cis- and *trans*-2-Mercaptocyclobutylamines, Their Benzylmercapto Analogs, and Aminomethyl Homologs. Influence on Bradykinin-Induced Contraction of the Guinea Pig Ileum†

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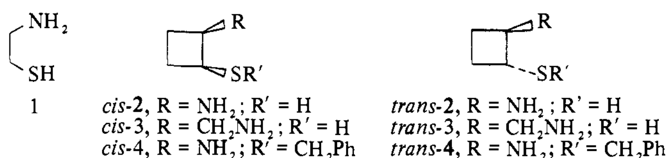
Received July 17, 1972

Many thio compounds, including 2-mercaptoethylamine (**1**), have been shown to potentiate the effect of bradykinin on smooth muscle. In this paper we describe the synthesis of cyclic analogs, *cis*- and *trans*-2-mercaptocyclobutylmethylamine (**3**), and the action of these stereoisomers as well as *cis*- and *trans*-2-mercaptocyclobutylamine (**2**) and *cis*- and *trans*-2-benzylmercaptocyclobutylamine (**4**) on bradykinin-induced contraction of the guinea pig ileum. Biological results are discussed in light of current proposals concerning the mechanism of action of thio analog influence on bradykinin contraction of the guinea pig ileum.

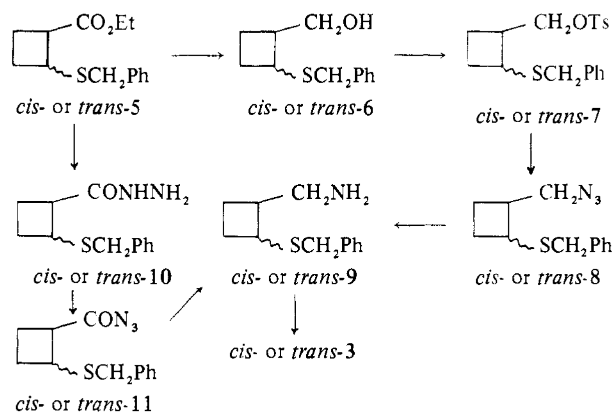
The action of bradykinin on smooth muscle is of considerable importance; this polypeptide is a potent stimulator of many smooth muscles including guinea pig ileum, rabbit intestine, and rat uterus, exhibits a relaxant action on the rat duodenum and colon, and produces a fall in blood pressure in a number of small animals.[1] In addition, a number of SH compounds, such as 2-mercaptoethylamine (**1**), glutathione, cysteine, 2,3-dimercaptopropanol, and 2-mercaptoethanol, are known to potentiate bradykinin-induced contraction of the guinea pig ileum.[2-5] Since Potter and Walaszek[6,7] concluded that the NH_2 and SH groups of cysteine are essential for the potentiating action of this amino acid, it seemed desirable to us to study the stereoselective effects of some recently synthesized[8] cyclobutane analogs of **1** in this system. Appropriately constructed compounds ultimately may serve as probes useful in elucidating structural requirements and mechanisms involved in bradykinin-induced responses of smooth muscle.

The comparative effects of *cis*- and *trans*-2-mercaptocyclobutylamine (**2**), the aminomethyl homologs (*cis*- and *trans*-**3**), and the benzyl thioethers (*cis*- and *trans*-**4**) on bradykinin-induced contraction of the guinea pig ileum are described in this article.



Synthetic Aspects. The synthesis for *cis*- and *trans*-**2** and the isomerically pure benzyl thioethers **4** previously have been described.[8] The isomeric homologs of **2**, namely **3**, were prepared from starting *cis*- or *trans*-2-benzylmercapto-1-carbethoxycyclobutane (**5**).[8] $LiAlH_4$ reduction of pure *cis*- or *trans*-**5** afforded the respective *cis*- or *trans*-2-benzylmercaptocyclobutylcarbinols (**6**) in 85-90% yield. The *cis*-tosylate **7** was obtained from carbinol **6** as a crystalline solid; the *trans*-tosylate **7** could not be crystallized. The latter isomer was purified by dissolving in a minimum amount of $HCCl_3$ followed by precipitation with a large volume of petroleum ether. Treatment of *cis*- or *trans*-**7** with a 10 *M* excess of NaN_3 in dry DMF afforded the respective *cis*-azide (N_3, ir at 2100 cm^{-1}) and *trans*-azide (N_3 at 2150 cm^{-1}) **8** in greater than 90% yield.

$LiAlH_4$ reduction[9] of *cis*- or *trans*-**8** afforded the respective *cis*- or *trans*-amines **9**. Alternatively, isomerically pure amines **9** were obtained by $LiAlH_4$ reduction of the respective cis and trans acid azides **11**. The alternate route is less satisfactory owing to considerably poorer yields, but the products **9** of these two reaction sequences (**8** → **9** and **11** → **9**) were identical in all respects. Pure *cis*- or *trans*-**3**, which were isolated as crystalline HCl salts, was obtained by debenzylation of the HCl salt of the appropriate isomeric amine **9** through use of Na in liquid NH_3.[10,11] This reaction fails if the free base **9** is employed and the NH_3 is not dried. When *cis*- or *trans*-**9** HCl are employed, the final products **3** are obtained in 85% yield.



The stereochemical assignment for pure *cis*- and *trans*-**3** was based on the observation that these amines could be obtained from isomerically pure *cis*- or *trans*-**5**, respectively, by way of pure *cis*- or *trans*-benzyl-protected analogs **9**. Gas-liquid partition chromatography (glpc) of these compounds confirmed that isomerization did not take place during the reaction sequence. Further, intermediate isomeric carbinols **6**, tosylates **7**, azides **8**, and amines **9** and **3** exhibit different physical and spectral properties.

Biological Aspects. The biological testing was carried out on the isolated guinea pig ileum suspended in Tyrode's solution at 37.5°. The drug-induced contractions were recorded *via* an isotonic myograph on a physiograph. During our initial studies it was observed that *cis*- and *trans*-**2**, in dose-dependent fashion, decreased the effects of bradykinin-

†The authors gratefully acknowledge support of this work through Contract No. DATA 17-72-C-2073 from the Department of the Army and U. S. Army Medical Research and Development Command, Grant No. N.S.-10203 from the National Institute of Neurological Diseases and Stroke, and GM-17859 from the National Institutes of General Medical Sciences, U. S. Public Health Service.

‡This work as well as ref 8 was abstracted in part from a dissertation presented by B. K. S., March 1972, to the Graduate School of The Ohio State University.

Table I. Effect of *cis*- and *trans*-**3** on Bradykinin-Induced Contraction of the Guinea Pig Ileum

Drug ($10^{-4} M$)[a]	*n*	Bradykinin, 5 ng/ml = 100% contraction of ileum[b]
cis-**3** HCl	5	81 ± 3.3%
trans-**3** HCl	5	99 ± 1.0%

[a]Incubation time = 2 min. [b]Guinea pig.

Table II. Effect of *cis*-**4** and Histamine in the Presence of Atropine on Guinea Pig Ileum Contraction

Drug	*n*	With atropine,[a] $3 \times 10^{-7} M$, % contraction[b]
cis-**4** HCl ($10^{-4} M$)	5	37 ± 16
Histamine ($10^{-7} M$)	5	93 ± 16

[a]Incubation time = 5 min. [b]Contraction of the ileum to the agonist in the absence of atropine was considered to be 100%.

Table III. Per Cent Reduction in Response to Bradykinin-Induced Contraction of the Guinea Pig Ileum by *cis*- and *trans*-**4** (5 ng/ml = 100%)

Antagonist ($10^{-4} M$)[a]	*n*	% reduction in the response to bradykinin
cis-**4** HCl	5	21 ± 6
trans-**4** HCl	5	31 ± 8

[a]Incubation time = 2 min. For *cis*-**4** the antagonism was studied during the relaxation phase.

induced contractions. At $3 \times 10^{-4} M$, the inhibition by *cis*- and *trans*-**2** was observed to be 25 and 29%, respectively. Since neither isomer potentiated bradykinin-induced contraction of the guinea pig ileum and showed little evidence of stereoselective antagonism, no further work was carried out with these drugs.

Alternatively, when the aminomethyl homologs, *cis*- and *trans*-**3**, similarly were assayed, *cis*-**3** at $10^{-4} M$ exhibited a small but significant reduction in bradykinin-induced contraction of the guinea pig ileum. At $10^{-4} M$ *trans*-**3** was ineffective as an antagonist (Table I). Two additional studies showed that *cis*-**3** ($10^{-4} M$) did not inhibit the effects of $10^{-7} M$ histamine or $3 \times 10^{-7} M$ acetylcholine. Thus, the stereoselective inhibitory effects of *cis*-**3** against bradykinin seem to be relatively specific for this agonist.

For these reasons we studied the action of the benzyl analogs of *cis*- and *trans*-**2** on bradykinin-induced contraction of the guinea pig ileum. We anticipated these compounds (*cis*- and *trans*-**4**) would exhibit even greater blocking effects. In fact, these compounds exhibited uniquely different biological activity. At $10^{-4} M$, *cis*-**4** showed marked intrinsic effects on the ileum. If the contraction to 5 ng/ml of bradykinin is considered as 100%, the stimulant activity of *cis*-**4** = 201 ± 3% (*n* = 3). The contractile effect is characterized by a rapid contraction and subsequent relaxation. If a second dose of *cis*-**4** was administered during the phase of relaxation, no further response was observed. In other words, tachyphylaxis was seen to the effects of *cis*-**4**. The rapid stimulatory effect of *cis*-**4** is stereospecific. *trans*-**4** does not cause a similar response. Analyses of the contractile effects to *cis*-**4** indicate that the drug-induced effects are cholinergic. One possibility is that *cis*-**4** causes the release of endogenous acetylcholine. The reasons for this suggestion are (1) a concentration of atropine which does not block the effects of histamine markedly reduced the effects of *cis*-**4** (Table II); (2) physostigmine (10^{-9} and $10^{-7} M$), an inhibitor of acetylcholinesterase, potentiates the effects of *cis*-**4**; (3) a concentration of antihistamine (chlorphenir-amine, $10^{-6} M$, 5-min incubation), which drastically reduces the effects of histamine, failed to alter the effects of *cis*-**4**.

In addition to the stimulatory effect of *cis*-**4**, both *cis*- and *trans*-**4** inhibit bradykinin-induced contraction of the guinea pig ileum. Since *cis*-**4** shows some intrinsic effects, the antagonism was studied during the relaxation phase. The effects of bradykinin in the presence of $10^{-4} M$ *cis*-**4** were reduced by 21%; *trans*-**4** caused a 31% reduction in response. These apparent stereoselective differences in blocking action (Table III) are statistically insignificant at the $P = 0.05$ level. In other tests, contractions induced by histamine and acetylcholine were equally blocked by *cis*-**4**. The results indicate that the antagonism by *cis*-**4** is nonspecific.

Discussion

Potter and Walaszek have suggested that cysteine potentiates the action of bradykinin on the guinea pig ileum *via* the release of acetylcholine.[6,7] Biological results obtained employing the *cis*-benzylmercapto ether **4** are consistent with an interpretation that this compound works by a similar mechanism. Since mercaptocyclobutylamines (*cis*- and *trans*-**2** or -**3**) did not exhibit intrinsic effects, but would be expected readily to form complexes with divalent metals such as Zn^{2+}, it seems unlikely that the potentiating effect of **1** on the guinea pig ileum is related to inactivation of carboxypeptidase B-type enzymes which contain Zn^{2+} in the active center and are responsible for the metabolism of bradykinin.[1] Sherman and Gautieri have proposed that 2-mercaptopropane is unable to potentiate the effect of bradykinin on the guinea pig ileum owing to a lack of an amino group, which, they suggest, reduces its ability to chelate Zn^{2+} in the active center of carboxypeptidase.[1] However, intrinsic effects obtained with *cis*-**4** and the lack of potentiation observed for *trans*-**4** or *cis*- and *trans*-**2** or -**3** are consistent with the notion that potentiators work primarily by another mechanism; *i.e.*, it is more likely that 2-mercaptopropane is inactive because it lacks appropriate structural requirements to effect the release of acetylcholine than because it cannot inactivate various polypeptidases.

Similarly, Ferreira and Silva[3] observed that the chelating agent 8-hydroxyquinoline, which fully protects bradykinin when added to rat plasma, exhibits no potentiating effect on bradykinin-induced contraction of the guinea pig ileum. While these investigators suggest that one reason for this discrepancy might be the low solubility of 8-hydroxyquinoline and its inability to reach the active sites of the enzyme *in vitro*, it would be particularly difficult to visualize why the bulky benzyl thioether *cis*-**4** could reach such a site and be a better chelating agent than either *cis*- or *trans*-**2** and -**3** or *trans*-**4**. A more likely explanation for the inactivity of 8-hydroxyquinoline is that this compound lacks appropriate structural requirements to effect release of acetylcholine. The observations of Tewksbury,[5] who showed that potentiation is obtained in the maximal response assay, and those of Cirstea,[4] who showed that the bradykinin destroying capacity of the guinea pig gut is not large enough to ascribe potentiation by thio compounds to inhibition of tissue polypeptidases, are in agreement with the conclusions derived from our studies. However, our results do not confirm the suggestion by Cirstea[4] that thio compounds potentiate the action of bradykinin by increasing the number of bradykinin receptors following the rupture of disulfide bridges and the reversible unfolding of protein complexes. If this mechanism were operative, it would be expected that

either *cis*- or *trans*-2 or -3 would be potentiators or have intrinsic activity and that *cis*-4, where the SH group is protected by a benzyl function, would be inactive.

Potter and Walaszek emphasized the importance of the basic amino group in cysteine; *N*-acetylcysteine is about $^1/_{10}$ as active as the unsubstituted amino acid.[6] These results are in agreement with our observation that *cis*-4, which contains a free NH_2 group, is a potent releaser of acetylcholine from the guinea pig ileum. Since the methyl ester of cysteine is four times as potent a potentiator as cysteine and the S-Me and S-CH_2Ph analogs have $^1/_{10}$ and $^1/_5$, respectively, the activity of cysteine, Potter and Walaszek concluded a free SH group also to be important for maximum potentiation. Large functions such as S-$CH(Ph)_2$ and S-$C(Ph)_3$ groups afforded cysteine analogs which blocked bradykinin-induced contraction of the guinea pig ileum. These structure-activity correlations are in agreement with results obtained utilizing benzyl thioethers *cis*- or *trans*-4 which also exhibit equal blocking activity against bradykinin. However, the free SH analogs (*cis*-3 or *cis*- or *trans*-2) also are antagonists. This may be a result of the bulky nature of the cyclobutane ring.

Relative to the antagonism of bradykinin-induced contraction by these analogs, the small but stereoselective antagonism observed for the aminomethyl homolog *cis*-3 is of greatest interest. Since *trans*-3 showed no antagonism at $10^{-4}M$ and *cis*-3 did not block the effects of histamine and acetylcholine, this isomer seems to have specific structural requirements for bradykinin antagonism. Therefore, *cis*-3 may serve as a lead compound for the preparation of more potent specific bradykinin antagonists.

Experimental Section§

***cis*-2-Benzylmercaptocyclobutylcarbinol (6).** To a suspension of 4.5 g (0.118 mol) of $LiAlH_4$ in 250 ml of dry Et_2O was added dropwise 6.0 g (0.024 mol) of *cis*-5[8] in 100 ml of dry Et_2O. After the addition, the reaction mixture was refluxed for 24 hr and cooled and the excess $LiAlH_4$ was decomposed. The Et_2O layer was collected, dried (Na_2SO_4), and concentrated under reduced pressure. Distillation of the residual oil afforded 4.5 g (90%) *cis*-6 as a colorless liquid, bp 126–127° (0.05 mm). Glpc analysis on 10% silicon gum rubber UC-W98 on diaport-S (80–100 mesh), 4 ft × 0.25 in. glass column with column temperature 175°, injection port temperature 250°, detector temperature 290°, inlet pressure of 40 psi, and carrier gas (He) flow rate of 60 ml/min showed one peak at 8.7 min.

***trans*-2-Benzylmercaptocyclobutylcarbinol (6)** was prepared from *trans*-5 according to the method described for *cis*-6. Distillation afforded 85% colorless *trans*-6, bp 122–125° (0.05 mm). The ir spectra for *cis*- or *trans*-6 were virtually identical, but glpc analysis under conditions described for *cis*-6 showed one peak at 8.5 min.

***cis*-2-Benzylmercaptocyclobutylcarbinol *p*-Toluenesulfonate (7).** To a well-cooled solution of 3.8 g (0.02 mol) of TsCl in 25 ml of dry pyridine was added 4.0 g (0.019 mol) of pure *cis*-6 in 25 ml of dry pyridine. The reaction mixture, which immediately turned yellow, was held at 0° for 12 hr, poured over ice-H_2O, and stirred for 1 hr. The resulting solid was filtered, washed with H_2O and petroleum ether, and dried affording 6.0 g (86%) *cis*-7, mp 55–57°. This compound was not further purified and was used immediately in the next reaction.

***trans*-2-Benzylmercaptocyclobutylcarbinol *p*-toluenesulfonate (7)** was prepared in 85–86% yield from *trans*-6 according to the method described for *cis*-7. The trans isomer was obtained as a highly viscous oil which could not be crystallized. Partial purification was achieved by dissolving *trans*-7 in a minimum amount of $HCCl_3$ followed by addition of a large excess of petroleum ether.

***cis*-2-Benzylmercapto-1-azidomethylcyclobutane (8).** To a suspension of 6.5 g (0.10 mol) of NaN_3 in 100 ml of dry DMF was added with stirring 5.0 g (0.014 mol) of *cis*-tosylate 7 in 50 ml of dry DMF. After the addition was complete, the reaction mixture was stirred vigorously at 80–90° for 18 hr, cooled, poured over ice-H_2O, and extracted (Et_2O). The Et_2O layer was dried (Na_2SO_4), filtered, and concentrated under reduced pressure affording 3.0 g (90–92%) of *cis*-8 as an oil, ir (neat, cm^{-1}) 2100 (N_3). This compound was not further purified for use in subsequent reactions.

***trans*-2-Benzylmercapto-1-azidomethylcyclobutane (8)** was prepared from *trans*-7 in 98% yield according to the method described for *cis*-8, ir (neat, cm^{-1}) 2150 (N_3).

***cis*-2-Benzylmercaptocyclobutylmethylamine (9) from *cis*-8.** To a suspension of 3.8 g (0.10 mol) of $LiAlH_4$ in 200 ml of dry Et_2O was added dropwise with stirring a solution of *cis*-8 (3.0 g, 0.013 mol) in 100 ml of dry Et_2O. After addition, the reaction mixture was refluxed for 18 hr and cooled and the excess $LiAlH_4$ was decomposed. The Et_2O layer was collected by filtration (solids washed with Et_2O), dried (Na_2SO_4), filtered, and concentrated under reduced pressure. Distillation of the residual oil afforded 2.5 g (96%) of a colorless liquid, bp 115–116° (0.20 mm). Glpc utilizing a column described for *cis*-6 with column temperature 190°, injection port temperature 265°, detector temperature 290°, inlet pressure of 40 psi, and carrier gas (He) flow rate of 60 ml/min showed one peak at 4.48 min.

The HCl salt was prepared by passing HCl gas into an Et_2O solution of *cis*-9; crystallization from *i*-PrOH–Et_2O afforded white crystals of *cis*-9 HCl, mp 106–107°. *Anal.* ($C_{12}H_{18}NSCl$) C, H, N, S.

An Alternative Synthesis for *cis*-9 from *cis*-11. To a suspension, maintained at 0–4°, of $LiAlH_4$ (3.8 g, 0.10 mol) in 200 ml of dry Et_2O was added dropwise with stirring a solution of *cis*-11 [prepared from 3.0 g (0.013 mol) of *cis*-10][8] in 100 ml of dry Et_2O. The reaction mixture was stirred for 2 hr at 0–5°, refluxed for 18 hr, cooled, and concentrated under reduced pressure. Distillation of the residual oil afforded 1.1 g (42% based on *cis*-9) of a colorless liquid, bp 115–117° (0.20 mm), which was identical with *cis*-9 prepared from *cis*-8 in every respect.

***trans*-2-Benzylmercaptocyclobutylmethylamine (9)** was prepared from *trans*-8 and *trans*-11 by methods described for the preparation of *cis*-9. From *trans*-8, *trans*-9 was obtained in 80% yield; from *trans*-11, *trans*-9 was obtained in 39–40% yield as a colorless oil, bp 109–110° (0.05 mm). Glpc analysis under conditions identical with those described for *cis*-9 showed one peak at 4.3 min. *Anal.* ($C_{12}H_{17}NS$) C, H, S; N: calcd, 6.76; found, 6.26. Crystallization of *trans*-9 HCl from *i*-PrOH–Et_2O afforded white crystals, mp 124–124.5°

***cis*-2-Mercaptocyclobutylmethylamine (3) HCl.** To a 250-ml three-necked flask equipped with a stirrer, gas inlet tube, and Dry Ice condenser was added 0.8 g (0.0032 mol) of *cis*-9 HCl. Dry liquid NH_3 (100 ml) was introduced and while N_2 was blown over the solution, Na (0.2 g, 0.09 g-atom) was added in small pieces until a permanent blue color remained for 45 min. The mixture was stirred for an additional 3 hr, the excess Na was decomposed by adding small portions of NH_4Cl, and NH_3 evaporated after adding 100 ml of dry Et_2O and heating the mixture gently over a hot H_2O bath. The stirred Et_2O suspension was cooled, 100 ml of dry Et_2O saturated with gaseous HCl was added, and the contents were stirred for 1 hr. The solids were filtered, washed with dry Et_2O, and treated with dry *i*-PrOH. The alcohol solution was concentrated to 25 ml under reduced pressure, dry Et_2O added, and crystallization induced in an ice bath (0–5°). The solid was filtered; three recrystallizations from *i*-PrOH–Et_2O afforded 0.41 g (85%) of pure *cis*-3 HCl as a white solid, mp 173.5–175.5°. *Anal.* ($C_5H_{12}NSCl$) C, H, N; S: calcd, 20.86; found, 20.31.

***trans*-2-Mercaptocyclobutylmethylamine (3) HCl** was prepared from *trans*-9 according to the method described for *cis*-3 HCl. Recrystallization from *i*-PrOH–Et_2O afforded 83% white solid, mp 97–98°. *Anal.* ($C_5H_{12}NSCl$) C, H, N, S.

Biological. Guinea pigs were killed by decapitation and segments of the ileum were isolated, cleaned, and refrigerated at 6° for 1.5 hr to decrease inherent rhythmicity. At the end of 1.5 hr, the tissues were suspended in a 10-ml tissue bath containing Tyrode's solution heated to 37–38° and continuously aerated with 95% O_2 and 5% CO_2. Tissue contraction was recorded using a physiograph *via* an isotonic transducer. The tension on the tissue was 0.5 g. Injection of bradykinin into the tissue bath was made at 5-min intervals and each injection was followed by a series of four washings. Drugs introduced into the tissue bath were allowed to incubate for 2 or 5 min prior to addition of bradykinin.

§Melting points were determined using a calibrated Thomas-Hoover melting point apparatus. Ir spectra were recorded utilizing a Perkin-Elmer 257 spectrophotometer and are in agreement with all structures proposed. Glpc analyses were determined using an F & M 402 biomedical gas chromatograph. Elemental analyses were performed by Clark Microanalytical Labs, Urbana, Ill. The correct melting point (reported in ref 8) for *trans*-2 to be 126–128° should be 150–151.5°.

References

(1) W. T. Sherman and R. F. Gautieri, *J. Pharm. Sci.*, **58**, 971 (1969).
(2) E. G. Erdos and J. R. Wohler, *Biochem. Pharmacol.*, **12**, 1193 (1963).
(3) S. H. Ferreira and M. Rocha E. Silva, *ibid.*, **11**, 1123 (1962).
(4) M. Cirstea, *Brit. J. Pharmacol.*, **25**, 405 (1965).
(5) D. A. Tewksbury, *Arch. Int. Pharmacodyn.*, **173**, 426 (1968).
(6) D. E. Potter and E. J. Walaszek, *Pharmacology*, 5, 359 (1971).
(7) D. E. Potter and E. J. Walaszek, *Fed. Proc., Fed. Amer. Soc. Exp. Biol.*, **28**, 799 (1969); **27**, 662 (1968).
(8) D. T. Witiak, B. K. Sinha, O. S. Lee, and D. R. Feller, *J. Med. Chem.*, **15**, 803 (1972).
(9) K. Ponsold, *Chem. Ber.*, 95, 1727 (1962); 96, 1411 (1963).
(10) F. I. Carroll, J. D. White, and M. E. Wall, *J. Org. Chem.*, **28**, 1236 (1963).
(11) J. Baddiley and E. M. Thain, *J. Chem. Soc.*, 800 (1952).

Synthesis and Biological Activity of Some Carbocyclic Analogs of Muscarine

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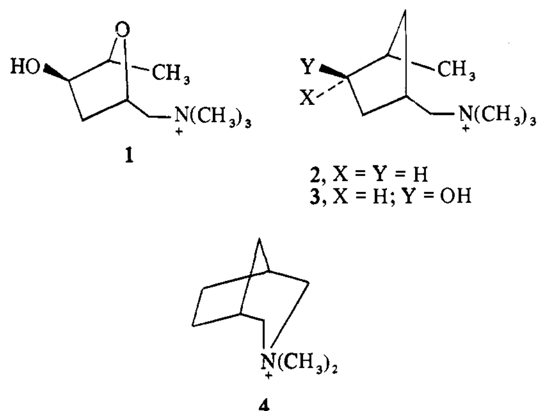
Optimum muscarinic potency has heretofore been assumed to require the presence in the drug of an oxygen atom more or less analogous to the ether oxygen of muscarine. This paper describes the synthesis of several carbocyclic muscarine analogs which lack this atom and yet retain very appreciable muscarinic potency. One, (±)-*trans*-1-hydroxy-*cis*-2-methyl-4-trimethylammoniomethylcyclopentane iodide (**3**), obtained *via* a stereospecific photochemical pathway, was shown to be a direct muscarinic agonist and to possess about five to ten times the potency of (±)-muscarine and acetylcholine. These results indicate a need to consider further muscarinic drug-receptor theory.

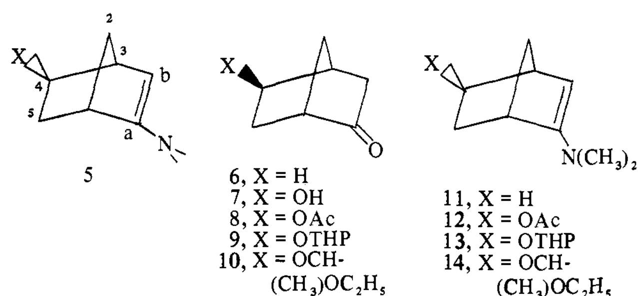
The various proposals put forward regarding the nature of the muscarinic receptor generally agree that, for maximum and selective muscarinic activity, an agonist must have a quaternary nitrogen, an ether oxygen, and an additional oxygen, all separated by critical spatial distances.[1-4] Even though it has been demonstrated that the quaternary nitrogen is essential in muscarine (**1**),[1] and that decreased activity is noted when the hydroxyl group is removed,[5] no one has heretofore replaced the ether oxygen with an isosteric unit



whose electronic properties differ radically from those of the ether oxygen, which has nevertheless been assumed necessary for optimum receptor interaction.[3]

This paper describes the syntheses and biological activity of compounds **2** and **3** which are carbocyclic analogs corresponding to deshydroxymuscarine and muscarine, respectively. Compound **4**, obtained fortuitously in one of the synthesis schemes, can be viewed as a conformationally biased analog of **2** and is therefore an interesting additional analog of deshydroxymuscarine. The cyclopentane ring was selected because it is isosteric with the tetrahydrofuran ring of muscarine (**1**), but the cyclopentane methylene cannot have the same electronic contribution toward receptor interaction postulated for oxygen in the tetrahydrofuran ring of muscarine.

Chemistry. A stereospecific synthesis was envisioned for compounds **2** and **3** from an appropriate norbornyl derivative, such as **5**. This would yield the desired stereochemical relationship among the 1, 3, and 4 substituents by cleavage



of the a–b bond. Synthetic approaches toward the desired compound began with norcamphor (**6**) and *exo*-5-hydroxybicyclo[2.2.1]heptan-2-one (**7**).[6,7]

Treatment of **6** with trisdimethylaminoborane as described for hindered ketones[8] produced the dimethylenamine **11**. This enamine could not be obtained by conventional methods, probably because of the strain produced in the bicyclic system by the introduction of the enamine double bond. The acetate **8**, an intermediate in the synthesis of **7**, afforded enamine **12**. The low yields obtained probably resulted from aminolysis of the ester. Dimethylacetamide, the expected aminolysis product, was also isolated. However, the THF adduct **9** and the ethyl vinyl ether adduct **10** produced good yields of the corresponding enamines **13** and **14**, respectively. From the yields of **13** and **14** and their subsequent reactions, **14** proved to be the more useful derivative.

The instability of these dimethylenamines made it impossible to effect their characterization by standard spectrometric and microanalytical methods. Characterization was carried out by obtaining the mass spectra of the major peak obtained when **11** and **14** were subjected to analytical gas chromatography, and the gas chromatographic effluent passed directly into the mass spectrometer. The spectra obtained were consistent with the structures assigned **11** and