Unsaturated Steroids. Part I. Synthesis of 22,23-Dihydroergosterol

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Bromination of 5α-ergost-7-en-3-one furnishes a mixture of the 2- and 4-bromo-derivatives which is readily dehydrobrominated to yield the isomeric 5α-ergosta-1,7- and ergosta-4,7-dien-3-ones. Reduction of the enol acetate of ergosta-4,7-dien-3-one yields 22,23-dihydroergosterol. Some reactions of the 22,23-epoxide of ergosterol are reported; the configurational assignment of the 22-hydroxy-group in 3,22-dihydroxyergosterols has been clarified.

THE first preparation,[1] in low yield, of 22,23-dihydroergosterol (1) was recorded by Windaus and Länger in 1934. Despite its potential interest as a D vitamin precursor, and its isolation as a metabolite from various fungi [2] and from a mutant yeast,[3] no alternative synthesis of this steroid was available until recently.[4] As part of an investigation concerning unsaturated steroids, we have devised an improved route to this material.

Thus 5α-ergost-7-en-3β-ol (5; R^1 = H,OH) was oxidised with modified Jones reagent to the ketone (5; R^1 = O) in 80% yield. Bromination of (5; R = O) with *NNN*-trimethylanilinium perbromide gave a mixture (1 : 1) of the isomeric α-bromo-ketones (6; R^1 = Br, R^2 = H) and (6; R^1 = H, R^2 = Br) which was not purified but was dehydrobrominated directly with lithium carbonate in dimethylformamide. The resultant mixture of 5α-ergosta-1,7-dien-3-one (7) and ergosta-4,7-dien-3-one (8) was separated by chromatography.

Acetylation of the dienone (8) with isopropenyl acetate in benzene (toluene-*p*-sulphonic acid as catalyst) gave the enol acetate (9), which was reduced immediately with an excess of sodium borohydride. The reduction product was essentially the dienol (1), contaminated by small amounts of the 3α-isomer. We were unable to obtain a sample of compound (1) which furnished satisfactory analytical results, although analytically satisfactory samples of the acetate and benzoate could be obtained from this material.

Alternatively, 5α-ergost-7-en-3-one (5; R^1 = O) was brominated and dehydrobrominated as before, and the mixture of dienones (7) and (8) acetylated and reduced as for (9). The product was dissolved in dichloromethane and treated with a solution of 4-phenyl-1,2,4-triazoline-3,5-dione to yield the adduct (10), from which (1) was regenerated by reduction with lithium aluminium hydride.

Again satisfactory analytical results could not be obtained, but samples of (1) and its acetate, benzoate, and adduct with 4-phenyl-1,2,4-triazoline-3,5-dione were identical with those obtained by Barton's group.[4]

During the investigation of an alternative route to the dienol (1), we reduced the 3-tetrahydropyranyl ether of 22,23-epoxy-22,23-dihydroergosterol, and obtained, by chromatography, three major products. Removal of the tetrahydropyranyl group gave (22*S*)-3β,22- (2), (22*R*)-3β,22- (3), and (23*RS*)-3β,23- (4) dihydroxy-

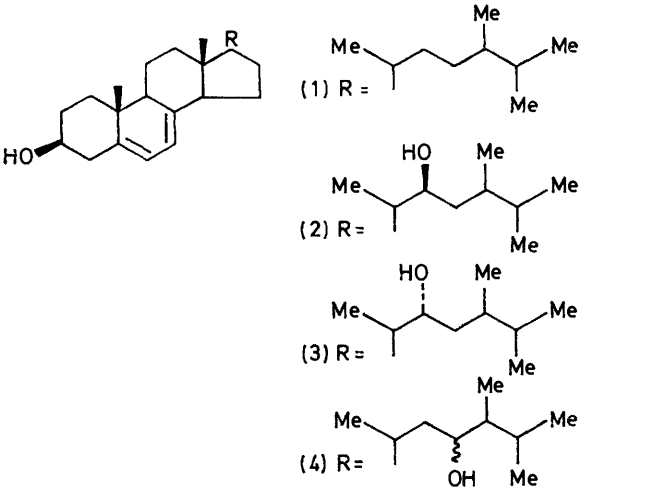
[1] A. Windaus and R. Länger, *Annalen*, 1934, **508**, 105.

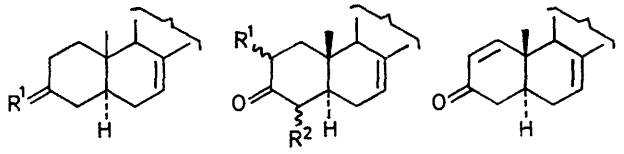
[2] N. J. McCorkindale, S. A. Hutchinson, B. A. Pursey, W. T. Scott, and R. Wheeler, *Phytochemistry*, 1969, **8**, 861; P. Singh and S. Rangaswami, *Current Sci.*, 1966, **35**, 515; A. Santos Ruiz, *Anales real acad. farm.*, 1943, **3**, 201.

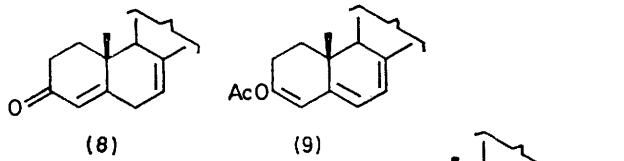
[3] D. H. R. Barton, J. E. T. Corrie, D. A. Widdowson, M. Bard, and R. A. Woods, *J.C.S. Perkin I*, 1974, 1326.

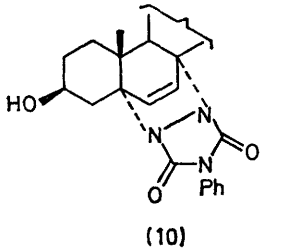
[4] D. H. R. Barton, A. A. L. Gunatilaka, T. Nakanishi, H. Patin, D. A. Widdowson, and B. R. Worth, preceding paper.

ergosta-5,7-diene, thereby demonstrating that the 22,23-epoxide is a mixture (as previously adumbrated)[5] of α- and β-epoxides. These three isomeric alcohols were characterised by mass spectrometric examination of their trimethylsilyl ethers.[5] The trimethylsilyl ether of the 22*S*-alcohol (2) showed *m/e* 187 (100%) and 173 (≪10%); that of the 22*R*-alcohol (3) showed *m/e* 187 (98%) and 173 (≪10%); and that of the 23*RS*-alcohol (4) showed *m/e* 187 (≪10%) and 173 (82%).









R as in (1) for formulae (5)—(10)

The assignments of configuration at C-22 are based on the following mutually self-consistent observations: (i) the relative specific rotations: Barton *et al.*,[6] state that for 22-alcohols, the 22*R*-epimers consistently possess a more highly positive rotation (no exceptions to this rule have been reported); (ii) oxidation of the 3-tetrahydropyranyl ethers of the 22*S*- and the 22*R*-alcohols furnished the same 22-ketone, which on reduction with lithium aluminium hydride generated predominantly, as required by theory,[6] the isomer of the 22*S*-configuration: this product was identified by the characteristic R_F value; and (iii) the i.r. spectrum of the alcohol designated as 22*S* has intense absorption at 1 064 [C(3)–O stretch] and bands of medium intensity at 1 040 [C(22)–O stretch] and 970 cm^{-1}. The i.r. spectrum of the alcohol designated 22*R* exhibited intense absorption at 1 062 [C(3)–O] and 1 035 [C(22)–O] and bands of low intensity at 980 and 970 cm^{-1}. These spectral characteristics are very similar to those reported for the isomeric (22*S*)- and (22*R*)-22-hydroxycholesterols,[7] respectively. We thus conclude that the provisional configurational assignment as *R* made by Williams *et al.*[5] to the 22-alcohol obtained during his work in this series may be incorrect.

Reduction of the mesylates of the 3-tetrahydropyranyl ethers of the isomeric 22- and 23-alcohols normally failed to yield significant quantities of 22,23-dihydroergosterol: instead elimination occurred to form ergosta-5,7,20(22)- and ergosta-5,7,23(24)-triene, respectively.

EXPERIMENTAL

Light petroleum refers to the fraction of b.p. 60—80°. Optical rotations were determined for solutions in chloroform. N.m.r. spectra were recorded with $CDCl_3$ as solvent and tetramethylsilane as internal standard. U.v. spectra were determined for solutions in ethanol unless stated otherwise and i.r. spectra for solutions in chloroform. T.l.c. and p.l.c. were carried out with Merck Kieselgel G 254.

5α-Ergosta-1,7- and Ergosta-4,7-dien-3-one.—5α-Ergost-7-en-3-one was prepared by the following improvement of the published method.[8] A stirred solution of 5α-ergost-7-en-3β-ol (2 g) in acetone (250 ml) was treated with Jones reagent (4N; 8.7 ml): 25 min later methanol (10 ml) was added, followed after a further 5 min by saturated sodium hydrogen carbonate solution (5 ml). Solvent was removed *in vacuo* and the product purified from ether–methanol to yield 5α-ergosta-7-en-3-one (1.7 g, 83%) as needles, m.p. 158—160°, $[\alpha]_D^{20}$ +15° (*c* 1.34) (lit.,[8] m.p. 159—161°, $[\alpha]_D^{20}$ +20°).

A solution of this ketone (2 g) in tetrahydrofuran (60 ml) was stirred with *NNN*-trimethylanilinium perbromide (1.85 g, 1 mol. equiv.) until reaction was complete (*ca.* 5 min). After the addition of water the mixture of 2- and 4-bromo-derivatives was isolated with ether. A solution of the bromo-compounds in freshly distilled dimethylformamide (50 ml) containing lithium carbonate (2 g) was refluxed for 45 min. The product (1.86 g) was purified by chromatography from light petroleum on silica to yield, on elution with light petroleum–ether (95 : 5), *5α-ergosta-1,7-dien-3-one* (1.0 g), which formed needles, m.p. 139—141° (from acetone), $[\alpha]_D^{20}$ 0° (*c* 0.86) (Found: C, 84.9; H, 10.8.

[5] D. R. Crump, D. H. Williams, and B. Pelc, *J.C.S. Perkin I*, 1973, 2731.

[6] D. H. R. Barton, J. P. Poyser, P. G. Sammes, M. B. Hursthouse, and S. Neidle, *Chem. Comm.*, 1971, 715; D. H. R. Barton, J. P. Poyser, and P. G. Sammes, *J.C.S. Perkin I*, 1972, 53.

[7] E. P. Burrows, G. M. Hornby, and E. Caspi, *J. Org. Chem.*, 1969, **34**, 103.

[8] C. Djerassi, G. W. Krakower, A. J. Lenin, L. H. Liu, J. S. Mills, and R. Villotti, *J. Amer. Chem. Soc.*, 1958, **80**, 6284.

$C_{28}H_{44}O$ requires C, 84.8; H, 11.2%), $\nu_{max.}$ 1 705 (C=O) and 1 675 cm^{-1} (C=C–C=O), $\lambda_{max.}$ 228 nm (ε 8 350), τ 9.43 (3 H, s, 18-H_3), 4.74br (1 H, s, 7-H), 4.12 (1 H, d, *J* 10.1 Hz, 2-H), and 2.99 (1 H, d, *J* 10.1 Hz, 1-H).

Continued elution with light petroleum–ether (4 : 1) gave *ergosta-4,7-dien-3-one* (0.65 g) as pale yellow needles, m.p. 112° (from acetone–methanol), $[\alpha]_D^{20}$ +17° (*c* 1.74) (Found: C, 84.8; H, 11.2%), $\nu_{max.}$ 1 664 (C=O) and 1 625 cm^{-1} (C=C–C=O), $\lambda_{max.}$ 238 nm (ε 11 250), τ 9.39 (3 H, s, 18-H_3), 4.8br (1 H, s, 7-H), and 4.19 (1 H, s, 4-H).

22,23-*Dihydroergosterol*.—A solution of ergosta-4,7-dien-3-one (0.9 g) in benzene (25 ml) containing isopropenyl acetate (3 ml) and toluene-*p*-sulphonic acid (0.25 g) was refluxed for 4 h; a solution of the crude enol acetate in a mixture of tetrahydrofuran (20 ml) and ethanol (70 ml) was stirred with an excess of sodium borohydride for 3 h. Purification of the product from benzene on silica (benzene as eluant) gave 22,23-dihydroergosterol (0.5 g) as needles (from ether–methanol), m.p. 132.5—134.5°, $[\alpha]_D^{20}$ −118° (*c* 1.40) (lit.,[3] m.p. 129—131°, $[\alpha]_D^{20}$ −131°), *m/e* 398 (M^+).

The *benzoate* formed plates (from ether–methanol), m.p. 155—157°, $[\alpha]_D^{20}$ −54° (*c* 1.2) (Found: C, 83.4; H, 9.8. $C_{35}H_{50}O_2$ requires C, 83.6; H, 10.0%), $\nu_{max.}$ 1 710 cm^{-1}, $\lambda_{max.}(CHCl_3)$ 282 (ε 10 800) and 272 nm (ε 10 800). The *acetate* formed plates (from ether–methanol), m.p. 152—154°, $[\alpha]_D^{20}$ −88° (*c* 0.95) (Found: C, 81.5; H, 11.1. $C_{30}H_{48}O_2$ requires C, 81.8; H, 11.0%), $\nu_{max.}$ 1 725 cm^{-1}, $\lambda_{max.}$ 281 nm (ε 11 300).

Prepared in the usual manner, the *adduct* with 4-phenyl-1,2,4-triazoline-3,5-dione separated from dichloromethane–methanol as needles, m.p. 192—194° (decomp.), $[\alpha]_D^{20}$ −166° (*c* 1.82) (Found: C, 75.3; H, 9.1; N, 7.5. $C_{36}H_{51}N_3O_3$ requires C, 75.4; H, 9.0; N, 7.3%), $\nu_{max.}$ 3 450, 1 750, and 1 688 cm^{-1}, $\lambda_{max.}$ 215 nm (ε 9 550).

Regenerated from this adduct (0.5 g) during 3 h by the action of lithium aluminium hydride (0.25 g) in boiling ether (25 ml), 22,23-dihydroergosterol (0.26 g) was identical (m.p., mixed m.p., i.r., n.m.r., and u.v. spectra, and $[\alpha]_D$) with the previously prepared specimen.

22,23-*Epoxy*-22,23-*dihydroergosterol*.—A solution of the non-crystalline adduct[5] of this epoxide and 4-phenyl-1,2,4-triazoline-3,5-dione (7 g) in ether (100 ml) containing lithium aluminium hydride (1 g) was refluxed for 3 h. Purification of the product from methanol gave the *epoxide* (4.4 g) as needles, m.p. 128—133°, as a mixture of diastereoisomers (Found: C, 78.0; H, 10.6%; M^+, 412. $C_{28}H_{44}O_2$ requires C, 81.5; H, 10.8%; *M*, 412. $C_{28}H_{44}O_2,H_2O$ requires C, 78.1; H, 10.8%; *M*, 412), $[\alpha]_D^{20}$ −135° (*c* 0.6), $\lambda_{max.}$ 292 (ε 7 066), 282 (12 566), and 271 nm (11 907).

(22S)-3β,22-, (23RS)-3β,23-, *and* (22R)-3β,22-*Dihydroxyergosta-5,7-diene*.—Prepared from 22,23-epoxy-22,23-dihydroergosterol (2 g), the 3β-*tetrahydropyranyl ether* separated from methanol containing 0.1% of pyridine as needles (1.9 g), m.p. 129—131°, $[\alpha]_D^{22}$ −68° (*c* 0.97) (Found: C, 79.8; H, 10.7%; M^+, 496. $C_{33}H_{52}O_3$ requires C, 79.8; H, 10.7%; M^+, 496). Reduction of this ether (2 g) in refluxing tetrahydrofuran (60 ml) with lithium aluminium hydride (1 g) was complete in 18 h. T.l.c. on neutral alumina [ethyl acetate–benzene–light petroleum (5 : 45 : 65)] gave fractions (a)—(c). Fraction (a), (23RS)-3β-*tetrahydropyranyloxyergosta-5,7-dien-23-ol* (0.5 g), formed needles (from methanol), m.p. 150—155°, R_F 0.26, $[\alpha]_D^{22}$ −49° (*c* 0.64) (Found: C, 79.5; H, 10.9. $C_{33}H_{54}O_3$ requires C, 79.5; H, 10.9%), $\nu_{max.}$ (Nujol) 3 600 cm^{-1} (OH), $\lambda_{max.}(CHCl_3)$ 296 (ε 8 086), 285 (15 199), and 275 nm (14 029). Hydrolysis of this ether (0.1 g) in boiling ethanol (10 ml) containing hydrochloric acid (N; 0.1 ml) during 45 min gave (quantitatively) (23RS)-*ergosta-5,7-diene*-3β,23-*diol* as needles, m.p. 160—168° (from cyclohexane), $[\alpha]_D^{22}$ −94° (*c* 1.4), M^+ 414, $\nu_{max.}$ 3 360 cm^{-1} (OH), $\lambda_{max.}$ 281 (ε 7 984) and 271 nm (7 392), τ 4.46 (2 H, ABq, *J* 6 Hz, 6- and 7-H). Fraction (b) was a mixture (R_F 0.24) of 3β-tetrahydropyranyloxyergosta-5,7-dien-22- and -23-ols which was hydrolysed to yield, after purification from methanol, (22R)-*ergosta-5,7-diene*-3β,22-*diol* as plates, m.p. 162—165°, $[\alpha]_D^{22}$ −117° (*c* 0.28), M^+ 414. Fraction (c), (22S)-3β-*tetrahydropyranyloxyergosta-5,7-dien-22-ol* (0.5 g), separated from methanol containing 0.1% pyridine as needles, m.p. 182—188°, R_F 0.22, $[\alpha]_D^{22}$ −92° (*c* 0.57) (Found: C, 79.4; H, 10.8%), $\nu_{max.}$ 1 715 cm^{-1} (C=O), $\lambda_{max.}$ 293 (ε 7 272), 282 (12 184), and 271 nm (11 615), τ 4.54 (2 H, ABq, *J* 6.7 Hz, 6- and 7-H). Hydrolysis of this ether as for the 23-isomers gave (quantitatively) (22S)-*ergosta-5,7-diene*-3β,22-*diol* as plates, m.p. 204—206°, $[\alpha]_D^{22}$ −152° (*c* 0.4) (Found: C, 77.6; H, 10.8%); M^+, 414. $C_{28}H_{46}O_2$ requires C, 81.1; H, 11.2%; *M*, 414. $C_{28}H_{46}O_2,H_2O$ requires C, 77.7; H, 11.2%; *M*, 414), $\nu_{max.}$ 3 040 (OH) and 1 715 cm^{-1} (C=O), $\lambda_{max.}$ 293 (ε 5 263), 282 (8 596), and 272 nm (8 263).

Oxidation of (22*S*)-3β-tetrahydropyranyloxyergosta-5,7-dien-22-ol (230 mg) with the Sarrett reagent gave 3β-*tetrahydropyranyloxyergosta-5,7-dien-22-one* (60 mg) as plates, m.p. 158—160° (from methanol), $[\alpha]_D^{20}$ −91° (*c* 1.15) (Found: C, 79.8; H, 10.1. $C_{33}H_{52}O_3$ requires C, 79.8; H, 10.6%). Obtained quantitatively from this tetrahydropyranyl ether, 3β-*hydroxyergosta-5,7-dien-22-one* separated from methanol as stout prisms, m.p. 139—141° (Found: C, 81.2; H, 10.5. $C_{28}H_{44}O_2$ requires C, 81.5; H, 10.8%). The same derivatives were obtained by oxidation of (22*R*)-3β-tetrahydropyranyloxyergosta-5,7-dien-22-ol.

Prepared as an unstable solid from (23*RS*)-3β-tetrahydropyranyloxy-5,7-dien-23-ol, the (23*RS*)-mesylate (0.3 g) was reduced with lithium aluminium hydride (0.3 g) in ether (60 ml) during 24 h to yield 3β-tetrahydropyranyloxyergosta-5,7,23-triene (75 mg), together with regenerated parent alcohol (100 mg). Hydrolysis of the triene (50 mg) with dilute acid gave ergosta-5,7,23-trien-3β-ol (25 mg) as needles, m.p. 147—150° (from methanol), M^+ 396, $\nu_{max.}$ 840 cm^{-1} (trisubstituted double bond). Similarly the mesylate (0.3 g) of (22*S*)-tetrahydropyranyloxyergosta-5,7-dien-22-ol gave ergosta-5,7,20(22)-trien-3β-ol (0.13 g) as plates, m.p. 123—130° (from ethanol), M^+ 396, $\nu_{max.}$ 840 cm^{-1} (trisubstituted double bond). These two trienes tenaciously retained solvent of crystallisation and failed to give acceptable elemental analyses.

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