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STEROIDS AND RELATED NATURAL PRODUCTS—XVII

CONVERSION OF LANOSTEROL TO 14α-METHYL STEROIDS[1-3]

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Abstract—The possibilty of abnormal steroid biosyntheses leading to *in vivo* formation of certain 14α-methyl steroids was discussed. In order to further evaluate this proposal, preparation of 14α-methyl steroids related to the androgenic hormones was undertaken. Initially, a thirteen-step degradation sequence was developed for converting isocholesterol (crude lanosterol) to 3-oxo-17β-hydroxy-4,4,14α-trimethyl-5α-androstane.

THE experimental support provided by Bloch *et al.*[4] for *in vivo* conversion of lanosterol (Ia) to cholesterol has stimulated a number of investigations directed at establishing the intermediates involved in this very important biosynthetic transformation. Evidence is now available which indicates that this segment of cholesterol biosynthesis proceeds from sterol Ia by, first, oxidative removal of the 14α-methyl substituent (cf. Ib) followed by three additional oxidation steps, a rearrangement ($\Delta^{8(9)} \rightarrow \Delta^{5}$) and, finally, reduction at Δ^{24} to yield cholesterol. The latter sequence proceeds from sterol Ib via 3-oxo-4α-methyl-5α-cholesta-8,24-diene (II), zymosterol (III) and 3β-hydroxy-cholesta-5,24-diene (desmosterol).[5]

The above considerations and significant evidence favoring the hypothesis that cholesterol is a precursor of the hormonally active steroids[6] suggested the desirability of preparing and submitting for biological investigation steroids which might be expected to form *in vivo* should a defect occur in the normal biogenetic pathway. Our initial studies in this area have been concerned with preparing 14α-methyl steroids which might arise following *in vivo* conversion of lanosterol to a 14α-methyl cholesterol.[7]

[1] Refer to G. R. Pettit, A. K. Das Gupta and U. R. Ghatak, *Steroids* **1**, 137 (1963), for the preceding contribution.

[2] A summary of this study was presented (August 27, 1962) at *The Second International Symposium on the Chemistry of Natural Products*, Prague, Czechoslovakia.

[3] This investigation was supported by PHS Research Grants CY-4074, CY-4074(Cl-C3), and CA 04074-05 from the National Cancer Institute, Public Health Service.

[4] For example, see: R. B. Clayton and K. Bloch, *J. Biol. Chem.* **218**, 319 (1956) and F. Gautschi and K. Bloch, *Ibid.* **233**, 1343 (1958).

[5] Excellent discussions of cholesterol biosynthesis have been presented by: A. Wettstein, *Experientia* **17**, 329 (1961); W. Cornforth, R. H. Cornforth, A. Pelter, M. G. Horning and G. Popjak, *Tetrahedron* **5**, 311 (1959); P. Crabbe, *Rec. Chem. Progr.* **20**, 189 (1959); and J. W. Cornforth, *J. Lipid Res.* **1**, 3 (1959).

[6] Refer to A. Wettstein, ref. 5 for a valuable review of this subject and leading literature citations.

[7] The physiological activity displayed by 11-oxo-14α-methyl-progesterone, *a*, W. Voser, H. Heusser, O. Jeger and L. Ruzicka, *Helv. Chim. Acta* **36**, 299 (1953), and several related biogenetic considerations inspired a study leading to synthesis of 3β-hydroxy-20-oxo-4,4,14α-trimethyl-5α-pregnane (Xa) by *b*, C. S. Barnes, *Austr. J. Chem.* **9**, 228 (1956). Both 14α-methyl steroids were obtained by degradation of lanosterol.

Although the unique synthesis of lanosterol[8] from cholesterol provides, in principle, an avenue to 14α-methyl steroids, it appeared desirable to explore further the lanosterol approach[7,9] for this purpose, since this substance is potentially one of the most abundant steroid precursors.[10] Furthermore, degradation of lanosterol would allow ready access, from common intermediates, to both androstane and pregnane derivatives of known stereochemistry at the C/D ring juncture. In order to evaluate the utility of this route, conversion of lanosterol to 4,4,14α-trimethyl steroids structurally related to the androgenic hormones was undertaken. The first objective was synthesis of 3-oxo-17β-hydroxy-4,4,14α-trimethyl-5α-androstane.

Conversion of lanosterol to 3β-hydroxy-20-oxo-4,4,14α-trimethyl-5α-pregnane[7b] appeared to offer the most useful approach to the androstane derivatives. For this reason, isocholesterol (crude lanosterol) was degraded to methyl 3β-acetoxy-7,11-dioxo-4,4,14α-trimethyl-5α-cholanate (IVa) essentially as outlined by Voser *et al.*[7a]

Several attempts to apply the vigorous Wolff-Kishner reduction of Barton *et al.*[11] to diketone IVa as previously described[7b] gave only traces of saturated methyl ester Va[12] Consequently, it appeared more promising to use an alternate procedure[7b] involving initial preparation of diphenyl olefin VIa followed by Wolff-Kishner reduction. In our hands, reaction between diketo methyl ester IVa and the Grignard reagent prepared from bromobenzene and magnesium in ether-benzene solution, followed by reacetylation, did not give satisfactory results. Several products were usually obtained from this reaction sequence.

Eventually, a dependable Grignard procedure employing tetrahydrofuran as solvent was found for selective conversion of diketone IVa to tertiary alcohol VIIa. The product (VIIa) was readily dehydrated by hydrochloric acid (6%) in methanol to yield (60% from IVa) styrene VIb. Wolff-Kishner[11] reduction of ketone VIb was routinely used to prepare alcohol VIIIa (ca. 90% yields).

While the above study was in progress a new synthesis of olefin VIIIa was developed. Reaction between diketone IVa and ethanedithiol, in boron trifluoride etherate solution, or in chloroform solution saturated with hydrogen chloride,[13] was used to prepare methyl 3β-acetoxy-11-oxo-4,4,14α-trimethyl-5α-cholanate 7-ethylenethioketal (Vb). A solution of the thioketal (Vb) in tetrahydrofuran was treated with phenylmagnesium bromide in the same solvent. Acetylating the crude Grignard product afforded tertiary alcohol VIIb. A prolonged (24-hr) period in refluxing acetic anhydride smoothly eliminated the 24-alcohol group to yield olefin VIc. A number of

[8] R. B. Woodward, A. A. Patchett, D. H. R. Barton, D. A. J. Ives and R. B. Kelley, *J. Chem. Soc.* 1131 (1957).

[9] After the present study was initiated in 1957, conversion of several other tetracyclic triterpenes to 4,4,14α-trimethylpregnane and 8β,14α-dimethyl-18-norandrostane derivatives was reported. See: W. O. Godtfredsen and S. Vangedal, *Tetrahedron* **18**, 1029 (1962); D. Rosenthal, J. Fried, P. Grabowich and E. F. Sabo, *J. Amer. Chem. Soc.* **84**, 877 (1962); E. Graf and H. J. Winckelmann, *Arch. Pharm.* **294**, 410 (1961); A. Melera, M. Gut and R. Noller, *Tetrahedron Letters*, 13 (1960); and P. Crabbé, G. Ourisson and T. Takahashi, *Tetrahedron* **3**, 279 (1958).

[10] Refer to E. V. Truter, *Wool Wax Chemistry and Technology* p. 319. Cleaver-Hume, London (1956).

[11] D. H. R. Barton, D. A. J. Ives and B. R. Thomas, *J. Chem. Soc.* 2056 (1955). See also, C. Djerassi and G. H. Thomas, *J. Amer. Chem. Soc.* **79**, 3835 (1957).

[12] Apparently the methyl ester side chain of IVa impedes the reactions necessary for complete reduction. Dr. C. S. Barnes has informed us, in a private communication, that poor yields of Va were also obtained in his laboratory.

[13] W. Voser, O. Jeger and L. Ruzicka, *Helv. Chim. Acta* **35**, 503 (1952).

other dehydration procedures (e.g., sodium hydrogen sulfate-acetic anhydride and thionyl chloride-pyridine) gave less pure specimens of the olefin (VIc). Vigorous Wolff-Kishner reduction of the dehydration product (VIc) followed by acetylation yielded olefin VIIIb.[14] Similarly, Wolff-Kishner reduction of ketone VIIb gave diol VIIc. Following monoacetylation the product (VIId) was dehydrated to olefin VIIIa using thionyl chloride in pyridine solution.

In order to verify further the structure of olefin VIIIb the ethylenethioketal derivative (Vb) of methyl ester IVa was successively: desulfurized with Raney nickel, allowed to react with phenylmagnesium bromide, dehydrated, and finally subjected to Wolff-Kishner reduction. The overall process is represented by intermediates Vc, VId and VIIIa. After acetylation, the product was identical with the diphenyl olefin (VIIIb) prepared directly from ethylenethioketal VIc.

Preparation of olefin VIIIb via ethylenethioketal VIc was discontinued when the more efficient procedure (*vide supra*) employing diketone VIb became available. A study of experimental conditions for the remaining Barbier-Wieland sequence led to selection of the following methods. Olefin VIIIa was acetylated, subjected to allylic bromination with N-bromosuccinimide, and the product was dehydrohalogenated using dimethylaniline. The resulting diene (IX) generally contained small amounts (10–20%) of styrene VIIIb. Chromium trioxide oxidation of the monoene-diene mixture, followed by saponification, yielded 20-ketone Xa (42% from monoene VIIIb). After the oxidation step, norcholanic acid XIa (9% yield based on olefin VIIIb) was isolated. Ozonolysis of diene IX in methylene chloride-pyridine solution provided acceptable yields of ketone Xb. However, the chromium trioxide technique was preferred when preparing large quantities of ketone Xa.

Trifluoroperoxyacetic acid oxidation[15] of 20-ketone Xa led to 3β-hydroxy-17β-acetoxy-4,4,14α-trimethyl-5α-androstane (XIIa) accompanied by a small amount of the corresponding diol (XIIb). Treating alcohol XIIa with an 8N chromium trioxide reagent[16] gave ketone XIIIa which was saponified to provide 3-oxo-17β-hydroxy-4,4,14α-trimethyl-5α-androstane (XIIIb).

The eleven-step reaction sequence developed for degrading lanosterol to 3β-hydroxy-17β-acetoxy-4,4,14α-trimethyl-5α-androstane illustrates the potential value of this route to 14α-methyl steriods.

EXPERIMENTAL

M.p.'s reported for analytical specimens were observed employing a Kofler m.p. apparatus and are uncorrected. All other m.p.'s were determined using a Fisher-Johns apparatus. Thin layer chromatograms were prepared on silica gel G and developed with conc sulfuric acid. Pet ether refers to petroleum ether boiling at 40–60°.

U.V. (ethanol solutions) and I.R. spectra were recorded by Dr. R. A. Hill of this laboratory. The microanalyses were provided by Dr. A. Bernhardt, Max-Planck Institut, Mulheim, Germany, and the optical rotation (chloroform solution) measurements by Drs. Weiler and Strauss, Oxford, England.

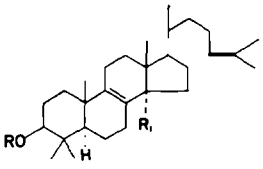
[14] The hydrazine-potassium hydroxide desulfurization method reported by V. Georgian, R. Harrison and N. Gubisch, *J. Amer. Chem. Soc.* **81**, 5834 (1959) involves reaction conditions reminiscent of the Wolff-Kishner reduction. Thus, it seemed reasonable to predict concomittant reduction and desulfurization and in the one example (VIc) investigated this was indeed the case.

[15] Cf., G. R. Pettit and T. R. Kasturi, *J. Org. Chem.* **26**, 4557 (1961).

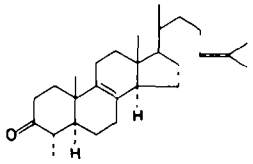
[16] K. Bowden, I. M. Heilbron, E. R. H. Jones and B. C. L. Weedon, *J. Chem. Soc.* 39 (1946).

Methyl 3β-acetoxy-7,11-dioxo-4,4,14α-trimethyl-5α-cholanate (IVa)[17]

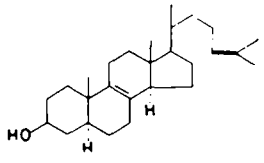
The following degradative procedure was found, after approximately 200 experiments, to be a dependable route to diketone IVa, and is based on the earlier studies of Voser *et al.*[7a]



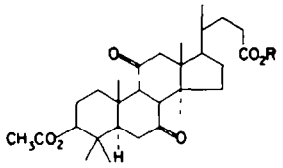
Ia, R = H, R = CH_3
b, R = R_1 = H



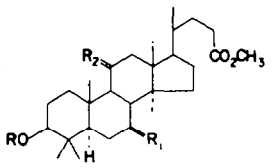
II



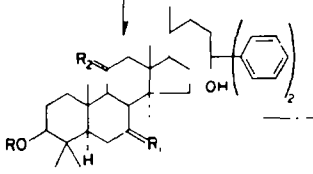
III



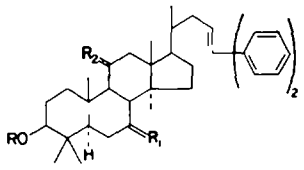
IVa, R = CH_3
b, R = H



Va, R = H, R_1 = R_2 = H_2
b, R = $COCH_3$, R_1 = ethylene dithioketal, R_2 = O
c, R = $COCH_3$, R_1 = H_2, R_2 = O



VIIa, R = H, R_1 = R_2 = O
b, R = $COCH_3$, R_1 = ethylene dithioketal, R_2 = O
c, R = H, R_1 = R_2 = H_2
d, R = $COCH_3$, R_1 = R_2 = H_2



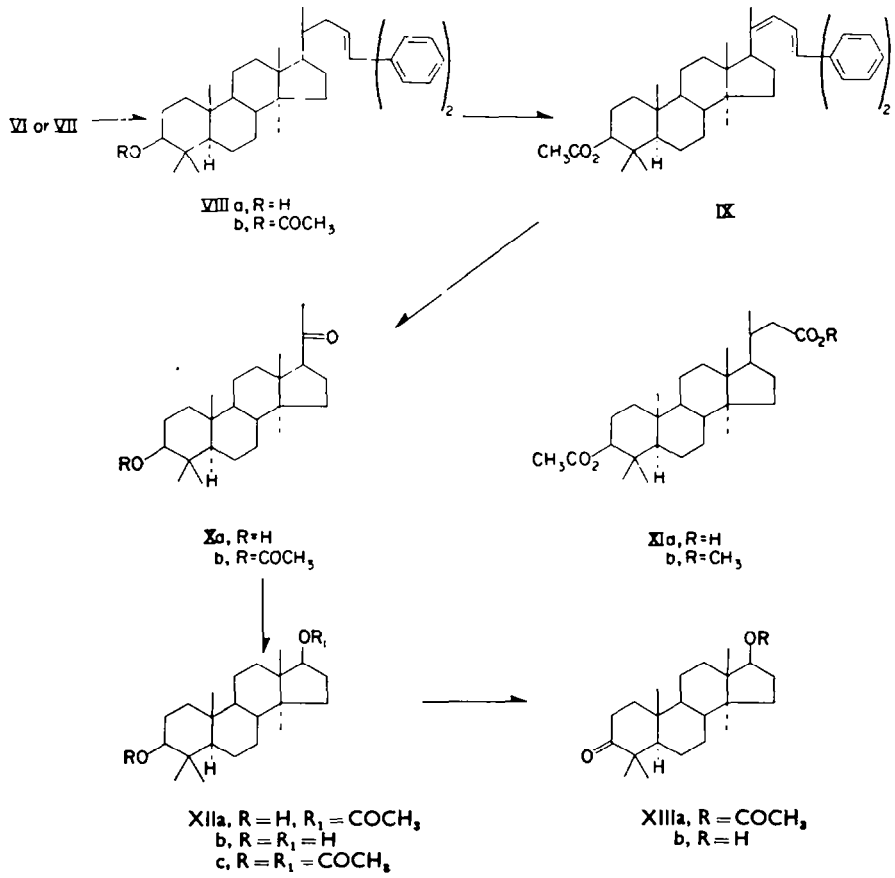
VIa, R = $COCH_3$, R_1 = R_2 = O
b, R = H, R_1 = R_2 = O
c, R = $COCH_3$, R_1 = ethylene dithioketal, R_2 = O
d, R = $COCH_3$, R_1 = H_2, R_2 = O

In a typical experiment, a solution of isocholesterol (1 kg)[18] and acetic anhydride (1·7 l.) was heated at reflux for 3 hr. The solid product, which separated on cooling, was collected and washed with cold methanol; yield, 960 g. Without further purification, the isocholesterol acetate (350 g) was dissolved in methylene chloride (350 ml)–glacial acetic acid (500 ml) and treated with a solution composed of chromium trioxide (400 g), water (330 ml) and glacial acetic acid (3·3 l.). The oxidizing agent was added over a *2-hr period while maintaining the reaction mixture at* 45–50°. The solution was quickly cooled to 40°, treated with methanol (350 ml), concentrated (steam bath) *in vacuo* to ca. 2·5 l. and then poured into 10 l. cold water. After standing at room temp for about 24 hr the solution was filtered and the green precipitate was washed with water (2 l.). The dry solid was next extracted with ethyl ether (5 × 1 l., at reflux temp). Residual solid (100–130 g) was discarded and the ether solution was extracted with 10 N KOH solution (300 ml followed by 2 × 100 ml). The interfacial solid and the aqueous phase were combined, treated with conc hydrochloric acid (400 ml) and extracted with ether (3 × 3 l.). Removal of solvent from the dry (sodium sulfate) ethereal extract gave 100–110 g of crude *3β-acetoxy-7,11-dioxo-4,4,14α-trimethyl-5α-Δ$^{8(9)}$-cholenic acid.*

[17] We wish to acknowledge the technical assistance provided by P. A. Whitehouse, J. A. Douville and M. A. Vickers during preparation of this substance.

[18] Provided by the Hugh J. Meeter Co., Grand Rapids 2, Mich. and D. S. Van Schuppen N.V., the Netherlands.

A solution of the cholenic acid (100–110 g) in methylene chloride (300 ml) was cooled (ice bath) and treated with an ethereal solution of diazomethane (from 100 g of nitrosomethylurea and 120 ml of 50% aqueous potassium hydroxide). After 10 min, excess diazomethane was removed with acetic acid and the solution was evaporated to dryness. A solution of the solid residue in 1:1 pet ether–benzene (500 ml) was chromatographed on activated alumina[18] (600 g). Elution with the same solvent



(4 l.) followed by benzene (2 l.) provided 75–80 g of yellow *methyl 3β-acetoxy-7,11-dioxo-4,4,14α-trimethyl-5α-Δ⁸⁽⁹⁾-cholenate.*

To a solution of the methyl ester (75–80 g) in refluxing glacial acetic acid (500 ml) was added (over 30 min) 80 g of zinc dust. The hot solution was poured into cold water (4 l.) and precipitated solid was collected. A chloroform solution of the solid was washed with water, aqueous sodium bicarbonate and again with water. Following removal of dry (sodium sulfate) solvent the residue was recrystallized from benzene–pet ether to yield 40–45 g, m.p. 202–205°, of colourless *methyl ester* IVa.

The corresponding carboxylic acid derivative (IVb) was prepared[20] as follows. A solution composed of methyl ester IVa (1·0 g), dry lithium iodide and redistilled (from potassium hydroxide) 2,4,6-trimethylpyridine (40 ml) was heated at reflux 8 hr. After cooling, the mixture was filtered; diluted with 2:1 chloroform–ethyl ether; washed with water and 2 N HCl; and concentrated to a crystalline solid. Recrystallization from methanol gave colorless needle clusters (0·60 g), m.p. 220–228°.

[18] Aluminum Co. of America's, Grade F-20 (80–200 mesh).

[20] Cf., F. Elsinger, J. Schreiber and A. Eschenmoser, *Helv. Chim. Acta* **43**, 113 (1960).

Another recrystallization from methanol led to a specimen (0·35 g) of 3β-*acetoxy*-7,11-*dioxo*-4,4,14α-*trimethyl*-5α-*cholanic acid* m.p. 222–228° (lit,[21] m.p. 223–230°).

Methylation of the acid (IVb, 0·15 g) in 1:1 chloroform–methanol with ethereal diazomethane and crystallization of the product from chloroform–methanol gave 0·14 g acetoxy methyl ester IVa, m.p. 200–202°. A mixture of the product and starting material (IVa) melted at 200–202°.

3β,24-*Dihydroxy*-7,11-*dioxo*-24,24-*diphenyl*-4,4,14α-*trimethyl*-5α-*cholane* (VIIa)

Bromobenzene (25 ml) was added to a mixture of magnesium (38 g) and dry tetrahydrofuran (1 l.) in a 5 l. 3-neck flask equipped with condenser, stirrer and dropping funnel. The reaction was initiated by warming and the remaining bromobenzene (125 ml) was added dropwise during 2·5 hr. A solution of diketone IVa (100 g) in tetrahydrofuran (1·5 l.) was slowly added (over 30–45 min) to the stirred Grignard reagent. Following a 2-hr reflux peroid the mixture was cooled and diluted with saturated ammonium chloride solution (300 ml). The organic phase was decanted and concentrated to dryness. A chloroform extract of the aqueous portion was combined with a chloroform solution of the tetrahydrofuran residue, and then washed with water and concentrated to dryness. Crystallization of the residue from acetone–ethyl ether gave 42·5 g (37%) of colourless solid, m.p. 228–232°. Recrystallization from chloroform–methanol led to a pure specimen; needles, m.p. 235–237°, $[\alpha]_D^{30}$ −42·5° (*c*, 1·34), ν_{max}^{KBr} 3500, 3400 and 1705 cm^{-1} (Found: C, 79·74; H, 8·86; O, 11·60. $C_{58}H_{52}O_4$ requires: C, 80·09; H, 8·96; O, 10·94%).

3β-*Hydroxy*-7,11-*dioxo*-24,24-*diphenyl*-5α-23-*cholene* (VIb)

Method A. A solution of tertiary alcohol VIIa (1·0 g) in methanol (50 ml)–conc. hydrochloric acid (3 ml) was heated at reflux during 1 hr. The crystalline olefin (VIb) began to separate during the first few min. After cooling, the product (0·78 g, 80·5%) was collected and washed with methanol–water (1:1). One recrystallization from chloroform–methanol gave colorless leaflets m.p. at 272–274° (lit,[7b] m.p. 275–277°). The purity of the specimen was confirmed by thin layer chromatography (7:3 hexane–ethyl acetate mobile phase).

Following recrystallization from chloroform–methanol the *acetate derivative* (VIa) melted at 221·5–222·5° (lit,[7b] m.p. 221–222°), $[\alpha]_D^{30}$ +67·6° (*c*, 2·41).

Method B. The following procedure was routinely used for preparing olefin VIb. The crude Grignard product from 100 g of methyl ester IVa (cf. VIIa) was dissolved in methanol (940 ml)–conc hydrochloric acid (60 ml) and dehydrated as described (with stirring) in method A. Following recrystallization from chloroform–acetone the product weighed 68 g (60%), m.p. 261–266°, and did not require further purification.

3β-*Hydroxy*-24,24-*diphenyl*-4,4,14α-*trimethyl*-5α-23-*cholene* (VIIIa)

In a typical experiment (employing a 5 l. 3-neck flask), a solution composed of redistilled diethylene glycol (1·5 l.) olefin VIb (115·5 g) and redistilled (from potassium hydroxide) anhydrous hydrazine (150 ml) was heated at reflux for 2 hr. After adding the deep straw-colored alkoxide solution prepared from sodium (64 g) and redistilled diethylene glycol (1 l.) the mixture was stirred and maintained at 170–185° for 24 hr. Volatile components were removed by distillation until the reaction mixture reached 215–220°. Before cooling the solution to room temp, heating at 215–220° was continued 24 hr. The solid (107 g) which separated upon adding the mixture to 4 l. ice–water was collected, washed with water and dried. Recrystallization from chloroform–acetone gave 100 g (91%), m.p. 244–247°, of colorless crystals. Another recrystallization from the same solvent led to needles m.p. 245–247° (lit,[7b] m.p. 236–238°).

Methyl 3β-*acetoxy*-11-*oxo*-4,4,14α-*trimethyl*-5α-*cholanate* 7-*ethylenethioketal* (Vb)

The 7,11-diketone (IVa, 10 g) in a solution composed of ethanedithiol (5 ml) and boron trifluoride etherate (200 ml) was allowed to remain at room temp for 2 hr before dilution with benzene. The benzene solution was repeatedly washed with N NaOH, followed by water, and dried (sodium sulfate). Removal of solvent *in vacuo* gave a pale cream-coloured solid (7·5 g) which recrystallized from chloroform–methanol as needles, m.p. 205–207°. Mixture m.p. determination with the product and a sample prepared according to the hydrogen chloride catalyzed procedure,[18] was undepressed.

[21] W. Voser, O. Jeger and L. Ruzicka, *Helv. Chim. Acta* **35**, 497 (1952).

Larger quantities of ethylenethioketal Vb were prepared using the hydrogen chloride technique.[18] Interestingly, a chloroform solution of the ethylenethioketal gave a deep straw color with tetranitromethane.

3β-Acetoxy-11-oxo-24-hydroxy-24,24-diphenyl-4,4,14α-trimethyl-5α-cholane 7-ethylenethioketal (VIIb)

A solution of monothioketal Vb (1·0 g) in dry tetrahydrofuran (20 ml) was added to the Grignard reagent from magnesium (0·55 g) and bromobenzene (3 ml) in 20 ml dry tetrahydrofuran. The reaction mixture was heated at reflux 2 hr (cf. VIIa) before adding ice and ammonium chloride solution. After extracting the aqueous mixture with chloroform the combined extract was concentrated to a viscous yellow oil. A solution of the crude product in pyridine (25 ml)–acetic anhydride (5 ml) was kept at room temp for 11 hr. Following removal of solvents, *in vacuo*, a chloroform solution of the residue was successively washed with 10% hydrochloric acid, dilute sodium bicarbonate solution and water. Removal (*in vacuo*) of dry (magnesium sulfate) solvent afforded 1·44 g yellow solid; m.p. 255–262° with sintering from 245°. The crude acetate dissolved in pet ether–benzene was chromatographed on 30 g of activated alumina.[19] Fractions eluted by benzene followed by benzene–chloroform (24:1) melted at 269–276° (0·33 g) and 272–279° (0·77 g) respectively. Five recrystallizations of the latter fraction from benzene–pet ether led to colorless crystals of *tertiary alcohol VIIb* m.p. 279–281°, $[\alpha]_D^{20}$ +34·8° (*c*, 0·258). (Found: C, 73·83; H, 8·16; S, 8·66. $C_{42}H_{58}O_4S_2$ requires: C, 73·44; H, 8·31; S, 9·12%).

3β-Acetoxy-11-oxo-24,24-diphenyl-4,4,14α-trimethyl-5α-23-cholene 7-ethylene-thioketal (VIc)

Method A. An acetic anhydride (5 ml) solution of teriary alcohol VIIb (0·19 g) was heated at reflux for 24 hr and then allowed to cool. The crystalline material (0·14 g, m.p. 235–250°) which separated was collected and washed with methanol. An analytical specimen was obtained by passing a benzene solution of the crude product through activated alumina[19] and recrystallizing eluted product from benzene–methanol. The pure colourless needles of olefin VIc melted at 257–259°, $[\alpha]_D^{23}$ +38·5° (*c*, 0·80). (Found: C, 75·74; H, 8·33; S, 9·05. $C_{42}H_{56}O_3S_2$ requires: C, 75·40; H, 8·24; S, 9·36%).

Method B. The crude Grignard product (cf. VIIb) from methyl ester Vb (29 g) was dissolved in acetic anhydride (250 ml). After adding fused sodium hydrogen sulfate (36 g) the mixture was heated (steam bath) for 1·5 hr. The dark red reaction mixture was diluted with water (3 l.) and the resulting solid phase was collected, dissolved in chloroform and washed with water. Following drying (calcium chloride) and removal of solvent the residue was dissolved in benzene and chromatographed on activated alumina (500 g).[22] Elution with the same solvent gave 19·3 g viscous oil which solidified on drying *in vacuo*. Further purification of this material by column chromatography on activated alumina or by recrystallization was unrewarding. However, Wolff-Kishner reduction (see VIIIa) of the crude solid (5 g) followed by acetylation and recrystallization led to a specimen (1·5 g) of olefin VIIIb m.p. 237–239° (lit,[7b] m.p. 235–237° and 243–246°).

3β,24-Dihydroxy-24,24-diphenyl-4,4,14α-trimethyl-5α-cholane (VIIc)

A 4·5-g specimen of 3β-acetoxy-11-oxo-24-hydroxy-24,24-diphenyl-4,4,14α-trimethyl-5α-cholane 7-ethylenethioketal (VIIb) was subjected to Wolff-Kishner reduction (as described above, see VIIIa) using anhydrous hydrazine (5·5 ml), diethylene glycol (40 ml) and the alkoxide from sodium (2·8 g) in diethylene glycol (35 ml). A pet ether–benzene (1:2) solution of the crude product was chromatographed on activated alumina.[19] Following crystallization from chloroform–acetone the fraction eluted with 3:2 benzene–chloroform weighed 1·59 g, m.p. 230–231°. Recrystallization from chloroform–methanol led to pure colorless needles of tertiary alcohol VIIc; m.p. 235–237°, $[\alpha]_D^{20}$ +24·8 (*c*, 2·41). (Found: C, 83·80; H, 10·13. $C_{39}H_{56}O_2$ requires: C, 84·12; H, 10·14).

3β-Acetoxy-24 24-diphenyl-4,4,14α-trimethyl-5α-23-cholene (VIIIb)

(a) *From 3β,24-dihydroxy-24,24-diphenyl-4,4,14α-trimethyl-5α-cholane* (VIIc). Monoacetylation (acetic anhydride-pyridine, 24 hr at 40°) of diol VIIc led to *3β-acetoxy-24-hydroxy-24,24-diphenyl-4,4,14α-trimethyl-5α-cholane* (VIId). A pure specimen recrystallized from chloroform–acetone as colorless leaflets: m.p. 234–236°, $[\alpha]_D^{20}$ +25·6° (*c*, 1·30) (Found: C, 81·97; H, 9·77; O, 8·44. $C_{41}H_{58}O_3$ requires: C, 82·22; H, 9·76; O, 8·02).

[22] Merck aluminum oxide, "suitable for chromatography".

A cold (ice bath) pyridine (20 ml) solution of tertiary alcohol VIId (1·5) g was treated with thionyl chloride (2 ml). After 4 hr of continued cooling the reaction mixture was diluted with ice and 2 N H_2SO_4. The crude olefin (VIIIb) was extracted with chloroform. Following removal of solvent the product was recrystallized from chloroform–acetone to afford 1·14 g colorless needles, m.p. 242–244° (lit,[7b] m.p. 235–237° and 243–246°).

(b) *From* 3*β-hydroxy*-24,24-*diphenyl*-4,4,14*α-trimethyl*-5*α*-23-*cholene* (VIIIa). Acetylation of alcohol VIIIa (100 g) was routinely accomplished employing a 16-hr period in refluxing acetic anhydride (2 l.). After cooling, the crystalline product was collected and washed with acetone; yield 94·8 g (87·5%), m.p. 235–239°. Recrystallization from chloroform–acetone led to colorless prismatic needles, m.p. 244–245°, λ_{max} 254 mμ (log ϵ = 4·19).

(c) *From* 3*β-acetoxy*-11-*oxo*-24,24-*diphenyl*-4,4,14*α-trimethyl*-5*α*-23-*cholene* 7-*ethylenethioketal* (VIc). A 5·5-g sample of ethylenethioketal VIc was subjected to Wolff-Kishner reduction essentially as described for preparation of diphenyl olefin VIIIa. Following the 24-hr period at 220°, the solution and a small quantity of precipitated solid was poured into water and the pale gray-colored solid was collected by filtration. A solution of the crude product was heated at reflux during 3 hr in acetic anhydride (150 ml). On cooling, the crystalline acetate separated (3·0 g) and recrystallized from chloroform–pet ether as colorless needles, m.p. 247–248°.

The olefin (VIIIa) was identical (mixture m.p. and I.R. spectral comparison) with the product of procedure d.

(d) *From* 3*β-acetoxy*-11-*oxo*-24,24-*diphenyl*-4,4,14*α-trimethyl*-5*α*-23-*cholene* (Vc). A solution of 7-monothioketal Vb (1 g) in ethanol (50 ml) containing W-4 Raney nickel[23] (10 ml) was heated at reflux during 15 hr. The mixture was next poured into 150 ml of 18% hydrochloric acid and extracted with ether. The dry (sodium sulfate) ethereal extract was concentrated to a colorless solid which recrystallized from methylene chloride as needles (0·6 g) to yield a pure sample of *methyl* 3*β-acetoxy*-11-*oxo*-4,4,14*α-trimethyl*-5*α-cholanate* (Vc) m.p. 176–177° (Voser[13] reports m.p. 176–177°).

The desulfurized 11-ketone Vc (0·35 g) in 7 ml dry tetrahydrofuran was added to the Grignard reagent from bromobenzene (4 ml) and magnesium (0·3 g) in 6 ml of the same solvent. Conversion to 3*β-acetoxy*-11-*oxo*-24,24-*diphenyl*-4,4,14*α-trimethyl*-5*α*-23-*cholene* (VId) was accomplished as described for preparation of diphenyl 7-ethylenethioketal VIc. The crude pale yellow crystalline solid recrystallized from methylene chloride–methanol as colorless crystals, m.p. 183–185°. A second recrystallization from the same solvent gave 0·15 g m.p. 191–192° (lit,[13] m.p. 190–191°).

The diphenyl 11-ketone (VId, 0·10 g) in diethylene glycol (4·5 ml) was reduced employing anhydrous hydrazine (0·7 ml total) and the alkoxide from sodium (0·15 g) and 3·5 ml diethylene glycol as described for Wolff-Kishner reduction of VIb. Following acetylation, the crude acetate (VIIIb) recrystallized from chloroform–pet ether as colorless needles (0·03 g) m.p. at 247–248°.

3*β-Acetoxy*-24,24-*diphenyl*-4,4,14*α-trimethyl*-5*α*-$\Delta^{20(22),23}$ *cholene* (IX)

A mixture composed of carbon tetrachloride (2 l.), olefin VIIIb (94·8 g) and N-bromosuccinimide (38·0 g) was heated at reflux and irradiated[24] during 14 min. After cooling, the solution was filtered and diluted with N,N-dimethyl aniline (160 ml). The carbon tetrachloride was then removed by distillation (Glas-Col heating mantle) through a Claisen flask. A chloroform solution of the residue was washed successively with 2 N H_2SO_4 and water. Following removal of solvent the crude product was dissolved in benzene and chromatographed on activated alumina (3 kg).[19] The fraction eluted with benzene (5 l.) was crystallized from chloroform–acetone; yield 69·8 g, m.p. 230–233°. A thin layer chromatogram (19:1 hexane–ethyl acetate mobile phase) of this material indicated that the diene (IX) was accompanied by a small quantity (ca. 10–20% on the basis of an U.V. special study) of starting olefin (VIIIb). However, the mixture was satisfactory for use in the oxidation step (cf. Xb).

Fractional recrystallization of the chloroform–acetone mother liquor residue using the same solvent mixture led to a pure (as evidenced by thin layer chromatography) specimen of diene IX: colorless needles, m.p. 236–238° (lit,[7b] m.p. 237–238°), λ_{max} 307 mμ (log ϵ = 4·42).

3*β-Acetoxy*-20-*oxo*-4,4,14*α-trimethyl*-5*α-pregane* (Xb)

Method A. The following procedure was employed for routine preparation of alcohol Xa. A sample of diene IX (69·8 g) containing 10–20% monoene (VIIIb) was dissolved in warm chloroform

[23] A. A. Pavlic and H. Adkins, *J. Amer. Chem. Soc.* **68**, 1471 (1946).

[24] General Electric 50–60 cycle, 110-125 V Sunlamp.

(700 ml). Following addition of glacial acetic acid (1·4 l.) the solution was maintained at 45° while slowly (1 hr) adding a solution of chromium trioxide (70 g) in water (340 ml)–acetic acid (680 ml) Stirring and warming (45°) were continued an additional 1·5 hr. The remaining oxidizing agent was reduced over a 30-min period with methanol (40 ml). Dilution with water gave a chloroform phase which was separated and combined with a chloroform extract of the aqueous layer. The combined extract was concentrated to ca. 1·5 l. and, successively, washed with 2 N Na_2CO_3 and water, dried (sodium sulfate) and concentrated to dryness. The residue was dissolved in methanol (2 l.) containing potassium hydroxide (100 g). After heating 1 hr at reflux the mixture was poured into water (2 l.), cooled and filtered. A chloroform solution of the solid product was washed with water, dried (sodium sulfate), concentrated to approximately 300 ml, diluted with benzene (1·2 l.), and chromatographed on activated alumina (1·8 kg).[19] The fraction eluted with chloroform was crystallized from chloroform–acetone to yield 24·8 g (42% from monoene VIIIa) m.p. 252–258°. Recrystallization from the same solvent mixture gave *3β-hydroxy-20-oxo-4,4,14α-trimethyl-5α-pregnane* (Xa) as colorless prisms, m.p. 259–262° (lit,[7b] m.p. 256–258°).

The *acetate derivative* (Xb prepared using acetic anhydride–pyridine) crystallized from ethyl ether–methanol as colorless crystals, m.p. 212–214° (lit,[7b] m.p. 210–211°). Acetate Xb was identical (mixture m.p. determination and I.R. spectral comparison) with a specimen (Xb, m.p. 207–209°) prepared by Dr. Barnes.[25]

Method B. The diene (IX, 0·42 g) was dissolved in methylene chloride (15 ml)–pyridine (5 ml), cooled to −25°, and treated with ozone until absorption appeared complete (ca. 30 min). After warming to room temp the solution was diluted with glacial acetic acid (6 ml) and treated (1 hr) with 1 g powdered zinc. Excess zinc was removed by filtration before washing with N HCl and water. Evaporation of the dry (sodium sulfate) solvent gave a light brown oil (0·35 g) which was chromatographed in 1:2 pet ether–benzene on activated alumina.[22] Elution with the same solvent followed by benzene afforded 0·143 g colorless crystals m.p. 209–210°. Mixture m.p. and I.R. spectral comparison with the sample[25] of acetate Xb, noted above, confirmed the identity of this product.

3β-Acetoxy-4,4,14α-trimethyl-5α-24-norcholanic acid (XIa)

The combined 2 N Na_2CO_3 wash liquor obtained during purification of 20-ketone Xa (see method A) was acidified with 2 N H_2SO_4 and extracted with chloroform. Removal of solvent from the chloroform extract gave 6·5 g crude acid (XIa). Recrystallization from chloroform–methanol afforded colorless needles (3·0 g) m.p. 257–259°. One additional recrystallization from this solvent led to a pure specimen; m.p. 258–259°, $[\alpha]_D^{20}$ −37·9° (*c*, 0·82) (Found: C, 74·86; H, 10·21. $C_{28}H_{46}O_4$ requires: C, 75·29; H, 10·38).

The chloroform–methanol mother liquors were treated with diazomethane and concentrated to dryness. A benzene solution of the crude ester was chromatographed on activated alumina.[19] The fraction (1·92 g) eluted with benzene crystallized from chloroform–methanol as colorless leaflets (1·26 g); m.p. 201–202°. Recrystallization from the same solvent raised the m.p. to 204–206°; $[\alpha]_D^{20}$ +35·8° (*c*, 2·65), ν_{max}^{KBr} 1732 and 1723 cm^{-1} (Found: C, 75·97; H, 10·46; O, 13·68. $C_{29}H_{48}O_4$ requires: C, 75·60; H, 10·50; O, 13·89%).

3β-Hydroxy-17β-acetoxy-4,4,14α-trimethyl-5α-androstane (XIIa)

Disodium hydrogen phosphate (8·5 g) was added to a stirred solution (at 20°) of alcohol Xa (4·3 g) in chloroform (260 ml) followed by a solution (10 ml) of trifluoroperoxyacetic acid. The peracid reagent[15] was prepared from trifluoroacetic anhydride (12 ml), 90% hydrogen peroxide (1·6 ml) and methylene chloride (10 ml). Following a 3-hr period at reflux, the reaction mixture was successively cooled, filtered, washed with water, dried (sodium sulfate) and concentrated to dryness. A solution of the residue in benzene was chromatographed on activated alumina (150 g). The fractions eluted with 3:2 benzene–chloroform and chloroform were combined and recrystallized from acetone to yield 2·1 g colorless prismatic needles (XIIa); m.p. 214–216°, $[\alpha]_D^{20}$ +8·1° (*c*, 1·12), ν_{max}^{KBr} 3450 and 1712 cm^{-1} (Found: C, 76·53; H, 10·44. $C_{34}H_{40}O_3$ requires: C, 76·55; H, 10·71).

Chromatograph fractions (0·77 g) obtained by continued elution with chloroform and ethyl acetate crystallized from chloroform–acetone as colorless needles; weight 0·60 g, m.p. 236–237°. Recrystallization from the same solvent gave a specimen of *3β,17β-dihydroxy-4,4,14α-trimethyl-5α-androstane*

[25] We are grateful to Dr. C. S. Barnes for providing this sample.

(XIIb); m.p. 241–242° (partial melting at 236–237°), $[\alpha]_D^{20}$ +41·5 (*c*, 0·179), ν_{max}^{KBr} 3300 and 3350 cm^{-1} (Found: C, 75·41 to 76·34; H, 10·94 to 11·12; O, 9·89 to 10·11. $C_{32}H_{58}O_2$ requires: C, 87·98; H, 11·45; O, 9·57%). Drying the analytical sample at 100° for 72 hr (0·1 mm) did not significantly alter the microanalytical data. The unsatisfactory carbon and hydrogen analyses obtained for diol XIIb suggested incomplete combustion. However, acetylation (acetic anhydridepyridine, 24 hr at 40°) led to *3β,17β-diacetoxy-4,4,14α-trimethyl-5α-androstane*. A pure sample of the diacetate (XIId) recrystallized from acetone as colorless plates; m.p. 208–210°, $[\alpha]_D^{20}$ +36° (*c*, 0·73). (Found: C, 74·43; H, 9·90; O, 15·64. $C_{36}H_{62}O_4$ requires C, 74·60; H, 10·11; O, 15·29%).

Saponifying (5% potassium hydroxide in methanol, 24 hr at 20°) acetate XIIa led to a sample of diol XIIb m.p. 244–245°. A mixture composed of both diol samples melted at 241–245° (sintering at 236–237°).

3-Oxo-17β-acetoxy-4,4,14α-trimethyl-5α-androstane (XIIIa)

A solution of alcohol XIIa (0·80 g) in acetone (50 ml) at 0° was treated with an 8 N chromium trioxide reagent (2·6 ml).[16] After 5 min the mixture was diluted with aqueous sodium acetate solution, concentrated to ca. 50 ml *in vacuo*, and extracted with chloroform. Following removal of solvent, the chloroform residue (0·82 g) was crystallized from ethyl ether. The resulting colorless plates weighed 0·37 g m.p. 163–165°. A thin layer chromatogram (4:1 hexane–ethyl acetate mobile phase) of this material showed, in addition to ketone XIIIa, a faint spot corresponding to alcohol XIIb. Chromatographing the product (0·37 g) on activated alumina (11 g)[19] and recrystallizing the fractions eluted with hexane–benzene (1:1) and benzene, from ethyl ether, yielded a pure specimen (0·20 g) of the ketone (XIIIa); m.p. 163–165°, $[\alpha]_D^{20}$ −1·9° (*c*, 1·56) (Found: C, 76·78; H, 10·15; O, 13·07. $C_{34}H_{56}O_3$ requires: C, 76·96; H, 10·23; O, 12·82).

3-Oxo-17β-hydroxy-4,4,14α-trimethyl-5α-androstane (XIIIb)

Saponification (1 hr, steam bath) of acetate XIIIa (0·43 g of crude material from the preceding experiment) using 5% sodium hydroxide in methanol (20 ml) led to 0·42 g crude product. A solution of this material in 1:1 hexane–benzene was chromatographed on activated alumina (12 g).[19] The fractions (0·27 g) eluted with benzene–chloroform (4:1) and chloroform were combined and recrystallized from ethyl ether. The pure alcohol (XIIIb) was obtained as colorless prisms (0·21 g) m.p. 197–199° (sublimation from 150°), $[\alpha]_D^{20}$ +11·1° (*c*, 0·749). (Found: C, 79·48; H, 11·04. $C_{32}H_{54}O_2$ requires: C, 79·46; H, 10·92%).