# X-RAY INVESTIGATION OF CRYSTAL AND MOLECULAR STRUCTURES IN THE CARYOPHYLLENE SERIES.

THESIS

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# PREFACE

This thesis embodies work carried out in the Chemistry Department of the University of Glasgow under the supervision of Professor J. Monteath Robertson. It is intended to publish the results.

I wish to thank my supervisor for suggesting the problem, and for constant encouragement and advice in the course of the work. My thanks are also due to Dr. V. Vand for helpful advice and discussion.

I am indebted to the Department of Scientific and Industrial Research for a maintenance allowance which enabled the work to be carried out.

## SUMMARY.

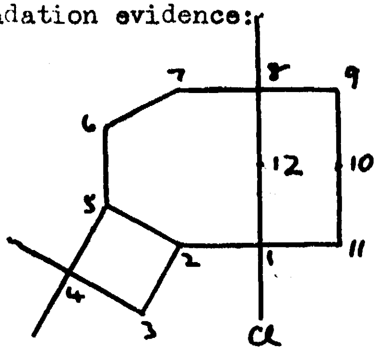
With a view to establishing directly the molecular constitution of the sesquiterpene, β-caryophyllene, an x-ray study has been made of certain of its crystalline derivatives.

β-caryophyllene dihydrochloride. This is a dicyclic compound containing the structure of the parent, β-caryophyllene, from which it is obtained by the addition of two molecules of HCl. The substance has been found to possess the space group $P2_1$ with two molecules per asymmetric unit. Attempts have been made to prepare the corresponding β-caryophyllene dihydrobromide derivative in order to obtain the structure of the compound directly by means of the isomorphous replacement method. It has not so far been possible to prepare this derivative.

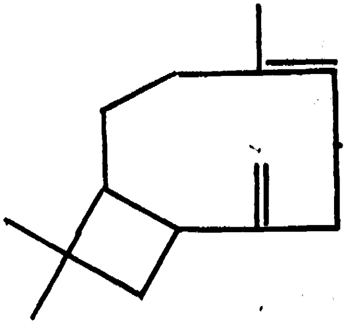
### β - caryophyllene bromide and chloride.

β - caryophyllene may be acid ring-closed and hydrated to form a tricyclic derivative, β-caryophyllene alcohol. This may be halogenated to yield isomorphous bromo - and chloro - derivatives without stereochemical or structural change.

$\beta$ - caryophyllene bromide and chloride have been studied by the method of x-ray crystal analysis, and the structure of the molecule obtained directly by means of the isomorphous replacement method. The results confirm the structure recently suggested on the basis of organic chemical degradation evidence:



$\beta$ - caryophyllene chloride



$\beta$ - caryophyllene

In addition, this work in establishing the structure of the chloride precludes all but the formulation shown for the parent compound — $\beta$-caryophyllene. Additional stereochemical information obtained from the x-ray analysis is as follows:

a) the six-membered ring possesses the trans-hexane skeleton.

b) the seven-membered ring is cis -, with atoms 5,6, and 12 on the same side.

c) the union of the four-membered ring to the seven-membered ring is trans -, with the hydrogen atoms on carbons 2 and 5 projecting in opposite directions.

# GENERAL INTRODUCTION.

The use of x-ray diffraction in solving problems of structural chemistry is dependent upon the fact that the intensities of x-ray reflections from a crystal are directly related to the positions in space of the atoms or diffracting centres. The solution of any particular crystal structure depends therefore upon finding a set of atomic positions which give calculated intensities in good agreement with those observed, and this is usually achieved by trial and error methods followed by Fourier refinement (1).

If molecular constitution is known, the problem resolves itself into positioning the molecular units rather than the independent atoms, and in this case supplementary geometrical and physical data can be very helpful in arriving at an intelligent trial structure.

One may quote, in this respect, the case of hexamethyl benzene (2) where a survey of the reflection intensities brought to light the regular decline in the (o o l) axial reflection intensities. This indicated that the molecule lay almost perfectly in the basal plane of the unit cell, and this fact, together with a consideration of the cell dimensions and some high order reflections was sufficient to give the approximate structure.

Another instance of the intelligent use of supplementary/

supplementary/ data to postulate a trial structure is found in the x-ray investigation of naphthalene and anthracene (3). Both compounds were monoclinic with the *a* and *b* axes almost equal, and it was possible to tell from the variations in the *C* axis in going from naphthalene to anthracene how the long axis of the molecules lay. A consideration of the magnetic and optical anisotropies of the crystals then provided the additional information that the plane of the molecules was at a smaller angle to the *bc* than to the *ac* face of the cell. This enabled the molecule to be very roughly positioned in the unit cell, and from this start subsequent Fourier refinement enabled the exact structure to be determined.

In a great number of cases, however, lack of a molecular model precluded analysis by trial and error methods. Occasionally the limited information *directly* obtained from x-ray data was effective in partly solving unknown structures. The work of Bernal and Crowfoot (4) showed, for instance, that the older model of the sterine molecule was too wide and too short for the unit cell to accommodate it, and that the present formulation was more acceptable. Apart from such exceptions, the method of trial and error broke down when presented with complex unknown structures, and since a large number of important compounds fall into this class, modern x-ray work tends towards finding new and powerful/

powerful/ methods of directly attacking a structure from a purely x-ray point of view and with the minimum of reference to supplementary data.

## TECHNIQUES IN DIRECT STRUCTURE ANALYSIS.

**Vector Methods:** In a study of crystal structure by means of x-rays, two kinds of measurement can be applied to the various diffracted beams. These refer to their position and to their intensity. The intensity of the x-ray beam enables us to obtain the square of the important quantity termed the structure factor which is defined as the ratio of the amplitude of the wave received from a unit cell in a given direction to that which would be received if the contents of the cell were replaced by a single electron. Bragg (5) first showed that the structure factors or F ( h k l) values could be used to give the electron density at any point in the unit cell if employed as the coefficients in the Fourier series:

$$\rho(x\,y\,z) = \sum_{-\infty}^{+\infty}\sum_{-\infty}^{+\infty}\sum_{-\infty}^{+\infty} \frac{F(h\,k\,l)}{V} \cos 2\pi \left[\frac{hx}{a} + \frac{ky}{b} + \frac{lz}{c}\right] \ldots\ldots (I:I)$$

where,

$\rho(x\,y\,z)$ = electron density at the point ( x, y, z)

F ( h k l) = structure factor referred to the plane ( h k l)

a, b, and c are the unit cell parameters.

In the above relationship (1:1) the values for F ( h k l) are complex quantities characterised by an amplitude and a phase constant. The experimental measurements of intensity, while defining the amplitudes of the structure factor, can give no information regarding these relative phase relationships. This provides the limitation of the x-ray method of analysis.

A. L. Patterson (6), however, showed that the $F^2$( h k l) values which are all positive and obtained directly from an x-ray photograph can be used in a function:

$$A(uvw) = \sum_{-\infty}^{+\infty} \sum_{-\infty}^{+\infty} \sum_{-\infty}^{+\infty} \frac{F^2(hkl)}{V} \cos 2\pi \left[ \frac{hu}{a} + \frac{kv}{b} + \frac{lw}{c} \right] \text{-----} \quad (1:2)$$

He showed that A ( u v w) will be large only when there are high values of ( x y z) both at ( x, y, z) and at (x + u, y + v, z + w). Thus a peak in the function A ( u v w) at ( $u_1$, $v_1$, $w_1$ ) corresponds to an inter-atomic distance in the crystal defined by the vector whose components are ( $u_1$ , $v_1$ , $w_1$ )

The usefulness of the $F^2$ synthesis is subject to the inherent limitation of a vector diagram where the vectors are all erected from a single point. For simple substances this presents no difficulty but for more complex structures interpretation become difficult, especially as the number of/

of/ vector peaks increases as the function n (n - 1) where n is the number of atoms.

To obtain maximum information from an $F^2$ synthesis, tedious three-dimensional work is required. To shorten the labour, Harker (7) pointed out that space group symmetry should specify on which plane or along which direction in vector space most vector information will be found. This reduces the work to that of a two dimensional or even of a line-synthesis but incorporating three-dimensional data. If for instance it is discovered that all peaks will be found on the a c face of a cell it is only necessary to evaluate the Patterson function on that face. The function simplifies to:

$$A(u\,0\,w) = \sum_{-\infty}^{+\infty}\sum_{-\infty}^{+\infty}\sum_{-\infty}^{+\infty} \frac{F^2(hkl)}{V} \cos 2\pi\left[\frac{hu}{a}+0+\frac{lw}{c}\right]\cdots(1:3)$$

and if all planes having the same h and l values are added up and the sums considered as coefficients we have:

$$A(u\,0\,w) = \frac{1}{V}\sum_{-\infty}^{+\infty}\sum_{-\infty}^{+\infty}\left[\left\{\sum_{-\infty}^{+\infty} F^2(hkl)\right\}\cos 2\pi\left(\frac{hu}{a}+\frac{lw}{c}\right)\right]\cdots(1:4)$$

The vector method in direct structure analysis has been expanded by Buerger (8) and employed among others by Beevers and Robertson(9)/

Robertson (9)/ who have adapted vector-set methods to the solution of Patterson synthesis of a more complex nature. Except in exceptional cases, however, the Patterson method has so far been most useful when certain simplifying conditions such as the presence of a heavy atom were operative.

Methods Based on Phase Relationships.

Although a vector map may be difficult to interpret, it is still the most explicit method of presenting inbiased reflection data. Taken in conjunction with the crude picture obtained when a Fourier synthesis is done on partial or partially correct data, it may serve to define the structure. Two methods claimed to yield such data directly from x-ray results without recourse to any trial structure are those of Harker-Kasper (10) and of Sayre (11).

Harker-Kasper Inequalities. By applying Schwarz's and Cauchy's Inequalities to the formulae for calculating F (h k l) from respectively, the density of scattering matter, and the atomic positions in a crystal, Harker and Kasper were able to deduce relations between the magnitudes of some $F_{(h\ k\ l)}$ values and the signs or phases of others. Gillies (12) applied the method to the data for oxalic acid obtained by Robertson and Woodward (13) and obtained a substantial number of correct signs.

Sayre Equalities. Briefly, these are based upon the fact that if we have an array of resolved, like atoms, squaring the electron density leaves the distribution unchanged, in that it still consists of equal atoms in the same position as before. The relationships make the same general statement about the structure factors as do the Harker-Kasper inequalities: namely, that the phase of F (a + b) is closely related to that of F(a). F(b). Applied to the data for hydroxy-proline (14), this method gave Sayre sufficient signs for a recognisable projection of the molecule.

Cochran (15) and Zachariasen (16) developed similar relationships, and with them, the latter was able to obtain the structure of metaboric acid where other methods had failed.

These methods, though still undeveloped, extend great hopes in the field of complex structure determination although the cases so far studied have possessed intensity distributions which were favourable.

## The Heavy Atom Technique.

The methods of direct crystal and molecular structure analysis, however, which have so far enjoyed most success involve modifications of the heavy atom technique. Over the past few years an imposing number of structures has been solved largely by means of the heavy atom method and making minimum reference to/

to/ other considerations such as chemical evidence.

The first organic structure to yield to an absolutely direct analysis by this method was that of the complex phthalocyanin molecule(17) . Use was made of the fact that a nickel atom could be located in the phthalocyanin complex to form nickel phthalocyanin without radically altering the crystal lattice constants.

| | a | b | c | β |
|---|---|---|---|---|
| Free phthalocyanin | 19.9 A | 4.7 | 14.8 | 122° |
| Nickel Derivative | 19.9 | 4.7 | 14.9 | 122° |

It follows that the substances are isomorphs, and we may assume that the atoms occupy approximately equivalent spatial positions. The space group is $P2_1/a$ and contains two molecules per unit cell,. Since there are only two nickel atoms in the cell, and the only twofold positions in the space group lie at centres of symmetry, each nickel atom lies on a centre of symmetry. It thus makes a positive contribution to each x-ray reflection, and inspection of the absolute F values of equivalent reflections from the isomorphs yields the sign of each reflection. The general equation for such cases of isomorphous replacement is written;

$$F_{M_1 + \text{residue}} - F_{M_2 + \text{residue}} = M_1 - M_2 \cdots (1:5)$$

where $M_1$ = contribution of heavier atom

$M_2$ = contribution of lighter substituent (in this case, hydrogen)

In this way, 132 out of 152 reflections from the free phthalocyanin were given signs, and these gave a well resolved projection of the molecule.

For such a beautiful solution of the first structure to be tackled by this method the conditions were ideal. The ease of solution of subsequent problems by this method has depended upon how far these conditions have been fulfilled.

Conditions.

A) the interchangeable atoms must differ sufficiently in diffracting power.

B) the two compounds must crystallise in isomorphous crystals of which the cell dimensions (and accordingly the atomic positions) agree sufficiently.

C) the compounds must yield good Fourier projections with good atomic resolution

D) the crystal must possess a centre of symmetry, at least in the projection involved.

E) it must be possible to locate the variable heavy atom.

The phthalocyanin problem was simplified by the fact that the heavy atom position was fixed by crystal symmetry, and was replaceable by hydrogen. This obviated some of the absorption/

absorption/ defects of the heavy atom technique, to be discussed later. It was fortunate, also, that the projection (010) which gave best resolution was the one in which no space group complications occur. In either of the other two projections, the heavy atom is effectively face centred and does not contribute to reflections where, for instance, h + k is odd. Thus, in copper tropolone (18) with space group $P2_{1/a}$ the best projection was in one such zone and the heavy copper atom phase determined planes only where h +k is even. A Fourier synthesis containing these planes only shows the molecule superimposed on its mirror image, and with complex structures such a situation is undesirable.

Heavy Atom in General Position.

When a heavy atom coexists in a structure with lighter atoms, its vector peaks are usually distinguishable in a vector diagram because of their greater height. The advent of the Patterson method thus enabled a heavy atom to be located in a structure when its position was not fixed by crystal symmetry, and by using the information so obtained in relation (13) the signs could be obtained as before.

The first example of this extended method is to be found in the work of Cox and Jeffrey (19) on the x-ray investigation of glucosamine - H Br and - H Cl. Sufficient signs were/

were/ obtained by the method of isomorphous replacement to yield a recognisable projection of the molecule on Fourier synthesis.

A rather elegant application of the method was used by Wiebenga and Krom (20) in obtaining the structure of d - $\alpha$ - Br - , Cl - and CN - camphor. It is convenient to mention, here, a few of the inherent difficulties of the heavy atom technique and to show how these were overcome in the case of this compound.

The space group of all three compounds was $P2_1$ and the lattice parameters were:

| | Br | Cl | CN |
|---|---|---|---|
| a | 7.4 A | 7.3 | 7.1 |
| b | 7.6 | 7.5 | 7.4 |
| c | 9.1 | 9.0 | 9.4 |
| $\beta$ | 94° | 93° | 94.5° |

The compounds are thus isomorphous with the CN - compound departing a little from true isomorphy.

<u>Accurate Absolute F Values.</u>

As a molecule becomes larger, the contributions of the various atoms to the structure amplitudes tend to cancel each/

each/ other out and we are left with a small residue. If we further introduce a heavy atom into the molecule, these small light atom contributions are swamped by the strong heavy atom reflections, and in order to reveal the light atoms on Fourier synthesis, a high numerical accuracy of reflection estimation is required. These intensities must be further corrected for absorption which in the case of heavy atom compounds is high, and methods of correction are never satisfactory.

Wiebenga and Krom used cylindrical crystals in their experiments in order to make easiest corrections for absorption. Absolute intensities were obtained by standardisation against rock salt using a novel two-crystal method which had been tested with resorcinol (21) and found reliable. Special precautions were taken to ensure that all reflections had the same spot size and by using both copper and molybdenum radiation, extinction coefficients for primary and secondary extinction were obtained and these errors corrected

Even with these precautions, the "absolute" intensities obtained with different crystals varied over a 10% range but the average values for the bromide and chloride were good enough to give a good number of signs in the centrosymmetric (h o l ) zone. Fourier syntheses on the bromide and chloride gave almost identical, projections, on which the atoms were sufficiently well resolved/

resolved/ to yield the structure.

## Fine Structure of the Molecule.

It has been already observed that with a heavy atom present, intensities are usually badly affected by absorption. A further complication is that incoherent scatter from the heavy atom produces a troublesome background which creates considerable termination of series errors. These factors operating, the data obtained from a heavy atom compound can rarely be used to obtain accurate coordinates, as was obvious in the case of Br - and Cl - camphor when the heavy atom contributions were subtracted from the reflection intensities.

| (h o l) | $F_{Br\text{-}camph.} - F_{Br}$ | $F_{Cl\text{-}camph.} - F_{Cl}$ |
|---|---|---|
| 001 | + 10 | + 17 |
| 100 | - 7 | - 7 |
| $10\bar{1}$ | - 67 | - 58 |
| 101 | + 46 | + 41 |
| 002 | - 8 | - 15 |

These low order residues should be almost identical for the two isomorphs, and the discrepancies show that the various corrections have not been entirely successful. Further refinement on the basis of these intensities would, therefore, be valueless.

The values were sufficiently close, however, for averages to be taken, and these were accepted as a good approximation to the residue reflections in CN - camphor. The molecule was completed by postulating the position of the CN - group and its contribution to the various reflections was calculated. These calculated values were added to the residue contributions.

| (h o l) | F CN - camph. calc. | F CN - camph. obs. |
|---|---|---|
| 001 | + 26 | 26 |
| 100 | - 8 | 8 |
| $10\bar{1}$ | - 46 | 45 |
| 101 | + 26 | 22 |
| 002 | - 20 | 25 |

98 out of 112 signs were obtained thus and the Fourier refined in the usual way. The absorption for this light atom compound was negligible and the coordinates of the atoms correspondingly more accurate. A longer series was available because of the light background, and no bad series terminations occurred.

We have here a case rather different from the phthalocyanin one, in which three isomorphous substances occur.

Lattice parameters indicate that the method of isomorphous replacement can best be applied to a particular pair, and as a result approximate coordinates of the atoms are obtained. The data is then applied with small adjustments to the third compound to give a good trial structure. Here the intensities are accurate and full, and allow of maximum refinement to give accurate coordinates.

It is seldom the case that the heavy atom technique adapts itself to fine structure determination as with CN - camphor and phthalocyanin. Demands are usually only made upon it to the extent of structure determination where relatively crude coordinates suffice.

## Suitable Heavy Atoms.

The possibility of finding suitable isomorphous compounds is not perhaps so small as might be feared. Isomorphs are often found with the substituents - Br, - Cl, - $CH_3$, - Se, - S, - O, - Cs, - Rb, - K, - Ba, - Pb, - Sr, - Ca. Also available are the Cs, Rb, K salts of acids and the halo-, selenic and sulphuric acid salts of bases.

If the values of reflections could be obtained accurately on an absolute scale, the choice would at all times be directed towards finding a pair of isomorphs where one of the replaceable atoms was particularly light. In this way, accurate intensities could be obtained for the light atom compound and/

and/ the structure determined accurately. Usually, because of the small magnitude of the light atom reflections together with scaling errors, only the signs of the heavy reflections are obtained with any certainty. If, for instance, we substitute in equation (15) a set of accurate data, and get the result:

$$|50| - |6| = +44$$

then the intensities are + 50 and + 6. If, however, the "absolute" F values are out by 10% we get:

$$|45| - |6| = +44$$

The strong reflection must still be positive, but the sign of the weak reflection is indefinite in this case. When this occurs, it may be possible should intensity errors not be too heavy, to perform a Fourier synthesis on the reflections for the heavier atom compound and obtain sufficient information to postulate a structure. The coordinates can then be transferred to the lighter atom isomorph, and a critical refinement made.

## Structure Determination with a Single Compound.

One of the cruder modifications of the heavy atom technique is applicable when a compound is available containing a very heavy atom which can be assumed to phase determine most reflections. This was done in the solution of the Pt-phthalo-/

Pt-phthalocyanin (22) problem, where the heavy atom lay on the origin and contributed to all the planes. When the heavy atom lies in a general position (or in certain special positions) it does not contribute substantially to all the reflections and a number of important planes may have to be omitted in a Fourier synthesis. The method was, however, employed in the solution of cholesteryl iodide (23) by Carlisle and Crowfoot, and strychnine hydrogen bromide (24) by Robertson and Beevers. Further difficulties are connected with the necessary presence of a very heavy (strongly diffracting and absorbing) constituent. Accurate intensity measurements are required, and thorough absorption corrections must be applied. Series termination is usually serious. A compensating advantage is that there is no need for absolute intensities.

## Non-centrosymmetric Case.

A final extension of the application of the heavy atom technique is to the case where the structure has no centre of symmetry. So it is with all optically active compounds so far studied and into this class falls practically the whole field of biochemically important substances. It is, of course, true that two dimensional projections of non-centrosymmetric crystals will be centrosymmetric when projected down an axis of even symmetry. In monoclinic/

monoclinic/ crystals, however, there is at most only one such projection and its value may be limited. Thus, in three dimensional work on non-centred space groups and in two dimensional work on non-centred projections, it is important that the heavy atom technique be applicable.

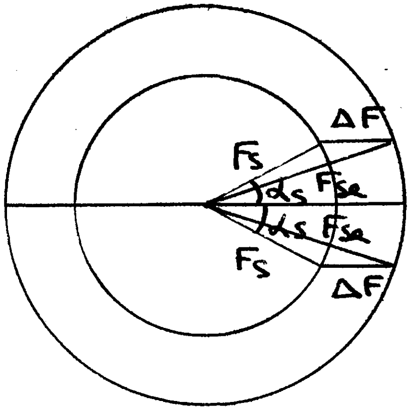
The general formula(15) is still valid if we consider all quantities as vector quantities and consider the phase angles, but it should be noted that, practically, unavoidable discrepancies between corresponding atomic positions in the two compounds and experimental errors in measuring intensities will in non-centred compounds cause serious inaccuracies in the phase constants.

The method was recently used in the solution of the isomorphous sulphate and selenate of strychnine pentahydrate(25) which crystallised in the monoclinic system. As the heavy atom lay on the symmetry centre of the (010) projection, the application of the heavy atom method for this projection followed the well known lines developed for the phthalocyanin structure. For the non-symmetrical (001) projection, the phase angles with the exception of their signs, were derived from the equation:

$$F_{\text{St.sel.}} - F_{\text{st. sulph.}} = F_{\text{sel.}} - F_{\text{sulph.}} = \Delta F$$

Since the heavy atom is located at the cell origin, the right/

right/ hand side has a phase angle zero for every reflection.



The phase angles are obtained either trigonometrically or graphically, but as the diagram shows, the equation is satisfied by two vector sets, one above and one below the axis, $\alpha = 0$. The magnitude only of the phase angles $\alpha_S$ and $\alpha_{S\ell}$ can thus be determined.

If, however, a Fourier synthesis is now done bringing in each term twice with $\alpha$ positive and $\alpha$ negative, the result is a projection of the molecule together with its mirror image. The (010) projection, in the case of the strychnine salt, was used to identify relevant peaks, and a Fourier done with the calculated phase angles.

## Estimation of Accuracy.

Several disadvantages of the heavy atom technique have been discussed but a further complication lies in the assessment of accuracy of results. Since the solution of any particular crystal structure depends upon finding a set of/

of/ atomic positions, which give calculated intensities identical with those observed, the criterion of accuracy has been taken to be the value of the ratio:

$$\frac{\sum |F_{obs.} - F_{calc.}|}{\sum |F_{obs.}|} = \frac{\sum |\Delta F|}{\sum |F_{obs}|} \quad \ldots\ldots (1:6)$$

In the case of relatively simple structures containing light atoms, a value of the ratio lying between 0.1 and 0.2 is generally taken to be indicative of a correct structure, although the refined work of Cochran on adenine hydrochloride(26) has shown that careful experimental work and thorough refining can reduce this ratio to an extremely small value.

When a heavy atom is introduced into the structure, however, the value of the ratio becomes less of a standard quantity for two reasons.

1) Due to absorption errors which can only be partially corrected, the threshold value of the ratio which in the case of a light atom compound is small and fairly constant, in the case of a heavy atom compound is relatively large and variable. This threshold value is independent of correctness of structure and is a measure of correctness of experimental data.

2) Due to the fact that the denominator in the expression (16) varies in an arbitrary way with the nature of the heavy atom introduced, the same value of $\sum|\Delta F|$ in the enumerator can give widely different values of the ratio. Because of the large numerical value of $\sum|F \text{ obs.}|$, a relatively large value of $\sum|\Delta F|$ signifying a poor accuracy may give a low value of the ratio. Thus, in the early stages of the investigation of penicillin (27) it was found that the value for the discrepancy of the rubidium salt was better than that for the potassium salt although the accuracy was no better. At one stage in the solution of the structure of $\beta$ -caryophyllene chloride (loc.cit), coordinates for a trial structure for the isomorphous <u>bromide</u> with a discrepancy of 21% were transferred to the <u>chloride</u>, and although the value of was practically unchanged, the value of the ratio for the chloride rose to 38%.

In general, when the heavy atom is potassium or lighter, the value of the ratio (16) seems to be still significant as a criterion of correctness of structure with the value lying around 0.2

| | | Discrepancies. | |
|---|---|---|---|
| Two dimensional data | Adenine H Cl(28) | 20 | 24% |
| | Guanine H Cl(29) | 17 | 24 |
| | Geranylamine H Cl(30) | 17 | 18 |
| Three dimensional data | Sodium benzyl penicillin(27) | | 22% |
| | Potassium benzyl penicillin(27) | | 19 |

Where the heavy atom is heavier than the range quoted, the magnitude of the overall discrepancy ceases to be a good criterion of accuracy and the individual discrepancies between the calculated and observed intensities are probably a better measure of the correctness of the structure.

Fig.(1.1) is an extract from the graphical representation of the observed (full line) and calculated (broken line) F values for the ( h o l) planes of strychnine hydrogen bromide(24). l begins with the value 0 at the left hand side.

The quantitative estimation of accuracy when dealing with a heavy atom compound is difficult and most authors merely claim bond lengths in completed structures to ± 0.1Å. If one takes the final bond lengths quoted by Cochran (27) for adenine H Cl/

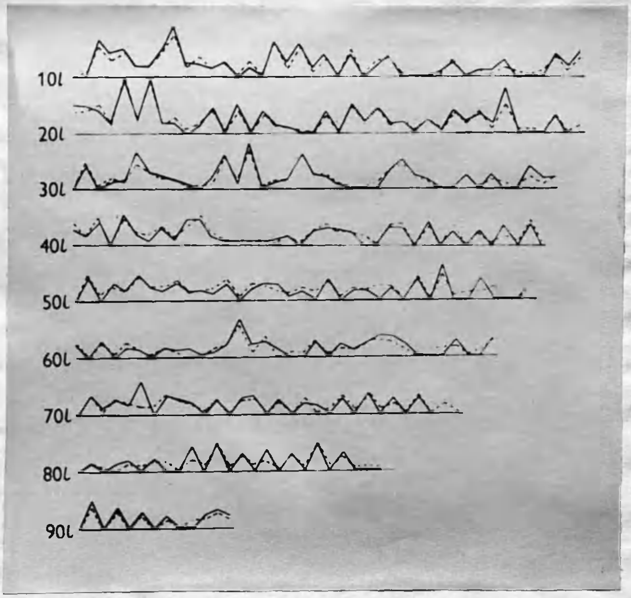


Fig.(1:1)

Graphical comparison of the observed ( full line) and calculated ( broken line ) F values for the (hol) planes of strychnine hydrogen bromide. l begins with the value 0 at the left hand side.

Cl/ as being correct, then the structure previously submitted by Broomhead (27) with a discrepancy of 20% and 24% possessed a mean error in bond length of 0.045 Å and a maximum error of 0.08 Å. Usually in structure determination this accuracy is more than sufficient. It is known that atoms bonded together in an organic molecule have their centres at distances of the order of 1.5 Å apart whereas if there is no primary valency bond between them, the distances are of the order of 2.5 Å or more. An accuracy of ± 0.5 Å in fixing an atomic position should therefore be sufficient to determine a chemical structure, so that the accuracies obtained in heavy atom analysis are usually sufficient.

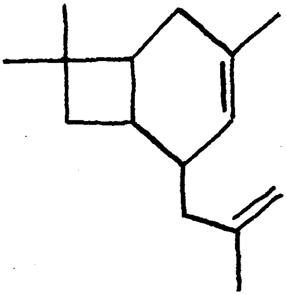
## THE CARYOPHYLLENES. § 2.

Caryophyllene, the principal sesquiterpene hydrocarbon of oil of cloves, has been the subject of numerous investigations since 1834, and for our knowledge of its chemistry, we are chiefly indebted to Simonsen (31), Ruzicka and their associates. The oil is difficult to fractionate by distillation but has been shown to contain probably two isomers and possible a third, all with the formula $C_{15}H_{24}$. This thesis embodies work done to establish the structure of one of them --- β-caryophyllene -- by direct x-ray crystal analysis of heavy atom derivatives of the sesquiterpene and of associated compounds, and the following is a brief summary of the purely chemical work done on the same problem.

Prior to 1950, all that had been established about the structure of the molecule was that the hydrocarbon was dicyclic with two double bonds, and that it contained a cyclo-butane ring with a gem.-dimethyl grouping. The former was readily established because of the facile formation of a dihydrochloride (32). The second fact was not finally established until 1936 when Rydon (33) synthesised norcaryophyllenic acid, an oxidation fragment of β -caryophyllene (34). Apart from this, much of the remaining information was speculative, and it is merely for interest that the following chronological survey of the early arguments is given.

<u>1911</u>. Semmler and Mayer (35) first studied the results of the mild oxidation of the β-caryophyllene molecule and isolated various important fragments. Two of these were the compounds $C_{11}H_{18}O_3$ (a keto acid) and $C_{14}M_{22}O_4$ (a diketo acid) both containing one methyl ketone grouping as demonstrated by the hypobromite reaction. Various smaller fragments were isolated.

<u>1935</u>. On the basis of studies on the ozonolysis products of β-caryophyllene, Ruzicka (36) postulated structure (2: 1) for caryophyllene:



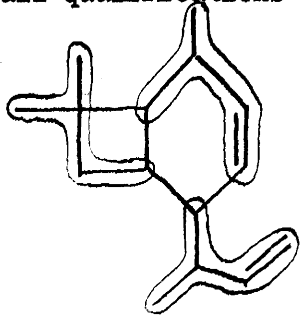
.......... (2:1)

Blair (37) pointed out that Ruzicka's formulation did not conform to the latter's postulate of 1922 that all sesquiterpenes consisted of a folded farnesol chain:

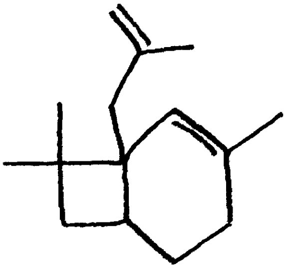
farnesol skeleton.

In addition, it was known that β-caryophyllene acid-ring-closed to form tricyclic clovene (38) ( $C_{15}H_{24}$ ), and/

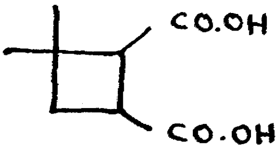
and/ that this gave a $C_{15}$ dicarboxylic acid on oxidation(39). A formula to fit all qualifications was suggested by Blair as:



Ramage (40) indicated that Ruzicka's chemical evidence was in any case not unambiguous and that the following structure was also permissible:



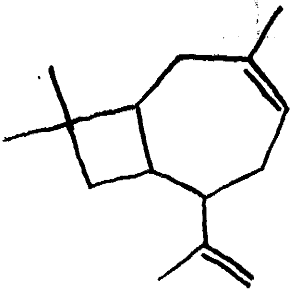
1936. A positive step was made by Rydon (33) in the synthesis of norcaryophyllenic acid, one of the oxidation fragments of β -caryophyllene. The existence of the four-membered ring and the gem.-dimethyl group was therefore established.



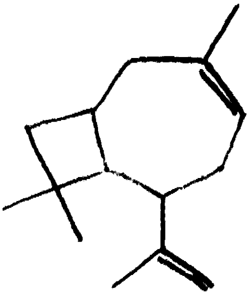
Norcaryophyllenic acid.

1938. Requoting Blair's objections to formula (2:1) Rydon(41) /

Rydon (41)/ by analogy with the azulene forming sesquiterpenes (42) suggested a seven membered ring system:



or

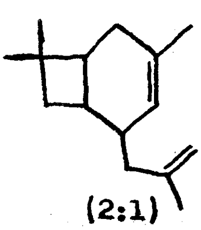


..... (2: 2)

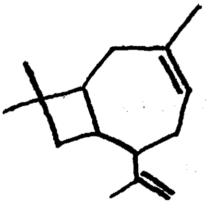
This structure would ring-close to give a formulation for clovene such as would oxidise to a $C_{15}$ dicarboxylic acid. The isopropylene side chain of formula (2: 2) was more acceptable than the isobutylene grouping of structure (2:1).

The existence of a diketone, $C_{13}H_{22}O_2$ which Ruzicka had obtained among the oxidation products of caryophyllene was explained by structure (2;2).

<u>1939</u>. Ruzicka (43) published work on dihydrocaryophyllene which seemed to indicate that β-caryophyllene contained a mixture of the two forms:



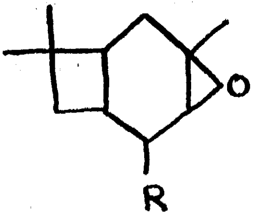
(2:1)



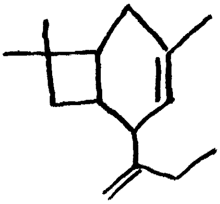
(2:2)

<u>1947</u>. Treibs (44) prepared β-caryophyllene monoxide, a/

a/ crystalline product eminently suited to stepwise degradation. This he postulated as:



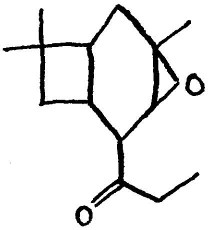
and from further oxidation studies he suggested for caryophyllene the structure:



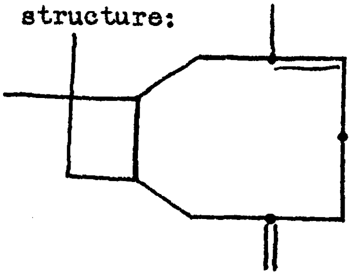
This was the extent of knowledge in 1949 when the x-ray investigation was begun. The dihydrochloride of β-caryophyllene is widely reported in the literature and is easily prepared. It had been intended to attempt the preparation of the dihydrobromide, and if it were isomorphous, to obtain the structure directly by the method of isomorphous replacement. The dihydrochloride is dicyclic and presumably possesses the β-caryophyllene framework. It was not, however, found possible to prepare the dihydrobromide, and so work was done directly on the chloro-compound keeping structures (2:1) and (2:2) in mind. The difficult task of postulating 68 parameters in the asymmetric/

asymmetric/ unit of the (h o 1) projection was suspended when a third structure was put forward by some Czeck workers.

In 1950, Sorm (45) and his co-workers examined the infra-red spectrum of an oxidoketone which Treibs had obtained by oxidising his $\beta$ -caryophyllene monoxide. This had been postulated as:

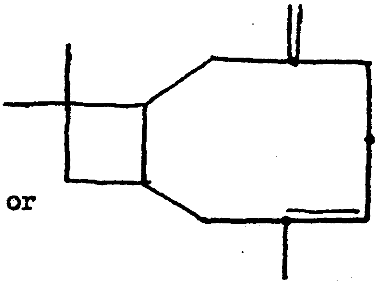


Sorm found the $>$C = 0 stretching frequency to be lower than for either aliphatic ketones or cyclohexanones, and noted that an analogous low-frequency shift had been observed in the case of cyclonones possessing greater than six membered rings (46). Further infra-red work on fission products of dihydro-$\beta$- caryophyllene oxide seemed to put forward a case for a nine membered ring for $\beta$ - caryophyllene. Sorm tentatively suggested the structure:



(2:3) (a)

or



(2: 3) (b)

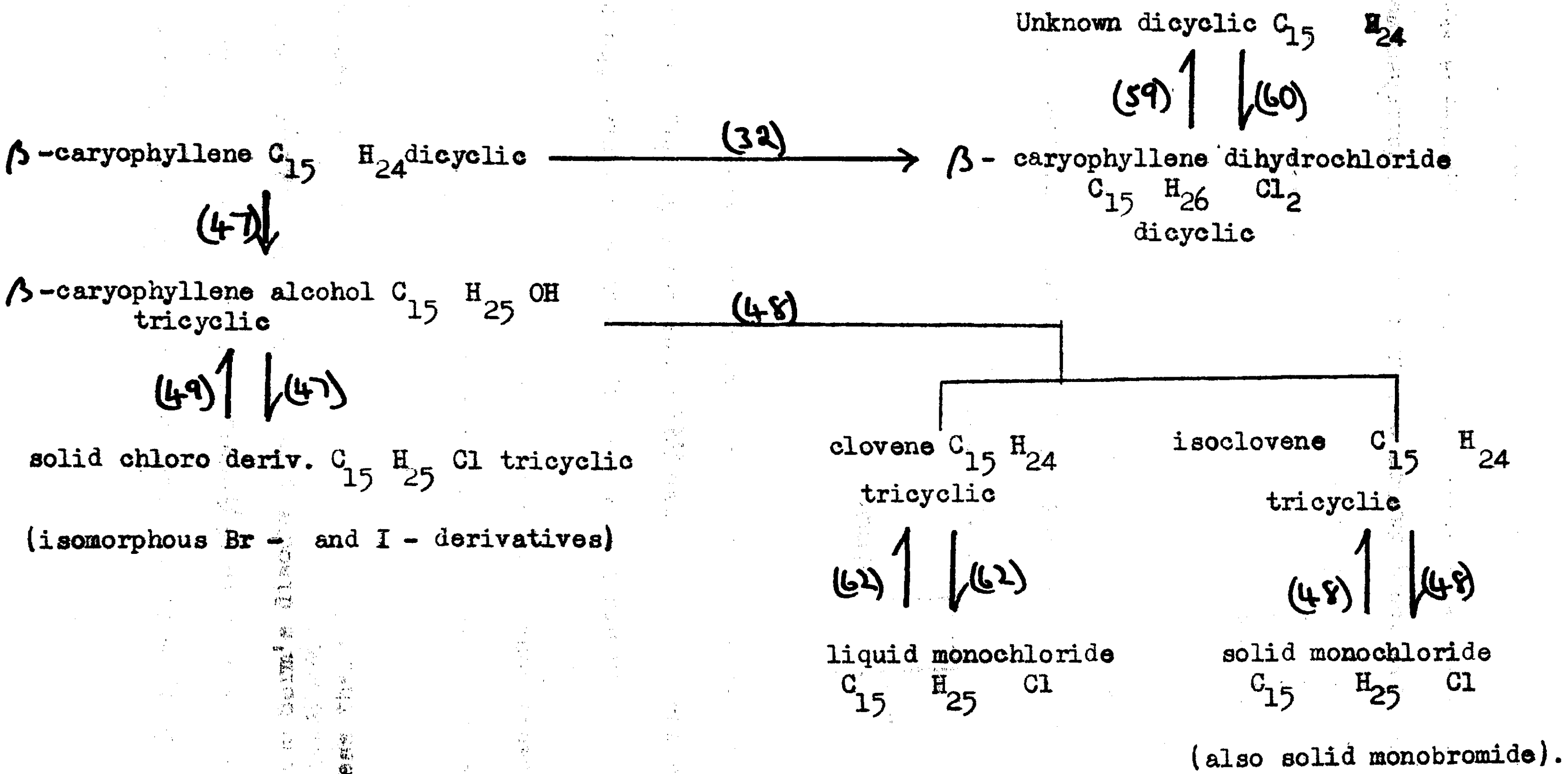
Although Sorm's work is now recognised as being the/

the/ turning point in the caryophyllene investigations, at the time it merely underlined the importance of solving the caryophyllene problem by direct x-ray methods without reference to the grossly conflicting chemical evidence. Since this had not been found possible with the only crystalline heavy atom derivative of caryophyllene itself, attention was turned to the closely allied compound, $\beta$-caryophyllene alcohol. This <u>tricyclic</u> alcohol, $C_{15}H_{25}OH$, does not contain the simple caryophyllene nucleus, but is one of the most important cyclisation products of caryophllene. It was first prepared by Wallach and Walker (47) by the hydration of caryophyllene, and is dehydrated with $P_2O_5$ to a mixture of tricyclic clovene and isoclovene (48). The structure of the alcohol is thus an important link between the unknowns, $\beta$-caryophyllene and clovene.

$\beta$-caryophyllene alcohol may be halogenated (47) to yield chloro-, bromo- and iodo- derivatives which are isomorphous and suitable for direct x-ray analysis by the method of isomorphous replacement. The chloride has been shown to be reconvertible to the alcohol (49), and although the possibility of reversible stereochemical (or even structural) change must be borne in mind, it is very likely that the structure of the alcohol and of its heavy atom derivatives are the same.

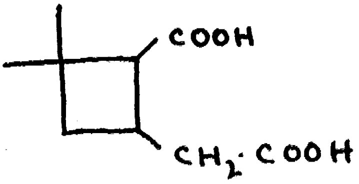
The inter-relation of some of these compounds is shown in Table (2:1):

TABLE (2:1)



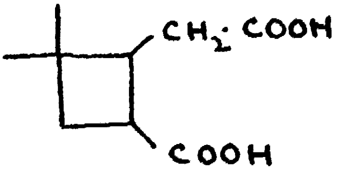
X-ray work was begun on the isomorphous chloro - and bromo - derivatives of β-caryophyllene alcohol, and in the meantime Sorm's discovery had enabled the organic chemists to reassess their data in the light of the new postulated nine-membered ring. Their work was put on a sounder footing by work establishing the relation of the cyclobutane ring to oxidisable centres in the molecule as follows:

1950. Dawson and Ramage (50) synthesised stepwise the compound a and showed that it was not identical with caryophyllenic acid, an oxidation fragment of β-caryophyllene:



........... a

1951. Campbell and Rydon (51) synthesised compound b and showed it to be identical with caryophyllenic acid:

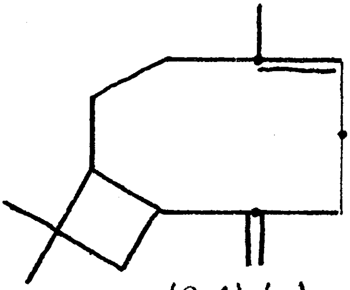


........... b

In the same year, Barton and Lindsay (52) published work on Treibs' oxidoketone which, they submitted, confirmed the structure (2: 3). They were as yet unable to distinguish between forms/

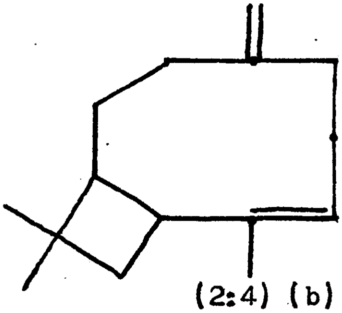
forms/ (2:3) (a) and (2:3) (b).

This was followed by a paper by Dawson, Ramage and Wilson (53) which described degradation work on the blue β -caryophyllene nitrosite (54). Structure (2: 3) was disputed by these authors who suggested structure (2:4)



(2:4) (a)

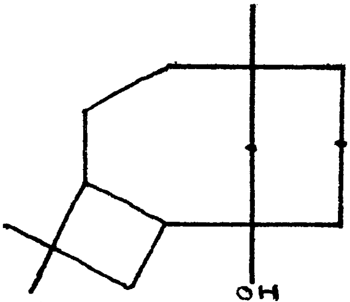
β - caryophyllene



(2:4) (b)

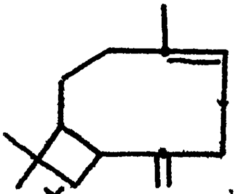
γ - caryophyllene

Barton, Brown and Lindsay (55) agreed with this formulation and suggested a structure for β - caryophyllene alcohol.

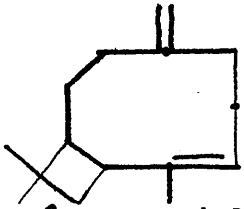


β - caryophyllene alcohol.

1952. Dawson, Ramage and Whitehead (56) re-examined the evidence and put forward an amendment to the previous postulate of Dawson, Ramage and Wilson, as follows:

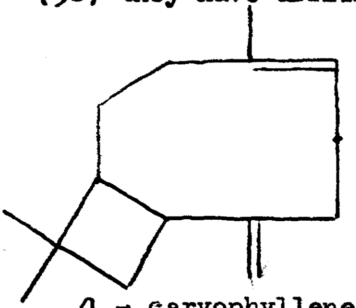


γ - caryophyllene

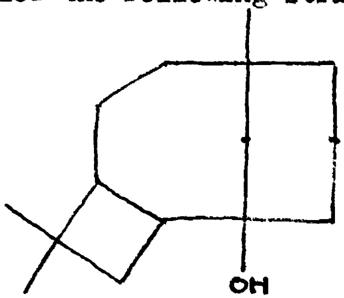


β - caryophyllene

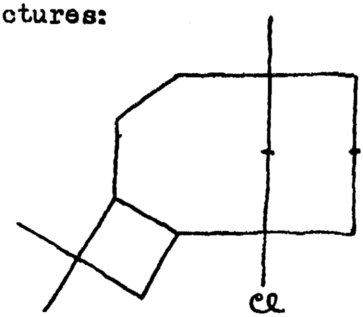
The school of Barton, Brown and Lindsay(57) did not accept this conclusion and preferred to retain (2:4) (a) as the structure of $\beta$ -caryophyllene. In a summarising article (58) they have affirmed the following structures:



$\beta$ - caryophyllene



$\beta$ - caryophyllene alcohol



$\beta$ -caryophyllene Chloride

§ 3.

## The structure of β-caryophyllene dihydrochloride and dihydrobromide.

### Chemical Synthesis of the Heavy-Atom Compounds.

A quantity of B.D.H. caryophyllene was distilled under reduced pressure, the fraction boiling between 127-129° C at 15 mm. pressure being taken as reasonably pure β-caryophyllene.

Simensen ( 61 ) states that β-caryophyllene boils at 129-130° C / 14 mm. Hg

### β-Caryophyllene Dihydrochloride.

This is obtained from β-caryophyllene by the addition of two molecules of hydrogen chloride.

A quantity of β-caryophyllene was dissolved in twice its volume of anhydrous ether and cooled to 0°C. Dry hydrogen chloride gas was passed in slowly in the cold until absorption was complete. The ether was removed under reduced pressure and a dark red oil remained. This was dissolved in a minimum of alcohol at 30-40°C and the solution cooled slowly to -10° C. Crude β-caryophyllene dihydrochloride was deposited as yellowish crystals and after repeated crystallisation from alcohol, a yield of clear, colourless crystals, melting at 69-70° C, was obtained.

Bell and Henderson ( 62 ) have shown that a liquid monochloride, yielding clovene on removal of hydrogen chloride is also/

also/ formed in substantial yield.

### β-Caryophyllene Dihydrobromide.

No information regarding this compound could be found in the literature. Two methods of preparation were attempted:

I. Pure dry hydrogen bromide gas was prepared according to the method of Houben (63). Liquid bromine was dropped into warm tetralin and the hydrogen bromide gas which was formed by reduction, was purified of bromide vapour and dried by passage through U-tubes containing red phosphorus and phosphorus pentoxide.

A quantity of β-caryophyllene was dissolved in ether and the hydrogen bromide gas bubbled through in the cold as in the preparation of β-caryophyllene dihydrochloride.

As in the latter case the ethereal solution changed colour through red to a deep purple. This is attributed by Bell (62) to the presence of azulene forming compounds in the caryophyllene.

The ethereal solution obtained after the passage of the gas was evaporated and the resultant oil dissolved in alcohol. No crystals were obtained on cooling. The experiment was repeated several times, care being taken to exclude water. No crystalline product was obtained.

The red oil obtained after the evaporation of the ether was placed in a desiccator containing caustic soda for several days until very little smell of hydrogen bromide remained. It was then dissolved in anhydrous ether and the solution introduced into an alumina chromatograph column. Development resulted in a shallow red stationary band at the top and a mobile yellow band. Further development and elution resulted in the collection of the yellow fraction, and the red band was only eluted by changing the solvent to alcohol.

Two fractions were thereby obtained, which on evaporation of the solvent yielded:

a) yellow viscous oil.

b) red brown tar.

Each was dissolved in the minimum amount of alcohol and kept over a period at -10°C. Fraction b) deposited a minute amount of yellow crystals which either absorbed water or melted while microscopic examination was being made.

Further examination of the fractions was not made.

II. Semmler (64) has prepared eudesmene dihydrobromide by shaking eudesmene with glacial acetic acid saturated with hydrogen bromide.

A quantity of β -caryophyllene was placed in a flask with five times its volume of glacial acetic acid saturated with pure, dry hydrogen bromide gas, and the mix shaken. A rise in temperature occurred and the flask was placed in a cooling mixture until all reaction had ceased. Thereafter, the flask was shaken mechanically for half an hour and then left in the cold for a few days. Two liquid layers resulted but no solid material. The upper layer containing the caryophyllene residues was dissolved in alcohol and cooled. No crystals were obtained.

This experiment was repeated, attention being paid to keeping the temperature low during reaction. No success was obtained.

Attempts to prepare the hydrobromide were suspended at this stage. Workers ( 62) stresses the fact that in the preparation of β -caryophyllene dihydrochloride large biproducts of a mono hydrochloride of clovene result, because of ring closure by the acid. It is felt that hydrogen bromide would be even more likely than hydrogen chloride to bring this ring closure about, and form the unwanted biproduct. Any decomposition of the hydrogen bromide to liberate free bromide would also readily form a dibromo derivative of β -caryophyllene ( 65 ). These reactions all yield liquid products which/

which/ probably account in part for the fractions obtained from the chromatograph.

---

## The x-ray investigation of the crystal and molecular structure of the dihydrochloride.

Crystal data.

$\beta$-caryophyllene dihydrochloride $C_{15}H_{26}Cl_2$

M, 277.3 m.p. 69.5°C

d, calc. 1.150 d, found 1.152

monoclinic prismatic $a = 19.98 \pm 0.04$ Å

$b = 6.16_0 \pm 0.00_7$

$c = 13.11 \pm 0.04$

$\beta = 100.25 \pm 0.25^0$

Absent spectra (0k0) when k is odd

Space group $C_2^2$ ($P2_1$) or $C_{2h}^2$ ($P2_1/m$) on purely x-ray evidence

The compound is optically active.

Hence a mirror plane is precluded and the space group is

$C_2^2$ ($P2_1$)

There are four molecules per unit cell( two per asymmetric unit) with no molecular symmetry.

The volume of the unit cell = 1587.9 Å$^3$.

The absorption coefficient for x-rays ( $\lambda = 1.54 \mathring{A}$ )

$$= 48.85 \ \mathrm{cm}^{-1}$$

Total number of electrons per unit cell = F (000) = 600

The crystals are colourless and are elongated along the b-axis. The (100), (001) and $(10\bar{1})$ faces are all well developed in specimens crystallised from alcohol.

## Experimental.

Crystal reflection data: copper $K_{\alpha}$ radiation, $\lambda = 1.54 \mathring{A}$, was employed in all the measurements. Rotation, oscillation and moving film Weissenberg photographs were used for space group determination and intensity records.

Crystal density measurements were made by flotation in solutions of calcium chloride at 25° C, a trace of detergent and air evacuation being used to avoid bubbles. Six crystals were employed and four agreed with d = 1.152 ± 0.005. Two results were grossly low due to air bubble formation.

The calculated value of the density ( 4 mols / unit cell) is 1.150

Measurement of reflection intensities: the multiple film technique ( 66 ) was used to correlate intensities in the effectively centrosymmetric (h o l) zone. The intensity range covered was 1000 to 1 and 186 reflections were observed out of/

of/ a possible 680.

These were corrected for Lorentz and polarisation effects and are listed in Table (3:1) on an approximate absolute scale. (This was obtained by scaling the F obs. values to the F calc. values in the trial structure attempts).

In calculating F values, the curves quoted by James and Waller factor, exp. $\left( - B\left\{\frac{\sin\theta}{\lambda}\right\}^2 \right)$, were used. B was,bye- as 2.0 x $10^{-16}$.

Waller factor, exp. $\left( - B\left\{\frac{\sin\theta}{\lambda}\right\} \right)$, were used. B was taken as 2.0 x $10^{-16}$.

The crystals were not appreciably volatile but were prone to twinning. Multiple fracture resulted from attempts at crystal cutting. No good photographs were obtained in either the (hko) or (okl) zones, although sufficient information was obtained for space group purposes.

The (hol) intensities were finally obtained with a cylindrical crystal of cross-section 0.2 mm. No absorption correction was applied.

## Analysis of structure.

Space group $P2_1$ has the general twofold position

$$x, y, z; \quad \bar{x}, \tfrac{1}{2} + y, \bar{z}$$

and the structure factor F is given by:

$$A = 2 \cos 2\pi (hx + lz + \frac{k}{4}) \cos 2\pi (ky - \frac{k}{4})$$

$$B = 2 \cos 2\pi (hx + lz + \frac{k}{4}) \sin 2\pi (ky - \frac{k}{4})$$

where $F^2 = A^2 + B^2$ and $\tan^{-1} \alpha = \frac{B}{A}$

Maximum resolution is to be expected for projection down the shorter b axis and this projection is effectively centro-symmetrical: <u>(hol) zone</u>

$$A = 2 \cos 2\pi (hx + lz)$$

$$B = 0, \therefore \alpha = 0^\circ \text{ or } 180^\circ$$

Vector map of (hol) projection.

The $F^2$ values obtained directly from the film and corrected for Lorentz and polarisation were introduced as positive coefficients into a Patterson synthesis.

An array of n atoms will yield in a Patterson synthesis a total number of peaks equal to n (n - 1) (discounting peak at origin which represents the distance of each atom from itself). If the array is in addition centro-symmetric, n peaks occur as single peaks while the rest are paired giving $\frac{n(n-2)}{2}$ doubled sized peaks.

Fig. (3:1) represents the vector map of sixty-eight atoms of which eight being chlorine have three times the scattering power of the others. If resolution of each vector peak were perfect, this would imply that each chlorine to chlorine vectorial peak should be nine times as large as each carbon to carbon peak, and three times as large as each carbon to chlorine peak, so that relative heights should indicate the nature of the vector. Unfortunately, the necessorily bad resolution of the atoms and probably of the vector peaks themselves makes identification of the vector peaks difficult.

As a guide to the interpretation of the Patterson vector map the following method was used.

In the (hol) zone of the space group $P2_1$, the geometric structure factor has the form:

$$S = 2 \cos 2 \pi (hx + lz)$$

where h and l are the indices of the plane we are considering, and ( x, z) are the coordinates of the atom whose contribution to the plane we are evaluating. S has its maximum numerical value for positions (x, z) which lie on the plane or on the interleaving planes. Accordingly a survey of the (hol) reflections/

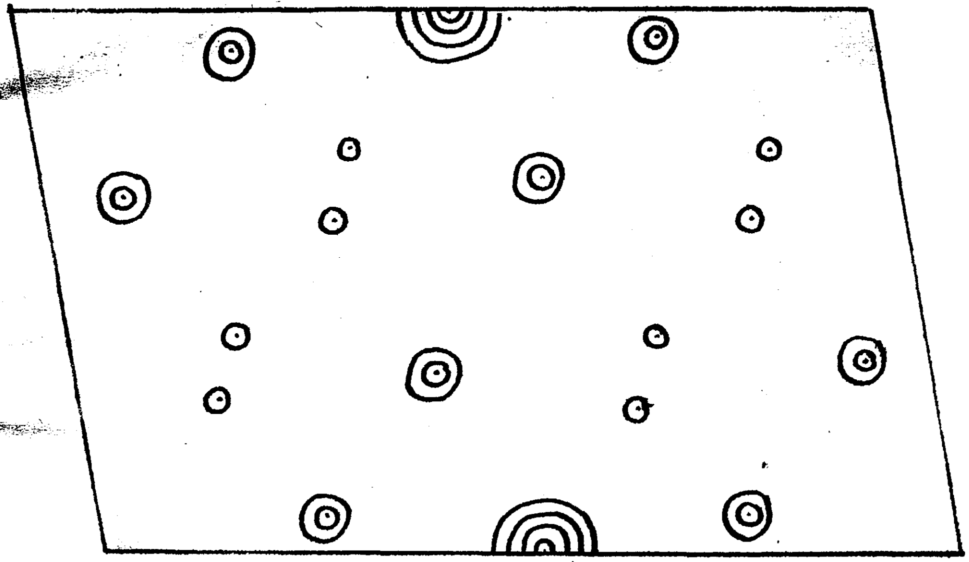


Fig. (3:1)

Patterson projection of the (hol) zone of $\beta$-caryophyllene dihydrochloride. Contour scale arbitrary.

Transparent overlay shows the theoretical vector map on the basis of the postulated chlorine positions listed in Table(3:2). Contours are proportional to multiplicity of peaks.

reflections/ was made and the following small spacing planes were selected as having well above average intensity:

$(\underline{h\ o\ l})$

$3\ 0\ 5$

$4\ 0\ 1$

$\bar{2}\ 0\ 6$

$\bar{4}\ 0\ 2$

$\bar{8}\ 0\ 1$

$\bar{8}\ 0\ 3$

$1\bar{6},\ 0\ 2$

These together with the interleaving planes were traced on a scale drawing of the (010) projection, and areas of high linear density were taken to indicate possible chlorine sites. The result was not unambiguous and several sites were obtained which were equally probable. Some time was spent in attempting to relate these to the vector map and eventually four "chlorine" positions were chosen as shown in Table(3:2.) The theoretical vector map obtained from these positions is shown in the transparent overlay to Fig. (3:1).

TABLE (3:2)

| Atom | x, (Å) | $\frac{2\pi x}{a}$ (deg.) | z, (Å) | $\frac{2\pi z}{c}$ (deg.) |
|---|---|---|---|---|
| $Cl_1$ | 1.83 | 33 | 9.29 | 255 |
| $Cl_2$ | 6.49 | 117 | 8.41 | 231 |
| $Cl_3$ | 3.50 | 63 | 4.70 | 129 |
| $Cl_4$ | 8.16 | 147 | 3.82 | 105 |

Coordinates of four chosen chlorine positions with the centre of symmetry as the origin and with respect to monoclinic axes.

Since the chlorine atoms provide 22.7% of the diffracting power of the $\beta$-caryophyllene dihydrochloride molecule, it had been hoped that the chlorine would phase determine the majority of the strong reflections. When structure factors were calculated for the chlorine alone, however, it was found that a large number of planes with high observed structure factors gave low calculated values. High order planes which came into this category were, as before, traced on a plan of the (010) projection and points of high linear density were considered to indicate centres of gravity of <u>groups</u> of carbon atoms. Eight such positions in the asymmetric unit were detected, and by assuming a centre of gravity of an arbitrary two carbons at each

each of these positions, structure factors were calculated and gave a discrepancy of 43% over 156 reflections.

The centre of gravity of these atom pairs had the coordinates shown in Table(3:3):

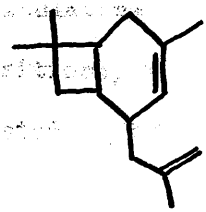
Table (3:3)

| Atom Pair | x, (Å) | $\frac{2\pi x}{a}$ (deg.) | z, (Å) | $\frac{2\pi z}{c}$ (deg.) |
|---|---|---|---|---|
| 1 | 3.00 | 54 | 11.69 | 321 |
| 2 | 5.49 | 99 | 10.93 | 300 |
| 3 | 3.33 | 60 | 9.40 | 258 |
| 4 | 8.16 | 147 | 9.83 | 270 |
| 5 | 3.16 | 57 | 6.01 | 165 |
| 6 | 8.82 | 159 | 7.10 | 195 |
| 7 | 6.33 | 114 | 5.24 | 144 |
| 8 | 3.33 | 60 | 3.17 | 87 |

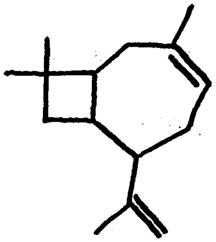
A double Fourier synthesis was performed using 156 planes whose signs had been obtained in this way. The result is shown in Fig. (3:2).

### Trial structures:

When the projection shown in Fig.(3:2) was obtained, several postulates for the structure of $\beta$-caryophyllene were considered equally valid. Trial structure attempts however were confined to structures (2:1) and (2:2):



(2:1)



(2:2)

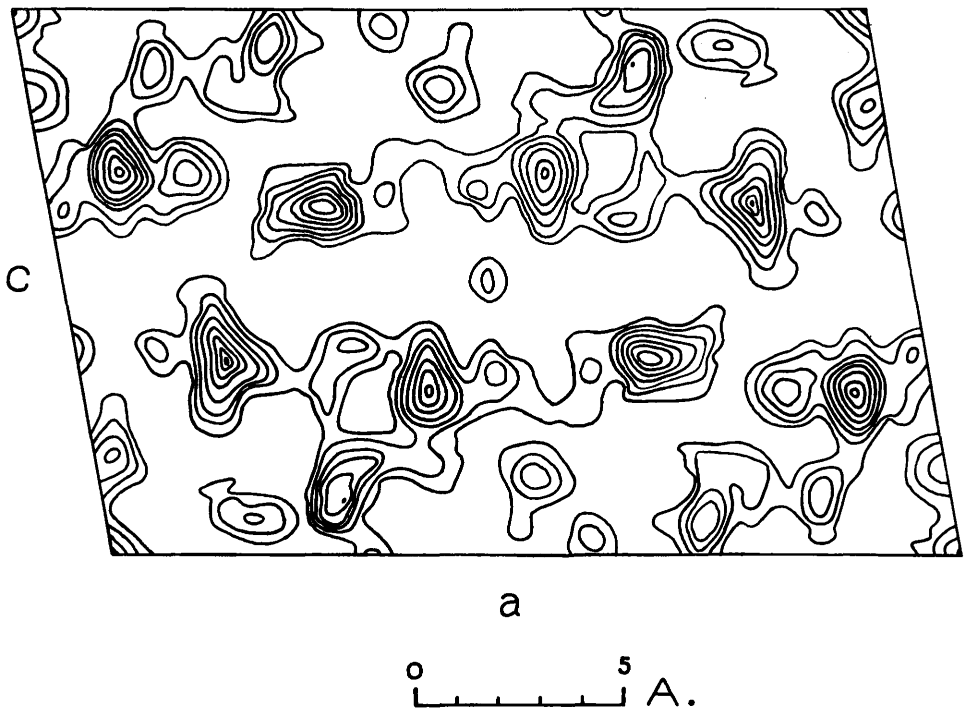


Fig. (3:2)

Fourier synthesis of 156 reflections in the (hol) zone of $\beta$-caryophyllene dihydrochloride. Signs obtained by calculating structure factors on the basis of the chlorine positions, together with the eight "atom pair" positions listed in Table (3:3)

The fact that the asymmetric unit contained two molecules appeared to complicate the problem hopelessly, since the mutual arrangement of these molecules and their orientation in the asymmetric unit is entirely speculative. No hydrogen bonding is possible and an examination of the three reflection zones failed to provide any clue as had been the case for instance with ascorbic acid ( 68 ).

A possible reason for the bimolecular asymmetric unit presents itself if we consider that the addition of a molecule of hydrogen chloride to a double bond can give rise, assuming no steric hindrance, to a cis- or trans - product. β -caryophyllene is itself optically active so that the two products would not be mirror images but a species of internal diastereo-isomer. In general such compounds would be physically distinct and would give different crystals ( 69 ) but it is conceivable that, with a complex molecule, they might be incorporated in the same crystal structure as a bimolecular asymmetric unit.

If steric hindrance, or any other factor caused only one species to result from the addition of H Cl, it is still possible that intermolecular forces and requirements of good packing would be better served by an asymmetric unit of two molecules.

Many trial structures were attempted on the basis of structures (2:1) and (2:2) and most discrepancies were over 60%.

The best of these was one of 48% over 116 planes but a Fourier synthesis was not satisfactory.

Since the method of trial and error was very laborious (68 parameters and 186 reflections) and did not appear promising, attempts were suspended at this point. It had not been found possible to prepare the dihydrobromide so attention was now turned to other compounds where the direct method of structure analysis could be employed.

## TABLE (3:1)

Measured values of the structure factor, on an approximately absolute scale, for β -caryophyllene dihydrochloride.

| hol | 2 sin θ | Fmeas. | hol | 2 sin θ | F meas. |
|---|---|---|---|---|---|
| 002 | 0.24 | 48 | 300 | 0.24 | 11 |
| 3 | 0.36 | 59 | 1 | 0.28 | 37 |
| 4 | 0.48 | 50 | 2 | 0.36 | 13 |
| 5 | 0.60 | 14 | 3 | 0.46 | 8 |
| 6 | 0.72 | 11 | 5 | 0.67 | 65 |
| 8 | 0.96 | 1 | 6 | 0.79 | 8 |
| 9 | 1.08 | 17 | 7 | 0.91 | 8 |
| 102 | 0.26 | 13 | ,10 | 1.52 | 25 |
| 3 | 0.38 | 13 | 400 | 0.31 | 11 |
| 4 | 0.49 | 13 | 1 | 0.35 | 105 |
| 5 | 0.62 | 15 | 2 | 0.42 | 43 |
| 6 | 0.73 | 31 | 3 | 0.51 | 30 |
| 200 | 0.16 | 25 | 4 | 0.61 | 27 |
| 1 | 0.21 | 55 | 5 | 0.72 | 8 |
| 2 | 0.30 | 40 | 6 | 0.83 | 34 |
| 3 | 0.41 | 67 | 7 | 0.94 | 20 |
| 4 | 0.53 | 52 | 501 | 0.43 | 8 |
| 6 | 0.76 | 21 | 3 | 0.57 | 25 |
| 7 | 0.88 | 32 | 7 | 0.98 | 13 |
| 8 | 0.99 | 21 | 9 | 1.20 | 11 |

| hol | 2 sin θ | F meas. | hol | 2 sin θ | F meas. |
|---|---|---|---|---|---|
| 601 | 0.50 | 13 | 900 | 0.71 | 30 |
| 2 | 0.56 | 8 | 1 | 0.73 | 21 |
| 3 | 0.64 | 42 | 2 | 0.78 | 56 |
| 4 | 0.72 | 31 | 3 | 0.83 | 20 |
| 5 | 0.82 | 23 | 5 | 1.00 | 8 |
| 7 | 1.03 | 13 | 10,00 | 0.78 | 30 |
| 700 | 0.55 | 45 | 2 | 0.85 | 20 |
| 1 | 0.58 | 13 | 3 | 0.91 | 36 |
| 2 | 0.64 | 8 | 6 | 1.15 | 13 |
| 4 | 0.79 | 17 | 11,00 | 0.86 | 21 |
| 5 | 0.88 | 19 | 1 | 0.89 | 8 |
| 6 | 0.97 | 20 | 2 | 0.93 | 21 |
| 7 | 1.08 | 21 | 12,00 | 0.93 | 37 |
| 9 | 1.29 | 15 | 1 | 0.96 | 20 |
| 800 | 0.63 | 15 | 3 | 1.06 | 23 |
| 1 | 0.65 | 50 | 5 | 1.20 | 30 |
| 3 | 0.77 | 18 | 13,02 | 1.08 | 11 |
| 5 | 0.94 | 11 | 3 | 1.13 | 11 |
| 6 | 1.03 | 11 | 14,01 | 1.12 | 23 |
| 7 | 1.13 | 27 | 2 | 1.16 | 25 |
| 8 | 1.23 | 15 | 15,02 | 1.23 | 15 |
| 9 | 1.34 | 11 | 4 | 1.34 | 15 |
|  |  |  | 16,00 | 1.25 | 15 |

| hol | 2 sin θ | F meas. | hol | 2 sin θ | F meas. |
|---|---|---|---|---|---|
| $\bar{1}02$ | 0.24 | 15 | $\bar{3}07$ | 0.83 | 18 |
| 3 | 0.35 | 20 | 8 | 0.95 | 8 |
| 4 | 0.47 | 24 | 9 | 1.06 | 17 |
| 5 | 0.59 | 27 | $\bar{4}00$ | 0.31 | 11 |
| 6 | 0.71 | 17 | 1 | 0.31 | 11 |
| 7 | 0.83 | 34 | 2 | 0.36 | 105 |
| 8 | 0.95 | 21 | 3 | 0.43 | 52 |
| $\bar{2}00$ | 0.16 | 25 | 4 | 0.52 | 53 |
| 1 | 0.18 | 52 | 5 | 0.63 | 11 |
| 2 | 0.26 | 50 | 6 | 0.73 | 11 |
| 3 | 0.37 | 49 | 7 | 0.84 | 20 |
| 4 | 0.47 | 47 | $\bar{5}01$ | 0.39 | 36 |
| 5 | 0.59 | 8 | 3 | 0.48 | 21 |
| 6 | 0.71 | 65 | 4 | 0.56 | 45 |
| 7 | 0.83 | 17 | 5 | 0.65 | 17 |
| $\bar{3}00$ | 0.24 | 11 | 7 | 0.86 | 35 |
| 1 | 0.24 | 27 | ,10 | 1.19 | 25 |
| 2 | 0.30 | 31 | ,11 | 1.31 | 8 |
| 3 | 0.39 | 21 | $\bar{6}00$ | 0.47 | 50 |
| 4 | 0.49 | 50 | 1 | 0.46 | 17 |
| 5 | 0.60 | 49 | 2 | 0.48 | 8 |
| 6 | 0.71 | 11 | 4 | 0.60 | 27 |

| hol | 2 sin θ | F meas. |
|---|---|---|
| $\bar{6}05$ | 0.69 | 17 |
| 6 | 0.78 | 32 |
| 7 | 0.88 | 24 |
| 8 | 0.99 | 13 |
| ,10 | 1.21 | 25 |
| $\bar{7}00$ | 0.55 | 45 |
| 1 | 0.54 | 32 |
| 2 | 0.55 | 8 |
| 4 | 0.65 | 17 |
| 5 | 0.74 | 17 |
| 7 | 0.92 | 11 |
| 8 | 1.02 | 8 |
| ,10 | 1.22 | 11 |
| $\bar{8}00$ | 0.63 | 15 |
| 1 | 0.61 | 65 |
| 2 | 0.63 | 8 |
| 3 | 0.66 | 62 |
| 6 | 0.86 | 11 |
| 7 | 0.95 | 24 |
| 8 | 1.05 | 13 |
| $\bar{9}00$ | 0.71 | 30 |
| 1 | 0.69 | 48 |
| $\bar{9}02$ | 0.70 | 52 |
| 3 | 0.73 | 17 |
| 4 | 0.78 | 8 |
| 5 | 0.84 | 58 |
| 9 | 1.18 | 13 |
| ,11 | 1.38 | 8 |
| $\overline{10},00$ | 0.78 | 30 |
| 2 | 0.78 | 11 |
| 5 | 0.89 | 36 |
| 9 | 1.22 | 13 |
| $\overline{11},00$ | 0.86 | 21 |
| 2 | 0.85 | 21 |
| 6 | 1.01 | 23 |
| $\overline{12},00$ | 0.93 | 37 |
| 1 | 0.92 | 21 |
| 3 | 0.94 | 37 |
| 4 | 0.97 | 24 |
| 8 | 1.21 | 15 |
| $\overline{13},03$ | 1.02 | 20 |
| 7 | 1.19 | 15 |
| 8 | 1.26 | 15 |

| hol | 2 sin θ | F meas. | hol | 2 sin θ | F meas. |
|---|---|---|---|---|---|
| $\overline{14}$,03 | 1.09 | 11 | $\overline{16}$,00 | 1.25 | 15 |
| 4 | 1.11 | 11 | 1 | 1.23 | 13 |
| 5 | 1.15 | 28 | 2 | 1.22 | 44 |
| $\overline{15}$,00 | 1.17 | 11 | 3 | 1.23 | 15 |
| | | | 5 | 1.29 | 15 |
| | | | $\overline{17}$,02 | 1.31 | 11 |

§ 4.

## The structure of $\beta$-caryophyllene chloride and bromide.

### Synthesis of the heavy-atom compounds:

This is a two stage process via $\beta$-caryophyllene alcohol.

$\beta$-caryophyllene alcohol: this was prepared according to the method of Asatina and Tsukamoto (70). A mixture of 80 mls. absolute ether and 30 mls. concentrated sulphuric acid were placed in a flask, and 100 mls. crude B.D.H. caryophyllene was added dropwise over $1\frac{1}{2}$ hours with shaking. The temperature was maintained at less than $10^\circ$ C. The mixture was allowed to stand for 2 hours at room temperature and then poured over a mixture of crushed ice and sodium carbonate. This was left overnight.

The alkaline mixture was steam distilled for 12 hours during which time the product came over, first as an oil and then as a waxy solid. The solid was filtered off and pressed dry on filter paper. The oily fraction consisted of unchanged caryophyllene and of clovene.

The yellowish solid material was dissolved in alcohol and cold treated with charcoal. Evaporation of the filtrate left a colourless oil which solidified. This was crystallised from acetone and gave 25 g. crude product, melting at $92^\circ$ C. (m.p. pure $\beta$-caryophyllene alcohol = $94^\circ$ C).

Bromo derivative of $\beta$ -caryophyllene alcohol. The method is due to Deussen ( 71 ). 2.2 g. $\beta$ -caryophyllene alcohol prepared as above were dissolved in a minimum of acetic acid and 3.2 g. (1 ml) of liquid bromine in acetic acid were added. The mixture was placed under an reflux with a tiny crystal of iodine and boiled for 3 hours. Hydrogen bromide was evolved and the bromine colour disappeared. A further addition of 1 ml. of bromine in acetic acid was made and boiling continued for a further 3 hours.

The mixture was cooled and added to much water. On rendering alkaline a brown oil separated which later solidified. This was filtered off and washed with water. It was then dissolved in ether, and dried with an-hydrous sodium sulphate. Evaporation of the ether yielded a brown oil which solidified. The yield of crude product was 2.5 g. Five successive crystallisations finally gave a crop of small, colourless crystals melting at 60-61° C.

### Chloro derivative of $\beta$ -caryophyllene alcohol ( 72 ).

2.2 g. of $\beta$ -caryophyllene alcohol and 2.1 g. of phosphorus pentachloride were placed in a flask which was then closed with a calcium chloride aspirator. After 2 hours the mixture melted and hydrogen chloride was evolved. When this evolution ceased the reaction mixture was allowed to stand for several hours/

hours/ and then the $POCl_3$ was removed by heating under reduced pressure.

The solid residue was ground with sodium carbonate solution and finally washed with water. It was dissolved in ether, and the mixture dried with sodium sulphate. The ether was removed and the solid washed by grinding with 2-3 mls. alcohol. The solid was filtered, dissolved in alcohol and charcoal treated. Three crystallisations from alcohol gave crystals similar to those of the bromide but melting at 62-63° C.

## X-ray investigation of the crystal and molecular structure of $\beta$-caryophyllene chloride and (in part) of the isomorphous $\beta$-caryophyllene bromide.

### Crystal data.

| | |
|---|---|
| $\beta$-caryophyllene bromide | $C_{15}H_{25}Br$ |
| M, 285.3 | m.p. 60-61° C. |
| d, calc. 1.292 | d, found 1.293 |
| $\beta$-caryophyllene chloride. | $C_{15}H_{25}Cl$ |
| M, 240.8 | m.p. 62-63° C |
| d, calc. 1.115 | d, found 1.135 |

Both crystals are orthorhombic bisphenoidal.

| | a (Å) | b | c |
|---|---|---|---|
| bromide | 16.41 | 10.77 | 8.37 |
| chloride | 16.47 | 10.54 | 8.27 |

these values being correct to ± 0.5%

The parameters give ratios of:

| | | | | | |
|---|---|---|---|---|---|
| bromide | 1.533 | : | 1.000 | : | 0.782 |
| | (1.531) | | (1.000) | | (0.778) |
| chloride | 1.563 | : | 1.000 | : | 0.787 |
| | (1.563) | . | (1.000) | | (0.785) |

The equivalent values quoted by Groth (73) are bracketted.

Absent spectra in each case:

(h o o) when h is odd, (o k o) when k is odd

(o o l) when l is odd

Space group $D_2^4$ ($P2_12_12_1$) with 4 symmetric units.

Molecules per unit cell = 4

Volume of unit cell: bromide 1467.1 $A^3$

chloride 1435.6 $A^3$

Absorption coefficients for x-rays, $\lambda$ = 1.54 Å

bromide, = 38.6 $cm^{-1}$

chloride, = 22.2 $cm^{-1}$

Total number of electrons per unit cell:

bromide, F (000) = 600

chloride, F (000) = 528

The crystals were mostly tabular with the (100) face well developed. Occasionally, however, the a - axis was found as the needle axis of a prismatic rhomb.

## Experimental.

Crystal data was obtained from rotation, oscillation and moving film photographs using copper $K_\alpha$ radiation, $\lambda = 1.54$ Å. Densities were obtained with calcium chloride solution at 25° C., several crystals being used in each case.

The values were: bromide $D_{25} = 1.293$
chloride $D_{25} = 1.135$

## Measurement of Intensities.

These were determined visually in each axial zone by applying the multiple film technique to moving film photographs of the equatorial layer lines. The reflection data is shown in Table(4:1)

TABLE(4:1)

| | Zone | Reflections Observed. | Total Possible Reflections | Percentage of Total Poss. | Intensity Range. |
|---|---|---|---|---|---|
| Bromide | h k o | 102 | 229 | 45 | 1000 : 1 |
| | h o l | 104 | 175 | 60 | 1000 : 1 |
| | o k l | 41 | 116 | 36 | 100 : 1 |
| Chloride | h k o | 118 | 229 | 52 | 3,300 : 1 |
| | h o l | 86 | 175 | 49 | 2,500 : 1 |
| | o k l | 64 | 115 | 56 | 1,100 : 1 |

In all, 232 bromide reflections and 254 chloride reflections were observed being 47% and 51% respectively of the 498 possible.

## Volatility.

The crystals of both substances were very volatile and special techniques had to be adopted in order to keep the crystal set over the several days required to take the crystal from the setting to the moving film stages. Coating the crystal with lacquer or with rubber cement was not a success. Gelatine capsules containing the crystal mounted in an atmosphere of its vapour were tried and finally narrow lithium borate tubes were found most suitable. These containers increased the background scatter considerably, and rather large crystals had to be used to obtain a reasonable number of reflections. The background effect was partly overcome by using ultra fast high contrast Ilford Industrial G film in certain cases.

For intensity measurements, crystals were chosen with a uniform cross section to make the path length through the crystal as uniform as possible.

The crystal dimensions of the bromide crystals were from (0.20 - 0.30) x (0.20 - 0.27) mms., and of the chloride crystals from (0.20 - 0.45) x (0.20 - 0.60) mms.

In order to detect if absorption errors were serious, a smaller crystal of the bromide was photographed and its (h k o ) reflections measured. These intensities were very similar to the previous set. The crystal was dipped in liquid air and rephotographed. No change in intensities could be detected, and so it/

it/ was assumed that extinction errors were negligible.

Note: evidence of extinction was apparent with the chloride when intensity values of (020) in the (h k o) and (o k 1) zones were compared at a later stage in the refinement. The higher value was taken as being more correct.

## Atomic scattering curves.

In initial calculation of intensities, James and Brindley theoretical curves for chlorine, bromine and carbon were used, each corrected for temperature by inserting a value of $B = 2.0 \times 10^{-16}$ in the Debye-Waller factor exp. $\left( -B \left\{ \frac{\sin\theta}{\lambda} \right\}^2 \right)$. Later, thermal vibration was shown to be above normal, and for the chloride the James and Brindley curve was amended by a factor $B = 4.4 \times 10^{-16}$ The carbon curve finally used was that given by McWeeny (91) for carbon (valence state) and with $B = 3.5 \times 10^{-16}$.

## Structure Determination.

The space group of both $\beta$ -caryophyllene bromide and chloride has been shown to be $P2_1 2_1 2_1$ and the cell parameters are sufficiently similar to warrant the assumption that the atoms of each compound occupy similar positions in the cell. This indicates that the method of isomorphous replacement may be applied.

## Space Group.

The space group $P2_1 2_1 2_1$ has no centre of symmetry but/

but/ each axial projection gives the plane group **Pba.** which is centro-symmetrical. Projecting on (001) for example, we get centres of symmetry at the points:

$(\frac{1}{4}, 0)$ $(\frac{3}{4}, 0)$ $(\frac{1}{4}, \frac{1}{2})$ $(\frac{3}{4}, \frac{1}{2})$ ——

– these coordinates being referred to the origin adopted in the International Tables (1935).

A diagram of such a projection is shown in Fig. (4:1)(a) where the post-script $\frac{1}{4}$ beside a screw axis indicates elevation of $\frac{1}{4}$ above the plane of the paper. The other horizontal screw axes are in the plane. A three dimensional diagram showing how the equivalent positions are related by screw axes is shown in Fig. (4:1) (b)

The origin of coordinates used in International Tables is not used in the calculation of structure factors. Simplification results if an origin is chosen to coincide with a zonal centre of symmetry in each zone. These origins are not spatially coincident and those chosen related to the origin shown in Figs. (4:1)(a) and (b) are as follows:

| Zone. | Coordinates. | | |
|---|---|---|---|
| h k o | $\frac{1}{4}$ | 0 | $\frac{1}{4}$ |
| h o l | $\frac{1}{2}$ | 0 | $\frac{1}{4}$ |
| o k l | $\frac{1}{4}$ | $\frac{1}{4}$ | $\frac{1}{2}$ |

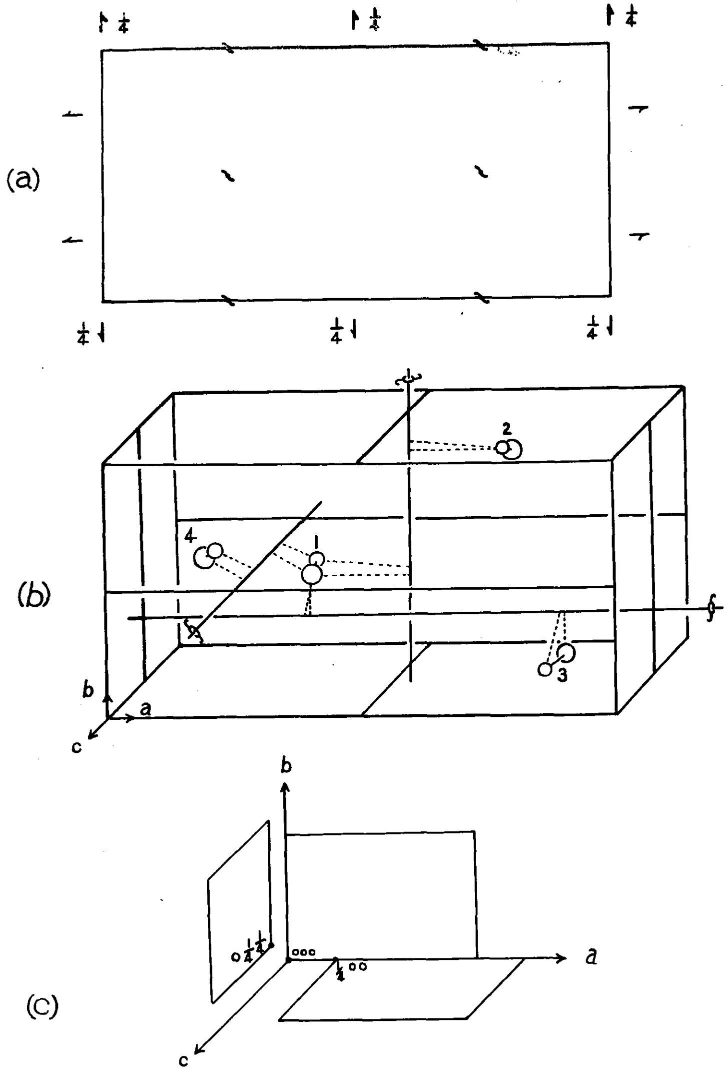


Fig.(4:1)

a) projection of the space group $P2_12_12_1$ showing relationship of the screw axes. The postscript $\frac{1}{4}$ indicates that the adjacent axis is $\frac{1}{4}$ of a translation above the plane of the paper. The other horizontal axes are in the plane.

b) a three dimensional diagram showing the equivalent positions of the space group. The origin is the general origin which is equidistant from all nearest screw-axes. It is not a centre of symmetry.

c) a diagram showing how the zonal origins used in the analysis are interrelated. The (hko) zone origin is arbitrarily selected as the cell origin.

If we arbitrarily select $(\frac{1}{4}\ 0\ \frac{1}{4})$ as the cell origin the zonal origins become:

| Zone. | Coordinates. |
|---|---|
| h k o | 0 0 0 |
| h o l | $\frac{1}{4}$ 0 0 |
| o k l | 0 $\frac{1}{4}$ $\frac{1}{4}$ |

The relationship of these origins is shown in Fig. (4:1)(c). Since a centre of symmetry is now the origin the two dimensional structure factors reduce to:

eg.( h k o) h + k even $S = 4 \cos h\theta_1 \cos k\theta_3$

h + k odd $S = 4 \sin h\theta_1 \sin k\theta_3$

Application of Method of Isomorphous Replacement.

This method depends upon the fact that in isomorphs the atoms preserve approximately the same relative positions although they may be of quite widely differing scattering power. In the application of the method to direct structure analysis it is usual to have in the two (or more) isomorphs only one atom of significantly differing diffracting power. If the absolute intensities of similar reflections in both compounds are known, and if the replaceable (heavy) atom can be located by space group requirements or by a vector map, it is usually possible to obtain the phases of many of the reflections by inspection.

In the case under examination, we may write the structure factors for any particular plane in the two isomorphs in the form:

$$F_{Br} = S.f_{Br} + \sum_{i} S_i \, . \, f_i$$

$$F_{Cl} = S.f_{Cl} + \sum_{i} S_i \, . \, f_i$$

where $f_{Br}$, $f_{Cl}$ and $f_i$ are the atomic scattering factors and subscript <u>i</u> denotes atoms other than bromine or chlorine. $\sum_{i} S_i \; f_i$ denotes the contribution of the carbon skeleton to each reflection, and because of the isomorphism is assumed constant for each compound.

It follows:

$$F_{Br} - F_{Cl} = S\,(f_{Br} - f_{Cl})$$

which in this centro-symmetrical case can be dealt with as a straightforward equation in which all but the signs of $F_{Br}$ and $F_{Cl}$ are known.

i.e. $$|F_{Br}| - |F_{Cl}| = S \; (f_{Br} - f_{Cl})$$

<u>Position of Replaceable Heavy Atom.</u>

The position of the heavy atom is not defined by the space group and was obtained by the use of the Patterson vector method in the case of the bromide. The vector method was not applied to the chloride where information could not be expected to be accurate when obtained frmm a vector map (Cf. β-caryophyllene dihydrochloride).

## Patterson synthesis.

A Patterson synthesis was applied to the (h k o) zone of the bromide using $F^2$ values obtained directly from the film and corrected only for Lorentz and polarisation effects. The resultant two dimensional vector map is shown in Fig. (4:2).

There are four heavy atoms in the projection and these will create in a vector map *four* peaks of double height and *four* of single height. Each asymmetric unit will thus contain one large and one small peak which are indicated by the letters *D* and *S*. The single peak is swamped to a large extent by other vectors.

The double peaks occur at positions (0.1333, 0.5000) and (0.5000, 0.0633), the coordinates expressed as fractions of the *a* and *b* axes. If a centre of symmetry is taken as the origin of coordinates, equivalent points in this projection of the space group occur at positions:

$$(x,y) \quad (\overline{\tfrac{1}{2} - x}, \tfrac{1}{2} + y) \quad (\overline{\tfrac{1}{2} + x}, \overline{\tfrac{1}{2} - y}) \quad (\bar{x}, \bar{y})$$

and the vectors between atoms not related by the centre of symmetry give peaks at the positions:

$$(\overline{\tfrac{1}{2} - 2x}, \tfrac{1}{2}) \quad \text{and} \quad (\tfrac{1}{2}, \overline{\tfrac{1}{2} - 2y})$$

Solution of the vector set gives four sets of coordinates which are equivalent points in the projection. Any one of these/

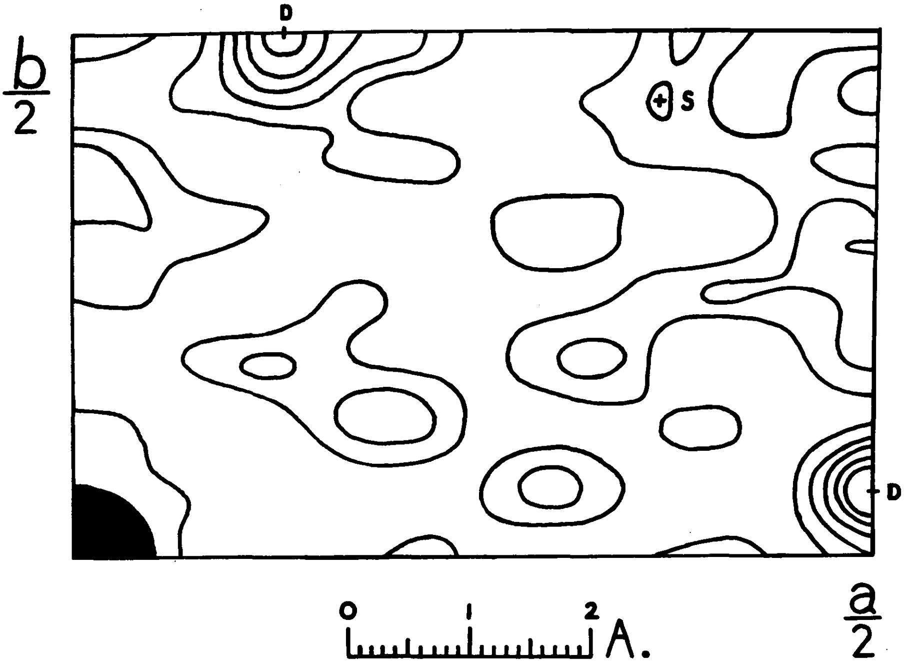


Fig.(4:2)

Asymmetric unit of the Patterson projection of the (hko) zone of Λ-caryophyllene bromide. The double peak, formed by the super-position of two equivalent vectors about the effective centre of symmetry in the projection, is lettered D, and the single peak indicating a unique vector is lettered S.

these/ may be taken as reference atom and the position (0.3167, 0.2183) was arbitrarily selected.

Similar syntheses were applied to the (h o l) and (o k l) data and the resultant coordinates of the reference atom are given in Table(4:2)

TABLE(4:2)

| Zone | Coords. separately referred to zone origin | | | Coords. mutually referred to cell origin | | |
|---|---|---|---|---|---|---|
| | | | | x/a | y/b | z/c |
| h k o | 0.3167 | 0.2183 | ------ | 0.3167 | 0.2183 | ------ |
| h o l | 0.0633 | ------ | 0.0400 | 0.3133 | ------ | 0.0400 |
| o k l | ------ | - 0.0317 | -0.2083 | ------ | 0.2183 | 0.0417 |
| | | Mean | ——— | 0.3150 | 0.2183 | 0.0409 |

Absolute values of $|F_{Br}|$ and $|F_{Cl}|$

By using the multiple film technique we are able merely to interrelate the F values i.e. we are able to measure $|F_{Br} . k_{Br}|$ and $|F_{Cl} . k_{Cl}|$ where $k_{Br}$ and $k_{Cl}$ are unknown scaling factors.

Before the method of isomorphous replacement can be used, the absolute values of $|F_{Br}|$ and $|F_{Cl}|$ must be obtained and this can be done (to a greater or less degree) in several ways.

1) The best method (74) is probably by measuring the intensity of the reflection by means of an ionisation spectrometer or Geiger counter, and relating this to the crystal and to the incident beam. This method, however, requires an elaborate experimental technique which was not available in the present instance.

2) By making use of the two crystal Weissenberg goniometer (75), it is possible to move the crystal under examination and a standard crystal alternately in and out of the x-ray beam. The two crystals are carefully weighed and in the case of the standard crystal, the integrated intensities of the various reflections can be calculated in absolute units. This is interrelated to the unknown reflections which can then be put on an absolute scale.

3) Approximately absolute reflections may be obtained by using any of the modifications of Wilson's (76) method. The accuracy of these methods is not particularly good.

4) Methods may be used depending upon special circumstances. For instance:

a) if by inspection, pairs of planes can be detected where one or other reflection is negligible, or absent, then the absolute intensity of the remaining reflection can be obtained directly:

$$\text{e.g.} \quad F_{Br} - 0 = S\ (f_{Br} - f_{Cl})$$

b) the relative scaling factor for the two series can be/

be/ obtained if large planes can be picked out where the halogen contribution is zero or negligible.

$$\text{Then}\quad \frac{\sum|F_{Br}|}{\sum|F_{Cl}|} = \frac{k_{Br}}{k_{Cl}}$$

---

In the case of the $\beta$-caryophyllene halides, a method suggested by J. C. Speakman was used. This method is based to a certain extent on the Wilson method, but its validity is mainly derived from experience in other cases.

For isomorphs containing a heavy atom we assume that

$$\frac{\sum|F_{Br}|}{\sum|F_{Cl}|} = \frac{\bar{f}_{Br}}{\bar{f}_{Cl}}$$

where $\bar{f}_{Br,Cl}$ are average atomic scattering factors over the range of $2 \sin \theta$.

This ratio $\frac{\bar{f}_{Br}}{\bar{f}_{Cl}}$ is found to be usually rather less than the ratio of $\frac{\text{at. no. Br.}}{\text{at. no. Cl.}} = 2.06$ and is obtained empirically from other data.

Scaling of (h k o) reflections of bromide and chloride.

It is assumed that $\frac{\sum|F_{Br}|}{\sum|F_{Cl}|} = 2.06$ where $|F_{Br}|$ and $|F_{Cl}|$ are absolute structure factors.

The ratio $$\frac{\sum|F^{1}_{Br}|}{\sum|F^{1}_{Cl}|} = \frac{\sum|F_{Br}|}{\sum|F_{Cl}|}\cdot\frac{k_{Br}}{k_{Cl}}$$ ( $F^{1}_{hal.}$ = observed )
( structor factor )

was evaluated for all planes where the halogen was thought to be phase determining as here the approximations are more liable to be justified. 32 planes were used.

$$\frac{\sum|F^{1}_{Br}|}{\sum|F^{1}_{Cl}|} = 1.55 = \frac{\sum|F_{Br}|}{\sum|F_{Cl}|}\cdot\frac{k_{Br}}{k_{Cl}} = 2.06.\ \frac{k_{Br}}{k_{Cl}}$$

whence $$\frac{k_{Br}}{k_{Cl}} = 0.75$$

Further we have:

$$\frac{\sum|F^{1}_{Br}|}{k_{Br}} - \frac{\sum|F^{1}_{Cl}|}{k_{Cl}} = S\ (f_{Br} - f_{Cl}).$$

or

$$\frac{1665}{k_{Br}} - \frac{1073 \times 0.75}{k_{Br}} = 1066$$

.............. substituting measured values.

The R.H.S. was obtained from a knowledge of the heavy atom position and by using James and Brindley scattering curves, $B = 2.0 \times 10^{-16}$.

The bromine and chlorine atoms were assumed to have the same coordinates.

Whence $k_{Br} = 0.81$
$k_{Cl} = 1.08$

As a check:

Six planes were observed in the bromide series which were absent in the chloride series. Using method 4(a):

$$k_{Br} = 0.89$$

It was not possible to calculate $k_{Cl}$ in a similar manner.

Result: the values were taken to be:

$$\left( \begin{array}{l} k_{Br} = 0.8 \\ k_{Cl} = 1.1 \end{array} \right.$$

Note: the scaling was not as straightforward as the above scheme seems to indicate. The value of the ratio $\frac{\sum|F_{Br}|}{\sum|F_{Cl}|}$ was at first obtained empirically from Binnie's results on hexamethylene diamine dihydrochloride and dihydrobromide (77) and found to be 2.16 instead of less than 2.06 as had been anticipated. In addition, in evaluating $\frac{\sum|F^1_{Br}|}{\sum|F^1_{Cl}|}$ for the $\beta$-caryophyllene halides, too many planes with a high carbon contribution were included and the ratio found to be 1.70 instead of 1.55. This resulted in values of $k_{Br}$ and $k_{Cl}$ about 10% different from those quoted above. This possible variation in the scaling/

scaling factors caused the writer to be conservative in fixing certain signs. It has been assumed at this stage that the bromine and chlorine atoms are in identical positions but this may not be so. Thus, small values of $S. (f_{Br} - f_{Cl})$ may not be correct and cannot be used to fix signs. The possible 10% variation in the scaling factor required that the important plane (400) be omitted at this stage. Other planes which have been square bracketted were also omitted for sundry reasons.

In all, 80 bromine reflections were given signs as shown in Table(4:3)and used to perform a two dimensional Fourier synthesis. The result is shown in Fig. (4:3).

Fig. (4:3)

Fourier synthesis of 80 reflections in the (h0l) zone of [illegible] [illegible]. [illegible] were obtained by the method of [illegible] [illegible] as shown in Table (4:3).

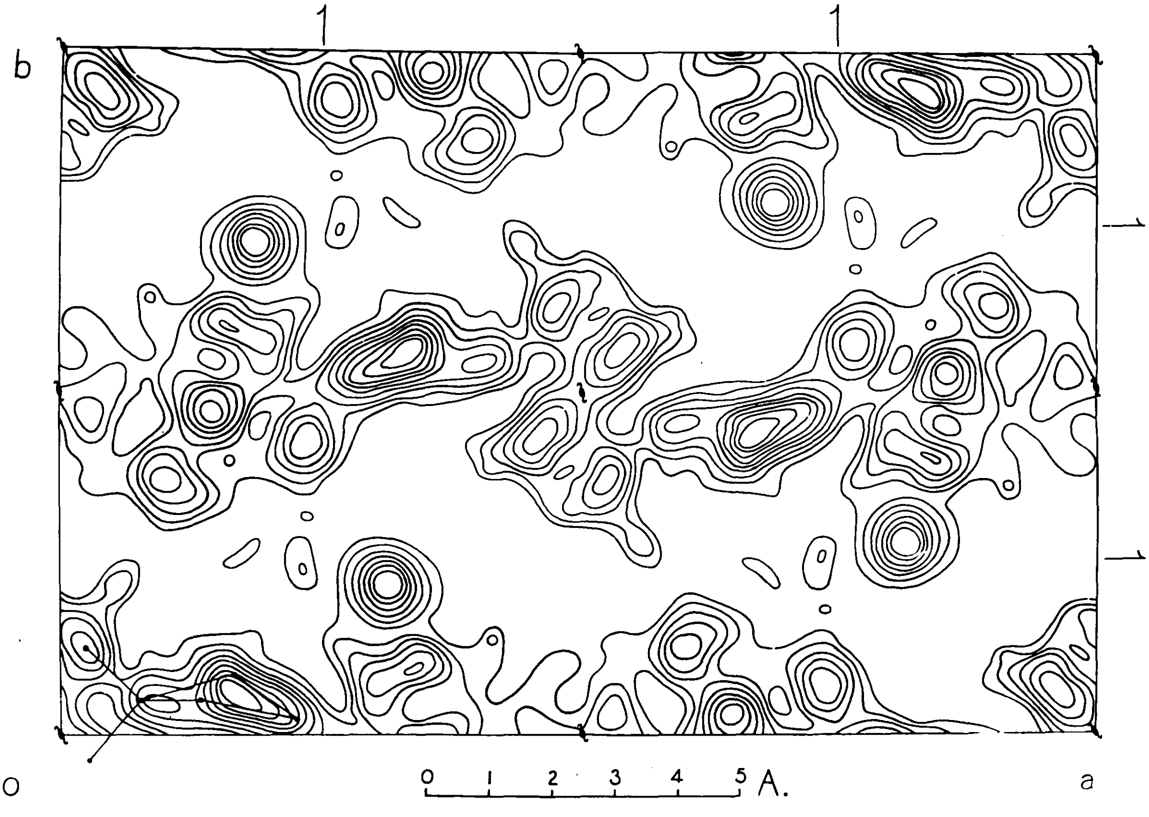


Fig.(4:3)

Preliminary Fourier synthesis of 80 reflections in the (hko) zone of $\beta$-caryophyllene bromide. Signs were obtained by the method of isomorphous replacement as shown in Table (4:3).

## TABLE (4:3)

| h k | $\lvert F_{Br}\rvert$ | $\lvert F_{Cl}\rvert$ | $F_{Br} - F_{Cl}$ calc. | Sign $F_{Br}$ | Sign $F_{Cl}$ | Brom. F.Carbon only | Chlor. F.Carbon only |
|---|---|---|---|---|---|---|---|
| 0,2 | 81 | 113 | $\overline{63}$ | + | + | +197 | +165 |
| 4 | 67 | 10 | $\overset{+}{42}$ | + | + | - 3 | - 19 |
| 6 | 44 | 16 | $\overline{18}$ | - | - | - 15 | - 5 |
| 8 | 23 | 23 | 0 | / | / | / | / |
| 1,1 | 15 | 9 | $\overline{6}$ | (-) | - | - 4 | - 4 |
| 2 | 85 | 67 | $\overline{24}$ | - | - | - 41 | - 47 |
| 3 | 33 | 16 | $\overset{+}{15}$ | + | + | + 7 | + 5 |
| 4 | 30 | 5 | $\overset{+}{39}$ | + | - | - 36 | - 32 |
| 5 | 34 | 12 | $\overline{19}$ | - | - | - 3 | 0 |
| 6 | 89 | 48 | $\overline{40}$ | - | - | - 15 | - 22 |
| 8 | 45 | 12 | $\overset{+}{32}$ | + | + | - 9 | - 9 |
| 9 | 32 | 15 | $\overline{12}$ | - | - | - 12 | - 7 |
| ,10 | 28 | 5 | $\overline{22}$ | - | - | + 9 | + 10 |
| 2, 0 | 121 | 72 | $\overline{47}$ | - | - | - 33 | - 31 |
| 1 | 59 | 0 | $\overset{+}{49}$ | + | / | - 34 | - 43 |
| 2 | 21 | 32 | $\overset{+}{42}$ | + | - | - 54 | - 65 |
| 3 | 105 | 63 | $\overline{40}$ | - | - | - 37 | - 34 |
| 4 | 81 | 44 | $\overline{28}$ | - | - | - 35 | - 25 |
| 5 | 16 | 9 | $\overset{+}{21}$ | (+) | - | - 19 | - 23 |
| 6 | 25 | 0 | $\overset{+}{11}$ | + | / | + 6 | - 7 |
| 7 | 28 | 15 | $\overline{5}$ | - | - | - 20 | - 12 |

| h k | $\lvert F_{Br} \rvert$ | $\lvert F_{Cl} \rvert$ | $F_{Br} - F_{Cl}$ calc. | Sign $F_{Br}$ | Sign $F_{Cl}$ | Brom. F Carbon only | Chlor. F.Carbon only |
|---|---|---|---|---|---|---|---|
| 2,9 | 25 | 15 | $\overline{5}$ | − | − | − 16 | − 11 |
| 3,1 | 87 | 69 | $\overset{+}{13}$ | + | + | + 64 | + 58 |
| 2 | 36 | 26 | $\overset{+}{8}$ | + | + | + 22 | + 20 |
| 3 | 104 | 68 | $\overline{33}$ | − | − | − 47 | −44 |
| 4 | 35 | 20 | $\overline{13}$ | − | − | − 14 | − 11 |
| 5 | 60 | 22 | $\overset{+}{42}$ | + | + | − 9 | − 6 |
| 6 | 14 | 0 | $\overset{+}{14}$ | + | / | − 8 | − 9 |
| 7 | 50 | 11 | $\overline{37}$ | − | − | + 11 | + 13 |
| 8 | 11 | 7 | $\overline{11}$ | / | / | / | / |
| 9 | 38 | 11 | $\overset{+}{28}$ | + | + | − 8 | − 8 |
| ,10 | 13 | 7 | $\overset{+}{7}$ | ( + ) | + | + 1 | + 2 |
| 4,0 | 95 | 76 | $\overline{7}$ | / | / | / | / |
| 1 | 131 | 60 | $\overline{64}$ | − | − | − 18 | − 11 |
| 2 | 52 | 51 | $\overset{+}{6}$ | / | / | / | / |
| 3 | 121 | 56 | $\overset{+}{49}$ | + | + | + 37 | + 21 |
| 5 | 21 | 14 | $\overline{26}$ | / | / | / | / |
| 7 | 22 | 8 | $\overset{+}{7}$ | + | + | + 11 | + 4 |
| 9 | 13 | 12 | $\overset{+}{6}$ | / | / | / | / |
| 5, 1 | 40 | 39 | $\overline{11}$ | / | / | / | / |
| 2 | 76 | 59 | $\overset{+}{12}$ | + | + | + 56 | + 50 |
| 3 | 51 | 20 | $\overset{+}{28}$ | + | + | + 4 | + 1 |
| 4 | 24 | 38 | $\overline{18}$ | + | + | + 56 | + 51 |

| h k | $\lvert F_{Br} \rvert$ | $\lvert F_{Cl} \rvert$ | $F_{Br} - F_{Cl}$ calc. | Sign $F_{Br}$ | Sign $F_{Cl}$ | Brom. F Carbon only | Chlor. F Carbon only |
|---|---|---|---|---|---|---|---|
| 5,5 | 49 | 20 | $\overline{35}$ | - | - | + 9 | + 3 |
| 6 | 32 | 12 | $\overset{+}{20}$ | + | + | 0 | - 1 |
| 7 | 67 | 33 | $\overset{+}{31}$ | + | + | + 15 | + 12 |
| 8 | 41 | 20 | $\overline{16}$ | - | - | - 14 | - 9 |
| 9 | 25 | 0 | $\overline{24}$ | - | / | + 15 | + 17 |
| ,11 | 11 | 0 | $\overset{+}{15}$ | (+) | / | - 15 | - 15 |
| 6,0 | 121 | 63 | $\overset{+}{48}$ | + | + | + 38 | ± 29 |
| 1 | 82 | 43 | $\overset{+}{35}$ | + | + | + 24 | + 19 |
| 2 | 56 | 11 | $\overline{43}$ | - | - | + 16 | + 18 |
| 3 | 29 | 0 | $\overline{27}$ | - | / | + 15 | + 18 |
| 4 | 45 | 10 | $\overset{+}{27}$ | + | + | - 1 | - 9 |
| 5 | 35 | 8 | $\overset{+}{15}$ | + | + | + 11 | - 2 |
| 7,1 | 29 | 23 | $\overset{+}{3}$ | / | / | / | / |
| 2 | 90 | 64 | $\overline{20}$ | - | - | - 56 | - 50 |
| 3 | 41 | 30 | $\overline{6}$ | - | - | - 31 | - 26 |
| 4 | 50 | 20 | $\overset{+}{34}$ | + | + | - 5 | - 1 |
| 6 | 42 | 8 | $\overline{34}$ | - | - | + 16 | + 15 |
| 7 | 31 | 24 | $\overline{7}$ | - | - | - 19 | - 19 |
| 8 | 42 | 10 | $\overset{+}{29}$ | + | + | - 7 | - 10 |
| ,10 | 13 | 0 | $\overline{20}$ | ( - ) | / | + 21 | + 20 |
| 8,0 | 81 | 28 | $\overline{51}$ | - | - | + 4 | + 6 |

| h k | $\lvert F_{Br}\rvert$ | $\lvert F_{Cl}\rvert$ | $F_{Br} - F_{Cl}$ calc. | Sign $F_{Br}$ | Sign $F_{Cl}$ | Brom. F Carbon only | Chlor. F Carbon only |
|---|---|---|---|---|---|---|---|
| 8,2 | 84 | 27 | $\overset{+}{45}$ | + | + | + 9 | - 3 |
| 3 | 14 | 16 | $\overline{8}$ | / | / | / | / |
| 4 | 57 | 17 | $\overline{30}$ | - | - | - 3 | + 2 |
| 6 | 32 | 12 | $\overset{+}{14}$ | + | + | + 10 | + 3 |
| 10 | 11 | 12 | $\overline{9}$ | / | / | / | / |
| 9,2 | 41 | 25 | $\overset{+}{15}$ | + | + | + 14 | + 10 |
| 3 | 14 | 0 | $\overline{14}$ | - | / | + 9 | + 9 |
| 5 | 44 | 22 | $\overset{+}{19}$ | + | + | + 14 | + 10 |
| 6 | 55 | 31 | $\overset{+}{24}$ | + | + | + 13 | + 14 |
| 8 | 18 | 0 | $\overline{21}$ | - | / | + 19 | + 15 |
| 10 | 11 | 00 | $\overset{+}{15}$ | ( + ) | / | - 15 | - 15 |
| 10,0 | 39 | 11 | $\overset{+}{22}$ | + | + | 0 | - 3 |
| 1 | 67 | 26 | $\overline{37}$ | - | - | - 6 | - 2 |
| 2 | 47 | 22 | $\overline{19}$ | - | - | - 15 | - 9 |
| 3 | 32 | 0 | $\overset{+}{29}$ | + | / | - 16 | - 19 |
| 4 | 27 | 0 | $\overset{+}{13}$ | + | / | + 6 | - 9 |
| 11,3 | 35 | 17 | $\overset{+}{20}$ | + | + | + 1 | + 3 |
| 4 | 12 | 0 | $\overset{+}{3}$ | / | / | / | / |
| 5 | 34 | 9 | $\overline{26}$ | - | - | + 10 | + 9 |
| 6 | 25 | 19 | $\overline{3}$ | / | / | / | / |
| 7 | 32 | 13 | $\overset{+}{25}$ | + | + | - 11 | - 4 |

| h k | $\lvert F_{Br}\rvert$ | $\lvert F_{Cl}\rvert$ | $F_{Br} - F_{Cl}$ calc. | Sign $F_{Br}$ | Sign $F_{Cl}$ | Brom. F.Carbon only | Chlor. F Carbon only |
|---|---|---|---|---|---|---|---|
| 11,9 | 16 | 7 | $\overline{19}$ | - | + | + 18 | + 21 |
| 12,1 | 47 | 13 | $\overset{+}{33}$ | + | + | - 9 | - 9 |
| 3 | 35 | 10 | $\overline{27}$ | - | - | + 9 | + 8 |
| 5 | 39 | 14 | $\overset{+}{16}$ | + | + | + 14 | + 4 |
| 13,2 | 24 | 14 | $\overline{9}$ | - | - | - 10 | - 8 |
| 3 | 21 | 12 | $\overline{13}$ | ( - ) | - | 0 | - 3 |
| 5 | 18 | 0 | $\overline{22}$ | + | / | $\overline{11}$ | $\overline{17}$ |
| 7 | 24 | 12 | $\overline{16}$ | - | - | + 4 | 0 |
| 14,0 | 35 | 12 | $\overline{27}$ | - | - | + 11 | + 7 |
| 1 | 30 | 15 | $\overline{12}$ | - | - | - 10 | - 7 |
| 2 | 30 | 11 | $\overset{+}{24}$ | + | + | - 11 | - 6 |
| 5 | 27 | 14 | $\overline{5}$ | - | - | - 18 | - 11 |
| 15,4 | 21 | 8 | $\overline{17}$ | - | - | + 8 | + 4 |
| 6 | 20 | 7 | $\overset{+}{19}$ | / | / | / | / |
| 8 | 13 | 6 | $\overline{18}$ | ( - ) | + | + 16 | + 18 |
| 16,2 | 30 | 10 | $\overline{20}$ | - | - | + 4 | + 4 |
| 4 | 11 | 0 | $\overset{+}{14}$ | + | / | - 13 | - 14 |

The bromide was chosen for preliminary work because the signs of this series of reflections are more likely to be correct. It is interesting to note for instance that only planes (020) and (540) are not phase determined by the bromine contribution.

## Scaling of the ( h o l) reflections of the bromide and chloride.

Methods 4(a) and (b) were used for scaling purposes. Nine relatively large planes were present in the bromide series and absent in the chloride series.

| (h o l) | ( h o l) |
|---|---|
| 101 | 605 |
| 103 | 607 |
| 304 | 10,05 |
| 403 | 15,01 |

The equation for isomorphous replacement reduces to:

$$F_{Br} = S\ (f_{Br} - f_{Cl})$$

Scaling factor $k_{Br} = \dfrac{\sum |F^{l}_{Br}|}{\sum |F_{Br}|} = 1.14$

To obtain the ratio $\dfrac{k_{Br}}{k_{Cl}}$

Seven planes were observed when the halogen contribution calculated low and could be neglected.

| ( h o l) | ( h o l) |
|---|---|
| 206 | 404 |
| 400 | 606 |
| 402 | 11,05 |
|  | 12,02 |

Then $$\frac{k_{Br}}{k_{Cl}} = \frac{\sum|F'_{Br}|}{\sum|F'_{Cl}|} = 0.87$$

when $k_{Cl} = 1.31$

$k_{Br} = 1.14$

The result of applying these factors to the observed reflections gave two series of "absolute" values, from which signs were given to 73 bromide reflections as in the ( h k o) zone. As before, several important omissions had to be made but the result of a Fourier synthesis is shown in Fig. (4:4).

It was observed at this stage that the (h 0 0) axial series which is common to both zones did not have the same values in each zone. The large plane (200) was found, in fact, to have been given different signs by the isomorphous method in each zone (allowing for the $\frac{\pi}{2}$ phase change by the change of origins). The majority of the planes, however, had been inserted in the Fourier with signs those of the bromine contribution and so were likely in the main to be correct.

Various attempts were made to obtain more accurate scaling factors but it was obvious that these could not be obtained by any of the easier methods to better than within ± 1%

## Analysis of the preliminary ( 0 0 1) and (010) projections.

It was realised at this stage that the method used to/

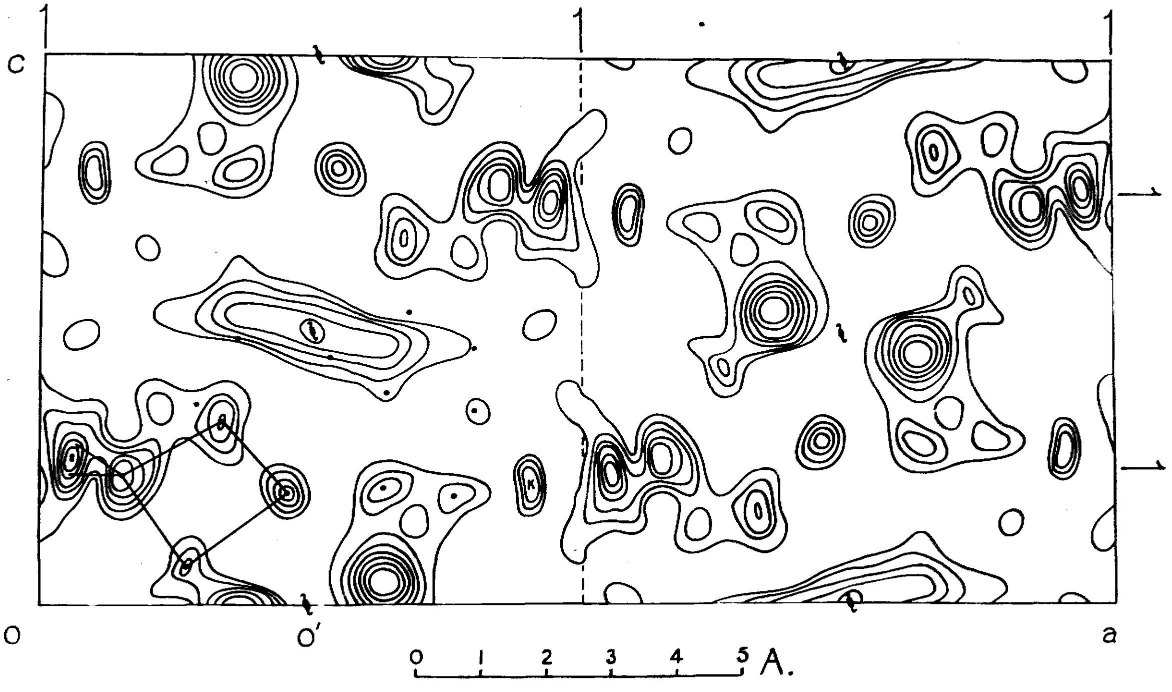


Fig.(4:4)

Preliminary Fourier Synthesis of 73 reflections in the (hol) zone of β-caryophyllene bromide. Signs obtained by a crude application of the method of isomorphous replacement but effectively determined by the bromine contribution.

to/ obtain absolute F values had not been sufficiently rigorous. Before embarking upon the laborious experimental work necessary to obtain the precise data which was obviously necessary for the isomorphous replacement method to be applied completely, it was decided to further examine the two projections of the unit cell so far obtained, although they were known to be very crude.

The sole feature of the $\beta$ -caryophyllene molecule which had been definitely established by this date was the four membered ring with the gem-dimethyl grouping. This was thought to be recognised at this early stage as shown [Figs. (4:3) and (4:4)] and the problem now resolved itself into fitting the rest of the molecule into the Fourier peaks. Some time was spent with a useful method to fit unknown molecules into direct Fourier data. The method consists of arranging the maps in space as the sides of a model of the unit cell. Parallel light is projected down the axes in such a way that a sphere placed inside the parallelpiped throws shadows on the Fourier maps. The spheres which are intended to represent atoms, are positioned so that their shadows coincide with maxima on all projections. In this way, a three dimensional model of the molecule can be built up.

Since a Fourier synthesis of the (o k l) zone data of the bromide gave practically no resolution, only the ( 0 0 1) and (0 1 0) projections were used. Starting from the four membered ring as an origin, a model was built up with spheres, stepwise, employing bond/

bond/ lengths and bond angles of the correct order. Several attempts were made and discrepancies calculated without any promising result. An unfortunate feature of the presence of the heavy atom at this stage was the fact that the discrepancy on the basis of the bromine alone, which made up 23% of the total scattering matter, was only 29%. Practically any structure was found to give a discrepancy only slightly higher than this:

Sharpened Fourier Syntheses.

It had been obvious early on from the relative sizes and areas of the various Fourier peaks, that in both principal projections many atoms were badly resolved. It has been suggested ( 78 ) that better resolution may be achieved by considering the atoms as point atoms and performing a Fourier synthesis on the geometric structure factors alone. These are obtained by dividing the observed F values by the atomic scattering factor for the particular value of 2 sin θ.

In the last two columns of Table(4:3)are listed the structure factors for the carbon residue without the heavy atom. The values were obtained by subtracting the calculated heavy atom contribution from the observed F value, and for the two isomorphs the resides agree fairly well. These values, where not incongruous, were averaged and divided by the appropriate atomic scattering factor (f) obtained from the standard diffraction curve. The/

The/ subtraction of the heavy atom contribution to the structure factor creates reflections previously absent, thus:

$$F_{Br} + F_{carb.} = 0$$

$$F_{carb} = - F_{Br}.$$

Where the heavy atom was found to give a considerable contribution to an absent plane, a value equal to $-\frac{(F_{Br} + F_{Cl})}{2}$ was taken to represent the carbon contribution. This is obviously a very crude approximation, but it is better than omitting a value altogether and the sign will be correct. Altogether, 107 reflections were obtained with their sign and employed in a two-dimensional "sharpened" Fourier synthesis. The result is shown in Fig. (4:5).

Little extra resolution was obtained from this synthesis which was, however, strongly reminiscent of the ( 0 0 1) projection already obtained. It did provide stronger evidence that the four membered ring had been located by the partial resolution of a further carbon atom (arrowed). The creation of such a huge trough at the site of the previous bromine position seemed to indicate a radical error in the diffraction curve which had been used for bromine. It was, however, decided to await a better series of absolute F values before formulating more accurate scattering curves.

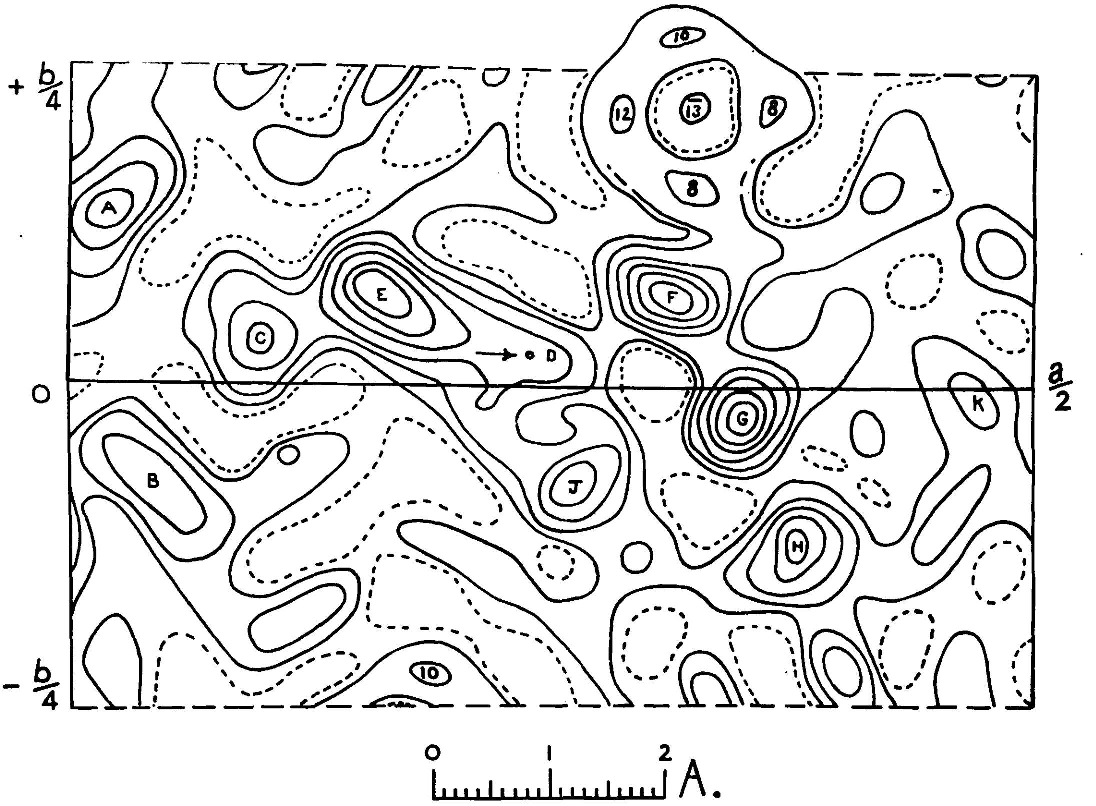
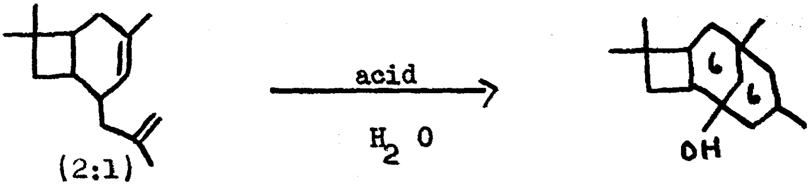


Fig.(4:5)
Asymmetric unit of a "sharpened" Fourier synthesis of the (hko) zone reflections of β-caryophyllene bromide. $F_{carbon}$ values were obtained by subtracting the bromine contributions from the F values, and these were converted to geometric structure factors by dividing by the appropriate <u>f</u> value for carbon. These values were introduced into the Fourier synthesis.

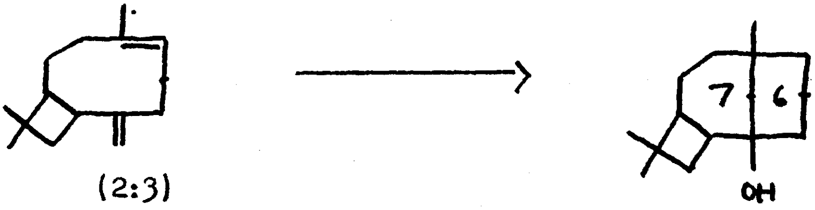
Trial structure attempts.

The three possible structures for $\beta$ -caryophyllene alcohol based on the three possibilities for $\beta$ - caryophyllene are shown below



(2:1)

(2:2)

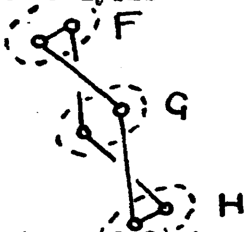


(2:3)

It will be seen that each of these postulates for the alcohol possesses, as well as the four membered ring of the parent, a six membered grouping as shown. It was conjectured that, if this could be found in the projections, Figs. (4:3) and (4:4), sufficient data would be known to bring in extra terms and locate the remaining three carbon positions by means of a Fourier synthesis. Various models of the alternative structures were made and it became apparent/

apparent/ that the hexane nucleus would be very badly resolved in the (0 0 1) zone if the four membered ring orientation were adhered to.

From this to choosing peaks F, G, and H as the hexane nucleus viewed endwise was a short step, and one which was to prove the turning point in the analysis



With the structure (2:2) in mind, carbons were roughly positioned as follows (these could only be very sketchily placed in the (0 1 0) projection but it was felt that in substance they were correct).

Gem.-dimethyl groups attached to cyclo-butane ring - A, B

Two resolved atoms of cyclo-butane ring - C, D

Two remaining atoms of cyclo-butane ring plus a possible other atom - E

Three doublets of end-on hexane nucleus - F, G, H

Single carbon atom - J

Single carbon atom - K

Atom K was put in with reluctance and could not be intelligently accounted for with any of the models because of its remoteness from and peculiar angle to the hexane ring. An unusually large/

large/ peak in the (0 1 0) projection with the same a - coordinate however, seemed to substantiate it, and on this account it was introduced.

On the basis of these carbon positions, a discrepancy was calculated for eleven planes not so far included in any Fourier on the ( h k o) zone data. The value of the ratio was 60% with (420) and (510) calculating very small, but (400) calculating high and negative. Introduced into the projection Fig. (4:3) it substantially "cleaned up" irregularities. Peak K however was severely reduced in size, and by using Bragg-Lipson charts ( 79 ) for planes (510) and (420), it became obvious that if (510) were introduced positively and (420) negatively, peak K would almost disappear whereas that a G would be substantially increased.

By switching the methyl group to this new position G, the discrepancy over the eleven planes previously alluded to fell from 60% to 36%, and it was possible to give signs to a further seven planes. These were introduced into the last Fourier on the (h k o) zone data and the resultant electron density map is shown in Fig.(4:6) The lower electron density contours have been omitted to make the picture clearer and the bromine atom contour intervals are reduced by ten.

Atoms were roughtly spotted as shown by the dots and a series of F values were calculated up to an index of ten along each reciprocal axis (89 reflections). The value of the discrepancy/

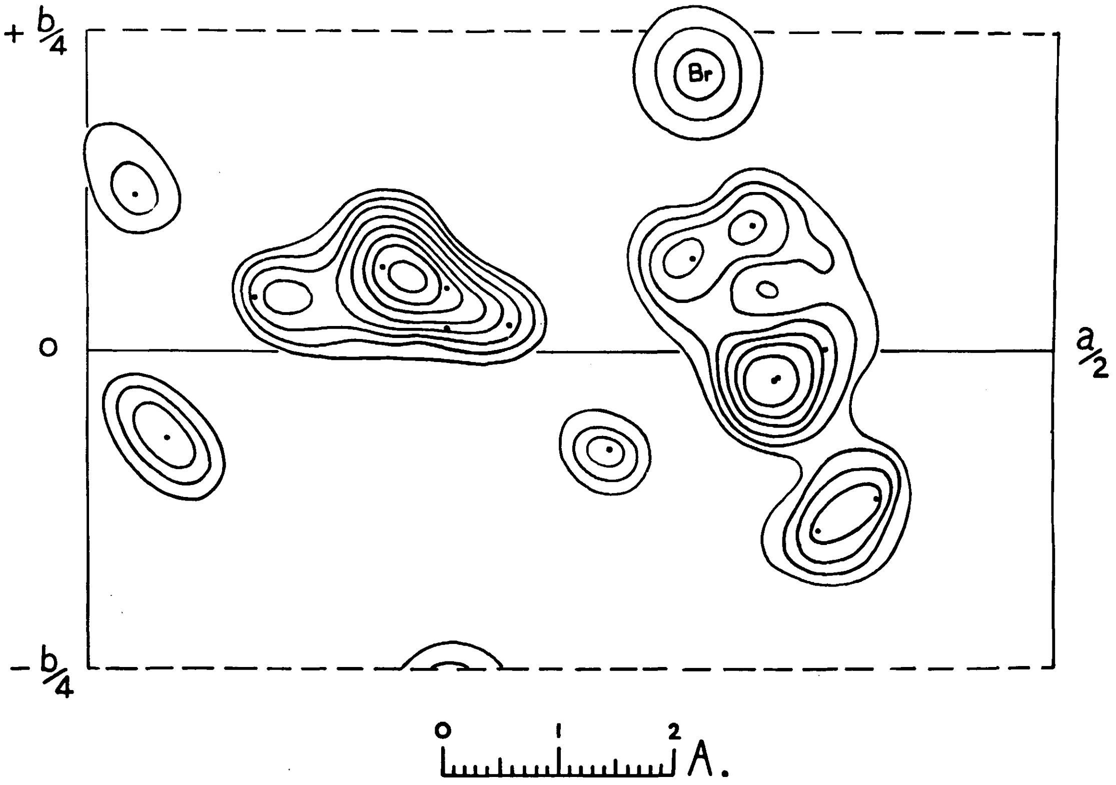


Fig.(4:6)

(001) projection of the $\beta$-caryophyllene bromide molecule. Atoms roughly spotted as shown gave a discrepancy of 21% over 89 reflections.

discrepancy/ between calculated and observed values was found to be 21%. Using the same coordinates for the light atoms and positioning a chlorine atom in place of the bromine atom a discrepancy was calculated over a similar range of intensities for the chloride. The value of the enumerator in the expression

$$\frac{\sum |F\ obs. - F\ calc.|}{\sum F\ obs.}$$

was almost identical with that obtained for the bromide but due to the increased size of the denominator, the value of the discrepancy was 38%

As it was obvious that the process of refinement could be better followed with the chloride, attention was now directed to that compound.

## β -caryophyllene chloride.

Using 91 terms whose signs had been obtained by using coordinates obtained from Fig. (4:6), a Fourier synthesis of the (h k o) reflections of the chloride was performed. Disappointing features were the small height of peak G where three atoms had been anticipated, and the presence of a substantial two electron irregularity. In any event, the treatment of this zone seemed to be as far advanced as resolution would allow and preliminary work on the (h o l) zone had indicated that here the resolution might be better.

## (h o l) zone of β -caryophyllene chloride.

Work on the (h k o) zones of the bromide and chloride was sufficiently advanced to warrant the assumption that the ( h 0 0)/

(h 0 0)/ reflections had been brought to an approximately absolute scale by scaling $\sum|F_{obs}|$ to $\sum|F_{calc}|$ It was decided to reattempt obtaining signs by the method of isomorphous replacement by scaling the (h o l) reflections to the absolute axial reflections obtained from the (h k o) zone. Thus if $\sum\left|F^{hko}_{hoo}\right|$ is the sum of the axial reflections on an sbolute scale from the (h k o) zone, then the scaling factor <u>k</u> to bring each observed (h o l) reflection to an absolute scale is given by $\sum\left|F^{h\,o\,l}_{h\,o\,o}\right| \div \sum\left|F^{h\,k\,o}_{h\,o\,o}\right|$ where $\sum\left|F^{h\,o\,l}_{h\,o\,o}\right|$ is the sum of the <u>observed</u> axial reflections in the (h o l) zone.

As a check, the same scaling factors were calculated using the statistical method previously employed to obtain the data for Fouriers (4:3) and (4:4) (Henceforth designated by the corresponding Fig. humber).

The ratio of the average structure factors $\frac{\bar{F}_{Br}}{\bar{F}_{Cl}}$ was taken to be 1.96 on the basis of the data now to hand for the (h k o) zone. The results were widely different from those obtained by axial scaling.

| | $k_{Br}$ | $k_{Cl}$ |
|---|---|---|
| Statistical method | 1.12 | 0.90 |
| Axial scaling | 0.94 | 0.62 |

The latter values were taken to be more probable.

Table(4:4) gives a selection of reflections whose signs were now obtained by comparing the absolute values of the bromide and chloride. Although yielding more signs than the initial attempt on which Fourier (4:4) was based, it was noted that all the terms which had been included in the latter had been inserted with the correct sign.

TABLE(4:4)

| h 0 l | $\lvert F_{Br}\rvert$ | $\lvert F_{Cl}\rvert$ | $F_{Br}-F_{Cl}$ | Sign $F_{Br}$ | Sign $F_{Cl}$ |
|---|---|---|---|---|---|
| 0 0 2 | 70 | 13 | $\overset{+}{60}$ | + | + |
| 4 | 66 | 32 | $\overset{+}{29}$ | + | + |
| 1 0 2 | 33 | 9 | $\overline{13}$ | - | - |
| 5 | 19 | 6 | $\overset{+}{12}$ | + | + |
| 7 | 32 | 19 | $\overset{+}{5}$ | - | - |
| 2 0 2 | 125 | 83 | $\overset{+}{38}$ | + | + |
| 3 | 85 | 48 | $\overline{29}$ | - | - |
| 4 | 33 | 13 | $\overset{+}{19}$ | + | + |
| 5 | 45 | 9 | $\overline{30}$ | - | - |
| 7 | 36 | 13 | $\overline{21}$ | - | - |
| 3 0 1 | 41 | 17 | $\overset{+}{24}$ | + | + |
| 2 | 65 | 33 | $\overline{29}$ | - | - |
| 6 | 38 | 8 | $\overline{33}$ | - | - |
| 9 | 11 | 7 | $\overline{5}$ | - | - |

**TABLE(4:4)(contd.)**

| h o l | $\lvert F_{Br} \rvert$ | $\lvert F_{Cl} \rvert$ | $F_{Br} - F_{Cl}$ | Sign $F_{Br}$ | Sign $F_{Cl}$ |
|---|---|---|---|---|---|
| 4 0 1 | 73 | 50 | $\overline{16}$ | - | - |
| 5 | 81 | 33 | $\overline{40}$ | - | - |
| 7 | 33 | 6 | $\overline{27}$ | - | - |
| 9 | 19 | 7 | $\overline{15}$ | - | - |
| 5 0 1 | 60 | 26 | $\overline{25}$ | - | - |
| 2 | 47 | 18 | $\overline{26}$ | - | - |
| 3 | 56 | 35 | $\overline{16}$ | - | - |
| 4 | 57 | 18 | $\overline{37}$ | - | - |
| 6 | 35 | 11 | $\overline{30}$ | - | - |
| 6 0 2 | 74 | 32 | $\overline{36}$ | - | - |
| 3 | 70 | 42 | $\overline{24}$ | - | - |
| 7 0 1 | 74 | 32 | $\overline{49}$ | - | - |
| 3 | 62 | 24 | $\overline{32}$ | - | - |
| 4 | 38 | 24 | $\overline{13}$ | - | - |
| 6 | 13 | 3 | $\overline{10}$ | - | - |
| 8 | 12 | 9 | $\overline{7}$ | - | - |
| 8 0 2 | 53 | 10 | $\overline{42}$ | - | - |
| 4 | 43 | 20 | $\overline{21}$ | - | - |
| 9 0 1 | 56 | 10 | $\overline{42}$ | - | - |
| 3 | 34 | 10 | $\overline{27}$ | - | - |

TABLE(4:4)(contd.)

| h o l | $\lvert F_{Br} \rvert$ | $\lvert F_{Cl} \rvert$ | $F_{Br}-F_{Cl}$ | Sign $F_{Br}$ | Sign $F_{Cl}$ |
|---|---|---|---|---|---|
| 9 0 5 | 29 | 14 | $\bar{9}$ | - | - |
| 9 | 11 | 7 | $\overset{+}{8}$ | + | + |
| 1 0,0 1 | 21 | 9 | $\overset{+}{8}$ | + | + |
| 3 | 35 | 12 | $\overset{+}{19}$ | + | + |
| 1 1,0 1 | 26 | 17 | $\overline{13}$ | - | - |
| 2 | 34 | 10 | $\overset{+}{17}$ | + | + |
| 1 2,0 1 | 21 | 6 | $\overset{+}{9}$ | + | + |
| 3 | 29 | 8 | $\overset{+}{22}$ | + | + |
| 7 | 23 | 7 | $\overset{+}{20}$ | + | + |
| 1 3,0 2 | 37 | 20 | $\overset{+}{13}$ | + | + |
| 3 | 26 | 9 | $\overset{+}{9}$ | + | + |
| 6 | 23 | 10 | $\overset{+}{18}$ | + | + |
| 1 4,0 1 | 16 | 11 | $\overset{+}{5}$ | + | + |
| 1 6,0 2 | 29 | 13 | $\overset{+}{21}$ | + | + |
| 1 7,0 1 | 28 | 14 | $\overset{+}{19}$ | + | + |
| 1 9,0 2 | 12 | 8 | $\bar{7}$ | - | - |

In all Fourier refinement work where a comparatively complex structure is involved, care must be taken to avoid manipulating the signs of the terms in order to "force" a pre-arranged structure into the projection which is not correct. The first Fourier synthesis of the ( h k o) zone data of the chloride gave a projection which possessed (as observed on p.84) a large two electron irregularity which could have been the residue of an atom forced out of existence by wrong term signs. Work on the (h k o) zone had so far been largely based on positioning the four and six membered ring systems but it was conceivable that those hypotheses were quite wrong.

With the facts in mind it was decided to experiment on the (h o l) zone with as little reference to the (h k o) zone as possible in order to see if the result would bear out any conclusions so far reached with reference to the (0 0 1) projection. Use was made of:

1) the scaling factors obtained by using (h k o) axial absolute values.

2) the fact that (400) was negative in zone (h k o) and if inserted thus into an (h o l) synthesis would dismiss peak K together with other maxima along the dotted line in Fig. (4:4).

With the latter proviso in mind, atoms were very roughly spotted as shown by dots in Fig. (4:4) and structure factors/

factors/ calculated for a few important planes whose sign had not been determined by the method of isomorphous replacement.

| h o l | G.S.F.* | $F_{carb}$ | $F_{calc.}$ | F.obs. | Sign |
|---|---|---|---|---|---|
| 0 0 6 | $\overline{1.36}$ | $\overline{2}$ | 0 | 12 | ? |
| 2 0 0 | 10.30 | 57 | 100 | 94 | + |
| 2 0 6 | 10.1 | 14 | 16 | 31 | + |
| 3 0 3 | $\overline{7.9}$ | $\overline{21}$ | $\overline{10}$ | 25 | - |
| 3 0 5 | 2.4 | 4 | 8 | 11 | + |
| 4 0 0 | $\overline{11.7}$ | $\overline{49}$ | $\overline{49}$ | 79 | - |
| 4 0 2 | $\overline{7.4}$ | $\overline{24}$ | $\overline{24}$ | 11 | - |
| 4 0 4 | 8.2 | 16 | 16 | 25 | + |
| 4 0 6 | $\overline{8.7}$ | $\overline{12}$ | $\overline{12}$ | 14 | - |
| 5 0 5 | 3.2 | 5 | 2 | 12 | ? |
| 6 0 1 | 8.4 | 24 | 13 | 24 | + |

* G.S.F. = geometric structure factor.

The additional 9 planes whose signs were thus obtained were taken in conjunction with data obtained by the direct isomorphous replacement method and introduced into a two dimensional Fourier synthesis. This is shown in Fig. (4:7). Taken in conjunction with Fig. (4:6) there did not seem to be any further/

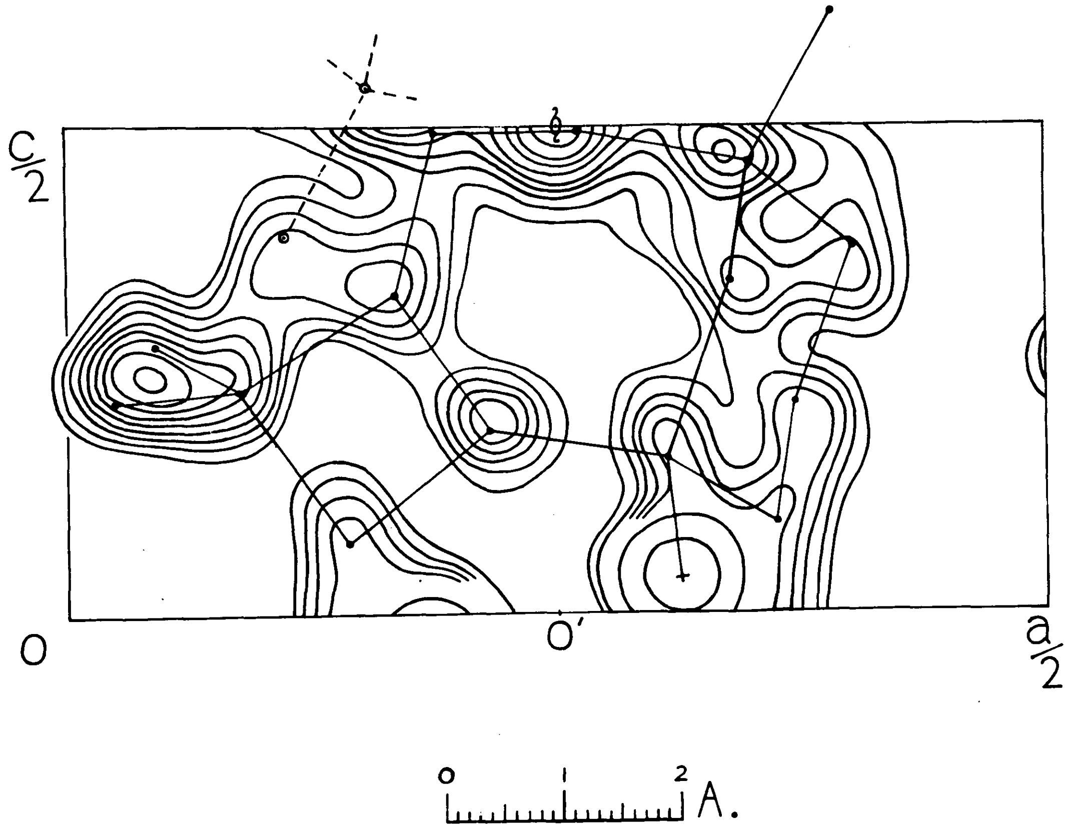


Fig.(4:7)

Direct Fourier synthesis of (hol) data for β-caryophyllene chloride.
Signs obtained: a) by the method of isomorphous replacement (Table 4:4)
b) for doubtful planes and those with a low heavy atom contribution, by spotting rough centres from the crude (010) projection already directly obtained. Cf. Fig.(4:4)

further/ doubt about the structure of the molecule.

Note: It must be noted that Fig. (4:6) is the (0 0 1) projection of the bromide molecule and Fig. (4: 7) the (0 1 0) projection of the chloride molecule. A (0 0 1) projection of the chloride molecule was available (Cf. p 84 ) but was very similar to that of the bromide and has not been reproduced here. An important difference lay in the shape of the central hexane peak G which had not the well defined bulge shown by the bromide. Thus when more precise centres were picked from the two projections, the methyl group was given a wrong position leading to the difficulties met with and discussed later.

The molecule is apparently as shown in the diagram by lines joining atomic centres. It is not completely contained by the area shown, having a methyl group projecting outside. The equivalent methyl group from another molecule related to the one shown by a centre of symmetry is projecting into the area of projection as shown by a dotted line. The centres obtained enabled a further 11 planes to be given signs and these terms were superimposed on Fourier (4:7). This addition gave a more precise version of the (0 1 0) projection but it was obvious that maximum resolution of the atoms had at this stage been reached. Only afew very small terms remained to be inserted, and it did not appear probable that many of the more/

more/ important terms would change sign by refinement.

### (o k l) zone of β -caryophyllene chloride.

Since the (h k o) and (h o l) projections so far obtained appeared to be in almost the final form, it was obvious that resolution would not allow precise spotting of atomic centres. Before going on to obtain these by means of $(F_o - F_c)$ syntheses, a brief reference was made to the badly resolved (1 0 0) projection to find if any information could be obtained with respect to coordinates.

Using axial values obtained from the other zones as a method of scaling, the isomorphous replacement method was applied to the (o k l) zone of the bromide and chloride. Two dimensional Fourier syntheses were done on each compound but at this crude stage no information was obtained. A preliminary set of (b,c) coordinates obtained from the (h k o) and (h o l) zones was used to calculate structure factors for a further 34 planes.in the (o k l) zone of the chloride. The discrepancy over these planes was 35%. On introducing them into a Fourier synthesis, however, spurious peaks appeared at centres of symmetry showing that a substantial number of signs were wrong. It was decided to leave this zone until fuller information was available from the principal zones.

### Refinement by $(F_o - F_c)$ Syntheses.

### Better atomic scattering curves:

Before refining the (h k o) and (h o l) zones by means/

means/ of $(F_o - F_c)$ syntheses, it was essential to obtain truer scattering curves for the atoms. Fig. (4:5), for instance, had indicated that the value of $B = 2.0 \times 10^{-16}$ in the Debye-Waller temperature factor might have been selected at too small a value. A better value was now obtained for the chlorine atom as follows:

From each (h k o) reflection ("absolute") was subtracted the calculated carbon contribution based on the diffraction curve already used $(B = 2.0 \times 10^{-16})$. The residual structure factor represented the chlorine contribution. Neglecting the reflections where 2 sin θ was less than 0.50 i.e. those most liable to hydrogen, and extinction errors, the reflections were divided up into groups according to sin θ with about 15 values per group. If $F_r$ is the value of aparticular structure factor, then the scattering factor $f_r$ is given by $f_r = \dfrac{F_r}{G_r}$.

The mean value of the scattering factor in any one of the reflection groups is thus given by the relation:

$$\bar{f} = \frac{F_1 + F_2 + \ldots + F_r \ldots + F_n}{G_1 + G_2 + \ldots + G_r \ldots + G_n}$$

where there are n reflections in the group.

In a particular excited state, the scattering factor $f_e$ of an atom is related to the James and Brindley value $f_o$ by the/

the/ relationship:

$$f_c = f_o e^{- B. \frac{\sin^2 \theta}{\lambda^2}}$$

Thus, $$\frac{\log_e \frac{f_c}{f_o}}{\frac{\sin^2 0}{\lambda^2}} = - B$$

Graphically the mean value of <u>B</u> was obtained as $4.4 \times 10^{-16}$

With this value, a new scattering curve was drawn up for chlorine and the F values for the reflections rescaled. This enabled an empirical curve to be drawn up for carbon. It was decided to employ purely empirical curves for carbon until the last refinement in coordinates had been made, a fresh empirical curve being drawn up after each $(F_o - F_c)$ synthesis.

<u>Difference Fourier Syntheses.</u>

The $(F_o - F_c)$ synthesis was used by Crowfoot and others in the penicillin investigation and treated fully by Cochran(90). The method is valuable in that it corrects for termination of series errors and helps to position atoms which are badly resolved. (Pitfalls, however, occur here as will be shown later). The method is pre-eminently suited to the case where a heavy atom or groups of/

of/ light atoms occur in a projection of a molecule. These large diffracting centres stabilise the signs of the terms and make the refinement a matter of adjusting magnitudes rather than signs.

Briefly, in any series of structure factor calculations based on a particular set of atomic coordinates, the discrepancies between calculated and observed intensities indicate the extent of errors in the coordinates. Theoretically, a Fourier synthesis of all the algebraic discrepancies between $F_o$ and $F_c$ should indicate the directions in which atoms should be moved in order to minimise the discrepancy.

The direction of shift in such a synthesis is along the steepest ascent and the magnitude of shift can be derived in the one dimensional case as follows:

The electron density distribution of an atom is approximately Gaussian and may be shown by the function:

$$\rho_o = \rho_M \cdot e^{-ax^2}$$

where $\rho_o$ is the electron density at x from the centre where the peak density is $\rho_M$

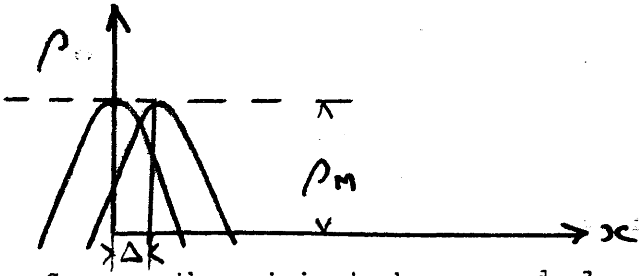
This may be written: $\rho_o = \rho_M(1 - ax^2 + \frac{a^2x^4}{2!} \text{------})$

$\doteqdot (1 - ax^2)$ since a is small and for small values of x eliminates terms containing its powers.

Thus we may write:

$$\rho_o = \rho_M - cx^2 \quad \text{where } c = (\rho_M \cdot \alpha).$$

and the electron distribution is parabolic.



Suppose the origin to be removed along the x axis a distance $\Delta$. The resultant electron distribution ( $\rho_c$ ) is given, related to the first origin, by the function:

$$\rho_c = \rho_M - c\,(x + \Delta)^2$$

$$\text{Since } \rho_o = \rho_M - cx^2$$

$$\text{Thus } \rho_o - \rho_c = -\cancel{cx^2} + \cancel{cx^2} + 2c.\Delta x + c.\Delta^2$$

$$\doteqdot 2c.\Delta.x \quad \text{if } \Delta \text{ is small}$$

$$\text{Whence } \frac{d(\rho_o - \rho_c)}{dx} = 2c.\Delta$$

$$\text{and } \Delta = \frac{d(\rho_o - \rho_c)}{dx} \Big/ 2c.$$

If The value of $\frac{d(\rho_o - \rho_c)}{dx}$ can be measured and the value of constant c is known, then $\Delta$ can be calculated.

Where $\rho_o$ and $\rho_c$ define the electron distribution of an/

an/ assemblage of atoms in projection or in three dimensions, and where $\rho_o$ is the true distribution and $\rho_c$ the chosen distribution (approximately correct), it may be shown that the above reasoning still holds.

The constant c is a function involving temperature and is best found by experiment.

$$\rho_o = \rho_m - cx^2$$

$$= \rho_m - c.n \quad \text{where } n = x^2$$

Hence $\frac{d\rho_o}{dn} = -c.$

The best resolved carbon in the (0 1 0) projection of the chloride was chosen, and with the centre as origin the radius x of the various contours was measured.

By graphing the value of the contour against the square of its radius, a value for c was obtained. of 0.62. This was later amended to 0.7

$$\therefore \quad \underline{2c = 1.4}$$

From the $(F_o - F_c)$ synthesis the direction of maximum gradient is measured at the site of each atom. This gives the direction of shift to minimise the discrepancy. The value of $\frac{d\rho}{dx}$ is measured as nearly as possible at the site and the magnitude of the shift is given by $\frac{d\rho}{dx} \Big/ 2c \,(= 1.4)$

Fig. (4:8) represents a typical ($F_o - F_c$) synthesis in the (h o 1) zone. The contours are at intervals of 0.3 ε. Negative contours are broken

Refinement of the (h o 1) zone of β-caryophyllene chloride, using successive ($F_o - F_c$) syntheses.

Operations were carried out in the following sequence :

1) $\sum|F_o|$ was scaled to $\sum|F_c|$ and the values ($F_o - F_c$) introduced algebraically into a two dimensional Fourier synthesis.

2). From the resultant map, $\frac{d\rho}{dx}$ was evaluated for each atom and shifts calculated.

3). Structure factors were evaluated on the basis of the new coordinates and $\sum|F_o|$ scaled to the new value of $\sum|F_c|$

4) Using the values for $F_o$ so obtained, an empirical curve for carbon was derived.

5). Better values of atomic scattering factors were thus obtained and used to calculate $F_c$ values.

These were then used to recommence the sequence.

Note: in doing these ($F_o - F_c$) syntheses, a certain amount of judgment was exercised in introducing the terms. Those where the difference between ($F_o - F_c$) was suspected of being due to absorption etc. were omitted from the syntheses.

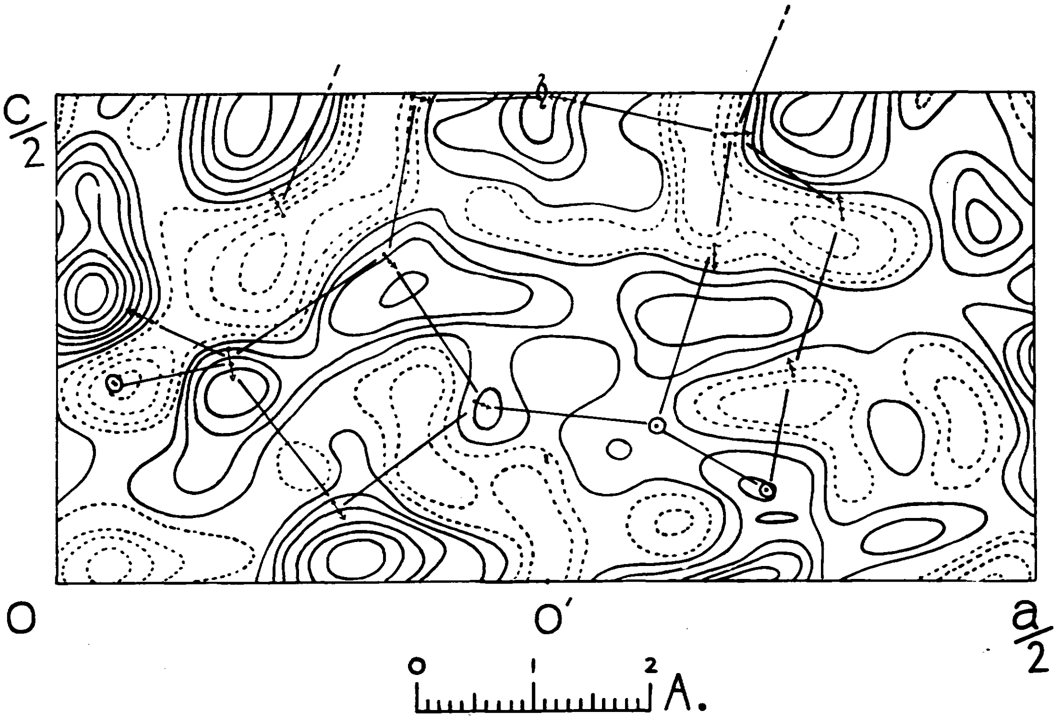


Fig.(4:8)

Second $(F_o - F_c)$ synthesis in the (hol) zone. Contours at intervals of 0.3ε. Negative contours broken. The direction and magnitude of calculated shifts are indicated.

From the best Fourier projection of the (h o l) zone, carbon centres were spotted. $F_c$ values were calculated and the value of the discrepancy over all the planes was 29.6%. An $(F_o - F_c)$ synthesis was done, and analysis of the results indicated atomic shifts of the order of 0.1 Å, ranging from zero to 0.23 Å. The discrepancy using the new coordinates was 23%, falling to 20.4% with a fresh empirical curve for carbon.

A second $(F_o - F_c)$ synthesis was performed and indicated shifts of the order of 0.07 Å, ranging from zero to 0.13 Å. Values of $F_c$ were not calculated at this stage.

### Refinement of the (h k o) zone of β-caryophyllene chloride by $(F_o - F_c)$ syntheses.

The positions of the atoms in the (o o l) projection were not so easy to spot as in the (0 1 0). Taking the <u>a</u> coordinate from the (0 1 0) projection as being a good first approximation in the (0 0 1), the <u>b</u> coordinates were arrived at with the aid of a model.

$F_c$ values were calculated and the discrepancy was found to be 31%. The first $(F_o - F_c)$ synthesis in this zone indicated atomic shifts of the order of 0.1 Å, and reduced the discrepancy to 24.2%. Using a better scattering curve for carbon this was further reduced to 22.5%. A second $(F_o - F_c)$ synthesis indicating shifts of about 0.06 Å was performed to give a further set of coordinates.

Synthesis of zonal coordinates.

Each zone, apart from the crude initial positioning of atoms, has been separately analysed by the $(F_o - F_c)$ synthesis method. The a coordinates which should be identical in both zones are shown in Table(4:5)

TABLE (4:5)

| a (Å) | | | a (Å) | | | |
|---|---|---|---|---|---|---|
| Atom | h k o | h o l | Atom | h k o | h o l | |
| 1 | 5.01 | 5.05 | 9 | 6.60 | 6.63 | |
| 2 | 3.58 | 3.58 | 10 | 6.13 | 6.17 | |
| 3 | 2.42 | 2.38 | 11 | 5.84 | 5.98 | * |
| 4 | 1.52 | 1.48 | 12 | 5.60 | 5.53 | |
| 5 | 2.76 | 2.80 | $8^{1}$ | 1.86 | 1.83 | |
| 6 | 3.14 | 3.10 | $4^{11}$ | 0.82 | 0.70 | * |
| 7 | 4.34 | 4.29 | $4^{1}$ | 0.36 | 0.49 | * |
| 8 | 5.67 | 5.72 | | | | |

These are in good agreement except for the atoms asterisked. Atoms $4^{11}$ and $4^{1}$ have associated with them each three hydrogen atoms which might falsify the $(F_o - F_c)$ shifts.

Atom 11 however has its hydrogen equally disposed in this projection and the discrepancy cannot be easily explained.

A synthesis of the data was now attempted by averaging the a coordinates and fixing this one parameter. The b and c coordinates were then obtained by reference to the ($F_o$ - $F_c$) maps. In the case of atoms 11, $4^{11}$ and $4^{1}$, a bond length of 1.54 Å was assumed and the best fit to the ($F_o$ - $F_c$) maps obtained. The resultant coordinates referred to the cell origin are shown in Table(4:6)

TABLE(4:6.)

| Atom | a(Å) | b | c | Atom | a(Å) | b | c |
|---|---|---|---|---|---|---|---|
| 1 | 5.04 | 0.61 | 1.30 | 9 | 6.63 | 1.04 | 3.19 |
| 2 | 3.58 | 0.28 | 1.50 | 10 | 6.16 | 1.42 | 1.80 |
| 3 | 2.40 | 0.56 | 0.62 | 11 | 5.98 | 0.38 | 0.74 |
| 4 | 1.50 | 0.51 | 1.84 | 12 | 5.57 | 1.12 | 2.72 |
| 5 | 2.78 | 0.94 | 2.70 | $8^{1}$ | 1.86 | 4.84 | 3.20 |
| 6 | 3.14 | 0.40 | 4.08 | $4^{11}$ | 0.76 | 0.82 | 2.24 |
| 7 | 4.32 | 0.68 | 4.98 | $4^{1}$ | 0.42 | 1.68 | 1.78 |
| 8 | 5.70 | 0.12 | 3.80 | | | | |

Structure factors for zones (h k o) and (h o l) were evaluated and empirical scattering curves obtained.for each zone. These were practically identical. The values of the discrepancy were/

were/ 22.7% and 14.3% in the (h k o) and (h o l) zones respectively.

Over the (o k l) reflections, the value of the ratio was 36%

Treatment of the (o k l) zone.

It had been anticipated that the (o k l) zone discrepancy would be higher than the others for two reasons.

1). since the zone with the smallest axes is more critical of coordinate accuracy, small errors which were negligible in the other zones are not so here.

2) hydrogen contributes 19% of the scattering power of the molecule but where the hydrogen atoms are well resolved can be conveniently neglected. In the smaller zone, however, bad resolution of the hydrogens may create quite large diffracting centres which cannot be neglected in calculating $F_c$ values.

Although it was anticipated that resolution would be too bad to adequately employ ($F_o - F_c$) syntheses in the (o k l) zone, a variation was now attempted aimed at eliminating coordinate errors. Since the discrepancies due to hydrogen would be most apparent, it was felt, in the low order reflections, an ($F_o - F_c$) synthesis was now done omitting planes where $2 \sin \theta$ was less than 0.40.

It had been hoped that the result of this synthesis would be to indicate quite small shifts not radically effecting the coordinates so far obtained in the two principal zones. In fact, peaks of/

of/ height two electrons together with a trough of minus four electrons were obtained and gross shifts of as much as 0.26 Å were indicated for atoms around this trough. Although it was realised that something was seriously wrong, $F_c$ values were calculated as a matter of form after the indicated shifts had been made and the discrepancy was still high at 33.5%.

A second ($F_o$ - $F_c$) synthesis was now done bringing in all reflection discrepancies over the whole 2 sin θ range. The result is shown in Fig.(4:9).

In this synthesis it was seen that the shifts which had been made had reduced the trough by only one electron from minus four to minus three.(the larger value is shown in sketch). A new feature, being a peak of height over four electrons, was however now apparent. Such a situation, it was felt, could only have been created if an atom had been positioned quite wrongly, the required large shift being 0.8 Å in the direction shown. The other zones were now examined to see if such a suggestion was tenable.

Any shift along the b axis, of course, would not affect any conclusions so far reached with respect to the (h o l) zone. The main problem was to resolve the shift with conclusions reached about the b coordinate in the (h k o) zone. Examination of the ($F_o$ - $F_c$) maps in this zone showed that in fact a situation had/

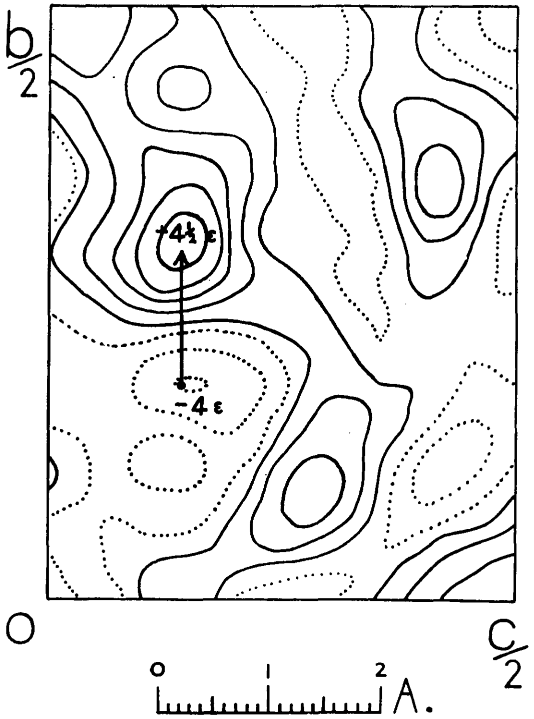


Fig.(4:9)

Second ($F_o$-$F_c$) synthesis in the (okl)zone. Contours at intervals of 1.0 ε Negative contours broken.

The electon density distribution indicates that in this case an atom has been wrongly positioned at a site indicated by a trough, and should be moved to the position indicated by the large peak.

had/ occurred analogous to that in the (o k 1) projection with the presence of the characteristic peak and trough. The effect was not so striking as in the (o k 1) case and had been partly compensated for by shifting atoms 8 and 11. Due to the bad resolution of atoms 8,11, and $8^1$ the error in positioning had not been so obvious in the discrepancy which stood at 22.5%. If refinement had been continued by the $(F_o - F_c)$ method long enough, it is probable that the correct positions would eventually have been found.

Review of the (h k o) zone.

Using the b coordinate obtained from the (1 0 0) projection, for atom $8^1$ the (h k o) zone was re-examined by the $(F_o - F_c)$ method. At the commencement, the discrepancy using the new position for atom $8^1$ was 24.2% but an $(F_o - F_c)$ synthesis gave shifts which dropped the ratio to 18.3%. A final $(F_o - F_c)$ synthesis indicated average shifts of $0.03_5$ $\overset{o}{A}$ to give a final list of a,b coordinates in this zone.

The a coordinates obtained independently from zones (h k o) and (h o 1) were as shown in Table(4:7).

TABLE.(4:7)

| | a(Å) | | | a(Å) | |
|---|---|---|---|---|---|
| Atom | h k o | h o l | Atom | h k o | h o l |
| 1 | 5.05 | 5.05 | 9 | 6.65 | 6.63 |
| 2 | 3.58 | 3.58 | 10 | 6.15 | 6.17 |
| 3 | 2.39 | 2.38 | 11 | 5.97 | 5.99 |
| 4 | 1.51 | 1.48 | 12 | 5.60 | 5.54 |
| 5 | 2.77 | 2.80 | $8^{1}$ | 6.37 | 6.39 |
| 6 | 3.10 | 3.10 | $4^{11}$ | 0.76 | 0.70 |
| 7 | 4.33 | 4.29 | $4^{1}$ | 0.42 | 0.49 |
| 8 | 5.72 | 5.72 | | | |

On average, these values agree to within $\pm\ 0.01_3$ Å, the maximum error being $\pm\ 0.03_5$ Å

In order to obtain the final list of the three dimensional coordinates of the atoms, the above values were averaged to a large extent, When however, either the (h o l) or (h k o) ($F_o - F_c$) synthesis map indicated a straightforward shift for an atom which was ill defined in the other map, the former a coordinate was taken, as being more probably correct. The b and c coordinates were then adjusted accordingly.

The final list of coordinates is given in Table(4:8)

TABLE(4:8)

| Atom | a(Å) | b | c | Atom | a(Å) | b | c |
|---|---|---|---|---|---|---|---|
| 1 | 5.06 | 0.72 | 1.33 | 9 | 6.67 | $\overline{1.09}$ | 3.16 |
| 2 | 3.58 | 0.18 | 1.55 | 10 | 6.16 | $\overline{1.46}$ | 1.80 |
| 3 | 2.39 | 0.56 | 0.60 | 11 | 6.00 | $\overline{0.24}$ | 0.78 |
| 4 | 1.50 | 0.54 | 1.86 | 12 | 5.60 | 1.16 | 2.81 |
| 5 | 2.78 | 0.95 | 2.70 | $8^{1}$ | 6.39 | 0.40 | 5.07 |
| 6 | 3.10 | 0.40 | 4.08 | $4^{11}$ | 0.76 | $\overline{0.84}$ | 2.27 |
| 7 | 4.30 | $\overline{0.68}$ | 4.08 | $4^{1}$ | 0.42 | 1.74 | 1.78 |
| 8 | 5.73 | $\overline{0.04}$ | 3.80 | | | | |

chlorine <u>5.165</u> (Å) <u>2.180</u> <u>0.294</u>

Final scattering curve for carbon.

Using the coordinates given in Table(4:8)(but corrected to refer to the origin used in each zone), structure factors were calculated for the (h k o) and (h o l) reflections. The $F_o$ values were then put on an absolute scale by scaling $\sum|F_o|$ to $\sum|F_c|$ and an empirical scattering curve for carbon obtained. The values of the empirical scattering factor ($f_c$) thus found are shown in Table(4:9). The most recent theoretical values for the scattering/

scattering power of carbon are given by McWeeny and are shown under $f_o$ in Table(4:9). By plotting $\log_e \frac{fe}{fo}$ against $\frac{\sin^2 \theta}{\lambda^2}$ a value of B was obtained of $3.5 \times 10^{-16}$. This gave calculated ($f_c$) values of the scattering factor as shown, indicating that the empirical curve was practically identical with the McWeeny curve, appropriately corrected for temperature.

TABLE(4:9)

| 2sinθ | 0.00 | 0.15 | 0.29 | 0.44 | 0.58 | 0.73 | 0.88 | 1.02 | 1.17 | 1.31 | 1.46 | 1.60 | 1.75 | 1.90 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| fe | (in electrons) | | | 4.38 | 3.45 | 2.63 | 1.98 | 1.53 | 1.18 | 0.90 | 0.68 | 0.50 | 0.40 | 0.33 |
| fo | 6.00 | 5.80 | 5.27 | 4.58 | 3.80 | 3.14 | 2.63 | 2.26 | 2.00 | 1.71 | 1.57 | 1.37 | 1.17 | 0.97 |
| fc | 6.00 | 5.76 | 5.11 | 4.27 | 3.35 | 2.58 | 1.93 | 1.54 | 1.20 | 0.91 | 0.72 | 0.53 | 0.38 | 0.26 |

Using the curve defined by the above fc values, final structure factors were calculated for all zones. They are listed in Table(4:10)(see p. 116).

Final discrepancies were:

| | |
|---|---|
| h k o | 15.7 % |
| h o l | 12.3 % |
| o k l | 24.0 % |
| overall | 17.5 % |

Final Fourier syntheses were performed on the (h k o) and (h o l) zones, bringing in 115 and 84 terms respectively. The resultant electron density maps are shown in Figs. (4:10) and (4:11).

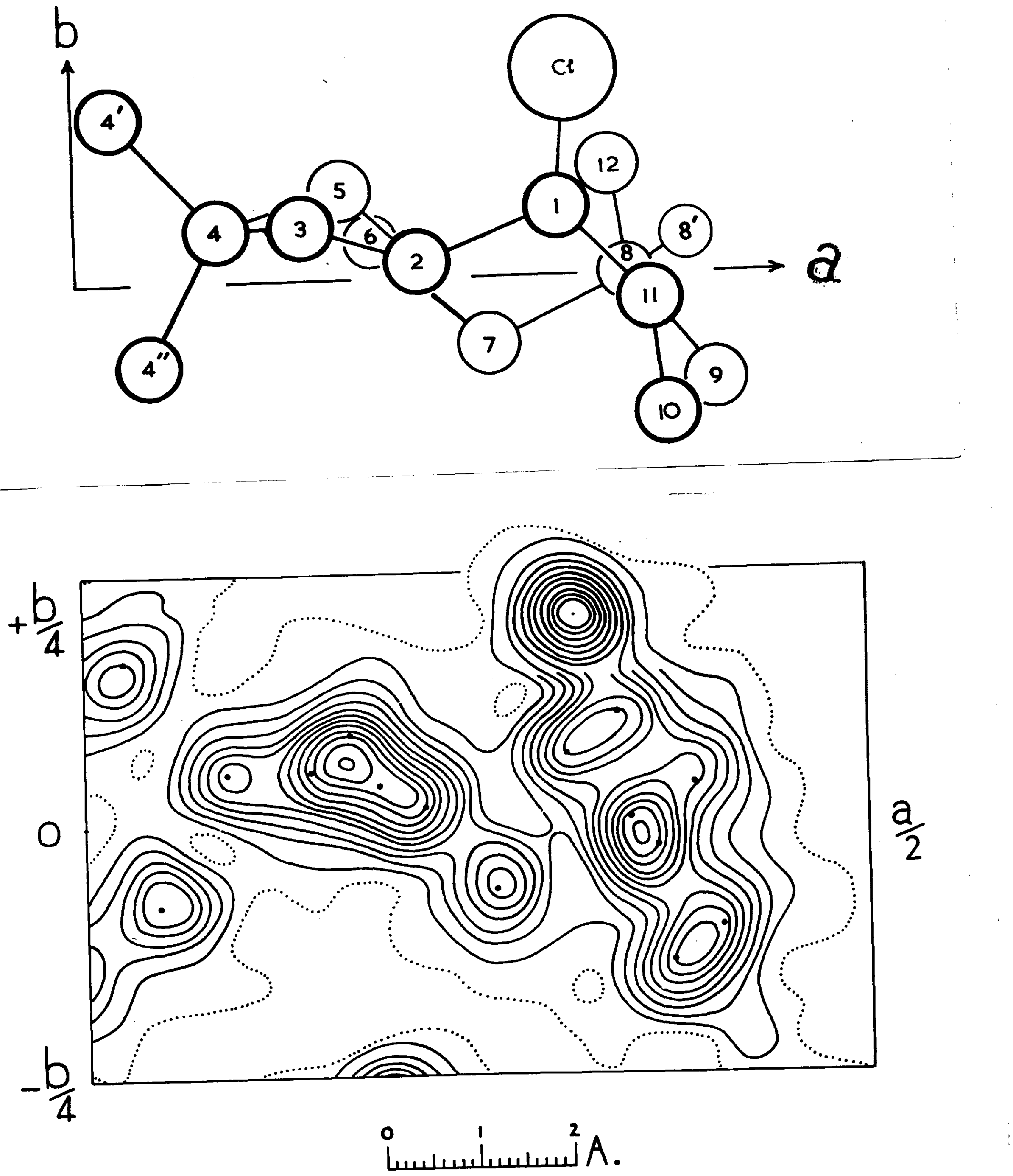


Fig.(4:10)
Final (001) projection of the β -caryophyllene chloride molecule as given by double Fourier synthesis of 115 (hko) reflections.
Contours at 1.0e intervals -- one electron line dotted.

Above is a diagrammatic representation of the same projection.

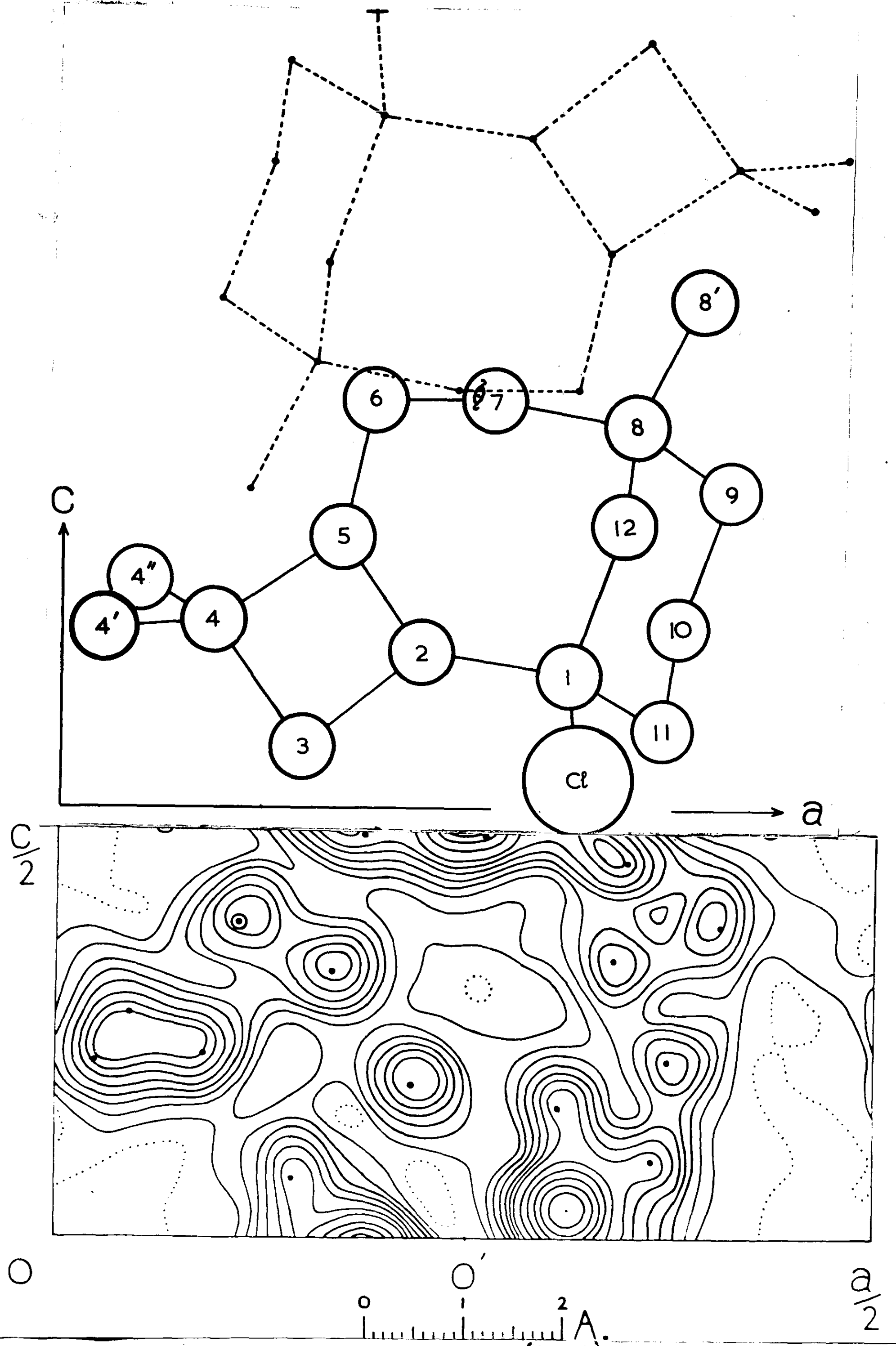


Fig.(4:10)

Final (010) projection of the β-caryophyllene chloride molecule as given by double Fourier synthesis of 84 (hol) reflections.
Contours at 1.0ε intervals -- one electron line dotted.

Above is a diagrammatic representation of the same projection.

## Discussion:

The relatively low value of the discrepancies together with the Fourier maps, seems to indicate that the correct spatial arrangement of the atoms in the crystal of β -caryophyllene chloride has been found. The shortest inter atomic distances are shown in Table(4:10) and all lie in the range 1.45 -- 1.62 Å . These are the distances found if a primary valence bond exists between carbon atoms. All other inter atomic distances have not been calculated but those so far determined have a closest approach of 4.2 - 4.3 Å compared with the $CH_3$ ..... $CH_3$ intermolecular distances for hexamethyl benzene of 4.0 - 4.1 Å .

### TABLE(4:10)

Interatomic distances in Å

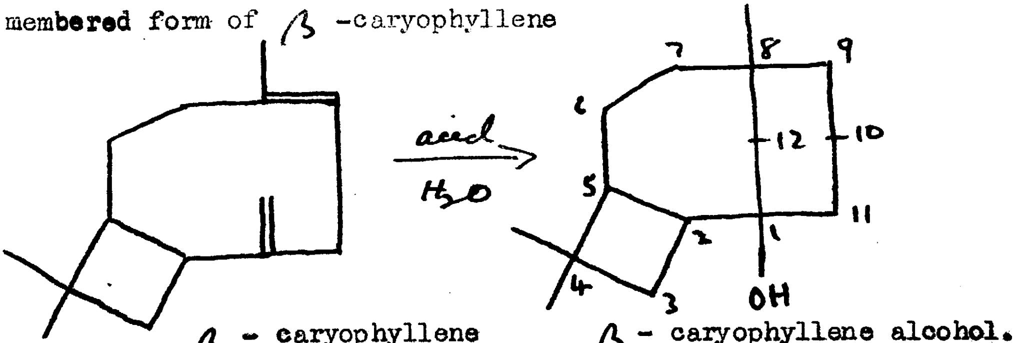
| | | | |
|---|---|---|---|
| C(1) — C(2) | 1.59 | C(7) -- C(8) | 1.59 |
| C(2) — C(3) | 1.57 | C(8) — $C(8^1)$ | 1.50 |
| C(3) — C(4) | 1.54 | C(8) — C(12) | 1.56 |
| C(4) — C(4 ) | 1.62 | C(8) -- C(9) | 1.55 |
| C(4) — C(4 ) | 1.62 | C(9) -- C(10) | 1.50 |
| C(4) — C(5) | 1.57 | C(10) - C(11) | 1.60 |
| C(2) — C(5) | 1.60 | C(11) - C(1) | 1.45 |
| C(5) — C(6) | 1.52 | C(1) - C(12) | 1.64 |
| C(6) — C(7) | 1.61 | | |

C(1) — chlorine: 1.79

These values vary substantially about the mean C - C distance of $1.56_6$ Å and the structure determination is not claimed as being complete. In order to completely define the structure in terms of accurate bond lengths, the hydrogen atoms would require to be considered before refinement by $(F_o - F_c)$ synthesis. The large amount of purely routine work necessary to take the structure to the present stage (40 two-dimensional Fourier syntheses) precluded further refinement in the present Ph. D. course.

Fig. (4:12) is a diagram of the molecule of β -caryophyllene chloride deduced from the x-ray evidence. The work of Barton (58) has shown that halogenation of the parent β -caryophyllene alcohol takes place without change of structure or stereochemical configuration so that Fig. (4:12) represents essentially the molecule of the alcohol.

The x-ray work therefore confirms the structure of β -caryophyllene alcohol and thereby precludes all but the 9-membered form of β -caryophyllene



β - caryophyllene β - caryophyllene alcohol.

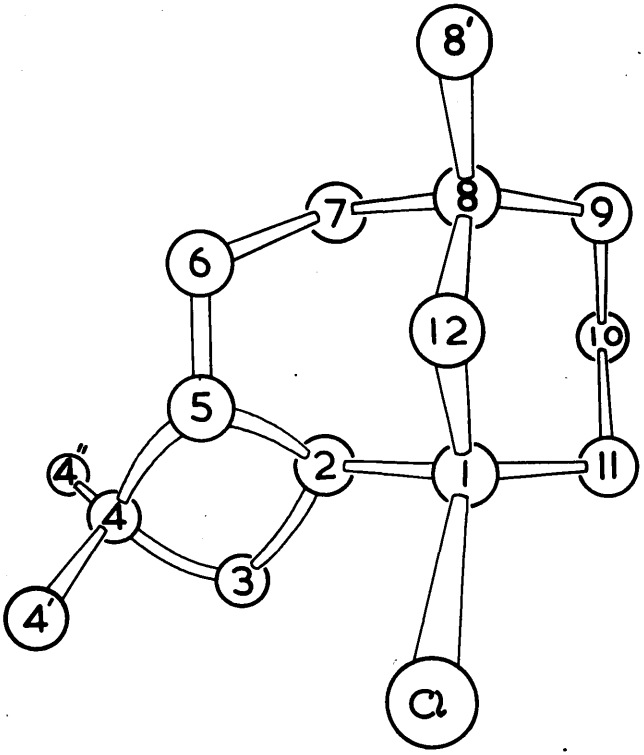


Fig.(4:12)

Perspective drawing of the β-caryophyllene chloride molecule as deduced from the (001) and (010) projections of the unit cell of the crystal.

The mechanism of this reaction is discussed by Barton in his summorising article (loc.cit). In this same article Barton discusses some stereochemical aspects of the molecule and concludes that the cyclo-butane ring is fused cis - to the larger ring. The x-ray determination of the structure of the chloride shows that in this compound the four membered ring is fused trans - to the larger ring. Reference to Fig. (4:12) shows that in addition, the hexane nucleus in the molecule is the trans- form whereas the seven membered ring is boat-shaped with atoms 6 and 5 cis - to atom 12.

## TABLE (4:10)

Measured and calculated values of structure factors for $\beta$ - caryophyllene chloride. The (h o o) and (o k o) axial values have signs related to the (h k o) zone origin. (o o l) values have signs related to the (h o l) zone origin. The others are referred to the zone in which they occur.

| h k l | 2sinθ | F. Meas. | F. Calc. | h k l | 2sinθ | F Meas. | F calc. |
|---|---|---|---|---|---|---|---|
| 2 0 0 | 0.19 | 87 | $\overline{86}$ | 1 4 0 | 0.59 | 4 | $\overline{8}$ |
| 4 | 0.38 | 73 | $\overline{76}$ | 5 | 0.74 | 11 | $\overline{14}$ |
| 6 | 0.56 | 56 | 57 | 6 | 0.88 | 45 | $\overline{45}$ |
| 8 | 0.75 | 25 | $\overline{22}$ | 7 | 1.03 | 4 | $\overline{7}$ |
| 10 | 0.94 | 11 | 10 | 8 | 1.17 | 11 | 10 |
| 12 | 1.13 | 6 | 0 | 9 | 1.31 | 13 | $\overline{11}$ |
| 14 | 1.31 | 12 | $\overline{16}$ | 10 | 1.46 | 5 | $\overline{6}$ |
| 16 | 1.50 | 5 | 4 | | | | |
| 18 | 1.69 | 7 | $\overline{8}$ | 2 1 0 | 0.24 | 2 | 4 |
| 20 | 1.88 | 3 | 1 | 2 | 0.34 | 29 | $\overline{28}$ |
| | | | | 3 | 0.47 | 58 | $\overline{61}$ |
| 0 2 0 | 0.29 | 105† | 123 | 4 | 0.61 | 40 | $\overline{37}$ |
| 4 | 0.58 | 9 | 18 | 5 | 0.75 | 9 | $\overline{5}$ |
| 6 | 0.88 | 15 | $\overline{10}$ | 6 | 0.89 | 4 | 4 |
| 8 | 1.17 | 21 | $\overline{18}$ | 7 | 1.04 | 14 | $\overline{7}$ |
| | | | | 8 | 1.18 | 7 | 6 |
| 0 0 2 | 0.37 | 12 | 14 | 9 | 1.32 | 13 | $\overline{17}$ |
| 4 | 0.74 | 31 | 30 | 10 | 1.47 | 6 | $\overline{8}$ |
| 6 | 1.12 | 11 | 11 | | | | |
| | | | | 3 1 0 | 0.32 | 64 | 62 |
| 1 1 0 | 0.18 | 9 | $\overline{10}$ | 2 | 0.40 | 24 | 22 |
| 2 | 0.30 | 62 | $\overline{62}$ | 3 | 0.52 | 63 | $\overline{49}$ |
| 3 | 0.44 | 16 | 7 | | | | |

| h k l | 2sinθ | F. Meas. | F calc. | h k l | 2sinθ | F. Meas. | F. calc. |
|---|---|---|---|---|---|---|---|
| 3 4 0 | 0.65 | 18 | $\overline{14}$ | 5 3 0 | 0.64 | 18 | 24 |
| 5 | 0.78 | 21 | 27 | 4 | 0.75 | 35 | 34 |
| 6 | 0.92 | 4 | $\overline{7}$ | 5 | 0.87 | 18 | $\overline{15}$ |
| 7 | 1.06 | 10 | $\overline{7}$ | 6 | 0.99 | 11 | 14 |
| 8 | 1.20 | 6 | $\overline{12}$ | 7 | 1.13 | 31 | 33 |
| 9 | 1.34 | 10 | 13 | 8 | 1.26 | 19 | $\overline{23}$ |
| 10 | 1.48 | 6 | $\overline{3}$ | 9 | 1.39 | 5 | $\overline{2}$ |
| 11 | 1.63 | 5 | 2 | 10 | 1.53 | 7 | $\overline{6}$ |
| 12 | 1.77 | 4 | 5 | 11 | 1.67 | 5 | 2 |
| 4 1 0 | 0.40 | 56 | $\overline{57}$ | 12 | 1.81 | 4 | $\overline{2}$ |
| 2 | 0.47 | 47 | 45 | 13 | 1.95 | 4 | 0 |
| 3 | 0.57 | 51 | 39 | 6 1 0 | 0.58 | 39 | 37 |
| 4 | 0.69 | 3 | $\overline{2}$ | 2 | 0.63 | 10 | $\overline{16}$ |
| 5 | 0.82 | 12 | 17 | 3 | 0.71 | 3 | 4 |
| 6 | 0.95 | 4 | $\overline{1}$ | 4 | 0.81 | 9 | 9 |
| 7 | 1.09 | 7 | 4 | 5 | 0.92 | 7 | 7 |
| 8 | 1.23 | 5 | $\overline{10}$ | 6 | 1.04 | 8 | 7 |
| 9 | 1.36 | 11 | 14 | 7 | 1.16 | 4 | $\overline{5}$ |
| 10 | 1.50 | 5 | $\overline{1}$ | 8 | 1.30 | 5 | $\overline{2}$ |
| 11 | 1.65 | 4 | $\overline{6}$ | 9 | 1.43 | 9 | $\overline{11}$ |
| 5 1 0 | 0.49 | 36 | 31 | 10 | 1.56 | 9 | 10 |
| 2 | 0.55 | 54 | 45 | 7 1 0 | 0.67 | 22 | $\overline{26}$ |

| h k l | 2sinθ | F Meas. | F calc. | h k l | 2sinθ | F Meas. | F calc. |
|---|---|---|---|---|---|---|---|
| 7 2 0 | 0.72 | 59 | $\overline{58}$ | 9 5 0 | 1.11 | 20 | 22 |
| 3 | 0.79 | 28 | $\overline{30}$ | 6 | 1.21 | 29 | 30 |
| 4 | 0.88 | 18 | 15 | 7 | 1.32 | 8 | 5 |
| 5 | 0.98 | 4 | $\overline{10}$ | 8 | 1.44 | 5 | $\overline{4}$ |
| 6 | 1.09 | 8 | $\overline{10}$ | 9 | 1.55 | 9 | 8 |
| 7 | 1.21 | 22 | $\overline{23}$ | 10,1 0 | 0.95 | 24 | $\overline{26}$ |
| 8 | 1.34 | 9 | 14 | 2 | 0.98 | 20 | $\overline{16}$ |
| 8 1 0 | 0.76 | 4 | 1 | 3 | 1.03 | 4 | 10 |
| 2 | 0.80 | 25 | 26 | 4 | 1.10 | 5 | 8 |
| 3 | 0.87 | 15 | 13 | 5 | 1.19 | 13 | 6 |
| 4 | 0.95 | 16 | $\overline{7}$ | 6 | 1.28 | 5 | 0 |
| 5 | 1.05 | 5 | 4 | 7 | 1.38 | 5 | $\overline{7}$ |
| 6 | 1.15 | 11 | 13 | 11,1 0 | 1.04 | 4 | $\overline{1}$ |
| 7 | 1.27 | 7 | $\overline{11}$ | 2 | 1.07 | 5 | $\overline{2}$ |
| 8 | 1.39 | 10 | 12 | 3 | 1.12 | 16 | 19 |
| 9 | 1.51 | 5 | $\overline{3}$ | 4 | 1.18 | 5 | 2 |
| 10 | 1.64 | 11 | $\overline{6}$ | 5 | 1.26 | 8 | $\overline{7}$ |
| 11 | 1.77 | 6 | 3 | 6 | 1.35 | 17 | $\overline{18}$ |
| 9 1 0 | 0.86 | 10 | $\overline{13}$ | 7 | 1.45 | 12 | 15 |
| 2 | 0.89 | 23 | 23 | 8 | 1.55 | 5 | $\overline{6}$ |
| 3 | 0.95 | 4 | $\overline{3}$ | 9 | 1.66 | 7 | $\overline{6}$ |
| 4 | 1.02 | 8 | 7 | | | | |

| h k l | 2sinθ | F meas. | F calc. | h k l | 2sinθ | F meas. | F calc. |
|---|---|---|---|---|---|---|---|
| 12, 1 0 | 1.13 | 12 | 14 | 15, 3 0 | 1.47 | 5 | 4 |
| 2 | 1.16 | 5 | $\overline{2}$ | 4 | 1.53 | 7 | $\overline{8}$ |
| 3 | 1.20 | 10 | $\overline{8}$ | 5 | 1.59 | 4 | $\overline{4}$ |
| 4 | 1.26 | 7 | $\overline{5}$ | 6 | 1.65 | 6 | 9 |
| 5 | 1.34 | 13 | 15 | 7 | 1.73 | 4 | 1 |
| 6 | 1.42 | 8 | $\overline{9}$ | 8 | 1.82 | 5 | $\overline{5}$ |
| 7 | 1.51 | 9 | 9 | 9 | 1.91 | 4 | 1 |
| 13, 1 0 | 1.23 | 5 | 3 | 16,1 0 | 1.51 | 10 | $\overline{11}$ |
| 2 | 1.25 | 13 | $\overline{13}$ | 2 | 1.54 | 10 | $\overline{11}$ |
| 3 | 1.29 | 11 | $\overline{9}$ | 3 | 1.56 | 5 | $\overline{2}$ |
| 4 | 1.35 | 5 | 4 | 4 | 1.61 | 5 | 5 |
| 5 | 1.42 | 5 | 10 | 5 | 1.67 | 5 | $\overline{2}$ |
| 6 | 1.50 | 5 | $\overline{4}$ | 6 | 1.74 | 4 | $\overline{1}$ |
| 7 | 1.58 | 11 | $\overline{10}$ | 7 | 1.81 | 6 | $\overline{4}$ |
| 14, 1 0 | 1.33 | 14 | $\overline{15}$ | 17,1 0 | 1.59 | 5 | $\overline{5}$ |
| 2 | 1.35 | 10 | 11 | 2 | 1.61 | 5 | $\overline{3}$ |
| 3 | 1.39 | 6 | $\overline{5}$ | 3 | 1.64 | 5 | 3 |
| 4 | 1.44 | 7 | $\overline{4}$ | 18,1 0 | 1.69 | 5 | 4 |
| 5 | 1.50 | 13 | $\overline{10}$ | 2 | 1.71 | 5 | 3 |
| 6 | 1.58 | 5 | 7 | 3 | 1.74 | 4 | $\overline{5}$ |
| 15, 1 0 | 1.41 | 5 | $\overline{4}$ | 20,1 0 | 1.88 | 3 | 3 |
| 2 | 1.43 | 5 | $\overline{4}$ | 2 | 1.89 | 5 | 3 |

| h k l | 2sinθ | F meas. | F calc. | h k l | 2sinθ | F meas. | F calc. |
|---|---|---|---|---|---|---|---|
| 1 0 1 | 0.20 | $\overset{*}{-}$ | 9 | 3 0 6 | 1.14 | 8 | $\overline{12}$ |
| 2 | 0.38 | 8 | $\overline{5}$ | 7 | 1.33 | 6 | 5 |
| 3 | 0.56 | 4 | $\overline{6}$ | 8 | 1.51 | 6 | 6 |
| 4 | 0.74 | 4 | $\overline{6}$ | 9 | 1.69 | 7 | $\overline{4}$ |
| 5 | 0.93 | 6 | 3 | 4 0 1 | 0.42 | 47 | $\overline{49}$ |
| 6 | 1.11 | 6 | $\overline{2}$ | 2 | 0.53 | 10 | $\overline{7}$ |
| 7 | 1.30 | 19 | $\overline{18}$ | 3 | 0.67 | 4 | $\overline{4}$ |
| 2 0 1 | 0.26 | 3 | $\overline{5}$ | 4 | 0.83 | 24 | 27 |
| 2 | 0.42 | 79 | 79 | 5 | 1.00 | 31 | $\overline{27}$ |
| 3 | 0.59 | 46 | $\overline{40}$ | 6 | 1.17 | 13 | 14 |
| 4 | 0.76 | 13 | 10 | 7 | 1.35 | 6 | $\overline{12}$ |
| 5 | 0.95 | 9 | $\overline{7}$ | 8 | 1.54 | 6 | 1 |
| 6 | 1.13 | 29 | 27 | 9 | 1.71 | 7 | $\overline{10}$ |
| 7 | 1.31 | 12 | $\overline{13}$ | 5 0 1 | 0.51 | 24 | $\overline{21}$ |
| 8 | 1.49 | 6 | $\overline{4}$ | 2 | 0.60 | 17 | 15 |
| 9 | 1.68 | 5 | $\overline{8}$ | 3 | 0.73 | 33 | $\overline{28}$ |
| 3 0 1 | 0.34 | 16 | 15 | 4 | 0.88 | 17 | $\overline{19}$ |
| 2 | 0.47 | 31 | $\overline{30}$ | 5 | 1.04 | 11 | 7 |
| 3 | 0.62 | 24 | $\overline{23}$ | 6 | 1.20 | 10 | $\overline{11}$ |
| 4 | 0.79 | 5 | $\overline{5}$ | 7 | 1.38 | 6 | 6 |
| 5 | 0.97 | 10 | $\overline{12}$ | 8 | 1.56 | 8 | $\overline{13}$ |

| h k l | 2sinθ | F meas. | F calc. | h k l | 2sinθ | F meas. | F calc. |
|---|---|---|---|---|---|---|---|
| 5 0 9 | 1.73 | 7 | $\overline{4}$ | 8 0 5 | 1.20 | 6 | $\overline{2}$ |
| 6 0 1 | 0.59 | 33 | 39 | 6 | 1.34 | 13 | $\overline{15}$ |
| 2 | 0.68 | 31 | $\overline{29}$ | 9 0 1 | 0.86 | 18 | $\overline{16}$ |
| 3 | 0.79 | 40 | $\overline{34}$ | 2 | 0.92 | 5 | 4 |
| 4 | 0.93 | 5 | $\overline{6}$ | 3 | 1.01 | 10 | $\overline{14}$ |
| 5 | 1.09 | 6 | $\overline{2}$ | 4 | 1.12 | 8 | $\overline{7}$ |
| 6 | 1.25 | 11 | $\overline{11}$ | 5 | 1.26 | 15 | $\overline{16}$ |
| 7 | 1.41 | 6 | $\overline{7}$ | 6 | 1.40 | 6 | 10 |
| 8 | 1.59 | 8 | 9 | 7 | 1.55 | 6 | $\overline{5}$ |
| 7 0 1 | 0.69 | 30 | $\overline{35}$ | 8 | 1.71 | 6 | 2 |
| 2 | 0.76 | 5 | $\overline{1}$ | 9 | 1.87 | 6 | 5 |
| 3 | 0.86 | 23 | $\overline{22}$ | 10 ,0 1 | 0.96 | 9 | 11 |
| 4 | 0.99 | 23 | $\overline{16}$ | 2 | 1.02 | 5 | $\overline{4}$ |
| 5 | 1.14 | 9 | 9 | 3 | 1.09 | 11 | 11 |
| 6 | 1.29 | 7 | $\overline{8}$ | 11 ,0 1 | 1.05 | 16 | $\overline{16}$ |
| 7 | 1.45 | 6 | $\overline{5}$ | 2 | 1.10 | 10 | 13 |
| 8 | 1.62 | 9 | $\overline{9}$ | 3 | 1.18 | 6 | 8 |
| 8 0 1 | 0.78 | 5 | 1 | 4 | 1.28 | 6 | 12 |
| 2 | 0.84 | 9 | $\overline{14}$ | 5 | 1.39 | 19 | $\overline{18}$ |
| 3 | 0.94 | 5 | 3 | 6 | 1.52 | 9 | 11 |
| 4 | 1.06 | 19 | $\overline{16}$ | 7 | 1.66 | 6 | 1 |
|  |  |  |  | 8 | 1.81 | 12 | 12 |

| h k l | 2sinθ | F meas. | F. calc. |
|---|---|---|---|
| 12,0 1 | 1.15 | 5 | $\overline{7}$ |
| 2 | 1.19 | 7 | 10 |
| 3 | 1.26 | 8 | 10 |
| 4 | 1.35 | 6 | 0 |
| 5 | 1.47 | 6 | 5 |
| 6 | 1.58 | 6 | 6 |
| 7 | 1.72 | 7 | 8 |
| 13,0 1 | 1.24 | 14 | $\overline{10}$ |
| 2 | 1.28 | 20 | 23 |
| 3 | 1.35 | 8 | 10 |
| 4 | 1.43 | 6 | 5 |
| 5 | 1.54 | 6 | 5 |
| 6 | 1.66 | 10 | 10 |
| 14,0 1 | 1.34 | 11 | 10 |
| 15,0 1 | 1.43 | 6 | 6 |
| 2 | 1.46 | 12 | 9 |
| 16,0 1 | 1.52 | 6 | 5 |
| 2 | 1.56 | 12 | 14 |
| 17,0 1 | 1.62 | 13 | 12 |
| 2 | 1.65 | 11 | $\overline{10}$ |
| 3 | 1.70 | 6 | $\overline{3}$ |
| 4 | 1.77 | 5 | $\overline{1}$ |
| 17,0 5 | 1.86 | 4 | 0 |
| 6 | 1.96 | 2 | $\overline{4}$ |
| 18,0 1 | 1.71 | 6 | $\overline{4}$ |
| 2 | 1.74 | 5 | 6 |
| 19,0 1 | 1.80 | 4 | 2 |
| 2 | 1.83 | 8 | $\overline{5}$ |
| 20,0 1 | 1.90 | 3 | $\overline{2}$ |
| 2 | 1.92 | 3 | 2 |
| 0 1 1 | 0.24 | 26 | 32 |
| 2 | 0.40 | 41 | $\overline{38}$ |
| 3 | 0.58 | 15 | $\overline{18}$ |
| 5 | 0.94 | 13 | 15 |
| 6 | 1.13 | 9 | $\overline{5}$ |
| 7 | 1.31 | 18 | $\overline{22}$ |
| 8 | 1.50 | 10 | $\overline{10}$ |
| 0 2 1 | 0.34 | 29 | $\overline{43}$ |
| 2 | 0.47 | 5 | $\overline{13}$ |
| 3 | 0.63 | 34 | 21 |
| 4 | 0.80 | 10 | 16 |
| 6 | 1.15 | 8 | 0 |
| 10 | 1.89 | 5 | 5 |

| h k l | 2sinθ | F meas. | F calc. | h k l | 2sinθ | F meas. | F calc. |
|---|---|---|---|---|---|---|---|
| 0 3 1 | 0.47 | 5 | $\overline{11}$ | 0 6 2 | 0.95 | 11 | 6 |
| 2 | 0.58 | 25 | 24 | 4 | 1.15 | 17 | $\overline{6}$ |
| 3 | 0.71 | 33 | $\overline{19}$ | 5 | 1.28 | 21 | $\overline{22}$ |
| 4 | 0.86 | 16 | 17 | 9 | 1.89 | 7 | 5 |
| 5 | 1.03 | 21 | 24 | 0 7 1 | 1.04 | 8 | $\overline{9}$ |
| 6 | 1.20 | 20 | $\overline{26}$ | 2 | 1.09 | 13 | $\overline{6}$ |
| 7 | 1.38 | 11 | 5 | 4 | 1.26 | 21 | 23 |
| 8 | 1.56 | 8 | 9 | 6 | 1.51 | 11 | $\overline{6}$ |
| 10 | 1.92 | 6 | $\overline{6}$ | 8 | 1.81 | 7 | 9 |
| 0 4 1 | 0.61 | 24 | $\overline{23}$ | 0 8 1 | 1.18 | 14 | $\overline{22}$ |
| 2 | 0.69 | 44 | $\overline{53}$ | 3 | 1.30 | 14 | 17 |
| 3 | 0.81 | 28 | 21 | 4 | 1.38 | 10 | $\overline{10}$ |
| 4 | 0.95 | 10 | 10 | 5 | 1.49 | 7 | 4 |
| 7 | 1.43 | 7 | 5 | 8 | 1.90 | 4 | $\overline{1}$ |
| 8 | 1.60 | 6 | $\overline{2}$ | 0 9 2 | 1.36 | 10 | 11 |
| 0 5 1 | 9.75 | 17 | 13 | 4 | 1.51 | 7 | 7 |
| 2 | 0.82 | 29 | $\overline{44}$ | 7 | 1.85 | 6 | 4 |
| 5 | 1.18 | 7 | 5 | 0,10,2 | 1.50 | 10 | 8 |
| 6 | 1.34 | 8 | $\overline{9}$ | 6 | 1.84 | 7 | 4 |
| 7 | 1.80 | 12 | $\overline{8}$ | 0,11,2 | 1.65 | 12 | $\overline{8}$ |
| 8 | 1.66 | 6 | 4 | 5 | 1.85 | 4 | $\overline{4}$ |
| 0 6 1 | 0.90 | 26 | $\overline{26}$ | 0,12,2 | 1.79 | 7 | 8 |

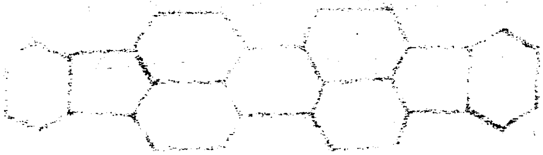
† F calc. used in Fourier synthesis.

* Intensity difficult to estimate because of spot shape.

[illegible]

[illegible] This [illegible] was prepared by [illegible] in an attempt [illegible] synthesise [illegible], and was [illegible] for x-ray study on the [illegible] compound. The substance [illegible]

[illegible]terylene (not so far synthesised).



periflanthene

[illegible]ts at cry[illegible]

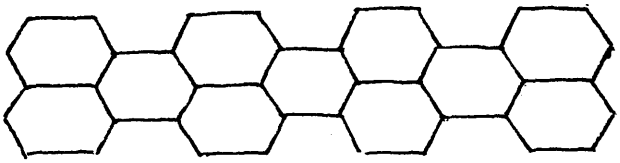
[illegible]pts to recrystallise from benzene, trichlorbenzene [illegible] results afforded no better crystals than the microscopic specimens received from E. Clar.

[illegible]imation was accordingly attempted, and since the

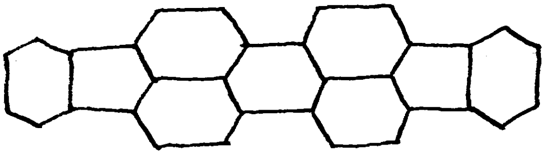
## APPENDIX.

Two additional compounds have been given preliminary examination with a view to x-ray study.

a) Periflanthene ( 82 ) This substance was prepared by E.Clar in an attempt to synthesise quaterylene, and was submitted for x-ray study as the latter compound. The substance was later shown to be periflanthene.



quaterylene (not so far synthesised).



periflanthene

Attempts at crystallisation.

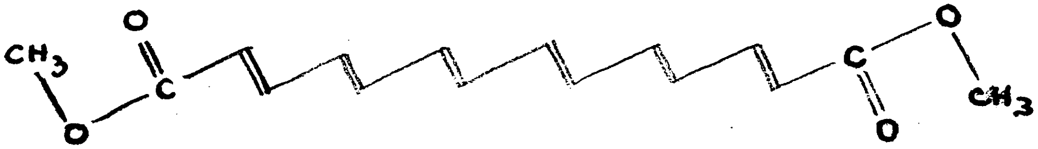
Attempts to recrystallise from benzene, trichlorbenzene and ethyl acetate afforded no better crystals than the microscopic speciments received from E. Clar.

Sublimation was accordingly attempted, and since the compound rapidly oxidises if heated in air, the following technique was adopted.

The substance was placed in a porcelain boat in a tube surrounded by an electric heating coil which maintained a uniform heating gradient. This could be varied quantitatively at will. All air was then evacuated from the apparatus and a dilute stream of carbon dioxide was allowed to flow over the boat at pressures of $\sim 10^{-3}$ mms. mercury. In this way controlled sublimation was achieved but no satisfactory crystals were obtained.

A few very small crystals (the largest available) were set up for rotation photography but no reflections could be recorded. The substance was passed on for electron microscope examination.

b) <u>Dimethyl corticrocin</u>. Corticrocin ( 83 ) is a compound of biological importance and crystals of its dimethyl ester were obtained from H. Erdtman, Royal Institute of Technology, Stockholm, Sweden



dimethyl corticrocin.

The substance has a melting point of 230-232° and a density of 1.228 g. /cc.

X-ray examination.

Dimethyl corticrocin crystallises from chloroform in needle shaped orange crystals. The length of the needle axis was determined from rotation photographs as 6.07Å, but initial Weissenberg moving film photographs indicated false symmetry because of crystal twinning and caused wrong indexing of the reflections.

Larger crystals were later obtained by very slow crystallisation from chloroform. These were found to be untwinned and showed the crystals to be triclinic. A second axial length of 16.6Å was further obtained by rotation photography.

The crystal analysis is being continued by A. H. McCallum in this department.

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