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ON THE USE OF VOLUME MAPS IN THE CONFORMATIONAL ANALYSIS OF VITAMIN D ANALOGS

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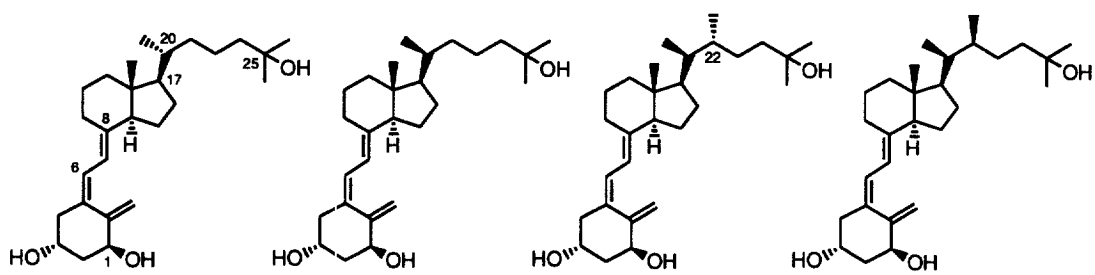
Abstract Dot maps used to represent the calculated conformations of the side chain of vitamin D derivatives are improved by replacing dots by coloured balls to create volume maps and to identify particular energy windows. Two procedures to define a relative activity volume, based on a pair of analogs with distinctly different biological activity and conformational behaviour, are presented.

The secosteroid hormone 1α,25-dihydroxyvitamin D_3 (**1**), the physiologically active form of vitamin D_3, elicits a variety of biological responses. The classical calciotropic activity, which involves intestinal calcium absorption and bone mineralisation and resorption, originates from a genomic pathway wherein the hormone binds to the intracellular vitamin D receptor (VDR) which triggers the synthesis of new proteins that are more directly responsible for the biological response.[1] An alternative non-genomic pathway, known as trans-caltachia, involves a membrane receptor and stimulates very rapid intestinal calcium transport.[2] Besides its traditional role in calcium homeostasis **1** induces cellular differentiation and inhibits cellular proliferation.[3] Its therapeutic utility in the treatment of certain cancers and skin diseases is however limited for effective doses provoke hypercalcemia. This has initiated in recent years a very active search for analogs of vitamin D which would possess a high cell differentiating ability but a low calciotropic action.[4] In the context of structure-function relation we report herein conformational studies which could help in defining biologically active shapes of the molecule.



The structure of the steroid hormone **1** is unique in that, during its biosynthesis, ring B underwent a photochemical cleavage, whereas the triterpene cholesterol-type side chain remained intact. Hence seco-steroid **1** appears as a molecule possessing three different parts.[5] To the central rigid *trans*-fused CD-ring system are connected two flexible moieties: the side chain with five essentially free rotatable carbon-carbon bonds along the C17-C25 chain, and the chair-interconverting six-membered A ring attached to C8 via the s-*trans* diene portion of the seco B-ring. In a first approximation the central part can be considered as an anchoring hydrophobic moiety whose structural function consists in isolating the two flexible parts of the molecule, each

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carrying one of the hydroxy groups, i.e. the 1α-OH and 25-OH groups, that have been shown to be essential for recognition by the receptor protein.

So far in the development of analogs of vitamin D one has concentrated essentially on the two flexible parts of the molecule. A few successful structural modifications that have led to the desired separation of activities include for the A-ring part 19-nor derivatives and for the side-chain part e.g. 22-oxa, 23-yne, 24-homo, 26,27-bishomo and 20-*epi* derivatives and combinations thereof.[4] In terms of structure-function analysis the *epi*-modification is of special interest since the inversion of one stereocenter is not expected to lead to major differences in the bulk properties of the molecule, yet may lead to a dramatic difference in shape. In this context the natural hormone **1** and its 20*S*-epimer (**2**) are of particular interest for the latter has been shown to be several orders of magnitude more efficient than the natural hormone in the inhibition of cellular proliferation and induction of cell differentiation.[6]

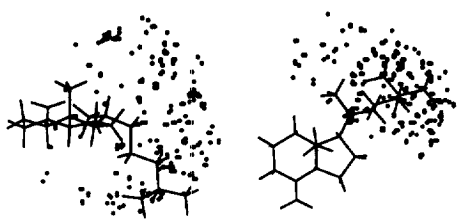


Figure 1

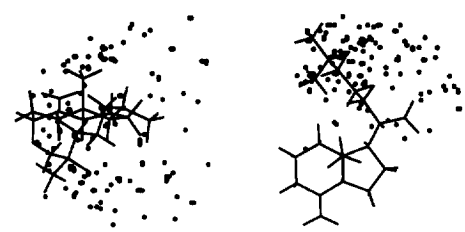


Figure 2

The effect of the above 20-*epi* modification on the conformational behaviour of the side chain can be visualized by a *dot map* approach that has been introduced in the field by Okamura and Midland.[7] In the latter approach force field calculations are performed so as to generate within a given energy window all possible local minimum energy conformations that the side chain may adopt. The orientation in space of each found conformation is further defined by a dot that corresponds to the position of the 25-oxygen atom in that particular conformation. Figures 1 and 2 represent dot maps of **1** and **2**, respectively, including a side and a top view of the molecule (see experimental). The line drawing corresponds to the global minimum energy form. In particular one notes that the combined inspection of the two perpendicular views provides an indication of which part of the 3D space is occupied by the side chain, and that the inspection of dot maps with gradually increasing energy windows (e.g. from 0 to 20 kJ/mole in 4 steps) as shown in Figure 3 for **1** further discloses the dynamic behaviour of the side chain. In the analysis of the Riverside group calculations were performed on a truncated model derivative in which the lower part of the molecule at C6 is replaced by H.

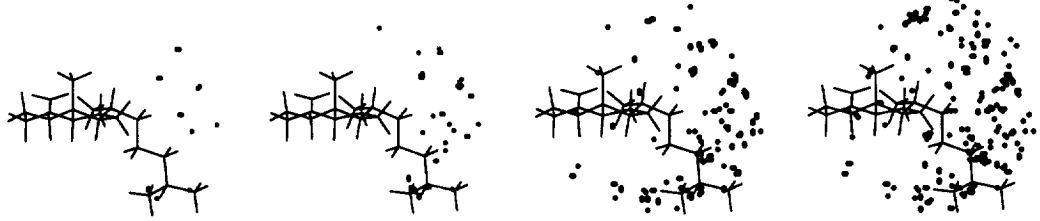
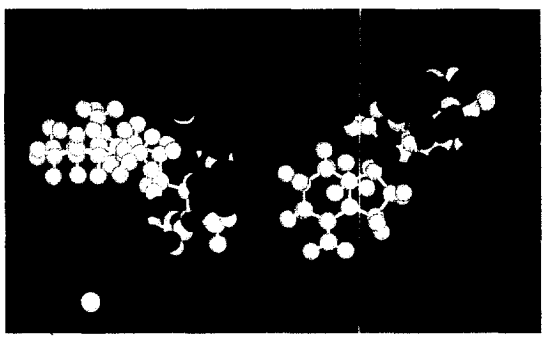


Figure 3

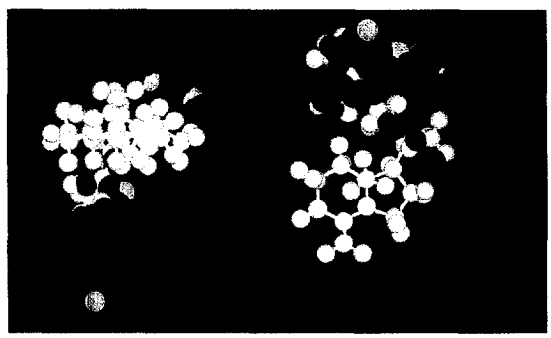
We further present a procedure, based on the dot map approach, that provides a substantial improvement in that one gets a far better feeling of (1) what effective volume is occupied by the side chain, and of (2) its dynamic conformational behaviour. In the procedure dots are replaced by balls, and the line drawing corres-

ponding to the global minimum energy form by a grey ball-and-stick model, hence creating a *volume map*. Combination of both features effectively generates an occupation volume for the side chain whose orientation relative to the central part of the molecule can be assessed. Another feature of the procedure resides in the introduction of colour to identify a particular energy window. Within each further used colour palette (pink-blue and yellow-brown) the four different nuances correspond to energy windows of 0-5, 5-10, 10-15 and 15-20 kJ/mole, respectively, the brightest colours indicating the most stable forms. Figures 4 ans 5 show perpendicular views of the corresponding volume maps of **1** and **2**, respectively; for the sake of illustration the two different colour palettes were used. Direct comparison of Figures 1 and 4, and of 2 and 5 illustrates how the volume map approach enables to visualize the space that is occupied by the side chain relative to the minimum energy conformation without the necessity of providing stereoviews. This effect is obtained by the overlapping of the balls that eventually creates a quasi continuous volume.



$1,25(OH)_2D_3$ (**1**)

Figure 4



(20*S*)-$1,25(OH)_2D_3$ (**2**)

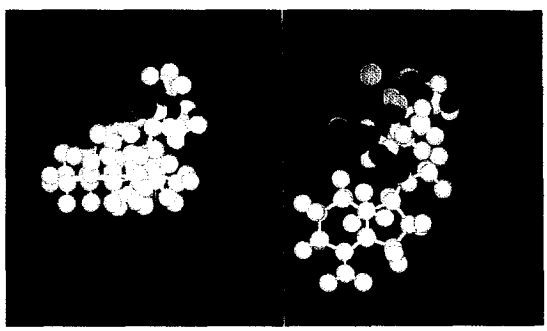
Figure 5

In the context of structure-function analysis it is clear that the conformation(s) of interest is (are) those that will be occupied by the molecule upon binding to the receptor protein and that those need not correspond to the global minimum or even local energy forms of the free ligand. In fact, in view of the small barriers for interconversion between the different rotameric states, one may reasonably expect any of these states to be sufficiently flexible to adopt a higher energy geometry if the latter affords a tighter binding to the receptor. Therefore, as long as the conformation(s) adopted by the natural hormone and active analogs upon binding to the receptor remain(s) unknown, the results of a rational design of new analogs, based on e.g. the conformational analysis of the side chain, should be unreliable. This aspect has been convincingly stressed by Jorgensen:[8] "A practical consequence is the frustration that will often accompany attempts to design drugs by analogy to the structures of flexible, unbound active substances". Nevertheless, in some particular cases where rather extreme differences in biological activity are observed within a limited set of structurally related analogs, it remains interesting to try to define structure-function relationships with the focus on *relative* differences. One way of doing so could consist in defining *relative activity volumes*. Such volume is a region in space, defined with respect to some points of reference (see experimental), corresponding to the preferred occupation of the side chain of the more active analog of a pair as compared to the inactive one. The way these relative activity volumes are defined is reminiscent of the procedure described by Marshall in the active analog approach (*vide infra*).[9]

A suitable pair of analogs for defining such a relative activity volume is available through a recent study of Yamada.[10] In this work a series of four 22-methyl substituted derivatives, diastereomeric at C20 and at C22, were conceived as analogs with a conformationally restricted side chain. Application of the dot map approach allowed the authors to define four rather different and confined areas that were each preferentially populated by

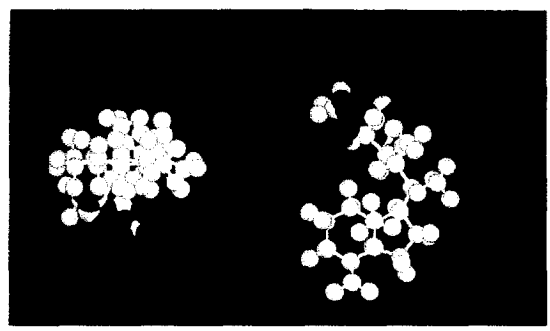
one of the four diastereomers. Interestingly, two of the four derivatives were shown to possess very different affinities for the VDR in that **3** showed a 20 times greater affinity and **4** only a 1/100 fraction, as compared to the natural hormone. Volume maps of both derivatives using perpendicular views are shown in Figure 6 and 7.

We hereby propose two procedures for the determination of a relative activity volume, illustrated on analogs **3** and **4**. In the first procedure it is assumed that the inactivity of **4** is due to the fact that its side chain cannot occupy the active site of the receptor. So one could define a relative activity volume as the volume which is left after subtracting the volume area accessible to the less active **4** from the volume area accessible to



(20*S*,22*R*)-22-Me-1,25(OH)$_2$D$_3$ (**3**)

Figure 6



(20*S*,22*S*)-22-Me-1,25(OH)$_2$D$_3$ (**4**)

Figure 7

the more active **3**. This subtraction procedure (see experimental) resulted in the relative activity volume shown as a stereo image in Figure 8 (side view). The reduced volume area is formed by 38 conformations of **3** (18 % of the original 210 conformations (0-20 kJ/mole), 88 mole% according to a Boltzmann distribution at 298K) and still includes 18 of its 20 most stable conformations (0-5 kJ/mole). The highly populated area in the figure could thus correspond to the orientation of the side chain upon binding of the ligand to the receptor. In contrast, in the case of the active analog approach a subtraction procedure is used in an opposite way, i.e. to define the receptor essential volume, a volume which is *not* available for binding.

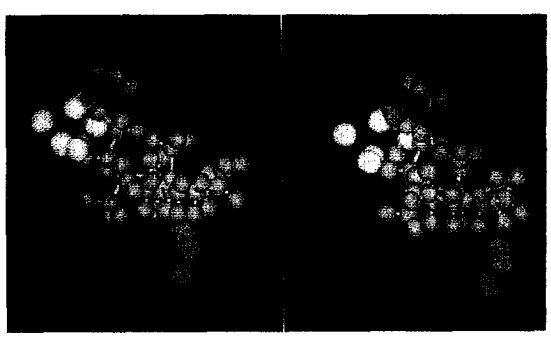


Figure 8

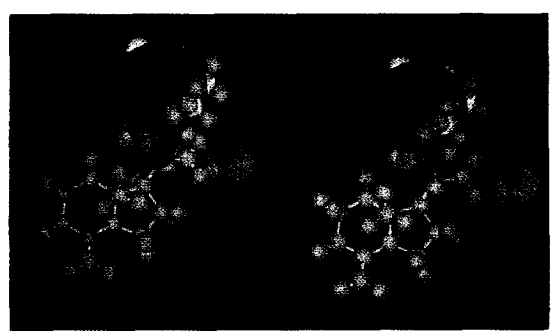


Figure 9

The second procedure consists of generating a volume, i.e. a sphere, with given dimensions that contains only a chosen mole fraction of the side chain conformations of the less active **4**, but at the same time contains the highest possible mole fraction of the conformations of the more active **3**. In this particular case, because of the extreme difference in affinity for the VDR, the fraction of the conformations of **4** allowed in the sphere was set to 0%. The result of this procedure (see experimental) is shown as a red ball. The ball has a radius of

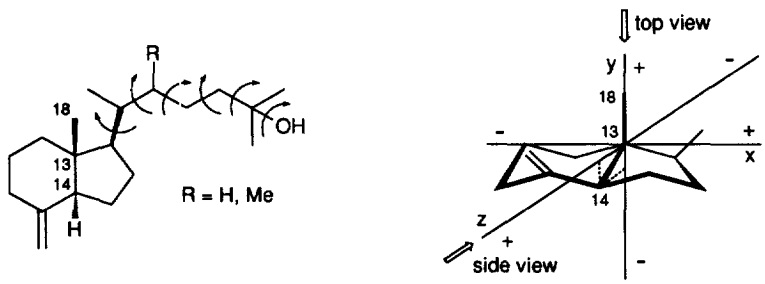
2.45 Å and center co-ordinates x = 1.4, y = 2.8 and z = -5.3 Å. It contains 60 conformations of **3** (29 % of the original 210 conformations (0-20 kJ/mole), 87 mole% according to a Boltzmann distribution at 298K). In the stereo image presented in Figure 9 the relative activity volumes resulting from both procedures are overlaid (top view). They are quite similar as 24 of the 38 conformations (including 18 of the 20 most stable ones) of **3** constituting the reduced volume area resulting from the above subtraction procedure lie inside the volume area generated by the variable volume search procedure. However, other pairs of (conformationally restricted) analogs may well lead to other relative activity volumes and are currently being investigated.

Relative activity volumes as determined for **3-4** might be useful in the study of vitamin D structure-function relationships. Indeed, biological testing of pairs of analogs designed to have a large and a small fraction, respectively, of side-chain conformations in a certain volume area might help to reveal the structural features that are required for triggering a certain biological response. The volume map and relative activity volume approaches presented here are believed to constitute a contribution to this study.

Experimental

Conformational Analysis, Dot Maps and Volume Maps

Conformational analysis of the side chain of compounds **1-4** was carried out using the MacroModel molecular modeling program of Still[11] run on a Digital VAXstation 4000-90A. Molecular mechanics calculations were carried out on model compounds in which the A ring and diene system up to C6 were replaced by a



H atom. Rotations with 60° increments were applied to the five rotatable C-C bonds of the side chain, while the 25-OH was rotated with increments of 120°, so generating 23,328 starting conformations. These were minimized using the MM2 force field implementation of MacroModel and led to 366, 280, 210 and 123 distinct conformations within 20 kJ/mole of the minimum energy conformation of **1-4**, respectively. Using a PC computer program all conformations of each compound were then overlaid using C13 as common origin (x, y, z = 0), C14 was positioned in the yz-plane (x = 0) and C18 was made to coincide with the positive y-axis (x, z = 0). A line drawing was generated of the minimum energy conformation and the position of O25 in each of the local energy minima within the given energy window was represented by a dot to obtain the dot maps. The volume maps were generated by importing the above dot maps into the CSC Chem3D molecular modeling program[12] for Macintosh, creating ball-and-stick models using van der Waals radii of 0.7 and 0.8 Å for C and O, respectively, and applying the appropriate colour to O (see text) according to the energy of the particular conformation.

Determination of a Relative Activity Volume by Subtraction

For subtraction of the dot map of **4** from the dot map of **3**, a PC computer program was developed which allowed for (1) the generation of a sphere centered around each O25 position in the dot map of **3** and (2) the determination of the presence of a O25 position of **4** in each of these spheres. For the procedure both dot maps were oriented and overlaid using C13, C14 and C18 as above. In case no O25 position of **4** was present in the sphere, the particular O25 position of **3** was retained. In case a O25 position of **4** was found in the sphere, the particular O25 position of **3** was either (a) retained if the energy category of the conformation of **3** was lower

(more stable) than the energy category of the conformation of **4**, or (b) rejected if the energy category of the conformation of **3** was equal or higher (less stable). For this, four energy categories of 0-5, 5-10, 10-15 and 15-20 kJ/mole were used. The radius of the sphere was varied so as to end up with 15-20% (arbitrarily chosen, but found to give a workable reduced volume area) of the original 210 conformations (0-20 kJ/mole) of **3**. In order to obtain the result discussed in the text and shown in Figure 8, the radius of the sphere had to be set to 4.1 Å. This rather large sphere reflects the large difference between the dot maps of **3** and **4**.

Determination of a Relative Activity Volume by Variable Volume Search

The variable volume search on the dot maps of **3** and **4**, oriented and overlaid as above, was carried out by means of a PC computer program developed to systematically search a volume in space with a sphere of varying size. As the purpose of the procedure is to find the sphere containing the highest possible mole fraction of **3**, but only a given small mole fraction (0% in this case) of **4**, the volume searched was constructed around the most densely populated area in the volume map of **3**, easily located upon simple inspection (see Figure 6). In practice, the center of the sphere was moved to all possible positions with co-ordinates x = 0.6 to 3.1 Å, y = 2.0 to 4.5 Å and z = -4.1 to -7.6 Å, in steps of 0.1 Å along the 3 axes, while in each of these positions the radius of the sphere was varied from 1.0 to 3.0 Å in steps of 0.05 Å. For each sphere the mole fractions of the conformations with O25 positioned in the sphere were summed for both **3** and **4**, the spheres containing 0 mole% of **4** were retained and sorted in order to obtain the sphere with the highest fraction of **3**.

Acknowledgement

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