Unsaturated Steroids. Part 3.[1] Synthesis of Steroidal 22,24(28)-Dienes,[2] Ergosta-5,7,22,24(28)-tetraen-3β-ol,[2] and Cholesta-5,7,22-trien-3β-ol

By **Alex B. Garry, John M. Midgley, W. Basil Whalley,* and Brian J. Wilkins,** The School of Pharmacy, The University, London WC1N 1AX

Dehydrobromination of 22.23-dibromo-5β-ergostane with 1.5-diazabicyclo[3.4.0]non-5-ene (DBN) gives 5β-ergosta-22.24(28)-diene (3). This structure has been confirmed by partial synthesis.

Ergosteryl acetate is readily regenerated from its adduct (8) with 4-phenyl-1.2.4-triazoline-3.5-dione by the action of warm DBN. The adduct reacts with bromine to yield the 22.23-dibromo-derivative. which forms ergosta-5.7.22,24(28)-tetraen-3β-ol (7) directly, in high yield, when heated with DBN.

The structure (9) (cholesta-5.7.22-trien-3β-ol) assigned to a metabolite of the protozoan. *Tetrahymena pyriformis*. has been confirmed by partial synthesis from stigmasterol.

DURING another investigation we planned to convert derivatives of ergosterol, *e.g.* 5β-ergost-22-ene (1) into the 22,23-dibromo-compound and thence into the 20(22),23-diene (2).

In fact, hydrogen bromide was readily abstracted from the 22,23-dibromo-steroid by 1,5-diazabicyclo-[4.3.0]non-5-ene (DBN) to yield, not the anticipated diene (2), but an isomer, 5β-ergosta-22,24(28)-diene (3). The n.m.r. spectrum exhibited signals at τ 5.12 (2 H, s, H_2-28) and 3.9—4.4 (2 H, m, H-22 and -23), and the i.r. spectrum showed $\nu_{max.}$ 1 604 (C:C stretch in a conjugated diene), 970 (out-of-plane deformation in a *trans*-di-substituted double bond), and 890 cm^{-1} (out-of-plane deformation of terminal CH_2).

Matter *et al.*[3] have developed a method for predicting the chemical shifts of olefinic protons by using additive, incremental data (*Z* values). From these *Z* values the chemical shifts were calculated for the four most probable isomeric structures (2)—(5) for our product. On this basis the most attractive candidate was (3); this assignment was confirmed (*a*) by ozonolysis to yield formaldehyde and the aldehyde (6), identical with the

[1] Part 2, N. Bosworth, A. Emke, J. M. Midgley, C. J. Moore, W. B. Whalley, G. Ferguson, and W. C. Marsh, preceding paper.

[2] Preliminary communication, A. B. Garry, J. M. Midgley, W. B. Whalley, and B. J. Wilkins, *J.C.S. Chem. Comm.*, 1972, 167.

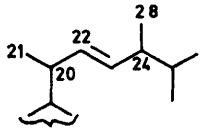
[3] U. E. Matter, G. Pascual, E. Pretsch, A. Pross, W. Simon, and S. Sternhell, *Tetrahedron*, 1969, **25**, 691, 2023.

ozonolysis product from 5β-ergost-22-ene (1), and (*b*) by partial synthesis from this hexanor-aldehyde. The configuration of (6) was deduced as 20*S* on the basis of n.m.r. data [τ 0.46 (1 H, d, $J_{20,22}$ 4 Hz)] (*cf.* ref. 4) and confirmed by the condensation of (6) with the ylide from(3-methyl-2-methylenebutyl)triphenylphosphonium bromide,[4] to give material (3) identical with the dehydrobromination product.

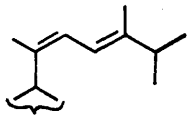
There appears to be little information available concerning the mechanism of dehydrohalogenation in aprotic solvents, but Cromwell *et al.*[5] have shown that the abstraction of halogen (to form a carbocation) occurs more readily than proton abstraction under such conditions.

Almost contemporaneously with our work, Barton *et al.*[4] showed that ergosta-5,7,22,24(28)-tetraen-3β-ol (7) is an intermediate in the biosynthesis of ergosterol by *Saccharomyces cerevisiae*. We have applied our observations to the development of a short synthesis of the tetraenol (7). Addition of bromine to the adduct (8) from ergosteryl acetate and 4-phenyl-1,2,4-triazoline-3,5-dione gave the 22,23-dibromo-derivative, which on treatment with DBN in boiling toluene formed the acetate of ergosta-5,7,22,24(28)-tetraen-3β-ol (7) directly, in high yield. The product was identical with a specimen derived from natural sources and provided by Professor Barton. The adduct (8) upon similar treatment readily gave ergosteryl acetate.

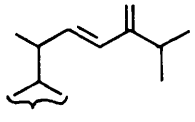
Another steroidal 5,7-diene, cholesta-5,7,22-trien-3β-ol (9) has been isolated[6] from the non-saponifiable lipids obtained when the protozoan, *Tetrahymena pyriformis*,



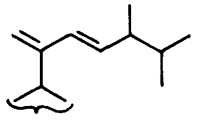
(1)



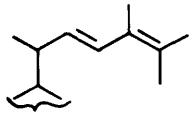
(2)



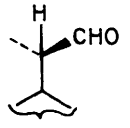
(3)



(4)

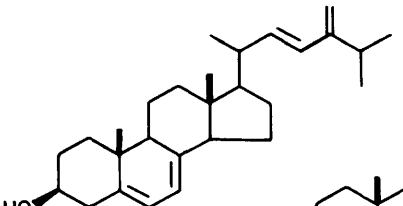


(5)

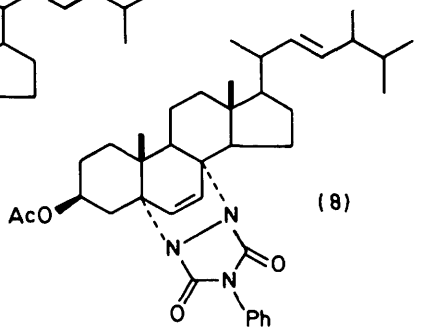


(6)

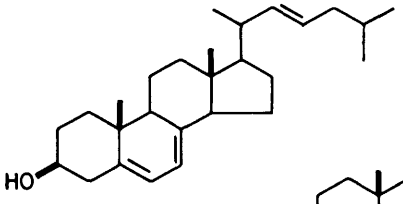
is cultured on a medium containing added cholesterol. Prior to this work the existence of (9) in biological systems had been postulated frequently,[7] but never substantiated. The structure was based upon analytical evidence, and has now been confirmed by a partial synthesis. Bromination of cholesta-5,22-dien-3β-yl



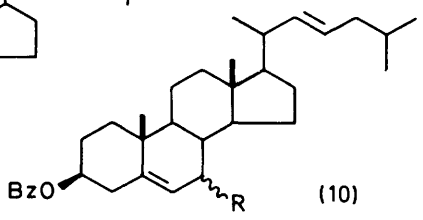
(7)



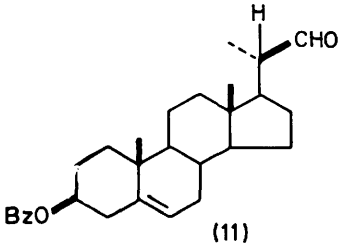
(8)



(9)



(10)



(11)

benzoate[8] (10; R = H) with 1,3-dibromo-5,5-dimethylhydantoin gave the 7-bromo-derivative (10; R = Br), from which hydrogen bromide was abstracted with triethyl phosphite to yield cholesta-5,7,22-trien-3β-yl benzoate, identical with a naturally derived specimen. A comparison of the corresponding acetates further confirmed the identity.

During the synthesis of (9) we experienced difficulty in reproducing[9] the ozonolysis of the acetate of 5,6-dibromostigmasterol. Much improved yields of the requisite aldehyde (11) were obtained from ozonolysis of the benzoate of 5,6-dichlorostigmasterol. The aldehyde (11) showed τ 0.42 (1 H, d, $J_{20,22}$ 5 Hz), thereby confirming[4] the configuration as 20*S*.

EXPERIMENTAL

Optical rotations were determined for solutions in chloroform. Light petroleum refers to the fraction of b.p. 40—60 °C.

[4] D. H. R. Barton, T. Shioiri, and D. Widdowson, *J. Chem. Soc.* (*C*), 1971, 1968.
[5] D. N. Kevill, E. D. Weiler, and N. H. Cromwell, *J. Amer. Chem. Soc.*, 1966, **88**, 4489.
[6] R. L. Conner, C. W. L. Iyengar, J. R. Landrey, and F. B. Mallory, *Tetrahedron Letters*, 1968, 6103; *J. Biol. Chem.*, 1969, **244**, 2325.
[7] *E.g.* J. van der Vliet, *Rec. Trav. chim.*, 1948, **67**, 265; T. Takagi and Y. Toyama, *Nippon Kagaku Zasshi*, 1958, **79**, 1236.
[8] W. Bergmann and J. P. Dusza, *J. Org. Chem.*, 1958, **23**, 1245.
[9] A. P. Centolella, M. E. Herand, and F. W. Heyl, *J. Amer. Chem. Soc.*, 1947, **69**, 1957.

5β-*Ergosta*-22,24(28)-*diene*.—A solution of 5β-ergost-22-en-3-one (12 g) in ethane-1,2-diol (150 ml) and ethanol (30 ml) containing hydrazine hydrate (100 ml) and potassium hydroxide (80 g) was refluxed for 0.5 h. Solvent was then distilled off until the temperature of the mixture was 200 °C. The mixture was then refluxed for 4.5 h, and the product isolated after dilution with water (1 500 ml). Purification from ether–methanol gave 5β-*ergost*-22-*ene* (6.5 g) in plates, m.p. 85—87°, $[\alpha]_D^{20}$ +0.51° (*c* 0.5) (Found: C, 87.3; H, 12.4%; M^+, 384. $C_{28}H_{48}$ requires C, 87.4; H, 12.6%; *M*, 384), τ 4.80 (2 H, m, H-22 and -23), $\nu_{max.}$ 972 cm^{-1}.

Hydrogenation of 5β-ergost-22-ene (0.1 g) dissolved in ethyl acetate (20 ml) over platinic oxide (20 mg) was complete during 2 h, to yield 5β-*ergostane* (90 mg) in plates (from acetone), m.p. 59°, $[\alpha]_D^{20}$ −0.2° (*c* 1.0) (Found: C, 86.6; H, 12.6%; M^+, 386. $C_{28}H_{50}$ requires C, 87.0; H, 13.0%; *M*, 386).

A solution of 5β-ergost-22-ene (1.55 g) in ether (30 ml) and acetic acid (8 ml) was cooled to 0 °C, and a solution of bromine (0.76 g, 1.2 mol) in carbon tetrachloride (25 ml) was added slowly (15 min) with stirring. After 3 h the mixture was diluted with ether (300 ml) and the product isolated to yield 22,23-*dibromo*-5β-*ergostane* (1.6 g), which formed prisms, m.p. 155° (from ether–methanol), $[\alpha]_D^{21}$ +3.43° (*c* 0.8), τ 5.57 (2 H, m, H-22 and -23) (Found: C, 61.9; H, 8.9; Br, 28.9%; M^+, 544. $C_{28}H_{48}Br_2$ requires C, 61.8; H, 8.9; Br, 29.4%; *M*, 544). Debromination of this compound with zinc dust regenerated, almost quantitatively, 5β-ergost-22-ene (m.p., mixed m.p., rotation, and i.r. and n.m.r. spectra).

A solution of 22,23-dibromo-5β-ergostane (1.3 g) in toluene (20 ml) containing 1,5-diazabicyclo[3.4.0]non-5-ene (DBN) (1 ml) was refluxed under nitrogen during 18 h. The cooled mixture was filtered; the filtrate was diluted with ether (100 ml) and benzene (20 ml), and the base was removed by washing (at 0 °C) with 0.5N-sulphuric acid. Purified from ether–methanol, 5β-*ergosta*-22,24(28)-*diene* (0.75 g) formed plates, m.p. 86°, $[\alpha]_D^{19}$ +12.2° (*c* 0.6) (Found: C, 87.8; H, 12.1%; M^+, 382. $C_{28}H_{46}$ requires C, 87.9; H, 12.1%; *M*, 382).

Partial Synthesis of 5β-*Ergosta*-22,24(28)-*diene*.—Ozonised oxygen was passed through a solution of 5β-ergost-22-ene (2 g) in dichloromethane (50 ml) and pyridine (0.5 ml) at −70 °C until t.l.c. (light petroleum) indicated completion of reaction. Zinc dust (3 g) and acetic acid (6 ml) were added and the mixture stirred at room temperature during 1 h. After isolation, t.l.c. showed the presence of only one compound; since the 23,24-bisnor-5β-cholan-22-al (6) (1.2 g) could not be induced to crystallise it was used directly for the next reaction. The 2,4-*dinitrophenylhydrazone* formed yellow needles, m.p. 158—161° (from ethanol), $[\alpha]_D^{20}$ +20.6° (*c* 0.4) (Found: C, 67.8; H, 8.0; N, 10.9%; M^+, 496. $C_{28}H_{40}N_4O_4$ requires C, 67.7; H, 8.1; N, 11.3%; *M*, 496).

When the crude ozonolysis mixture (after hydrolysis) was treated with Brady's reagent and the resultant 2,4-dinitrophenylhydrazones were separated by chromatography on silica, this same 2,4-dinitrophenylhydrazone was obtained, together with the 2,4-dinitrophenylhydrazone of (−)-α-methylisovaleraldehyde, m.p. 121—122° (lit.,[10] 124—124.5°), $[\alpha]_D^{20}$ −33.8° (*c* 0.75) (lit.,[10] −37.7°), M^+ 280.

A solution of (3-methyl-2-methylenebutyl)triphenylphosphonium bromide (0.2 g) in tetrahydrofuran (20 ml) was added to a solution of methyl-lithium (2.0M; 0.3 ml) under nitrogen at 0 °C. After 1 h a solution of the 22-aldehyde (6) (250 mg) in tetrahydrofuran (10 ml) was added. The solution was stirred for 24 h, and then refluxed for 1 h. The product was purified by t.l.c. (Kieselgel G254; light petroleum) and the band of R_F 0.9 separated to yield 5β-ergosta-22,24(28)-diene (30 mg), in plates, m.p. 87° (from ether–methanol), $[\alpha]_D^{20}$ +12.2° (*c* 1.0) (Found: C, 87.8; H, 11.0%; M^+, 382. Calc. for $C_{28}H_{46}$: C, 87.9; H, 12.1%; *M*, 382), identical (m.p., mixed m.p., rotation, i.r., u.v., and mass spectrum) with that obtained previously.

Ergosta-5,7,22,24(28)-*tetraen*-3β-*yl Acetate*.—To a solution of the adduct (1 g) from ergosterol acetate and 4-phenyl-1,2,4-triazoline-3,5-dione, in ether (30 ml) and acetic acid (8 ml) at 0 °C, was added dropwise with stirring a solution of bromine (420 mg) in carbon tetrachloride (25 ml). After 2 h the mixture was diluted with ether (100 ml) and the product isolated. Purified from methanol the *adduct* from 4-phenyl-1,2,4-triazoline-3,5-dione and 22,23-dibromoergosteryl acetate formed needles (1.1 g), m.p. 182—183°, $[\alpha]_D^{20}$ −40° (*c* 1.0), $\nu_{max.}$ 1 750, 1 735, 1 700, 1 252, and 772 cm^{-1}, τ 5.52 (2 H, m, H-22 and -23), 4.6 (1 H, m, H-3α), and 3.7 (2 H, q, *J* 8 Hz, H-6 and -7) (Found: C, 58.4; H, 6.5; Br, 19.2; N, 5.3. $C_{38}H_{51}Br_2N_3O_4$ requires C, 58.8; H, 6.6; Br, 20.6; N, 5.4%).

A solution of this dibromo-adduct (0.5 g) in toluene (30 ml) containing DBN (0.5 ml) was refluxed under nitrogen during 78 h; ergosta-5,7,22,24(28)-tetraen-3β-yl acetate was obtained in plates (0.16 g), m.p. and mixed m.p. 140—142° (from ethanol), $[\alpha]_D^{20}$ −42° (*c* 1.0) (lit.,[4] −41.7°), $\lambda_{max.}$ 230 (ε 21 000), 271 (11 000), and 294 nm (6 900), τ 5.5 (1 H, m, H-3α), 5.15 (2 H, s, H_2-28), and 4.7—4.1 (4 H, m, H-6, -7, -22, and -23) (Found: C, 82.2; H, 10.6. Calc. for $C_{30}H_{44}O_2$: C, 82.5; H, 10.2%).

When the adduct (0.5 g) from 4-phenyl-1,2,4-triazoline-3,5-dione and ergosteryl acetate was treated similarly, ergosteryl acetate (0.27 g, 73%), identical with an authentic specimen, was regenerated.

Ozonolysis of 5β-*Ergosta*-22,24(28)-*diene*.—Ozonolysis of this diene (0.62 g) as for 5β-ergost-22-ene followed by isolation of the products as 2,4-dinitrophenylhydrazones gave (i) the 2,4-dinitrophenylhydrazone of the hexanor-22-al (0.45 g; calc. wt. for production of 1 mole of derivative from 1 mole of diene 0.42 g), identical (i.r., u.v., n.m.r., rotation, and m.p. and mixed m.p.) with authentic specimen; (ii) the 2,4-dinitrophenylhydrazone of formaldehyde, identical with an authentic specimen; and (iii) the 2,4-*dinitrophenylhydrazone* of 28-*nor*-5β-*ergost*-22-*en*-24-*one* (3 mg) in orange needles, m.p. 153° (from ethanol), $\lambda_{max.}$ 358 (ε 22 000), 263 (46 000), and 258 nm (58 000) (Found: M^+, 564.3703. $C_{33}H_{48}N_4O_4$ requires *M*, 564.3675).

3β-*Benzoyloxypentanorcholest*-5-*en*-22-*al*.—(i) Prepared by dropwise addition of a solution of bromine (4 g) in chloroform (50 ml) to a stirred solution of stigmasteryl benzoate (10 g) in chloroform (100 ml) at 0 °C, 5α,6β-*dibromostigmasteryl benzoate* formed prisms (12 g), m.p. 135—139°, $[\alpha]_D^{20}$ −38° (*c* 1.0), $\nu_{max.}$ 1 705 cm^{-1} (ester C=O), τ 5.52 (1 H, s, H-6), 5.12 (1 H, s, H-3α), and 4.85 (2 H, m, H-22 and -23) (Found: C, 63.6; H, 8.0; Br, 22.8. $C_{36}H_{52}Br_2O_2$ requires C, 63.9; H, 7.7; Br, 23.6%). A solution of this benzoate (3.5 g) in dichloromethane (50 ml), cooled to −70 °C, was added to a solution in dichloromethane (100 ml) at −70 °C, containing ozone (0.04M). After 5 min, zinc dust (15 g) and acetic acid (50 ml) were added and the mixture was stirred at room temperature during 3 h. The product was

[10] W. Bergmann and H. A. Stansbury, *J. Org. Chem.*, 1944, **9**, 281.

purified by chromatography from benzene–light petroleum (1 : 1) on silica. Elution with benzene–light petroleum (1 : 1) gave stigmasteryl benzoate. Elution with benzene gave 3β-*benzoyloxypentanorcholest-5-en-22-al* in prisms (0.65 g), m.p. 180—183° (from aqueous acetone), $[\alpha]_D^{20}$ −42° (*c* 1.0), $\nu_{max.}$ 1 710—1 700 and 1 605 cm^{-1}, τ 9.25 (3 H, s, H_3-18), 8.91 and 8.82 (6 H, 2 s, H_3-19 and -21), 5.24 (1 H, m, H-3α), 4.54 (1 H, m, H-6), and 0.42 (1 H, d, $J_{20,22}$ 5 Hz) (Found: C, 79.8; H, 8.9. $C_{29}H_{38}O_3$ requires C, 80.1; H, 8.8%).

(ii) Iodobenzene dichloride (5 g) was added to a solution of stigmasteryl benzoate (10 g) in chloroform (100 ml); the mixture was maintained at 40 °C during 40 min, and the product purified from ethanol to give 5α,6α-*dichlorostigmasteryl benzoate* (5.2 g) as plates, m.p. 231°, $[\alpha]_D^{20}$ −12.8° (*c* 1.0), $\nu_{max.}$ 1 705 cm^{-1} (ester C=O), τ 5.72 (1 H, t, $J_{6,7}$ 8.0 Hz), 4.75 (2 H, m, H-22 and -23), and 4.60 (1 H, m, H-3α) (Found: C, 73.6; H, 8.8; Cl, 12.1. $C_{36}H_{52}Cl_2O_2$ requires C, 73.6; H, 8.9; Cl, 12.1%). The mother liquors deposited 5β,6β-*dichlorostigmasteryl benzoate*, which was purified from chloroform and then ethanol to yield needles (1.2 g), m.p. 148—150°, $[\alpha]_D^{20}$ −35° (*c* 1.0), $\nu_{max.}$ 1 705 cm^{-1} (ester, C=O), τ 5.60 (1 H, t, $J_{6,7}$ 3.1 Hz), 4.75 (2 H, m, H-22 and -23), and 4.60 (1 H, m, H-3α) (Found: C, 73.2; H, 8.6; Cl, 13.1%).

Ozonolysis of the mixed *cis*-5,6-dichloro-derivatives (4.0 g) in dichloromethane (200 ml) and pyridine (2 ml) during 30 s, by rapid addition to a solution of ozone in methylene chloride (50 ml) at −70 °C, followed by addition of zinc dust (15 g) and acetic acid (50 ml), gave 3β-benzoyloxypentanorcholest-5-en-22-al (0.66 g), identical with the specimen described in (i).

3β-*Acetoxycholesta-5,7,trans-22-triene*.—A solution of *trans*-cholesta-5,22-dien-3β-yl benzoate [8] (0.2 g) in light petroleum (20 ml) containing 1,3-dibromo-5,5-dimethylhydantoin (95 mg) and benzoyl peroxide (2 mg) was refluxed until t.l.c. [benzene–light petroleum (1 : 4)] indicated completion of reaction (½ h). The solution was then cooled, filtered, diluted with xylene (10 ml), and added to boiling xylene (40 ml) containing triethyl phosphite (0.8 ml). The product was purified from acetone to yield 3β-*benzoyloxycholesta-5,7,trans-22-triene* (42 mg) in plates, m.p. 129—131°, $[\alpha]_D^{25}$ −35° (*c* 1.0), $\nu_{max.}$ 1 710 cm^{-1} (ester C=O), $\lambda_{max.}$ 235 (ε 20 500), 271 (12 100), 281.5 (12 600), and 293 nm (6 900), τ 9.38, 9.21, 9.07, 8.97, and 8.77 (5 × 3 H, s, CMe), 4.7 (5 H, m, H-3, -6, -7, -22, and -23), 2.6 (3 H, m, ArH), and 2.1 (2 H, m, ArH) (Found: C, 83.5; H, 9.6%; M^+, 486.3478. $C_{34}H_{46}O_2$ requires C, 83.9; H, 9.5%; *M*, 486.3498).

Hydrolysis of this benzoate during 1 h with methanolic 5% potassium hydroxide gave the corresponding alcohol, which was directly acetylated (pyridine–acetic anhydride) to yield 3β-acetoxycholesta-5,7,*trans*-22-triene in plates, m.p. 136—138° (from methanol), m.p. of a mixture with a natural sample of m.p. 142°, 138—140°, M^+ 424, R_F values of natural acetate 0.09 [light petroleum–benzene (4 : 1)], 0.39 [light petroleum–benzene (1 : 1)], and 0.49 (benzene); corresponding R_F values of synthetic acetate 0.09, 0.39, and 0.49.

One of us (A. B. G.) thanks the School of Pharmacy for a Research Studentship. We acknowledge gifts of ergosterol from Organon Limited, and stigmasterol from Glaxo Research Limited and from the Upjohn Company (Kalamazoo). Professor F. B. Mallory generously provided a sample of the acetate derived from natural cholesta-5,7,22-trien-3β-ol.

[6/1116 *Received*, 11*th June*, 1976]