Theses and Dissertations                                                                 Graduate School

1982

# Synthesis of 3-(Substituted-aryl)-1,2,3,4-oxatriazolium-5-olates As Potential Hypotensive Agents

Mary Quinn Lund

Synthesis of 3-(Substituted-aryl)-1,2,3,4-oxatriazolium-5-olates

As Potential Hypotensive Agents


BY

Mary Quinn Lund

B.S., S.U.N.Y. Stony Brook, 1972


Thesis

Submitted in partial fulfillment of the requirements for the

Degree of Master of Science in the Department of

Pharmaceutical Chemistry at the Medical College of Virginia

Health Sciences Division, Virginia Commonwealth University

Richmond, Virginia

This thesis by Mary Quinn Lund is accepted in
its present form as satisfying the thesis requirement for the
Master of Science.

Date:                                           Approved:

12/16/81


Advisor, Chairman Graduate
Committee

12/16/81

12/17/81

12/17/81

12/18/81

APPROVED

Chairman, MCV Graduate Council
Dean, School of Basic Sciences

Curriculum Vitae

ACKNOWLEDGEMENTS

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION


A.  Hypertension


Hypertension or high blood pressure is a major cause of illness and death in the United States today.[1,2]  An estimated thirty-six million people suffer from this disease.[3]  Prolonged hypertension and its attendant strain on various organs may cause heart failure, brain stroke or kidney damage.[4]  Usually, a pressure of 140/90 mm Hg is taken to be the dividing line between normotension and hypertension.[5,6]

The hypertensive condition is generally characterized as either primary (essential) hypertension or secondary hypertension.[1]  A specific cause can be identified in secondary hypertensive patients, such as, pheochromocytoma or adrenal tumor.  These causes can typically be corrected by surgery.  Essential hypertension occurrs in about 90% of the hypertensive population.  The etiology of essential hypertension is unknown.[1,3,6]  The increase in diastolic pressure in primary hypertension may take on either a gradual (benign) or accelerated (malignant) course.  The prognosis of gradual hypertension is more favorable than that of the accelerated.  Blood pressure in the essential hypertensive patient can almost always be controlled by drug therapy.

It is currently accepted that in most hypertensive cases the primary abnormality is due to a high peripheral resistance.[7]  Numerous

biological feedback mechanisms interact in trying to return the body to the normotensive state.[8] Some factors involved in the maintenance of homeostasis are central and peripheral sympathetic activity, renal pressor and depressor mechanisms, antidiuretic hormone, sodium balance, baroreceptors, small blood vessel resistance, blood volume and viscosity.[1,4,7]

Attempts have been made to rationalize the cause of the increase in peripheral resistance in essential hypertension as due to either humoral or neurogenic factors.[6,7] DeQuattro et al.[9] have classified the humoral causes into nephrogenic and steriodogenic agents. The humoral factors involve the regulation of salt and water content, which are mainly under control of the kidneys and adrenal cortex. These factors have been implicated in the sensitivity of the small resistive arterioles.[10] Freis has suggested, based on epidemiology, the existance of a critical maximum dietary sodium daily intake of 60 mmol.[11] He found that hypertension was common in communities with an average sodium intake above this level, but on an individual basis, blood pressure has not been correlated to the amount of salt.

Neurogenic factors have been cited because experimental lesions of the central nervous system have produced hypertension.[12,13] These lesions have resulted in the resetting of the barostat to a higher level.

Many physiological structures are involved in the neural regulation of blood pressure as can be seen in Figure 1.[6] Peripheral vascular resistance, mainly due to arterial contraction is under the control of

Figure 1.[6]   Physiological structures involved in neural regulation of
blood pressure.



Higher centers

Medulla, vasomotor centers

IX

Carotid sinus

X

Aortic arch

α-Adrenergic
receptor

Sympathetic
ganglia

β-Adrenergic
receptor

Arteriole smooth
muscle

Adrenal medulla

Renal renin
release

β-type
receptor
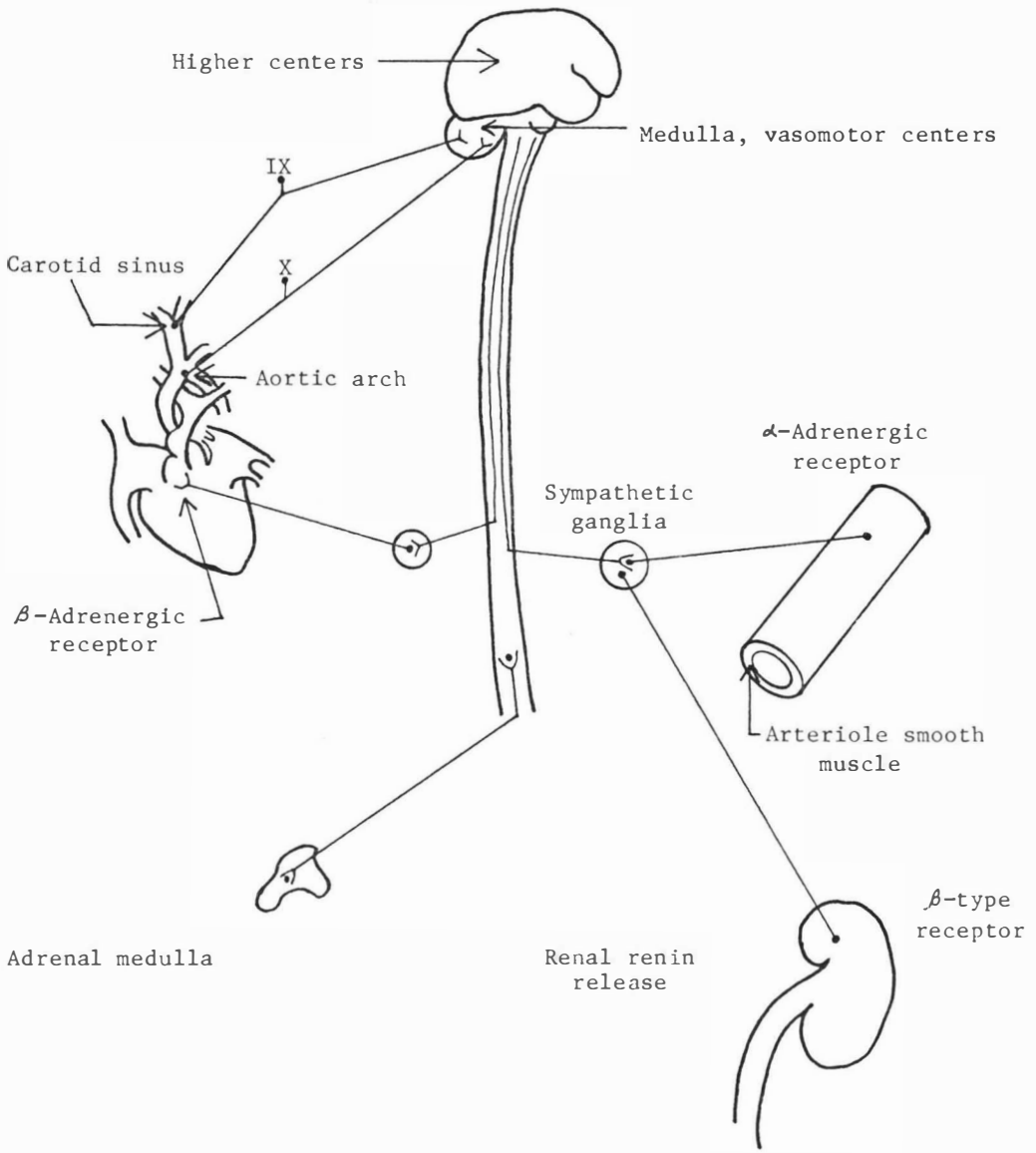
the vasomotor center located in the medulla. This center is innervated from other central areas as well as peripheral baroreceptors. Some baroreceptors are located in the carotid sinus and aortic arch. An increase in arterial pressure as sensed by these baroreceptors causes afferent traffic along cranial nerves IX and X, to initiate a decrease in the sympathetic outflow from the vasomotor centers. Also, there is an increase in vagal outflow to cause a decrease in the heart rate which will tend to lower blood pressure. Efferent nerve fibers travel down the spinal cord and synapse via connector cells at the sympathetic ganglia. Stimulation of the postganglionic fibers results in norepinephrine release, causing vasoconstriction through $\alpha$-adrenergic interaction on the arterioles and tachycardia mediated by $\beta$-adrenoceptors. Stimulation of the preganglionic fiber innervating the adrenal medulla results in the release of epinephrine into the systemic circulation resulting in a general adrenergic response. The sympathetic nervous system also exerts fine control of renin release from the kidney via a $\beta$-type adrenergic receptor.[14]

An increase in either cardiac output or sympathetic activity will cause an apparent increase in plasma volume and pressure in the glomerular capillaries. In the normal individual, this results in an increase in the glomerular filtration rate and an increased excretion of sodium due to reduced amounts of the humoral factors, aldosterone and antidiuretic hormone (ADH).

Sodium reabsorption is controlled by the hormone aldosterone which is released by the adrenal cortex. Aldosterone secretion is controlled

by reflexes initiated by the kidney. Cells within the kidney detect the amount of sodium in the tubules. A reduction in tubular sodium concentration stimulates the secretion of the protein renin into the blood. Renin enzymatically cleaves circulating angiotensinogen to the smaller polypeptide angiotensin I. Angiotensin I is cleaved to angiotensin II by the converting enzyme. Angiotensin II is a potent stimulator of the adrenal cortex, causing it to secrete aldosterone. This results in the tubular reabsorption of sodium. The sequence can be seen in Figure 2.

Changes made in sodium excretion must be accompanied by changes in water excretion in order to regulate extracellular volume. As blood pressure increases, baroreceptors located in the left atrium are stimulated to cause inhibition of the ADH neurons in the hypothalmus. Thus, as blood pressure rises in a normotensive person, a number of neurogenic and humoral factors interact to return the pressure to normal. In the hypertensive patient, at least one of these factors is at fault and homeostasis cannot be maintained.

Figure 2.  Sequence of events leading to an increase in aldosterone
secretion.

Kidney

Liver                    ($\downarrow Na^+$)          Adrenal Cortex

Renin

Aldosterone

Converting
Enzyme

Angiotensinogen ⟶ Angiotensin I ⟶ Angiotensin II
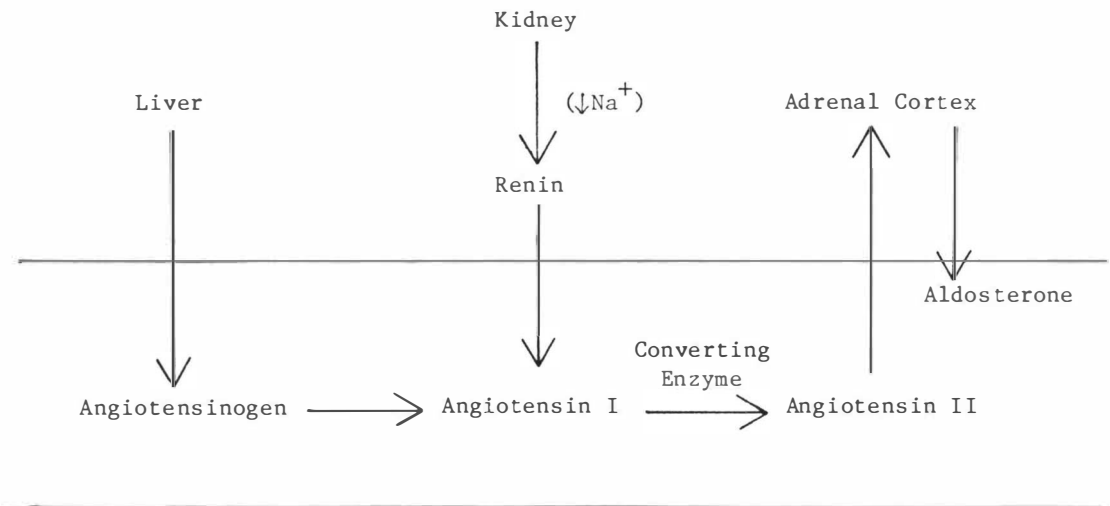
B.  Drug Therapy

Since correction of the underlying cause of essential hypertension is not possible at the present time, it has been accepted practice to treat the disease with antihypertensive agents.[15]  These drugs help to reduce the severity of the hypertensive condition and its complications by acting upon physiological mechanisms which regulate the control of blood pressure.

Antihypertensive agents can be classified into five groups based on the primary mechanism involved to elicit an antihypertensive response: diuretics, direct-acting vasodilators, angiotensin analogues, converting enzyme inhibitors, and sympathetic antagonists.[5,7]

There are three major classes of diuretics, the thiazide diuretics, the 'loop' diuretics, and the potassium-sparing agents.  The thiazide diuretics (e.g. chlorothiazide, 1) interfere with the active chloride reabsorption in the distal convoluted segment of the renal tubule. The 'loop' diuretics (e.g. furosemide, 2) block the active transport of chloride ions in the ascending limb of the loop of Henle.  The potassium-sparing diuretics (e.g. triamterene, 3) cause a decrease in the amount of potassium secreted in the distal convoluted tubule.[1]
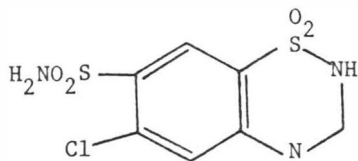
1
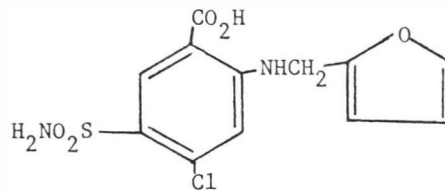


2
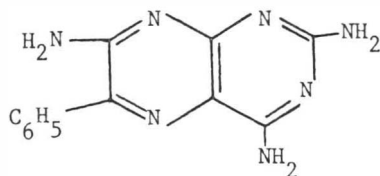


3

Direct-acting vasodilators produce relaxation of the vascular smooth muscle. These agents exclude those compounds whose main mechanism for effect is mediated by the adrenergic nervous system. The precise biochemical mechanism for the vasodilators, such as hydralazine (4), has not been elucidated.[16]
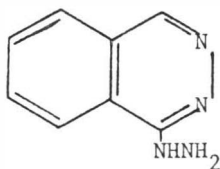


4

The enzymatic conversion of inactive angiotensin I to angiotensin II is inhibited by converting enzyme inhibitors such as captopril. Synthetic analogs, of the potent vasoconstrictor angiotensin II, (e.g. saralasin) compete with circulating angiotensin II for receptor sites within vascular smooth muscle, adrenal cortex and neural tissue.[1]

Sympathetic inhibition may occur through a single or a combination of four main sites of action: central, ganglionic block, blockade of neuroeffector transmission, or adrenergic receptor block. Adrenoceptors are classified as $\alpha$- or $\beta$-receptors.

Ganglionic blocking agents decrease cardiac output as well as total peripheral resistance.[17,18] This occurs by the blockade and the displacement of acetylcholine from nicotinic receptor sites postjunctional to the sympathetic ganglia. Ganglionic blocking drugs, such as hexamethonium (5), have severe side effects associated with parasympathetic blockade and their use has been superseded by more selective agents.

$$(CH_3)_3\overset{+}{N}-(CH_2)_6-\overset{+}{N}(CH_3)_3 \qquad 2Cl^-$$
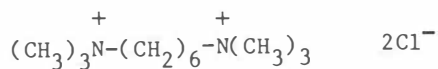
5

Adrenergic neurone blocking drugs, such as guanethidine (6), selectively inhibit the sympathetic nervous system. These agents inhibit the release of norepinephrine at the neuroeffector junction.

They are actively taken up and stored within the intraneuronal storage vesicles, causing a depletion of norepinephrine. The adrenergic neurone blockers are very potent antihypertensive drugs, but unfortunately they have many side effects.[5]



$$\text{CH}_2\text{CH}_2\text{NHCNH}_2 \quad (NH)$$

**6**
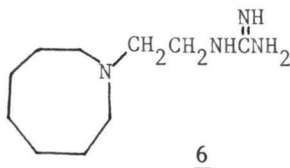
The main site of antihypertensive action of $\alpha$-methyldopa (7) is within the central nervous system. Alpha-methyldopa crosses the blood-brain barrier and is converted to $\alpha$-methylnorepinephrine within the central adrenergic neurons. Stimulation of these central neurons results in the release of both norepinephrine and $\alpha$-methylnorepinephrine. The $\alpha$-methylnorepinephrine stimulates central $\alpha$-adrenergic receptors causing a decrease in sympathetic efferent activity to result in the lowering of arterial pressure.[5,6]



**7**
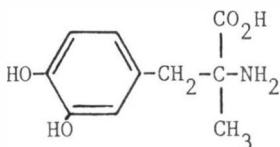
In response to a decrease in cardiac output by $\beta$-adrenergic
blocking agents, such as propranolol (8), peripheral resistance is
initially increased.  If the $\beta$-blockade is maintained, cardiac output
remains at the lowered level and peripheral resistance decreases
resulting in a lowering of blood pressure.[5]



8
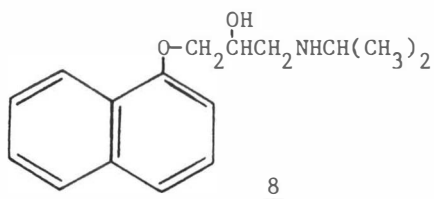
Agents which block the $\alpha$-adrenergic receptor also have peripheral
vasodilator properties.  Prazosin (9), a post-synaptic $\alpha$-adrenergic
antagonist, lowers arterial pressure by decreasing peripheral vascular
resistance.[19]



9
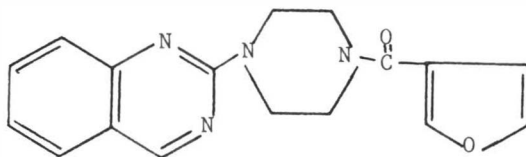
The above antihypertensive agents are currently being used in the
stepped treatment of hypertension.[15]  This empirical approach has been

initiated because a specific pathophysiological abnormality is rarely identified in the hypertensive patient. As the severity of the hypertensive disorder increases, a drug from the next step, as shown in Figure 3, is added.

Unfortunately, none of the agents used are without undesirable side effects.[16] Consequently, the search continues for antihypertensive agents with fewer side effects and with a longer duration of action.

Figure 3.[15]   The stepped care approach to antihypertensive drugs.

```
Step 1
     Diuretics
          thiazides
     'Loop' diuretics
          chloruretic sulfonamides
     Potassium-sparing diuretics
          triamterene
          spironolactone
Step 2
     Adrenergic inhibitors
          propranolol
          methyldopa
          reserpine
Step 3
     Vasodilators
          hydralazine
     Others
          prazosin
          clonidine
Step 4
     Postganglionic neuron blocking agents
          guanethidine
```

C.  Mesoionic Compounds


In 1935, Earl and Mackney found that the treatment of
$\underline{N}$-nitroso-$\underline{N}$-phenylglycine ($\underline{10}$, R=C$_6$H$_5$, R'=H) with acetic anhydride,
(Ac$_2$O), yielded a neutral anhydro compound.[20]  The structure of the
stable compound was tentatively proposed by Earl and Mackney to contain
a three-membered ring fused to a four-membered ring ($\underline{11}$, R=C$_6$H$_5$, R'=H).




|       10        |                 |       11        |
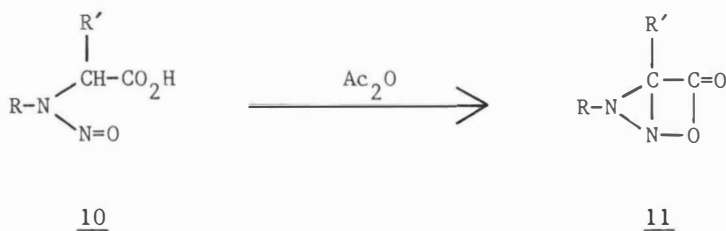
This bridged ring, containing a $\beta$-lactone type structure, was
found by Baker et al.[21] to be an unacceptable representation.  The
system as proposed would be highly strained and formation would require
more drastic conditions than treatment with acetic anhydride.  Evidence
against the strained $\beta$-lactone type structure includes the fact that
the compound does not readily decompose with heat.  Also, the compound
as drawn could be optically-active.  Compounds made from optically-
active starting material were not optically-active.  Electrophilic
aromatic substitution occurred on the carbon adjacent to the carbonyl
and not as expected on the benzene nucleus.  To account for these

properties, Baker et al.[21] described the compound as a resonance hybrid
of a large number of contributing forms. A few of these forms (12a-e)
can be seen in Figure 4. The term "mesoionic" was coined to denote the
contributing dipolar and tetrapolar forms. Ollis and Ramsden[22] in 1976
suggested that the definition include the fact that a sextet of
electrons is associated with a five- or six-membered ring where the
ring carries a small positive charge and the exocyclic atom or group
carries the corresponding negative charge. Ramsden[23] in 1979 proposed
that the term mesoionic should be restricted in its use to only
five-membered ring systems.

A single structure, 13, is used to represent these contributing
resonance forms. This planar representation is incapable of
optical-activity and the characteristic properties of $\beta$-lactones are
not expected. The benzene ring is deactivated toward electrophilic
substitution because of the positive charge delocalized about the ring.

R     R′
 \   /
  N — C
 /    \
N  (+)  C — O⁻
 \    /
   O
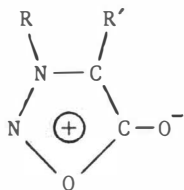
13

The mesoionic compound, 13, is systematically named as a 3-R-4-R′-
1,2,3-oxadiazolium-5-olate. This basic ring system, the oxadiazole

Figure 4.  A few forms contributing to the resonance hybrid structure of mesoionic sydnones, 12a-e.
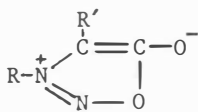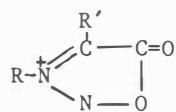


12a                                        12b
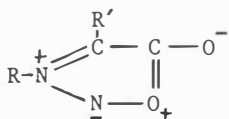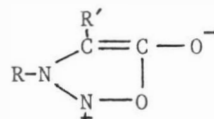


12c                                        12d
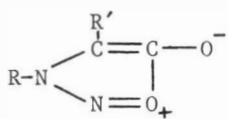


12e

with an exocyclic oxygen, has been given the trivial name "sydnone."
Other nomenclatures can be found in the literature.  It has been
suggested that the symbol $\Psi$ be used to represent the hybrid structure
of mesoionic compounds.  Compound 13 would thus be named as $\Psi$-5-keto-
3-R-4-R′-3,5-dihydro-1-oxa-2,3-diazole or $\Psi$-3-R-4-R′-1,2,3-oxadiazole-
5-one.  This later nomenclature has been reduced to the trivial name
$\Psi$-oxadiazolone.  Katritzky[24] has recommended the use of the betaine
nomenclature.  The compound would then be named anhydro(5-hydroxy-3-R-
4-R′-1-oxa-2,3-diazolinium hydroxide).

The major thrust in the investigation of the five-membered
mesoionic compounds has been in the areas of synthesis, structural
proof, chemical and physical properties.[25-32]  Excellent reviews are
available.[22,23,33,34]

Initial pharmacological investigation of mesoionic compounds began
in the early 1960′s with the report of antitumor activity by Greco[35]
and the subsequent report by Kier et al.[36] of central nervous system
stimulation.  The medicinal chemistry was reviewed by Ackermann[37] and
Kier and Roche[38] in 1967.

The structure of the N-alkylsydnones (14, R′=H), which possessed
convulsive activity, was compared by Kier and Dhawan[39] to the
anticonvulsives 15-17.  It was possible to reverse the action of the
anticonvulsives by alkylation of the nitrogen.  The 3,4-dialkylsydnones
were also shown to have convulsive properties.[40]

In 1964, Fregly et al.[41] synthesized and investigated three 3-
alkylsydnones for biological activity.  It was found that 3-butyl-4-
ethylsydnone (18) had three times the naturetic and chloruretic effect
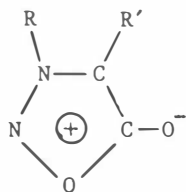
14
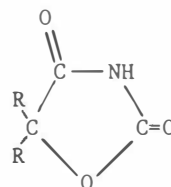


15
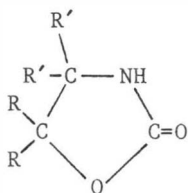


16
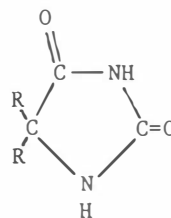


17

of the monoalkylated 3-(sec-butyl)sydnone (19) or the sodium salt of

3-isopropyl-4-carboxysydnone (20). A dose response curve was obtained

for the moderate depressor activity of 3-(sec-butyl)sydnone but as the

dose increased so did the stimulation of the central nervous system.

The reduction in blood pressure was immediate upon administration to

normotensive rats but the blood pressure returned to pre-injection

levels in a matter of minutes. Sodium 3-isopropylsydnone carboxylate

had no hypotensive activity while 3-butyl-4-ethylsydnone could not be

tested because of the strong convulsive activity. Greco and Kier[42]

synthesized some 3-phenyl-4-acylsydnones, 21. These compounds exhibited

a moderate hypotensive activity which was greater than that observed for

3-(sec-butyl)sydnone (19).

Bu    Et  
N—C  
N  (+)  C—O⁻  
O

**18**

s-Bu  
N—CH  
N  (+)  C—O⁻  
O

**19**

i-Pr   $CO_2^-Na^+$  
N—C  
N  (+)  C—O⁻  
O

**20**

$C_6H_5$  $\overset{O}{CR}$  
N—C  
N  (+)  C—O⁻  
O
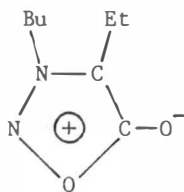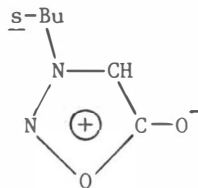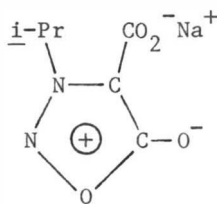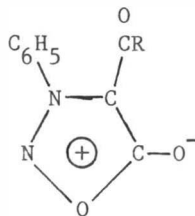
**21**

Kier et al.[43,44] synthesized a number of 3-alkyl-1,2,3,4-oxatri-azolium-5-olates, **22**. Hypotensive activity was measured in the anesthetized dog. Varying the R group, the following order of hypotensive activity was observed:  t-Bu > s-Bu ≅ 3-pentyl ≅ i-Pr > Et > Me.  Pretreatment of the dogs with atropine or pyribenzamine did not influence the depressor effects of the Δ-oxatriazoles, **22**. The 3-alkyl-Δ-oxatriazoles appeared to potentiate responses to epinephrine.
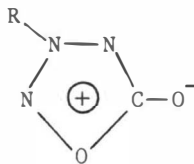
$$\underline{22}$$

Kier and Keating[45] synthesized 4-oxatriazoles with larger
substituents. The results of some compounds having fair hypotensive
activity in the anesthetized dog can be seen in Table 1. The onset of
effect was noted to be within minutes.

Thomas et al.[46] synthesized higher homologs and dimers (bis
compounds) of the 3-alkyl-1,2,3,4-oxatriazolium-5-olates. The
compounds in Table 2 were evaluated for possible hypotensive activity
in mice and dogs. Active compounds had a rapid onset of action after
an oral or intravenous administration. Maximum activity was attained
within thirty minutes. A dose-response curve was obtained for 1-10
mg/kg whereupon further increases in the dosage did not increase the
response. These compounds were found to decrease systolic, diastolic
and pulse pressures.

Table 1.[45]  Blood pressure response and duration of effect of 3-substi-
tuted-1,2,3,4-oxatriazolium-5-olates, 22.
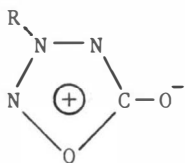


22
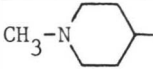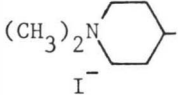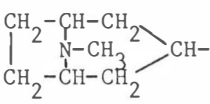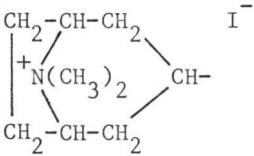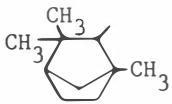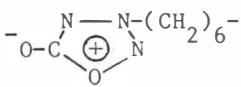
| R | Dose (mg/kg) | % Decrease in Blood Pressure[a] | Duration (hr) |
|---|---|---|---|
| $CH_3CH_2CHCH_3CH_2-$ | 25-40 | 0 | 0 |
| (see structure) CH— | 10-50 | (10-70) | 0.5 |
| $C_6H_5CH_2CHCH_3$ | 10-25 | 20-30 | 1-2 |
| $CH_3-N$ (ring) | 5-20 | 25-30 | 1-2 |
| $(CH_3)_2CHCH_2CHCH_3$ | 10-50 | 40-50 | 3 |
| $(CH_3)_2NCH_2CHCH_3$ | 50 | 25-30 | 4.5 |
| $4-CH_3C_6H_4CH_2-$ | 50 | 25 | 6 |

[a] No control reported.

Table 2.[46]  Percent decrease in blood pressure for 5 mg/kg intravenous dose of 3-substituted-1,2,3,4-oxatriazolium-5-olates, 22.

$$\begin{array}{c} R-N-N \\ |\;\oplus\;\;C-O^- \\ N-O \end{array}$$

<u>22</u>

| R | % Decrease in Blood Pressure[a] |
|---|---|
| $CH_3-N\hspace{-0.5em}\bigcirc\hspace{-0.5em}-$ | 15-30 |
| $(CH_3)_2N\hspace{-0.5em}\bigcirc\hspace{-0.5em}-$ $I^-$ | Negligible |
| $\begin{array}{c}CH_2-CH-CH_2 \\ |\hspace{2em}N-CH_3\hspace{0.5em}\rangle CH- \\ CH_2-CH-CH_2\end{array}$ | 0-15 |
| $\begin{array}{c}CH_2-CH-CH_2 \hspace{2em} I^- \\ \overset{+}{N}(CH_3)_2 \hspace{1em} CH- \\ CH_2-CH-CH_2\end{array}$ | Negligible |
| $\begin{array}{c}CH_3\\CH_3\hspace{-1em}\diagdown\hspace{-1em}\diagup CH_3 \\ \hspace{2em}-CH_3\end{array}$ | Negligible |
| $4-CH_3C_6H_5CH_2\overset{|}{C}HCH_3$ | 15-30 |
| $C_6H_5CH_2CH_2-$ | Negligible |
| $\begin{array}{c}\;\;\;\;N-N-(CH_2)_6- \\ ^-O-C\;\oplus\;\;N \\ \;\;\;\;O\end{array}$ | 15-30 |
| $(CH_3)_2CHCH_2\overset{|}{C}HCH_3$ | 0-15 |
| $CH_3(CH_2)_3CHCH(CH_2CH_3)CH_2-$ | 0-15 |

[a]No control reported.

The mesoionic oxadiazole compound 23a ($R=C_6H_{11}$, R'=morpholine) has been reported to lower blood pressure in man at low doses by a vasodilator mechanism.[47] This structure is an analog of compound 23b which had been reported earlier to be active in dogs.[48-51] The activity in spontaneous hypertensive rats of analogs of the mesoionic oxadiazoles has been compared.[52] The antihypertensive activity of the oxadiazole was retained when the 4-position hydrogen was replaced with a halogen. This activity was lost when the ring hydrogen was replaced by a methyl group.



23

| | R | R' |
|---|---|---|
| 23a | $\underline{c}-C_6H_{11}$ | N O (morpholine) |
| 23b | $-OC_2H_5$ | N O (morpholine) |
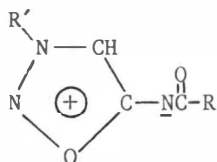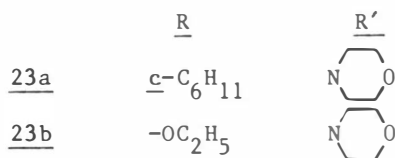
Many inquiries have focused on the synthesis of drugs which would have potent hypotensive activity. Investigation of the therapeutic potential of mesoionic compounds is in its infancy.

RESEARCH AIM


The high incidence of hypertension of unknown etiology and poor
compliance using existing agents make it desirable to investigate new
compounds for hypotensive activity.

The following order of hypotensive activity was observed in three
groups of mesoionic compounds:[43]   3-alkyl-$\psi$-oxatriazole > 4-acyl-
sydnone > 3-alkylsydnone.  A correlation was found between the increase
in hypotensive activity and the decrease in the charge density on the
number three nitrogen atom.  The calculated charge density on the
number three nitrogen atom of 3-phenyl-$\psi$-oxatriazole was found to be
less than that of 3-alkyl-$\psi$-oxatriazole.

One goal of this research is to synthesize various substituted aryl
analogs of the $\psi$-oxatriazolone system.  Three of the aryl compounds,
3-phenyl-[53-57], 3-(4-nitrophenyl)-[54,55], and the 3-(4-chloro-
phenyl)-[55,57] $\psi$-oxatriazolone have previously been synthesized.  The
substituted aryl-1,2,3,4-oxatriazolium-5-olates, 24a-h are seen in
Figure 5, will be prepared according to Scheme 1.[53,57-59]

The structure of the proposed compounds will be confirmed by
infrared spectral analysis, ultraviolet spectral analysis and carbon,
hydrogen, and nitrogen analyses.  Integrated carbonyl absorption

intensities will be calculated and mass spectra fragmentation patterns investigated.

It is a purpose of this research to add to the knowledge gained from Kier[45] and Thomas[46] by pharmacologically testing these mesoionic compounds for hypotensive activity. Possible vehicles for administration of the mesoionic $N$-oxatriazolones will be examined. The $LD_{50}$ will be estimated[60] in the male Sprague-Dawley rat. A dose-response curve will be obtained for 3-(4-methyl)phenyl-1,2,3,4-oxatriazolium-5-olate. The time course of action of the aryl analogs will be compared. The various substituents on the aryl ring will provide some information on the steric and electronic requirements for activity.

Figure 5.  Substituted 3-aryl-1,2,3,4-oxatriazolium-5-olates, <u>24</u>,
to be synthesized.
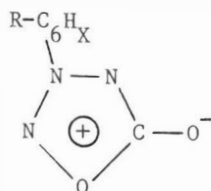


<u>24</u>

| No. | R | X |
|---|---|---|
| 24a | H | 4 |
| 24b | 4-Cl | 4 |
| 24c | 3,4-Cl$_2$ | 3 |
| 24d | 4-NO$_2$ | 4 |
| 24e | 4-CH$_3$ | 4 |
| 24f | 4-OCH$_3$ | 4 |
| 24g | 4-F | 4 |
| 24h | 2,6-Cl$_2$ | 3 |

Scheme 1.

$$K_2S_2O_5 \quad + \quad KCN \quad \xrightarrow{\hspace{3cm}}$$



$$\underline{25}$$

$$\xrightarrow{KNO_2} \qquad \bar{N}=\overset{+}{N}=C(SO_3K)_2 \quad \xrightarrow{K_2S_2O_5} \quad KO_3S-\overset{K}{\underset{|}{N}}-N=C(SO_3K)_2$$

$$\underline{26} \qquad\qquad\qquad \underline{27}$$

$$\xrightarrow{AcOH} \quad (KO_3S)_2C=NNHSO_3K \quad \xrightarrow{R-C_6H_XN_2^+} \quad R-C_6H_X-NHN=C(SO_3K)_2$$

$$\underline{28} \qquad\qquad\qquad\qquad \underline{29}$$

$$\xrightarrow{H^+}$$



$$\underline{24}$$
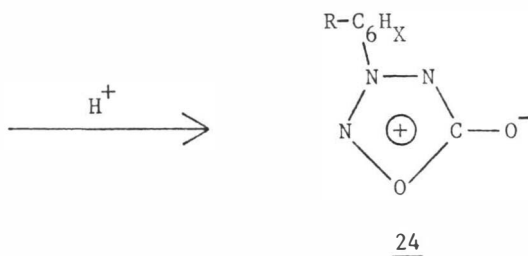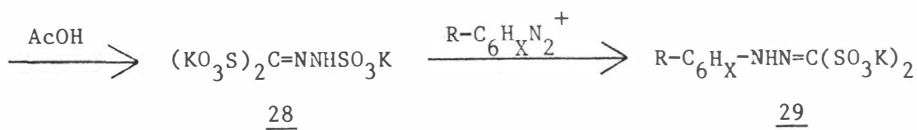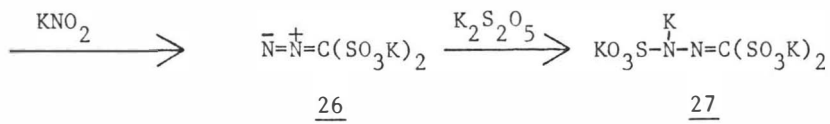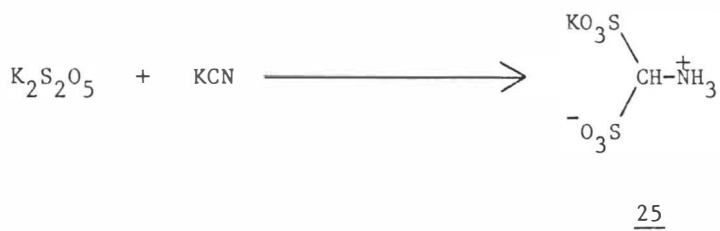
RESULTS AND DISCUSSION

A. Chemistry

The synthesis of monopotassium aminomethanedisulfonate (25) was
effected by the addition of an aqueous solution of potassium cyanide to
an aqueous solution of potassium metabisulfite at 55°C as described by
von Pechmann and Manck.[58]  The potassium salts were used for ease of
isolation.  An infrared spectral band at 3160 cm$^{-1}$ indicated the
presence of $NH_3^+$ group and the broad strong band between 1260–1210 cm$^{-1}$
was characteristic of the sulfonate group.

$$K_2S_2O_5 \quad + \quad KCN \quad \longrightarrow \quad H_3N^+-\overset{\displaystyle SO_3K}{\underset{\displaystyle SO_3^-}{CH}}$$

25

A proposed mechanism for the formation of aminomethanedisulfonate
is seen in Figure 6.  The hydrogen of the bisulfite ion (25a) is
subject to displacement by carbon of the nucleophile, cyanide (25b).
Evidence indicates that the hydrogen of the bisulfite ion is bonded to
the sulfur atom instead of the oxygen atom.[61-65]  The resulting
carbanion, 25c, transposes a hydrogen ion from another bisulfite ion to

Figure 6.   Proposed mechanism for the formation of potassium amino-
            methanedisulfonate.
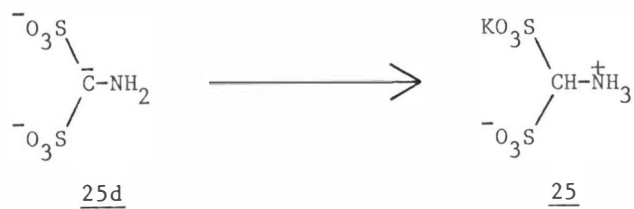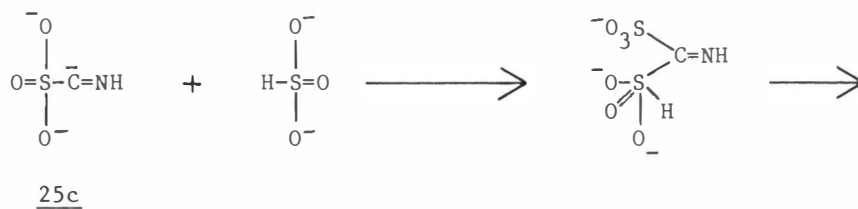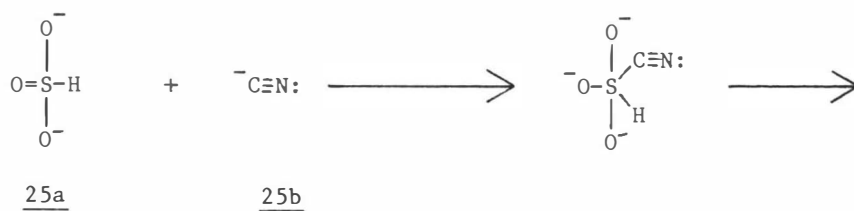
yield the intermediate 25d. Subsequent acidification results in the conversion of excess bisulfite to sulfur dioxide and the precipitation of the desired amine salt, 25.

The method of von Pechmann and Manck[58] afforded dipotassium diazomethanedisulfonate (26) by pouring cold aqueous potassium nitrite into a cold slurry of the previously synthesized amine. By quenching the reaction at 45°C high yields of the yellow product were obtained. The ultraviolet spectrum and the presence of a diazo band at 2100 cm$^{-1}$ in the infrared spectrum agreed with literature values.[66]

$$H_3\overset{+}{N}-CH\overset{\displaystyle SO_3K}{\underset{\displaystyle SO_3^{-}}{}} \quad + \quad KNO_2 \longrightarrow \quad \bar{N}=\overset{+}{N}=C(SO_3K)_2$$

25                                                26
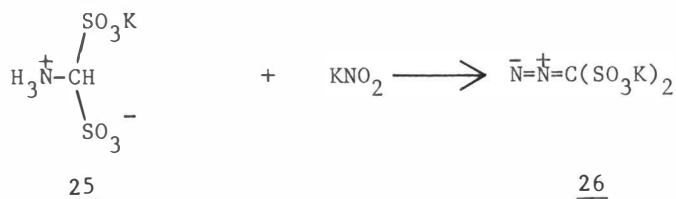
Tetrapotassium sulfohydrazonomethane disulfonic acid (27) was prepared in quantitative yield by treating the diazo compound with alkaline potassium metabisulfite as described by von Pechmann and Manck.[58] The structure of 27 was supported by the lack of the diazo band in the infrared spectrum. It was not necessary to isolate this white tetrapotassium salt. Treatment of the tetrapotassium slurry with concentrated acetic acid to pH 6.5 yielded quantitatively the tripotassium sulfohydrazonomethanedisulfonate (28).[58] In the infrared spectrum, a band characteristic of N-H stretch was seen at 3340 cm$^{-1}$.

$$\bar{N}=N=C(SO_3K)_2 \quad + \quad K_2S_2O_5 \quad \longrightarrow \quad KO_3S-\overset{\overset{\displaystyle K}{|}}{N}-N=C(SO_3K)_2$$

$$\underline{26} \qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad \underline{27}$$

$$\underline{27} \quad \longrightarrow \quad KO_3S-NH-N=C(SO_3K)_2$$

$$\underline{28}$$
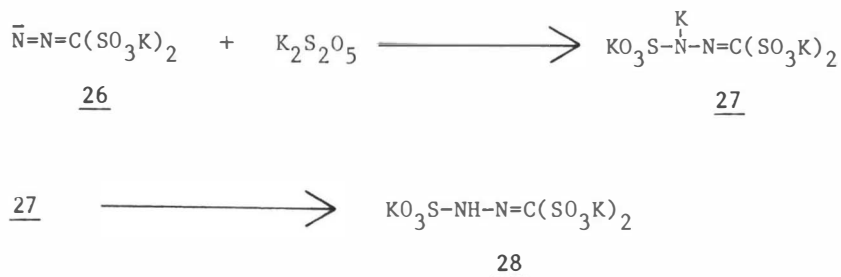
The next step involved the coupling of the neutral salt, $\underline{28}$, to an aryl diazonium acetate according to the procedure of von Pechmann.[53] The use of the neutral salt was preferred over the yellow diazomethane-disulfonate, $\underline{26}$, because of its greater stability.[57]

The appropriately substituted, recrystallized or freshly distilled, anilines were diazotized. The pH was adjusted to 5 by the addition of granular potassium acetate. The aryl diazonium acetate, $\underline{30}$, was added to an aqueous solution of the neutral salt. The colored effervescent solution was maintained at pH 5 by the addition of granular potassium acetate. The desired water-soluble product, $\underline{29}$, was isolated by concentration in vacuo then precipitation with ethanol. Support for the assigned structure was obtained using infrared spectral data. One spot was observed on thin layer chromatography. The dry, unsubstituted aryl hydrazone was also characterized by the violet color formed on treatment with concentrated sulfuric acid and ferric chloride as reported by von Pechmann.[53]

$$KO_3S-NH-N=C(SO_3K)_2 \quad + \quad R-C_6H_XN_2^+ \ {}^-OAc \longrightarrow R-C_6H_X-NHN=C(SO_3K)_2$$

$$\underline{28} \qquad\qquad\qquad \underline{30} \qquad\qquad\qquad\qquad \underline{29}$$
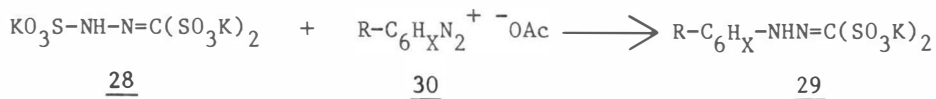
Potassium arylhydrazonomethanedisulfonate ($\underline{29}$) was treated with potassium nitrite. The water-soluble product which was not isolated may be represented as structure $\underline{31}$. The nitroso compound, $\underline{31}$, was added to 2N hydrochloric acid to effect cyclization. Fair yields of the desired aryl-1,2,3,4-oxatriazolium-5-olates, $\underline{24}$, were obtained. The assigned structure was supported by infrared spectra, melting point, elemental analysis and a negative Liebermann nitroso test. A single spot was seen on TLC. Additional structural support was gained on some of the mesoionic compounds using ultraviolet spectra, mass spectra and integrated carbonyl absorption intensities. Properties of the mesoionic compounds, $\underline{24a-h}$, are listed in Table 3.

$$R-C_6H_X-N-N=C(SO_3K)_2$$
$$\underset{N=O}{|}$$
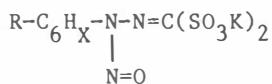
$$\underline{31}$$
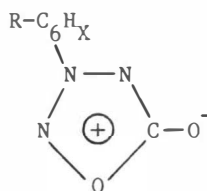
$$\underline{24}$$

Table 3. Properties of 3-(substituted-aryl)-1,2,3,4-oxatriazolium-5-olates, 24.

$$\text{R-C}_6\text{H}_X\text{-N}\overset{\displaystyle N-C-O^-}{\underset{\displaystyle N-O}{\underset{\displaystyle \oplus}{\Big|}}}$$
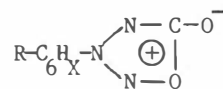
24

| no. | R | X | formula | mp$^a$,°C | lit val | % yield | anal. | calc (%) | found (%) | IR (CCl$_4$) C=O (cm$^{-1}$) | $\lambda_{max}$, nm ($\epsilon$) |
|-----|---|---|---------|-----------|---------|---------|-------|----------|-----------|----------|-------|
| 24a | H | 4 | C$_7$H$_5$N$_3$O$_2$ | 83–84 | 89[53] | 52 | | | | 1785 | 215 (7286) |
| | | | | | | | | | | | 265 (9643) |
| 24b | 4-Cl | 4 | C$_7$H$_4$ClN$_3$O$_2$ | 135–136 | 133[55] | 30 | | | | 1800 | 216 (8933) |
| | | | | | | | | | | | 273 (13267) |
| 24c | 3,4-Cl$_2$ | 3 | C$_7$H$_3$Cl$_2$N$_3$O$_2$ | 113–116 | | 35 | C | 36.21 | 36.32 | 1805 | 271 (12287) |
| | | | | | | | H | 1.29 | 1.38 | | |
| | | | | | | | N | 18.10 | 18.09 | | |
| 24d | 4-NO$_2$ | 4 | C$_7$H$_4$N$_4$O$_4$ | 164–165 | 166[54] | 25 | C | 40.38 | 40.34 | 1800 | |
| | | | | | | | H | 1.92 | 1.95 | | |
| | | | | | | | N | 26.92 | 26.97 | | |
| 24e | 4-CH$_3$ | 4 | C$_8$H$_7$N$_3$O$_2$ | 96–97 | | 50 | C | 54.24 | 53.99 | 1795 | 218 (8430) |
| | | | | | | | H | 3.95 | 4.04 | | 278 (12714) |
| | | | | | | | N | 23.73 | 23.63 | | |
| 24f | 4-OCH$_3$ | 4 | C$_8$H$_7$N$_3$O$_3$ | 135–136 | | 40 | C | 49.74 | 49.76 | 1790 | 220 (6929) |
| | | | | | | | H | 3.63 | 3.67 | | 305 (11929) |
| | | | | | | | N | 21.76 | 21.73 | | |
| 24g | 4-F | 4 | C$_7$H$_4$FN$_3$O$_2$ | 122–123 | | 33 | C | 46.41 | 46.52 | 1795 | 220 (8311) |
| | | | | | | | H | 2.21 | 2.30 | | 295 (12147) |
| | | | | | | | N | 23.21 | 23.19 | | |
| 24h | 2,6-Cl$_2$ | 3 | C$_7$H$_3$Cl$_2$N$_3$O$_2$ | 99–100 | | 18 | C | 36.21 | 36.26 | 1795 | |
| | | | | | | | H | 1.29 | 1.34 | | |
| | | | | | | | N | 18.10 | 18.11 | | |

$^a$Recrystallization solvent – methanol.

The fragmentation pattern, seen in Figure 7, of 3-phenyl-1,2,3,4-oxatriazolium-5-olate in the mass spectrometer was predicted by Doughery et al.[67] and was similar to that reported by Christophersen and Treppendahl[68] for structure 32.

$$C_6H_5$$

N——N

N $\oplus$ C—$\bar{N}$—CH$_2$CH$_3$

O

32
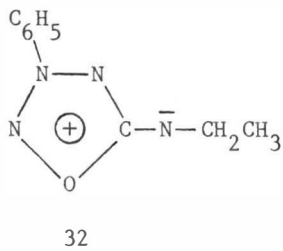
Integrated carbonyl absorption intensities for various types of carbonyl groups are reported in the literature and can be seen in Table 4. The integrated carbonyl absorption intensities of the 3-aryl-1,2,-3,4-oxatriazolium-5-olates were measured according to the procedure of Ramsey.[69] The absorption intensities of the carbon-oxygen bond were found to range from 10-12.7 indicating a high degree of molecular polarization as is represented by their mesoionic structure. These values are reported in Table 5.

Figure 7. Mass spectrum of 3-phenyl-1,2,3,4-oxatriazolium-5-olate, 24a.
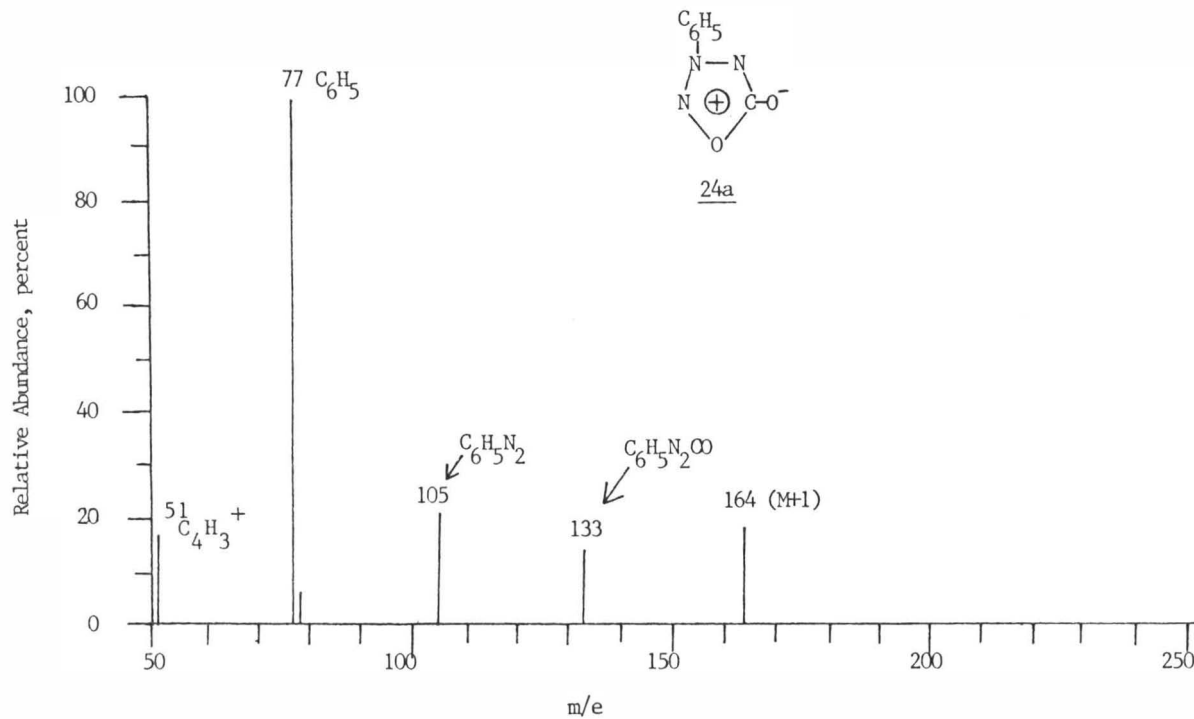
Table 4.  Integrated carbonyl absorption intensities.

| Compound | Absorption Intensity (A) |
|---|---|
| aldehydes, ketones[34] | 1.5–2.4 |
| esters[34] | 2.5–4.1 |
| amides[34] | 3.7–5.7 |
| butyrolactones[34] | 4.2 |
| sydnone[70] | 7.0–12.0 |
| mesoionic thiazolo[3,2-a]-pyrimidine-5,7-diones[71] | 9.9–11.7 |
| isosydnone[72] | 12.0–12.5 |

Table 5.  Integrated carbonyl absorption intensities for
substituted 3-aryl-1,2,3,4-oxatriazolium-5-olates, 24.

$$R\text{-}C_6H_X$$
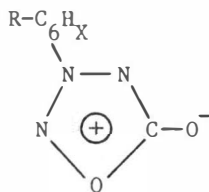


24

| no. | R | X | conc. ($\times 10^{-3}$ M) | $N^a$ | Absorption Intensity[b] (A) |
|-----|-----|-----|-----|-----|-----|
| 24a | H | 4 | 23.56 | 1 | 12.2 |
|     |   |   | 50.31 | 4 | 12.3 $\pm$0.2 |
| 24b | 4-Cl | 4 | 50.63 | 4 | 12.48$\pm$0.65 |
| 24c | 3,4-Cl$_2$ | 3 | 43.79 | 5 | 10.21$\pm$0.42 |
| 24e | 4-CH$_3$ | 4 | 27.51 | 4 | 12.96$\pm$0.45 |
|     |   |   | 39.30 | 3 | 12.59$\pm$0.13 |
|     |   |   | 53.11 | 4 | 13.71$\pm$0.28 |
| 24f | 4-OCH$_3$ | 4 | 41.55 | 5 | 11.27$\pm$0.82 |
| 24g | 4-F | 4 | 39.01 | 5 | 10.46$\pm$0.62 |

[a]No. of observations.  [b]Control was phenylacetate, lit. val. 3.1,
found 3.07$\pm$0.05 at conc. 58.76$\times 10^{-3}$ M.

B. Pharmacology


 Preliminary toxicity studies were undertaken to determine the dose
range to test for hypotensive activity in the male normotensive
Sprague-Dawley rat (175-240 g). Dosages ranging from 50-500 mg/kg of
3-(4-methylphenyl)-1,2,3,4-oxatriazolium-5-olate were administered in
corn oil by gavage. The animals were continually observed during the
first hour, hourly for the next eight hours, then every twelve hours
for 14 days. An increase in respiration rate was observed in the four
rats dosed above 200 mg/kg. The animal which received 209 mg/kg
expired after 2.5 days while the animal dosed at 224 mg/kg survived the
14 day observation period. After seven hours, the animal dosed at 360
mg/kg expired. A rat dosed at 500 mg/kg exhibited tonic convulsions and
expired 5 hours after receiving the test compound. Three animals
exhibited signs of cyanosis prior to death. Gross necropsy findings
were negative. The $LD_{50}$ was estimated[60] to be 210 mg/kg.

 Intravenous administration of the test compounds was precluded by
their insufficient solubility in Emulphor, polypropylene glycol 300,
and the Tweens 20, 40, and 80. Thus, the oral route of administration
was chosen to test for hypotensive activity in the rats using corn oil
as the vehicle.

 A femoral artery in a pentobarbital anesthetized male Sprague-
Dawley rat was cannulated. The blood pressure of the restrained
animal, upon recovery from anesthesia, was continuously observed for
one hour prior to the administration of the experimental compound. The
desired dose was always dissolved in less than one mL of corn oil.

Blood pressure readings were recorded continuously for the first thirty

minutes after oral administration of the test compound.  In longer

studies, blood pressure recordings were taken at two hour intervals.

The experimental parameters, vehicle, time of day of blood

pressure recordings and the length of time of unanesthetized restraint

did not significantly affect the blood pressure readings.

Administration of corn oil to three rats did not affect their blood

pressure at thirty minutes.  However, a mean hypotensive response of

1.7% was observed in these restrained rats at seven hours.

A typical hypotensive response for the reference compound,

clonidine, at a dose of 0.1 mg/kg can be seen in Table 6.  Both

systolic and diastolic pressures were depressed equally in two of the

four rats dosed with clonidine.  The maximum hypotensive response

occurred at two hours.  The systolic pressure was quicker to return to

pre-administration levels.

The maximum effect on the mean arterial blood pressure by varying

the dose of 3-(4-methylphenyl)-1,2,3,4-oxatriazolium-5-olate is shown

in Table 7.  The percent decrease of both the systolic and diastolic

pressures were equal.  The onset of pressure reduction was observable

after 2-3 minutes and the maximum effect was attained within thirty

minutes for doses less than 15 mg/kg.  For higher doses, the maximum

effect was achieved within one hour.  The duration of activity was dose

dependent.  It can be seen in Table 8 that substantial decreases in

blood pressures at five hours exist after single doses of 15 and 25

mg/kg of the 4-methylphenyl analogue.  A typical time course for the

hypotensive response can be seen in Table 9 for a dose of 15 mg/kg of

Table 6.  Hypotensive response of rats[a] to the oral administration of
0.1 mg/kg[b] clonidine.[c]

|                  | % Decrease in Blood Pressure (mm Hg) | |
| Time (hr)        | Systolic        | Diastolic       |
|------------------|-----------------|-----------------|
| 0.0              | 0[d]            | 0[d]            |
| 0.6              | 12.0±0.0        | 13.5±0.4        |
| 1.5              | 13.5±1.8        | 15.5±1.1        |
| 2.0              | 14.5±1.8        | 15.0±1.4        |
| 3.0              | 4.5±0.4         | 11.5±1.8        |
| 4.0              | 0±0             | 9.0±1.4         |

[a]Two animals showed response, data of 2 rats showing no activity
not included.  [b]Volume of total dose was always less than 1 mL.
[c]Dissolved in corn oil.  [d]Baseline blood pressure after recovery from
anesthesia, 128/115.

Table 7.  Maximum response of 3-(4-methylphenyl)-1,2,3,4-oxatriazolium-
          5-olate, <u>24e</u>.
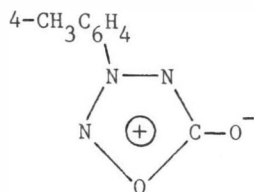


<u>24e</u>

| Dose[a] (mg/kg) | N[b] | % Decrease in Blood Pressure[c] (mm Hg) |
|---|---|---|
| 0[d] | 3 | 0.0[e] |
| 1 | 5 | 13.9±3.0 |
| 3 | 9 | 23.5±6.2 |
| 10 | 12 | 28.0±8.3 |
| 15 | 8 | 37.6±7.0 |
| 25 | 1 | 48.1 |
| 45 | 1 | 53.0 |
| 90 | 2 | 75.3±5.4 |

[a]Dose given in less than 1 mL corn oil by gavage.  [b]No. of rats
observed.  [c]Mean change for the no. of animals observed, mean arterial
pressure.  [d]Control, corn oil administered.  [e]Baseline blood pressure
after recovery from anesthesia.

Table 8.  Hypotensive effect at 2-5 hours of 3-(4-methylphenyl)-1,2,3,4-
          oxatriazolium-5-olate, 24e.

$$4\text{-}CH_3C_6H_4$$

$$\begin{array}{c} N-N \\ N \quad \oplus \quad C-O^- \\ O \end{array}$$
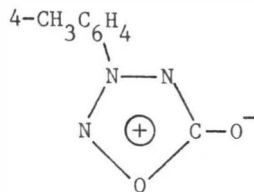
24e

| Dose[a] (mg/kg) | N[b] | Time (hr) | % Decrease[c] in Blood Pressure |
|---|---|---|---|
| 90 | 2 | 5 | 80±5.4 |
| 45 | 1 | 5 | 32 |
| 25 | 1 | 5 | 25 |
| 15 | 8 | 5 | 21±5.8 |
| 1 | 5 | 2[d] | 0±0.2 |

[a]Less than 1 mL volume of test compound in corn oil by gavage, a
single dose. [b]No. of rats observed. [c]Mean decrease in the mean
arterial pressure for no. of animals observed. [d]Blood pressure
returned to pre-injection levels and observation was terminated.

Table 9.  A typical time course for a single dose of 15 mg/kg of
3-(4-methylphenyl)-1,2,3,4-oxatriazolium-5-olate, 24e.
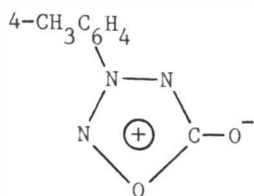


24e

| Time[a] (min) | Blood Pressure (mm Hg) | |
| | Systolic | Diastolic |
| --- | --- | --- |
| 0[b] | 135[c] | 117[c] |
| 3 | 125 | 115 |
| 5 | 115 | 100 |
| 10 | 103 | 90 |
| 20 | 85 | 73 |
| 30 | 80 | 70 |
| 60 | 92 | 75 |
| 180 | 105 | 80 |
| 300 | 125 | 90 |

[a]Time lapsed after a single dose for one animal.  [b]One hour after
recovery from anesthesia, pentobarbital (35 mg/kg, ip).  [c]Baseline
blood pressure.

the 4-methylphenyl analogue.

Because the maximum effect at 10 mg/kg occurred within thirty minutes, this dose was chosen for single dose studies for seven analogues of the 3-aryl-1,2,3,4-oxatriazolium-5-olates. Table 10 compares the hypotensive response for a single dose at thirty minutes in the rat. In the limited number of observations, the unsubstituted compound, 24a, was found to have the greatest activity which was approximately twice the activity of the 4-methylphenyl analogue. The hypotensive effect of 3-(4-methylphenyl)-1,2,3,4-oxatriazolium-5-olate when given at a dose of 1 mg/kg was found to equal the hypotensive response elicited by half the animals administered clonidine at a dose of 0.1 mg/kg. Substituents on the aromatic ring resulted in a lowering of the hypotensive activity. Thus, optimal steric, lipophilic and electronic requirements for the series are met in the unsubstituted phenyl mesoionic compound.

The duration of effect of six of the analogues did not follow the same ordering as the maximum effect at thirty minutes following a single dose. The order observed for the duration of the response was: $4\text{-Cl} \cong 4\text{-Me}$ ($\approx 12$ hr) $> 4\text{-NO}_2 \cong C_6H_5$ ($\approx 8$ hr) $> 4\text{-MeO} \cong 3,4\text{-Cl}_2$ ($\approx 6$ hr).
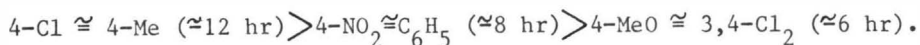
The 3-phenyl-$\psi$-oxatriazole was tested for its ability to inhibit cyclic 3′,5′-monophosphate (cAMP) phosphodiesterase (PDE) in an assay described by Klee[73] using bovine heart PDE and 1 $\mu$M cAMP. Because of poor solubility in the buffer solution, ethanol was used in a 5% final concentration to solubilize the test compound. The mesoionic compound was found to be inactive three times at 60 $\mu$M with respect to the theophylline control.

Table 10.  Response at 30 minutes of a single 10 mg/kg dose of
Ψ-oxatriazolones, 24.

$$R-C_6H\begin{matrix} X \\ \backslash \\ N-N \\ / \quad \oplus \quad \backslash \\ N \quad C-O^- \\ \backslash \quad / \\ O \end{matrix}$$
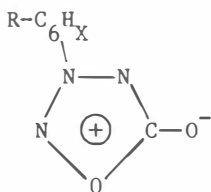
24

| No. | R | X | N[b] | % Decrease[a] in Blood Pressure (mm Hg) Systolic | Diastolic | mM[c] |
|---|---|---|---|---|---|---|
| 24a | H | 4 | 4 | 51.7±3.4 | 52.3±3.2 | 61.0 |
| 24b | 4-Cl | 4 | 2 | 47.0±2.0 | 47.9±1.5 | 50.4 |
| 24c | 3,4-Cl$_2$ | 3 | 2 | 31.7±0.1 | 30.9±0.6 | 42.9 |
| 24e | 4-CH$_3$ | 4 | 12 | 28.5±8.1 | 27.5±8.8 | 47.8 |
| 24d | 4-NO$_2$ | 4 | 1 | 24.8 | 23.8 | 56.2 |
| 24f | 4-OCH$_3$ | 4 | 1 | 22.2 | 20.0 | 51.6 |
| 24g | 4-F | 4 | 0 | not tested | | |
| 24h | 2,6-Cl$_2$ | 3 | 1 | 11.5 | 8.3 | 42.9 |
| Clonidine[d] | | | 4[e] | 7.2±6.1 | 7.9±6.8 | 0.44 |
| | | | 2[f] | 12.0±0.0 | 13.5±0.4 | 0.44 |

[a]Mean decrease at 30 minutes.  [b]No. animals given test compound.
[c]Millimolar conc.  [d]Reference compound given at 0.1 mg/kg in corn oil by gavage, max. effect at 2 hours.  [e]No effect was observed in 2 rats.
[f]The 2 animals exhibiting activity.

This research has shown that the 3-aryl-1,2,3,4-oxatriazolium-5-olates possess a moderate hypotensive effect in the normotensive male white rat.  These orally active compounds were found to have a rapid onset of action and a long duration of hypotensive activity.  Data obtained in this research does provide a useful basis for further work in this area.  Further investigation is needed to define the site and mechanism of action at the molecular level.

EXPERIMENTAL

Elemental analyses were performed by Atlantic Microlabs, Atlanta, Georgia. The infrared spectra of the compounds were obtained on a Perkin-Elmer Model 257 spectrophotometer and a Beckman Acculab 8 spectrophotometer. The ultraviolet spectra of the compounds were obtained on a Beckmann Model 25 spectrophotometer. Mass spectrometer fragmentation pattern was obtained by electron-impact technique on the Finnegan 4000 series GC/Mass spectrometer. All melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. TLC were performed on silica gel sheets without fluorescent indicator.

**Monopotassium** **Aminomethanedisulfonate** **(25).** The methods of von Pechmann and Manck[58] and Bannard and Ross[59] were used for the preparation of **25**. Potassium metabisulfite (111 g, 0.5 mol) was dissolved in water (200 mL) at 55°C. A solution of potassium cyanide (65 g, 1.0 mol) in water (50 mL) was added to the warm bisulfite solution. The solution was heated at 75°C for 3 hours. The pH of the solution was maintained at 10 by the addition of concentrated hydrochloric acid. As the brown solution was cooled to room temperature, the pH was adjusted to 2.5 with concentrated hydrochloric acid. The filtrate was washed twice with water to give 129 g (60%) of the pure white inorganic powder. IR(KBr): 3160 $cm^{-1}$($NH_3$), 2950 $cm^{-1}$ (CH), 1585 and 1510 $cm^{-1}$($NH_3$), 1260–1210 $cm^{-1}$($SO_3$).

**Dipotassium** **Diazomethanedisulfonate** **(26).** The procedure of von Pechmann and Manck[58] afforded the synthesis of **26**. A slurry of monopotassium aminomethanedisulfonate (23 g, 0.1 mol) and water (34 mL) was prepared and cooled to 7°C. Potassium nitrite (9.4 g, 0.11 mol) was dissolved in water (20 mL) and cooled to 7°C. Then, all at once the nitrite solution was added to the slurry with stirring. It was removed from the ice bath and the effervescent exothermic reaction was allowed to attain a maximum temperature of 45°C. After cooling the yellow mixture to room temperature, a few drops of a 10% potassium hydroxide solution were added to pH 9. The pure yellow inorganic product was collected by filtration, yielding 23.5 g (90%). IR(KBr): 2080 $cm^{-1}$(C=N=N), 1270–1220 $cm^{-1}$($SO_3$). UV(1N–KOH)[66]: $\lambda_{max}$ 233 ($\epsilon$ 4755).

**Tetrapotassium Sulfohydrazonomethane Disulfonic Acid (27).** Following
the procedure of von Pechmann and Manck[58], potassium metabisulfite
(28.9 g, 0.13 mol) and potassium hydroxide (7.3 g, 0.13 mol) were
dissolved in water (80 mL). Potassium diazomethanedisulfonate (28 g,
0.1 mol) was added to the solution. With gentle warming,
decolorization occurred within 1/2 hour. After cooling and filtration
of the thick mixture, a quantitative yield of the white inorganic
precipitate was obtained. IR(KBr): 1260-1230 $cm^{-1}(SO_3)$.

**Tripotassium Sulfohydrazonomethanedisulfonate (28).** The method of von
Pechmann and Manck[58] was used for the preparation of **28**. An aqueous
slurry of tetrapotassium sulfohydrazonomethanedisulfonate was made, pH
14. Glacial acetic acid was added to pH 6.5. Quantitative yields of
the pure inorganic white product were obtained. IR(Nujol): 3340 $cm^{-1}$
(NH), 1260-1230 $cm^{-1}(SO_3)$.

**Diazotization of Substituted Anilines.** All anilines used were
recrystallized or freshly distilled. To the appropriate aniline (1
mol) was added concentrated hydrochloric acid (2.3 mol). Enough water
was added to effect solution of the salt. The solution was cooled to
0°C. An aqueous solution of potassium nitrite (1.1 mol) was added at a
rate such that the temperature always remained between 0-5°C.
Diazotization was completed in 15 minutes. Granular potassium acetate
was added to adjust to pH 5.5 while maintaining 0-5°C.

**Potassium Hydrazonomethanedisulfonate (29).** The procedure of von
Pechmann[53] afforded the synthesis of **29**. To an aqueous solution of
tripotassium sulfohydrazonomethanedisulfonate (0.1 mol) was added the
cold diazobenzene acetate (0.15 mol). The pH of the colored

effervescent mixture was maintained at 5 by the addition of granular

potassium acetate. The reaction was followed by TLC, the product

fluoresced. The brown tar was filtered from the desired aqueous layer

and washed with hot water twice. The water was removed in vacuo until

precipitation began. The colored product was obtained by precipitation

from water with ethanol. Yields ranged 50-90% depending on reaction

conditions and substituents. The dry phenyl hydrazone reacted with

concentrated sulfuric acid and ferric chloride to give a violet color.

TLC: $CHCl_3/MeOH/AcOH=6/3/1$. IR(KBr): 3270 $cm^{-1}$(NH), 3020 $cm^{-1}$(CH),

1545 $cm^{-1}$(C=N), 1250-1220 $cm^{-1}$ ($SO_3$).

General Procedure for the Preparation of Substituted-3-aryl-1,2,3,4-

oxatriazolium-5-olates (24). The procedures of von Pechmann[53] and

Farrar[57] were used in the preparation of 24. Granular sodium nitrite

(55 mmol) was added to an aqueous solution of potassium arylhydrazono-

methanedisulfonate (50 mmol). Nitrosation was effected in 30 minutes.

The aqueous portion was added to 2N hydrochloric acid (300 mL). The

formation of the fluorescent product was followed by TLC: $CCl_4/CHCl_3/$-

MeOH=5/3/1. The precipitate was collected in fair yields and

recrystallized from methanol. All compounds exhibited a negative

Liebermann nitroso test. Data for these compounds can be found in

Table 3.

3-Phenyl-1,2,3,4-oxatriazolium-5-olate (24a). Sodium nitrite (3.8 g,

55 mmol) was added to a solution of potassiumphenylhydrazonomethane-

disulfonate (17.85 g, 50 mmol) in water (145 mL). Nitrosation was

effected in 30 minutes. The aqueous portion was added to 2N

hydrochloric acid (300 mL). Production of the fluorescent product from

the orange mixture was followed by TLC: $CCl_4$/$CHCl_3$/MeOH=5/3/1. The precipitate was collected after 5 hours. Recrystallization from MeOH gave 4.2 g (52%) of 24a as yellow needles: mp 83–84°C, lit val. 89°C[53]; IR($CCl_4$): 3060 $cm^{-1}$(CH), 1785 $cm^{-1}$(C=O); NMR ($CDCl_3$) $\delta$ 7.9–8.4 (m, 5H, aromatic protons); UV(EtOH): $\lambda_{max}$ 215 ($\epsilon$ 7286), 265 ($\epsilon$ 9643); mass spectrum, m/e (relative intensity) 164 (17), 133 (14), 105 (23), 77 (100). The Liebermann nitroso test was negative.

**Integrated Carbonyl Absorption Intensity.** Cell thickness was calculated prior to the experiment. Spectrograde chloroform was the solvent for the test compounds. Concentrations of compounds ranged from 20–60X10$^{-3}$ M. The infrared spectra was scanned between 1900–1700 $cm^{-1}$ a minimum of 3 times per test compound. The method of Ramsay[69] was used to calculate the integrated carbonyl absorption intensity. Phenylacetate was used as a control, literature value[74] 3.1, found 3.07±0.05. See appendix.

**Pharmacology.** Eight male Sprague-Dawley rats were administered by gavage 3-(4-methylphenyl)-1,2,3,4-oxatriazolium-5-olate in dosages of 50–500 mg/kg dissolved in 0.7–0.8 mL corn oil. $LD_{50}$ was estimated.[60] Male Sprague-Dawley rats (250–350 g) were administered pentobarbital (35 mg/kg, ip) and a femoral artery cannulated. The animal was restrained in a supine position and the excision area was bathed in normal saline for the duration of the experiment. A 10% heparin saline solution (0.01 mL) was injected into the cannulated artery 30 seconds before recording the blood pressure. Blood pressure readings were taken by Statham transducer interfaced with a Grass Model 7 Polygraph. After the animal recovered from anesthesia, the blood pressure was recorded continuously for one hour. The test compound dissolved in

0.7-0.8 mL corn oil was administered by gavage. Blood pressure readings were continuously recorded for 30 minutes, then, at various time intervals. The animal was sacrificed at the conclusion of each experiment.

## APPENDIX

The integrated absorption intensity, A, of an infrared band is
proportional to the square of the oscillating dipole for the vibration
of the molecule giving rise to that band as seen in Equation 1.[74]

$$A = N\pi(3c^2)^{-1}(\partial\mu/\partial Q)^2 \qquad\qquad \text{Eq. 1}$$

where N = Avagodros number          $\mu$ = molecular dipole moment
    c = velocity of light          Q = normal coordinate
                                                                 describing the vibration

The molecular dipole will vary with bond stretching and bending as a
result of a change in the total molecular electronic configuration.
That is, dipole moment fluctuations are dependent on the contribution
of charge separated resonance forms, the nonbonding electrons and the
dependence of the nonbonding orbitals on the hybridization.[75]

If the electronic structure of a molecule can shift to accommodate
a change in the length of the carbonyl bond, the value of $\partial\mu/\partial Q$ for
the carbonyl vibration is expected to be greater than for a molecule
where the bond is localized.

Ramsay[69] found that the true absorption intensity, A, of an
infrared band could be approximated by the integration of a Lorentz
equation, Equation 2.

$$A = \int \alpha_\nu \, d\nu = (cl)^{-1} \ln(I_0/I)_\nu \, d\nu \qquad\qquad \text{Eq. 2}$$

where $\alpha_\nu$ = adsorption coefficient at frequency, $\nu$
   c = concentration (moles/liter)
   l = cell length (cm)

Due to finite slit widths, the apparent integrated absorption intensity was measured. In order to obtain the true integrated absorption intensity, A, the Ramsay slit correction factor, K, was used. See Equation 3. A sample calculation of the integrated carbonyl absorption intensity of the 3-phenyl-$\psi$-oxatriazole is seen in Figure 8.

$$A = K(cl)^{-1}(\ln T_0/T)_\nu \Delta\nu_{\frac{1}{2}}^{a} \qquad\qquad \text{Eq. 3}$$

where $\Delta\nu_{\frac{1}{2}}^{a}$ = apparent half band width ($cm^{-1}$).

Figure 8. A sample calculation of the integrated carbonyl absorption intensity of 3-phenyl-$\Delta\psi$-oxatriazole, 24a.
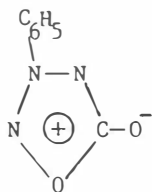


$$A = K(cl)^{-1}(\ln T_o/T)_\nu \Delta\bar{\nu}^a_{1/2}$$
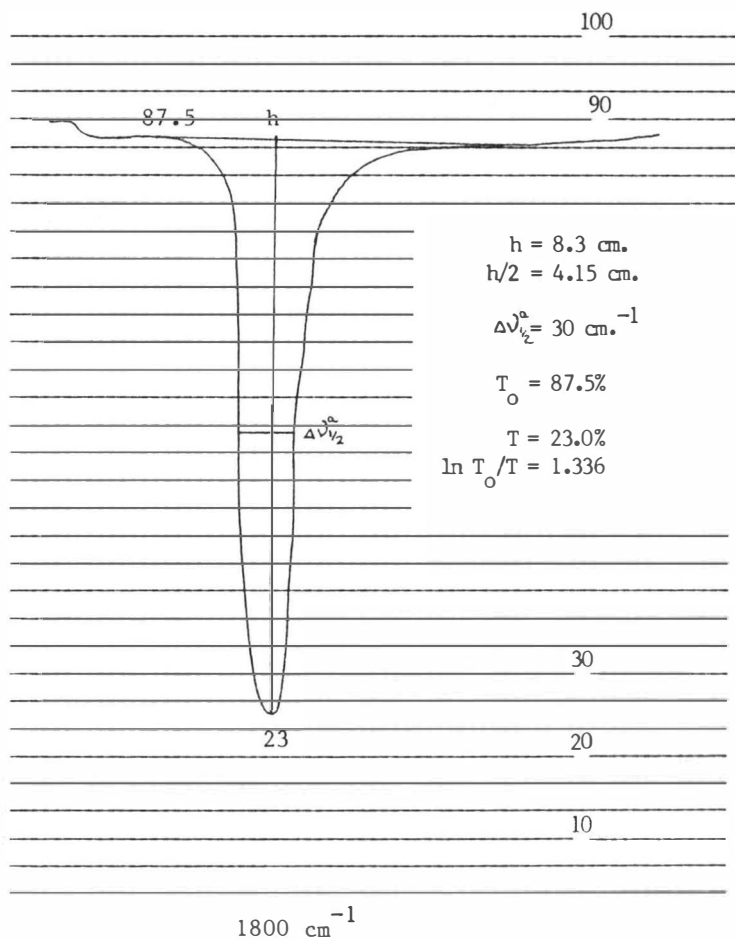
$$A = \frac{(1.57)(1.336)(30\ cm^{-1})}{(50.306\times10^{-3}\ m/1)(0.01051\ cm)}$$

where K=1.57

$$A = 11.9\times10^4\ moles^{-1}\text{-liter-}cm^{-2}$$

or

$$A = 11.9\ \text{intensity units}$$

h = 8.3 cm.

h/2 = 4.15 cm.

$\Delta\bar{\nu}^a_{1/2} = 30\ cm.^{-1}$

$T_o = 87.5\%$

T = 23.0%

$\ln T_o/T = 1.336$

1800 cm$^{-1}$

REFERENCES

1.  Nickerson, M; Ruedy, J. In "The Pharmacological Basis of Therapeutics", 5th ed.; Goodman, L. S.; Gilman, A. G., Eds.; MacMillan: New York, 1975; p 717, Chap. 8.

2.  Hammer, R. H. In "Principles of Medicinal Chemistry", Foye, W. O., Ed.; Lea and Febiger: Philadelphia, 1975; p 377.

3.  "Heart Facts", Am. Heart Assoc., 1981.

4.  Vander, A. J.; Sherman, J. H.; Luciano, D. S. "Human Physiology, The Mechanisms of Body Function", McGraw-Hill: New York, 1970; p 297.

5.  Brogden, R. N., Ed. "Cardiovascular Drugs", University Park Press: Baltimore, 1979; Vol. 4.

6.  Bender, A. D. In "Topics in Medicinal Chemistry", Rabinowitz, J. L.; Myerson, R. M., Eds.; Interscience: New York, 1967; Vol. 1, p 178.

7.  Simpson, F. O. In "Cardiovascular Drugs", Avery, G. S., Ed.; University Park Press: Baltimore, 1978; p 37.

8.  Taylor, S. H. In "Theories and Use of $\beta$-Blockade in Hypertension and Angina", Roberts, R. H., Ed.; Symposia Specialists: Miami, 1979; p 240.

9.  DeQuattro, V.; Campese, V.; Antonaccio, M. J. In "Cardiovascular Pharmacology", Antonaccio, M. J., Ed.; Raven: New York, 1977; p 198-202.

10. Mendlowitz, M.; Naftchi, N.; Gitlow, S. E.; Weinreb, H. L.; Wolf, R. L. Ann. N.Y. Acad. Sci. 1960, 88, 964.

11. Freis, E. D. Circulation 1976, 53, 589.

12. Heymans, C. Perspectives Biol. Med. 1960, 3, 409.

13. McCubbin, J. W.; Green, J. W.; Page, I. H. Cir. Res. 1956, 4, 205.

14. Clarkson, R. In "Antihypertensive Agents", Engelhardt, E. L., Ed.; ACS Symposium Series 27: Washington, D.C., 1976; p 19.

15. Moser, M.; Guyther, J. R.; Finnerty, F., Jr.; Richardson, D. W.; Langford, H.; Perry, H. M., Jr.; Wood, D. E.; Krishan, I.; Branche, G. C., Jr.; Smith, W. M. J. Am. Med. Assoc. 1977, 237, 255.

16. Scriabine, A., Ed. "Pharmacology of Antihypertensive Drugs", Raven: New York, 1980.

17. Freis, E. D.; Rose, J. C.; Pantenope, E. A.; Higgins, T. F.; Kelly, R. T.; Schnaper, H. W.; Johnson, R. L. J. Clin. Invest. 1953, 32, 1285.

18. Tarazi, R. C.; Dustan, H. P. Am. J. Cardiolo. 1972, 29, 633.

19. Wood, A. J.; Phelan, E. L.; Simpson, F. O. Clin. and Exp. Pharm. and Phys. 1975, 2, 297.

20. Earl, J. C.; Mackney, A. W. J. Chem. Soc. 1935, 399.

21. Baker, W; Ollis, W. D.; Poole, V. D. J. Chem. Soc. 1949, 307.

22. Ollis, W. D.; Ramsden, C. A. In "Advances in Heterocyclic Chemistry", Katritzky, A. R.; Boulton, A. J., Eds.; Academic Press: New York, 1976; Vol. 19.

23. Ramsden, C. A. In "Comprehensive Organic Chemistry", Sammes, P. G., Ed.; Pergamon Press: London, 1979; p 1173.

24. Katritzky, A. R. Chem. and Ind. 1955, 521.

25. Potts, K. T.; Armbruster, R.; Houghton, E.; Kane, J. Org. Mass Spectrometry 1973, 7, 203.

26. Hanley, R. N.; Ollis, W. D.; Ramsden, C. A. J. Chem. Soc. Chem. Comm. 1976, 306.

27. Hanley, R. N.; Ollis, W. D.; Ramsden, C. A. J. Chem. Soc. Chem. Comm. 1976, 307.

28. Boyer, J. H.; Hernandez, J. A. J. Amer. Chem. Soc. 1956, 78, 5124.

29. Christophersen, C.; Treppendahl, S. Acta Chem. Scand. 1972, 858.

30. Isukul, E. A.; Ranson, R.; Tillett, J. G. J. Org. Chem. 1976, 41, 3040.

31. Talukdar, P. B.; Chakraborty, A. Indian J. Chem. 1973, 11, 556.

32. Christophersen, C.; Treppendahl, S. Acta Chem. Scand. 1971, 25, 625.

33. Stewart, F. H. C. Chem. Rev. 1964, 64, 129.

34. Ohta, M.; Kato, H. In "Nonbenzenoid Aromatics", Snyder, J. P., Ed.; Academic Press: New York, 1969; Vol. 1.

35. Greco, C. V.; Nyberg, W. H.; Cheng, C. C. J. Med. Pharm. Chem. 1962, 5, 861.

36. Kier, L. B.; Fox, L. E.; Dhawan, D.; Waters, I. W. Nature 1962, 195, 817.

37. Ackermann, E. D. Pharmazie 1967, 10, 537.

38. Kier, L. B.; Roche, E. B. J. Pharm. Sci. 1967, 56, 149.

39. Kier, L. B.; Dhawan, D. J. Pharm. Sci. 1962, 51, 1058.

40. Dhawan, D.; Kier, L. B. J. Pharm. Sci. 1964, 53, 83.

41. Fregly, M. J.; Kier, L. B.; Dhawan, D. Toxicol. Appl. Pharmacol. 1964, 6, 529.

42. Greco, C. V.; Tobius, J.; Kier, L. B. J. Hetero. Chem. 1967, 4, 160.

43. Kier, L. B.; Al-Shamma, A.; Hahn, R.; Tye, A. J. Pharm. Sci. 1966, 55, 1467.

44. Kier, L. B.; Al-Shamma, A.; Campbell, D.; Patil, P. H.; Tye, A. Nature 1966, 210, 742.

45. Kier, L. B.; Keating, J. W. U.S. Patent 3 427 317, 1969.

46. Thomas, T. L.; Fedorchuk, M.; Shetty, B. V.; Anderson, F. E. J. Med. Chem. 1970, 13, 196.

47. Ryan, J.; Blumenthal, H; McMahon, F. Clin. Pharm. Ther. 1975, 17, 243.

48. Takenada, F.; Takeya, N.; Ishihara, T.; Inoue, S.; Tsutsumi, E.; Nakamura, R.; Mutsufiyi, Y.; Sumie, M. Jap. J. Pharmacol. 1970, 20, 253.

49. Nitz, R. E. In "Medicinal Chemistry V, Proceedings of the 5th International Symposium on Medicinal Chemistry", Mathieu, J., Ed.; Elsevier Scientific: New York, 1977; p 233.

50. Masuda, K.; Kamitani, T. Jap. Patent 7021 102, 1969; Chem. Abstr. 1970, 73, 87922f.

51. Masuda, K.; Kamitani, T. Jap. Patent 7020 904, 1969; Chem. Abstr. 1970, 73, 87930g.

52. Goetz, M.; Grozinger, K,; Oliver, J. J. Med. Chem. 1973, 16, 671.

53. v. Pechmann, H. Berichte 1896, 29, 2161.

54. Ponzio, G. Gazzetta 1915, 45, 12.

55. Quilico, A. Gazzetta 1932, 62, 912.

56. Ponzio, G. Gazzetta 1933, 63, 471.

57. Farrar, W. V. J. Chem. Soc. 1964, 906.

58. v. Pechmann, H.; Manck, Ph. Berichte 1895, 28, 2374.

59. Bannard, R. A. B.; Ross, J. H. Can. J. Chem. 1954, 32, 49.

60. Weil, C. S. Biometrics 1952, 8, 249.

61. Betts, R. H.; Voss, R. H. Can. J. Chem. 1970, 48, 2035.

62. Bourne, D. W. A.; Higuchi, T.; Pitman, I. H. J. Pharm. Sci. 1974,
    63, 865.

63. Meyer, B. "Sulfur, Energy and Environment"; Elsevier Scientific:
    Amsterdam, 1977; p 77.

64. Herlinger, A. W.; Long, T. V., III. Inorg. Chem. 1969, 8, 2661.

65. Meyer, B.; Peter, L.; Spitzer, K. Inorg. Chem. 1977, 16, 27.

66. Young, J. M. J. Chem. Soc. Perkin I 1974, 2541.

67. Doughterty, R. C.; Foltz, R. L.; Kier, L. B. Tetrahedron 1970, 26,
    1989.

68. Christophersen, C.; Treppendahl, S. Acta Chem. Scan. 1971, 25,
    625.

69. Ramsay, D. A. J. Amer. Chem. Soc. 1952, 74, 72.

70. Zaitsev, B. E.; Sheinker, Y. N. Bull. Acad. Sci. U.S.S.R. Div.
    Chem. Sci. 1962, 378.

71. Coburn, R. A.; Glennon, R. A. J. Hetero. Chem. 1973, 10, 487.

72. McCarthy, A. R.; Ollis, W. D.; Barnes, N. M.; Sutton, L. E.;
    Ainsworth, C. J. Chem. Soc. (B) 1969, 1167.

73. Klee, C. B. Biochem. 1977, 16, 1017.

74. Barrow, G. M. J. Chem. Phys. 1953, 21, 2008.

75. Brown, T. L. Chem. Rev. 1958, 58, 581.