$Ba(OH)_2$ in a 300-ml flask equipped with a 20-cm column. On heating, some H_2O distilled above the melting point of the acid and active pyrolysis started at about 290°. The pressure was reduced to 250 mm and gradually to 2–3 mm as the reaction proceeded. A small amount of froth passed over into the receiving flask as the process continued. When no more distillate was obtained, the distillate was dissolved in 300 ml of Et_2O and washed (H_2O, $KHCO_3$, H_2O, NaCl). After drying (Na_2SO_4), the Et_2O was removed *in vacuo*, and the residue was distilled, bp 97–100° (0.6 mm), 17.3 g (50%). *Anal.* ($C_{12}H_{17}F_3O$) C, H.

8-Trifluoromethylspiro[5.5]undecane-3-ketoxime (XVIII).—A mixture of 10 g of $NH_2OH \cdot HCl$ and 12 g of NaAc was dissolved in the smallest volume of H_2O to give a clear solution. Compound XVII (9 g) was added with stirring and the mixture was shaken vigorously for 1 hr. After filtering, the crude product (9.5 g, mp 109–110°) was recrystallized from MeOH and then from EtAc, mp 115.5–116.5°. *Anal.* ($C_{12}H_{18}F_3NO$) C, H, N.

3-Amino-8-trifluoromethylspiro[5.5]undecane (XIX).—The oxime XVIII (8 g, 0.03 mole) was dissolved in anhydride Et_2O and was slowly added to a solution of 7 g (0.15 mole) of $LiAlH_4$ in 500 ml of anhydrous Et_2O. After stirring and refluxing for 6 hr the mixture was decomposed and filtered. The ethereal solution was dried (Na_2SO_4) and the Et_2O was removed *in vacuo*. Vacuum distillation of the residue gave the product (6 g, 86%), bp 77–79° (0.75 mm). Conversion in the usual manner gave the hydrochloride (alcoholic HCl), mp 293–296° (EtAc and EtOH). *Anal.* ($C_{12}H_{21}ClF_3N$) C, H, Cl, N.

Acknowledgment.—The authors wish to thank the General Chemical Division of the Allied Chemical Corp. for a generous gift of 3-trifluoromethylcyclohexanone.

Studies on the Cholinergic Receptor. II.[1] Monosubstituted and Bicyclic Derivatives of *cis*-2-Methyl-4-dimethylaminomethyl-1,3-dioxolane Methiodide[2,3]

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In continuation of our studies on rigid analogs of acetylcholine, some 7-aza-2,4-dioxa-*cis*-bicyclo[3.3.0]octane methiodides (III) have been prepared. These are bicyclic analogs of the potent muscarinic agent *cis*-2-methyl-4-dimethylaminomethyl-1,3-dioxolane methiodide (VI). For comparative purposes the *cis*- and *trans*-2-methyl-*cis*-4-dimethylaminomethyl-5-methyl-1,3-dioxolane methiodides (IV) have been prepared so that the effects of 5-methyl substitution could be determined. Muscarinic activities of these compounds were low but an inversion of geometric specificity, relative to *cis*- and *trans*-2-methyl-4-dimethylaminomethyl-1,3-dioxolane methiodide, was noted with the 3,7-dimethyl-7-aza-2,4-dioxa-*cis*-bicyclo[3.3.0]octane methiodides, the *exo* isomer being more potent than the *endo* isomer by a factor of ten. This observation may be rationalized by assuming that the quaternary ammonium group constitutes the primary binding site in these compounds and thus determines the general mode of binding of the remaining molecular structure.

A fundamental problem in the analysis of structure–activity relationships is that of relating molecular structure to the relative geometry of the binding sites on the macromolecular receptive surface: this problem is particularly acute with small flexible molecules such as acetylcholine and some of its congeners where, despite the existence of preferred conformations in the crystalline and solution state, it is not possible to assume that these are also the conformations involved in binding at the receptor surface.[1,4] A partial solution to this problem may be obtained by the use of analogs of active molecules in which conformational rigidity predetermines the relative geometry of the proposed binding groups.

An important consideration in this general approach is that the molecular modification required to convert a flexible molecule into a rigid or semirigid analog should involve the minimum structural change in the active molecule. This point was discussed in part I of this series of papers and forms the basis for our choice of analogs of *cis*-2-methyl-4-dimethylaminomethyl-1,3-dioxolane methiodide[5,6] (VI) in which reduced conformational flexibility may be achieved without the incorporation of additional potential binding groups.

In this paper we report the synthesis and muscarinic activities of 7-aza-2,4-dioxa-*cis*-bicyclo[3.3.0]octanes (III) which can duplicate one of the limiting conformations of the highly active agent VI. Since, in some ways, III is more analogous to a 2,4,5-trisubstituted 1,3-dioxolane, we have prepared for comparative purposes the 5-methyl derivative of VI, 2-methyl-*cis*-4-dimethylaminomethyl-5-methyl-1,3-dioxolane methiodide (IV), so that the effects of 5-methyl substitution on the activity of VI can be determined.

Chemistry.—The bicyclooctanes (III) were prepared by reaction between methylamine and the corresponding 4,5-*cis*-bischloromethyl-1,3-dioxolane (II) and subsequent quaternization of the formed tertiary amine with methyl iodide. The intermediate dioxolanes (II) were obtained[7] from *meso*-1,4-dichloro-2,3-dihydroxybutane[8] (I) and the appropriate aldehyde or ketone (Scheme I).

The chloromethyldioxolanes IIc and IId were obtained in admixture. Gas chromatography revealed two compounds in the ratio of 1:4 (peak areas) and this was confirmed by nmr spectroscopy. The nmr spec-

(1) Part I of this series, M. May and D. J. Triggle, *J. Pharm. Sci.*, **57**, 511 (1968).

(2) This work was supported by grants from the U. S. Public Health Service (GM 11603) and NASA (NGR-33-015-016).

(3) Presented in part at the 155th National Meeting of the American Chemical Society, San Francisco, Calif., April 1968 (Abstract No. 3 of Medicinal Chemistry Section).

(4) P. Pauling in "Structural Chemistry and Molecular Biology," A. Rich and N. Davidson, Ed., W. H. Freeman & Co., San Francisco, Calif., 1968, p 555.

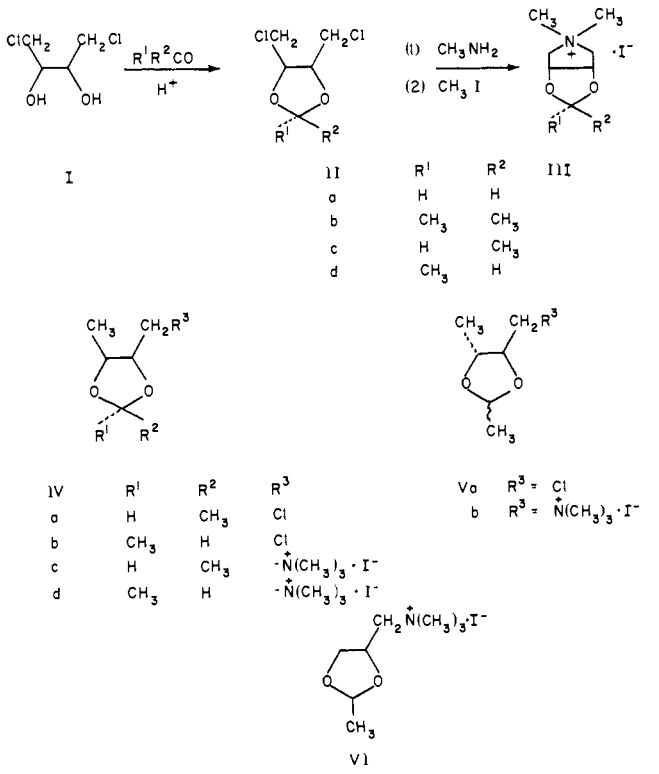
(5) D. J. Triggle and B. Belleau, *Can. J. Chem.*, **40**, 1201 (1962).

(6) D. J. Triggle, "Chemical Aspects of the Autonomic Nervous System," Academic Press, London, 1965, p 91.

(7) J. F. W. McOmie, *Advan. Org. Chem.*, **3**, 264 (1963).

(8) L. N. Owen, *J. Chem. Soc.*, 243 (1949).

SCHEME I



trum also showed that the major component was the *cis* isomer IIc. Pure samples of IIc and IId were isolated by repeated fractional distillation through a spinning-band column. Initially we had converted the mixture of IIc and IId to a mixture of the quaternary compounds IIIc and IIId, which, on careful fractional crystallization, yielded the pure *cis* isomer IIIc. However, the pure *trans* isomer (IIId) could not be isolated. Melting point and nmr data indicated that, in common with other 1,3-dioxolanes,[5] a 1:1 molecular complex of IIIc and IIId had been produced.

Similar results were obtained in the synthesis of IV. Reaction between *erythro*-4-chloro-2,3-dihydroxybutane and acetaldehyde gave a 3:1 mixture of IVa and IVb, which were separated, with considerable difficulty, by fractional distillation. Reaction of the separated isomers with dimethylamine and methyl iodide gave pure IVc and IVd. Similar treatment of the 3:1 mixture of IVa and IVb gave a 3:1 mixture of IVc and IVd which could not be separated into its components.

Gryszkiewicz-Trochimowski, *et al.*, have reported[9] mp 133° for the methiodide of a 4-dimethylaminomethyl-2,5-dimethyl-1,3-dioxolane methiodide of unassigned geometry; this is in contrast to the 187–189° and 157–160° observed by us for IVc and IVd, respectively. However, the method of preparation[9] of Gryszkiewicz-Trochimowski's compound suggests a *trans* disposition of the 4 and 5 substituents about the 4,5 bond of the 1,3-dioxolane. In agreement with this we have obtained a mixture of methiodides (Vb), which melts at 132–134°, from the 4-chloromethyl-1,3-dioxolane (Va) in which the 4 and 5 substituents have the *trans* orientation and the C_2 substituent is *cis* or *trans* to the C_4 and C_5 substituents.

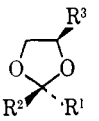
(9) E. Gryszkiewicz-Trochimowski, O. Gryszkiewicz-Trochimowski, and R. Levy, *Bull. Soc. Chim. France*, 610 (1958).

Assignment of Structure.—Baggett, *et al.*,[10] have shown that the geometry of 2,4-disubstituted 1,3-dioxolanes may be determined by nmr spectroscopy. They found that the signal from the C-2 proton of the *trans* isomers occurs at lower field than the corresponding signal from the *cis* isomer and attributed this to transannular deshielding of the C-2 proton, in the *trans* isomer, by the 4 substituent. However, Baggett's compounds, which did not include quaternary ammonium derivatives, were not necessarily suitable models for our purposes. Accordingly, we have examined (Table I) the nmr spectra of *cis*-2-methyl-4-dimethylaminomethyl-1,3-dioxolane methiodide (VI), its *trans* isomer (XIII), and several precursors in the synthetic route to these compounds to which the relative geometry at the 2 and 4 positions had previously been assigned by unambiguous synthesis.[5]

The data of Table I show that, in agreement with Baggett's observations, the signal of the C-2 proton occurs at lower field when *cis* to a 4-methyl (VIII) or a 4-trimethylammoniummethylene group (XIII); however, the inversion of this relationship which occurs with the 2-substituted 4-tosyloxymethyl-1,3-dioxolanes (IX, XII) of previously unambiguously determined geometry[5] shows that caution is needed in the application of nmr spectroscopy to structure assignment in this type of compound. Examination of the nmr data of VI and XIII (Table I) confirms[10,11] that the relative chemical shifts of 2-methyl groups in 2-methyl-4-substituted 1,3-dioxolanes can also provide information about the relative geometry of the 2 and 4 substituents. Deshielding of the 2-methyl group by the C-4 substituent occurs in the *cis* isomers so that the signal from the 2-methyl group (VI) occurs at lower field than in the *trans* isomers (XIII). However, the 4-tosyloxy derivatives (XI and XII) again show inversion of this relationship (see above discussion of IX and XII).

TABLE I

NMR SPECTRA OF 2,4-DISUBSTITUTED 1,3-DIOXOLANES



Compd	R^1	R^2	R^3	Chemical shifts, τ^a R^1	R^2	Spectral solvent
VII	H	CH_3	CH_3	5.08 q[b]	...	CCl_4
VIII	CH_3	H	CH_3	...	4.97 q[b]	CCl_4
IX	H	CCl_3	CH_2OTs	4.64 s	...	$CDCl_3$
X	CCl_3	H	CH_2OTs	...	4.67 s	$CDCl_3$
XI	H	CH_3	CH_2OTs	5.01 q	8.72 d	$CDCl_3$
XII	CH_3	H	CH_2OTs	8.67 d	5.07 q	$CDCl_3$
VI	H	CH_3	$CH_2{}^+N(CH_3)_3$	4.81 q	8.49 d	D_2O
XIII	CH_3	H	$CH_2{}^+N(CH_3)_3$	8.53 d	4.75 q	D_2O

[a] Relative to Me_4Si ($CDCl_3$ or CCL_4) or 3-(trimethylsilyl)-propanesulfonic acid sodium salt (D_2O) as internal references. d = doublet, q = quartet, s = singlet. [b] After Baggett, *et al.*[10]

The assignments of the geometry to compounds II, III, and IV were based on the interpretation of their

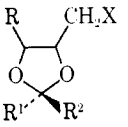
(10) N. Baggett, K. W. Buck, A. B. Foster, M. H. Randall, and J. M. Webber, *J. Chem. Soc., C*, 3394, 3401 (1965); N. Baggett, J. M. Duxbury, A. B. Foster, and J. M. Webber, *ibid.*, 208 (1966).

(11) F. A. L Anet, *J. Amer. Chem. Soc.*, **84**, 747 (1962); M. Anteunis and F. Aldorwereldt, *Bull. Soc. Chim. Belges*, **73**, 889, 903 (1964); **74**, 488 (1965); N. Baggett, K. W. Buck, A. B. Foster, R. Jeffries, B. H. Rees, and J. M. Webber, *J. Chem. Soc.*, 3382 (1965); J. Chuche, G. Danna, and M. R. Monot, *Bull. Soc. Chim. France*, 3300 (1967).

nmr spectra (Tables II and III) according to the principles discussed above. In the *cis*-4,5-disubstituted 1,3-dioxolanes (Table II) the deshielding effect on the *cis*-2 substituent (R^2) is magnified so that the differential chemical shifts between the *cis*-2,4,5-trisubstituted 1,3-dioxolanes and the *trans*-2-*cis*-4,5-trisubstituted 1,3-dioxolanes are greater than in the 2,4-disubstituted 1,3-dioxolanes (Table I). In the 7-aza-2,4-dioxa-*cis*-bicyclo[3.3.0]octanes (Table III), *endo*- and *exo*-methyl groups on both nitrogen and C-3 may be distinguished readily. The deshielding effect of the *cis*-pyrrolidine ring structure causes the signal of the *endo*-3 substituent (R^2, *endo*) to appear at lower field. Similarly the differential deshielding effect of the *cis*-dioxolane ring structure in the rigid bicyclo[3.3.0]octane structure on the N-methyl groups causes the *endo*-methyl group to appear at lower field relative to the *exo*-methyl group. Similar observations have been made[12] by Sable and coworkers for the *endo*- and *exo*-methyl substituents in a number of bicyclic derivatives of 1,3-dioxolanes.

TABLE II

NMR SPECTRA OF 4,5-DISUBSTITUTED 1,3-DIOXOLANES

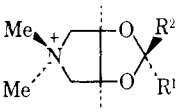


Compd	R	X	R^1	R^2	Chemical shifts, τ[a] R^1	R^2
IIa	CH_2Cl	Cl	H	H	5.30 s	5.00 s
IIb	CH_2Cl	Cl	CH_3	CH_3	8.66 s	8.56 s
IIc	CH_2Cl	Cl	H	CH_3	4.90 q	8.60 d
IId	CH_2Cl	Cl	CH_3	H	8.73 d	4.55 q
IVa	CH_3	Cl	H	CH_3	5.15 q	...
IVb	CH_3	Cl	CH_3	H	...	4.73 q
IVc	CH_3	$N^+(CH_3)_3\ I^-$	H	CH_3	4.85 q	...
IVd	CH_3	$N^+(CH_3)_3\ I^-$	CH_3	H	...	4.53 q

[a] Compounds IIa-d and IVa,b were measured on pure material, Me_4Si as internal reference; IVc,d were measured in D_2O with 3-(trimethylsilyl)propanesulfonic acid sodium salt as internal reference. d = doublet, q = quartet, s = singlet.

TABLE III

NMR SPECTRA OF 7-METHYL-7-AZA-2,4-DIOXA-*cis*-BICYCLO-[3.3.0]OCTANE METHIODIDES



Compd	R^1	R^2	Chemical shift, τ[a] R^1	R^2	*endo*	*exo*
IIIa	H	H	5.33 s	4.78 s	6.76 s	6.86 s
IIIb	CH_3	CH_3	8.67 s	8.47 s	6.73 s	6.84 s
IIIc	H	CH_3	5.00 q	8.59 s	6.70 s	6.84 s
IIId	CH_3	H	8.72 s	4.42 q	6.76 s	6.90 s

[a] Measured in D_2O with 3-(trimethylsilyl)propanesulfonic acid sodium salt as internal reference. s = singlet, q = quartet.

The substituents and protons on C-4 and C-5 in the monocyclic (Table II) and C-1 and C-5 in the bicyclic (Table III) series of compounds give rise to complex spectra with unresolved multiplets in the range τ 5.5–6.5 (II, IVa, and IVb) and 6.0–6.5 (III, IVc, and IVd), presumably as a consequence of long-range coupling effects. We have made no attempt to analyze these portions of the spectra.

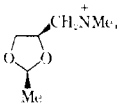
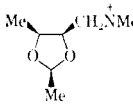
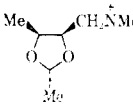
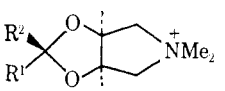
Experimental Section

Biological Tests.—Muscarinic activities of the compounds were determined using the rat jejunum preparation and nicotinic activities were determined using the frog rectus abdominis preparation, as previously described.[1]

The muscarinic activities of the compounds are listed in the Table IV. The nicotinic properties of these compounds were negligible.

TABLE IV

MUSCARINIC ACTIVITIES OF 1,3-DIOXOLANES

No.	Compd	ED_{50}, mole/20 ml rat jejunum	Moles/mole of VI
	Acetylcholine	$5.4 \pm 3.0 \times 10^{-9}$	1
VI		$7.0 \pm 5.0 \times 10^{-9}$	1
IVc		$1.1 \pm 2.0 \times 10^{-6}$	280
IVd		$4.4 \pm 3.0 \times 10^{-6}$	700
			
	R^1 R^2		
IIIa	H H	$8.3 \pm 2.0 \times 10^{-6}$	1200
IIIb	Me Me	Inactive at 10^{-3}	
IIIc	H Me	$1.7 \pm 3.0 \times 10^{-5}$	2400
IIId	Me H	$1.6 \pm 3.0 \times 10^{-6}$	240

Chemistry.—Melting points were determined on a Thomas–Kofler hot stage and are corrected. Ir spectra were recorded on a Beckman I.R 8 spectrophotometer and nmr spectra on a Varian A-60; glpc analyses were carried out on a 10% Carbowax column using an F & M scientific research chromatogram (Model 5750). Analyses were performed by Dr. A. E. Bernhardt, Mulheim, West Germany; where analyses are indicated only by symbols of the elements, the analytical results obtained for those elements are within ±0.4% of the theoretical values.

***erythro*-4-Chloro-2,3-dihydroxybutane.**—Performic acid oxidation[13] of *trans*-crotyl chloride[14] (9 g, 0.1 mole) yielded 12.7 g (82%) of a **monoformyl ester** of the desired diol, bp 66° (0.1 mm), ir (capillary film) 1740 (ester C=O) and 3480 cm^{-1} (OH). *Anal.* ($C_5H_9ClO_3$) C, H.

Hydrolysis of the ester in boiling MeOH–HCl yielded 7.2 g (80%) of product, bp 63–65° (1.5 mm). *Anal.* ($C_4H_9ClO_2$) C, H, Cl.

***threo*-4-Chloro-2,3-dihydroxybutane.**—Oxidation of *trans*-crotyl chloride (9 g, 0.1 mole) with neutral $KMnO_4$[15] yielded 5.4 g (43%) of product, bp 74° (1.5 mm). *Anal.* ($C_4H_9ClO_2$) C, H, Cl.

Substituted 4-Chloromethyl-1,3-dioxolanes.—The compounds described in Table V were prepared, by standard procedures,[4,5,16] from the appropriate 2,3-dihydroxybutane and an aldehyde or ketone. Purification was effected by single or repeated fractional distillation through a Teflon spinning-band column (Nester Faust) until homogeneous by glpc.

***cis*-2-Methyl-*cis*-4-dimethylaminomethyl-5-methyl-1,3-dioxo-**

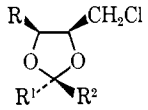
(12) H. Z. Sable, W. M. Ritchey, and J. E. Norlander, *Carbohydrate Res.*, **1**, 70 (1965); *J. Org. Chem.*, **31**, 3771 (1966); H. Z. Sable, Western Reserve University, personal communication.

(13) D. Swern, *Org. Reactions*, **7**, 378 (1953).

(14) L. F. Hatch and S. S. Nesbit, *J. Amer. Chem. Soc.*, **72**, 727 (1950).

TABLE V

4-CHLOROMETHYL-1,3-DIOXOLANES. PHYSICAL DATA



Compd	R	R¹	R²	Yield, %	Bp, °C (mm)	Formula	Analyses
IVa	CH_3	H	CH_3	47[a]	38–39 (15)	$C_6H_{11}ClO_2$	C, H, Cl[b]
IVb	CH_3	CH_3	H	47[a]	39–41 (15)	$C_6H_{11}ClO_2$	C, H, Cl[b]
IIa	$ClCH_2$	H	H	60	55 (0.8)[c]	$C_5H_8Cl_2O_2$	
IIb	$ClCH_2$	CH_3	CH_3	59	58–60 (0.6)	$C_7H_{12}Cl_2O_2$	C, H, Cl
IIc	$ClCH_2$	H	CH_3	70[d]	34–35 (0.1)	$C_6H_{10}Cl_2O_2$	C, H, Cl[e]
IId	$ClCH_2$	CH_3	H	70[d]	34–35 (0.1)	$C_6H_{10}Cl_2O_2$	C, H, Cl[e]
Va[f]	See Scheme I			43	48 (15)	$C_6H_{11}ClO_2$	C, H, Cl

[a] Yield of 3:1 mixture of IVa and IVb. [b] Analysis performed on 3:1 mixture of IVa and IVb. [c] J. B. Miller, *J. Org. Chem.*, **25**, 1279 (1960), gives bp 94–100° (13 mm). [d] Yield of 4:1 mixture of IIc and IId. [e] Analysis performed on 4:1 mixture of IIc and IId. [f] Mixture of two components in the ratio 1:1.

lane **Methiodide (IVc)**.—Dry C_6H_6 (15 ml) containing *cis*-2,5-dimethyl-*cis*-4-chloromethyl-1,3-dioxolane (3.3 g, 0.022 mole) and Me_2NH (0.99 g, 0.044 mole) was heated at 90–100°, in a sealed metal tube, for 48 hr. After being cooled to 0°, C_6H_6 was filtered from $Me_2NH_2^+ \cdot Cl^-$ and evaporated at atmospheric pressure. The residue was treated with an excess of MeI in Et_2O to give the crude product. Crystallization (EtOH) gave 1.6 g (24%) of pure product as colorless prisms, mp 187–189°. *Anal.* ($C_9H_{20}INO_2$) C, H, I, N.

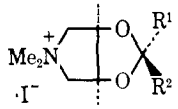
***trans*-2-Methyl-*cis*-4-dimethylaminomethyl-5-methyl-1,3-dioxolane methiodide (IVd)** was obtained in 10% yield from IVb by the method described above for IVc and had mp 157–160° from EtOH. *Anal.* ($C_9H_{20}INO_2$) C, H, I.

4-Dimethylaminomethyl-*trans*-5-methyl-*cis*-2-methyl-1,3-dioxolane methiodide (Vb) was prepared in 10% yield from Va as described for IVc and had mp 132–133° (lit.[8] mp 132°) from EtOH. *Anal.* ($C_9H_{20}INO_2$) C, H, N.

7-Aza-7-methyl-2,4-dioxa-*cis*-bicyclo[3.3.0]octane methiodides (III) (Table VI) were prepared from the appropriate 4,5-bischloromethyl-1,3-dioxolane (Table V) and 3 molar equiv of $MeNH_2$ as described for IVc.

TABLE VI

7-METHYL-7-AZA-2,4-DIOXA-*cis*-BICYCLO[3.3.0]OCTANE METHIODIDES. PHYSICAL DATA



Compd	R¹	R²	Yield, %	Mp, °C	Formula[f]
IIIa	H	H	51	221–223[a]	$C_7H_{14}INO_2$
IIIb	CH_3	CH_3	64	218–220[a]	$C_9H_{18}INO_2$
IIIc	H	CH_3	60[d]	237–239[b,e]	$C_8H_{16}NO_2$
IIId	CH_3	H	20	231–233[c,e]	$C_8H_{16}NO_2$

[a] From EtOH. [b] From MeOH. [c] From MeCN. [d] Isolated from mixture with IIId. [e] Mmp (IIIc + IIId) 205–207°. [f] All compounds analyzed correctly for C, H, N, I.

Discussion

The data presented in Table IV indicate that the structural variations of *cis*-2-methyl-4-dimethylaminomethyl-1,3-dioxolane methiodide (VI) that we have studied lead to significant depression of cholinomimetic activity. Nevertheless, a closer examination of the data reveals some points of interest. The effects of 2 substitution in the 7-methyl-7-aza-2,4-dioxa-*cis*-bicyclo[3.3.0]octane methiodides (III) are generally similar to the same substitutions in the 4-dimethylaminomethyl-1,3-dioxolane methiodide series,[5,6] activity increasing with the introduction of the 2-methyl

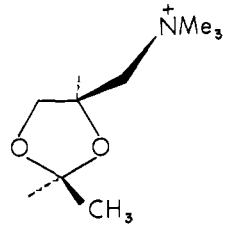


Figure 1.

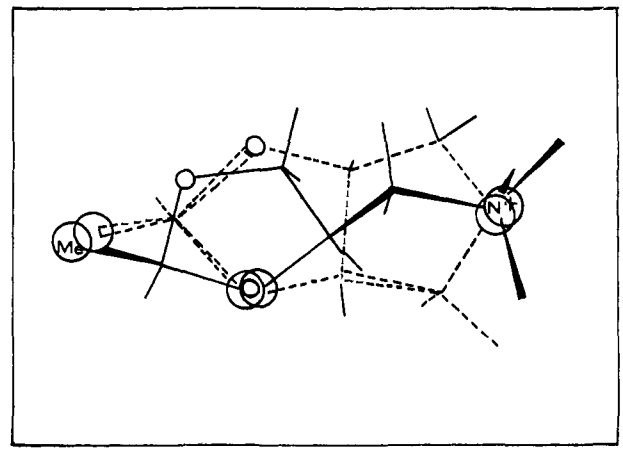


Figure 2.

substituent and dramatically decreasing with 2,2-dimethyl substitution. However, an important difference between these two series of compounds does exist since, in contrast to the 2-methyl-4-dimethylaminomethyl-1,3-dioxolane methiodides where the *cis* isomer is more potent,[5] the *exo* isomer (IIId) of 3,7-dimethyl-7-aza-2,4-dioxa-*cis*-bicyclo[3.3.0]octane methiodide is ten times more active than the *endo* isomer (IIIc).

In order to determine whether this inversion in geometric specificity could be attributed to the effects of ring closure across the 4,5 position of the monocyclic 1,3-dioxolanes the 2-methyl-*cis*-4-dimethylaminomethyl-5-methyl-1,3-dioxolane methiodides (IVc, IVd), in which the 5-methyl substitution might be anticipated to generate a closer structural analogy between the mono- and bicyclic compounds, were prepared and evaluated. However, the 5-methyl substituent, while reducing activity considerably, did not invert the order of activity, *cis*-2-methyl-*cis*-4-dimethylaminomethyl-5-methyl-1,3-dioxolane methiodide (IVc) being more active than the *trans*-2 isomer (IVd) by approximately the same factor previously observed for the 2-methyl-4-dimethylaminomethyl-1,3-dioxolane methiodides.

A more probable rationalization of this phenomenon is afforded by the data of Table VII, which show that the one conformation of VI which cannot be adopted by the conformationally restricted molecules reported here or previously is that in which the quaternary head is maximally extended away from the dioxolane ring (Figure 1). The quaternary ammonium function is known to be the minimum structural requirement for cholinomimetic activity[15–17] and its binding, in simple compounds, is likely to determine the binding confor-

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(17) H. L. Friedman in "Drugs Affecting the Peripheral Nervous System," A. Burger, Ed., M. Dekker, New York, N. Y., 1967, Chapter 2, p 116.

mation of cholinomimetic molecules. Superimposition of molecular (Dreiding) models of *cis*-2-methyl-4-dimethylaminomethyl-1,3-dioxolane methiodide (VI), in the extended configuration, and *exo*-3-methyl-7-methyl-7-aza-2,4-dioxa-*cis*-bicyclo[3.3.0]octane methiodide (IIId) initially at the quaternary groups results in further superimposition of one ring oxygen and, most importantly, the C-methyl groups (Figure 2). The C-methyl group of the *endo* isomer (IIIc) is not superimposable on the C-methyl of VI but is, however, superimposable on the C-methyl of *trans*-2-methyl-4-dimethylaminomethyl-1,3-dioxolane methiodide. The tenfold difference in activity between IIIc and IIId is approximately that observed between the *cis* and *trans* isomers of VI.[5]

It is perhaps pertinent also to note that the MO calculations on muscarine also suggest[18] the biological significance of the conformation in which the quaternary head is maximally extended away from the tetrahydrofuran ring. Further discussion of the implications of this conformation in the general interpretation of the structure–activity relationship of muscarinic agents will be presented in a subsequent publication.

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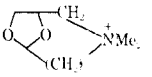
TABLE VII

INTERATOMIC DISTANCES IN SOME MUSCARINIC AGENTS

Compd	Distances, Å: $(CH_3)_3{}^+N \rightarrow O_1$	$(CH_3)_3{}^+N \rightarrow O_2$
VI	3.6	4.6[a]
IIIa–d	3.2	3.2 (min)
	3.5	3.5 (max)
		
$n = 1$	2.5	3.3[b]
	2.8	2.8[c]
$n = 2$	2.5	3.9[d]
	2.85	3.5[e]

[a] Measured for conformation of VI in which ^+N is at maximum distances from both oxygen atoms. Measurements also apply to IVc and IVd. [b] Morpholine ring in boat conformation. [c] Morpholine ring in chair conformation. [d] 1,4-Oxaazacycloheptane ring in boat conformation. [e] 1,4-Oxaazacycloheptane ring in chair conformation.

Choline Acetyltransferase Inhibitors. Configurational and Electronic Features of Styrylpyridine Analogs

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A variety of molecular modifications were made of styrylpyridine prototype choline acetylase (choline acetyltransferase) inhibitors. Among these, enzyme inhibitory activity is favored by the presence of an aromatic ring system conjugated to a pyrido ring through an exocyclic unsaturated bond in such a manner as to provide an over-all coplanar molecule with minimal third dimensional structure. Optimum size appears to be provided by linkage of fused bicyclic and of monocyclic ring systems through either a double or triple bond. One of the cyclic structures should contain at least a weakly basic moiety; quaternization generally increases choline acetylase inhibitory potency. Acetylcholinesterase inhibitory activities of most of these compounds are relatively low and bear no relationship to the activities against choline acetylase.

A review on choline acetylase (ChA) (choline acetyltransferase, acetyl CoA, choline O-acetyltransferase, EC 2.3.1.6), published 5 years ago, concludes with the statement,[1] "A really potent and specific inhibitor of ChA has not been found as yet. Such a compound obviously would be of great interest." Recently, potent and selective inhibitors (reversible, noncompetitive) of this enzyme system have been discovered among some congeners of styrylpyridine.[2] The present report describes a variety of molecular modifications designed to provide further insight as to the steric and electronic features of this type of compound which are conducive to choline acetylase inhibitory properties. Inhibitory activities against acetylcholinesterase (AChE) (acetylcholine acetylhydrolase, EC 3.1.1.7) also were determined in order to assess specificities.

A variety of styrylpyridines have been prepared in the past, particularly for studies of physical-chemical characteristics of position and of *cis–trans* isomers. The most general synthetic route involves condensation of an arylaldehyde with a methylpyridine to yield the *trans*-stilbazole derivatives.[3–6] Additional literature sources may be derived from the cited references. Appropriate methylquinolines form analogous compounds.[7] Quaternary pyridinium or quinolinium derivatives may be formed by quaternizing the condensation product or, preferably, the heterocyclic base component is quaternized prior to condensation.[8–11] Most

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