sterilized nutrient soln (200 ml) were inoculated with B. theobromae Pat.<sup>8</sup> and then incubated on a rotary shaker at 25°. After 3 days 1 (50 mg) in Me<sub>2</sub>CO (1 ml) was added to each flask. Four days later the contents of 50 flasks (pH 6) were combined, adjusted to pH 2 with concd HCl (40 ml), shaken with EtOAc (31.), and then filtered through Celite to remove mycelium. The filtrate was sepd and the aq layer was washed with EtOAc (21.). The aq layer was adjusted to pH 11 with NaOH (8 N) and then extd 3 times with EtOAc (2 l. each time). The combined exts were washed twice with brine (500 ml each time), dried  $(MgSO_4)$ , and then evapd to a gum (2.35 g). Tlc (silica gel GF; Et<sub>2</sub>NH-EtOAc-C<sub>6</sub>H<sub>6</sub>, 5:77.5:17.5) showed a new product,  $R_I$  0.15, containing a trace of starting material,  $R_{\rm f}$  0.38, visible at 254 m $\mu$ . This gum was chromatographed on Al<sub>2</sub>O<sub>3</sub> (50 g, Grade III, neutral) and eluted with petr ether (bp  $60-80^\circ$ ) contg increasing amts of  $C_6H_6$ , and then  $C_6H_6$  contg increasing amounts of CHCl<sub>3</sub>. The (-) isomer of 2 (2.2 g) was eluted in the range petr ether  $(60-80^{\circ})-C_{6}H_{6}$  (4:1) to  $C_{6}H_{6}-CHCl_{3}$  (9:1): mp 107-108° from EtOAc-petr ether (bp 60-80°),  $[\alpha]^{21}$ D – 41.5° (c 1.1, EtOH);  $\tau$  (CDCl<sub>3</sub>) 5.38 (singlet, CHOH, 1); no molecular ion, m/e 121  $[C_{5}H_{4}N \cdot CH(CH_{3})_{2}]^{+}$ , 108  $[C_{5}H_{4} \cdot CHOH]^{+}$ . Anal.  $(C_{14}H_{16}N_{2}O)$ C, H, N.

Check on Optical Purity of (-)-2-Methyl-1,2-di-(3-pyridyl)-1propanol.—A soln of (-)-2 (1.035 g, 0.0045 mole) and (-)-O, Odi-p-toluoyltartaric acid (1.72 g, 0.0045 mole) in MeOH (35 ml) at 50° was cooled slowly to room temp. The crystals which sepd were isolated, mp 155–156°,  $[\alpha]^{21}D - 109.1°$  (c 0.99, MeOH), and recrystd 3 times from MeOH to give (-)-2 hydrogen (-)-O,O-di-p-toluoyltartrate hemihydrate (950 mg): mp 155–156°;  $[\alpha]^{21}D - 109.1°$  (c 0.99, MeOH). Anal. (C<sub>34</sub>H<sub>34</sub>H<sub>2</sub>O<sub>9</sub>·0.5H<sub>2</sub>O) C, H, N. (-)-2-Hydrogen (-)-O,O-di-p-toluoyltartrate hemi-

(8) Imperial Chemical Industries Ltd., A.C.C. 3121, kindly supplied by Royal Netherlands Fermentation Industries Ltd., Delft.

hydrate (890 mg) was shaken with EtOAc (50 ml) and NaOH (0.5 N, 25 ml). The EtOAc extract gave (-)-2 free base, mp 108-9° from EtOAc,  $[\alpha]^{24}D - 42.3^{\circ}$  (c 0.99, EtOH).

(+)-2-Methyl-1,2-di-(3-pyridyl)-1-propanol (2).—A soln of racemic 2, mp 100° (1.7 g, 0.0075 mole), and (+)-0,0-di-p-toluoyltartaric acid (2.8 g, 0.0073 mole) in MeOH (50 ml) at 50° was cooled slowly to room temp. The solid which sepd was recrystd 6 times from MeOH to give (+)-2 hydrogen (+)-0,0-di-p-toluoyltartrate hemihydrate (1.3 g, 1st and 2nd crops) of constant rotation: mp 155–156°;  $[\alpha]$  b + 108.6° (c 1.0, MeOH). Anal. (C<sub>34</sub>H<sub>34</sub>N<sub>2</sub>O<sub>9</sub>·0.5H<sub>2</sub>O) C, H, N. This salt gave (+)-2 free base: mp 107–8°;  $[\alpha]$ <sup>21</sup>b + 42.7° (c 1.1, EtOH). Anal. (C<sub>14</sub>H<sub>16</sub>N<sub>2</sub>O) C, H, N.

**1,2-Dipyrid-3-yl-2-methylpropyl Dihydrogen Borate** (3). NaBH<sub>4</sub> (1 g) was added during 3 hr to a stirred soln of 2-methyl-1,2-di-3-pyridyl-1-propanone (2 g) in MeOH (30 ml) at 0°, and then kept for 2 hr. MeOH (15 ml) was removed *in vacuo*, brine (15 ml) was added, and the mixt was extd with EtOAc. The ext gave 3, mp 192°, mass spectrum identical with that of (-)-1,  $\tau$  (DMSO-d<sub>6</sub>) 4.57 (broad singlet exchanged by D<sub>2</sub>O, OH), 5.12 (singlet, CHO, 1). *Anal.* (C<sub>14</sub>H<sub>17</sub>BN<sub>2</sub>O<sub>3</sub>) H, N; C: calcd, 61.8; found 62.3.

Isolation of 2-Methyl-1,2-di-(3-pyridyl)-1-propanol from Urine Extract.—The crude ext (2.4 g) supplied by Dr. Sprunt was dissolved in H<sub>2</sub>O (50 ml) and EtOAc (50 ml) and then the pH was adjusted to 2.0 with conced HCl. The mixt was shaken and then the aq acid layer was sepd, washed with EtOAc (50 ml), adjusted to pH 11 with NaOH (8 N), and then extd 3 times with EtOAc (100 ml each time). The combined exts were washed twice with brine (50 ml each time), dried (MgSO<sub>4</sub>), and then evapd to a gum (1.18 g). This was chromatographed on Al<sub>2</sub>O<sub>8</sub> as described above. The pure product was eluted in the range CHCl<sub>5</sub>-C<sub>6</sub>H<sub>6</sub> (1:19 to 1:3), (0.65 g), mmp 100° from EtOAc-petr ether (bp 60–80°), [ $\alpha$ ]<sup>21</sup>D  $\pm$  0° (c 1.04, EtOH).

# Stereochemical Studies on Medicinal Agents. 9.<sup>1,2</sup> Bicyclic Bases.<sup>3</sup> Synthesis and Biological Activities of Epimeric Quaternary Derivatives of 2-Oxa-5-azabicyclo[2.2.1]heptane<sup>4</sup>

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Optically active N-Me-N-benzyl quaternary derivatives of (1S,2S)-2-oxa-5-azabicyclo[2.2.1]heptane were synthesized in order to investigate the effect of an asymmetric quaternary N on anticholinergic activity. Nmr studies indicate that the N-substituted bicyclic system undergoes highly stereoselective quaternization. Configurations have been tentatively assigned to the N epimers. The *cxo*-5-methyl-*endo*-5-benzyl and *exo*-5-benzyl*endo*-5-methyl N epimers possess comparable antagonistic activities on the guinea pig ileum. The possible implications of the biological data are discussed.

Although the chiralities of ligands at cholinergic receptors have been investigated extensively,<sup>5</sup> little is known about the influence of an enantiomeric quaternary N on anticholinergic potency. Such information might complement existing data and provide a more coherent view of the interaction of anticholinergic ligands with cholinergic receptors.

Our approach to investigating this problem was to utilize the 2-oxa-5-azabicyclo[2.2.1]heptane system (1) as a probe, since endo-exo isomerism about the quater-



nary N in optically active 1 gives an enantiomeric N atom. Substituents (R, R') not favorable for agonist activity would be expected to give antagonist, partial agonist, or inactive compounds.

**Chemistry.**—The bicyclic intermediate **2** for the preparation of the desired compounds has been reported recently.<sup>3</sup> The absolute configuration of this compound is as depicted, since it was prepared from hydroxy-L-proline. Reduction of **2** with LAH failed to give optimal yields of the desired benzyl derivative **3** due to the

<sup>(1)</sup> We gratefully acknowledge support of this work by Public Health Service Grant GM 09402.

<sup>(2)</sup> Part VIII of this series: P. S. Portoghese and D. A. Williams, J. Med. Chem., 13, 626 (1970).

<sup>(3)</sup> Previous paper: P. S. Portoghese and J. G. Turcotte,  $\mathit{Tetrahedron},$  in press.

<sup>(4)</sup> Presented in part at the 154th National Meeting of the American Chemical Society, Chicago, Ill., Sept 1967, Abstract P-17.

<sup>(5)</sup> P. S. Portoghese, Annu. Rev. Pharmacol., 10, 51 (1970), and ref cited therein.

formation of cleavage product 4. It subsequently was found that diborane<sup>6</sup> cleanly converted the amide into 3 in 90% yield. The identity of 4 was confirmed by catalytic hydrogenolysis of 3.



Reaction of **3** with MeI afforded the methiodide which was converted into methochloride **7**. Catalytic hydrogenolysis of **7** gave **5**  $\cdot$  HCl which was obtained also from **4** using the Leuckart reaction. Reaction of **5** with either PhCH<sub>2</sub>Br or with MeI afforded the corresponding quaternary salts, which were converted into **8** and **9**, respectively.

Deuterated quaternary ammonium chlorides 10-14 were prepared using the same synthetic procedure except that  $CD_{\vartheta}Br$  was employed instead of MeI in appropriate alkylation steps. The deuteromethyl tertiary amine 6 was prepared by hydrogenolysis of 10.



The fact that the *exo*-and *endo*-Me groups of 9 possessed different chemical shifts (Table I) enabled us to

TABLE I	
CHEMICAL SHIFTS OF N EPIMERIC QUATER	NARY
2-Oxa-5-Azoniabicyclo[2.2.1]heptane Chlor	IDES
Chemical	Chemical

S

alt	Exo	$shift^a$	$\mathbf{Endo}$	$shift^a$
7	$\mathrm{CH}_3$	184.0	$CH_2Ph$	286.0
8	$CH_2Ph$	279.0	$CH_3$	193.2
9	$CH_3$	196.8	$CH_3$	199.0
10	$CD_3$		$\mathrm{CH}_2\mathrm{Ph}$	285.0
11	$\mathrm{CH}_{2}\mathrm{Ph}$	270.0	$CD_3$	
12	$CD_3$	b	$CH_3$	198.8
13	$CH_3$	196.0	$CD_3$	с

<sup>a</sup> Expressed in Hz at 60 MHz. <sup>b</sup> A low intensity signal having a chemical shift identical with that of the *exo*-Me protons of **13** and contg approx 5% of the endo proton integral was observed. <sup>c</sup> A low intensity signal having a chemical shift identical with that of the *endo*-Me protons of **12** and contg approx 5% of the exo proton integral was observed.

determine, by measurement of the N-Me nmr peak areas, the isomeric purities of 12 and 13 derived from trideuteromethylation of 5 and methylation of 6, respectively. It was found that N-alkylation occurred on the same side of the molecule with about 95% stereoselectivity. Comparison of the chemical shifts of the benzylic protons (Table I) in the benzylmethyl derivatives

(6) H. C. Brown and P. Heim, J. Amer. Chem. Soc., 86, 3566 (1964).

(7, 8) and in the benzyltrideuteromethyl derivatives (10, 11) showed that these quaternizations were essentially stereospecific. Although the chances of separating one of a pair of stereoisomeric quaternary ammonium salts by fractional crystallization was greater with the benzylmethyl quaternary salts than with the methyltrideuteromethyl salts, the ir spectra of the major crops of benzylmethyl salts which crystallized directly from the reaction mixtures, were identical with the spectra of the respective mother liquors.

While it can be concluded that the bridged system exhibits a high degree of stereoselectivity toward quaternization, unequivocal stereochemical assignment to the exo and endo substituents could not be made. The nmr data showed conclusively that N-alkylation took place almost quantitatively from the same side of the molecule in these systems, but did not reveal from which side alkylation took place. A reasonable assignment, however, can be made on the basis of reported studies on the stereochemical course of quaternization in a number of bicyclic bases.<sup>7-13</sup> Of the bicyclic derivatives that have been investigated, the racemic 2-azabicyclo [2.2.1]heptanes prepared by Gassman and Heckert<sup>7</sup> most closely resemble the 2-oxa-5-azabicyclo [2.2.1] heptanes. These workers reported that alkylation of N-methyl-2azabicyclo[2.2.1]heptane and N-ethyl-2-azabicyclo[2.-2.1]heptane with EtI and MeI, respectively, produced two different quaternary salts, and that alkylation proceeded with exceptional stereospecificity. Configurational assignment of N-alkyl substituents in these salts was made specifically on the assumption that the endo position is more sterically hindered than the exo position, and hence alkylation would be more likely to take place from the relatively unhindered exo side of the molecule. Our tentative stereochemical assignments are based on the same reasoning.

**Pharmacology.**—On the guinea pig ileum 7 and 8 were found to be 1/750 and 1/500 as active as atropine sulfate  $(pA_2 = 8.1)$  in their effect upon antagonizing the stimulant action of methacholine as suggested by parallel shifts of dose-response curves to higher concentration. The analysis of covariance showed that the difference in the slopes of dose-response curves for 7 (6.94) and 8 (6.86) were not statistically significant. These data are indicative of competitive postganglionic blockade and show that in the bicyclic system the effects of enantiomeric quaternary N on anticholinergic activity were comparable.

The agonist activities of **9** and **14** were approximately  $1/_{2500}$  that of methacholine. It is of interest that there was no significant difference in potencies between the  $+NMe_2$  and  $+N(CD_3)_2$  groups. This is in accord with the work of Belleau<sup>14</sup> who observed that the cholinergic activities of acetylcholine and its deuterium-substituted analogs were the same.

- (7) P. G. Gassman and D. C. Heckert, Tetrahedron, 21, 2725 (1965).
- (8) H. O. House and C. G. Pitt, J. Org. Chem., 31, 1062 (1966).
- (9) H. O. House and B. A. Tefertiller, ibid., 31, 1068 (1966).
- (10) D. R. Brown, J. McKenna, J. M. McKenna, J. M. Stuart, and G. B. Hutley, Chem. Commun., 380 (1967).
  - (11) G. Fodor, J. D. Medina, and N. Mandava, ibid., 581 (1968).
  - (12) C. C. Thut and A. T. Bottini, J. Amer. Chem. Soc., 90, 4752 (1968).
  - (13) D. R. Brown and J. McKenna, J. Chem. Soc., 571 (1969).
- (14) B. Belleau in "Isotopes in Experimental Biology," L. S. Roth, Ed., University of Chicago Press, Chicago, Ill., 1965, p 458.

#### Discussion

The ratio of antagonistic activities between 7 and 8 is  $1.5:1.^{15}$  This would suggest that the anonic site which associates with the enantiomeric quaternary groups in 7 and 8 is not located in a highly dissymmetric environment. It should be recognized, however, that the potencies of these stereoisomers are low relative to that of atropine, and consequently more conclusive evidence regarding this point must be obtained with asymmetric quaternary antagonists possessing much higher affinities.

Ellenbroek, *et al.*,<sup>16</sup> have reported low stereoselectivities associated with the  $\beta$ -C of the choline moiety in esters having antagonistic properties. Our study complements these data<sup>16</sup> and suggests that the *entire* choline moiety is relatively insensitive to steric effects in the antagonist-receptor interaction. It appears that the topographic features of the sites that bind common structural elements of agonist and antagonist molecules differ substantially, since it is well known that chiral muscarinic compounds show a high order of stereoselectivity.<sup>5</sup>

#### **Experimental Section**<sup>17</sup>

(1S,4S)-N-Benzyl-2-oxa-5-azabicyclo[2.2.1]heptane (3).-To 75 ml of a 1 M soln of diborane in  $THF^{18}$  (0.075 mole) was added 5.82 g (0.028 mole) of 2 in 25 ml of anhydrous THF under  $N_2$ . The temp was maintained at approximately 0° during the 15-min addition period, after which the soln was refluxed for about 1 hr, cooled to room temp, and treated cautiously with 5 ml of 6 N HCl. The THF was removed by heating on a steam bath and NaOH pellets were added to the residue which was then extd with several 50-ml portions of  $Et_2O$ . The exts were combined and dried  $(Na_2SO_4)$  and the solvent was removed in vacuo to give an oil. The oil was treated with 10% HCl and the resultant soln was extd with Et<sub>2</sub>O. The aq phase was neutralized with KOH pellets in the cold and was extd with several portions of Et<sub>2</sub>O. The exts were combined and treated as above to furnish 5.40 g (quantitative yield) of a colorless oil, which was chromatographically homogeneous and was employed without further purification in the prepn of quaternary salts.

(1S,4S)-2-Oxa-5-azabicyclo[2.2.1] heptane Hydrochloride (4-HCl).—A soln contg 2.0 g (0.009 mole) of 3 dissolved in 2 N HCl (20 ml) was shaken at an initial pressure of 3.87 kg/cm<sup>2</sup> in the presence of 0.5 g of 10% Pd/C until no further uptake of H<sub>2</sub> was noted. The reaction mixt was filtered and the solvent removed *in vacuo*. The solid residue was azeotroped several times with  $C_8H_6$  and crystn (MeOH-Et<sub>2</sub>O) gave 1.02 g (85%) of product, mp 154-156°. Anal. (C<sub>5</sub>H<sub>10</sub>ClNO) C, H, N.

(1S,4S)-exo-5-Methyl-endo-5-benzyl-2-oxa-5-azoniabicyclo-[2.2.1]heptane Chloride (7).—MeI (25 ml) was added to a solu of 1.0 g (0.0053 mole) of intermediate 3 dissolved in 10 ml of abs EtOII and the mixt was allowed to stand at room temp for 24 hr. The cryst solid was collected by filtration, rinsed with several portions of anhyd Et<sub>2</sub>O, and dried to give 1.16 g of methiodide, mp 217–218.5° dec. An additional 0.46 g of product, mp 217– 218°, was obtained from the mother liquor. The ir spectra of each fraction were identical. The combined fractions accounted

(16) B. W. J. Ellenbroek, R. J. F. Nivard, J. M. Van Rossum, and E. J. Ariëns, J. Pharm. Pharmacol., 17, 303 (1965).

(17) Melting points (Thomas-Hoover capillary melting point apparatus) of all quaternary ammonium chloride salts are corrected. All other melting points are not corrected. The ir data are expressed in cm<sup>-1</sup> (Perkin-Elmer 237B or Perkin-Elmer 621 spectrophotometers, mull or KBr disc). The nmr data ( $\tau$ ) were obtained with a Varian A-60 spectrometer using D<sub>2</sub>O as solvent and DSS as internal standard, unless otherwise indicated. Specific rotations were determined with a Perkin-Elmer 141 polarimeter. The ir and nmr data of all of the compds were consistent with the proposed structures.

(18) Alfa Inorganics, Inc., Beverly, Mass.

for 93%. The product was recrystd (twice) (abs EtOH) to yield colorless crystals of  $3 \cdot \text{MeI}$ , mp 218-222°. Anal. (C<sub>13</sub>-H<sub>1s</sub>INO) C, H, N.

To 2.55 g (0.007 mole) of the methiodide dissolved in 10 ml of H<sub>2</sub>O, was added 1.43 g (0.01 mole) of freshly prepd AgCl. The suspension was stirred vigorously for 10 min and then was heated on a steam bath for several min. After decolorizing (Norit) the soln was filtered through Celite, the solvent removed in vacuo, and the residue recrystd from EtOH-Et<sub>2</sub>O to give 1.67 g (91%) of 7, mp 215-216° dec,  $[\alpha]^{26}$  D + 17.2° (c 1.47, EtOH). Nmr included signals at 6.93 (3 H, singlet, NCH<sub>3</sub>) and 5.23 (2 H, singlet NCH<sub>2</sub>Ph). Anal. (C<sub>13</sub>H<sub>18</sub>ClNO) C, H, N.

(15,4*S*)-*exo*-5-Trideuteriomethyl-*endo*-5-benzyl-2-oxa-5-azoniabicyclo[2.2.1]heptane Chloride (10).—To 1.97 g (0.01 mole) of intermediate 3 dissolved in 10 ml of anhyd EtOH in a cooled Carius tube was introduced 2.03 g (0.021 mole) of  $CD_3Br.^{19}$ The Carius tube was immediately sealed, and the reaction mixt was allowed to stand at room temp for 24 hr. During this period, a product crystd from the soln. The material was collected by filtration from the reaction tube and was washed with anhyd Et<sub>2</sub>O. A second crop of product was recovered from the Et<sub>2</sub>Otreated mother liquor. After recrystn (EtOH-Et<sub>2</sub>O, 10:1), there was obtained 2.55 g (85%) of a 3  $\cdot$  CD<sub>3</sub>Br, mp 228-235° dec.

The quaternary bromide salt (2.50 g 0.0087 mole), was treated with 1.43 g (0.01 mole) of freshly prepared AgCl as described for 7 to give 2.14 g of 10: recrystn (EtOH-Et<sub>2</sub>O); mp 242-245°,  $[\alpha]^{26}D + 18.2^{\circ}$  (c 1.15, EtOH). The nmr spectrum was identical with that of 7 except for the absence of the signal at 6.93 (Table I).

(1S,4S)-N-Methyl-2-oxa-5-azabicyclo[2.2.1]heptane (5) from 7.—A soln of 1.67 g (0.0069 mole) of 7 dissolved in 10% HCl was hydrogenated in the presence of Pd/C (0.5 g) at an initial pressure of 3.87 kg/cm until no additional uptake of H<sub>2</sub> occurred. After filtering through Celite and removing the solvent *in vacuo*, the hygroscopic product was azeotroped with  $C_6H_6$ several times to give 1.07 g of cryst solid, mp 275° dec. The free base was generated in abs EtOH by treatment with 0.5 equiv of Ag<sub>2</sub>O.

 $(1\overline{S},4S)$ -*N*-Methyl-2-oxa-5-azabicyclo[2.2.1]heptane (5) from 4.—To 0.29 g (0.0029 mole) of 4 was added 0.25 ml (0.0032 mole) of a 37% CH<sub>2</sub>O soln followed by dropwise addition of 0.16 ml (0.0032 mole) of a 90% HCOOH with a Hamilton syringe at a rate which maintained spontaneous reflux. The reaction mixt was refluxed for 1 hr, cooled, and then satd with solid KOII. The mixt was extd with Et<sub>2</sub>O, and the exts were dried (Na<sub>2</sub>SO<sub>4</sub>). The solvent was removed *in vacuo* to give an oil having spectral characteristics identical with those of the product obtained from hydrogenolysis of 7.

(1S,4S)-N-Trideuteroimethyl-2-oxa-5-azabicyclo[2.2.1]heptane (6).—Intermediate 10 (2.14 g, 0.008 mole), was subjected to catalytic hydrogenolysis in the same manner as was employed in the preparation of 5. The HCl salt (1.33 g, 99%) was converted directly into the base with Ag<sub>2</sub>O and used without further purification for prepn of quaternary salts.

(1S,4S)-exo-5-Benzyl-endo-5-methyl-2-oxa-5-azoniabicyclo-[2.2.1]heptane Chloride (8),—An EtOH soln (2 ml) contg 0.2 g of 5 was treated with 5 ml of PhCH<sub>2</sub>Br and allowed to stand 24 hr during which time crystn of product took place. There was obtained 0.4 g of the bromide salt and an additional 0.03 g of product from the mother liquor. The ir spectrum of each fraction was identical. The salt was crystd from EtOH–Et<sub>2</sub>O, mp 228° dec.

The bromide salt (0.4 g, 0.00085 mole) was dissolved in 5 ml of H<sub>2</sub>O and treated with 0.12 g (0.001 mole) of AgCl as described for 7: crystn (EtOH-Et<sub>2</sub>O 10:1); 0.19 g (94%); mp 212-213°;  $[\alpha]^{26}D + 35.5^{\circ}$  (c 1.62, EtOH). The nmr spectrum included resonances at 6.77 (3 H, singlet, NCH<sub>3</sub>) and 5.35 (2 H, singlet, NCH<sub>2</sub>-Ph) (Table I). Anal. (C<sub>13</sub>H<sub>18</sub>ClNO) C, H, N.

(1S,4S)-exo-5-Benzyl-endo-5-trideuteriomethyl-2-oxa-5-azoniabicyclo[2.2.1]heptane Chloride (11.)—PhCH<sub>2</sub>Br (5 ml) was added to an EtOH soln (2 ml) contg 0.2 g of 6 and the reaction mixt was allowed to stand 24 hr. There was obtained 0.33 g (combined fractions) of quaternary salt, mp 228° dec. The ir spectra of the major and minor fractions were identical. The

<sup>(15)</sup> It is noteworthy that differences in the neuromuscular blocking potencies of N epimers of conline and of conhydrine also are of a low order of magnitude [J. B. Stenlake, *Progr. Med. Chem.*, **3**, 12 (1963), and ref cited therein].

<sup>(19)</sup> An ampule contg 5 ml of  $CD_{\theta}Br$  (Isotopes Specialties Co., Burbank, Calif.) was fitted with a break-seal, solid glass slug, and a generator tube interposed with a Teilon valve stopcock. The liquid was allowed to volatilize spontaneously at room temp into a previously cooled and tared Carius tube.

quaternary bromide salt (0.27 g, 0.001 mole) was treated with an equiv amt of freshly prepared AgCl to furnish 0.23 g of 11: crystd (EtOH-Et<sub>2</sub>O 15:1), mp 223-235°,  $[\alpha]^{26}D$  +41.4 (c 1.15, EtOH). The nmr spectrum included a signal at 5.35 (2 H, singlet, NCH<sub>2</sub>-Ph) and was identical with the spectrum of 8 except for the absence of the N-Me resonance at 6.77 (Table I).

(15,4S)-N,N-Dimethyl-2-oxa-5-azoniabicyclo[2.2.1]heptane Chloride (9).—An EtOH soln (4 ml) contg 0.4 g of 5 was mixed with 10 ml of MeI and allowed to stand for 24 hr during which time crystn of product (0.50 g) took place. Two recrystns (ab EtOH) afforded the pure methiodide, mp 292-294° dec. This salt (0.50 g, 0.002 mole) was dissolved in 10 ml of H<sub>2</sub>O and treated with 0.35 g (0.0025 mole) of freshly prepared AgCl to give 0.29 g (92%) of product after crystn (EtOH-Et<sub>2</sub>O 10:1): mp 292-294° dec; [ $\alpha$ ]<sup>26</sup>D +59.0° (c 1.1, EtOH). The nmr spectrum included signals at 6.67 and 6.71 (6 H, two singlets, N-(CH<sub>4</sub>)<sub>2</sub>) (Table I). Anal. (C<sub>7</sub>H<sub>14</sub>ClNO) C, H, N.

(1S,4S)-exo-5-Trideuteriomethyl-endo-5-methyl-2-oxa-5-azoniabicyclo[2.2.1]heptane Chloride (12).—An EtOH soln (6 ml) contg 0.6 g of 5 was treated with 1.48 of CD<sub>3</sub>Br in a sealed Carius tube for 24 hr. The yield of product (mp 300° dec) which crystd spontaneously from soln was 0.44 g. The Et<sub>2</sub>O treated mother liquor yielded an additional 0.07 g of product which had an ir spectrum identical with that of the major fraction of product. The bromide salt (0.40 g, 0.0019 mole) was dissolved in about 10 ml of H<sub>2</sub>O and treated with 0.35 g (0.0025 mole) of freshly prepd AgCl to obtain after recrystn (EtOH-Et<sub>2</sub>O) 0.27 g (86%) of product, mp 300° dec,  $[\alpha]^{26}$ D +58.0° (c 1.21, EtOH). The nmr spectrum was identical with that of **9** except that the signal corresponding to exo N-Me was of very low intensity (Table I).

(1S,4S)-exo-5-Methyl-endo-5-trideuteriomethyl-2-oxa-5-azoniabicyclo[2.2.1]heptane Chloride (13).—An EtOH soln (25 ml) contg 0.25 g of 6 was mixed with 5 ml of MeI and allowed to stand for 24 hr during which time crystn of a product occurred. The crude (0.44 g) was twice crystd (abs EtOH), mp 297° dec. Material obtained from the mother liquor was identical in all respects with the product that crystd. The quaternary iodide salt (0.35 g, 0.0013 mole) was dissolved in 10 ml of distd H<sub>2</sub>O and the soln treated with 0.35 g (0.0025 mole) of freshly prepared AgCl. Crystn (EtOH-Et<sub>2</sub>O 10:1) afforded 0.21 g (93%) of **13**, mp 300° dec,  $[\alpha]^{26}D + 53.6^{\circ}$  (c 1.24, EtOH). The nmr spectrum was identical with that of **9** except that the peak corresponding to endo N-Me was of very low intensity (Table I).

(1S,4S)-N,N-Ditrideuteriomethyl-2-oxa-5-azoniabicyclo[2.-2.1]heptane Chloride (14).—An EtOH soln (7 ml) contg 0.7 g of 6 was treated with 1.20 g of CD<sub>3</sub>Br in a Carius tube as described for the prepn of 12. The crude product (0.74 g) was crystd (abs EtOH), mp 297° dec. The bromide salt (0.40 g, 0.0019 mole) was treated with AgCl as previously described to obtain 0.29 g of 14: crystn (EtOH-EtOAc); mp 300° dec; [a]<sup>26</sup>D +56.1° (c 1.04, EtOH). The nmr spectrum was identical with those of 9, 12, and 13 except for the absence of N-Me resonances.

**Pharmacological Testing.**—Testing was carried out with isolated guinea pig ileum obtained from freshly sacrificed animals (av wt, 300 g). Pieces of ileum were sutured at each end through the mesenteric side of the organ. The intestinal strips were suspended in a thermostated muscle bath  $(37.5^{\circ})$  contg 16 ml of modified Tyrode soln.<sup>20</sup> through which was bubbled a continuous flow of Carbogen (95/5). Recording of muscle contractions were made with a lightly loaded (*ca.* 500 mg) isotonic lever attached to a C. F. Palmer Super 10 recording drum and stand. In studies with antagonists, drugs were allowed to remain in contact with the ileum for 1 min prior to the introduction of an agonist. Ileum strips were rinsed 3 times between administration of doses of agonist compounds.

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## Synthesis of Some 6-Chloro-3,7-dihydroxy-∆⁵-pregnene Derivatives

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The progestational activities and syntheses of the 6-chloro-3,7-dihydroxy- $\Delta^{5}$ -pregnene derivatives **4a,b,c,d**, and **5** as well as their 16-methylene analogs are reported. Several of these compounds exhibited high progestational activity when tested in the rabbit.

It is well known that cholesterol is converted into  $3\beta$ -hydroxycholest-5-en-7-one, cholest-5-ene- $3\beta$ , $7\beta$ -diol, and the corresponding  $7\alpha$ -hydroxy isomer by different fractions of rat liver homogenate.<sup>1</sup> Cholest-5-ene- $3\beta$ , $7\alpha$ -diol is also converted by these homogenates into  $7\alpha$ -hydroxycholest-4-en-3-one<sup>2</sup> probably *via* the intermediate formation of a 3-keto- $\Delta^5$ -steroid. If 3-hydroxy- $\Delta^5$ -pregnenes are metabolized in this manner, dehydration of the resultant 7-hydroxy metabolite would lead to the 4,6-dien-3-one system. The high activity of such progesterone derivatives, incorporating the 6-chloro-4,6-diene system, is well known.<sup>3</sup> It is also reported that various 3-hydroxy- $\Delta^5$ -pregnenes have the same activity as the corresponding  $\Delta^4$ -3-ketones.<sup>4</sup> We therefore felt

it to be of interest to prepare some  $\Delta^5$ -pregnenes incorporating the 6-chloro-3,7-dihydroxy system.

Chlorination of  $3\beta$ ,  $17\alpha$ -diacetoxypreg-5-ene-7, 20-dione<sup>5</sup> (1) followed by dehydrochlorination with pyridine gave an inseparable mixture of 2 and the 8-Cl impurity 3 (Scheme I). Purification was accomplished by treatment of the mixture with Zn in HOAc which converted 3 into 2. Reduction of 2 with LiAl(*t*-BuO)<sub>8</sub>H gave the desired 7-OH isomers 4a and 5 in 53 and 7% yield, respectively, after column chromatography.

The stereochemistry at C-7 in 4a and 5 was assigned on the basis of the nmr spectra. In 4a the C-7 H appeared as a broad signal at  $\delta$  3.92 (half-band width ~11 Hz), which is consistent with axial-axial coupling with the C-8 H.<sup>6</sup> The broadening of the signal is prob-

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