

TABLE XII

PARTIAL COMPOSITION OF TWO HIGHEST OCCUPIED MOLECULAR ORBITALS IN TERMS OF  $F_{ij}$  (EQ. 7)

$i$	$C_{2v}$		$C_s$	
	( $b_1$ ) $j = 14$	( $a_1$ ) $j = 15$	( $a''$ ) $j = 14$	( $a'$ ) $j = 15$
$d_z^2$	0.000	0.000	0.000	0.000
$d_x^2 - y^2$	0.000	0.080	0.000	0.001
$d_{xy}$	0.029	0.000	0.000	0.000
$d_{xz}$	0.000	0.000	0.026	0.000
$d_{yz}$	0.000	0.000	0.000	0.048

TABLE XIII

ELEMENTS OF THE OVERLAP MATRIX INVOLVING THE SULFUR d-ORBITALS<sup>a</sup>

	$d_{xz}$	$d_{xz} - y^2$	$d_{xy}$	$d_{xz}$	$d_{yz}$
Nitrogen					
s	-0.1368	0.0909	0.2187	0	0
px	0.0945	0.0402	-0.1939	0	0
py	0.0630	-0.1962	-0.0367	0	0
pz	0	0	0	0.1671	0.1116
Oxygen					
s	0.2052	-0.0721	0	0	-0.2484
px	0	0	-0.1168	0.2012	0
py	0.2221	0.0625	0	0	0.0140
pz	-0.0340	0.0936	0	0	0.2053

<sup>a</sup> These values apply to all three conformations, but the designation of orbitals for the lone pair of N will vary according to the model.

We have neglected the atomic cores in this analysis. These core repulsions have effectively been included in the calculation by the choice of Coulomb integrals and by the proportionality of resonance integrals

( $H_{ij}$ ) to overlap, and hence are largely cancelled by electron-nuclear attractions. The resulting bonding energy is, in fact, too large by about a factor of two because the electron-electron repulsion energies are not completely cancelled. Similar results have been described elsewhere,<sup>19</sup> and provide a basis for an extensive and successful application of LCAO-MO methods to conformations and bonding in organic systems. The magnitude of the core repulsions, neglected here for the above reasons, is so great that the core interaction for the  $C_{2v}$  case is 7938 e.v., about six times greater than the total orbital energy, and hence leads to no bonding at all! This question has also been discussed by Ruedenberg,<sup>20</sup> who also justifies the neglect of core repulsions in a somewhat different, but related, analysis of the various energy contributions to the stabilities of molecules.

Finally, partial support of these conclusions comes from recent experiments<sup>10</sup> which eliminate the possibility of a barrier to inversion of a pyramidal configuration about  $C\alpha^-$  as a cause of retention of configuration in carbanions of this type.

**Acknowledgments.**—We acknowledge suggestion of this problem by Professor E. J. Corey with whom we have enjoyed many discussions. This research was supported by the National Institutes of Health, the Air Force Office of Scientific Research, and by award of a Fellowship to T. J. by the Socony Mobil Company. We also thank Mr. R. M. Stevens for use of molecular integral programs, and the Computation Centers at Harvard and M.I.T. for use of facilities.

- (19) R. Hoffmann and W. N. Lipscomb, *J. Chem. Phys.*, **37**, 2872 (1962).  
 (20) K. Ruedenberg, *ibid.*, **34**, 1861 (1961).

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## The Crystal Structure of Aureomycin (Chlortetracycline) Hydrochloride. Configuration, Bond Distances and Conformation

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The crystal structure of aureomycin hydrochloride has been refined with X-ray data used in a previous study and also with independent, more extensive data. The stereochemistry 1 was confirmed, and the bond distances are now in much better accord with the chemical structure. The conformation about C(4)–C(4a) is eclipsed, contrary to expectation, and is expected to remain so in this compound and in other tetracyclines. A reinterpretation of the chemistry involving this bond is indicated. In the  $\beta$ -tricarbonylmethane system at C(2) the hydrogen atom appears to be localized on the oxygen atom of the amide group. The hydrogen bond system is satisfactory, all eight of the possible hydrogen atoms of the cation entering into hydrogen bonds, four of which are intramolecular. The configuration at C(5) in the related compound terramycin is indicated to be OH(5) *cis* to OH(6).

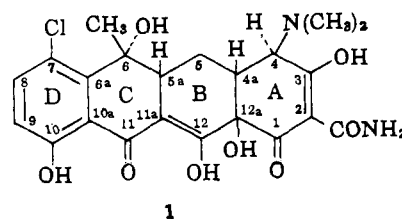
### Introduction

A determination of the crystal structure of aureomycin<sup>1</sup> hydrochloride, based on three dimensional X-ray data, has been reported by Hirokawa, Okaya, Lovell and Pepinsky.<sup>2a,b</sup> This work confirmed the constitution that had been derived on chemical grounds for aureomycin<sup>3</sup> and at the same time established the relative configurations of the five asymmetric carbon atoms in the molecule as shown in 1.

(1) Aureomycin is the registered trade name of the American Cyanamid Co. for the compound having generic name 7-chlortetracycline. A related antibiotic is Terramycin, which is the registered trade name of Chas. Pfizer and Co. for 5-hydroxytetracycline.

(2) (a) S. Hirokawa, Y. Okaya, F. M. Lovell and R. Pepinsky, *Acta Cryst.*, **12**, 811 (1959); (b) *Z. Krist.*, **112**, 439 (1959).

(3) C. R. Stephens, L. H. Conover, R. Pasternack, F. A. Hochstein, W. T. Moreland, P. P. Regna, F. J. Pilgrim, K. J. Brunings and R. B. Woodward, *J. Am. Chem. Soc.*, **76**, 3568 (1954).



Some of the conclusions drawn by these authors appear, however, to conflict with 1. For example, it was stated that: "(a) ring D takes a partially quinone structure; (b) C(11) and O(11) are single bonded; (c) no double bond is localized between C(11a) and C(12)." These conclusions, which were drawn largely on the basis of the observed bond lengths, do not seem justifiable in view of the rather large standard errors of

the positional parameters of the atoms. Certain other features of the discussion of the molecular structure are unsatisfactory. These include obvious numerical errors in the calculation of the best planes through groups of atoms and, more important, a quite erroneous description of the conformation about the C(4)–C(4a) bond. Finally, the identification of the oxygen and nitrogen atoms of the amide group on C(2) was left open.

Thus it seemed worthwhile to refine the structure further, in the hope that the uncertainties in the positional parameters, and hence in the bond lengths, could be reduced. These calculations have now been carried out, not only with the X-ray data used previously<sup>4</sup> but also with an independent and more extensive set of experimental data. The complete description of these calculations, together with a detailed discussion of the procedures used and the results obtained with them, will be presented elsewhere.<sup>5</sup> The present report describes those results which are of prime interest to chemists.

### Experimental

Recrystallization of a commercial sample of aureomycin hydrochloride (Lederle Laboratories) from water gave diamond-shaped plates which, on X-ray examination, proved to be orthorhombic,  $a = 11.2$ ,  $b = 12.8$ ,  $c = 15.5$  Å. The observed density, 1.52 g. cm.<sup>-3</sup>, corresponds to a molecular weight of  $515 \pm 5$  for 4 molecules of C<sub>22</sub>H<sub>24</sub>N<sub>2</sub>O<sub>8</sub>Cl<sub>2</sub> (molecular weight: 515.3) per unit cell. The systematic absences show the space group to be  $P2_12_12_1$  ( $D_2^4$ , no. 19). These results<sup>6</sup> agree with those of Hirokawa, *et al.*,<sup>7</sup> to prevent confusion we shall use their lattice constants  $a = 11.20$ ,  $b = 12.89$ ,  $c = 15.47$  Å. The intensities were recorded in a Weissenberg camera and estimated visually with the multiple film technique. All reflections accessible with CuK $\alpha$  radiation were sought. A total of 2057 unique reflections was observed and, aside from the space group extinctions, 682 accessible reflections were too weak to be observed. These numbers compare with the 1348 observed reflections used in the previous study. The main reason for the increase of more than 50% in the number of observed reflections lies in the fact that larger crystals (*ca.* 0.4 mm. maximum dimension) were used in the present study. Neither set of intensity data was corrected for absorption. However, it has been shown<sup>7</sup> that moderate absorption errors do not significantly affect positional parameters obtained from a refined structure, and this observation was borne out again in the present analysis. The differences in the positional parameters derived from the two independent sets of data were not statistically significant. On the other hand, the differences in the derived "thermal vibration parameters" were significant, as was to be expected. However, since our main interest lies in the space relationships of the atoms in the crystal rather than in their thermal motion, the increase in precision in the positional parameters attainable with the more extensive data caused us to focus most attention on these results.

**Identification of Material.**—Aureomycin readily undergoes reversible epimerization at C(4) in the pH range of approximately 2 to 6; the antibacterial activity (*vs.* *S. aureus*) of 4-epiaureomycin is less than 5% of that of aureomycin itself.<sup>8</sup> In the preliminary note<sup>2a</sup> of Hirokawa, *et al.*, it was stated that as a result of single crystal examination of fresh samples of the hydrochlorides of both aureomycin and epiaureomycin, the structure reported was that of aureomycin; but no other details were given and the point is not mentioned again in the fuller report.<sup>2b</sup> Comparison of the two sets of X-ray data leaves no doubt whatsoever that our data and the previous data come from the same material. Since, however, it seems imperative that the identity of this material be established unequivocally, the following tests were made on portions of the original sample from which the crystals used in recording our diffraction data had been selected earlier (10 years): (1) The ultraviolet spectrum (0.307 mg. in 10 ml. of 0.1 *N* H<sub>2</sub>SO<sub>4</sub>) is identical with that of aureomycin hydrochloride as given by McCormick, *et al.*,<sup>8</sup> and different from that of the 4-epimer. (2) The X-ray powder

diagram agrees with that published by Krc and McCrone<sup>9</sup> who obtained their crystals by evaporation of a saturated solution of aureomycin hydrochloride in 0.02 *M* HCl. Under these conditions, epimerization cannot be detected.<sup>8</sup> Krc and McCrone also reported that the substance was orthorhombic with cell dimensions 11.27, 12.89, 15.48 Å., values in excellent agreement with both our results and those of Hirokawa, *et al.* Our crystals were grown by evaporation of an aqueous solution of the hydrochloride; those of Hirokawa, *et al.*, were grown from aqueous ethanol of unstated composition. We conclude that since the epimer *could not* be formed under the conditions used by Krc and McCrone, it *was not* formed in either of the more extensive X-ray studies. (3) A commercial (Lederle) sample of aureomycin hydrochloride gives the same X-ray powder lines as our sample, and in addition shows a set of strong lines which presumably arise from the inorganic salts added for buffering. (4) The antibacterial activity of our sample, in HPO<sub>4</sub><sup>3-</sup>/H<sub>2</sub>PO<sub>4</sub><sup>-</sup> buffer, is twice that of the commercial sample, measured against *S. aureus* and *B. subtilis*. In addition: (5) A preliminary X-ray study of single crystals of epiaureomycin hydrochloride<sup>10</sup> shows that that substance, when crystallized from 95% ethanol–5% water, is trigonal, the unit cell being utterly dissimilar in dimensions, shape and symmetry to that of aureomycin hydrochloride.

In view of the above evidence, we consider it established that our X-ray data (and those of Hirokawa, *et al.*) come from aureomycin hydrochloride and not from its 4-epimer.

**Confirmation of the Structure.**—Because of the complexity of the crystal structure of aureomycin hydrochloride and because of possible doubt about the configuration at C(4) (*vide infra*), we began by verifying the over-all correctness of the atomic positions cited by Hirokawa, *et al.*, in order to ensure that no atoms had been incorrectly located in the gross sense. We first used the new data in the calculation of a three-dimensional electron density synthesis from which the contributions of the two chlorine atoms and the eighteen carbon atoms of the hydronaphthacene nucleus had been subtracted. This function revealed the presence of the remaining fourteen heavy atoms in the molecule as clearly defined peaks at, or close to, the reported positions and contained no other significant peaks.

**Refinement of the Structure.**—The method of least squares was used to refine the positional and thermal parameters of the 34 heavy atoms in the molecule. Three different refinements were carried out: (1) with the newer, more extensive X-ray data, refining 34  $\times$  3 positional parameters plus 34 individual isotropic thermal vibration parameters, and calculating the full matrix of the corresponding normal equations, a procedure which allows interaction of any variable with any other; (2) also with the newer data, refining the 34  $\times$  3 positional parameters plus 34  $\times$  6 anisotropic thermal vibration parameters by the use of the block diagonal approximation which allows interaction between the nine variables of any atom but not for interatom interactions; (3) with the data of Hirokawa, *et al.*, again allowing for anisotropy and using the block diagonal approximation. These calculations were carried out on an IBM 7090.

All of these procedures led to essentially the same results within their own standard errors, and the detailed comparisons among them as well as with the previously published set<sup>2b</sup> are made elsewhere.<sup>5</sup> The final interatomic distances and their standard errors resulting from refinement 2 are presented in Table I.

**Identification of the Atoms of the Amide Group.**—The code numbers which were assigned to the various atoms for computational purposes were not those of the conventional numbering system of 1. In particular, in the amide group bonded to C(2), the carbon atom is no. 21, its ligand nearer to O(1) is no. 22, and its ligand nearer to O(3) is no. 23. At an early stage in our work, the identification of atoms 22 and 23 was tested by two independent refinements of their isotropic thermal vibration parameters,  $B_i$ , first with 22 as ni-

(4) We are indebted to Professor R. Pepinsky for placing his X-ray data at our disposal.

(5) J. Donohue, J. D. Dunitz, K. N. Trueblood and M. S. Webster, *Acta Cryst.*, to be published.

(6) J. D. Dunitz and J. E. Leonard, *J. Am. Chem. Soc.*, **72**, 4276 (1950).

(7) F. Jellinek, *Acta Cryst.*, **11**, 677 (1958).

(8) J. R. D. McCormick, S. M. Fox, L. L. Smith, B. A. Bitler, J. Reichen-thal, V. E. Origoni, W. H. Muller, R. Winterbottom and A. P. Doerschuk, *J. Am. Chem. Soc.*, **79**, 2849 (1957).

(9) J. Krc and W. C. McCrone, *Anal. Chem.*, **22**, 1576 (1950).

(10) K. Folting and W. N. Lipscomb, personal communication.

TABLE I  
 BOND DISTANCES<sup>a</sup>

C—C (formal single bond)	C=C	
Tetrahedral-tetrahedral	C(11a)—C(12)	1.36 Å.
C(4)—C(4a)	1.57 Å.	
C(4a)—C(12a)	1.51	
C(4a)—C(5)	1.56	
C(5)—C(5a)	1.53	
C(5a)—C(6)	1.58	
C(6)—CH <sub>3</sub> (6)	1.51	
Trigonal-tetrahedral	C—OH	
C(6)—C(6a)	1.56	
C(3)—C(4)	1.54	
C(5a)—C(11a)	1.50	
C(12)—C(12a)	1.50	
C(12a)—C(1)	1.57	
C—C (conjugated)	C—OH	
Trigonal-trigonal	C(10)—OH(10)	1.37
C(10a)—C(11)	1.46	
C(11)—C(11a)	1.45	
C—C (tricarbonyl)	C(12)—OH(12)	1.35
C(1)—C(2)	1.44	
C(2)—C(3)	1.40	
C(2)—C(am)	1.43	
C—C (benzene)	C(am)—OH-(am)	1.32
C(6a)—C(7)	1.40	
C(7)—C(8)	1.41	
C(8)—C(9)	1.34	
C(9)—C(10)	1.41	
C(10)—C(10a)	1.42	
C(10a)—C(6a)	1.39	
	C=O	
	C(1)—O(1)	1.24
	C(3)—O(3)	1.25
	C(11)—O(11)	1.28
	C—N	
	N(4)—CH <sub>3</sub> <sup>I</sup>	1.52
	N(4)—CH <sub>3</sub> <sup>II</sup>	1.51
	N(4)—C(4)	1.49
	C—N	
	C(am)—N(am)	1.30
	C—Cl	
	C(7)—Cl	1.75

<sup>a</sup> A reasonable limit of error ( $2\sigma$ ) is 0.03–0.04 Å. for these distances.

trogen and 23 as oxygen, and second with the converse identification. Refinement of  $B$  of atom 21 was included as a control. The results are shown in Table II where it is seen that with 22 as oxygen an abnormally large apparent  $B$ -value (tending to reduce the effective number of electrons in the atom) is obtained; at the same time, identification of 23 as nitrogen gives a low  $B$ -value (tending to concentrate its electrons to render them more effective in scattering).

TABLE II

IDENTIFICATION OF ATOMS 22 AND 23. LEAST SQUARES ON  $B_i$ 

Atom	$B$ (in)	22 = N, 23 = O		22 = O, 23 = N	
		$B$ (out)		$B$ (out)	
21	3.37	3.26		3.28	
22	3.97	3.98		5.04	
23	3.85	4.31		3.18	

Finally, two refinement cycles were made starting with both atoms 22 and 23 identified as nitrogen and refining not only the  $B_i$  but also scale factors  $k_i$  which multiply the atomic scattering factor. The results, shown in Table III, confirm those obtained above, *i.e.*, that no. 22 is nitrogen and no. 23 is oxygen.

TABLE III

IDENTIFICATION OF ATOMS 22 AND 23. LEAST SQUARES ON  $B_i$  AND  $k_i$ 

Atom	$B$ (in)	$k$ (in)	$B$ (out)	$k$ (out)
22	3.97	1.00	4.26	1.05
23	3.85	1.00	4.54	1.26

**Configuration of Aureomycin.**—The configuration of aureomycin established by Hirokawa, *et al.*, is com-

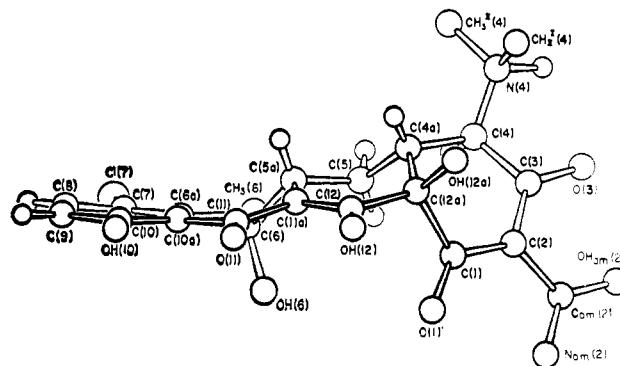


Fig. 1.—The aureomycinium cation, viewed in an arbitrary direction to show its general shape. The positions of some of the hydrogen atoms (smallest circles) were assumed; methyl and hydroxyl hydrogen atoms are not shown.

pletely confirmed by our new calculations. The spatial relationships between the atoms are evident in Fig. 1 which is a projection derived from our final positional parameters, in an arbitrary direction chosen to show the general shape of the entire molecule. This figure should not be taken as implying anything whatsoever about the chirality of aureomycin. As far as we know, the chirality has not been established.

Hirokawa, *et al.*, stated in their preliminary report<sup>2a</sup> that the configuration at C(4) is the converse of that assigned chemically. Various interpretations<sup>11–14</sup> of the chemical behavior of tetracyclines and their derivatives have been advanced in connection with the configuration at C(4). They are, however, tentative, inconclusive and sometimes contradictory. On the other hand, the X-ray analyses have shown in a perfectly straightforward and unequivocal manner that in aureomycin, the dimethylamino group at C(4) is *cis* with respect to the hydrogen atom at the ring junction C(4a). The relevant chemical reactions should now, therefore, be reconsidered in the light of this result.

**Bond Distances in Aureomycin.**—Bond distances in the aureomycin molecule, calculated from our final positional parameters, are shown in Fig. 2 and are listed in Table I. The standard errors are about one-

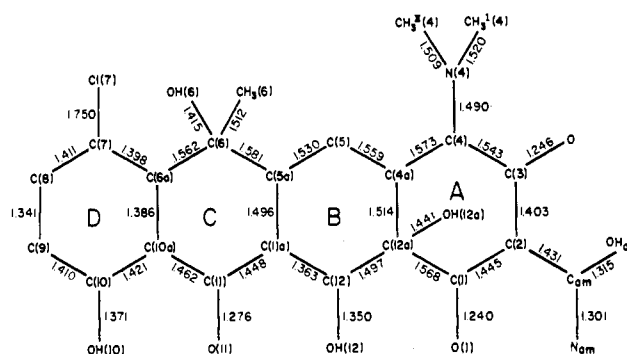


Fig. 2.—Bond distances in aureomycin.

half to one-third of those cited by Hirokawa, *et al.*, for corresponding bonds; the improvement in accuracy that has actually been attained is even better than would be indicated by this comparison for the standard

(11) F. A. Hochstein, C. R. Stephens, L. H. Conover, P. P. Regna, R. Pasternack, P. N. Gordon, F. J. Pilgrim, K. J. Brunings and R. B. Woodward, *J. Am. Chem. Soc.*, **75**, 5455 (1953).

(12) C. Waller, B. L. Hutchings, R. W. Broschard, A. A. Goldman, W. J. Stein, C. F. Wolf and J. H. Williams, *ibid.*, **74**, 4981 (1952).

(13) A. Green and J. H. Boothe, *ibid.*, **82**, 3950 (1960).

(14) A. S. Kende, T. L. Fields, J. H. Boothe and S. Kushner, *ibid.*, **83**, 440 (1961).

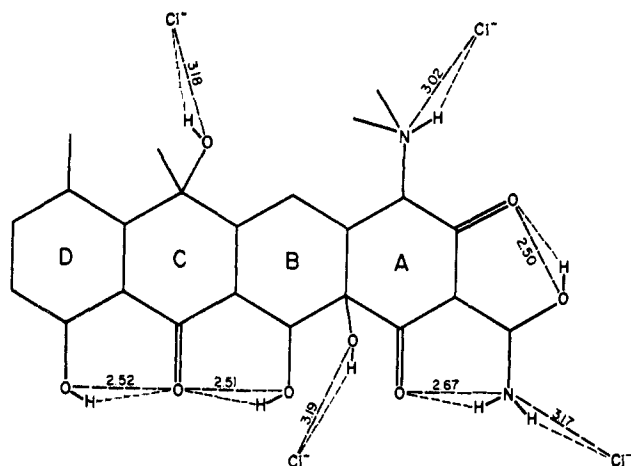


Fig. 3.—The hydrogen bonding, shown schematically.

errors cited previously<sup>2b</sup> were calculated by a method<sup>15</sup> that has been shown<sup>16</sup> to underestimate standard errors by a factor which increases with the proportion of unobserved reflections. Our results thus provide a much more reliable basis for the discussion of the bond distances than those of the previous work.

Differences of more than 0.1 Å. between the bond distances reported by Hirokawa, *et al.*, and those of Table I occur for three bonds; C(10)–O(10) has changed from 1.25 to 1.37 Å, C(11)–O(11) from 1.38 to 1.28 Å, and N(4)–CH<sub>3</sub><sup>1</sup> from 1.41 to 1.52 Å. In each case, the change is to a more reasonable value. The localized double bond C(11a)–C(12), previously reported to be 1.43 Å., is now found to be 1.36 Å. Thus, three of the conclusions drawn by Hirokawa, *et al.*, namely, that ring D is quinonoid, that C(11)–O(11) is a single bond, and that no double bond is localized between C(11a) and C(12), are all found to be untenable.

The carbon–carbon bonds may be considered to fall into five groups, as separated in Table I. The average of the six formally single bonds between tetrahedral carbon atoms is 1.545 Å., a value only slightly and not significantly higher than the average of 1.533 Å. for the five single bonds between trigonal and tetrahedral carbon atoms. The other carbon–carbon bonds are all shorter and have lengths in agreement with the expected values.<sup>17</sup> The C–Cl, C=O, C–N and C–OH bond lengths are also normal, the C(trigonal)–OH bonds being somewhat shorter than the C(tetrahedral)–OH bonds, as expected.

The dimensions of the tricarbonylamide system require special comment. In a normal amide, the

resonance form  $\text{--}\overset{\text{O}^-}{\text{C}}=\overset{+}{\text{N}}\text{H}_2$  contributes to an extent such that the carbon–oxygen bond length is increased from the double bond value of 1.20 Å. to about 1.23 Å. and the carbon–nitrogen distance is decreased from the trigonal–trigonal single bond value of 1.41 Å. to about 1.32 Å. In no case has it been observed that the C–N bond is shorter than the C–O bond. The results for the aureomycinium ion, with C–O = 1.32 Å. and C–N = 1.30 Å., clearly indicate that this is no normal amide, and the comparison of the three C–O bond lengths in the tricarbonyl system strongly suggests that the hydrogen atom is associated with the amide oxygen atom, at least in this crystal.

(15) D. W. J. Cruickshank, *Acta Cryst.*, **2**, 65 (1949).

(16) R. A. Sparks, Thesis, University of California, Los Angeles, 1958.

(17) I. E. Sutton, editor, "Tables of Interatomic Distances and Configuration in Molecules and Ions," Special Publication No. 11, The Chemical Society, London, 1958.

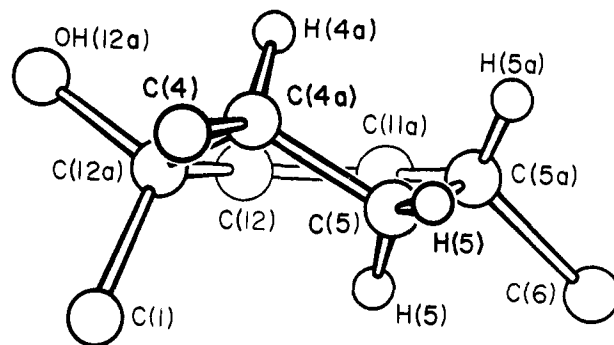


Fig. 4.—Ring B, viewed parallel to the plane of C(5a)–C(11a)–C(12)–C(12a).

**Hydrogen Bonds.**—There are eight hydrogen atoms in the aureomycinium cation which are capable of entering into hydrogen bond formation. These are: five hydroxyl, two on the nitrogen atom of the amide group and one dimethylammonium. Examination of the structure shows that all eight do in fact enter into hydrogen bonds; four of these are intramolecular, to neighboring oxygen atoms, and the remainder are to four different chloride ions. The structure is thus held together by a three dimensional network of hydrogen bonds. The hydrogen bonding is shown schematically in Fig. 3.

**Conformation of Aureomycin.**—Certain features of the conformation of aureomycin are evident from Fig. 1. Ring D and its ligands are, as expected, nearly coplanar. Considerable distortion of the bond angles at C(7) occurs, clearly as a result of steric repulsion between the chlorine atom and the methyl group on C(6); the Cl...CH<sub>3</sub> distance is 2.99 Å. which is still much less than 3.8 Å., the sum of the van der Waals radii.<sup>18</sup>

Ring C tends to adopt a somewhat boat-like form with the four atoms C(6), C(6a), C(10a) and C(11) more or less coplanar with ring D; C(5a) is considerably displaced from this plane, C(11a) less so but in the same sense, as can be seen from Fig. 1.

Ring B assumes the half-chair conformation. This is not so apparent from Fig. 1 but it is brought out more clearly in Fig. 4. We have here the third experimental verification by means of X-ray crystallography of this conformation for a cyclohexene ring, the other two examples occurring in 2,3,4,5,6-pentachlorocyclohexene-1<sup>19</sup> and in *trans*-β-ionylideneacetic acid.<sup>20</sup> This conformation has been predicted<sup>21</sup> to be more stable than the half-boat form by 2.7 kcal. mole<sup>-1</sup>.

Ring A, like ring B, contains a double bond. However, the double bond in A, unlike that in B, is not localized. Three tautomeric forms occur but none of these provides a ready explanation of the actual conformation, which is shown most clearly in Fig. 5. It is seen to be that of a rather flattened boat with C(3) and C(12) in the flagpole positions. It is also obvious from Fig. 5 that the conformation about the bond C(4)–C(4a) is eclipsed, with the dimethylammonium group at C(4) and the hydrogen atom at C(4a) in an almost *syn*-planar relationship.<sup>22</sup> There are in the molecule five other single bonds between tetrahedral atoms, C(4a)–C(5), C(5a)–C(6), C(5)–C(5a), C(4a)–C(12a)

(18) I. Pauling, "The Nature of the Chemical Bond," Cornell University Press, Ithaca, N. Y., 3rd Edition, 1960, p. 260.

(19) R. A. Pasternak, *Acta Cryst.*, **4**, 318 (1951).

(20) E. L. Eichhorn and C. H. MacGillavry, *ibid.*, **12**, 872 (1959). The conformation of the cyclohexene ring in this compound is not referred to in the text. The atomic positions cited lead, however, to the half-chair conformation.

(21) C. W. Beckett, N. K. Freeman and K. S. Pitzer, *J. Am. Chem. Soc.*, **70**, 4227 (1948).

(22) See W. Klyne and V. Prelog, *Experientia*, **16**, 521 (1960), for a recommended terminology of steric relationships across single bonds.

and C(4)–N(4), whose conformations can be found from this study. Projections along each of these bonds have been prepared and all five show the expected staggered conformation and remarkably well too, considering the distortions in the rings caused by the presence of double bonds.

The conformation about C(4)–C(4a) was misrepresented in the paper by Hirokawa, *et al.*, where a drawing<sup>23</sup> indicates a staggered arrangement. The discussion of this conformation in the text (where it is stated, for example, that "the hydrogen atom on C(4a) is 'gauche' to the dimethyl group") is also erroneous. A projection down the C(4)–C(4a) bond, prepared from their published positional parameters, shows a nearly eclipsed arrangement, very similar to that seen in Fig. 5.

The eclipsed conformation about the C(4)–C(4a) bond is unusual. It is certainly not due to purely geometrical restraints and the problem arises of whether it is nevertheless an intrinsic characteristic of the tetracyclines and of their 4-epimers and persists under a wide range of environments or whether it occurs only in special circumstances such as pertain in crystals of aureomycin (and presumably terramycin, *vide infra*) hydrochlorides. In the absence of reliable potential curves for estimating the complex variations in energy that will occur in the process of changing the conformation, it is not possible to provide a definite answer to this question. Nevertheless, some ideas can be gained from model considerations.

If the conformation about C(4)–C(4a) were staggered, two possibilities arise, depending on the sense of rotation from the observed eclipsed arrangement. Rotation of the dimethylammonium group into the pseudo-equatorial position of the ring does not seem to be possible without introducing other conformational changes which include conversion of ring B into the energetically unfavorable boat form by eclipsing of the bond C(4a)–C(5). It seems unlikely that the net energy change for such a rotation will be favorable under any circumstances and we shall not consider it further.

The only serious effect of a rotation of the dimethylammonium group in the opposite sense, into the pseudo-axial position of the ring, is to introduce a new 1,3-diaxial interaction between this group and the hydroxyl group at position 12a. In aqueous media, where both of these groups will be hydrated, a strong repulsive interaction seems certain. The repulsive energy may be reduced only in circumstances where an intramolecular hydrogen bond can be formed between these groups, *i.e.*, in the absence of solvating molecules or ions. But the crystal itself provides just such an environment and here the extra intramolecular hydrogen bond is not formed: the eclipsed conformation with hydrogen bonding of both groups to chloride ions is preferred. For the 4-epimers, however, it is quite another story. Here the corresponding rotation produces a 1,3-diaxial interaction between OH(12a) and H(4) and the net energy balance on going to this conformation may not be so unfavorable.

For the tetracyclines themselves, we therefore expect the unusual eclipsed conformation about C(4)–C(4a) to persist under a rather wide range of conditions; for the 4-epimers, on the other hand, the staggered arrangement with H(4) in the pseudo-axial position of the ring may be preferred. In any case, a reinterpretation of those chemical reactions that have been used to infer the stereochemistry at C(4) would seem highly desirable.

**Configuration of Terramycin.**—The isomorphism that exists between crystals of aureomycin hydrochloride and terramycin hydrochloride, and the striking

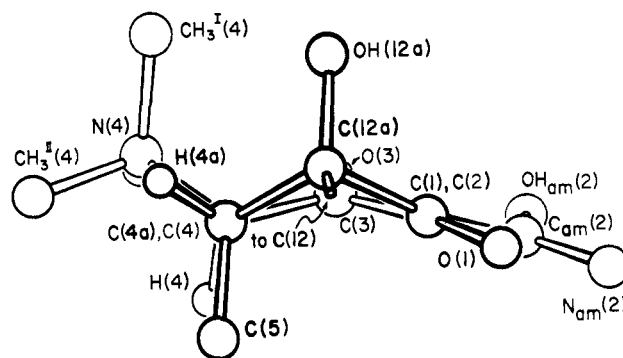


Fig. 5.—Ring A, viewed along the direction of the bonds C(1)–C(2) and C(4a)–C(4).

resemblance between the intensities of the X-ray diffraction maxima from the two substances led early to the suggestion that the two molecules must be closely similar in shape and orientation in closely related unit cells and, further, that the non-ionic chlorine atom in aureomycin was replaced by a hydroxyl group in terramycin.<sup>24,25</sup> While the latter suggestion has proved to be incorrect, the similarity in shape and orientation must be retained in order to explain the concordance of the X-ray data. Supporting evidence on this point is found in the similarity between the preliminary positional parameters of the chloride ion in terramycin hydrochloride<sup>26</sup> and the final positional parameters of this ion in aureomycin hydrochloride,<sup>2b,5</sup> and also in the close similarity of the optical properties of the two salts.<sup>9,26</sup> It may therefore be concluded that the relative configurations at the asymmetric atoms which are common to both substances (*viz.*: C(4), C(4a), C(5a), C(6) and C(12a)) are the same in both molecules. Inversion at any of the three bridgehead atoms C(4a), C(5a), or C(12a) would alter drastically the molecular shape, while inversion at C(4) or C(6), although not changing too much the over-all molecular shape, would alter the directions of hydrogen-bond-forming groups which determine the packing of the ions in the crystal and would thus lead to a change in the relative orientation of the cations. The same conclusion has been arrived at from the close similarity of the chemical and biological properties of the two substances.<sup>14</sup>

Considerations of the same kind allow the assignment to be made of the configuration at C(5) in terramycin. If OH(5) is *cis* to OH(6), a new intramolecular hydrogen bond almost identical in geometry to those between OH(10) and O(11) and between OH(12) and O(11) can be formed without disturbing the OH(6) . . . Cl<sup>-</sup> hydrogen bond. This condition would allow the molecules to assume the same orientation in the crystalline hydrochloride as in aureomycin hydrochloride. On the other hand, if OH(5) is *trans* to OH(6), it would be too far for hydrogen bond formation, not only from OH(6) but also from any other acceptor atom in the structure. In order to conform to the principles of maximum hydrogen bonding<sup>27,28</sup> the molecules would then be forced to assume a different orientation, if not a different crystal structure.

Takéuchi and Buerger<sup>29</sup> have recently proposed a crystal structure for terramycin hydrochloride in which the orientation of the terramycin molecule is indeed very

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similar to that of the aureomycin molecule in aureomycin hydrochloride. The hydroxyl group OH(5) has, however, been placed equatorially with respect to ring B, *i.e.*, *trans* to OH(6). The published set of positional parameters leads to distances of about 3.75 Å. from OH(5) to the nearest chloride ion and of more than 4 Å. for OH(5) . . . OH(6); both of these distances are much too long for hydrogen bonding to take place. The evidence presented by Takéuchi and Buerger consists of a minimum function that contains many spurious peaks and of an electron density projection down the *a*-axis (11.2 Å.) in which severe overlapping of many of the atoms occurs. This evidence cannot be regarded as constituting conclusive proof of the correctness of their proposed structure. The structure is probably correct in its general lines but the published projection is just as compatible with the alternative *cis* relationship of OH(5) and OH(6) which would allow a satisfactory intramolecular hydrogen bond to be formed.

Thus although the X-ray information on the configuration at C(5) in terramycin is incomplete and does not permit an unequivocal conclusion to be drawn, a strong case can be made for the *cis* relationship between OH(5) and OH(6), in agreement with the tentative chemical assignment.<sup>11</sup>

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[CONTRIBUTION FROM THE EVANS CHEMICAL LABORATORY, THE OHIO STATE UNIVERSITY, COLUMBUS, 10, OHIO]

## A Study of the Ethyl-Oxygen Reaction by Flash Photolysis

BY DAVID P. DINGLEY AND JACK G. CALVERT

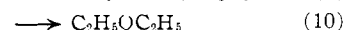
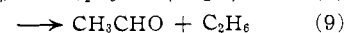
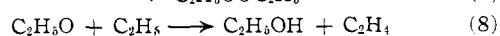
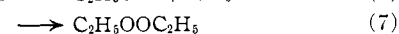
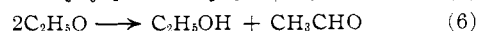
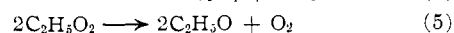
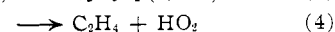
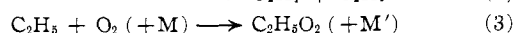
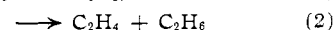
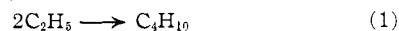
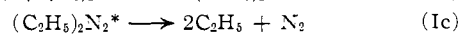
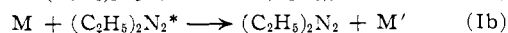
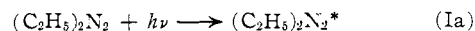
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Ethyl radicals were formed by the flash photolysis of azoethane in both fused quartz and Pyrex systems and with and without additions of moderating gas and oxygen. Flash photolyses in the fused quartz system formed thermally non-equilibrated radicals even with the addition of relatively large quantities of moderating gas (up to 9.5 cm. of methanol). Similar photolyses in the Pyrex system with moderating gas resulted in thermal equilibration of the ethyl radicals as judged by the normal, thermal value obtained for the disproportionation to combination ratio of the ethyl radicals ( $0.11 \pm 0.01$ ). The "hot" radicals in the flash photolyses in the fused quartz system have their origin in the absorption of the short wave length ultraviolet by azoethane.

For the pressure range 0.48–10.3 cm. at 22°, the reaction  $C_2H_5 + O_2 \rightarrow C_2H_5O_2$  (3) was shown to be second order with a rate constant,  $k_3 = 6.9 \times 10^{-12}$  cc./molecule-sec. The values of this rate constant derived from previous work on diethyl ketone-oxygen mixture photolyses are considerably lower than the present estimate probably because of the importance of  $C_2$ -product forming reactions other than H-abstraction from ketone. The present work shows the possible reaction  $C_2H_5 + O_2 \rightarrow C_2H_4 + HO_2$  (4) to be unimportant compared to reaction 3 for thermally equilibrated radicals at 22°.

The association reaction of the methyl radical with oxygen was shown to obey third-order kinetics<sup>1–3</sup> in the usual pressure range encountered (up to 31 cm. of neopentane). It is of both theoretical and practical interest to establish the degree of complexity in the initial reaction product ( $RO_2$ ) of a radical (R) with oxygen for the transition to the region of second-order kinetics to occur. Estimates of the rate constant for the ethyl-oxygen reaction are available<sup>4,5</sup>; however, the reaction system from which these were derived was very complex, and the results could not be expected to be of high accuracy. The previous estimates lie in the range  $k_3 = 10^{-13} - 10^{-14}$  cc./molecule-sec.<sup>4,5</sup> These values are low in comparison with the rate constants calculated for the assumed second-order kinetics in the methyl-oxygen reaction in the same pressure range:  $7 \times 10^{-12}$  cc./molecule-sec.<sup>3</sup> Therefore in this study we have attempted to redetermine both the order and the rate constant for the ethyl-oxygen reaction by measurement of the competitive reaction of radical combination following the flash photolysis kinetic techniques employed by Sleppy and Calvert.<sup>3</sup> Since the radical concentration must be very high for the combination reaction to proceed measurably when in competition with the reaction with oxygen, flash photolysis generation of the radicals is an essential

part of the method. Azoethane was our choice of ethyl radical source because of its convenient absorption region and the demonstrated simplicity of the photochemistry of this compound. However, in view of the previous studies of the alkyl and alkoxy radical reactions, it was anticipated that the reactions involved in the photooxidation of azoethane could be much more complex than those encountered in the azomethane photooxidation; in fact, it seemed likely that consideration of the following rather involved reaction sequence would be necessary in this case.



Other reactions involving hydroperoxide formation may also contribute to the mechanism. Although the complexity of the possible reaction scheme is great, it has been possible to sort out the kinetic data for

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