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Stereochemical Analogs of a Muscarinic, Ganglionic Stimulant. *cis*- and *trans*-4-[*N*-(3-Chlorophenyl)carbamoyloxy]-2-butenyltrimethylammonium Iodides[1,†]

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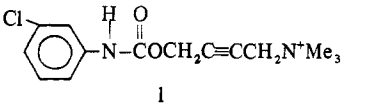
Synthesis and pharmacological properties of *cis*- and *trans*-4-[*N*-(3-chlorophenyl)carbamoyloxy]-2-butenyltrimethylammonium iodides (**2** and **3**) are reported. The trans compound **3** shows atropine-sensitive ganglion-stimulating properties similar to those previously reported for the acetylenic analog **1**. The cis compound **2** shows much less ganglion-stimulating activity than **3**. Muscarinic ganglion-stimulating properties of **1** and **3** are interpreted in terms of similar fit at the receptor level of the alkyltrimethylammonium ion, unsaturation at C-2, and the ether oxygen. Both **2** and **3** show certain nonmuscarinic properties of **1**.

Receptor categories based on classical autonomic agents are rather accommodating. Thus, for example, the population of β-adrenergic receptors blocked by propranolol may now be subdivided into β_1 and β_2 on the basis of specific agonists and antagonists.[2] Likewise, we have for years differentiated the nicotine-sensitive receptors into ganglionic nicotinic and neuromuscular nicotinic receptors, recognizing that these receptors, although related, are not identical. A similar subdivision of atropine-sensitive or so-called muscarinic receptors may be forthcoming based on work with specific subclasses of muscarinic agonists. The present work represents an attempt to explore one possible avenue toward more specific muscarinic agonists and/or antagonists.

A detailed pharmacological study by Roszkowski[3] of 4-[*N*-(3-chlorophenyl)carbamoyloxy]-2-butynyltrimethylammonium chloride, McN-A-343 (**1**), showed that the compound possessed unique ganglionic-stimulant properties. Compound **1** excited ganglia, especially sympathetic ganglia, at muscarinic (*i.e.*, atropine-sensitive) sites. Other less potent muscarinic effects noted were vasodilitation and stimulation of intestinal smooth muscle. Direct nicotinic effects at the neuromuscular junction were also observed. More recently, other nonmuscarinic effects of **1** have been noted by other workers. These include antagonism of the amine uptake pump of the sympathetic nerve terminal[4,5] and a local anesthetic effect.[6]

Early work concerning structure-activity relationships in this series showed the olefinic analog to possess only weak pressor effects.[7] Since no stereochemistry was specified, our study began with preparation and testing of the cis and trans olefinic analogs of **1**, compounds **2** and **3**.

Synthesis. The cis olefinic analog **2** was prepared from 4-[*N*-(3-chlorophenyl)carbamoyloxy]-1-chlorobutyne (barban)‡ by reaction with dimethylamine, quaternization



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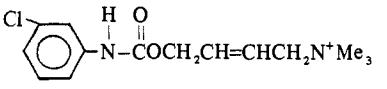
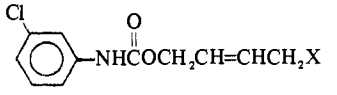


2 (cis)
3 (trans)

with iodomethane, and catalytic reduction on quinoline poisoned Pd·$BaSO_4$. Alternatively, *cis*-2-butene-1,4-diol (**8**) was converted to *cis*-4-chloro-2-buten-1-ol (**10**), treated with dimethylamine and iodomethane, and followed by esterification with 3-chlorophenyl isocyanate. The instability of the intermediate, *cis*-4-[*N*-(3-chlorophenyl)carbamoyloxy]-1-dimethylamino-2-butene (**6**), required this sequence of reactions to circumvent its preparation.



cis	trans
4, X = Cl	**5**, X = Cl
6, X = NMe_2	**7**, X = NMe_2

$HOCH_2CH{=}CHCH_2X$

cis	trans
8, X = OH	**9**, X = OH
10, X = Cl	**11**, X = Cl
12, X = NMe_2	**13**, X = NMe_2
14, X = $N^+Me_3I^-$	

The trans isomer **3** was prepared from *trans*-2-butene-1,4-diol (**9**), which is available from 1,3-butadiene by the method of V'yunova.[8] The diol was converted to chloro alcohol **11** and esterified with 3-chlorophenyl isocyanate to afford **5**. Displacement with dimethylamine and quaternization completed the sequence.

All nmr data were consistent with the geometric assignment of cis and trans in each series of precursors. The cis compounds show non-first-order multiplets near δ 6.0 for the olefinic protons. The olefinic protons of the trans compounds show similar chemical shift and appear as multiplets resembling quintets. Similar observations were reported by Willette and Driscoll[9] in a series of 4-amino-2-buten-1-ol esters.

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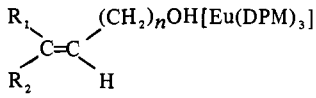
‡Acquired through the courtesy of Dr. T. R. Hopkins, Gulf Research and Development Co.

The isomeric chloro alcohols, **10** and **11**, were subjected to decoupling studies after signal separation using a shift reagent, $Eu(DPM)_3$.[10] In the cis compound, at a molar ratio of 10:1 (compound to shift reagent), the olefinic protons were separated into two overlapping doublets of triplets, $J_{cis} = 11$ Hz.

In the trans compound downfield shifts occurred but no separation of the olefinic signals was noted. A difference in the magnitude of induced shifts between the cis and trans compounds was noted. Richey and Von Rein[11] studied these effects on a series of alkenols where R_1 and R_2 are alkyl. They defined θ_{R_1/R_2} as the change in chemical shift of the R_1 signal divided by the change in R_2. For all compounds θ_{R_1/R_2} was greater than 1, suggesting the group closer to the paramagnetic ion is influenced to a greater extent. They did, however, note values θ_{R_1/R_2} of 2.4-3.1 in many trans olefins; *e.g.*, for *cis*- and *trans*-3-hexen-1-ol, $\theta_{R_1/R_2} = 1.0$ and 2.5, respectively, for *trans*-2-buten-1-ol, $\theta_{R_1/R_2} = 2.9$. Values for **10** and **11** were obtained and were 1.2 and 3.2, respectively, quite consistent with the results of Richey.[11]

Pharmacology. ***In Vivo* Experiments.** As reported by Roszkowski,[3] **1** produces a brief initial depressor response



and bradycardia followed by a sustained (5 min) pressor response and tachycardia. Compound **3** produced an essentially identical pattern with an estimated potency approximately 0.5 that of **1**. Compound **2** produced an initial depressor response and, at high doses, did produce a small pressor response (Figure 1) and slight bradycardia.

The initial depressor response to **1**, **2**, and **3** is presumed to be due to interaction with muscarinic receptors in peripheral blood vessels. A similar response is produced by acetylcholine (ACh) and the depressor effects of ACh, **1**, **2**, and **3** were all blocked by atropine (1 mg/kg).

The pressor response produced by **1** and **3** is the most unique and interesting effect. In each animal employed, the pressor and heart rate responses to **3** and to **1** were affected similarly by various treatments. For example, the pressor response and tachycardia to each was absent in a cat pretreated with reserpine (2.5 mg/kg, 24 hr prior to the experiment). In another animal these were blocked by pentolamine. Both the depressor and pressor responses to **1**, **2**, and **3** were blocked by atropine (1 mg/kg). On the other hand, the pressor responses to **1** and **3** were not blocked by hexamethonium. Thus, the pressor response and tachycardia appear to depend on stores of sympathetic transmitter and excitation of sympathetic ganglia *via* atropine-sensitive rather than hexamethonium-sensitive sites. The results are consistent with the proposition that **3** acts like **1**, *i.e.*, as a muscarinic ganglionic stimulant.

Why **2** is so much less potent than **3** is not yet clear. Theoretically, differences in *in vivo* distribution could account for the observed potency difference. The octanol-water partition coefficients (Table I) show that **1** and **3** are similar in behavior, with **2** being slightly more water soluble. This small difference may partially explain some of the differences in biological activity noted. Differential uptake, based on a specific carrier-mediated process, could account for the data and would be presumed to depend on the different structure of the two compounds. Possibly **2** is rapidly hydrolyzed or metabolized, so that only a small amount reaches the receptor. All of the carbamates were stable in aqueous solution during the duration of pharmacological testing, suggesting that non-enzyme-catalyzed hydrolysis is not a significant factor. No metabolism experiments were performed; however, the rapid rate of onset suggests metabolism is not an important factor prior to action. Structural specificity of the muscarinic ganglionic receptor, in which both **1** and **3** "fit" but in which **2** does not "fit," could also account for the data. We consider this to be the most attractive interpretation.

Table I. Octanol-H_2O Partition Coefficients

Compd	*p*	Log *p*
1	0.116	−0.94
2	0.067	−1.17
3	0.114	−0.94

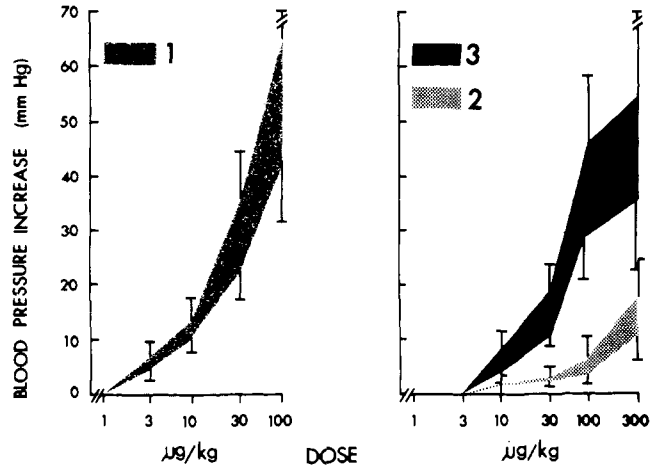
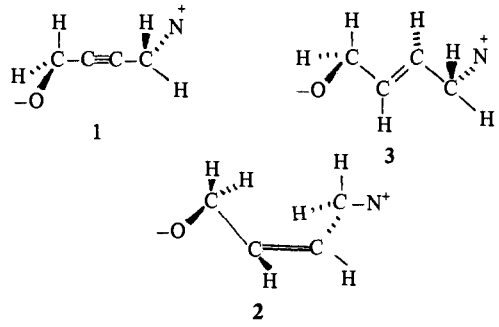


Figure 1. Influence of experimental compounds on arterial blood pressure. Semilogarithmic plots of the maximum increase in systolic and diastolic blood pressure *vs.* dose. Data were obtained from five anesthetized cats given intravenous injections, in a randomized sequence, of 1 (McN-A-343) (left-hand panel) and **2** (cis) and **3** (trans) (right-hand panel). Upper border of the shaded area indicates the maximum increase in systolic blood pressure, the lower border, diastolic blood pressure. Bars represent the respective values of S.E.M.

The similarities in the effects of **1** and **3** at this receptor are thus interpreted in terms of a similar distribution of functional groups in the drug-receptor complex. The obvious groups on which to focus attention are the quaternary nitrogen, the unsaturation at C-2, and the ether oxygen. Models show that certain conformations of **1** can be closely approximated by **3**, in which the quaternary ammonium ion and the carbamate ether oxygen are approximately 5.7 Å apart in a nearly fully extended conformation. In this conformation, the double bond of **3** closely approximates the location of the triple bond in **1**, suggesting a similar fit.

Attempting to fit **2** to this pattern shows that in order for the distance between the ether oxygen and quaternary nitrogen to approximate 5.7 Å, the double bond occupies space considerably above or below the plane formed by the three functional groups in **1**. When two of these functional groups are located in space similar to **1** and **3**, the third seems not to be compatible with the occupation of a similar position. This is not necessarily meant to imply that a preferred conformation, such as that illustrated, is required for the specific muscarinic effect. It is, however, suggested as a possible basis fot the potency difference between **2** and **3**. Further studies are necessary before these possible explanations can be accepted or rejected.



One nonmuscarinic effect of **1**, **2**, and **3**, that is, the excitation of the somatic neuromuscular junction, was noted *in vivo*. The effect was short-lived and not sensitive to atropine. There were no obvious differences in potency among the compounds. Roszkowski[3] reported that **1** produced contracture by acting as a depolarizing agent in the frog rectus muscle *in vitro* and that the effect was blocked by *d*-tubocurarine. We did not test the influence of *d*-tubocurarine.

In Vitro **Experiments**. Other nonmuscarinic effects of **2** and **3** were noted using the isolated sinoatrial node. In this system **2** and **3**, up to $2 \times 10^{-4} M$ (the highest concentration tested), produced little slowing of spontaneous rate. This indicates that these compounds exert little direct muscarinic action in this system.

Both **2** and **3** initially prolonged the duration of the accelerator (adrenergic) response to transmural stimulation of the sinoatrial node. Since it has recently been reported that **1** blocks the amine uptake pump,[5] it is suggested that **2** and **3** share this action. Prolongation of the accelerator response to transmural stimulation, which presumably depends on block of reuptake of norepinephrine in sympathetic nerve terminals, was not sensitive to atropine, and there was no obvious potency difference between **2** and **3**.

Continued exposure of the isolated sinoatrial node to higher concentrations of **2** or **3** resulted in gradual loss of both cholinergic and adrenergic responses to transmural stimulation. In the presence of $2 \times 10^{-4} M$ **2** or **3**, the dose-response curve to ACh was not changed but cholinergic responses to transmural stimulation were blocked. This demonstrates that neither **2** nor **3** exerted significant atropine-like action in this system but that parasympathetic neuroeffector transmission was blocked. The results suggest that **2** and **3** block transmitter release, possibly by a local anesthetic effect on autonomic nerve terminals. The suggestion is reinforced by the recent report that **1** exerts a significant local anesthetic effect which is atropine resistant.[6] In the presence of atropine **2** or **3** blocked the adrenergic response to transmural stimulation (after the initial prolongation of the response, as noted above). Neither the initial prolongation of adrenergic response nor the eventual blockade was an atropine-sensitive effect. Thus, both adrenergic potentiation and decreased neuroeffector transmission appear to be rather nonspecific effects of **2** and **3** (*i.e.*, requiring rather high concentrations and not blocked by atropine). In contrast to the atropine-sensitive ganglionic-stimulant properties of **2** and **3** (in which there was a large difference in potency), these nonspecific, atropine-insensitive effects did not differentiate between **2** and **3**.

Using the isolated rabbit ileum, **1**, **2**, and **3** were compared to ACh. The results, presented in Figure 2, illustrate that the experimental compounds were only weakly agonistic. Quantitative comparison of the compounds with ACh is probably not warranted. Qualitatively speaking, the results

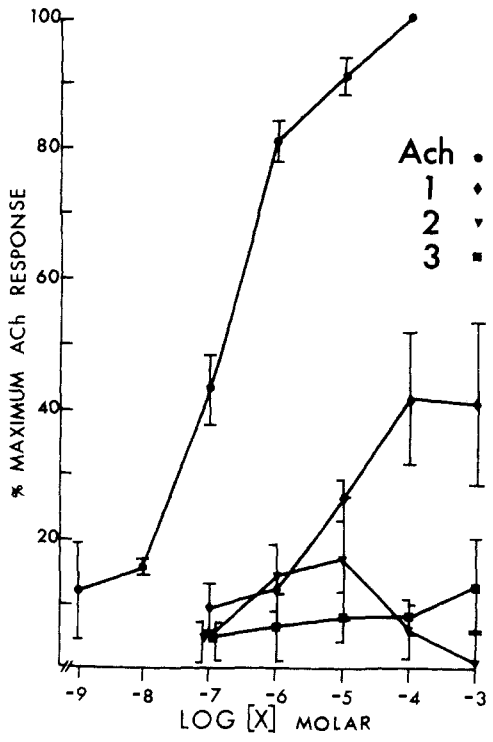


Figure 2. Influence of experimental compounds on isolated rabbit ileum. Semilogarithmic plots of isotonic contraction *vs.* molar concentration. Data are expressed as a per cent of the maximum ACh response, defined as that produced by $10^{-4} M$ ACh in each preparation. **1** (McN-A-343), **2** (cis), and **3** (trans) were weak agonists. Vertical bars represent ±S.E.M., $n = 3$.

emphasize the selectivity of both **1** and **3** which are potent agonists of sympathetic ganglionic muscarinic receptors but only weak agonists of classical muscarinic receptors. Since none of the compounds shifted the dose-response curve to ACh, it is also concluded that none exerted significant antagonistic (*i.e.*, atropine-like) action in the rabbit ileum system.

In summary, the data demonstrate that the unique pharmacological properties of **1** are shared by **3**. The nonspecific properties of **1** appear to be shared by both **2** and **3**. It is suggested that muscarinic receptor specificity accounts both for the unique pharmacology of **1** and **3** and for the differing ganglion-stimulating potencies of **2** and **3**.

Experimental Section

Infrared data were recorded on a Beckman IR-5A spectrophotometer. Liquid samples were run neat using NaCl plates and solid samples were recorded as KBr pellets. Nmr data were determined from Varian A-60 and T-60 spectrometers. The solvents and internal standards used are stated. Tetramethylsilane (TMS) and sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) were the internal standards. Ultraviolet spectra were recorded on a Perkin-Elmer Coleman 101 uv-visible spectrophotometer. Mass spectra were determined using the AEI-MS9 mass spectrometer and a DEC PDP-12 equipped with suitable programs for data collection and reduction, Mass Spectrometry Laboratory, Department of Chemistry, University of Washington. Melting points were taken on a Thomas-Hoover capillary melting point apparatus and are corrected. Microanalyses were performed by Dr. F. B. Strauss, Oxford, England. Where analyses are indicated only by symbols of the elements, analytical results obtained for these elements were within ±0.4% of theoretical values.

4-[*N*-(3-Chlorophenyl)carbamoyloxy]-2-butynyltrimethylammonium Iodide (1). Technical grade 4-chloro-2-butynyl *N*-(3-chlorophenyl)carbamate[7] (barban) was dissolved in refluxing cyclohexane and the solution decanted from dark impurities. Upon cooling, fibrous crystals were collected, mp 73–74 [lit.[12] mp 75–76° (EtOH–H_2O)].

A solution of Me_2NH, 3.40 g (80 mmol), in 40 ml of C_6H_6 was

cooled to 10°, and a solution of barban, 2.60 g (10 mmol), in 50 ml of C_6H_6 was added. The solution was refluxed 4 hr, cooled, and extracted with 3 × 50 ml aqueous 1 *N* HCl. The combined aqueous extracts were made alkaline with aqueous 50% NaOH solution and extracted with $CHCl_3$, 3 × 50 ml. The combined organic extracts were washed with H_2O, dried (Na_2SO_4), and evaporated. The residue was crystallized from hexane–C_6H_6 affording 1.43 g (54%), mp 104–106°.

The crude tertiary amine, 590 mg (2.2 mmol), and 2.28 g (16 mmol) of CH_3I in 30 ml of C_6H_6 were stirred at room temperature for 2 hr affording a precipitate, 770 mg (86%), of the white salt of 1: mp 164–166°; nmr (CD_3OD–TMS) δ 7.0–7.7 (m, Ar), 4.98 (t, $-CH_2O$, J = 2 Hz), 4.55 (t, $-CH_2N^+$, J = 2 Hz), 3.33 (s, $(CH_3)_3N^+$).

The chloride salt was prepared by passing a methanol–H_2O solution of iodide salt through an Amberlite IRA-400 ion exchange resin, chloride form, mp 179–180° (lit.[13] mp 182–183°).

4-[*N*-(3-Chlorophenyl)carbamoyloxy]-*cis*-2-butenyltrimethylammonium Iodide (2). (a) Reduction of 1. A solution of 1, 0.34 g (0.8 mmol), 40 ml of MeOH, 4 drops of synthetic quinoline, and 50 ml of 5% Pd·$BaSO_4$ was hydrogenated at an initial pressure of 1.3 kg/cm². After 25 hr hydrogen uptake was slightly greater than 100% of theory. The mixture was filtered through a Celite pad and the solvent removed by rotary evaporation. The crude product was recrystallized from an EtOH–Et_2O mixture affording 0.32 g (94%) of fine white granular crystals: mp 129–131° dec; nmr (CD_3OD–TMS) δ 7.65 (m, H-2′ Ar proton), 6.90–7.40 (m, 3 Ar protons), 5.70–6.50 (m, complex, HC=CH, overlapping signals), 4.88 (d, $-CH_2O$, J = 6 Hz), 4.25 ($-CH_2N^+$, J = 7 Hz), 3.25 (s, $(CH_3)_3N^+$). *Anal.* Calcd for $C_{14}H_{20}N_2O_2ClI$ (410.684) C, H, N.

(b) Reaction of 14 with 3-Chlorophenyl Isocyanate. A mixture of **14**, 500 mg (1.9 mmol), and 25 ml of dioxane was stirred at room temperature while 500 mg (3.3 mmol) of 3-chlorophenyl isocyanate was added dropwise to this reaction mixture. After stirring for 20 hr, the solvent was removed by rotary evaporation and the residue was crystallized from a MeOH–Et_2O mixture to yield 600 mg (75%) of light tan crystals. Recrystallization from MeOH–Et_2O gave fine, white crystals, mp 126–129° dec, spectrally identical with that prepared from 1.

***trans*-4-Chloro-2-buten-1-ol (11).** To a solution of *trans*-2-butene-1,4-diol[8] (**9**), 40 g (0.45 mol), 50 ml of C_6H_6, and 38.3 g (0.49 mol) of pyridine was added dropwise, over a period of 1.5 hr and with vigorous stirring, 58 g (0.49 mol) of $SOCl_2$, while the reaction solution was maintained at 10–15°. The mixture was stirred for an additional 4 hr at room temperature and then allowed to stand overnight. The mixture was then poured into 100 ml of ice–H_2O, the C_6H_6 layer separated, and the aqueous phase extracted with Et_2O, 4 × 50 ml. The organic extracts were combined and washed with two portions of a $NaHCO_3$ solution and two portions of cold H_2O and then dried (Na_2SO_4). The solvents were removed by rotary evaporation and the residue was vacuum distilled through a Vigreux column affording 27.9 g (58%) of a clear colorless liquid: bp 50–51° (0.2–0.3 mm); nmr ($CDCl_3$–TMS) δ 5.78–6.00 (m, overlapping pattern of olefinic protons having nearly identical chemical shifts), 4.00–4.23 (m, 4, $-CH_2$ protons); mass spectrum (70 eV) calcd for $C_4H_7O^{35}Cl$ and $C_4H_7O^{37}Cl$, 106.0186 and 108.0156; found, 106.0192 and 108.0166.

***trans*-4-Chloro-2-butenyl *N*-(3-Chlorophenyl)carbamate (5).** A solution of 11, 27.4 g (0.26 mol), 125 ml of C_6H_6, and 40 g (0.26 mol) of 3-chlorophenyl isocyanate was gently refluxed for 3 hr and allowed to stand overnight at room temperature. A portion of the C_6H_6 solvent was removed from the reaction solution by rotary evaporation and the residue mixed with 100 ml of hexane. The brown viscous oil layer which formed was separated, washed with 4 × 50 ml of hexane, and dried by rotary evaporation yielding 52.0 g (77%) of a dark brown, viscous oil. The crude material was used without further purification.

***trans*-4-Dimethylamino-2-butenyl *N*-(3-Chlorophenyl)carbamate (7).** A solution of Me_2NH, 2.4 g (53 mmol), and 30 ml of C_6H_6 was maintained at 0° while, with stirring, a solution of 2.0 g (8.0 mmol) of **5** in 40 ml of C_6H_6 was added dropwise over a period of 30 min. The reaction mixture was then refluxed for 4 hr and after cooling was filtered to remove the precipitated Me_2NH_2Cl. The filtrate was washed three times with H_2O and dried (Na_2SO_4), and the solvent was removed by rotary evaporation. The residue was washed with two portions of hexane and dried by rotary evaporation to yield 1.45 g (68%) of a viscous light brown oil which was used without further purification.

4-[*N*-(3-Chlorophenyl)carbamoyloxy]-*trans*-2-butenyltrimethylammonium Iodide (3). To a solution fo 1.13 g (4.2 mmol) of 1 and 50 ml of C_6H_6 was added, with stirring, 4.0 g (28 mmol) of CH_3I. The mixture was stirred for 2 hr and allowed to stand overnight at room temperature. The white precipitate which formed was collected by suction filtration and recrystallized from an EtOH–Et_2O mixture affording 1.30 g (75%) of white fibrous crystals: mp 168–170° dec; nmr (CD_3OD–TMS) δ 7.66 (m, H-2′ Ar proton), 7.00–7.42 (m, complex, 3 Ar protons), 6.17–6.40 (m, 2 olefinic protons, HC=CH), 4.80 (d, $-CH_2O$, J = 6 Hz), 4.10 (d, $-CH_2N^+$, J = 6 Hz), 3.18 (s, $(CH_3)_3N^+$). *Anal.* ($C_{14}H_{20}N_2O_2ClI$) C, H, N.

***cis*-4-Chloro-2-buten-1-ol (10).** Compound **10** was prepared by a procedure analogous to that reported for 11, using *cis*-2-butene-1,4-diol (**8**) (Aldrich) as starting material. The halo alcohol was obtained in 49% yield: bp 50–52° (0.4–0.5 mm); mass spectrum (70 eV) calcd for $C_4H_7O^{35}Cl$ and $C_4H_7O^{37}Cl$, 106.0186 and 108.0156; found, 106.0186 and 108.0174.

***cis*-4-Dimethylamino-2-buten-1-ol (12).** To a 0° solution of Me_2NH, 4.8 g (0.11 mol), in 20 ml of C_6H_6 was added dropwise a solution of *cis*-4-chloro-2-buten-1-ol (**10**), 1.0 g (9.4 mmol), in 20 ml of C_6H_6. The solution was allowed to warm to room temperature and then refluxed for 4.5 hr. After cooling, the reaction mixture was filtered to remove the precipitated Me_2NH_2Cl and the solvent was evaporated yielding 0.55 g (51%) of a viscous, brown oil which was used without further purification.

***cis*-4-Hydroxy-2-butenyltrimethylammonium Iodide (14).** To a solution of 12, 0.55 g (4.8 mmol), in 20 ml of C_6H_6 was added, with stirring, 10.6 g (75 mmol) of CH_3I. The reaction mixture was stirred for 3 hr at room temperature. The light tan precipitate which formed was collected by suction filtration and recrystallized from a MeOH–Et_2O mixture affording 1.15 g (93%) of white crystals, mp 91–93° dec.

Partition Coefficients. The quaternary ammonium compounds (4–10 mg) were dissolved in H_2O and diluted to 50 ml (H_2O). Aliquots of 1-octanol, 5 or 10 ml, were pipetted into culture tubes containing 2 ml of the aqueous solution. The mixtures were stirred using a submersion rotator at 30 rpm for 30 min. The mixtures were centrifuged to ensure complete phase separation. Concentrations in the octanol phase were determined spectrophotometrically at 242.5 nm using a previously determined standard curve.

***In Vivo* Pharmacology.** Cats weighing approximately 3–4 kg were anesthetized with pentobarbital (38 mg/kg ip). Tracheal, venous, and arterial cannulae were inserted and both vagi were cut. Cats were prepared for recordings of ECG, heart rate, and direct arterial blood pressure by standard methods. All drugs were administered intravenously in normal saline.

***In Vitro* Pharmacology.** The isolated, spontaneously beating sinoatrial node of rabbit heart was prepared as previously described.[14] The isolated nodal preparation acts as a model of a dually innervated tissue in which both postganglionic cholinergic (parasympathetic) and adrenergic (sympathetic) nerve fibers may be excited *in vitro* by transmural stimulation. The respective slowing and accelerator responses of the node to nerve stimulation are taken as a measure of neuroeffector transmission of the two types of nerves.

The isolated terminal ileum of the rabbit was prepared for measurement of isotonic contraction of longitudinal smooth muscle in a 5-ml chamber maintained at 37°. The nutrient solution was the same as that used for the isolated sinoatrial node. Quantification of contractions was based on electronic integration of the area under the contraction curve taking the response to $10^{-4}M$ ACh as the 100% response in each preparation.

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Some 2-Amino-5-substituted Oxazolines and Intermediates as Potential Anorectants

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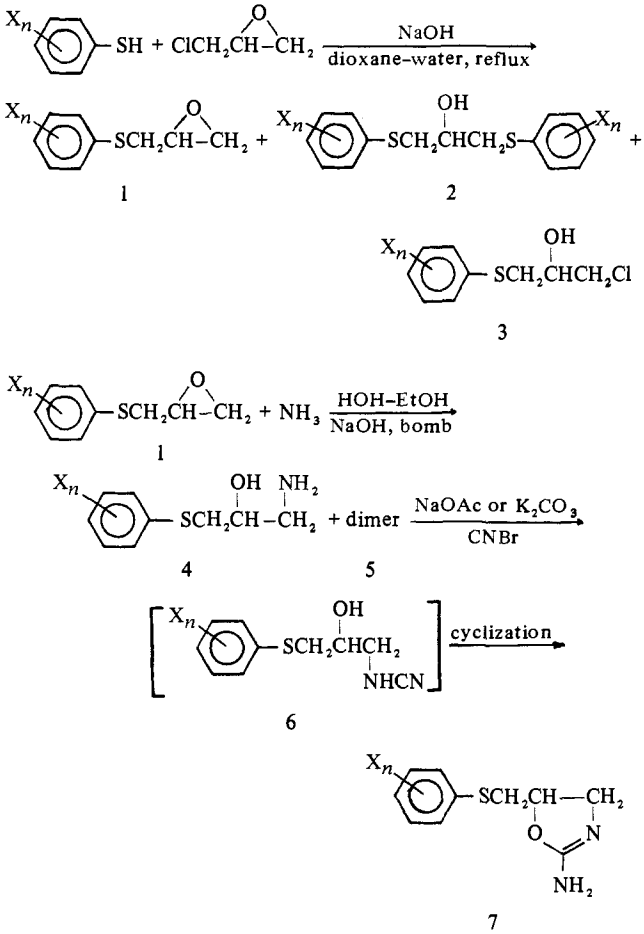
The synthesis of some 2-amino-5-arylthiomethyloxazolines and intermediates is reported. These compounds and some of their intermediates although less active than *d*-amphetamine were found to have significant anorectant activity. The most active in this series was found to be 2-amino-5-(phenylthiomethyl)oxazoline (7a) and caused a significant decrease in the food intake of male albino rats when compared to controls.

The search for anorectants has covered a wide range of chemical structures including numerous heterocyclic ring systems.[1] Poos, *et al.*,[2] have previously synthesized a series of 2-amino-5-phenyloxazolines which have exhibited anorectant and CNS activity. The work reported here differs from the above in that the 5 position on the 2-aminooxazolines is substituted by various arylthiomethyl derivatives. These compounds along with the 1-amino-3-arylthio-2-propanol intermediates have exhibited significant anorectant activity.

Chemistry. The route by which the oxazolines and amino alcohols were synthesized is shown in Scheme I. The reaction of epichlorohydrin with mercaptans is documented in the literature[3,4] and proceeds as illustrated in Scheme I. In our hands it was found that the desired epoxide **1** could be formed free of the dimer **2** and the chlorohydrin **3** by a slight modification of previously reported experimental conditions. The reaction mixture was changed to include a large excess of epichlorohydrin and a slight excess or equivalent of NaOH (see Experimental Section). The epoxide **1** although stable at room temperature had a tendency to decompose at higher temperatures. Decomposition was quite evident when running vpc and was also obvious during distillation.

Scheme I



There are several literature references pertaining to the opening of epoxides with amines.[5-7] It was found that good yields of 1-amino-3-thioaryl-2-propanol (**4**) could be obtained (with negligible amounts of dimer) by using a large excess of NH_3 in water with a trace of NaOH as catalyst.

The final step in the synthesis was formation of the oxazoline ring by treatment of the amino alcohol **4** with cyanogen bromide[2,8] in the presence of an acid acceptor. The reaction proceeded through an intermediate (probably the corresponding hydroxycyanamide, **6**). This intermediate did not cyclize spontaneously as previously reported[2] and could be detected by following the reaction on tlc. Attempts to isolate this open-chain intermediate by removing the solvent under vacuum, however, were unsuccessful and resulted only in the isolation of end product **7**.

Biological Activity and Discussion. Table I shows that the substitution of 4-chloro (**7b**) and 3,4-dichloro (**7c**) on the phenyl ring caused a decrease in the oral and intraperitoneal acute toxicities of the phenylthiooxazolines. On the other hand, the 4-methoxy substitution (**7d**) increased toxicity. However, in the case of the open-ring intermediates, the 3,4-dicholoro (**4c**) was more toxic than either the 4-chloro (**4b**) or the unsubstituted (**4a**) compound.

All compounds tested caused some degree of anorexia in mice. When compared on a milligram per kilogram basis their anorectant activity was less than that of *d*-amphetamine or of the related compound 2-amino-5-phenyloxazoline (aminorex).[2] The 46 mg/kg doses of compounds **7a-d** caused significant ($p < 0.05$) reduction of food intake only at the 1-hr interval. Our results show that the halogenation of the phenyl ring in the 4 or 3,4 positions decreased acute toxicity without significantly reducing anorectic activity. In equipotent anorectant doses with *d*-amphetamine, the oxazolines **7a-d** caused moderate increases in spontaneous motor activity which was less than that of *d*-amphetamine.

Experimental Section

Pharmacology. Groups of three male, albino mice (25-30 g) (Harlan Industries, Cumberland, Ind.) were placed in suspended