Conformational Analysis of Model Compounds of Vitamin D by Theoretical Calculations

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ABSTRACT: A conformational analysis of two model compounds of vitamin D was carried out by means of theoretical computations, *Ab initio* calculations were carried out using the standard 6-31G* basis set at the Hartree–Fock (HF) level of theory. In addition, the Møller–Plesset (MP2) correlation treatment was applied on the simplest model. Semiempirical calculations were also performed using the AM1 Hamiltonian. The results predict stable A-ring twist forms with energies in the order of 4–6 kcal/mol relative to the global minimum, significantly higher than those reported from molecular mechanics calculations. In addition, a folded conformation was found by the HF optimizations; however, its stability is predicted to be very poor. Comparison of the theoretical results with experimental data is discussed. © 1997 by John Wiley & Sons, Inc. *J Comput Chem* **18:** 1647–1655, 1997

Keywords: vitamin D; steroid hormones; conformational analysis; *ab initio* calculations; semiempirical calculations

Introduction

The importance of computational chemistry in understanding structure–activity relationships in biological systems has been recognized in recent years. Different approaches can be used depending on the nature of the problem considered and/or the size of the system studied. For very large biological compounds, calculations are limited to the less expensive computational treatment: molecular mechanics. Semiempirical or, more increasingly, *ab initio* methods may be preferred if the system under investigation is relatively small or if the interest is in modeling a concrete part of the system.

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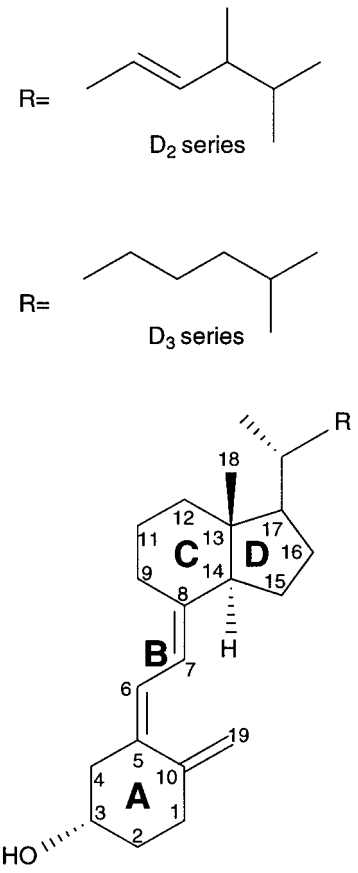

FIGURE 1. Numbering and ring nomenclature for vitamin D.

Attention has been focused on the conformational properties of the vitamin D group, a family of vitamins that includes the natural vitamins D_2 (ergocalciferol) and D_3 (cholecalciferol), as well as a long series of synthetic derivatives, some of which have therapeutic properties. Figure 1 shows a picture of vitamin D; its structure can be regarded as two conformationally independent flexible components connected to a central CD ring system. These two flexible components, the side chain on the one hand, and the A-ring together with the triene system on the other hand, make vitamin D a complex system for conformational studies.

The early experimental work aimed to examine the conformational behavior of vitamin D, primarily using NMR spectroscopy, had shown the existence in solution of a dynamic equilibrium between nearly equimolar populations of two chair A-ring conformations[1–5] (see Fig. 2). This observation was subsequently supported by molecular mechanics calculations on a series of vitamin D_3

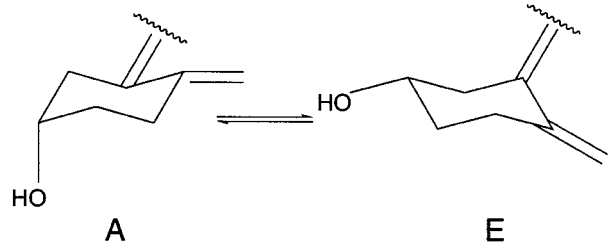

FIGURE 2. Conformational equilibrium between A and E forms of the A-ring.

analogues[6,8] and by *ab initio* computations employing the STO-3G and 3-21G basis sets on 1,2-dimethylene-cyclohexane,[9] a model compound for A-ring mobility of vitamin D. Besides this, the theoretical results predicted another type of A-ring conformation: the twist form, recently detected by Hofer et al.,[10] based on a combination of LIS measurements, LIS simulations, and force field calculations.

The most stable conformation of the intercyclic diene unit B is the *s*-trans (transoid or extended) arrangement. This has been shown in the experimental studies of vitamin D metabolites carried out in solution by NMR spectroscopy[1–5,10,11] or circular dichroism,[12] and in crystal state by x-ray diffraction.[13–16] In fact, there is no direct evidence for detectable levels of the *s*-cis (cisoid or folded) conformation. However, it is well accepted that the energy barrier associated with the torsion around the C6—C7 bond should not be very high so that the cisoid conformation can be accessible for all vitamins D, a requirement for the known vitamin–previtamin D equilibrium.[17,18] The published theoretical calculations[6–8,10,19] are not quite in accordance with the experimental observations. Thus, the molecular mechanics results obtained by Hofer et al.[10] showed stable *s*-cis conformations with quite low relative energies. More surprisingly, calculations on 1,25-dihydroxyvitamin D_3, based on a simulated annealing process,[19] have predicted the *s*-cis conformation to be the global energy minimum. This result prompted Zhu et al.[11] to investigate the diene system B conformation in solution using ^{1}H-NMR spectroscopy and molecular mechanics calculations. The latter considered 11-fluorinated vitamin D_3 derivatives, wherein hydrogen bonding could favor the folded conformations. Their NMR analysis has not shown evidence for the presence of the *s*-cis form, although their calculations on the 11-β-1-α-OH-D_3 derivative predicted this arrangement to be the most stable one.

The side chain is the most flexible component of the vitamin D structure. Due to the very large number of conformational orientations that the steroid side chain can adopt, it is more appropriately analyzed via molecular mechanics calculations; some studies based on this methodology have appeared in the literature.[20, 21]

Obviously, the conformational problem of vitamin D is quite complex; this is the main reason why experimental work has encountered several difficulties in arriving at quantitative results for this system. On the other hand, the published theoretical studies, most of them based on molecular mechanics calculations, might not be rigorous enough to model all the conformational features of vitamin D with high accuracy. This fact, together with the importance that the conformation has on the biological activity of vitamin D, has prompted us to carry out this theoretical study chiefly by means of the *ab initio* MO methodology. Our main purpose was to investigate the conformational properties of the ring A and system B to obtain a major complement to the experimental findings.

Computational Details

Because vitamin D is a very large system to be investigated by *ab initio* theory, we had to handle model compounds for which the calculations were computationally less expensive. As mentioned before, our goal did not deal with the conformational properties associated with the side chain so this fragment could be neglected in the computations. However, even without the side chain the structure we had to analyze was quite large considering the conformational wealth of ring A. Taking this into account we regarded two model compounds, shown in Figures 3 and 4, respectively.

The model compound **I** (Fig. 3) was chosen to examine the conformational features of ring A in detail. For the sake of convenience the atom numbering used in the computations parallels that of vitamin D. To obtain the conformers of this model full geometry optimizations were performed using the standard 6-31G* basis set at both the Hartree–Fock (HF) level of theory and Møller–Plesset perturbation theory with the inclusion of energy corrections through the second order (MP2). The location of twist forms was facilitated through a reaction coordinate driving method in which the dihedral angle of interest was constrained step by step in a series of geometry optimizations. The optimized geometries are identified using an acronym in which the first (capital) letter (A, E, or T) has the following meaning. The letter ''A'' refers to a geometry wherein the ring has a chair shape with the 3-OH group in axial orientation; ''E'' refers to a chair conformation with the 3-OH group in equatorial orientation; and ''T'' refers to a twist conformation. A number after the letter T (1 to 6) is used to distinguish the different twist conformations found in this work. Finally, the small letter in the acronym specifies the orientation of the O—H bond as shown in Figure 5.

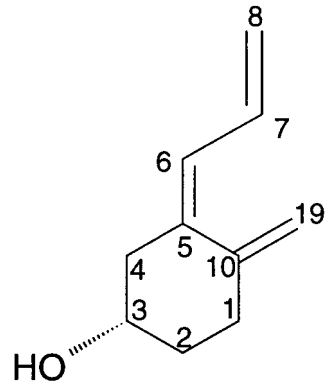

FIGURE 3. Model compound **I**.

The conformational characteristics of diene system B were analyzed from the model compound **II** (Fig. 4), first by semiempirical calculations using the AM1 Hamiltonian and subsequently by the *ab initio* MO methodology. In general, the latter calculations involved full geometry optimizations at the HF level of theory with the 6-31G* basis set,

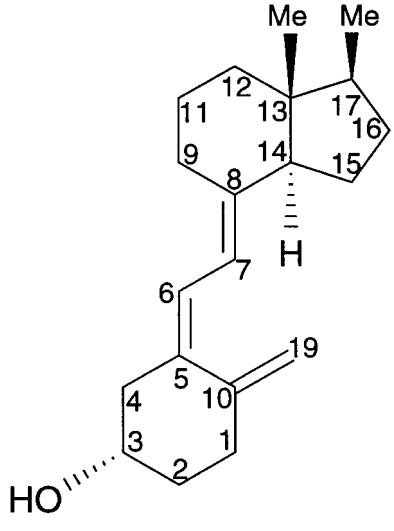

FIGURE 4. Model compound **II**.

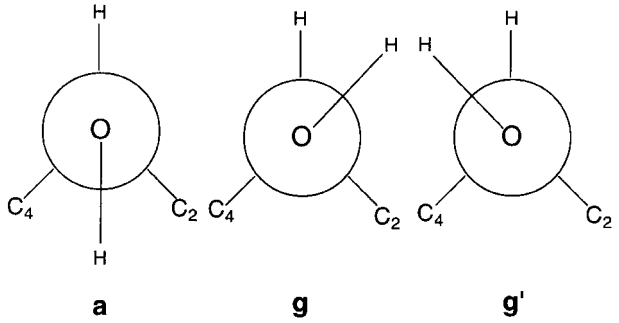

FIGURE 5. The a, g, and g′ rotamers for the 3-OH group.

using the AM1 geometries as starting points. Nevertheless, due to computational limitations we considered only the following A-ring conformations in the calculations: Aa, Ea, T4a, and T6a. Two different forms, distinguished by the sign of the dihedral angle C11—C9—C8—C14, were found for the CD hydrindane fragment and were named using capital letters: P for the conformation with a positive dihedral angle, and N for the other. To label the optimized geometries of model compound **II** an acronym was composed by preceding one of the above letters to the acronym employed for model **I**. Finally, the torsional barrier around the C6—C7 bond was examined by means of the reaction coordinate driving procedure mentioned previously.

The *ab initio* calculations on these models were undertaken with the Gaussian-92 and -94 suites of programs[22] implemented on a Fujitsu vp2400/10 supercomputer. The AM1 semiempirical calculations were executed on an Indigo2 Silicon Graphics workstation employing the Insight II program package.[23]

Results and Discussion

MODEL COMPOUND I

Our calculations on the model compound **I** yielded two chair forms (E and A) and the six possible twist forms that could be expected (T1, T2, . . . , T6). Rotation around the C3—O bond produced a family of three rotamers for each form, so 24 conformers were optimized in all. Due to computational limitations the MP2 optimizations on the twist forms were only undertaken on the most stable rotamer obtained previously at the HF level. The relative energies of all conformations studied with the MP2 procedure are given in Table I,

TABLE I.
Relative Energies (E_{rel}, in Kilocalories per Mole) and Selected Torsion Angles (in Degrees) for Conformers of Model I Optimized at the MP2 level.[a]

	Aa	Ag	Ag′	Ea	Eg	Eg′	T1a	T2a	T3a	T4g′	T5g	T6g′
E_{rel}	0.00	0.89	1.30	1.54	1.43	1.52	4.81	4.39	5.02	6.19	6.26	5.33
	(0.03)	(0.30)	(0.72)	(0.20)	(0.00)	(0.03)	(5.22)	(5.18)	(5.11)	(5.17)	(5.40)	(5.01)
C4—C5—C10—C1	52.20	49.94	50.14	−50.27	−49.98	−49.95	−55.43	−32.07	30.27	53.20	31.96	−33.53
C5—C10—C1—C2	−49.16	−50.07	−51.56	50.60	50.24	50.48	27.02	61.98	32.51	−22.14	−61.56	−28.40
C10–C1—C2—C3	51.95	53.41	54.64	−53.89	−54.06	−54.29	32.45	−26.34	−60.33	−36.66	25.34	65.49
C1—C2—C3—C4	−57.60	−57.02	−56.70	56.69	57.60	57.62	−67.78	−33.15	22.40	69.06	34.34	−37.13
C2—C3—C4—C5	58.01	55.36	53.53	−54.55	−55.66	−55.02	40.07	63.42	38.64	−38.56	−64.38	−23.59
C3—C4—C5—C10	−55.49	−51.59	−50.21	51.43	51.01	51.33	18.97	−29.52	−68.53	−20.18	29.62	61.91
C6—C5—C10—C19	60.19	55.47	55.28	−56.48	−56.16	−55.63	−59.93	−41.10	42.05	56.70	40.29	−41.69
H—O—C3—H	153.74	75.14	−61.71	−179.84	60.21	−60.75	170.53	−178.66	174.26	−57.51	54.96	−55.71
O—C3—C4—C5	−64.96	−62.16	−67.29	−179.75	−174.11	−176.63	−83.58	−60.82	−84.81	−160.53	177.05	−144.48

[a] Values in parentheses correspond to the HF/6-31G* optimizations.

TABLE II.
HF / 6-31G* Relative Energies for Conformations Excluded in Table I.

Conformer	E_{rel}(kcal mol^{-1})
T1g	5.66
T1g′	5.81
T2g	6.46
T2g′	6.81
T3g	5.90
T3g′	5.87
T4a	5.51
T4g	5.39
T5a	5.85
T5g′	5.56
T6a	5.97
T6g	5.19

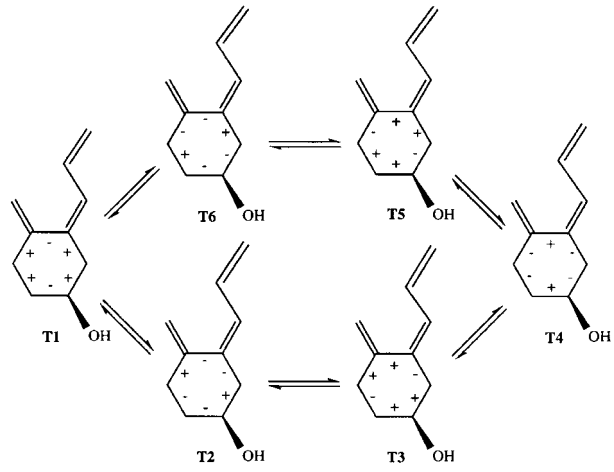

FIGURE 6. Pseudorotation process for ring A.

together with the more significant dihedral angles. The HF/6-31G* relative energies for those conformations are included in that table as well, whereas the values for the remaining conformers are listed in Table II.

The MP2 results show the Aa conformation as the most stable one. The energies of the other chair conformers are found to be higher by at least 0.9 kcal/mol than that computed for the above conformation. Conversely, the HF treatment slightly favors the equatorial (E) over the axial form (A). On the other hand, our results predict a significant influence of the O—H bond orientation (a, g, or g′) on the conformational stabilities; in general, the energy differences in a family of rotamers are within 1 kcal/mol.

The twist arrangement is in a much more shallow energy minimum than the (rigid) chair, and interconversion of the several twist forms by a pseudorotation process requires a small activation energy. A picture of this process, showing the signs of the internal torsion angles of the A-ring, is depicted in Figure 6. The relative energies of the twist conformers vary from 4.39 kcal/mol (T2a) to 6.26 (T5g) in the MP2 calculations and from 5.19 kcal/mol (T6g) to 6.81 (T2g′) in the HF results. These values are far different from those reported from molecular mechanics calculations,[7, 10] which can be as low as 0.35 kcal/mol.

The experimental estimates from NMR spectroscopy[3, 5, 10] reveal that, in solution, the total Boltzmann population of the vitamin D_3 conformers with the hydroxyl group in the equatorial position is similar to that of the conformers with the hydroxyl group in axial position. Apart from that, Hofer et al.,[10] from their force-field calculations, experimental LIS data, and LIS-simulation, concluded that a conformation with the A-ring in a twist form is significantly populated, up to $\sim$20%, in the equilibrium mixture of vitamin D. However, they admitted that the quantitative evaluation of LIS data in a multicomponent conformational equilibrium of the type they studied is close to the limits of the method. Using the HF/6-31G* results shown in Tables I and II, and the Boltzmann distribution law, one obtains, at the standard ambient temperature, a population of 59.0% for the E form and 40.9% for the A form (all the 3-OH rotamers were included in both cases). From the MP2 results we have estimated a population of 15.1% for the E group of conformations and 84.6% for the A group. Regarding the axial/equatorial population ratio, the results obtained at the HF level are in closer agreement with experiment than those achieved with the MP2 treatment. On the other hand, the total population of the twist forms computed with the HF results and the one obtained with the MP2 method are insignificant; this accounts for the difficulty involved in its experimental detection. Our calculations, therefore, suggest that the population of the twist form reported by Hofer et al.[10] might be overestimated. However, it is important to keep in mind that, besides using a model compound to mimic the conformational features of ring A, our theoretical results correspond to gas phase behavior, so a direct comparison should be taken with caution.

MODEL COMPOUND II

The relative energies calculated at the HF/6-31G* and AM1 levels of theory for the optimized

conformations of the model compound **II** are presented in Table III, together with some selected torsion angles. Several conclusions can be drawn from the table. The HF/6-31G* relative energies computed for the N conformations, those in which the dihedral angle C11—C9—C8—C14 has a negative value, parallel in general the results obtained on the model compound **I** at the same level of theory. The same is true for the P conformations and so the model compound **I** is valuable for analyzing the conformational features of ring A. Concurrently, the HF/6-31G* calculations predict a systematic change of 4.2 kcal/mol between conformers differing on the CD-ring conformation, with the N conformation favored over the P. Therefore, our results point out that the CD-ring system has no great influence on the conformational stabilities of ring A, as could be expected. Overall, structure **II** can be regarded as two quite independent moieties joined by the diene arrangement.

On the other hand, the energy results achieved with the AM1 semiempirical methodology are quite distinct from the *ab initio* outcomes. The values for the N conformers are similar to those for their corresponding counterparts, the P conformers. In addition, the AM1 twist–chair energy difference is much lower than that computed by the *ab initio* treatment; for instance, $N\Delta E$(NT4a − NAa) = 1.1 kcal/mol (AM1) vs. 5.7 kcal/mol (HF/6-31G*).

We paid special attention to the torsion around the C6—C7 bond. Experimentally, the extended conformation is the only one observed. However, the molecular mechanics calculations undertaken by Hofer et al.[10] yielded stable *s*-cis conformations, the lowest having a steric energy of 0.85 kcal/mol relative to the global minimum. More unexpectedly, Wilson et al.,[19] using simulated annealing, found the *s*-cis form of 1,25-dihydroxyvitamin D_3 as the most stable arrangement. A plot of the relative energy as a function of the dihedral angle C5—C6—C7—C8 for our most stable conformation (NAa) of model compound **II** is shown in Figure 7. The AM1 profile is somewhat more shallow than the HF/6-31G* curve, but both treatments predict that the *s*-trans conformation is the preferred one. A local minimum at 65°, associated with a folded conformation, is observed in the HF/6-31G* curve; however, its high relative energy (4.7 kcal/mol) and the extremely low cis-to-trans barrier point to a very poor conformational stability. The highs of both asymmetric potential wells appear to be reasonable for the molecule to assume a cisoid conformation necessary for establishing the vitamin–previtamin equilibrium.

TABLE III.
Selected Torsion Angles (in Degrees) and Relative Energies (E_{rel}, in Kilocalories per Mole) for Conformations of Model II Optimized at HF/6-31G* and AM1 Levels of Theory.[a]

Conformer	C1—C2—C3—C4	C5—C6—C7—C8	C6—C5—C10—C19	C11—C9—C8—C14	E_{rel}
NAa	−54.98	−174.43	59.64	−51.78	0.00
	(−53.13)	(−160.83)	(45.46)	(−47.74)	(0.48)
NEa	55.06	173.50	−57.44	−51.87	0.30
	(57.29)	(160.36)	(−45.24)	(−48.16)	(0.00)
NT4a	65.80	−174.18	60.19	−51.75	5.69
	(64.89)	(−157.94)	(44.24)	(−48.03)	(1.62)
NT6a	−29.34	167.32	−40.67	−51.82	5.90
	(−31.32)	(152.97)	(−34.27)	(−48.01)	(3.69)
PAa	−54.94	−175.35	59.87	13.30	4.19
	(−52.82)	(−168.39)	(47.64)	(12.31)	(0.79)
PEa	55.11	173.98	−57.20	13.61	4.43
	(57.06)	(166.68)	(−46.68)	(12.48)	(0.26)
PT4a	65.90	−175.09	60.30	13.39	9.91
	(64.60)	(−167.03)	(47.28)	(12.35)	(1.96)
PT6a	−29.33	168.41	−40.66	13.66	10.02
	(−35.75)	(160.91)	(−38.04)	(12.53)	(2.17)

[a] Values in parentheses correspond to AM1 optimizations.

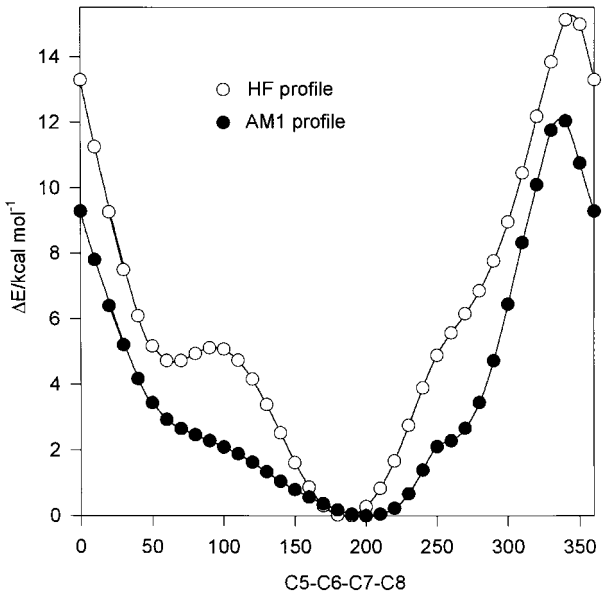

FIGURE 7. Energy profiles computed for the torsion around the C6 — C7 bond of model **II**.

Clearly, our results contrast with those obtained by Hofer and Wilson, and are in closer agreement with the experimental observations.

To know whether the side chain could affect the torsional barrier around the C6—C7 bond we carried out, using the AM1 method, a similar reaction coordinate run on the complete vitamin D_3 structure.[24] The curve obtained for vitamin D_3 was very close to that of model **II**. Clearly, the results support our initial hypothesis that the side chain should not have an important influence on the main fragment of the molecule.

Because of an exposition of the geometrical features of all conformers optimized in this work is out of place, we will only present the results achieved for the NEa conformation, for which room temperature x-ray diffraction data [13,14] are available from the literature. Our intention, however, is not to make a direct comparison between the experimental and theoretical results: the latter concern a gas phase structure, whereas the x-ray data

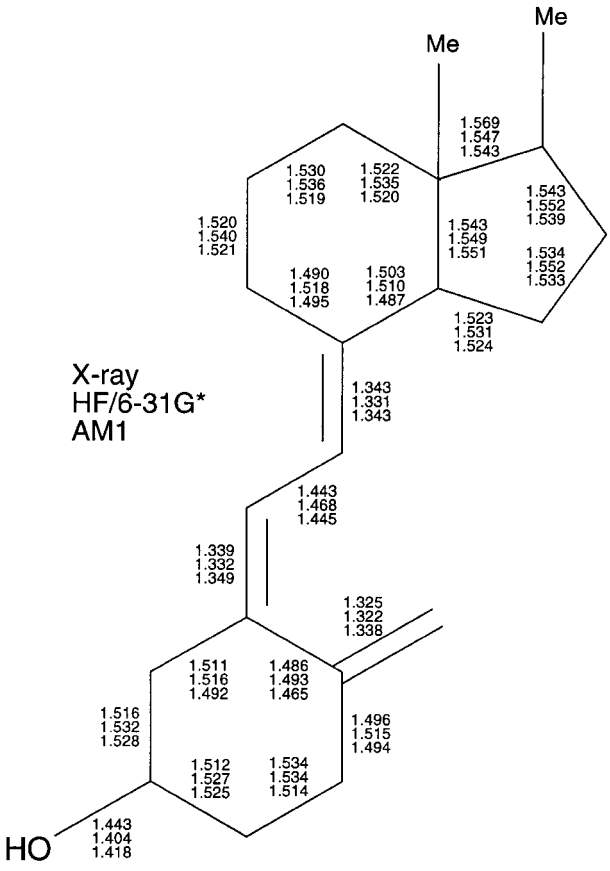

FIGURE 8. Comparison of x-ray bond lengths (angstroms) in 25-hydroxyvitamin D_3 monohydrate[13] with our HF / 6-31G* and Am1 results fro model **II**.

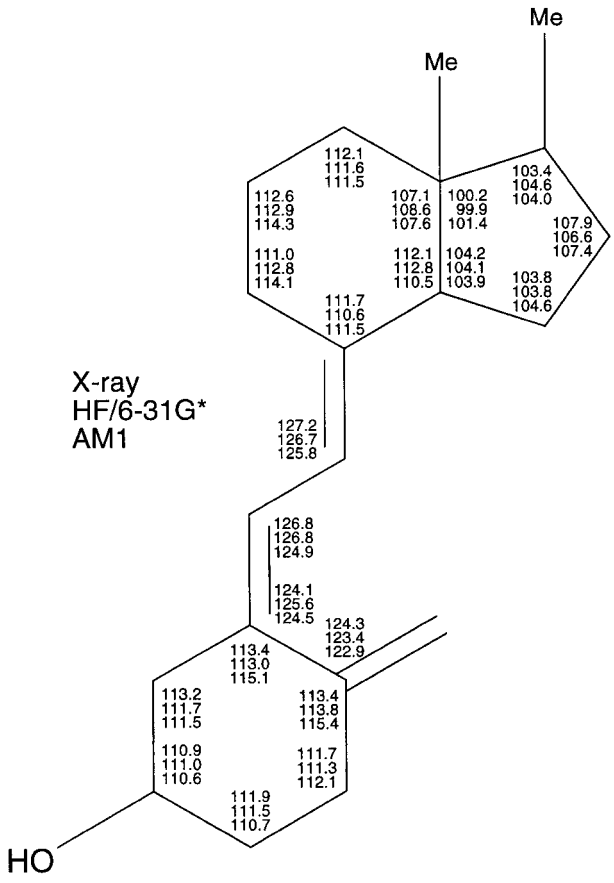

FIGURE 9. Comparison of x-ray bond angles (degrees) in 25-hydroxyvitamin D_3 monohydrate[13] with our HF / 6-31G* and AM1 results for model **II**.

refer to a crystal structure. The bond lengths and the bond angles for the skeleton of the above conformation are shown in Figures 8 and 9. The dihedral angles are given in Table IV. The differences between the experimental and the theoretical bond lengths are, in most cases, less than 0.015 Å. The lengths computed for the C3—O bond (1.404 Å for HF/6-31G* and 1.418 for AM1) greatly disagree with the x-ray value (1.443 Å). The experimental figure may be quite uncertain because x-ray diffraction spectroscopy maps the electron density; therefore, an apparent distortion may result from lone pairs present on the oxygen. For the bond angles most of the deviations between the experimental and the theoretical values are within 2°.

The dihedral angles shown in Table IV ensure that we are comparing corresponding conformations. In general, the HF/6-31G* figures compare quite well with the x-ray data. The agreement between the AM1 results and the experimental figures is not so good. The largest deviation is found for the diene torsional angle C5—C6—C7—C8, for which the AM1 value differs from the others by at least 10°. Another important AM1 discrepancy, in the order of 10°, is found for the dihedral angle C6—C5—C10—C19. Finally, there are also some significant deviations between the experimental data and our theoretical results that may have arisen from reasons mentioned before.

Conclusions

Ab initio and semiempirical theories were applied on two model compounds to get insight into the conformational features of vitamin D associated mainly to ring A and diene system B. Model compound **I**, chosen to study the A-ring properties in detail, was examined by the 6-31G* basis set at both the HF and MP2 levels of theory. The results show two chair forms as the most stable conformations, one with the hydroxyl group in axial and the other with the group in equatorial orientation. The six possible twist forms were localized and their relative energies are significantly higher than those obtained previously by molecular mechanics calculations.[7, 10]

The total Boltzmann population for the twist family of conformers, evaluated at 25°C with the *ab initio* energies, is insignificant at both levels of theory applied (HF and MP2). This result agrees

TABLE IV.
Comparison Between Calculated Torsion Angles (in Degrees) for the NEa Conformer and Experimental Data.

Torsion angle	x-ray[a]	x-ray[b]	HF / 6-31G*	AM1
C3 — C2 — C1 — C10	−53.8	−54.0	−53.3	−53.4
C4 — C3 — C2 — C1	57.0	53.8	55.1	57.3
C5 — C4 — C3 — C2	−58.2	−51.8	−54.1	−54.2
C10 — C5 — C4 — C3	53.1	50.0	52.2	48.3
C1 — C10 — C5 — C4	−49.3	−49.8	−51.3	−45.5
C2 — C1 — C10 — C5	51.6	51.1	51.4	47.6
C6 — C5 — C10 — C19	−53.6	−56.7	−57.4	−45.2
C7 — C6 — C5 — C10	1.4	2.6	−1.4	−2.0
C8 — C7 — C6 — C5	170.3	−177.0	173.5	160.4
C6 — C7 — C8 — C9	3.6	1.8	−3.0	−4.5
C11 — C9 — C8 — C14	−50.8	−54.1	−51.9	−48.2
C12 — C11 — C9 — C8	49.6	51.9	50.2	44.7
C13 — C12 — C11 — C9	−54.7	−54.5	−52.4	−50.4
C14 — C13 — C12 — C11	57.3	55.9	55.3	58.4
C8 — C14 — C13 — C12	−61.7	−58.6	−59.0	−63.7
C9 — C8 — C14 — C13	58.6	59.1	57.4	58.3
C17 — C13 — C14 — C15	49.9	45.6	46.3	42.9
C13 — C14 — C15 — C16	−40.4	−35.4	−33.9	−33.4
C14 — C15 — C16 — C17	14.2	11.0	8.2	10.9
C15 — C16 — C17 — C13	16.3	17.0	20.4	15.9
C16 — C17 — C13 — C14	−39.1	−37.6	−40.2	−35.6

[a] Vitamin D_3.[13]
[b] 25-Hydroxyvitamin D_3.[14]

with the fact that no twist forms have been observed in most experimental investigations. The twist population reported by Hofer et al. [10] (up to ~20%) in their combined study of molecular mechanics calculations, LIS measurements, and LIS simulation might be overestimated.

Model compound **II** was mainly selected to analyze the conformational properties of diene system B. The *ab initio* computations carried out on this compound were done at the HF/6-31G* level. Semiempirical calculations were also undertaken with the AM1 Hamiltonian. Both methods predict that vitamin D adopts an extended conformation, although the *ab initio* results achieved on the NAa conformation show a local minimum (at C5—C6—C7—C8 = 65°) corresponding to a folded arrangement. Its high relative energy, about 5 kcal/mol, and the very low cis-to-trans barrier agree with the experimental fact that no folded conformer was observed. Thus, our results corroborate the experimental findings and contrast with molecular mechanics[10] or simulated annealing outcomes.[19] Apart from that, both methods predict two stable forms for the fused CD ring (i.e., N and P) in this study. When we compared the results of both theoretical methods, we found a significant discrepancy in the conformational stabilities yielded by each treatment for the last forms as well as for the chair and twist conformations of ring A.

Finally, the geometrical features calculated on the NEa conformer of model **II** at both HF/6-31G* and AM1 levels of theory are compared with available x-ray data. In general, the HF/6-31G* results are more similar to the x-ray data than those computed with the AM1 method.

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