SYNTHESIS AND BIOLOGICAL ACTIVITY OF 24ξ[1]- AND 24ξ[2]-HYDROXYVITAMIN D_3[+]

by

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Stereoisomers of 24-hydroxyvitamin D_3 were synthesized from fucosterol obtained from marine brown algae. Both isomers are equally active in stimulating intestinal calcium transport in vitamin D-deficient rats. Nephrectomy abolishes the intestinal calcium responses which suggests that these compounds must be hydroxylated on carbon 1 for biological activity.

In recent years, the unexpected finding that vitamin D must be metabolically activated before it can function has appeared. Thus vitamin D_3 is metabolized to 25-hydroxyvitamin D_3 (25-OH-D_3)[1] in the liver (1) and subsequently in the kidney to 1,25-dihydroxyvitamin D_3 (1,25-$(OH)_2D_3$) before it carries out its function in bone and intestine. This hydroxylation is under strict feedback control by parathyroid hormone (2), serum calcium (3), serum phosphorus (4) and 1,25-$(OH)_2D_3$ itself (5). When 1,25-$(OH)_2D_3$ synthesis is retarded by such regulation, another metabolite is synthesized. This metabolite was isolated and identified as 24,25-dihydroxyvitamin D_3 (24,25-$(OH)_2D_3$) (6) and its biological activity determined (7). However, the physiological

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The configuration of the 24-hydroxyl group of 24-hydroxycholesterol has been determined by Klyne and Stokes in 1954 (13). However, it was suggested by Van Lier and Smith (14) in 1970 that this assignment should be reversed and still some uncertainty remains. Therefore, we retain the original nomenclature (15) for the present paper.

[1]Abbreviations: 25-hydroxyvitamin D_3, 25-OH-D_3; 1,25-dihydroxyvitamin D_3, 1,25-$(OH)_2D_3$; 24,25-dihydroxyvitamin D_3, 24,25-$(OH)_2D_3$; 24-hydroxyvitamin D_3, 24-OH-D_3.

function of the 24,25-$(OH)_2D_3$ is still unknown although it was found that further hydroxylation is required at C-1 in the kidney to achieve biological activity (7). Since 24,25-$(OH)_2D_3$ is the major dihydroxy metabolite of vitamin D_3 found in normal rats (8) as well as man (9), continued investigation of the significance of 24-hydroxylation appears important. Furthermore, the stereochemical configuration of the hydroxyl group at C-24 of 24,25-$(OH)_2D_3$ and its possible biological significance is not yet determined.

The present report describes the chemical synthesis of both stereoisomers of 24-hydroxyvitamin D_3 (24-OH-D_3) and their biological activities.

Synthesis

Melting points were determined on a hot-stage microscope and are uncorrected. UV spectra in ethanol solution were recorded with a Hitachi ESP-3T spectrophotometer. ^1H nmr spectra were run on a Varian T-60 or JNM-MH-100 with $CDCl_3$ as solvent and with TMS as internal reference. Mass spectra were obtained with Shimadzu LKB-9000S; ionization voltage, 70 eV, ion source temperature, 80°.

Column chromatography was effected with silica gel (Wakogel C-200). Thin-layer chromatography (tlc) was carried out on Merck silicagel F_{254} (0.25 nm thick). High pressure liquid chromatography was performed with a Dupont 840 instrument equipped with Zorbax SIL column (25 cm x 2.1 mm), using hexane-CH_2Cl_2 (2:1) as eluant unless otherwise cited.

24ξ[1]- and 24ξ[2]-Hydroxycholesterol dibenzoates (IIa and IIb).[2] Treatment of 3β-acetoxy-24-hydroxycholest-5-ene (I) (2.10 g) with $LiAlH_4$ (0.5 g) in an ether-tetrahydrofuran (10:1) (30 ml) at room temperature for 30 min gave the 3,24-diol (1.77 g). The product was heated at 60° for 4 hours in pyridine (20 ml) containing benzoylchloride (2 ml). Then the mixture was stirred with water at room temperature overnight. The precipitate was filtered and washed

[2]The synthesis of 3β,24ξ-dihydroxycholest-5-ene-3-acetate from fucosterol has been described previously (16).

with warm water. Crystallization from acetone 4 times gave 24ξ[1]-dibenzoate (IIa) (898 mg), mp 182-184° (cf. Ercoli and Ruggieri (15); 179-181°). The mother liquor was crystallized from hexane 3 times to afford 24ξ[2]-dibenzoate (IIb) (497 mg), mp 144-146.5 (cf. 141-142°). Compound IIa is more polar than IIb on tlc as well as high pressure liquid chromatography.

3β,24ξ[1]-Dihydroxycholesta-5,7-diene dibenzoate (IIIa). Compound IIa (800 mg) was refluxed with N-bromosucinimide (240 mg) in CCl_4 (48 ml) for 10 min. After cooling with ice, the precipitate was removed by filtration. The evaporated filtrate was dissolved in xylene (15 ml) and was added, during 10 min, to refluxing xylene containing trimethylphosphite (0.8 ml). Refluxing was continued for 90 min and the solvent was evaporated in vacuo. The residue was crystallized from acetone to give the 5,7-diene (IIIa) (165 mg): mp 146-147°; δ, 0.56 (3H, s, 18-Me), 4.9 (2H, m, C-3 and 24-Hs) and 5.5 (2H, dd, J = 6 Hz, C-6,7-Hs); λ_{max}, 299, 272, 282, and 294 nm. Saponification of IIIa gave the corresponding 3β,24ξ[1]-diol, mp 173-174°.

3β,24ξ[2]-Dihydroxycholesta-5,7-diene dibenzoate (IIIb). By the same manner as described above, compound IIb (602 mg) yielded the 5,7-diene (IIIb) (194 mg), mp 155-157°; δ 0.59 (3H, s, 18-Me), 5.0 (2H, m, C-3- and 24-Hs) and 5.5 (2H, dd, J = 6 Hz, C-6,7-Hs). Saponification of IIIb gave the corresponding 3β,24ξ[2]-diol, mp 161-162.5°.

24ξ[1]-Hydroxyvitamin D_3 (IVa). The solution of the 5,7-diene (IIIa) (50 mg) in benzene (150 ml) was irradiated with a high pressure mercury lamp. (Ushio UM-102) at 5° for 10 min and then refluxed for 1 hour. After evaporation of solvent, the residue was chromatographed on a silica gel column using high pressure liquid chromatography. From the fraction eluted with hexane-benzene (8:3), the vitamin dibenzoate (8 mg) was obtained. This was treated with 10% KOH in tetrahydrofuran-methanol at room temperature overnight. Final purification of the product was carried out by preparative liquid chromatography using solvent of 1% methanol in CH_2Cl_2. Pure IVa (3 mg) had λ_{max} 265 nm,

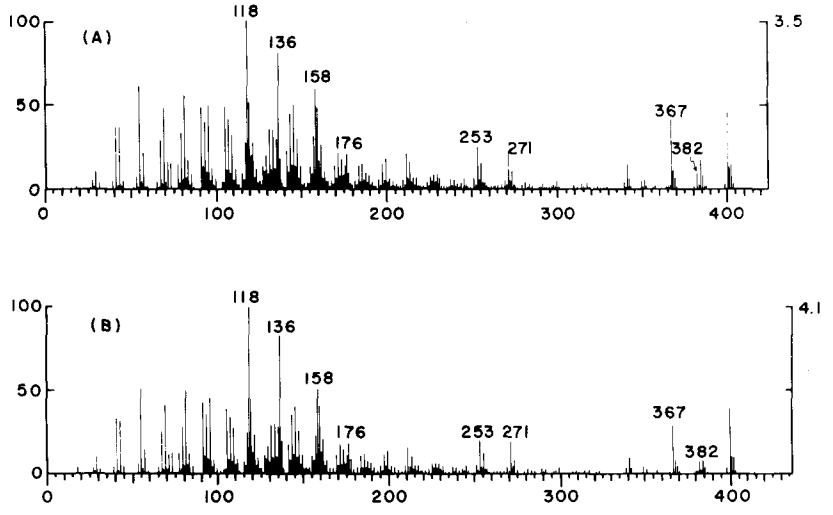

Figure 1. Mass spectrum of (A) $24\xi^1$-OH-D_3 and (B) $24\xi^2$-OH-D_3.

λ_{min} 228 nm; δ, 0.58 (3H, s, 18-Me), 3.40 (1H, m, C-24-H), 3.95 (1H, m, C-3-H), 4.86 and 5.08 (2H, two broad s, 19-CH_2) and 6.2 (2H, AB q, J = 11 Hz, C-6,7-Hs).

$24\xi^2$-Hydroxyvitamin D_3 (IVb). By the analogous manner described above, the 5,7-diene (IIIb) afforded the vitamin (IVb), mp 183-184.5° (from CH_2Cl_2).

Table 1. Intestinal Calcium Transport Response to the 24-OH-D_3 Isomers

Compound	Intestinal Calcium Transport Ca^{45} serosal/Ca^{45} mucosal
Ethanol	1.6 ± 0.1
$24\xi^1$-OH-D_3	4.2 ± 0.4*
$24\xi^2$-OH-D_3	4.5 ± 0.6*
25-OH-D_3	4.9 ± 0.7*

The compounds (650 pmoles) were dissolved in 0.05 ml of ethanol and dosed intrajugularly 48 hr prior to sacrifice. Data are expressed as mean ± standard error of the mean. There were 5 to 6 rats in each group.

*Significantly different from control $p < 0.005$ (Student's t test).

Table 2. The Elimination of Intestinal Calcium Transport Response to 24-OH-D_3 by Nephrectomy

Compound	Surgery	Intestinal Calcium Transport Ca^{45} serosal/Ca^{45} mucosal
Ethanol	Sham	1.6 ± 0.2^b
$24\xi^1$-OH-D_3	Sham	3.2 ± 0.2^a
$24\xi^2$-OH-D_3	Sham	3.2 ± 0.4^a
$24\xi^1$-OH-D_3	Nephrectomized	1.8 ± 0.5
$24\xi^2$-OH-D_3	Nephrectomized	1.8 ± 0.2

Vitamin D-deficient rats given a low calcium diet were either sham operated or nephrectomized. At the time of surgery 325 pmoles of either compound in 0.05 ml ethanol was injected intrajugularly 18 hr prior to sacrifice. Data are expressed as the mean of 6 rats $\pm$ standard error of the mean.

a is significantly different from b $p < 0.005$

Uv, nmr and mass spectra (Figure 1) were superimposable with those of IVa.

Biological Activity

Weanling male rats were obtained from the Holtzman Company (Madison, Wis.) and maintained in overhanging wire cages. They were fed a low calcium-vitamin D-deficient diet supplied ad libitum (10). After the animals had been on the diet for two weeks, they were considered calcium and vitamin D deficient and were used for the determination of biological activity. Compounds were dissolved in 0.05 ml of 95% ethanol and dosed intrajugularly. Intestinal calcium transport was measured by the everted gut sac technique as described by Martin and DeLuca (11). As shown in Table 1, both stereoisomers of 24-OH-D_3 are active in stimulating intestinal calcium transport.

Since it has been shown that C-1 hydroxylation of vitamin D compounds or existence of a hydroxyl group at the geometric position normally occupied by

the 1-hydroxyl of 1,25-$(OH)_2D_3$ is essential for the biological activity (12), it might be expected that 24-OH-D_3 must undergo further hydroxylation(s) to stimulate intestinal calcium transport. As shown in Table 2, nephrectomized animals do not respond to either of the 24-OH-D_3 isomers.

Although it is not known if 24-OH-D_3 occurs naturally, the present investigation illustrates that it clearly possesses biological activity. Of importance is the fact that 1-hydroxylation is probably necessary for activity. It is not known, however, whether 25-hydroxylation is necessary or whether the active species is 1,24-dihydroxyvitamin D_3. It is interesting that the 24-hydroxy group does not interfere with intestinal calcium transport activity and furthermore that this system does not discriminate between the two 24-hydroxy stereoisomers. This suggests that the intestinal calcium transport system is not very specific in the vitamin D side chain structural requirements.

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