Synthesis of 25-Hydroxyprovitamin D_3 and 25ξ,26-Dihydroxyprovitamin D_3

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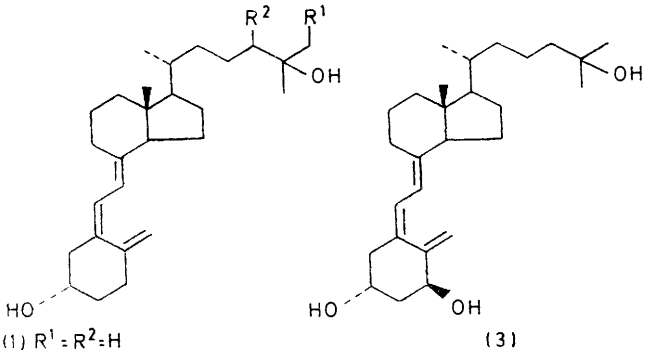
The known C-22 aldehyde derived by degradation of ergosterol (in which the ring B diene system has been protected by reaction with 4-phenyl-1.2.4-triazoline-3.5-dione) reacts with the Grignard reagent derived from 4-chloro-2-methylbut-1-ene. The mesylate of the resulting C-22 alcohol undergoes reductive elimination, and the side chain double bond has been elaborated to give provitamins D_3 hydroxylated at C-25 or at C-25 and C-26. These provitamins are the precursors of known metabolites.

25-HYDROXYCHOLECALCIFEROL (1) is an obligatory metabolic intermediate in the formation of 24,25-dihydroxycholecalciferol (2) and in the formation of 1α,25-dihydroxycholecalciferol (3), the hormonally active form of vitamin D_3 in the intestine.[1] A fourth metabolite, 25,26-dihydroxycholecalciferol (4),[2] has been the subject of little investigation to date. The configuration at C-25 in natural 25,26-dihydroxycholecalciferol is unknown.

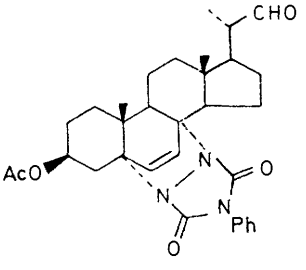


(1) $R^1 = R^2 = H$
(2) $R^1 = H$, $R^2 = OH$
(4) $R^1 = OH$, $R^2 = H$

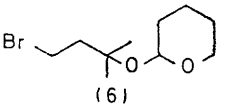
(3)

The preceding paper [3] indicated the general strategy on which syntheses of the metabolites (1), (2), and (4) have been based, *i.e. via* hydroxylated cholesterol intermediates, and described our approach to the provitamin form of 24ξ,25-dihydroxycholecalciferol (2) *via* the key intermediate aldehyde (5), derived from ergosterol.[4]

This methodology has been extended to provide syntheses of the provitamins corresponding to the metabolites 25-hydroxycholecalciferol (1) and 25,26-dihydroxycholecalciferol (4), the latter as a 1 : 1 mixture of C-25 epimers.



(5)



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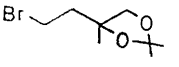


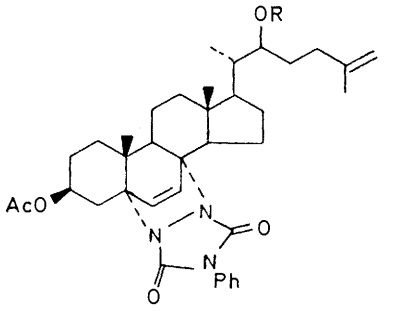
(7)

A route which appears attractive centres on the reaction between the aldehyde (5) and metal derivatives of halides which bear the desired hydroxy-substitution in a protected form. This approach, with the halides (6) and (7), seems to offer rapid routes to 25-hydroxycholecalciferol (1) and 25,26-dihydroxycholecalciferol (4), respectively, only reductive elimination of the C-22 alcohol function and removal of the protecting groups being required to obtain the provitamins corresponding to these metabolites. Such a route to 25,26-dihydroxycholecalciferol offers an additional advantage in that the stereochemistry at C-25 in the final metabolite would be embodied in the side chain halide (7). The synthesis could thus be controlled to yield either C-25 epimer of the metabolite by using the appropriate enantiomer of the halide.

[1] A. W. Norman and H. Henry, *Recent Progr. Hormone Res.*, 1974, **30**, 431; H. F. DeLuca, *Fed. Proc.*, 1974, **33**, 2211.
[2] T. Suda, H. F. DeLuca, H. K. Schnoes, Y. Tanaka, and M. F. Holick, *Biochemistry*, 1970, **9**, 4776.
[3] S. C. Eyley and D. H. Williams, preceding paper.
[4] D. H. R. Barton, T. Shioiri, and D. A. Widdowson, *J. Chem. Soc.* (*C*), 1971, 1968.

The bromo-acetal (7) was synthesised in five steps from 2-methylallyl chloride in 31% overall yield, but this halide proved to be extremely unreactive towards magnesium and lithium. Variations in solvent (diethyl ether, tetrahydrofuran, benzene, or dimethoxymethane), in the surface area of the metal, and in methods employed to activate the metal did not produce conditions under which the required organometallic reagent could be formed in significant quantity. Similar difficulties were encountered with the tetrahydropyranyl ether (6).[5]

As attempts to add fully substituted side chains were not successful, a side chain bearing a terminal double bond was introduced. The Grignard reagent derived from 4-chloro-2-methylbut-1-ene reacted with the aldehyde (5) below −20 °C to give the C-22 alcohol (8), in 82% yield. If the reaction was performed at temperatures above −15 °C, loss of the 3β-acetate became

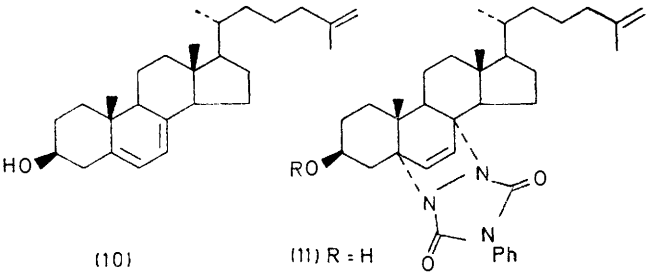


(8) R = H
(9) R = SO_2Me

significant. The doublet resonances due to the C-21 methyl protons in the C-22 alcohol (8), the C-22 mesylate (9), and the C-22 *p*-nitrobenzoate indicated that the material was predominantly one C-22 epimer,[6] duplication of signals not being detected. This also suggested that epimerisation at C-20 had not taken place during the Grignard addition.

Reductive elimination of the C-22 hydroxy-function was performed prior to elaboration of the terminal double bond to obviate the selective protection of hydroxy-groups required for the target compounds. Methanesulphonyl chloride in pyridine transformed the alcohol (8) into the mesylate (9), which on reduction with lithium aluminium hydride in tetrahydrofuran at 65 °C afforded cholesta-5,7,25-trien-3β-ol (10). The 5,7-diene was titrated with 4-phenyl-1,2,4-triazoline-3,5-dione to give the adduct (11) in 55% overall yield from the alcohol (8).

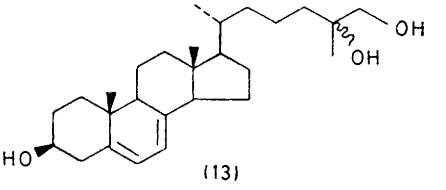
Re-protection of the diene system was necessary to allow elaboration of the terminal double bond. Selective reduction elimination of the mesylate group in the presence of the triazolinedione adduct was preferable to this sequence. The copper–aluminium hydride complex derived from copper(I) iodide and lithium trimethoxyaluminium hydride [7] was without effect on the mesylate (9). Sodium borohydride in dipolar aprotic solvents has been reported to reduce halides and



(10) (11) R = H (12) R = Ac

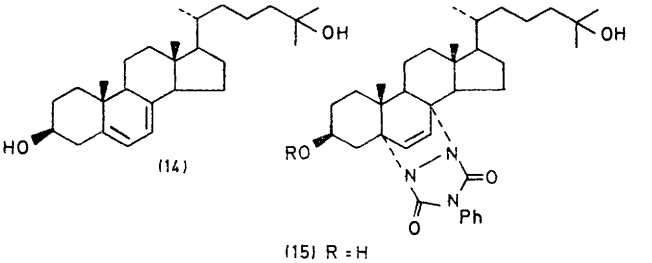
tosylates efficiently.[8] Treatment of the mesylate (9) with sodium borohydride in anhydrous dimethyl sulphoxide (90 °C; 1 h) afforded an 85% yield of the desired product (12), both the diene and acetate protecting groups remaining intact.

Reduction of the product from exposure of the adduct (11) to osmium tetraoxide with lithium aluminium hydride afforded a 37% yield of 3β,25ξ,26-trihydroxycholesta-5,7-diene (13), the provitamin of the metabolite 25,26-dihydroxycholecalciferol (4). Seeking higher yields of hydroxylation products with cheaper oxidising agents, we studied oxidations by peroxy-acid. Oxidation of (11) with performic acid was rapid and



(13)

occurred exclusively in the side chain to yield two compounds. Reduction of this mixture with lithium aluminium hydride to regenerate the 5,7-diene, and isolation of the two products by chromatography, gave comparable amounts of 3β,25ξ,26-trihydroxycholesta-5,7-diene (13) and 3β,25-dihydroxycholesta-5,7-diene (14), the provitamin form of 25-hydroxycholecalciferol



(14) (15) R = H (16) R = Ac

(1). The latter was the result of a direct hydration of the terminal double bond under the acidic conditions employed for the oxidation. Treatment of the adduct

[5] D. R. Crump and D. H. Williams, unpublished results.
[6] H. Mori, K. Shibata, K. Tsuneda, and M. Sawai, *Chem. and Pharm. Bull.* (*Japan*), 1969, **17**, 690; see also D. H. R. Barton, P. G. Feakins, J. P. Poyser, and P. G. Sammes, *J. Chem. Soc.* (*C*), 1970, 1584.
[7] S. Masamune, P. A. Rossy, and G. S. Bates, *J. Amer. Chem. Soc.*, 1973, **95**, 6452.
[8] R. O. Hutchins, D. Hoke, J. Keogh, and D. Koharski, *Tetrahedron Letters*, 1969, 3495.

(11) with formic acid gave the adduct (15) of 3β,25-dihydroxycholesta-5,7-diene.

Conversion of the olefin (12) into the alcohol (16) was achieved efficiently by reaction with mercury(II) acetate in aqueous tetrahydrofuran, followed by demercuriation with sodium borohydride.[9] Cleavage of the heterocyclic ring in the usual manner gave the prometabolite, 3β,25-dihydroxycholesta-5,7-diene (14).

To avoid formation of the 25-hydroxy-derivative in the oxidation with peroxy-acid, *m*-chloroperbenzoic acid was employed to form the 25,26-epoxides (17) from the olefin (12). The epoxides were cleaved to the 25,26-diols (18) in aqueous tetrahydrofuran, with perchloric acid as catalyst. Stereoselective epoxidation of the (13), the provitamin corresponding to the metabolite 25,26-dihydroxycholeciferol (4). This material could be resolved into its two components by high-pressure liquid chromatography.

The intermediate olefin (12), readily available in 79% yield from the aldehyde (5), may thus be conveniently transformed into the provitamins of the metabolites 25-hydroxycholecalciferol (1) and 25,26-dihydroxycholecalciferol (4).

EXPERIMENTAL

Instrumental techniques, *etc.*, were as described in the preceding paper.[3]

3β-*Acetoxy*-3′,5′-*dioxo*-4′-*phenyl*-5α,8α-[1′,2′]-1′,2′,4′-*tri*-

δ($CDCl_3$) 5.4—5.6 (2 H, m, 6- and 7-H), 4.66 (2 H, m, 26-H), 3.6 (1 H, m, 3α-H), 1.70 (s, 27-H), 0.99 (d, *J* 4 Hz, 21-H), 0.92 (s, 19-H), and 0.60 (s, 18-H); *m/e* 382 (M^+, 71%), 349 (100), 323 (46), and 253 (16).

3′,5′-Dioxo-4′-phenyl-5α,8α-[1′,2′]-1′,2′,4′-triazolidinocholesta-6,25-dien-3β-ol (11).—A solution of 4-phenyl-1,2,4-triazoline-3,5-dione in acetone was added to a solution of cholesta-5,7,25-triene-3β-ol (10) (600 mg) in acetone until a faint pink colour persisted for 30 s. The solvent was removed under reduced pressure, and the residue crystallised from ethanol to give the *adduct* (11) (670 mg, 77%), m.p. 147—151°; $\lambda_{max.}$ 255 nm (ε 4 300); $\nu_{max.}$ (Nujol) 3 500, 1 750, and 1 700 cm^{-1}; δ($CDCl_3$) 7.42 (5 H, m, Ph), 6.41 and 6.23 (2 H, ABq, J_{AB} 8 Hz, 6- and 7-H), 4.70 (2 H, m, 26-H), 4.45 (1 H, m, 38-H), 3.20 (1 H, m, 9-H), 1.72 (s, 27-H), 0.95 (s, 19-H), and 0.82 (s, 18-H); *m/e* 557 (M^+), 382 (M^+ — RDA), 380 (M^+ — RDA — H_2), 349, 323, 251, and 177 (100).

3′,5′-Dioxo-4′-phenyl-5α,8α-[1′,2′]-1′,2′,4′-triazolidinocholesta-6,25-dien-3β-yl Acetate (12).—Sodium borohydride (700 mg) was added to a stirred solution of the 22-mesylate (9) (2 g) in dry dimethyl sulphoxide (75 ml) at 90 °C. After 1 h at this temperature, the mixture was cooled to 20 °C and poured slowly into water (1.5 l). The mixture was stirred for 2 h and the product isolated by filtration. The yield of fully dried *adduct* (12), homogeneous by t.l.c. (R_F 0.7 in acetone–petroleum, 1 : 3), was 1.47 g (85%); $\nu_{max.}$ (Nujol) 1 750, 1 730, and 1 700 cm^{-1}; δ($CDCl_3$) 7.4 (5 H, m, Ph), 6.42 and 6.22 (2 H, ABq, J_{AB} 8 Hz, 6- and 7-H), 5.5 (1 H, m, 3α-H), 4.7 (2 H, m, 26-H), 3.3 (1 H, m, 9-H), 2.00 (s, 3β-OAc), 1.71 (s, 27-H), 0.99 (s, 19-H), and 0.82 (s, 18-H); *m/e* 599 (M^+, *ca.* 0.01%), 442 (M^+ — RDA — H_2, 10), 364 (M^+ — RDA — AcOH, 80), 362 (M^+ — RDA — H_2 — AcOH, 100), 253 (30), 251 (35), and 177 (95).

Oxidation of the Dienol (11) *with Osmium Tetraoxide.*—The dienol (11) (215 mg, 0.38 mmol) in pyridine (3 ml) was treated with osmium tetraoxide (100 mg, 0.39 mmol) at room temperature for 48 h. The pyridine was removed under reduced pressure and the crude product taken up in dry tetrahydrofuran (30 ml). Lithium aluminium hydride (713 mg, 19 mmol) was added and the mixture heated at reflux temperature for 16 h. The excess of hydride was decomposed with wet tetrahydrofuran, and the mixture filtered. The filtrate was concentrated and the residue dissolved in chloroform (50 ml); the solution was washed with N-hydrochloric acid and water, dried (Na_2SO_4), and evaporated. The red residue was purified on silica preparative thin-layer plates, to give *cholesta-5,7-diene-3β,25ξ,26-triol* (13) (R_F 0.5 in 4% MeOH–$CHCl_3$) (60 mg, 37%), m.p. 165—168°; $\lambda_{max.}$ 272, 282, and 294 nm (ε_{282} 11 400); $\nu_{max.}$ (Nujol) 3 480 cm^{-1}; δ($CDCl_3$) 5.65—5.38 (2 H, m, 6- and 7-H), 3.6 (1 H, m, 3α-H), 3.48 (2 H, s, 26-H), 1.22 (s, 27-H), 0.98 (s, 19-H), and 0.60 (s, 18-H); *m/e* 416 (M^+, 100%), 383 (M^+ — H_2O — CH_3, 45), 357 (M^+ — 59, 20), and 269 (61).

Treatment of the sterol with an excess of chloro(trimethyl)silane–*N*-trimethylsilylacetamide (2 : 1) in the presence of pyridine gave the tristrimethylsilyl ether; *m/e* 632 (M^+, 100%), 529 (62), 439 (529 — Me_3SiOH, 65), 413 (39), and 219 (15).

Oxidation of the Dienol (11) *with Performic Acid.*—The dienol (11) (397 mg) in 98% formic acid (10 ml) was treated with performic acid [from formic acid (5 ml) and 30% hydrogen peroxide (1 ml)]. After 1 h at room temperature, the mixture was diluted with water (15 ml) and ether layer separated, washed with saturated sodium hydrogen carbonate solution until the washings remained alkaline, dried (Na_2SO_4), and concentrated. The crude residue (341 mg) was dissolved in anhydrous tetrahydrofuran (50 ml) and heated to reflux temperature with lithium aluminium hydride (1 g) for 22 h. The reaction was worked up by the addition of ethyl acetate (2 ml) followed by dilute hydrochloric acid and ether to dissolve all solid. The organic layer was separated, washed with 5% sodium hydrogen carbonate solution and water, and dried (Na_2SO_4). Concentration gave a mixture of dienes (200 mg). Preparative t.l.c. (silica; 5% MeOH–$CHCl_3$) gave cholesta-5,7-diene-3β,25ξ,26-triol (13) (R_F 0.3; 60 mg, 20%) and cholesta-5,7-diene-3β,25-diol (14) (R_F 0.5; 50 mg, 17.5%), m.p. 150—151° (from methanol) [lit.,[10] 169—171° (from aqueous methanol)]; $\lambda_{max.}$ 272, 282, and 293 nm (ε_{282} 11 300); $\nu_{max.}$ (Nujol) 3 400 cm^{-1}; δ($CDCl_3$) 5.64—5.30 (2 H, m, 6- and 7-H), 3.44 (1 H, m, 3α-H), 1.20 (s, 26- and 27-H), 1.0 (d, *J* 4 Hz, 21-H), 0.94 (s, 19-H), and 0.56 (s, 18-H); *m/e* 400 (M^+, 100%), 382 (M^+ — H_2O, 21), 367 (M^+ — H_2O — CH_3), 271 (M^+ — side chain, 32), and 253 (18).

Cholesta-5,7-diene-3β,25-diol (14).—A solution of mercury(II) acetate (58 mg, 0.18 mmol) in water–tetrahydrofuran (1 : 1; 0.3 ml) was stirred at room temperature while a solution of the diene (12) (100 mg, 0.18 mmol) in tetrahydrofuran (1 ml) was added. After 4 h, 3N-sodium hydroxide (0.2 ml) was added, followed by sodium borohydride solution (0.5M-$NaBH_4$ in 3M-NaOH; 0.5 ml). The mixture was partitioned between water (5 ml) and chloroform (15 ml) and filtered from mercury; the organic phase was dried (Na_2SO_4) and concentrated to give *3β-acetoxy-3′,5′-dioxo-4′-phenyl-5α,8α-[1′,2′]-1′,2′,4′-triazolidinocholest-6-en-25-ol* (16) (95 mg, 92%); δ($CDCl_3$) 7.4 (5 H, m, Ph), 6.42 and 6.22 (2 H, ABq, J_{AB} 8 Hz, 6- and 7-H), 5.5 (m, 3α-H), 3.25 (1 H, m, 9-H), 1.98 (s, 3β-OAc), 1.17 (s, 26- and 27-H), 0.97 (s, 19-H), and 0.80 (s, 18-H).

Reduction with lithium aluminium hydride in the usual manner afforded cholesta-5,7-diene-3β,25-diol (14) (59 mg, 89%), m.p. 150—152° (from methanol), with spectral characteristics as above.

3′,5′-Dioxo-4′-phenyl-5α,8α-[1′,2′]-1′,2′,4′-triazolidinocholest-6-ene-3β,25ξ,26-triol (18).—A solution of the diene (12) (600 mg, 1 mmol) and *m*-chloroperbenzoic acid (1 mol. equiv.) in dichloromethane (30 ml) was left at 0 °C for 24 h, then extracted with sodium carbonate solution. The organic phase was washed and dried (Na_2SO_4); concentration gave the epoxides (17) as a non-crystalline foam (541 mg, 88%); δ($CDCl_3$) 5.4 (5 H, m, Ph), 5.34 and 5.19 (2 H, ABq, J_{AB} 8 Hz, 6- and 7-H), 5.4 (1 H, m, 3α-H), 3.15 (1 H, m, 9-H), 2.54 (s, 26-H), 2.01 (s, 3β-OAc), 1.23 (s, 27-H), 0.92 (s, 19-H), and 0.79 (s, 18-H).

The crude epoxides (520 mg) were dissolved in tetrahydrofuran (50 ml), water (10 ml), and 60% perchloric acid (0.3 ml) were added, and the mixture was kept at 0 °C for 30 h. Sodium carbonate was added to neutralise the acid, and the bulk of the solvent was removed under reduced pressure. The residue was partitioned between chloroform and water and the organic layer was separated, washed with water, dried, and concentrated to give an oil (550 mg), which was crystallised from aqueous methanol to give the *triols* (18) (402 mg, 75%), m.p. 145—150°; $\lambda_{max.}$ 255 nm (ε 4 200); $\nu_{max.}$ (Nujol) 3 350, 1 750, and 1 700 cm^{-1}; δ($CDCl_3$) 7.39 (5 H, m, Ph), 6.35 and 6.19 (2 H, ABq,

[10] J. W. Blunt and H. F. DeLuca, *Biochemistry*, 1969, **8**, 671.

J_{AB} 8 Hz, 6- and 7-H), 4.4 (1 H, m, 3α-H), 3.37 (2 H, s, 26-H), 3.14 (1 H, m, 9-H), 1.1 (s, 27-H), 0.92 (s, 19-H), and 0.78 (s, 18-H); *m/e* 591 (M^+) absent, 416 (M^+ — RDA, 43%), 515 (M^+ — RDA — H_2, 39), 396 (M^+ — RDA — H_2 — H_2O, 28), 383 (35), 251 (37), and 177 (100).

Treatment of the triols (18) (80 mg) with acetic anhydride (0.5 ml) and pyridine (0.5 ml) at room temperature for 15 h gave the *diacetates* (19) (83 mg), homogeneous by t.l.c.; δ($CDCl_3$) 7.4 (5 H, m, Ph), 6.32 and 6.13 (2 H, ABq, 6- and 7-H), 5.5 (1 H, m, 3α-H), 3.98 (2 H, s, 26-H), 3.3 (1 H, m, 9-H), 2.08 (s, 26-OAc), 2.00 (s, 3β-OAc), 1.19 (s, 27-H), 0.98 (s, 19-H), and 0.82 (s, 18-H).

Cholesta-5,7-dien-3β,25ξ,26-triol.—A mixture of the triol (18) (170 mg), lithium aluminium hydride (270 mg), and anhydrous tetrahydrofuran (30 ml) was heated at reflux temperature for 18 h under nitrogen, then cooled to room temperature, and the excess of hydride was decomposed with ethyl acetate. 2N-Hydrochloric acid and ether were added to dissolve all solid material, the layers were separated, and the aqueous layer was extracted with more ether. The combined organic extracts were washed with 5% sodium carbonate solution and water, and dried (Na_2SO_4). Concentration gave the crude product (130 mg). Preparative t.l.c. (silica; 10% MeOH–$CHCl_3$) gave the prometabolite (13), crystallised from methanol (75 mg, 73%) with spectral characteristics as recorded previously, m.p. 174—178° (lit.,[11] for a 55 : 45 mixture of C-25 epimers, 170—172°). High-pressure liquid chromatographic separation of the two epimeric components of this material was performed on ODS-Permaphase with solvent gradients from water to methanol and from water–methanol (7 : 3) to water–methanol (2 : 8).

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[11] J. Redel, P. Bell, F. Delbarre, and E. Kodicek, *Compt. rend.*, 1973, **276**, 2907.