Synthesis of 24ξ,25-Dihydroxyprovitamin D_3

By **Stephen C. Eyley** and **Dudley H. Williams,*** The Chemical Laboratories, Lensfield Road, Cambridge CB2 1EW

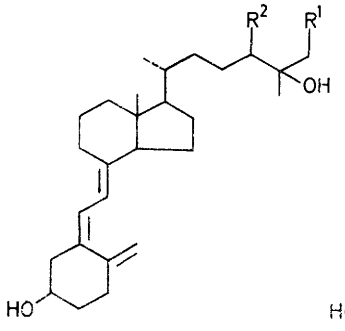
The known C-22 aldehyde obtained by degradation of ergosterol acetate (in which the ring B diene system has been protected by Diels–Alder addition of 4-phenyl-1,2,4-triazoline-3,5-dione) undergoes aldol condensation with 3-methyl-3-tetrahydropyranyloxybutan-2-one. The resulting enone gives 24ξ,25-dihydroxyprovitamin D_3 (the provitamin of the metabolite 24,25-dihydroxycholecalciferol) upon successive reductions with sodium borohydride in pyridine and lithium aluminium hydride.

INVESTIGATIONS into the mode of action of vitamin D have revealed the requirement for metabolic hydroxylation of the vitamin before manifestation of biological activity.[1] Metabolites of vitamin D may be divided into two groups, *viz.* (i) those formed from the vitamin by hydroxylation solely in the side chain, comprising 25-hydroxycholecalciferol (1),[2] 24,25-dihydroxycholecalciferol (2),[3] and 25,26-dihydroxycholecalciferol (3),[4]

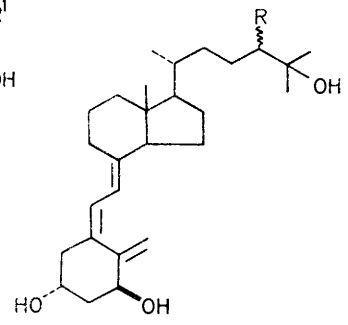
[1] (*a*) H. F. DeLuca, *Fed. Proc.*, 1974, **33**, 2211; (*b*) A. W. Normana nd H. Henry, *Recent Progr. Hormone Res.*, 1974, **30**, 431.
[2] J. W. Blunt, H. F. DeLuca, and H. K. Schnoes, *Biochemistry*, 1968, **7**, 3317.
[3] M. F. Holick, H. K. Schnoes, F. H. DeLuca, R. D. Gray, I. T. Boyle, and T. Suda, *Biochemistry*, 1972, **11**, 4251.
[4] T. Suda, H. F. DeLuca, H. K. Schnoes, Y. Tanaka, and M. F. Holick, *Biochemistry*, 1970, **9**, 4776.

and (ii) the metabolites bearing a 1α-hydroxy-group, *i.e.* 1α,25-dihydroxycholecalciferol (4) [5] and 1α,24,25-trihydroxycholecalciferol (5).[6] As all the known metabolites bear hydroxy-groups in the side chain, syntheses of metabolites in the first group are of relevance for all metabolites.

Evidence has recently been presented [7] indicating that natural 24,25-dihydroxycholecalciferol (2) possesses the 24*R*-configuration. Resolution of the epimers at C-24 of synthetic material has been achieved.[8]



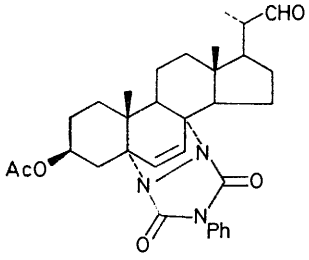
(1) $R^1 = R^2 = H$
(2) $R^1 = H, R^2 = OH$
(3) $R^1 = OH\ R^2 = H$



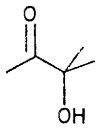
(4) R = H
(5) R = OH

Synthetic routes to 25-hydroxycholecalciferol (1),[9] 24,25-dihydroxycholecalciferol (2),[10] and 25,26-dihydroxycholecalciferol (3) [11] have appeared, all involving the production of the provitamin metabolites *via* the classical sequence of bromination–dehydrobromination to introduce the 7,8-double bond into a hydroxylated cholesterol at a late stage in the synthesis. Yields for this step are seldom reported to be above 30%. We wished to develop a general strategy towards syntheses of metabolites solely hydroxylated in the side chain which bypassed this relatively inefficient step. Barton, in studies of ergosterol biosynthesis,[12] had succeeded in protecting the 5,7-diene system of ergosteryl acetate by formation of the Diels–Alder adduct with 4-phenyl-1,2,4-triazoline-3,5-dione, and in cleaving the side chain double bond, by ozonolysis, to give the aldehyde (6). Efficient regeneration of the 5,7-diene system was also described. The C-22 aldehyde (6) becomes a key intermediate in syntheses of metabolites of vitamin D.

The readily available 3-hydroxy-3-methylbutan-2-one (7) contains both the carbon skeleton and the oxygenation pattern required for a synthesis of 24,25-dihydroxycholecalciferol (2) *via* a C_{22} steroidal intermediate. Alkylation of derivatives of the ketone with the C-22



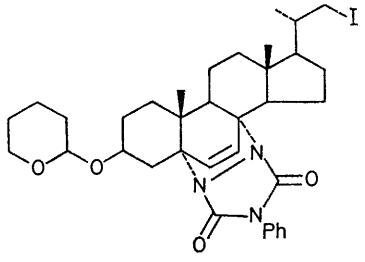
(6)



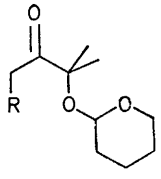
(7)

iodide (8) was investigated. This iodide was synthesised in high yield from the aldehyde (6) by standard chemical manipulations.

The sodium enolate of the β-oxo-ester (9a) did not undergo alkylation by the C-22 iodide (8) in a variety of solvents. The magnesium enolate of the β-oxo-acid (9b), formed from the ketone and 'methoxymagnesium carbonate' [13] was similarly unreactive towards the steroidal iodide. The magnesium chelate derived from 3-hydroxy-3-methylbutan-2-one was alkylated efficiently by simple aliphatic iodides, but no alkylation was observed with the C-22 iodide (8). The preformed sodium and lithium enolates of the methyl ketone (9c) were not alkylated by the iodide in a series of solvents, even after long reaction times.



(8)



(9) a; R = CO_2Et
b; R = CO_2H
c; R = H

Attempts to displace the halogen with the lithium salt of 3-methyl-3-tetrahydropyranyloxybut-1-yne, under conditions which had been described for another C-22 iodide,[14] resulted in loss of the triazoline group in preference to reaction in the side chain. The product, isolable in *ca.* 60% yield, was 22-iodo-3β-tetrahydropyranyloxydinorchola-5,7-diene.

[5] (*a*) D. E. M. Lawson, D. R. Fraser, E. Kodicek, H. R. Morris, and D. H. Williams, *Nature*, 1971, **230**, 228; (*b*) M. F. Holick, H. K. Schnoes, H. K. DeLuca, T. Suda, and R. J. Cousins, *Biochemistry*, 1971, **10**, 2799.

[6] M. F. Holick, A. Kleiner-Bossaller, H. K. Schnoes, P. M. Kasten, I. T. Boyle, and H. F. DeLuca, *J. Biol. Chem.*, 1973, **248**, 6691.

[7] N. Koizumi, M. Morisaki, N. Ikekawa, A. Suzuki, and T. Takeshita, *Tetrahedron Letters*, 1975, 2203, and references therein.

[8] M. Seki, N. Koizumi, M. Morisaki, and N. Ikekawa, *Tetrahedron Letters*, 1975, 15.

[9] J. W. Blunt and H. F. DeLuca, *Biochemistry*, 1969, **8**, 671; see also T. A. Narwid, K. E. Cooney, and M. R. Uskoković, *Helv. Chim. Acta*, 1974, **57**, 771; M. Morisaki, J. Rubio-Lightbourn, and N. Ikekawa, *Chem. and Pharm. Bull.* (*Japan*), 1973, **21**, 457.

[10] (*a*) J. Redel, P. Bell, F. Delbarre, and E. Kodicek, *Compt. rend.*, 1974, **278**, 529; (*b*) H-Y. Lam, H. K. Schnoes, H. F. DeLuca, and T. C. Chen, *Biochemistry*, 1973, **12**, 4851.

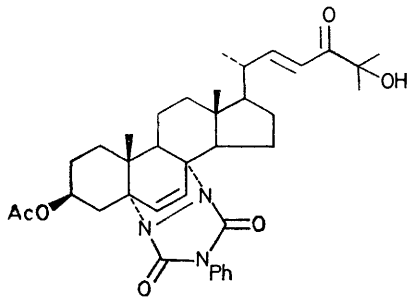
[11] J. Redel, P. Bell, E. Kodicek *Compt. rend.* 1973, **276**, 2907; see also M. Seki, J. Rubio-Lightbourn, M. Morisaki, and N. Ikekawa, *Chem. and Pharm. Bull.* (*Japan*), 1973, **21**, 2783.

[12] D. H. R. Barton, T. Shioiri, and D. A. Widdowson, *J. Chem. Soc.* (*C*), 1971, 1968.

[13] L. Crombie, P. Hemesley, and G. Pattenden, *Tetrahedron Letters*, 1968, 3021.

[14] J. J. Partridge, S. Faber, and M. R. Uskoković, *Helv. Chim. Acta*, 1974, **57**, 764.

An electrophilic C_{22} component alternative to the iodide (8) is the aldehyde. An aldol condensation

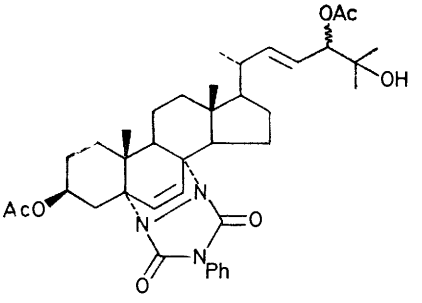


(10)

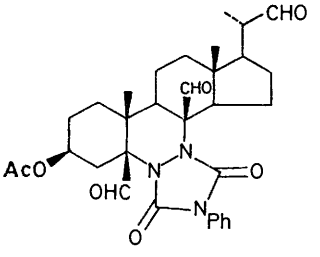
between the preformed enolate of 3-methyl-3-tetrahydropyranyloxybutan-2-one (9c) (generated by treatment of the ketone with lithium di-isopropylamide in tetrahydrofuran) and the aldehyde (6) proceeded smoothly to give, after acidic work-up, the enone (10).

It was necessary to prove that stereochemical integrity at C-20 had been maintained in the aldol condensation. Reduction of the enone (10) with lithium aluminium hydride at −70 °C afforded, after acetylation, the diene (11). Attempts to ozonolyse this compound to the aldehyde (6) to check stereochemistry at C-20 by n.m.r. comparison with authentic material indicated that the 6,7-double bond underwent preferential reaction with ozone. An excess of ozone was therefore used to obtain the trialdehyde (12).

The pure (20*S*)-trialdehyde (12) was prepared by treatment of the triazolinedione adduct of ergosteryl acetate with an excess of ozone, followed by a reductive



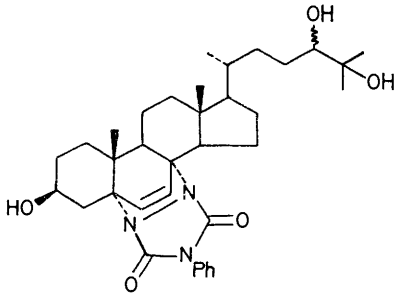
(11)



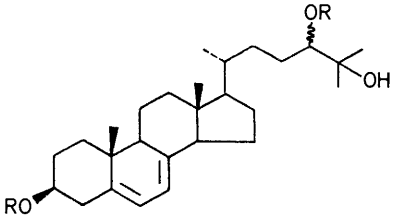
(12)

work-up under conditions which resulted in no epimerisation. Chromatography of a sample of this trialdehyde over alumina resulted in partial epimerisation. Further epimerisation was not observed on treatment with triethylamine. The two C-20 epimers give rise to distinct 22-H resonances, the chemical shift of the (presumed) 7-H also proving sensitive to configuration at C-20. The 21-Me doublet also appears at higher field in the spectrum of the 20*R*-isomer. The n.m.r. spectrum of the trialdehyde obtained from the aldol product showed no resonances due to the 20*R*-isomer, proving that the desired stereochemistry at C-20 was present in the enone (10).

Reduction of the enone (10) with sodium borohydride in pyridine solution, an efficient reagent for reduction of αβ-unsaturated ketones to saturated alcohols,[15] gave the 3β,24ξ,25-triol (13) in high yield.



(13)



(14) R = H
(15) R = Ac

Generation of 3β,24ξ,25-trihydroxycholesta-5,7-diene (14), the provitamin of the metabolite 24,25-dihydroxycholecalciferol (2), from the adduct (13), was accomplished by treatment with lithium aluminium hydride in tetrahydrofuran.

Evidence that the expected mixture of C-24 epimers had been formed in the reduction of the enone (10) came from the n.m.r. spectrum of the prometabolite 3,24-diacetate (15). The 26- and 27-methyl groups are accidentally equivalent, giving rise to a singlet at δ 1.27. As the chiral centre at C-24 is relatively remote from the steroid nucleus, the mixture of diastereoisomeric diacetates (15) would be expected effectively to behave as a mixture of enantiomers towards a paramagnetic shift reagent. The chiral shift reagent tris-[3-(2,2,2-trifluoro-1-hydroxyethylidene)camphorato]europium(III) separated the terminal methyl groups into two pairs of signals, appearing at δ 1.9 and 1.65 after the addition of 0.78 mol. equiv. of shift reagent. The dual nature of

[15] W. R. Jackson and A. Zurqiyah, *J. Chem. Soc.* (*C*), 1965 5280.

these resonances and the C-18 protons' signal at δ 0,7 indicated that a mixture of C-24 epimers was present. The signal due to the C-24 acetoxy-group was also a composite of two resonances. The relative intensities of the various dual resonances indicated that in twice crystallised 3,24-diacetate (15) the 24*S*- and 24*R*-isomers were present in similar amounts.

The overall yield of 3β,24ξ,25-trihydroxycholesta-5,7-diene (14) from the aldehyde (6) was 38%. This three-step sequence represents a reasonably efficient, convenient synthesis of the prometabolite.

EXPERIMENTAL

N.m.r. spectra were recorded at 100 MHz on a Varian HA 100 spectrometer at normal probe temperatures and at 80 MHz spectra on a Varian CFT-20 spectrometer, with Me_4Si as internal standard. I.r. spectra were recorded on a Perkin-Elmer 257 spectrophotometer. U.v. spectra were recorded for solutions in ethanol. Mass spectra were obtained with an A.E.I. MS9, MS12, or MS30 spectrometer (—RDA refers to retro-Diels–Alder loss of 4-phenyl-1,2,4-triazoline-3,5-dione). Silica GF_{254} adsorbent was used for t.l.c.

3-Methyl-3-(tetrahydropyran-2-yloxy)butan-2-one (9c).—A mixture of 3-hydroxy-3-methylbutan-2-one (33 g, 0.32 mol) and 2,3-dihydropyran (35 g, 0.42 mol) was treated with concentrated hydrochloric acid (0.2 ml), the temperature being maintained close to 20 °C by a cooling bath. After 12 h at room temperature solid sodium carbonate was added to neutralise the acid, the mixture was filtered, and the excess of dihydropyran was evaporated off under reduced pressure. Distillation gave *compound* (9c) (49.1 g, 82.5%), b.p. 61—63° at 0.15 mmHg; $\nu_{max.}$ (film) 1 720 cm^{-1}; δ($CDCl_3$) 4.6 (1 H, m, O·CH·O), 4.0—3.3 (2 H, 2m, CH_2·O), 2.18 (3 H, s, Ac), and 1.30 and 2.34 (2s, 2Me); *m/e* 186 (M^+, 0.07%), 143 (15), 128 (6), 101 (7), and 85 (100).

3β-Acetoxy-25-hydroxy-4′-phenyl-5α,8α-[1′,2′]-1′,2′,4′-triazolidinocholesta-6,22-diene-3′,5′,24-trione (10).—Butyllithium (1.5M-solution in hexane; 2.68 ml) was added to a stirred, cooled (0 °C) solution of di-isopropylamine (400 mg, 4 mmol) in dry tetrahydrofuran (5.4 ml). 3-Methyl-3-(tetrahydropyran-2-yloxy)butan-2-one (9c) (683 mg, 3.67 mmol) in dry tetrahydrofuran (2 ml) was added dropwise at 0 °C over 15 min. The solution was stirred at 0 °C for a further 1 h, then cooled to —70 °C, and a solution of the aldehyde (6) (1.67 g, 3.06 mmol) in dry tetrahydrofuran (20 ml) was added. The temperature was raised to —20 °C, and held there for 3 h. Glacial acetic acid (0.5 ml) was added at —20 °C, and the solution was brought to room temperature. Ether (200 ml) and water (100 ml) were added and the organic layer was separated and washed with dilute hydrochloric acid, sodium hydrogen carbonate solution, and water. Concentration gave the crude product (1.95 g), which was dissolved in tetrahydrofuran (25 ml) containing 1.5N-hydrochloric acid (3 ml). After 3 h at room temperature, the mixture was diluted with ether (150 ml), washed with dilute sodium carbonate solution (5%; 50 ml) and water, and dried (Na_2SO_4). Concentration gave the crude product (1.85 g). Chromatography over silica (petroleum–acetone as eluant) gave the *enone* (10) (935 mg, 48%) as a foam homogeneous by t.l.c. (R_F 0.3 in acetone–petroleum, 2 : 3); $\lambda_{max.}$ 255 nm (ε 4 400); $\nu_{max.}$ (Nujol) 3 400, 1 750, 1 730, and 1 700 cm^{-1}; δ($CDCl_3$) 7.4 (5 H, m, Ph), 7.07 (1 H, dd, *J* 15 and 8 Hz, 22-H), 6.4 (d, *J* 15 Hz, 23-H), overlapping 6.43 and 6.29 (ABq, J_{AB} 8 Hz, 6- and 7-H), 5.5 (1 H, m, 3α-H), 3.3 (1 H, m, 9-H), 2.03 (s, 3β-OAc), 1.4 (s, 26- and 27-H), 1.18 (d, *J* 6 Hz, 21-H), 1.02 (s, 19-H), and 0.88 (s, 18-H); *m/e* 629 (M^+) absent, 452 (M^+ — RDA — H_2), 394 (M^+ — RDA — AcOH, 94%), 392 (M^+ — RDA — H_2 — AcOH, 65), 379 (8), 253 (22), 251 (29), and 177 (100).

Degradation of the Enone (10) *to 3β-Acetoxy-3′,5′-dioxo-4′-phenyl-5α,8α-[1′,2′]-1′,2′,4′-triazolidino-6,7-seco-23,24-dinorcholane-6,7,22-trial* (12).—The enone (10) (150 mg, 0.238 mmol) in dry ether (15 ml) was cooled to —70 °C and lithium aluminium hydride (15 mg, 0.4 mmol) was added. After 3 h at —70 °C, wet ether (20 ml) was added, followed by water (10 ml). The organic layer was separated, filtered, and dried (Na_2SO_4). The crude product (135 mg) obtained by concentration was acetylated (acetic anhydride–pyridine) to give, after preparative t.l.c. (silica; petroleum–acetone) *3′,5′-dioxo-4′-phenyl-5α,8α-[1′,2′]-1′,2′,4′-triazolidinocholesta-6,22-diene-3β,24ξ,25-triol 3β,24-diacetate* (11) (113 mg, 70%); δ($CDCl_3$) 7.4 (5 H, m, Ph), 6.39 and 6.24 (2 H, ABq, J_{AB} 8 Hz, 6- and 7-H), 5.5 (2 H, m, 3α- and 24-H), 5.1 (2 H, m, 22- and 23-H), 3.25 (1 H, m, 9α-H), 2.04 (s, 24-OAc), 1.99 (s, 3β-OAc), 1.17 (s, 26- and 27-H), 0.98 (s, 19-H), and 0.81 (s, 18-H).

The diacetate (100 mg, 0.147 mmol) was treated with an excess of ozone at —70 °C in 1% pyridine–methylene chloride (50 ml). Dimethyl sulphide (1 ml) was added at —70 °C and the mixture warmed to room temperature, extracted with 1% hydrochloric acid, washed with water, and dried (Na_2SO_4). Evaporation gave the product as a foam which was purified by preparative t.l.c. to give the *(20S)-trialdehyde* (12) (74 mg, 86%), δ($CDCl_3$) 9.98 (1 H, s, 7-H), 9.72 (s, 6-H), 9.58 (d, *J* 3 Hz, 22-H), 7.42 (5 H, m, Ph), 5.1 (1 H, m, 3α-H), 2.15 (s, 3β-OAc), 1.19 (s, 19-H), 1.15 (d, *J* 6 Hz, 21-H), and 0.97 (s, 18-H); *m/e* 577 (M^+, 4%), 548 (26), 520 (31), 504 (56), 488 (100), 459 (46), and 177 (75).

Preparation of the Trialdehyde (12).—A solution of 3′,5′-dioxo-4′-phenyl-5α,8α-[1′,2′]-1′,2′,4′-triazolidinoergosta-6,22-dien-3β-yl acetate (300 mg) in 1% pyridine–methylene chloride (40 ml) was cooled to —70 °C and ozonised oxygen was passed through until a blue colour appeared. The solution was purged with nitrogen for 5 min and dimethyl sulphide (1 ml) was added at —70 °C. The solution was warmed to room temperature and washed with 1% hydrochloric acid (10 ml) and water (10 ml). The dried (Na_2SO_4) organic phase was concentrated to give a white solid (322 mg), which was purified by preparative t.l.c. (silica; acetone–petroleum, 1 : 1) to give the pure (20*S*)-trialdehyde (253 mg, 90%). Chromatography over alumina gave a mixture of C-20 epimers (*S* : *R* *ca.* 4 : 1); δ($CDCl_3$) (resonances due to 20*R*-epimer) 9.94 (s, 7-H), 9.52 (d, *J* 3 Hz, 22-H), and 1.07 (d, *J* 6 Hz, 21-H).

3′,5′-Dioxo-4′-phenyl-5α,8α-[1′,2′]-1′,2′,4′-triazolidinocholest-6-ene-3β,24ξ,25-triol (13).—The enone (10) (570 mg, 0.905 mmol) was dissolved in a solution of sodium borohydride (140 mg, 3.7 mmol) in dry pyridine (15 ml). After 48 h the mixture was poured into water (100 ml) and the aqueous layer extracted twice with ether (100 ml). The combined organic phases were washed with 2N-hydrochloric acid (50 ml), sodium carbonate solution (50 ml), and water, dried (Na_2SO_4), and concentrated, and the residue (652 mg) was chromatographed over silica to give the *triol* (13) (452 mg, 84%), homogeneous by t.l.c. (R_F 0.3 in acetone–petroleum, 1 : 1), as crystals (from benzene), m.p. 148—152° (monobenzene solvate); $\lambda_{max.}$ 255 nm (ε 4 600); $\nu_{max.}$ (Nujol) 3 500, 1 750, and 1 700 cm^{-1}; δ($CDCl_3$) 7.42

(11 H, m, Ph and C_6H_6), 6.40 and 6.25 (ABq, J_{AB} 8 Hz, 6- and 7-H), 5.4 (1 H, m, 3α-H), 3.2 (2 H, m, 9- and 24-H), 1.20 and 1.15 (2s, 26- and 27-H), 0.97 (s, 19-H), and 0.83 (s, 18-H); *m/e* 593 (M^+) absent, 416 (M^+ − RDA, 20%), 414 (M^+ − RDA − H_2, 15), 398 (416 − H_2O, 12), 383 (14), 383 (14), 381 (8), 253 (11), 251 (17), and 177 (100).

Cholesta-5,7-diene-3β,24ξ,25-triol (14).—The triol (13) (400 mg, 0.675 mmol) was reduced with lithium aluminium hydride (300 mg) in tetrahydrofuran (50 ml) at reflux temperature for 12 h. Preparative t.l.c. gave *cholesta-5,7-diene-3β,24ξ,25-triol* (14) (264 mg, 94%), m.p. 164—166° (from methanol); $\lambda_{max.}$ 272, 282, and 294 nm (ε_{282} 11 800); $\nu_{max.}$ (Nujol) 3 450 cm^{-1}; δ($CDCl_3$) 5.40—4.65 (2 H, 2m, 6- and 7-H), 3.61 (1 H, m, 3α-H), 3.33 (1 H, m, 24-H), 1.27 (s, 26- and 27-H), 1.02 (s, 19-H), and 0.70 (s, 18-H); *m/e* 416 (M^+, 100%), 398 (M^+ − H_2O, 13), 383 (M^+ − H_2O − CH_3, 61), 357 (26), and 253 (17). Acetylation with acetic anhydride–pyridine gave the *diacetate* (15), homogeneous by t.l.c., m.p. 153—159° (from methanol); $\lambda_{max.}$ 272, 282, and 293 nm (ε_{282} 11 600); δ($CDCl_3$) 5.67—5.10 (2 H, 2m, 6- and 7-H), 4.75 (2 H, m, 3α- and 24-H), 2.12 (s, 24-OAc), 2.05 (s, 3β-OAc), 1.27 (s, 26- and 27-H), 1.00 (s, 19-H), and 0.62 (s, 18-H); *m/e* 500 (M^+, 2%), 440 (32), 398 (27), 380 (100), 365 (37), and 253 (55).

We thank Philips-Duphar for a gift of ergosterol. S. C. E. thanks the S.R.C. for financial support.

[5/1616 *Received*, 15*th August*, 1975]