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A CONFORMATIONAL STUDY OF R-ALAPROCLATE, A NEW SELECETIVE INHIBITOR OF NEURONAL 5-HYDROXYTRYPTAMINE UPTAKE

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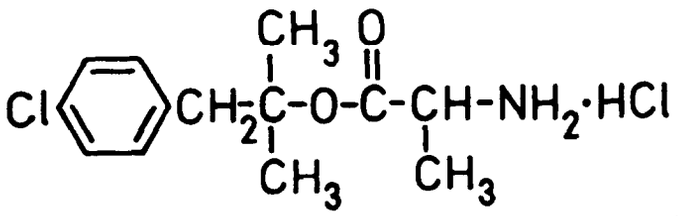
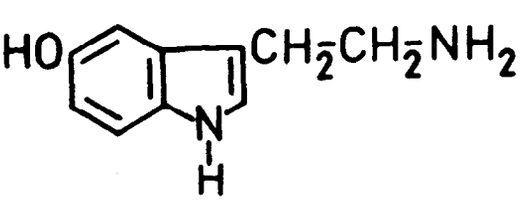
In the search for new drugs, inhibiting the neuronal uptake of various monoamines, it was observed that alaproclate (pINN), 2-(4-chlorophenyl)-1,1-dimethylethyl 2-aminopropanoate hydrochloride (I), is a potent, selective and competitive inhibitor of the accumulation of 5-hydroxytryptamine (5-HT, II) in brain slices and homogenates.[1] The racemic alanine ester was resolved into its enantiomers.[1] Following separation of diastereomeric derivatives of racemic alaproclate by gas chromatography it was shown that the dextrorotamer is the R-alanine ester.[2] In a rat hypothalamic synaptosomal preparation, R-alaproclate was found to be three times more potent than the S-form in inhibiting the accumulation of 5-HT (*Table 1*). The enantiomers of alaproclate were about 270 times more potent in blocking the uptake of 5-HT than that of noradrenaline (NA). The corresponding selectivity factor for chlorimipramine was about 2 (*Table 1*).



I



II

One important way of physiological inactivation of 5-HT at the receptor level, is the re-uptake into the presynaptic nerve. This transport of 5-HT through the cell membrane is believed to involve an energy dependent carrier mechanism. The structure-activity relationship in the series of alaproclate analogues shows three molecular features to be important for the inhibition of 5-HT upatke;[1] A) a terminal primary amino group positively charged at physiological pH, B) an aromatic benzene nucleus influenced by substituents and C) an electron-rich position (in alaproclate represented by the ester carbonyl oxygen). In 5-HT it is obvious that at least the elements A) and B) are present. Further, it is known that in charge transfer complexes the indole 2-carbon can act as a local electron-donor.[4,7] Thus,it is possible that in relation to the carrier the element C as well is present in 5-HT. These elements A, B and C define a so-called pharmacophore which is postulated to account for the affinity of both agonist and antagonist drugs to the same carrier site on the presynaptic side of the nerve terminal.[1]

An attempt to verify this supposition would be to elucidate the conformation in which alaproclate might compete with 5-HT for the carrier site. Therefore an X-ray analysis as well as energy calculations were performed.

Table 1: Inhibition of the accumulation of ^{14}C-5-hydroxytryptamine and ^{3}H-(-)-noradrenaline in synaptosomes of rat hypothalamic homogenate.[3]

	Inhibition of accumulation [a] IC_{50} µM (95 % confidence limits)	
Compound	^{14}C-5-HT	^{3}H-NA
R-alaproclate	0.090(0.074-0.106)	25
S-alaproclate	0.297(0.222-0.387)	81 [b]
chlorimipramine	0.027(0.021-0.035)	0.052(0.041-0.068)

a) Incubation mixture: 100 µl of hypothalmic homogenate (1:10 w/v in 0.25 mM sucrose), $5x10^{-8}$ M ^{14}C-5-HT (specific acitvity 52 mCi/mmol), $5x10^{-8}$ M ^{3}H-(-)-NA (specific activity 3.8 Ci/mmol), 0.24 mM pargyline, 0.13 mM EDTA, 1.1 mM ascorbic acid, 5.6 mM glucose and the inhibitor tested in 1.9 ml Krebs-Henseleit's buffer, pH 7.4. The incubation was performed at 37° and 0° for 4 minutes and the difference in radioactivity in the pellet obtained after centrifugation at 16000 x g for 20 minutes was taken as the active accumulation of the amines. IC_{50} values were calculated from log dose inhibition curves based on at least 5 different concentrations. 95 % confidence limits were calculated by linear regression analysis. b) Extrapolated value.

Alaproclate hydrochloride hydrate, $C_{13}H_{18}ClNO_2 \cdot HCl \cdot H_2O$, crystallizes in space group $P2_1$ with a = 15.247(7), b = 7.036(2), c = 7.916(3) Å, β = 97.81(4)° and Z = 2. Intensity data were collected on a computer controlled Philips PW1100 diffractometer. The crystal structure was solved by the heavy atom method. The Cl atomic positions were determined from a three-dimensional Patterson synthesis. The remaining non-hydrogen atomic positions were found by subsequent Fourier calculations and anisotropic refinement resulted in an R value of 0.071. At this stage all hydrogen atomic positions but five (belonging to the water molecule and the protonated nitrogen) were revealed from difference Fourier syntheses. Anisotropic refinement for the non-hydrogen atoms and isotropic for the hydrogen atoms ended with an R value of 0.045.

The molecular structure and bond lengths are shown in *Figs. 1 and 2*. The average estimated standard deviation in bond distances involving non-hydrogen atoms is 0.010 Å.

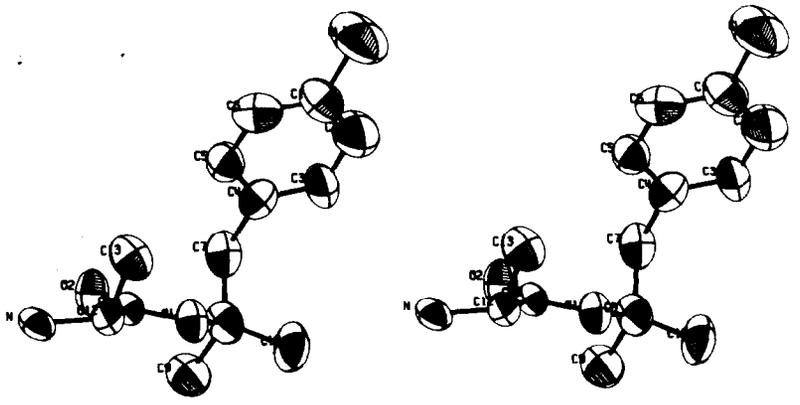


Fig. 1: A stereo view of R-alaproclate.

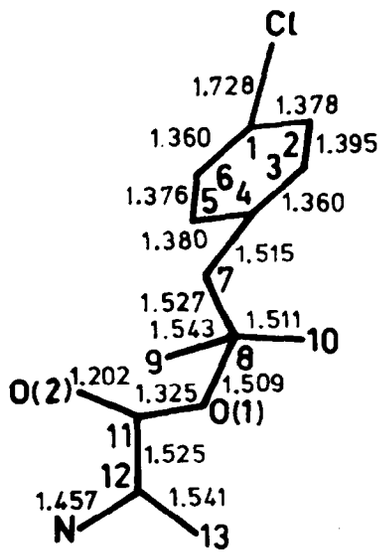


Fig. 2: Bond lengths and numbering system.

Potential energy maps for a free molecule were calculated for five different torsion angles, $T_1 - T_5$, along the side chain (*cf. Table 2*). The energy was calculated using the Lennard-Jones 6-12 potential function.[5] These calculations show that the conformation found in the crystal state corresponds to a minimum energy conformation for a free molecule.

The energy minima are well defined for the torsion angles T_1, T_2 and T_3 but more extended for $T_4 - T_5$. The results of the calculations are shown in *Figs. 3a - 3d*. The $T_4 - T_5$ map (*Fig. 3d*) indicates that the terminal part of the side chain is relatively flexible. Taking the flexibility of the side chain into account, a superimposition of the three pharmacophore moities A, B and C in molecular models of 5-HT and alaproclate can be made. From these matching mo-

dels of agonist and antagonist a hypothetic model of the carrier site can be deduced. A more detailed discussion is presented in Ref. 1. Full details of the X-ray determination as well as energy calculations will be published elsewhere.[6]

Table 2: Conformation angles in the side chain.

Bond	Angles	Atoms involved
T_1	90.°	C(3)-C(4)-C(7)-C(8)
T_2	61.3°	C(4)-C(7)-C(8)-O(1)
T_3	65.2°	C(7)-C(8)-O(1)-C(11)
T_4	-7.2°	C(8)-O(1)-C(11)-O(2)
T_5	17.6°	O(2)-C(11)-C(12)-N

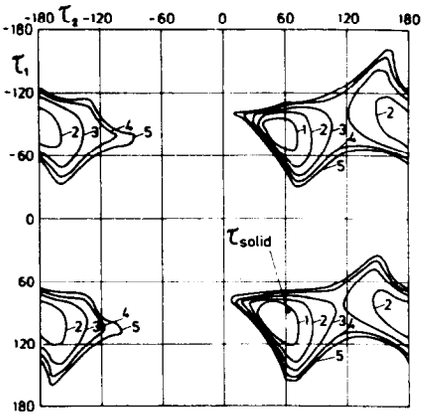


Fig. 3a.

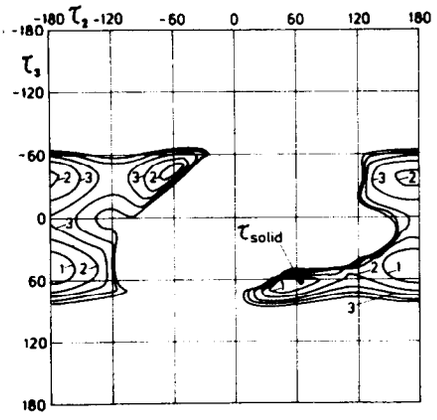


Fig. 3b

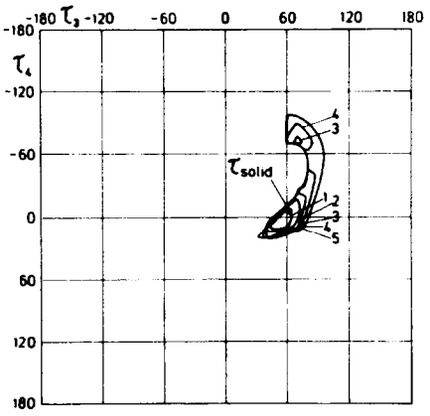


Fig. 3c

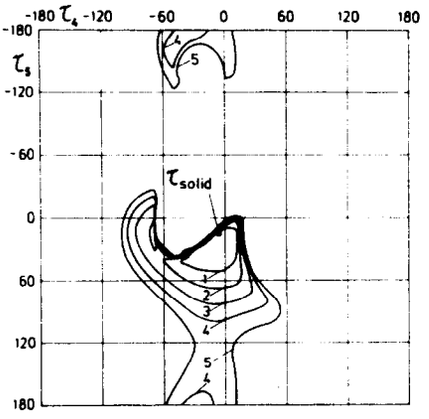


Fig. 3d.

Results of the potential energy calculations in kcal mol^{-1}. Solid state is marked with ● . Torsion angles τ_i as defined in Table 2.

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