

Synthesis and Determination of the Configuration of 23,25-Dihydroxyvitamin D₃; a New Metabolite of Vitamin D₃; X-Ray Crystal Structure of a 3,23,25-Triol Precursor

By NOBUO IKEKAWA,* TADASHI EGUCHI, and YUTAKA HIRANO

(Laboratory of Chemistry for Natural Products, Tokyo Institute of Technology,
Nagatsuta, Midori-ku, Yokohama 227, Japan)

YOKO TANAKA and HECTOR F. DELUCA

(Department of Biochemistry, College of Agricultural and Life Sciences, University of
Wisconsin-Madison, Madison WI 53706)

and AKIKO ITAI and YOICHI IITAKA

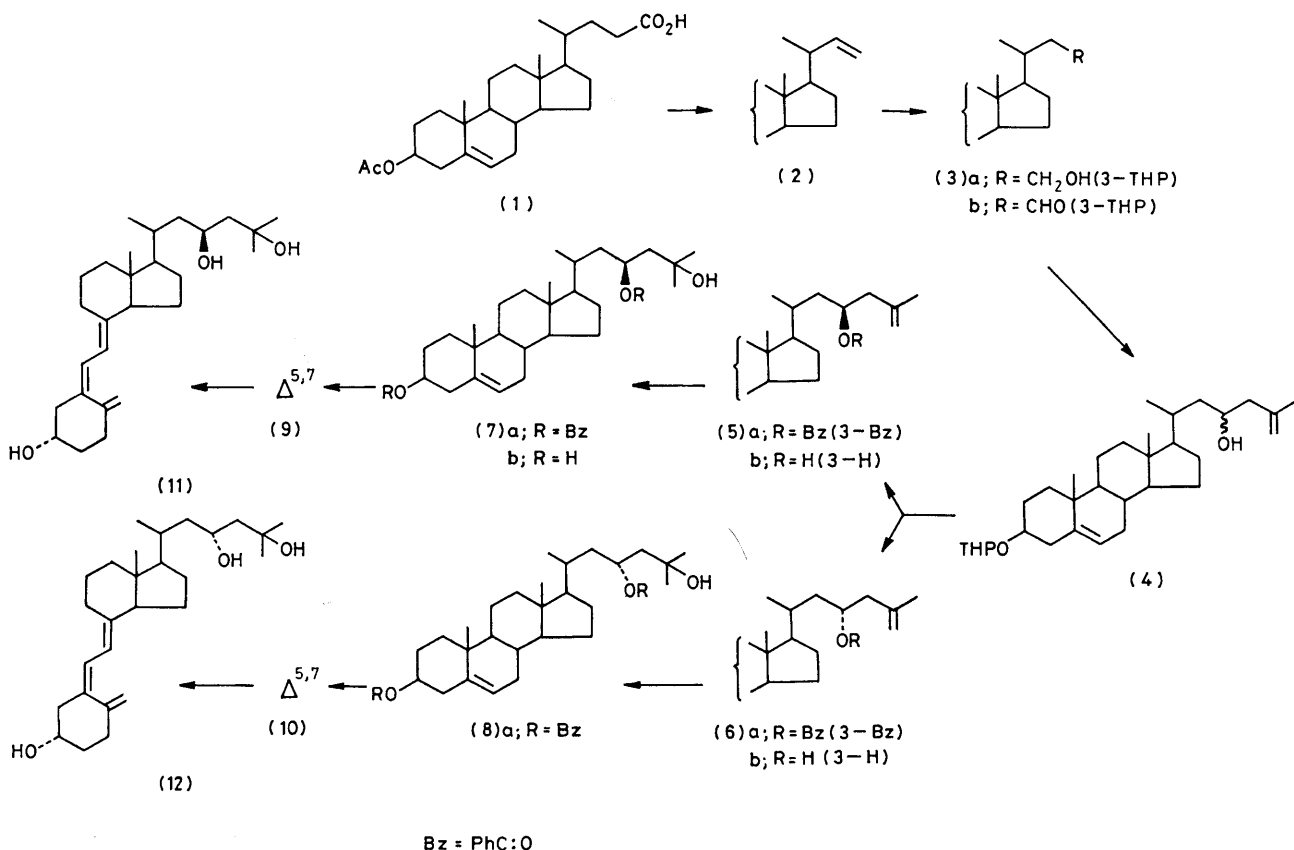
(Department of Pharmaceutical Sciences, University of Tokyo, Bunkyo-ku, Tokyo 113, Japan)

Summary The configuration at the C-23 position of 23,25-dihydroxyvitamin D₃, a new metabolite of vitamin D₃, 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 D₃ has recently been isolated and positively identified as 23,25-dihydroxyvitamin D₃ [23,25-(OH)₂D₃].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)₂D₃ 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 (BH₃-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)



SCHEME

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 (CDCl₃) 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 (CDCl₃) 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); $[\alpha]_D^{25} - 30.2^\circ$ (*c* 1, CHCl₃), and (6a) gave the less polar (6b), m.p. 126–128.5 °C (from ether–hexane); $[\alpha]_D^{25} - 31.0^\circ$ (*c* 1, CHCl₃). 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 NaBH₄ reduction. Thus, (5a) afforded the 25-hydroxy-3,23-dibenzoate (7a), m.p. 221–222 °C (from acetone–hexane); $[\alpha]_D^{25} - 7.1^\circ$ (*c* 0.67, CHCl₃); δ (CDCl₃) 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); $[\alpha]_D^{25} + 9.2^\circ$ (*c* 0.79, CHCl₃); δ (CDCl₃) 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–H₂O); $[\alpha]_D^{25} - 2.2^\circ$ (*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 *P*2₁, *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,

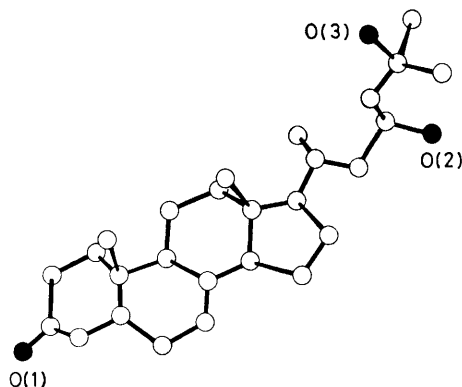


FIGURE 1. Perspective view of the molecular structure of compound (7b).

† 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.

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.†

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 CCl₄ 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

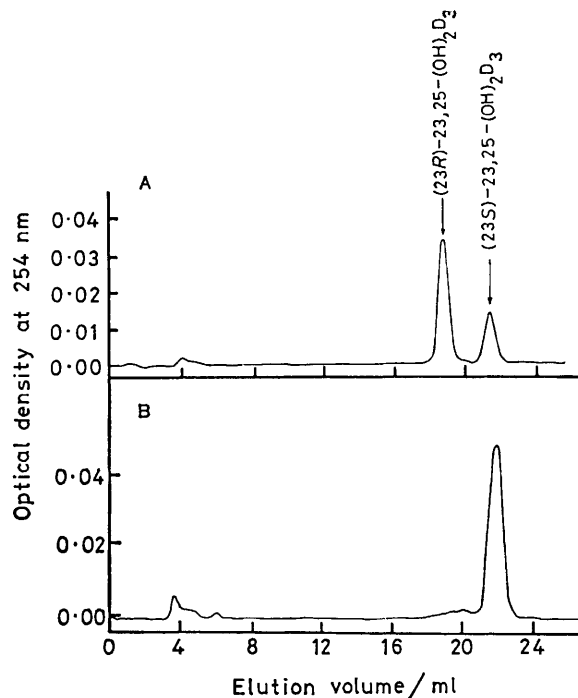


FIGURE 2. Separation of epimers of synthetic 23,25-(OH)₂D₃ and co-chromatography of natural 23,25-(OH)₂D₃ with synthetic isomers by h.p.l.c. The h.p.l.c. using a Zorbax-Sil column (4.6 mm × 25 cm) was performed at 1300 lb in⁻² pressure and a flow rate of 2 ml min⁻¹ with 2% MeOH in CH₂Cl₂ as solvent. (A) Separation of (23*R*)-23,25-(OH)₂D₃ and (23*S*)-23,25-(OH)₂D₃. (B) Co-chromatography of a mixture of synthetic (23*S*)-23,25-(OH)₂D₃ and natural 23,25-(OH)₂D₃.

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)₂D₃ (**11**) from (**9**) and (23*R*)-23,25-(OH)₂D₃ (**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)₂D₃ (ref. 1) had an identical elution time to that of synthetic (23*S*)-23,25-(OH)₂D₃. Thus the configuration of the 23-hydroxy-group of natural 23,25-(OH)₂D₃ was clearly determined to be *S*. Evidence that this compound is a natural precursor

in the biosynthesis of 25-hydroxyvitamin D₃ 26,23-lactone will be reported elsewhere.²

This work was supported in part by a program project grant from the National Institutes of Health, a U.S.–Japan Co-operative grant from the National Science Foundation and the Japan Society for the Promotion of Science, and by the Harry Steenbock Research Fund of the Wisconsin Alumni Research Foundation.

(Received, 6th May 1981; Com. 532.)

¹ Y. Tanaka, J. K. Wickmann, H. K. Schnoes, and H. F. DeLuca, *Biochemistry*, 1981, **20**, 3875.

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