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# 24(R), 25-DIHYDROXYVITAMIN $D_2$ SYNTHESIS, DETERMINATION OF ABSOLUTE CONFIGURATION BY X-RAY ANALYSIS AND IDENTIFICATION AS A KIDNEY METABOLITE OF VITAMIN $D_2$

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Vitamin $D_3$ is known to undergo functional metabolism first in the liver, resulting in 25-hydroxy vitamin $D_3$ which is transformed latter in the kidney to 1α,25- and 24(R),25-dihydroxy vitamin $D_3$, both hormonally active in calcium homeostasis.[1] The activity of $D_2$ in mammals resembles that of vitamin $D_3$, and it has been recently found that it also undergoes similar metabolism to the hormonally active 1α,25-dihydroxy vitamin $D_2$.[2] However, the identification of the other metabolite of vitamin $D_2$, analogous to the 24(R),25-dihydroxy vitamin $D_3$, has not been established yet, although there is evidence of its formation in kidneys of rats fed with vitamin $D_2$.[3]

We report here on the synthesis of both epimers of 24, 25-dihydroxy vitamin $D_2$, 1R and 1S, establishing their absolute configuration at C24, and the identification of the 24(R) epimer as a natural metabolite of vitamin $D_2$.[4]

Our synthesis starts from the known C22-aldehyde,[5] the degradation product of stigmastery acetate, using the codensation procedure previously described by D.H. Williams.[6] The synthetic pathway is shown in Scheme I.

The epimeric mixture of 24, 25-dihydroxy vitamin $D_2$, 1R and 1S, was separated by high pressure liquid chromatography into its components in a 65:35 ratio. Co-chromatography with a purified kidney metabolite obtained from rats fed with vitamin $D_2$ proved the minor component of the synthetic mixture to be identical with the natural metabolite. Quantitative

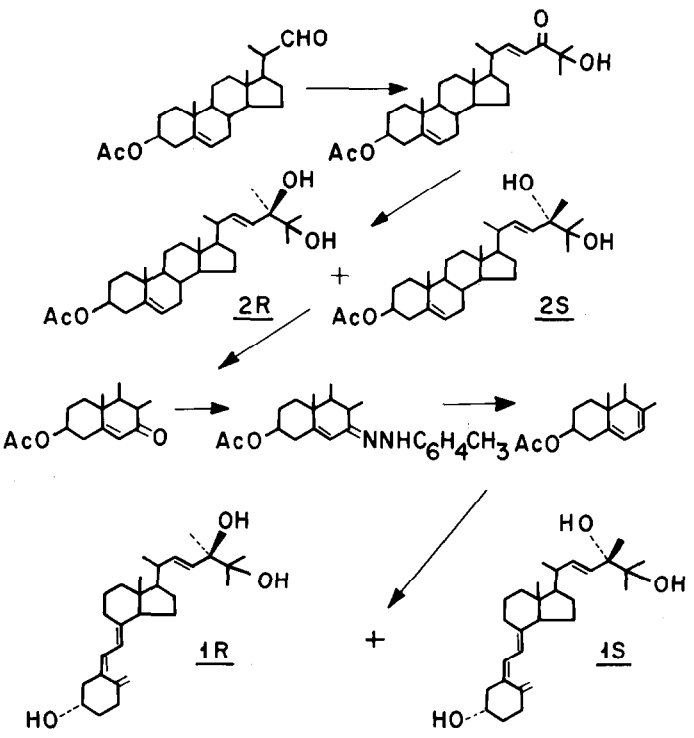
separation of the mixture of the 24,25-dihydroxycholesterol acetates, 2R and 2S, by column chromatography on silica gel type H, gave the two epimers in 65:35 ratio. The minor epimer was converted to the corresponding 24,25-dihydroxy vitamin $D_2$ repeating the sequence described in Scheme I, and was found to be identical with the natural metabolite.

The configuration at C24 was established by X-ray analysis of the cholesterol derivative 2R. The crystals (mp 164-165° from acetone) are monoclinic, space group *C2*. Cell dimensions are $a$=73.60, $b$=7.57, $c$=10.26Å, β=91.9°, Z=8. Intensity data were collected on a Siemens diffractometer with Nickel filtered Cu-$K\alpha$ radiation. Positions of the tetracyclic systems of the two independent molecules were determined by means of packing considerations, using the observed parameters of cholesteryl myristate[7] which is isomorphous in two directions with the present structure. The coordinates of the ester and side chain atoms were located from a difference Fourier synthesis. Hydrogens were inserted in geometrical positions, and the structure was refined unisotropically to the discrepancy factor, R=6%, for 5700 observed reflections.

The two molecules adopt a similar geometry, differing mainly in the conformation about the C24-C25 bond; the two hydroxyl groups being *+gauche* oriented in one molecule, and *-gauche* in the other. In both molecules, the C24-OH bond is nearly synplanar with the adjacent C22-C23 double bond. Such an arrangement, which seems most favorable from energy considerations, leads to an extended conformation of the side chain similar to that observed in vitamin $D_2$[8] and its 4-iodo-5-nitrobenzoate ester.[9] The observed C17...C25 intramolecular distance (6.0Å) resembles that observed in vitamin $D_3$[10] and its 25-hydroxy derivative[11] where the side chain adopts an extended zig-zag form.

This X-ray determination proves that the synthetic precursor of the natural kidney metabolite of vitamin $D_2$ has 24(R) configuration in agreement with the corresponding metabolite of vitamin $D_3$, 24(R),25-dihydroxy vitamin $D_3$.[12] It is important to note that the configuration of the methyl at C24 in vitamin $D_2$ and its 24(R),25-dihydroxy derivative is the same, proving that the enzymatic hydroxylation at this C-atom proceeds with retention of configuration.

The fact that 24,25-dihydroxycholesterol (2) crystallizes with two independent molecules differing only in their rotameric conformations about the C24-C25 s-bond (2a, 2b) suggests a similar ground-state energy for both. These two conformations probably exist in solution in a dynamic equilibrium, as implied by our observation on the absence of the exciton chirality effect[13] in the CD spectrum of its 24,25-dibenzoate.


CHO
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2R
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1R
1S
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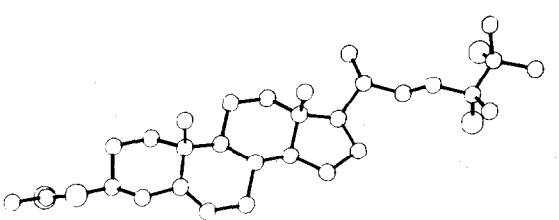
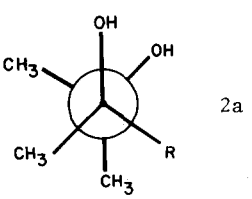


2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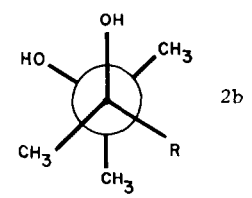
CH3
CH3
R

2a


OH
HO
CH3
CH3
CH3
R

2b

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