Synthesis and Determination of the Configuration of 23,25-Dihydroxyvitamin D_3; a New Metabolite of Vitamin D_3; *X*-Ray Crystal Structure of a 3,23,25-Triol Precursor

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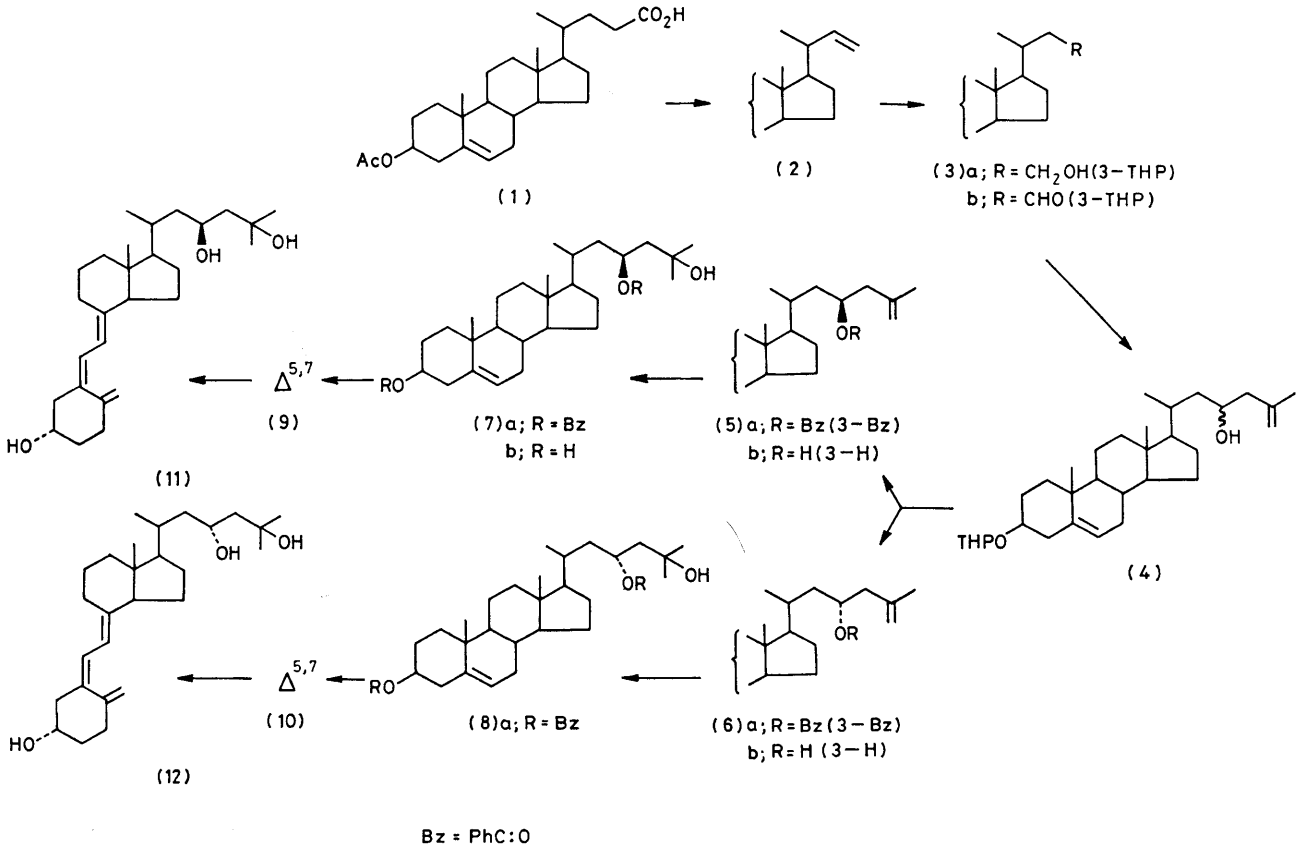
Summary The configuration at the C-23 position of 23,25-dihydroxyvitamin D_3, a new metabolite of vitamin D_3, was determined as *S* by comparison with the synthetically prepared C-23 *S*- and *R*-isomers and an *X*-ray crystallographic determination of the structure of the 3,23,25-triol (**7b**).

A NEW metabolite of vitamin D_3 has recently been isolated and positively identified as 23,25-dihydroxyvitamin D_3 [23,25-$(OH)_2D_3$].[1] The structure was elucidated by mass spectrometry and chemical synthesis but the configuration at the C-23 position was not determined. We have now synthesized both isomers of 23,25-$(OH)_2D_3$ and determined their configuration by *X*-ray crystallography. The C-23 configuration of the natural metabolite was determined by means of co-chromatography with the synthetic products.

Cholenic acid acetate (**1**) was converted into the 22-olefin (**2**) by oxidative decarboxylation with lead tetra-acetate, copper(II) acetate, and pyridine in refluxing benzene in 61% yield. After changing the protecting group of the 3-hydroxy-group to tetrahydropyran-2-yl (THP), the 22-olefin was treated with boron hydride–tetrahydrofuran (BH_3–THF) complex at 0 °C and then with alkaline hydrogen peroxide to give the 23-alcohol (**3a**), which was oxidized with pyridinium chlorochromate in methylene dichloride containing sodium acetate to afford the 23-aldehyde (**3b**)



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in 45% yield from (**2**). The aldehyde was coupled with methallyl chloride in THF at 0 °C by a Grignard reaction to give a 1:1 mixture of the stereoisomers of the 23-alcohol (**4**) in 96% yield. To separate the C-23 isomers, (**4**) was converted into the 3,23-dibenzoate and recrystallized from methanol to give the more polar dibenzoate (**5a**), m.p. 151—153 °C (from acetone); δ ($CDCl_3$) 0·70 (s, 18-H), 1·02 (s, 19-H), 1·76 (s, 27-H), 4·50—5·00 (m, 3-H, 26-H), and 5·10—5·60 (m, 6-H, 23-H). After removal of most of (**5a**), the less polar dibenzoate was purified by preparative t.l.c. (benzene–hexane, 1:1; developed five times) to give the amorphous (**6a**), δ ($CDCl_3$) 0·62 (s, 18-H), 1·79 (s, 27-H), 4·60—5·10 (m, 26-H), and 5·20—5·60 (m, 6-H, 23-H). On treatment with MeOH–KOH (**5a**) afforded the more polar 3,23-diol (**5b**), m.p. 153—154 °C (from acetone); $[\alpha]_D^{25}$ −30·2° (*c* 1, $CHCl_3$), and (**6a**) gave the less polar (**6b**), m.p. 126—128·5 °C (from ether–hexane); $[\alpha]_D^{25}$ −31·0 ° (*c* 1, $CHCl_3$). It should be noted that the isomeric 23-benzoates exhibited clearly different chemical shifts for the C-18 methyl resonance. Introduction of a hydroxy-group at the C-25 position of (**5a**) or (**6a**) was performed by oxymercuration–demercuration with $Hg(OAc)_2$ followed by $NaBH_4$ reduction. Thus, (**5a**) afforded the 25-hydroxy-3,23-dibenzoate (**7a**), m.p. 221—222 °C (from acetone–hexane); $[\alpha]_D^{28}$ −7·1° (*c* 0·67, $CHCl_3$); δ ($CDCl_3$) 0·72 (s, 18-H), 1·27 (s, 26-H, 27-H), 4·60—5·00 (m, 3-H), and 5·20—5·60 (m, 23-H, 6-H), and (**6a**) gave (**8**), m.p. 176—177 °C (from acetone–hexane); $[\alpha]_D^{28}$ +9·2° (*c* 0·79, $CHCl_3$); δ ($CDCl_3$), 0·62 (s, 18-H), 1·20 (s, 26-H), 1·25 (s, 27-H), 4·60—5·10 (m, 3-H), and 5·30—5·60 (m, 6-H, 23-H). The 3,23,25-triol (**7b**) of the more polar series was obtained by treatment of (**7a**) with alkali, m.p. 218—210 °C (decomp.) (from MeOH–H_2O); $[\alpha]_D^{28}$ −2·2° (*c* 0·2, EtOH). The configuration at C-23 of this compound was established as *S* by *X*-ray diffraction.

Crystal data: monoclinic, space group $P2_1$, a = 16·367(7), b = 11·632(5), c = 6·473(3) Å, β = 97·04(4)°, Z = 2. Intensity data were measured on a Philips PW1100 four-circle diffractometer using Cu-K_α radiation monochromated by a graphite plate. A total of 1122 non-zero, independent reflections were used for the structure determination. The structure was solved by direct methods using MULTAN and refined by block-diagonal least-squares assuming anisotropic temperature factors. The final *R*-index was 0·096, taking no account of hydrogen atoms. Further refinement was not attempted, since the crystals were very thin flakes and the number of observed reflections was almost half the theoretically possible number, within a 2θ angle of 120°. Furthermore, it was not possible to collect the data beyond this angle.†

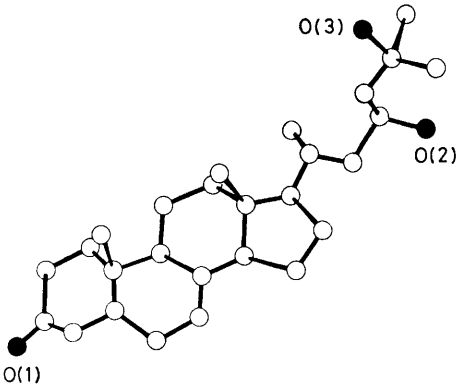

FIGURE 1. Perspective view of the molecular structure of compound (**7b**).

The molecular configuration is shown in Figure 1. The side chain adopts a rather extended conformation and all the hydroxy-groups are turned outside to interact with neighbouring molecules. Thus the molecules are strongly connected to each other by the two intermolecular hydrogen bonds: O(1) . . . O(2) (2·77 Å) and O(1) . . . O(3) (2·77 Å). No intramolecular hydrogen bonds are observed. A *gauche* conformation about the C(22)–C(23) bond along the C(20)–C(22)–C(23)–C(24) chain may be a consequence of the aforementioned intermolecular hydrogen bonds. Thus the more polar series (**5a**) and (**7a**) have the 23*S*- and the less polar series (**6a**) and (**8**) have the 23*R*-configuration.

Conversion of (**7a**) and (**8**) into the corresponding vitamin D form was carried out by the standard procedure as follows. Allylic bromination of (**7a**) and (**8**) with *N*-bromosuccinimide in CCl_4 followed by dehydrobromination with 2,4,6-trimethylpyridine in xylene and saponification with 5% KOH–MeOH gave the 5,7-dienes (**9**) and (**10**), respectively, in 35% yield. Both compounds show λ_{max} (EtOH) 292, 282, and 272 nm. They were irradiated with a medium-pressure

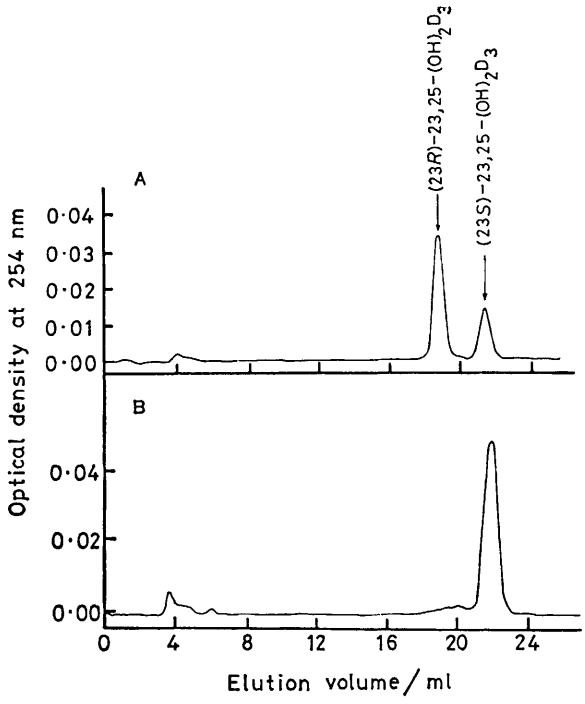

FIGURE 2. Separation of epimers of synthetic 23,25-$(OH)_2D_3$ and co-chromatography of natural 23,25-$(OH)_2D_3$ with synthetic isomers by h.p.l.c. The h.p.l.c. using a Zorbax-Sil column (4·6 mm × 25 cm) was performed at 1300 lb in^{-2} pressure and a flow rate of 2 ml min^{-1} with 2% MeOH in CH_2Cl_2 as solvent. (A) Separation of (23*R*)-23,25-$(OH)_2D_3$ and (23*S*)-23,25-$(OH)_2D_3$. (B) Co-chromatography of a mixture of synthetic (23*S*)-23,25-$(OH)_2D_3$ and natural 23,25-$(OH)_2D_3$.

† The atomic co-ordinates for compound (**7b**) are available on request from the Director of the Cambridge Crystallographic Data Centre, University Chemical Laboratory, Lensfield Road, Cambridge CB2 1EW. Any request should be accompanied by the full literature citation for this communication.

mercury lamp in ethanol–benzene and refluxed for 1 h. Purification by high-pressure liquid chromatography (h.p.l.c.) gave (23*S*)-23,25-$(OH)_2D_3$ (**11**) from (**9**) and (23*R*)-23,25-$(OH)_2D_3$ (**12**) from (**10**). These compounds exhibited λ_{max} 265 and λ_{min} 228 nm; *m/e* 416 (*M*), 383, 271, 253, 136, and 118, and can be separated cleanly by h.p.l.c.

As shown in Figure 2, the natural 23,25-$(OH)_2D_3$ (ref. 1) had an identical elution time to that of synthetic (23*S*)-23,25-$(OH)_2D_3$. Thus the configuration of the 23-hydroxy-group of natural 23,25-$(OH)_2D_3$ was clearly determined to be *S*. Evidence that this compound is a natural precursor in the biosynthesis of 25-hydroxyvitamin D_3 26,23-lactone will be reported elsewhere.[2]

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[2] Y. Tanaka, H. F. DeLuca, H. K. Schnoes, N. Ikekawa, and T. Eguchi, *Proc. Natl. Acad. Sci. USA*, 1981, in the press.