PART I

4-OXASTEROIDS

PART II

EXTRACTIVES OF EUPHORBIA PULCHERIMMA

by

J.M. Ferland

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

Department of Chemistry,
McGill University,
Montreal, Quebec.

April, 1963

SUMMARY

PART I

A. Ozonization of nortestosterone acetate, followed by alkaline hydrolysis and acidification, gave 17β-hydroxy-5-oxo-3,5-seco-4-norestrane-3-oic acid.

B. Reduction of 17β-hydroxy-5-oxo-3,5-seco-4-norestrane-3-oic acid with sodium borohydride or lithium tri-t-butoxyaluminum hydride gave 17β-hydroxy-3-oxo-4-oxa-5α-estrane. The 5α configuration was shown by studies of the nuclear magnetic resonance and optical rotation of the lactone.

C. The same lactone was obtained by Baeyer-Villiger oxidation of 19-nortestosterone acetate. A consideration of the mechanism of this reaction again indicates a 5α configuration in the product.

D. Hydrogenation of this and of two other steroidal δ-lactones over Adam's catalyst in acid solution was found to give tetrahydropyrans. This reaction was shown to be responsible in part for the low yield of δ-lactones from the catalytic reduction of some steroidal keto-acids.

PART II

A. The wood of poinsettia (euphorbia pulcherrimma) were found to contain arabinose and sucrose and ten of the common aminoacids. They were free of phenolic acids.

B. An acetate, $C_{30}H_{408}O_2$, m.p. 279 - 281°, was obtained from the petroleum ether extracts. An alcohol, m.p. 170 - 174°C was obtained from this compound by treatment with lithium aluminum hydride.

C. The acetate and the alcohol appeared from infrared and n.m.r. studies to have an trialkyl-substituted double bond and from ultraviolet studies a second tetraalkyl-substituted double bond.

Y a-t-il au monde rien de plus grand et de plus désintéressé que le coeur d'une mère, d'un père et d'une fiancée?

REMERCIEMENTS

Je voudrais remercier les autorités de l'université McGill pour le support financier qu'elles m'ont accordé durant les années 1961-1963.

Mes remerciements à la compagnie Ayerst-McKenna pour l'échantillon d'Oestrone qu'elle m'a offerte. Mes remerciements aux docteurs A. Taurins et C. Stammer pour les spectres de résonnance nucléomagnétique, à mesdames Edward et Bahr pour les chromatographies sur papier.

J'exprime ma gratitude à tous les membres du département de chimie de McGill et spécialement à mes confrères au laboratoire pour leur gentillesse et leur compréhension alors qu'ils ont eu à m'écouter à un moment où je devais m'exprimer dans un langage qui m'était à peu près inconnu.

Le Dr. Edward m'a continuellement secondé et encouragé au cours de ce travail; ses conseils se sont avérés des plus utiles et des plus éclairants au moment d'écrire cette thèse. Pour ces raisons, je lui offre toute ma reconnaissance.

PART I

TABLE OF CONTENTS

LIST OF FIGURES

PART II

TABLE OF CONTENTS

4-OXASTEROIDS

HISTORICAL INTRODUCTION

As part of a general investigation into the effects of changes in the basic carbon skeleton of steroids on their physiological and chemical properties, a number of androstane and cholestane derivatives having an oxygen in place of a carbon at the 4-position have been prepared since 1950.

Turner (1) obtained by catalytic hydrogenation of 5-oxo-3,5-seco-A-norcholestane-3-oic acid (IIa) (2), two lactones, m.p. 116-116.5°C and m.p. 109.5-110°C, the latter in large amount. He provisionally assigned to these compounds the structures IXa and VIIIa respectively because of their optical rotations and because hydrogenation of 4-oxacholest-5-en-3-one (IIIa) (3), which should be expected to give lactone IXa, gave the higher melting isomer. The fact that the lower melting lactone was the major product of the hydrogenation reaction (4) was also in agreement with Turner's proposed configuration at carbon 5.

Klyne (5) found further evidence for these structures from the molecular rotations of the lactones by an extension of Hudson's "Lactone Rule" (6,7) which states that the stereochemistry of the carbon atom carrying the potential hydroxyl group determines the sign of the rotational contribution of the lactone ring.

Unequivocal proof for the correctness of Turner's assignment was afforded by the Baeyer-Villiger oxidation of A-nor-5β-cholestan-3-

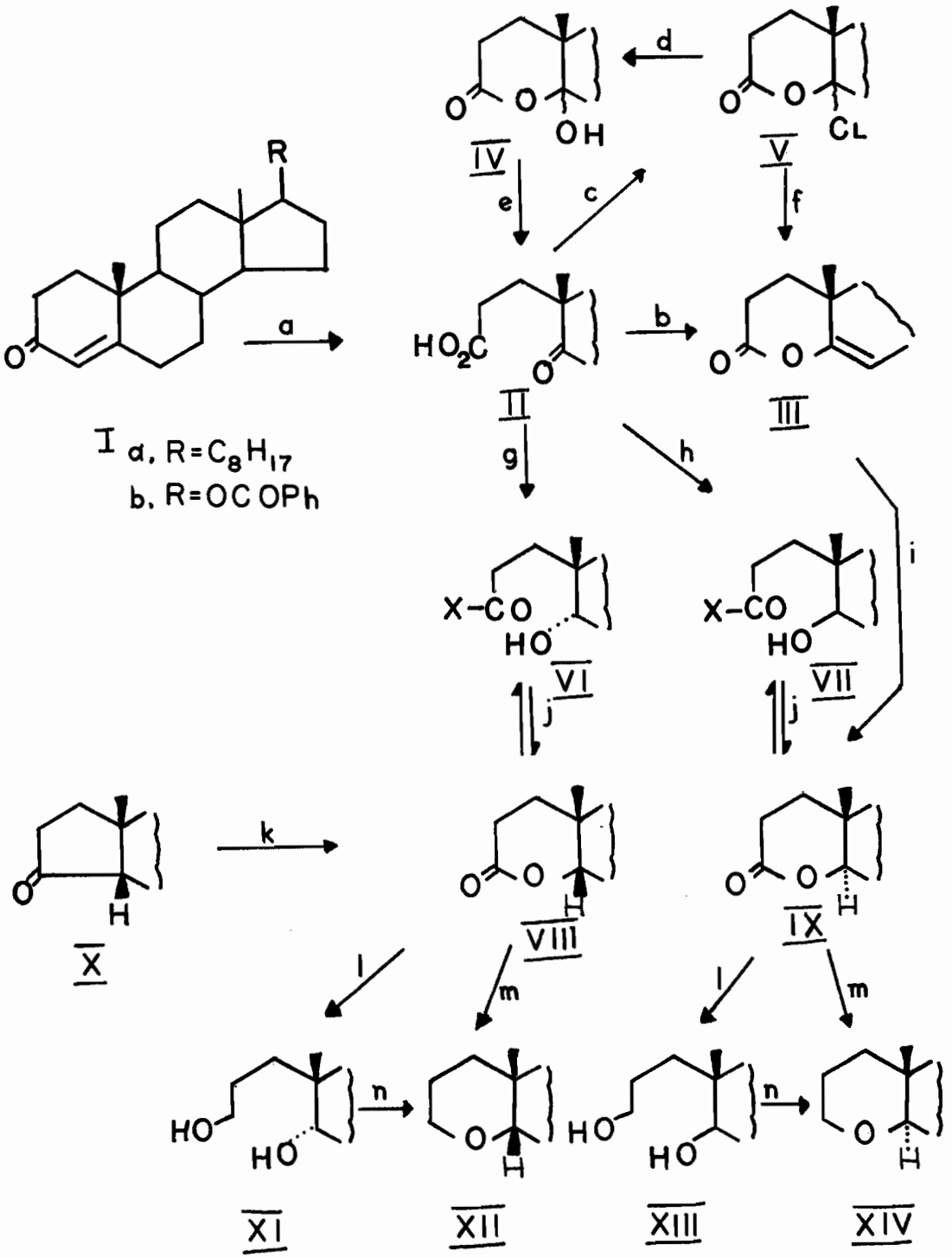
one (Xa) (8) with organic peracids to the lactone with the lower melting point. Since the configuration at the 5-position of Windaus' norketone (Xa) (9) is well established and since it is known that this reaction proceeds with retention of configuration (10,11,12), the lactone obtained must have the structure VIIIa.

As expected (8,13,14) the lactone IXa was the preponderant epimer in the borohydride reduction of the keto-acid IIa.

Atwater and Rolls (14) found that ozonolysis of testosterone-17β-benzoate (Ib) gives not only 17β-benzoyloxy-5-oxo-3,5-seco-4-nor-androstane-3-oic acid (IIb) but also an isomeric neutral compound 17β-benzoyloxy-3-oxo-4-oxa-5-hydroxy-androstane (IVb). Turner (1) assumed it to be IIb. Borohydride reduction (14) of IIb and IVb gave a 59% yield of a lactone assumed to be IXb from the known course of borohydride reductions. Similarly catalytic reduction of IIb gave the expected VIIIb.

Reductions of lactones VIIIa and IXa with lithium aluminum hydride gave the diols XIa and XIIIa from which the 4-oxacholestanes XIIa and XIVa were obtained by treatment with benzene sulfonyl chloride in pyridine or lutidine (8). As expected the 4-oxacholestane having the trans fusion of rings A and B had a higher melting point than the cis compound (8,15).

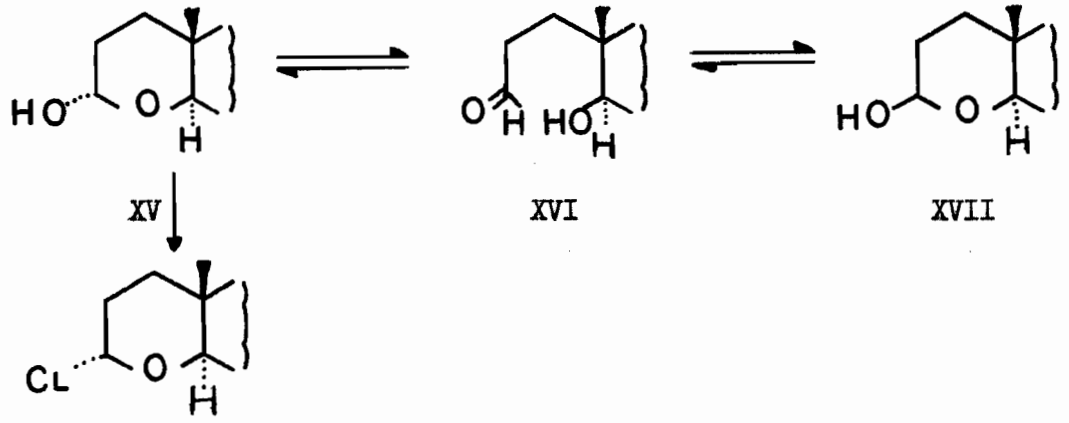
This transformation can now be done more conveniently using a novel method discovered by Pettit et al. (16,17,18,19) in which lactones such as VIIIa and IXa are reduced directly to their corresponding ethers with a mixture of boron trifluoride and lithium aluminum hydride.



Reagents: a, ozone; b, AcCl, Ac_2O; c, $SOCl_2$; d, H_2O; e, Heat; f, pyridine; g, H_2/Pt (X = OH); h, $NaBH_4$ (X = OH); i, H_2/Pt; j, NH_3 (H = NH_2) k, $PhCO_3H$; l, $LiAlH_4$; m, $LiAlH_4$ + BF_3; n, $ArSO_2Cl$ in pyridine or lutidine.

This method seems to be general for the direct transformation of esters and lactones to ethers and has been used on β, γ, and δ lactones of steroids (19). It does however fail with lactones having a primary carbon atom attached to the ether oxygen of the ester group (19).

Reduction of the lactone IXa in tetrahydrofuran with one-quarter of a mole of lithium aluminum hydride (8) or with diborane (20,21) gives 4-oxa-5α-cholestan-3α-ol (XV). The sterochemistry of this molecule had been studied by Edward et al. (22), who proved the tautomerism of the lactol ring:



XV XVI XVII

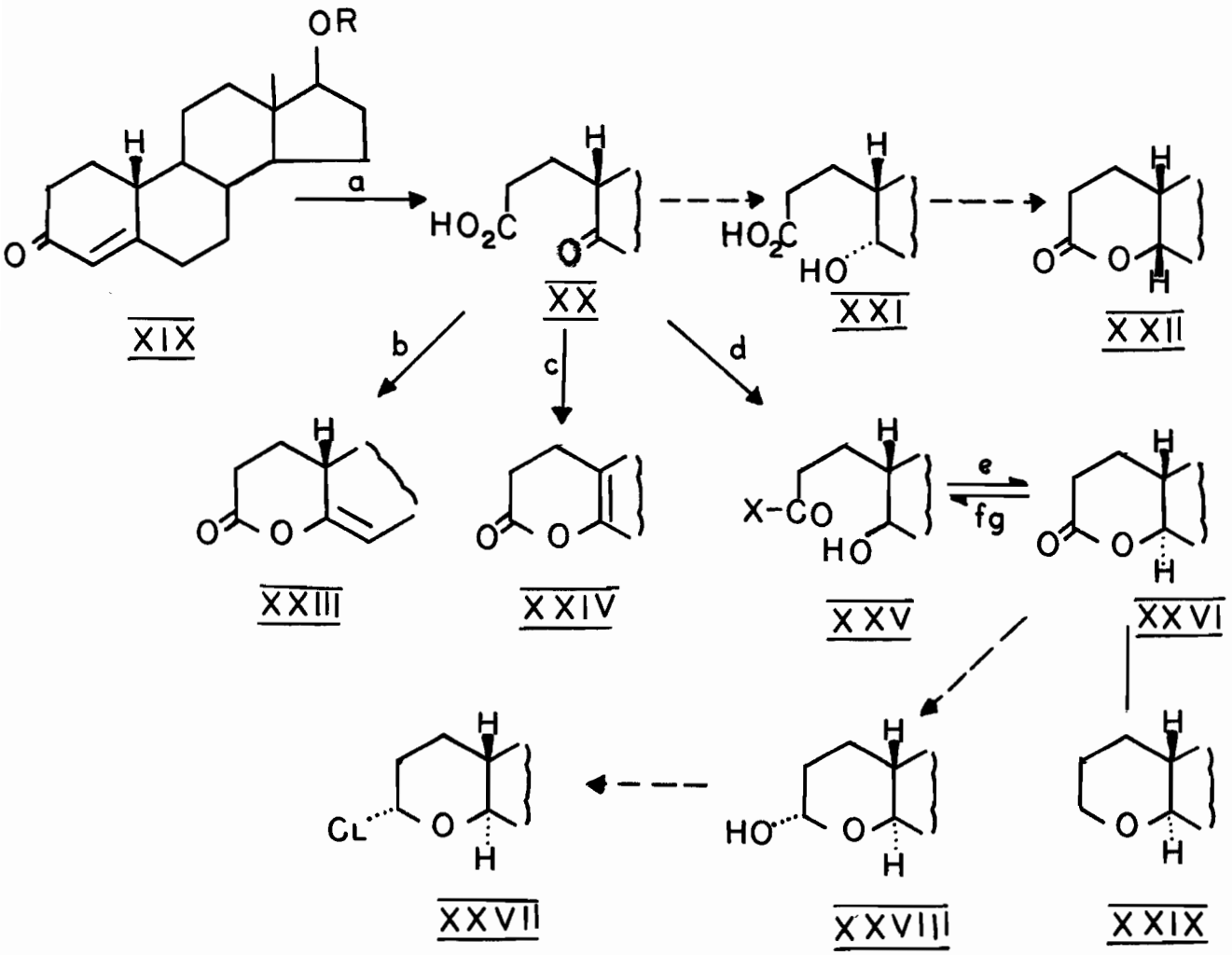
XVIII

The reactions of 3α-chloro-4-oxa-5α-cholestane (XVIII), obtained from XV with phosphorus oxychloride and pyridine, were also investigated as model studies of similar carbohydrate reactions (22). It was found that the replacement of the chlorine atom by alkosyl groups takes place, contrary to expectation, with predominant retention of configuration. This result was explained by postulating steric hindrance in the reaction

from the 19-methyl group.

RESULTS AND DISCUSSION

In order to assess steric importance of the 19-methyl group in the reactions of 3α-chloro-4-oxa-steroids, an attempt was made to synthesize several representative 4-oxaestranes which lack the 19-methyl group.



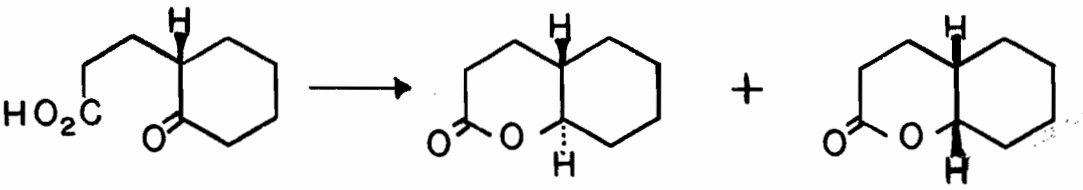
Reagents: a, O_3; b, Ac_2O + AcONa; c, Ac_2O + AcCl; d, $NaBH_4$ or H_2/Pt; e, H^+; f, OH^- for X = O^-; g, NH_3 for X = NH_2; h, $LiAlH_4$ + BF_3 or H_2/ Pt.

Compounds of this type have been prepared previously as intermediates in the series of reactions leading to the introduction of radioactive carbon into the steroid molecule. Dreiding et al. (23) obtained the keto acids (XX; R = COMe, COPh and $CO.C_6H_4.NO_2$-p) by ozonization of the appropriate 19-nortestosterone esters (XIX). The keto acids were converted into isomeric unsaturated lactones, assigned the structures XXIII and XXIV on the basis of their optical rotations and infrared spectra, by acetic anhydride with basic and acid catalysts respectively.

Our own researches started with 17β-acetoxy-5-oxo-3,5-seco-A-norestran-3-oic acid (XX; R = Ac), which was obtained in 88% yield by an improvement of the ozonization procedure of Dreiding et al. (23). From this compound the synthesis of the 3-chloro-4-oxasteroid (XXVII) was envisaged by the route indicated; this synthesis has been carried as far as the lactone (XXVI; R = H and Ac), and the remaining reactions (indicated by broken arrows) have yet to be carried out. However, the work of this part of the thesis is concerned with an exploration of the stereochemistry and the side-reactions accompanying the reduction of the keto acid (XX; R = H and Ac).

(a) Hydride Reduction of 17β-Hydroxy-3,5-seco-4-norestran-3-oic Acid

Sodium borohydride reductions of ketones produce predominantly the more stable isomer, provided that addition from one side of the carbonyl group is not excessively hindered (4,24,25,26). This is attributed to "product development control", i.e. to the fact that the transition states for these reductions resemble the products (24, 25,26). Thus sodium borohydride reduction of Windaus' keto acid (IIa) (38) afforded about three times as much of the equatorial hydroxy acid (VIIa; X = OH) as the axial hydroxy acid (VIa; X = OH), as evidenced by the isolation of the corresponding lactones IXa and VIIIa in 64% and 17.5% yields respectively (13). Similarly House et al. (29) found that borohydride reduction of the keto acid (XXVIII) gave a mixture made up of about two-thirds of the lactone (XXIX), arising from reduction of the carbonyl to an equatorial hydroxyl, and one-third of the isomeric lactone (XXX). It should accordingly be expected that



XXVIII XXIX XXX

borohydride reduction of the keto acid (XX; R = H) would give mainly the hydroxy acid (XXV; R = H, X = OH), which would cyclize readily to the 5α-lactone (XXVI; R = H).

In fact, from this reaction, one lactone, m.p. 131-132°C was

isolated in 52% yield, which is shown by the evidence cited below to be the 5α-lactone. No other crystalline product could be isolated, although a small amount of a second compound was detected as a faster-moving spot by thin-layer chromatography of the crude reaction products (Fig. 1). This could be (XXVI; R = H) since the 5β-lactone VIII moves faster than its 5α-isomer IXa under the same conditions in thin-layer chromatography (Fig.3).

The use of lithium tri-t-butoxy aluminum hydride which has been shown by Wheeler and Mateos (27,28) to give a higher proportion of equatorial hydroxyl than borohydride in the reduction of keto-steroids, raised the yield of 5α-lactone to 61%; in this case the spot attributed to the 5β-lactone was no longer visible on thin-layer chromatography (Fig. 1).

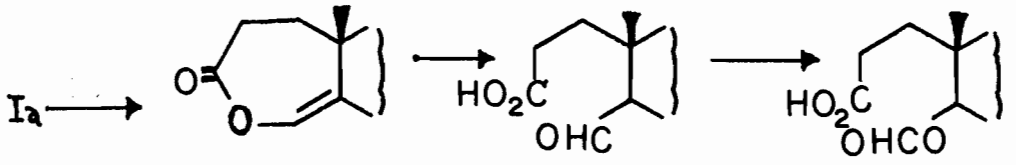
The evidence for the 5α-configuration (XXVI; R = H) of the major reduction product is as follows:

1. Lactonization produces a positive shift in molecular rotation, in accordance with the Klyne-Hudson Rule (5,6,7). Strictly speaking, this rule should apply to the shift in rotation in going from the hydroxy acid (XXV; R = H, X = OH) to the lactone (XXVI; R = H). In practise (5), it is often difficult to isolate the pure hydroxy acid, and it is often necessary to determine the rotation of some isolable derivative of the hydroxy acid, such as the amide (8) with the assumption that this has almost the same molecular rotation. This has been done in the present instance, the amide (XXV; R = H, X = NH_2) being obtained as a nicely crystalline product from the reaction of the lactone (XXVI; R = H)

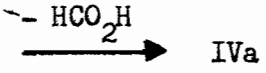
with ammonia in methanol. The difference between the molecular rotations of the lactone and the amide ($[M]_D$ = + 55°) and ($[M]_D$ = + 200°) is + 145°.

An attempt was made to obtain the rotation of the hydroxy acid (XXV; R = H, X = OH) without isolating it from solution. However, it was found that while the lactone ring of XXVI (R = H) opened almost instantaneously in 0.01 N sodium hydroxide, in methanol-water (99:1 v/v) as shown by a drop in molecular rotation to a constant value of + 8° within 1-2 minutes, the hydroxy acid obtained by acidifying this alkaline solution cyclized back to the lactone in less than one minute as shown by the rise in rotation of the solution ($[\alpha]_D^{24}$ + 65.2 unchanged on further standing; the lactone (XXVI; R = H) had $[\alpha]_D^{27}$ + 69.8 in the same solvent). However, the changes again are in the direction predicted by the Klyne-Hudson Rule if it is assumed that the hydroxy acid and its sodium salt have almost the same value. The shift is + 192°.

2. The lactone, m.p. 131-132°C was also obtained by oxidation of the unsaturated ketone (XIX; R = $OCOCH_3$) with persulfuric acid. This reaction would be expected to give the 5α-lactone (XXVI; R = H) as it has already been found in a similar oxidation of cholest-4-en-3-one (Ia) (19) from Pettit's mechanism (20) for this reaction.



XXXI XXXII



This mechanism involves the formation of the aldehyde (XXXI) as an intermediate which is then oxidized by a second Baeyer-Villiger reaction to the formate (XXXII). Since this is a slow reaction, the aldehyde group of XXXI can be expected by equilibration in the acidic solution to be preponderantly in the equatorial configuration, and hence also the formate group of XXXII (remembering the stereospecific character of the Baeyer-Villiger oxidation (10,11,12)).

Several authors (19,20,30,31,32) have applied this reaction to the oxidation of steroidal Δ^4-3-ketones, and have assumed the δ-lactones thus obtained to have the 5α-configuration but this configuration has been proved only in the case of the oxidation of cholest-4-en-3-one.

3. The compound shows a peak (τ = 6.23) in its n.m.r. spectrum having a chemical shift to be expected for an axial rather than an equatorial hydrogen on the alcohol carbon of the lactone (39); about the same chemical shift is shown by the hydrogen of IXa (τ = 6.16), while the equatorial hydrogen of VIIIa absorbs at a lower field (τ = 5.91). House (29) found that the axial hydrogen of XXIX absorbed at about the same field (τ = 6.18) as the steroidal 5α-lactones, but the equatorial hydrogen of XXX absorbed at considerably lower field (τ = 5.57).

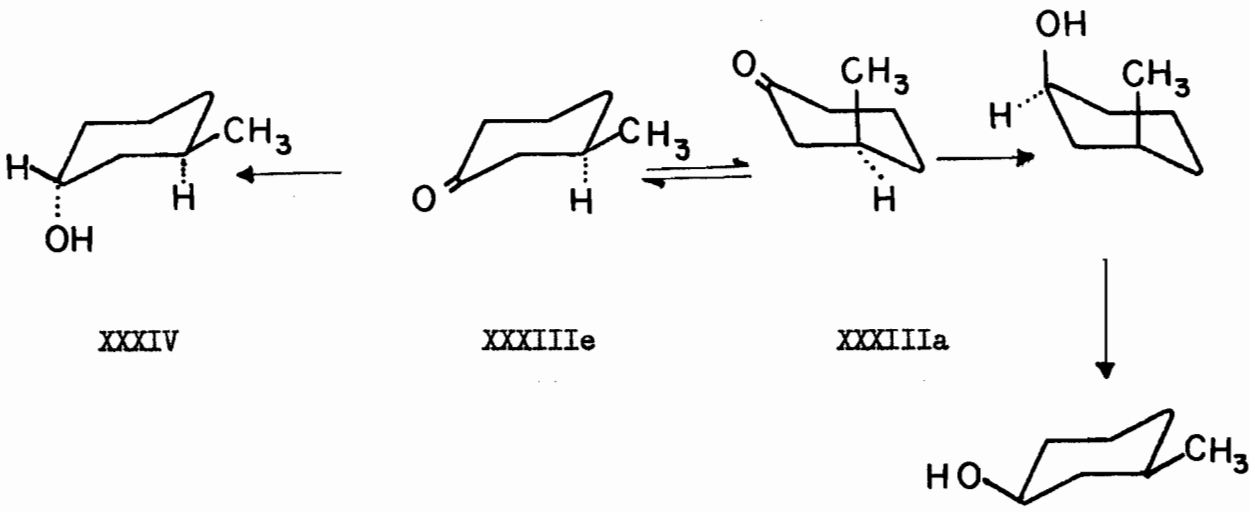
(b) <u>Hydrogenation of 17β-Hydroxy-3,5-seco-4-norestrane-3-oic Acid</u>

Hydrogenation of cyclohexanones of fixed conformation over platinum in acetic acid gives usually a preponderance of the axial

---

N.m.r. spectra were taken on a Varian H.R. 6 spectrophotometer apparatus in deuterated chloroform. Tetramethylxylene (τ = 10.00) was used as an internal standard.

alcohol (33,34) because the hydrogen must approach the absorbed ketone from the catalyst surface, and because the ketone is more easily absorbed on the less-hindered equatorial side (33,34). However, 3-methylcyclohexanone (XXXIII), which should be about 90% in the conformation (XXXIIIe) and 10% in the conformation XXXIIIa (35), and hence which should be expected to be hydrogenated mainly to the trans



XXXIV XXXIIIe XXXIIIa XXXV

alcohol XXXIV, gives 73% of the cis alcohol XXXV (33). This isomeric composition of the hydrogenation product was determined from its density; a reinvestigation using other methods of analysis (e.g. vapor phase chromatography) would be indicated. The effect of having an acid or alkaline solvent on the proportions of isomeric alcohols formed (33) is also difficult to explain and worthy of reinvestigation.

Hydrogenation of the keto acid (IIa) over platinum in acetic acid would be expected to give mainly the 5β-lactone (VIIIa). The yield of crude 5β-lactone, however, was low (about 34%) and several re-

crystallizations were necessary to obtain a pure compound. (The reasons for the low yield of lactone become apparent from later work in this thesis).

In similar fashion, hydrogenation of the keto acid XX (R = H) over platinum in acetic acid would be expected to give chiefly the hydroxy acid XXI (R = H) and hence the 5β-lactone XXII (R = H). However, hydrogenation with the theoretical amount of hydrogen gave a 26% yield of the crude 5α-lactone (XXVI; R = H), 14% of 17β-hydroxy-4-oxa-5α-estrane (XXIX; R = H) (the formation of which is discussed in the next section), and 40% of starting material. Thin-layer chromatography of the crude reaction product revealed, besides these three compounds, a trace of the compound (mentioned previously) (Fig. 2) tentatively identified as the 5β-lactone (XXII; R = H).

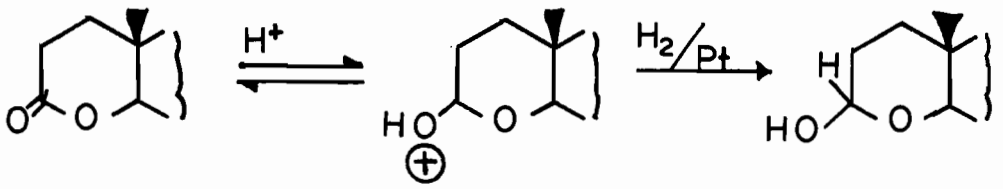
The results indicate that the presence of an angular methyl group in place of a hydrogen atom at the 10-position makes it <u>easier</u> to add hydrogen from the catalyst surface to the topside of the 5-carbonyl group. It is usually assumed that the angular methyl groups make reaction with the topside of steroid molecules more <u>difficult</u> (36). These results are most surprising. While various possible explanations can be offered for the reversal of the expected stereochemistry of hydrogenation of the keto acid XX (R = H), these must remain highly speculative until more experimental work has been done with a variety of compounds related to the keto acid.

Hydrogenolysis of δ-Lactones to Tetrahydropyrans

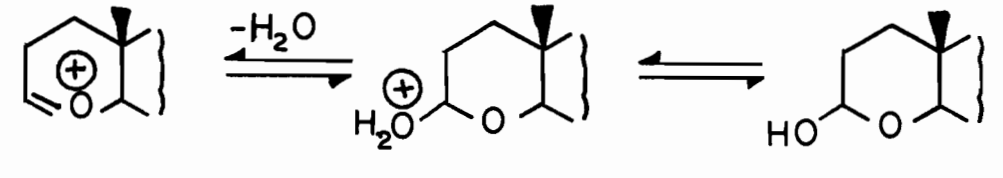
17β-Hydroxy-4-oxa-5α-estrane (XXIX; R = H) is readily obtained in 86% yield from the 5α-lactone (XXVI; R = H) by reduction with lithium aluminum hydride in the presence of boron trifluoride according to Pettit (19,20). Very surprisingly, this ether is also obtained in 14% yield when the keto acid (XX; R = H) is hydrogenated with one mole of hydrogen. The latter reaction must proceed through the 5α-lactone (XXVI; R = H) as an intermediate, and indeed the ether can be obtained by hydrogenation of the lactone in 90% yield. Hydrogenation of the keto acid (XX; R = H) with an excess of hydrogen gave the ether in 74% yield.

Similarly, hydrogenation of the lactones VIIIa and IXa gave the ethers XIIa and XIVa in 89 and 92% yields respectively. The poor yields of the lactone VIIIa obtained by Turner (1) and by Morand (8) from the hydrogenation of the keto acid IIa thus receive a possible explanation. An examination by thin-layer chromatography (Fig. 3) of the crude product obtained from IIa with one mole of hydrogen indicated the presence of the lactones VIIIa and IXa and the ethers XIIa and XIVa.

The catalytic reduction of lactones to cyclic ethers has not previously been reported in the literature, though catalytic reduction of one of the carbonyl groups of phthalic anhydride to phthalide has been reported (37). A possible mechanism for the lactone reduction is:

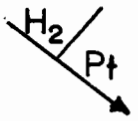


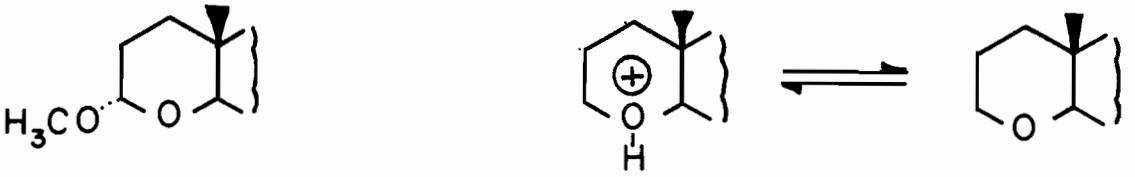
XXXVI



XXXVIII

XXXVII





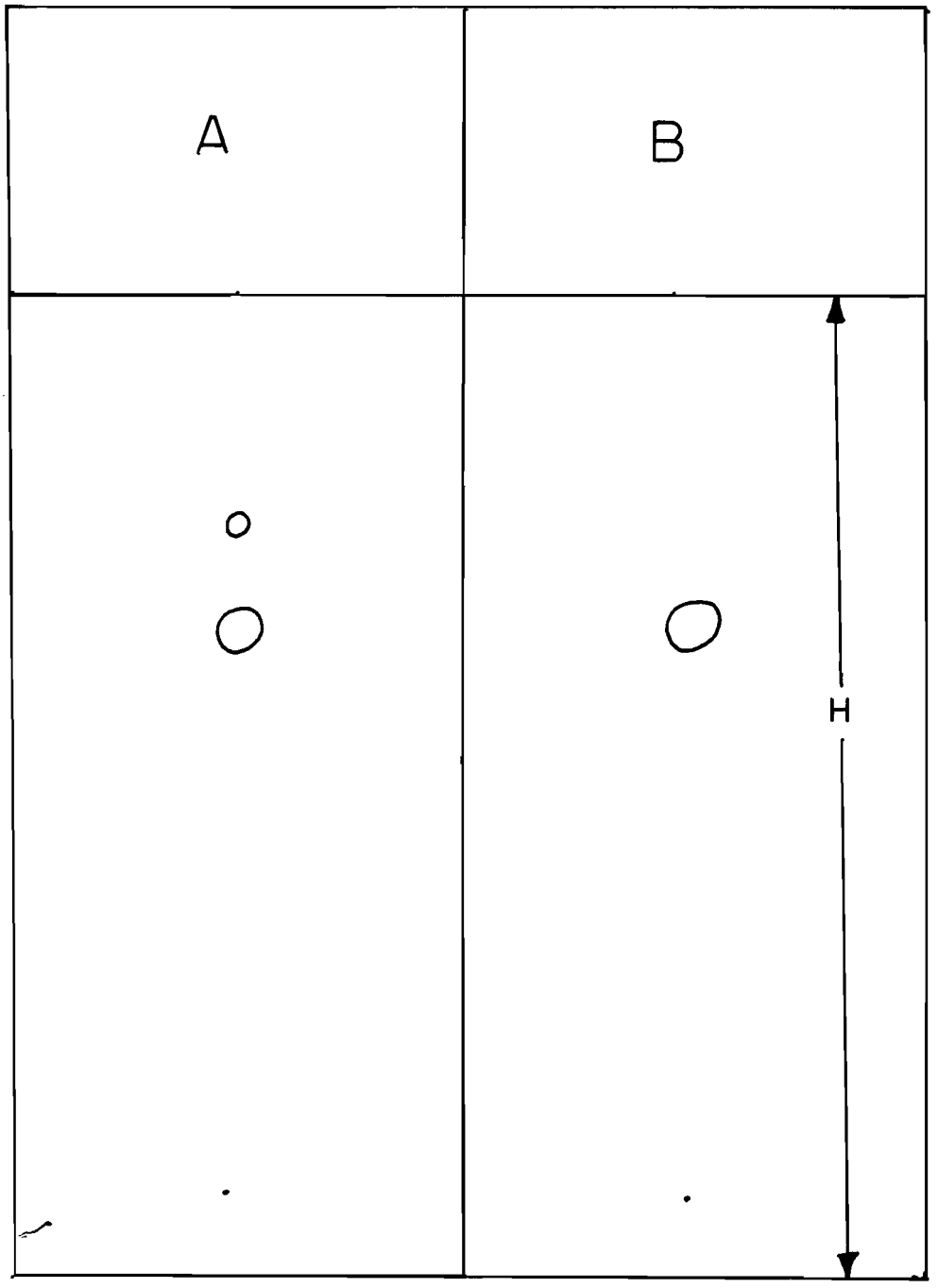
XXXIX

An attempt to trap the intermediate XXXVII by carrying out the hydrogenation of IXa in methanol containing hydrogen chloride, in which XXXVII should be converted to XXXIX, was unsuccessful, only the tetrahydropyran being obtained. This is perhaps to be expected because XXXIX like XXXVII, should be in equilibrium with XXXVIII in acid solution and hydrogenation of the latter should give the ether.

Further investigation with other lactones and esters is required to define the scope of the reaction; the mechanism outlined above suggests that the ability of lactones to undergo catalytic reduction may be related (among other things) to their basicity and hence ability to form the intermediate ion XXXVI.

FIG. 1

Thin-layer chromatography of crude product isolated (a) after sodium borohydride reduction and (b) after lithium tri-t-butoxy aluminum hydride reduction of 17β-hydroxy-5-oxo-3,5-seco-4-norestrane-3-oic acid.

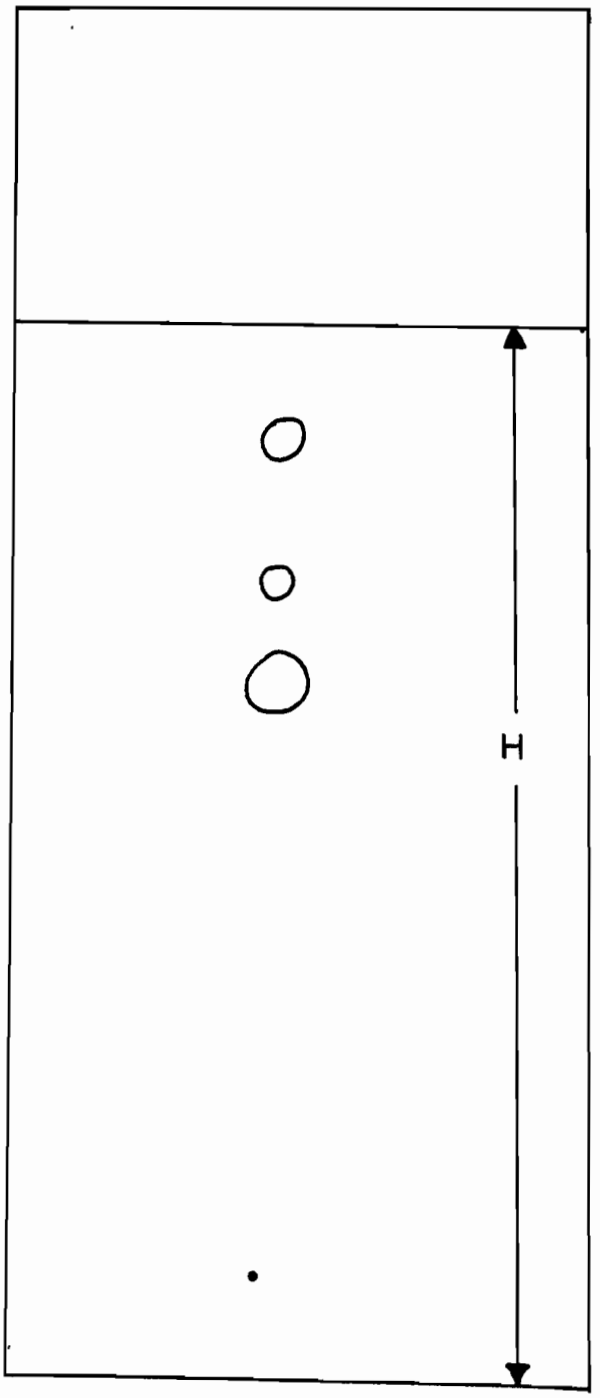


Solvent System: Ethyl Acetate-Ether (75/25, v/v)

H = Solvent Front

FIG. 2

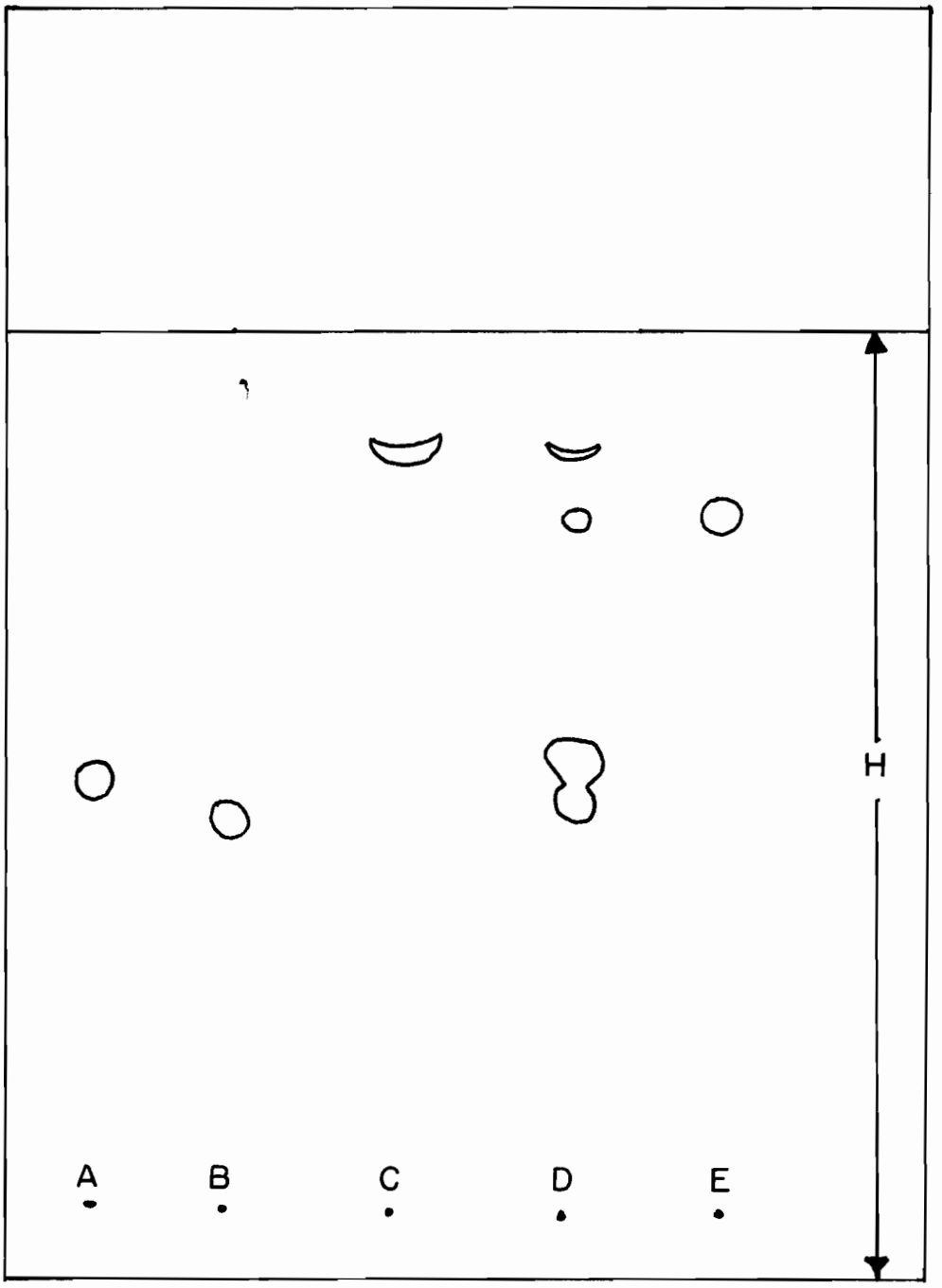
Thin-layer chromatography of crude product from the hydrogenation of 17β-hydroxy-5-oxo-3,5-seco-4-norestrane-3-oic acid with one mole of hydrogen.



Solvent System: Ethyl Acetate-Ether (75/25, v/v)

FIG. 3

Thin-layer chromatography of crude product isolated from hydrogenation of 5-oxo-3,5-seco-4-norcholestane-3-oic acid with one mole of hydrogen.



Solvent System: Ether-Ethyl Acetate (90/10, v/v)

A - Pure 3-oxo-4-oxa-5β-cholestane

B - Pure 3-oxo-4-oxa-5α-cholestane

C - Pure 4-oxa-5β-cholestane

D - Mixture after Hydrogenation

E - Pure 4-oxa-5α-cholestane

H = Solvent Front.

FIG. 4

Infrared spectrum of chloroform solutions of 17β-hydroxy-3-oxo-4-oxa-5α-estrane from sodium borohydride reduction (-) and from hydrogenation (••).

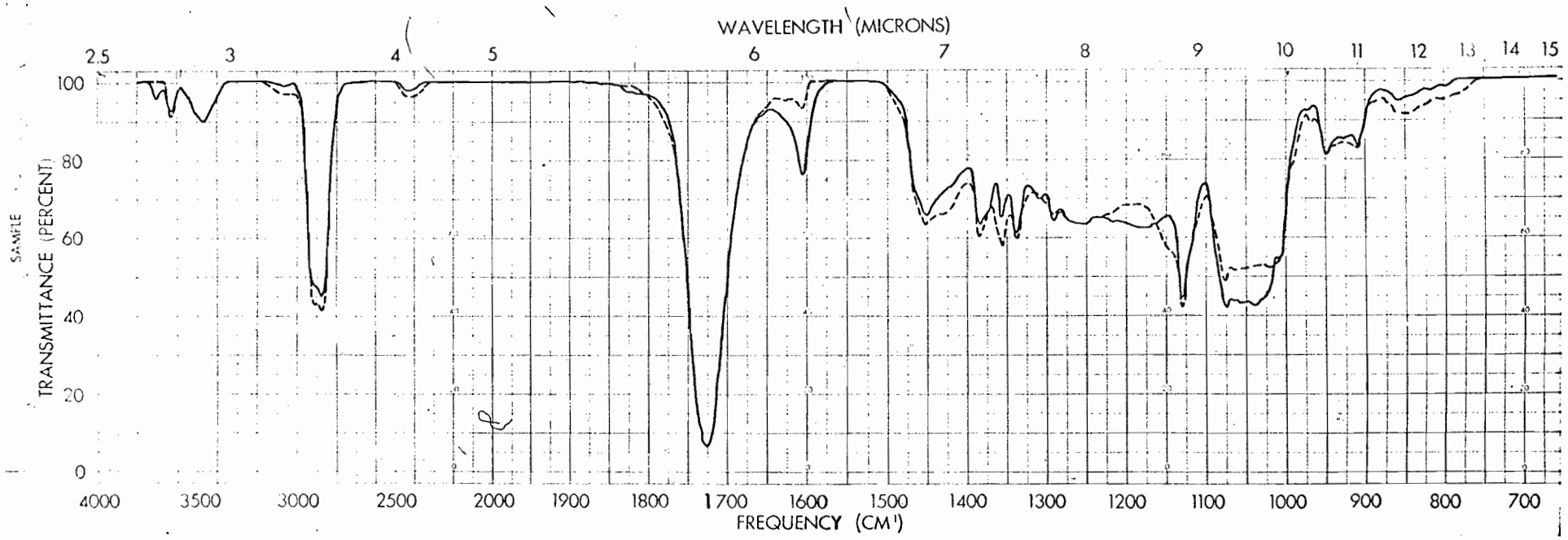

WAVELENGTH (MICRONS)
2.5
3
4
5
6
7
8
9
10
11
12
13
14
15
TRANSMITTANCE (PERCENT)
100
80
60
40
20
0
4000
3500
3000
2500
2000
1900
1800
1700
1600
1500
1400
1300
1200
1100
1000
900
800
700
FREQUENCY (CM⁻¹)

FIG. 5

Infrared spectrum of chloroform solutions of 17β-hydroxy-4-oxa-5α-estrane from boron trifluoride-lithium aluminum hydride (-) and from hydrogenation (••).

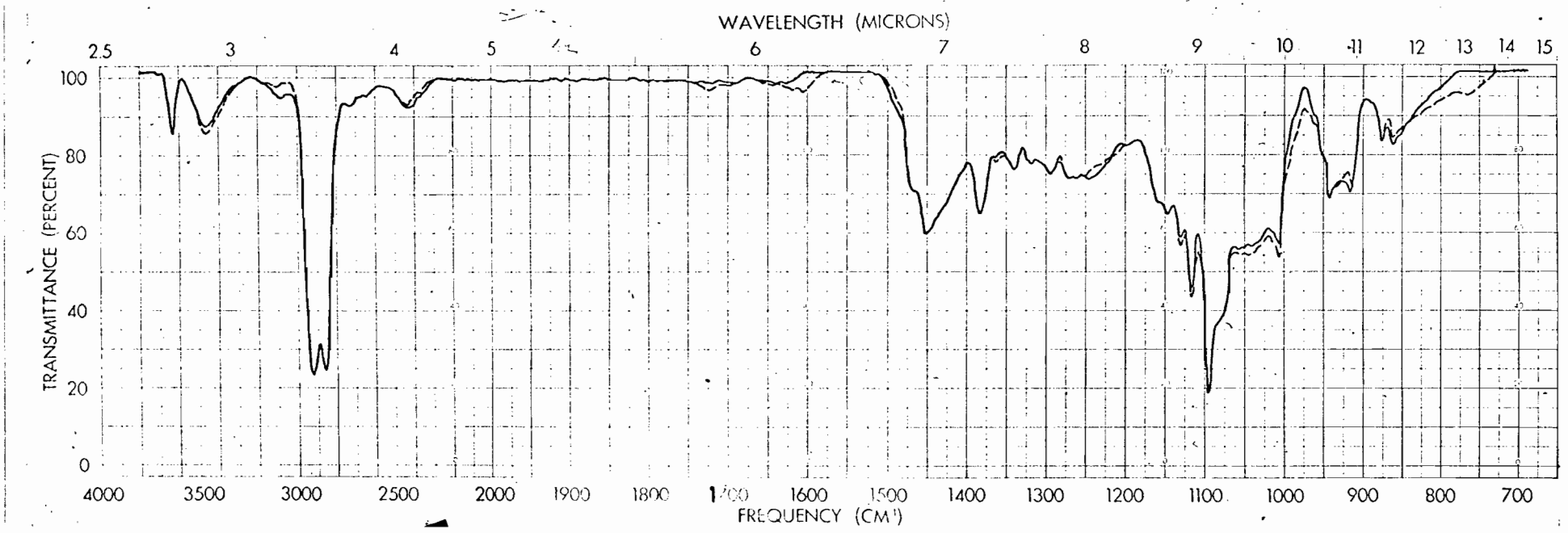

WAVELENGTH (MICRONS)
2.5
3
4
5
6
7
8
9
10
11
12
13
14
15
TRANSMITTANCE (PERCENT)
100
80
60
40
20
0
4000
3500
3000
2500
2000
1900
1800
1700
1600
1500
1400
1300
1200
1100
1000
900
800
700
FREQUENCY (CM⁻¹)

FIG. 6

Infrared spectrum in KBr of 17β,5β-dihydroxy-3,5-oxo-4-norestrane-3-carboxamide.

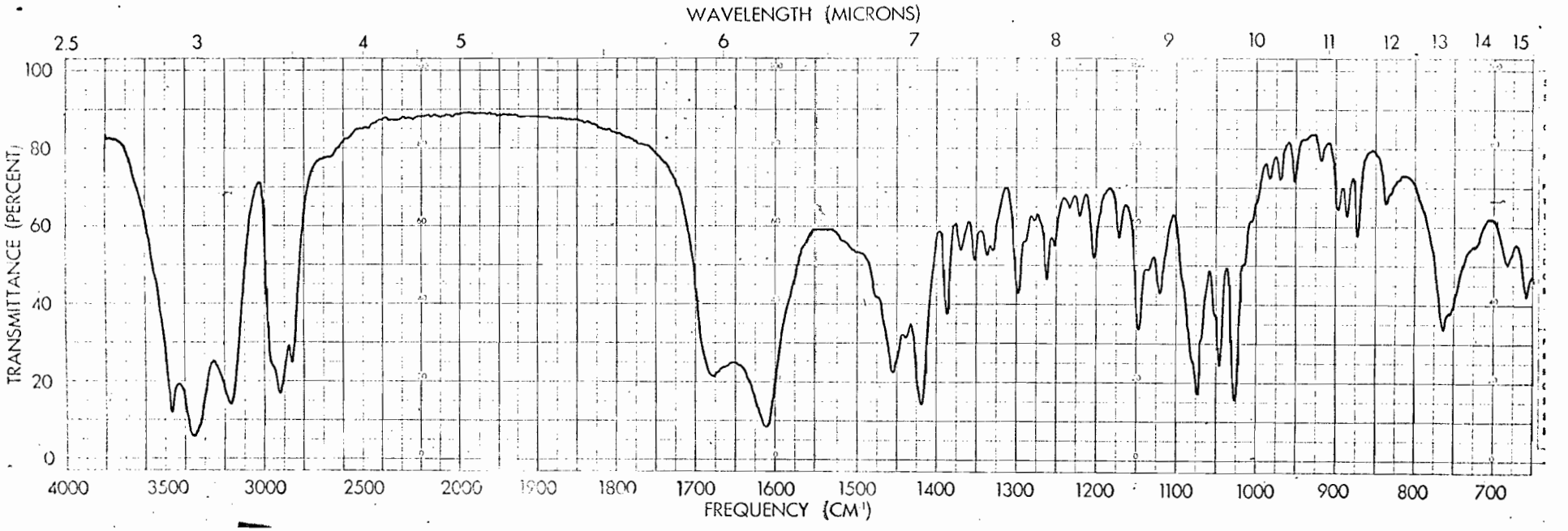

WAVELENGTH (MICRONS)
2.5
3
4
5
6
7
8
9
10
11
12
13
14
15
TRANSMITTANCE (PERCENT)
100
80
60
40
20
0
4000
3500
3000
2500
2000
1900
1800
1700
1600
1500
1400
1300
1200
1100
1000
900
800
700
FREQUENCY (CM⁻¹)

FIG. 7

Infrared spectrum of carbon tetrachloride solutions of 4-oxa-5α-cholestane from benzene-sulfonyl chloride-pyridine (-) and from hydrogenation (••).

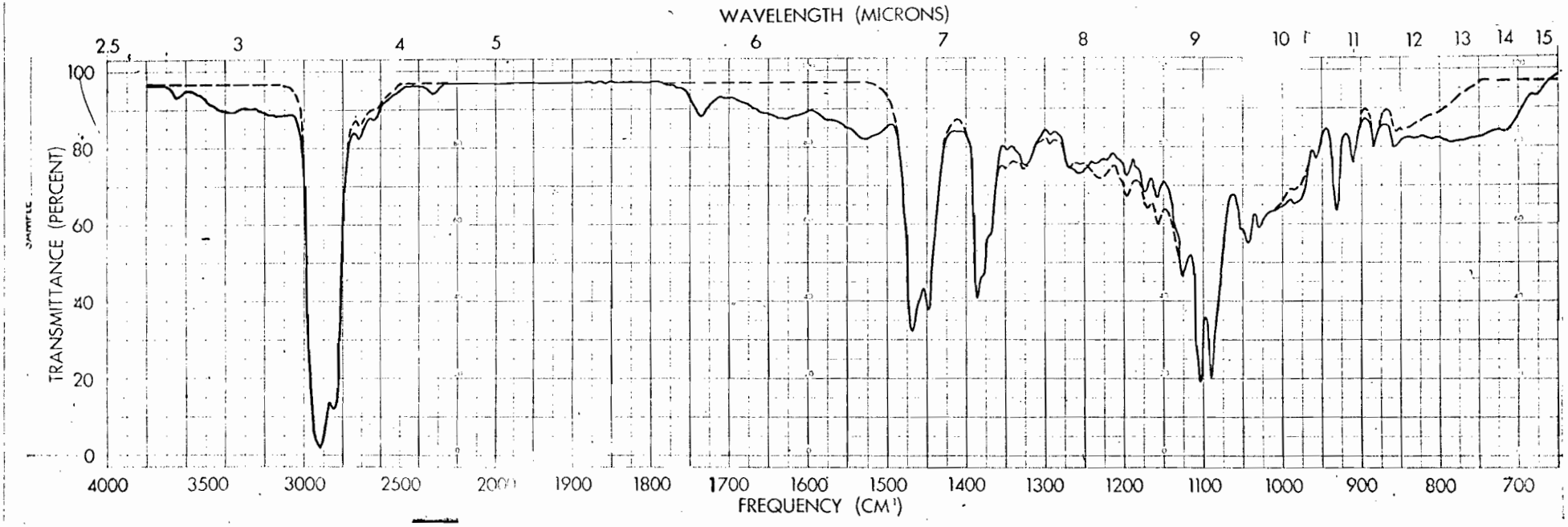
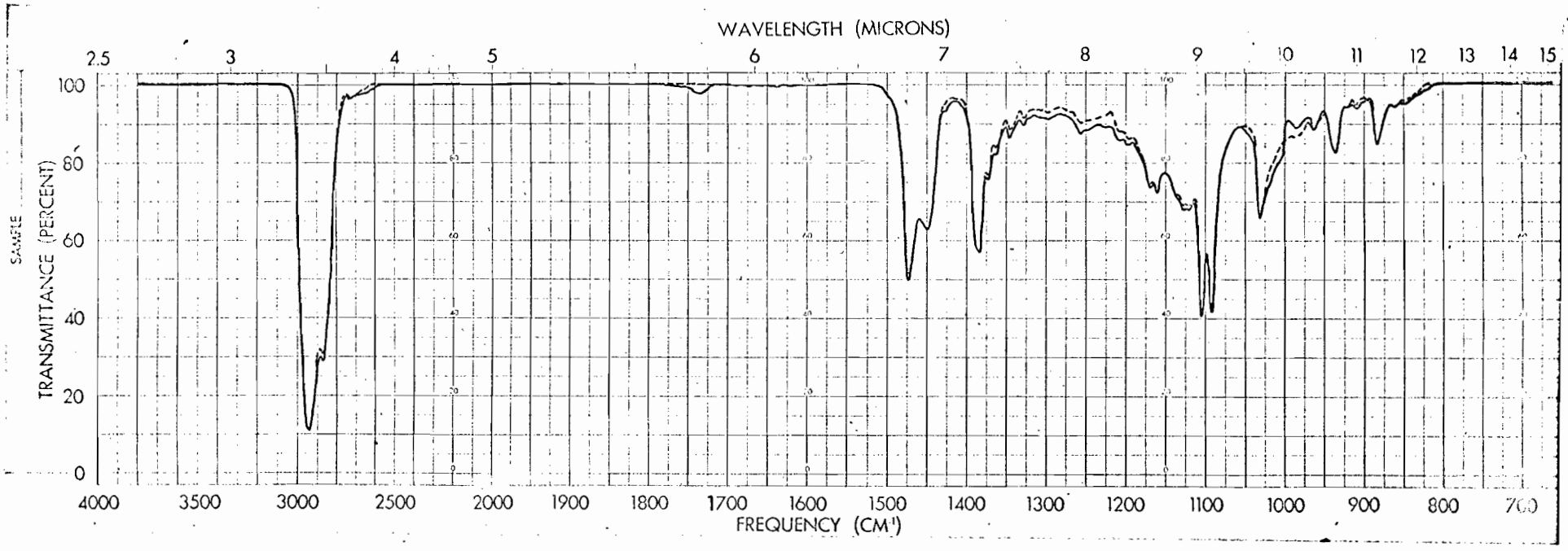

WAVELENGTH (MICRONS)
2.5
3
4
5
6
7
8
9
10
11
12
13
14
15
TRANSMITTANCE (PERCENT)
100
80
60
40
20
0
4000
3500
3000
2500
2000
1900
1800
1700
1600
1500
1400
1300
1200
1100
1000
900
800
700
FREQUENCY (CM⁻¹)

FIG. 8

Infrared spectrum of carbon tetrachloride solution of 4-oxa-5β-cholestane from benzene-sulfonyl chloride-pyridine (-) and from hydrogenation (••).


WAVELENGTH (MICRONS)
2.5
3
4
5
6
7
8
9
10
11
12
13
14
15
TRANSMITTANCE (PERCENT)
100
80
60
40
20
0
4000
3500
3000
2500
2000
1900
1800
1700
1600
1500
1400
1300
1200
1100
1000
900
800
700
FREQUENCY (CM⁻¹)

EXPERIMENTAL

Chromatography was done on Grace and Davidson activated silica gel. Thin-layer chromatography plates were developed with concentrated sulfuric acid. Magnesium sulfate was used to dry all solvent extracts before concentration.

The melting points reported for analytical samples were observed on a Gallenkamp melting point apparatus under reduced pressure but are not corrected. Other melting points were performed using open capillary tubes and are also uncorrected.

Infrared spectra were recorded on a Perkin-Elmer Model 21 spectrophotometer. Optical rotation measurements (in chloroform solution unless otherwise noted) were taken on a Carl Zeiss Apparatus (No. 367732). Microanalyses are by Dr. C. Daessle of Montreal, Canada. Each compound was proved to be homogeneous by thin-layer chromatography before analysis.

17β-Acetoxy-5-oxo-3,5-seco-4-norestrane-3-oic acid (XX; R = $COCH_3$)

Potassium carbonate (0.28 g) in water (8 ml) was added with vigorous stirring to a solution of the 17β-acetate of 19-nortestosterone (0.5 g) in t-butanol-water azeotrope (30 ml), followed by 5 ml of a solution prepared from sodium metaperiodate (2 g) and water (25 ml) and then 0.5 ml of 0.8% aqueous potassium permanganate. The remainder of the periodate was added at a rate of 5 ml/minute for 2 minutes and then 2 ml/minute for 5 minutes. Permanganate solution was added when necessary to maintain the purple color.

After one hour, the excess permanganate was destroyed with sodium bisulfite. The resulting iodine-colored solution was concentrated at reduced pressure to a volume of 30 ml, cooled at 4°C, acidified with ice-cold 50% sulfuric acid, and extracted with ether. The ethereal extract was washed with sodium bisulfite until free from iodine, with water until neutral and dried.

The oil (0.446 g) obtained on evaporation was chromatographed on 3% deactivated silica gel and a crystalline fraction (0.068 g; 1.2% yield) m.p. 80-89°C was obtained on elution with ether. Two recrystallizations from methanol-water gave 17β-acetoxy-5-oxo-3,5-seco-4-norestrane-3-oic acid (XXb) (0.032 g) m.p. 114-115°C, $[\alpha]_D^{24}$ - 3.8 (c 0.457). (Lit. m.p. 113-115°C, $[\alpha]_D^{28}$ - 4.08 ($CHCl_3$) (23). $\nu_{max}^{CCl_4}$ 1708 and 1733 cm^{-1}.

Calc. for $C_{19}H_{28}O_5$: C: 67.83%; H, 8.38%

Found: C: 67.52%; H, 8.33%.

17β-Hydroxy-5-oxo-3,5-seco-4-norestrane-3-oic acid (XX; R = H)(23)

19-Nortestosterane-17β-acetate (1.007 g) was dissolved in ethyl acetate (60 ml) and treated with oxygen-containing ozone (6%) during one hour at room temperature. After that period, 2 ml of hydrogen peroxide (30%) in 4 ml of water and 10 ml of methanol was added to the mixture which was allowed to stand overnight.

After its volume had been reduced to one-half under reduced pressure, the solution was diluted with ether, extracted with sodium

hydroxide (2%), and left at room temperature for 12 hours. The sodium hydroxide extract was then acidified to pH 3 with conc. hydrochloric acid and extracted with ether. After being washed with water and dried the ethereal solution was taken to dryness to give a crystalline solid (0.813 g; yield 95%) m.p. 85-92°C. Two recrystallizations from acetone-hexane gave the pure 17β-hydroxy-5-oxo-3,5-seco-4-norestrane-3-oic acid (XXa), m.p. 108-109°C, $[\alpha]_D^{24}$ + 1.8 (c 0.449) $\nu_{max}^{CHCl_3}$ 3538 and 1710 cm^{-1}.

Calc. for $C_{17}H_{26}O_4$: C, 69.36%; H, 8.9%

Found: C, 69.27%; H, 8.79%.

17β-Hydroxy-3-oxo-4-oxa-5α-estrane (XXVIa; R = H)

A. By reduction of 17β-hydroxy-3,5-seco-4-norestrane-3-oic acid with sodium borohydride

To the keto acid (1.00 g) dissolved in 25% ethanol (50 ml), sodium borohydride (1.0 g) in water (15 ml) was added. The solution was left overnight, then heated under reflux until no more decomposition of sodium borohydride was noted. The aqueous ethanol solution was acidified to pH 3 and concentrated under reduced pressure to one-half of its initial volume. It was then poured into ice-cold water and left at room temperature for 12 hours. A crystalline solid (0.404 g; yield 52%) m.p. 105-115°C was collected. Recrystallizations from ethyl ether yielded the pure 17β-hydroxy-3-oxo-4-oxa-5α-estrane, m.p. 131-132°C, $[\alpha]_D^{24}$ + 70.1 (c 0.502) $\nu_{max}^{CHCl_3}$ 3630 and 1725 cm^{-1}.

Calc. for $C_{17}H_{26}O_3$: C, 73.31%; H, 9.41%

Found: C, 73.09%; H, 9.13%.

B. By reduction of 17β-hydroxy-5-oxo-3,5-seco-4-norestrane-3-oic acid with lithium aluminum tri-t-butoxy hydride

The keto acid (0.972 g) dissolved in tetrahydrofuran (15 ml) was added to a suspension of lithium aluminum tri-t-butoxy hydride (6 equi.) in the same solvent. The mixture was allowed to stand at 0°C for half an hour and at room temperature for one hour, and the mixture was then poured in excess dilute hydrochloric acid. On standing overnight the solution deposited a solid product (0.575 g; 61% yield). Recrystallizations from ether yielded pure 17β-hydroxy-4-oxa-3-oxo-5α-estrane, m.p. 131-132°C, $[\alpha]_D^{24}$ + 70.1 (c 0.504), $\nu_{max}^{CHCl_3}$ 3630 and 1725 cm^{-1}.

C. By oxidation of 19-nortestosterone-17β-acetate with potassium persulfate

Potassium persulfate (1.3 g) and conc. sulfuric acid (1.4 g) were mixed in a mortar and diluted with glacial acetic acid (22 ml). The resulting mixture was added to a solution of 19-nortestosterone-17β-acetate (0.999 g) in glacial acetic acid (22 ml). Following a seven-day period of intermittent shaking at room temperature in the absence of light, the mixture was cooled and treated with aqueous 5% potassium hydroxide. Precipitated salts were removed by filtration and the solution was evaporated to dryness under reduced pressure at 60°C. A solution of the residue in ether was washed successively with water, 5% sodium carbonate and water. The solvent was removed and residual solid saponified (1 hour) in a refluxing solution of potassium hydroxide (2 g) in dioxane (12 ml) and water (12 ml).

The mixture, after being acidified with dilute hydrochloric acid, was extracted with ether. The extract was washed with water, dried, and concentrated. It yielded an oil (0.158 g) which was crystallized from ethyl ether to give the pure 17β-hydroxy-3-oxo-4-oxa-5α-estrane, m.p. 131-132°C, $[\alpha]_D^{24}$ + 70.4 (c 0.517). This compound was shown to be identical with the product obtained in (a) and (b) above by mixed melting point and infrared spectrum.

17β-Acetoxy-3-oxo-4-oxa-5α-estrane (XXVI; R = $COCH_3$)

17β-Hydroxy-3-oxo-4-oxa-5α-estrane (0.200 g) was acetylated with a mixture of acetic anhydride (2 ml) and pyridine (2 ml). Following recrystallizations from ether-acetone, 17β-acetoxy-3-oxo-4-oxa-5α-estrane (0.095 g) was collected, m.p. 208-209°C, $[\alpha]_D^{20}$ + 40.2 (c 0.501) $\nu_{max}^{CCl_4}$ 1733 cm^{-1}.

Calc. for $C_{19}H_{28}O_4$: C, 71.22%; H, 8.81%

Found: C, 71.31%; H, 8.69%.

17β,5β-Dihydroxy-3,5-oxo-4-norestrane-3-carboxamide (XXVII; R = NH_3)

A solution of 17β-hydroxy-3-oxo-4-oxa-5α-estrane (0.200 g) in methanol saturated with ammonia gas deposited needles of 17β,5β-dihydroxy-3,5-oxo-4-norestrane-3-carboxamide (0.165 g; yield 83%), which after recrystallizations from methanol melted at 298-300°C, $[\alpha]_D^{24}$ + 18.8 (c 0.461 in methanol) ν_{max}^{KBr} 3465, 3360, 3180, 1680, 1610 cm^{-1}.

Calc. for $C_{17}H_{29}O_3N$: C, 69.11%; H, 9.90%; N, 4.74%

Found: C, 69.31%; H, 9.73%; N, 4.91%.

After treatment with dilute hydrochloric acid the amide regenerated the starting lactone identified by melting point, mixed melting point, infrared spectrum and optical rotation.

Hydrogenation of 17β-hydroxy-5-oxo-3,5-seco-4-norestrane-3-oic acid (XX; R = H)

The 17β-hydroxy keto acid (1.142 g) was dissolved in glacial acetic acid (15 ml) and hydrogenated in the presence of Adam's catalyst (0.420 g) at room temperature and atmospheric pressure. At the end of two and a half hours, 99% of the theoretical amount of hydrogen had been absorbed.

The catalyst was removed by filtration and the filtrate diluted with ether and washed with 2% sodium hydroxide. The ethereal fraction was dried over magnesium sulfate. The oil (0.142 g; yield 13.8%) collected after evaporation of ether, slowly crystallized from ether, and was identified as 17β-hydroxy-4-oxa-5α-estrane by comparison with authentic material described below.

The sodium hydroxide solution was acidified with dilute hydrochloric acid and extracted with ether. The ethereal solution was washed with 5% sodium carbonate, with water, and dried. Evaporation of the ether left an oil (0.487 g) which was chromatographed over activated silica gel. The fractions (0.287 g; 26.2%) eluted by benzene-ether (50/50) were crystallized from ether to give 17β-hydroxy-3-oxo-4-oxa-5α-estrane, m.p. 125-129°C, $[\alpha]_D^{24}$ + 67.6 (c 0.492). The identity of the compound was established by mixed melting point, thin-layer

chromatography (Fig. 2), infrared spectrum.

The sodium carbonate fraction was acidified and after the usual treatment gave the starting material (0.456 g; 40%).

17β-Hydroxy-4-oxa-5α-estrane (XXIX; R = H)

A. From reduction of 17β-hydroxy-3-oxo-4-oxa-5α-estrane with lithium aluminum hydride-boron trifluoride

17β-Hydroxy-3-oxo-4-oxa-5α-estrane (0.262 g) dissolved in boron trifluoride-ether complex (0.840 g) was added to a suspension of lithium aluminum hydride (0.080 g; 2 equivs.). The mixture was left at 0°C for one hour and refluxed for 2 hours. It was then poured into excess dilute hydrochloric acid and the mixture was extracted with ether. The ethereal fraction was washed with 2% sodium hydroxide, water, and dried. Evaporation of ether gave a crystalline solid (0.216 g; yield 86%) melting at 166-168°C. Two recrystallizations from ether gave pure 17β-hydroxy-4-oxa-5α-estrane, m.p. 173-175°C, $[\alpha]_D^{24}$ + 42.8 (c 0.492).

Calc. for $C_{17}H_{28}O_2$: C, 77.22%; H, 10.67%

Found: C, 77.15%; H, 10.63%.

B. From hydrogenation of 17β-hydroxy-5-oxo-3,5-seco-4-norestrane-3-oic acid

17β-Hydroxy-5-oxo-3,5-seco-4-norestrane-3-oic acid (0.831 g) dissolved in glacial acetic acid (10 ml) was hydrogenated over Adam's catalyst (0.413 g) at normal temperature and pressure until no more

hydrogen was absorbed (234 cc after 48 hours 370%).

The catalyst was removed by filtration and the solution diluted with ether. The ethereal solution was washed with 2% sodium hydroxide, water, and dried. Evaporation of ether gave a white crystalline solid (0.545 g; yield 74%) m.p. 168-172°C. Recrystallization from ether gave needles melting at 173-175°C, $[\alpha]_D^{24}$ + 42.6 (c 0.496).

Calc. for $C_{17}H_{28}O_2$: C, 77.22%; H, 10.67%

Found: C, 77.17%; H, 10.61%.

The compound was identical with that prepared above, as shown by mixed melting point and infrared spectrum.

C. From hydrogenation of 17β-hydroxy-3-oxo-4-oxa-5α-estrane

17β-Hydroxy-3-oxo-4-oxa-5α-estrane (0.210 g) dissolved in 15 ml of glacial acetic acid was hydrogenated over Adam's catalyst (0.090 g) until no more hydrogen was absorbed.

The solution was diluted with ether and filtered. The ethereal solution was washed with 2% sodium hydroxide, with water and dried. Evaporation of ether gave a white crystalline solid (0.185 g; yield 90%) m.p. 173-175°C, $[\alpha]_D^{24}$ + 42.8 shown by mixed melting point and infrared spectrum to be identical with the products above.

4-Oxa-5β-cholestane (VIII)

3-Oxo-4-oxa-5β-cholestane (0.200 g) was dissolved in glacial

acetic acid (15 ml) and hydrogenated over Adam's catalyst (0.090 g) until no more hydrogen was absorbed. The product obtained after the the usual work-up gave, after one crystallization from methanol, cosettes of white needles (0.175 g; yield 89%) m.p. 51-52°C, $[\alpha]_D^{23}$ + 3.5 (c 0.629) (Lit. m.p. 51-52°C, $[\alpha]_D^{17}$ + 3.8 ($CHCl_3$) (12)). The compound was identified as 4-oxa-5β-cholestane (12) by mixed melting point, thin-layer chromatography (Fig. 3) and by comparison of infrared spectra.

4-Oxa-5α-cholestane (XIV)

3-Oxo-4-oxa-5α-cholestane (0.201 g) dissolved in glacial acetic acid and hydrogenated over Adam's catalyst (0.090 g) until no more hydrogen was absorbed, gave a product which after one crystallization from methanol afforded white colorless plates (0.187 g; yield 92%) at 89-90°C $[\alpha]_D^{23}$ + 43.4 (c 0.577) (Lit. m.p. 89-90°C, $[\alpha]_D^{23}$ + 42.3 (12)). The compound had the same infrared spectrum as that reported for 4-oxa-5α-cholestane (12).

BIBLIOGRAPHY

1. R.B. Turner, J. Am. Chem. Soc., 72, 579 (1950).
2. A. Windaus, Ber., 39, 2008 (1906).
3. R. Willstätter and E.W. Mayer, Ber., 41, 2199 (1908).
4. D.H.R. Barton, J. Chem. Soc., 1027 (1953).
5. W. Klyne, Chem. and Ind., 1198 (1954).
6. C.S. Hudson, J. Am. Chem. Soc., 32, 338 (1910).
7. C.S. Hudson, J. Am. Chem. Soc., 61, 1529 (1939).
8. J.T. Edward and P.F. Morand, Can. J. Chem., 38, 1325 (1960).
9. L.F. Fieser and M. Fieser, "Steroids" Rheinhold, New York. 1959. p. 80.
10. T.F. Gallaher and T.H. Ktritchesky, J. Am. Chem. Soc., 72, 882 (1950).
11. R.B. Turner, J. Am. Chem. Soc., 72, 879 (1950).
12. K. Mislow and J. Brenner, J. Am. Chem. Soc., 75, 2319 (1953).
13. J.T. Edward, W.H. Lunn, Ph.D. Thesis, McGill University (1961).
14. N.W. Atwater and J.W. Rolls, J. Am. Chem. Soc., 82, 2011 (1960).
15. E.L. Eliel, "Stereochemistry of Carbon Compounds" McGraw-Hill, New York. 1962. p. 326.
16. G.R. Pettit and T.R. Kasturi, J. Org. Chem., 25, 875 (1960).
17. G.R. Pettit and U.R. Ghatak, B. Green, T.R. Kasturi and D.M. Piatak, J. Org. Chem., 26, 1685 (1961).
18. G.R. Pettit and T.R. Kasturi, J. Org. Chem., 26, 4553 (1961).
19. G.R. Pettit, B. Green, T.R. Kasturi, U.R. Ghatak, Tetrahedron, 18, 953 (1962).
20. G.R. Pettit and T.R. Kasturi, J. Org. Chem., 26, 4557 (1961).
21. G.R. Pettit, T.R. Kasturi and J.C. Knight, J. Org. Chem., 26, 4773 (1961).

22. J.T. Edward, P.F. Morand and I. Puskas, Can. J. Chem., 39, 2069

23. J.A. Hartman, A.J. Tomasewski and A.S. Dreiding, J. Am. Chem. Soc., 78, 5662 (1956).

24. W.G. Dauben, G.F. Fouken and D.S. Noyce, J. Am. Chem. Soc., 76, 2579 (1956).

25. W.G. Dauben, E.J. Blanz, Jr., J. Jiu and R.A. Micheli, J. Am. Chem. Soc., 78, 3752 (1956).

26. O.R. Vail and D.M.S. Wheeler, J. Org. Chem., 27, 3803 (1962).

27. O.H. Wheeler and J.L. Mateos, Chem. and Ind., 395 (1957).

28. O.H. Wheeler and J.L. Mateos, Can. J. Chem., 36, 325 (1958).

29. H.O. House, H. Badad, R.B. Toothill and A.W. Nottes, J. Org. Chem., 27, 4141 (1962).

30. C.H. Hassall. "Organic Reactions". Vol IX, John Wiley and Sons, New York. 1957. p. 81

31. A. Salaman, Z. Physiol. Chem., 272, 61 (1941).

32. E. Caspi, Y.W. Chang and R.I. Dorfman, J. Med. Pharm. Chem., 5, 714 (1962).

33. R.J. Wicker, J. Chem. Soc., 2165 (1956).

34. E.L. Eliel. "Stereochemistry of Carbon Compounds" McGraw-Hill, New York. 1962. p. 328.

35. E.L. Eliel. "Stereochemistry of Carbon Compounds" McGraw-Hill, New York. 1962. p. 211.

36. L.F. Fieser and M. Fieser. "Steroids" Rheinhold, New York. 1959. p. 271.

37. R. McCrindle, K.H. Overton and R.A. Raphael, J. Chem. Soc., 4798 (1962).

38. J.T. Edward, D. Holder, W.H. Lunn and I. Puskas, Can. J. Chem., 39, 599 (1961).

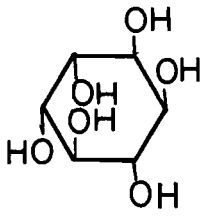
39. J.I. Musher, J. Am. Chem. Soc., 83, 1146 (1961).

EXTRACTIVES OF EUPHORBIA PULCHERIMMA

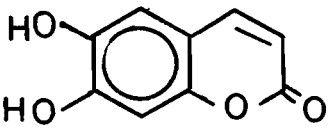
HISTORICAL INTRODUCTION

A great variety of compounds have been extracted from the leaves and stems of various species of euphorbia such as sugars, fatty acids, proteins and amino-acids (1,2,3,4,5,6). The flowers of these species have furnished alcohols such as L-inositol (7,8) (I), coumarin derivatives such as aesculetin (II) (9) and daphnetin (III) (10), acids such as ellagic acid dimethyl ether (IV) (11) and glucosides such as quercitin (V) (12,13), xanthoramnin (VI) (14,15), and aucubin (VII) (16,17,18). However, the latex of these plants has been most intensively investigated. It has furnished β-ethyl malic acid (19), biglandulinic acid (VIII) (20,21), methyl gallate (19) and several important triterpenes: cycloartenol (IX) (22,23,24,25,26), lanosterol (X) (27,28,29,30), euphol (XI) (31,32,33,34), taraxasterol (XII) (35,36,37), tinucallol (XIII) (38,39,40), euphorbol (XIV) (41,42,43) and isolupeol (XV) (44,45,36).

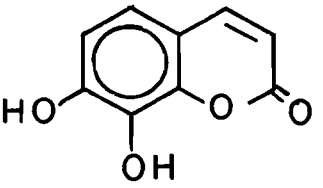
While many species of euphorbia have been intensively investigated, very few studies have been made of the chemical constituents of euphorbia pulcherimma (poinsettia). Our own work originated in an attempt to extract from the wood of this plant the chromogen responsible for an orange coloration when the wood is treated with a solution of concentrated aqueous hydrochloric acid in methanol (1.5/100 v/v). This attempt proved fruitless but a number of other materials were obtained by extraction of the wood with petroleum ether, followed by benzene, ether, ethanol, water, and acetic acid.



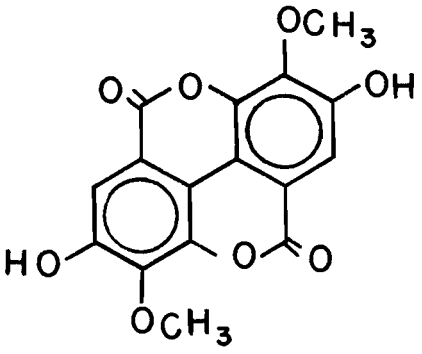
I



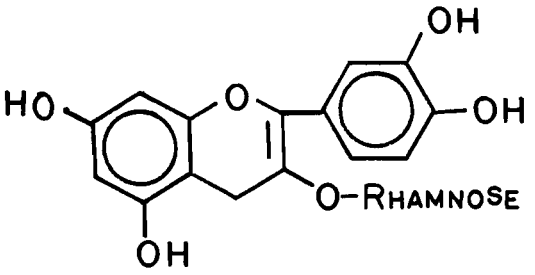
II



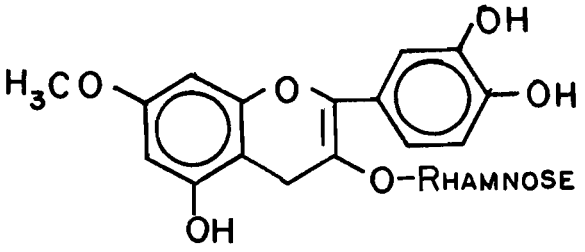
III



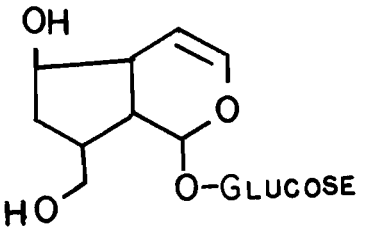
IV



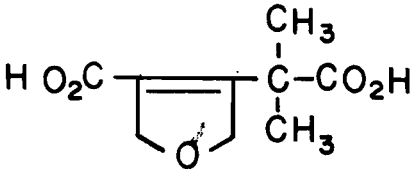
V



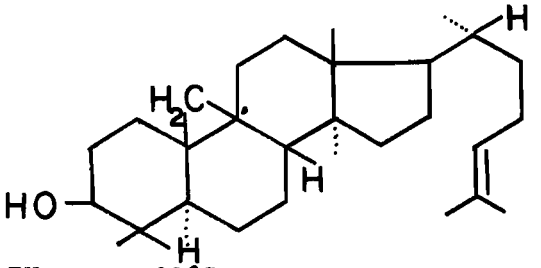
VI



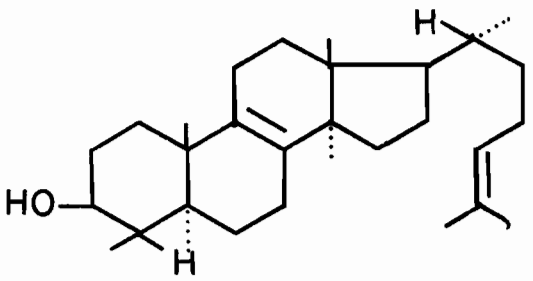
VII



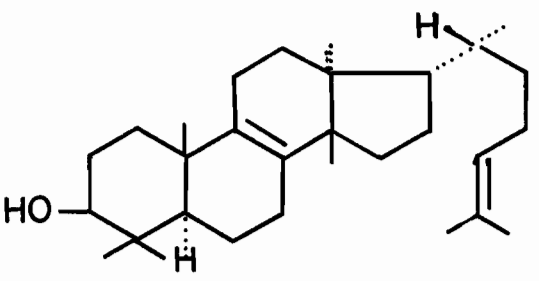
VIII



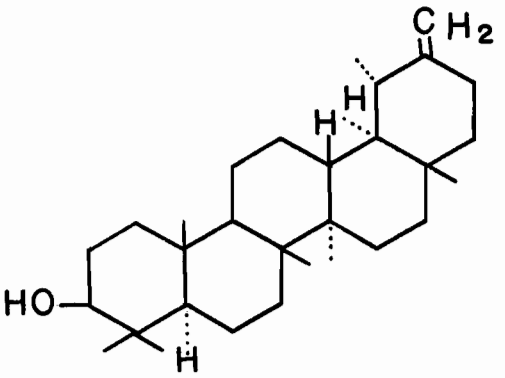
IX m.p. 99°C
$[\alpha]_D$ = + 54 ($CHCl_3$)



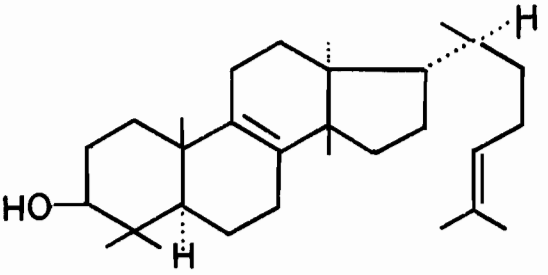
X m.p. 138-140°C
$[\alpha]_D$ = + 61°($CHCl_3$)



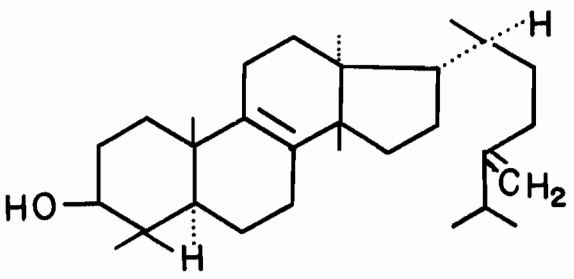
XI m.p. 115-117°C
$[\alpha]_D$ = + 32° ($CHCl_3$)



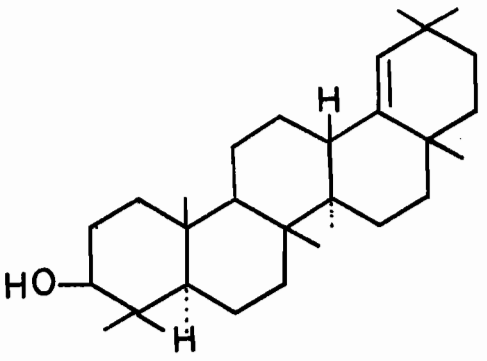
XII m.p. 220-226°C
$[\alpha]_D$ = + 97° ($CHCl_3$)



XIII m.p. 133-134.5°C
$[\alpha]_D$ = + 4.5° (C_6H_6)



XIV m.p. 126-127°C



XV m.p. 177-178°C
$[\alpha]_D^{17}$ = + 5.8° ($CHCl_3$)

RESULTS AND DISCUSSION

Montant (5) reported 17 common amino-acids in 19 species of euphorbia (not including poinsettia) with a preponderance of aspartic acid. Examination by paper chromatography of ethanol and water extracts of the wood of euphorbia pulcherimma proved the presence of 10 common amino-acids with a preponderance of serine. Sucrose and arabinose (13) were also found in these fractions by the same method. No phenolic acids could be detected by paper chromatography in any of the extracts, even after acid or alkaline hydrolysis.

Chromatography on 3% deactivated silica gel of the solid obtained from the petroleum ether extracts gave, on elution with benzene, a crystalline solid. The recrystallized product A, m.p. 279-281°C, analyzed for $C_{30}H_{48}O_2$, and had the general characteristics of a steroid or a triterpene in its infrared and n.m.r. spectra. Peaks in the infrared spectrum at 840 cm^{-1} (47) and in the n.m.r. spectrum (τ = 5.1) (48,49) showed a vinyl hydrogen. However, the double bond could not be brominated by the procedure of Fieser (52).

A proved to be an acetate, since treatment of it with lithium aluminum hydride gave a compound B, $C_{28}H_{46}O$, m.p. 170-174°C, and reacetylation of B in pyridine-acetic anhydride solution gave back the starting product A. B showed a peak at τ = 8.1 in its n.m.r. spectrum which can be attributed to an hydroxy group, since it disappeared on treating the compound with deuterium dioxide. Shoolery and Rogers (48) reported a chemical shift at τ = 8.0 for β-hydroxy steroids. A triplet at τ = 6.8

($w/2 = 6.5$ c/s*) can be assigned to the hydrogen adjacent to the hydroxyl group, indicating probably a secondary alcohol. Musher (50) gives values from $\tau = 6.62$ to $\tau = 6.10$ in 10-methyl-decalols-2 for the 2-hydrogen atom, with a half width equal to 6.7 c/s when this 2-hydrogen atom is equatorial.

A peak in ultraviolet spectrum at 208 mμ ($\varepsilon = 14{,}100$) indicated a tetraalkyl substituted double bond (51).

* $w/2$ = half-width in cycles per second. Solvent: spectroanalyzed carbon tetrachloride.

EXPERIMENTAL*

A. The chopped wood of poinsettia (4 kg) was extracted in a Soxhlet apparatus with four liters of various boiling solvents. Petroleum ether (60-80°C) removed 22.1 g of a yellow solid (yield: 0.5%); benzene, 79 g of a brown oil (yield: 1.5%); ether, 31 g of a brown oil (0.6%); ethanol (95%), 57 g of a brown oil (1.1%). Extractions with acetic acid and water are still in progress.

B. The ethanol fraction was submitted to two-dimensional ascending paper chromatography on Whatman No. 1 filter paper using the following solvent systems: butanol-acetic acid-water (5:1:4) (vols.) and water saturated phenol and containing 3% sodium cyanide. Spraying with 1% nynhydrin in butanol-ethanol (1:1, v/v) gave ten, well-defined blue zones having R_F values for leucine, phenylalamine, valine, tyrosine, alanine, threosine, glycine, serine, aspartic acid and arginine.

C. Portions (100 mg) of each fraction were hydrolyzed with 10% hydrochloric acid and with 10% sodium hydroxide at 0°C for 12 hours. After acidification of the alkaline solutions to pH 2, each portion was extracted with ether. The residues from evaporation of the ether were submitted to descending paper chromatography using the following solvent systems: benzene-acetic acid-water (6:7:3) (vols) and 2% formic acid. It was impossible to detect any phenolic acids after ultraviolet examination or after spraying with ferric chloride or diazotized sulfanilic acid solutions.

---

* Ultraviolet measurements were recorded on a Beckman D.K.1 spectrophotometer (solutions in spectroanalyzed cyclohexane).

Product A

The solid extracted with petroleum ether (60-80°C) was chromatographed on 3% deactivated silica gel. A crystalline compound eluted by benzene and was recrystallized from ether-methanol to give transparent plates (1.21 g; yield 0.03%), m.p. 279-281°C, $[\alpha]_D^{27} = +27.3°$ ($\underline{c} = 0.463$).

Calc. for $C_{30}H_{48}O_2$: C, 81.76%; H, 10.98%

Found: C, 81.76%; H, 10.90%.

Product B

A solution of product A (0.080 g) in ether (15 ml) was added to a suspension of lithium aluminum hydride (0.15 g) in ether (20 ml) over two minutes. The mixture was refluxed for 1-1/2 hours and then 10% aqueous sodium hydroxide (1 ml) was added dropwise with continued stirring. The precipitated inorganic salts were removed by filtration and washed with more ether. Evaporation of the dry solvent gave a white crystalline material (0.073 g; yield 89%), m.p. 164-169°C. Several recrystallizations from methanol-water gave needles melting at 170-174°C, $[\alpha]_D^{27} = 19.3°$ ($\underline{c} = 1.094$).

Calc. for $C_{28}H_{46}O$: C, 84.35%; H, 11.63%

Found: C, 83.81%; H, 11.60%.

Acetylation of this compound with a mixture of acetic anhydride (1 ml) and pyridine (1 ml) gave, after several recrystallizations from ether-methanol an acetate, m.p. 268-271°C, $[\alpha]_D^{27} = +27.5°$ ($\underline{c} = 0.512$)

shown by mixed melting point and infrared spectrum to be identical with product A.

BIBLIOGRAPHY

1. P. Gillot, Mat. Grasses, 21, 8390 (1929).
2. K. Huzii, H. Huzikawa and Y. Kuelo, J. Pharm. Soc. Japan (in German), 88 (1937).
3. M. Zimmerman, Ber. Schweig. Botan. Ges., 63, 402 (1953).
4. P. Matile, Ber. Schweig. Botan. Ges., 66, 237 (1956).
5. C. Montant, Comp. rend., 245, 1454 (1957).
6. L. Crombie and A.G. Jacklin, Chem. and Ind., 1186 (1955).
7. E.P. Hallet and L.M. Parks, J. Am. Pharm. Assoc., 40, 474 (1951); Chem. Abstr., 45, 10507 (1951).
8. E.J. Rower, A.A. Harwood and D.B. Myers, J. Am. Pharm. Assoc., 44, 308 (1955); Chem. Abstr., 49, 10586 (1955).
9. R. Jaretzky and W. Köhler, Chem. Zentr., I, 303 (1943).
10. M. Rindl, Chem. Zentr., I, 715 and 1991 (1934).
11. E. Lederer, J. Chem. Soc., 2115 (1949).
12. R. Kuhn and T. Löw, Ber., 82, 474 (1945).
13. F. Moewus, Chem. Abstr., 44, 9007 (1950).
14. G. Fattree and A.G. Perkin, J. Chem. Soc., 234 (1927).
15. H. Veda and C.H. Hsu, Chem. Abstr., 45, 7306 (1951).
16. P. Paris and M. Chaslot, Ann. Pharm. franç., 13, 648 (1955). Chem. Abstr., 50, 10201 (1956).
17. S. Fujise, H. Obara and H. Uda, Chem. and Ind., 289 (1960).
18. S. Fujise, H. Uda, T. Ishikawa, H. Obara and A. Fujino, Chem. and Ind., 454 (1959).
19. N.P. Kirjalov, Chem. Zentr., II, 773 (1940).
20. Ibid., Chem. Zentr., II, 4479 (1939).
21. Ibid., Chem. Zentr., I, 563 (1940).
22. D.H.R. Barton, J. Chem. Soc., 1444 (1951).

23. S. Chapon and S. David, Bull. soc. chim. France, 456 (1952).

24. J.A. Henry and F.S. Spring, Chem. and Ind., 185 (1954).

25. D.H.R. Barton and E.W. Warnhoff, Chem. and Ind., 220 (1954).

26. D.H.R. Barton and F.S. Spring, J. Chem. Soc., 1316 (1955).

27. D.H.R. Barton, J.S. Fawcett and B.R. Thomas, J. Chem. Soc., 3147 (1951).

28. W. Woser, H.H. Günthard, O. Jeger and L. Ruzicka, Helv. Chim. Act., 35, 66 (1952).

29. C.S. Barnes, D.H.R. Barton, A.R.H. Cole, J.S. Fawcett and B.R. Thomas, J. Chem. Soc., 51 (1953).

30. Ibid., J. Chem. Soc., 576 (1953).

31. G.T. Newbold and F.S. Spring, J. Chem. Soc., 249 (1944).

32. G. Dupont, W. Kopaczewski and Brodski, Bull. soc. chim. France, 1068 (1947).

33. D.H.R. Barton, J.F. McGhie, M.K. Pradhan and S.A. Knight, Chem. and Ind., 1325 (1954).

34. Ibid., J. Chem. Soc., 876 (1955).

35. S. Burrows and J.C.E. Simpson, J. Chem. Soc., 2042 (1938).

36. T.R. Ames, J.L. Beton, A. Bowers, T.G. Halsall and E.R.H. Jones, J. Chem. Soc., 1905 (1954).

37. W. Fruhstorfer, Ber., 87, 423 (1954).

38. D.W. Haines and F.L. Warren, J. Chem. Soc., 2554 (1949).

39. D.H.R. Barton and E. Sloan, J. Chem. Soc., 4150 (1956).

40. F.L. Warren and K.H. Walting, J. Chem. Soc., 179 (1958).

41. J.B. Barbour, F.L. Warren and P.A. Wood, J. Chem. Soc., 2537 (1951).

42. P. Arigoni, R. Viterbo, M. Dünnenberger, O. Jeger and L. Ruzicka, Helv. Chim. Act., 37, 2306 (1954).

43. Ibid., Helv. Chim. Act., 38, 222 (1955).

44. J.C.E. Simpson, J. Chem. Soc., 283 (1944).

45. S. David, Bull. soc. chim. France, 155 (1949).

46. S. Chapon and S. David, Bull. soc. chim. France, 456 (1952).

47. N. Sheppard and D.M. Simpson, Quart. Rev., 6, 1 (1952).

48. J.N. Shoolery and M.T. Rogers, J. Am. Chem. Soc., 80, 5121 (1958).

49. Varian, N.M.R. Spectra Catalog, No. 272.

50. J.I. Musher, J. Am. Chem. Soc., 83, 1146 (1961).

51. R.A. Micheli and T.H. Applewhite, J. Org. Chem., 27, 345 (1962).

52. L.F. Fieser "Experiments in Organic Chemistry". 3rd ed. D.C. Heath and Company, Boston (1957). p. 68.