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STEROIDS AND RELATED NATURAL PRODUCTS—XXII

OXIDATION OF 3β-ACETOXY-5α-LANOSTANE[1,2]

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Abstract—Chromium trioxide oxidation of 3β-acetoxy-5α-lanostane (IVa) has been evaluated as a route to 3β-acetoxy-20-oxo-4,4,14α-trimethyl-5α-pregnane (VI) and 3β-acetoxy-17-oxo-4,4,14α-trimethyl-5α-androstane (VIIa). One of the principal oxidation products was 3β-acetoxy-25-oxo-5α-27-norlanostane (V). Transformation of 25-ketone V to 3β,24-dihydroxy-4,4,14α-trimethyl-5α-cholane (VIIIa) confirmed the structural assignment. Although small amounts of 20-oxo-pregnane VI and 17-oxo-androstane VIIa were detected among the oxidation products, stepwise degradation of lanosterol was considered a more satisfactory route to these substances.

THE oxidative degradation of dibromocholesterol acetate to dehydroepiandrosterone (1) was first achieved approximately 30 years ago.[3] Chromium trioxide was employed as oxidizing agent and the 17-ketone (1) was subsequently obtained in 1–3% yields. One of the commercially important procedures was developed at Schering AG in Berlin employing 1,2-dichloroethane–acetic acid as solvent.[4] Somewhat improved procedures were devised in several other industrial laboratories and eventually yields corresponding to ca. 8% of 17-ketone I were realized.[3b] In 1958, one of the studies based on 1,2-dichloroethane–acetic acid as solvent was summarized by Maas and DeHeus.[5] During the intervening period, oxidative degradation of several compounds related to cholesterol was also reported. For example, a chromic acid reagent, in water–acetic acid, was found to convert lanostane derivative II to 17-oxosteroid III[6] and oxidize 3β-acetoxy-5α-lanostane (IVa) to a substance tentatively assigned[7] 3β-acetoxy-25-oxo-5α-27-norlanostane (V).

[1] Part XXI, G. R. Pettit, B. Green, A. K. Das Gupta and G. L. Dunn, *Experientia*, in press.

[2] This investigation was supported by PHS Research Grants CY-4074 to CA-04074-06 from the National Cancer Institute, Public Health Service.

[3] For example, refer to: A. Butenandt, H. Bannenbaum, G. Hanisch and H. Kudzsus, *Z. Physiol. Chem.* **237**, 57 (1935); L. Ruzicka and A. Wettstein, *Helv. Chim. Acta* **18**, 986 (1935); and E. S Wallis and E. Fernholz, *J. Amer. Chem. Soc.* **57**, 1504 (1935). Reviews of these studies have been prepared by [a] H. I. Ruiz, *Anales Inst. Farm. Espan.* **4**, 289 (1955) [*Chem. Abstr.* **51**, 6670 (1957)] and [b] L. F. Fieser and M. Fieser, *Steroids* p. 507. Reinhold, New York (1959). Oxidative degradation of 3β-acetoxy-5α-cholestane to 3β-acetoxy-17-oxo-5α-androstane, employing CrO_3, was reported the preceding year: L. Ruzicka, M. W. Goldberg and H. Brüngger, *Helv. Chim. Acta* **17**, 1389 (1934).

[4] The first report of these investigations was summarized by W. Schoeller, A. Serini and M. Gehrke, *Naturwissenschaften* **23**, 337 (1935) [*Chem. Abstr.* **35**, 3771 (1941)].

[5] S. P. J. Maas and J. G. DeHeus, *Rec. Trav. Chim.* **77**, 531 (1958).

[6] C. S. Barnes, D. H. R. Barton, A. R. H. Cole, J. S. Fawcett and B. R. Thomas, *J. Chem. Soc.* 571 (1953). Related studies leading to the now accepted structure of lanosterol have been summarized in an interesting review by E. R. H. Jones and T. G. Halsall, *Progress in the Chemistry of Organic Natural Products* (Edited by L. Zechmeister) Vol. XII; p. 44. Springer-Verlag, Vienna (1955).

[7] C. S. Barnes, *Austr. J. Chem.* **9**, 228 (1956).

At an early stage in our investigation of stepwise degradation of lanosterol to 3β-acetoxy-20-oxo-4,4,14α-trimethyl-5α-pregnane (VI) and androstane derivatives,[8] it seemed likely that chromium trioxide[9] oxidation of 3β-acetoxy-5α-lanostane might provide a more useful route to these intermediates. An attempt was then made to apply the Maas techniques[10] to lanosterol derivative IVa.[11] First, oxidation of sterol IVa was performed at 0–5°, 25–30° and 82–84°. The rapid decrease in ketone-containing neutral products with increasing temperature led us to limit extensive separation efforts to the low temperature (0–5°) reaction product. Following initial separation of neutral and acidic products, the neutral components were treated with Girard's reagent T and the ketone fraction was isolated. Using a series of column and thin layer[12] chromatographic techniques combined with fractional recrystallization procedures, both 20- and 25-ketones VI and V were isolated. The structure assigned 20-oxo-pregane VI was easily confirmed by comparison with an authentic sample.[8] The 27-nor-ketone (V) was found to be identical with the corresponding ketone isolated by Barnes[7,13] and the structure was confirmed as follows: Baeyer–Villiger oxidation[14] followed by saponification resulted in loss of a 2-carbon fragment and formation of 3β,24-dihydroxy-4,4,14α-trimethyl-5α-cholane (VIIIa). The course of peracid oxidation was anticipated on the basis of model experiments starting with 3β-acetoxy-25-oxo-27-norcholest-5-ene (IX). Palladium–charcoal catalyzed hydrogenation of olefin IX yielded 5α,27-norcholestane X. Treating 25-ketone X with trifluoroperoxyacetic acid led to 3β,24-diacetate XIa. Isolation of 3β,24-dihydroxy-5α-cholane (XIb) following saponification of ester XIa, completed the reaction sequence.

When further investigation of the low temperature ketone fraction failed to reveal the presence of 17-ketone VII, attention was next directed at ketone fractions from the medium (25–30°) and high (82–84°) temperature oxidation products. Again, only very small quantities (less than 1% yield) of ketones V and VI were detected. Each of

[8] G. R. Pettit, P. Hofer, W. J. Bowyer, T. R. Kasturi, R. C. Bansal, R. E. Kadunce and B. Green, *Tetrahedron* **19**, 1143 (1963).

[9] Initial attack by CrO_3 on the sterol side-chain is believed to occur at position 25: A. L. J. Beckwith, *J. Chem. Soc.* 3162 (1961). Other recent studies pertinent to the mechanism of this reaction have been described by: M. C. R. Symons, *Ibid.* 4331(1963); N.Venkatasubrananian, *Indian J. Chem.* **1**, 48 (1963); and F. Mareš and J. Roček, *Coll. Czech. Chem. Comm.* **26**, 2389 (1961).

[10] Meanwhile, the oxidative degradation of various protected cholesterol esters was continued in the laboratories of D. S. van Schuppen, N. V. The results of these carefully conceived experiments were recently summarized by S. P. J. Maas, M. J. D. Van Dam, J. G. DeHeus and D. Mulder, *Bull. Soc. Chim. Belges* **72**, 239 (1963). Under certain conditions 3β-acetoxy-5β,6α-dibromocholestane can be converted to the semicarbazone derivative of ketone I in 21·3% yield. The exact amount of solvent (1,2-dichloroethane–acetic acid), reaction time (9 hr), temp (0°) and protecting substituents are of particular importance. We wish to thank Dr. M. J. D. Van Dam for allowing us to review the manuscript prior to publication.

[11] In 1959 when the present study was already underway, Dr. C. S. Barnes (Ref. 7) kindly informed us that he had isolated a small quantity of the 20-oxo-pregnane (VI) following oxidation of sterol IVa with CrO_3. However, the expected product of oxidation, 17-oxo-androstane VIIa, was not found.

[12] See for example, M. J. D. Van Dam, G. J. De Kleuver and J. G. DeHeus, *J. Chromatog.* **4**, 26 (1960); J. Avignan, D. S. Goodman and D. Steinberg, *J. Lipid Res.* **4**, 100 (1963); and a review by J. H. Russel, *Rev. Pure Appl. Chem.* **13**, 15 (1963).

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

[14] C. H. Hassall, *Organic Reactions* Vol. IX; p. 73. J. Wiley, New York (1957).

the oxidation reactions led to a variety of ketones; however, the exact composition of the mixtures was not determined as the principal objective was isolation of ketones VI and VII. By this time an efficient eleven-step reaction sequence for degrading lanosterol to 3β,17β-dihydroxy-4,4,14α-trimethyl-5α-androstane (XII) became available,[8] and it was decided to meet requirements for 17-ketone VIIb by selective oxidation of diol XII. Mild chromium trioxide oxidation of the diol (XII) yielded 17-ketone VIIb (30%) accompanied by starting material, 3-oxo-17β-hydroxy-4,4,14α-trimethyl-5α-androstane,[8] and the corresponding 3,17-dione (XIII).

After preparing a pure specimen of the 17-oxo-steroid (VIIa), the low temperature ketone fraction was reinvestigated. The ketone subfractions obtained by column chromatographic separation were subjected to further separation on thin layer chromatographic plates using the 17-ketone as a reference. No substance corresponding to androstane derivative VIIa was observed. The problem was eventually resolved when it was ascertained that the 17-ketone was unreactive toward Girard's reagent T, thereby explaining its absence in the ketone fraction and failure of earlier attempts to isolate the 17-ketone as a semicarbazone derivative. Consequently, the fraction of neutral material unreactive toward Girard's reagent T was next studied and a very small quantity (ca. 0·2% overall yield) of the 17-ketone was isolated from this neutral fraction by preparative thin layer chromatography. At this point, the stepwise sequence[8] from isocholesterol to ketones VI and VIIa was considered more practical on a laboratory scale; and our investigation of the direct oxidation route was discontinued. By employing somewhat modified conditions (Ref. 9), the oxidation route might prove superior for commercial-type production of the 17-ketone (VIIa).

One oxidation reaction of potential biological interest, but incidental to the above program was also undertaken. In this phase of the study, 3β-hydroxy-4,4,14α-trimethyl-5α-24-norcholanic acid (XIVa)[8] was methylated with diazomethane and the resulting ester (XIVb) was oxidized, using an 8N chromium trioxide reagent, to 3-oxo-norcholanic acid XV. Further oxidation of ketone XV by molecular oxygen in the presence of potassium t-butoxide afforded diosphenol XVI.[15] Concomitant formation of the carboxylic acid (XVI) during the reaction sequence was confirmed by results of a mass spectral analysis.

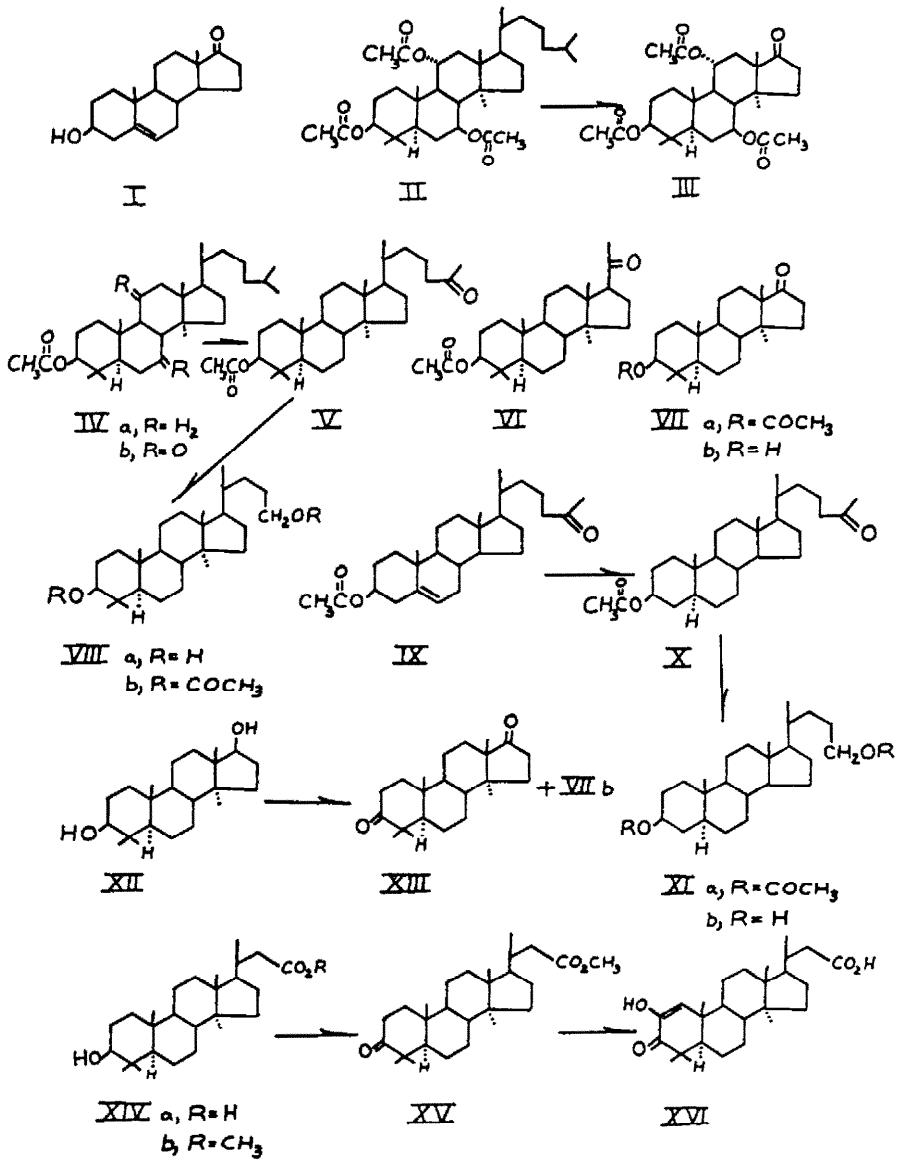
EXPERIMENTAL

Diethylene glycol was redistilled and the fraction boiling at 243–245° was collected. Hydrazine (95+% from Eastman Kodak Co.) was heated at reflux for 6 hr in the presence of KOH pellets and then distilled from the two-phase liquid system. The hydrazine distillate was heated at reflux for 0·5 hr over KOH and redistilled. The fraction of anhydrous hydrazine, b.p. 113–113·5°, was collected and employed in the Wolf–Kishner reduction experiments. Pet. ether was redistilled and refers to a fraction boiling at 40–50°. The benzene was also redistilled. All solvent extracts were dried (Na_2SO_4).

Unless otherwise noted, activated alumina refers to Merck aluminum oxide, "suitable for chromatography". Thin layer chromatograms were prepared on silica gel G (E. Merck, AG) and developed using a spray of conc. H_2SO_4. Preparative thin layer chromatograms were also performed on silica gel G and products were located using a water spray.

M.ps were observed employing a Kofler m.p. apparatus. Elemental analyses were performed in the laboratories of Dr. A. Bernhardt, Max Planck Inst., Mülheim, Germany. The IR spectra

[15] The corresponding diosphenol prepared from 3-oxo-5α-lanost-8,24-diene has been found to exhibit tumor inhibitory properties: H. Mori, V. S. Gandhi and E. Schwenk, *Chem. Pharm. Bull., Tokyo* **10**, 842 (1962).



were recorded by Dr. R. A. Hill of these laboratories and optical rotation ($CHCl_3$ solution) measurements were provided by the microanalytical laboratories of Dr. C. Janssen, Beerse, Belgium and Drs. Weiler and Strauss, Oxford, England.

3β-Acetoxy-7,11-dioxo-5α-lanostane (IVb)

Isocholesterol acetate was subjected to CrO_3 oxidation as described in an earlier study.[8] Following separation of the acidic products, the crude yellow neutral component (87 g) was dissolved in glacial acetic acid (2 l.). The yellow solution was heated at reflux during 2 hr while 87 g of Zn dust was added in 5-g increments. After pouring the warm and nearly colorless solution onto approximately 4 kg of ice chips, the resulting mixture was allowed to stand approximately 24 hr. The colorless solid was dissolved in chloroform and the resulting solution washed successively with water, $NaHCO_3$ aq and water. Following distillation of a major portion of the chloroform, the residue was diluted

with methanol. Upon cooling, 81 g, m.p. 221–222° (lit.[16] m.p. 222–224°), of colorless crystalline dione IVb was collected. Preparation of the diketone (IVb) was repeated a number of times with reproducibly good results.

3β-Acetoxy-5α-lanostane (IVa)

The following procedure is based on experience[8] with Wolf–Kishner reduction of 3β-hydroxy-7,11-dioxo-24,24-diphenyl-4,4,14α-trimethyl-5α-23-cholene and is a modification of methods employed by Barton[17] and Barnes[18] and their colleagues for synthesis of 3β-acetate IVa. A solution of dione IVb (100 g) in diethylene glycol (1300 ml) was prepared by heating the mixture to 175°. After adding anhydrous hydrazine (90 ml) the temp was maintained at 175–180° for 2 hr. The alkoxide solution prepared from diethylene glycol (1300 ml) and Na (37 g) was added and the mixture was stirred and heated at 175–180° for 24 hr. The reaction mixture temp was next raised to 210–225° by distillation of volatile components. Before cooling the solution to room temp, heating was maintained above 210° for 24 hr. The solution was added to approximately 4 kg of ice chips and the solid which separated was collected, washed with water and dried. A solution of the product in 1·4 l. of 1:1 acetic anhydride–pyridine was heated at reflux 3 hr. Following concentration, *in vacuo*, to ½ volume, the mixture was cooled and the colorless solid (m.p. 154·5–155°, 85–88% yields) was collected, washed with water, and dried. M.ps of 137–140 and 156–157° have been reported[17,18] for 3β-acetoxy-5α-lanostane.

In another experiment, an 82-g sample of diketone IVb was converted to 3β-hydroxy-5α-lanostane and the crude product was recrystallized from chloroform–methanol. The colorless crystalline alcohol weighed 63·5 g (85%), m.p. 168–169° (lit.,[16] m.p. 171–172°). The above Wolf–Kishner procedure was found uniformly dependable.

Oxidation of 3β-acetoxy-5α-lanostane (IVa)

Oxidation at 0–5°. A solution of 3β-acetoxy-5α-lanostane (50 g) in 1,2-dichloroethane (800 ml)–glacial acetic acid (600 ml) was maintained at 0–5° while adding, over 2·5 hr, a solution prepared from water (310 ml), CrO_3 (100 g), H_2SO_4 (93 ml), and glacial acetic acid (310 ml). Before reducing excess oxidizing agent by dropwise addition of methanol (250 ml, during 0·5 hr) stirring was continued at 0–5° for a 1·5 hr period. The reaction mixture was poured onto approximately 3 kg of ice. When the mixture warmed to room temp, the organic phase was separated and the aqueous portion was extracted with chloroform. The chloroform extract was added to the 1,2-dichloroethane solution and the combined solvent was washed with water and dried. Removal of solvent *in vacuo* led to 50 g of green waxy solid. The crude product was dissolved in diethyl ether (1 l.) and slowly mixed with 10% NaOH aq (200 ml). The yellow ethereal layer was separated and the aqueous phase was washed (3 × 500 ml) with diethyl ether. After drying, the organic phase was concentrated *in vacuo* to afford 24·5 g of neutral material. Acidifying the aqueous mixture with 12% HCl aq yielded 20·8 g of an acid fraction.

The following method was employed for separating ketone components from the neutral product and is based on a procedure described by Callow.[19] A mixture of the neutral product (24·5 g), glacial acetic acid (55 ml), Girard's reagent T (45·0 g), and 95% ethanol (500 ml) was heated at reflux for 2 hr. The solution was diluted with 500 ml of propylene glycol and the lower boiling components were removed by evaporation *in vacuo*. After cooling to room temp, the glycol solution was extracted with benzene (6 × 500 ml) and the benzene extract was washed with propylene glycol, and water and dried. Removal of solvent from the benzene solution yielded 14 g of material unreactive toward Girard's reagent T. The propylene glycol solutions were combined and residual benzene was removed *in vacuo*. Following dilution with water (1 l.) and glacial acetic acid (1 l.) the glycol-mixture was heated at reflux for 3 hr. The warm solution was poured into 5 l. ice-water and after approximately 24 hr, 1·0 g of colorless solid was collected. The remaining aqueous mixture was extracted (3 × 1 l.) with diethyl ether and the combined ethereal extract was washed successively with water, Na_2CO_3 aq and water. Removing the solvent *in vacuo* provided 8·5 g of ketonic components as a yellow waxy solid.

[16] W. Voser, H. Heusser, O. Jeger and L. Ruzicka, *Helv. Chim. Acta* **33**, 1893 (1950).
[17] D. H. R. Barton, D. A. J. Ives and B. R. Thomas, *J. Chem. Soc.* 2056 (1955).
[18] C. S. Barnes and A. Palmer, *Austr. J. Chem.* **10**, 334 (1957).
[19] R. K. Callow and P. N. Massy-Bevesford, *J. Chem. Soc.* 4482 (1957).

A thin layer chromatogram (9:1 pet. ether–ethyl acetate mobile phase) indicated that the 1-g solid sample (noted in the previous paragraph) represented 1 principal component and 5 minor constituents. The crude solid, m.p. 120–140°, was recrystallized from methanol to yield colorless crystals (0·50 g), m.p. 176–178°. Repeated recrystallization from methanol afforded a pure sample subsequently shown to be *3β-acetoxy-25-oxo-5α-27-norlanostane* (V): m.p. 185°, $[\alpha]_D^{22}$ +42·6° (*c*, 1·2), ν_{max}^{KBr} 1724, 1710 and 1255 cm^{-1}. (Found: C, 78·66; H, 10·94; O, 10·39. $C_{32}H_{52}O_3$ requires: C, 78·76; H, 11·09; O, 10·15%). The specimen of 25-ketone V was found[20] to be identical with a sample provided by Barnes.[21]

The remaining ketonic fraction (8·5 g) was dissolved in 1:1 acetic anhydrine–pyridine (150 ml) and heated at reflux 3 hr. Following removal of solvent *in vacuo*, the residue was dissolved in diethyl ether and washed successively with 1% HCl, 5% $NaHCO_3$ aq and water. The ethereal solution was concentrated to dryness and the residue was dissolved in 6:1 pet. ether–benzene and chromatographed on activated alumina (260 g). A series of 86 fractions were collected employing pet. ether with increasing quantities of benzene as eluent. Nearly all the fractions were found (by thin layer chromatograms) to represent mixtures of closely related substances. However, a series of fractions eluted by 1:2 pet. ether–benzene were relatively homogeneous and 6 of these were combined (0·37 g) and rechromatographed in 6:1 pet. ether–benzene on activated alumina (10 g). Several fractions eluted by 1:2 pet. ether–benzene were found to be *3β-acetoxy-20-oxo-4,4,14α-trimethyl-5α-pregnane* (VI). The pure 20-ketone (VI, 0·024 g) exhibited one spot on a thin layer chromatogram (9:1 pet. ether–ethyl acetate mobile phase) and m.p. 211–213°, following recrystallization from methanol. Confirmation of the structural assignment was obtained by comparison[20] with an authentic specimen.[8]

The 14·0-g fraction of presumed non-ketonic components from the Girard separation was next considered. Attempted isolation of 17-ketone VIIa from this material by chromatography on neutral alumina (Fisher, Brockmann Activity I) was unrewarding. Thin layer chromatograms indicated that the ketone was distributed along with other components over a number of the hexane–benzene fractions. The presence of ketone VIIa was verified as follows: A 4-g sample of the crude material was dissolved in diethyl ether and the solution was allowed to evaporate slowly. The solid (1·8 g, predominantly *3β-acetoxy-5α-lanostane*, IVa) which separated was collected and the mother liquor was concentrated to a viscous oil. A 0·10-g portion of the oily residue was separated by preparative thin layer chromatography (4:1 hexane–ethyl acetate mobile phase). The procedure was repeated 3 more times and zones corresponding to the 17-ketone (VIIa) were combined and the product was eluted with 1:1 acetone–chloroform. Recrystallizing the product from methanol afforded 4 mg of colorless crystalline *3β-acetoxy-17-oxo-4,4,14α-trimethyl-5α-androstane* (VIIa), m.p. 148–150°. The ketone was identical[20] with an authentic sample prepared as described in the sequel.

Oxidation at 25–30°. Except for maintaining a reaction mixture temp 25–30°, the 0–5° oxidation experiment was repeated exactly as summarized. The oxidation product was separated into neutral (14·8 g) and acetic (32·3 g) fractions and the neutral components were further separated using the Girard T procedure. The ketone separation led to 4·0 g of unreacted material and 7·2 g of ketone components. The solid ketone mixture weighed 3·8 g, and the oily ketone fraction extracted with diethyl ether weighed 3·5 g. The solid (3·8 g) ketone fraction in 2:1 pet. ether–benzene was chromatographed on activated alumina (380 g). During elution the concentration of benzene was gradually increased and a total of 56 pet. ether–benzene fractions were collected. As in the previous case, a majority of the fractions represented mixtures composed of a variety of ketones. A series of fractions (0·50 g total) eluted by 1:2 pet. ether–benzene were, however, obtained in reasonably pure form. Recrystallization from methanol led to 0·31 g *20-ketone* VI, m.p. 202–205°. Recrystallization from the same solvent raised the m.p. to 209–211°. The *25-ketone* (V) was obtained in small amounts accompanied by other components. A similar chromatographic study of the oily (3·5 g) ketone fraction indicated that comparatively small amounts of ketones V and VI resided in this fraction.

Oxidation at 82–84°. Chromium trioxide oxidation of *3β-acetoxy-5α-lanostane* (IVa) was repeated at 82–84° (reflux temp) as described in the preceding experiments (0–5° and 25–30°). The following quantities of partially separated products were obtained: 8·5 g neutral, 37·3 g acid; 4·3 g recovered from the Girard sequence, 0·34 g solid ketones and 1·4 g of oily ketones. The ketonic

[20] Identity was established by mixture m.p. determination and IR spectral comparison (in KBr).
[21] C. S. Barnes, *Austr. J. Chem.* **9**, 228 (1956).

fractions were studied employing column and thin layer chromatographic techniques. Again small amounts of the 20- and 25-ketones (V and VI) were detected. The two approximately 4-g specimens (unreactive toward Girard's T reagent from the medium and high temp oxidation reactions) were not further investigated once the presence of 17-ketone VIIa was detected in the low temp oxidation product.

3β-Acetoxy-25-oxo-5α-27-norcholestane (X)

A sample of 3β-acetoxy-25-oxo-27-norcholest-5-ene (IX, 0·60 g, from Mann Research Laboratories) in glacial acetic acid (125 ml) was subjected to a slight positive pressure of hydrogen during 6 hr in the presence of 10% Pd-C (0·06 g). After filtration, the solution was diluted with water (1 l.) and the solid which separated was collected, washed with water and dried: yield 0·45 g, m.p. 147·5–149°. Recrystallization from methyl alcohol gave pure (negative tetranitromethane test) colorless crystals, m.p. 148–149° (lit.,[22] m.p. 135–136°). (Found: C, 78·18; H, 10·81. $C_{30}H_{46}O_3$ requires: C, 78·09; H, 10·77%).

3β-24-Diacetoxy-5α-cholane (XIa)

A stirred suspension of Na_2HPO_4 (2 g) in chloroform (25 ml) containing 25-ketone X (0·36 g) was treated with 2·5 ml of a trifluoroperoxyacetic acid solution (prepared[23] from 12 ml of trifluoroacetic anhydride, 1·6 ml 90% H_2O_2, and 12 ml methylene chloride). The mixture was stirred and maintained at ice-bath temp for 30 min. After cessation of cooling, the reaction was continued 2·5 hr. The solution was filtered and washed with Na_2CO_3 aq and water. Following removal of solvent *in vacuo*, the residue was allowed to crystallize from methanol: yield 0·19 g, colorless needles, m.p. 122·5–123·5°. Two recrystallizations from methanol raised the m.p. to 129·5–130°. (Found: C, 75·11; H, 10·10; O, 14·65. $C_{32}H_{48}O_4$ requires: C, 75·29; H, 10·38; O, 14·33%).

The corresponding diol derivative (XIb) was obtained by saponifying (1N methanolic KOH) diacetate XIa. Three recrystallizations from methanol–water gave an analytical sample as colorless crystals, m.p. 202–203°, $[\alpha]_D^{20}$ +26° (*c*, 0·95). (Found: C, 79·58; H, 11·20; O, 8·92. $C_{24}H_{42}O_2$ requires: C, 79·50; H, 11·68; O, 8·83%).

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

A 0·27-g specimen of 25-ketone V was treated with trifluoroperoxyacetic acid as illustrated in the preceding experiment (XIa). Following recrystallization from methanol, the Baeyer–Villiger product weighed 0·14 g, m.p. 133–137°. Two recrystallizations from methanol yielded an analytical specimen of *3β-24-diacetoxy-4,4,14α-trimethyl-5α-cholane* (VIIIa) as colorless needles, m.p. 142–143°, $[\alpha]_D^{20}$ +35·0° (*c*, 0·40), ν_{max}^{KBr} 1735 and 1250 cm^{-1}. (Found: C, 75·44; H, 10·86; O, 13·50. $C_{31}H_{52}O_2$ requires: C, 76·18; H, 10·72; O, 13·10%).

The diacetate (VIIIb) was saponified as noted in a previous (XIb) experiment. Recrystallizing the diol (VIIIa) derivative from diethyl ether led to a pure specimen of colorless plates, m.p. 211–212°. (Found: C, 79·99; H, 11·95; O, 7·92. $C_{27}H_{46}O_2$ requires: C, 80·14; H, 11·96; O, 7·91%).

3β-Acetoxy-17-oxo-4,4,14α-trimethyl-5α-androstane (VIIa)

An acetone (500 ml) solution of 3β,17β-dihydroxy-4,4,14α-trimethyl-5α-androstane (XII, 2·53 g)[8] was treated, at ice-bath temp, with 3·0 ml of an 8N CrO_3 reagent.[24] After 5 min, sodium acetate was added and the mixture was diluted with methanol–water. The organic solvents were removed *in vacuo* and the aqueous residue extracted with chloroform. Evaporating the combined chloroform extract to dryness yielded 2·52 g of product corresponding (as evidenced by a thin layer chromatogram: 65:35 hexane–ethyl acetate mobile phase) to a 4-component mixture. A solution of the residue in 1:1 hexane–benzene was chromatographed on activated alumina (75 g).[25] The fractions eluted with 1:1 and 1:3 hexane–benzene were combined and recrystallized from acetone–hexane to yield 0·79 g of *3,17-dioxo-4,4,14α-trimethyl-5α-androstane* (XIII). A pure specimen recrystallized from the same solvent as colorless prismatic needles, m.p. 171–173°, $[\alpha]_D^{20}$ +36·4° (*c*, 1·45). (Found: C, 79·78; H, 10·33; O, 9·80. $C_{22}H_{34}O_2$ requires: C, 79·95; H, 10·37; O, 9·68%).

[22] L. Ruzicka, M. Oberlin, H. Wirz and J. Meyer, *Helv. Chim. Acta* **20**, 1283 (1937).
[23] G. R. Pettit and T. R. Kasturi, *J. Org. Chem.* **26**, 4557 (1961).
[24] K. Bowden, I. M. Heilbron, E. R. H. Jones and B. C. L. Weedon, *J. Chem. Soc.* **39** (1946).
[25] Aluminum Co. of America's Grade F-20 (80–100 mesh).

The fractions eluted with benzene and 1:1 benzene–chloroform weighed 1·01 g and crystallized from acetone–hexane as colorless needles to yield (0·78 g, 31%) *3β-hydroxy-17-oxo-4,4,14α-trimethyl-5α-androstane* (VIIb); m.p. 231–232°, $[\alpha]_D^{30}$ +35·5° (*c*, 0·7), ν_{max}^{KBr} 3410 and 1730 cm^{-1}. (Found: C, 79·81; H, 10·82. $C_{22}H_{36}O_2$ requires: C, 79·46; H, 10·92%).

Acetylation (1:1 acetic anhydride–pyridine) of 3β-alcohol VIIb gave *3β-acetoxy-17-oxo-4,4,14α-trimethyl-5α-androstane* (VIIa). Crystallization from methanol provided a lower melting (140–142°) form of the acetyl derivative while crystallization from hexane afforded a higher melting, 151–152°, analytical sample as colorless needles; $[\alpha]_D^{30}$ +57·8 (*c*, 0·45), ν_{max}^{KBr} 1730 cm^{-1}. (Found: C, 76·74; H, 10·04; O, 13·01. $C_{24}H_{38}O_3$ requires: C, 76·96; H, 10·23; O, 12·82%).

Methyl 3β-hydroxy-4,4,14α-trimethyl-5α-24-norcholanic acid (XIVb)

A specimen of 3β-acetoxy-4,4,14α-trimethyl-5α-24-norcholanic acid[8] was saponified and the product (XIVa, 7·0 g, m.p. 245–249° following recrystallization from glacial acetic acid–chloroform) was methylated with ethereal diazomethane. A benzene solution of the methyl ester (XIVb) was chromatographed on activated alumina and the fractions eluted with benzene were crystallized from acetone; yield 1·95 g, colorless leaflets, m.p. 189–190°, $[\alpha]_D^{30}$ +23·2° (*c*, 1·12). (Found: C, 77·61; H, 10·99; O, 11·61. $C_{27}H_{46}O_3$ requires: C, 77·46; H, 11·08; O, 11·44%).

The 3β-alcohol (XIVb) was acetylated (1:1 acetic anhydride–pyridine) and the resulting specimen of *methyl-3β-acetoxy-4,4,14α-trimethyl-5α-24-norcholanic acid* was identical[20] with a sample prepared by methylating 3β-acetoxy-4,4,14α-trimethyl-5α-24-norcholanic acid.[8]

Methyl 3-oxo-4,4,14α-trimethyl-5α-24-norcholanic acid (XV)

To a cool (ice-bath) solution of 3β-alcohol XIVa (1·7 g) in acetone (400 ml) was added an 8N CrO_3 solution (7·5 ml).[24] After a 10-min period, excess oxidizing agent was eliminated by adding sodium acetate and methanol. The mixture was diluted with water and the organic solvents were removed *in vacuo*. Following removal of solvent from a chloroform extract of the aqueous phase, the crude product was crystallized from acetone; yield 1·12 g, m.p. 164–165°. Recrystallization from methanol afforded an analytical sample as colorless leaflets; m.p. 163–164°, $[\alpha]_D^{30}$ +6·8° (*c*, 1·32), ν_{max}^{KBr} 1735 and 1700 cm^{-1}. (Found: C, 77·88; H, 10·45. $C_{27}H_{44}O_3$ requires: C, 77·83; H, 10·65%).

2,3-*Dioxo-4,4,14α-trimethyl-5α-24-norcholanic acid* (XVI)

Methyl ester XV (1·12 g) was added to a solution of 1N potassium t-butoxide in t-butanol (100 ml). The yellow solution was saturated, at room temp, with oxygen and shaken in an oxygen atmosphere (7·5 p.s.i.) for 2·5 hr. Next, the reaction mixture was poured into cold 2N H_2SO_4 (50 ml) and the solid (1·02 g) which separated was collected, washed with water and dried. Crystallization from chloroform–acetone yielded 0·25 g of colorless crystals, m.p. 253–259°. An analytical sample crystallized from methanol as prisms, m.p. 264–267°, $[\alpha]_D^{30}$ +54·0° (*c*, 1·07), $\lambda_{max}^{ethanol}$ 270 mμ (log ε 3·97), ν_{max}^{KBr} 3200, 1730, 1705, 1655 and 1625 cm^{-1}. (Found: mol. wt. 416 (by mass spectrometry)[26] C, 74·83; H, 9·52; O, 15·57. $C_{26}H_{40}O_4$ (416) requires: C, 74·96; H, 9·68; O, 15·36%).

[26] We are indebted to John Occolowitz for the mass spectral determination.