ACh. *trans*-3-Trimethylammonium-2-acetoxybicyclo-[2.2.2]octane iodide (**2**) showed muscarinic activity at 1.1×10^{-4} *M* equivalent to 3×10^{-7} *M* ACh chloride, equipotent molar concentration equal to 370. This activity was blocked by atropine, and not by hexamethonium. Repeated experiments at close intervals gave reproducible dose–response curves. These data indicate the activity of **2** is muscarinic, not nicotinic, and provides no evidence for ACh-releasing activity.[6c] Compound **1** showed no muscarinic activity at concentrations up to 10^{-3} *M*. The difference in activity of **1** and **2** is compatible with the hypothesis that the muscarinic receptor is most complementary to a transoid arrangement of the AcO function and the quaternary ammonium head.

Both the cis and trans analogs are substrates for eel AChE. Hydrolysis rates were *ca.* 0.33% ACh ($K_m = 4.3 \times 10^{-4}$) for **1** and 13.6% ACh for **2** ($K_m = 1.2 \times 10^{-3}$); K_m for ACh $= 1.2 \times 10^{-4}$.[15,16] Both also were inhibitors of eel AChE showing $K_i = 1.0 \times 10^{-5}$ for **1** and 1.8×10^{-5} for **2**, indicating each is more tightly bound to the enzyme than the substrate, ACh, but not nearly as active as competitive inhibitors like physostigmine ($K_i = 4.25 \times 10^{-8}$).[16]

The activity of the trans compound **2**, being a better substrate for AChE by some 40-fold, is more consistent with an eclipsed conformation of the AcO group and quaternary ammonium head ($\theta \simeq 120°$) of ACh analogs in the enzyme–substrate complex of eel AChE than the totally eclipsed conformation. Dreiding models indicate considerable flexibility in the molecule allowing θ to vary from *ca.* 95 to 145°. The upper limit of this range is consistent with the conformation suggested by Chothia and Pauling[17] for the AChE site, on the basis of X-ray data. However, since there is some flexibility in molecular models of the compounds, no absolute analogy can be made. In addition speculation concerning these results and the conformation of ACh at its site on AChE may be misleading because of possible allosteric interactions of the bicyclooctane analogs at sites adjacent to the esteratic site. However, the comparison of cis and trans analogs, **1** and **2**, suggests the latter is a more suitable model for the ACh–AChE interaction than the former.

Experimental Section[18]

***cis*-3-Trimethylammonium-2-acetoxybicyclo[2.2.2]octane Iodide (1).**—A mixture of 618 mg (3.0 mmoles) of *cis*-3-dimethylamino-2-hydroxybicyclo[2.2.2]octane·HCl,[7] 20 ml of pyridine, and 10 ml of Ac_2O was allowed to stand overnight. Excess reactants were removed utilizing a rotary evaporator 20 ml of aq 3% HCl was added, and the mixture allowed to stand at room temp for 20 min. The aq soln was washed with $CHCl_3$, made alk with aq 10% NaOH, and extd 3 times with EtOAc. The combined organic exts were washed with H_2O, satd NaCl and dried ($MgSO_4$), and the solvent was removed, affording a yellow oil.

The yellow oil was dissolved in 5 ml of Me_2CO, 5 ml of MeI added, and the solution was allowed to stand at room temp for 5 hr. The solvent was removed, and the residue crystd from EtOAc–MeOH affording 385 mg (36%): mp 206–208°; nmr (D_2O), δ 5.50 (**H**COAc, broadened triplet, line separation *ca.* 7 Hz), 3.75 (**H**CN$^+$, broadened doublet, $J_{32} = 6.5$ Hz, $J_{34} =$ 0–1 Hz), 3.30 [(**H**$_3$C)$_3$N$^+$, singlet], 2.32 (**H**$_3$CCOO, singlet), 2.45 (H-4 methine, multiplet, W_h *ca.* 10 Hz), 1.5–2.2 (CH_2–CH envelope). *Anal.* ($C_{13}H_{24}INO_2$): C, H, N.

***trans*-3-Trimethylammonium-2-acetoxybicyclo[2.2.2]octane Iodide (2).**—Crude 2,3-epoxybicyclo[2.2.2]octane,[8] 3.40 g (27 mmoles), obtained from the reaction of bicyclo[2.2.2]oct-2-ene (Chemical Samples Co., Columbus, Ohio) and *m*-$ClC_6H_4CO_3H$, was heated with 33.8 g (0.75 mole) of anhyd $HNMe_2$ in 50 ml of C_6H_6 in a stainless steel autoclave at 160° for 3 days. After cooling to 0° the autoclave was opened, and the contents were removed by washing the bomb with several portions of C_6H_6. C_6H_6 and excess $HNMe_2$ were removed on a rotary evaporator, and the residue was dissolved in aq 10% HCl, washed with C_6H_6, made alk with aq 10% NaOH, and extd with several portions of C_6H_6. The combined org exts were dried ($MgSO_4$) and the solvent removed (vac) affording 1.70 g (37%) of a brown viscous liquid which was not further purified.

The crude trans amino alcohol was acetylated and allowed to react with MeI as described for the cis compound affording colorless needles: mp 209–210° (MeOH–EtOAc); nmr (D_2O), δ 5.17 (**H**COAc, multiplet, W_h = *ca.* 12 Hz), 3.73 (**H**CN$^+$, doublet of doublets, $J_{32} = 6.5$ Hz, $J_{34} =$ 0–1 Hz), 3.17 [(**H**$_3$C)$_3$N$^+$, singlet], 2.12 (**H**$_3$CCOO, singlet), 2.42 (H-4 methine multiplet, W_h = *ca.* 7 Hz), 1.5–2.2 (CH_2–CH envelope). *Anal.* ($C_{13}H_{24}INO_2$): C, H, N.

Enzyme-catalyzed hydrolyses of the compounds and their inhibition of ACh hydrolysis were determined at pH 7.2 using a Radiometer TTT-1 Titrator pH-Stat. Eel AChE (Sigma, type III) was used in the presence of 0.160 *M* NaCl, 0.002 *M* $MgCl_2$, and 0.05% bovine serum albumin. Inhibitor concns were 5×10^{-6} and 5×10^{-7} *M*. Reaction rates were determined at 25° and were linear. A computer program was used to determine K_m and K_i.

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(16) G. H. Cocolas, E. C. Robinson, and W. L. Dewey, *ibid.*, **13**, 299 (1970).
(17) C. Chothia and P. Pauling, *Nature (London)*, **223**, 919 (1969).
(18) Melting points were obtained on a calibrated Thomas-Hoover Uni-Melt and are corrected. Ir data were recorded on a Beckman IR-5A spectrophotometer and were as expected. Nmr spectra were determined with a Varian A-60 spectrometer in CD_3OD (Me_4Si). Decoupling experiments were obtained by frequency sweep, double resonance procedure using a Varian DA-60IL spectrometer. Microanalyses were conducted by Drs. G. Weiler and F. B. Strauss, Oxford, England. Where analyses are indicated only the symbols of the elements, analytical results were obtained for those elements within ±0.4% of the theoretical values.

Antimalarials Related to Aminopyrocatechol Dialkyl Ethers. Conformational Effects[1,2]

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Many of the common antimalarial agents, particularly the polynuclear types such as chloroquine, may function biologically *via* an intercalation of the drug with DNA.[3] The basic amino side chain of this type of antimalarials interacts ionically with the phosphoric acid groups of the complementary strands of DNA

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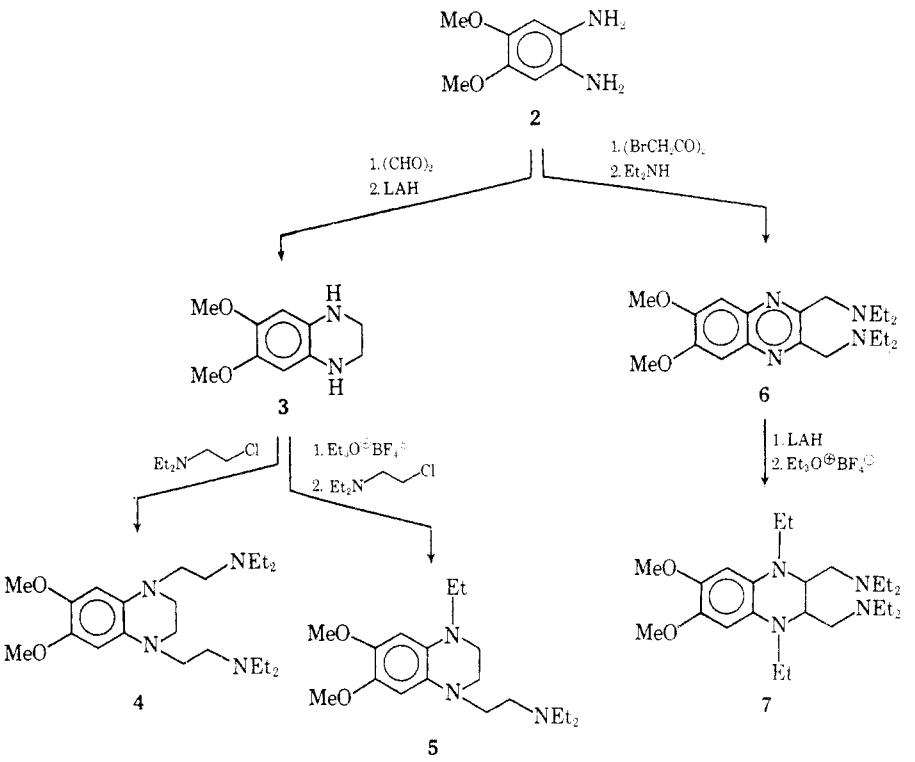
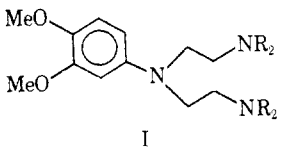
(2) For part 3 of this series see E. L. Stogryn, *J. Med. Chem.*, **13**, 1106 (1970).
(3) F. E. Hahn, R. L. Obrien, J. Ciak, J. L. Allison, and J. G. Olenick, *Mil. Med. Suppl.*, **131**, 1071 (1966); J. Ciak and F. E. Hahn, *Science*, **156**, 655 (1967).

SCHEME I



across the minor groove. It can be inferred from this that the structure of the basic side chain particularly with regard to the allowable number of C atoms between the basic sites must lie within a rather narrow range. Indeed, each drug type appears to exhibit a C chain distance between basic sites characteristic of its own class.[4] However, recent studies with chloroquine side chain variations by Singh and coworkers[5] have indicated that simple additive bond distances between the basic N atoms may not be as important as the manner in which the side chain can adjust itself to bridge the phosphate groups of the complementary strands of DNA. Thus the biological activity would be expected to reflect the energetic permissibility of certain conformations of the basic side chain.

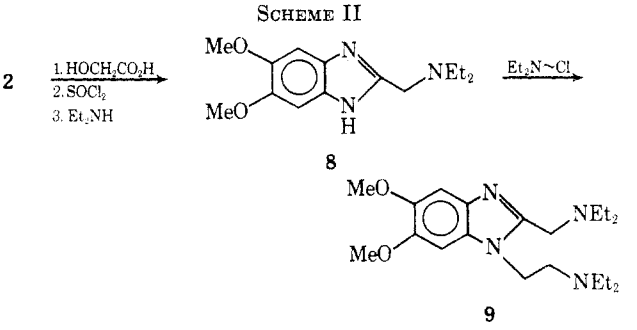
The present study was an attempt to uncover such a relationship between conformation of the basic side chain and activity of the aminopyrocatechol[6] class of antimalarials. Structure I is illustrative of the structural features of the side chain found to be necessary for this antimalarial class. This paper describes the synthesis and evaluation of structures related to I in which the rotational and vibrational degrees of freedom of the side chain have been restricted.



I

Chemistry.—Activity[6,7] of the aminopyrocatechol class of antimalarial agents has only been observed when the N atoms are separated by a 2-C fragment. Thus, our synthetic studies have been confined to systems in which this structural features has been maintained. Variations in side chain conformation have been imposed as a consequence of incorporation of the side chain into two ring systems, *i.e.*, the quinoxaline and benzimidazole rings. The sequence of synthetic steps leading to the target compounds are outlined in Schemes I and II.

SCHEME II



Formation of both ring systems, from 4,5-dimethoxy-*o*-phenylenediamine,[8a,b] was accomplished according to classical techniques. The diamine **2** was best prepared by the low-pressure hydrogenation of 4,5-dinitroveratrole over Ra Ni. Details of the synthetic steps utilized to obtain the target compounds are given in the Experimental Section.

LAH reduction of **6** gave the corresponding tetrahydroquinoxaline **6a**. The sharp 3-line Me resonance at

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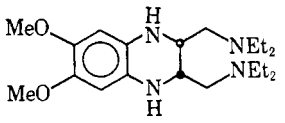

6a

τ 9.1 suggested that **6a** was stereochemically homogenous. By analogy with the reported LAH reduction of 2,3-dimethylquinoxaline[9] **6a** can be assigned the meso-(cis) configuration. Ethylation of **6a** with triethyloxonium tetrafluoroborate was effected under very mild conditions and thus the stereochemical integrity about C_2–C_3 can be presumed to have been maintained. On this basis **7** should also have the meso-(cis) configuration.

Biological Evaluation.[10]—Target compounds **4–9** were assessed for antimalarial activity in mice infected with *Plasmodium berghei*[11] and in chicks and *Aedes aegypti* mosquitos[12] infected with *P. gallinaceum*. Compounds **5–9** showed insignificant activity at the maximum dose levels. In the bird screen, **4** effected one cure/5 test animals at the 120 mg/kg level. However, at the next dose level, 240 mg/kg, **4** was lethal to all test animals.

It is interesting to note that the only compound exhibiting biological response to the activity screens appears structurally to possess the greatest degree of conformational mobility of the basic side chain.

Experimental Section

6,7-Dimethoxy-1,2,3,4-tetrahydroquinoxaline (3).—Reduction of 6,7-dimethoxyquinoxaline with LAH in refluxing Et_2O gave **3** in 63% yield as a white solid, mp 134–134.5° (C_6H_6–hexane) (lit.[13] mp 133–134°).

1,4-Bis(diethylaminoethyl)-6,7-dimethoxy-1,2,3,4-tetrahydroquinoxaline (4).—A soln of 2.6 g of **3** and 7.2 g of 2-chlorotriethylamine in 100 ml of dry DMF was heated on a steam bath overnight. Removal of DMF, *in vacuo*, gave a dark residue which was dissolved in H_2O, made basic with Na_2CO_3, and extracted with pentane. A dark viscous oil, 3.2 g, was isolated from the pentane. Distn of the oil gave a yellow–brown oil, bp 185–187° (0.04 mm), (lit.[13] bp 200° (0.5 mm)). *Anal.* ($C_{22}H_{40}N_4O_2$) C, N.

6,7-Dimethoxy-1-ethyl-1,2,3,4-tetrahydroquinoxaline.—To the tetrahydroquinoxaline **3** (3 g) in 20 ml of CH_2Cl_2 was added 2.85 g of triethyloxonium tetrafluoroborate in 25 ml of CH_2Cl_2. After 2 hr reflux the dark-colored soln was shaken with aq Na_2SO_3 and dried over Na_2CO_3. The viscous residue isolated from the CH_2Cl_2 soln (3.1 g) was chromatographed through a base-treated silica gel[2] column. The 1,4-diethylated material (0.8 g) was eluted with Et_2O and 1.4 g of the titled compound eluted with Me_2CO. The starting material (0.8 g) was removed with THF.

4-Diethylaminoethyl-6,7-dimethoxy-1-ethyl-1,2,3,4-tetrahydroquinoxaline (5).—The monoethylated tetrahydroquinoxaline (1.3 g) in 50 ml of THF was treated with 6.7 mmoles of EtMgBr in Et_2O. 2-Chlorotriethylamine (0.95 g) in 2 ml of THF was added, and the mixture was refluxed overnight. A dark brown oil was isolated from the THF. Chromatography through base-treated silica gel[2] gave **5** (from the Et_2NH eluate) as a yellow oil. Distn gave 0.75 g (38%) of a light brown oil, bp 160° (bath temp) (0.1 mm). *Anal.* ($C_{18}H_{31}N_3O_2$) C, H, N.

2,3-Bis(bromomethyl)-6,7-dimethoxyquinoxaline.—This prepn is an adaption of the reported synthesis of 2,3-bis(bromomethyl)-quinoxaline.[14] A mixture of 21 g of **2** and 22.5 g of 1,4-dibromo-2,3-butanedione in 300 ml of EtOH was stirred, with cooling, for 3 hr, and then allowed to stand overnight at room temp. The reddish ppt was filtered and recrystd from Me_2CO–H_2O. The title compound was obtained in 62% yield as fine white needles, mp 191.5–192.5°. *Anal.* ($C_{12}H_{12}Br_2N_2O_2$) C, H, N.

2,3-Bis(diethylaminomethyl)-6,7-dimethoxyquinoxaline (6).—The bis(bromomethyl)quinoxaline (8.5 g) was dissolved in 100 ml of refluxing THF. Et_2NH (30 ml) was added and the mixture stirred and refluxed for 3 hr. Filtration of the hot reaction mixture gave 6.2 g of $Et_2NH_2^+Br^-$. The solvent was evapd and the residue dissolved in pentane and made basic with aq Na_2CO_3. Distn through a short-path still gave a pale yellow oil (7 g, 85% yield), bp 180° (bath temp) (0.01 mm). *Anal.* ($C_{20}H_{32}N_4O_2$) C, H; N: calcd N, 15.55; found, 16.07.

2,3-Bis(diethylaminomethyl)-6,7-dimethoxy-1,2,3,4-tetrahydroquinoxaline (6a).—To 0.95 g of LAH in 300 ml of Et_2O was added 5.4 g of **6** in 30 ml of Et_2O. The reaction mixture was refluxed overnight, cooled, and worked up in the usual manner. Distn gave 4.5 g (82% yield) of a light red oil, bp 190–195° (bath temp) (0.04 mm).

1,4-Diethyl-2,3-bis(diethylaminomethyl)-6,7-dimethoxy-1,2,3,4-tetrahydroquinoxaline (7).—A soln of 1.56 g of **6a** and 3.3 g of triethyloxonium tetrafluoroborate in 50 ml of CH_2Cl_2 was stirred at ambient temp overnight. The CH_2Cl_2 soln was washed with aq Na_2SO_3, dried, and concd, yielding a dark brown oil. Short-path distn gave 0.4 g, bp 160–165° (bath temp) (0.01 mm). *Anal.* ($C_{24}H_{44}N_4O_2$) C, H, N.

2-Diethylaminomethyl-5,6-dimethoxybenzimidazole (8).—A soln of 6.5 g of 2-chloromethyl-5,6-dimethoxybenzimidazole and 6.4 g of Et_2NH in 15 ml of EtOH was refluxed for 3 hr. The EtOH was removed *in vacuo*, and the residue was dissolved in H_2O, treated with dil Na_2CO_3, and extracted with Et_2O. Removal of Et_2O gave a pale yellow oil, 5.5 g. This oil was converted into the HCl salt and recrystd twice from EtOH. The HCl salt was dissolved in H_2O, basified, and extracted with Et_2O. Removal of the last traces of Et_2O under high vacuum was required to induce solidification, mp 72–73° (C_6H_6–pet ether). *Anal.* ($C_{14}H_{21}N_3O_2$) C, H, N.

1-Diethylaminoethyl-2-diethylaminomethyl-5,6-dimethoxybenzimidazole (9).—A soln of 5 g of **8** in 75 ml of THF was treated with 19 mmoles of MeMgCl in Et_2O. 2-Chlorotriethylamine (2.6 g) was added and the reaction mixture refluxed for 3 days. A small quantity of H_2O was added, the THF was evapd, and the residue extracted with pentane. The pale colored oil, 2.5 g, isolated from the pentane was chromatographed through base-treated silica gel (product eluted with Et_2NH) and distd, bp 200° (0.01 mm). *Anal.* ($C_{20}H_{34}N_4O_2$) C, H, N.

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(10) The mouse and avian activity screens were performed by Dr. L. Rane of the University of Miami. The mosquito activity screen was performed by Dr. E. Gerberg. The test results were made available through the courtesy of Dr. D. P. Jacobus, formerly of the Walter Reed Army Research Institute, Washington, D. C.

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