Tetrahedron Letters,Vol.25,No.30,pp 3239-3242,1984 0040-4039/84 $3.00 + .00
Printed in Great Britain

STEREOSELECTIVE SYNTHESIS OF (25R)-25,26-DIHYDROXY-23-OXOVITAMIN D_3 AND ITS ENZYMATIC CONVERSION TO (25R)-1α,25,26-TRIHYDROXY-23-OXOVITAMIN D_3, A PUTATIVE METABOLITE OF VITAMIN D_3

Sachiko Yamada, Keiko Nakayama, and Hiroaki Takayama*

Faculty of Pharmaceutical Sciences, Teikyo University, Sagamiko, Kanagawa 199-01, Japan

Toshimasa Shinki and Tatsuo Suda

The Department of Biochemistry, School of Dentistry, Showa University,

1-5-8 Hatanodai, Shinagawa-ku, Tokyo 142, Japan

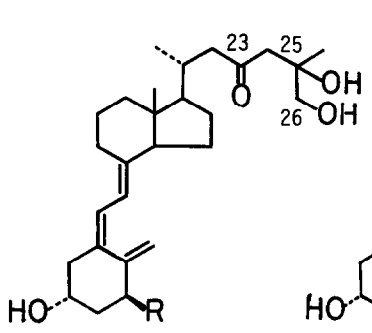
Summary: (25R)-25,26-Dihydroxy-23-oxovitamin D_3 was synthesized efficiently and stereoselectively, and it was converted enzymatically to (25R)-1α,25,26-trihydroxy-23-oxovitamin D_3, a putative metabolite of 1α,25-dihydroxyvitamin D_3. The spectral and chemical properties of (25R)-25,26-dihydroxy-23-oxovitamin D_3 and its 1α-hydroxylated derivative disagree with those reported for the isolated metabolite.

Hemiacetal form (2a) of 1α,25,26-trihydroxy-23-oxovitamin D_3 (1a)[1] was assigned to the new major metabolite of 1α,25-dihydroxyvitamin D_3[2] produced in vitro by incubation of 1α,25-dihydroxyvitamin D_3 with homogenate of small intestinal mucosa from vitamin D repleted chicks. The R configuration of 1a was suggested for the stereochemistry at C-25 of the metabolite on the basis of the biogenesis of the metabolite.[1,3] We successfully synthesized 1α,25R,26-trihydroxy-23-oxovitamin D_3 (1a, 25R) using a combination of chemical synthesis and enzymatic transformation. Here we report the first and stereoselective synthesis of 25R,26-dihydroxy-23-oxovitamin D_3 (1b, 25R) and 1α,25R,26-trihydroxy-23-oxovitamin D_3 (1a, 25R).

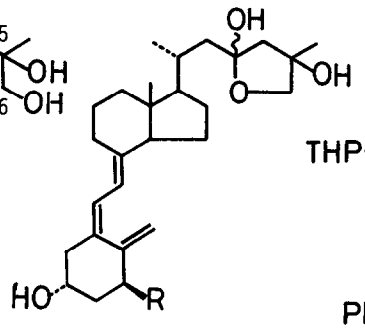
We designed the stereoselective synthesis of 1α,25R,26-trihydroxy-23-oxo-vitamin D_3 (1a) as follows; (i) the synthesis is divided into two parts, chemical synthesis of 25R,26-dihydroxy-23-oxovitamin D_3 (1b) and its enzymatic transformation to the corresponding 1α-hydroxylated derivative (1a) by using 1α-hydroxylase in the kidney from vitamin D-deficient chicks,[4] (ii) the skeleton of the target molecule (1b) of the chemical synthesis is constructed from two segments, C(22) steroid synthon (3)[3a,5] and C(5) chiral synthon (4)[3a,5b,5c] possessing the desired functionality and stereochemistry, (iii) the 23-oxo group is introduced by oxidative desulfonylation[6] of the phenylsulfonyl group, which serves to couple the two segments. The chiral C(5) sulfone (4) was synthesized from (R)-(-)-citramalic acid.[7] Coupling of the sulfone 4 with C(22) bromide 3 (LDA, THF-HMPA, -20 °C) gave the 23-phenyl-

sulfonyl derivative 5a in high yield (95%). Oxidative desulfonylation[6] of 5a with MoO_5·pyridine·HMPA (MoOPH) in the presence of LDA failed to give the corresponding 23-oxo derivative 6a but gave a compound oxidized at the phenyl group. However, the required oxidation proceeded in fairly good yield when oxygen was used instead of bulky MoOPH yielding 6a. Removal of the acetonide protecting group of 6a was rather difficult because of acid sensitiveness of the resulting β,γ-dihydroxyketone 6c. Thus, deprotection of 6a under acidic conditions (PPTS/EtOH, reflux) yielded the furan 7 (quantitative yield)[8] as a result of the cyclization between the 23-ketone and the 26-hydroxyl groups of the β,γ-dihydroxy ketone 6c formed and the subsequent dehydration. To avoid an exposure of 6c to acidic conditions, the protecting groups of the hydroxyl groups in 5a were exchanged to t-butyldimethylsilyl (TBDMS) giving 5b, and the same oxidation (LDA, O_2, THF-HMPA, -20°C) was carried out on the sulfone 5b affording the 23-ketone 6b [MS m/z 658 (M^+); ^1H NMR ($CDCl_3$) δ 2.45 (1 H, d, J = 16 Hz, H-24), 2.79 (1 H, d, J = 16 Hz, H-24); IR ($CHCl_3$) 1695 cm^{-1}] (54%). Attempted removal of the TBDMS group with n-Bu_4NF (THF), however, caused retro aldol reaction giving the methyl ketone 8 (quantitative yield).[9] The deprotection was achieved only when a combination of KF and 18-crown-6 (1:1, DMF, r.t.) was used as the reagent giving the desired provitamin D (6c) in 60% yield [MS m/z 430 (M^+), 394, 356; ^1H NMR ($CDCl_3$) δ 1.21 (3 H, s, H-27), 2.52 (1 H, d, J = 17 Hz, H-24), 2.78 (1 H, d, J = 17 Hz, H-24), 3.42 (2 H, m, H-26); IR ($CHCl_3$) 1695 cm^{-1}]. Irradiation of the provitamin D (6c) followed by thermal isomerization afforded 25R,26-dihydroxy-23-oxovitamin D_3 (1b, 25R) [high- resolution MS, $C_{27}H_{42}O_4$ requires m/z 430.3083; found m/z 430.3078; MS, m/z 430 (M^+), 412 (M - H_2O), 394 (M - $2H_2O$), 361 (M - $3H_2O$ - CH_3), 356 {M - $C_3H_6O_2$ (McLafferty rearrangement)}, 323 (356 - H_2O - CH_3), 298 {M - $C_6H_{12}O_3$ (McLafferty rearrangement)}, 265 (298 - H_2O - CH_3), 253 (M - side chain - H_2O), 136 (M - side chain and CD-ring), 118 (136 - H_2O); IR ($CHCl_3$) 1695 cm^-; ^1H NMR ($CDCl_3$) δ 1.20 (3 H, s, H-27), 2.56 (1 H, d, J = 17 Hz, H-24), 2.74 (1 H, d, J = 17 Hz, H-24), 3.42 (2 H, br s, H-26); UV (95% EtOH) 265 nm (max), 228 nm (min)] in 27% overall yield.

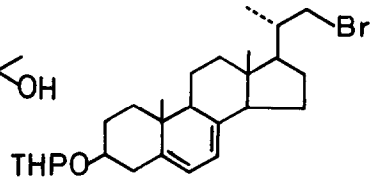
The vitamin D (1b, 25R) thus obtained was subjected to enzymatic 1α-hydroxylation by using kidney homogenate from vitamin D deficient chicks.[4] Chloroform-methanol extracts of the incubation mixture of 1b and kidney homogenate were purified on Sephadex LH-20 column followed by two straight phase HPLC columns to give homogeneous metabolite (about 3.5% yield based on the UV spectrum). The structure of the isolated metabolite was determined to be 1α,25,26-trihydroxy-23-oxovitamin D_3 (1a, 25R) on the basis of the UV [20% 2-PrOH/hexane 264 nm (max), 229 nm (min)] and the MS spectra [m/z 446 (M^+), 410 (M - $2H_2O$), 392 (M - $3H_2O$), 374



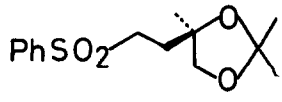
1a: R = OH
1b R = H



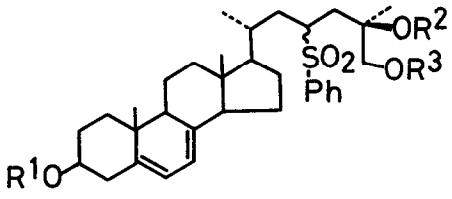
2a: R = OH
2b: R = H



3

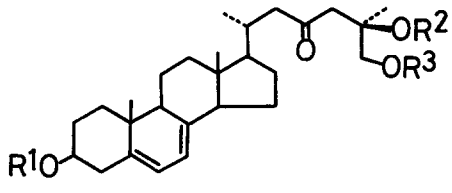


4



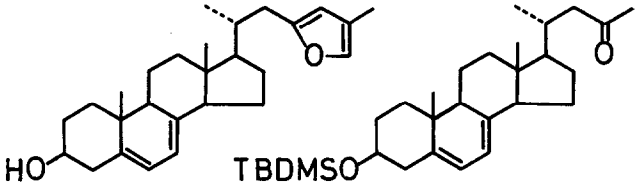
5a: R^1 = THP R^2,R^3 = $>C<^{CH_3}_{CH_3}$

5b: $R^1 = R^3$ = TBDMS R^2 = H



6a: R^1 = THP R^2,R^3 = $>C<^{CH_3}_{CH_3}$

6b: R^1,R^3 = TBDMS R^2 = H
6c: R^1,R^2,R^3 = H



7

8

(M - $4H_2O$), 354 {M - $C_3H_6O_2$ (McLafferty rearrangement) - H_2O}, 336 (354 - H_2O), 321 (336 - CH_3), 278 {M - $C_6H_{12}O_3$ (McLafferty rearrangement) - $2H_2O$}, 263 (278 - CH_3), 152 (M - side chain and CD-ring), 134 (152 - H_2O)], and the fact that the kidney of vitamin D-deficient chicks contains 25-hydroxyvitamin D_3-1α-hydroxylase.[10]

Ohnuma et al. claimed in their report[1a] that the isolated metabolite exists as a single anomer of the hemiacetal form (2a) in a neutral medium and that the anomer isomerizes to a

mixture of two anomers when it was warmed. The synthetic compound (1b) with the same side chain structure does not form hemiacetal under neutral conditions, although the provitamin D 6c does cyclizes between the 23-ketone and 26-hydroxyl groups under acidic conditions (PPTS/dichloromethane, r.t.) but the hemiacetal formed undergoes dehydration to yield the furan 7 (quantitative yield) under the conditions. The fragmentation pattern of the MS spectrum of 1a produced enzymatically from 1b (25R) is quite different from that reported for the metabolite;[1a] the characteristic fragment ions (354, 336, 321, and 278) due to McLafferty rearrangement of the side chain part are negligible in the MS spectrum of the metabolite and characteristic peaks found in the MS spectrum of the metabolite (428, 415, 388, 370, 357, 352, 334, 322, 251 etc.) are almost not detectable in our spectrum. As a conclusion, we suggest that the structure proposed for the major metabolite of 1α,25-dihydroxyvitamin D_3 is not 1α,25,26-trihydroxy-23-oxovitamin D_3 as reported.

In accord with our conclusion, revision of the proposed structure for the metabolite of 1α,25-dihydroxyvitamin D_3 was reported from the same laboratory after we completed this work; 1α,25,26-trihydroxy-23-oxovitamin D_3 previously proposed being revised to 1α,23,25-trihydroxy-24-oxovitamin D_3.[11]

References and Notes

1. a) N. Ohnuma, J. R. Kruse, G. Popjak, and A. W. Norman, J. Biol. Chem., 257, 5097 (1982); b) N. Ohnuma and A. W. Norman, J. Biol. Chem., 257, 8261 (1982).
2. H. F. DeLuca and H. K. Schnoes, Annu. Rev. Biochem., 45, 631 (1976).
3. a) S. Yamada, K. Nakayama, and H. Takayama, Chem. Pharm. Bull., 29, 2393 (1981); b) S. Yamada, K. Nakayama, H. Takayama, T. Shinki, Y. Takasaki, and T. Suda, J. Biol. Chem., 259, 884 (1984); c) T. Eguchi, S. Takatsuto, M. Ishiguro, N. Ikekawa, Y. Tanaka, and H. F. DeLuca, Proc. Natl. Acad. Sci. USA, 78, 6579 (1981).
4. a) S. Yamada, M. Ohmori, H. Takayama, Y. Takasaki, and T. Suda, J. Biol. Chem., 258, 457 (1983); b) R. W. Gray, J. L. Omdahl, J. G. Ghazarian, and H. F. DeLuca, J. Biol. Chem., 247, 7528 (1972); c) Y. Tanaka, R. S. Lorenc, and H. F. DeLuca, Arch. Biochem. Biophys. 171, 521 (1975).
5. a) H. Takayama, M. Ohmori, and S. Yamada, Tetrahedron Lett., 5027 (1980); b) S. Yamada, K. Nakayama, and H. Takayama, Tetrahedron Lett., 22, 2591 (1981); c) S. Yamada, K. Nakayama, and H. Takayama, J. Org. Chem., 47, 4770 (1982). (d) S. Yamada, M. Ohmori, H. Takayama, T. Suda, and Y. Takasaki, Chem. Pharm. Bull., 29, 1187 (1981); (e) Y. Takasaki, T. Suda, S. Yamada, M. Ohmori, H. Takayama, and Y. Nishii, J. Biol. Chem., 257, 3732 (1982).
6. R. D. Little and S. O. Myong, Tetrahedron Lett. 21, 3339 (1980).
7. H. A. Barker, Biochem. Prep. 9, 25 (1962); Y. Fujimoto, J. S. Yadav, and C. J. Sih, Tetrahedron Lett., 21, 2481 (1980).
8. 7: MS m/z 394 (M^+); ^{1}H NMR ($CDCl_3$) δ 2.00 (3 H, s, H-27), 5.89 (1 H, s, H-24), 7.10 (1 H, s, H-26); UV (EtOH) λmax 293, 281.5, 271, 215 nm.
9. 8: MS m/z 470 (M^+), 338, 323, 297.
10. The enzyme has been known to hydroxylate other vitamin D metabolites at the 1α-position; Y. Tanaka, J. K. Wichmann, H. E. Paaren, H. K. Schnoes, and H. F. DeLuca, Proc. Natl. Acad. Sci. USA, 77, 6411 (1980); R. L. Horst, T. A. Reinhardt, and J. L. Napoli, Biochem. Biophys. Res. Commun., 107, 1319 (1982); J. L. Napoli and R. L. Horst, Biochem. J., 214, 261 (1983); see also ref. 4a.
11. E. Mayer, J. E. Bishop, R. A. S. Chandraratna, W. H. Okamura, J. R. Kruse, G. Popjak, N. Ohnuma, and A. W. Norman, J. Biol. Chem., 258, 13458 (1983).

(Received in Japan 10 April 1984)