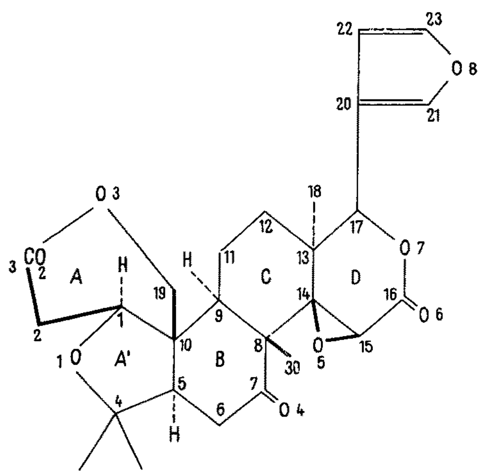
worden ist. Die Umwandlung von Desoxylimonin (XIV) in Desoxylimonsäure (XXVII) gestattet, die Beziehung zwischen dem Lactonring D und dem Ketoncarbonyl festzulegen. Zu gleichen Schlussfolgerungen führt auch die Untersuchung des Merolimonols (XXXIII), welches als Produkt des alkalischen Abbaus von Limonol (XII) anfällt. Der oxydative Abbau von Ring B gibt Auskunft über die Substitution der C-Atome 5 und 6, während die Beziehung zwischen dem Ketoncarbonyl und dem Lactonring A durch die Bildung der Limonilsäure (LVI) festgelegt ist. Der drastische Abbau des Limonins mit Alkali führt zur Limoclastsäure (XLIV). Aus dieser Umwandlung ergibt sich die Natur des Lactonringes A sowie eine Haftstelle der verbleibenden Sauerstoffunktion. Die Integration der somit abgeleiteten Strukturelemente mit früheren Dehydrierungsversuchen erlaubt die für Limonin in Frage kommenden Strukturen auf wenige Möglichkeiten zu beschränken, unter welchen lediglich (LXVI) biogenetisch plausibel erscheint.

Anschliessend werden andere Aspekte der Chemie des Limonins und verwandter Bitterstoffe kurz erläutert.

The Structure of Limonin

By S. ARNOTT, A. W. DAVIE, J. M. ROBERTSON, G. A. SIM, and D. G. WATSON[1]

X-ray studies in this department have led to the elucidation of the crystal structure of *epi*-limonol iodoacetate and hence to the derivation of the molecular structure of limonin, $C_{26}H_{30}O_8$, the bitter principle of citrus fruits. Our results are summarised in formula I for limonin and in Figure 5 which shows the arrangement of the atoms in the crystal asymmetric unit.



I

The X-ray work commenced in 1956 and at that time our knowledge of the chemical structure was confined to an enumeration of the functional groups and the fact that two carbocyclic rings were necessary. There was insufficient basis on which to build any plausible trial structure, and it was clear that we could only hope to proceed by means of some phase determining heavy atom technique[2,3].

Professor BARTON *et al.* supplied us with a variety of derivatives, and a preliminary study of these[4] indicated that the esters of *epi*-limonol were the most promising. The chloroacetate, which has two molecules in a monoclinic cell, space group $P2_1 - C_2^2$, was first studied. However, the presence of a screw axis introduces the ambiguity of a false symmetry centre between the two phase determining chlorine atoms, and work on this derivative was later abandoned in favour of the iodo-acetate of *epi*-limonol, $(C_{26}H_{31}O_8)COCH_2I$. This crystallises in the monoclinic system with cell dimensions $a = 15{\cdot}03$, $b = 12{\cdot}36$, $c = 15{\cdot}93$ Å, $\beta = 95° 12'$. There are four molecules in the unit cell, $D_m = 1{\cdot}426$, $D_x = 1{\cdot}441$ g·cm^{-3}, and the space group is again $P2_1 - C_2^2$.

In this space group the general positions are twofold, and hence, with four molecules in the unit cell, the asymmetric crystal unit consists of two chemical molecules. This is a formidable complication, because it means that the coordinates of 76 atoms other than hydrogen must be determined from the X-ray data. At a later stage of our analysis, however, this circumstance has been helpful. There is no symmetry relationship between the two molecules and the positions of all the atoms have been found and refined independently. The fact that these positions are now found to conform quite precisely to two chemically identical but differently oriented molecules is an important verification of the structure and the stereochemistry.

MoKα radiation was employed and the reflections were recorded photographically with Weissenberg and precession cameras from specimens measuring about 1·0×0·2×0·02 mm. Absorption corrections are small and were not applied. The extremely thin, flaky crystals made the collection of accurate data a very lengthy and tedious operation, but in the end 2927 structure factors were evaluated.

The positions of the iodine atoms were determined initially by means of two-dimensional and three-dimensional sharpened Patterson syntheses. A section of the three-dimensional Patterson function at $y = {}^1/_2$ is shown in Figure 1.

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[1] Chemistry Department, The University, Glasgow (Scotland).

[2] J. M. ROBERTSON, J. chem. Soc. *1935*, 615; *1936*, 1195.

[3] J. M. ROBERTSON and I. WOODWARD, J. chem. Soc. *1937*, 219; *1940*, 36.

[4] S. ARNOTT and J. M. ROBERTSON, Acta cryst. *12*, 75 (1959).

The iodine coordinates were obtained from the vector maps without ambiguity, and the fractional values, after refinement by later Fourier syntheses, are

	x	y	z
I(1)	0·1333	0·0367	0·3658
I(2)	0·0617	0·4633	0·9708

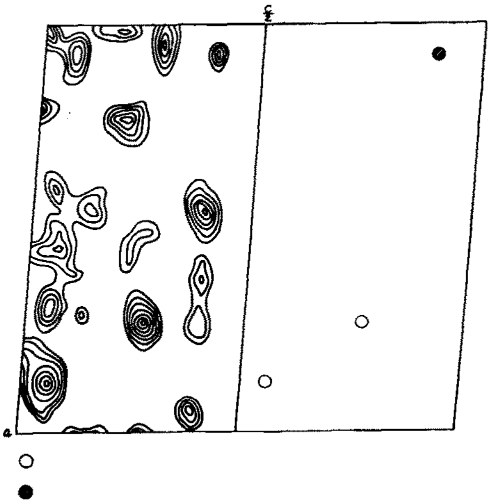


Fig. 1.—Harker section at $y = {}^1/_2$

With phase angles calculated from these coordinates (before refinement) various Fourier syntheses were now evaluated. Although it was not possible to interpret the two-dimensional projections obtained in this way, they led to a useful refinement of the iodine positions. The projection along the b axis has an effective centre of symmetry, and this is shown in Figure 2. The interpretation of this projection given in Figure 3 is based on our later results. It shows the complexity of the structure and the impossibility of attempting any definitive solution by these methods.

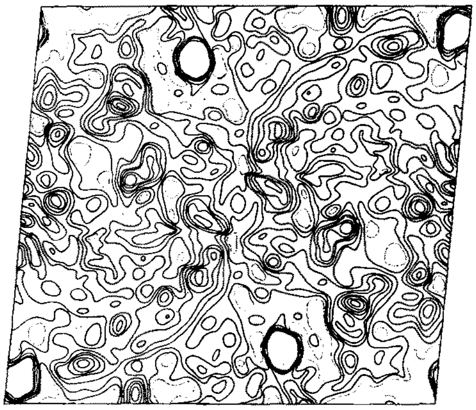


Fig. 2.—*Epi*-limonol iodoacetate. Electron density projection on (010)

In 1958 the first three-dimensional Fourier synthesis with phase angles based on the iodine atoms alone was evaluated and drawn out. Unfortunately a mistake in the programme for the electronic computer partially vitiated the results at this stage and much time was wasted in fruitless attempts at interpretation. When the error was detected, the synthesis was repeated. The coefficients of the Fourier series now employed were the observed structure amplitudes weighted according to the method proposed by Sim[5]. The adoption of this procedure permitted the inclusion at this early stage of all the structure-amplitude data rather than merely part of the data as is normally the case with other methods of assessing the reliability of the phase-angle information.

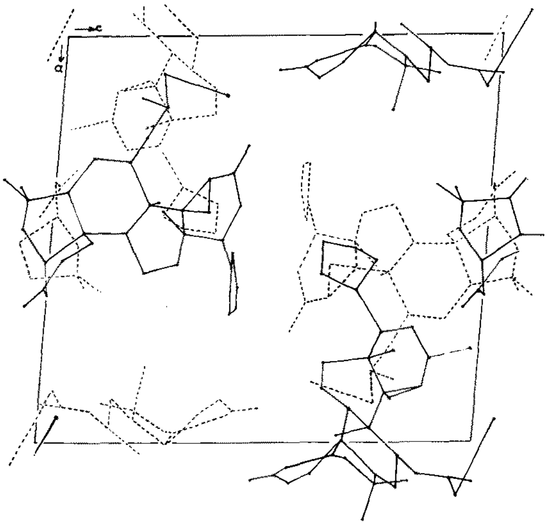


Fig. 3.—The final structure as projected on (010)

This approximate electron-density distribution was now compared with a function prepared from the three-dimensional Patterson synthesis by a superposition method[6,7], and a total of 50 atoms other than iodine were located with sufficient certainty to be included in the next structure-factor calculations. The phase angles obtained in these calculations were then used to derive an improved electron-density distribution in which all 76 atoms (C, O, and I) in the asymmetric unit could be placed. These atoms were now found to group themselves into two distinct and well separated molecules which were mutually consistent in the chemical sense. Part of this electron density distribution is shown by means of superimposed contour sections in Figure 4. The arrangement of the atoms in the asymmetric unit of the crystal structure is shown more clearly in Figure 5. This should be compared with Figure 3, which gives some idea of the mutual arrangement of the molecules in the unit cell.

[5] G. A. Sim, Acta cryst. *12*, 813 (1959).

[6] C. A. Beevers and J. H. Robertson, Acta cryst. *3*, 164 (1950).

[7] M. J. Buerger, Acta cryst. *4*, 531 (1951).

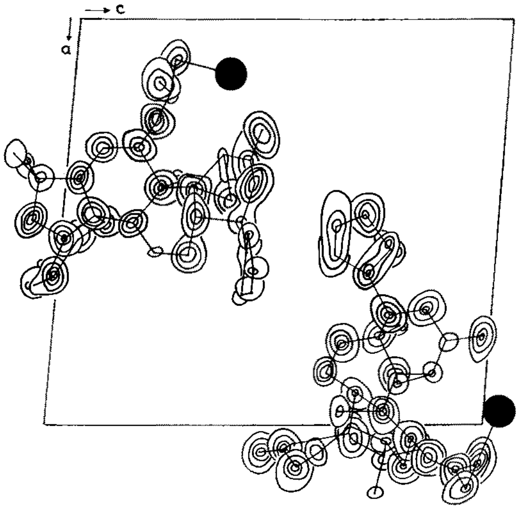
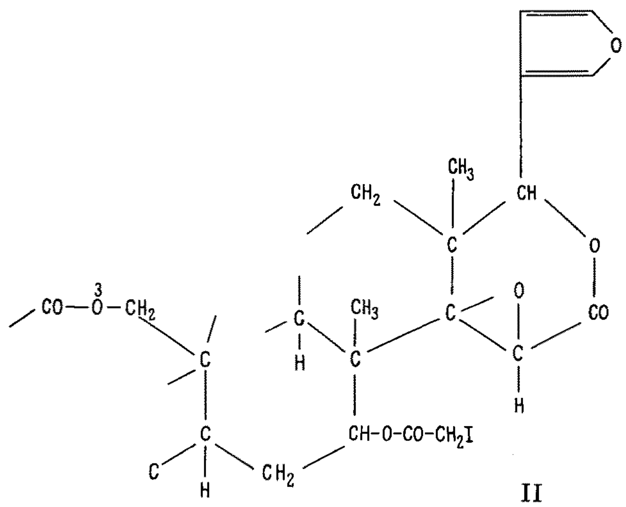


Fig. 4.—The third three-dimensional electron density distribution for *epi*-limonol iodoacetate shown by means of superimposed contour sections projected on (010).

At this stage it was not possible to distinguish beyond all doubt between oxygen and carbon atoms. In particular in the lactone ring A it was not possible on the basis of observed peak heights to rule out the possibility that O(3) might be carbon and C(2) oxygen. This alternative, however, is eliminated by the chemical evidence (see previous communication) which establishes unambiguously the partial structure II.



II

The approximate atomic positions are now being refined by least-squares and Fourier techniques. Two further three-dimensional electron density distributions have now been computed, with an increasing number of well defined atoms inserted in the phasing calculations. The furan ring and carbon atom 17 of one molecule, which are distinctly resolved but still rather weak, have not yet been used in the structure factor calculations. The mean discrepancy between observed and calculated structure amplitudes is at present 22%. From the X-ray point of view a most important verification of the structure lies in the fact that the 76 independently resolved peaks are found to group themselves into two separate but chemically identical molecules.

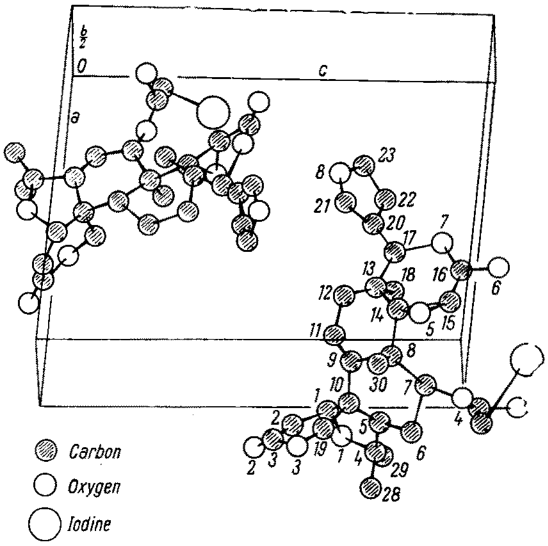


Fig. 5.—The arrangement of the atoms in the crystal asymmetric unit (two chemical molecules)

The stereochemistry of limonin is as indicated in formula I. In *epi*-limonol the hydroxyl group O(4) at C(7) is equatorial and the methyl group, C(30), at ring junction B/C is axial. Ring B has a chair and ring C a boat conformation. A full description with the coordinates of each atom will be given after further refinement has been completed. At the present stage of the work errors and uncertainties of a few tenths of an Angstrom may exist in some parts of the field.

We are grateful to Professor D. H. R. BARTON for first suggesting this problem, and for making available supplies of the iodoacetate. Dr. O. S. MILLS computed one electron-density distribution on the Manchester University Mercury computer. All the later calculations were carried out on the Glasgow University Deuce computer. Three of us (S. A., A. W. D., and D. G. W.) are also indebted to the Gourock Rope Work Company, the Department of Scientific and Industrial Research, and the Carnegie Trust for scholarships.

Zusammenfassung

Die Struktur des *epi*-Limonol-jodoacetates $(C_{26}H_{31}O_8)$ $COCH_2I$, wurde durch dreidimensionale Röntgenanalyse bestimmt. Es gelang, die Lage sämtlicher Atome in zwei kristallographisch unabhängigen, jedoch chemisch identischen Molekeln zu ermitteln. Dieses Resultat stellt eine vollständige Bestimmung der Konstitution und der Stereochemie des Limonins dar.