Central Nervous Depressants VIII

Structure-Activity Relationship of Some New Azlactones

By P. K. SHARMA, M. K. MENON, and P. C. DANDIYA

Five azlactones having trimethoxybenzene structure were synthesized and their influence on blood pressure of anesthetized dogs, spontaneous motor activity of rats, pentobarbital hypnosis, and conditioned avoidance response in trained rats were studied. All the compounds possessed mild central nervous system depressant action. Two compounds—namely, azlactone of 3,4,5-trimethoxybenzylamino-2,3,5-trimethoxycinnamic acid and azlactone of 3,4,5-trimethoxybenzylamino-*p*-dimethylaminocinnamic acid—exerted a transient hypotensive action. A structure-activity relationship has been indicated.

SINCE THE structure of reserpine was established, the problem of synthesis of reserpine analogs has been investigated by various workers, to search for compounds of simpler chemical structure having similar pharmacological actions but devoid of undesirable side effects. The reserpine molecule possesses an indole nucleus and a trimethoxybenzene group in its chemical structure; it was assumed that compounds built upon these structures may reproduce the pharmacological activities of reserpine. Lassalo and Jordan (6), Ramasastry and Lassalo (9), Borsy (2), and Dandiya *et al.* (4) have synthesized some compounds having trimethoxybenzene as the basic structure. Dandiya *et al.* (4) have also studied the structure-activity relationship of the compounds synthesized by them. Another interesting development in this field was the isolation of two active principles of *Acorus calamus*, a plant indigenous to India. Both of these principles, asarone and β-asarone, are simple trimethoxybenzene derivatives (1) and exerted marked central nervous depressant effects (3).

Lately, in the course of investigations of new compounds with actions on the central nervous system, trimethoxybenzoyl-glycine-diethylamide (Riker 548, trimeglamide) has also been shown to possess tranquilizing properties (11).

Since Riker 548 could be considered as a derivative of a saturated azlactone, Robison and Schueler (10) have worked out the pharmacological activity of a number of azlactone derivatives and reported that they were capable of prolonging the duration of hexobarbital-induced hypnosis in mice, although by themselves, these compounds exhibited convulsant properties.

Since most of the compounds of the azlactone series have a prominent action on the central nervous system, it was considered desirable to synthesize a few azlactones possessing a trimethoxy group in the benzene ring. These azlactones (Table I) were prepared by reacting the substituted glycine with the appropriate aldehyde in the presence of acetic anhydride and sodium acetate. 2,4,5-Trimethoxy- and 2,3,4-trimethoxybenzaldehyde were prepared by the method described by Deohra (5).

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EXPERIMENTAL

Chemical.—The following four intermediary compounds were prepared.

2,4,5-Trimethoxybenzaldehyde.—Freshly distilled $POCl_3$ (15 ml.) was added to dimethylformamide (10 ml.) previously cooled to 5°. The mixture was then added to 1,2,4-trimethoxybenzene (16.7 Gm.), dissolved in dimethylformamide (10 ml.), and heated on a steam bath for 4 hours. The mixture was diluted with water, neutralized with sodium bicarbonate, and allowed to stand for 1 hour. It was then filtered and washed with water and recrystallized from hot water (m.p. 114°, 2,4-dinitrophenylhydrazone, m.p. 252°).

2,3,4-Trimethoxybenzaldehyde.—A mixture of $POCl_3$ (15 ml.), dimethylformamide (10 ml.), and 1,2,3-trimethoxybenzene (16.7 Gm.) in dimethylformamide (10 ml.) was heated on a steam bath for 2 hours, then diluted with water and neutralized with sodium bicarbonate. It was filtered quickly after refrigerating overnight, washed with cold water, and recrystallized with hot water (m.p. 37°, 2,4-dinitrophenylhydrazone, m.p. 177–178°).

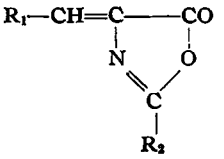
Preparation of Substituted Azlactone.—The following five compounds were synthesized.

Compound I.—(Azlactone of α-Acetylamino-2,4,5-trimethoxy cinnamic Acid).—A mixture of 2,4,5-trimethoxybenzaldehyde (10 Gm.), acetylglycine (7 Gm.), acetic anhydride (5 ml.), and anhydrous sodium acetate (4.1 Gm.) was warmed on a water bath with occasional shaking for 15–20 minutes, and the whole mixture was boiled for 1 hour and cooled by refrigeration for 4 hours. The mass was then stirred with 60 ml. of cold water, filtered, and recrystallized from carbon tetrachloride (m.p. 106–108°, yield 7.5 Gm.).

Anal.—Calcd. for C, 60.64; H, 5.41; N, 5.05. Found: C, 61.25; H, 5.93; N, 5.68.

Compound II.—(Azlactone of 3,4,5-Trimethoxybenzoyl-α-amino-2,4,5-trimethoxycinnamic Acid).—A mixture of 2,4,5-trimethoxybenzaldehyde (10

TABLE I.—SUBSTITUTED AZLACTONE DERIVATIVES AND RESULTS OF THEIR PHARMACOLOGICAL TESTING



Compd.	Substitution at R_1	Substitution at R_2	M.p., (uncorrected), °C.	Emperical Formula	Mean Sleeping Time (in Min. ± S.E.) in Mice Due to 20 mg./Kg. of Compd. +40 mg./Kg. of Pentobarbital Sodium (Mean Sleeping Time Due to Pentobarbital Alone 62.4 ± 13.5), Min.	Conditioned Avoidance Response 50 mg./Kg. Loss of CAR, %	Conditioned Avoidance Response 50 mg./Kg. Loss of Escape Phenomenon, %	Spontaneous Activity[a] in Rats, 50 mg./Kg.	Blood Pressure of Anesthetized Dogs, 10 mg./Kg. Blood Pressure Decrease, mm. Hg	Blood Pressure of Anesthetized Dogs, 10 mg./Kg. Duration of Action, Min.
I	2,4,5-Trimethoxyphenyl	Methyl	106–108	$C_{14}H_{15}NO_5$	94.8 ± 12.0	20	20	−	No effect	
II	2,4,5-Trimethoxyphenyl	2,4,5-Trimethoxyphenyl	195–197	$C_{22}H_{23}NO_8$	80.6 ± 9.6	20	0	0	No effect	
III	2,3,4-Trimethoxyphenyl	Methyl	198–199	$C_{14}H_{15}NO_5$	112.1 ± 10.0 ($P < 0.05$)	30	0	0	20–30	2–3
IV	Phenyl	3,4,5-Trimethoxyphenyl	158–159	$C_{19}H_{17}NO_5$	58.0 ± 10.2	20	0	0	...	...
V	*p*-Dimethylaminophenyl	3,4,5-Trimethoxyphenyl	194–195	$C_{21}H_{22}NO_5$	70.2 ± 8.6	20	0	+	20–30	2–3

[a] Key: 0, no effect; −, reduction; +, increase.

Gm.), 3,4,5-trimethoxybenzoylglycine (12.4 Gm.), acetic anhydride (7 ml.), and anhydrous sodium acetate (4.5 Gm.) was warmed with shaking for 10 minutes, then boiled for 1 hour, cooled in the refrigerator overnight, stirred with cold water, and filtered. Recrystallized from carbon tetrachloride (m.p. 195–197°, yield 6 Gm.).

Anal.—Calcd. for C, 61.53; H, 5.36; N, 3.26. Found: C, 60.97; H, 5.75; N, 3.15.

Compound III.—(Azlactone of α-Acetylamino-2,3,4-trimethoxycinnamic Acid).—A mixture of 2,3,4-trimethoxybenzaldehyde (10 Gm.), acetylglycine (7 Gm.), acetic anhydride (5 ml.), and anhydrous sodium acetate (4.1 Gm.) was taken and subjected to the procedure mentioned above (m.p. 198–199°, yield 8 Gm.).

Anal.—Calcd. for C, 60.64; H, 5.41; N, 5.05. Found: C, 60.96; H, 5.53; N, 5.25.

Compound IV.—(Azlactone of 3,4,5-Trimethoxybenzoylaminocinnamic Acid).—A mixture of redistilled benzaldehyde (4 Gm.), 3,4,5-trimethoxybenzoylglycine (8.5 Gm.), acetic anhydride (7 ml.), and anhydrous sodium acetate (2.5 Gm.) was warmed for 15–20 minutes with occasional shaking and boiled for 45 minutes. It was then cooled in a refrigerator for 4 hours, stirred with 100 ml. of water, filtered, and recrystallized from ethyl acetate and liq. petroleum (m.p. 158–159°, yield 2.5 Gm.).

Anal.—Calcd. for C, 67.25; H, 5.01; N, 4.12. Found: C, 66.98; H, 5.03; N, 4.25.

Compound V.—(Azlactone of 3,4,5-Trimethoxybenzoyl-α-amino-*p*-dimethylaminocinnamic Acid).—A mixture of *p*-dimethylaminobenzaldehyde (7.5 Gm.), 3,4,5-trimethoxybenzoylglycine (12.5 Gm.), acetic anhydride (7 ml.), and anhydrous sodium acetate (4 Gm.) was warmed for 10 minutes, boiled for 1 hour, cooled in the refrigerator overnight, stirred with a minimum volume of water, filtered with suction and recrystallized from carbon tetrachloride (m.p. 194–195°, yield 3.8 Gm.).

Anal.—Calcd. for C, 68.47; H, 5.97; N, 3.80. Found: C, 68.40; H, 5.88; N, 3.56.

Pharmacological Study.—The compounds were administered as a fine suspension in 3% polysorbate 80. Control experiments were always performed with the solvent-treated animals.

Blood Pressure of Anesthetized Dogs.—Ten mongrel dogs were anesthetized with morphine (5 mg./Kg.) and urethane (1.4 Gm./Kg.) administered intramuscularly. The left common carotid artery was cannulated, and blood pressure was recorded on a slowly moving kymograph. The compounds (10 mg./Kg.) were administered through the cannulated femoral vein; changes in blood pressure were observed.

Spontaneous Motor Activity.—Fifteen albino rats (Haffkine) were employed for each experiment. They were divided into three groups of five animals each and kept in individual cages. The first group of animals was treated with the compound (50 mg./Kg.), the second group received 3 mg./Kg. of chlorpromazine, and the third group was treated with the solvent which served as control. The animals were observed for 4 hours; changes in spontaneous activity and effect of tactile stimuli on their activity were compared with the control animals and to chlorpromazine-treated animals. All injections were made intraperitoneally.

Pentobarbital Hypnosis.—A set of 30 albino mice (Haffkine) was employed for each experiment. They were divided into three groups of 10 animals each. Group I was treated with pentobarbital sodium (40 mg./Kg.). Groups II and III were pretreated with the solvent and the compound (20 mg./Kg.), respectively. Pentobarbital was injected 15 minutes after the pretreatment. The injections were made intraperitoneally. The sleeping time of each individual mouse was the time taken by the animal to regain the righting reflex from the time it was lost after the administration of the anesthetic agent. The average sleeping time of the control group of animals was compared with the sleeping time of the group which received the compound in addition to pentobarbital sodium.

Conditioned Avoidance Response.—This experiment was similar to that employed by Dandiya and Sharma (3). Albino rats were trained to jump on the wall of a special cage when a bell rang to avoid an electric shock which followed. Two types of responses were observed—the conditioned avoidance response (CAR), in which the animal jumped after hearing the bell, and the escape response, in which the animal jumped only after the electric shock. Ten trained rats in which CAR was developed were employed for each experiment. The compound was administered intraperitoneally in a dose of 50 mg./Kg., and the effect on the CAR was observed at 1-hour intervals for 4 hours.

RESULTS

The results of pharmacological screening are given in Table I.

Maximum potentiation of pentobarbital hypnosis was shown by compound III, in which the sleeping time was increased by 80%, which was statistically significant. Although compounds I and II raised sleeping time, due to pentobarbital, the effect was not significant. The other two compounds (IV and V) were comparatively ineffective in influencing the sleeping time of mice.

All of the compounds exerted a moderate influence on the conditioned avoidance response of trained rats. In this experiment maximum effect was shown by compound III. Thirty per cent of the animals lost CAR, the escape response being unaffected. Other compounds blocked CAR in 20% of the animals. In addition to the loss of CAR in 20% of the animals, compound I also blocked the escape response of these animals.

There was no marked change in the spontaneous activity of rats treated with these compounds. Moderate depression, indicated by decreased alertness to tactile and auditory stimulus, was more marked in animals treated with compound I. Compound V had a slight stimulant action in rats.

None of these compounds showed marked effect on blood pressure of anesthetized dogs. A transient hypotensive effect was observed in dogs in which either compound III or V was administered.

DISCUSSION

Compared to many other trimethoxybenzene derivatives, the azlactones exerted only minimal effect on the cardiovascular system. Compounds like 2,4,5-trimethoxy-1-propenylbenzene (12), *N*-acetyl 3,4,5-trimethoxybenzamide (7), and 3,4,5-

trimethoxy amphetamine (8) have been reported to exert moderate hypotensive action in anesthetized animals. Trimeglamide, which can be considered as an analog of azlactone, also exerts only a moderate and transient fall in blood pressure (11). The relative absence of cardiovascular action may be advantageous because these compounds may be more specific in their central effect.

The effect of these compounds on spontaneous motor activity, pentobarbital hypnosis, and conditioned avoidance response shows that all of the five compounds exerted a mild central nervous system depressant action. Compound III was more potent and, in addition to its effect on the three above-mentioned experiments, it also exerted a mild and transient hypotension. The effect on conditioned response is noteworthy because of the specific blockage of CAR without affecting escape response. Only compound I acted in a nonspecific way in blocking both CAR and escape response.

A study of the structure activity relationship has shown that substitution of R_1 and R_2 by trimethoxyphenyl group does not bring about marked change in pharmacological activity. Substitution of the methyl group at position R_2 and the 2,4,5-trimethoxyphenyl group at position R_1 prolongs the pentobarbital sleeping time. A study of compounds I and III has shown that trimethoxy groups at 2,3,4-position in R_1 substitution exert a hypotensive effect as opposed when trimethoxy groups are attached to 2,4,5-position. Substitution of 3,4,5-trimethoxyphenyl group at R_2 position does not bring about change in the biological activity in the molecule. Substitution of *p*-dimethylaminophenyl group at position R_1 with 3,4,5-trimethoxyphenyl group at position R_2 in the compound exerts a hypotensive effect with slight increase in spontaneous activity. These properties are not present when the substituent is the phenyl group at the R position.

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Incorporation of Proline-C^{14} into the Principal Alkaloids of *Datura stramonium* var. *tatula* and *Datura innoxia*

By GERALD SULLIVAN and MELVIN R. GIBSON

Proline-C^{14} made available to root cultures of *Datura stramonium* L. variety *tatula* Torrey and *Datura innoxia* Miller was incorporated into hyoscyamine and scopolamine. Root growth was affected in both species.

EXTENSIVE RESEARCH has been accomplished on the genus *Datura* in an attempt to elucidate the biosynthetic pathway or pathways to alkaloid production. Various amino acids have been used to gain clearer and more precise information concerning the production of alkaloids. James (1) reported significant increases in alkaloid content when *Atropa belladonna* L. leaves were supplied *l*-arginine and *l*-ornithine. Later, van Haga (2), working with sterile root cultures of *A. belladonna*, confirmed that additional amounts of arginine and ornithine yielded increased alkaloid production. However, these and other investigators (2–4) found proline to be ineffective in increasing the plant production of hyoscyamine. Work in this laboratory (5) on *D. stramonium* variety *tatula* in which glutamic acid was added to isolated root cultures caused an increase in growth of the roots and a small but statistically significant decrease in alkaloid content of the roots. In this instance it is possible that the glutamic acid entered the citric acid cycle, contributing to growth, but not the ornithine cycle, leaving unaffected tropane alkaloid production. Other work in this laboratory (6) on the same species showed that tested quantities of *l*-proline inhibited total alkaloid production in isolated root culture, but in one concentration appeared to change relative concentrations of hyoscyamine and scopolamine in favor of scopolamine. This latter work suggested that proline plays a dynamic role in the synthesis of

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