Crystal Structures of the Dihydro- and 2α-Hydroxydihydro-derivatives of Phytuberin

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The *X*-ray crystal structure analyses of two derivatives of the potato stress metabolite phytuberin, are reported.

Dihydrophytuberin (III) {8-(1-acetoxy-1-methylethyl)-3a,5a-dimethylperhydrofuro[3,2-*c*]isobenzofuran} has space group $P2_1$, a = 9.723(2), b = 10.722(5), c = 8.107(5) Å, β = 101.29(3)°, and its structure was derived by direct methods. Full-matrix least-squares refinement, to R 0.060 for 1 650 reflections, showed a very compact, rigid molecule, with considerable conformational strain within the three-ring system.

2α-Hydroxydihydrophytuberin (II) has two independent molecules in the triclinic $P1$ cell, a = 10.887(3), b = 10.057(8), c = 8.661(5) Å, α = 111.85(5), β = 89.63(4), γ = 99.43(5)°. This structure was determined from the alignment of the rigid skeletal structure of (III) with small ring fragments in an E map. The final refined structure, R 0.134 for 2 845 measured reflections, shows a pseudo-two-fold screw axis closely relating the two molecules. There are negligible differences in the conformations of the two molecules, and only in the rings adjoining the hydroxy-groups do they differ from (III).

The packing arrangements of the two derivatives are quite different. It is suggested that, in (II) there is a hydrogen-bonding scheme spiralling parallel to the *a* axis; in (III), no such interactions are possible and all inter-molecular contacts are at van der Waals distances.

PHYTUBERIN is a liquid antifungal sesquiterpenoid which accumulates in potato tubers inoculated with the late blight fungus *Phytophthora infestans* [1] or the bacterium *Erwinia carotovora* var. *atroseptica*.[2]

The structure of phytuberin (I) has been established from the crystal structure of its hydrogenated derivative, derivative, (II), 2α-hydroxydihydrophytuberin, the first crystalline derivative of phytuberin to be prepared and examined; the successful determination of its structure had to wait, however, until that of the dihydro-derivative (III) was complete.

The crystallographic, chemical, and spectral,[4] data

(I)   (II)   (III)
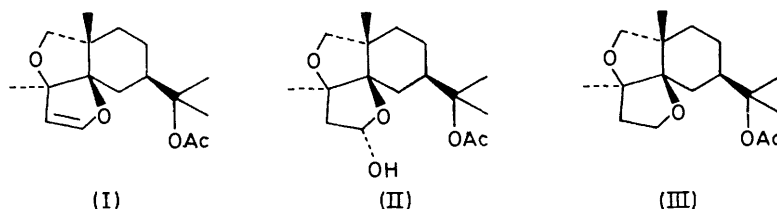
briefly reported recently.[3] This analysis is now complete and is reported here in more detail, together with the results of the crystal structure analysis of another relate the structures of phytuberin (I) and its hydroxy-dihydro- (II) and dihydro- (III) derivatives. The absolute configurations shown here have been suggested

[1] J. Varns, ' Biochemical Response and its Control in the Irish Potato Tuber (*Solanum tuberosum* L.): *Phytophthora infestans* Interaction,' Ph.D. Thesis, Purdue University, Lafayette, Indiana, 1970.

[2] G. D. Lyon, *Physiological Plant Pathology*, 1972, **2**, 411.

[3] D. L. Hughes and D. T. Coxon, *J.C.S. Chem. Comm.*, 1974, 822.

[4] D. T. Coxon, R. F. Curtis, K. R. Price, and B. Howard, *Tetrahedron Letters*, 1974, 2363.

from the postulated biogenetic relationships between phytuberin and other stress metabolites in potato.[3]

EXPERIMENTAL

Structure of Dihydrophytuberin (III)

Dihydrophytuberin was prepared by the catalytic hydrogenation of phytuberin, and this sample was crystallised by slow evaporation of the solvent, n-pentane. The product, m.p. 66 °C, comprised colourless masses from which crystals suitable for X-ray analysis were cut. Some of these fragments showed one good face but generally had no distinct shape. The first selected crystal was mounted on a fibre but, after some days of exposure to air and X-rays, appeared to be covered by bubbles. A second crystal, $0.12 \times 0.57 \times 0.19$ mm³, was mounted in a fine glass tube for the collection of intensity data; the longest dimension, the b axis, was arranged to be close to the rotation axis.

Crystal Data.—$C_{17}H_{28}O_4$, $M = 296.41$. Monoclinic, $a = 9.723(2)$, $b = 10.722(5)$, $c = 8.107(5)$ Å, $\beta = 101.29(3)°$, $U = 828.7$ Å³, $D_c = 1.188$ g cm⁻³, $Z = 2$, $F(000) = 324$. $\mu(\text{Cu-}K_{\bar{\alpha}}) = 6.8$ cm⁻¹, $\lambda(\text{Cu-}K_{\bar{\alpha}}) = 1.5418$ Å (1 Å $\equiv 10^{-10}$ m). Space group $P2_1$ (No. 4); since the compound is optically active; $[\alpha]_D$ 41.1°.

Preliminary cell dimensions were determined from oscillation, Weissenberg, and precession photographs, and later refined from θ measurements of 39 reflections on the diffractometer. Intensities of 1 657 independent reflections in the range $2.5 \leqslant \theta \leqslant 70°$ were measured on an Enraf-Nonius CAD 4 Kappa diffractometer by use of monochromated Cu-$K_{\bar{\alpha}}$ radiation. Measurement was by forward and return scan in the 2θ—ω scan mode at varying scanning speeds to obtain an approximately constant counting rate through the peak within a maximum scan time of 60 s. The 2θ scan angle was set to $1.0 + 0.5 \tan \theta°$; background counts were recorded from the first and last quarters of each scan.

Seven reflections from a confined region in the reciprocal lattice were later found to have extraordinarily large and irregular background counts, suspected to result from beams scattered by the mounting table set on the goniometer arcs; these reflections were omitted from the refinement of the structure. Of the 1 650 remaining reflections, 93 had a net intensity of zero. Lorentz and polarisation factors were applied to the net intensity values, but no absorption corrections were made.

Structure Analysis.—The structure of (III) was solved by direct methods. E Values were calculated from the K curve method,[5] and their statistics [6] were consistent with the non-centrosymmetric space group $P2_1$ for the optically active phytuberin derivatives. The 160 reflections with $|E| \geqslant 1.50$ were used in the automatic multisolution tangent-formula program MULTAN,[7] which initially was allowed to select a starting set of reflections. E Maps based on those sets of phases which showed the highest figures of merit, ABSFOM, and lowest residual factors, RESID, all showed one outstanding peak, which was two to three times as large as any other and one of a zig-zag fragment comprising eight of the strongest peaks. Varying amounts of pseudosymmetry persisted around this fragment. Eventually, another set of starting reflections was

selected and input to MULTAN; it was in an E map from a set of phases with only moderately high ABSFOM (1.18, *vs.* the highest value 1.25), but with the lowest RESID, that a fragment of the molecule, involving a five-membered ring, could be identified. Fourteen peaks were taken, as carbon atoms, for a structure-factor calculation, and the whole molecule showed clearly in a succeeding electron-density map.

On the first refinement of the parameters of the twenty-one atoms (all designated carbon atoms), four had distinctly lower temperature factors and were shown to be the oxygen atoms. The molecular skeleton found agreed with one suggested by Coxon,[4] except that the carbon and oxygen atoms indicated as C(1) and O(1) in Figure 1 were interchanged.

With all the data (equally weighted) and scattering factors from ref. 8, refinement of the atomic parameters was rapid. All hydrogen atoms were located in a Fourier
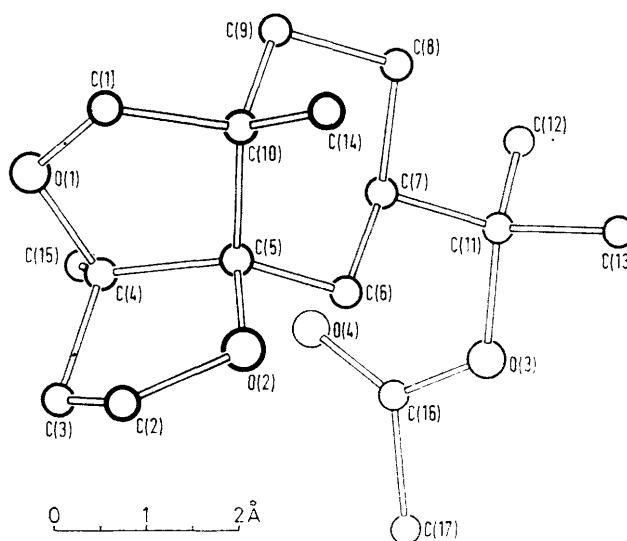

FIGURE 1 Projection of a molecule of dihydrophytuberin (III) showing the atom numbering scheme

difference synthesis and were included, with scattering factors of ref. 9, in further calculations. In alternate cycles of least-squares refinement, (i) the carbon and oxygen atoms were refined anisotropically, (ii) the hydrogen atom parameters and the parameters of their bonded carbon atoms were refined. The y co-ordinate of O(2) was not refined: this defined the origin of the unit cell, as required by the space group $P2_1$. In the final cycles, a weighting scheme was introduced: $w = 1/\sigma^2$ where $\sigma^2 = 0.234 - 0.037 \, 4|F_o| + 0.002 \, 04|F_o|^2 + 0.000 \, 096|F_o|^3$. The final R was 0.060 and R' 0.066 for the 1 650 reflections. In a Fourier difference synthesis, based on the 1 557 observable reflections, the highest peaks were 0.20 Å⁻³.

Final atomic parameters for (III) are in Table 1, and a projection of the molecule (with the atom numbering scheme) is in Figure 1. In the numbering of the hydrogen atoms of the methylene groups the first digit, and of the

⁵ After J. and I. L. Karle, in ' Computing Methods in Crystallography,' ed. J. S. Rollett, Pergamon Press, Oxford, 1965, ch. 17.

⁶ I. L. Karle, K. S. Dragonette, and S. A. Brenner, *Acta Cryst.*, 1965, **19**, 713.

⁷ G. Germain, P. Main, and M. M. Woolfson, ' MULTAN: A Computer Programme for the Automatic Solution of Crystal Structures,' University of York, 1971.

⁸ ' International Tables for X-Ray Crystallography,' vol. III, Kynoch Press, Birmingham, 1962, p. 202.

⁹ R. F. Stewart, E. R. Davidson, and W. T. Simpson, *J. Chem. Phys.*, 1965, **42**, 3175.

methyl groups the first two digits, indicate the carbon atoms to which the hydrogen atoms are bonded. Standard deviations for bond lengths and angles (not involving hydrogen atoms; Tables 2 and 3) were calculated from the variance–covariance matrix, with inclusion of cell-parameter errors. Where hydrogen atoms are involved, errors

TABLE 1

Atomic parameters for dihydrophytuberin (III), with estimated standard deviations in parentheses. The absolute configuration, as predicted in ref. 3, is defined with respect to a right-handed system of axes

(*a*) Non-hydrogen atoms: co-ordinates (fractional $\times 10^4$)

	x	y	z
O(1)	598(3)	−1 087(4)	4 063(3)
O(2)	820(2)	−3 719(−)	2 428(3)
O(3)	−4 327(2)	−4 780(3)	1 791(3)
O(4)	−4 536(3)	−3 602(4)	4 034(4)
C(1)	887(4)	−947(4)	2 421(5)
C(2)	1 825(4)	−3 600(5)	3 979(5)
C(3)	1 003(4)	−3 173(5)	5 232(5)
C(4)	−59(4)	−2 277(4)	4 207(4)
C(5)	−324(3)	−2 858(4)	2 408(4)
C(6)	−1 652(3)	−3 634(4)	1 840(4)
C(7)	−3 013(3)	−2 897(4)	1 276(4)
C(8)	−2 778(4)	−1 903(4)	−16(5)
C(9)	−1 567(4)	−1 047(5)	751(5)
C(10)	−169(3)	−1 752(4)	1 252(4)
C(11)	−4 287(3)	−3 736(5)	614(4)
C(12)	−5 676(4)	−3 028(5)	401(6)
C(13)	−4 198(5)	−4 412(5)	−1 013(5)
C(14)	389(4)	−2 111(5)	−310(4)
C(15)	−1 302(4)	−2 041(5)	5 042(5)
C(16)	−4 465(3)	−4 597(5)	3 389(5)
C(17)	−4 499(6)	−5 822(6)	4 265(6)

(*b*) Hydrogen atoms: co-ordinates (fractional $\times 10^3$), and isotropic thermal parameters *

	x	y	z	$B/\text{Å}^2$
H(11)	189(4)	−118(4)	238(5)	3.8(8)
H(12)	82(4)	4(5)	211(5)	4.5(9)
H(21)	226(5)	−443(5)	417(6)	5.6(11)
H(22)	263(5)	−297(5)	381(6)	5.4(10)
H(31)	152(7)	−264(7)	613(8)	7.7(15)
H(32)	55(6)	−376(6)	561(6)	5.9(12)
H(61)	−173(4)	−405(4)	279(5)	3.9(9)
H(62)	−144(4)	−407(5)	84(6)	4.5(9)
H(7)	−326(4)	−247(4)	225(5)	3.9(8)
H(81)	−363(5)	−142(5)	−24(5)	5.1(10)
H(82)	−252(4)	−230(4)	−107(5)	3.3(7)
H(91)	−181(4)	−63(4)	181(4)	2.9(7)
H(92)	−147(4)	−31(4)	−11(5)	4.0(8)
H(121)	−577(7)	−263(7)	136(9)	7.5(17)
H(122)	−581(6)	−237(7)	−66(8)	8.5(15)
H(123)	−648(6)	−369(6)	15(7)	6.6(12)
H(131)	−419(5)	−373(6)	−196(6)	5.9(11)
H(132)	−495(6)	−491(6)	−124(7)	6.5(13)
H(133)	−336(5)	−481(5)	−97(6)	4.4(10)
H(141)	−19(5)	−279(5)	−103(5)	4.6(9)
H(142)	52(6)	−133(6)	−103(7)	6.4(13)
H(143)	129(7)	−256(7)	−13(8)	8.3(15)
H(151)	−195(5)	−147(5)	431(6)	5.7(12)
H(152)	−179(5)	−282(5)	514(6)	5.5(11)
H(153)	−98(5)	−157(6)	612(7)	6.9(12)
H(171)	−361(12)	−598(13)	433(13)	17.4(35)
H(172)	−509(8)	−647(9)	363(9)	10.2(20)
H(173)	−462(7)	−575(6)	532(9)	6.9(15)

* From the expression: $\exp[-B(\sin^2\theta)/\lambda^2]$.

in bonds were calculated from the atomic variances in the bond direction, but spherical atomic variances were assumed in the calculation of bond angle σ values.

The absolute configuration of (III) has been predicted [3] from the suggested biogenetic relationship of the parent compound phytuberin with other compounds isolated from

microbially stressed potatoes. By use of the anomalous dispersion factors of ref. 10 for the oxygen atoms, structure factors of the 1 475 acentric reflections were calculated for the predicted configuration, using the final atomic co-ordinates (x, y, z), and for the structure inverted through the origin, with co-ordinates ($\bar{x}$, $\bar{y}$, $\bar{z}$). On comparison, no significant difference between the $|F_c|$ values were found, and the difference between the R values was negligible, 0.063 46 *vs.* 0.063 55. Distinction between the two

TABLE 2

Bond lengths (Å) in the phytuberin derivatives, with standard deviations in parentheses

		(II)	
	(III)	Molecule (A)	Molecule (B)
O(1)–C(1)	1.421(5)	1.425(12)	1.388(13)
O(1)–C(4)	1.441(5)	1.439(12)	1.444(12)
O(2)–C(2)	1.439(5)	1.409(12)	1.378(12)
O(2)–C(5)	1.444(4)	1.448(11)	1.402(11)
O(3)–C(11)	1.476(4)	1.509(11)	1.503(11)
O(3)–C(16)	1.343(5)	1.275(12)	1.314(11)
O(4)–C(16)	1.196(5)	1.206(12)	1.210(12)
O(5)–C(2)		1.397(13)	1.395(13)
C(1)–C(10)	1.522(5)	1.515(13)	1.517(14)
C(2)–C(3)	1.483(6)	1.525(16)	1.508(17)
C(3)–C(4)	1.531(6)	1.571(15)	1.518(15)
C(4)–C(5)	1.560(5)	1.548(12)	1.591(11)
C(4)–C(15)	1.518(5)	1.490(13)	1.492(13)
C(5)–C(6)	1.529(5)	1.539(12)	1.540(13)
C(5)–C(10)	1.536(5)	1.557(12)	1.570(13)
C(6)–C(7)	1.532(4)	1.553(12)	1.540(12)
C(7)–C(8)	1.542(5)	1.511(12)	1.501(13)
C(7)–C(11)	1.540(5)	1.533(12)	1.547(12)
C(8)–C(9)	1.526(5)	1.508(13)	1.513(13)
C(9)–C(10)	1.539(5)	1.520(13)	1.510(13)
C(10)–C(14)	1.521(5)	1.519(13)	1.532(13)
C(11)–C(12)	1.529(5)	1.527(14)	1.499(13)
C(11)–C(13)	1.523(6)	1.467(13)	1.485(13)
C(16)–C(17)	1.496(7)	1.529(16)	1.530(16)
C(1)–H(11)	1.02(4)	0.93(7)	1.02(9)
C(1)–H(12)	1.09(5)	1.02(7)	1.09(9)
C(2)–H(2)		0.94(7)	1.00(8)
C(2)–H(21)	0.99(6)		
C(2)–H(22)	1.06(5)		
C(3)–H(31)	0.99(7)	1.06(10)	1.05(11)
C(3)–H(32)	0.86(6)	0.87(8)	0.96(9)
C(6)–H(61)	0.91(4)	0.98(6)	1.05(6)
C(6)–H(62)	0.99(5)	0.97(5)	1.05(5)
C(7)–H(7)	0.98(4)	0.98(6)	0.95(7)
C(8)–H(81)	0.96(5)	0.89(8)	1.02(7)
C(8)–H(82)	1.03(4)	1.08(7)	0.99(4)
C(9)–H(91)	1.04(4)	0.93(6)	1.04(4)
C(9)–H(92)	1.08(4)	0.97(6)	0.87(6)
C(12)–H(121)	0.91(7)	1.07(9)	1.02(8)
C(12)–H(122)	1.10(7)	0.95(13)	0.99(11)
C(12)–H(123)	1.05(6)	1.15(8)	0.95(10)
C(13)–H(131)	1.06(6)	1.02(8)	1.06(11)
C(13)–H(132)	0.90(6)	1.08(10)	0.96(10)
C(13)–H(133)	0.91(5)	0.89(11)	1.07(9)
C(14)–H(141)	1.03(5)	1.03(6)	1.01(10)
C(14)–H(142)	1.05(6)	0.99(8)	1.12(11)
C(14)–H(143)	0.99(7)	1.01(8)	1.03(11)
C(15)–H(151)	0.98(5)	1.23(10)	0.95(9)
C(15)–H(152)	0.97(6)	1.04(13)	1.01(11)
C(15)–H(153)	1.00(6)	0.87(10)	0.92(9)
C(17)–H(171)	0.88(12)	0.98(12)	1.02(10)
C(17)–H(172)	0.98(9)	0.84(10)	0.88(12)
C(17)–H(173)	0.89(7)	0.91(10)	1.19(11)

enantiomers is thus not possible, and, by this method, we are not able to confirm the absolute configuration of phytuberin.

[10] D. T. Cromer and D. Liberman, *J. Chem. Phys.*, 1970, **53**, 1891.

TABLE 3

Valence angles (°) in the phytuberin derivatives

	(III)	(II) Molecule (A)	(II) Molecule (B)
C(1)–O(1)–C(4)	109.9	109.0	108.7
C(2)–O(2)–C(5)	110.1	109.3	111.5
C(11)–O(3)–C(16)	122.2	120.2	119.3
O(1)–C(1)–C(10)	106.9	104.8	107.1
O(2)–C(2)–O(5)		112.6	112.6
O(2)–C(2)–C(3)	105.1	105.0	104.6
O(5)–C(2)–C(3)		112.5	109.7
C(2)–C(3)–C(4)	102.6	104.5	106.8
O(1)–C(4)–C(3)	109.7	108.8	110.6
O(1)–C(4)–C(5)	105.8	104.2	104.3
O(1)–C(4)–C(15)	107.0	107.3	107.8
C(3)–C(4)–C(5)	103.2	103.3	102.0
C(3)–C(4)–C(15)	112.0	112.1	113.0
C(5)–C(4)–C(15)	118.9	120.4	118.8
O(2)–C(5)–C(4)	105.0	106.2	104.9
O(2)–C(5)–C(6)	105.0	105.8	107.5
O(2)–C(5)–C(10)	109.4	107.3	108.1
C(4)–C(5)–C(6)	118.3	118.8	118.2
C(4)–C(5)–C(10)	104.1	105.5	103.9
C(6)–C(5)–C(10)	114.5	112.6	113.6
C(5)–C(6)–C(7)	116.0	115.1	114.6
C(6)–C(7)–C(8)	109.0	108.2	110.9
C(6)–C(7)–C(11)	113.1	111.3	110.9
C(8)–C(7)–C(11)	112.5	111.5	112.6
C(7)–C(8)–C(9)	110.1	110.1	110.2
C(8)–C(9)–C(10)	112.5	113.7	114.2
C(1)–C(10)–C(5)	101.1	98.9	98.4
C(1)–C(10)–C(9)	109.8	110.1	109.7
C(1)–C(10)–C(14)	110.8	112.2	113.4
C(5)–C(10)–C(9)	110.7	110.3	110.7
C(5)–C(10)–C(14)	114.0	113.7	112.4
C(9)–C(10)–C(14)	110.2	111.1	111.5
O(3)–C(11)–C(7)	109.9	108.6	108.8
O(3)–C(11)–C(12)	108.3	107.8	110.6
O(3)–C(11)–C(13)	102.3	101.0	101.5
C(7)–C(11)–C(12)	112.4	112.9	111.4
C(7)–C(11)–C(13)	113.7	115.6	114.7
C(12)–C(11)–C(13)	109.7	110.1	109.4
O(3)–C(16)–O(4)	125.2	131.1	131.1
O(3)–C(16)–C(17)	110.2	109.2	107.7
O(4)–C(16)–C(17)	124.6	119.7	121.1
Range of σ	0.25—0.38	0.69—1.00	0.72—1.00
Mean σ	0.29	0.79	0.81

The values of angles involving hydrogen atoms all lie in the normal ranges.

Structure of 2α-Hydroxydihydrophytuberin (II)

2α-Hydroxydihydrophytuberin was prepared by acid-catalysed hydration of phytuberin. The major α-epimer was separated by fractional crystallisation. Recrystallisation from n-hexane yielded needles, some almost fibrous, m.p. 119 °C. Several crystals were mounted before a suitable single specimen was found; this had dimensions $0.35 \times 0.10 \times 0.12$ mm³ and was enclosed in a fine capillary tube.

Crystal Data.—$C_{17}H_{28}O_5$, $M = 312.4$. Triclinic, $a = 10.887(3)$, $b = 10.057(8)$, $c = 8.661(5)$ Å, $\alpha = 111.85(5)$, $\beta = 89.63(4)$, $\gamma = 99.43(5)°$, $U = 866.8(8)$ Å³, $D_m = 1.16$ g cm⁻³ (by flotation), $Z = 2$, $D_c = 1.20$ g cm⁻³, $F(000) = 340$, $\mu(Mo-K_{\bar{\alpha}}) = 0.9$ cm⁻¹, $\lambda(Mo-K_{\bar{\alpha}}) = 0.710\,69$ Å. Space group $P1$ (No. 1).

Preliminary oscillation, Weissenberg, and precession photographs indicated the triclinic crystal system and the cell dimensions. The dimensions were determined more accurately by refinement from Guinier powder photograph measurements. The non-centrosymmetric space group was considered more probable since the suggested molecular formula of phytuberin [4] had four asymmetric centres.

Intensity data were collected on a Stoe STADI 2 diffractometer which has Weissenberg equi-inclination geometry. The crystal was mounted with the a axis coincident with the rotation (ω) axis. Monochromated Mo-$K_{\bar{\alpha}}$ radiation (with attenuators) and an ω-scan were used. Of the 3 021 reflections with $2\theta \leqslant 50°$, 176 were considered weak from prescan searches, and intensity measurements were not made; these reflections are included in the data-set with F_0 0. Of the remaining 2 845 reflections, 1 647 were considered observed, having $I \geqslant 2\sigma_I$, where I is the net intensity count, and $\sigma_I^2 = S^2(T + (b_1 + b_2)n/1\,600)$ where S = attenuation factor, T = scan count, b_1 and b_2 are background counts, each 20s, n = number of scan steps, each step being 0.01° in ω and 1.0s. Reflections having $I \leqslant 0$ were given the value $I = 1$. Corrections for Lorentz and polarisation effects, but not for absorption, were made, and structure amplitudes calculated. The design of the diffractometer provides a constant crystal-to-detector distance, and all data are therefore scaled by the same factor.

Some reflections with low Υ (*i.e.* 2θ′) values could not be measured accurately in this setting on the diffractometer. For purposes of structure determination only, these reflections were added to the data-set with intensities estimated visually from photographs.

Structure Analysis.—After normalisation of structure amplitudes, the E statistics confirmed the non-centrosymmetric space group $P1$. The high $|E|$ value reflections, plus 50 low $|E|$ value, ψ_0, reflections (taken from the weak unmeasured reflections) were entered into the program MULTAN.[7] In several runs, normally with 32 sets of phases produced, there was very little variation in the consistency indicators of the different sets. E Maps calculated from sample sets showed little sign of any likely molecules. Eventually, in a run of MULTAN using 210 reflections with $|E| \geqslant 1.70$ and in which the starting set of reflections was selected manually, more variation in the consistency indicators was achieved; the set of phases having the lowest absolute figure of merit (1.21 compared with all the rest in the range 1.31—1.38), the lowest ψ_0-value and the highest RESID value, yielded the most promising E map in which substituted six-membered rings were recognisable. A pseudo-centre of symmetry confused the map; a sufficient proportion of the atoms of the two molecules was not found, and electron-density maps based on these fragments did not yield a complete solution.

The solution of the crystal structure of dihydrophytuberin had by this time shown that the molecules of phytuberin and its derivatives should all be rigid and of similar conformations, except perhaps in the acetate group. Consequently the co-ordinates of the atoms of the six-membered ring (Figure 1) were aligned with the peaks of a ring in the most promising E map (see earlier) and structure factors were calculated for 18 atoms, O(1)—(3) and C(1)—(15), in each of the six possible conformations. One arrangement looked more feasible than the others and, after a cycle of rigid-body refinement of this whole fragment, structure factors were again calculated and an electron-density map drawn. Two possible substituted five-membered rings were found in this map and from electron-density maps based on one of these fragments (plus the first molecule fragment), the second molecule of the cell eventually emerged, and the remaining atoms of the first molecule were located.

In the course of a lengthy refinement process, 54 of

the hydrogen atoms of the two molecules were located in difference maps, or their co-ordinates calculated in idealised tetrahedral positions about the carbon atoms. The two missing hydrogen atoms are of the hydroxy-groups in each molecule; they could not be positively identified in any electron-density or difference map, and their co-ordinates could not be calculated. From examination of inter-molecular contacts, it is thought that these hydrogen atoms should be involved in hydrogen bonds between the hydroxy-groups and the carbonyl groups of neighbouring acetate groups, but this has not been proved from the refinement process. The numbering of the hydrogen atoms follows the same pattern as that in dihydrophytuberin.

In the least-squares refinement, all the carbon and oxygen atoms were refined with anisotropic vibration parameters and the hydrogen atoms with isotropic para-meters. Scattering factors were as for (III). The refine-ment of 613 parameters was performed in stages, refining
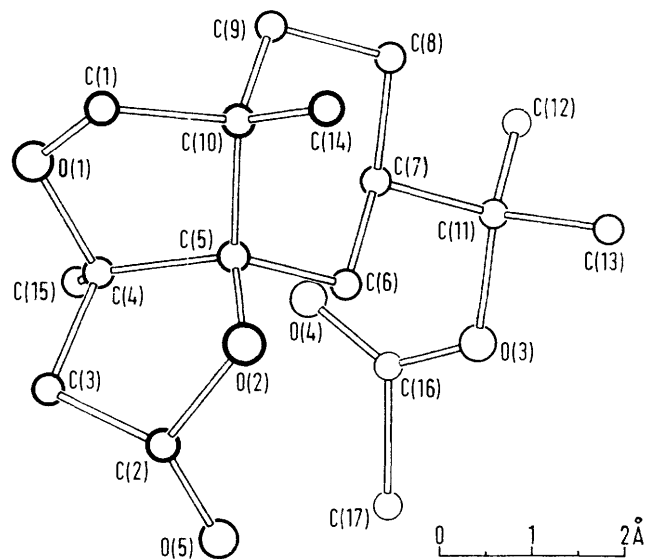

FIGURE 2 Projection of one molecule of 2α-hydroxydihydro-phytuberin (II). This is molecule (A), with atomic labels as shown; molecule (B), has an almost identical view, in projec-tion, and its atoms are asterisked C*(1), O*(1), etc.

three groups of atoms in successive cycles: in the first group atoms O(1)—(2) and C(1)—(10) of both molecules, in the second group atoms O(3)—(5) and C(11)—(17) of both molecules, and in the third group all the hydrogen atoms (refer to Figure 2 for the atom numbering scheme). It was not necessary to maintain one atom's co-ordinates constant (in order to define the cell origin) throughout the refinement process, since at no time were all the heavier atoms' parameters being refined simultaneously.

In the final data set of 3 021 reflections, the weak re-flections were given zero-weighting and effectively excluded from the refinement process. The 2 845 diffractometer measurements were initially given weights derived from intensity counts, $w_c = 1/\sigma_c^2$ where $\sigma_c^2 = \sigma_I^2/(4I.Lp)$ and Lp is the Lorentz-polarisation factor; in the final stages of refinement, the weights were modified so that the mean

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

[11] IBM 360 and ICL 4/70 programs: NUCLS, R. J. Doedens and J. A. Ibers; ORFFE, W. R. Busing, K. O. Martin, and H. A. Levy.

values of $w(|F_o| - |F_c|)^2$ in several ranges of $|F_o|$ were approximately constant. The scheme used was: $w = w_c/(-0.145 + 0.221|F_o| + 0.051|F_o|^2)$, but for $|F_o| < 1.0$ for the calculation of w, $|F_o|$ was adjusted to 1.0.

The refinement was concluded with R 0.134 and R' 0.087 for the 2 845 measurements, and R 0.072 for the 1 647 observed measurements. Final atomic parameters are in Table 4. Errors in bond lengths (Table 2) were calculated from the atomic variances in the direction of the bonds; in the bond-angle σ calculations (for Table 3) symmetrically spherical atomic variances were assumed.

Measured and calculated structure amplitudes for the analyses of both derivatives, and the anisotropic thermal parameters, U_{ij} values, for the carbon and oxygen atoms in both structures are listed in Supplementary Publications No. SUP 21697 (25 pp., 1 microfiche).*

Computing.—The full-matrix least-squares refinement program NUCLS,[11] the program ORFFE [11] for determining statistical errors from the full correlation matrix, and the multisolution program MULTAN [7] for phase determination by the tangent formula, have been adapted for use on the IBM 360/65X system of University College, London, and

TABLE 4

Atomic parameters for (II), with estimated standard deviations in parentheses. The absolute configuration, as predicted in ref. 3, is defined with respect to a right-handed system of axes

(a) Non-hydrogen atoms: co-ordinates (fractional × 10^4)

	x	y	z
O(1)	−28(5)	−1 428(7)	1 866(8)
O(2)	2 561(6)	−1 192(7)	146(8)
O(3)	2 635(6)	4 215(7)	1 464(8)
O(4)	550(6)	4 104(8)	1 586(10)
O(5)	2 164(7)	−904(8)	−2 346(8)
C(1)	968(9)	−1 732(10)	2 669(12)
C(2)	1 895(10)	−1 781(12)	−1 417(13)
C(3)	525(10)	−1 971(11)	−1 024(13)
C(4)	479(7)	−754(11)	743(11)
C(5)	1 871(8)	−204(9)	1 345(11)
C(6)	2 476(8)	1 332(10)	1 477(11)
C(7)	2 144(6)	2 595(9)	3 021(10)
C(8)	2 430(9)	2 307(10)	4 561(11)
C(9)	1 685(8)	871(10)	4 459(11)
C(10)	1 998(8)	−429(10)	3 017(11)
C(11)	2 856(7)	4 070(10)	3 108(11)
C(12)	2 432(10)	5 353(11)	4 476(14)
C(13)	4 220(10)	4 235(11)	3 213(14)
C(14)	3 263(9)	−755(12)	3 347(13)
C(15)	−342(7)	286(10)	713(13)
C(16)	1 533(9)	4 188(10)	950(12)
C(17)	1 566(13)	4 231(14)	−796(16)
O*(1)	−4 052(6)	4 745(8)	−2 884(9)
O*(2)	−1 551(6)	4 399(7)	−1 314(8)
O*(3)	−3 203(5)	−1 025(7)	−2 753(8)
O*(4)	−5 299(6)	−1 002(8)	−3 024(9)
O*(5)	−1 999(7)	4 095(8)	1 153(9)
C*(1)	−2 994(10)	5 029(11)	−3 691(13)
C*(2)	−1 991(11)	4 989(11)	249(13)
C*(3)	−3 309(12)	5 149(12)	−73(14)
C*(4)	−3 749(7)	4 035(10)	−1 798(12)
C*(5)	−2 500(7)	3 450(10)	−2 473(12)
C*(6)	−2 394(7)	1 890(10)	−2 659(11)
C*(7)	−3 104(7)	688(9)	−4 216(11)
C*(8)	−2 784(9)	991(10)	−5 756(12)
C*(9)	−3 068(8)	2 464(10)	−5 575(11)
C*(10)	−2 345(8)	3 722(10)	−4 140(11)
C*(11)	−2 878(8)	−830(10)	−4 354(12)
C*(12)	−3 629(9)	−2 015(10)	−5 804(12)
C*(13)	−1 550(9)	−1 012(11)	−4 443(13)
C*(14)	−973(9)	4 043(11)	−4 508(12)
C*(15)	−4 873(9)	2 969(12)	−1 786(15)
C*(16)	−4 366(9)	−1 074(11)	−2 327(13)
C*(17)	−4 424(10)	−1 323(15)	−687(15)

TABLE 4 (Continued)

(b) Hydrogen atoms: co-ordinates (fractional × 10³) and isotropic thermal parameters†

	x	y	z	$B/\text{Å}^2$
H(11)	121(6)	−260(7)	200(9)	3.5(16)
H(12)	58(5)	−197(7)	362(8)	2.8(14)
H(2)	227(6)	−260(8)	−197(9)	4.1(18)
H(31)	30(8)	−299(10)	−89(11)	5.8(25)
H(32)	7(7)	−195(8)	−184(10)	4.7(19)
H(61)	229(5)	141(7)	41(8)	2.1(14)
H(62)	336(4)	136(6)	163(6)	0.5(10)
H(7)	126(5)	256(7)	275(8)	1.5(13)
H(81)	223(7)	300(9)	548(10)	5.4(19)
H(82)	342(7)	231(8)	469(10)	5.3(19)
H(91)	83(6)	82(7)	428(9)	3.1(16)
H(92)	184(5)	77(6)	551(7)	2.4(12)
H(121)	144(9)	503(10)	436(12)	6.0(25)
H(122)	280(11)	541(14)	549(16)	9.6(35)
H(123)	289(6)	641(8)	434(10)	3.3(19)
H(131)	456(6)	408(9)	422(10)	5.0(20)
H(132)	475(8)	529(11)	337(12)	7.5(25)
H(133)	449(9)	359(11)	232(14)	10.2(26)
H(141)	403(6)	1(7)	339(9)	3.4(15)
H(142)	341(6)	−80(8)	445(10)	4.7(17)
H(143)	350(7)	−166(9)	248(11)	4.5(19)
H(151)	−17(8)	133(10)	206(13)	6.3(26)
H(152)	−13(12)	91(14)	−1(16)	12.3(36)
H(153)	−113(9)	−4(11)	40(13)	9.3(28)
H(171)	111(10)	327(13)	−150(14)	10.4(33)
H(172)	231(9)	443(10)	−103(12)	7.8(25)
H(173)	93(8)	464(12)	−99(13)	5.5(27)
H*(11)	−242(8)	595(10)	−294(10)	8.5(23)
H*(12)	−343(8)	516(10)	−474(12)	6.9(24)
H*(2)	−138(7)	583(9)	101(11)	4.8(21)
H*(31)	−318(9)	625(11)	5(12)	8.2(27)
H*(32)	−396(8)	488(10)	57(11)	4.9(23)
H*(61)	−258(5)	181(6)	−150(7)	1.5(11)
H*(62)	−143(5)	194(6)	−277(7)	2.0(13)
H*(7)	−399(7)	53(8)	−432(10)	4.0(18)
H*(81)	−329(6)	22(8)	−678(9)	4.7(17)
H*(82)	−189(4)	96(5)	−596(6)	0.8(9)
H*(91)	−402(4)	246(5)	−550(6)	0.6(10)
H*(92)	−295(5)	251(7)	−655(8)	3.1(14)
H*(121)	−448(7)	−183(10)	−609(11)	4.6(20)
H*(122)	−333(9)	−244(11)	−694(14)	8.3(28)
H*(123)	−366(9)	−278(11)	−540(13)	5.3(28)
H*(131)	−116(9)	−64(12)	−537(14)	10.3(32)
H*(132)	−141(8)	−197(10)	−460(11)	7.8(24)
H*(133)	−92(7)	−34(9)	−340(11)	5.3(21)
H*(141)	−55(8)	316(11)	−489(12)	7.8(26)
H*(142)	−94(9)	458(11)	−543(13)	8.8(28)
H*(143)	−36(9)	472(12)	−352(14)	7.9(21)
H*(151)	−504(7)	231(10)	−291(11)	6.7(21)
H*(152)	−465(10)	269(12)	−84(14)	9.6(32)
H*(153)	−552(8)	346(10)	−140(12)	7.7(25)
H*(171)	−465(8)	−51(11)	32(12)	7.0(25)
H*(172)	−380(11)	−170(13)	−49(15)	13.5(35)
H*(173)	−521(10)	−233(12)	−80(13)	10.4(32)

† See Table 1.

on the ICL 4/70 computer at this Station. An IBM 1130 computer and our X-RAY ARC [12] library of programs were used for the remainder of the computing for these analyses.

DISCUSSION

The two independent molecules in the crystal form of (II) are remarkably similar in conformation, and a pseudo-twofold-screw axis, parallel to the a axis, closely relates the two molecules. Figure 2 is a projection of molecule (A); the corresponding projection of molecule (B) differs only slightly from that shown, in the acetate group. Comparison with the projection of (III) (Figure 1) shows that differences in conformation are small, and only about C(2) is there any significant difference.

These two Figures show the atomic numbering schemes which are based on that of eudesmane to which, it has been suggested,[3] phytuberin is related.

TABLE 5

Torsion angles (°) in the rings of the phytuberin derivatives

		(II)	
	(III)	Molecule (A)	Molecule (B)
C(9)−C(10)−C(5)−C(6)	44.4	45.3	43.6
C(10)−C(5)−C(6)−C(7)	−45.0	−47.1	−43.9
C(5)−C(6)−C(7)−C(8)	50.3	53.4	50.7
C(6)−C(7)−C(8)−C(9)	−57.6	−59.1	−57.1
C(7)−C(8)−C(9)−C(10)	61.7	62.9	60.7
C(8)−C(9)−C(10)−C(5)	−53.7	−54.2	−52.7
C(1)−C(10)−C(5)−C(4)	30.0	29.6	28.8
C(10)−C(5)−C(4)−O(1)	−16.1	−8.5	−8.6
C(5)−C(4)−O(1)−C(1)	−6.0	−18.4	−18.2
C(4)−O(1)−C(1)−C(10)	26.3	39.1	39.7
O(1)−C(1)−C(10)−C(5)	−34.9	−41.2	−42.1
C(4)−C(5)−O(2)−C(2)	−7.0	28.2	28.2
C(5)−O(2)−C(2)−C(3)	28.5	−36.0	−33.9
O(2)−C(2)−C(3)−C(4)	−37.7	28.7	24.9
C(2)−C(3)−C(4)−C(5)	32.6	−11.8	−8.5
C(3)−C(4)−C(5)−O(2)	−16.3	−8.5	−10.4
Range of σ	0.35—0.43	0.87—1.05	0.88—1.12
Mean σ	0.39	0.97	1.01

TABLE 6

Mean planes. Deviations (Å) of atoms from mean planes in the phytuberin derivatives. Values in italics indicate atoms used to define the plane

		(II)	
	(III)	Molecule (A)	Molecule (B)
Plane (A)			
C(5)	−0.54	−0.57	−0.54
C(6)	−0.010	−0.002	−0.007
C(7)	0.011	0.002	0.007
C(8)	0.71	0.71	0.68
C(9)	−0.012	−0.003	−0.009
C(10)	0.010	0.002	0.009
Plane (B)			
O(3)	−0.001	−0.001	−0.002
O(4)	−0.001	−0.002	−0.003
C(16)	0.004	0.010	0.013
C(17)	−0.002	−0.005	−0.005
C(11)	0.026	−0.095	−0.027
C(13)	0.080	−0.004	−0.201

			(II)			
	(III)		Molecule (A)		Molecule (B)	
Planes (C, D)						
O(1)	0.025	−0.78	0	−0.52	0	−0.51
C(1)	−0.028	0	0.42	0	0.41	0
C(10)	0.52	0	−0.22	0	−0.23	0
C(5)	0.019	0	0	0.50	0	0.51
C(4)	−0.037	−0.76	0	0	0	0
Planes (E, F)						
O(2)	−0.014	0.85	−0.21	0	−0.24	0
C(2)	0.046	0	0.30	0	0.21	0
C(3)	−0.54	0	0	−0.46	0	−0.39
C(4)	−0.031	0	0	0	0	0
C(5)	0.045	0.82	0	0.41	0	0.41

The molecular dimensions of all three molecules are in Tables 2, 3, and 5. Mean planes, describing the conformations of the six- and five-membered rings and the acetate groups are in Table 6.

12 ' X-Ray ARC,' Library of Programs for the IBM 1130 Computer, J. Appl. Cryst., 1973, 6, 309.

In the molecules of both derivatives, the skeletal structures are rigid, consisting of three rings which have common edges or vertices. There is considerable conformational strain in each system; all the bond lengths

the same molecule, $ca.$ 3.00 Å in every case. Similarly the O(4) $\cdots$ H(7) and O(4) $\cdots$ H(121) distances lie in the range 2.37—2.66 Å. The dimensions of the acetate groups are normal,[13] and in each molecule, the four
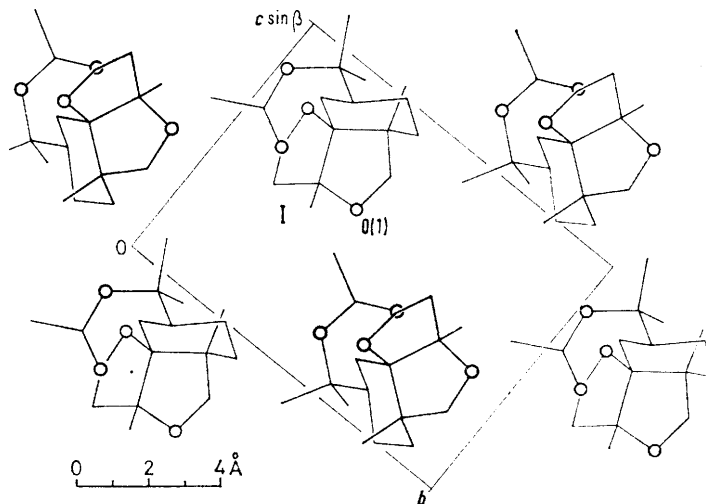

FIGURE 3   The packing arrangement of (III) looking down the a axis

are as expected but the valence angles show the distortions of strain.

The cyclohexyl ring in each molecule has a chair conformation; the angles within the rings range from 108.2 to 116.0°, and the torsion angles (60° in an ideal system) vary from 44 to 63°. The variation in angles follows very similar patterns in the three molecules. From the best-mean-plane of those of four ideally-planar carbon atoms in the six-membered rings, C(5) and C(8) are displaced $ca.$ 0.55 and 0.70 Å in each molecule. From the cyclohexyl ring, the substituent bonds C(5)–O(2), C(7)–C(11), and C(10)–C(1) are equatorially arranged; C(5)–C(4) and C(10)–C(14) are axial substituents.

In (III) the two five-membered rings are each best described as having envelope shapes. One atom in each ring [$viz.$ C(10) and C(3)] is $ca.$ 0.5 Å displaced from the mean plane of the other four, but in each case, the four atoms defining the plane cannot be considered strictly planar, some deviations from the plane being $ca.$ 10 σ. The torsion angles around each ring also illustrate the lack of planarity. The angles between the normals to the four-membered planes and their envelope-flap complement planes are 32.9 and 36.0° in the two rings.

The hydroxy-group substitution in the molecules of (II) affects the shapes of the five-membered rings; all now have conformations closer to 'half-chairs.' In the O(1) rings of both molecules, O(1) and C(5) are $ca.$ 0.5 Å above and below the plane of the other three atoms, whilst in the O(2) rings, C(3) and C(5) are $ca.$ 0.4 Å, either side of the corresponding plane.

The acetate group in each of the three molecules folds around, so that O(4) lies close to both C(7) and C(12) of

13 J. F. McConnell and J. D. Stevens, $J.C.S.$ $Perkin$ II, 1974, 345.

atoms O(3), O(4), C(16), and C(17) form a good plane; C(11) and C(13) are not far displaced from this plane.

TABLE 7

Some shorter intramolecular non-bonded interactions (Å) in the phytuberin derivatives

	(III)	(II) Molecule (A)	(II) Molecule (B)
O(4) $\cdots$ C(7)	3.009	3.026	3.100
O(4) $\cdots$ C(12)	3.000	2.987	2.977
C(1) $\cdots$ C(2)	3.173	3.659 *	3.606 *
C(6) $\cdots$ C(16)	3.386	3.386	3.480
C(7) $\cdots$ C(15)	3.308	3.356	3.346
O(1) $\cdots$ H(91)	2.72 *	2.48	2.56
O(2) $\cdots$ H(143)	2.53	2.50	2.39
O(4) $\cdots$ H(7)	2.41	2.37	2.49
O(4) $\cdots$ H(121)	2.49	2.37	2.66 *
C(6) $\cdots$ H(133)	2.84	2.77	2.84
C(7) $\cdots$ H(151)	2.91	2.62	2.85
C(8) $\cdots$ H(131)	2.71	2.77	2.70
C(12) $\cdots$ H(81)	2.76	2.79	2.65
C(13) $\cdots$ H(62)	2.83	2.70	2.76
C(13) $\cdots$ H(82)	2.79	2.73	2.83
C(14) $\cdots$ H(82)	2.79	2.83	2.88
C(15) $\cdots$ H(7)	2.70	2.67	2.92
H(11) $\cdots$ H(22)≡H(2)	2.29	3.61 *	3.66 *
H(32) $\cdots$ H(152)	2.44 *	2.76 *	2.10
H(32) $\cdots$ H(153)	2.85 *	2.68 *	2.30
H(62) $\cdots$ H(133)	2.28	2.24	2.31
H(7) $\cdots$ H(121)	2.41 *	2.33	2.26
H(7) $\cdots$ H(151)	2.18	1.77	2.25
H(82) $\cdots$ H(131)	2.24	2.17	2.13
H(91) $\cdots$ H(151)	2.25	2.47 *	2.54 *

* These distances are not considered short but are included for comparison.

Space-filling models show each molecule to be very compact with only the C(17) methyl groups and, in (II), the hydroxy-groups capable of any uninhibited

vibration. Some short intramolecular distances are in Table 7.

The two compounds differ most in their packing arrangements. Figure 3 shows the packing of the

TABLE 8

Intermolecular distances (Å) in (III)

O(4) · · · C(17$^{\text{I}}$)	3.489
O(4) · · · C(2$^{\text{II}}$)	3.530
C(14) · · · C(15$^{\text{III}}$)	3.806
C(2) · · · C(15$^{\text{IV}}$)	3.828
C(2) · · · C(16$^{\text{V}}$)	3.880
C(12) · · · H(143$^{\text{II}}$)	2.94
C(14) · · · H(153$^{\text{III}}$)	3.00
C(15) · · · H(21$^{\text{VI}}$)	3.05
H(123) · · · H(143$^{\text{II}}$)	2.45
H(11) · · · H(133$^{\text{VII}}$)	2.48
H(142) · · · H(153$^{\text{III}}$)	2.50

Roman numeral superscripts denote the following equivalent positions relative to the reference molecule at x, y, z:

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

TABLE 9

Intermolecular contacts in (II)

(a) Dimensions of suggested hydrogen-bonding scheme (distances Å, angles °)

Atoms a–b · · · c–d		Dimensions	
	b · · · c	a–b · · · c	b · · · c–d
C(2)–O(5) · · · O*(4)–C*(16)	2.830	103.9	137.4
C*(2)–O*(5) · · · O(4)–C(16)	2.800	101.9	143.3

(b) Other short intermolecular distances (Å)

Atoms a · · · b	Distance a · · · b	Cell translations of atom b from		
		x	y	z
O(2) · · · C*(17)	3.370	1	0	0
O(4) · · · C*(2)	3.376	0	0	0
O*(4) · · · C(2)	3.443	−1	0	0
O*(2) · · · C(17)	3.466	0	0	0
C(1) · · · C*(13)	3.676	0	0	1
C(2) · · · C(12)	3.768	0	−1	−1
C*(3) · · · C*(12)	3.793	0	1	1
C(13) · · · C*(1)	3.839	1	0	1
C(13) · · · C*(12)	3.894	1	1	1
C*(12) · · · H(132)	2.82	−1	−1	−1
C*(3) · · · H*(122)	2.89	0	1	1
C(13) · · · H*(12)	2.93	1	0	1
C*(13) · · · H(12)	2.93	0	0	−1
C(14) · · · H*(121)	2.95	1	0	1
C*(14) · · · H(121)	2.95	0	0	−1
C(1) · · · H*(131)	2.98	0	0	1
H(132) · · · H*(123)	2.29	1	1	1
H(131) · · · H*(12)	2.30	1	0	1
H*(12) · · · H*(123)	2.39	0	1	0

dihydro-derivative (III) in which the binding of molecules is solely by van der Waals interactions; shorter intermolecular contacts are in Table 8. In the hydroxy-dihydro-derivative (II), however, there is a possibility

of hydrogen bonding, and, although the hydroxy-group hydrogen atoms have not been located, intermolecular dimensions (Table 9) indicate that there should be hydrogen bonding between each O(5) atom and the O(4) atom of the other molecule; the hydrogen bonding is shown in Figure 4, spiralling parallel to the *a* axis (the needle axis of the crystal). Other short intermolecular distances are also in Table 9.

Although the molecular structures are not readily apparent in either packing diagram, each arrangement
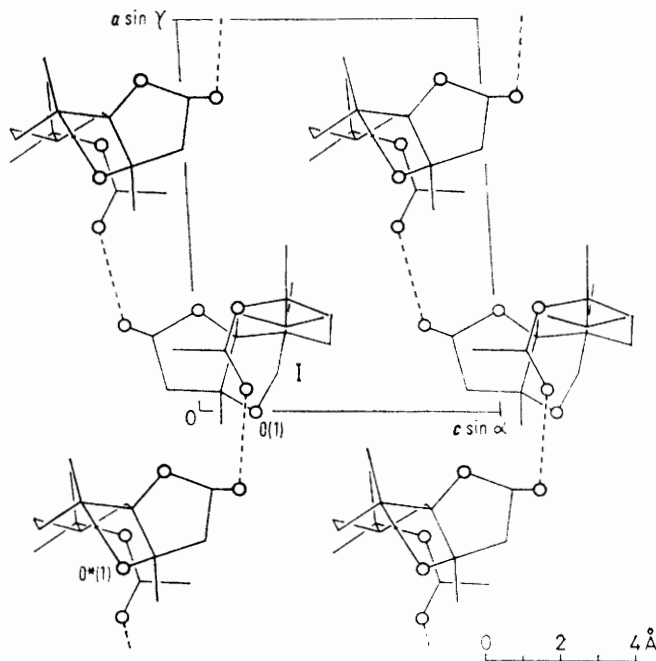

FIGURE 4 The packing arrangement of (II), showing the spiralling hydrogen-bonding system and the pseudo-two-fold screw symmetry parallel to the *a* axis. The molecule labelled I, which is a type (A) molecule, is arranged in a similar orientation to that marked I in Figure 3. The projection is down the *b* axis

has the acetate group lying roughly parallel to the plane of the drawing, and the molecules labelled I in each diagram are in similar orientations; the intermolecular contacts and packing arrangements are thus shown to be quite different.