AN X-RAY CRYSTALLOGRAPHIC STUDY
OF SOME WOOD EXTRACTIVES

A Thesis Submitted for the Degree

of

Doctor of Philosophy

in the

UNIVERSITY OF IBADAN

by

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I certify that the work here reported has been largely carried out under my supervision.

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GENERAL INTRODUCTION

The discovery of X-ray was first reported[1] in 1895 and in 1913 its diffraction by crystals was also discovered by a number of workers including Bragg[2] and Laue[3]. The use of this property of X-ray to determine the structure of some simple crystals was reported[4] the same year. This property has become a powerful tool in modern science particularly in the fields of Physics, Chemistry, Metallurgy and Mineralogy. It has provided an easy approach to the study of the morphology of crystals and has led to methods of finding the locations of the atoms in the unit cell of the crystal of any compound.

The determination of the atomic positions leads to a detailed knowledge of the geometry of the molecule, the spatial distribution of the atoms and information such as bond distances and angles and the forces (intra-molecular and intermolecular) that may be inferred from the spatial distribution of the atoms in the crystal.

The X-ray crystallographic method was applied to mostly inorganic structures at the early stages of its development. Recently the application to organic chemistry has become pronounced and it has been used to elucidate the structure of many complex organic substances such as haemoglobin[5] and vitamin B_{12}[6,7].

In this work an attempt is made to determine the structure of two wood extractives: Cedrela Odorata Substance B and Turraeanthin.

CEDRELA ODORATA SUBSTANCE B

INTRODUCTION

Early in 1963, Bevan[8] and others carried out a light petroleum extraction of a number of samples of West African timbers. One of them was a species of the genus Cedrela which is not normally grown in West Africa. The species Cedrela Odorata is cultivated. The timber of the different specimens were examined and the light petroleum extract of each gave colourless crystals from methanol. These crystals had ranges of melting points varying with the specimen from which they were isolated. Whatever the source, they were shown to be a mixture of two compounds which are referred to subsequently as COA and COB. The proportions of the two components varied with the source of the crystals, hence the varying ranges of melting points.

The separation of the two compounds was based on the following observations:

(i) That COB is very easily hydrolysed by alkali to form the compound COB (I) now recognised[9] as I or II in figure I. COA is not affected and remains in crystalline form. COB (I) goes into solution.

(ii) COA reacts readily with hydroxylamine hydrochloride to give a non-crystalline product, whilst COB does not react, remaining in the crystalline form.

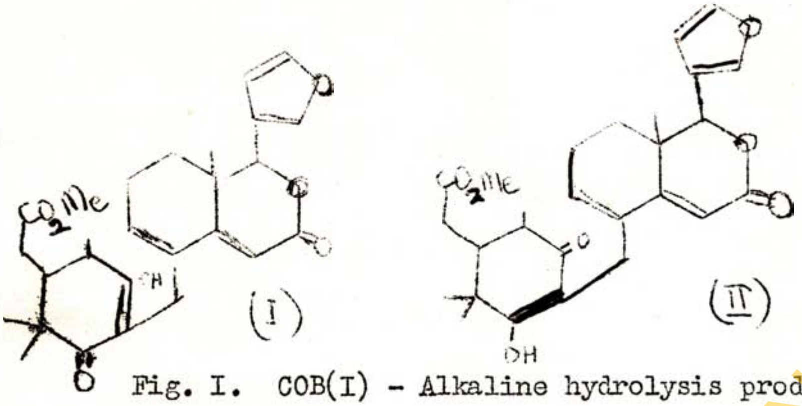


Fig. I. COB(I) - Alkaline hydrolysis product of COB.

COA crystallises from methanol as colourless prismatic crystals melting at 262°. The results of its chemical analysis were consistent with the formula $C_{26}H_{30}O_6$. It was later identified as 7 - deacetoxy-7-oxogedunin (Fig. 2), a compound

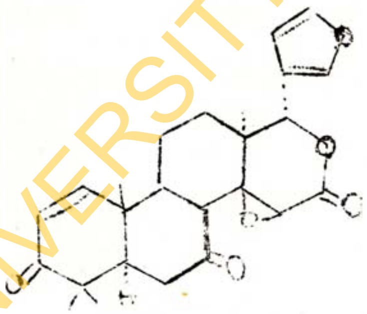


Fig. 2

which had earlier been obtained in the same laboratory by A. Akisanya in an attempt to establish the structure of gedunin[10,11].

COB, crystallised from methanol, melted at 228° - 232° and had $[\alpha]_D^{20}$ of + 41° in chloroform. Analysis gave the formula $C_{27}H_{32}O_7$.

Bevan[8] and others also reported the extraction of COB from the timber of "carapa procera". The petroleum extract crystallised from methanol, gave a substance of which different samples melted between 180° and 210°. In spite of the difference in melting point the infra-red spectrum was similar to that of COB. Rapid chromatography of a sample gave a pure specimen with m.p. 226° to 228°.

A mixture of this with COB as obtained from Cedrela Odorata gave no depression of melting point, and the infra-red spectra of both samples were identical.

From the identification of COA as 7-deacetoxy-7-oxogedunin (Fig. 2), it was assumed as a working hypothesis that COB might be related to gedunin. Early stages of the investigations on the structure of COB was carried out on this basis. J.W. Powell and others[12] pursued the chemical degradation together with spectral studies. Chemical analysis showed the presence of one methoxy group and a carbonyl group which was later confirmed by the infra-red spectrum. The assumed similarity in structure to gedunin and related compounds - cedrelone[13] and Limonin[14], would suggest the presence of the following features:

(a) furan ring

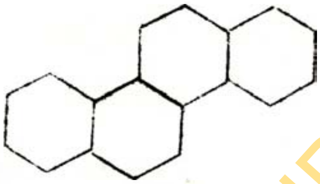
(b) lactone ring

(c) epoxide ring

(d) keto-group or groups

(e) four or five angular methyl groups and

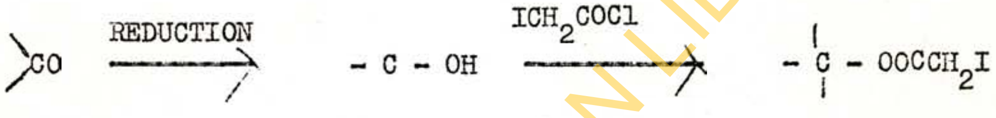
(f) four skeleton rings fused thus



However, the rather complex nature of the reaction of COB on treatment with alkali suggested some kind of departure.

The alkaline hydrolysis product, COB (I), later shown to have structure I or II of figure 1 was found stable and isomeric with COB, formula $C_{27}H_{32}O_{7}$. The infra-red spectra of both COB and COB (I), showed the presence of a furan ring. From the nuclear magnetic resonance spectrum[15] it was possible to recognise a furan ring, an ester group of the type - COOMe, and the presence of four angular methyl groups in COB. Thus whilst a fundamental difference between gedunin and COB has been indicated by the presence of the ester group, it still seemed to possess some similarity by the presence of four angular methyl groups, the keto group and the furan ring.

It was then thought that X-ray analysis might provide a definite clue to the structure of COB and to this end a procedure whereby a heavy atom would be introduced into COB was outlined. The keto-group would be reduced to the alcohol which in turn would be treated with iodoacetyl chloride to obtain the iodoacetate.



COB

COB
ALCOHOL

COB
IODOACETATE

The reduction of the keto-group gave a sticky mass which was not possible to crystallise and which thin layer chromatography proved to be a mixture of about four substances. Since it is now known that COB contains a β -dicarbonyl system, it can be inferred that the four substances are the α or β reduction products of each of the two carbonyl groups. There is of course in addition to this, the slight possibility of having the two carbonyl groups reduced assuming that there is no unreduced COB in the sticky mass. As the presence of the β -dicarbonyl system was then not known, it was not possible to draw these inferences. There was therefore no attempt to isolate one of the reduction products in this mixture and continue the programme for the preparation of the iodoacetate. A similar compound - Mexicanolide[9] with the structure recently formulated for it, is most probably a stereoisomer of COB. Like COB, it is obtained from the timber of a species of Cedrela - Cedrela Mexicana. The same process of reduction, with sodium borohydride, has been reported to yield one major product, implying the presence of other products.

Connolly and his co-workers were virtually certain they were reducing a compound containing a β -dicarbonyl system and hence must have expected a mixture of products which they proceeded to separate. Latest work[16] on the reduction of COB with the knowledge of the presence of the β -dicarbonyl system suggests as many as nine reduction products in which the lactone ring is affected in some instances.

The next line of approach was to methylate COB in the hope that the new compound might yield a purer reduction product. Chemical analysis of COB (II) (the methylation product), showed the presence of three methoxy-groups which meant that the process of methylation added two more methoxy - groups to COB. COB (II), did indeed give one major reduction product, the alcohol COB (III). The iodoacetate of COB (III) was then prepared. The preparation of the iodoacetate from COB was first carried out successfully by J.W. Powell.

The work here reported consists of a repetition of the preparation of iodoacetate and its subsequent X-ray analysis to obtain the molecular and crystal structures. From the molecular structure of the iodoacetate, the molecular structure of COB is derived.

To sum up, the following chemical information was available at the beginning of the X-ray analysis:

(a) a probable furan ring

(b) iodoacetate

(c) three methoxy groups

(d) four methyl groups

(e) an ester group of the form - COOMe.

PREPARATION OF THE IODOACETATE OF CEDRELA ODORATA SUBSTANCE B

Methylation of COB

Mixed extract crystals of cedrela odorata (40 g.) were added to methanol (2 lit.) in a 5 lit. flange flask. The mixture was refluxed until the crystals were all dissolved and to this was added 40 ml. conc. sulphuric acid in about 330 ml. methanol. Some methanol was distilled off in 40 minutes and 2 lit. water was added. There was a white precipitate which was extracted with chloroform in bits of 200, 100, 50 and 25 mls. The chloroform extract was washed with some sodium carbonate solution and then with water. The chloroform extract was evaporated until crystals could form on cooling the remaining solution.

The crystals in ethylacetate/benzene mixture were passed down a chromatographic column of about 750 ml. alumina, 5 per cent deactivated with 10 per cent acetic acid. The elute was collected in fractions the first crop being the methylation product of COB. An elute of 2.4 lit. yielded 13.7 gm. m.p. 170°C.

Both substances A and B, and of course, the methylation product were shown by the I.R. to contain carbonyl groups on the rings.

Reduction of the Methylation Product

The methylation product (4.0 g.) in 40 ml. chloroform and 200 ml. ethanol were mixed up in 500 ml. conical flask. Sodium borohydride (0.8 g.) dissolved in 10 ml. cold water was added and the whole mixture was mechanically stirred for one hour. More sodium borohydride (0.4 g.) in 5 ml cold water was added and the mixture stirred for another hour. Water was added in portions until the solution was clear and it was stirred further for 3 hours. 100 ml. water was added and the reduction product was extracted with chloroform in bits of 100, 35, 35 and 35 mls. The extract was washed with a little quantity of water and dried with some quantity of magnesium sulphate. It was then evaporated to a greenish yellow oily product.

Benzene/petroleum ether (60°-80°) mixture was added until it all dissolved. The solution looking cloudy was covered loosely and left to crystallise by evaporation for over 24 hours. This reduction process was repeated a number of times. The reduction product consists of yellowish crystals, yields vary from 1.6 to 2.8 gm. for each 4 gm. of the methylation product. In each instance only a range of m.p. point (130 to 157°C) could be obtained but the I.R. gave an absorption band at about 3,700 cm^{-1} for the presence of the -OH group.

Chloroacetylating the Reduction Product

Chloroacetylchloride was prepared by heating equimolecular quantities of chloroacetic acid and thionyl chloride on a water bath until the production of hydrogen chloride slackened. The product was then distilled through a fractionating column and chloroacetyl chloride collected between 99° to 105°.

Using the method of Barton[17] and others, 8.6 gm. of the reduction product in 250 ml. chloroform to which had been added 55 ml. chloroacetyl

X-RAY CRYSTALLOGRAPHIC ANALYSIS

Recrystallisation

The iodoacetate separated from the reaction mixture as clumps of plates which proved difficult to separate. It was therefore necessary to recrystallise the product. A few clups of about one milligram were put in a micro-specimen tube which had a tight-fitting cover. To this was added the same number of drops of benzene and petroleum ether (60° - 80°). The mixture was warmed on a waterbath until the crystals dissolved in the minimum quantity of benzene/petrol mixture. An extra drop of each solvent was added and the solution warmed for a further ten seconds. The tube was immediately covered and thermally insulated to prevent rapid cooling. It cooled slowly for several hours. When the solution attained the room temperature, the cover was removed and the solution was left standing overnight.

The symmetry of the reflections obtained from the two photographs indicated that the crystal belongs to the orthorhombic system. This was later confirmed by the symmetry of the Weissenberg equi-indination photographs for the general reflections hkl.

From these photographs and the zero layer photograph about the a axis, the following systematic absences were observed:

hoo with h odd

oko with k odd

ool with l odd

These absences implied the presence of two-fold screw axes parallel to each of the crystal axes. Since there were no other absences, the space group was determined unambiguously as $P2_12_12_1$. This has four equivalent general positions and hence four asymmetric units in the unit cell.

In determining the densities of crystals in this type of work, it is the practice to use a mixture of two solvents or a solution in which the crystals are insoluble. Since the crystals are insoluble in water, it was considered safe to use a salt solution and zinc sulphate solution was found dense enough to keep the crystals floating. The density was determined as 1.432 ± 0.005 gm. per c.c. by flotation in this solution. The calculated density based on the final structure is 1.422 gm per c.c.

Assuming that the asymmetric unit contained one molecule, an assumption which is usually true for natural products, the molecular weight was calculated to be 693± 7.

The coefficient of the linear absorption from the present accepted formula of $C_{30}H_{38}O_8$ ClI with molecular weight of 688.4 can be calculated thus:

$$\mu = \frac{\rho \sum n \; \mu_g}{\sum n \; W}$$

where μ is the linear absorption coefficient

ρ is the density of the iodoacetate

μ_g is the gram-atomic absorption coefficient for each atom.

n is the number of atoms of atomic weight W in the molecule.

$$\therefore \mu = \frac{1.432\,(30 \times 66.1 + 38 \times 0.435 + 8 \times 203 + 39900)}{688.4}$$

$$= 90.5 \text{ per cm}$$

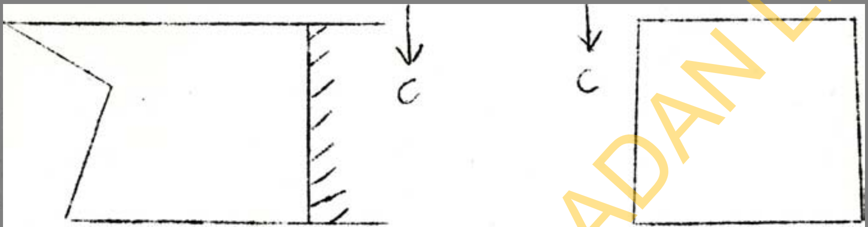
for copper Kalpha radiation.

It was calculated from this value that absorption varied by as much as 28% over the range of θ values 0° is 90°.

The gram-atomic absorption coefficients for carbon, oxygen chlorine and iodine were from the tables of Henry, Lipson and Wooster[19] and that of hydrogen was equated to the mass absorption coefficient obtained from the International Tables (1952). The linear absorption coefficient was previously calculated based on the molecular weight as known at the time to be 97 cm^{-1}. It was clear then that there would be considerable errors ~~with~~ in the structure factors owing to absorption. But as the object of the analysis was to obtain the gross molecular structure rather than accurate bond lengths and angles, and as an absorption correction programme was not immediately available, anyway, no corrections for absorption were made.

Collection of Intensity Data

The intensities were collected from Weissenberg photographs taken about both a and b axes. The exposure for each photograph was roughly 50 hours of Copper Kα radiation. In order to reduce absorption errors to a minimum, attempts were made to obtain smaller crystals of almost cylindrical shape. To achieve this the crystals were re-grown as described a number of times. A second crystal of dimensions 0.15 by 0.20 by 0.35 mm was mounted about an axis which was found to be b. It is expedient to take photographs of reciprocal layers about the shortest axis i.e. a. Because of its shape it was not reasonable to expect much from remounting this crystal. And so while a search was going on for an approximately cubic shaped crystal which could be mounted about any of the three axes, multiple film equi-inclination Weissenberg photographs of the layers (hol), (h1l) and h2l) were taken. The (hol) photograph was taken with unfiltered copper radiation. Copper Kα radiation was used for the other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Intensity Measurement

Of the varying methods of measuring intensities from single crystal photographs, visual estimation[20] is the least accurate. It has, however proved satisfactory at least for structural purposes and has been employed in the determination of as complex a structure as the hexacarboxylic acid of Vitamin B_{12}[7] as well as for Vitamin B_{12}[6,7] itself. It is the most commonly used method. This is obviously due to its simplicity and the fact that it is often the only available method in many laboratories. For a very accurate work, one would require the quantum counter. Diffractometers which use radiation counters for measuring intensities, have been designed for completely automatic operation.

Visual estimation consists of measuring the intensity of the photographically recorded reflection with the aid of a standard comparison intensity strip, prepared by recording the same reflection from a given crystal for different lengths of time. The exposure times represent the relative values of the intensities on the strip. This was the only available method. To increase the range of measurable intensities, the multiple film technique[21] was used. With only four films for each photograph, it was still not possible to estimate the intensities of 0 2 0 and 0 2 3 which were among the strongest reflections.

The following five reflections, having the least Sin θ values, were cut off by the beam trap and were not recorded: (0 1 1, 1 0 1, 1 1 0, 1 1 1, 1 2 0).

There were not as many reflections as was expected with Copper $K\alpha$ radiation; there are 3673 reflections within the copper sphere. The recorded (hol) reflections were only 178 in number out of which only four had sin θ values above 0.8; the maximum sin θ value was 0.8507. Similarly there were 147 (h11) reflections, with only three reflections having sin θ values above 0.8; the highest value was 0.83 (7 1 16). For the (h21), there were 129 reflections with a maximum sin θ value of 0.765 (4 2 17).

From the photographs about the <u>a</u> axis, there were: 217 (okl) reflections with a maximum sin θ value of 0.788; 199 (1kl) with a maximum sin θ value of 0.73; 194 (2kl) with a maximum sin θ value of 0.788; 170 (3kl) with sin θ of 0.72. An average of about 140 maximum reflections, the actual number for each layer decreasing with increasing value of h, were obtained for the other layers. The maximum sin θ value was about 0.70.

In measuring the intensities, spot extension and contraction were observed. The extended spots reflected from only one quadrant of the reciprocal lattice were estimated. There were no systematic search for the effects of anomalous scattering but one reflection (4 4 2) was observed to show some departure from Friedel's law. Two of its equivalent reflections had different intensities. The two were estimated and averaged.

Correction of Intensities

Disregarding errors of absorption, extinction and spot-extension[22], the measured relative intensity is related to the structure amplitude F(hkl) by[23] the equation. $I(hkl) = kLpF^2(hkl)$

where k is a constant

p the polarization correction is given by

$$p = (1 + \text{Cos}^2 2\theta) / 2$$

and L the Lorentz correction by

$$L = \frac{\text{Sin}\,\theta}{\text{Sin}\,2\theta\,(\text{Sin}^2\theta - \text{Sin}^2\mu)^{\frac{1}{2}}}$$

This is Tunnell's[24] method of expressing the Lorentz correction. θ is the Bragg angle and μ the equi-inclination angle. When $\mu = 0$, i.e. for equatorial reflections, this reduces to $L = (\text{Sin}\,2\theta)^{-1}$. These expressions give

$$(Lp)^{-1} = \frac{2\,\text{Sin}\,2\theta\,(\text{Sin}^2\theta - \text{Sin}^2\mu)^{\frac{1}{2}}}{(1 + \text{Cos}^2 2\theta)\cdot\text{Sin}\,\theta} \quad \text{---------} \ (1)$$

$$= \frac{2\left[(1 - \text{Sin}^2\theta)(\text{Sin}^2\theta - \text{Sin}^2\mu)\right]^{\frac{1}{2}}}{1 - 2\,\text{Sin}^2\theta\,(1 - \text{Sin}^2\theta)} \quad \text{-----} \ (2)$$

$$= \frac{2\,\text{Sin}\,2\theta}{1 + \text{Cos}^2 2\theta} \quad \text{when } \mu = \text{zero}$$

Values of Sin θ were calculated on a FACIT desk calculating machine for all the (hol) reflections and from the values of $\frac{1 + \text{Cos}^2 2\theta}{\text{Sin}\,2\theta}$ as a function of Sin θ, obtained from the International Tables, Lp and hence $\{F^2\}$ values were derived for all these reflections.

For the general reflections (hkl), the Lorentz Polarization corrections and hence $\{F^2\}$ were desk calculated using equation (1) above for a few of the (1kl) reflections.

The results were compared with those computed on the IBM 1620. Corrected intensities $\{Fo^2\}$ for all the measured reflections were then calculated on the computer.

Scaling and Temperature Factors

To put the corrected intensities on the same scale, the first approach was to correlate the eight layers about the a axis by the use of common reflections on one of the layers about the b axis. The second layer was arbitrarily chosen.

TABLE 1

h k l	I (2b)	I (2a)	Correlation Factor $I(2b)/I(2a)$
2 2 1	4.13	5.16	0.800
2 2 2	8.07	4.39	1.838*
2 2 3	4.76	2.99	1.592*
2 2 4	4.56	5.12	0.891
2 2 5	7.54	5.93	1.272
2 2 6	21.38	18.92	1.130
2 2 7	8.59	9.56	0.898
2 2 8	9.75	11.68	0.835
2 2 9	7.87	6.81	1.156
2 2 10	6.33	7.26	0.872
2 2 11	3.39	2.03	1.670
2 2 12	5.26	4.58	1.149
2 2 13	2.92	2.39	1.222

* Not included in the average value in tables 1 to 3.

I(2b) are the intensities from (h2l) reflections.

I(2a) are the intensities from (2kl) reflections.

Average Correlation Factor = 1.02.

Table 1 shows the range of correlation factors of each reflection obtained for the second layer about *a* axis. The lowest (0.800) for 2 2 1 was fairly acceptable but the highest ones for reflections 2 2 2, 2 2 3 and 2 2 11 were far from the others and were ruled out in calculating an average value.

Tables 2 and 3 show similar calculations for the third and fourth layers respectively. The reflections 3 2 1 and 3 2 11 in Table 2 and 4 2 1, 4 2 7, 4 2 8, 4 2 9 and 4 2 10 in Table 3, were ruled out in calculating the average value of the correlation factors.

This was done for the eight layers and as the range of value for the individual reflection correlation factors for each layer was so large as to necessitate cancelling some of them, the results did not inspire confidence. As a check the eight layers were correlated twice again just as above by comparing intensities of common

TABLE 2

h k l	I (2b)	I (3a)	Correlation Factor $I(2b)/I(3a)$
3 2 1	11.55	14.69	0.786*
3 2 2	18.33	12.52	1.464
3 2 3	5.59	3.52	1.587
3 2 4	13.42	10.8	1.242
3 2 5	6.99	6.36	1.099
3 2 7	4.56	4.01	1.138
3 2 8	5.95	5.12	1.161
3 2 9	10.19	9.83	1.036
3 2 10	3.65	2.52	1.449
3 2 11	2.81	1.43	1.965*

I(3a) are the intensities from (3kl) reflections.
Average correlation factor = 1.235.

TABLE 3

h k l	I (2b)	I (4a)	Correlation Factor I(2b)/I(4a)
4 2 1	4.81	3.67	1.311*
4 2 2	1.36	1.31	1.038
4 2 3	17.54	16.71	1.049
4 2 4	12.58	15.62	0.805
4 2 5	6.73	8.45	0.796
4 2 7	7.36	15.26	0.482*
4 2 8	3.56	7.88	0.452*
4 2 9	1.78	2.60	0.684*
4 2 10	4.07	6.77	0.601*
4 2 11	5.56	6.29	0.884

I(4a) are the intensities from (4kl) reflections.

Average correlation factor = 0.8403.

reflections on the zero and first layer photographs about the b axis with those on photographs about the a axis.

Bearing in mind that the reciprocal layers (hol) to (h2l) are themselves to be correlated, the ratios obtained are shown in Table 4 for the eight layers.

TABLE 4

RATIO OF AVERAGE CORRELATION FACTORS OBTAINED BY COMPARISON

	hol	h11	h21
okl	1.00	1.00	1.00
1kl	0.74	0.95	1.15
2kl	0.88	1.36	1.31
3kl	1.14	1.63	1.62
4kl	0.56	0.98	1.10
5kl	0.92	1.43	1.15
6kl	1.03	2.60	1.20
7kl	1.96	3.69	2.69

The figures obtained from hol reflections appear generally lower and it may be better to average the figures from the h11 and h21

TABLE 4A

SCALE FACTORS

obtained by two methods

	Comparison	Wilson Plot
0kl	1.00	1.000
1kl	1.15	1.151
2kl	1.31	1.508
3kl	1.62	1.861
4kl	1.10	1.222
5kl	1.15	1.539

reflections. The depression in the hol figures is rather difficult to explain, but it may be pointed out that the photograph was taken with unfiltered radiation. The variation for the 6kl ratios is the largest and thus any factor, used for this set of reflections, based on this approach is most unreliable. The 7kl reflections undoubtedly must have the highest factor. These figures seemed so unreliable that it was decided to adopt another procedure. This was the statistical method of Wilson's[25].

Within a narrow range of $\mathrm{Sin}^2\,\theta$

$$S\bar{I}_o = \bar{I}_{abs}\, e^{-2B(\overline{\mathrm{Sin}^2\,\theta})/\lambda^2} \text{ -------- } (3)$$

where S is the scale factor, Io, I_{abs}, the measured and absolute intensities respectively, λ , the wavelength of the rediation used and B the temperature factor. The equation is used to derive the scale factor (correlation factor) S and the temperature factor B. The absolute intensity is unknown but estimated by using the assumption that within the narrow range of $\mathrm{Sin}^2\theta$ (or θ value)

$$\overline{I(hkl)} = \sum_n f_n^2 ,$$ where f_n the atomic scattering curve of n^{th} atom in the molecule, $\overline{I(hkl)}$ is the mean absolute intensity. The theoretical form of the f curve is assumed. Neglecting hydrogen atoms, the formula of the iodoacetate as presumed at the time was $C_{31}O_{10}$ I.

A graph of log $\frac{\langle Io\rangle}{\bar{I}abs}$ against $\mathrm{Sin}^2\,\theta$ was plotted for each zone of reflections, (see figs. 2 to 12 and tables 5 to 9), $\langle Io\rangle$ is the mean observed intensity for the narrow range of $\mathrm{Sin}^2\theta$ and $\bar{I}abs = \frac{\sum_n fn}{42}$.

TABLE 9 - 7kl

$\langle \sin\theta \rangle$	$\sin^2\theta$	$\sum I$	N(I)	$\sum \bar{f}^2$	$R=I/\sum \bar{f}^2$	$\log_e R$
0.10	0.0100	-	-	100.96	-	-
0.15	0.0225	-	-	90.76	-	-
0.20	0.0400	-	-	80.37	-	-
0.25	0.0625	-	-	70.76	-	-
0.30	0.0900	-	-	62.01	-	-
0.35	0.1225	-	-	54.53	-	-
0.40	0.1600	-	-	48.18	-	-
0.45	0.2025	-	-	42.67	-	-
0.50	0.2500	18.70	25	38.13	0.020	- 3.9313
0.55	0.3025	39.11	48	34.23	0.024	- 3.7378
0.60	0.3600	37.20	44	30.90	0.027	- 3.5987
0.65	0.4225	30.14	31	28.09	0.035	- 3.3636
0.70	0.4900	13.25	10*	25.63	0.052	- 2.9548
0.75	0.5625	-	-	23.51	-	-
0.80	0.6400	-	-	21.71	-	-

* Not reliable (tables 5 to 9).

$\langle \sin\theta \rangle$ is the mean value of sin θ range. $\sum I$ is the sum of measured intensity in the range of sin θ, and N(I) is the number of reflections summed for the range. $\sum \bar{f}^2$ is the estimated absolute intensity for the range. $\bar{I}$ is the mean observed intensity.

Table 5 illustrates the sort of results obtainable for the reciprocal layers about b axis. Table 6 gives the results for one of the two sets of equatorial reflections. Table 7 gives the best results and Tables 8 and 9 give the results for layers 6 and 7 about the a axis. Figures 2 to 10 show the plots of the results from which the scale and temperature factors were derived. From the tables, the number of reflections summed at low values of $\sin^2\theta$ is usually low and as the relationship (3) is a statistical one, depending on the average of large numbers of reflection the figures for these low angle reflections will be of low reliability. The same applies at high angles of $\sin^2\theta$. The results are reliable only for those ranges of $\sin^2\theta$ values in which the number of reflections is large.

On plotting the points, the best linearity was shown for the (3kl) reflections in fig. 8. Since the expected temperature factor was isotropic, the gradient of this line was assumed as a rough approximate value in drawing the remaining plots. This procedure was applicable to all but the (6kl) and (7kl) reflections. There were only four points on the (5kl) graph that would lie on the straight line of the given gradient; five other points, two with low values and three with high values of $\sin^2\theta$, were ignored.

The gradient of the plots for the (6kl) and (7kl) reflections would not give a meaningful temperature factor. Just before the photographs of these layers were taken, a steady change in colour of the crystal had been observed. This could lead to changes in the intensity of reflections. Measurable changes, sometimes due to decomposition of the crystal, in relative intensities after an exposure to radiation for a length of time, are known to occur. Such changes after a ten hour exposure have been reported by Cullis[5] and others. Moreover, the number of reflections can hardly stand the test of a statistical law. The calculations could give a better results by including a mean intensity of half the threshold value for those reflections whose intensities were too weak to be estimated. The reflections (6kl) and (7kl) were finally ignored.

Table 10 (p.26) gives the scale and temperature factors derived from the plots - fig. 2 to 10. The numbers in brackets are in the same ratio, relative to S(okl) as 1.00. The scale factor ratios are not far off those obtained from the comparison of common reflections on intersecting layers particularly those based on the h2l layer. (See Table 4A). This statement is not applicable to the (6kl) and (7kl) reflections.

Observed Structure Amptitude

The corrected intensities were put on a common scale by multiplying each zone of reflections by its scale factor-s. The reflections, common to reciprocal planes about both <u>a</u> and <u>b</u> axes, were given average values of the two structure amplitudes. These operations reduced the total number of independent reflections to 1303, of which 991 were measurable and the remaining 312, too weak to be measured.

STRUCTURE ANALYSIS

Location of Iodine Atom

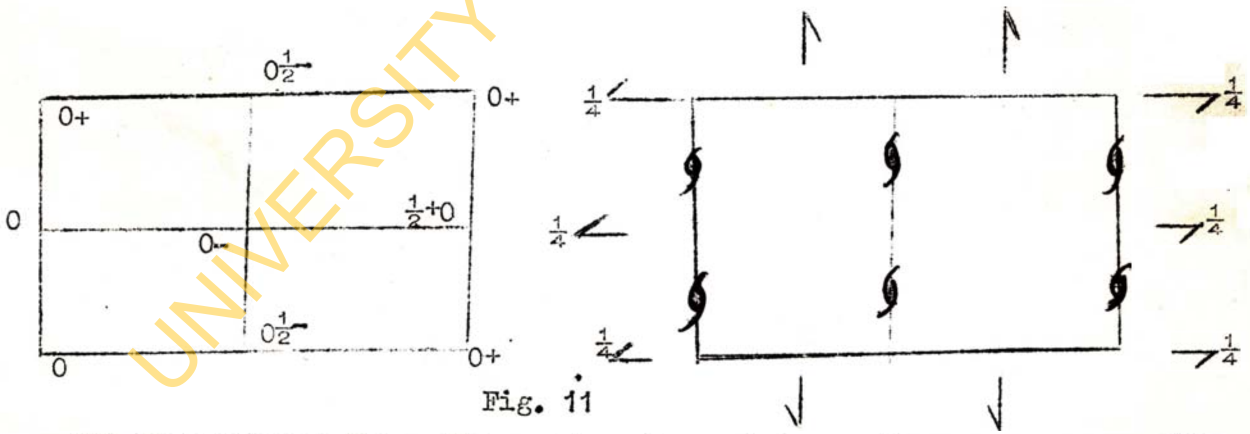
With the assumption that each molecule constitutes an asymmetric unit and therefore accupies a general position, there should be four iodine atoms in each unit cell. This is a relatively small number of heavy atoms[27] and hence it should be possible to locate them. They occupy a set of four equivalent general positions (fig. 11) with co-ordinates

(i) $x, y, z.$

(ii) $\frac{1}{2}-x, \bar{y}, \frac{1}{2}+z$

(iii) $\frac{1}{2}+x, \frac{1}{2}-y, \bar{z}$

(iv) $\bar{x}, \frac{1}{2}+y, \frac{1}{2}-z.$

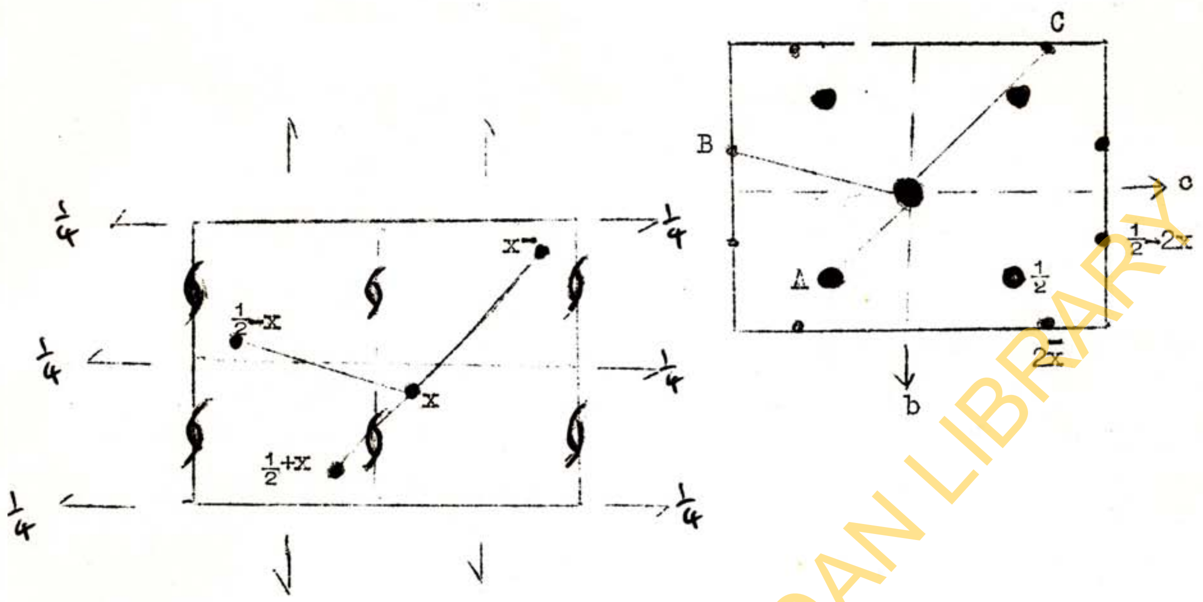


Equivalent positions

Symmetry Elements

Fig. 11

Origin halfway between three pairs of non-intersecting screw axes. (The symbols are on the same convention as in International Tables 1952. vol.1). Vectors between pairs of these positions will be represented essentially by the following points in the Patterson map:



A. Real Space B. Vector Space

Fig 13

Origin at the centre of drawing still halfway between three pairs of non-intersecting screw axes

(i) $\frac{1}{2}, \frac{1}{2} - 2y, 2z$

(ii) $\frac{1}{2} - 2x, 2y, \frac{1}{2}$

(iii) $2x, \frac{1}{2}, \frac{1}{2} - 2z$.

These points, others related to them by symmetry, are shown in the vector space diagram of fig. 13B.

Two forms of the Patterson function were used in order to locate the iodine atom.

Two dimensional Patterson

The two dimensional Patterson function, P(uv), for any space group is given by

$$P(uv) = \frac{1}{A} \sum_{h=-\infty}^{\infty} \sum_{k=-\infty}^{\infty} F(hko)^2 \exp 2\pi i \; (hu + kv)$$

For the space group $P2_12_12_1$ in which

$$|F(hko)|^2 = |F(\bar{h}ko)|^2$$
$$= |F(h\bar{k}o)|^2$$

this reduces to

$$P(uv) = \frac{4}{A} \sum_{h=o}^{\infty} \sum_{k=o}^{\infty} |F(hko)|^2 \, \mathrm{Cos}\, 2\pi hu \; \mathrm{Cos}\, 2\pi kv$$

In practice, this becomes

$$P(uv) = \frac{4}{A} \sum_{h=o}^{H} \sum_{k=o}^{K} |F(hko)|^2 \, \mathrm{Cos}\, 2\pi hu \; \mathrm{Cos}\, 2\pi kv$$

where H and K are the highest values of h and k.

A Patterson projection on the (100) plane

$$P(vw) = \frac{4}{A} \sum_{k=o}^{K} \sum_{l=o}^{L} |F(okl)|^2 \, \mathrm{Cos}\, 2\pi kv \; \mathrm{Cos}\, 2\pi lw$$

was computed at intervals of 1/50 of the unit cell side in v and w. The coefficients $|Fo|^2$ were unsharpened and the highest values of k and l were both 16. $|F(000)|^2$ was omitted. The results are shown in fig. 12 A

The contours are drawn at arbitrary intervals. Comparing this map with the vector space projection shown in figure 13, it is possible to locate peaks equivalent to peaks B and C along the lines $w = \frac{1}{2}$ and $v = \frac{1}{2}$ respectively in projection, but the peak equivalent to A at the position marked A, is blurred. The co-ordinates y and z are, however, obtainable from the two easily recognised peaks. For the <u>a</u> axis projection, the peaks are

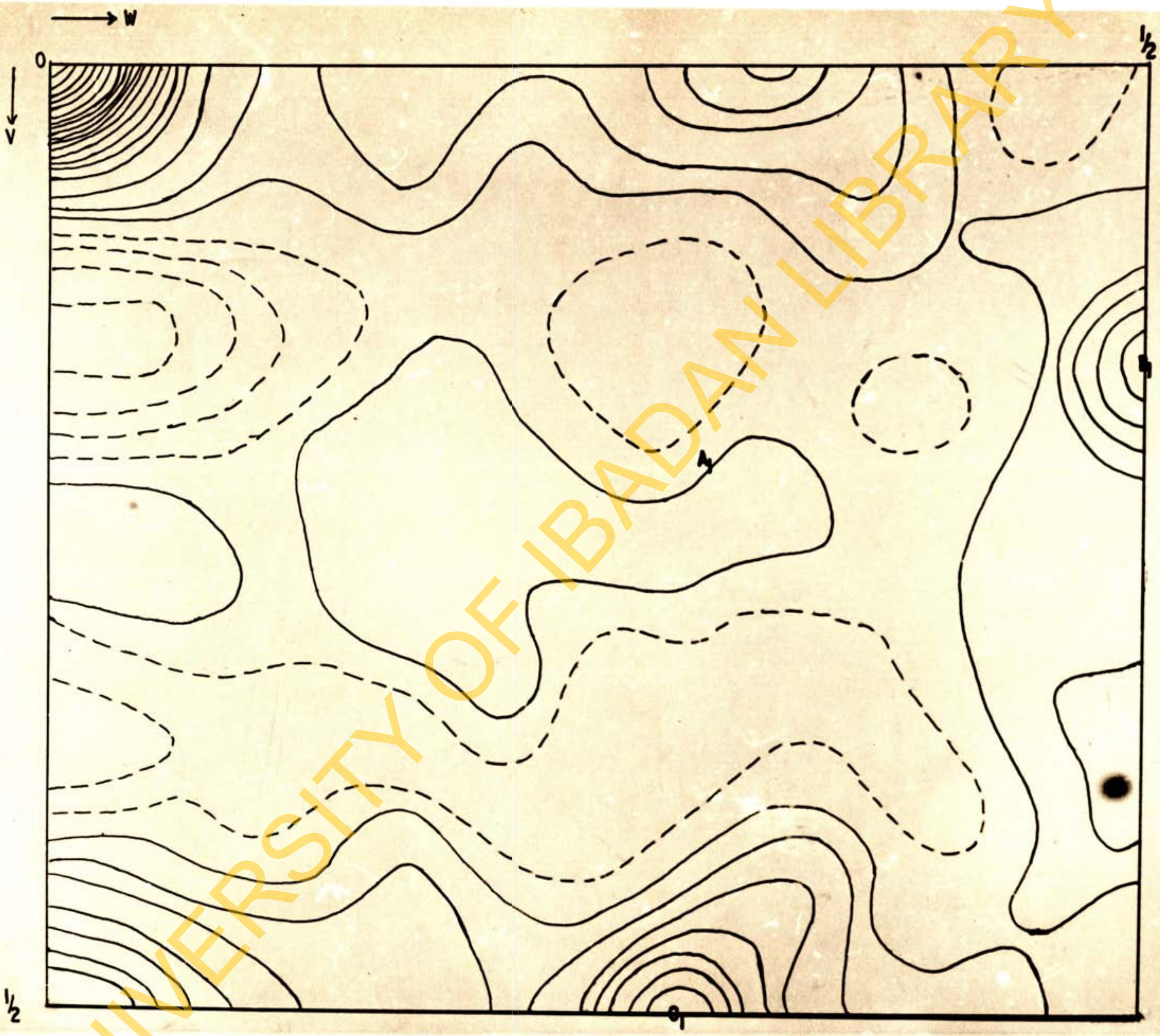


Figure 12A

$(\frac{1}{2} - 2y,\ 2z;)$

$(2y,\ \frac{1}{2};)$

$(\frac{1}{2},\ \frac{1}{2} - 2z)$

Thus $2y = 7.8/50$

$y = 0.078$

$\frac{1}{2} - 2z = 28.8/100$

$z = 0.106$

To test the reliability of these parameters, they were used to calculate the structure amplitudes of a few of the (okl) reflections.

The general expression for the structure factor is given by

$$F(hkl) = \sum_{n=1}^{N} fn \exp \left\{ 2\pi i (hx_n + ky_n + lz_n) \right\}$$

where F(hkl) is the structure factor of the reflection (hkl).

N is the number of atoms in the unit cell, fn the atomic scattering curve of the n^{th} atom and x_n, y_n, z_n; are the position parameters of the n^{th} atom.

In two dimensions, this becomes

$$F(okl) = \sum_{n=1}^{N} fn \exp \left\{ 2\pi i (ky_n + lz_n) \right\}$$

But $F(okl) = A + iB$

$F(o\bar{k}\bar{l}) = A - iB$

and $F(okl) = F(o\bar{k}\bar{l})$, by Friedel's law.

Hence B = zero and

$$F(okl) = A$$
$$= \sum_{n=1}^{N} fn \cdot \cos 2\pi (ky_n + lz_n)$$

where $N = 4$ for the iodine atoms only.

The general equivalent positions for the plane group Pgg are:

(i) y, z;

(ii) $\bar{y}, \bar{z}$;

(iii) $\frac{1}{2} + y, \frac{1}{2} - z$;

(iv) $-\frac{1}{2}-y, -\frac{1}{2}+z$;

and F(okl) simplifies to

$$F(okl) = 4f \operatorname{Cos} 2\pi \left(ky + \frac{k + l}{4}\right) \operatorname{Cos} 2\pi \left(lz - \frac{k + l}{4}\right)$$

If $k + l = 2n$ (even)

$$F(okl) = 4f \operatorname{Cos} 2\pi ky \operatorname{Cos} 2\pi lz.$$

If $k + l = 2n + 1$ (odd),

$$F(okl) = - 4f \sin 2\pi ky \sin 2\pi lz.$$

Table 11 shows the structure amplitudes calculated on a FACIT desk calculating machine using the tabulated sine and cosine values from the international tables. The iodine f- curve was the same as before (page 25). An isotropic temperature factor of $\exp(- 2B \sin^2\theta / \lambda^2)$, where $B = 4.40A^2$, was applied.

TABLE 11

0 K l	F(CALC)	F(OBS)
0 1 11	+ 0.884	1.634
0 6 6	- 4.734	4.202
0 6 8	- 5.618	2.980
0 6 10	+ 5.208	6.304
0 9 9	- 5.301	5.913
0 9 11	- 1.093	1.857
0 12 4	- 4.430	4.22
0 12 8	+ 2.840	1.992
0 14 6	+ 1.283	2.352
0 1 8	+ 5.92	7.736
0 1 10	- 4.249	3.884
0 1 12	- 5.316	4.627
0 10 7	- 5.307	3.811
0 10 13	+ 1.329	2.269
0 14 3	- 2.288	2.005

The F(CALC.) was scaled so that the sum of the calculated F's was the same as that of the observed values. There is a fairly high degree of agreement. This indicates that the parameters are quite reasonable.

A Patterson projection (fig. 14) along the b-axis was also calculated and the calculations following the same arguments as outlined above gave the results

$$\tfrac{1}{2} - 2x = 0.26$$

$$x = 0.12$$

$$2z = 0.22$$

$$z = 0.11$$

In spite of the good agreement shown between the observed and calculated structure factors as above, it was considered desirable to check the co-ordinates from a three dimensional Patterson. The co-ordinates from this would be more reliable in consequence of the less overlap of vectors.

<u>Three dimensional Patterson</u>

The three dimensional Patterson function P(uvw), for any space group is given by

$$P(uvw) = \frac{1}{V} \sum_{h} \sum_{k} \sum_{l=-\infty}^{\infty} |F^2(hkl)| \exp 2\pi i\,(hu+kv+lw).$$

By Friedel's law, this simplifies to

$$P(uvw) = \frac{2}{V} \sum_{h=0}^{\infty} \sum_{k=-\infty}^{\infty} \sum_{l=-\infty}^{\infty} |F^2(hkl)| \cos 2\pi\,(hu+kv+lw)$$

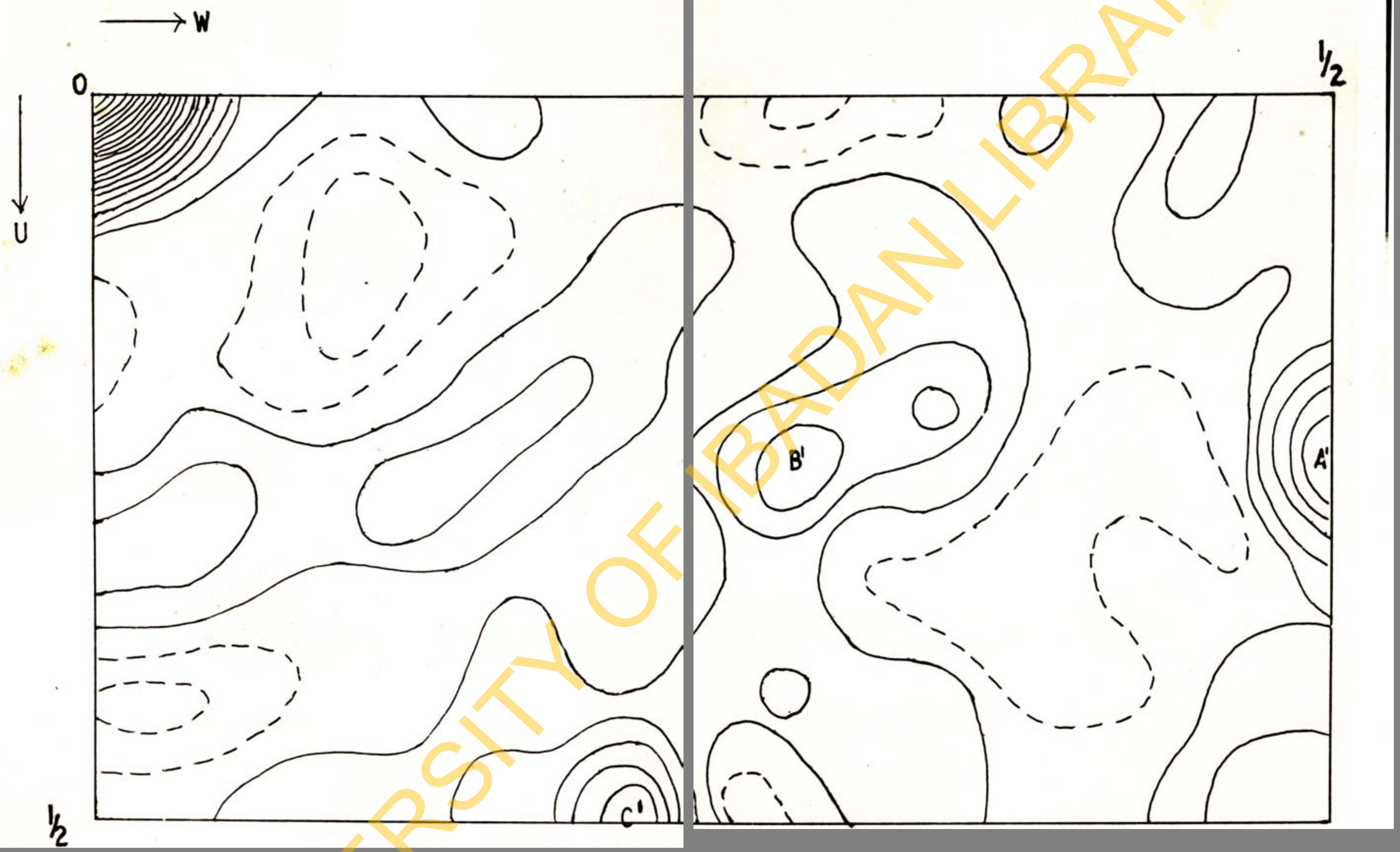
which is real for all values of u,v, and w.

For the space group $P2_1\,2_1\,2_1$

$$|F(hkl)|^2 = |F(\bar{h}kl)|^2$$

$$= |F(h\bar{k}l)|^2$$

$$= |F(hk\bar{l})|^2$$


W
U
0
½
½
B'
A'
C'

and the equation simplifies to

$$P(uvw) = \frac{8}{V}\sum_{h=0}^{\infty}\sum_{k=0}^{\infty}\sum_{l=0}^{\infty}\left|F(hkl)\right|^{2} \cos 2\pi hu \cos 2\pi kv \cos 2\pi lw.$$

A general reflection, hkl has a multiplicity of eight; any reflection with a zero index, e.g. okl, a multiplicity of four and those with two zero indices, a multiplicity of two. If all the indices are zero, the multiplicity is reduced to ONE. But $|F(000)|^2$ cannot be measured and must be calculated and added to the experimental data.

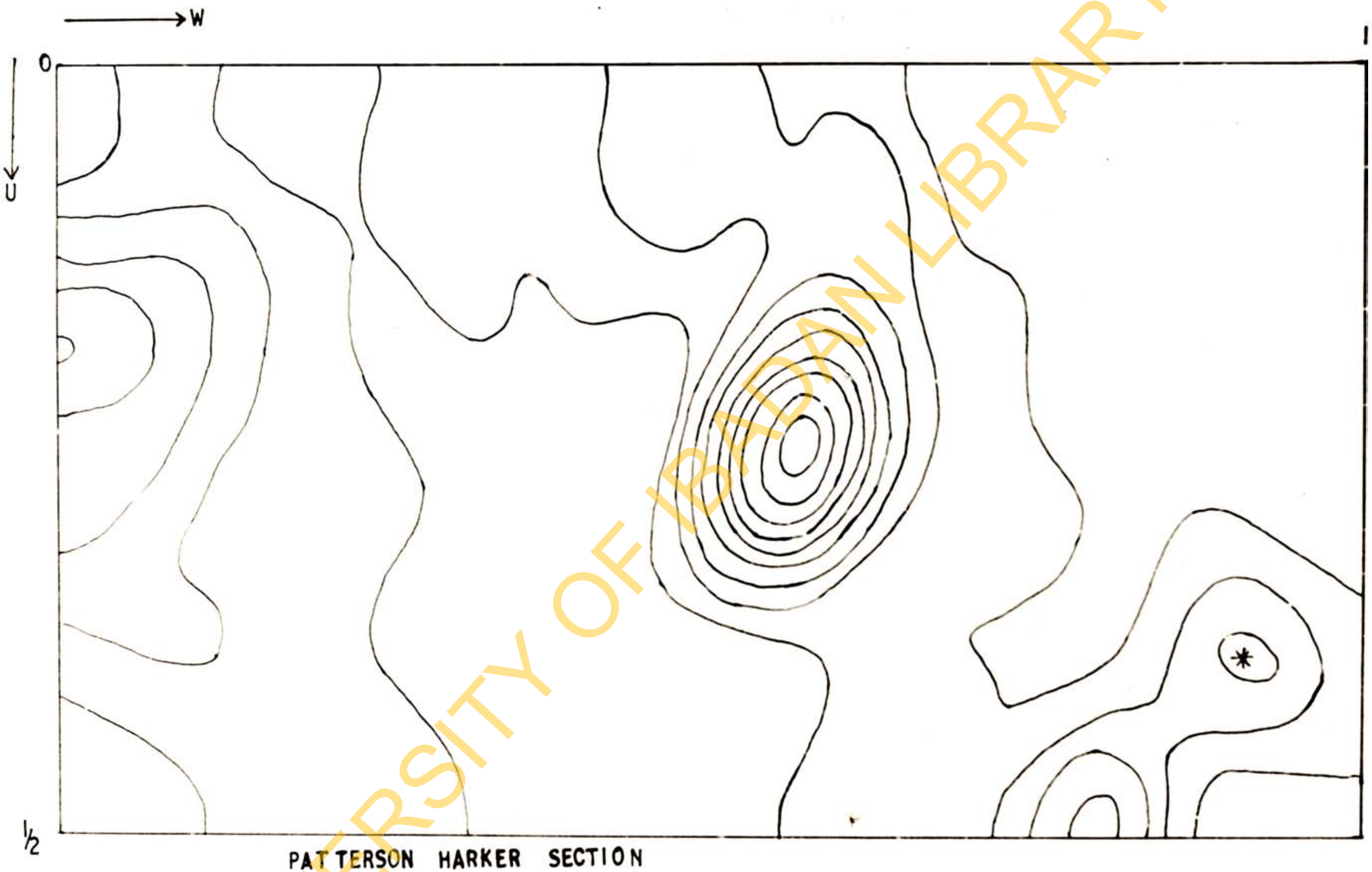
The three dimensional Patterson summations were computed using the 991 measurable reflections at intervals of $1/_{60}$ th along u, v, and w, to a maximum of $\frac{1}{2}$ in each direction. The intensities were on an arbitrary scale and $|F(000)|^2$ was not included. The figures for the Harker[28] sections of planes

$u = \frac{1}{2}$

$v = \frac{1}{2}$ and

$w = \frac{1}{2}$

were plotted on tracing paper to obtain the maps shown in figures 15, 16, and 17. The Iodine-Iodine vectors were quite easily distinguished and in agreement with vector space diagram figure 13. The positions of the peaks in each axial direction were determined by graphical interpolation of the Harker peaks and assuming that the highest peak on each section represents an iodine-iodine vector, the co-ordinates were derived as follows:



PATTERSON HARKER SECTION

Figure 15

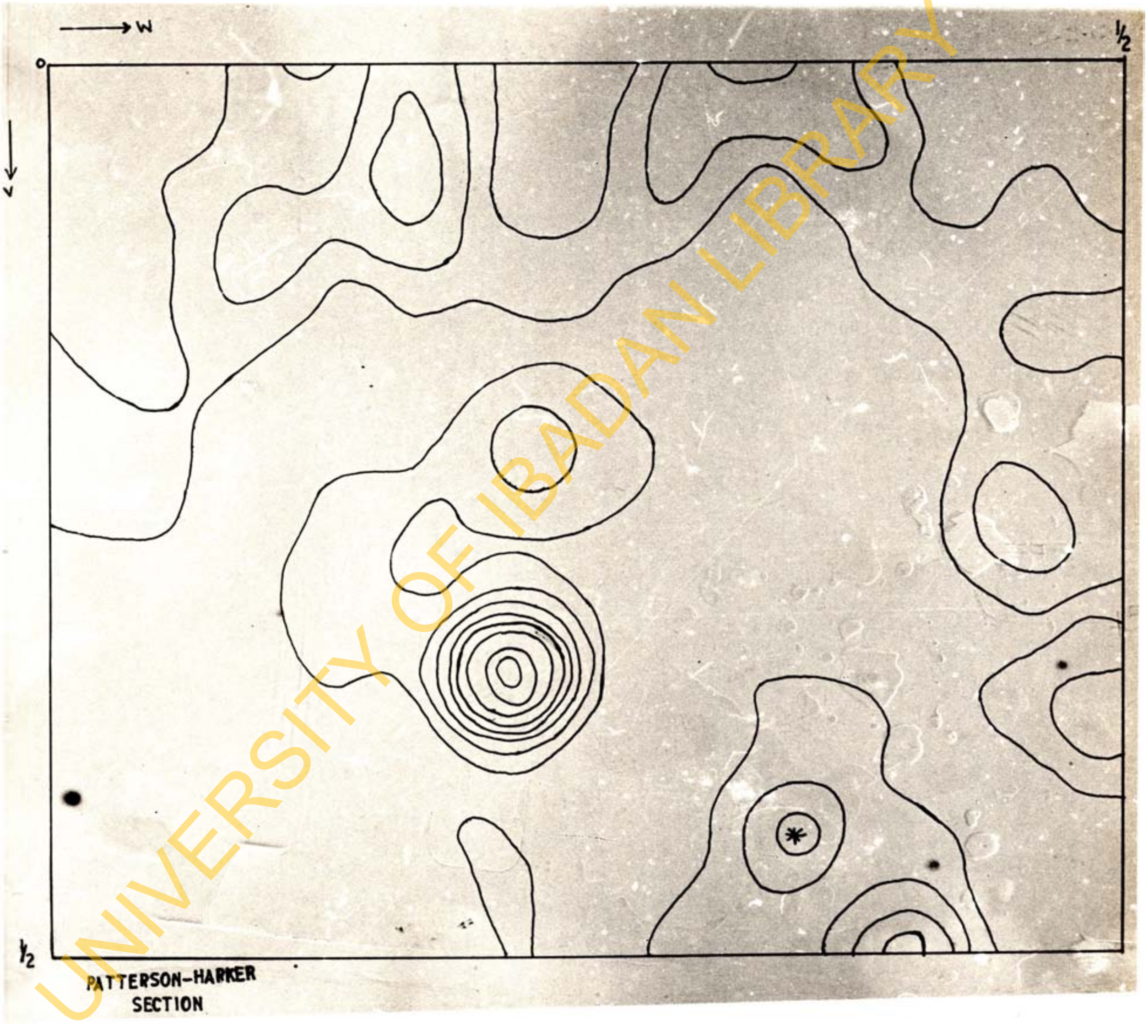
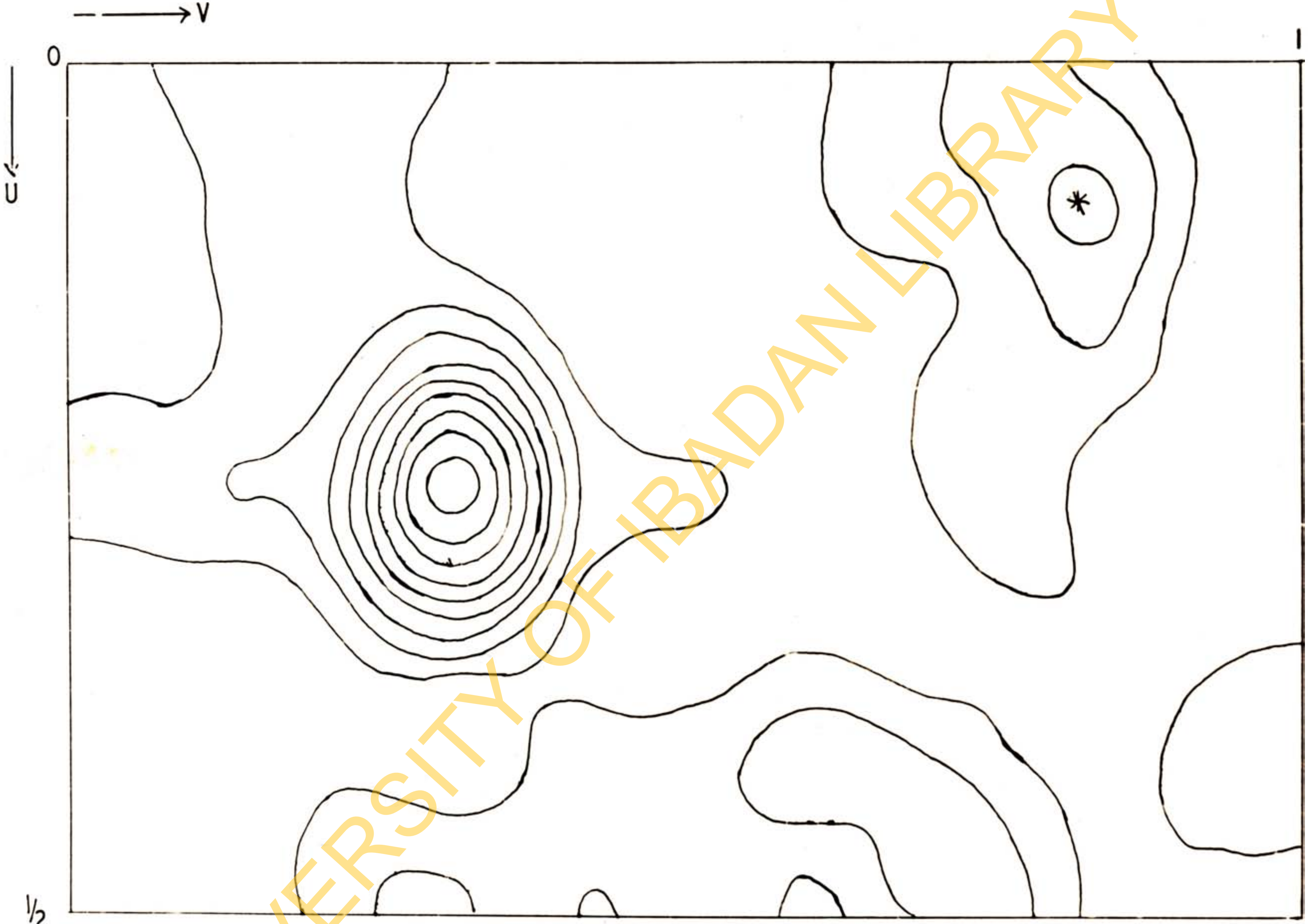


Figure 16



PATTERSON–HARKER SECTION

Figure 17

For the plane $u = \frac{1}{2}$, the co-ordinates (v,w) of the peak were 40.8/120, 258/1200;

Hence (i) $\frac{1}{2} - 2y = 40.8/120$

$y = 0.080$

(ii) $2z = 258/1200$

$z = 0.108$

For the plane $w = \frac{1}{2}$

(i) $v = 189/1200$

$\therefore 2y = 189/1200$

$y = 0.079$

(ii) $u = 3035/12000$

$\frac{1}{2}-2x = 3035/12000$

$x = 0.124$

For the plane $v = \frac{1}{2}$

(i) $u = 3015/12000$

$2x = 3015/12000$

$\therefore x = 0.126$

(ii) $w = 344/1200$

$\therefore \frac{1}{2}-2z = 344/1200$

$z = 0.107.$

The following average values were obtained

$x = 0.125$ (0.12)

$y = 0.079$ (0.078)

$z = 0.107$ (0.106).

These compare very favourably with the values obtained in the two dimensional Patterson syntheses shown in parenthesis and were employed as the iodine parameters in the next calculation.

First Phasing Calculation

The heavy atom method[27] is most successful[29] when $\sum f_H^2 = \sum f_L^2$ ——(a) where f_H and f_L are the atomic scattering factors for the heavy atom and any other atoms respectively in the unit cell; the summation is over the total content of the unit cell.

In order to better estimate the degree of success that attended the first phasing calculation, it is reasonable to assume the accepted formula (see page 112) $C_{30}H_{38}O_8ClI$ instead of $C_{31}H_xO_{10}I$ which was thought to be the formula (x was unknown).

$$\text{At } \sin\theta = \text{zero}$$

$$\sum f_I^2 = 11236$$

$$\sum f_L^2 = 7676.$$

The excessive contribution of the iodine atom beyond what equation (a) requires, would make the diffraction effects arising from the "finite series" errors fairly serious. This necessarily makes difficult the location of atoms close to iodine. At low angles, the phasing is just dominated by the contributions from the heavy atoms. But at higher angles of diffraction, the phasing is entirely dominated by these contributions because the scattering factor for the heavy atom decreases much more slowly than the f-curves for the light elements present. Hence a good degree of agreement for the structure factors of reflections at high glancing angles may be expected with phasing based on iodine atoms only.

The structure factor is given by

$$F(hkl) = \sum_{n=1}^{N} f_n \exp\left\{ 2\pi i\,(hx_n + ky_n + lz_n)\right\}$$

$$= A + iB$$

where

$$A = \sum_{n=1}^{N} f_n \operatorname{Cos}\left\{ 2\pi\,(hx_n + ky_n + lz_n)\right\}$$

$$B = \sum_{n=1}^{N} f_n \operatorname{Sin}\left\{ 2\pi\,(hx_n + ky_n + lz_n)\right\}$$

the summation being taken over all N atoms in the unit cell

$$\tan\alpha\,(hkl) = \frac{B(hkl)}{A(hkl)}$$

The substitution of the co-ordinates of the equivalent general positions gives

$$A(hkl) = \sum_{n=1}^{N/4} A'(hkl)\; f_n \exp\,(-2B \operatorname{Sin}^2 \theta/\lambda^2)$$

$$B(hkl) = \sum_{n=1}^{N/4} B'(hkl)\; f_n \exp\,(-2B \operatorname{Sin}^2 \theta/\lambda^2)$$

where $A'(hkl) = 4 \operatorname{Cos} 2\pi(hx_n - \frac{h-k}{4}) \operatorname{Cos} 2\pi(ky_n - \frac{k-l}{4}).\ \operatorname{Cos} 2\pi(lz_n - \frac{l-h}{4})$

$$B'(hkl) = -4 \operatorname{Sin} 2\pi(hx_n - \frac{h-k}{4}) \operatorname{Sin} 2\pi(ky_n - \frac{k-l}{4}) \operatorname{Sin} 2\pi(lz_n - \frac{l-h}{4})$$

the summation being over ~~atoms~~ the N/4 atoms in the asymmetric unit only.

Further simplification occurs when the reflection are divided into classes. Here, the expressions used for A' and B' become:

In addition to the results shown in Table 12, the structure factor programme types out the co-ordinates of the given atom or atoms, the total number of reflections, R or Reliability factor, the sum of F(OBS) and $|F(CALC)|$ and the sum of DELTA.

DELTA is the absolute value of the difference between $|F(OBS)|$ and $|F(CALC)|$ and R-factor is given by

$$R = \frac{\sum \left| |F(OBS)| - |F(CALC)| \right|}{\sum |F(OBS)|}$$

$$A(OBS) = F(OBS) \cos \alpha$$

$$B(OBS) = F(OBS) \sin \alpha$$

SCALE FACTOR = 1.360.

TABLE 12

H	K	L	FOBS	ACALC	BCALC	FCALC	DELTA	AOBS	BOBS
0	1	11	2.5	0.0	2.1	2.1	0.4	0.0	2.5
0	6	6	5.7	7.5	0.0	7.5	2.2	5.7	0.0
0	6	8	4.0	-6.5	0.0	6.5	2.5	4.0	0.0
0	6	10	8.7	-8.0	0.0	8.0	0.7	-8.7	0.0
0	9	9	8.0	0.0	-7.0	7.0	1.0	0.0	-8.0
0	9	11	2.5	0.0	-2.6	2.6	-0.1	0.0	-2.5
0	12	4	5.7	-5.8	0.0	5.8	-0.1	-5.7	0.0
0	12	8	2.7	3.3	0.0	3.3	-0.6	2.7	0.0
0	14	6	3.2	-2.2	0.0	2.2	1.0	-3.2	0.0
0	1	8	10.6	0.0	-9.3	9.3	1.3	0.0	-10.6
0	1	10	5.6	0.0	4.3	4.3	1.4	0.0	5.6
0	1	12	7.3	0.0	7.8	7.8	-0.5	0.0	7.3
0	10	7	5.2	-7.3	0.0	7.3	-2.1	-5.2	0.0
0	10	13	3.1	2.7	0.0	2.7	0.4	3.1	0.0
0	14	3	2.7	-3.0	0.0	3.0	-0.3	-2.7	0.0

The results of table 12, showing a considerable degree of agreement indicated fairly good position parameters for iodine. The reflections given in this table are the same as those used in testing the parameters obtained from the two dimensional syntheses. The reliability factor of 0.373 was considered satisfactory. As expected, the low angle reflections showed the highest disagreement.

First Three Dimensional Fourier Summation

The electron density at any point (x, y, z) is given by

$$\rho(x\ y\ z) = \frac{1}{V} \sum_{h} \overset{\infty}{\underset{-\infty}{\sum_{k}}} \sum_{l} F(hkl) \exp\left\{-2\pi i(hx + ky + lz)\right\}$$

$$= \frac{1}{V} \sum_{h} \overset{\infty}{\underset{-\infty}{\sum_{k}}} \sum_{l} F(hkl) \cos\left\{2\pi(hx + ky + lz) - \pi(hkl)\right\} \qquad \text{........ (F.1)}$$

For space group $P2_1\ 2_1\ 2_1$

$$F(hkl) = F(\bar{h}\bar{k}\bar{l})$$
$$= F(\bar{h}kl)$$
$$= F(h\bar{k}l)$$
$$= F(hk\bar{l})$$

And the phase angles are related thus for the following classes of reflections

(1) for $h + k = 2n$

$k + l = 2n$

$$\alpha(hkl) = -\alpha(\bar{h}\bar{k}\bar{l})$$
$$= -\alpha(\bar{h}kl)$$
$$= -\alpha(h\bar{k}l)$$
$$= -\alpha(hk\bar{l})$$

(2) for $h + k = 2n$

$k + l = 2n + 1$

$$\alpha(hkl) = -\alpha(\bar{h}\bar{k}\bar{l})$$
$$= -\alpha(\bar{h}kl)$$
$$= \pi - \alpha(h\bar{k}l)$$
$$= \pi - \alpha(hk\bar{l})$$

(3) for $h + k = 2n + 1$

$$k + l = 2n$$

$$\begin{aligned} \alpha(hkl) &= -\alpha(\bar{h}\bar{k}\bar{l}) \\ &= \pi - \alpha(\bar{h}kl) \\ &= -\alpha(h\bar{k}l) \\ &= \pi - \alpha(hk\bar{l}) \end{aligned}$$

(4) for $h + k = 2n + 1$

$$k + l = 2n + 1$$

$$\begin{aligned} \alpha(hkl) &= -\alpha(\bar{h}\bar{k}\bar{l}) \\ &= \pi - \alpha(\bar{h}kl) \\ &= \pi - \alpha(h\bar{k}l) \\ &= -\alpha(hk\bar{l}). \end{aligned}$$

For this space group equation (F.1) becomes

$$\rho(xyz) = \frac{8}{V}\Big[\,CCCSSS - CSSSCC - SCSCSC - SSCCCS\,\Big] \qquad \text{.... (F.2)}$$

where CCCSSS

$$= \sum \sum_{0}^{\infty} \sum |F(hkl)| \Big[\cos 2\pi hx \cos 2\pi ky \cos 2\pi lz \cos\alpha(hkl) - \sin 2\pi hx \sin \pi ky \sin 2\pi lz \sin\alpha(hkl)\Big]$$

for reflections with $h + k = 2n$, $k + l = 2n$,

CSSSCC

$$= \sum \sum_{0}^{\infty} \sum |F(hkl)| \Big[\cos 2\pi hx \sin 2\pi ky \sin 2\pi lz \cos\alpha(hkl) - \sin 2\pi hx \cos 2\pi ky \cos 2\pi lz \sin\alpha(hkl)\Big]$$

for reflections with $h + k = 2n$, $k + l = 2n + 1$,

SCSCSC

$$= \sum \sum_{0}^{\infty} \sum |F(hkl)| \Big[\sin 2\pi hx \cos 2\pi ky \sin 2\pi lz \cos\alpha(hkl) - \cos 2\pi hx \sin 2\pi ky \cos 2\pi lz \sin\alpha(hkl)\Big]$$

for reflections with h + k = 2n + 1, k + l = 2n,

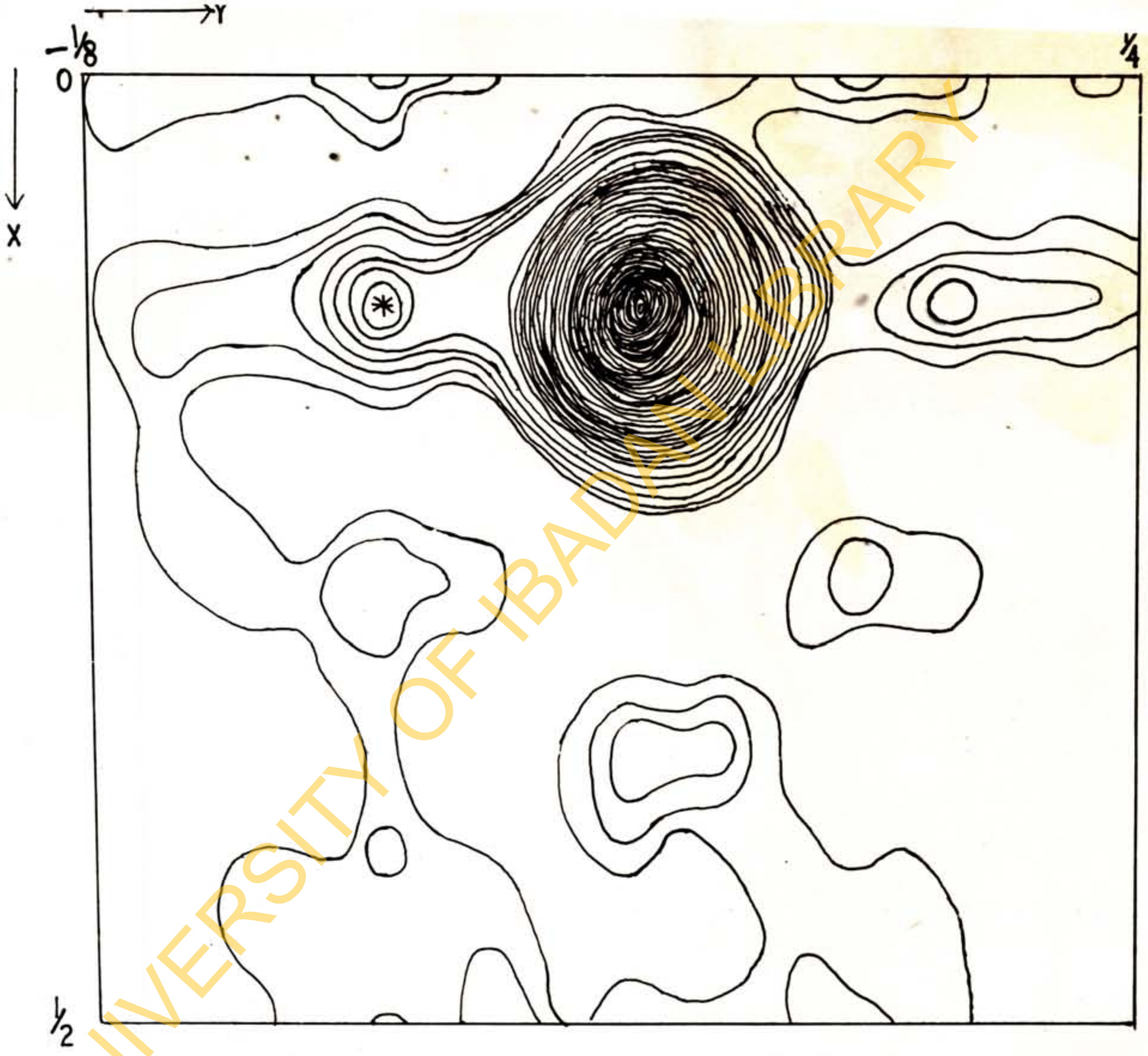
$$SSCCCS = \sum \sum_{0}^{\infty} \sum |F(hkl)| \Big[\text{Sin } 2\pi hx \text{ Sin } 2\pi ky \text{ Cos } 2\pi lz \text{ Cos } \alpha(hkl) - \text{Cos } 2\pi hx \text{ Cos } 2\pi ky \text{ Sin } 2\pi lz \text{ Sin } \alpha(hkl) \Big].$$

The approximate electron density disbritution $\rho(xyz)$ of equation (F.2) was computed at intervals of 1/60 allowing for the lower multiplicities of reflections with zero indices and leaving out the F(000) term. The results were on an arbitrary scale but were satisfactory for the purposes of locating atomic centres. They were plotted on tracing paper in sheets of constant (z) on a scale of 4 cm to 1Å at intervals of 1/60 from zero to $z = \frac{1}{2}$.

There were a number of peaks one of which was the highest, around the iodine atom. This high peak starred in fig. 18 was 2.1 A° from iodine and it was considered the carbon bonded to the iodine. The second highest peak starred in fig. 19 was also far above the others but rather isolated and difficult to imagine linked to any nearby atom.

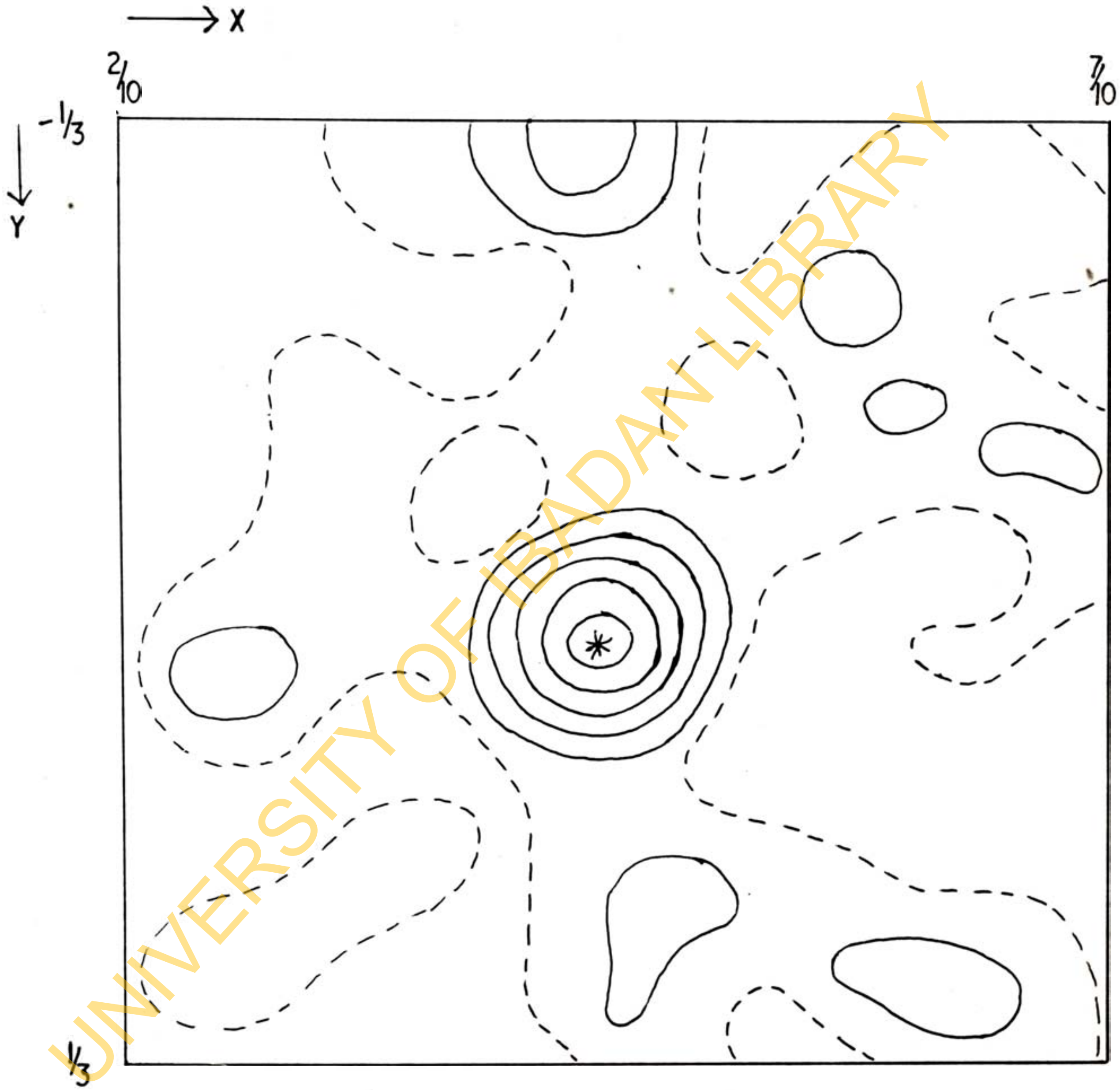
Fig. 20 illustrates the random distribution of electron density with two starred points representing peaks on this plane whilst the positions marked with a cross are shadows of atomic peaks slightly distant from this plane.

In all, the co-ordinates of 34 peaks shown in fig. 21 were determined. Out of these, the highest 15 were treated as carbon atoms and included in the next structure factor calculation. The final results show that



$z = 1/10 \quad \rho_1$

Figure 18



$z = 1/15 \quad \rho_1$

Figure 19

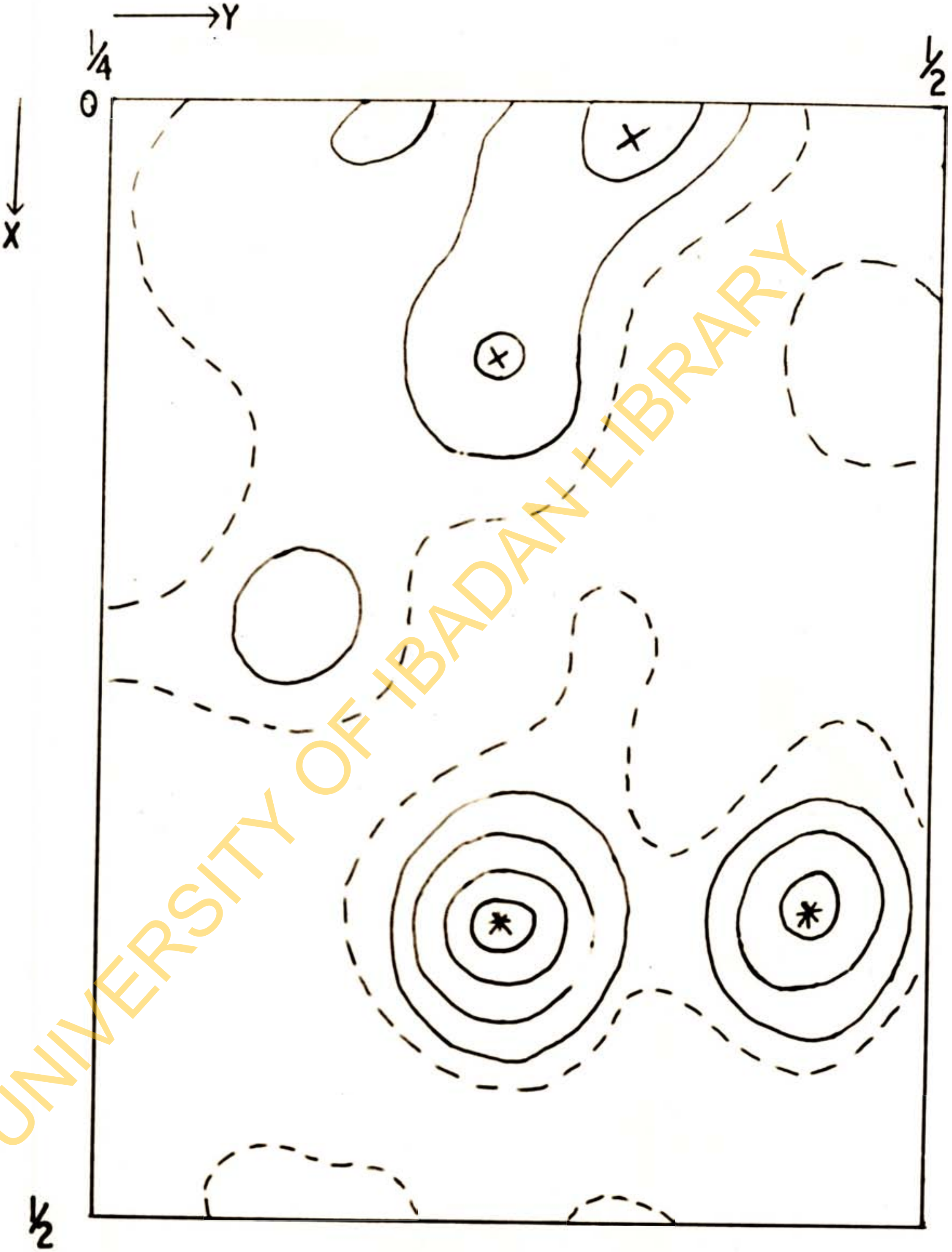


Figure 20

$z = 1/10 \quad \rho_1$

Peak centres from the ~~second~~ 1ST Fo Fourier

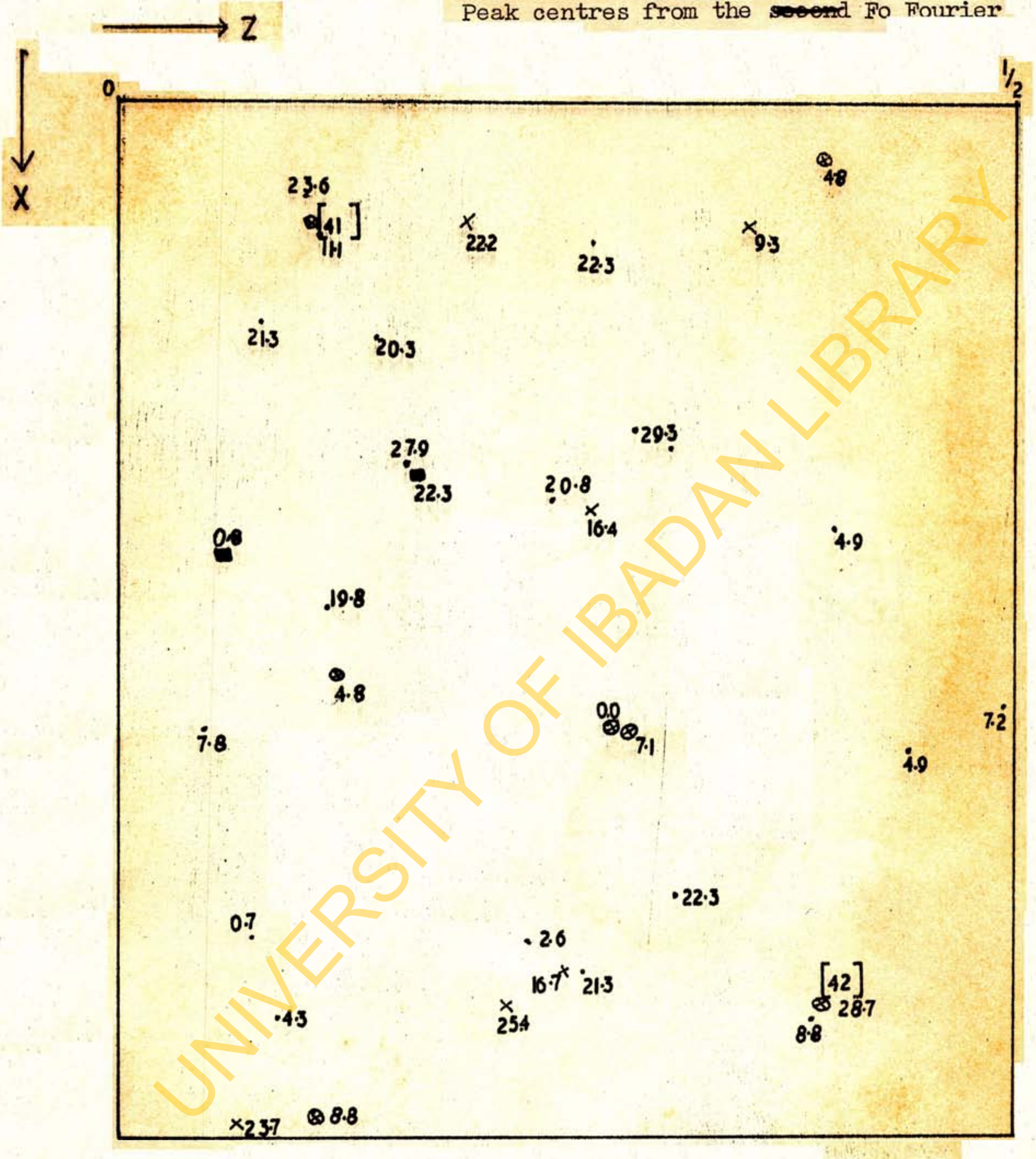


Figure 21

■ Inserted under 0.1Å from correct position.

× Inserted 0.1 to 0.4Å from correct position.

⊗ More than 0.4Å away or spurious peaks inserted.

Other peaks (other peaks from not inserted)

(Figures 21 - 23)

as coefficients combined with the appropriate phase angles. The unobserved reflections may be included in the calculations and their co-efficients may be treated in one of the following ways:

(1) They may be completely ignored. In this instance, only the differences for the 991 measurable reflections are used. The remaining three involves including approximate values - usually half the threshold, for these reflections. They can be given a scale factor in three ways.

(2) The structure factors of these unobserved reflections are given the same scale factor as for the 991 measurable reflections and their difference coefficients are included in the calculations.

(3) The structure factors are given a scale factor based on equalizing the sums of their own F(OBS) and F(CALC), and adding their difference coefficients to those 991 above.

(4) These structure factors are scaled together with the 991 observed structure factors so that $\sum Fo = \sum Fc$.

The first "difference synthesis" was calculated with method (1) using the results of the second phasing involving iodine and 15 supposed carbon atoms.

The "difference synthesis" was originally put forward as a means of attaining further refinement where the structure was partially known. A variation of this, named the "error synthesis" was used by Bunn to look for atoms wrongly placed and where they should rightly be placed, in the structure determination of sodium benzyl penicillin[31]. Later, Cochran giving it the name "Difference Synthesis" showed that it could be used also to find the correction Δx_n for the x-coordinate of the n^{th} atom

$$x_n = -\left(\frac{\partial D}{\partial x}\right)_n \Big/ \left(\frac{\partial^2 \rho_c}{\partial x^2}\right)_n \quad \ldots\ldots\ldots\ldots \text{(i)}$$

where $\frac{\partial D}{\partial x}$ is the slope of the electron density at the point x in the difference synthesis.

Assuming[32] that $\rho_c = \rho_o$; $\left(\frac{\partial^2 \rho_c}{\partial x^2}\right)_n$

can be replaced by $\left(\frac{\partial^2 \rho_o}{\partial x^2}\right)_n$,

which can be determined from the F(OBS) synthesis. Using the approximations of Costain[33] and Booth[34] that

$$\rho = Z \left(\frac{p}{\pi}\right)^{3/2} . \exp(-\ pr^2)$$

is the electron density near the centre of an atom where Z is the atomic number of the atom, r the distance to the centre of the atom and p is a constant, eqn (i) above simplifies to

$$\Delta x_n = \left(\frac{\partial D}{\partial x}\right)_n \Big/ \ 2P(\rho_o)_n \quad \ldots\ldots\ldots \text{(ii)}$$

P has been reported[32,34,35] constant for some light atoms and is usually given the value of about 5.0 in practice[36]. It can however, be obtained from a graph of log ρ against r^2 and it depends on the temperature factor of the atom concerned, its value is also affected by the point at which the Fourier series for ρ_o is terminated.

In this analysis, the difference syntheses are used to find

(i) changes in atomic and temperature parameters for correctly placed atoms

(ii) wrongly placed atoms indicated by relatively high negative peaks at supposed atomic positions and

(iii) atomic positions not yet known, indicated by highly positive peaks.

Two of the atoms inserted marked (41) and (42) in fig. 21 had strongly negative peaks of 4.3 e/Å^3 and 4.32 e/Å^3 respectively and were eliminated as spurious. The rather isolated peak - in ρ_1 (fig. 19), starred in table 13, which had the highest density, beside iodine, of 8.5 e/Å^3 in the second Fo Fourier summation, had a positive density of 2.3 e/Å^3 in this difference synthesis. Its temperature factor was decreased to 4.0 Å^2. The iodine peak had - 5.8 e/Å^3 suggesting a higher temperature factor than that given. Its temperature factor was increased from 4.0 to 4.2 Å^2. The remaining 14 atoms were shifted according to their density gradients along each axial direction, using ρ_o obtained in the ~~last~~ 2nd Fourier in equation (ii) above. For example, iodine had $\Delta x = 0.0004$, $\Delta y = 0.0002$, $\Delta z = 0.0$ Their temperature factors were also adjusted in such a way as to make the peak densities in the difference synthesis approach zero. Trueblood[7] recommends the introduction of difference synthesis into structure determination as early as possible. This is mainly to guide against using positions of diffraction ripples and local density maxima in the phasing and Fourier calculations. Lipson and Cochran[36] hold the view that while the difference synthesis is not free from errors inherent in the Fo synthesis, it gives a greater accuracy in the sense that the point of maximum electron density is precisely located without recourse to interpolation.

On the assumption of a greater accuracy from difference synthesis, parameters of peaks corresponding to those previously sorted out from the two Fo syntheses were determined. There were 17 such peaks, the highest being 2.3 e/Å^3 and the lowest 1.1 e/Å^3. If all these were atoms, then all other peaks above 1.1 e/Å^3 could also be atoms. There were 17 new peaks with densities higher than 1.1 e/Å^3, the highest being 2.0 e/Å^3. The eleven highest of these were selected. The total number of atoms at this stage was 42. The lowest of the latest peaks was 1.27 e/Å^3. The main use of the second Fo Fourier summation was reduced to finding the ρ_o value to be used in calculating Δx_n etc. as above.

In preference to the atomic co-ordinates obtained from this second Fo 'Fourier', those obtained from the 'Difference' were used in the next phase calculation. Final parameters show (fig. 22) that five of these atoms were well placed, being less than 0.1 Å from their correct positions; eleven others were 0.1 to 0.4 Å from their correct positions whilst 18 were 0.4 to 1.0 Å off the mark. Others were spurious peaks.

Third Phasing and First Model

Another set of structure factors was calculated with iodine and 41 other atoms which were assumed to be carbon. All the newly inserted atoms were given a temperature factor of 4.5 Å^2. For some reason, the structure factor programme would not work for reflection, (4 3 12) and so the calculations were done for only 990 of the measurable reflections. The scale factor was 1.620 and the Reliability factor became 0.257. The reflections having differences between FOBS and FCALC of 3.0 and above reduced to 87 out of which 25 were above 5.0. The highest difference of 14.5 was for (0, 6, 0).

The Reliability factor of 0.257 was low enough to try the construction of a model to see what features of the structure could be got out of the present position parameters for the 42 atoms. The first model was made up of small wooden balls hanging on threads suspended from a cork mat. The x and y axes were in the plane of the mat and the z axis was perpendicular to this plane. Thus the x and y co-ordinates of an atom were fixed by the point of attachment of the thread to the mat and the z co-ordinate was determined by the length of the thread. The scale of the model was 4 cm to 1Å.

The most noticeable and disappointing feature in this model was the presence of three "atoms" around the iodine atom all within a distance of about 2.0 to 2.5 Å. From all considerations, this is an impossible situation and some of the supposed atoms must be due to diffraction ripples around the iodine. One of them "atom 43", table 13, had a peak height of only 1.28 $e/\text{Å}^3$ in the difference map and it seemed most probable that it was due to the diffraction ripples around the iodine. It was eliminated. A second, "atom 44" had a peak height of 2.03 $e/\text{Å}^3$, and was about 2.01 Å from the iodine. This is only slightly less than the normal carbon iodine bond distance[37], (2.08). But considering that the carbon atom bonded to the iodine must also be bonded to C(27), the C(27) - C(44) - I bond angle would be acute. The third atom, 28, 42 peak centres got from the first difference synthesis

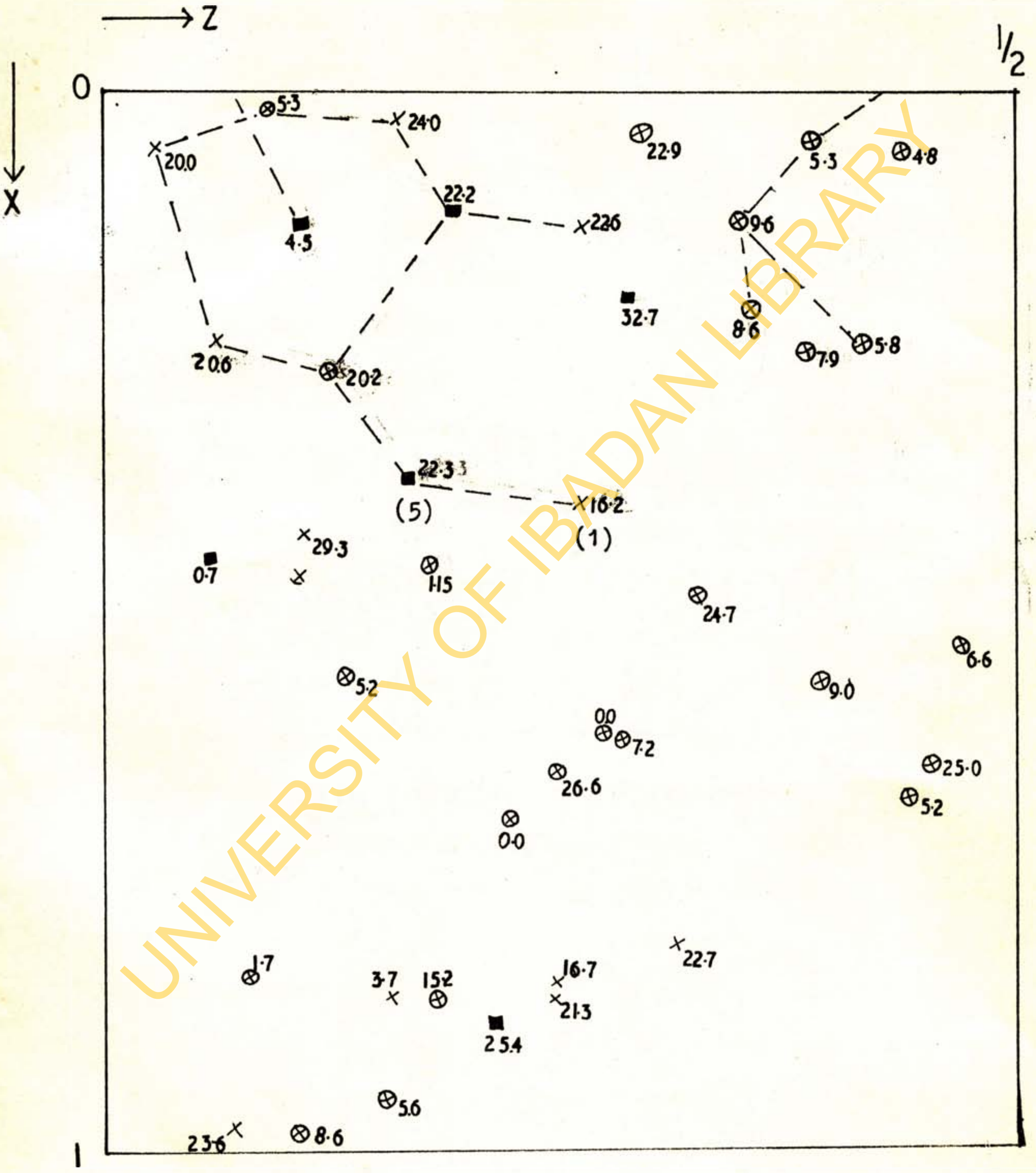


Figure 22

had been sited in the first and second Fo Fourier summations. There was no definite indication in the difference map that it had been wrongly placed. The corresponding C(27) - C(28) - I angle was much more acceptable. A movement of this atom in the direction of the other peak ("atom" 44) would make for a still better bond angle. On these grounds, it was considered reasonable to eliminate "atom" 44 and move atom 28 in the direction of "atom" 44. The bond distance between iodine and "atom" 28 was about 2.1 Å and this was kept constant when the bond angle changed. Having got an iodine-carbon link, the stereochemical implications of the neighbouring atoms were carefully considered. Assuming an approximate bond length of between 1.3 to 1.5 Å for all other bonds, a tetrahearal angle of 109° or trigonal angle of 120° around carbon atoms, it was possible to make out an iodoacetyl radical. This meant the conversion of two of the supposed carbon atoms (32 and 33) to oxygen.

The peak height of atom 39 was persistently above the others, excepting iodine, suggesting that it was very probably oxygen. It was rather isolated and did not make sense stereochemically. It was therefore left on as carbon for the time being.

The lowest density of 1.27 e/Å^3 ("aton" 45) was within a distance of 1Å from two other atoms (34 and 46). The three atoms were considered for an epoxide ring which was suggested from the assumed similarity to gedunin, (Page 3). They were rather isolated and to some degree it seemed plausible to connect the ring with the rest of the molecule through a peak of 1.2 e/Å^3 at about 2.0 Å from "atom" 45. But a decrease of the distance between this peak and "atom" 45 implied a decrease of the distance between "atom" 45 and atoms 24 and 46. Since the peak height of "atom" 45 was the lowest, it was suspected to be spurious and eliminated.

Another peak, 47, of height 1.9 e/Å^3, 0.9 Å from atom 23, was eliminated on the grounds that it was part of the gradient through atom 23, which indicated the direction in which this atom was to be moved.

It was difficult to make atomic shifts in space with this 'mat and string' model and another model was constructed with the remaining 38 atoms. This was made by the method of Carlisle and Crowfoot[38]. It consists of metal spokes stuck into the cork mat at the appropriate yz positions with short lengths of sleeving at the height of the x-coordinate. The scale was reduced to 2 cm to I Å and it represented the section of the unit cell from x=0 to 1, y=0 to $\frac{1}{2}$ and z=0 to $\frac{1}{2}$. This gave small sections of the molecule from which it was possible to recognise the iodoacetyl radical, (fig. 22) a six-membered ring, 4 connected atoms which presumably constituted part of the molecule containing the enolic - OMe. An enolic - OMe had been suggested[39] for one of the three methoxy groups. Atom 39, the presence of which was never in doubt, was still isolated. The model gave bond distances between atoms 4 - 20, 20 - 34, 36 - 48, 48 - 39, 39 - 49, 36 - 50, of the order of 2.0 Å. Apart from this, the distribution of atoms around atoms 34, 7, 1 and 20 did not reflect the tetrahedral nature of carbon. It was therefore strongly felt that many of these atoms should be removed and only those that have shown fairly sensible linkages with other atoms should be left. Implementing this decision would affect atom 39. This atom had shown fairly spherical electron density and had given a positive electron density even in the difference synthesis. The model was adjusted to make it possible to try all equivalent positions for each atom. Atom 39 and a few others which had been selected from the iodine phased Fourier, could not be satisfactorily placed. It was therefore decided to plot another difference map based on the phasing with all the remaining 38 atoms.

Fourth Phasing and Second Difference Synthesis

As a result of defficiencies that are inevitably present in any Fourier synthesis, it is sometimes difficult to decide which atoms are least well represented. It is therefore not easy to decide what changes to make as regards real and spurious peaks. To overcome this difficulty, Bunn suggested the difference synthesis which makes particular use of of the reflections that are of negligible observed intensities and fairly high calculated structure amplitudes (Error Synthesis[31]). Bunn also showed by calculation that for a centro-symmetric structure, it is advisable to omit those reflections with strong observed structure amplitudes and weak calculated structure amplitudes. This applies even for non-centrosymmetric structures because of the uncertainty in the phase angle.

To calculate the difference synthesis by method (1) (page 51) above without including the 312 unobserved reflections, implies neglecting many of the finer features of the structure which this synthesis is designed to bring out. While the result would not be entirely wrong, it is not quite correct, as assumed, that atomic centres brought out in such a synthesis would be more accurate than those obtained from a similar Fo synthesis. Besides at this stage of the phasing when many phase angles were likely to be incorrect, more accurate atomic centres from the difference synthesis is not probable. The synthesis at this stage and with measurable reflections only, has been quite useful in detecting spurious peaks as advocated by Trueblood[7]. The final position parameters show that most atomic centres obtained from this synthesis are 0.4 to 1.0 Å from the correct positions. Subsequent difference syntheses included contributions from the unobserved reflections.

The structure factors were calculated for the fourth time with the parameters for iodine, two oxygen atoms and 35 other atoms all assumed to be carbon. Two of the atoms inserted in the first difference summation had been eliminated and the remaining fourteen had different temperature factors derived from the first difference. All other atoms had a temperature parameter of 4.5 $Å^2$. It was possible to calculate for reflection (4 3 12) this time and so there were 991 measurable reflections. The scale factor was 1.600 and the reliability index reduced very little to 0.251. Like the last structure factor calculation, there were 87 differences of F(OBS) and F(CALC) from 3.0 and above. Out of this, 24 were above 5.0. The highest difference of 10.8 was for (0 6 0).

The phase angles and structure factors were then separately calculated for the 312 unobserved reflections. They were mistakenly assumed to have zero observed structure amplitudes and together with the 991 measurable reflections, they were used in calculating a difference synthesis. The results show greater negative densities than before. The iodine had a height of -7.38 e/Å^3 and there were twelve other atoms with negative densities of 1.0 e/Å^3 and above. They were all eliminated in the next calculations, but four of them came out again later as atoms, (table 13). One of the eliminated atoms was the one (atom 28) supposed to be connected to iodine. It was at the peak of a negative density of 2.49 e/Å^3. The final position of this atom is about 0.6 Å from this position. Atom 39 came out with the highest positive density of 2.90 e/Å^3 and in spite of a lack of understanding of the stereochemical implication of its position, it was decided to regard it as an oxygen atom.

One of the two oxygen atoms (33) put in, came out at a height of -1.81 e/Å^3 and was eliminated. It came out later at a distance of 0.5 Å away. Thus the 38 atoms were reduced to 26 in number, one oxygen atom and 11 carbons being eliminated. But atom 39 now regarded as oxygen still kept the number of oxygen atoms at 2.

Among the highest positive peaks, three, 1, 5, and 17 were found to have come out in the second Fo Fourier (table 13) at heights of 2.67, 2.7 and 2.2 e/Å^3. The last peak height was not recorded among the highest 33.

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They here had peaks of 2.8, 2.2 and 1.84 $e/\AA^3$ respectively, and were found in space at good bonding distances and angles with nearby atoms. The last of them provided a link between atom 39 and the rest of the molecule. The other two connected atoms 51 and 31, and atoms 51 and 8 respectively, (fig. 23). There were in addition three other peaks of height 1.81, 1.7 and 1.73 $e/\AA^3$ that were stereochemically well placed and these were added to make a total of 32 atoms. The co-ordinates and temperature constants of the first 26 atoms were adjusted according to their density gradients and peak heights bearing in mind the greater negative peaks resulting from zero F(OBS) values mistakenly assigned to 312 plane

Fifth Phasing

With the latest parameters for iodine two oxygen atoms and 29 carbon atoms, a set of structure factors was calculated. The scale factor was 1.424. The reflections having differences between $|F(OBS)|$ and $|F(CALC)|$ greater than 3.0 decreased from 87 to 69, out of which only 18 were above 5.0. The reflection 0 6 0, which had constantly showed the highest difference, improved, the difference being reduced to 6.3. But others had worsened and the highest difference of 9.6 was for 4 0 0. The reliability index rose from 0.251 to 0.253.

Although no appreciable fall in this factor had been expected, a rise, as small as it was, constituted a disappointment. Every atom had been carefully selected, with some justification for including it in the calculation. The highest negative density at atomic sites in

32 peak centres used in the 5th Phasing with the atom numbers of the last six added from the second difference synthesis in parentheses.

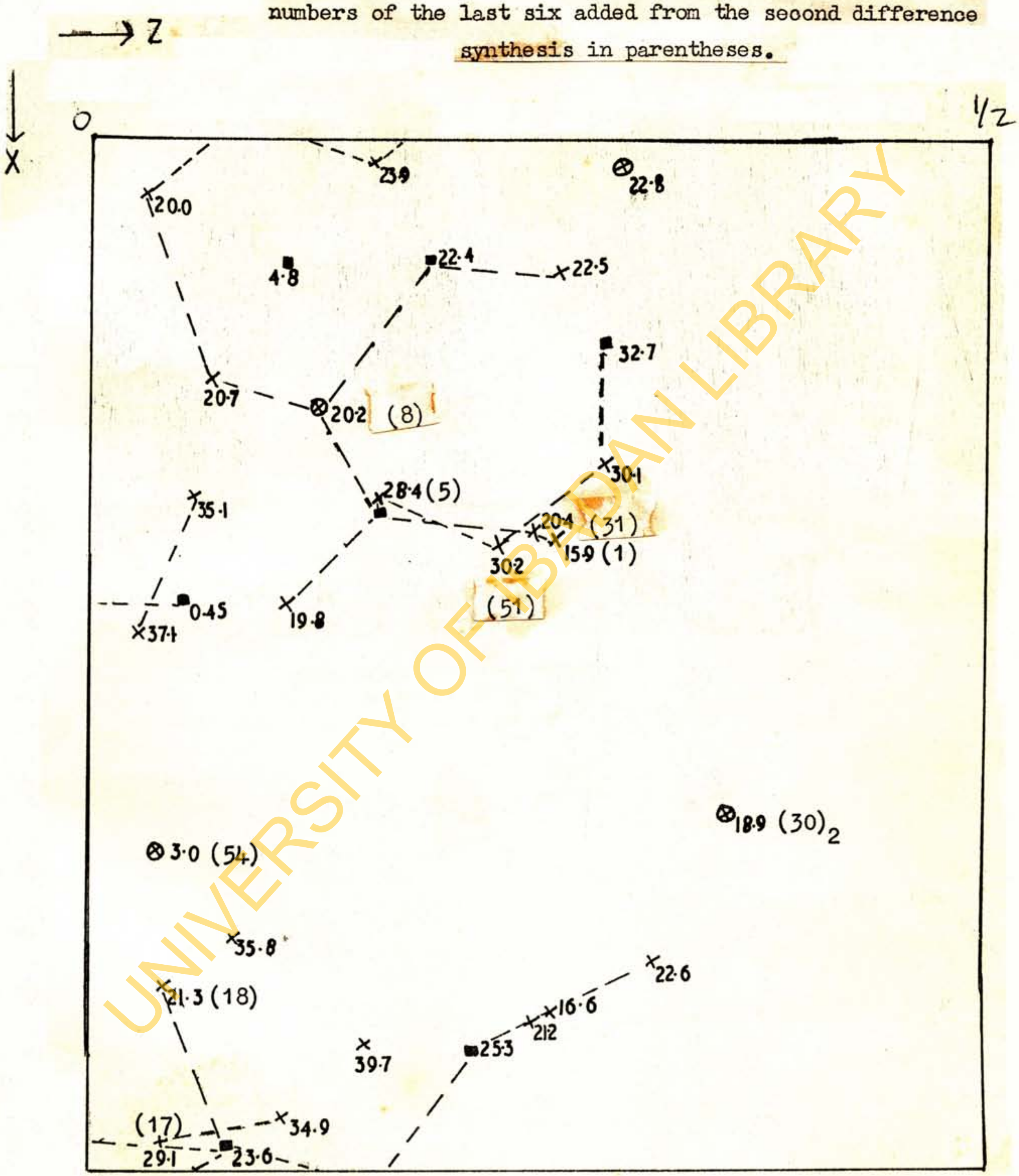


Figure 23

the difference, apart from iodine, was 0.79 e/Å^3. Since the density gradients of wrongly placed atoms affect the densities at the sites of well placed atoms, this negative density is not too high for a real atom bearing in mind the possible effects of temperature. At the end of the analysis, the atom with this peak height was in fact found to be rightly placed. However, two of these peaks were shown to be spurious.

A suggestion[40] that the second Fo synthesis had more in it than had been used, led to the construction of a plausible model with iodine and 31 other atoms. This model showed a six-membered ring but no other distinctive chemical entity. The position parameters in this model were used in a phase calculation. Two atoms, 32 and 39, were assumed to be oxygen atoms. There were varying temperature factors all in the neighbourhood of 4.5 Å^2. The resulting reliability index was 0.253. This was not better than the latest R-factor and it was decided to continue the analysis with the parameters used for the fifth phasing.

Third Fo Synthesis

The third approximate distribution of electron density was calculated with the scaled observed structure factors and calculated phase angles from the fifth phasing. The results were plotted on maps of constant z at intervals of 1/60 up to z = $\frac{1}{2}$. A new model was built to show the distribution of peaks in this synthesis.

It has been suggested[36] that if an atomic co-ordinate is taken as (x_n, y_n, z_n) in calculating phase angles, but the point of maximum electron density occurs at $(Xn + \Delta Xn$, etc.) in the subsequent electron density map, the process of refinement would be considerably speeded up by taking the centre of this atom to be at $(Xn + 2\Delta Xn$, etc.) when phase angles are recalculated. A shift of about four times that suggested by the change of peak centre has also been put[7] forward. In deciding new atomic co-ordinates from the contour maps, there is no definite rule. The shape of the contours around an incorrectly placed atom is often a guide to the magnitude of the shift required. These contours show a greater or lesser departure from spherical symmetry depending on the amount of displacement. An additional criterion for deciding the magnitude of the shift is the stereochemical considerations. While using the maps to decide the direction and probable magnitude of the shift of atomic centre, the model served to indicate whether such a movement would improve the bond distances and angles. Until there was certainty about the chemical nature of the atom and the type of bonding surrounding it, the bond distances except for the one involving iodine, were assumed to be about 1.5 Å.

For the iodine (atom 40), (fig. 24), the peak height of 44.0 e/Å^3, left no doubt about the singular nature of the atom. The contours were, however, not sufficiently spherically symmetrical. This departure from spherical symmetry of the contours for an evidently correctly placed atom is due to the anisotropy of its temperature vibration. Further, The contours around the iodine are at intervals of 5 e/A^3.

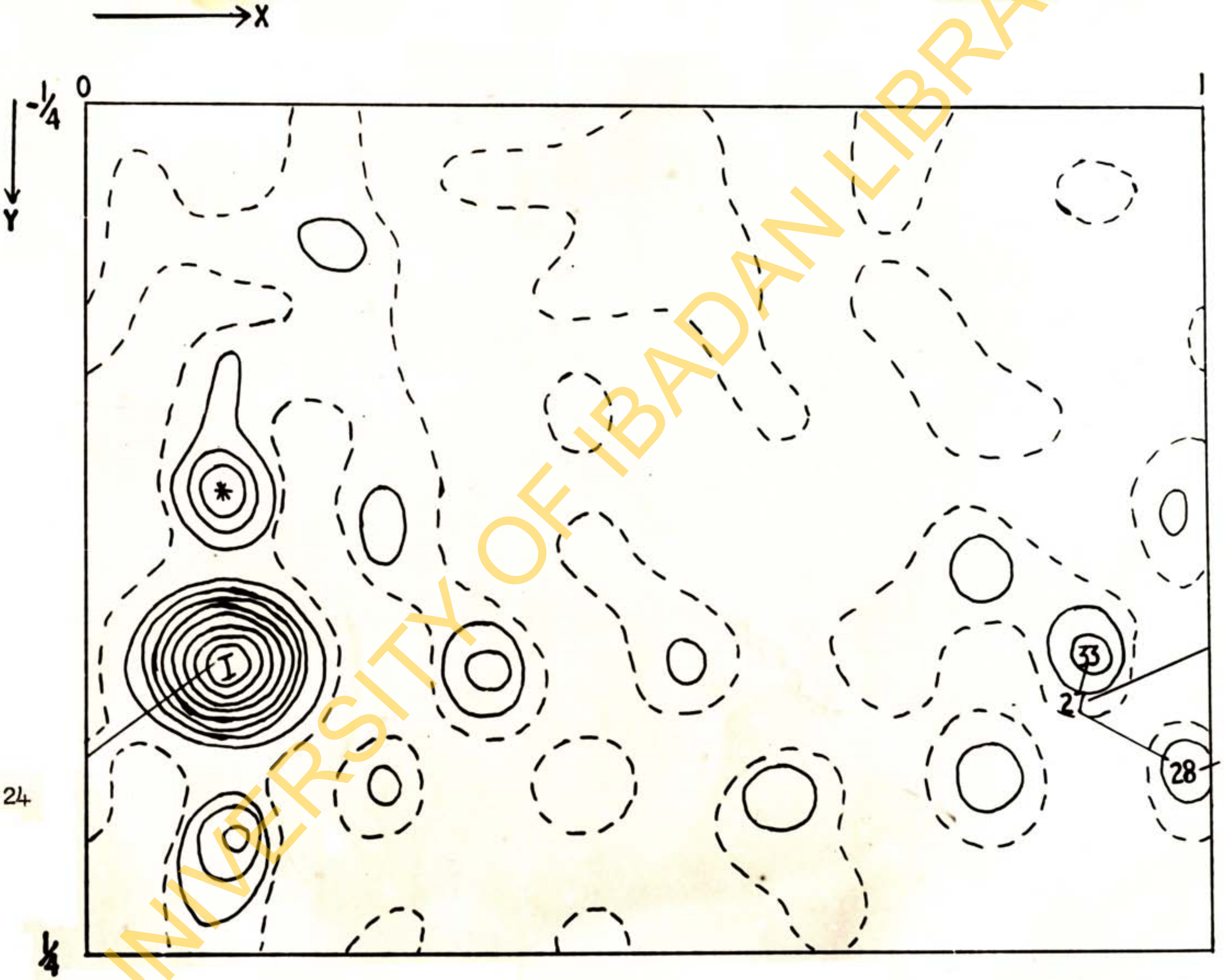
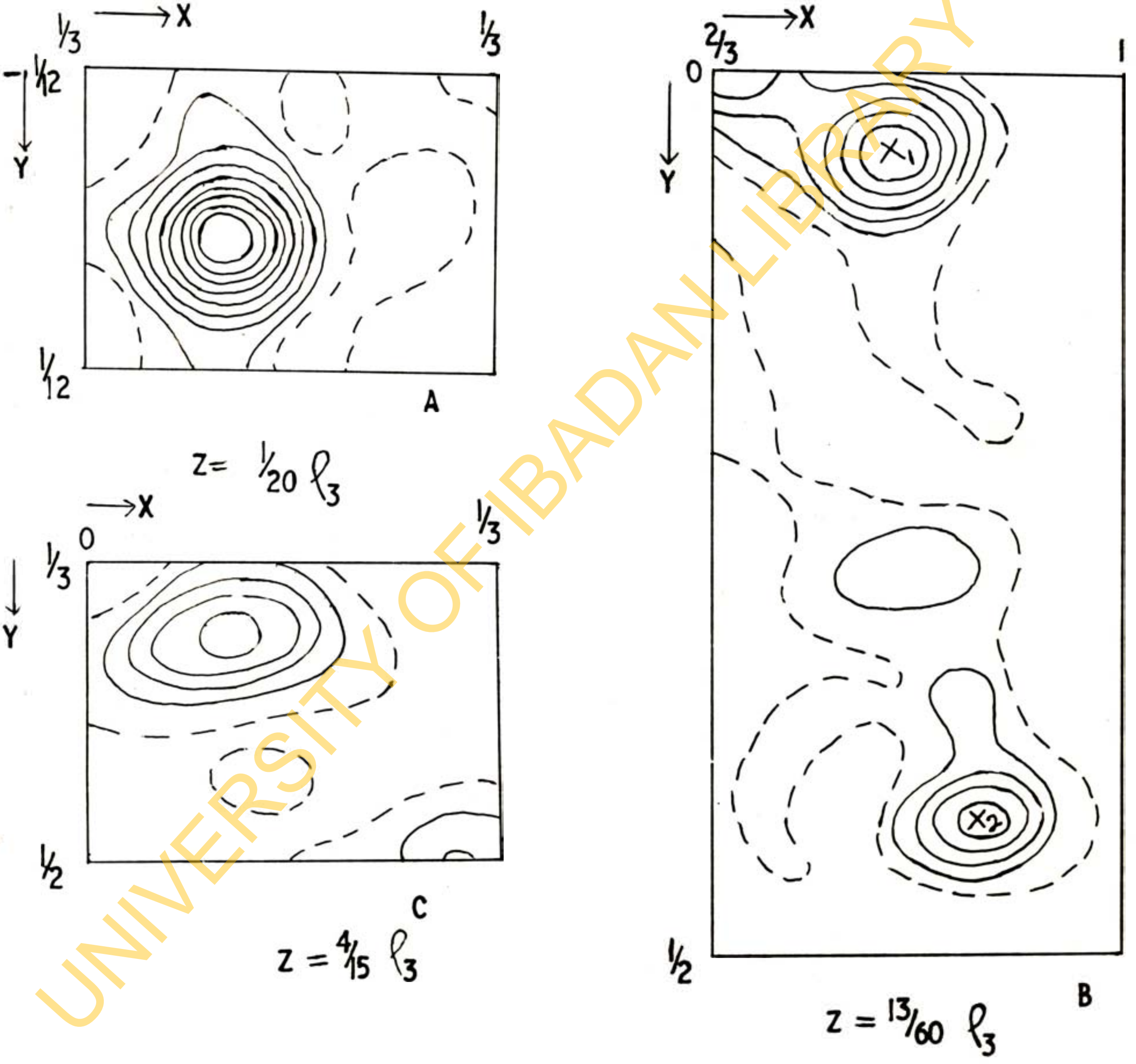


Figure 24

$z = 1/10$ ρ_3

there were, on opposite sides, parallel to the y-axis, spurious peaks of as high a density as 3.8 e/Å^3. One of these, starred in fig. 24, had the highest density apart from the iodine, in the 'Iodine phased' Fourier (fig. 18). It was shown to be false in the first difference synthesis. Parallel to the x-axis and on opposite sides of the iodine peak were two other spurious peaks of height 2.0 and 2.4 e/Å^3. The contours can only suggest a higher degree of vibration in the y-axial direction. These spurious peaks must be due to a combination of the effects of the diffraction ripples and the anisotropic temperature vibrations [41,42]. The iodine co-ordinates were therefore left unaltered.

Atom 39 (fig. 25A) now assumed to be oxygen still gave a distinctly higher peak of 8.9 e/Å^3 than all other atoms beside iodine. The second atom assumed oxygen, 32, marked XI in fig. 25B, had a peak height of 5.9 e/Å^3. Only a very small shift in position was indicated along the x direction. In the model, atom 22 (fig. 25C) was seen to be much too close to "atom" 53 (fig30) and farther than required from atom 8. There was no indication of a y or z directional change. From the shape of the contours, however, a shift in the x direction was a possibility. The x co-ordinate was therefore changed from 0.1246 to 0.1000 in order to improve the bond distances to 'atoms' 8 and 53. The final x-coordinate of atom 22 came out to be 0.1209. The misfortune of this particular shift is that 'atom' 53 was spurious and merely served to deceive at this stage.



1/3
X
1/3
1/12
Y
1/12
A
$Z = \frac{1}{20}\rho_3$
0
X
1/3
1/3
Y
1/2
C
$Z = \frac{4}{15}\rho_3$
2/3
X
1
0
Y
X_1
X_2
1/2
B
$Z = \frac{13}{60}\rho_3$

Figure 25

The shape of the contours around atom 36, marked X1 in Fig. 26A was not easy to explain. A close examination suggested very slight movements in the x and z directions only. The innermost contour line was symmetrical about the y direction but the outer lines were not. The deviation from spherical distribution was attributed to the anisotropy of the temperature vibration.

The lowest peak heights were those of atoms 18 (fig. 26B), 2.9 e/A^3 30 (fig. 26C) $2.\overset{e}{9}/A^3$ and 54 (marked X2 in fig. 26A) 2.6 e/A$^{\cdot 3}$. They were the last three just selected from the second difference synthesis. Since it is possible to introduce peaks, quite comparable in height with true atomic peaks, into the electron density distribution, by phasing with spurious "atoms", peak height by itself cannot be used as a criterion of the correctness or otherwise of a chosen site. Such peaks have been observed to have come out, however, lower than most true atomic peaks. These three peaks were therefore strongly suspected to be false. The contours of atom 18 (fig. 26B) were only slightly distorted and it could be an atom needing a shift. It was at a distance of 1.85 Å from another atom (13). In this position it would provide a fourth angular methyl group. Moreover, it was found to have come up as a peak in each of the previous Fourier calculations, being 0.8 3/Å^3 in the first Fo synthesis, 1.3 e/Å^3 in the second (Table 13). It was therefore included as a real atom with slightly changed coordinates and noted for further scrutiny.

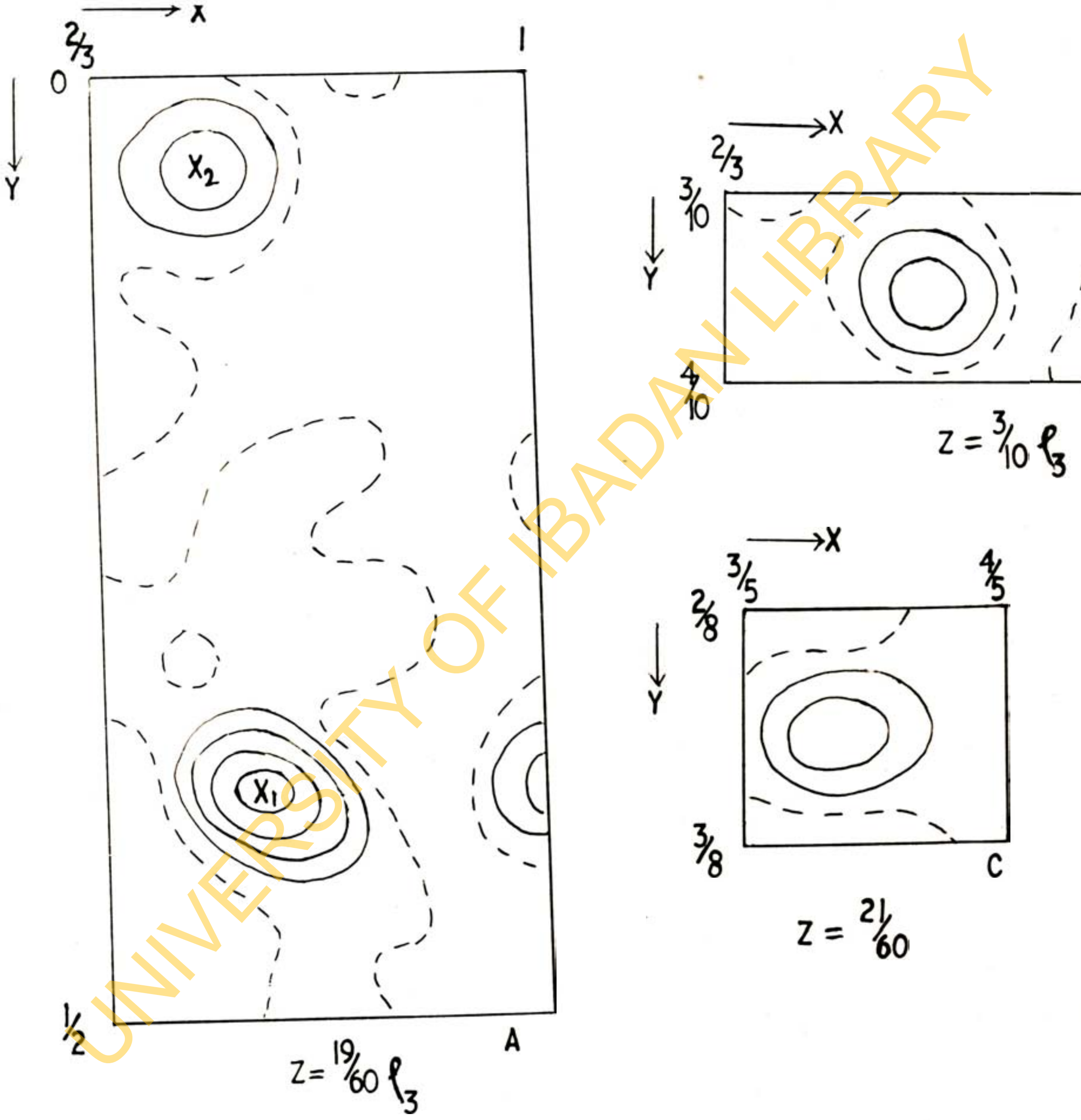

X
2/3
0
Y
1
X2
X1
1/2
A
Z = 19/60 ℓ3
X
2/3
3/10
Y
1
4/10
B
Z = 3/10 ℓ3
X
3/5
4/5
2/8
Y
3/8
C
Z = 21/60

Figure 26

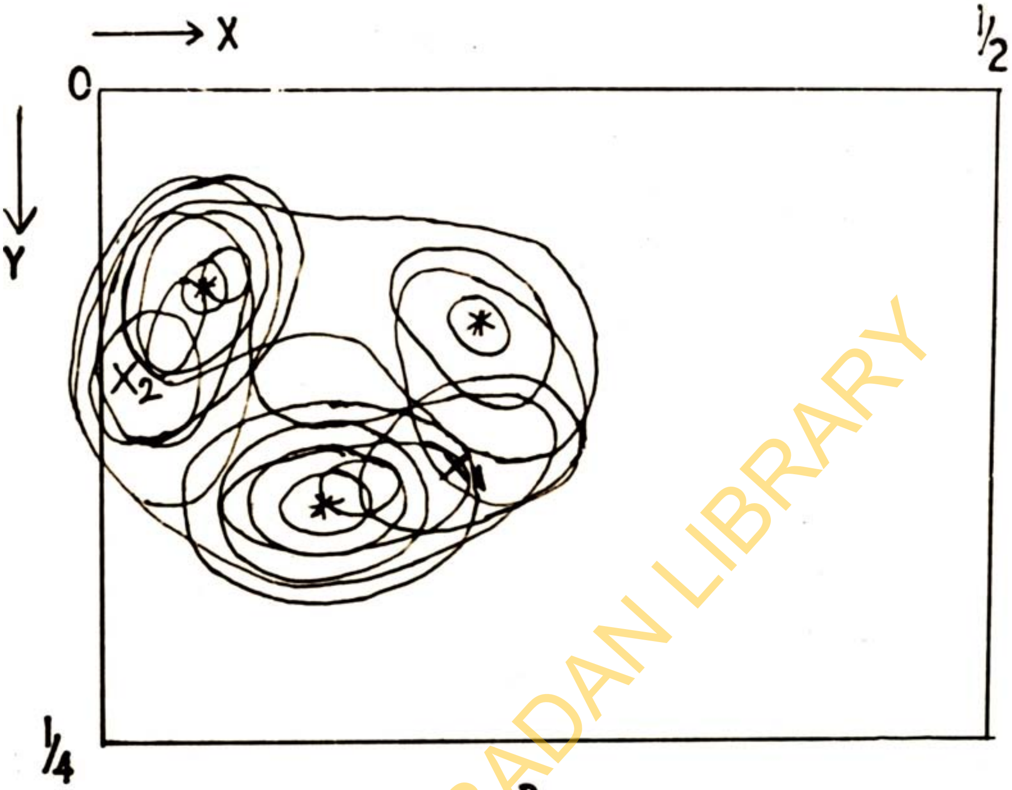
The contours for atom 30 (Fig. 26C) could be that of a real atom under the influence of anisotropic temperature vibration or that of an atom needing shift or just that of a local electron density maximum wrongly assumed to be an atom in phasing. It was difficult to take it for a fictitious peak because the model showed that it was only 1.6 Å from atom 36 but the bond angle so formed was slightly different from what was expected. In the previous calculations, it was a peak in each Fourier with 1.7 e/Å^3 in the second difference, 1.15 e/Å^3 in the first difference, 1.6 e/Å^3 in the second Fo synthesis and 1.0 e/Å^3 in the first Fo synthesis. (Table 13). It was also marked down for scrutiny and included as a real atom with adjusted coordinates in the next calculation.

'Atom' 54, marked X2 in Fig. 26 A, though not far below atoms 18 and 30, had the lowest peak height. Its co-ordinates showed no signs of change and the contours were reasonably spherically symmetrical. In the model, it was about 2.1 Å from the nearest atom, 5, and the bond angles at atom 5 involving atom 54, were quite good for sp^3 hybridization around carbon. In the previous calculations, it showed up as a peak in all but the first difference, where it was on a gradient. It had heights of 1.73, 0.65, 0.60 and 0.55 e/Å^3 in the second difference, first difference, second Fo Fourier, and first Fo Fourier respectively. This range of densities was rather low and it was therefore decided to leave it out.

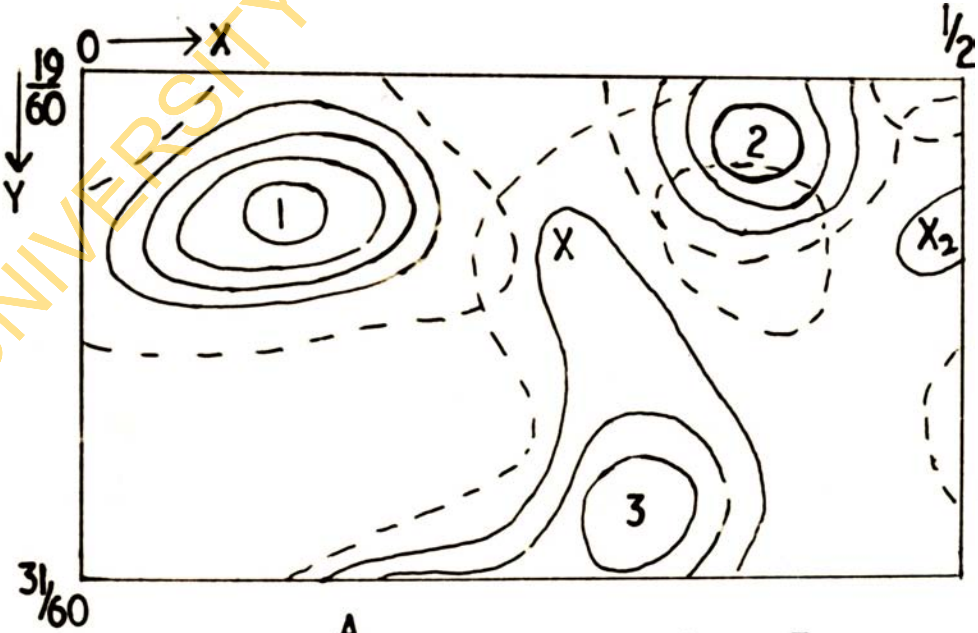
It is possible to calculate[43] the peak heights to be expected both for the atoms used in phasing and for those omitted at each stage. The results of such calculations predicts a gradual increase in peak heights for real atoms as the phasing improves from stage to stage. The results of most experimental work also give a gradual improvement in the relative peak heights of the omitted atoms as the phasing improves. Simultaneously, therefore, the relative heights of the spurious peaks are expected to decrease. However, there are sometimes departures from these generalisations. Some spurious peaks have been reported[47] to have improved their heights at the early stages and some real atomic peaks have shown little or no improvement at the later stages. Such atomic peaks, however, usually persist throughout the course of the structure analysis.

With these guiding principles more peaks were sorted out as atomic sites. The first, atom 2, marked X in fig. 27A, had a peak height of 1.7 e/A^3 and the contours suggested linkage to atoms 22, (marked 1 in fig. 27A), 1 (marked 2 in fig. 27A) and 3, (marked 3 in fig. 27A). But the distance from 3 was of the order of 2.0 Å and so it was not connected to this atom in the model. In the previous Fourier calculations, it had peaks of 1.1, 1.1, 1.5, 0.9 e/A^3 in the second difference, first difference, second Fo Fourier and first Fo Fourier respectively. In the model, it was stereochemically well placed, although the distance to atom 3 was rather long.

The next two peaks, selected, 25 and 26, were in the region of an unresolved density that later resolved into the atoms of the furan ring. By superposing the contour maps for Z = $\frac{21}{60}$, $\frac{22}{60}$, $\frac{23}{60}$, $\frac{24}{60}$, and $\frac{25}{60}$, one could immediately make out a ring as seen in fig. 27B.



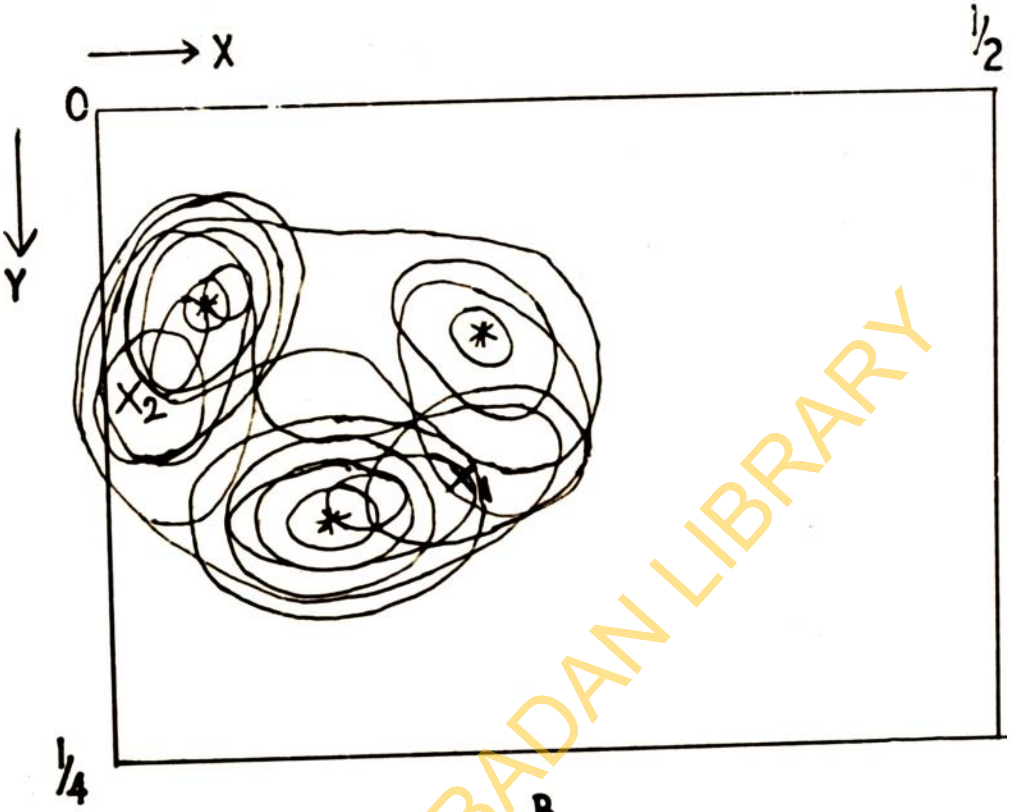
B



A

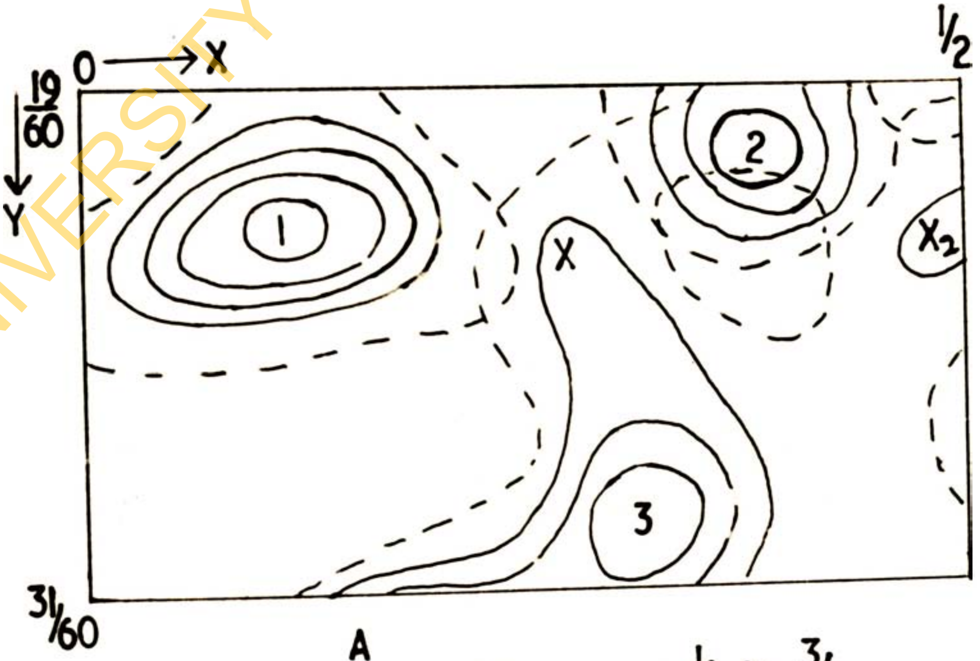
Figure 27

planes $Z = 1/4$ to $3/10$



B

Figure 27



A

planes Z = 1/4 to 3/10

The possibilities of this being a four-, five- or six-membered ring were examined. Three points starred in fig. 27B, had been selected and included in the last calculation as atoms 23, 24 and 38 on the basis of points of maximum electron density previously observed in this region. In the model they were too far off from one another to make chemical sense. The contours around each of them were all distorted. There was nothing to suggest that any of the three atoms was spurious. 23 and 38 had been sighted in the first Fo Fourier, and the two difference syntheses did not suggest the removal of any of them. Atom 24 sighted in the first difference, retained its identity in the second difference. From the positions of these three atoms, and the distribution of the density within the ring, it was not possible to select a fourth point to make a good four-membered ring. Also from the available chemical evidence, there was no four-membered ring. It was therefore considered reasonable to discard this idea.

In favour of a five membered ring, there was the chemical evidence for a furan ring which was reported[39] probably affected during the introduction of the chloroacetyl group. A new peak-25, of height 2.0 e/Å^3, marked XI in fig. 27B, was recognised about 1.1 Å away from both atoms 38 and 24. Since the contours of these two atoms make room for shifting the atomic centres, they were both moved away from position 25, to improve the bond distances. Another hitherto, unrecognised peak - 26, marked X2 in fig. 27B, of height 2.5 e/Å^3, was immediately obvious. It was at a distance of 1.25 A from atom 38 and 1.1 Å from atom 23. Atom 23 itself was only 1.5 Å away from atom 24 and its co-ordinates were therefore left unaltered. In the previous Fourier calculations atom 25 had heights of 1.1, 1.3, 2.4, 0.9 $e/\text{Å}^3$ in the second difference, first difference, second Fo Fourier, and first Fo Fourier respectively. These heights were on density gradients. Similarly atom 26 had heights of 1.65, 1.0, 3.0 and 1.5 $e/\text{Å}^3$ as above, (Table 13). The distribution of these peaks, 23, 24, 25, 26 and 38 was consistent with the presence of a FURAN RING.

The peak height of atom 38, 4.7 $e/\text{Å}^3$ was much higher than those of atoms 23 and 24, which were both 3.0 $e/\text{Å}^3$. It was suggested as the oxygen atom. Besides, neither atom 23 nor 24 could be the oxygen atom because 23 was seen in the model to be bonded to atom 24 and two other atoms (17 and 26), (Fig. 28).

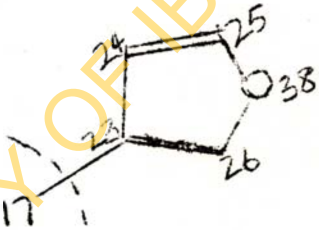
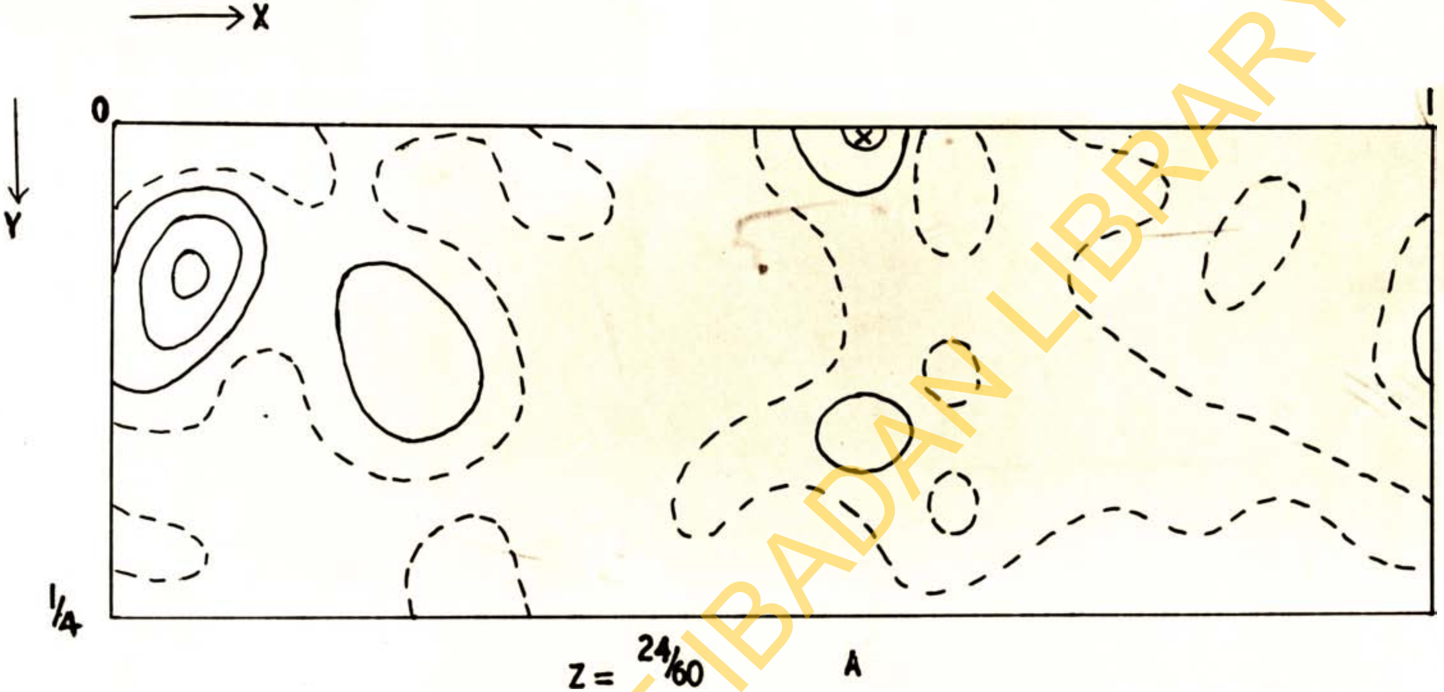


Figure 28.

Thus only atom 25 could be considered as the alternative to atom 38 as the possible oxygen atom of the furan ring. Atom 38 had been observed in the first Fo Synthesis and had been a more prominent peak than any other in the ring. It was therefore decided to regard atom 38 as oxygen in the next calculation.

The next atom selected, (6) marked X in fig. 28A was readily recognised on the section Z = 24/60. It had a comparatively good peak height of 2.1 $e/\text{Å}^3$. It previously had heights of 0.5, 1.4, 2.4 and 1.4 $e/\text{Å}^3$ in the second difference, first difference, second Fo Fourier and first Fo Fourier respectively. In space, it seemed to connect atoms 7



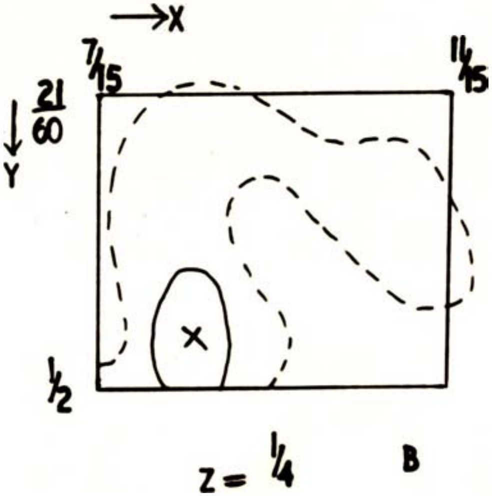


Figure 28

and 34 to the rest of the molecule. Atom 21 (marked X in fig. 28B), with a peak height of 1.7 e/Å^3 was included mainly because of its stereochemical significance. The shape of the contours around this point was not encouraging. Its previous heights were 0.8, 0.95, 1.8, 0.7 e/Å^3 in the same order as above. The increase in density from the first to the second Fo Fourier suggested reality for this peak. The next peak (55) marked X2 in fig. 27A and also chosen because of its location in the model had been assumed an atom in the second Fo Fourier. In the present Fourier, it had only a peak height of 1.1 e/Å^3 and it was about 1.8 Å from both atoms 21 and 1. It was decided to test its reality in the next difference Fourier calculation.

Around the iodine atom, it was still quite difficult to decide the positions of the atomic centres. A peak (28), marked 28 in fig. 24, of height 1.2 e/Å^3 was recognised at 2.1 Å away from the iodine and this was assumed to be the carbon bonded to the iodine. At a distance of about 1.5 Å from this atom was a peak of 1.7 e/Å^3, marked 27 in fig. 24, but not in the same Z plane. This peak was also about 1.5Å from atom 32, an atom already assumed oxygen. It was observed that peak 33, marked 33 in fig. 24, was roughly in the same plane as 28, 27 and 32. If atom 27 were real, it must be the carbon of the carbonyl group of the iodoacetate. It must then be trigonally bonded to three other atoms. The angles made at atom 27 by the "bonds" to these other atoms were not too far off 120°. The general variation of the peak heights of these atoms close to the iodine in the previous calculations were not considered because it was very probable that peaks chosen were a combination of diffraction ripples around the iodine and its anisotropy of vibration. They were selected only for trial.

At this stage, it was possible from the model to recognise the ester group - COOMe as part of a sidechain - CH_2 COOMe. There were three rings in addition to the furan. There was another sidechain which was considered to be - CH_2 OMe attached to ring A. This would then account for two of the three methoxy groups expected in the structure from chemical evidence. Thus the structure was written out as shown in fig. 30.

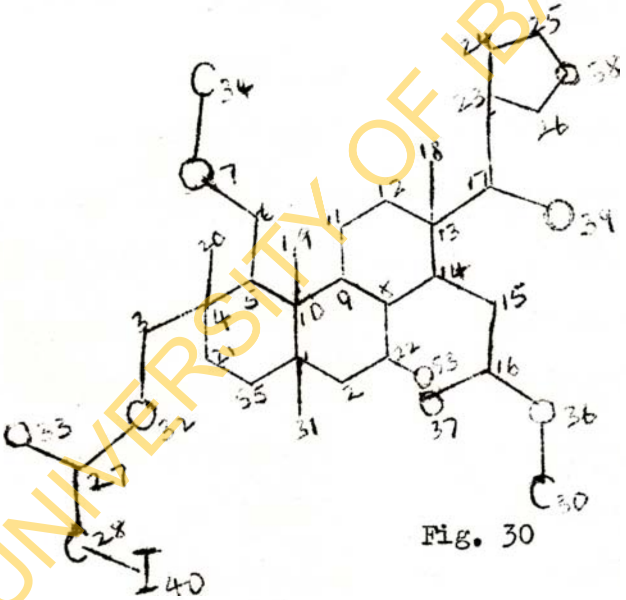
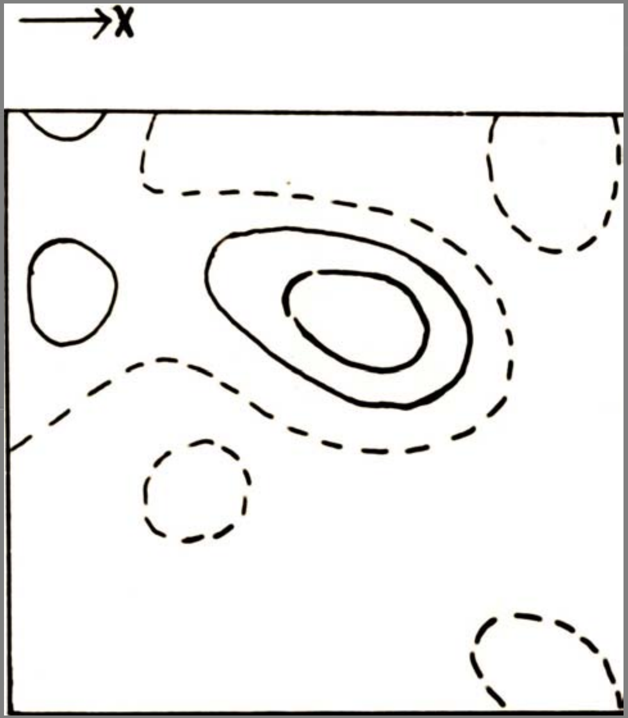
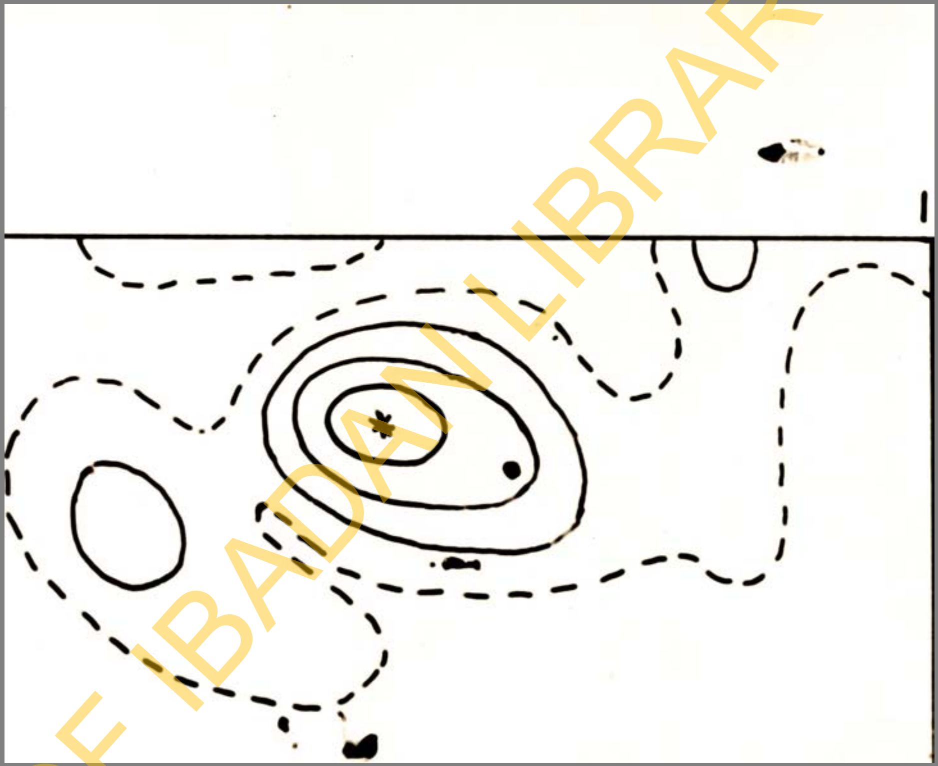


Fig. 30

Atom 53 was made oxygen on the basis of the assumed similarity of the structure to gedunin (page 3).

In the model, there were still a number of dubious points which could alter some parts of the structure. The first was that atom 3 was only about 2.0 Å from 2, suggesting a possible linkage between them. To determine this and other distances more accurately, it was decided to calculate all the bond lengths in the molecule. The second was that the pronounced departure from spherical symmetry of the contours around atom 7 (starred in fig. 29) had not been explained. It could be explained in terms of an atom needing some drastic shift or one that is seriously affected by a high anisotropic temperature effect. If either of this is true, the peak height would be lower than for most others inserted into the previous calculation. But its peak height of 3.8 e/Å^3, was about the average height for the atoms included in the calculation. It was therefore considered to be two unresolved peaks probably representing two dissimilar atoms. A point (dotted in fig. 29) of density 2.4 e/Å^3 was chosen on the lowest density gradient as the position of the omitted atom (35). Atom 7 was therefore moved to a point roughly 1.2 Å from atom 35. In the model it was immediately recognised that these new positions with two other atoms, 6 and 34, were all planar, with the bond angles around atom 7 about 120°. This suggested a trigonal system with a probable double bond between the atoms in the unresolved peak (7 and 35). If the new peak (35) is assumed to be an oxygen atom, then the spatial arrangement suggests the presence of a carbonyl group or if atom 6 is also an oxygen an acetate group. There was no evidence to support the presence of an acetate group. However it had been established from chemical evidence that there were three methoxy groups in COB (II). Assuming that the conversation of COB(II) to the iodoacetate of the reduction product COB(III)


X


1

did not affect these methoxy groups, the structure so far arrived at leaves two of them unaccounted for. If the group of atoms (6, 7, 34, 35) was part of an ester group another methoxy group would have been accounted for. It was shown later that this, in fact, was the case.

A meticulous search of the maps showed a peak (29) of height 1.7 $e/\AA^3$ at a point 1.6 Å from atom 34. The bond angle so formed was about 109°. Thus a second ester grouping for which there had been no chemical evidence was suggested.

The last dubious point was the problem of atom 39. It had consistently come up with a peak height about twice that expected for oxygen, and the distance from the nearest atom (17) was about 1.9 Å.

All the bond lengths were calculated and the results suggested bonding between atoms 2 and 3. They were 1.82 Å apart. The structure was therefore rewritten as in fig. 31.

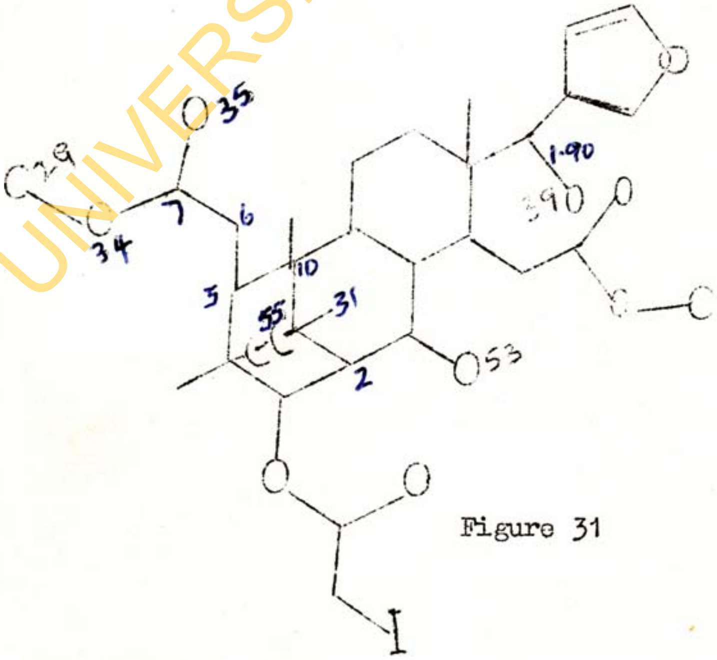


Figure 31

Atom 39 was at first considered the oxygen atom of the third methoxy group but there was no peak nearby that could be the carbon of the methyl group bonded to it. Besides this, with slight error in the number of hydrogen atoms, the formula of $C_{32}H_{40}O_9I$ for the present structure would give a molecular weight of 697. This is slightly above the experimental value. If atom 39 is the oxygen of a third methoxy group, the addition of a methyl group to this formula would increase the molecular weight even further. Also, if atom 39 were not oxygen but a heavier atom, the molecular weight would again be much greater than the experimental value. In either case, it was obvious that the structure as known at this time must include one or two fictitious atoms.

Sixth Phasing

With 42 atoms, 9 of which were weighted as oxygen and 32 as carbon and the last as iodine, the latest co-ordinates were used in another phase calculation. The new atoms were all given a temperature constant of 4.5 $Å^2$. The programme would not work for reflection (1 9 4) and this was omitted. The scale factor for the remaining 990 reflections was 1.459. The reliability factor reduced only slightly to 0.231. But the agreement between the individual experimental and calculated structure factors improved considerably. The reflections with differences greater than 3.0 reduced from 69 to 41 out of which 11 were still above 5.0. In spite of this general improvement, a few had worsened and the highest difference of 11.5 was for reflection 0 2 3. This was not surprising because the intensity of this reflection was too strong to be measured and the given experimental value was known to be low.

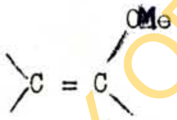
The structure factor calculations were then repeated for the 312 unobserved reflections each having half the minimum measurable intensity. The Lorentz and polarization corrections were applied and the same scale factors for the different zones, as used for the measurable reflections, were applied.

Review of Chemical Evidence

Since COB occurs alongside COA (fig. 2), it was assumed to be similar in general structure. The nuclear magnetic resonance spectrum of COB(II) suggested[39] that the three methoxy groups were present as

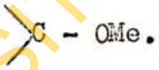
(a) - COOMe

(b) an enol OMe



$>C=C(OMe)<$ and

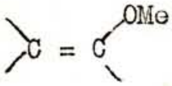
(c) a questionable ordinary



$>C-OMe$.

There was a furan ring and most probably a lactone. This meant a ring D probably similar to that of COA (fig. 2) but without the epoxide ring. It was not clear what happened in the formation of the chloroacetyl derivative from the analytical results. It seemed that it was not a simple chloroacetylation. There was some evidence from the spectra that the furan was affected. There was, however no certainty on any of these suggestions.

With an ester grouping of the type - CH_2 COOMe found on ring C (fig. 30), the second side-chain on ring A, was considered to be of the enol type



Hence the structure of fig. 30 was first put forward and sent to Dr. Powell for his comments in the light of more up to date chemical evidence. The oxygen of atom 39 was also considered to be that of the third methoxy group, but there was no peak stereochemically well placed to be considered bonded to this atom.

A discussion [44] of the structure of fig. 30 led to the following suggestions[40]:

(1) That the side chain in position marked (1) fig. 31A) should be as shown (i.e. a methyl ester).

(2) That there should be a ring in position marked (2).

(3) That the iodoacetate group would prove to be attached as at (3) with a probable hydrogen bonding connection with the furan ring.

(4) That ring A is without doubt not normal.

(5) That the possible existence of an epoxide ring should not be ruled out.

(6) That the atom marked OZ should be an oxygen.

These suggestions were considered as follows:

Suggestion 1

From the second Fo Fourier model, there were four atoms in the circled region. They were not well placed and hence difficult to interprete. ~~They~~

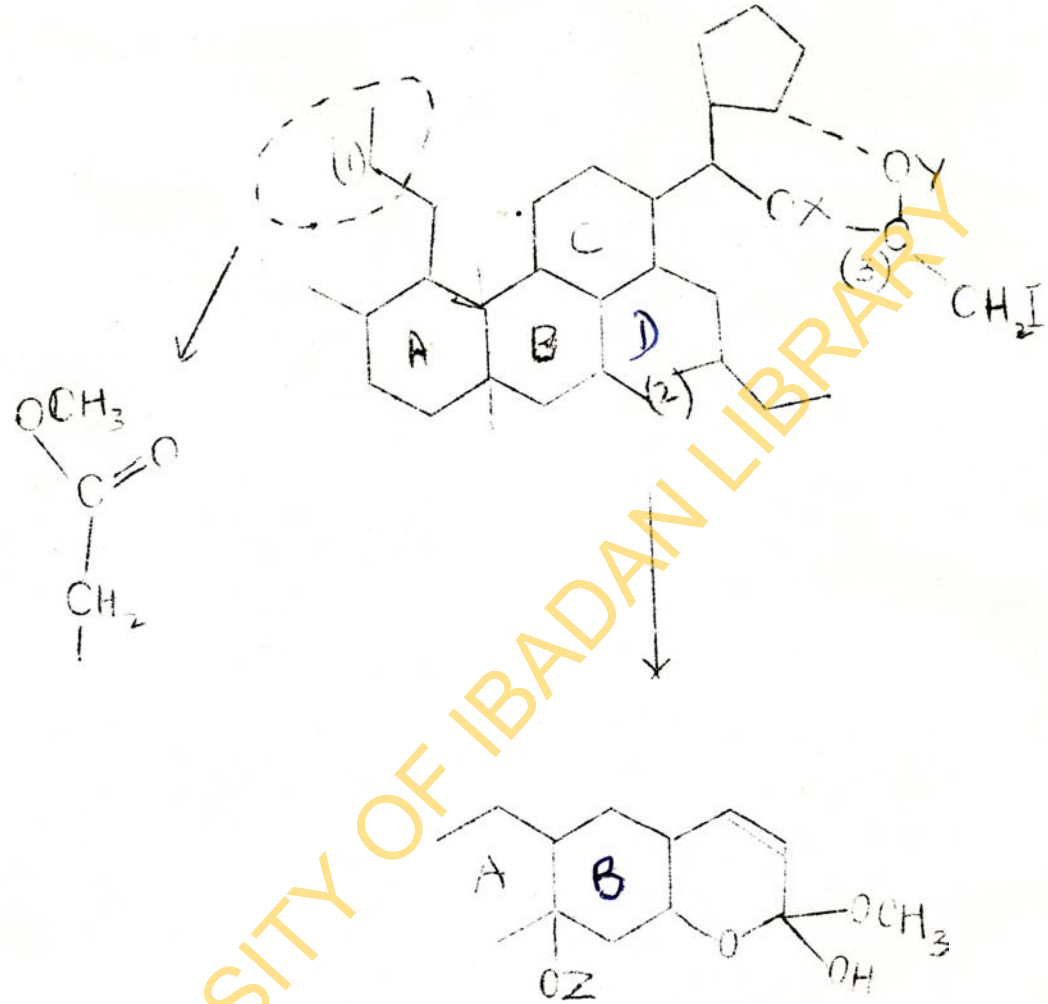
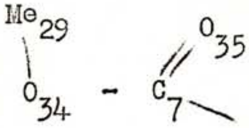


fig 31 A.

They could be regarded as



of the $-CH_2 - COOMe$

but the peak corresponding to atom 6 ($-CH_2-$) had not then been observed. Hence the connection of these four atoms with the rest of the molecule was missing. The situation was further complicated by the fact that the peaks representing the carbon and oxygen atoms of the carbonyl in this group were only about 1 $^{\circ}$A apart. They were however quite well resolved, unlike the situation in the third Fo Fourier.

In the third Fo Fourier, two of these atoms (7 and 34) were included in the calculation. The result was that atoms 7 and 35 were unresolved (fig. 29). As already stated atom 35 was postulated only after a close examination of the shape of the contours around atom 7. The presence of an ester group - CH_2 COOMe, in this region, had been arrived at independently before the suggestion from Dr. Powell. But there were now two such ester groups in the structure. This was in conflict with the N.M.R. evidence.

Suggestion 2

A ring had been inserted in the position marked (2) in the second Fo Fourier. This was arrived at by supposing that "atom" 53 was bonded to atoms 16 and 22 (fig.30). The distance from 16 had been shown to be well above 2.0 Å in the third Fo Fourier and hence the ring was opened. It was however suggested[40] that the increase in this distance was accidental and the ring should be closed. It was difficult to make a decision on this especially at this stage when the side chain was being regarded as a second ester group of the type $-CH_2$ COOMe. A convincing argument in favour of opening the ring was the planar configuration of atoms 15, 37, 16 and 36. The bond distance between 16 and 37 of about 1.3 Å, and the relatively large peak height of atom 37 suggested that it was the carbonyl oxygen of an ester group. It could not therefore be bonded to another atom. As already stated "atom" 53 was only about 2.0 Å from atom 16, and it was not clear whether it was linked to the ester group or not. It was decided to leave it for the time being.

Suggestion 3

That the iodoacetate should be close to the furan as shown was considered possible because there was some uncertainty around the iodine and it was difficult to locate the atoms close to it. The iodine atom was at a distance of 4.5 to 5.0 Å away from atom 39 or OX, which is close to the furan ring. Since the positions of the atoms around iodine were uncertain, the proximity of the iodoacetate to the furan ring could not be ruled out. This suggestion was left as an open question.

Suggestion 4

That ring A is not normal was a happy confirmation of the results obtained at this stage, namely, that ring A was part of a bi-cyclo octane system as shown in fig. 31.

Suggestion 5

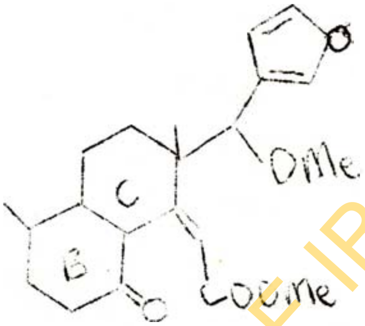
The idea of an epoxide had arisen mainly from the assumed similarity of COB to COA (fig.2). From the first model, a spurious peak of height 1.27 e/Å^3 ("atom" 45) taken together with atoms 24 and 25 gave the impression of a three-membered ring. This had been considered for an epoxide ring and rejected (page 56). The three peaks were in the region of the furan ring and two of them at present constitute members of the ring. From another model constructed with the parameters obtained from the second Fo Fourier another set of three atoms (7, 48 and 34) were supposed to make an epoxide ring. They were about 1.2 Å from one another but rather isolated. Atom 6, bonded to atoms 5 and 7 was then not recognized. Hence atom 7 together with the others connected with it did not make any chemical sense. Later on, "atom" 48 gave a negative peak of 3.2 e/Å^3 in the second difference synthesis and was discarded. The other two now constitute members of the ester group attached to ring A. There was therefore no evidence in favour of an epoxide ring.

Suggestion 6

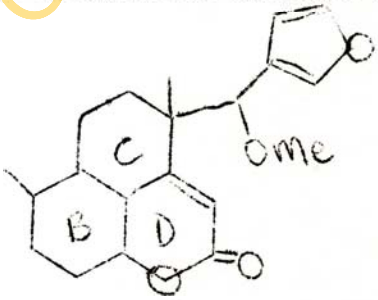
The last suggestion that OZ or atom 31 should be an oxygen, seemed reasonable from the point of view of the peak height of this atom in all the calculations. But the structure at this stage could only admit a single bond between this atom and the carbon atom, C(1), to which it was bonded. This was already bonded to three other atoms (10, 55 and 2) (fig. 31). Such an oxygen atom could only be in the form of hydroxyl group. Since there was no evidence of such a group, the atom was left on as carbon. In fact atom 31 or OZ is an oxygen. Had it been realised that "atom" 55 was spurious, this suggestion might have been more easily acceptable at this stage.

Further correspondence on the structure of fig. 30 led to the following suggestions[39]:

(a) That assuming that COB(II) has the partial structure,



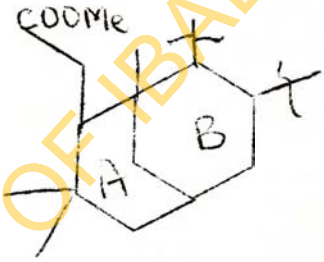
the rings C - D part of the iodoacetate molecule could be



This would agree with the hypothesis that on reduction of the 7-keto, followed by treatment with acid (present in the acetylating agent) the carbo-methoxyl was hydrolysed and recyclised. This was put forward because the N.M.R. had shown loss of one methoxy group on formation of the chloroacetyl derivative. However the lost band was more what would be expected for a $-\overset{|}{\underset{|}{C}}-$ OMe group. Alongside this, it was suggested that the hydroxyl that was chloroacetylated was around Ring A.

The arguments for and against Ring D have already been stated (page 77). Only two methoxy group had appeared in the structure so far; if the third one was to be ruled out, then the idea of atom 39 being the possible oxygen of the third methoxy group would have to be ruled out. That the iodoacetyl group is attached to Ring A would agree with the present structure.

(b) That the carbon skeleton in the Ring A/B region could be of the type:

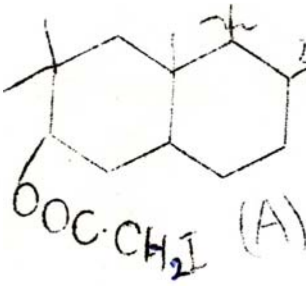


This would be analogous to that in Swietenine[45]. There was also a definite statement that COB and derivatives contain the ester grouping in the form $-CH_2$. COOMe. This carbon skeleton agreed with what was on hand except for the presence of a bi-cyclo octane system formed by including "atom" 55 (fig. 31). This "atom" was included only to test its reality and its removal would immediately give the above carbon skeleton with the gem-di-methyl groups.

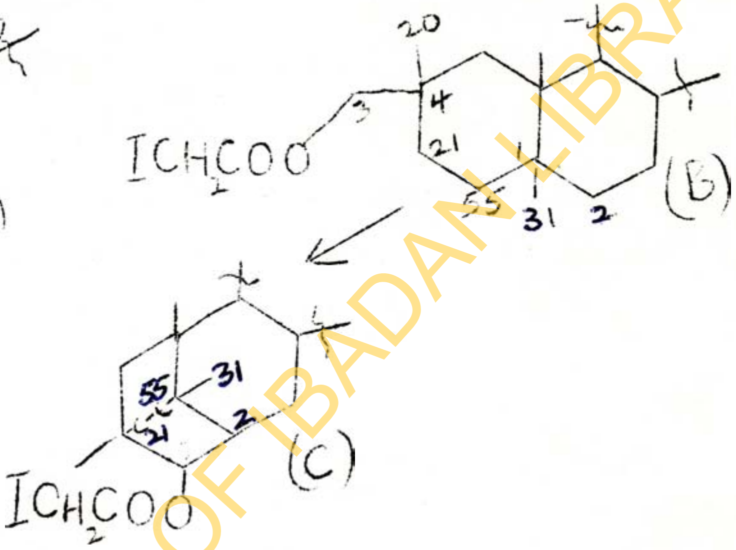
That COB and derivatives contain a $-CH_2COOMe$ group was a satisfactory confirmation of the interpretation of the map in fig. 29 (page 72). The second ester group was not known to be present in COB. It was formed by methylating COB to give COB(II) containing two more methoxy groups.

(c) That from the chemical point of view, it would seem more likely that the iodoacetate is attached to C(3), rather than one of the gem-dimethyl groups.

That is, (A) is more likely than (B):



(A)



(B)

(C)

(B) was the structure proposed from X-ray results before it was realised that atoms 2 and 3 were bonded as in (C). This was not easily perceptible from the model and the conclusion was made only after calculating the bond distances. Suggestion (c) is therefore in agreement with the x-ray results.

The suggestions which could not be dealt with unambiguously at this stage were borne in mind as possible aids in the interpretation of the next electron density map. There was still no chemical evidence about the nature of atom 39; the X-ray evidence suggested it was heavier than oxygen. It was therefore considered desirable to re-analyse COB and derivatieves chemically. At this stage, when the chemical nature of most of the atoms were known with a fair amount of confidence, it should be possible to correlate the peak heights with chemical nature.

Third Difference Synthesis

The set of phase angles just calculated for all the 1303 independent, measurable and estimated reflections, were used in calculating a difference synthesis. By far the most outstanding feature of the results was the elimination of "atom" 53. The peak starred in fig. 32, had come out fairly spherically symmetrical in the previous Fo Synthesis calculation at a height of 3.9 e/Å^3 when weighted as carbon with a temperature factor of 4.7 Å^2. In this calculation, it was weighted as oxygen with the same temperature factor. It came out, starred in fig. 33, close to a negative peak density of - 2.2 e/Å^3 and was therefore eliminated.

With the elimination of this atom, the absence of a ring D (fig. 31A) which had been ruled out on other grounds was finally confirmed.

The atoms of the ester group in this region indicated the need for small adjustments of the co-ordinates. Atom 36 (fig. 31) had a peak of - 1.3 e/Å^3 with a temperature constant of 5.0 Å^2. It was also weighted as oxygen. There was no doubt about its reality and this negative peak was attributed to a probably wrong chemical identity. In the third Fo synthesis where it was weighted as carbon, it had a peak height of 4.6 e/Å^3. In other calculations, it had peak heights of 0.61 e/Å^3 with B value of 4.5 Å^2 in the second 'difference', 1.41 e/Å^3 in the first 'difference', 3.1 e/Å^3 in the second Fo synthesis and 1.80 e/Å^3 in the first Fo synthesis. It was therefore changed to carbon. Unlike this atom, atom 15 which was weighted as carbon had a positive peak of

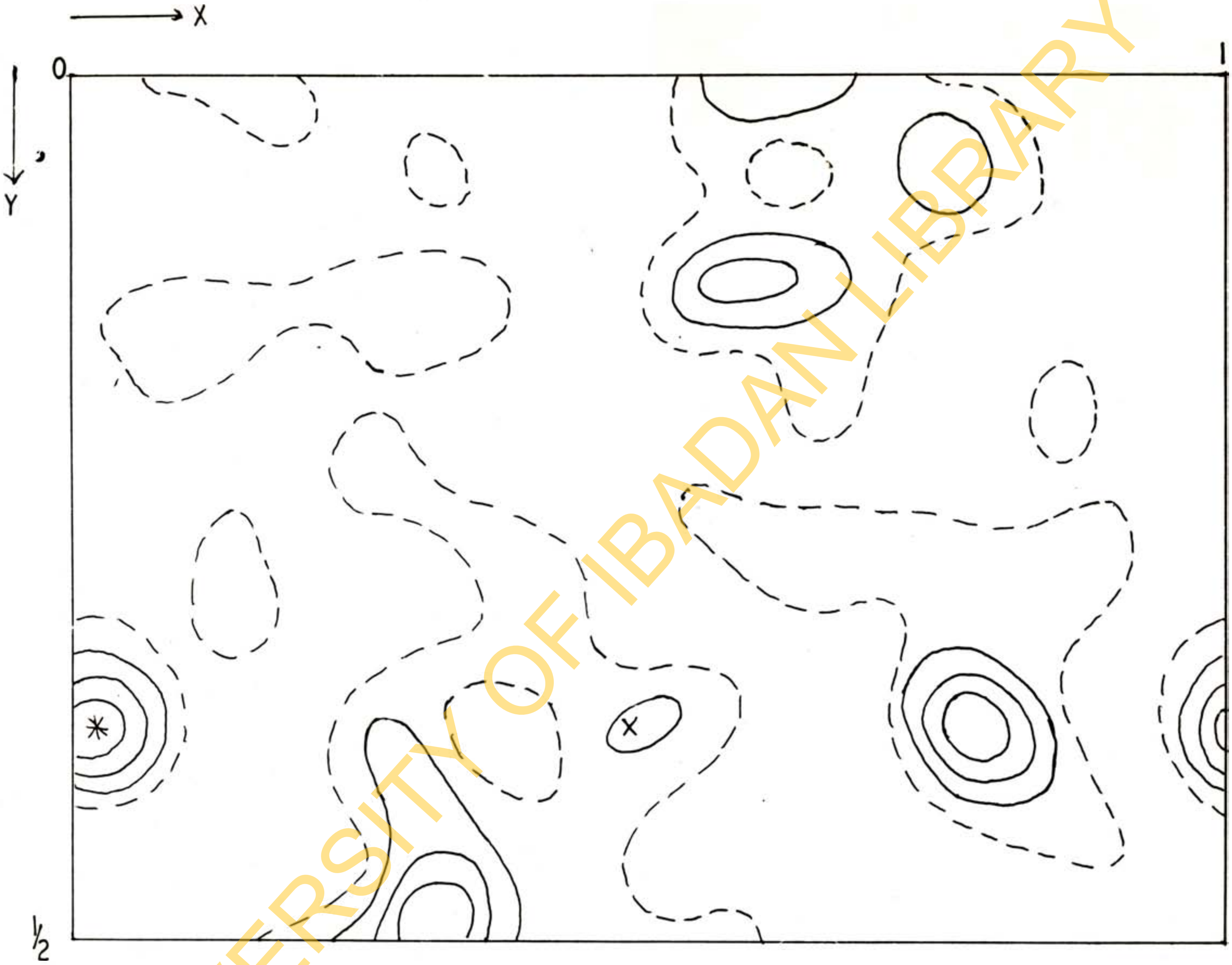
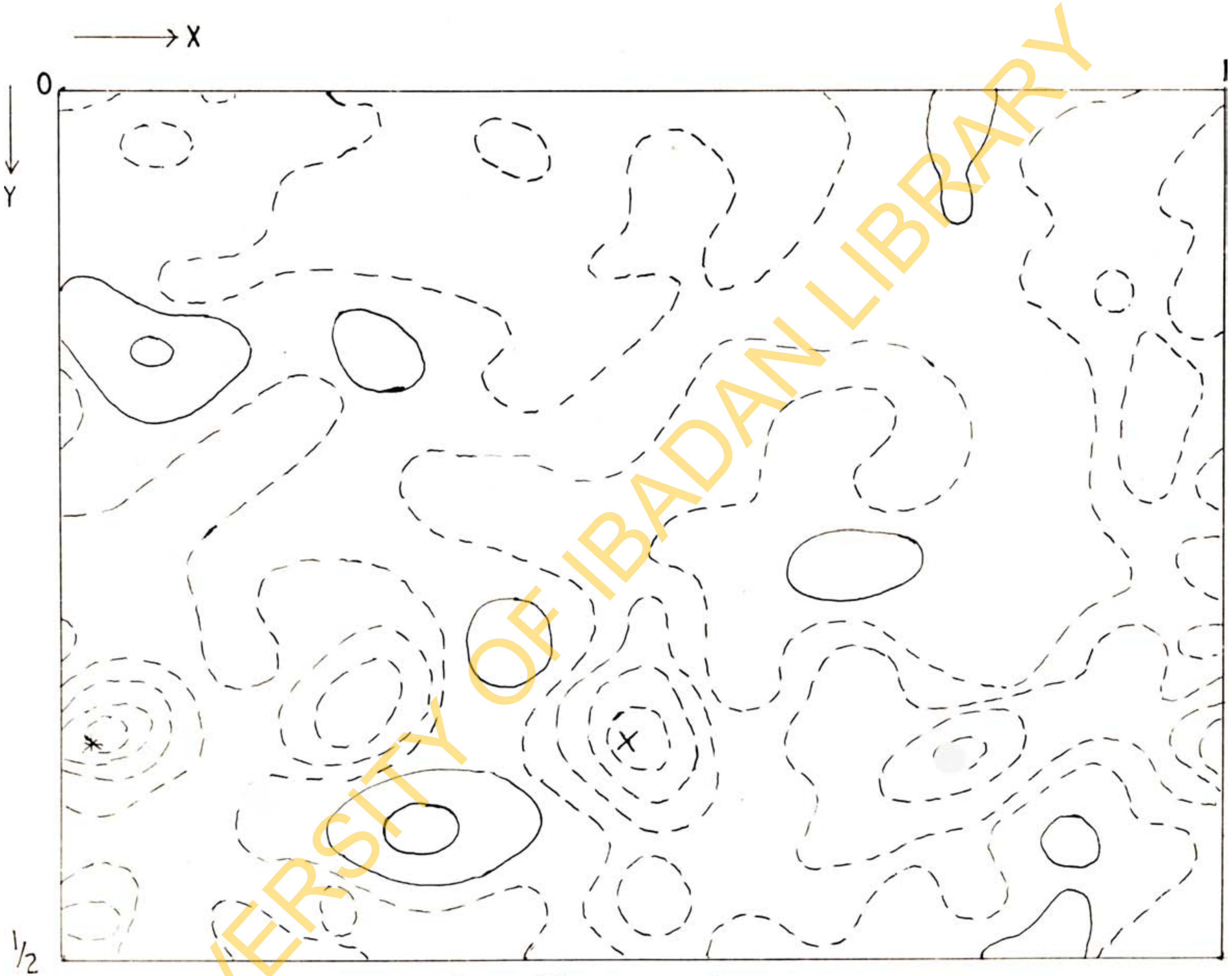


Figure 32

Z= 18/60 ρ_3



3RD DIFF Z = 18/60

Figure 33

0.3 e/Å^3 with a temperature constant of 4.3 Å^2. In the third Fo synthesis, it had a peak density of 4.5 e/Å^3 which was only slightly less than that of atom 36. In other calculations, it had peak heights of 0.31 e/Å^3 with a temperature constant of 4.5 Å^2 in the second 'difference', 0.18 e/Å^3 with B = 4.5Å^2 in the first 'difference', 5.4 e/Å^3 in the second Fo sunthesis, and 2.4 e/Å^3 in the first Fo synthesis. The early peak heights were higher than those of atom 36 and so atom 15 was changed to oxygen. It was noted however, that the peak height of atom 36 was higher than that of atom 15 in the third Fo synthesis where the phasing should be better than in the first two. This change of the chemical identity of two atoms would then bring in a propionate side chain.

The negative peak of iodine decreased from - 7.38 to - 3.82 e/Å^3 with B = 6.0 Å^2. B was therefore increased to 6.7 Å^2. The immediate neighbourhood of iodine was marked out with ripples of positive and negative densities. Atom 28, marked X in fig. 34 to which the iodine atom was attached, was at the centre of a trough 2.37 electrons/Å^3 deep. This might suggest increasing the temperature factor but B was so generally high that the peak heights whether positive or negative were rather low. A negative density of value 2.0 electrons/Å^3 was therefore very likely to mean a wrongly located atom. At a distance of about 0.8 Å from this position was a positive density, 1.83 e/Å^3, marked X in fig. 35. It was also 2.1 Å away from the iodine atom. It was therefore considered to be the correct position of this atom. Atom 27, (C(27)), seemed to have x and y co-ordinates correct but showed a high density gradient in the z direction. It was shifted accordingly on the basis of a qualitative judgment.

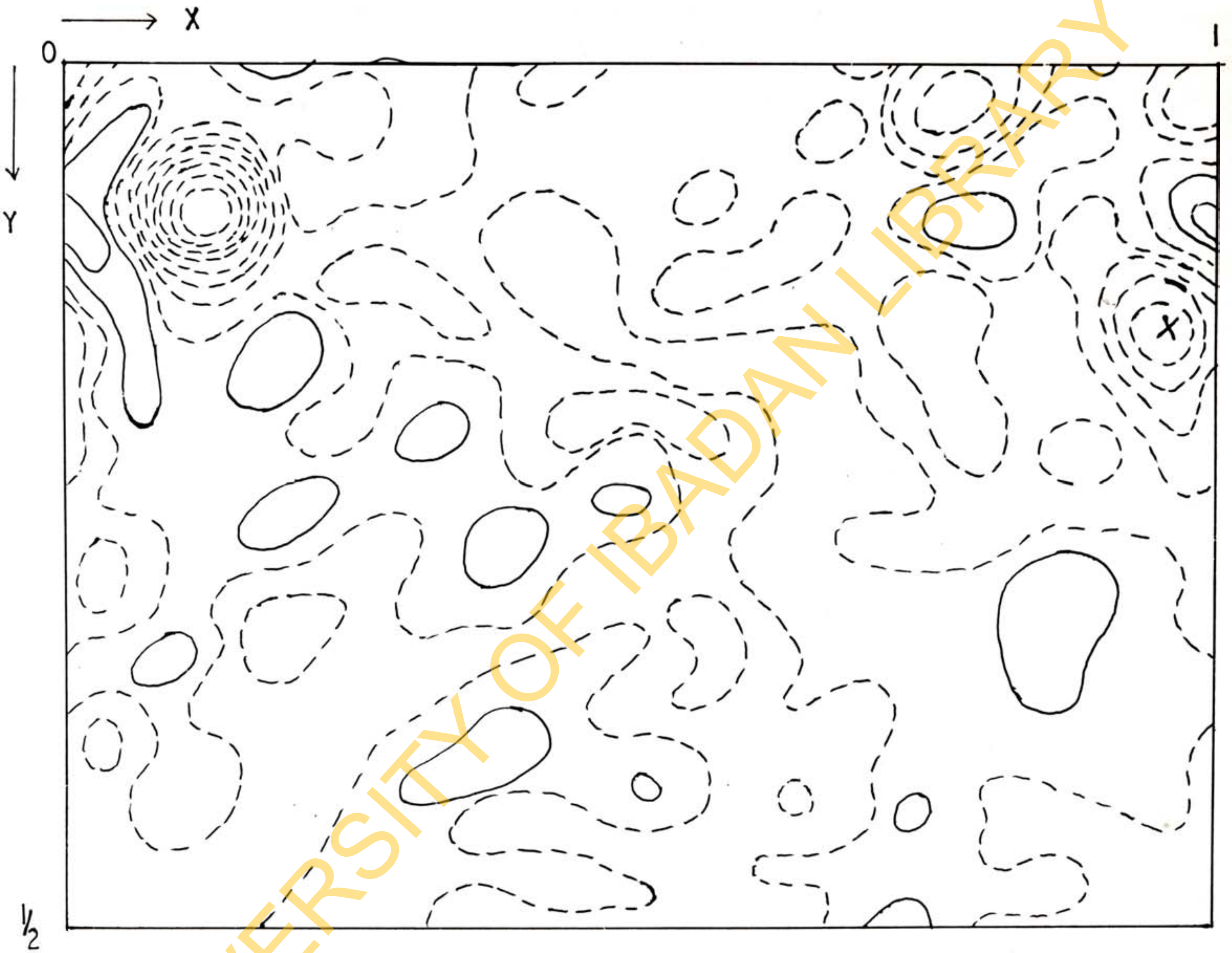


Figure 34 3RD DIFF $Z = 6/60$

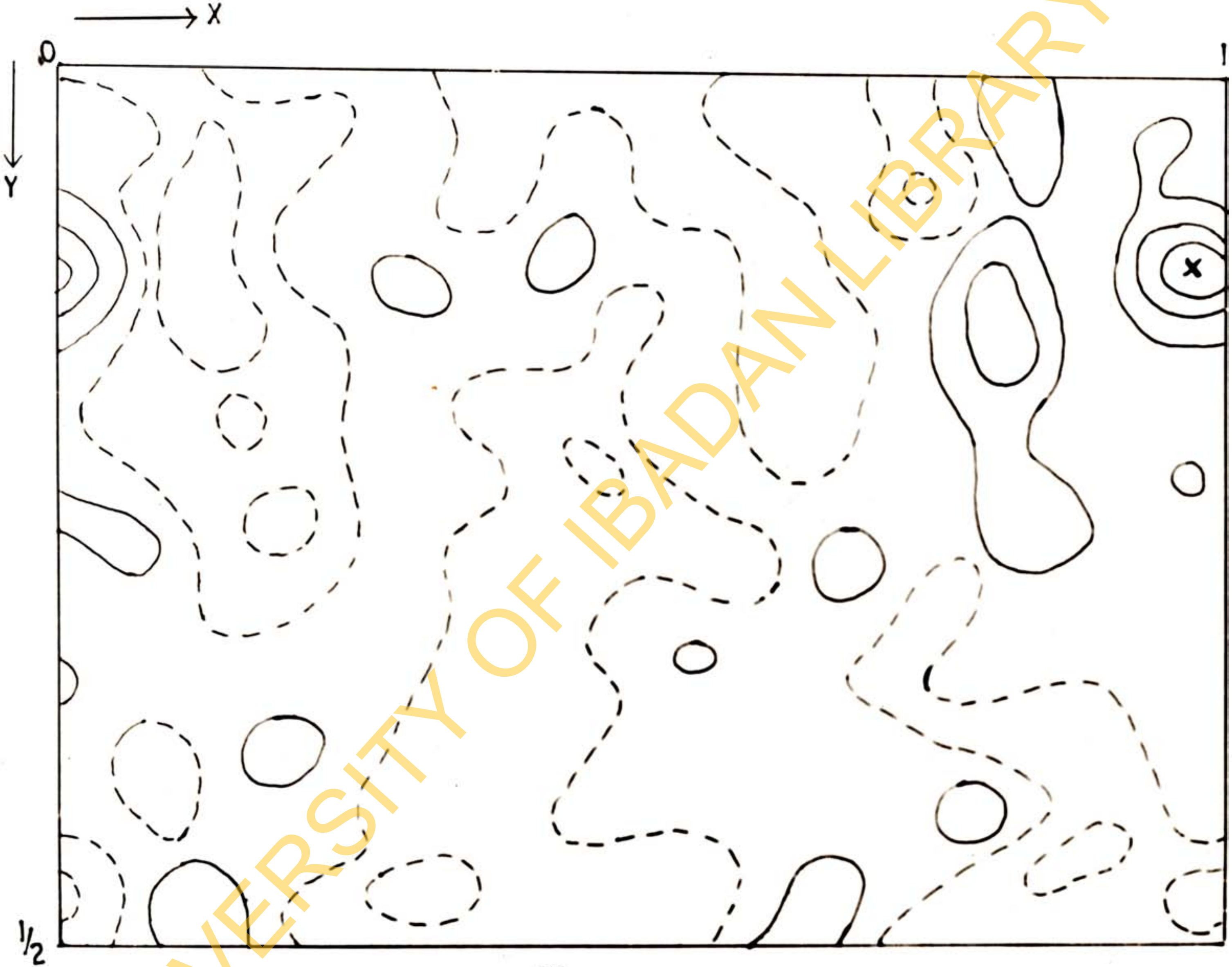


Figure 35 3RD DIFF $Z = 10/60$

But the new position was only 1.27 Å from atom 28. This was worse than the previous distance of 1.58 Å. Hence, these new positions of the two atoms were questionable. They were however left alone for the time being.

Atom 33, marked x in fig. 36, was on a high density gradient the peak of which was - 2.70 e/Å^3. Appropriate changes in co-ordinates were made. The new position was distant 1.28 Å from atom 27. The co-ordinates of these three atoms, close to the iodine were rather unsatisfactory but they were included in the next structure factor and bond length calculations.

Atoms 30 and 2, which in the previous Fo Fourier had the lowest peak heights, and were included only because of their stereochemical implications, showed only slight negative densities. Atom 2, marked X in fig. 37, was in a trough of depth 0.85 e/Å^3 and its temperature factor B was increased from 4.5 to 4.8 Å^2. There were two positive peaks of about 0.9 e/Å^3 on opposite sides of it and its co-ordinates were not altered.

Atom 30 was on a low negative density gradient and its temperature factor B was increased from 4.5 to 4.6 Å^2. Its co-ordinates were also slightly adjusted.

"Atom" 55, marked X in fig. 32, which had the lowest density of the atoms inserted in the last phasing, was in a trough, marked X in fig. 33, of depth 1.71 e/Å^3. Since the previous peak height was only 1.1 e/Å^3, increasing the temperature factor would be inadequate to account for this trough. Elimination of this atom would rule out the bi-cyclo octane formed within ring A. The structure would change from (A) to (B) in fig. 38.

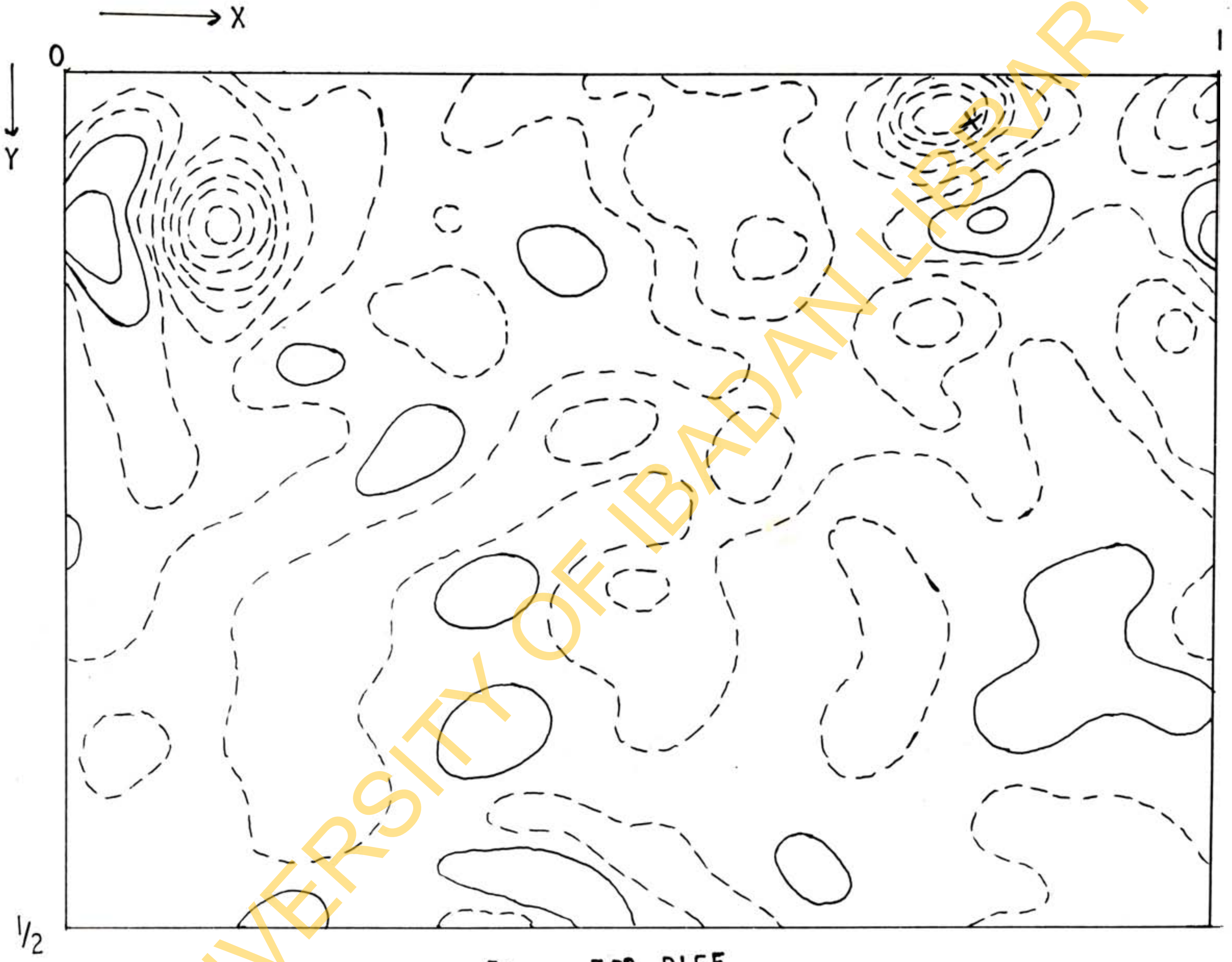


Figure 36 Z = 5/60 3RD DIFF

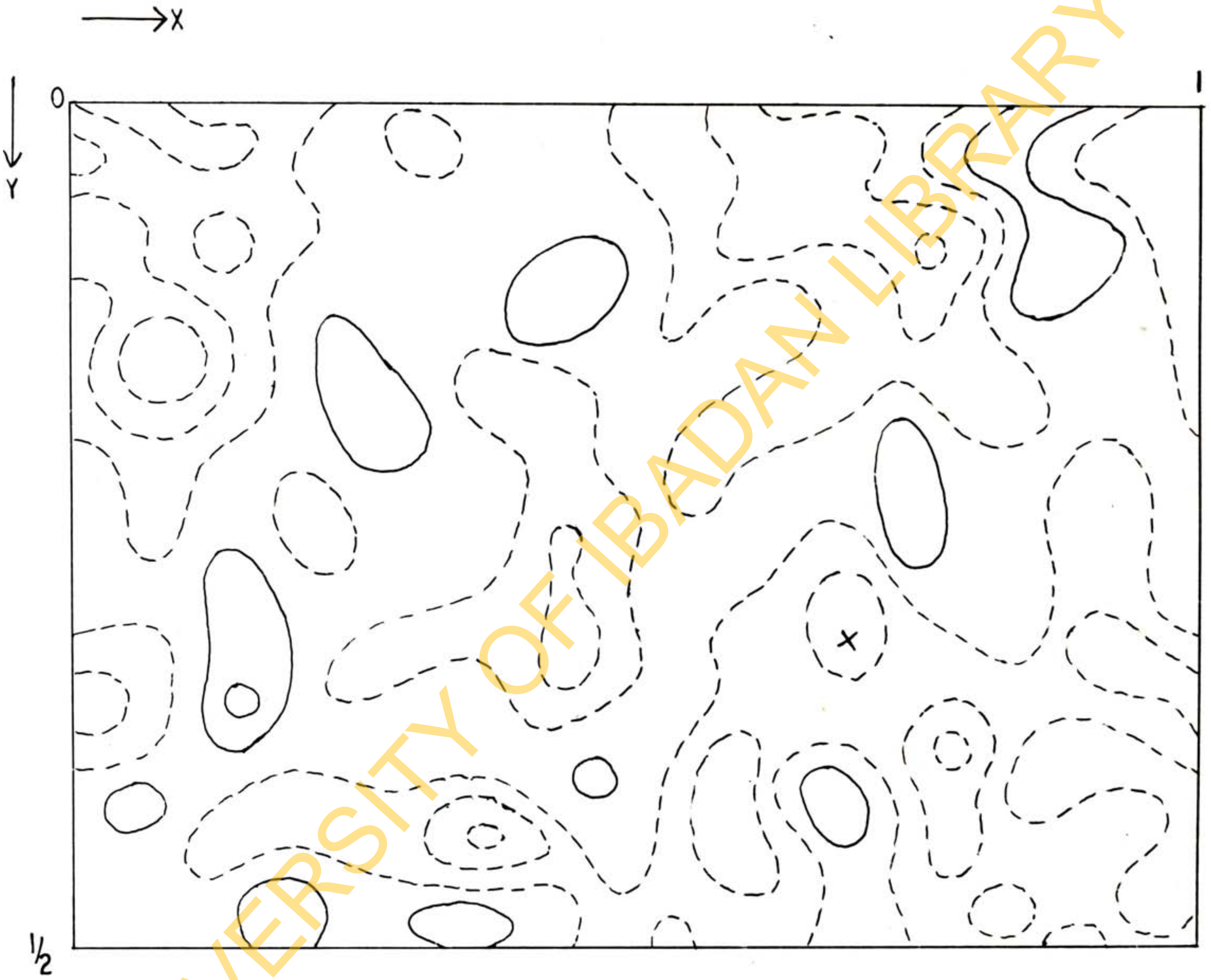


Figure 37

3RD DIFF Z = 21/60

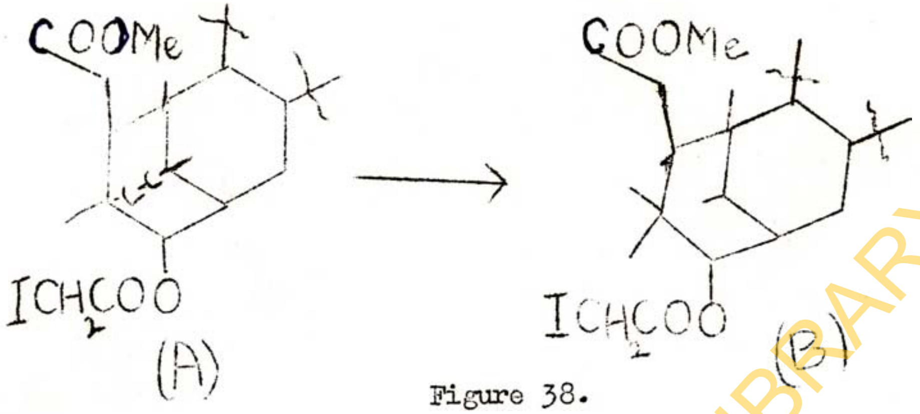


Figure 38.

In this form, atom 31 could be made oxygen, as suggested by chemical evidence, and the bond distance between atoms 1 and 31, (1.1 Å) would be acceptable for a double bond. An adjustment of the position of atom 31, marked X in fig. 39, indicated by its density gradient in the x direction, brought it back to its former position. In this position, it was co-planar with atoms 1, 2 and 10, suggesting a double bond between atoms 1 and 31.

Various shifts were made for the atoms constituting the furan ring. None of them was at the peak of a negative density but two atoms, 26 and 38, were on negative gradients and their temperature factors were increased from 4.0 to 5.0 Å^2 and 4.5 to 5.4 Å^2 respectively.

The last feature of this difference synthesis was the peak height of atom 39, marked XI in fig. 40A. It was 1.69 e/Å^3 even when the temperature factor B of 4.0 Å^2 was among the lowest for all the atoms. At a distance of 2.1 Å away from it was another peak, marked X2 in fig. 40A, of height 1.39 e/Å^3. This was a position in which an atom had been inserted in the first and second Fo syntheses ("atom" 42) and had proved spurious in the first difference synthesis with a trough of - 4.32 e/A^3. It had

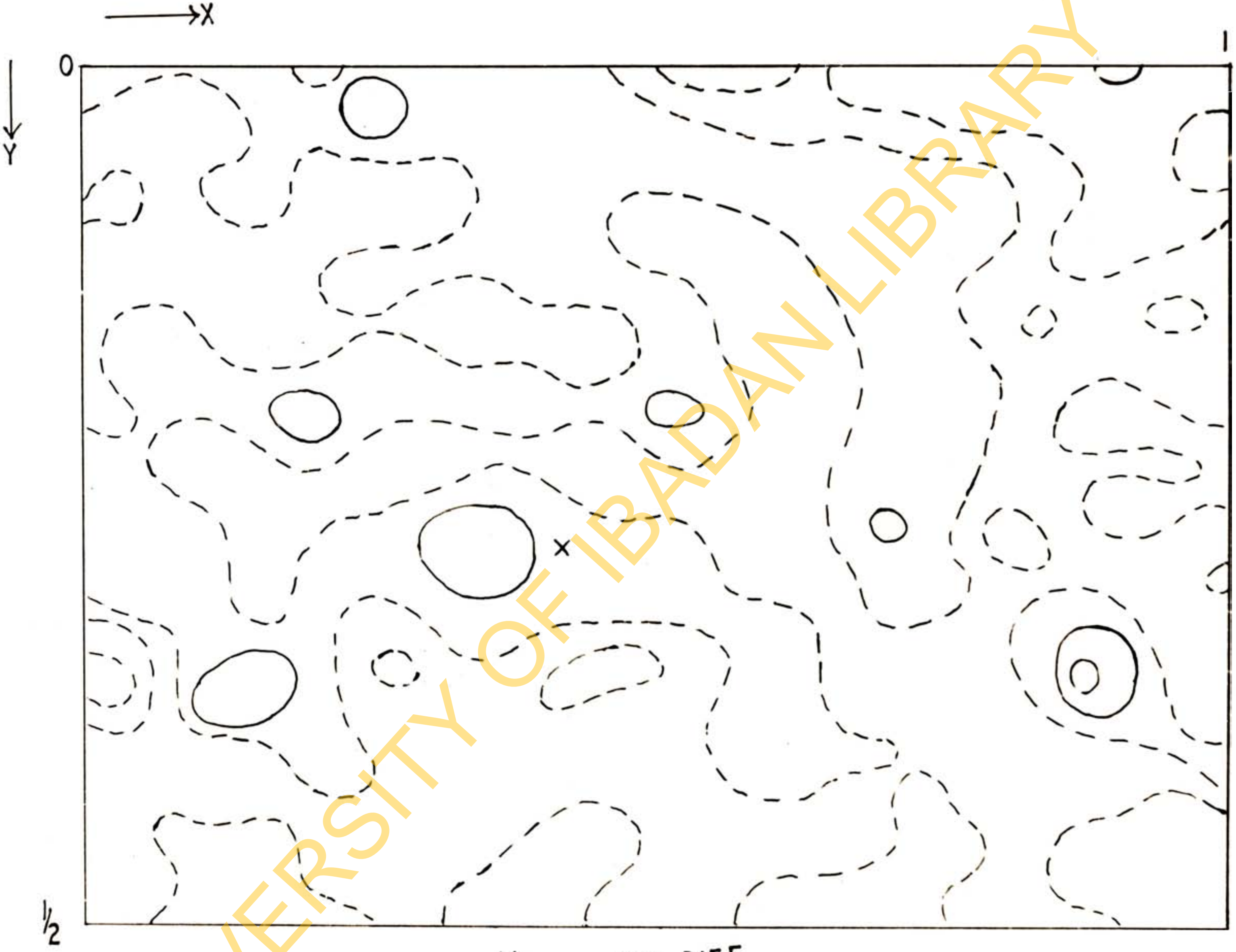


Figure 39 Z = 16/60 3RD DIFF

therefore been eliminated. But it was again included in the next calculation as a probable carbon to which atom 39 was attached to make a methoxy group. This was to decide if atom 39 was part of a methoxy group as suggested[39]. Assuming that such an atom was slightly displaced from its real atomic centre, or that the value of temperature factor was accountable for the negative peak, then inserting it in the calculation should lead to some improvement of the reliability index.

With the adjustment of the co-ordinates in the model, it was very easy to note the planarity of atoms 8, 9, 22, 14, 13 and 15. This established the existence of a double bond between atoms 8 and 14. Hence the structure at this stage was as seen in fig. 41 with molecular formula $C_{31}O_9I$.

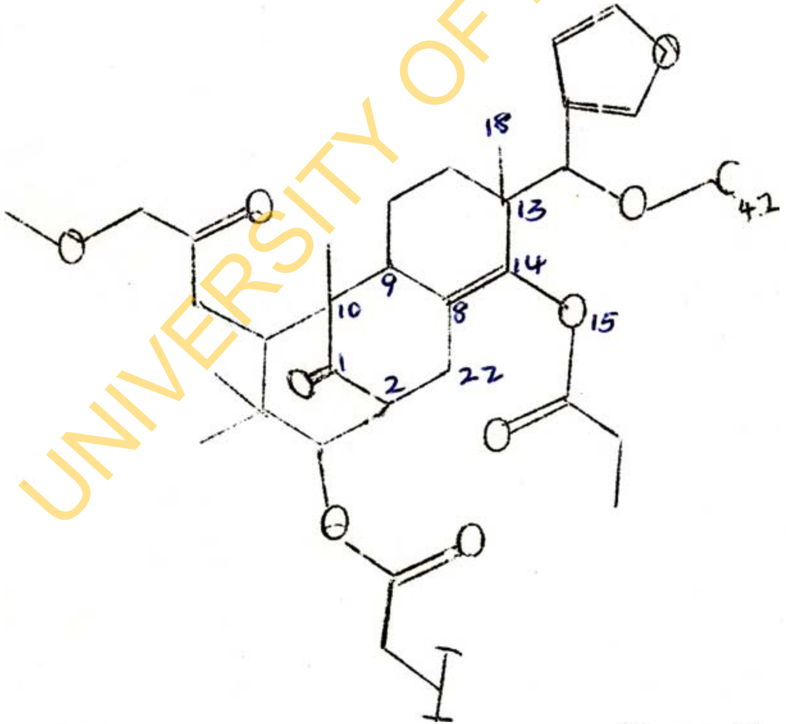
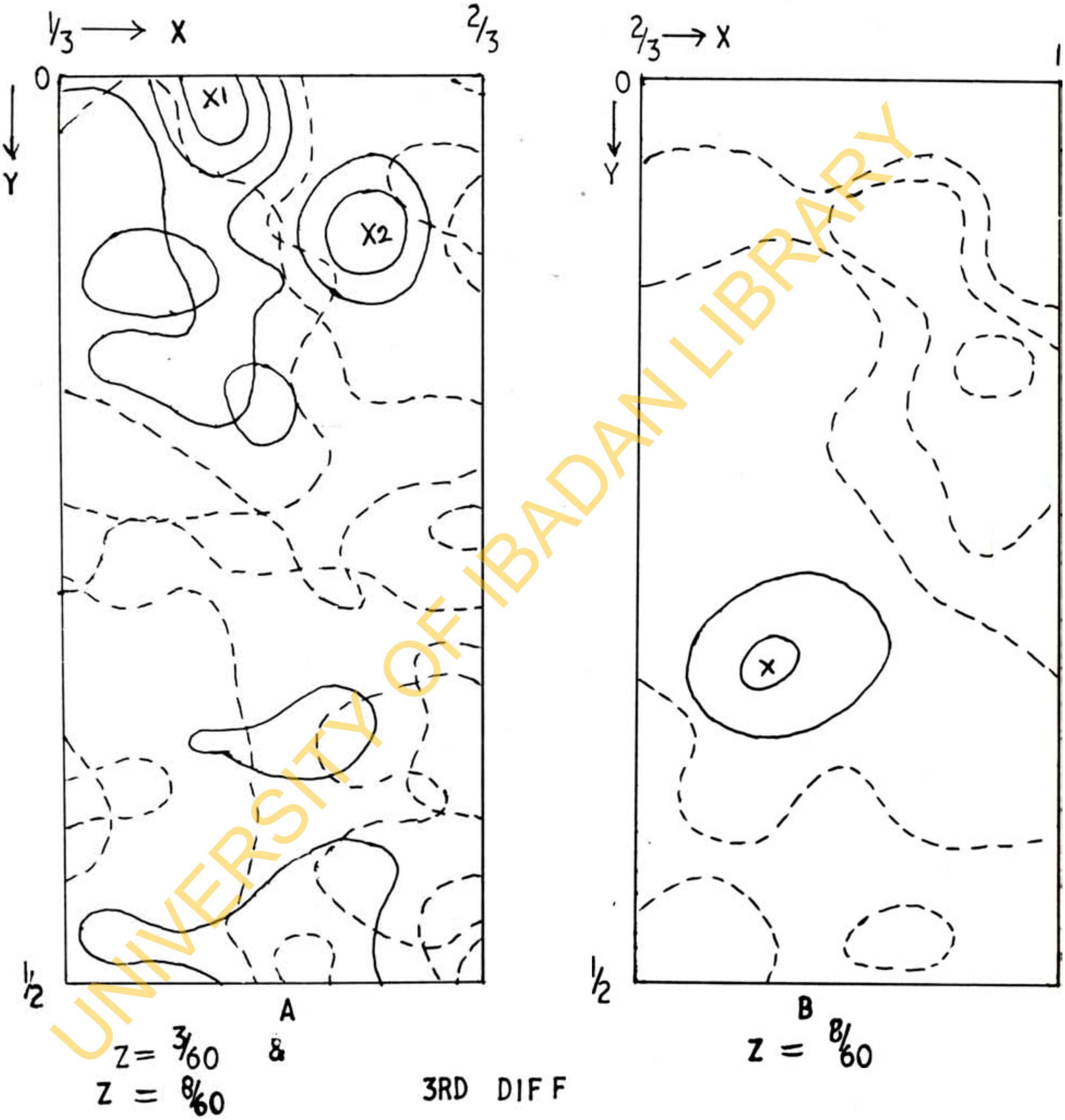


Figure 41



3RD DIFF

Figure 40

The structure as written with formula $C_{31}H_{40}O_9I$, has a molecular weight of 683. This is about one carbon atom, short of the experimental value of 693 ± 7. Besides one of these atoms (42) was still suspected to be spurious.

The maps were re-examined and it was noted that there were 13 other peaks with heights greater than 1.0 e/Å^3. A number of these were peaks towards which atomic centres should be moved. Others whose spherical nature suggested them to be atoms were examined in space with the aid of the model. One such peak marked X in fig. 40B, of height 1.07 e/Å^3, seemed to join C(18) and C(15). But it was only 1.1 Å from C(18) and about 2.1 Å from C(15). It was probably a hydrogen atom attached to C(18). Some of the other peaks were positions of local electron density maxima. For example the point marked X in fig. 42A, had a peak height of 1.35 e/Å^3 but the contours do not suggest an atomic peak.

Seventh Phasing

Structure factor calculations

(i) With the latest co-ordinates for 41 atoms and varying isotropic temperature factors, structure factors were calculated again. The scale factor was 1.398. The highest difference between the structure factors, observed and calculated was 9.8 and for reflection 0 2 3. The intensity for this reflection was too high to be estimated and this result was not surprising. The reliability index R reduced to 0.223.

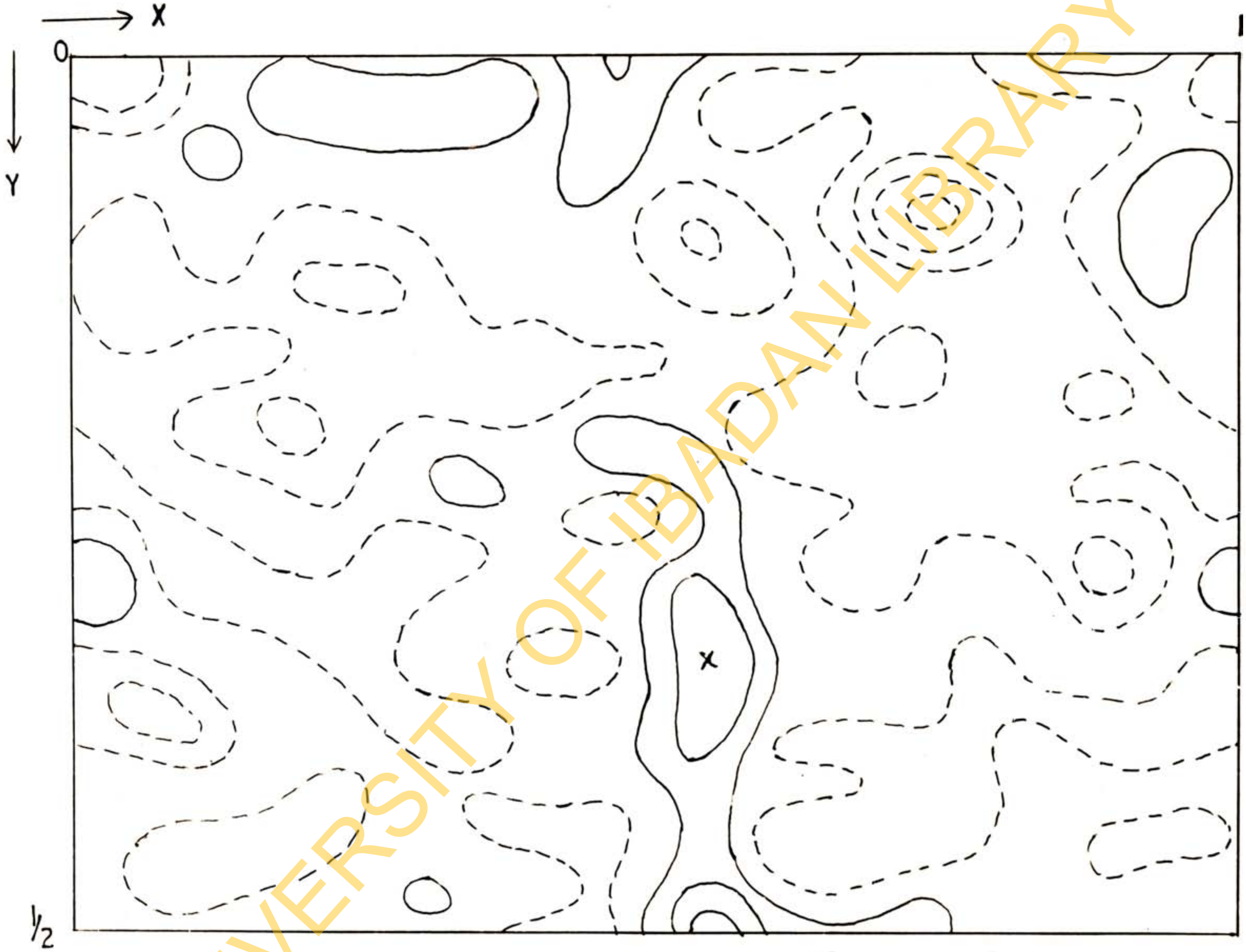


Figure 42A 3RD DIFF $Z = {}^{27}/_{60}$

(ii) "Atom" 42, supposed bonded to atom 39, was removed together with C(28), C(27) and atom 33, these three constituting the CH_2CO group to which the iodine atom was supposed to be bonded. The remaining position and temperature parameters were used in calculating all the structure factors as above. The scale factor was 1.372 and the highest difference between the structure factors was 8.7 and for reflection 4 0 0. The reliability index R reduced to 0.216. This showed that some or all of these four atoms were probably incorrectly located. "Atom" 42, was strongly suspected to be spurious. To test this suspicion, this atom alone was removed and the calculations, carried out for the (okl) reflections alone, showed a marked improvement of the reliability index, ($0.225 \longrightarrow 0.21$). The possibility of atom 39 being the oxygen of a methoxy group was then ruled out.

(iii) Another possibility was that atom 39 was heavier than oxygen and was an atom which needed to be bonded to only one other atom. Chlorine was such an atom. At least, in part to satisfy the need to put more electrons in its position as required by the difference syntheses, it was decided to test this possibility by inserting two oxygen atoms with the same temperature constant $B = 4.0Å^2$ in its position. And with parameters for $C_{28}O_9I$, the resulting calculation reduced the reliability index R to 0.204. The scale factor was 1.405 and the highest difference of structure factors, 8.1, was still for reflection 4 0 0.

Chlorine Atom

Table 13 shows the peak densities of the atoms for all the Fourier calculations. Though they were generally low due to the high temperature constants, the figures for atom 39 had been consistently and remarkably higher than those for carbon and oxygen. It was on the basis of this 'far above the average' argument that its identity was changed from carbon to oxygen after the second difference synthesis. It still persisted as the highest peak apart from the iodine in the Fo syntheses and showed a high positive density in the difference syntheses. The improvement in the reliability factor, R, for the set of structure factors just calculated supported the suggestion that it was chlorine. A chlorine atom in this compound should show a chlorine-chlorine vector peak in the Patterson synthesis. This should be higher than all others but the iodine-iodine and chlorine-iodine vector peaks. With the known co-ordinates of the iodine and supposed chlorine, the co-ordinates of the corresponding vector peak positions were calculated. Some of these are starred in the Patterson - Harker maps of figs. 15, 16 and 17.

Those peaks on the Patterson, higher than those starred are tails of the iodine-iodine or iodine-chlorine vectors. Thus the Patterson synthesis indicated the presence of an element heavier than either oxygen or carbon but lighter than iodine.

The chemical analysis of COB had shown[39] beyond doubt that the compound contains only the elements, carbon, hydrogen and oxygen.

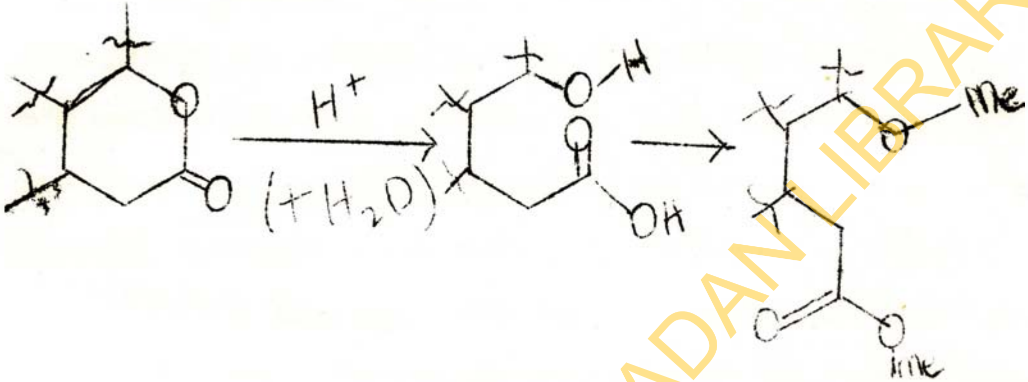
Its accurate mass spectrum agrees[39] with the formula $C_{27}H_{32}O_7$. The methylation product COB (II) contains[39] the same elements. But the chloroacetyl derivative of COB(II) was not, until this stage, analysed to find the chlorine content. This was due to the poor yield of the reduction product of COB(II). The yield of the chloroacetyl derivative was itself poor. With the indication in the infra-red spectrum of the introduction of the chlorine atom, it was supposed to be present only in the form of the chloroacetyl radical.

A quantitative analysis of the chloroacetyl derivative was carried out at this stage. The results indicated two chlorine atoms per molecule. This provided chemical confirmation of the suggestion that atom 39 was a chlorine atom. It was later established[16] that the methoxy group attached to atom 17 in COB(II) is labile and easily replaced by chlorine on treating COB(II) with HCl.

Further Structure Factor Calculations

A chlorine atom was inserted into the position of atom 39 in the model and with the same position and temperature parameters, the structure factor for the 991 reflections were calculated for formular $C_{28}O_7ClI$. The scale factor was 1.416 and the reliability index R remained as in the last calculation 0.204. The highest difference between the structure factors, 8.0, was still for reflection 4 0 0 Though there was no improvement in the R-factor, there was a slight improvement in the degree of agreement of the independent values of the structure factors as indicated, for instance, by that of 4 0 0

It was at this stage suggested[39] that the introduction of two methoxy groups on methylating COB involved a break down of the lactone ring by acid hydrolysis followed by methylation with the resultant addition of the elements of dimethylether.



But a propionate group had been introducted into the structure by the interchange of the chemical identity of atoms 15 and 36 as a result of the previous difference synthesis. In the light of the new evidence, this change was reversed producing a - CH_2COOMe ester. The temperature constant of atom 36 was increased from 5.0 Å^2 to 5.7 Å^2 because of its negative density in the "difference". That of atom 15 remained unaltered.

The structure became as seen in fig 42.

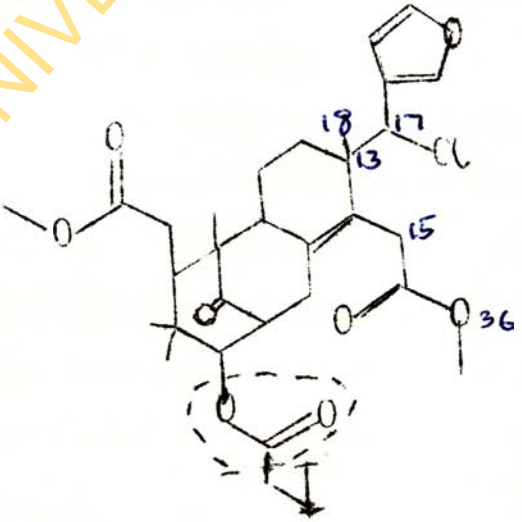


Figure 42.

Without including the parameters of atoms 27, 28 and 33, circled with a broken line in fig. 42, close to the iodine atom, the structure factors were calculated for the 991 independent measurable reflections. The scale factor was 1.416 and the reliability index reduced to 0.198. This decrease could be attributed to the change in chemical identity of atoms 15 and 36 and perhaps to the unsatisfactory parameters of some or all of atoms 27, 28 and 33.

There were now 29 reflections with difference in structure factors above 3.0 of this number, 10 had differences above 5.0 and the highest difference of 7.9 was for reflection 2 1 11

Fourth Fo and Difference Syntheses

The phasing was now based on 37 atoms, $C_{28}O_{7}ClI$. But there were three other atoms around the iodine atom which were known to be there but were not included in the calculation. Assuming the presence of these atoms, a structure written in fig. 42, has the formula $C_{30}H_{38}O_{8}ClI$. This gives a molecular weight of 688.4 which agrees well with the experimental value. This was further evidence in support of the correctness of the present structure, but there were still a few points to be clarified.

From the stereochemical point of view, there were fairly large deviations from the accepted bond lengths and angles. The worst was the bond length of 1.85 Å between C(13) and C(18). From the crystallographic point of view, there were:

(a) the propriety of eliminating atom 53 (p.73)

(b) the reality or otherwise of a peak of 1.06 e/Å^{3} that seemed to link atoms 15 and 18

(c) the problem of accounting for those peaks with heights above $1.0\ e/\AA^3$.

(d) the determination of more correct co-ordinates for the atoms around iodine.

From the chemical point of view, it was suggested[46] that the present structure was entirely acceptable except for the probable migration of C(18) from attachment at C(13) to C(17). In the X-ray analysis, there had been nothing to suggest such an attachment so far but it was rather disturbing that C(18) was so distant from C(13).

A difference synthesis would serve to refine the structure and an Fo synthesis combined with this would clarify those points that were still in doubt.

The approximate electron density distribution were calculated with the observed structure factors and phase angles in the seventh phasing. The assumed values of the structure factors of the 312 unobserved reflections were also scaled by 1.416 - the same as used for the 991 observed reflections. All the 1303 reflections were used in calculating a difference synthesis.

The results show that "atom" 53 could at best be considered a hydrogen atom. In the Fo synthesis, it had a height of $1.0\ e/\AA^3$, marked X in fig.43A. In the 'difference', there was nothing remarkable about its omission. It had a density of $0.65\ e/\AA^3$, marked X in fig. 43B. These proved unequivocally that this "atom" should be omitted and completely ruled out a possible ring D as proposed in fig. 31A. (p.76A)

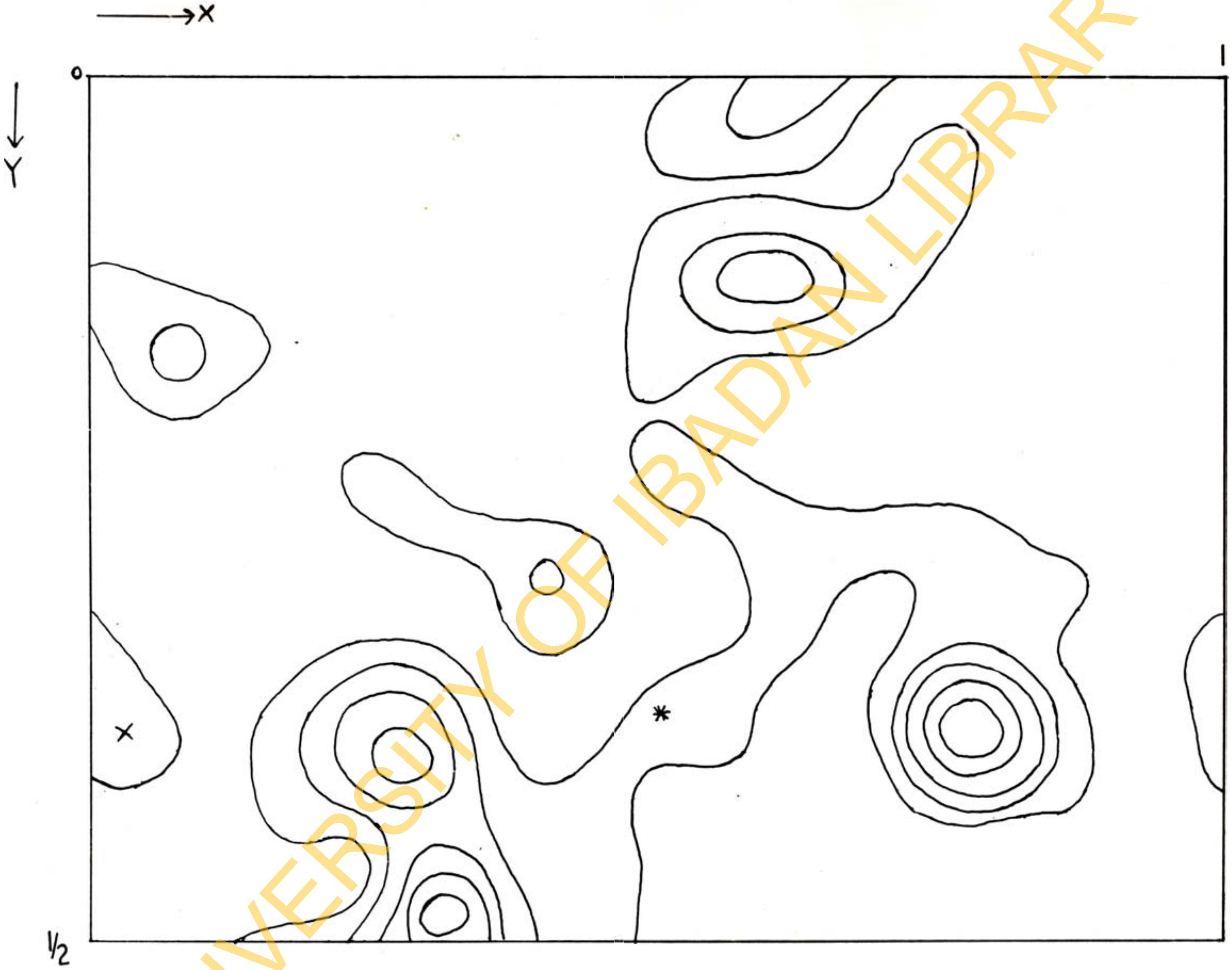


Figure 43A

Z = 18/60 ρ₄

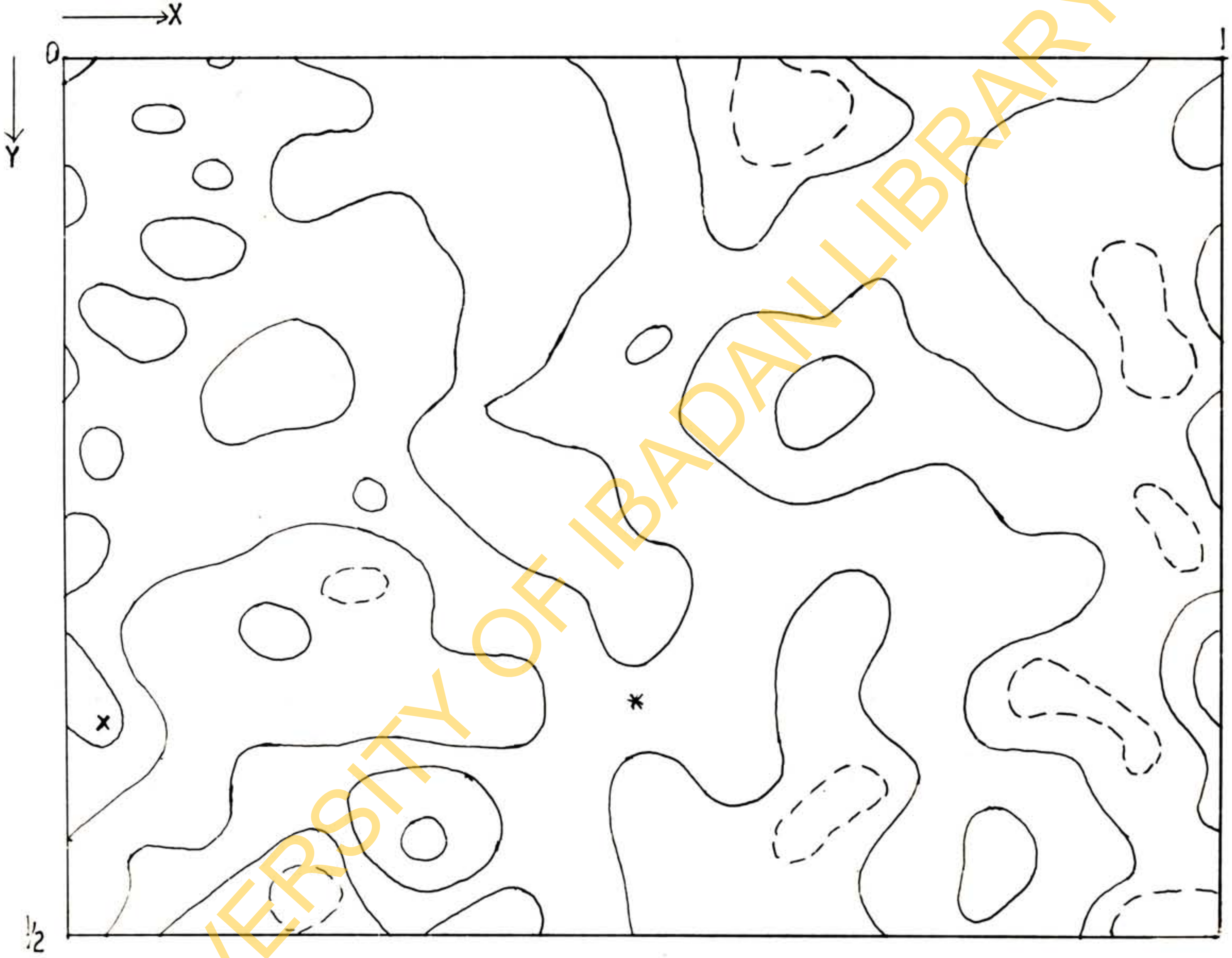


Figure 43B

4th DIFF Z = 18/60

On the same maps could be seen, starred, the site of "atom" 55, the existence of which led to the formation of a bicyclo octane system. In the Fo synthesis, the peak height became 0.4 $e/\AA^3$ and in the difference it was 0.26 $e/\AA^3$. Thus its elimination was proper. The peak that was regarded as "atom" 42, marked X in figs. 44A and 44B, decreased from 1.7 $e/\AA^3$ in the previous Fo synthesis to 1.4 $e/\AA^3$ in the present. In the 'difference' it decreased from 1.39 $e/\AA^3$ in the third to 1.16 $e/\AA^3$ in the fourth. It also moved farther away from atom 39. With atom 39, as chlorine and bonded to C(17), a bond distance of 2.2 Å between 'atom' 42 to 'atom' 39 was inconceivable. "Atom" 42, peak 18 in Table 14, must be a product of errors. Starred on the same maps, fig. 44, is the peak that seemed to link atoms 15 and 18. In the Fo synthesis, it decreased from 1.0 $e/\AA^3$ in the third to 0.9 $e/\AA^3$ in the fourth. In the difference, it decreased from 1.07 $e/\AA^3$ in the third to 1.02 $e/\AA^3$ in the fourth. It previously had peak heights of 0.4, 1.60, 0.65, and 1.02 $e/\AA^3$ in the first and second Fo syntheses, first and second difference syntheses respectively. It was still the same distance of 1.1 Å from C(18) and 2.1 Å from C(15). The distance of 1.1 Å would indicate a carbon-hydrogen bond. The fall in peak height from $\rho 2$ to $\rho 4$ does not suggest a real carbon atom. Besides, a density of 1.1 $e/\AA^3$ inserted as "atom" 55 in $\rho 3$ was shown to be spurious. To insert a lower density which does not show any tendency to increase with improved phasing is not reasonable. It was therefore concluded that this peak, number 19 in Table 14, was probably a hydrogen atom.

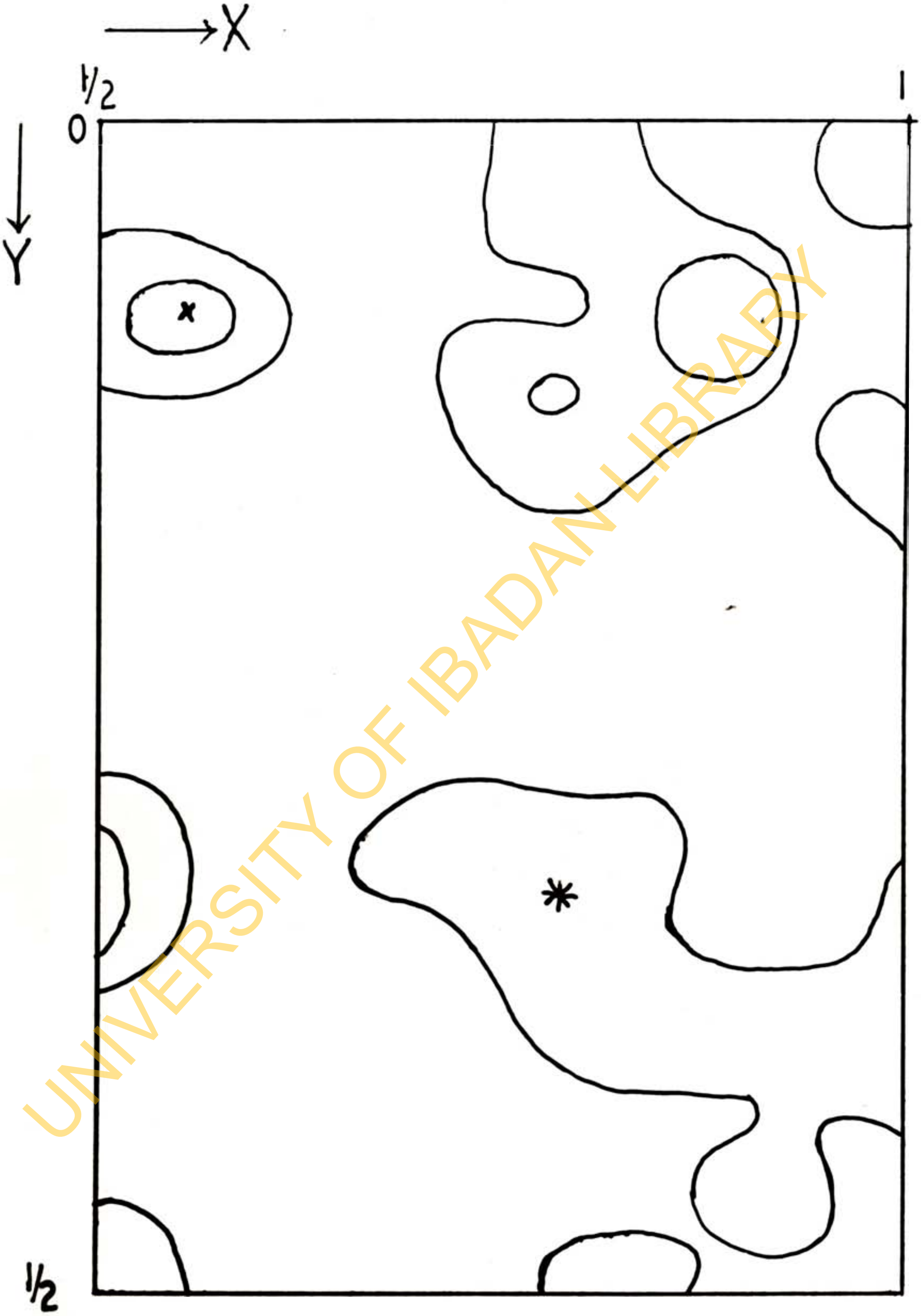


Figure 44A

Z = 8/60 ρ_4

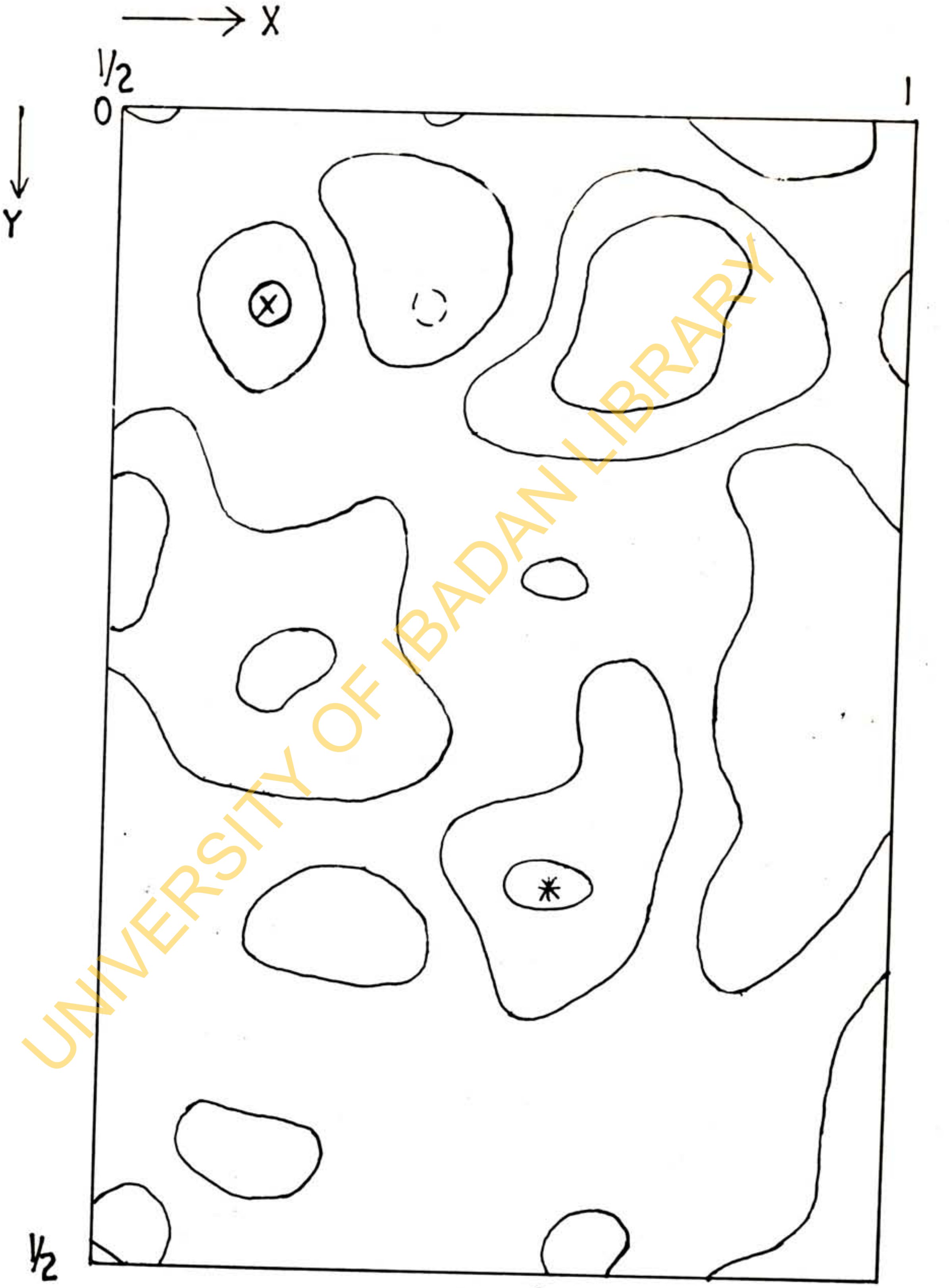


Figure 44B

$Z = 8/60$ 4TH DIFF

TABLE 14

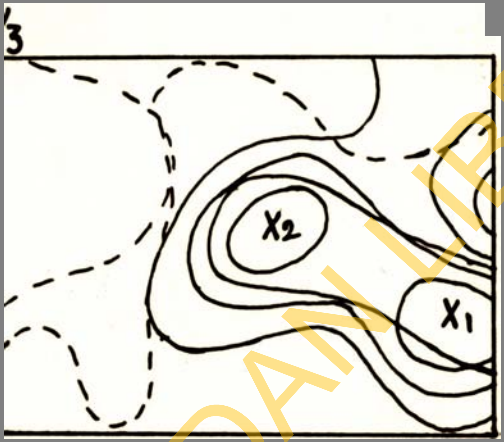
	Co-ordinates in 60th			Peak Heights	Peak Heights
	x	y	z	in ρ4 in e/Å^3	in 4th Diff.
1	40	30	4	1.0	1.13
2	44	5	5	1.1*	-0.03
3	48	9	6	1.3*	0.47
4	8	11	6	3.1*	0.41
5	22	5	7	2.2*	0.03
6	16	10	7	1.1*	0.32
7	16	1	7	1.5*	0.21
8	4	15	7	1.1*	0.40
9	38	9	7	1.0	0.0
10	25	5	10	1.2	1.11
11	34	2	12	1.1	0.77
12	48	8	13	1.2	0.32
13	2	23	18	1.0	0.65
14	40	25	21	1.2	0.67
15	52	25	23	1.0*	0.28
16	52	29	24	3.3*	0.41
17	32	24	27	1.2	0.57
18	34	5	8	1.40	1.16
19	48	20	8	0.9	1.02
20	24	21	29	0.8	1.0

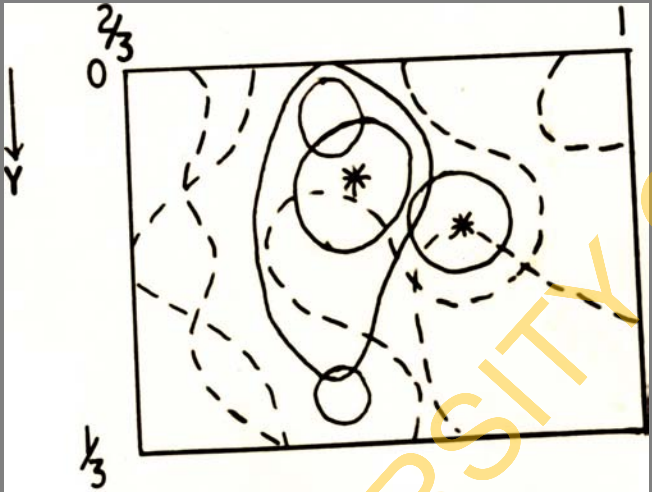
* Iodine satelites.

In the Fo synthesis, apart from those peaks obviously representing the three atoms close to the iodine atom, there were 18 others, still greater than 1.0 $e/\text{Å}^3$ and not regarded as atoms. These are shown in Table 14. Nine of them were easily seen, by symmetry to be iodine satelites. The seventeenth was most probably a ripple produced by the anisotropic temperature vibration of the chlorine atom. The densities of the remaining ten but numbers 1, 10 and 18, fall below 1.0 $e/\text{Å}^3$ in the "difference". Less significance was therefore attached to these. The difference synthesis showed five peaks of heights 1.0 $e/\text{Å}^3$ and above. Two of these, numbered 19 and 20 in Table 14, had densities below 1.0 $e/\text{Å}^3$ in the Fo synthesis. The nineteenth or "atom" 55 had been discussed and shown to be spurious (p. 95) while the twentieth had a density of only 0.5 $e/\text{Å}^3$ in the third Fo synthesis. It was very probably therefore of no significance.

Of the remaining three, the eighteenth or "atom" 42 had been considered and shown to be spurious (p. 95). The first was at a distance of 1.95 Å from C(17). In the Fourier calculations, this position had densities of 1.13, 0.84, 0.38, 1.12, 1.15 and 0.8 $e/\text{Å}^3$ in the third, second and first difference syntheses and third, second and first Fo syntheses respectively. The tenth was quite isolated and its contours were irregular. It was decided to leave these out .

The peaks around the iodine were many, as can be seen from Table 14. In the Fo synthesis, the real atomic peaks were not significantly higher than the spurious ones. But with the aid of the difference synthesis, the real atomic peaks were readily discerned. However, the peak centres in both syntheses were not the same. For C(28) and C(27), marked X1 and X2 in fig. 45A, the peak centres in the two syntheses were not significantly





$Z = 1/10$ ρ_4 & 4TH DIFF

different. But the peak centre of atom 33, starred in fig. 45B, came out in two different positions, about 1.0 Å apart in the two syntheses. The centre in the Fo synthesis was about 2.8 Å away from the iodine atom. Such iodine-oxygen distance in the acetate group was given as 5.7 Å by Fridrichsons and Mathieson[47] and as 4.02 Å by Robertson[14] and others. The peak centre in the difference was about 4.0 Å. But in the Fo synthesis, this atom had fairly spherically symmetrical contours and the contours in the "difference" were rather distorted. Also the peak centre in the "difference" was more consistent with a trigonal configuration about the carbonyl carbon. In fig. 45, the "difference" contours, at intervals of 0.5 e/Å^3 are superposed on the Fo Fourier contours at intervals of 1.0 e/Å^3.

Table 15 shows the peak densities of these three atoms in the fourth Fo and difference syntheses.

TABLE 15

	Densities in 4th Fo Synthesis	Densities in 4th "Difference"
C(27)	2.1 e/Å^3	2.14 e/Å^3
C(28)	1.7 e/Å^3	1.94 e/Å^3
Atom 33	1.9 e/Å	1.35 e/Å^3

The peak height of atom 33 was about average in the Fo synthesis but it was the lowest in the "difference". However spurious peaks of density as high as 1.3 e/Å^3 exist in this neighbourhood (around the iodine atom) in the Fo synthesis. It was therefore decided to make use of the position parameters obtained from the difference synthesis.

Eighth Phasing

This consists of a series of trial calculations of structure factors.

(i) In order to test the reliability of the new co-ordinates of atoms 27, 28 and 33, around the iodine atom, their parameters were added to those of the 37 atoms used in the previous phasing and the structure factors were caculated. They were given a temperature constant of 4.5 $Å^2$ each. The scale factor was 1.437 and the reliability index reduced from 0.198 to 0.190. This decrease indicated that a fairly good judgment of the location of the atoms had been made.

(ii) In the difference synthesis, it was noticed that the iodine atom was still in a trough of depth - 3.61 $e/Å^3$. Its temperature factor was consequently increased from 6.70 to 7.50 $Å^2$. The position of the chlorine atom had a density of -2.46 $e/Å^3$. Its temperature factor was increased from 4.0 to 5.5 $Å^2$. With these two changes, the position and temperature parameters of the previous 40 atoms - $C_{30}O_8ClI$, were used in calculating the structure factors. The scale factor was 1.358 and the reliability index rose to 0.205.

(iii) The parameters of the three atoms (27, 28, 33) around the iodine were removed and the remaining parameters with increased B for iodine and chlorine were used in calculating the structure factors. The scale factor was 1.340 and the reliability index increased to 0.212. This result confirms that to a fairly good degree the input co-ordinates for these three atoms were reasonable and that there was not enough justification for increasing the temperature constants for iodine and chlorine.

If iodine is present in all its sites then the percentage of iodine in the sample should be 18.4. With the possible interference of the chlorine atom during the analysis, the sample analysis[18,48], showed the following percentages for iodine.

(a)[18]	17.62
	17.04
(b)[48]	14.75

In (b), no duplicate determination was performed and the result is very much lower than the others. Even so the more reliable figures are appreciably less than the calculated 18.4%.

A possible explanation for this lower percentage is that the iodine sites might not all be occupied by iodine in all the molecules. The chlorine could be left unsubstituted in a few molecules. This would then reduce the effective scattering contribution of the heavy atom by a "Site Occupation Factor"[5]. In this circumstance, it would not be correct to account for the valley in which the iodine was found in terms of thermal vibration alone. It was therefore decided to leave B at 6.7 $Å^2$ for iodine.

(iv) After a close study of the maps of both the Fo and difference synthesis appropriate shifts of atomic co-ordinates were made, based on a qualitative judgment only for all but ONE of the 37 atoms used in the seventh phasing calculation. The co-ordinates of the remaining atom was changed altogether. Atom 30, C(30) had the lowest peak density of 2.6 e/Å^3 in the fourth Fo synthesis. In the difference, it was at the centre of a valley of -1.25 e/Å^3 with a temperature constant of 4.8 Å^2. A further increase in B to account for this negative density would decrease the peak in the Fo synthesis further. This was considered rather undesirable. In the previous Fourier calculations, this position had peak densities of 2.9 e/Å^3 with B = 4.5 Å^2 in the third Fo, - 0.85 e/Å^3 with B = 4.5 Å^2 in the third difference, 1.7 e/Å^3 in the second difference, 1.15 e/Å^3 in the first difference, 1.6 e/Å^3 in the second Fo and 1.0 e/Å^3 in the first Fo. It had been suspected to be a spurious peak or an atom which was not well located. There was at a distance of 0.8 Å to this position another peak of height 1.5 e/Å^3 and 1.0 e/Å^3 in the Fourth Fo and difference syntheses respectively. Previously, this new position had densities of 1.12, 1.3, 1.33, 0.80, 1.5 and 0.90 e/Å^3 in the third difference, third Fo, second difference, first difference, second Fo and first Fo syntheses respectively. The peak height in the Fo Fourier summations, had been above 1.3 e/Å^3 from the second one and this was considered a favourable factor to make this site the atomic position. The co-ordinates of C(30) were therefore changed and it was given a temperature constant of 4.5 Å^2. The temperature constant of chlorine was also decreased to 5.0 Å^2 and the structure factors were calculated again. The scale factor was 1.427 and the reliability index reduced to 0.183. The number of differences in structure factors above 3.0 decreased to 21 of which only 6 were above 5.0. The highest difference of 9.9, was for reflection 0 2 0 The intensity of this reflection was originally too large to be estimated accurately and so this great difference was not surprising.

The main features of the structure were fairly evident at this stage. A satisfactory structural formula could be written. The refinement by difference syntheses was therefore terminated.

FURTHER REFINEMENT

Apart from the difference synthesis, methods of refinement consist of minimizing a function (Rf) of Fo and Fc. The least squares[49] method makes use of a principle first enunciated by Legendre in 1806 and later subjected to rigorous mathematical proof by Laplace (1811) and Gauss (1821). It gives Rf as follows:

$$Rf = \sum_{N} w(|Fo| - |Fc|)^2 \text{ where}$$

w is a weighting factor, and the sum is taken for all the observed structure factors. The method of steepest descent[50], based on the same principle[49] gives Rf as follows:

$$Rf = \sum W(Fo^2 - Fc^2)^2 .$$

To carry out a least square refinement, the structure factors must be roughly on the absolute scale and the phasing must be approximately correct. These conditions were roughly satisfied at this stage when the structure was already known. In addition it is essential that an appropriate weighting scheme is used.

The process of refinement involves the determination of the changes in position and temperature parameters necessary to bring about the minimization of Rf. These parameters determine the calculated structure factors and so each reflection provides an observational equation of the form

$$\sqrt{W}\,Fo(hkl) - \sqrt{W}\,Fc(hkl) = \sqrt{W}\,E$$

where $Rf = \sum w\,E^2.$

The weight w is introduced to account for the degree of reliability of each equation. This is considered proportional to the accuracy with which the observed structure factor is known. The accuracy with which each intensity is known depends on the magnitude of the intensity, the background intensity, spot shape and other factors depending on experimental efficiency. Another rough indication of this accuracy is the degree of agreement of the observed and calculated structure factors when the phasing is fairly correct. A range or point of highest degree of agreement is then selected and the reflexions are weighted according to one of the following schemes[51]:

(i) $\sqrt{w} = |Fo| / F_1$ if $Fo < F_1$

$\sqrt{w} = F_1 / |Fo|$ Otherwise, where F_1 is the point of highest degree of agreement.

(ii) $\sqrt{w} = I$ if $|Fo| < F_1$

$\sqrt{w} = F_1/|Fo|$ otherwise, where $|Fo| = 0$ to F_1 is the range of highest degree of agreement.

(iii) $w = 1/(a + |Fo| + c\,|Fo|^2)$ where a and c are constants of the order of 2Fmin and 2/Fmax respectively. This scheme essentially puts less weight on very large and very small $|Fo|$. Besides this expression for w, there are[52] a few others, all functions of $|Fo|$ designed to reduce w for very large and very small Fo. It has been suggested[51,52] that the weight w may also depend on sin θ/λ, but no weighting scheme has yet been designed which is a function of both $|Fo|$ and Sin θ/λ.

A modified form of Hughes[53] weighting scheme (Scheme 2) was considered suitable for the 991 observed reflections. F_1 was 12.0 which is approximately 8 Fmin. For the first cycle of refinement, 161 parameters made up of x, y and z co-ordinates and temperature constants for the 40 atoms together with the absolute scale factor were refined. The reliability index decreased from 0.183 to 0.156. The greatest shifts were the absolute scale factor (from 1.427 to 1.200) and the temperature parameters for atoms 2, (from 4.6 to - 0.12), 8, (from 3.6 to - 1.6), 30, (from 4.5 to 8.99) and 33, (from 4.5 to 9.01).

That atoms 30 and 33 should develop such high temperature factors suggested that they were not well located. Atom 30 was put in one of two possible sites, both being under 1.0 Å apart and about 1.5 Å distant from adjoining atom 36. The peak centre of atom 33 was uncertain. Two different peak centres were observed in the fourth Fo and difference syntheses. The site from the difference synthesis was geometrically more reasonable and had been inserted for refinement.

Considering the positions of C(2) and C(8) in the structure, a change of chemical identity, as a possible explanation for the negative temperature factors was not feasible. It was therefore decided to keep the previous temperature factors constant and refine the occupation numbers. After another two cycles, the reliability index decreased to 0.138. The occupation numbers of C(2) and C(8) became 1.195 and 1.362 respectively. Assuming some mesomerism within the molecule, resulting in C(2) and C(8) becoming negative centres, one would still not expect the occupation number of either of these atoms to be as high as 1.362. The occupation numbers were therefore reduced to 1 and thermal parameters of 4.5 Å^2 refined.

The temperature constants of the carbon atom (atom 30) in the methoxy group of the ester attached to ring C and atom 33 (carbonyl oxygen of the iodoacetate) rose to 9.04 Å^2 and 15.2 Å^2 respectively. During the course of the analysis, atom 30 had alternated between two positions each about 1.55 Å away from the adjacent oxygen atom and about 0.7 Å away from each other. It was suggested[54] that atom 30 could partly occupy the two positions. A partial occupation of the two positions would induce serious vibrations in the adjacent oxygen atom, resulting in a low electron density at the atomic centre. This had been found true to the extent that the oxygen atom was at one stage temporarily assumed carbon. In the following cycles of refinement, atom 30 was given an occupation number of 0.5 in the two positions. Its thermal parameter was kept constant at 4.5 Å^2 and the occupation numbers were refined.

The temperature constants of the other two atoms around the iodine, atoms 27 (carbonyl carbon in the iodoacetate group) and 28 (carbon bonded to iodine) had also increased from 4.5 Å^2 to 6.88 Å^2 and 4.64 Å^2 respectively. These values suggest that the two atoms were approximately correctly located. Their positions together with that of atom 32 (the oxygen atom in the iodoacetate bonded to ring A) necessarily fix the position of atom 33 since the four atoms must be in the same plane. However, the location of atom 33 in this plane was adopted with reservation as two well defined peak centres were observed in the fourth Fo and difference syntheses (fig. 45). Its high temperature factor was indicative of the unsatisfactory nature of the present position. However, with the same position parameters and the temperature constant changed to 4.5 Å^2, another three cycles of refinement resulted in a B of 24.2 Å^2 for atom 33. It then became obvious that the process of refinement had completely removed atom 33 from its location in the fourth 'difference' synthesis.

A partial occupation of two positions by an atom should show considerable densities in the two types of synthesis. This was not true for atom 33. However, in favour of the idea of partial occupation was the high thermal parameter of the adjacent carbonyl carbon (atom 27). A double bond between atoms 27 and 33 would induce serious vibrations in atom 27 if atom 33 were to partially occupy two positions. Also the direction of vibration of atom 27 should also be the direction of the vector between the two partially occupied positions. However, the second position was considered too close to the iodine atom and would further increase the O-C-O angle (32, 27, 33). The initial position and thermal parameters were therefore inserted again for atom 33 in the next cycle of refinement. Its temperature constant was left unrefined and the occupation number was refined. The reliability index before this was 0.1292 and the refined occupation numbers of atom 30 were 0.584 and 0.416.

The last cycle of refinement gave a reliability index of 0.1298 and occupation numbers of atom 30 became 0.574 and 0.426. It also reduced the occupation number of atom 33 to 0.586.

The deficiencies shown by the results of this refinement must be due to the degree of accuracy of the experimental data. Apart from the fact that there was no absorption correction, the scaling of the intensities obtained from the different layer photographs was merely an approximate one. This could be improved by correlating the Fo and Fc for each layer separately.

With the last set of position and thermal parameters(table 17) structure factor bond angles and bond distances were calculated. These are shown in tables 16 and 18 and figures 46 a and b. A last set of Fo and difference syntheses were calculated. Table 13 shows the peak densities obtained in these syntheses These are discussed later, (page 112).

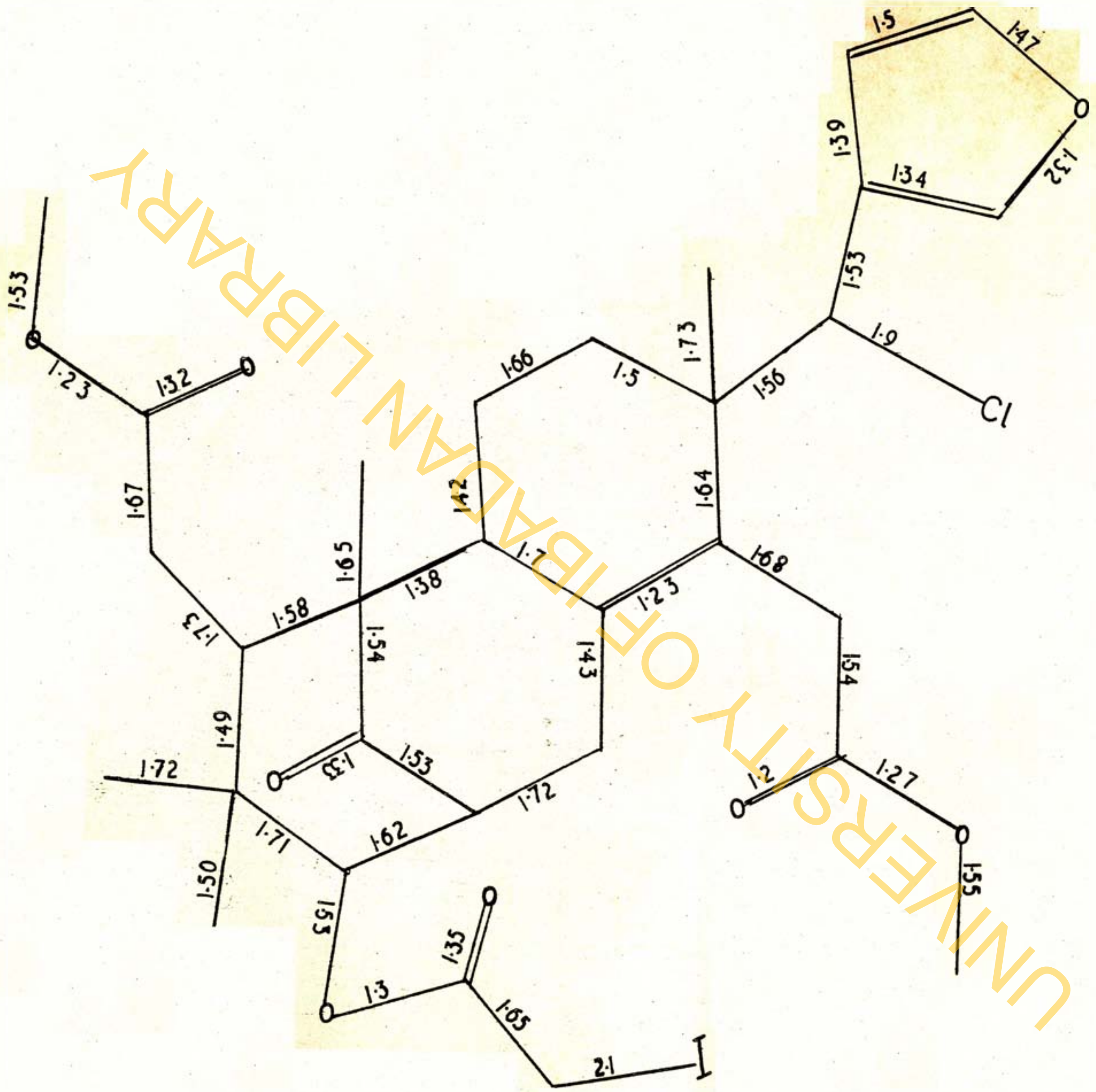
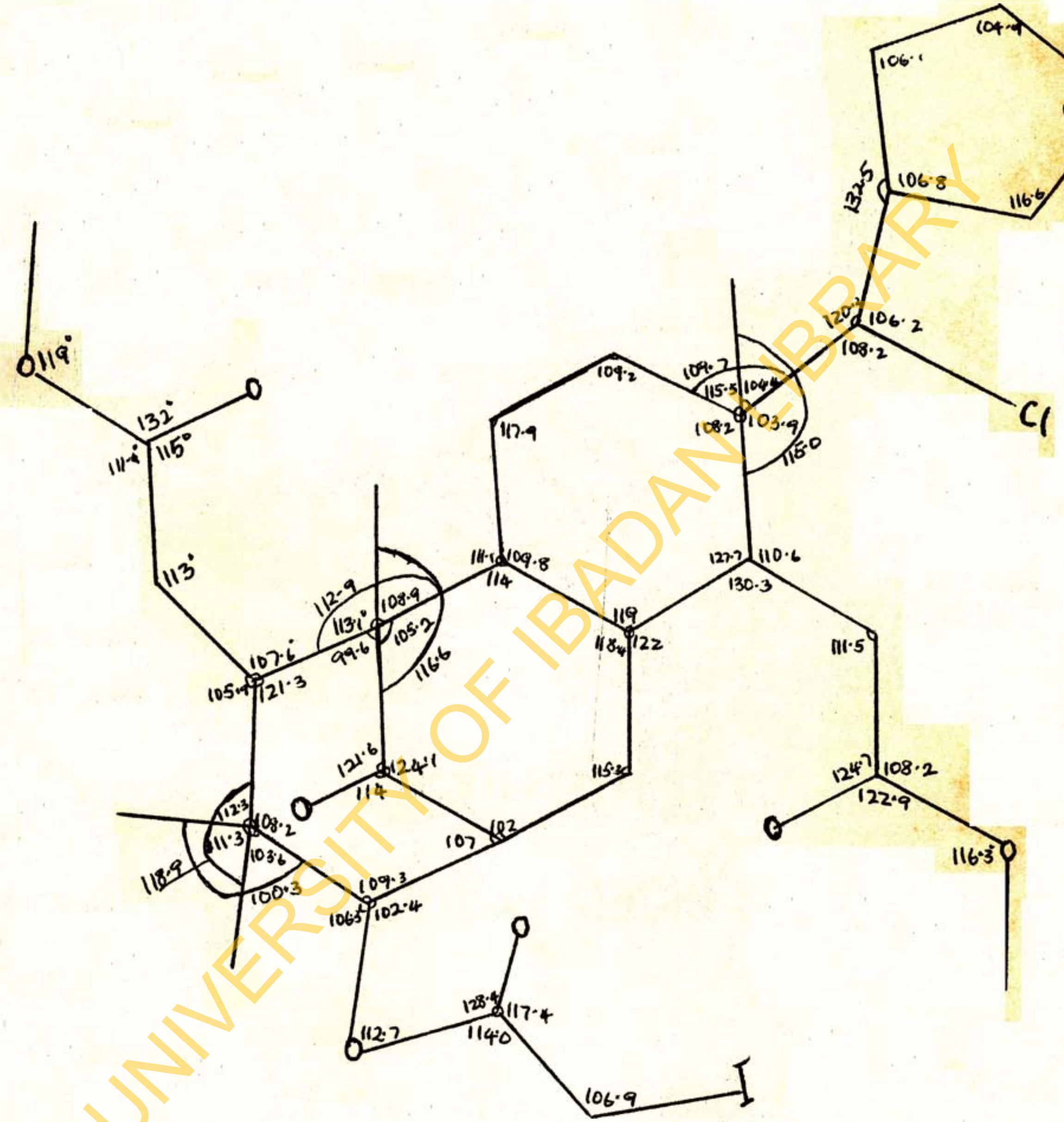

1·5
1·47
O
1·39
1·34
1·32
1·53
1·53
1·9
Cl
1·73
1·66
1·5
1·56
O
1·23
1·32
O
1·67
1·42
1·64
1·65
1·7
1·38
1·68
1·23
1·73
1·58
1·54
1·43
1·54
1·49
1·53
1·72
O
1·33
1·72
1·2
1·27
O
O
1·71
1·62
1·50
1·53
1·55
O
1·35
1·3
O
1·65
2·1
I

Figure 46A



119°
132°
115°
111.4°
113°
107.6
105.4
121.3
112.9
113.1°
108.9
99.6
105.2
116.6
111.1
109.8
114
117.9
109.2
109.7
115.5
104.4
108.2
103.9
115.0
120.2
106.2
108.2
Cl
132.5
106.8
106.1
104.9
104.6
116.6
127.7
110.6
130.3
119
118.4
122
111.5
124.7
108.2
122.9
116.3
115.3
121.6
124.1
114
107
102
112.3
108.2
111.3
103.6
118.9
100.3
109.3
106.5
102.4
128.4
117.4
114.0
112.7
106.9
I

Figure 46B

DISCUSSION

The structure of iodoacetate derivative of Substance B (Cedrela Odorata)

Molecular Structure

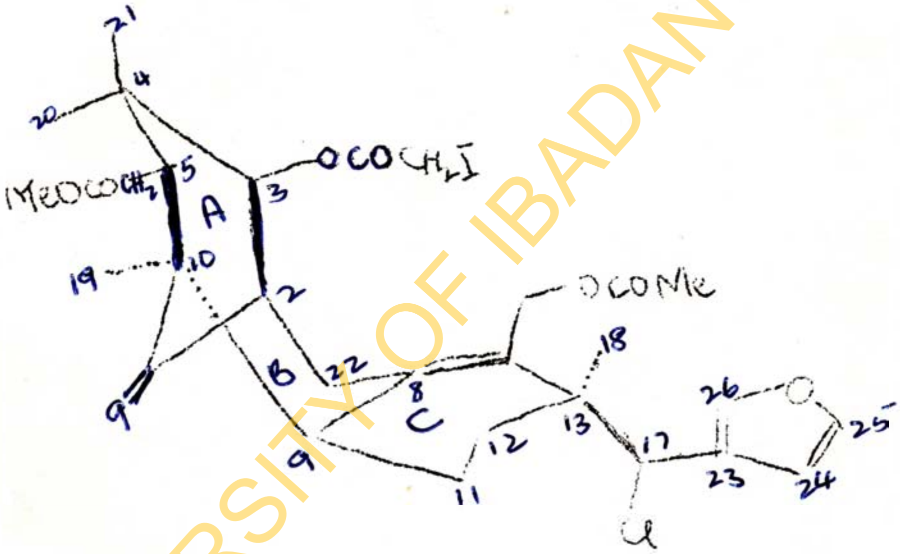
The structure derived from the X-ray analysis is defined by the set of position parameters listed in table 17. Atoms 1 to 30 are carbon and atoms 31 to 38 are the oxygen atoms. Atom 39 is chlorine and atom 40 is the iodine Others given in table 13 are the fictitious atoms that came up during the course of the analysis. The resulting interatomic distances and valency angles are not considered to be individually accurate, but table 16, gives the ranges and mean values, which are comparable with accepted values.

The molecule is folded into two layers, seen in projection in fig.47b, one layer being distinctly marked out by the double bond between C(8) and C(14)togethe with rings B and C. Ring B, in the chair conformation, is fused to ring A in the boat conformation at C(2) and C(10). The molecule folds over at ring A to which the iodoacetate group is attached. This substituent forms part of the second layer.

TABLE 16

Region of Molecule	Type of bond or angles	No.	Range	Mean Value
Rings	C - C	15	1.39 - 1.75	1.56
	C = C	3	1.23 - 1.50	1.36
	C - O	2	1.32 - 1.47	1.40
Side Chains	C - I	1	2.10	2.10
	C - Cl	1	1.89	1.89
	C - O	7	1.23 - 1.75	1.49
	C = O	4	1.20 - 1.35	1.29
	C - C	11	1.50 - 1.73	1.54
Molecule without furan ring	Trigonal	18	111.4 - 132°	121.2
	Tetrahedral	33	102.4 - 114.1°	108.3

The ester group attached to C(5) and the furan ring are also in the second layer. The carbo methoxyl group attached to C(14) lies approximately in the same plane as rings B and C projecting out of this plane with its carbonyl oxygen to the opposite side of the furan ring. Each molecule has the conformation



The stereochemical configuration of the molecule largely conforms with the staggered trans concept, accepted[55] for complex organic structures but for the limitations imposed by the distortion produced by the double bond and the peculiar linkage of rings A and B. Ring A perhaps has to adopt the boat conformation in order to reduce possible intra molecular repulsive forces between the substituents on C(3), C(4) and C(5). Moreover, a chair conformation for ring A, would so distribute the gem dimethyl groups in space that the present cis-conformation of the iodoacetate group to C(22) would not be feasible. The trans-conformation, on the other hand, would result in creating a vast volume of empty space, which could possibly be filled by a change of space group for the crystal. Such space-filling consideration would not arise in solution and ring A could then have the chair conformation.

Arrangement of Molecules in the Crystal

The packing is illustrated by the projection diagrams of fig. 47 (a) and (b). There is no possibility of intermolecular hydrogen bonding, since none of the highly electro-negative atoms in the molecule is bonded to hydrogen. The molecules are in fact, held together by Van der Waals forces and oriented to give maximum packing efficiency. Since a molecule roughly occupies a rectangular box (of dimension one by half by half of those of the unit cell) with an empty space between ring A, connecting the two layers of the molecule and ring B in one layer, the packing seemed, to some extent, dependent on the attempt to fill this space. This seems best achieved by arranging the molecules around sets of two-fold screw axes such that the side chain in one molecule projects into the empty space in the rectangular box containing the adjacent molecule. The intermolecular distances are in all cases above 3.0 Å. The closest contact of 3.5 Å and 3.51 are (1) between carbon C(30) of the methoxy group of the ester attached to ring C at C(14) and oxygen (atom 35) of the carbonyl group of the ester attached to ring A at C5, shown with dotted lines in fig. 47(a) and (2) between C(29)(carbon of the methoxy group of ester attached at C(5)) and O(1) (atome31) shown in dotted lines in fig.47b respectively.

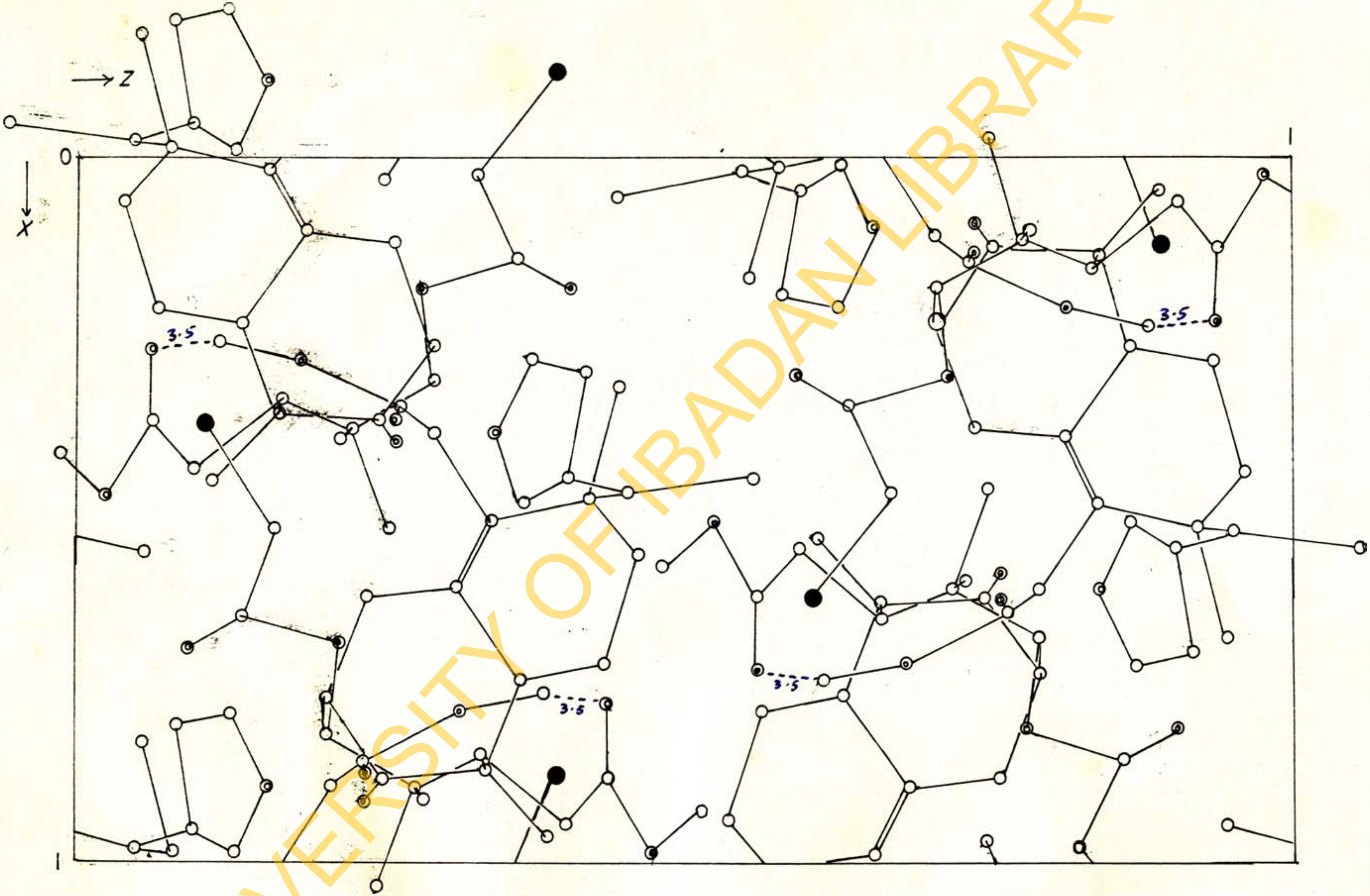


Figure 47A

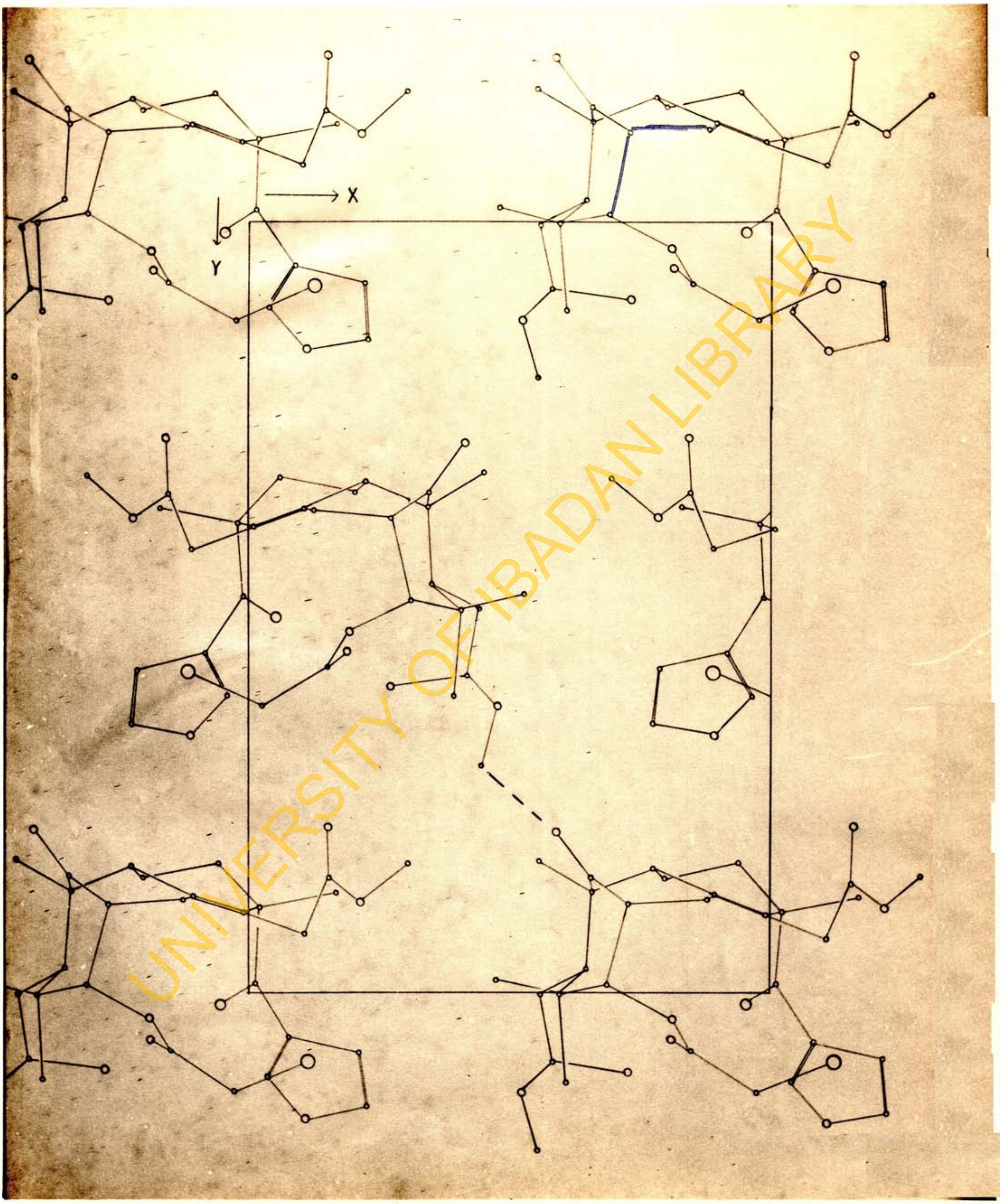


Figure 47B

The low melting point of 220°C for a structure of molecular weight 690 is consistent with Van der Waals bonding for the molecules.

Reliability of Structure

The analysis began with very little knowledge of the actual structure but uppermost in the assumption that was employed for the analysis was the idea that each molecule contains an iodine atom. There was no chemical determination of the molecular weight. The crystallographic measurements gave it as 693.0 ± 7. The final calculation from the molecular formula of $C_{30}H_{38}O_{8}ClI$, corresponding to a molecular weight of 688.4, is reasonable when compared with the experimental value. This means that there is very little probability of having left out an atom or that a fictitious atom had been included.

Moreover, the final Fourier calculations (fifth Fo and Difference syntheses) confirm the accuracy of the number of atoms. In the Fo synthesis, there were still a few density peaks above 1.0 e/Å^3, produced no doubt by the specific diffraction effects[27] resulting from the presence of the iodine atom. In the difference synthesis, the highest density, apart from iodine, was 0.67 e/Å^3. There is therefore no density peak that could conceivably be an atom.

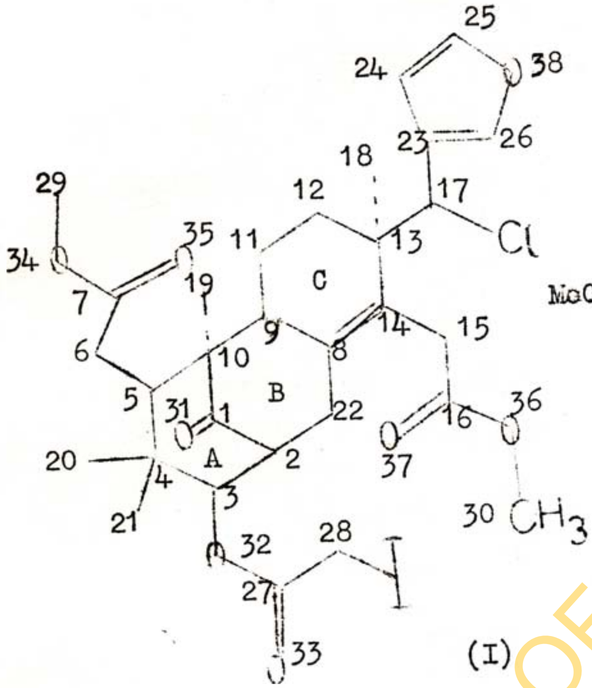
The chemical identity of the atoms were all along correlated with chemical evidence. In two instances, one involving the chlorine atom and the other a trial exchange of the identities of atoms 15 and 36 (Oxygen of the methoxy group in the ester attached to ring C) mistakes had been made in the course of the analysis, and these have been corrected.

One feature of the intensity photograph was the general weakness of the reflections from planes with small spacings. Consequently the resolution of the electron density peaks is limited. The position parameters of the atoms are therefore of limited reliability. The rather low number of observed reflections indicates some disorder in the crystal structure. Correlating this with the complexity of the molecule it has been suggested[38] that where, as in this case, complicated molecules are held in the crystal by comparatively weak intermolecular forces, it is unlikely that the atoms will be so precisely ordered throughout space as they are in simpler crystal structures. As a result of this effect together with specific diffraction rings around the iodine atom, it had not been possible to determine the position parameters of atom 33 (carbonyl oxygen in the iodoacetate) at least to the same degree of accuracy as the other atomic positions. Its final position is 3.36 Å from the iodine atom.

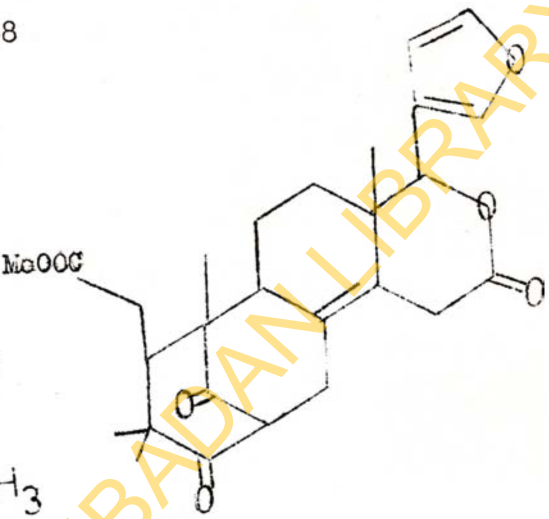
Thus accurate measurements of inter-atomic distances and valency angles could not be obtained from the experimental data, but the X-ray analysis has revealed the most essential geometrical features of the molecule.

The Structure of Cedrela Odorata Substance B

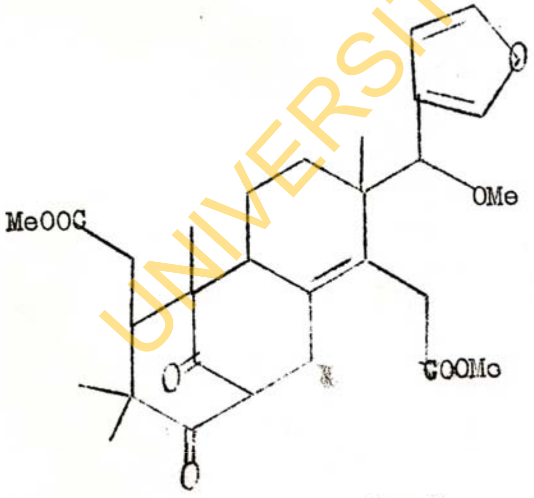
On the basis of the crystallographic evidence for the iodoacetate[56] and chemical evidence[12], it has been suggested[56] that COB has the structure (II) on page 113A. It was broken down to (III) during the process of methylation. The methylation product (III) was further reduced to (IV) which reacted with chloroacetyl chloride to give a chloroacetate. In the same step, the methoxy group on C(17) was replaced by a chlorine atom. The chloroacetate was then converted into the iodoacetate.


25
24
38
23
26
18
17
29
12
Cl
35
11
13
34
19
C
7
15
14
9
6
10
8
36
5
31
1
B
16
22
20
A
2
37
4
21
3
30 CH3
32
28
27
33

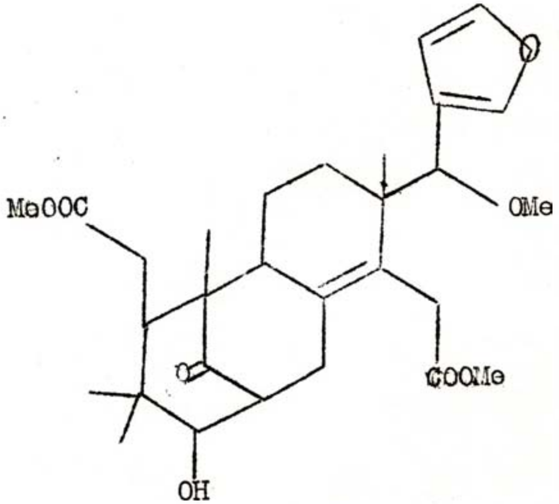
(I)


MeOOC

(II)


MeOOC
OMe
COOMe

(III)


MeOOC
OMe
COOMe
OH

(IV)

Flow Sheet

Patterson
↓
1st Phase Calculation with I. only; R = 0.37
↓
1st Fo Fourier giving 15 atoms regarded as carbon
↓
2nd Phase Calculation with $C_{15}I$; R = 0.322
↓
2nd Fo Fourier giving I + 31 atoms all carbon
↓
Phase Calculation, R = 0.253
→ 1st Difference giving I + 41 atoms all carbon
↓
3rd Phase Calculation with $C_{41}I$, R = 0.257
↓
4th Phase Calculation with $C_{35}O_2I$; R = 0.251
↓
2nd Difference giving $C_{29}O_2I$
↓
5th Phase Calculation with $C_{29}O_2I$; R = 0.253
[Phase Calculation from 2nd Fo, R = 0.253]
↓
3rd Fo Fourier giving $C_{32}O_9I$
↓
6th Phase Calculation with $C_{32}O_9I$; R = 0.23
↓
3rd Difference giving $C_{31}O_9I$
↓
7th Phase Calculation with $C_{31}O_9I$; R = 0.223
with $C_{28}O_8I$; R_2 = 0.216
with $C_{28}O_9I$; R_3 = 0.204
with $C_{28}O_7ClI$; R_4 = 0.204
with $C_{28}O_7ClI$; R_5 = 0.198
↓
8th Phasing with $C_{30}O_8ClI$; R = 0.183
↓
Least Squares Refinement

R = 0.129

TABLE 13

Atom No.	Atom Type	Densities in e/Å^3									
		ρ_1	ρ_2	D_1	D_2	ρ_3	D_3	ρ_4	D_4	ρ_5	D_5
1	Carbon		2.67	0.65	2.2	3.3	$\bar{0}.40$	3.5	$\bar{0}.51$	4.9	0.31
2	"	0.9	1.5	1.1	1.1	1.4	$\bar{0}.20$	3.6	$0.\bar{0}6$	5.1	0.16
3	"		3.15	1.41	$\bar{0}.27$	3.6	$\bar{0}.39$	4.2	0.05	5.5	$0.\bar{4}1$
4	"	2.84	5.5	$\bar{0}.12$	0.54	4.0	$\bar{0}.55$	3.8	$\bar{0}.28$	4.5	$\bar{0}.44$
5	"		2.7	0.10	2.8	4.4	0.05	4.5	$\bar{0}.05$	4.9	$0.\bar{4}9$
6	"	1.4	2.4	1.4	0.5	2.0	$1.\bar{0}6$	3.9	$\bar{0}.34$	4.6	0.39
7	"		2.83	2.28	$0.\bar{0}5$	3.60	$0.\bar{3}9$	3.7	$0.\bar{2}9$	4.1	0.53
8	"	2.89	5.5	1.07	0.06	4.4	0.25	4.8	0.40	6.1	0.37
9	"		3.73	1.97	$0.\bar{3}3$	4.5	0.30	4.4	0.20	4.67	0.32
10	"	3.18	5.8	0.40	0.87	4.0	$0.\bar{4}7$	4.3	$0.\bar{0}7$	4.9	0.14
11	"		2.7	1.84	$\bar{0}.32$	3.4	$\bar{0}.25$	3.5	$0.\bar{3}6$	3.5	0.38
12	"			1.41	$0.\bar{2}4$	3.5	0.02	3.8	0.05	4.10	0.2
13	"	2.80	5.5	0.05	0.08	4.0	0.05	4.0	0.15	4.3	0.28
14	"			1.50	$0.\bar{1}5$	3.9	0.30	3.7	$0.\bar{3}1$	4.5	$\bar{0}.23$
15	"	3.18	5.2	0.18	0.31	4.2	0.30	4.1	0.02	4.9	0.32
16	"		3.86	1.46	0.75	4.4	0.10	4.3	$0.\bar{4}0$	4.6	0.31
17	"		2.2		1.84	3.7	$\bar{0}.30$	4.0	$\bar{0}.52$	4.3	$\bar{0}.30$
18.	"				1.81	2.9	$\bar{0}.20$	3.7	$\bar{0}.19$	4.2	0.11
19	"		2.84	1.70	$\bar{0}.99$	3.6	$\bar{0}.32$	3.3	$\bar{0}.50$	3.55	0.22
20	"	2.80	5.06	$\bar{0}.48$	0.39	4.3	$\bar{0}.10$	4.4	$\bar{0}.10$	5.4	$\bar{0}.43$
21	"	0.7	1.8	0.95	0.80	1.60	$\bar{0}.5$	3.9	$\bar{0}.16$	4.2	$\bar{0}.23$
22	"		2.87	1.52	$\bar{0}.32$	4.2	0.52	4.2	0.02	5.3	$\bar{0}.15$
23	"	3.25	3.8	$\bar{0}.59$	$\bar{0}.27$	3.0	$\bar{0}.70$	3.3	$\bar{0}.55$	4.2	0.42
24	"	1.14	2.87	$\bar{1}.06$	$\bar{0}.79$	3.0	$\bar{0}.54$	3.4	$\bar{0}.39$	4.1	0.37

(Table 13 Contd.)

Atom No.	Atom Type	Densities in e/Å^3									
		ρ_1	ρ_2	D_1	D_2	ρ_3	D_3	ρ_4	D_4	ρ_5	D_5
25	Carbon	0.9	2.4	1.3	1.1	1.9	$1\bar{.}20$	3.2	$0\bar{.}56$	5.6	0.31
26	"	1.5	3.0	1.0	1.65	2.3	$0\bar{.}44$	3.8	$0.\bar{2}3$	4.5	$0.\bar{0}4$
27	"							1.6	2.05	2.67	0.51
28	"							1.7	1.94	2.98	0.37
29	"					1.6	$0\bar{.}40$	3.1	$0\bar{.}90$	3.7	0.56
30	"	1.0	1.6	1.15	1.70	2.9	$0\bar{.}85$	2.6	1.02	2.9/2.1	0.63
31	Oxygen	2.8	4.4	$0\bar{.}17$	0.15	3.9	0.15	5.1	$1\bar{.}30$	6.2	0.24
32	"		3.56	1.83	$0\bar{.}14$	5.8	$0.\bar{3}0$	6.0	$0.\bar{2}6$	7.12	0.16
33	"							2.1	1.35	2.6	0.60
34	"		2.67	2.28	0.25	4.9	$0.\bar{2}5$	5.8	$0.\bar{4}3$	7.2	$0.\bar{2}1$
35	"					2.3	$1.\bar{2}9$	4.8	$1.\bar{0}2$	5.2	$0.\bar{5}3$
36			2.80	1.89	$0,\bar{6}1$	4.3	$1.\bar{3}0$	5.5	$0.\bar{3}5$	7.0	0.56
37	"	2.97	5.2	0.48	0.15	4.8	$0.\bar{7}6$	5.5	$0.\bar{9}6$	6.0	0.42
38	"	2.86	5.5	0,14	0.63	4.4	$1.\bar{0}2$	5.2	$0.\bar{6}6$	5.7	$0.\bar{4}0$
39	Chlorine	4.3*	8.5*	2.3	2.90	8.6	1.69	12.6	$2.\bar{4}6$	13.7	0.25
40	Iodine	29.8	48.1	$5\bar{.}8$	$7\bar{.}38$	43.4	4.35	41.3	$3\bar{.}7$	49.1	$1.\bar{1}8$
41	Spurious	4.63*	5.8	$4\bar{.}\underline{3}$							
42	"	2.92	4.0	$4.\bar{3}2$							
43	"			1.28							
44	"			2.03							
45	"			1.27							
46	"			1.30	$1.\bar{5}0$						
47	"			1.90							
48	"			1.36	$3\bar{.}2$						
49	"			1.40	$1.\bar{1}6$						
50	"			1.41	$2\bar{.}55$						
51	"	3.10	4.0	$0\bar{.}61$	$1\bar{.}33$						
52	"	2.84	4.4	$1\bar{.}75$	$2\bar{.}49$						
53	"		2.74	1.41	$0\bar{.}35$	3.6	$2\bar{.}21$				
54	"	0.55	0.60	0.65	1.73	2.6					
55	"		1·60			1.1					

TABLE 17

Position and Temperature Parameters

Atom Type and No.	x/a	y/b	z/c	B
C(1)	0.3454	0.3476	0.2421	4.46
C(2)	0.2704	0.3887	0.2974	0.35
C(3)	0.3116	0.4897	0.2995	2.18
C(4)	0.5980	0.0074	0.2735	2.14
C(5)	0.3506	0.4668	0.1642	4.63
C(6)	0.4372	0.4977	0.0910	2.81
C(7)	0.6112	0.0843	0.4441	4.10
C(8)	0.1066	0.3708	0.1899	0.24
C(9)	0.2265	0.3376	0.1380	1.44
C(10)	0.3415	0.3669	0.1594	3.00
C(11)	0.1975	0.3480	0.0629	6.38
C(12)	0.0598	0.3283	0.0398	1.66
C(13)	0.9747	0.3906	0.0755	3.50
C(14)	0.0099	0.3959	0.1604	2.32
C(15)	0.8871	0.4215	0.2090	2.10
C(16)	0.8462	0.3472	0.2584	3.37
C(17)	0.9908	0.4833	0.0487	3.80
C(18)	0.8244	0.3644	0.0564	2.82
C(19)	0.4508	0.3211	0.1087	4.86
C(20)	0.6089	0.1137	0.2789	1.71
C(21)	0.5266	0.4798	0.2523	5.25
C(22)	0.1209	0.3687	0.2660	2.10
C(23)	0.0867	0.0536	0.4166	1.70
C(24)	0.2136	0.0755	0.4211	3.86

(Table 17 Contd.)

Atom Type and No.	x/a	y/b	z/c	B
C(25)	0.2318	0.1477	0.3693	4.58
C(26)	0.0348	0.1094	0.3746	3.21
C(27)	0.8537	0.0747	0.1578	5.8
C(28)	0.9747	0.1262	0.1690	4.50
C(29)	0.0555	0.2983	0.4805	4.59
$C(30)_1$	0.7457	0.3074	0.3792	4.48
$C(30)_2$	0.6923	0.3271	0.3579	4.48
$O_1(31)$	0.4148	0.2823	0.2659	5.05
$O_2(32)$	0.8107	0.0320	0.2150	2.20
$O_3(33)$	0.8136	0.0568	0.0938	9.06
$O_4(34)$	0.5257	0.1242	0.4735	2.65
$O_5(35)$	0.7325	0.1002	0.4411	5.91
$O_6(36)$	0.7853	0.3788	0.3103	2.88
$O_7(37)$	0.8386	0.2774	0.2397	5.26
$O_8(38)$	0.1109	0.1604	0.3397	4.47
Cl(39)	0.4498	0.0129	0.0536	4.94
Br(40)	0.1241	0.0801	0.1077	5.06

TABLE 18

Comparison of Fo and Fc

*	H	K	
	L	Fo	Fc
*	0	0	
	2	91	69
	4	116	103
	6	181	198
	8	29	96
	10	32	16
	12	36	32
	14	47	56
	16	23	6
	18	43	33
	20	28	21
*	0	1	
	2	36	40
	3	61	57
	4	59	25
	5	126	115
	6	97	84
	8	131	115
	10	69	54
	11	30	29
	12	89	91
	13	32	30
	14	30	30
	16	43	44
	18	24	16
*	0	2	
	0	>207	271
	1	42	20
	2	61	61
	3	>233	262
	4	86	94
	5	72	58
	6	114	100
	7	127	112
	8	78	68
	9	18	16
	10	59	61
	11	88	76
	12	36	28
	13	29	30
	17	43	48
	18	37	29
*	0	3	
	1	145	146
	2	143	144
	3	134	126
	4	72	59
	5	137	133
	6	33	30
	7	42	33
	8	45	43
	9	77	80
	11	47	46
	13	30	41
	14	43	36
	15	43	50
*	0	4	
	0	248	226
	1	232	213
	2	16	7
	3	225	215
	4	144	123
	5	93	83
	6	54	44
	7	150	161
	9	24	6
	10	35	32
	11	52	57
	15	31	34
*	0	5	
	1	74	79
	2	71	57
	3	92	82
	4	26	21
	5	37	26
	6	148	146
	8	73	80
	9	81	84
	10	33	23
	12	68	85
	13	32	29
	15	33	34
	16	33	43

(Table 18 Contd.)

*	L	H	Fo	K	Fc
*		0		6	
	0		38		43
	1		26		39
	2		25		31
	3		25		25
	4		114		128
	6		70		69
	8		49		45
	9		75		77
	10		107		114
	14		28		33
*		0		7	
	1		32		33
	2		144		150
	3		49		36
	4		39		45
	5		67		55
	6		115		115
	7		42		45
	8		40		31
	9		24		18
	10		44		54
	11		45		54
	12		59		62
	14		20		16
*		0		8	
	0		55		35
	1		44		43
	2		55		56
	3		118		114
	4		94		113
	6		40		39
	7		68		70
	8		75		74
	9		28		7
	10		53		51
	11		27		28
	14		33		19

*	L	H	Fo	K	Fc
*		0		9	
	1		31		28
	3		64		59
	4		22		23
	5		86		75
	6		26		28
	7		23		25
	8		23		20
	9		99		108
	11		31		28
	13		33		34
*		0		10	
	0		59		51
	1		82		84
	2		26		23
	3		50		46
	7		64		64
	13		38		44
*		0		11	
	1		36		41
	2		55		48
	3		28		31
	4		31		27
	5		32		18
	6		40		40
	9		73		77
	12		36		39
	15		32		6
*		0		12	
	0		63		50
	2		47		41
	3		46		39
	4		71		72
	5		51		42
	6		52		52
	8		33		33
*		0		13	
	1		39		32
	2		39		38

*	L	H	Fo	K	Fc
	3		17		18
	4		31		38
	5		24		20
	6		46		46
*		0		14	
	0		36		40
	3		33		30
	6		39		36
*		0		15	
	1		36		44
	6		31		22
*		0		16	
	4		35		2
*		1		0	
	2		148		152
	3		158		146
	4		95		93
	5		121		111
	6		94		92
	7		79		65
	8		68		1
	9		60		40
	10		29		15
	11		57		53
	12		56		54
	13		55		40
	14		38		17
	15		16		19
	16		63		56
*		1		1	
	2		135		126
	3		121		114
	4		136		131
	5		100		90
	6		83		73
	7		57		55
	8		80		72
	9		137		131
	10		56		52

(Table 18 Contd.)

*		H		K	
	L		Fo		Fc
	11		67		48
	12		22		18
	14		64		53
	15		65		58
*		1		2	
	1		30		28
	2		49		50
	3		94		93
	4		211		216
	5		94		97
	6		58		50
	7		113		105
	8		93		88
	9		98		87
	10		60		69
	11		52		50
	12		20		21
	13		40		43
	14		29		39
	15		26		20
	16		44		43
*		1		3	
	0		24		11
	1		76		81
	2		173		179
	3		126		128
	4		77		48
	5		78		69
	6		42		38
	7		104		119
	8		61		44
	9		30		18
	10		35		31
	11		55		51
	12		56		68
	13		36		45
*		1		4	
	0		106		105
	1		183		190
	2		85		83
	3		95		80
	4		84		82

*		H		K	
	L		Fo		Fc
	5		87		89
	6		61		55
	7		43		40
	8		62		71
	9		50		57
	10		67		68
	11		27		18
	12		32		25
	13		51		53
	14		41		41
*		1		5	
	0		83		80
	1		104		102
	2		65		58
	3		82		69
	4		92		82
	5		30		39
	6		74		76
	7		51		42
	8		73		77
	9		42		32
	10		83		87
	11		32		36
	13		40		46
	15		35		36
*		1		6	
	0		72		80
	1		120		112
	2		73		62
	3		95		108
	4		27		30
	5		24		16
	6		63		66
	7		100		109
	8		33		36
	9		31		31
	10		31		28
	11		44		43
	12		46		56
	13		29		29
*		1		7	
	0		121		105

*		H		K	
	L		Fo		Fc
	1		94		99
	2		81		84
	3		28		14
	4		70		58
	5		84		91
	6		59		56
	7		59		53
	8		40		44
	10		52		65
	11		39		38
	13		43		59
*		1		8	
	0		37		37
	1		102		103
	2		60		53
	3		90		92
	4		52		43
	5		46		48
	6		77		74
	7		33		31
	8		39		33
	9		44		47
	10		41		41
	11		37		35
	12		23		36
	14		36		37
*		1		9	
	0		35		27
	1		28		27
	2		77		78
	3		53		43
	4		17		28
	5		15		26
	6		53		50
	7		65		63
	8		41		37
	11		55		44
	13		30		25
*		1		10	
	0		91		93
	1		39		29
	2		47		50

(Table 18 Contd.)

*	L	H	Fo	K	Fc
	3		52		39
	4		48		54
	5		42		50
	6		41		39
	8		30		21
	9		34		34
	10		25		26
	11		24		38
	13		37		36
*		1		11	
	0		38		34
	1		40		38
	2		33		29
	3		47		47
	4		48		51
	5		51		52
	6		33		35
	7		38		37
	8		25		22
	9		41		43
	11		34		26
*		1		12	
	0		28		37
	2		41		53
	3		38		30
	4		38		43
	6		33		26
	8		34		36
	10		36		34
*		1		13	
	0		55		55
	4		23		16
	5		55		54
*		2		0	
	0		38		34
	1		74		42
	2		130		109
	3		17		24
	4		80		41
	5		132		139
	6		18		4
	7		64		47
	9		112		119

*	L	H	Fo	K	Fc
	10		45		35
	11		76		74
	12		22		13
	13		103		112
	14		32		35
	15		30		15
	16		16		16
	17		25		12
	18		23		15
	19		24		9
*		2		1	
	0		81		74
	1		178		191
	2		128		115
	3		126		134
	4		125		129
	5		28		26
	6		19		22
	7		138		135
	8		29		44
	9		43		38
	10		62		53
	11		165		70
	12		46		44
	13		51		48
*		2		2	
	0		58		55
	1		59		51
	2		68		70
	3		54		51
	4		60		62
	5		71		76
	6		123		138
	7		83		77
	8		90		83
	9		74		67
	10		72		71
	11		45		45
	12		61		59
	13		44		47
	16		41		46
*		2		3	
	0		174		187

*	L	H	Fo	K	Fc
	1		69		64
	2		29		29
	3		39		40
	4		89		107
	5		55		44
	6		152		140
	7		64		41
	8		109		103
	9		43		44
	10		74		70
	11		38		34
	14		61		65
*		2		4	
	0		84		71
	1		66		68
	2		99		104
	4		24		19
	5		62		55
	6		112		115
	8		50		60
	9		34		34
	10		61		48
	11		39		42
	12		64		68
	13		43		39
*		2		5	
	0		8		6
	1		57		59
	2		63		58
	3		170		172
	4		91		81
	6		71		77
	7		45		44
	8		55		51
	9		43		31
	10		56		49
	11		61		68
	14		36		38
*		2		6	
	0		28		31
	1		113		115
	2		59		57
	3		74		74

(Table 18 Contd.)

*		H		K	
	L		Fo		Fc
	4		46		42
	5		121		113
	6		31		38
	7		34		29
	8		42		38
	9		70		79
	12		48		38
	13		39		35
*		2		7	
	0		69		73
	1		76		72
	3		98		106
	5		37		34
	6		48		48
	7		100		94
	8		28		25
	9		33		26
	10		36		29
	11		63		59
	12		23		29
	13		20		24
*		2		8	
	0		28		37
	1		62		60
	2		98		95
	3		31		34
	4		26		29
	5		66		65
	6		41		38
	8		52		49
	9		51		46
	10		45		37
	11		40		44
	12		38		41
	13		30		8
*		2		9	
	0		122		118
	2		48		47
	3		33		31
	4		86		82
	6		54		49
	8		35		27
	10		16		31

*		H		K	
	L		Fo		Fc
*		2		10	
	0		45		48
	1		14		19
	2		75		80
	3		39		40
	5		24		21
	6		35		27
	7		32		34
	8		62		73
	10		42		46
	12		42		54
*		2		11	
	0		71		65
	1		34		42
	2		34		30
	3		44		38
	4		70		61
	6		40		25
	7		44		48
	9		45		41
	11		44		46
*		2		12	
	1		41		47
	5		47		53
	9		44		45
*		2		13	
	1		61		62
	7		58		53
*		2		15	
	4		45		45
*		2		16	
	2		26		34
*		3		0	
	1		145		130
	2		113		128
	3		91		93
	4		96		86
	5		51		66
	6		24		19
	7		119		124
	8		99		82
	10		35		40
	11		73		56

*		H		K	
	L		Fo		Fc
	12		66		61
	13		15		27
	15		46		29
	16		50		39
	18		30		26
*		3		1	
	0		104		110
	1		145		154
	2		91		81
	3		82		85
	4		83		103
*	7		37		41
	8		56		62
	9		38		40
	10		56		46
	11		39		25
	12		41		32
	13		40		36
	14		45		30
	15		46		47
	17		34		32
*		3		2	
	0		90		110
	1		92		96
	2		113		131
	3		61		76
	4		100		106
	5		75		75
	6		13		5
	7		60		50
	8		68		59
	9		92		97
	10		50		49
	11		41		33
	16		44		43
*		3		3	
	0		111		110
	1		102		98
	2		85		99
	3		111		123
	4		63		72
	5		38		44
	6		59		65

* Omission (see page 127).

(Table 18 Contd.)

*	L	H	Fo	K	Fc
	7		62		72
	8		81		88
	9		59		37
	10		57		39
	11		48		40
	12		51		48
	13		50		35
	14		41		41
*		3		4	
	0		149		159
	1		73		77
	3		48		49
	4		79		88
	5		67		68
	6		70		79
	7		39		27
	8		50		33
	9		53		45
	10		44		55
	11		46		50
	12		49		44
*		3		5	
	0		80		76
	1		67		65
	2		74		86
	3		115		110
	4		66		72
	5		75		81
	6		79		62
	7		72		66
	8		68		78
	9		62		61
	10		69		71
*		3		6	
	0		58		73
	1		38		47
	2		57		60
	3		34		43
	5		47		28
	6		82		95
	7		61		62
	8		53		40
	11		59		56
	12		42		45
*		3		7	
	0		80		74
	1		74		67
	3		37		36
	4		57		64
	5		76		81
	6		55		47
	7		43		39
	8		42		36
	9		39		31
	10		69		73
	13		42		40
	14		30		35
*		3		8	
	0		15		12
	1		61		60
	3		38		48
	4		69		62
	5		54		57
	6		54		63
	7		43		47
	8		44		37
	9		42		47
	11		31		34
*		3		9	
	0		33		38
	1		34		23
	2		84		76
	3		65		60
	6		52		51
	7		64		66
	9		18		30
	10		22		29
	11		42		42
*		3		10	
	0		46		46
	1		38		32
	2		28		33
	3		28		40
	4		39		44
	5		48		55
	6		39		36
	8		48		40
	11		19		34
*		3		11	
	0		48		52
	1		38		42
	2		30		36
	5		40		34
	7		41		28
*		3		12	
	0		28		15
	1		28		22
	2		28		43
	3		52		48
	4		45		53
	5		42		32
	7		30		37
	9		42		19
*		3		13	
	0		44		44
	3		42		33
	5		26		28
*		3		14	
	0		27		31
	1		38		38
*		4		0	
	0		122		138
	1		62		65
	2		103		88
	4		51		59
	6		103		94
	7		21		17
	8		94		87
	9		23		6
	10		69		68
	11		29		10
	12		31		14
	13		34		19
	14		53		52
	15		27		17
	16		16		22
	17		16		2
	18		37		40
*		4		1	
	0		123		66
	1		65		69
	2		104		112

(Table 18 Contd.)

*	H	K	
	L	Fo	Fc
	3	39	29
	4	119	125
	5	49	49
	6	117	124
	7	74	60
	8	45	44
	9	60	50
	10	59	47
	11	40	35
	12	57	48
	13	53	55
	15	49	36
	16	31	35
*	4	2	
	0	54	52
	1	59	69
	2	31	19
	3	108	126
	4	97	101
	5	71	66
	7	87	90
	8	61	61
	9	38	41
	10	60	63
	11	63	58
	17	37	34
*	4	3	
	0	51	65
	1	98	124
	2	45	47
	3	78	88
	4	36	38
	5	71	90
	7	12	7
	9	87	88
	10	47	29
	11	35	39
	12	34	26
	13	64	73
	14	14	11

*	H	K	
	L	Fo	Fc
*	4	4	
	1	84	93
	2	17	13
	3	79	77
	4	26	27
	5	48	32
	6	59	56
	7	108	104
	8	50	30
	9	42	38
	11	40	43
	13	37	40
	14	23	26
	15	24	29
*	4	5	
	1	53	48
	2	103	107
	3	59	65
	4	91	83
	5	85	70
	6	85	86
	7	32	20
	8	31	37
	9	48	54
	10	35	36
*	4	6	
	0	99	111
	1	44	44
	2	35	43
	3	43	36
	4	88	92
	5	17	19
	6	64	62
	7	44	25
	8	57	55
	9	22	21
	10	55	58
	12	44	40
	14	34	36

*	H	K	
	L	Fo	Fc
*	4	7	
	0	23	6
	1	77	64
	2	97	103
	3	24	15
	5	18	24
	6	53	54
	8	37	46
	9	23	21
	12	43	49
	13	35	37
*	4	8	
	0	78	86
	1	49	57
	3	57	50
	4	69	64
	5	15	19
	6	28	17
	7	49	52
	10	51	58
	13	18	23
	14	15	23
*	4	9	
	0	39	32
	1	71	66
	2	45	47
	4	15	24
	5	76	72
	8	24	25
	9	63	65
*	4	10	
	1	74	64
	3	70	71
	4	23	23
	5	35	29
	7	44	47
	8	25	24
	10	37	19
*	4	11	
	2	53	60

(Table 18 Contd.)

*	L	H	Fo	K	Fc
	5		14		46
	6		37		39
	9		24		30
*		4		12	
	0		53		49
	3		39		38
	4		39		40
	5		23		16
	6		23		20
	7		24		14
	12		36		15
*		4		13	
	2		43		53
	4		18		10
	6		24		29
*		4		14	
	0		48		57
	3		33		26
*		4		15	
	1		23		18
	4		23		9
*		5		0	
	1		11		12
	2		54		44
	3		52		57
	4		33		22
	5		35		50
	6		97		92
	7		46		43
	8		59		55
	9		23		10
	10		16		14
	11		66		65
	12		48		47
	13		43		43
	19		14		17
*		5		1	
	1		65		74
	2		29		30
	3		34		39
	4		54		50
	5		87		95
	6		29		32
	7		19		9
	8		78		69
	9		47		54
	10		64		64
	12		45		34
	14		37		35
			38		17
*		5		2	
	0		69		71
	1		53		56
	2		68		68
	3		40		54
	4		62		79
	5		78		87
	6		64		68
	7		51		49
	9		64		60
	10		28		17
	11		30		23
	12		31		32
	13		46		38
	14		28		32
*	0		80		82
	1		55		55
	2		55		74
	3		41		50
	4		34		41
	5		45		32
	6		64		61
	7		56		52
	8		65		55
	10		53		44
	11		33		34
	12		55		50
	13		42		48
*		5		4	
	2		78		78
	4		74		78
	5		59		48
	6		38		25
	7		21		26
	8		41		55
	9		61		57
	10		41		34
	14		47		43
*		5		5	
	0		54		57
	1		44		41
	2		55		56
	3		40		51
	4		44		42
	5		79		74
	6		41		42
	7		43		42
	11		35		30
	12		42		24
*		5		6	
	1		28		32
	2		95		97
	3		60		59
	4		58		59
	6		48		34
	7		54		60
	8		50		43
	9		25		18
	10		26		20
	11		42		49
*		5		7	
	0		73		80
	1		48		43
	2		45		48
	3		54		61
	5		48		45
	8		54		39
	9		18		14
	11		51		39
*		5		8	
	0		42		47
	1		57		46
	2		23		33
	3		50		48
	4		56		48
	5		45		28
	6		32		26
	7		37		31
	10		19		27

(Table 18 Contd.)

*		H		K	
	L		Fo		Fc
*		5		9	
	2		55		63
	3		53		56
	4		46		36
	7		55		51
	8		45		37
*		5		10	
	0		45		47
	1		58		60
	2		71		27
	4		34		37
	5		35		33
	8		20		26
	9		37		17
*		5		11	
	0		37		35
	1		25		42
	4		45		42
	8		28		21
*		5		12	
	1		36		33
*		6		0	
	1		106		129
	2		29		16
	5		116		138
	6		36		14
	8		28		6
	9		67		60
	10		37		26
	11		32		28
	13		50		49
*		6		1	
	0		41		46
	1		78		77
	2		28		26
	3		55		69
	4		53		45
	7		62		67
	8		21		35
	10		57		48
	15		34		16
	17		37		45

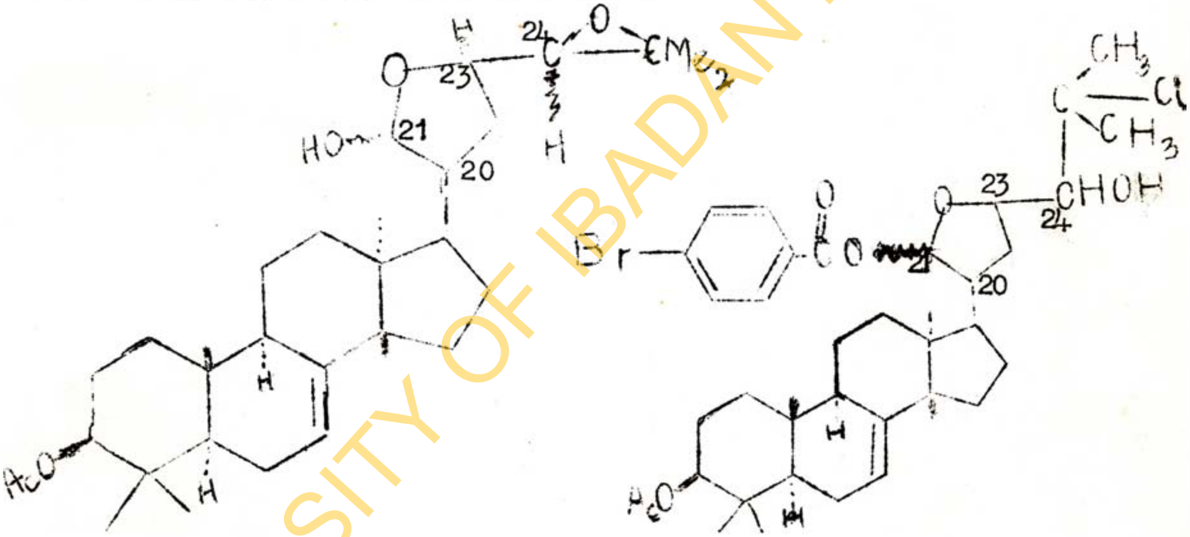
*		H		K	
	L		Fo		Fc
*		6		2	
	1		69		74
	2		97		116
	4		29		27
	5		63		68
	6		51		44
	8		42		43
	10		35		37
	11		46		46
	12		41		41
*		7		0	
	1		54		51
	2		58		60
	3		30		35
	4		14		11
	6		23		33
	7		49		57
	8		66		60
	11		35		18
	12		28		30
	14		29		20
*		7		1	
	1		66		71
	3		39		25
	4		53		65
	5		38		43
	10		67		57
	12		24		29
	13		34		20
	16		34		28
*		7		2	
	0		40		42
	1		35		39
	2		40		40
	3		43		68
	4		44		49
	5		35		35
	8		41		34
	9		40		53
*		8		0	
	0		57		60
	1		30		32
	2		21		18

*		H		K	
	L		Fo		Fc
	4		49		63
	8		36		42
	13		35		26
*		8		1	
	0		22		33
	4		37		37
	5		41		26
	6		62		73
	8		43		36
	9		24		28
	10		24		21
	12		41		41
*		8		2	
	2		38		35
	3		55		68
	7		26		34
	8		26		30
*		9		0	
	1		29		51
	2		40		42
	3		39		52
	5		25		36
	9		29		17
	10		12		14
	11		12		7
*		9		1	
	0		33		38
	1		23		28
	2		24		31
	6		34		35
	8		34		22
*		10		0	
	0		16		10
	3		23		33
	5		25		34
	8		26		181
	9		31		25
	10		21		21
	11		24		24
*		10		1	
	9		22		9
*		11		0	
	8		24		10
* *		3		1	
	5		75		81
	6		97		83

TURRAEANTHIN

Introduction

Turraeanthin was isolated[57] from Turraeanthus africanus and its structure has been put forward[58] as (I) below. A few mg. of the p-bromo benzoate derivative of its hydrochloride



(I)

(II)

supposed[58] to have the structure (II) was supplied for X-ray examination by T.G. Halsall and P. Toft of Oxford University. The purpose of the X-ray crystallographic study is to confirm this structure and to determine the configuration at C(21), C(23) and C(24).

The preliminary investigation of the material was performed by R.D. Gordon of the Department of Chemistry, University of Ibadan. This included crystallisation of the original powder and the determination of cell dimensions, space group and molecular weight. The cell dimensions were determined from rotation and Weissenberg photograph calibrated with copper, aluminium and sodium chloride powder lines.

Some of the later work, in the collection of intensity data, was also performed independently by R.D. Gordon. The set of intensities that was finally used included some of the (hhl) and (hk6) data collected by him.

The cell dimensions and space group determinations were repeated in order to check the results of R.D. Gordon. No significant differences were observed.

Crystallisation and Crystal Data

Small colourless lath-shaped crystals grew from the powder supplied from a benzene/methanol mixture. It was not possible to recrystallise many times in order to obtain ~~fairly~~ more suitable crystal for single crystal photographs.

From rotation and Weissenberg photographs, taken about the unique monoclinic c axis, and the molecular formula of the compound given as

$C_{39}H_{54}O_6$ Br Cl, the following crystal data were deduced:

$a = 16.743 \pm 0.06$ Å

$b = 16.473 \pm 0.06$ Å

$c = 7.589 \pm 0.03$ Å

$\beta = 118.95 \pm 0.2^\circ$

$V = abc \sin\beta$

$= 1831.0$ Å^3

$M = 734.2$

$\mu(CuK\alpha) = 37$ cm^{-1}

$F(000) = 776$

Space Group Determination

The only systematic absences were 00l with $l \neq 2n$. Thus the space group could be $P2_1$ (No.4) or $P2_1/m$ (No.11). Space group $P2_1$ has $Z = 2$ and $P2_1/m$ has $Z = 4$. The density of 1.34 ± 0.05 g/ml measured by flotation in aqueous KI solution, agrees with a calculated value of 1.331 g/ml for two molecules per unit cell. Since the molecule as a natural product does not have a plane or centre of symmetry, the space group was unambiguously decided as $P2_1$.

Measurement of intensities

From Weissengerg photographs taken with the best two crystals, of dimensions 0.9 by 0.08 by 0.03 m. and 0.4 by 0.12 by 0.03 m., 752 reflections were recorded and estimated visually as before. Another 200 were observable but too weak to be estimated. This total number of observed reflections (952) represents less than 20 per cent of the total number of accessible reflection (5037) with CuKα radiation. Very few reflections were observed beyond Sin θ value of 0.7. The repetition of the hko layer photograph revealed that the quality of photographs deteriorated with exposure to X-rays.

The intensities were corrected for Lorentz and Polarization effects as before and put on a common scale with the aid of a Wilson plot.[25] A temperature factor of $7.5\ \text{Å}^2$ was determined. This high temperature factor partially accounts for the small number of observed reflections. A complex structure such as this, usually held together in the crystal by Van der Waals forces, is prone to oscilate and thus increasing the effect of atomic vibrations.

The scale factors for the highest layers, hk5 and hk6 were rather unreliable but because of the rather small number of available reflections they were included all the same. The number of available data is considered small for the complexity of the structure but the problem can be lessened by improving the accuracy of the data. To this end, the data obtained from each layer photograph would be correlated from stage to stage.

There was no absorption correction.

Determination of the Bromine and Chlorine Positions

3-D Patterson: A three dimensional summation using the observed $|F(OBS)|^2$ as co-efficients gave the map of fig. 48 as the Harker section of $w = \frac{1}{2}$. The bromine-bromine vector was quite prominent and from its u and v values, the x and y co-ordinates for bromine were calculated as

$$x = 0.4817 \text{ and } y = 0.1367$$

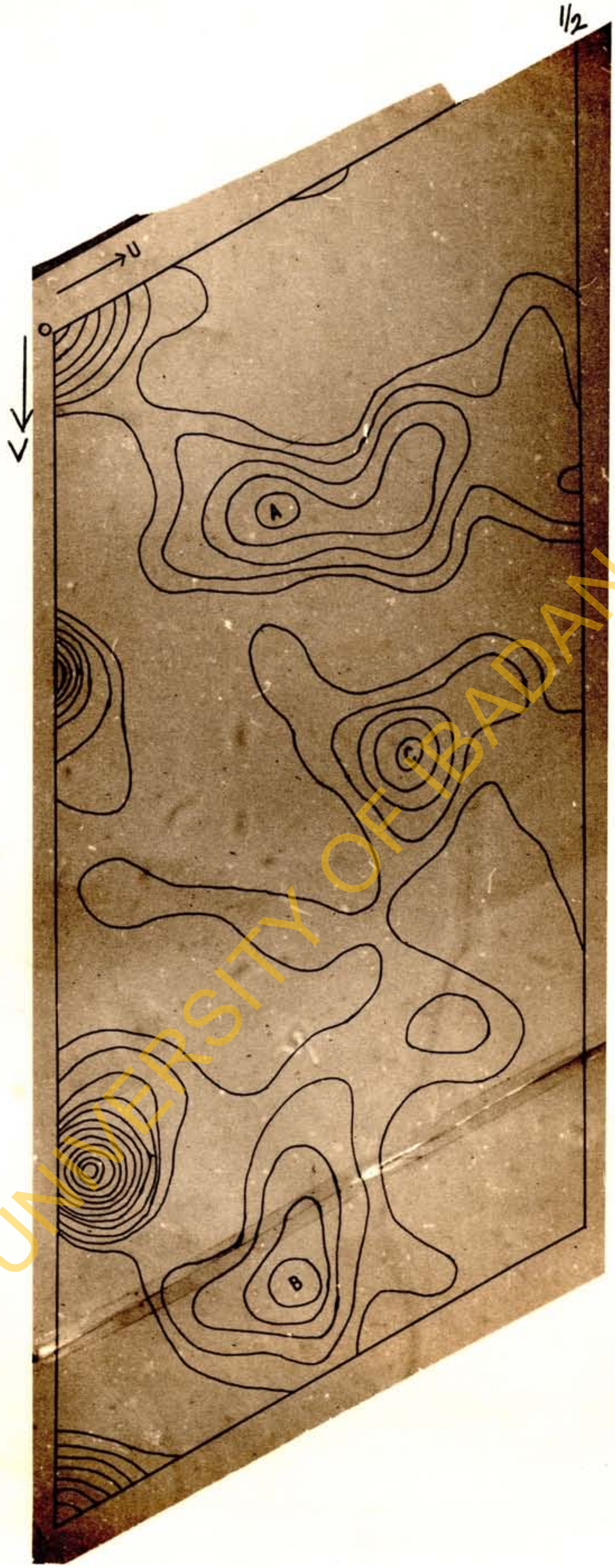


Figure 48

There were three other peaks A, B, and C, all having approximately the same weight on this section. One of this must be the chlorine-chlorine vector. In order to decide the actual Cl - Cl vector peak, the x and y co-ordinates derived from the three positions were separately combined with those for the bromine atom and structure factors were calculated for all the hko reflections. The reliability index obtained are as follows:

Br and Cl(A); R = 55.16%

Br and Cl(B); R = 52.03%

Br and Cl(C); R = 52.53%

Br only; R = 49.6%

The remarkable feature of this result was that the insertion of a chlorine atom in each of the three possible positions increased the reliability index.

2-D Patterson: A two-dimensional Patterson summation was next calculated and the results show the bromine-bromine vector peak becoming much less prominent. In fact, position A (fig. 48) of the probable chlorine-chlorine vectors became most prominent. Position C had approximately the same weight as the Br - Br vector peak and position B was much smaller than in the Harker section. Structure factors were calculated for the hko reflections with the bromine position parameters derived from vector peak A. The R-factor was 48.2%. This is smaller than that calculated from the bromine position which was deduced from the Harker section. Chlorine was then inserted in turn in the other two positions and in the former bromine position, together with bromine in its new position (A). The resulting structure factor calculations gave R-factors which were higher than 48.2%.

Bromine was then inserted in turn in the positions deduced from vector peaks B and C. The structure factors caculated for the hko reflections gave R-factors of 56.6 and 54.8% respectively. Similar calculations for bromine in each of these positions and chlorine in one of the other three positions showed a worsening of the R-factor. It therefore appears that whilst bromine could be in the position (1) deduced from the prominent Patterson - Harker peak or (2) deduced from the vector peak A, it is uncertain that chlorine could be located from any of the vector peaks A, B and C.

2-D Fourier Summations: To locate the chlorine atom otherwise, two two-dimentional Fo Fourier summations were calculated. The phases were those calculated with bromine in the two alternative positions. The first summation with bromine in (1) showed two prominent peaks apart from that of bromine. Structure factors calculated for hko reflections with the bromine (1), and chlorine in one of the two positions gave an R-factor of 48.2% ; with chlorine in the other position the R-factor was 51.3%. The second chlorine position was therefore rejected.

The second summation with bromine in (2) had four prominent peaks apart from that of bromine. Structure factors for the hko reflections with bromine (2) and each of the four probable positions for chlorine were calculated. The R-factors were: 47.0, 50.3, 51.3 and 50.6 per cent. Three of these positions were accordingly rejected. There were therefore two sets of x and y co-ordinates for the bromine and chlorine given by

	Br	Cl
(i)	$x = 0.4825$	$x = 0.2717$
	$y = 0.1362$	$y = 0.8750$
(ii)	$x = 0.1041$	$x = 0.4750$
	$y = 0.1291$	$y = 0.3617$

Neither of the two positions for chlorine corresponded with any of those derived from the Patterson. To decide which set of parameters should be used for further calculations, the three-dimensional Patterson was re-examined. Since the z (unique axis) parameter of the bromine atoms in the structure may be assigned arbitrarily, it was chosen as 0.000. That of chlorine could be decided by finding the vector peak for the corresponding bromine-chlorine positions. In each case, such a peak should lie on a line parallel to the unique axis. There was no such peak for the second set of parameters and the first set was therefore employed for further calculations with z-parameter for chlorine equal to 0.3550. The structure factor calculation for the hkl reflections gave a reliability index of 44.55% with bromine alone and 43.48% with bromine and chlorine.

Structure Analysis

A three dimensional Fourier summation with Fo coefficients phased on the bromine and chlorine positions, was calculated. The two atoms were given a temperature factor of $7.5\ \text{Å}^2$. The resulting densities were generally low. Apart from the two atoms inserted the highest density was $1.9\ e/\text{Å}^3$. Twenty-four peaks of densities $1.0\ e/\text{Å}^3$ and above were selected as carbon atoms. These together with the bromine and the chlorine were used in calculating another set of structure factors. The R-factor was 40.5%. The second Fo Fourier summation gave six new peaks which did not make much chemical sense and therefore a 'difference' synthesis in which the 200 unobserved reflections had Fo values of half the threshold, based on the previous 26 atoms was calculated.

This led to the removal of ten atomic centres with negative densities of 1.0 $e/\text{Å}^3$ and the addition of seven new centres which were stereochemically sensible. No definite feature of the molecule was still recognisable. The previous position and temperature paranters were adjusted and the third structure factor calculations gave a reliability index of 37.06%

This was followed by another difference synthesis. From this it was observed that a peak came out persistently highly positive in the difference syntheses being 1.4 $e/\text{Å}^3$ in the first one with a temperature factor of 7.5 Å^2 and 1.0 $e/\text{Å}^3$ in the second one with $B = 8.0\ \text{Å}^2$. It was about 1.9 Å from the present chlorine position. All other atoms inserted had densities of value between 0.5 to - 0.5 $e/\text{Å}^3$. Their temperature and position parameters were adjusted. There were ten new peaks which were stereochemically acceptable as atomic positions. With these 33 atoms, the model shows a six-membered ring attached to a five-membered ring. This is probably the section of rings C and D in the suggested structure.

The fourth structure factor calculation based on these 33 atoms made up bromine, chlorine and 31 carbon atoms gave a reliability index of 34.3%. The calculated structure factors are compared with observed ones in Table 20 and the co-ordinates of the 33 atoms are shown in Table 19.

No further work was done on this structure.

Discussion

The solution of the crystal structure is beset by the quality and quantity of the available data. The relative intensities obtained from each layer photograph are within experimental error reliable but the method of scaling the different layers together (Wilson's method) depends on a statistical law. Since this requires a large number of data, (which were not available particularly from hk5 and hk6 layers), the scale factors initially used are not altogether trustworthy and must be adjusted from stage to stage. In addition, there was no correction for absorption.

The quantity of the data would make the resolution of peaks rather low. In a three-dimensional synthesis, however, distances of 1.1 to 1.2 Å should be resolved.

An isotropic temperature factor of 7.5 Å^2 is not likely to represent thermal vibrations alone. There is probably a certain amount of disorder. The high value may also be partly due to compensation for the uncorrected absorption effects. The diminishing values of the R-factor is an indication that the correct structure is being approached. The small amount of data is likely, however, to require many more cycles of difference syntheses before the final structure is arrived at. Carlisle and Ladd[59] recently found that, working with a slightly better reflections to number of atoms ratio (947; 35) in the same space group, several difference syntheses were required to bring the R-factor from 50% to 37% (Least square refinement reducing R-factor to 25%).

At the moment, all atoms, other than bromine and chlorine, are being treated as carbon. The only chemical feature recognisable is a cyclohexane ring, formed by atoms 15, 16, 17, 32, 4 and 30 of table 19. The ring is fused to a five-membered ring formed by atoms 17, 32, 5, 31 and 13. After a few more cycles, it should be possible to recognize the whole molecule and hence replace supposed carbon atoms by oxygen at the appropriate positions. This should lead to a more drastic fall in the R-factor than has been found so far.

Flow Chart

1st Phase Calculation with Br + Cl; R = 43.5%

↓

1st Fo Fourier giving 24 atoms regarded as carbon

↓

2nd Phase Calculation with $C_{24}ClBr$; R = 40.5%

↓

2nd Fo Fourier

↓

1st Difference giving $C_{24}ClBr-10C+7C$

↓

3rd Phase Calculation with $C_{21}ClBr$; R = 37.1%

↓

2nd Difference giving $C_{31}ClBr$

↓

4th Phase Calculation with $C_{31}ClBr$; R = 34.3%

↓

TABLE 19

	x/a	y/b	z/c	B	
1.	0.4867	0.1383	0.000	8.50	Br
2.	0.2750	0.8817	0.3634	7.50	Cl
3.	0.1700	0.8033	0.0100	8.50	C
4.	0.8100	0.6517	0.1500	7.50	C
5.	0.2700	0.2100	0.4767	7.00	C
6.	0.2283	0.4350	0.3733	7.50	C
7.	0.4150	0.3300	0.3267	7.40	C
8.	0.5100	0.3283	0.3400	7.00	C
9.	0.5950	0.3717	0.4900	7.80	C
10.	0.5983	0.5100	0.0067	8.80	C
11.	0.9717	0.6783	0.1633	7.30	C
12.	0.9467	0.3550	0.0600	7.30	C
13.	0.8700	0.8533	0.1333	7.50	C
14.	0.0650	0.6317	0.2600	7.00	C
15.	0.0417	0.3300	0.3533	7.10	C
16.	0.0467	0.2250	0.2833	8.00	C
17.	0.1300	0.1717	0.3500	8.00	C
18.	0.5083	0.1383	0.4867	8.30	C
19.	0.4350	0.0933	0.1117	7.50	C
20.	0.1307	0.0741	0.2000	7.50	C
21.	0.2117	0.5750	0.1367	7.80	C
22.	0.7117	0.1517	0.2767	7.70	C
23.	0.7067	0.3567	0.3217	7.10	C
24.	0.0233	0.2650	0.0367	7.50	C
25.	0.6400	0.7583	0.0133	7.50	C
26.	0.8500	0.3966	0.0133	7.50	C
27.	0.3242	0.9783	0.0683	7.50	C
28.	0.1050	0.7383	0.2867	7.50	C
29.	0.6633	0.0050	0.3500	7.50	C
30.	0.1283	0.3850	0.4900	7.50	C
31.	0.7800	0.8417	0.1467	7.50	C
32.	0.7867	0.7217	0.0500	7.50	C
33.	0.6700	0.2633	0.3433	7.50	C

TABLE 20

Comparison of Fo and Fc

H	K	L	Fo	Fc	H	K	L	Fo	Fc
0	-11	0	108	103	1	-11	0	118	59
0	-10	0	134	179	1	-10	0	123	22
0	-8	0	251	436	1	-10	1	134	141
0	-8	2	190	258	1	-9	0	101	187
0	-8	4	188	163	1	-9	1	113	157
0	-7	0	180	317	1	-9	2	142	165
0	-7	1	235	333	1	-9	3	113	169
0	-7	2	210	228	1	-8	0	273	335
0	-7	3	166	173	1	-8	1	253	86
0	-6	0	71	343	1	-8	2	256	203
0	-6	1	307	479	1	-8	4	144	110
0	-6	2	164	77	1	-7	0	84	180
0	-6	3	215	221	1	-7	2	310	332
0	-5	0	117	165	1	-7	3	119	77
0	-5	1	321	311	1	-7	4	221	286
0	-5	2	290	55	1	-6	0	521	275
0	-5	3	400	408	1	-6	1	465	474
0	-4	0	944	1020	1	-6	2	175	285
0	-4	1	139	109	1	-6	3	136	51
0	-4	2	245	261	1	-6	4	132	270
0	-4	5	110	73	1	-5	0	525	443
0	-3	0	510	283	1	-5	1	500	451
0	-3	1	570	476	1	-5	2	224	361
0	-3	2	645	504	1	-5	3	255	324
0	-3	3	227	117	1	-5	4	214	208
0	-3	4	263	251	1	-5	5	80	171
0	-2	0	657	810	1	-4	0	527	343
0	-2	1	839	852	1	-4	1	139	132
0	-2	2	84	128	1	-4	2	411	337
0	-2	3	249	273	1	-4	3	185	309
0	-2	4	93	185	1	-4	4	137	288
0	-2	5	109	172	1	-4	6	114	79
0	-1	1	256	346	1	-3	0	778	362
0	-1	2	251	184	1	-3	1	822	586
0	-1	3	507	450	1	-3	2	357	454
0	-1	4	210	39	1	-3	3	213	129
0	-1	5	51	41	1	-3	4	149	336
0	1	6	54	46	1	-3	5	111	96
0	2	6	100	70	1	-3	6	81	60
0	3	6	73	75	1	-2	0	461	310
0	4	6	113	126	1	-2	1	699	1088
0	6	6	102	69	1	-2	2	282	278

H	K	L	Fo	Fc
1	-2	3	312	383
1	-2	4	122	177
1	-2	5	119	161
1	-2	6	109	73
1	-1	1	499	604
1	-1	2	509	337
1	-1	3	71	280
1	-1	4	285	199
1	-1	5	59	121
1	-1	6	42	39
1	0	1	427	513
1	0	2	517	796
1	0	4	111	231
1	0	6	79	132
1	1	1	284	216
1	1	2	733	746
1	1	3	255	166
1	1	4	86	173
1	1	5	56	67
1	1	6	78	65
1	2	0	757	334
1	2	1	376	670
1	2	2	311	430
1	2	3	212	357
1	2	4	206	66
1	2	5	103	156
1	3	0	805	859
1	3	1	251	195
1	3	2	592	643
1	3	3	279	321
1	3	4	295	282
1	4	0	191	422
1	4	1	263	428
1	4	2	541	451
1	4	3	336	201
1	4	4	96	173
1	5	0	125	246
1	5	1	293	507
1	5	4	120	12
1	5	6	98	77
1	6	0	187	242
1	6	1	363	388
1	6	2	370	250
1	6	3	251	268
1	6	4	132	99
1	7	0	497	474
1	7	[illegible]	130	24
1	8	1	134	173
1	8	2	223	208
1	9	1	104	94
1	10	1	117	125
1	11	0	152	203
2	-12	1	122	34
2	-11	0	163	189
2	-10	0	283	206
2	-10	1	160	141
2	-10	3	162	208
2	-9	0	268	50
2	-9	1	242	147
2	-8	0	80	269
2	-8	1	314	281
2	-8	2	308	389
2	-8	4	113	195
2	-7	1	365	107
2	-7	2	374	364
2	-7	3	127	75
2	-7	4	201	237
2	-6	0	546	342
2	-6	1	126	49
2	-6	2	208	272
2	-6	3	265	386
2	-6	4	149	84
2	-5	0	610	617
2	-5	1	311	269
2	-5	2	174	286
2	-5	3	146	343
2	-5	4	141	59
2	-5	6	143	121
2	-4	0	434	277
2	-4	1	573	791
2	-4	2	206	453
2	-4	3	140	220
2	-4	4	168	251
2	-4	5	131	163
2	-4	[illegible]	106	57
2	-3	0	758	186
2	-3	1	481	414
2	-3	2	547	585
2	-3	4	316	266
2	-3	5	85	84
2	-3	6	88	43
2	-2		186	179

H	K	L	Fo	Fc
2	-2	1	167	550
2	-2	2	409	280
2	-2	3	381	612
2	-2	4	173	55
2	-2	5	70	180
2	-2	6	120	52
2	-1		348	298
2	-1	1	693	777
2	-1	2	190	200
2	-1	3	266	520
2	-1	4	166	105
2	-1	5	96	106
2	-1	6	70	81
2	0	0	503	264
2	0	1	595	636
2	0	2	134	498
2	0	3	201	279
2	0	4	215	431
2	0	5	63	31
2	0	6	84	77
2	1	0	609	158
2	1	1	642	668
2	1	2	569	605
2	1	3	430	481
2	1	4	380	357
2	1	6	61	62
2	2	0	443	423
2	2	1	547	373
2	2	2	333	362
2	2	3	142	216
2	2	4	100	148
2	2	5	75	235
2	3	0	332	139
2	3	2	380	434
2	3	3	177	110
2	3	4	196	288
2	4	0	494	442
2	4	1	103	261
2	4	2	496	616
2	4	3	321	331
2	4	4	222	306
2	5	0	148	180
2	5	1	230	224
2	5	2	205	223
2	5	3	212	220
2	5	4	102	120

H	K	L	Fo	Fc
2	6	0	167	230
2	6	1	295	269
2	6	3	159	139
2	7	1	169	124
2	7	2	249	291
2	8	0	118	37
2	8	1	82	84
2	9	0	102	43
2	9	1	139	189
2	10	0	122	90
3	-12	1	120	218
3	-10	0	204	306
3	-10	1	152	165
3	-10	2	152	54
3	-9	0	237	194
3	-9	1	236	362
3	-9	2	91	169
3	-8	0	162	42
3	-8	1	198	212
3	-8	2	246	138
3	-8	3	167	99
3	-7	0	247	434
3	-7	1	309	174
0	-9	0	117	100
0	-9	1	196	163
3	-7	2	303	415
3	-7	4	140	122
3	-7	5	115	87
3	-6	0	501	508
3	-6	1	180	317
3	-6	2	340	343
3	-6	3	198	118
3	-6	4	219	81
3	-6	6	96	88
3	-5	0	291	138
3	-5	1	636	294
3	-5	2	240	393
3	-5	3	241	258
3	-5	6	61	61
3	-4	0	930	1107
3	-4	1	422	270
3	-4	2	226	576
3	-4	3	394	240
3	-4	4	212	147
3	-4	5	100	79
3	-4	6	130	155
3	-3	0	1074	1220
3	-3	1	480	439
3	-3	2	165	462

H	K	L	Fo	Fc
3	-3	2	165	462
3	-3	3	262	415
3	-3	4	159	80
3	-2	0	558	695
3	-2	1	278	368
3	-2	2	379	211
3	-2	3	115	150
3	-2	4	272	107
3	-2	5	80	148
3	-1	0	228	161
3	-1	1	748	634
3	-1	2	482	488
3	-1	3	314	155
3	-1	4	248	333
3	-1	5	125	210
3	-1	6	81	42
3	0	0	122	514
3	0	1	166	360
3	0	2	512	641
3	0	3	352	287
3	0	4	229	292
3	0	6	79	121
3	1	0	263	553
3	1	1	343	249
3	1	2	350	152
3	1	3	601	237
3	1	4	216	105
3	1	5	72	128
3	1	6	101	110
3	2	0	455	521
3	2	1	531	527
3	2	2	254	82
3	2	3	360	534
3	2	4	218	29
3	2	5	61	132
3	3	0	263	349
3	3	1	311	344
3	3	2	357	327
3	3	4	213	166
3	3	5	100	103
3	4	0	272	560
3	4	1	230	143
3	4	2	547	486
3	4	4	138	243
3	4	6	129	86
3	5	0	101	198
3	5	2	151	188
3	5	3	178	280
3	6	1	117	87
3	6	2	130	56
3	7	0	236	181
3	8	1	86	36
3	8	2	151	191
4	-12	1	145	145
4	-11	0	197	211
4	-11	2	165	118
4	-10	0	152	151
4	-10	2	154	256
4	-10	4	172	98
4	-9	0	165	47
4	-9	1	397	520
4	-9	2	124	136
4	-9	5	105	88
4	-8	0	147	53
4	-8	1	293	213
4	-8	2	104	91
4	-8	3	166	120
4	-7	0	282	603
4	-7	1	309	301
4	-7	2	256	316
4	-7	4	171	130
4	-7	6	103	96
4	-6	0	397	559
4	-6	1	449	316
4	-6	2	281	275
4	-6	3	205	86
4	-6	4	139	214
4	-6	5	123	61
4	-6	6	106	99
4	-5	0	577	64
4	-5	1	542	493
4	-5	2	245	353
4	-5	3	212	115
4	-5	4	75	51
4	-4	0	807	1009
4	-4	1	682	585
4	-4	2	382	298
4	-4	3	105	115
4	-4	4	162	167
4	-4	5	111	134
4	-4	6	76	94
4	-3	0	883	1019

H	K	L	Fo	Fc
4	-3	1	326	164
4	-3	2	608	454
4	-3	3	309	75
4	-3	4	321	250
4	-3	5	89	75
4	-2	0	788	591
4	-2	1	624	444
4	-2	2	444	357
4	-2	3	97	104
4	-2	4	130	69
4	-2	5	106	98
4	-1	0	227	68
4	-1	1	680	613
4	-1	2	390	617
4	-1	3	454	412
4	-1	4	483	337
4	-1	5	83	157
4	0	0	693	596
4	0	1	194	198
4	0	2	171	321
4	0	3	370	133
4	0	6	113	80
4	1	0	550	698
4	1	1	161	237
4	1	2	321	339
4	1	3	321	218
4	1	4	193	190
4	2	1	480	644
4	2	2	118	71
4	2	2	118	71
4	2	3	130	96
4	3	1	242	400
4	3	2	279	254
4	3	3	174	121
4	3	4	195	172
4	4	0	242	291
4	4	1	187	185
4	4	2	95	238
4	4	3	164	54
4	5	0	238	454
4	5	1	115	193
4	5	2	129	146
4	5	3	118	173
4	5	4	169	79
4	6	0	79	12
4	6	1	199	169
4	7	1	85	78
4	8	0	176	104
4	9	0	123	247
5	-12	1	105	85
5	-11	0	221	173
5	-11	2	225	272
5	-10	0	309	285
5	-10	2	154	83
5	-9	1	126	133
5	-9	3	256	219
5	-8	0	288	334
5	-8	1	304	305
5	-8	2	218	135
5	-7	0	183	297
5	-7	1	179	224
5	-7	2	229	293
5	-7	4	153	197
5	-6	0	176	322
5	-6	1	434	498
5	-6	2	152	289
5	-6	3	97	162
5	-6	5	61	88
5	-5	0	741	348
5	-5	1	438	392
5	-5	2	332	250
5	-5	3	412	341
5	-5	5	67	82
5	-4	0	598	587
5	-4	1	411	405
5	-4	2	158	340
5	-4	3	197	251
5	-4	4	128	147
5	-4	5	101	87
5	-3		492	220
5	-3	1	309	80
5	-3	2	456	494
5	-3	3	246	229
5	-3	4	219	177
5	-3	5	66	10
5	-2	-	187	128
5	-2	1	275	277
5	-2	2	98	225
5	-2	3	267	218
5	-2	4	214	234
5	-2	5	109	127
5	-1		166	537

H	K	L	Fo	Fc
5	-1	1	444	405
5	-1	2	155	143
5	-1	3	230	202
5	-1	4	60	19
5	-1	5	63	100
5	0	0	493	644
5	0	1	111	264
5	0	2	187	398
5	0	3	354	337
5	0	4	208	186
5	0	5	91	98
5	1	0	547	807
5	1	1	189	357
5	1	2	366	377
5	1	3	143	67
5	1	6	107	103
5	2	1	251	250
5	2	2	432	364
5	2	3	153	220
5	3	0	275	479
5	3	1	342	325
5	3	2	119	111
5	3	4	94	70
5	4	0	287	391
5	4	1	133	64
5	4	2	252	301
5	4	4	266	196
5	5	1	101	182
5	5	2	138	85
5	6	1	188	165
5	6	2	175	71
5	8	0	150	198
6	-13	1	150	87
6	-11	0	150	166
6	-11	2	210	140
6	-10	1	159	137
6	-10	2	145	274
6	-9	1	247	206
6	-9	2	79	144
6	-8	0	156	68
6	-8	1	281	231
6	-8	2	117	249
6	-7	0	206	425
6	-7	1	176	139
6	-7	2	159	340
6	-7	3	264	145
6	-7	4	133	204
6	-6	0	152	86
6	-6	1	297	115
6	-6	3	124	211
6	-5	1	441	495
6	-5	2	339	305
6	-5	3	167	330
6	-5	4	97	178
6	-5	5	102	173
6	-4	0	206	141
6	-4	1	362	277
6	-4	2	380	261
6	-4	3	104	111
6	-4	4	104	313
6	-3	0	488	472
6	-3	1	189	200
6	-3	2	559	481

H	K	L	Fo	Fc	H	K	L	Fo	Fc
6	3-	3	231	183	7	5-	3	224	252
6	3-	4	186	397	7	4-	1	239	173
6	2-	0	349	344	7	4-	2	282	327
6	2-	1	280	99	7	4-	3	176	139
6	2-	2	243	253	7	4-	4	225	184
6	2-	3	337	292	7	4-	5	64	69
6	1-	0	205	573	7	3-	0	269	55
6	1-	1	367	373	7	3-	1	365	335
6	1-	2	106	152	7	3-	2	192	398
6	1-	3	325	479	7	3-	3	216	175
6	1-	4	141	141	7	3-	4	179	192
6	0	1	291	222	7	2-	0	357	246
6	0	2	386	372	7	2-	1	137	184
6	0	3	180	132	7	2-	2	204	223
6	0	4	136	166	7	2-	3	183	186
6	0	5	81	88	7	2-	4	162	154
6	1	0	226	132	7	2-	5	93	42
6	1	1	291	130	7	1-	0	538	131
6	1	2	357	408	7	1-	1	297	323
6	2	0	292	55	7	1-	2	133	246
6	2	1	199	151	7	1-	3	129	217
6	2	2	172	45	7	0	0	246	133
6	2	3	137	133	7	0	1	450	423
6	3	0	94	93	7	0	2	184	232
6	3	3	158	285	7	0	3	190	100
6	3	4	161	76	7	0	4	136	129
6	4	0	181	175	7	0	5	79	91
6	4	2	152	216	7	1	0	129	115
6	5	0	84	51	7	1	1	102	155
6	5	2	148	170	7	1	2	194	256
6	6	1	141	160	7	1	3	152	115
7	13-	1	123	130	7	1	4	156	199
7	11-	3	137	71	7	2	1	75	137
7	10-	0	187	381	7	2	4	119	38
7	10-	1	153	178	7	3	1	145	238
7	10-	2	208	197	7	5	0	134	74
7	9-	1	208	178	7	5	1	89	141
7	9-	2	238	92	8	-13	0	180	215
7	9-	3	186	88	8	-12	3	203	85
7	8-	0	231	123	8	-11	0	208	278
7	8-	1	311	257	8	-10	1	169	164
7	8-	3	117	31	8	-10	2	196	176
7	7-	0	354	546	8	-10	3	148	46
7	7-	1	376	414	8	-9	1	268	312
7	7-	2	310	354	8	-8	0	193	
7	7-	4	138	144	8	-8	2	162	177
7	6-	0	507	446	8	-8	3	149	190
7	6-	1	258	187	8	-7	0	577	567
7	6-	2	223	345	8	-7	2	156	238
7	6-	3	141	80	8	-6	0	110	220
7	6-	4	119	126	8	-6	2	178	179
7	5-	1	428	484	8	-6	3	247	101
7	5-	2	86	81	8	-5	0	158	237

H	K	L	Fo	Fc	H	K	L	Fo	Fc
8	-5	1	185	255	9	-3	1	213	180
8	-5	2	147	110	9	-3	2	218	230
8	-5	3	111	174	9	-3	3	245	161
8	-5	4	95	47	9	-3	4	118	146
8	-4	0	316	62	9	-2	0	170	286
8	-4	1	209	269	9	-2	1	205	164
8	-4	2	146	247	9	-2	2	189	131
8	-4	3	165	217	9	-2	3	122	125
8	-3	0	698	788	9	-1	0	151	208
8	-3	1	234	134	9	-1	1	229	259
8	-3	2	104	293	9	-1	3	112	135
8	-3	3	110	138	9	0	0	213	245
8	-2	0	509	385	9	0	2	134	202
8	-2	1	189	285	9	0	4	211	128
8	-2	3	221	266	9	1	0	180	269
8	-2	5	90	102	9	1	2	147	165
8	-1	0	296	62	10	-11	0	118	87
8	-1	1	227	305	10	-9	0	161	182
8	-1	2	168	154	10	-7	0	368	218
8	-1	3	141	93	10	-7	1	246	186
8	-1	4	114	30	10	-6	2	162	152
8	0	1	264	282	10	-6	3	128	107
8	0	2	143	252	10	-6	4	171	200
8	1	0	255	282	10	-5	0	232	237
8	1	2	212	192	10	-5	1	153	153
8	1	3	165	49	10	-5	2	170	149
8	1	4	153	93	10	-5	3	179	176
8	3	1	175	132	10	-4	1	161	281
8	3	2	150	132	10	-4	2	171	95
8	3	3	180	153	10	-3	1	163	191
8	4	0	135	204	10	-3	2	211	262
9	-13	0	153	57	10	-3	3	170	123
9	-10	1	110	19	10	-3	4	125	149
9	-9	1	225	182	10	-2	0	111	70
9	-9	2	111	165	10	-2	1	139	144
9	-8	0	78	83	10	-2	3	160	137
8	-8	1	245	300	10	-1	0	121	143
9	-8	1	134	324	10	-1	3	177	166
9	-8	2	152	66	10	0	1	186	83
9	-7	1	165	92	10	0	2	132	75
9	-7	2	164	169	10	0	3	194	81
9	-6	0	287	322	10	1	2	141	150
9	-6	1	267	135	11	-12	1	176	110
9	-6	2	176	161	11	-11	0	166	95
9	-6	3	147	108	11	-11	1	121	145
9	-5	1	344	364	11	-9	0	115	221
9	-5	3	178	222	11	-9	1	140	35
9	-5	4	101	151	11	-9	2	220	182
9	-4	1	267	129	11	-8	1	159	123
9	-4	3	168	40	11	-7	1	135	101
9	-3	0	143	275	11	-7	2	177	173

H	K	L	Fo	Fc
11	-6	0	129	225
11	-5	0	134	143
11	-4	1	173	270
11	-3	3	153	78
11	-1	3	141	218
11	-1	4	116	17
11	0	1	92	82
12	-12	0	113	31
12	-11	1	124	154
12	-9	0	107	68
12	-9	1	134	132
12	-9	2	161	63
12	-8	1	144	196
12	-7	0	117	105
12	-7	2	141	90
12	-6	0	116	304
12	-6	2	244	165
12	-6	3	158	29
12	-5	0	116	90
12	-5	1	193	147
12	-5	2	194	119
12	-4	2	200	71
12	-3	0	106	104
12	-3	2	160	164
12	-3	3	203	80
12	-2	0	108	186
13	-6	1	122	98
13	-6	3	145	22

APPENDIX: COMPUTING

Apart from the use of a FACIT desk calculating machine for minor work, calculations were carried out on IBM 1620 computers at the Universities of Ghana, Lagos and Ibadan and a KDF 9 at the University of Oxford.

Fourier Synthesis: This was calculated on the IBM 1620 with programmes obtained from Laboratorium fur Organische Chemie, Eidg. Technische Hochschule, Zurich through Dr. Max Dobler. There were two programmes, "FSI.D" and "2DFOU", both written in the Symbolic Programming System.

Because of the limited memory capacity of the 1620, 3-dimensional syntheses had to be performed in two phases:

(1) Summation over the first dimension using programme 'FSI.D' (Fourier Synthesis 1st Dimension) is carried out along the Z-direction. The programme is applicable to the following systems with the limitations stated:

(a) Triclinic. No limitations

(b) Monoclinic. The unique axis must be c

(c) Orthorhombic. Orthorhombic space groups must not contain any glide planes.

The programme may be used for other space groups with some modifications.

(2) Summation over the second and third dimensions using programme '2DFOU' uses the output from the FSI.D to calculate in x and y directions at a given z value. The product (Hmax + 1) X (the number of y values for which the density is to be calculated) should not exceed the value 1260.

Hmax is the maximum value of h. Since Hmax from the two crystallographic analyses was 20, the programme was applicable to as much as 60 y values. In all calculations, y had a maximum value of 31.

The programme has no other limitations.

A 2-dimensional syntheses would use programme 2DFOU. alone with some adjustment of the INPUT data when used for the 3-dimensional summation.

A programme in FORTRAN, written by Professor D.A. Bekoe was also used for the two dimensional Patterson summations to locate the iodine in Cedrela Odorata Substance B derivative.

The last three-dimensional Fo and difference summations were also carried out on the KDF9 with a programme written in ALGOL by Dr. J.S. Rollett.

Structure Factor Calculation: A general structure factor programme also obtained from the Zurich Laboratory was used to calculate structure factors on the IBM 1620. The calculation is in two steps

(1) Preparation of Input Cards for the Structure Factor programme.

(2) The Structure Factor programme.

The "preparation" cards (output from first step) contain h, k, l, Fo, $\mathrm{Sin}\ \theta/\lambda$, f_1, f_2, f_3, f_4, f_5, f_6, $\mathrm{Sin}^2\theta/\lambda^2$. The f's are the scattering factors for a maximum of 6 atoms at the sin θ/λ value corresponding to h,k,l, θ is the Bragg angle and λ, the wave length. $\mathrm{Sin}^2\theta/\lambda^2$ was calculated from the formula

$$\mathrm{Sin}^2\theta/\lambda^2 = R_{11}.h^2 + R_{12}.hk + R_{13}.hl + R_{22}.k^2 + R_{23}.kl + R_{33}.l^2$$

where R_{11}, R_{12}, R_{13}, R_{22}, R_{23} and R_{33} are the reciprocal cell constants.

A and B value, as previously expressed, are then caculated for each reflection by the structure factor programme.

The Least Squares programme used was that written by J.S. Rollett in ALGOL for the KDF9.

Other Programmes:

Programmes for Weissenberg Lp corrections, Wilson Plot, and Bond Angles and Distances were written for the IBM 1620 by Professor D.A. Bekoe.

Final bond distances and angles were calculated on KDF9 with a programme written by Dr. J.S. Rollett.

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