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The Crystal Structure of 1-Ethyl-5-bromouracil. II. The Crystal Structure of the Form II Crystal of 1-Ethyl-5-bromouracil

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Two crystalline forms of 1-ethyl-5-bromouracil were found. The crystal structure of the form II has been determined. The dimension of the tetragonal unit cell are $a=b=17.13$ Å, $c=5.36$ Å, and $Z=8$. The space group is $P4_2/n$. The final R is 0.064. The hydrogen bond scheme in this crystal, N(3)–H(3)$\cdots$O(2), is the first finding in alkylated pyrimidine dimers. The carbon atom binding to the nitrogen atom of the pyrimidine ring deviates significantly from the pyrimidine ring plane.

Two crystalline forms of 1-ethyl-5-bromouracil were recently found. The study of these structures showed the presence of different modes of hydrogen bond.[2] The detail of the structure of the form I is presented in the preceeding paper.[3]

The present paper deals with details of the crystal structure of the form II, in which a new hydrogen-bonding type in the alkylated pyrimidine dimers has been found.

<div align="center">

TABLE 1. CRYSTAL DATA

</div>

$C_6H_7BrN_2O_2$	M.W.$=219.05$
Tetragonal	$P4_2/n$
$a=b=17.13$ Å	$c=5.36$ Å
$D_{cal}=1.85$ g/cm³	
$D_{obs}=1.84$ g/cm³ (by flotation)	

Experimental

Two types of crystals were grown from a dimethyl sulfoxide solution at room temperature. From I crystal is a colorless plate-like crystal and the form II is a transparent needle. Preliminary Weissenberg and Precession photographs established that the form II was tetragonal with the c axis along the needle axis. The systematic extinction of (00l) for l odd and (hk0) for $h+k$ odd restricted the space group to $P4_2/n$. The crystal data are listed in Table 1.

A nickel filtered Cu-$K\alpha$ radiation was used to collect intensity data for all the reflections in the range $0<\sin\theta/\lambda<0.55$ Å^{-1} in octant (hkl) and ($\bar{h}k$0). All the intensities were measured on a Rigaku Denki computer-controlled four-circle diffractometer (AFC-II). A scintillation counter was used with a pulse-height discriminator. A crystal of approximate dimension, 0.02 mm$\times 0.02$ mm$\times 0.10$ mm ($0.14<\mu r<0.16$), was mounted with the c axis parallel to the ϕ-axis of the diffractometer. The ω-2θ scan technique was employed with a scan speed of 2°/min by ω, and backgrounds were measured for 6.00 sec at each start and end points of the scan range. A scan range of ω for each reflection was calculated by the formula;[4] scan range$=1.00°+0.15°\times\tan\theta$. Attenuators were automatically inserted when the maximum

1) Present address: Faculty of Engineering, Tottori University, Tottori.
2) H. Mizuno, N. Nakanishi, T. Fujiwara, K. Tomita, T. Tsukihara, T. Ashida, and M. Kakudo, *Biochem. Biophys., Res. Commun.*, **41**, 1161 (1970).
3) H. Mizuno, T. Fujiwara, and K. Tomita, This Bulletin, **45**, 905 (1972).
4) T. C. Furnas, (1957), Single Crystal Orienter Instruction Manual. Milwaukee: General Electric Co.

count rate exceeded 8000 cps. The intensities were corrected only for Lorentz and polarization factors.

Measurements of two reference reflections, (400) and (200), were repeated at every fifty reflections. For 35 time repetition of the measurements, $|F_o(400)|=149.50\pm0.57$ and $|F_o(002)|=93.14\pm0.56$. The standard deviation of $|F_o(hk0)|$ were assigned on the basis of the following equation

$$\sigma^2(|F_o|) = \langle(((|F_o(hk0)| - |F_o(\bar{k}h0)|)/2)^2\rangle_{av} \quad (1)$$

where $\langle\ \rangle_{av}$ represents the average of 10 reflections of similar magnitude of F_o. The standard deviations of $|F_o(hkl)|$ were substituted for $\sigma(|F_o(hk0)|)$ of similar $|F_o|$. An index of experimental accuracy

$$\sum_{h,k}(|F_o(hk0)| - |F_o(\bar{k}h0)|)/\sum_{h,k}(|F_o(hk0)| + |F_o(\bar{k}h0)|) \quad (2)$$

is 0.0119, where the summation is over all reflections, of which $|F_o|$ are greater than 10.0. Figure 1 shows the distribution of $\sigma(|F_o(hk0)|)$ versus $|F_o|$.
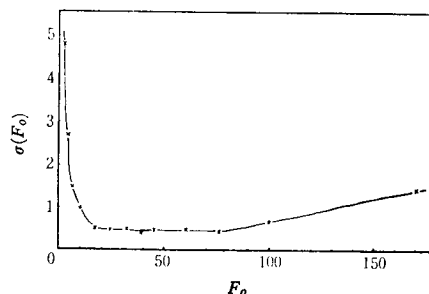
Fig. 1. The standard deviations of $|F_o(hk0)|$. Each symbol (x) represents the average of 10 reflections of similar $|F_o|$.

Structure Determination and Refinement

The position of the bromine atom was found from the three dimentional Patterson function. A Fourier synthesis was computed on the basis of the position of bromine atom. Eight extra atoms except ethyl group showed up on the first map. The definite location of the ethyl group could not be found on the electron density map. The peak which corresponds to the carbon atom C(7) binding to N(1) is too much elongated along the direction perpendicular to the pyrimidine ring plane.

Structure refinements were carried out by the block diagonal least-squares method. The function, $\sum w(|F_o|-|F_c|)^2$, was minimized for the least-squares refinements. The atomic scattering factors were taken from "Inter-

TABLE 2. FINAL ATOMIC COORDINATES AND THERMAL PARAMETERS WITH THEIR ESTIMATED STANDARD DEVIATIONS

	x	e.s.d. (x)	y	e.s.d. (y)	z	e.s.d. (z)
Br	0.3591	0.0007	0.6928	0.0006	1.3252	0.0007
C2	0.4139	0.0049	0.4906	0.0046	0.7649	0.0053
C4	0.4448	0.0045	0.6244	0.0047	0.9259	0.0047
C5	0.3841	0.0045	0.6125	0.0046	1.1045	0.0052
C6	0.3451	0.0049	0.5465	0.0052	1.1099	0.0057
N1	0.3587	0.0044	0.4860	0.0043	0.9462	0.0052
N3	0.4556	0.0036	0.5600	0.0036	0.7689	0.0039
O2	0.4279	0.0035	0.4397	0.0033	0.6139	0.0038
O4	0.4860	0.0034	0.6819	0.0031	0.9021	0.0035
H3	0.4962	0.0360	0.5649	0.0346	0.6603	0.0412
H6	0.2979	0.0393	0.5415	0.0388	1.2066	0.0444
C7	0.2884	0.0098	0.4258	0.0097	0.8958	0.0105
C8	0.3069	0.0121	0.3654	0.0120	1.0861	0.0118
C7'	0.3313	0.0109	0.3983	0.0107	1.0249	0.0116
C8'	0.2618	0.0126	0.3926	0.0126	0.8818	0.0134

	$B_{11} \times 10^5$	$B_{22} \times 10^5$	$B_{33} \times 10^4$	$B_{12} \times 10^5$	$B_{13} \times 10^4$	$B_{23} \times 10^4$
Br	663 (3)	459 (3)	485 (2)	174 (6)	6 (2)	−70 (2)
C2	383 (21)	342 (21)	436 (26)	−89 (37)	58 (12)	7 (13)
C4	317 (20)	321 (19)	349 (22)	109 (34)	−44 (12)	40 (12)
C5	308 (19)	323 (20)	358 (23)	−15 (33)	−8 (11)	−11 (12)
C6	338 (22)	532 (26)	470 (29)	20 (40)	92 (14)	−38 (15)
N1	515 (22)	416 (20)	708 (27)	−386 (35)	230 (14)	−79 (13)
N3	304 (15)	348 (16)	366 (19)	−22 (26)	55 (9)	16 (9)
O2	520 (17)	370 (15)	620 (21)	−233 (26)	163 (11)	−68 (10)
O4	481 (16)	320 (14)	501 (19)	−195 (25)	12 (10)	14 (9)

	B					
H3	3.500 (0.897)					
H6	3.500 (0.903)					
C7	4.616 (0.233)					
C8	6.747 (0.299)					
C7'	5.445 (0.263)					
C8'	7.499 (0.331)					

Temperature factor
$$= \exp(-(B_{11} \times h^2 + B_{22} \times k^2 + B_{33} \times l^2 + B_{12} \times hk + B_{13} \times hl + B_{23} \times kl))$$
or
$$= \exp(-B(\sin\theta/\lambda)^2)$$

national Table for X-ray Crystallography (1962)." To take into account of the anomalous scattering by bromine atom, $\Delta f'(-0.95)$ and $\Delta f''$ (1.40) were included in the calculations. The weighting scheme employed was

$w = 0.0$ $(|F_o| < 10.0$ or attenuated reflection)

$w = \sigma^{-1}(|F_o|)$ (others).

Five cycles of least-squares refinement of positional para-

Fig. 2. The composite difference electron density map, for which the contribution of the hydrogen atoms and ethyl group carbon atoms were excluded from the calculated structure factors. Contours are drown at interval of 0.5 e/Å³, starting at 0.5 e/Å³.

meters and anisotropic thermal parameters for the nine atoms except ethyl group resulted an a value of R of 0.1394. An $(F_o—F_c)$ synthesis then revealed the two possible site of the ethyl group (Fig. 2).

After three cycles of least-squares refinements of the positional parameters, the anisotropic thermal parameters for the nine atoms except ethyl group and the isotropic thermal parameters for the four atoms of the two sites of the ethyl group, the R value decreased to 0.068. The ethyl group was supposed as equally distributed to the two sites, that is, the occupancies of the both sites are 0.5. In the course of the refinement,

Fig. 3. Structural formula of 1-ethyl-5-bromouracil.

the temperature factor of an atom of the ethyl group compensated for particular choice of the occupancy of the same atom. Accurate occupancies of two ethyl group sites could not be determined, partly because only about 60% of reflections within the limiting sphere of Cu-$K\alpha$ radiation was used in the determination. For several sets of occupancies of two ethyl group sites, similar refinements were tried. These calculations suggested that two ethyl group sites should be multiplied by 0.60—0.45 and 0.45—0.60 respectively. The $(F_o—F_c)$ synthesis at this stage revealed two hydrogen atoms bound to the pyrimidine ring.

The final refinement included all atoms except for the hydrogen atoms of the ethyl group. The final R is 0.064.*

Molecular Structure

Tables 4 and 5 give the bond distances in the pyrimidine ring of this crystal and some other crystals respectively. Comparison with these crystals shows that structure of the pyrimidine ring are in good agreement with each others. The molecule except ethyl group is nearly planar (Table 6), bromine atom being displaced from the plane by 0.04 Å. The carbon atom C(7) and C(7') deviate significantly from the plane by 0.53 Å and 0.57 Å, respectively. This displacement suggests an increase of p-character in the bond

TABLE 4. BOND LENGTHS AND ANGLES WITH THEIR ESTIMATED STANDARD DEVIATIONS

Bond	Length	e.s.d.	Angle	θ	e.s.d.
C6–N1	1.376 Å	0.008 Å	C6–N1–C2	121.8°	0.5°
N1–C2	1.360	0.007	C6–N1–C7	117.7	0.6
C2–N3	1.383	0.007	C7–N1–C2	116.1	0.6
N3–C4	1.399	0.006	C6–N1–C7'	118.8	0.6
C4–C5	1.429	0.007	C7'–N1–C2	116.1	0.6
C5–C6	1.313	0.008	N1–C2–N3	113.7	0.5
C2–O2	1.216	0.007	N1–C2–O2	124.7	0.5
C4–O4	1.218	0.006	O2–C2–N3	121.7	0.5
Br–C5	1.864	0.005	C2–N3–C4	128.7	0.4
N1–C7	1.599	0.012	C2–N3–H3	118.8	3.2
C7–C8	1.495	0.016	H3–N3–C4	113.2	3.2
N1–C7'	1.626	0.013	N3–C4–C5	112.7	0.4
C7'–C8'	1.425	0.018	N3–C4–O4	119.9	0.4
N3–H3	0.916	0.041	O4–C4–C5	127.4	0.5
C6–H6	0.963	0.045	C4–C5–C6	120.6	0.5
			C4–C5–Br	119.1	0.5
			Br–C5–C6	120.3	0.4
			C5–C6–N1	123.2	0.5
			C5–C6–H6	123.4	3.3
			H6–C6–N1	112.5	3.3
			N1–C7–C8	100.4	0.8
			N1–C7'–C8'	99.6	0.9

* Table 3 which gives a complete list of the observed and the calculated structure factors has been submitted to, and is kept as Document No. 7202 by, the office of the Bulletin of the Chemical Society of Japan, 1-5 Kanda-Surugadai, Chiyoda-ku, Tokyo. A copy may be secured by citing the Document number and by remitting, in advance, ¥400 for photo prints. Pay by check or money order payable to: Chemical Society of Japan.

TABLE 5. BOND LENGTHS AND ANGLES OF URACIL OR THYMINE DERIVATIVES

Bond	T[7]	TD[8]	MT[9]	EBU (form I)[3],
C(6)–N(1)	1.382 Å	1.374 Å	1.383 Å	1.384 Å
N(1)–C(2)	1.355	1.385	1.379	1.361
C(2)–N(3)	1.361	1.381	1.379	1.400
N(3)–C(4)	1.391	1.378	1.375	1.381
C(4)–C(5)	1.447	1.453	1.432	1.432
C(5)–C(6)	1.349	1.343	1.346	1.339
C(2)–O(2)	1.234	1.206	1.214	1.212
C(4)–O(4)	1.231	1.230	1.237	1.226
σ	0.005	0.006	0.004	0.008

a) T, TD, MT and EBU represent thymine, thymidine, 1-methylthymine, and 1-ethyl-5-bromouracil respectively.

TABLE 6. THE EQUATION OF THE LEAST-SQUARES PLANE THROUGH ATOMS

Equation	Atom	Deviation
$-0.6617X+0.3866Y$	Br	0.042 Å
$-0.6425Z+4.0882=0$	C2	0.016
	C4	−0.012
	C5	−0.010
	C6	−0.030
	N1	−0.014
	N3	−0.017
	O2	0.034
	O4	−0.010
	*H3	0.041
	*H6	0.092
	*C7	0.545
	*C8	−0.709
	*C7'	−0.563
	*C8'	0.660

where $X=ax$, $Y=by$, $Z=cz$

* Atom not included in the least-squares calculation.

orbitals of C(7)–N(1) and C(7')–N(1). Triethylpyrazine[5] and bromodihydroacromycine[6] have a similar structure. In form I, the carbon atom C(7) does not displace from the pyrimidine ring plane. In form II, if the carbon atom C(7) is placed on the plane, the distance between the carbon atom C(8) and the oxygen atom O(2) or O(4) of the neighboring molecule is shorter than 3.2 Å.

Molecular Packing

There are four intermolecular contact regions. Bromine atoms pack closely around a 4_2-axis. Hydrogen bonds, N–H···O, are formed around a center of symmetry. Disordered ethyl groups surround a $\bar{4}$-axis. The hydrogen atom H(6) and the oxygen atom O(4) contact closely with each other. One of the interesting

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6) J. Z. Gougoutas and B. A. Kaski, *ibid.*, **B26**, 853 (1970).
7) R. Gerdil, *ibid.*, **14**, 333 (1961).
8) D. W. Young, P. Tollin, and H. Wilson, *ibid.*, **B25**, 1423 (1969).
9) K. Hoogsteen, *ibid.*, **16**, 28 (1963).

TABLE 7. INTERMOLECULAR CONTACTS

Atom	Neighbor atom		Distance	Atom	Neighbor atom		Distance
Br	Br	g	4.012 Å	C(7)	C(8)	e	4.296 Å
Br	Br	h	4.012	C(7)	C(8)	f	4.360
Br	Br	j	4.012	C(7)	C(7')	e	4.328
Br	Br	k	4.012	C(7)	C(8')	c	3.999
Br	Br	i	4.223	C(8)	C(8)	b	4.435
				C(8)	C(8)	d	3.553
O(2)	N(3)	a	2.866	C(8)	C(7')	d	4.288
O(2)	H(3)	a	1.958	C(8)	C(7')	f	4.158
				C(8)	C(8')	b	4.323
O(4)	C(6)	k	3.249	C(8)	C(8')	c	3.680
O(4)	H(6)	k	2.268	C(8)	C(8')	d	3.857
				C(7')	C(8')	c	3.711
				C(7')	C(8')	d	4.357
				C(8')	C(8')	e	3.781

Key for molecule position

a ($1.0-x$ $1.0-y$ $1.0-z$)
b (x y $1.0+z$)
c (y $0.5-x$ $1.5-z$)
d (y $0.5-x$ $2.5-z$)
e ($0.5-y$ x $1.5-z$)
f ($0.5-y$ x $2.5-z$)
g ($-0.5+y$ $1.0-x$ $0.5+z$)
h ($-0.5+y$ $1.0-x$ $-0.5+z$)
i ($0.5-x$ $1.5-y$ z)
j ($1.0-y$ $0.5+x$ $0.5+z$)
k ($1.0-y$ $0.5+x$ $-0.5+z$)

Fig. 4. The crystal structure viewed along the c axis. Hydrogen bonds are indicated by broken lines.

aspects of this crystal structure is the packing of bromine atoms. Two non-equivalent distances between bromine atoms are 4.22 Å and 4.01 Å respectively (Table 7). This closest packing region elongates along the needle axis.

The hydrogen bond scheme in this crystal, N(3)–H(3)···O(2), is the first finding in the alkylated pyrimidine dimers. While the hydrogen bond scheme in form I, N(3)–H(3)···O(4), is usually found in the self-dimer of uracil and thymine derivatives, for example, uracil,[10,11] 1-methyluracil,[12] 5-ethyl-6-methyl uracil[13] and 1-me-

thylthymine.[14] It seems that a change of electron distribution in pyrimidine ring by a substitution of a bromine atom or van der Waals interaction between closest packed bromine atoms perturbs the construction of the hydrogen bond scheme. Table 7 gives a list of all the intermolecular contacts between the disordered carbon atoms of the ethyl group. The distances of C(8)–C(8d)[15] and C(8)–C(8'c)[15] are shorter than usual, but a similar intermolecular contact between methyl groups in a crystal of octamethyltetraamidodiphosphono-2,3-butadiene-1,3.[16] The distance between H(6) and O(4), 2.27 Å, is fairly short, and the angle C(6)–H(6)–O(4) is 178.0°. In a thymine or uracil molecule, C(6) has a fairly positive charge.[17] Thus C(6)–H(6)···O(4) may be a hydrogen bond. Similar hydrogen bonds were reported in form I crystal and some other crystals, barium uridine-5'-phosphate,[18] β-adenosine-2'-β-uridine-5'-phosphoric acid[19] and calcium thymine phosphate.[20]

10) G. S. Parry, *Acta Crystallogr.*, **7**, 313 (1954).
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12) D. W. Green, F. S. Mathews, and A. J. Rich, *J. Biol. Chem.*, **237**, 3572 (1962).
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15) The symbols d and c are defined in Table 7.
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17) C. Nagata and A. Imamura, *Kagaku*, **24**, 13 (1969).
18) E. Shefter and K. N. Trueblood, *Acta Crystallogr.*, **18**, 1067 (1965).
19) E. Shefter, M. Barlow, R. A. Sparks, and K. N. Trueblood, *ibid.*, **B25**, 895 (1969).
20) K. N. Trueblood, P. Horn, and V. Luzzati, *ibid.*, **14**, 965 (1961).

The Crystal and Molecular Structure of Averufin

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The crystal structure of averufin has been determined by means of the X-ray diffraction method. The space group is P2₁/a, with $a=23.70$ Å, $b=7.24$ Å, $c=9.48$ Å, and $\beta=105.5°$. The structure was solved by the interpretation of the three-dimensional Patterson function and was refined by the least-squares method. The molecular structure is in agreement with that proposed by Roffey and Grandjean. In the crystal, the molecules are linked to form a molecular pair by a OH----O hydrogen bond across the center of symmetry.

Aspergillus versicolor (Vuillemin) Tiraboschi produces a number of xanthones and anthraquinones. The fact that all these metabolites are produced by the same mold leads to the postulate that some of them may be biogenetic precursors of the carsinogenic sterigmatocystin. In a previous paper, the crystal structure of *p*-bromobenzoate of sterigmatocystin was reported by Tanaka *et al.*[1])

Averufin was isolated as the metabolite of this mold by Pusey *et al.*,[2]) and its chemical structure was proposed by Holker *et al.*[3]) and Roffey and Sargent.[4])

The present paper will describe the crystal and molecular structure of averufin, as analyzed by means of the X-ray diffraction method, as one of a series of studies of the crystal structures of some metabolites from this mold. After this study was completed, it was discovered that the structure of averufin had been elucidated by Grandjean by means of NMR spectroscopy.[5]) The molecular structure determined by us is in agreement with that proposed by Roffey and Sargent[4]) and Grandjean.[5])

Experimental

The crystal grown from an acetone solution was in the form of a reddish, flat, rectangular plate elongated in the *a*-axis direction. The unit cell dimensions are shown in Table 1.

TABLE 1. CRYSTAL DATA OF AVERUFIN

Molecular formula; $C_{20}H_{16}O_7$
$M=368.328$
Monoclinic; space group P2₁/a
$a=23.704$Å
$b=7.239$Å
$c=9.483$Å
$\beta=105.46°$
$V=1568.3$Å³
$d(\text{obs})=1.562$ g/cm³
$d(\text{cal})=1.558$ g/cm³
$Z=4$

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1) N. Tanaka, Y. Katsube, Y. Hatsuda, T. Hamasaki, and M. Ishida, This Bulletin, **43**, 3635 (1970).

2) D. F. G. Pusey and J. C. Roberts, *J. Chem. Soc.*, **1963**, 3542.

3) J. S. E. Holker, S. A. Kagal, L. J. Mulheirin, and P. M. White, *Chem. Commun.*, **1966**, 911.

4) P. Roffey and M. V. Sargent, *ibid.*, **1966**, 913.

5) J. Grandjean, *ibid.*, **1971**, 1060.

Mo-K_α radiation filtered by means of a zirconium foil was used to collect all the reflections in the $0<\sin\theta/\lambda<0.651$ range, within the (*hkl*) and (*hkī*) octants. All the intensities were measured on a Rigaku Denki computer-controlled four-circle diffractometer (AFC-II). A scintillation counter with a pulse-height discriminator was used. Altogether, 2909 independent reflections were collected. A crystal of the approximate dimensions of $0.23 \times 0.22 \times 0.13$ mm³ ($0.964 < e^{-\mu r} < 0.968$) was mounted with the *b*-axis parallel to the ϕ-axis of the diffractometer. The ω-2θ scan technique was employed, with a scan speed of 1°/min by ω, backgrounds were measured for 10 seconds at each start and end points of the scan range. The scan range for each reflection was calculated by means of the formula indicated by Alexander *et al.*[6]: ω scan range$=0.70°+0.40°\tan\theta$. Attenuators were automatically inserted when the maximum counting rate exceeded 5000 cps. The intensities were corrected only for Lorentz and polarization factors. Measurements of three reference reflections, (14 0 0), (0 0 8), and (0 4 0), were repeated every fifty reflections; for 62 repetitions of the measurements, $|F_0(14\ 0\ 0)|=144.26\pm0.33$, $|F_0(0\ 0\ 8)|=42.78\pm0.11$, and $|F_0(0\ 4\ 0)|=56.37\pm0.10$.

Determination of the Structure

The angular coordinates defining the orientation of the planar anthraquinone group in the molecule were readily established by means of the vector-set-seeking method;[7]) the vector set of the anthraquinone skeleton was superposed on the three-dimensional Patterson function and rotated until the best fit was obtained. The location of this planar group in the unit cell was determined from the Patterson function, in which a large non-Harker peak is expected corresponding to the anthraquinone-anthraquinone vector from molecules related by a center of symmetry. Of several possible peaks, the one which satisfied a reasonable packing condition in the crystal and which gave the best match with the Patterson function was selected. This interpretation was proved to be correct. The 780 largest structure factors were calculated by the use of the atomic positions of the anthraquinone group only, and the R value defined by R$=\sum||F_o|-|F_c||/\sum|F_o|$ was 0.69. The Fourier synthesis phased by the anthraquinone group was computed. The peaks corresponding to some additional atoms were clearly visible, their peak-heights were approximately

6) T. C. Furnas, "Single Crystal Orienter Instruction Manual" Milwaukee, General Electric Co. (1957).

7) Y. Katsube, Y. Sasada, and M. Kakudo, This Bulletin, **39**, 6108 (1966).

Table 2. Final parameters of carbon and oxygen atoms

The anisotropic temperature factors are of the form

$$\exp[-(\beta_{11}h^2+\beta_{22}k^2+\beta_{33}l^2+2\beta_{12}hk+2\beta_{13}hl+2\beta_{23}kl)]$$

| | x | y | z | $\beta_{ij}\times10^4$ | | | | | |
				β_{11}	β_{22}	β_{33}	β_{12}	β_{13}	β_{23}
C (1)	−0.1346	0.0387	−0.0667	13	120	65	0	10	−1
C (2)	−0.1123	−0.1356	−0.0806	13	123	62	−7	17	−17
C (3)	−0.0610	−0.1911	0.0203	14	118	70	9	19	−5
C (4)	−0.0320	−0.0741	0.1348	12	121	59	6	13	−9
C (5)	0.0199	−0.1364	0.2438	12	122	80	−2	17	−23
C (6)	0.0468	−0.0149	0.3653	11	133	75	−3	17	1
C (7)	0.0953	−0.0737	0.4775	11	160	86	2	14	−12
C (8)	0.1202	0.0404	0.5952	12	180	71	0	4	−6
C (9)	0.0978	0.2156	0.6008	14	153	72	−21	15	−26
C (10)	0.0507	0.2794	0.4908	13	127	85	−9	10	−19
C (11)	0.0248	0.1635	0.3747	11	129	68	−2	11	8
C (12)	−0.0280	0.2293	0.2655	14	116	77	−1	18	2
C (13)	−0.0559	0.1022	0.1443	13	109	67	0	11	−14
C (14)	−0.1061	0.1587	0.0441	15	101	86	13	9	−4
C (15)	−0.2183	−0.0273	−0.2706	14	138	73	−7	7	−13
C (16)	−0.1461	−0.2590	−0.2015	17	129	77	5	8	−49
C (17)	−0.1867	−0.3891	−0.1489	20	120	120	−16	−8	21
C (18)	−0.2312	−0.2773	−0.0949	19	212	110	−24	18	87
C (19)	−0.2606	−0.1351	−0.2086	14	174	85	−12	4	−4
C (20)	−0.2480	0.0933	−0.3976	18	206	87	0	−13	53
O (1)	−0.1850	0.1001	−0.1588	15	123	91	12	−11	−11
O (2)	−0.1789	−0.1426	−0.3168	16	171	59	1	7	−7
O (3)	−0.0410	−0.3615	0.0034	18	132	108	25	2	−84
O (4)	0.0404	−0.2946	0.2355	16	138	111	27	−3	−41
O (5)	0.1184	−0.2443	0.4770	16	184	114	35	−10	−31
O (6)	0.1228	0.3207	0.7189	15	210	90	−9	−5	−83
O (7)	−0.0491	0.3800	0.2774	21	131	105	16	−3	−55

Table 3. Atomic parameters of hydrogen atoms

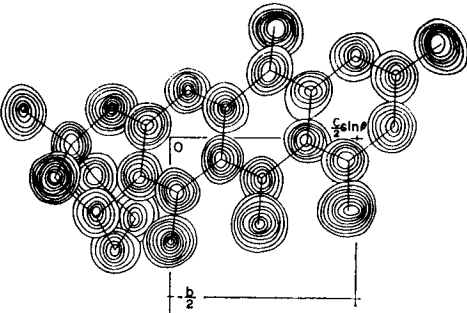| Atom	x	y	z	B	Atom	x	y	z	B
H (1)	−0.124	0.279	0.050	1.3Å²	H (9)	−0.208	−0.464	−0.228	3.5Å²
H (2)	0.032	0.411	0.488	1.9	H (10)	−0.212	−0.208	−0.002	2.2
H (3)	0.099	0.421	0.717	5.1	H (11)	−0.260	−0.350	−0.068	2.8
H (4)	0.155	−0.002	0.674	2.9	H (12)	−0.284	−0.046	−0.170	3.5
H (5)	0.096	−0.300	0.400	5.3	H (13)	−0.285	−0.194	−0.288	2.2
H (6)	−0.008	−0.390	0.085	5.2	H (14)	−0.273	0.181	−0.355	3.4
H (7)	−0.115	−0.330	−0.242	3.0	H (15)	−0.275	0.025	−0.461	3.9
H (8)	−0.162	−0.458	−0.067	3.1	H (16)	−0.216	0.154	−0.444	4.9

Fig. 1. The electron density map.

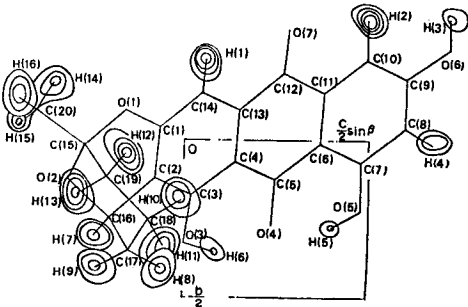The contours are at equally spaced intervals on an arbitrary scale.

Fig. 2. The difference electron density map, for which the contributions of hydrogen atoms were excluded from the calculated structure factors.

TABLE 4. INTERATOMIC DISTANCES AND ANGLES

Bond	Length (Å)	Bond	Length (Å)	Bond	Length (Å)
C (1)–C (2)	1.387(5)	C (1)–C (14)	1.392(5)	C (1)–O (1)	1.354(4)
C (2)–C (3)	1.393(5)	C (2)–C (16)	1.504(5)	C (3)–C (4)	1.406(5)
C (3)–O (3)	1.344(4)	C (4)–C (5)	1.456(5)	C (4)–C (13)	1.404(5)
C (5)–C (6)	1.455(5)	C (5)–O (4)	1.252(4)	C (6)–C (7)	1.410(5)
C (6)–C (11)	1.407(5)	C (7)–C (8)	1.385(5)	C (7)–O (5)	1.353(5)
C (8)–C (9)	1.382(5)	C (9)–O (6)	1.353(4)	C (9)–C (10)	1.388(5)
C (10)–C (11)	1.386(5)	C (11)–C (12)	1.474(5)	C (12)–C (13)	1.485(5)
C (12)–O (7)	1.219(4)	C (13)–C (14)	1.375(5)	C (15)–C (19)	1.510(5)
C (15)–C (20)	1.505(5)	C (15)–O (1)	1.467(4)	C (15)–O (2)	1.403(4)
C (16)–C (17)	1.521(5)	C (16)–O (2)	1.435(4)	C (17)–C (18)	1.523(6)
C (18)–C (19)	1.518(6)	C (14)–H (1)	1.00 (4)	C (10)–H (2)	1.11 (4)
O (6)–H (3)	0.96 (5)	C (8)–H (4)	1.04 (4)	O (5)–H (5)	0.92 (5)
O (3)–H (6)	0.96 (5)	C (16)–H (7)	1.08 (5)	C (17)–H (8)	0.97 (5)
C (17)–H (9)	0.96 (5)	C (18)–H (10)	1.00 (4)	C (18)–H (11)	0.98 (4)
C (19)–H (12)	1.02 (5)	C (19)–H (13)	0.97 (4)	C (20)–H (14)	1.01 (5)
C (20)–H (15)	0.85 (5)	C (20)–H (16)	1.03 (5)		

	Angle(°)		Angle(°)
C (2)–C (1)–C (14)	121.4(3)	C (2)–C (1)–O (1)	122.0(3)
C (14)–C (1)–O (1)	116.6(3)	C (1)–C (2)–C (3)	118.9(3)
C (1)–C (2)–C (16)	118.3(3)	C (3)–C (2)–C (16)	122.8(3)
C (2)–C (3)–C (4)	120.8(3)	C (2)–C (3)–O (3)	117.1(3)
C (4)–C (3)–O (3)	122.2(3)	C (3)–C (4)–C (5)	120.4(3)
C (3)–C (4)–C (13)	118.5(3)	C (5)–C (4)–C (13)	121.0(3)
C (4)–C (5)–C (6)	118.9(3)	C (4)–C (5)–O (4)	120.7(3)
C (6)–C (5)–O (4)	120.4(3)	C (5)–C (6)–C (7)	120.9(3)
C (5)–C (6)–C (11)	121.1(3)	C (7)–C (6)–C (11)	118.0(3)
C (6)–C (7)–C (8)	121.1(3)	C (6)–C (7)–O (5)	121.4(3)
C (8)–C (7)–O (5)	117.5(3)	C (7)–C (8)–C (9)	119.4(3)
C (8)–C (9)–C (10)	121.1(3)	C (8)–C (9)–O (6)	117.3(3)
C (10)–C (9)–O (6)	121.6(3)	C (9)–C (10)–C (11)	119.7(3)
C (6)–C (11)–C (10)	120.7(3)	C (6)–C (11)–C (12)	120.6(3)
C (10)–C (11)–C (12)	118.6(3)	C (11)–C (12)–C (13)	117.7(3)
C (11)–C (12)–O (7)	121.0(3)	C (13)–C (12)–O (7)	121.3(3)
C (4)–C (13)–C (12)	120.6(3)	C (4)–C (13)–C (14)	121.1(3)
C (12)–C (13)–C (14)	118.3(3)	C (1)–C (14)–C (13)	119.4(3)
C (19)–C (15)–C (20)	113.4(3)	C (19)–C (15)–O (1)	109.0(3)
C (19)–C (15)–O (2)	112.5(3)	C (20)–C (15)–O (1)	104.9(3)
C (20)–C (15)–O (2)	107.7(3)	O (1)–C (15)–O (2)	109.0(3)
C (2)–C (16)–C (17)	112.0(3)	C (2)–C (16)–O (2)	107.6(3)
C (17)–C (16)–O (2)	110.6(3)	C (16)–C (17)–C (18)	109.7(3)
C (17)–C (18)–C (19)	110.6(3)	C (15)–C (19)–C (18)	113.6(3)
C (1)–O (1)–C (15)	118.1(3)	C (15)–O (2)–C (16)	112.4(3)
C (1)–C (14)–H (1)	118 (2)	C (13)–C (14)–H (1)	122 (2)
C (9)–C (10)–H (2)	123 (2)	C (11)–C (10)–H (2)	116 (2)
C (9)–O (6)–H (3)	106 (3)	C (7)–C (8)–H (4)	118 (3)
C (9)–C (8)–H (4)	122 (3)	C (7)–O (5)–H (5)	104 (3)
C (3)–O (3)–H (6)	109 (3)	C (2)–C (16)–H (7)	108 (3)
C (17)–C (16)–H (7)	109 (3)	O (2)–C (16)–H (7)	108 (3)
C (16)–C (17)–H (8)	106 (3)	C (18)–C (17)–H (8)	108 (3)
H (9)–C (17)–H (8)	116 (4)	C (16)–C (17)–H (9)	110 (3)
C (18)–C (17)–H (9)	104 (3)	C (17)–C (18)–H (10)	109 (3)
C (19)–C (18)–H (10)	105 (3)	H (11)–C (18)–H (10)	105 (4)
C (17)–C (18)–H (11)	112 (3)	C (19)–C (18)–H (11)	112 (3)
C (15)–C (19)–H (12)	111 (3)	C (18)–C (19)–H (12)	109 (3)
H (13)–C (19)–H (12)	108 (4)	C (15)–C (19)–H (13)	104 (3)
C (18)–C (19)–H (13)	110 (3)	C (15)–C (20)–H (14)	104 (3)
H (15)–C (20)–H (14)	102 (4)	H (16)–C (20)–H (14)	118 (4)
C (15)–C (20)–H (15)	108 (3)	H (16)–C (20)–H (15)	115 (4)
C (15)–C (20)–H (16)	109 (3)		

Intramolecular hydrogen bond:
O (3)⋯⋯⋯⋯O (4) 2.561(4)Å   O (4)⋯⋯⋯⋯O (5) 2.554(4)Å   O (4)⋯⋯⋯⋯H (6) 1.72 (6)Å
O (4)⋯⋯⋯⋯H (5) 1.70 (5)Å   O (3)–H (6)⋯O (4) 151 (6)°Å   O (5)–H (5)⋯O (4) 151 (5)°Å
Estimated standard deviations shown in parentheses refer to the last decimal positions.

half of those of the atoms of the anthraquinone group. The whole structure of the molecule except for the hydrogen atoms was revealed after three successive Fourier synthesis. At this stage, the R value was 0.46. The structure was refined by a diagonal-matrix least-squares procedure with the isotropic temperature factors, until the R value of 0.15 was reached. Further refinement was carried out by the least-squares of block-diagonal matrix approximations with anisotropic temperature factors, using all the reflections. The R value was thus reduced to 0.12. At this stage, the difference Fourier synthesis showed all the hydrogen atoms. The final refinement was made by including the positional parameters and the isotropic temperature factors of the hydrogen atoms, where upon the R value decreased to 0.083. The final atomic parameters are listed in Tables 2 and 3. The final electron density map is shown in Fig. 1. The difference electron density map, for which the contributions of the hydrogen atoms were excluded from the calculated structure factors, is shown in Fig. 2.

A list of the observed and calculated structure factors is available from the authors on request.

Most of the calculations were done on the TOSBAC-3400 by the use of the programs written by the authors. The block-diagonal least-squares refinement was performed on the HITAC 5020E of the university of Tokyo using a program written by T. Ashida, the weighting scheme in the calculation was $w=1.0$ for reflections with $|F_o| \geq 1.5$ and $w=0.5$ for the others. The atomic scattering factors were taken from Ref. 8.

Description of the Structure and Discussion

The bond lengths and bond angles are given in Table 4. The mean bond length of the carbon-carbon bonds in the benzenoid rings is 1.393 Å, which is the same as the length, 1.397 Å, formed in the benzene ring[9] within the limits of error. However, the lengths of C–C bonds adjacent to the quinonoid carbonyl bonds, C(4)–C(5), C(5)–C(6), C(11)–C(12), and C(12)–C(13), are considerably longer than those in the benzenoid rings. The lenghts of the C(12)–O(7) and C(5)–O(4) of the quinonoid carbonyl bonds are 1.219 Å and 1.252 Å respectively. The former agrees well with the C–O bond length in acetaldehyde (1.215)[9] and may be a normal double bond. The latter is longer than the former, the elongation of the latter bond may be due to the influence of the adjacent hydroxyl groups. The lengths of the C–O bonds attached to the anthraquinone skeleton, C(1)–O(1), C(3)–O(3), C(7)–O(5), and C(9)–O(6), are almost constant and have a mean of 1.351 Å. All are in agreement with the length of a single bond between an oxygen and an sp^2-hybridized carbon atom.

The dimensions of the quinonoid group in the averufin show features similar to those in other related

molecules whose structures have been studied: p-benzoquinone,[10] anthraquinone,[11] 1,5-dihydroxy-anthraquinone,[12] 1:2,5:6-dibenzanthraquinone,[13] and some derivatives of anthraquinone.[14]

There are no significant differences among the five bond lenghts of $C(sp^3)$–$C(sp^3)$, and the mean value (1.515 Å) is shorter than that of a pure single C–C bond. The lengths of the C–O bonds involving $C(sp^3)$ have a mean of 1.435 Å and agree with the C–O single bond distance.

The least-squares plane for the aromatic condensed ring including O(3) to O(7) can be represented by the equation:

$$Z = 1.3981X + 0.6983Y + 3.2390$$

where $X = ax + cz\cos\beta$, $Y = by$, and $Z = cz\sin\beta$. The perpendicular displacements of the atoms from the plane are given in Table 5. As may be seen in Table 5, these atoms are coplanar.

TABLE 5. DEVIATIONS FROM THE LEAST-SQUARES PLANE

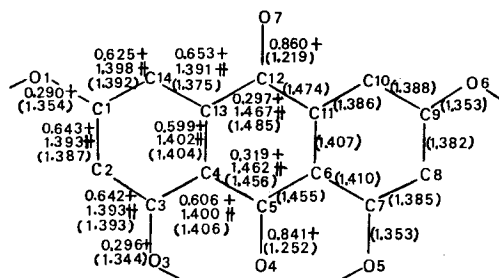| Atom	Deviation	Atom	Deviation
Atoms included in plane			
C(1)	−0.098Å	C(2)	−0.079Å
C(3)	−0.005	C(4)	0.051
C(5)	0.065	C(6)	0.045
C(7)	−0.014	C(8)	−0.062
C(9)	−0.023	C(10)	0.061
C(11)	0.076	C(12)	0.056
C(13)	0.039	C(14)	−0.020
O(3)	0.004	O(4)	0.058
O(5)	−0.063	O(6)	−0.098
O(7)	0.009		
Atoms not included in plane			
O(1)	−0.202	C(16)	−0.194
H(1)	−0.05	H(2)	−0.10
H(3)	−0.24	H(4)	−0.10
H(5)	−0.09	H(6)	−0.04

Fig. 3. The π-bond orders and the bond lenghts (Å).
  + the π-bond orders
  ++ the bond lenghts calculated from Coulson's equation
  The values in the parentheses are the observed lengths (Å).

10) J. Trotter, *Acta Crystallogr.*, **13**, 86 (1960).
11) B. V. R. Murty, *Z. Kristallogr.*, **113**, 445 (1960).
12) D. Hall and C. L. Nobbs, *Acta Crystallogr.*, **21**, 927 (1966).
13) R. F. Entwistle, J. Iball, W. D. S. Motherwell, and B. P. Thompson, *ibid.*, **B25**, 770 (1969).
14) J. Gaultier and C. Hauw, *ibid.*, **B25**, 419 (1969).

8) "International Tables for X-ray Crystallography", Vol. III, Kynoch Press, Birmingham (1962), p. 202.
9) L. E. Sutton, "Tables of Interatomic Distance (Suppl.)" The Chemical Society, London (1965).
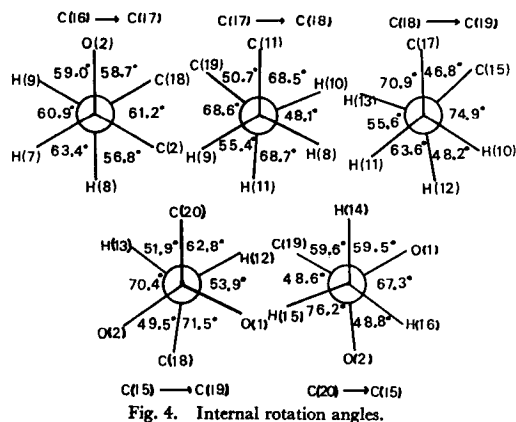
Fig. 4. Internal rotation angles.

The π-bond orders were obtained from a simple LCAO molecular orbital treatment (simple Hückel's method) for the π-electron system alone. The set of Coulomb and exchange integral values suggested by Pullman[15] were used. There are:

$$\alpha(C)=\alpha_0, \ \alpha(=O)=\alpha_0+1.2\beta_0, \ \alpha(-O-)=\alpha_0+2.0\beta_0,$$
$$\beta(C-C)=\beta_0, \ \beta(C=O)=2.0\beta_0, \ \beta(C-O)=0.9\beta_0$$

where α_0 is the Coulomb integral of the carbon atom in the benzene and whose β_0 is the exchange integral of the C–C bond in the benzene. The lengths of the C–C bonds were estimated from the existing bond order–bond length curve.[16] In the C–C bonds, the agreement between the calculated and observed bond lengths is within 0.02 Å. The lengths of the C–O bonds were not predicated in the present work, because the C–O bond order–bond length data generally have a much wider range of values than the corre sponding curve for the C–C bond. The results of the MO calculation are given in Fig. 3.

The conformation of the six-membered ring, C(16)–C(17)–C(18)–C(19)–C(15)–O(2), is a chair form. The internal rotation angles around the $C(sp^3)$–$C(sp^3)$ bonds are illustrated in Fig. 4. They show a stable, stag gered conformation. The average bond angle at the $C(sp^3)$ is 109.8°, and that at the $C(sp^2)$ is 119.9°. The C(15)–O(2)–C(16) angle in the six-membered ring of the chair form is 112.4°; this agrees well with the corresponding angles in sugars.[17] There are two
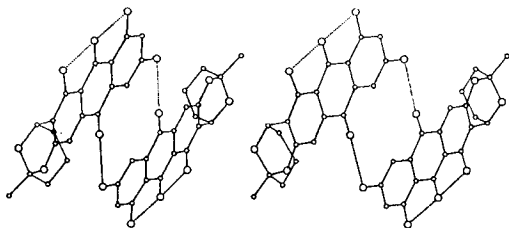

Fig. 5. Stereoscopic drawing of a pair of molecules.

15) B. Pullman and A. Pullman, "Quantum Biochemistry," Interscience Publishers, N. Y., (1963), pp. 108, 356.
16) C. A. Coulson, Proc. Roy. Soc., A169, 413 (1939).
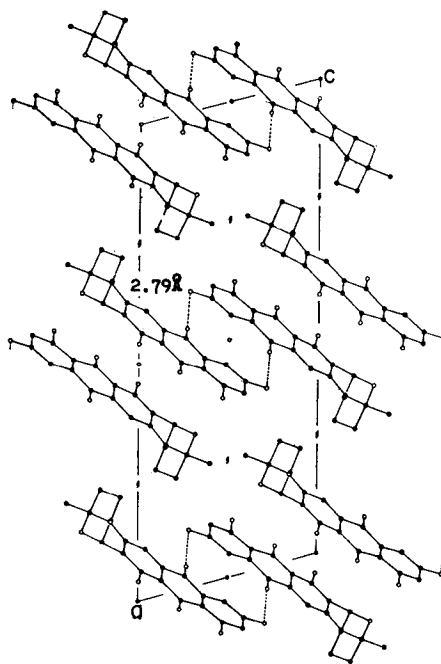17) C. A. Beevers and H. N. Hansen, Acta Crystallogr., B27, 1323 (1971).

Fig. 6. The crystal structure projected along the b-axis.

intramolecular hydrogen bonds of the OH···O type: O(3)···O(4)=2.56 Å and O(4)···O(5)=2.55 Å. The molecule has, therefore, the conformation which might be expected to have the lowest energy. A stereoscopic drawing of the molecule is given in Fig. 5.

The crystal structure projected along the b-axis is shown in Fig. 6. The closest intermolecular distances are listed in Table 6. The reference molecule at (x, y, z) is connected by the intermolecular hydrogen bond of OH···O of 2.79 Å with the molecule at (−x, 1−y, 1−z), thus forming a pair of molecules across the center of symmetry. Although the molecule itself has two asymmetric centers, the crystal of averufin is racemized by the molecules related by the centers of symmetry. The intermolecular approach distance between O(3) and O(3) in the "b-molecule" is 2.81 Å. Although this distance corresponds to the sum of the van der Waals radii, it is less than the intermolecular O···O distances found in common compounds and compares with the rather long intermolecular hydrogen bonds. Such a close distance has been found in 1,5-dihydroxy-anthraquinone: according to Hall et al.,[12] this inter-molecular interaction is due to the proton of the hydrogen atom in the hydroxyl group, which may participate in the interactions among the closest oxygen atoms. However, in averufin, it appears that the approach of these oxygen atoms may correspond to the van der Waals interaction, because the hydrogen atom attached to the O(3) atom may participate only in the formation of the intramolecular hydrogen bond.

The other intermolecular distances require no special comment; all correspond to the normal van der Waals

TABLE 6. INTERMOLECULAR CONTACTS

Atom	Neighbour atom	Distance	Atom	Neighbour atom	Distance
C(1)	C(5) a	3.63 Å	C(7)	C(12) c	3.44Å
C(2)	C(5) a	3.59	C(8)	C(12) c	3.45
C(2)	C(6) a	3.62	C(8)	C(13) c	3.39
C(3)	C(4) a	3.53	C(9)	C(13) c	3.66
C(3)	C(13) a	3.59	C(11)	C(16) a	3.74
C(4)	C(4) a	3.47	C(11)	C(11) c	3.76
C(4)	C(9) c	3.44	O(6)	C(3) c	3.33
C(4)	C(13) a	3.79	O(6)	C(4) c	3.37
C(5)	C(9) c	3.53	O(7)	C(10) d	3.31
C(5)	C(10) c	3.53	O(2)	C(7) a	3.21
C(6)	C(16) a	3.72	O(2)	C(8) a	3.38
C(6)	C(10) c	3.54	O(2)	C(20) e	3.39
C(6)	C(11) c	3.51	O(3)	O(3) b	2.81
C(7)	C(11) c	3.56	O(7)	O(6) d	2.79

Key for position of molecules
  a ($-x$ $-y$ $-z$)
  b ($-x$ $-1.0-y$ $-z$)
  c ($-x$ $-y$ $1.0-z$)
  d ($-x$ $1.0-y$ $1.0-z$)
  e ($-0.5-x$ $-0.5+y$ $-1.0-z$)

The Crystal Structure of Bonito (Katsuo) Ferrocytochrome *c* at 4 Å Resolution

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The crystal structure of bonito ferrocytochrome *c* has been studied at 4 Å resolution on the basis of two isomorphous heavy atom derivatives (K₃UO₂F₅ and K₂PtCl₄). The crystal of bonito ferrocytochrome *c* belongs to an orthorhombic system with a space group of $P2_12_12_1$. The unit cell dimensions are: $a=57.54$, $b=84.71$ and $c=37.74$ Å. The crystal contains two kinds of molecules which are nearly equivalent to each other *via* the pseudo-twofold axis along the a axis.

The main structural features of bonito ferrocytochrome *c* molecule, such as the size and shape, the pathway of the polypeptide chain, and the orientation and the environment of the heme group, appear to be similar to those of horse ferricytochrome *c*, although it is suggested that the side-chain conformation at the surface of ferrocytochrome *c* is different from that of ferricytochrome *c*. The molecule is a prolate spheroid of a dimension of about $30 \times 30 \times 35$ Å. Its heme group sits in the center of the molecule, with one corner exposed to the surroundings. In the crystal, the molecules are in close contacts with one another; usually, the residues present in the contact regions have long polar side chains.

In the crystal, the PtCl₄²⁻ ion is located closely at Met 65 as in the case of horse ferricytochrome *c*. The reagent, K₂HgI₄, deteriorates the crystal structure, and it cannot be used for the structure analysis. On a difference Fourier map, however, the Hg group is located closely at Cys 17, which links the heme group to the polypeptide chain.

It is well known that cytochrome *c* is a one-electron carrier in the oxidation-reduction system in mitochondria. The protein is a molecule made up of one linear polypeptide chain carrying one heme group. The polypeptide chains of the proteins found in vertebrates usually consist of 104 amino acid residues, and their amino ends are acetylated. The heme group is covalently bonded to the polypeptide chain by thioether links to the two cysteinyl residues in the invariable sequence, -Cys-X-Y-Cys-His-, which is usually

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