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Procaine: A Comparative Study of Two Independent Structure Determinations; Conformations in Different Solid-State Environments

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The results of a second, and independent, study of the procaine hydrochloride crystal structure are compared with a prior determination by means of a half-normal probability plot. The coordinate standard deviations obtained from the least-squares refinements appear to be underestimated by a factor of two. An examination of two solid-state conformations of procaine shows that the major conformational features are preserved in the different crystal environments.

Introduction

The solid-state conformation of the local anesthetic procaine (2-diethylaminoethyl *p*-aminobenzoate) has been determined in a *p*-nitrophenyl phosphate complex (Sax, Pletcher & Gustaffson, 1970) and in the hydrochloride salt (Beall, Herdklotz & Sass, 1970). The results of an independent determination of the procaine HCl structure are compared with those of Beall, Herdklotz & Sass (1970), (hereafter referred to as BHS) by means of a half-normal probability plot (Abrahams & Keve, 1971) and the conformations of procaine found in two different solid-state environments are examined.

Structure determination and refinement

The crystal data of procaine HCl and a comparison of cell dimensions determined here with those of BHS and of Rose (1958) are summarized in Table 1. For the present study a crystal, approximately 0·18 × 0·22 × 0·23 mm, was cut from a large needle and mounted on a computer-controlled GE diffractometer with **b** along

Table 1. *Crystal data of procaine* HCl *and comparison of cell dimensions*

Formula	$C_{13}H_{20}N_2O_2.HCl$		
M.W.	272·77		
Systematic absences	$0kl$ for l odd, $h0l$ for h odd		
Space group	*Pcab*		
Cell volume	2948·6 Å		
ϱ (X-ray)	1·229 g.cm^{-3}		
ϱ (flotation; Rose, 1958)	1·232 g.cm^{-3}		
Z	8		
μ	22·7 cm^{-1}		
	This work*	BHS (1970)	Rose (1958)
a	25·017 ± 0·001 Å	25·023 Å	25·04 Å
b	8·305 ± 0·0005	8·280	8·28
c	14·192 ± 0·001	14·157	14·35

* Lattice parameters and standard deviations were obtained from a least-squares calculation with 2θ values of general high-angle reflections.

Table 2. *Final coordinates and thermal parameters* of procaine hydrochloride*

	x	y	z	U_{11}	U_{22}	U_{33}	U_{12}	U_{13}	U_{23}
C(1)	2986 (2)	1136 (5)	3510 (3)	6·6 (2)	8·1 (3)	5·8 (2)	0·5 (2)	0·0 (2)	1·5 (2)
C(2)	3101 (1)	9360 (4)	3614 (2)	5·3 (2)	6·5 (2)	4·7 (2)	−0·9 (2)	−0·5 (1)	−0·1 (2)
C(3)	4270 (1)	9327 (5)	5382 (3)	4·3 (2)	7·8 (3)	7·7 (2)	0·2 (2)	−0·5 (2)	−2·2 (2)
C(4)	3980 (1)	9575 (4)	4462 (2)	3·9 (1)	5·1 (2)	6·5 (2)	−0·7 (1)	0·6 (1)	−1·0 (2)
C(5)	3349 (1)	7260 (4)	4801 (2)	3·9 (1)	5·6 (2)	5·4 (2)	−0·2 (1)	0·5 (1)	0·3 (1)
C(6)	3545 (1)	6018 (4)	4112 (3)	4·5 (2)	4·9 (2)	7·1 (2)	−0·5 (1)	−0·6 (2)	−0·4 (2)
C(7)	4356 (1)	4887 (4)	3556 (2)	5·3 (2)	4·8 (2)	5·0 (2)	0·0 (1)	−0·1 (1)	0·0 (1)
C(8)	4935 (1)	5049 (4)	3533 (2)	4·9 (2)	4·5 (2)	4·2 (1)	0·3 (1)	0·4 (1)	0·4 (1)
C(9)	5238 (1)	4034 (4)	2942 (2)	6·1 (2)	5·2 (2)	5·2 (2)	−0·1 (2)	0·5 (1)	−0·7 (2)
C(10)	5776 (1)	4179 (4)	2869 (2)	6·1 (2)	5·5 (2)	5·4 (2)	0·8 (2)	1·3 (1)	−1·2 (2)
C(11)	6058 (1)	5370 (4)	3369 (2)	4·5 (2)	5·5 (2)	4·8 (2)	0·7 (1)	0·6 (1)	0·8 (1)
C(12)	5762 (1)	6330 (4)	3995 (2)	4·9 (2)	4·8 (2)	4·7 (2)	0·4 (1)	−0·1 (1)	−0·4 (1)
C(13)	5218 (1)	6182 (4)	4062 (2)	4·8 (2)	4·7 (2)	4·3 (2)	0·4 (1)	0·5 (1)	0·0 (1)
N(1)	3410 (1)	8990 (3)	4504 (2)	3·7 (1)	4·6 (1)	4·3 (1)	−0·1 (1)	0·4 (1)	−0·3 (1)
N(2)	6597 (1)	5529 (4)	3295 (2)	4·8 (2)	7·2 (2)	7·1 (2)	0·6 (1)	0·9 (1)	−0·9 (2)
O(1)	4115 (1)	6060 (3)	4067 (1)	4·0 (1)	4·9 (1)	6·0 (1)	0·1 (1)	0·1 (1)	−0·8 (1)
O(2)	4095 (1)	3857 (3)	3175 (2)	6·1 (1)	6·1 (1)	9·4 (2)	−1·1 (1)	−1·1 (1)	−3·3 (1)
Cl	29008 (3)	07854 (11)	61733 (5)	4·73 (4)	7·79 (6)	4·78 (4)	1·62 (4)	−0·11 (3)	−1·10 (4)

* Positional parameters for Cl are $\times 10^5$, for H, $\times 10^3$ and for all others, $\times 10^4$. The U_{ij}'s are $\times 10^2$. E.s.d.'s are those obtained from the least-squares refinement and refer to the last decimal place given. The form of the anisotropic temperature factor is: $\exp[-2\pi^2(U_{11}h^2a^{*2}+U_{22}k^2b^{*2}+U_{33}l^2c^{*2}+2U_{12}hka^*b^*+2U_{13}hla^*c^*+2U_{23}klb^*c^*)]$.

Table 2 (*cont.*)

	x	*y*	*z*
H(1)	272 (1)	134 (4)	299 (2)
H(2)	276 (1)	151 (4)	406 (2)
H(3)	332 (1)	160 (4)	334 (2)
H(4)	272 (1)	388 (4)	127 (2)
H(5)	333 (1)	397 (4)	192 (2)
H(6)	041 (1)	004 (4)	038 (2)
H(7)	061 (1)	320 (4)	456 (2)
H(8)	094 (1)	−024 (4)	087 (2)
H(9)	416 (1)	409 (4)	107 (2)
H(10)	392 (1)	078 (4)	434 (2)
H(11)	298 (1)	212 (4)	008 (2)
H(12)	149 (1)	219 (4)	462 (2)
H(13)	346 (1)	514 (4)	437 (2)
H(14)	345 (1)	104 (4)	151 (2)
H(15)	005 (1)	188 (4)	260 (2)
H(16)	098 (1)	143 (4)	249 (2)
H(17)	095 (1)	285 (4)	064 (2)
H(18)	000 (1)	303 (4)	050 (2)
H(19)	174 (1)	377 (4)	156 (2)
H(20)	173 (1)	−011 (4)	284 (2)
H(21)	173 (1)	458 (4)	506 (2)

the φ axis. Peak heights of 3033 unique reflections with $2\theta < 150°$ (Cu $K\bar{\alpha}$; $\lambda = 1{\cdot}5418$ Å) were measured using the stationary-crystal stationary-counter technique and a take-off angle of 4°. The 2486 reflections for which $I_p > 2\sigma(I_p)$ were treated as observed. Approximate integrated intensities were obtained from the peak heights, as described by Alexander & Smith (1962), and were corrected for Lorentz and polarization factors but not for absorption.

The structure was determined routinely after chloride ion coordinates were deduced from an E^2-1 vector map. Refinement by least-squares methods with isotropic and then anisotropic temperature factors led to a final R of 0·072. Hydrogen atoms were included in the refinement, with isotropic temperature factors fixed at 4·0 Å^2. Before the last three cycles 15 reflections which had been mismeasured were removed from the reflection list. In the last cycle the average shift for all 227 variables was $0{\cdot}2\sigma$ and the maximum shift was less than $1{\cdot}0\sigma$. A final difference map showed no positive peak larger than about 0·3e.

Final coordinates and thermal parameters are presented in Table 2. Bond distances and angles are listed in Table 3. Observed and calculated structure factors are given in Table 4. Fig. 1(*a*) is an *ORTEP* (Johnson, 1965) drawing of the molecule.

Most calculations were carried out with the *XRAY*67 programs (Stewart, 1967) and their 1970 revisions. The quantity minimized in the least-squares refinement was $\sum w(|F_o|-|F_c|)^2$ with all reflections receiving unit weight. Mean planes were computed using the routine of Ahmed, Hall, Pippy & Saunderson (1966). Scattering factors for carbon, nitrogen, and oxygen were taken from *International Tables for X-ray Crystallography* (1962), for hydrogen from Stewart, Davidson & Simpson (1965), and for chlorine from Cromer & Waber (1965).

Table 3. *Bond distances and angles of procaine hydrochloride*

C(1)–C(2)	1·511 (6) Å	C(6)—H(14)	0·91(3)Å
C(1)–H(1)	1·01 (3)	C(7)—O(1)	1·356 (4)
C(1)–H(2)	1·01 (3)	C(7)—O(2)	1·210 (4)
C(1)–H(3)	0·95 (3)	C(7)—C(8)	1·457 (4)
C(2)–N(1)	1·512 (4)	C(8)—C(9)	1·409 (4)
C(2)–H(4)	1·07 (3)	C(8)—C(13)	1·396 (4)
C(2)–H(5)	1·02 (3)	C(9)—C(10)	1·356 (5)
C(3)–C(4)	1·508 (5)	C(9)—H(15)	1·01 (3)
C(3)–H(6)	0·99 (3)	C(10)–C(11)	1·407 (5)
C(3)–H(7)	0·98 (3)	C(10)–H(16)	0·90 (3)
C(3)–H(8)	0·95 (3)	C(11)–C(12)	1·405 (4)
C(4)–N(1)	1·506 (4)	C(11)–N(2)	1·359 (4)
C(4)–H(9)	0·98 (3)	C(12)–C(13)	1·367 (4)
C(4)–H(10)	1·02 (3)	C(12)–H(17)	0·98 (3)
C(5)–N(1)	1·505 (4)	C(13)–H(18)	1·05 (3)
C(5)–C(6)	1·503 (5)	N(1)—H(21)	0·87 (3)
C(5)–H(11)	0·93 (3)	N(2)—H(19)	0·72 (4)
C(5)–H(12)	0·92 (3)	N(2)—H(20)	0·81 (3)
C(6)–O(1)	1·427 (4)		
C(6)–H(13)	0·85 (3)		

C(1)—C(2)—N(1)	112·2 (3)°
C(3)—C(4)—N(1)	112·2 (3)
C(2)—N(1)—C(4)	112·7 (2)
C(2)—N(1)—C(5)	112·1 (2)
C(4)—N(1)—C(5)	114·5 (2)
C(5)—N(1)—C(6)	116·1 (3)
C(5)—C(6)—O(1)	104·7 (3)
O(1)—C(7)—O(2)	121·0 (3)
O(1)—C(7)—C(8)	112·9 (3)
O(2)—C(7)—C(8)	126·2 (3)
C(7)—C(8)—C(9)	119·5 (3)
C(7)—C(8)—C(13)	123·7 (3)
C(4)—C(8)—C(13)	116·8 (3)
C(8)—C(9)—C(10)	121·7 (3)
C(9)—C(10)–C(11)	121·5 (3)
C(10)–C(11)–C(12)	116·9 (3)
C(10)–C(11)–N(2)	121·8 (3)
C(12)–C(11)–N(2)	121·2 (3)
C(11)–C(12)–C(13)	121·1 (3)
C(8)—C(12)–C(13)	121·9 (3)

Comparison of the two procaine HCl structures

The same 227 variables were refined in both studies, but data sets differed in size and in collection technique. BHS based their analysis on 883 reflections measured by the fixed-counter moving-crystal method using silicon monochromatized Mo $K\alpha$ radiation on an equi-inclination diffractometer.

Abrahams & Keve (1971) have recently described how independent determinations of the same structure may be compared by means of a half-normal probability plot. The ordered statistic δp_i is plotted against the expected normal distribution

$$\delta p_i = ||p(\mathrm{DDD})_i| - |p(\mathrm{BHS})_i||/[\sigma^2 p(\mathrm{DDD})_i + \sigma^2 p(\mathrm{BHS})_i]^{1/2}, \quad (1)$$

where p_i are the positional parameters obtained from the respective determinations (DDD are the initials of the present author) and σp_i are the associated standard deviations. For correctly estimated σp_i and random distribution of errors, this plot is linear with a slope of unity and an intercept of zero.

The individual $\sigma p(\mathrm{BHS})_i$ are not given by BHS and have been estimated as follows: (*a*) since $\sigma p_i \propto$ [no. of degrees of freedom]$^{1/2}$, the larger number of reflections used in the present refinement would be expected to lead to standard deviations approximately one-half those of BHS; (*b*) BHS quote 0·01 Å as the estimated standard deviation for their bond distances and the corresponding standard deviations found here (Table 3) are roughly one-half as large. Fig. 2 shows the half-normal probability plot that results, assuming

$$\sigma p(\mathrm{BHS})_i = 2\sigma p(\mathrm{DDD})_i$$

for the 117 positional parameters of procaine HCl.

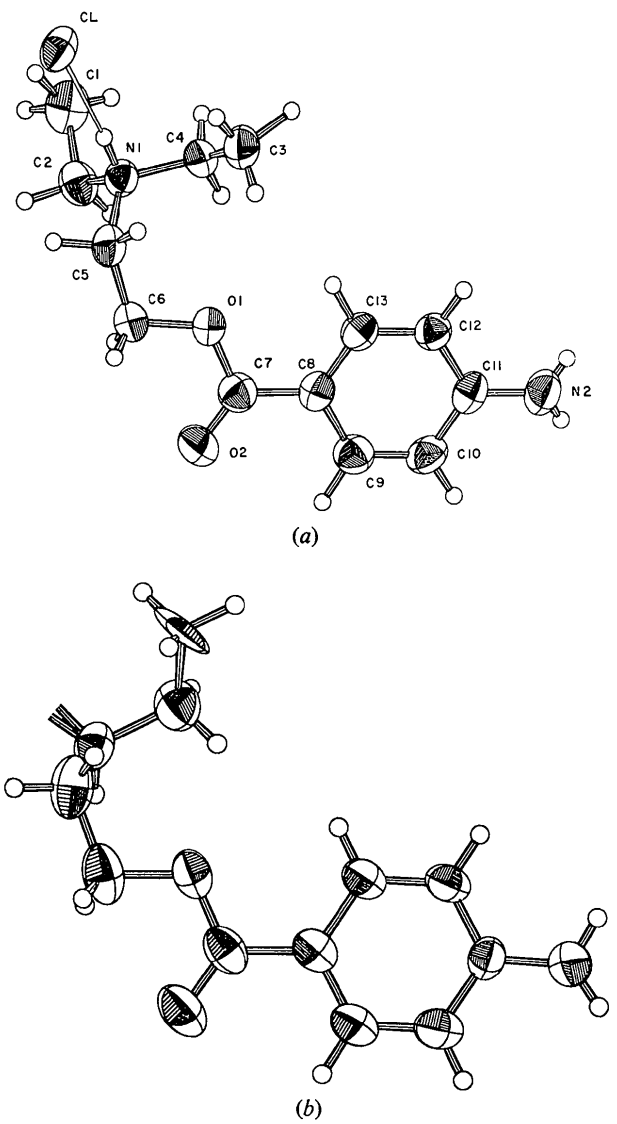

Fig. 1. (*a*) Perspective drawing of procaine hydrochloride. (*b*) Perspective drawing of procaine in the 1:1 procaine bis-*p*-nitrophenyl phosphate complex. One ethyl group is disordered and has not been drawn. In (*a*) and (*b*) molecules are viewed normal to the benzene ring, and thermal ellipsoids are plotted at the 50% level.

The array of points in Fig. 2 is nearly linear and has an intercept of approximately zero. This indicates (Abrahams & Keve, 1971) that the two crystals studied are samples of the same material and that the errors in the data are mostly random. The slope of the array is approximately two, and Abrahams & Keve have shown that the most likely explanation for this is that the denominator of equation (1) has been underestimated by about one half. If the above estimate of the $\sigma p(\mathrm{BHS})_i$ is appropriate, and both data sets are equally affected, then the standard deviations derived from the least-squares refinement are small by a factor of two.

After adjusting the scale of the σ's, only five of the 117 parameters differ by more than 2σ. The only discrepancies between the two structures are associated with the ends of the molecule: three with the hydrogen atoms in the ethyl groups, one with a hydrogen atom in the *p*-amino group, and one with a terminal carbon atom in an ethyl group.

Comparison of procaine conformations

Fig. 1(*b*) shows the procaine molecule of the 1:1 procaine – bis-*p*-nitrophenyl phosphate complex (Sax *et al.*, 1970). Major differences between procaine molecules in the salt and in the phosphate complex are: (*a*) a 145° relative rotation about the C(5)–N(1) bond, and (*b*) the quinonoid character of the *p*-aminobenzoate group. Some torsion angles along the side chain in the two molecules are compared in Table 5. Despite the rotation about the C(5)–N(1) bond, the molecules maintain largely similar conformations.

A packing diagram of the hydrochloride salt (Fig. 3) shows layers of procaine molecules, their phenyl rings stacked in the familiar herringbone array, alternating with layers of chloride ions. The protonated tertiary amino nitrogen N(1) is hydrogen-bonded to a chloride ion 3·08 Å distant. Each chloride ion also interacts with two *p*-amino groups, 3·39 and 3·44 Å away. These nitrogen–chlorine interactions affect not only the conformation of the alkylamino end of the molecule, but

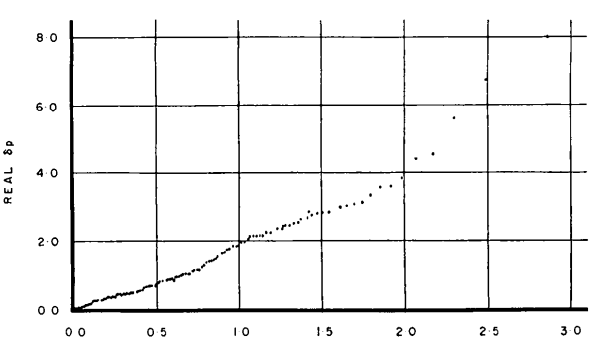

Fig. 2. Half-normal probability plot comparing the results of Beall, Herdklotz & Sass (1970) for procaine HCl with the present work.

also the quinonoid character of the *p*-aminobenzoate group. The N(2)–C(11), C(9)–C(10), and C(12)–C(13) distances of 1·359 (4), 1·356 (5), and 1·367 (4) Å, respectively, tend to be shorter than the corresponding distances in the phosphate complex [1·377 (7), 1·381 (8), 1·369 (7) Å]. In the hydrochloride salt, the average of the interior angles at the *para* positions of the phenyl ring is 5·0° less than the average of the other interior

Table 4. *Observed and calculated structure factors for procaine* HCl

Columns are h, $10F_o$ and $10F_c$. Unobserved reflections are marked with an asterisk and the extinguished reflection by *E*.

Table 4 (*cont.*)

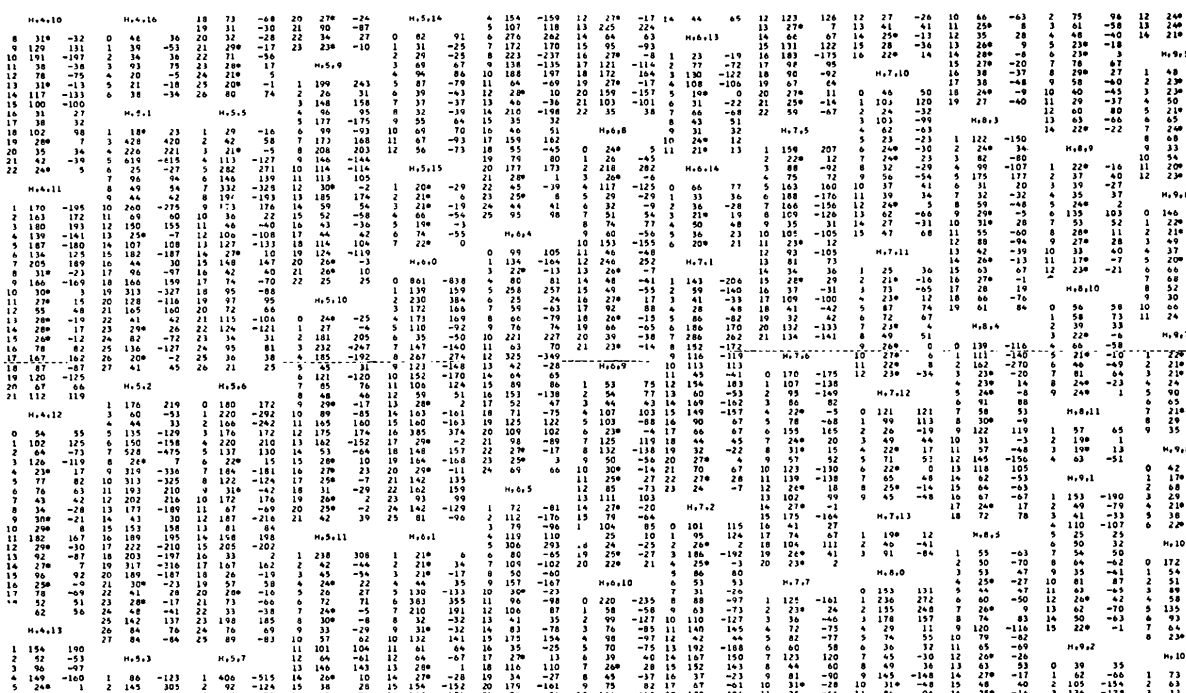

Table 5. *Torsion angles in procaine*

Atoms involved (procaine HCl numbering)	τ (°) Procaine HCl (DDD)	τ (°)* Phosphate complex
O(2)–C(7)–O(1)–C(6)	1·0	2·8
C(7)–O(1)–C(6)–C(5)	173·3	178·8
O(1)–C(6)–C(5)–N(1)	70·1	61·6
C(6)–C(5)–N(1)–C(4)	69·0	92·2
C(6)–C(5)–N(1)–C(2)	−61·4	†
C(5)–N(1)–C(4)–C(3)	54·6	70·2
C(5)–N(1)–C(2)–C(1)	158·2	†
C(6)–C(5)–N(1)–H(21)	175·8	31·0

* Calculated from the coordinates of Sax *et al.* (1970).

† These angles involve the disordered part of the structure [See Fig. 1(*b*)] and were not calculated.

angles, while this difference is only 2·5° in the complex. In both structures, the benzene ring adopts a slight boat form; however, the deviations of C(8) and C(11) from the mean plane of the ring in the salt are twice those of their counterparts in the complex. The mean plane of the phenyl ring ($0{\cdot}1183X+0{\cdot}6228Y-0{\cdot}7394Z+2{\cdot}4035=0$) makes an angle of 28° with the plane of the *p*-amino group ($0{\cdot}5672X+0{\cdot}5904Y-0{\cdot}5742Z+0{\cdot}3296=0$) and an angle of 7·4° with the plane of the carboxyl group ($0{\cdot}0655X+0{\cdot}5746Y-0{\cdot}8158Z+2{\cdot}4946=0$). For the phosphate complex, these angles are 1·6 and 2·8° respectively. (The coefficients in the mean plane equations are in Å and refer to an orthogonal set of axes where X lies along a, Y is in the (a,b), plane and Z lies along c^*.)

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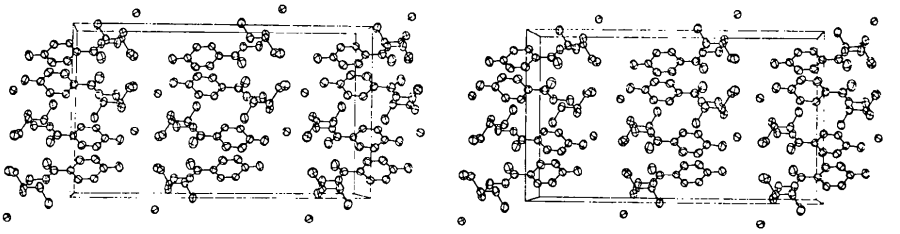

Fig. 3. Stereodiagrams showing packing of procaine HCl. $a\rightarrow$, $c\uparrow$, and b into the paper.

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References

ABRAHAMS, S. C. & KEVE, E. T. (1971). *Acta Cryst.* A**27**, 157.

AHMED, F. R., HALL, S. R., PIPPY, M. E. & SAUNDERSON, C. P. (1966). *NRC Crystallographic Programs for the IBM/360 System, IUCr World List of Crystallographic Computer Programs.* 2nd ed. Appendix, p. 52.

ALEXANDER, L. E. & SMITH, G. S. (1962). *Acta Cryst.* **15**, 983.

BEALL, E., HERDKLOTZ, J. & SASS, R. L. (1970). *Biochem. Biophys. Res. Comm.* **39**, 329.

CROMER, D. T. & WABER, J. T. (1965). *Acta Cryst.* **18**, 104.

International Tables for X-ray Crystallography (1962). Vol. III. Birmingham: Kynoch Press.

JOHNSON, C. K. (1965). *ORTEP, A Fortran Ellipsoid Plot Program for Crystal Structure Illustrations.* Report ORNL-3794, Oak Ridge National Laboratory, Oak Ridge, Tennessee.

ROSE, H. A. (1958). *Acta Cryst.* **11**, 300.

SAX, M., PLETCHER, J. & GUSTAFFSON, R. (1970). *Acta Cryst.* B**26**, 114.

STEWART, J. M. (1967). Technical Report 67-58, Computer Science Center, Univ. of Maryland.

STEWART, R. F., DAVIDSON, R. E. & SIMPSON, W. T. (1965). *J. Chem. Phys.* **42**, 3175.

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The Crystal and Molecular Structure of Serotonin Picrate Monohydrate

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Red crystals of serotonin picrate monohydrate ($C_{10}H_{13}N_2O.C_6H_2N_3O_7.H_2O$) are monoclinic, space group $P2_1/c$, with $a=14{\cdot}172$, $b=6{\cdot}908$, $c=18{\cdot}749$ Å, and $\beta=101{\cdot}65°$. Data were collected on an automated diffractometer; the structure was solved by the symbolic-addition procedure and was refined by block-diagonal least-squares methods to $R=0{\cdot}073$. The crystal structure features continuous columns of approximately parallel hydroxyindole and picrate moieties, intimately stacked with interplanar spacings of 3·3–3·4 Å. The stacking interaction appears to be of the donor–acceptor (charge-transfer) type. Bond lengths within the picrate ion are not significantly different from those found for other picrate salts. The serotonin cation assumes a conformation which is different from that found in the crystal structure of serotonin creatinine sulphate.

Introduction

Serotonin (5-hydroxytryptamine) is an indolealkylamine found in all vertebrate and some invertebrate systems (Erspamer, 1961). Although the exact physiological functions of serotonin are unknown, there is evidence that the compound mediates a number of processes, including smooth muscle contraction (Erspamer, 1961) and synaptic transmission (Chase, Breese, Carpenter, Schanberg & Kopin, 1968; Fuxe, Hökfelt & Ungerstedt, 1968; Bradley, 1968). In humans, serotonin affects the central nervous system, and abnormal metabolism of brain serotonin has been implicated in mental disorders (Woolley, 1962).

Little is known about the specific mechanisms by which serotonin affects biological systems; but it has been suggested that many of the physiological properties of the compound might be related to its propen-