The Crystal and Molecular Structure of the Serotonin–Creatinine Sulphate Complex

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Serotonin (5-hydroxytryptamine) is a vasoconstrictor found in the sera of mammals. It is isolated as a complex with creatinine sulfate, and one molecule of water crystallizes with each complex. The material crystallizes in the monoclinic system with unit cell parameters $a = 10.75$, $b = 9.70$, $c = 35.26$ Å°, $\beta = 99°\ 20'$, $Z = 8$. The space group may be Cc, although all the components except the water molecules satisfy the centrosymmetric space group $C2/c$.

The phases were obtained directly by the symbolic addition procedure applied to space group $C2/c$. In this manner, the approximate positions of all the heavy atoms except the H_2O molecules were located. All the members of the complex are tightly bound by an intricate network of hydrogen bonds. Most of the hydrogen bonding is between the sulfate groups and the serotonin or creatinine molecules. There is only one hydrogen bond directly between the serotonin and creatinine.

Introduction

Serotonin or 5-hydroxytryptamine is an animal hormone concerned with physiological, neurophysiological, and psychological processes. Its property of causing smooth muscle contraction prompted its discovery and isolation from blood serum. In mammals, serotonin is found chiefly in the gastric and intestinal mucosa, the spleen, blood platelets, and the hypothalamus and pineal gland of the brain. It inhibits nerve impulse transmission. Serotonin and many of its structural relatives (*i.e.* LSD, ibogaine, bufotenine) produce mental changes. Abnormal serotonin metabolism in the brain is believed to result in personality changes in the direction of schizophrenia and perhaps schizophrenia itself (Woolley, 1962).

The free base is quite unstable and sensitive to light, heat, and *p*H changes. Unless samples are to be immediately analyzed or used, they are generally converted to a stable crystalline form as the sulphate complex with creatinine. Biological samples are usually isolated as the complex. The complex crystallizes as a monohydrate:
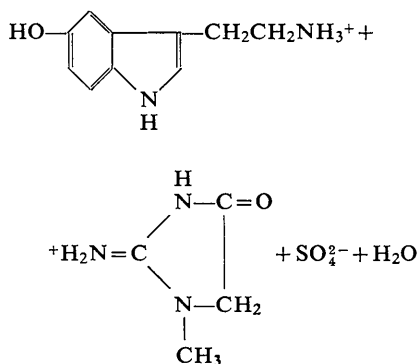
The crystal structure of creatinine itself has been determined by du Pré & Mendel (1955).

Experimental

The serotonin sulphate complex crystallizes from warm water to form slightly brownish plates elongated parallel to [$\bar{1}$10]. The material appears to be stable. The unit-cell parameters as determined from $hk0$ and $0kl$ precession data using copper radiation are:

$$a = 10.75 \pm 0.03 \text{ Å}$$
$$b = 9.70 \pm 0.02$$
$$c = 35.26 \pm 0.08$$
$$\beta = 99°20' \pm 10'$$
$$Z = 8$$

The computed density is 1.485 g.cm^{-3} and the measured density 1.489 g.cm^{-3}. The latter was determined by flotation in mixed solvents.

Multiple film equi-inclination Weissenberg photographs were taken with the rotation axis parallel to [$\bar{1}$10]. Layers 0 through 5 were recorded. The systematic extinctions implied that the space group was either $C2/c$ or Cc.

Intensities from the Weissenberg photographs were estimated visually by comparison with a calibrated film strip. Utilizing a revision of an earlier data reduction program (Norment, 1962) for the IBM 7030 computer (STRETCH), intensities were corrected for spot size and Lorentz and polarization factors. No absorption corrections were made. The data were adjusted to an absolute scale and structure factor magnitudes $|F_h|$ and normalized structure factor magnitudes $|E_h|$ were determined. The $|E_h|$ are defined:

$$E_h^2 = F_h^2 / \varepsilon \sum_{j=1}^{N} f_{jh}^2 \tag{1}$$

where the F_h^2 have been corrected for vibrational motion, $\varepsilon = 2$ when **h** is $h0l$ and $\varepsilon = 1$ otherwise for both space groups $C2/c$ and Cc, f_{jh} is the atomic scattering factor for the *j*th atom in the unit cell, and N is the

number of atoms in the unit cell. Of the total 3360 independent reflections, 2680 were judged to have intensity values greater than zero.

The statistical averages involving the $|E_h|$ are shown in Table 1. They correspond to those for a centrosym-

Table 1. *Statistical averages*

	Experimental	Centro-symmetric	Noncentro-symmetric
$\langle|E|\rangle$	0·766	0·798	0·886
$\langle|E^2-1|\rangle$	1·023	0·968	0·736
$\langle|E|^2\rangle$	1·021	1·000	1·000

metric crystal and accordingly space group $C2/c$ was assigned at this point. The values in Table 2 describe the actual and theoretical distribution of $|E|$ magni-

Table 2. *Distribution of normalized structure factors*

	Experimental	Centro-symmetric	Noncentro-symmetric
$|E|>3$	0·5 %	0·3 %	0·01 %
$|E|>2$	4·7	5·0	1·8
$|E|>1$	26·6	32·0	37·0

tudes in a crystal. The theoretical values were computed on the basis of a random distribution of atoms. The comparison of the theoretical and experimental distributions indicate a reasonable centrosymmetric distribution.

Phase determination

The symbolic addition procedure (Karle & Karle, 1963, 1964; Karle, Britts & Gum, 1964) was used to determine the phases. For the non-primitive space

group $C2/c$, only two reflections are required to specify the origin (Hauptman & Karle, 1958). Three vectors having large $|E|$ magnitudes and a large number of interactions in the Σ_2 relationship:

$$sE_h \sim s \sum_k E_k E_{h-k} \tag{2}$$

where s means sign of, were assigned letter phases. These five reflections were used to initiate the symbolic addition procedure and they are listed in Table 3.

Table 3. *Starting set for the application of Σ_2*

Phase	h	k	l	$|E|$
+	2	6	19	2·50
+	3	7	1	2·86
b	0	4	27	3·59
a	6	2	$\bar{26}$	2·95
c	1	1	$\bar{28}$	3·15

Approximately two hundred and sixty phases for reflections with $|E| \geq 2\cdot0$ were determined by hand in terms of the initial signs and letters a, b, and c. A computed listing of interactions was used to expedite the determination. Relationships occurred among the letters since many terms entered into each phase determination [equation (2)]. The indications were very numerous that $a=-b$, $a=-$ and $b=+$. A few indications suggested that $c=-$.

The number of known phases was rapidly expanded to approximately 400 by a computer program. Two possibilities were tried where the symbols a, b, and c had the values $(-+-)$ and $(-++)$. The first combination was considered somewhat more probable since
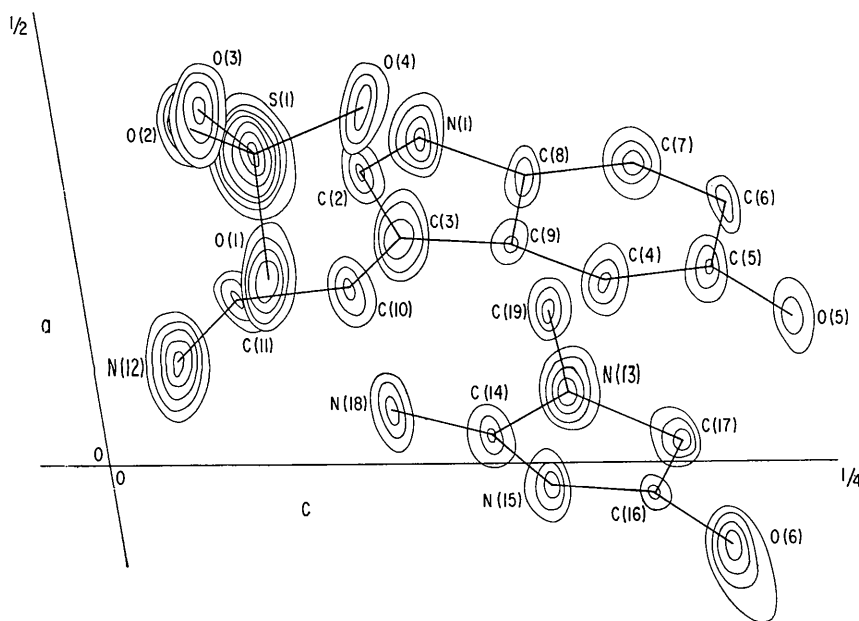

Fig. 1. A three-dimensional E-map projected on (010) computed from 402 phases which were determined by the symbolic addition procedure. Contours are at equally spaced, arbitrary levels. Every other contour for the sulfur atoms has been omitted.

there were fewer exceptions to the Σ_2 formula. However, the E-map (a Fourier map computed with E values rather than F values for the coefficients) for the first combination placed the sulfur atom too near a center of symmetry. The E-map computed from the second combination revealed the structure. It is illustrated in Fig. 1.

The structure

The initial E-map shown in Fig. 1 clearly revealed the positions of the 26 atoms in the creatinine, sulphate and serotonin groups. Of the several weaker peaks, only one was sufficiently reasonably situated to be the water of hydration. However, in a preliminary least-squares refinement (Busing, Martin & Levy, 1962), the value of the isotropic temperature factor of the water oxygen diverged. Therefore a difference map based on the 26 determined atoms was computed and it revealed two peaks at one-half weight for the water oxygen. One was too close to a center of symmetry; hence a second refinement was attempted using only the other. Again the value of the water temperature factor diverged. At this point, a possible alternative could be a noncentrosymmetric arrangement for the water. Two possibilities exist. Either the space group is Cc with an approximate center of symmetry for the serotonin, creatinine and sulfate, or the space group is $C2/c$ with the water molecules disordered. With both possibilities, the initial refinement proceeded normally. The quantity which was minimized in the refinement was $\Sigma w (F_o - F_c)^2$, where $w = 1$ for all $|F_o|$ observed to be greater than zero.

The large number of parameters and the great number of data involved made the computing time per cycle rather prohibitive. The coordinates and isotropic temperature factors have been refined at present only until the approximate bond distances confirmed the chemical reliability of the structure and indicated that continued cycling, especially with anisotropic temperature factors and the inclusion of hydrogen atoms, would probably complete the refinement. The approximate coordinates obtained at $R = 18.5\%$ for all the

data are given in Table 4.* The refinement will be completed in the future when the requirements for this large computation can be more readily fulfilled.

Table 4. *Fractional coordinates and isotropic temperature factors for serotonin for space group $C2/c$*

Atom	x	y	z	β
S	0·3390	0·5497	0·0657	2·6
O(1)	0·2025	0·5644	0·0621	3·6
O(2)	0·3710	0·4257	0·0446	4·1
O(3)	0·3928	0·6765	0·0513	3·4
O(4)	0·3976	0·5324	0·1072	3·7
N(1)	0·3659	0·2449	0·1248	3·8
C(2)	0·3213	0·1488	0·0968	3·7
C(3)	0·2558	0·0427	0·1102	3·2
C(4)	0·2011	0·0127	0·1797	3·4
C(5)	0·2165	0·0793	0·2159	3·8
C(6)	0·2880	0·1971	0·2234	4·2
C(7)	0·3439	0·2631	0·1952	4·1
C(8)	0·3235	0·2009	0·1579	3·7
C(9)	0·2555	0·0781	0·1507	3·1
C(10)	0·1907	−0·0778	0·0904	3·4
C(11)	0·1836	−0·0663	0·0469	3·3
N(12)	0·1016	−0·1819	0·0285	3·0
O(5)	0·1616	0·0144	0·2440	4·7
N(13)	0·0752	0·4605	0·1605	3·2
C(14)	0·0362	0·3668	0·1345	3·0
N(15)	−0·0326	0·2632	0·1484	3·2
C(16)	−0·0377	0·2930	0·1873	3·9
C(17)	0·0319	0·4243	0·1973	3·4
N(18)	0·0574	0·3654	0·0982	3·3
C(19)	0·1503	0·5841	0·1564	4·0
O(6)	−0·0926	0·2192	0·2073	4·7
O(7)	0·3542	0·1867	−0·0046	5·6
O(8)	0·5405	−0·0616	−0·0031	7·4

An electron density map was computed on the basis of these coordinates and is shown in Fig. 2. Bond distances and angles are tabulated in Table 5.

Discussion of the structure

A discussion of individual bond lengths is not warranted since the refinement process was terminated before completion. Nevertheless, there are some gross features of the structure which merit some discussion. If the space group is $C2/c$ and the water molecules are disordered, the disorder must not be completely random. In any particular (001) plane (the two water oxygen sites lie very near to the (001) planes), the water molecules must be ordered in a noncentrosymmetric array so that the intermolecular distances between the water molecules are not too small. However, disorder can exist in parallel (001) planes in that the water molec-
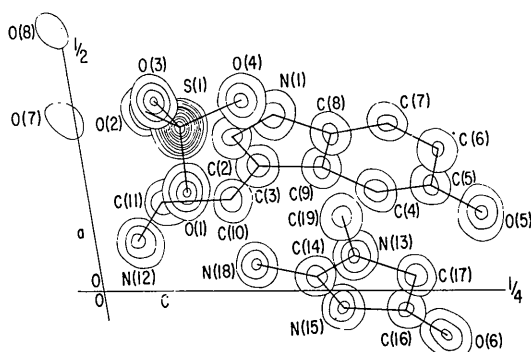

Fig. 2. The final electron-density map projected on (010). Contours are at intervals of 2 e.Å^{-3}, beginning with the 2 e.Å^{-3} contour.
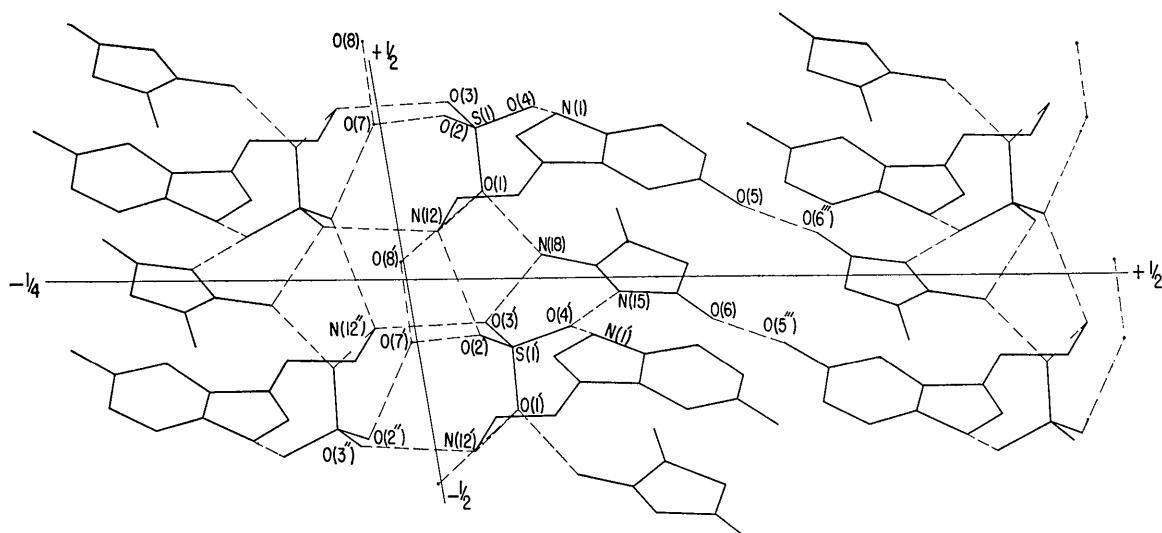
Fig. 3. Possible hydrogen bonding viewed along the *b* axis. Hydrogen bonds are indicated by dashed lines.

Table 5. *Bond distances and angles*

Bond	Length	Angle	
S(1)–O(1)	1·46 Å	O(1)–S(1)–O(2)	110°
S(1)–O(2)	1·48	O(1)–S(1)–O(3)	109
S(1)–O(3)	1·48	O(1)–S(1)–O(4)	111
S(1)–O(4)	1·50	O(2)–S(1)–O(3)	111
		O(2)–S(1)–O(4)	108
		O(3)–S(1)–O(4)	108
N(1)–C(2)	1·39	N(1)–C(2)–C(3)	113
C(2)–C(3)	1·38	C(2)–C(3)–C(9)	104
C(3)–C(9)	1·47	C(3)–C(9)–C(8)	107
C(9)–C(8)	1·40	C(9)–C(8)–N(1)	110
C(8)–N(1)	1·39	C(8)–N(1)–C(2)	106
C(8)–C(7)	1·43	C(8)–C(7)–C(6)	116
C(7)–C(6)	1·40	C(7)–C(6)–C(5)	122
C(6)–C(5)	1·38	C(6)–C(5)–C(4)	122
C(5)–C(4)	1·42	C(5)–C(4)–C(9)	117
C(4)–C(9)	1·41	C(4)–C(9)–C(8)	121
		C(9)–C(8)–C(7)	122
C(5)–O(5)	1·38	O(5)–C(5)–C(6)	122
C(3)–C(10)	1·48	O(5)–C(5)–C(4)	116
C(10)–C(11)	1·53	C(9)–C(3)–C(10)	136
C(11)–N(12)	1·51	C(2)–C(3)–C(10)	131
		C(3)–C(10)–C(11)	108
		C(10)–C(11)–N(12)	108
		C(3)–C(9)–C(4)	132
		N(1)–C(8)–C(7)	128
N(13)–C(14)	1·31	N(13)–C(14)–N(15)	113
C(14)–N(15)	1·38	C(14)–N(15)–C(16)	108
N(15)–C(16)	1·41	N(15)–C(16)–C(17)	108
C(16)–C(17)	1·49	C(16)–C(17)–N(13)	100
C(17)–N(13)	1·49	C(17)–N(13)–C(14)	110
N(13)–C(19)	1·46	C(19)–N(13)–C(14)	128
C(14)–N(18)	1·34	C(19)–N(13)–C(17)	122
C(16)–O(6)	1·22	N(18)–C(14)–N(13)	126
		N(18)–C(14)–N(15)	121
		O(6)–C(16)–N(15)	122
		O(6)–C(16)–C(17)	130

ules can occupy either the sites shown in Figs. 2 and 3 or those related by a center of symmetry.

The creatinine molecule is nearly planar as is the indole portion of the serotonin. In the chain, C(11) and N(12) are progressively further out of the plane of the indole group.

There is an intricate network of hydrogen bonding in all three dimensions. The probable hydrogen bonds are indicated in Fig. 3. All possible hydrogen atoms available for hydrogen bonding have been utilized. The distances between pairs of atoms which could act as donors and acceptors are reasonable, 2·7–3·0 Å, and the angles are reasonable as well. The possible hydrogen bonding arrangement suggests an =NH$_2$$^+$ group on the creatinine and an —NH$_3$$^+$ group on the serotonin.

References

Busing, W. R., Martin, L. O. & Levy, H. A. (1962). ORFLS, Oak Ridge National Laboratory, Oak Ridge, Tennessee, U.S.A.

Hauptman, H. & Karle, J. (1958). *Acta Cryst.* **12**, 93.

Karle, I. L. & Karle, J. (1963). *Acta Cryst.* **16**, 969.

Karle, I. L. & Karle, J. (1964). *Acta Cryst.* **17**, 1356.

Karle, I. L., Britts, K. & Gum, P. (1964). *Acta Cryst.* **17**, 496.

Norment, H. G. (1962). U.S. Naval Research Laboratory Report No. 5739,Washington, D.C., U.S.A.

Pré, S. du & Mendel,H. (1955). *Acta Cryst.* **8**, 311.

Wooley, D. W. (1962).*The Biochemical Bases of Psychoses or the Serotonin Hypothesis about Mental Diseases*. New York: John Wiley.