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Stereochemistry and Physiological Significance of Hydroxylation of Vitamin D Side Chain

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Abstract: Stereochemical aspect of side chain hydroxylation (C-23, C-24, and/or C-26 positions) of vitamin D_3 is described. The effect of the configuration of hydroxy group on biological activities is emphasized. Stereochemistry of vitamin D hydroxylation is compared with that of other natural hydroxylated steroids. Conformation of steroid side chain is also discussed.

The discovery of physiologically active hydroxylated metabolites of vitamin D prompted us to consider in depth the relationship between the biological activity and the three-dimensional arrangement of hydroxy group on the steroidal side chain.[1] Until that time, little had been known on the stereochemical importance of the side chain, except for ecdysone, in which 22*S*-epimer is much less active than the natural 22*R*-ecdysone.[2] Besides the hydroxylation at the C-1 and C-25 positions to form 1,25-dihydroxyvitamin D_3 [1,25-$(OH)_2D_3$], a hormonal metabolite of vitamin D_3, the hydroxylation was known to take place to afford 23,25-, 24,25- and 25,26-$(OH)_2D_3$. Our primary concern was devoted to study the relationship of the hydroxylation and the biological activity. Due to the scarcity of isolated metabolites, we took the following strategy for the stereochemical determination of these hydroxy-bearing carbons of vitamin D metabolites. All the possible stereoisomers were to be synthesized first, their configurations were then determined by unequivocal methods, and finally those stereoisomers were compared with the natural metabolites by a chromatographic method. All the stereoisomers were also subjected to the biological assays.

In the initial stage of our synthetic work on vitamin D metabolites, we focused on the elucidation of the C-24 stereochemistry of 24,25-$(OH)_2D_3$. Thus, both epimers of 24-OH-D_3 as well as 24,25-$(OH)_2D_3$ were synthesized and their biological activities were examined.[3,4] Surprisingly, the 24*S*-epimers exhibited almost no activity, but the 24*R*-isomers were as active as 25-hydroxyvitamin D_3.[5] This finding demonstrated the importance of the orientation of hydroxy group on the steroidal side chain. In this paper, we describe the previous and recent results on the stereochemical aspects of the hydroxylated sites and the biological activities.

24-Hydroxylation

Subsequently the configuration of naturally occurring 24,25-$(OH)_2D_3$ was determined as *R* by HPLC comparison with the synthetic samples.[6] The metabolic studies using [^{3}H]-(24*R*)- and (24*S*)-24-OH-D_3 revealed that the former epimer was converted into 1,24,25-$(OH)_3D_3$.[7] The fact implies that 1-hydroxylase in kidney is specific for the 24*R*-isomer. In agreement with this result, the (24R)-OH-D_3 has marked ability to cure rickets in rats, whereas no activity was observed for (24S)-OH-D_3. 1,24,25-$(OH)_3D_3$ is thought to be the active substance in healing the rickets.

23-Hydroxylation

23,25-$(OH)_2D_3$, a major product formed upon incubation of 25-OH-D_3 with the homogenate of kidney from the vitamin D fed chicks, was isolated and the structure was determined by Tanaka in 1981.[8] The C-23 stereochemistry was determined as *S* by the HPLC comparison with the stereochemically defined synthetic compounds.[9] The affinity of both (23*S*)- and (23*R*)-23,25-$(OH)_2D_3$ for plasma vitamin D binding protein was

similar to vitamin D_3. The binding affinity of 23,25-$(OH)_2D_3$ to the chick intestinal cytosol receptor for 1,25-$(OH)_2D_3$ was weaker than that of 24*R*,25-$(OH)_2D_3$ or 25,26-$(OH)_2D_3$.[10] 23*S*,25-$(OH)_2D_3$ is also metabolized to (23*S*,25*R*)-25-OH-D_3 26,23-lactone by the kidney enzyme preparation. The C-23 epimer, 23*R*,25-$(OH)_2D_3$ cannot be involved in the lactone formation. 25*R*,26-$(OH)_2D_3$ is similarly metabolized to give the same lactone much less effectively than 23*S*,25-$(OH)_2D_3$.[11]

26-Hydroxylation

The C-25 stereochemistry of 25,26-$(OH)_2D_3$, which is formed by 26-hydroxylation of 25-OH-D_3, had been controversial for several years. This was mainly due to the difficulty in the separation of 25,26-$(OH)_2D_3$ epimers. While an effective chromatographic separation of the C-24 epimers of 24,25-$(OH)_2D_3$ was achieved in the form of tris-trimethylsilyl derivatives, the TMS derivatives of the 25-epimers of 25,26-$(OH)_2D_3$ could not, in contrast, be separated effectively. We found that the separation of the epimers is much improved in the form of bis-(+)-α-methoxy-α-(trifluoromethyl)phenylacetyl ester derivatives. Thus, co-chromatography of biologically produced 25,26-$(OH)_2D_3$ in this form with the synthetic isomers on HPLC established that the natural 25,26-$(OH)_2D_3$ was a C-25 epimeric mixture (1:1).[12]

The binding affinity of the C-25 epimeric 25,26-$(OH)_2D_3$ for the chick intestinal receptor for 1,25-$(OH)_2D_3$ is almost the same as, and slightly weaker than that for 24*R*,25-$(OH)_2D_3$.[10] When the both epimers were incubated with the kidney homogenate from vitamin D-deficient chicks, 25*S*,26-$(OH)_2D_3$ was readily converted into 1,25*S*,26-$(OH)_3D_3$, whereas 1-hydroxylation of 25*R*,26-$(OH)_2D_3$ isomer found to be negligible.[13] This is in agreement with the finding that 1,25*S*,26-$(OH)_3D_3$ is the natural metabolite of vitamin D_3.[14]

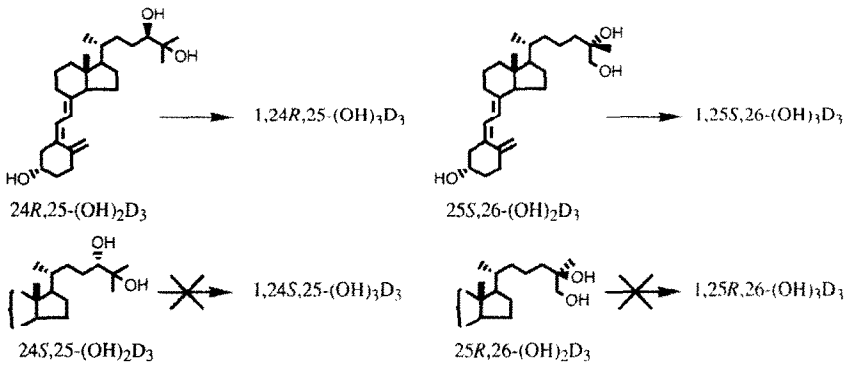


Figure 1

25-OH-D_3 26,23-lactone and 1,25-$(OH)_2D_3$ 26,23-lactone

Configuration of the natural 25-OH-D_3 26,23-lactone was determined to be 23*S*,25*R* by the HPLC comparison with four synthetic diastereomers.[15]

1,25-$(OH)_2D_3$ 26,23-lactone was isolated as a major metabolite of 1,25-$(OH)_2D_3$. The configuration was also determined as 23*S*,25*R* in the same way.[16] The lactone is produced from 1,25-$(OH)_2D_3$ through 1,23*S*,25-$(OH)_3D_3$ as well as 1,25*R*,26-$(OH)_3D_3$.[17] It was demonstrated that the lactone stimulates bone formation by increasing bone matrix content.[18]

The four synthetic 26,23-diastereomers of 1,25-$(OH)_2D_3$ 26,23-lactone show different biological properties. The receptor affinity (1,25-$(OH)_2D_3$, 100) was as follows: 23*S*,25*S*-isomer, 7.9; 23*R*,25*R*-isomer, 2.27; 23*S*,25*R*-isomer (natural), 0.17; 23*R*,25*S*-isomer, 0.22. The 23*S*,25*S*- and 23*R*,25*R*-lactones were estimated to be 3 and 20 times less active than 1,25-$(OH)_2D_3$ in elevation of serum calcium concentration. However, the 23*S*,25*R*- and 23*R*,25*S*-lactones decreased the serum calcium level. The 23*S*,25*R*-lactone reduced serum calcium concentration to a greater extent than the 23*R*,25*S*-lactone.[19]

Stereochemical aspects of vitamin D_3 metabolism discussed above, is summarized in Fig. 2.

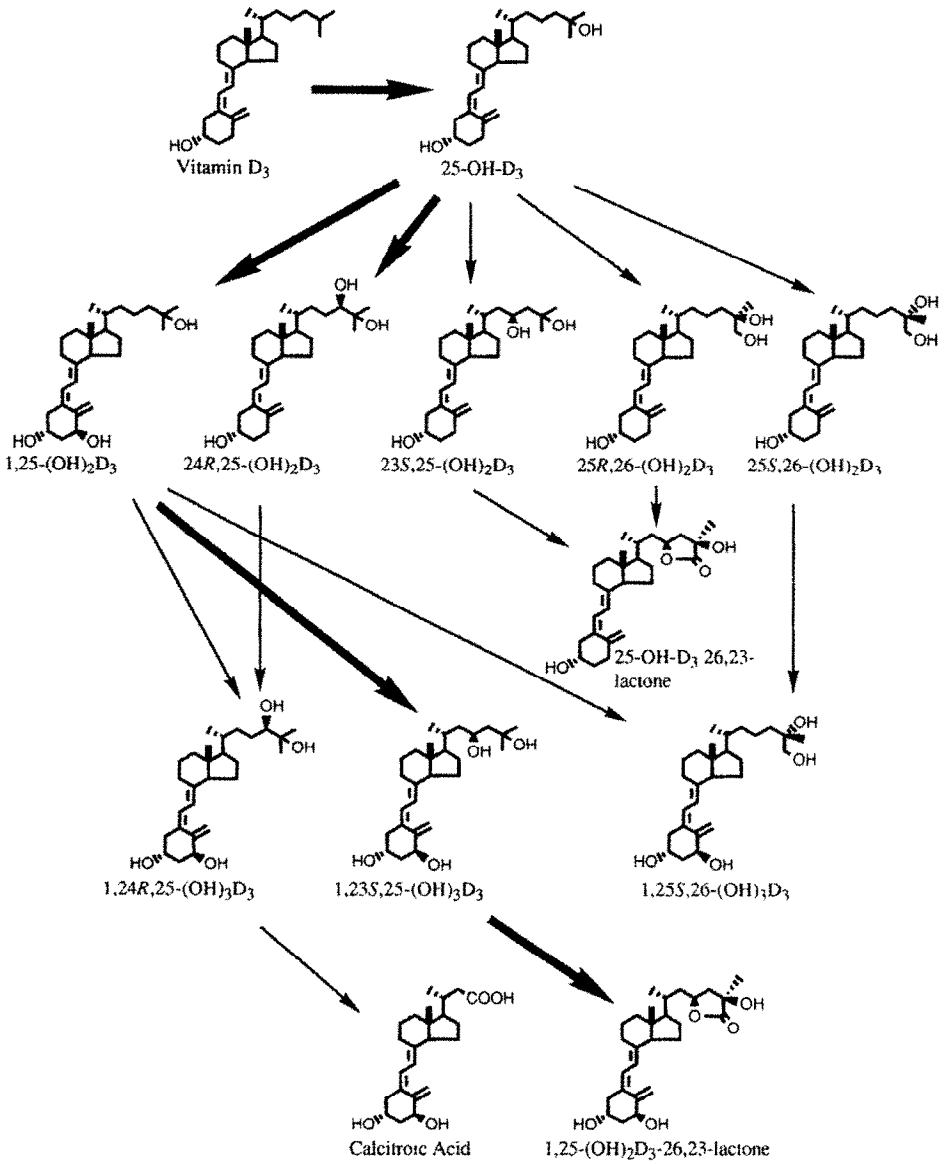


Figure 2

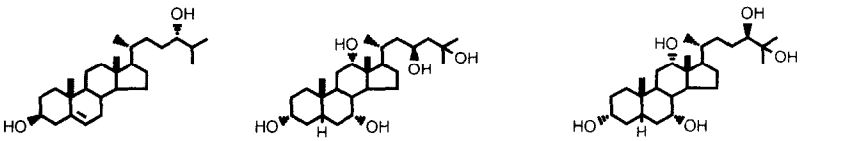
(Major metabolic pathways are shown in bold arrows)

Comparison of the stereochemistry with other hydroxylations in different biological systems

The configuration of 24-hydroxycholesterol (cerebrosterol), isolated from brain,[20] was determined as 24*S*.[21] Occurrence of 24-hydroxylase in bovine cerebral cortex has been reported.[22]

In cerebrotendious xanthomatosis, bile acid biosynthesis is disturbed due to lack of 26-hydroxylase, resulting in accumlation of bile alcohols. The major components of the bile alcohols are 25-hydroxy, 23,25-dihydroxy-, and 24,25-dihydroxy-5β-cholestan-3α,7α,12α-triols. In the course of our synthetic studies on vitamin D_3 metabolites, we prepared stereochemically defined 23,25-dihydroxy- and 24,25-dihydroxycholesterols. Comparison of the molecular rotation of these sterols allowed us to conclude that the bile alcohols have 23*S*- and 24*R*-configurations.[23] These stereochemistries coincide with vitamin D metabolites.

There are examples of nonstereospecific hydroxylation at C-26 or C-27 position. 26-Hydroxycholesterol isolated from human aortas was reported as a mixture of 25*S* and 25*R*, the latter being the major (90%).[24] 26-Hydroxylation in liver is an initiation step of C-24/C-25 bond cleavage reaction in the bile acid formation. C-27 (*pro-S* methyl group on the C-25 prochiral center) is hydroxylated in rat mitochondoria,[25] whereas the C-26 (*pro-R* methyl) is hydroxylated in rat liver microsomes.[26] Inokosterone, one of the common phytoecdysone, is a 1:2 mixture of the *R* and *S* isomers.[27] Thus, the formation of both 25*S*,26-$(OH)_2D_3$ and 25*R*,26-$(OH)_2D_3$ is not an exception.



Cerebrosterol 5β-cholestane-3α,7α,12α,23,25-pentol 5β-cholestane-3α,7α,12α,24,25-pentol

Figure 3

Conformation of the side chain

In order to investigate the effect of 22-hydroxy group on the biological activity of vitamin D, we synthesized 22*R*- and 22*S*-22,25-$(OH)_2D_3$. Both isomers exhibited neither vitamin D or antivitamin D activities.[28]

In general, a zig-zag conformation for the steroidal side chain is thought to be energetically favored from the studies of X-ray crystallography of several steroids. 25-Hydroxyvitamin D_3 is also reported to have a similar zig-zag conformation in a crystalline form. According to the results of molecular mechanics calculations (the MM2 calculation was carried out for the model compounds), the most stable conformer of the 22*S*-isomer was found to have about -170° of the dihedral angle for C17-C20-C22-C23. The rotamer showing the lowest energy of 22*R*-isomer was found to be about 70°. As shown in Fig. 4B, the most stable side chain conformation is zig-zag conformation for the 22*S*-isomer. For the 22*R*-isomer, such a zig-zag conformation is sterically unfavorable, because of an interaction between the 22-substituent and the 16-methylene group, and the conformation depicted in Fig. 4A becomes the most energetically stable. The conformation of the 22*S*- and 22*R*-isomers was supported by the ^{1}H-NMR studies of 23,23,24,24-$^2H_4$-labeled derivatives.

The epimers of 1α-hydroxylated 22,25-$(OH)_2D_3$ exhibited different biological activities in *in vitro* test. The effect on cell differentiation of HL-60 leukemia cells, the 22*S*-isomer was at least 30 times as effective as the corresponding 22*R*-isomer. The binding affinities to the 1,25-$(OH)_2D_3$ receptor of a chick intestinal cytosol showed that 22*S*-isomer was about three times as potent as the 22*R*-isomer.[29]

It is not clear why the 22-hydroxy derivatives showed no activities in the *in vivo* experiments. The difference of the biological activities observed in the *in vitro* experiments may be attributed to their side chain conformations. The zig-zag conformation seems to play an important role in exerting biological function.

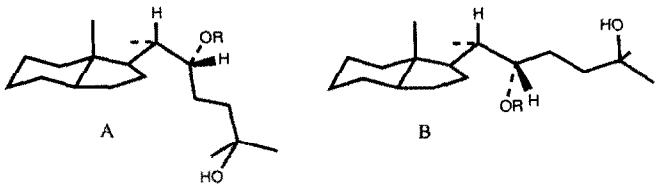
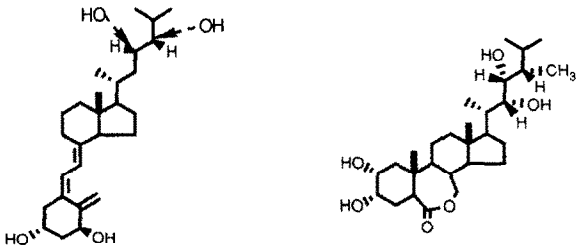


Figure 4

If one assumes such a zig-zag conformation for most of the vitamin D metabolites, above mentioned hydroxylation reactions at the C-23 or C-24 position have to take place from the rear of the side chain. In this context, brassinolide, a recently isolated plant growth promoting steroid, is interesting because of its 22*R*,23*R*,24*S*-configuration.[30] The C-22 and C-23 hydroxylations in the biosynthesis of the brassinolide side chain may be similar to the vitamin D_3 hydroxylations in that the reactions take place from the rear side of the side chain in a zig-zag conformation as shown in Fig. 5. It thus deserves considering to note from the evolutionary viewpoints that the stereochemical consequences of these biological hydroxylations may reflect the similarity of the enzymes responsible for these reactions and may suggest a common ancestral enzyme.



Vitamin D side chain hydroxylation

Brassinolide

Figure 5

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