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The Crystal Structure of Chloramphenicol[1] and Bromamphenicol[2]

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The crystal and molecular structures of chloramphenicol and bromamphenicol have been determined by X-ray analysis. The structure derived by chemical methods is confirmed. The molecules adopt a curled configuration in the crystal. Certain anomalous variations in the intensities of the X-ray reflections are observed; these are discussed and an explanation proffered.

A preliminary X-ray investigation of some antibiotic substances has been described in a previous communication.[4] The crystal and molecular structure of bromamphenicol has now been determined from X-ray data; from the close similarity which exists between crystals of bromamphenicol and those of chloramphenicol, and from simultaneous analysis of portions of their X-ray diffraction patterns, the structure of chloramphenicol may also be regarded as established.

Chloramphenicol was recrystallized from water in the form of yellowish-white needles or laths which tended to form agglomerates. The crystals are orthorhombic with unit cell dimensions, a = 17.6 Å., b = 7.35 Å., c = 22.3 Å. The probable space group, assigned on the basis of absent spectra is D_2^5 – $C222_1$, and the observed density, 1.49 g. cm.$^{-3}$, indicates the presence of eight molecules in the unit cell. The crystals have strong negative birefringence, with the direction of the lowest refractive index along the b axis, suggesting that the p-nitrophenyl groups lie roughly parallel to one another and perpendicular to the b axis. No detailed structural information has been obtained from X-ray analysis of chloramphenicol alone. The Patterson projection $P(uw)$ and the sections $P(0vw)$, $P(u0w)$, $P(uv\frac{1}{2})$ were computed but it was not found possible to identify even the chlorine–chlorine interactions.[5]

Crystals of bromamphenicol are similar in appearance and in optical properties to those of chloramphenicol and were found to be isomorphous with them. The cell dimensions differ only slightly being, a = 17.9 Å., b = 7.4 Å., c = 22.1 Å., and the observed density 1.87 g. cm.$^{-3}$ shows the presence of 8 molecules in the unit cell. On exposure to X-rays, the crystals slowly decompose, finally disintegrating into a viscous mass in which the presence of free bromine is indicated by a deep brown color. The intensities of the X-ray reflections gradually diminish until the original pattern disappears leaving only a weak amorphous pattern.

It was also observed that the *relative* intensities of certain reflections varied from one crystal to another. The intensities of the $(h0l)$ reflections from two crystals (designated A and B), representing more or less extreme examples of the variations observed,[6] were measured and Patterson $P(uw)$ functions calculated. These two functions are shown in Fig. 1 along with the corresponding function for chloramphenicol. The scales are adjusted so that $P(00)$ is approximately the same in all three cases. It is clear that the plot for crystal A can be described as an interpolation between those for chloramphenicol and crystal B.

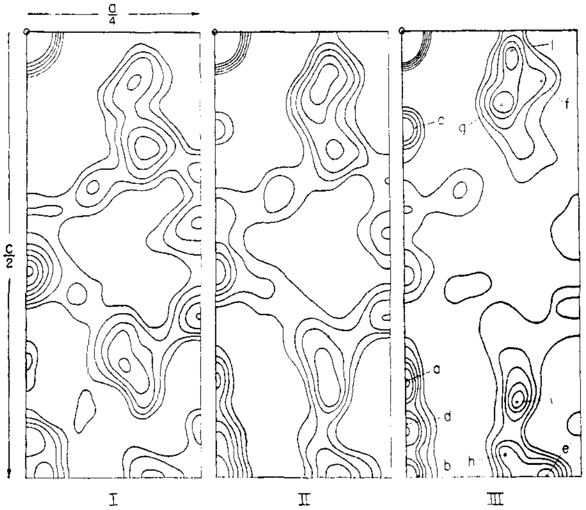

Fig. 1.—Patterson $P(uw)$ projection of (I) chloramphenicol, (II) bromamphenicol A, and (III) bromamphenicol B. In all three functions $P(00)$ is approximately equal and contour lines are drawn at the same intervals. Only one asymmetric unit ($\frac{1}{8}$ unit cell) bounded by mirror lines is shown.

By comparison of these three plots, the bromine–bromine interactions can be identified and the x and z coördinates of both bromine atoms inferred. The eight general positions of the space group $C222_1$ are[7]

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

The Patterson $P(uvw)$ function corresponding to two independent atoms at $(x_1y_1z_1)(x_2y_2z_2)$ will

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[1] Chloramphenicol has been assigned as a generic name for the compound D-*threo*-N-(1,1'-dihydroxy-1-p-nitrophenylisopropyl)-dichloroacetamide for which Parke, Davis and Co. has adopted "Chloromycetin" as its trademark.

[2] Aided by grants from the National Foundation for Infantile Paralysis, Inc. and from Parke, Davis and Co.

[3] Laboratory of Chemical Crystallography, University Museum, Oxford, England.

[4] J. D. Dunitz and J. E. Leonard, THIS JOURNAL, **72**, 4276 (1950).

[5] A large temperature factor contributes to this difficulty; the corresponding diffuseness of the electron-density peaks is especially troublesome in the interpretation of Patterson functions where lack of resolution is often the principal barrier to the correct analysis.

[6] The variations observed were larger than could be accounted for by the differences in size and shape of the crystals. In six different crystals of bromamphenicol the observed ratio of intensities of the (004) and (006) reflections was 2.5 (B), 1.9, 1.7, 1.2, 1.0, 1.0 (A). From final structure factor calculations the ratio is 3.6 for bromamphenicol and 0.2 for chloramphenicol.

[7] The expressions for the structure factor for the space group $C222_1$ given both in "Internationale Tabellen zur Bestimmung von Kristallstrukturen," Vol. I, Berlin, 1935, and in K. Lonsdale, "Structure Factor Tables," London, 1936, are in error. The correct expressions may be obtained from them by interchanging the letters h (and hx) for k (and ky) throughout.

thus have peaks situated at (000) ± ± ± . . . and ($\frac{1}{2}\frac{1}{2}$ 0) ± ± ± . . .

(a)	$0, 2y_1, 2z_1$	(d)	$0, 2y_2, 2z_2$
(b)	$2x_1, 2y_1, \frac{1}{2}$	(e)	$2x_2, 2y_2, \frac{1}{2}$
(c)	$2x_1, 0, \frac{1}{2} + 2z_1$	(f)	$2x_2, 0, \frac{1}{2} + 2z_2$

(g) $x_1 - x_2, y_1 - y_2, z_1 - z_2$
(h) $x_1 - x_2, y_1 + y_2, z_1 + z_2$
(i) $x_1 + x_2, y_1 + y_2, \frac{1}{2} + (z_1 - z_2)$
(j) $x_1 + x_2, y_1 - y_2, \frac{1}{2} + (z_1 + z_2)$

These ten peaks, projected on (0*l*0), may be identified by their increase in height from chloramphenicol through bromamphenicol A to bromamphenicol B, and are shown in the Patterson projection of bromamphenicol B. It is not possible to identify them uniquely owing to ambiguities resulting from the presence of symmetry elements, but the set of values

$x_1 = 0.242$	$z_1 = 0.196$
$x_2 = 0.100$	$z_2 = 0.279$

provides one solution which was shown to be correct in subsequent calculations.

The contributions of the halogen atoms to $F(h0l)$ were calculated and the three sets of relative F values were placed on approximately the same scale by consideration of reflections for which the halogen atom contribution was negligibly small. The signs to be associated with the F's could now be determined and the three $\rho(xz)$ projections calculated. They are shown in Fig. 2 and are seen to be strikingly similar, excepting the heights of the heavy-atom peaks. The approximate positions of some of the light atoms may be inferred from the recurrence of certain features in all three functions; the *p*-nitrophenyl group can be located as shown and also the carbon atom of the terminal CHX_2 group. The other atoms project close to one another and form a composite peak in which they cannot be resolved.

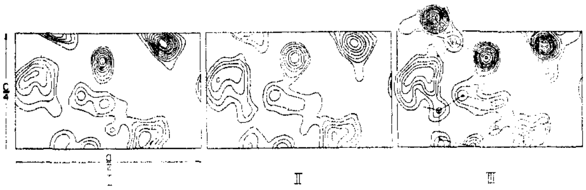

Fig. 2.—Fourier $\rho(xz)$ projections of (I) chloramphenicol, (II) bromamphenicol A and (III) bromamphenicol B. Contour lines are drawn at the same intervals throughout except for the bromine atoms in (II) and (III) where the interval is increased by a factor of 2.5 for clarity.

Contamination of the bromamphenicol crystals with chloramphenicol or chlorbromamphenicol seemed a possible explanation of the observed variations in intensity, if the amount of impurity were assumed to vary from one crystal to another depending on the exact conditions of crystallization. Microanalysis, however, showed absence of chlorine and a slightly but significantly low bromine content (Calcd. for $C_{11}H_{12}N_2O_5Br_2$: Br, 38.8. Found: Br, 36.0).

Further investigation revealed another type of intensity variation. On exposure to X-rays, the *relative* intensities of certain reflections changed,[8] every observed example being consistent with a decrease in effective bromine scattering power. Such a decrease would occur, if, in randomly distributed molecules, a carbon–bromine bond were broken, the bromine atom diffusing away while the result of the molecule remained essentially unchanged.[9] Ultimately, of course, the crystals disintegrate and the original diffraction pattern disappears.

The Patterson function $P(uvw)$ for bromamphenicol was computed. This function will not be reproduced here in its entirety as its complete representation in a concise form is not possible. Certain portions which are particularly significant are shown in Fig. 3. Ten peaks, (a)–(j), defined previously, are expected to occur in the asymmetric unit of the Patterson function for two atoms situated at (x_1,y_1,z_1), and (x_2,y_2,z_2). All of these peaks are seen in Fig. 3 where their identification follows from the internal consistency of the relations exist-

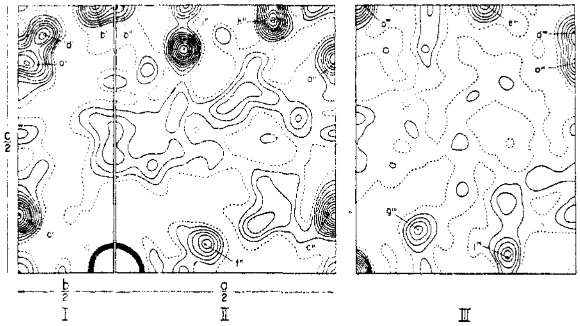

Fig. 3.—Some portions of the three-dimensional Patterson function $P(uvw)$ for bromamphenicol with bromine–bromine peaks marked.

I $P(0vw)$ II $P(u0w)$ III $P(u\frac{3}{30}w)$

All boundaries of figures are mirror planes. The peaks marked may be identified as the following Br–Br interactions:

(a′) $0, \overline{2y_1}, 2z_1$
(b′) $\frac{1}{2} \pm 2x_1, \frac{1}{2} + 2y_1, \frac{1}{2}$
(c′) $\frac{1}{2} \pm 2x_1, \frac{1}{2}, \frac{1}{2} - 2z_1$
(d′) $0, 2y_2, \overline{2z_2}$
(e″) $\frac{1}{2} - 2x_2, \frac{1}{2} - 2y_2, \frac{1}{2}$
(f″) $2x_2, 0, \frac{1}{2} + 2z_2$
(g″) $x_1 - x_2, y_1 - y_2, \overline{z_1 - z_2}$
(h″) $\frac{1}{2} - (x_1 - x_2), \frac{1}{2} \pm (y_1 + y_2), z_1 + z_2$
(i″) $\frac{1}{2} - (x_1 + x_2), \frac{1}{2} \pm (y_1 + y_2), \frac{1}{2} + (z_1 - z_2)$
(j″) $x_1 + x_2, y_1 - y_2, \frac{1}{2} - (z_1 + z_2)$
(a″) $\frac{1}{2}, \frac{1}{2} \pm 2y_1, 2z_1$
(b″) $\frac{1}{2} - 2x_1, \frac{1}{2} \pm 2y_1, \frac{1}{2}$
(c″) $2x_1, 0, \frac{1}{2} - 2z_1$
(d″) $\frac{1}{2}, \frac{1}{2} - 2y_2, \overline{2z_2}$
(a″) $\frac{1}{2}, \frac{1}{2} + 2y_1, 2z_1$
(b″) $\frac{1}{2} - 2x_1, \frac{1}{2} + 2y_1, \frac{1}{2}$

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[8] This effect, although quite definite, is difficult to measure because it occurs together with the general decrease of the intensities caused by crystal decomposition.

[9] Dilute aqueous solution of bromamphenicol decomposes in sunlight with liberation of bromide ion (private communication from H. M. Crooks to Professor V. Schomaker). It is tentatively proposed that the original variations observed in the intensities of reflection patterns from *different* crystals may be due to similar photo-decomposition by ultraviolet light to which the crystals may have been exposed in the ordinary course of events.

ing among them. From the positions of the peaks the y-coördinates of the bromine atoms may be established and the x- and z-coördinates confirmed. The values adopted were

$$\begin{array}{lll} x_1 = 0.242 & y_1 = 0.300 & z_1 = 0.195 \\ x_1 = 0.102 & y_2 = 0.182 & z_2 = 0.277 \end{array}$$

An attempt was made to locate the other atoms of the molecule by superimposing the Patterson function on itself with its origin transferred to the positions found for the two bromine atoms—the Vector Convergence method[10]—but without success.

A three-dimensional electron density function $\rho_1(xyz)$ was computed with phase angles calculated from the contributions of the bromine atoms alone. In this function the molecule could be recognized and approximate atomic positions determined. The phase angles were recalculated, taking account of contributions from all atoms and a second function, $\rho_2(xyz)$ calculated. Corresponding portions of $\rho_1(xyz)$ and $\rho_2(xyz)$ are shown in Fig. 4, to demonstrate the greatly improved resolution in the latter. In Fig. 5 a bromamphenicol molecule, formed by superposition of the peaks in $\rho_2(xyz)$, is shown.

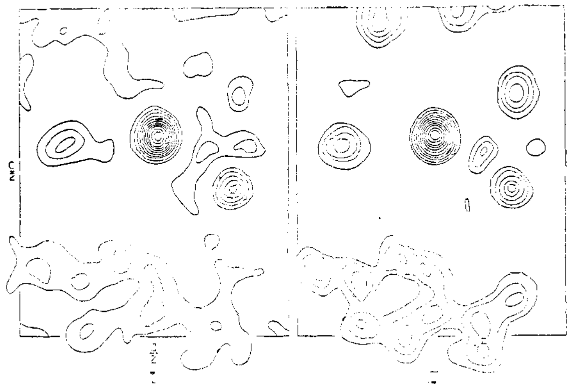

Fig. 4.—Comparison of first (I) and second (II) three dimensional Fourier series $\rho(xyz)$ for bromamphenicol illustrated by a portion of the respective sections at $y = -7/30$. Contour lines are drawn at the same intervals in both plots, those for the bromine atoms being drawn at 2.5 times the normal interval.

From the isomorphism of chloramphenicol and bromamphenicol and from the similarity of their Fourier projections it can be inferred that the chloramphenicol structure is the same as that found for bromamphenicol, apart from changes in dimension resulting from substitution of chlorine for bromine. Because of the change in position of the halogen atoms (corresponding to the difference of 0.2 Å. between the carbon–chlorine and carbon–bromine bond lengths), a separate calculation at this stage for chloramphenicol would require recalculation of the structure factors as well as a further three-dimensional Fourier series. While these additional calculations would lead to somewhat improved accuracy in the atomic positions, it is felt that the labor involved is of such proportion as to outweigh the additional information which might be gained. Accordingly, they have not been performed.

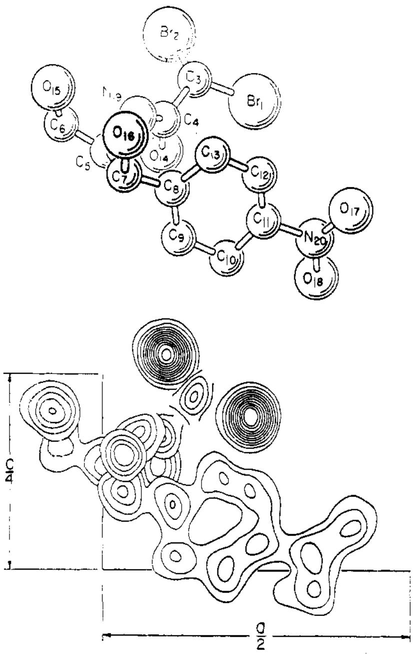

Fig. 5.—Superposition of peaks in the second three-dimensional Fourier series $\rho_2(xyz)$ to form a bromamphenicol molecule, with explanatory diagram to show the identification of the atoms. Contour lines for the bromine atoms are drawn at 2.5 times the normal interval.

Experimental

All X-ray diffraction experiments were performed with copper $\mathrm{Cu}K\alpha$ radiation ($\lambda = 1.542$ Å.) on a Buerger type equi-inclination camera. Lattice constants were determined from oscillation and zero-layer photographs. The crystal densities were determined by flotation methods.

Intensity measurements were made using the multiple film technique, by visual estimation with the aid of film strips on which calibrated exposures had been made of some convenient reflection. Although care was taken to select crystals for which absorption effects would be minimized, the absorption errors implicit in the intensities must be considerable, especially for bromamphenicol for which the linear absorption coefficient is about 76 cm.$^{-1}$. Decomposition of the crystals introduces further errors in the intensities; in particular the correlation of sets of relative intensities on different photographs is extremely uncertain. The intensity estimates for bromamphenicol must therefore be regarded as highly inaccurate. For the final intensity estimates, only crystals approximating to the B type were used; these were rejected as soon as signs of severe decomposition became evident on the photographs. Intensities of all reflections with $k < 6$ and with $\sin\theta \leqslant 0.675$ were measured on equi-inclination photographs. For most of these the crystal was rotated about b, other orientations being used only for correlation purposes. This choice was dictated by consideration of crystal shape and the necessity of minimizing absorption errors. Intensities were placed on an absolute scale by the method proposed by Wilson.[11] The constant B in the expression $\exp\{-B(\sin\theta/\lambda)^2\}$ was assigned the relatively high value 7.5×10^{-16} cm.^{2} for both substances.

Summation for Patterson and Fourier series and calculation of structure factors were carried out on I.B.M. punched card machines by methods which have not yet been fully described.[12] In the final three-dimensional Fourier series the function $\rho_2(wyz)$ was tabulated at intervals of $a/60$, $b/30$, $c/60$ over the asymmetric unit, one-eighth of the unit cell. In the structure factor calculation, Hartree scattering curves were used and the temperature factor was included. The ratio $R = \Sigma|F_{\text{obsd.}} - F_{\text{calcd.}}|\Sigma|F_{\text{obsd.}}|$ is 0.21 for the structure on which the phase angles for the final Fourier series were calculated.

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

[11] A. J. C. Wilson, *Nature*, **150**, 151 (1942).

[12] V. Schomaker, unpublished work.

Discussion

The principal result of this analysis is the confirmation of the structure for chloramphenicol established by chemical studies and synthesis.[13] In a strict sense, this result is not completely independent of chemical information. The X-ray analysis shows that the molecule contains two atoms of comparatively large scattering power and eighteen others[14] of smaller and approximately equal scattering power. Identification of these as carbon, nitrogen and oxygen atoms rests largely on previous chemical information although in a more favorable case (*i.e.*, if better intensity data were available) this distinction could be made independently. Likewise, in a more favorable case the distinction could be drawn between single and double bonds from the electron-density maps (taking into account the atomic species involved, the number of ligands and the observed bond distances) but this is not possible in the present analysis. In one respect the X-ray analysis provides more direct evidence than the chemical work. The shape of the molecule is obtained without the need for any chemical assumptions; the configuration about the two asymmetric carbon atoms is seen directly from the Fourier plots and it is especially satisfactory that they confirm the previous assignment of the molecule as a threose derivative.

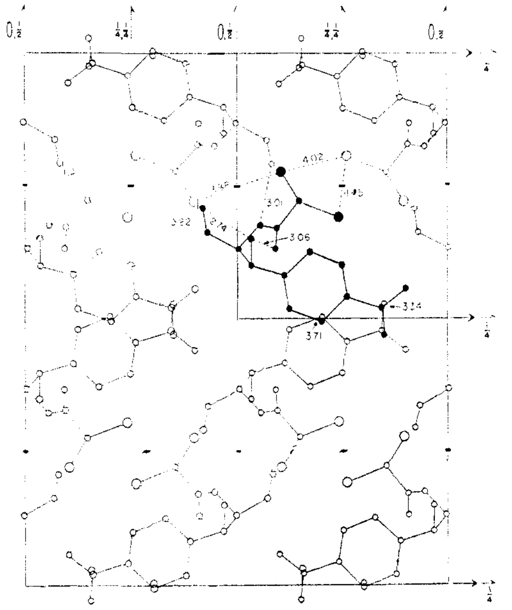

Fig. 6.—One unit cell of bromamphenicol projected down the *b*-axis to show the relationships between the eight molecules in the cell. The standard molecule (at x,y,z) is drawn in shaded circles, the others in open circles. Shorter intermolecular approaches are denoted (in Å.) together with the intramolecular hydrogen bridge of 2.74 Å. between O_{15} and O_{16}. The distance of 3.06 Å. between O_{16} and O_{14} is not intramolecular; it is between O_{16} in the standard molecule and O_{14} in a molecule one translation removed along the *b* axis.

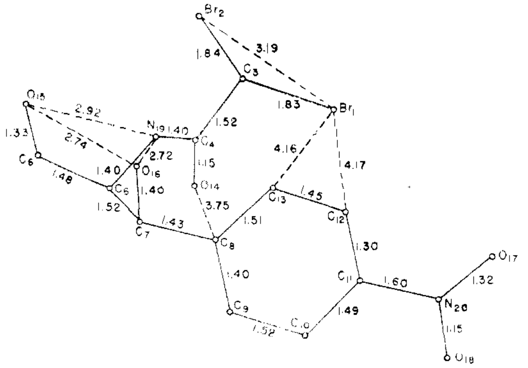

Fig. 7.—Interatomic distances in Å. in bromamphenicol molecules as measured from the 2nd three-dimensional Fourier plot.

Parameters from which phase angles for the final three-dimensional Fourier series were calculated are given in Table I, together with the final parameters. The poor quality of the available intensity data and the diffuseness resulting from the high temperature factor make these parameters rather imprecise and the resulting interatomic distances (shown in Figs. 6 and 7) are of only a low order of accuracy. It is seen, for example, that the observed bond distances around the benzene ring vary from 1.3 to 1.5 A.; this variation is certainly spurious and we may estimate that the bond distances may be in error by at least 0.1 Å. It is therefore profitless to engage in detailed discussion of the observed bond distances. Some significance may nevertheless be attached to the longer distances found between non-bonded atoms.

Table I

Parameters of Atoms in Bromamphenicol

I, Parameters used for structure factor calculation assigned on basis of first three-dimension Fourier series. II, Parameters assigned on basis of second three-dimensional Fourier series.

	I			II		
	x	*y*	*z*	*z*	*y*	*z*
Br_1	0.242	0.297	0.194	0.242	0.297	0.194
Br_2	.103	.180	.277	.103	.180	.277
C_3	.145	.333	.217	.146	.333	.221
C_4	.097	.400	.170	.095	.400	.170
C_5	.000	.640	.133	.003	.634	.132
C_6	.067	.667	.175	.070	.634	.163
C_7	.033	.800	.100	.033	.800	.100
C_8	.117	.783	.083	.110	.784	.082
C_9	.125	.733	.017	.121	.767	.018
C_{10}	.200	.767	.000	.200	.760	.007
C_{11}	.258	.767	.042	.258	.767	.042
C_{12}	.250	.767	.100	.250	.784	.100
C_{13}	.183	.800	.117	.175	.800	.125
O_{14}	.092	.300	.133	.092	.300	.130
O_{15}	.092	.717	.217	.083	.750	.208
O_{16}	.042	.900	.150	.033	.917	.150
O_{17}	.400	.800	.067	.400	.784	.057
O_{18}	.342	.700	.025	.346	.700	.028
N_{19}	.050	.550	.167	.053	.560	.173
N_{20}	.342	.767	.025	.344	.750	.020

Molecules of bromamphenicol adopt a "curled" configuration in the crystals. They are probably

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(13) M. C. Rebstock, H. M. Crooks, J. Controulis and Q. R. Bartz, This Journal, **71**, 2458 (1949); J. Controulis, M. C. Rebstock and H. M. Crooks, *ibid.*, **71**, 2463 (1949).

(14) The analysis is not sufficiently refined for resolution of hydrogen atoms.

maintained in this configuration by an internal hydrogen bridge. The presence of hydrogen bonds is generally inferred in this type of work from the existence of a closer approach between two nonbonded atoms than would be predicted from the van der Waals radii. Three intermolecular nonbonded distances, $O_{15}\ldots O_{16}$, $O_{15}\ldots N_{19}$, $O_{16}\ldots N_{19}$, are sufficiently short to justify the inference that hydrogen bridges might exist between the atoms in question.

The close approach of O_{15} and O_{16} is probably due to a fairly strong hydrogen bridge. A six-membered ring is formed by O_{15}, C_6, C_5, C_7, O_{16}, H, O_{15} and the hydrogen atoms of either O_{15} or O_{16} are suitably oriented to take part in the hydrogen bond. It seems most probable that the hydrogen atom attached to O_{15} is involved in the bond as this would leave the hydrogen atom attached to O_{16} free to form an intermolecular hydrogen bond with O_{14} of a molecule one translation removed along the *b* axis—the distance $O_{16}\ldots O_{14}$ being 3.05 Å. corresponding to a somewhat weak interaction—but this argument is not altogether convincing in view of the comparatively short distance of 3.22 Å. separating O_{15} (in the standard molecule at (x,y,z)) and Br_1 in the adjacent molecule at $(\bar{x},y,\frac{1}{2}-z)$, which distance is also perhaps ascribable to the presence of a weak hydrogen bridge.

The close approaches of 2.71 Å. between N_{19} and O_{16} and 2.92 Å. between N_{19} and O_{15} are not to be interpreted as being due to strong hydrogen bonds. The hydrogen atom attached to N_{19} is not suitably directed for hydrogen bonding with either O_{15} or O_{16} and, moreover, the above distances are close to the normal approach of atoms connected by a two-carbon atom bridge in the staggered configuration which is found for the pairs of atoms C_5 and C_6, and C_5 and C_7. This hydrogen probably participates in an intermolecular hydrogen bond between N_{19} in the standard molecule and O_{16} in the adjacent molecule at $(\bar{x},y,\frac{1}{2}-z)$, the distance between these atoms being 3.01 Å. Other intermolecular distances of 3.05 and 3.22 Å. have been noted above and the only additional close approach is one of about 3.3 Å. between the nitro groups of molecules related to one another by the twofold rotation axis [100]. The closest distances between bromine atoms of different molecules are found to be 4.01 and 3.88 Å., close to double the van der Waals radius of bromine,[15] 1.95 Å.

The explanation proposed for the anomalous intensity changes on exposure to X-rays does not appear unreasonable. The bromine atoms are not involved in the intermolecular binding to any great extent; they are situated in channels along which they may diffuse with a minimal amount of interference from other groups in other molecules. Thus, on rupture of a carbon–bromine bond the bromine atom may move into a new environment while the rest of the molecule may be expected to retain its original position and configuration, at least for some time. The final decomposition of the molecule is certainly accompanied by other chemical changes but no attempt has been made to study these so far.

The configuration adopted by molecules of bromamphenicol and of chloramphenicol in the crystal is especially suitable for interaction with the polar groups of a protein chain. The two hydroxyl groups at O_{15} and O_{16} and also the nitrogen atom N_{19} of the peptide link may all be described as pointing outward from the molecule; furthermore, the distances N_{19}–O_{15} and N_{19}–O_{16} are close to the distance between the nitrogen atom and the carbonyl oxygen atom on the same side of a fully extended polypeptide chain. Thus, two-point attachment of a chloramphenicol molecule to a protein chain is possible. If adsorption of the antibiotic onto bacterial protein in this manner is assumed to be the first step in their interaction it is perhaps significant that the unusual terminal groups of the antibiotic are held comparatively far away from the protein and are thus unlikely to interfere with the adsorption process. They may, however, interfere with any subsequent action at the adsorption site. It does not seem profitable to develop this speculation at present since we are ignorant both of the actual mode of action of the antibiotic and of its configuration in solution.

Acknowledgments.—The author takes this opportunity of expressing his indebtedness to many members of these laboratories for helpful discussion. Special acknowledgment is due to Professors Robert B. Corey and Verner Schomaker. Without the help of the latter many calculations necessary for this work would not have been started, far less completed. Thanks are also due to Professor A. J. Haagen-Smit for a microanalysis, to Dr. John E. Leonard for assistance with some preliminary stages of the work, and to Mrs. B. M. Bell and Miss Lillian Casler for their assistance with several figures.

PASADENA, CALIFORNIA RECEIVED FEBRUARY 19, 1951

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[15] L. Pauling, "The Nature of the Chemical Bond," The Cornell University Press, Ithaca, N. Y., 1940, p. 189.