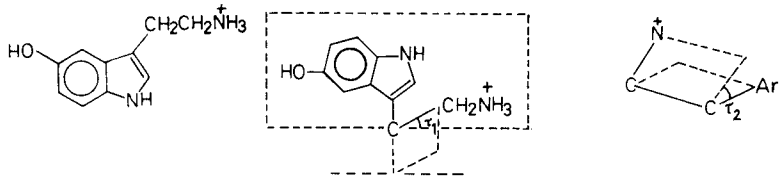
The conformation of 5-hydroxytryptamine in solution

There have been a number of studies in recent years of the conformation of 5-hydroxytryptamine (5-HT; I). On the basis of molecular orbital calculations using the extended Hückel method, Kier (1968) suggested that the molecule exists in a single preferred conformation in which the angles τ_1 and τ_2 (see Ia and Ib) have values of 90° and 180° respectively. Both Kier (1968) and Chothia & Pauling (1969) have commented on the similarity between this extended conformation of 5-HT and part of the molecule of lysergic acid diethylamide, a powerful antagonist of the central and peripheral actions of 5-HT, and have suggested that this is the conformation of 5-HT which interacts with its receptor. Other calculations using the PCILO molecular orbital method (Courrière, Coubeils & Pullman, 1971) have suggested two low energy forms with τ_1, τ_2 values of −140°, 20° and 100°, 60°. Some years earlier, Greenberg (1960) had postulated an active conformation for 5-HT with $\tau_1 = 0°$ and $\tau_2 = 180°$ after a pharmacological study of tryptamine analogues on the *Venus mercenaria* heart.



I Ia Ib

In the crystal state X-ray studies have shown that $\tau_1 = 9°$, $\tau_2 = 173°$ for the creatinine sulphate (Karle, Dragonette & Brenner, 1965) and $\tau_1 = -115°$, $\tau_2 = 61°$ for the picrate (Bugg & Thewalt, 1970). We have now determined the conformation about the CH_2CH_2 bond of 5-HT in aqueous solution, using high resolution nuclear magnetic resonance spectroscopy.

The 100 MHz ^1H spectrum of 5-hydroxytryptamine oxalate (Sigma; 2·0 g/100 ml in 99·8% D_2O) shows a multiplet at 7·3 ppm (relative to external HMS) due to the indole ring protons, and one at 3·5 ppm due to the CH_2CH_2 fragment. In the latter multiplet shown in Fig. 1(a) the lower-field group of lines was somewhat broadened, probably by an unresolved coupling to the proton on C-2 of the indole ring. The higher-field group of five resolved lines and the most intense of the lower-field lines were used in the analysis of the AA′BB′ spin system. Estimates of N and L (the sum and difference of the vicinal coupling constants) were obtained from appropriate line separations in the AA′XX′ approximation (Garbisch, 1968), and estimates of the geminal coupling constants were taken from previous data (Partington, Feeney & Burgen, 1971). Initial values for the spectral parameters were then obtained by plotting trial spectra (using the Varian Associates 620i spectrum simulation program), and these values were refined using the iterative program SXLAOCOONOR (Partington, 1971). The final parameters obtained were

ν_A	= 334·30 Hz	ν_B	= 356·93 Hz
$J_{AA'}$	= −15·47 Hz	$J_{BB'}$	= −13·00 Hz
J^1_{VIC}	= 7·88 Hz	J^2_{VIC}	= 6·30 Hz

The spectrum simulated using these parameters is shown in Fig. 1 (b).

The observed vicinal coupling constants, J^1_{VIC} and J^2_{VIC}, are the weighted averages of the couplings in the three classical staggered rotamers, II–IV.

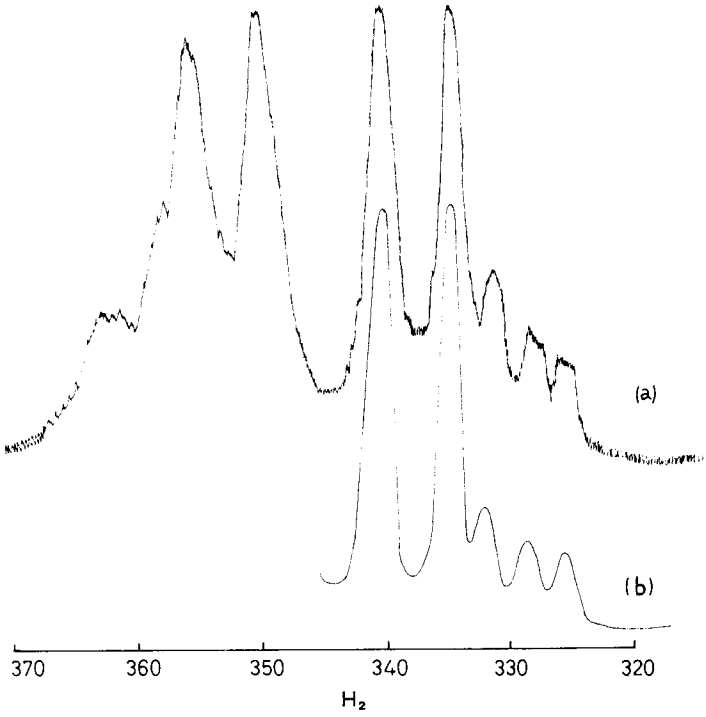
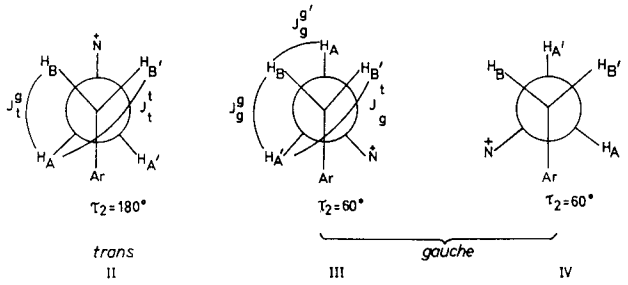


FIG. 1(a). Pmr spectrum (100 MHz) of the bimethylene protons of 5HT, and (b) the simulated AA'BB' spectrum using the iterative parameters.



The nomenclature of the individual couplings in rotamers II–IV is that of Abraham & Gatti (1969), and we shall use their approach, which has been successfully applied to the acetylcholine series (Partington & others, 1971), to derive rotamer populations from the observed vicinal couplings. For the rotamers depicted above

$$J = n_t J_t^g + n_g(J_g^t + J_g^{g'})/2 \quad (1)$$

$$J' = n_t J_t^t + n_g J_g^g \quad (2)$$

$$n_t + n_g = 1 \quad (3)$$

where n_t and n_g are the fractional populations of the *trans* and *gauche* rotamers respectively, and J and J' are appropriate vicinal coupling constants. The coupling constant J is rather insensitive to changes in rotamer distribution and equation (1) can give misleading results (Feeney, 1971: personal communication); we have therefore used only equation (2). Values of J_t^t and J_g^g for 5-HT can be calculated using

the relation given by Abraham & Gatti (1969) between these couplings and the electronegativity of the substituents in molecules of the type XCH_2CH_2Y; we find $J_t^t = 13{\cdot}10$ and $J_g^g = 3{\cdot}53H_2$. A decision about which of the observed vicinal coupling constants, J_{VIC}^1 and J_{VIC}^2, corresponds to J' in equation (2), can be made if the sign of L ($= J_{AB} - J_{AB'}$) is known. Although this cannot be obtained from the spectrum, Abraham & Pachler (1964) have shown that it can be derived in principle from a relation between (1/2 N + 1/6 L) and substituent electronegativity. Because in the present case the value of L (1·58 Hz) is too small to be meaningfully distinguished from zero, this relation cannot be used and we are unable to assign one of the observed vicinal couplings unequivocally to J'. Rotamer populations calculated from equation (2) for *both* the alternative assignments are:

		Hz	n_t	n_g
$J' = J_{VIC}^1$	=	7·88	0·45	0·55
$J' = J_{VIC}^2$	=	6·30	0·28	0·72

Since the values when all three rotamers are of equal energy are $n_t = 0{\cdot}33$, $n_g = 0{\cdot}67$, the first assignment indicates a slight preponderance of the *trans* rotamer, and the second, a slight preponderance of the two *gauche* rotamers. On steric grounds one would expect the *trans* rotamer to be somewhat preferred, as in phenethylamine (Ison & Roberts, 1971: unpublished observations) and a series of 2-haloethylbenzenes (Bodot, Leroy & Pujol, 1967; Abraham & Gatti, 1969). We suggest, therefore, that the first assignment is the correct one, and that the rotamer populations for 5-HT are $n_t = 0{\cdot}45$, $n_g = 0{\cdot}55$.

In either case, however, it is quite clear that the energy difference between the *trans* and *gauche* rotamers is small [for $n_t = 0{\cdot}45$, $n_g = 0{\cdot}55$, $(E_g - E_t) = 1{\cdot}3$ kJ/mol (0·3 kcal/mol)] and in contrast to the predictions from molecular orbital calculations, the nmr results show unequivocally that 5-HT in solution cannot be considered to exist in a single conformation. The solution conformation, therefore, is not useful as a constraint when discussing either the possible conformations of 5-HT in the drug-receptor complex, or the energetics of formation of such a complex.

It should be emphasized that the extrapolation of preferred conformations determined by crystallographic or molecular orbital studies to the solution state or to the situation in a drug-receptor complex should be approached very cautiously as generally discussed by Portoghese (1970) and Shefter (1971).

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Centrally mediated contraction of the lower eyelid elicited by anticholinesterases in anaesthetized rats

In anaesthetized rats, centrally acting anticholinesterases elicit a rise in blood pressure (Dirnhuber & Cullumbine, 1955; Varagić, 1955) and augmentation of the cervical sympathetic outflow (Kuga & Erdmann, 1967; Nakagawa, 1968; Stemanović & Varagić, 1970). We have found that the rat lower eyelid, as a sympathetic effector organ (Gertner, 1956; Spriggs, 1966; Morpurgo, 1968), offers an easy approach to indicate central sympathetic hyperactivity. Recording the movements of the rat lower eyelid may provide reliable information about the central excitatory action of anticholinesterases.

Male white rats, 270 to 370 g, anaesthetized with urethane (1·3–1·5 g/kg, s.c.), immobilized by gallamine and artificially ventilated, were used. Before each experiment, bilateral adrenalectomy was performed. Blood pressure was measured in

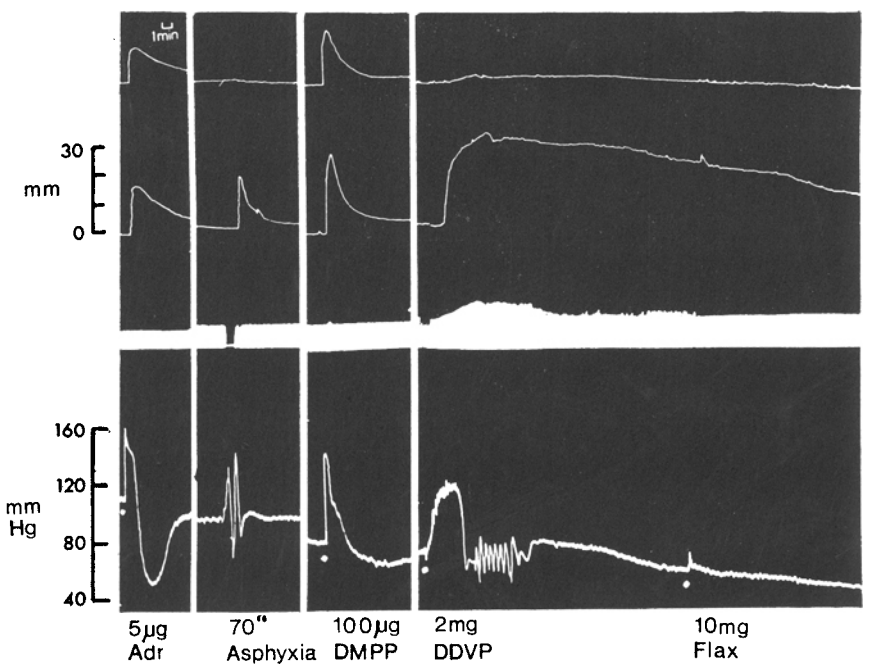

FIG. 1. Reactions of an anaesthetized rat (290 g) upon adrenaline hydrochloride, asphyxia, dimethylphenylpiperazinium iodide, and dichlorvos. Traces from top to bottom: decentralized lower eyelid, innervated lower eyelid, intratracheal pressure, femoral blood pressure. Doses were given per kg body weight intravenously. Asphyxia was elicited by stopping the artificial ventilation for 70 s.