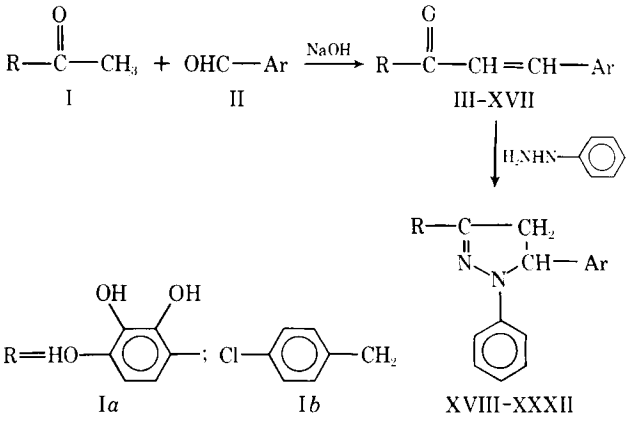
Anticonvulsant Activity and Monoamine Oxidase Inhibitory Properties of 1,3,5-Trisubstituted Pyrazolines

SURENDRA S. PARMAR*x, B. R. PANDEY*, C. DWIVEDI‡, and RAYMOND D. HARBISON‡

Abstract □ Several substituted pyrazolines were synthesized and characterized, and all of these pyrazolines inhibited rat brain monoamine oxidase. Evaluation of these compounds revealed appreciable anticonvulsant activity, reflected by their ability to afford protection against pentylenetetrazol-induced seizures in mice. The anticonvulsant activity possessed by these pyrazolines was unrelated to their monoamine oxidase inhibitory effectiveness.

Keyphrases □ Pyrazolines, 1,3,5-trisubstituted—synthesis, relationship between anticonvulsant and monoamine oxidase inhibitory properties □ Monoamine oxidase inhibitors—substituted pyrazolines, synthesis, anticonvulsant activity □ Anticonvulsant activity—substituted pyrazolines, relationship to monoamine oxidase inhibitory properties □ Structure-activity relationships—monoamine oxidase inhibitory property-anticonvulsant activity

The ability of pyrazole derivatives to exhibit tranquilizing, muscle relaxant, psychoanaleptic (1), hypnotic (2), and anticonvulsant (3, 4) activities prompted the synthesis of some 1,3,5-trisubstituted pyrazolines. The anticonvulsant activity of these pyrazolines was investigated against pentylenetetrazol-induced seizure in albino mice. The monoamine oxidase [EC 1.4.3.4-monoamine: O_2 oxidoreductase (deaminating)] inhibitory property possessed by these pyrazolines was also investigated to study the biochemical mechanism of action for the anticonvulsant activity of pyrazolines. The various pyrazolines were synthesized following the methods outlined in Scheme I.



Scheme I

EXPERIMENTAL

Chemistry—Appropriate ketones (I) were condensed with arylaldehydes (II) to yield chalcones (III-XVII). These chalcones (III-XVII), on treatment with phenylhydrazine hydrochloride in acetic acid, were cyclized to the corresponding pyrazolines (XVIII-XXXII).

Analyses for carbon, hydrogen, and nitrogen were performed[1]. Melting points were taken in open capillary tubes and are uncorrected. These results are presented in Tables I and II.

Gallacetophenone (Ia)—This compound was prepared by the Friedel-Crafts reaction with acetic anhydride on pyrogallol using anhydrous zinc chloride as the catalyst, mp 171° (5).

Chalcones III-VIII—A mixture of gallacetophenone (0.043 mole), an appropriate aromatic aldehyde (0.041 mole), sodium hydroxide (0.21 mole), ethanol (13 ml), and water (50 ml) was stirred vigorously until a thick paste formed and stirring was no longer effective. The reaction mixture was kept in a refrigerator overnight, and the solid mass was dissolved in water and neutralized with hydrochloric acid. The separated chalcones were collected by filtration, recrystallized from ethanol, and then characterized by their sharp melting points and elemental analyses (Table I).

Chalcones IX-XVII—4-Chlorobenzyl methyl ketone (0.043 mole), a suitable aromatic aldehyde (0.041 mole), sodium hydroxide (0.054 mole), ethanol (13 ml), and water (50 ml) were mixed and stirred vigorously. The reaction mixture, after cooling overnight in a refrigerator, was neutralized with hydrochloric acid. The resulting yellow oil, which could not be crystallized, was used for subsequent reactions without further purification.

1,3,5-Trisubstituted Pyrazolines (XVIII-XXXII)—A mixture of chalcone (III-XVII) (0.01 mole) and phenylhydrazine hydrochloride (0.02 mole) was refluxed in acetic acid (20 ml) for 4–5 hr. The reaction mixture was cooled and poured in ice water. The solid mass which separated was collected by filtration, washed with water, and recrystallized from benzene. These trisubstituted chalcones were characterized by their sharp melting points and elemental analyses (Table II).

Determination of Monoamine Oxidase Activity—A spectrophotofluorometric method was used for the determination of monoamine oxidase activity of rat brain homogenate, using kynuramine as the substrate (6). The 4-hydroxyquinoline formed during oxidative deamination of kynuramine was measured fluorometrically[2] using activating light of 315 nm and measuring fluorescence at the maximum of 380 nm.

Male adult rats, weighing approximately 150–200 g, were killed by decapitation. Brains were quickly removed and homogenized[3] in ice-cold 0.25 *M* sucrose. The reaction mixture, in a final concentration, consisted of 0.5 ml of phosphate buffer (0.2 *M*, pH 7.5), 1×10^{-4} *M* kynuramine, and 0.5 ml of brain homogenate (equivalent to 10 mg of wet weight of the tissue). The monoamine oxidase activity of the brain homogenate was determined by incubation at 37° in air for 30 min. The various substituted pyrazolines were added to the brain homogenate to produce a final concentration of 5×10^{-5} *M* and incubated for 10 min before adding kynuramine. The mixture was then incubated for an additional 30 min. The reaction was stopped by the addition of 1 ml of 10% trichloroacetic acid (w/v), and the precipitated proteins were removed by centrifugation. Suitable 1-ml aliquots of the supernate were taken in 2 ml of 1 *N* NaOH solution and were assayed for 4-hydroxyquinoline. An increase in absorbance provided a direct measurement of the 4-hydroxyquinoline formation, which was taken as an index of enzyme activity. The percent inhibition was calculated from the decrease observed in the absorbance, and this

[1] Central Drug Research Institute, Lucknow, India.

[2] Aminco Bowman spectrophotofluorometer.

[3] Potter-Elvehiem homogenizer.

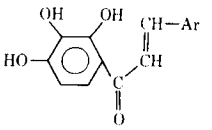
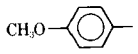
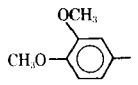
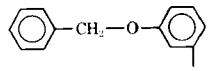
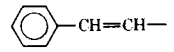


Table I—Physical Constants of 2,3,4-Trihydroxychalcones

Compound	Ar	Melting Point[a]	Yield, %	Formula	Analysis, % Calc.	Analysis, % Found
III[b]		112°	64	—	—	—
IV		225°	62	$C_{15}H_{11}ClO_4$	C 61.85 H 3.72	61.72 3.49
V		140°	58	$C_{16}H_{14}O_5$	C 67.13 H 4.89	67.38 4.72
VI		105°	59	$C_{17}H_{16}O_6$	C 64.55 H 5.06	64.31 4.99
VII		120°	67	$C_{22}H_{18}O_5$	C 72.92 H 4.97	73.10 5.23
VIII		125°	63	$C_{23}H_{18}O_4$	C 77.09 H 5.02	77.18 5.18

[a] All melting points were taken in open capillary tubes and are uncorrected. [b] *Curr. Sci.*, **34**, 18(1965).

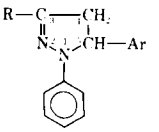
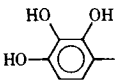
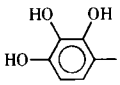
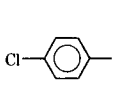
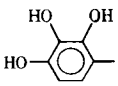
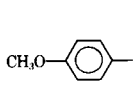
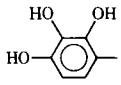
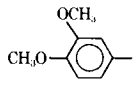
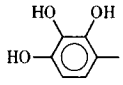
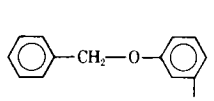
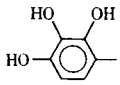
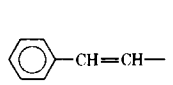
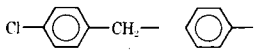
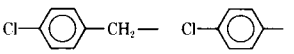
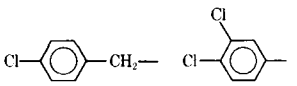
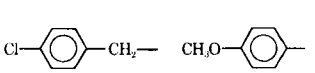
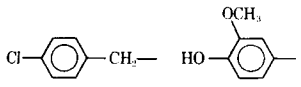
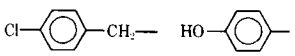
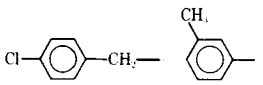
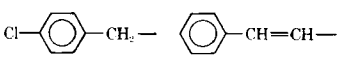
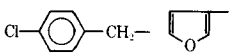


Table II—Physical Constants of 1,3,5-Trisubstituted Pyrazolines

Compound	R	Ar	Melting Point[a]	Yield, %	Formula	Analysis, % Calc.	Analysis, % Found
XVIII			150°	61	$C_{21}H_{18}N_2O_3$	C 72.83 H 5.20 N 8.09	72.62 5.00 8.31
XIX			215°	59	$C_{21}H_{17}ClN_2O_3$	C 66.14 H 4.46 N 7.34	66.44 4.58 7.28
XX			108°	62	$C_{22}H_{20}N_2O_4$	C 70.21 H 5.31 N 7.44	70.10 5.52 7.31
XXI			142°	65	$C_{23}H_{22}N_2O_5$	C 67.98 H 5.42 N 6.89	68.28 5.22 6.58
XXII			137°	62	$C_{28}H_{24}N_2O_4$	C 74.33 H 5.31 N 6.19	74.01 5.58 6.01
XXIII			130°	59	$C_{23}H_{20}N_2O_3$	C 74.19 H 5.37 N 7.52	74.31 5.30 7.22
XXIV			128°	60	$C_{22}H_{19}ClN_2$	C 76.08 H 5.46 N 8.06	76.31 5.63 8.35
XXV			115°	68	$C_{22}H_{18}Cl_2N_2$	C 69.29 H 4.72 N 7.34	69.00 4.51 7.64
XXVI			133°	64	$C_{22}H_{17}Cl_3N_2$	C 63.53 H 4.09 N 6.73	63.33 4.39 6.60
XXVII			122°	65	$C_{23}H_{21}ClN_2O$	C 73.30 H 5.57 N 7.43	73.01 5.50 7.62

(continued)

Table II—(*Continued*)

Compound	R	Ar	Melting Point[a]	Yield, %	Formula	Analysis, % Calc.	Analysis, % Found
XXVIII			110°	69	$C_{23}H_{21}ClN_2O_2$	C 70.31 H 5.35 N 7.13	70.55 5.40 7.38
XXIX			165°	68	$C_{22}H_{19}ClN_2O$	C 72.82 H 5.24 N 7.72	73.00 5.13 7.46
XXX			126°	64	$C_{23}H_{21}ClN_2$	C 76.56 H 5.82 N 7.76	76.31 5.90 7.99
XXXI			135°	63	$C_{24}H_{21}ClN_2$	C 77.31 H 5.63 N 7.54	77.51 5.70 7.44
XXXII			103°	67	$C_{20}H_{17}ClN_2O$	C 71.32 H 5.05 N 8.32	71.62 5.09 8.21

[a] All melting points were taken in open capillary tubes and are uncorrected.

provided an index of the inhibitory property of these substituted pyrazolines.

Determination of Anticonvulsant Activity—Anticonvulsant activity was determined (7) in mice of either sex weighing 25–30 g. The mice were divided in groups of 10, keeping the group weights as near the same as possible. Each pyrazoline was suspended in 5% aqueous gum acacia to give a concentration of 0.25% (w/v). The test compound was injected intraperitoneally in a group of 10 animals at a dose of 100 mg/kg. Four hours after the administration of the substituted pyrazolines, the mice were injected with pentylenetetrazol (90 mg/kg sc). This dose of pentylenetetrazol has been shown not only to produce convulsions in almost all untreated mice but also to exhibit 100% mortality during 24 hr. On the other hand, no mortality was observed during 24 hr in animals treated with 100 mg/kg alone of the test compounds. The mice were then observed for 60 min for seizures. An episode of clonic spasm that persisted for a minimum of 5 sec was considered a threshold convulsion. Transient intermittent jerks or tremulousness was not counted. Animals devoid of threshold convulsions during 60 min were considered protected. The number of animals protected in each group was recorded, and the anticonvulsant activity of these substituted pyrazolines was represented as percent protection. The animals were then observed for 24 hr and their mortality was recorded.

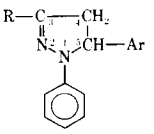
RESULTS AND DISCUSSION

The ability of these substituted pyrazolines to inhibit rat brain monoamine oxidase, using kynuramine as the substrate, and their anticonvulsant activity are recorded in Table III. All 3-(2,3,4-trihydroxyphenyl)-substituted pyrazolines produced inhibition of rat brain monoamine oxidase at a final concentration of 5×10^{-4} *M*, while the presence of a 3,4-dimethoxyphenyl substituent at position 5 of the pyrazoline ring (XXI) produced maximum inhibition of monoamine oxidase of 74.28%. However, the presence of a 4-chlorophenyl substituent at position 5 of the pyrazoline ring (XIX) produced minimum inhibition of 17.14% of monoamine oxidase. The presence of a styryl group at position 5 (XXIII) also produced a greater degree of monoamine oxidase inhibition as compared to a pyrazoline possessing an unsubstituted phenyl ring (XVIII).

These results indicated that the presence of an electron-donating substituent on the phenyl group present at position 5 of the pyrazoline ring (XX–XXII) produced a relatively higher degree of enzyme inhibition while an electron-withdrawing atom (XIX) produced a lesser degree of monoamine oxidase inhibition. Among the 3-(4-chlorobenzyl)-substituted pyrazolines, the compound possessing a 3-methoxy-4-hydroxyphenyl substituent at position 5 (XXVIII) produced the maximum inhibition of 90.00% of monoamine oxidase. The pyrazolines possessing an electron-donating substituted phenyl group at position 5 (XXVII–XXX) showed an increased degree of enzyme inhibition, while an electron-withdrawing substituted phenyl group at position 5 (XXV and XXVI) decreased the degree of enzyme inhibition in a similar manner as was observed with 3-(2,3,4-trihydroxy)phenyl-substituted pyrazolines. The presence of two electron-withdrawing atoms at the 5-phenyl group (XXVI) decreased further their ability to inhibit monoamine oxidase.

Results of anticonvulsant activity studies (Table III) indicate significant protection of 50–90% by 3-(2,3,4-trihydroxyphenyl)-substituted pyrazolines against pentylenetetrazol-induced seizures at a dose of 100 mg/kg. The presence of a 4-chlorophenyl substituent at position 5 of the pyrazoline nucleus (XIX) produced maximum anticonvulsant activity. The protection afforded by 3-(4-chlorobenzyl)-5-substituted pyrazolines ranged from 30 to 70%, and 3-(4-chlorobenzyl)-5-phenylpyrazoline (XXIV) was the

Table III—Monoamine Oxidase Inhibition and Anticonvulsant Properties of 1,3,5-Trisubstituted Pyrazolines



Compound	Monoamine Oxidase Inhibition[a], %	Anticonvulsant Activity[b], Protection, %	Pentylenetetrazol Mortality, %
XVIII	32.86 ± 0.56	50	50
XIX	17.14 ± 0.44	90	Nil
XX	32.86 ± 0.52	80	20
XXI	74.28 ± 0.98	60	30
XXII	47.71 ± 0.86	80	20
XXIII	44.28 ± 0.75	80	10
XXIV	46.66 ± 0.78	70	30
XXV	22.22 ± 0.42	60	30
XXVI	18.59 ± 0.38	50	40
XXVII	41.11 ± 0.46	60	40
XXVIII	90.00 ± 1.25	40	50
XXIX	48.57 ± 0.98	30	70
XXX	32.22 ± 0.62	40	60
XXXI	50.00 ± 0.46	40	50
XXXII	56.66 ± 0.98	50	50

[a] Assay procedure and contents of the reaction mixture are as indicated in the text. Inhibitors were used at a final concentration of 5×10^{-4} *M*. Each experiment was done in duplicate, and figures indicate mean values of three separate experiments with ± standard error of the mean. [b] Screening procedure was as indicated in the text. Each compound was used at the dose of 100 mg/kg ip, and pentylenetetrazol mortality was observed during 24 hr. In the present study, administration of an equivalent amount of 5% gum acacia solution was found to possess no anticonvulsant activity.

most active compound. As is evident from Table III, all pyrazolines exhibiting a greater degree of protection elicited a lower pentylenetetrazol-induced mortality during 24 hr in the experimental animals.

These studies, exhibiting pronounced anticonvulsant activity of substituted pyrazolines, were unable to provide a correlation between their anticonvulsant activity and their ability to inhibit monoamine oxidase activity as a biochemical basis of their anticonvulsant activity. Further studies dealing with the synthesis of other related structures carrying different substituents and the determination of their ability to inhibit purified enzyme preparations may possibly reflect the biochemical basis of the anticonvulsant activity of substituted pyrazolines.

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Novel Synthesis of Bifunctional Acid-Esters

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Abstract □ A novel method was developed for the synthesis of acid-esters *via* monodemethylation of a dimethyl ester by 1,1-dimethylhydrazine and subsequent conversion of the resulting trimethylhydrazonium ester to the desired acid-ester. The method is facile and seems to have a wide range of applicability.

Keyphrases □ Acid-esters, bifunctional—novel synthesis using 1,1-dimethylhydrazine and a dimethyl ester and conversion of the resulting trimethylhydrazonium ester □ Trimethylhydrazonium ester—intermediate in synthesis of bifunctional acid-esters □ 1,1-Dimethylhydrazine—utilized in novel synthesis of bifunctional acid-esters

Quite often the synthesis of compounds containing both an acid and an ester moiety (acid-ester) requires tedious experimental design and manipulation. This is particularly true with cyclic systems from which no anhydride (a widely used precursor for the acid-ester) can be synthesized, due either to nonproximity of the functional groups or to development of severe strain if the anhydride is formed.

This paper describes a novel and efficient solution to the problem of preparing bifunctional compounds containing both a carboxy and a carbomethoxy functional group.

DISCUSSION

The developed method utilizes 1,1-dimethylhydrazine to effect the monodemethylation of an aliphatic or aromatic dimethyl ester and subsequent conversion of the resulting trimethylhydrazonium ester to the desired acid-ester (Scheme I). Either the addition of dilute hydrochloric acid to the trimethylhydrazonium salt followed by ether extraction or the use of silica gel column chromatography provided the free acid-ester. The latter method, although laborious, is an attractive feature, particularly for compounds containing acid-sensitive moieties or whose acid-esters exist in zwitterion forms and are, therefore, highly water soluble and ether insoluble.

That this method of synthesis of acid-esters is facile and has broad application in both aliphatic and aromatic systems is shown by the examples in Table I. A possible exception seems to be five-membered heterocyclic rings with vicinal methyl ester substituents. Under the generally applied reaction conditions, no bifunctional acid-ester compounds were afforded from the dimethyl esters of either 4,5-imidazoledicarboxylic acid or 3,4-pyrazoledicarboxylic acid[1]. However, more five-membered rings with vicinal diesters must be investigated before generalizations can be made concerning their reactions with 1,1-dimethylhydrazine.

The reaction of dimethyl 3,5-pyridinedicarboxylate with 1,1-dimethylhydrazine (Scheme I) exemplifies the general procedure. The structural formulas of all synthesized compounds as well as their melting points, spectral data, and elemental analyses are shown in Table I.

The molecular structures of the reaction intermediates, the bifunctional trimethylhydrazonium esters, and the final products, the acid-esters, were elucidated by using IR, NMR, and mass spectrometry. Elemental analyses were carried out for only a few crystalline hydrazonium salts[2], in an effort to establish an unequivocal correlation between molecular structures and spectral

[1] The reaction product is somewhat complex and is currently being investigated.

[2] Some hydrazonium salts were observed to be semisolid and extremely hygroscopic, and no elemental analyses were attempted for them. Spectral data of the given trimethylhydrazonium and its corresponding acid-ester were used for determining molecular structure.