Metabolites of Pathogenic Fungi.
IX. The Crystal and Molecular Structure of Floccosin, $C_{30}H_{26}O_{14}$, and Some of Its Degradation Products*

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Floccosin, $C_{30}H_{26}O_{14}$, is the main pigment of *Epidermophyton floccosum* (Harz) which parasitizes the skin only. The crystals of floccosin are tetragonal with $a = b = 7.762$ (2) and $c = 45.326$ (10) Å. There are four molecules per cell and the space group is $P4_32_12$. The structure was solved by direct methods from 1303 measured reflections. The final R value reached is 0.044. The molecule consists of two nearly planar three-ring systems, perpendicular to each other and related by a crystallographic twofold axis of rotation. There is an epoxide ring on one edge of each of the three-ring systems. Based on this determination, the structures of two degradation products have been reassigned. Each floccosin molecule is connected through hydrogen bonds to four neighbouring molecules.

Introduction

Epidermophyton floccosum (Harz) Langeron and Milochevitch 1930 is an anthropophilic dermatophyte which parasitizes the skin only. When grown on Sabouraud's media, its culture is olive green. The function of the coloured metabolite is not yet understood. It was therefore attractive to isolate and analyse the main pigment, floccosin, in order to gain some insight into the formation of the coloured metabolite.

Floccosin was isolated some time ago by Blank, Buxtorf, Chin, Just & Tredor (1969). The molecular formula of floccosin, $C_{30}H_{26}O_{14}$, a yellow crystalline magnesium-free pigment, was based on elemental analysis, NMR, proton count and osmometric weight determination of the parent compound and some of its derivatives. Blank *et al.* (1969) concluded that the molecular structure consisted of two identical halves, joined by an odd number of bonds. Because of the complexity of the ring *C* substituents, summarized at the time as (I), it was decided to determine the crystal structure of floccosin by X-ray diffraction. The molecular structure found is shown by (II).

(I)   (II)
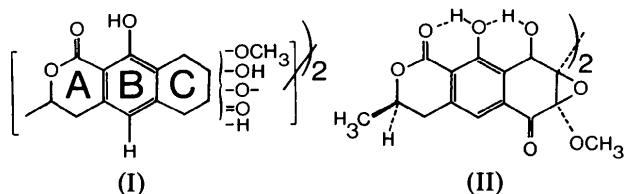
Experimental

Floccosin was crystallized from chloroform–methanol solution in yellow tetragonal bipyramids. Precession photographs revealed that the crystals belonged to the tetragonal system and the systematic absences $h00$, $h \neq 2n$; $0k0$, $k \neq 2n$; $00l$, $l \neq 4n$ corresponded to the enantiomorphous space groups $P4_12_12$ or $P4_32_12$. The unit-cell dimensions were obtained at 20 (1)°C by least-squares refinement of the angular measurements of 12 reflections centered on the Picker diffractometer. These and other crystal data are given in Table 1.

Table 1. *Crystal data*

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* This paper is dedicated to Dr Rachel Brown, Albany, NY, USA.

Since the general space-group position is eightfold and the density indicates the presence of only four molecules, each molecule must possess a twofold axis of symmetry. All the crystals at our disposal were rather large. Attempts to cut them to smaller dimensions usually destroyed the crystal completely. An irregular fragment of approximate dimensions 0·32 × 0·32 × 0·50 mm was mounted with its c axis aligned along the φ axis of the diffractometer. Three-dimensional intensity data were collected with an automatic Picker diffractometer using graphite mono-chromatized Cu $K\alpha$ radiation and the θ–2θ scan technique with a 1° (2θ) min^{-1} scan speed and a scan width varying from 1·70 to 2·80° (2θ) as θ increased. Backgrounds were measured for 20 s on each side of the peak. Three standard reflections, measured after each group of 30 reflections, revealed no significant deterioration of the crystal over the duration of the data collection. All the reflections were corrected for back-ground and placed on a common scale. The standard deviation $\sigma(I)$, for the net intensity, I_N, of a reflection was calculated by the relation given by Brisse, Lectard & Schmidt (1974). Of the 1303 measured reflections [$F(hkl)$, $h \le k$] within the Cu sphere limited by $2\theta <$ 125°, there were only 100 for which $I_N/\sigma(I) \le 2\cdot50$. The data were corrected for Lorentz, polarization and absorption effects in the usual manner with the programs of Ahmed, Hall, Pippy & Huber (1973).† The transmission factor ranged from 0·68 to 0·75. The absorption-correction program also evaluated the quantity dA^*/ dμ for secondary-extinction corrections according to Åsbrink & Werner (1966).

Structure determination

A set of normalized structure amplitudes |E_h| was obtained after isotropic temperature factor correction. The statistical averages and the distribution of the normalized structure factors strongly indicated a centrosymmetrical structure. This indication, however, is contrary to the space-group determination but might be consistent with the fact that the molecule of floccosin is on a crystallographic twofold axis of symmetry. The

Fig. 1. Stereoscopic pair showing one complete molecule of floccosin.

† The computer programs used were locally modified versions of the following: NRC-2: data reduction, modified to include the polarization due to the monochromator; NRC-3: absorption correction; NRC-14: error analysis and agreement summary; NRC-22: least-squares plane (Ahmed et al.); FORDAP: Fourier and Patterson maps (Zalkin); NUCLS: least-squares refinement (Doedens & Ibers), with block-diagonal approximation option introduced by Sygusch; MULTAN: multisolution program (Germain, Main & Woolfson); ORTEP: stereo drawings (Johnson).

Table 2. *Positional parameters for one half of the molecule of floccosin*

The coordinates are ×10⁴ for the heavy atoms and ×10³ for the hydrogen atoms. The coordinates of the atoms in the other half of the molecule are given by: $y,x,\bar{z}$.

	x	y	z		x	y	z
O(1)	−4033 (4)	−3803 (4)	−1768 (1)	C(12)	−6893 (6)	−3505 (6)	−1565 (1)
O(2)	−1710 (4)	−3413 (6)	−1504 (1)	C(13)	−5794 (5)	−4461 (1)	−1782 (1)
O(3)	−1782 (4)	−3895 (5)	−945 (1)	C(14)	−6338 (6)	−4304 (7)	−2101 (1)
O(4)	−2266 (3)	−2769 (4)	−376 (1)	C(15)	−8856 (5)	−3605 (6)	168 (1)
O(5)	−5672 (3)	−2429 (3)	−120 (1)	H(O3)	−138 (5)	−366 (5)	−113 (1)
O(6)	−7469 (3)	−4687 (3)	62 (1)	H(O4)	−137 (5)	−298 (5)	−45 (1)
O(7)	−8914 (3)	−4397 (5)	−494 (1)	H(51)	−293 (4)	−520 (4)	−41 (1)
C(1)	−3262 (6)	−3646 (7)	−1511 (1)	H(101)	−833 (4)	−387 (4)	−100 (1)
C(2)	−4321 (5)	−3701 (6)	−1240 (1)	H(121)	−799 (5)	−424 (5)	−157 (1)
C(3)	−3508 (5)	−3799 (6)	−965 (1)	H(122)	−697 (4)	−232 (4)	−160 (1)
C(4)	−4483 (4)	−3881 (5)	−705 (1)	H(131)	−559 (4)	−568 (4)	−172 (1)
C(5)	−3528 (4)	−4070 (5)	−413 (1)	H(141)	−553 (5)	−514 (5)	−222 (1)
C(6)	−4684 (4)	−3999 (4)	−144 (1)	H(142)	−743 (5)	−478 (5)	−212 (1)
C(7)	−6563 (4)	−4034 (4)	−170 (1)	H(143)	−631 (5)	−304 (5)	−214 (1)
C(8)	−7396 (5)	−4102 (5)	−468 (1)	H(151)	−907 (5)	−393 (5)	34 (1)
C(9)	−6257 (4)	−3862 (5)	−729 (1)	H(152)	−979 (5)	−341 (5)	0 (1)
C(10)	−7070 (5)	−3719 (5)	−1005 (1)	H(153)	−843 (6)	−217 (6)	19 (1)
C(11)	−6116 (5)	−3635 (5)	−1259 (1)				

phases of 156 reflections with $|E| \geq 1\cdot50$ were determined by the multisolution method described by Germain, Main & Woolfson (1970). The E map for the set of phases having the highest figure of merit, revealed all the 22 non-hydrogen atoms among the highest peaks. A structure factor calculation based on the coordinates of these 22 'C' atoms and an overall isotropic temperature factor of $4\cdot0$ Å^2 gave an $R = \Sigma \Delta F/\Sigma F_o$ of $0\cdot42$. The seven O atoms were identified from the behaviour of their temperature factors. Four cycles of isotropic refinement followed by six cycles of anisotropic refinement decreased R to $0\cdot089$. The least-squares program included the refinement of an isotropic extinction correction as described by Coppens & Hamilton (1970). This parameter was now refined. Ten of the thirteen H atoms were located on a Fourier difference synthesis. The least-squares refinement was continued with the inclusion of the newly found H atoms, with isotropic temperature factors, by the block-diagonal approximation. The last H atoms were revealed in a new Fourier difference map. Three reflections were removed from the last four cycles of isotropic refinement. The final R value reached $0\cdot044$. When all measured reflections were included $R = 0\cdot055$.* A weight of unity was taken for each reflection.

At the end of the refinement, the average shift-to-σ ratio was less than $0\cdot4\sigma$ and the isotropic extinction coefficient was $g = 0\cdot85 \times 10^3$. The standard deviation of unit weight $\sigma(F) = [\Sigma_H w_H \Delta F^2/(m - n)]^{1/2}$, where m is the number of reflections and n the number of parameters, was $1\cdot62$. A final difference electron density map showed no significant residual density, the maximum and minimum values being $0\cdot13$ and $-0\cdot17$ e Å^{-3} respectively. The scattering factors for the heavy atoms were those of Cromer & Waber (1965) and those of Stewart, Davidson & Simpson (1965) were used for the H atoms.

Results and discussion

The final refined atomic coordinates and their standard deviations are listed in Table 2. The molecular structure is represented by the pair of stereoscopic drawings in Fig. 1.

The results of this study indicate that floccosin has structure (II). The absolute stereochemistry of floccosin is based on the absolute stereochemistry of its oxidation product (V), which had been related by comparison of the respective optical rotatory dispersion curves to that of (VI). The latter (VI) had been degraded to $(-)$-(R)-β-hydroxybutyric acid of known absolute stereo-

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* Lists of structure factors and thermal parameters have been deposited with the British Library Lending Division as Supplementary Publication No. SUP 33031 (10 pp.). Copies may be obtained through The Executive Secretary, International Union of Crystallography, 13 White Friars, Chester CH1 1NZ, England.

(III) R = Ac
(IIIa) R = H

(IV) R = H
(IVa) R = Ac
(IVb) R = CH$_3$

(V)

(VI)
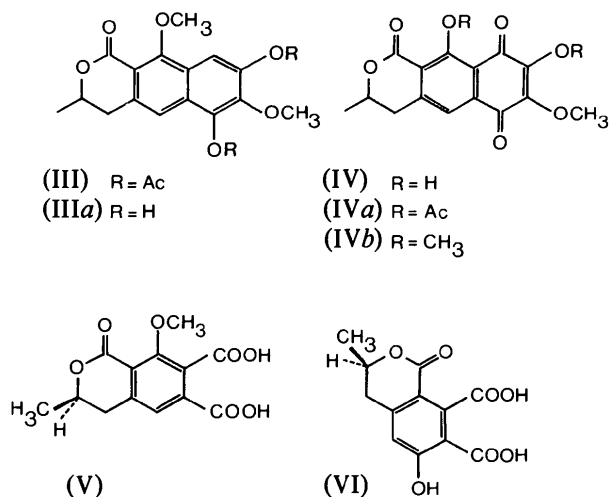
chemistry (Ng, Just & Blank, 1969). As a consequence, it is possible to assign the space group $P4_32_12$ to the floccosin crystals. Based on this structure, the product of reductive acetylation (Zn–acetic anhydride) of tetramethylfloccosin is now assigned structure (III), and its hydrolysis product (IIIa).

The product obtained by treatment of floccosin with sodium iodide in boiling acetic acid is now reassigned structure (IV). The products of acetylation and methylation are (IVa) and (IVb) respectively.

Description of the structure

The molecule of floccosin consists of two nearly planar three-ring systems perpendicular to each other. These two systems are connected together through the C(6)–C(6') bond. Primed atoms refer to atoms related to those in Table 2 by the crystallographic and molecular twofold axis of rotation. This axis relating the two halves of the molecule goes through the middle of the C(6)–C(6') bond.

An unusual feature of the ring system, which had not been detected in the earlier structural study (Blank et al., 1969), is the presence of an epoxide ring on the edge of ring C. The epoxide ring plane through atoms C(6), C(7) and O(5) is nearly perpendicular (94°) to ring C. A similar situation has already been observed for epimonol iodoacetate (Grant, Hamilton, Hamor, Robertson & Sim, 1963) where the angles between the six-membered ring and the epoxide plane are $88\cdot6$ and $94\cdot2°$, respectively, for two molecules in the asymmetric unit. The same angle in a derivative of 1,2-epoxycyclohexane (Merlino, Lami, Macchia, Macchia & Monti, 1972) has a value of 80°.

The C(14) methyl group and the O(4)–CH$_3$ methoxy group are both attached to the ring system in equatorial positions.
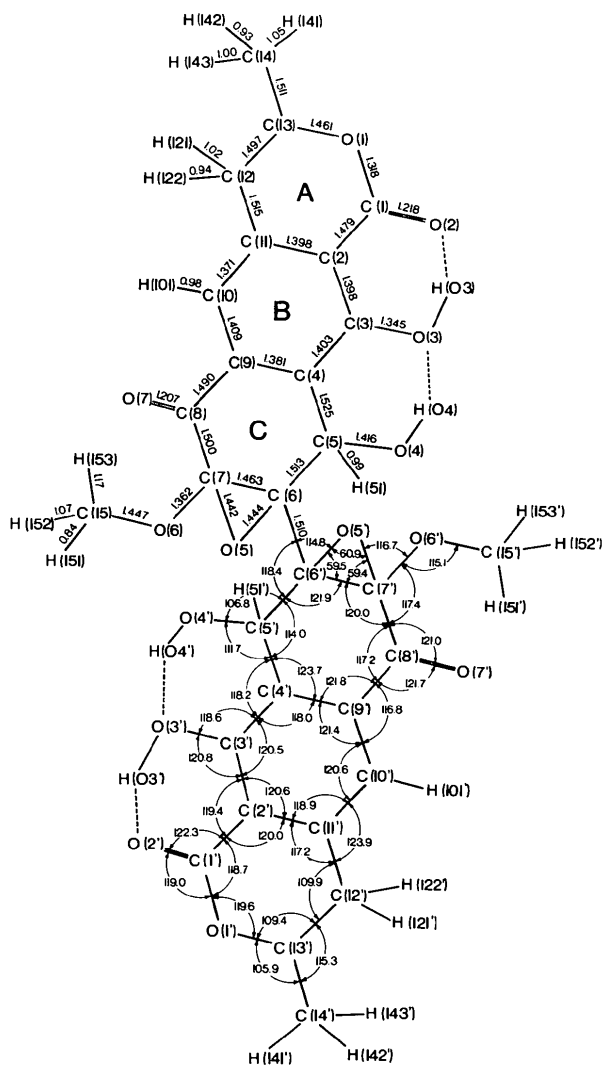
Fig. 2. Numbering of the atoms and bond distances and angles of floccosin. Primed atoms are related to those of Table 2 with the same number by the transformation $y,x,\bar{z}$.

Bond distances and angles

The bond distances shown in Fig. 2 are calculated from the final refined coordinates and have not been corrected for thermal motion. The estimated errors for distances between non-hydrogen atoms are of the order of 0·002 Å while they reach 0·02 Å when an H atom is involved. The average bond distances in the molecule compare well with the values given by Sutton (1965).

The individual distances are all reasonable except for the $C(sp^3)$—$C(sp^3)$ type bonds which are significantly shorter than the expected value. The extremely short C(6)—C(7) bond, 1·463 Å, involving tetracoordinated C atoms is due to the presence of the three-atom epoxide ring. Such short bonds, as compared in Table 3

Table 3. *Comparison of bond distances (Å) and angles (°) in epoxide rings*

Average C—O	C—C	Average C—C—O	C—O—C	References
1·443 (2)	1·463 (2)	59·5 (1)	60·9 (1)	This work
1·436 (2)	1·472 (2)	59·3	61·4	1
1·424 (2)	1·496 (2)	58·3	63·4	2
1·465 (20)	1·480 (20)	59·7 (10)	60·7 (10)	3
1·43	1·485	58	62	4
1·435 (22)	1·500 (25)	58·5 (11)	63·0 (11)	5
1·465 (30)	1·47 (3)	59·5 (18)	60·0 (18)	6
1·46 (7)*	1·60 (9)*	57 (4)*	66 (4)*	7
1·52 (11)*	1·57 (11)*	59 (6)*	62 (6)*	8
1·458 (6)	1·460 (6)	60·0 (4)	60·1 (4)	9
1·444 (3)	1·473 (3)	59·3 (2)	61·3 (2)	10
1·437 (6)	1·460 (6)	59·5 (2)	61·0 (2)	11

Average values of the bond distances and angles in epoxide rings

1·433	1·476	59·2	61·4

References: (1) Cunningham, Boyd, Myers, Gwinn & LeVan (1951). (2) Mathews, Swanson, Mueller & Stucky (1971). (3) Merlino, Lami, Macchia, Macchia & Monti (1972). (4) Mackay & Mathieson (1965). (5) Craven (1962). (6) Craven (1964). (7) Grant, Hamilton, Hamor, Robertson & Sim (1963). (8) Arnott, Davie, Robertson, Sim & Watson (1961). (9) Birnbaum (1973). (10) Riche (1973). (11) Berking & Seeman (1971).
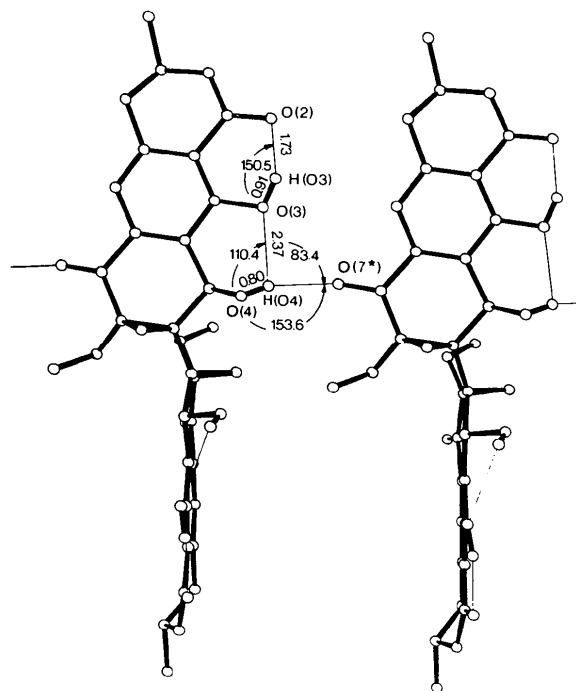
* Not used in the final averages.

Fig. 3. Hydrogen bonding in the crystal of floccosin.

with similar values in other epoxide rings, seem to be the rule for such rings. The C(3)—O(3)H distance of 1·345 Å and the C(4)—O(4)H bond length of 1·416 Å are comparable to C—OH bond distances in other compounds (Dusausoy, Protas, Besancon & Tirouflet, 1973). The C(sp^3)—H bond distances, which range from 0·84 to 1·17 Å, have an average value of 1·01 Å. The two O—H distances have a slightly shorter average of 0·85 Å.

The bond angles are shown on the lower part of Fig. 2. The estimated errors for the angles are of the order of 0·2°. The average bond angle around C(sp^3) atoms is 113·6° [again excluding the angles at C(6) and C(7)]. The individual angle values vary from 105·9 to 121·9°. The bond angles around C(sp^2) atoms are in the range of 116·8 to 123·7° and average 119·9°.

The angles of the epoxy ring are all close to 60°. A comparison of bond angles in other epoxide rings, in Table 3, reveals that the C—O—C angles are always larger than 60° and average 61·4° while the two C—C—O angles average 59·2°. The C(7)—O(6)—C(15)H₃ angle of 115·1° compares well with other methoxy angles.

There is one intramolecular hydrogen bond between O(3) and O(2). The distances and angles of interest are shown on Fig. 3.

The plane passing through ring B and the deviations of the atoms of rings A and C from that plane are listed in Table 5. As can be seen from its equation this plane is nearly parallel to the ac plane.

The C(14) methyl group is nearly staggered with respect to the C(13)—O(1), C(13)—C(12) and C(13)—H(131) bonds. The average deviation from the perfectly staggered orientation is 5°.

Packing of the molecules and hydrogen bonding

None of the intermolecular distances between C—C or C—O atoms are shorter than 3·30 Å. However, there is one short O—O distance, implying hydrogen bonding between O(4) and O(7*),† 2·942 Å. The distance H(O4)···O(7*) is 2·21 Å and the bond angle O(4)—H(O4)···O(7*) is 153·6 (18)°. This O(4)—O(7*) hydrogen bond may be considered a bifurcated one, the other branch would then be the intramolecular bond O(4)—H(O4)···O(3). It should be noted that the H(O4)···O(3) distance of 2·37 Å is significantly longer than the value of 1·73 Å observed for H(O3)···O(2). The hydrogen bonding of the molecules of floccosin is shown in Fig. 3.

† The asterisked atoms have the symmetry position $-1 + x, y, z$.

Conformation

The ring torsion angles are shown in Fig. 4, while some others that could not be shown are listed in Table 4. The three-ring system is nearly planar, except in the vicinity of atoms O(1), C(12) and C(13).
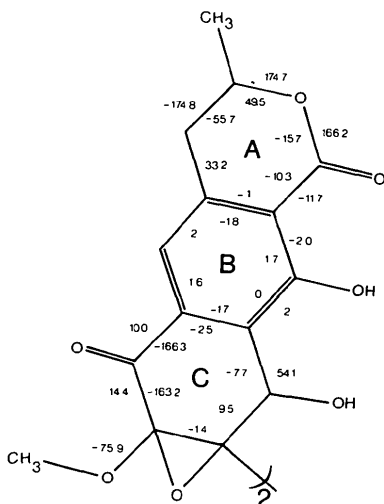
Table 4. Torsion angles

C(4)—C(5)—C(6)—O(5)	−58·2°
C(9)—C(8)—C(7)—O(5)	57·6
C(4)—C(5)—C(6)—C(6')	162·2
O(6)—C(7)—C(6)—C(6')	−0·9
C(5)—C(6)—C(7)—O(6)	152·7
C(5)—C(6)—C(6')—C(5')	126·8

Table 5. Mean plane

Equation for the least-squares plane:
$$-0.0156X - 0.9978Y - 0.0648Z + 3.2676 = 0.$$
The plane is expressed by $lX + mY + nZ - p = 0$ in Å. The orthogonal system of axes has X,Y,Z parallel to the a, b, and c axes respectively.

Atom	Deviation from plane (Å)	Atom	Deviation from plane (Å)
C(2)*	−0·006 (2)	C(1)	−0·039 (3)
C(3)*	0·003 (2)	C(5)	−0·048 (2)
C(4)*	0·003 (2)	C(6)	0·072 (2)
C(9)*	−0·006 (2)	C(7)	0·018 (2)
C(10)*	0·004 (2)	C(8)	−0·139 (2)
C(11)*	0·002 (2)	C(12)	0·006 (2)
O(1)	−0·244 (2)	C(13)	−0·790 (2)
O(2)	0·152 (2)	H(O3)	0·117 (27)
O(3)	−0·013 (2)	H(O4)	0·792 (21)
O(4)	1·015 (2)	H(101)	−0·061 (20)
O(7)	−0·395 (2)		

* Atoms included in the mean-plane calculation.

Fig. 4. Ring torsion angles. The e.s.d.'s for the torsion angles are of the order of 3°.

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The Crystal Structure of 3-(*p*-Chlorophenyl)-4-oxo-3a,5,6,6a-tetraphenyl-3a,4-dihydrocyclopenta[2,3-*d*]isoxazoline

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The crystal structure of 3-(*p*-chlorophenyl)-4-oxo-3a,5,6,6a-tetraphenyl-3a,4-dihydrocyclopenta[2,3-*d*]-isoxazoline, $C_{36}H_{24}NO_2Cl$, has been determined from three-dimensional intensities, measured with an automated Philips PW 1100 single-crystal diffractometer (1603 independent non-zero reflexions). The structure was solved by direct phase determination with *MULTAN*. The cell constants, obtained by least-squares calculations from direct θ-value measurements on the diffractometer, are: $a = 14 \cdot 373$ (4), $b = 21 \cdot 084$ (7), $c = 9 \cdot 261$ (3) Å, $\beta = 99 \cdot 27$ (3)°, $Z = 4$; the space group is $P2_1/n$. The positional and vibrational parameters, with anisotropic temperature factors for the non-hydrogen atoms, were refined by full-matrix least-squares calculations to a final $R = 0 \cdot 060$. Correction for anomalous scattering of the Cl atom was applied. A molecule of the compound has a cyclopentenone–isoxazoline structure. To the two central five-membered isoxazoline and cyclopentenone rings, at an angle of 107°, are linked five benzene rings with different orientations. A characteristic feature is the coplanar system formed by the isoxazoline ring and the *p*-chloro-substituted benzene ring.

Introduction

Reactions yielding isoxazoline derivatives are of considerable theoretical interest as regards regioselectivity owing to the influence of the frontier molecular orbitals of the reacting systems. To this end a series of new compounds were prepared at the Laboratory of Organic Chemistry, Aristotle University of Thessaloniki (Alexandrou & Argyropoulos, 1977). Since chemical and spectroscopic data were in most cases