

438. Wool Wax. Part VII.* *The Autoxidation of Dihydrolanosteryl Acetate.*

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On autoxidation, dihydrolanosteryl acetate (I) yielded the 7 β -hydroperoxy-† and 7 β :11 α -dihydroperoxy-derivatives † (III) and (II), together with smaller quantities of 7-oxo- and 7:11-dioxo-derivatives (VIII) and (V). The 7 β -hydroperoxide (III) was decomposed by aqueous ferrous ammonium sulphate into the 7-oxo-, 7 β -hydroxy-,† and 7:9-diene-derivatives (VIII), (IV), and (VII), respectively.

In earlier communications¹ it was shown that commercial wool wax contains a complex group of largely unidentified substances which are autoxidation products of cholesterol, lanosterol, and dihydrolanosterol. The structures of the major primary autoxidation products of cholesterol have been elucidated,² principally by Bergström and Wintersteiner,³ and some of these characteristic autoxidation products, absent in significant quantities from freshly secreted wax, have been isolated from the commercial wax by Daniel *et al.*³ and Milburn *et al.*⁴ A study has now been made of the autoxidation of dihydrolanosteryl acetate (I) to assist the identification of some of the remaining wool-wax autoxidation products.

Lanost-8-en-3 β -yl acetate (dihydrolanosteryl acetate) in ethyl acetate readily absorbed oxygen at 50°; and, after 48 hours, the peroxide content of the solute, determined by Sully's method,⁵ had reached a value of 1.6 milliequiv./g., or 80% hydroperoxide (calculated as C₃₂H₅₄O₄). On chromatography on alumina, this material was partly decomposed into 7-oxolanost-8-en-3 β -yl acetate (VIII), 7:11-dioxolanost-8-en-3 β -yl acetate (V), and lanosta-7:9-dien-3 β -yl acetate (VII) and unidentified resin. Separation of the autoxidation products was effected with the minimum of decomposition by means of counter-current distribution, or more conveniently by reversed-phase partition chromatography,⁶ into two hydroperoxide fractions together with very small quantities of unsaturated ketones.

The second hydroperoxide fraction yielded a pure monoperoxide, whose structure, formulated as (III), was elucidated by the following series of reactions: on catalytic hydrogenation, the hydroperoxide yielded an alcohol (IV), which on mild oxidation with

* Part VI, *J.*, 1954, 1460.

† The infrared spectra of compounds thus marked have been offered for inclusion in the D.M.S. collection (cf. *J.*, 1955, 4501).

¹ Horn and Ilse, *Chem. and Ind.*, 1956, 524; Horn, *Manuf. Chem.*, 1955, No. 4, p. 3.

² Bergström and Wintersteiner, *J. Biol. Chem.*, 1941, **141**, 596; 1942, **143**, 503; **145**, 309, 327; Mosbach, Nierenberg, and Kendall, *J. Amer. Chem. Soc.*, 1953, **75**, 2358; Fieser, *Science*, 1954, **119**, 710.

³ Daniel, Lederer, and Velluz, *Bull. Soc. Chim. biol.*, 1945, **27**, 218.

⁴ Milburn, Truter, and Woodford, *J.*, 1956, 1740.

⁵ Sully, *Analyst*, 1954, **79**, 86.

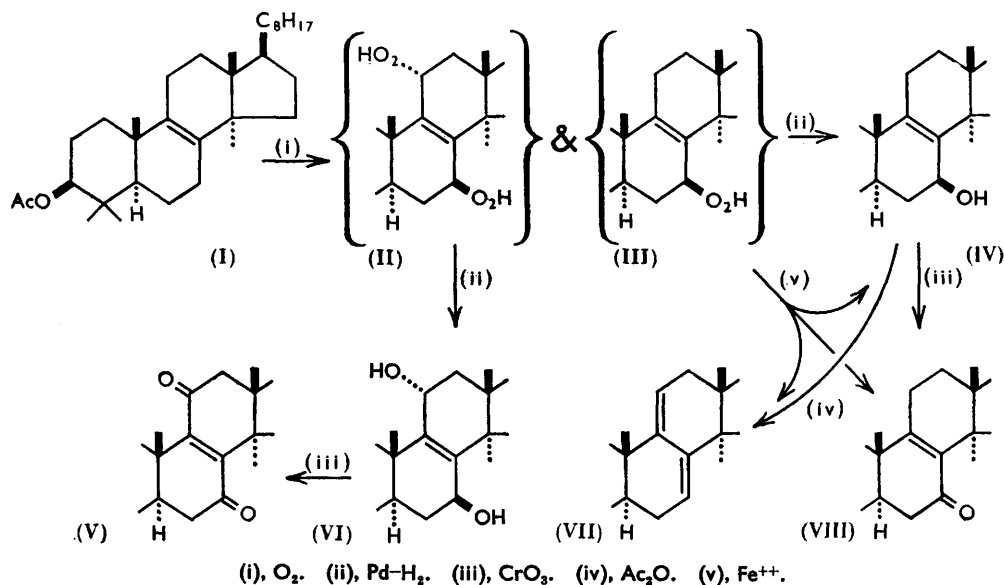
⁶ Howard and Martin, *Biochem. J.*, 1950, **46**, 532; Sephton and Sutton, *J. Amer. Oil Chemists' Soc.*, 1956, **33**, 263.

chromic oxide in acetone according to the method of Bowers *et al.*⁷ yielded 7-oxolanost-8-en-3 β -yl acetate (VIII). Attempted mild acetylation of the alcohol (IV) yielded lanosta-7:9-dien-3 β -yl acetate (VII).⁸

The 7-hydroxyl group of compound (IV), and hence the 7-hydroperoxyl group of (III), is assigned the β -configuration on the basis of molecular-rotation evidence, the contribution (ΔM_D , in CHCl_3) of the 7-hydroxyl group in the 7 β -hydroxy-acetate being $+97^\circ$, those of 7 α - and 7 β -hydroxycholestane being -59° and $+110^\circ$, respectively.⁹

In the autoxidation of cholesterol, attack takes place at both 7 α - and 7 β -positions.¹⁰ The absence of the epimeric 7 α -hydroperoxy-derivative or of a 7:9-diene in the autoxidation products, suggests that the 7 α -position is sufficiently hindered sterically by the 14 α -methyl group in the lanost-8-ene series to prevent significant autoxidative attack at the 7 α -position.¹¹

The hydroperoxide (III) with aqueous ferrous ammonium sulphate gave 7-oxolanost-8-en-3 β -yl acetate (VIII), 7-hydroxylanost-8-en-3 β -yl acetate (IV), and lanosta-7:9-dien-3 β -yl acetate (dihydroagnosteryl acetate) (VII). The 7:9-dienyl artefacts (esters of agnosterol and dihydroagnosterol) present in autoxidised wool wax, are probably formed by the decomposition of the corresponding 7-hydroperoxides by metal catalysts, particularly manganese salts known to be present in the wax.¹² In view of the ease of formation



of this heteroannular conjugated diene system on autoxidation, other analogous dienes accompanying naturally occurring lanost-8-enes in small amounts, might also be artefacts (*e.g.*, dehydroeburicoic acid).

The first hydroperoxide fraction to be eluted from the column yielded a crystalline dihydroperoxide formulated as (II). On catalytic hydrogenation it gave an acetoxy-diol

⁷ Bowers, Halsall, Jones, and Lemin, *J.*, 1953, 2548.

⁸ Cf. also Marker, Whittle, and Mixon, *J. Amer. Chem. Soc.*, 1937, **59**, 1368.

⁹ Braude and Nachod, "Determination of organic structures by physical methods," Academic Press Inc., New York, 1955, p. 111.

¹⁰ Bergström and Wintersteiner, *ref. 2*.

¹¹ Fieser and Fieser, "Organic Chemistry," 3rd edn., Reinhold Publishing Corp., New York, 1956, p. 965.

¹² von Rudloff, "A Study of the Saponifiable and the Unsaponifiable Components of Wool Wax," D.Sc. Thesis, Pretoria Univ., 1952, 80-81, 140-141; Truter and Woodford, *Chem. and Ind.*, 1954, 1323.

(VI), which was acetylated to a triacetate. Mild chromic oxidation of the acetoxy-diol yielded 7 : 11-dioxolanost-8-en-3 β -yl acetate (V), in support of the structure (II) assigned to the dihydroperoxide.

The formation of the 7 : 11-dihydroperoxide is surprising in view of the absence of detectable amounts of the 11-hydroperoxide. However, as the 11-position is less easily attacked than the 7-position, it is possible that at the advanced stages of autoxidation reached, most of the small amount of 11-hydroperoxide formed had been converted into the dihydroperoxide, as the dihydroperoxide should be more rapidly formed from the 11- than the 7-hydroperoxide. Alternatively the 11-position may be activated by a 7-hydroperoxide substituent or by an unstable 7-substituted-radical intermediate.

Too little of the acetoxy-diol (VI) was available for molecular-rotation measurements, but it is reasonable to assume the 7-hydroperoxyl group of the dihydroperoxide to have the β -configuration as hydroperoxide in (III), and following the ready acetylation of the 11-hydroxyl group of the acetoxy-diol (VI) and on steric grounds,¹³ the 11-hydroxyl group is assigned the α -configuration.

Besides the hydroperoxides isolated, very small amounts of unsaturated ketones, probably decomposition products of the two hydroperoxides, were present. These ketones were not isolated, but from ultraviolet and infrared absorptions measurements are considered to be 7-oxolanost-8-en-3 β -yl acetate (VIII) and 7 : 11-dioxolanost-8-en-3 β -yl acetate (V).

EXPERIMENTAL

M. p.s are uncorrected. Ultraviolet spectra were determined in 96% ethanol with a Unicam (S.P.500) spectrophotometer. Infrared spectra were determined in chloroform with a Hilger (H.800) instrument. Rotations were determined in chloroform at room temperature, a 0.5-decimetre polarimeter tube being used.

Autoxidation of Lanost-8-en-3 β -yl Acetate.—Lanost-8-en-3 β -yl acetate {5 g., m. p. 120—121°, $[\alpha]_D +57.3$ (c, 1.24)} in ethyl acetate (100 ml.) was heated to 50° and oxygen was passed through for 48 hr. Removal of the solvent in a vacuum at 40° gave a solid (5.2 g.; peroxide content 1.6 milliequiv./g.). This material in suspension with non-wetting Supercel⁶ (15 g.) and *iso*-heptane (15 g.) was poured on to a reversed-phase partition column (800 mm. \times 34 mm.), prepared by pouring a suspension of non-wetting Supercel (240 g.) and *iso*heptane (240 g.), in aqueous ethanol (80%), into an unpacked column containing ethanol (80%). The column was eluted with ethanol (80%), and the eluate divided into fractions with a syphon (36 c.c.). The peroxide content⁸ of each fraction was determined and a curve plotted of the peroxide value against the volume of eluate. The curve showed two peaks, the first corresponding to a peroxide value of 2.0 milliequiv./g., the second to 1.25 milliequiv./g.

7 β -Hydroperoxy lanost-8-en-3 β -yl Acetate.—Evaporation of the fractions corresponding to the second peroxide maximum gave a residue (2.2 g.) of 7 β -hydroperoxy lanost-8-en-3 β -yl acetate as needles (from methanol), m. p. 159—160°, $[\alpha]_D +67.4$ ° (c, 0.6) (Found : C, 76.4; H, 11.1. C₃₂H₅₄O₄ requires C, 76.4; H, 10.8%).

7 β -Hydroxy lanost-8-en-3 β -yl Acetate.—Reduction of 7 β -hydroperoxy lanost-8-en-3 β -yl acetate (1.5 g.) in ethanol (300 c.c.; 96%) with hydrogen for 3 hr. in presence of palladium-calcium carbonate catalyst (1.5 g.) gave 7 β -hydroxy lanost-8-en-3 β -yl acetate (1.2 g.), flat needles (from methanol), m. p. 163—164°, $[\alpha]_D +76.3$ ° (c, 1.0) (Found : C, 79.1; H, 11.3. C₃₂H₅₄O₃ requires C, 79.0; H, 11.2%).

Attempted Acetylation of 7 β -Hydroxy lanost-8-en-3 β -yl Acetate.—The hydroxy-acetate (450 mg.) in acetic anhydride-pyridine (1 : 1; 30 c.c.) was kept overnight at room temperature. After being worked up in the usual way the product was adsorbed from hexane on alumina (20 g.). Elution with hexane-benzene (1 : 1; 100 c.c.) gave a solid (355 mg.), which after one crystallisation gave pure lanosta-7 : 9-dien-3 β -yl acetate, λ_{max} 243 m μ (log ϵ 4.2), m. p. 167—168°, undepressed on admixture with an authentic specimen prepared from wool wax. Further elution with ether gave a yellowish-brown gum (75 mg.).

¹³ Fieser and Fieser, *loc. cit.*, p. 979.

7-Oxolanost-8-en-3 β -yl Acetate.—7 β -Hydroxy lanost-8-en-3 β -yl acetate (500 mg.) in acetone (200 c.c.) was oxidised with aqueous chromic acid as described by Bowers *et al.*⁷ The crude product, in hexane-benzene (1 : 1), was chromatographed on acid-washed alumina and yielded 7-oxolanost-8-en-3 β -yl acetate (240 mg.). Recrystallised from ethanol it had $[\alpha]_D +21.3^\circ$ (*c.* 1.0), λ_{\max} 253 m μ (log ϵ 4.06), m. p. 151–152°, undepressed on admixture with an authentic specimen¹⁴ (Found : C, 79.0; H, 10.5. Calc. for C₃₂H₅₂O₃ : C, 79.3; H, 10.8%).

Decomposition of 7 β -Hydroperoxy lanost-8-en-3 β -yl Acetate with Ferrous Ammonium Sulphate.—The pure hydroperoxide (1.0 g.) in ether-ethanol (1 : 1; 100 c.c.) was shaken overnight with aqueous ferrous ammonium sulphate (5%; 50 c.c.). Extraction of the product with ether gave a yellow glass which was adsorbed from hexane-benzene (1 : 1; 10 c.c.) on to acid-washed alumina (30 g.). Elution with the same solvent and crystallisation from ethanol-ethyl acetate (1 : 1) gave lanosta-7 : 9-dien-3 β -yl acetate (309 mg.), $[\alpha]_D +90^\circ$ (*c.* 1.09), λ_{\max} 242 m μ (log ϵ 4.2), m. p. 169° undepressed on admixture with an authentic sample obtained from wool wax (Found : C, 82.1; H, 11.2. Calc. for C₃₂H₅₂O₂ : C, 82.0; H, 11.2%).

Further elution of the column with benzene gave 7-oxolanost-8-en-3 β -yl acetate (174 mg.), which after repeated crystallisation from ethanol had λ_{\max} 253 m μ (log ϵ 4.03), $[\alpha]_D +20.5^\circ$ (*c.* 1.0), m. p. and mixed m. p. 151–151.5° (Found : C, 79.6; H, 10.9. Calc. for C₃₂H₅₂O₃ : C, 79.3; H, 10.8%).

Elution with ether then gave 7 β -hydroxy lanost-8-en-3 β -yl acetate (251 mg.), m. p. 163° (from methanol) (Found : C, 79.0; H, 10.8. Calc. for C₃₂H₅₄O₃ : C, 79.0; H, 11.2%).

7 β : 11 α -Dihydroperoxy lanost-8-en-3 β -yl Acetate.—Evaporation of the fractions corresponding to the first peroxide maximum gave pale yellow 7 β : 11 α -dihydroperoxy lanost-8-en-3 β -yl acetate (750 mg.), m. p. 210° (from aqueous methanol) (Found : C, 71.5; H, 10.2. C₃₂H₅₄O₆ requires C, 71.9; H, 10.2%).

7 β : 11 α -Dihydroxy lanost-8-en-3 β -yl Acetate.—Reduction of 7 β : 11 α -dihydroperoxy lanost-8-en-3 β -yl acetate (250 mg.) in ethanol (300 c.c.; 96%) with hydrogen for 3 hr. in the presence of palladium-calcium carbonate (250 mg.) gave 7 β : 11 α -dihydroxy lanost-8-en-3 β -yl acetate (240 mg.), m. p. 234° (*in vacuo*) (from methanol) (Found : C, 76.5; H, 11.0. C₃₂H₅₄O₄ requires C, 76.4; H, 10.8%).

3 β : 7 β : 11 α -Triacetoxylanost-8-ene.—7 β : 11 α -Dihydroxy lanost-8-en-3 β -yl acetate (54 mg.) and acetic anhydride-pyridine (1 : 1, 10 c.c.) at 45° for 15 hr. gave a yellow glass, which on recrystallisation from methanol yielded 3 β : 7 β : 11 α -triacetoxylanost-8-ene, m. p. 192–193° (Found : C, 73.6; H, 9.9. C₃₆H₅₈O₆ requires C, 73.7; H, 10.0%).

Oxidation of 7 β : 11 α -Dihydroxy lanost-8-en-3 β -yl Acetate.—7 β : 11 α -Dihydroxy lanost-8-en-3 β -yl acetate (324 mg.) in pyridine (4.25 c.c.) was added to chromic oxide (425 mg.)-pyridine (4.25 c.c.). After being kept overnight at room temperature, the mixture was diluted with water and extracted with ether. Evaporation gave a yellow glass, which was adsorbed from benzene on alumina (50 g.). Elution with benzene and crystallisation of the residue (40 mg.) from ethanol gave 7 : 11-dioxolanost-8-en-3 β -yl acetate, λ_{\max} 270 m μ (log ϵ 3.98), m. p. 156°, undepressed on admixture with an authentic specimen.¹⁴

Elution with benzene-ether (1 : 1) then gave unchanged 7 β : 11 α -dihydroxy lanost-8-en-3 β -yl acetate (98 mg.) and elution with ether, a yellow resin (80 mg.).

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¹⁴ Ruzicka, Rey, and Muhr, *Helv. Chim. Acta*, 1944, **27**, 472.