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On the Conformational Flexibility of Vitamin D

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Abstract. The conformational flexibility of vitamin D was investigated by a combination of force field calculations, LIS measurements and LIS simulations. Besides the two A-ring chair forms, A-ring twist forms were detected and were attributed to steric interactions arising from the substitution pattern of this class of seco-steroids.

Keywords. Vitamin D; Force-field calculations; LIS-simulation; NOE-measurements.

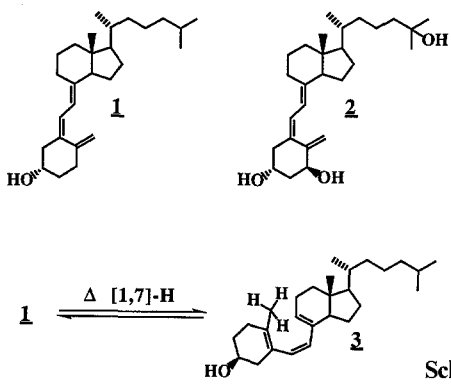
Über die konformationelle Beweglichkeit von Vitamin D

Die konformationelle Beweglichkeit von Vitamin D wurde mittels einer Kombination aus Kraftfeld Rechnungen, LIS-Messungen und LIS-Rechnungen untersucht. Neben den beiden A-Ring Sesselformen wurden auch A-Ring Twistformen aufgefunden. Die Ursache für das Vorhandensein von diesen Twistformen liegt in sterischen Wechselwirkungen, verursacht durch das Substitutionsmuster des A-Ringes in diesem seco-Steroid.

Introduction

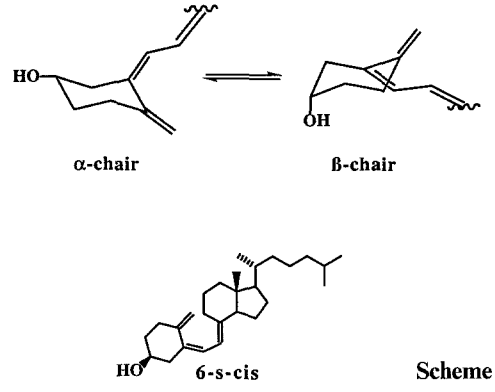
It is now well established that vitamin D (**1**) is not the physiological active form of this steroid hormone and has to be further metabolised to 1α,25-dihydroxyvitamin D (**2**) to exhibit its regulatory effects upon the calcium homeostasis [1]. Besides of the biological importance of this steroid hormone, this class of molecules possesses certain unique structural features. First of all it is a highly flexible molecule, able to adopt a wide range of geometric structures, due to the open B-ring and second having a conjugated triene system in which all double bonds are exocyclic and still being the thermodynamic more favourable structure in comparison to previtamin D (**3**), its [1,7-H] shifted isomer (Scheme 1). Therefore the conformational behaviour of vitamin D has attracted considerable attention over the past 20 years. The pioneering work by Okamura [2] and La Mar [3] established the existence of a dynamic equilibrium of two A-ring chair conformations (α and β; Scheme 2) using qualitative ^{1}H-NMR-LIS measurements. These findings were subsequently supported by analysing the ^{1}H- and ^{13}C-spectra of various vitamin D derivatives and analogs [4] as well as by force field calculations [5].

In addition a conformational study of flexible trans dienes by polarisation spectroscopy by Mazur [6] suggested that vitamin D oscillates around the 6–7 single bond adopting also cisoid conformations. Rotation around this bond creates



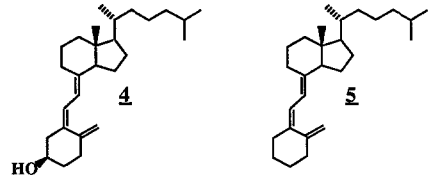
Scheme 1

conformations with a more "steroid like" shape of this molecule (Scheme 2) besides the fact that this conformations are important for the thermal interconversion: previtamin D–vitamin D. A most recent computer simulation [7] by a simulated annealing process found a 6–7 s-cis conformation as the global minimum of 1α,25-dihydroxyvitamin D.

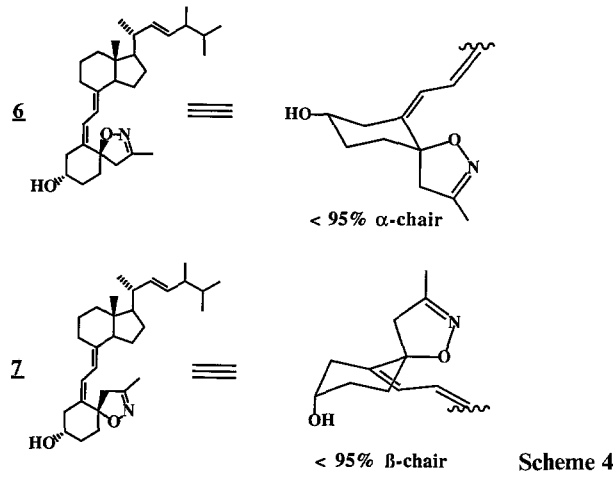


Scheme 2

The purpose of our reinvestigation of the conformational flexibility of vitamin D was to find experimental proof or disproof of the existence of such conformations (as well as other potential A-ring conformations) in solution. The method used was a combination of force-field calculations, quantitative lanthanide induced chemical NMR-shift measurements (LIS) and simulation of such shifts on the basis of the calculated geometries [8]. Due to the method used (LIS) only molecules with one hydroxyl group could be investigated. To get a clearer picture on the influence of the C-3 hydroxyl group upon the conformational behaviour of vitamin D (**1**), 3-epi-vitamin D (**4**) was included in this study as well as 3-desoxy-vitamin D (**5**), the latter one only in the computational study (Scheme 3). For comparison the A-ring rigid isoxazolin-adducts **6** and **7** of vitamin D [9] were taken into account as well. Compounds **6** and **7** are vitamin D analogs having predominately fixed A-ring chair conformations (>95%) and do not interconvert (Scheme 4).



Scheme 3



Scheme 4

Results and Discussion

Force-Field Calculations

For the calculation of individual geometries of vitamin D conformers and their relative steric energies Allinger's MMP2 force field [10] was chosen. The side-chain of vitamin D (**1**) and of its analogs were substituted by a methyl group for simplicity. Initial conformations with regard to the shape of ring A and the diene bridge were fed into the programme and minimised. In all the cases investigated eight local minima within the range of 1.8 kcal could be found. Distorting these geometries or changing other geometric features never led to new conformers. In the cases of vitamin D (**1**) and 3-epi vitamin D (**4**) each of this conformations could be split further into a family of three due to the different gauche orientations of H-C-3-O-H, but this conformers were considered of no importance for this study since no change in other geometric features were noted. The most striking feature in the outcome of our calculations is the apparent stability of A-ring twist conformations in solution.

Vitamin D

The two lowest energy conformations found are the two ring A chair conformations Ceq(+s-tr)* and Cax(−s-tr), but the ring A twist from Teq(−s-tr) lies only ~0.35 kcal above the global minimum. The lowest s-cis conformation is Ceq(−s-cis) wherein the exomethylene group lies above the plane of the C- and D-ring. As mentioned above, each conformation is split into a family of three by the different orientation of the 3-OH. We attribute this fact to the nature of the parameterisation of the force field used. In the cases of conformations having an equatorial OH, gauche orientation where the oxygen H points toward C-2 is always the minimum within the family. In the conformations with axial OH the gauche orientation in which the hydrogen points towards C-4 is favoured. The s-trans orientations of

* C denotes A ring chair-, T=A ring twist conformation; s-cis, s-trans refers to the conformation of the C-6–C-7 single bond; +/− indicates the sign of this torsion angle.

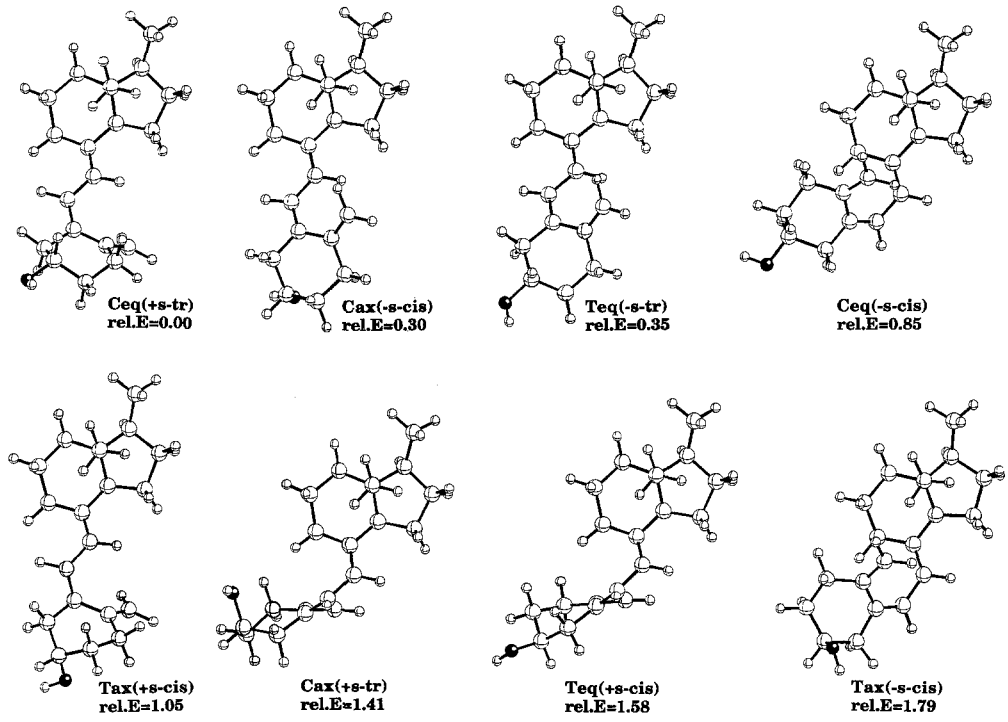
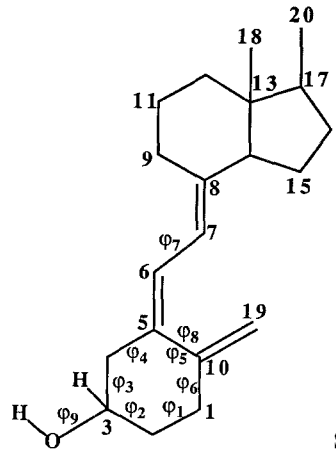


Fig. 1. Ball and Stick representations of the computed conformations of vitamin D (**1**)

Table 1. Relative steric engergies and Boltzmann distribution of calculated vitamin D (**1**) conformations at 298 K

Conformation	ΔE (kcal)	%
Ceq (+s-tr)	0.00	35.87
Cax (-s-tr)	0.30	21.78
Teq (-s-tr)	0.35	19.90
Ceq (-s-cis)	0.85	8.60
Tax (+s-tr)	1.05	6.14
Cax (+s-cis)	1.41	3.38
Teq (+s-cis)	1.58	2.55
Tax (-s-cis)	1.79	1.77
Sum of all conformations with 3-OHeq = 66.92 %		

H-2-C-3-O-H is always ~0.7 to 0.9 kcal higher in energy. Ball and Stick pictures and the relative steric energies of the eight local minima are shown in Fig. 1. A Boltzmann distribution of the population of these conformers at 298 K is given in Table 1 (note that the sum of all conformers with 3-OH equatorial is 66.92%). Selected torsion angles and intramolecular distances as well as the pseudorotations parameter of the D-ring as a criteria for the distortion of the CD-ring, are given in Table 2 and 3 (for the definition of given torsion angles see Scheme 5; bold face numbers in the tables indicate distances, which may give rise to NOE-effects).



Scheme 5

Table 2. Selected torsion angles [± 180°] of calculated vitamin D (**1**) conformations

Conf.	φ_7	φ_8	φ_9	φ_1	φ_2	φ_3	φ_4	φ_5	φ_6	Δ^a
Ceq (+s-tr)	175.93	-45.07	-53.18	-52.49	60.81	-55.34	44.33	-39.04	42.20	-3.61
Cax (-s-cis)	-177.39	46.86	52.50	51.49	-58.38	53.58	-44.8	40.70	-42.92	-4.12
Teq (-s-tr)	-172.66	41.20	-51.58	-42.45	69.42	-43.85	-5.78	33.19	-7.32	-4.20
Ceq (-s-cis)	-60.07	-37.88	-53.45	-52.33	61.83	-54.95	41.22	-34.48	39.26	-7.72
Tax (+s-tr)	169.71	-40.28	49.57	41.68	-66.97	41.64	6.15	-32.23	6.86	-4.50
Cax (+s-cis)	53.64	35.25	52.47	50.18	-60.13	54.32	-41.08	33.11	-36.86	-4.91
Teq (+s-cis)	53.65	34.28	-41.40	-46.33	68.93	41.43	-6.11	28.91	-1.11	-4.76
Tax (-s-cis)	-58.19	-35.60	50.72	42.46	-66.94	41.94	4.78	-29.96	4.96	-7.78

[a] Pseudorotation parameters of ring D; calculated according to Ref. [11]

Table 3. Selected distances [Å] in calculated vitamin D (**1**) conformations

Conf.	$C_{18}H_{19Z}$	$C_{18}H_{19E}$	$C_{18}H_7$	$C_{18}H_6$	$C_{18}H_{9\beta}$	$H_{9\beta}H_{1\alpha}$	$H_{9\beta}H_{1\beta}$	$H_{9\alpha}H_{1\alpha}$	$H_{9\alpha}H_{1\beta}$
Ceq (+s-tr)	6.147	6.987	**3.786**	4.955	4.397	6.538	7.361	7.488	8.123
Cax (-s-tr)	**4.355**	5.871	**3.875**	5.238	4.358	7.354	6.448	8.055	6.960
Teq (-s-tr)	**4.083**	5.667	**3.828**	5.221	4.365	7.277	6.956	8.246	7.573
Ceq (-s-cis)	**3.585**	**3.599**	**4.118**	6.006	4.413	**2.629**	**4.086**	**4.264**	5.722
Tax (-s-tr)	6.123	7.169	**3.879**	5.005	4.387	6.978	7.259	7.736	7.964
Cax (+s-cis)	5.816	6.862	**3.807**	5.037	4.339	**4.325**	**3.095**	**3.881**	**2.817**
Teq (+s-cis)	5.860	6.870	**3.816**	5.044	4.328	**4.095**	**2.655**	**4.338**	**2.877**
Tax (-s-cis)	**3.468**	**3.671**	**4.112**	6.018	4.419	**2.998**	**4.280**	**4.774**	5.915

3-Epi-vitamin D

In 3-epi-vitamin D (**4**) also eight conformations are found. The global minimum is the A-ring chair conformation with an equatorial OH. The chair form with the axial OH orientation lies ~0.6 kcal above this minimum. But the lowest twist conformation lies only ~0.7 kcal above the global minimum. The lowest 6 s-cis form Cax(−s-cis) is with 1.2 kcal somewhat higher in energy as the corresponding s-cis conformation in vitamin D. Again each of the conformations were split into a

Table 4. Relative steric energies and Boltzmann distribution of calculated 3-epi-vitamin D (**4**) conformations at 298 K

Conformation	ΔE (kcal)	%
Ceq (- s-tr)	0.00	45.74
Cax (+ s-tr)	0.59	16.92
Teq (+ s-tr)	0.71	13.79
Tax (- s-tr)	1.01	8.34
Cax (- s-cis)	1.22	5.94
Ceq (+ s-cis)	1.40	4.36
Teq (- s-cis)	1.46	3.96
Tax (+ s-cis)	2.31	0.95
Sum of all conformations with 3-OHeq = 67.85 %		

Table 5. Selected torsion angles [$\pm$ 180°] of calculated 3-epi-vitamin D (**4**) conformations

Conf.	φ_7	φ_8	φ_9	φ_1	φ_2	φ_3	φ_4	φ_5	φ_6	Δ^a
Ceq (- s-tr)	-174.63	44.47	54.25	52.77	-60.76	54.94	-43.85	38.90	-42.45	-4.2
Cax (+ s-tr)	177.86	-47.43	-52.42	-51.27	58.48	-53.86	45.14	-40.81	42.70	-3.2
Teq (+ s-tr)	171.56	-41.55	51.49	42.29	-69.44	43.98	5.69	-33.27	7.52	-4.1
Tax (- s-tr)	-176.14	43.76	-49.43	-42.42	67.04	-39.72	-9.13	34.51	-7.12	-4.7
Cax (- s-cis)	-58.62	-37.74	-51.83	-51.56	59.63	-52.90	40.75	-35.08	39.64	-7.5
Ceq (+s-cis)	54.72	33.28	53.28	53.37	-62.00	53.55	-38.47	31.95	-38.90	-4.4
Teq (- s-cis)	-60.39	-37.71	51.79	45.61	-69.06	41.22	7.11	-30.74	2.66	-8.0
Tax (+ s-cis)	54.27	31.71	-49.78	-43.26	66.81	-42.51	-3.00	27.24	-2.91	-4.5

[a] Pseudorotation parameters of ring D; calculated according to Ref. [11]

Table 6. Selected distances [Å] in calculated 3-epi-vitamin D (**4**) conformations

Conf.	$C_{18}H_{19Z}$	$C_{18}H_{19E}$	$C_{18}H_7$	$C_{18}H_6$	$C_{18}H_{9\beta}$	$H_{9\beta}H_{1\alpha}$	$H_{9\beta}H_{1\beta}$	$H_{9\alpha}H_{1\alpha}$	$H_{9\alpha}H_{1\beta}$
Ceq (- s-tr)	**4.216**	5.715	**3.864**	5.277	4.360	7.352	6.536	5.081	**4.250**
Cax (+ s-tr)	6.154	6.909	**3.758**	4.949	4.396	6.475	7.349	**4.238**	5.174
Teq (+ s-tr)	6.119	7.115	**3.865**	5.043	4.387	6.958	7.287	4.519	4.469
Tax (- s-tr)	**4.395**	5.844	**3.889**	5.272	4.353	7.296	6.914	4.543	4.469
Cax (- s-cis)	**3.540**	**3.634**	**4.103**	6.006	4.425	**2.588**	**4.020**	**3.886**	4.572
Ceq (+ s-cis)	5.789	6.877	**3.791**	5.041	4.346	4.177	**2.781**	4.614	**3.860**
Teq (- s-cis)	**3.581**	**3.579**	**4.132**	6.010	4.405	**3.083**	**4.324**	**3.315**	4.446
Tax (+ s-cis)	5.750	6.888	**3.800**	5.041	4.344	4.423	**3.186**	4.457	**3.474**

family of three. In contrast to the computed vitamin D conformations, the oxygen hydrogen is pointing now towards C-4 in all of the lowest energy forms with equatorial OH gauche orientations and pointing towards C-2 in all of the conformations with axial OH. The s-trans orientation of the OH is still being the highest energy conformation in all the families. The relative energies of the conformations and the Boltzmann distribution of the populations of these conformers at 298 K is given in Table 4. Selected torsion angles and intramolecular distances are given in Table 5 and Table 6.

Table 7. Relative steric energies and Boltzmann distribution of calculated 3-desoxy-vitamin D (**5**) conformations at 298 K

Conformation	ΔE (kcal)	%
Cax (- s-tr)	0.00	32.42
Ceq (+ s-tr)	0.14	25.87
Teq (- s-tr)	0.47	14.80
Tax (+ s-tr)	0.58	12.31
Ceq (- s-cis)	0.99	6.13
Cax (+s-cis)	1.31	3.60
Tax (- s-cis)	1.38	3.22
Teq (+ s-cis)	1.77	1.66
Sum of all conformations with 3ß-Heq = 48.46 %		

Table 8. Selected torsion angles [± 180°] of calculated 3-desoxy-vitamin D (**5**) conformations

Conf.	φ_7	φ_8	φ_1	φ_2	φ_3	φ_4	φ_5	φ_6	Δ[a]
Cax (- s-tr)	-176.72	45.97	53.15	-60.09	54.21	-44.04	40.10	-43.74	-4.4
Ceq (+ s-tr)	177.67	-46.64	-52.65	60.05	-54.71	44.71	-40.33	43.33	-3.4
Teq (- s-tr)	-175.70	42.77	-42.58	69.24	-43.26	-6.45	33.60	-7.37	-4.7
Tax (+ s-tr)	173.72	-41.29	43.46	-69.12	42.87	6.21	-32.30	5.81	-3.5
Ceq (- s-cis)	-59.35	-37.21	-52.41	61.46	-54.42	40.82	-34.31	39.37	-7.6
Cax (+ s-cis)	53.24	34.27	53.00	-61.54	53.82	-39.73	33.42	-39.46	-5.1
Tax (- s-cis)	-58.78	-35.47	44.26	-68.99	43.32	4.36	-29.42	3.57	-7.5
Teq (+ s-cis)	53.44	33.10	-43.33	68.85	-44.61	-2.83	28.79	-4.25	-4.7

[a] Pseudorotation parameters of ring D; calculated according to Ref. [11]

Table 9. Selected torsion angle data from published x-ray structures and force field calculations on vitamin D derivatives

Entry [Ref.]	φ_7	φ_8	φ_1	φ_2	φ_3	φ_4	φ_5	φ_6	Δ
$VitD_3$[12] α	170.3	-53.6	-53.8	57.0	-58.2	53.1	-49.3	51.6	-4.3
ß	-174.3	55.2	51.9	-51.2	48.0	-48.3	50.0	-51.2	-6.6
25OH [13]	177.0	-56.7	-54.0	53.8	-51.8	50.0	-49.8	51.1	11.9
$VitD_2$[14] α	175	-49	-59	58	-52	43	-43	51	-26.5
ß	-164	46	54	-58	54	-49	46	-48	-0.7
ECF [15]	-168.9	56.3	55.1	-53.3	50.8	-49.1	51.6	-55.0	-19.6
INC [16]	167	-61	-57	57	-52	49	-49	54	-6.5
MMP1[17] α	163	-46.6			-54.2			44.7	
ß	-165	45.8			51.4			-47.2	
MM [5] α	176	-55	-55	60	-56	51	-50	51	
ß	-173	53	52	-56	53	-50	50	49	
STO-3G [18]		-43.7	-54.3	58.6	-54.3	47.0	-43.9	47.0	
STO 3-21 [18]		-53.1	-55.7	58.4	-55.7	51.9	-50.8	51.9	

3-Desoxy-vitamin D

Again eight conformations were found in 3-desoxy-vitamin D (**5**). Due to the lack of the A-ring hydroxyl the steric energy differences between the corresponding two chair conformations are small ($\sim$0.05 kcal for the s-trans conformations and 0.4 kcal for the s-cis conformations). The conformational behaviour of 3-desoxy vitamin D follows more closely the pattern of vitamin D (**1**) than 3-epi vitamin D (**4**). The relative steric energies of these conformers and their population at 298 K are given in Table 7 and selected torsional data are given in Table 8.

For the purpose of comparison selected data of published x-ray work and calculation work on vitamin D are given in Table 9. Some geometric features of the calculated conformers should be emphasised: (i) the deviation of the diene bridge from planarity in the s-trans conformations is ca. $\pm 10°$; (ii) the torsion angle 6-5-10-19 is somewhat smaller than in the x-ray structures, probably due to the parameterisation of the force field tending to bring the triene moiety into planarity; (iii) the differences in torsion angles of ring A twist conformers compared to the chair forms are significant only for φ_4 and φ_6. This has the consequence that this twist forms do not have any significant influence upon the H-3 coupling pattern in the ^{1}H-NMR spectra of **1** and **4**. It should be pointed out that twist conformations were found in a recent dynamic study of 1,2-dimethylencyclohexane [18]. The geometric features (torsion angles) of these twist forms found by STO-3G and STO-3-21G computations are very similar to our twist geometries.

From the data found in the literature and in comparison with our calculated structures we conclude that our geometries are sufficiently accurate for the LIS simulations.

LIS-Simulation

For the LIS-simulations a special programme was designed allowing to mix 2–4 components with systematically varying populations [8]. For four conformers a four-fold set of coordinates had to be entered in the input, all four components were then orientated in the same way in a Cartesian coordinate system (e.g. coordinating oxygen in the origin, the next C along the negative z-axis, etc., compare Refs. [8] and [19]). Now the lanthanide ion position could be optimised and the population ratios (via introduction of weights for the particular contributions to the final averaged LIS values) could also be optimised. The lanthanide ion position was assumed to be very similar for all conformers of a substrate. This is an approximation, in our case however, it was justified by model calculations using axial and equatorial 4-tert.butylcyclohexanols which *both* gave identical lanthanide ion positions with polar coordinates $d = 2.9$ Å, $\rho = 60°$, $\phi = 0°$ (Ref. [19], ϕ with reference to the symmetry plane through C1 and C4). The corresponding data obtained in the best fits for the three-component mix of vitamin D were $d = 2.9 \pm 0$ Å, $\rho = 60°$, $\phi = 6 \pm 2°$. Therefore the average lanthanide ion positions in the conformational mixtures of complexes seem to be simulated satisfactorily.

The further procedure is illustrated in Fig. 2 for vitamin D (**1**) in $CDCl_3$ at 23 °C: In the first step the optimal mixture for the expected conformers Cax(−s-tr) + Ceq(+ s-tr) was calculated in a two component mixture; the result was a 72:28% mixture with $R = 6.7\%$ (which is the average deviation between experi-

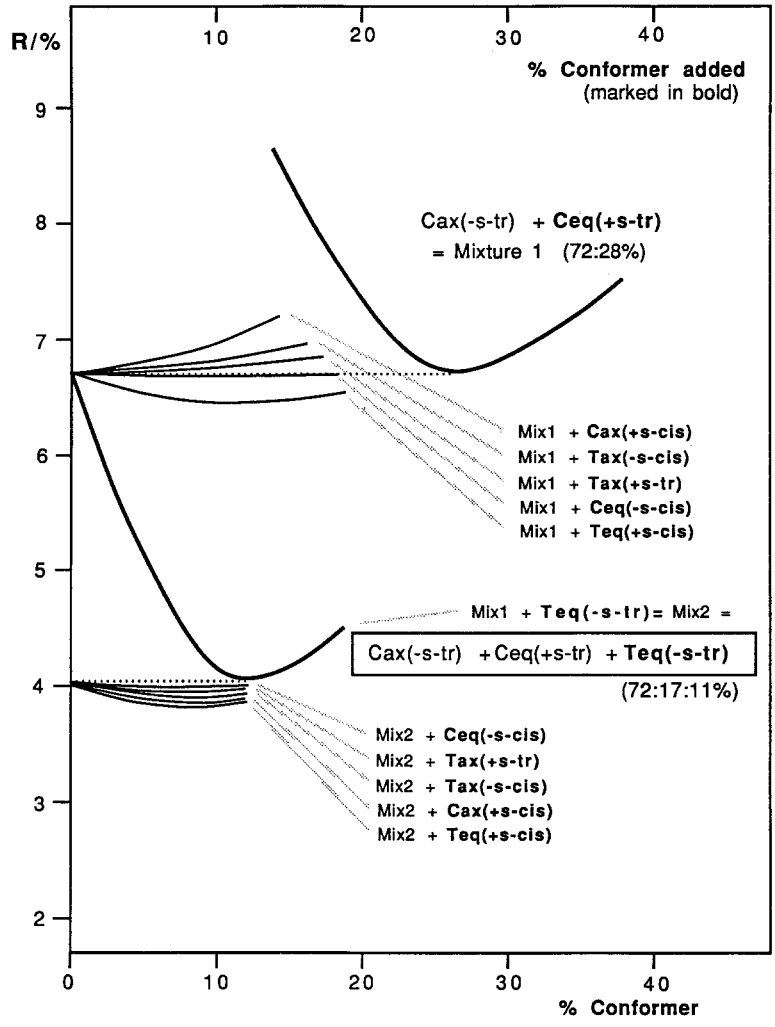


Fig. 2. Dependence of the *R*-factor from the components used in the LIS-simulation of vitamin D [Eu(*fod*)$_3$, $CDCl_3$, 23 °C]. Note that Mixture 1 is a two-component mixture and Mix 2 a three-component mixture; the % Conformer scale is valid for the addition of one further component; the *R*-factor is taken as a measure for the quality of the fit

mental and calculated LIS values, for the use of the *R*-factor compare Ref. [19]). Taking into account the preferred complexing of axial cyclohexanols compared to analogue equatorial compounds (compare competition experiments of vitamin D isoxazoline derivatives; Table 10), one obtains a corrected ratio of 42:58%. The latter values are very close to the commonly assumed 1:1 conformer mixture. In the next step we checked if addition of a further conformer to the two-component mixture (Mix1 in Fig. 2) would improve the fit. Only one of the six possible conformers improved the *R*-factor significantly: the addition of Teq(−s-tr) lowered the *R*-factor to a value of 4.03%. The final distribution Cax(+s-tr):Ceq(−s-tr):Teq(−s-tr) = 72:17:11 (corrected 58:25:17) shows that the twist form with equatorial OH takes part in the equilibrium. All other tested components did either

Table 10. LIS Competition Experiments. Equimolar amounts of OH-eq and OH-ax vitamin D isoxazoline derivatives **6** and **7** were dissolved in $CDCl_3$ or toluene-d_8 and the LIS data were determined at different temperatures. Based on the relative LIS shifts of the isomers in the mixture and taking into account the LIS of the pure compounds the listed ratios of the complex formation constants were obtained. For direct comparison of the complexing abilities of the two compounds the lanthanide induced shifts of the proton of C-3 (geminal to the coordinating OH) is best suited, because these shifts should be equal in both 1:1 complexes (identical relative position to OH); however, all other shifts can be scaled to this proton and used as well

	$CDCl_3$		Toluene		
	23°C	55°C	23°C	55°C	80°C
Complex ratio axial : equatorial	1.9 : 1	1.5 : 1	3.8 : 1	2.9 : 1	2.4 : 1

Table 11. Experimental and calculated LIS data for Vitamin D (**1**) in dependence of solvent and temperature. Eu(*fod*)$_3$; normalized values in ppm (extrapolated to the 1:1 complex and scaled to the values $CDCl_3$/23 °C)

Protons	LIS in $CDCl_3$				LIS in Toluene					
	23°C		55°C		23°C		55°C		80°C	
	exp.	calc.	exp.	calc.	exp.	calc.	exp.	calc.	exp.	calc.
1α	6.20	6.37	6.19	6.26	6.59	6.52	6.51	6.37	6.48	6.32
1β	10.37	10.76	9.98	10.34	10.95	11.21	10.43	10.77	10.01	10.59
2β	14.10	13.45	14.33	13.42	14.26	13.61	14.36	13.46	14.47	13.43
2α	9.97	10.11	10.13	10.33	9.85	10.04	9.94	10.11	10.06	10.20
O-H	20.50	—	20.71	—	23.87	—	23.57	—	22.98	—
4β	15.49	15.12	15.29	15.11	15.38	15.16	15.22	15.13	15.14	14.99
4α	9.42	9.93	9.51	10.17	9.24	9.82	9.24	9.93	9.33	10.01
=C6-H	5.17	5.37	5.02	5.21	5.37	5.45	5.22	5.37	5.04	5.20
=C7-H	3.26	3.73	3.16	3.58	3.34	3.86	3.22	3.73	3.07	3.65
C19-H_E	3.54	3.48	3.50	3.42	3.63	3.56	3.56	3.48	3.49	3.46
C19-H_Z	3.43	3.47	3.41	3.40	3.55	3.58	3.46	3.48	3.39	3.45
9β	1.11	0.84	1.07	0.79	1.24	0.88	1.20	0.84	1.14	0.80
C18-Me	0.71	0.79	0.74	0.69	0.77	0.85	0.78	0.79	0.76	0.73
R factor (%)		4.03		4.64		4.30		4.74		5.40
% Ceq(s-tr)		17		20		14		16		18
% Cax(s-tr)		72		68		76		72		70
% Teq(s-tr)		11		12		10		12		12
Ratio (C+T)eq:ax		28:72		32:68		24:76		28:72		30:70
corr. (s.Tab.10)		42:58		41:59		55:45		53:47		51:49

increase the average error in the calculation or gave only nonsignificant improvements (Fig. 2, series of curves originating from $R = 6.7\%$). In a further step a fourth component was added in the simulation (Fig. 2, series of curves originating from the best three-component fit at $R = 4.03\%$); however, no significant improvement was observed for any one of the conformers tested. The three-component mixture (Mix 2 in Fig. 2) remained the best fit.

Table 12. Experimental and calculated LIS data for epi-vitamin D (**4**) in dependence of temperature. Eu(*fod*)$_3$; toluene; normalized values in ppm (extrapolated to the 1:1 complex and scaled to the values of vitamin D in $CDCl_3$/23 °C for better comparison with Table 11)

Protons	23°C		55°C		80°C	
	exp.	calc.	exp.	calc.	exp.	calc.
1α	6.36	6.24	6.28	6.07	6.20	5.90
1β	10.48	10.82	9.72	10.20	9.21	9.76
2β	14.77	14.09	14.78	14.02	14.75	13.92
2α	10.61	11.08	10.73	11.33	10.84	11.45
O-H	22.52	—	23.49	—	23.66	—
4β	14.85	14.76	14.82	14.39	14.85	14.38
4α	9.99	10.45	10.08	10.61	10.12	10.76
C6-H	4.94	4.78	4.70	4.51	4.55	4.46
C7-H	3.20	3.71	3.11	3.49	2.94	3.38
C19-H_a	3.55	3.52	3.42	3.39	3.36	3.28
C19-H_b	3.41	3.46	3.17	3.33	3.27	3.24
2β	1.27	1.21	1.20	1.09	1.14	1.13
C18-Me	0.57	0.76	0.54	0.74	0.47	0.78
R factor (%)		4.30		4.80		5.34
% Ceq(s-tr)		20		24		26
% Cax(s-tr)		64		58		54
% Teq(s-tr)		16		18		20
Ratio (C+T) eq:ax		36:64		42:58		46:54
corr. (s.Tab.10)		68:32		68:32		67:33

In principle, the results for all other temperatures and solvents were similar. However, following trends should be emphasised: (i) higher temperatures shift the complex equilibrium towards the equatorial conformers and (ii) in toluene the complexed conformers with axial OH are favoured more than in $CDCl_3$. The latter may be derived from the results listed in Table 11 and the independent LIS-competition experiments with suitable model compounds, listed in Table 10.

The LIS results for the 3-epi-compound (**4**) (Table 12) agree well with the conclusions drawn from the corresponding data for vitamin D (**1**) (Table 11 and Fig. 2). At 23 °C in toluene a two-component mixture Cax(+s-tr):Ceq(−s-tr) = 64:36% gave a *R*-factor minimum of 4.80%. Adding Teq(+ s-tr) lowered the *R*-factor to 4.30% giving a final population distribution Cax(+s-tr):Ceq(−s-tr):Teq(+s-tr) = 64:20:16. A correction of these ratios based on the results of the competitions experiments of the isoxazoline derivatives **6** and **7** gave a shift in the distribution in favour of the equatorial conformers Ceq(−s-tr) and Teq(+s-tr) (see Table 10) indicating that the equatorial forms are favoured in the substrate equilibrium and/or in the complexing ability (at least in comparison with vitamin D and the rigid test compounds **6** and **7**).

NOE-Experiments

With the geometries of the calculated conformations in hand, intermolecular hydrogen distances have been selected, which may give rise to an NOE effect

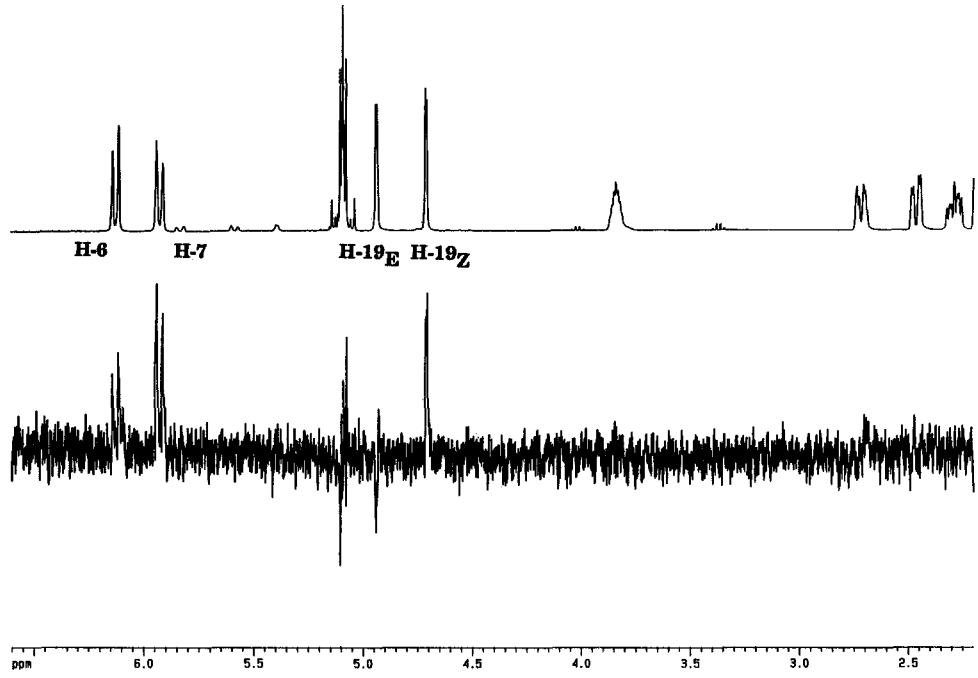


Fig. 3. Part of the ^{1}H-NMR spectrum (~2.5 to 6.6 ppm) of vitamin D_2 and the corresponding NOE trace upon irradiating the C-18 methyl group

depending on their population in solution. Vitamin D (**1**) and 3-epi vitamin D (**4**) were investigated. Standard NOE measurements were done by variation of irradiation time (minimum time four times the longest T_1 value of the vitamin D_2 protons under consideration (Ref. [20]). In order to detect small NOE enhancements at the maximum of build up, several transient NOE measurements (1 D NOESY; Ref. [21]) were employed by selective inversion with a 80 ms half-gaussian pulse followed by a 90° hard pulse and varying the mixing time between 150 to 300 ms. In all the s-cis conformations protons at C-1 are in close proximity to the protons of C-9. A similar situation can be found for the C-18 methyl group and H_{19Z} in Cax(−s-tr), Teq(−s-tr) and Ceq(−s-cis). Therefore the following protons have been selected for NOE measurements: 1α, 1β, 9α, 9β, $19H_E$, $19H_Z$ and the C-18 methyl group. In Tables 3 and 6 distances judged to be of potential significance marked in bold. The only NOE effect [22] which could be detected in this set of experiments was between C-18 CH_3 and $H19_Z$ and is shown in Fig. 3. No NOE could be detected in the reverse direction. The results of the NOE studies were essential the same for vitamin D (**1**) and 3-epi-vitamin D (**4**). From this result we conclude that s-cis conformations are very minor components in the equilibrium mixture at 298 K. The only NOE found may also arise from the s-trans conformations indicated in Tables 3 and 6 and therefore does not proof significant populations of s-cis conformations at 298 K, even so we are confident that they are populated ($\geqslant 5\%$).

Conclusion

Our data from force-field calculations, experimental LIS-data and LIS-simulation establish clearly the existence of a third type of conformations of vitamin D in solution, beneath the two A-ring chair forms. If should be emphasised that the twist form improves significantly the outcome of the LIS simulation. This is in full agreement with the relative steric energies of these conformations derived from force field calculations. The Teq(−s-tr) form is populated up to ~20% in the equilibrium mixture of vitamin D. For 3-epi-vitamin D the contribution of the twist forms to the equilibrium mixture is appr. in the same range. It should be pointed out the steric energy difference between the lowest twist form and the axial chair is very small ($\leqslant 0.1$ kcal) for vitamin D (**1**) and the 3-epi isomer (**4**).

Due to the two exocyclic double bonds severe steric interactions between H-19$_Z$ and H-7 arise in this class of compounds, thus facilitating the adoption of twist forms. If is obvious that the interconversion of the two chair forms proceed via a variety of distorted conformations. This includes also skewed conformations about the C-6–C-7 single bond of the diene system. Note that for the chair–chair interconversion C-19 has to go through the plane defined by the diene bridge. Such a movement is restricted because of the 19-H$_Z$ and H-C-7 interactions. This steric interaction forces now the twist form into an accessible local minimum and therefore into a significantly populated conformer on the pathway of the chair interconversion. To overcome this interaction a movement of the sp^2–sp^2 single bond out of planarity of the diene is necessary. However, this movement is not wide enough to allow significant populations of s-cis conformations in the equilibrium mixture at least at room temperature.

Our NOE experiments do not proof the existence of significantly populated s-cis conformations. The observed NOE between CH$_3$-18 and C-19H$_Z$ may arise from such a conformation, but certain s-trans geometries contribute to the same effect as well.

In conclusion our data show clearly that the conformational flexibility of vitamin D (**1**), 3 epi-vitamin D (**4**) and 3-desoxy vitamin D (**5**) is governed by an interplay of A-ring mobility and distortions of the diene bridge. We are aware that the quantitative evaluation of LIS-data in a multi-component conformational equilibrium of this type is close to the limits of the method. However, LIS-and force-field calculations support each other rather convincingly.

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Experimental Part

All force field computations were done on the IBM 3090 of the computing centre of the University of Vienna. The LIS-simulations were run on an Apple Macintosh computer using a programme of the PDIGM type [19], but extended to handle a mixture [8]. For the LIS-measurments a Bruker WM 250 instrument was used. Increasing amounts of the shift reagent, Eu(*fod*)$_3$, were added to solutions

of the compounds studied in the appropriate solvent ($CDCl_3$ and toluene-d_8) and at the appropriate temperature (23 °C, 55 °C, and 80 °C). 2D-experiments and the NOE studies were done on a Bruker AM 400 WB instrument. Complete assignment of the protons of vitamin D_2 has been achieved by phase sensitive double quantum filtered H,H-COSY as well as proton detected ^1H, ^{13}C-COSY (with BIRD pulse sequence and GARP ^{13}C decoupling).

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