Degradation of the Lanosterol Side-chain [1]

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3β-Acetoxylanosta-8,24-diene (1) has been converted by a six-step degradation into 3β-acetoxy-4,4,14α-trimethyl-5α-pregn-8-en-2-one (14).

DERIVATIVES of lanosterol, such as 4,4,14α-trimethyl-5α-pregn-8-ene-3,20-dione (16), were required as substrates for microbial transformation. The following degradation of the lanosterol side-chain was made as a contribution to the general problem of degradation of steroid side-chains.[2]

Epoxidation of 3β-acetoxylanosta-8,24-diene (1) with monoperphthalic acid gave 3β-acetoxy-24,25-epoxylanost-8-ene (2) in 85% yield. Treatment of the epoxide (2) with boron trifluoride–ether afforded two isomeric compounds, $C_{32}H_{52}O_3$, separated by column chromatography. That these isomers were the ketone, 3β-acetoxylanost-8-en-24-one (3) and the aldehyde, 3β-acetoxy-24,24-dimethyl-26,27-dinorlanost-8-en-25-al (4), respectively, was deduced from their spectral properties and application of the mechanism for boron trifluoride-catalysed rearrangements of epoxides.[3] Attempts to improve the yield of the ketone (3) by using magnesium bromide as catalyst resulted in the formation of appreciable amounts of unsaturated alcohols. However, an improved yield of the ketone was obtained by oxidation of the boron trifluoride-catalysed reaction mixture with Jones reagent followed by extraction of the acid (5) formed from the aldehyde (4).

Bromination of the ketone (3) with one mol. equiv. of bromine in acetic acid afforded three products (R_F 0·37, 0·46, and 0·55 on t.l.c. in benzene). With two mol. equiv. of bromine, however, an aliquot portion showed that a new product (R_F 0·62) appeared, the spot at

R_F 0·55 had almost disappeared but the spots at R_F 0·37 and 0·46 were still present. After addition of a

[1] Preliminary publication, L. H. Briggs, J. P. Bartley, and P. S. Rutledge, *Tetrahedron Letters,* 1970, 1237.

[2] (a) W. Voser, M. V. Mijovic, O. Jeger, and L. Ruzicka, *Helv. Chim. Acta,* 1951, **34,** 1585; (b) C. S. Barnes, *Austral. J. Chem.,* 1956, **9,** 228; (c) D. Rosenthal, P. Grabowich, E. F. Sabo, and J. Fried, *J. Amer. Chem. Soc.,* 1963, **85,** 3971; (d) D. H. R. Barton, D. Giacopello, P. Manitto, and D. L. Struble, *J. Chem. Soc. (C),* 1969, 1047; (e) Y. Yanuka, R. Katz, and S. Sarel, *Chem. Comm.,* 1968, 849, 851; Y. Yanuka, R. Katz, and S. Sarel, *Tetrahedron Letters,* 1968, 1725.

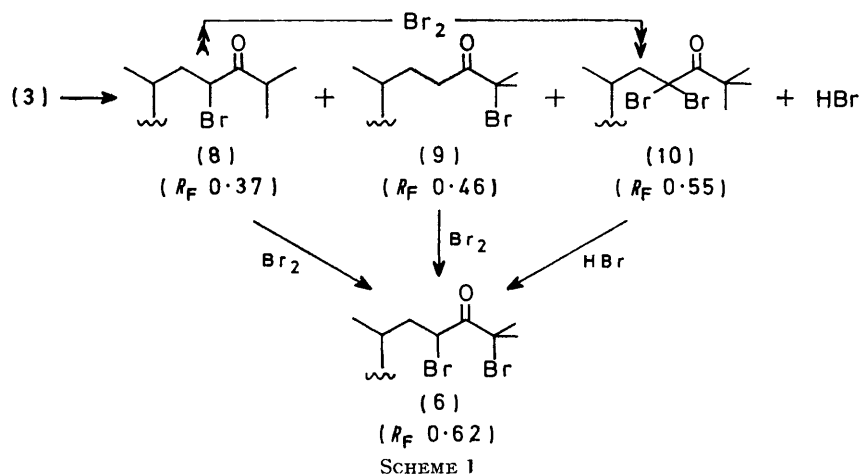[3] D. N. Kirk and M. P. Hartshorn, 'Steroid Reaction Mechanisms,' Elsevier, Amsterdam, 1968, ch. 8.

further 0·25 mol. equiv. of bromine the product with R_F 0·62 predominated with only traces of the other three products. Work-up then afforded a dibromo-ketone, $C_{32}H_{52}Br_2O_3$, (6) in 72% yield. The structure (6) is supported by the n.m.r. spectrum, particularly when compared with that of 3β-acetoxy-24,25-dibromo-lanost-8-ene (7) [4] (see Experimental section). A doublet (see later Discussion) at δ 5·18 was assigned to the proton of the group −CHBr− at C-23 while the signals at δ 1·91 and 2·06 were assigned to the protons of the −CMe₂Br group. Since these signals are further down-field than those (δ 4·1 and 1·80 respectively) of the corresponding groups in 3β-acetoxy-24,25-dibromo-lanost-8-ene (7) this can be explained by both groups flanking a carbonyl group as in (6).

Since the products formed by the addition of 1 mol. equiv. of bromine to the ketone (3) are all converted by excess of bromine to the dibromoketone (6) they are probably the two bromoketones (8) and (9), and the *gem*-dibromoketone (10). The reaction probably follows the course shown in Scheme 1.

Cotton effect at 342 nm. Since Cotton effects are not normally observed in non-rigid molecules this also suggests a relatively stable conformation about C-20, -22, -23, and -24.

Attempted conversion of the dibromoketone (6) to the dienone (11) by elimination of hydrogen bromide with collidine gave a mixture of products from which only 10% of the required dienone could be isolated.

Treatment of the dibromoketone (6) with a mixture of lithium bromide and lithium carbonate in dimethyl-formamide at 110° for 1 h, however, afforded 3β-acetoxy-lanost-8,22,25-trien-24-one (11) mixed with the Δ^7 isomer, in 67% yield. The u.v. and i.r. spectra clearly indicated a dienone system. A tertiary methyl reson-ance at δ 1·92 is consistent with the presence of the O=C−CMe=C− group in the side chain. The n.m.r. spectrum of the dienone, however, also exhibits two sets of singlet resonances at δ 0·68 and 0·73, and 0·98 and 1·01, respectively, each set of which together integrates for three protons. Now the equilibrium mixture of 3β-acetoxylanost-8-ene and 3β-acetoxylanost-7-ene

SCHEME 1

The *gem*-dibromoketone is apparently formed under conditions of kinetic control but under thermodynamic control it isomerises to the more stable dibromoketone (6). Similar conversions of *gem*-dibromoketones to flanking dibromoketones in the presence of acid have been observed in bicyclic compounds [5] and steroids.[6]

The C-23 methine proton of (6) gives rise to a broad doublet (J 12 Hz) in the n.m.r. spectrum instead of the expected triplet suggesting that there is no free rotation about the C-22−C-23 bond. The preferred conformation would probably be as shown in the Newman projection with the bromine atom and C-20 in staggered position. If one of the C-22 protons makes an angle of 90° with H-23 then its spin contribution to the coupling would be negligible [7] and the observed coupling would be expected.

The dibromoketone (6) also exhibits a strong positive

(formed by passing dry hydrogen chloride into a chloro-form solution of the former compound) [8] shows two 18-H_3 resonances at δ 0·65 (Δ^7-isomer) and 0·69 (Δ^8-isomer) and two 19-H_3 resonances at δ 0·99 (Δ^7-isomer)
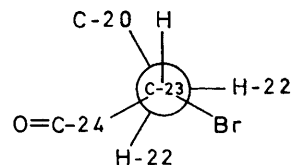
and 1·00 (Δ^8-isomer). Hence it appears that the product from the dehydrobromination reaction is the Δ^8-isomer, accompanied by the Δ^7-isomer. This is further supported by the broad resonance at δ 5·18 which

[4] K. Bloch, F. Gautschi, and J. D. Johnson, *J. Biol. Chem.*, 1957, **224**, 185.

[5] C. H. Heathcock, R. A. Badger, and J. W. Patterson, *J. Amer. Chem. Soc.*, 1967, **89**, 4133.

[6] H. O. House, 'Modern Synthetic Reactions,' Benjamin, New York, 1965, ch. 6.

[7] M. Karplus, *J. Phys. Chem.*, 1959, **30**, 11.

[8] R. E. Marker, E. L. Wittle, and L. W. Mixon, *J. Amer. Chem. Soc.*, 1937, **59**, 1368.

integrates for a fractional proton and can be assigned to the C-7 vinyl proton of the Δ^7-isomer.

Oxidation of the dienone (11) with potassium permanganate under neutral conditions afforded the aldehyde (12), with spectral data consistent with this structure. The mass spectrum showed a molecular ion at m/e 414 and a strong peak at m/e 356 corresponding to the loss of a C_3H_6O fragment by a McLafferty type rearrangement (Scheme 2).

SCHEME 2
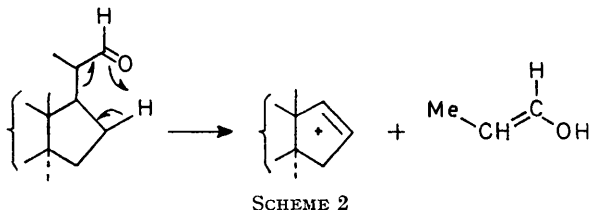
The aldehyde (12) was converted into the ketone (14) by van Rheenan's method [9] in 39% yield, a method which was found superior to Sucrow's.[10] A by-product in the reaction was an acid, probably (13), since it was shown by t.l.c. to be identical with that obtained by Jones oxidation of (12).

By standard methods, the acetoxyketone (14) was hydrolysed with alkali to the alcohol [2c] (15) which, in turn, was oxidised with Jones reagent to the required ketone (16), identical with that obtained [2c,d] from eburicoic acid.

EXPERIMENTAL

U.v. spectra were determined for solutions in ethanol with Unicam SP 800A or Perkin-Elmer 137 spectrophotometers, i.r. spectra for solutions in chloroform with a Perkin-Elmer 237 spectrophotometer, n.m.r. spectra for solutions in deuteriochloroform with a Varian A 60 spectrometer, with tetramethylsilane as internal reference, and optical rotations for solutions in chloroform with a JASCO ORD-UV 5 spectrometer.

3β-Acetoxy-24,25-epoxylanost-8-ene (2).—A 0·39M solution of monoperphthalic acid in ether (60 ml, 23 mmol) was added in three portions over 45 min to a solution of 3β-acetoxylanosta-8,24-diene [4] (10 g, 23 mmol) in chloroform (400 ml) at 5°. After standing for 2·5 h at 5° the precipitated phthalic acid was filtered off and the solution was washed with saturated sodium hydrogen carbonate solution. The product (10 g) from the work-up gave 3β-*acetoxy-24,25-epoxylanost-8-ene* (7·5 g, 75%) as plates, m.p. (from chloroform–methanol) 175—179°, $[\alpha]_D$ +74° (c 1·1), $\nu_{max.}$ 3010 (epoxide C–H), 1720 (acetate C=O), and 1250 (acetate C–O) cm⁻¹, δ 0·68 (3H, s, 18-H_3), 1·28 and 1·30 (6H, 2s, 26- and 27-H_3), 2·05 (3H, s, 3-OAc), 2·7br (1H, 24-H), and 4·56 (1H, t, J 7 Hz, 3-H) (Found: C, 79·05; H, 10·8; O, 9·8. $C_{32}H_{52}O_3$ requires C, 79·3; H, 10·8; O, 9·9%).

Boron Trifluoride-catalysed Rearrangement of 3β-Acetoxy-24,25-epoxylanost-8-ene (2).—Boron trifluoride–ether complex (6 ml, 47 mmol) was added to a solution of 3β-acetoxy-24,25-epoxylanost-8-ene (8 g, 16·5 mmol) in dry benzene (800 ml). T.l.c. of an aliquot portion indicated that no starting material remained after 2 min. Water (100 ml) was then added to quench the reaction, the organic phase was separated, diluted with ether (500 ml), and worked-up to yield an oil (8·0 g) which showed two spots (R_F 0·64 and 0·58) on t.l.c. in chloroform–benzene (2 : 1). The oil was chromatographed on deactivated alumina (320 g).

(i) Elution with light petroleum–benzene (19 : 1, 150 ml) gave a solid (0·58 g) which crystallised from chloroform–methanol as needles of 3β-*acetoxy-24,24-dimethyl-26,27-dinorlanost-8-en-25-al* (4) (0·42 g), m.p. 121—123°, $[\alpha]_D$ +62° (c 3·1), $\nu_{max.}$ 2820, 2710 (aldehyde C–H), 1725 (acetate and aldehyde C=O), and 1255 (acetate C–O) cm⁻¹, δ 0·68 (3H, s, 18-H_3), 2·08 (3H, s, 3-OAc), 4·54 (1H, m, 3-H), and 9·16 (1H, s, 24-CHO) (Found: C, 79·0; H, 10·4; O, 9·85. $C_{32}H_{52}O_3$ requires C, 79·3; H, 10·8; O, 9·9%).

(ii) Elution with light petroleum–benzene (19 : 1, 9 : 1, 17 : 3, and 4 : 1) gave 38 fractions (totalling 5·8 g) shown by t.l.c. in benzene to be a mixture of (4) and (3) (see following).

(iii) Further elution with light petroleum–benzene (4 : 1) gave a solid (0·55 g) which crystallised from chloroform–methanol as needles of 3β-*acetoxylanost-8-en-24-one* (3), m.p. 121—123°, $[\alpha]_D$ +69° (c 2·4), $\nu_{max.}$ 1730 (acetate C=O), 1715 (aliphatic C=O), and 1255 (acetate C–O) cm⁻¹, δ 0·69 (3H, s, 18-H_3), 1·08 (6H, d, J 7 Hz, 26- and 27-H_3), 2·06 (3H, s, 3-OAc), and 2·45 (3H, m, 25-H and 23-H_2) (Found: C, 79·0; H, 10·9; O, 9·9. $C_{32}H_{52}O_3$ requires C, 79·3; H, 10·8; O, 9·9%).

Oxidation of the Mixture from the Boron Trifluoride-catalysed Rearrangement of 3β-Acetoxy-24,25-epoxylanost-8-ene.—Jones reagent was added dropwise to a solution of the mixture of products from the above reaction (5·8 g) in acetone (150 ml); purified by distillation from potassium permanganate) until an orange colour persisted (2·64 ml). Water (300 ml) was then added and the precipitated solid was dissolved in ether. Extraction with 2N-sodium hydroxide solution gave, after acidification and work-up, an acidic fraction (2·1 g, 35%) and a neutral fraction (3·8 g).

The neutral fraction afforded needles (chloroform–methanol) of 3β-acetoxylanost-8-en-24-one (3·1 g) identical (m.p., i.r., and n.m.r.) with that obtained above.

The acidic fraction crystallised from methanol in plates of 3β-*acetoxy-24,24-dimethyl-26,27-dinorlanost-8-en-25-oic acid* (5) (1·5 g), m.p. 200—202°, $[\alpha]_D$ +73° (c 2·9), $\nu_{max.}$ 3500 (carboxylic acid, free OH), 2400—3100 (carboxylic acid, bonded OH), 1725 (acetate C=O), and 1700 (carboxylic acid C=O) cm⁻¹, δ 0·68 (3H, s, 18-H_3), 1·00 (3H, s, 19-H_3), 1·19 (6H, s, 24-CMe_2), 2·03 (3H, s, 3-OAc), 4·50br (1H, 3-H), and 9·31br (1H, 24-CO₂H) (Found: C, 77·1; H, 10·3; O, 13·0. $C_{32}H_{54}O_4$ requires C, 76·75; H, 10·6; O, 12·8%).

Bromination of 3β-Acetoxylanost-8-en-24-one (3).—Bromine (0·22 ml, 4·1 mmol) in acetic acid (10 ml) was added dropwise over 15 min to a stirred solution of 3β-acetoxylanost-8-en-24-one (2 g, 4·1 mmol) in chloroform (1 ml) and acetic acid (100 ml). T.l.c. of an aliquot portion in benzene indicated the presence of starting material (R_F 0·33) and three other compounds (R_F 0·37, 0·46, and 0·44). After the addition of more bromine (0·22 ml, 4·1 mmol) in acetic acid (10 ml), t.l.c. in benzene indicated the disappearance of most of the starting material and the appearance of a fourth product (R_F 0·62). The addition of excess of bromine (0·055 ml, 1·03 mmol) in acetic acid (2·5 ml) resulted in the slow conversion (30 min) of the other products into that with R_F 0·62. The yellow solution was then poured into water and the precipitated solid (85%

⁹ Y. van Rheenan, *Tetrahedron Letters*, 1969, 985.
¹⁰ W. Sucrow, *Chem. Ber.*, 1968, **101**, 2326.

yield) dissolved in ether. Work-up gave 3β-*acetoxy*-23,25-*dibromolanost*-8-*en*-24-*one* (6) (1·91 g, 72%) as needles (chloroform–methanol), m.p. 215—218°, [α]_D +97° (*c* 1·21), $v_{max.}$ 1725 (acetate C=O and α-bromoketone C=O) and 1255 (acetate C–O) cm⁻¹, δ 0·75 (3H, s, 18-H₃), 1·91 (3H, s, 26- or 27-H₃), 2·06 (6H, 2s, 3-OAc and 27- or 26-H₃), 4·54br (1H, 3-H), and 5·17 (1H, d, *J* 14 Hz, 23-H), o.r.d. (*c* 0·352; MeOH; 25°) [φ]₄₀₀ +398°, [φ]₃₄₂ +1395°, [φ]₃₁₈ 0°, [φ]₂₈₈ −1481° (Found: C, 60·4; H, 7·9; Br, 24·85. C₃₂H₅₀O₃Br₂ requires C, 59·8; H, 7·85; Br, 24·9%).

3β-Acetoxy-24,25-dibromolanost-8-ene (7) prepared by Bloch's method [4] had $v_{max.}$ 1725 (acetate C=O) and 1250 (acetate C–O) cm⁻¹, δ 0·70 (3H, s, 18-H₃), 1·01 (3H, s, 19-H₃), 1·80 (6H, 2s, 26- and 27-H₃), 1·98 (3H, s, 3-OAc), 4·1 (1H, m, 24-H), and 4·41 (1H, m, 3-H).

3β-*Acetoxylanosta*-8,22,25-*trien*-24-*one* (11).—(*a*) A solution of 3β-acetoxy-23,25-dibromolanost-8-en-24-one (1 g, 1·7 mmol) in dry dimethylformamide (20 ml) was added under nitrogen to a stirred suspension of dry lithium bromide (0·4 g, 46 mmol) and fused lithium carbonate (0·46 g, 62 mmol) in dry dimethylformamide (25 ml). The mixture was heated under reflux in a nitrogen atmosphere for 1 h and then poured into an aqueous solution of acetic acid (10%, 50 ml). The precipitated solid was dissolved in ether and worked-up to yield a yellow solid (0·63 g) which crystallised from chloroform–methanol in plates of 3β-*acetoxylanosta*-8,22,25-*trien*-24-*one* (0·5 g, 67%), m.p. 180—184°, [α] +90° (*c* 1·5), $v_{max.}$ 3080 (C=CH₂), 3020 (CH=CH), 1725 (acetate C=O), 1665 (conjugated dienone C=O), 1610 (C=C), and 1255 (acetate C–O) cm⁻¹, $λ_{max.}$ 244 (ε 13,100) nm, δ 0·68 and 0·73 (3H, 2s, 18-H₃ of Δ⁷- and Δ⁸-isomers respectively), 0·98 and 1·01 (3H, 2s, 19-H₃ of Δ⁷- and Δ⁸-isomers respectively), 1·18 (3H, d, *J* 7 Hz, 21-H₃), 1·92 (3H, s, 27-H₃), 2·04 (3H, s, 3-OAc), 5·80 (1H, d, *J* 8 Hz, 23-H), 6·68 (1H, d, *J* 8 Hz, 22-H), 6·60 (1H, s, 26-H), and 5·71 (1H, s, 26-H) (Found: C, 79·8; H, 10·0; O, 10·1. C₃₂H₄₈O₃ requires C, 79·95; H, 10·1; O, 10·0%).

(*b*) The dibromoketone (0·5 g, 8·5 mmol) was heated under reflux with collidine (5 ml) for 5 h. The precipitated collidine hydrobromide was then filtered off and the filtrate was diluted with ether (20 ml). Work-up gave a red oil (0·375 g) which was dissolved in benzene and filtered through alumina to give a yellow oil (0·15 g). Two crystallisations from chloroform–methanol gave plates of 3β-acetoxylanosta-8,22,25-trien-24-one (50 mg, 10%), m.p. 179—182°, identical with that obtained above.

3β-*Acetoxy*-4,4,14α-*trimethyl*-5α-*pregn*-8-*en*-20α-*carbaldehyde* (12).—Potassium permanganate (0·88 g, 5·6 mmol) was added slowly over 50 min to a stirred solution of 3β-

acetoxylanosta-8,22,25-trien-24-one (1 g, 2 mmol) in redistilled acetone (150 ml) containing water (2 drops) and the mixture was stirred for 15 min. The precipitated manganese dioxide was filtered off through Celite and the solvent was removed from the filtrate to yield a yellow oil (0·75 g). Preparative t.l.c. in benzene–chloroform (1 : 1) gave the *aldehyde* (0·57 g, 65%) as plates, m.p. 110—111° (from acetone), [α]_D +32° (*c* 2·9), $v_{max.}$ 2720 (aldehyde C–H) and 1725 (acetate and aldehyde C=O) cm⁻¹, δ 0·77 (3H, s, 18-H₃), 1·01 (3H, s, 19-H₃), 2·04 (3H, s, 3-OAc), and 9·22 (1H, d, *J* 3·5 Hz, 20-CHO), *m/e* 414, 399, 386, 371, 356, 339, and 311 (Found: C, 78·6; H, 10·2; O, 11·4. C₂₇H₄₂O₃ requires C, 78·2; H, 10·2; O, 11·6%).

Jones oxidation of the aldehyde afforded what is probably the acid, 3β-*acetoxy*-4,4,14α-*trimethyl*-5α-*pregn*-8-*ene*-20α-*carboxylic acid*, needles, m.p. 200—205° (from methanol).

Oxygenation of 3β-*Acetoxy*-4,4,14α-*trimethyl*-5α-*pregn*-8-*en*-20α-*carbaldehyde* (12).—(*a*) van Rheenan's method.[9] Oxygen was bubbled for 20 h through a solution of 3β-acetoxy-4,4,14α-trimethyl-5α-pregn-8-en-20α-carbaldehyde (0·1 g, 0·24 mmol), 2,2'-dipyridylcopper(II) diacetate (3 mg), and pyridine (0·02 g) in dimethylformamide (0·3 ml) at 40°. Water (1 ml) was then added and the precipitate extracted with ether. Extraction with 2N-sodium hydroxide solution gave an acidic fraction (0·04 g) which was shown by t.l.c. to contain 3β-acetoxy-4,4,14α-trimethyl-5α-pregn-8-ene-20α-carboxylic acid (13) by comparison with the acid prepared above.

The neutral fraction, after work-up, yielded an oil (0·06 g) which crystallised from methanol in needles of 3β-acetoxy-4,4,14α-trimethyl-5α-pregn-8-en-20-one (14) (0·04 g, 39%), m.p. 166—169° (lit.,[11] 169—170°), [α]_D +115° (*c* 1·3), $v_{max.}$ 1725 (acetate C=O) and 1710 (aliphatic C=O) cm⁻¹, δ 2·12 (3H, s, 21-H₃).

(*b*) *Sucrow's method*.[10] Oxygen was bubbled for 20 min through a solution of the aldehyde (12) (0·1 g, 0·24 mmol) and potassium t-butoxide (0·1 g, 0·9 mmol) in t-butyl alcohol (9 ml). After pouring into water the mixture was extracted with ether and worked up to yield a yellow oil (0·1 g). Chromatography on alumina (15 g) with light petroleum–ether (99 : 1) gave an oil (13 mg) shown by t.l.c. in benzene to contain mostly the ketone (14).

[11] H. R. Bently, J. A. Henry, D. S. Irvine, and F. S. Spring, *J. Chem. Soc.*, 1953, 3673.