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SYNTHESIS OF ERGOSTANE DERIVATIVES OXYGENATED IN RING D*

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SUMMARY

The preparation and physical constants of 14α,15α-oxido-5α-ergostan-3β-ol acetate; 5α-ergostane-3β,14α,15β-triol 3-acetate and 3β,14α-dihydroxy-5α-ergostan-15-one 3-acetate is described.

INTRODUCTION

For studies of the biosynthesis of sterols in yeast homogenates we required samples of C_{28} sterols oxygenated in ring D. For the record, we report the preparation of several such compounds.

Hydrogenation of ergosteryl acetate in glacial acetic acid—PtO_2 [1] yielded 5α-ergost-8(14)-en-3β-ol acetate which was isomerized to 5α-ergost-14-en-3β-ol acetate by treatment with dry hydrogen chloride in chloroform [2, 3]. Treatment of the 5α-ergost-14-en-3β-ol acetate with *m*-chloroperbenzoic acid in chloroform [4] resulted in 14α,15α-oxido-5α-ergostan-3β-ol acetate. Hydrolysis of the 14,15-oxide with H_5JO_6 in aq. acetone [5] gave 5α-ergostane-3β,14α,15β-triol 3-acetate. Oxidation of this triol with Sarett reagent [6] resulted in 3β,14α-dihydroxy-5α-ergostan-15-one 3-acetate.

The assignment of the chemical shifts of the methyls of the ergostane derivatives together with those of other sterols is described elsewhere [7].

EXPERIMENTAL [8]

Ergosterol (25 g, Aldrich Chemical Co.) was purified by crystallization from MeOH–$CHCl_3$ and acetylated [pyridine (100 ml)-acetic anhydride (100 ml), 4 h at room temperature]. The sterol acetate was crystallized from MeOH–$CHCl_3$.

5α-*Ergost*-8(14)-*en*-3β-*ol acetate*

A mixture of ergosteryl acetate (6.5 g), PtO_2 (200 mg) and glacial acetic acid was vigorously stirred in an atmosphere of hydrogen. The course of the reaction was followed by argentation t.l.c. [9] (chloroform, freed of alcohol) and by U.V. on aliquots removed at various intervals.

When t.l.c. showed a single spot with a mobility similar to that of cholesteryl acetate, the reaction was terminated (4 h). The PtO_2 was removed by filtration on cellite and the filtrate was poured into ice. The white solid was collected by filtration, washed with hot water, and crystallized from MeOH–$CHCl_3$. The obtained 5α-ergost-8(14)-en-3β-ol acetate (5.75 g) showed m.p. 116–118°; n.m.r. spectrum, 0.845 (*s*, 3H, 18-CH_3, calc. 0.842), 0.710 (*s*, 3H, 19-CH_3, calc. 0.708), 0.878 (*d*, J = 6.5 Hz, 3H, 21-CH_3), 0.789 (*d*, J = 6.8 Hz, 6H, 26, 27-CH_3), 0.940 (*d*, J = 6.0 Hz, 3H, 28-CH_3), 2.00 (*s*, 3H, 3β-OAc), ca. 4.68 (*m*, 1H, 3α-H); m.s. *m/e* 442 (M^+, 100%), 427 (M-15, 12%), 315 (M-127, 11%), 255 (M-(127 + 60), 11%), 229 (22%), 213 (27%).

5α-*Ergost*-14-*en*-3β-*ol acetate*

A solution of 5α-ergost-8(14)-en-3β-ol acetate (2.5 g) in chloroform was cooled to −35° and then a stream of dry HCl was admitted. The reaction was followed by argentation t.l.c. (chloroform, freed of alcohol). After 4.5 h the reaction was stopped and allowed to warm up slowly to room temperature. The excess of HCl was removed in a stream of N_2.

Aqueous $NaHCO_3$ (0.5 M, 10 ml) was added and the mixture was stirred for 30 min. The aqueous layer was separated and the chloroform solution was washed with water and dried ($MgSO_4$). Removal of solvent gave an oily residue which resisted crystallization and was purified by argentation t.l.c. to yield 5α-ergost-14-en-3β-ol acetate (1.73 g). The product was crystallized from MeOH–$CHCl_3$ (1.49 g) and showed m.p. 108–110.5°; n.m.r. spectrum 0.885 (*s*, 3H, 18-CH_3), 0.825 (*s*, 3H, 19-CH_3), 0.855 (*d*, J = 6.0 Hz, 3H, 21-CH_3), 0.778 (*d*, J = 6.5, Hz, 6H, 26, 27-CH_3), 0.905 (*d*, J = 6.0 Hz, 3H, 28-CH_3), 2.000 (*s*, 3H, 3β-OAc), ca. 4.68 (*m*, 1H, 3α-H), 5.13 (15-H); m.s. *m/e* 442 (M^+, 20%), 316 (M-126, 38%), 315 (M-127, 100%), 314 (M-128, 10%), 257 (17%), 256 (316-60, 14%) 255 (315-60, 93%).

14α,15α-*Oxido*-5α-*ergostan*-3β-*ol acetate*

The epoxidation of 5α-ergost-14-en-3β-ol acetate was carried out in three batches of 570 mg each. The 14-olefin (570 mg) was dissolved in $CHCl_3$ (9 ml) and a solution of *m*-chloroperbenzoic acid (405 mg) in $CHCl_3$ (7.5 ml) was added. The mixture was stirred at 22° in the dark and the reaction was followed by

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t.l.c. (hexane: EtOAc (19:1, v/v)). After 2 h the reaction was terminated and ethyl ether (100 ml) was added. The solution was washed with NaOH (aq. 0.5 N, 2 × 10 ml), water, dried, and the solvent was removed to yield the crude 14α,15α-oxido-5α-ergostan-3β-ol acetate. The crude product from the three runs was crystallized from MeOH–$CHCl_3$ to give homogeneous 14α,15α-oxido-5α-ergostan-3β-ol acetate (0.75 g) m.p. 112.5–114.5°; n.m.r. spectrum 0.848 (*s*, 3H, 18-CH_3), 0.848 (*s*, 3H, 19-CH_3), 0.790 (*d*, J = 6.0 Hz, 3H, 21-CH_3), 0.776 (*d*, J = 6.8 Hz, 6H, 26,27-CH_3), 0.845 (*d*, J = 6.0 Hz, 3H, 28-CH_3), 1.99 (*s*, 3H, 3β-OAc), ca. 4.67 (*m*, 1H, 3α-H) 3.30 (15β-H); I.R. ν(KBr) 1722 cm^{-1} (3β-OAc); m.s. *m/e* 458 (M^+, 14%), 440 (M-18, 18%), 380 (M-(60 + 18), 10%), 365 (380-15, 13%), 332 (13%), 331 (29%), 292 (15%), 224 (34%), 223 (100%).

5α-Ergostane-3β,14α,15β-triol 3-acetate

To a magnetically stirred solution of 14α, 15α-oxido-5α-ergostan-3β-ol acetate (250 mg) in acetone (7.5 ml), a solution of periodic acid (125 mg) in water (0.5 ml) was added. The mixture was stored at r.t. and after 16 h t.l.c. (hexane: EtOAc, 4:1, v/v) indicated the presence of only traces of starting material. Most of the acetone was then removed in a stream of N_2, ether (2 ml) was added and the obtained mixture was fractionated by preparative t.l.c. (hexane: EtOAc, 4:1, v/v) to yield 5α-ergostane-3β,14α,15β-triol 3-acetate (86 mg). The product was crystallized from MeOH–H_2O (or from hexane) and showed m.p. 162.5–164°; n.m.r. spectrum 0.778 (*s*, 3H, 18-CH_3), 0.990 (*s*, 3H, 19-CH_3), 0.850 (*d*, 3H, J = 6.5 Hz, 21-CH_3), 0.780 (*d*, 6H, J = 6.5 Hz, 26, 27-CH_3), 0.918 (*d*, 3H, J = 6 Hz, 28-CH_3), 2.000 (*s*, 3H, 3β-OAc), 4.67 (*m*, 1 H, 3α-H), 4.02 (15α-H); I.R. ν(Kbr) 3490 and 3400 cm^{-1} (14α-OH and 15β-OH), 1710 cm^{-1} (3β-OAc); m.s. *m/e*, 458 (M-18, 24%), 440 (M-(18 + 18), 23%), 332 (M-(126 + 18), 22%), 331 (M-(127 + 18), 34%), 313 (M-(127 + 18 + 18), 20%), 305 (35%), 293 (35%), 292 (100%), 223 (76%).

3β,14α-Dihydroxy-5α-ergostan-15-one 3-acetate

A solution of 5α-ergostane-3β,14α,15β-triol 3-acetate (40 mg) in pyridine (0.4 ml) was added to a complex formed from CrO_3 (40 mg) in pyridine (0.4 ml). The reaction mixture was stored at room temperature and the progress of the reaction was followed by t.l.c. (hexane:EtOAc (17:5, v/v). When the reaction was essentially completed (90 min), methanol (3 drops) was added and the mixture was poured into water (15 ml). The product was extracted with ether (3 × 5 ml) and processed in the conventional manner to yield a white solid. Crystallization from methanol gave the 15-ketone (32 mg) m.p. 161–62°; n..m.r. spectrum 0.798 (*s*, 3H, 18-CH_3), 1.030 (*s*, 3H, 19-CH_3), 0.860 (*d*, J = 6.5 Hz, 3H, 21-CH_3), 0.790 (*d*, J = 6.5 Hz, 6H, 26, 27-CH_3), 0.948 (*d*, J = 6.0 Hz, 3H, 28-CH_3), 1.98 (*s*, 3H, 3β-OAc), ca. 4.68 (*m*, 1H, 3α-H); I.R. ν(KBr) 3515 cm^{-1} (14α-OH), 1740 cm^{-1} (15-ketone), 1715 cm^{-1} (3β-OAc); m.s. *m/e* 474 (M^+, 19%), 456 (M-18, 10%), 431 (M-43, 10%), 330 (M-126, 18%), 329 (M-(127 + 18), 10%). 293 (51%), 292 (100%), 239 (59%).

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