Isolation, and Crystal and Molecular Structure of Cytochalasin G: an [11]Cytochalasan containing an Indole Group

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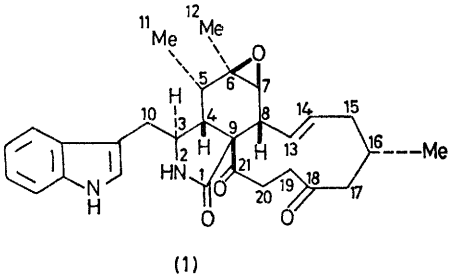
The structure of cytochalasin G, an [11]cytochalasan containing a 10-(indol-3-yl) group, and produced by an unidentified *Nigrosabulum sp.*, has been determined by *X*-ray methods, by use of 1656 independent data collected on a diffractometer. Crystals are orthorhombic, $a = 25{\cdot}437(2)$, $b = 7{\cdot}480(1)$, $c = 13{\cdot}458(1)$ Å, space group $P2_12_12_1$, with $Z = 4$. The structure was solved by the symbolic-addition method and refined by least-squares techniques to R 0·102.

CULTURE filtrates from an unidentified *Nigrosabulum sp.* produce cytochalasan-like effects [1-3] on mouse ' L ' cells growing in tissue culture.[4] The major active component, for which we suggest the trivial name cytochalasin G,[5] has been shown to possess structure (1) by *X*-ray analysis. Although the absolute stereochemistry of cytochalasin G was not determined by the *X*-ray analysis, the stereochemistry shown in all representations of the molecule is in accord with the absolute stereochemistries of other cytochalasans,[3] in particular that of cytochalasin E[6]. Cytochalasin G is 6,7-epoxy-10-(indol-3-yl)-16-methyl[11]cytochalas-13^{t}-ene-1,18,21-trione.[7]



(1)

Cytochalasin G may be derived formally from one molecule of tryptophan, one acetate unit, seven malonate

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[1] S. B. Carter, *Nature*, 1967, **213**, 261.

[2] For a recent review of cytochalasan biology see S. B. Carter, *Endeavour*, 1972, **113**, 77.

[3] For a recent review of cytochalasan chemistry see M. Binder and C. Tamm, *Angew. Chem. Internat. Edn.*, 1973, **12**, 370.

[4] S. B. Carter, personal communication.

[5] For cytochalasins E and F see D. C. Aldridge, D. Greatbanks, and W. B. Turner, *J.C.S. Chem. Comm.*, 1973, 551.

[6] G. Büchi, Y. Kitaura, S.-S. Yuan, H. E. Wright, J. Clardy, A. L. Demain, T. Glinsukon, N. Hunt, and G. N. Wogan, *J. Amer. Chem. Soc.*, 1973, **95**, 5423.

[7] M. Binder, C. Tamm, W. B. Turner, and H. Minato, *J.C.S. Perkin I*, 1973, 1146.

units, and two C_1 residues from methionine, and is the first example of an [11]cytochalasan containing a 10-(indol-3-yl) group. The recently reported chaetoglobosins A and B are examples of [13]cytochalasans containing 10-(indol-3-yl) groups.[8] It was apparent from a t.l.c. examination of culture filtrates and mycelial extracts of the aforementioned *Nigrosabulum sp.* that this organism produces other indole-containing metabolites.

Molecular Geometry.—In view of the early termination of the least-squares refinement, we limit discussion of the molecular geometry to a brief mention of the salient features of the conformation.

The eleven-membered ring, which contains a *trans*-double bond, possesses a conformation which in general terms may be described as chair-like. However, it is probably more meaningful to regard this ring as being composed of the three linked planar elements of the two carbonyl functions and the atoms of the double-bond system. The two carbonyl-containing planes are inclined at 85·2°, while the planes containing O(21′) and O(18′) are inclined at 46·9 and 38·6° to that of the double-bond system.

The six-membered ring [C(4)—(9)] adopts a slightly twisted boat conformation in which C(5) and C(8) are −0·62 and 0·69 Å distant from the least-squares plane of atoms C(4), C(6), C(7), and C(9). Whereas the eleven- and six-membered rings are *trans*-fused, the six-membered ring is *cis*-fused to the five-membered ring containing N(2). The conformation of the latter ring is that of a distorted envelope, defined by the approximately planar set of atoms C(1), N(2), C(3), and C(9), with C(4) lying 0·22 Å from this plane.

The indole group is planar, and for the most part bond lengths and interbond angles are in agreement with literature values for similar bonding environments. The main feature of the molecular packing is the existence of fairly short N–H ··· O contacts [N(2) ··· O(6′) 2·92, N(1″) ··· O(21′) 2·82 Å], which indicate that the molecules are hydrogen-bonded in columns extending along *b*, with additional hydrogen-bonding between columns.

EXPERIMENTAL

Isolation and Spectroscopic Data.—N.m.r. spectra were measured at 220 MHz for solutions in [$^2H_5$]pyridine. Mass spectrometric data was determined by use of an A.E.I. MS 9 spectrometer. M.p.s were recorded on a Kofler hot-stage apparatus.

The unidentified *Nigrosabulum sp.* (CMI 171,019, No. 4895 in our collection) was grown as surface culture for 28 days in glass vessels each containing 1 l of the following medium (g l^{-1}) adjusted to pH 5·5: Cerelose (50), sodium nitrate (2), potassium dihydrogen orthophosphate (1), magnesium sulphate heptahydrate (0·5), potassium chloride (0·5), iron(II) sulphate (0·01), Oxoid yeast extract (1), and 0·1% (v/v) minor element concentrate.[9] The culture filtrate (65 l) was extracted at pH 6·8 with chloroform (2 × 18 l) to afford an amorphous solid (5 g), a part of which (3·8 g) was dissolved in the two-phase system toluene–methanol–chloroform–water (40·5 : 40·5 : 9·5 : 9·5, 100 ml). The mixture was placed in equal portions in the first four separators of a Gallenkamp automatic counter-current apparatus programmed to perform 120 solvent transfers with partition period 1 min and settling time

TABLE 1

Fractional co-ordinates and isotropic temperature factors ($Å^2$)

(*a*) Non-hydrogen atoms

	x/*a*	*y*/*b*	*z*/*c*	U_{iso}
C(1)	0·3302(4)	0·1470(14)	0·7901(8)	0·041(3)
C(3)	0·2520(4)	0·2574(14)	0·7110(8)	0·045(3)
C(4)	0·2497(4)	0·3275(14)	0·8212(7)	0·038(2)
C(5)	0·2036(4)	0·2416(15)	0·8816(8)	0·044(3)
C(6)	0·2146(4)	0·0472(15)	0·9031(7)	0·041(3)
C(7)	0·2646(4)	0·0151(14)	0·9530(8)	0·042(3)
C(8)	0·2982(4)	0·1797(14)	0·9706(3)	0·035(2)
C(9)	0·3045(4)	0·2797(12)	0·8661(7)	0·032(2)
C(10)	0·2588(4)	0·4075(15)	0·6345(8)	0·048(3)
C(11)	0·1495(5)	0·2792(17)	0·8351(9)	0·062(3)
C(12)	0·1893(5)	0·9007(18)	0·8441(9)	0·065(4)
C(13)	0·3493(4)	0·1388(15)	1·0221(7)	0·044(3)
C(14)	0·3672(4)	0·2261(14)	1·1004(7)	0·042(3)
C(15)	0·4177(4)	0·1862(15)	1·1551(8)	0·047(3)
C(16)	0·4542(4)	0·3519(16)	1·1622(8)	0·049(3)
C(17)	0·4666(4)	0·4313(16)	1·0635(8)	0·054(3)
C(18)	0·4311(4)	0·5763(17)	1·0253(9)	0·057(3)
C(19)	0·4237(5)	0·6039(17)	0·9149(9)	0·058(3)
C(20)	0·3949(4)	0·4474(15)	0·8659(7)	0·044(3)
C(21)	0·3377(4)	0·4462(13)	0·8878(7)	0·033(2)
C(16′)	0·5051(5)	0·2932(18)	1·2161(9)	0·069(4)
C(2″)	0·3116(4)	0·6839(15)	0·6909(8)	0·050(3)
C(3″)	0·3071(4)	0·5182(14)	0·6428(7)	0·042(3)
C(4″)	0·3789(5)	0·3453(18)	0·5454(9)	0·065(3)
C(5″)	0·4313(6)	0·3562(23)	0·5203(12)	0·095(5)
C(6″)	0·4639(6)	0·4969(22)	0·5501(11)	0·089(4)
C(7″)	0·4490(5)	0·6362(20)	0·6070(10)	0·075(4)
C(3*a*″)	0·3589(4)	0·4866(15)	0·6035(8)	0·048(3)
C(7*a*″)	0·3912(4)	0·6290(16)	0·6300(8)	0·053(3)
N(2)	0·2967(3)	0·1378(11)	0·7099(6)	0·041(2)
N(1″)	0·3610(3)	0·7511(13)	0·6861(7)	0·058(3)
O(1′)	0·3703(2)	0·0639(10)	0·8010(5)	0·047(2)
O(6′)	0·2163(3)	0·0074(10)	1·0009(5)	0·044(2)
O(18′)	0·4075(3)	0·6736(14)	1·0841(7)	0·085(3)
O(21′)	0·3152(2)	0·5717(10)	0·9253(5)	0·048(2)

(*b*) Calculated hydrogen atom positions

	x/*a*	*y*/*b*	*z*/*c*
H(3)	0·223	0·211	0·709
H(4)	0·244	0·480	0·816
H(5)	0·208	0·313	0·947
H(7)	0·283	−0·091	0·942
H(8)	0·278	0·281	1·008
H(10)	0·264	0·318	0·567
H(10)	0·226	0·456	0·638
H(13)	0·372	0·096	0·995
H(14)	0·343	0·271	1·122
H(15)	0·436	0·120	1·119
H(15)	0·413	0·162	1·239
H(16)	0·438	0·409	1·203
H(17)	0·472	0·339	0·998
H(17)	0·502	0·464	1·038
H(19)	0·457	0·590	0·883
H(19)	0·410	0·727	0·889
H(20)	0·407	0·330	0·887
H(20)	0·392	0·459	0·797
H(2″)	0·286	0·662	0·745
H(4″)	0·358	0·242	0·525
H(5″)	0·449	0·256	0·482
H(6″)	0·503	0·498	0·527
H(7″)	0·470	0·728	0·638

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[8] S. Sekita, K. Yoshihira, S. Natori, and H. Kuwano, *Tetrahedron Letters*, 1973, 2109.

[9] P. W. Brian, P. J. Curtis, and H. G. Hemming, *Trans. Brit. Mycol. Soc.*, 1946, **29**, 173.

10 min. Additional lower-solvent phase (12·5 ml) was placed in each of the remaining separators and upper-solvent phase was placed in the reservoir. At completion the contents of separators 56—80 were pooled and concentrated *in vacuo* to *ca*. 50 ml, then extracted with ethyl acetate (3 × 100 ml) to afford a solid (0·81 g) which was recrystallised from acetone–ethyl acetate. Two further crystallisations of the product from methanol yielded 6,7-*epoxy*-10-(*indol*-3-*yl*)-16-*methyl*[11]*cytochalas*-13^t-*ene*-1,18,21-*trione* (*cytochalasin G*) (350 mg) as needles, m.p. 255—257 °C, $[\alpha]_D^{24}$ −99° (*c* 0·35, methanol) (Found: C, 73·1; H, 7·2; N, 5·9%; M^+, 474·2504. $C_{29}H_{34}O_4N_2$ requires C, 73·1; H, 7·2; N, 6·1%; *M*, 474·2518); τ 3·33 [dd, C(13)-H], 4·80 [m, C(14)-H], 5·86 [m, C(3)-H], 6·17 [A part of ABX system, C(17)-H_A], 8·75 [s, C(6)-Me], 8·97 [d, C(5) or C(16)-Me], and 9·20 [d, C(5) or C(16)-Me]; $\lambda_{max.}$ (methanol) 222 and 283 nm (ε 3250 and 535); $\nu_{max.}$ (Nujol) 3360s, 3200m, 1716s, 1678s, 1112m, 978m, 878m, and 740s cm^{-1}.

Crystal Data.—$C_{29}H_{34}N_2O_4$, *M* = 474·6. Orthorhombic, *a* = 25·437(2), *b* = 7·480(1), *c* = 13·458(1) Å, *U* = 2560·7 Å^3, D_m = 1·21 (by flotation), *Z* = 4, D_c = 1·23, *F*(000) = 1016. Space group $P2_12_12_1$ from systematic absences. Cu-K_α *X*-radiation, λ = 1·5418 Å; μ(Cu-K_α) = 6·66 cm^{-1}.

Crystallographic Measurements and Structure Determination.—Initial unit-cell parameters were determined photographically, and were subsequently refined by least-squares calculations.

Intensity measurements were made on a Hilger and Watts Y 290 computer-controlled diffractometer. Exposure of a flat plate (0·4 × 0·2 × 0·1 mm) to Cu-K_α radiation yielded 1656 [$I > 2\sigma(I)$; $\sigma(I) = \sqrt{I + B_1 + B_2}$] independent reflections, using the θ—ω scan technique in the range 2θ 0—57°. The intensities were modified by the appropriate Lorentz and polarisation factors, but absorption effects were considered small and were ignored.

The structure was solved by application of the symbolic-addition method, using the programs SINGEN and TANGEN incorporated in the '*X*-Ray' suite of programs.[10] An *E* map based on 273 reflections ($|E| > 1·30$) revealed the complete structure.

Positional and isotropic temperature factors for all non-hydrogen atoms were refined during the course of 7 cycles of full-matrix least-squares calculations, which converged when *R* was 0·102. Hydrogen-atom positions were calculated after cycle 3, and their contributions were included (U_{iso} 0·05 Å^2), but not refined, in the remaining 3 cycles of least-squares.

At this stage it was decided to terminate the refinement calculations since only a definitive molecular structure, and not precise geometrical details, was required from the analysis. Moreover, the limited number of data in conjunction with a relatively large number of atomic parameters, suggested that further refinement would be of

TABLE 2

Bond lengths (Å), interbond angles (°), and pertinent intra- and inter-molecular non-bonded distances (Å)

(*a*) Bond lengths

C(1)–C(9)	1·57(1)	C(14)–C(15)	1·51(2)
C(1)–O(1′)	1·21(1)	C(15)–C(16)	1·56(2)
C(1)–N(2)	1·37(1)	C(16)–C(17)	1·49(2)
C(3)–C(4)	1·57(1)	C(16)–C(16′)	1·55(2)
C(3)–C(10)	1·54(2)	C(17)–C(18)	1·51(2)
C(3)–N(2)	1·44(1)	C(18)–C(19)	1·51(2)
C(4)–C(9)	1·55(1)	C(18)–O(18′)	1·23(2)
C(4)–C(5)	1·57(1)	C(19)–C(20)	1·53(2)
C(5)–C(11)	1·54(2)	C(20)–C(21)	1·48(1)
C(5)–C(6)	1·51(2)	C(21)–O(21′)	1·21(1)
C(6)–C(12)	1·50(2)	C(2″)–N(1″)	1·36(2)
C(6)–O(6′)	1·47(1)	C(2″)–C(3″)	1·40(2)
C(6)–C(7)	1·46(1)	C(3″)–C(3*a*″)	1·44(2)
C(7)–O(6′)	1·45(1)	C(4″)–C(3*a*″)	1·41(2)
C(7)–C(8)	1·52(1)	C(4″)–C(5″)	1·37(2)
C(8)–C(9)	1·60(1)	C(5″)–C(6″)	1·40(2)
C(8)–C(13)	1·50(1)	C(6″)–C(7″)	1·38(2)
C(9)–C(21)	1·54(1)	C(7″)–C(7*a*″)	1·40(2)
C(10)–C(3″)	1·47(2)	C(3*a*″)–C(7*a*″)	1·39(2)
C(13)–C(14)	1·32(1)	C(7*a*″)–N(1″)	1·41(2)

TABLE 2 (*Continued*)

(*b*) Interbond angles

O(1′)–C(1)–N(1)	126·5(9)	C(8)–C(13)–C(14)	124·7(9)
O(1′)–C(1)–C(9)	126·4(9)	C(13)–C(14)–C(15)	126·1(9)
N(2)–C(1)–C(9)	107·0(8)	C(14)–C(15)–C(16)	112·5(9)
N(2)–C(3)–C(4)	104·5(8)	C(15)–C(16)–C(17)	113·1(9)
N(2)–C(3)–C(10)	110·9(8)	C(15)–C(16)–C(16′)	107·4(9)
C(4)–C(3)–C(10)	113·0(9)	C(17)–C(16)–C(16′)	111·3(9)
C(3)–C(4)–C(5)	112·3(8)	C(16)–C(17)–C(18)	117·8(9)
C(3)–C(4)–C(9)	104·9(8)	C(17)–C(18)–C(19)	120·4(9)
C(5)–C(4)–C(9)	111·8(8)	C(17)–C(18)–O(18′)	120·0(9)
C(4)–C(5)–C(6)	110·7(8)	C(19)–C(18)–O(18′)	119·5(9)
C(4)–C(5)–C(11)	112·8(9)	C(18)–C(19)–C(20)	111·9(9)
C(6)–C(5)–C(11)	114·8(9)	C(19)–C(20)–C(21)	112·9(9)
O(6′)–C(6)–C(12)	112·5(9)	C(9)–C(21)–C(20)	120·7(8)
C(5)–C(6)–O(6′)	112·8(8)	C(9)–C(21)–O(21′)	116·3(8)
C(5)–C(6)–C(7)	114·3(9)	C(20)–C(21)–O(21′)	122·9(9)
C(7)–C(6)–O(6′)	59·2(6)	C(3″)–C(2″)–N(1″)	112·3(9)
C(5)–C(6)–C(12)	121·7(9)	C(10)–C(3″)–C(2″)	126·6(9)
C(7)–C(6)–C(12)	119·7(9)	C(10)–C(3″)–C(3*a*″)	129·4(9)
C(6)–C(7)–O(6′)	60·9(6)	C(2″)–C(3″)–C(3*a*″)	103·9(9)
C(6)–C(7)–C(8)	115·4(9)	C(5″)–C(4″)–C(3*a*″)	116·5(9)
C(8)–C(7)–O(6′)	115·2(8)	C(4″)–C(5″)–C(6″)	123·1(9)
C(7)–C(8)–C(9)	107·4(7)	C(5″)–C(6″)–C(7″)	121·3(9)
C(7)–C(8)–C(13)	113·3(8)	C(6″)–C(7″)–C(7*a*″)	115·8(9)
C(9)–C(8)–C(13)	114·6(7)	C(3″)–C(3*a*″)–C(7*a*″)	108·6(9)
C(1)–C(9)–C(4)	104·9(7)	C(3″)–C(3*a*″)–C(4″)	131·3(9)
C(1)–C(9)–C(8)	108·6(7)	C(4″)–C(3*a*″)–C(7*a*″)	120·1(9)
C(1)–C(9)–C(21)	114·1(8)	C(7″)–C(7*a*″)–N(1″)	128·7(9)
C(4)–C(9)–C(8)	111·4(7)	C(7″)–C(7*a*″)–C(3*a*″)	123·0(9)
C(4)–C(9)–C(21)	112·5(7)	C(3*a*″)–C(7*a*″)–N(1″)	108·2(9)
C(8)–C(9)–C(21)	105·4(7)	C(2″)–N(1″)–C(7*a*″)	106·9(9)
C(3)–C(10)–C(3″)	117·0(9)	C(6)–O(6′)–C(7)	59·9(6)

(*c*) Pertinent intramolecular non-bonded distances ⩽3·40 Å

C(1) · · · C(6)	3·39	C(4″) · · · C(10)	3·31
C(1) · · · C(7)	2·92	O(1′) · · · C(7)	3·40
C(1) · · · C(10)	3·38	O(1′) · · · C(8)	3·05
C(1) · · · C(13)	3·17	O(1′) · · · C(13)	3·07
C(1) · · · C(20)	2·97	O(1′) · · · C(20)	3·06
C(13) · · · C(21)	3·26	O(1′) · · · C(21)	3·20
C(13) · · · C(27)	3·33	O(18′) · · · C(16)	2·88
C(14) · · · C(17)	3·00	O(21′) · · · O(18′)	3·26
C(14) · · · C(18)	3·24	O(21′) · · · C(4)	2·84
C(14) · · · C(21)	3·38	O(21′) · · · C(8)	3·03
C(17) · · · C(20)	3·23	O(21′) · · · C(18)	3·24
C(18) · · · C(21)	3·16	O(21′) · · · C(19)	2·78
C(2″) · · · C(21)	3·26	O(21′) · · · C(2″)	3·26

(*d*) Intermolecular distances <3·50 Å *

N(1″) · · · O(1′^{I})	2·82	C(10) · · · O(21′^{III})	3·40
N(1″) · · · N(2^{I})	3·34	C(2″) · · · O(6′^{III})	3·43
C(2″) · · · N(2^{I})	3·43	N(2) · · · O(6′^{IV})	2·92
C(1) · · · N(1″^{II})	3·37	C(3) · · · O(6′^{IV})	3·45

* Roman numerals as superscripts refer to the following equivalent positions:

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

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[10] 'The *X*-Ray System of Crystallographic Programs,' eds. J. M. Stewart, G. J. Kruger, H. L. Ammon, C. Dickinson, and S. R. Hall, Technical Report TR-192, Computer Science Center, University of Maryland, June 1972.

doubtful value. Calculation of an electron-density distribution and a difference synthesis revealed no errors in the structure. Throughout the refinement it was not found necessary to apply weights other than unity to the data, and accordingly no weighting scheme was used.

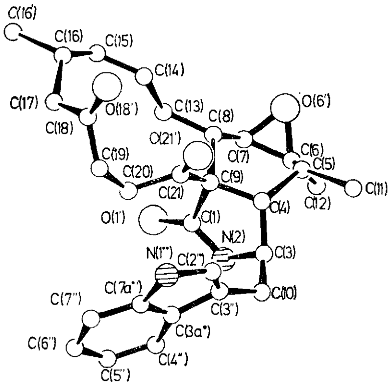

FIGURE 1 A projected view of one molecule showing the atomic numbering. Hydrogen atoms are numbered according to the carbon atoms to which they are bonded

TABLE 3

Equations of least-squares planes in the form $lX' + mY' + nZ' = d$, where X', Y', and Z' are co-ordinates in Å, and in square brackets, deviations (Å) of atoms from the planes

Plane (1): $-0{\cdot}239X' + 0{\cdot}489Y' - 0{\cdot}839Z' = -7{\cdot}207$
[C(2'') 0·012, C(3'') −0·024, C(4'') 0·005, C(5'') 0·019, C(6'') −0·005, C(7'') −0·025, C(3*a*'') −0·009, C(7*a*'') 0·015, N(1'') 0·013]

Plane (2): $-0{\cdot}195X' + 0{\cdot}357Y' - 0{\cdot}914Z' = -11{\cdot}408$
[C(9) −0·002, C(20) −0·002, C(21) 0·007, O(21') −0·003, C(8)* −1·522, C(19)* −0·327]

Plane (3): $0{\cdot}768X' + 0{\cdot}640Y' - 0{\cdot}005Z' = 11{\cdot}112$
[C(17) 0·000, C(18) 0·000, C(19) 0·000, O(18') 0·000, C(16)* −0·622, C(20)* −1·313]

Plane (4): $0{\cdot}482X' + 0{\cdot}638Y' - 0{\cdot}601Z' = -3{\cdot}334$
[C(8) −0·007, C(13) 0·007, C(14) 0·007, C(15) −0·007, C(9)* 1·390, C(16)* 1·183]

Plane (5): $-0{\cdot}522X' - 0{\cdot}746Y' + 0{\cdot}413Z' = -0{\cdot}801$
[C(1) −0·007, C(3) −0·031, C(9) 0·015, O(1') −0·018, N(2) 0·041, C(4)* 0·219]

Plane (6): $0{\cdot}253X' - 0{\cdot}559Y' - 0{\cdot}790Z' = -8{\cdot}450$
[C(4) −0·035, C(6) 0·037, C(7) −0·037, C(9) 0·035, C(8)* −0·695, C(5)* −0·617]

* Atom not included in definition of plane.

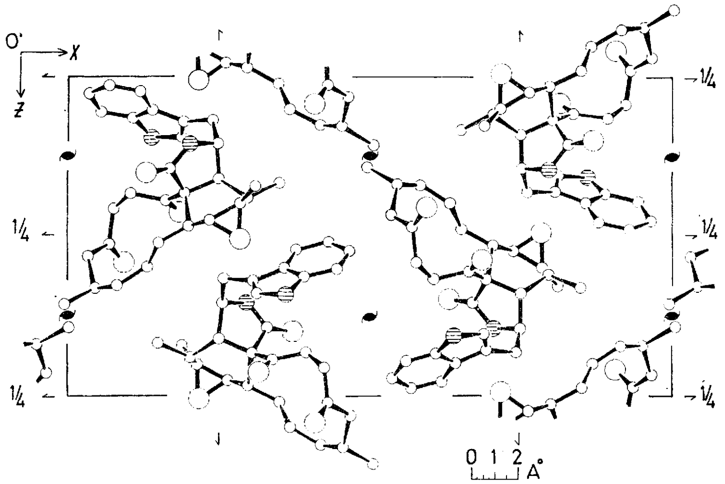

FIGURE 2 A projected view of the molecular packing

Final atomic co-ordinates and isotropic thermal parameters are in Table 1, bond lengths, angles, and non-bonded distances in Table 2, and least-squares planes calculated for various portions of the molecular framework in Table 3. The estimated standard deviations in Tables 1 and 2, are derived from the inverse of the least-squares normal-equation matrix, and are best regarded as minimum values.

Figure 1 shows the atomic numbering, and Figure 2 a projected view of the molecular packing.

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