

STUDIES ON CENTRAL NERVOUS SYSTEM DEPRESSANTS (XIII) "STRUCTURE ACTIVITY RELATIONSHIP OF SOME 3:4:5 TRIMETHOXY BENZAMIDES"

By

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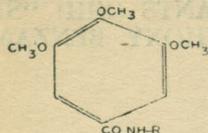
The importance of trimethoxybenzene moiety of reserpine molecule in influencing the pharmacological actions of reserpine has been stressed by Bein (1). Large number of trimethoxybenzene derivatives have been synthesised and studied by several workers (3, 4, 11, 14, 15, 18, 22, 24). Some of the compounds synthesised by these workers have shown central nervous system depressant properties. The two active principles, asarone and β -asarone isolated from the volatile oil of *Acorus calamus* were found to be simple trimethoxybenzene derivatives. These compounds also showed well defined tranquillizing effect in various experimental animals.

In contrast to this, other trimethoxybenzene derivatives like mescaline and trimethoxyamphetamine are potent hallucinogens. These interesting findings led us to the synthesis of a large number of trimethoxybenzene derivatives in this laboratory in the hope that pharmacological investigations of a large number of these compounds, would, in addition to the development of a reserpine analogue, also provide clue to the essential structural features which will confer hallucinogenic property to the compounds possessing this basic structure. Some of the compounds synthesised and evaluated in this laboratory exhibited marked central nervous system depressant effect and a structure activity relationship could be established between the compounds belonging to the same series (8), (19), and (21). Of these N-acetyl 3:4:5 trimethoxybenzamide showed promising effects (13) and, therefore, large number of substituted amides were synthesised (20), (21). In the present study preliminary investigations of the central nervous system action of four trimethoxybenzamides were undertaken and the structure activity relationships of these and other trimethoxy benzene derivatives were noted.

MATERIALS AND METHODS

The compounds under investigation

Four compounds with the following general formula were investigated for their pharmacological actions :

Substitution at 'R'	Symbol assigned
	$-\text{C}_6\text{H}_4\cdot\text{CH}_3$ (O) 'S'A' $-\text{CH}_2\cdot\text{C}_6\text{H}_5$ 'SB' $-\text{C}_6\text{H}_4\text{CH}_3$ (P) 'SC' $-\text{C}_6\text{H}_4\cdot\text{Cl}$ (P) 'SD'

Compounds 'SA', 'SB', 'SC' and 'SD' were synthesised in this laboratory as reported earlier by Sogani *et. al.* (20) and Sogani and Dandiya (21).

These compounds were administered in the form of a fine suspension in three percent Tween-80. Solutions of other drugs employed during this study were prepared in distilled water. The administration of drugs was made intraperitoneally unless otherwise stated. Control experiments were performed simultaneously using equivalent quantities of Tween-80 solution in test animals.

SPONTANEOUS MOTOR ACTIVITY

The procedure followed was similar to that employed by Menon and Dandiya (13) in all essential details.

A set of thirty albino mice (Haffkine strain) of either sex weighing between 35 to 40 gm. were divided into six groups of five animals each. The first group of animals served as control and received the solvent. The second group of animals were administered chlorpromazine HCl in a dose of 3 mg/kg. The compounds 'SA', 'SB', 'SC' and 'SD' were administered in doses of 50 mg/kg to the other groups of animals.

DRUG INDUCED HYPNOSIS

The influence of the test compounds on drug induced hypnosis was studied by the method of Dandiya and Cullumbine (5). To the hypnotic agents employed were pentobarbitone sodium (40 mg/kg), or hexobarbitone sodium (80 mg/kg), or ethanol (3 g/kg).

CONDITIONED AVOIDANCE RESPONSE

The influence of the test compounds on conditioned avoidance response was studied as described by Dandiya and Sharma (8). Chlorpromazine HCl was used for comparison.

RECTAL TEMPERATURE OF MICE

The experiments were performed at room temperature of $20^{\circ}\text{C}\pm 2^{\circ}\text{C}$. Thirty albino mice (Haffkine strain) of either sex weighing between 35 to 40 gms. were employed. The compound under investigation was given in a dose of 50 mg/kg. Rectal temperature of mice was recorded by inserting lubricated bulb of clinical thermometer inside the rectum and keeping it for ninety seconds.

MORPHINE ANALGESIA

Albino mice (Haffkine strain) of either sex seighing 35 to 40 g were employed. The hot wire method was employed for testing the analgesic effect, the method being similar to that employed by Dandiya and Menon (10) in all essential details. After recording the normal reaction time of the animals, the compound under investigation was administered (50 mg/kg), following fifteen minutes later by the sub-analgesic dose of morphine.

ELECTRO-CONVULSIONS

Convulsions were induced in albino rats (Haffkine strain) weighing between 125 and 150 gm by application of a current of 150mA for 0.2 second through corneal electrodes. The reduction in the duration of extensor tonic spasm and death was accepted as the criterion of protection from electroshock seizure. The prolongation of extensor tonic spasm or the increase in percentage mortality of animals in 24 hours was taken as the criterion in estimating the potentiation of the electro-convulsion by the compounds.

RESULTS

In control experiments using 3 percent Tween-80 only, no effect was observed hence the results are not reported under individual experiments.

INFLUENCE ON SPONTANEOUS MOTOR ACTIVITY

All the four compounds studied were able to reduce spontaneous motor activity of mice. Compounds 'SA' and 'SB' made the animals sedate and reduced their spontaneous motor activity significantly. The tested animals were very much less responsive to tactile stimulus. The action of the compounds 'SA' and 'SB' started in about half an hour and lasted for three hours, and was almost similar to the effect produced by chlorpromazine. In this respect the compounds 'SC' and 'SD' were found to be less potent since these made the animals only slightly sedate and caused moderate reduction in their spontaneous motor activity.

EFFECT OF THE COMPOUNDS ON DRUG INDUCED HYPNOSIS

The results are given in Table I

TABLE I

*Effect of the compounds 'SA', 'SB', 'SC', and 'SD' on drug induced hypnosis*SLEEPING TIME IN MINUTES (Mean \pm S.E.)

Drug	Dose (mg/kg)	SLEEPING TIME IN MINUTES (Mean \pm S.E.)		SLEEPING TIME IN MINUTES (Mean \pm S.E.)		SLEEPING TIME IN MINUTES (Mean \pm S.E.)	
		Control Pentobarbitone sodium only	Test Pentobarbitone sodium + Drug	Control Hexobarbitone sodium only	Test Hexobarbitone sodium + Drug	Control Ethanol	Test Ethanol + Drug
'SA'	10	67.9 \pm 16.0	107.4 \pm 14.4	26.0 \pm 7.7	59.6 \pm 7.8 (P 0.01)	10.7 \pm 3.1	27.4 \pm 4.8 (P < 0.01)
'SB'	10	45.2 \pm 6.3	73.3 \pm 8.7 (P < 0.025)	35.1 \pm 4.6	41.1 \pm 5.0	13.1 \pm 3.4	50.2 \pm 39.8 (P < 0.01);
'SC'	10	79.1 \pm 17.0	99.6 \pm 15.9	33.9 \pm 7.0	31.1 \pm 5.6	9.4 \pm 2.5	18.0 \pm 5.1
'SD'	10	74.5 \pm 20.0	94.1 \pm 15.7	33.9 \pm 7.0	70.1 \pm 10.2 (P < 0.01);	17.2 \pm 2.9	25.8 \pm 5.8

*Probability (P) calculated by 't' test

EFFECT ON THE CONDITIONED BEHAVIOUR OF RATS

Results are given in Table II.

TABLE II

Effect of the compounds on the conditioned avoidance response ('CAR') and escape response ('ER') of trained rats

Drug;	Dose; (mg/kg)	No. of rats	PERCENTAGE OF RATS:		Duration of action (Hours)
			Showing loss of 'CAR'	Showing loss of 'ER'	
Chlorpromazine HCL	3	10	50	..	5-6
'SA'	50	10	40	..	1-3
'SB'	50	10	10	..	1-2
'SC'	50	10	30	10	1-3
'SD'	50	10	30	..	3-4

EFFECT ON RECTAL TEMPERATURE OF MICE

Compounds 'SA', 'SB', 'SC' and 'SD' caused only insignificant rise in the rectal temperature of mice.

EFFECT ON MORPHINE ANALGESIA

Results are given in Table III.

TABLE III

*Effect of the compounds on analgesia due to subanalgesic dose of morphine
(1 mg/kg)*

Drug	Dose mg/kg	No. of mice used	REACTION TIME IN SECONDS (Mean \pm S.E.);				Percentage increase in reaction time at peak effect	No. of animals showing definite* analgesia
			Before drug treatment	After drug treatment				
				15 minutes	30 minutes	60 minutes		
Control	—	20	2.6 \pm 0.11	3.4 \pm 0.13	2.8 \pm 0.10	2.69 \pm 0.09	30	2/20
'SA'	50	10	2.0 \pm 0.19	2.7 \pm 0.023	3.0 \pm 0.44	2.2 \pm 0.19	50	3/10
'SB'	50	10	2.4 \pm 0.28	3.8 \pm 0.39	3.7 \pm 0.14	2.8 \pm 0.35	58	3/10
'SC'	50	10	2.6 \pm 0.21	4.1 \pm 0.35	3.7 \pm 0.25	2.9 \pm 0.2	57	3/10
'SD'	50	10	2.4 \pm 0.25	3.2 \pm 0.33	3.2 \pm 0.19	2.6 \pm 0.24	33	2/10

*Animals showing at-least double the average normal reaction time.

EFFECT ON ELECTRO-SHOCK SEIZURES IN RATS

Results are shown in Table IV. All the control rats subjected to electro-shock exhibited extensor spasm but none of them died. All the compounds offered protection to the animals as indicated by the absence of extensor spasm in some of the animals treated with these compounds. A reduction in the duration of tonic convulsions was also observed in the drug treated animals.

TABLE IV

Effect of the compounds on electro-shock seizures in rats

Drug	Dose (mg/kg)	No. of rats	TONIC CONVULSIONS		
			No. of rats getting extensor spasm	Duration of tonic convul- sions (Flexion + Extension) Seconds (Mean \pm S.E.)	No. of deaths
Control	..	20	100%	17.3 \pm 1.0	Nil
'SA'	50	10	50%	8.0 \pm 2.7	Nil
'SB'	50	10	60%	9.5 \pm 3.1	Nil
'SC'	50	10	90%	12.6 \pm 2.2	Nil
'SD'	50	10	50%	9.0 \pm 2.2	Nil

DISCUSSION

The effect of each one of the four benzamides on spontaneous motor activity, drug induced hypnosis, and conditioned avoidance behaviour resembled to some extent with major tranquillizers, namely, reserpine and chlorpromazine and asarone (2:4:5-trimethoxy-1-propenyl benzene). These actions were exhibited by these compounds in a dose much higher than asarone (6) and, therefore, these were less potent as compared to asarone which has been claimed as a potent tranquillizer (7). But in their effect on morphine analgesia and pattern of electroconvulsions these compounds were found to be more like minor tranquillizers, like meprobamate and hydroxyzine.

These observations, together with our earlier findings (9) that asarone (2:4:5 trimethoxy-1-propenylbenzene) is a very potent central nervous system depressant as compared to 3:4:5 trimethoxy and 2:3:4 trimethoxy-1-propenyl benzene (Though the nature of side chain in all the three compounds remains the same), led us to conclude that asymmetric substitution of the methoxy groups as in asarone enhance the central nervous system depressant activity in comparison to the vicinally substituted ones as in 3:4:5 trimethoxybenzamides studied at present. It might be due to the fact that out of the three vicinally substituted methoxy groups, the middle one is twisted out of the plane of the ring under the influence of the steric effects exerted by the other two bulky methoxy groups present in both the ortho positions. Thus this group is removed from conjugation with the rest of the system due to the steric interference in the planarity of the molecule. This steric inhibition of resonance will result in a decreased contribution of the resonating structures and so diminishes the electron repelling power of this group and also the degree of aromatic conjugation of the whole system, as compared to that which is coplanar. This reduced degree of aromatic conjugation is probably inversely proportional to the central nervous system depressant potency. This view is further supported by the fact that the removal of a methoxy group in position 5 of mescaline results in 50 percent loss of stimulant activity (23).

There are certain factors other than aromatic conjugation which might also play an important role. In asarone due to the +M effect of the methoxy groups favoured by the +E effect and electromeric displacement due to the double bond in side chain, the β carbon atom in the side chain becomes a centre of high electron density. The hyperconjugative effect due to the methyl group in the side chain and -I effect due to methoxy groups in the ring are dominated by the above mentioned effects. In case of these amides studied here, the vicinally situated methoxy groups make the ring itself mesomeric, of course the carbonyl oxygen in the side chain here, also serves as the negative centre, but here it is due to -I and -E effect of the carbonyl group which withdraws electrons from the nitrogen of the amido group. The magnitude of the negative centre in the benzamides is less in comparison to asarone and thus the binding

capacity of asarone to the receptor surface, which is probably electrophilic in nature, is greater than those of amides and hence is more potent.

The substitution of $-\text{CH}_2 \cdot \text{C}_6 \text{H}_5$ in place of R (Compound 'SB') results in increased potency which could be due to the fact that the amino group in benzylamino substituted compound resembles the aliphatic amino group *i.e.* it is more basic than aromatic amino group. Here the lone pair of 2p electrons on the nitrogen atom are unable to conjugate with the pz electrons of the benzene ring, thereby making the lone pair of electrons more available for the carbonyl oxygen resulting in the formation of negative centre with comparatively greater electron density than to normal benzamides. The depressant activity of trimethoxybenzamide with $\text{C}_6\text{H}_4 \cdot \text{CH}_3(\text{O})$ in place of R (Compound 'SA') was found to be greater than $\text{C}_6\text{H}_4 \cdot \text{CH}_3(\text{P})$ (Compound 'SC'). This may be due to the fact that aromatic amines with ortho substituted methyl groups, are more basic than para due to the steric effect exerted by the methyl group present in the O-position, which reduces the degree of aromatic conjugation and the electron repelling power of the amino group thus making the lone pair of electrons more available for carbonyl oxygen resulting in the formation of a negative centre with comparatively greater electron density than to compound 'SC'. Substitution of $-\text{C}_6\text{H}_4 \cdot \text{Cl}(\text{P})$ for R (Compound 'SD') did not increase the potency which could be due to the fact that presence of p-halogen in the benzene ring decreases the basic property of nitrogen of the amido group and thus making the lone pair of electrons less available for the carbonyl oxygen resulting in the formation of a negative centre with lesser magnitude.

SUMMARY

Four trimethoxybenzamides synthesised in this laboratory were investigated for their actions on central nervous system. All these compounds reduced spontaneous motor activity of mice, potentiated hypnosis due to various anaesthetic agents, caused specific blockade of conditioned avoidance response in rats, protected rats subjected to electro-shock seizures, and potentiated morphine—analgesia to some extent. Attempt has been made to establish structure activity relationship.

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