A Modified Synthesis of 1α-Hydroxyvitamin D_3

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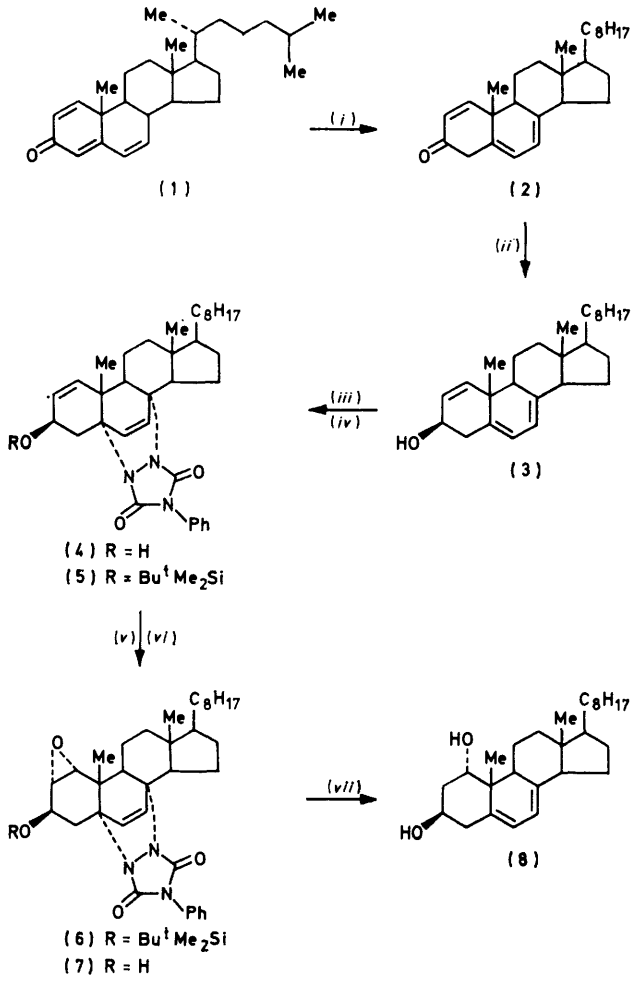
Base-catalysed deconjugation of cholesta-1,4,6-trien-3-one to cholesta-1,5,7-trien-3-one is a key step in a reported route to 1α-hydroxyvitamin D_3. A procedure for greatly improving the yield of the product, which allows its isolation *via* direct crystallisation, is reported. This procedure, used in conjunction with a recently described method for α-epoxidation of protected Δ^1-3β-ols, has been employed in a relatively efficient synthesis of 1α-hydroxyvitamin D_3.

In 1974, we commenced a proposed synthesis of 1α-hydroxyvitamin D_3 in which we intended to deconjugate cholesta-1,4,6-trien-3-one (1) to cholesta-1,5,7-trien-3-one (2) (Scheme) and to subsequently protect the 5,7-diene as a Diels–Alder adduct with 4-phenyl-1,2,4-triazoline-3,5-dione. It was planned to convert the Δ^1-double bond, *via* an epoxide, into the required 1α-hydroxy group, and to regenerate the 5,7-diene system of a 1α-hydroxyprovitamin D_3 by removal of the diene protecting group.



SCHEME (*i*) NaOMe–DMSO, then 2M $MeCO_2H$. (*ii*) $CaBH_4$. (*iii*) 4-Phenyl-1,2,4-triazoline-3,5-dione. (*iv*) Bu^{t}Me$_2$SiCl. (*v*) *m*-$ClC_6H_4CO_3H$. (*vi*) $MeCO_2H$–THF–H_2O. (*vii*) $LiAlH_4$

RESULTS AND DISCUSSION

Shortly after we commenced our work, a route based on the same principles was published by Kaneko *et al.*[1] In their route, (2) was reduced to (3) with calcium borohydride and the diene protected to give (4) (Scheme). Epoxidation of (4) with *m*-chloroperbenzoic acid unfortunately gave more of the unwanted β-epoxide than of the desired α-epoxide (7), but a synthesis of (8) was nevertheless successfully completed by $LiAlH_4$ reduction of (7). The overall yield of the provitamin (8) from (1) was 4%.

In the hope of improving this type of route to 1α-hydroxyvitamin D_3 (which is important since it may be used clinically as a convenient precursor of the human hormone 1α,25-dihydroxyvitamin D_3 [2]), we continued our studies. Deconjugation of (1) to (2) using the method of Kaneko *et al.*[1] (potassium t-butoxide in DMSO–ether, and quenching the reaction in ice-cold, CO_2-saturated water) did not, in our hands, give the desired product, but gave a product which had $\lambda_{max.}$ at 290 nm in the u.v. Reaction in which the anion, generated by sodium methoxide, was quenched in ice-cold, CO_2-saturated water gave significant quantities of (2) [5—10%, isolable as the adduct (4)] in only 3 out of 30 reactions. Kaneko *et al.*[1] were able to obtain (2) in 12% yield from (1), but the reaction is clearly a capricious and low-yield step.

The conversion (1)→(2) was therefore investigated under a wide range of conditions. An early finding was that the KOBu^t deconjugation was catalysed by traces of methanol, presumably since some MeO^- is generated, and this species suffers less steric hindrance in abstracting the C-8 hydrogen. Deprotonation conditions investigated included NaOMe, KOBu^t with a trace of MeOH, and NaOMe–KF–18-crown-6 (all in DMSO). The anion was quenched under a wide range of conditions including dilute acid, CO_2 saturated water, pH 4 buffer, aqueous sodium hydrogencarbonate, and aqueous sodium carbonate. Examination of the u.v. absorption spectra of the crude products showed that deconjugation with NaOMe in DMSO and subsequent quenching of the anion into 2M acetic acid gave the best yield of (2). Crystallisation of the crude product, obtained from an acetic acid quench, from ether at low temperature gave (2) in reproducible yields of 40—50%. These yields were reduced if the reaction was 'scaled-up' beyond that reported in the Experimental section. The isolation of

(2) in moderate yield by direct crystallisation from the reaction mixture represents a major simplification and improvement over the original route [1] to 1α-hydroxy-7-dehydrocholesterol.

Attention was next turned to epoxidation of the allylic alcohol (4). In the earlier work,[1] the unwanted β-isomer was obtained as the main product (α : β ratio of 2 : 3) when *m*-chloroperbenzoic acid in chloroform was employed as the oxidising agent. It has been reported [3] that the stereochemical course of epoxidation of double bonds can be reversed (*i.e.* to the face opposite to that occupied by neighbouring hydroxyl groups) by using ether as solvent. However, in the conversion of (4)→(7), the use of ether, or of benzene or dioxan, as solvents did not significantly increase the proportion of the α-epoxide in the reaction product. We therefore considered the alternative possibility of protecting the 3β-OH group with a bulky protecting group. During the course of these studies, Whalley and his co-workers [4] reported the protection of the 3β-OH group as a silyl ether prior to selective α-epoxidation of a Δ^1-double bond. This procedure was therefore adopted in our synthesis. Treatment of (4) with t-butyldimethylsilyl chloride in dimethylformamide in the presence of imidazole gave (5). Reaction was complete in a much shorter time (5 min) than that used in the literature procedure (18 h). Epoxidation of (5) with *m*-chloroperbenzoic acid in chloroform gave a single epoxide (6), from which the silyl group was removed by treatment with acetic acid–THF–water. T.l.c. and n.m.r. spectroscopy established that the product (7) was not contaminated with the β-epoxide. The synthesis of 1α-hydroxy-7-dehydrocholesterol (8) was completed by reduction of (7) with lithium aluminium hydride.

The 1α-hydroxyprovitamin (8), so obtained, was converted into 1α-hydroxyvitamin D_3. An ethereal solution of (8) was irradiated under nitrogen for 25 min, after which time fluorenone was added [5] as a triplet sensitizer to increase the quantum yield of 1α-hydroxyprevitamin D_3. Isolation of the vitamin in the usual way [5] gave 1α-hydroxyvitamin D_3 in 15% yield from (8).

By use of the described route, the 1α-hydroxyprovitamin (8) has been obtained in 14% yield from cholesterol.* This represents a marked improvement over that earlier described for a similar route,[1] largely due to changes in steps (1)→(2) and (4)→(7) (Scheme).

EXPERIMENTAL

Except for trace contaminants in (2), all compounds described were pure according to t.l.c. (carried out on silica GF_{254} or alumina F_{254} adsorbants).

Cholesta-1,5,7-trien-3-one (2).—Cholesta-1,4,6-trien-3-one (1) (1.0 g) in dry DMSO (10 ml) at 60 °C was added dropwise over 5 min to a stirred mixture of sodium methoxide (1.5 g) and dry DMSO (10 ml) at 60 °C under a nitrogen atmosphere. After stirring for 10 min at 60 °C, the solution was quenched by pouring into rapidly stirred 2M acetic acid (250 ml). The precipitate was extracted into ether and the organic layer was washed with sodium hydrogencarbonate solution and dried over anhydrous sodium sulphate. The oil obtained by evaporation of the solvent was crystallised from ether at −78 °C to give the product (450 mg), m.p. 120—125 °C (lit.,[1] 136—146 °C); $\lambda_{max.}$ (EtOH) 231 (ε 11 700), 268, and 277 (8 420) nm (lit.,[1] 231, 268, and 277 nm); $\lambda_{min.}$ (EtOH) 251 nm; δ [$(CD_3)_2CO$] (100 MHz) 6.86, 5.82 (AB quartet, *J* 10 Hz, 2 H, H-1, H-2), 5.75—5.40 (m, 2 H, H-6, H-7), 3.40, and 2.96 (AB quartet, *J* 18 Hz, 2 H, H-4).

Cholesta-1,5,7-trien-3β-ol (3).—Calcium chloride (1.75 g) was dissolved in methanol (50 ml) and cooled to −15 °C. Sodium borohydride (0.875 g), in ethanol (50 ml) cooled to −15 °C, was added dropwise to the calcium chloride solution, and the resulting mixture was stirred for 20 min. Cholesta-1,5,7-trien-3-one (1.75 g) in ether (50 ml) was cooled to the same temperature and added dropwise. The mixture was stirred for 1.75 h while the temperature rose from −15 to 0 °C. An excess of 50% aqueous acetone was added and the excess removed under reduced pressure; 50% aqueous acetic acid was then added to give a clear solution. This solution was extracted with ether and the ethereal extract washed with sodium hydrogencarbonate solution, dried over anhydrous sodium sulphate, and evaporated under reduced pressure. The residue was crystallised from methanol to give *cholesta-1,5,7-trien-3β-ol* (1.60 g, 90%), m.p. 125—127 °C (lit.,[1] 128—129 °C); $\lambda_{max.}$ 271, 281, and 292 nm; δ ($CDCl_3$) 5.67 (br s, 3 H), 5.45 (br s, 1 H), 4.30 (dd, *J* 10 and 6 Hz, 1 H), 1.50 (s, H-19), 1.0 (d, *J* 8 Hz, H-26, H-27), 0.90 (d, *J* 6 Hz, H-21), and 0.67 (s, H-18).

The Diels–Alder Adduct of Cholesta-1,5,7-trien-3β-ol and 4-Phenyl-1,2,4-triazoline-3,5-dione (4).—Cholesta-1,5,7-trien-3β-ol (1.6 g) was dissolved in dichloromethane (25 ml), and 4-phenyl-1,2,4-triazoline-3,5-dione (0.8 g) in acetone (10 ml) was added slowly until a faint pink colour persisted. The solvent was removed under reduced pressure, and the product was crystallised from aqueous ethanol and dried over phosphorus pentaoxide to give the Diels–Alder adduct (4) (2.34 g), m.p. 175—178 °C (lit.,[1] 178—182 °C); δ ($CDCl_3$) 7.38 (m, 5 H, Ph), 6.44 and 6.24 (AB quartet, *J* 8 Hz, 2 H, H-6, H-7), 5.70 (s, 2 H, H-1, H-2), 5.02 (t, *J* 6 Hz, 1 H, H-3α), 3.34 (dd, *J* 14 and 7 Hz, 1 H, H-9), 1.07 (s, H-19), 0.95 (d, *J* 6 Hz, H-21), 0.85 (d, *J* 6 Hz, H-26, H-27), and 0.81 (s, H-18).

The 3β-(t-Butyldimethyl)silyl Derivative (5) *of the Diels–Alder Adduct* (4).—The Diels–Alder adduct (4) (700 mg) was dissolved in dimethylformamide (1.5 ml), the solution warmed to 35 °C, and imidazole (310 mg) and t-butyldimethylsilyl chloride (310 mg) were added to the stirred solution. This solidified rapidly but was set aside for 30 min. The product was partitioned between ether and water. The ether layer was dried over anhydrous sodium sulphate, evaporated to dryness, and the residue was crystallised from ether–methanol (748 mg, 89%), m.p. 159—161 °C (lit.,[4] 163—165 °C); δ ($CDCl_3$) 7.38 (m, 5 H, Ph), 6.46 and 6.24 (AB q, *J* 8 Hz, 2 H, H-6, H-7), 5.68 (s, 2 H, H-1, H-2), 4.98 (t, *J* 7 Hz, 1 H, H-3a), 3.33 (dd, *J* 14 and 8 Hz, 1 H, H-9), 1.12 (s, H-19), 0.92 (s, 9 H, t-Bu), 0.84 (intense singlet), 0.13, and 0.11 (6 H, Me_2Si).

The 1α,2α-Epoxy-derivative (6).—The alkene-silyl ether (5) (748 mg) was dissolved in chloroform (22.5 ml) and *m*-chloroperoxybenzoic acid (450 mg) was added. After 21 h a further quantity of *m*-chloroperoxybenzoic acid

* We thank a referee for drawing to our attention a publication (A. Mourino, *Synth. Comm.*, 1978, **8**, 127) which appeared almost concurrently with submission of our paper. It describes a modification of the Barton procedure [6] for conversion of cholesterol to (8) in 20% yield.

(450 mg) was added and the solution was set aside for a further 22 h. It was then diluted, washed twice with 10% sodium carbonate solution, dried over anhydrous sodium sulphate, and evaporated to dryness to give the crude product (830 mg); δ ($CDCl_3$) 7.42 (m, 5 H, Ph), 6.47 and 6.21 (AB q, *J* 8 Hz, 2 H, H-6, H-7), 5.00 (m, 1 H, H-3α), 3.23 (m, 3 H, H-1, H-2, and H-9), 1.16 (s, H-19), and 0.97 (s, t-Bu).

The 1α,2α-*Epoxy-alcohol* (7).—The epoxy-silyl ether (6) (830 mg) was dissolved in tetrahydrofuran (12 ml), acetic acid (12 ml), and water (6 ml), and the solution was set aside at 40 °C for 3 d. The resulting solution was extracted with dichloromethane, and the extract was washed with saturated sodium hydrogencarbonate solution and water, dried over anhydrous sodium sulphate, and evaporated to dryness. Chromatography on silica (80 g) (eluant acetone) gave the α-epoxide (400 mg, 63% from the alkene), m.p. 148—150 °C (lit.,[1] 152—154 °C); δ ($CDCl_3$) 7.40 (m, 5 H, Ph), 6.44 and 6.14 (AB q, *J* 8 Hz, 2 H, H-6, H-7), 5.05 (t, *J* 8 Hz, 1 H, H-3α), 3.23 (s, 2 H, H-1β, H-2β), 1.13 (s, H-19), 0.94, and 0.87 (intense, sharp signals).

1α-*Hydroxy*-7-*dehydrocholesterol* (8).—The α-epoxide (400 mg) in tetrahydrofuran (30 ml) was added to lithium aluminium hydride (400 mg) in tetrahydrofuran (30 ml). The mixture was heated under reflux for 90 min, then cooled to room temperature and saturated sodium sulphate solution added. The aqueous layer was separated, washed with tetrahydrofuran, and the washings combined with the separated organic layer. The combined solution was dried over anhydrous magnesium sulphate and evaporated to dryness to yield the crude product (375 mg). Chromatography on silica (40 g) (eluant ether) gave the product (150 mg), which was crystallised from methanol (112 mg, 40%), m.p. 150—160 °C (lit.,[1] 155—158 °C); $\lambda_{max.}$ 271, 282, and 294 nm (ε 10 300, 11 100, and 6 520 respectively); δ ($CDCl_3$) 5.75, 5.40 (m, 2 H, H-6, H-7), 4.07 (m, 1 H, H-3α), 3.77 (br s, 1 H, H-1β), 1.30 (s, H-19), 0.98 (d, *J* 4 Hz, H-20), 0.90 (d, *J* 7 Hz, H-26, H-27), 0.68 (s, H-18); *m/e* 400 (M^+) (60%), 382 ($[M - H_2O]^+$) (40%), 364 ($[M - 2H_2O]^+$) (50%), and 157 (100%).

1α-*Hydroxyvitamin* D_3.—The 1α-hydroxyprovitamin (8) (20 mg) was dissolved in methanol (1.5 ml) and the solution diluted with ether (250 ml). The solution was irradiated (Hanovia medium-pressure mercury vapour lamp) for 25 min under a nitrogen atmosphere while the temperature was maintained below 5 °C. Fluorenone (22 mg) was added and the solution was irradiated for a further 15 min. Preparative t.l.c. (silica, ether) gave the previtamin.[5] This was dissolved in ethanol and the solution was heated at 80 °C under a nitrogen atmosphere for 1 h. The solvent was removed under reduced pressure and the residue was chromatographed on silica (eluant ether). The band containing the vitamin was removed and extracted with chloroform–methanol, giving the product (3 mg) on evaporation of solvent; $\lambda_{max.}$ 265 nm; δ ($CDCl_3$) 6.40, 6.00 (AB q, *J* 10 Hz, H-6, H-7), 5.33, 5.01 (m, 2 H, H-19), 4.55—4.00 (m, 2 H, H-1β, H-3α), 1.30 (s), 0.90 [d, *J* 6 Hz, H-26, H-27 (?)], and 0.58 (s, H-18); *m/e* 400 (M^+) (50%), 382 ($[M - H_2O]^+$) (100%), 364 ($[M - 2H_2O]^+$) (50%), and 134 (cleavage of 7,8-bond — H_2O) (25%).

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REFERENCES

[1] C. Kaneko, A. Sugimoto, Y. Egudic, S. Yamada, M. Ishikawa, S. Sasaki, and T. Suda, *Tetrahedron*, 1974, **30**, 2701.

[2] S. A. Holick, M. F. Holick, T. E. Tavela, H. K. Schnoes, and H. F. DeLuca, *J. Biol. Chem.*, 1976, **251**, 1025; M. Fukushima, Y. Suzuki, Y. Tohira, Y. Nishii, M. Suzuki, S. Sasaki, and T. Suda, *F.E.B.S. Letters*, 1976, **65**, 211, and references cited therein.

[3] R. Zurfluh, E. N. Wall, J. B. Siddall, and J. A. Edwards, *J. Amer. Chem. Soc.*, 1968, **90**, 6224.

[4] A. Emke, D. Hands, J. M. Midgley, W. B. Walley, and R. Ahmad, *J.C.S. Perkin I*, 1977, 820.

[5] S. C. Eyley and D. H. Williams, *J.C.S. Chem. Comm.*, 1975, 858.

[6] D. H. R. Barton, R. H. Hesse, M. Pechet, and E. J. Rizzardo, *J. Amer. Chem. Soc.*, 1973, **95**, 2748.