after each washing: yield 20 mg; mp >260°; uv pH 13 [λ_{max} in mμ ($\epsilon \times 10^{-3}$)], 253 (31.3), 363 (7.5); nmr, 2.6 (multiplet, CH_2-CH_2CO), 3.4 (pyrazine CH_2CH_2), 5.1 (NHCH), 7.35, 7.45, 7.8, 7.9 (phenyl), 8.7 (pyrazine). *Anal.* ($C_{20}H_{20}N_6O_6$) C, H, N.

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Muscarinic Receptors. Derivatives of 7-Oxabicyclo[2.2.1]heptane[1]

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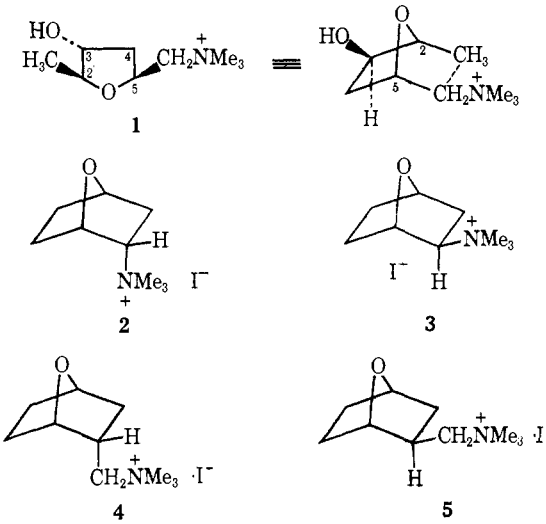
Syntheses of muscarinic analogs *endo-* and *exo*-2-trimethylammonium-7-oxabicyclo[2.2.1]heptane iodides and *endo-* and *exo*-2-dimethylaminomethyl-7-oxabicyclo[2.2.1]heptane methiodides are described. Muscarinic assays are reported.

Steric and electronic effects in the drug, as well as conformational differences in drug and in the drug–receptor interaction, have been suggested as major reasons why certain analogs of acetylcholine (ACh) are more active than others at various cholinergic sites.[2] Preparation and screening of conformationally rigid or semirigid analogs of ACh has met with some success in applications to muscarinic and AChE sites.[2]

In this study of analogs of various conformers of cholinergic agents, muscarine (**1**) was chosen as a model, and analogs in the 7-oxabicyclo[2.2.1]heptane series were prepared. The C-5 methylene ammonium side chain of muscarine has considerable flexibility in models, and various conformations of it and of muscarone have been suggested to explain the difference in absolute stereochemistry of the most active isomer in each case.[3,4] Calculations (extended Hückel theory)[5–7] are not consistent with earlier drug–receptor proposals.[3]

In this series of compounds the position of the N^+Me_3 is restricted to only certain distances from the ether O, and the agents incorporate few or no additional atoms in the C skeleton, which potentially allows for accumulation of some evidence concerning the conformation of muscarine in this drug–receptor interaction, although separation of optical isomers would be necessary to obtain data concerning the muscarine–muscarone controversy.



1 **2** **3** **4** **5**

Bicyclic analogs **2**, **3**, **4**, and **5** were prepared as rigid desoxymuscarine analogs. Compounds **2** and **3** differ from desoxymuscarine only by connection of the C-2 methyl and C-5 CH_2 groups through a single C–C bond. Compounds **4** and **5**, being homologs of **2** and **3**, were prepared because the necessary starting materials were intermediates in the preparation of **2** and **3**. These compounds (**4** and **5**) have the disadvantages of conformational freedom of the quaternary head with respect to the O, but could provide information concerning possible steric interaction between the N^+Me_3 cation and portions of the bicyclic skeleton when compared to **2** and **3**.

Initial attempts were made to find a facile route to 7-oxabicyclo[2.2.1]hept-2-ene (**8**) through Diels–Alder reaction of furan and maleic anhydride to form 7-oxabicyclo[2.2.1]hept-5-ene-*exo*-2,3-dicarboxylic anhy-

(1) (a) An account of this work was presented to the 161st National Meeting of the American Chemical Society, Los Angeles, Calif., March 1971, Abstract MEDI 32. (b) This work was supported in part by Grant NS-08121 from the National Institute of Neurological Diseases and Stroke, U. S. Public Health Service, Bethesda, Md.

(2) For recent reviews see: (a) M. Friedman, *Drugs Affecting Peripheral Nerv. Syst.*, **1**, 79 (1967); (b) S. Ehrenpreis, J. H. Fleisch, and T. W. Miggag, *Pharmacol. Rev.*, **21**, 131 (1969); (c) P. S. Portoghese, *Annu. Rev. Pharmacol.*, **10**, 51 (1970).

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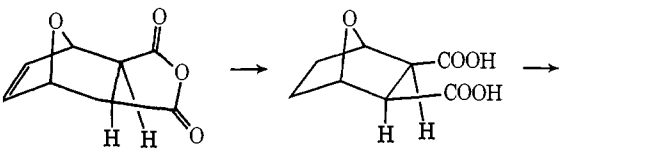
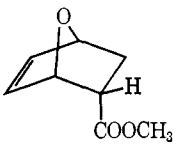
dride (**6**).[8] Although **8** has been prepared by Diels–Alder addition of ethylene and furan directly,[9] the conditions of temp and pressures required for this process were not readily available in our laboratory. Anhydride adduct **6** was hydrolyzed and reduced to **7**[10] which was subjected to various conditions to bring about bisdecarboxylation (Scheme I). Reported procedures

SCHEME I

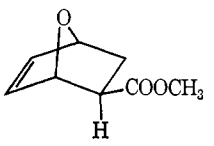


6 **7** **8**

using $Pb(OAc)_4$ in various solvents[11–13] or PbO_2[14,15] failed to provide more than trace amounts of **8**, and in one case a small explosion was encountered. Other routes were sought.



9



10

The mixture of *endo*- and *exo*-2-carbomethoxy-7-oxabicyclo[2.2.1]hept-5-enes (**9** and **10**) has been prepared by Diels–Alder addition of furan to methyl acrylate in generally poor yield after refluxing for several weeks,[16] or at 40° for periods of a month or more.[17] However, starting materials for this process are readily available and at room temp for 30–60 days yields of up to 50% were obtained by us.

Separation of the endo isomer from the mixture of unsaturated carboxylic acids by column chromatography has been reported,[17] although only minimal amounts of the exo isomer are available by this method. Considerable losses were encountered using alumina. However, if reduction of the olefin was performed on the mixture of isomers, facile separation of esters **13** and **14** was accomplished by chromatography on silica gel. Further experimentation showed the unsaturated esters **9** and **10** were readily separable on $AgNO_3$-impregnated silica gel. This separation will facilitate further work to compounds closer to muscarine, with oxygen functions in the C-5 and C-6 positions.

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(9) W. Nudenberg and L. Butz, *J. Amer. Chem. Soc.*, **66**, 307 (1944).
(10) K. Alder and K. H. Backendorf, *Justus Liebigs Ann. Chem.*, **535**, 113 (1938).
(11) E. E. van Tamelen and S. P. Pappas, *J. Amer. Chem. Soc.*, **85**, 3297 (1963).
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(13) C. M. Cimarusti and J. Wolinsky, *J. Amer. Chem. Soc.*, **90**, 113 (1968).
(14) W. von E. Doering, M. Farber, and A. Sayigh, *ibid.*, **74**, 4370 (1952).
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(16) R. J. Ouellette, A. Rosenblum, and G. Booth, *J. Org. Chem.*, **33**, 4302 (1968).
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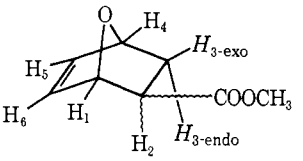
This separation was readily followed by nmr spectroscopy. The difference in *O*-Me positions in the nmr spectra of the mixture of unsaturated esters has been described by Ouellette, and provided a monitoring system for determination of the equilibrium for the isomerization of **9** to **10**. Separation of the endo and exo isomers permitted complete nmr characterization and the obtainment of data useful in determining the relative stereochemistry at C-2 throughout the series (Table I).

TABLE I
60-MC NMR DATA



	endo-COOMe (**9**) δ	*endo*-COOMe (**9**) *J* in Hz	*exo*-COOMe (**10**) δ	*exo*-COOMe (**10**) *J* in Hz
H_6	6.43	q, $J_{6,5} = 6$, $J_{6,1} = 2$	6.37	s, broadened
H_5	6.21	q, $J_{5,6} = 6$, $J_{5,4} = 2$		
H_1	5.16	d, broadened, $J_{1,2\text{-exo}}$	5.18	s, broadened, $J_{1,2\text{-endo}} \simeq 0$, $J_{1,6} \simeq 0$
H_4	5.00	d, broadened, $J_{4,3\text{-exo}} = 4$	5.06	d, $J_{4,3\text{-exo}} \simeq 4$
CH_3	3.63	s	3.71	s
H_2	3.17	quintet, $J_{2\text{-exo},3\text{-exo}} = 8$, $J_{2\text{-exo},1} = 4$, $J_{2\text{-exo},3\text{-endo}} = 4$	2.43	q, $J_{2\text{-endo},3\text{-exo}} = 12$, $J_{2\text{endo},3\text{-exo}} = 4$
$H_{3\text{-exo}}$	2.09	octet, $J_{gem} = 10$, $J_{3\text{-exo},4} = 4$, $J_{3\text{-exo},2\text{-exo}} = 8$	2.16	d or triplets, $J_{gem} = 12$, $J_{3\text{-exo},4} = 4$, $J_{3\text{-exo},2\text{-endo}} = 4$
$H_{3\text{-endo}}$	1.55	q, $J_{gem} = 10$, $J_{3\text{-endo},2\text{-exo}} = 4$	1.57	q, $J_{gem} = 12$, $J_{3\text{-endo},2\text{-endo}} = 8$

Notable differences were seen in the vinyl region (H_5 and H_6), the H_2 and $H_{3\text{-exo}}$ and $H_{3\text{-endo}}$ areas. Deshielding effects of the *endo*-CO_2H probably account for the downfield position of H_6 with respect to H_5 in **9**. Decoupling experiments confirmed the assignment of the downfield signal to H_6, as H_1 is readily identifiable by decoupling of H_2, which then was irradiated to assign H_6.

The positions and multiplicity of the H_2 protons in the endo and exo ester also varied. The large difference in chemical shifts probably results from a deshielding effect of the bridge O on the exo proton (in the endo ester) and/or a shielding effect of the 5,6-double bond on the endo proton in the exo ester. The former explanation is most consistent with the noted similar difference in chemical shifts in the reduced esters **13** and **14**. The differences in multiplicity result from a $J_{2\text{-endo},1} \simeq 0$, consistent with torsion angle measurement of *ca.* 90°, giving a quartet for $H_{2\text{-endo}}$ in **10**, and $J_{2\text{-exo},1} \simeq J_{2\text{-exo},3\text{-endo}} \simeq (1/2)\ J_{2\text{-exo},3\text{-exo}}$ giving a quintet for $H_{2\text{-exo}}$ in **9**.

The signal of $H_{3\text{-exo}}$ is *ca.* δ 0.6 downfield from $H_{3\text{-endo}}$ probably as a result of the bridge O deshielding effect. Multiplicity differences are a result of $J_{3\text{-endo},4} \simeq 0$, as well as torsion angle dependent coupling constants.

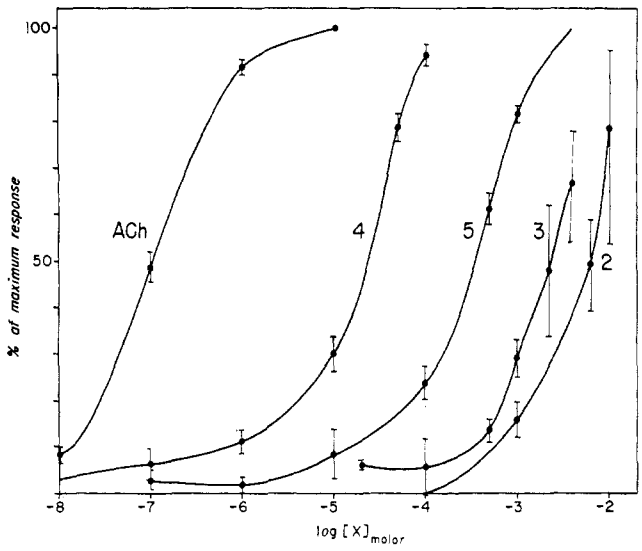
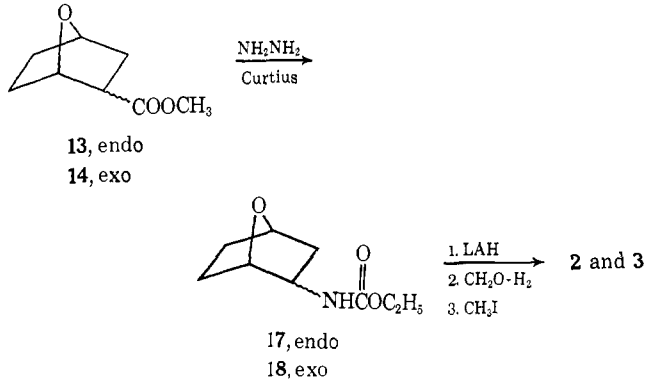


Figure 1.—Concentration–effect relationships of 2-substituted-7-oxabicyclo[2.2.1]heptane derivatives. Isometric contraction of guinea pig ileum *vs.* concentration (logarithmic scale) of various compounds as indicated by the numbers. Data are expressed as a percentage of the maximal tissue response, defined as that produced by 10^{-5} *M* ACh. Each point (with one exception, see Experimental Section) represents the mean from at least 3 separate experiments. Bars represent 2 standard errors.

Bridgehead protons H_1 and H_4 show differences in multiplicity based on the torsion angle dependent $J_{1,2}$ and $J_{3,4}$ coupling constants, and the difference in chemical shifts is probably due to the effect of the CO_2H on H_1 in both isomers.

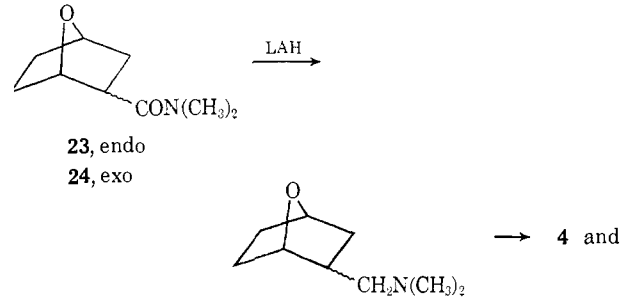
Conversion of the isomeric esters **13** and **14** to **2** and **3** was accomplished by formation of the hydrazides **15** and **16**, Curtius rearrangement, LAH reduction, N-alkylation using CH_2O, and hydrogenation (Pd/C), and quaternization (Scheme II). No change in the

SCHEME II



shape of the nmr signal of the H_2 proton was observed after hydrazide formation confirming the lack of isomerization at C-2. In samples of the individual hydrazides signals for the respective H_2 protons were similar to those of unsaturated esters **9** and **10**. In the rearrangement products (carbamates **17** and **18**) and LAH reduction products (secondary amines **19** and **20**) the nmr spectra were characteristic of the $H_{2\text{-exo}}$ as being present in one series and $H_{2\text{-endo}}$ in the other. In the quaternary ammonium salts (**2** and **3**) similar patterns were observed.

The homologs **4** and **5** were also prepared from esters **13** and **14**. Amide formation with Me_2NH afforded **23** and **24**. Isomerization of endo to exo isomer was noted under the conditions of the reaction, Me_2NH and a trace of HCl at 100° in a sealed autoclave. As the conditions for the Diels–Alder addition formation gave a preponderance of endo ester **9**, this process provided a convenient method to obtain the exo isomers, precluding tedious separation of small amounts of material from the mixture. Separation could be delayed until after amide formation, where column chromatography proved to be very successful. The nmr spectra of amides **23** and **24** showed different sets of *N*-Me groups for each isomer (two each) as well as similar differences in the H_2 and H_3 protons to **9** and **10**. Alternatively, the amides were available from the free acids **11** and **12** by formation of the acyl halide followed by reaction with anhyd Me_2NH, without isomerization. Reduction (LAH) afforded the corresponding tertiary amines **25** and **26** which were quaternized with MeI to give **4** and **5**.



Pharmacology.—All compounds were tested for muscarinic and antimuscarinic activity on guinea pig ileum. Each compound tested produced muscarinic effects as evidenced by concn–effect curves approximately parallel to that of ACh, lack of blockade by hexamethonium, and blockade by atropine. Intrinsic activity of the 4 compounds is assumed to be 1.0, although supportive graphical evidence (Figure 1) is presented only for **4** and **5**. Low potency of **2** and **3** prevented completion of the respective concn–effect curves. However, the slopes of these curves are approximately parallel to those for the other agonists, and lack of any significant atropine-like action on the part of **2**, **3**, **4**, or **5** seems to justify this assumption.

Comparison of the potencies with ACh (Table II)

TABLE II

MUSCARINIC ACTIVITY OF 2-SUBSTITUTED-7-OXABICYCLO[2.2.2.1]HEPTANE DERIVATIVES

Compound (X)	$[X]_{50} = K_X$	Affinity constant	$[X]_{50}/[ACh]_{50}$
ACh	1.06×10^{-7}	9.4×10^{3}	1.0
4, *endo*-$CH_2N^+Me_2$	2.0×10^{-5}	5.0×10^{4}	190
5, *exo*-$CH_2N^+Me_3$	3.0×10^{-4}	3.3×10^{3}	2,800
2, *endo*-N^+Me_3	6.3×10^{-3}	1.6×10^{2}	59,000
3, *exo*-N^+Me_3	2.2×10^{-3}	4.5×10^{2}	21,000

suggests that only the endo analog, **4**, of the CH_2 compounds has significant activity, being about 1.5 times as potent as its exo isomer **5**, and about 0.5% as potent as ACh. Compounds **2** and **3** showed very

weak activity, and speculation concerning any differences between them is not warranted.

The approximately 300-fold difference in potency between **2** and its CH_2 homolog **4** could be related to hindrance of the 2-*endo*-N^+Me_3 cation by the $H_{6\text{-endo}}$ proton on the C_5–C_6 bridge. However, the low level of activity of the exo compound **3** does not support this suggestion.

Models of **4** and **5** can be made to assume a 3.6–3.9 Å $N^+ \rightarrow O$ distance, similar to the dioxolanes[18-20] and extended ACh conformations.[21,22] However, those made of the less active compound **5** are less constrained in this conformation than those of **4**, although the considerable flexibility of the models of both these agents prevents further speculation. The influence of the 7-oxygen atom must be explored to allow consideration of additional factors, as well as 5- and 6-hydroxylated compounds.

Experimental Section[23,24]

***endo-* and *exo*-2-Carbomethoxy-7-oxabicyclo[2.2.1]hept-5-ene (9 and 10).**[17]—A mixt of 30.0 g (0.035 mole) of furan and 21.5 g (0.41 mole) of freshly distd methyl acrylate (Eastman Organic) was allowed to stand at room temp for 1 month or longer. Excess reactants were removed *in vacuo* and the residue was distd affording 22.0 g (50%) of a mixt of endo and exo isomers (6:1) as shown by redn and glpc expts, bp 60° (0.15 mm) (lit.[17] bp 50° (0.10 mm)].

A 3.0-g sample of this mixt was chromatographed on 100 g of silica gel impregnated with 20% Ag^+ using *ca.* 1500 ml of $CHCl_3$ as eluent. The silica gel was prepd from an aq slurry of Silicar-CC-7 (Mallinckrodt) and an aq soln of $AgNO_3$, followed by evapn of the H_2O, and activation at 110° to Brockmann activity I. The sepn was monitored by obtg the nmr spectra of the fractions removed from the column (Table I). This process afforded, in the first 500 ml, the endo ester **9**, followed by a small amt of a mixt of both isomers and, in the last 700 ml, by 0.5 g of exo ester **10**.

***endo-* and *exo*-2-Carbomethoxy-7-oxabicyclo[2.2.1]heptane (13 and 14).**[17]—A mixt of esters **9** and **10**, 10.0 g (0.065 mole), was dissolved in 100 ml of 95% EtOH and hydrogenated (0.5 g 10% Pd/C as catalyst) at 2.45 kg cm^{-2}. About 4 hr was required for H_2 uptake. The soln was filtered (Celite) and evapd. The residue (10.0 g) was placed on a 300-g silica gel (Merck) column and eluted with 8 l. of a mixt of 1:6 Et_2O–pet ether (bp 30–60°). In the first 5.5 l., 5.1 g of endo ester **13** was obtained, followed by 1.5 g of a mixt of esters, predominantly endo, in the next 1 l., followed by 1.5 g of pure exo ester **14** in the final 1.5 l. Fractions were analyzed by glpc at 160°.[23] The retention times were 2.1 min for **13** and 2.7 min for **14**.

***endo-* and *exo*-2-Carboxy-7-oxabicyclo[2.2.1]heptane (11 and 12).**[17]—To 5.0 g (0.0324 mole) of a mixt of esters **13** and **14** was added 20 ml of aq 10% NaOH soln, and the mixt was stirred at room temp for 24 hr. After acidification (concd aq HCl) the mixt was continuously extd with Et_2O for 24 hr. The org layer was dried and evapd affording 4.0 g (94% of theory) of crude acids **11** and **12**. Sepn was accomplished by the method of Kunstmann, *et al.*,[17] by chromatog on neutral alumina (Woelm) deactivated with HOAc. Chromatog afforded 1.0 g of endo acid **11**, mp 76–77° [lit.[17] mp 78–79.5°], and 0.25 g of exo acid **12**, mp 76–77°, indicating considerable losses on the column were encountered.

***endo*-2-Dimethylamino-7-oxabicyclo[2.2.1]heptane Methiodide (2).**—A mixt of 2.0 g (12.8 mmoles) of endo ester **13** and 3.03 g (94.0 mmoles) of 99% hydrazine hydrate was refluxed 15 min. EtOH (50 ml) was added and refluxing contd an addl 45 min. After cooling and drying (Na_2SO_4), the soln was passed through a column of 5.0 g of neutral alumina (Woelm) and evapd affording a yellow oil: 1.9 g (95%); mass spectrum (70 eV) *m/e* calcd, 156.0898; found, 156.0910.

A soln of hydrazide **15** (1.69 g, 11 mmoles) in 25 ml of 50% aq HOAc was cooled to 0° in an ice–salt bath. A soln of 0.90 g (13 mmoles) of $NaNO_2$ in 10 ml of H_2O was added dropwise with stirring followed by stirring at 0° for 45 min. H_2O and Et_2O were added and the mixt was partitioned. The aq layer was extd with 3 addl portions of Et_2O, the exts were combined, washed twice with cold H_2O and twice with aq 10% K_2CO_3 soln, dried (Na_2SO_4), and then evapd *in vacuo* (bath temp 35°) affording a yellow oil (crude azide, ir 4.65 μ). EtOH (20 ml) was added and the mixt was refluxed for 15 min and evapd. The crude carbamate was dissolved in $CHCl_3$, dried (Na_2SO_4), and evapd affording a light yellow oil: 1.73 (98%); mass spectrum (70 eV *m/e* calcd, 185.1051; found, 185.1051.

To a suspension of 1.20 g (31.7 mmoles) of LAH in 250 ml of freshly distd THF (from CaH) was added dropwise a soln of 1.70 g (9.2 mmoles) of carbamate **17** dissolved in 20 ml of THF. The mixt was stirred at room temp for 24 hr and then 2 ml of aq 40% Rochelle salt soln was added dropwise to destroy excess LAH. After suction filtration and drying (Na_2SO_4) the solvent was removed *in vacuo* affording 1.10 g (97%) of **19** of a pale yellow oil: mass spectrum (70 eV) *m/e* calcd, 127.0996, found, 127.0996.

To a soln of 500 mg (3.8 mmoles) of amine **19** in 50 ml of EtOH was added 500 mg of 10% Pd/C and 5.0 g of aq 37% formalin soln. Hydrogenation was performed at 2.10 kg cm^{-2} for 4 hr. Filtration (Celite) removed the catalyst. HOAc (3 ml) was added to prevent volatilization of the amine, and the solvents were evapd. The residue was mixed with 15 ml of aq 10% NaOH soln and extd 3 times with Et_2O. The exts were combined, dried (Na_2SO_4), and evapd (bath 35°) affording 450 mg (85%) of a colorless oil (**2**), which was converted to **2** without further purification.

A mixt of 200 mg of amine **21** and 5.0 ml of MeI was allowed to stand at room temp overnight. The salt was collected: 204 mg (57%); mp 262–268° (Me_2CO–MeOH). *Anal.* ($C_9H_{18}INO$) C, H, N.

The exo quaternary ammonium salt **3** was prepared in an analogous series of steps from exo ester **14** (24% overall yield), mp 205–206° (Me_2CO–MeOH). *Anal.* ($C_9H_{18}INO$) C, H, N.

***endo-* and *exo*-2-Carboxy-7-oxabicyclo[2.2.1]heptane *N,N*-Dimethylamides (23 and 24). A. Ester Aminolysis.**—A 3.0-g sample (19 mmoles of the mixt of endo and exo esters **13** and **14**, prepd by redn of the 6:1 mixt of Diels–Alder adducts **9** and **10**), and 25 ml of anhyd $HNMe_2$ (bp 6°) and 15 mg of $HNMe_2 \cdot HCl$ (hygroscopic) was sealed in a 0° Parr bomb and heated at 125° for 1 to 3 days, depending on the proportion of exo amide desired. Longer runs produce a greater proportion of exo amide **24**.

After cooling, the bomb was opened and excess $HNMe_2$ was allowed to evap. The residue was partitioned between $CHCl_3$ and aq 5% NaOH soln. The aq layer was extd with addl portions of $CHCl_3$, the org layers were combined, dried (Na_2SO_4), and evapd affording 2.10 g (65%, a mixt of amides). The alk layer was acidified with aq 6% HCl and extd with several portions of $CHCl_3$. These exts were combined, dried (Na_2SO_4), and evapd affording 0.50 g of a mixt of acids **11** and **12**.

A 2.0-g portion of the amide mixt was chromatographed on 300 g of silica gel using $CHCl_3$ as eluent, using an automatic fraction collector. Elution was complete with 15 l. of $CHCl_3$ affording 480 mg of pure endo amide **23** as an oil and 580 mg of pure exo amide **24** also an oil. This sepn was readily followed by glpc

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(23) Melting points were determined using a Thomas-Hoover Uni-Melt and are corrected. Ir spectra were recorded on Beckman IR-5A and IR-20 spectrophotometers and were as expected. Nmr spectra were obtained on Varian A-60 and T-60 spectrometers and were consistent with structures assigned. Analysis of the nmr spectra of **9** and **10** is given in Table I. Similar differences were observed in the nmr signals of the C-2 proton of other derivatives in this series. Gas–liquid partition chromatographic data were obtained on a Hewlett-Packard 5752 chromatograph, H_2 flame detector, helium flow rate of 50 cm^3/min. All sepns were done isothermally on a 1.88 m × 0.317 cm column packed with 5% SE-30 silicon gum rubber on 80–100 mesh Chromosorb W. Mass spectra were determined on an AEI MS-9 mass spectrometer. Microanalyses were conducted by Dr. F. B. Strauss, Oxford, England. Where analyses are indicated only by symbols of the elements, analytical results obtained for those elements were within ±0.4% of theoretical values.

(24) In cases where analogous procedures were applied to endo and exo isomers of 2-substituted-7-oxabicyclo[2.2.1]heptanes, only the procedure for the former is given.

at 200.[23] The retention time was 1.3 min for **23** and 2.0 min for **24**.

B. Acid Chloride Aminolysis.—Carboxylic acids **11** and **12** were separately converted to their resp halides using $SOCl_2$ in C_6H_6. The addn of aq $HNMe_2$ to the crude acid chloride afforded an aq soln of the amide which was continuously extd with Et_2O for 24 hr. Evapn of the dry Et_2O ext afforded the amide which was chromatographed on silica gel, and then distd evaporatively. *Anal.* ($C_9H_{15}NO_2$) C, H, N.

Exo amide **24** was prepared from **12** by a similar procedure. *Anal.* ($C_9H_{15}NO_2$) C, H, N.

endo-**2-(N,N-Dimethylamino)methyl-7-oxabicyclo[2.2.1]heptane Methiodide (4) (25).**—Amide **23** was reduced by the method described for redn of carbamate **17,** affording amine **25** as an oil in 85% yield which was converted to **4** (85%) by the method described for **2,** mp 274–275° (Me_2CO–MeOH). *Anal.* ($C_{10}H_{20}INO$) C, H, N.

Exo amine **26** was prepd from **24** by LAH redn (75%) and converted to **5** (70%), mp 233–234° (Me_2CO–MeOH). *Anal.* ($C_{10}H_{20}INO$) C, H, N.

Pharmacological Testing.—Compds were tested on isolated guinea pig ileum perfused in a 5-ml chamber at 37° with the physiol soln described by Blinks and Koch-Weser.[25] Generally, test compds were dissolved at a concn of 10^{-1} *M* in this soln. Appropriate amts were then added to achieve a given final concn in the tissue bath. Concn–effect curves were detd for each compd, and for ACh by exposing the tissue to the drug for 30 sec and then washing out the drug at least 4 times. The max contraction occurring during the 30-sec exposure was taken as the response. The interval between exposure to different concns of a given compd, or to different compds was not less than 4 min. In each tissue preparation a maximal (100%) contraction was defined as that isotonic contraction produced by 10^{-5} *M* ACh. All other response were then expressed as a percentage of the maximal contraction of the tissue as so defined.

At least 3, and usually 5 or more separate experiments (tissues) were employed for each compd at various concns. One exception was **2** at 10^{-2} *M*, where only 2 experiments were performed. When more than one response was elicited in a given tissue, with a given concn of a particular compd the individual responses were averaged and were counted as 1 observation. Observations from sep experiments (tissues) were then averaged and the standard error of the mean was calcd using standard statistical methods for small groups.

For each compd the concn producing a 50% contraction ($[X]_{50}$) was estimated from the standard semilog plot of the concn–effect data.

To test for possible nicotinic actions each compd was tested in the presence of 10^{-5} *M* hexamethonium which was added 1 min prior to the test compd. This concn of hexamethonium blocked the response to dimethylphenylpiperazinium iodide (DMPP) (10^{-6} *M*). To test for possible atropine-like action of the compds another procedure was employed. A low, or just no effect, concn of the test compd was introduced for 1 min, followed by a test dose of 10^{-7} *M* ACh. The resulting response was compared with the response to 10^{-7} *M* ACh in the absence of the compd. None of the compds exhibited significant atropine-like action in the concns tested.

The muscarinic nature of the responses was determined by treating prepns with 10^{-7} *M* atropine. In the presence of atropine, responses to **2, 3, 4, 5,** and ACh were completely blocked.

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Conformational Study of Catecholamine Receptor Sites. 7. Syntheses of *erythro*- and *threo*-3-Amino-2-(3,4-dihydroxyphenyl)-2-butanol Hydrochlorides and *erythro*- and *threo*-2-Amino-3-(3,4-dihydroxyphenyl)butane Hydrochlorides[1]

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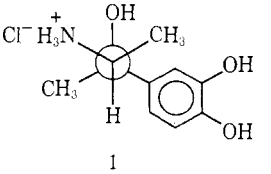
The syntheses of the semirigid analogs of norepinephrine, *threo*- and *erythro*-3-amino-2-(3,4-dihydroxyphenyl)-2-butanol·HCl (**1, 2**), and the analogs of dopamine, *erythro*- and *threo*-2-amino-3-(3,4-dihydroxyphenyl)butane·HCl (**3, 4**), are described. The results of O-methylation by catechol-*O*-methyltransferase (COMT) of these norepinephrine and dopamine analogs are discussed.

In earlier publications[3,4] the synthesis and preliminary testing of the decalin analogs of α-methylnorepinephrine and α-methyldopamine were reported. The rigid analogs of α-methylnorepinephrine exhibited marked differences as substrates for catechol-*O*-methyltransferase (COMT), whereas, the differences in activity of the α-methyldopamine analogs were significantly less. These findings indicated a primary role for the β-OH group in the determination of the preferred conformation for COMT activity. To explore further the importance of the β-OH group and the preferred conformations of α-methylnorepinephrine and α-methyldopamine on COMT activity, the synthesis and preliminary testing of the semirigid analogs **1, 2, 3,** and **4** were undertaken and are the subject of this paper.



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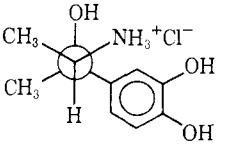

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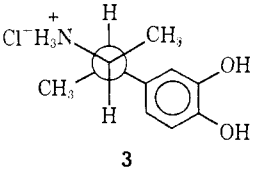

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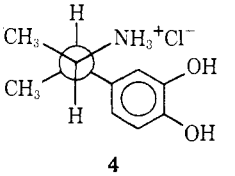

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(1) Presented in part before the 90th Annual Meeting of the Pharmaceutical Society of Japan, Sapporo, Japan, July 29, 1970.

(2) Taken in part from the dissertation presented by R. T. Borchardt, April 1970, to the Graduate School of the University of Kansas in partial fulfillment of the requirements for the Doctor of Philosophy Degree.

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