PREPARATION

OF

SOME UNSATURATED BILE ACIDS

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MUSA Z. NAZER

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ABSTRACT

Methyl- Δ^4-cholenate and methyl- Δ^1-cholenate have been prepared from hyodesoxycholic acid and lithocholic acid respectively.

A convenient method has been developed for the preparation of methyl-3-keto- Δ^1-cholenate from lithocholic acid.

A procedure has been found for the reduction of unsaturated ethylenethioketals with Raney nickel. This procedure has proved to be far superior to the ones previously described in the literature.

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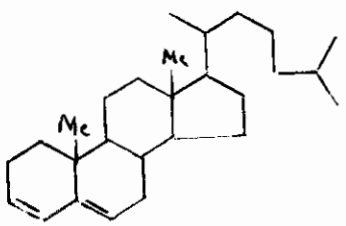
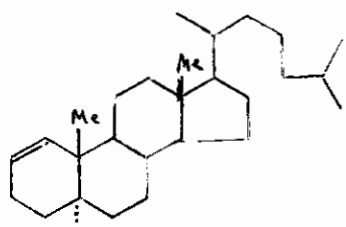
INTRODUCTION

Purpose of the work:

$\Delta^{3,5}$-cholestadiene (I)[1,2,3,4,5,6], Δ^{1}-cholestene (II)[7,8,9], Δ^{2}-cholestene (III)[8,9,10], Δ^{3}-cholestene (IV)[11,12] and Δ^{4}-cholestene (V)[13,14,15], are well known compounds in the cholestane series.



I



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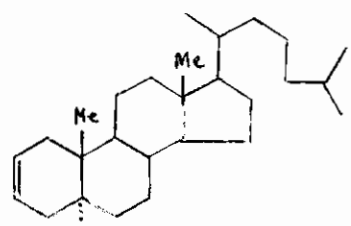

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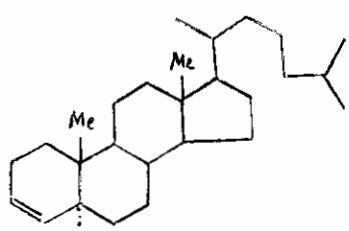
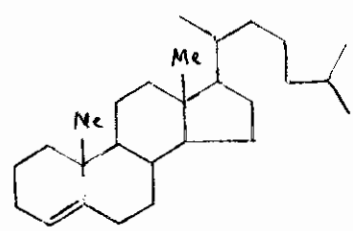
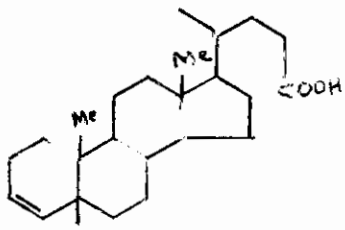


IV



V

Of the corresponding compounds in the bile acid series, only Δ^{3}-cholenic acid (VI)[16] and $\Delta^{3,5}$-choladienic acid (VII)[17,18] are known in a pure state.



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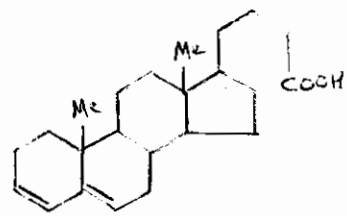
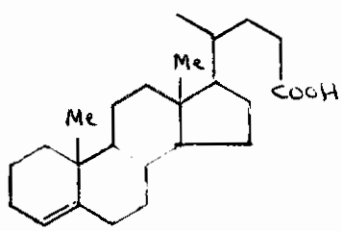
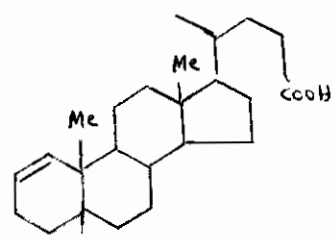


VII

The purpose of the present work was to prepare two unsaturated bile acids: Δ^4 cholenic acid (VIII) from hyodesoxycholic acid and Δ^1 cholenic acid (IX) from lithocholic acid.



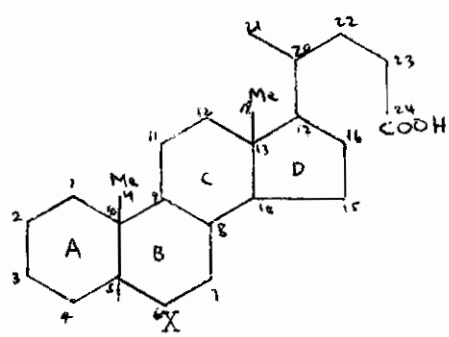
VIII



IX

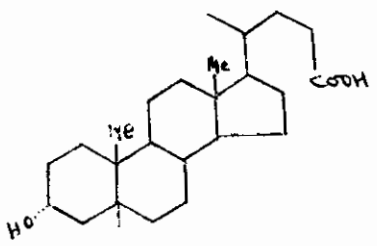
Historical

The naturally occuring bile acids are generally mono, di or trihydroxy derivatives of cholanic acid (X), where the A/B ring junction is cis[19,20,21]. Some of the common bile

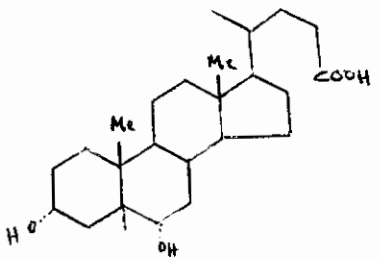


X

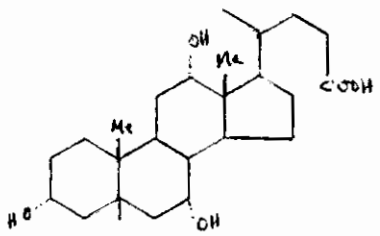
acids are lithocholic acid (XI), hyodesoxycholic acid (XII) and cholic acid (XIII). They all possess a 3 α-hydroxyl group. Related oxoacids are often found in traces in natural sources, while the unsaturated acids are rarely encountered.



XI



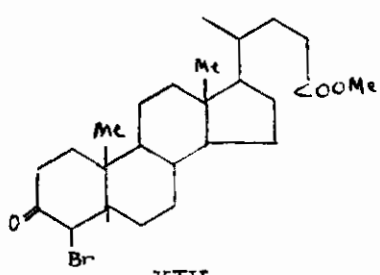
XII



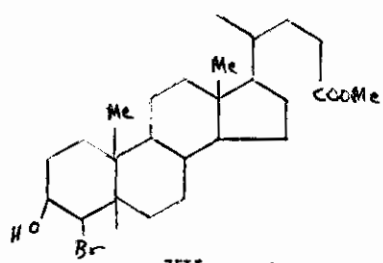
XIII

Δ^3 cholenic acid (VI) and $\Delta^{3,5}$ choladienic acid (VII) are the only ring A unsaturated bile acids that have been prepared in a pure state.

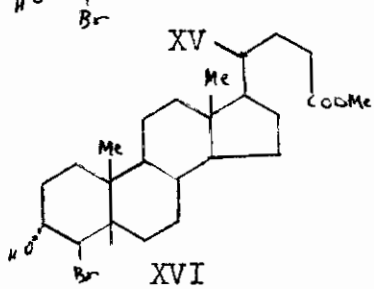
Fieser and Ettore[16] prepared pure methyl Δ^3-cholenate (XVII) from the 4-bromoderivative of methyl 3-ketocholanate (XIV) by reduction with sodium borohydride followed by dehypohalogenation of the resulting bromohydrins (XV, XVI).



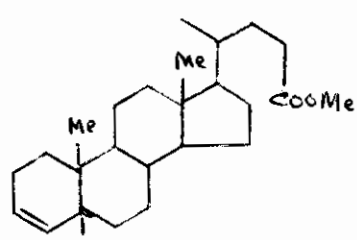
XIV



XV

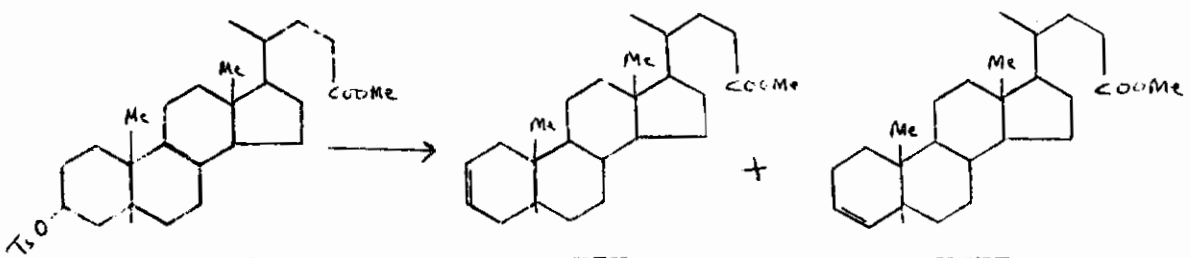


XVI



XVII

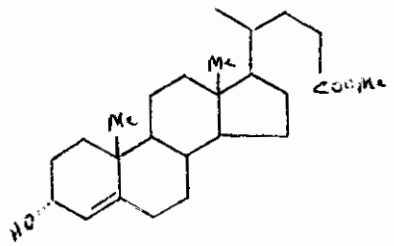
Later on, Chang, Blickenstaff, Feldstein, Gray, McCaleb and Sprunt[22], in an attempt to obtain a large quantity of methyl Δ^{3}-cholenate for the study of its serofluccculating action, dehydrotosylated the suitably constituted methyl 3α-tosyloxycholanate (XVIII) to get an unsaturated product which they regarded as methyl Δ^{3}-cholenate. This product, however, was later found[17] to be a mixture of methyl Δ^{2}-cholenate (XIX) and methyl Δ^{3}-cholenate (XVII).



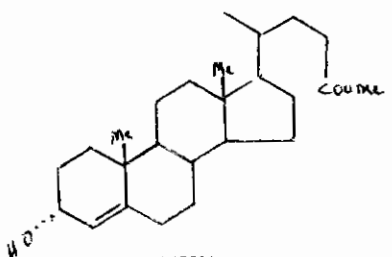
XVIII XIX XVII

In 1920, Wieland and Weyland[23] obtained as a major product of pyrolysis of lithocholic acid an unsaturated acid which could be purified by regeneration from the dibromides. This acid was tentatively formulated by Wieland[24] as Δ^{2}-cholenic acid but found, later on[16,25], to be identical with Δ^{3}-cholenic acid prepared by Fieser and Ettore.

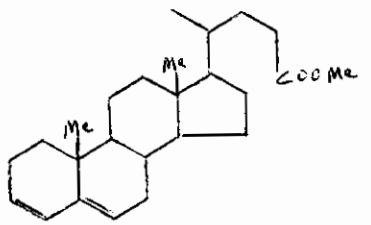
Methyl $\Delta^{3,5}$ choladienate (XXII) was prepared by the action of acids on the methyl esters of the epimeric 3-hydroxy Δ^{4}-cholenic acids (XX, XXI)[17],



XX

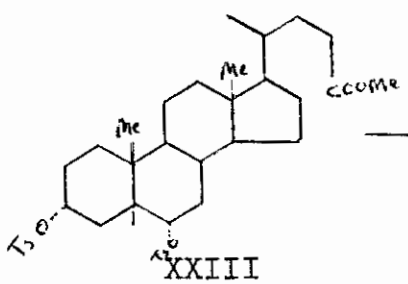


XXI

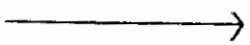


XXII

or by the didehydrotosylation of methyl 3α : 6 α ditosyloxy-cholanate (XXIII)[18]. This acid is also formed as a by-product



XXIII

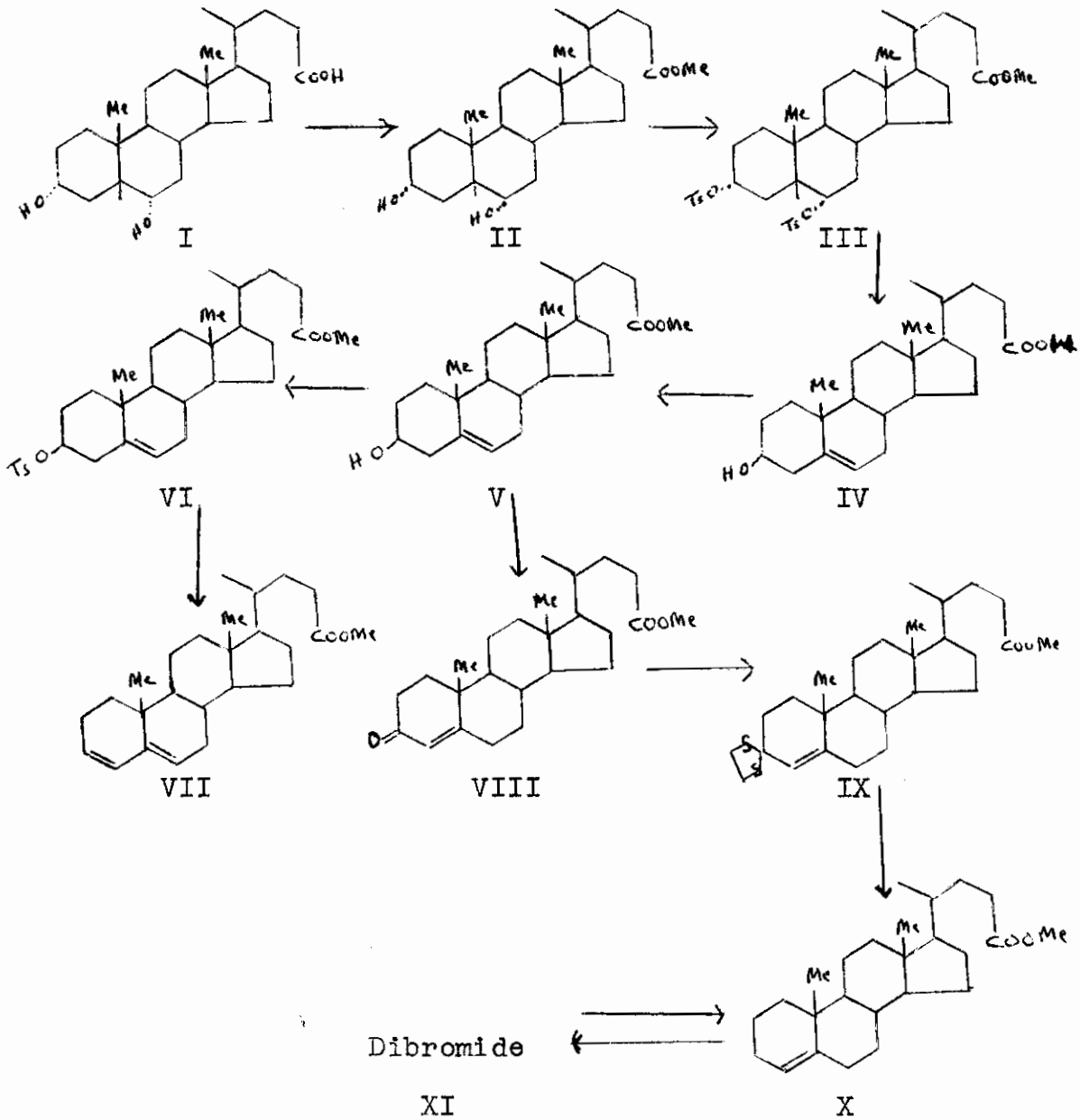


XXII

of selenium dioxide oxidation of methyl Δ^{3}-cholenate[17].

SUMMARY OF EXPERIMENTAL

A. Preparation of methyl Δ^4 cholenate:



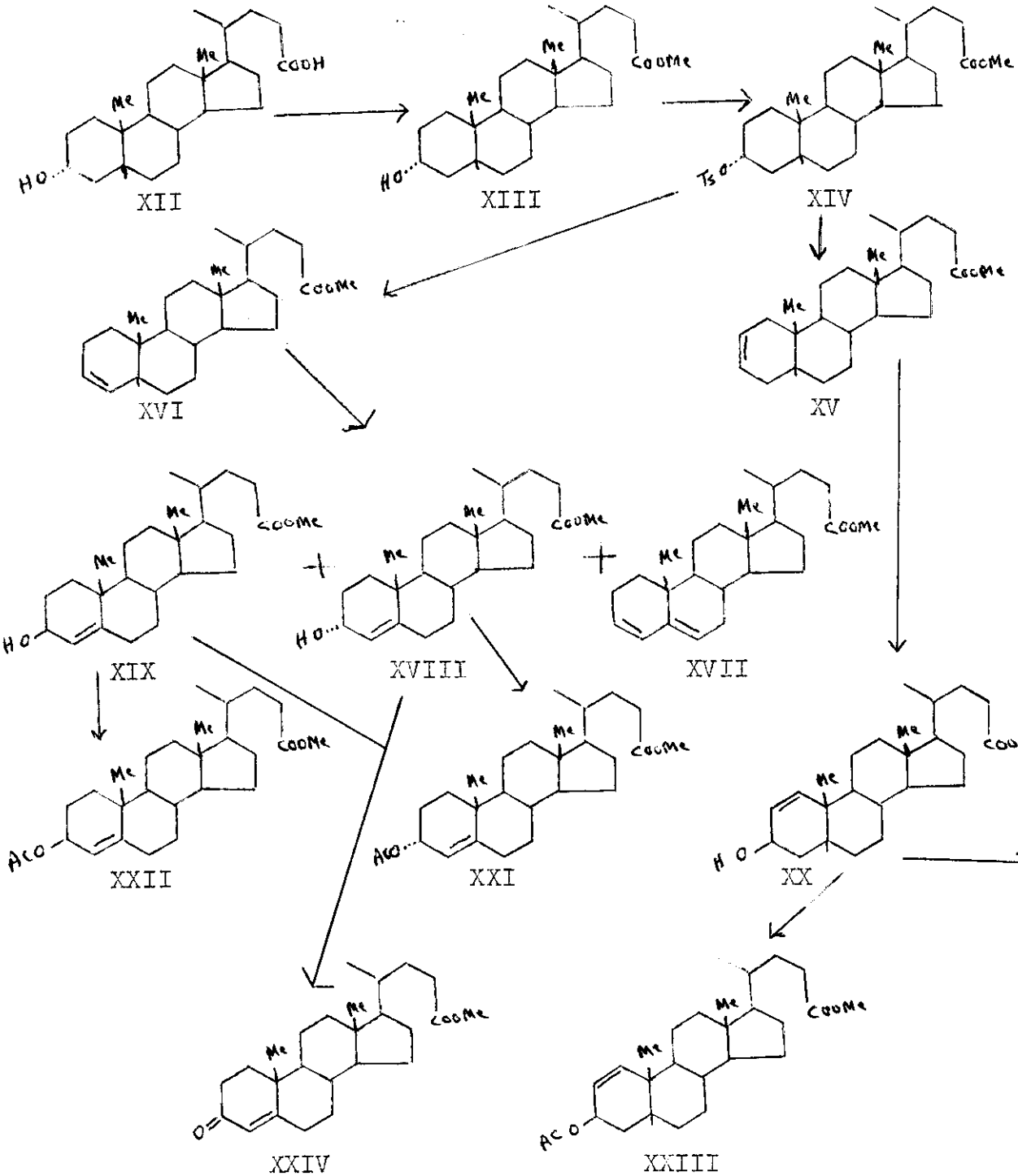
Hyodesoxycholic acid (I) was methylated by an ethereal solution of diazomethane. The resulting methylhyodesoxy-

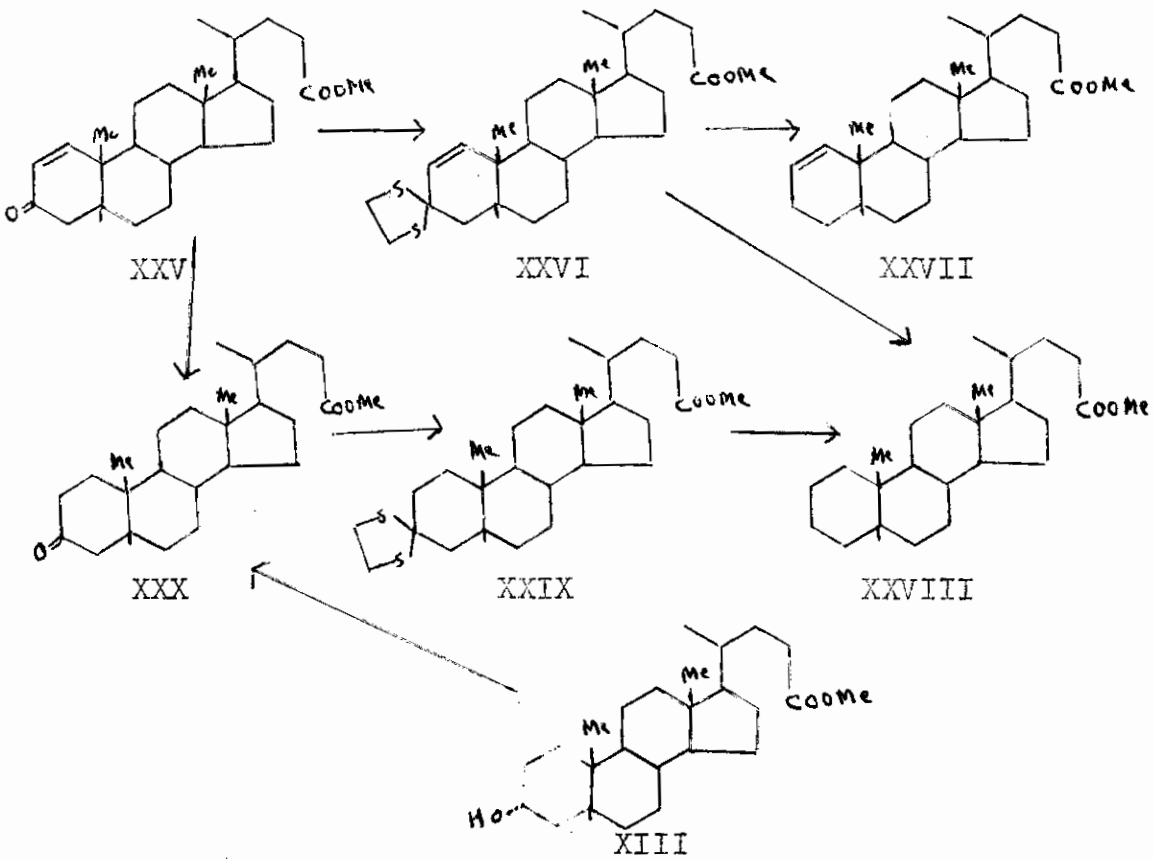
cholate (II), upon treatment with pyridine and p-toluene-sulfonyl chloride, gave methyl 3∝ : 6 ∝ ditosyloxycholanate (III). Selective dehydrotosylation of (III)[26] in dimethyl-formamide and potassium acetate followed by hydrolysis with 4% methanolic potassium hydroxide, furnished 3β-hydroxy Δ^{5}-cholenic acid (IV). Methylation of (IV) with diazomethane produced methyl 3β-hydroxy Δ^{5}-cholenate (V). From this point the work was divided into two parts:

Part 1: Tosylation of (V) in pyridine gave methyl 3β-tosyloxy Δ^{5}-cholenate (VI) which, upon dehydrotosylation with 2,6 lutidine and working up of the resulting mixture, furnished methyl $\Delta^{3,5}$-choladienate (VII).

Part 2: Oppenaur oxidation of (V) (cyclohexanone and aluminum isopropoxide) gave methyl 3-keto Δ^{4}-cholenate (VIII). Compound (VIII) was converted to the thioketal (IX) with ethanedithiol in presence of boron fluoride etherate. Hydrogenolysis of (IX) with W-2 Raney nickel in acetone-methanol gave the desired methyl Δ^{4}-cholenate (X). Bromination of an ethereal solution of (X) with bromine in acetic acid produced the dibromide (XI), which, upon treatment with sodium iodide in acetone, regenerated methyl Δ^{4}-cholenate (X).

B. Preparation of methyl Δ^{1}-cholenate:


XII
XIII
XIV
XV
XVI
XVII
XVIII
XIX
XX
XXI
XXII
XXIII
XXIV



Methyl lithocholate (XIII) was obtained by methylation of lithocholic acid (XII) with excess methanol in presence of hydrochloric acid. When (XIII) was treated with pyridine and p-toluenesulfonyl chloride, methyl 3α-tosyloxycholanate (XIV) resulted. Compound (XIV), upon refluxing with 2,6 lutidine, gave a mixture of methyl Δ^2 and Δ^3-cholenate (XV, XVI). The mixture was purified (without separation) by elution with petroleum ether from a column of alumina. Treatment of the eluted mixture with selenium dioxide in acetic acid followed by acetylation with acetic anhydride in pyridine gave a mixture of (XVII), (XXI), (XXII) and (XXIII). Methyl $\Delta^{3,5}$ choladienate (XVII) was removed together with

some of the unreacted material by chromatography. Hydrolysis of XXI, XXII and XXIII with 2.5 N methanolic potassium hydroxide gave the free hydroxy acids which, upon methylation with diazomethane, produced the methyl esters XVIII, XIX and XX. Oxidation of the mixture of XVIII, XIX and XX with manganese dioxide in chloroform at room temperature gave the two ketones XXIV and XXV, which were separated by chromatography. Treatment of methyl 3 keto-Δ^1-cholenate XXV with ethanediethiol and boron fluoride etherate produced the thioketal (XXVI) which, upon hydrogenolysis with W-2 Raney nickel in acetone-methanol, furnished methyl-Δ^1-cholenate (XXVII). When the thioketal (XXVI) was treated with W-2 Raney nickel in methanol, it gave methyl cholanate (XXVIII). Product XXVIII was also prepared by Raney nickel reduction of the thioketal (XXIX) of methyl 3 keto-cholanate (XXX), obtained from methyl lithocholate (XIII) by Oppenaur oxidation. The hydrogenation of XXV over 10% palladium-charcoal catalyst produced methyl 3-keto-cholanate XXX.

DISCUSSION

A. Methyl Δ^4 cholenate:

For the preparation of methyl Δ^4 cholenate (X) we elected to start with methyl 3-keto- Δ^4-cholenate (VIII), readily obtainable from hyodesoxycholic acid by a five step synthesis[18].

The next step in the synthesis involved the preparation of the ethylene thioketal (IX) of VIII. Fieser recommends two procedures[27] for effecting the condensation of ethanedithiol with ketosteroids. The first involves addition of excess ethanedithiol and boron fluoride etherate to an acetic acid solution of the ketone. The second requires addition of boron fluoride etherate to a suspension of a solution of the compound in ethanedithiol. In applying either procedure for the preparation of methyl 3-keto- Δ^4-cholenate ethylene thioketal (IX), the isolation of a solid product was not easy, probably due to the solubility of the thioketal in the reaction mixture. Addition of methanol or water to the mixture, resulted in an oil which solidified only on prolonged cooling. However, it was found that the pure thioketal could be prepared easily in high yield by treatment of a methanolic solution of the ketone with ethanedithiol and borom-fluoride etherate. This procedure gives excellent results also with 3-keto- Δ^4-cholestene and methyl 3-ketocholanate.

The following step was the selective desulfurization of the thioketal (IX), leaving the double bond unaffected. W-2 Raney nickel catalyst[28] was chosen for this purpose. Different types of Raney nickel catalysts with varying activity are known[29]. The most active is W-7 Raney nickel catalyst and the least is W-1. Fully active W-2 catalyst has been reported to effect desulfurization of unsaturated cyclic ethylenethioketals without reduction of the double bond in some cases[14,30,31] and with reduction of the double bond in others[27]. The very active W-7 catalyst has also been used to desulfurize unsaturated ethylene thioketals without saturation of the double bond[32].

In attempting to find out the specific action of W-2 Raney nickel catalyst on our thioketal (IX), three procedures were tried out:

Procedure 1: The thioketal in methanol was refluxed with W-2 catalyst of full activity. The product obtained was found to be a saturated mixture. It gave a negative sulfur test and a negative tetranitromethane test (yellow complex with unsaturated compounds). The melting point after one crystallization was in the range 56-62°C. and went up to 75-80 upon chromatography and repeated crystallization. The specific rotation ($[\alpha]_D$ = + 19) corresponded to the saturated compounds: methyl 5α and 5β cholanate. Complete saturation was also confirmed by the disappearance of the infrared band at 810 cm^{-1} due to a non-conjugated Δ^4 steroid[33] (Appendix I)

Procedure 2: The thioketal was treated with partially deactivated W-2 Raney nickel according to the method of Spero, McIntosh and Levin[34]. Spero and his co-workers refluxed an acetone suspension of the catalyst during two hours and subsequently added the compound, completing the reaction by further refluxing. This method gave an impure unsaturated product (positive tetranitromethane). Attempts to purify it by recrystallization failed. Only refrigeration could bring about a solid (M.P. 79-83, α_D = + 60, $\lambda_{cyclohexane}$ a broad band at 805-795 cm^{-1} (Appendix II)). Purification via the dibromide gave methyl Δ^4-cholenate in low yield, needles M.P. 91-92. $\lambda_{cyclohexane}$ a sharp band at 810 cm^{-1} (Appendix III).

Procedure 3: The thioketal (IX) dissolved in 1:1 acetone: methanol was refluxed with fully active W-2 catalyst. Working up of the mixture gave in high yield an unsaturated compound which after two recrystallizations from methanol furnished needles M.P. 90-92, α_D = + 68. After six more recrystallizations the compound melted 92-93°C. α_D = + 70. Bromination of the compound after the second crystallization, purification of the dibromide by recrystallization and debromination with sodium iodide in acetone at room temperature (mild elimination condition) regenerated the unsaturated product: methyl Δ^4-cholenate (X). M.P. 92-93, α_D = + 71.

Comparable results were obtained when the last two methods were employed for the preparation of Δ^4-cholestene

from the ethylene thioketal of 3-keto- Δ^4-cholestene. Procedure 2 gave a product inferior to the one obtained by procedure 3 (Table I).

Table I

	M.P. (After two crystallizations)	D	I.R. band
Procedure 2	68-72	+ 58	810 cm^{-1} broad (Appendix IV)
Procedure 3	79-81	+ 72	810 cm^{-1} sharp (Appendix IV)
Pure Δ^4-cholestene[35]	82.5	+ 77	810 cm^{-1} sharp[33]

The findings reported above demonstrate clearly the sensitivity of reductions by Raney nickel to experimental conditions. Several other examples may be cited from the literature[36]. The effect of temperature has been shown in a striking manner by Zafiriadis in the hydrogenation of cinnamylidenemethylhexyl ketone with Raney nickel. At 40°C. the ethylenic bonds were reduced; at 130°C. the carbonyl group was reduced to the alcohol and at 260°C. the phenyl ring was reduced. Cornubert and Phelisse showed that the reduction of benzalacetone gave ω-phenyl-2-pentanol when the reaction was run in absolute ethanol; when, however, the reaction was run in a solution of ethanol containing chloroform in a concentration of 2 g./l., or HCl in a concentra-

tion of 1.1 g./l., benzyl acetone was obtained. Even the presence of an isolated group in the molecule may have sometimes an effect on the course of reduction. Rosenkranz, Kaufman and Romo[37] found that desulfurization of the thioenol ether of Δ^4 androstene 3,17 dione gave a mixture of $\Delta^{3,5}$ androstadiene-17-one and androstane-17β-ol. The carbonyl group of the thioether molecule apparently acted as a partial deactivator of the catalyst, hindering the saturation of the double bonds, but when it was reduced to the alcohol group the influence disappeared and the double bonds were saturated.

Further evidence for structure X assigned to our unsaturated compound is furnished by the molecular rotations. The shift in molecular rotation in passing from VIII(M_D 336 chloroform) to X (M_D 265 chloroform) is found to be -71, which is in excellent agreement with the value -72 for the corresponding difference in molecular rotation between 3-keto-Δ^4-cholestene (M_D 354 chloroform[38]) and Δ^4-cholestene (M_D 282 chloroform[39]). The shift in passing from IX (M_D 505 chloroform) to X (M_D 265 chloroform) is found to be -240, which is in close agreement with the value -232 for the corresponding difference in molecular rotation between 3-keto-Δ^4-cholestene ethylenethioketal (M_D 514 chloroform[27]) and Δ^4-cholestene (M_D 282 chloroform[39]). Furthermore the molecular rotation difference between X (M_D 265 chloroform) and methyl cholanate (M_D 79 chloroform) is found to be + 186 which is in agreement with the average value + 194[40] for the effect on molecular rotation of the introduction of a double bond at position 4.

B. Methyl Δ^1 cholenate:

For the synthesis of methyl Δ^1 cholenate (XXVII), methyl 3-keto- Δ^1-cholenate (XXV) was elected as the starting material.

Compound (XXV) was prepared recently[17] from lithocholic acid by the following method: A mixture of methyl-Δ^2 and Δ^3-cholenate (XV, XVI) (prepared conveniently[22] by the action of lutidine on the tosylate of methyl lithocholate (XIV)) was oxidized by selenium dioxide to give a mixture of methyl $\Delta^{3,5}$ choladienate (XVII), 3β and 3α-hydroxy Δ^4-cholenates (XIX, XVIII) and 3β-hydroxy- Δ^1-cholenate (XX). The allylic alcohols were separated from the diene by chromatography as the acetates. Treatment of the alcohols with digitonin precipitated the 3β isomers, separable by fractional crystallization. Oxidation of methyl 3β-hydroxy- Δ^1-cholenate (XX) gave XXV.

The successive chromatography, digitonin precipitation and fractional crystallization steps of this method are lengthy and tedious.

During this investigation, the same approach was essentially followed for the preparation of XXV, but the procedure was considerably simplified by omitting the digitonin precipitation and the fractional crystallization steps. The method recommended is as follows: The mixture obtained from the selenium dioxide oxidation is treated with acetic anhydride and then subjected to preliminary

purification by chromatography. Hydrolysis of the allylic acetates followed by manganese dioxide oxidation of the 3-hydroxy unsaturated acids (as the methyl esters), gives a mixture of two isomeric ketones separable by careful chromatography. The ketones were shown to be methyl 3-keto-Δ^1-cholenate (XXV) and methyl 3-keto-Δ^4-cholenate (XXIV). The latter was found to be identical (M.P., M.M.P and α_D) with methyl 3-keto-Δ^4-cholenate obtained by the Oppenaur oxidation of methyl 3β-hydroxy-Δ^5-cholenate (V). An authentic sample of methyl 3-keto-Δ^1-cholenate was not available for direct comparison with ketone XXV. However, the structure was established by the following evidence: The ketone exhibits an absorption band $\lambda^{MeOH}_{231-232}$ mu (Appendix VIII) characteristic of a conjugated unsaturated ketone of the β-monosubstituted type. Hydrogenation of XXV over 10% palladium-charcoal catalyst at room temperature and one atmospheric pressure readily yielded the corresponding saturated ketone[17] XXX which proved to be identical (M.P., M.M.P., α_D and infrared comparison (Appendix V)) with methyl 3-keto-cholanate (prepared by the Oppenaur oxidation of lithocholic acid). Moreover, the infrared spectrum of the unsaturated ketone XXV showed a band at 1675 cm^{-1} (Appendix VI) characteristic of α-β unsaturated ketones in six numbered rings of steroids, while the hydrogenation product had a band at 1705 cm^{-1} (Appendix V) corresponding

to the carbonyl stretching frequency of a saturated ketone[41].

In a subsequent step, ketone XXV was conveniently transformed to the cyclic ethylenethioketal XXVI. This was accomplished by treatment of a solution of the ketone in ethanedithiol with boron fluoride etherate followed by dilution with methanol. Previous attempts to condense a similar ketone, 3-keto-Δ^1-cholestene with ethanedithiol in the presence of hydrogen chloride gave inconsistent results[42], or, a mixture where the main product was the cyclic ethylene thioketal[43]. In the present case no such difficulty was experienced. The condensation product XXVI showed a sharp melting point after one crystallization and no considerable change in melting point was observed on repeated recrystallizations. The elemental analysis corresponded to the formula $C_{27}H_{42}O_2S_2$, excluding the possibility of addition of ethanedithiol to the double bond, a phenomena previously reported[42,43] for the condensation of 3-keto-Δ^1-cholestene with benzyl mercaptan.

Hydrogenolysis of the thioketal XXVI with W-2 Raney nickel in methanol resulted in complete saturation of the double bond (negative tetranitromethane test). The product isolated was pure methyl cholanate, identical (M.P., M.M.P., α_D and infrared spectrum (Appendix VII)) with an authentic sample prepared by Raney nickel reduction of the thioketal of methyl 3-keto-cholanate(XXX). On the other hand, when treated with W-2 Raney nickel in acetone-methanol, (as

previously described for the preparation of methyl Δ^4-cholenate), the thioketal XXVI gave an unsaturated low melting product (yellow color with tetranitromethane).

In view of the finding of Striebel and Tamm[43] that reductive desulfurization of 3-keto- Δ^1-cholestene ethylenethioketal with partly deactivated Raney nickel yielded Δ^2-cholestene instead of the expected Δ^1 isomer, the possibility that our unsaturated product is methyl Δ^2-cholenate cannot be excluded. However, the molecular rotation difference between our product (M_D 209 chloroform) and methyl cholanate (M_D 79 chloroform) is + 130, which is appreciably different from the value -24[40] for the effect on molecular rotation of the introduction of a double bond at position 2 in the 5β series. In view of this, we would like to assign tentatively to this product structure XXVII.

EXPERIMENTAL*

Ethanedithiol[44]

(i) Ethylene diisothiuronium bromide:

A mixture of thiourea (300 g.) and 95% ethanol (1.4 lit.) was heated on a water bath in a 3-lit. round-bottomed flask (fitted with a reflux condenser) until it was almost clear. The water bath was removed and ethylene dibromide (330 g.) was added. A vigorous exothermic reaction started, which required external cooling of the flask. The precipitated ethylene diisothiuronium bromide (500 g.) was collected by filtration.

(ii) Hydrolysis of the salt:

Ethylene diisothiuronium bromide (150 g.) in water (800 ml.) was placed in a 3-lit. round-bottomed three-necked flask. Potassium hydroxide (380 g.) was added and the mixture refluxed for 3 hours.

(iii) Liberation of ethanedithiol:

Caution: Vapours of ethanedithiol may cause nausea and headache. The reaction at this stage should be carried out under an efficient hood.

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* Melting points are uncorrected. Rotations (rounded off to the nearest integer) were measured in chloroform solution. Alumina used for chromatography was neutral, grade I "Woelm" to which 3% water was added. Ultraviolet spectra were determined in a Beckman DU quartz spectrophotometer. Infra-red Spectra were determined with a Perkin-Elmer Model 137 Infracord double beam Spectrophotometer.

At the end of the reflux period the resulting alkaline solution was allowed to cool to room temperature. The flask was equipped with a separatory funnel (fitting the middle neck of the flask) containing a solution of concentrated sulfuric acid (180 ml.) in distilled water (320 ml.). One of the other two necks was provided with a gas inlet tube and the third neck with a condenser set for steam distillation. The acid was added dropwise while nitrogen was passed through the solution. When all the acid had been added the nitrogen was replaced by steam. Two liters of distillate were collected and transferred to a separatory funnel. The oil was separated and the aqueous milky layer was extracted with three 300 ml. portions of ether. The ether was distilled and the residue added to the oil. The crude combined product was dried over calcium chloride and distilled under reduced pressure through a 10-inches Vigreaux column. The colorless fraction (25 g.) distilling 60-63/43 mm was collected, transferred to a glass stoppered flask and stored in a refrigerator.

Raney Nickel Catalyst, W-2[28]:

To a solution of sodium hydroxide (95 g.) in water (380 ml.) in a 1-lit. beaker at 10°C. was added nickel Aluminum alloy (75 g.) in small portions with stirring (by means of an air stirrer) over a period of 1½ hr., keeping the temperature below 25°C by cooling in an ice bath. After

the addition of all the alloy, the beaker was removed from the ice bath and the contents were allowed to come to room temperature. When the evolution of hydrogen became slow, the mixture was heated on a steam bath for 8 hr. maintaining the volume of the mixture constant by addition of distilled water. The nickel was allowed to settle and most of the solution was decanted. Distilled water (400 ml.) was added to the residue, the mixture was stirred and the solution was decanted after the nickel had settled. The catalyst at this stage transferred by means of distilled water to a 500-ml graduate, the solution was decanted and sodium hydroxide (13 g.) in water (130 ml.) was added, the mixture stirred, allowed to settle and then decanted. The nickel was stirred, with (300 ml.) of water, allowed to settle for 10 min., stirred again and the wash water was decanted as soon as the catalyst settled. This process was repeated thirty times with distilled water, three times with 100 ml. of 95% ethanol and finally three times with 100 ml. of absolute ethanol. The catalyst was then stored under absolute ethanol.

Manganese dioxide[45]:

A solution of manganese sulfate tetrahydrate (70 g.) in water (150 ml) was heated to about 90°C by means of a water bath. To the hot solution was added a concentrated solution of potassium permanganate (38 g.) with efficient mechanical stirring. Stirring was continued for 15 min. at

about 90°C. Filtration, followed by thorough washing with hot water and drying to constant weight, gave a brown solid which could be powdered easily*.

Palladium on carbon catalyst (10% Pd)[46]:

Palladium chloride (0.42 g.), hydrochloric acid (0.3 ml.) and water (2.5 ml.) were heated on a steam bath until solution was complete. The resulting solution was poured into a solution of 7 g. of sodium acetate in 25 ml. of water, and placed in a 100-ml round bottomed flask to fit in the hydrogenation system (Appendix IX). Norite (2.3 g.) (previously heated on a steam bath with 10% nitric acid for 3 hr., washed with water and dried at 110-120°C for 2 hr.) was introduced in the flask and the mixture hydrogenated at one atmospheric pressure by means of the hydrogenation system. When absorption of hydrogen ceased, the catalyst was collected by filtration, washed with 500 ml. of distilled water in five portion, dried in air and then over calcium chloride.

Methyl 3β-hydroxy-Δ^5-cholenate (V)

(i) Methylation of hyodesoxycholic acid[18]:

An ethereal solution of diazomethane[47] (prepared from p-tolylsulfonyl methyl nitrosamide[48]) was added to a suspension of hyodesoxycholic acid (I) (30 g.) in ether until the yellow color of diazomethane persisted and evolution of

* Occasionally, under these conditions a black hard solid was obtained which was found to be unsuitable as a selective oxidizing agent.

nitrogen ceased. Evaporation of the solution under reduced pressure gave a residue which solidified on addition of a small amount of benzene. Recrystallization from benzene gave fibers of methyl hyodesoxycholate (II) (29 g.) M.P. 119-120°C (Lit.: 120[17], 114[49], 86[50]*, 110-112[51]).

(ii) Tosylation of methyl hyodesoxycholate[26]:

A solution of methyl hyodesoxycholate (II) (25 g.), dry pyridine** (50 ml.) and p-toluene sulfonyl chloride (29 g.) was kept at room temperature for two days, then poured into ice-cold dilute hydrochloric acid with stirring. The solid was collected on a Buchner funnel and washed several times with cold water. The dry product, methyl 3α:6α ditosyloxycholanate, (III) (45 g.) gave hard prisms from ethyl acetate, M.P. 164-166 (Lit.: 156-157[18] and 165-167[26]).

(iii) 3β-hydroxy- Δ^5-cholenic acid (IV)[26]:

Potassium acetate (44 g.) was dissolved in water (12 ml.) and dimethylformamide (240 ml.) by heating in an oil bath at 100-105°C. Methyl 3α: 6α ditosyloxycholanate (III) (30 g.) was added and the resulting solution was kept at 100-102°C for 5 hr. The cooled solution was poured into ice-cold dilute hydrochloric acid with manual stirring. The

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* Gallagher and co-workers[51] point out that this material (M.P. 86) is undoubtedly a coordination compound with 1 mole of benzene.

** Obtained by distillation of pyridine over potassium hydroxide.

white precipitate was filtered, washed with water and then refluxed with 4% methanolic potassium hydroxide (420 ml.) for 2 hr. After cooling, the mixture was poured into dilute hydrochloric acid and the precipitate was collected, washed with water and air dried to constant weight. Recrystallization from ethyl acetate afforded (12 g.) of 3β-hydroxy- Δ^5-cholenic acid, M.P. 221-227°C with decomposition, (Lit.: 222-227[18]; and 220-227[26]).

(iv) Methylation of 3β-hydroxy- Δ^5-cholenic acid[18]:

Treatment of 3β-hydroxy- ^{5}-cholenic acid (12 g.) with diazomethane gave a solid residue upon evaporation of the ethereal solution. Recrystallization of the solid from afforded needles of methyl 3β-hydroxy- Δ^5-cholenate (V) (12 g.) M.P. 143-144 (Lit.: 143-144[18]; 144[26]).

Methyl 3-keto- Δ^4-cholenate (VIII):

Dry, sulfur-free toluene[12*] (200 ml.) and dry methyl 3β-hydroxy- Δ^5-cholenate (V) were placed in a 500 ml round-bottomed three-necked flask equipped with a sealed stirrer, dropping funnel and a take off condenser with a receiver protected by a calcium chloride tube. Twenty five milliliters of toluene were distilled and cyclohexanone (60 ml.) was added to the reaction flask. After distillation of a

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* Toluene (1 lit.) was shaken in a separatory funnel with concentrated sulfuric acid (80 ml.) for ½ hr. with occasional cooling to keep the temperature below 30°C. Shaking was repeated twice with fresh portion of sulfuric acid and the toluene was distilled.

further 10 ml. portion of toluene, a solution of aluminum isopropoxide (5 g.) in sulfur free toluene (30 ml.) was added dropwise by means of the dropping funnel over a period of 30 min. and distillation continue at the same rate of addition. The cold solution was treated with a saturated solution of potassium sodium tartrate (150 ml.), the resulting mixture was steam distilled and the residue extracted several times with chloroform. The combined chloroform extracts were washed with water, dried over anhydrous sodium sulfate and evaporated under reduced pressure. Recrystallization of the residue from methanol gave hard prisms (5 g.) M.P. 123-125. After further crystallizations from methanol, the product melted at 126-127. The mother liquor and washings from the recrystallizations were concentrated to give a second crop (2.2 g.) M.P. 118-122. repeated crystallizations from methanol raised the M.P. to 126-127. Further concentration of the mother liquor and addition of hot water to the point of incipient cloudiness gave an oil which solidified on standing in the refrigerator for several days (1.5 g.). This crop was dissolved in 2 ml of benzene and applied to an alumina column. Elution with 7:3 - 6:4 petroleum ether-benzene gave a solid (800mg.) which upon recrystallization from methanol melted at 126-127 (Lit.: 125[16], 126-127[17,18], 124-125[51,53]. The total yield of product of M.P. 123-125 or better was 7.9 g. (79%).

Methyl 3-keto- Δ^4-cholenate ethylenethioketal (IX)

A solution of methyl 3-keto- Δ^4-cholenate(VIII) (2 g.) in methanol (30 ml.) was treated with ethanedithiol (1 ml.) and freshly distilled boron fluoride etherate (1 ml.). When cooled in an ice-salt bath, the turbid solution deposited a solid which was collected and washed with cold methanol, (2.2 g., 92% yield) M.P. 107-109. Repeated crystallizations from methanol afforded feathery needles M.P. 110-111, α_D + 109 (C 1.1).

Anal. Calcd. for $C_{26}H_{42}O_2S_2$ (462.61): C, 70.07; H, 9.15; S, 13.86. Found: C, 70.05; H, 9.19; S, 13.80.

Methyl Δ^4-cholenate (X)

Methyl 3-keto- Δ^4-cholenate ethylenethioketal (IX) (2 g.) was refluxed with W-2 Raney nickel catalyst (ca 25 g.) in methanol (50 ml.): acetone (50 ml.) with efficient stirring for 12 hr. The hot reaction mixture was filtered through a sintered glass suction funnel and the clear filtrate was taken up to dryness under reduced pressure. Recrystallization of the residue from methanol gave needles of methyl- ^{4}-cholenate (X) (1.3 g., 81% yield) M.P. 89-91. The analytical sample melted 92-93, α_D = + 70 (C 1.2). Tetranitromethane test was positive. $\lambda_{cyclohexane}$ 810 cm^{-1}.

Anal. Calcd. for $C_{25}H_{40}O_2$ (372.57): C, 80.59; H, 10.82. Found: C, 80.59; H, 10.84.

Product X (235 mg.) (M.P. 89-91) in ether was treated with excess bromine in acetic acid. The resulting yellow solution was diluted with ether, washed with 5% sodium bicarbonate and evaporated to dryness under reduced pressure. Two crystallizations of the solid residue from acetone gave fibrous needles of the dibromide XI (250 mg.) (M.P. 130-132. The dibromide was shaken with excess sodium iodide in acetone (1.5 g./ 10 ml.) at room temperature, diluted with water and extracted with ether. Addition of few drops of sodium thiosulfate, washing with water and evaporation of ether gave a solid which upon recrystallization from methanol furnished pure methyl- Δ^4-cholenate (X), (M.P. 92-93, α_D + 71).

When the Raney nickel reduction of methyl 3-keto- Δ^4-cholenate ethylenethioketal was carried out under the same conditions as above but in a methanolic solution instead of a solution in methanol-acetone, a product was obtained which, upon one crystallization from methanol, melted at 56-62. Repeated crystallizations and chromatography raised the melting point to 75-80; α_D + 19 (C 1.4); negative tetranitromethane test; $\lambda_{cyclohexane}$ no band at 810 cm^{-1} (Appendix I).

Another sample of the thioketal IX dissolved in acetone, upon treatment with previously deactivated W-2 Raney Nickel afforded, in low yield, a product which could not be purified by crystallizations. Purification via the dibromide gave needles of methyl- Δ^4-cholenate X.

Methyl 3β-tosyloxy- Δ^5-cholenate (VI)

Treatment of methyl 3β-hydroxy- Δ^5-cholenate (5 g.) with dry pyridine (10 ml.) and p-toluenesulfonyl chloride (3 g.) at 0°C for 20 hr., as previously described for the preparation of methyl 3 : 6 ditosyloxy cholanate, gave a sticky product which, after drying in a vacuum desiccator and recrystallization from ethyl acetate, afforded large prisms of VI (5 g.) M.P. 120-122, α_D -42 (C 1.2).

Anal. Calcd. for $C_{31}H_{44}O_5S$ (528.6): C, 70.42; H, 8.39; S, 6.06. Found: C, 70.90; H, 8.64; S, 6.16.

Methyl- $\Delta^{3,5}$-choladienate (VII)

Methyl 3β-tosyloxy- Δ^5-cholenate (VI) (3 g.) was refluxed for 2 hr. in 30 ml. of 2,6 lutidine. The cold mixture was added to chips of ice and extracted with ether. The ether was washed in order with ice-cold 3% hydrochloric acid, 10% sodium bicarbonate solution and water. The dried ethereal solution left a residual oil on evaporation under reduced pressure. The oil was dissolved in benzene (1 ml.) and chromatographed on alumina (50 g.). Elution with petroleum ether-benzene (9:1) and evaporation of the solvent gave methyl- $\Delta^{3,5}$-choladienate (1.3 g.). Recrystallization from methanol gave needles M.P. 96-97.5 (Lit.: 96-98[17,18]), α_D -125 (C 1.12) (Lit.: -124[17], -128[18])
λ_{max}^{MeOH} 227.5, 235, 243.5 mu.

Methyl lithocholate (XIII)[17]

Lithocholic acid (XII) (50 g.), methanol (500 ml.) and concentrated hydrochloric acid (5 ml.) were refluxed for ½ hr. The hot solution was filtered, left overnight, iced, and the precipitated product was collected, washed with cold methanol and air dried. Concentration of the mother liquor gave a second crop. The total product weighed 48 g. M.P. 126-128 (Lit.: 126.5-128[17], 125-127[16], 129-130[54] and 125-127.5[22]).

Methyl 3α-tosyloxycholanate (XIV)[17]

Treatment of methyl lithocholate (XIII) with dry pyridine and p-toluenesulfonyl chloride (as previously described for methyl 3α: 6α ditosyloxycholanate (III)) gave methyl 3α-tosyloxycholanate (XIV) M.P. 110-116. Repeated recrystallization from methanol-acetone afforded plates M.P. 118-120 (Lit.: 119-121[17], 120-121.5[22], 110-112[55]).

Dehydrotosylation of methyl 3α-tosyloxycholanate[22]

Methyl 3α-tosyloxycholanate (XIV) (25 g.) and 2,6 lutidine (250 ml.) were refluxed for 4½ hr. The cooled solution was poured into cracked ice with continuous stirring. The precipitate was collected on a Buchner funnel, washed with cold dilute hydrochloric acid then cold water. A portion of the dry solid (10 g.) was dissolved in petroleum

ether (B.P. 40-70) and applied to an alumina column. Elution with petroleum ether, followed by crystallization from methanol resulted in shiny rectangular plates of XV and XVI (5 g.) M.P. 74-75 (Lit.: 73.5-74.5[17], 74.5-75[22]).

Selenium dioxide oxidation of the dehydrotosylate[17]

Selenium dioxide (1.3 g.) dissolved in water (3.7 ml.) and acetic acid (25 ml.) was added to a solution of the dehydrotosylate (5 g.) (produced in the previous step) in acetic acid (55 ml.), and the mixture was stirred, by a magnetic stirrer, at room temperature for 35 hr. The resulting red mixture was poured into ether (200 ml.) placed in a separatory funnel, and washed with sodium carbonate (5%) until no more carbon dioxide was evolved, then with water. The coagulated selenium was removed by filtration and the clear yellow ether filtrate was left over anhydrous sodium sulfate for 15 hr. During this period some of the colloidal selenium coagulated on the sodium sulfate. The ether was evaporated under reduced pressure at room temperature or below. The dry residue was dissolved in dry pyridine (60 ml.), treated with acetic anhydride (30 ml.) and allowed to stand overnight. The solution was poured on cracked ice with stirring; the solid was collected by filtration, washed with water and dried. This product, dissolved in benzene (5 ml.), was adsorbed on a column of alumina (150 g.). The column was washed slowly with petroleum ether (200 ml.) and

then eluted fast with 1:4 P.E-B. The combined P.E-B. fractions solidified immediately after evaporation of the solvent. The solid residue was refluxed with 2.5 N methanolic potassium hydroxide (70 ml.) for 1½ hr., cooled, transferred to a separatory funnel and acidified, under ether, with cold dilute sulfuric acid. The ether layer was washed thoroughly with water and evaporated to a solid residue which upon treatment with diazomethane in the cold gave a mixture (3 g.) of the methyl esters of 3-hydroxy unsaturated acids XVIII, XIX and XX.

Manganese dioxide oxidation of the 3-hydroxy unsaturated methyl esters[17]

A solution of the product (5 g.) (obtained in the last step described above) in chloroform (500 ml.) was stirred at room temperature for 3½ hr. with freshly prepared manganese dioxide (50 g.). The manganese dioxide was removed by filtration and the chloroform filtrate was evaporated under reduced pressure.

The dry solid (3.5 g.) was taken up in benzene (7 ml.) and chromatographed on alumina (100 g.) in a column (25 mm. in diam. and 300 mm. in height). The column was eluted slowly with P.E. (40-70°) and ten fractions, 75-80 ml. each, were collected. At this stage, slow chromatography was interrupted and the column was eluted fast with 4:1 P.E-B. to collect fifteen more fractions (total: 25 fractions). The

first two fractions were discarded and fractions 3-10 were combined, evaporated, taken up in 2 ml. of benzene and rechromatographed on 30 g. of alumina combined in a column (20 mm. in diam. and 260 mm. in height). The column was washed with P.E. (150 ml.) and eluted with 500 ml. of 4:1 P.E-B. Evaporation of the P.E-B. eluants left a crystalline solid (600 mg.) M.P. 130-134. Recrystallization from methanol gave needles of methyl 3-keto- Δ^1-cholenate (XXV), M.P. 137-138 (Lit.: 138.5-139.5[17]) $[\alpha]_D$ + 113 (C 1.2)
λ_{max}^{MeOH} 231.5 mu.

Fractions 11-25 on evaporation gave a solid (1.5 g.) M.P. 120-124. Recrystallization from methanol afforded small prisms M.P. 125-126.5 $[\alpha]_D$ + 87 (C 1.25). This product was found to be identical with a sample of methyl 3-keto- Δ^4-cholenate prepared by the Oppenaur oxidation of methyl 3β-hydroxy- Δ^5-cholenate. (M.P. M.M.P. and $[\alpha]_D$).

Methyl 3 keto- Δ^1-cholenate ethylenethioketal (XXVI)

A mixture of methyl 3-keto- Δ^1-cholenate (XXV) (200 mg.), ethanedithiol (0.2 ml.) and boron fluoride etherate (0.2 ml.) was allowed to stand at room temperature for 20 min. Methanol (20 ml.) was added and the mixture was cooled in the refrigerator for several hours. The deposited solid (200 mg.) was filtered, washed with cold methanol and recrystallized from methanol M.P. 109-110

α_D + 125 (C 1.13).

Anal. Calcd. for $C_{27}H_{42}O_2S_2$ (462.6): C, 70.07; H, 9.15; S, 13.86. Found: C, 69.96; H, 9.07; S, 13.49.

Methyl- Δ^1-cholenate (XXVII)

Treatment of methyl 3-keto- Δ^1-cholenate ethylene-thioketal (XXVI) (100 mg.) with W-2 Raney nickel catalyst (ca 8 g.) in acetone (25 ml.) methanol (25 ml.), as previously described for the preparation of methyl- Δ^4-cholenate, gave a solid which, upon crystallization from aqueous methanol, afforded crystals M.P. 42-44, unchanged on further crystallizations α_D + 56 (c 1.2).

Anal. Cald. for $C_{25}H_{40}O_2$ (372.6): C, 80.59; H, 10.82; Found: C, 80.34; H, 11.18.

Methyl 3-keto-cholanate (XXX)

(i) From methyl 3-keto- Δ^1-cholenate[17]

Thirty milligrams of 10% palladium on charcoal were added to a solution of methyl 3-keto- Δ^1-cholenate (110 mg.) in absolute ethanol (30 ml.) and the mixture was placed in a hydrogenation flask (50 ml. round bottomed flask). The flask was connected to the hydrogenation system (Appendix IX) and hydrogenation was conducted at room temperature and one atmospheric pressure. Filtration of the mixture and evaporation of the filtrate gave a solid which upon crystallization from petroleum ether afforded flat hard needles (70 mg.)

M.P. 117-119, α_D +31.6 (C 1.3).

(ii) From methyl lithocholate (XIII)

Oppenaur oxidation of methyl lithocholate (10 g.), as described for the preparation of methyl 3-keto- Δ^4-cholenate, gave a solid (8.5 g.) which upon recrystallization from petroleum ether melted 117.5-118.5 (Lit.: 117.5-118.5[16]), α_D +32.5 (C 1.3). The product was found to be identical with that in (i) (M.P., M.M.P. and infrared spectrum (Appendix VI)).

Δ^4-cholestene

(i) Cholestenone[56]

Cholesterol was oxidized by the Oppenaur oxidation, as previously described for methyl 3β-hydroxy- Δ^5-cholenate (V), to give cholestenone M.P. 79-80.

(ii) 3-keto- Δ^4-cholestene ethylenethioketal

Cholestenone (2 g.) in methanol (20 ml.) was treated with ethanedithiol (1 ml.) and boron fluoride etherate (1 ml.) at room temperature. The deposited solid (2.3 g.) was filtered, washed with methanol and recrystallized from acetone to give colorless plates M.P. 106-107 (Lit.: 106-107[14], 118.5-119.5[27]).

(iii) Δ^4-cholestene

A sample of 3-keto- Δ^4-cholestene ethylene thioketal (2 g.) was treated with Raney nickel (ca 30 g.) in acetone (50 ml.) methanol (50 ml.), as previously described, furnished

Δ^4-cholestene. Recrystallization from ethyl acetate-methanol gave needles M.P. 78-80 (Lit.: pure Δ^4-cholestene 82.5[35]), α_D + 72 (C 1.3) (Lit.: + 77[35]) λ cyclohexane 810 cm^{-1}.

When 3-keto- Δ^4-cholesteneethylenethioketal was treated with deactivated W-2 Raney nickel, the product, upon crystallization from ethylacetate-methanol, melted 68-72, α_D + 58.

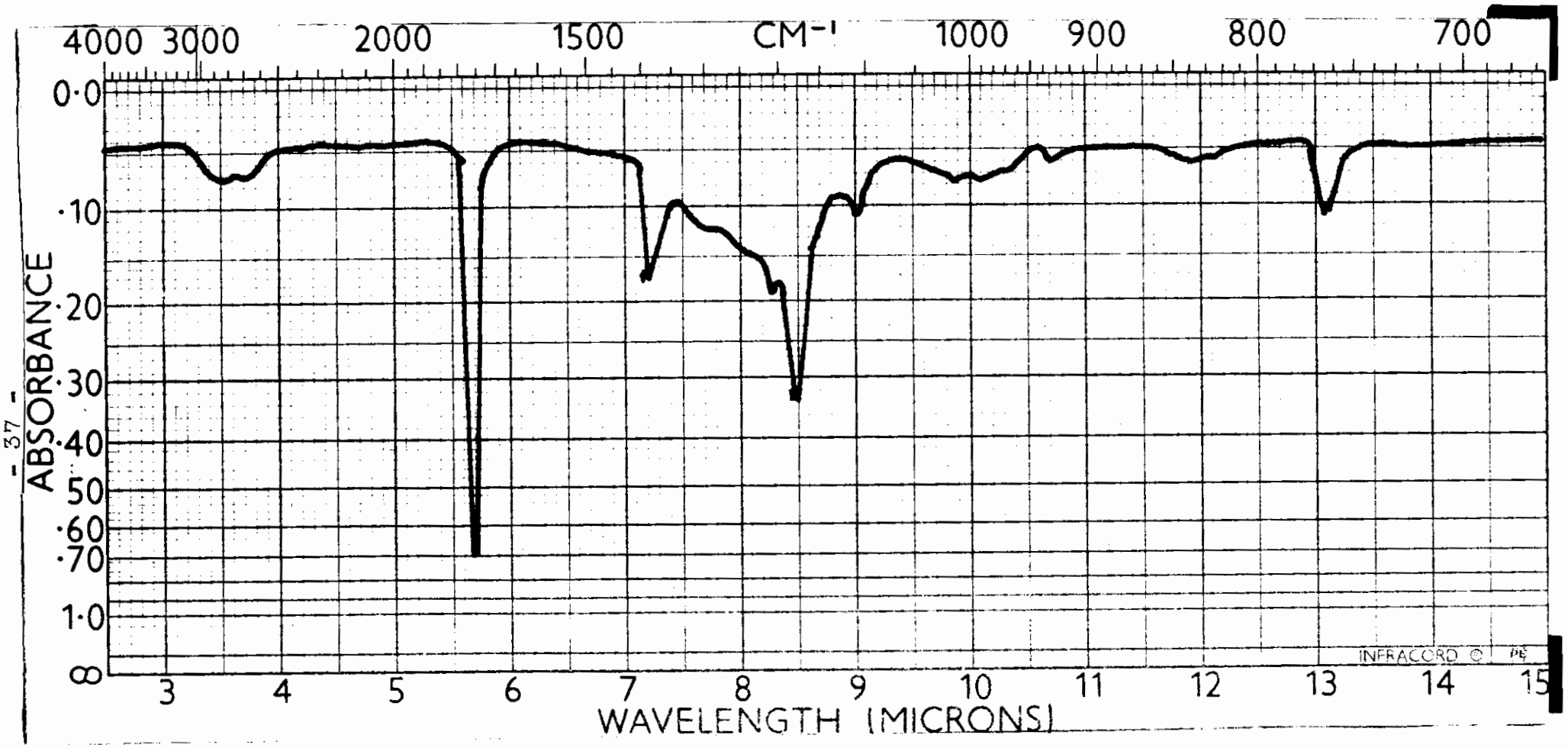
Methyl cholanate (XXVIII)

(i) From methyl 3-keto- Δ^1-cholenate ethylene-thioketal

Reduction of the thioketal XXVI with fully active W-2 Raney nickel in methanol gave a solid which upon crystallization from methanol furnished needles of methyl cholanate M.P. 86-87, α_D + 21 (C 1.31).

(ii) From methyl 3-keto-cholanate

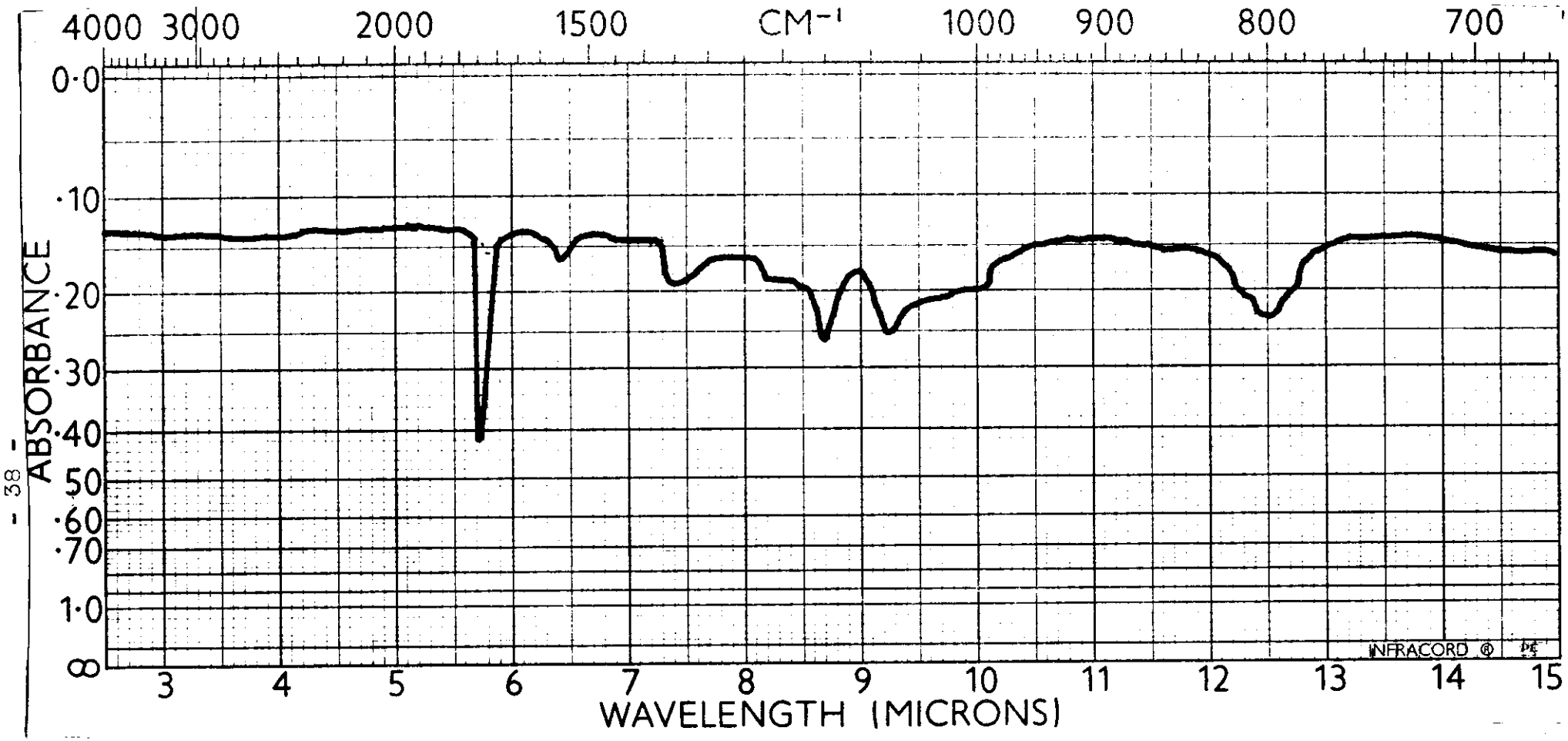
The thioketal XXIX of methyl 3-keto-cholanate (XXX) was prepared as usual, recrystallized from methanol and treated with active W-2 Raney nickel catalyst in methanol. Working up the reaction mixture, recrystallization of the product from methanol afforded needles of methyl cholanate XXVIII identical (M.P., M.M.P., α_D and infrared spectrum (Appendix VII)) with product prepared as in (i).



Appendix I

Reduction product of methyl 3-keto-Δ^4-cholenate ethylenethioketal

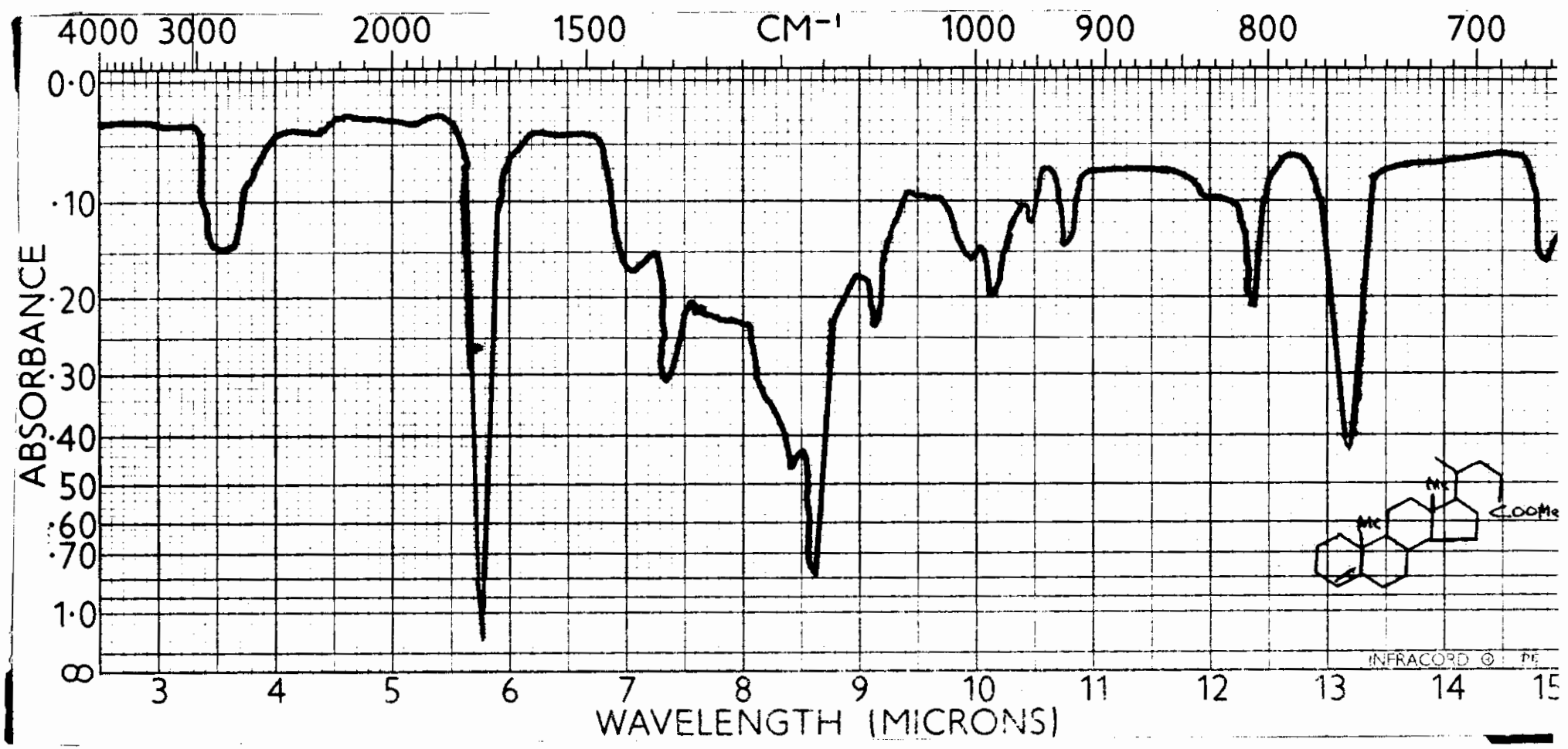
(Procedure 1)



Appendix II

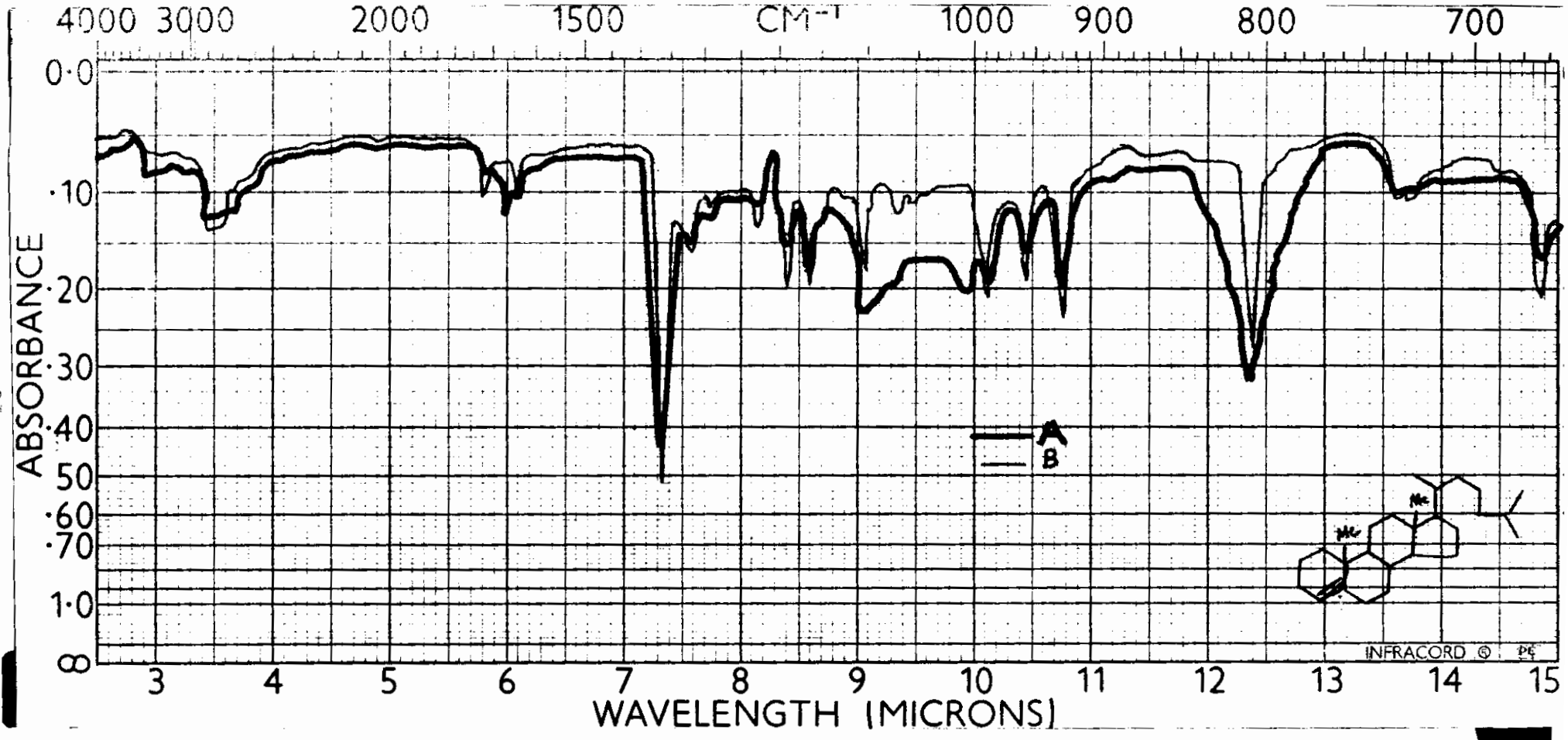
Reduction product of methyl 3-keto- Δ^4-cholenate ethylenethioketal

(Procedure 2)



Appendix III

Methyl- Δ^4-cholenate (Procedure 3)
or
Purified product of procedure 2

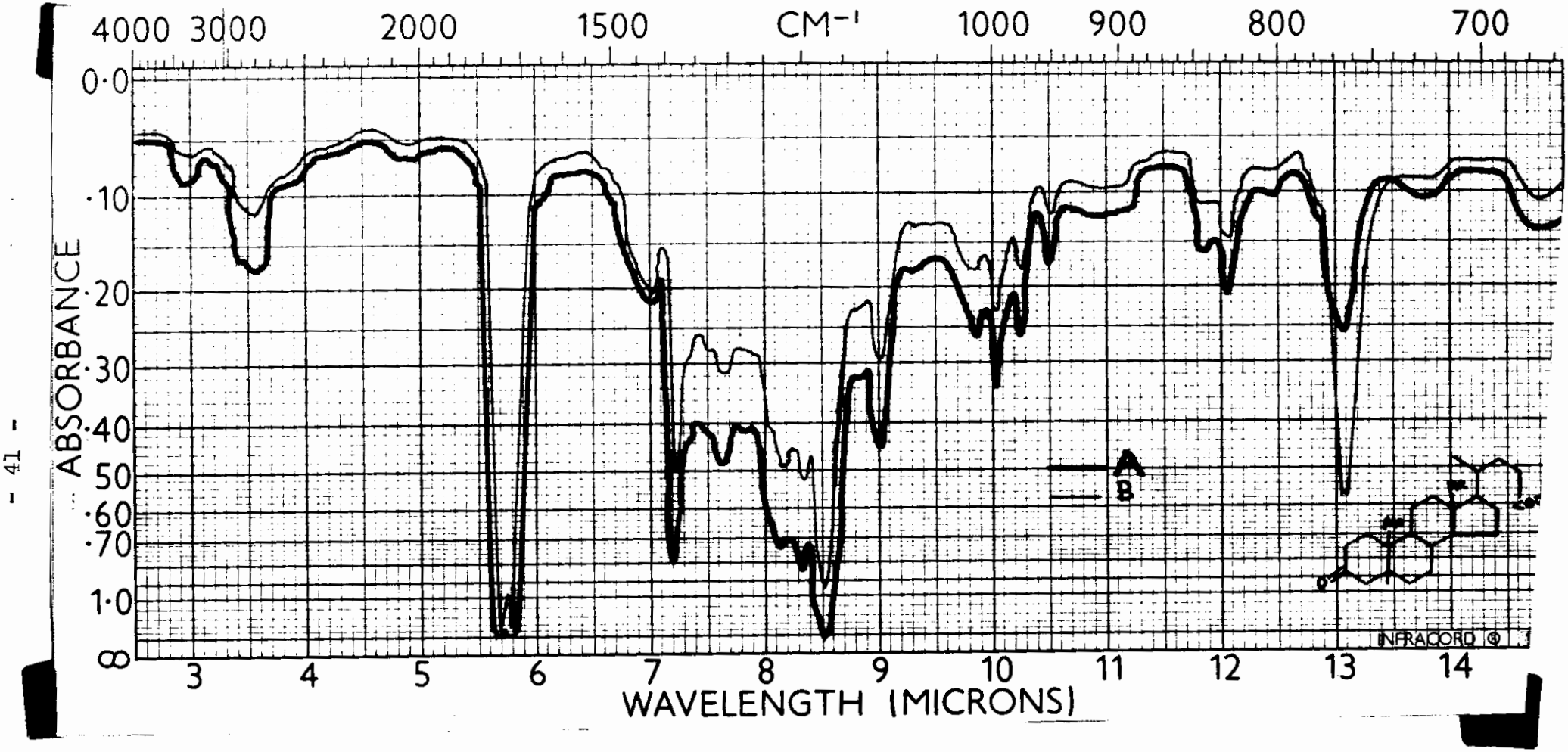


Appendix IV

Δ^4-cholestene

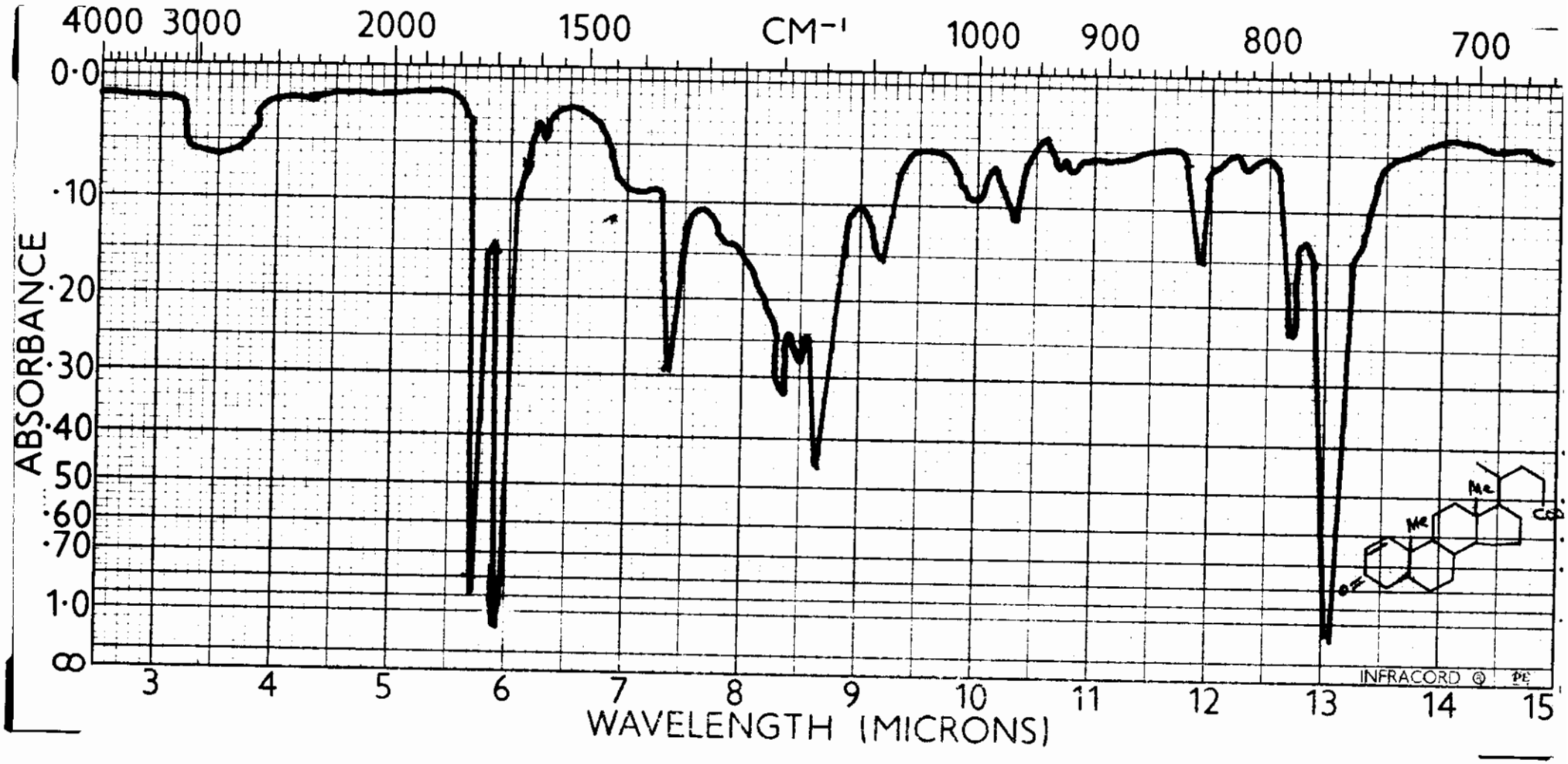
A- Procedure 2

B- Procedure 3



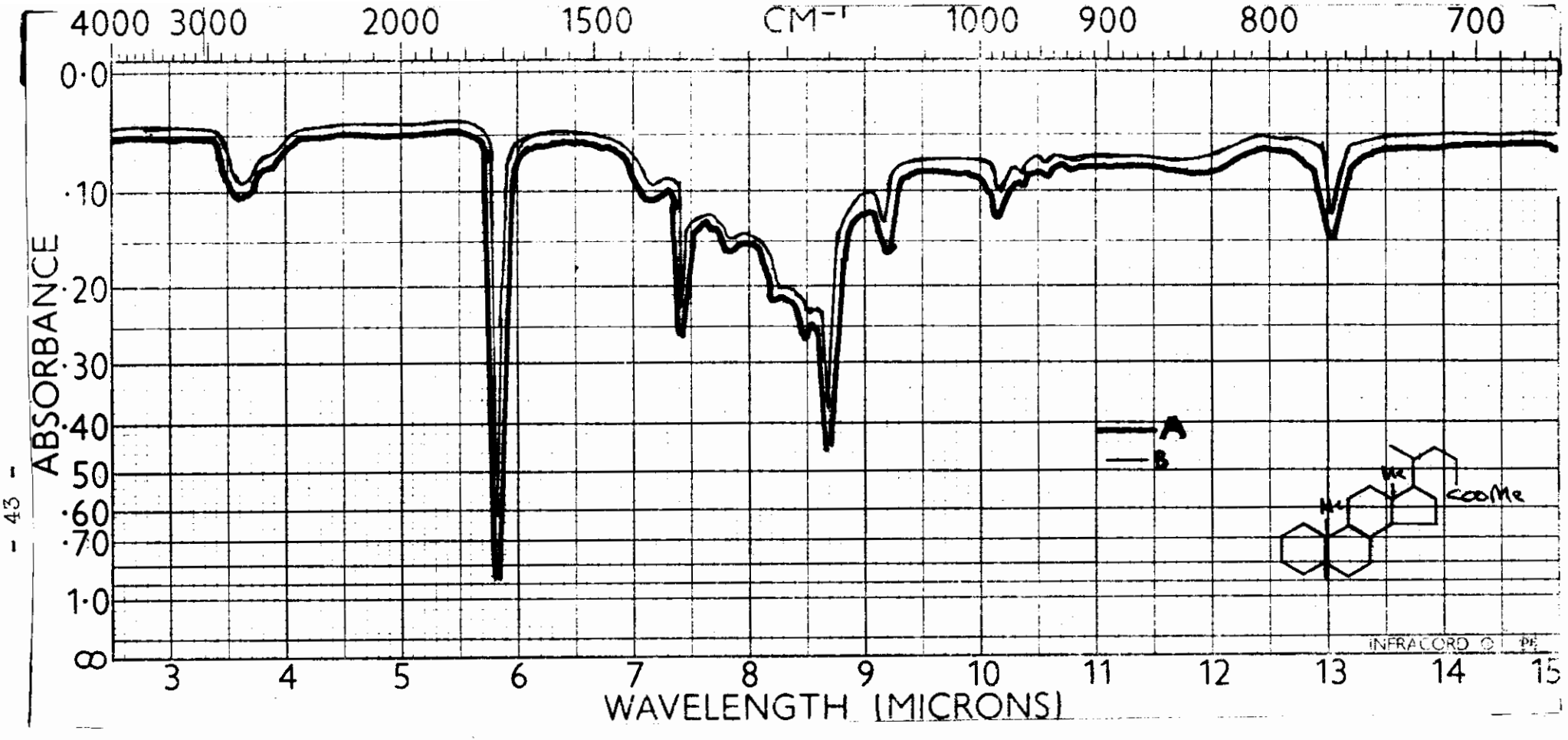
Appendix V

Methyl 3-keto-cholanate
A- From methyl 3-keto-Δ^1-cholenate
B- From lithocholic acid



Appendix VI

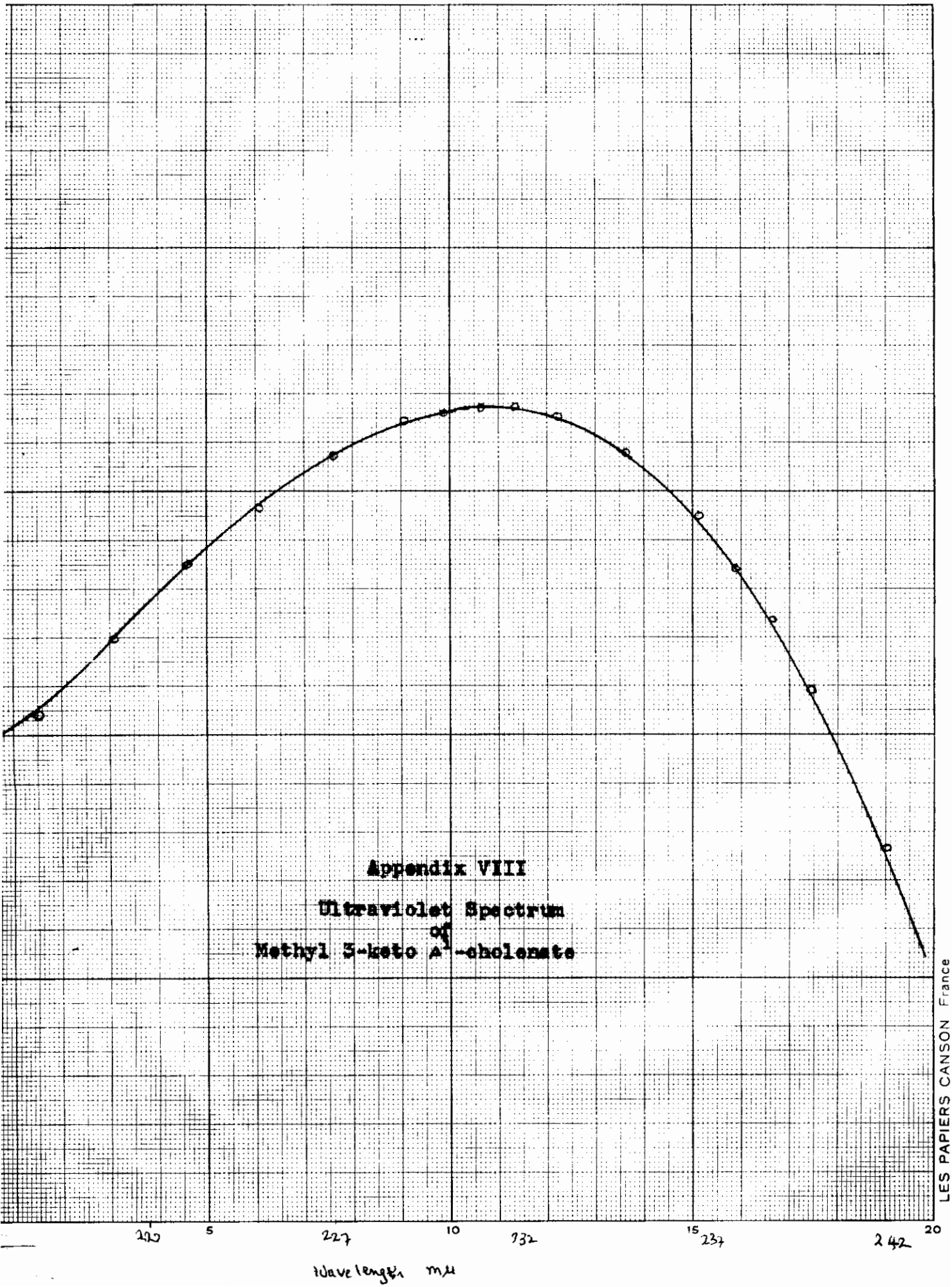
Methyl 3-keto- Δ^1-cholenate



Appendix VII

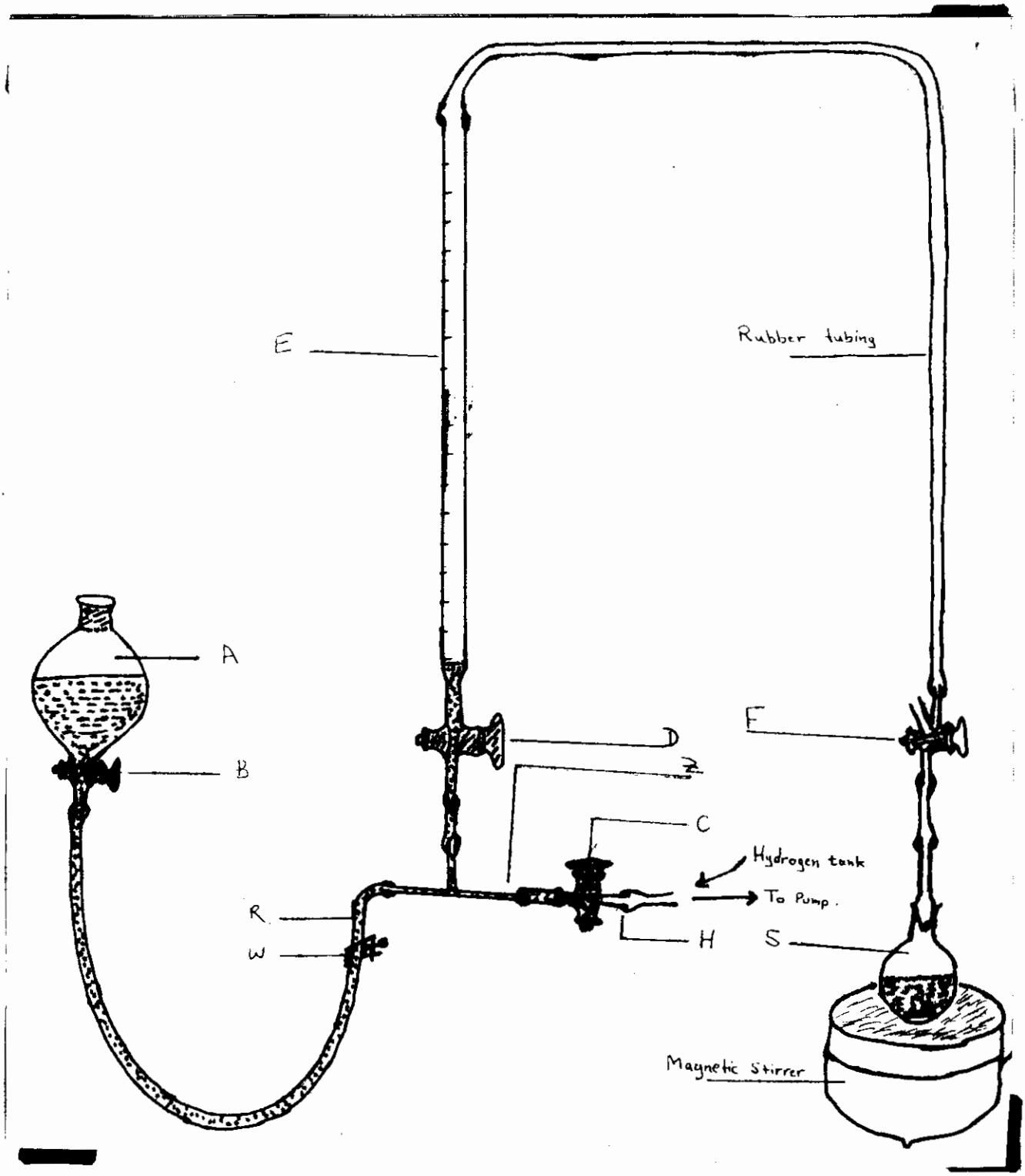
Methyl cholanate

A- From methyl 3-keto-Δ^1-cholenate
B- From methyl 3-keto cholanate



Appendix VIII

Ultraviolet Spectrum
of
Methyl 3-keto Δ^4-cholenate



Appendix IX

Hydrogenation Apparatus

(Refer to figure)

A: Separatory funnel containing enough mercury. B, C and D : Two-way stopcocks. E: Buret. F: Three-way stopcock connecting: (i) S to rest of system; or (ii) S to atmosphere; or (iii) E to atmosphere. S: Hydrogenation flask. W: Screw clamp.

With C, D, F and W open, lower A so that mercury level is at point R. Close W and connect S (containing the solvent and the catalyst to be prereduced) to the system. Attach a pump to H and evacuate the system. Close C, disconnect the pump and connect H to a hydrogen cylinder. Open C and admit a slow stream of hydrogen to fill the system. Close C, disconnect the hydrogen cylinder, connect the pump (in operation) and re-evacuate the system. Repeat filling with hydrogen and evacuation a few times to ensure complete removal of air. Finally fill system with hydrogen and close C. Open W and raise A so that mercury passes through D and rises a little in E. Open C carefully and let mercury fill out the horizontal portion completely, even if a little mercury enters the bore of stopcock C. Close C and raise A to bring the level of mercury in E on the graduated portion. Turn F very quickly one complete turn to connect system momentarily to atmosphere in order to equilibrate the system and bring the hydrogen pressure to one atmosphere. Start stirring and allow catalyst to be prereduced. When hydrogen uptake stops, lower A so that mercury level is at

point R and close W. Connect pump (in operation) to H, open C and evacuate the system. Close C, disconnect the pump and open C to atmosphere. Disconnect S, add to it the solution containing the substance to be reduced, connect S again to the system and evacuate. Fill with hydrogen and evacuate a few times to ensure removal of air. Fill the system finally with hydrogen and allow hydrogenation to proceed as above (for catalyst) raising A occasionally to keep up with uptake of hydrogen. At the end of reaction proceed as before (lower A, close W ..... etc.)

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