4α-Hydroxycholecalciferol and an Attempted Synthesis of its 4β-Hydroxy-epimer

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4α-Hydroxycholecalciferol has been prepared from cholest-5-ene-3β,4α-diyl diacetate *via* its Δ^7-derivative. Attempts to convert cholest-5-ene-3β.4β-diyl diacetate into its Δ^7-derivative were unsuccessful.

THE increased clinical significance of hydroxylated derivatives of vitamin D has led recently to the synthesis and biological testing of various derivatives, hydroxylated in ring A or in the side chain. Preparations of compounds with hydroxy-groups in positions 1α;[1-3] 22;[4] 25;[5,6] 20,25;[7] 1α,25;[1,8] and 25,26[9] have been described. The present paper reports the synthesis of a

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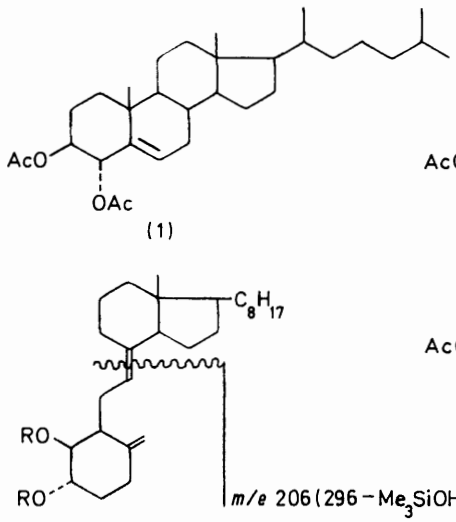
ring-A-hydroxylated derivative, 4α-hydroxycholecalciferol.

The readily available 3β,4α-diacetate (1)[10] was treated with *N*-bromosuccinimide, and the resulting 7-bromo-derivative was dehydrobrominated with trimethyl phosphite in xylene under reflux.[11] U.v. analysis of the t.l.c.-purified product showed the presence of only 17% of the expected 5,7-diene (2), with no 4,6-diene. Attempts to brominate with dibromodimethylhydantoin, followed by treatment with trimethyl phosphite, did not give a greater yield of the 5,7-diene. Models show that there is considerable α-face repulsion by the 4α-acetoxy-group in the bromination step.



Irradiation of an ethereal solution of the diene (2) in quartz apparatus with a 100 W medium-pressure lamp gave a mixture of products from which the corresponding precholecalciferol and tachysterol derivatives were obtained as a mixture by preparative t.l.c. on silica gel. The final separation of 4α-hydroxycholecalciferol (3)* and 4α-hydroxytachysterol was achieved by alkaline hydrolysis and reaction with maleic anhydride.[12] The mass spectrum of the bistrimethylsilyl ether (4) showed the expected loss of one and two trimethylsilanol molecules. The fragment containing ring A C-6, C-7, and C-19 gave a weak peak at *m/e* 296 and an intense peak at *m/e* 206. This fragmentation is typical of a ring-A-hydroxylated derivative, *e.g.* 1α,25-dihydroxycholecalciferol.[13] The presence of an abundant ion at *m/e* 147 (75% intensity of the peak at 206) confirms the presence of two vicinal trimethylsilyl groups[14] [$(Me_3Si-O-SiMe_2)^+$].

Synthesis of 4β-hydroxycholecalciferol was tried as early as 1946,[15] but an attempted pyrolysis of Δ^5-3β,4β,7α- and Δ^5-3β,4β,7β-tribenzoates did not afford any 5,7-diene. In the present work, the allylic bromination of the 3β,4β-diacetate (5) gave the bromo-derivative (6) in high yield. This was confirmed by conversion of the bromo-derivative into the 7α-hydroxy-compound (8) (*ca.* 70%) and recovery of the diacetate (5) (23%). The 7α-configuration of the hydroxy-group was verified by conversion of compound (8) into the known triacetate (9) and triol (10).[16] Dehydrobromination of (6) with collidine or trimethyl phosphite did not give any 5,7-diene owing to β-face steric hindrance by the 4β-acetoxy-group, blocking approach to the 8β-proton. Attempts to eliminate a molecule of water from the 7α-hydroxy-compound (8) with dimethylaniline under reflux[17] did not afford any diene (95% recovery of starting material). The reaction of compound (8) with phosphoryl chloride in pyridine[17,18] gave a 1 : 1 mixture of 7-chloro-derivatives (7) with no 5,7-diene, as demonstrated by u.v. and n.m.r. analysis. A similar reaction of 7α-hydroxycholesteryl acetate with phosphoryl chloride in pyridine gave *ca.* 10% of 3β-acetoxycholesta-4,6-diene with no formation of 5,7-diene.

EXPERIMENTAL

M.p.s were determined with a Kofler hot-stage apparatus. U.v. spectra were recorded with a Unicam SP 800 spectrometer, n.m.r. spectra with a Varian HA-100 instrument (tetramethylsilane as internal standard), and mass spectra with a G.E.C.–A.E.I. MS-902 instrument.

Cholesta-5,7-diene-3β,4α-diyl Diacetate (2).—The diacetate (1) (106 mg) and *N*-bromosuccinimide (55 mg) in petroleum (b.p. 60—80°; 10 ml) and benzene (2 ml) were heated under reflux for 30 min. The succinimide was filtered off and washed with petroleum and the solution was evaporated to dryness. The residue in xylene (5 ml) was heated with trimethyl phosphite (1 ml) under reflux for 90 min, and the solution was evaporated under vacuum. The product was purified by t.l.c. (silica gel Merck F 254; dichloromethane). U.v. analysis ($\lambda_{max.}$ 272, 282, and 293 nm) showed the presence of the 5,7-diene (2) (17·5 mg).

4α-Hydroxycholecalciferol (3).—A solution of the diacetate (2) (53 mg) in ether (100 ml) was irradiated in a quartz

* 4α-Hydroxycholecalciferol has 3% of the activity of vitamin D in promoting the absorption of calcium in rachitic chicks.

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apparatus (cooled in an ice-bath; solution agitated with a stream of nitrogen) for 15 min. The solvent was evaporated off and the residue in chloroform was chromatographed on a silica gel plate, developed twice with petroleum (b.p. 60—80°)–dichloromethane (1 : 1). The previtamin and tachysterol fractions were combined and the starting material was irradiated once again for 15 min. The combined previtamin and tachysterol fractions were evaporated and the residue, dissolved in ethanolic potassium hydroxide (100 mg in 10 ml), was heated under reflux for 90 min. The product was extracted with ether, and, after the usual work-up, tachysterol was separated as a maleic anhydride adduct.[12] Extraction with ether gave impure compound (3), which was finally purified on a silica gel plate (developed in chloroform for *ca.* 3 h) to give 4α-*hydroxycholecalciferol* (3) (9·5 mg), $\lambda_{max.}$ (EtOH) 265 nm (95% purity on the basis of ε 18,300 [19]), *m/e* 400, 382, 287, 152, 134, and 117 (Found: M^+, 400·3356. $C_{27}H_{44}O_2$ requires *M*, 400·3341), δ (CCl_4) 0·90 (18-H_3, s), 3·26 (4β-H, m), 3·80 (3α-H, m), 4·78 (1H, d) and 5·02 (1H, d) (19-H_2), and 6·23 (ABq, *J* 10 Hz, 6- and 7-H). The 3β,4α-bistrimethylsilyl ether (4) showed *m/e* 544, 454, 364, 296, 206, and 147.

7α-*Hydroxycholest-5-ene*-3β,4β-*diyl Diacetate* (8).—The diacetate (5) (1 g) and *N*-bromosuccinimide (1 g) in hexane (30 ml) were heated under reflux for 15 min. The succinimide was filtered off and the filtrate evaporated under vacuum. A solution of the bromo-derivative (6) in benzene was adsorbed on an alumina column (activity II; 30 g) and, on the following day, the column was eluted with benzene [to give the diacetate (5) (235 mg)], chloroform, and ethyl acetate (to give 780 mg of material). Crystallisation of the most polar fraction gave the *diacetate* (8) (720 mg), m.p. 238—240° (from acetone–ethanol), $[\alpha]_D$ −107° ($CHCl_3$) (Found: C, 74·2; H, 9·9. $C_{31}H_{50}O_5$ requires C, 74·1; H, 10·0%), *m/e* 502, 442, 400, 383, and 382, δ ($CDCl_3$) 0·85 (18-H_3, s), 1·10 (19-H_3, s), 1·98 (s) and 2·04 (s) (2 OAc), 3·90 (7β-H, m), 4·76 (3α-H, m), 5·53 (6-H, d), and 5·98 (4α-H, d) [$J_{3\alpha,4\alpha}$ 5 (vicinal); $J_{4\alpha,6}$ 4 Hz (allylic)].

Hydrolysis of compound (8) gave the known triol (10), m.p. 198—200° (from methanol) (lit.,[17] 195—197°); acetylation afforded the known triacetate (9), m.p. 169—171° (from ethanol) (lit.,[17] 172—174°).

In a similar manner, the chloro-derivative (7) was converted into the diacetate (8), m.p. 232—235°.

7α- *and* 7β-*Chlorocholest-5-ene*-3β,4β-*diyl Diacetate* (7).—The diacetate (8) (260 mg) in pyridine (5 ml) was cooled in an ice-bath and phosphoryl chloride (0·3 ml) was added dropwise. After 1 h the solution was poured onto ice and extracted with ether. After the usual work-up, the product in ether was filtered through a short column of silica gel and gave a mixture of 7α- and 7β-chloro-derivatives (7), m.p. 150—152° (from ethanol), $[\alpha]_D$ −20° ($CHCl_3$) (Found: C, 71·3; H, 9·5. Calc. for $C_{31}H_{49}ClO_2$: C, 71·4; H, 9·5%), *m/e* 522 (25%)/520 (75%), 484, 424, 366, and 364, δ (CCl_4) 0·88 (18-H_3, s), 1·17 (19-H_3, s), 1·91 (s) and 2·01 (s) (2 OAc), 4·08 (7β-H, d), 4·15 (7α-H, d), 4·60 (3α-H, m), 5·53 (6-H, d), and 5·71 (4α-H, d) ($J_{6,7\alpha}$ 3, $J_{6,7\beta}$ 3, $J_{3\alpha,4\alpha}$ 3, $J_{4\alpha,6}$ 3 Hz) (ratio 7α-H : 7β-H = 1 : 1). U.v. analysis did not show the presence of any 5,7-diene.

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