(22*S*)-Hydroxyvitamin D_4

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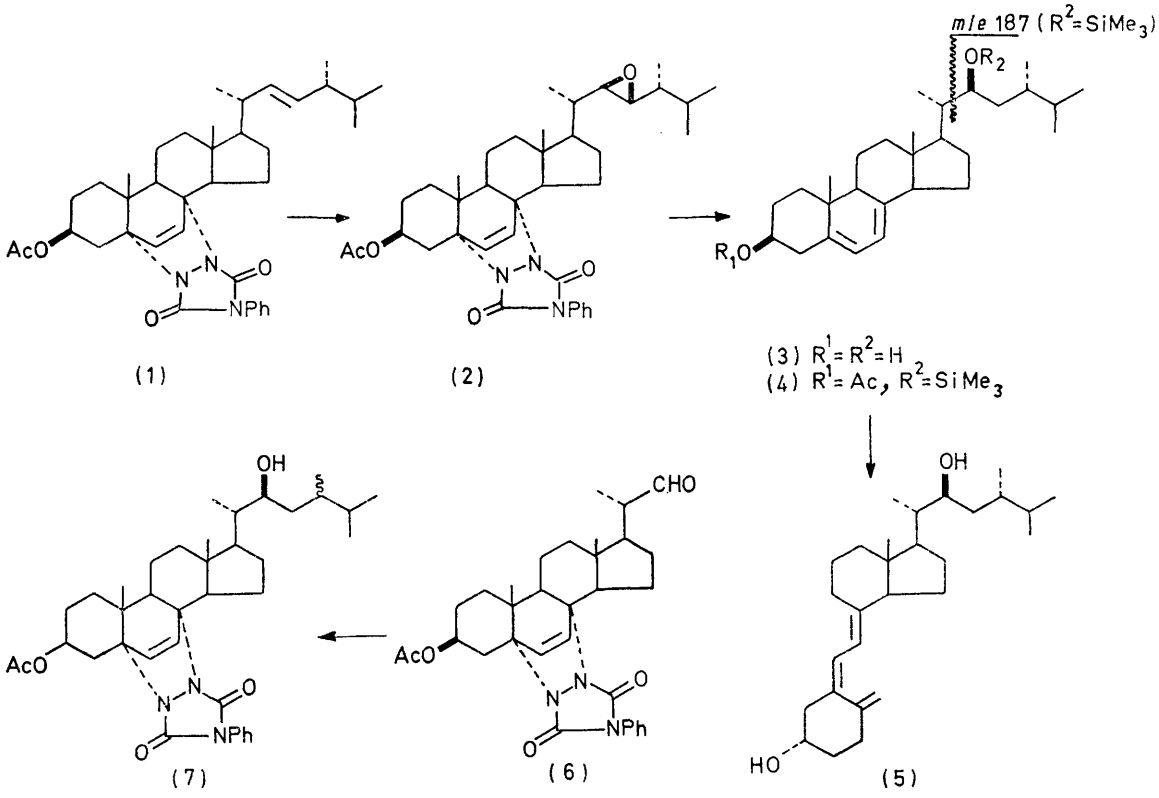
Using ergosterol acetate as starting material, 22-hydroxylated analogues of 22,23-dihydroergosterol have been prepared *via* (i) selective epoxidation of the 22,23-double bond and (ii) Grignard reaction on the hexanor-22-aldehyde. (22*S*)-Hydroxyvitamin D_4, produced upon irradiation of (22*S*)-22,23-dihydro-22-hydroxyergosterol, showed no significant antirachitic activity in rats.

RECENT studies indicate that vitamin D must be hydroxylated in the body before it produces its characteristic physiological effects. Thus 25-hydroxycholecalciferol (25-HCC),[1] 24,25-dihydroxycholecalciferol (24,25-DHCC),[2] and 25,26-dihydroxycholecalciferol (25,26-DHCC)[3] have been isolated from pig plasma, and show biological activity. 1,25-Dihydroxycholecalciferol (1,25-DHCC) has been isolated as a product of hydroxylation of 25-HCC in kidney,[4] and from chicken intestines;[5] 1,25-DHCC acts as a hormone on target tissues.[4] The possibility exists that other hydroxylated derivatives of vitamin D could exhibit useful specific and/or enhanced activities. We now report the synthesis and biological testing of (22*S*)-hydroxyvitamin D_4 [(22*S*)-22,23-dihydro-22-hydroxyergocalciferol (5)].

Ergosterol acetate was treated with 4-phenyl-1,2,4-triazoline-3,5-dione to give the cyclic adduct (1),[6] which was then treated with *m*-chloroperbenzoic acid to give the epoxide (2) as the major product in high yield. Epoxidation of a 22,23-double bond of the ergosterol type has been shown previously to give the isomer corresponding to (2) as the major product.[7] If the stereochemistry of the epoxide function in (2) is that assumed, then reduction of the epoxide function with lithium aluminium hydride should lead to (22*S*)-22,23-dihydro-22-hydroxyergosterol (3).[7] Following reduction of (2) with lithium aluminium hydride (which also removes the protecting triazolinedione),[6] the position of the hydroxy-function in the side-chain was indeed established as C-22 from the mass spectral fragmentation



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of the 3β-acetoxy-22-trimethylsilyl derivative (4). The base peak in the spectrum was at *m/e* 187, and no significant ion was detected at *m/e* 173 (the latter corresponding to the ion anticipated from a 23-trimethylsilyl derivative).

To confirm independently the position of the hydroxy-group in (3), the aldehyde (6) was prepared *via* ozonolysis of (1) [6] and then treated with the Grignard reagent derived from 2,3-dimethylbutyl bromide. The resulting mixture of (24*R*)- and (24*S*)-22,23-dihydro-22-hydroxyergosterols (7) was treated with lithium aluminium hydride and then converted into the 3β-acetoxy-22-trimethylsilyl derivative, which exhibited the same mass spectral fragmentation pattern as (4). The product (7) is tentatively assigned the 22*S* stereochemistry by analogy to the stereoselectivity displayed in other Grignard reactions on a C-22 aldehyde,[8] but it is emphasised that the important property of (7) in the present context is unambiguous functionalisation at C-22 (irrespective of stereochemistry).

Photolysis [9] of (22*S*)-22,23-dihydro-22-hydroxyergosterol (3) gave (22*S*)-22,23-dihydro-22-hydroxyergocalciferol (5), characterised *via* its ^{1}H n.m.r., mass, and u.v. spectra, and the mass spectrum of its bistrimethylsilyl ether. The product (5) showed no measurable antirachitic activity (<4% relative to vitamin D_3) in rats. In earlier work to obtain hydroxylated analogues of the D vitamins which were not known metabolites, five substances hydroxylated in the side chain have been synthesised, and none showed any antirachitic activity.[10]

EXPERIMENTAL

Unless otherwise stated, m.p.s were determined on a Kofler hot stage apparatus, optical rotations were determined in AnalaR methanol, and n.m.r. spectra in deuteriochloroform at 100 MHz. Mass spectra were run on an A.E.I. MS 902 mass spectrometer, operating at an accelerating voltage of 8 kV and an electron beam energy of 70 eV.

(22S)-22,23-*Dihydro*-22-*hydroxyergosterol* (3).—*m*-Chloroperbenzoic acid (0·4 g) was added to the ergosterol acetate adduct (1) (1 g) in dichloromethane (10 ml) and the mixture was kept at 20° for 18 h. The solution was then filtered through Woelm neutral alumina and the filtrate was concentrated to yield the epoxide (2) (1 g) as a foam, δ (CCl_4) 0·89 (m, Me groups), 1·95 (3H, s, OAc), 5·25 (1H, m, 3α-H), 6·14 and 6·33 (2d, AB pattern, J_{AB} 8 Hz, 6·33 further split, *J* 2 Hz, 6- and 7-H), and 7·37 (5H, m, Ph).

The crude epoxide (2) (0·5 g) and lithium aluminium hydride (1 g) in tetrahydrofuran (20 ml) and ether (30 ml) were heated under reflux for 3 days. Work-up in the usual manner gave a solid (0·4 g). Crystallisation from methanol gave (22S)-22,23-*dihydro*-22-*hydroxyergosterol* (3) (170 mg, 52%), m.p. 163—165°, $[\alpha]_D$ −94° (*c* 0·6), δ 0·91, 1·08, 1·14, 1·19, and 1·22 (Me groups), 3·98 (m, 3- and 22-H), and 5·68 and 5·82 (2m, 6- and 7-H), *m/e* 414 (M^+) (Found: C, 80·7; H, 11·2. $C_{28}H_{46}O_2$ requires C, 81·1; H, 11·1%).

The diol (3) (30 mg) was heated on a steam-bath for 2 h with acetic anhydride (1 ml), pyridine (1 drop), and tetrahydrofuran (3 ml). The mixture was evaporated to dryness under vacuum and the residue afforded, after preparative t.l.c. [development with light petroleum–acetone (3 : 2)], the diacetate of (3) (20 mg) and the 3β-monoacetate (6 mg). The diacetate, crystallised from methanol, had m.p. 150—152°.

Alternatively, the diol (3) (40 mg) was kept at room temperature for 22 h with acetic anhydride (1 ml), pyridine (1 drop), and tetrahydrofuran (3 ml). Work-up and separation as above gave the 3β-monoacetate (28 mg). This monoacetate in tetrahydrofuran was treated with a few drops of hexamethyldisilazane and one drop of trimethylsilyl chloride and then left at room temperature overnight. The mixture was evaporated to dryness under vacuum and the residue used directly for mass spectrometry, *m/e* 460 (M^+ −60), 396, and 187 (base peak).

(22S,24R)- *and* (22S,24S)-22,23-*Dihydro*-22-*hydroxyergosterol*.—2,3-Dimethylbutylmagnesium bromide [from magnesium (55 mg) and 2,3-dimethylbutyl bromide (380 mg)] in ether (5 ml) was added to the hexanoraldehyde (6) (410 mg) in dry tetrahydrofuran (10 ml) at −20°. After 30 min at −15 to −20° the mixture was worked-up in the usual manner. Isolation of the major component, by preparative t.l.c. [dichloromethane–ethyl acetate (9 : 1)], gave the alcohols (7) (140 mg) as a gum. The alcohols (7) and lithium aluminium hydride (100 mg) in tetrahydrofuran (7 ml) were heated under reflux for 18 h. Work-up in the usual manner and crystallisation of the product from methanol gave a mixture of (22S,24R)- and (22S,24S)-22,23-*dihydro*-22-*hydroxyergosterol* (100 mg), m.p. 177—183°, $[\alpha]_D$ −74° (*c* 0·6), δ 0·91, 1·05, 1·13, 1·19, 1·22 (Me groups) 3·98 (m, 3- and 22-H), and 5·68 and 5·82 (2m, 6- and 7-H), *m/e* 414 (M^+) (Found: C, 79·4; H, 10·8. $C_{28}H_{46}O_2$,0·5CH_3OH requires C, 79·5; H, 11·1%).

The foregoing diol was converted into the epimeric mixture of diacetates using the conditions previously described in the preparation of the diacetate of (3). The resulting epimeric mixture had m.p. (from MeOH) 121—122°.

Irradiation of (22S)-22,23-*Dihydro*-22-*hydroxyergosterol* (3).—A solution of (22*S*)-22,23-dihydro-22-hydroxyergosterol (3) (50 mg) in ether (100 ml) was irradiated in a quartz apparatus placed in an ice-bath. The solution was agitated by a stream of oxygen-free nitrogen, dispersed through a sintered disc in the base of irradiation vessel. The solution was irradiated for 15 min and evaporated to dryness under vacuum below 30°. The residue was applied as a streak to a silica gel plate (Merck GF_{254}) and the plate developed for *ca.* 3 h with chloroform containing 3% methanol.

The bands corresponding to hydroxylated previtamin and tachysterol (R_F *ca.* 0·4) were scraped off, and extracted with ether to give a mixture of these two substances (28 mg). A more polar band consisted of the starting material (7·5 mg). Material from the combined (22*S*)-22-hydroxyprevitamin and (22*S*)-22-hydroxytachysterol fractions was dissolved in ethanol (5 ml) and heated under reflux for 90 min. The ethanol was evaporated, maleic anhydride (100 mg) in benzene (5 ml) was added to the residue, and the solution was kept at 70° for 30 min. A solution of potassium hydroxide (120 mg) in ethanol (5 ml) was added and after 1 h at room temperature the product was extracted into ether. The ether layer was washed three times with water, dried, and evaporated and the residue (8 mg) finally purified on a silica gel column (20 g; deactivated with 10% water).

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The column was eluted with a linear gradient (300 ml) from ether–light petroleum (1 : 1) to absolute ether, 20 ml fractions being collected. The u.v. spectrum of each fraction was measured and pure (22*S*)-22,23-dihydro-22-hydroxyergocalciferol [(22*S*)-hydroxyvitamin D_4] (5·75 mg) was obtained, $\lambda_{max.}$ (ethanol) 266 nm (ε 18,300 taken as standard for calculation [11]); the ^1H n.m.r. spectrum (CCl_4 solution) of the product showed the characteristic resonances of the olefinic protons associated with the vitamin D chromophore, with chemical shifts and coupling constants identical with those observed in vitamin D_3 itself, δ 5·94 (1H, d, J_{AB} 11 Hz) and 6·11 (1H, d, J_{AB} 11 Hz) (6- and 7-H), 4·74 (1H, d, *J ca.* 2 Hz) and 4·97 (1H, d, *J ca.* 2 Hz) (19-H_2), 3·80 (1H, m, 3α-H) (*cf.* vitamin D_3 δ 3·80), and 3·60 (1H, m, 22-H). The mass spectrum of (5) showed a molecular ion at *m/e* 414, and intense fragment ion peaks at *m/e* 136 and 118 [*m** 136 ⟶ 118 (−H_2O), calc. and obs. at *m/e* 102·4]. The bistrimethylsilyl ether of (5) showed *m/e* 558 (M^+) and abundant fragment ions at *m/e* 208 and 118 (208 − Me_3SiOH). A g.l.c. check on this product on a 3% OV-1 column gave two peaks, corresponding to the pyrocalciferol and isopyrocalciferol analogues with the retention times 3·50 and 3·92 (cholestane as standard, t_R 1·00).

The test for physiological activity was done by radiographical biological assay,[12] on rachitic rats using the high calcium–low phosphorus rachitic diet described by Numerof *et al.*[13]

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