Calciferol and its Relatives. Part XVIII.[1] Total Synthesis of 1α-Hydroxyvitamin D_3[2]

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(3*S*,5*R*)-1-Ethynyl-2-methyl-3,5-bistrimethylsilyloxycyclohexene (7),† prepared from the optically active lactone 2-methylcyclohexene-3,5-carbolactone (1), was combined with 9α-chloro-des-AB-cholestan-8-one {5α-chloro-1β-[(1*R*)-1.5-dimethylhexyl]-7aβ-methyl-*trans*-perhydroindan-4-one} (8) to give the chlorohydrin (9), from which, in turn, 1α-hydroxyprecalciferol$_3$ diacetate (11), and 1α-hydroxyvitamin D_3 (12; R = H) were obtained.

RECENT studies on the metabolism of vitamin D_3 have shown that it is converted in the liver into its 25-hydroxy-derivative,[3] and then, by a second hydroxylation which can only be effected in the kidney, into 1α,25-dihydroxyvitamin D_3 (1α,25-dihydroxycholecalciferol, 1α,25-DHCC) (12; R = OH).[4,†] The formation of this most potent and rapidly-acting metabolite is necessary before the vitamin can exert its full biological activity; it stimulates the cells of the gastrointestinal tract to increase calcium absorption,[5] and those of the bone to increase calcium resorption.[6] It is of interest as a tool in the further study of calcium metabolism, and of possible clinical value in, for example, cases of vitamin D-resistant osteodystrophy due to renal failure.

At the outset of the present work, 1α,25-DHCC had only been obtained [4] from biological sources in microgram amounts and few of its physical constants were known; a chemical synthesis was clearly desirable. It seemed likely that a partial synthesis, employing 1α,25-dihydroxycholesterol (at that time unknown), and methods similar to those normally used for the preparation of vitamin D_3 from cholesterol, would provide the most convenient approach.[7] Nevertheless, it remained of interest to test whether methods of total synthesis, devised for vitamin D_3, were also applicable to its active metabolite. We therefore undertook the total synthesis of 1α-hydroxyvitamin D_3 (12; R = H), and now report our results.

In the route [8] chosen for extension, precalciferol$_3$ was obtained by union of 9α-chloro-des-AB-cholestan-8-one (8) with an acetylenic fragment representing ring A and the bridging carbon atoms 6 and 7, and was then transformed by thermal equilibration into vitamin D_3. For the present work the same chloroketone (8), which has been obtained [9] by total synthesis, as well as by degradation of vitamin D_3, was required. The earlier synthesis used as the ring A fragment the trimethylsilyl ether of (*S*)-3-ethynyl-4-methylcyclohex-3-en-1-ol, which was prepared [10] from the optically active lactone (1); [11] for the present extension we required the protected dihydroxy-enyne (7). It seemed likely that this, too, could be obtained conveniently from the lactone (1).

Reaction of the lactone and *m*-chloroperbenzoic acid gave in good yield a single epoxide; consideration of the steric effect of the β-carbolactone bridge suggested that this was the α-epoxide (2). It reacted with methanolic sodium methoxide to give an ester which, on hydrolysis, provided a crystalline dihydroxy-αβ-unsaturated acid, presumably (3). This assignment was supported by two observations. First, the acid showed $[\alpha]_D$ −156° (EtOH). Since the monohydroxy-analogue (6) has $[\alpha]_D$ −52°

† All structures in the present paper represent absolute configurations. Racemates are denoted by the prefix *rac*; thus *rac*-(4) means the racemate corresponding to the optically active compound (4).

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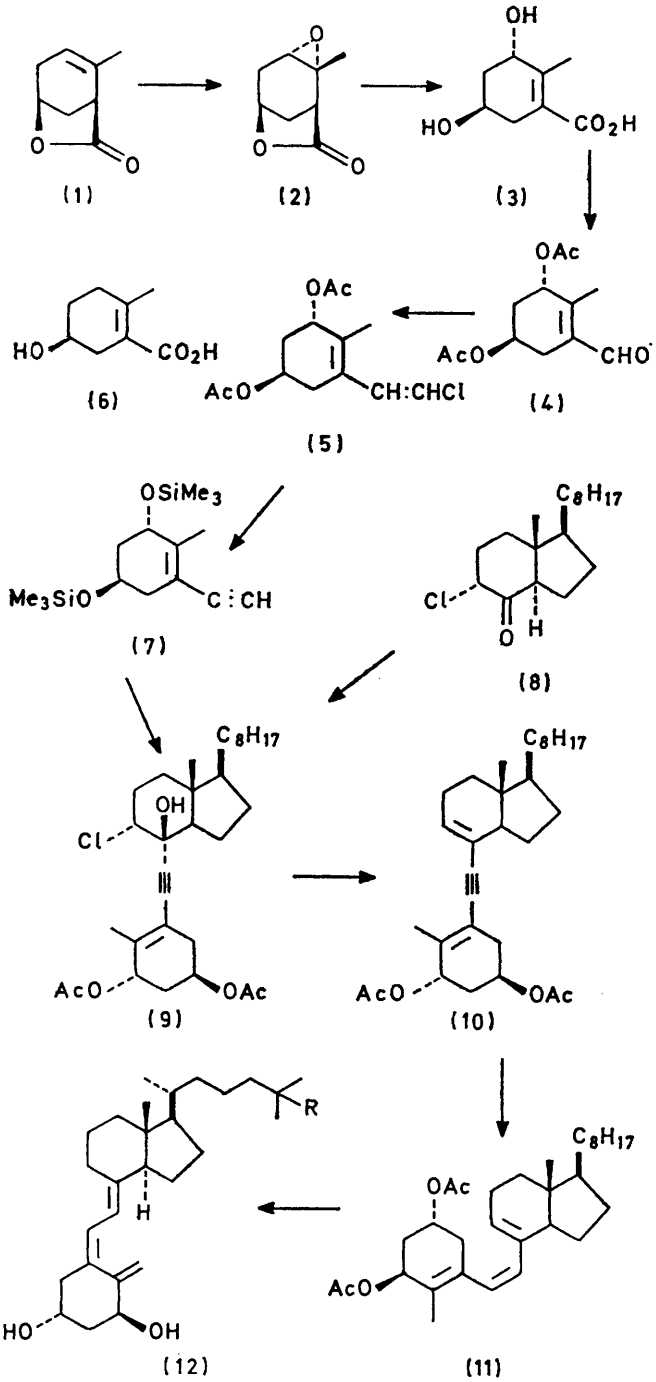
(EtOH), Mills' rule [12] indicates the α-configuration shown for the allylic hydroxy-group in (3). Secondly,



the diacetoxy-aldehyde (4), obtained from the diacetoxy-acid chloride by reduction with lithium hydridotri-t-butoxyaluminate [13] at −78°, displayed n.m.r. signals indicating that the allylic acetoxy-group occupied a quasi-axial, and the non-allylic acetoxy-group an equatorial conformation, in agreement with the postulated configurations.

The diacetoxy-aldehyde (4) was converted by reaction with chloromethylenetriphenylphosphorane [14] into the chlorodiene (5), obtained as a mixture of *E*- and *Z*-isomers. Treatment with sodamide in liquid ammonia, followed by removal of the protecting acetate groups, and their replacement by trimethylsilyl groups, gave the enyne (7) as an oil in *ca.* 18% yield from the lactone (1).

Reaction of the lithio-derivative of the enyne (7) with 9α-chloro-des-AB-cholestan-8-one (8) gave, after replacement of the protecting ether groups with acetate groups, the chlorohydrin (9), from which the synthesis was completed by existing methods. Treatment with bisethylenediaminechromium(II) in dimethylformamide [15] gave the dienyne (10), which was semi-hydrogenated with Lindlar catalyst in light petroleum to give the precalciferol derivative (11). After thermal equilibration in benzene, and methanolysis with sodium methoxide to remove the acetate residues, unisomerised 1α-hydroxyprecalciferol$_3$ was separated chromatographically, and 1α-hydroxyvitamin D$_3$ was obtained as a crystalline solid. The spectral data confirmed the assigned structure (12; R = H). Taking into account recovered starting materials, it was obtained from the 9α-chloro-compound (8) in >20% yield.

Before our work was completed, two groups reported the preparation of 1α-hydroxyvitamin D$_3$ by partial synthesis from cholesterol *via* 1α-hydroxycholesterol and its 7-dehydro-derivative. DeLuca's [16] publication did not present the physical data necessary for a satisfactory characterisation of their product, but Barton's group,[17] whose methods seem particularly convenient, presented data which, apart from minor discrepancies, are in satisfactory agreement with our own. Since the submission of our preliminary report, Fürst and his co-workers [18] have also described briefly a partial synthesis from 1α-hydroxycholesterol; the physical constants which they quote for 1α-hydroxyvitamin D$_3$ are in very good agreement with ours.

Our synthetic 1α-hydroxyvitamin D$_3$ has been used successfully by Peacock and his co-workers [19] for the treatment of patients with conditions in which calcium malabsorption is a recognised feature, including some showing vitamin D resistance. They report that it is very potent and rapidly-acting, increasing calcium absorption, and levels of plasma calcium and phosphate, in all patients; these effects are regarded as being due to its conversion in the body into 1α,25-dihydroxyvitamin D$_3$.

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EXPERIMENTAL

Unless otherwise specified, u.v. data relate to solutions in EtOH, n.m.r. data to solutions in $CDCl_3$ and $[\alpha]_D$ values to solutions in $CHCl_3$; light petroleum unless further qualified refers to the fraction b.p. 60—80°.

(3S,5R)-3,5-*Dihydroxy*-2-*methylcyclohex*-1-*enecarboxylic Acid* (3).—To the (−)-lactone (1) (2·1 g) in methylene chloride (10 cm³) at 0° a solution of *m*-chloroperbenzoic acid (2·5 g) in methylene chloride (10 cm³) was added with stirring during 10 min, and the mixture was then kept at 22° for 12 h. Excess of peracid was destroyed by the addition of aqueous sodium sulphite, the mixture was extracted with ether, and the extract was washed with aqueous sodium carbonate and with water, and then dried and evaporated. Recrystallisation of the residue from ether–light petroleum (b.p. 40—60°) gave 5α,6α-*epoxy*-6-*methylcyclohexane*-1β,3β-*carbolactone* (2) as needles (1·9 g), m.p. 68—69°, $[\alpha]_D^{22}$ −218°, $\nu_{max.}$ (Nujol) 971s and 1766s cm⁻¹, τ 5·30 (1H, m, >CH·O·CO), 6·99 (1H, d, *J* 2 Hz, >CH·O−), 7·21 (1H, d, *J* 5 Hz, >CH·CO), and 8·44 (3H, s, Me) (Found: C, 62·35; H, 6·35. $C_8H_{10}O_3$ requires C, 62·3; H, 6·5%).

The epoxide (0·93 g) and sodium methoxide [from sodium (0·3 g)] were kept together in dry methanol (30 cm³) at 22° for 20 h. The solution was concentrated to 15 cm at 15° under reduced pressure, water (10 cm³) was added, and the solution was kept at 22° for 24 h. Water (25 cm³) was then added, the methanol was removed under reduced pressure, and the solution was passed through a column of Amberlite 1R-120 resin in its acid form, the resin then being washed with water (35 cm³). Evaporation of the filtrate and washings, and crystallisation from acetone–light petroleum gave the *dihydroxy-acid* (3) as needles (0·87 g), m.p. 148—149°, $[\alpha]_D^{22}$ −165° (EtOH), $\nu_{max.}$ (Nujol) 1049s, 1642m, 1688s, 3230s, and 3420s cm⁻¹, τ [$(CD_3)_2CO$] 5·70—6·17 (2H, m, >CHOH) and 7·92 (3H, s with fine splitting, Me) (Found: C, 56·2; H, 6·9. $C_8H_{12}O_4$ requires C, 55·8; H, 7·0%).

In a similar manner, the *epoxide rac*-(2), m.p. 80—81° (from ether—light petroleum) (Found: C, 62·5; H, 6·3%) was obtained from the lactone *rac*-(1), and was converted into the *acid rac*-(3), m.p. 159—160° (needles from acetone) (Found: C, 56·0; H, 6·95%).

3α,5β-*Diacetoxy*-2-*methylcyclohex*-1-*enecarbaldehyde* rac-(4).—Acetylation of the dihydroxy-acid *rac*-(3) (0·29 g) in the usual way with acetic anhydride and pyridine gave the corresponding *diacetoxy-acid* (0·23 g), m.p. 140—142° (from chloroform–light petroleum), $\nu_{max.}$ (Nujol) 1218s, 1650m, 1680s, 1718s, and 1733s cm⁻¹, τ 4·39 (1H, t, *J* 5 Hz, >CHOAc), 4·6—5·1 (1H, m, >CHOAc), 7·85 (3H, s, Ac), and 7·92 (3H, s, Ac) (Found: C, 56·45; H, 6·4. $C_{12}H_{16}O_6$ requires C, 56·2; H, 6·3%). It was converted into the acid chloride with oxalyl chloride in benzene. Resublimed lithium hydridotri-t-butoxyaluminate (0·5 g) in dry diglyme (2 cm³) was added during 1 h to a stirred solution of the acid chloride (0·55 g) in diglyme (2 cm³) at −78° under nitrogen. The solution was brought to 20° during 2 h, water (25 cm³) was then added, followed by dilute hydrochloric acid to dissolve the gelatinous precipitate. The mixture was extracted with ether, and the extract was washed with aqueous sodium carbonate and then with water to remove the diacetoxy-acid which was recovered (100 mg; m.p. 140°). Evaporation of the dried neutral ethereal layer and crystallisation from ether–light petroleum gave the *diacetoxy-aldehyde rac*-(4) (208 mg), m.p. 59—60° (Found: C, 59·95; H, 6·5. $C_{12}H_{16}O_5$ requires C, 59·9; H, 6·7%).

In a similar manner the optically active acid (3) was converted (yield 60%) into the *diacetoxy-aldehyde* (4), which formed an oil, homogeneous by t.l.c., $[\alpha]_D^{22}$ −185°, $\nu_{max.}$ (film) 1040s, 1235s, 1638m, 1670s, 1720s, 1734s, and 2760w cm⁻¹, $\lambda_{max.}$ 241 nm (ε 10,800), τ −0·28 (1H, s, −CHO), 4·32 (1H, t, *J* 5 Hz, >CHOAc), 4·55—4·95 (1H, 5 line m, >CHOAc), 7·82 (3H, s, Ac), and 7·92 (3H, s, Ac) (Found: M^+, 240·09823. $C_{12}H_{16}O_5$ requires *M*, 240·09976).

(3S,5R)-1-*Ethynyl*-2-*methyl*-3,5-*bistrimethylsilyloxycyclohex*-1-*ene* (7).—A solution of chloromethylenetriphenylphosphorane was prepared from chloromethyltriphenylphosphonium chloride (4·1 g), piperidine (1·01 g), and n-butyl-lithium (0·85M; 12·4 cm³) in dry ether (100 cm³) under nitrogen, and to it the aldehyde (4) (900 mg) in dry ether (15 cm³) was added dropwise with stirring at 20°. The mixture was heated under reflux for 12 h, and then cooled, treated with water (1 cm³), and filtered. It was washed with dilute hydrochloric acid, and brine, and then dried and evaporated; t.l.c. showed the presence of some deacetylated material, so the product was re-acetylated with acetic anhydride and pyridine, and then chromatographed on silica gel (100 g). Elution with 10% ethyl acetate–benzene gave the crude mixture of chlorodienes (5) as an oil (765 mg); g.l.c. (5% Carbowax at 166°) showed peaks corresponding to the *E*-chlorodiene (major product), *Z*-isomer, and ω-dichlorodiene (minor product). The mixture showed $\nu_{max.}$ (film) 740m, 845m, 1050s, 1240s, 1580m, and 1732s cm⁻¹, τ 3·19 and 3·72 (both d, *J* 13 Hz, *trans* −CH=CH−), 3·70 and 3·90 (both d, *J* 8 Hz, *cis* −CH=CH−), 4·57 (1H, t, *J* 4 Hz, >CHOAc), 4·72—5·10br (1H, m, >CHOAc), 7·91 (3H, s, Ac), and 7·96 (3H, s, Ac).

The chlorodiene mixture (730 mg) in dry ether (20 cm³) was added to a stirred suspension of sodamide [from sodium (550 mg)] in liquid ammonia (500 cm³); the mixture was stirred for 3 h, and kept for a further 12 h, after which ammonium chloride (3 g) was added cautiously, and the ammonia was evaporated in a current of nitrogen. After addition of water, the product was isolated with ether, and was deacetylated by treatment with 0·2N-methanolic sodium methoxide (1 cm³) at 0° for 3 h. Acidification with glacial acetic acid, followed by removal of the solvent gave the crude acetylene, which was dissolved in ether (6 cm³) and kept at 0° for 12 h with hexamethyldisilazane (350 mg), trimethylsilyl chloride (190 mg), and pyridine (0·4 cm³) under nitrogen. The filtered solution was evaporated, and the residue, dissolved in benzene, was passed through a short column of neutral alumina (grade III). Evaporation of the eluate gave the *disilyl ether* (7) as an oil (480 mg), $[\alpha]_D^{22}$ −90°, $\lambda_{max.}$ 230 nm (ε 11,300), $\nu_{max.}$ (film) 750m, 850s, 898s, 995s, 1080s, 1257s, and 2100s cm⁻¹, τ 5·70—6·18 (2H, complex m, >CH·O), 6·99 (1H, s, ⋮CH), 9·83 (9H, s, $OSiMe_3$), and 9·86 (9H, s, $OSiMe_3$) (Found: M^+, 296·16185. $C_{15}H_{28}O_2Si_2$ requires *M*, 296·16277). It was stored in the dark at −40° under nitrogen.

1α,3β-*Diacetoxy*-9,10-*secocholesta*-5(10),8-*dien*-6-*yne* (10).—The acetylene (7) (680 mg) in dry ether (10 cm³) was treated with 2·1M-n-butyl-lithium (1·12 cm³; in hexane) during 15 min, and the mixture was stirred at 20° for 1 h, after which it was cooled to 0°, and the chloroketone (8) (700 mg) in ether (12 cm³) was added, stirring being continued for 2·5 h at 0°. The mixture was then stirred with 2N-hydrochloric acid (5 cm³) for 3 h at 22°. The product was isolated with ether, and acetylated with acetic anhydride and pyridine (16 h at 22°) to give an oil which was chromatographed on Kieselgel GF with 2% ethyl acetate–benzene. The early eluates contained the chloroketone (8) (144 mg);

next eluted was the acetoxyacetylene [as (7), Ac instead of $SiMe_3$] (100 mg), and lastly the diacetate (9) (400 mg), $[\alpha]_D^{23}$ −130°, $\lambda_{max.}$ 234 nm (ε 15,000), $\nu_{max.}$ (film) 1050s, 1245s, 1720s, 1738s, 2210w, and 3470s cm^{-1}, τ 4·53 (1H, t, *J* 4 Hz, >C*H*OAc), 4·70—5·05 (1H, m, >C*H*OAc), 5·83 (1H, t, *J* 3 Hz, >CHCl), 7·95 (3H, s, Ac), 7·98 (3H, s, Ac), and 8·12br (3H, s, :CMe).

The diacetate (9) (400 mg) and bisethylenediaminechromium(II) [from 1·07M-chromium(II) perchlorate (6·33 cm^3) and ethylenediamine (10·5 cm^3) in oxygen-free dimethylformamide (90 cm^3)] were kept together under nitrogen for 16 h; the solvent was then removed, ether was added, and the solution was washed successively with dilute hydrochloric acid, aqueous sodium carbonate, and water, and then evaporated. The residue was acetylated with acetic anhydride and pyridine, and the product was chromatographed on plates of Kieselgel GF_{254} with 2% ethyl acetate–benzene as the eluant, giving the *dienyne* (10) as an oil (277 mg), $[\alpha]_D^{20}$ −108°, $\lambda_{max.}$ 273 nm (ε 20,000), $\nu_{max.}$ (film) 1030m, 1228s, 1741s, and 2180w cm^{-1}, τ 4·00 (1H, m, =CH−), 4·52 (1H, t, *J* 4 Hz, >CH·O), 4·72—5·12 (1H, m, >CH·O), 7·95 (3H, s, Ac), 7·98 (3H, s, Ac), and 8·12br (3H, s, CMe) (Found: M^+, 482·338877. $C_{31}H_{46}O_4$ requires *M*, 482·339591).

1α-*Hydroxyprecalciferol*$_3$ *Diacetate* (11).—Lindlar's catalyst (200 mg) in purified light petroleum (3 cm^3) containing quinoline (2 mg) was equilibrated with hydrogen at room temperature; the dienyne (10) (275 mg) in light petroleum (4 cm^3) was then added, and hydrogenation was continued until 1 mol. equiv. had been absorbed (35 min). The solution was filtered and evaporated at 20°, and the oily residue was chromatographed on plates of Kieselgel GF_{254} with 2% ethyl acetate–benzene. Evaporation of the first eluates gave the *diacetate* (11) as an oil (193 mg), $[\alpha]_D^{22}$ −50°, $\lambda_{max.}$ (Et_2O) 260 nm (ε 8700), $\nu_{max.}$ (film) 1028s and 1738s cm^{-1}, τ 4·17br (2H, s, −CH=CH−), 4·40—4·80 (2H, m, =CH− and >CH·O), 4·80—5·20 (1H, m, >CH·O), 7·95 (3H, s, Ac), 7·98 (3H, s, Ac), 8·36 (3H, s, CMe), and 9·30 (3H, s, 13-Me) (Found: M^+, 484·355240. $C_{31}H_{48}O_4$ requires *M*, 484·353917), *m/e* 424 (*M* − AcOH) and 364 (*M* − 2AcOH).

Evaporation of the later eluates gave the unchanged dienyne (10) (60 mg), $\lambda_{max.}$ 273 nm (ε 20,000).

1α-*Hydroxycholecalciferol* (12; R = H).—A solution of the diacetate (11) (190 mg) in benzene (5 cm^3) was heated under nitrogen at 75° for 24 h. Removal of the solvent and Zemplén methanolysis of the residue gave an oil (150 mg) which was chromatographed on plates of Kieselgel GF_{254} with 20% ethyl acetate–benzene as eluant. The more polar fraction (57 mg) was the diol corresponding to the diacetate (11). The fraction first eluted was 1α-hydroxyvitamin D_3, which separated from ether–light petroleum (b.p. 40—60°) as fine white needles (79 mg), m.p. 134—136°, $[\alpha]_D^{25}$ +28° (*c* 0·6 in Et_2O), $\lambda_{max.}$ (Et_2O) 264—265 nm (ε 18,000), $\nu_{max.}$ ($CHCl_3$) 895m, 912m, 952m, 1040s, 1645m, 3450m, and 3600s cm^{-1}, τ 3·58 (1H, d, *J* 12 Hz, H-6 or -7), 3·99 (1H, d, *J* 12 Hz, H-6 or -7), 46·6 (1H, m, H-19), 4·99 (1H, m, H-19), 5·45—6·05 (2H, m, >CH·O), 9·12 (9H, d, *J* 6 Hz, *Me*CH<), and 9·45 (3H, s, H-18) (Found: C, 80·85; H, 11·3%; M^+, 400·33297. Calc. for $C_{27}H_{44}O_2$: C, 80·9; H, 11·1%; *M*, 400·33411), *m/e inter alia* 382 (5·2%), 364 (16·2), 149 (26·0), 134 (6·1), 128 (30·4), 121 (67·5), 119 (32·0), 95 (49·4), 83 (50·5), 81 (59), 71 (58·5), 69 (76·3), and 57 (100).

These data agree satisfactorily with those of Barton *et al.*,[17] apart from the value of $\varepsilon_{max.}$ (ether), for which they quote 20,200, and from the position of the C-19 proton resonances, for which they quote δ 5·30 and 4·85 (τ 4·70 and 5·15). Our data are in good agreement with those of Fürst.[18]

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