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*Research Article*

# Structure–Activity Relationship Studies of Hemicholinium (HC-3) Congeners

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In a continuing investigation of structural requirements for hemicholinium-like activity (inhibition of neuromuscular transmission due to inhibition of uptake of choline into nerve terminals), some additional molecular modifications of hemicholinium ("HC-3"; structure **1**) were made. The target compounds were prepared by standard one- or two-step sequences. Noncyclic acetal moieties in general permitted retention of pharmacological activity, as did concomitant replacement of the central biphenyl "spacer" by other bulky cyclic groupings and replacement of the oxazinium rings by piperidine or 4-methylpiperidine. However, these modifications generally produced compounds of a lower potency. Replacement of the biphenyl moiety of HC-3 with polyalkylene chains permitted retention of a considerable degree of activity. In these target compounds, the two quaternary nitrogens can exist the same distance apart (approximately 14 Å) as in the hemicholinium molecule. The ditertiary amino congener of a pharmacologically active bis-quaternary oxazinium compound was almost completely inactive. To date, only one tertiary amine has been found which displays a significant degree of hemicholinium-like activity.

**KEY WORDS:** hemicholinium; inhibition of choline uptake; neuromuscular inhibition.

## INTRODUCTION

A prior communication (1) described hemicholinium-like actions (inhibition of neuromuscular transmission due to inhibition of choline uptake into nerve terminals) of a series of congeners of hemicholinium (**1**) in which the chemical nature of the central biphenyl portion of the molecule was varied, while retaining the oxazinium rings and maintaining the interquaternary nitrogen distances the same as in an analogous conformation of hemicholinium. It was concluded that the pharmacologically critical portions of the hemicholinium molecule are the quaternary nitrogens or perhaps the entire oxazinium rings and that the biphenyl portion of the molecule serves as a spacer, to maintain the quaternary nitrogens or the entire oxazinium rings optimal distances apart. The present communication describes a variety of congeners of hemicholinium, in which chemical variations of the oxazinium rings were made, designed to assess structural requirements in these portions of the hemicholinium molecule for effects on choline uptake and for other related pharmacologic actions. In addition, some of these variations of the quaternary nitrogen-containing rings were combined with structural variations of the biphenyl "spacer," to result in congeners of some biphenyl-derived molecules which the previous literature has reported to have interesting pharmacological properties. Structures **2–12** represent the subject compounds of the present study. Although there are various theoretical mechanisms for inhibition of cholinergic transmission, other than inhibition of choline uptake, the activities of the compounds in the present study correlate closely when comparing *in vivo* and *in vitro* results. Inhibition of transport of choline into synaptosomes is a direct measure of activity involving sodium-dependent, high-affinity transport of choline (2).

Design of target compounds **2–5** was stimulated by the discovery of the complex spectrum of pharmacological effects of a noncyclic acetal congener, "DMAE" (**13**), which includes blockade of neuromuscular transmission (which is only in part due to a hemicholinium-like mechanism) (3), antagonism of the auricular stimulating action of nicotine (4), antagonism of ganglionic stimulants (5), potentiation of pressor and contractile responses to exogenous norepinephrine (6), and enhancement of response to tyramine and angiotensin (7). Compound **2** is based upon *trans,trans*-4,4′-disubstituted bicyclohexyl, whose exact hemicholinium congener possesses a high degree of hemicholinium-like activity (1). In **3** and **4** the ketonic group characteristic of DMAE has been reduced to secondary alcohol. In **5** the diethyl acetal moiety of DMAE has been replaced by an ethyl ester.

In **6–9**, the oxazinium ring characteristic of hemicho-

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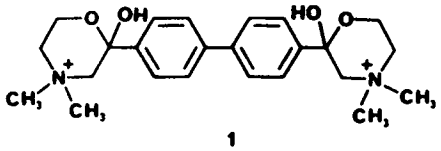

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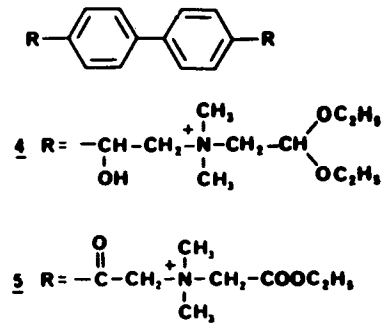
linium has been replaced by a piperidine ring system. Tedford *et al.* (8) have reported potent hemicholinium-like activity of a biphenyl derivative containing 4-methylpiperidine ring instead of oxazinium (compound 14). The unsubstituted piperidine derivative (15) was decidely less potent in an assay for its ability to inhibit choline uptake in synaptosomes (Table 1). The 2,2′-dimethylbiphenyl spacer moiety of 8 and 9 was reported (1) to permit retention of high potency and activity in hemicholinium itself. Some pharmacological properties of the phenanthrene derivatives 6 and 7 have been described recently in the literature (9), but details of their synthesis have not been previously published.

Compound 10 is the tertiary amine analogue of the potent diquaternary hemicholinium-like bicyclohexyl derivative (1), and it was prepared to assess the necessity for permanent positive charges in the oxazine rings. Attempts to prepare the biphenyl analogue, the tertiary amine congener of hemicholinium (1) itself, were unsuccessful; the compound was unstable and it could not be isolated in analytically pure state.

Compounds 11 and 12 are a continuation of previous studies (1) on the role of the central spacer for hemicholinium-like activity, and these compounds are the first in which the central spacer moiety is noncyclic. Analysis of molecular models suggests that, in the same reasonable conformation of the molecules (polyalkylene chain staggered and maximally extended), the interquaternary distance is approximately 14 Å in 11 and 15 Å in 12. In hemicholinium, this distance is approximately 14.4 Å. On the basis of previous studies (1) it was predicted that 11 and 12 would exhibit hemicholinium-like activity.



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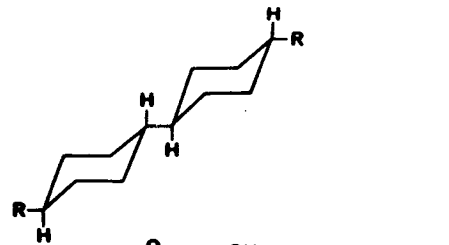
The target compounds were prepared by simple one- or two-step sequences utilizing standard methods and reactions. Spectral (IR, NMR, MS) data on all intermediates and final compounds were consistent with the proposed structures.

## EXPERIMENTAL

*Pharmacology. Methods. Inhibition of Neuromuscular Transmission.* Inhibition of neuromuscular transmission was determined using the rabbit sciatic nerve–gastrocnemius muscle preparation (10). Dutch rabbits weighing 1.5–2.0 kg were anesthetized with pentobarbital Na (250 mg/kg, i.v.). The trachea was isolated and respiration was supported by a Harvard respiration pump. The jugular vein was cannulated for intravenous administration of drugs. One of the sciatic nerves was isolated, sectioned centrally, and bipolar Ag electrodes were placed on the distal end of the sciatic nerve and attached to a Grass S4C stimulator. The ankle was attached to a solid mount and the Achilles tendon was isolated and sectioned. Resting tension (10 g) was applied to the tendon and contractions were recorded using a Beckman R-611 recorder. To quantify the neuromuscular blocking activity of the compounds and to compare with hemicholinium, the following parameters of stimulation were used: every 10 sec interrupted tetanic stimulation was delivered for 0.2 sec at 200 Hz. The pulse duration was 0.2 msec and maximal voltage was applied (usually 20 V). The antago-



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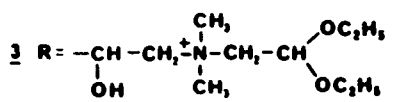

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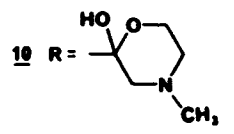

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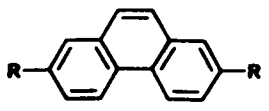

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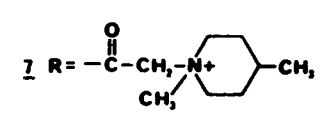

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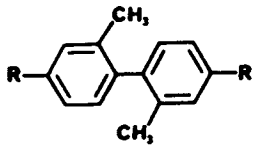
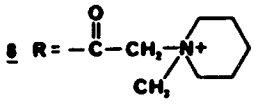
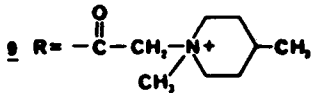




8 R = $-\overset{O}{\overset{\|}{C}}-CH_2-N^+$ ($CH_3$)



9 R = $-\overset{O}{\overset{\|}{C}}-CH_2-N^+$ ($CH_3$)($CH_3$)

nistic properties of choline chloride (5 mg/kg, i.v.) were evaluated.

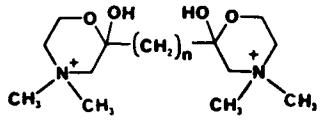
*Effects on [$^{14}$C]Choline Uptake.* The effect of the test compounds on choline uptake was determined in synaptosomal preparations from rat striatum.

*Preparation of Rat Synaptosomes.* Rats were killed by decapitation, and brains were removed and placed upon a cold Al plate. Striatal regions were rapidly dissected out, weighed, and homogenized in 1:20 vol (wet weight/volume) of ice-cold 0.32 *M* sucrose solution containing 10 m*M* glucose (pH was adjusted to 7.4 with Tris buffer) with six up-and-down strokes in a glass–Teflon homogenizer (Potter–Elvehjem type). The homogenate was centrifuged in the cold at 900*g* for 10 min (Sorvall RC-2B refrigerated centrifuge) to obtain a crude nuclear pellet, which was discarded. The supernatant was centrifuged in the cold at 18,000*g* for 10 min, and the pellet was collected, resuspended in the original volume of homogenizing medium, and used for uptake studies. A part of this suspension was stored at −80°C for future determination of protein according to the method of Lowry *et al.* (11) using bovine serum albumin as the standard.

*Procedure for Uptake Studies.* Synaptosomal uptake of [$^{14}$C]choline was determined by a modification of the method of Simon *et al.* (12). The uptake was followed at pH 7.4 in both Na-containing and Na-free medium. The uptake value obtained with the latter medium was taken as Na-independent uptake and was subtracted in all experiments as blank value from the value obtained with Na-containing medium, to obtain Na-dependent uptake. The composition of the Na-containing medium was as follows: NaCl, 157 m*M;* KCl, 5.6 m*M;* $MgCl_2$, 1.1 m*M;* $CaCl_2$, 0.9 m*M;* $Na_2HPO_4$, 1.1 m*M;* glucose, 11.2 m*M;* Tris buffer, 22.5 m*M;* and neostigmine, 11 μ*M*. The composition of the Na-free medium was the same except that NaCl and $Na_2HPO_4$ were replaced by sucrose (314 m*M*) and Tris phosphate (1.1 m*M*). The pH of both media was adjusted to 7.4 (at 25°C) with HCl. Routine assay for uptake was done as follows: polypropylene tubes, while kept in ice, received 880 μl of the appropriate medium, 10 μl of appropriate concentrations of test drugs or buffer, and 100 μl of the synaptosomal suspension. Tubes were vortexed and were allowed to preincubate for 5 min at 37°C. After exactly 5 min, 10 μl of radiolabeled choline of appropriate final concentration (1 μ*M* [$^{14}$C]choline) was added and the incubation was continued for another 4 min. The incubation was terminated by adding 5.0 ml of ice-cold 0.9% NaCl solution, and the sample was immediately filtered through a Millipore membrane filter (type DAWP 02500; 0.65-μm pore size) followed by a 5.0-ml rinse of the filter. The filter was kept overnight in 10 ml of Instagel (Packard) in a scintillation vial before counting in the liquid scintillation spectrometer. Total radioactivity was determined by counting 30 μl of the assay mixture.

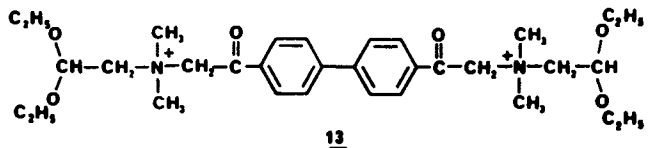
*Chemistry.* Melting points were determined in open glass capillaries with a Thomas-Hoover Uni-Melt apparatus and are uncorrected. Elemental analyses were performed by Galbraith Laboratories, Knoxville, Tennessee.

trans,trans-*4-4'-bis-[*N,N-*Dimethyl*-N-*(2,2-diethoxyethylammoniumacetyl]Bicyclohexyl Dibromide (2).* A solu-



11 n = 6

12 n = 7



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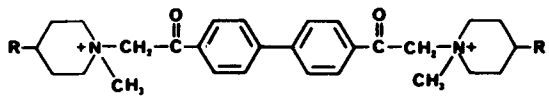


14 R = $CH_3$

15 R = H

Table I. Inhibition of Rabbit Neuromuscular Transmission and Inhibition of Choline Transport

| Compound No. | Neuromuscular inhibition, $ID_{50}$ (μmol/kg) (95% C.L.) | Reversed by choline chloride? | Inhibition of choline uptake, $IC_{50}$ (n*M*)[a] |
|---|---|---|---|
| Hemicholinium | 0.007 (0.006–0.011) | Yes, rapid[b] | 18.28 ± 1.34 |
| 1 | | | |
| 2 | 0.6 (0.31–0.86) | Yes, slow[c] | >200 |
| 3 | 0.08 (0.01–1.3) | Yes, slow | 5.58 ± 0.11 |
| 4 | 1.18 (1.05–1.36) | Yes, rapid | >200 |
| 5 | 1.22 (0.91–1.89) | Yes, rapid | >200 |
| 6 | 0.08 (0.076–0.084) | Yes, rapid | 13.06 |
| 7 | 0.023 (0.022–0.026) | No | 0.96 ± 0.06 |
| 8 | 0.022 (0.018–0.028) | Yes, rapid | 28.91 ± 2.78 |
| 9 | 0.045 (0.04–0.06) | Yes, rapid | 5 |
| 10 | >>20 | — | >200 |
| 11 | 0.2 (0.15–0.32) | Yes, rapid | 75.16 ± 24.56 |
| 12 | 0.3 (0.2–0.7) | Yes, rapid | 106.86 ± 24 |
| 13 (DMAE) | 0.058 (0.033–0.10) | Partial, slow | 28.89 ± 5.24 |
| 14 | 0.050 (0.03–0.07) | Yes, rapid | 3.29 ± 1.03 |
| 15 | — | — | 39.8 ± 3.18 |

[a] Values indicate mean ± SE as determined from at least three separate experiments.
[b] Inhibition reversed within 5 min.
[c] Inhibition reversed within 5–10 min.

tion of 1 g (0.002 mol) of *trans, trans*-4,4′-bis-(bromoacetyl)bicyclohexyl (1) and 1.29 g (0.01 mol) of *N,N*-dimethylaminoacetaldehyde diethyl acetal in 50 ml of cyclohexanone was heated under reflux overnight. The reaction mixture was cooled and the solid which separated was collected on a filter, washed with $Et_2O$, air-dried, and recrystallized from 2-PrOH–cyclohexane to afford 1.5 g (84%) of white crystals, m.p. 226–227°C. *Anal.* Calcd for $C_{32}H_{62}Br_2N_2O_6$: C, 52.60; H, 8.55; N, 3.83. Found: C, 52.62; H, 8.74; N, 3.45.

trans,trans-*4,4′-bis-[1-Hydroxy-2-(*N,N-*dimethyl*-N-*{2,2-diethoxyethylammonium}*)-ethyl]Bicyclohexyl Dibromide (3).* Compound **2** (0.3 g, 0.4 mmol) in 20 ml of $H_2O$ was hydrogenated over 0.2 g of $PtO_2$ at 25°C for 24 hr at an initial pressure of 55 psig. The hydrogenation mixture was filtered through Celite, then the filtrate was carefully diluted with $Me_2CO$ until a white solid separated. This material was recrystallized from MeOH–$Me_2CO$–$Et_2O$ (1:1:2) to yield 0.28 g (92%) of white crystals, m.p. 209–210°C. *Anal.* Calcd for $C_{32}H_{66}Br_2N_2O_6$ (Karl Fischer, 0.53% $H_2O$): C, 52.15; H, 9.02; N, 3.80. Found: C, 52.42; H, 9.21; N, 3.83.

*4,4′ - bis-[1-Hydroxy-2-(*N,N - *dimethyl*-N-*(2,2 - diethoxyethylammonium}*)-ethyl]Biphenyl Dibromide (4).* 4,4′-bis-[*N,N* - Dimethyl - *N* - (2,2 - diethoxyethyl)ammoniumacetyl]biphenyl dibromide (**13**; DMAE) (13) (0.85 g, 1.18 mmol) in 50 ml of $H_2O$ was hydrogenated over 0.02 g of $PtO_2$ at an initial pressure of 40 psig. After 1.5 hr, TLC analysis (silica, MeOH, + 1 drop of AcOH) indicated that no starting material remained. The reduction mixture was filtered, and the filtrate was evaporated under reduced pressure. The oily residue solidified on standing, and it was recrystallized from MeOH–$Me_2CO$ to yield 0.74 g (86%) of a white solid, m.p. 207–208°C. *Anal.* Calcd for $C_{32}H_{54}Br_2N_2O_6$: C, 53.18; H, 7.48; N, 3.88. Found: C, 53.25; H, 7.41; N, 3.75.

*4,4′-bis-[*N,N-*dimethyl*-N-*(carbethoxymethyl)ammoniumacetyl]Biphenyl Dibromide (5).* bis-(Bromoacetyl)biphenyl (1 g, 2.5 mmol) (14) and 0.726 g (5.5 mmol) of *N,N*-dimethylglycine, ethyl ester, in 15 ml of 99% EtOH and 10 ml of tetrahydrofuran were stirred at ambient temperature overnight, then were heated under reflux for 3 hr. The cooled reaction suspension was diluted with 100 ml of dry $Et_2O$ and the resulting mixture was filtered through a Büchner funnel. The solid on the filter was washed with dry $Et_2O$ and dried in a stream of $N_2$. This material was crystallized thrice from EtOH–$Et_2O$ (charcoal) to yield 1.5 g (91%) of an off-white solid, m.p. 182–183°C (dec). *Anal.* Calcd for $C_{28}H_{38}Br_2N_2O_6$ (Karl Fischer, 0.50% $H_2O$): C, 50.81; H, 5.84; N, 4.23. Found: C, 50.95; H, 6.20; N, 4.25.

*2,7-bis-(1-Piperidylacetyl)phenanthrene Dimethbromide (6).* 2,7-bis-(Bromoacetyl)phenanthrene (0.42 g, 1 mmol) (1) and 0.297 g (3 mmol) of 1-methylpiperidine were stirred at room temperature in 7 ml of MeOH and 3 ml of tetrahydrofuran until all of the bromoketone dissolved. Volatiles were then removed under reduced pressure and the solid residue was recrystallized from MeOH–$Me_2CO$–$Et_2O$ to yield 0.49 g (80%) of material, m.p. 205–207°C. *Anal.* Calcd for $C_{30}H_{38}Br_2N_2O_2$ (Karl Fischer, 8.76% $H_2O$): C, 56.54; H, 7.30; N, 4.13. Found: C, 53.35; H, 7.52; N, 4.50.

*2,7-bis-[1-(4-Methylpiperidyl)acetyl]phenanthrene Dimethbromide (7).* 2,7-bis-(Bromoacetyl)phenanthrene (0.30 g, 0.7 mmol) (1) and 1.2 g (10 mmol) of 1,4-dimethylpiperidine in 6 ml of MeOH and 2 ml of tetrahydrofuran were stirred at 45°C for 24 hr. Volatiles were removed under reduced pressure and the residue was recrystallized from EtOH–$Me_2O$–$Et_2O$ to yield 0.278 g (61%) of fluffy yellow crystals, m.p. 198–199°C (dec). *Anal.* Calcd for $C_{32}H_{42}Br_2N_2O_2$ (Karl Fischer, 5.88% $H_2O$): C, 55.95; H, 6.82; N, 4.08. Found: C, 56.29; H, 6.90; N, 4.23.

*4,4′-bis-(1-Piperidylacetyl)-2,2′-dimethylbiphenyl Dimethbromide (8).* The method described for **6** was used with 0.424 g (1 mmol) of 4,4′-bis-(bromoacetyl)-2,2′-dimethylbiphenyl (1) and 0.297 g (3 mmol) of 1-methylpiperidine. The product (0.466 g, 75%) showed m.p. 255°C (dec) (from EtOH–$Me_2CO$–$Et_2O$). *Anal.* Calcd for $C_{30}H_{42}Br_2N_2O_2$ (Karl

Fisher, 0.80% $H_2O$): C, 57.42; H, 6.88; N, 4.46. Found: C, 57.54; H, 7.04; N, 4.21.

*4,4'-bis-[1-(4-Methylpiperidyl)acetyl]-2,2'dimethylbiphenyl Dimethbromide (9).* The method described for **6** was used with 0.424 g (1 mmol) of 4,4'-bis-(bromoacetyl)-2,2'-dimethylbiphenyl (1) and 0.339 g (3 mmol) of 1,4-dimethylpiperidine. The product (0.533 g, 82%) showed m.p. 184–188°C (from EtOH–$Me_2CO$–$Et_2O$). *Anal.* Calcd for $C_{32}H_{46}Br_2N_2O_2$ (Karl Fischer, 5.88% $H_2O$): C, 55.95; H, 6.82; N, 4.08. Found: C, 56.29; H, 6.90; N, 4.08.

trans, trans-*4,4'-bis-[2-(2-hydroxy-4-methyl-1,4-tetrahydrooxazinyl)]bicyclohexyl Dihydrobromide (10). trans, trans*-4,4'-bis-(Bromoacetyl)bicyclohexyl (1) (0.5 g, 1.22 mmol), 0.3 g (4 mmol) of *N*-methylethanolamine, and 15 ml of 2-PrOH were heated under reflux for 12 hr. The reaction solution was cooled to room temperature and the solid which separated was recrystallized from EtOH–$Et_2O$ to afford 0.52 g (78%) of white crystals, m.p. 235–236°C. *Anal.* Calcd for $C_{22}H_{42}Br_2N_2O_4$: C, 47.52; H, 7.46; N, 5.29. Found: C, 47.63; H, 7.48; N, 5.31.

*1,6-bis-[2-(2-Hydroxy-4,4-dimethyl-1,4-tetrahydrooxazinyl)]hexane Dibromide (11).* 1,10-Dibromodecane-2,9-dione (15) (0.41 g, 1.25 mmol) and 0.7 g (7.9 mmol) of *N,N*-dimethylaminoethanol were heated under reflux overnight in 20 ml of $Me_2CO$. The solid which separated from the cooled reaction mixture was collected on a filter, washed with cold $Me_2CO$, and dried overnight at room temperature in a vacuum desiccator, to yield 0.60 g (95%) of a white solid, m.p. 185–186°C (dec). *Anal.* Calcd for $C_{18}H_{40}Br_2N_2O_4$: C, 42.52; H, 7.93; N, 5.51. Found: C, 42.70; H, 7.88; N, 5.51.

*1,7-bis-[2-(2-Hydroxy-4,4-dimethyl-1,4-tetrahydrooxazinyl)]heptane Dibromide (12).* 1,11-Dibromoundecane-2,10-dione (15) (1.3 g, 3.8 mmol), 1.2 g (13.5 mmol) of *N,N*-dimethylaminoethanol, and 40 ml of $Me_2CO$ were treated as described for *11*. Yield, 1.84 g (93%) of a white solid, m.p. 202–203°C (dec). *Anal.* Calcd for $C_{19}H_{42}Br_2N_2O_4$ (Karl Fischer, 1.26% $H_2O$): C, 42.13; H, 8.14; N, 5.29. Found: C, 43.51; H, 7.96; N, 5.23.

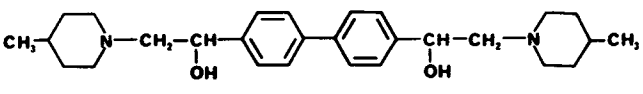
## RESULTS AND DISCUSSION: PHARMACOLOGY

Compounds were evaluated for their ability to inhibit neuromuscular transmission in rabbits and to inhibit choline transport in synaptosomes. The results were summarized in Table I. The mechanism of action of **4** and **5** is unclear. In *in vivo* experiments, antagonism of effects by choline is suggestive of hemicholinium-like activity, since choline is inactive as an antagonist of competitive neuromuscular blocking agents and it enhances the neuromuscular blocking properties of noncompetitive agents. However, **4** and **5** exhibited a very weak ability to inhibit choline transport in synaptosomes. The piperidine and 4-methylpiperidine derivatives **6–9**, having either a phenanthrene or a 2,2'-dimethylbiphenyl central spacer, retain some neuromuscular blocking effect, but all were decidedly less potent than the biphenyl-derived 4-methylpiperidine derivative (**14**). The neuromuscular blocking effect of the phenanthrene-derived 4-methylpiperidine compound (**7**) was not reversed by choline, suggesting a different mechanism of action for this compound. The mechanism of action of **7** is unknown. In *in vivo* experiments the duration of action was actually shorter than that of hemicholinium, which may suggest that high-energy bonding to the transport site may not be the mechanism for the noncompetitive inhibition exhibited by **7**. In this piperidine-derived series of compounds, there is no apparent advantage in replacement of the central biphenyl portion with other spacer moieties; indeed, this change was deleterious to pharmacological activity. Also, the remarkable enhancement of activity noted for the 4-methylpiperidine substituent in the biphenyl system (**16**) was not noted in **7** and **9**.

bis-Diethylacetal congeners of hemicholinium (compounds **2–4 and 13**, which is DMAE) demonstrated a considerable range of potency for inhibition of neuromuscular transmission. The inhibition produced by these compounds was reversed by choline chloride (5 mg/kg), indicating that the mechanism of inhibition was probably hemicholinium-like. Compound **3**, with a 4,4'-bicyclohexyl central spacing moiety, exhibited activity of the same order of magnitude as DMAE (**13**), which has the biphenyl spacer. These studies did not reveal a consistent effect of the nature of the oxygen functions (secondary alcohol or ketone) on pharmacological actions. The 4,4'-bicyclohexyl alcohol derivative (**3**) is not statistically more active than its carbonyl analogue (**2**), but in the case of the biphenyl series, the carbonyl analogue (**13**) was significantly more active than its secondary alcohol analogue (**4**). Thus, potent neuromuscular inhibiting activity is obtained both with *trans, trans*-bicyclohexyl- and with biphenyl derivatives. Both ketonic carbonyl and secondary hydroxyl moieties in the side chain are capable of producing potent pharmacological effects in some molecules. Replacement of the diethyl acetal moiety of DMAE (**13**) by the structurally similar ethyl ester moiety (compound **5**) permitted retention of some degree of choline-reversible neuromuscular inhibitory action, but remarkably, this compound (like **2** and **4** exhibited little or no ability to inhibit choline uptake. No explanation for these observations is apparent.

Compound **10** is the first tertiary amino congener of a bis-quaternary 1,4-oxazine-derived hemicholinium system described in the literature; **10** was inactive at a dose level of 20 μmol/kg, whereas its *N,N*-dimethyl quaternary homologue showed an $ID_{50}$ or 0.03 (0.02–0.05) μmol/kg, being approximately one fourth as potent as hemicholinium (**1**) (1). Previous work (8,9) revealed other examples of lower neuromuscular blocking activity of hemicholinium-like ditertiary amines, compared with their bis-quaternary ammonium congeners. The only tertiary amino hemicholinium analogue having significant hemicholinium-like activity discovered thus far is the 4-methylpiperidine derivative (**16**) (17). Repeated attempts in our laboratory to prepare the tertiary amino analogue of hemicholinium itself have not been successful.

Compounds **11** and **12** permitted evaluation of the necessity for bulky spacing groups (such as biphenyl or bicyclohexyl) between the quaternary heads of hemicholinium-like compounds. Compounds **11** and **12** exhibited neuromus-




**16**

cular inhibitory actions which were slow in onset and of a long duration (1–2 hr). The effect was reversed by choline chloride. However, 11 and 12 were less potent than hemicholinium. It may be concluded that polyalkylene spacing groups permit retention of hemicholinium-like activity in a molecule, and these results further support a previous proposal (1) that a critical structural parameter in the hemicholinium series is the ability of the molecule to exist in an energetically favorable conformation such that the two quaternary ammonium heads exist approximately 14 Å apart.

In summary, although it is not possible to define all of the structural features which determine prejunctional inhibition of neuromuscular transmission and inhibition of choline transport, several observations can be cited in the limited series of compounds reported herein: (i) the hemiacetal moiety of hemicholinium is not essential for inhibition of choline transport or for inhibition of neuromuscular transmission; (ii) the biphenyl moiety of hemicholinium may be replaced by phenanthrene, 2,2′-dimethylbiphenyl, or *trans, trans*-bicyclohexyl with retention of a high degree of pharmacological activity and potency, whereas the compounds containing polyalkylene spacers are somewhat less potent; and (iii) both ketonic carbonyl and secondary alcohol moieties in the side chains provide compounds effective in the synaptosomal preparation. Although the effect(s) of amine structure on activity remains to be defined, it is noted that incorporation of 4-methylpiperidine moieties produces very active agents. The very active and potent agent 7 is the only compound in the entire series of hemicholinium congeners studied thus far for which choline was an ineffective antagonist both in synaptosomes and in the rabbit neuromuscular junction.

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