Amine Oxide Analogs of Certain Cholinergic Agents[1]

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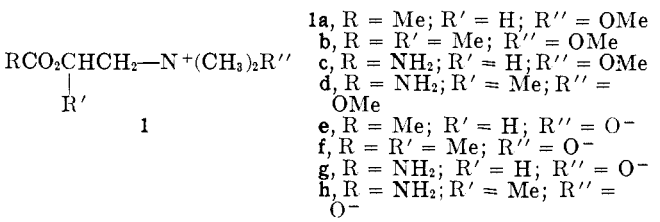
A prior communication from this laboratory[2] described preparation and cholinergic properties of N-methoxylated analogs of acetylcholine and of related structures (**1a–d**). As an extension of this study, prep-



1a, R = Me; R′ = H; R″ = OMe
b, R = R′ = Me; R″ = OMe
c, R = NH_2; R′ = H; R″ = OMe
d, R = NH_2; R′ = Me; R″ = OMe
e, R = Me; R′ = H; R″ = O^-
f, R = R′ = Me; R″ = O^-
g, R = NH_2; R′ = H; R″ = O^-
h, R = NH_2; R′ = Me; R″ = O^-

aration of the *N*-oxide analogs **1e–h** was undertaken. Jones and Major[3] prepared the *N*-oxide of diethylaminoethanol by treating the tertiary amine with aq H_2O_2. However, such amine oxide alcohols cannot be esterified conveniently, due to the attack of acyl halides and anhydrides upon the amine oxide moiety, with cleavage of the N–O bond.[4] Therefore, the appropriate amino alcohols were esterifed with Ac_2O or carbamoyl chloride, and these esters were converted into their amine oxides. H_2O_2 cannot be utilized to form the amine oxide if the molecule contains an ester link, due to the powerful hydrolyzing effect of H_2O_2;[5] *m*-chloroperbenzoic acid in organic solvent was satisfactory. **1h** was isolated in the crystalline, anhydrous state; all other amine oxide products were crystallized by conversion into their HCl salts. All amine oxides were extremely hygroscopic.

The tertiary amino ester precursors to **1e** and **1f** were quite unstable in aq media, being subject to autocatalytic hydrolysis. As determined by nmr spectroscopy, the half-life of 1-dimethylamino-2-propyl acetate in H_2O was approximately 30 min. **1e** and **1f** were considerably more stable, presumably because of the difference in basicity between a tertiary amine and its *N*-oxide.[6] **1f** was found to have a half-life of greater than 80 days in D_2O.

Results and Discussion

Muscarinic Activity on Guinea Pig Ileum.—All of the amine oxides were extremely weak muscarinics (Table I). The amine oxide derivative of ACh (**1e**), however, was 10–20 times more potent than the other amine oxides. The amine oxide induced contraction of the ileum was abolished by atropine (10^{-6} g/ml) in the superfusate. Neostigmine (10^{-8} g/ml) in the superfusate did not decrease the muscarinic activity of **1e**, and it is therefore unlikely that the activity of **1e** is due to inhibition of AChE. Hexamethonium (10–50 × 10^{-6} g/ml) in the superfusate, which decreased the ileum's responsiveness to nicotine by 75%, did not alter the response to ACh or to **1e**. It was observed that the responses to ACh were transiently potentiated following the administration of the amine oxides, especially **1f**. Since this was observed only after very high doses (greater than 10^{-5} mole), its significance remains in doubt.

TABLE I
CHOLINERGIC ACTIVITIES OF AMINE OXIDES

Compd	Muscarinic potency[a]	Nicotinic potency[b]
Acetylcholine	1.00	1.00
1e	2.1×10^{-5} (1.5–2.8)	9.4×10^{-4} (6.8–12.6)
1f	1.2×10^{-6}[c] (0.8–1.6)	Inactive[d]
1g	9.0×10^{-7}[c] (7.0–11.0)	Inactive[d]
1h	3.2×10^{-6}[c] (3.1–3.3)	Inactive[d]

[a] Guinea pig ileum. [b] Frog rectus abdominis. [c] Dose ratio estimate of relative potency. See text for details of calculation. [d] Inactive at 4×10^{-5} mole (approximately 8 mg).

Nicotinic Activity on Frog Rectus Abdominis.—Only **1e** possessed the ability to contract the rectus muscle (Table I). This activity could be abolished by superfusing the muscle with *d*-tubocurarine (5×10^{-5} *M*). Compounds **1f–h** were ineffective in doses up to 4×10^{-5} mole. Acetylcholine responses, subsequent to the administration of the highest doses of **1f–h**, were unimpaired, and thus any significant curare-like activity of the amine oxides is unlikely.

Enzyme Studies.—None of the compounds served as substrates for either acetyl- or butyrylcholinesterase in the concentration range 10^{-4}–10^{-2} *M*. **1f–h** also did not inhibit ACh hydrolysis by either enzyme in concentrations up to 10^{-2} *M*. **1e** caused approximately 10–20% inhibition of AChE at 10^{-2} *M*. Choline iodide under similar conditions had an I_{50} of 2×10^{-3} *M* and caused virtually 100% inhibition at 10^{-2} *M*.

The cholinergic inactivity and the refractoriness toward cholinesterases of all of the amine oxides is striking, in view of their close structural similarity to potent ACh-like drugs, and in view of the pronounced cholinergic activity observed in the N-methoxylated congeners (**1a–d**),[2] which also bear an O on the quaternary N. The inability of the amine oxides to inhibit cholinesterases suggests that, unlike the parent trimethylammonium cholinergics and their N-methoxylated congeners, they do not interact with the cholinergic receptors. While this proposal remains to be proven, possible explanations may be cited for noninteraction of the amine oxide esters with cholinergic receptors and with cholinesterase catalytic surfaces. Amine oxides (both in the free and in the protonated state) form extremely strong H bonds with H_2O (structures **3** and **4**).

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(2) L. L. Darko, J. G. Cannon, J. P. Long, and T. F. Burks, *J. Med. Chem.*, **8**, 841 (1965).

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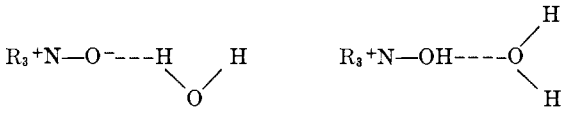
(6) P. Nylen, *Tidsskr. Kjemi Bergv. Met.*, **18**, 48 (1938).

TABLE II
TERTIARY AMINOESTER *N*-OXIDES
$RCO_2CHR'CH_2N^+(CH_3)_2O$

Compd	R	R′	Mp, °C	Reaction solvent (time, days)	Yield, %	Formula	Analyses
1e·HCl	Me	H	73–74.5[a]	C_6H_6 (2)	30	$C_9H_{14}ClNO_3$	CHClN
·picrate			115–116.5[b]			$C_{12}H_{16}N_4O_{10}$	CHN
1f·HCl	Me	Me	111–112[a]	Et_2O (4)	26	$C_7H_{16}ClNO_3$	CHClN
1g·HCl	H_2N	H	85–86[c] dec	Et_2O–EtOAc 1:1 (4)	44	$C_5H_{13}ClN_2O_3$	CHClN
1h	H_2N	Me	159–161[d] dec	Et_2O–EtOAc 1:1 (5)	48	$C_6H_{14}N_2O_3$	CHN

[a] From *n*-BuOH–hexane. [b] From EtOH. [c] From MeOH–Et_2O. [d] From *i*-PrOH–hexane.



3

4

It might be inferred that in solution, both **3** and **4** would be surrounded by an electrostatically held shell of H_2O molecules, as has been proposed for R_4N^+ cations.[7] This water shell, if held strongly, could shield the quaternary N of the amine oxide from interaction with an anionic moiety on a receptor. The compounds reported herein formed nonstoichiometric hydrates from which it was impossible to remove all of the water without causing decomposition of the amine oxide. Thus, it would appear that these systems hold multiple numbers of H_2O molecules tightly; the energy advantage resulting from interaction of the positively charged N with a cholinergic receptor would not compensate for the energy required to dislodge the water shell from the amine oxide, in order that it could approach the receptor.

Bickel[8] has cited several reports of reduction of trimethylamine oxide by biological systems *in vivo* and *in vitro*. These studies were neither exhaustive nor definitive; nevertheless, they introduce the possibility that the amine oxide analogs of cholinergic drugs were inert because the biological systems employed in the assays induced a rapid and facile reduction to tertiary amines.

It might be speculated that the full negative charge on the O adjacent to the N cation reduces the affinity of the *N*-oxide group for an anionic site on a receptor. Long and Lands[9] have presented results of conductance studies on amine oxides which seem to make this explanation unlikely.

These amine oxides may be useful for further and more detailed study of *in vivo* synthesis and metabolic disposition of the amine oxide moiety.

Experimental Section[10]

1-Dimethylamino-2-propanol was prepared by the method of Goldfarb.[11]

2-Dimethylaminoethyl acetate (**2**) was prepared in 56% yield by treating 2-dimethylaminoethanol with an equimolar amount of Ac_2O in Et_2O; the reaction mixture was basified with 6 *N* NaOH, the Et_2O was removed from the organic layer, and the residue was distilled, bp 148–149° (750 mm).[12]

1-Dimethylamino-2-propyl acetate was prepared in 66% yield as described for **2**, bp 152–153° (750 mm).[13]

2-Dimethylaminoethyl carbamate was prepared in 22% yield from carbamoyl chloride[14] as described for **2**, bp 98–99° (0.2 mm).[15]

1-Dimethylamino-2-propyl carbamate was prepared in 4% yield from carbamoyl chloride[14] as described for **2**, bp 81–82° (0.25 mm).[16]

Amine Oxides of Tertiary Aminoesters 1e–h.—A mixture of 0.025 mole of the appropriate tertiary aminoester and an equimolar amount of *m*-chloroperbenzoic acid (Aldrich Chemical Co.) in 50 ml of a suitable solvent was permitted to stand at room temperature. **1h** sepd as a crystalline solid and was recrystd. The other amine oxides were isolated by treating the reaction mixture with anhyd HCl to form a solid salt which was recrystd (see Table II). Ir and nmr spectra of all amine oxide products were consistent with the proposed structures.

Pharmacology.—Guinea pigs weighing 200–300 g were sacrificed by cervical dislocation. The terminal portion of the ileum, 3–5 cm in length, was removed, threaded at both ends, and superfused with Tyrode's soln oxygenated with 95% O_2–5% CO_2 at 37°. The superfusion rate was 3–4 ml/min. Drugs were injected directly into the superfusate in vols not more than 0.1 ml. The pH of the amine oxide solns was adjusted to 7.4 with NaOH.

Frog Rectus Abdominis Preparation.—The reactus abdominis muscle (*Rana pipiens*) was dissected as described by Burn.[17] The muscle was superfused with frog Ringer's soln oxygenated with 95% O_2–5% CO_2 at room temp. The method of drug administration and measurement were as described above.

Relative Potency Calculations.—A dose ratio ACh:amine oxide for equally responsive doses was calcd for each tissue. This dose ratio was replicated 3–6 times. For **1e**, a 2 × 2 parallel line bioassay[18] was utilized. The reliability of the dose ratio estimate of potency for **1f-h** was substantiated by the fact that a dose ratio estimate of potency for **1e** was within the 95% fiducial limits of the potency ratio calcd in the 2 × 2 bioassay.

Enzyme Studies.—The compounds were examined as substrates and inhibitors of both acetyl- and butyrylcholinesterase. AChE from the electric eel (Sigma Chemical Co., Type V) with a specific activity of 2.5 mmoles of ACh hydrolyzed/mg per min and

(7) C. J. Cavallito and A. P. Gray, *Progr. Drug Res.*, **2**, 141 (1960).
(8) M. H. Bickel, *Pharmacol. Rev.*, **21**, 325 (1969).
(9) J. P. Long and A. M. Lands, *J. Pharmacol. Exp. Ther.*, **120**, 46 (1957).
(10) All boiling points are uncorrected. Melting points were determined in open glass capillaries using a Thomas-Hoover Uni-Melt apparatus and are corrected. Ir spectra were recorded on Beckman IR5-A and IR-10 instruments and nmr spectra on a Varian T-60 instrument. Microanalyses were performed by Galbraith Laboratories, Knoxville, Tenn. Where analyses are indicated only by symbols of the elements, analytical results were within ±0.4% of the theoretical values.
(11) A. R. Goldfarb, *J. Amer. Chem. Soc.*, **63**, 2280 (1941).
(12) K. N. Campbell, C. J. O'Boyle, and B. K. Campbell, *Proc. Indiana Acad. Sci.*, **58**, 120 (1949), reported bp 150° (740 mm).
(13) L. E. Tammelin, *Acta Chem. Scand.*, **11**, 487 (1957), reported bp 152° (750 mm).
(14) L. Gatterman, *Chem. Ber.*, **23**, 1190 (1890).
(15) R. Hazard, J. Cheymol, P. Chabrier, A. Sekera, and R. Eche-Fialaire, *Bull. Soc. Chim. Fr.*, 2087 (1961), reported bp 135° (16 mm).
(16) R. T. Major and H. T. Bonnett, U. S. Patent 2374367 (1945); *Chem. Abstr.*, **39**, 4721 (1945), reported prepn and distn of this compound, but listed no boiling point.
(17) J. H. Burn, "Practical Pharmacology," Blackwell Scientific Publications, Oxford, 1952.
(18) D. J. Finney, "Experimental Design and its Statistical Basis," University of Chicago Press, Chicago, Ill., 1955.

butyrylcholinesterase from horse serum with a specific activity of 2.5 mmoles of ACh hydrolyzed/ml per min were used. The acid produced during hydrolysis of the esters was titrated with standardized 0.001 *N* NaOH at 37.5° under N_2 with a Radiometer titrator, type TTTld, titrigraph type SBR2c, and a syringe buret SBU1a. The reaction medium consisted of 0.03 *M* NaCl and 0.02 *M* $MgCl_2$. The ester substrate (0.8 ml) was placed in the reaction vessel and the pH and vol were adjusted to 7.4 and 0.9 ml, respectively, with NaOH. Spontaneous hydrolysis was measured for 5 min, whereupon 0.1 ml of the enzyme soln was added. The final concns of the enzyme were 4.64×10^{-6} g/l. and 2.5×10^{-3} g/l. for AChE and butyrylcholinesterase, respectively. After measuring enzymatic hydrolysis for 5 min, ACh (0.1 ml) was added and the inhibition of ACh hydrolysis was measured. The final concns of ACh were 10^{-3} *M* for AChE and 10^{-2} for butyrylcholinesterase. Appropriate ACh control rates were measured separately.

Phenothiazines as Local Anesthetics

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The possibility that *N*-(acylamino)phenothiazines and iminodibenzyls reported herein, might possess local anesthetic properties similar to those of corresponding phenothiazines[1] led us to undertake their synthesis and pharmacological evaluation as local anesthetics. These compounds, referred to in Chart I, were prepared using the procedure[2] reported for the synthesis of related phenothiazines. Thus, appropriately substituted phenothiazines or iminodibenzyls when treated with haloacylchlorides afforded the corresponding *N*-haloacyl derivative. The latter when condensed with *t*-$BuNH_2$ or *N*-β-hydroxyethylpiperazine gave the respective 10-*N*-aminoacylphenothiazine or *N*-aminoacyliminodi-

TABLE I

RELATIVE CORNEAL ANESTHETIC ACTIVITY OF THE COMPOUNDS[a]

Compd	Minimum effective concn (%)[b]	Median effective concn (%)[c] (ME_{50})	Relative potency (intensity)
A	0.2	0.12	0.46
B	0.2	0.11	0.50
C	0.1	0.06	0.92
D	0.1	0.05	1.10
E	0.1	0.10	0.55
F	0.1	0.09	0.61
G	0.5	0.355	0.155
H	0.5	0.50	0.11
I	0.2	0.16	0.34
J	0.2	0.18	0.31
K	0.5	0.50	0.11
Lidocaine[e]	0.075	0.055	1.00

[a] Three guinea pigs of either sex were used for each concn of test compd. [b] Different concn (%) were used and thereby the minimal effective concn (%) that produced complete loss of blink reflex in all 3 animals was detd. Opposite eye of each animal served as control. The onset of anesthesia for such concn of each compd (A–K) was 0.7, 0.8, 1.3, 3.5, 4.2, 4.2, 4, 4.3, 3.2, 3.5, 4.5 and 3.5 min, while duration of activity lasted for 39, 42, 10, 33, 15, 12, 10, 15, 17, 15, 10, and 7 min, respectively. [c] Average per cent loss of corneal reflex was noted for different concn of each drug. On plotting the log concn against its per cent response, a median effective concn (ME_{50}) which caused 50% loss of blink reflex was calcd. [d] Obtd by dividing ME_{50} of lidocaine by ME_{50} of test compound. [e] Standard drug for comparison.

TABLE II

RELATIVE RATING (PROCAINE) INDICES FOR INFILTRATION ANESTHESIA OF THE COMPOUNDS[a]

Compd	Relative potency[b] Intensity	Relative potency[b] Duration	Relative toxicity[c]	Relative rating[b] Intensity	Relative rating[b] Duration
A	10	10	0.56	17.92	17.92
B	10	10	0.66	15.60	15.60
C	5	2	0.56	8.96	3.58
D	10	2	0.498	20.10	4.10
E	5	2.5	0.56	8.96	4.48
F	10	10	0.498	20.10	20.10
G	2	2	0.33	6.02	6.02
H	2	1	0.29	6.70	3.35
I	2	2	0.29	6.82	6.82
J	5	2	0.33	15.01	6.03
K	1	1	0.29	3.41	
Procaine[d]	1	1	1.00	1.00	1.00

[a] Guinea pigs of either sex were used. Six tests were performed. for each concn. [b] The relative potency (regarding intensity) is a ratio of the concns, behaving alike, of the standard drug for comparison and of the test compd. Similarly relative potency (regarding duration) is a ratio of the concn of the standard drug and that of the test compd, producing anesthesia for nearly the same duration. Hence the relative rating (regarding intensity) would be obtd by dividing relative potency (regarding intensity) by relative toxicity; while relative rating (regarding duration) would be a ratio of relative potency (regarding duration) and the relative toxicity exhibited by the test compound. For details refer to Hamilton, *et al.*[3b] [c] Refer to Table IV for relative toxicity. [d] Standard drug for comparison.

TABLE III

RELATIVE PLEXUS ANESTHETIC ACTIVITY OF THE COMPOUNDS

Compd	Time (min) for a 0.5% soln of the drug to cause anesthesia[a]	Relative potency[b]
A	6.16	0.81
B	8.00	0.625
C	5.00	1.00
D	3.60	1.40
E	6.33	0.79
F	6.00	0.835
G	10.00	0.50
H	11.30	0.44
I	5.66	0.88
J	11.60	0.43
K	11.00	0.45
Cocaine[c]	5.00	1.00

[a] For each concn of every compd 3 frogs were used. Criteria for anesthesia was the abolishment of the stimulatory reaction due to 0.2 *N* HCl. [b] Refer to footnote *a* of Table II. [c] Standard drug for comparison.

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