STEREOCHEMISTRY OF 25-HYDROXYVITAMIN D_3–26,23-LACTONE AND 1α,25–DIHYDROXYVITAMIN D_3–26,23-LACTONE IN RAT SERUM

Seiichi ISHIZUKA, Hisao YAMAGUCHI, Sachiko YAMADA*, Keiko NAKAYAMA* and Hiroaki TAKAYAMA*

Department of Biochemistry, Teijin Institute for Bio-Medical Research, 4-3-2 Asahigaoka, Hino-Shi, Tokyo 191 and **Faculty of Pharmaceutical Sciences, Teikyo University, Sagamiko, Kanagawa 199-01, Japan*

Received 20 August 1981

1. Introduction

Two new vitamin D_3 metabolites bearing a lactone moiety in the side chain have been isolated: 25-OH-D_3–26,23-lactone [1]; and 1α,25-$(OH)_2D_3$–26,23-lactone [2,3]. The stereochemistry of both metabolites is still unknown. Although 4 possible diastereoisomers of 25-OH-D_3–26,23-lactone have been synthesized [4–8], they have not been directly compared to the natural metabolite. We have reported the isolation of a new metabolite, Dxl and proposed that its structure was tentatively determined to be 1α,25-$(OH)_2D_3$–26,23-lactone without measurement of IR spectrum [3].

We now report the definite determination of stereochemical configuration of 25-OH-D_3–26,23-lactone and the structural determination including stereochemistry of the metabolite Dxl, 1α,25-$(OH)_2D_3$–26,23-lactone.

2. Materials and methods

2.1. *Compounds*

We synthesized 25-OH-D_3, 24,25-$(OH)_2D_3$, 1α,25-$(OH)_2D_3$ and 1α,24,25-$(OH)_3D_3$ as in [10].

Abbreviations: 23(*S*)25(*R*)-25-OH-D_3–26,23-lactone, 23(*S*)25(*R*)-25-hydroxyvitamin D_3–26,23-lactone; 23(*R*)25(*S*)-25-OH-D_3–26,23-lactone, 23(*R*)25(*S*)-25-hydroxyvitamin D_3–26,23-lactone; 23(*R*)25(*R*)-25-OH-D_3–26,23-lactone, 23(*R*)25(*R*)-25-hydroxyvitamin D_3–26,23-lactone; 23(*S*)25(*R*)-1α,25-$(OH)_2D_3$–26,23-lactone, 23(*S*)25(*R*)-1α-25-dihydroxyvitamin D_3–26,23-lactone; 23(*R*)25(*S*)-1α,25-$(OH)_2D_3$–26,23-lactone, 23(*R*)25(*S*)-1α,25-dihydroxyvitamin D_3–26,23-lactone; 23(*S*)25-$(OH)_2D_3$, 23(*S*)25-dihydroxyvitamin D_3; 25(*S*)26-$(OH)_2D_3$, 25(*S*)26-dihydroxyvitamin D_3; HPLC, high-pressure liquid chromatography

23(*R*)25(*S*)-25-OH-D_3–26,23-lactone, 23(*S*)25(*R*)-25-OH-D_3–26,23-lactone and 23(*R*)25(*R*)-25-OH-D_3–26,23-lactone were chemically synthesized as in [7,8].

2.2. *Preparation and purification of 25-OH-D_3–26,23-lactone in rat serum*

Male weanling rats of the Wistar strain were fed a normal vitamin D_3-containing diet (Nippon Clea Corp. CE-2: Ca, 1.0%; P, 1.0%; D_3, 2000 IU/kg) for 8 weeks ad libitum. The 150 rats were then dosed 2 times intramuscularly with 4×10^5 IU vitamin D_3 in 100 μl ethanol at 3 day intervals. Three days after the second dose, the rats were anesthetized with ether and their blood was withdrawn from the abdominal aorta. The serum (600 ml) was diluted with the same volume of water and then extracted with 2 vol. chloroform:methanol (1:1). The chloroform extracts of serum was chromatographed on a 1.5 × 25 cm Sephadex LH-20 column eluted with chloroform: *n*-hexane (65:35). The 24,25-$(OH)_2D_3$ fraction from the Sephadex LH-20 column was then subjected to HPLC on a Hitachi Model 635 equipped with a 4.6 × 250 mm Zorbax Sil column eluted with 9% isopropanol in *n*-hexane at a flow rate of 1 ml/min. 25-OH-D_3–26,23-lactone fraction was rechromatographed by using the same solvent. 25-OH-D_3–26,23-lactone was further purified by HPLC using a Zorbax Sil column eluted with 1.5% methanol in dichloromethane at a flow rate of 1 ml/min.

2.3. *Preparation and purification of 1α,25-$(OH)_2D_3$–26,23-lactone in rat serum*

Male weanling Wistar rats were maintained as above. The 150 rats were each given orally by stomach tubes 100 μg 1α,25-$(OH)_2D_3$ in 1 ml 5% ethanol

Published by Elsevier/North-Holland Biomedical Press
00145793/81/0000–0000/$02.75

in 0.2% Triton X-100 solution. At 7–8 h after dosing, the rats were anesthetized with ether and their blood was withdrawn from the abdominal aorta. The extraction and purification of 1α,25-$(OH)_2D_3$–26,23-lactone was done as in [2,3].

2.4. *1α-Hydroxylation of 23(R)25(S)-25-OH-D_3–26,23-lactone and 23(S)25(R)-25-OH-D_3–26,23-lactone by chick kidney homogenate*

Four-week-old White Leghorn cockerels were raised on a vitamin D-deficient diet [11]. The chicks were sacrificed, their kidneys were taken and 10% tissue homogenate in 0.25 M sucrose was prepared with the aid of a Potter-Elvehjem homogenizer fitted with a Teflon pestle. To 24 ml homogenate (13 mg protein/ml) in a 300 ml flask, 56 ml reaction mixture containing 30 mM Tris–HCl (pH 7.4), 3.6 mM $MgCl_2$, 50 mM sucrose and 20 mM sodium succinate was added as in [10]. The incubation was initiated by addition of 25 nmol 23(*R*)25(*S*)-25-OH-D_3–26,23-lactone or 23(*S*)25(*R*)-25-OH-D_3–26,23-lactone in 0.5 ml ethanol. The incubations were carried out at 37°C for 60 min with shaking; 160 ml chloroform:methanol (1:1) was added to each flask to terminate the reaction. The metabolites of 25-OH-D_3–26,23-lactone were separated and purified by Sephadex LH-20 column chromatography and HPLC using a Zorbax Sil column as described above.

2.5. *Spectroscopy*

Mass spectra were determined with a Simadzu-LKB mass spectrometer Model 9000 in the direct probe inlet mode. The Fourier transform infrared (FT-IR) spectra were obtained by using a JEOL Model JIR-40X (Japan Electric Optical Lab. Ltd.).

3. Results and discussion

We obtained the highly purified 25-OH-D_3–26,23-lactone from rat serum as in [9] with slight modifications. The natural metabolite was directly compared to 2 diastereoisomers, 23(*S*)25(*R*)-25-OH-D_3–26,23-lactone and 23(*R*)25(*S*)-25-OH-D_3–26,23-lactone stereospecifically synthesized by [7,8]. Both compounds were chosen for comparison because of higher similarity of NMR spectrum of either compound to that of natural metabolite than the other 2 diastereoisomers [1,4,6]. The 2 synthetic diastereoisomers, 23(*S*)25(*R*)-25-OH-D_3–26,23-lactone and 23(*R*)25(*S*)-25-OH-D_3–26,23-lactone, could be separated into each peak by HPLC (fig.1A). The former isomer comigrated with the natural metabolite (fig.1B). The IR spectra and mass spectra of the natural metabolite and those of 23(*S*)25(*R*)-25-OH-D_3–26,23-lactone were completely superimposable (fig.2,3). Thus, the stereochemistry of the natural metabolite was definitely determined to be 23(*S*)25(*R*)-25-OH-D_3–26,23-lactone.

The other metabolite, Dxl was obtained from

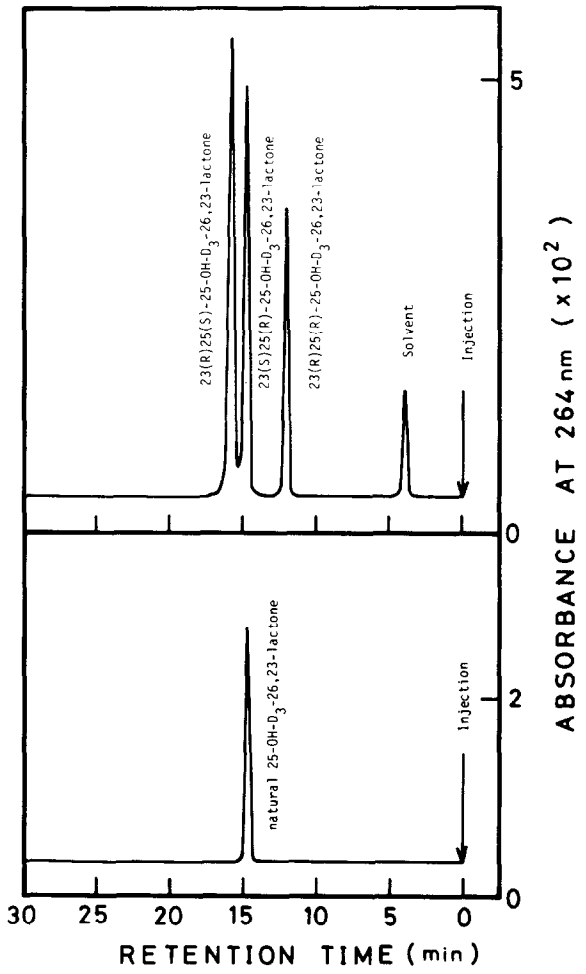

Fig.1. High-pressure liquid chromatographic profiles of diastereoisomers of synthetic 25-OH-D_3–26,23-lactone and natural 25-OH-D_3–26,23-lactone: (A) synthetic 25-OH-D_3–26,23-lactones; (B) natural 25-OH-D_3–26,23-lactone from rat serum. 25-OH-D_3–26,23-Lactones were subjected to high pressure liquid chromatography on a 4.6 × 250 mm Zorbax Sil column eluted with 9% isopropanol in *n*-hexane at a flow rate of 1 ml/min.

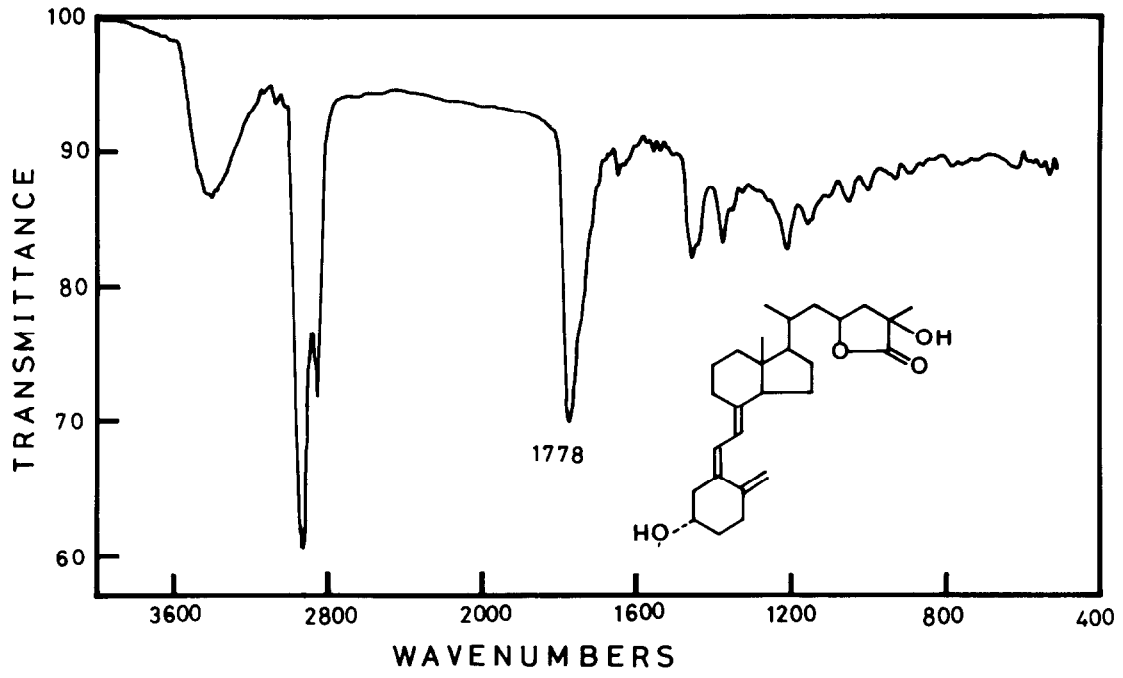

Fig.2. Fourier transform infrared spectrum of natural 25-OH-D_3–26,23-lactone from rat serum.

rat serum by the HPLC method in [2,3]. The structural elucidation of Dxl was made by spectroscopic data (IR and mass) and by direct comparison with 23(*S*)25(*R*)-1α,25-$(OH)_2D_3$–26,23-lactone and 23(*R*)25(*S*)-1α,25-$(OH)_2D_3$–26,23-lactone, which were bioconverted from the synthetic diastereoisomers of 25-OH-D_3–26,23-lactone. The IR spectrum of the natural metabolite, Dxl, is shown in fig.4. It indicates the presence of an absorption at 1776 cm^{-1} due to γ-lactone moiety. So, the metabolite, Dxl, was confirmed to have γ-lactone in the side chain. The mass spectrum of Dxl gave 444, 426, 408, 393, 287,

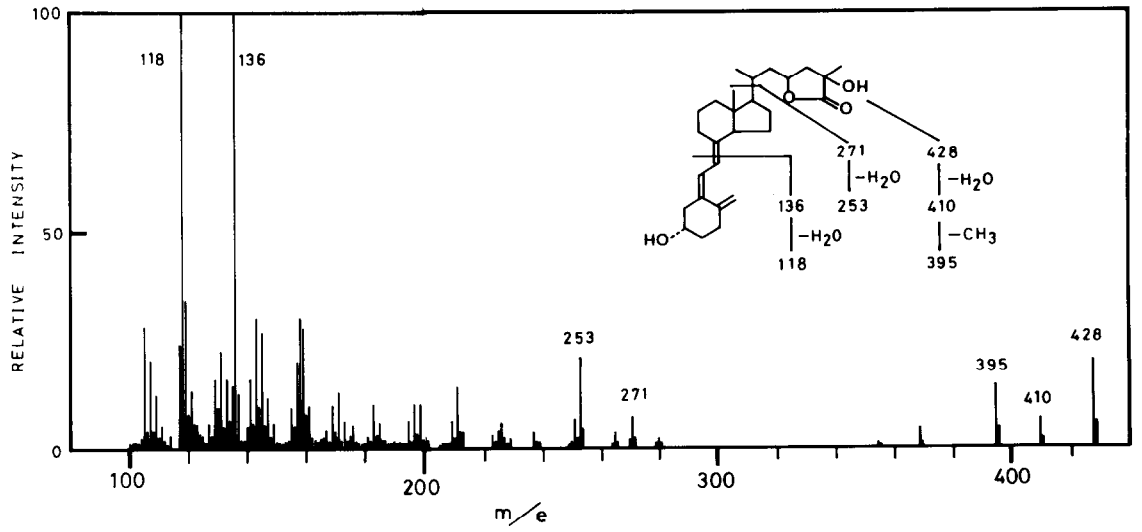

Fig.3. Mass spectrum of natural 25-OH-D_3–26,23-lactone from rat serum.

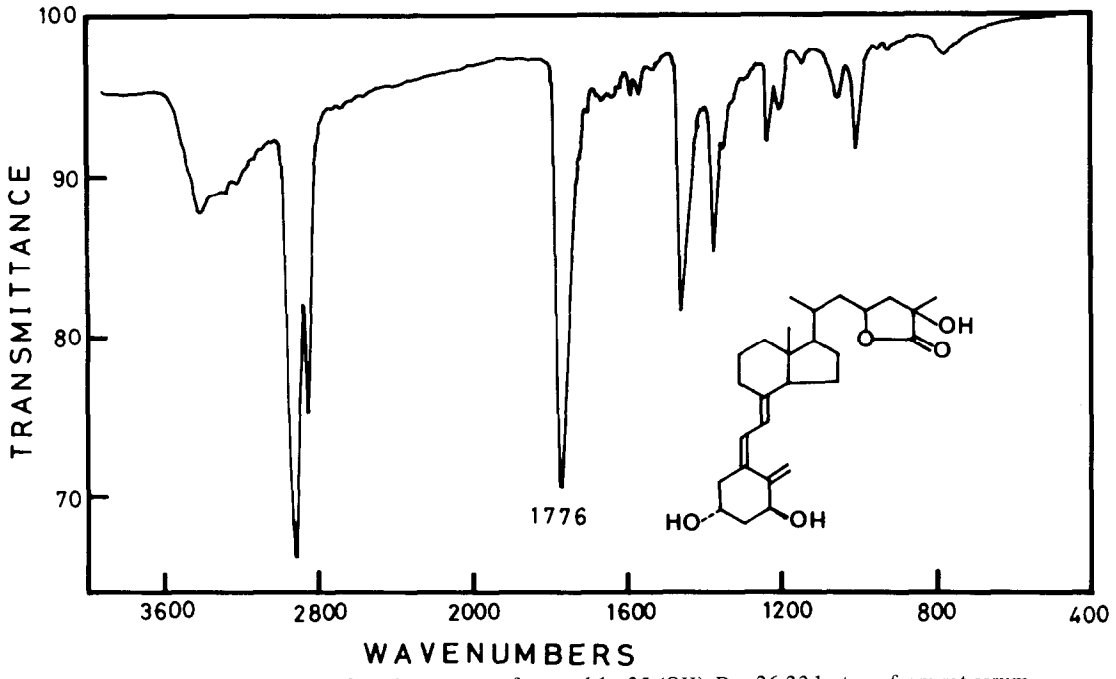

Fig.4. Fourier transform infrared spectrum of natural 1α,25-$(OH)_2D_3$–26,23-lactone from rat serum.

269, 251, 152 and 134 as in [3]. It shows the very similar fragmentation pattern to that of the natural 25-OH-D_3–26,23-lactone.

Each diastereoisomer, 23(*S*)25(*R*)-1α,25-$(OH)_2D_3$–26,23-lactone and 23(*R*)25(*S*)-1α,25$(OH)_2D_3$–26,23-lactone, prepared from the corresponding synthetic isomers by 1α-hydroxylation with kidney homogenate, could be separable into peaks by HPLC (fig.5). The former isomer comigrated together with the natural metabolite Dxl. Thus, the structure of Dxl was identified unequivocally to be 23(*S*)25(*R*)-1α,25-$(OH)_2D_3$–26,23-lactone.

These results demonstrate that 2 lactonic vitamin D_3 metabolites have the same stereochemical configuration 23(*S*)25(*R*) at C-23 and C-25 positions. It can be considered that a particular enzymatic system may be involved in the biotransformation from 25-OH-D_3 or 1α,25-$(OH)_2D_3$ to be corresponding lactonic metabolites.

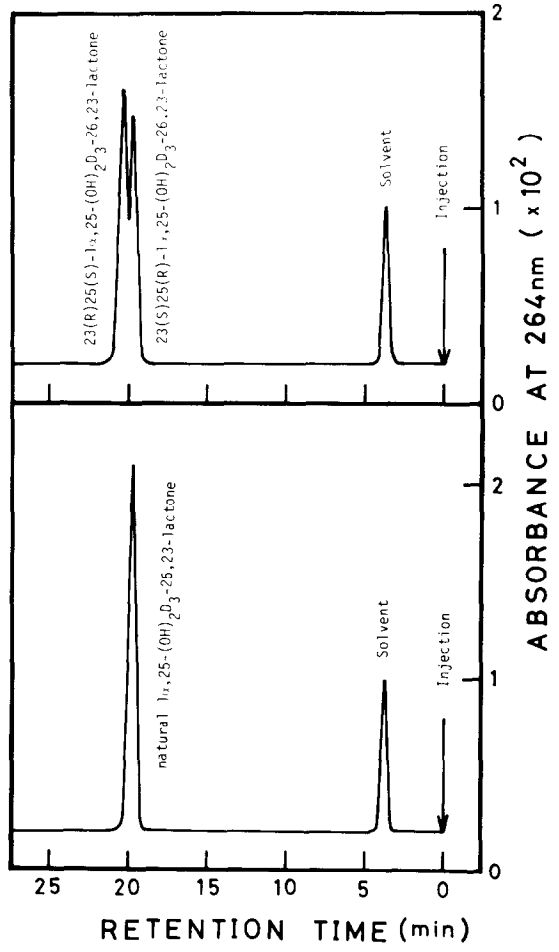

Fig.5. High-pressure liquid chromatographic profiles of diastereoisomers of bioconverted 1α,25-$(OH)_2D_3$–26,23-lactone and natural 1α,25-$(OH)_2D_3$–26,23-lactone: (A) bioconverted 23(*S*)25(*R*)- and 23(*R*)25(*S*)-1α,25-$(OH)_2D_3$–26,23-lactone by chick kidney homogenate; (B) natural 1α,25-$(OH)_2D_3$–26,23-lactone from rat serum. 1α,25-$(OH)_2D_3$–26,23-Lactones were subjected to high-pressure liquid chromatography on a 4.6 × 250 mm Zorbax Sil column eluted with 20% isopropanol in *n*-hexane at a flow rate of 1 ml/min.

This enzymatic system may be activated when the levels of some vitamin D_3 metabolites in plasma or the tissues reach far higher than normal physiological levels. The biotransformation pathways of lactonic metabolites are also very interesting.

It can be assumed that metabolites such as 23(*S*)25-$(OH)_2D_3$ [12] or 25(*S*)26-$(OH)_2D_3$ [13] may be involved as one of the precursors of 23(*S*)25(*R*)-25-OH-D_3–26,23-lactone. With 25(*S*)26-$(OH)_2D_3$ as a precursor, the stereochemical inversion of hydroxy group at C-25 position should occur during the course of lactone formation. Thus, it might be reasonable to consider that oxidation reactions such as as hydroxylation take place initially on the C-23 methylene group, followed by oxidation of the C-26 methyl group.

The biological and physiological functions of the lactonic metabolites have been investigated, but they remain still undefined. Further investigations on these problems are now in progress.

Acknowledgements

The authors would like to express their deep thanks to Mr S. Yamazaki for the measurement of mass spectroscopy, IR spectroscopy and for valuable discussions.

References

[1] Wichmann, J. K., DeLuca, H. F., Schnoes, H. K., Horst, R. L., Shepard, R. M. and Jorgensen, N. A. (1979) Biochemistry 18, 4775–4780.
[8] Ohnuma, N., Kiyoki, M., Bannai, K., Naruchi, T., Hashimoto, Y., Noguchi, T. and Norman, A. W. (1980) Steroids 36, 27–39.
[3] Ohnuma, N., Bannai, K., Yamaguchi, H., Hashimoto, Y. and Norman, A. W. (1980) Arch. Biochem. Biophys. 204, 387–391.
[4] Wichmann, J. K., Paaren, H. E., Fivizzanni, M. A., Schnoes, H. K. and DeLuca, H. F. (1980) Tetrahedron Lett. 21, 4667–4670.
[5] Ikekawa, N., Hirano, Y., Ishiguro, M., Oshida, J., Eguchi, T. and Miyasaka, S. (1980) Chem. Pharm. Bull. 28, 2852–2854.
[6] Morris, D. S., Williams, D. H. and Norris, A. F. (1981) J. Chem. Soc. Commun. 424–425.
[7] Yamada, S., Nakayama, K. and Takayama, H. (1981) Tetrahedron Lett. 22, 2591–2594.
[8] Yamada, S., Nakayama, K. and Takayama, H. (1981) Chem. Pharm. Bull. in press.
[9] Horst, R. L. and Littledike, E. T. (1980) Biochem. Biophys. Res. Commun. 93, 149–154.
[10] Ishizuka, S., Bannai, K., Naruchi, T. and Hashimoto, Y. (1981) Steroids 37, 33–43.
[11] Omdahl, J., Holick, M. F., Suda, T., Tanaka, Y. and DeLuca, H. F. (1971) Biochemistry 10, 2935–2940.
[12] Ikekawa, N., Eguchi, T., Hirano, Y., Tanaka, Y. and DeLuca, H. F. (1981) Folia Endocrinol. Jap. 57, 675.
[13] Hollis, B. W., Roos, B. A. and Lambert, P. W. (1980) Biochem. Biophys. Res. Commun. 95, 520–528.