

Life Sciences Vol. 7, pp. 267-277, 1968. Pergamon Press
Printed in Great Britain.

STRUCTURE-ACTIVITY RELATIONSHIPS AMONG 5-METHOXY-N,N-DIMETHYLTRYPTAMINE,
4-HYDROXY-N,N-DIMETHYLTRYPTAMINE (PSILOCIN) AND OTHER SUBSTITUTED
TRYPTAMINES*

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(Received 7 October 1966; in final form 7 December 1967)

In several respects, the substituted tryptamines constitute an interesting family of pharmacologically active compounds. Many occur in plant materials which have either been associated with the preparation of psychotropic snuffs, potions, etc., or are known for their toxic properties. Thus 5-methoxy-N,N-dimethyltryptamine (5MeO-DMT), 5-methoxy-N-methyltryptamine (5MeO-MT), 5-hydroxy-N,N-dimethyltryptamine (5HO-DMT; bufotenine), N,N-dimethyltryptamine (DMT) and N-methyltryptamine (MT) are all present in Piptadenia peregrina Benth (1-4) which has been used in the making of the psychotropic Cohoba snuff by the Central American Indians. 5MeO-DMT is also the main component of the psychotropic Epena snuff which contains in addition small amounts of DMT and 5HO-DMT and is used by the Waica tribes of South America (5). The presence of 5MeO-DMT has also been shown in Desmodium pulcellum Benth, a plant used in the Ayurvedic system of medicine in India (6) and in the Brazilian plant Dictyoloma incones-cens D. C. (7). 5MeO-MT is present in Phalans arundicea L. (8) and it is found together with 5MeO-DMT, 5HO-DMT and DMT in Phalaris tuberosa L. (9) grasses known to induce "staggers" in sheep. 4-Hydroxy-N,N-dimethyltryptamine

*This research was supported by United States Public Health Service Grant 1 RO1 MH-07575, from the National Institute of Mental Health.

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(4HO-DMT; psilocin) and its phosphate (psilocybin) are present in Psilocybe mexicana Heim (10) and in at least four other fungal species (11).

Some of these tryptamines are known to induce hallucinations in man, while others not yet tested for this property have been shown to exert powerful actions on the central nervous system of experimental animals. Thus 5HO-DMT was described as hallucinogenic by Fabing and Hawkins (12), DMT and N:N-diethyl-tryptamine (DET) by Szara (13) and 4HO-DMT and its phosphate by Hofmann et al. (10). Gessner and Page reported in 1962 (14) on the marked central nervous system effects in animals of 5MeO-DMT, a finding confirmed in 1964 by Gallagher et al. (9). The identification of 5MeO-DMT as the main component of the psychotropic Epena snuff (5) suggests this compound possesses similar activity in man.

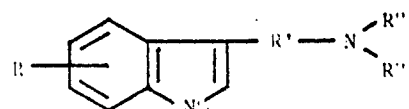
Little, however, is known regarding statistically significant differences in activity among substituted tryptamines. It has been claimed that 6-hydroxy-N:N-dimethyltryptamine is more potent than DMT (15); however, general agreement on this point has not been forthcoming (16,17). In this paper we report on statistically significant differences in activity, as characterized by the ability to disrupt the conditioned avoidance response of trained rats, among a number of naturally occurring and synthetic tryptamines.

Materials and Methods

Materials: 4-Hydroxy-N:N-dimethyltryptamine (psilocin) was a gift from Sandoz Pharmaceuticals. N:N-diethyltryptamine was obtained from Calbiochem Corporation. All other drugs were synthesized in this laboratory and are listed together with yields, physical constants, analytical data and synthetic methods in Table 1.

Methods: The apparatus used was a shuttle box similar to that described by Gessner and Page (14). Female rats (150-250g) were trained to cross from one chamber of the shuttle box to the other in response to a conditioned

TABLE I



R	R'	R''	R'''	Yield	Form	Formula	% Calcd			% Found			m.p.
							C	H	N	C	H	N	
5-OCH ₃	CO.CO	CH ₃	C ₂ H ₅			C ₁₄ H ₁₆ N ₂ O ₃	64.60	6.20	10.76	64.87	6.06	10.67	141-145
5-OCH ₃	CO.CO	C ₂ H ₅	C ₂ H ₅	82%		C ₁₅ H ₁₈ N ₂ O ₃	65.70	6.62	10.20	65.48	6.60	10.12	156-158
4-OCH ₃	CH ₂ CH ₂	CH ₃	CH ₃	85%	bioxalate	C ₁₅ H ₂₀ N ₂ O ₅	58.43	6.54	9.09	58.65	6.70	9.24	163.5-164.5
5-OCH ₃	CH ₂ CH ₂	CH ₃	CH ₃	81%	bioxalate	C ₁₅ H ₂₀ N ₂ O ₅							173 (lit(14): 174)
5-OCH ₃	CH ₂ CH ₂	CH ₃	C ₂ H ₅	57%	bioxalate	C ₁₆ H ₂₂ N ₂ O ₅	59.60	6.88	8.69	59.88	6.88	8.64	116-120
5-OCH ₃	CH ₂ CH ₂	C ₂ H ₅	C ₂ H ₅	37%	hydrochl.	C ₁₅ H ₂₃ N ₂ OCl	63.70	8.20	9.91	64.00	8.38	10.07	194-195
6-OCH ₃	CH ₂ CH ₂	CH ₃	CH ₃	65%	bioxalate	C ₁₅ H ₂₀ N ₂ O ₅	58.43	6.54	9.09	57.69	6.59	9.65	220-223
7-OCH ₃	CH ₂ CH ₂	CH ₃	CH ₃	62%	hydrochl.	C ₁₃ H ₁₉ N ₂ OCl	61.30	7.52	11.00	61.53	7.77	10.81	200-204
5-OCOCH ₃	CH ₂ CH ₂	CH ₃	CH ₃	100%	base	C ₁₄ H ₁₈ N ₂ O ₂	68.27	7.36	11.38	68.14	7.50	11.25	150-160
					bioxalate	C ₁₆ H ₂₀ N ₂ O ₆	57.13	5.99	8.33	57.46	6.11	8.54	158-161

The methoxy ring substituted tryptamines were synthesized by the procedure of Speeter and Antony (18). Typically the respective methoxyindole was reacted with oxalyl chloride and the precipitated methoxyindole-3-glyoxylyl chloride was treated with the proper dialkylamine to obtain the desired glyoxamide. Reduction of the glyoxamide with lithium aluminum hydride furnished the requisite methoxyalkyltryptamine which were isolated as bioxalates or hydrochlorides. The acetyl ester of bufotenine was synthesized by the addition of acetic anhydride to a solution of bufotenine in sodium hydroxide kept at 5° C.

stimulus which consisted of a 0.5 mA shock derived from the intermittent (100 msec on, 200 msec off) electrification of the chamber floor for a period of 5 sec. Conditioned stimuli were presented every 25 seconds. The animals used in the experiments were those which could be trained to respond to the conditioned stimulus in at least 9 of 10 consecutive trials on two consecutive days.

The effects of the drugs tested were evaluated on the basis of the number of failures of the conditioned response occurring in the first half hour (exclusive of the first 2.5 minutes) following intraperitoneal administration of the test drug. The rats were all first tested after treatment with saline. Following this, the effects of drugs were determined, the animals being tested with different drugs or different doses of the same drug at five day intervals. With experiments involving drug comparisons all the drugs were administered in equimolar quantities. Solutions of drugs were made up in isotonic saline, care being taken that all the solutions were of the same molar concentration (40 mmoles/liter) with respect to the drug tested.

Fully-orthogonal, randomized, Latin-square, experimental designs were used throughout. Analysis of variance was applied to the results of drug comparisons and significance was tested for by the method of Hartley (19). When comparing different doses of the same drug, all the results were subjected to the probit transformation (20) and analysis of variance used to determine the significance of the regression of the resulting values.

Chloroform-water partition coefficients were determined in triplicate. The concentration of the compound in solution in sodium monohydrogen phosphate-sodium hydroxide buffer at pH 7.4 was determined spectrophotometrically both before and after equilibration with chloroform. The equilibration was achieved by shaking a 10 ml aliquot of the aqueous solution with 10 ml of chloroform in 40 ml glass-stoppered centrifuge tubes for 15 minutes and allowing the two liquids to separate. Since the partition coefficients thus obtained (Table 5) differ substantially from ones determined using a spectrophotofluorometer (14),

the stability of these compounds when exposed to the spectrophotofluorometer light beam was investigated. It was found that some of these compounds are degraded when exposed to the spectrophotofluorometer light beam resulting in readings whose value increased as a function of time. In contrast the spectrophotometer gave constant and steady readings.

Results

In the first experiment the effects of five substituted tryptamines on the conditioned avoidance response were determined following i.p. administration of doses of 10 μ moles/kg. As can be seen from the results listed in Table 2, this experiment established that 5MeO-DMT and 5-methoxy-N:N-diethyltryptamine (5MeO-DET) have a significantly greater effect on the conditioned avoidance response than do 4HO-DMT (psilocin), DET or 6-methoxy-N:N-dimethyltryptamine (6MeO-DMT). In the second experiment (Table 3) the effects of four substituted

Table 2

Experiment 1: Comparison of the effects of 5-Methoxy-N:N-dimethyltryptamine (5MeO-DMT), 5-Methoxy-N:N-diethyltryptamine (5MeO-DET), 6-Methoxy-N:N-dimethyltryptamine (6MeO-DMT), 4-Hydroxy-N:N-dimethyltryptamine (4HO-DMT) and N:N-Diethyltryptamine (DET) on the Conditioned Avoidance Response (CAR) of Trained Rats

Drug 10 μ moles/kg	CAR failures %	Interdrug Differences			
		6MeO DMT	DET	4HO DMT	5MeO DET
5MeO-DMT	29.3	26.3*	24.6*	23.3*	0.9
5MeO-DET	28.4	25.4*	23.7*	22.4*	
4HO-DMT	6.0	3.0	1.3		
DET	4.7	1.7			
6MeO-DMT	3.0				

*Significant differences ($P < 0.05$)

Table 3

Experiment 2: Comparison of the Effects of 5-Acetoxy-N:N-dimethyltryptamine (5AcO-DMT), 4-Methoxy-N:N-dimethyltryptamine (4MeO-DMT), 7-Methoxy-N:N-dimethyltryptamine (7MeO-DMT) and 6-Methoxy-N:N-dimethyltryptamine (6MeO-DMT) on the Conditioned Avoidance Response (CAR) of Trained Rats

Drug 20 μ moles/kg	CAR failures %	Interdrug Differences		
		6MeO DMT	7MeO DMT	4MeO DMT
5AcO-DMT	40.0	35.0*	28.8*	0.8
4MeO-DMT	39.2	34.2*	28.0*	
7MeO-DMT	11.2	6.2		
6MeO-DMT	5.0			

*Significant differences ($P < 0.05$)

Table 4

Experiment 3: Comparison of the Effect of 5-Methoxy-N:N-dimethyltryptamine (5MeO-DMT), 5-Methoxy-N-methyl-N-ethyltryptamine (5MeO-MET), 4-Methoxy-N:N-dimethyltryptamine (4MeO-DMT), 5-Acetoxy-N:N-dimethyltryptamine (5AcO-DMT) and N:N-Diethyltryptamine (DET) on the Conditioned Avoidance Response (CAR) of Trained Rats

Drug 10 μ moles/kg	CAR failures %	Interdrug Differences			
		DET	5MeO DMT	4MeO DMT	5AcO DMT
5MeO-MET	76.6	59.3*	55.3*	51.0*	35.6
5AcO-DMT	41.0	23.7	20.7	15.4	
4MeO-DMT	25.6	8.3	4.3		
5MeO-DMT	21.3	4.0			
DET	17.3				

*Significant Differences ($P < 0.05$)

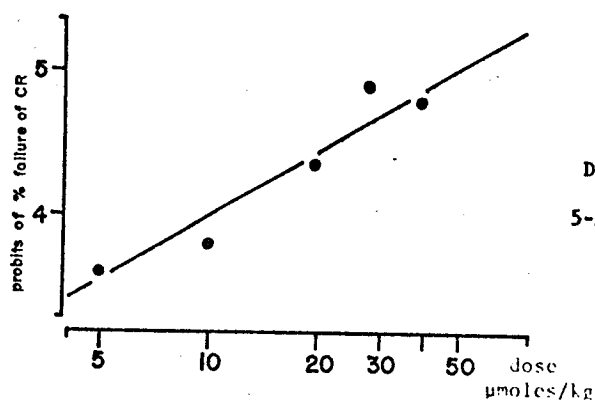


Fig. 1

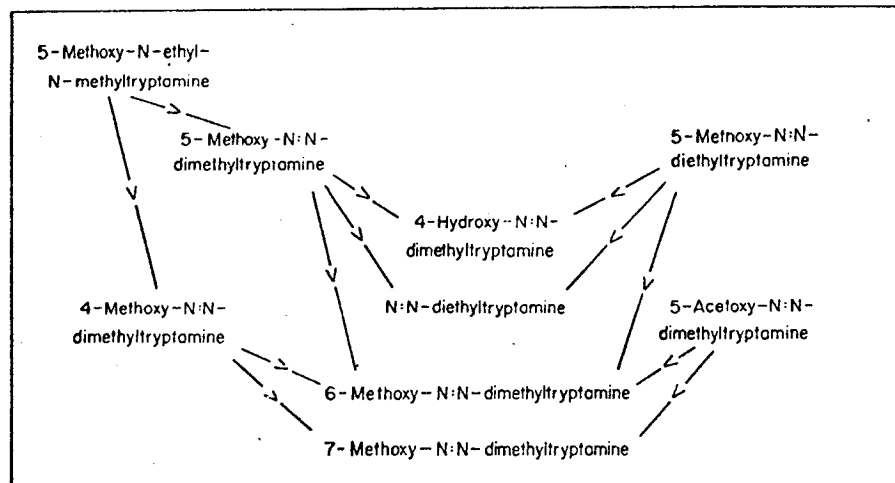
Dose Response Relationship for
5-Acetoxy-N,N-dimethyltryptamine

tryptamines given i.p. at a dose of 20 μ moles/kg were compared. This experiment established that 5-acetoxy-N,N-dimethyltryptamine (5AcO-DMT: acetyl bufotenine) and 4-methoxy-N,N-dimethyltryptamine (4MeO-DMT) have a significantly greater effect than 7-methoxy-N,N-dimethyltryptamine (7MeO-DMT) or 6MeO-DMT. In the third experiment (Table 4) doses of 10 μ moles/kg were used. This experiment demonstrated that 5-methoxy-N-methyl-N-ethyltryptamine (5MeO-MET) has a significantly greater effect than 5AcO-DMT, 4MeO-DMT, 5MeO-DMT and DET.

5AcO-DMT is of particular interest since its lipid solubility characteristics should permit it to cross the blood brain barrier where by the action of tissue esterase it could give rise to 5HO-DMT (bufotenine) a compound which would otherwise not be expected to enter the central nervous system too readily (14). Accordingly, an attempt was made to establish the dose-response curve for the behavioral effects of this compound. Thus, the effect of several doses of 5-acetoxy-N,N-dimethyltryptamine was investigated, using again a Latin-square experimental design ($N = 6$: 6 animals $\times$ 5 dose levels and saline). The conditioned avoidance response failure frequencies were converted first into percentage of total responses possible in the experimental period and then into probit units. The resulting regression line was calculated by the method of Finney (20) and is shown in Fig. 1. Analysis of variance shows that there is significant regression. Interestingly, even 5 μ moles/kg, the lowest dose given, differed from saline in its behavioral effect at the 0.01 level of significance.

Fig. 2

Significant Differences in Activity as Established by This Study

Key: A $\longrightarrow$ B Signifies that A is significantly more active ($P < 0.05$) than B.

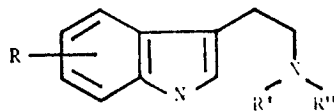
Discussion

Although complete ranking of the investigated compounds is not possible, a number of conclusions can be made regarding structure-activity relationships with respect to the ability of these compounds to cause failure of the conditioned avoidance response in rats. Thus it is evident, from a comparison of the activity of 4MeO-DMT and 5MeO-DMT with that of 6MeO-DMT and 7MeO-DMT respectively, that substitution in either the 4 or 5 position of the indole ring leads to compounds which are more active than those substituted in either position 6 or 7. This difference in activities is made all the more striking by the fact that the partition coefficients and thus the lipid solubility of the 6 and particularly of the 7 substituted compounds is considerably higher than that of either the 4 or the 5 substituted homologues (Table 5).

No detectable increase in activity is observed when the alkyl substituents on the side chain nitrogen are changed from dimethyl, as in 5MeO-DMT, to diethyl, as in 5MeO-DET, although this leads to a twofold increase in the

partition coefficient. A significant increase in activity relative to 5MeO-DMT is obtained when one of the methyl groups on the side chain nitrogen is replaced with an ethyl group as in 5MeO-MET. This increase in potency could simply reflect the increased lipid solubility of the latter compound.

Table 5
Chloroform-water Partition Coefficients



Compound	<u>R</u>	<u>R''</u>	<u>R'''</u>	<u>Partition Coeff.</u>
4MeO-DMT	4-OCH ₃	CH ₃	CH ₃	2.28
5MeO-DMT	5-OCH ₃	CH ₃	CH ₃	3.30
5MeO-MET	5-OCH ₃	CH ₃	C ₂ H ₅	5.72
5MeO-DET	5-OCH ₃	C ₂ H ₅	C ₂ H ₅	7.89
6MeO-DMT	6-OCH ₃	CH ₃	CH ₃	3.68
7MeO-DMT	7-OCH ₃	CH ₃	CH ₃	9.54
5AcO-DMT	5-OCOCH ₃	CH ₃	CH ₃	2.31
5HO-DMT	5-OH	CH ₃	CH ₃	0.06
DET	--	C ₂ H ₅	C ₂ H ₅	1.85
4HO-DMT	4-OH	CH ₃	CH ₃	5.52

The partition coefficient for 4HO-DMT (Psilocin) is remarkably high, particularly in comparison to that of 5HO-DMT (bufotenine). The almost hundred-fold difference in partition coefficients may be responsible for the reported wide differences in the hallucinogenic activity of these compounds in man.

It is of interest that 5MeO-DMT, 5MeO-DET and 5MeO-MET are all more active than the established hallucinogens, 4HO-DMT and DET. While the mental condition brought about by such as 4HO-DMT, DMT and DET is now recognized to differ from

a psychosis, Sai Halasz has shown that under certain circumstances DMT can lead to a state in which insight is lost and which does not differ significantly from the schizophrenic syndrome (21). Enzymes capable of both N- and O-methylating hydroxyindolealkylamines are present in the mammalian body (22,23). Gessner and Page suggested that the formation of 5MeO-DMT, which may be viewed as a fully methylated derivative of 5-hydroxytryptamine, may occur under either physiological or pathological conditions. The formation of 5MeO-DMT has now been shown to occur in at least one animal species, namely the toad Bufo alvarius. Furthermore, the presence of 5-methoxytryptamine, 5HO-DMT and DMT in human blood and urine has been reported (24), though Siegel was not able to confirm this with respect to 5HO-DMT (25). It is noteworthy that several investigators have shown that conditions which could be expected to lead to increased cerebral levels of methylated amines cause an exacerbation of the schizophrenic syndrome in hospitalized patients (26-31). It would appear desirable to investigate the mechanism whereby the more active of these amines exert their central effects.

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