

## The Constitution of (1*R*)-Sequirin-D (*Sequoia sempervirens*), a Biogenetically Novel Norlignan, by Direct X-Ray Analysis

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*Sequoia sempervirens* heartwood is shown to contain a new metabolite, sequirin-D[(1*R*)-1-(*p*-hydroxybenzyl)-6-hydroxy-1,2-dihydronaphthalene], whose constitution was determined by X-ray analysis (direct methods) of its di-*O*-methyl ether (4; R = Me); the structure was refined to *R* 6.4%. The chirality and biosynthesis of this norlignan, with a carbon skeleton new in nature, are discussed.

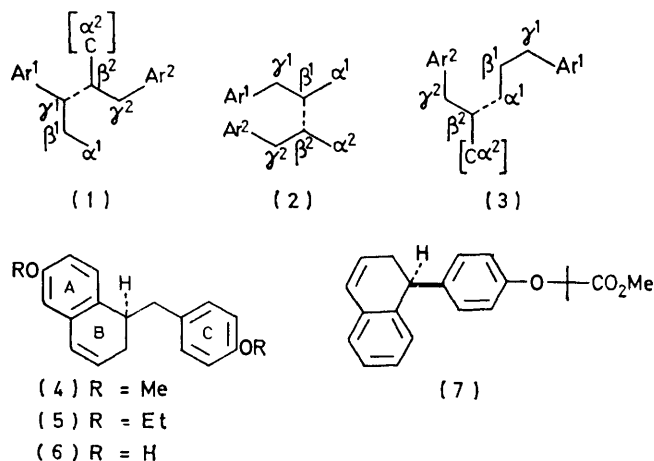
THE heartwoods of *Sequoia sempervirens* (D. Don) Endl. and the related *Sequoiadendron gigantea* Lindl. are well known for their durability and rot resistance, suggesting the potential value of examination of their extractives. Although no single factor with marked antifungal action has been recognised, a series of phenolic norlignans has

been extracted and characterised.<sup>1</sup> These are of biogenetic interest since they contain a basic carbon

<sup>1</sup> (a) N. A. R. Hatam and D. A. Whiting, *J. Chem. Soc. (C)*, 1968, 1921; (b) R. Riffer and A. B. Anderson, *Phytochemistry*, 1967, **6**, 1557; (c) P. Henley-Smith and D. A. Whiting, *Phytochemistry*, 1976, **15**, 1285.

skeleton (1) which appears to derive from  $\gamma^1$ - $\beta^2$  linking of phenylpropanoid units, in contrast to the common  $\beta^1$ - $\beta^2$  union (2) exhibited by the natural lignans.<sup>2</sup> In this paper we report the constitution of a new metabolite (sequirin-D) of *S. sempervirens* which contains a carbon skeleton (3) with the key phenylpropanoid junction  $\alpha^1$ - $\beta^2$ ; to the best of our knowledge sequirin-D is the first example of the occurrence of this carbon skeleton in nature.

Sequirin-D dimethyl ether (4) was isolated after extensive chromatography of methylated aqueous extracts of heartwood, as a minor component (0.002% of dry wood),  $C_{19}H_{20}O_2$  (mass spectrometry). That neither this dimethyl ether nor a monomethyl ether was the true natural product was demonstrated by the exclusive



isolation of the diethyl ether (5),  $C_{21}H_{24}O_2$ , after ethylation of the extracts; sequirin-D is thus the free hydroxy-compound (6). The paucity of samples available led us to attempt direct crystallographic analysis of dimethyl sequirin-D, as needles, m.p. 107.5–109 °C, from hexane. The structure, solved by direct methods and refined by least-squares was revealed as (4) and is shown in general view in Figure 1 which also includes the numbering used for crystallographic purposes. The bond lengths and angles are displayed in Figure 2 with an indication of

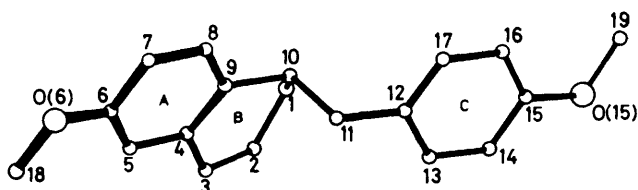


FIGURE 1 Dimethylsequirin-D. General view of the molecule and showing the crystallographic numbering system

standard deviation; in all cases these are similar to the expected values. Mean bond lengths for rings A and C are 1.390 and 1.386 Å respectively, with mean bond angle 120.0° in each case. Both aryl rings are planar

<sup>2</sup> K. Weinges and R. Spänig, in 'Oxidative Coupling of Phenols,' Edward Arnold, London, 1967, p. 323.

with maximum deviations from mean planes 0.007 and 0.012 Å for rings A and C respectively. Bond lengths and angles to hydrogen are not shown but C-H lengths were in the range 0.90–1.09 Å (largest standard deviation 0.05 Å) and bond angles involving hydrogen ranged between 117 and 124° for  $sp^2$  and 103 and 118° for  $sp^3$  carbon atoms (largest standard deviation 4°). The torsion angles are given in Table 1 and show that the

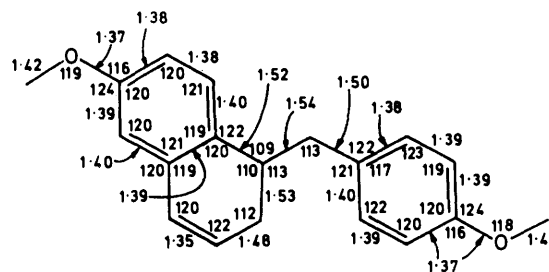


FIGURE 2 Dimethylsequirin-D. Bond lengths (Å) and bond angles (°): largest standard deviations 0.006 Å and 0.3°

double bond in ring B is not coplanar with ring A but twisted at a mean angle between planes of 13°. The *p*-methoxybenzyl group at C(10) adopts the *quasi*-axial conformation. Data on comparable dihydronaphthalenes is sparing but in  $\alpha$ -(+)-methyl-2-*[p*-(1,2-dihydro-1-naphthyl)phenoxy]propionate (7) the aryl substituent in ring B takes the *quasi*-equatorial dis-

TABLE 1  
Torsion angles (°)

C(10)-C(1)-C(2)-C(3)	31
C(1)-C(2)-C(3)-C(4)	-1
C(2)-C(3)-C(4)-C(5)	165
C(2)-C(3)-C(4)-C(9)	-12
C(4)-C(9)-C(10)-C(1)	37
C(4)-C(9)-C(10)-C(11)	-88
C(8)-C(9)-C(10)-C(1)	-147
C(8)-C(9)-C(10)-C(11)	+88
C(9)-C(10)-C(1)-C(2)	-47
C(11)-C(10)-C(1)-C(2)	76
C(1)-C(10)-C(11)-C(12)	57
C(9)-C(10)-C(11)-C(12)	-180
C(10)-C(11)-C(12)-C(13)	-100
C(10)-C(11)-C(12)-C(17)	+79

Max.  $\sigma$  0.4

position.<sup>3</sup> Compound (4) differs from (7) in that the substituent in ring B is benzyl rather than aryl. It appears from molecular models that in (4) with *quasi*-equatorial orientation the benzylic hydrogens [H(11A) and H(11B)] would involve in steric interaction with H(8). However in the *quasi*-axial disposition, steric repulsions around the C(10)-C(11) bond are minimised by the conformation adopted in the solid state (see Figures 1 and 3) with C(9)-C(10) nearly parallel to C(11)-C(12), and the 11 hydrogens flanking the C(9)-C(10) bond.

The chromophore of rings A,B is thus seen to comprise a styrene system, with a mean skew between aryl and olefinic components of 13°. The absolute chirality of such chromophores has been empirically correlated with their

<sup>3</sup> W. L. Bencze, B. Kisis, R. T. Puckett, and N. Finch, *Tetrahedron*, 1970, **26**, 5407.

chiroptical properties for many twisted styrene units and in all cases negative Cotton effects near 270 nm are

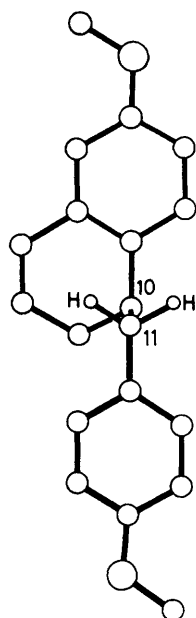


FIGURE 3 Dimethylsequirin-D. Conformation about C(10)-C(11) bond

associated with righthand helical chromophores and *vice versa*.<sup>4</sup> Di-*O*-methylsequirin-D shows a strong positive Cotton effect in the o.r.d. and c.d. spectra (Figure 4), with  $\Delta\epsilon +10.3$  (MeOH) indicating the ring AB unsaturation to be skewed in the absolute sense of (8). The

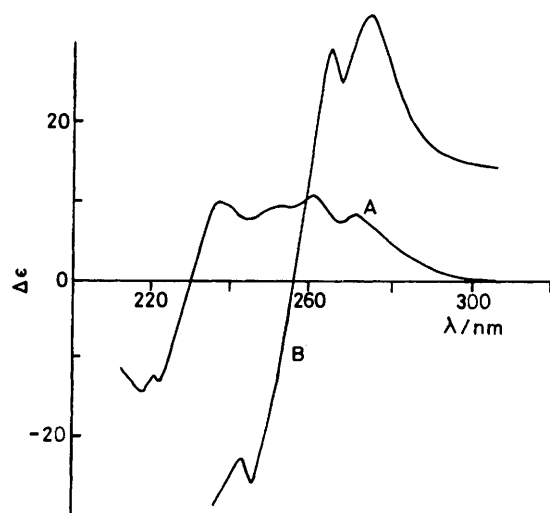
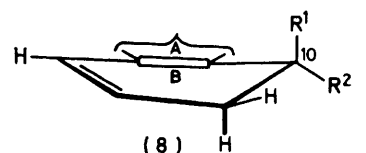


FIGURE 4 Chiroptical data for dimethylsequirin-D: A, c.d. in MeOH; B, o.r.d. in EtOH

absolute configuration at C(10) then depends on the disposition of the substituent at that centre. In the solid

<sup>4</sup> P. Crabbé, 'Optical Rotatory Dispersion and Circular Dichroism in Organic Chemistry,' Holden-Day, San Francisco, 1965, p. 253; H. G. Leeman and S. Fabbri, *Helv. Chim. Acta*, 1959, **42**, 2696; P. Crabbé, *Chem. and Ind.*, 1969, 917.

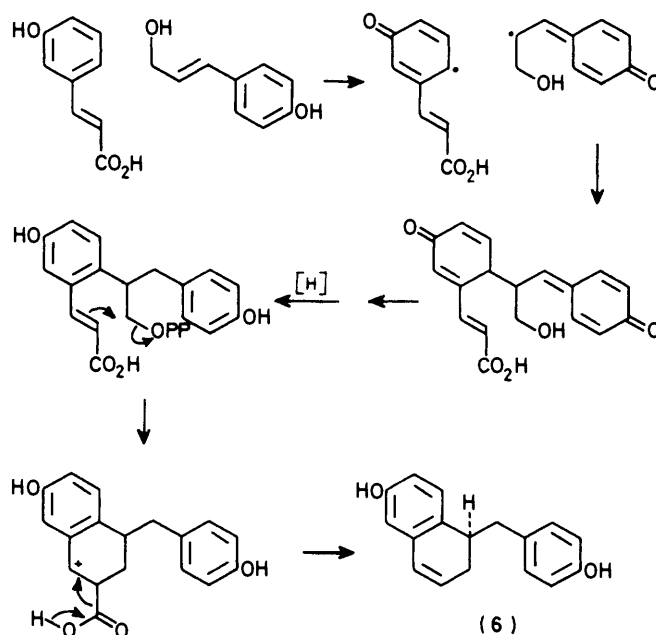
state, the *p*-methoxybenzyl group has been shown to prefer a *quasi*-axial orientation, *i.e.* (8a). It is possible that a different conformation might be adopted in solution, but since we consider the 10-axial arrangement to be the thermodynamically preferred form for a single molecule, it seems more likely that this orientation is retained in solution; in this case the absolute chirality is represented by (8a), (4), *etc.* <sup>1</sup>H N.m.r. data were ob-



a; R<sup>1</sup> = *p*-methoxybenzyl, R<sup>2</sup> = H

b; R<sup>1</sup> = H, R<sup>2</sup> = *p*-methoxybenzyl

tained but the lack of sufficient sample precluded studies detailed enough to reveal the coupling constants needed



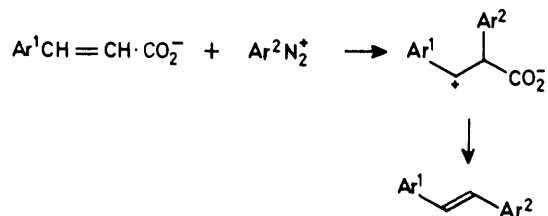
SCHEME 1 Hypothetical biogenesis of sequirin-D

to define the conformations in solution; work is in progress to produce synthetic sequirin-D to supply the deficiency.

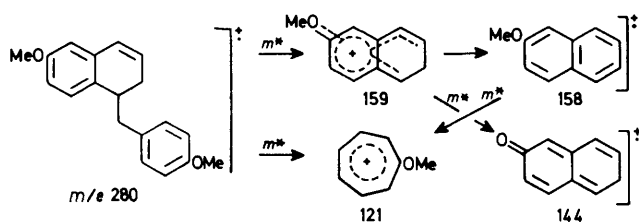
The biosynthesis of sequirin-D looks to involve two phenylpropane units (the alternative, cinnamate extended by acetate, leads to a different oxygenation pattern). A plausible mechanism (Scheme 1) is shown which involves oxidative coupling between *p*-hydroxycinnamyl alcohol and *m*-hydroxycinnamic acid. Metabolites arising from the latter are rare in nature, but examples<sup>5</sup> have been discovered in both higher and lower plants and animals.

<sup>5</sup> D. L. Dreyer, *J. Org. Chem.*, 1968, **33**, 3577; W. J. Irvine and M. J. Saxby, *Phytochemistry*, 1968, **7**, 278; P. V. Divekar, G. Read, and L. C. Vining, *Chem. and Ind.*, 1959, 731; (d) E. Lederer, *Trav. Soc. chim. biol.*, 1941, **23**, 1457.

Addition by an electrophilic carbon of the allylic alcohol, or a derivative, to the double bond of the  $\alpha$ -unsaturated acid, followed by decarboxylation produces the sequirin-D system. Chemical precedent for this type of addition is found in Bergmann and Shapiro's stilbene synthesis.<sup>6</sup> The mass spectra of di-*O*-methyl- and

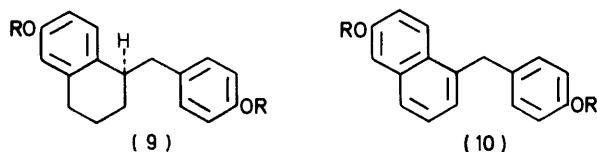


di-*O*-ethyl-sequirin-D showed the expected major cleavages (Scheme 2). Some samples purified by t.l.c. but



SCHEME 2 Major electron-impact induced fragmentation of di-*O*-methylsequirin-D

not crystallised showed additional peaks at  $M + 2$  and  $M - 2$ ; since sequirin-D is a dihydronaphthalene, it is tempting to infer the presence of the corresponding tetrahydronaphthalene (9) and naphthalene (10) respectively. A very small specimen (<1 mg) of the



former was obtained (of the diethyl compound), and had appropriate mass spectrometry and n.m.r. parameters (see Experimental section).

#### EXPERIMENTAL

*Extraction of Sequoia sempervirens.*—The general procedure has been described.<sup>1c</sup> An aqueous extract (ca. 0.8% of dry wood) was allowed to crystallise from acetone-benzene and the crystalline sequirin-B removed by filtration. The mother liquors were evaporated and treated as follows.

*Di-O-methylsequirin-D* (4; R = Me).—The residue (8.5 g) from extraction was heated under reflux in acetone (75 cm<sup>3</sup>) over anhydrous potassium carbonate (25 g), with stirring. Methyl iodide (20 cm<sup>3</sup>) was added to the mix initially, and another portion (10 cm<sup>3</sup>) was added dropwise over 6 h. After 48 h the reaction mixture was diluted with water (750 cm<sup>3</sup>) and extracted with ether. The extracts were dried, evaporated, and chromatographed on a silica

column (Hopkin & Williams MFC) by use of benzene-acetone (4:1), with t.l.c. monitoring (silica gel G plates). The first fraction ( $R_F$  0.61, benzene-acetone 4:1) was rechromatographed on a silica column with benzene elution. The least-polar fraction was repurified by p.l.c. (HF<sub>254</sub>), by use of light petroleum-benzene (1:2); the main component, *di-O-methylsequirin-D*, crystallised (30 mg) from n-hexane, (Found:  $M$ , 280.149. C<sub>19</sub>H<sub>20</sub>O<sub>2</sub> requires  $M$ , 280.146);  $\lambda_{\text{max}}$ , (EtOH) 230 (4.40), 254i (3.61), 264 (3.68), 272i (3.64), 286 (3.32), 302 (3.23), and 310i nm (3.15);  $\tau$  (CDCl<sub>3</sub>) 3.01d, 3.20 d (4 H,  $J$  8.8 Hz, ArH of ring c), 3.10–3.40m (3 H, ArH of ring a), 3.54d (1 H,  $J$  10.7 Hz, 3\*-H), 4.08m (1 H, 2-H), 6.21s (6 H, 2  $\times$  OCH<sub>3</sub>), 7.17–7.38m (3 H, 11-H<sub>2</sub>, 10-H), and 7.72m (2 H, 1-H<sub>2</sub>); c.d. (MeOH) 238 (+1.58), 253 (+8.53), 260 (+10.3), 271 (+8.37), and 299 nm (+1.42);  $m/e$  280 (23%), 161, 160, 159 (100%), 158 (100%), 145, 144, 143, 129, 128, 127, 122, 121 (51%), 116, and 115.

*Di-O-ethylsequirin-D* (4; R = Et).—The residue (18 g) from extraction was vigorously stirred in ethanol (150 cm<sup>3</sup>) while sodium hydroxide (20 g) in water (50 cm<sup>3</sup>) with diethyl sulphate (50 g) were added in portions over 1 h. More sodium hydroxide (5 g) in water (10 cm<sup>3</sup>) was added and the mixture heated under reflux for 15 h, cooled, and poured into

TABLE 2

Atom co-ordinates, with standard deviations in parentheses

Atom	$x/a$	$y/b$	$z/c$
C(1)	0.159 1(3)	0.765 9(8)	1.093 1(2)
C(2)	0.150 0(3)	0.535 5(8)	1.147 2(2)
C(3)	0.252 2(3)	0.453 8(7)	1.222 0(2)
C(4)	0.377 0(2)	0.594 5(6)	1.250 6(2)
C(5)	0.470 9(3)	0.545 8(7)	1.340 8(2)
C(6)	0.585 3(3)	0.689 4(7)	1.369 9(2)
C(7)	0.605 8(3)	0.878 4(7)	1.309 9(2)
C(8)	0.513 7(3)	0.924 2(6)	1.220 8(2)
C(9)	0.398 4(2)	0.782 2(6)	1.189 6(2)
C(10)	0.301 8(3)	0.816 8(6)	1.088 3(2)
C(11)	0.344 8(3)	0.653 6(6)	1.012 5(2)
C(12)	0.255 1(2)	0.675 3(6)	0.909 7(2)
C(13)	0.157 7(3)	0.498 3(6)	0.871 5(2)
C(14)	0.069 8(3)	0.522 3(6)	0.778 2(2)
C(15)	0.078 0(3)	0.721 3(6)	0.720 6(2)
C(16)	0.176 2(3)	0.897 7(6)	0.754 6(2)
C(17)	0.262 2(3)	0.871 5(6)	0.848 5(2)
C(18)	0.669 2(3)	0.459 0(10)	1.519 7(2)
C(19)	-0.018 8(4)	0.945 9(9)	0.572 5(2)
O(6)	0.683 0(2)	0.654 5(6)	1.456 5(2)
O(15)	-0.014 1(2)	0.729 5(5)	0.630 5(1)
H(1A)	0.134(4)	0.914(9)	1.127(3)
H(1B)	0.100(3)	0.760(8)	1.022(2)
H(2)	0.058(2)	0.433(9)	1.125(3)
H(3)	0.240(3)	0.299(9)	1.262(2)
H(5)	0.451(3)	0.433(6)	1.382(2)
H(7)	0.687(3)	0.962(8)	1.329(2)
H(8)	0.533(3)	1.046(8)	1.175(2)
H(10)	0.310(3)	0.986(8)	1.065(2)
H(11A)	0.440(2)	0.695(6)	1.016(2)
H(11B)	0.349(3)	0.478(7)	1.040(2)
H(13)	0.152(3)	0.358(6)	0.912(2)
H(14)	-0.007(3)	0.400(9)	0.754(2)
H(16)	0.183(3)	1.025(9)	0.711(2)
H(17)	0.332(3)	1.000(7)	0.873(2)
H(18A)	0.748(3)	0.466(8)	1.578(2)
H(18B)	0.674(4)	0.309(15)	1.484(3)
H(18C)	0.583(3)	0.478(8)	1.545(2)
H(19A)	-0.082(4)	0.916(9)	0.511(3)
H(19B)	0.077(4)	0.974(11)	0.557(3)
H(19C)	-0.049(3)	1.064(9)	0.609(3)

water. The organic products were collected in diethyl ether, washed, dried, and the solvent evaporated. The product (11 g) was rechromatographed ( $\times 2$ ) on silica columns

\* Crystallographic numbering system used to avoid confusion.

<sup>6</sup> F. Bergmann and D. Shapiro, *J. Org. Chem.*, 1947, **12**, 57.

as before, the parallel fraction being collected. The component corresponding in chromatographic behaviour to the dimethyl ether was twice repurified by p.l.c. to yield *di-O-ethylsequirin-D*, an amorphous solid (Found:  $M$ , 308.176.  $C_{21}H_{24}O_2$  requires  $M$ , 308.177);  $\tau$  ( $CDCl_3$ ) 3.03d, 3.21d (4 H,  $J$  9 Hz, ArH of ring c), 3.35 (3 H, ArH of ring a), 3.55 d (1 H,  $J$  10 Hz, 3-H), 4.1m (1 H, 2-H), 5.99q (4 H,  $J$  7 Hz,  $2 \times OCH_2$ ), 7.1–7.9m (5 H), and 8.60t (6 H,  $J$  7 Hz,  $2 \times CH_3$ );  $m/e$  308 (4%) ( $M^+$ ), 173 (100%), 172 (45%), and 135 (35%). An incompletely purified sample of this compound also displayed ions at 306.161 a.m.u. [ $C_{21}H_{22}O_2$ , (10), requires  $M$  306.162] and 310.192 [ $C_{21}H_{26}O_2$ , (9), requires 310.193]. On repeated p.l.c. the *benzyltetralin* (9) was obtained,  $\tau$  ( $CDCl_3$ ) 2.8–3.3 (7 H, ArH), 5.99q (4 H,  $J$  7 Hz,  $2 \times OCH_2$ ), 7.48q (2 H,  $J$  6 Hz), and 8.60t (6 H,  $J$  7 Hz,  $2 \times CH_3$ ); hydrocarbon impurities (arising from silica) masked other signals  $\tau$  8.6–9.2:  $m/e$  310 (64%) ( $M^+$ ), 175 (66%), and 135 (100%).

**Crystallography.**—A suitable specimen was obtained by crystallisation from n-hexane. Oscillation and Weissenberg photographs taken about the needle ( $b$ ) axis established unit-cell dimensions and space group. For intensity measurements a crystal was mounted on a Hilger and Watts four-circle diffractometer. Unit-cell dimensions were refined by a least-squares fit on the positions of 12 reflections measured on the diffractometer. Intensity data were collected with Cu- $K_\alpha$  radiation for  $2\theta < 156^\circ$  by use of the  $\omega$ - $2\theta$  scan. Of 1 819 independent reflections measured, 1 622 were considered observed (net count  $> 3\sigma$ ) and were used in the subsequent structure refinement. No absorption corrections were made. Data reduction and subsequent crystallographic calculations were performed using the 'X-Ray '70' system of programs. Normalised structure factors were calculated using the SAP programs of the National Research Council (Ottawa) with a manually plotted  $K(s)$  curve. Atomic scattering factors were taken from ref. 7.

**Crystal Data.**— $C_{19}H_{20}O_2$ ,  $M = 280$ . Monoclinic,  $a = 10.432 \pm 0.004$ ,  $b = 5.440 \pm 0.001$ ,  $c = 14.046 \pm 0.004$  Å,  $\beta = 105.64 \pm 0.03^\circ$ ,  $U = 767.60$  Å,  $Z = 2$ ,  $D_c = 1.21$ ,  $F(000) = 300$ . Space group  $P2_1$  uniquely from systematic absences and observed optical activity. Cu- $K_\alpha$  radiation,  $\lambda = 1.54178$  Å;  $\mu(\text{Cu-}K_\alpha) = 4.73$  cm $^{-1}$ .

The structure was determined by direct methods by use of the MULTAN program.<sup>8</sup> Eight sets of phases were calculated and several of the corresponding  $E$  maps were investigated without success. Finally an  $E$  map computed on the set of phases with the worst figure-of-merit (FOM 1.05) showed 19 large peaks, 18 of which corresponded to chemically reasonable atomic positions.

A structure-factor calculation based on these 18 atomic positions showed an agreement factor  $R$  of 0.47. A sub-

sequent Fourier synthesis revealed the positions of the remaining 3 atoms. Four cycles of block-diagonal least-squares refinement of spatial co-ordinates with isotropic temperature factors followed, using all data and unit weight. The agreement factor  $R$  was lowered to 0.15 and in subsequent calculations the thermal parameters were allowed to vary anisotropically. Two further cycles of refinement were carried out, and analysis of the agreement between  $F_o$  and  $F_c$  suggested the adoption of a weighting scheme of the form  $w = 1$  for  $|F_o| < 6.0$  and  $w = (6.0/|F_o|)^2$  for  $|F_o| > 6.0$ . Two more cycles reduced  $R$  to 0.10. A difference-Fourier synthesis was next calculated which revealed the approximate positions of all 20 hydrogen atoms.

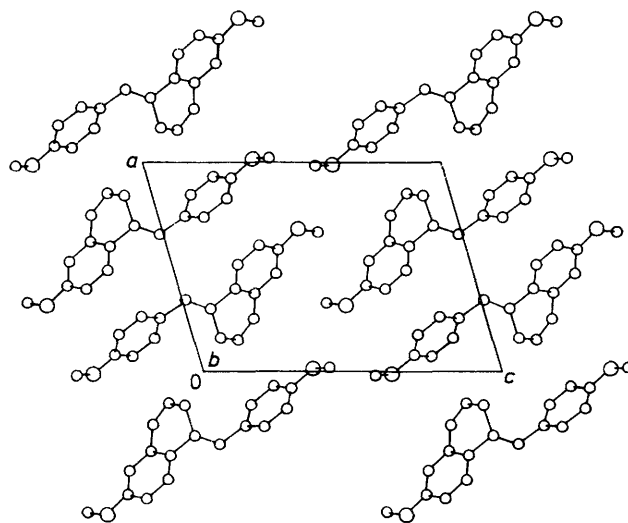


FIGURE 5 Dimethylsequirin-D. Packing in the unit cell projected down the  $b$  axis

The positions of the hydrogen atoms were then calculated from bond-length and -angle considerations, and included in the structure-factor calculations. Four further cycles of refinement, including hydrogen atoms with isotropic temperature factors, lowered  $R$  to 0.064 when the largest parameter shifts were  $< 0.7\sigma$ , indicating that refinement had converged after a total of 12 cycles. The accuracy of the structure was confirmed by computing a final difference map which showed no peaks or depressions  $> 0.3$  eÅ $^{-3}$ . Final atomic co-ordinates are listed in Table 2; temperature factors and observed and calculated structure factors are listed in Supplementary Publication No. 22237 (12 pp., 1 microfiche).<sup>\*</sup> Molecular packing in the crystalline state is shown in Figure 5. No intermolecular contact less than the sums of appropriate van der Waals' radii were observed.

[7/1776 Received, 10th October, 1977]

\* For details see Notice to Authors No. 7 in *J.C.S. Perkin I*, 1975, Index issue.

<sup>7</sup> 'International Tables for X-Ray Crystallography,' vol. III, Kynoch Press, Birmingham, 1962.

<sup>8</sup> G. Germain, P. Main, and M. M. Woolfson, *Acta Cryst.*, 1971, **A27**, 360.