342. *The Components of Wool Wax. Part IV.* The Identification of Some Steroidal Derivatives.*

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Cholestane-3β : 5α : 6β-triol, 7-oxocholesterol, and 3β-hydroxylanost-8-en-7-one and 3β-hydroxylanost-8-en-7 : 11-dione have been isolated from commercial wool-wax alcohols by chromatography on alumina.

The alcoholic fraction of wool wax contains about 20% of long-chain aliphatic alcohols which can be easily extracted by treatment with urea.[1, 2] The residue, which consists, as far as is known, of steroids and trimethyl-steroids, was resolved on a large-scale, somewhat unorthodox, chromatographic "column." Alumina, instead of being packed in a vertical column, was placed in stoppered bottles, and the solvent was progressively decanted through the series. Seventy-nine fractions were eventually obtained by development of the chromatogram with benzene, the course of the separation being followed by means of the optical rotation. Although each fraction still contained a mixture, it was found that different components were concentrated in particular fractions. Detailed examination of some of these fractions has, so far, led to the identification of four new wool-wax components, namely, cholestane-3β : 5α : 6β-triol, 7-oxocholesterol, 3β-hydroxylanost-8-en-7-one and 3β-hydroxylanost-8-en-7 : 11-dione.

The diketone has been described by Ruzicka *et al.*[3] It has been characterised through its acetate [3, 4] and the acetate of the corresponding saturated ketol.[5, 6] 3β-Hydroxylanosten-7-one has been prepared by Mijovic *et al.*[7] and by Knight *et al.*[8] by different routes. Neither of these compounds has been isolated from natural sources hitherto. Because very mild oxidation of lanostenyl acetate gives the 7-oxo-derivative which can in turn be oxidised to 7 : 11-dioxolanostenyl acetate, both compounds may be the products of autoxidation.

The presence of 7-oxocholesterol was suspected by Daniel *et al.*[9] They found that the quantity of cholesta-3 : 5-dien-7-one that could be isolated varied with the conditions of hydrolysis of the wax. Hot alkaline saponification gave high yields whereas hydrolysis in the cold gave very low yields of the dienone. They postulated that a 7-oxocholesteryl ester was the precursor, but 7-oxocholesterol itself was not isolated. After the conclusion of the work described here, Panizo and Bilbao [10] reported the isolation of 7-oxocholesterol from Spanish merino wool wax. Contrary to our experience, and also that of Daniel *et al.*[9] and of Mauthner and Suida,[11] these authors state that the higher the temperature at which the wax is saponified the greater is the yield of 7-oxocholesterol.

The preceding paper describes the isolation of 7-oxocholesterol by means of its digitonin complex. In the present study it was easily obtained by chromatography on alumina.

The cholestane-triol was isolated from the most strongly adsorbed fractions. Its physical constants and those of its derivatives agreed well with those of a synthetic sample, but the elementary analysis, despite careful drying, persistently showed a low carbon content. As no difficulty was encountered in the analysis of the synthetic compound, it seemed that the impurity in the natural triol might be a congener which is not removed by recrystallisation. After purification *via* its diacetate, the natural triol gave a satisfactory analysis. The possibility that the natural product was contaminated with traces of

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* Part III, preceding paper.

[1] von Rudloff, *Chem. and Ind.*, 1951, 338.

[2] Tiedt and Truter, *ibid.*, p. 911.

[3] Ruzicka, Rey, and Muhr, *Helv. Chim. Acta*, 1944, **27**, 472.

[4] Cavalla and McGhie, *J.*, 1951, 834.

[5] Dorée, McGhie, and Kurzer, *J.*, 1948, 988.

[6] Voser, Montavon, Gunthard, Jeger, and Ruzicka, *Helv. Chim. Acta*, 1950, **33**, 1893.

[7] Mijovic, Voser, Heusser, and Jeger, *ibid.*, 1952, **35**, 964.

[8] Knight, McGhie, and Birchenough, *Chem. and Ind.*, 1953, 822.

[9] Daniel, Lederer, and Velluz, *Bull. Soc. Chim. biol.*, 1945, **27**, 218.

[10] Panizo and Bilbao, *Publ. Inst. Quim. Alonso Barba*, 1954, **8**, 181.

[11] Mauthner and Suida, *Monatsh.*, 1896, **105**, 565.

aluminium compounds which had been eluted from the chromatographic column, was also examined; no aluminium could be detected. Apart from this observation the wool-wax compound appeared to be identical with the synthetic one. The mixed melting point of the diacetates was undepressed, and the infrared spectra of the natural triol and its diacetate were identical with those of the synthetic specimens. The triol was characterised by oxidation to the 5-hydroxy-diketone which was dehydrated to the 4 : 5-unsaturated diketone.

EXPERIMENTAL

Optical rotations were measured in $CHCl_3$, and ultraviolet spectra in EtOH.

Removal of the Long-chain Alcohols.[2]—Commercial wool-wax alcohols (300 g.; " Hartolan," Messrs. Croda, Ltd.) were dissolved in benzene–chloroform (1500 : 750 ml.) and the solution was poured on finely powdered urea (450 g.) which had been moistened with methanol (90 ml.). After 19 days, during which the mixture was occasionally stirred, the solids were filtered off and washed with benzene (250 ml.). The filtrate and washings were combined and treated with more urea (100 g.) moistened with methanol (20 ml.). Nine days later the solids were filtered off, washed with benzene (100 ml.), and united with the solids from the previous treatment with urea. The filtrate was washed with water (3 × 500 ml.), and the solvent was distilled off. The residue, consisting of polycyclic alcohols, was a red glass. The urea complex was decomposed with water and the long-chain alcohols were filtered off (yield 64 g.; m. p. 59—63°).

Separation by Adsorption.—Activated alumina was placed in 79 stoppered bottles of 450 ml. capacity. The first 25 bottles each contained 100 g. of alumina of low activity (Brockmann V); the next 25 each contained 50 g. of alumina (Brockmann V), and each of the remaining bottles held 50 g. of alumina (Brockmann I). The polycyclic alcohols (300 g.) were dissolved in benzene (500 ml.) to give a clear solution. Subsequent dilution with benzene precipitated the more polar alcohols which did not re-dissolve, even after dilution to 8 l. The precipitate (21 g.) was filtered off and an aliquot part of the solution (400 ml.) was poured into bottle 1. After the alumina had been thoroughly stirred and allowed to settle, the supernatant liquid was poured into bottle 2, and a further aliquot part of solution (400 ml.) into bottle 1. In this manner the entire volume was distributed in 16 bottles; later, benzene was used to develop the system until it occupied 79 bottles. The optical rotation of the supernatant liquids was used as a guide to the course of the separation. Each bottle yielded two fractions for more detailed examination : the mixture of alcohols dissolved in the supernatant liquid (recovered by evaporation to dryness), and the mixture of alcohols adsorbed on the alumina (recovered by extraction with chloroform in a Soxhlet apparatus). In some cases these fractions were combined.

7 : 11-*Dioxolanost*-8-*en*-3-*ol*. The canary-yellow solid from bottle 47 (5 g.) was dissolved in light petroleum–benzene (9 : 1) and adsorbed on alumina (Brockmann IV; 1·5 × 85 cm. column). Elution with increasingly polar solvents gave nine fractions : A, 256 mg., $[\alpha]_D$ +9°, was eluted with light petroleum–benzene (9 : 1); B, 33 mg., $[\alpha]_D$ −85°, light petroleum–benzene (4 : 1); C, 755 mg., $[\alpha]_D$ +14°; D, 417 mg., $[\alpha]_D$ +29°; E, 1481 mg., $[\alpha]_D$ +33°; F, 1125 mg., $[\alpha]_D$ 0° (all light petroleum–benzene; 1 : 1); G, 312 mg., $[\alpha]_D$ +57, benzene; H, 799 mg., $[\alpha]_D$ +54°, benzene–ether (4 : 1); I, 208 mg., $[\alpha]_D$ 0°, ether. Fractions G and H were combined and adsorbed on a second column of alumina (Brockmann IV; 1 × 10 cm.). The first fraction was eluted with benzene, the next five with benzene–ether (9 : 1), and the last two with benzene–ether (1 : 1). The amounts were : GH1, 31 mg., $[\alpha]$ 0°; GH2, 48 mg., $[\alpha]$ −30°; GH3, 238 mg., $[\alpha]$ +103°; GH4, 373 mg., $[\alpha]$ +42°; GH5, 175 mg., $[\alpha]$ +63°; GH6, 59 mg., $[\alpha]$ +25°; GH7, 85 mg., $[\alpha]$ +33°; GH8, 132 mg., $[\alpha]$ +25°.

Fraction GH3 was 3β-hydroxylanost-8-ene-7 : 11-dione (pale yellow needles from methanol). It appeared to melt at 112—114°, solidify as the temperature was raised, and melt again at 142·5—143·5° (Ruzicka *et al.*[3] give m. p. 113—115°), $[\alpha]_D$ +98° (*c* 0·71), $\lambda_{\text{max.}}$ 272 mμ (log ε 3·9) (Found : C, 78·4; H, 10·6. Calc. for $C_{30}H_{48}O_3$: C, 78·8; H, 10·6%). Acetylation with acetic anhydride at 50° gave a monoacetate, m. p. 157·5—158°, $[\alpha]_D$ +90° (*c* 0·318), $\lambda_{\text{max.}}$ 272 mμ (log ε 3·94) {lit., m. p. 157°, $[\alpha]$ +90°, $\lambda_{\text{max.}}$ 273 mμ (log ε 3·95)}. The acetate (50 mg.) was dissolved in boiling glacial acetic acid (10 ml.), and zinc dust (0·6 g.) was slowly added. After the mixture had been heated under reflux for 1 hr. it was cooled and diluted with water, and the ester was extracted with ether. The ethereal solution was washed with water and dried (Na_2SO_4), and after evaporation to dryness the residue was recrystallised from methanol, to give plates of 7 : 11-dioxolanostanyl acetate, m. p. 223° (lit., 223°), $[\alpha]_D$ +65° (*c* 0·198) (Found : C, 76·9; H, 10·5. Calc. for $C_{32}H_{52}O_4$: C, 76·75; H, 10·5%).

3β-*Hydroxylanost*-8-*en*-7-*one*. The alcohols from bottle 43 (2·74 g.) were combined with fractions GH 4—8 and adsorbed on alumina (Brockmann IV; 2 × 90 cm.), and the eluates were combined into fractions: B1, 755 mg., $[\alpha]$ −40°, eluted with light petroleum–benzene (2 : 1); B2, 177 mg. $[\alpha]$ +83°, and B3, 241 mg., $[\alpha]$ +31°, with light petroleum–benzene (1 : 1); B4, 461 mg., $[\alpha]$ +30°, with light petroleum–benzene (1 : 3).

Fraction B1 consisted mainly of cholesterol, and fraction B2 of 3β-hydroxylanost-8-en-7 : 11-dione. Fraction B3 yielded 3β-hydroxylanost-8-en-7-one (white needles from methanol), m. p. 136, $[\alpha]_D$ +30° (*c* 0·406), $\lambda_{max.}$ 255 mμ (log ε 3·94) (Found: C, 81·0; H, 11·1. Calc. for $C_{30}H_{50}O_2$: C, 81·4; H, 11·4%). The acetate (needles from acetonitrile) had m. p. 151°, $[\alpha]_D$ +22° (*c* 0·45), $\lambda_{max.}$ 255 mμ (log ε 3·99) {values recorded in the literature are: m. p. 151°;[4] 151° and 157°;[3] 155°,[12] $[\alpha]_D$ +20°, $\lambda_{max.}$ 255 mμ (log ε 4·1)} (Found: C, 79·4; H, 10·7. Calc. for $C_{32}H_{52}O_3$: C, 79·3; H, 10·8%).

Fraction B4 was obtained as needles (from ether–hexane), m. p. 223°, $\lambda_{max.}$ 259 mμ (log ε 2·5). It formed a monoacetate (in acetic anhydride at 50° for 30 min.), m. p. 185° (Found: C, 76·6; H, 10·5. Calc. for $C_{32}H_{52}O_4$: C, 76·75; H, 10·5%). The diacetate (obtained by acetic anhydride–pyridine) had m. p. 132°. This substance appears to be a mixture of 3β : 11α-dihydroxylanost-8-en-7-one, m. p. 228°, $\lambda_{max.}$ 252 mμ (log ε 3·95), which gives a monoacetate, m. p. 185°, and a diacetate, m. p. 132°, and 3β : 11α-dihydroxylanostan-7-one, m. p. 216° (diacetate, m. p. 134°).

Cholestane-3β : 5α : 6β-*triol*. (*a*) From wool wax: The alcohols extracted from the alumina of bottles 5—10 were re-adsorbed on alumina (Brockmann V) and, after extensive washing of the column with chloroform, 1 : 9 methanol–ether eluted the triol (needles from methanol), m. p. 238° (decomp.), $[\alpha]_D$ 0° (in MeOH) (Found, after drying *in vacuo* at 30° for 48 hr.: C, 76·3; H, 11·15; after purification *via* the acetate: C, 77·0; H, 11·4. Calc. for $C_{27}H_{48}O_3$: C, 77·1; H, 11·5%). The diacetate (needles from aqueous methanol) had m. p. and mixed m. p. 167°, $[\alpha]_D$ −40° (*c* 0·63) (Found: C, 73·6; H, 10·8. Calc. for $C_{31}H_{52}O_5$: C, 73·75; H, 10·4%).

(*b*) Synthetic cholestanetriol, prepared by the method of Pickard and Yates,[13] had m. p. 238° (decomp.), $[\alpha]_D$ 0° (in MeOH) (Found, after drying *in vacuo* at room temperature: C, 77·2; H, 11·6%). The diacetate had m. p. 165—166°, $[\alpha]_D$ −39° (Found: C, 73·5; H, 10·6%).

5α-*Hydroxycholestane*-3 : 6-*dione*. (*a*) Wool-wax cholestanetriol (405 mg.) in 90% acetic acid (20 ml.) and benzene (4 ml.) was oxidised with sodium dichromate dihydrate (191 mg.) at 0° for 22 hr. After dilution with water the product was extracted with ether. Removal of the solvent afforded the dione (needles from ethanol; 355 mg.), m. p. 231° (decomp.), $[\alpha]_D$ −21° (*c* 4·8). In agreement with Fieser's observation[14] it was found that very slow cooling of the solution gave needles having a higher m. p., *e.g.*, 242° and 248° (Found: C, 77·4; H, 10·4. Calc. for $C_{27}H_{44}O_3$: C, 77·8; H, 10·6%). There was no ultraviolet absorption band.

(*b*) Synthetic triol (2 g.) was oxidised with chromium trioxide (1·4 g.) in glacial acetic acid (185 ml.) at 5° for 16 hr. Purification of the product, as above, gave the dione, m. p. 231° (decomp.), $[\alpha]_D$ −19° (*c* 9·6) (Found: C, 77·3; H, 10·8%).

Cholest-4-*ene*-3 : 6-*dione*. (*a*) Synthetic dione (516 mg.) was dissolved in dry chloroform (60 ml.), and dry hydrogen chloride was bubbled through the solution at 0° for 30 min. The yellow product afforded pale yellow plates (296 mg.) (from aqueous ethanol), m. p. 130°, $[\alpha]_D$ −14°, $\lambda_{max.}$ 252 and 307 mμ. Fractional crystallisation from ethanol gave cholest-4-ene-3 : 6-dione as yellow plates, m. p. 120°, $\lambda_{max.}$ 251 mμ (log ε 4·02). The less soluble fraction was obtained as yellow needles, m. p. 164—165°, $[\alpha]_D$ +1°, $\lambda_{max.}$ 308 and 251 mμ (log ε 4·18 and 3·89).

(*b*) Hydroxycholestadione from wool wax was dehydrated as described under (*a*). The product was a yellow glass, $[\alpha]_D$ −23°, $\lambda_{max.}$ 252 (principal) and 308 mμ (weak).

7-*Oxocholesterol*. The contents of bottles 32 and 33 were chromatographed separately. Fractions rich in 7-oxocholesterol were combined (567 mg.; $[\alpha]_D$ −38°) and re-adsorbed on another alumina column (Brockmann V; 1·4 × 55 cm.). Four fractions were eluted with benzene: U1, 9 mg.; U2, 105 mg., $[\alpha]$ −87°; U3, 131 mg. $[\alpha]$ −94°; and U4, 20 mg. $[\alpha]$ −79°. Fractions U2 and U3 afforded 7-oxocholesterol (needles from light petroleum), m. p. 169—170°, $[\alpha]_D$ −103° (*c* 1·021), $\lambda_{max.}$ 238 mμ (log ε 4·13) (Found: C, 80·8; H, 11·0. Calc. for $C_{27}H_{44}O_2$: C, 80·95; H, 11·1%). It gave an acetate, m. p. and mixed m. p. 155—156°, $[\alpha]_D$ −95° (*c* 1·0), $\lambda_{max.}$ 237 mμ (log ε 4·09) (Found: C, 78·3; H, 10·2. Calc. for $C_{29}H_{46}O_3$: C, 78·6; H, 10·45%). The 2 : 4-dinitrophenylhydrazone had m. p. and mixed m. p. 246—247°,

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[12] Barton, Fawcett, and Thomas, *J.*, 1951, 3147.

[13] Pickard and Yates, *J.*, 1908, 1678.

[14] Fieser, *J. Amer. Chem. Soc.*, 1953, **75**, 4386.

$[\alpha]_D$ −600°, $\lambda_{max.}$ 258 and 385 mμ (log ε 4·24 and 4·46) (Found : C, 67·9; H, 8·4; N, 9·8. Calc. for $C_{33}H_{48}O_5N_4$: C, 68·2; H, 8·3; N, 9·65%).

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