This method with its sensitivity, rapidity, and color stability provides a significant improvement over the procedures mentioned in the literature.

SUMMARY

Pyridoxine hydrochloride, steroid, and morphine sulfate which contain either phenol c, or similar-to-phenolic hydroxyl groups, form colored azo dyes with diazotized disulfonamide.

The color intensity was significantly increased when coupling occurred *para*, rather than *ortho*, to the phenolic hydroxyl group. Certain compounds showing group similarities, as well as commonly used excipients in pharmaceutical formulations, do not interfere in this determination.

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Keyphrases

Pyridoxine HCl—analysis
Morphine—analysis
17β-Hydroxy-7aα-1,7α-dimethyl-B-homo-A-norestrane-3,6-dione—analysis
Colorimetric analysis—spectrophotometer
4-Amino-6-chloro-*m*-benzenedisulfonamide, diazotized—color reagent

In Vivo and *In Vitro* Activity of a Vasoactive Drug 9-(3,5-Dimethylpyrazole-1-carboxamido)-7-methyl-4,6,6*a*,7,8,9,10,10*a*-octahydroindolo[4,3-*fg*] Quinoline Maleate

By PAUL W. WILLARD and CLARENCE E. POWELL

An indoloquinoline compound prepared from lysergic acid base has been found to have an α-blocking action on *in vivo* and *in vitro* preparations. The compound reverses the blood pressure response to epinephrine from pressor to depressor in pithed and decerebrate cats. In renal hypertensive dogs, the compound produced a significant sustained decrease in blood pressure. On rat vas deferens preparations, the spasmogenic effect of epinephrine and norepinephrine was blocked by the compound similar to, but not in the same degree as that observed with dibenamine. The primary effect of the compound appears to be an interference with sympathetic transmission postsynaptically at the α-receptor sites resulting in peripheral dilatation.

COMPOUNDS DERIVED from lysergic acid are known to have a variety of pharmacological properties. Among these properties are effects on blood pressure and other cardiovascular parameters (1). Recently, a chemically unique series of indoloquinoline compounds prepared from the lysergic acid base[1] produced a sustained lowering of blood pressure in unanesthetized and anesthetized animals. This study was made to determine the mechanism of actions whereby one of the members of the series lowered blood pressure.

The compound, 9-(3,5-dimethylpyrazole-1-carboxamido)-7-methyl-4,6,6*a*,7,8,9,10,10*a*-

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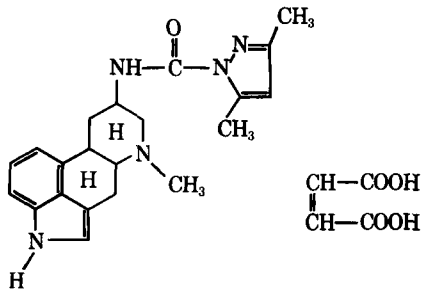
[1] Compounds were prepared by Dr. William L. Garbrecht of the Process Research and Development Division, Eli Lilly and Co., Indianapolis, Ind.

octahydroindolo [4,3-*fg*] quinoline maleate (compound PAI), has the following structural formula:



METHODS

In Vivo Preparations of Unanesthetized Animals—Renal hypertension was produced in adult male dogs by the Goldblatt procedure (2). Blood pressure responses were measured from a direct arterial puncture by a mercury manometer. Following control blood pressure and heart-rate recordings, the drug was administered orally to each of three hypertensive dogs at 5.0, 7.5, and 10.0 mg./kg., respectively. Blood pressure and heart rate were measured at 30-, 60-, 120-, 180-, and 360-min. intervals.

Subacute toxicity studies of the compound were made in adult white roosters (3). One group of three roosters received 2.0 mg. of compound PAI intramuscularly, b.i.d. for 21 days. Another group of three roosters was given 5.0 mg. of compound PAI intramuscularly b.i.d. for 31 days. In a third group of nine roosters, 5.0 mg. of the drug was given intramuscularly daily 30 min. prior to a 1.0-mg. dose of ergotamine tartrate intramuscularly for 21 days. An additional group of nine roosters received only 1.0 mg. of ergotamine tartrate intramuscularly for 21 days.

Anesthetized Animals—Ganglionic inhibition and blood pressure effects were evaluated in four cats anesthetized with chloralose (50 mg./kg.) following administration of progressively larger doses of compound PAI (0.1, 0.25, 0.5, 1.0, 2.0, 5.0, and 10.0 mg./kg.). The superior cervical sympathetic nerve was stimulated for 30 sec. by a 2- or 20-c.p.s. (9.0 v.-5 msec.) square wave from a Grass stimulator. Four minutes were allowed between stimulations for recovery of the preparation. Epinephrine (2.5 mcg./kg.) and norepinephrine (2.5 mcg./kg.) were administered to chloralose anesthetized cats following intravenous administration of compound PAI (5.0 mg./kg.).

In Vitro Preparations—The adrenergic blockade of compound PAI was compared with dibenamine using a method modified from that described by Leach (6). Isolated vas deferens from adult rats, 250–600 g., were suspended in either 2-ml. or 10-ml. muscle chambers containing Tyrode's solution warmed to 36° and gassed with 95% oxygen and 5% carbon dioxide. Responses of the muscles were recorded electronically by either isometric or isotonic myographs with 0.5-g. tension. Epinephrine and norepinephrine were used as the agonists. Doses of the antagonists which would block muscle response to the agonists by 50% were determined. The β-receptor action of dichloroisoproterenol against epinephrine- and norepinephrine-induced contractions of the rat vas deferens was also investigated.

In another series of experiments, the protective action of PAI against dibenamine blockade of norepinephrine-induced contractions of rat vas deferens was studied by a method modified from that of Furchgott (7). In protection experiments a Vickers biological assay apparatus was used and muscle responses were recorded electronically by isotonic myographs with 0.5-g. tension.

The test procedure was to first give a series of norepinephrine doses (1.0 mcg./ml.) at 5-min. intervals until the muscle response was quantitated. The muscle strip was then treated with a dose (100 mcg./ml.) of PAI and 5 min. later dibenamine (0.1 mcg./ml.) was added. Following a 5-min. exposure the muscle was washed three times and allowed to rest 10 min. Norepinephrine in the same dose as control was then given at 5-min. intervals for a period of 30–60 min.

The contractile force and coronary flow was tested on four rabbit hearts suspended in an Anderson-Carver perfusion chamber (4). Chenoweth's solution was used as the perfusion fluid (5). Doses of compound PAI were injected directly into the perfusate as it entered the aortic cannula. The heart-muscle response was recorded electronically with an isometric myograph.

RESULTS

In Vivo Preparations of Unanesthetized Animals—When compound PAI was administered orally to renal hypertensive dogs, blood pressure was lowered within 1 hr. of dosing (Table I). The blood pressure returned to near normal 3 hr. following the 5.0-mg./kg. dose. With 7.5 and 10.0 mg./kg., the blood

TABLE I—CARDIOVASCULAR RESPONSES OF RENAL HYPERTENSIVE DOGS FOLLOWING TREATMENT WITH PAI

	Days			
	1	2	1	2
Dog	ZH116	ZH116	ZH120	ZH120
Dog weight, kg.	8.5	—	14.2	—
Dog dose, mg./kg.	5	10	7.5	—
Control blood pressure	210	210	190	185
Control pulse	120	128	100	112
Compound 39985 oral (capsule), mg.	42.5	85.0	106.5	—
Blood pressure, 30 min.	215	200	175	—
Pulse, 30 min.	120	160	112	—
Blood pressure, 1 hr.	190[a]	170	140[b]	—
Pulse, 1 hr.	128	200	136	—
Blood pressure, 2 hr.	175	130	110	—
Pulse, 2 hr.	120	200+	176	—
Blood pressure, 3 hr.	195	120	140	—
Pulse, 3 hr.	136	200+	138	—
Blood pressure, 6 hr.	200	165	150	—
Pulse, 6 hr.	112	176	144	—

[a] Vomited. [b] Diarrhea (slight).

pressure was still reduced significantly at 6 hr. However, in dog ZH-120, there was no residual effect 24 hr. following drug administration. The heart rate increased in each instance and appeared to be a function of the dose. The dogs vomited after receiving the drug, and a slight diarrhea was evident with the 7.5-mg./kg. dose. Emesis was a frequent occurrence with the drug when administered orally or i.v. to other dogs, however, there was no apparent relationship to dose or to blood pressure decreases. Emesis generally only occurred once about 1 hr. following treatments. Emetic or other apparent central nervous system activity was not produced in unanesthetized cats.

The rooster combs appeared normal following 21 days of treatment with compound PAI (2.0 mg. b.i.d.) and the general physical condition of the birds was good. During the treatment period, the roosters had an average weight gain of 0.3 kg. All roosters receiving 5.0-mg. b.i.d. doses were also in good condition with normal combs.

Seven of nine roosters dosed only with ergotamine tartrate (1.0 mg.) had definite bluing of the comb followed by dry gangrene on the sixth day of treatment. One rooster died on the seventh day and the remaining bird survived with no bluing of the comb.

In the group of roosters receiving 5.0 mg. of compound PAI 30 min. prior to ergotamine treatment, none of the six surviving birds developed gangrenous combs. One rooster died on the third day, one on the tenth day, and one on the twentieth day following initiation of the regimen. Cause of death was not apparent. There was no weight change in this group.

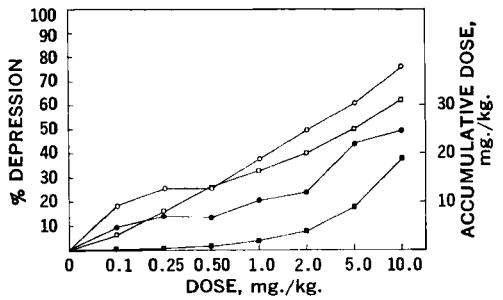


Fig. 1—Four ether–chloralose anesthetized, normotensive cats. Effect of accumulation of larger doses of PAI on blood pressure and nictitating membrane contractile response. Stimulation of nictitating membrane preparation with a square wave (9 v.) stimulation at 2 and 20 c.p.s. following each dose. Key: ○, 2 c.p.s.; ●, 20 c.p.s.; □, B.P.; ■, accumulative dose.

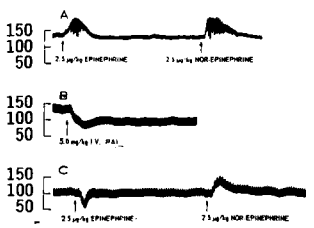


Fig. 2—Example of in vivo *effects of PAI (5.0 mg./kg.) on equal doses of (2.5 mcg./kg. i.v.) of epinephrine and norepinephrine in an ether–chloralose anesthetized cat.*

Anesthetized Animals—The action of compound PAI administered in graded doses of 0.1, 0.25, 0.50, 1.0, 2.0, 5.0, and 10.0 mg./kg. to chloralose anesthetized cats is shown in Fig. 1. A maximum decrease of 62% in blood pressure was produced with 10.0 mg./kg. (accumulated dose = 18.85 mg./kg.). The nictitating membrane was blocked 76% with the 2 c.p.s. stimulation and 50% with the 20 c.p.s. stimulation. "Neural fatigue," defined as a gradual decay of the sustained contractile response, was not observed with any dose of compound PAI.

Following a 5.0 mg./kg. dose of compound PAI to chloralose anesthetized cats, the pressor response to epinephrine was reversed to depressor (Fig. 2). Blood pressure response to norepinephrine was only slightly inhibited.

In Vitro Preparation—Doses of 0.1, 0.2, and 0.3 ml. of a 1-mg./ml. stock solution reduced the isolated heart contractile force by 5.0, 7.0, and 75%, respectively. A dose of 0.5 mg. (0.5 ml.) completely blocked the heart beat with no recovery. The heart rate was not altered nor was the coronary flow changed by smaller doses.

Compound PAI was found to antagonize epinephrine- and norepinephrine-induced spasms of the rat vas deferens. Figure 3 shows a typical response of the muscle to 10 mcg./ml. of the compound. Following matching contractions from doses of 0.3 mcg./ml. of epinephrine, compound PAI (10 mcg./ml.) was injected into the tissue chamber and allowed to act 1 min. Following this procedure, an 0.3-mcg./ml. dose of epinephrine was added to the bath. The stimulating action of epinephrine was reduced 50%. Even with repeated change of bathing fluid and exposure to epinephrine, the muscle failed to recover the pretreatment response after five trials. Thus, compound PAI has a sustained blocking action on epinephrine. The lower tracing on

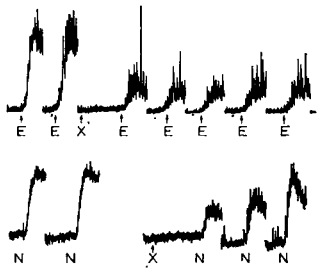


Fig. 3—Comparison of the stimulating action of epinephrine (E) (0.3mcg./ml.) and norepinephrine (N) (0.3 mcg./ml.) on isolated vas deferens of the rat, before and after compound PAI (X) (10 mcg./ml.).

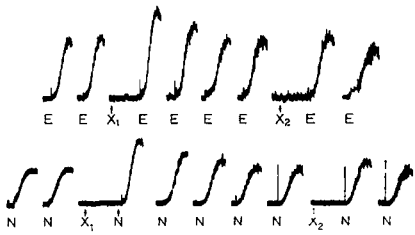


Fig. 4—Potentiation of the spasmogenic activity of epinephrine (E) (0.4 mcg./ml.) and norepinephrine (N) (0.4 mcg./ml.) by DCI (X_1 = 5.0 mcg./ml.) and (X_2 = 10 mcg./ml.) on the isolated vas deferens of the rat.

Fig. 3 shows a similar treatment of the muscle using norepinephrine in doses of 0.25 mcg./ml. Ten mcg./ml. of compound PAI blocked the muscle response to norepinephrine by 50%, but the time of recovery was somewhat less than was required for epinephrine.

In the study with DCI on spasms induced by epinephrine or norepinephrine of the rat vas deferens, it was found that an additive or enhancing action resulted (Fig. 4). DCI was added to the muscle chamber in doses of 5.0 and 10.0 mcg./ml. following matching contractions to 0.4-mcg./ml. doses of epinephrine and norepinephrine. When DCI had been in contact with the tissue for 1 min., a 0.4-mcg/ml. dose of either epinephrine or norepinephrine was added to the chamber. As shown in the tracing, the muscle contracted higher following both catecholamines, indicating an enhancing effect, since DCI in the doses given produced no activity alone.

Although it could be demonstrated in isolated rat vas deferens preparations that compound PAI left in contact with the muscle strip partially blocked norepinephrine-induced contractions (Fig. 3), such was not the case in protection experiments. From control protection tests in which compound PAI alone (100 mcg./ml.) was in contact with the muscle strip 5 min. then washed three times and dosed with norepinephrine (1.0 mcg./ml.), no significant adrenergic receptor blockade was noted. An average of results on three muscle-strip preparations showed response to norepinephrine to be reduced by less than 20%. However, when muscle strips were treated in the same manner with dibenamine (0.1 mcg./ml.), contractile responses to norepinephrine (1.0 mcg./ml.) were reduced by more than 95% through eight doses over a period of 40 min. (Fig. 5).

The protection experiments indicate compound PAI partially protects adrenergic receptors from dibenamine blockade. This confirms the suggestion that PAI reacts with α-adrenergic receptors.

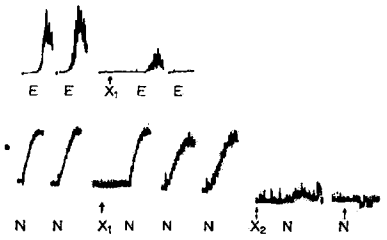


Fig. 5—Antagonism of dibenamine (X_1 = 0.4 mcg./ml.) and (X_2 = 1.0 mcg./ml.) on the stimulation action of epinephrine (E) (0.2 mcg./ml.) and norepinephrine (N) (0.2 mcg./ml.) on the isolated vas deferens of the rat.

DISCUSSION

The *in vivo* and *in vitro* activity of compound PAI suggests that the hypotensive response can be related or ascribed to α-adrenergic blockade. Epinephrine reversal occurs in pithed and decerebrate cats (Fig. 2) and inhibition of epinephrine and norepinephrine contraction of the vas deferens is a prominent feature of the drug (Fig. 3).

It has been clearly demonstrated that compound PAI, when left in contact with rat vas deferens 5 min., protects adrenergic receptors against the contractile action of norepinephrine (Fig. 3). However, when large doses of the compound (100 mcg./ml.) are incubated 5 min. and washed 3 times, most of the blocking action to norepinephrine stimulation disappears. This would indicate the compound does not adhere to the adrenergic receptors as tightly as that of dibenamine. It is well known that dibenamine blockade of epinephrine stimulation of isolated muscle persists up to 2 or more hr. (7). In this study control experiments show dibenamine (0.1 mcg./ml.) to inhibit almost 100% the contractile action of norepinephrine through three initial washes and eight doses of the agonists given at 5-min. intervals (total time 55 min.).

Compound PAI does protect adrenergic receptors against dibenamine blockade of the contractile response of smooth muscle to norepinephrine as demonstrated when compound PAI was incubated with dibenamine and then washed three times. From these experiments it was estimated that PAI protected 38% of the adrenergic receptors from the effectiveness of dibenamine.

The hypotensive response in renal hypertensive dogs was accompanied by an increased heart rate which indicates an attempted compensation by the unblocked β-receptors at the terminals of the cardiac sympathetic nerves (Table I). This response is consistent with the peripheral dilating action of α-blockade (8). Blockade of ergotamine-induced gangrene of rooster combs by PAI further indicates a competitive block at the site of peripheral vasoconstriction. In anesthetized dogs, the heart rate generally decreased. The anesthetic probably depressed the cardiac center and effectively inhibited a compensatory chronotropic response. Compound PAI does produce a blockade of the sympathetic nervous system (Fig. 1). From the data obtained in the above studies, α-receptor blockade probably accounts for a large portion of the hypotensive activity.

SUMMARY

1. Compound PAI produced a significant sustained decrease in blood pressure orally in hypertensive dogs (5.0, 7.5, and 10.0 mg./kg.). Emesis occurred in one hypertensive dog and slight diarrhea in a second.

2. On the rat vas deferens preparation, PAI was observed to block the spasmogenic effect of epinephrine and norepinephrine similar to, but not in the same degree as that observed with dibenamine. Protection experiments show that PAI partially protected the α-receptors from dibenamine blockade of the contractile response to norepinephrine. Thus, compound PAI is primarily an α-blocking agent, since DCI potentiated the spasmogenic effect of epinephrine and norepinephrine. Additional evidence in favor of α-blockade is provided by the fact that epinephrine is reversed in spinal cats by compound PAI.

3. In anesthetized cats, sympathetic block is evidenced following PAI administration. Ergotamine toxicity is inhibited in roosters following prophylactic treatment with PAI.

4. It has been concluded that a primary effect of PAI is interference with sympathetic transmission postsynaptically at the α-receptor which results in peripheral dilatation.

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Keyphrases

Indoloquinoline derivatives of lysergic acid
Hypotensive activity—lysergic acid derivatives
Mechanism—hypotensive activity
α-Receptor blockade—lysergic acid derivative

Micellar Solubilization of Testosterone II

In Aqueous Solutions of Some Ionic Surfactants

By ARVIND L. THAKKAR* and NATHAN A. HALL

The solubility of testosterone at 25° was determined in aqueous solutions containing varying concentrations of dodecyltrimethylammonium bromide (DTAB), hexadecyltrimethylammonium bromide (HTAB), and potassium laurate (KL). After the initial stages of solubilization, a linear relationship was observed between the amount of testosterone solubilized and the molar concentration of the surfactant. The order of increasing solubilizing capacity was DTAB < HTAB < KL. The environment of solubilized testosterone, as investigated by the *Z*-value method, was found to be quite polar in all cases. At low concentrations of DTAB and HTAB the *Z* value was similar to that in water. As the concentration of the quaternary ammonium bromides increased, a precipitous drop in *Z* value, corresponding to a sudden decrease in environmental polarity, was observed in the region of the critical micelle concentration. With further increase in the concentration of DTAB and HTAB the *Z* value remained reasonably constant. In low KL concentrations the *Z* value of the testosterone environment was higher (more polar) than in water. With increasing concentrations of KL the *Z* value displayed behavior similar to that in DTAB and HTAB. In general, solutions of ionic surfactants showed higher *Z* values than those of the nonionic surfactants examined previously.

THE MICELLAR solubilization of testosterone by solutions of the nonionic surfactants, polysorbates 20, 40, and 60, was the subject of a prior report from this laboratory (1). The solubilizing capacities of the three polysorbates were determined and the polarity of the chromophore of testosterone examined in these systems. A description of the *Z*-value method for empirically measuring the environmental polarity of the solubilized testosterone was included and the results were discussed in the light of current micellar theories.

In 1949 Ekwall and Sjöblom (2) prepared clear aqueous solutions of testosterone in 10% sodium oleate, 20% sodium myristyl sulfate, and 20% sodium cholate solutions, but they did not study the micellar solubilization of this steroid in detail. Recently Lach and Pauli (3) reported the solubilizing action of aqueous sodium desoxycholate solutions upon this steroid. These workers attributed the solubilizing action of sodium desoxycholate, at least in part, to channel-like inclusion complex formation. Besides these two reports no data regarding the solubilizing action of other anionic or cationic agents for testosterone are available. The investigations reported here include the solubilizing capacities of dodecyltrimethylammonium bromide, hexadecyltrimethylammonium bromide, and potassium laurate, as well as the effect of varying concentrations of these surfactants upon the environmental polarity of solubilized testosterone as determined by the *Z*-value method.

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