Biotransformation of the Antiarrhythmic Agent 2-(o-{[3-(Dimethylamino)propyl]thio}phenyl)-3-methylurea in the Dog

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Six metabolites were isolated chromatographically from the urine of dogs that had been given oral doses of 1-[^{14}C]o-{[3-(dimethylamino)propyl]thio}phenyl-3-methylurea (I). Metabolites 1 and 2 were identified as unchanged I and mono-N-demethylated I, respectively. Electron impact mass spectrometry (EIMS) of metabolites 3 and 5 yielded the same apparent molecular compositions as I and mono-N-demethylated I, respectively, but, in addition, produced olefinic products by the Cope elimination. Metabolites 4 and 6 yielded M^+ that were weak or absent; however, $M^+ - 17$ fragments were present for both. Downfield chemical shifts for all *N*-Me proton resonances of metabolites 3, 4, and 5 are consistent with N-oxidation of the aminopropylthio side chain. Chemical ionization mass spectrometry (CIMS) greatly enhanced the intensity of the high-mass fragment ions, including the MH^+ of 3, 4, and 6, but not of 5. Metabolite 5, like 3, produced olefinic fragments in both EIMS and CIMS, but possessed 3 fewer *N*-Me protons. It is concluded that 3 and 5 are the *N*-oxide and aliphatic *N*-OH derivatives, respectively, of I and 2, whereas 4 and 6 are the sulfoxide and *N*-oxide *S*-oxide derivatives, respectively, of I. Altered chemical shifts of the 4 aromatic protons of 4 and 6 also identify these compounds as *S*-oxides. CIMS is a useful supplement for the elucidation of structure of those compounds that produce EIMS spectra of low intensity.

Salts of I (carbonyl-^{14}C; see structure in Table I) have exhibited antiarrhythmic activity in pharmacological tests in animals without being depressant to cardiac function.[1] I contains a dimethylaminopropyl*thio* side chain, rather than dimethylaminopropyl, a side chain more commonly found in a variety of drugs. This communication reports on the products of biotransformation of I by the dog as studied by electron impact (EIMS) and chemical ionization mass spectrometry (CIMS). The data indicate that the thermolytically induced Cope elimination[2] can provide useful structural information for the characterization of the products of N-oxidation and that the additional presence of an *S*-oxide function on I decreases the tendency for the Cope elimination to occur.

Experimental Section

Isolation of Metabolites. Urine was pooled from 2 sources. On the one hand, dogs had been given a single dose of 40 mg/kg of [^{14}C]I as its cyclohexanesulfamic acid salt (II) by gavage. Urine collected during the first 24 hr was saved. Other dogs had been given chronically 120 mg/kg per day of nonradioactive II in the diet; urine was collected for each 24-hr period. All the urine from the dogs that had been dosed with [^{14}C]II was combined with several liters of urine from the dogs that had been dosed with nonradioactive II and evapd under reduced pressure at 40–50° to about 10% of its original vol. The concentrate was mixed with sufficient neutral alumina [Woelm, prewashed with $CHCl_3$–MeOH–NH_3 (75:25:5)] to form a thick paste, which was taken to dryness *in vacuo* at 40–50°. A portion of this powder was placed on top of a 5-cm wide by 13-cm high column of neutral alumina, grade III, and eluted with $CHCl_3$–MeOH (10:1), according to the method of Loev and Goodman.[3] This procedure recovered 86% of the radioactivity applied to the column.

The eluate was concd to a suitable vol and streaked on silica gel PQ1-F plates (Quantum Industries, Fairfield, N. J.), which were developed with MeCN–28% NH_3–H_2O (60:10:3). This procedure sepd the radioactivity into 4 uv-pos zones, identified, in the order of their decreasing R_f values, as zones 1, 2, 3, and 4. These 4 zones were isolated by elution of the silica gel with $CHCl_3$–MeOH–NH_3 (75:25:5). The metabolites of zones 1, 2, 3, and 4 were rechromatographed in (A) MeCN–28% NH_3–H_2O (60:10:3) and (B) PhH–28% NH_3–dioxane (60:5:35). In these systems, zones 3 and 4 remained as single components and were designated as metabolites 3 and 4. On the other hand, zone 1 sepd into 2 radioactive zones in solvent system B, one of which, designated as metabolite 1, corresponded in its R_f value to I. The other metabolite, which was slightly more polar, was designated as metabolite 5. Similarly, zone 2 also sepd into several components in solvent system B. In this system, the major component is designated at metabolite 2 and the next most abundant component, which was slightly less polar, as metabolite 6.

In a control experiment, where [^{14}C]II was added to the urine of undosed dogs and carried through the column and tlc procedures, 97% of the radioactivity was eluted from the alumina column, and 95% of the radioactivity applied to the plate had the same R_f value as authentic I.

Instrumentation. Nmr spectra were obtd at 100 MHz with a Model XL-100 spectrometer (Varian Associates). EIMS were obtd with a Model MS902 spectrometer (Associated Electrical Industries). All EIMS were obtd at both low and high resoln. CIMS were obtd with a modified Model MS902 equipped with an SRI (Scientific Research Instruments, Baltimore, Md.) chemical ionization source, using isobutane as the reagent gas. All samples were admitted with a direct insertion probe.

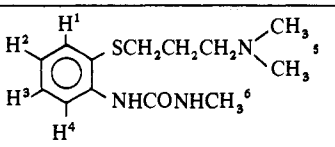
Mass Spectra of Metabolites 2 and 5. The fragment ions produced in the EIMS for metabolite 2 and their intensities (in parentheses) are: 41 (7.2), 42 (7.3), 43 (3.5), 44 (100), 45 (3.0), 58 (5.6), 60 (4.3), 70 (14.8), 71 (10.6), 72 (6.8), 80 (4.9), 83 (5.9), 93 (2.8), 98 (2.2), 111 (5.7), 124 (6.2), 125 (15.9), 126 (3.2), 136 (2.6), 139 (1.7), 149 (1.7), 150 (1.8), 151 (4.5), 152 (2.8), 154 (6.8), 177 (3.1), 182 (2.8), 192 (4.9), 215 (1.4), 238 (0.3), 253 (7.7), 254 (1.4).

The fragment ions produced in the EIMS for metabolite 5 are: 39 (18.5), 40 (7.0), 41 (38.8), 42 (26.1), 43 (100), 44 (75.8), 45 (69.7), 55 (10.3), 56 (11.3), 57 (9.1), 58 (20.0), 59 (7.9), 60 (38.2), 61 (8.8), 68 (4.8), 69 (7.0), 70 (17.9), 71 (13.1), 72 (8.1), 73 (8.2), 74 (19.4), 80 (8.2), 84 (6.9), 85 (11.5), 87 (10.9), 88 (4.7), 93 (8.5), 98 (4.8), 99 (10.6), 114 (7.0), 115 (38.2), 124 (8.8), 125 (14.2), 136 (5.3), 139 (2.4), 150 (4.2), 151 (3.5), 152 (7.2), 153 (6.3), 192 (2.4), 209 (1.2), 222 (4.2), 239 (1.2), 253 (2.4), 265 (1.2).

Results

Data from the nmr spectra of the 6 isolated metabolites and the parent compound, I, are shown in Table I. The main features of the spectra demonstrated that metabolites 2 and 5 contained 3 fewer *N*-Me protons than did any of the other compounds. The nmr spectra of I and metabolite 1, as well as their mass and ir spectra, were identical, identifying this

Table I. Chemical Shifts of Selected Protons of I and Its Metabolites



Metabolite	Chemical shift (τ) in CD_3OD[a]					
	H^1	H^2	H^3	H^4	H^5	H^6
I	2.55 (q, 2, 8)	3.06 (h, ~1.5, 8)	2.78 (h, 2, 8)	2.07 (q, 1.5, 8)	7.82	7.21
1	2.53 (q, 2, 8)	3.05 (h, 2, 8)	2.78 (h, 2, 8)	2.10 (q, 2, 8)	7.64	7.23
2	2.54 (q, 2, 8)	3.03 (h, 1.5, 8)	2.77 (h, 2, 8)	2.10 (q, 2, 8)	7.62[b]	7.22
3	2.51 (q, 2, 8)	3.03 (h, 1.5, 8)	2.77 (h, 2, 8)	2.09 (q, 1.5, 8)	6.90	7.21
4[d]	2.71 (h, 2, 8)[c]	2.53 (q, 2, 8)[c]	2.45 (q, 2, 8)[c]	2.28 (q, 2, 8)[c]	6.82	7.2
5	2.51 (q, 2, 8)	3.03 (h, ~1, 8)	2.79 (h, 2, 8)	2.07 (q, 1, 8)	7.16[b]	7.21
6	2.71 (q, 2, 8)[c]	2.57 (m)[c]	2.42 (s)[c]	2.27 (q, 1.5, 8)	7.40	7.22

[a] Internal reference tetramethylsilane; s = singlet, t = triplet, q = quartet, p = pentuplet, h = hextuplet, coupling constants in hertz. [b] Three protons. [c] Not assigned. [d] 2.13 (s); 2 protons.[c]

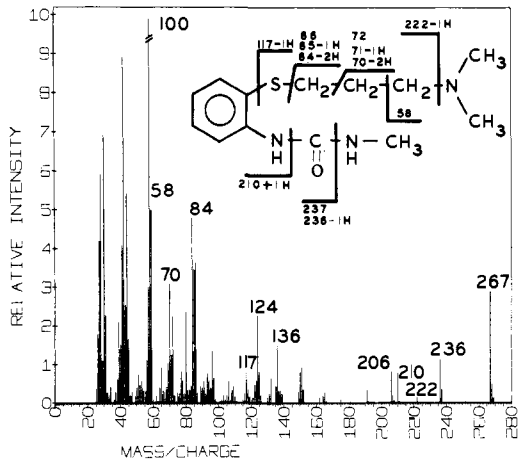


Figure 1. Electron impact mass spectrum of I.

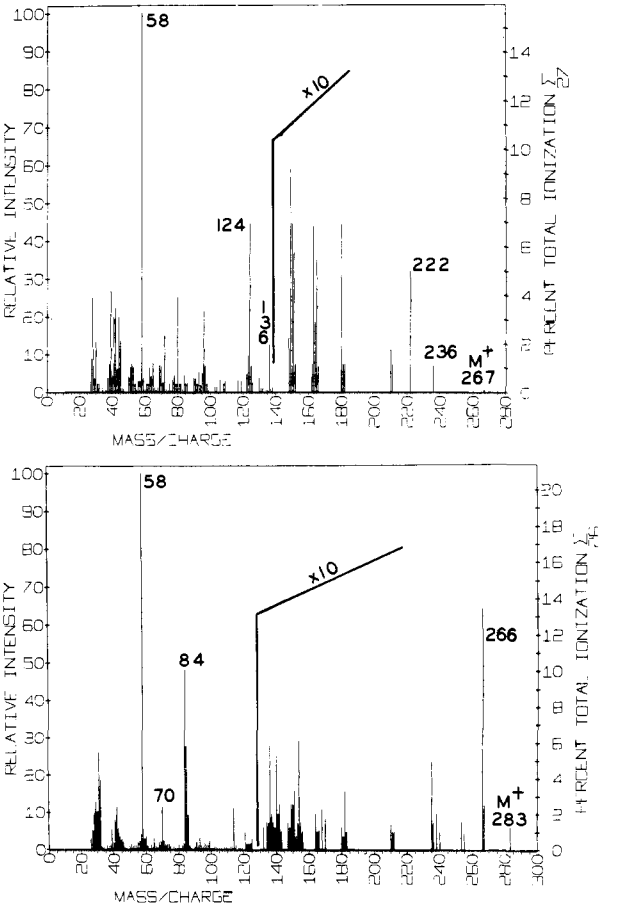


Figure 2. Electron impact mass spectra of metabolites **3** (top) and **6** (bottom).

metabolite. Metabolites **2**, **3**, and **5** had the same aromatic proton resonances as did I, but metabolites **4** and **6** showed significant differences in the chemical shifts of their aromatic protons, even though 4 protons were still present on the Ph group of all the metabolites. The *N*-Me protons of the methylaminopropylthio group of metabolite **5** occur 0.5 ppm downfield from those of metabolite **2**. This difference between metabolites **2** and **5** could be explained by the presence of an additional electronegative group that is substituted on the alkyl N of **5**. Metabolite **5** was the only compound that contained OH, as shown by ir spectroscopy. The chemical shifts of the *N*-Me protons of the dimethylaminopropylthio groups of **3** and **4** also are downfield from that of I, and suggest that, as in metabolite **5**, an electronegative group is attached to the alkyl N. The ureido Me protons appear to be present in all of the metabolites, since 3 protons are found in the spectrum of each metabolite at essentially the same τ value.

Figure 1 shows the EIMS of I; as expected, the molecular ion for I is found at *m/e* 267. The base peak occurs at *m/e* 58, resulting from α cleavage of the C-C bond adjacent to the N of the alkyl side chain. Cleavage of the other C-C bond results in the ions at *m/e* 70-72 and cleavage of the C-S bond in the ions at *m/e* 84-86. The ion at *m/e* 236 results from the loss of $MeNH_2$ from the methylureido side chain. The ion at *m/e* 210 results from the loss of the elements of methyl isocyanate and transfer of the proton. The ion at *m/e* 206, containing 3 N atoms but no S, arises through the elimination of C_2H_5S and transfer of the CH_2 dimethylamino group to the aromatic ring. This rearrangement appears to be unique to aminopropylthio derivatives, since it is not observed in the aminoethylthio or in the corresponding aminopropyloxo analogs.† The weak ion at *m/e* 222 arises by the elimination of Me_2NH_2 from the alkyl side chain.

The EIMS spectrum of metabolite **2** shows a M^+ at *m/e* 253, 14 amu less than that of I; the base peak has also been shifted from *m/e* 58 to *m/e* 44. Other homologous mass shifts are found (*m/e* 192, 177, 72, 71, and 70) that support its structure as the mono-N-demethylated derivative of I, substantiating the structure that had been obtained from nmr.

Figure 2 shows the EIMS of metabolites **3** and **6**. The EIMS

†A. I. Cohen and P. T. Funke, unpublished data.

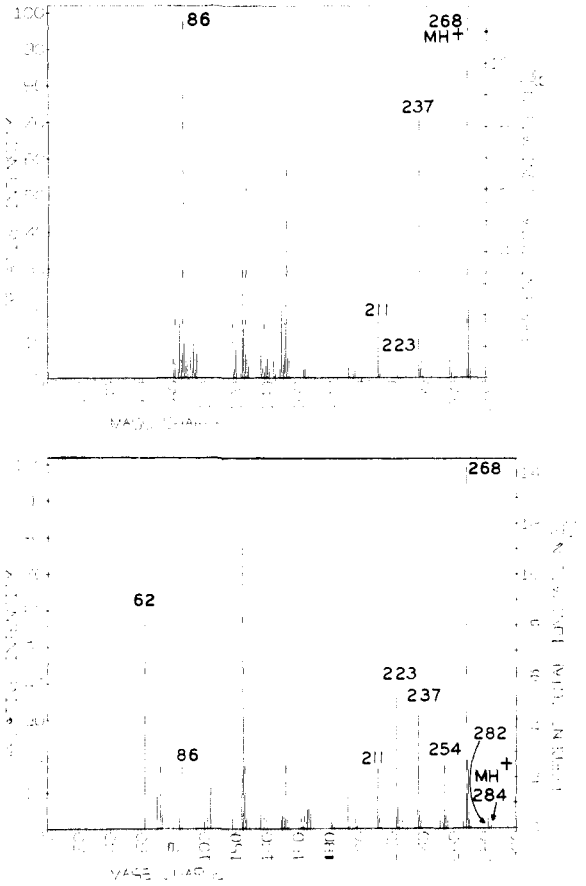


Figure 3. Chemical ionization mass spectra of I (top) and metabolite **3** (bottom).

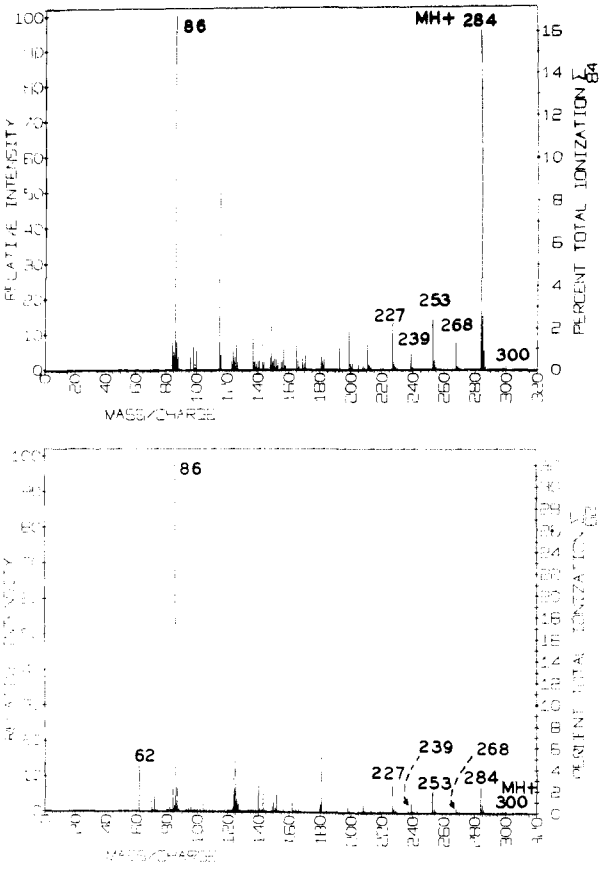
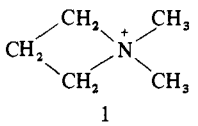


Figure 4. Chemical ionization mass spectra of metabolites **6** (top) and **4** (bottom).

spectrum of **3** generally resembles that seen of I, except that peaks present in the high-mass region are generally very weak; no obvious parent ion for **3** was found, but there is an ion at *m/e* 222 that is considerably more abundant that that found for I. If metabolite **3** were an *N*-oxide, the fragment ion at *m/e* 222 could correspond to that resulting from the thermolytic elimination of dimethylhydroxylamine (Cope elimination). The EIMS of metabolite **6** shows a very weak spectrum in the high-mass region, except for the fragment ion at *m/e* 266, which may be due to loss of OH from the very weak M^+ at *m/e* 283.

The CIMS of I and metabolite **3** are shown in Figure 3. The CIMS of I shows a markedly enhanced MH^+ (protonated M^+) at *m/e* 268 and an ion at *m/e* 237 (loss of $MeNH_2$) resulting from protonation of the amino N and subsequent displacement by the adjacent S. This process is similar to that which occurs in the elimination of NH_3 from S-containing amino



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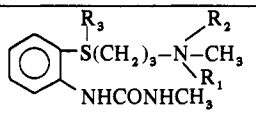
acids.[4] The base peak in the EIMS of I, which occurred at *m/e* 58, is replaced by the ion at *m/e* 86 (**1**) in the CIMS due to protonation of the S atom and displacement by the amine,[4] the reverse of the aforementioned process. Correspondingly, the ion at *m/e* 206 in the EIMS, resulting from α cleavage with charge retention on the aromatic portion, is not found at *m/e* 207 in the CIMS.

The CIMS of metabolite **3** (Figure 3) likewise is greatly enhanced in the high-mass region, as compared with the EIMS. Now, the ion at *m/e* 268 is the base peak and a weak peak is found at *m/e* 284, 16 amu higher than that of I, suggesting that **3** is an oxygenated derivative of I. The ion of metabolite **3** in the CIMS at *m/e* 223 is considerably enhanced over that seen in the CIMS spectrum of I, and its intensity is found to be a function of the temp of the source. The intensity of the ion at *m/e* 86 is greatly diminished for **3**, compared with that found for I. A significant ion at *m/e* 62 appears in the spectrum of **3**, but not in that of I, that corresponds to protonated dimethylhydroxylamine, *i.e.*, the MH^+ ion of the product of the Cope elimination occuring on the alkylamino end of the dimethylaminopropylthio side chain. The ion at *m/e* 268 corresponds to the MH^+ found for I, but, if this is its source, it is difficult to rationalize the weak intensity of the *m/e* 86 ion compared to that found in the CIMS of I. This ion most probably arises from the direct loss of O from the MH^+ ion.

The CIMS of metabolites **6** and **4** are shown in Figure 4. Metabolite **6** exhibits a strong MH^+ at *m/e* 284, which was weak in the EIMS. The CIMS of **6** also exhibits a strong ion at *m/e* 86 resulting from attack of the amino group on the C adjacent to the protonated SO group,[4] demonstrating that the dimethylaminopropyl side chain was unaltered. The small peak at *m/e* 300 is attributed to an impurity. In the CIMS of **6**, an ion is present at *m/e* 268, corresponding to the loss of O from the MH^+, whereas in the EIMS of **6** a similar peak is found at *m/e* 266 corresponding to the loss of OH. The abstraction of H as OH in the EIMS of **6** is considered to represent the removal of a proton from the C on the aromatic ring adjacent to the SO oxygen atom.

The CIMS of metabolite **4** (Figure 4) shows only a slight enhancement in the intensity of the ions seen in the EIMS, except that the ions at *m/e* 284 and 300 are now visible in the CIMS. It is typically observed in CIMS that some ions are formed by alkylation. Thus, the ion at *m/e* 298 is the result

Table II. Urinary Metabolites of [^{14}C]I in the Dog



Metabolite	R_1	R_2	R_3	% of dose
I	CH_3			6.8
2	H			8.1
3	CH_3	0		35.6
4	CH_3	0	0	3.3
5	OH			2.7
6	CH_3		0	2.3
				58.8

of methylation of the molecule of 283 amu that arises by the thermal elimination of oxygen from the molecule of 299 amu.

An ion is found in the CIMS of metabolites **4** and **6** at *m/e* 227 (Figure 4) that is not found for these metabolites in the EIMS. On the other hand, there is an ion at *m/e* 211 in the CIMS of I that corresponds to the protonated ion at *m/e* 210 in the EIMS. It is reasonable to assume that the ion at *m/e* 227 of metabolites **4** and **6** is the oxygenated analog of the ion at *m/e* 211 for I, a consequence of the formation of a sulfoxide. A small ion is present for **4** at *m/e* 239 that corresponds to the MH^+ of the Cope elimination product, which was absent in the EIMS of **4**; the ion at *m/e* 62 corresponds, again, to Me_2NOH. Interestingly, thermolysis occurs by both the loss of O and the elimination of Me_2NOH, but more readily by the former, since ions are found at *m/e* 86 (for the loss of O) and at *m/e* 62 and 239 (for the Cope elimination). Metabolites **4** and **6**, but none of the others nor I, exhibited a band in the ir spectra that was indicative of the presence of the SO function. A summary of the metabolites of I isolated and identified from dog urine is shown in Table II.

Discussion

Metabolic products resulting from the N-oxidation or N-hydroxylation of methylamino alkyl side chains can be difficult to identify. Thus, the *N*-oxide function is not readily detected by uv or ir spectroscopy. Nmr spectrometry of these compounds does produce characteristic downfield shifts of neighboring protons, but the significance of these shifts is difficult to interpret in the absence of spectra of authentic *N*-oxides. Mass spectrometry, however, can provide diagnostic information on the presence of *N*-oxy- or *N*-OH-containing metabolites if certain fragments characteristic of the Cope elimination are found, even if the M^+ or MH^+ are not observed. The presence of such olefinic fragment ions can be further studied in the EIMS or CIMS by varying the temperature of the source.

The thermolysis of *N*-oxides by gas chromatography has been employed as a technique to detect the presence of *N*-oxides of chlorpromazine.[5] Unlike chlorpromazine, I bears a dimethylaminopropyl*thio* side chain. Apparently, the presence of the S atom does not influence the formation of olefinic products from a simple *N*-oxy or *N*-OH compound, but when an SO function is present together with NO, the tendency for the Cope elimination to occur is markedly reduced. Thus, the absence of olefinic products does not rule out the presence of a metabolite due to N-oxidation, particularly if a neighboring SO function is also present. In conclusion, CIMS appears to be a very useful supplementary technique for determining the structure of those compounds that produce weak or nonexistent fragment ions in the high-mass region of the mass spectrum.

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Conformational Aspects of Systems Related to Acetylcholine. 4. The Syntheses of the *dl*-2-Dimethylamino-*trans*-decalin Methiodides

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In order to ascertain if the muscarinic activity of ACh analogs in the *trans*-decalin series was due only to the quaternary function and if the inability of certain of these analogs to act as substrates for AChE was due to selective inhibition by the quaternary group, the axial and equatorial 2-dimethylamino-*trans*-decalin methiodides were prepared and tested. Neither of the compounds prepared inhibited AChE to the degree necessary to account for the inability of certain of the decalin ACh analogs to act as substrates. The pharmacologic results indicate that 4° N alone was not responsible for the action observed with the decalin ACh analogs but that the activity was due to conformational relationship between both ACh pharmacophoric functions.

Earlier reports[1,2] from this laboratory indicate that a conformational preference exists at the various receptor sites for acetylcholine (ACh). Marked differences were found in the rigid analogs of ACh in the *trans*-decalin[1] and *trans*-decahydroquinoline[2] series with respect to acetyl cholinesterase (AChE) substrate activity and muscarinic activity. The possibility existed that the muscarinic activity was due only to the quaternary function and that the inability of certain isomers to act as substrates for AChE was due to selective inhibition by the quaternary group with the

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