169. Natural Abundance ^{13}C-NMR. of Vitamin D_3 Metabolites

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Summary

Chemical shifts of ^{13}C- and ^{1}H-NMR. spectra of vitamin D_3 metabolites (**2**–**7**) are assigned. Substituent effect parameters due to hydroxyl groups are deduced by comparison with vitamin D_3.

Introduction. – The discovery of hormonal activity of hydroxylated vitamin D_3 has stimulated interest in the chemistry of vitamin D_3 (**1**) and its metabolites. Natural abundance ^{13}C-NMR. spectra of vitamin D_3 and related compounds have been published (1) (2), but none are reported for the metabolites. This article presents ^{13}C-NMR. assignments of metabolites **2**–**7** based upon comparisons with vitamin D_3 itself. One of the metabolites, 1α-hydroxycholecalciferol (**2**) [3], is derived from plant sources while 25-hydroxycholecalciferol (**3**) [4], 1α,25-dihydroxycholecalciferol (**4**) [5], 24*R*,25-dihydroxycholecalciferol (**5**) [6] [7], 1α,24*R*,25-trihydroxycholecalciferol (**6**) [8] [9] and 25*S*,26-dihydroxycholecalciferol (**7**) [10] [11][1]) are found in humans [12]. The substituent effects of hydroxyl groups in the 1α, 24*R*, 25*S*, and 26 positions were determined and should prove useful in structural elucidation of new metabolites or steroids with hydroxylated cholesteryl side chains.

The spectra were obtained in CD_3OD, since the metabolites were not very soluble in $CDCl_3$. The chemical shifts of **1** were assigned based on those published for a $CDCl_3$ solution [1] [2]. The ambiguity of assignment of C(14) *versus* C(17) in $CDCl_3$ [1] [2] was elucidated in CD_3OD by consideration of the single-frequency off-resonance (SFOR) residual couplings. In general, in steroids C(14) has been assigned at lower field than C(17) [18]. Consideration of substituent effects of the 1α-hydroxyl group of **2** on the chemical shift of vitamin D_3 confirmed the assignment of *Tsukida et al.* [1], but not the reverse assignments of *Berman et al.* [2] for C(5) and C(10). The chemical shift data for vitamin D_3 (**1**) and for the metabolites (**2**-**7**) are compiled in *Table 1*.

[1]) The configuration of this metabolite was erroneously reported as 25*R* [13]. This misassignment by the *Redel* group [11] [14] was caused by an error in the X-ray structure determination of one of their intermediates [15]. The correct absolute configuration was shown to be 25*S* by high pressure liquid chromatographic comparison of our unambiguously prepared samples of the 25*R*,26- and 25*S*,26-dihydroxycholecalciferols and the human metabolite [16]. The error in the configuration at C(25) was also independently detected by *Barner & Hübscher* [17].

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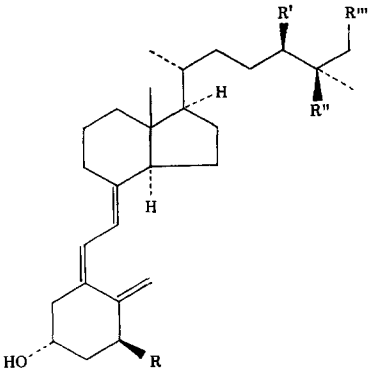

	R	R′	R″	R‴	
1	H	H	H	H	Cholecalciferol (vitamin D_3)
2	OH	H	H	H	1α-Hydroxycholecalciferol
3	H	H	OH	H	25-Hydroxycholecalciferol
4	OH	H	OH	H	1α,25-Dihydroxycholecalciferol
5	H	OH	OH	H	24*R*,25-Dihydroxycholecalciferol
6	OH	OH	OH	H	1α,24*R*,25-Trihydroxycholecalciferol
7	H	H	OH	OH	25*S*,26-Dihydroxycholecalciferol

Results. – *1α-Hydroxycholecalciferol* (**2**). Chemical shift considerations and use of the multiplicity of the signals in the SFOR spectrum of **2** indicated that the C-atoms C(6) to C(9) and C(11) to C(27) were within ±0.4 ppm of those of vitamin D_3 (**1**). Among the remaining six C-atoms of the ring A, the sp^2-hybridized C(5) and C(10) gave bands at lowest field, while the oxygenated sp^3-hybridized C(1) and C(3) were at lower field than the non-oxygenated C(2)

Table 1. *^{13}C-NMR. Data for Vitamin D_3 and Metabolites*[a])

	Cholesterol	**1**	**2**	**3**	**4**	**5**	**6**	**7**
C(1)	38.5	33.5	71.2	33.4	71.0	33.5	71.3	33.5
C(2)	32.3	36.4	43.5	36.3	43.4	36.4	43.6	36.5
C(3)	72.3	70.1	67.2	70.1	67.1	70.2	67.3	70.3
C(4)	43.0	46.8	46.0	46.7	45.9	46.8	46.1	46.9
C(5)	142.0	136.8	135.4	136.7	135.3	136.8	135.4	137.0
C(6)	122.0	122.3	124.6	122.2	124.5	122.3	124.7	122.4
C(7)	33.2	118.8	118.8	118.7	118.6	118.7	118.8	118.8
C(8)	33.0	141.7	142.1	141.7	141.9	141.9	142.3	142.1
C(9)	51.7	29.8	29.9	29.7	29.8	29.8	29.9	29.8
C(10)	37.6	146.3	149.4	146.3	149.3	146.4	149.5	146.6
C(11)	22.2	23.1	23.2	23.0	23.1	23.1	23.3	23.2
C(12)	41.1	41.7	41.8	41.6	41.7	41.7	41.8	41.6
C(13)	43.5	46.6	46.8	46.5	46.7	46.7	46.9	46.7
C(14)	58.1	57.7	57.8	57.6	57.7	57.8	58.0	57.8
C(15)	25.3	24.4	24.6	24.3	24.5	24.4	24.6	24.3
C(16)	29.3	28.7	28.7	28.5	28.6	28.6	28.7	28.5
C(17)	57.5	57.2	57.4	57.2	57.3	57.3	57.5	57.3
C(18)	12.4	12.5	12.4	12.4	12.4	12.4	12.4	12.4
C(19)	19.9	112.5	111.8	112.4	111.8	112.5	111.9	112.5
C(20)	37.1	37.2	37.4	37.1	37.3	37.0	37.2	37.1
C(21)	19.3	19.5	19.4	19.3	19.3	19.2	19.3	19.4
C(22)	37.4	37.2	37.2	37.5	37.6	34.0	34.1	37.7
C(23)	24.9	24.9	24.9	21.7	21.8	28.6	28.6	21.0
C(24)	40.6	40.5	40.6	45.0	45.1	79.4	79.6	39.8
C(25)	29.1	28.9	29.1	71.0	71.1	73.6	73.8	73.5
C(26)	22.9	23.0	22.9	29.0	29.1	25.0	24.9	70.3
C(27)	23.2	23.3	23.2	29.2	29.2	25.4	25.6	23.6

[a]) Measured in ppm downfield from internal tetramethylsilane.

Table 2. *C-Substituent effects*[a])

	2	3	4	5	6	7
C(1)	+37.7		+37.5		+37.8	
C(2)	+ 7.1		+ 7.0		+ 7.2	
C(3)	− 2.9		− 2.9		− 2.8	
C(4)	− 0.8		− 0.9		− 0.7	
C(5)	− 1.4		− 1.5		− 1.4	
C(6)	+ 2.3		+ 2.2		+ 2.4	
C(10)	+ 3.1		+ 3.0		− 3.2	
C(19)	− 0.7		− 0.7		− 0.6	
C(22)		+ 0.3	+ 0.4	− 3.2	− 3.1	+ 0.5
C(23)		− 3.2	− 3.1	+ 3.7	+ 3.7	− 3.9
C(24)		+ 4.5	+ 4.6	+38.9	+39.1	− 0.7
C(25)		+42.1	+42.2	+44.7	+44.9	+44.6
C(26)		+ 6.0	+ 6.1	+ 2.0	+ 1.9	+47.3
C(27)		+ 5.9	+ 5.9	+ 2.1	+ 2.3	+ 0.3

[a]) Effects in ppm (positive values represent downfield shifts) obtained by comparing the shieldings for each C-atom of the metabolites *versus* vitamin D_3.

and C(4). The substituent effects of 4β-hydroxylation [18] upon the chemical shifts of ring A C-atoms of 5α steroids should be similar to those of 1α-hydroxylation upon the ring A C-atoms of vitamin D_3. Thus, C(5) can be distinguished

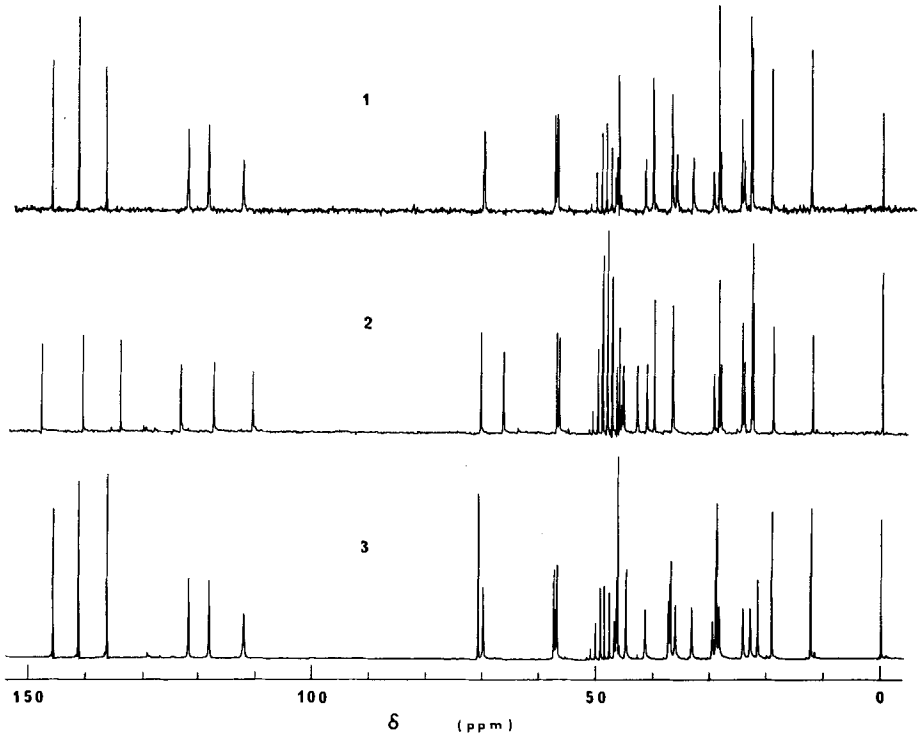

Fig. 1. *Natural abundance proton noise decoupled ^{13}C-NMR. spectra of vitamin D_3* (**1**) *and metabolites* **2** *and* **3**

from C(10), C(1) from C(3) and C(2) from C(4). These substituent effect parameters are given in *Table 2*.

25-Hydrocholecalciferol (**3**). The chemical shifts of the C-atoms C(1) to C(22) were the same as those of vitamin D_3 (**1**) within ± 0.3 ppm. The low field doublet at δ 71.0 is readily assigned to the hydroxylated C(25), while the two high field quartets at δ 29.0 and δ 29.2 were assigned to C(26) and C(27), respectively. The remaining two signals, triplets at δ 21.7 and δ 45.0, were assigned to C(23) and C(24) respectively, based on a comparison with **1** and taking into account the deshielding β-effect and shielding γ-effect of the hydroxyl group [18] [19].

1α,25-Dihydroxycholecalciferol (**4**). Because of the rapid attenuation of substituent effects on ^{13}C chemical shifts with increasing distance from the site of substitution, metabolite **4** can be readily assigned by comparison with the ring A and side chain ^{13}C-resonances in **2** and **3**, respectively, and with the remaining C-atoms in **1**. Specifically, the C-atoms C(1) to C(6), C(10) and C(19) of **4** *versus* **2** and the C-atoms C(22) to C(27) of **4** *versus* **3** showed chemical shift identity within ± 0.2 ppm. The remaining C-atoms in between these two hydroxylated regions, viz. C(7) to C(9), C(11) to C(18), C(20), and C(21) showed agreement of ± 0.2 ppm in comparison with the analogous C-atoms of vitamin D_3 itself.

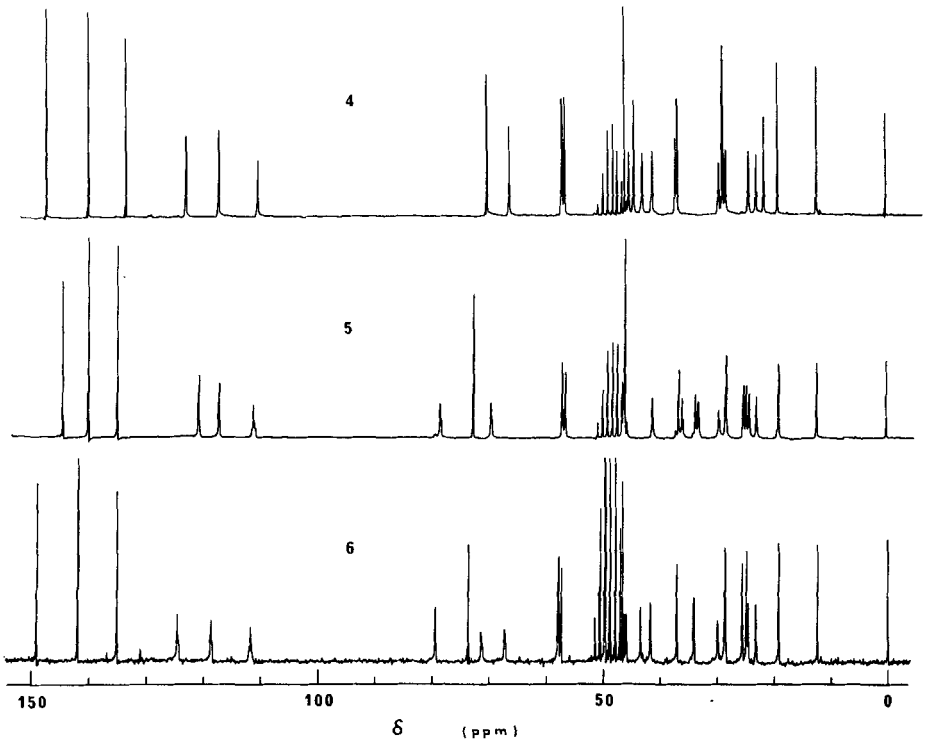

Fig. 2. *Natural abundance proton noise decoupled ^{13}C-NMR. spectra of metabolites* **4–6**

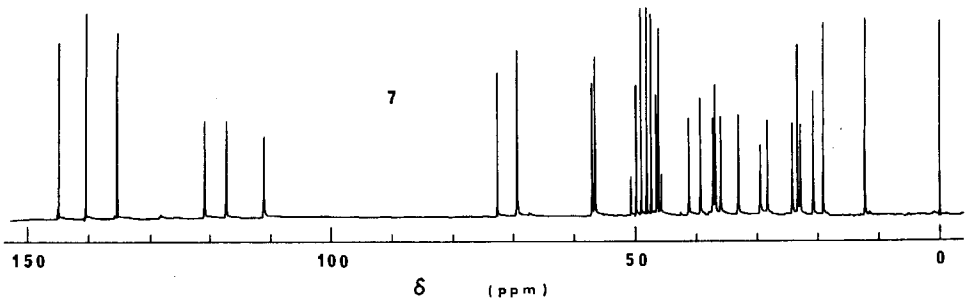

Fig. 3. *Natural abundance proton noise decoupled ^{13}C-NMR. spectrum of metabolite* **7**

*24*R,*25-Dihydroxycholecalciferol* (**5**). The chemical shifts of the C-atoms C(1) to C(21) were identical, within ±0.3 ppm, with vitamin D_3 (**1**). The rest of the side chain C-atoms were assigned by consideration of multiplicity in the SFOR spectrum and by comparison with the 25-hydroxylated metabolite **3**. Accordingly, the hydroxylated C-atoms C(24) and C(25) were readily assigned based on their downfield chemical shifts and their respective doublet and triplet multiplets, whereas C(26) and C(27) exhibited high field quartets at δ 25.0 and δ 25.4, respectively. The remaining signals, triplets at δ 34.0 and δ 28.6, were assigned to C(22) and C(23), respectively, based on the hydroxyl substituent effects [19] on the chemical shifts of C(22) and C(23) in **5** *versus* **1** and **3**.

*1α,24*R,*25-Trihydroxycholecalciferol* (**6**). Assignment of C-chemical shifts for **6** were straightforward based on comparisons with **4** and **5**. The chemical shifts of the C-atoms C(1) to C(19) were identical, within ±0.2 ppm, with metabolite **4**, whereas the C-atoms C(20) to C(27) showed agreement, within ±0.1 ppm, with metabolite **5**.

*25*S,*26-Dihydroxycholecalciferol* (**7**). Identity within ±0.4 ppm was observed in comparison of the chemical shifts of the C-atoms C(1) to C(21) for **7** with vitamin D_3 (**1**). The side chain C(25), C(26) and C(27) were assigned on the basis of the SFOR spectrum; the hydroxylated C(25) and C(26) lowfield at δ 73.5 (singlet) and δ 70.3 (triplet), respectively, and C(27) highfield at δ 23.6

Table 3. *^1H-NMR. Chemical shifts for vitamin D_3 and metabolites*[a])

	1		2	3	4	5	6	7
H–C(1)			4.33		4.33		4.33	
H–C(3)	3.94[b])	3.74	4.10	3.74	4.10	3.74	4.10	3.74
H–C(6)	6.25	6.17	6.29	6.17	6.29	6.17	6.29	6.17
H–C(7)	6.03	5.98	6.04	5.98	6.04	5.98	6.04	5.98
H–C(18)	0.54	0.56	0.56	0.56	0.56	0.56	0.56	0.56
$H_{(E)}$–C(19)	4.82	4.72	4.87	4.72	4.87	4.72	4.87	4.72
$H_{(Z)}$–C(19)	5.05	4.99	5.26	4.99	5.26	4.99	5.26	4.99
H–C(21)	0.92	0.96	0.96	0.96	0.96	0.96	0.96	0.96
H–C(24)						3.16	3.16	
H–C(26)	0.87	0.87	0.87	1.16	1.16	1.12	1.12	3.34
H–C(27)	0.87	0.87	0.87	1.16	1.16	1.14	1.14	1.11

[a]) Measured in MeOD in δ-values downfield from TMS. [b]) Reference [20].

(quartet). The remaining signals, triplets at δ 37.7, δ 21.0, and δ 39.8 were assigned to C(22), C(23) and C(24), respectively, based on comparison of **7** with **3** recognizing the small long-range substituent effects [18] [19] due to hydroxylation at C(26).

Table 3 summarizes the proton chemical shifts of vitamin D_3 and of the metabolites. The substituent effect of the 1α-hydroxyl group on the chemical shift of H–C(6), $H_{(E)}$–C(19), $H_{(Z)}$–C(19) and H–C(3) is shown to be 0.1, 0.2, 0.3 and 0.4 ppm, respectively, while the 25-hydroxyl group deshields the geminal dimethyl groups by 0.3 ppm.

Experimental Part

The ^{1}H- and ^{13}C-NMR. spectra were run in continuous-wave and *Fourier*-transform modes, respectively, on a *Varian* XL-100 spectrometer at a probe ambient temperature of 27°. Samples (100 mg each, but a saturated solution in the case of **2**, **6**, and **7**) were dissolved in 0.35 ml of CD_3OD containing TMS as internal reference.

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