REDUCTIONS

OF

STEROIDAL α - β UNSATURATED KETONES

BY

YUSUF J. ABUL-HAJJ

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This work is dedicated

to

Fadia

ACKNOWLEDGMENT

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ABSTRACT

The effect of changing the solvent on the relative amounts of the C_3 epimeric alcohols obtained by sodium borohydride reduction of Δ^4- cholestene-3-one, methyl 3-keto- Δ^4- cholenate, and methyl 3-keto- Δ^1- cholenate has been studied.

Reduction of 3 keto- Δ^4- steroids by sodium borohydride in methanol or pyridine gives predominantly the 3β-isomer while reduction of methyl 3-keto- Δ^1- cholenate gives predominantly the 3α-isomer.

The cleavage of digitonides of allylic 3β-hydroxy steroids by dimethyl sulfoxide has been found to give almost quantitative yields of the sterol.

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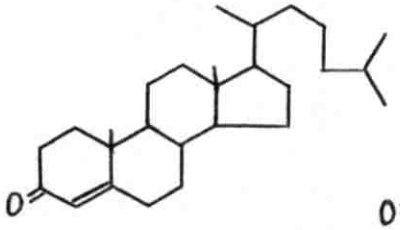
INTRODUCTION

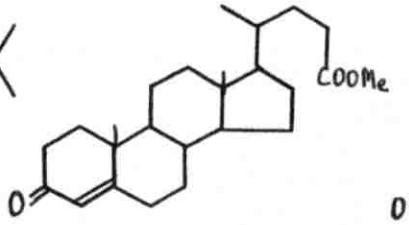
Purpose of the work:

A considerable amount of work has been done on the reduction by complex metal hydrides of 3 keto- Δ^1 - steroids[1] and 3 keto- Δ^4 - steroids[2,3,4,5] in the cholestane series, and on 3 keto- Δ^4 - steroids[6,7,8,9] in the bile acid series. However, no work has been done on the reduction of 3 keto- Δ^1 - steroids in the bile acid series.

During this investigation we have attempted to make a comparative study of the reduction by sodium borohydride of Δ^4 - cholestene-3-one (I), methyl 3-keto- Δ^4 - cholenate (II), and methyl 3-keto- Δ^1 - cholenate (III) in methanol and pyridine.



(I)



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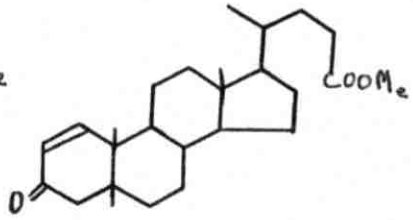


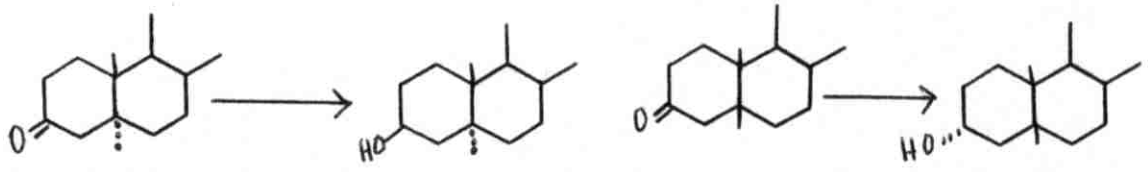
(III)

Historical

The use of complex metal hydrides for the selective reduction of various polar functional groups has found wide applications in steroid chemistry[10]. The fact that sodium borohydride can selectively reduce the carbonyl group in the presence of other

reactive functional groups led many investigators to use this reagent for the preparation of some steroidal alcohols of medicinal importance.[11,12] This method gives excellent yields and is superior to that of Sondheimer and Evans employing Meerwin-Pondorf reduction.[13]

Reduction of 3-ketosteroids by sodium borohydride has been found to be stereospecific[1,4,14,15]: 3-ketosteroids of the cholestane series (IV) give 3β-ols as the sole product, while 3-ketosteroids of the coprostane series (V) give the 3α-ols.[16]



(IV) (V)

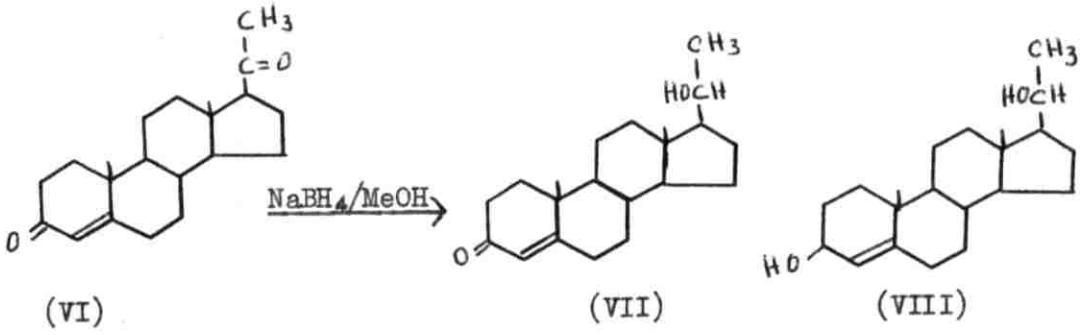
Reduction of 3 keto-Δ^4- steroids with complex metal hydrides has been reported to give contradictory results concerning the amounts of 3α- and 3β-hydroxy-Δ^4- epimers. Table I summarizes some of these results:

Table I

Compound	% 3β	% 3α	Ref.
Δ^4- cholestene-3-one	44	44	2
Δ^4- cholestene-3-one	50	50	3
Δ^4- cholestene-3-one	69	24	4
Methyl-3-keto-Δ^4-cholenate	78	10	9
17α-ethinyl testosterone	70	--	7
Methyl-3-keto-Δ^4-etiocholenate	91	absent	6
Methyl-3 keto-19-acetoxy-Δ^4-etiocholenate	major	minor	17

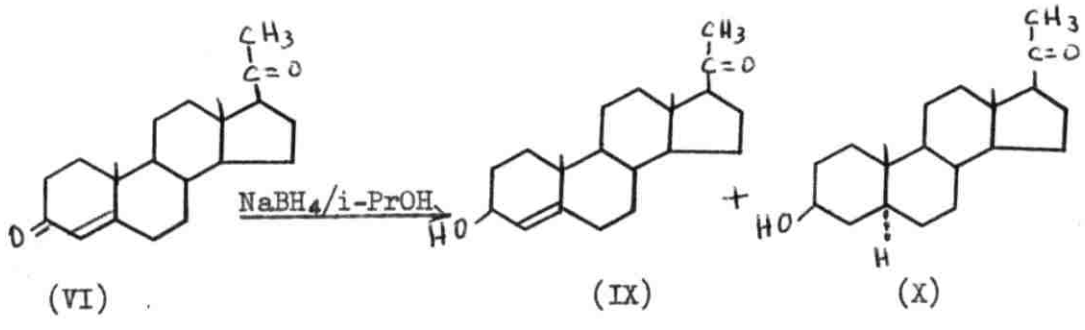
Bergman[1] reported that reduction of Δ^1 - cholestene-3-one with lithium aluminium hydride gave exclusively 3β hydroxy- Δ^1 - cholestene (90%) with remarkable absence of the 3 alpha isomer.

Solvent effects in the reductions of 3 keto- Δ^4 - steroids by sodium borohydride were reported by Kupfler[8] in 1961. Thus, reduction of progesterone (VI) with sodium borohydride in methanol yielded VII and VIII; in isopropanol IX and X; and in pyridine X and IX.



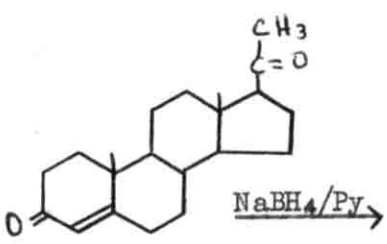
(VI) (VII) (VIII)

major product minor product

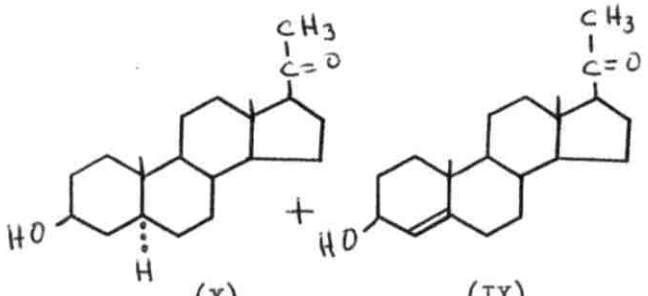


(VI) (IX) (X)

major product minor product



(VI)

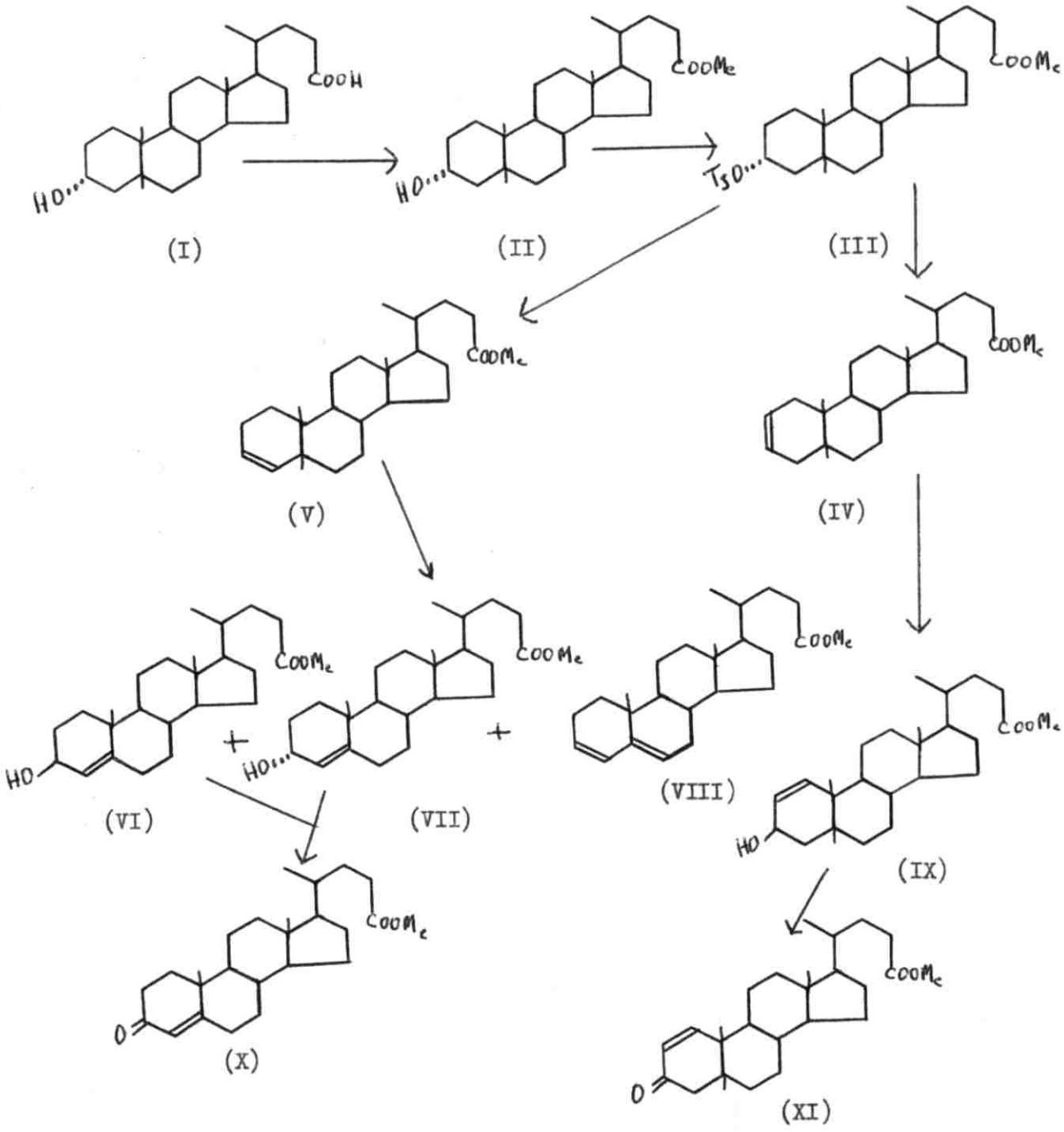


(X) major product

(IX) minor product

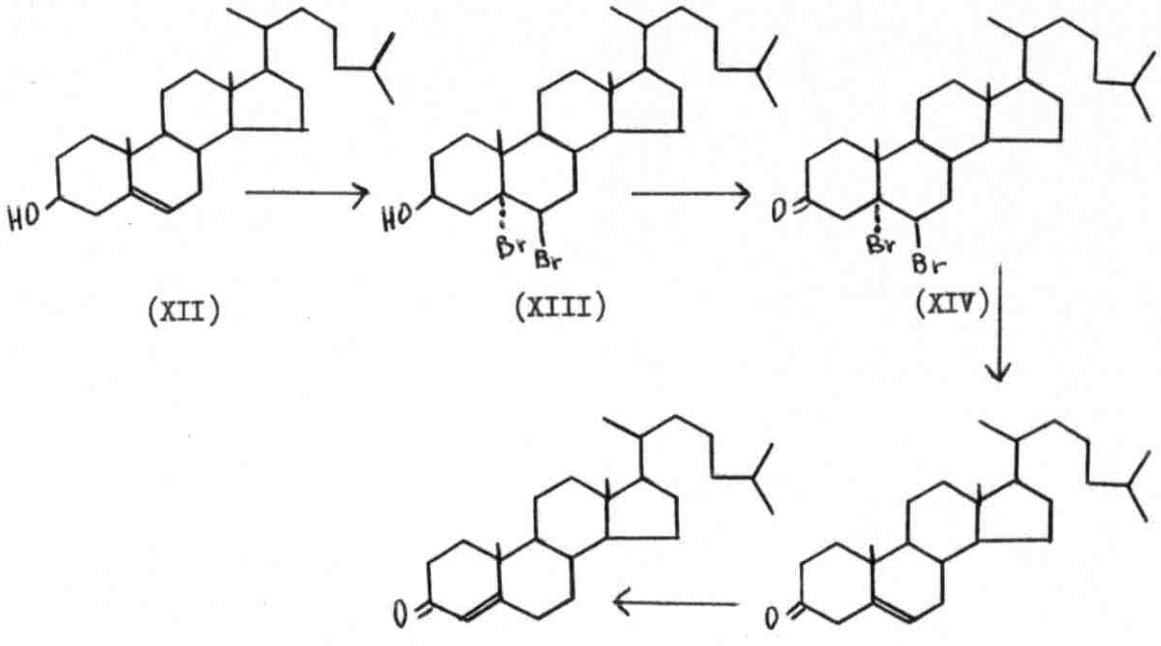
RESULTS

A. Preparation of methyl 3-keto-Δ^4- cholenate and methyl 3-keto-Δ^1- cholenate.



Methyl lithocholate (II) was obtained by esterification of lithocholic acid (I) with excess methanol in the presence of concentrated hydrochloric acid. Conversion to methyl 3α-tosyloxy-cholanate (III) was accomplished by adding 1.1 equivalents of p-toluenesulfonyl chloride to a solution of methyl lithocholate (II) in dry pyridine. Dehydrotosylation of III by 2,6 lutidine gave a brown oil which was chromatographed on a column of alumina. Elution with P.E. and P.E.-B (9:1) gave an intractable mixture of Δ^{2}- and Δ^{3}-cholenate (IV, V). The mixture was treated with selenium dioxide in acetic acid, and then acetylated. Chromatography on alumina removed methyl $\Delta^{3,5}$-choladienate (VIII) (early fractions). Saponification of the late fractions with 2.5 N methanolic potassium hydroxide gave the free hydroxy acids which, upon treatment with diazomethane in the cold, gave the methyl esters of VI, VII and IX. The mixture (VI, VII, and IX), upon oxidation at room temperature with freshly prepared manganese dioxide in chloroform, gave methyl 3-keto-Δ^{1}- cholenate (XI) m.p. 138 - 139^{0} λ_{max}^{MeOH} 231 - 232 mμ, and methyl 3-keto-Δ^{4}- cholenate (X) m.p. 126 - 127^{0} λ_{max}^{MeOH} 241 - 242 mμ, which were separated by chromatography over alumina.

B. Preparation of Δ^4- cholestene-3-one.



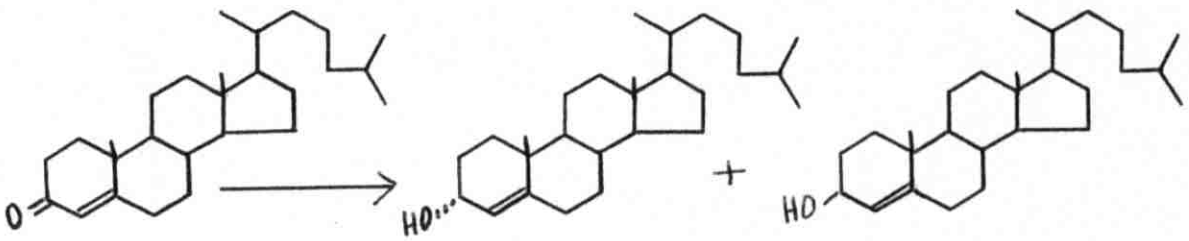
(XII) (XIII) (XIV)

(XVI) (XV)

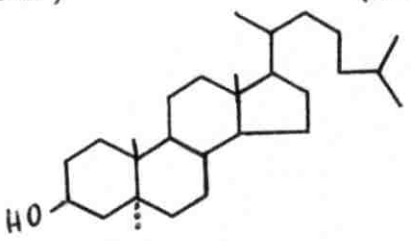
Cholesterol dibromide (XIII) was obtained by adding bromine and anhydrous sodium acetate in acetic acid to an ethereal solution of cholesterol (XII). When XIII was treated with a hot solution of sodium dichromate in acetic acid, 5α, 6β -dibromo-cholestane-3-one (XIV) resulted. Treatment of XIV with zinc dust gave Δ^5-cholestene-3-one (XV). Compound XV, in ethanol, was isomerized by oxalic acid to Δ^4-cholestene-3-one (XVI) m.p. 81 - 82^0.

C. Reductions.

1. Reduction of Δ^4- cholestene-3-one.



(XVI) (XVII) (XVIII)



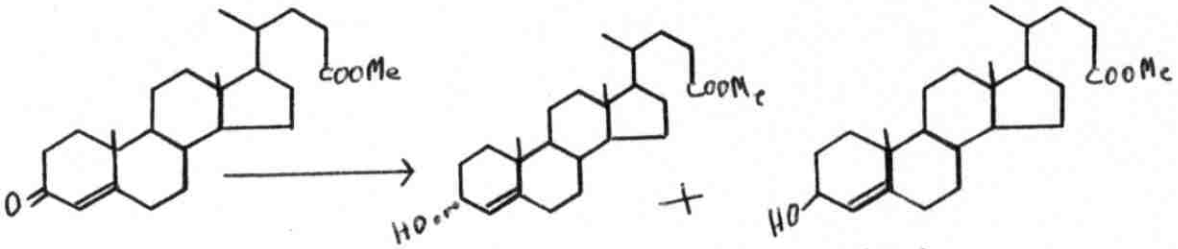
(XIX)

Reduction of XVI with sodium borohydride in methanol gave a mixture of 3α-hydroxy- Δ^4- cholestene(XVII), 15%, and 3β-hydroxy- Δ^4- cholestene (XVIII), 75%. The mixture of the epimeric alcohols was separated by the following general procedure: the reduction mixture was treated with a 1% solution of digitonin in 90% ethanol. The precipitated digitonide was collected by filtration, dried, and cleaved by dimethyl sulfoxide. Recovery of the 3β -isomer was almost quantitative. Evaporation of the filtrate and extraction of the residue in a Soxhlet extractor gave 3α-hydroxy- Δ^4- cholestene (XVII).

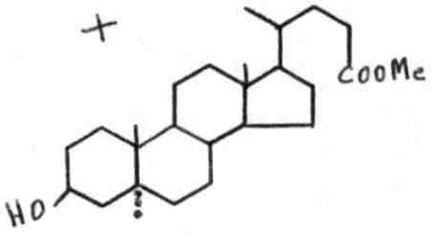
Reduction of XVI with sodium borohydride in pyridine gave 68%

of the 3β-isomers (XVIII and XIX), and 22% of the 3α-isomer (XVII).

2. Reduction of methyl 3-keto-Δ^4- cholenate.



(X) (XX) (XXI)



(XXII)

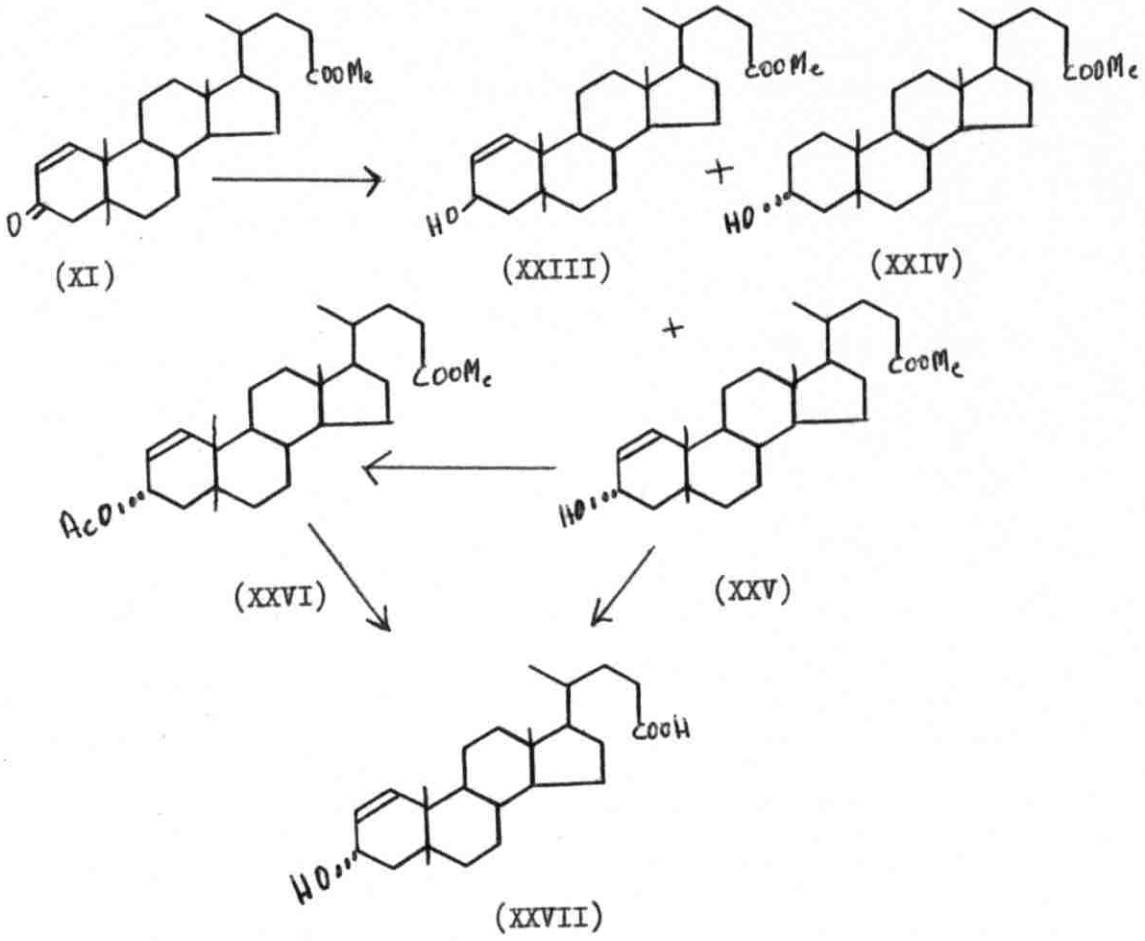
Reduction of X with sodium borohydride in methanol gave methyl-3α-hydroxy-Δ^4- cholenate (XX), 15%, and methyl-3β-hydroxy-Δ^4-cholenate (XXI), 82%.

Reduction of X with sodium borohydride in pyridine gave 74% of the 3β-isomers (XXI and XXII), and 20% of the 3α-isomer (XX).

3. Reduction of methyl 3-keto-Δ^1- cholenate.

Reduction of XI with sodium borohydride in methanol gave 84% of XXV and 9% of XXIII.

Reduction of XI with sodium borohydride in pyridine gave 92% of the 3α-isomers (XXIV and XXV) and traces of the 3β-isomer (XXIII).



Compound XXV, after acetylation and recrystallization from methanol, gave needles of methyl-3α-acetoxy-Δ^1-cholenate (XXVI) m.p. 116 - 118°. Hydrolysis of XXVI and XXV in 2.5 N methanolic potassium hydroxide, and recrystallization from aqueous methanol gave needles of 3α-hydroxy-Δ^1- cholenic acid (XXVII) m.p. 190 - 192°, α_D + 47.

DISCUSSION

Several methods have been described in the literature for the preparation of Δ^4- cholestene-3-one.[18,19,20] During this investigation we followed the procedure described by Fieser.[18]

Lithocholic acid (I) was the starting material for the preparation of methyl 3-keto- Δ^1- cholenate (XI) and methyl 3-keto- Δ^4- cholenate (X), by the following method[21]: Selenium dioxide oxidation of methyl Δ^2- and Δ^3- cholenate (prepared[22] by the action of lutidine on the tosylate of methyl lithocholate) gave a mixture of methyl $\Delta^{3,5}$- choladienate (VIII), methyl 3α- and 3β- hydroxy- Δ^4- cholenate (VII, VI), and methyl-3β-hydroxy- Δ^1- cholenate (IX). The mixture was acetylated and the diene separated from the allylic alcohols by chromatography. Hydrolysis of the acetates followed by methylation with diazomethane gave a mixture of VI, VII, and IX. Oxidation of VI, VII, and IX with manganese dioxide gave a mixture of methyl 3-keto- Δ^4- cholenate (X) and methyl 3-keto- Δ^1- cholenate (XI), separable by chromatography.

The chromatographic separation of the diene from the allylic alcohols (as the acetates), and of methyl 3-keto- Δ^4- cholenate from methyl 3-keto- Δ^1- cholenate, described above, was found to be time consuming and tedious. We are at present investigating a considerable simplification of this procedure, but are in no position to report definite results yet.

The reduction of Δ^4- cholestene-3-one, methyl 3-keto- Δ^4-

cholenate, and methyl 3-keto-Δ^1- cholenate was carried out with sodium borohydride in methanol and pyridine at room temperature. Sodium borohydride rather than lithium aluminium hydride was used since the former is milder and does not attack the ester group.[23] The results of the present work are summarized in Table II.

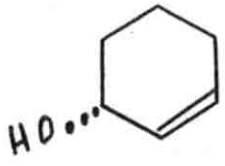
Table II

Compound	Solvent	Time(hrs.)	% β	% α
Δ^4- cholestene-3-one	MeOH	16	75	15
	Pyridine	16	68	22
Methyl 3-keto-Δ^4-cholenate	MeOH	16	82	15
	Pyridine	16	74	20
Methyl 3-keto-Δ^1-cholenate	MeOH	16	9	84
	Pyridine	16	traces	92

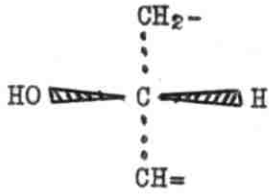
Inspection of Table II reveals the following striking feature: reduction of Δ^4-3-keto-steroids gives predominantly the 3β epimer, while reduction of Δ^1-3-keto-steroids of the 5β series gives predominantly the 3α epimer. This remarkable reversal in the isomer ratios may be attributed to the shape of the molecules in question. The ring system of Δ^4-3-keto-steroids is nearly flat and the predominance of the 3β isomer is not surprising, since the angular methyl group at C_{10} hinders frontal attack by the reducing agent. In Δ^1-3-keto-steroids of the 5β series, however, the rearward bending of ring A shields the α-side of the molecule and invites frontal attack.

Changing the solvent does not alter appreciably the ratios of 3 β and 3 α epimers. However, saturation of the double bond has been observed in these reductions when pyridine was used as a solvent. This is evidenced by the depression of the melting point and the diminished optical rotation of the predominant isomer. Saturation of the double bond in the reduction of progesterone with sodium borohydride in pyridine has been observed by Kupfler[8] in 1961.

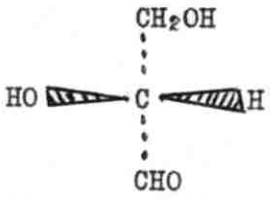
Optical rotation studies in the terpene series[24] reveal that allylic alcohols of type XXVIII (of projection formula XXVIIIa corresponding to that of D-glyceraldehyde) are found to be more dextrorotatory than their epimers of type XXIX (belonging to the L-series of projection formula XXIXa).



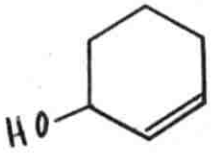
(XXVIII)



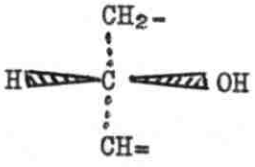
(XXVIIIa)



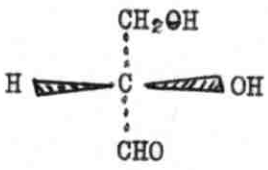
D-(+) glyceraldehyde



(XXIX)



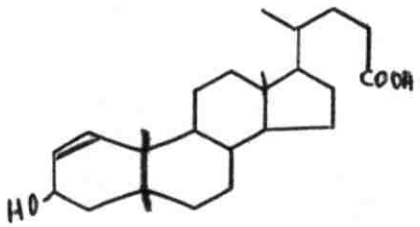
(XXIXa)



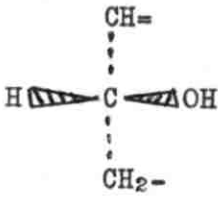
L-(-) glyceraldehyde

Similar considerations apply to allylic alcohols in the steroid series. Thus, comparison of the optical rotations of the epimeric 3-hydroxy- Δ^4- cholenic acids[25] shows that the 3αepimer

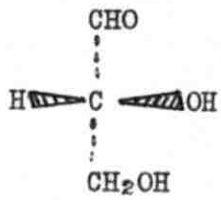
(α_D + 120) is more dextrorotatory than the 3β epimer (α_D + 51). On this basis, it is expected that 3β-hydroxy-Δ^1-cholenic acid (XXX) (α_D + 135[25]) (of projection formula XXXa corresponding to that of D-glyceraldehyde) should be more dextrorotatory than 3α-hydroxy-Δ^1-cholenic acid (XXVII) (α_D found during this investigation: + 47) (belonging to the L-series of projection formula XXVIIa)



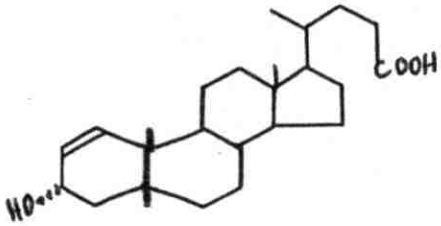
(XXX)



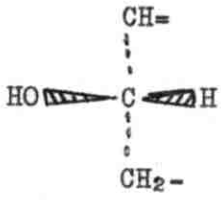
(XXXa)



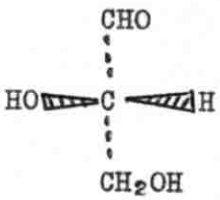
D-(+) glyceraldehyde



(XXVII)



(XXVIIa)



L-(-) glyceraldehyde

The use of dimethyl sulfoxide for the cleavage of steroidal digitonides was introduced recently by Issidorides, Kitigawa, and Mosettig.[26] During this investigation we found that dimethyl sulfoxide is an effective and safe reagent for the cleavage of digitonides of allylic 3β steroidal alcohols. Compared to the method introduced by Schoenheimer[27] using pyridine as a solvent, the dimethyl sulfoxide method has the advantage of convenience and speed in the isolation of the sterols; the yields are almost quantitative.

EXPERIMENTAL*

p-tolylsulfonyl nitrosamide[28]

Aqueous methylamine (210 ml., 33%) was placed in a 1-liter round-bottomed flask, and p-toluenesulfonyl chloride (190 g.) was added in portions (with swirling) for five minutes. The mixture was heated to 80 - 90° in order to maintain the sulfonylmethylamide (m.p. 78) in a molten state. Boiling was prevented by cooling the flask with water. When the mixture became acid to litmus, sodium hydroxide (50 ml., 50%) was added slowly, followed immediately by gradual addition of p-toluenesulfonyl chloride (90 g.). Sodium hydroxide (25 ml., 50%) was added to the acid mixture, followed by a third portion of p-toluenesulfonyl chloride (40 g.). When the mixture was acid to litmus, sufficient amount of sodium hydroxide (50%) was added to make the final solution alkaline.

The walls of the flask were rinsed with water, and the mixture was heated on a water bath for fifteen minutes with vigorous mechanical stirring. The hot mixture was poured in 1.5 liters of glacial acetic acid contained in a 5-liter round-bottomed flask. The small flask was rinsed with 250 ml. of acetic acid.

The mixture was kept at a temperature between 0 - 5° in an ice bath. A solution of sodium nitrite (124 g.) in water (250 ml.) was

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*Melting points are uncorrected. Alumina used for chromatography was neutral, grade I "Woelm" to which 3% waterwas added. Solvents for chromatography are indicated thus; P.E. = 40-70°pet.ether, B = Benzene, E = Ether. U.V. spectra were determined in Zeiss Spectrophotometer PMQII.

added dropwise over a period of forty five minutes, with vigorous mechanical stirring. The mixture was stirred for fifteen more minutes, with the temperature kept below 10^{0}C. The product, which separated as a yellow crystalline solid, was diluted with water (1 liter), filtered, washed with water (500 ml.) and dried in a vacuum desiccator (40 mm. Hg). Weight 280 g. m.p. 52 - 56^{0}. The product was recrystallized by dissolving it in petroleum ether - ether (1:1) and cooling in the refrigerator. Hard orange crystals were obtained, melting at 58 - 60^{0}.

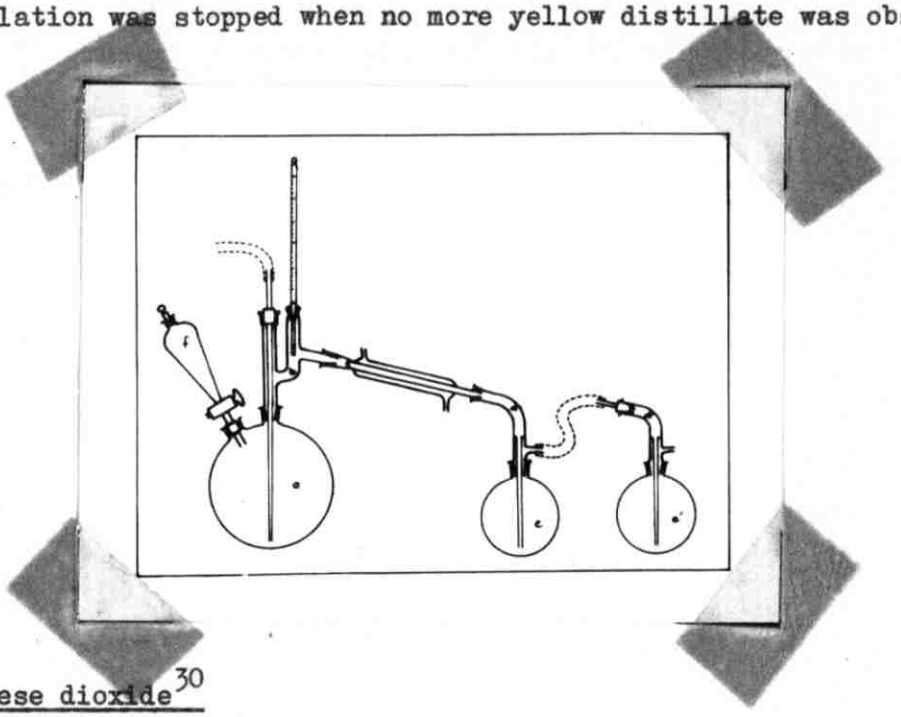
Diazomethane[29]

Caution: Diazomethane is very toxic and explosive. Its preparation should be carried out under the hood. The use of a safety glass window is recommended. The apparatus used should have ungreased glass joints with fire polished edges.

A Claisen head (b), equipped with a thermometer (g) and condenser (c), was fitted into a 250 ml. round-bottomed flask (a) immersed in an oil bath. A 250 ml. round-bottomed flask (e) was connected to the condenser by means of a vacuum adapter with a side connection (d). Another flask (e') was joined to (e) through a rubber tubing and a similar adapter (d'). Flasks (e & e') were placed in an ice-salt bath.

A mixture of potassium hydroxide (6 g.) in water (10 ml.), 2-ethoxy ethanol (35 ml.) and ether (10 ml.) was placed in flask (a). During the preparation a gentle stream of nitrogen was allowed to flow through a capillary immersed below the surface of the solution

in flask (a). The temperature of the oil bath was kept at 70 - 75°. From a dropping funnel, a solution of p-tolylsulfonyl nitrosamide (21.5 g.) in ether (125 ml.) was added slowly (fifteen minutes). The distillation was stopped when no more yellow distillate was observed.



Manganese dioxide[30]

A solution of manganese sulfate monohydrate (55 g.) in water was placed in a 2-liters 3-necked flask and heated with stirring at 86°C. Potassium permanganate (240 ml.) (prepared by mixing 60 g. of the salt in 400 ml. of water and heating to 70 - 85°) was added to the hot manganese sulfate solution in portions, (30 - 35 ml. each), and the mixture stirred for five minutes. Another portion (100 ml.) of potassium permanganate was added and this time the mixture was stirred for twenty minutes. The remainder of the potassium permanganate was added to the manganese sulfate solution and stirring was continued for thirty minutes at 86°C. Filtration, followed by thorough washing of the precipitate with hot water

(700 ml.) and drying at 110 - 120° for two days to constant weight (74 g.) gave a brown solid which could be powdered easily.

Cleavage of digitonides[26]

The standard dimethyl sulfoxide method for the cleavage of digitonides was used for recovery of the 3β-isomer. The following is a typical run: the digitonide of Δ^4-cholestene-3β-ol (1.023 g.) in dimethyl sulfoxide (20 ml.) was heated on a steam bath for five minutes, cooled to room temperature, and extracted with n-hexane (70 ml.). The dimethyl sulfoxide layer was extracted with four 30 ml. portions of n-hexane. The combined extracts were dried for thirty minutes over sodium sulfate (15 g.), filtered, and evaporated to dryness. The residue was dried to constant weight (100°/1 mm) giving Δ^4-cholestene-3β-ol (0.232 g., 96% recovery) melting at 128 - 131°. Recrystallization from methanol gave needles m.p. 131 - 132°.

Cleavage of the other digitonides by the above procedure gave values ranging from 89 - 99% recovery.

Methyl lithocholate (II)[25]

A mixture of lithocholic acid (I) (50 g.), methanol (500 ml.) and concentrated hydrochloric acid (5 ml.) was refluxed for thirty minutes. The hot solution was filtered, left overnight, cooled to -2°, and the precipitated product (38 g. m.p. 126 - 128°) was collected, washed with cold methanol and dried. Concentration of the mother liquor and washings gave a second crop (8 g.) m.p. 126 - 128°. (Lit.: 126 - 128[21], 126.5 - 128[25], 125 - 127.5[22], 125 - 127[32]).

Methyl 3α-tosyloxycholanate (III)[25]

A solution of methyl lithocholate (II) (25 g.) in dry pyridine (50 ml.) was treated with p-toluenesulfonyl chloride (29 g.) and kept at room temperature for two days, then poured into ice-cold dilute hydrochloric acid with stirring. The white precipitate was filtered and washed with cold water. The dry, methyl 3α-tosyloxycholanate (III) (33 g.) gave shiny plates from methanol-acetone mp. 116 - 118° (Lit.: 118 - 120[21], 119 - 121[25], 120 - 121.5[22], 110 - 112[31]).

Dehydrotosylation of methyl 3α-tosyloxycholanate (III)[22]

Methyl 3α-tosyloxycholanate (III) (25 g.) was refluxed with 2,6 lutidine (125 ml.) for 4½ hours. The cooled solution was poured into cracked ice and extracted repeatedly with ether. The combined ethereal extracts were washed thoroughly with hydrochloric acid (3%) and water, dried over sodium sulfate, filtered, and evaporated to dryness under reduced pressure. A portion (7 g.) of the brown oily residue was dissolved in benzene (6 ml.) and applied to a column of alumina (100 g. of alumina in a column 25 mm. in diameter and 300 mm. in height). Elution with P.E. and P.E.-B. (9:1) and evaporation of the fractions gave an oily product which solidified when rubbed with methanol. Repeated recrystallizations from methanol gave shiny plates of a mixture of methyl-Δ^2-cholenate and methyl-Δ^3-cholenate (IV, V) (3 g.) m.p. 73.5 - 75° (Lit.: 74 - 75[21], 73.5 - 74.5[25], 74.5 - 75[22]).

Selenium dioxide oxidation of the dehydrotosylate[21]

A solution of selenium dioxide (1.3 g.) in water (3.7 ml.) and acetic acid (25 ml.) was added to a solution of methyl- Δ^2-cholenate and methyl- Δ^3-cholenate (5 g.) (obtained in the last step described above) in acetic acid (55 ml.), and the mixture was stirred (magnetic stirrer) at room temperature for 35 hours. The resulting red mixture was diluted with water, filtered, washed with sodium carbonate (5%) until no more carbon dioxide was evolved, then washed with water and finally treated with charcoal (0.2 g.) and filtered. The yellow clear filtrate was dried over sodium sulfate for 3 hours, filtered, and evaporated to dryness under reduced pressure at room temperature. The residue was dissolved in dry pyridine (60 ml.) and acetic anhydride (30 ml.). The solution was allowed to stand overnight at room temperature, then poured onto cracked ice with stirring. The yellow precipitate was filtered, washed thoroughly with water and dried in a vacuum desiccator (40 mm. Hg). The product, dissolved in benzene (5 ml.), was adsorbed on a column of alumina (150 g.). The column was washed with P.E. (200 ml.) and then eluted fast with P.E.-B. (1:4). The combined P.E.-B. fractions were evaporated to dryness. The product was refluxed with 2.5 N methanolic potassium hydroxide (70 ml.) for 1½ hours. The saponification mixture was cooled, diluted with water (40 ml.) and ether (75 ml.), and acidified with cold 1 N sulfuric acid. The ether layer was separated and the aqueous layer extracted repeatedly with ether. The combined ether extracts were washed thoroughly with water and dried over sodium sulfate. The solution was filtered, evaporated

to dryness under reduced pressure, and treated with an ethereal solution of diazomethane in the cold. The excess diazomethane and ether were evaporated on a water bath, giving a white solid (3.8 g.) of the methyl esters of isomeric 3-hydroxy unsaturated acids VI, VII, and IX.

Manganese dioxide oxidation of the 3-hydroxy unsaturated methyl esters[21]

A solution of the product (3.8 g.) (obtained in the last step described above) in chloroform (380 ml) was stirred with freshly prepared manganese dioxide (38 g.) for 3½ hours at room temperature. The reaction mixture was filtered and the manganese dioxide was washed with chloroform (100 ml.). The filtrate was evaporated to dryness under reduced pressure.

The dry solid (2.4 g.) was dissolved in benzene (6 ml.) and applied to an alumina column (100 g. of alumina in a column 25 mm. in diameter and 300 mm. in height). The column was eluted fast with P.E.-B. (9:1, 8:2 respectively) and five fractions were collected. Evaporation of these fractions gave an oil of methyl- $\Delta^{3,5}$ - choladienate (VIII) (110 mg.) which solidified when rubbed with methanol. Recrystallization from methanol afforded lancets m.p. 96 - 98°

λ_{max}^{MeOH} 227.5, 236, 243 mμ. (Lit.: 96 - 98[25], 96 - 98[9])

Further elution with P.E.-B. (1:9) gave no product. The column was then eluted with benzene (150 ml.) and B.-E. (9:1) and six fractions, 75 - 80 ml. each, were collected. At this stage the column was eluted with B.-E. (8:2) and eight more fractions were collected.

The first six fractions were combined, evaporated, dissolved in benzene (2 ml.) and rechromatographed on an alumina column (30 g. of alumina in a column 20 mm. in diameter and 260 mm. in height). The column was eluted with P.E.-B. varying from (9:1) to (1:9) to collect five fractions which were discarded. Elution with B. (100 ml.) and B.-E. (9:1, 300 ml.), followed by evaporation of the combined fractions gave a white solid (500 mg) m.p. 128 - 134°. Repeated recrystallization from methanol gave shiny needles of methyl-3-keto-Δ^1- cholenate (XI) m.p. 138 - 139°, α_D + 109 Chf. (c 1.4), λ_{max}^{MeOH} 231 - 232 mμ (7,600) (Lit.: m.p. 137 - 138[21], 138.5 - 139.5[25]; α_D + 113[21] λ_{Max}^{MeOH} 231 - 232 mμ (9,000)[25]).

The B.-E. (8:2) fractions were combined and evaporated. The white residue (1.6 g.) was recrystallized twice from methanol, giving small prisms of methyl-3-keto-Δ^4- cholenate (X) m.p. 126 - 127°; α_D + 86 Chf. (c 0.25); λ_{max}^{MeOH} 241 mμ (16,800) (Lit.: m.p. 125 - 126.5[21], 125 - 126[9], 126 - 127[25]; α_D + 90[9], + 87[25,21], + 66.13[33]; ϵ = 15,800[9], 16,800[25]).

Δ^5-cholestene-3-one (XV)[18]

A mixture of cholesterol (XII) (150 g.) in absolute ether (1 liter) was placed in a 4-liter beaker and brought into solution by warming on a steam bath with stirring. A solution of anhydrous sodium acetate (5 g.) and bromine (68 g.) in acetic acid (600 ml.) was added to the cooled cholesterol solution with stirring. A stiff paste of the

dibromide (XIII) was formed in three minutes. The mixture was cooled to 20° in an ice bath, filtered, and the precipitate was washed with acetic acid (500 ml.).

A suspension of the dibromide in acetic acid (2 liters) was placed in a 5-liter round-bottomed flask and stirred at room temperature for fifteen minutes. To this was added a solution, preheated to 90°, of sodium dichromate dihydrate (80 g.) in acetic acid (2 liters). While stirring vigorously, the temperature rose to 55 - 58° and all the solid dissolved in 3 - 5 minutes. Stirring was continued for another two minutes. The solution was cooled in an ice bath for ten minutes without stirring, diluted with water (400 ml.), and stirred vigorously to bring the temperature to 15°. The product was collected on a 21-cm Buchner funnel, washed repeatedly with methanol (800 ml.) and transferred to a 3-liter round-bottomed flask containing ether (2 liters) and acetic acid (25 ml.). The mixture was stirred in an ice bath till the temperature was 15°. The ice bath was lowered and fresh zinc dust (5 g.) was added. The temperature was kept between 15 - 20° and zinc dust (35 g.) was added in portions over a five minute interval. The solution was stirred for ten more minutes; pyridine (40 ml.) was added, and the mixture filtered. The filtrate was washed with water (three 600 ml. portions) and sodium bicarbonate (5%) until no more carbon dioxide was evolved; it was then dried over magnesium sulfate and filtered. The filtrate was evaporated to a volume of one liter, and diluted with methanol (500 ml.). The solution was concentrated to about 1.2 liters, cooled, iced, and filtered. The solid Δ^5 - cholest-

ene-3-one (XV) (104 g.) melted at 115 - 122°.

Δ^4 - cholestene-3-one (XVI)[18]

Δ^5 - cholestene-3-one (XV) (100 g.), anhydrous oxalic acid (10 g.) and 95% ethanol (800 ml.) were heated on a steam bath for twenty five minutes. The hot solution was left overnight and then cooled to -2°. The precipitated product was collected, washed with cold 95% ethanol (200 ml.), and dried. The mother liquor and washings were combined and concentrated to give a second crop. The combined product weighed 87 g. m.p. 76 - 80°. Two recrystallizations from 95% ethanol gave a product (73 g.) melting at 81 - 82° (Lit.: 81 - 82[18], 78.5 - 80.5[19], 79.5 - 80.5[20]).

Reduction of Δ^4 - cholestene-3-one by $NaBH_4$ in methanol

Sodium borohydride (330 mg.) was added to a solution of Δ^4 - cholestene-3-one (XVI) (1 g.) in methanol (75 ml.). The solution was kept at room temperature for sixteen hours, poured into water (75 ml.) and extracted with ether (150 ml.). The aqueous layer was extracted with three 50 ml. portions of ether. The combined ether extracts were dried over sodium sulfate, filtered, and evaporated to dryness.

The white residue was dissolved in 90% ethanol and poured into a hot solution of digitonin (370 mg.) in 90% ethanol (400 ml.). The mixture was allowed to stand at room temperature for twenty four hours. The digitonide was filtered, washed with cold 90% ethanol and dried in a vacuum desiccator for twenty four hours (40 mm. Hg). The digitonide was cleaved with dimethyl sulfoxide, and extracted with n-hexane. The n-hexane extracts were combined, dried over sodium sulfate, and

evaporated to dryness. The white product (0.75 g., 75%) melted at 126 - 130°. Recrystallization from methanol gave needles of Δ^4- cholestene-3β-ol (XVIII) m.p. 131 - 132° α_D + 46 Chf. (c 1.02) (Lit.: m.p. 131 - 132[13] α_D + 44[13] α_D + 44[2]).

The digitonide filtrate was evaporated to dryness under reduced pressure. The dry residue was extracted with ether in a Soxhlet extractor for four hours. When evaporated, the ether extract gave a crude product (0.15 g., 15% yield) which was dissolved in dry pyridine (6 ml.) and acetic anhydride (3 ml.), kept overnight, and poured onto cracked ice with stirring. The white precipitate was filtered, washed with water, and dried in a vacuum desiccator. The dry product was taken up in benzene (2 ml.) and chromatographed on an alumina column (a 50 ml. burette containing 10 g. of alumina). The column was eluted with P.E. (100 ml.) and P.E.-B. (9:1, 8:2; 100 ml. and 50 ml. respectively). The combined fractions were evaporated and the residue recrystallized from methanol giving long needles of 3α-acetoxy- Δ^4- cholestene m.p. 81 - 82° (Lit.: m.p. 82.5[13]).

Reduction of Δ^4- cholestene-3-one by $NaBH_4$ in pyridine

A mixture of Δ^4- cholestene-3-one (500 mg.) and sodium borohydride (165 mg.) in pyridine (35 ml.) was kept at room temperature for sixteen hours. The solution was acidified with 1 N hydrochloric acid (25 ml.) and extracted repeatedly with ether. The combined ether extracts were washed with water, dilute hydrochloric acid, and finally water. The solution was dried over sodium sulfate, filtered, and evaporated to dryness. Precipitation with digitonin was carried out as

described for the reduction of Δ^4- cholestene-3-one in methanol. Cleavage of the digitonide with dimethyl sulfoxide gave 0.34 g. (68%) of the 3β-isomers. Repeated recrystallization from methanol, gave needles m.p. 120 - 122° α_D + 42 Chf. (c 0.84). (Lit.: 131 - 132[13], α_D + 46). The product was acetylated and recrystallized from methanol affording tiny needles of the 3β-acetate m.p. 72 - 76° (Lit.: m.p. 85[13]).

The digitonide filtrate gave a crude product (0.11 g., 22%) which was acetylated, chromatographed on alumina and recrystallized repeatedly from methanol giving long prismatic needles of the 3α-acetate m.p. 78 - 84° (Lit.: m.p. 82.5[14]).

Reduction of methyl 3-keto-Δ^4- cholenate by $NaBH_4$ in methanol

Sodium borohydride (35 mg.) was added to a solution of methyl 3-keto-Δ^4-cholenate (X) (104 mg.) in methanol (25 ml.). The solution was kept at room temperature for sixteen hours and worked-up as described for the reduction of Δ^4- cholestene-3-one in methanol. This gave a crude product of methyl 3β hydroxy-Δ^4- cholenate (XXI) (85 mg., 82%). Recrystallization from n-hexane gave rosettes m.p. 132 - 133° α_D + 49 Chf. (c 0.67) (Lit.: m.p. 131 - 133[9] α_D + 48[9]).

The digitonide filtrate gave a crude product of methyl 3α-hydroxy-Δ^4- cholenate (XX) (14 mg., 15%). This product was acetylated, chromatographed over alumina, and recrystallized from methanol. Shiny plates of methyl 3α-acetoxy-Δ^4- cholenate m.p. 141 - 142° were obtained (Lit.: 143 - 145[9]).

Reduction of methyl-3 keto- Δ^4 - cholenate by $NaBH_4$ in pyridine

A mixture of methyl-3 keto- Δ^4 - cholenate (100 mg.) and sodium borohydride (35 mg.) in pyridine (25 ml.) was kept at room temperature for sixteen hours. The mixture was worked-up as described for the reduction of Δ^4 - cholestene-3-one in pyridine, giving a crude product (74 mg., 74%) of the 3β-isomers. Recrystallization from n-hexane gave rosettes m.p. 125 - 129°, α_D + 38 Chf. (c 0.98) (Lit.: m.p. 131 - 133[9] α_D + 48[9]).

The digitonide filtrate gave a crude product (20 mg, 20%) of the 3α-isomer, which was acetylated, chromatographed over alumina, and recrystallized repeatedly from methanol yielding plates of the 3α-acetate. m.p. 133 - 136° (Lit.: 143 - 145[9]).

Reduction of methyl-3 keto- Δ^1 - cholenate by $NaBH_4$ in methanol

Sodium borohydride (35 mg.) was added to a solution of methyl-3 keto- Δ^1 - cholenate (XI) (111 mg.) in methanol (25 ml.). The solution was kept at room temperature for sixteen hours and the mixture was worked-up as described for the reduction of Δ^4 - cholestene-3-one in methanol. This gave a crude product of methyl 3β-hydroxy- Δ^1 - cholenate (XXIII) (10 mg., 9%). The yield was based on the weight of the digitonide.

The digitonide filtrate was evaporated and extracted with ether in a Soxhlet extractor for four hours. When evaporated, the ether extract gave crude methyl 3α-hydroxy- Δ^1 - cholenate (XXV) (93 mg., 84%). This product was acetylated, and chromatographed on an alumina column (a 50 ml. burette containing 10 g. of alumina). Elution with

P.E. (100 ml.) and P.E.-B. (9:1, 8:2; 100, and 200 ml. respectively) and evaporation of the combined fractions gave a white residue which was recrystallized from methanol yielding shiny needles of methyl 3α-acetoxy- Δ^1- cholenate (XXVI) m.p. 116 - 118° α_D + 113° Chf. (c 0.93).

Anal. Calcd. for $C_{27}H_{42}O_4$ (430.61): C, 75.31; H, 9.83

Found:

Determination (1): C, 75.49; H, 9.78

Determination (2): C, 75.05; H, 9.61

Reduction of methyl-3 keto- Δ^1- cholenate by $NaBH_4$ in pyridine

A mixture of methyl-3 keto- Δ^1- cholenate (106 mg.) and sodium borohydride (35 mg.) in pyridine (25 ml.) was kept at room temperature for sixteen hours. The mixture was worked-up as described for the reduction of Δ^4- cholestene-3-one in pyridine. Traces of the digitonide were formed.

The digitonide filtrate was worked-up as described in the last above procedure to give the 3α-hydroxy isomer. (98 mg., 92%). This product was acetylated, chromatographed over alumina, and recrystallized three times from methanol to give the 3α-acetoxy isomer m.p. 110 - 115° α_D + 98° Chf. (c 0.74). Further recrystallization did not affect the melting point. Admixture of this compound with methyl 3α-acetoxy- Δ^1- cholenate obtained from the methanol run melted at 110 - 115°.

3α-hydroxy- Δ^1- cholenic acid (XXVII)

Methyl 3α-acetoxy- Δ^1- cholenate (XXVI) (120 mg.) was refluxed

with 2.5 N methanolic potassium hydroxide (10 ml.) for 1½ hours. The saponification mixture was cooled, diluted with water (20 ml.) and ether (40 ml.), and acidified with cold 1 N sulfuric acid. The ether layer was separated and the aqueous layer extracted repeatedly with ether. The combined ether extracts were washed thoroughly with water, dried over sodium sulfate, filtered, and evaporated to dryness. Repeated recrystallizations from aqueous methanol gave tiny needles (86 mg.) m.p. 190 - 192° α_D + 47 Di (c 0.84) .

Anal. Calcd. for $C_{24}H_{38}O_3$ (374.54): C, 76.96; H, 10.23

Found:

Determination (1): C, 77.17; H, 10.20

Determination (2): C, 76.93; H, 10.06

Manganese dioxide oxidation of the allylic alcohols obtained from the reduction of Δ^4- cholestene-3-one, methyl-3 keto- Δ^4- cholenate, and methyl-3 keto- Δ^1- cholenate

a) Oxidation of the epimeric 3-hydroxy- Δ^4- cholestenes

The epimeric 3-hydroxy- Δ^4- cholestenes obtained from the reduction of Δ^4- cholestene-3-one (0.8 g.) were dissolved in chloroform (80 ml.) and stirred with freshly prepared manganese dioxide (8 g.) for 3½ hours. The mixture was filtered and the solvent was evaporated to dryness under reduced pressure. Recrystallization from 95% ethanol gave prismatic needles of Δ^4- cholestane-3-one (0.5 g.) m.p. 81 - 82° (Lit.: m.p. 81 - 82[18]).

b) Oxidation of methyl 3α-hydroxy- Δ^4 - cholenate and methyl 3β-hydroxy- Δ^4 - cholenate

Oxidation of the methyl 3α-hydroxy- Δ^4 - cholenate and methyl 3β-hydroxy- Δ^4-cholenate (run separately) with manganese dioxide as described in the above procedure gave a product melting at 120 - 124°. Recrystallization from methanol gave small prisms of methyl-3 keto- Δ^4 - cholenate m.p. 126 - 127°, λ_{max}^{MeOH} 242 mμ. (Lit.: 126 - 127[25], 125 - 126[9]).

c) Oxidation of methyl 3α-hydroxy- Δ^1 - cholenate and methyl 3β-hydroxy- Δ^1 - cholenate

Oxidation of the methyl 3α-hydroxy- Δ^1 - cholenate and methyl 3β-hydroxy- Δ^1 - cholenate (run separately) with manganese dioxide gave a product melting at 126 - 132°. Recrystallization from methanol gave needles of methyl-3 keto- Δ^1 - cholenate m.p. 133 - 136°, λ_{max}^{MeOH} 233 mμ. (Lit.: 138.5 - 139.5[25], 137 - 138[21]).

BIBLIOGRAPHY

1. W. Bergman, M. Kita, and D. Diancola; J. Am. Chem. Soc., 76, 4974 (1954).
2. H. McKennis, and G.W. Gaffney; J. Biol. Chem., 175, 217 (1948).
3. M.J. Haddadin; M.S. Thesis, Am. Univ. Beirut., 1959, p. 14; P.L. Plattner, H. Heusser, and A. Kulkarni; Helv. Chim. Acta, 32, 266 (1949).
4. W. Dauben, R.A. Micheli, and J.F. Eastham; J. Am. Chem. Soc., 74, 3852 (1952).
5. N.G. Gaylord, Reduction with Complex Metal Hydrides, Interscience Publishers, Inc., New York (1956), p. 236; P.L. Plattner, H. Heusser, and A. Kulkarni; Helv. Chim. Acta, 32, 1020 (1949).
6. W.W. Zorback; J. Am. Chem. Soc., 75, 6344 (1953).
7. F. Sondheimer, and Y. Klibansky; Tetrahedron, 5, 15 (1959).
8. D. Kupfler; Tetrahedron, 15, 193 (1961).
9. M. Haddadin, and C.H. Issidorides; J. Org. Chem., 25, 403 (1960).
10. C. Djerassi, editor; Steroid Reactions, Holden-Day, Inc., San Francisco (1963), p. 135.
11. O. Mancera, H. Ringold, C. Djerassi, G. Rosenkranz, and F. Sonheimer; J. Am. Chem. Soc., 75, 1286 (1953).
12. J. Norymberski, and G.F. Woods; J. Chem. Soc., 3426 (1955).
13. R. Sondheimer, and E.A. Evans; J. Biol. Chem., 114, 567 (1936).
14. C. Shopee, and G. Summers; J. Chem. Soc., 687 (1950).
15. D.H. Barton; J. Chem. Soc., 1027 (1953).

16. N.G. Gaylord; Reduction with Complex Metal Hydrides, Interscience Publishers, Inc., New York (1956), p. 236.

17. P. Th. Herzig, and M. Ehrenstein; J. Org. Chem., 17, 713 (1952).

18. L.F. Fieser; Org. Synth., 35, 43 (1953).

19. R.V. Oppenauer; Org. Synth., Coll. Vol. 3, 207 (1955).

20. J.F. Eastham, and R. Teranishi; Org. Synth., 35, 39 (1955).

21. M. Z. Nazer, and C. H. Issidorides; J. Org. Chem., 26, 839 (1961).

22. F. Chang, R. Blickenstaff, A. Feldstein; J. Gray, G. McCaleb, and D. Sprunt; J. Am. Chem. Soc., 79, 2164 (1957).

23. H. Heymann, and L.F. Fieser; J. Am. Chem. Soc., 73, 5252 (1951).

24. J. Mills; J. Chem. Soc., 4976 (1952).

25. C.H. Issidorides, M. Fieser, and L.F. Fieser; J. Am. Chem. Soc., 82, 2002 (1960).

26. C.H. Issidorides, I. Kitigawa, and E. Mosettig; J. Org. Chem., 27, 4693 (1962).

27. R. Schoenheimer, and H. Dam; Z. Physiol., 215, 59 (1933).

28. T.J. DeBoer, and H. Backer; Org. Synth., 34, 99 (1954).

29. H.J. Backer, and T.J. DeBoer; Proc. Konikl. Nederland. Acad. Wetenschap, 54B, 191-193 (1953); Chem. Abs. 46, 1961h.

30. E.P. Papadopoulos, and A. Shamma'a, unpublished results.

31. C. Babcock, and L.F. Fieser; J. Am. Chem. Soc., 74, 5472 (1952).

32. L.F. Fieser, and R. Ettorre; J. Am. Chem. Soc., 75, 1700 (1953).

33. A. Petit, J.P. Mathiew; Pouvoir Rotatoire Naturel, I Steroides (1956); H. Takeda, and J. Kawanami; J. Biol. Japan, 40, 477 (1953).