Communications to the Editor

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SYNTHESIS OF DIASTEREOMERIC 24,25-DIHYDROXYVITAMIN D_2 AND SEPARATION OF ITS (24R)- AND (24S)-ISOMERS

Kotomi Katsumi[a], Toshio Okano[a], Tadashi Kobayashi[*,a], Okiko Miyata[b], Takeaki Naito[b] and Ichiya Ninomiya[b]

Departments of Hygienic Sciences[a] and Medicinal Chemistry[b], Kobe Women's College of Pharmacy, Motoyamakita-machi, Higashinada-ku, Kobe 658, Japan

Diastereomeric 24,25-dihydroxyvitamin D_2 (I) was synthesized from ergosterol (II) through an efficient route and successfully separated into the (24R)- and (24S)-isomers by high-performance liquid chromatography (HPLC). The absolute configurations of the isomers were determined by co-chromatography with the authentic respective specimens.

KEYWORDS ——— 24,25-dihydroxyvitamin D_2; 24R,25-dihydroxyvitamin D_2; 24S,25-dihydroxyvitamin D_2; ergosterol; high-performance liquid chromatography; vitamin D_2; vitamin D

It has been documented that vitamin D is metabolized to 25-hydroxyvitamin D (25-OH-D) in the liver and subsequently to 1α,25-dihydroxyvitamin D [1α,25-$(OH)_2$-D] or 24R,25-dihydroxyvitamin D [24R,25-$(OH)_2$-D] in the kidney according to respectively lower or higher plasma calcium levels than normal.[1] There are two groups of vitamin D, namely D_2 and D_3, which are different only in the structures of side chain but they are known to have practically the same biological activity for mammals including humans. Though a number of syntheses of various metabolites of vitamin D_3 have been reported, only few reports have appeared on the synthesis of vitamin D_2 metabolites due to difficulties in the synthesis by inserting a double bond into the 22- and a methyl group into the 24S-positions and also to the difficulty of securing synthetic compounds in vitamin D_2 series. Though the synthesis of 24,25-$(OH)_2$-D_2 from stigmasterol was reported by Jones *et al.*,[2,3] their synthetic route is apparently complicated, resulting in only a poor overall yield. Therefore, we have investigated the modification of the synthesis of vitamin D_2 metabolites and succeeded in the establishment of an improved synthesis of a vitamin D_2 metabolite, 24,25-$(OH)_2$-D_2, its separation into (24R)- and (24S)-isomers, and the confirmation of their absolute configuration.

As shown in Chart 1, ergosterol (II) was converted into the known 20-aldehyde (IV) *via* the route involving the protection of the 5,7-diene group and ozonolysis according to the procedure given by Barton *et al.*[4] The 20-aldehyde (IV) was then converted to the enone (V) by the condensation with 3-methyl-3-(tetrahydropyran-2-yloxy)butan-2-one according to the procedure given by Eyley and Williams[5] in 36% yield. Methylation of the enone (V) with methyllithium afforded the methylated 24,25-glycol (VI) as a mixture of diastereomers in 60% yield, which without separation was refluxed

with lithium aluminum hydride in tetrahydrofuran to afford the desired 24,25-dihydroxyprovitamin D_2 [24,25-$(OH)_2$-pro-D_2 (VII)] also as a mixture of diastereomers in 70% yield [MS: m/z, 428 (M^+); ^{1}H-NMR ($CDCl_3$): δ 0.64 (3H, s, 13-Me), 1.07 (3H, d, J= 6Hz, 20-Me), 1.20, 1.22 and 1.26 (each s, 24-Me and 25-Me_2), 5.34~5.64(m, 6-, 7-, 22- and 23-H); UV $\lambda_{max}^{ethanol}$: 272, 281 and 292 nm].

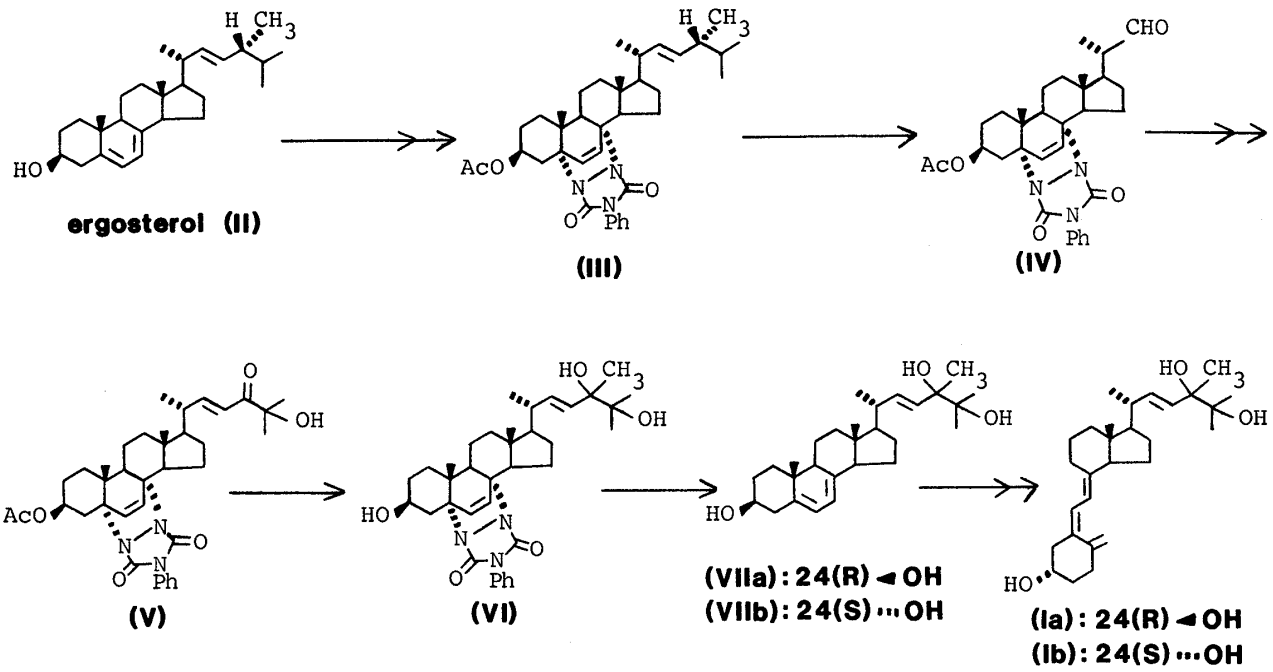

Chart 1. Our Synthetic Course of 24,25-Dihydroxyvitamin D_2

Separation of two diastereomers (VIIa and VIIb) was performed by HPLC using a Zorbax SIL column with 2.5% isopropanol in n-hexane as a mobile phase. As shown on the profile of HPLC in Fig. 1, two peaks were clearly separated with nearly same peak heights; the first peak was confirmed as (24S)-24,25-$(OH)_2$-pro-D_2 (VIIb) and the second peak as (24R)-isomer (VIIa) by converting the respective fractions into the corresponding 24,25-$(OH)_2$-D_2 (Ib and Ia) upon ultraviolet (UV) irradiation followed by thermal isomerization. The UV irradiation was carried out by using a monochromatic ray at 295 nm obtained from a spectroirradiator (Japan Spectroscopic Co., CRM-FA type) and thermal isomerization was performed by refluxing the UV irradiated ethanolic solution for 2 h. Each isomerized product was applied to HPLC which clearly separated respective products as shown in Fig. 2, thus identifying the former peak as (24S)-24,25-$(OH)_2$-D_2 (Ib) and the latter as (24R)-24,25-$(OH)_2$-D_2 (Ia). [(Ib) MS: m/z, 428 (M^+); ^{1}H-NMR ($CDCl_3$): δ 0.50 (s, 13-Me), 0.98 (d, J=6Hz, 20-Me), 1.14, 1.16 and 1.20 (s, 24-Me and 25-Me_2), 3.88 (m, 3-H), 4.76 and 4.99 (br s, 19-methylene), 5.50 (m, 22- and 23-H), 5.97 and 6.20 (d, J=11Hz, 6- and 7-H); UV $\lambda_{max}^{ethanol}$: 265 nm and $\lambda_{min}^{ethanol}$: 228 nm].

The identity of respective products (Ia and Ib) was established by co-chromatography with authentic samples of (24R)- and (24S)-24,25-$(OH)_2$-D_2 kindly donated by Dr. G. Jones, as shown in Fig. 2, thus unambiguously confirming not only their structures but their absolute configurations.

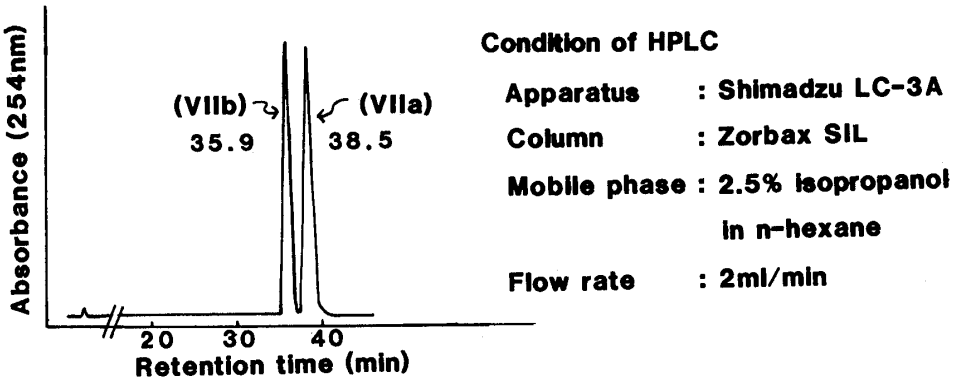

Fig. 1. Profile of HPLC on (VIIa) and (VIIb)

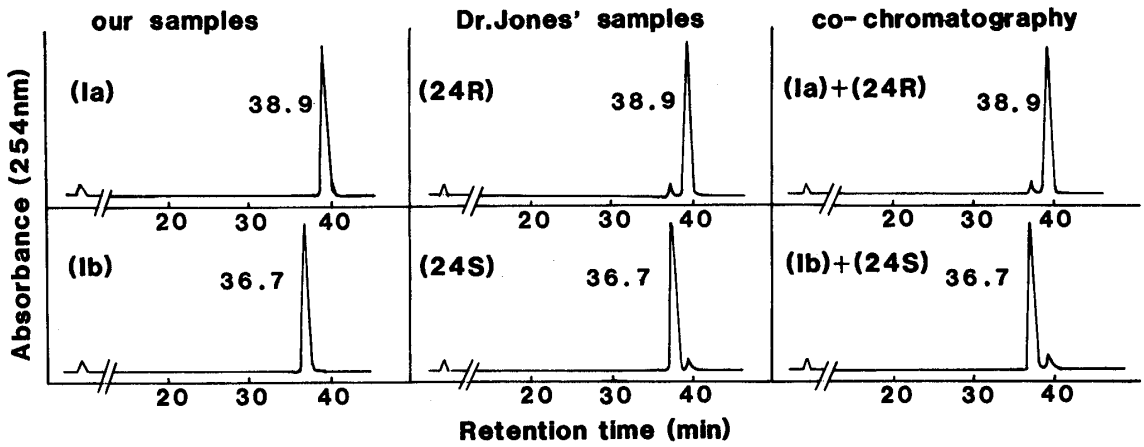

Fig. 2. Co-chromatography of Our Synthetic and Isolated Compounds (Ia and Ib) with the Authentic Isomers Kindly Donated by Dr. Jones. (The conditions of HPLC were the same as described in Fig.1.)

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