our attempts to develop a route to Heard's lactone, alternative (B) was tentatively discarded for the following reasons. The most obvious way to prepare a Δ^{14} androstene compound such as XIX would seem to be through a series of acid catalysed bond migrations from the Δ^{7-8} position through Δ^{8-14} to the Δ^{14-15}, especially in view of the fact that a convenient starting compound Δ^{7-8} androsten-3β,17β-diol XXIV

is fairly readily prepared (25). Thus, for example, Δ^{7}-cholestenol XXV, on shaking with platinum and acetic acid in an atmosphere of hydrogen, does not take up a molecule of hydrogen but rearranges to the $\Delta^{8(14)}$ cholestenol XXVI. When the latter is treated with hydrogen chloride in chloroform the double bond again migrates to yield the Δ^{14}-cholestenol XXVII (26).



XXV XXVI XXVII

A similar series of bond migrations takes place in the case of Δ^{7}-ergostenol (27). However, in turning now to an analagous androstene compound, it was of significance to us to note that Bernstein and Sax (28) failed to effect migration of the $\Delta^{8(14)}$ bond to Δ^{14} in the androstane series. This fact was unfortunate not only because the starting material would be readily available, as has been mentioned already, but also because the well-known difference in reactivity between substituents at C_3 and substituents at C_{17} would make the selective removal of a 17-keto or 17-hydroxy grouping in the route to XIX an easy matter. Although this route involving the crucial Δ^{14}-androstenol-3β seemed unprofitable and was thus discarded, it

will be referred to again because of the fact that results presented in this thesis as well as more recent published information open further avenues of approach in this direction.

PART B

ATTEMPTED SYNTHESIS OF ANDROSTANOL-3β-ONE-15 "XVIII" BY CYCLISATION

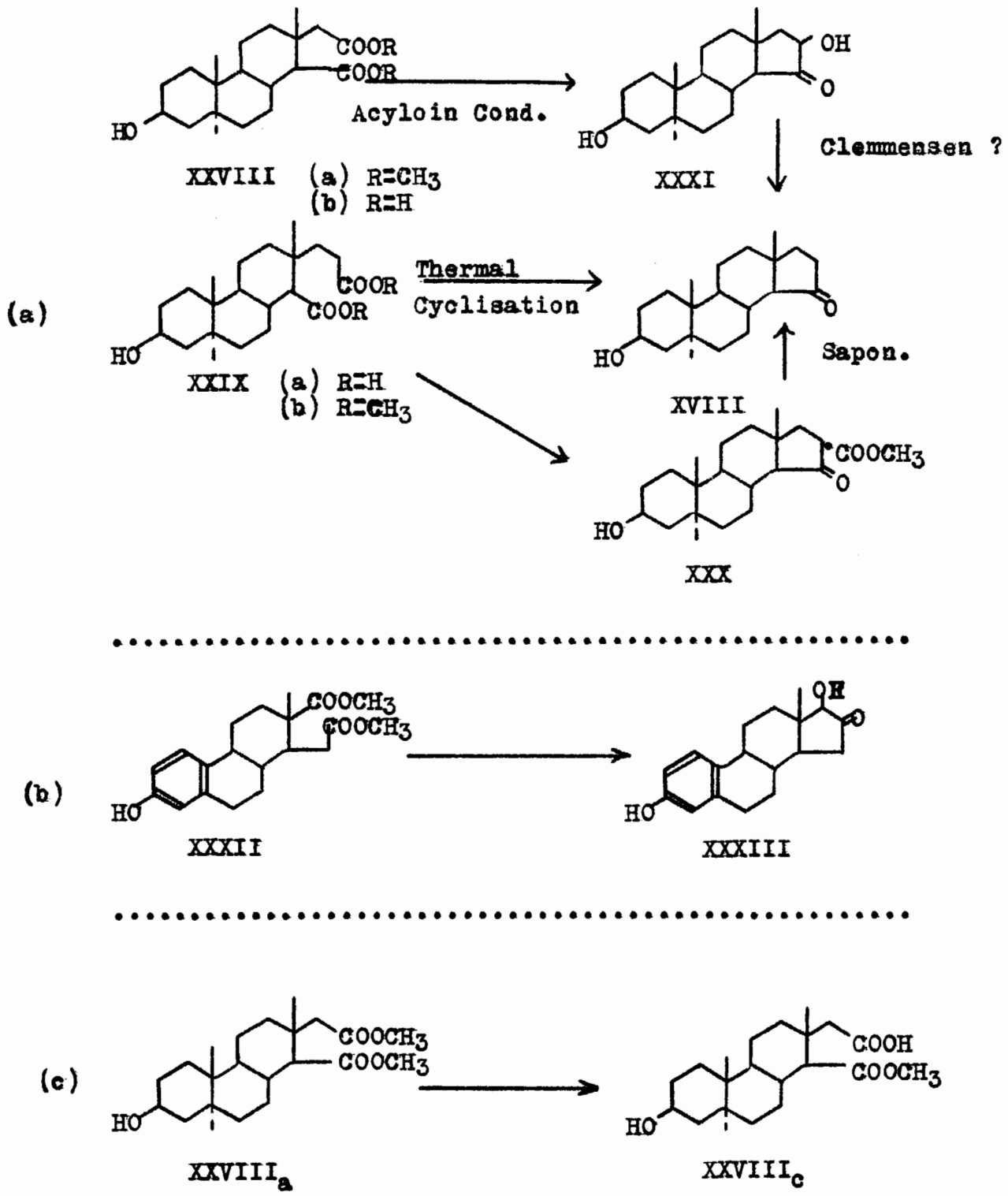
1. Theoretical

The approach to such a steroid ketone of this type might be realised by the acyloin condensation of the dimethyl ester of the appropriate diacid ($XXVIII_a$), or preferably by the thermal cyclisation of the lead salt or Dieckman cyclisation of the dimethyl ester of the homologue of this diacid ($XXIX_a$). (See Chart IIIa.)

Such methods of ring D closure in steroid compounds have been found to proceed in excellent yields in many instances. Examples are almost too numerous to mention and need no special comment here, but they have been used to advantage by Bachman, Anner and Miescher in total synthesis involving homomarrianolic acid type compounds. The older methods of Dieckman and lead salt cyclisation have the advantage in our particular case that either should lead unequivocably to the 15-ketone (XVIII) directly, as in the thermal decomposition of the lead salt of ($XXIX_a$), or indirectly through the 16-carbomethoxy compound (XXX) in the Dieckman cyclisation of ($XXIX_b$).

In the field of steroid chemistry the application of the Dieckman or lead salt cyclisation has invariably involved

CHART III


COOR
COOR
HO
Acyloin Cond.
OH
O
HO
Clemmensen ?
XXVIII (a) R=CH3
(b) R=H
XXXI
(a)
COOR
COOR
HO
Thermal
Cyclisation
O
HO
Sapon.
XXIX (a) R=H
(b) R=CH3
XVIII
COOCH3
O
HO
XXX
COOCH3
COOCH3
HO
OH
O
HO
XXXII
XXXIII
(b)
(c)
COOCH3
COOCH3
HO
COOH
COOCH3
HO
XXVIIIa
XXVIIIc

a sequence of homologation steps in the conversion of the available diacid to the homo-diacid - as in the conversion of marrianolic acid to homomarrianolic acid in the synthesis of estrone - due to the expulsion of one carbon atom in this cyclisation. The homologation step itself poses certain problems in our case which will be referred to shortly. It was in an attempt to avoid this wasteful homologation step and to cyclise directly the available diacid itself rather than its homologue that Sheehan et al. (29) applied the acyloin condensation in the closure of ring D. Thus by the addition of an ethereal solution of marrianolic acid dimethyl ester - 3 methyl ether (XXXII) to an excess of sodium in a medium of 60% liquid ammonia and 40% anhydrous ether they obtained the ketol 16-keto-estradiol-17β-3 methyl ether (XXXIII) in 96% yield.(See Chart III$_b$).

Application of this to the diacid ester (XXVIII$_a$) should lead to the 15-keto androstan-3β,16-diol (XXXI). Conversion of this to the 15-keto androstanol-3β involves the selective removal of the 16-hydroxy grouping. The work of Huffman (22; see part C,II) would suggest here that Clemmensen reduction of this ketol might effect conversion of (XXXI) to the desired androstanol-3β-one-15 (XVIII).

The key compound in the cyclisation reactions outlined above is the diacid (XXVIII$_a$) required in the acyloin condensation from which would be obtained by homologation the diacid (XXIX$_a$) required in the Dieckman cyclisation. In the field of steroid chemistry the series of reactions involved in this homologation step has most commonly been done on marrianolic

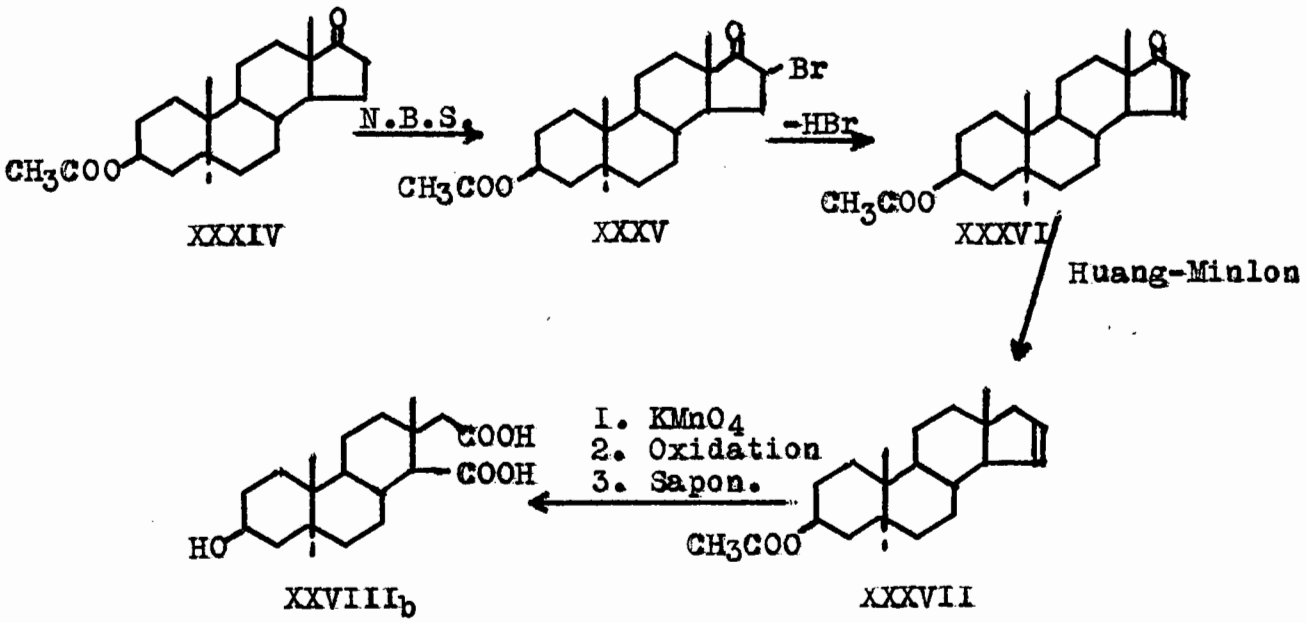
acid type compounds, i.e. acids where the carboxyl groups are derived from carbons 16 and 17 of the original steroid nucleus, and involves the selective extension of the "C_{17}" chain, by means of the Arndt-Eistert reaction, while the carboxyl at carbon 16 is maintained unreactive in the form of its methyl ester. The factor which allows of the preparation of this monomethyl ester is the marked difference in reactivity between the sterically hindered tertiary carboxyl at C_{17} and the unhindered primary carboxyl at C_{16} so that mild saponification of the dimethyl ester yields the monomethyl ester.

In preparing the homodiacid ($XXIX_a$) from the diacid ($XXVIII_a$) the critical step in the overall series of reactions involving the Arndt-Eistert reaction would be the partial saponification of ($XXVIII_b$) to the monomethyl ester ($XXVIII_c$). (See Chart III_c).

The difference in reactivity between the carboxyls of this compound is that between a primary and secondary carboxyl, and hence is not as great as that between the carboxyls of marrianolic acid. Because of this, the behaviour of ($XXVIII_b$) towards partial saponification is unforeseeable.

To obtain this key acid ($XXVIII_a$) required as the starting point in either of the condensations outlined above it was proposed to follow the series of reactions outlined on Chart IV: i.e. allylic bromination of epiandrosterone acetate (XXXIV) followed by dehydrobromination to yield the unsaturated ketone (XXXVI), removal of the 17-ketone by the procedure of Huang-Minlon with subsequent glycollisation and oxidation of

CHART IV

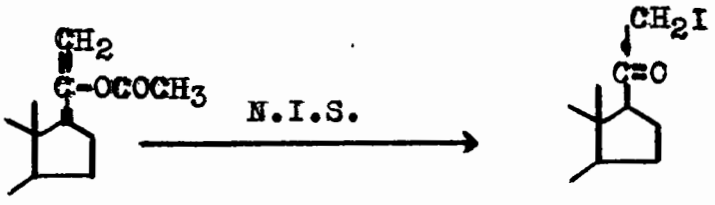


XXXIV XXXV XXXVI

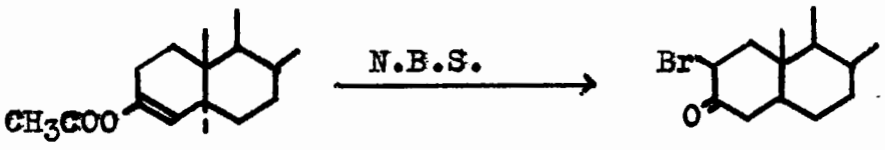
XXVIIIb XXXVII

the resultant androstenol acetate (XXXVII) to yield the diacid ($XXVIII_b$).

The steps from (XXXVI) to ($XXVIII_b$) represent reactions well established in steroid chemistry, and should present no special difficulties in these compounds. Since the introduction of N-bromosuccinimide by Ziegler et al. (30) as a general reagent for the allylic bromination of olefins, carbonyls etc., many steroidal alpha bromo ketones have been prepared by this procedure, which has many advantages over the older bromine-acetic acid method. Djerassi (31) has reviewed the scope and application of this reaction in general organic chemistry. There is, however, no published illustration of the preparation of a 16-bromo 17-keto steroid although many 2-bromo 3-keto, 4-bromo 3-keto, 11-bromo 12-keto compounds have been prepared. Djerassi and Link (32), and Rubin and Armbrecht (33) have further modified the reaction with ketones as they have shown that the enol acetates of saturated keto steroids react with N-bromosuccinimide and N-iodosuccinimide to give the corresponding alpha bromo and alpha iodo ketones.



Enol-Acetate Iodo-Ketone



Enol-Acetate Bromo-Ketone

2. Experimental

Some Properties of Heard's Lactone

Crystallisation. Brown crystals which had separated out at the bottom of a "neutral non-hydroxy ketonic oil fraction" were removed with a spatula and placed on a porous plate in a desiccator until the oil adhering to the surface of the crystals was absorbed onto the plate. Six hundred milligrams of the brownish crystals thus obtained were recrystallised three times from 95% ethanol, yielding 233 mg. melting at 260°-262°C.(Fisher Johns). Two more recrystallisations raised the melting point to 264°-265°C.

Colour Tests

Zimmerman .....positive
Ferric Chloride.....negative
TetraNitroMethane.....negative
Silver Diamine.....non-reducing
Sodium Bicarbonate.....insoluble

Spectrum No absorption between 2200 A° and 3200 A°

Mixed Melting Points

Melting point of pure specimen.....264°-265°C.
Melting point of authentic specimen.....268°-270°C.
Mixed melting point.....261°-264°C.
Mixed melting point with estrone.....212°-221°C.
Mixed melting point with equilenin..... - 215°C.

Rotation 19.78 mg. dissolved in 2.854 ml. (corrected volume) of purified dioxane gave:

$[\alpha]^{D} = +70.4$ (authentic $[\alpha]^{D} = +70.0$)

Analysis

	C	H
Found:	75.36%	8.51%
	75.48%	8.52%
Calculated: ($C_{19}H_{26}O_3$)	75.46%	8.67%

Preparation of the Oxime.

To a solution of 53 mg. of the ketolactone in 2.5 ml. of ethanol was added 53 mg. of hydroxylamine hydrochloride and 120 mg. of sodium acetate in 1.6 ml. of water. The precipitate which formed was taken into solution by adding ethanol and heating. The solution was refluxed for 1½ hours. After standing for 12 hours it was concentrated down under nitrogen. The precipitated material was then centrifuged and after the supernatant was removed, it was washed with 10% ethanol. The crystals were then dissolved in hot 75% ethanol, and after having been cooled to room temperature were placed in the refrigerator. Filtration and drying yielded 31 mg. of crystalline material. After three recrystallisations from aqueous alcohol 15 mg. of crystalline material melting at 249°-250°C. (Fisher Johns) was obtained. On admixture with authentic material it did not depress the melting point. It gave a negative Zimmerman reaction.

Analysis:

	C	H	N
Found	71.66% 71.65%	8.48% 8.49%	4.41%
Calculated	71.89%	8.57%	4.59%

Preparation of the Hydroxy Acid of the Lactone

Seventeen milligrams of the lactone was refluxed in 3 ml. of 2 N ethanolic potassium hydroxide for 50 minutes and then cooled and washed into a separatory funnel with water and diluted to 15 ml. This was extracted with 1, ½ volumes of ether to remove the insoluble crystalline material and the aqueous phase acidified to pH 2 with 6 N hydrochloric acid when fine needles separated out. After filtration and drying this melted at 214.5°-216.5°C. (Fisher Johns) and gave no depression on admixture with a sample of authentic material. It was not purified further but was used for preparation of the methyl ester.

Preparation of the Methyl Ester of the Hydroxy Acid

To 50 mg. of the hydroxy acid dissolved in 5 ml. of cold ether an excess of a solution of diazomethane in ether was added and stirred continuously for 4 hours. It was then placed in the refrigerator for 12 hours. At the end of this time the ethereal solution was washed once with 0.1 N hydrochloric acid, three times with 5% sodium carbonate, and finally five times with water. The ether solution was dried over anhydrous sodium sulphate and evaporated to dryness to

yield 36.5 mg. of a yellowish oil. This was sublimed in vacuo at 140-145°C. and 4 mm. pressure yielding 22 mg. of crystalline material. After two recrystallisations from benzene/ligroin 13 mg. of the methyl ester melting at 171-173°C. (Fisher Johns) was obtained.

Report on Infra Red Spectra of the Lactone.

"Spectrum done in potassium bromide cell, concentration 2 mg./300 and 0.7 mg./300.

Findings: Very strong carbonyl band at 1730 (with shoulder at 1737; perhaps a non resolved doublet ?) with satellite at 1697. Low band at 1545, bands at 1480, 1455 (shoulder at 1448), 1412, 1382 & 1367, 1342, 1295, 1257 & 1247, 1207, 1185, 1140, 1100-1105, 1067, 1042, 1020, 972, 923, 895, 840 (with satellites at 857, 830, 820), 780 and several weaker peaks.

Interpretation

The carbonyl band shows conclusively that the lactone ring cannot be five-membered (γ-lactones 1770 cm^{-1}, δ-lactones 1740,) .....

On the other hand the expected band at 1700-1710 for a 3-ketone is missing, which is very surprising on the basis of the structure given. The double peaks at 1257 & 1247 could perhaps be due to the -C-O- group of the lactone ring since esters generally have their -C-O- stretching vibration in this region. ..... Of the other bands present, 1412 could agree with the bands due to the $-CH_2-$ of a $-CO-CH_2$ group; the double

peak at 1455 and 1447 agrees well with the data for the bands due to $-CH_2-$ groups.

Conclusion: The spectrum definitely shows that the lactone ring is six-membered (or larger) but not five-membered. The absence of a peak due to the 3-keto group is surprising."

Report on the Infra Red Spectrum of the Hydroxy Acid Methyl Ester.

"Investigated in potassium bromide; concentration 1 mg./300 mg.

Findings: Very strong hydroxyl band at 3440 cm^{-1}. Strong peak at 1725, with shoulder at 1710-15; 1450 (shoulders around 1465, 1440), 1412, 1375, 1345, 1307 & 1298, 1215 (strong), 1180, 1163, 1125, 1082, 1042, 1020, 996, 825, and many minor ones.

Interpretation: The -O-H band at 3440 is in agreement with the formulation as a hydroxy acid. ..... The carbonyl (band) at 1725 agrees with the value given for the carbonyl band of non-conjugated acids in the solid state, viz. 1725 - 1705 cm^{-1}. The shoulder at 1710 - 1715 would then be due to the 3-carbonyl, in much better agreement with expectation than was found in the lactone.

The band at 1412 is present here as in the lactone; the absence of the strong double peak around 1250, which is present in the lactone seems to agree with the assignment of this band to the -C-O- vibration of the lactone group. The band at 1215 might be the one given by carboxylic acids between 1210 - 1320.

Conclusion: The spectrum agrees well with the expected one."

Purification of Dehydro Epiandrosterone Acetate Semicarbazone

Six grams of potassium hydroxide pellets was dissolved by refluxing in 800 ml. of 95% ethanol. To the boiling solution 20 grams of the crude semicarbazone was added and refluxing continued for 2 hours. The suspension was filtered hot, washed with 100 ml. of boiling ethanol and 400 ml. of cold distilled water, dried on suction and in vacuum desiccator overnight.

Weight of white powder.....16.9 grams (95%)

Hydrolysis of the Semicarbazone.

The above was refluxed for 2½ hours in 253 ml. ethanol, 338 ml. benzene, 338 ml. water and 67.5 ml. of concentrated hydrochloric acid and then allowed to stand overnight. After separation of the phases, the benzene layer was allowed to stand over potassium hydroxide pellets for thirty minutes and then washed free of alkali with water. Most of the water was removed by drying over anhydrous sodium sulphate for about an hour and the solution was then concentrated down to about 100 ml. on a water bath under nitrogen. On cooling, this solidified to a hard cake which was broken up and dried out in a vacuum oven at 65°C. for 2½ hours yielding 10.6 grams of dehydro epiandrosterone.

Acetylation was effected by dissolving in a mixture of 50 ml.

anhydrous pyridine and 50 ml. of acetic anhydride and allowing to stand overnight. The product was worked up by pouring into 400 ml. of distilled water with vigorous stirring. After being allowed to stand for several hours it was filtered, washed well with dilute hydrochloric acid and water and finally dried on suction and in a vacuum desiccator over calcium chloride. Recrystallisation of the 11.9 grams thus obtained (75% overall recovery) from 250 ml. of boiling ligroin gave 9.33 grams of pure dehydro epiandrosterone acetate melting at 176-177°C. (Fisher Johns).

Preparation of Epiandrosterone Acetate.

Preparation of 10% Palladium - Charcoal Catalyst. Norit charcoal was heated on a steambath with 10% nitric acid for 5 hours, then filtered and washed exhaustively with distilled water. It was dried on suction and in a vacuum oven at 100° C.

To 0.42 grams of palladium chloride in a mixture of 2.50 ml. water and 0.3 ml. concentrated hydrochloric acid, cooled in an ice-bath and stirred, was added 2.25 ml. of 37% formaldehyde and 2.5 grams of the acid washed charcoal. Stirring was continued and a solution of 2.5 grams potassium hydroxide in 2.5 ml. of water was added slowly over a period of 15 minutes. At the end of this period the suspension was heated in a water bath at 60° C. for 15 minutes and then washed by decantation with three 50 ml. volumes of distilled water. After one washing by decantation with 1% acetic acid it was collected on a suction filter and washed exhaustively with hot distilled water

until free of alkali and finally dried in vacuo at 100° C. (N.B. -- The washing with acetic acid was found to be critical for the activity of the catalyst in this hydrogenation and the procedure described above was found to give the best results.)

Hydrogenation. (33)

Two grams of purified dehydro epiandrosterone acetate, dissolved in 35 ml. of distilled ethanol, was placed in the hydrogenation flask and 0.5 gm. of the palladium catalyst placed in the sidearm. The entire apparatus was flushed with hydrogen by evacuating five times with a water pump and refilling with hydrogen. The catalyst was then tipped in and shaking commenced. After five hours, hydrogen uptake had ceased after the absorption of 196 ml. (theoretical for one double bond: 143 ml; 50 mg of palladium : 50 ml.). The solution was then filtered free of catalyst and evaporated down in vacuo to a colourless syrup. This was dissolved in hot neohexane and concentrated down by boiling. On cooling at room temperature and then in the refrigerator, 1.39 grams of hard plates melting at 123-125°C. (115-117°C. corrected) was obtained. By concentration of the mother liquors, up to 85-90% yields of pure epiandrosterone acetate could be obtained.

Reaction of Epiandrosterone Acetate and N-Bromosuccinimide

Purification of N-Bromosuccinimide: Crude amorphous N-bromosuccinimide was dissolved in rapidly boiling water to give a concentrated solution, then cooled rapidly and the crystals filtered, washed with cold water, dried on suction and in a vacuum desiccator.

Standardisation of N-bromosuccinimide: About 100 mg. of N-bromosuccinimide was dissolved in 7 ml. of 3 N hydrochloric acid and 10 ml of 10% potassium iodide added with shaking. The liberated iodine was titrated with standard sodium thiosulphate solution.

$$C_4H_4O_2NBr + 2KI + 2HCl = C_4H_4O_2NH + 2KCl + HBr + I_2$$

(1) 100.37 mg. N-bromosuccinimide =
8.7 ml. of 0.I N (F 1.277) Sodium thiosulphate
= 11.12 ml. of 0.I N Sodium thiosulphate
178 mg. (1 mM) = 19.81 ml. (Theor. 20 ml.)
Purity = 99%

(2) 101.52 mg. N-bromosuccinimide =
8.78 ml. of 0.I N (F 1.277) sodium thiosulphate
=11.21 ml. of 0.I N sodium thiosulphate
1 mM = 19.64 ml.
Purity = 98.2%

Bromination:

One hundred and eleven milligrams (0.3 mM) of epiandrosterone acetate was dissolved in 5 ml. of purified, dried, carbon tetrachloride and 62 mg. of N-bromosuccinimide (0.3 mM) added. The suspension was refluxed under anhydrous conditions for varying periods of time using an infra red heating lamp as a source of heat and light activation. When the reaction was stopped, the solution was cooled and the crystalline material in suspension filtered, dried and weighed. As judged by the weight of succinimide recovered after varying

reaction times, the reaction was apparently completed in six minutes heating under the infra red lamp.

The pale orange carbon tetrachloride solution obtained on filtration was evaporated down under nitrogen to a slightly yellow oil. This was placed in a vacuum desiccator over paraffin to remove the last traces of solvent.

Wt. of oil = 143 mg.

After failing to achieve crystallisation by the usual solvents, the oil was treated with about 15 ml. of hot neohexane and the supernatant siphoned off from the insoluble material. The solution was concentrated down to about 3 ml. and placed in the refrigerator. A yellowish oily solid separated out after thirty-six hours which, on removing the supernatant and drying in air, weighed 47 mg. This crop of material was then dissolved in three or four drops of acetone and placed in the ice-box. Some fine crystalline material separated out, which was removed by centrifugation and washed with ice cold acetone. After air-drying, this melted at 142-147°C. and on admixture with starting material melted at 80-100°C. However, when heated in an Abderhalden drying pistol the melting point dropped considerably until it no longer depressed the melting point of epiandrosterone acetate. Thus the isolated material was solvated starting material. Many attempts were made to obtain a brominated product by crystallisation from solvents and solvent mixtures but to no avail.

Chromatogram of the "Bromination Oil"

One hundred and forty-three milligrams of a "Bromination Oil" was placed on a column of 9 grams of I:I magnesium silicate/celite mixture with the aid of 10 ml. petroleum ether and 5 ml. of petroleum ether containing a little benzene. Fractions were collected as follows:

(1) Petroleum Ether, petroleum ether/benzene.....-------

(2) Benzene.....20 mg. oil and crystals

(3) Benzene and Bz/ether 50:I.....Oil (colourless)

(4) Benzene/ether 50:I and 20:I.....Oil "

(5) Benzene/ether 20:1 and 9:1 ..... " "

(6) Benzene/ether 9:I and 4:1 ..... " "

(7) Benzene/ether I:I and Ether..... " "

Recrystallisation of (2) from acetone-neohexane yielded crystals melting 144-152°C. but which on drying out thoroughly in the Abderhalden proved again to be starting material. The other oils resisted all attempts at crystallisation.

Dehydrobromination of the "Bromination Oil"

(a) With Collidine:

The oil obtained from the bromination of 111 mg. of epiandrosterone acetate was dissolved in 3 ml. of γ-collidine which had been freshly distilled from potassium hydroxide, and refluxed for 70 minutes. A crystalline precipitate of collidine hydrobromide formed during the course of the heating and this, after removal of the supernatant and washing of the precipitate with ether, weighed 45 mg.

The collidine solution was poured into 100 ml. of 6 N hydrochloric acid and stirred well. The aqueous oily suspension was then extracted three times with an equal volume of ether and the combined ether extracts washed four times with 5% potassium hydroxide, five times with water and finally dried with anhydrous sodium sulphate. The dried ether solution yielded, on evaporation under nitrogen, 100 mg. of a brownish oil. An aliquot of this oil was taken and dissolved in methanol and examined in the spectrophotometer for absorption in the ultraviolet. A definite peak at 241 mu was observed. (See fig. 1).

The remainder of the oil was dissolved in a small volume of methanol and placed in the ice-box. Thirteen milligrams of brownish needle crystals was obtained which was recrystallised from aqueous methanol and yielded, after washing and drying, 6 mg. of crystals melting at 131-133°C. (Fisher Johns; uncorrected). On admixture with authentic epiandrosterone acetate (123-125°C. uncorrected) it melted at 123-125°C. Hence the material was starting material.

(b) <u>With Dimethylaniline</u>:

The above procedure was repeated using dimethylaniline as the dehydrobrominating agent. Thirty-seven milligrams of a crystalline hydrobromide salt was formed but no crystalline material could be isolated from the "Dehydrobromination Oil". When examined in the spectrophotometer a broad flat peak with a maximum at 260 mu was observed. (See fig. 1).

(c) With Pyridine:

On substituting pyridine as the dehydrobromination agent, only 18% of the bromine could be recovered as hydrobromide and the oil obtained could not be crystallised. No absorption was evident in the ultraviolet.

Chromatography of the Oil after Dehydrobromination.

The oil obtained (91 mg.) after bromination and dehydrobromination with collidine and which showed a peak in the ultraviolet at 241 mu was placed on a column of 9 grams of activated alumina by dissolving in 5 ml. of a mixture of 40% benzene and 60% petroleum ether. Elution was commenced with petroleum ether and the following fractions obtained.

(1) Fractions 6-16 (benzene/pet. ether 1:1) :

Thirty-six milligrams of a white oil which crystallised on standing. It showed no absorption in the ultraviolet.

(2) Fractions 21-50 (benzene/ether 9:1, 6:1, 4:1) :

Twelve milligrams of a yellowish oil which showed no absorption in the ultraviolet.

(3) The column was extracted for eight hours in a Soxhlet extractor with chloroform but this yielded only a trace of oily material.

(I) could not be recrystallised at first. This was placed in a mixture of four drops of pyridine plus four drops of acetic anhydride. Having been allowed to stand overnight it was diluted with water, washed by centrifugation with dilute hydrochloric acid and water, and then dried first in the vacuum

desiccator and finally in the Abderhalden. Recrystallisation of this from methanol yielded fine needles melting at 122.5-124.5°C. (Fisher Johns) but which on admixture with authentic epiandrosterone acetate melted at 123-124°C.

(2) could not be crystallised.

Forty-three milligrams was retained on the column.

Attempted Bromination of Epiandrosterone Benzoate.

Preparation of the Benzoate:

Epiandrosterone acetate (0.5 gm.) was saponified by standing overnight in 6.5 ml. of 4% ethanolic potassium hydroxide. It was crystallised from the reaction mixture by the addition of 12 ml. of water to the boiling solution and allowing to cool to room temperature. This yielded 366 mg. of pure epiandrosterone.

Benzoylation was effected by allowing to stand overnight in a mixture of 6.5 ml. of pyridine and 1.4 ml. of redistilled benzoyl chloride. The excess benzoyl chloride was destroyed by the addition of methanol and the precipitated benzoate filtered off, washed with methanol, dilute hydrochloric acid and water. After drying, it was recrystallised from chloroform-methanol yielding 331 mg. melting at 229-230°C. (Fisher Johns).

Bromination:

This was done in the manner described for the acetate and, after removal of the succinimide (27 mg.) and evaporation

of the carbon tetrachloride, an amorphous white powder was obtained which melted at 208-218°C. (Fisher Johns). On re-crystallisation from acetone the melting point was 226-232°C. and there was no depression on admixture with the starting material. A test for halogens by sodium fusion was negative.

Dibromination of Epiandrosterone Acetate

Epiandrosterone acetate (550 mg.) was dissolved in 13 ml. of carbon tetrachloride and refluxed for 8 minutes with 960 mg. of N-bromosuccinimide. The weight of recovered succinimide indicated a bromine uptake of 250 mg. (theor. for dibromination: 250 mg.). However, working up the oil resulted in the isolation of only 16 mg. of crystalline material which again proved to be unchanged epiandrosterone acetate.

Saponification of the Dibromination Oil

The "dibromination oil" from 220 mg. of epiandrosterone acetate was refluxed for two hours in 10 ml. of 2% methanolic potassium hydroxide. The cooled solution was poured into water with stirring and then ether extracted. The ether extract was washed free of alkali and dried over sodium sulphate. Evaporation yielded 217 mg. of an oil.

Chromatography of the oil:

The oil obtained above was dissolved in 5 ml. of benzene and placed on a column of 3 gm. activated alumina and fractions collected in 5 ml. aliquots and combined as follows.

(1) Benzene, 40 ml. (total) .....101 mg. of oily crystals.

(2) Benzene 10 ml.; Bz/ether 9:1 (30 ml.)...22 mg. oily crystals.

No further material could be recovered from the column. Fractions (1) and (2) were combined (M.Pt. 161-168°C.) and on recrystallisation from methanol yielded fine needles of epiandrosterone M.Pt. 175-177°C. (Fisher Johns). One hundred milligrams of material was unaccounted for.

Large Scale Chromatography of the Dibromination Oil

A "dibromination oil" (prepared from 2 grams of epiandrosterone acetate and representing 2.82 grams of steroid) was dissolved in 20 ml. of benzene, placed on a column of 35 grams of 1:1 silica - celite mixture and fractions collected as follows:

COLUMN I

Fraction	Eluant	Vol. (per fraction)	Wt.	Nature
1-6	Benzene	20 ml.	1.195	yellow oil
7-11	"	"	0.590	white oil
12-14	"	"	0.050	oily crystals
15-18	Bz/ether 20:1	"	0.050	" "
19-22	" 3:1	"	0.075	oil
23-27	" 1:1	"	0.100	oil
28-32	Ether	"	0.030	oil
33	Ether/$CHCl_3$ (1:1)	40 ml.	0.035	crystals
34-36	Chloroform	40 ml.	0.020	"
37	$CHCl_3$/MeOH (1:1)	40 ml.	-	-
38-40	Methanol	120 ml.	0.470	Amorphous inorganic material

Fractions were combined as follows:

(A) Fractions 6 to 11 inclusive: 1.75 grams of oil and crystals.

(B) Fractions 12-24 inclusive: oil and crystals -0.323 grams.

(C) Fractions 33-34 inclusive: 55 mg. of crystals M.Pt. 104-108°C. (Kofler).

(D) Fractions 38-40 inclusive: 470 mg. of amorphous inorganic material.

On twice recrystallising (C) from cold ether a small quantity of gritty crystals was obtained which melted at 111.5-114°C. (Kofler). On admixture with epiandrosterone acetate the melting point was 75-115°C. The material gave a positive sodium fusion test for halogens, but apart from the determination of the ultra-violet and infra red spectra (see figs. 2 and 3) there was insufficient material for further examination.

Weight of organic material recovered from column.....2.13 gm.

Weight of unrecovered material.....0.69 gm.

Rechromatogram of Fraction (A) from Column I

Fraction (A) (1.75 gm.) from Column I was dissolved in 43 ml. of a benzene - pet. ether mixture and placed on a column of 35 gm. of 1:1 silica/celite and fractions collected as follows.

COLUMN II

Fraction	Eluant	Vol. (per fraction)	Wt.	Nature
1-5	Pet. ether	40 ml.	-	-
6-13	Pet. ether/Bz (40:1)	40 ml.	0.215	oil & cryst.
14-17	" "9:1	40 ml.	0.090	" "
18-21	" " 4:1	40 ml.	0.100	" "
22-37	" " 1:1	40 ml.	0.390	" "
38-45	Benzene	40 ml.	0.137	" "
46-49	Bz/ether (9:1)	40 ml.	0.100	" "
50-51	" " 4:1	40 ml.	0.080	" "
52-57	" " 1:1	40 ml.	0.114	" "
58-61	Ether	40 ml.	-	- -
62	Ether/$CHCl_3$ (1:1)	40 ml.	-	- -
63	Chloroform	40 ml.	-	- -

Weight of recovered material 1.27 grams

Weight unrecovered material 0.58 grams

"Spot" recrystallisations along the column from acetone - neohexane yielded, in each case, material melting at 149-150°C. On drying thoroughly in Abderhalden the melting point dropped and there was no depression on admixture with authentic epiandrosterone acetate.

There was an overall loss of 1.34 grams of the oil placed originally on column I (representing 2.822 grams - based on bromine uptake in the bromination reaction) due to retention on both Column I and Column II. The oils (combined fraction B from Column I and all the oils from Column II) from

which starting material was isolated amount to 1.59 grams. The material retained on the column must represent the main bromination product - the small amount of crystalline bromo compound obtained from Column I being only a minor product. No further attempts were made to isolate the material.

Attempted Preparation of Androstan-3βol,17-enol Diacetate

(a) Six hundred and sixty milligrams of epiandrosterone acetate and 548 mg. of purified p-toluenesulphonic acid were dissolved in 75 ml. of redistilled acetic anhydride. The acetic anhydride was distilled off, over a period of three hours, through a short unpacked column. When most of the acetic anhydride had been removed the dark brown residue was chilled in an ice bath and diluted with 50 ml. of distilled water. After standing for 15 minutes it was extracted twice with a total of 75 ml. of ether and the combined ether extracts were washed first with dilute sodium hydroxide and finally with water until neutral. Evaporation of the dried extract yielded an oil which on recrystallisation from aqueous methanol gave fine needles melting at 92.5-93.5°C. On admixture with epiandrosterone acetate it melted at 104-105°C.

(b) Six hundred and sixty milligrams of epiandrosterone acetate was dissolved in a mixture of 7.5 ml. of acetic anhydride and 10 ml. of acetyl chloride. This was refluxed for five hours in an atmosphere of nitrogen under anhydrous conditions. After the first fifteen minutes the stream of

nitrogen was turned off to prevent entraining of the acetyl chloride. At the end of five hours most of the solvent was removed by distillation and ethyl alcohol was added to the residue in the flask. This was now distilled in vacuo resulting in a crystalline mass. Recrystallisation from aqueous ethanol yielded 578 mg. of material melting at 106-109°C. (Fisher Johns) which on admixture with epiandrosterone acetate melted at 106-107°C.

Reaction of Cholestanone and N-Bromosuccinimide (34)

Cholestanone (474 mg.) was dissolved in 10 ml. of carbon tetrachloride and refluxed under anhydrous conditions with 218 mg. of N-bromosuccinimide. After five minutes an amber colour developed and gradually deepened. An abrupt disappearance of the colour took place at twenty-nine minutes when the solution was cooled and the succinimide filtered off (129 mg.). The solution was concentrated down in vacuo to a brown crystalline mass which on recrystallisation from acetone yielded authentic 2-bromo cholestanone melting 167.5-168.5°C. (Kofler). Literature melting point is 169°C.

3. Discussion

In the bromination of epiandrosterone acetate (XXXIV), the typical "bromination colour" appeared within one minute of the application of heat and light activation - indicating that a reaction was taking place. As judged by the recovery of succinimide, the reaction was apparently complete in six minutes, although no sharp disappearance of the bromination colour was evident as happens, for example, in the termination of the reaction between N-bromosuccinimide and cholestanone. Initial attempts to isolate a bromo compound from the "bromination oil" by means of crystallisation from various solvents invariably led to the isolation of starting material. Chromatographic analysis on a magnesium silicate - celite mixture yielded mainly uncrystallisable oils with no appreciable demarcation between fractions, while at the same time a considerable amount of material was held on the column.

Attempts to isolate a bromo compound having thus failed, it was then expected that dehydrobromination might remove those properties - apparently due to bromine substitution - which were rendering isolation difficult and that the α,β unsaturated ketone (XXXVI) (Chart IV) might prove more amenable to crystallisation. In addition it was expected that compound (XXXVI) would show an absorption peak in the ultraviolet and thus serve as a guide to this stage of the experiments.

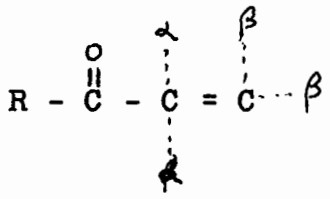
Dehydrobromination of the "bromination oil" was tried

using γ-collidine, pyridine and dimethyl aniline. While the crude oily products of these reactions exhibited an ultraviolet absorption peak, all attempts to isolate a product either by crystallisation or chromatography yielded only starting material. Again about 40% of the dehydrobrominated material placed on a column of activated alumina could not be recovered in the elution procedure - even after the column was extracted for eight hours in a Soxhlet extractor. It should be mentioned that aluminium oxide was chosen as the adsorbant because it was expected that its known dehydrobrominating properties (35) would help to increase the yield of bromine-free product.

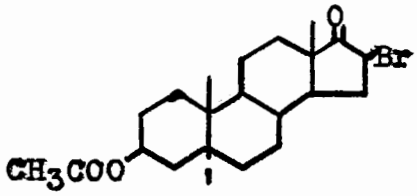
The most efficient dehydrobrominating reagent - as judged by the recovery of bromine as the insoluble hydrobromide salt - proved to be γ-collidine where 79% of the collidine hydrobromide was obtained. With dimethyl aniline and pyridine the values were 60% and 18% respectively. Moreover, while no product was isolated from these reactions, there is some suggestion from the ultraviolet spectra (See Fig. 1) that the products formed by the action of γ-collidine and dimethyl aniline are not the same.

Ultraviolet Spectra.

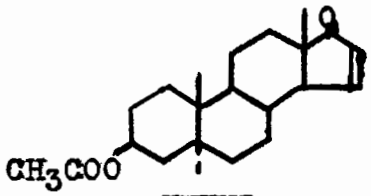
Using the empirical rules devised by Woodward for calculating the "theoretical" position of absorption peaks due to conjugated unsaturated ketones, where the parent system represented as



has a λ max. at 215 mu and the bathochromic contribution of an alkyl substituent in the β position is 12 mu, one arrives at the value of 227 mu for the "theoretical" position of the maximum of the absorption peak due to the compound (XXXVI) to be expected in the dehydrobromination of (XXXV).

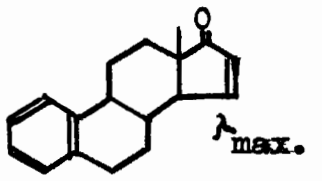


XXXV



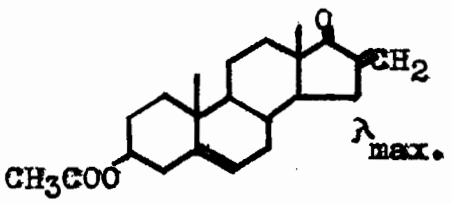
XXXVI

While these empirical rules have been well substantiated in the case of conjugated ketone groupings located in rings A, B and C of the steroid nucleus, very few compounds analagous to (XXXVI) have been prepared. Dorfman (36), in his extensive review entitled "Ultraviolet Absorption Spectra of Steroids", lists only one compound with a chromophoric grouping analagous to that of (XXXVI) - the compound (XXXVIII) (a product of total synthesis) which has three peaks: 230 mu, 270 mu, and 321 mu. The first of these at 230 mu - due to the conjugated ketone - is in good agreement with the calculated value of 227 mu. In addition the 16-methylene derivative of 3β-acetoxy Δ^5-androstene-17-one (XXXIX) has an absorption peak at 228 mu (36).



$\lambda_{max.}$ 230 mu
270 mu
321 mu

XXXVIII

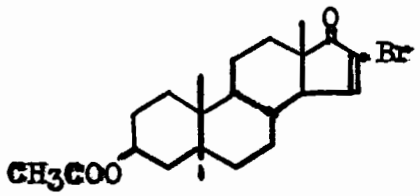


$\lambda_{max.}$ 228 mu

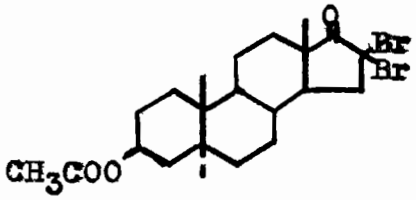
XXXIX

The product obtained by refluxing the "bromination oil" with γ-collidine showed an absorption peak at 241 mu (alcohol) with an additional peak of low intensity at 275 mu. (See fig. 1). The curve obtained on the product formed by dimethylaniline was broad and flat with an ill-defined maximum in the neighbourhood of 260 mu (alcohol).

These spectral properties of the oils formed by dehydrobromination strongly suggested that the reaction between equimolar quantities of N-bromosuccinimide and epiandrosterone acetate (XXXIV) did not produce simply the 16-bromo compound (XXXV). While the spectra unquestionably indicated that there is an α,β unsaturated ketone formed in the dehydrobromination reaction, the strong bathochromic displacement of the observed maximum from the calculated one pointed to the presence of a substituent in the chromophoric system of this unsaturated ketone. As the only substituent possible as a consequence of the two reactions involved is a bromo radical, the interpretation of the spectrum would best fit the formula (XL).

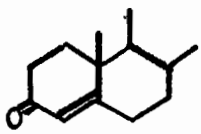


XL



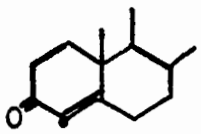
XLIII

This is in agreement with the fact that bromo substitution at C_4 of 3-keto-Δ^4-steroids (37) shifts the absorption maximum to the longer wavelength by as much as 13-16 mu, as is seen on comparing the λ max. of structures (XLI) and (XLII).



$\lambda_{max.}^{(ether)}$ 234 mu

XLI



$\lambda_{max.}^{(ether)}$ 250, 248, 247

XLII

On this basis the calculated λ max. of compound (XL) would be 215 + 12 + (13-16) = 240-243 mu, in very good agreement with the observed value.

The implication of the above is that the bromination of epiandrosterone acetate (XXXIV) yields directly the dibromo (XLIII) and not the mono-bromo compound (XXXV). A search of the literature did not reveal any published 16,16'-dibromo, 17-keto-steroid, but, in a footnote to their paper, Jones et al. (16) refer to the infra red spectrum of ..... "one dibrominated 17-keto steroid, believed to be 16,16' - dibromo androstanol-3 -one-17".....

Although it was now realized that this approach to

the synthesis of the lactone was unprofitable, a further effort was made to isolate a bromination product of the reaction. To this end, a comparatively large (2 gm.) amount of epiandrosterone acetate (XXXIV) was allowed to react with an amount of N-bromosuccinimide sufficient to result in dibromination - the reaction taking just slightly longer (8 minutes) than when a 1:1 mole ratio of the reactants was used. The "dibromination oil" resulting from this was then chromatographed on two successive columns of silica-celite mixture, as described in the experimental section. However, the only crystalline material isolated from this was:

(a) starting material obtained in very low yield in the crystallisation of the oils from Column II;

(b) a small amount of crystalline compound melting point 111.5-114°C. (Kofler) which gave a positive halogen reaction after sodium fusion.

There was an overall loss of material due to retention on Columns I & II (representing about 46% of the 2.82 gm. placed on Column I) and this material retained on the columns presumably corresponds to the main product of the reaction. It is possible that the use of some adsorbant other than aluminium oxide or silica-celite might facilitate the isolation of this material.

The crystalline bromo compound obtained was present in only very small quantity (55 mg. from the 2.82 gm.) and, as such, must represent only a minor by-product of the reaction. Due to this limitation of the available material, it was impossible to

do any chemical investigation on it, although the infra-red and ultraviolet spectra, which are presented here, supply some information.

Dr. Weiss, in his interpretation of the infra-red spectrum (See fig. 2) of this compound, states:

> "... the carbonyl band at 1765 (1745 in potassium bromide) could be due to the 16-dibromo, 17-keto group, which is listed by Jones, Herling at 1764 in CS_2. One would, however, expect to find it 5-15 cm^{-1} lower in chloroform.
>
> The 1690 band in chloroform is surprising: a 3-acetate band in chloroform should have a 1719-1728 (band); .....This discrepancy is serious and together with the absence of a strong acetate band in the potassium bromide spectrum at 1240 makes it doubtful that an acetoxy band can be present. The location of bands at 1690 (chloroform), 1658 (potassium bromide) and the bands at 1630 and 1600 in potassium bromide, rather suggest some α, β unsaturated carbonyl ....."

That the compound is not the 16,16' dibromo epiandrosterone acetate (XLIII) seems clear from this interpretation, although the absence of either a 3-acetoxy or 3-hydroxy band is unexplainable in terms of the experimental procedures involved in its preparation. The fact that certain bands indicate the presence of an α, β unsaturated ketone finds some support in the ultraviolet spectrum where there is an intense band with a maximum at 214 mu (alcohol), (see fig. 3) - although readings at these low wavelengths are admittedly un-

reliable. As the compound also contains bromine, it is impossible to formulate a structure on the basis of this contradictory information.

Because of the failure to prepare the desired 16-bromo epiandrosterone acetate (XXXV) and also the 17-enol acetate of epiandrosterone acetate (see experimental), this approach to the problem was dropped. No explanation for the failure of the 17-ketone to react normally towards N-bromosuccinimide can be advanced, although it brings to mind the well-known phenomenon of the highly strained nature of ring D and the steric factors associated with it - a feature encountered more than once in the work described in this thesis.

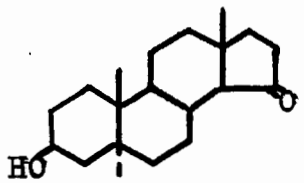
PART C

ATTEMPTED SYNTHESIS OF ANDROSTANOL-3β-one-15 (XVIII) BY DIRECT SUBSTITUTION ON C_{15}

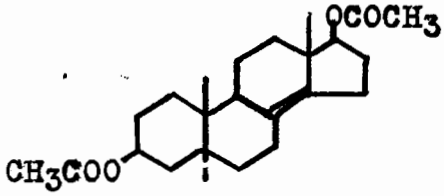
I ..... via Δ^{16}-Androstenol-3β(LIII)

1. Theoretical

After the failure of the method attempted in Part B, the possibility of achieving the synthesis of androstanol-3β-one-15 (XVIII) by direct substitution on C_{15} was sought. Such a process would also obviate the lengthy route involved in the opening and closing of ring D. The substituent of choice being a halogen, the initial step would involve a structure in which C_{15} was activated towards a halogenation reagent such as N-bromosuccinimide. The double bond of $\Delta^{8,14}$-androstan-3β,17β-diol diacetate ($XXIV_a$) (25)



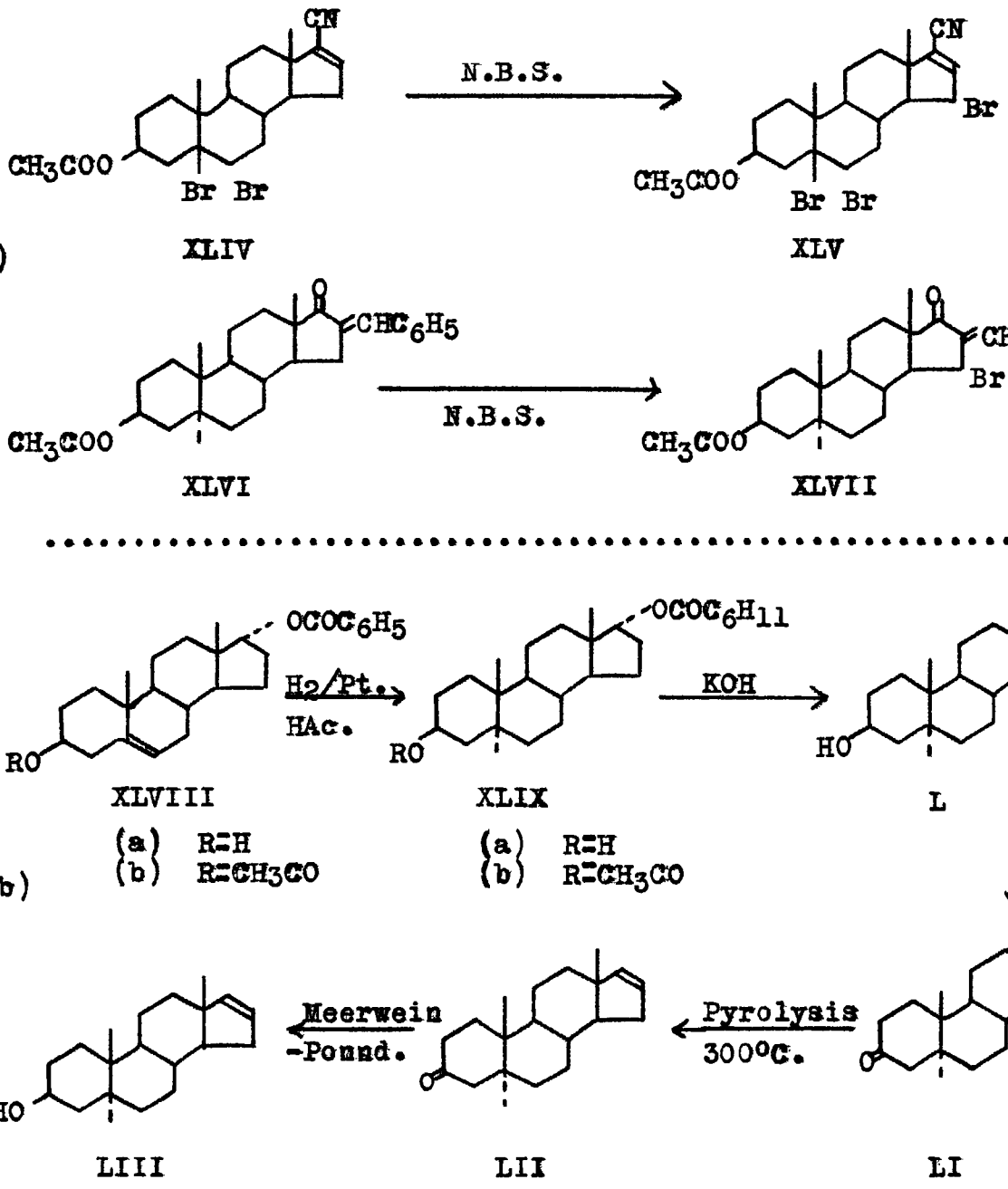
XVIII



$XXIV_a$

would fulfil these requirements were it not for both the alternative possible site of bromination in this compound and the difficulty, referred to previously, of saturating this bond by hydrogenation. For this reason a compound was sought in which the source of activation of C_{15} might come from a direction

CHART V

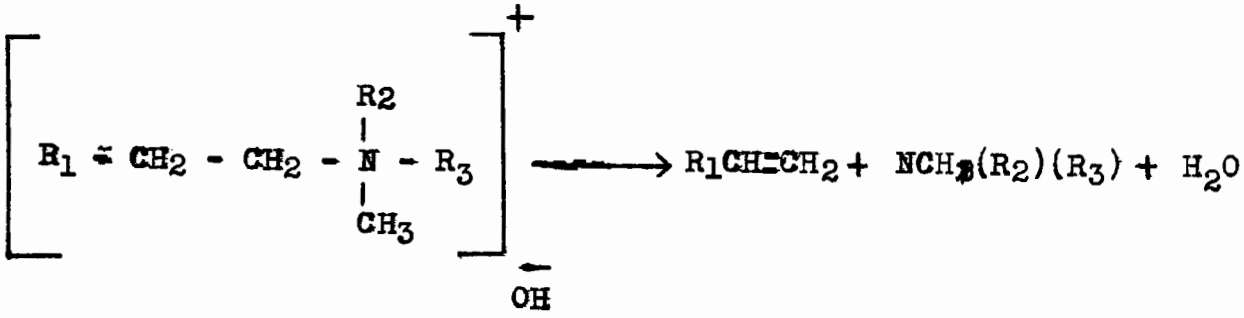

CN
CH3COO
Br Br
N.B.S.
Br
XLIV
XLV
(a)
CHC6H5
O
Br
XLVI
XLVII
OCOC6H5
OCOC6H11
H2/Pt.
HAc.
KOH
RO
HO
XLVIII
XLIX
L
(a) R=H
(b) R=CH3CO
(b)
CrO3
Meerwein
-Ponnd.
Pyrolysis
300°C.
LIII
LII
LI

within the molecule which would be compatible both with the lack of an alternate site for bromination and the subsequent facile removal of the activating group. There are a number of examples in the literature of bromination at C_{15} - as for example the conversion of 3β-acetoxy choladienenitrile 5,6 dibromide (XLIV) to the 15-bromo-3β-acetoxy-choladienenitrile 5,6 dibromide (XLV) by Plattner et al. (38) and also the preparation of the 15-bromo compound (XLVII) from 16-benzylidine epiandrosterone acetate (XLVI) by Scholz et al. (39). (See Chart V_a). Hence a suitable starting compound would be a Δ^{16}-androstenol-3β(LIII) or a structure analagous to the 16-benzylidine epiandrosterone acetate (XLVI).

Prelog, Ruzicka et al. (40, 41), in the process of identifying steroids obtained from testicular extracts, have synthesised Δ^{16}-androstenol-3β (LIII) by a route involving a series of reactions starting from Δ^{5}-androsten-3β,17α-diol (so-called "cis" diol) 17-benzoate as outlined in Chart V_b. This involves complete saturation of ($XLVIII_b$) to the hexahydrobenzoate ($XLIX_b$), partial saponification of this to the free 3-hydroxy compound (L) which after chromic acid oxidation and pyrolysis in nitrogen yields the Δ^{16}-androstenone-3 (LII). Meerwein-Ponndorf reduction of this leads to both the 3α and 3β-androstenol. The crucial step in this sequence of reactions is the hydrogenation of (XLVIII) to the hexahydrobenzoate (XLIX) which in our hands was found to behave capriciously. A more serious disadvantage is that the "cis" diol represents the minor product of the Raney nickel reduction of dehydro epiandrosterone acetate. Isolated by tedious recrystallisations from

the mother liquors after the main product has been removed, it is not a readily available compound. The 17β-isomer of (LI) does not undergo pyrolysis to the Δ^{16}-compound. While we were fortunate in obtaining some of the "cis diol" -17-monobenzoate, it was considered desirable to develop a new route to (LIII) especially in view of the erratic behaviour of the hydrogenation reaction.

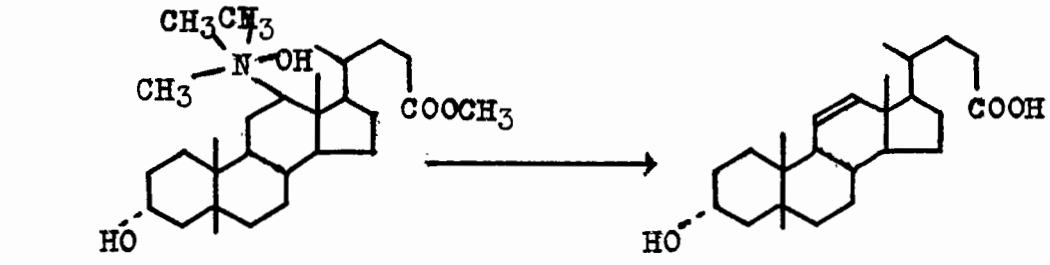
The Hofmann Degradation or "Exhaustive Methylation" of amines through the quaternary ammonium hydroxides is a reaction which has been employed extensively in many fields of organic chemistry - particularly in the realm of alkaloid chemistry where it has often been the major tool to provide chemical inroad into the molecule. Based on the instability of quaternary ammonium hydroxides towards heat and their tendency to revert to the tertiary base with the loss of a molecule of water, the reaction leads to the rupture of a -C-N bond.



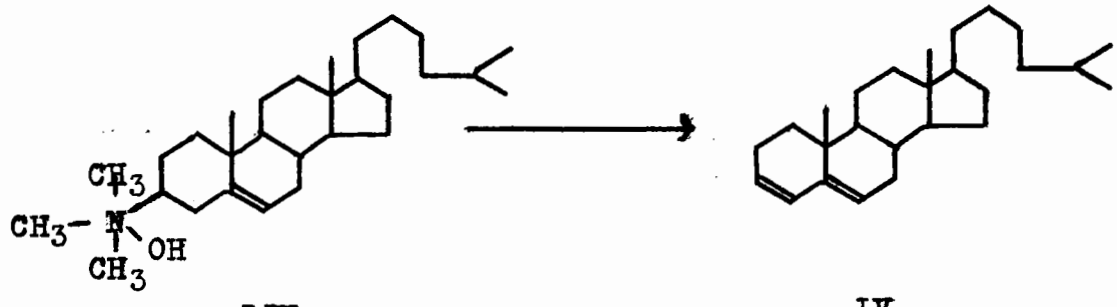
If R_2 and R_3 are methyl groups - i.e. if the original amine is primary - the "N", in reverting to the tertiary base

stage, splits out of the molecule as the volatile trimethylamine, together with a molecule of water, leaving the olefin R_1-CH=CH_2. If the amine is secondary or tertiary - as in alkaloids - the reaction results in a rupture of the nitrogen ring and a second stage of "Exhaustive Methylation" is necessary to remove the N from the molecule.

While this reaction is essentially a degradative tool for the study of natural compounds, it finds its application as a method for introducing unsaturation into intermediates required in synthetic work. However, it is only in more recent years that it has been used in steroid chemistry where, for example, McPhillamy and Scholz (42) have patented a process in which 3α-hydroxy-12-aminocholanic acid ester trimethyl ammonium hydroxide (LIV) is converted in high yield, by the action of sodium hydroxide and heat, into 3α-hydroxy-$\Delta^{11,12}$-cholenic acid (LV).



LIV LV



LIV_a LV_a

More recently Haworth et al. (43) have converted cholest-5-en-3β-trimethyl ammonium hydroxide (LIV_a) into cholesta-3:5 diene (LV_a) by vacuum distillation.

The initial step in this method of introducing a double bond involves the attachment of the amine group to the steroid molecule. Marker (44), Ruzicka and Goldberg (45) and McPhillamy and Scholz (42) have prepared steroid amines by sodium in alcohol reduction of the corresponding oximes as in the conversion of dehydro epiandrosterone oxime (LVI) to 17-amino Δ^5-androstenol-3β (LVII) (45). (See Chart VI_a). The displacement of the p-toluenesulphonyl radical by ammonia or amines has also been used (43), a reaction which in certain cases leads to Walden inversion.

On this basis it was intended to attempt the preparation of Δ^{16}-androstenol-3β (LIII) by means of the steps given in Chart VI_b. It was decided to form the amine (LIX) by reduction of the oxime (LVIII) and to convert to the methiodide (LX) by refluxing in the presence of methyl iodide with the addition of potassium carbonate to neutralise the hydriodic acid formed. While this method suffers from the disadvantage that a large excess of methyl iodide (100/1) and prolonged refluxing has to be used owing to the fact that methanol is used as solvent, the 3-hydroxyl group is not affected and does not have to be protected in this procedure for introducing the double bond (42). The conversion of the salt to the unsaturated compound has been done in one step by McPhillamy and Scholz (42), without isolation of the base, by

heating in aqueous sodium hydroxide. However, conversion of the salt to the base by treatment with silver oxide, followed by heat decomposition of the base, seems more efficient. Other methods of decomposing the base have been tried in the experiments described in this thesis with results which will be referred to later.

2. Experimental.

Preparation of Δ^5-Androsten-3β,17β-diol-3-monoacetate (46)

One gram of dehydro epiandrosterone acetate (172-173°C. Fisher Johns) was dissolved in 40 ml. of 95% alcohol and shaken in a hydrogenation apparatus with 800 mg. of freshly prepared Raney nickel (72). After 30 minutes, absorption was complete at 35 ml. The suspension was filtered free of catalyst and washed with 95% ethanol and the combined filtrate and washings evaporated *in vacuo* to an oil. Recrystallisation of the oil from hexane yielded 900 mg. of needles melting at 148-150°C. (Fisher Johns).

Benzoylation of Δ^5-Androsten-3β,17β-diol-3-monoacetate (47)

Four hundred and fifty-six milligrams of the diol monoacetate was dissolved in 2.5 ml. of pyridine and 2.5 ml. of freshly distilled benzoyl chloride added and the solution

allowed to stand overnight. It was then shaken with an ether-water mixture and the ether solution washed with dilute hydrochloric acid, sodium hydroxide and water until neutral. The dried ethereal solution was evaporated in vacuo and residue recrystallised twice from ethanol yielding 450 mg. of plates melting at 175-177°C. (Fisher Johns).

Hydrogenation of Δ^5-Androsten-3β,17β-diol-3-acetate, (48, 41) 17-benzoate to Androstan-3β,17β-diol-3-acetate 17-hexahydrobenzoate.

The androstendiol-3-acetate 17 benzoate (450 mg.) was dissolved in 20 ml. of purified glacial acetic acid and shaken with 40 mg. of pre-reduced Adams platinum oxide. After 40 minutes only 10 ml. of hydrogen had been absorbed; then an additional 45 mg. of catalyst was tipped in from a side-arm when a rapid uptake of hydrogen commenced and then slowed down again after 1 hour. Finally an additional 45 mg. of catalyst was added and shaking continued until hydrogen uptake had stopped. The catalyst was removed by filtration and the acetic acid solution diluted with water and saturated with sodium chloride. After standing for an hour it was filtered, washed well with water and dried in a vacuum desiccator over potassium hydroxide and calcium chloride. The crude product melted at 120-127°C. and on recrystallisation from methanol melted at 131-133°C. (Fisher Johns).

Preparation of Androstan-3β,17α-diol-17-hexahydrobenzoate (XLIX$_a$) (48, 41)

Six hundred milligrams of Δ^5-androsten-3β,17α-diol-

17-benzoate (XLVIII$_a$) was dissolved in purified glacial acetic acid (25 ml.) and placed in a hydrogenation flask containing 50 mg. of Adams platinum oxide. An additional 50 mg. of catalyst was placed in a side-arm in the flask and the entire apparatus evacuated 5 times and refilled with hydrogen. After shaking for 40 minutes, the hydrogen uptake was 35 ml. and began to slow down. Then the catalyst was tipped in from the side-arm and shaking continued. After 3 hours hydrogen uptake was complete at 210 ml. The catalyst was removed by centrifugation and washed with acetic acid. The combined filtrate and washings were diluted with water and after standing for 1 hour the precipitated material was filtered, washed, and dried in a vacuum desiccator over calcium chloride and potassium hydroxide. This crude material melted at 200-205°C. (Fisher Johns) and on recrystallisation the melting point was 201-204°C.

Preparation of Androstanol-17α-one-3, 17-hexahydrobenzoate (LI)(41)

Androstan-3β,17α-diol, 17-hexahydrobenzoate (0.5 gm.) was dissolved in 7.5 ml. of purified glacial acetic acid and a solution of 0.125 grams of chromic anhydride in 1.85 ml. of 90% acetic acid added. The mixture was let stand for 24 hours at room temperature during which time it gradually turned green. It was then poured into a saturated solution of sodium chloride with stirring. After extraction with two 50 ml. portions of ether, the ether extract was washed with 5% sodium carbonate, to remove the slight amount of gummy acidic material, and then finally with water until neutral. Evaporation of the solvent yielded

366 mg. of a colourless oil which, on crystallisation from methanol, gave beautiful needles melting at 137-137.5°C. (Fisher Johns).

Pyrolysis of Androstanol-17α -one-3,17-Hexahydrobenzoate (LI) to Δ^{16}-Androstenone-3 (LII) (40)

Five hundred milligrams of the hexahydrobenzoate was placed in a pyrolysing tube which was encased in a resistance wire embedded in asbestos. The tube was divided into two parts by means of a fitted glass disc of coarse porosity and was fitted with a thermometer. The temperature was controlled by means of a variable transformer inserted between the mains and the 22 ohm resistance wire. A slow current of nitrogen was passed through the tube and the temperature raised to 300°C. when a colourless oil with a strong musk odour collected in the outlet tube. This oil (496 mg.) was then refluxed for 3 hours in 22 ml. of 5% methanolic potassium hydroxide. On cooling, the alcoholic solution was diluted with water and ether extracted. The washed and dried ether extract yielded 307 mg. of a semi-solid oil after evaporation of the solvent.

Chromatography: The oil was dissolved in a mixture of 10 ml. pet. ether and 6 ml. benzene and placed on a column of 9 grams of alumina and the following fractions collected and combined.

1. Pet. Ether/Benzene: 1/1:: 111 mg. of oily crystals
2. Benzene/Ether: 3/1 & 1/1:: 80 mg. of oily crystals
3. Ether: 100% :: 40 mg. of oils

1. was sublimed in vacuo at 10 mm, 122°C. and yielded 56 mg. of Δ^{16}-androstenone-3 melting at 142-144°C. (Fisher Johns).

2. was presumed to be the androstanol-17α-one-3.

Difficulties in Hydrogenation:

When difficulties were encountered in the hydrogenation of the benzoate, various steps were taken to try to overcome them. Two preparations of Adams platinum oxide catalyst were made; one from ammonium chloroplatinate obtained by reworking platinum residues and the other from commercial C.P. chloroplatinic acid. In addition, a commercial preparation of Adams catalyst was used. All three were extremely active in reducing nitrobenzene to aniline. The catalyst made from ammonium chloroplatinate was completely inactive in the hydrogenation of the benzoates. Both the commercial catalyst and the one made from chloroplatinic acid behaved erratically on the 17β-benzoate (sometimes effective and sometimes not) and both were ineffective in the hydrogenation of the 17α-isomer. In the case of the latter isomer, there was occasionally an apparent hydrogen uptake (the apparatus was repeatedly checked for leaks) but on subsequent dilution of the acetic acid solution gas bubbles seemed to be evolved while the very sticky precipitate obtained always proved to be starting material. (It seemed as if some hydrogen was "absorbed" but was unable to react and was thus subsequently released). The solvent, hydrogen, and apparatus were checked for impurities (two different apparata and both electrolytic and purified "zinc/hydrochloric acid" hydrogen were used) but the source of the

trouble could not be found. In one instance the reaction was found to give the hydrogenated α-isomer when a little air was admitted to the flask during the course of the shaking. There was an overconsumption of hydrogen due to the oxygen but the material isolated and crystallised was the authentic androstan-3β,17α-diol 17-hexahydrobenzoate melting at 201-204°C. While the procedure used in the hydrogenation is that of Djerassi (48), use of Ruzicka's (#1) original procedure was equally unsuccessful. The samples of the 17α-isomer were taken from the same bottle as those used in the first successful hydrogenations. While the catalyst prepared from chloroplatinic acid behaved in the manner described in the hydrogenation of these compounds, it was quite efficient in the hydrogenation of Δ^5 etiocholenic acid - a compound difficult to hydrogenate. Finally a commercial preparation of 5% Rhodium on alumina (catalyst #310 of Baker & Co., Newark, N.J.), which is claimed to be especially efficient in the hydrogenation of aromatic compounds, was tried - both alone and in conjunction with platinum oxide - but it was found to be useless in this reaction.

As the amount of the Δ^5-androsten-3β,17α-diol 17-benzoate was not sufficient to warrant any further attempts to solve this difficulty in the hydrogenation reaction, this series of reactions had to be abandoned.

Preparation of Epiandrosterone Oxime (LVIII)

Epiandrosterone acetate oxime (11.7 gm.) was dissolved in 200 ml. of 2% methanolic potassium hydroxide and 4 ml. of water added. After 20 minutes some material started to come out of solution, so an additional 220 ml. of methanol was added. It was let stand for 4 hours and then brought to the boiling point. Four hundred and twenty millilitres of distilled water was then added slowly, the solution being kept at its boiling point. On cooling to room temperature it was placed in the refrigerator overnight. The crystals were filtered, washed with 10% aqueous methanol, and dried 2 hours in vacuo at 75°C. This yielded 10.9 grams of material melting at 190-191°C. (Kofler).

Preparation of 17ξ-Amino-Androstanol-3β(LIX)

Epiandrosterone oxime (LVIII)(10.6 gm.) was dissolved in 900 ml. of dry iso-amyl alcohol. The solution was heated and a total of 20 grams of sodium metal was added gradually so as to maintain a brisk reaction. Heating was continued until all the sodium had dissolved. The solution was then allowed to cool to about 70-80°C. and 900 ml. of distilled water added. On being allowed to stand overnight all the sodium amyloxide had decomposed and the alcohol layer was separated from the aqueous phase and repeatedly washed with water until neutral. This was evaporated in vacuo to yield a slightly yellow oil. This oil was taken up in dry ether and a stream of anhydrous hydrochloric acid passed through until precipitation of the amine hydro-

chloride was complete. The precipitate was filtered and washed with ether and then dried on suction and in a vacuum desiccator over calcium chloride. This yielded 10.75 grams of a white amorphous powder melting at 320°C. with decomposition.

$$[\alpha]_D = +7.2$$
(Ethanol)

Analysis:

	C	H	N	Cl
Calculated ($C_{19}H_{33}ONCl$)	68.9%	10.36%	4.26%	10.8%
Found	68.42%	10.47%	4.12%	10.45%
	68.12%	10.42%		

Report on the Infra Red Spectrum of 17 -Amino-Androstanol-3 Hydrochloride

"Investigated in potassium bromide (not soluble in chloroform) 1 mg. in 300 mg.

Findings: Major peaks at 1620 (broad), 1578-80 (low), 1520, 1460, 1405 & 1390 (double peak), 1160 & 1138, 1190, 1050 (strong, sharp), 982, 960 cm^{-1}.

Interpretation: 1050 is the -C-O- stretching band of the hydroxyl group; 1620 could be the N-H band of the NH_2 group; the bands near 1400 could be C-N vibrations (Bellamy, p.213); the bands at 1460 and 1390 belong to the $-CH_2-$ and $-CH_3-$ groups.

The peaks, except the one at 1050, are rather broad,

suggesting that the substance may be a mixture of C_{17} stereoisomers.

Conclusion: The spectrum agrees well with expectation."

Reaction of 17ξ-Amino-Androstanol-3β(LIX) with Nitrous Acid.

One hundred milligrams of the amine hydrochloride (= 88 mg. of amine), which had been recrystallised from ether-alcohol, was dissolved in 4.4 ml. of glacial acetic acid, diluted with water, and 2 drops of concentrated sulphuric acid added. The solution was cooled well in packed ice (0-5°C.) and 1.32 grams of sodium nitrite added gradually over a period of 1½ hours. On adding the sodium nitrite a precipitate appeared which gradually turned yellow during the course of the reaction. The reaction mixture was allowed to stand for 3 hours when the precipitate became white again and the suspension was then heated to 60 C., the precipitate now becoming quite oily. After being allowed to come to room temperature, the reaction mixture was diluted with water and the resulting aqueous suspension was ether-extracted and the ether extract washed with alkali and then with water until neutral. After drying over sodium sulphate, evaporation yielded 87 mg. of a semi-crystalline oil. This was dissolved in a little methanol and placed in the ice box. After several months some crystals appeared which were removed by centrifugation and recrystallised from methanol yielding plates melting at 163-165°C.(Kofler). On admixture with an authentic sample of androstan-3β,17β-diol

("isoandrostanediol") (M.Pt. 163-165°C.) it melts at 163°C., recrystallises immediately and melts again at 167-169°C. (Kofler).

Acetylation of 17ξ -Amino-Androstanol-3β (LIX)

One hundred and twenty milligrams of the amine hydrochloride was converted to the free base by dissolving in alcohol, alkalising, diluting with water and extracting with ether. This yielded 109 mg. of oily crystals which could not be recrystallised. It was dissolved in 15 ml. of refluxing ether and 1 ml. of acetic anhydride added when a white crystalline precipitate formed immediately. Refluxing was continued for 15 minutes and the crystalline material was filtered off and washed with ether and dried in air. This yielded 61 mg. of crystals melting at 268-269°C. (Kofler) with decomposition.

This material was soluble in glacial acetic acid and precipitated out on dilution with water. It is believed to be the N-acetyl 17β-amino-androstanol-3β (LXII) which is borne out by the infra red spectrum (see fig. 4).

Acetylation of N-Acetyl, 17β-Amino-Androstanol-3β (LXII)

Twenty milligrams of the N-monoacetate was dissolved in 2½ ml. of glacial acetic acid and 0.5 ml. of acetic anhydride plus a trace of p-toluenesulphonic acid added. After being allowed to stand overnight it was diluted with water and the precipitate centrifuged and washed with dilute sodium hydroxide and water until neutral. After drying and recrystallisation

from aqueous alcohol it melted at 202.5-205°C. and on admixture with the monoacetate melted at 186-247°C. (Kofler). The infra-red spectrum (see fig. 5) shows that the hydroxyl band is no longer present so that this material is the 3β-acetoxy, N-acetyl, 17β-amino-androstan (LXIII).

Preparation of N,N Dimethyl 17ξ-Amino-Androstanol-3β-Methiodide(LX)

Ten grams of 17ξ-amino-androstanol-3β-hydrochloride was dissolved in 500 ml. of absolute methanol (dried with magnesium) and to it was added 137 grams of anhydrous potassium carbonate and 48 ml. of purified methyl iodide. The mixture was refluxed on a water bath, care being taken to exclude moisture. At 12 hour intervals 23 grams of potassium carbonate and 24 ml. of methyl iodide were added until a total of 230 grams of the salt and 144 ml. of methyl iodide had been added. After 48 hours the reaction was stopped and the methanol solution filtered from the large deposit of salts which was then washed with methanol. The combined filtrate and washings were then evaporated in vacuo to dryness. The residue thus obtained (which still consisted largely of inorganic salts) was collected and placed in the thimble of a large Soxhlet extractor and extracted continuously with chloroform. The quaternary salt, only slightly soluble (about 100 mg./100 ml.), separated out of the chloroform solution in the form of fine needles as the extraction proceeded. Successful extraction necessitated frequent interruption of the process in order to repulverise the caked salts in the thimble. After

6 days, a total of 14 grams of quaternary salt was obtained, which was filtered from the chloroform and dried. The slightly coloured salt melted at 248-250°C. and after one recrystallisation from methanol this was raised to 270-272°C. (Kofler). It gave a very intense colour in the Beilstein Test. Optical rotation was $[\alpha]_{(Ethanol)}^{D} = +14.8$. The compound decomposed even on slight heating and no sample could be prepared for analysis.

Preparation of 17ξ-Amino-Androstanol-3β,N-trimethyl Ammonium Hydroxide (LXI)

One hundred and fifty milligrams of the quaternary salt was dissolved in 6 ml. of methanol and silver oxide, prepared from 105 mg. of silver nitrate, added. The suspension was shaken for 1½ hours at room temperature and the solution then filtered from the greyish precipitate. Evaporation yielded a colourless oil which on drying in a vacuum oven (water pump) at 100°C. was transformed into a buff coloured powder. After two recrystallisations from benzene, plates melting at 210-214°C. (Kofler) were obtained. On standing, the melting point dropped considerably. The combustion analysis results agree with those calculated from the empirical formula of the quaternary base.

Analysis:	C	N	N
Calculated ($C_{22}H_{41}O_2N$)	75.21%	11.73%	3.98%
Found	75.09%	11.65%	3.98%
	75.35%	11.71%	

Attempted Degradation of the Quaternary Base (LXI)

(I) .....by Vacuum Sublimation

One hundred and fifteen milligrams of a colourless oil, prepared as described above, was transferred to a small vacuum sublimator. The apparatus was placed in an oil bath and evacuated to 10-15 mm pressure. The temperature was slowly raised and at 145°C. a crystalline sublimate appeared on the condenser. After 6 hours, all had sublimed and the light feathery crystalline material was removed by washing with ether. On evaporation of the ether solution 60 mg. of amorphous material melting at 100-120°C. (Kofler) was obtained. It gave a yellow colour with tetranitromethane (not regarded as significant, as the base also gives this colour). It could not, however, be recrystallised and hence was dissolved in 20 ml. of 1:1 petroleum ether/benzene and placed on a column of 1.8 grams of alumina. Elution of this column yielded two fractions as follows:

(1) Pet. Ether/Benzene 1:1 :: 28 mg. of oily crystalline material m.pt. 99-110°C.(Kofler)

(2) Benzene; Benzene/Ether 1:1 :: 21.3 mg. of starting material melting at 212°C. (Kofler)

After two recrystallisations from acetone (1) melted at 107.5-109°C. (Kofler). It gave a yellow colour with tetranitromethane and failed to give a precipitate with digitonin. The infra-red spectrum suggests that there is no free hydroxyl grouping at C_3. (see below)

Analysis:		C	H	N
1.	Calculated for $C_{22}H_{41}O_2N$	75.21%	11.73%	3.98%
2.	for $C_{22}H_{40}O_2N$	75.42%	11.42%	4.00%
	Found	75.89%	11.49%	3.90%
		75.54%	11.37%	

Attempted Degradation of the Quaternary Base (LXI)

(2) .....in Alkali

Three grams of the quaternary salt was converted to the crude oily base which was then treated with 4.5 ml. of water and 4.5 ml. of a solution of 10 grams of sodium hydroxide in 8 ml. of water. This mixture was stirred well, placed in an oil bath and the temperature gradually raised. At about 140°C. a volatile base could be detected. The temperature was increased to 180°C. and kept there for two hours when no more base was evolved. On cooling, the mixture was diluted with water, filtered, and washed thoroughly with water to remove all the alkali. After drying in a vacuum desiccator, 1.77 grams of powdery material was obtained.

This product was placed in a sublimator, evacuated to 10 mm. and heated to 100°C. Initially, (a) 450 mg. of a colourless oil collected on the condenser and was removed as soon as crystalline material began to appear. The temperature was then raised to 140-145°C. and after another 7 hours, 390 mg. of a semi-crystalline oil (b) was removed. There remained

1 gram of material in the sublimator which after recrystallisation from benzene proved, by melting point and mixed melting point, to be undecomposed base.

Recrystallisation of the oily crystalline fraction (b) from cold acetone yielded only 30 mg. of crystals, melting at 105-107°C. (Kofler), and which proved, by mixed melting point, to be identical with the product previously obtained.

Attempt to increase the yield: Because of the low yield in the crystallisation, an attempt was made to purify the crude product prior to this operation. Hence the entire sublimate was recombined (about 600 mg.) and treated with 50 ml. of warm 3 N hydrochloric acid. On cooling, this suspension was extracted with ether, which removed 105 mg. of a yellow oil. This was discarded. The aqueous phase was then made basic with sodium hydroxide and again extracted with ether. Washing, drying, and evaporation yielded 340 mg. of crystalline material, melting at 99-104°C. (Kofler). However, recrystallisation of this from acetone (the only effective solvent) again yielded only 27 mg. of material melting at 107.5-108°C. (Kofler).

Chromatography of the crude product on alumina did not prove to be more effective than the procedure described above.

As mentioned in the discussion at the end of this section this material is not the Δ^{16}-androstenol-3β (LIII) (which melts at 127°C.) and its actual structure is very much a matter for conjecture.

Infra-Red Spectrum of Compound M.Pt. 107.5-109°C.

"Investigated in potassium bromide, 2 mg./300 mg. potassium bromide; spectrum #463; good spectrum.

Findings: bands at 2750, 1640 (very weak), 1475 & 1454, 1392 & 1381, 1360, 1328, 1275, 1257, 1213, 1195, 1160, 1115 (strong, sharp), 1048 & 1035, 970, 950, 935, 900 cm^{-1}, and many weaker ones.

Interpretation

...the low-intensity band at 1640 could be due to a Δ^4-bond (Δ^3 is listed (Jones and Healing, l.c.) 1647 cm^{-1}). The absence of a strong band around 1050 cm^{-1} speaks for the correctness of the assumption that this compound arises from (LXI) by loss of H_2O; (the band at 1048 cm^{-1}) in the present spectrum, the somewhat weaker one of a doublet 1048 & 1035, seems too weak for an (OH)-band, which is usually the strongest peak in this region of the spectrum."

Attempted Degradation of the Quaternary Base (LXI)

(3) .....in Collidine.

It was thought possible that refluxing the quaternary base in a high boiling basic organic solvent might effect the Hofmann Degradation. Hence, one hundred and fifty milligrams of the base was dissolved in 2.5 ml. of collidine and a small crystal of sodium hydroxide added. This was refluxed for 3

Hours, then cooled and poured into 3 N hydrochloric acid and allowed to stand. Some crystalline material separated out of solution. The mixture was then extracted with ether and the ether extracts washed with dilute hydrochloric acid, sodium hydroxide and water until neutral. Drying and evaporation yielded 35 mg. of a slightly yellow oil, which crystallised slowly on standing. Recrystallisation from cold acetone yielded 11 mg. of crystals which melted at 125-127.5°C. (Kofler) with slight softening at 122°C. On admixture with the unidentified product previously obtained, the melting point was 70-110°C. (Kofler). This material gave a typical colour with tetranitromethane (as compared to that given by a sample of Δ^{16}-androstenone-3 (LII) and also gave a precipitate with digitonin. On the basis of this and also its infra-red spectrum (see fig.6), it can be accepted as the authentic Δ^{16}-androstenol-3β (LIII).

However, all attempts to increase the very low yield of the reaction in collidine failed, as did also the substitution of a much stronger base (hexylamine) for collidine. Moreover, the Δ^{16}-compound did not always separate as neatly in the hydrochloric acid solution as in the instance described above. Occasionally, unchanged starting material tended to precipitate out of the solution making recrystallisation difficult.

Attempted Degradation of the Quaternary Base (LXI)

(4) .....Summary of Other Methods Tried.

In view of the above failures, a number of other methods were tried in order to effect splitting of the base. All of these returned starting material only.

(a) High vacuum sublimation at 100°C.

(b) Distillation in a current of nitrogen.

(c) Refluxing the base in solutions of sodium hydroxide in ethylene glycol and propylene glycol.

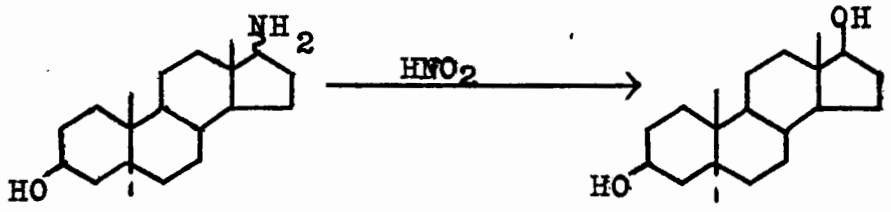
Thus, as this series of reactions did not provide a good route to the required Δ^{16}-androstenol-3β (LIII) they had to be abandoned.

3. Discussion

The first attempts to convert the Δ^{5}-androstendiol-3β 17α,17-benzoate ($XLVIII_a$) to the Δ^{16}-androstenone-3 (LII) by the sequence of reactions of Prelog et al. (40,41,47) were successful on small scale runs. However, at the point where a large scale (5 grams) hydrogenation of Δ^{5}-androstendiol-3β17α, 17-benzoate ($XLVIII_a$) had to be undertaken, freshly prepared Adams catalyst was employed (an old preparation was used in the previous runs) and the reaction immediately caused trouble. Despite the preparation of several batches of catalyst (which were

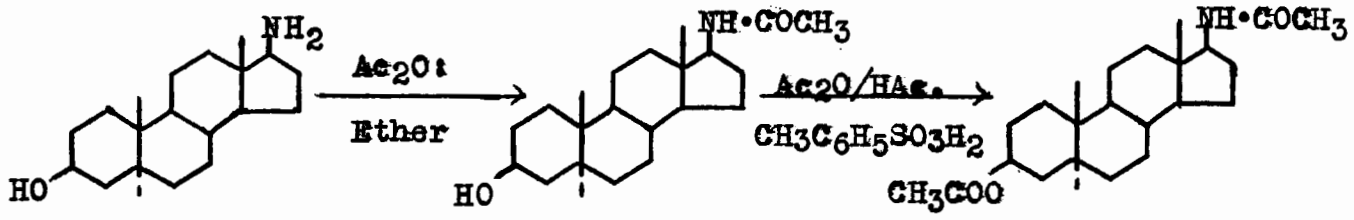
extremely active on other compounds), repeated checking of the solvent, hydrogen, and apparatus and also the use of a new rhodium catalyst, both alone, and in conjunction with platinum oxide, the difficulty could never be successfully overcome. An amount of the 17β-benzoate isomer was prepared in order to spare the limited quantity of the 17α-compound. In a few instances the hydrogenation could be effected by the device of allowing a small amount of air into the apparatus (which also resulted in an over consumption of hydrogen) yielding the authentic cis androstandiol, 17-hexahydrobenzoate. But this was never reliable. The capriciousness of the reaction is undoubtedly associated with the benzoyl radical as hydrogenation of aromatic compounds at atmospheric pressure is difficult. In this connection it is worth mentioning that Hickinbottom states (49_a) "... the efficiency of hydrogenation (of aromatic compounds) is dependent on the presence of oxygen in the colloidal platinum or palladium..."

In the projected alternate route to Δ^{16}-androstenol-3β (LIII), reduction of the epiandrosterone oxime (LIX) proceeded smoothly, yielding an oil which could not be crystallised and which undoubtedly contained a mixture of the isomeric 17-amines. The material was converted to the hydrochloride and was re-crystallised as such from ether - alcohol. The authenticity of the amine was established by combustion analyses, by the infra-red spectrum, and especially by the action of a cold solution of nitrous acid on the amine hydrochloride, which led to the isolation - in low yield - of androstan-3β,17β-diol ("isoandro-standiol").



LIX "Isoandrostandiol"

As the oily mixture of the amine isomers did not yield pure material by crystallisation and since it was not considered significant for the reactions to be subsequently employed, the bulk of the oil was methylated directly. However, at a later stage in the experiments it was found that one of the isomers could be separated from the oily mixture in the form of its N-acetyl derivative. When acetic anhydride was added to a refluxing ether solution of the base, a white crystalline material, which melted at 268-269°C. (Kofler), separated out immediately. This material was insoluble in dilute acetic acid, indicating the loss of the basic characteristics and, as the infra-red spectrum confirmed the presence of both the amide linkage and the free hydroxyl group (see fig.4), the compound is formulated as the N-acetyl,3β-hydroxy-androstanyl-17β-amine (LXII).



LIX_a LXII LXIII

The sharpness and regularity of the peaks in the infra red spectrum confirm that the compound is a single isomer and the abundant yield points to the orientation of the amine group as being 17β. Further evidence for the claim that it is a mono-acetate of (LIX) is supplied by the fact that reacetylation yielded the N-acetyl,3-β-acetoxy-androstanyl-17β-amine (LXIII) with a simultaneous disappearance of the hydroxyl band in the infra-red spectrum (see fig.5).

A forty-eight hour reaction time in the presence of a large excess of methyl iodide and potassium carbonate effected the complete conversion of the amine (LIX) to the corresponding trimethyl ammonium iodide (LX) (See Chart VI_b). This material, which proved to be only slightly soluble in chloroform - the most convenient solvent for separating organic from inorganic salts - required a prolonged extraction in a Soxhlet extractor in order to effect isolation of the compound in high yield. The compound was quite unstable and although it could be recrystallised readily from chloroform-methanol, it decomposed on slight heating and could not be subjected to microanalysis. The methiodide was converted readily and quantitatively to the quaternary base (LXI). (See Chart VI_b). This compound retained alcohol tenaciously and under these conditions it was an oil. Thorough drying yielded a powder which was recrystallised from benzene yielding plates whose microanalyses agree with the empirical formula $C_{22}H_{41}O_2N$ for structure (LXI). The infra-red spectrum (see fig.7) of this substance is anomalous as the hydroxyl band seems displaced from its usual position at 1055 cm^{-1} to 1067 cm^{-1}. In fact Dr. Weiss concludes from his

examination of this spectrum: "... The spectrum does not seem to agree too well with the postulated structure ..." However, from its mode of preparation alone there can be little doubt that the compound has the structure assigned.

The Hofmann Degradation

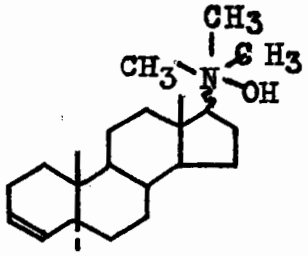
The degradation of quaternary bases is most commonly accomplished by vacuum distillation or sublimation, in which the volatile decomposition product trimethylamine is continuously removed, while the unsaturated product either sublimes or distills from the reaction site. McPhillamy and Scholz (39) have also employed heating in aqueous sodium hydroxide at high temperature (150-200°C.) and have obtained the unsaturated compounds in high yield.

When the quaternary base was sublimed in low vacuum at 140°C., fine feathery crystals collected on the condenser. This could not be recrystallised directly and was obviously a mixture. After chromatography on alumina, two fractions were obtained; one consisting of unchanged starting material melting at 212°C. and the other being a very oily crystalline fraction. On recrystallisation of the latter from acetone, there was obtained, in very low yield, a compound melting at 107.5-109°C.(Kofler). That this was not the required Δ^{16}-androstenol-3β (LIII) was evident from its melting point, its solubility in acid, and the failure to give a precipitate with digitonin. Its basic character was later confirmed by the combustion analyses which showed the presence of nitrogen.

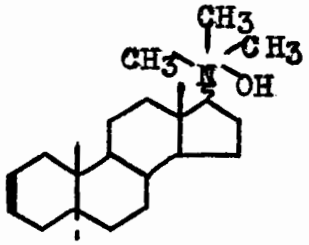
When the degradation was attempted by heating in aqueous sodium hydroxide, followed by chromatography, the same product was again isolated. Although some of the accompanying oil could be removed by partitioning between ether and aqueous hydrochloric acid, the yield in the recrystallisation could not be improved, so that only a small amount of the pure material was available for study.

Structure of the Compound M.Pt. 107.5-109°C.

This material failed to give a precipitate with digitonin and,on this basis, attempts to assign a structure involved consideration of the possible loss of the hydroxyl group at C_3. This seemed to find some support in the infra-red spectrum (see Experimental), where the 1067 cm^{-1} band (assigned to the 3-hydroxy group - though displaced) of the quaternary base (see fig.7) is missing - the 1048 cm^{-1} band being considered too weak for a hydroxyl band. The possible structures arising by elimination of water at C_3 would be the isomers (LXIV),(LXV).



LXIV



LXV

However, the carbon, hydrogen, and nitrogen analyses are completely contradictory to such a formulation and suggest the empirical formula $C_{22}H_{40}O_2N$. While the compound gives a

colour with tetranitromethane, no reliance can be placed on this, as the quaternary base itself (and also the salt) gives an intense atypical colour. Hence it is not possible to formulate a structure that would be in keeping with the data.

Degradation of the Base in Collidine.

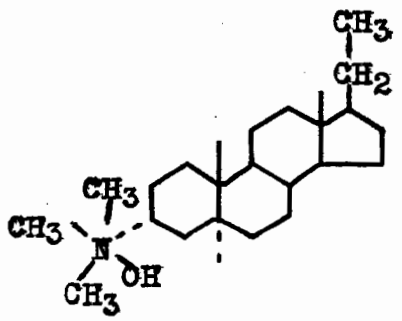
After various other methods (described in the experimental section) were tried unsuccessfully in an attempt to convert the quaternary base to the Δ^{16}-androstenol-3β (LIII), it was finally considered that refluxing the base in a high boiling basic organic solvent might effect the degradation. Hence the base was refluxed in collidine and the crystalline product which settled out of solution after pouring the reaction mixture into dilute hydrochloric acid melted at 125-127°C. As it gave a precipitate with digitonin and also produced a typical colour with tetranitromethane, it was formulated as the Δ^{16}-androstenol-3β (LIII). A comparison of the infra-red spectrum of this compound (see fig. 6) with the published spectrum of the 3α epimer (50) shows that the 1500-1350 cm^{-1} regions agree very well. The typical hydroxyl band is at 1048 cm^{-1} (1000 cm^{-1} for the 3α epimer) and the group 745-720-709 cm^{-1} is identical in both curves. This leaves little doubt that the structure of the compound is in fact as formulated. No authentic sample was available for direct comparison.

However, the yield was exceedingly low and all attempts to increase it failed. Moreover, the isolation of the andro-

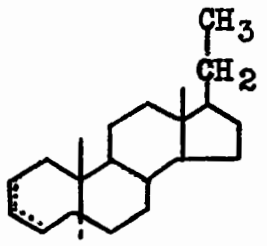
stenol was not always as neat as in the instance reported in the experimental section. Hence this approach had to be abandoned again.

The Failure of the Hofmann Degradation.

It has been shown in recent years that the Hofmann Degradation proceeds by means of a bimolecular elimination reaction (51). In alicyclic compounds the quaternary centre must be trans to a hydrogen on a β-carbon, and the state most favourable to elimination is where the quaternary N, the β-H and the two carbons form a coplanar arrangement (43,52). Thus it has been shown recently by Haworth et al. (43) that, whereas the 3α-dimethylamino allopregnane methohydroxide decomposes readily on vacuum distillation to give the allopregn-2 or 3-ene the 3β-epimer merely returns starting material.



3α-Dimethylamino Allopregnane Methohydroxide



Allopregn-2 or 3-ene

Owing to the A/B trans conformation of the steroid nucleus, the axial 3α-bond, in the representation of the steroid nucleus due to Barton (52) (Fig.8), forms a coplanar arrangement with C_2, C_4 and either trans hydrogen thus favouring elimination. This is not true for a 3βsubstituent, where neither hydrogen on a β-carbon allows of such a favourable coplanarity. Arnold and

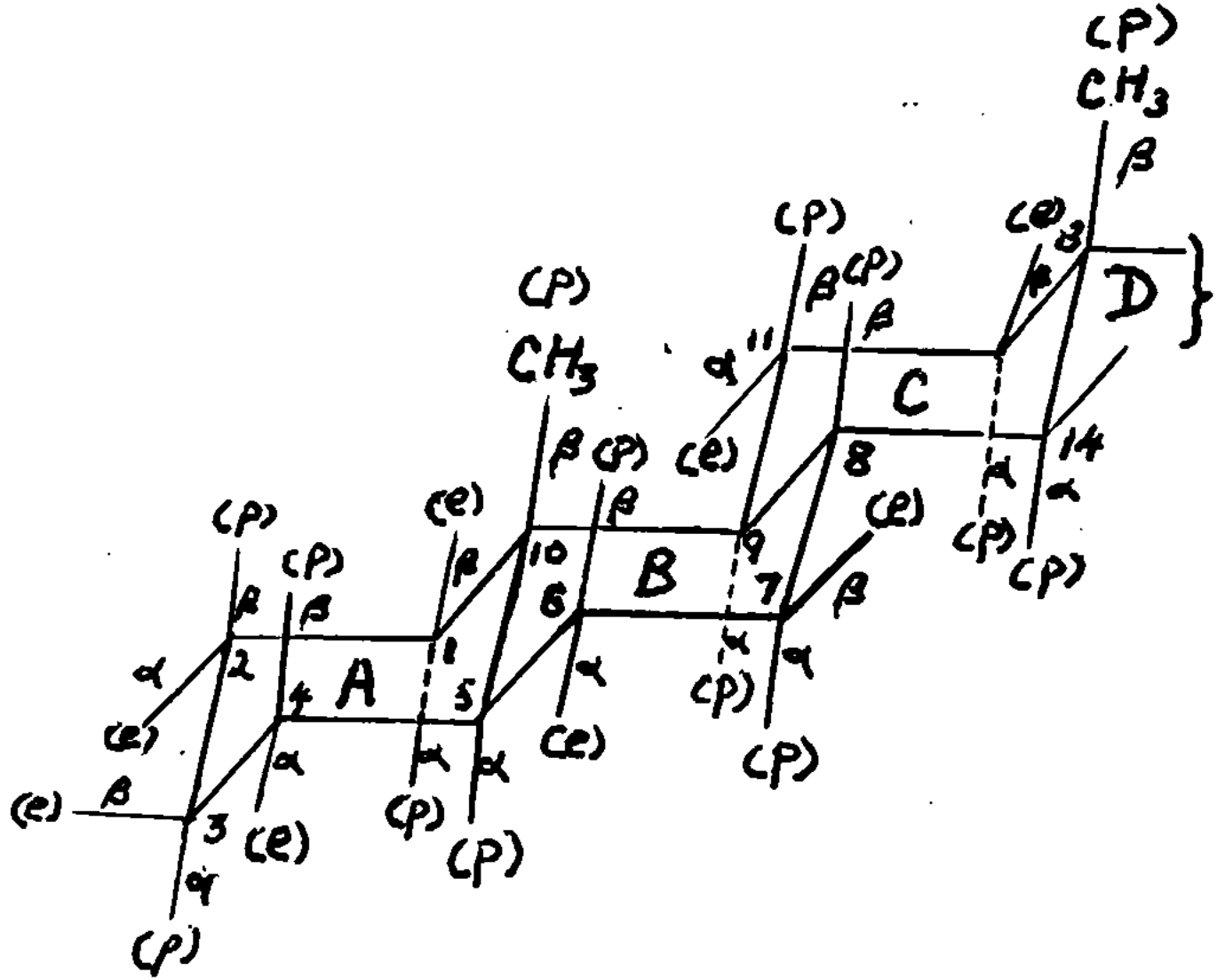


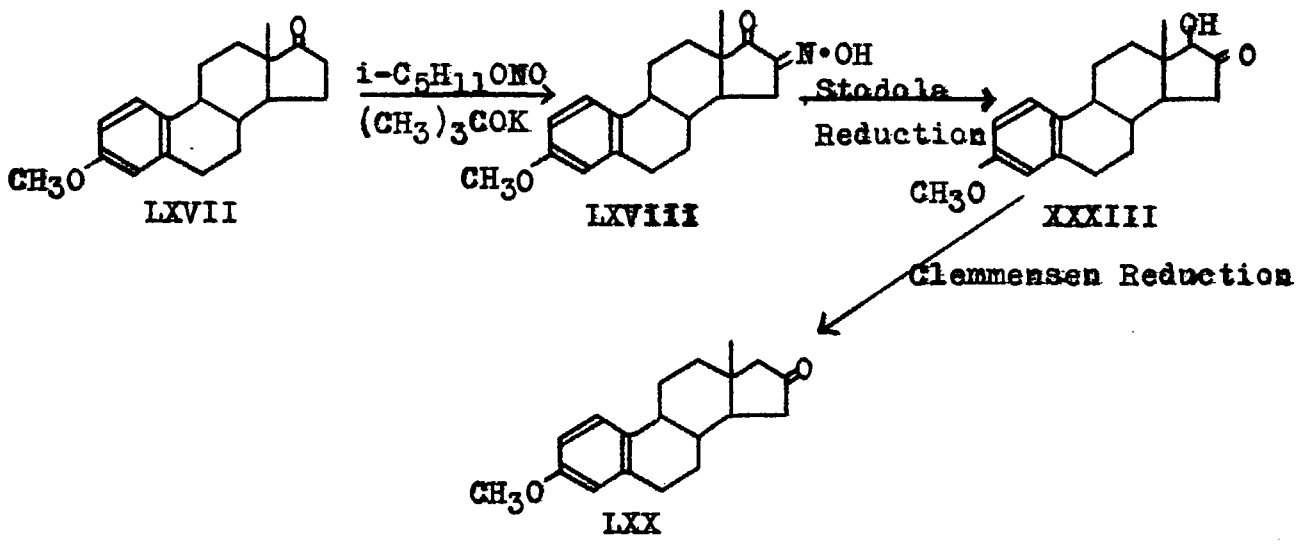
Figure 8

Richardson (53) have explained the Hofmann Degradation of 2-phenyl cyclohexylamines in similar terms.

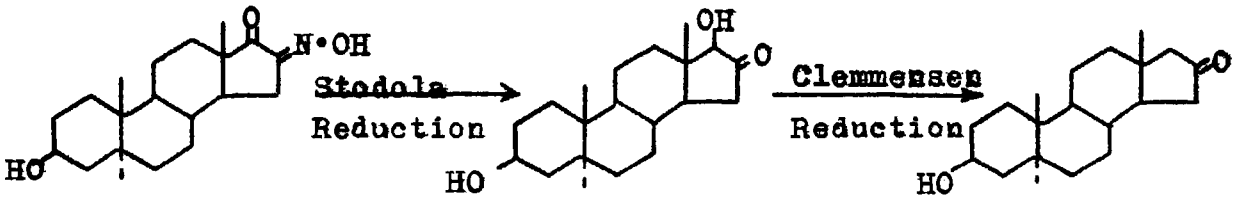
Examination of steroid molecular models with a view to explaining the failure of the quaternary nitrogen centre at C_{17} to be eliminated readily, reveals that both the 17β and 17α bonds have the same spatial arrangement relative to carbons 16 and 17 and the trans hydrogen on C_{16}. Neither of these is coplanar. A study of the steroid nucleus as represented by Barton (see fig. 8) shows that in rings A, B, C there is at every carbon one epimeric orientation which furnishes such an arrangement with a trans hydrogen on a β -carbon. However, the attachment of ring D to ring C is of such a strained nature that for substituents at C_{16} and C_{17} such an orientation favourable to bimolecular elimination reactions is precluded.

Hence, the resistance of this quaternary base to undergo degradation is in conformity with the current views of the mechanism of the Hofmann Degradation.

CHART VII



LXVII LXVIII XXXIII LXX



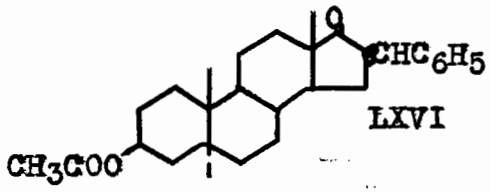
LXXII LXIX LXXI

PART C

II ..... via 16-Oximino Epiandrosterone (LXXII)

1. Theoretical.

Activation of C_{15} of an androstane compound is also furnished in the 16-benzylidine epiandrosterone acetate (LXVI)



LXVI

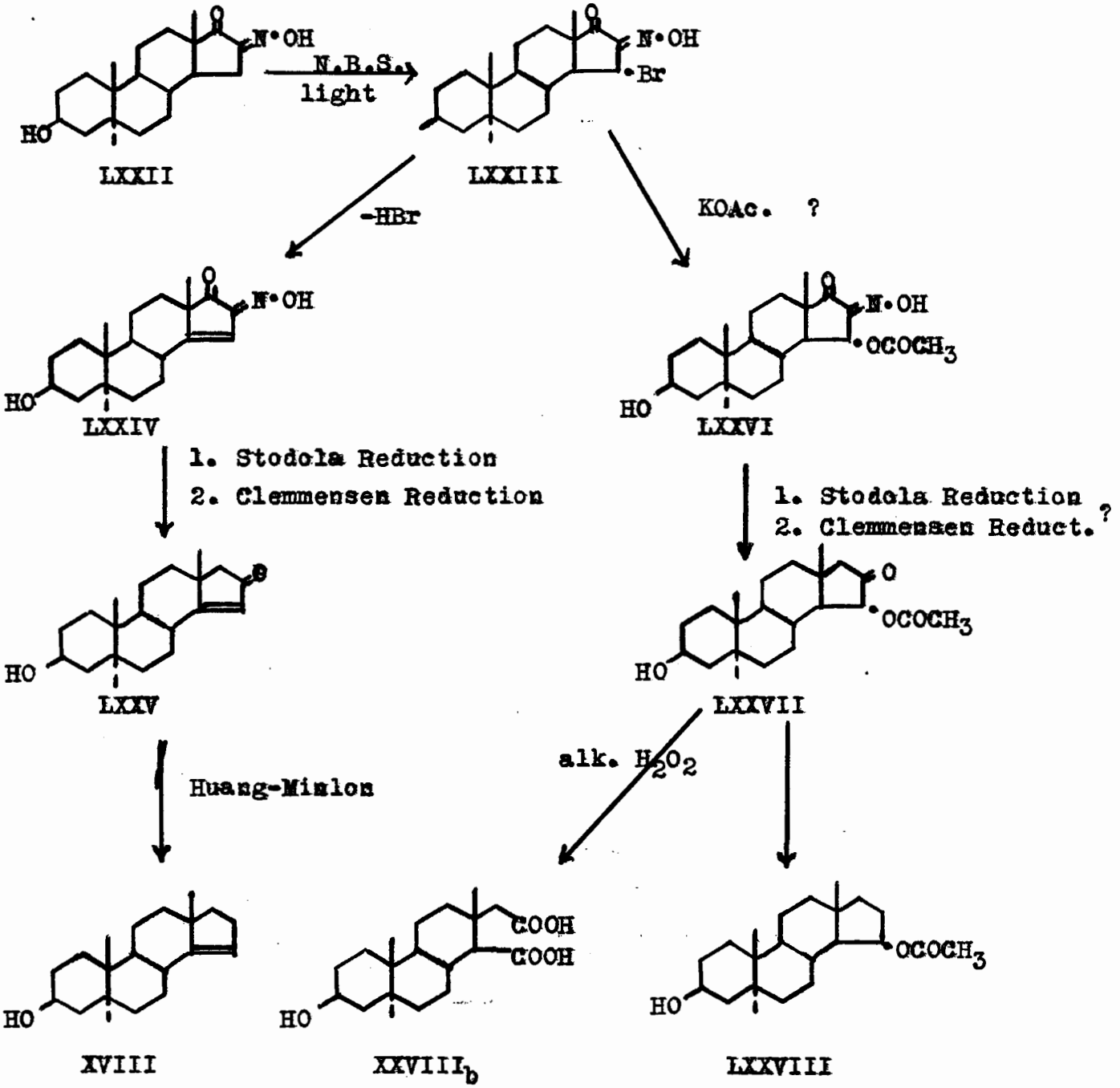
of Scholz et al. (39). Some analogous structure would be suitable for our purposes provided that the activating group could be subsequently removed. In 1938 Litvan and Robinson (54), seeking an improved route to marrianolic acid, were successful in preparing 16-oximino estrone-3-methyl ether (LXVIII) by the potassium tertiary.butoxide catalysed condensation of isoamyl nitrite and estrone 3-methyl ether (LXVII)(See Chart VII). This reaction has been extended to other steroids mainly by Huffman and Lott (22) who have been able to make profitable use of this reaction in leading to significant new compounds. By the zinc-acetic acid treatment of 16-oximino estrone-3-methyl ether (LXVIII) these authors (55) obtained, not an amine, but the ketol, 16-keto-estradiol-3,17β-3-methyl ether (XXXIII).

This reaction of oximino ketones was first observed by Stodola et al. (57) and later termed Stodola reduction. Such ring D ketols have been used for the preparation of 16,17 diketones (58) 16,17 diols (59), lactones (15) but more especially it has been observed by the same authors that Clemmensen reduction of, for example, 16-keto estradiol-3,17β-3-methyl ether (XXXIII) and 16-keto androstandiol-3β,17β (LXIX) yielded estrone-16,3-methyl ether (LXX) (60) and androstanol-3-β-one-16 (LXXI) (22) (See Chart VII), the latter proving identical with the androstanolone isolated from mare pregnancy urine by Heard and McKay (21).

The surprising elimination of the nitrogen atom in the Stodola reduction suggested to us that 16-oximino-epiandrosterone (LXXII) would be a compound capable of fulfilling the requirements of a suitable precursor to the 15-keto-androstanol-3β (XVIII). It was believed that a series of reactions such as those outlined on Chart VIII might provide a route either to (XVIII) or (XIX), referred to in the introduction of this thesis as the most desirable starting compounds in a route to Heard's lactone.

The hypothetical 15-bromo-16-oximino epiandrosterone (LXXIII) could result in two possible approaches:-

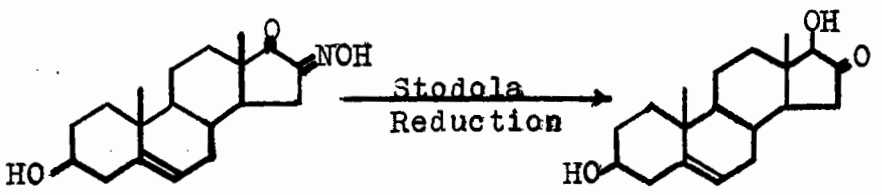
CHART VIII


N·OH
N.B.S.
light
Br
HO
LXXII
LXXIII
-HBr
KOAc. ?
OCOCH3
LXXIV
LXXVI
1. Stodola Reduction
2. Clemmensen Reduction
1. Stodola Reduction
2. Clemmensen Reduct.?
O
LXXV
LXXVII
Huang-Minlon
alk. H2O2
COOH
XVIII
XXVIIIb
LXXVIII

(A) proceeding through the unsaturated oximino ketone (LXXIV) offers the possibility of achieving - by what is essentially a duplication of Huffman's reactions - the synthesis of (XVIII);

(B), involving substitution of the Br by acetoxyl and leading ultimately to the acid (XXVIII$_b$) or the diol monoacetate (LXXVIII), is very much open to question both because of the uncertainty of the reactions leading from (LXXIII) to (LXXVII) and also because of the doubtful stability of (LXXVI) and hence will not be discussed.

In developing route (A) the only reaction whose behaviour is unforeseeable is the bromination of 16-oximino epiandrosterone (LXXII) to the 15-bromo compound (LXXIII). Steps from (LXXIV) to (LXXV) should not differ from the analogous reactions carried out by Huffman on a variety of 16-oximino,17-keto-steroids. The Δ^{14} double bond would probably be resistant to any tendency towards saturation in the Stodola and Clemmensen reductions. In this connection it may be remembered that Stodola reduction of 16-oximino, Δ^5-androstenol-3β-one-17 (LXXIX) yields the Δ^5-androstendiol-3β,17β-one-16 (LXXX)(61).



LXXIX LXXX

The reaction of oximes towards N-bromosuccinimide does not seem to have been studied at the time these investigations were undertaken and no reference to it is made by Djerassi (31) in his comprehensive review of the subject. For this reason and also because of the nature of the results obtained on first attempting to prepare the 15-bromo compound (LXXIII), some preliminary studies were made on the reaction between N-bromosuccinimide and steroid oximes. Subsequently, one paper appeared dealing with this reaction, which will be discussed, together with the experimental results found by us, in the discussion at the end of the experimental section.

2. Experimental

Preparation of 16-Oximino Epiandrosterone (LXXII)

One gram of potassium was dissolved in 35 ml. of dry tertiary butyl alcohol and the solution poured off from the slight sediment. Epiandrosterone (3.86 gm.) was then added and the mixture stirred for one hour under nitrogen by which time solution had been effected. To this was added dropwise 3.9 ml. of freshly prepared isoamyl nitrite. An orange colour developed almost immediately and a sediment gradually formed. After the addition of the isoamyl nitrite the flask was stoppered and stirring continued for a further 2 hours. It was then diluted with water and any insoluble material removed by ether extraction. After acidification of the yellow aqueous phase and allowing to

stand for a while, the precipitated material was extracted with a total of 500 ml. of chloroform. The combined chloroform extracts were then washed once with 250 ml. of 1% potassium carbonate and then the oximino ketone was extracted with 0.5 N potassium hydroxide (250, 200, 100 ml.). The combined potassium hydroxide extracts were then acidified with 6 N hydrochloric acid and allowed to stand for 3 hours. The precipitate was filtered, washed well with water, and dried in a vacuum desiccator over calcium chloride. It was finally dried in an Abderhalden at 85°C. for 1½ hours. This yielded 2.95 grams of a creamy white powder melting at 213-218°C. (Kofler) with decomposition.

The product thus obtained was dissolved in a mixture of 40 ml. of methanol and 10 ml. of water and boiled with charcoal for 10 minutes. After removal of the charcoal by hot filtration, the filtrate was concentrated down and allowed to crystallise. This yielded 2.435 grams of greyish white crystals melting at 220°C. (Kofler) with decomposition (Literature M.Pt. 218-219.5°C. (22)). By concentration of the mother liquor a further crop of 0.345 grams of equally pure material was obtained. The compound has an absorption peak at 240 mu (alcohol) in the ultraviolet (see fig.9).

Acidification of the potassium carbonate extract yielded a yellow oil which was discarded.

Report on the Infra-Red Spectrum of 16-Oximino Epiandrosterone

"Investigated in potassium bromide. Spectrum #497. Good spectrum.

Findings: Strong -(OH) peaks at 3410 and 3180-3205, weak band at 3030.

Strong band at 1700, band at 1635 (strong), broad band with peak at 1460, shoulders on both sides of it; bands at 1382 & 1365, 1308, 1278, 1215.

Series of bands of about equal strength at 1170, 1155, 1135, 1110, 1085; strong band at 1040; very strong band at 950, bands at 920, 880, etc.

Interpretation: 3410 and 1040 correspond to the 3-OH, 1730 to the 17-keto group. 3200 should be due to the hydroxyl of =N-OH, and 950 to the -N-O- of the oxime group. 1635 could perhaps be the -C=N- stretching vibration; this is listed at 1670 cm^{-1} for aliphatic oximes (probably for oximes not adjacent to an aromatic ring.) A shift to 1635 cm^{-1} in an α- oximino ketone like (LXXII) might not be unreasonable.

Conclusion.

The spectrum seems in reasonable agreement with expection."

Reaction Between N-Bromosuccinimide and 16-Oximino Epiandrosterone

(I) .....in Carbon Tetrachloride/Ethanol

The 16-oximino epiandrosterone (LXII) was completely insoluble in carbon tetrachloride and so 120 mg. of the compound was dissolved in a mixture of 10 ml. of carbon tetrachloride and 0.5 ml. of absolute ethanol. To this sixty-three milligrams of N-bromosuccinimide was added. A pale yellow colour appeared immediately, which deepened to orange on refluxing the mixture, and the N-bromosuccinimide gradually dissolved. After 5 minutes refluxing time, the colour disappeared abruptly and the solution was cooled. Evaporation of the solvent yielded a brownish oil which could not be crystallised. The oil was triturated with water and thus became powdery. The powder was filtered and washed thoroughly with water and dried in a vacuum desiccator yielding 126 mg. of material melting at 108-118°C. (Fisher Johns). The material showed no absorption in the ultraviolet and although it gave a positive Beilstein test for halogens, neither halogen nor nitrogen could be detected by sodium fusion. However a Dumas nitrogen determination indicated a nitrogen content of 4.48%. The material gave a slightly positive Zimmerman reaction but all attempts to obtain a pure compound failed, even after removing the small amount of starting material by washing an ether solution of the product with alkali.

Reaction Between N-Bromosuccinimide and 16-Oximino Epiandrosterone (LXXII)

(2) .....in Carbon Tetrachloride/Pyridine

One hundred and twenty-one milligrams of 16-oximino epiandrosterone (LXXII) was dissolved in 5 ml. of carbon tetrachloride and 1 ml. of pyridine. To this was added 63 mg. of N-bromosuccinimide and the solution refluxed under anhydrous conditions. An orange colour developed immediately and disappeared abruptly at the end of 15 minutes when the solution was cooled and filtered free of the dark precipitate (45 mg.). The pale yellow solution was evaporated in vacuo to an orange oil. On triturating this with ether a white sediment settled out which was removed by centrifugation and dried. This weighed 15 mg., melted at 212-220°C. (Fisher Johns) and showed an absorption peak at 224 mu (see fig. 10).

The ether supernatant was washed with 0.3 N hydrochloric acid, 0.1 N sodium hydroxide, and finally with water and then dried over sodium sulphate. On evaporation, a brownish amorphous powder was obtained which melted at 180-200°C. (Fisher Johns). The presence of nitrogen or halogens could not be detected by sodium fusion and the material defied attempts at recrystallisation. It exhibited a peak in the ultraviolet at 221 mu (see fig.10).

Preparation of Cholestanone (61)

Two grams of β-cholestanol (M.Pt. 139.5-141°C.) was

dissolved in 80 ml. of glacial acetic acid and a solution of 0.515 grams of chromic anhydride in 7.7 ml. of 90% acetic acid added slowly and the mixture shaken until the precipitated cholestanol had gone back into solution. When all the chromic anhydride had been added, the solution was allowed to stand, with occasional shaking, for 12 hours. At the end of this time the solution was completely green and it was poured into 400 ml. of water with stirring and then extracted with ether. The ether solution was washed free of acidic material with 1% sodium carbonate and then finally with water until neutral. Evaporation of the dried ether solution yielded 1.63 grams of a white powder melting at 126-129°C. (Fisher Johns). After one recrystallisation from acetone the melting point was 130-131°C. (Fisher Johns).

Oxime: this was prepared in alcoholic solution in the usual manner yielding material which, after recrystallisation from ethyl acetate, melted at 200-201°C.(Kofler); $[\alpha]_{(chloroform)}^{D} = +46.5$

Bromination of Cholestanone Oxime ($LXXXI_a$) Followed by Oxidation

Eight hundred milligrams of cholestanone oxime ($LXXXI_a$) was dissolved in 70 ml. of carbon tetrachloride in a flask previously flushed out with nitrogen. N-bromosuccinimide (354 mg.) was added and the flask again flushed out with nitrogen and finally stoppered. It was then placed on a shaking apparatus and as the reaction proceeded a bluish green colour was apparent

in the solution. After 6½ hours the reaction was stopped and the now amber coloured solution was filtered from the succinimide (184 mg.). Evaporation of the solvent in vacuo yielded a greenish-yellow oily solid with a pungent odour.

Oxidation:

This product was dissolved in 10 ml. of carbon tetrachloride and 24 ml. of glacial acetic acid. To this was added a solution of 200 mg. of chromic anhydride in 10 ml. of acetic acid and the solution allowed to stand for 30 hours. It was then diluted with water and extracted twice with 50 ml. portions of chloroform. The combined chloroform extracts were washed three times with water, three times with 1% sodium carbonate and finally with water again until neutral. It was then dried over sodium sulphate and evaporated to dryness to yield 715 mg. of slightly oily crystalline material melting at 135-160°C.(Kofler). Recrystallisation of this from ligroin yielded 288 mg. of feathery crystals melting at 164.5-167°C.(Kofler) and a further recrystallisation raised the melting point to 167-168.5°C. (Kofler). Sodium fusion showed the presence of halogen and the surprising absence of nitrogen. The optical rotation was $[\alpha]_{D}^{(chloroform)} = +44.7$. On admixture with a sample of authentic 2-bromo-cholestanone (LXXXIII) (M.Pt. 168-169°C.-Kofler) the melting point was 168-169°C.(Kofler).

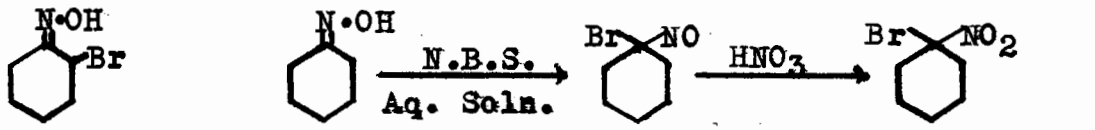
Reaction Between N-Bromosuccinimide and Epiandrosterone Acetate Oxime Followed by Oxidation

This reaction was carried out on 200 mg. of epiandrosterone acetate oxime using 165 mg. of N-bromosuccinimide. As no reaction seemed to take place at room temperature the solution was refluxed and a yellow colour appeared. The bluish colour observed with cholestanone oxime ($LXXXI_a$) was not evident. After 45 minutes the reaction mixture was cooled and the product worked up in the usual manner. It was then oxidised with chromic anhydride in glacial acetic acid and on working up the product 182 mg. of a pale yellow oil was obtained. This gave a very intense Beilstein test but could not be crystallised.

3. Discussion

As there was no previous report in the literature on the reaction between oximes and N-bromosuccinimide, this approach was undertaken with some reservations. The 16-oximino epiandrosterone (LXXII), whose preparation has recently been reported by Huffman (22), proved to be completely insoluble in carbon tetrachloride and solution could only be effected by the addition of a small amount of absolute alcohol. On refluxing this solution an obvious reaction took place as shown by the appearance

of a typical "bromination colour". However, examination of the crude product obtained showed that the ultraviolet absorption peak had disappeared completely, indicating some rearrangement of the oxime grouping. The product could not be purified. At this time a publication appeared (63), dealing with the reaction between N-bromosuccinimide and simple alicyclic oximes in aqueous solution, where it was shown that the product of the reaction was not the α-bromo oxime but the bromonitroso compound which could be oxidised to the bromonitro compound. Thus, for example, cyclohexanone oxime was converted to 1-bromo,1'-nitro cyclohexane by the action of N-bromosuccinimide followed by oxidation with nitric acid.



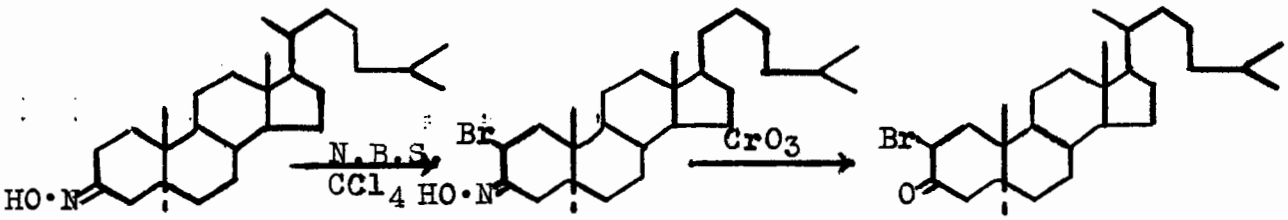
2-Bromo Cyclohexanone -1-Oxime | Cyclohexanone Oxime | Bromo-Nitroso Cyclohexane | Bromo-Nitro Cyclohexane

In an attempt to repeat this reaction on a steroid oxime in carbon tetrachloride solution, the reaction between cholestanone oxime ($LXXXI_a$) and N-bromosuccinimide was studied.

Bromination and Oxidation of Cholestanone Oxime ($LXXXI_a$)

When a solution of cholestanone oxime ($LXXXI_a$) in carbon tetrachloride was shaken in an atmosphere of nitrogen at room temperature an immediate reaction ensued which was completed in $6\frac{1}{2}$ hours. Oxidation of the product thus obtained was effected by allowing it to stand in chromic acid for 30 hours.

This resulted in the isolation - by crystallisation - of a compound melting sharply at 167-168.5°C.(Kofler) and which, because of the absence of nitrogen and presence of halogen, was obviously not the 3-bromo, 3-nitro cholestane expected. The melting point suggested that it might be the 2-bromo-cholestanone-3 (LXXXIII), - a not unreasonable possibility envisaged in the introduction to this section - and a mixed melting point with a sample of authentic 2-bromo-cholestanone-3 (LXXXIII) confirmed this. This was not substantiated further as the problem of interest to us at this time led in other directions. However, it would seem justifiable to say that the -C=N- bond has the same directing influence with respect to bromination by N-bromosuccinimide as the -C=C- bond (a fact not hitherto known) when the reaction is carried out in carbon tetrachloride solution. The reaction prevailing here in the case of cholestanone oxime ($LXXXI_a$) can be represented as



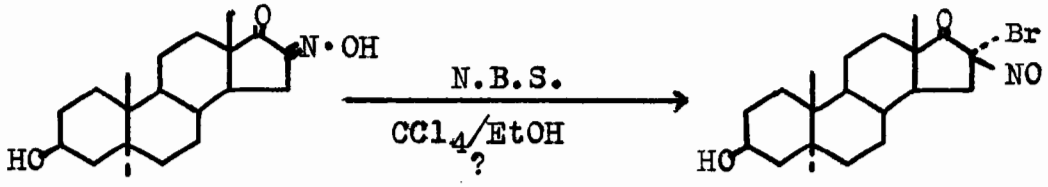
$LXXXI_a$ LXXXII LXXXIII

The overall yield of purified material was about 30% and would probably have been higher if the ratio of chromic anhydride to steroid had been greater than 1:1. As judged by by the development of a blue colour in the bromination reaction there is undoubtedly some bromo-nitroso compound formed also.

One attempt was made to repeat this reaction on epiandrosterone acetate oxime and while bromination and oxidation obviously took place no crystalline product could be isolated. This is not too surprising in view of our experiences in part B of this section of the thesis.

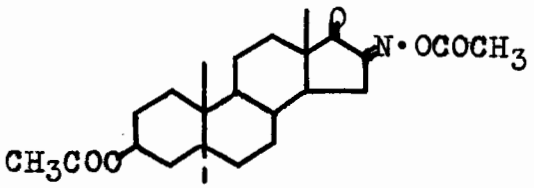
Bromination of 16-Oximino Epiandrosterone (LXXII)

Due to its extreme insolubility in carbon tetrachloride it was impossible to carry out this reaction on 16-oximino epiandrosterone (LXXII) itself. It is very likely that the product obtained by us, when the reaction was carried out in the presence of alcohol, was the 16-bromo-16-nitroso epiandrosterone which would explain the disappearance of the ultraviolet absorption peak, and is analogous to the products obtained by Iffland and Criner (63) from simple alicyclic oximes in aqueous solution. The strong tendency of nitroso compounds to dimerise would make crystallisation of this compound very difficult.



LXXII 16-Bromo-16'-Nitroso-Epiandrosterone

In an attempt to overcome the difficulty caused by the insolubility of the 16-oximino epiandrosterone (LXXII), it was decided to acetylate it in the expectation that the oxime acetate (LXXIX)

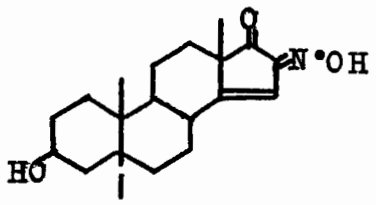


LXXIX

would be more soluble in carbon tetrachloride. However, the product obtained was not the oxime acetate and, in fact, the reaction which prevailed on attempted acetylation, together with the experimental work subsequently carried out, forms the topic of SECTION III of this thesis.

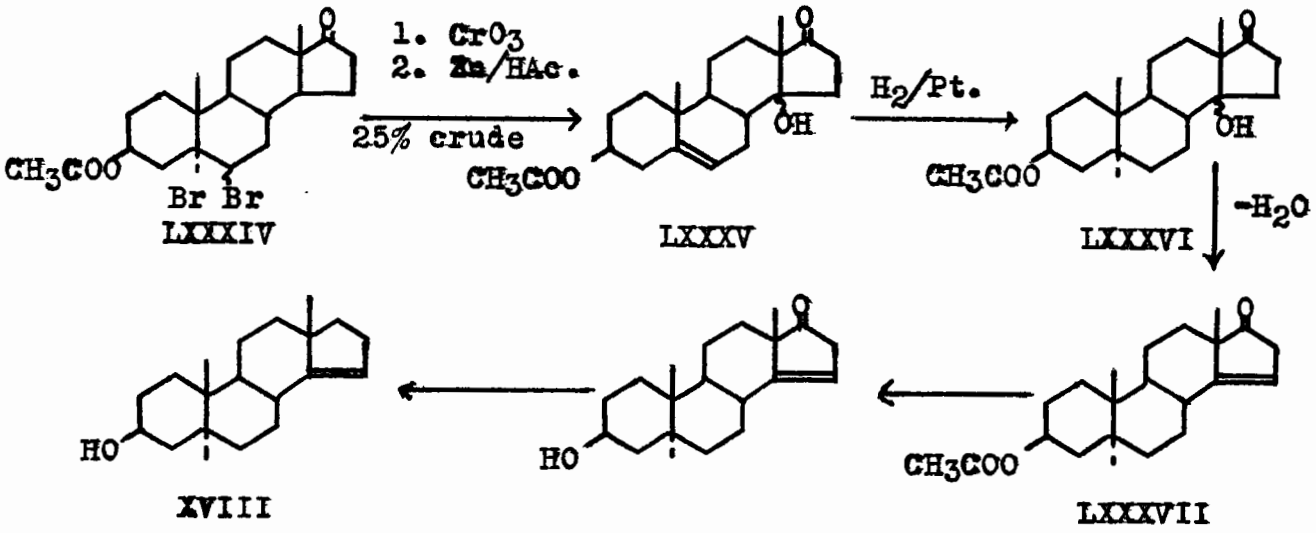
Conclusion:

One further avenue of appraach to the problem through the 16-oximino epiandrosterone (LXXII) remains and though based on a single experiment it is included here only as a possible starting point for future work. Refluxing the oximino ketone (LXXII) in carbon tetrachloride/pyridine with added N-bromo-succinimide resulted in two crude products - differing in their solubility in ether. While they could not be purified readily by crystallisation, their spectra in the ultraviolet, with sharp peaks at 221 and 224 mu respectively (see fig.10) suggest that a process of bromination and dehydrobromination may have taken place. If such is the case, the product of the reaction may be the Δ^{14}-16-oximino epiandrosterone (LXXIV), one of the goals in this approach - as outlined in the introduction.



LXXIV

Finally a recent publication by McPhillamy and Scholz et al. (39) affords an approach to the Δ^{14}-androstenol-3β (XVIII). The principal disadvantage is the very low yield in the initial step - the conversion of dehydroepiandrosterone acetate dibromide (LXXXIV) to the 3β-acetoxy,14ξ-hydroxy-Δ^{5}-androstene-17-one (LXXXV) by chromic acid oxidation and debromination.



LXXXIV LXXXV LXXXVI

XVIII LXXXVII

The remaining steps to the 3β-acetoxy,Δ^{14}-androstenone-17 (LXXXVII) have been carried out by these authors in good yield and the conversion of this to the Δ^{14}-androstenol-3β (XVIII) should present little difficulty.

SECTION III

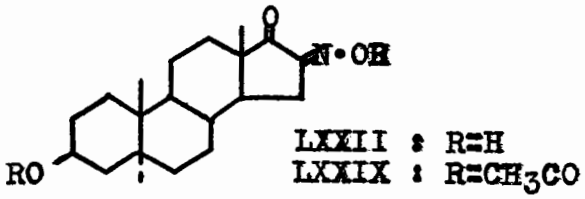
STUDIES IN THE BECKMANN REARRANGEMENT

STUDIES

ON THE BECKMANN REARRANGEMENT

1. Introduction

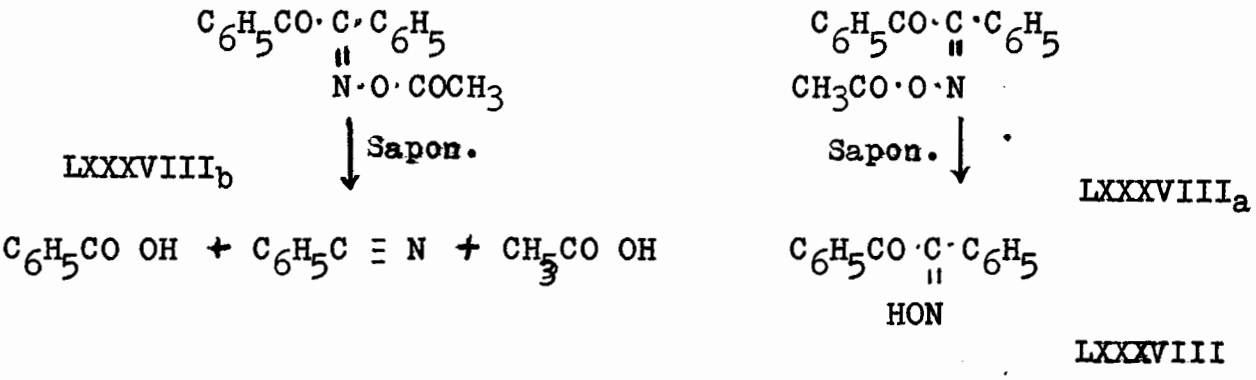
The work described in this section of the thesis arose from an observation in the studies on the bromination of 16-oximino epiandrosterone (LXXII). Realising that the solubility of this compound was a limiting factor in the attempts to carry out the above reaction, it was decided to subject it to acetylation in the hope that the expected 16-oximino epiandrosterone acetate oxime acetate (LXXIX) would be more soluble in carbon tetrachloride.



LXXII : R=H
LXXIX : R=CH_3CO

Acetylation of oximes - especially under the mild conditions used (acetic anhydride/pyridine at room temperature) - is a standard reaction and the oxime acetates are relatively stable compounds from which the original oxime can be generated by mild saponification. The only exceptions to the latter are the oximino ketones such as benzil monoxime (LXXXVIII). It was shown by Blatt et al. (64) that, whereas treatment of β benzil monoxime acetate ($LXXXVIII_a$) with alkali results in

saponification to original oxime, the α benzil monoxime acetate ($LXXXVIII_b$) undergoes the so-called "Second Order Beckmann Rearrangement" i.e. hydrolytic fission of the molecule into benzonitrile, benzoic acid and acetic adid.



Regardless of what the possible behaviour of 16-oximino epiandrosterone acetate oxime acetate (LXXIX) might be towards alkali, little doubt was entertained but that (LXXIX) would result from (LXXII) by treatment with acetic anhydride and pyridine at room temperature. That this was not so became apparent from a study of the product obtained and is exemplified in the fact that the reaction product exhibited a shift in the ultraviolet absorption peak from the 240 mu of 16-oximino epiandrosterone (LXXII) (see fig.9) to 223 mu. The explanation for this will be dealt with, together with the series of reactions subsequently carried out, in the discussion presented after the report of the experimental work.

2. Experimental

Reaction of Acetic Anhydride/Pyridine with 16-Oximino Epiandrosterone (LXXII)

One hundred milligrams of pure 16-oximino epiandrosterone (LXXII) (M.Pt. 220°C. with decomposition - Kofler; $\lambda_{max.}^{(alcohol)}$ 240 mu) was dissolved in 1 ml. of pyridine (freshly distilled from barium oxide) and 1 ml. of acetic anhydride (twice distilled from fused sodium acetate; B.Pt. 137-138°C.) and allowed to stand overnight. It was then poured, with stirring, into 50 ml. of 3 N hydrochloric acid. After filtration, it was washed thoroughly with dilute hydrochloric acid and water and then dried on suction and in a vacuum desiccator over calcium chloride. This yielded 102 mg. of colourless powdery material M.Pt. 161.5-163°C. (Fisher Johns). Recrystallisation from cold pure anhydrous ether (Merck) yielded 82 mg. of long feathery crystals, M.Pt. 163-165°C. (Fisher Johns), $\lambda_{max.}^{(alcohol)}$ 223 mu (see fig.9). The combustion analyses indicate the empirical formula $C_{23}H_{33}O_5N$.

Analysis	C	H	N
Calculated ($C_{23}H_{33}O_5N$)	68.48%	8.18%	3.47%
Found	68.49% 68.39%	8.38% 8.23%	3.38%

Crystallisation from Merck Reagent Ether yielded material melting at 155-164°C. (Fisher-Johns) in lower yield. The compound is

unstable - especially in the presence of light - and begins to turn yellow within one week of its preparation. It is especially unstable in solution in alcohol where the absorption peak at 223 mu gradually disappears, particularly in the presence of acid. This compound will be hereinafter referred to as "X".

Attempted Bromination of "X"

One hundred milligrams of "X" was dissolved in 13 ml. of pure, anhydrous, carbon tetrachloride and 46.5 mg. of N-bromosuccinimide added. Refluxing was commenced and after 10 minutes a yellow colour appeared which gradually deepened. After 45 minutes all the succinimide appeared to have reacted, so the reaction mixture was cooled, allowed to stand for 2 hours, and filtered from the succinimide (wt. 27 mg.). An aliquot of the filtrate was taken and, after evaporation to an oil, was dissolved in alcohol and examined in the spectrophotometer where a peak at 223 mu - of lessened intensity - was evident. The remainder of the filtrate was evaporated down under nitrogen to yield 134 mg. of a yellow oil. On recrystallisation from cold anhydrous ether, 39 mg. of crystalline material melting at 148-153°C. (Fisher Johns) was obtained. On admixture with "X" the M.Pt. was 159-163°C. Examination of the mother liquor in the spectrophotometer showed the peak in still lower intensity. Dehydrobromination of the oil obtained on evaporation of the mother liquor resulted in the recovery of some bromine as collidine hydrobromide, but no attempt was made to recrystallise

the oily product as the indications were that any brominated product formed had rearranged spontaneously and hence was useless for characterisation purposes.

Preparation of Epiandrosterone Acetate Oxime Acetate (CV)

One hundred and ten milligrams of epiandrosterone acetate oxime was dissolved in 1.5 ml. of anhydrous pyridine and 1.5 ml. of acetic anhydride and allowed to stand overnight. It was then poured into dilute hydrochloric acid solution with stirring and the precipitate collected on a filter, washed thoroughly with water and dried on the Abderhalden at 80°C. Recrystallisation from methanol yielded crystals melting at 180-180.5°C.(Fisher-Johns) and on admixture with epiandrosterone acetate oxime (M.Pt. 192-193°C.) melted at 148-158°C.

Partial Saponification of the Diacetate (CV) to Epiandrosterone Oxime Acetate (CVI)

Sixty milligrams (0.154 m moles) of the diacetate was dissolved in 5 ml. of methanol and 0.154 m moles of aqueous sodium hydroxide added. The pH was 10 and after standing 3 hours at room temperature fell to 8, then the solution was poured into 50 ml. water, acidified, and the precipitate collected on a suction filter. It was washed thoroughly with water and then dried on suction and in the Abderhalden. This yielded the epiandrosterone oxime acetate (CVI) M.Pt. 106-109°C. (Fisher Johns).

Saponification of Epiandrosterone Oxime Acetate (CVI)

Forty-three milligrams (0.121 m moles) of the oxime acetate was dissolved in 4 ml. of methanol and 0.121 m moles of aqueous sodium hydroxide added and the solution allowed to stand for 20 hours at room temperature. At the end of this time crystalline material had separated out and after filtration, washing and drying, melted at 192-194°C.(Fisher Johns). On admixture with authentic epiandrosterone oxime (M.Pt. 192-193°C.) it melted at 190-193°C. (Fisher Johns).

.....

M. Pt. of cholestanone oxime is .....200-201°C.(Kofler)
M. Pt. of cholestanone oxime acetate is.....124-138°C.(Kofler)

Preparation of Testosterone Acetate Oxime Acetate (CVIII)

Five hundred milligrams of testosterone oxime (M.Pt. 222-223°C.; Kofler; $\lambda_{max.}^{(alcohol)}$ 242.5 mu) was dissolved in 3 ml. of anhydrous pyridine and 3 ml. of acetic anhydride added. After 6 hours it was poured into 50 ml. of 3 N hydrochloric acid and the solid triturated well. Filtration, washing and drying yielded 610 mg. of material melting 113-131°C.(Kofler), $\lambda_{max.}^{(alcohol)}$ was 246.5 mu (see fig. 11). Since this was obviously a mixture of isomers no attempt was made to crystallise it.

Saponification:

On refluxing 150 mg. in 10 ml. of 1% alcoholic potas-

sium hydroxide for 1½ hours and crystallising directly from the hot solution, 85 mg. of authentic testosterone oxime (CVII), melting at 222-223°C. (Kofler) was obtained.

Rearrangement of "X"

As mentioned previously, "X" is fairly stable in anhydrous ether but rearranges spontaneously in wet ether and especially in alcohol. To observe the kinetic nature of the rearrangement the disappearance of the peak at 223 mu was followed with time in the Beckman Spectrophotometer* under various conditions. Solutions were made up in alcohol and in all cases zero time is the time of addition of the solvent to the material in the volumetric flask, although the material does not go into solution instantaneously. In tables I, II and III density values are converted to the value "E", where "E" = $D/M \times 10^3$. D is the observed density and M the initial molarity of "X". The curves on figures 13,14 are plots of "E" v Time. Photometric studies were also made to examine the stability of both testosterone oxime acetate (CVIII) and 16-oximino epiandrosterone (LXXII) under the same conditions which were used in following the rearrangement of "X". All this spectrophotometric data is tabulated in the following pages together with relevant experimental detail. The plots of the data on "X" are inserted in the discussion. (figures 13 and 14).

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* All spectrophotometric studies were carried out with the Beckman DU Quartz Spectrophotometer with photomultiplier attachment.

TABLE I

Rearrangement of "X" in Presence of Acid

Effect of increasing concentrations of hydrochloric acid at "high" concentration of "X"

	0.0152 m mole in 100 ml EtOH		plus 1 m mole HCl/m mole "X"		plus 2 m mole HCl/m mole "X"		plus 4 m mole HCl/m mole "X"		plus 8 m mole HCl/m mole "X"	
Time (mins.)	D	"E"	D	"E"	D	"E"	D	"E"	D	"E"
29	.....		.....	acid added	.....		.....		.....	
42	1.330	8750	1.326	8724	1.288	8474	1.101	7243	0.980	6447
47	1.277	8401	1.286	8461	1.237	8138	0.995	6546	0.825	5428
52	1.258	8277	1.258	8277	1.188	7816	0.909	5980	0.704	4632
57	1.234	8118	1.221	8033	1.120	7368	0.815	5362	0.595	3914
64	1.222	8039	1.194	7855	1.075	7072	0.705	4638	0.475	3125
69	1.212	7974	1.155	7599	1.020	6711	0.618	4060	0.393	2586
74	1.194	7855	1.138	7487	0.987	6493	0.563	3704	0.342	2250
79	1.165	7664	1.094	7197	0.923	6072	0.501	3296	0.291	1914
89	1.137	7480	1.063	6993	0.874	5750	0.423	2783	0.241	1586
94	1.127	7414	1.031	6783	0.838	5513	0.379	2493	0.214	1408
99	1.108	7289	1.020	6711	0.800	5263	0.347	2283	0.196	1289
104	1.104	7263	1.000	6645	0.780	5132	0.325	2138	0.180	1184
109	1.087	7112	0.975	6415	0.744	4895	0.292	1921	0.163	1072
114	1.077	7082	0.950	6250	0.716	4711	0.269	1770	0.154	1013
119	1.065	7007	0.930	6118	0.682	4487	0.250	1645	0.152	1000
124	1.047	6888	0.908	5974	0.658	4329	0.225	1480	0.138	908
129	1.038	6829	0.885	5822	0.631	4151	0.211	1388	-	-
134	1.034	6803	0.870	5724	0.610	4013	0.198	1303	0.130	855
147	0.980	6447	0.810	5329	0.545	3586	0.159	1046	0.116	763
157	0.960	6316	0.765	5033	0.487	3204	0.137	901	-	-
167	0.939	6178	0.731	4809	0.454	2987	-	-	-	-
179	0.924	6079	0.714	4697	0.438	2855	-	-	-	-
189	0.904	5947	0.685	4507	0.400	2632	0.131	862	-	-
199	0.880	5789	0.654	4303	0.373	2454	0.121	796	0.114	750
210	0.864	5684	0.643	4230	0.343	2257	0.110	724	-	-
220	0.842	5539	0.619	4072	0.321	2112	0.107	704	-	-
230	0.828	5447	0.598	3934	0.304	2000	0.101	665	0.114	750
240	0.810	5329	0.574	3776	0.281	1849	0.101	665	0.114	750
250	0.800	5263	0.550	3618	0.264	1737	0.101	665	0.114	750
260	0.779	5125	0.524	3447	0.246	1618	0.095	625	0.110	724
270	0.762	5013	0.500	3289	0.232	1526	0.095	625	0.107	704
290	0.676	4447	0.397	2546	0.168	1105	0.095	625	-	-
366	0.594	3908	0.308	2026	0.132	868	0.092	605	0.105	691
475	0.500	3289	0.225	1480	0.100	658	0.082	539	0.099	651
1209	0.167	1099	0.038	250	0.041	270	0.059	388	0.064	421

See fig. 13.

TABLE II

Rearrangement of "X" in Presence of Acid

Effect of increasing concentrations of hydrochloric acid at "low" concentration of "X"

Conc. of "X" : 0.0051 m moles in 100 ml alcohol

	Plus 1 m mole HCl/m mole "X"		Plus 2 m moles HCl/m mole "X"		Plus 4 m moles HCl/m mole "X"		Plus 8 m moles HCl/m mole "X"	
Time (mins.)	D	"E"	D	"E"	D	"E"	D	"E"
20	.....		acid added		.....		.....	
33	0.445	8725	0.455	8922	0.425	8333	0.421	8255
38	0.440	8627	0.457	8961	0.417	8176	0.407	7980
43	0.440	8627	0.450	8824	0.414	8119	0.390	7647
48	0.432	8471	0.439	8609	0.390	7647	0.379	7431
53	0.429	8412	0.438	8588	0.383	7570	0.363	7118
61	0.429	8412	0.430	8431	0.364	7137	0.338	6627
67	0.419	8216	0.421	8255	0.354	6941	0.323	6333
72	0.418	8196	0.414	8119	0.340	6667	0.306	6000
77	0.413	8096	-	-	0.337	6609	0.302	5922
82	0.402	7882	0.400	7843	0.324	6353	0.282	5529
87	-	-	0.395	7745	0.312	6118	0.267	5235
97	0.401	7863	0.386	7569	0.298	5843	0.249	4882
107	0.398	7804	0.375	7353	0.284	5569	0.220	4333
117	0.386	7569	0.366	7176	0.265	5159	0.210	4118
127	0.385	7549	0.360	7059	0.257	5039	0.200	3922
137	0.364	7137	0.341	6686	0.239	4686	-	-
147	-	-	0.338	6627	0.237	4647	0.173	3392
159	-	-	0.336	6582	0.230	4510	0.163	3294
169	0.359	7039	0.333	6529	0.220	4314	0.154	3020
189	0.350	6862	0.314	6157	0.198	3882	0.134	2627
264	0.320	6275	0.270	5294	0.156	3059	0.102	2000
334	0.295	5784	0.245	4804	0.126	2471	0.092	1804
392	0.274	5373	0.214	4000	0.108	2118	0.074	1450
459	0.235	4609	0.185	3627	0.093	1824	0.064	1255

See fig. 14.

TABLE III

Rearrangement of "X" in Boiling Alcohol

Conc. of "X" : 100 mg. in 10 ml. alcohol

Time	"E"
0	9,400
1 hour	1,410
3 hours	427
4 hours	427

.....

TABLE IV

Stability of 16-Oximino Epiandrosterone (LXXII) to Various Concentrations of Acid

$D_1^{240\ mu}$: 0.0167 m moles/100 ml. alcohol plus 1 m mole HCl/m mole.
D_2 : " " " " plus 2 " " "
D_3 : " " " " plus 4 " " "
D_4 : " " " " plus 8 " " "

Time	$D_1^{240\ mu}$	$D_2^{240\ mu}$	$D_3^{240\ mu}$	$D_4^{240\ mu}$
0	1.620	1.590	1.600	1.600
1 hour	1.615	1.600	1.580	1.600
22 hours	1.595	1.592	1.565	1.560
39 hours	1.620	1.620	1.580	1.575

TABLE V

Stability of Testosterone Acetate Oxime Acetate (CVIII)

to Various Concentrations of Acid

$D_1^{246.5\ mu}$: 0.008 m moles in 100 ml. alcohol
D_2 : " " " " plus 1 m mole HCl/m mole
D_3 : " " " " plus 2 " " "
D_4 : " " " " plus 4 " " "
D_5 : " " " " plus 8 " " "

Time	$D_1^{246.5\ mu}$	$D_2^{246.5\ mu}$	$D_3^{246.5\ mu}$	$D_4^{246.5\ mu}$	$D_5^{246.5\ mu}$
0	1.842	1.842	1.800	1.800	1.795
1 hour	1.842	1.842	1.820	1.820	1.820
18 hours	1.785	1.760	1.760	1.710	1.710

Saponification of the Rearrangement Product of "X"

Five hundred milligrams of freshly prepared "X" was dissolved in 50 ml. of 95% ethanol. It was refluxed for 6 hours at the end of which time the rearrangement was complete. Then 2.5 grams of potassium hydroxide was added and refluxing was continued for 20 hours. On cooling it was evaporated in vacuo and the residue dissolved in 50 ml. of water. Ether extraction removed 60 mg. of a yellow oil. The clear aqueous phase was then placed in a separatory funnel and acidified with concentrated hydrochloric acid. It was then shaken with two 30 ml. portions of chloroform which dissolved some material but left most of the solid in suspension. The entire chloroform and aqueous phases were then combined in one flask and the insoluble material re-

moved by filtration. After washing and drying this yielded 220 mg. of amorphous material melting at 214-218°C.(Fisher Johns).

The chloroform layer was then separated from the aqueous phase in a separatory funnel and was washed and dried. Evaporation of the chloroform solution in vacuo yielded 136 mg. of material melting 100-110°C.(Fisher Johns). This was now dissolved in 50 ml. of 10% aqueous potassium hydroxide and refluxed for 24 hours. Acidification, followed by filtration, washing and drying, yielded material identical with the product obtained above.

The combined product was recrystallised six times from methanol and after drying in vacuo over phosphorous pentoxide at 120°C., it melted at 218.5-220.5°C. (Fisher Johns). It was completely insoluble in chloroform, ether, ethyl acetate, benzene etc. Sodium fusion indicated the presence of nitrogen and the material was soluble in sodium carbonate. Titration indicated that it was a monocarboxylic acid (based on an equivalent weight of 338). Results of combustion analysis correspond to the formula $C_{19}H_{29}O_4N$.

Titration:

(a) The compound (10.8 mg.) was dissolved in 1 ml. of 0.1 N sodium hydroxide, diluted with water, and titrated while boiling with 0.01 N hydrochloric acid using phenolphthalein as indicator. This required 6.69 ml. of acid.

10.8 mg. of compound ≡ 3.31 ml. 0.01 N sodium hydroxide

1 m mole (338 mg.) ≡ 105.9 ml. " "

≡ 1.06 m moles sodium hydroxide

(b) 9.1 mg. of compound required 6.8 ml. of 0.01 N hydrochloric acid

1 m mole of compound ≡ 1.05 m moles sodium hydroxide

Analysis:

	C	H	N
Calculated ($C_{19}H_{29}O_4N$)	67.62%	9.26%	4.15%
Found	67.67% 67.61%	9.36% 9.40%	4.25%

The above material is the 3β-hydroxy-etioallobilianic acid semi-amide ($XCIV_a$). When this was dissolved in cold acetic acid and treated with a cold sodium nitrite solution, no evolution of nitrogen was evident. The unpurified chloroform-soluble product is presumably the 3β-hydroxy-etioallobilianic acid imide (XCV).

Saponification of the Semi-Amide at High Temperature

Forty milligrams of the pure semi-amide was added to 5 ml. of a solution of 20% potassium hydroxide in glycerol. The temperature of the oil bath was gradually raised to 200°C. when a copious evolution of bubbles of a basic gas took place. After 2½ hours, heating was stopped. The material came out of

solution on cooling, but on dilution with water a clear, slightly yellow, solution was obtained. This was acidified and after standing for 2 hours it was filtered and washed well with water. It was dried on suction and finally in vacuo over phosphorous pentoxide at 120°C. This yielded 20 mg. of material softening at 225°C. and melting at 234-237°C.(Fisher Johns). After one recrystallisation from methanol the melting point was 237.5-238.5°C.(Fisher Johns). (Literature melting point of 3 β-hydroxy-etioallobilianic acid is 238°C.) On admixture with the semi-amide it softens at 211°C. and melts at 216-238°C. Sodium fusion indicated the absence of nitrogen and it titrated as a dicarboxylic acid (based on an equivalent weight of 336) - 1.89 milliequivalents/milli mole.

3. Discussion

Treatment of 16-oximino epiandrosterone (LXXII) with acetic anhydride and pyridine at room temperature resulted in a compound which could be crystallised from pure anhydrous ether to yield long feathery crystals melting at 163-165°C.(Fisher Johns). The most striking property of this compound was its absorption peak in the ultraviolet with a $\lambda_{max.}^{(alcohol)}$ at 223 mu whereas the oximino ketone (LXXII) has a peak at 240 mu (see fig.9) i.e. a hypsochromic shift of 17 mu.

The compound is quite unstable even when stored in the dry state and begins to turn yellow after about one week. It is extremely unstable in alcohol or reagent grade ether - this instability being manifested in a disappearance of the absorption peak on standing in alcohol solution.

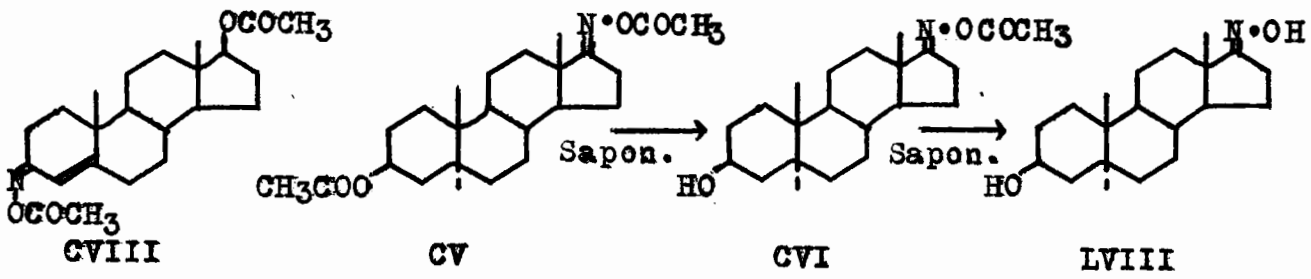
A search of the literature did not yield any information on the effect of acetylation on the absorption curves of α,β unsaturated oximes or of oximino ketones. In fact comparatively little information is available on the absorption due to such a chromophoric grouping as

$$-\overset{O}{\overset{\|}{C}}-C=N- \qquad \text{or} \qquad -C=C-C=N-$$

It seemed extremely improbable that the compound obtained was the acetate of the oximino ketone, as it is unlikely that mere acetoxyl substitution could have such a marked effect on any chromophoric system. However, it was decided to prepare testosterone oxime and testosterone acetate oxime acetate (CVIII) and to compare their ultraviolet spectra.

As is seen in figure 11, acetylation of the oxime-hydroxyl of testosterone oxime causes a bathochromic displacement of 5 mu of the position of the λ max. With this evidence which tended to eliminate an oxime acetate structure for "X", attention was directed towards an understanding of the transformation involved in the reaction and of the chemical nature of this unexpected compound. Because of the nature of the starting compound, and of the reaction conditions, the answer which first presents itself is that the transformation is a <u>partial</u> Beckmann

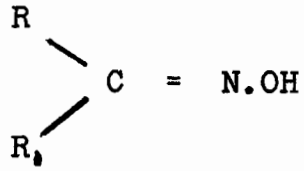
Rearrangement yielding as an end-product, in a relatively stable crystalline form, a true intermediate of the normal reaction - but taking place here under conditions which should properly yield the oxime acetate. Thus, for example, both testosterone oxime and epiandrosterone oxime, when subjected to the same conditions, yield in each case an oxime acetate (CVIII and CV) from which the original oxime can be regenerated by saponification.



CVIII CV CVI LVIII

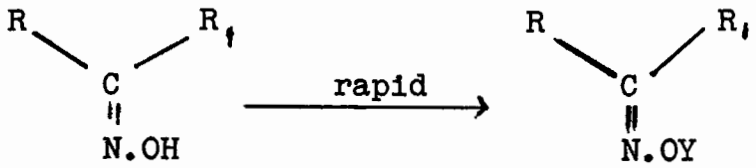
Structure of "X"

The mechanism of the Beckmann Rearrangement, in which oximes of the type



are transformed into amides by the action of strong acids or acyl chlorides, has been the subject of much study since the reaction was first discovered in 1866. As reviewed by Jones (66) and based on the structural investigations of Mesenheimer and especially the kinetic studies of Kuhara and of Chapman, the reaction can be formulated briefly from a structural point of view as:-

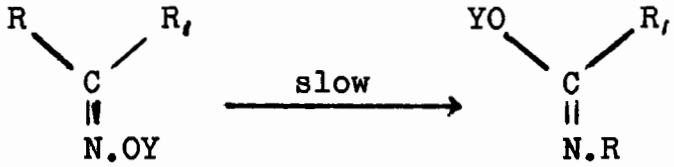
(a) conversion of an oxime (LXXXIX) into a derivative ($LXXXIX_a$)



LXXXIX $LXXXIX_a$

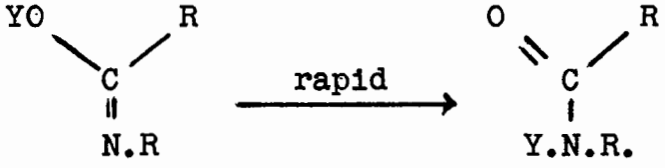
where Y may be an acyl or alkyl radical either simple or complex.

(b) a process of <u>trans</u> interchange between the group R and the group OY - caused by conditions which may be an intrinsic property of the Y grouping or which may be "supplied" in the reaction medium (which will be dealt with later) - leading to the imino structure (XC).



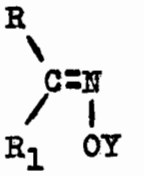
$LXXXIX_a$ XC

(c) the imino compound then rearranges to the amide (XC_a)

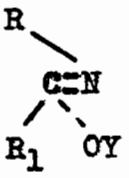


XC XC_a

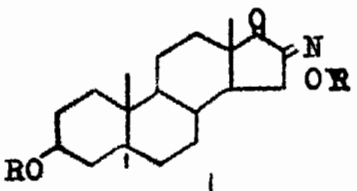
It must be noted here that the geometric isomers of the same oxime yield different amides due to the compulsorily



LXXXIX$_a$



XC

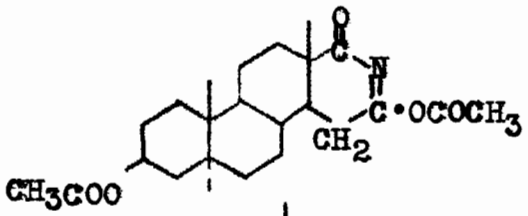


LXXII : R=H

LXXIX : R=CH_3CO

Beckmann Rearrangement

(First Stage)

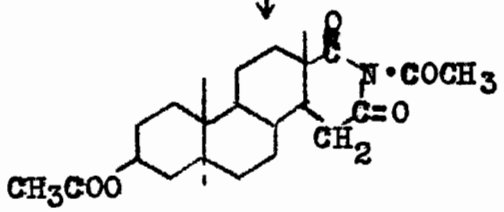


XCI

(Compound "X")

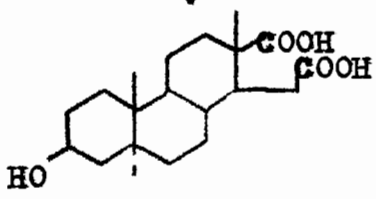
Beckmann Rearrangement

(Second Stage)



XCIII

Saponification



XCIV

trans nature of the interchange.

Within this framework of structural changes at present accepted as being the mechanism of the Beckmann Rearrangement, and making the assumption (which will be justified later) that the oxime hydroxyl of 16-oximino epiandrosterone (LXXII) is orientated "cis" to C_{15} and trans to C_{17}, - as shown - an intermediate in the Beckmann Rearrangement of the oxime acetate (LXXIX) would be the structure (XCI) (analogous to (XC)), (See Chart IX), which is hence postulated as the structure of compound "X" derived from (LXXII) by acetylation.

Evidence

(A) Chemical:

(1) Completed Beckmann Rearrangement:

The formal Beckmann Rearrangement of a 16-oximino-17-keto-steroid, presuming the oxime hydroxyl is orientated "cis" to C_{15}, should yield a diacid of the type (XCII) after saponification of the Beckmann end-product:-

XCII

That this in fact is so, is demonstrated by the preparation of marrianolic acid-3-methyl ether from 16-oximino estrone-3-methyl ether by treatment with phosphorous pentachloride in acetyl chloride followed by prolonged alkaline hydrolysis (54). This justifies the orientation assigned above to the oxime hydroxyl. Moreover, such a preferred orientation of the oxime hydroxyl in the case of 16-oximino compounds is logical in view of the sterically "cluttered" nature of this region of the molecule and one would expect the oxime grouping to adopt the configuration which allows more "space" to the hydroxyl grouping. This is in sharp contrast to ring A oximes. Thus testosterone oxime acetate and cholestanone oxime acetate (and hence the oxime itself) are obviously mixtures of geometric isomers, which accounts for the wide range of the melting points (See Experimental). On the other hand, epiandrosterone acetate oxime acetate (CV) is quite probably a single isomer - as judged from its sharp melting point.

If "X" has the structure assigned to it (XCI), it should readily undergo a transformation involving migration of the acetyl radical from the carbon to the nitrogen yielding the N-acetyl imide (XCIII)(See Chart IX). Furthermore, saponification of this imide should lead ultimately to the 3β-hydroxyetioallobilianic acid (XCIV).

As mentioned previously "X" is unstable in alcohol solution. When such a solution is allowed to stand at room temperature there is a gradual decline in the value of "E_{223}" and after about 24 hours the absorption peak disappears com-

pletely (see fig.13). The rate of this decline is dependent on a number of factors which will be dealt with later (see "Kinetics"). When the solution in alcohol is refluxed, only 3-4 hours are required for the complete transformation of (XCI) to (XCIII). Evaporation of this solution in vacuo yielded a pale yellow oil which defied all attempts at crystallisation.

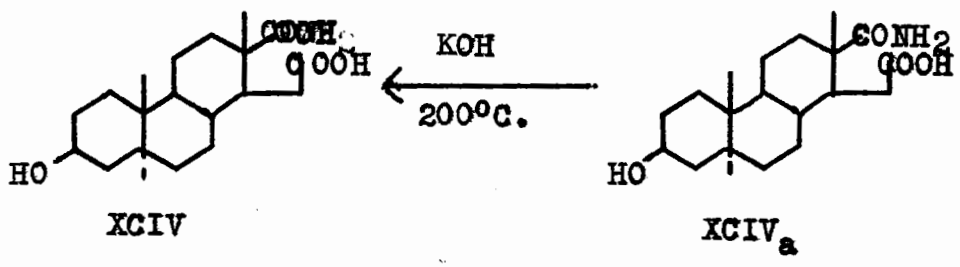
(2) Saponification of the Beckmann End-Product

When the oil obtained above - which showed no absorption spectrum - was refluxed for 16 hours in alcoholic potassium hydroxide, two substances were isolated:

(i) an alkali-soluble, chloroform-insoluble, material;

(ii) an alkali-soluble, chloroform-soluble, material.

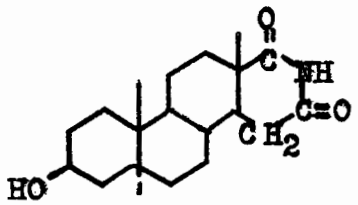
The compound (i) was completely insoluble in most of the solvents tried but could be recrystallised from methanol yielding fine needles melting 218.5-220.5°C. From its properties and the results of the combustion analysis it was formulated as the semi-amide ($XCIV_a$) of 3β-hydroxy-etioallobilianic acid (XCIV).



XCIV $XCIV_a$

Assignment of the amide grouping to the tertiary carboxyl is in keeping with the extreme resistance of the compound to hydrolysis and is borne out by the fact that it failed to react with a cold solution of nitrous acid - a reagent to which even some sterically hindered amides respond. The semi-amide structure is further supported by the fact that high temperature hydrolysis leads to the known 3β-hydroxy-etioallobilianic acid (XCIV).

The substance (ii) obtained in conjunction with (i) in the low temperature saponification melted at 100-110°C. (Fisher Johns). Unlike (i) it was quite soluble in chloroform and from its solubility in alkali and its convertibility to ($XCIV_a$) by heating in 15% aqueous potassium hydroxide it was presumed to be the 3β-hydroxy-etioallobilianic acid imide (XCV). However, no attempt was made to purify it.

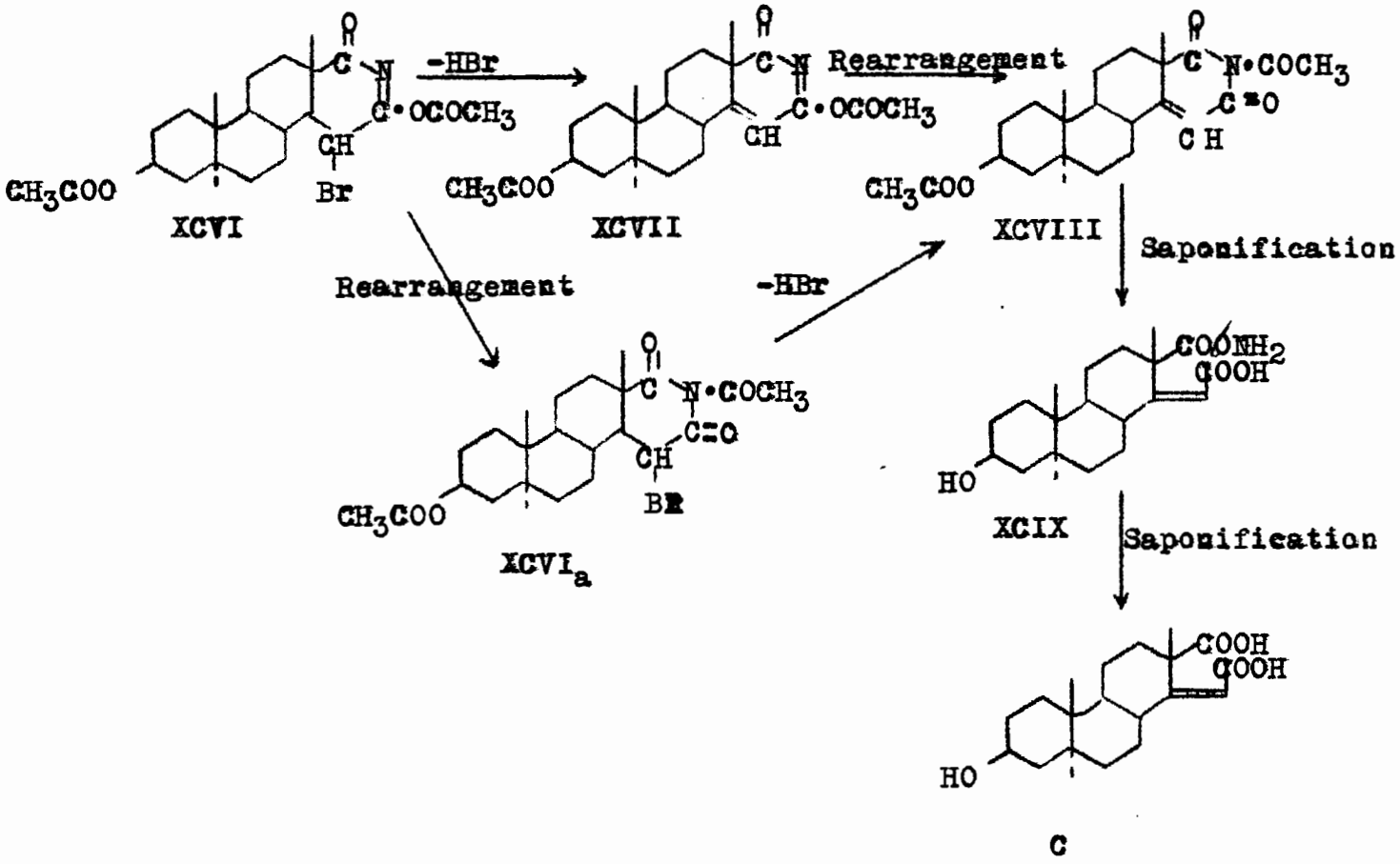


XCV

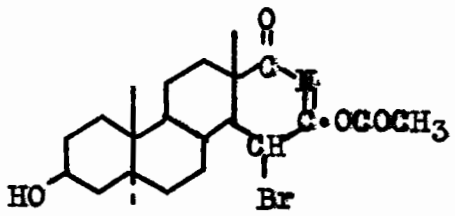
(3) Attempted Bromination:

The inherent instability of "X" (XCI) and its tendency to transform into (XCIII) makes consideration of any attempt at proving the structure directly rather difficult, as it would be a necessary prerequisite that this spontaneous transformation should not take place during the course of whatever chemical

CHART X


-HBr
Rearrangement
Rearrangement
-HBr
Saponification
Saponification
XCVI
XCVII
XCVIII
XCVIa
XCIX
C

reaction is applied. However, it was decided to attempt allylic bromination on C_{15} of "X" in the hope of obtaining the bromo compound (XCVI).



XCVI

The criterion essential to the reasoning here is that the compound (XCVI) should have essentially the same absorption spectrum as (XCI) and should also be capable of undergoing a transformation analogous to (XCI). The rationale of the application of the N-bromosuccinimide reaction is based on our own single observation that -C=N- bond has essentially the same "allylic activating power" as a -C=C- bond (see the bromination of cholestanone oxime - Section II) when the reaction is done in carbon tetrachloride.

The proof of the structure of the bromo compound (XCVI) would rest on subsequent dehydrobromination, rearrangement and saponification to yield the unsaturated acid 3β-hydroxy-Δ^{14}-etioallobilienic acid (XCIX) (See Chart X).

When this reaction was carried out, the red oil obtained on working up the carbon tetrachloride solution showed the same absorption peak as "X" (XCI) but in lower intensity. However, crystallisation from ether and re-examination of the mother liquors in the spectrophotometer showed that this peak was almost entirely due to the starting material isolated in the

crystallisation in about 40% yield. The indications are that (XCI) brominated to give (XCVI) but then rearranged spontaneously to give ($XCVI_a$)(CHart X).

It is hoped that, in the future, hydrogenation of the compound (XCI) with platinum black in anhydrous ether may lead to the saturation of the -C=N- bond. By thus stabilising the position of the acetyl radical on C_{16} its migration back to the nitrogen atom would be prevented. In this manner it may be possible to prove directly the structure of compound "X".

(B) Spectra

(1) Ultraviolet:

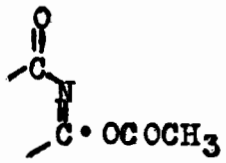
The relatively few studies that have been made (see, for example, refs.68, 69, 36) on the chromophoric systems such as,

$$-C=C-C=N- \quad ; \quad -\overset{O}{\overset{\|}{C}}-C=N-$$

have involved compounds where the "N" is derived from a semicarbazone, thiosemicarbazone, or phenylhydrazine moiety. It seems clear from these studies that the conjugating power of a -C=N- bond is of the same order of activity as a -C=C- bond so that α, β unsaturated ketones and their oximes have essentially the same absorption curves (36). The position of the $\lambda_{max.}$ of such compounds is interpreted as being due to a bathochromic displacement effected by the -C=N- group on the -C=C- ethylenic bond (which normally absorbs at 180-200 mu).

However, as the C=O group absorbs at 280-300 mu and the -C=N- does not apparently absorb between 200 and 400 mu, it is difficult, from an empirical point of view, (lacking other experimental information on the absorption of oximino ketones) to form some rationale as a basis for discussing the experimental results.

Taking the $\lambda_{max.}$ of the oximino ketone (see fig.9)(LXXII) as a starting point, it is clear that in this case the "activity" of a -C=N- group is not the same as a -C=C- in the sense that, whereas the 16'-methylene-3β-acetoxy-Δ^5androsten-17-one (XXXIX) (36) has an absorption peak at 228 mu, this oximino ketone has a peak at 240 mu (see fig.9). Furthermore, assuming the structure assigned to "X" is correct (i.e. (XCI)), the change to the new chromophoric system, which is

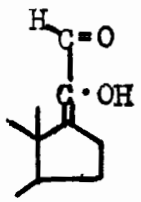


causes a hypsochromic shift of 17 mu. There are a number of factors which, although they cannot perhaps be directly integrated into an understanding of these spectra, are probably of significance and should be mentioned here. These are:-

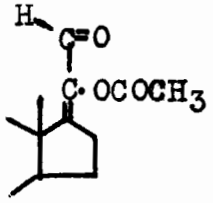
(a) In the new chromophoric system the -C=N- group is now conjugated to the carbonyl through the nitrogen atom rather than through the carbon.

(b) Acetoxyl substitution on an α,β unsaturated carbonyl system, while usually not very great, can

sometimes cause a displacement to the shorter wavelength of as much as 36 mu - as for example (taken from ref. 36),



$\lambda_{max.}$ 281 mu



$\lambda_{max.}$ 246 mu

(c) Conditions of steric hindrance, tending to distort the coplanarity of the absorbing system, can have a marked effect on both the intensity and position of the absorption peak (70). As considerable steric hindrance is known to prevail at least in the region of C_{17} and C_{16} of the steroid nucleus, and as the conversion of (LXXII) to (XCI) is also associated with an expansion of ring D to a six membered ring, this may be the chief factor associated with the shift in the absorption maximum.

In view of these points it is believed that the formation of (XCI) from (LXXII) should be accompanied by a pronounced shift in the $\lambda_{max.}$ although it is impossible to predict what the magnitude or direction of the displacement might be.

(2) Infra Red

Due to the lack of reference spectra little can be said. However Dr. Weiss, in his interpretation of these curves, states ... "in the case of the 16-oximino epiandrosterone (LXXII)

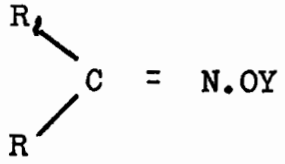
the spectrum (see Experimental) seems in reasonable agreement with expectation..." and for compound "X" (XCI); "except for the apparent absence of a band due to the ring carbonyl, the spectrum seems to agree reasonably well with expectation for a compound of such unusual type...". (See fig.12).

(C) Kinetics

The rate of completion of the second stage of the transformation (i.e. the disappearance of the absorption peak) was shown to be dependent on certain factors. For example, elevation of the temperature of the ethanolic solution of "X" from room temperature to the boiling point diminishes the time for the disappearance of $\lambda_{max.}$ from 24 hours (see fig.13) to 3 hours (see Table III). Furthermore, the rate of the rearrangement - as expressed in figures 13, 14 by a plot of "E" v Time - is dependent both on the presence of added hydrochloric acid and on its concentration (expressed on these curves as m moles hydrochloric acid/m mole of "X"). The rate is also dependent on the absolute concentration of "X" itself. This is especially marked in the catalytic effect exerted by added hydrochloric acid at two different concentrations of "X" and is evident from a cursory comparison of figures 13 and 14.

These experimental findings on the behaviour of "X", which were undertaken in order to supply some corroborative evidence for the structure postulated, are in agreement with the ideas established by the kinetic studies of Kuhara (see ref.66) who showed that polar effects, manifested either in the dielectric

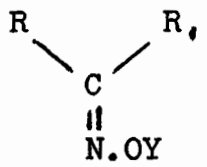
constant of the reaction medium or in the electron-attracting or electron-repelling nature of the substituent in the oxime derivative,



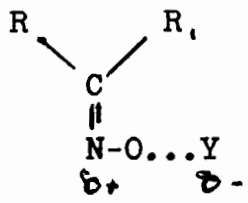
were an intimate part of the mechanism of this reaction and thus are a determining factor in the rate of the rearrangement. It should be emphasized that the work of Kuhara was done on the oxime itself - or oxime derivative (ester or ether) and much of the modern interpretation of the reaction is based on it. Hence the dependence of the rate of the rearrangement of "X" on these same factors points to it as an authentic intermediate in a normal Beckmann Rearrangement and argue in favour of the structure (XCI) assigned to "X".

Some General Comments on the Significance of Compound "X"

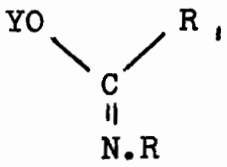
The presently accepted interpretation of the mechanism of the Beckmann Rearrangement postulates the initial formation of an ester or ether of the oxime of the type ($LXXXIX_a$), which then rearranges spontaneously, provided that the group Y is sufficiently electronegative in character (e.g. when Y is a picryl residue) as to create a dipole on the -N-O- bond as in ($LXXXIX_b$), to an intermediate structure of the type (XC).



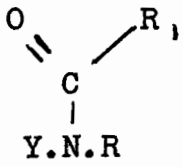
LXXXIX$_a$



LXXXIX$_b$

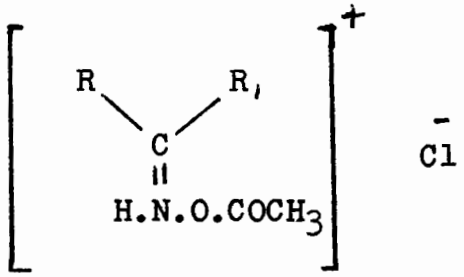


XC



XC$_a$

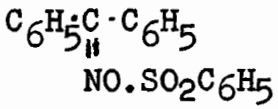
leading ultimately to the true Beckmann end-product (XC$_a$). Oxime acetates - normally stable compounds, because the acetyl radical is not sufficiently electronegative - can, by the addition of acid, be caused to undergo a rearrangement through the ammonium salt form (LXXXIX$_c$).



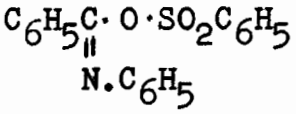
LXXXIX$_c$

Within the framework of these statements the significance of compound "X" is believed to be twofold:

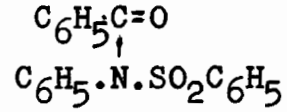
(1) As far as the author is aware no such intermediate as (XC) has ever been obtained or isolated in a pure crystalline form. Kuhara (quoted in ref.66) noted that the colourless benzenesulphonate of benzophenone oxime (CI) transformed on standing to a yellow oil which he represented as (CII).



CI



CII



CIII

However, Chapman (71) suggested that (CIII) was the more probable structure of this oil. Most of the oxime derivatives employed classically rearranged through intermediates which were too unstable to provide even evidence of their existence and it is only a fortuitous set of conditions obtaining in the case of the compound studied here which made possible the isolation of what is believed to be such an intermediate.

(ii) Oximes and oximino-ketones (e.g. benzil monoxime) form stable acetates which can be saponified to the original oxime (except in the case of the α benzil monoxime acetate, studied by Blatt et al., which undergoes the so-called "Second Order Beckmann Rearrangement"). The rearrangement of oxime acetates in the presence of acid is also explained as operating through an initial polarisation of the -N-O-bond by means of salt formation. In the formation of "X" from 16-oximino epiandrosterone (LXXII) another factor - apparently not hitherto encountered in studies on the Beckmann Rearrangement - presents itself. It is presumed that the oxime acetate (LXXIX) is a precursor to "X" (XCI) and the causative force in this transformation is the considerable strain present in ring D which now comes into play by means of a ring expansion - awakening an otherwise dormant Beckmann Mechanism. From the point of view of mechanism, it would seem that this strain can create a dipole on the -N-O- bond - the

pivotal structure through which all Beckmann Rearrangements pass - in the same way that the introduction of a highly electrophilic alkyl residue can - as in the spontaneous rearrangement of the oxime picryl ethers. However, the strain in itself is not sufficient to cause the rearrangement. Thus for example, 16-oximino epiandrosterone (LXXII) is quite stable to those concentrations of hydrochloric acid which markedly catalyse the rearrangement of (XCI) (see Table IV). There is a purely structural requirement in that the H of =N-OH must be substituted by an acetyl radical which presumably contributes some electrophilic character to the overall balance of forces.

Once the rearrangement to (XCI) takes place however, the balance of forces is restored and the rearrangement stops at the intermediate stage. Completion of the rearrangement requires the addition of either a polar solvent or of acid. It is almost certain that the employment of acetyl chloride in the initial step would not have led to the intermediate compound "X" but to a completed rearrangement.

Finally, it was rather paradoxical for us that this phenomenon of strain - attributable both to the fact that ring D is five membered and also that it is attached to the perhydrophenanthrene nucleus in the vicinity of the angular methyl group - should now be largely responsible for the isolation of "X"

having previously interfered with all attempts to develop a successful approach to Heard's Lactone.

SUMMARY

The work described in this thesis, while initially aimed at synthesising Heard's lactone, may be summarised by expressing it as an experimental encounter with certain features of ring D of the steroid nucleus. This is seen, first of all, in the failure of some classical reactions of organic chemistry to take place when the reacting groups are located in ring D. This failure is attributed to the strained nature of ring D and to the sterically "cluttered" nature of the region of the molecule in the vicinity of C_{16} and C_{17}. Thus, for example, monobromo substitution could not be effected at C_{16} and a quaternary base grouping at C_{17} could not be forced to undergo Hofmann Degradation except in very low yield. This special nature of ring D precludes the type of bond orientation at C_{16} and C_{17} that is favourable to bimolecular elimination.

On the other hand, this phenomenon of strain was manifested in a more positive fashion in that it was the causitive force leading to the spontaneous Beckmann Rearrangement of 16-oximino epiandrosterone acetate oxime acetate and led to the isolation, in a relatively stable crystalline form, of what is believed to be the first authentic intermediate of a Beckmann Rearrangement.

CLAIMS TO ORIGINAL RESEARCH

The following points in this thesis are claimed to be of an original nature:

1. Evidence indicating that the reaction between equimolar quantities of N-bromosuccinimide and epiandrosterone acetate (XXXIV) leads to the 16-dibromo (XLIII) and not the 16-monobromo epiandrosterone acetate (XXXV).

2. The isolation of a by-product of the above reaction: an unidentified bromo-compound possessing the spectral properties of an α,β unsaturated ketone.

3. The preparation, isolation and characterization of:

   (a) 17ξ-amino-androstanol-3β (LIX)

   (b) N-acetyl,17β-amino-androstanol-3β (LXII)

   (c) 3β-acetoxyl,N-acetyl-17β-amino-androstan (LXIII)

   (d) N,N dimethyl,17ξ-amino-androstanol-3β methiodide (LX)

   (e) 17ξ-amino-androstanol-3β,N trimethyl ammonium hydroxide (LXI)

4. The conversion of 17ξ-amino-androstanol-3β to androstandiol-3β,17β ("isoandrostandiol") by the action of nitrous acid.

5. The isolation of an unidentified compound, m.pt. 107.5-109°C.,

$C_{22}H_{40}O_2N$, after vacuum sublimation and sodium hydroxide-heat treatment of the quaternary base (LXI).

6. The Hofmann Degradation- by refluxing in collidine - of the quaternary base (LXI) yielding the Δ^{16}-androstenol-3β (LIII) in low yield.

7. Evidence in support of the current view of the Hofmann Degradation as a 1,2 bimolecular elimination reaction.

8. The observation that the reaction between an oxime (exemplified by cholestanone-3-oxime (LXXXI$_a$)) and N-bromosuccinimide yields the bromo oxime (2-bromo-cholestanone-3-oxime (LXXXII)) when carried out in carbon tetrachloride solution. When the reaction is performed in the presence of a small amount of alcohol, it takes a course in accordance with that published for bromination of oximes in aqueous solution.

9. (a) The isolation of a compound (Compound "X" (XCI)) which was characterized, chemically, spectroscopically and by kinetic studies, as a relatively stable crystalline intermediate in a Beckmann Rearrangement. Much significance is attached to this compound since it is believed to be the first time that such an intermediate in this reaction has been obtained. It thus provides direct chemical evidence of the Beckmann Rearrangement as a two-stage, rather than a one-stage,process. It also means that Beckmann Rearrangement of other 16-oximino 17-keto steroids

should be possible under less drastic conditions than those used classically.

(b) The isolation and characterization of 3β-hydroxy-etioallobilianic acid semi-amide ($XCIV_a$).

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Figure 1.

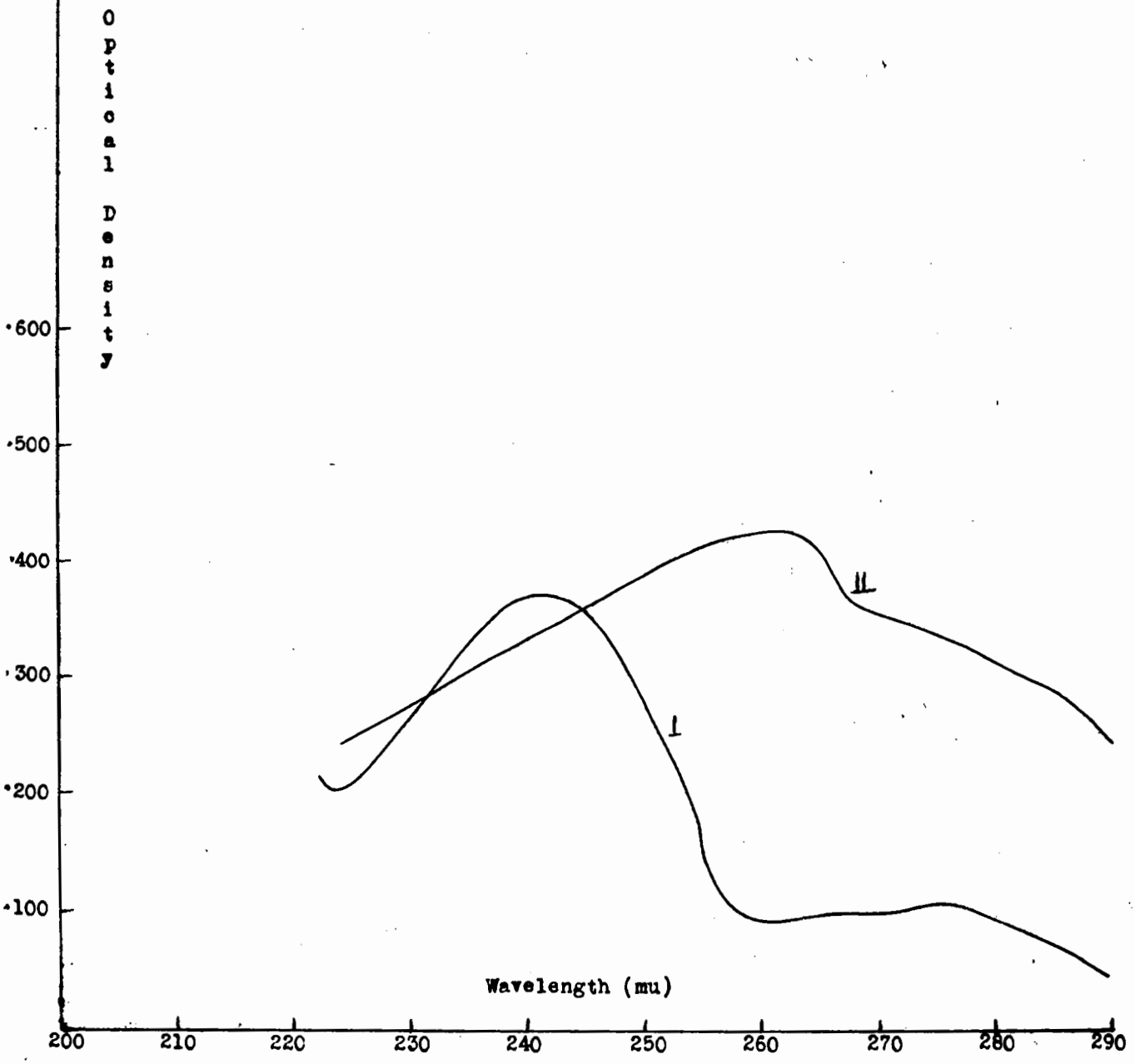
ULTRAVIOLET SPECTRA

OF

"DEHYDROBROMINATION OILS"

I Collidine: conc. 3.3 mg./100 ml. methanol.

II Dimethylaniline: conc. 3.74 mg./100 ml. methanol.



INFRA-RED SPECTRUM OF THE CRYSTALLINE BROMINATION PRODUCT OF EPIANDROSTERONE ACETATE

(in potassium bromide)

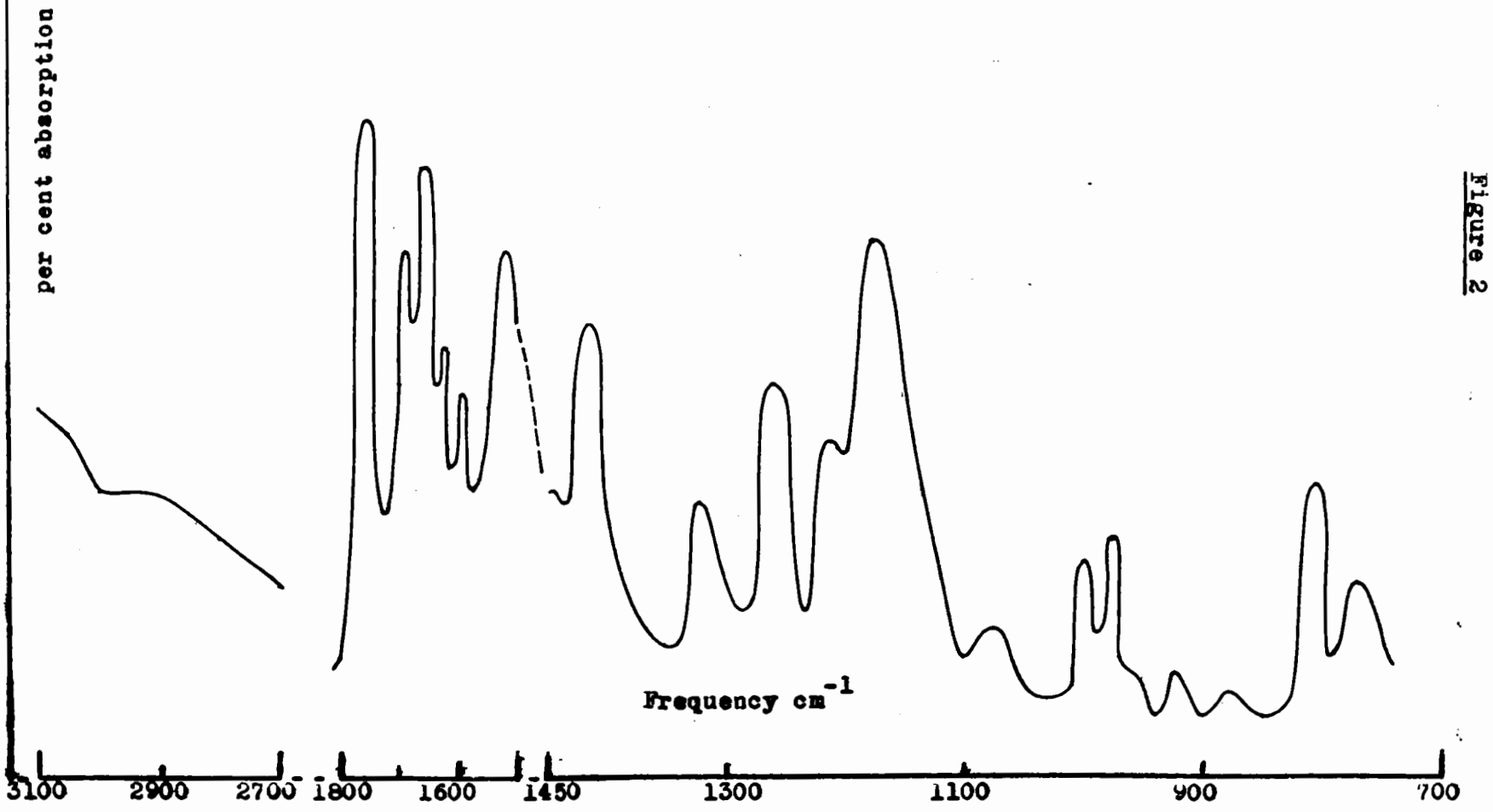


Figure 2

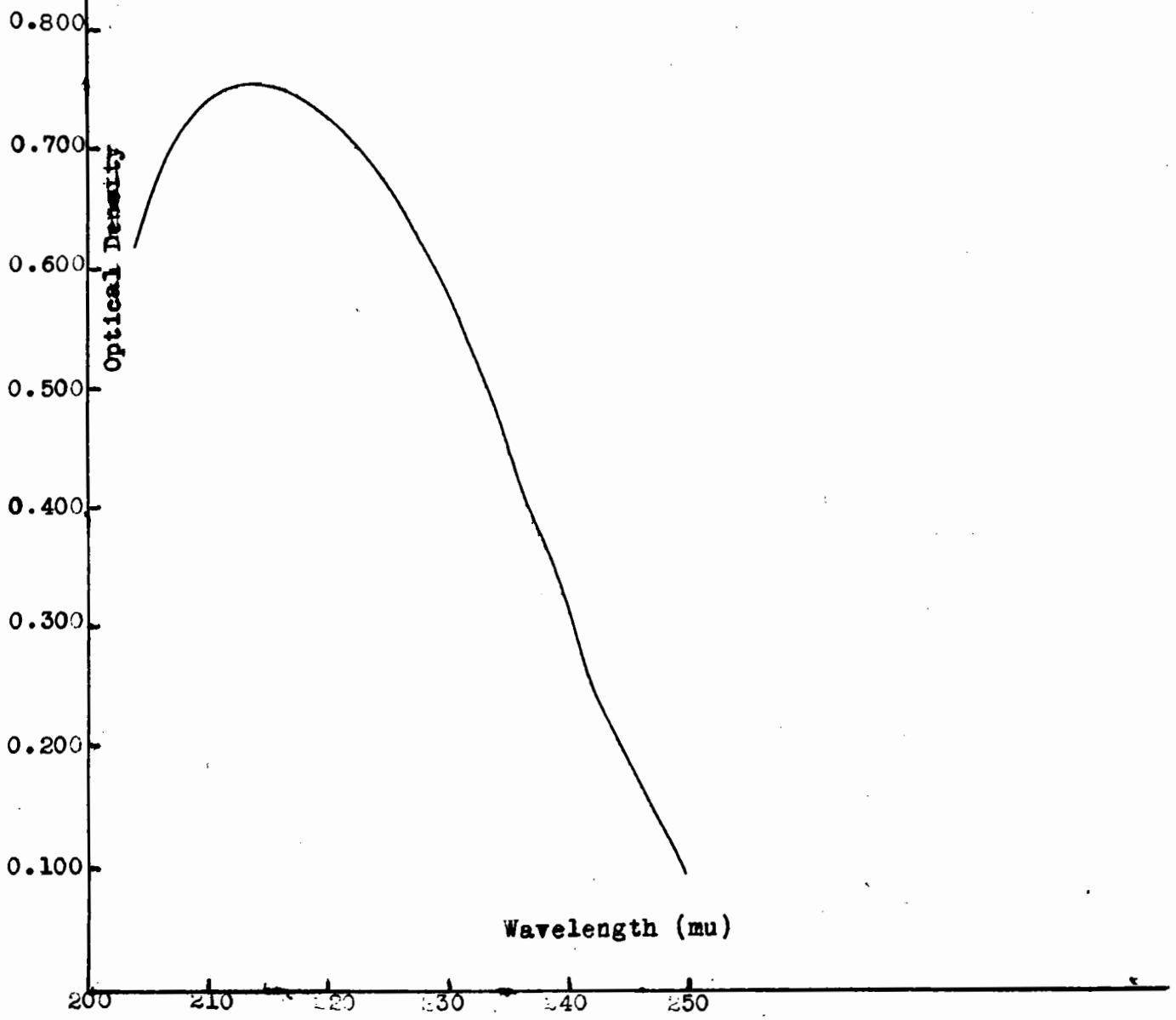
Figure 3

ULTRAVIOLET SPECTRUM OF

CRYSTALLINE BROMINATION PRODUCT

OF EPIANDROSTERONE ACETATE

Concentration 1 mg./78 ml. Ethanol.



INFRA-RED SPECTRUM OF N-ACETYL, 17β-AMINO-ANDROSTANOL-3β (LXII)

(in potassium bromide)

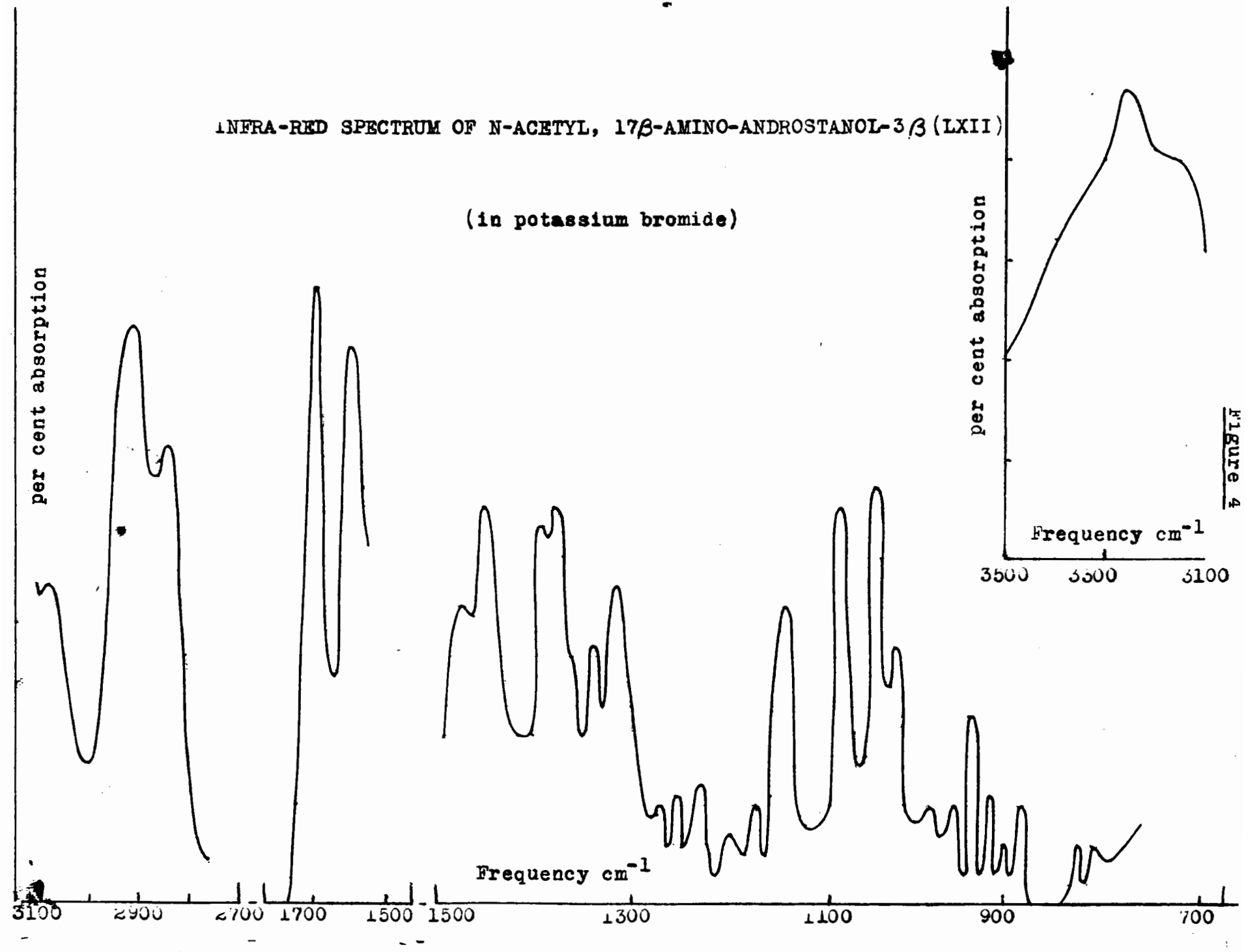


Figure 4

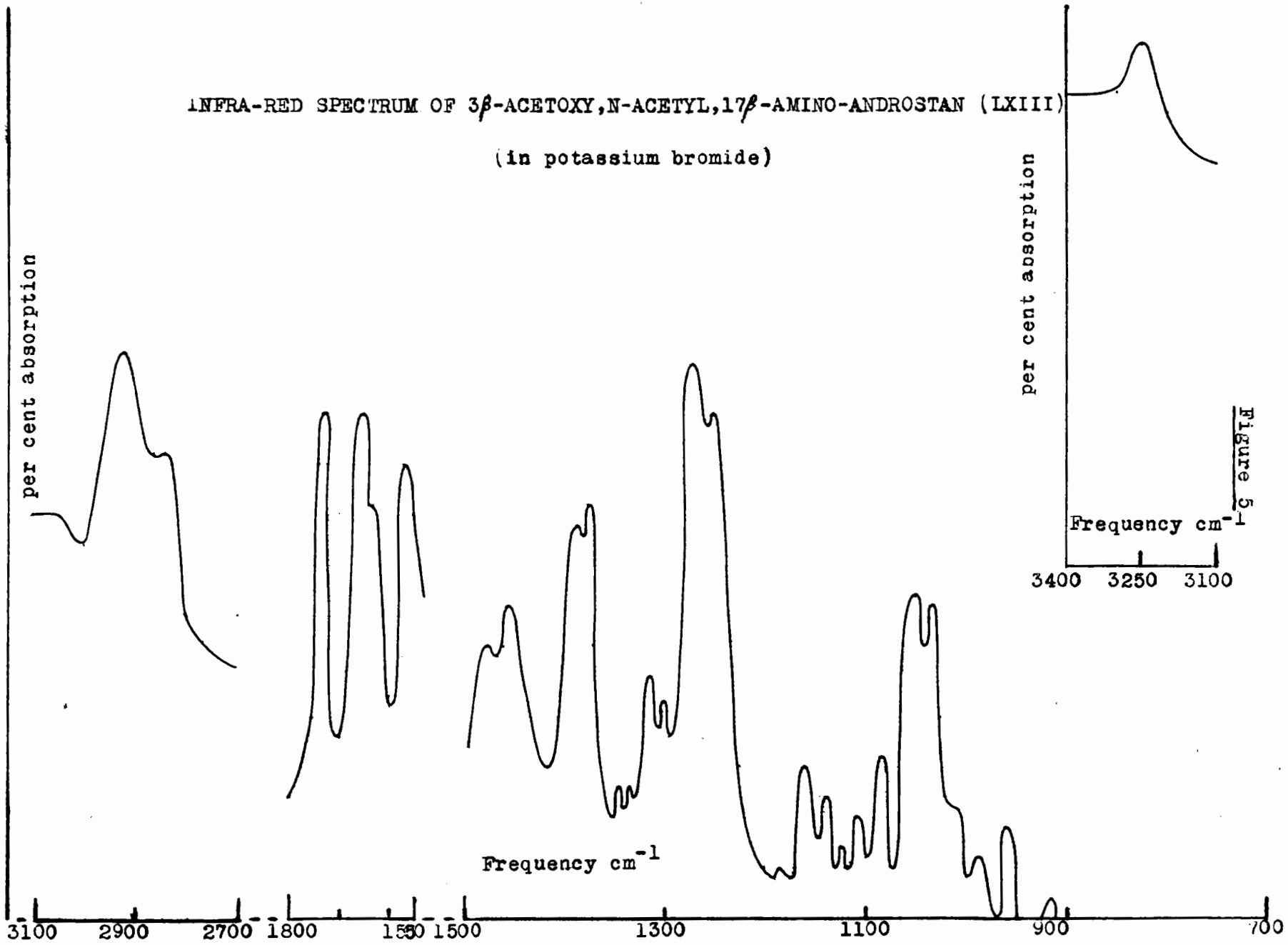

INFRA-RED SPECTRUM OF 3β-ACETOXY,N-ACETYL,17β-AMINO-ANDROSTAN (LXIII)
(in potassium bromide)
per cent absorption
Frequency cm-1
3100
2900
2700
1800
1550
1500
1300
1100
900
700
per cent absorption
Frequency cm-1
3400
3250
3100

Figure 51

INFRA-RED SPECTRUM OF Δ^{16}-ANDROSTENOL-3β (LII).

(in potassium bromide)

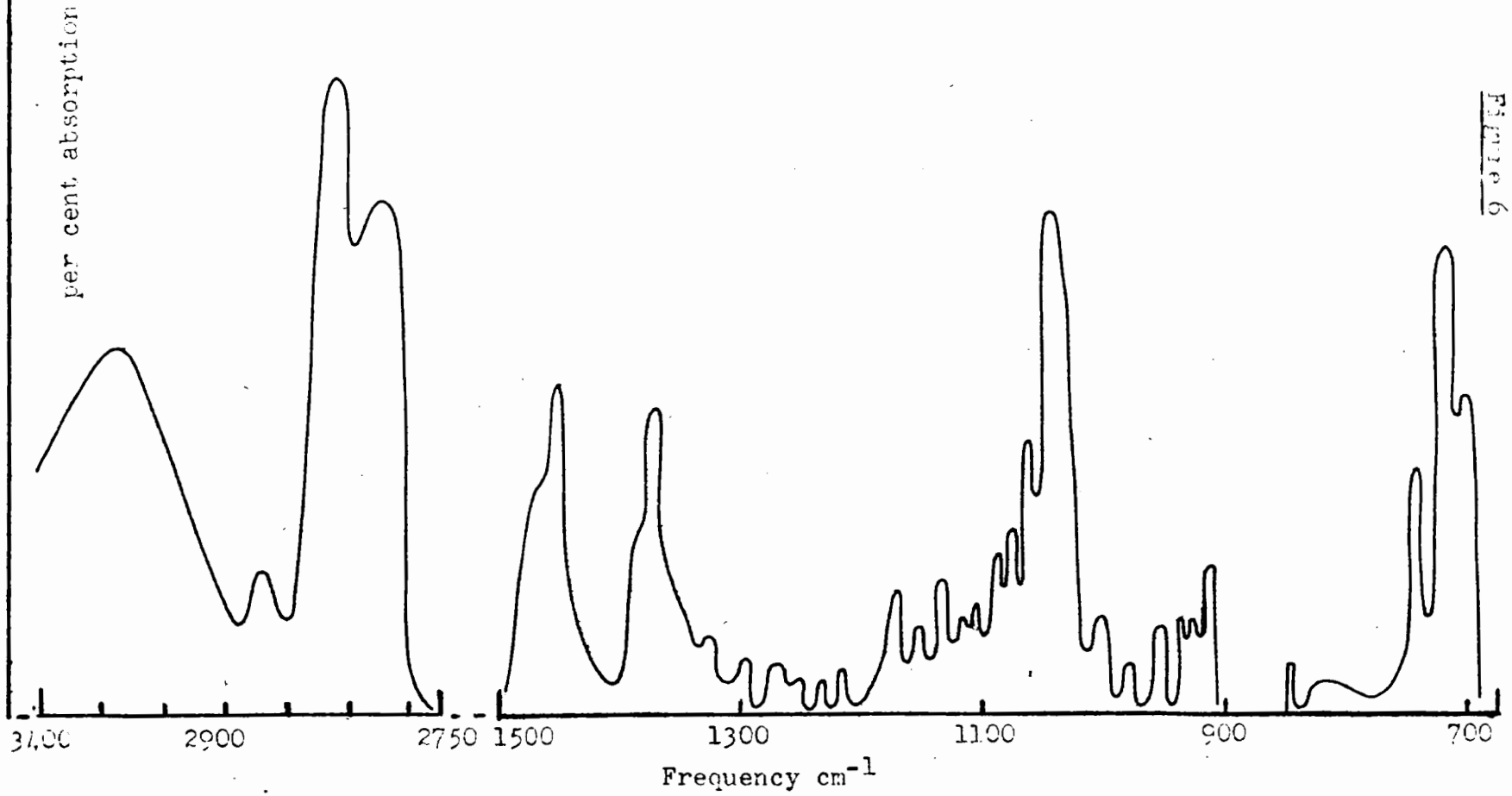


Figure 6

INFRA-RED SPECTRUM OF 17ξ-AMINO-ANDROSTANOL-3α,N-TRIMETHYL AMMONIUM HYDROXIDE (LXI).

(in potassium bromide)

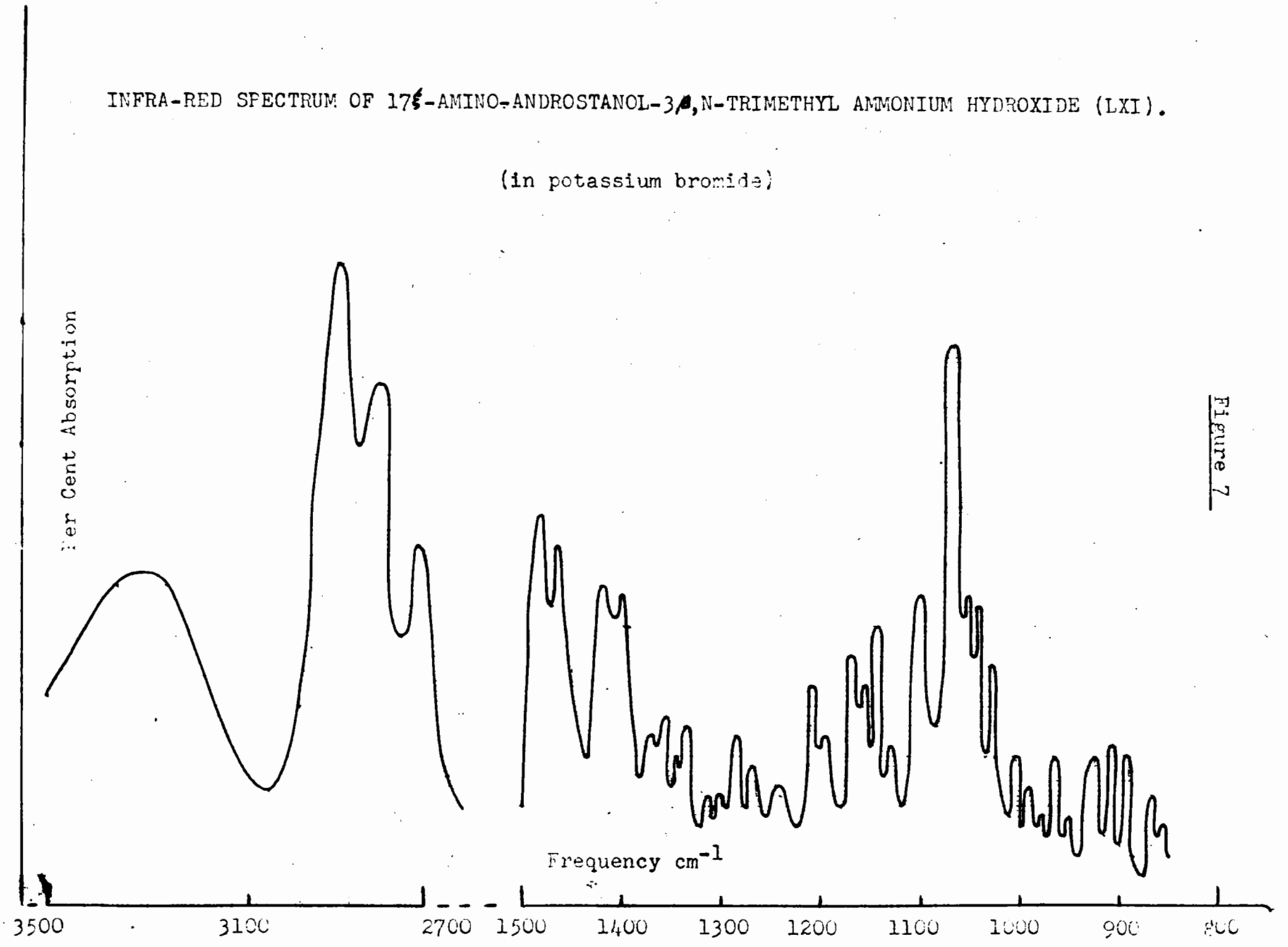


Figure 7

Figure 9

ULTRAVIOLET SPECTRA OF:

I 16-Oximino Epiandrosterone (LXXII)

II "X"

Concentrations.

I 3 mg./100 ml. methanol

II 4.7 mg./100 ml. ethanol

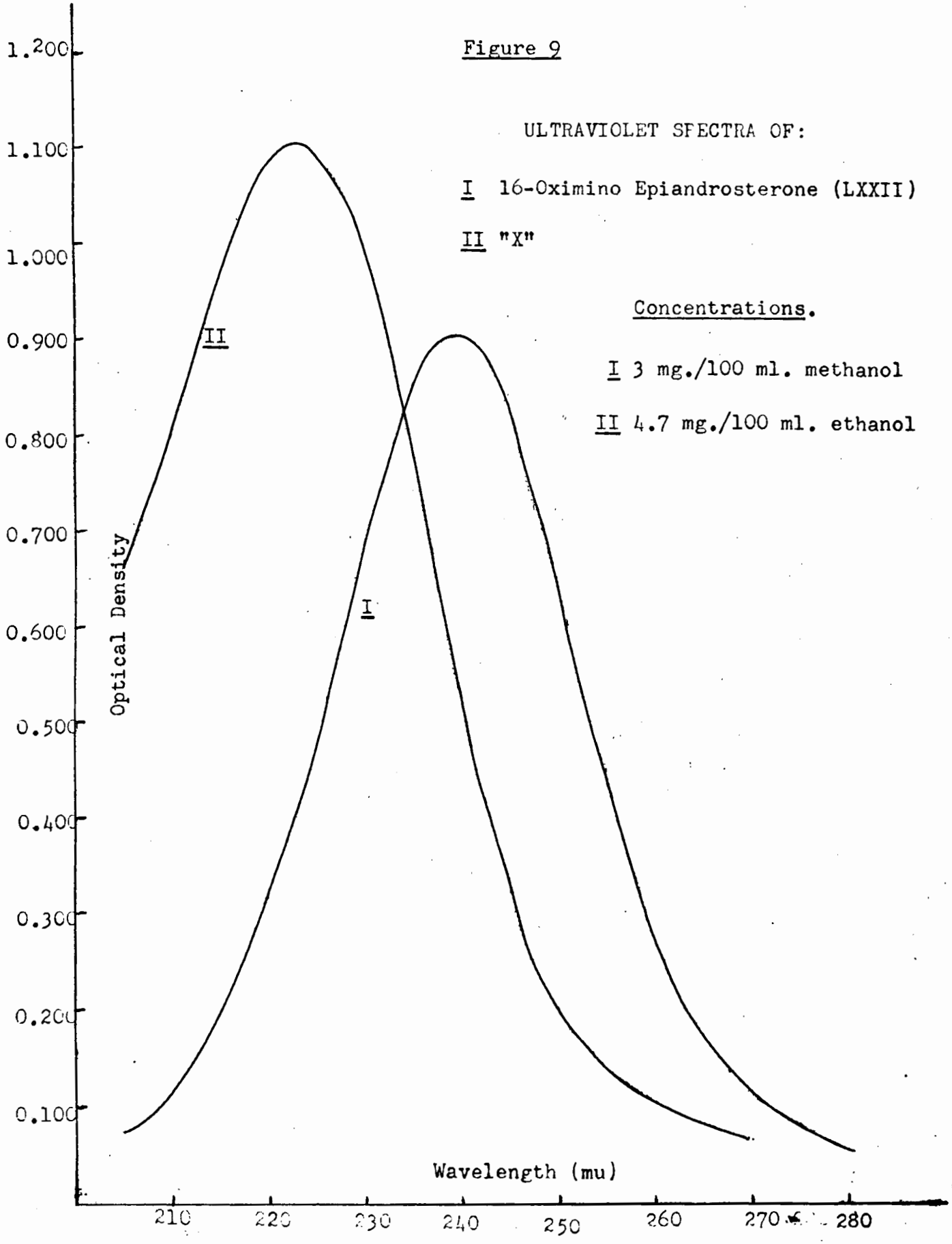


Figure 10.

ULTRAVIOLET SPECTRA OF THE PRODUCTS OF BROMINATION

OF

EPIANDROSTERONE ACETATE IN CCl_4/PYRIDINE.

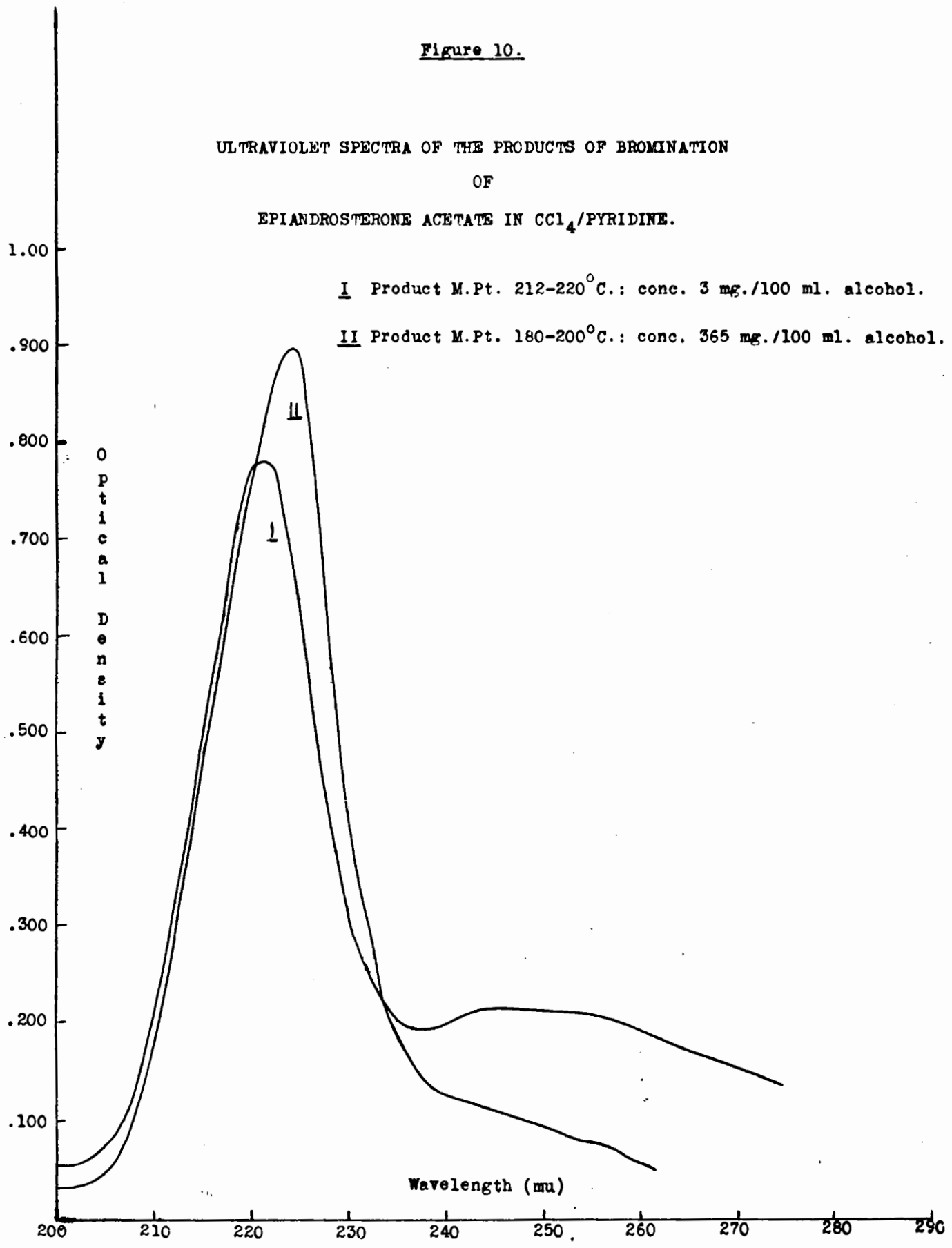


Figure 11.

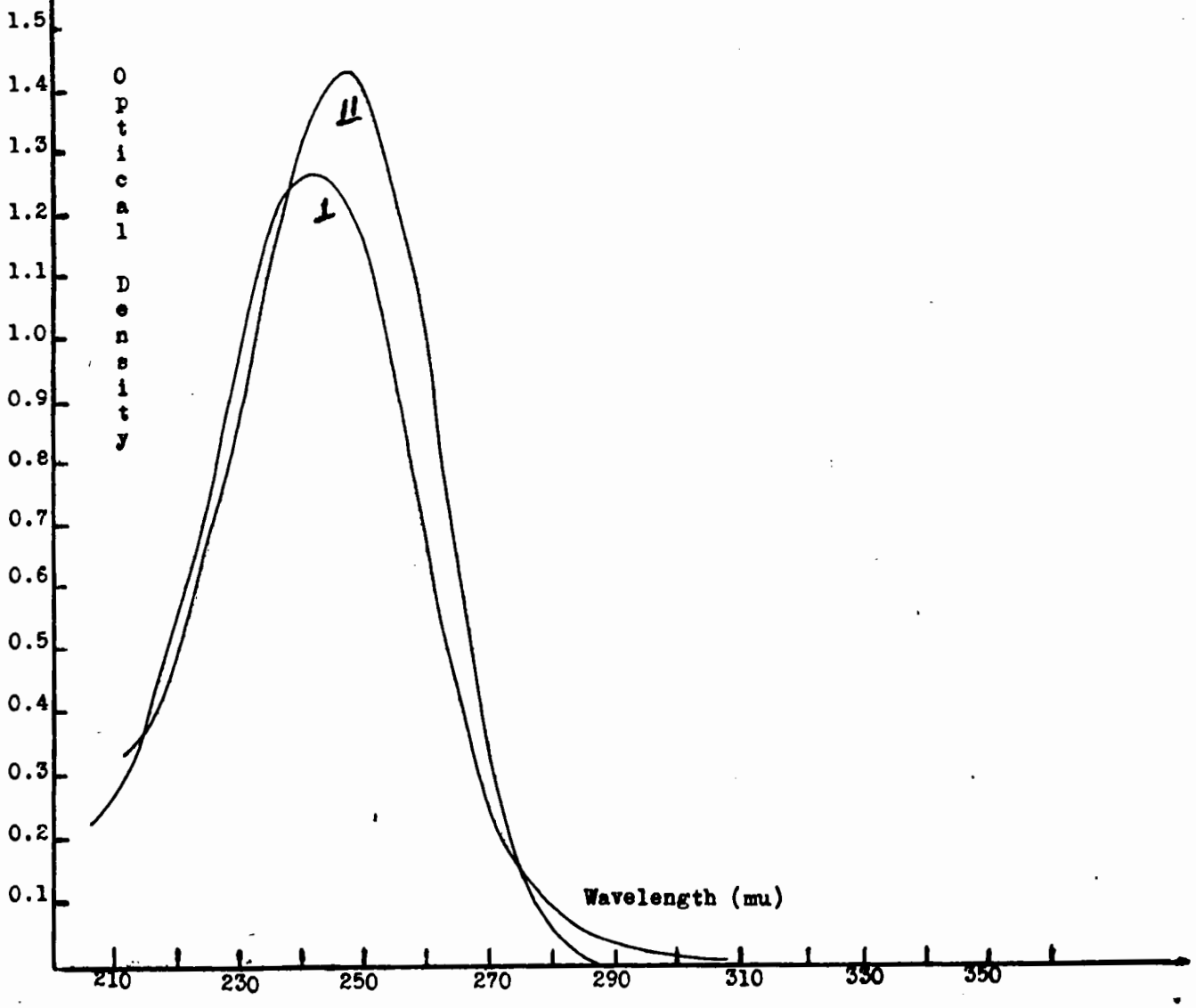
ULTRAVIOLET SPECTRA OF TESTOSTERONE OXIME

AND

TESTOSTERONE ACETATE OXIME ACETATE.

I Testosterone Oxime: conc. 2 mg./100 ml. alcohol.

II Testosterone Acetate Oxime Acetate: conc. 2.32 mg./100 ml. alcohol.



INFRA-RED SPECTRUM OF COMPOUND "X"

(in potassium bromide)

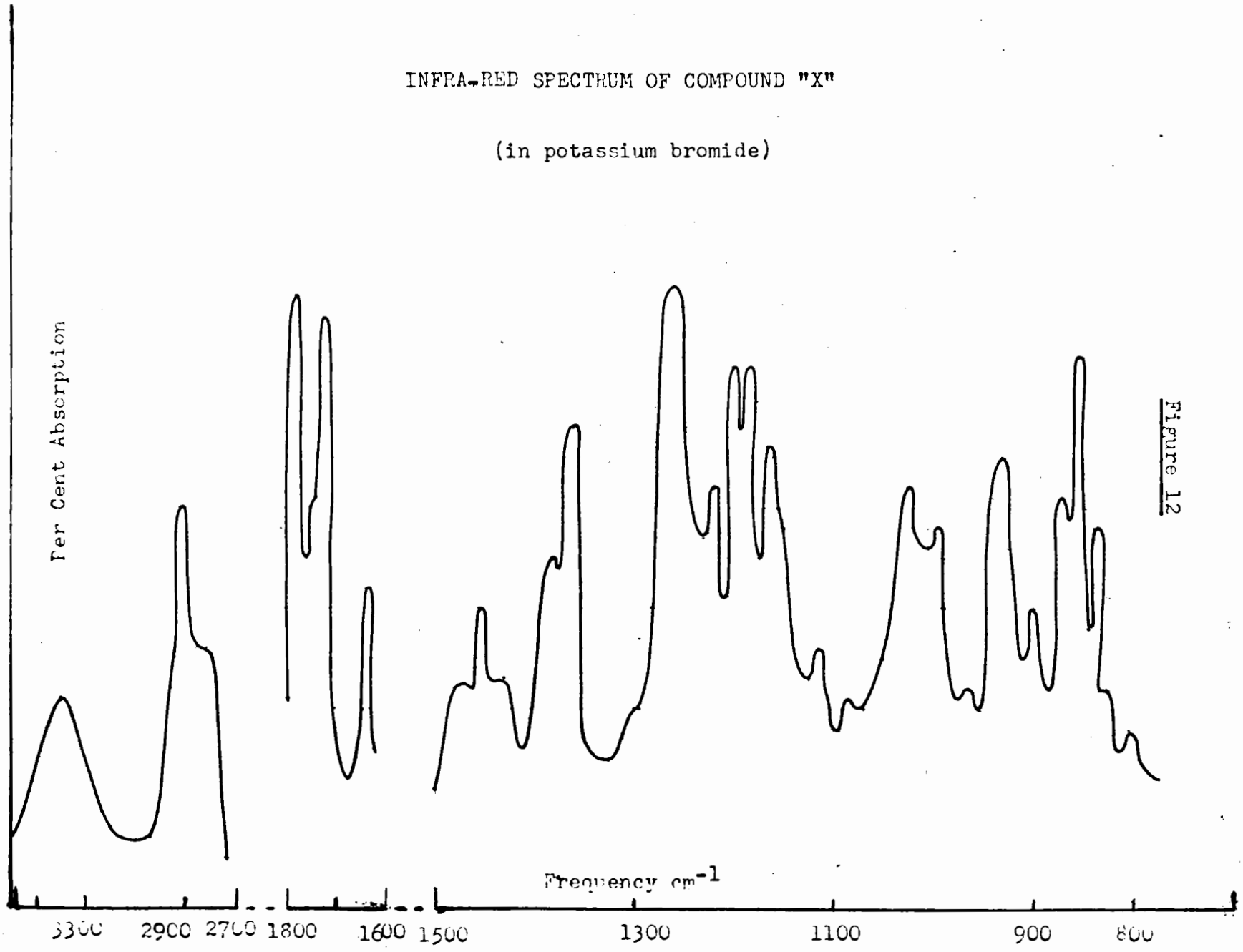


Figure 12

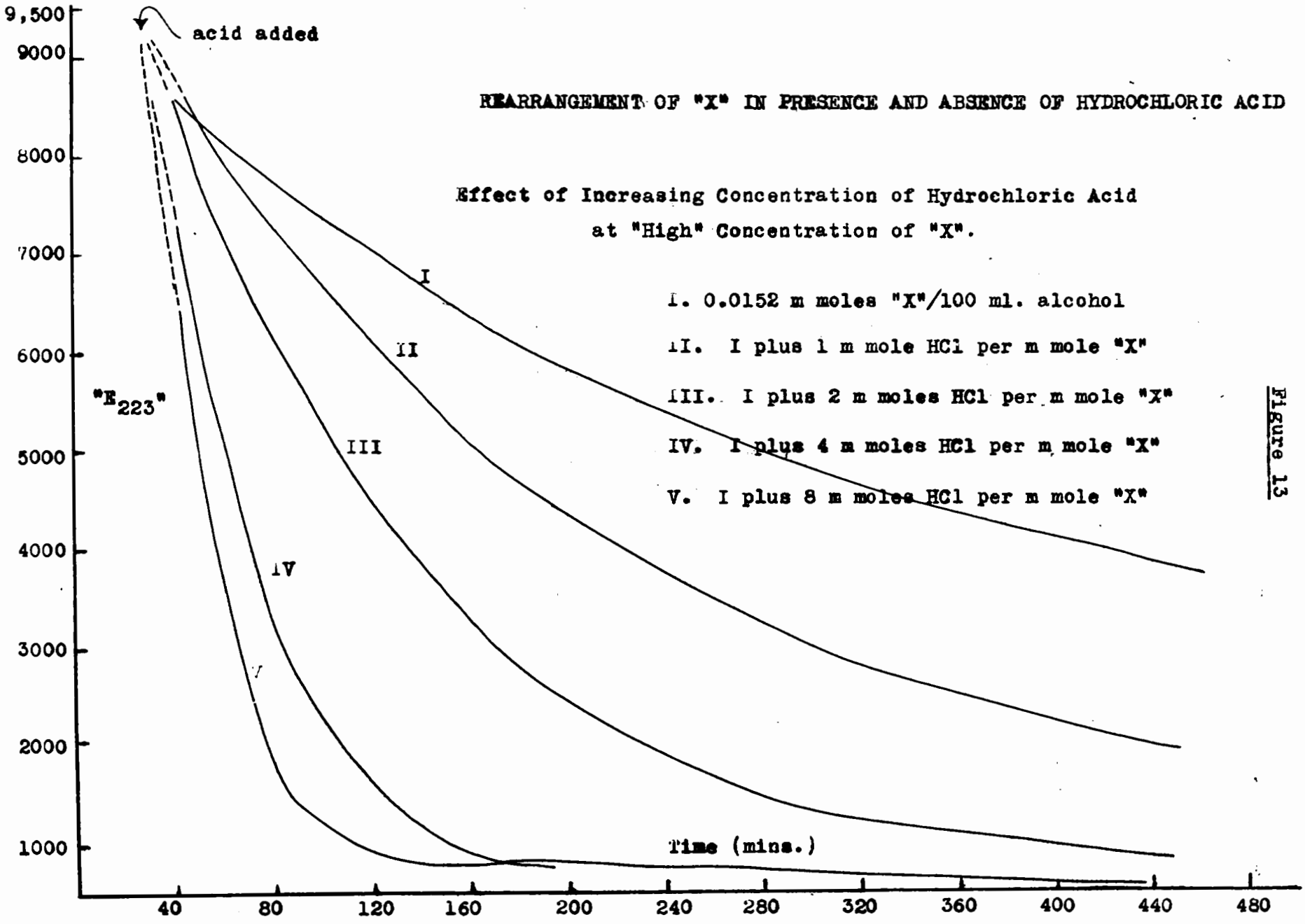

9,500
9000
8000
7000
6000
5000
4000
3000
2000
1000
"E223"
acid added
REARRANGEMENT OF "X" IN PRESENCE AND ABSENCE OF HYDROCHLORIC ACID
Effect of Increasing Concentration of Hydrochloric Acid
at "High" Concentration of "X".
I. 0.0152 m moles "X"/100 ml. alcohol
II. I plus 1 m mole HCl per m mole "X"
III. I plus 2 m moles HCl per m mole "X"
IV. I plus 4 m moles HCl per m mole "X"
V. I plus 8 m moles HCl per m mole "X"
I
II
III
IV
V
Time (mins.)
40
80
120
160
200
240
280
320
360
400
440
480

Figure 13

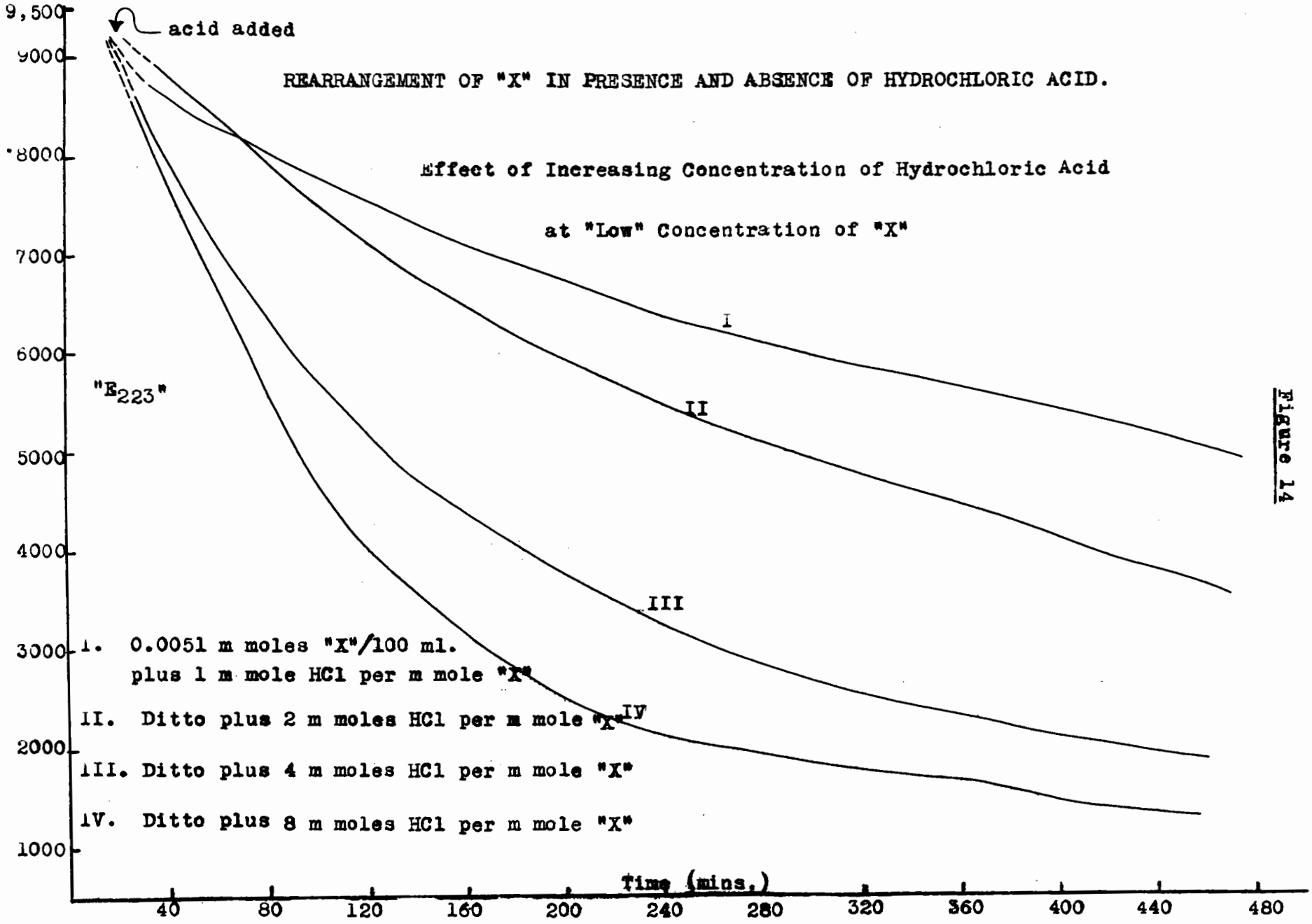

acid added
REARRANGEMENT OF "X" IN PRESENCE AND ABSENCE OF HYDROCHLORIC ACID.
Effect of Increasing Concentration of Hydrochloric Acid
at "Low" Concentration of "X"
"E223"
9,500
9000
8000
7000
6000
5000
4000
3000
2000
1000
I
II
III
IV
I. 0.0051 m moles "X"/100 ml.
plus 1 m mole HCl per m mole "X"
II. Ditto plus 2 m moles HCl per m mole "X"
III. Ditto plus 4 m moles HCl per m mole "X"
IV. Ditto plus 8 m moles HCl per m mole "X"
Time (mins.)
40
80
120
160
200
240
280
320
360
400
440
480

Figure 14