

161. Lanosterol. Part X. Ketodihydrolanosterol (continued).

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Oxidation of γ -lanostadienyl acetate with chromic acid gave ketolanostenyl acetate, identical with that from lanostenyl acetate. This product was dehydrogenated by selenium dioxide to ketolanostatrienyl acetate which has also been isolated from the mother-liquors obtained in the preparation of γ -lanostadienyl acetate from lanostenyl acetate with selenium dioxide. Oxidation of the triene gave triketolanostadienyl acetate, the presence of an α -diketo-grouping in which was shown by oxidation with alkaline hydrogen peroxide to a dibasic acid. Ketolanostenyl acetate has been reduced to γ -lanostadienyl acetate.

MARKER, WITTLE, AND MIXON (*J. Amer. Chem. Soc.*, 1937, **59**, 1368) reported that oxidation of lanostenyl acetate with chromic acid under comparatively mild conditions gave rise to two isomeric monoketolanostenyl acetates, designated by the prefixes α and β . Subsequent work (Birchenough and McGhie, *J.*, 1950, 1249) showed that only the β -isomer was a true keto-acetate, the supposed α -compound being impure γ -lanostadienyl acetate.* The isolation of only one keto-acetate is in conformity with the results of Ruzicka *et al.* (*Helv. Chim. Acta*, 1944, **27**, 472) who were also unable to obtain evidence for the formation of more than one ketolanostenyl acetate in the chromic acid oxidation of lanostenyl acetate.

Birchenough and McGhie (*loc. cit.*) also reported that under the conditions used by Marker *et al.* for the oxidation of lanostenyl acetate to ketolanostenyl acetate, γ -lanostadienyl acetate is rapidly oxidised to diketolanostenyl acetate; we have found that ketolanostenyl acetate behaves similarly.

γ -Lanostadienyl acetate on oxidation with perbenzoic acid or with ozone is readily converted into ketolanostenyl acetate (Birchenough and McGhie, *loc. cit.*; Birchenough, Thesis, London, 1950). We have now found it possible to oxidise γ -lanostadienyl acetate to ketolanostenyl acetate, in 20% yield, with chromic acid, using only one equivalent of oxidant and a short reaction time. This procedure is the most convenient for the rapid preparation of ketolanostenyl acetate. The identity of this product with that obtained by Ruzicka *et al.* or Birchenough and McGhie (*loc. cit.*) followed from physical properties and by dehydrogenation with selenium dioxide to the same ketolanostatrienyl acetate. Birchenough and McGhie (*loc. cit.*) showed that this compound was in all probability identical with a substance isolated by Muhr (Thesis, Zurich, 1945) from the mother-liquors left after the removal of γ -lanostadienyl acetate from the selenium dioxide oxidation of lanostenyl acetate. Although Muhr had formulated his compound as an unsaturated diketo-alcohol $C_{30}H_{44}O_3$, or less probably $C_{30}H_{46}O_3$, it being tentatively suggested that in the course of chromatographic purification deacetylation had occurred, all attempts to form an acetate failed. We have isolated Muhr's compound from the mother-liquors remaining after the conversion of lanostenyl acetate into γ -lanostadienyl acetate by selenium dioxide. It was identical with the ketolanostatrienyl acetate obtained from ketolanostenyl acetate according to Birchenough and McGhie (*loc. cit.*), and both were

* We revert to the nomenclature first used by Petrow (Thesis, London, 1935) in describing compounds obtained in reactions on lanosterol (now lanostadienol) derivatives.

oxidised by chromic acid to triketolanostadienyl acetate, m. p. 189—190°, $[\alpha]_D^{20} - 60^\circ$ (Dorée, McGhie, and Kurzer, *J.*, 1949, 570).

Recently Ruzicka and his collaborators (*Helv. Chim. Acta*, 1950, **33**, 1893) have reported failure to obtain consistent yields of triketolanostadienyl acetate following the directions of Dorée, McGhie, and Kurzer (*loc. cit.*): the main product was reported to be a diketolanostadienyl acetate, m. p. 122°, $[\alpha]_D + 77^\circ$, which on further treatment with selenium dioxide in dioxan at 180° was converted into triketolanostadienyl acetate identical with that obtained by Dorée *et al.*, although formulated as containing an additional double bond. We have, however, always obtained consistent yields of 30—35% of the triketoacetate, but no indication of the existence of an intermediate form as reported by Ruzicka *et al.* From our results, it appears that the controlling factor is the quantity of water used to dissolve the selenium dioxide. Dorée, McGhie, and Kurzer on the one hand state "a saturated aqueous solution," whilst Ruzicka *et al.* specify the amount of water used as 8 ml./3 g. of selenium dioxide. We have found that under anhydrous conditions (see McGhie, Thesis, London, 1947) consistent yields of triketolanostadienyl acetate (30%) can be obtained, even at the expense of cutting down the amounts of acetic anhydride used. However, we have found that as the amount of water used to dissolve the selenium dioxide increased, so the yields of the triketoacetate decreased and the quantity of diketolanostadienyl acetate increased until in the conditions used by Ruzicka it becomes the main product. In repeating this work we have isolated a product which appears to be identical with diketolanostadienyl acetate, having m. p. 128.5—129.5°, $[\alpha]_D 74.9^\circ$.

The triketo-acetate had the properties described by Dorée *et al.*, giving no colour with ferric chloride and failing to react with *o*-phenylenediamine even under forcing conditions. The presence of an α -diketo-grouping postulated by McGhie (*loc. cit.*) has been confirmed by oxidation with alkaline hydrogen peroxide in dioxan to an acid, m. p. 244°, $[\alpha]_D^{20} - 57.5^\circ$, which gave analytical figures for a hydroxy-dibasic acid formed by simultaneous oxidation and hydrolysis.

Marker *et al.* first reported the conversion of lanostenyl acetate into γ -lanostadienyl acetate. Their method involved oxidation to ketolanostenyl acetate, followed by reduction to the diol with sodium and *isopropyl* alcohol and subsequent dehydration and acetylation with acetic anhydride. Since Birchenough and McGhie (*loc. cit.*) have already shown that only one of the two keto-acetates isolated by Marker was a true ketolanostenyl acetate, it was uncertain which of the two "isomers" was converted into γ -lanostadienyl acetate. Reduction of ketolanostenyl acetate (Birchenough and McGhie, *loc. cit.*) by Marker's method, followed by crystallisation, gave a product, the melting point of which slowly rose on repeated crystallisation to 156.5—157.5° (Marker *et al.* give m. p. 165°). Our product was identical with γ -lanostadienol, m. p. 157—158°. It is possible that during the purification continuous dehydration of the intermediate allylic alcohol takes place. All attempts to isolate the intermediate diol in a pure state by chromatography failed, even when the reduction was carried out with lithium aluminium hydride. Direct treatment of the reduction product with acetic anhydride gave γ -lanostadienyl acetate.

EXPERIMENTAL.

All m. p.s are uncorrected. Specific rotations were measured in chloroform solution at 20°. Aluminium oxide used in chromatographic work was from Messrs. Peter Spence Ltd. (Grade "0"). All analyses are by Drs. Weiler and Strauss, Oxford.

Ketolanostenyl Acetate.— γ -Lanostadienyl acetate (3 g.) in glacial acetic acid (100 ml.) was treated at 95° with a solution of chromic acid (0.75 g.) in 90% acetic acid (7.5 ml.) for 2 minutes, whereafter the mixture was quickly poured into water and the excess of chromic acid destroyed with dilute sulphurous acid, and the precipitated solid was taken up in ether. The ethereal solution was washed with water, then dilute sodium carbonate solution and water until neutral, and dried (Na_2SO_4), and the solvent removed to leave a light yellow solid. Crystallisation from ethyl acetate (12 ml.) gave impure ketolanostenyl (0.9 g.), m. p. 140—143°; this was adsorbed from light petroleum (b. p. 60—80°; 150 ml.) on a column of alumina (12 × 1.3 cm.). Elution with light petroleum (b. p. 60—80°)—benzene (1 : 1; 300 ml.) and benzene (300 ml.) gave, after crystallisation from acetone-methanol, glistening needles (0.6 g.) of ketolanostenyl acetate, m. p. 152—153°, $[\alpha]_D^{20} + 18.5^\circ$ (*c.* 0.040) (Found: C, 79.2; H, 10.9. Calc. for $\text{C}_{32}\text{H}_{52}\text{O}_3$: C, 79.3; H, 10.8%). Ultra-violet absorption spectrum: Max., 246 $\mu\mu$.; log ϵ , 4.06 in cyclohexane; corresponding to λ_{max} 257 $\mu\mu$. in alcohol. Authentic material is stated to have m. p. 151—152.5°, $[\alpha]_D^{18} + 21.3^\circ$, and λ_{max} 255 $\mu\mu$. (log ϵ 4.08) in alcohol. From the ethyl acetate mother-liquors, diketolanostenyl acetate was obtained.

Ketolanostatrienyl Acetate.—Ketolanostenyl acetate (4 g.) in glacial acetic acid (100 ml.) was refluxed for 3 hours with a solution of selenium dioxide (2 g.) in water (2 ml.). The product was worked up by Birchenough and McGhie's method (*J.*, 1950, 1249), yielding pure ketolanostatrienyl acetate (2.8 g.), m. p. 198—199°, $[\alpha]_D^{20} + 70.2^\circ$ (*c.* 0.046) (Found: C, 80.0; H, 10.2. Calc. for $\text{C}_{32}\text{H}_{48}\text{O}_3$: C, 80.0; H, 10.1%). Absorption: Max., 256 and 328 $\mu\mu$.; log ϵ , 3.89 in alcohol.

Examination of the Mother-liquors remaining from the Oxidation of Lanostenyl Acetate to γ -Lanostadienyl Acetate (Dihydroagnosteryl Acetate).—The mother-liquors (3 l.) corresponding to the oxidation of approx. 200 g. of lanostenyl acetate were evaporated in a vacuum to 100 ml. and then poured into water. The precipitated solids were extracted with ether, and the ethereal layer was washed with water and sodium carbonate solution until neutral. Removal of the ether gave a red solid. Passage of its deep-red solution in benzene through alumina (25×4 cm.) removed most of the colloidal selenium, leaving a light brown solid on removal of the solvent. Crystallisation from acetone-methanol gave an ill-formed product (3 g.), m. p. 140–150°, which was dissolved in light petroleum (b. p. 60–80°) (150 ml.) and adsorbed on alumina (15×1.3 cm.). Elution with light petroleum (b. p. 60–80°; 500 ml.) gave γ -lanostadienyl acetate, m. p. 168–169° (0.5 g.); benzene (500 ml.) eluted ketolanostatrienyl acetate (1.3 g.), m. p. 196–199°, one crystallisation from ethyl acetate-methanol giving the pure compound, m. p. 198–199°, $[\alpha]_D^{20} + 68.9^\circ$ (c, 0.081) (Found: C, 80.1; H, 10.1%). Absorption: Max., 256 and 328 μ .; log ϵ , 3.89 in alcohol. A mixed m. p. with authentic material gave m. p. 198–199°.

Triketolanostadienyl Acetate.—Ketolanostatrienyl acetate (0.65 g.) in glacial acetic acid (50 ml.) was treated at 95° for 1 hour with chromic acid (0.65 g.) in 90% acetic acid (6.5 ml.) and then poured into water; the excess of chromic acid was destroyed with dilute sulphurous acid. The precipitated solids were extracted with ether, the ethereal layer was washed with water and dilute sodium carbonate solution until neutral, then dried (Na_2SO_4), and the solvent removed. The residual orange solid was crystallised from methanol (20 ml.), giving fine orange needles (0.45 g.), m. p. 186–188°. Two further recrystallisations gave pure triketolanostadienyl acetate, m. p. 189–190°, $[\alpha]_D^{20} - 60.0^\circ$ (c, 0.033) (Found: C, 75.2; H, 9.2. Calc. for $\text{C}_{32}\text{H}_{46}\text{O}_5$: C, 75.3; H, 9.1%). Absorption: Max., 284 μ .; log ϵ , 4.01 in alcohol.

A similar experiment with ketolanostatrienyl acetate obtained from the mother-liquors from γ -lanostadienyl acetate gave triketolanostadienyl acetate, m. p. 189–190°, alone and on admixture with an authentic specimen.

Dorée, McGhie, and Kurzer (*J.*, 1949, 570), and Voser *et al.* (*Helv. Chim. Acta*, 1950, **33**, 1893) respectively report m. p. 184–187°, 189–190°, $[\alpha]_D - 50.4^\circ$, -61° to -63° , λ_{max} . 280 (log ϵ 4.10), 285 (log ϵ 4.0) in alcohol.

Treatment of Diketolanostenyl Acetate with Selenium Dioxide.—(a) *Conditions described by Ruzicka et al.* (loc. cit.). Diketolanostenyl acetate (3 g.) dissolved in glacial acetic acid-acetic anhydride (50 ml.; 1:1) was refluxed for 3½ hours with a solution of selenium dioxide (3 g.) in water (8 ml.). Chromatographic purification of the product gave lemon-yellow needles (1.3 g.), m. p. 128.5–129.5°, $[\alpha]_D^{20} + 74.9^\circ$ (c, 0.038) (Found: C, 77.2; H, 9.6. Calc. for $\text{C}_{32}\text{H}_{48}\text{O}_4$: C, 77.4; H, 9.7%), as described by Ruzicka *et al.* A mixed m. p. of this compound with the starting material was 128–140°. From the benzene chromatographic eluates, triketolanostadienyl acetate, m. p. 188–189°, was obtained in small yield.

(b) *Anhydrous conditions.* Diketolanostenyl acetate (3 g.) was added to a suspension of selenium dioxide (3 g.) in acetic acid-acetic anhydride (50 ml.; 1:1) and refluxed for 3½ hours. The precipitated black selenium was filtered off and the filtrate poured into water. Extraction with ether and removal of solvent gave a red solid, crystallising from acetone-methanol in red needles (1.5 g.), m. p. 170–176°. Adsorption of this product on alumina (15×1.3 cm.) from light petroleum (b. p. 60–80°; 100 ml.) gave the following fractions on elution: (a) light petroleum (b. p. 60–80°; 400 ml.), trace of oil; (b) light petroleum (b. p. 60–80°)-benzene (2:1; 300 ml.), 0.15 g. of yellow oil; (c) light petroleum (b. p. 60–80°)-benzene (1:2; 300 ml.), 0.2 g. of orange solid; (d) benzene (300 ml.), 0.9 g. of orange solid; (e) chloroform (300 ml.), 0.1 g. of orange solid. Fraction (b), crystallised from methanol, gave diketolanostadienyl acetate as lemon-yellow needles, m. p. 127–128°, brought by two recrystallisations from methanol to m. p. 128.5–129.5° alone or on admixture with material prepared as above. Fraction (c), crystallised from methanol, gave slightly impure triketolanostadienyl acetate, m. p. 184–185°. Fractions (d) and (e) crystallised from acetone-methanol to give triketolanostadienyl acetate as fine orange needles, m. p. 188–190°, brought by one crystallisation to m. p. 189–190°, $[\alpha]_D^{20} - 60.5^\circ$ (c, 0.062) (Found: C, 75.2; H, 9.2. Calc. for $\text{C}_{32}\text{H}_{46}\text{O}_5$: C, 75.3; H, 9.1%).

Treatment of Triketolanostadienyl Acetate with Hydrogen Peroxide.—To a solution of triketolanostadienyl acetate (0.45 g.) in dioxan (25 ml.) was added aqueous potassium hydroxide (8 ml.; 10%) and then, at 50°, "Perhydrol" (4 ml.). The colour of the solution faded and after 15 minutes was almost nil. A further ml. of "Perhydrol" was added and the solution left for 10 minutes, poured into water, and extracted with ether. The ethereal layer was further extracted with 10% aqueous potassium hydroxide, then washed with water, and the solvent removed to leave a negligible neutral fraction. Acidification of the alkaline fraction gave a white solid which, on extraction with ether and crystallisation from ethyl acetate, gave cubic crystals (0.3 g.), m. p. 244° (decomp.), $[\alpha]_D^{20} - 57.5^\circ$ (c, 0.022 in alcohol). This was a *hydroxy-dibasic acid* (Found: C, 71.5, 71.4; H, 9.2, 9.0. $\text{C}_{30}\text{H}_{46}\text{O}_6$ requires C, 71.7; H, 9.2%). Absorption: Max., 251 μ .; log ϵ , 4.03 in alcohol.

Reduction of Ketolanostenyl Acetate.—(a) *Sodium and isopropyl alcohol.* Sodium (3 g.) was added slowly during 1 hour to a refluxing solution of ketolanostenyl acetate (0.5 g.) in dry isopropyl alcohol (50 ml.). The solution was boiled for a further 2 hours and then poured into water. Extraction with ether, followed by crystallisation from methanol-acetone, gave felted white needles (0.33 g.), m. p. 140–146°. Recrystallisation of this product six times from the same solvents raised the m. p. to a constant value of 156.5–157.5°, undepressed on admixture with an authentic specimen of γ -lanostadienol, m. p. 157–158°. Concentration of the mother-liquors from this purification to 30 ml. gave felted needles (0.1 g.), m. p. 128–130°. Further concentration of the mother-liquor gave a semicrystalline material (0.08 g.), m. p. 196–202°, which by repeated crystallisation from aqueous methanol gave fine needles of a *diol*, m. p. 207–208° (Found: C, 81.0; H, 11.9. $\text{C}_{30}\text{H}_{52}\text{O}_2$ requires C, 81.0; H, 11.8%).

(b) *Lithium aluminium hydride*. A solution of ketolanostenyl acetate (0.5 g.) in absolute ether (50 ml.) was added dropwise to a suspension of lithium aluminium hydride (1.3 g.) in absolute ether (100 ml.), with mechanical stirring and under reflux. A white solid was precipitated and the ether boiled gently. Stirring was continued for 1 hour, and the excess of lithium aluminium hydride then decomposed by cautious addition of ice-water, followed by dilute sulphuric acid. Extraction of the mixture with ether gave, on removal of the solvent, a colourless solid which was adsorbed from light petroleum (b. p. 60–80°; 250 ml.) on a column of alumina (12 × 1.3 cm.). Elution with light petroleum (b. p. 60–80°)–benzene (1 : 1; 500 ml.) and benzene (500 ml.) gave a white solid (0.35 g.), crystallising from acetone–methanol in felted needles, m. p. 153–155°. Three recrystallisations from the same mixture gave felted needles of γ -lanostadienol, m. p. 156–157°, $[\alpha]_D^{20}$ 67.8° (*c*, 0.057), undepressed on admixture with authentic material (Found : C, 84.9; H, 11.7. Calc. for $C_{30}H_{50}O$: C, 84.4; H, 11.8%).

Acetylation. The crude products from both the above reductions were severally boiled with acetic anhydride (25 ml.) for 2 hours. Pouring into water and extraction with ether in the usual manner gave a white solid which crystallised from acetone–methanol in glistening plates, m. p. 167–168°, in each case, undepressed on admixture with authentic γ -lanostadienyl acetate, $[\alpha]_D^{20}$ 91.0° (*c*, 0.033) (Found : C, 82.1; H, 11.0. Calc. for $C_{32}H_{52}O_2$: C, 82.0; H, 11.2%). Absorption : Max. 244 ($E_{1\text{cm}}^{1\%}$, 360) and 251 μ . ($E_{1\text{cm}}^{1\%}$, 224).

Diketolanostenyl Acetate.—Ketolanostenyl acetate (0.5 g.), in glacial acetic acid (50 ml.) was treated at 90° with a solution of chromic acid (0.5 g.) in 90% acetic acid (5 ml.) for 1 hour, with mechanical stirring, and then poured into water; the excess of chromic acid was then destroyed with dilute sulphurous acid. Extraction of the precipitated solids with ether in the usual manner and removal of the solvent gave a yellow solid crystallising from methanol in glistening yellow plates (0.35 g.), m. p. 155–157°. Two recrystallisations from methanol gave diketolanostenyl acetate, m. p. 157–158° (undepressed on admixture with authentic material), $[\alpha]_D^{20}$ +91.6° (*c*, 0.024) (Found : C, 77.1; H, 10.1. Calc. for $C_{32}H_{50}O_4$: C, 77.1; H, 10.1%).

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