The Structure of Muscimol, a GABA Analogue of Restricted Conformation

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Intensive studies in recent years have furnished evidence of the role of γ-aminobutyric acid (GABA) as an inhibitory transmitter in the mammalian central nervous system.[1-4] Some conceptions concerning the structural basis of GABA-receptor interactions have been provided by investigations of the specific GABA antagonist bicucullin.[2,5] Preliminary X-ray analyses indicate that GABA exists in a partially folded conformation in the solid state,[5,6] while the results of molecular orbital calculations on GABA,[7] supported by the GABA-like activity of 4-aminotetrolic acid,[8] seem to indicate that GABA is in an extended conformation, when it acts on the receptors.

Muscimol, an isoxazole enol-betaine isolated from *Amanita muscaria*, has a pronounced and multifarious effect, including psychogenic activity, upon the human central nervous system.[9,10] On certain central interneurons in cats, however, muscimol has been shown to exert a depressant activity very similar to that of GABA.[11] Furthermore muscimol, like GABA, is antagonized by bicucullin.[2] Thus it is likely to be a GABA analogue of restricted conformation. Correlation of the detailed structures of muscimol and analogues and their corresponding physiological action seems to be a rational approach to elicit the structural characteristics of the GABA receptors.

As part of our investigations an X-ray analysis of muscimol has been performed. Structural determinations of muscimol analogues, and the syntheses of isoxazole enol-betaines with additional restrictions of the conformation are in progress.

Crystals of muscimol, $C_4H_6N_2O_2$, were obtained by diffusion of ethanol in aqueous solutions of the compound. By varying the relative concentrations, the compound was obtained in the monoclinic form and the triclinic form. A structure determination has been made on the monoclinic form, which has space group $P2_1/n$, $a = 10.738(9)$, $b = 6.950(4)$, $c = 6.794(7)$ Å, $\beta = 98.18(7)°$, $Z = 4$. Three-dimensional diffraction data in the range $2.5 \leq \theta \leq 25°$ were measured on a Nonius three-circle automatic diffractometer using graphite monochromated Mo$K\alpha$ radiation.

The structure was solved using a modified symbolic addition procedure[12] and refined using the full-matrix least-squares method. The present R value is 0.069.

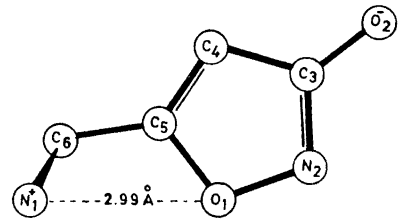

Fig. 1.

The figure illustrates the conformation of the molecules and shows the interatomic distance $N(1)^+ \cdots O(1)$ of 2.99 Å. The muscimol molecule adopts a conformation in which the side chain is directed toward the ring oxygen. This is in accordance with molecular orbital calculations.[7] On the other hand the side chain is not coplanar with the ring plane, and this observation differs from the molecular orbital predictions. The torsion angle $O(1)-C(5)-C(6)-N(1)^+$ found (60°) is similar to the corresponding torsion angle of muscarine (73°) and to those of other muscarinic agonists.[13] The intramolecular $O(2)^- \cdots N(1)^+$ distance is 5.77 Å. The molecular orbital calculations[7] predict $O(2)^-$ and $N(1)^+$ to be 5.0 – 6.0 Å apart in both muscimol and GABA.

The detailed crystal structure of muscimol will be described elsewhere.

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Formation of 2-Oxoisovalerate Dehydrogenase in *Pseudomonas fluorescens*

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Decarboxylation of branched chain 2-oxo acids, 2-oxoisovalerate, 2-oxoisocaproate, and 2-oxo-3-methylvalerate has been detected in animal tissues,[1,2] and in *Pseudomonas aeruginosa*,[3] *Bacillus subtilis*,[4] and *Pseudomonas putida*.[5] 2-Oxoisovalerate dehydrogenase from *Bacillus subtilis* has been purified about 40-fold and it catalyses oxidative decarboxylation of all the three branched chain 2-oxo acids, thus yielding the related acyl coenzyme A esters.[4]

The enzyme is highly stereospecific and the L-isomer is the active substrate. Sulphhydryl reactants inhibit the activity of 2-oxoisovalerate dehydrogenase.[4] The enzyme was induced during growth on valine on *Pseudomonas putida*.[5]

The present investigation shows that 2-oxoisovalerate dehydrogenase is formed in the presence of valine, isoleucine, leucine, and 2-oxo acids derived from these amino acids in *Pseudomonas fluorescens* (strains P-2 and UK-1).

Materials. L-Amino acid oxidase and catalase were purchased from Calbiochem, Los Angeles, and 1-^{14}C-L-valine from the New England Nuclear Corporation, Boston. 1-^{14}C-2-Oxoisovalerate was prepared as described earlier by Meister[6] and purified by ion exchange chromatography.

Cultures. Pseudomonas fluorescens P-2 and UK-1 were used as test organisms. *Ps. fluorescens* P-2 was grown with aeration in the salt solution described by Goodhue and Snell[7] with 10 mM of various carbon sources. The strain UK-1 was cultured in the basal salt solution containing 0.817 g of KH_2PO_4, 0.247 g of $MgSO_4.7H_2O$, and 2.8 mg of $FeSO_4.7H_2O$ per litre.

Growth was estimated from turbidity measurements made with a Klett-Summerson colorimeter, employing filter 62. Cultures were grown as described elsewhere, with some modifications.[8]

Enzyme preparation and assay. The samples (about 4 mg dry weight) withdrawn from the