The Direct Determination of the Crystal Structure of p-Bromobenzoate–Glaucarubin[1]

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p-Bromobenzoate-glaucarubin, $C_{32}H_{39}O_{11}Br$, forms monoclinic crystals with space group $P2_1$ and lattice constants: $a = 17.35$ Å., $b = 6.99$ Å., $c = 13.79$ Å., $\beta = 110.80°$. The crystal structure was determined by computing three-dimensional electron density maps using the heavy atom method and refined, by least squares, to an R value of 0.14. The molecule was found to consist of three six-membered carbon rings, a lactone ring, and a ring containing a hemiacetal group in addition to various side groups. There is one intermolecular hydrogen bond per molecule which holds the molecules in spirals around the twofold screw axis.

The bitter lactone glaucarubin is found in *Simaruba glauca*[4] and has been used for a number of years as an intestinal amoebacide. Because of its chemical similarities to chaparrin[5] and quassin,[6] chemists have been studying it for some time in the hope·of determining the structures of this group of compounds.

The empirical formula of glaucarubin was known to be $C_{25}H_{36}O_{10}$. Neither the number of rings nor the positions of the methyl groups or oxygen atoms (except the oxygen atoms in the lactone ring) had been definitely established before this structural analysis.[7,8] A p-bromobenzoate derivative of glaucarubin was used for the X-ray analysis; the results showed that its structure was not that of any of the previously proposed structures for the molecule. The complete structural analysis is presented below.

Experimental

Crystals of p-bromobenzoate–glaucarubin were prepared and crystallized from a water–methanol solution by slow evaporation to dryness. The crystals are colorless and grow as flat needles along the (010) direction.

Preliminary X-ray crystallographic examination established that the crystals belong to the monoclinic system with space group $P2_1$ the lattice constants for these air-dried crystals are given in Table I.

TABLE I[a]

DRY CRYSTAL

$a = 17.35$ Å.
$b = 6.99$ Å.
$c = 13.79$ Å.
$\beta = 110.80°$
$\rho_{obsd} = 1.43$ g./cc.
$\rho_{calcd} = 1.44$ g./cc.

[a] Even though the accuracy of measurement of the lattice constants is better than ±0.01 Å., changes up to 0.1 Å. occurred during the measurements.

The volume of the unit cell and the density established that there are two molecules of p-bromobenzoate–glaucarubin per cell.

Three-dimensional X-ray intensities were collected on the GE-XRD5 with Single Crystal Orienter using Cu Kα radiation "monochromatized" by a balanced pair of cobalt and nickel filters. As the lattice constants and intensities were found to change during the collection of data, a different crystal was used after about 400 reflections were measured. A scale factor was

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obtained for each partial set of intensities, which allowed us to place all intensities on the same scale. In all, 2919 intensities were measured and these were converted to $|F|^2$ in the usual way and placed on an approximate absolute scale by the Wilson method.

Determination of the Crystal Structure

The crystal structure was solved by the heavy atom method using bromine as the heavy atom. The analysis proceeded through the computation of a series of three-dimensional electron density syntheses using more and more atoms in the phase angle calculations as they became established without ambiguity on inspection of these maps. No chemical knowledge of the structure, except the presence of one bromine atom per molecule, was assumed until all the nonhydrogen atom positions were revealed in the electron density syntheses. Up to that time all atoms, except the bromine, were represented as carbon atoms in the phase angle calculations. Thereafter, peak heights, as well as interatomic distances and bond angles, were used to identify the atoms and their corresponding scattering curves were used in later structure factor calculations and refinement cycles.

The starting point of the structure determination was, of course, the determination of the heavy atom (*i.e.*, bromine) positions in the unit cell. It was first hoped that the comparatively short rotation axis would enable this to be done in projection and attempts were made to determine the x and z parameters of the bromine atoms using data from the $h0l$ zone only. However, due to the large number of additional vectors, it was not possible to locate the bromine atoms unambiguously from the projected vector map; and it was decided to use the complete three-dimensional data to compute several vector maps with $|F|^2$ suitably modified to enhance the bromine–bromine vector appearing in the Harker section $(U\ ^1/_2\ W)$. From this section of these maps it was possible to determine the coordinates of the bromine atoms.

For the space group $P2_1$, to which the present crystal belongs, the position of the origin along the b axis is arbitrary. For convenience, the b axis origin was chosen as the midpoint between the two bromine atoms related by the twofold screw axis. With $y = 0.25$ and 0.75 for the two bromine atoms, the origin becomes a pseudo-center of inversion for these two atoms and the electron density synthesis computed with the bromine phases alone will result in a pseudo-mirror plane at $y = 0.25$. This results in the superposition of the molecule onto its mirror image, causing duplication of the atoms in the cell. It was necessary to remove this ambiguity in order to isolate and identify the atoms corresponding to a single molecule.
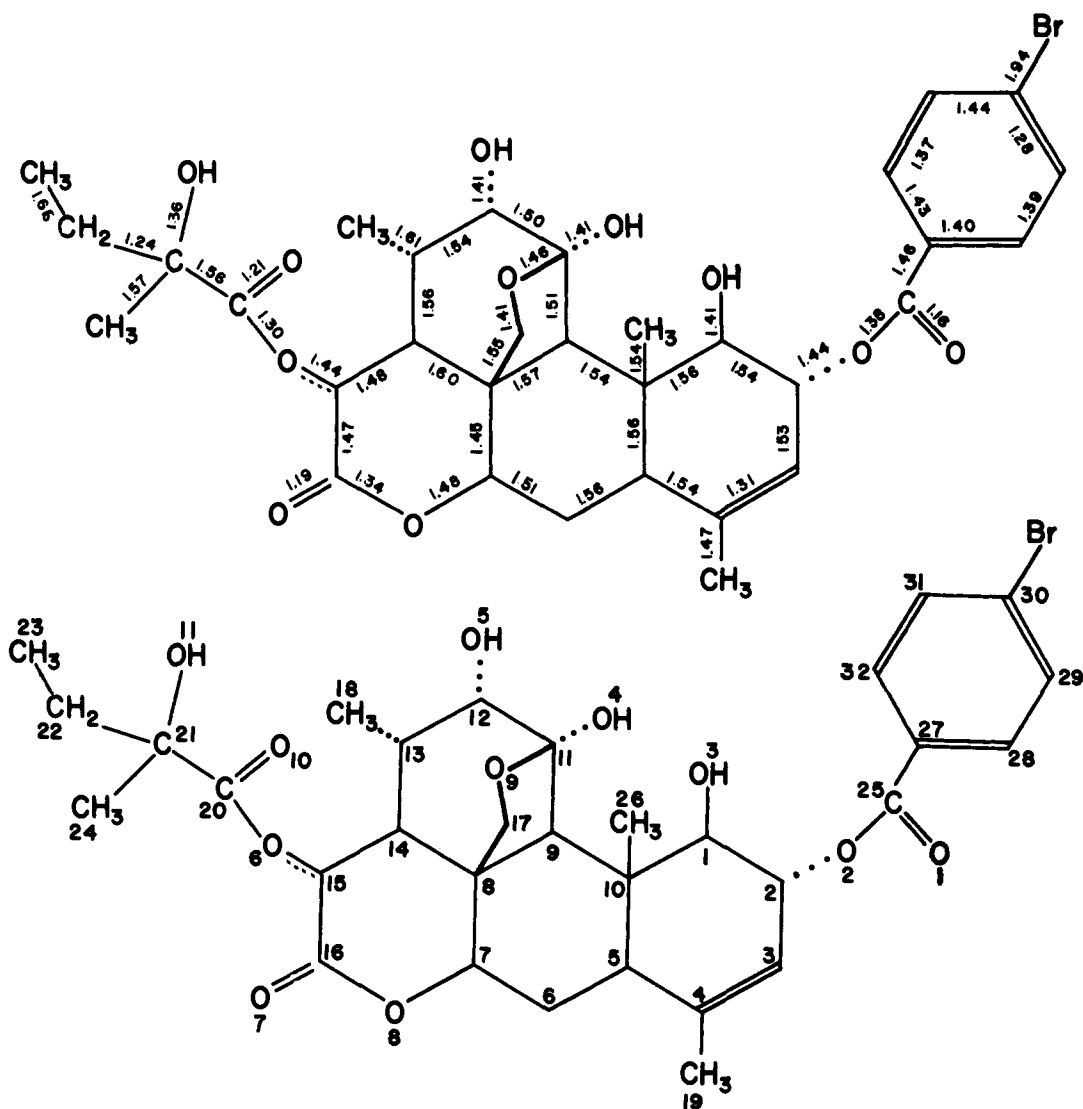
Fig. 1.—(a) Interatomic distances (top) and (b) chemical configuration (bottom) for the molecules; the eight-membered side chain has inaccurate values, as explained in the text.

This was done by the following procedures. In the bromine-phased electron density synthesis it was found that there were a large number of peaks, about 24, near the pseudo-mirror plane and some peaks further away. As the introduction of atoms on the pseudo-mirror plane would not help resolve this ambiguity, it was necessary to remove the additional symmetry by introducing atoms belonging to a single molecule and having y parameters significantly different from 0.25. However, care was necessary in choosing a group of atoms away from the mirror plane to make sure that all of these belonged to the same molecule and were not part of the superimposed image. For this, a group of four peaks having y parameters near zero was located in the electron density map which resembled a chemically sensible group, i.e., having reasonable interatomic distances and bond angles. It was assumed that these atoms would introduce enough asymmetry into the phase angles, which, in turn, would raise the value of peaks in the same molecule and lower those of the corresponding atoms of the superimposed ghost molecule. In fact, to increase and magnify the effectiveness of these four atoms in removing the mirror symmetry, they were introduced in the phase angle calculation

with about double their normal scattering power. A few cycles of structure factor calculations and least squares using these four atoms and all the atoms at the y = 0.25 pseudo-mirror plane showed that the asymmetry introduced by the four atoms was enough to move the atoms near the mirror plane away from it and in the direction to correspond to their positions in a single molecule. These shifted positions resulted in further removal of the pseudo-symmetry.

At the end of four cycles of least-squares calculations, it was possible to compute a second electron density map from which 22 sites were accepted as possible atomic positions. A structure factor calculation with the bromine and 21 additional atoms represented by carbon atoms gave a discrepancy factor of 0.39.

A third electron density synthesis was computed with these 22 atoms in the phase angle calculation, and this showed an additional 18 possible atomic positions. A phase angle calculation with these 40 atoms followed by a three-dimensional electron density and difference electron density synthesis revealed four more possible positions. A structure factor calculation with these 44 atoms, followed by electron density and difference electron density syntheses, showed that there were no
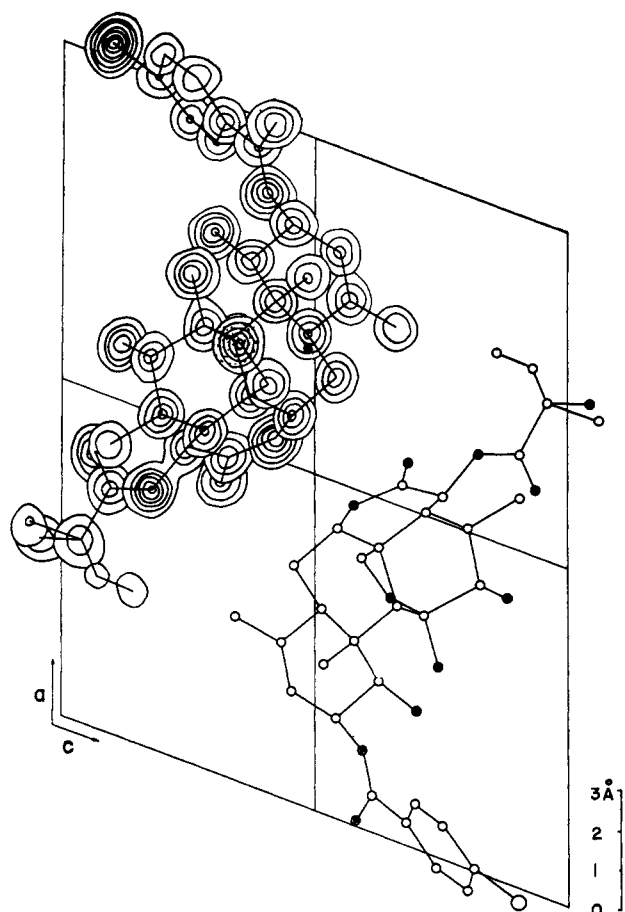
Fig. 2.—Composite projection of the electron density sections through the centers of the atoms; molecule on the right is the chemical interpretation of the contoured molecule. Open circles represent carbon atoms, double circles oxygen atoms, and the large circle the bromine atom.

additional atoms other than hydrogen in the unit cell. The discrepancy factor at this stage, with the molecule

TABLE II
PROGRESS OF THE REFINEMENT

Stage		R
1	Calculation of Patterson function to locate bromine atoms	
2	SFC^a with bromine atoms only	
3	EDS^b using phases from stage 2	
4	Four cycles of least squares and SFC with bromine plus 28 possible atoms	0.46
5	EDS with phases from stage 4	
6	SFC with bromine plus 21 of the 28 possible atoms	0.39
7	EDS using phases from stage 6	
8	SFC with bromine plus 39 possible atoms	0.30
9	EDS and $(F_o - F_c)$ EDS using phases from stage 8	
10	SFC with bromine plus 43 possible atoms	0.24
11	EDS and $(F_o - F_c)$ EDS using phases from stage 10	
12	Carbon and oxygen atoms identified, weak intensities removed	
13	Eight cycles of least-squares refinement and SFC with bromine, 32 carbon, and 11 oxygen atoms	0.14
14	EDS and $(F_o - F_c)$ EDS using phases from stage 13	

^{a} SFC, structure factor calculation. ^{b} EDS, electron density synthesis.

TABLE III

	x/a	y/b	z/c	σ	B
Br	0.0184	0.2563	0.0947	0.011	7.2
O$_1$	0.9933	0.9900	0.4130	0.021	9.0
O$_2$	0.8868	0.8163	0.4014	0.013	5.7
O$_3$	0.7988	0.1390	0.2948	0.013	5.7
O$_4$	0.7254	0.4700	0.2561	0.016	6.7
O$_5$	0.5825	0.2773	0.1189	0.020	5.5
O$_6$	0.3824	0.1124	0.1762	0.011	4.3
O$_7$	0.4360	0.8298	0.3117	0.016	5.1
O$_8$	0.5242	0.0095	0.4236	0.011	4.6
O$_9$	0.6477	0.5855	0.3450	0.014	5.9
O$_{10}$	0.4096	0.9226	0.0631	0.015	6.5
O$_{11}$	0.2483	0.8500	0.9583	0.037	13.1
C$_1$	0.7770	0.0396	0.3706	0.017	4.8
C$_2$	0.8574	0.9645	0.4523	0.018	5.1
C$_3$	0.8403	0.8747	0.5440	0.020	5.8
C$_4$	0.7729	0.9080	0.5637	0.019	5.7
C$_5$	0.7022	0.0287	0.4891	0.015	4.1
C$_6$	0.6487	0.1354	0.5419	0.017	4.8
C$_7$	0.5686	0.1927	0.4585	0.016	4.4
C$_8$	0.5791	0.3014	0.3743	0.016	4.3
C$_9$	0.6505	0.2458	0.3353	0.014	3.8
C$_{10}$	0.7292	0.1716	0.4207	0.016	4.1
C$_{11}$	0.6539	0.4290	0.2784	0.019	5.4
C$_{12}$	0.5817	0.4407	0.1780	0.021	6.1
C$_{13}$	0.5001	0.4604	0.1975	0.016	4.5
C$_{14}$	0.4938	0.3097	0.2775	0.015	4.1
C$_{15}$	0.4681	0.1114	0.2425	0.015	4.0
C$_{16}$	0.4742	0.9759	0.3261	0.015	3.9
C$_{17}$	0.6034	0.5126	0.4054	0.019	5.4
C$_{18}$	0.4258	0.4537	0.0875	0.020	5.8
C$_{19}$	0.7598	0.8246	0.6545	0.021	6.1
C$_{20}$	0.3614	0.0097	0.0925	0.019	5.4
C$_{21}$	0.2667	0.9867	0.0333	0.038	10.1
C$_{22}$	0.2217	0.0800	0.0685	0.042	10.7
C$_{23}$	0.2267	0.8867	0.1400	0.132	22.0
C$_{24}$	0.2617	0.1167	0.9383	0.054	12.9
C$_{25}$	0.9568	0.8588	0.3806	0.023	6.7
C$_{26}$	0.7858	0.3300	0.4864	0.020	5.7
C$_{27}$	0.9710	0.7067	0.3165	0.018	5.1
C$_{28}$	0.0209	0.7472	0.2588	0.024	6.7
C$_{29}$	0.0346	0.6144	0.1920	0.023	6.4
C$_{30}$	0.9995	0.4504	0.1840	0.021	6.0
C$_{31}$	0.9534	0.3795	0.2447	0.022	6.2
C$_{32}$	0.9406	0.5146	0.3088	0.021	6.0

represented by a bromine and 43 carbon atoms for 2919 reflections, was 0.24.

At this time interatomic distances and bond angles were calculated; all atoms were identified as either carbon or oxygen atoms from peak heights in the electron density maps and the distances and angles to their neighboring atoms. Thus, the molecule was represented by a bromine plus 32 carbon and 11 oxygen atoms in computing a series of diagonal least-squares refinements of positional and isotropic thermal parameters. After eight cycles of refinement using 1843 reflections (weak intensities removed), the R value fell to 0.14. An electron density synthesis at this stage showed no spurious peaks. A difference electron density synthesis showed no high values, either positive or negative, except near the bromine atom and the tail end of the eight-membered side chain attached to carbon 15, both of which are no larger than could reasonably be expected since the possible anisotropy of the atomic vibration terms was neglected in the phase angle calculations.

An outline of the progress of the refinement is given in Table II. The final coordinates, as well as the esti-
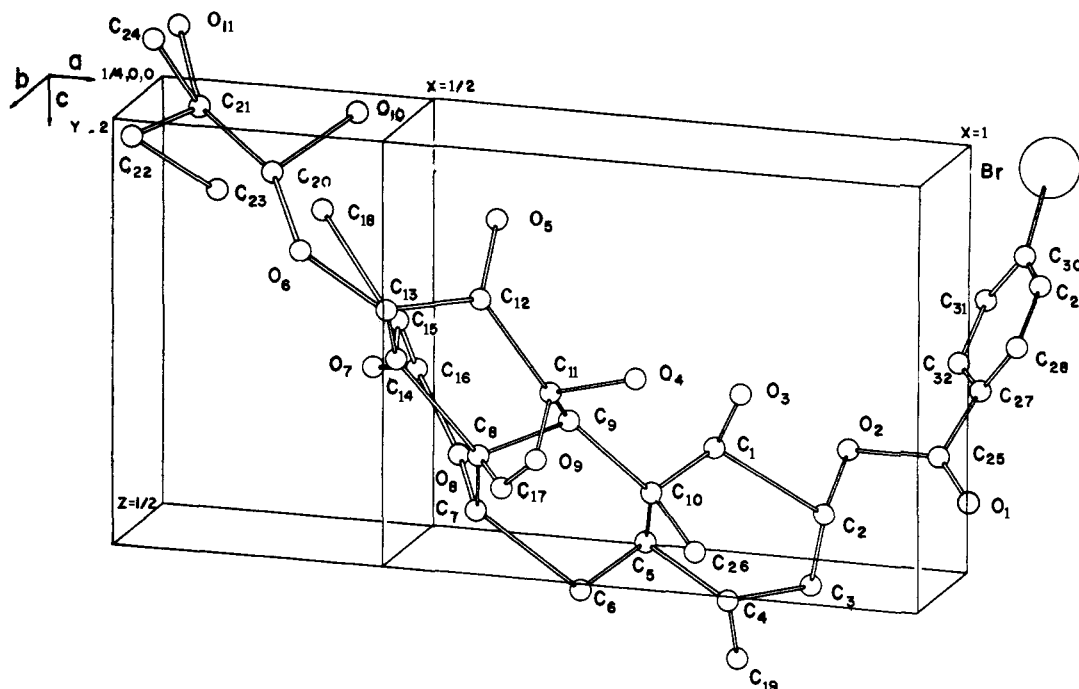
Fig. 3.—Perspective diagram of the molecule; note the chair form of the lactone and two adjoining rings.

mated standard deviations, are given in Table III, significant interatomic distances in Fig. 1a, and bond angles in Table IV. A composite drawing of the elec-

TABLE IV
BOND ANGLES

	Angle, deg.		Angle, deg.		Angle, deg.
C(2)C(1)C(10)	111	C(1)C(10)C(26)	111	C(21)C(20)O(10)	120
C(2)C(1)O(3)	107	C(5)C(10)C(9)	108	O(6)C(20)O(10)	124
C(10)C(1)O(3)	111	C(5)C(10)C(26)	112	C(20)C(21)C(22)	116
C(1)C(2)C(3)	111	C(9)C(10)C(26)	114	C(20)C(21)C(24)	96
C(1)C(2)O(2)	106	C(9)C(11)C(12)	111	C(20)C(21)O(11)	113
C(3)C(2)O(2)	108	C(9)C(11)O(4)	119	C(22)C(21)C(24)	100
C(2)C(3)C(4)	123	C(9)C(11)O(9)	106	C(22)C(21)O(11)	131
C(3)C(4)C(19)	122	C(12)C(11)O(4)	107	C(24)C(21)O(11)	81
C(5)C(4)C(19)	117	C(12)C(11)O(9)	108	C(21)C(22)C(23)	84
C(4)C(5)C(6)	115	O(4)C(11)O(6)	105	C(27)C(25)O(1)	128
C(4)C(5)C(10)	115	C(11)C(12)C(13)	111	C(27)C(25)O(2)	109
C(6)C(5)C(10)	109	C(11)C(12)O(5)	108	O(1)C(25)O(2)	123
C(5)C(6)C(7)	108	C(13)C(12)O(5)	112	C(25)C(27)C(28)	118
C(6)C(7)C(8)	114	C(12)C(13)C(14)	111	C(25)C(27)C(32)	126
C(6)C(7)O(8)	104	C(12)C(13)C(18)	108	C(28)C(27)C(32)	116
C(8)C(7)O(8)	113	C(14)C(13)C(18)	115	C(27)C(28)C(29)	122
C(7)C(8)C(9)	119	C(13)C(14)C(13)	110	C(28)C(29)C(30)	118
C(7)C(8)C(17)	112	C(13)C(14)C(15)	119	C(29)C(30)C(31)	127
C(7)C(8)C(14)	110	C(8)C(14)C(15)	109	C(29)C(30)Br	120
C(9)C(8)C(17)	99	C(14)C(15)C(16)	115	C(31)C(30)Br	113
C(9)C(8)C(14)	109	C(14)C(15)O(6)	109	C(30)C(31)C(32)	113
C(14)C(8)C(17)	106	C(16)C(15)O(6)	106	C(27)C(32)C(31)	124
C(8)C(9)C(10)	115	C(15)C(16)O(7)	123	C(2)O(2)C(25)	116
C(8)C(9)C(11)	99	C(15)C(16)O(8)	121	C(15)O(6)C(20)	118
C(10)C(9)C(11)	120	O(7)C(16)O(8)	116	C(7)O(8)C(16)	124
C(1)C(10)C(5)	102	C(8)C(17)O(9)	109	C(11)O(9)C(17)	106
C(1)C(10)C(9)	109	C(21)C(20)O(6)	116		

tron density sections through the centers of the atoms is given in Fig. 2 with the chemical interpretation in Fig. 1b.

Discussion of the Structure

The glaucarubin nucleus consists of five rings, one of which is a hemiacetal ring out of the plane of the six-membered hydrocarbon and lactone rings. In addition to the *p*-bromobenzoate group attached to carbon 2 of the nucleus, the molecule has three hydroxyl groups on carbons 1, 11, and 12; three methyl groups on carbons

4, 10, and 13; and an eight-membered side chain attached to carbon 15. The lactone ring and the two adjoining six-membered rings are in the chair form (Fig. 3). The distance between carbons 3 and 4 is significantly shorter than 1.54 Å. and has been interpreted as a carbon–carbon double bond. This is in agreement with the bond angles formed by these atoms. Some of these structural features had been established by chemical methods prior to this analysis and have been discussed in a previous publication.[9]

In the crystal, the eight-membered side chain has either considerable freedom of movement or some positional disorder; this results in a decrease in the electron density at the time average center of the atoms. For this reason, the positions of atoms O_{11}, C_{21}, C_{22}, C_{23}, and C_{24} were not well established so that distorted bond angles and inaccurate bond lengths resulted. However, the chemical structure of this side chain was known from previous chemical work[10] and was identical with the structure obtained from the X-ray analysis. Positioning of the atom C_{30}, which happens to be directly attached to the heavy atom, is also susceptible to unusually large errors; and bond lengths and angles involving this atom of the benzene ring are also less accurate.

The crystal is held together by van der Waals attractions and one intermolecular hydrogen bond per molecule; this hydrogen bond is between atoms O_5 and O_{10}' (2.76 Å.). As one can see from the packing diagram (Fig. 4), the hydrogen bonds of the molecules lie around the twofold screw axis and form spirals parallel to the *b* axis. van der Waals' attractions hold these spirals of hydrogen-bonded molecules together.

In addition to the single intermolecular hydrogen bond, there may be intramolecular hydrogen bonds between some of the oxygen atoms. Since the poor intensity data did not allow us to refine the structure

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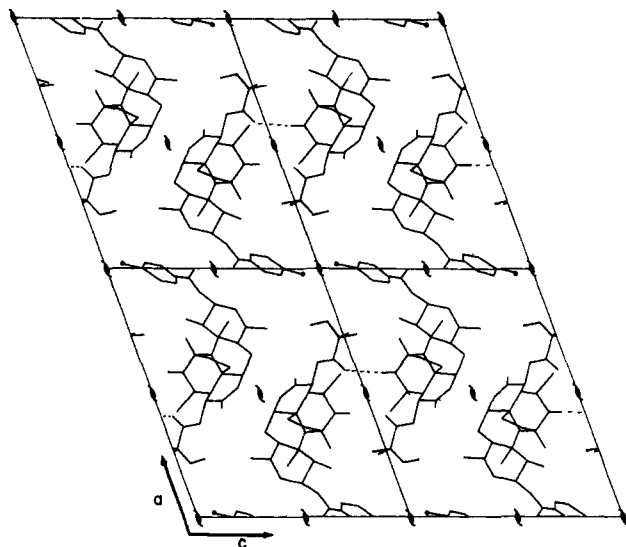(10) H. J. Schaeffer, private communication.

Fig. 4.—Packing diagram of the molecule. The hydrogen-bonded molecules form spirals parallel to the b axis; the spirals are held together by van der Waals' attractions.

sufficiently, we have listed in Table V all the oxygen atoms near enough to one another to permit internal hydrogen bonds.

TABLE V
CLOSE OXYGEN–OXYGEN DISTANCES AND PERTINENT ANGLES

	Units, Å.
O_2–O_3	2.83
O_3–O_4	2.60
O_4–O_5	2.86
O_{10}–O_{11}	2.70
	Units, deg.
C_1–O_3...O_2	57.3
C_1–O_3...O_4	110.4
C_2–O_2...O_3	57.4
C_{11}–O_4...O_3	100.3
C_{11}–O_4...O_5	55.0
C_{12}–O_5...O_4	54.5
C_{20}–O_{10}...O_{11}	64.4
C_{21}–O_{11}...O_{10}	62.5

On the Interaction of Magnesium with Deoxyribonucleic Acid[1a]

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Counterion activity coefficients of the pure sodium and magnesium salts of DNA (NaDNA and MgDNA, respectively) were measured by means of ion-exchange membrane electrodes. Viscosity measurements and determination of the molar extinction coefficients at 258 mμ were carried out on the same material. The following conclusions have been reached: (1) The magnesium activity coefficient of thermally denatured Mg-DNA is slightly lower than that of the native salt, whereas the sodium activity coefficient of thermally denatured NaDNA is substantially higher than that of the corresponding native salt. (2) A correlation is made between the magnitude and the concentration dependence of the activity coefficient and the thermal stability of the native structure. (3) Evidence is provided which suggests that the modes of binding of sodium and magnesium ions by DNA are quite similar, being diffuse electrostatic attractions typical of similar salts of a wide variety of synthetic polyelectrolytes. Evidence for some site binding is discussed in relation to the behavior of MgDNA when heated in solutions of simple magnesium salts.

Introduction

The importance of magnesium in many biological processes is well known. To cite several well-documented examples, there is a magnesium requirement both in the enzymatic synthesis of deoxyribonucleic acid (DNA)[2] and in the function of ATP-ase.[3] It has also been suggested that magnesium plays a significant role in holding ribosomal nucleoprotein together.[4] However, the nature of the interaction of magnesium with nucleic acids has been unclear with regard to

molecular interpretation and, indeed, with regard to phenomenological description of the interaction. Shack and co-workers have reported that native DNA binds magnesium more strongly than does denatured DNA on the basis of conductivity data[5] and titration with eriochrome black T.[6] Zubay and Doty have concluded precisely the opposite based on conductivity data.[7] The latter attributed the stronger binding by denatured DNA to the availability of purine and pyrimidine bases as possible binding sites. However, recent magnetic resonance studies of 0.1–0.2 M adenosine triphosphate[8a] and deoxyadenosine and cytidine[8b] have shown that there is no detectable interaction be-

(1) (a) Presented in part at the 145th National Meeting of the American Chemical Society, Sept. 8–13, 1963, New York, N. Y.; (b) to whom correspondence should be addressed at the Monsanto Co., St. Louis, Mo. 63166.

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