Table III—Rotational Effect on Dissolution in the Various Vessels

Vessel	Percent Hydrochlorothiazide Dissolved in 30 min.—Rotational Speed of Basket			
	50	100	150	200
Pyrex	21	69	91	96
	22	74	92	98
Kimble	36	78	87	94
	18	70	80	96
Fabricated	22	69	55	63
	24	68	68	70

[a] Values are for individual samples.

During the test, one could observe particles of the disintegrated tablet forming a ring at the bottom of the fabricated flask (Fig. 3).

It is not likely that one would use the type of dissolution vessel shown in Fig. 2 when the other two types are commercially available. There are, however, other commercially available resin pots or dissolution vessels and possible differences of the type illustrated could occur with these vessels. These could be important to a formulator or a quality control investigator working with other products and under varying conditions. Finally, the testing discussed here was done at 150 r.p.m., as called for in the USP XVIII monograph for hydrochlorothiazide tablets. The data presented in Table III appear to indicate that the magnitude of the difference between the various flasks is a function of the basket's rotational speed. This phenomenon will be dependent not only on the hydrodynamics in the bulk of the solution but, more importantly, in the concavity at the base of the flask where the granules tend to accumulate and lie relatively undisturbed.

Each product having a dissolution test in its USP XVIII or NF XIII monograph should be investigated in this manner. Such studies are underway in these laboratories.

CONCLUSIONS

These observations led to the following conclusions:

1. The shape of the dissolution flask, which is not clearly defined in USP XVIII and NF XIII, can significantly affect dissolution patterns (ostensibly by affecting hydrodynamics).
2. The magnitude of these differences is a function of the rotational speed of the USP basket.
3. Formulators should be aware that these differences exist, and it is recommended that there be more definitive specifications for the dissolution vessel in the compendia.

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NOTES

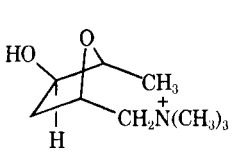
Muscarinic Receptors: 2-Trimethylammonium-7-oxabicyclo[2.2.1]heptane Iodide Epoxides and 2-Trimethylammoniumbicyclo[2.2.1]heptane Iodides

WENDEL L. NELSON▲, DAVID R. ALLEN, and FRANK F. VINCENZI

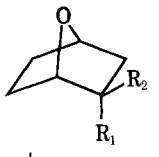
Abstract □ The syntheses of *endo-* and *exo*-2-trimethylammonium-*exo*-5,6-epoxy-7-oxabicyclo[2.2.1]heptane iodides are reported. Muscarinic assay results are reported and compared with *endo-* and *exo*-2-trimethylammoniumbicyclo[2.2.1]heptane iodides. Of the compounds tested, only *exo*-2-trimethylammoniumbicyclo-[2.2.1]heptane iodide demonstrated muscarinic activity, but it was only marginally active.

Keyphrases □ 2-Trimethylammonium-7-oxabicyclo[2.2.1]heptane iodide epoxides—synthesized and screened for muscarinic activity □ 2-Trimethylammoniumbicyclo[2.2.1]heptane iodides—synthesized and screened for muscarinic activity □ Muscarinic activity—2-trimethylammonium-7-oxabicyclo[2.2.1]heptane iodide epoxides and 2-trimethylammoniumbicyclo[2.2.1]heptane iodides

In a previous study (1), conformationally rigid analogs of the cholinergic agonist muscarine (I) in the 7-oxabicyclo[2.2.1]heptane system were reported. In that report, *endo-* and *exo*-2-trimethylammonium-7-oxabicyclo[2.2.1]heptane iodides (II and III) showed only marginal muscarinic activity. This report describes an effort to prepare additional compounds in the series, namely the 5,6-epoxides and 6-oxygenated species, which are more closely related to muscarine. To compare II and III with their carbon analogs, *endo-* and *exo*-2-tri-

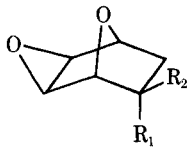


I: muscarine



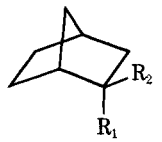
II: $R_1 = \overset{+}{N}(CH_3)_3I^-$, $R_2 = H$

III: $R_1 = H$, $R_2 = \overset{+}{N}(CH_3)_3I^-$



IV: $R_1 = \overset{+}{N}(CH_3)_3I^-$, $R_2 = H$

V: $R_1 = H$, $R_2 = \overset{+}{N}(CH_3)_3I^-$



VI: $R_1 = \overset{+}{N}(CH_3)_3I^-$, $R_2 = H$

VII: $R_1 = H$, $R_2 = \overset{+}{N}(CH_3)_3I^-$

methylammoniumbicyclo[2.2.1]heptane iodides were also screened for muscarinic activity.

SYNTHESIS

Epoxides IV and V were prepared from *endo*- and *exo*-2-(*N*-methyl-*N*-carbomethoxyamino)-7-oxabicyclo[2.2.1]hept-5-enes, VIII and IX, respectively, which are available by Diels-Alder condensation of furan and methyl acrylate, followed by Curtius degradation and alkylation (2) (Scheme I). Peracid oxidation with *m*-chloroperbenzoic acid afforded a single epoxide from each olefinic carbamate, which was assigned the *exo*-configuration based on similar results in the closely related 7-oxabicyclo[2.2.1]heptane and bicyclo[2.2.1]heptene systems (3–8) and on NMR evidence.

In the NMR spectrum of X, both H_1 and H_4 appear as doublets at δ 4.65 ($J_{1,2\text{-}exo}$ = 5 Hz.) and 4.48 ($J_{4,3\text{-}exo}$ = 5 Hz.) (2). In these cases, coupling is not observed between bridgehead protons and adjacent *endo*-protons. H_5 and H_6 appear as a singlet. These data are consistent with the expected small, or nearly zero, coupling constants based on dihedral angle considerations.

The stereochemistry at C-2 is maintained in X and XI, respectively, during this process, as determined from the lack of change in multiplicity of $H_{2\text{-}exo}$ in X and of $H_{2\text{-}endo}$ in XI, respectively. Kunstmann *et al.* (9) reported isomerization at C-2 under epoxidation conditions on a similar system, 2-hydroxymethyl-7-oxabicyclo-[2.2.1]hept-5-ene, using monoperphthalic acid.

Lithium aluminum hydride reduction of X and XI afforded XII and XIII, respectively, which were converted to their respective quaternary salts with iodomethane. Under these conditions, no reduction of the 5,6-epoxide was encountered.

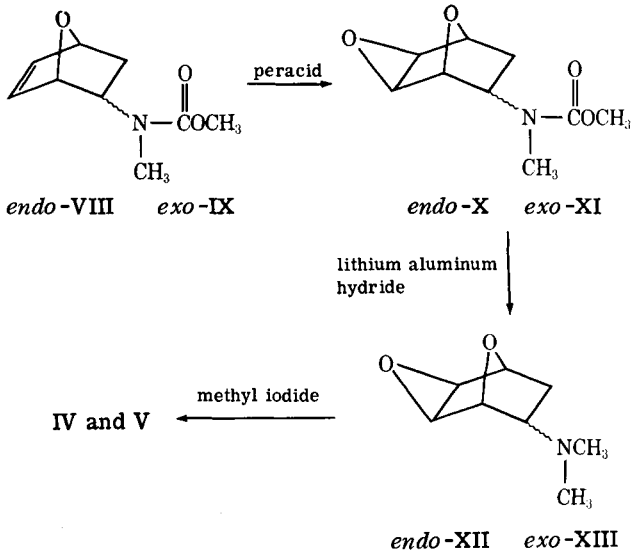
In an effort to prepare 6- (or 5-) hydroxylated analogs, more strenuous reduction conditions were attempted, including lithium aluminum hydride in diglyme (100°) which was unsuccessful. Similar systems have been reduced under these conditions (7, 9). More drastic conditions, including aluminum hydride (10), resulted in decomposition. Several hydroxylation methods, including hydroxymercuration (10–17), also failed. Hydroboration–oxidation (14) afforded only small amounts of hydroxyl-containing material.

An attempt was also made to prepare the corresponding *endo*-epoxides by the addition of hypobromous acid (18–20) using *N*-bromosuccinimide and sodium hydroxide. Only methyl *N*-methylcarbamate was isolated, evidently a result of ring cleavage under the reaction conditions. Isolation of fragments from the remainder of the molecule failed.

PHARMACOLOGY

Compounds IV, V, VI, and VII were tested for muscarinic activity (Fig. 1) on guinea pig ileum by a method described previously (1). Only VI showed marginal activity, producing half-maximal contraction of acetylcholine at about 6.3×10^{-4} *M*. This compares to the half-maximal concentration of acetylcholine of about 7×10^{-8} *M*. At 10^{-4} *M*, each of the other compounds showed only weak smooth muscle contraction. Contractile effects of IV, V, VI, and VII were blocked by atropine. None of the compounds demonstrated significant atropinic effects.

In view of the low level of muscarinic activity and differences in structure, further speculation is not warranted.



Scheme I

EXPERIMENTAL[1]

***endo*-2-(*N*-Methyl-*N*-carbomethoxyamino)-*exo*-5,6-epoxy-7-oxabicyclo[2.2.1]heptane (X)**—*endo*-2-(*N*-Methyl-*N*-carbomethoxyamino)-7-oxabicyclo[2.2.1]hept-5-ene, VIII [prepared by the method of Nelson and Allen (2)], 0.7 g. (9.2 mmoles), and 2.5 g. (80%, 14.6 mmoles) of *m*-chloroperbenzoic acid[2] were dissolved in 25 ml. of

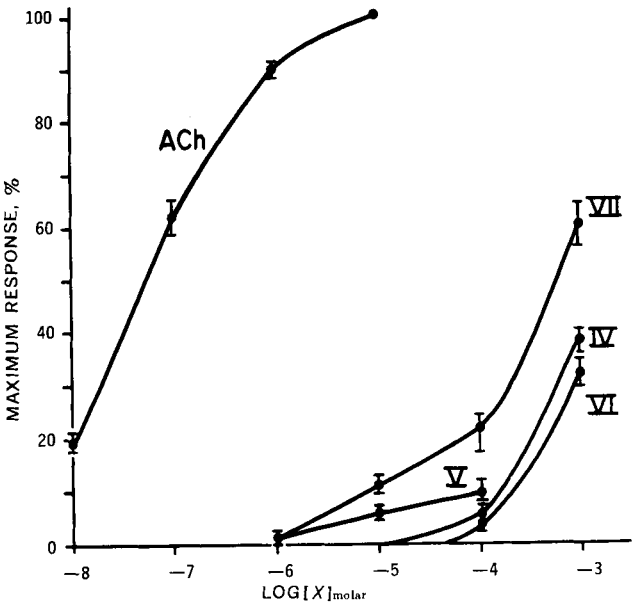


Figure 1—*Muscarinic assay data (ACh = acetylcholine).*

[1] Melting points were obtained on a calibrated Thomas-Hoover Unimelt melting-point apparatus and are corrected. IR spectra were recorded on Beckman IR-5A and IR-20 spectrophotometers. NMR spectra were determined with Varian A-60 and T-60 MHz. spectrometers, using tetramethylsilane and sodium 2,2-dimethyl-2-silapentane-5-sulfonate as internal standards. In NMR descriptions, the following notations are used: s = singlet, d = doublet, t = triplet, q = quartet, dd = doublet of doublet, dt = doublet of triplet, and m = multiplet. Microanalyses were conducted by Dr. F. B. Strauss, Oxford, England.

[2] Research Organic and Inorganic.

chloroform. The solution was stirred (magnetic) for 2 hr. at room temperature and for 30 min. at reflux, transferred to a separator, and washed once with 15 ml. of 5% aqueous sodium hydroxide solution. After drying (magnesium sulfate), the chloroform was evaporated to leave 1.6 g. (95%) of pale-yellow oil. To remove impurities in the material, the epoxide was passed through a 50-g. silica gel column by ether elution. The unidentified impurity was eluted in the first 390 ml., and the epoxide was eluted in the next 350 ml.; IR (neat): 3.39 (aliphatic C—H stretching), 5.90 (carbamate C=O stretching), 6.50, 6.85, 7.45, 7.70, 7.90, 8.40, 8.60, 9.45, 9.80, 10.00, 10.80, 11.65, 12.50, 13.00, 14.25, and 14.85 μ; NMR ($CDCl_3$): δ 4.65 (d, 1, H_1, $J_{1,2\text{-}exo}$ = 4 Hz.), 4.48 (d, 1, H_4, $J_{4,3\text{-}exo}$ = 4 Hz.), 4.23 (dt, overlapping quintet, $H_{2\text{-}exo}$, $J_{2\text{-}exo,3\text{-}exo}$ = 10 Hz., $J_{2\text{-}exo,3\text{-}endo}$ = 4 Hz., $J_{2\text{-}exo,1}$ = 4 Hz.), 3.70 (s, 3, —OCH_3), 3.40 (s, $H_{5\text{-}endo}$ and $H_{6\text{-}endo}$, W/2 = 2 Hz.), 2.90 (s, 3, —NCH_3), 2.10 (dq, 1, $H_{3\text{-}exo}$, J_{gem} = 12 Hz., $J_{3\text{-}exo,2\text{-}exo}$ = 10 Hz., $J_{3\text{-}exo,1}$ = 5 Hz.), and 1.56 (dd, 1, $H_{3\text{-}endo}$, J_{gem} = 12 Hz., $J_{3\text{-}endo,2\text{-}exo}$ = 5 Hz.).

***exo*-2-(*N*-Methyl-*N*-carbomethoxy)amino-*exo*-5,6-epoxy-7-oxabicyclo[2.2.1]heptane (XI)**—The *exo*-epoxide, XI, was prepared from *exo*-2-(*N*-methyl-*N*-carbomethoxyamino)-7-oxabicyclo[2.2.1]-hept-5-ene, IX (2), in quantitative yield by the same method as was used for X; IR (neat): 3.35 (aliphatic C—H stretching), 5.93 (carbamate C=O stretching), 6.90, 7.15, 7.47, 8.40, 8.63, 8.73, 9.20, 9.53, 9.73, 10.02, 10.26, 10.55, 10.80, 11.20, 11.69, 12.05, 12.25, 12.60, 13.35, 14.20, and 15.69 μ; NMR ($CDCl_3$): δ 4.49 (d, 1, H_4, $J_{4,3\text{-}exo}$ = 5 Hz.), 4.37 (s, 1, H_1), $H_{2\text{-}endo}$ buried under H_1 and H_4, 3.67 (s, 3, —OCH_3), 3.29 (s, 2, $H_{5\text{-}endo}$ and $H_{6\text{-}endo}$, W/2 = 2 Hz.), 2.85 (s, 3, —NCH_3), and 1.30–2.00 (m, 2, $H_{3\text{-}exo}$ and $H_{3\text{-}endo}$).

***endo*-2-(*N*,*N*-Dimethylamino)-*exo*-5,6-epoxy-7-oxabicyclo[2.2.1]-heptane Methiodide (IV)**—*endo*-Carbamate epoxide, X, 0.12 g. (0.64 mmole), dissolved in 5 ml. of anhydrous tetrahydrofuran (distilled from calcium hydride), was added dropwise over 3 min. to a stirring (magnetic) suspension of 50 mg. (1.3 mmoles) of lithium aluminum hydride suspended in 50 ml. anhydrous tetrahydrofuran. The reaction mixture was stirred at room temperature under a positive nitrogen pressure for 3 hr. and then refluxed for 30 min. before the dropwise addition of 3 ml. of 40% Rochelle salt solution. Lithium aluminate was removed by filtration, and the filtrate was dried (sodium sulfate) and evaporated. The residue was dissolved in 5 ml. of acetone, and 10 drops of iodomethane was added. The solution was allowed to stand at room temperature for 48 hr. The crystalline material was removed by filtration (100 mg., 53%) and recrystallized from acetone–methanol, m.p. 190° dec.; IR (KBr): 3.35 (aliphatic C—H stretching), 6.85, 7.10, 7.27, 7.65, 8.05, 8.40, 9.19, 9.40, 9.80, 10.01, 10.60, 10.95, 11.65, 12.10, and 13.80 μ; NMR (CD_3OD): δ 4.73 (m, 3, $H_{2\text{-}exo}$, H_1, H_5), 4.10 (d, 1, $H_{6\text{-}endo}$, $J_{6\text{-}endo,5\text{-}endo}$ = 4 Hz.), 3.80 (d, 1, $H_{5\text{-}endo}$, $J_{5\text{-}endo,6\text{-}endo}$ = 4 Hz.), 3.25 [s, 9, $N^+(CH_3)_3$], and 2.75–1.60 (m, 2, $H_{3\text{-}exo}$ and $H_{3\text{-}endo}$).

Anal.—Calc. for $C_9H_{16}INO_2$: C, 36.58; H, 5.43; N, 4.71. Found: C, 36.54; H, 5.52; N, 4.62.

***exo*-2-(*N*,*N*-Dimethylamino)-*exo*-5,6-epoxy-7-oxabicyclo[2.2.1]-heptane Methiodide (V)**—The *exo*-quaternary salt, V, was obtained in 40% yield by the procedure used for IV. It was recrystallized from methanol, m.p. 214° dec.; IR (KBr): 3.35 (aliphatic C—H stretching), 6.80, 7.30, 7.65, 7.85, 8.10, 8.30, 8.55, 8.90, 9.70, 9.87, 10.40, 10.85, 11.65, 12.23, 12.57, and 14.47 μ; NMR (CD_3OD): δ 5.12 (s, 1, H_1), 4.62 (d, 1, H_4, $J_{4,2\text{-}exo}$ = 4 Hz.), 3.58 (s, 2, $H_{5\text{-}endo}$ and $H_{6\text{-}endo}$, W/2 = 3 Hz.), 3.18 [s, 9, $N^+(CH_3)_3$], and 2.25 (m, 2, $H_{3\text{-}endo}$ and $H_{3\text{-}exo}$).

Anal.—Calc. for $C_9H_{16}INO_2$: C, 36.58; H, 5.43; N, 4.71. Found: C, 36.91; H, 5.48; N, 4.88.

***endo*-2-Trimethylammoniumbicyclo[2.2.1]heptane Iodide (VI)**—*endo*-2-Dimethylaminobicyclo[2.2.1]heptane was prepared by the method of Bach (21) and converted to V using iodomethane according to Cope *et al.* (22), m.p. 291° dec. [lit. (22) m.p. 290° dec.].

***exo*-2-Trimethylammoniumbicyclo[2.2.1]heptane Iodide (VII)**—*exo*-2-Aminobicyclo[2.2.1]heptane was prepared by the method of Rathke *et al.* (23), reductively methylated utilizing formaldehyde and formic acid, and converted to VI according to the method of Cope *et al.* (22), m.p. 297° dec. [lit. (22) m.p. 295° dec.].

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