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Conformational Analysis of Vitamin D and Analogues. ^{13}C and ^{1}H Nuclear Magnetic Resonance Study

Elisha Berman, Zeev Luz, Yehuda Mazur,* and Mordechai Sheves

Departments of Isotopes and Organic Chemistry, The Weizmann Institute of Science, Rehovot, Israel

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Complete and self-consistent assignment of the ^{13}C NMR spectra of the C_3-epimeric pairs of vitamin D_3, *trans*-vitamin D_3, its keto analogue, and dihydrotachysterol$_2$ were made. The conformational flexibility of ring A in these compounds and their esters was investigated using the ^{13}C as well as ^{1}H NMR data. The merits and the complementary nature of each approach are discussed in terms of the accuracy and reliability of the results. The relative excess of the equatorial conformer in the C_3 epimeric pairs of vitamin D_3 and its analogues was determined by both methods. This analysis indicates that in the case of vitamin D_3 and its C_3 epimer the methylene group affects the equilibrium population by stabilizing the chair conformation where the hydroxy group is axially oriented.

The recent discoveries of hormonal activity of vitamin D metabolites[1] have renewed the interest in the structure and chemistry of vitamin D and their analogues.[2] These ring B secosteroids exist as a mixture of two ring A conformations, which in solution are in dynamic equilibrium.

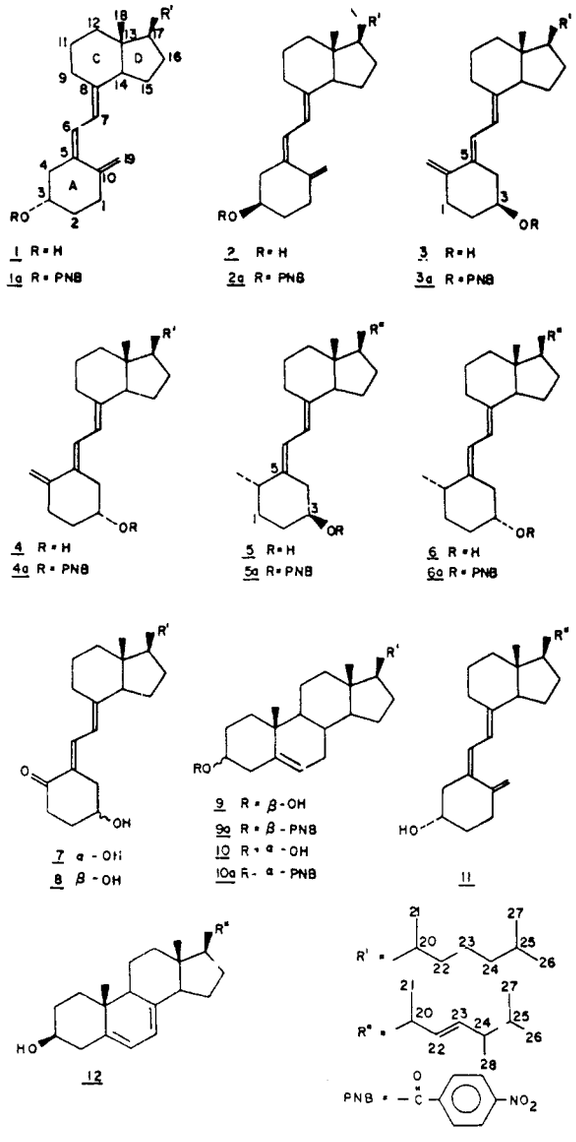
Detailed analysis of this equilibrium in vitamin D_2 (**11**) using ^{1}H NMR spectroscopy was performed by La Mar and Budd. This analysis was based on the correlation between the observable coupling constants of the proton at C_3–OH (J = 7.4 Hz)[3] and the limiting values of the axial–axial and equatorial–equatorial coupling constants for the cyclohexanol proton (J = 11.1 and 2.7 Hz).[4] Accordingly, the ratio of the two conformers, the one with the OH group in an equatorial and the other in an axial orientiation (Figure 1), was calculated to be ca. 50:50.[3] Using lanthanide-induced shifts this ratio was established to be 45:55 in favor of the OH-axial conformer, which, according to the temperature dependance of these shifts, was also the thermodynamically more stable conformer.[3] On the other hand, Wing et al.,[5,6] analyzing the conformational equilibrium of vitamin D_3 (**1**) by similar methods, established this ratio to be 54:43 in favor of the OH-equatorial conformer. This divergency of the results was attributed by the latter authors to the perturbation of the equilibrium ratio toward the OH-axial conformer by the complexing of the shift reagent with the substrate.[5,6]

The method of correlation of the observed spin-coupling constants with the Karplus equation was extended to ring A substituted vitamin D_3 derivatives, as well as to the 10,19-dihydrovitamin analogues, possessing a diene instead of a triene chromophore.[6-9] For some of these compounds this method gave consistent results, but for others it failed, since the multiplet structure due to the proton at C_3 was insufficiently resolved to allow such analysis.[8] Even in favorable cases, the use of the Karplus relation has a relatively large experimental error, up to 10% of the value obtained for the population ratio. As a result, the interpretation of the differences in the conformational equilibria of compounds, whose population ratio varies slightly, will be difficult. An additional error may be introduced by using cyclohexanol as a model for all the compounds investigated, although some of these possess at least one exocyclic double bond which may change the limiting coupling-constant values.

Our decision to utilize the ^{13}C NMR data for the conformational analysis stems from the fact that there are intrinsically large ^{13}C chemical-shift differences[10] between ring A carbon atoms in the respective conformers. The use of the sophisticated NMR techniques enables us to obtain ^{13}C chemical shifts with a high degree of precision, better than 0.03 ppm. Thus, a suitable choice of conformational analysis utilizing these ^{13}C chemical shifts may yield results with relatively small experimental error. For example, the limiting range of ^{13}C chemical shifts used in the present work was 4.62 ± 0.07 ppm (ca. 1.5% error) as compared to the limiting range of 8.4 ± 0.4 Hz[4] (ca. 5% error) for the proton coupling constant observed in vitamin D_3 (**1**) (vide infra).

In the present work, we have investigated the ^{13}C NMR spectra of vitamin D_3 (**1**) and its C_3 epimer **2**. Other compounds studied include *trans*-vitamin D_3 (**3**), its keto analogue **7**, and dihydrotachysterol$_2$ (**5**) as well as their respective C_3 epimers **4**, **8**, and **6**. In addition, the ^{13}C NMR spectra of the *p*-nitrobenzoate esters of these compounds (**1a** to **6a**) were recorded and analyzed. To help in the analysis we have also studied the NMR spectra of cholesterol (**9**), epicholesterol (**10**), and their *p*-nitrobenzoate esters **9a** to **10a** and vitamin D_2 (**11**).

The ^{13}C NMR spectra of all the compounds studied gave well-resolved lines which could be fully assigned. We have first described in detail the assignments for the various com-



pounds, for which we have used several techniques including ^{13}C chemical-shift additivity parameters, effects of lanthanide shift reagents (LIS), single-frequency off-resonance decoupling (SFORD), and comparison with known structures.[11] We have then interpreted the ^{13}C resonances of the A ring in terms of the conformational equilibria between the two chair conformers, and use the experimental results to determine the relative populations of the epimeric pairs. Finally, we have compared our results with those obtained from other sources and discussed them in terms of the possible steric factors which affect the conformational equilibria.

While this work was in progress the ^{13}C NMR of vitamin D analogues, including vitamin D_2 and D_3, *trans*-vitamin D_2, isotachysterol$_2$, isovitamin D_2 and their acetates were reported by Tsukida et al.[12] They have also estimated the conformational equilibrium of the two chair forms by an approach which differs from ours.

Experimental Section

With the exception of the following compounds, the samples used in this study were either commercially available or synthesized by previously described methods.

Epivitamin D_3 (2). A solution of 0.136 g (0.78 mmol) of diethyl azocarboxylate in 3 mL of dry tetrahydrofuran (THF) was added dropwise, under nitrogen atmosphere, to a cold solution (0 °C) of 0.200 g of vitamin D_3 (1) (0.52 mmol), 0.205 g of triphenylphosphine (0.78 mmol), and 0.130 g of *p*-nitrobenzoic acid (0.78 mmol) in 8 mL of dry THF. The resulting mixture was stirred at room temperature for ca. 6 h. The solvent was evaporated at room temperature, and the residue was chromatographed on silica gel using ether–hexane (1:9 mixture) as eluent. The product was crystallized from a methanol–ether solution to give 0.050 g of *p*-nitrobenzoate of epivitamin D_3 (**2a**): mp 117–118 °C; $[\alpha]_D$ −44° (*c* 0.89 in $CHCl_3$), −1° (C_6H_6). To a solution of 0.040 g of **2a** in 2 mL of methanol and 1 mL of ether a 5% KOH in methanol solution (2 mL) was added. The resulting mixture was stirred for 3 h at room temperature. The organic material was extracted with ether. The organic layer was washed three times with saturated NaCl solution and dried over $MgSO_4$. The solvent was evaporated to dryness and the residue was chromatographed on silica gel, using a ether–hexane (3:7) mixture as eluent, to afford 0.025 g of epivitamin D_3 (**2**): $[\alpha]_D$ −0.5° (*c* 0.9 in C_6H_6); UV λ_{max} 264 nm (ϵ 18 000).[9b,13]

Epi-*trans*-vitamin D_3 (4). The synthesis of **4** from **3** followed the same procedure as outlined above to give the *p*-nitrobenzoate epi-*trans*-vitamin D_3 (**4a**): mp 104–105 °C; $[\alpha]_D$ −95° (*c* 0.9 in $CHCl_3$). After hydrolysis, **4a** yielded the required product, epi-*trans*-vitamin D_3 (**4**): $[\alpha]_D$ −30° (*c* 0.85 in C_6H_6); UV λ_{max} 272 nm (ϵ 22 000).[9b,13]

Epidihydrotachysterol$_2$ (6). **6** was obtained by the same method from dihydrotachysterol$_2$ (**5**). The *p*-nitrobenzoate ester **5a**, mp 100–101 °C, $[\alpha]_D$ = −141° (*c* 1.0 in $CHCl_3$), was hydrolyzed to give epidihydrotachysterol$_2$ (**6**); UV λ_{max} 242, 251, and 261 nm (ϵ 34 500, 40 000, and 26 000); exact mass calcd for $C_{28}H_{46}O$: 398.6782; found; 398.6784.

NMR Spectra. All ^{13}C NMR spectra were recorded on a Varian CFT-20 spectrometer, operating at 20 MHz. Flip angles of 45° or less were employed with 8K transform which gave ca. 1 Hz per data point for a 4000-Hz sweep width. Peak positions were determined by a software control and are considered to be accurate to within 0.03 ppm. The LIS experiments were done by adding small portions of Eu(fod)$_3$[14] (ca. 50 mg) to a $CDCl_3$ solution containing the compound under investigation (ca. 200 mg/mL), which was then filtered. All ^1H NMR spectra were recorded on a Bruker HFX-90 spectrometer operating in FT mode with a 8K transformer which gave ca. 0.2 Hz per data point for a 900-Hz sweep width. Peak positions are considered to be accurate to within 2×10^{-3} ppm.

Results

(a) Assignment of the ^{13}C Resonances. The ^{13}C chemical shifts for all observed peaks and their assignments are summarized in Table I. In the table, the numbering of the carbon atoms are given in the first column to the left. The other columns give the chemical shifts for the various compounds studied in ppm relative to Me_4Si as internal standard. All measurements were performed in deuterated chloroform and recorded at room temperature. The concentration of the solution ranged between 0.5 and 0.1 M.

To illustrate the procedure of assignment, we discuss in some detail the analysis of vitamin D_3. The relevant information is summarized in Table II. The carbon atoms of the side chain and of rings C and D were generally assigned by comparison with previous results for ergosterol (**12**) and cholesterol (**9**), discussed in detail in ref 11. Olefinic carbon atoms (i.e., C_5, C_6, C_7, C_8, C_{10}, C_{19}) could be easily distinguished from the rest of the carbon atoms by their large downfield shifts, and were further subdivided according to the multiplicity of the signals in the coupled spectra (C_{19} triplet (t), C_6 and C_7 doublets (d), and C_5, C_8, and C_{10} singlets (s), see column 2 in Table II). Within each subdivision, the peaks were finally assigned using single-frequency off-resonance proton-decoupling experiments and by the effect of lanthanide shift reagents (column 4 in Table II).

Carbon-3 is readily identified by its unique chemical shift and by its multiplicity in the coupled spectrum and by its large LIS. The rest of the ring A carbon atoms were assigned by their LIS, and the characteristic α, β, γ, and δ shifts caused by esterification at position 3 (column 5 in Table II).[15,16] The assignments were tested by comparing spectra of epimeric pairs and by comparison with calculated shifts (column 6, in Table II) based on ^{13}C chemical-shift additivity parameters.[17] Good agreement was obtained for the alkane carbon atoms (standard deviation of 1.7 ppm), although the agreement for the olefinic carbon atoms proved less satisfactory (standard deviation 3.0 ppm). Similar analysis, although less extensive,

Table I. ^{13}C NMR Data for the Vitamins D and Analogues[a,b]

C no.	1 (1a)	2 (2a)	3 (3a)	4 (4a)	5 (5a)	6 (6a)	7	8	9 (9a)	10 (10a)	11
1	32.05	32.40	31.20	31.15	33.20	31.30	30.90	30.95	37.35	33.30	32.00
	(−0.05)[c]	(−0.35)[c]	(−0.35)	(−0.25)[c]	(0.65)	(0.75)			(−0.3)	(0.75)	
2	35.25	35.60	34.80	34.75	34.95	32.05	35.30[c]	35.35[c]	31.70	28.95	35.25
	(−3.15)[c]	(−3.25)[c]	(−3.8)	(−3.65)[c]	(−4.6)	(−2.95)			(−3.85)	(−2.6)	
3	69.20	69.70	69.00	68.95	70.90	69.65	66.55	66.65	71.75	67.15	69.20
	(4.35)	(4.15)	(4.0)	(4.05)	(3.8)	(4.5)			(4.1)	(5.4)	
4	46.00	46.25	37.25	37.10	38.30	35.65	35.65[c]	35.65[c]	42.35	39.95	46.00
	(−3.85)	(−3.95)	(−4.05)	(−3.85)	(−4.75)	(−3.2)			(−4.2)	(−3.4)	
5	145.20	145.05	149.25	149.20	139.75	139.30	128.80	178.80	140.85	138.60	145.15
	(−0.8)	(−0.75)	(−0.85)	(−0.8)	(−1.6)	(−0.8)			(−1.55)	(−0.04)	
6	122.40	122.25	121.05	121.05	117.05	119.15	133.15	133.15	121.65	124.05	122.45
	(0.55)	(0.7)	(0.55)	(0.5)	(1.25)	(−0.5)			(1.55)	(−1.37)	
7	117.65	117.65	116.0	115.9	115.75	115.60	115.65	115.65	31.95	32.05[c]	117.60
	(−0.2)	(−0.25)	(−0.4)	(−0.35)	(−0.2)	(−0.05)			(0.0)	(0.1)	
8	142.10	142.15	144.55	144.65	142.15	142.20	156.45	156.45	31.95	31.95[c]	142.10
	(0.7)	(0.8)	(0.65)	(0.6)	(0.6)	(0.0)			(0.0)	(0.0)	
9	29.10	29.10	29.15	29.10	29.00	28.95	29.85	29.85	50.25	50.45	29.05
10	135.15	135.40	134.95	134.80	37.80	38.40	199.35	199.50	36.55	37.40	135.20
	(−1.35)	(−1.45)	(−1.3)	(−1.45)	(0.2)	(−0.05)			(0.15)	(−0.2)	
11	22.30	22.35	22.40	22.35	22.40	22.35	22.20	22.20	21.15	20.85	22.25
12	40.65	40.65	40.70	40.60	40.55	40.45	40.45	40.45	39.90	39.85	40.50
13	45.90	45.95	46.00	46.00	45.70	45.65	47.10	47.10	42.35	42.40	45.80
14	56.40	56.45	56.70	56.60	56.60	56.55	57.30	57.35	56.30[d]	56.25[d]	56.50
15	23.60	23.70	23.65	23.65	23.60	23.50	23.95	24.00	24.30	24.30	23.60
16	27.70	27.65	27.70	27.70	27.80	27.80	27.60	27.65	28.25	28.25	27.80
17	56.75	56.80	56.85	56.75	56.60	56.55	56.90	56.95	56.85[d]	56.85[d]	56.60
18	12.00	12.00	12.20	12.15	12.40	12.40	12.15	12.20	11.90	11.85	12.30
	(0.05)	(−0.05)	(−0.25)		(−0.2)	(−0.1)				(−0.05)	
19	112.35	112.50	108.15	108.2	17.85	(18.70	(—)	(—)	19.40	18.70[e]	112.35
	(0.7)	(0.6)	(0.3)	(0.4)	(0.0)	(−0.2)			(0.05)	(0.25)	

[a] Shifts are given relative to Me_4Si and are accurate within ±0.05 ppm. The side-chain carbon resonances not listed in the table for compounds 1 to 4, 7 to 10 and their esters are: C_{20}, 36.20; C_{21}, 18.90; C_{22}, 36.20; C_{23}, 23.95; C_{24}, 39.60; C_{25}, 28.05; C_{26}, 22.60; C_{27}, 22.80; and for compounds 5, 6 and 11: C_{20}, 40.35; C_{21}, 19.70; C_{22}, 135.65; C_{23}, 132.05; C_{24}, 42.90; C_{25}, 33.15; C_{26}, 19.95; C_{27}, 21.15; C_{28}, 17.65. [b] The values in parentheses are $\Delta\delta = \delta_{ester} - \delta_{OH}$; estimated error ± 0.07 ppm; any $\Delta\delta < 0.1$ was neglected altogether for all carbons other than ring A and B carbon atoms. [c] Assignment may be reversed down any column. [d] Original assignments have been reversed. [e] Appears as a shoulder on the C_{21} resonance peak.

Table II. ^{13}C-Chemical Shift Assignments of Carbons 1 to 19 for Vitamin D_3

C no.	Vit D_3(1) shifts, ±0.05 ppm	Multiplicity[a]	LIS (Δδ),[b] ±0.15 ppm	$\delta_{ester} - \delta_{OH}$[c] ppm ± 0.08 ppm	Calcd[d] shifts, ppm	Ergosterol,[e] 0.1 ± ppm
1	32.05	t	2.9	−0.0[5]	33.1	38.4
2	35.25	t	4.2	−3.5	35.7	32.0
3	69.20	d	24.4	4.3[5]	71.1	69.5
4	46.00	t	2.8	−3.8[5]	43.6	40.5
5	145.20	s	2.6	−0.8	144.9	140.5
6	122.40	d	3.0	0.5[5]	122.6	119.5
7	117.65	d	0.9	−0.2	122.6	116.5
8	142.10	s	1.2	0.7	143.2	140.4
9	29.10	t	0.3	—[f]	31.4	46.4
10	135.25	s	2.1	−1.3[5]	142.3	37.0
11	22.30	t	0.3	—	22.0	21.0
12	40.65	t	—[f]	—	39.8	39.2
13	45.90	s	—	—	46.0	42.8
14	56.40	d	0.5	—	52.6	54.4
15	23.60	t	0.2	—	23.0	22.9
16	27.70	t	—	—	26.4	28.1
17	56.75	d	—	—	52.7	55.8
18	12.05	q	0.2	—	13.9	11.6
19	112.35	t	1.7	0.7	112.0	15.8

[a] The multiplicity was determined from off-resonance measurements at various offsets. [b] Gradual increase of the concentration of $Eu(fod)_3$ up to ca. 1:1 molar ratio. [c] Negative sign indicates an upfield shift. [d] Calculated from ref 15, using $\delta = B + \sum_i A_{ij}\eta_i$ for $j = \alpha, \beta$, and γ carbons where B(alkyl) = −2.5 ppm and B(alkene) = 122.1 ppm relative to Me_4Si. [e] Taken from ref 16. [f] Bars indicate that observed differences are within the experimental error.

were made for the rest of the compounds studied and the results are given in Table I.

For those compounds which were also studied by Tsukida et al.[12] there is complete agreement in the assignment of the carbon resonances except for the reversal of the resonances due to C_5 and C_{10}. Our assignment is mainly based on the LIS effect. We performed careful shift measurements on these two signals as a function of added $Eu(fod)_3$[14] and have assigned

Table III. ^{1}H NMR Results

Compd[a]	H(6), ppm	H(7), ppm	$^3J_{H6,H7}$, Hz	$H(19)_Z$, ppm	$H(19)_E$, ppm	H(3), ppm	$^3J_{H(3)}$, Hz	H(18), ppm
1[c]	6.2 (6)	6.0 (6)	11.2	4.9 (6)	4.7 (3)	3.9 (6)	3.8	0.5 (5)
2	6.2 (1)	6.0 (1)	11.2	5.0 (1)	4.8 (0)	3.9 (6)	4.1	0.5 (5)
3[c]	6.5 (7)	5.8 (8)	11.1	4.9 (8)	4.6 (9)	3.8 (8)	4.3	0.5 (6)
4	6.5 (6)	5.8 (7)	11.1	4.9 (7)	4.6 (8)	3.8 (8)	4.2	0.5 (6)
5[c]	6.1 (5)	5.9 (0)	11.1			3.6 (0)	4.8	0.5 (6)
6	6.2 (8)	5.8 (6)	11.2			3.8 (5)	3.7	0.5 (6)
7	8.4 (4)[d]	4.9 (9)	12.0			4.1 (9)	3.8	0.5 (4)
8	8.4 (4)[d]	5.0 (0)	12.1			4.1 (8)	3.8	0.5 (4)
1a	6.2 (5)	6.0 (6)	11.1	5.1 (2)	4.9 (0)	5.2 (6)	3.8	0.5 (5)
2a	6.2 (7)	6.0 (8)	11.3	5.1 (2)	4.9 (2)	5.1 (6)	4.1	0.5 (5)
3a	6.5 (7)	5.7 (9)	11.1	5.0 (0)	4.7 (1)	5.2 (3)	(—)[b]	0.5 (6)
4a	6.5 (9)	5.8 (0)	11.0	3.0 (3)	4.7 (3)	5.2 (8)	4.2	0.5 (6)
5a	6.2 (1)	5.8 (0)	11.0			4.9 (3)	4.8	0.5 (7)
6a	6.2 (0)	5.7 (0)	11.0			5.1 (7)	(—)[b]	0.5 (4)

[a] Chemical shifts are given in ppm downfield from Me_4Si as an internal standard, SD = ±0.03 ppm; the coupling constants are given in Hz and believed to be accurate to ±0.2 Hz. [b] The fine structure of the multiplet was too distorted to permit analysis. [c] See also ref 3. [d] This part of the multiplet seemed to have a secondary splitting to a triplet probably arising from the H(4) protons.

Table IV. The Trans Vicinal Coupling Constants of H at C_3, $^{13}C_3$ Chemical Shift Differences, and the Relative Excess of the Equatorial Chair Conformers at Room Temperature[a]

Compd	^3J_t,[b] Hz	N_q, %[c]	ΔN(H), %[d]	Δδ (^{13}C), ppm[e]	ΔN (^{13}C), %[d]
1 (1a)	7.6 (7.6)	57	−8 ± 12	−0.48 (−0.27)	−10 (−8) ± 2
2 (2a)	8.2 (8.2)	65			
3 (3a)	8.6	70	1 ± 14	0.07 (0.01)	1 (0) ± 1
4 (4a)	8.5 (8.5)	69			
5 (5a)	9.7 (9.3)	84	26 ± 15	1.29 (0.56)	27 (17) ± 3
6 (6a)	7.7 (7.7)	58			
7	7.6	57	−2 ± 11	−0.07	−2 ± 1

[a] Values in parentheses are for the corresponding *p*-nitrobenzoate esters. [b] Trans vicinal proton spin–spin coupling constant at C_3. [c] Percentage of the OH-equatorial conformer calculated using the Karplus relation with $J_{ax,ax}$ = 11.1 ± 0.2 Hz and $J_{eq,eq}$ = 2.7 ± 0.2 Hz; errors estimated to be ca. ±10% of the value quoted. [d] Difference between the percentages of the equatorial conformer in the C_3-epimeric pairs. [e] Difference in the $^{13}C_3$ chemical shifts of the epimeric pairs.

the resonance that was more affected by the shift reagent to C_5, since this carbon is closer to the hydroxyl group which is expected to be the binding site for the LIS reagent.

(b) Conformational Analysis. In addition to the ^{13}C NMR measurements, we have also studied the proton spectra of all compounds, some of which (**1, 3, 5**) have been previously analyzed.[3,5] The chemical shift of the protons bonded to C_3, C_{18}, C_6, and C_7, as well as the proton–proton spin–spin couplings of the C_3 multiplet, $^3J_{H(3)}$, and the vicinal couplings, $^3J_{H(6),H(7)}$, are summarized in Table III. In all spectra, the C_3 protons exhibited a heptet indicating fast dynamic equilibrium between the two possible ring A conformers. We have employed these coupling constants to determine the equilibrium population of the conformers using the Karplus equation, as was done by La Mar et al. for vitamin D_2.[3] The results are summarized in column 2 of Table IV.

We attempted a similar conformational analysis on the ^{13}C NMR data. The analysis of ^{13}C NMR data (Table I) shows that the resonance shifts of ring D and the side-chain carbon atoms are insensitive to changes in the structure of ring A, unlike those of ring C and the butadiene bridge. It also appears that all these resonances are similar in the C_3-epimeric pairs (**1,2; 3,4; 5,6; 7,8**). In contrast, the resonances of the ring A carbon atoms differ significantly, not only among the compounds having dissimilar ring A structure but also between the pairs of epimers (e.g., C_3 shifts in the pairs **1,2;** or **3,4**). These differences are attributed to the chair–chair conformational equilibrium of ring A,[18] in which one conformer has an equatorial and the other an axial OH group at C_3 (Figure 1).

In principle, these differences can be used to determine the equilibrium constants between the two conformers, provided the chemical shifts δ^{eq} and δ^{ax} of the OH-equatorial and OH-axial conformers were known for a given atom. The observed shift δ is then given by:

$$\delta = \delta_0 + (\Delta/2)(N_{eq} - N_{ax}) \quad (1)$$

$$N_{eq} + N_{ax} = 1 \quad (2)$$

where $\delta_0 = \frac{1}{2}(\delta^{eq} + \delta^{ax})$ is the average of δ^{eq} and δ^{ax}, $\Delta = \delta^{eq} - \delta^{ax}$, and N_{eq}, N_{ax} are the fractional populations of the OH-equatorial and OH-axial conformers, respectively. This approach cannot be utilized directly, since the values of δ_0 are unknown and cannot be ascertained.

We may, however, obtain more reliable information on the relative ratios of conformers within epimeric pairs. Corresponding conformers in C_3 epimeric pairs have their ring A in a similar conformational relationship with respect to the rest of the molecule and are thus expected to show identical shifts (Figure 2). In particular, we may assume that $\Delta = \delta^{eq} - \delta^{ax}$ is identical for an epimeric pair and interpret the ^{13}C shift between the C_3 epimers as solely reflecting the different populations of the two conformers. Thus, from eq 1 and 2

$$(\delta^{epi} - \delta)/\Delta = N_{eq}{}^{epi} - N_{eq} = \Delta N \quad (3)$$

where the superscript epi refers to the epimeric compound.

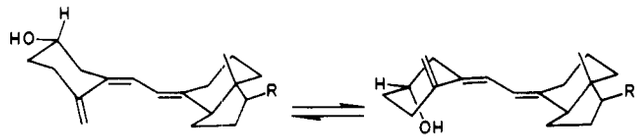


Figure 1. Conformational equilibria in vitamin D_3.

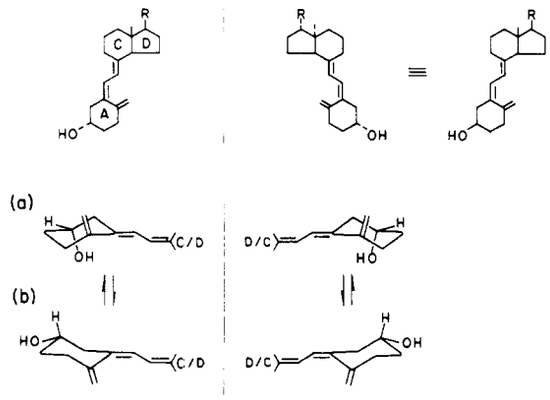


Figure 2. Conformational equilibria in vitamin D_3 and epivitamin D_3. (a) OH-axial conformers; (b) OH-equatorial conformers. Epivitamin D_3 formulas are drawn inverted in the middle row to show the pseudoenantiomeric relationship of rings A in vitamin D_3 and epivitamin D_3.

Consequently, if Δ is known, we may determine the excess of one of the conformers in the epimeric compound from the relative shift ($\delta^{epi} - \delta$).

For the structures under consideration, we have chosen Δ = 4.62 ppm for the C_3 resonances, which was calculated from the C_3 shift of cholesterol (**9**) and epicholesterol (**10**). These two compounds have the C_3 carbon with an equatorial-OH and an axial-OH conformation, respectively, and resemble the structure of the vitamin systems. Similar Δ values may be derived from compounds having analogous substitution patterns as the ring B secosteroids. For example, we calculate Δ = 4.9, 4.6, and 4.4 ppm for the carbinol carbon in cholestanol,[19] androstanol,[19] and 10-methyl-*trans*-decal-3-ol,[20] respectively.[21] For the ester derivatives, we use Δ = 3.27 ppm which was derived from the *p*-nitrobenzoic esters of cholesterol and epicholesterol.

Discussion

In this work we have determined the equilibrium population of the ring A conformers of vitamin D analogues using both ^1H and ^{13}C NMR spectra. The first method relies on the spin–spin coupling of the protons at C_3 using the Karplus relation,[3,4] while the second approach uses the ^{13}C chemical shift differences between an axial and equatorial carbinol groups.

The conformational analysis using ^{13}C NMR data as utilized by us is based on the γ-shift effect. This γ effect is mainly a steric effect and is directly involved with the carbon under consideration (C_3). This method does not give the absolute equilibrium ratios, but, nevertheless, provides reliable information about the conformational equilibria.

In Table IV we have summarized the pertinent results used for the conformational analysis. The first column shows the vicinal coupling constants for the protons at C_3, and the second the calculated populations of the OH-equatorial conformers, using these values. In the fourth column are listed the differences between ^{13}C chemical shifts of the C_3 atoms in the C_3-epimeric pairs. The third and the fifth columns show the differences between the percentage of the equatorial conformers of these epimeric pairs, calculated from the ^1H and ^{13}C NMR data, respectively.

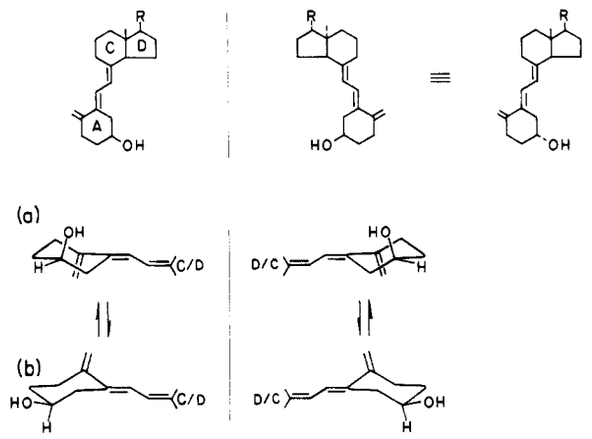


Figure 3. Conformational equilibria in *trans*-vitamin D_3 and epi-*trans*-vitamin D_3. (a) OH-axial conformers; (b) OH-equatorial conformers. Epi-*trans*-vitamin D_3 formulas are drawn inverted in the middle row to show the pseudoenantiomeric relationship of rings A in *trans*-vitamin D_3 and epi-*trans*-vitamin D_3.

As may be seen from Table IV, similar results are obtained for the relative excess of the equatorial conformers in the epimeric pairs (ΔN) using both the ^{13}C and ^1H NMR methods. However, the errors in the calculation of the ΔNs using the ^1H NMR method are of the same order of magnitude as the ΔNs themselves. On the other hand, the ^{13}C results are of much higher accuracy.

In the following, we discuss the four pairs of C_3-epimeric compounds in order to determine the various factors that influence the conformational equilibrium in the vitamin D system.

Dihydrotachysterol$_2$ (5). The population ratios of conformers in dihydrotachysterol$_2$ (**5**) and its epimer **6**, obtained from the coupling constants analysis of the C_3 proton, were found to be 84:16 and 58:42, respectively. Accordingly, **5** possesses 26% more of the equatorial conformer than its epimer **6**. A similar value was calculated from the corresponding ^{13}C NMR data (Table IV).

Dihydrotachysterol$_2$ (**5**) and its epimer **6** may be regarded as analogues of *trans*- and *cis*-4-methylcyclohexanol, respectively. However, it was found that **5** and **6** have a higher proportion of the conformer with an axial CH_3 group than the corresponding 4-methylcyclohexanols (16 and 58% in **5** and **6** vs. <2 and 10% *trans*- and *cis*-4-methylcyclohexanol,[22] respectively). This difference in population ratios is due to the 1:3 peri interaction between the C_6–H and C_{10}–CH_3 groups, which destabilizes the conformers having an equatorial CH_3 group by about 1 kcal/mol.[8a]

The same ratios of conformers were found from the coupling-constant analysis of the C_3 protons of the corresponding *p*-nitrobenzoate esters **5a** and **6a**. However, the relative excess of the OH-equatorial conformer, as calculated from the ^{13}C spectra of **5a** and **6a**, was found to be lower than the corresponding excess in the alcohols (17 vs. 27%).[23]

***trans*-Vitamin D_3 (3) and its Keto Analogue 7.** In both epimeric pairs the coupling constant of the C_3 protons as well as the ^{13}C chemical shifts of epimers were similar (see Tables IV and I). This similarity indicates that the OH-equatorial and OH-axial conformers in these pairs are not only magnetically equivalent, but are also in the pseudoenantiomeric relationship in respect to their ring A and C_6 atoms (Figure 3). However, we attribute the difference of 11% in the population ratios between the *trans*-vitamin D_3 and its keto analogue (69 vs. 58%) to the change in ring A geometry caused by substituting the methylene by a carbonyl group.

The *p*-nitrobenzoate esters **3a** and **4a** have identical ^1H and

^{13}C NMR spectra and a similar ratio of the conformers as the corresponding alcohols **3** and **4**.

Vitamin D_3 (1). The ^1H NMR spectra of **1** and **2** are similar and the only observable difference lies in their C_3-proton spin–spin coupling constants (7.6 vs. 8.2 Hz).[9b] The calculated ratio of the conformers in **1** was 57:43 and in **2** was 65:35, i.e., an excess of 8% of the OH-axial conformer in vitamin D_3 (**1**). Similar results were obtained from the analysis of their ^{13}C NMR spectra. The corresponding *p*-nitrobenzoate esters **1a** and **2a** have almost the same population ratios as their parent alcohols.

The difference in the population ratio of the OH-equatorial and OH-axial conformers in **1** and **2**, respectively, is surprising as they are expected to be identical due to the pseudoenantiomeric relationship of their rings A and C_6 [as found in *trans*-vitamin D_3 (**3**) and the keto analogue **7** systems (Figure 2)]. Therefore, it must be assumed that there are different steric interactions between ring A and C_6 and rings C/D in the respective conformers of **1** and **2**. These interactions are responsible for the predominance of the β conformation[2] in which the methylene group lies above the plane of the butadiene bridge.

X-ray single crystal-structure determination of vitamin D_2 (**11**)[24] and D_3(**14**)[25] have shown that two types of molecules, each with a different ring A chair conformation, are present in a unit cell. One molecule has the OH group in an equatorial orientation and the methylene group below the plane of the butadiene bridge (α conformation), while the other has the OH group in an axial orientation and the methylene group above the plane of the butadiene bridge (β conformation). It was also found that in the vitamin D_2 the two molecules differ not only in the ring A conformations but also in their C_6–C_7 torsional angle, this angle being +175° for the α conformation and −164° for the β conformation.

Similar differences may also exist in the solution conformations of vitamin D_3 (**1**) and its epimer **2**. Thus, respective ring A conformers in the two epimers do not have a pseudoenantiomeric relationship, as the butadiene bridge is more distorted in the β than in the α conformation.

A different approach to the conformational analysis of vitamin D analogues, using ^{13}C NMR spectroscopy, was recently described by Tsukida et al.[12] These authors have derived the C_3 chemical shifts of the OH-equatorial and the OH-axial conformers using the respective chemical in shifts of the *cis*- and *trans*-4-*tert*-butylcyclohexanol and correcting for the substituent effects. In order to test this approach we have calculated the equilibrium populations in the compounds investigated by us, using our experimental data for the $^{13}C_3$ chemical shifts.[26] However, these calculations resulted in population ratios which sometimes differed significantly from the respective ratios derived from the $^3J_{H(3)}$ data. Thus, the OH-equatorial conformer populations found in *trans*-vitamin D_3 (**3**) and its C_3 epimer **4** were 46 and 44% as compared with 70 and 69%, respectively, as established from the proton spin–spin coupling-constant values.

Registry No.—**1**, 67-97-0; **1a**, 6183-79-5; **2**, 57651-82-8; **2a**, 60413-84-5; **3**, 22350-41-0; **3a**, 62743-69-5; **4**, 57651-83-9; **4a**, 60413-85-6; **5**, 67-96-9; **5a**, 62743-70-8; **6**, 62777-58-6; **6a**, 60503-42-6; **7**, 62743-71-9; **8**, 62743-72-0; **9**, 57-88-5; **9a**, 23838-12-2; **10**, 474-77-1; **10a**, 62743-73-1; **11**, 50-14-6; *p*-nitrobenzoic acid, 62-23-7.

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