AN EXPERIMENTAL INVESTIGATION OF THE CHOLINOLYTIC ACTIVITY OF SOME NEW SYNTHETIC ESTERS OF AMINO-ALCOHOLS

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Numerous publications during the last quarter of a century have described a large number of esters of basic amino-alcohols which have significant antispasmodic activity (Blicke, 1944; Lévy, 1948; Lévy and Tchoubar, 1952). More recently, an attempt has been made to describe the relationship between structural configurations favorable for stimulation and blockade at the acetylcholine sensitive receptors of cholinergically innervated organs (Lands, 1951; Luduena and Lands, 1954; Long *et al.*, 1956) and these communications have provided evidence which indicates that the muscarine-sensitive receptors are of a single type with similar if not identical properties.

Our investigations now have been extended to the study of the antispasmodic activity of a large number of new compounds. A considerable portion of these have been supplied by Blicke[1] and the synthetic methods involved are described elsewhere (Blicke *et al.*, 1944, 1952, 1955). This communication presents various basic screening data and discusses some of the relationhips between molecular structure and cholinolytic activity which these data suggest.

METHODS. The screening of antispasmodic compounds has been carried out with isolated segments of rabbit ileum suspended in Tyrode solution maintained at 38°C. (Miller, Becker, and Tainter, 1948). In some instances, a more recent modification (Luduena and Lands, 1954) was used. In this modified method, the experimental drug was added to the bath solution two minutes after ACh and the resultant reduction in contracture occurring within two minutes after addition of the spasmolytic drug expressed in per cent of the total contracture. After two minutes of exposure to the antispasmodic drug, the bath solution was removed, the intestinal segment washed twice with fresh Tyrode solution and again after a five-minute period of immersion. The spasmolytic effect of four or five concentrations, graded at 0.3 logarithmic intervals, was determined for intestinal segments from at least eight rabbits. The concentration that would be expected to produce a 50 per cent reduction was estimated from the dose-response curves. We have considered an activity of $1/100$ that of atropine sulfate as significant spasmolytic activity. Less potent compounds may have an important papaverine-like component of action.

Effect on pilocarpine-induced salivary flow in adult albino rabbits has been determined by the method described by Luduena and Lands (1954).

Acute toxicity was determined in albino mice by intravenous injection. The approximate dose that would be expected to kill 50 per cent of the injected mice within 24 hours (LD_{50}) has been determined in most instances by a short method in which three mice were injected at three or more dose levels, the doses graded at 0.3 log intervals. In some instances, a more exact value was obtained by injecting intravenously 10 mice at each of three or more dose levels, the doses being graded at 0.1 log intervals. In these instances, mortality at 24 hours has been computed and expressed as the $LD_{50} \pm$ S.E., in mgm./kgm. (Hoppe *et al.*, 1949).

[1] University of Michigan.

RESULTS. 1. *2-Diethylaminoethyl 3-hydroxy-2-phenylalkanoates.* Previous investigation (Lands, 1951) has shown that relatively small changes in the structure of the acetate portion of acetylcholine-like compounds will bring about a change in the character of the response elicited in parasympathetically innervated organs, from excitation to inhibition. This may be illustrated by 2-diethylaminoethyl acetate, in which substitution of the terminal methane of the acid portion of the ester by a hydroxyl and/or cyclic groups converts the ester to a compound devoid of cholinomimetic properties (Lands *et al.*, 1946; Lands, 1951). The presence of two aryl, alkyl or aralkyl groups in addition to the hydroxyl leads to an enhancement of cholinolytic action. The investigations of Halpern (1938) and Dupre, Lévy and Tchoubar (1946) have shown the effectiveness of aliphatic groups larger than ethyl as one of the substituents on the acetate portion of the ester.

We have extended our investigation of cholinolytic activity to include a large number of 3-hydroxy-2-phenylalkanoates, synthesized by Blicke *et al.* (1952, 1955), and made available to this laboratory for pharmacologic evaluation. Important spasmolytic activity was obtained with 2-diethylaminoethyl β-hydroxy-α-phenylbutyrate HCl (No. 1, table 1). This action was greatest with Nos. 15, 16 and 21 among the hydrochloride salts; Nos. 5a, 16a and 20a among the methohalide salts. The alkyl substituent on the acetate (R, R′) may be as large as cyclooctyl (No. 21) or as small as propyl (No. 5) without a change in the order of magnitude of cholinolytic potency. In general, the methobromide quaternaries are more potent than the corresponding hydrochlorides. Quaternization causes a distinct increase in acute toxicity, as determined in mice by intravenous injection.

Structural variation of the quaternizing group leads to important differences in cholinolytic potency. This is illustrated by the results obtained with the quaternary salts of 2-diethylaminoethyl α-phenyl-α-(1-hydroxycyclohexyl)-acetate HCl (No. 16) as shown in table 2-A. Maximum activity was obtained with the metho- and ethobromides, the latter having a potency of 118 per cent that of atropine sulfate. The benzyl- and phenacylbromide quaternaries have cholinolytic potencies distinctly less than that of the ethobromide.

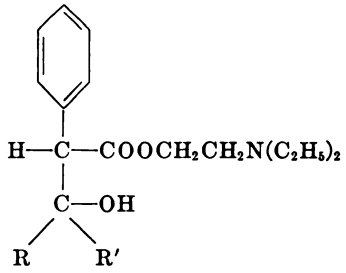
When cholinolytic activity of the hydrochloride salt is of the same order as that of atropine sulfate, quaternization may cause little or no further increase in potency. Thus, with 2-diethylaminoethyl α-cyclopentyl α-hydroxy-α-(2-thienyl)-acetate HCl (No. 64) in which cholinolytic potency exceeds that of atropine, quaternization to form the metho- or ethobromide results in no further increase (table 2-B).

Acute intravenous toxicity in mice was greatest for the allylbromide (No. 16c) and least for the benzylbromide (No. 16d) quaternary salts of 2-diethylaminoethyl-α-phenyl-α-(1-hydroxy-cyclohexyl) acetate. The values obtained do not correlate with those for cholinolytic action and this suggests that lethality resulted largely from effects in some physiologic system other than that involved in this action.

2. *2-Diethylaminoethyl 3-hydroxy-2-cyclohexylalkanoates.* The previous section

TABLE 1

A comparison of cholinolytic activity on the rabbit isolated intestinal segment and acute toxicity in mice of a series of 2-diethylaminoethyl 3-hydroxy-2-phenylalkanoates



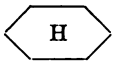
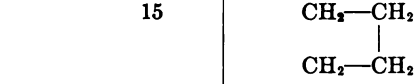
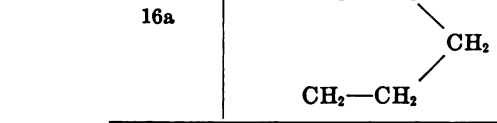
Compound No.	Structure		Salt	Cholinolytic Activity* (Atropine = 100)	Toxicity, i.v. Mouse† LD_{50}
	R	R'			
					mgm./kgm.
1	H	CH_3	HCl	15	56.0 ± 6.0
1a			CH_3Br	33	21.0 ± 2.0
2	CH_3	CH_3	HCl	10	
3	CH_3	C_2H_5	HCl	9	53
4	H	C_2H_5	HCl	35	70
5	H	C_3H_7	HCl	25	60
5a			CH_3Br	100	18
6	H	$CH(CH_3)_2$	HCl	12	60
6a			CH_3Br	11	15
7	H	$CH(C_2H_5)_2$	HCl	3	
8	H		HCl	4	44
9	H		HCl	10	28
9a			CH_3Br	44	8
10	H		HCl	15	36
10a			CH_3Br	28	9
11	H		HCl	23	62
11a			CH_3Br	62	12
12	H	C_6H_{11}(n)	HCl	5	31
12a			CH_3Br	53	6
13	H		HCl	28	6
13a			CH_3Br	60	
14	C_2H_5	C_2H_5	HCl	3	
15			HCl	112	
16			HCl	43	50.0 ± 4.0
16a			CH_3Br	83	14.5 ± 0.9

TABLE 1—*Continued*

Compound No.	Structure R | R′	Salt	Colinolytic Activity* (Atropine = 100)	Toxicity, i.v. Mouse† LD_{50}
				mgm./kgm.
17	$CH_2—CH_2—CH_2$	HCl	27	47.0 ± 3.5
17a	$CH_2—CH_2—CH_2$	CH_3Br	41	10.9 ± 1.1
18	$CH_2—CH_2$	HCl	11	
18a	CH_2 / $CH—CH_2$ / CH_3	CH_3Br	32	9
19	$CH_2—CH_2$	HCl	5	38
19a	CH_2 / $CH_2—CH$ / CH_3	CH_3Br	27	16
20	$CH_2—CH_2$	HCl	29	38
20a	$CHCH_3$ / $CH_2—CH_2$	CH_3Br	80	9
21	$CH_2—CH_2—CH_2$	HCl	41	44
21a	CH_2 / $CH_2—CH_2—CH_2$	CH_3Br	53	1

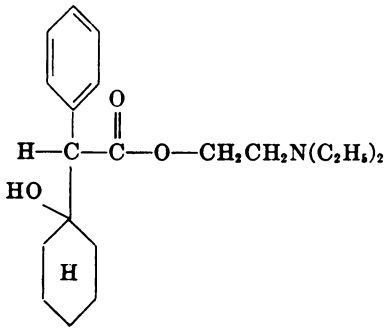
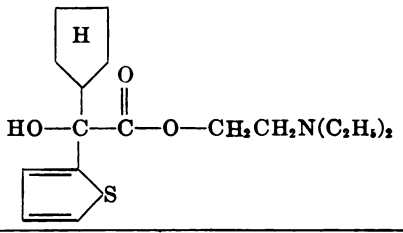
* Values tabulated are based upon the effective concentration of drug (Luduena and Lands, 1954) in comparison with that of atropine sulfate. Contracture was induced by ACh, added to give a final concentration of 1:1,000,000.

† Acute intravenous toxicity in mice. The tabulated value is an approximate LD_{50}, unless given with the standard error.

described a series of esters in which a phenyl group is one of the substituents in the acid portion of the ester. Lévy *et al.* (1948) and Buchel *et al.* (1948) have reported that the spasmolytic activity of cyclohexyl- and cyclohexenylalkanoate esters is comparable to that of the corresponding phenyl analogs. We have been able to examine this matter further by the use of a series of cycloalkyl substituted esters (Blicke *et al.*, 1952, 1955). Results obtained are shown in table 3. Significant spasmolytic action (more than 1 per cent that of atropine sulfate) was observed with most of these compounds. It is interesting to note that No. 30 and the

TABLE 2

The influence of the quaternizing group on the cholinolytic activity and toxicity of esters of 2-diethylaminoethanol

Compound No.	Salt	Cholinolytic Activity‡ (Atropine = 100)	Toxicity, i.v. Mouse LD_{50}
	A. 		
			mgm./kgm.
16†	HCl	43	50.0 ± 4.0
16a	CH_3Br	83	14.5 ± 0.9
16b	C_2H_5Br	118	17.0 ± 1.2
16c	$CH_2{:}CHCH_2Br$	70	12.0 ± 0.9
16d	$C_6H_5CH_2Br$	28	51.0 ± 3.6
16e	$C_6H_5COCH_2Br$	33	26.0 ± 1.8
	B. 		
64*†	HCl	200	80.0 ± 6.0
64a*	CH_3Br	210	16.0 ± 1.4
64b	C_2H_5Br	200	18.0 ± 0.8

* Luduena and Lands, 1954.

† Blicke, F. F., U. S. Patent 2,541,634 (Feb. 13, 1951).

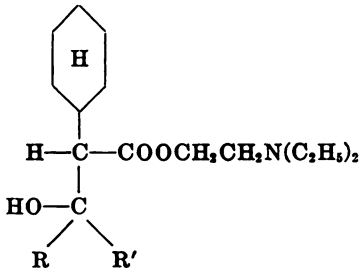
‡ ACh contracture, rabbit isolated intestinal segment.

corresponding phenyl analog (No. 15) are the most potent hydrochloride salts. Conversion to the methobromide quaternary salt resulted in a distinct increase in this activity. The quaternary salts also were distinctly more toxic than the corresponding hydrochlorides, but there does not appear to be any direct relationship between spasmolytic potency and acute toxicity.

3. *2-Diethylaminoethyl cyclopentylalkylacetates.* The preceding section has

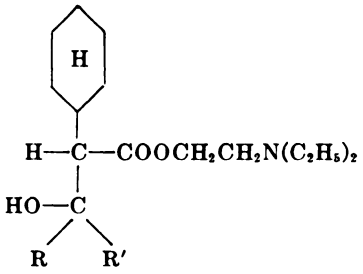
TABLE 3

A comparison of the cholinolytic activity on the rabbit isolated intestinal segment and acute toxicity in mice of a series of 2-diethylaminoethyl 3-hydroxy-2-cyclohexylalkanoates



Compound No.	Structure		Salt	Cholinolytic Activity* (Atropine = 100)	Toxicity, i.v. Mouse LD_{50}
	R	R'			
					mgm./kgm.
22	H	H	HCl	6	88.0 ± 7.0
22a			CH_3Br	19	23.0 ± 1.9
23	CH_3	CH_3	HCl	5	75
23a			CH_3Br	14	22
24	H	C_2H_5	HCl	7.1	63
24a			CH_3Br	23.2	22
25	H	C_3H_7	HCl	6	42
25a			CH_3Br	44	19
26	CH_3	C_2H_5	HCl	21	60
26a			CH_3Br	48	18
27	H	$CH(CH_3)_2$	HCl	3	38
27a			CH_3Br	14	16
28	C_2H_5	C_2H_5	HCl	7	50
28a			CH_3Br	32	16
29	C_3H_7	C_3H_7	HCl	0.2	31
			CH_3Br	0.8	5
30	$CH_2—CH_2$		HCl	33	37.0 ± 2.0
	$CH_2—CH_2$				
30a			CH_3Br	104	16
31	$CH_2—CH_2$		HCl	9	44
		CH_2			
31a	$CH_2—CH_2$		CH_3Br	62	16
32	$CH_2—CH_2—CH_2$		HCl	8	44.0 ± 2.6
32a	$CH_2—CH_2—CH_2$		CH_3Br	50	10.2 ± 0.5
33	$CH_2—CH_2$		HCl	3	44
		$CH—CH_3$			
33a	$CH_2—CH_2$		CH_3Br	21	8

TABLE 3—*Continued*



Compound No.	Structure		Salt	Cholinolytic Activity† (Atropine = 100)	Toxicity, i.v. Mouse† LD_{50}
	R	R'			
					mgm./kgm.
34	$CH_2—CH_2—CH_2$	CH_2	HCl	1.8	25
34a	$CH_2—CH_2—CH_2$	CH_2	CH_3Br	7.7	8
35	H	C_6H_{11}	HCl	2	42
35a			CH_3Br	13.5	11
36	H	C_5H_{11}	HCl	1.3	30
36a			CH_3Br	18.5	13
37	H	C_6H_{13}	HCl	0.5	8
37a			CH_3Br	18.5	4
38a	CH_3	H	CH_3Br	3.2	4

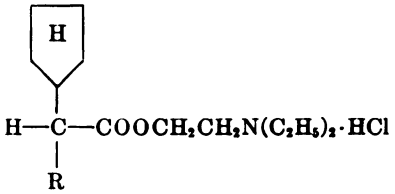
* See footnote, table 1.

described high spasmolytic potency with 2-diethylaminoethyl esters of aliphatic acids indicating that the aromatic group is not essential. The cyclohexyl ring may also be replaced by a cyclopentyl ring and still provide esters of aliphatic acids with significant spasmolytic activity. Moffett *et al.* (1950) have synthesized and made available to this laboratory the series of cyclopentyl substituted compounds shown in table 4. Although they do not have a hydroxyl and are not quaternized, spasmolytic potencies of 2 to 38 per cent that of atropine sulfate were obtained. The most active compound, No. 41, has a potency comparable to that of 2-diethylaminoethylphenylcyclohexylacetate HCl (No. 48, Trasentine 6-H).

4. *2-Dialkylaminoethylphenyl- or thienylalkylacetates and -glycolates.* From the results presented in the preceding sections, it is clearly apparent that potent spasmolytic compounds may be obtained from 2-diethylaminoethyl esters of a large variety of aliphatic and arylaliphatic acids. Lévy *et al.* (1948) have shown the 2-diethylaminoethyl esters of cyclohexyl- and cyclohexenylpropionic, -butyric and -hexanoic acids are potent spasmolytic agents and more potent than the corresponding phenyl analogs. We have extended our investigation of

TABLE 4

*A comparison of the cholinolytic activity on the rabbit isolated intestinal segment and acute toxicity in mice of a series of 2-diethylaminoethyl cyclopentylalkylacetates**



Compound No.	Structure R	Cholinolytic Activity† (Atropine = 100)	Toxicity, i.v. Mouse† LD_{50}
			mgm./kgm.
39	$CH(CH_3)_2$	6	50
40	C_4H_9	6	34
41	$CH_2CH(CH_3)_2$	38	47
42	$CH_2C(CH_3):CH_2$	6	
43	$CH(CH_3)CH(CH_3)_2$	2	
44	$CH_2CH_2CH(CH_3)_2$	8	
45	$CH(CH_3)CH_2CH_2CH_3$	2	31
46	$CH_2CH(CH_3)CH_2CH_3$	4	
47	$CH_2CH(C_2H_5)_2$	12	25
48	2-diethylaminoethyl phenylcyclohexylacetate	26	
49	2-diethylaminoethyl dicyclohexylacetate	16	

* Moffett, R. B., and Hart, C. A., U. S. Patent 2,538,792 (Jan. 23, 1951.)

† See footnote, table 1.

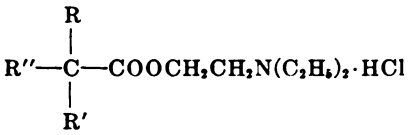
spasmolytic agents to include esters of thienylaliphatic (Blicke and Tsao, 1944) as well as cycloalkyl- and phenyl-substituted esters. Results obtained are shown in table 5.

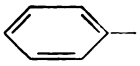
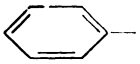
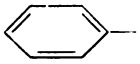
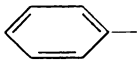
The presence of a hydroxyl group on either the first or second carbon from the carboxyl greatly increases cholinolytic action. A comparison of the spasmolytic potency of No. 51 (table 5) with No. 6 (table 1) shows that while the former is more potent than the latter, their potencies are of the same order of magnitude. The phenyl group may be replaced by a thienyl group without important changes in potency as is shown by a comparison of No. 54 (phenyl) and No. 56 (thienyl). These compounds have spasmolytic potencies of 36 and 47 per cent that of atropine sulfate. The spasmolytic values obtained from No. 51 (phenyl) and No. 53 (thienyl) are equally close. This generalization appears to be applicable also to the analogs in which a cycloalkyl has been substituted for an alkyl group. No. 60 (thienyl) has a spasmolytic potency of 80 per cent that of atropine sulfate which is comparable to that of the corresponding phenyl analog, No. 61 (2-diethylaminoethyl phenylcyclohexylhydroxyacetate) with a potency of 60 per cent.

Although the major portion of the data presented here was obtained in screening tests on the isolated rabbit ileum, a few of the more potent compounds were examined for cholinolytic action in the intact animal. Blockade of pilocarpine-induced salivation in rabbits was determined by the method described by

TABLE 5

A comparison of the cholinolytic activity on the rabbit isolated intestinal segment and acute toxicity in mice of a series of 2-diethylaminoethyl phenyl- or thienylalkylacetates and -glycolates



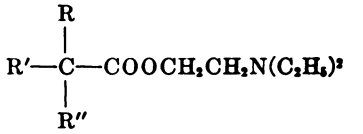
Compound No.	Structure			Cholinolytic Activity* (Atropine = 100)	Toxicity, i.v. Mouse* LD_{50}
	R	R′	R″		
					mgm./kgm.
50		$CH_2CH(CH_3)_2$	H	4	37.0 ± 2.0
51			OH	32	72.0 ± 4.0
52		$CH_2CH(CH_3)_2$	H	2	50.0 ± 4.0
53			OH	40	66.0 ± 2.0
54		$CH_2CH_2CH(CH_3)_2$	OH	36	
55		$CH_2CH_2CH(CH_3)_2$	H	4	
56			OH	47	
57		$CH(CH_3)_2$	H	<1	
58			OH	15	
59			H	6	42.0 ± 3.0
60			OH	80	58.0 ± 5.0
48			H	13	
61			OH	60	
Antrenyl†			OH (CH_3Br)	100†	13.2†
62			OH	62	21
62a			OH (CH_3Br)	53	
62b			OH (C_2H_5Br)	26	

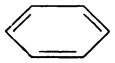
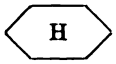
* See footnote, table 1.

† Values taken from Plummer, Barrett, Rutledge and Yonkman, 1953.

TABLE 6

Blockade of pilocarpine induced salivation in rabbits



Compound No.	Structure			Salt	Blockade of Salivation i.v. ED_{50}
	R	R'	R''		
					microgm./kgm.
1		H	$CHOHCH_3$	HCl	520.0
1a				CH_3Br	5.2
63a		OH		CH_3Br	1.5
64a*		OH		CH_3Br	2.5
65		OH		HCl	110.0
Homatropine				HCl	250.0
				CH_3Br	11.0
Atropine*				sulfate	8.0
				methyl nitrate	1.0
Scopolamine					3.2

* Luduena and Lands, 1954.

Luduena and Lands (1954) with the results shown in table 6. The quaternary methobromides are distinctly more effective than the corresponding tertiary amines. The potency of 2-diethylaminoethyl 3-hydroxy-2-phenylbutanoate hydrochloride (No. 1) is of the same order of magnitude as that of homatropine, the corresponding methobromide being comparable in potency to homatropine methobromide.

The cholinolytic actions of 2-diethylaminoethyl cyclopentylhydroxy-(2-thienyl)acetate (No. 64) and the corresponding methobromide salt have been described in a previous publication (Luduena and Lands, 1954). The ethobromide (No. 64b), shown in table 2, has been examined for cholinolytic action in anesthetized dogs with results as follows, expressed as ED_{50} values in microgm./kgm. for intravenous administration: spasmolysis of the small intestine, 1.88; blockade of carbamylcholine induced salivation, 1.62; blockade of carbamylcholine induced vasodepression, 1.33. The corresponding approximate values for No. 60a are 4.0, 2.0 and 2.0 microgm./kgm. These data support the view that the intestinal screening data provide an indication of the general order of cholinolytic activity in the various compounds described in this communication.

5. *Miscellaneous esters of 2-diethylaminoethanol.* This exploration of the esters of 2-diethylaminoethanol in an effort to determine the optimum configuration of the acid portion for effective blockade of the acetylcholine-sensitive receptors of

parasympathetically innervated organs led to the evaluation of several compounds wherein the structure was too varied for tabulation in the first six tables of the preceding sections. The compound obtained by partial saturation of the ring (2-diethylaminoethyl Δ^2-cyclopentenyl-isoamylacetate HCl) is not significantly different in spasmolytic potency from the close phenyl and cyclopentyl analogs shown in tables 4 and 5. It was found that 2-diethylaminoethyl Δ^2-cyclohexenyl-Δ^2-cyclopentenylacetate has spasmolytic potency comparable with that of 2-diethylaminoethylphenyl-cyclohexylacetate HCl (Trasentine 6-H, No. 48).

Some modification of the structure of the acid portion of the ester, as represented by No. 8 and 2-diethylaminoethyl α-(2-hydroxyhydrenyl)-phenylacetate hydrochloride, wherein the position of the hydroxyl is varied relative to the carboxy group is permissible without a large loss of activity. However, a similar lengthening of this distance by interposing $-CH_2-$ between the hydroxyl and the carbon atom bearing the cyclic substituents as illustrated by 2-diethylaminoethyl α-phenyl-α-cyclohexyl-β-hydroxypropionate hydrochloride and 2-diethylaminoethyl α-benzyl-α-phenyl-β-hydroxypropionate hydrochloride does not favor spasmolytic action.

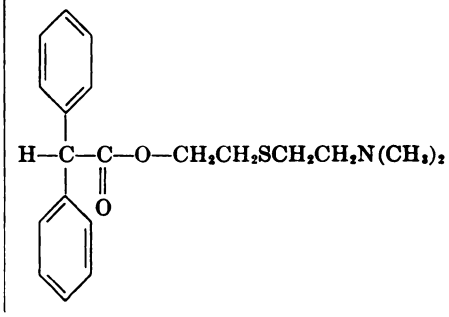
a. *Dialkylaminoalkylmercapto esters of organic acids.* Benoit *et al.* (1951) have described the spasmolytic activity of diphenyl-1,1-diethylamino-ω-alkanes in which the distance between the terminal methane and the amino group varied from 1–6 carbons. They reported maximal activity at 4 and 6 carbon distances, these compounds being 6–15 times more potent than 2-diethylaminoethyl diphenylacetate (Trasentine). We have investigated the spasmolytic activity of a series of long chain mercapto derivatives shown in table 7-A. Spasmolytic activity of these esters is not great, the most active having a potency of less than 1 per cent that of atropine. These compounds are slightly longer than the longest member of the series described by Benoit *et al.* (1951) and may be too long for proper attachment to the parasympathetic receptor site. Also, the large sulfur atom may not favor attachment. We have included for comparison two compounds of similar length, 2-diethylaminoethoxyethyl diphenylacetate hydrochloride and 4-diethylamino-2-butenyl diphenylacetate hydrochloride, in which the sulfur moiety is not present. They appear to be somewhat more spasmolytic (activity ratios of 1 per cent) than the thiol analogs but with activity that is at the lower limit of significance.

b. *Alkylaminoalkanoic acid esters.* Schueler *et al.* (1951) have shown that methyl-2-trimethylammonium propionate bromide (reversed ester of ACh) has muscarinic activity of a high order. The corresponding triethylammonium analog is considerably less stimulating, requiring doses of 1–2 mgm./kgm. intravenously in dogs to elicit vasodepressor responses. We have included in this investigation the spasmolytic data obtained with a series of diphenyl- and phenylcyclohexyl substituted benzohydryl alkanoates in which the terminal carbon of these "reversed esters" have been modified as in the antispasmodic groups described in preceding sections. Results obtained are shown in table 7-B. None of these compounds were found to have significant spasmolytic action.

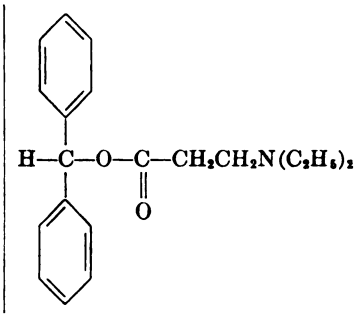
c. *Dialkylaminoalkanoate esters.* It has been shown that the addition of a

TABLE 7

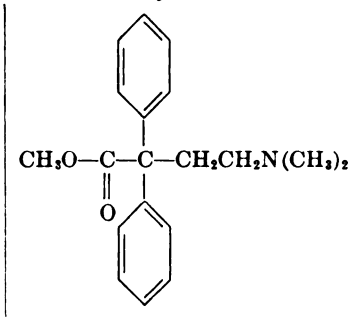
A comparison of the cholinolytic activity on the rabbit isolated intestinal segment of a miscellaneous group of synthetic esters of basic amines

Compound No.	Structure	Salt	Cholinolytic Activity* (Atropine = 100)
A.		citrate	0.2

3-(2-Dimethylaminoethylmercapto)-2-propyl diphenylacetate citrate, 2-(2-diethylaminoethylthio)ethyl fluorene-9-carboxylate citrate, 3-(2-dimethylaminoethylthio(-propylfluorene-9-carboxylate citrate, 3-(2-diethylaminoethylthio)-2-propyl phenylcyclohexylacetate citrate, diethylaminoethylthioethyl phenylcyclohexylacetate citrate have cholinolytic activities of 0.1–0.4. The cholinolytic activities of 2-diethylaminoethoxyethyl diphenylcetate hydrochloride and 4-diethylamino-2-butenyl diphenylacetate hydrochloride are 1.0.

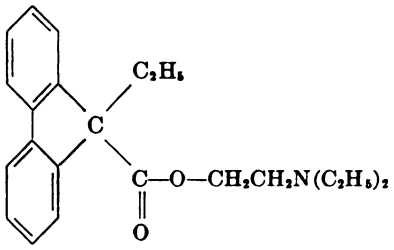
B.		HCl	0.3

Benzohydryl diethylaminoacetate hydrochloride, benzohydryl 3-piperidinopropionate hydrochloride, benzohydryl 3-morpholinopropionate hydrochloride have cholinolytic activities of 0.1 or less. Similarly, cyclohexylphenylmethyl 3-(N-2-methylpiperidyl)-propanoate hydrochloride and the corresponding diethylaminopropionate hydrochloride are weak antispasmodics with cholinolytic acitivities of less than 2.

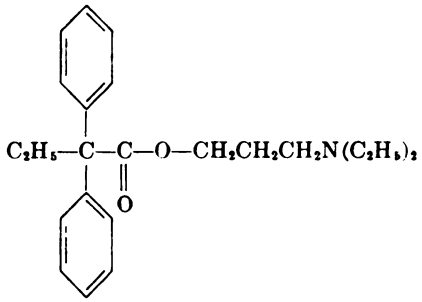
C.		HCl	<2

Isopropyl 2,2-diphenyl-4-dimethylaminobutanoate hydrochloride, ethyl 2,2-diphenyl-4-dimethylaminobutanoate methiodide, ethyl 2,2-diphenyl-4-N-morpholinylbutanoate methiodide, ethyl 4-dimethylamino-2,2-diphenylpentanoate hydrochloride all have cholinolytic activities of less than 2.

TABLE 7—*Continued*

Compound No.	Structure	Salt	Cholinolytic Activity* (Atropine = 100)
D.		HCl	2.5

Ethyl 9-(2-N-piperidylethyl)-fluorene-9-carboxylate hydrochloride has a cholinolytic activity of 2.5.

E.		HCl	0.9

Diethylaminoethyl diphenylethylacetate hydrochloride has a cholinolytic activity of 3. 3-N-Piperidylpropyl 2,2-diphenylbutanoate hydrochloride, 1-methyl-2-dimethylaminoethyl 2,2-diphenylbutanoate hydrochloride, dimethylaminoethyl 2,2-diphenylbutanoate hydrochloride, dimethylaminoethyl 2,2-diphenylbutanoate methiodide, N-piperidylisopropyl 2,2-diphenylbutanoate methiodide and 2-N-piperidylethyl 2,2-diphenylbutanoate hydrochloride all have cholinolytic activity of less than 2.

* See footnote, table 1.

cyano group to the aryl substituted carbon does not diminish significantly spasmolytic potency (Lands *et al.*, 1949). Substitution of larger groups, as shown in table 7-C, does not favor spasmolytic activity. These compounds are weak and appear to be somewhat less effective than the corresponding aminoalkane and -alkanols (Lands and Luduena, 1956). The somewhat more compact structure in which the benzene rings are incorporated into the fluorene structure (table 7-D) have spasmolytic potencies comparable to that of the corresponding diphenylacetates.

A series of dialkylaminoalkyl 2,2-diphenylbutanoates (table 7-E) were examined for spasmolytic potency with results similar to those previously described. Activity was not great in any instance and was greatest with 2-diethylaminoethyl diphenylethylacetate HCl which has a spasmolytic potency of 3 per cent that of atropine sulfate. This compound is not more spasmolytic than the corresponding diphenylacetate ester.

DISCUSSION. In previous communications from this laboratory, we have suggested that the ACh sensitive receptor surface of parasympathetically innervated organs have quite similar or identical properties; that the reactive surface lies within an area about 10 Å in length (measured from the nitrogen center) by 5 Å in width; that greatest activity is obtained when there is a hydroxyl group at a distance of 4-7 Å from the cationic head; that there should be present on the carbon near the hydroxyl an umbrella-like mass which may form a protecting shield and/or an additional attracting force for atachment to the receptor surface (Lands, 1951; Lands and Cavallito, 1954; Luduena and Lands, 1954; Lands and Luduena, 1956). The large number of structurally diverse esters described in this communication provides an additional opportunity for evaluating this concept.

The influence of chain length (distance between N and terminal methane of the hydrocarbon chain) is most simply determined by the series of 1,1-diphenyldiethylamino-ω-alkanes described by Benoit *et al.* (1951). Two optimal chain lengths were reported at 7.5 and 10.5 Å from the N. There was no hydroxyl group present, so one may assume that the additional attracting forces were supplied by the phenyl rings substituted on the terminal carbon. Inasmuch as the unsubstituted five carbon analog is cholinomimetic rather than cholinolytic, the phenyl rings determined the nature of the action of the molecule. Introduction of a hydroxyl into the appendage group at a distance of 4.5 Å (or on the third carbon from the N) in the four carbon analog (McManus *et al.*, 1954; Schultz *et al.*, 1952) results in a compound with cholinolytic potency not appreciably greater than that of the corresponding alkane. However, when one phenyl group is replaced by a propyl or isopropyl group, cholinolytic potency is one to five per cent that of atropine sulfate. Reduction of the appendage group to three carbons and substitution of the terminal carbon with a hydroxyl and two phenyl rings results in a compound with cholinolytic potency of 50–100 per cent that of atropine sulfate (White *et al.*, 1951). Inasmuch as the three carbon distance is near the critical limit (Lands and Luduena, 1956), relatively small structural changes may prevent close approach of the hydroxyl to the reactive site. The observation that a carbamyl group may replace the hydroxyl without a resultant loss of significant activity is of interest (Schaumann and Lindner, 1951; Cheney *et al.*, 1952; de Jong *et al.*, 1955; van Proosdij-Hartzema *et al.*, 1955). This group also appears in the potent cholinomimetic drug, carbaminoylcholine (Doryl, Lentin, Carcholin).

The Blicke modifications shown in tables 1 and 2 indicate the large variety of structures which may be substituted on the hydroxyl bearing carbon atom without loss of significant cholinolytic activity. These results are similar to those obtained with comparable glycolates (Lands *et al.*, 1946, 1951) and indicate that the nature of these modifications are less critical than chain length or the presence of a hydroxyl group.

Schueler and Keasling (1951) described potent cholinomimetic compounds in their series of "reversed ester" analogs of ACh. One might reasonably expect that appropriate substitution of the terminal carbon would provide potent

cholinolytic compounds. This was not realized with the compounds shown in table 7-B. The reversed ester analog of Trasentine is clearly less potent than the latter. The —C:O— at a distance of 3 Å from the N is not opposite the positive center involved in attachment (Long *et al.*, 1956) and may provide steric hindrance to receptor attachment.

We have previously referred to the carbamyl substituted compounds. A modification of several potent cholinolytic esters is shown in table 7 (D and E) wherein the terminal hydroxyl or carbamyl group has been replaced by an ethyl group; the resultant compounds are either low in potency or are below the level of significant action. However, the size of the ethyl substitutent is comparable to that of the carbamyl group suggesting some specific importance for the latter similar to that represented by a hydroxyl.

Quaternization of effective cholinolytic compounds to give the methyl- or ethylhalide salt produces the expected increase in potency and is in agreement with the findings of Fromherz (1933), Meier and Hoffmann (1941), Ing *et al.* (1945) and many others. Recently, similar results have been described for a series of optically active dialkylaminoalkanols, the cholinolytic activity being due almost entirely to the *levo* component (Duffin and Green, 1955). The effect of quaternization is amply illustrated by data given in tables 1–3. A four- or five-fold increase in spasmolytic potency is not uncommon. These quaternary salts also have curare-like actions (Fromherz, 1933) and are markedly more toxic than the corresponding hydrochloride salts.

This communication provides data on a large number of new synthetic compounds. It is the authors' hope that the additional information will prove helpful in the further elucidation of the relationship between chemical structure and the nature of the receptor surface involved in the mediation of cholinergic nerve impulses.

SUMMARY

Various phenyl, cycloalkyl and alkyl substituted β-hydroxybutyric acid esters of 2-diethylaminoethanol have cholinolytic action comparable to that of the corresponding hydroxyacetate esters.

The most effective compounds, within a variety of structurally diverse esters, have a hydroxyl group at a distance of 4–7 Å from the amine group. The replacement of the hydroxyl by a hydrogen atom greatly reduces cholinolytic potency in most instances.

The various effective substitutions of the carbon atoms near the hydroxyl provide an umbrella-like mass which may form a protecting shield over the receptor surface thereby preventing close approach of stimulating molecules such as acetylcholine. The data suggest that other effects also may be important and that these groups may in some specific way increase affinity for the receptor site.

Quaternization of the amine to form the methyl- or ethylhalide salts increases cholinolytic potency and toxicity. This is similar to the increase in cholinomimetic potency and toxicity which is associated with methylhalide quaternization of

various tertiary amines and may result, in part, from an increased reactivity of the cationic group.

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