Structure of a Main Metabolite of Piroheptine Excreted in Urine of Rabbits; X-Ray Analysis of Its Hydrobromide

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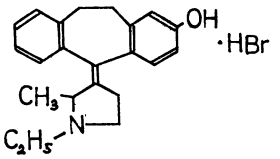
The structure of a main metabolite of piroheptine excreted in urine after administered to rabbits has been determined by X-ray analysis of its hydrobromide, $C_{22}H_{26}NOBr$. The crystals are monoclinic with space group $P2_1/n$, the unit-cell dimensions are: $a=14.28$, $b=12.50$, $c=10.97$ Å, $\beta=96.06°$. The intensities were estimated visually from multiple-film equi-inclination Weissenberg photographs. The structure was solved by the heavy atom method. The atomic parameters were refined by the block-diagonal least-squares method, and the final R index was 0.11. The established structure is shown by the following formula (hereafter, named as piroheptine metabolite HBr)



It has been cleared that the hydroxylation in the metabolic process takes place at the 2-position in the benzene ring located far from the N-ethyl group on pyrrolidine ring, and piroheptine metabolite HBr and piroheptine HBr differ considerably in their conformations in the crystalline state.

Piroheptine[1] has been found to show potent antagonism against tremorine-induced tremor in mice.[2] Nojima *et al.*[3] have suggested that 3-(10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5-ylidene)-1,2-dialkylpyrrolidines and their salts seemed to have more than two conformations in solution on the basis of interpretation of PMR spectra. The conformation of piroheptine hydrobromide in the crystal has been found to correspond to the most stable one suggested by PMR study in solution.[4]

In the research of piroheptine metabolism, the main metabolite in urine of rabbits administered piroheptine was isolated by thin layer and gas liquid chromatography. High resolution mass spectrographical study revealed the mono-hydroxylation on benzene ring in the metabolic process, but not the position of hydroxylation. The X-ray analysis of the crystal of piroheptine metabolite hydrobromide was undertaken to elucidate the precise molecular structure and to compare it with piroheptine hydrobromide in the crystal.

Experimental

Piroheptine hydrochloride suspended in deionized water was orally given to rabbits in dose of 200 mg/kg. The urine collected for 24 hr was refluxed for 1 hr in 2 M HCl in order to hydrolyze the conjugated compounds. The main metabolite extracted from $CHCl_3$ was isolated by thin layer and gas liquid chromatography. After the main metabolite was dissolved in a slight excess of 50% HBr, it was heated in a water bath at 50 °C for 45 min and then was evaporated *in vacuo*. The residual oil was recrystallized twice in ethanol. The suitable crystals for X-ray work were obtained from the ethanol in the form of colorless prisms elongated along the *b* axis.

Unit-cell dimensions were determined by the least-squares calculation using the high angle spots of $CuK\alpha_1$ and $CuK\alpha_2$ on zero-layer Weissenberg photographs superimposed by the Al wire patterns for calibration. The density was measured by floatation in a benzene–carbon tetrachloride mixture. Crystal data are summarized in Table 1. The three dimensional intensity data were collected from multiple-film equi-inclination Weissenberg photographs for the layer lines 0 to 9 on the b axis and 0 on the a axis using nickel-filtered $CuK\alpha$ radiation. The intensities of reflection were visually estimated by comparison with a standard scale. The values of intensity ranged from 1 to 8600. Corrections were made for Lorentz, polarization factors and variation in spot size on higher layer photographs.[5] The intensity data from the a axis photograph were used only for layer scale adjustment of b axis photographs. Thus 2413 intensity data were obtained of which 379 were too weak to be observed.

TABLE 1. CRYSTAL DATA

$C_{22}H_{26}NOBr$; F.W.=400.4, mp=258 °C.
Monoclinic; $a=14.28\pm0.01$, $b=12.50\pm0.01$, $c=10.97\pm0.01$ Å, $\beta=96.06\pm0.06°$, $V=1948$ Å^3.
$D_m=1.36$, $Z=4$, $D_x=1.365$ g/cm^3.
$F(000)=832$.
Absent spectra; $h0l$ when $h+l$ is odd, $0k0$ when k is odd.
Space group; $P2_1/n$.

Structure Determination

The coordinates of a bromide ion were deduced from the Patterson function. Fourier synthesis based on the coordinates of the bromide ion clearly revealed all the 24 light atoms. The positional and thermal parameters of the atoms were refined by the block-diagonal least-squares method which minimized $\sum w(|F_o|-|F_c|)^2$. Two cycles of the least-squares refinement were carried out, using isotropic temperature factors to all the atoms and ion. Four more cycles of the refinement were performed, assigning anisotropic temperature factors to only bromide ion. The R index was reduced to 0.11, excluding non-observed reflections ($R=0.13$ for all 2413 reflections). The weighting scheme adopted in the

Table 2. The fractional atomic coordinates, isotropic temperature factors (Å²) and their standard deviations in parentheses

Atom	x	y	z	B
Br	0.2053(1)	0.1952(1)	−0.1549(1)	
N(1)	0.2476(5)	−0.0313(7)	−0.0340(7)	2.4(1)
C(2)	0.2927(6)	0.0017(8)	0.0943(8)	2.1(2)
C(3)	0.2059(6)	0.0374(8)	0.1557(8)	2.1(2)
C(4)	0.1169(7)	−0.0029(9)	0.0814(9)	2.7(2)
C(5)	0.1527(7)	−0.0800(10)	−0.0133(10)	3.4(2)
C(6)	0.3026(8)	−0.1030(10)	−0.1098(10)	4.0(2)
C(7)	0.3925(9)	−0.0476(12)	−0.1418(12)	4.8(3)
C(8)	0.3452(8)	−0.0910(10)	0.1608(10)	3.9(2)
C(9)	0.4262(7)	0.1092(10)	0.4939(10)	3.4(2)
C(10)	0.4803(8)	0.1820(10)	0.4381(10)	4.0(2)
C(11)	0.4479(7)	0.2273(9)	0.3260(9)	3.3(2)
C(12)	0.3600(6)	0.1997(9)	0.2703(9)	2.7(2)
C(13)	0.3048(6)	0.1229(8)	0.3243(7)	1.9(2)
C(14)	0.2114(6)	0.0897(8)	0.2622(8)	2.3(2)
C(15)	0.1265(6)	0.1193(8)	0.3238(8)	2.3(2)
C(16)	0.0518(6)	0.1707(8)	0.2569(8)	2.3(2)
C(17)	−0.0267(7)	0.2107(9)	0.3098(9)	3.0(2)
C(18)	−0.0303(7)	0.1902(10)	0.4351(10)	3.4(2)
C(19)	0.0411(8)	0.1394(10)	0.5012(10)	3.9(2)
C(20)	0.1201(6)	0.1014(9)	0.4509(8)	2.8(2)
C(21)	0.1918(8)	0.0433(10)	0.5403(10)	4.0(2)
C(22)	0.2781(8)	−0.0064(10)	0.4910(10)	3.9(2)
C(23)	0.3386(7)	0.0790(9)	0.4386(9)	3.0(2)
O(24)	−0.1044(6)	0.2291(8)	0.4929(8)	5.4(2)

Table 3. Anisotropic temperature factors for bromide ion (their standard deviations)

The anisotropic temperature factors are expressed in the form of $\exp[-(\beta_{11}h^2+\beta_{22}k^2+\beta_{33}l^2+\beta_{12}hk+\beta_{13}hl+\beta_{23}kl)]$

Atom	β_{11}	β_{22}	β_{33}
Br	0.00561 (0.00007)	0.00721 (0.00013)	0.00933 (0.00011)

Atom	β_{12}	β_{13}	β_{23}
Br	0.00144 (0.00013)	0.00352 (0.00014)	0.00447 (0.00017)

final least-squares calculation was; $w=0.5$ when $|F_o|=0$, $w=1.0$ when $|F_o|<24.0$ and $w=24.0/|F_o|$ when $|F_o|\geq 24.0$. The scattering factors for C, N, O, and Br^- were taken from International Tables for X-ray Crystallography.[6]

The final atomic coordinates and isotropic temperature factors are given in Table 2, and the anisotropic temperature factors for the bromide ion in Table 3. The complete F_o-F_c table are kept at the office of the Bulletin of the Chemical Society of Japan. (Document No. 7501).

Most of the computer programs used were in the UNICS.[7] All the programs were modified by the authors to allow calculations on middle size of computer, HITAC 8400 at Nippon Business Consultant and Fujisawa Pharmaceutical Co., Ltds.

Results and Discussion

Piroheptine metabolite hydrobromide viewed along the c axis is shown in Fig. 1. The bond lengths and angles are shown in Fig. 2. The averaged estimated

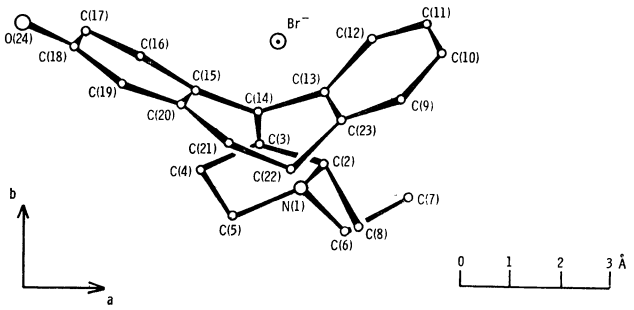


Fig. 1. Piroheptine metabolite hydrobromide viewed along the c axis.

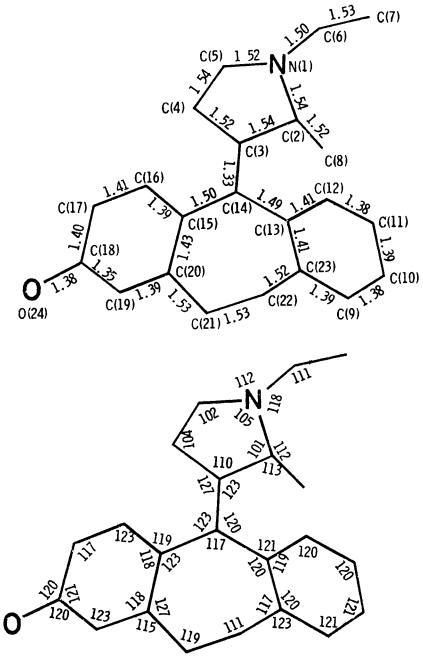
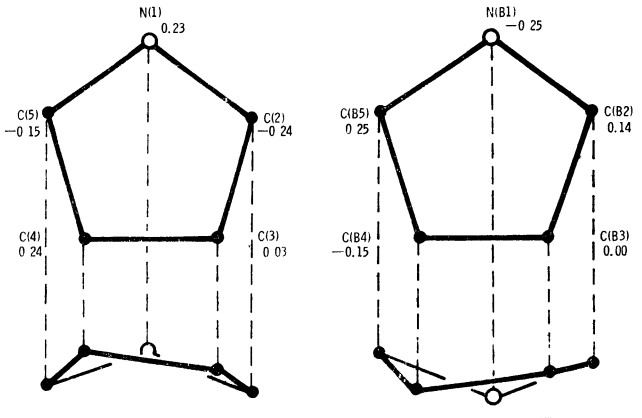
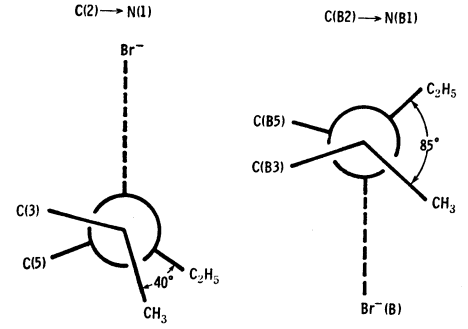


Fig. 2. Bond lengths and angles.



piroheptime metabolite·HBr piroheotine·HBr

Fig. 3. Conformation of the pyrrolidine ring. Arabic numerals show the displacements (in Å) of the atoms from the best plane of the pyrrolidine ring. For comparison, that of piroheptine hydrobromide B[4] is shown on the right.



piroheptine metabolite·HBr piroheptine·HBr

Fig. 4. Stereographic projection of the conformation about C(2)–N(1) bond. For comparison, one of piroheptine hydrobromide B[4] is shown on the right.

standard deviations in the bond lengths and angles are about 0.016 Å and 1.0°, respectively. Carbon atoms C(22) and C(21) are located on 0.96 and 0.28 Å respectively out of the best plane through the four carbon atoms, C(13), C(15), C(20), and C(23), of the seven-membered ring which are jointed in the benzene rings connected in both sides. The C(20)–C(21)–C(22) angle, 119°, in the seven-membered ring has a large deviation from the tetrahedral angle. The similar angular deviation in seven-membered ring was also found in the crystal structure of piroheptine hydrobromide[4] and 5-(bromomethylene)-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cycloheptene.[8]

Table 4. Best planes

(1) Equations[a]

Benezne plane I

$$0.474X - 0.742Y - 0.474Z = -0.945$$

Benzene plane II

$$0.441X + 0.873Y + 0.208Z = 2.658$$

Ylidene plane

$$-0.004X + 0.862Y - 0.507Z = -0.497$$

a) X, Y, and Z are the rectangular coordinates in Å unit.

(2) Displacements of atoms from the planes

Benzene plane I		Benzene plane II		Ylidene plane	
C(9)	−0.01 Å	C(15)	−0.01 Å	C(2)	−0.02 Å
C(10)	0.01	C(16)	0.02	C(3)	0.03
C(11)	0.01	C(17)	−0.02	C(4)	0.01
C(12)	−0.02	C(18)	0.01	C(14)	0.00
C(13)	−0.02	C(19)	0.00	C(13)	0.01
C(23)	0.00	C(20)	0.00	C(15)	−0.02
C(14)*	0.05	C(14)*	−0.11		
C(22)*	0.08	C(21)*	0.03		
		O(24)*	−0.05		

The atoms with asterisk were not included in the least-squares calculations.

(3) Dihedral angles between the planes

	Piroheptine metabolite HBr	Piroheptine[4] HBr
Benzene plane I and Benzene plane II	58°	63°
Benzene plane I and Ylidene plane	66	59
Benzene plane II and Ylidene plane	50	76

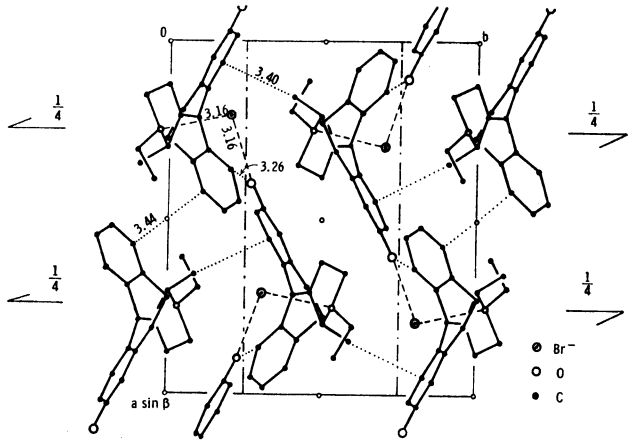


Fig. 5. A drawing of the structure viewed along the c axis. Hydrogen bonds and van der Waals contacts are indicated by broken and dotted lines, respectively.

The conformation of the pyrrolidine ring is shown in Fig. 3 with one of piroheptine hydrobromide.[4] Arabic numerals denote the displacements (in Å) of the atoms from the best plane of the pyrrolidine ring.

The conformation about the C(2)–N(1) bond is compared with one of piroheptine hydrobromide[4] in Fig. 4. The relation between the ethyl and methyl group is *cis*-position in contrast with the case of piroheptine hydrobromide that corresponds to the most stable one suggested by PMR study in solution.[4]

The 2-hydroxy-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5-ylidene part consists of the three planes. The equations for these planes, displacements of the individual atoms from the planes and dihedral angles between the planes are given in Table 4. The oxygen atom O(24) of hydroxyl group is situated on benzene plane II within the limits of error.

The drawing of the structure viewed along the c axis is shown in Fig. 5. It seems that two hydrogen bondings (N(1)–H···Br⁻ and O(24)–H···Br⁻) are formed in this crystal structure, and the N(1) is protonated. These hydrogen bondings are:

N(1)–H···Br⁻		O(24)–H···Br⁻	
N(1)···Br⁻	3.16 Å	Br⁻···O(24)*	3.16 Å
Br⁻···N(1)–C(2)	101°	Br⁻···O(24)*–C(18)*	122°
Br⁻···N(1)–C(5)	107		
Br⁻···N(1)–C(6)	113,		

asterisks denote the (1/2+x, 1/2−y, −1/2+z) transformation relative to the reference molecule at (x,y,z). These hydrogen bonds are indicated by the broken lines in Fig. 5. Some shorter intermolecular distances are indicated by dotted lines in Fig. 5. No unusually short contact is found in this structure.

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References

1) Piroheptine: 3-(10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5-ylidene)-1-ethyl-2-methylpyrrolidine.

2) S. Umio, M. Hitomi, H. Nojima, N. Kumadaki, I. Ueda, T. Kanaya, and Y. Deguchi, *J. Med. Chem.*, **15**, 891 (1972).
3) H. Nojima, S. Umio, Y. Morimoto, and H. Tamura, private communication.
4) Y. Tokuma, H. Nojima, and Y. Morimoto, This Bulletin, **44**, 2665 (1971).
5) D. C. Phillips, *Acta Crystallogr.*, **7**, 746 (1954).
6) "International Tables for X-ray Crystallography," Vol. III, Kynoch Press, Birmingham (1962), p. 202.
7) "Universal Crystallographic Computation Program System (UNICS)," ed. by T. Sakurai, The Crystallographic Society of Japan, Tokyo (1967).
8) K. Larsson, *Acta Chem. Scand.*, **24**, 1503 (1970).