Monoamine Oxidase Inhibitory and Anticonvulsant Properties of 1,2,4-Trisubstituted 5-Imidazolones

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Abstract □ Several 1,2,4-trisubstituted 5-imidazolones were synthesized and characterized by their sharp melting points and elemental analyses. All substituted imidazolones inhibited activity of monoamine oxidase in rat brain homogenate. Preincubation of the brain homogenates with these substituted imidazolones, prior to the addition of the substrate (kynuramine), in no way altered the degree of enzyme inhibition. On further evaluation, one of these compounds indicated the competitive nature of inhibition of monoamine oxidase. All substituted imidazolones possessed anticonvulsant activity ranging from 20 to 70% at a dose of 100 mg/kg; however, this activity was unrelated to their monoamine oxidase inhibitory effectiveness.

Keyphrases □ 5-Imidazolones, 1,2,4-trisubstituted—synthesis, anticonvulsant activity and relationship to inhibition of monoamine oxidase □ Structure–activity relationships—5-imidazolones, anticonvulsant activity, rats, inhibition of brain monoamine oxidase □ Anticonvulsant activity—1,2,4-trisubstituted 5-imidazolones □ Monoamine oxidase inhibition—1,2,4-trisubstituted 5-imidazolones

Hydrazine derivatives (1), cinnamides (2), and styrylquinoliniums (3) have been shown to inhibit monoamine oxidase [EC 1.4.3.4 monoamine: O_2 oxidoreductase (deaminating)] *in vitro*. The high degree of inhibition possessed by styrylquinoliniums was proposed to be related to the presence of the styryl group and, more specifically, to the ethylenic moiety (3, 4). Inhibitors of the enzyme monoamine oxidase have also been reported to possess antidepressant (5) and anticonvulsant (6, 7) properties. Certain derivatives of imidazole have also been reported to possess anticonvulsant activity (8, 9).

The ability of imidazolecarboxyhydrazides to manifest monoamine oxidase inhibitory activity during their evaluation as possible psychopharmacological agents (10–12) led to the synthesis of 1,2,4-trisubstituted 5-imidazolones, having a substituted benzylidene moiety at position 4 of the imidazolone nucleus, as monoamine oxidase inhibitors. In the present study, attempts were made to correlate the *in vitro* monoamine oxidase inhibitory effectiveness of these imidazolones with their behavioral effects and anticonvulsant activity as a function of their chemical structure.

CHEMISTRY

The various 1,2,4-trisubstituted 5-imidazolones were synthesized by the route outlined in Scheme I. Aceturic acid (I*a*), synthesized by the acetylation of glycine, was treated with acetic anhydride in the presence of anhydrous sodium acetate and then with suitable aromatic aldehydes to obtain the corresponding substituted oxazolones (I*e*). The reaction proceeds by an initial cyclization of the acylaminoacetic acid (I*b*), which tautomerizes into an intermediate (I*c*) and is followed by the Perkin-type condensation of the aldehyde with the active methylene unit of I*d*. The oxazolones (I*e*) were heated in equimolar ratio with the appropriate ethyl *p*-aminobenzoate in an oil bath at 140°; this procedure resulted in the formation of the substituted benzylidene-5-imidazolones (Compounds I–IX), which were converted into their corresponding hydrazides (Compounds X–XVIII) by refluxing with hydrazine hydrate (99–100%) in absolute ethanol.

EXPERIMENTAL[1]

Aceturic Acid (I*a*)—Compound I*a* was synthesized by the acetylation of glycine according to a method reported earlier (13).

2-Methyl-4-substituted Benzylidene-5-oxazolones (I*e*)—A mixture of the appropriate aromatic aldehyde (0.38 mole), powdered aceturic acid (0.24 mole), freshly fused and powdered sodium acetate (0.27 mole), and acetic anhydride (0.65 mole) was heated on an electric hot plate with occasional shaking until all solids went into solution. The resulting solution was warmed on the steam bath for 1 hr, cooled, and allowed to stand overnight in a refrigerator. The solid mass which separated was collected, washed with 60 ml of cold water, dried, and used without further purification for subsequent reaction (13).

1-Carboethoxy-2-methyl-4-substituted Benzylidene-5-imidazolones (I–IX)—The appropriate oxazolone was heated with an equimolar quantity of the suitable ethyl *p*-aminobenzoate in an oil bath at 140° for 10–20 min. The jelly-like mass which separated (14) was recrystallized from suitable solvents. The various substituted imidazolones were characterized by their sharp melting points and elemental analyses (Table I).

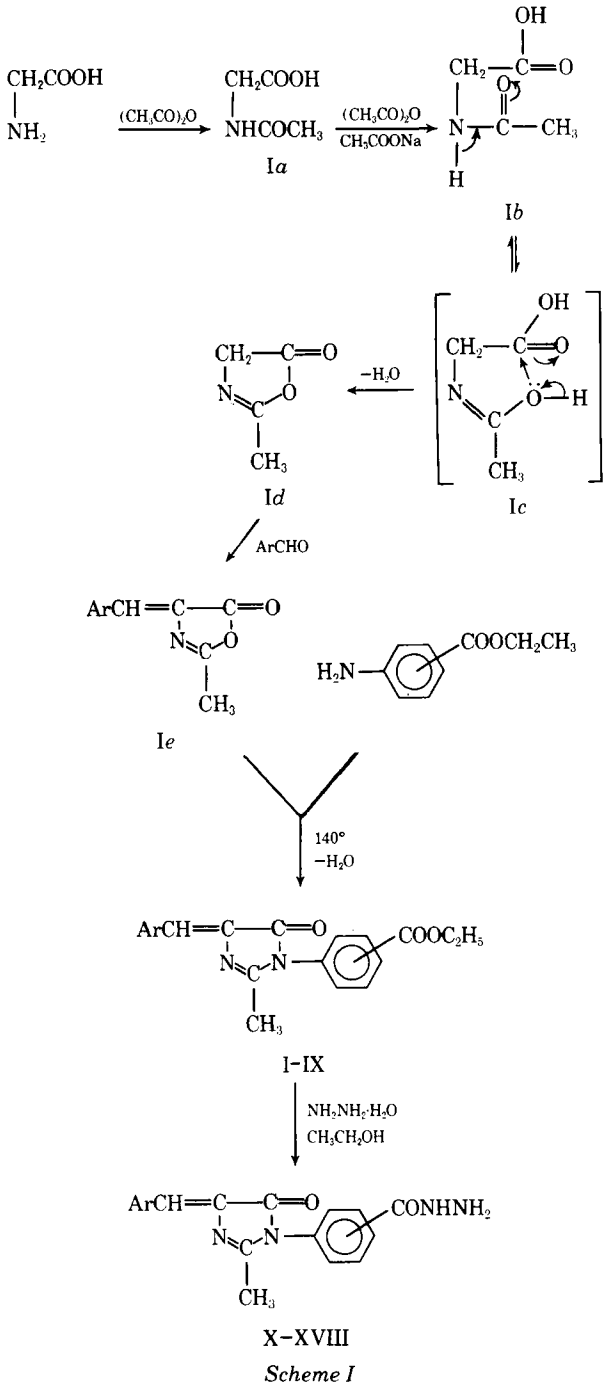
1-Carbohydrazino-2-methyl-4-substituted Benzylidene-5-imidazolones (X–XVIII)—A mixture of 0.1 mole of the appropriate 1-carboethoxy-2-methyl-4-substituted benzylidene-5-imidazolone (I–IX) and 0.2 mole of hydrazine hydrate (99–100%) in absolute ethanol was refluxed on a steam bath for 8–10 hr. Excess ethanol was removed by distillation under reduced pressure, and the hydrazides which separated were collected by filtration, dried, and recrystallized from ethanol. The various 1-carbohydrazino-2-methyl-4-substituted benzylidene-5-imidazolones (Table I) were characterized by their sharp melting points and elemental analyses.

Determination of Monoamine Oxidase Activity—Monoamine oxidase activity of rat brain homogenate was determined by a spectrophotofluorometric method, using kynuramine as the substrate (15). Male albino rats, 100–150 g, were allowed food and water *ad libitum* and were sacrificed by decapitation. The brains were removed immediately and homogenized[2] in the ratio of 1:9 (w/v) in ice-cold 0.25 *M* sucrose. The monoamine oxidase activity of rat brain homogenate was determined by incubation at 37° in air for 30 min. The reaction mixture in a total volume of 3 ml consisted of 0.5 ml of phosphate buffer (0.2 *M*, pH 7.5), 0.1 m*M* kynuramine, and 0.5 ml of brain homogenate (equivalent to 10 mg wet weight of the tissue).

The various 1-carboethoxy-2-methyl-4-substituted benzylidene-5-imidazolones and 1-carbohydrazino-2-methyl-4-substituted benzylidene-5-imidazolones were dissolved in propylene glycol (100%) and added to the brain homogenate to produce a final concentration of 0.25 m*M*. An equivalent amount of propylene glycol was added to the control tubes and the mixture was incubated for 10 min before the addition of kynuramine. The mixture, after addition of kynuramine, was further incubated for 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.

[1] All compounds were analyzed for carbon, hydrogen, and nitrogen. Melting points were taken in open capillary tubes with partial immersion thermometer and are corrected.

[2] Potter–Elvehjem homogenizer.



Scheme I

Suitable 1-ml aliquots of the supernatant solution were taken in 2 ml of 1 *N* NaOH solution and were assayed for 4-hydroxyquinoline, formed during oxidative deamination of kynuramine by rat brain homogenate. The 4-hydroxyquinoline formed was measured fluorometrically[3], using activating light of 315 nm and measuring fluorescence at the maximum of 380 nm. An increase in absorbance provided a direct measurement of 4-hydroxyquinoline formation, which was taken as an index of the monoamine oxidase activity. The percentage inhibition was calculated from the decrease observed in absorbance, and this provided an index of the inhibitory property of these substituted imidazolones.

In preincubation studies, rat brain homogenate, in the incubation mixture, was incubated with or without substituted imidazolones at 37° for 10, 20, and 30 min prior to the addition of kynuramine. The zero-time experiments represent those in which substituted imidazolones and kynuramine were added simultaneously to the reaction mixture containing appropriate brain homogenate preparation. The I_{50} values (concentrations producing 50% inhibition) were determined graphically from the values obtained for the inhibition of rat brain monoamine oxidase by the use of different concentrations of these imidazolones. In the present study, the nature of the enzyme inhibition caused by XVII was evaluated by the graphic method of Lineweaver and Burk (16) as modified by Dixon (17).

[3] Aminco-Bowman.

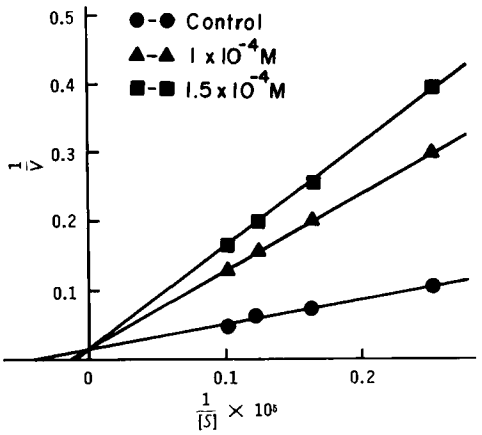


Figure 1—*Competitive inhibition of rat brain monoamine oxidase by 1-*m*-carbohydrazino-2-methyl-4-*p*-chlorobenzylidene-5-imidazolone (Compound XVII). Assay procedure and the contents of the reaction mixture are described in the text.* [S] *denotes molar concentration of kynuramine, and 1/V represents reciprocal of the change in percent absorbance/10 mg fresh tissue wet weight/30 min. Key:* ●, *control;* ▲, *0.1 m*M *imidazolone; and* ■, *0.15 m*M *imidazolone. The* K_m *value for kynuramine was found to be 0.22 m*M.

Determination of Anticonvulsant Activity—Anticonvulsant activity was determined against pentylenetetrazol-induced seizures in mice, 25–30 g, of either sex. The mice were divided into groups of 10, keeping the group weights as near the same as possible. All compounds were suspended in 5% aqueous gum acacia, devoid of anticonvulsant activity, to give a concentration of 0.25% (w/v). An arbitrary dose of 100 mg/kg ip of imidazolones was administered to 10 mice. The mice were then injected with pentylenetetrazol (90 mg/kg sc) 4 hr (2) after the administration of the test compounds. This dose of pentylenetetrazol has been shown to produce convulsions in almost all untreated mice and to exhibit 100% mortality during 24 hr. No mortality was observed during 24 hr in animals treated with 100 mg/kg of the imidazolones alone.

The mice were observed for 60 min for the occurrence of seizures. An episode of clonic spasm persisting for a minimum of 5 sec was considered a threshold convulsion. Transient intermittent jerks and tremulousness were not counted. Mice devoid of threshold convulsions during 60 min were considered protected. The number of mice protected in each group was recorded, and the anticonvulsant activity of these imidazolones was represented as percent protection. The animals were then observed for 24 hr, and the mortality of mice in each group was recorded.

Determination of Behavioral Effects—The effects of these imidazolones were investigated in albino mice, 25–30 g, by administration of a dose of 100 mg/kg ip.

RESULTS AND DISCUSSION

All imidazolones except III inhibited rat brain monoamine oxidase when used at a final concentration of 0.25 m*M* (Table II), where most of the 1-carbohydrazino-2-methyl-4-substituted benzylidene-5-imidazolones exhibited greater inhibition as compared to their corresponding precursors, 1-carboethoxy-2-methyl-4-substituted benzylidene-5-imidazolones. Such an increase in the inhibitory effectiveness of hydrazide derivatives was also reflected by their I_{50} values. In both of these series of imidazolones, with the exception of XI, the imidazolones possessing the *m*-$COOC_2H_5$ or *m*-$CONHNH_2$ substituent at position R′ of their structure produced higher inhibition as compared to their corresponding *ortho*-

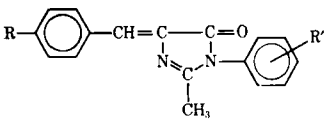


Table I—Physical Constants of 1-Carboethoxy/Carbohydrazino-2-methyl-4-substituted Benzylidene-5-imidazolones

Compound	R	R′	Recrystallization Solvent	Melting Point[a]	Yield, %	Molecular Formula	Analysis, %	
							Calc.	Found
I	H	*o*-$COOC_2H_5$	Benzene–petroleum ether (60–80°)	185°	42	$C_{20}H_{18}N_2O_3$	C 71.85 H 5.38 N 8.38	72.11 5.77 8.54
II	H	*m*-$COOC_2H_5$	Benzene–petroleum ether (60–80°)	113–115°	43	$C_{20}H_{18}N_2O_3$	C 71.85 H 5.38 N 8.38	71.71 5.45 8.58
III	H	*p*-$COOC_2H_5$	Ethanol	197–199°	47	$C_{20}H_{18}N_2O_3$	C 71.85 H 5.38 N 8.38	71.53 5.21 8.34
IV	OCH_3	*o*-$COOC_2H_5$	Acetone–ethanol	215–216°	40	$C_{21}H_{20}N_2O_4$	C 69.23 H 5.49 N 7.69	69.37 5.67 7.54
V	OCH_3	*m*-$COOC_2H_5$	Acetone–ethanol	211–214°	54	$C_{21}H_{20}N_2O_4$	C 69.23 H 5.49 N 7.69	69.43 5.31 7.79
VI	OCH_3	*p*-$COOC_2H_5$	Acetone–ethanol	220–223°	53	$C_{21}H_{20}N_2O_4$	C 69.23 H 5.49 N 7.69	69.48 5.53 7.34
VII	Cl	*o*-$COOC_2H_5$	Ethanol	203–206°	43	$C_{20}H_{17}ClN_2O_3$	C 65.12 H 4.61 N 7.59	64.97 4.35 7.68
VIII	Cl	*m*-$COOC_2H_5$	Ethanol	210–211°	57	$C_{20}H_{17}ClN_2O_3$	C 65.12 H 4.61 N 7.59	65.00 5.01 7.44
IX	Cl	*p*-$COOC_2H_5$	Ethanol	199–202°	61	$C_{20}H_{17}ClN_2O_3$	C 65.12 H 4.61 N 7.59	65.34 4.52 7.50
X	H	*o*-$CONHNH_2$	Ethanol	154°	40	$C_{18}H_{16}N_4O_2$	C 67.50 H 5.00 N 17.50	67.32 4.98 17.47
XI	H	*m*-$CONHNH_2$	Ethanol	199–202°	46	$C_{18}H_{16}N_4O_2$	C 67.50 H 5.00 N 17.50	67.42 5.37 17.71
XII	H	*p*-$CONHNH_2$	Ethanol	215–218°	56	$C_{18}H_{16}N_4O_2$	C 67.50 H 5.00 N 17.50	66.92 4.75 17.32
XIII	OCH_3	*o*-$CONHNH_2$	Ethanol	89°	38	$C_{19}H_{18}N_4O_3$	C 65.14 H 5.14 N 16.00	65.45 5.43 15.88
XIV	OCH_3	*m*-$CONHNH_2$	Ethanol	68°	50	$C_{19}H_{18}N_4O_3$	C 65.14 H 5.14 N 16.00	65.08 5.35 15.78
XV	OCH_3	*p*-$CONHNH_2$	Ethanol	78–80°	57	$C_{19}H_{18}N_4O_3$	C 65.14 H 5.14 N 16.00	65.54 5.03 15.78
XVI	Cl	*o*-$CONHNH_2$	Ethanol	114°	40	$C_{18}H_{15}ClN_4O_2$	C 60.93 H 4.23 N 15.79	60.72 4.52 15.72
XVII	Cl	*m*-$CONHNH_2$	Ethanol	125–127°	53	$C_{18}H_{15}ClN_4O_2$	C 60.93 H 4.23 N 15.79	61.21 4.37 15.85
XVIII	Cl	*p*-$CONHNH_2$	Ethanol	108–111°	50	$C_{18}H_{15}ClN_4O_2$	C 60.93 H 4.23 N 15.79	61.32 4.33 15.67

[a] Melting points were taken in open capillary tubes with a partial immersion thermometer and are corrected.

and *para*-substituted imidazolones. In general, substitution of a OCH_3 (IV–VI and XIII–XV) or Cl (VII–IX and XVI–XVIII) substituent at position R increased the monoamine oxidase inhibitory property of these imidazolones; with VII, however, the degree of enzyme inhibition decreased by the introduction of Cl substituent at the R position.

The monoamine oxidase inhibitory property of 1-carboethoxy-2-methyl-4-substituted benzylidene-5-imidazolones could presumably account for the presence of the benzylidene group, *i.e.*, the styryl group or, more specifically, the ethylenic moiety, at position 4 of the imidazolone nucleus (3). Increased activity of 1-carbohydrazino-2-methyl-4-substituted benzylidene-5-imidazolones may presumably be due to the additive monoamine oxidase inhibitory effectiveness of both the styryl and the hydrazide groups. Preincubation of these imidazolones for varying times prior to the addition of the substrate in *in vitro* studies in no way altered the degree of monoamine oxidase inhibition. These studies thus indicated a rapidly reversible and competitive nature of the inactivation by imidazolones (Table III). This finding was further supported by kinetic studies with XVII, which revealed the competitive nature of inhibition of rat brain monoamine oxidase (Fig. 1). The intercept at the $1/S$ axis was taken as $-1/K_m$; the value of 0.22 mM was obtained as the Michaelis constant (K_m) in these experiments, using kynuramine as the substrate.

All 1,2,4-trisubstituted 5-imidazolones possessed anticonvulsant activity, and the protection of pentylenetetrazol-induced convulsions ranged from 10 to 70%, with maximum anticonvulsant activity observed with VI (Table II). These results, unlike the monoamine oxidase inhibitory effects, indicated a decrease in anticonvulsant activity of 1-carboethoxy-2-methyl-4-substituted benzyli-

Table II—Monoamine Oxidase Inhibitory, Anticonvulsant Activity, and Behavioral Effects of 1-Carboethoxy/Carbohydrazino-2-methyl-4-substituted Benzylidene-5-imidazolones

Compound	Monoamine Oxidase Inhibition[a]		Anticonvulsant Activity[b], % Protection	Pentylenetetrazol Mortality[b], % Protection after 24 hr	Behavioral Effects[c]
	% Inhibition	I_{50} Values, mM			
I	41.2 ± 0.4	0.32	50	20	A,B,C
II	54.4 ± 0.6	0.22	40	40	C
III	Nil	—	40	70	A,B
IV	60.7 ± 0.1	0.20	20	70	A,C
V	69.2 ± 0.2	0.19	40	50	A,C
VI	65.0 ± 0.2	0.17	70	50	A
VII	20.6 ± 0.5	0.62	20	80	A,C
VIII	64.2 ± 0.6	0.17	40	70	A,B,C
IX	52.5 ± 0.4	0.23	60	40	B,C
X	46.8 ± 0.3	0.29	40	80	C
XI	28.0 ± 0.5	0.47	30	60	Nil
XII	35.5 ± 0.4	0.38	20	80	C
XIII	66.2 ± 0.1	0.16	40	80	A,C,D
XIV	72.5 ± 1.0	0.14	50	80	A,C,D
XV	60.7 ± 0.8	0.20	40	70	A,C,D
XVI	73.0 ± 0.4	0.13	10	80	Nil
XVII	91.7 ± 0.5	0.09	20	70	A,C,E
XVIII	61.7 ± 0.3	0.21	30	70	Nil

[a] Vessel contents and assay procedures are described in the text. Each experiment was done in triplicate, and the values are the mean values of three separate experiments with ±standard error of the mean. Kynuramine and imidazolones were used at a final concentration of 0.1 and 0.25 mM, respectively. The enzyme activity observed in the absence of imidazolones ranged from 20.1 to 24.7% change in transmission/10 mg fresh tissue weight/30 min. [b] Screening procedures for the determination of anticonvulsant activity are described in the text. Mortality in pentylenetetrazol-treated animals was observed during 24 hr. [c] Behavioral effects were observed at a dose of 100 mg/kg where A = general excitation, B = increased motor activity, C = circling movement, D = ataxia, and E = slight depression of spontaneous motor activity.

Table III—Preincubation Studies with 1-Carboethoxy/Carbohydrazino-2-methyl-4-substituted Benzylidene-5-imidazolones to Study Monoamine Oxidase Inhibition

Compound	Preincubation Time, min			
	0	10	20	30
	Monoamine Oxidase Inhibition[a], %			
I	40.3 ± 0.4	40.9 ± 0.4	39.5 ± 0.2	41.8 ± 0.4
II	54.0 ± 0.3	55.1 ± 0.2	54.9 ± 0.1	54.9 ± 0.3
III	Nil	Nil	Nil	Nil
IV	59.8 ± 0.7	59.9 ± 0.6	60.5 ± 0.2	61.0 ± 0.1
V	69.2 ± 0.2	69.4 ± 0.3	70.0 ± 0.7	69.8 ± 0.3
VI	65.4 ± 0.3	65.6 ± 0.4	64.9 ± 0.8	64.7 ± 0.6
VII	20.7 ± 0.4	20.5 ± 0.4	20.9 ± 0.4	21.0 ± 0.1
VIII	63.9 ± 0.2	64.1 ± 0.3	64.3 ± 0.5	64.7 ± 0.5
IX	52.3 ± 0.1	52.6 ± 0.6	53.0 ± 0.3	53.2 ± 0.4
X	46.7 ± 0.4	46.5 ± 0.3	46.2 ± 0.3	46.7 ± 0.2
XI	28.0 ± 0.7	28.8 ± 0.2	27.9 ± 0.4	28.4 ± 0.3
XII	35.1 ± 0.7	36.2 ± 0.4	35.4 ± 0.2	36.2 ± 0.1
XIII	65.9 ± 0.5	66.1 ± 0.3	65.8 ± 0.5	66.7 ± 0.9
XIV	72.7 ± 0.6	71.9 ± 0.4	72.3 ± 0.2	72.9 ± 1.0
XV	59.9 ± 0.4	60.3 ± 0.8	60.8 ± 0.7	60.5 ± 0.9
XVI	72.9 ± 0.1	73.4 ± 0.4	72.7 ± 0.3	72.5 ± 0.4
XVII	90.8 ± 0.8	91.2 ± 0.3	91.8 ± 0.6	92.0 ± 0.2
XVIII	61.2 ± 0.3	61.7 ± 0.2	62.0 ± 0.4	62.3 ± 0.8

[a] Contents of the reaction mixture and the assay procedures are as indicated in Table II. The enzyme preparations were incubated with the imidazolones for varying times before the addition of kynuramine. Zero-time experiments indicate that both kynuramine and the imidazolones were added to the reaction mixture containing enzyme preparation simultaneously.

dene-5-imidazolones by their conversion into their corresponding 1-carbohydrazino-2-methyl-4-substituted benzylidene-5-imidazolones, with the exception of IV and V where a slight increase in anticonvulsant activity was observed (XIII and XIV). Data on anticonvulsant activity of these compounds and their 24-hr pentylenetetrazol-induced mortality did not indicate an association between increased protection from convulsions and decreased mortality in experimental animals.

With many of these imidazolones, the behavioral effects indicated were general excitation, increased motor activity, and the circling movement. But XI, XVI, and XVIII were devoid of any appreciable behavioral effects at the dose tested. Ataxia was observed only with XIII–XV, while slight depression of the spontaneous motor activity was observed with XVII.

The results from this study demonstrate, with only a few exceptions, some correlation between the biological activities and the chemical structures of these 1,2,4-trisubstituted 5-imidazolones. The hydrazides are better inhibitors than the esters for monoamine oxidase, whereas the reverse is true for anticonvulsant activity. In addition, there is a clear indication that the monoamine oxidase inhibitory activity of the *meta*-compounds is greater than that of the corresponding *ortho*- and *para*-compounds and that the enzyme inhibitory activity increases by substitution of OCH_3 or Cl. However, these studies failed to provide any definite correlation between monoamine oxidase inhibitory property of these imidazolones and their anticonvulsant activity. It is hoped that detailed pharmacological and toxicological studies and investigations of the effects of these compounds on other enzyme systems may reflect a biochemical basis for the anticonvulsant activity of these 1,2,4-trisubstituted imidazolones.

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Comparison of Procainamide Analyses in Plasma by Spectrophotofluorometry, Colorimetry, and GLC

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Abstract □ A procedure for analysis of procainamide in plasma based on the reaction of procainamide with fluorescamine was developed. The accuracy and precision of results obtained using this method were compared to those of results obtained using colorimetric and GLC methods. The utility of the three procedures in the routine determination of procainamide in plasma is discussed.

Keyphrases □ Procainamide—comparison of spectrophotofluorometric, colorimetric, and GLC analyses, plasma samples □ Methodology—procainamide in plasma, comparison of spectrophotofluorometric, colorimetric, and GLC analyses

Procainamide appears to be one of the numerous drugs (1) for which the monitoring of drug plasma concentration is often necessary to ensure effective therapy. Large variations in procainamide plasma concentration resulting from a given daily dose have been noted (2, 3), and plasma concentration appears to relate predictably to the patient's response to the drug (4). Thus, rapid and accurate procedures for determination of procainamide in plasma are necessary.

Procainamide (as well as other drugs containing a primary amine on an aromatic ring) has most often been determined by diazotization, coupling with *N*-(1-naphthyl)ethylenediamine, and subsequent colorimetric analysis (5). While this procedure has been used for a long time (6), it requires 1–2 hr for a single assay, sodium nitrite and ammonium sulfamate solutions must be prepared at the time of analysis, and the color produced is unstable (7). The utility of direct spectrophotofluorometry for this determination has been investigated (3). However, a metabolite of procainamide (*N*-acetylprocainamide) interfered in the analysis, and accuracy and precision were not determined. Finally, GLC has been used for the analysis of procainamide in plasma (8) and undoubtedly has utility, although nonlinear calibration curves and relatively unstable column packing material limit its usefulness.

Spectrophotofluorometric procedures have a high daily capacity for sample analysis, are more amenable to application by less sophisticated personnel than are GLC techniques, and generally are more precise. Therefore, the development of such an assay for procainamide as a possible substitute for the colorimetric assay is of interest.

A general procedure for the microanalysis of drugs containing the primary aromatic amino substituent was presented previously (9). This procedure is based on selective reaction of fluorescamine with the primary aromatic amino substituent and subsequent de-