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THE SYNTHESIS OF 24,25-DIHYDROXYCHOLECALCIFEROL, A METABOLITE OF VITAMIN D_3

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SUMMARY

The synthesis of 24,25-dihydroxycholecalciferol, a polar metabolite of cholecalciferol (vitamin D_3) is described. 5,24-Cholestadien-3β-yl acetate (I) was converted in two steps to 5,7-cholestadiene-3β,24(RS),25-triol 3,24-diacetate (III). This compound was irradiated with U.V. light to give the 3,24-diacetate of 24(RS),25-dihydroxy-precholecalciferol (IV), which in turn was subjected to thermal isomerization to give 24(RS),25-dihydroxycholecalciferol-3,24-diacetate (Va). Saponification of the latter compound afforded 24(RS),25-dihydroxycholecalciferol (Vb). The structure (Vb) was confirmed by periodate degradation and by mass spectroscopy.

INTRODUCTION

Recent studies of the metabolism of cholecalciferol (vitamin D_3) have led to the isolation and identification of a number of its polar and biologically active metabolites. In view of the difficulties encountered in obtaining these metabolites from biological sources, their preparation by chemical synthesis is necessary in order to permit a detailed study of their biological activities.

Our objective has been the preparation of the two polar metabolites of cholecalciferol isolated by Suda *et al.*[1, 2], 24,25-dihydroxycholecalciferol and 25,26-dihydroxycholecalciferol. Our synthesis of 25,26-dihydroxycholecalciferol has been published [3, 4] and has enabled us to confirm that the preponderant action of that metabolite is on the intestinal absorption of calcium [2].

In the course of that synthesis, it was verified that the osmylation of 5,25-cholestadiene-3β-yl acetate affords selective hydroxylation at carbons 25 and 26. We report here that similar selectivity applies to the osmylation of 5,24-cholestadiene-3β-yl acetate, and that the product of this reaction was converted by an established reaction sequence to 24,25-dihydroxycholecalciferol. A preliminary report of this work has already appeared [5]. 24,25-dihydroxycholecalciferol has also been synthesized by a different route [6], and in addition, after the publication of our preliminary note we became aware of the work of Seki *et al.*[7] on the synthesis of 24,25- and 25,26-dihydroxycholesterol. The physical constants reported by these latter authors are however not in agreement with our findings.

EXPERIMENTAL

Melting points were measured in open capillary tubes and are uncorrected. Ultra-violet spectra were determined in ethanol with a Perkin–Elmer 402 Spectrometer, I.R. spectra in KBr pellets with a Perkin–Elmer Infracord spectrophotometer, and NMR spectra in deuterochloroform with tetramethylsilane as internal standard using a Varian HA 100 spectrometer. Gas chromatography was performed using a Pye Model 104 gas chromatograph with flame ionization detection. The column (1500 mm glass) contained 3% OV-1 on Gas Chrom Q and was run at 240°C with an argon flow rate of 80–100 ml/min. All retention times are quoted relative to 5α-cholestane, approximately 6 min. Mass spectra were recorded on an AEI MS9 spectrometer using a direct insertion probe. Microanalyses were performed with a Technicon CHN-Analyser. Optical rotations were measured in chloroform (Uvasol, Merck). Thin layer chromatography was performed on 200 × 200 × 0·25 mm plates of Merck silica gel GF_{254} using the following solvent systems: (a) benzene; and (b) ethyl-acetate–*n*-heptane (1:1, v/v). Spots were visualized by spraying with 5% w/v phosphomolybdic acid in ethanol, and heating.

5,24-*Cholestadien-3β-yl acetate* (*I*)

(I) was obtained from 3β-hydroxy-27-nor-5-cholesten-25-one by the method of Bergmann and Dusza[10], and was purified by chromatography on a

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column of silicic acid impregnated with silver nitrate (20% w/w) [8]. The yield was 30%, m.p. 93–94°C. [8]: m.p. 96–97°C. $[\alpha]_D^{27°C} = 42{\cdot}5°$ (c 1·0). [8]: $\alpha_D = -43°$. R_f 0.70 (b).

5-Cholestene-3β,24(RS), 25-triol 3-acetate (IIa)

Osmic acid (0·18 g) in benzene (18 ml) was slowly added to a stirred solution of I (0·3 g) in dry benzene (5 ml). The black solution was stirred for 12 h and a solution of sodium hydrogensulphite (0·5 g) in water (7 ml) and ethanol (40 ml) was then added. Stirring was continued for a further 5 h. The brown precipitate was filtered off and the clear filtrate was concentrated under reduced pressure and poured into water. The precipitate which formed was extracted with chloroform, dried over anhydrous Na_2SO_4, and evaporated under reduced pressure. The crystalline residue (0·31 g, 95%) was recrystallized from methanol–*n*-hexane, m.p. 152–155°C, [7] m.p. 153·5–154·5°C. $[\alpha]_D^{24°C} = -35{\cdot}8°$ (c 1·0), [7] $[\alpha]_D = -13°$ (chloroform). R_f 0,29 (b). NMR: Me-18, 0·67 (s); Me-21, 0·92 (d); Me-19, 1·0 (s); Me-26, 27, 1·14 and 1·19; OAc-3, 1·98 (s); H-3, 4·55 (m); H-6, 5·32 ppm (d).

5-Cholestene-3β,24(RS),25-triol 3,24-diacetate (IIb)

Crude IIa (0·15 g) was treated with acetic anhydride (1 ml) and pyridine (2 ml) at room temperature overnight. After the usual isolation, the product was chromatographed on neutral alumina (activity I). Elution with ether gave IIb (0·138 g, 84%). The analytical sample was crystallized from chloroform–*n*-hexane, m.p. 155–157°C. Seki *et al.*[7] gave m.p. 167·5–168°C. $[\alpha]_D^{27} = -37{\cdot}3°$ (c 1·0). R_F 0,43 (b). IR: 3480 cm^{-1} (OH) and 1740 cm^{-1} (acetate). Calc. for $C_{31}H_{50}O_5$ (502·7): C, 74·06; H, 10·02%. Found: C, 73·87; H, 9·89. NMR: Me-18, 0·68 (s); Me-21, 0·92 (d); Me-19, 1·0 (s); Me-26, 27, 1·13 (s); OAc-3, 1·95 (s); OAc-24, 2·02 (s); H-3, 4·60 (m); H-6, 5·32 ppm (d).

Addition of the chiral europium compound, tris-[3-(tri-fluoromethyl-hydroxymethylene)-*d*-camphorato]-europium [9] to a solution of IIb in carbon tetrachloride resulted in the appearance of two acetate-24 n.m.r. signals of equal intensity, due to the presence of both epimers, 24R and 24S, in equal amounts.

5,7-Cholestadiene-3β,24(RS),25-triol 3,24-diacetate(III)

IIb (0·13 g) and 1,3-dibromo-5,5-dimethylhydantoin (0·052 g, 25% excess) were suspended in a mixture of cyclohexane (2·5 ml) and light petroleum (b.p. 60–80°C, 5 ml) and heated under reflux, with stirring, for 30 min. After cooling to room temperature, the reaction mixture was filtered and the residue washed with light petroleum. Collidine (1,8 ml) was added to the filtrate, the low boiling solvents were evaporated *in vacuo*, and the remaining solution heated at 140°C for 20 min under nitrogen. After cooling, ether was added and the precipitate filtered off and washed with ether. Work-up of the ether extract in the usual manner gave a yellow residue (mixture of isomers $\Delta^{5,7}$ and $\Delta^{4,6}$) which crystallized from methanol to give III (0·021 g, 16%). The analytical sample was recrystallized from acetone–light petroleum, m.p. 176–177°C (capillary tube *in vacuo*). λ_{max} 272,282 (ϵ = 11·000), 293 nm. $[\alpha]_D^{24°C} = -63°$ (c 0·73), R_F 0·42 (b). Calc. for $C_{31}H_{48}O_5.CH_3.CO.CH_3$ (558·8), C, 73·10; H, 9·74%. Found, C, 72·66; H, 9·51%.

24(RS),25-dihydroxycholecalciferol (Vb)

III (0·050 g) in diethyl ether–ethanol (9:1, v/v, 100 ml) was irradiated with a medium-pressure mercury arc lamp for 30 min at 0°C in the apparatus described previously [11]. The solution was evaporated to dryness under reduced pressure at 5°C, and the residue, in a small volume of chloroform, was applied to a silica gel thin-layer plate (Merck silica-gel GF_{254} 200 × 200 × 1 mm) and developed by continuous elution for 6 h with chloroform. At the end of this period, the least polar U.V.-absorbing band was scraped off and eluted with diethyl ether–ethanol (3:1, v/v; 100 ml). The eluate contained IV, λ_{max} 262 nm, contaminated by traces of the tachysterol analogue of IV. The eluate was evaporated to dryness, and the residue refluxed in ethanol (25 ml) for 1 h to effect thermal isomerization to Va. The solution was again evaporated to dryness, and to the residue was added dry benzene (5 ml) and powdered maleic anhydride (0·120 g). The mixture was refluxed for 30 min, the benzene removed under reduced pressure, methanolic potassium hydroxide (2*N*, 5 ml) added and the mixture left at room temperature for 1 h. After the usual isolation, the product, in chloroform–hexane (65:35, v/v), was applied to a 50 × 1·5 cm. column of Sephadex LH-20 packed in chloroform–*n*-hexane (65:35, v/v). The column was eluted with the same solvent mixture, 8 ml-fractions being collected. Each fraction was taken to dryness in a stream of nitrogen and redissolved in ethanol (10 ml) for measurement of ultraviolet absorbance. Fractions 37–43, with λ_{max} 265 nm were combined, to give 24(RS),25-dihydroxy-cholecalciferol (Vb). The yield was 4·5 mg (calculated assuming that $\epsilon_{265} = 18{\cdot}300$).

R_F 0,17 (b)

R_F of 25,26-dihydroxycholecalciferol is 0·10 in the same solvent system [4].

RESULTS AND DISCUSSION

5,24-Cholestadiene-3β-yl acetate (desmosterol acetate, I) was prepared from 3β-hydroxy-27-nor-5-cholesten-25-one and purified on a column of silicic acid containing silver nitrate, according to Svoboda and Thompson[8]. Treatment of desmosterol acetate selectively gave 5-cholestene-3β,24,25-triol 3-acetate (IIa) in almost quantitative yield. The structure of the product was confirmed by n.m.r. spectroscopy.

Acetylation of IIa with acetic anhydride and pyridine at room temperature gave 5-cholestene-3β,24,25-triol 3,24-diacetate (IIb) which was purified by chromatography on alumina. The optical purity of the mixture of epimers at carbon-24 obtained was determined

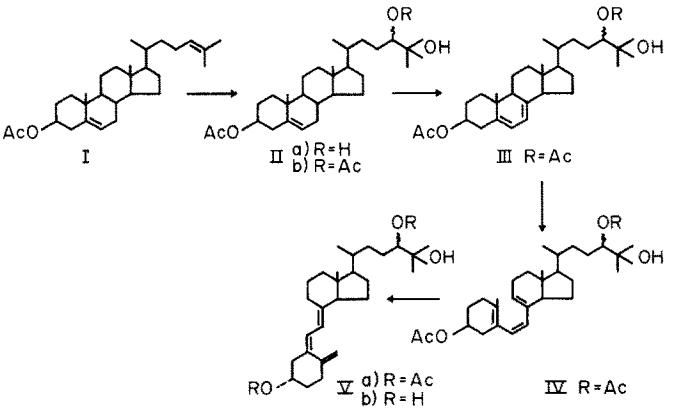

Fig. 1. Synthesis of 24(RS),25-Dihydroxycholecalciferol.

by the addition of a chiral europium complex, Tris-[3-(tri-fluoromethyl-hydroxymethylene)-*d*-camphorato]-europium [3, 4, 9] to a solution of IIb in carbon tetrachloride. This addition resulted in the n.m.r. signal due to the acetate group at carbon-24 being resolved into two peaks of equal intensity which indicated that approximately equal proportions of the 24R- and 24S-diastereoisomers were present. Thus IIa and IIb are 5-cholestene-3β,24(RS),25-triol 3-acetate and 5-cholestene-3β,24(RS), 25-triol 3,24-diacetate, respectively.

It is unlikely that the relative proportions of the diastereoisomers were changed in the course of the successive reactions.

Bromination of IIb with 1,3-dibromo-5,5-dimethylhydantoin and dehydrobromination with collidine [3] gave a mixture of isomers 5,7-diene and 4,6-diene. 5,7-Cholestadiene-3β,24(RS), 25-triol 3,24-diacetate (III) was isolated from this mixture in 16% yield by crystallization.

Irradiation of III with U.V. light gave a mixture of irradiation products from which 24(RS),25-dihydroxyprecholecalciferol 3,24-diacetate was isolated in an impure form, contaminated with the tachysterol analogue. Thermal isomerization to Va was followed by removal of the tachysterol analogue by adduct formation with maleic anhydride and saponification to give 24(RS),25-dihydroxycholecalciferol (Vb). The structure (Vb) was confirmed in the following way. Vb gave two gas chromatographic peaks corresponding to pyro- and isopyro-derivatives, with relative retention times of 4·24 and 4·70, respectively. The separation factor 1·11 is characteristic of derivatives of vitamin D unmodified in the nucleus [4]. The structure of Vb was further confirmed by derivative formation and mass spectroscopy; Vb had peaks at *m/e* (relative intensity): 416 (13; M^+), 398 (4, M—H_2O), 271 (12), 253 (16), 136 (96), 118 (100). These data are in agreement with the findings of Lam *et al.*[6]. The intense peaks at m/e 136 and 118 arise by cleavage of the C_7—C_8 bond with subsequent loss of H_2O, and are characteristic of all vitamin D derivatives having an intact triene system [12]. The fragments at m/e 271 and 253, derived by loss of the side chain, demonstrate that the hydroxyl substituents are in the side chain. With N,O-*bis* (trimethylsilyl)-acetamide Vb gave a di-TMS derivative, M^+ 560, and with hexamethyldisilazane and trimethylchlorosilane Vb gave a tri-TMS derivative, M^+ 632.

Conclusive evidence for structure Vb was provided by periodate degradation. With periodate, Vb gave a single product having mass spectral peaks at m/e (relative intensity): 356 (9, M^+), 338 (3, M—H_2O), 271 (3), 253 (6), 136 (79), 118 (100). This product could only have arisen by cleavage of a 24,25-diol.

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