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Self-association and Binding Sites of Some Psychotomimetic Tryptamine Derivatives and Related Compounds: Nuclear Magnetic Resonance Investigations

H. SAPPER AND W. LOHMANN

Institut für Biophysik der Universität, D-63 Giessen, and Institut für Biochemie, GSF, D-8042 Neuherberg, Federal Republic of Germany

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SUMMARY

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The nature of the binding sites in the self-association of some psychotomimetic drugs has been investigated by NMR techniques. In the case of *d*-lysergic acid diethylamide the interaction of the aromatic ring systems was found to be the basis of self-association ($\Delta H = -8.7 \pm 0.7$ kcal mole^{-1}). In self-associates of the tryptamine derivatives and mescaline ($\Delta H = -3$ to -4 kcal mole^{-1}) the ring overlap of associated molecules may be replaced by an alignment of the amino group located at the end of the side chain with the ring system of a neighboring molecule. The influence of substitutions on the ring systems and special solvent-solute interactions is discussed. Whereas the amino group at the end of the side chain seems to be the preferred site of solvent-solute interactions, the locus of a hydroxyl substitution on the ring system seems to modify the stereochemistry of the self-associates.

INTRODUCTION

It has been proposed repeatedly that the structural similarities between *d*-lysergic acid diethylamide and psilocin, mescaline, and other psychotomimetic drugs might be responsible for the similar psychic activity of these substances. Because LSD[1] is the most effective hallucinogen known, it has been suggested that other drugs simulate its conformation (1). Theoretical calculations support such an assumption (2, 3). X-ray crystallographic studies, however, have revealed certain differences in the distances between the binding sites (4).

Possible binding sites might be the aromatic ring system, substitutions on this ring system (e.g., OH– or OCH_3– groups), and a protonated nitrogen corresponding to N-6 of LSD.

In this study possible binding sites have been investigated. Various compounds have been compared in regard to their binding sites and structural characteristics by means of NMR spectroscopy. Moreover, their self-association has been studied in terms of thermodynamic parameters and stereochemistry, because knowledge of the self-association of drugs is indispensable for evaluating spectroscopic data on their complexes with receptor molecules.

METHODS

N,N-Dimethyltryptamine, 5-hydroxy-*N,N*-dimethyltryptamine (bufotenine), 5-

[1] The abbreviations used are: LSD, *d*-lysergic acid diethylamide; DMSO, dimethyl sulfoxide; DMT, *N,N*-dimethyltryptamine; 5-HT, 5-hydroxytryptamine (serotonin); 6-HT, 6-hydroxytryptamine.

hydroxytryptamine, mescaline, and gramine were purchased from Fluka, Buchs, Switzerland. 6-Hydroxytryptamine was obtained from Labkemie, Stockholm. We gratefully acknowledge the generosity of the National Institute of Mental Health in supplying 4-hydroxy-*N*,*N*-dimethyltryptamine (psilocin), and of Sandoz, Nürnberg, in supplying *d*-lysergic acid diethylamide. Figure 1 shows the structures of these compounds.

The buffer components and reference substances, *tert*-butyl alcohol and 2,2,3,3-tetradeutero-3-(trimethylsilyl)propionic acid sodium salt, were purchased from Merck-Schuchardt, Darmstadt. The solvents D_2O and dimethyl sulfoxide-d_6 were obtained from Sharp and Dohme, München. All substances used were of reagent-grade quality and were used without further purification.

The self-association of molecules A (total concentration, a_0) can be described in general by

$$A_{n-1} + A_1 \leftrightharpoons A_n \qquad (n \geqq 2)$$

with the association constants

$$K_n = \frac{a_n}{a_{n-1} \cdot a_1}$$

where a_i is the concentration of A_i. With $n \to \infty$, $K \cdot a_1 < 1$, and assuming that successive association constants are equal, Dimicoli and Hélène (5), investigating the self-association of purine and indole derivatives by means of NMR spectroscopy, derived the equation

$$\frac{\Delta}{\Delta_2} = 2 \cdot K \cdot a_1 \qquad (1)$$

with Δ as the chemical shift observed and Δ_2 as the complex shift of a dimer proton, both measured relative to the extrapolated ($a_0 \to 0$) monomer shift δ_M. Because the total concentration a_0 can be written as

$$a_0 = \sum_{n=1}^{\infty} n \cdot a_n = a_1 \sum_{n=1}^{\infty} n(K \cdot a_1)^{n-1} = \frac{a_1}{(1 - K \cdot a_1)^2}$$

it follows from Eq. 1 that

$$\frac{\Delta}{\Delta_2} = 2 + \frac{1 - \sqrt{1 + 4 \cdot K \cdot a_0}}{K \cdot a_0} \qquad (2)$$

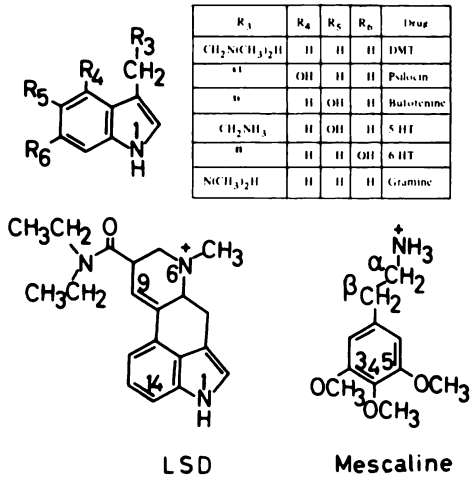
If the product $K \cdot a_0$ is small enough, the root in Eq. 2 can be approximated by

$$\sqrt{1 + 4 \cdot K \cdot a_0} \approx 1 + 2 \cdot K \cdot a_0 - 2 \cdot K^2 \cdot a_0^2$$

and Eq. 2 can then be written simply as

$$\frac{\Delta}{\Delta_2} = 2 \cdot K \cdot a_0 \qquad (3)$$

The NMR spectra were recorded with a Varian HA-100 spectrometer equipped with a variable temperature system. Deuterated phosphate buffer (pD 7.4 with 20 mM *tert*-butyl alcohol as an internal reference) was used as a solvent for all substances investigated, with the exception of LSD, which was measured at a lower pD value in acetate buffer (pD 5.4 with 2,2,3,3-tetradeutero-3-(trimethylsilyl)-propionate as a reference in a separate probe) because of its lower pK value of protonation (pK 7.8 of LSD corresponds to pK values of the tryptamine derivatives near 10). The calculations of K and Δ_2 according to Eq. 2 were performed with a Wang 600-14 computer. The errors in the chemical shifts measured are $\pm 10^{-3}$ ppm.



R_3	R_4	R_5	R_6	Drug
$CH_2N(CH_3)_2H$	H	H	H	DMT
"	OH	H	H	Psilocin
"	H	OH	H	Bufotenine
CH_2NH_3	H	OH	H	5-HT
"	H	H	OH	6-HT
$N(CH_3)_2H$	H	H	H	Gramine

FIG. 1. *Structures of compounds studied*

RESULTS

The PMR spectra of the ring protons of DMT and gramine were analyzed on the basis of the tryptophan spectrum reported by Gerig (6); in the case of the hydroxyl-substituted tryptamine derivatives, coupling of the protons was used. The LSD spectrum was analyzed according to Cohen *et al.* (7) and based on results obtained with the other compounds investigated.

The methylene protons of the side chain of the tryptamine derivatives and mescaline resemble an A_2B_2 NMR spectrum as shown for 5-HT by Nogrady *et al.* (8), using a 220-MHz spectrometer. They obtained a value of about 0.15 for the quotient $J^*/\Delta\delta \cdot \nu_0$ (for explanation of the symbols, see Fig. 2). In the present investigation, using a 100-MHz spectrometer, the $J^*/\Delta\delta \cdot \nu_0$ value is about 0.29 (Table 1). Therefore only approximate values for the chemical shifts of CH_2-α and CH_2-β, as well as for $\Delta\delta$ and J, can be obtained from the spectra. The estimated values of the vicinal coupling constant J (denoted by J^*), which increase with methylation of the amino group of the tryptamine derivatives (Table 1), indicate, however, that the bimethylene side chain seems to be more extended in the case of the methylated derivatives. This is in agreement with results obtained by crystallographic investigations (4).

The chemical shift observed depends on temperature as well as concentration. The extrapolated monomer shifts ($a_0 \rightarrow 0$; T = 25°) are shown in Table 2. It can be seen that a hydroxyl substitution results in an increased electron density of the aromatic ring system and, thus, in enhanced shielding of the protons relative to the unsubstituted ring system of DMT. This enhancement, being extended over the entire ring system, is most pronounced, however, at H-5 of psilocin, at H-4 of bufotenine and 5-HT, and at H-7 of 6-HT. This again is in good agreement with calculations by Kumbar and Sankar (10). It should be noted that H-9 of LSD exhibits a relatively large upfield shift, although it is located outside the indole ring system.

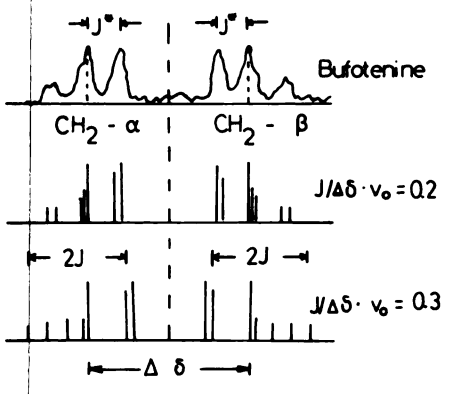


FIG. 2. *A_2B_2 spectrum observed for bufotenine and calculated (9) for $J/\Delta\delta \cdot \nu_0 = 0.2$ and 0.3*

TABLE 1

Difference ($\Delta\delta$) between chemical shifts of CH_2-α and CH_2-β and coupling constant observed (J^*)

ν_0 was 100 MHz.

Drug	$\Delta\delta$ ppm	J Hz	$J^*/\Delta\delta\nu_0$
DMT	0.231	6.2	0.27
Psilocin	0.224	6.1	0.27
Bufotenine	0.267	6.2	0.23
5-HT	0.193	5.5	0.29
6-HT	0.188	5.3	0.28
Mescaline	0.312	6.7	0.22

The temperature dependence of the monomer shifts, most pronounced for the side chain protons and for H-2 among the ring protons, seems to be caused by a solvent effect, that is, mainly by hydrogen bonds between solvent and solute molecules. Since water acts as both a proton donor and acceptor in the case of hydrogen bond interactions, the temperature dependence of δ_M was also determined in DMSO-d_6. H-2 and $N(CH_3)_2$ of bufotenine are shifted in opposite directions (Fig. 3), but more strongly in DMSO than in aqueous solution. This finding suggests that the proton donor function in regard to hydrogen bridges seems to be the preferred solute-solvent interaction of the substances investigated. The side chain protons [only $N(CH_3)_2$ is shown in Fig. 3] are more affected than H-2. The other ring protons are modified just as much as H-2 (not shown). In accordance with the crystallographic results obtained by Falkenberg (4), therefore, the nitrogen atom of the side chain seems to be the site preferred

TABLE 2

Monomer shifts at 25° ($\delta_M^{25°}$) of drug protons investigated (pD 7.4, except for LSD)

Proton	Monomer shift ($\delta_M^{25°}$)						
	DMT	Psilocin	Bufotenine	5-HT	6-HT	LSD[a]	Mescaline
	ppm	*ppm*	*ppm*	*ppm*	*ppm*	*ppm*	*ppm*
H-2	7.361	7.206	7.318	7.298	7.186	7.197	
H-4	7.72		7.115	7.116	7.545		
H-5	7.25	6.57			6.785		
H-6	7.25	7.08	6.868	6.865			
H-2/6							6.716
H-7	7.59	7.08	7.418	7.421	6.992	6.545	
H-9						7.33	
H-12						7.20	
H-13						7.48	
H-14							2.962
CH_2-β	3.276	3.302	3.207	3.115	3.129		3.274
CH_2-α	3.507	3.526	3.474	3.308	3.317		
CH_3-6						3.286	
$N(CH_3)_2$	2.924	2.913	2.920				
OCH_3-3/5							3.878
OCH_3-4							3.780

[a] pD 5.4

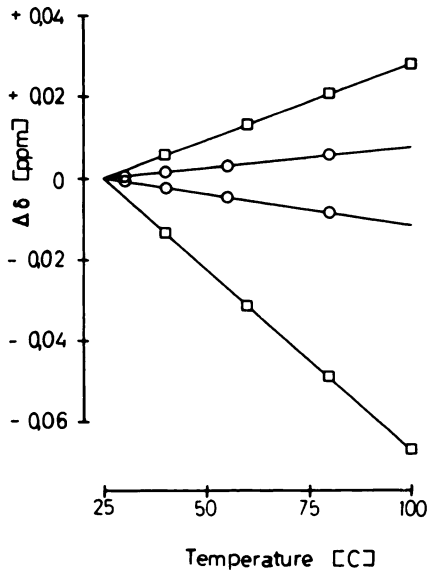


FIG. 3. *Temperature dependence of monomer shift [e.g., H-2 (upward) and $N(CH_3)_2$ (downward) of bufotenine] in aqueous solution (○) and DMSO (□), relative to values at 25°*

for hydrogen bond interactions, whereas the H-1 proton or hydroxyl substitution may be less involved in such interactions.

Over the concentration range used, the concentration dependence of the chemical shifts is nearly linear for the tryptamine derivatives, gramine, and mescaline (as an example, this dependence is shown in Fig. 4 for the H-2 protons of bufotenine and 5-HT). In the case of LSD, however, a curved function is observed (only H-2 is shown in Fig. 4), with a slope at zero concentration greater than those of the other substances. From these results it can be concluded that the self-association of LSD

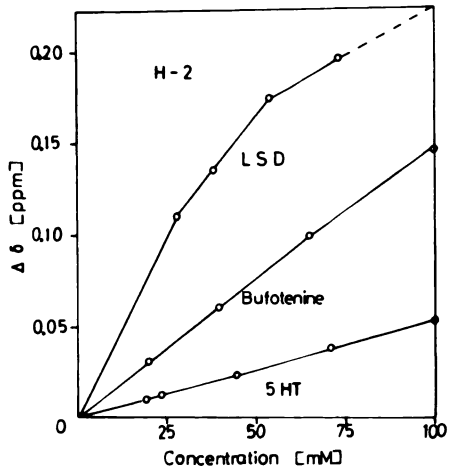


FIG. 4. *Concentration dependence of H-2 chemical shift of LSD, bufotenine, and 5-HT at 25°*

is more pronounced than that of the other compounds. Whereas only the product $K \cdot \Delta_2$ of each proton can be evaluated according to Eq. 3 from the linearity of the plots for the tryptamine derivatives, gramine, and mescaline, K and Δ_2 can be evaluated separately from the curvature observed in the case of LSD by fitting the experimental data according to Eq. 2.

In Table 3 the values of $K \cdot \Delta_2$ are given as a percentage of the maximum value for each compound. These values should be independent of K and therefore should correlate with the complex shifts Δ_2 of any proton. In the case of LSD the Δ_2 values of the various protons are compared with that of H-12, which exhibited the maximum Δ_2 value. If one assumes that the ring current effect due to the indole ring system of all the compounds investigated (6-membered ring in the case of mescaline) is quite similar to that of LSD, the self-association constants of these compounds can be estimated. They should be on the order of 0.5 M^{-1} (at 25°) in the case of the tryptamine derivatives, gramine, and mescaline, and 1 M^{-1} in the case of the dimethylated tryptamine derivatives. The K value of LSD at 25° was determined to be about 8 M^{-1} (Table 4). The Δ_2 values near 0.8 ppm, as assumed in the estimation above, are in good agreement with the shielding values calculated for the indole ring system of tryptophan at distances near 0.4 nm (11).

The differences in the complex shifts reflect the influence of self-association with the various protons. From the results obtained for self-association it can be concluded that the methylene protons of the tryptamine derivatives are affected more strongly than the protons of the 6-membered ring. Only H-4 of 6-HT is shifted more extensively than other ring protons. In the case of mescaline H-2 and H-6, and in the case of LSD H-9 and H-12, are shifted the most.

From the temperature dependence of the K values the enthalpies of self-association can be calculated. The ΔH value of LSD was found to be -8.7 kcal $mole^{-1}$ (Table 4). Since the complex shifts Δ_2 are dependent on temperature linearly, if at all, (12), and the K values rise exponentially with temperature, the temperature dependence of the product $K \cdot \Delta_2$ can be used to calculate

TABLE 3

$\Delta_2^{25°}$ (LSD) and $K \cdot \Delta_2^{25°}$ (other compounds) as a percentage of the maximum value observed

The maximum value for each compound is given in parentheses and is expressed as parts per million per mole $liter^{-1}$ except for LSD, which is given in parts per million.

Proton	DMT (0.88)	Psilocin (0.68)	Bufotenine (1.05)	5-HT (0.29)	6-HT (0.36)	Gramine (0.50)	LSD[a] (0.79)	Mescaline (0.22)
			% maximum value observed					
H-2	51	100	72	93	72	78	54	
H-4			37	47	100			
H-2/6								100
H-5/6	14		11	28	48	54		
H-7			21	67	30			
H-9							96	
H-12							100	
H-13							63	
H-14							43	
CH_2-β	86	72	82	100	83			37
CH_2-α	100	92	100	90	47			0
CH_3-6							67	
$N(CH_3)_2$	51	82	57			100		
$-CH_2CH_3$							0	
OCH_3-3/5								49
OCH_3-4								67

[a] pD 5.4.

TABLE 4

Association constants (at 25°) and enthalpies of self-association (pD 7.4, except for LSD)

Drug	$K^{25°}$ M^{-1}	$-\Delta H$ kcal mol^{-1}
DMT	~1	3.1 ± 0.5
Psilocin	~1	3.5 ± 0.5
Bufotenine	~1	3.5 ± 0.5
5-HT	~0.5	2.9 ± 0.4
6-HT	~0.5	3.2 ± 0.5
Gramine	~0.5	2.1 ± 0.4
LSD[a]	8 ± 2	8.7 ± 0.7
Mescaline	~0.5	4.0 ± 0.6

[a] pD 5.4.

enthalpy values. They were found to be about -2 kcal mole^{-1} for gramine, about -3 kcal mole^{-1} for the tryptamine derivatives, and about -4 kcal mole^{-1} for mescaline (Table 4).

DISCUSSION

The self-association of LSD is more pronounced than that of the tryptamine derivatives and mescaline. This may be caused by the fact that the protonated N-6 of LSD is locked in a ring system, whereas the protonated N-12 of the tryptamine derivatives, as well as N-9 of mescaline, is placed at the end of a rather freely rotating side chain. The LSD molecules therefore are able to associate in such a way that the positive charges are separated sufficiently and yet the ring systems interact with each other. The rotation ability of the side chains of the other compounds, however, may impede direct ring-ring interactions, and from the $K \cdot \Delta_2$ values observed it may be concluded that the ring overlap of associated molecules is replaced by an alignment of the polar end of the side chains with the ring systems.

The enthalpies of self-association of the tryptamine derivatives and mescaline are quite similar (-3 to -4 kcal mole^{-1}), but the ΔH value of gramine is somewhat smaller (-2 kcal mole^{-1}). Therefore alignment of the cationic side chain with the ring system of an adjacent molecule may not be completely responsible for self-association. Because the upfield shifts described above are observed only in aqueous solutions, it can be assumed that solvent-solute interactions also contribute to the stabilization of the associates. The voluminous CH_3 groups at the side chains seem to lengthen the distance between the rings of associated molecules, since the difference between the lowest and highest $K \cdot \Delta_2$ values is greater for the methylated compounds. On the other hand, methylation seems to enhance the association (the estimated K values are higher), an effect which has also been observed in the case of stacking association between nucleobases in aqueous solution (12). The association might therefore be caused by van der Waals interactions between solute molecules as well as between the solute molecules and the surrounding water shell.

Substitutions on the ring system of the tryptamine derivatives e.g., by a hydroxyl group, influence the total π-electron density of the entire ring system and the local electron density distribution as well. Hence it is diffucult to interpret the different complex shifts of different protons. The total π-electron density should correspond to the ring current intensity and, therefore, to the shielding caused by the ring current effects during association. The local π-electron density around a proton, however, should correspond to the sensitivity of this proton.

If one assumes that the ring current intensities of 5-HT and 6-HT are about the same, the site of hydroxyl substitution should influence the stereochemistry of the associates. Although the H-5 and H-7 protons of 6-HT are influenced most by hydroxyl substitution (δ_H exhibits the largest upfield shift relative to DMT) and therefore seem to have the highest sensitivities, they are influenced least by self-association. The H-2 and H-4 protons, however, which are less influenced by hydroxyl substitution, show the largest Δ_2 values of all the ring protons. In the case of 5-HT, on the other hand, the H-4 and H-6 protons are influenced most by hydroxyl substitution, and the H-2 and H-7 protons exhibit the largest Δ_2 values. The center of interaction during self-association therefore might be shifted from an area between H-7 and H-2 to one between H-4 and H-2 by shifting the hydroxyl group from C-5 to C-6.

In the case of LSD the electron density distribution (13) is in agreement with the Δ_2 values. Therefore it can be assumed that the entire ring system of LSD participates in the interaction. This confirms the statement that the LSD self-associates are stabilized by ring-ring interactions more fully than those of tryptamine or phenylethylamine derivatives. It can also be concluded that if the psychotomimetic drugs do act on the same receptor, the mechanism of action may be different for LSD than for the other psychotomimetics studied here. Haigler and Aghajanian (14) were led to the same conclusion by comparing the action of LSD and mescaline upon raphe cells.

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