CONCLUSIONS

The yields of *N*-[indenyl (3)] ureas varied from 8–38% as can be seen from the table. With increase in the chain length of the alkyl group as the substituent on the urea nitrogen atom, the yield decreased. β-Methoxyethyl substituent on the urea gave maximum antitussive activity. *n*-Propyl and isopropyl substituents imparted activity of a lower order. 3,4-Dimethoxybenzyl-substituted urea showed some activity. Other compounds showed no activity at all. The compounds, however, do not warrant any further studies as regards the mechanism of action, because of low oral absorption and high toxicity.

REFERENCES

(1) G. Drefahl and K. Ponsold, *Ber.*, **93,** 519(1960).

(2) R. R. Wittekind, J. D. Rosenau, and G. I. Poos, *J. Org. Chem.*, **26,** 444(1961).

(3) A. Hassner, M. E. Lorber, and C. Heathcock, *ibid.*, **32,** 540(1967).

(4) A. Hassner and C. Heathcock, *ibid.*, **30,** 1748(1963).

(5) C. Heathcock and A. Hassner, *Angew. Chem. Intern. Ed. Engl.*, **2,** 213(1963).

(6) A. Hassner and C. Heathcock, *Tetrahedron*, **20,** 1037(1964).

(7) C. F. Huebner, E. M. Donoghue, P. L. Strachen, P. Beak, and E. Wenkert, *J. Org. Chem.*, **27,** 4465(1962).

(8) D. H. R. Barton and R. C. Cookson, *Quart. Rev. (London)*, **10,** 44(1956).

(9) A. F. Green and N. B. Ward, *Brit. J. Pharmacol.*, **10,** 418 (1955).

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Aryloxyacetamidines of Medicinal Interest

WILLIAM J. HAGGERTY, JR.* and WILLIAM J. ROST

Abstract □ The synthesis of new aryloxyacetamidines of potential medicinal interest as antihypertensives was undertaken. The preparation of the unsubstituted, *N*-methyl and *N,N*-dimethyl amidines was carried out by the Pinner synthesis or a modification of this process. The *N,N,N'*-trimethylaryloxyacetamidines necessitated preparation by another procedure. Fifteen amidines and nine intermediates not previously reported in the literature have been prepared and characterized. 2-Thymoloxyacetamidine, 2-(2,6-xylyloxy)acetamidine, 2-(2,6-dichlorophenoxy)acetamidine, 2-(2,6-dimethoxyphenoxy)acetamidine, 2-benzyloxyacetamidine, and their *N*-methyl and *N,N*-dimethyl derivatives were evaluated for their potential cardiovascular effects. None of these compounds showed any potent pharmacological activities in a general screen or when tested as norepinephrine-depleting agents or as adrenergic neuron-blocking agents.

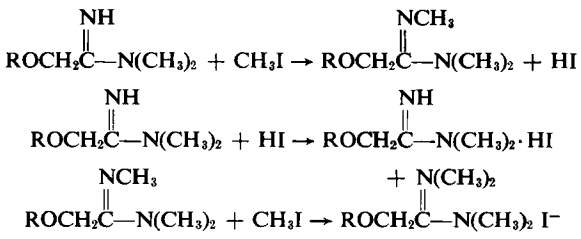
Keyphrases □ Aryloxyacetamidines—synthesis □ NMR spectroscopy—identity, structure □ Pharmacological screening—aryloxyacetamidines

Although the literature describes the adrenergic neuron-blocking effects of guanidines, aminoguanidines, amidoximes, and quaternary ammonium compounds with bulky substituents, it appears that aryloxyamidines have received little attention as potential antihypertensive agents (1). Previous research has shown that molecules consisting of a strongly basic group attached to a suitable ring by a short alkylene or oxyalkylene chain can give rise to compounds with considerable hypotensive effects (2).

The purpose of this research was to investigate the synthesis of variously substituted aryloxyacetamidines and to have them screened as potential adrenergic neuron-blocking agents and anticholinergics. The synthesis of the amidines and substituted acetamidines of the thymoloxy, 2,6-xylyloxy, 2,6-dichlorophenoxy, 2,6-dimethoxyphenoxy, and the 2-benzyloxy series would provide structural analogs of active compounds and provide new information for the design of drugs which act on the autonomic nervous system.

Although there are many synthetic methods for preparing amidines, the most versatile method for preparing these compounds appeared to be the well-known Pinner synthesis (3). This method was used for the preparation of the amidines and *N,N*-dimethyl amidines. However, it was noted that yields were lowered unless the intermediate imidate salts were freshly prepared. In one case, the 2-(2,6-dimethoxyphenoxy)acetimidate hydrochloride could not be obtained, but instead 2-(2,6-dimethoxyphenoxy)acetamide was isolated. It has been reported that some acetimidate salts which contain electronegative groups on the β carbon will decompose to an amide and alkyl halide at room temperature (4, 5). Because of this difficulty, an alternate synthetic approach to the desired amidine was tried. Schaefer has noted that many electronegatively substituted nitriles may be converted to the imidates by alcohol in the presence of catalytic amounts of sodium. Reaction of the imidates with amine salts or ammonium chloride yielded the amidine salts (6). This method was also used and proved superior to the Pinner synthesis in both yields and facility.

The synthesis of *N,N,N'*-trisubstituted aryloxyacetamidines proved to be more difficult (Scheme I). Using the Pyman procedure (7) for alkylation of *N,N*-dimethyl aryloxyacetamidines gave very low yields. Whether no solvent, a minimum amount of solvent, or a large amount of solvent was used in the alkylation procedure, from 40–45% of the starting material was isolated as the hydroiodide salt. In order for this to occur, the same amount of *N,N,N'*-trimethylaryloxyacetamidine must have formed. Since the hydrogen iodide formed in this reaction combined with the free base starting material more readily than the trimethylamidine, it would then be possible for the excess methyl iodide to attack the trimethylamidine to form tetramethylamidinium iodide. If 45% of the starting material was recovered as the hydrogen iodide salt, only 10% of the desired trimethylamidine could then be obtained provided the trimethylamidine was further attacked by the methyl iodide.



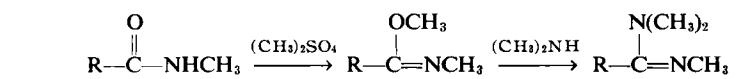
Scheme I

The best yield of *N,N,N'*-trimethylaryloxyacetamidine that was obtained by alkylation of the *N,N*-dimethylaryloxyacetamidine in this fashion was 7%. Since Pyman's procedure was not satisfactory for the synthesis of *N,N,N'*-trimethylaryloxyacetamidines, alternate procedures were tried.

Treatment of an imidoyl chloride with a secondary amine (8, 9) did not yield the desired products, but instead gave mostly decomposition products.

Reaction of an *N*-substituted amide with dimethylcarbamoyl chloride (10) gave either low yields or no product.

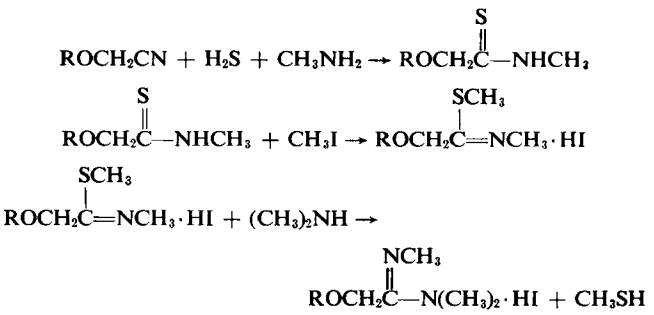
Bredereck has also described the preparation of trisubstituted amidines (11). This method involves the reaction of dimethyl sulfate and an *N*-methyl amide to give an intermediate imidate which is then converted to the trisubstituted amidine (Scheme II).



Scheme II

Even heating to 140° with dimethylsulfate, *N*-methyl thymoloxyacetamide failed to react. A stronger alkylating agent, triethyloxonium fluoroborate, has recently been described by Meerwein (12). Despite the superior alkylating powers of this reagent, no reaction could be effected when *N*-methyl-2-(2,6-xylyloxy)acetamide was reacted in the manner described by Paquette (13).

The most satisfactory route to the trisubstituted amidines was a modification of procedures found in the literature (14, 15). The aryloxyacetonitriles were reacted with methylamine and hydrogen sulfide to yield *N*-methyl-2-aryloxythioacetamides in excellent yields. Alkylation of the *N*-methylthioamides with methyl iodide converted the amides to thioimidates. When the thioimidates were treated with excess dimethylamine in methanol, the amidines were obtained in fair yields (Scheme III).



Scheme III

One compound, methyl *N*-methyl-2-(2,6-dichlorophenoxy)thioacetimidate hydroiodide, failed to give the desired *N,N,N'*-trimethyl-2(2,6-dichlorophenoxy)-acetamidine hydroiodide under these conditions.

EXPERIMENTAL

All melting points were taken on a melting point apparatus[1] and were corrected. All microanalyses were run on an analyzer.[2] The NMR spectra were obtained on a spectrometer.[3]

The aryloxyacetonitriles were prepared according to Djerassi (16) from commercially available phenols and chloroacetonitrile with the exception of 2-benzyloxyacetonitrile, which was prepared after the method of Quarterman (17).

Unsubstituted, N-Methyl and N,N-Dimethylaryloxyacetamidines

The following amidines were prepared by the Pinner synthesis (Method A) (3) or by the method of Schaefer (Method B) (6).

Method A—*2-Thymoloxyacetamidine Hydrochloride (I)*—A cold solution of 3.4 g. (0.2 mole) of anhydrous ammonia in 100 ml. of absolute methanol was cooled to about 10° and then 10 g. (0.04 mole) of freshly prepared methyl 2-thymoloxyacetimidate hydrochloride was quickly added in small portions while swirling the reactants. The flask was stoppered and allowed to stand at room temperature for 3 days. A small amount of ammonium chloride was removed by filtration, and then the filtrate was reduced to dryness *in vacuo* to give 8.5 g. (90%) of crude amidine, m.p. 180–183°. Recrystallization from a mixture of *n*-butanol and ethyl ether (1:1) gave an analytical sample, m.p. 184–185°.

Method B—*2-(2,6-Dimethoxyphenoxy)acetamidine Hydrochloride (IV)*—A solution of 0.12 g. (0.005 g. atom) sodium in 75 ml. of absolute methanol was stirred with 9.7 g. (0.05 mole) of 2-(2,6-pimethoxyphenoxy)acetonitrile for 16 hr. The reactants were then treated with 2.7 g. (0.05 mole) of ammonium chloride and stirred an additional 20 hr. Filtration of sodium chloride followed by evaporation of the filtrate *in vacuo* afforded crystals. The crude amidine was recrystallized from *n*-butanol and ethyl ether (1:1) to give platelets, m.p. 180–181°, in an 85% yield.

Several attempts to prepare the above amidine (IV) by Method A failed. Apparently, the desired imidate hydrochloride, once formed, is very unstable and spontaneously decomposed to the amide (4, 5). The amide, m.p. 126–128°, was recrystallized from *n*-butanol.

Anal—Calcd. for $C_{10}H_{13}NO_4$: C, 56.87; H, 6.20; N, 6.63. Found: C, 56.82; H, 6.15; N, 6.85.

See Tables I and II for analytical data of amidines prepared by Methods A and B.

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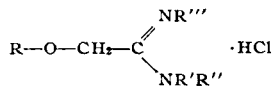
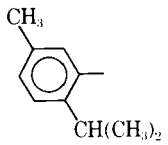
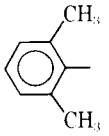
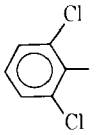
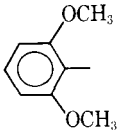
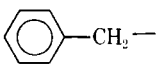
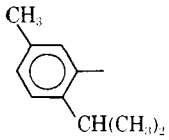
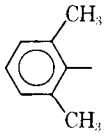
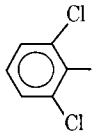
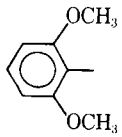
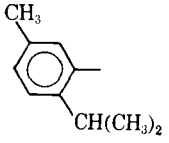
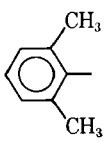
[1] Thomas-Hoover Unimelt.
[2] F & M model 185.
[3] Varian model A-60 The authors are deeply indebted to Midwest Research Institute for providing the analytical services.

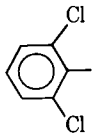
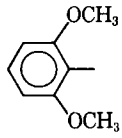
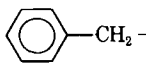
Table I—Unsubstituted, *N*-Methyl and *N,N*-Dimethylaryloxyacetamidines



Compd.	R	R′	R″	R‴	Method	Yield, %	M.p., °C.	Anal. Calcd.	Anal. Found
I		H	H	H	A	90	184–185[a]	—	—
II		H	H	H	A	67	193–194	C, 55.95 H, 7.03 N, 13.04	C, 55.84 H, 7.52 N, 12.73
III		H	H	H	A	61	192–194	C, 37.61 H, 3.52 N, 10.96	C, 37.80 H, 3.28 N, 10.77
IV		H	H	H	B	85	180–181	C, 48.68 H, 6.12 N, 11.35	C, 48.54 H, 6.19 N, 11.38
V		H	H	H	A	75	117–119	C, 53.89 H, 6.53 N, 13.96	C, 53.80 H, 6.86 N, 13.90
VI		CH_3	H	H	B	62	139–140	C, 60.79 H, 8.26 N, 10.90	C, 61.09 H, 8.10 N, 10.84
VII		CH_3	H	H	B	91	175–177	C, 57.76 H, 7.48 N, 12.24	C, 57.56 H, 7.74 N, 12.48
VIII		CH_3	H	H	B	80	200–201	C, 40.10 H, 4.12 N, 10.38	C, 40.17 H, 4.12 N, 10.28
IX		CH_3	H	H	B	82	176–177	C, 50.67 H, 6.56 N, 10.74	C, 50.82 H, 6.57 N, 10.56
X		CH_3	CH_3	H	A	78	196–197[a]	—	—
XI		CH_3	CH_3	H	B	92	194–195	C, 59.37 H, 7.89 N, 11.54	C, 59.33 H, 8.14 N, 11.29

(*Continued on next page*)

Table I (*Continued*)

Compd.	R	R′	R″	R‴	Method	Yield, %	M.p., °C.	Anal. Calcd.	Anal. Found
XII		CH_3	CH_3	H	A	68	185–186	C, 42.35 H, 4.62 N, 9.87	C, 42.37 H, 4.73 N, 9.64
XIII		CH_3	CH_3	H	B	81	177–179	C, 52.46 H, 6.95 N, 10.19	C, 52.30 H, 7.20 N, 10.10
XIV		CH_3	CH_3	H	A	83	128.5–129	C, 57.78 H, 7.47 N, 12.24	C, 57.70 H, 7.74 N, 12.45

[a] Known compounds, see *Reference 16.*

Aryloxy-N,N,N′-trimethylacetamidines

N,N,N′-Trimethyl-2-thymoloxyacetamidine Hydroiodide (XV)—A cold solution of 8.1 g. (0.18 mole) dimethylamine in 200 ml. of anhydrous methanol was stirred while 32.7 g. (0.09 mole) of methyl-*N*-methyl-2-thymoloxythioacetimidate hydroiodide (XXII) was added in one portion. The reactants were stirred overnight at room temperature and then 2 hr. at 40–45°. Removal of the solvent followed by trituration with anhydrous ether gave 26.5 g. of crystalline product which melted with decomposition over a wide range (100–140°). The crude product was extracted with warm benzene and the filtrate reduced to dryness. The residue was recrystallized from ethyl acetate to give 8.7 g. (27%) of pure cotton-like needles, m.p. 155–156°. See Tables II and III for analytical data.

Table II—NMR Absorption Frequencies for Amidines

Compd.	Solvent	N—H (δ, p.p.m.)	N—CH_3 (δ, p.p.m.)
I	DMSO	9.10	
II	DMSO	9.23	
III	DMSO	9.23	
IV	DMSO	9.13	
V	DMSO	9.10	
VI	DMSO	9.60	2.93
VII	DMSO	9.48	2.83
VIII	DMSO	9.57	2.88
IX	DMSO	9.33	2.93
X	$CDCl_3$	9.85, 10.2	3.30, 3.45
XI	$CDCl_3$	8.96, 10.2	3.27, 3.42
XII	DMSO	9.33	3.20
XIII	$CDCl_3$	8.87, 10.3	3.27, 3.48
XIV	$CDCl_3$	9.17, 9.87	3.17, 3.33
XV	$CDCl_3$	8.47	3.17, 3.25; 3.37, 3.45
XVI	$CDCl_3$	8.33	3.00 3.08; 3.33 3.37
XVII	$CDCl_3$	8.27	3.17, 3.23; 3.33, 3.43

Aryloxy-N-methylthioacetamides

N-Methyl-2-(thymoloxy)thioacetamide (XVIII)—A solution of 7.8 g. (0.25 mole) of methylamine in 200 ml. of dry methanol was chilled while saturating with hydrogen sulfide. This solution was placed in a stainless steel bomb with 47.3 g. (0.25 mole) of 2-thymoloxyacetonitrile and the reactants were warmed at 90–100° for 14 hr. When cooled, the thioamide (51.0 g., 86%) precipitated as long needles. The analytical sample was recrystallized from a mixture of aliphatic naphthas[4] (1:1) to give long needles, m.p. 105–106°. The NMR spectra and elemental analyses were consistent for the desired structures (see Table IV).

[4] Skellysolves B and F, Skelly Oil Co., Kansas City, Mo.

Aryloxythioacetimidate Hydroiodides

Methyl N-Methyl-2-(thymoloxy)thioacetimidate Hydroiodide—A 1-l., three-necked flask was equipped with stirrer, addition funnel, and reflux condenser and then a solution of 400 ml. of dry acetone, 40.0 g. (0.17 mole) of *N*-methyl-2-(thymoloxy)thioacetamide (XVIII), and 72.0 g. (0.51 mole) of methyl iodide was stirred at reflux for 5 hr. After standing overnight, 48.0 g. (76%) of long needles precipitated. The product which decomposed (gassing) between 150–155° was used without further purification. This type of compound is reported to decompose to the methyl iodide and the starting amide by Peak (15). Therefore, elemental analyses gave inconclusive results. The NMR spectra were consistent for the desired structures, however. See Table V for additional details.

Nuclear Magnetic Resonance Spectral Data

The NMR spectra of the compounds prepared in this research effort were recorded on a spectrometer[3] operating at a frequency of 60 Mc.p.s. The chemical shifts of the protons were referenced internally to tetramethylsilane (TMS) at 0 c.p.s.

The unsubstituted amidines (I–V) all showed a broad singlet at 9.10–9.23 δ. Hammond (18) has reported that amidine protons will appear as a doublet in deuterated anhydrous DMSO with absorption frequencies of 9.23 and 10.13 δ. However, he also noted that this doublet coalesced into a broad singlet when a small amount of water was added to the solvent. Since no care was taken to specially dry the DMSO used in these measurements it is assumed that the broad singlet noted for Compounds I–V is due to the presence of traces of water in the solvent.

The *N*-methylamidines (VI–IX) had two characteristic absorption bands when measured in DMSO. The N—H absorption band ap-

Table III—Aryloxy-*N*,*N*,*N*′-trimethylacetamidines

$$R{-}O{-}CH_2C(=NCH_3){-}N(CH_3)_2 \cdot HI$$

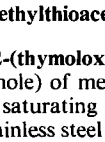
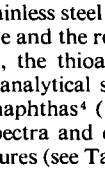
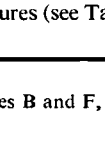
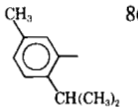
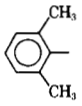
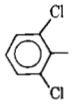
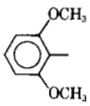
Compd.	R	Yield, %	M.p., °C.	Anal. Calcd.	Anal. Found
XV		27	155–156	C, 47.89 H, 6.70 N, 7.44	C, 47.98 H, 6.69 N, 7.31
XVI		37	176–178	C, 44.83 H, 6.08 N, 8.04	C, 44.66 H, 5.77 N, 7.89
XVII		36	166–168	C, 41.06 H, 5.58 N, 7.36	C, 40.86 H, 5.39 N, 7.08

Table IV—Aryloxy-*N*-methylthioacetamides $RO-CH_2-\overset{\overset{S}{\|}}{C}-NHCH_3$

Compd.	R	Yield, %	M.p., °C.	Anal. Calcd.	Anal. Found
XVIII		86	105–106	C, 65.81 H, 8.07 N, 5.90	C, 65.99 H, 8.19 N, 5.95
XIX		76	59–60	C, 63.15 H, 7.22 N, 6.69	C, 63.44 H, 7.36 N, 6.60
XX		66	91–92	C, 43.22 H, 3.60 N, 5.60	C, 43.67 H, 3.30 N, 5.52
XXI		87	87–89	C, 54.77 H, 6.26 N, 5.80	C, 55.15 H, 6.27 N, 5.80

peared as a singlet between 9.33 and 9.60 δ, while the N—CH_3 absorption band appeared as a singlet at about 2.88 δ. Both of these values are in good agreement with the recorded MR characteristics of protons bound to nitrogen (18).

The use of $CDCl_3$ as a solvent for the *N,N*-dimethyl and *N,N,N'*-trimethylamidines changed the spectral characteristics of the amidines. The N—H absorption band for the *N,N*-dimethylamidines appeared as broad doublets between 8.87–10.30 δ, while the N—CH_3 bands appeared as doublets at approximately 3.23 and 3.42 δ. This type of splitting in anhydrous solvents is consistent with the data reported by Hammond (18). One compound (XII) was not sufficiently soluble in $CDCl_3$. Measurement of its NMR in DMSO gave singlets for both the N—H and N—CH_3 protons. This variance may once again be due to the presence of trace amounts of water in the DMSO.

The *N,N,N'*-trimethylamidine moiety appeared as a pair of doublets between 3.00–3.45 δ with the integrated areas for the

Table V—Aryloxythioacetimidates

$$R-O-CH_2-\overset{\overset{SCH_3}{|}}{C}=NCH_3\cdot HI$$

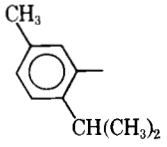
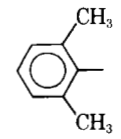
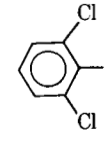
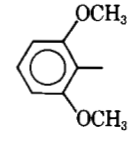
Compd.	R	Yield	M.p., °C.
XXII		76	150–155
XXIII		85	175–180
XXIV		49	127–132
XXV		64	144–148

Table VI—Effect of Aryloxyacetamidines on the Release of Norepinephrine

Compd.	NE Control, %
I	32 ± 2[a]
II	78 ± 5[a]
III	86 ± 1[a]
IV	94
V	75 ± 2[a]
VII	89
VIII	107
IX	90
X	96 ± 3
XI	101 ± 6
XII	98 ± 2
XIII	110
XIV	94 ● 5

[a] $p < 0.95$.

N—CH_3 *versus* the N'—CH_3 in the proper 2 to 1 ratio. The N'—CH_3 signal was upfield to the N—CH_3 signal. The N—H proton signal of the trimethylamidines appeared as a broad band at about 8.33 δ.

All the NMR frequency absorption bands for the amidine regions of compounds reported in this paper are tabulated in Table II.

Pharmacological Evaluation of Amidines

Thirteen of the aryloxyacetamidines (I–V, VII–XIV) were screened for potential pharmacological activities by the Cardiovascular Group of the Lilly Research Laboratories, Indianapolis, Ind., under the direction of Dr. J. A. Leighty. The authors are most grateful to the Eli Lilly Company for providing these test results.

Mouse Behavior Screen—Each of the compounds was injected intraperitoneally into mice. Changes in the behavior of these animals were observed over a period of several hours. Survivors were kept for 1 week. Most of the compounds did not show patterns of behavior of great interest. In most cases the animals convulsed and died within 10 min. after the injection of a dose of 100 mg./kg. intraperitoneally. Other animals treated with 25 mg./kg. showed no change in spontaneous activity and the various other signs and symptoms examined for the usual mouse behavior test. Only one compound, *N,N*-dimethyl-2-thymoloxyacetamidine hydrochloride, showed any significant pharmacological activity. It appeared to be an anticholinergic compound, an effect previously noted by Djerassi (16).

Norepinephrine-Releasing Properties—To test for this type of activity, each of the acetamidines was injected intraperitoneally into male Sprague-Dawley rats weighing approximately 200 g. The dose administered was 0.1 mole/kg. or about 20 mg./kg. for most of the compounds. The animals were sacrificed 8 hr. later, the hearts removed, and total catecholamines were determined by the method of Chang (19). None of the compounds tested caused a remarkable decrease in catecholamines although a few did cause some significant decreases. The results of this experiment are summarized in Table VI.

Adrenergic Neuron-Blockade Evaluation—Several of the compounds submitted were screened as adrenergic neuron blocking agents by the method of Finkleman (20). This preparation records the spontaneous activity of an isolated strip of the small intestine of the rabbit. Stimulation of the sympathetic nerve leading to the gut segment causes inhibition of contractions. Compounds II, III, V, and XIV were tested for their ability to block inhibitions of the intestines mobility when stimulated. None of these compounds were active when applied to the organ bath in a concentration of 6×10^{-7} *M*.

REFERENCES

(1) F. C. Copp, *Advan. Drug Res.*, **1**, 161(1964).

(2) J. Augstein, S. M. Green, A. M. Munro, G. W. H. Potter, C. R. Worthing, and T. I. Wrigley, *J. Med. Chem.*, **8**, 446(1965).

(3) A. Pinner, "Die Imidoather und ihre Derivate," R. Oppenheim, Berlin, Germany, 1892.

(4) W. Steinkopf and W. Malinowski, *Ber.*, **44**, 2898(1911).
(5) C. A. McKenzie and L. R. Webb, *J. Org. Chem.*, **18**, 594 (1953).
(6) F. C. Schaefer and G. A. Peters, *ibid.*, **26**, 412(1961).
(7) F. L. Pyman, *J. Chem. Soc.*, **1923**, 3359.
(8) J. von Braun and W. Pinkernelle, *Ber.*, **67B**, 1218(1934).
(9) M. Grdinic and V. Hahn, *J. Org. Chem.*, **30**, 2381(1965).
(10) M. Seefelder, German pat. 1,078,568 (Mar. 31, 1960).
(11) H. Bredereck, F. Effenberger, and E. Henseleit, *Ber.*, **98**, 2754(1965).
(12) H. Meerwein, *Org. Syn.*, **46**, 113(1966).
(13) L. A. Paquette, *J. Am. Chem. Soc.*, **86**, 4096(1964).
(14) M. Rogers, *J. Chem. Soc.*, **1950**, 3350.
(15) D. Peak and F. Stansfield, *ibid.*, **1952**, 4067.
(16) C. Djerassi and C. R. Scholz, *J. Am. Chem. Soc.*, **69**, 1688(1947).
(17) J. Quarterman and T. S. Stevens, *J. Chem. Soc.*, **1955**, 3292.
(18) G. S. Hammond and R. C. Neumann, Jr., *J. Phys. Chem.*, **67**, 1655(1963).
(19) C. C. Chang, *Int. J. Neuropharmacol.*, **3**, 643(1964).
(20) B. Finkleman, *J. Physiol.*, **70**, 145(1930).

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Colorimetric Determination of Some *N*-1-Substituted Nitroimidazoles

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Abstract □ A colorimetric method is presented for the determination of some *N*-1-substituted nitroimidazoles. The method utilizes diazotization of a sulfanilamide with the nitrite ions produced during the alkaline hydrolysis of the respective imidazole. Subsequent coupling is then carried out with Bratton-Marshall reagent. The sensitivity of this method of analysis is approximately 0.1 mcg./ml. This procedure can be used for the determination of one of the *N*-1-substituted nitroimidazoles, 1-methyl-2-isopropyl-5-nitroimidazole, in feed premixes containing multiple vitamins, and is herein presented.

Keyphrases □ Nitroimidazoles, *N*-1-substituted—analysis □ Sulfanilamide reagent—diazotization, imidazole hydrolysis □ Bratton-Marshall reaction—analysis □ Colorimetric analysis—spectrophotometer □ 1-Methyl-2-isopropyl-5-nitroimidazole—determination, feed premix

An important class of compounds which are effectively used as antiprotozoal agents are substituted nitroimidazoles. Nitroimidazoles can be analyzed by polarography (1, 2) and by reduction of the nitro group to the corresponding amine, which is subsequently determined by diazotization and coupling reaction (3). A rapid and sensitive colorimetric procedure based on the hydrolysis of the nitroimidazoles was developed for the quantitative determination of *N*-1-substituted nitroimidazoles and the method of determination of one of these compounds in feed premix is described.

Although quantitative spot tests have been reported (4) for the alkaline hydrolysis of aliphatic nitro and aromatic polynitro compounds, a quantitative procedure for the determination of heterocyclic nitro compounds such as the nitroimidazoles based on the alkaline hydrolysis of the molecule was not found in the literature.

In the course of these kinetic studies (5) pertaining to the hydrolysis of nitroimidazoles, it was observed that the nitrite ion was found in the reaction. Controlled conditions for the hydrolysis permitted stoichiometric liberation of nitrite ion which can be used for the determination of the corresponding nitroimidazoles. A number of procedures for the determination of nitrite ion which involve diazotization and coupling reactions have been reported (6, 7). A very convenient method, with good sensitivity, is the Bratton-Marshall color reaction (3). This procedure involves the diazotization of sulfanilamide with nitrous acid produced to form a diazonium salt in acidic medium. The diazonium salt is then treated with an aromatic coupling compound forming a colored substance suitable for spectrophotometric measurement.

The concentration of the *N*-1-substituted 5-nitroimidazoles after alkaline hydrolysis, diazotization, and coupling was found to be proportional to absorbance.

The rate of hydrolysis and the formation of nitrite ions from the substituted 5-nitroimidazoles depends upon the structural configuration. Graphic presentation of the preliminary kinetic data illustrates the substituent effect for the alkaline hydrolysis of this type of compound as shown in Fig. 1 and the corresponding structures as given in Table I. Compound I exhibited good stability and did not hydrolyze in 0.1 *N* sodium hydroxide to form nitrite ions. As is evident from Table I, it appears that