

## The Chemistry of Pseudomonic Acid. Part 1. The Absolute Configuration of Pseudomonic Acid A

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The configuration of the double bond in pseudomonic acid A (1a) is shown to be *E* by comparison of the spectroscopic properties of its methyl ester (1b) with those of methyl isopseudomonate A (2) obtained from (1b) by photolysis. Ozonolysis of methyl pseudomonate A (1b) afforded the crystalline ketone (3a). An X-ray analysis of the *o*-bromophenylhydrazone derivative of ketone (3a) confirmed the structural assignment and provided the absolute stereochemistry at each of the eight chiral centres. Pseudomonic acid A, therefore, may be formulated as (4), 9-{4-[5*S*-(2*S*,3*S*-epoxy-5*S*-hydroxy-4*S*-methylhexyl)-3*R*,4*R*-dihydroxytetrahydropyran-2*S*-yl]-3-methylbut-2(*E*)-enoxy}nonanoic acid.

PSEUDOMONIC ACID A, a naturally occurring antibiotic produced by fermentation of a strain of *Pseudomonas fluorescens*<sup>1</sup> was assigned the gross structure (1a) by Chain and Mellows on the basis of spectroscopic and degradative evidence.<sup>2</sup> In this paper we present our findings in support of the *E*-configuration of the olefinic double bond in pseudomonic acid A (1a), together with an X-ray analysis of a derivative of (1a) which confirms the structural assignment and also provides the absolute stereochemistry.

The configuration of the double bond of the  $\alpha\beta$ -unsaturated ester linkage in (1a) was designated *E* from the chemical shift of the vinylic methyl group in the <sup>1</sup>H and <sup>13</sup>C n.m.r. spectra of its methyl ester (1b).<sup>2</sup> In order to confirm this assignment we prepared the geometrical isomer (2) of methyl pseudomonate A (1b) as follows. A 0.01M solution of (1b) in benzene was irradiated with a Hanovia low pressure mercury lamp and after 2 h worked up to give unchanged (1b) and an oily new product,  $[\alpha]_D +25.8^\circ$  (*c* 1.5 CHCl<sub>3</sub>), which was separated by silica gel chromatography. The molecular formula of the new product, C<sub>27</sub>H<sub>46</sub>O<sub>9</sub> (M<sup>+</sup>), indicated that it was isomeric with methyl pseudomonate A and on the basis of its spectroscopic properties,  $\lambda_{\max}$  (C<sub>2</sub>H<sub>5</sub>-OH) 221 nm ( $\epsilon$  11 600),  $\nu_{\max}$  (CHCl<sub>3</sub>) 3 400, 1 730, 1 690, and 1 650 cm<sup>-1</sup>, <sup>1</sup>H and <sup>13</sup>C n.m.r., it was formulated as

(2), with the C-15 vinylic methyl group *cis* to the olefinic proton. The name methyl isopseudomonate A was ascribed to (2).

The geometry of the 2,3-double bond, *E* in (1b) and *Z* in (2), was assigned by comparison of the <sup>1</sup>H and <sup>13</sup>C n.m.r. spectra for the two isomers and confirmed by NOE measurements. These data are summarised in Table 1. In the 90 MHz <sup>1</sup>H spectra the chemical shift

TABLE I

<sup>1</sup> H and <sup>13</sup> C chemical shifts ( $\delta$ ) and NOE enhancement				
Compound	15-CH <sub>3</sub>	15-CH <sub>3</sub>	4-CH <sub>2</sub>	% NOE <sup>a</sup>
(1b)	2.21	19.1	42.7	4
(2)	2.01	27.4	35.6	23

<sup>a</sup> Percentage enhancement of vinylic proton signal,  $\delta$  5.73 (1b),  $\delta$  5.82 (2), by irradiation of 15-CH<sub>3</sub> signal.

of the C-15 vinylic methyl group,  $\delta$  2.21 in (1b) and 2.01 in (2), indicated that this group was *cis* to the ester function in (1b) and *trans* in (2).<sup>3</sup> Major shift differences in the <sup>13</sup>C n.m.r. spectra of (1b) and (2) were observed for C-15 and -4 resonances only, while the excellent agreement between the other carbon resonances (Table 2) confirmed that the compounds were geometrical isomers. These shift differences were consistent with a

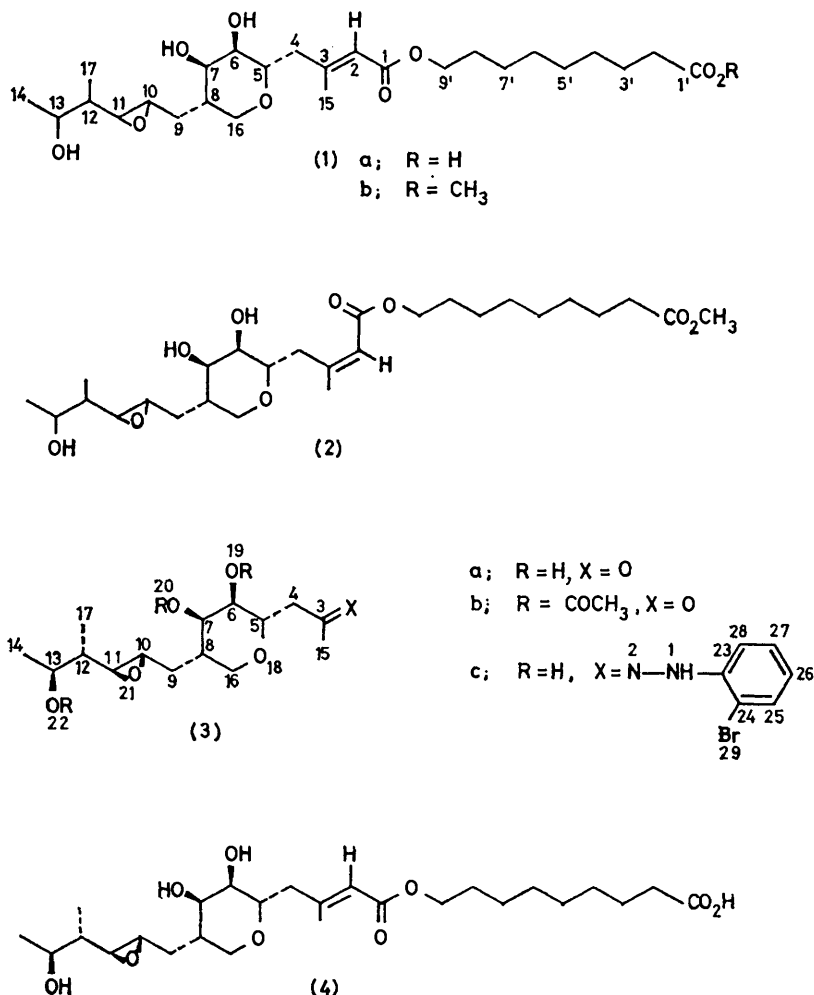
<sup>2</sup> E. B. Chain and G. Mellows, *J.C.S. Chem. Comm.*, 1974, 847; *J.C.S. Perkin I*, 1977, 294.

<sup>3</sup> L. M. Jackman, 'Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry,' Pergamon Press, London, 1959, 1st edn., pp. 119-125.

<sup>1</sup> A. T. Fuller, G. Mellows, M. Woolford, G. T. Banks, K. D. Barrow, and E. B. Chain, *Nature*, 1971, **234**, 416; Beecham Group Ltd., B.P. 1,395,907.

dominant steric interaction of the ester function exerting a shielding effect on C-15 in (1b) and C-4 in (2).<sup>4</sup> The <sup>1</sup>H and <sup>13</sup>C chemical shift assignments of the C-15 methyl groups in (1b) and (2) were supported by similar values obtained for the *cis*- and *trans*-methyl groups of 3,3-dimethylacrylic acid.<sup>5,6</sup> Finally, unambiguous configurational assignments were made from NOE measurements, where a significant 23% enhancement of the C-2

proved unsuccessful. However, ozonolysis of methyl pseudomonte A (1b) followed by reductive work up with triethyl phosphite afforded the crystalline methyl ketone (3a) quantitatively. The similarities of the carbon resonances of (3a) (Table 2) with the corresponding carbons in (1b), indicated that the stereochemical integrity of the molecule had been preserved during the ozonolysis. The oily triacetate (3b) of ketone (3a) was



vinyl proton signal intensity on low power irradiation of the C-15 methyl protons was observed for the *Z*-isomer (2) only.

It was of interest to monitor the light induced isomerisation of methyl pseudomonte A and this was conveniently done by means of h.p.l.c. (Spherisorb silica column, 4 × 200 mm), when it was observed that photolysis in benzene of either methyl pseudomonte A (1b) or its isomer (2) resulted after 4 h in an equilibrium mixture of 55% (1b) and 45% (2). This ratio did not change on further irradiation.

Attempts to obtain suitable crystals of pseudomonic acid A or a derivative for X-ray analysis have so far

<sup>4</sup> J. B. Stothers, 'Carbon-13 N.M.R. Spectroscopy,' Academic Press, London, 1972, p. 406.

shown to be identical with a sample of (3b), kindly provided by Dr. Mellows and prepared by *cis*-hydroxylation of methyl pseudomonte A triacetate with osmium tetroxide followed by sodium periodate cleavage.<sup>2</sup>

An X-ray analysis of the *o*-bromophenylhydrazone (3c) of ketone (3a) confirmed the assigned structure and established the chirality at each of the eight asymmetric centres in (3c) as 5*S*, 6*R*, 7*R*, 8*S*, 10*S*, 11*S*, 12*S*, and 13*S*. Bond lengths and angles are given in Figure 1, and the conformation in the crystal lattice is illustrated by means of the stereoscopic drawings in Figure 2. It

<sup>5</sup> F. A. L. Anet and A. J. R. Bourn, *J. Amer. Chem. Soc.*, 1965, **87**, 5250.

<sup>6</sup> E. Lippmaa, T. Pehk, K. Andersson, and C. Rappe, *Org. Magnetic Resonance*, 1970, **2**, 109.

will be observed that the pyran ring adopts a chair conformation in which the C-6 and -7 hydroxys are *cis*

4*S*-methylhexyl)-3*R*,4*R*-dihydroxytetrahydropyran-2*S*-yl]-3-methylbut-2(*E*)-enoxy}nonanoic acid.

TABLE 2

Comparison of  $^{13}\text{C}$  n.m.r. spectra of the esters (1b), (2) and the ketone (3a) <sup>a,b</sup>

Carbon	(1b)	(2)	(3a)	Multiplicity
1	166.7	168.5		s
2	117.5	117.9		d
3	156.6	159.4	192.2	s
4	42.7	35.6	46.9	t
5	74.8	76.6	72.7	d
6	68.8	67.4	69.0	d
7	70.1	70.2	70.1	d
8	39.4	38.9	39.6	d
9	31.6	31.8	31.6	t
10	55.5	56.0	55.6	d
11	61.2	61.5	61.1	d
12	42.7	43.0	42.8	d
13	71.0	71.5	71.2	d
14	20.7	20.7	20.8	q
15	19.1	27.4	30.9	q
16	65.4	65.6	65.5	t
17	12.7	12.8	12.7	q
1'	174.2	174.5		
2'	34.1	34.1		
3'	24.9	25.0		
4'				
5'	29.0	29.1		
6'				
7'	25.9	25.9		
8'	28.7	28.6		
9'	63.8	64.7		
OMe	51.4	51.5		

<sup>a</sup> P.p.m. to low field of  $\text{Me}_4\text{Si}$ ; solvent  $\text{CDCl}_3$ . <sup>b</sup> Carbon assignments made by reference to T. C. Felton, R. B. Jones, G. Mellows, and L. Phillips, *J.C.S. Perkin I*, 1977, 309.

to one another and *trans* to the C-5 and -8 carbon substituents. The epoxide ring is shown to be *trans*-substituted in accord with the coupling constant ( $J$  2.2 Hz) for the C-10 and -11 protons. The conformation adopted by (3c) in the crystal lattice closely parallels that for the predominant solution conformer of the ketone-triacetate (3b) deduced by Chain and Mellows from  $^1\text{H}$  n.m.r. measurements.<sup>2</sup>

## EXPERIMENTAL

Mass spectra were obtained at 70 eV using an AEI MS9 instrument operating at 8 kV.  $^1\text{H}$  n.m.r. data were recorded at 90 MHz on a Perkin-Elmer R32 instrument and  $^{13}\text{C}$  measurements using a Varian CFT20 spectrometer. Both

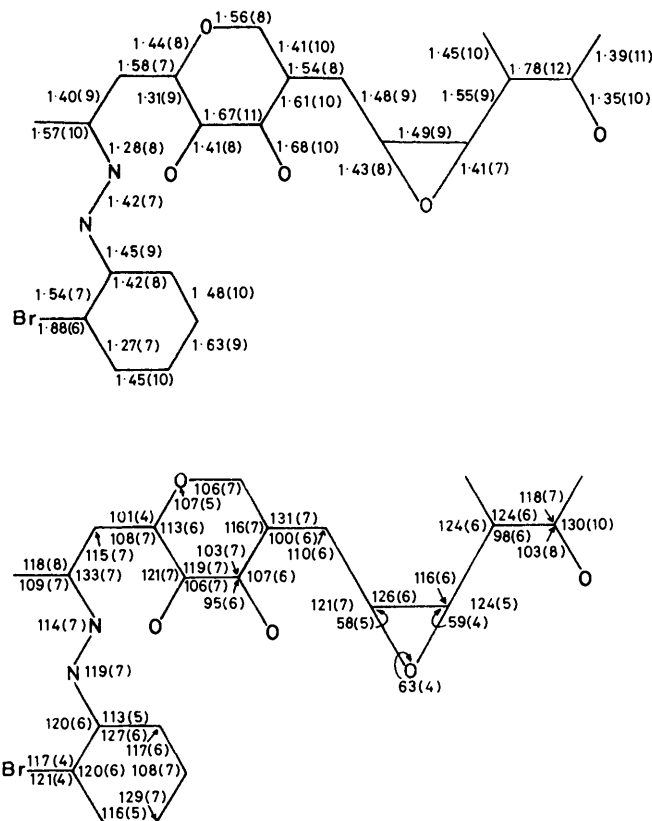


FIGURE 1 Bond lengths (Å) and angles ( $^\circ$ ) of compound (3c) with standard deviations in parentheses

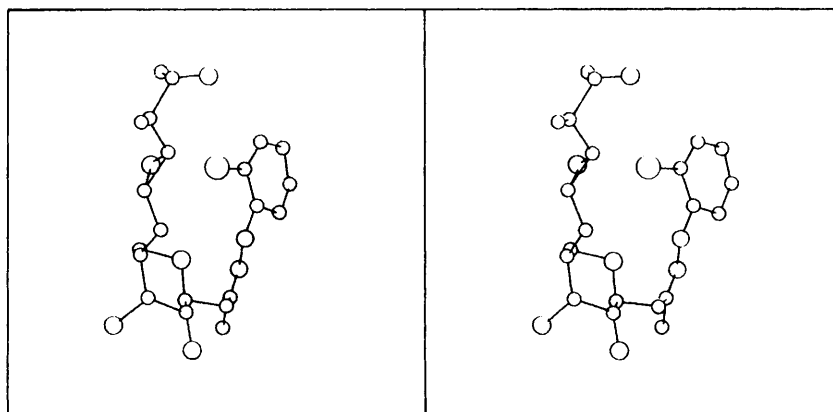


FIGURE 2

In conclusion therefore the results of these studies permit the total structure of pseudomonic acid A to be formulated as (4), 9-[4-[5*S*-(2*S*,3*S*-epoxy-5*S*-hydroxy-

$^1\text{H}$  and  $^{13}\text{C}$  n.m.r. spectra were recorded at ambient temperatures in  $\text{CDCl}_3$  solutions with tetramethylsilane as internal standard.

*Methyl Isopseudomonate A* (2).—A 0.01M solution of methyl pseudomonate A (1b) in benzene (400 ml) was irradiated in a Hanovia  $2 \times 45$  W low pressure mercury lamp for 2 h. The benzene was removed and the residual oil chromatographed over silica gel. The first fraction isolated was an oil (0.92 g, 45%), identified as *methyl isopseudomonate A* (2),  $[\alpha]_D^{20} +25.8^\circ$  ( $c$  1.5 CHCl<sub>3</sub>),  $\nu_{\max}$  (CHCl<sub>3</sub>) 3 400br, 1 730, 1 690, 1 650, 1 195, 1 155, and 1 085 cm<sup>-1</sup>,  $\lambda_{\max}$  (EtOH) 221 nm ( $\epsilon$  11 600),  $\delta_{\text{H}}$  (CDCl<sub>3</sub>) 5.82 (1 H, m, 2-H), 2.80 (1 H, m, 10-H), 2.71 (1 H, m, 11-H), 2.01 (3 H, d,  $J$  1.5 Hz, 15-CH<sub>3</sub>), 1.20 (3 H, d,  $J$  7 Hz, 14-CH<sub>3</sub>), and 0.93 (3 H, d,  $J$  7 Hz, 17-CH<sub>3</sub>),  $m/e$  514 ( $M^+$ , 1.5%), 496 (2.5), 412 (6.5), 366 (44), 270 (87), 227 (100), and 209 (32) (Found: C, 62.7; H, 8.8. C<sub>27</sub>H<sub>46</sub>O<sub>9</sub> requires C, 63.0; H, 9.0%). The second fraction isolated (1.12 g) was identified as unchanged methyl pseudomonate A (1 b).

3R,4R-Dihydroxy-5S-(2S,3S-epoxy-5S-hydroxy-4S-methylhexyl)tetrahydropyran-2S-ylacetone (3a).—Ozonised oxygen (ca. 1%) was bubbled through a solution of methyl pseudomonate (1b) (0.514 g) in methanol (8 ml) and pyridine (2 drops) at  $-78^\circ\text{C}$  for 0.5 h (when a blue colour developed). Excess of ozone was blown off by dry nitrogen at  $-78^\circ\text{C}$ . Triethyl phosphite (80%; 0.3 ml) was then added and the reaction mixture was allowed to come to room temperature. The solvent was removed at room temperature *in vacuo* and the residue was chromatographed over silica gel (20 g). Elution of the column with chloroform-methanol (93:7) at the rate of 2 ml min<sup>-1</sup> gave the ketone (3a) (0.299 g), m.p. 85–86° (from chloroform),  $[\alpha]_D^{20} +11.9^\circ$  ( $c$  1.0 CHCl<sub>3</sub>),  $\nu_{\max}$  (CHCl<sub>3</sub>) 1 708, 1 112, 1 080, and 1 050 cm<sup>-1</sup>,  $\delta_{\text{H}}$  (CDCl<sub>3</sub>) 2.72 (4 H, m), 2.19 (3 H, s, COCH<sub>3</sub>), 1.96 (1 H, m), 1.69 (2 H, m), 1.35 (1 H, m), 1.20 (3 H, d,  $J$  6 Hz, 14-CH<sub>3</sub>), and 0.92 (3 H, d,  $J$  6.5 Hz, 17-CH<sub>3</sub>),  $m/e$  302 ( $M^+$ , 1%), 284 (1), 269 (3), 266 (1), 259 (2), 253 (3), 244 (3), 241 (9), 227 (24), 154 (100), and 112 (63) (Found: C, 59.45; H, 8.65. C<sub>15</sub>H<sub>26</sub>O<sub>6</sub> requires C, 59.6; H, 8.65%).

3R,4R-Diacetoxy-5S-(2S,3S-epoxy-5S-acetoxy-4S-methylhexyl)tetrahydropyran-2S-ylacetone (3b).—A solution of ketone (3a) (0.025 g) in acetic anhydride (3 ml) and pyridine (2 drops) was stirred at room temperature overnight. The mixture was then poured into 5% sodium hydrogen carbonate, extracted with chloroform and the extract washed with brine, dried, and evaporated to afford the oily triacetate (3b) (0.018 g) spectroscopically identical with a sample of (3b) provided by Dr. Mellows.

*o*-Bromophenylhydrazone of 3R,4R-Dihydroxy-5S-(2S,3S-epoxy-5S-hydroxy-4S-methylhexyl)tetrahydropyran-2S-ylacetone (3c).—A solution of ketone (3a) (0.1 g) in water (1 ml) was added dropwise to a hot solution of *o*-bromophenylhydrazine hydrochloride (0.09 g) and sodium acetate (0.07 g) in water (8 ml). The mixture was kept at 60 °C for 5 min and then cooled to room temperature. The precipitate was filtered, washed with water, and dried to give the *o*-bromophenylhydrazone (3c) (0.12 g), m.p. 120–121° (from acetonitrile-water),  $\nu_{\max}$  (Nujol) 3 370br, 3 230sh, 1 590, 1 500, 1 300, 1 285, 1 110, 1 070, 1 030, 955, 915, and 755 cm<sup>-1</sup>;  $\lambda_{\max}$  (EtOH), 278 nm ( $\epsilon$  14 400),  $\delta_{\text{H}}$  ([CD<sub>3</sub>]<sub>2</sub>CO) 8.89 (1 H, s, NH), 7.00–7.45 (3 H, m, ArH), 6.60 (1 H, m, ArH), 2.74 (3 H, s, 15-CH<sub>3</sub>), 2.65 (2 H, m, 10- and 11-H), 1.14 (3 H, d,  $J$  7 Hz, 14-CH<sub>3</sub>), and 0.88 (3 H, d,  $J$  7 Hz, 17-CH<sub>3</sub>),  $m/e$  470 ( $M^+$ , 30%), 452 (4), 279 (10), 267 (11), 250 (37), 237 (25), and 226 (100) (Found:  $M^+$ , 470.142. C<sub>21</sub>H<sub>31</sub>BrN<sub>2</sub>O<sub>5</sub> requires  $M$ , 470.141).

*Crystal Structure Determination of the o*-Bromophenylhydrazone (3c).—*Crystal data*. C<sub>21</sub>H<sub>31</sub>BrN<sub>2</sub>O<sub>5</sub>,  $M$  471.4,

hexagonal  $a = b = 24.769(3)$ ,  $c$  7.467(2),  $\alpha = \beta = 90^\circ$ ,  $\gamma$  120°,  $U$  3 967.3 Å<sup>3</sup>,  $D_c$  1.18,  $D_m$  1.17 g cm<sup>-3</sup>,  $Z$  6,  $F(000)$  1 476. Space group  $P6_5$  from systematic absences and anomalous dispersion results, Mo- $K_\alpha$  radiation (graphite monochromator)  $\lambda$  0.710 69 Å,  $\mu$  16.8 cm<sup>-1</sup>.

The crystals were long but very thin, flattened needles. The cell parameters were initially found from oscillation and Weissenberg photographs and then refined by least squares from the setting angles of 14 reflections on a Hilger-Watt four-circle diffractometer. Reflections were scanned ( $\omega$ —2 $\theta$  mode) for  $\theta \leq 18^\circ$  and 634 which were found to have a net count  $\geq 2\sigma$  were deemed observed and used in the refinement.

The position of the bromine atom was readily deduced by the Patterson method which also defined the space group as belonging to one of the two enantiomeric arrangements  $P6_1$  or  $P6_5$ . The development of the total structure by Fourier techniques phased on the bromine atom was slow but eventually successful. Least-squares refinements were carried out with the bromine treated anisotropically but there were insufficient data to justify refining the other atoms in this mode. Taking all the factors into account, the final  $R$  factor of 14.5% is regarded as satisfactory. Figure 1 shows in diagrammatic form, the bond lengths and angles found. Although some of these vary considerably from the normal values the standard deviations of the quantities are also large and none of the parameters differ from a reasonable value by more than two standard deviations. Formula (3c) indicates the crystallographic numbering.

The problem of the connectivity of the molecule having been settled there remained the question of the absolute configuration. The statistical approach to the determination of this depends on quite small differences in  $R$  for enantiomeric molecules when the calculation is performed using anomalous dispersion correction for a heavy atom. This method assumes that there are no systematic errors in the data and is less convincing if agreement between observed and calculated structure factors is relatively poor, as is the present case.

Application of this method did, however, provide an answer which by generally accepted criteria could be regarded as conclusive. The ratio of the weighted  $R$  values for the two enantiomorphs (corrected for anomalous dispersion) was  $17.59/17.33 = 1.015$ . Reference to the Tables given by Hamilton<sup>7</sup> shows that for the assignment of configuration to be significant at the 0.005 level this ratio should be  $>1.010$ .

However, in view of the inaccuracy of the structure it was felt that this evidence was insufficient. Accordingly, 22 reflections were chosen which calculation showed should exhibit the largest difference in intensity between the Friedel pairs. The crystal was realigned on the diffractometer and for each of these 22 reflections five measurements, under slow counting conditions, were made for the reflection and its Friedel pair. In every case a significant difference was found in the pair of intensities for every measurement, and the sense of this difference was in agreement with the computed difference. The results obtained in this way confirmed the earlier statistical derivation. The conclusion is therefore that the true space-group is  $P6_5$  and that the configuration of the molecule is as shown in Figure 2.

<sup>7</sup> W. C. Hamilton, *Acta Cryst.*, 1965, **18**, 502.

Figure 3 shows the crystal packing viewed down the  $c$  axis. The secondary hydroxy-groups at the ends of the molecular chains are close to the three-fold screw axis and

TALS package,<sup>8</sup> and perspective drawings were prepared using PLUTO.<sup>9</sup> A list of observed and calculated structure factors together with fractional co-ordinates and thermal

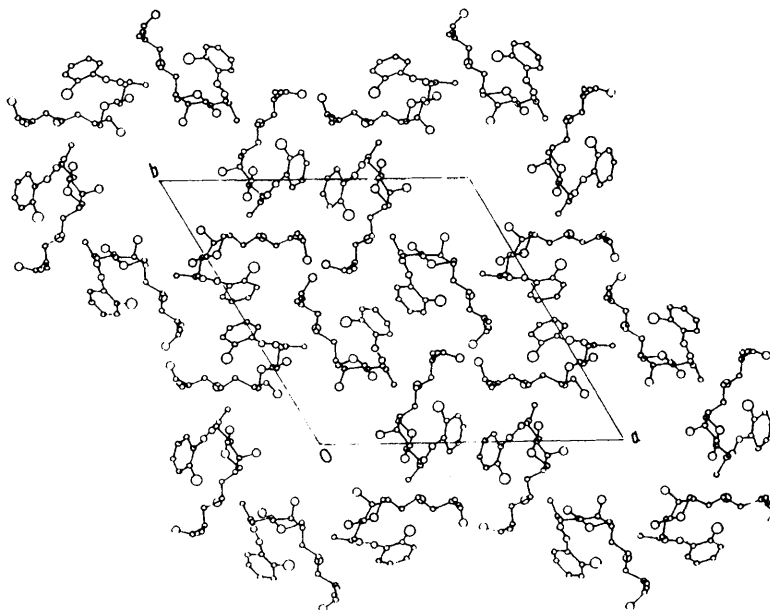


FIGURE 3

appear to form a hydrogen bonded spiral (O—O distance 2.93 Å). Notable also is the large empty channel round the origin which probably is the reason for the low density (1.18 g cm<sup>-3</sup>) of the crystal.

Computations were carried out using the Oxford CRYSTALS

package, and perspective drawings were prepared using PLUTO.<sup>9</sup> A list of observed and calculated structure factors together with fractional co-ordinates and thermal parameters are available in Supplementary Publication No. SUP 22219 (9 pp.).\*

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\* For details of Supplementary Publications see Notice to Authors No. 7 in *J.C.S. Perkin I*, 1977, Index issue.

<sup>8</sup> W. R. Carruthers, personal communication.

<sup>9</sup> Cambridge Data Centre, W. D. S. Motherwell, personal communication.