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Effect of C2O Stereochemistry on the Conformational Profile of the Side Chains of Vitamin D Analogs.[1]

M. Mark Midland*, Joaquin Plumet and William H. Okamura
Department of Chemistry, University of California
Riverside, California 92521

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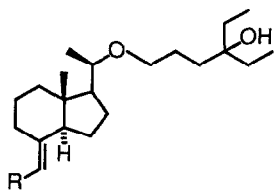
Abstract: The occupancy volumes have been explored using molecular mechanics based conformational analysis for the side chain of vitamin D analogs KH 1060, its C2O epimer, 1α,25-dihydroxyvitamin D_3 and its C2O epimer. Dot maps reflect the sites most accessible by the hydroxyl group. The stereochemistry at C2O causes the hydroxyl group to preferentially populate different (but not exclusively different) regions of space.

Side chain analogs of 1α,25-dihydroxyvitamin D_3 [1α,25-$(OH)_2$-D_3] have recently been shown to exhibit a bewildering array of biological activities of potential interest in biomedical applications. Of considerable interest is an analog developed by Leo Pharmaceutical Products in Denmark, KH 1060 (**1**), which exhibits extraordinarily potent effects in inhibiting T-cell activation *in vitro*, several orders of magnitude more active than cyclosporin A.[2,3] Because of the potent immunosuppressive effects of KH 1060 *in vitro*, this side chain analog is of considerable interest for the prevention of graft reject and treatment of autoimmune disorders. From a structural and drug development standpoint, KH 1060 is especially remarkable when compared to 1α,25-$(OH)_2$-D_3 because it possesses the unnatural configuration at C20 (in addition to the formal insertion of an oxygen atom between C20 and C22 as well as two extra methyl groups at C25 and C26 of the side chain). Because several structural changes are involved in relating the side chain of KH 1060 to that of 1α,25-$(OH)_2$-D_3 (**4**), this finding would not have been so remarkable were it not for the additional finding that a family of analogs possessing the unnatural C20 stereochemistry exhibit high potency in the above assay as well as in other biological activities characteristic of 1α,25-$(OH)_2$-D_3. Most notably, 20-epi-1α,25-$(OH)_2$-D_3 (Leo MC-1288, **3**) has been shown using human histiocytic lymphoma cell line U 937 to be several orders of magnitude more effective than 1α,25-$(OH)_2$-D_3 in inhibition of cellular proliferation and induction of cell differentiation, characteristics which may be of great value in the design of a cancer chemopreventive agent. Thus, there is some justification in the claim that no future vitamin D analog study would be complete without an evaluation of both C20 epimeric series, both natural and unnatural.[4]



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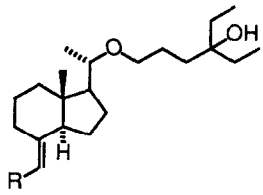

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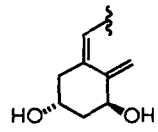

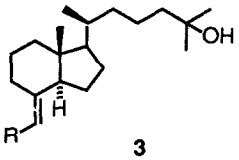

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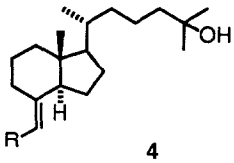

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The unusual biological properties of the C2O epimers of vitamin D analogs have prompted us to explore the conformational profile of these side chains. Herein we focus on KH 1060 (**1**) and its C2O epimer **2**, which has the "natural" C2O steroidal stereochemistry. We will also present results for the 2O-epi-1α,25-$(OH)_2$-D_3 (**3**) and 1α,25-$(OH)_2$-D_3 (**4**). To simplify the calculations, we have used a hydrogen in place of the R group in **1**-**4**.

Since the side chains of these analogs are flexible, it would be expected that numerous low energy conformations would be accessible. In order to evaluate the volume of space occupied by the side chain and locate possible visitation sites for the hydroxyl functional group, we have constructed dot maps for the side chain.[5] Dot maps are constructed in the following manner. All accessible low energy conformations for the side chain are generated using a Monte-Carlo conformational searching routine.[6] The CD hydrindane moiety is very rigid and exhibits only very minor deformations in the structures of interest. All conformations are then superimposed on the global minimum structure using only the carbons of the CD ring for the alignment. The coordinates of a selected atom in the side chain (in these cases the tertiary alcohol oxygen atom) are then recorded. The final output is a picture of the global minimum overlaid with dots mapping out the sites visited by the selected atom in the remaining conformations.

The Monte-Carlo conformational search using the MM2 force field was performed for compounds **1** and **2** using the 9 rotatable bonds of the side chain. In theory this could generate 19,683 (3^9) *gauche* and *anti*-conformations. Many of these are of high energy because of long-range Van der Walls interactions or numerous *gauche* interactions. Nevertheless, several thousand low energy conformations are possible. We have selected a window of 4 kcal above the lowest energy conformation as a cutoff. Higher energy species are of negligible population and only serve to fill in the gaps in the dot map. The energy window may be closed to 1, 2 or 3 kcal to see how the dot map distribution changes with energy. This distribution is listed for compounds **1**-**4** in Table I.

Table I. Conformational Distribution for Vitamin D Analogs

Energy Window	Number of Conformers for 1	2	3	4
0-1	72	152	16	29
0-2	376	765	39	63
0-3	701	1624	148	251
0-4	1140	2365	329	394

As can be seen in Table I, compound **2** exists in about twice as many conformations as does **1**. This is partly due to the fact that **2** has two low energy conformations about the C17-C20 bond, one in which the hydrogens at C17 and C20 are *anti* to one another, and one in which the oxygen on C20 is *anti* to the hydrogen on C17. This latter conformation is higher in energy for structure **1** since the C21 methyl group would then buttress into the C ring. Molecular mechanics calculations show that these two conformations differ by less than 0.1 kcal for **2** but by more than 2 kcal for **1**. Conformations **1** and **2** in which the C21 methyl group is *anti* to the C17 hydrogen are disfavored by more than 2.7 kcal. In both **1** and **2** the methyl group would suffer a 1,4 interaction with the C18 angular methyl group.

Dot maps indicate that the tertiary alcohol oxygen of compound **1** is largely localized above and to the north of the C ring as depicted in Figure 1. For compound **2**, the oxygen is largely above and to the northeast of the D ring. Comparison of the volumes occupied by the two dot maps indicates that the oxygen for the most part occupies different regions for the two isomers. At low energy (0-1 kcal) there is no overlap. However, at higher energy there is a considerably higher probability that the two epimers may place the oxygen in the same locale.

The natural hormone, 1α,25-$(OH)_2$-D_3 (**4**), shows less conformational flexibility since the side chain is one atom shorter and is missing the rotatable ethyl groups on the end of the chain. With 6 rotatable bonds there are 729 (3^6) possible *gauche* and *anti* conformations. Like compounds **1** and **2** the C2O epimer **3** has fewer conformations available than the natural isomer **4**. However, the difference is not so pronounced and levels off at higher energy. The epimer **3** probably has fewer degrees of freedom because some bond rotations will place the chain very near the CD rings. For both **3** and **4** there is only one low energy rotamer about the C17-C20 bond. This places the two hydrogens on C17 and C20 *anti* to one another.

The dot maps of **3** and **4** indicate that the C25 oxygen is located both above and below the plane of the CD ring. For the C20 epimer case (**3**) the oxygen is largely located towards the northwest (Figure 3) while with the natural vitamin (**4**) it is largely located towards the northeast. As in compounds **1** and **2**, compounds **3** and **4** localize the oxygen in different, but not exclusively different regions in the higher energy diagrams. On comparing **1** to **3** and **2** to **4** one sees that **1** and **2** tend to place the tertiary oxygen above the plane of the CD ring more than in **3** and **4**. This may be attributed to the fact that O22 may be placed *anti* to the C17 hydrogen (and thus above the CD ring) in **1** and **2** whereas this conformation is not favorable in the carbon analogs **3** and **4**.

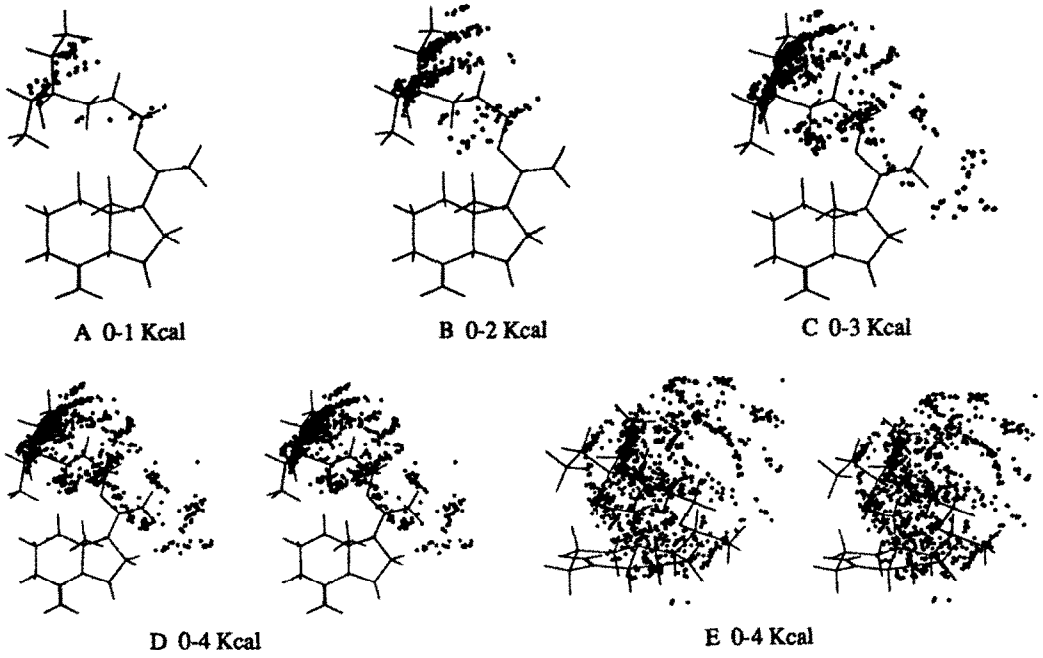

Figure 1. Dot maps for KH 1060 analog 1; D, E are stereoviews

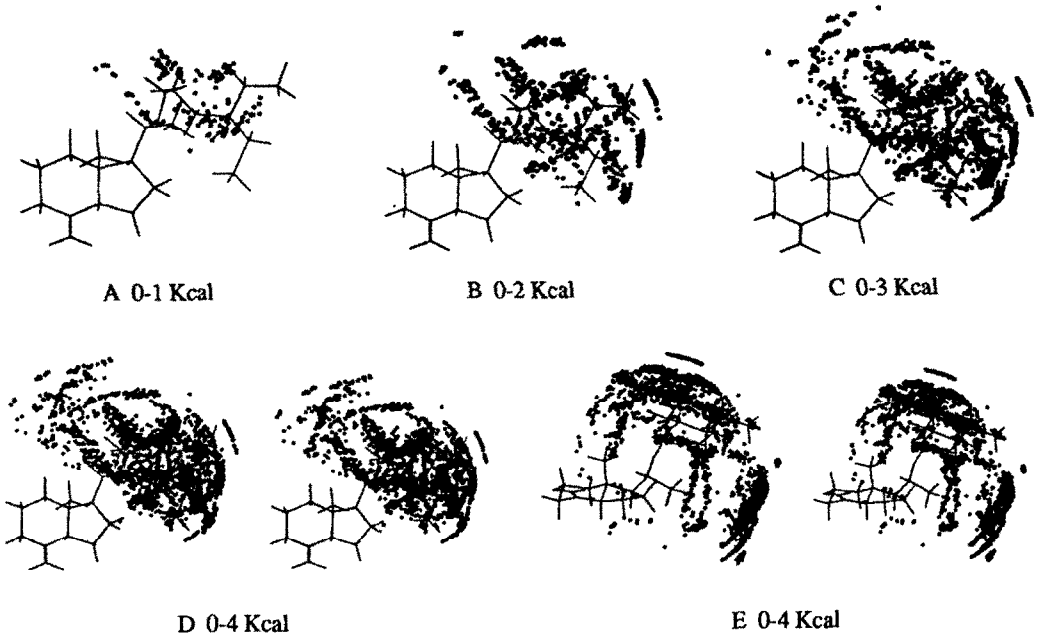

Figure 2. Dot maps for 20-epi KH 1060 analog 2; D, E are stereoviews

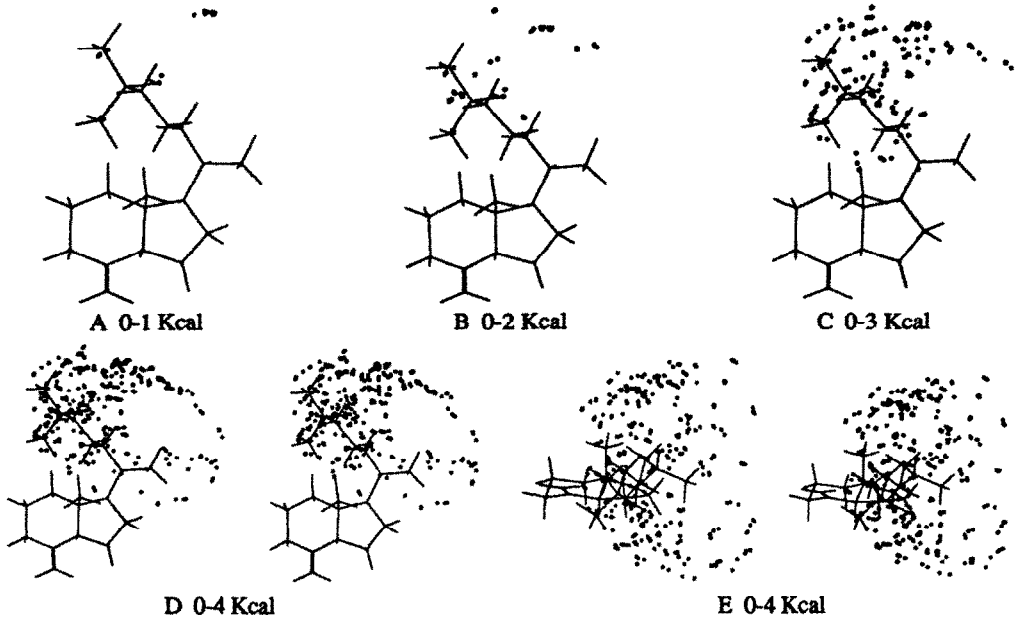

Figure 3. Dot maps of 20-epi-1α,25-$(OH)_2$-D_3 analog **3**

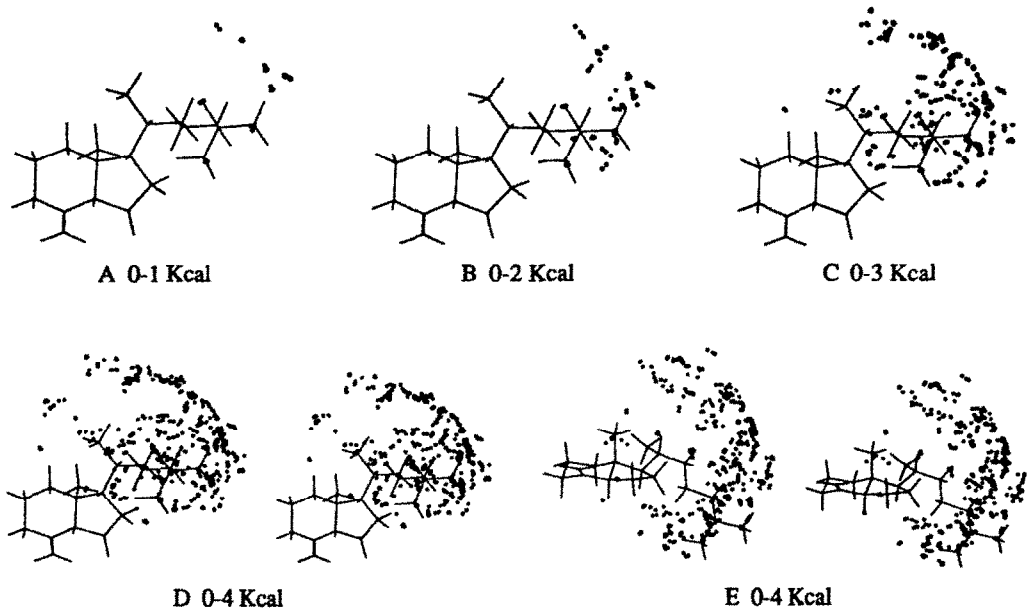

Figure 4. Dot maps of 1α,25-$(OH)_2$-D_3 analog **4**

In summary, the stereochemistry at C20 causes the side chain to be directed to the left in **1** and **3** and to the right in the "natural" isomers **2** and **4**. At low energy (0-1 kcal) two distinct regions are occupied by the tertiary oxygen of the side chain. However, at higher energies the two regions overlap. Since the energy differences dealt with in this study are quite modest in terms of dynamics (i.e., the barriers to their interconversion are small), it is quite reasonable to expect that virtually any of the species considered in this study are sufficiently flexible to distort to a higher energy conformer to form optimal interactions with a putative receptor.[7] Accordingly, it is concluded that in order to determine whether the orientation to the 'left' as in **1** and **3** or to the 'right' as in **2** and **4** offer significant differences in terms of biological selectivities would best be evaluated through developing rigid isosteric analogs. That is, the synthesis and study of a family of rotationally restricted side chain analogs should provide valuable insight into developing analogs of 1α,25-$(OH)_2$-D_3 for biomedical applications.

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(1) (a) This is paper 46 in the series Studies of Vitamin D (Calciferol) and Its Analogs. For paper 45, see: Muralidharan, K.R.; de Lera, A.R.; Isaeff, S.D.; Norman, A.W.; Okamura, W.H. J. Org. Chem. **1993**, 58, in press. (b) This study was supported by grants from the National Institutes of Health (NIH grant CA-43277 and in part by NIH DK-16595).

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