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CARBON-13 NUCLEAR MAGNETIC RESONANCE SPECTRA OF VITAMINS D AND RELATED COMPOUNDS[1]

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Summary 1. The natural abundance carbon-13 nmr of vitamins D (D_2 and D_3) and several isomers (5, 6-*trans*-vitamin D_2, isotachysterol$_2$ and isovitamin D_2) have been completely assigned by employing off-resonance noise-decoupling, acetylation shifts, and lanthanide-induced shifts experiments. The last two techniques were especially useful for the present study.

2. Carbon-13 nmr spectral characteristics of the three main conjugated triene moieties (*sE-Z-sZ*, *sE-E-sZ*, or *sE-E-sE*), involved in the molecules of vitamin D and its isomers, were revealed. Thus, the striking dependence of the shieldings on molecular geometries and high sensitivity of the resonances to the environments of conjugated systems were surveyed.

3. Conformational preferences in solutions of the hydroxyl groups in vitamins D_2 and D_3 as well as 5,6-*trans*-vitamin D_2 were conveniently determined.

Although carbon-13 nmr spectra of steroids were investigated extensively (*1,2*), surprisingly no information has been available on natural abundance carbon-13 spectra of the important 9,10-secosteroids vitamins D. By using off-resonance decoupling, acetylation shifts, and lanthanide-induced shifts (LIS) techniques, we now present unequivocal and complete assignments of the carbon-13 spectra of vitamin D_2 (I), vitamin D_3 (II), 5,6-*trans*-vitamin D_2 (III), isotachysterol$_2$ (IV), isovitamin D_2 (V) and their respective acetates (Ia, IIIa, IVa and Va) (Fig. 1). Together with the data of the parent steroids ergosterol (VI), dehydrocholesterol (VII) and their acetates (VIa and VIIa), our experimental results clarified for the first time the carbon-13 nmr spectral characteristics of the three representatives

[1] Application of a Shift Reagent in Nuclear Magnetic Resonance Spectroscopy. Part V. Part IV in this series, see *Chem. Pharm. Bull.*, **21**, 248 (1973).

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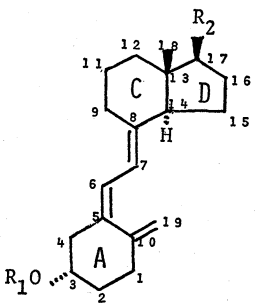
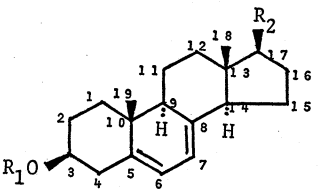
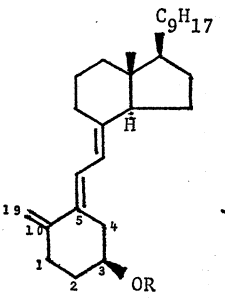


(I) R_1=H, R_2=C_9H_{17}
(Ia) R_1=Ac, R_2=C_9H_{17}
(II) R_1=H, R_2=C_8H_{17}



(VI) R_1=H, R_2=C_9H_{17}
(VIa) R_1=Ac, R_2=C_9H_{17}
(VII) R_1=H, R_2=C_8H_{17}
(VIIa) R_1=Ac, R_2=C_8H_{17}



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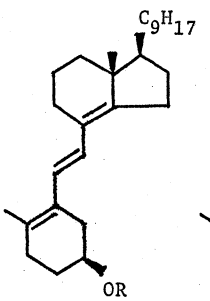

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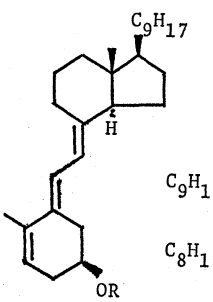

(V) R=H
(Va) R=Ac

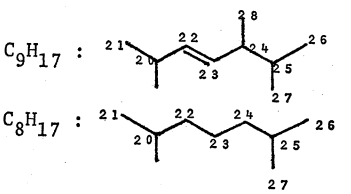

Fig. 1. Vitamins D and isomers.

in the vitamin D chemistry, possessing either *sE-Z-sZ* (in I and II), *sE-E-sZ* (in III) or *sE-E-sE* (in IV and V) conjugated triene system.

EXPERIMENTAL

Crystalline vitamins D_2 and D_3 were purchased from Philips-Duphar Co., Holland. Ergosterol and dehydrocholesterol were also commercial products. The 5,6-*trans* isomer of vitamin D_2 was prepared according to the literature (*3*). Isotachysterol$_2$ was produced by vigorous shaking a hexane solution of vitamin D_2 with 60% sulfuric acid for 10 min, followed by chromatographic purification through a silica-gel column employing acetone-benzene (5: 95) as a developer. Isovitamin D_2 was obtained from vitamin D_2 by our new method employing N-chlorosuccinimide as a reagent.[3] All acetylation was performed with acetic anhydride-pyridine. Authenticity of the sample materials was certified by uv,

[3] Details will be described elsewhere.

Table 1. Carbon-13 chemical shifts (δ) of vitamins D and parent steroids.[a]

Carbon	E (VI) [EA[b] (VIa)]			D_2 (I) [D_2A (Ia)]	DC (VII) [DCA (VIIa)]	D_3 (II)
	Ref. 2	Ref. 1	this work			
1	38.9	38.4	38.4 [−1.7[d]]	32.0 t[c]	38.5	32.1
2	32.5	32.0	32.0 [−3.8]	35.3 t [−3.0]	32.0 t [−3.5]	35.3
3	70.0	69.5	70.4 [+2.5]	69.2 d [+2.6]	70.5 d [+2.4]	69.2 d
4	41.4	40.5	40.8 [−2.9]	46.2 t [−3.9]	40.8 [−3.7]	46.0
5	141.0	140.5	141.4	135.4 s [−0.9]	141.3 s	135.3 s
6	119.7	119.2	119.6 [+0.8]	122.4 d	119.8 d [+0.9]	122.5 d
7	117.0	116.5	116.4	117.8 d	116.5 d	117.8 d
8	140.9	140.4	139.9 [−1.3]	142.2 s	140.0 s [−1.2]	142.2 s
9	46.8	46.3	46.3	29.1*t?	46.5	29.1*
10	37.5	37.0	37.0	145.3 s [−0.5]	37.2 s	145.3 s
11	21.5	21.0	21.1	22.4 t?	21.2	22.4
12	28.6	39.2[e]	39.1	40.4 t	39.2	40.6
13	43.3	42.8	42.9	46.0 s	43.1 s	46.0
14	54.9	54.4	54.6	56.5 d	54.6	56.5**
15	23.4	22.9	23.0	23.7 t?	23.1	23.7
16	39.7	28.1[e]	28.3	27.8*t?	28.1	27.8*
17	56.3	55.8	55.8	56.5 d	56.2	56.7**
18	12.1	11.6	11.9	12.3 q	11.9 q	11.9 q
19	16.3	15.8	16.3	112.5 t	16.4 q	112.5 t
20	40.8	40.3	40.4	40.4 d	36.3	36.2
21	19.7	19.2	19.7	19.7 q	18.9 q	18.9 q
22	136.3	132.0[f]	132.1	132.0 d	36.3	36.2
23	132.5	135.8[f]	135.8	135.7 d	24.0	24.0
24	43.3	42.8	42.9	42.8 d	39.6	39.6
25	33.5	33.0	33.1	33.2 d	28.1	28.1*
26	20.0	19.5	19.9	20.0 q	22.6	22.7
27	21.3	20.8	21.1	21.1 q	22.8	22.9
28	17.7	17.2	17.6	17.7 q		

[a] E, ergosterol; EA, ergosteryl acetate; D_2, vitamin D_2; D_2A, vitamin D_2 acetate; D_3; vitamin D_3; DC, dehydrocholesterol; DCA, dehydrocholesteryl acetate.

[b] EA, 170.4 and 21.1 ppm; D_2A, 170.2 and 21.1 ppm; DCA, 169.4 and 20.7 ppm (CO and acetyl Me, respectively).

[c] Splitting in off-resonance. s, singlet; d, doublet; t, triplet; q, quartet; br, broad.

[d] Values for the acetates are given in parentheses as shifts from the corresponding alcohols values only when they offer significant values. + downfield shift, − upfield shift.

[e,f] Reversed from Ref. 2; see Ref. 1.

*,** Assignments may be interchanged.

proton nuclear magnetic resonance (pmr) and mass spectra.

Carbon-13 spectra were run on a NEVA-NV 21 spectrometer at 22.6 MHz (sample: *ca.* 0.7 M solution in $CDCl_3$; sample tube: 8 mm o.d.; internal reference: tetramethylsilane; 5 KHz spectrum width). Typically 5,000 transients were accumulated at room temperature. The breakdown into quaternary,

Table 2. Carbon-13 chemical shifts (δ) of vitamin D_2 isomers.[a]

Carbon	*trans*-D_2 (III)	IT_2 (IV)	ID_2 (V)
	[*trans*-D_2A[b] (IIIa)]	[IT_2A (IVa)]	[ID_2A (Va)]
1	31.2	31.3	122.8 d[c]
2	34.7	31.3	35.0
	[−3.5][d]	[−3.8]	[−3.5]
3	68.9 d	67.5 d	67.0 d
	[+2.5]	[+2.9]	[+3.0]
4	37.2	34.8	35.2
	[−3.9]	[−4.0]	[−3.5]
5	135.0 s	125.9* s	134.4 s
6	121.0 d	123.7 d	120.6 d
7	116.0 d	125.6 d	116.6 d
8	144.5 s	124.6 s	144.4 s
9	29.1*	27.5**	29.2
10	149.3 s	131.2* s	132.2 s
11	22.4	19.1	22.4
12	40.4	37.1	40.6*
13	45.9 s	43.8 s	46.0 s
14	56.7 d	149.6 s	56.8 d
15	23.6	24.3	23.7
16	27.8*	26.0**	27.8
17	56.7 d	56.5 br	56.8 d
18	12.4 q	18.5	12.5
19	108.3 t	19.1	19.2
20	40.4	39.3 d	40.4*
21	19.7 q	19.7	19.7
22	132.2 d	132.3 d	132.2 d
23	135.7 d	135.4 d	135.8 d
24	42.9 d	42.9 d	43.1 d
25	33.2	33.2	33.2
26	20.0	20.0	20.0
27	21.1	21.3	21.2
28	17.7 q	17.7	17.7

[a] *trans*-D_2, 5,6-*trans*-vitamin D_2; *trans*-D_2A, 5,6-*trans*-vitamin D_2 acetate; IT_2, isotachysterol$_2$; IT_2A, isotachysteryl$_2$ acetate; ID_2, isovitamin D_2; ID_2A, isovitamin D_2 acetate.

[b] *trans*-D_2A, 170.3 and 21.2 ppm; IT_2A, 170.7 and 21.3 ppm; ID_2A, 170.7 and 21.5 ppm (CO and acetyl Me, respectively).

[c,d] See Table 1.

*,** Assignments may be interchanged.

tertiary, secondary, and primary carbon atoms was achieved by the use of an off-resonance noise-decoupling technique. LIS were produced by the addition of an appropriate amount of the shift reagent Eu $(dpm)_3$[4] to the sample solution.

RESULTS AND DISCUSSION

Assignments

The full assignment of the observed resonances to particular carbon atoms is the most important task because of an increasing availability of the recording of cmr spectra as a routing matter. This was done throughout the present series with the aid of off-resonance proton decoupling, acetylation shifts, and LIS experiments together with the previous results in steroids (*1,2*).

1) *Steroids* (VI, VIa, VII and VIIa) (Table 1). The shifts and assignments for ergosterol (VI), dehydrocholesterol (VII) and their acetates (VIa and VIIa) are presented together with previous results (*1,2*). Typical acetylation shifts (downfield shifts [*ca.* 2.5 ppm] of the C-α signals and upfield shifts [*ca.* 3–4 ppm] of the C-β signals) are indicated.

2) *Vitamins D_2 and D_3* (I, II and IIa) (Tables 1 and 3, Figs. 2 and 3). Vitamin D_2 possesses 28 carbons in total, 8 sp^2 and 20 sp^3 carbons. Among the latter, the assignment of C-3 is straightforward from its peculiar chemical shift, doublet splitting in off-resonance, downfield acetylation shift and the largest LIS of all. In a characteristic chemical shift region where sp^2 carbon signals should locate, 8 signals (1 triplet, 4 doublets and 3 singlets) are clearly observed. Of these, the triplet signal is firstly attributed to C-19. Then, from the splitting patterns and

Table 3. LIS ($\Delta\delta$ Hz) in vitamins D and related compounds.

Carbon	Vitamin D_3 (200 mg)		Vitamin D_2 (277 mg)		5,6-*trans*-vitamin D_2 (140 mg)		Isotachysterol$_2$ (277 mg)		Isovitamin D_2 (190 mg)	
	Reagent (mg)									
	60	80	120	180	50	100	120	180	50	100
1	32	40	50	77	27	59	(55)	(83)	40	78
2	52	68	80	119	57	124	93	138	58	98
3	171	214	268	400	155	345	307	468	148	348
4	45	55	63	97	42	89	83	123	(33)	83
5	29	37	47	72	24	52	(58)	(86)	30	67
6	25	33	38	60	20	45	31	46	23	53
7	7	12	15	25	14	29	25	38	13	28
8	4	10	5	10	7	12	11	18	7	15
10	27	37	45	67	24	54	55	83	27	62
14	0	0	0	2	0	4	5	8	7	10
19	14	17	22	34	14	29	25	40	25	42

Reagent, Eu $(dpm)_3$; Carbon atoms not listed show negligible changes.

[4] Tris (dipivaloylmethanato) europium

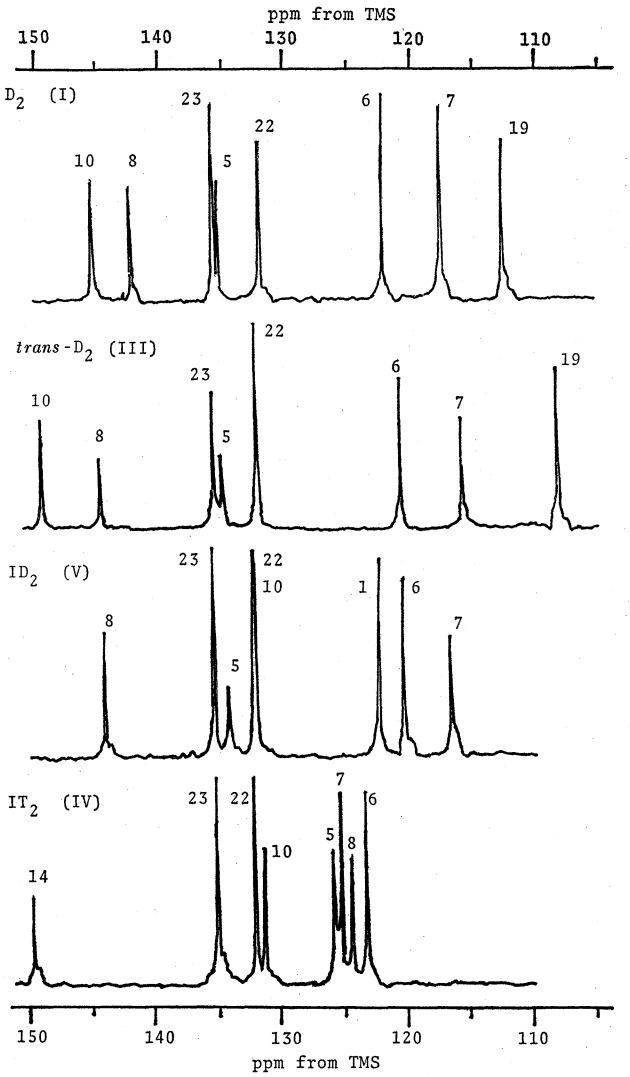

Fig. 2. Carbon-13 nmr spectra (sp^2 region) of the four vitamin D_2 isomers. [22.6 MHz, in $CDCl_3$]
D_2, vitamin D_2; *trans*-D_2, 5,6-*trans*-vitamin D_2; ID_2, isovitamin D_2; IT_2, isotachysterol$_2$.

LIS data, C-5 to C-8 and C-10 can be assigned without difficulties. Finally, C-22 and C-23 are assigned by analogy with the assignment of ergosterol (VI). Regarding sp^3 carbons, the side-chain assignments (C-20, C-21 and C-24 to C-28) also follow precisely those of ergosterol (VI). Among the remaining sp^3 carbons, three carbons in the A-ring (except C-3 already assigned) which are expected to have larger LIS than the other carbons can be easily picked up. From comparison of the acetylation shifts of these three carbons, C-1, which should possess the

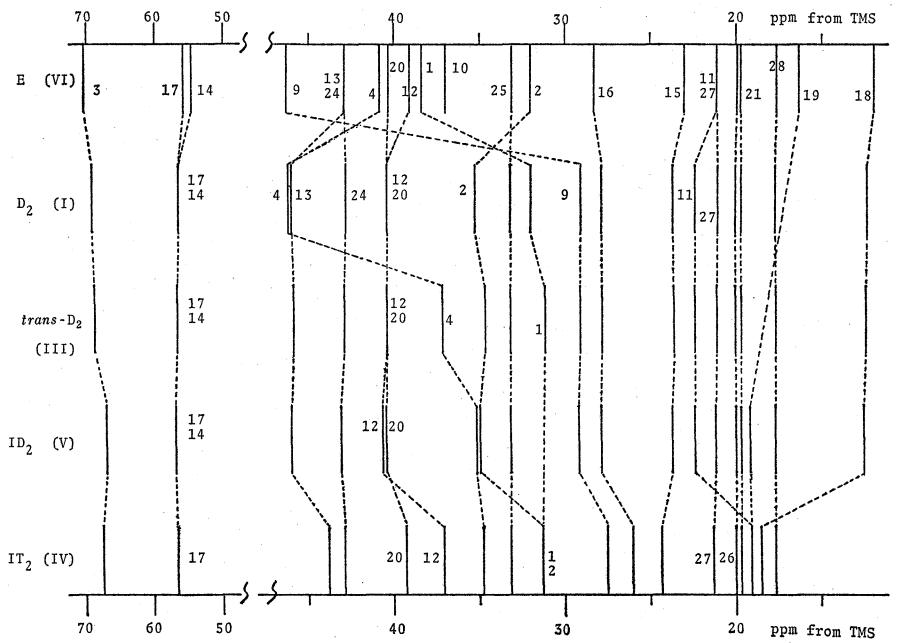

Fig. 3. Correlation diagram for carbon-13 chemical shifts (sp^3 carbon region) of isomeric vitamin D_2. [22.6 MHz, in $CDCl_3$]
E, ergosterol; D_2, vitamin D_2; *trans*-D_2, 5,6-*trans*-vitamin D_2; ID_2, isovitamin D_2; IT_2, isotachysterol$_2$.

smallest value, is immediately apparent while differentiation between C-2 and C-4 presents some difficulties. However, since the vitamin D molecule is essentially plannar and the LIS of C-1 is greater than that of C-5 under the experimental conditions employed, C-2 should indicate a larger LIS than C-4. This interpretation is well supported by their chemical shifts which should reflect the allylic or non-allylic character of the carbon atoms. In an off-resonance spectrum of the remaining 9 sp^3 carbons in the C- and D-rings, only one singlet and one quartet signal exist. They are unambiguously assigned to C-13 and C-18, respectively. Considering their splitting patterns in off-resonance and the result with ergosterol (VI) mentioned above, the remainder can be easily assigned as shown in Table 1, though the C-17 signal is hidden under the C-14 signal in the spectrum and the assignments of C-9 and C-16 are not unambiguous and could be interchanged.

The assignments of C-1 to C-19 of vitamin D_3 are made by comparison with those for vitamin D_2, and the side-chain assignments (C-20 to C-27) follow those of dehydrocholesterol (VII) and its acetate (VIIa). Further, there is substantial evidence obtained from a detailed inspection of the off-resonance decoupling and LIS experimental results.

3) Vitamin D_2 isomers (III–V and IIIa–Va) (Tables 2 and 3, Figs. 2 and 3).

All carbons constituting 5,6-*trans*-vitamin D_2 (III), isotachysterol$_2$ (IV) and isovitamin D_2 (V) molecules can be easily assigned by taking essentially the same procedure as adopted for vitamin D_2 analysis. For assigning the spectra of these seco-steroids, LIS and acetylation shift techniques exhibit a potential advantage of effective extracting particular carbons out of 28 carbons in the molecule, *i.e.* the former technique can pick out ten carbons (C-1 to C-8, C-10 and C-19) and the latter three (C-2 to C-4).

Sensitivity to the structural environments

The pronounced dependence of carbon-13 shieldings on molecular geometry and high sensitivity of the resonances to the environments of conjugated triene systems are surveyed as follows.

1) Vitamins D and provitamins D. When the spectrum of either vitamin D_2 or D_3 is compared with that of ergosterol or dehydrocholesterol, all side-chain resonances are unchanged to within 0.1 ppm. However, because of a cleavage of the C_9–C_{10} bonding of the parent provitamin molecule, reasonable changes are now induced on ring-A and -C resonances of the vitamin (Table 4, Fig. 3), though ring-D signals are affected to a lesser extent. When chemical shifts of the sp^3 carbons in ring-A of vitamin D_2 are calculated roughly, approximate consistencies with the experimental values are demonstrated (Fig. 4).

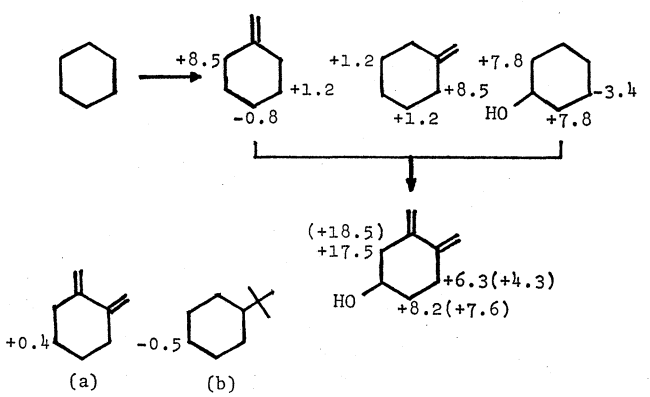

Fig. 4. Approximate substitution effects on the cyclohexane moiety. [( ): observed]

2) Vitamin D_2 isomers. When the spectrum of 5,6-*trans*-vitamin D_2 or isovitamin D_2 is compared with that of vitamin D_2, all resonances except those of C-1 to C-8, C-10 and C-19 remain virtually constant to within 0.3 ppm. As is expected, a sensitive dependence of the resonances on the conjugated triene environments is clearly observed on the above ten carbons in the neighborhood of the triene system, especially on C-4, 10, and 19. In isotachysterol$_2$, which possesses no exo carbon-carbon double bond, chemical shift changes are especially significant and are noticeable in varieties of carbon atoms (Table 4, Fig. 3). The

Table 4. Chemical shift changes* ($\Delta\delta$ ppm) of isomeric vitamin D to the parent compounds.

Carbon	DC→D_3	E→D_2	D_2→tr. D_2	D_2→ID_2	D_2→IT_2
1	−6.4	−6.4			
2	+3.3	+3.3			−4.0
3	−1.3	−1.2		−2.2	−1.7
4	+5.2	+5.4	−9.0	−11.0	−11.4
5	−6.0	−6.0		−1.0	−9.5
6	+2.7	+2.8	−1.4	−1.8	+1.3
7	+1.3	+1.4	−1.8	−1.2	+7.8
8	+2.2	+2.3	+2.3	+2.2	−17.6
9	−17.4	−17.2			−1.6
10			+4.0	−13.1	−14.1
11	+1.2	+1.3			−3.3
12	+1.4	+1.3			−3.3
13	+2.9	+3.1			−2.2
14	+1.9	+1.9			
16					−1.8
18					+6.2
19			−4.2		
20					−1.1

* Changes in $sp^3 \rightarrow sp^2$ (*vice versa*) are omitted. Insignificant values (<1 ppm) are neglected.

DC, dehydrocholesterol; E, ergosterol; tr. D_2, 5,6-*trans*-vitamin D_2; ID_2, isovitamin D_2; IT_2, isotachysterol$_2$.

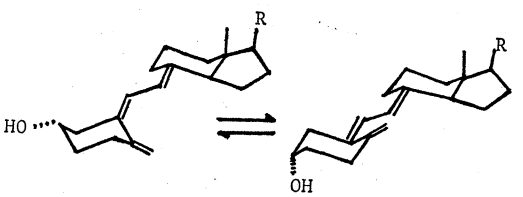

Fig. 5. Solution conformation of vitamin D.

C-18 atom in isotachysterol$_2$ is now released from a steric compression shift and appears in a lower field than in vitamin D_2 and isovitamin D_2.

Acetylation shifts

It has been noted for cyclohexanols and steroids (*2*) that acetylation of a hydroxyl group produces a characteristic downfield shift of the C-α and upfield shifts of the C-β and C-γ signals. This is sufficiently valid for present series of 9,10-secosteroids (C-α: +2.5 to +3.0 ppm, C-β: −3.0 to −4.0 ppm, C-γ: negligible) and is invaluable for permitting an immediate extraction of the two β-carbons (C-2 and C-4) out of all carbons in the molecule.

Conformational preference in solution

For vitamins D, dynamic equilibria are possible in solutions between the two chair conformations as shown in Fig. 5. It has been revealed from pmr studies that the relative proportions of the two conformers in vitamin D_2 (*4*), vitamin D_3, 1α-hydroxy- and 1α,25-dihydroxy-vitamin D_3 (*5*) are approximately 1: 1. Since the shieldings of the readily identifiable carbinol carbons in cyclohexanols are markedly dependent on the orientation of the oxygen functions, a convenient approach for determining quantitative distribution of the above two conformers can be expected using cmr analysis (*2,6*). As is generally agreed, the values for the *cis*- and *trans*-4-*tert*-butyl-cyclohexanols (65.7 and 70.9 ppm, respectively (*7*)) may be employed as a first approximation for characterizing the carbinol shieldings in the extreme axial and equatorial conformers. Then, taking the values (a) and (b) in Fig. 4 into consideration, the relative proportions of the two conformers in vitamins D_2 and D_3 as well as 5,6-*trans*-vitamin D_2 are estimated as follows:

Equatorial OH: Axial OH=50: 50 (vitamins D_2 and D_3)

Equatorial OH: Axial OH=44: 56 (5,6-*trans*-vitamin D_2)

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