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The Crystal and Molecular Structure of γ-Aminobutyric Acid Determined at Low Temperature

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γ-Aminobutyric acid (GABA), $C_4H_9NO_2$, m.p. 203 °C. Space group and cell dimensions at room temperature (22 °C): $P2_1/a$, $a=8{\cdot}285$ (5), $b=10{\cdot}224$ (12), $c=7{\cdot}203$ (5) Å, $\beta=110{\cdot}79$ (9)°. Space group and cell dimensions at low temperature (*ca.* −135°C): $P2_1/a$, $a=8{\cdot}228$ (5), $b=10{\cdot}036$ (9), $c=7{\cdot}210$ (4) Å, $\beta=110{\cdot}63$ (5)°. $Z=4$, ϱ_{calc} at room temperature $=1{\cdot}201$ g cm^{-3}, $\varrho_{meas}=1{\cdot}21$ g cm^{-3}. Crystals were grown from ethanol solution. Final $R=0{\cdot}068$. The molecules are zwitterions, partially folded, and held in a cross-linked chain arrangement by hydrogen bonds.

Introduction

A tape-controlled automated Stoe–Güttinger diffractometer and associated low-temperature attachment were used for data collection. Liquid nitrogen was used as coolant and the temperature at the crystal adjusted by mixing with nitrogen at room temperature. Temperature was measured with a conventional 'T_1T_2' thermocouple attached to the goniometer arcs, the junction of the thermocouple being placed about 2 mm from the crystal. The lowest temperature most stably achieved was −130 °C at the thermocouple junction and exploratory tests gave an estimated temperature at the crystal of *ca.* −135 °C. It was found that this temperature could be held to ±3 °C throughout data collection. Because of the solubility of the crystals in water great care was taken to isolate them from the atmosphere before, during and after low-temperature cycles by keeping them in a nitrogen gas environment. During protracted low-temperature periods some slight icing of crystals was observed, but repeat observations of particular reflections showed this to have a negligible effect on measured intensities.

Systematic absences from Weissenberg photographs were $0k0$, $k=2n+1$ and $h0l$, $h=2n+1$, uniquely defining the space group $P2_1/a$. Accurate cell dimensions at low temperature were obtained from a least-squares refinement using 2θ values of 48 reflexions. Data were collected from the *hkn*, *hnl*, *nkl* ($n=0,1,2$) layers up to $\sin\theta/\lambda=0{\cdot}62$, using Ni-filtered Cu $K\alpha$ radiation (1·5418 Å) and a pulse-height analyser. 890 reflexions were measured, of which 61 were systematically absent, and 74 designated as unobserved, *i.e.* with integrated intensity less than 100 counts above background. The remaining reflexions were regarded as 'observed'.

The X-RAY 70 system of programs (Stewart, Kundell & Baldwin, 1970), implemented on the CDC 6600 and 7600 computers at the University of London Computer Centre, was used for data processing and structure determination. Atomic scattering factor data were taken from *International Tables for X-ray Crystallography* (1962).

An initial set of 152 phases was obtained using the conventional symbolic addition procedure of Karle & Karle. Fourier synthesis then enabled the heavy atoms to be positioned. This model gave a residual $R=\sum||F_o|-|F_c||/\sum|F_o|$ of 0·34. Three cycles of full-matrix least-squares refinement, minimizing the function $\sum\omega(|F_o|-|F_c|)^2$ with an overall temperature factor and unit weights for all reflexions, reduced R to 0·165. Anisotropic temperature factors and a weighting scheme were then introduced. Reflexions having $\sin\theta<0{\cdot}35$ and/or $F_o>10$ were assigned reduced weights according to the scheme

$$\omega=X\,.\,Y,$$

where if $\sin\theta>0{\cdot}35$, $X=1$, otherwise $X=\sin\theta/0{\cdot}35$; if $F_o<10$, $Y=1$, otherwise $Y=10/F_o$.

The largest reflexion, 120, was thought to be affected by extinction and was given zero weight. Two further cycles of refinement then gave $R=0{\cdot}11$. Hydrogen-atom positions were obtained from a ΔF synthesis and two further cycles of refinement carried out. The data were insufficient, however, for determination of the isotropic temperature factors for the hydrogen atoms. Instead, the overall temperature factor for the molecule was assigned to the hydrogen atoms: this was not refined further. Under these conditions $R=0{\cdot}068$ was

Table 1. *Fractional atomic coordinates*

	x	*y*	*z*
N	0·0701 (3)	0·8412 (3)	−0·3190 (4)
C(1)	0·1652 (4)	0·8010 (4)	−0·1086 (5)
C(2)	0·2942 (4)	0·9086 (4)	−0·0019 (5)
C(3)	0·3836 (4)	0·8773 (4)	0·2181 (5)
C(4)	0·2682 (4)	0·8948 (3)	0·3414 (5)
O(1)	0·1225 (3)	0·9481 (3)	0·2652 (3)
O(2)	0·3282 (3)	0·8525 (3)	0·5184 (3)
H(1)	0·154 (6)	0·857 (5)	−0·382 (7)
H(2)	0·002 (6)	0·920 (5)	−0·316 (7)
H(3)	−0·004 (6)	0·778 (5)	−0·380 (7)
H(4)	0·075 (6)	0·785 (5)	−0·042 (7)
H(5)	0·232 (6)	0·712 (5)	−0·104 (7)
H(6)	0·233 (6)	0·998 (5)	−0·004 (8)
H(7)	0·381 (6)	0·921 (5)	−0·066 (7)
H(8)	0·490 (6)	0·938 (5)	0·285 (7)
H(9)	0·439 (6)	0·786 (5)	0·236 (7)

Table 2. *Final thermal parameters* $\times 100$ Å^2

The temperature factor is of the form:

$$T=\exp\{-2\pi^2[(ha^*)^2U_{11}+(kb^*)^2U_{22}+(lc^*)^2U_{33}+2hka^*b^*U_{12}+2hla^*c^*U_{13}+2klb^*c^*U_{23}]\}.$$

$U_{HYD}=2{\cdot}18$.

	U_{11}	U_{22}	U_{33}	U_{12}	U_{13}	U_{23}
N	1·8 (1)	2·5 (1)	1·4 (1)	−0·1 (1)	0·6 (1)	−0·3 (1)
C(1)	2·3 (1)	2·2 (2)	1·3 (2)	−0·3 (1)	0·6 (1)	−0·0 (2)
C(2)	2·4 (1)	2·7 (2)	1·5 (1)	−0·6 (1)	0·6 (1)	0·1 (2)
C(3)	2·0 (1)	3·1 (2)	1·4 (2)	0·1 (1)	0·8 (1)	−0·0 (2)
C(4)	1·9 (1)	2·0 (2)	1·5 (1)	0·2 (1)	0·5 (1)	−0·2 (2)
O(1)	3·2 (1)	5·1 (2)	1·8 (1)	2·4 (1)	0·7 (1)	0·3 (1)
O(2)	2·3 (1)	3·7 (2)	1·3 (1)	0·5 (1)	0·7 (1)	0·5 (1)

obtained for the observed reflexions and $R=0{\cdot}071$ for all measured reflexions.*

Details of all atomic parameters are given in Tables 1 and 2. Bond lengths and angles for the heavy atoms are given in Table 3. Fig. 1 shows the arrangement of the four molecules of GABA in the unit cell.

* A table of observed and calculated structure factors has been deposited with the National Lending Library, England, as Supplementary Publication No. 30138. Copies may be obtained through the Executive Secretary, International Union of Crystallography, 13 White Friars, Chester CH1 1NZ, England.

Table 3. *Bond lengths* (Å) *and bond angles* (°)

N—C(1)	1·497 (4)
C(1)–C(2)	1·520 (5)
C(2)–C(3)	1·528 (5)
C(3)–C(4)	1·522 (6)
C(4)–O(1)	1·249 (4)
C(4)–O(2)	1·268 (4)
N—C(1)–C(2)	110·1 (3)
C(1)–C(2)–C(3)	112·0 (3)
C(2)–C(3)–C(4)	114·3 (3)
C(3)–C(4)–O(1)	119·5 (3)
C(3)–C(4)–O(2)	116·6 (3)
O(1)–C(4)–O(2)	124·0 (4)

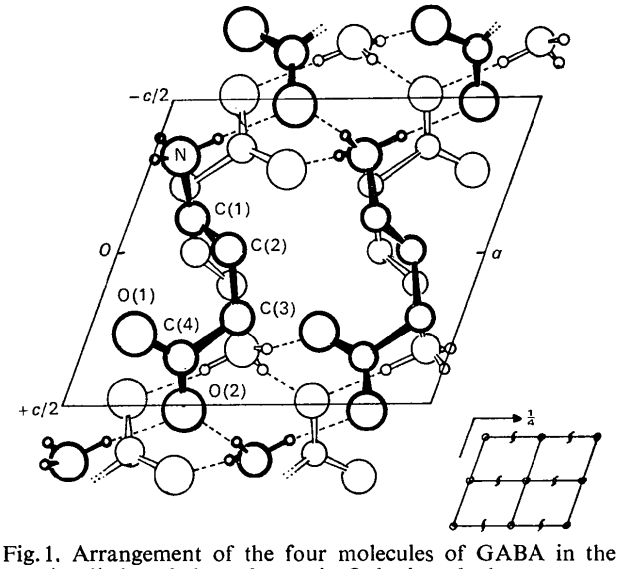

Fig. 1. Arrangement of the four molecules of GABA in the unit cell viewed along the *y* axis. Only those hydrogen atoms involved in hydrogen bonding are shown.

Discussion

There is strong evidence that GABA is the natural inhibitory transmitter at synaptic junctions in some regions of the mammalian brain (Curtis & Johnston, 1970). Its molecular structure may help to resolve its mode of action at these sites.

Preliminary low-temperature studies had indicated that the advantage of the increased number of reflexions obtainable using Mo radiation compared with Cu radiation was offset by the reduction in the number of statistically acceptable reflexion intensities when limited to a maximum working voltage of 50 kV. Only Cu radiation was therefore used.

The structure shown in Fig. 1 and the C–C bond lengths appear consistent with the general pattern of amino acids (Marsh & Donohue, 1967), but the N–C(1) bond length is slightly greater than expected.

Three hydrogen atoms were found to be tetrahedrally bonded to the nitrogen atom, confirming the expected presence of the zwitterion. These hydrogen atoms are suitably positioned to form intermolecular hydrogen bonds with oxygen atoms in neighbouring molecules. One of the hydrogen bonds links the molecules in chains in the **c** direction. These chains are cross-linked to form a three-dimensional structure by the remaining two hydrogen bonds. The N–H···O bonds, which have lengths of 2·767 (5), 2·751 (4), 2·733 (4) Å, may in part explain the extension of the N–C(1) bond.

The molecule is found to be in the *gauche* form about the C(2)–C(3) bond, in contrast to the structure of GABA HCl which is found to be fully *trans* (Tomita, 1965; Steward, Player & Warner, 1973). The *gauche* structure is not the conformation predicted by molecular orbital calculations to be the lowest energy state for the conservative molecule (Kier & Truitt, 1970). This suggests the possibility that more than one conformer of GABA may exist in solution, which may be of importance in relation to the physiological activity of the molecule (Warner, Player & Steward, 1973).

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Geometry of the Unperturbed Flavin Nucleus. The Crystal Structure of 10-Methylisoalloxazine

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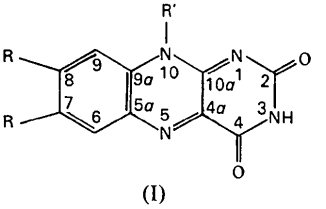
The structure of a simple analogue of quinoid flavin, 10-methylisoalloxazine, which is unperturbed by charge-transfer complexing or protonation, has been determined by X-ray crystallographic methods. The crystals have triclinic symmetry $P\bar{1}$, with a lattice having constants $a = 7{\cdot}179$ (3), $b = 6{\cdot}725$ (4), $c = 10{\cdot}857$ (3) Å, $\alpha = 103{\cdot}10$ (1)°, $\beta = 75{\cdot}08$ (2)°, $\gamma = 109{\cdot}07$ (2)°, $Z = 2$, $\varrho_{obs} = 1{\cdot}61$ g cm^{-3}, and $\varrho_{calc} = 1{\cdot}60$ g cm^{-3}. The final residual, R, based on 947 counter-measured reflections is 6·0 %. Heavy atoms were given ellipsoidal thermal parameters and hydrogen atoms given fixed isotropic thermal parameters. Comparison with N(1)-protonated flavins shows that protonation leads to extensive bond-length changes in the pyrimidinoid ring and in the bond N(10)–C(10*a*). In contrast, the bonds in the yellow molecular complex lumiflavin bis(naphthalene-2,3-diol) agree well with those in this structure except for a possible general contraction in the regions of C(4*a*) and C(9*a*) and in the bond C(4)–C(4*a*) in the complex.

Introduction

Isoalloxazine, (I) (shown in oxidized or quinoid form)) is the aromatic moiety of riboflavin, the cofactor of flavoenzymes.



(I)

These redox enzymes occur widely in biological systems, most importantly in the electron transport chain which links foodstuff oxidation with production of adenosine triphosphate, ATP (Ehrenberg & Hemmerich, 1968; Wainio, 1970). The extensive flavin structural studies so far published deal with either N(1)-protonated forms (Tanaka, Ashida, Sasada & Kakudo, 1969; Fritchie & Trus, 1968; Trus & Fritchie, 1969; Bear, Waters & Waters, 1970; Tillberg & Norrestam, 1972; Karlsson, 1972), metal complexes (Fritchie, 1972*a*, *b*; Wade & Fritchie, 1973), molecular complexes (Langhoff & Fritchie, 1970; Bear, Waters & Waters, 1970; Trus, Wells, Johnston, Fritchie & Marsh, 1971; Voet & Rich, 1971; Kuo, Dunn & Fritchie, 1972; Tillberg & Norrestam, 1972; Karlsson, 1972), or compounds highly modified by chemical substitution (von Glehn, Kierkegaard & Norrestam, 1970; Kierkegaard 1971; von Glehn & Norrestam, 1972; Norrestam, 1972). Because the rationale of many of the above investigations is determining the effect on the isoalloxazine moiety of the particular perturbation, it is highly desirable, even essential, to study an unperturbed model compound. Since isoalloxazine itself (I, with R′ = R=H) tautomerizes immediately to alloxazine by a 10 → 1 proton shift and the latter compound has quite different electronic properties, the 10-methyl derivative is one of the simplest derivatives suitable for flavin model studies, and we report here its crystal structure. Another essentially unperturbed isoalloxazine, 3-methyl-lumiflavin, has also recently been studied (Norrestam & Stensland, 1972).

Experimental

Yellow crystals of 10-methylisoalloxazine were grown by very slow (6 months) evaporation of concentrated solutions of the flavin in dimethyl sulfoxide. Most were twinned, but one untwinned blade-shaped crystal was mounted on the blade axis (*b*) and used for all further

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