Crystal Structure of 25-Hydroxy-vitamin D_3 Monohydrate: A Stereochemical Analysis of Vitamin D Molecules

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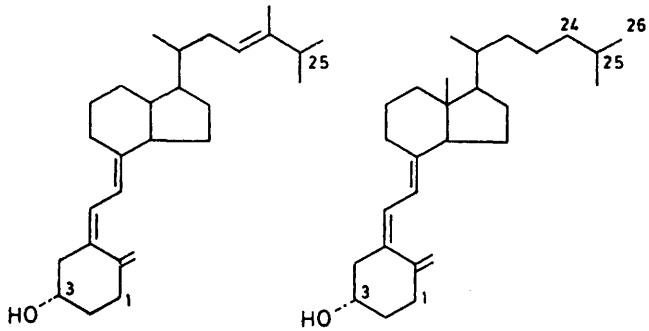
A three-dimensional *X*-ray diffraction study on the monohydrate of the 25-hydroxy-vitamin D_3, the predominant metabolite of vitamin D_3 in the blood and the immediate precursor of 1α,25-dihydroxy-vitamin D_3, has provided the first high-precision molecular parameters for this class of molecules. A detailed comparative analysis of the solid-state conformations of the rings and side-chain is made with those of other stucturally known vitamin D molecules, and the resulting stereochemical implications are discussed with respect to biological activities of various metabolites and active analogues. Crystals are monoclinic with $Z = 2$ in a unit cell of space group $P2_1$ and dimensions $a = 10.600(1)$, $b = 6.798(1)$, $c = 18.142(2)$ Å, $\beta = 103.75(2)°$. The structure was solved by direct methods and refined to R 3.4% based on 2 012 independent intensities.

THE existence of vitamin D, the antirachitic vitamin, has long been known [1,2] and the great importance of this family of compounds including the two primary sources, vitamin D_2 (ergocalciferol, $C_{28}H_{44}O$) and vitamin D_3 (cholecalciferol, $C_{27}H_{44}O$), to medicine and nutrition has long been recognized and recently well-documented.[3] For thirty years after the chemical identification [4,5] of vitamins D_2 (I) and D_3 (II) it was presumed that they



(I) (II)

acted directly in accounting for the known physiological actions attributed to vitamin D. This belief was overturned with the discovery of metabolites of vitamin D possessing marked biological activity.[6] In rapid succession 25-hydroxy-vitamin D_3 (25-OH-D_3),[7] 1α,25-dihydroxy-vitamin D_3 [1α,25-$(OH)_2$-D_3],[8] 24,25-dihydroxy-vitamin D_3 [24,25-$(OH)_2$-D_3],[9] 25,26-dihydroxyvitamin D_3 [25,26-$(OH)_2$-D_3],[10] and 1α,24,25-trihydroxyvitamin D_3 [1α,24,25-$(OH)_3$-D_3],[11] as well as 25-hydroxyvitamin D_2 (25-OH-D_2) [12] were isolated and chemically identified. It is currently accepted that vitamin D_3 must first be hydroxylated in the liver to 25-OH-D_3 which is then further hydroxylated in the kidney to 1α,25-$(OH)_2$-D_3 before it can initiate physiological reponses including the stimulation of intestinal calcium transport, bone calcification, bone calcium mobilization, and phosphate transport.[3] Other significant developments include recent ^{1}H n.m.r. studies on the solution conformations of vitamins D_2 [13] and D_3 and several metabolites including 1α,25-$(OH)_2$-D_3 [14] which have not only substantiated Havinga's suggestion [15] of a dynamic equilibrium existing in solution between two chair conformations of the cyclohexane-like A ring of a vitamin D molecule but also have led to a proposal [16] of a topological model for vitamin D activity.

In order to assess structure–function inter-relationships of various vitamin D metabolites and analogues we have examined their molecular geometries in the solid state. Before our present *X*-ray diffraction study of 25-OH-D_3 monohydrate there were only two reports of crystallographic work on vitamin D derivatives.[17,18]

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An early *X*-ray photographic investigation by Hodgkin and co-workers [17] of the 4-iodo-5-nitrobenzoate ester of vitamin D_2 (abbreviated INC) revealed the overall spatial arrangement of the non-hydrogen atoms, but the low precision of the atomic parameters (*i.e.*, σ 0.03—0.05 Å for non-hydrogen atom co-ordinates) due to a lack of sufficiently accurate intensity data precluded any meaningful analysis of distances and angles. A subsequent *X*-ray examination [18] of a vitamin D analogue, 3,20-bis-(ethylenedioxy)-9,10-secopregna-5,7,10(19)-triene (abbreviated ECF) produced a structure which concurred with the INC structure with respect to an analogous *cis*-Z-*trans* arrangement [15] of the triene fragment and a *trans*-fusion of the C and D rings but differed in the form

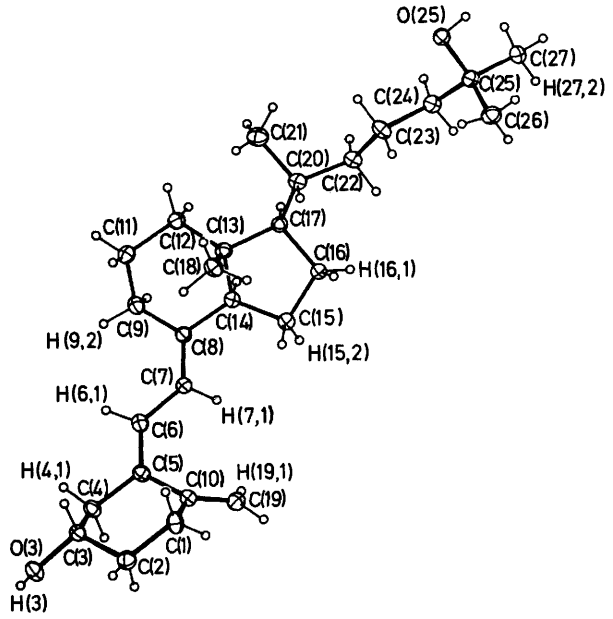

FIGURE 1 View of the molecular geometry of 25-hydroxycholecalciferol (25-OH-D_3) showing the numbering system used in the analysis

of the chair conformation of ring A, *viz.*, in the solid-state INC structure ring A has the α form (with the exocyclic CH_2 substituent below the ring plane), while in the solid-state ECF structure ring A possesses the β form (with the CH_2 substituent above the ring plane). Despite the use of three-dimensional diffractometry data in the refinement of the ECF structure, the presence of unusually large molecular thermal motion at room temperature resulted in a sufficiently low precision of the atomic co-ordinates (*i.e.*, σ 0.03—0.06 Å). Recently, *X*-ray diffraction investigations have been carried out on vitamin D_2 [19] and vitamin D_3.[20] Although the abstract [19] reporting the solution by direct methods of the crystal structure of vitamin D_2 provided no description of the molecular geometry, the fact that vitamins D_2 and D_3 possess the same space group with similar lattice parameters strongly indicates that these two vitamins, which differ only in the nature of the side chain, must have analogous solid-state structures. Our crystallographic investigation [20] revealed vitamin D_3 (cholecalciferol) to exist in the solid state in an equimolar ratio of two conformers, one with ring A in the α and the other with ring A in the β chair form (denoted D3α and D3β, respectively).

We now describe the structural features of crystalline 25-OH-D_3 monohydrate together with resulting stereochemical implications based upon a comparative analysis of the solid-state conformation of 25-OH-D_3 with those of D3α, D3β, INC, and ECF. Detailed and accurate structural properties of this important metabolite, the immediate precursor of the steroid hormone 1α,25-$(OH)_2$-D_3, may in future provide some understanding in structural terms of biochemical studies.[21-27]

TABLE 1

Atom positional and isotropic thermal parameters of 25-hydroxycholecalciferol monohydrate [a,b]

Atom	*x*	*y*	*z*
O(3)	0.653 8(2)	−0.421 7(4)	1.386 7(1)
O(25)	0.675 1(1)	0.2448 [d]	0.485 9(1)
O(W)	0.450 5(2)	0.357 2(4)	0.539 4(1)
C(1)	0.780 8(3)	−0.055 3(5)	1.263 8(2)
C(2)	0.745 2(2)	−0.140 9(5)	1.334 4(1)
C(3)	0.674 6(2)	−0.335 1(5)	1.317 9(1)
C(4)	0.751 7(2)	−0.478 8(5)	1.282 1(1)
C(5)	0.792 2(2)	−0.395 0(5)	1.214 0(1)
C(6)	0.765 9(2)	−0.485 4(5)	1.146 5(1)
C(7)	0.797 8(2)	−0.416 2(5)	1.077 9(1)
C(8)	0.767 5(2)	−0.500 3(5)	1.008 9(1)
C(9)	0.690 0(2)	−0.683 6(5)	0.988 6(1)
C(10)	0.855 7(2)	−0.199 3(5)	1.228 5(1)
C(11)	0.575 6(2)	−0.649 5(5)	0.921 3(1)
C(12)	0.616 2(2)	−0.555 8(5)	0.853 9(1)
C(13)	0.692 2(2)	−0.366 2(4)	0.875 8(1)
C(14)	0.809 1(2)	−0.416 0(5)	0.941 8(1)
C(15)	0.895 3(2)	−0.234 4(5)	0.949 4(1)
C(16)	0.878 7(2)	−0.163 8(5)	0.867 3(1)
C(17)	0.764 8(2)	−0.279 4(4)	0.817 1(1)
C(18)	0.607 2(2)	−0.207 8(5)	0.899 1(1)
C(19)	0.970 3(2)	−0.156 0(5)	1.215 5(1)
C(20)	0.690 5(2)	−0.159 8(5)	0.749.6(1)
C(21)	0.574 5(3)	−0.272 0(5)	0.701 8(1)
C(22)	0.781 9(2)	−0.100 5(5)	0.698 4(1)
C(23)	0.734 5(3)	0.063 2(5)	0.641 6(1)
C(24)	0.825 2(2)	0.088 0(5)	0.587 7(1)
C(25)	0.804 7(2)	0.262 8(5)	0.534 5(1)
C(26)	0.813 9(3)	0.459 1(5)	0.575 8(1)
C(27)	0.902 1(2)	0.255 1(5)	0.484 2(1)

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TABLE 1 (*Continued*)

Atom	*x*	*y*	*z*	*B*/Å²
H(3)	0.607 9	−0.323 3	1.417 3	6.2
H(25)	0.669 1	0.330 0	0.447 5	4.4
H(W,1)	0.535 2	0.375 1	0.527 8	9.2
H(W,2)	0.415 6	0.509 3	0.535 4	4.1
H(1,1)	0.695 5	−0.023 3	1.225 0	4.3
H(1,2)	0.814 3	0.078 0	1.269 0	8.2
H(2,1)	0.684 6	−0.057 0	1.358 9	5.2
H(2,2)	0.816 1	−0.168 3	1.369 1	5.0
H(3,1)	0.583 8	−0.313 9	1.280 7	3.4
H(4,1)	0.697 7	−0.584 6	1.265 1	4.3
H(4,2)	0.835 0	−0.500 0	1.319 1	3.7
H(6,1)	0.720 8	−0.618 3	1.143 4	3.7
H(7,1)	0.845 5	−0.293 4	1.083 9	3.4
H(9,1)	0.758 6	−0.784 1	0.976 9	4.4
H(9,2)	0.658 5	−0.748 6	1.027 0	4.1
H(11,1)	0.528 5	−0.774 2	0.907 5	4.2
H(11,2)	0.510 5	−0.574 0	0.934 8	4.4
H(12,1)	0.535 7	−0.540 9	0.810 8	4.0
H(12,2)	0.675 5	−0.647 8	0.833 4	4.3
H(14,1)	0.851 1	−0.523 6	0.918 7	3.4
H(15,1)	0.862 4	−0.132 6	0.981 3	4.5
H(15,2)	0.980 9	−0.280 6	0.973 0	4.7
H(16,1)	0.964 9	−0.167 3	0.855 4	5.4
H(16,2)	0.862 7	−0.037 4	0.867 1	3.8
H(17,1)	0.802 0	−0.397 7	0.795 9	3.5
H(18,1)	0.650 6	−0.079 1	0.903 0	6.6
H(18,2)	0.534 6	−0.191 5	0.867 8	5.2
H(18,3)	0.579 7	−0.251 3	0.944 4	4.4
H(19.1)	1.019 0	−0.253 9	1.201 8	5.6
H(19,2)	1.002 5	−0.019 4	1.228 5	4.0
H(20,1)	0.655 8	−0.028 7	0.768 7	3.1
H(21,1)	0.535 0	−0.191 0	0.653 8	6.0
H(21,2)	0.519 6	−0.237 4	0.728 3	8.7
H(21,3)	0.591 9	−0.397 7	0.690 1	3.6
H(22,1)	0.870 0	−0.058 9	0.733 5	3.6
H(22,2)	0.802 2	−0.214 7	0.673 3	4.4
H(23,1)	0.734 8	0.189 1	0.670 4	4.7
H(23,2)	0.640 1	0.040 5	0.608 3	5.8
H(24,1)	0.824 2	−0.024 2	0.558 2	3.9
H(24,2)	0.912 3	0.107 1	0.618 0	4.7
H(26,1)	0.889 4	0.469 3	0.603 3	6.7
H(26,2)	0.743 2	0.465 4	0.598 3	5.8
H(26,3)	0.802 4	0.568 9	0.542 7	6.5
H(27,1)	0.891 1	0.132 6	0.452 7	5.7
H(27,2)	0.890 6	0.371 6	0.450 4	4.2
H(27,3)	0.992 4	0.256 1	0.516 0	6.0

[a] Here and in subsequent Tables estimated standard deviations are given in parentheses. [b] In the final refinement cycle only the positional parameters of non-hydrogen atoms were varied, while all anisotropic thermal parameters of these atoms as well as positional and isotropic thermal parameters of hydrogen atoms are assigned as fixed contributors (see Experimental section).

EXPERIMENTAL

Crystal Isolation.—Transparent needle-like crystals of 25-OH-D_3 containing one water of crystallization per formula species were obtained by slow evaporation from a methanolic solution. A single crystal of dimensions 0.20 × 0.20 × 0.40 mm was mounted with epoxy glue to a thin glass fibre such that the needle axis (corresponding to the *b* axis) was approximately parallel to the spindle axis of the goniometer.

Crystal Data.—$C_{27}H_{44}O_2,H_2O$, M = 418.7. Monoclinic, a = 10.600(1), b = 6.798(1), c = 18.142(2) Å, β = 103.75(2)°, U = 1 268.8 Å³, Z = 2, D_c = 1.09 g cm⁻³, $F(000)$ = 464. Cu-K_α radiation, λ = 1.541 8; μ(Cu-K_α) = 5.43 cm⁻¹. Space group $P2_1$ (C_2^2, No. 4, *b* axis unique).

Preliminary Weissenberg and precession photographs showed monoclinic C_{2h} 2/m Laue symmetry. Systematic absences of {0*k*0} for $k = 2n + 1$ denoted the probable space group of this substance to be $P2_1$ by virtue of its

TABLE 2

Bond distances (Å) and angles (°)

(*a*) Interatomic distances

C(3)–O(3)	1.443(3)	C(12)–C(13)	1.522(3)
C(25)–O(25)	1.451(3)	C(13)–C(14)	1.543(3)
C(1)–C(2)	1.534(4)	C(13)–C(17)	1.569(3)
C(1)–C(10)	1.496(4)	C(13)–C(18)	1.527(3)
C(2)–C(3)	1.512(4)	C(14)–C(15)	1.523(4)
C(3)–C(4)	1.516(4)	C(15)–C(16)	1.534(3)
C(4)–C(5)	1.511(3)	C(16)–C(17)	1.543(3)
C(5)–C(6)	1.339(3)	C(17)–C(20)	1.524(3)
C(5)–C(10)	1.486(4)	C(20)–C(21)	1.529(4)
C(6)–C(7)	1.443(3)	C(20)–C(22)	1.548(3)
C(7)–C(8)	1.343(3)	C(22)–C(23)	1.519(4)
C(8)–C(9)	1.490(4)	C(23)–C(24)	1.535(3)
C(8)–C(14)	1.503(3)	C(24)–C(25)	1.513(4)
C(9)–C(11)	1.520(3)	C(25)–C(26)	1.522(4)
C(10)–C(19)	1.325(3)	C(25)–C(27)	1.534(3)
C(11)–C(12)	1.530(3)		
O(3)–H(3)	1.06	O(W)–H(W,1)	0.98
O(25)–H(25)	0.90	O(W)–H(W,2)	1.10
C(1)–H(1,1)	1.03	C(18)–H(18,1)	0.98
C(1)–H(1,2)	0.97	C(18)–H(18,2)	0.85
C(2)–H(2,1)	1.04	C(18)–H(18,3)	0.98
C(2)–H(2,2)	0.88	C(19)–H(19,1)	0.91
C(3)–H(3,1)	1.05	C(19)–H(19,2)	1.00
C(4)–H(4,1)	0.93	C(20)–H(20,1)	1.05
C(4)–H(4,2)	0.98	C(21)–H(21,1)	1.03
C(6)–H(6,1)	1.02	C(21)–H(21,2)	0.87
C(7)–H(7,1)	0.97	C(21)–H(21,3)	0.91
C(9)–H(9,1)	1.06	C(22)–H(22,1)	1.04
C(9)–H(9,2)	0.95	C(22)–H(22,2)	0.95
C(11)–H(11,1)	0.99	C(23)–H(23,1)	1.00
C(11)–H(11,2)	0.94	C(23)–H(23,2)	1.05
C(12)–H(12,1)	1.02	C(24)–H(24,1)	0.93
C(12)–H(12,2)	1.02	C(24)–H(24,2)	0.97
C(14)–H(14,1)	1.00	C(26)–H(26,1)	0.84
C(15)–H(15,1)	1.02	C(26)–H(26,2)	0.94
C(15)–H(15,2)	0.96	C(26)–H(26,3)	0.95
C(16)–H(16,1)	0.99	C(27)–H(27,1)	1.00
C(16)–H(16,2)	0.88	C(27)–H(27,2)	0.99
C(17)–H(17,1)	1.01	C(27)–H(27,3)	0.99

(*b*) Inter- and intra-molecular nonbonding distances [a]

O(3[I]) · · · O(25)	2.871	O(3[I]) · · · H(25)	2.00
O(25) · · · O(W)	2.880	O(25) · · · H(W,1)	2.03
O(25[II]) · · · O(W)	2.939	O(25[II]) · · · H(W,2)	1.86
O(W[II]) · · · O(3[I])	2.705	O(W[II]) · · · H(3[I])	1.65
H(6,1) · · · H(4,1)	2.29	H(14,1) · · · H(15,2)	2.22
H(6,1) · · · H(9,2)	2.24	H(14,1) · · · H(17,1)	2.33
H(7,1) · · · H(19,1)	2.48	H(15,1) · · · H(7,1)	2.20
H(11,2) · · · H(18,3)	2.31	H(15,1) · · · H(16,2)	2.17
H(12,2) · · · H(17,1)	2.36	H(15,1) · · · H(18,1)	2.38
H(14,1) · · · H(9,1)	2.39	H(16,1) · · · H(15,2)	2.24
H(14,1) · · · H(12,2)	2.28	H(16,1) · · · H(17,1)	2.39

(*c*) Bond angles

C(2)–C(1)–C(10)	111.7(2)	C(14)–C(13)–C(17)	100.2(2)
C(1)–C(2)–C(3)	111.9(2)	C(14)–C(13)–C(18)	111.0(2)
C(2)–C(3)–C(4)	110.9(2)	C(17)–C(13)–C(18)	109.9(2)
C(2)–C(3)–O(3)	110.8(2)	C(8)–C(14)–C(13)	112.1(2)
C(4)–C(3)–O(3)	108.5(3)	C(8)–C(14)–C(15)	121.5(3)
C(3)–C(4)–C(5)	113.2(2)	C(13)–C(14)–C(15)	104.2(2)
C(4)–C(5)–C(6)	122.4(2)	C(14)–C(15)–C(16)	103.8(2)
C(4)–C(5)–C(10)	113.4(2)	C(15)–C(16)–C(17)	107.9(2)
C(6)–C(5)–C(10)	124.1(2)	C(13)–C(17)–C(16)	103.4(2)
C(5)–C(6)–C(7)	126.8(2)	C(13)–C(17)–C(20)	120.2(2)
C(6)–C(7)–C(8)	127.2(2)	C(16)–C(17)–C(20)	112.6(2)
C(7)–C(8)–C(9)	125.6(2)	C(17)–C(20)–C(21)	112.5(2)
C(7)–C(8)–C(14)	122.8(2)	C(17)–C(20)–C(22)	110.1(2)
C(9)–C(8)–C(14)	111.7(2)	C(21)–C(20)–C(22)	108.8(2)
C(8)–C(9)–C(11)	111.0(2)	C(20)–C(22)–C(23)	116.4(2)
C(1)–C(10)–C(5)	113.4(2)	C(22)–C(23)–C(24)	110.9(2)
C(1)–C(10)–C(19)	122.2(3)	C(23)–C(24)–C(25)	118.4(2)
C(5)–C(10)–C(19)	124.3(3)	C(24)–C(25)–C(26)	113.1(2)
C(9)–C(11)–C(12)	112.6(2)	C(24)–C(25)–C(27)	109.8(2)
C(11)–C(12)–C(13)	112.1(2)	C(24)–C(25)–O(25)	107.1(2)
C(12)–C(13)–C(14)	107.1(2)	C(26)–C(25)–C(27)	110.4(2)
C(12)–C(13)–C(17)	117.1(3)	C(26)–C(25)–O(25)	108.3(2)
C(12)–C(13)–C(18)	111.0(2)	C(27)–C(25)–O(25)	108.0(2)

TABLE 2 (*Continued*)

H(W,1)–O(W)–H(W,2)	100.5
H(19,1)–C(19)–H(19,2)	124.0
C(25)–O(25) · · · O(3^{I})	103.7
C(25)–O(25) · · · O(W)	120.8
C(25)–O(25) · · · O(W^{III})	119.9
C(3^{I})–O(3^{I}) · · · O(25)	149.2
C(3^{I})–O(3^{I}) · · · O(W^{II})	107.9
O(25)–H(25) · · · O(3^{I})	162.7
O(W)–H(W,1) · · · O(25)	145.0
O(W)–H(W,2) · · · O(25^{II})	167.6
O(3^{I})–H(3^{I}) · · · O(W^{II})	171.1
O(W) · · · O(25) · · · O(3^{I})	93.1
O(W) · · · O(25) · · · O(W^{III})	84.9
O(3^{I}) · · · O(25) · · · O(W^{III})	130.2
O(25) · · · O(3^{I}) · · · O(W^{II})	102.9
O(25) · · · O(W) · · · O(25^{II})	124.4
O(25) · · · O(W) · · · O(3^{IV})	116.7
O(25^{II}) · · · O(W) · · · O(3^{IV})	118.8

(*d*) Ring torsion angles

ϕ(3–2–1–10)	−54.0	ϕ(12–11–9–8)	51.9
ϕ(4–3–3–1)	53.8	ϕ(13–12–11–9)	−54.5
ϕ(5–4–3–2)	−51.8	ϕ(14–13–12–11)	55.9
ϕ(10–5–4–3)	50.0	ϕ(8–14–13–12)	−58.6
ϕ(1–10–5–4)	−49.8	ϕ(9–8–14–13)	59.1
ϕ(2–1–10–5)	51.1	ϕ(17–13–14–15)	45.6
ϕ(6–5–10–19)	−56.7	ϕ(13–14–15–16)	−35.4
ϕ(7–6–5–10)	2.6	ϕ(14–15–16–17)	11.0
ϕ(8–7–6–5)	−177.0	ϕ(15–16–17–13)	17.0
ϕ(6–7–8–9)	1.8	ϕ(16–17–13–14)	−37.6
ϕ(11–9–8–14)	−54.1		

[a] Roman numeral superscripts refer to the following positions:

I $x, 1 + y, -1 + z$ IV $1 - x, \frac{1}{2} + y, 2 - z$
II $1 - x, \frac{1}{2} + y, 1 - z$ V $x, 1 + y, z$
III $1 - x, -\frac{1}{2} + y, 1 - z$

known chirality and optical activity, and this choice was substantiated from the successful refinement.

After optical and *X*-ray alignment [28] on a Datex-controlled General Electric diffractometer equipped with an E & A full circle, 20 independent reflections were carefully centred [29a] to give the above lattice constants. Two equivalent sets of intensity data were collected (*viz.*, *hkl*, $\bar{h}kl$, and $\bar{h}\bar{k}l$, $h\bar{k}l$) *via* the θ—2θ scan method to $2\theta \leqslant 120°$ at 2° min^{-1} with a constant scan width of 2.2° and with stationary-crystal–stationary-counter background measurements of 15 s made on each side of the scan. Three standard reflections monitored every 80 reflections revealed no indication of electronic instability and/or crystal decay or movement during data collection. After corrections for Lorentz and polarization effects, data were converted to structure-factor amplitudes and then merged [29b] to yield 2 012 independent reflections for which $I > 2\sigma(I)$. The small linear absorption coefficient did not necessitate a correction of the intensities for absorption.

Determination and Refinement of the Structure.—Wilson-type statistics expectedly indicated a non-centrosymmetric distribution of intensities in harmony with the polar space group $P2_1$. After considerable difficulty which involved the extensive use of several direct-method programs, the crystal structure was finally solved by the application of MULTAN [30] to the largest 395 $|E|$ values ($\geqslant 1.3$). The correct solution had an absolute figure of merit of 1.13 and an estimated residual error of 36.4%. An *E* map assigned with these phases revealed the positions of 27 of the 30 non-hydrogen atoms. A conventional Fourier synthesis [29d] provided the positions of the two remaining carbons and the oxygen atom of the water molecule of crystallization. An isotropic full-matrix least-squares refinement [29e] converged

TABLE 3

Equations of least-squares planes [a] in the form $AX + BY + CZ + D = 0$ where *X*, *Y*, and *Z* are orthogonal (Å) co-ordinates.[b] Deviations (Å) of relevant atoms from the planes are given in square brackets

A	*B*	*C*	*D*
Plane (1): C(4)—(10), C(14)			
−0.828 2	0.502 2	−0.248 5	9.260 2
[C(4) −0.010, C(5) −0.025, C(6) −0.048, C(7) −0.036, C(8) −0.002, C(9) 0.070, C(10) 0.075, C(14) −0.025, C(19) −0.773]			
Plane (2): C(4)—(8), C(14)			
−0.820 7	0.515 9	−0.245 8	9.246 4
[C(4) 0.011, C(5) 0.007, C(6) −0.028, C(7) −0.008, C(8) 0.015, C(14) 0.002]			
Plane (3): C(5)—(8)			
−0.832 8	0.497 8	−0.242 1	9.164 1
[C(5) 0.014, C(6) −0.014, C(9) −0.015, C(8) 0.014]			
Plane (4): C(1), C(5), C(10), C(19)			
−0.256 1	0.310 5	−0.915 4	21.222 4
[C(1) −0.005, C(5) −0.005, C(10) 0.017, C(19) −0.007]			
Plane (5): C(1), C(5), C(19)			
−0.255 6	0.310 6	−0.915 6	21.229 1
[C(10) 0.023]			
Plane (6): C(4)—(6), C(10)			
−0.846 1	0.459 8	−0.269 5	9.656 8
[C(4) 0.006, C(5) −0.020, C(6) 0.008, C(10) 0.006]			
Plane (7): C(4), C(6), C(10)			
−0.846 2	0.460 0	−0.268 9	9.637 6
[C(5) −0.027]			
Plane (8): C(7)—(9), C(14)			
−0.806 5	0.541 5	−0.237 4	9.114 0
[C(7) 0.001, C(8) −0.002, C(9) 0.001, C(14) 0.001]			
Plane (9): C(7), C(9), C(14)			
−0.806 5	0.541 5	−0.237 5	9.114 5
[C(8) −0.002]			
Plane (10): C(15)—(17)			
0.692 4	−0.706 0	−0.148 9	−2.369 5
[C(13) −0.445, C(14) 0.281]			
Plane (11): C(14)—(17)			
0.766 6	−0.611 1	−0.197 1	−1.877 9
[C(13) 0.041, C(14) −0.064, C(15) 0.064, C(16) −0.041, C(17) −0.668]			
Plane (12): C(17), C(20), C(22)—(25), C(27)			
−0.303 2	−0.715 8	−0.629 1	8.921 0
[C(13) −0.086, C(14) −0.865, C(17) −0.167, C(20) 0.150, C(21) 1.536, C(22) 0.068, C(23) −0.020, C(24) 0.094, C(25) −0.171, C(27) 0.046]			

[a] Unit weights were used for all atoms in the application of the Smith least-squares planes program.[29h] [b] *X*, *Y*, *Z* are related to the monoclinic fractional unit cell co-ordinate system (*x*,*y*,*z*) by the transformation $X = ax + cz\cos\beta$, $Y = by$, and $Z = cz\sin\beta$.

[28] T. C. Furnas, 'Single-Crystal Orienter Instruction Manual,' General Electric Co., Milwaukee, 1957.

[29] (*a*) A. S. Foust, ANGSET, Ph.D. Thesis (Appendix), University of Wisconsin-Madison, 1970; J. C. Calabrese, (*b*) SORTMERGE, and (*c*) MIRAGE, Ph.D. Thesis, 1971, (*d*) MAP, and (*e*) LSQ, University of Wisconsin-Madison, 1972; W. R. Busing, K. O. Martin, and H. A. Levy, (*f*) ORFLS Report ORNL TM 305, and (*g*) ORFFE, Report ORNL TM 306, Oak Ridge National Laboratory, Oak Ridge, Tennessee, 1962; (*h*) D. L. Smith, PLANES, Ph.D. Thesis, University of Wisconsin-Madison, 1962.

[30] G. Germain, P. Main, and M. M. Woolfson, *Acta Cryst.*, 1971, **A27**, 368.

at R 7.9 and R' 10.3%.* At this stage the locations of the 46 hydrogen atoms were determined either from difference-Fourier synthesis or from idealized configuration [29c] based upon the carbon positions. Further least-squares refinement, performed *via* a block-diagonal approximation [29e] involved the use of anisotropic thermal parameters for the non-hydrogen atoms with fixed-parameter contributions from the hydrogen atoms. Peaks for all 46 hydrogen atoms were then resolved from a difference Fourier map, after which the positional and isotropic thermal parameters of the hydrogen atoms were refined separately to yield R 3.4 and R' 3.9%. Attempts to determine the absolute configuration from the very small anomalous dispersion corrections [32] to the atomic scattering factors (*i.e.*, for Cu-K_α radiation, $\Delta f'$ 0.05, $\Delta f''$ 0.03 for oxygen; $\Delta f'$ 0.02, $\Delta f''$ 0.01 for carbon) were unsuccessful, and our tabulated co-ordinates represent those in agreement with the accepted absolute configuration.

The output of the final full-matrix cycle [29f] in which only co-ordinates of the non-hydrogen atoms were varied is listed in Table 1. Interatomic distances and angles together with estimated standard deviations calculated from the variance–covariance matrix [29g] are provided in Table 2. Least-squares planes of selected atoms and perpendicular distances of these and other atoms from these planes were calculated [29h] and presented in Table 3. Observed and calculated structure factor amplitudes and anisotropic thermal parameters are listed in Supplementary Publication No. SUP 21864 (17 pp.).†

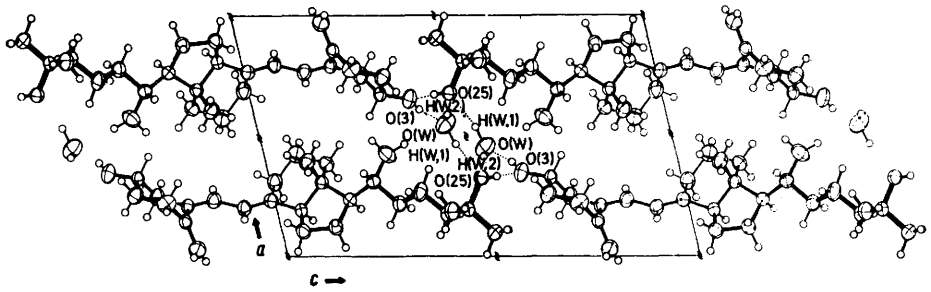


FIGURE 2 An [010] projection of the monocline unit cell

RESULTS AND DISCUSSION

Structure of 25-Hydroxy-vitamin D_3 *and its Stereochemical Relationship with Other Vitamin* D *Molecules.*—The overall features of the molecular structure are in accord with those of other vitamin D molecules.[17,18,20] Since the structural parameters of 25-OH-D_3 represent a higher level of precision (as reflected by the close conformity of the bond lengths and angles to expected values and by the corresponding σ values of only 0.003—0.004 Å and 0.2°, respectively), a close examination of its structural features is worthwhile. The relatively precise structural determination at room temperature of this hydrated crystalline compound may be largely attributed to its hydrogen-bonding network markedly dampening the atomic thermal motion (as evidenced by the relatively small thermal ellipsoids of the non-hydrogen atoms) and thereby providing well-defined atomic co-ordinates. Furthermore, in that vitamins D_2 and D_3 differ only in the nature of the side chain, a comparison of corresponding ring conformations in 25-OH-D_3 with those in the two conformers of vitamin D_3 and ECF and INC is especially informative.

The molecular structure of 25-OH-D_3 is illustrated in Figure 1. A prominent structural feature is the elongation of the molecule with the longest intramolecular distance being 18.9 Å between H(3) and H(27,2); the longest non-hydrogen intramolecular distance is O(3) · · · C(27) 17.8 Å. Other significant structural features include the exocyclic triene system, and the conformations of ring A, of the fused C,D-ring system, and of the side chain attached to C(17).

The packing of the 25-OH-D_3 and water molecules in the unit cell (Figure 2) is primarily dictated by hydrogen-bonding interactions which result in reasonably close O–H · · · O distances (*vide infra*). There appear to be no other unusual interactions in that there are no H · · · H separations between molecules less than the van der Waals contact distance of 2.4 Å. Closest intramolecular non-bonding distances are given in Table 2.

Conformation of the Triene System.—The exocyclic C(5):C(6)·C(7):C(8) *trans*-diene fragment and exocyclic C(10):C(19) double bond may be considered conjointly in a vitamin D molecule as a triene system in a *cis-Z-trans* conformation.[15] This system in 25-OH-D_3 involving C(4)—(10), C(14), and C(19) deviates significantly from planarity. However, all these carbon atoms except for C(19) are coplanar within 0.08 Å, while C(19) is 0.77 Å out of the same mean plane defined by the other carbon atoms. This large displacement of C(19) from a planar arrangement is readily attributed to the steric constraints imposed by the chair-shaped ring A. The near planarity in 25-OH-D_3 of the C(4)·C(5):C(6)·C(7):C(8)·C(14) *trans*-diene fragment, which gives rise to close H(4,1) · · · H(6,1) (2.29 Å) and H(6,1) · · · H(9,2) (2.24 Å) distances is also demonstrated from the fact that these six carbon atoms lie within 0.03 Å of the mean carbon plane and that the

* $R' = [\Sigma w_i||F_o| - |F_c||^2/\Sigma w_i|F_o|^2] \times 100$. All least-squares refinements were based on the minimization of $\Sigma w_i||F_o| - |F_c||^2$ with the individual weights $w_i = 1/\sigma_c^2(F_o)$. Atomic scattering factors were taken from ref. 31*a* for non-hydrogen and from ref. 31*b* for hydrogen atoms.

† See Notice to Authors No. 7 in *J.C.S. Perkin II*, 1975, Index issue.

[31] (*a*) D. T. Cromer and J. B. Mann, *Acta Cryst.*, 1968, **A24**, 3219; (*b*) R. F. Stewart, E. R. Davidson, and W. T. Simpson, *J. Chem. Phys.*, 1965, **42**, 3175.

[32] D. T. Cromer and D. Liberman, *J. Chem. Phys.*, 1970, **53**, 189.

C(5)–C(6)–C(7)–C(8) torsional angle is 177.0°. The corresponding *trans*-diene fragments in other vitamin D molecules are likewise almost planar, although less so than that in 25-OH-D_3, as can be seen from the larger deviation from 180° of the C(5)–C(6)–C(7)–C(8) torsional angle in D3α (170.3°),[20] D3β (174.3°),[20] ECF (169°),[18] and INC (167°).[17]

Figure 1 clearly reveals a twisting of the planar C(1)·C(10):C(19)H_2 fragment about the C(5)–C(10) axis from coplanarity with the *trans*-diene fragment such that the C(10):C(19)H_2 double bond is largely isolated from the conjugated *trans*-diene fragment. That the trigonal character of C(10) persists is manifested in its position less than 0.02 Å from the plane defined by its three attached carbon atoms C(1), C(5), and C(19). Figure 1 further shows that the observed orientation of the C(19)-H_2 substituent due to this highly nonplanar but more favourable C(6):C(5)·C(10):C(19) *cis*-diene arrangement gives rise to an H(7,1) · · · H(19,1) contact distance of 2.50 Å which is sufficiently long to imply no significant repulsive interaction. The large degree of distortion of the exocyclic C(6):C(5)·C(10):C(19) *cis*-diene fragment from planarity is evidenced by its torsional angle of 56.7° (*vs.* 0° for a planar system). The fact that similar angles of twisting of the corresponding *cis*-diene fragment are found in D3α (53.6°),[20] D3β (55.2°),[20] ECF (56°),[18] and INC (61°),[17] and that this torsional angle is essentially invariant to the two different chair-shaped conformations adopted by ring A (*vide infra*) indicates that the effect of the non-planarity of ring A on the twisting of the exocyclic *cis*-diene system is practically the same in both conformations of ring A.

Although the variations are expectedly small and on the edge of statistical significance, the bond-length distribution in the triene system of 25-OH-D_3 is in accord with the occurrence of considerable electron delocalization in the planar C(5):C(6)·C(7):C(8) *trans*-diene part relative to that in the twisted C(6):C(5)·C(10):C(19) *cis*-diene part. The C(10)–C(19) distance [1.325(3) Å *vs.* normal double-bond value 1.334 Å] is shorter than the C(5)–C(6) [1.339(3) Å] and C(7)–C(8) [1.343(3) Å] distances, while the C(5)–C(10) distance [1.486(4) Å] is longer than C(6)–C(7) [1.443(3) Å].

Conformation of Ring A.—The geometry of ring A is of particular interest in that crystallographic studies have uncovered its two chair conformations (readily differentiated from each other by the torsion angles) corresponding to the two rapidly equilibrating solution conformations which were deduced [13,14] from recent analyses by ^{1}H n.m.r. spectroscopy for vitamin D_2 [13] and for vitamin D_3, 1,25-$(OH)_2$-D_3, and other metabolites.[14] Figure 3 shows that the solid-state conformation of ring A in 25-OH-D_3 corresponds to the α-chair form, also found [20] subsequently in D3α, with the exocyclic CH_2 group situated below the mean cyclohexane-like ring plane and with the 3-hydroxy-group occupying the equatorial position. This conformation was initially discovered [17] for ring A in INC with the bulky 4-iodo-5-nitrobenzoate substituent at C(3) equatorial. The other conformation of ring A (β chair form), with the CH_2 group situated above the mean ring plane and with the 3-hydroxy-group in the axial position was first observed [18] in the crystalline state in ECF [which has a dioxolan ring substituted at C(3)] and later in D3β.

The existence of two adjacent exocyclic double bonds at C(5) and C(10) leads to a pronounced deformation of ring A from an idealized cyclohexane conformation. Because of the steric constraints imposed on the six-membered ring A, the endocyclic angles subtended at C(5) and C(10) are markedly reduced in 25-OH-D_3 from an unconstrained trigonal value of 120° to 113.4°. Nevertheless, the fact that these values are *ca.* 2.4° greater than

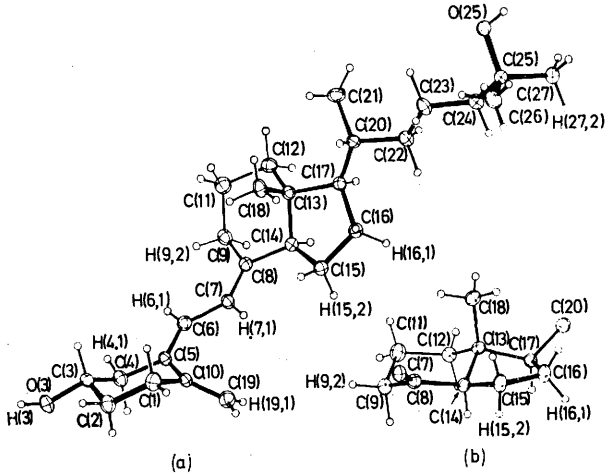


FIGURE 3 Solid-state conformations of (a) the entire 25-OH-D_3 molecule and (b) the C,D-ring system

the average intra-ring angle of 111.0° determined [33] for gaseous cyclohexane produces a significant decrease in ring puckering from that in regular cyclohexane ring, with the greatest geometrical deformation involving C(5) and C(10). This flattening effect for ring A in 25-OH-D_3 is manifested by the resulting endocyclic torsional angles (Table 2) which show a range from 49.8 to 54.0° with the actual values related in pairs by an idealized vertical mirror plane which passes through C(2) and C(5). The relative flatness of ring A in 25-OH-D_3 is also apparent from its mean torsion angle of 51.8°, which is significantly less than that of 55.9° in gaseous cyclohexane. Analogous ring flattening from a regular cyclohexane geometry (but with irregular trends) occurs in other vitamin D molecules with corresponding mean torsion angles of 53.8° in D3α, 50.1° in D3β, 52.5° in ECF, and 53° in INC.

Conformation of the C,D-*Ring System.*—The overall conformation of the C,D-ring system is analogous to those of D3α, D3β, INC, and ECF in that all corresponding torsional angles possess the same signs, although the corresponding magnitudes of individual torsional angles vary somewhat from one another. In 25-OH-D_3, ring C adopts a somewhat irregular chair conformation as can

[33] H. J. Geise, H. R. Buys, and F. C. Mijlhoff, *J. Mol. Structure*, 1971, **9**, 447.

be seen in Table 2 from the rather large range in torsion angles (51.9—59.1°). This geometrical distortion of ring C is due to its fusion with the five-membered ring D at C(13) and C(14) coupled with the steric constraints imposed by the planarity within 0.01 Å of the exocyclic double-bond fragment [at C(8)] comprising C(7)—(9) and C(14).

A geometrical constancy (to a first approximation) in the *trans*-fusion of the cyclopentane ring D to ring C at C(13) and C(14) (Figure 3) is apparently retained in both vitamin D molecules and steroids as evidenced from a comparison of the ring torsional angles $\phi_C \equiv \phi$ (8–14–13–12) and $\phi_D \equiv \phi$ (17–13–14–15). The ϕ_C and ϕ_D magnitudes of 58.6 and 45.6°, respectively, in 25-OH-D_3 are in reasonably close accord not only with those in ECF (58, 46.5°), D3α (61.7, 49.9°), D3β (62.0, 44.9°), and INC (49, 42°)* but also with those in a number of crystallographically analysed steroids (means 60.8° and 46.3° for eight compounds).[34] This relative invariance in the geometry of the C/D junction is also reflected by the experimental agreement within 2.5° of the values of the ring angles about C(13) and C(14) in 25-OH-D_3 with corresponding means [34] in the eight steroids. The *trans*-fusion of rings C and D in 25-OH-D_3 results in the adoption by ring D of an intermediate conformation between an ideal C(13)-envelope form (with mirror symmetry) and an ideal half-chair form (with two-fold symmetry). The ring-junction C(13) and C(14) atoms are perpendicularly displaced on opposite sides by −0.45 and 0.28 Å from the plane of the other three atoms of ring D. A gross representation of the conformation of ring D to a C(13)-envelope is indicated by the coplanarity of the other four ring atoms [C(14)—(17)] within 0.06 Å in contrast to C(13) which is 0.65 Å out of the mean plane of these four carbon atoms. However, a more precise geometrical description [34] of ring D in terms of a maximum torsion angle $\phi_{max.}$ and a conformational parameter Δ (denoted as the phase angle of pseudorotation) reveals ring D in the solid-state structure of 25-OH-D_3 to conform much more closely to a standard half-chair form. This analysis is based upon the computed Δ value of 6.7° being much nearer to zero (for a regular half chair) than to either 36° for a regular C(13) or −36° for a C(14)-envelope. A comparison of the $\phi_{max.}$ and Δ values in 25-OH-D_3 (45.7, 6.7°) with those in D3α (49.9, 0.6°), D3β (45.5°, 19.1°), and ECF (46.5, 3.8°) is especially informative. Whereas the narrow range of $\phi_{max.}$ values indicates a similar puckering of ring D, the wide variation in their pseudorotation parameters Δ indicates the flexibility of ring D to assume a variety of conformations in the solid-state ranging from an essentially half-chair form in D3α to much more of a C(13)-envelope form in D3β.

Conformation of the Side Chain.—The prime structural characteristic of the side chain of 25-OH-D_3 is the close conformity of its carbon skeleton to a planar zigzag conformation. The near planarity of this carbon backbone containing C(17), C(20), C(22)—(25), and C(27) is indicated by the maximum deviation of these atoms being only 0.17 Å from their mean carbon plane, from which C(13) is displaced by only 0.09 Å while C(14) is displaced by 0.87 Å. The extended zigzag arrangement adopted by the almost planar carbon framework of the side chain results in a very long distance of 7.48 Å between C(17) and C(27). Although this side-chain conformation in the solid state may be rationalized in part by the crystal packing (*vide infra*), its extended nature may also be expected to have biological implications.

The side chain on C(17) of each of the other compounds (except in ECF where it is substituted by a five-membered heterocyclic ring) also exists in an extended fashion. Whereas the nonhydroxylated side chain in D3α also possesses a nearly planar zigzag conformation (except for the crystal-disordered isopropyl fragment at the end of the side chain), the corresponding non-hydroxylated side chain in D3β is not in such a regular form. Although this difference in side chain conformations for the D3α and D3β molecules shows that non-bonded repulsive interactions can cause a change in geometry of the side chain, nevertheless, the similarity in corresponding C(17) · · · C(27) non-bonding distances in D3α and D3β of 7.24 and 6.74 Å, respectively, reflects the extended nature of their side chains in the crystalline state. The solid-state conformation of the vitamin D_2 side chain in INC is likewise not in a planar zigzag form. However, its particular arrangement, which is partly influenced by the steric requirements of its double bond between C(22) and C(23) and a methyl group substituent at C(24), also produces an extended conformation as indicated by the C(17) · · · C(27) separation of 6.61 Å.

Intermolecular Hydrogen Bonding.†—The molecular crystal packing of 25-OH-D_3 (Figure 2) is controlled mainly by hydrogen-bonding forces. Infinite chains (or strings) of hydrogen-bonded molecules are formed along the [O11] direction in one sheet (or layer) and along the [O$\bar{1}$1] direction in an adjacent sheet which forms a double layer with the first sheet through hydrogen bonding with water molecules. Each string consists of identically oriented molecules which are interconnected *via* an O–H · · · O tail-to-head linkage from an O(25)–H(25) group of one molecule to the O(3) atom from the adjacent molecule. Rows of these infinite chains (mutually related by translational symmetry in the *b* direction, and hence having the same projection down the *b* axis in Figure 2) are interconnected by water molecules to give a two-dimensional infinite hydrogen-bonded sheet (parallel to the *bc* plane) with each linkage along the *b* direction comprised of a spiral-like O(25)–H(25) · · · O(3^{I})–H(3^{I}) · · · O(W^{II})–H(W,2^{II}) · · · O(25^{V}) unit. In turn, two of these adjacent sheets (which are related by a screw axis) are joined together within each unit cell by O(W)–H(W,1) · · · O(25) hydrogen bonds. The observed values for the four independent O(25) · · · O(3^{I}), O(3^{I}) · · ·

* These values are probably not significantly different from the others owing to the much lower reliability of the torsion angles in INC (*vide supra*).

† In this section, Roman numeral superscripts refer to the positions defined in Table 2.

[34] C. Altona, H. J. Geise, and C. Romers, *Tetrahedron*, 1968, **24**, 13.

$O(W^{II})$, $O(W^{II}) \cdots O(25^{V})$, and $O(W) \cdots O(25)$ distances (2.87, 2.71, 2.94, and 2.88 Å) indicate reasonably strong hydrogen bonding interactions. Hence, all the hydrogen atoms of the two hydroxy-groups per 25-OH-D_3 molecule and of the one independent water molecule participate in some form of hydrogen bonding in the infinite double layer such that there is no interaction between double layers (Figure 2) other than normal van der Waals contacts (*i.e.*, the closest $H \cdots H$ separations are $\geqslant 2.5$ Å). This particular packing arrangement within the double layers apparently has the effect of shaping the side chain of 25-OH-D_3 into the observed extended planar zigzag conformation.

Relationship between Structure and Activity of Vitamin D Metabolites and Analogues.—Although it is currently recognized that 25-OH-D_3 must be converted into 1,25-$(OH)_2$-D_3 before it becomes physiologically active,[3] the present structural analysis may make some of the structure–function relationships of 25-OH-D_3 and other vitamin D metabolites and analogues more understandable. In the following discussions both the structural requirements of 25-OH-D_3 for either the 25-OH-D_3-1α-hydroxylase enzyme or the plasma transport proteins, and the requirements of vitamin D metabolites and analogues for the biological functions attributed to vitamin D will be considered.

Requirements of the Side Chain.—The fully extended side-chain feature of 25-OH-D_3 correlates with the markedly reduced physiological activity of the 24-nor-25-OH-D_3 [35] and the lack of activity of 22-27-hexanor-25-OH-D_3.[36] It may be that analogues of 25-OH-D_3 with shorter side chains are not transported as effectively on the 25-OH-D_3 binding protein to the kidney for 1α-hydroxylation. Apparently the 25-hydroxy-group must also be at the spatial position elicited by a fully extended side chain for the conversion into the more polar 1-hydroxylated metabolite. Substitutions on the side chains such as a methyl group on C(24) (as in the case of 25-OH-D_2) apparently make little difference to biological activity.[37] The $C(17) \cdots C(25)$ intramolecular distance observed in crystalline INC is 5.81 Å, even though the side chain is not fully extended. It is expected that at full extension, the side chain of 25-OH-D_2 would become longer and closer to the value of 6.41 Å found for the corresponding $C(17) \cdots C(25)$ distance in the fully extended side chain of 25-OH-D_3. On the other hand, the $C(17) \cdots C(25)$ distance at full extension of the side chain in 24-nor-25-OH-D_3 [corresponding to the $C(17) \cdots C(24)$ distance in 25-OH-D_3 as the side chain contains one less carbon atom] is estimated to be only 5.0 Å, which represents an apparent shortening by *ca.* 1.4 Å compared to that in 25-OH-D_3.

The direct participation of the 25-hydroxy-group of the side chain in performing the known biological functions of vitamin D appears to be very probable since the peak activity of the non-25-hydroxylated 1α-OH-D_3 (which occurs later than that of 1α,25-$(OH)_2$-D_3 [38]) is found [38a-c] to be preceded by a conversion of 1α-OH-D_3 into 1α,25-$(OH)_2$-D_3.

Requirements of the Ring System.—The structural requirements of the rings and substituents on the rings necessary for the optimization of biological activities of vitamin D metabolites and analogues in general, and for the metabolism of 25-OH-D_3 in particular, are more complex to analyse.

The fact that the C,D-ring system and the seco-B ring fragment which bridges rings A and C of all five structurally known vitamin D derivatives display similar solid-state conformations (reflecting a relatively rigid framework) leads to the conclusion that this part of the molecule is conformationally invariant in all biologically active vitamin D molecules. There remain several salient structural features for further consideration in connection with the physiological activities, *viz.* the importance of the 1α-hydroxy-group and its orientation relative to the two known chair conformations of ring A, the possible function of the 3β-hydroxy-group on ring A, and the role of the methylene $C(19)H_2$ substituent at C(10).

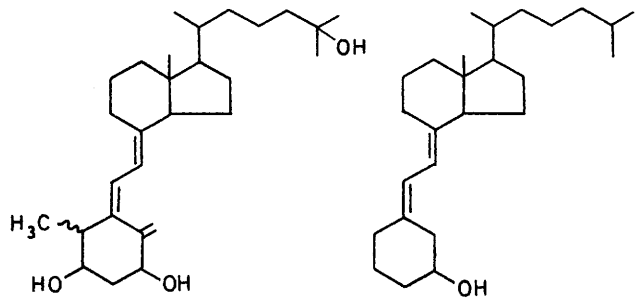
It has been recently emphasized that the 1α-hydroxy-group and concomitantly the conformation of ring A play a key role in producting all the biological functions attributed to vitamin D_3.[16] This conclusion was based on the observation that the 1α- or pseudo-1α-hydroxylation has enabled metabolites and analogues of vitamin D_3 with slightly different ring A structures [*viz.* 1α,25-$(OH)_2$-D_3,[11] 1α-hydroxyvitamin D_3 (1α-OH-D_3),[3a] 3-deoxy-1α-hydroxyvitamin D_3 (3-d-1α-OH-D_3),[39,40] 25-hydroxy-5,6-*trans*-vitamin D_3 (25-OH-5,6-*trans*-D_3),[35] 25-hydroxydihydrotachysterol$_3$ (25-OH-DHT_3),[41] and 1,24,25-trihydroxyvitamin D_3 [1,24,25-$(OH)_3$-D_3) [3a]] to possess in different degrees the biopotency to stimulate intestinal calcium transport. These considerations resulted in a suggestion [16] that the 1α-hydroxy-groups of 1α,25-$(OH)_2$-D_3 or its geometrical equivalent in analogues must occupy the equatorial orientation, which requires ring A to adopt the β conformation for the optimization of biological activity.

The possible biological functions of the 3β-hydroxy- and methylene $C(19)H_2$-groups appear to be unresolved as yet. Okamura *et al.*[16] suggested that these substituent groups are not necessary for vitamin-D biological activities because active analogues such as [3-^{2}H]-1α-OH-D_3,

[35] M. F. Holick, M. Garabedian, and H. F. DeLuca, *Biochemistry*, 1972, **11**, 2715.
[36] M. F. Holick, M. Garabedian, H. K. Schnoes, and H. F. DeLuca, *J. Biol. Chem.*, 1975, **250**, 226.
[37] T. Suda, H. F. DeLuca, and Y. Tanaka, *J. Nutrition*, 1970, **100**, 1049.
[38] (*a*) M. F. Holick, S. A. Holick, T. Tavela, B. Gallagher, H. K. Schnoes, and H. F. DeLuca, *Science*, 1975, **190**, 576; (*b*) M. F. Holick, T. F. Tavela, S. A. Holick, H. K. Schnoes, H. F. DeLuca, and B. M. Gallagher, *J. Biol. Chem.*, 1976, **251**, 1020; (*c*) S. A. Holick, M. F. Holick, T. E. Tavela, H. K. Schnoes, and H. F. DeLuca, *ibid.*, 1976, **251**, 1025; (*d*) A. W. Norman, M. N. Mitra, W. H. Okamura, and R. M. Wing, *Science*, 1975, **188**, 1013.
[39] H. Y. Lam, B. L. Onisko, H. K. Schnoes, and H. F. DeLuca, *Biochem. Biophys. Res. Comm.*, 1974, **59**, 845.
[40] W. H. Okamura, M. N. Mitra, R. M. Wing, and A. W. Norman, *Biochem. Biophys. Res. Comm.*, 1974, **60**, 179.
[41] R. B. Hallick and H. F. DeLuca, *J. Biol. Chem.*, 1972, **247**, 91.

DHT_3, 5,6-*trans*-D_3, and isotachysterol$_3$ (iso-T_3) [42] do not possess a hydroxy-substituent at the 3β-position, while the latter three compounds do not possess a methylene group at the carbon atom which geometrically corresponds to the C(10) position of ring A. However, the three latter compounds are at least two orders of magnitude less active than 1α,25-$(OH)_2$-D_3, and furthermore, while the [3-^{2}H]-1α-OH-D_3 is found to be active on the intestine,[38d] it is relatively inactive on calcium mobilization or mineralization of bone.[43] The 25-OH-5,6-*trans*-D_3 was also found to be unable to stimulate bone calcium mobilization.[35] These considerations lead us to suggest that the 3-hydroxy-group may be important for all functions.

The very large reduction, but not complete elimination of biological activity in the 5,6-*trans*-D_3, DHT_3, and iso-T_3 which do not possess a methylene group at the pseudo-19 position indicates that either this methylene group is important or the substituent group (methyl in DHT_3 and iso-T_3, and methylene in 5,6-*trans*-D_3) which occupies the pseudo-4 position sterically hinders an interaction with the receptor. To differentiate between these two possibilities, biological assays with compounds such as the epimeric pair of 4α- and 4β-methyl-1α,25-dihydroxyvitamin D_3 (III) which have both a methylene group at



(III) (IV)

the 10- and a methyl substituent at the 4-position, and the 19-nor-3-deoxy-1α-hydroxyvitamin D_3 (IV) which has neither group at the 4- and 10-positions, should be carried out. If the two former compounds are active then the C(10):C(19)H_2 fragment is necessary, while the 4-methyl group definitely constitutes a steric hindrance if the biological activities of these two compounds are much reduced. Similarly, if the 19-nor-3-deoxy-1α-hydroxyvitamin D_3 is not active, then the C(19)H_2 substituent of vitamin D molecules is important for physiological responses, while if the 19-nor-3-deoxy-1α-hydroxyvitamin D_3 (which is also the 19-nor-5,6-*trans*-vitamin D_3, *i.e.*, the 5,6-*trans*-D_3 with the methylene group at the pseudo-4-position eliminated) is more active than the 5,6-*trans*-D_3, then a methylene or methyl substituent at the pseudo-4-position of 5,6-*trans*-D_3 and DHT_3 is not desirable.

The structural requirements of ring A in 25-OH-D_3 (and 25-OH-D_2) for renal conversion to 1,25-$(OH)_2$-D_3 are more difficult to assess owing to lack of existing information. N.m.r. proton spin–spin splitting studies have suggested that in solution, ring A of vitamins D_2 and D_3 (which should be very similar if not identical in behaviour to that of 25-OH-D_3) exhibits a dynamic equilibrium in approximately equimolar mixtures of the α- and β-conformations [13,14] which were later found in the solid state by *X*-ray diffraction analyses.[19,20] The present work has shown that in crystalline 25-OH-D_3, ring A possesses the α-conformation with the 3β-OH-group occupying the equatorial position. With the assumption that the activities of these metabolites are related to the ability of their hydroxy-groups to form hydrogen bonds with receptor active sites, it is expected that this α conformation (in which the 3β-OH-group occupies the more exposed equatorial position) would be preferred, and hence that required by the plasma transport proteins,[25] and/or by the active site of the renal 25-OH-vitamin-D_3-1α-hydroxylase. This speculation is consistent with the suggestion [16] that for the optimization of the biological response the 1α-hydroxy-group in 1α,25-$(OH)_2$-D_3 must likewise occupy the more exposed equatorial orientation which necessitates ring A to possess the α-conformation. If this hypothesis is correct, then the ease of interconversion of ring A between the α- and β-conformations is a key factor in providing an efficient method for ring A to adopt the α-conformation in 25-OH-D_3 and the β-conformation in 1α,25-$(OH)_2$-D_3.

We thank the National Science Foundation and the National Institutes of Health for grants (to L. F. D. and H. F. D.), and the Ford Foundation for a Postdoctoral Fellowship (to T.-T.) and the Graduate School of the University of Wisconsin-Madison for partial support (of T.-T.). The use of the UNIVAC 1110 computer at the Academic Computing Center, University of Wisconsin–Madison was made available through partial support of the National Science Foundation and the Wisconsin Alumni Research Foundation administered through the University Research Committee. We thank the Philips–Duphar Company for a sample of 25-hydroxyvitamin D_3.

[6/627 *Received*, 1*st April*, 1976]

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[43] L. Reeve, P. H. Stern, H. Y. Lam, H. K. Schnoes, and H. F. DeLuca, unpublished results.