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Crystal and Molecular Structure of 1,25-Dihydroxycholecalciferol

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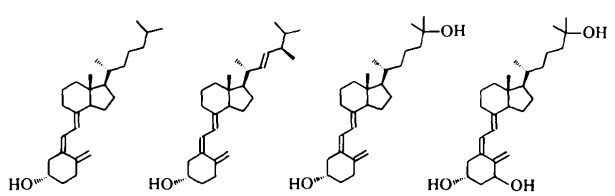
Abstract

The structure of 1,25-dihydroxycholecalciferol, 1,25-dihydroxy-9,10-secocholesta-5,7,10(19)-trien-3-ol, has been solved by direct methods and refined against F^2 to R_1 = 0.08 for 2637 reflections with $F > 4\sigma(F)$ and 0.222 for all 3412 data. Crystal data: $C_{27}H_{44}O_3.3H_2O$, M_r = 470.67, orthorhombic, $P2_12_12_1$, a = 6.234 (2), b = 15.628 (3), c = 30.749 (10) Å, V = 2995.7 (15) Å^3, Z = 4, D_x = 1.044 Mg m^{-3}, (Cu $K\alpha$) = 1.54178 Å, μ = 0.572 mm^{-1}, $F(000)$ = 1040, room temperature. Of the three water molecules present in the crystals of this compound, one is involved in a network of hydrogen bonds formed with the hydroxyl groups of the compound, the remaining two are disorderly arranged in channels formed along the crystallographic x axis with no hydrogen-bonding interactions with the compound molecules. The conformation of the cyclohexane-like ring A was found to be exclusively in the β form. Stabilization of the conformer in the solid state may be due to the formation of hydrogen bonds to water molecules, as well as to the symmetry-related 1,25-$(OH)_2$-vitamin D_3 molecules.

1. Introduction

The recent discovery of new areas of the biological activity of the D vitamins has greatly stimulated further interdisciplinary research in this field (DeLuca, 1992). Recently, new synthetic analogues of 1,25-dihydroxycholecalciferol [1,25-dihydroxy-vitamin D_3, 1,25-$(OH)_2D_3$, calcitriol] were obtained with the reversed profile of biological activity.



D_3 D_2 25-OH-D_3 1,25-$(OH)_2D_3$

These analogues showed a reduced or abolished regulatory effect on calcium and phosphorous homeostasis combined with an enhanced activity in the stimulation of cell differentiation and in the inhibition of cell proliferation (Perlman, Kutner, Prahl, Smith, Inaba, Schnoes & DeLuca, 1990). Current research in the field is mainly directed to the development of new analogues of 1,25-$(OH)_2D_3$ with a selective activity profile as potential therapeutic agents in a number of hyperproliferative diseases. Attempts are also being made to determine the three-dimensional structure of the vitamin D receptor (VDR) and to elucidate the molecular mechanism of the vitamin D unique dual activity (calcitropic and cell differentiation). Due to our still underdeveloped knowledge of the structure of the active site of VDR, various indirect approaches were developed, enabling the design of new analogues of 1,25-$(OH)_2D_3$. Molecular modelling accompanied by semi-empirical calculations is at present one of the leading approaches in this strategy (Choliński, Midland & Kutner, 1995; Wilson & Guanieri, 1991; Hofer & Kahling, 1991; Okamura, Palenzuela, Plumet & Midland, 1992; Midland, Plumet & Okamura, 1993). There was, therefore, a need to determine the X-ray structure of 1,25-$(OH)_2D_3$, which might serve as a set of starting parameters for molecular modelling and quantum chemistry calculations. The crystal structure was determined for the main D vitamins (D_2 and D_3), the circulating metabolite of vitamin D (25-OH-D_3), a synthetic intermediate (PTAD adduct of pre-25-OH-D_3) and an analogue (calcipotriol) over the last two decades (Hull, Leban, Main, White & Woolfson, 1976; Trinh-Toan, DeLuca & Dahl, 1976; Trinh-Toan, Ryan, Simon, Calabrese & Dahl, 1977; De Clercq, Zhu, van Haver & Jurriaans, 1994; van Meersche, Tinant, Germain, Declercq, Vanmaele, De Clercq & Vandewalle, 1982; Larsen, Hansen, Hoffmeyer & Rastrup-Andersen, 1993). However, the X-ray structure of the most active hormonal form of vitamin D_3, *i.e.* 1,25-$(OH)_2D_3$, was still missing. One reason might be that 1,25-$(OH)_2D_3$, available mainly as a pharmaceutical substance in the non-solvated form, resists the formation of crystals large enough for X-ray diffraction studies.

As part of our search for new therapeutic agents in the vitamin D group (Kutner, Zhao, Fitak & Wilson, 1995) we prepared, and first describe in the present paper, the single crystal structure of 1,25-$(OH)_2D_3$, which is a biologically important molecule.

2. Experimental

2.1. *Preparation of the crystals*

A sample of 1,25-dihydroxycholecalciferol was obtained from INFARM (Warsaw, Poland) by the previously described method (Ryznar, 1988). The non-solvated crystals of 1,25-$(OH)_2D_3$, not suitable for X-ray crystal structure determination, were dissolved in ethylformate, which was proved not to form solvates, and left to slowly evaporate at room temperature. After 2 weeks needle-shaped transparent colourless crystals had grown.

2.2. *Data collection and structure refinement*

The details of experimental conditions and structure refinement are summarized in Table 1.* Atomic scattering factors were from *International Tables for Crystallography* (1992). The *SDP* system (B. A. Frenz & Associates Inc., 1982) was used for data reduction. The structure was solved by direct methods (*SHELXS*86; Sheldrick, 1990) and refined on F^2 by full-matrix least-squares with *SHELXL*93 (Sheldrick, 1993). The H atoms were included into calculations in idealized geometrical positions, except those of hydroxyl groups and water molecules which were located on difference electron-density maps and were refined as riding atoms on their C or O atoms. The final refinement was performed using anisotropic thermal parameters (isotropic for H atoms). The final atomic parameters are given in Table 2.* Due to the two disordered water molecules the ratio of observed:unobserved reflections is 2:1 and the quality of data is rather poor, but the biological importance of the compound convinced us to solve the structure and refine it as well as possible.

Table 1. *Experimental details*

Crystal data	
Chemical formula	$C_{27}H_{44}O_3.3H_2O$
Chemical formula weight	470.67
Cell setting	Orthorhombic
Space group	$P2_12_12_1$
a (Å)	6.234 (2)
b (Å)	15.628 (3)
c (Å)	30.749 (10)
V (Å^3)	2995.7 (15)
Z	4
D_x (Mg m^{-3})	1.044
Radiation type	Cu $K\alpha$
Wavelength (Å)	1.54178
No. of reflections for cell parameters	20
θ range (°)	8–16
μ (mm^{-1})	0.572
Temperature (K)	293 (2)
Crystal form	Needle
Crystal size (mm)	0.65 × 0.20 × 0.15
Crystal colour	Colourless
Data collection	
Diffractometer	Enraf–Nonius CAD-4
Data collection method	ω–2θ
Absorption correction	None
No. of measured reflections	3412
No. of independent reflections	3412
No. of observed reflections	1436
Criterion for observed reflections	$I > 2\sigma(I)$
θ_{max} (°)	76.61
Range of h, k, l	$-7 \rightarrow h \rightarrow 0$ $0 \rightarrow k \rightarrow 19$ $-38 \rightarrow l \rightarrow 0$
No. of standard reflections	3
Frequency of standard reflections (min)	60
Intensity decay (%)	±0.5
Refinement	
Refinement on	F^2
$R[F^2 > 2\sigma(F^2)]$	0.0803
$wR(F^2)$	0.1854
S	1.212
No. of reflections used in refinement	2637
No. of parameters used	308
H-atom treatment	See text
Weighting scheme	$w = 1/[\sigma^2(F_o^2) + (0.1038P)^2 + 0.0000P]$, where $P = (F_o^2 + 2F_c^2)/3$
$(\Delta/\sigma)_{max}$	−0.001
$\Delta\rho_{max}$ (e Å^{-3})	0.350
$\Delta\rho_{min}$ (e Å^{-3})	−0.238
Extinction method	*SHELXL*93 (Sheldrick, 1993)
Extinction coefficient	0.0029 (5)
Source of atomic scattering factors	*International Tables for Crystallography* (1992, Vol. C, Tables 4.2.6.8 and 6.1.1.4)

3. Results and discussion

Fig. 1 shows the *ORTEP*II (Johnson, 1976) representation of the solid-state conformation of 1,25-$(OH)_2D_3$, together with the numbering scheme. The bond lengths and bond angles are in ranges typical for steroid-type compounds. Due to the low accuracy of the structure determination, tables of bond distances, angles and torsion angles are available as supplementary material.* Both cyclohexane-like rings (*A* and *C* in steroid notation) adopt a slightly distorted chair conformation. The magnitude of distortion is 7 (1)° in the twist-boat direction and 6.6 (9)° in the boat direction for the rings *A* and *C*, respectively (Cremer & Pople, 1975). The cyclopentane-like ring (*D* in steroid notation) adopts a half-chair conformation with the psuedo-twofold axis of symmetry passing through C(16) and bisecting the C(13)—C(14) bond. The r.m.s. plane through the atoms C(17), C(20), C(22), C(23), C(24) and C(25) shows the aliphatic side chain to be approximately planar. In the vitamin D structures the geometry of ring *A* is of particular interest as it is believed to be responsible for binding the vitamin molecule to the VDR binding site. Comparison with other D vitamins, *i.e.* vitamins D_2 and D_3, vitamin D metabolite, 25-OH-D_3, and the 1,24-dihydroxylated analogue of vitamin D_3, reveals the different conformational behaviour of ring *A* in these five compounds. In the case of vitamin D_2 and D_3, which

* Lists of atomic coordinates, anisotropic displacement parameters and structure factors have been deposited with the IUCr (Reference: NA0072). Copies may be obtained through The Managing Editor, International Union of Crystallography, 5 Abbey Square, Chester CH1 2HU, England.

Table 2. *Fractional atomic coordinates and equivalent isotropic displacement parameters (Å²)*

$$U_{eq} = (1/3)\Sigma_i\Sigma_j U_{ij} a_i^* a_j^* \mathbf{a}_i.\mathbf{a}_j.$$

	x	y	z	U_{eq}
O(1)	0.8885 (10)	0.7624 (4)	0.8743 (2)	0.058 (2)
C(1)	1.0838 (13)	0.7214 (5)	0.8613 (2)	0.041 (2)
C(2)	1.1356 (14)	0.6473 (5)	0.8916 (2)	0.046 (2)
C(3)	1.3466 (16)	0.6024 (5)	0.8799 (2)	0.051 (2)
O(3)	1.5256 (10)	0.6569 (5)	0.8875 (2)	0.066 (2)
C(4)	1.3460 (16)	0.5765 (5)	0.8324 (2)	0.051 (2)
C(5)	1.2797 (13)	0.6465 (5)	0.8011 (2)	0.036 (2)
C(10)	1.0814 (14)	0.6936 (5)	0.8141 (2)	0.041 (2)
C(19)	0.9152 (16)	0.7070 (6)	0.7887 (3)	0.065 (3)
C(6)	1.3858 (13)	0.6611 (5)	0.7650 (2)	0.039 (2)
C(7)	1.3465 (14)	0.7280 (5)	0.7330 (2)	0.042 (2)
C(8)	1.4507 (14)	0.7408 (5)	0.6954 (2)	0.043 (2)
C(9)	1.6378 (14)	0.6868 (6)	0.6792 (2)	0.050 (2)
C(11)	1.6088 (16)	0.6600 (6)	0.6316 (2)	0.055 (2)
C(12)	1.5495 (12)	0.7330 (5)	0.6008 (2)	0.039 (2)
C(13)	1.3562 (12)	0.7808 (5)	0.6175 (2)	0.034 (2)
C(14)	1.4018 (13)	0.8132 (4)	0.6642 (2)	0.037 (2)
C(15)	1.2195 (16)	0.8739 (5)	0.6751 (2)	0.055 (3)
C(16)	1.1714 (16)	0.9171 (5)	0.6301 (2)	0.051 (2)
C(17)	1.3069 (14)	0.8687 (5)	0.5953 (2)	0.041 (2)
C(18)	1.1547 (13)	0.7240 (5)	0.6154 (3)	0.050 (2)
C(20)	1.2025 (14)	0.8695 (5)	0.5494 (2)	0.039 (2)
C(21)	1.3518 (18)	0.8323 (6)	0.5149 (2)	0.073 (3)
C(22)	1.1324 (18)	0.9607 (5)	0.5378 (2)	0.061 (3)
C(23)	1.0152 (16)	0.9700 (5)	0.4947 (2)	0.053 (2)
C(24)	0.9042 (16)	1.0561 (5)	0.4905 (2)	0.052 (2)
C(25)	0.8154 (14)	1.0808 (5)	0.4465 (2)	0.040 (2)
O(25)	0.7027 (10)	1.1619 (3)	0.4541 (2)	0.053 (2)
C(26)	0.9829 (19)	1.0936 (7)	0.4133 (3)	0.080 (3)
C(27)	0.6504 (18)	1.0180 (6)	0.4304 (3)	0.075 (3)
O(w1)	1.5800 (13)	0.6838 (4)	0.9751 (2)	0.089 (3)
O(w2)*	0.022 (8)	0.5564 (26)	0.2094 (10)	0.273 (18)
O(w3)*	0.232 (7)	0.5641 (21)	0.2269 (11)	0.251 (15)
O(w4)*	0.449 (8)	0.5219 (27)	0.2028 (11)	0.290 (19)
O(w5)*	0.704 (8)	0.5511 (23)	0.1978 (11)	0.265 (16)

* s.o.f. = 0.5.

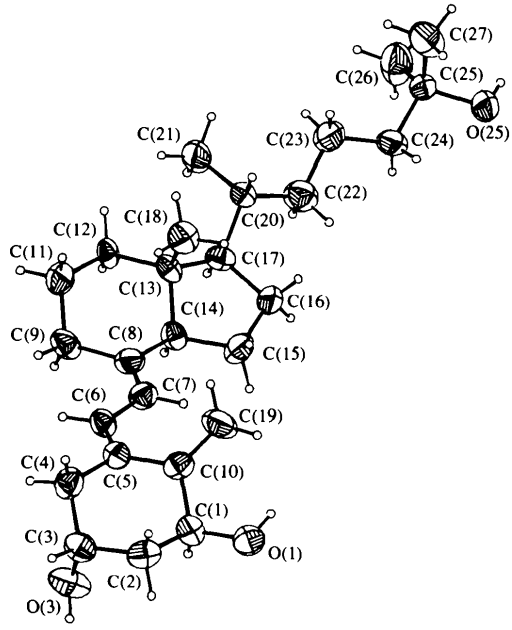


Fig. 1. The molecular structure of 1,25-$(OH)_2D_3$. Thermal displacement ellipsoids are drawn at the 50% probability level (*ORTEP*II; Johnson, 1976).

appear to be isostructural, two independent molecules in the unit cell were found: one molecule with ring *A* in the α-chair form in which the hydroxyl group at C(3) is in an axial position, whereas in the second molecule it is equatorial – β-chair form [Fig. 2 (Hull, Leban, Main, White & Woolfson, 1976; Trinh-Toan, DeLuca & Dahl, 1976)]. For 25-OH-D_3, only the α conformer was observed in the solid state (Trinh-Toan, Ryan, Simon, Calabrese & Dahl, 1977), while the 1,24-dihydroxylated analogue of vitamin D_3 (calcipotriol) exists in crystals as the conformer only [notation α given by authors is incorrect (Larsen, Hansen, Hoffmeyer & Rastrup-Andersen, 1993)]. In the structure of 1,25-$(OH)_2D_3$ reported here ring *A* exists exclusively in the β-chair form with the hydroxyl groups at C(1) and C(3) in equatorial and axial positions, respectively (as found for calcipotriol). Stabilization of the β conformer in the solid state can be due to both the presence of the hydroxyl group at C(1) and the formation of the hydrogen bond to O(*w*1) of the water molecule. The three hydroxyl groups of 1,25-$(OH)_2D_3$ are involved in a network of hydrogen bonds to the O(*w*1) water molecule and symmetry-related 1,25-$(OH)_2D_3$ molecules (Fig. 3). Detailed geometry of the hydrogen bonds formed is summarized in Table 3.

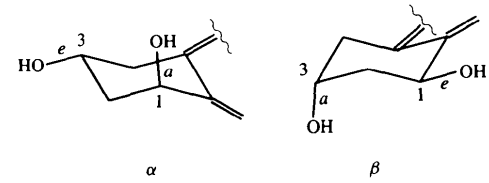


Fig. 2. Two different chair-like conformations of ring *A* existing in the D vitamins and their derivatives.

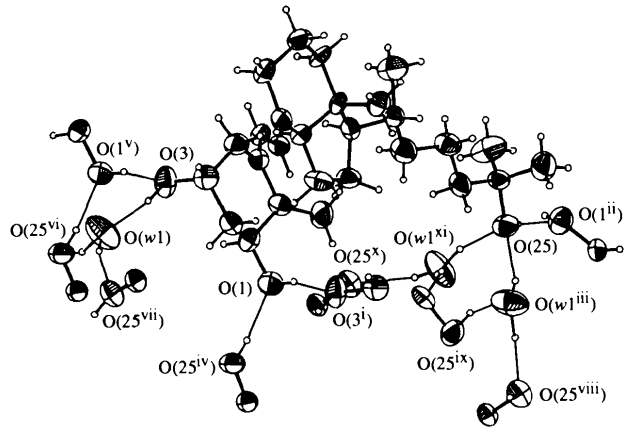


Fig. 3. The network of hydrogen bonds formed by the 1,25-$(OH)_2D_3$ molecule to adjacent water and symmetry-related 1,25-$(OH)_2D_3$ molecules. Symmetry codes: (i) $x-1, y, z$; (ii) $\frac{3}{2}-x, 2-y, z-\frac{1}{2}$; (iii) $2-x, \frac{1}{2}+y, \frac{3}{2}-z$; (iv) $\frac{3}{2}-x, 2-y, \frac{1}{2}+z$; (v) $1+x, y, z$; (vi) $\frac{5}{2}-x, 2-y, \frac{1}{2}+z$; (vii) $2-x, y-\frac{1}{2}, \frac{3}{2}-z$; (viii) $x-\frac{1}{2}, \frac{5}{2}-y, 1-z$; (ix) $2-x, \frac{1}{2}+y, \frac{3}{2}-z$; (x) $\frac{1}{2}+x, \frac{5}{2}-y, 1-z$; (xi) $\frac{5}{2}-x, 2-y, z-\frac{1}{2}$ (*ORTEP*II; Johnson, 1976).

Contrary to the single conformation in the solid state, 1,25-$(OH)_2D_3$, in solution a dynamic equilibrium exists between almost equimolar populations of both α and β conformers of the *A* ring (Wing, Okamura, Rego, Pirio & Norman, 1975) and a similar distribution of conformers was also obtained from our molecular mechanics modelling. However, the NMR experiments were performed in non-aqueous solvent ($CDCl_3$) and the molecular mechanics calculations were performed for the isolated molecule. The solid-state conformation of 1,25-$(OH)_2D_3$ described here indicates the easy formation of hydrogen bonds between molecules of the compound, as well as to the water molecules.

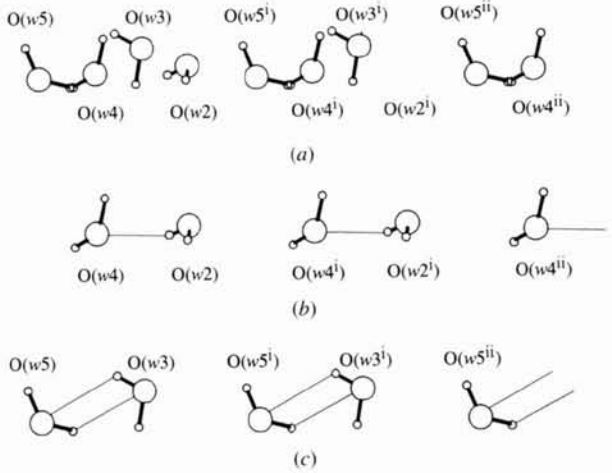


Fig. 4. Arrangement of water molecules in molecular channels: (*a*) positions of water molecules as found during crystal structure analysis; (*b*) chain formed by water molecules O(*w*2) and O(*w*4) with hydrogen bonds; (*c*) chain formed by water molecules O(*w*3) and O(*w*5) with hydrogen bonds (each water molecule with s.o.f. = 0.5). Symmetry codes: (i) $x-1, y, z$; (ii) $x-2, y, z$.

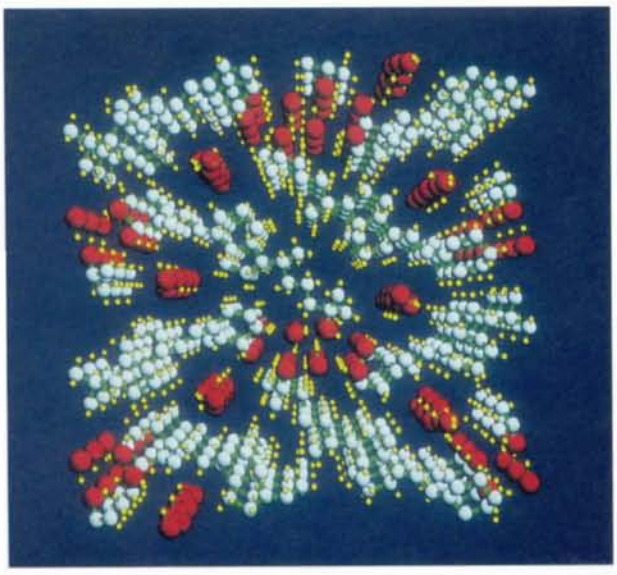


Fig. 5. View of the structure along the molecular channels 'filled' with water molecules (*ATOMS*; Dowty, 1994).

Table 3. *Hydrogen-bonding geometry (Å, °)*

D—H···*A*	*D*···H	H—*A*	*D*···*A*	*D*—H···*A*
O(1)—H(1)···O(3^{i})	0.76 (10)	2.14 (10)	2.829 (9)	152 (10)
O(3)—H(3)···O(w1^{ii})	0.92 (9)	1.86 (9)	2.748 (8)	162 (9)
O(25)—H(25)···O(1^{iii})	0.82 (3)	1.96 (3)	2.782 (8)	175 (6)
O(w1)—H(w1a)···O(25^{iv})	0.937 (8)	1.912 (9)	2.820 (9)	162.9 (7)
O(w1)—H(w1b)···O(25^{v})	0.922 (9)	1.961 (8)	2.840 (9)	158.8 (8)
O(w2)—H(w2a)···O(w4^{ii})	0.63 (6)	2.16 (7)	2.72 (7)	151 (5)
O(w3)—H(w3a)···O(w5^{ii})	0.90 (6)	2.57 (6)	3.08 (6)	117 (4)
O(w4)—H(w4a)···O(w2^{ii})*	1.14 (5)	2.69 (7)	2.72 (7)	80 (1)
O(w5)—H(w5b)···O(w3^{ii})	1.02 (6)	2.33 (6)	3.08 (6)	129 (3)

Symmetry codes: (i) $x-1, y, z$; (ii) x, y, z; (iii) $\frac{3}{2}-x, 2-y, z-\frac{1}{2}$; (iv) $2-x, y-\frac{1}{2}, \frac{3}{2}-z$; (v) $\frac{5}{2}-x, 2-y, \frac{1}{2}+z$. * Not considered as a hydrogen bond due to an unfavourable *D*—H···*A* angle.

The 1,25-$(OH)_2D_3$ molecules are packed in the unit cell in such a way so as to form channels along the crystallographic *x* axis. The channels are filled with the remaining two disordered water molecules not involved in hydrogen bonding to the dihydroxylated vitamin molecules. The distances between water O atoms are in the range 1.42 (6)–2.01 (7) Å; the accepted model for the disorder postulates two independent chains of water molecules, with one dislocated with respect to another by the distance *a*/4 and the occupancy factor 0.5 (Fig. 4). One chain is formed by the O(*w*4)···O(*w*2)···O(*w*4^{i}) water molecules [O···O distances equal to 2.72 (7) and 3.62 (7) Å, respectively] and the other consists of O(*w*5)···O(*w*3)···O(*w*5^{i}) water molecules [O···O distances equal to 3.08 (6) and 3.42 (6) Å, respectively; symmetry code: (i) $-1+x, y, z$]. From the distances between O atoms, as well as from the intermolecular O···H distances in each channel (Table 3), it is clear that the water molecules in channels exist as dimers rather than forming the continuous hydrogen-bonded chain. The packing along the channels filled with water molecules is illustrated in Fig. 5.

Work is under way in these laboratories to determine the structure of the non-solvated (and non-hydrated) form of 1,25-$(OH)_2D_3$, as well as the structures of synthetic analogues selected for pre-clinical studies.

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References

B. A. Frenz & Associates Inc. (1982). *SDP Structure Determination Package*. College Station, Texas, USA.

Choliński, J., Midland, M. & Kutner, A. (1995). *Pol. J. Chem.* **69**, 229–234.

Cremer, D. & Pople, J. A. (1975). *J. Am. Chem. Soc.* **97**, 1354–1358.

De Clercq, P. J., Zhu, G.-D., van Haver, D. & Jurriaans, H. (1994). *Vitamin D: A Pluripotent Hormone*, edited by A. W. Norman, R. Bouillon & M. Thomasset, p. 311. Berlin, New York: Walter de Gruyter.

DeLuca, H. F. (1992). *Drug News Perspect.* **5**, 8792.

Dowty, E. (1994). *ATOMS. A Computer Program for Displacing Atomic Structures*. IBM-PC Version 2.3, 32-bit. Kingsport, Tennessee: SHAPE Software.

Hofer, O. & Kahling, H. (1991). *Proceedings of the Eighth Workshop on Vitamin D*, edited by R. Bouillon, A. W. Norman & M. Thomasset, pp. 190–191. Berlin, New York: Walter de Gruyter.

Hull, S. E., Leban, I., Main, P., White, P. S. & Woolfson, M. M. (1976). *Acta Cryst.* **B32**, 2374–2381.

Johnson, C. K. (1976). *ORTEP*II. Report ORNL-5138. Oak Ridge National Laboratory, Tennessee, USA.

Kutner, A., Zhao, H., Fitak, H. & Wilson, S. R. (1995). *Bioorg. Chem.* **23**, 22–32.

Larsen, S., Hansen, E. T., Hoffmeyer, L. & Rastrup-Andersen, N. (1993). *Acta Cryst.* C**49**, 618–621.

Meersche, M. van, Tinant, B., Germain, G., Declercq, J. P., Vanmaele, L. J., De Clercq, P. J. & Vandewalle, M. E. (1982). *Bull. Soc. Chim. Belg.* **91**, 205–211.

Midland, M. M., Plumet, J. & Okamura, W. H. (1993). *Bioorg. Med. Chem. Lett.* **3**, 1799–1804.

Okamura, W. H., Palenzuela, A., Plumet, J. & Midland, M. M. (1992). *J. Cell Biochem.* **49**, 1018.

Perlman, K. L., Kutner, A., Prahl, J. M., Smith, C., Inaba, M., Schnoes, M. K. & DeLuca, H. F. (1990). *Biochemistry*, **29**, 190–196.

Ryznar, T. (1988). *Acta Pol. Pharm.* **45**, 214–218.

Sheldrick, G. M. (1990). *Acta Cryst.* A**46**, 467–473.

Sheldrick, G. M. (1993). *SHELXL*93. *Program for the Refinement of Crystal Structures*. University of Göttingen, Germany.

Trinh-Toan, DeLuca, H. F. & Dahl, L. F. (1976). *J. Org. Chem.* **41**, 3476–3478.

Trinh-Toan, Ryan, R. C., Simon, G. L., Calabrese, J. C. & Dahl, L. F. (1977). *J. Chem. Soc., Perkin Trans. II*, pp. 393–401.

Wilson, S. R. & Guanieri, E. (1991). *Proceedings of the Eighth Workshop on Vitamin D*, edited by R. Bouillon, A. W. Norman & M. Thomasset, pp. 186–187. Berlin, New York: Walter de Gruyter.

Wing, R. M., Okamura, W. H., Rego, A., Pirio, M. R. & Norman, A. W. (1975). *J. Am. Chem. Soc.* **97**, 4980–4985.