Synthesis and Screening of Cyclic Benzylhydrazine Congeners as Antidepressants

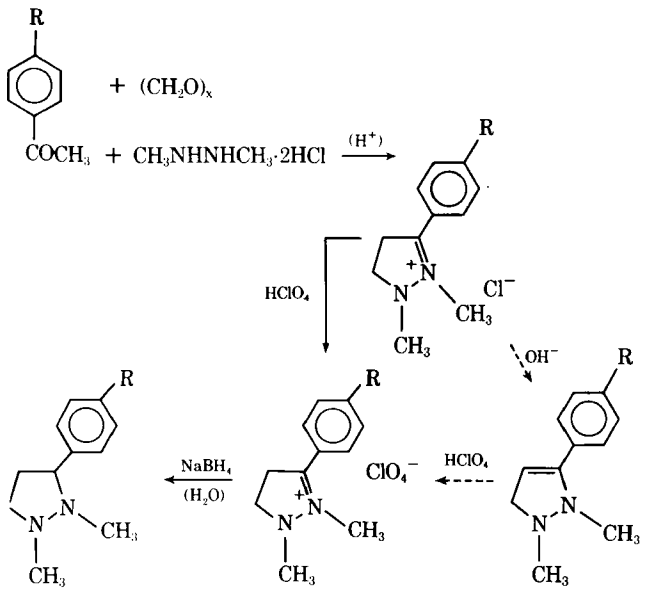
A. M. TAHA*x, N. M. OMAR*, S. M. BAYOMI*, E. M. AMMAR‡, and A. AFIFI‡

Abstract □ Several 2-pyrazolinium salts and their corresponding pyrazolidines, congeners of a cyclic benzylhydrazine structure, were synthesized and their pharmacological activity as antidepressant agents was evaluated. The 2-pyrazolinium salts were obtained through the interaction of 1,2-dimethylhydrazine dihydrochloride, paraformaldehyde, and the appropriately substituted aralkyl ketone *via* a Mannich reaction. The corresponding pyrazolidines were synthesized by reduction of the pyrazolinium perchlorates with sodium borohydride in aqueous medium. The antidepressant activity of both series was evaluated in mice in comparison with nialamide. As indicators of antidepressant action, use was made of the antagonism of reserpine-induced ptosis and the antagonism of reserpine protection against amphetamine lethality in mice. The possible correlations between structure and antidepressant activity are suggested.

Keyphrases □ Benzylhydrazine congeners (cyclic)—synthesized and screened as antidepressants, structure-activity relationships □ 2-Pyrazolinium perchlorates—synthesized and screened as antidepressants, structure-activity relationships □ Antidepressants, potential—synthesis and screening of cyclic benzylhydrazine congeners

The established monoamine oxidase inhibitory activity of iproniazid was demonstrated clinically as an antidepressant effect (1). This led to the synthesis and screening of several hydrazines in search of analogous antidepressant agents useful in treating psychic disturbances associated with overactivity of monoamine oxidase (2, 3).

Of the several hydrazines tested, the aralkyl derivatives were found to possess marked activity (4), a fact that justified the clinical adoption of such monoamine oxidase inhibitors as phenelzine[1] (phenethylhydrazine) and pheniprazine[2] (β-phenylisopropylhydrazine) as well as the unsubstituted benzylhydrazine (5).



Scheme I

Although the 3-aryl pyrazolines may be regarded as the cyclic analogs of benzylhydrazine, a recent survey of the diverse monoamine oxidase inhibitor hydrazines revealed that all derivatives cited belong to the class of open-chain hydrazines (6).

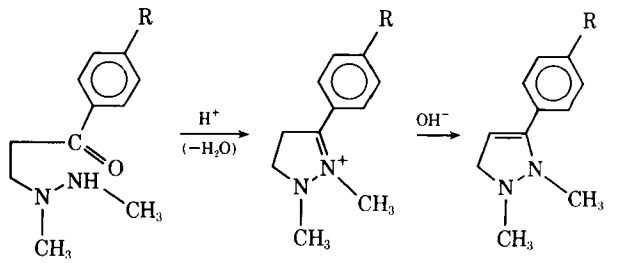
The work presented is a preliminary report on the synthesis and pharmacological evaluation of a series of new 2-pyrazolinium perchlorates and some of their corresponding pyrazolidines incorporating the basic benzylhydrazine skeleton —C_6H_4—C—N—N— in a cyclic form.

CHEMISTRY

The substituted 2-pyrazolinium perchlorates and the corresponding pyrazolidinium perchlorates, synthesized by the route outlined in Scheme I, are recorded in Tables I and II, respectively.

The procedure employed for the synthesis of the new 2-pyrazolinium perchlorates was that of Omar and Eltsov (7), which is a modification of the original method of Hinman *et al.* (8). It involves a one-step Mannich reaction of 1,2-dimethylhydrazine dihydrochloride, paraformaldehyde, and a substituted acetophenone. In support of the previous findings of Omar and Eltsov (7), which were based on spectrophotometric monitoring of the reactions, a 2-hr reflux period and a hydrazine–aldehyde–acetophenone mole ratio of 1:1:1 were again found adequate for completion of the reaction. These findings contradict some recent reports (9) where other authors used a 24-hr reflux time and a 1:2:2 mole ratio of reactants. The latter specifications were originally reported by Hinman *et al.* (8) for a different product, which is disubstituted.

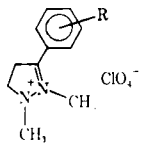
In previous reports (7–9), it was the common practice to isolate the 3-pyrazolines as end-products of the Mannich reaction. From the 3-pyrazoline bases, which are usually oils, the 2-pyrazolinium perchlorates were prepared. In this work the isolation of the base was found unnecessary. Direct workup of the reaction mixture with perchloric acid afforded the desired salts in good yields and relatively pure state, and without the need of the nitrogen atmosphere recommended previously (9). This may be taken to suggest that cyclization of the intermediate Mannich base to give rise to



Scheme II

[1] Nardil.
[2] Catron.

Table I—Physical Constants of 3-Aryl Pyrazolinium Perchlorates



Compound	R	Melting Point	Yield, %		Analysis, % Calc.	Found
I	H	134°	65	C	48.09	48.09
				H	5.50	4.97
				N	10.19	9.88
II	*p*-CH_3	97°	55	C	49.91	49.66
				H	5.93	5.89
				N	9.70	9.68
III	*p*-OCH_3	154°	60	C	47.29	47.53
				H	5.62	6.36
				N	9.19	8.93
IV	*p*-OC_2H_5	85–86°	72	C	48.98	49.37
				H	6.01	6.60
				N	8.78	8.37
V	*p*-Cl	152°	55	C	42.73	42.36
				H	4.56	4.96
				N	9.06	8.94
VI	*p*-Br	169°	40	C	37.36	37.46
				H	3.99	4.35
				N	7.92	7.34
VII	*m*-NO_2	163°	40	C	41.32	41.38
				H	4.41	4.80
				N	13.14	12.65

2-pyrazolinium salts precedes the formation of the 3-pyrazolines (Scheme II) under the influence of the acid catalyst.

In the preparation of the pyrazolidines by metal hydride reduction, it was found that replacement of the popularly used (7, 9, 10) lithium aluminium hydride–ether system by a sodium borohydride–aqueous system leads to an improvement in yields and a significant reduction in reaction time—from 12 (7) or 18 (9) hr to 15 min. These improvements may be attributed to the homogeneous phase reduction system of the aqueous borohydride, as contrasted to the lithium aluminium hydride–ether system in which the perchlorate salts are insoluble.

PHARMACOLOGY

Antagonism of reserpine-induced ptosis in mice has been employed frequently as an initial procedure for testing antidepressant activity. Under various experimental conditions, all current antidepressant drugs will give this antireserpine effect (11).

Antidepressant agents are fairly capable of counteracting the sedative effect of reserpine. This sedative effect is expressed as the protective action of reserpine against amphetamine-induced lethality in mice (12). The antagonism of this effect by antidepressants is specific for monoamine oxidase inhibitors and is parallel to inhibition of brain monoamine oxidase as well as reserpine-induced hypothermia and ptosis (13).

Using these techniques, the cyclic aralkyl hydrazine congeners synthesized were found to possess antidepressant activity comparable to that of nialamide.

EXPERIMENTAL[3]

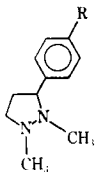
Aralkyl Ketones—Substituted acetophenones were synthesized according to reported methods (14, 15).

1,2-Dimethyl-3-arylpyrazolinium Perchlorates (I–VII)—As a general procedure, a mixture of equimolar amounts (0.1 mole) of 1,2-dimethylhydrazine, paraformaldehyde, and the appropriately substituted acetophenone was heated under reflux in 100 ml absolute ethanol for 2 hr. The reaction mixture was concentrated *in vacuo* to near dryness and triturated with 20-ml portions of ether; then the ether was removed and the residue was dissolved in 50 ml water. The clear aqueous solution was treated with 5 ml of 40% perchloric acid while stirring and cooling. The separated crystalline salts were filtered, washed with ether, and purified by recrystallization from an absolute ethanol–ether mixture. The perchlorate salts were characterized by sharp melting points, elemental analyses, and IR spectra. The spectra showed a moderate to strong absorption of a conjugated C=N$^+$ stretching vibration in the 1600–1640-cm^{-1} range and, more significantly, the absence of any absorption at or near 2200 cm^{-1} (N—H$^+$) (16).

1,2-Dimethyl-3-arylpyrazolidines and Their Perchlorates (VIII–XIII)—As a general procedure, an excess of sodium borohydride (0.075 mole) was added to the appropriate pyrazolinium perchlorate (0.025 mole) in 50 ml water with continuous stirring. The vigorous exothermic reaction subsided within 10 min, and continuous stirring was performed for 5 min longer at 60–65° on a water bath. The reaction mixture was cooled and made strongly alkaline by the addition of 10 ml of 30% sodium hydroxide solution, and the light-yellow oily layer that separated was taken up with 25-ml portions of ether. The washed ethereal extract was dried and the ether was distilled, followed by distillation of the oily residue *in vacuo*. The title pyrazolidines were obtained as colorless to pale-yellow liquids in almost quantitative yields. They were characterized by boiling points, specific gravity values, and refractive indexes. The latter values were used to compute the molecular refraction (MR_D) values, which were compared with calculated values from atomic refraction (Table II).

The perchlorate salts were obtained by mixing equimolar amounts (0.005 mole) of the pyrazolidines in absolute ethanol and 50% perchloric acid with cooling. The corresponding salts separated and were filtered, washed with ether, and recrystallized from an absolute ethanol–ether mixture. They were characterized

Table II—Physical Constants of 3-Aryl Pyrazolidines and Perchlorates



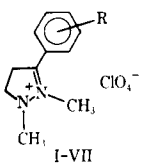
Compound	R	Physical Constants	Yield, %			
		Pyrazolidines				MR_D[b]
VIII	H	bp 83°/2 mm[a]	90.8			55.08
		n_D^{20} 1.5290[a]				55.31
		Specific gravity, d_4^{20} 0.9815				
IX	CH_3	bp 82°/2 mm	92.3			
		n_D^{20} 1.5255				59.69
		Specific gravity, d_4^{20} 0.9721				59.80
X	Cl	bp 98°/2 mm	91.6			
		n_D^{20} 1.5415				59.94
		Specific gravity, d_4^{20} 1.0962				60.33
		Perchlorates			Analysis, % Calc.	Found
XI	H	mp 106°		C	47.77	47.25
				H	6.19	5.71
				N	10.12	10.07
XII	CH_3	mp 152°		C	49.57	50.07
				H	6.59	6.50
				N	9.63	9.46
XIII	Cl	mp 172°		C	42.45	42.22
				H	5.18	5.47
				N	9.00	8.72

[a] Lit. (8) bp 72° (1.2 mm), n_D^{21} 1.5318. [b] Molecular refraction found from the equation:

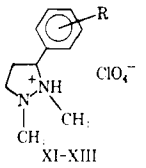
$$MR_D = \frac{n^{2-1}}{n^{2+2}/d_4^{20}} \times \text{molecular weight}$$

and calculated from atomic refraction after Eisenlor (18).

[3] Melting points were taken in an open capillary and are uncorrected; IR spectra were recorded by a Beckman IR-20 spectrophotometer.



I-VII



XI-XIII

Table III—Antidepressant Activity of Cyclic Benzylhydrazine Congeners

Preparation	R	Mortality %	p	Antagonism of Reserpine-Induced Ptosis	Percent Antagonism[a]
Amphetamine sulfate	—	50	<0.005	—	—
Reserpine + amphetamine sulfate	—	0	—	—	—
Nialamide + reserpine + amphetamine sulfate	—	50	<0.005	++++	100
I + reserpine + amphetamine sulfate	H	30	<0.005	++++	90
II + reserpine + amphetamine sulfate	p-CH_3	40	<0.005	++++	95
III + reserpine + amphetamine sulfate	p-OCH_3	40	<0.005	++++	100
IV + reserpine + amphetamine sulfate	p-OC_2H_5	40	<0.005	++++	100
V + reserpine + amphetamine sulfate	p-Cl	20	<0.05	++	45
VI + reserpine + amphetamine sulfate	p-Br	30	<0.01	+++	75
VII + reserpine + amphetamine sulfate	m-NO_2	20	<0.05	++	50
XI + reserpine + amphetamine sulfate	H	30	<0.01	+++	70
XII + reserpine + amphetamine sulfate	p-CH_3	30	>0.01	+++	75
XIII + reserpine + amphetamine sulfate	p-Cl	10	>0.25	+	20

[a] Expressed as percent of animals protected, taking nialamide protection as 100%. All preparations are dissolved in "water for injection."

by sharp melting points, elemental analyses, and IR spectra (strong absorption at 2200 cm^{-1} of the $N—H^+$).

Pharmacological Examination—The antidepressant activity of the perchlorate salts was compared with that of nialamide in mice of either sex weighing 18–26 g. The antagonism of reserpine-induced ptosis (11, 12) and the antagonism of reserpine protection against amphetamine lethality in mice (12) were used for evaluating the antidepressant action.

In a group of 20 mice, amphetamine sulfate (30 mg/kg) was injected intraperitoneally and the percent mortality was determined within the first 24 hr.

In another group of 20 mice, reserpine (1.5 mg/kg) was injected intraperitoneally 2 hr prior to the above-mentioned dose of amphetamine. The degree of reserpine-induced ptosis was noted, and the mortality percentage was calculated after amphetamine injection.

Other groups of 20 mice each were used for evaluating the different compounds under investigation in comparison with nialamide as a reference compound. Nialamide and the new compounds were injected intraperitoneally in doses of 30 mg/kg, followed after 2 hr with 1.5 mg/kg ip reserpine. The degree of ptosis was noted and, 3 hr after reserpine administration, 30 mg/kg amphetamine was injected. The percent mortality within 24 hr was determined.

In another set of experiments, groups of 10 mice were used as the control for injecting the compounds under investigation in the same dose (30 mg/kg) used in the experiment. Any lethality due to the administration of the new compounds was noted.

The significance of the results was statistically evaluated by calculation of χ^2 and the corresponding p values.

RESULTS AND DISCUSSION

Amphetamine sulfate (30 mg/kg) was found to cause 50% mortality in mice within 24 hr of the intraperitoneal injection. Pretreatment of mice with reserpine (1.5 mg/kg ip) 2 hr prior to the administration of amphetamine protected mice against lethality and reduced the mortality to 0%. Reserpine at this dose caused marked ptosis. Nialamide (30 mg/kg), which was used as a reference compound possessing antidepressant activity, was found to counteract the protective action of reserpine; 50% of the animals injected with nialamide, reserpine, and amphetamine died within 24 hr. Furthermore, nialamide prevented the ptosis caused by reserpine administration. The compounds under investigation were found to possess an activity similar to nialamide when administered in 30-mg/kg doses (Table III). The activity varied according to variation in the chemical structure. Control experiments showed that the investigated compounds, in the doses used, were devoid of any lethal effect.

Data in Table III reveal that pyrazolinium and pyrazolidinium perchlorates, as cyclic congeners of benzylhydrazine, possess an antidepressant activity comparable to that of nialamide.

Regarding the structure–activity relationships, certain suggestions may be advanced as follows.

1. Pyrazolinium compounds substituted in the 3-phenyl moiety by electron-donating groups (II, III, and IV) are more potent than those substituted by electron-attracting centers (V, VI, and VII). Such a variation in activity may be attributed to differences in oxidation–reduction potentials of these systems. Pyrazolinium compounds (I–VII), particularly I–IV, were characterized by strong reducing activity as demonstrated by the reduction of an alcoholic solution of 1,3,5-triphenyltetrazolium chloride as well as the rapid reduction of silver nitrate solution at room temperature. Quantitative differences in the reducing power were observed in the polarographic half-wave potentials of these compounds[4].

2. The pyrazolidinium compounds (XI–XIII) may be regarded as having generally weaker activity as antidepressants than the corresponding pyrazolinium salts (I, II, and V). This could be a direct result of saturation of the former compounds, thus bringing about a marked decrease in the electron density on the nitrogen atoms of the free base. This is in accord with the fact that amines are less basic than eneamines (17) with the overall result of a decrease in the reducing power of the carrier compounds. This was demonstrated practically in the finding that the pyrazolidines (VIII–XIII) slowly reduced silver nitrate at room temperature but could not affect an alcoholic solution of 1,3,5-triphenyltetrazolium chloride even after prolonged boiling.

3. It may be inferred that the monoamine oxidase inhibitory activity of the compounds synthesized is dependent mainly upon their reducing potentials.

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Sodium–Calcium-Ion Exchange in Phosphatidyl Serine Monolayer

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Abstract □ The effect of replacement of sodium chloride subphase solution with calcium chloride subphase solution and the effect of replacement of calcium chloride subphase solution with sodium chloride subphase solution were studied on monolayers of phosphatidyl serine. When the sodium chloride subphase was replaced with calcium chloride solution, the π–A curve shifted to almost the same position as the π–A plot of a film spread on calcium chloride without subphase replacement. This showed that almost all sodium ions had been replaced by calcium ions in the film. When the calcium chloride subphase was replaced by a sodium chloride solution, the film expanded somewhat, showing that some calcium ions had been replaced by sodium ions. This study gives evidence for the existence of cation exchange in phosphatidyl serine films, and sodium seems to be in competition with calcium for the negatively charged sites in the film. It is possible that this competition is concentration dependent.

Keyphrases □ Sodium-calcium subphase exchange—effects on phosphatidyl serine monolayers □ Calcium-sodium subphase exchange—effects on phosphatidyl serine monolayers □ Phosphatidyl serine monolayers—effects of sodium-calcium and calcium-sodium subphase exchange, sodium-calcium competition, cation-exchange evidence □ Monolayers, phosphatidyl serine—effects of sodium-calcium and calcium-sodium subphase exchange, sodium-calcium competition, cation-exchange evidence

A previous article (1) reported an apparatus and technique for studying monomolecular films by which the subphase can be exchanged without disturbing the film. Data were presented to demonstrate the utility of this system for studying protein and enzyme monolayers. This article will show the applicability of the system to the study of ion-exchange properties of phospholipid monolayers.

DISCUSSION

Binding of cations by phospholipids and fatty acids has been studied extensively (2–26). Calcium ions were found (3) to produce little reduction in area per molecule of cephalin, spread at an air–water interface at a given surface pressure. Studies (4, 5) on the dependence on surface pressure of the penetration rate of water molecules through lipid monolayers found that calcium ions, at low concentration, decreased such penetration. The association of synthetic lecithin with ions was studied (6), showing indirectly that, with dilute solutions, there is no binding of sodium, potassium, or lithium. However, the same study reported that magnesium seemed to be adsorbed from dilute solutions. Vilallonga *et al.* (7) observed that the presence of monovalent ions increases the area per molecule, the surface potential, and the surface dipole moment of dipalmitoyl lecithin monolayers in the order sodium > potassium ≈ lithium > water. It was reported (8) that lecithin monolayers bind calcium (0.1 mM) and that sodium or potassium ions (112 mM) each displace the same fraction of this calcium (about 50%). Many workers reported interaction of divalent and monovalent cations with phosphatidyl serine (2, 9–13).

Shah and Schulman (14) reported that the π–A curves of lecithins, phosphatidyl choline, and dicetyl phosphate are not affected by the presence of divalent metal ions. However, dipalmitoyl lecithin shows a higher surface potential in the presence of divalent metal ions than in the presence of monovalent ions; the increase is greatest for the fully saturated dipalmitoyl lecithin and the smallest for a highly unsaturated lecithin. They suggested that the divalent metal ion–lecithin monolayer interaction is dependent on the packing of the hydrocarbon chains. The large intermolecular separation permits water, hydrated monovalent ions, and nitrogen ion of the same molecule to associate with the phosphate ion; this prevents the calcium interaction with the phosphate ion. On compression, some water molecules and hydrated ions are squeezed out and the conditions become more favorable for calcium, because one calcium ion between two phosphate groups is smaller than two monovalent ions. These authors also postulated a position for calcium in dicetyl phosphate monolayers that would not affect the area of the film but would increase the surface potential.

In a later publication, Shah and Schulman (15) suggested that there is an internal salt linkage between the phosphate and trimethylammonium group on the same lecithin molecule, which prevents the interaction of the phosphate group with calcium. An increase in unsaturation of the fatty acyl chains increases the intermolecular spacing, which reduces the ionic repulsion between polar groups and hence strengthens the internal salt linkage. Thus, increasing unsaturation decreases calcium binding in lecithin monolayers. They suggested a vertical rather than a coplanar orientation of the phosphoryl choline group with respect to the interface and proposed a position for calcium in the lecithin films. It was suggested, based on surface potential studies, that calcium forms an ionic dipole ($Ca^{+2} \leftrightarrow O^{-}$).

Colacicco (16) showed that the reported (14, 15) surface poten-