evident: the positive charge for each oxygen atom is generally slightly higher than 2, but for three oxygen atoms is much lower: 1·6 for O(3) and O(10), 1·2 for O(4). This last case is represented by the oxygen not bonded with silicon and with a possible covalent bond with titanium. Generally it is rather difficult to verify the electrostatic equilibrium in a crystal containing titanium. It is very probable that the whole electrostatic equilibrium is affected by the distortion of the TiO_6 octahedron. It is a matter of fact that considering the bonds as purely ionic, potassium and, in part, also the other alkaline ions would be in contact with oxygen atoms which are apparently neutral because they are shared by two SiO_4 tetrahedra.

However, conclusive statements will be possible only after a refinement of the non-centrosymmetrical structure, by using a greater amount of experimental data.

We wish to thank Prof. F. Sgarlata, who kindly made available to us his program for three-dimensional refinement.

References

AHMED, F. R. & CRUICKSHANK, D. W. J. (1953). *Acta Cryst.* **6**, 385.
BELOV, N. V. (1963). *Acta Cryst.* **16**, A7.
BERRY, L. G. (1963). *Canad. Min.* **7**, 679.
BRADLEY, W. M. (1909). *Amer. J. Sci.* **28**, 15.
CANNILLO, E., MAZZI, F. & ROSSI, G. (1965). *Rendiconti della S. M. I.* **21**, 348.
CRUICKSHANK, D. W. J. (1949). *Acta Cryst.* **2**, 65, 154.
DARLOW, S. F. (1960). *Acta Cryst.* **13**, 683.
FERSMAN, A. E. (1926). *Amer. Min.* **11**, 289.
FLINK, I. G. (1895). *Neues Jahrb. Min. Geol. Pal.* Band 1, 452 (abstract).
FORD, W. E. (1909). *Amer. J. Sci.* **27**, 235.
GÖSSNER, B. (1925). *Z. Min. Geol. Pal.* Abt. A, 73.
GÖSSNER, B. & MUSSGNUG, F. (1928). *Z. Min. Geol. Pal.* Abt. A, 274.
HEINRICH, E. W. M. & QUON, S. H. (1963). *Canad. Min.* **7**, 650.
JONA, F. & SHIRANE, G. (1962). *Ferroelectric Crystals*, pp. 108, 235. Oxford: Pergamon Press.
LOUDERBACK, G. D. (1910). *Neues Jahrb. Min. Geol. Pal.* Band 2, 15 (abstract).
MOORE, F. H. (1963). *Acta Cryst.* **16**, 1169.
NOCKOLDS, S. R. (1950). *Miner. Mag.* **29**, 27.
PEACOR, D. R. & BUERGER, M. J. (1962). *Amer. Min.* **47**, 539.
SAKURAI, T. (1962). *Acta Cryst.* **15**, 443.
SAYRE, D. (1952). *Acta Cryst.* **5**, 60.
SJÖSTRÖM, D. A. (1895). *Neues Jahrb. Min. Geol. Pal.* Band 1, 457 (abstract).
STRUNZ, U. (1957). *Mineralogische Tabellen*, p. 286. Leipzig: Akad. Verlag Ges. Geest & Portig K.-G.
WOOLFSON, M. M. (1961). *Direct Methods in Crystallography*, Chap. 3. Oxford: Clarendon Press.
ZACHARIASEN, W. (1930). *Z. Kristallogr.* **73**, 7.

Acta Cryst. (1966). **21**, 208

A Discussion of the Distribution of Bonded Electron Density

BY A. M. O'CONNELL*, A. I. M. RAE† AND E. N. MASLEN

Department of Physics, University of Western Australia, Nedlands, Western Australia

(*Received* 14 *December* 1965)

The final difference syntheses on a number of previously published structures are examined for evidence of bonding electrons. It is found that the aromatic C–C bond contains a residual peak of maximum height about 0·2 e.Å^{-3} with half height extensions of about 0·3 Å in, and 0·75 Å perpendicular to, the trigonal plane. In some cases direct evidence is also obtained for charge movements in the molecule. The experimental results are compared with those calculated from Slater wave functions. The agreement is generally good, but discrepancies remain which can be attributed to the neglect of σ orbital contraction and electron correlation in the theoretical calculations. It is found that refinement of structures by use of isolated atom wave functions leads to significant errors in the parameters, not only of terminal atoms, but also of trigonally bonded atoms with hydrogen substituents.

1. Introduction

The structure analysis of β-sulphanilamide (O'Connell & Maslen, 1966) has provided detailed information on the electron density distribution in the C–C, C–N and C–S bonds in this molecule. The difference synthesis shows peaks in the bonds which reach maxima near the bond centres and which extend a considerable distance above and below the plane of the molecule. The existence of such residual features in difference maps has been known for some time and detailed projection studies were carried out by Cochran (1953, 1956) and Mason (1960). However, since the advent of highly accurate three-dimensional structure analysis, remarkably little attention has been paid to features in the residual electron density maps. In fact, an extensive

---

* Present address: Institute of Medical Biochemistry, University of Göteborg, Göteborg SV, Sweden.

† Present address: Department of Chemistry, The University, Sheffield 10, England.

search of the recent literature revealed very few cases where any part of the final difference synthesis had been published; and what results were available were almost entirely confined to sections through the molecular planes of aromatic compounds.

In view of the considerable value of information on residual electron densities in the study of molecular wave functions, a number of published structures were selected for detailed examination. The present study was confined to structures containing trigonally bonded carbon atoms which were chosen on the basis of their being light atom centrosymmetric structures with final R values generally less than 6%, so that effects of phase and data errors would be minimized as much as possible. The structures studied were triaminotrinitrobenzene (Cady & Larsen, 1965), 2-amino-3-methylbenzoic acid (Brown & Marsh, 1963), salicylic acid (Sundaralingam & Jensen, 1965), *p*-nitroaniline (Trueblood, Goldish & Donohue, 1961) and ammonium oxamate (Beagley & Small, 1963). These are referred to below as TATNB, AMBA, SA, NA and AMOX respectively. Their molecular formulae are shown in Fig. 1 and details of the methods for data collection and refinement and the accuracy of the structures are summarized in Table 1. The analysis of NA is much less accurate than that of the others and was chosen primarily because of its formal similarity to sulphanilamide. Unfortunately, what are probably the two most accurate structure analyses performed to date – those of cyanuric acid (Verschoor, 1964) and diphenyl (Robertson, 1961) – have been published as only preliminary communications and the intensity data are not available.

2. Experimental results

TATNB

The difference synthesis evaluated near the molecular plane is shown in Cady & Larsen (1965). Figs. 2 and 3 show the synthesis evaluated in sections which are perpendicular to the molecular plane and pass through the eighteen bonds that do not include hydrogen atoms (sections of this type are referred to as 'perpendicular planes' below). The symmetry of the molecule is $P\bar{6}2m$, which implies six chemically equivalent regions of the molecular plane. The result of averaging these regions is shown in Fig. 4(*a*). Similarly, Fig. 5(*b*) is the average of the twelve equivalent sectors of the C–C perpendicular planes while the sixfold averages for the C–N bonds and the twelvefold for the N–O bonds are included in Fig. 3. This averaging procedure reduces the standard deviation from 0·07 e.Å^{-3} in the original synthesis to 0·028 e.Å^{-3} in the molecular plane and the C–N perpendicular plane, and 0·020 in the C–C and N–O perpendicular planes.

The maximum electron density in the C–C bond has the value 0·22 e.Å^{-3} at a point near the centre of the bond. The contour at 0·10 e.Å^{-3} extends 0·3 Å from the bond in the molecular plane and 0·75 Å in the perpendicular plane. The shape and magnitude of the C–N(O_2) bond peak is like that in the C–C bond, while there is less density in the C–N(H_2) bond and very little in the N–O bonds. The last effect can be explained in terms of shifts of terminal atoms from their true positions discussed by Dawson (1964*a*, 1965). A terminal atom has only one bond and the least-squares refinement shifts such an atom towards the

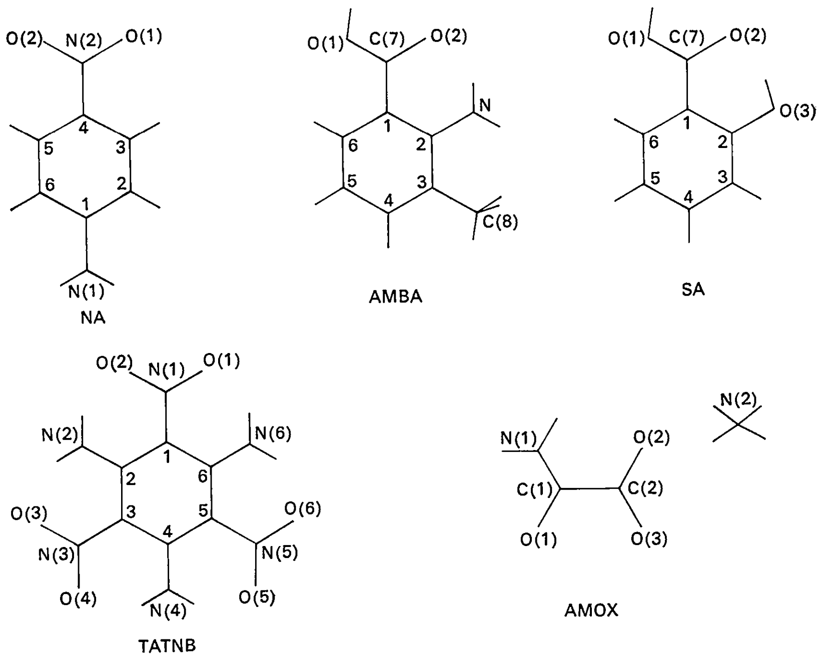


Fig. 1. The molecular formulae of the compounds studied.

Table 1. *Details of the molecules studied*

Compound	Space-group	Method of data collection	Maximum $\sin\theta/\lambda$	Number of reflexions		Method of refinement	Final R index	$\sigma(\varrho)$ (e.Å^{-3})	Amount of final difference synthesis published	Remarks
				Possible	Measured					
TATNB	$P\bar{1}$	Scintillation counter	0·70	2597	928	Full-matrix least-squares	5·3 %	0·07	Section near molecular plane	
AMBA	$P2_1/c$	Photographic	0·65	1597	1394	Semi-block-diagonal least-squares	5·6	0·06	None	7 terms which appeared to suffer seriously from extinction were omitted from the present calculations.
SA	$P2_1/a$	Photographic	0·65	1452	732	Full-matrix least-squares	5·9	0·05	None	24 terms were omitted from the refinement and the present calculation because of extinction.
NA	$P2_1/n$	Photographic	0·65	1100	826	Block-diagonal least-squares	9·6	0·10	None	
AMOX	$P2_1/n$	Proportional counter	0·65	941	929	Block-diagonal least-squares	4·3	0·08	A composite projection contoured at an arbitrary interval	4 terms were omitted from the present analysis because of extinction. The contribution to the structure factors of a 'half-hydrogen' which had been added to the structure to compensate for bonding electrons was subtracted.

bond peak, thus greatly reducing the height of the residual density. The partial reduction of the C–N(H_2) peak implies that the electron density in the N–H bond is less than that in the C–N bond causing a small shift in the nitrogen which, in turn, somewhat reduces the C–N bond peak. This is analogous to the effect in benzene discussed in § 4.

The major regions of negative electron density lie in positions antisymmetric to the bond peaks, supporting the predictions of Dawson (1965), although the depth of the hollows is greater than would be expected from this work.

The resonance structures of Cady & Larsen (1965, Fig. 5) predict electron deficiencies on both the nitrogen atoms and excesses on the oxygen positions and the nitro-substituted carbon atoms. It is seen from Fig. 4(*a*) that the nitro-substituted carbon atom does indeed lie on a positive region of height 0·035 e.Å^{-3} while N(O_2) lies on a minimum of −0·055 e.Å^{-3}. The lack of any appreciably positive regions at the oxygen positions can probably be attributed to the terminal atom effects discussed above. The absence of a hollow on the amino nitrogen atom may be similarly caused although it should be noted that this atom is charged in only one of the resonance structures whereas N(O_2) is positively charged in all three. One of the two remaining peaks

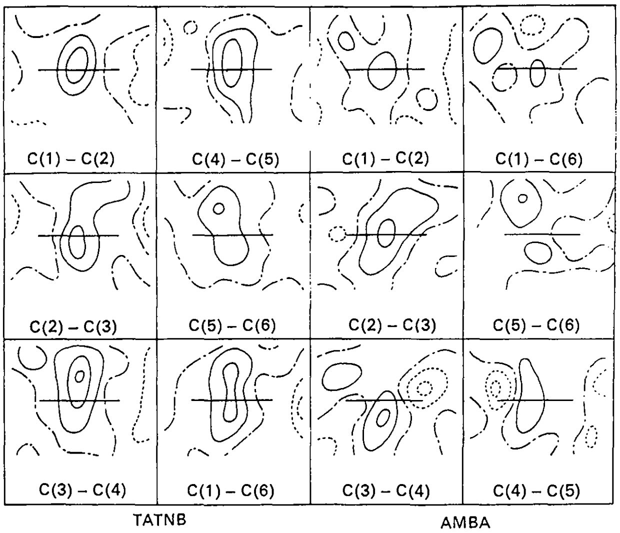


Fig. 2. The residual electron density in the individual C–C perpendicular planes of TATNB and AMBA. Contour interval 0·1 e.Å^{-3}, negative contours broken and zero contours chain-dotted.

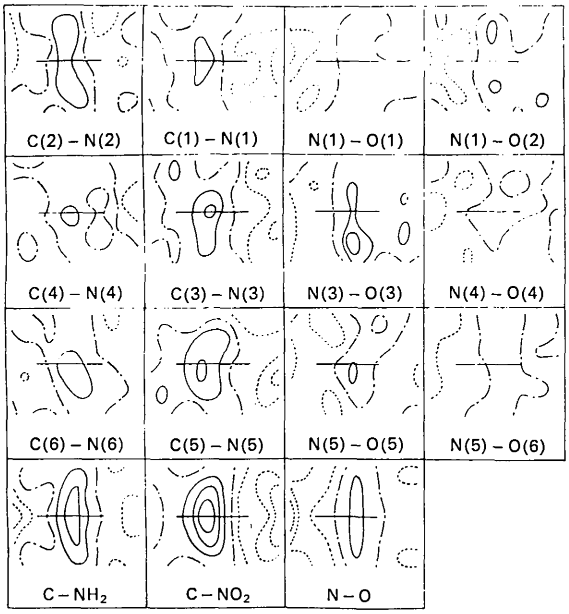


Fig. 3. The individual and average residual electron densities in the C–NH_2, C–NO_2, and N–O perpendicular planes of TATNB. Contour interval 0·10 e.Å^{-3} in the individual and 0·05 e.Å^{-3} in the average densities. Negative contours broken and zero contour chain-dotted.

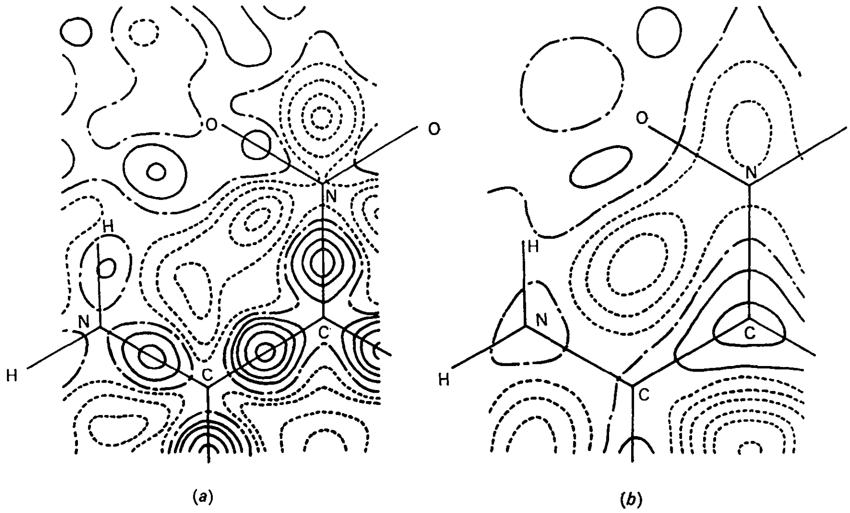


Fig. 4. The averaged density in the molecular plane of TATNB calculated (*a*) with the use of all available data and (*b*) omitting terms with sin $\theta/\lambda < 0·4$. Contour interval 0·05 e.Å^{-3}, negative contours broken, zero contour chain-dotted.

in the synthesis has a maximum height of 0·11 e.Å^{-3} and lies between the oxygen and the amino group, confirming the importance of the resonance form shown in Fig. 5(*c*) of Cady & Larsen (1965). The second (of height 0·10 e.Å^{-3}) lies in the direction of the intermolecular hydrogen bond at a distance of about 1 Å from the oxygen atom. This would appear to result from electron withdrawal along the hydrogen bond in a manner discussed by Verschoor (1964) in the case of cyanuric acid.

Some of these points concerning the details of the electron density have been briefly discussed by Cady & Larsen (1965), but the level of significance has been greatly increased by the process of averaging used in the present study.

AMBA

The difference synthesis was evaluated in the molecular and perpendicular planes (Figs. 6(*b*), 2 and 8). This molecule does not have the high symmetry of TATNB. However, if the effects of different environments on the C–C bonds are considered to be small, all these bonds may be taken as being equivalent and each perpendicular plane as having the symmetry *p*4. This provides 24 sectors to be averaged in obtaining a mean C–C perpendicular plane and the result of this process is shown in Fig. 5(*e*). The nominal standard deviation in the electron density is then 0·013 e.Å^{-3} compared with 0·06 e.Å^{-3} in the original map. This mean bond contains a positive region of electron density of central height 0·14 e.Å^{-3} and the half-height extensions from the bond are 0·20 Å and 0·40 Å in and perpendicular to the molecular plane respectively. The amount of electron density in the bond and the extension out of the plane are both less than those observed in the case of TATNB. An unusual feature of the perpendicular plane density is the presence of positive regions above and below the atoms. This, coupled with the fact that the atoms lie in negative regions of −0·05 e.Å^{-3}, indicates that the refinement may not have been complete and that these features result from residual errors in the scale and temperature factors or from the effects of extinction on the data.

Another noticeable feature in the molecular plane section is the occurrence of peaks of average magnitude 0·1 e.Å^{-3} at or near the positions of the hydrogen atoms. This is almost certainly due to the fact that the hydrogen temperature factors used were those of the associated carbon atoms incremented by 0·5 Å^2. Recent work (Jensen & Sundaralingam, 1965; Mason, Phillips & Robertson, 1965) has shown that, when hydrogen atom temperature factors are refined, they reach values of about 2·5 Å^2 lower than those expected, because of the difference between the scattering curves of isolated and bonded hydrogen.

The perpendicular planes of the bonds outside the benzene ring are shown in Fig. 7. These have the same

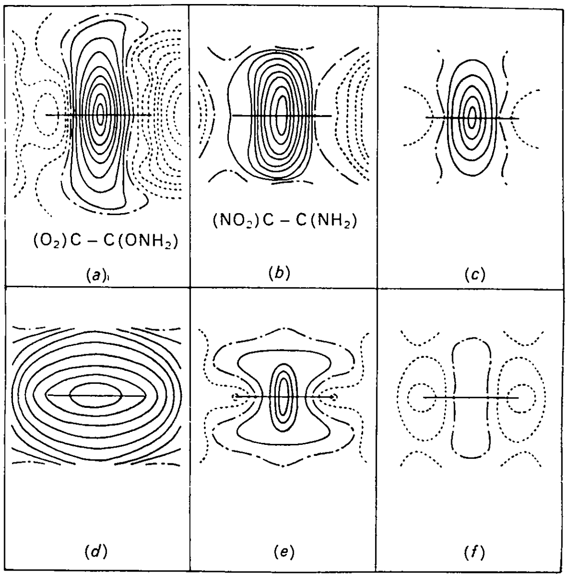


Fig. 5. The average density in the C–C perpendicular planes of (*a*) AMOX, (*b*) TATNB, (*c*) SULPH, (*d*) NA, (*e*) AMBA and (*f*) SA. Contour interval 0·025 e.Å^{-3}, negative contours broken, zero contour chain-dotted.

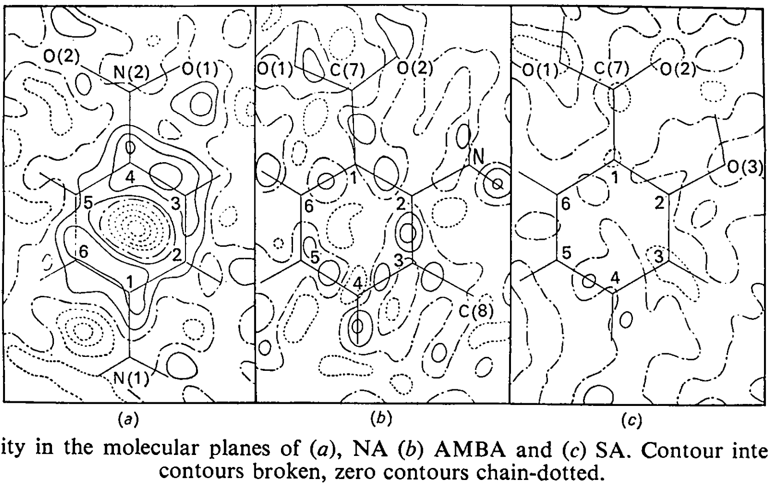


Fig. 6. The residual density in the molecular planes of (*a*), NA (*b*) AMBA and (*c*) SA. Contour interval 0·10 e.Å^{-3}, negative contours broken, zero contours chain-dotted.

general form as expected, but the lack of symmetry does not allow them to be averaged and the high standard deviation precludes any detailed discussion.

SA

The difference synthesis evaluated in the molecular plane is shown in Fig. 6(*c*) while the result of averaging the C–C perpendicular planes in the same way as was used for AMBA is shown in Fig. 5(*f*). The distribution of electron density in this plane is similar in shape to that observed in the other structures, but its height is very much lower (maximum 0·02 e.Å^{-3}). The mean density at the atomic positions is $-0{\cdot}10$ e.Å^{-3}, which indicates that the synthesis may well be in error due to bad refinement or inaccurate data. The latter reason is probably to be preferred because, although the 24 strongest structure factors were omitted from both the original refinement and the present analysis, a large proportion of the remaining terms of high intensity have $|F_o|$ less than $|F_c|$, suggesting that these, too, suffer from extinction.

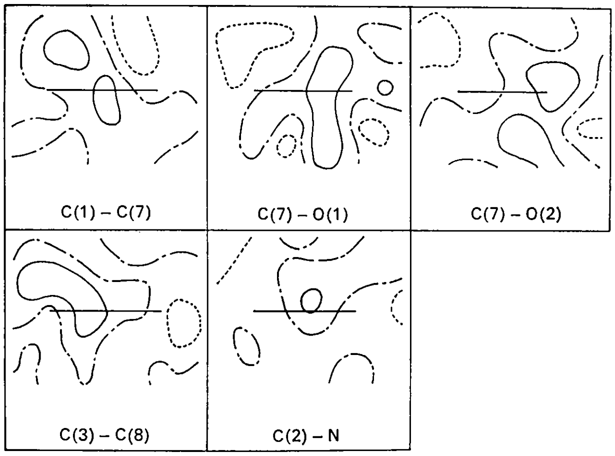


Fig. 7. The residual electron density in the individual C–X perpendicular planes of AMBA. Contour interval 0·1 e.Å^{-3}, negative contours broken, zero contours chain-dotted.

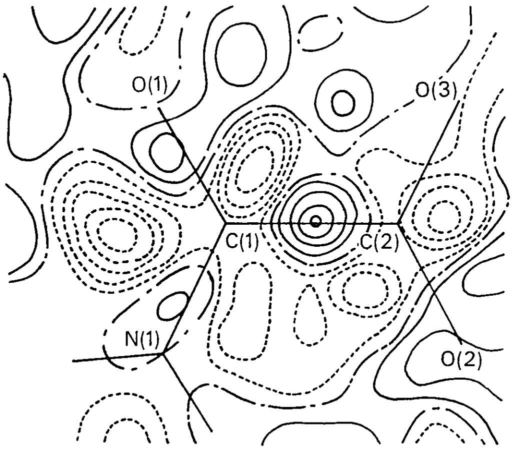


Fig. 8. The residual electron density in the plane of the oxamate ion of AMOX. Contour interval 0·05 e.Å^{-3}, negative contours broken, zero contours chain-dotted.

NA

The sections of the difference synthesis evaluated in the molecular plane and averaged in the C–C perpendicular planes are shown in Figs. 6(*c*) and 5(*d*) respectively. As mentioned above, the accuracy of this structure is considerably lower than that of the others considered and, although the electron density reaches a maximum of 0·21 e.Å^{-3} at the centre of the bond, this effect is almost concealed by the fact that the whole ring lies in a region of positive density which has an average value of 0·175 e.Å^{-3} at the atomic positions. It seems likely that this structure has not been correctly refined, particularly with respect to scale and temperature factor.

AMOX

The sections of the difference synthesis calculated in the plane of the oxamate group and the C–C perpendicular plane are shown in Figs. 8 and 5(*a*) respectively. In the latter section, the two sectors above and below the bond have been averaged. The residual electron density reaches a maximum of 0·21 e.Å^{-3} and extends 0·3 Å in the oxamate plane and 0·75 Å in the perpendicular plane. Both carbon atoms lie in regions of negative electron density of about $-0{\cdot}08$ e.Å^{-3}. This latter result may well arise from extinction because, although the four terms most seriously affected were removed from the analysis, examination of the data revealed a general tendency for $|F_c|$ to be greater than $|F_o|$ over the other large terms.

There are regions of negative density in the oxamate plane which correspond in position on the antisymmetric hollows predicted by Dawson (1965). However, as in the case of TATNB, their magnitude is greater than expected.

The appearance of this difference synthesis is quite different from that of the composite map published by Beagley & Small (1963). Although the shape of the peak in the molecular plane is similar to that in the composite synthesis, a direct comparison is impossible because the latter is drawn on an arbitrary scale. Moreover, Beagley & Small state that this peak is the only significant feature in the synthesis whereas examination of Fig. 8 shows that the antisymmetric hollows are of even greater magnitude than the residual positive region. Furthermore, there are significant peaks close to the oxygen atoms and it is possible that a further study of the three-dimensional synthesis might well lead to a clearer understanding of the ionic character of the molecule. Such an analysis is outside the scope of the present work, but the presence of these features serves to emphasize the need for careful examination of final difference syntheses as well as the dangers of relying on composite maps.

3. Discussion of experimental results

Fig. 5 shows the mean electron density distributions in the perpendicular planes of the C–C bonds for the five compounds studied as well as that for β-sulphanilamide (O'Connell & Maslen, 1966). This last structure is referred to below as SULPH. Allowing for the probable errors due to extinction and incorrect refinement discussed above, each distribution has the same general form. The residual density reaches a maximum of about 0·2 e.Å^{-3} near the centre of the bond and the perpendicular extension is between 0·5 and 0·8 Å, being double that observed in the planes of the trigonal bonds.

The three most accurate structure determinations appear to be those of TATNB, SULPH and AMOX. Of these, SULPH has the highest π bond order, being close to that of benzene, and AMOX has the lowest, having a purely single bond (Beagley & Small, 1963). It is interesting to note, therefore, that the amount of electron density in the bond and the perpendicular extension is greatest for AMOX and lowest for SULPH, which is the reverse of what would be expected if the extension were due to the π electrons. This anomaly is discussed in more detail in § 4.

The two structures (TATNB and AMOX) for which the data were collected by counter techniques yield the most sensible and apparently most accurate electron density distributions. On the other hand, the reliability of the results does not appear to depend strongly on the method used for refinement. The structure of SA was refined by full-matrix least squares but the results obtained seem to be in error owing to incorrect refinement and extinction. This latter effect appears to be a particularly serious barrier to the obtaining of accurate electron densities.

Reference has been made to the processes of averaging used in the above investigations. The validity of this depends on a number of factors. Firstly, the quantities averaged must be formally equivalent and have similar features. That this is generally the case can be seen from examination of the figures showing the individual regions which were averaged. In the cases of TATNB and AMOX there can be little doubt that the procedure was justified. In the other structures, there is the implicit assumption that the effects of bond environment on the electron density are negligible compared with the basic bond-electron configuration. This appears to be a reasonable assumption in general, although the discussion in § 4 shows that caution must be exercised in combining bonds between carbon atoms having hydrogen substituents on one hand and carbon or nitrogen subsituents on the other. In calculating the standard deviations on the averaged maps, the original standard deviation was divided by the square root of the number of regions averaged. It must be emphasized that this measure of accuracy covers only random error and that if there are overall systematic errors in the map due to factors such as extinction or incorrect scale, these will not be eliminated by the averaging procedure, and their effect will not be allowed for in the standard deviations.

4. A theoretical calculation of the electron density in benzene

The results obtained for the residual bond densities are among the most accurate available at present and it therefore seemed worth while to compare these with theoretical predictions. Two methods of approach have been used for such calculations. The first is the derivation of atomic scattering factors from the wave functions of bonded atoms. These are used to calculate structure factors and then a difference map which should, in principle, contain no significant features. Scattering factors of this type have been developed by McWeeny (1951, 1952, 1953, 1954) and Dawson (1964*b*). Rae & Maslen (1965) have made some necessary corrections to McWeeny's results and have applied them to the structure of diphenylbenzene. The alternative approach which has been favoured by March (1952), Cochran (1956) and Mason (1960) has been to examine directly the electron density derived from molecular wave functions and the difference density obtained by subtracting the electron density of an isolated atom model from this. This theoretical difference synthesis is then compared with one obtained experimentally.

As the structures discussed above contain a number of different bonded atom types, the scattering factor approach was considered impracticable. The greater part of the information obtained was related to the residual density in the aromatic carbon–carbon bond. It was therefore thought that a theoretical analysis of the difference density for the benzene molecule would provide a useful model for comparison with experimental results. Two calculations of this type have been carried out (Cochran 1956, Mason 1960), but both are confined to an analysis of a projection onto the molecular plane. It was therefore decided to extend the calculations to the three-dimensional case and, in particular, to examine the perpendicular planes of the bonds.

Although the results of Mason (1960) are based on wave functions which allow for σ-orbital contraction, the final results correspond less well to experimental observations than those of Cochran (1956), which are based on simple Slater wave functions. In particular, the residual density obtained from Mason's calculations consists basically of a ridge of density surrounding each carbon atom rather than a peak in the carbon–carbon bond. This calculation does lead to a hollow within the benzene ring which corresponds to experiment better than Cochran's prediction of zero inner ring density. However, thermal motion has not been allowed for and the low temperature results on cyanuric acid (Verschoor, 1964) combined with the qualitative theoretical approach of Dawson (1965) indicate that, at 0°K, the centre of the ring would contain not one negative region, but six nearly resolved hollows antisymmetric to the C–H bond peaks. A similar indication is obtained from an examination of the mean molecular plane difference synthesis of TATNB [Fig. 4(*a*)]. It was therefore decided to proceed using Slater wave functions and the probable errors arising from this approximation are discussed below.

The procedure of Cochran (1956) was followed except that his accurate formula (1) was used in preference to the approximate formula (2) in the calculation of ϱ^{σ} and the approximation to ϱ^{π} was similarly eliminated. At each stage the syntheses were evaluated in three dimensions. This involved Fourier transformation of the whole molecule when the temperature factor was applied. In the transformation from reciprocal space back to real space, the data outside what would correspond to the Cu $K\alpha$ sphere of X-ray diffraction was neglected.

The section of the difference synthesis in the plane of the molecule, with an isotropic temperature factor coefficient of 3·0 Å^2 applied, is given in Fig. 9(*a*). This corresponds to the projection shown in Fig. 4(*a*) of Cochran (1956). The densities in the C–C and C–H perpendicular planes are shown in Fig. 9(*c*) and (*e*).

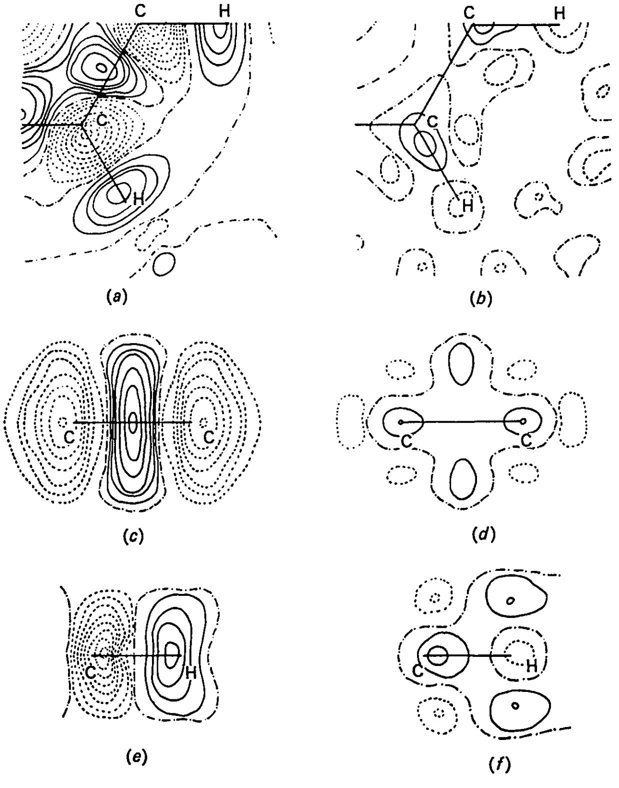


Fig. 9. Theoretical residual electron densities for benzene. (*a*) Molecular plane before refinement; (*b*) molecular plane after refinement; (*c*) C–C perpendicular plane before refinement; (*d*) C–C perpendicular plane after refinement; (*e*) C–H perpendicular plane before refinement; (*f*) C–H perpendicular plane after refinement. Contour interval 0·025 e.Å^{-3}. Negative contours broken, zero contours chain-dotted.

Comparison of Fig. 9(*c*) with the experimental results in Fig. 5 reveals quite a close similarity. The height of the bond peak is 0·15 e.Å^{-3}, which is similar to that of the more reliable experimental measurements, and the extension at half height (0·75 Å) is very close to that found in these cases. The comparison in the molecular plane is, however, not as encouraging. The hollow in the benzene ring is too low and the peak in the carbon–carbon bond is more extended than the experimental results would indicate. Moreover, there are hollows of depth $-0{\cdot}20$ e.Å^{-3} at the carbon positions in the theoretical picture whereas the best experimental results show these atoms on regions of practically zero density.

There is still, however, a further effect to be considered. If Fig. 9(*a*), (*c*) and (*e*) represents a true picture of the electron density, then it is clear that, on refinement, the atomic parameters will converge to values different from their true values so as to compensate for the residual features. To test this hypothesis, the theoretical model was 'refined' by the following procedure. The Fourier transform of the aspherical electron density was sampled at points corresponding to the reciprocal lattice of a cell of dimensions 6 Å × 6 Å × 3 Å where the origin of this cell is taken at the centre of the benzene ring, the *c* axis is perpendicular to the molecular plane, the *b* axis intersects the centre of the C–C bond and the *a* axis lies along a C–H bond. The symmetry is then *Pmmm* with one-eighth of the molecule in the asymmetric unit. The temperature factor coefficient of 3·0 Å^2 was applied to this data and spherical scattering factors were obtained by taking the Fourier transform of the electron density of carbon in the prepared valence state and hydrogen in the ground state.

Structure factors calculated from true positions and temperature factors yielded an *R* value of 3·61% and a difference synthesis evaluated on these structure factors was, as expected, identical with that shown in Fig. 9(*a*) and (*c*). The atomic parameters were then refined by block-diagonal least squares, care being taken to preserve the symmetry of the molecule*. After three rounds, the refinement had converged to an *R* value of 3·16%. The following effects were then observed.

(i) The carbon and hydrogen atoms were shifted radially towards the ring centre by 0·0075 Å and 0·032 Å respectively.

(ii) The temperature factor coefficient of the carbon increased by 0·15 Å^2 and that of the hydrogen decreased by 1·28 Å^2.

(iii) Fig. 9(*b*), (*d*) and (*f*) shows sections of the difference synthesis, evaluated on the final structure fac-

---

* The procedure described was used in preference to refinement in a hexagonal cell only because of the limitations of the programs available. In fact, the ratios between the changes in the coordinates were very close to those expected and corrections were applied at each round of refinement to keep the molecular symmetry exact.

tors, through the molecular plane and the C–C and C–H perpendicular planes. The shape of the synthesis has changed quite radically. The carbon and hydrogen atoms now lie on regions of practically zero electron density, but the C–H bond peak has almost disappeared and the C–C bond peak has vanished completely in the molecular plane, although it still reaches a maximum of 0·035 e.Å^{-3} at a distance of 0·75 Å above the bond centre. The shape of this peak is now quite different from the experimental results shown in Fig. 4.

What appears to be an alarming discrepancy between theory and experiment is, however, largely resolved by a closer examination of the results. The shift in the carbon atom, which causes the near elimination of the C–C bond peak, is a direct result of its asymmetric environment. Clearly, there would be no shift if the carbon atom had complete trigonal symmetry – as, for example, in graphite. One would therefore expect a much smaller shift and much less reduction in the electron density if the hydrogen substituent were replaced by an atom, such as nitrogen or oxygen, which had bonding properties close to those of carbon.

Most of the C–C bonds in the structures examined above have non-hydrogen substituents and would therefore be expected to have residual bond densities nearer to that in the unrefined theoretical maps. An exception is AMBA where an examination of the molecular plane [Fig. 6(*b*)] and the individual perpendicular planes (Fig. 2) shows that the bonds between hydrogen substituted carbons [C(4), C(5), C(6)] have densities generally less than the other C–C bonds and in some cases [notably C(5)–C(6)] the hydrogen substituted bonds have an approximately dumbell shape similar to the theoretical density in Fig. 9(*d*). The theoretical results are confirmed even more strongly by a recent analysis of diphenyl (Mason & Robertson, 1965). In this structure, the inter-ring C–C bond has a high residual density whereas the hydrogen substituted bonds have very little density in the molecular plane, but possess small peaks above and below the bond centres. A similar, but less accurate result has been obtained for diphenylbenzene (Rietveld & Maslen, 1966) and the structure of orthanilic acid (Hall & Maslen, 1966) provides confirmation similar to that obtained from AMBA. The absence of significant bond peaks in benzene (Cox, Cruickshank & Smith, 1958) can also be attributed to the effects discussed above.

Still further confirmation is obtained by comparing the results of X-ray measurements of bond lengths between hydrogen substituted carbons and values obtained by other methods. The mean C–C bond in benzene, for example, is measured by X-ray methods (Cox, Cruickshank & Smith, 1958) as 1·392 Å while the values obtained by Raman spectroscopy (Stoicheff, 1954), electron diffraction (Almenningen, Bastiansen & Fernholt, 1958) and neutron diffraction (Bacon, Curry & Wilson, 1964) are 1·397, 1·397 and 1·398 Å respectively. Although there is a considerable spread in the individual values, it is probably significant that the means of the hydrogen substituted bonds in naphthalene and anthracene are found by X-ray diffraction measurements to be 0·003 Å and 0·002 Å respectively less than the corresponding values obtained by electron diffraction (Coulson & Golebiewski, 1961).

Individually these results have limited significance in comparison with the corresponding estimated standard deviations, but when taken together, their consistency provides strong qualitative confirmation of the theoretical results. It seems probable, however, that the shift in the carbon atom is somewhat smaller and the elimination of the residual bond density less complete than predicted theoretically.

The shortening of the C–H bond length when measured by X-ray diffraction has been known for some time but the present theoretical estimate of 0·03 Å is considerably less than that generally observed. The low thermal parameters obtained for bonded hydrogen have also been reported (Jensen & Sundaralingam, 1965, Mason, Phillips & Robertson, 1965) although, in this case also, the change predicted here (1·28 Å^2) is less than that measured experimentally (2·5 Å^2).

Verification of the changes in carbon temperature factors would require accurate X-ray and neutron analyses of the same compounds conducted under similar conditions, but unfortunately, no such investigation has been conducted on a suitable aromatic compound. However, projection neutron analyses of both β-sulphanilamide (O'Connell & Maslen, 1966) and *p*-diphenylbenzene (Rietveld & Maslen, 1966) provide carbon temperature factors which are almost invariably lower than those obtained from the X-ray analyses while the hydrogen temperature factors are consistently higher.

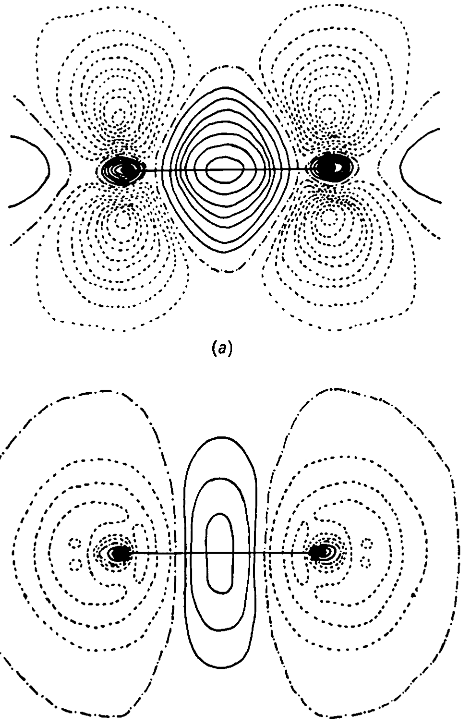
Despite the general agreement between theory and experiment discussed above, one major discrepancy remains. This is the failure of the theoretical analysis to predict the hollows antisymmetric to the bond peaks, particularly as displayed in the negative area almost always found at the centre of the benzene ring. This anomaly can probably be attributed to the neglect of the effect of σ-orbital contraction and electron correlation on the molecular wave functions. Although, as indicated above, the results of Mason (1960) agree less well with experiment than the present calculations, the former indicate that an accurate correction for σ-orbital contraction might well lead to a greater concentration of electrons in the bonds. This, in turn, would cause sharper features in the difference maps, probably accompanied by the correct distribution of negative regions. The errors in the carbon positions would be smaller and the hydrogen shifts larger. The effects of electron correlation cannot be predicted qualitatively and only a thorough quantitative analysis would show whether these are important.

There can, however, be little doubt that the general features of the present calculations are correct and that the effects of bonding introduce errors in the atomic positional and thermal parameters which are not confined solely to terminal atoms and which are significant when compared with the accuracy of modern structure analyses.

The variation of residual bond density with π-bond order

One of the most surprising experimental results (§ 3) it that the amount of electron density in the bond and the extension out of the plane are both greater for bonds of lower π-bond order. The former discrepancy between SULPH and TATNB can be partly attributed to the reduction in density in bonds between hydrogen substituted carbons discussed above, but the relative extensions remain an anomaly, as do the relative amounts of electron density in the TATNB and AMOX bonds. A full theoretical study would require the evaluation of the electron density for molecules containing bonds of different π-bond order. However, a rough picture can be obtained by examination of the C–C bond density of benzene without including the π electrons in the calculation. The results of such an evaluation are shown for the perpendicular C–C bond plane in Fig. 10(*a*) while Fig. 10(*b*) shows the same situation, but with the π electrons added*. Fig. 10(*a*) is then complementary to Fig. 3 of Cochran (1956) which shows a section through the molecular plane. It must be emphasized that Fig. 10(*a*) has limited physical significance, and must be considered as either the difference between two five-electron atoms or the difference between a carbon atom with three valence electrons in σ orbitals with the fourth in a prepared state and a prepared state carbon. Nevertheless, it is probably significant that the height of the bond peak is decreased by about one half when the π electrons are included. The relative extensions do not, however, agree with experiment and, although the effects of thermal motion have not been investigated, this anomaly remains unexplained.

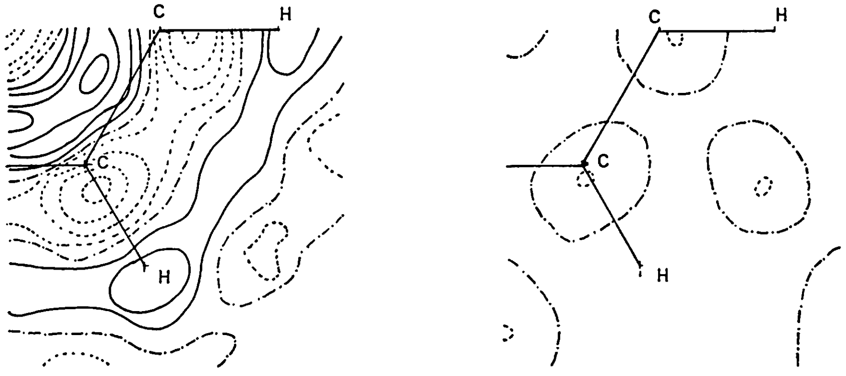
Comparison of Fig. 10(*a*) and (*b*) also illustrates why, even when the π electrons are taken to be localized above the carbon atoms, no peak is observed at this point in the difference synthesis. The formation of the σ bonds illustrated in Fig. 10(*a*) leaves a large negative



(*a*)

(*b*)

Fig. 10. Theoretical residual electron density for C–C perpendicular bond with no temperature factors applied. (*a*) shows density before the π electron contribution is included while (*b*) includes the π electrons. Contour interval 0·10 e.Å^{-3}, negative contours broken, zero contour chain-dotted.



(*a*) (*b*)

Fig. 11. Theoretical electron density in the molecular plane of benzene calculated omitting data with sin $\theta/\lambda < 0{\cdot}4$ Å^{-1}. (*a*) shows density before and (*b*) after refinement. Contour interval 0·025 e.Å^{-3}, negative contours broken, zero contour chain-dotted.

---

* Fig. 10(*b*) differs from Fig. 9(*c*) because, firstly, the electron density has not been subjected to Fourier tranformation and retransformation so the very sharp peak at the carbon atom which arises from the (1*s*) electrons has not been removed and, secondly, no temperature factor has been applied and consequently the height of the bond peak is greater.

area above the atom, which is then partly filled when the contribution of the π electrons is added. In other words the introduction of the π electrons at this point renders the atom more nearly spherical than it was before. This serves to emphasize the dangers of directly identifying residual features in difference maps with the actual disposition of bonding electrons.

5. The Bragg angular dependence of the scattering from valence electrons

It has been generally accepted (*e.g.* McWeeny, 1954; Dawson, 1964*a*) that the effects of the scattering from bond electrons are largely confined to the structure factors with low Bragg angle ($\sin\theta/\lambda \lesssim 0{\cdot}4$ Å^{-1}). To test this proposition, difference maps were evaluated for the theoretical model using these data only, before and after refinement (Fig. 11) and also for TATNB [Fig. 4(*b*)]. If the scattering were confined to low angle, these syntheses would be very like those calculated using the full data. In fact, a comparison of Figs. 9 and 11 shows that, although there is a general similarity, the features of the theoretical difference synthesis have changed quite appreciably both before and after refinement. This indicates that, in fact, there is significant scattering by the valence electrons above $\sin\theta/\lambda = 0{\cdot}4$. Examination of Fig. 4 shows that the experimental synthesis on TATNB has altered even more radically. In this case, the bond peaks have disappeared completely and the main features remaining are those associated with the charge movements discussed in § 2. In fact, these latter features are considerably enhanced in Fig. 4(*b*).

The experimental results then suggest that the scattering from the valence electrons, far from being confined to the region with $\sin\theta/\lambda < 0{\cdot}4$ Å^{-1}, is in fact predominantly a higher-angle effect. This result is consistent with the greater sharpness of the features in the experimental, as compared with the theoretical, results, and is probably a property of σ-orbital contraction discussed above. The change in scattering power due to charge movement is, however, primarily a low angle phenomenon. Since this is not observed to the same extent in the synthesis calculated from the full data, it must have been partly compensated for in the refinement by changes in the atomic temperature factors.

6. Conclusion

The survey of experimental results on residual electron density has shown that it is possible to obtain useful information on bonding electron density with the use of modern techniques of data collection and structure refinement. In particular, the details of the magnitude and shape of the residual bond density can be obtained and, in favourable cases, charge movement in the molecule can be observed. However, it has been seen that, even in structures which have been refined to high accuracy, there are often residual errors in scale which, along with extinction, seriously limit the usefulness of the results. Nevertheless, significant information on bonding electrons has been overlooked in a number of cases. This is probably a by-product of the widespread use of least-squares refinement techniques, which has resulted in an unfortunate tendency to give inadequate attention to the final difference synthesis.

The theoretical analysis has extended the results of Cochran (1956) to three dimensions and has confirmed his conclusion that the Slater wave functions give a reliable qualitative picture of the electron density. The neglect of σ-orbital contraction and also, possibly, electron correlation gives rise to discrepancies which are significant when compared with the experimental results. The refinement of structures with the use of isolated atom models may yield positional and thermal parameters which are in error by amounts which are significant compared with the accuracy of modern X-ray structure analysis.

It can be concluded that in order to obtain better experimental results, accurate structure analyses should be conducted with both neutron and X-ray diffraction under similar conditions. The parameters obtained by neutron diffraction could be used in the calculation of final X-ray difference syntheses, and the latter would then be directly comparable with theoretical difference syntheses evaluated with the best molecular wavefunctions available.

All the calculations were performed on the University of Western Australia's P.D.P.-6 computer with programs written by one of the authors (AIMR). The advice and assistance of the director and staff of the University's computing centre are gratefully acknowledged. The research described in this paper was performed while one of the authors (AMO'C) was in receipt of an Australian Commonwealth Postgraduate Award.

References

Almenningen, A., Bastiansen, O. & Fernholt, L. (1958). *Kgl. Norske Viedenskab. Selskab. Schrifter,* No. 3.

Bacon, G. E., Curry, N. A. & Wilson, S. A. (1964). *Proc. Roy. Soc.* A, **279**, 98.

Beagley, B. & Small, W. H. (1963). *Proc. Roy. Soc.* A, **275**, 469.

Brown, G. M. & Marsh, R. E. (1963). *Acta Cryst.* **16**, 191.

Cady, H. & Larsen, A. C. (1965). *Acta Cryst.* **18**, 485.

Cochran, W. (1953). *Acta Cryst.* **6**, 260.

Cochran, W. (1956). *Acta Cryst.* **9**, 924.

Coulson, C. A. & Golebiewski, A. (1961). *Proc. Phys. Soc.* **78**, 1316.

Cox, E. G., Cruickshank, D. W. J. & Smith, J. A. S. (1958). *Proc. Roy. Soc.* A, **247**, 1.

Dawson, B. (1964*a*). *Acta Cryst.* **17**, 990.

Dawson, B. (1964*b*). *Acta Cryst.* **17**, 997.

Dawson, B. (1965). *Aust. J. Chem.* **18**, 595.

Hall, S. R. & Maslen, E. N. (1966). *Acta Cryst.* To be published.

Jensen, L. H. & Sundaralingam, M. (1964). *Science*, **145**, 1185.
March, N. H. (1952). *Acta Cryst.* **5**, 187.
Mason, R. (1960). *Proc. Roy. Soc.* A, **258**, 302.
Mason, R., Phillips, D. C. & Robertson, G. B. (1965). *Mol. Phys.* **9**, 277.
Mason, R. & Robertson, G. B. (1965). Private communication.
McWeeny, R. (1951). *Acta Cryst.* **4**, 513.
McWeeny, R. (1952). *Acta Cryst.* **5**, 463.
McWeeny, R. (1953). *Acta Cryst.* **6**, 631.
McWeeny, R. (1954). *Acta Cryst.* **7**, 180.
O'Connell, A. M. & Maslen, E. N. (1966). *Acta Cryst.* To be published.
Rae, A. I. M. & Maslen, E. N. (1965). *Acta Cryst.* **19**, 1061.
Rietveld, H. & Maslen, E. N. (1966). To be published.
Robertson, G. B. (1961). *Nature, Lond.* **191**, 593.
Stoicheff, B. P. (1954). *Canad. J. Phys.* **32**, 339.
Sundaralingam, M. & Jensen, L. H. (1965). *Acta Cryst.* **18**, 1053.
Trueblood, K. N., Goldish, E. & Donohue, J. (1961). *Acta Cryst.* **14**, 1009.
Verschoor, G. C. (1964). *Nature, Lond.* **202**, 1206.

Acta Cryst. (1966). **21**, 219

The Crystal Structures of Derivatives of Tetrodotoxin.
I. Tetrodonic Acid Hydrobromide

By Chihiro Tamura and Osamu Amakasu
Research Laboratories, Sankyo Co. Ltd, Hiromachi, Shinagawa-ku, Tokyo, Japan

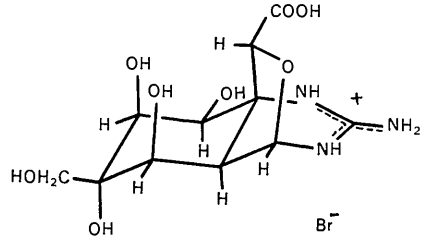
Yoshio Sasada
Institute for Protein Research, Osaka University, Joancho, Kita-ku, Osaka, Japan

and Kyosuke Tsuda
Institute of Applied Microbiology, University of Tokyo, Yayoicho, Bunkyo-ku, Tokyo, Japan

(*Received* 6 *August* 1965 *and in revised form* 12 *January* 1966)

The structure of tetrodonic acid, one of the derivatives of the toxic substance tetrodotoxin, has been determined by X-ray analysis of the hydrobromide. The crystals are orthorhombic with four molecules in a unit cell of dimensions $a=20{\cdot}231$, $b=10{\cdot}590$, $c=6{\cdot}813$ Å, with space group $P2_12_12_1$. The position of the bromine atom was determined by three-dimensional Patterson methods.

After four cycles of three-dimensional Fourier refinement, the structure of the molecule was found to be



Atomic coordinates and anisotropic temperature factors were further refined by the block-diagonal matrix least-squares method. The final R value decreased to 0·129. Intra- and intermolecular bond lengths are within the range of normal values.

Introduction

Tetrodotoxin, one of the most toxic substances of low molecular weight, was found in the ovaries and livers of pufferfish (Tahara, 1909). Tsuda & Kawamura (1952) obtained it in crystalline form after chromatographic purification. This poisonous compound has attracted the attention of chemists and biologists, and many investigations on it have been carried out by several authors (Yokoo, 1950; Tsuda & Kawamura, 1953; Kakisawa, Okumura & Hirata, 1959).

Tsuda, Ikuma, Kawamura, Tachikawa, Miyadera & Baba (1962) proved by total chemical synthesis that an alkaline degradation compound from tetrodotoxin is 2-amino-6-hydroxymethyl-8-hydroxyquinazoline. It was impossible, however, to deduce uniquely the structure of tetrodotoxin from this result because some migration might occur in the course of the vigorous reaction. X-ray crystallographic analysis, therefore, was attempted for the determination of the molecular structure. The crystals of tetrodotoxin itself were not obtained in a suitable form for X-ray work. Several