TABLE IV
PHARMACOLOGICAL ACTIVITIES

No.	Ulcer index[a] A	B	C	TC	Dose, mg/kg, po	LD_{50}, mg/kg, ip
41	1.7	3.0	1.0	2.0	20	>1000
61	1.6	1.7	2.5	2.0	20	
42	2.1	1.4	2.5	1.9	20	>2000
35	2.2	1.6	2.1	1.9	20	>1000
31	1.4	2.5	1.2	1.8	15	>1000
58	1.7	1.5	2.0	1.7	20	>1000
68	1.6	1.5	2.0	1.7	20	
69	2.7	1.3	1.3	1.7	20	>1000
24	2.2	1.6	1.6	1.7	20	
28	2.2	1.2	2.2	1.7	20	>1000
63	1.6	1.5	2.0	1.7	20	
48	1.8	1.6	1.4	1.6	20	>1000
59	1.3	3.4	1.1	1.5	20	
39	0.9	4.0	1.5	1.5	20	
37	1.4	1.0	2.5	1.5	20	
18	1.2	1.2	2.3	1.5	20	
70	1.9	1.5	1.1	1.5	20	
33	1.6	1.1	2.0	1.4	20	
62	1.0	1.1	2.2	1.4	20	
76	1.1	1.0	2.2	1.3	20	
71	1.2	0.9	1.5	1.2	20	
57	0.0	1.0	2.6	1.1	20	
56	0.3	1.8	1.6	1.0	20	
Oxymethalone	2.2	1.2	1.5	1.7	50	

[a] Values indicate the ratio to the value of the control animals without receiving drugs for ulcer remedy.

the same manner as XIII from XII, was dissolved in 22 ml of EtOH containing 7 ml of H_2O and concd HCl (1.0 g, 0.01 mole) and was hydrogenated in the presence of PtO_2 (1.0 g) at 40–50° and 6 atm pressure. After H_2 uptake was completed, the mixt was cooled and filtered from the catalyst. EtOH was removed under reduced pressure, and the solid which sepd from the soln was filtered off. To the filtrate was added 10 ml of 10% aq Na_2CO_3, and the soln was extd with $CHCl_3$ and dried (Na_2SO_4). After the removal of the solvent, the resulting solid was recrystd to give **27**.

1-(3,4,5-Trimethoxybenzoyl)-3-aminopiperidine (XVIIb).—A soln of 2.2 g (0.0095 mole) of 3,4,5-trimethoxybenzoyl chloride in 5 ml of MeCN was added gradually to a soln of 2.2 g (0.0095 mole) of **27** and 0.6 g (0.0113 mole) of Na_2CO_3 in 6 ml of H_2O with vigorous stirring and cooling with an ice bath. After stirring 2 hr at room temp, the soln was extd with $CHCl_3$. The solvent was removed under reduced pressure, and the residue was hydrogenated with 10% Pd/C (0.2 g) in 100 ml of EtOH and concd HCl (0.7 ml) at ordinary temp and pressure. After H_2 uptake was completed, the mixt was filtered from the catalyst, the solvent was removed under reduced pressure, and the resulting solid was recrystd from EtOH–MeCN to give 1.6 g (65.2%) of an amorphous powder, mp 239–242°. *Anal.* ($C_{15}H_{22}N_2O_4 \cdot HCl \cdot H_2O$) C, H, N.

1-(3,4,5-Trimethoxybenzoyl)-3-(*p*-nitrobenzamido)piperidine [**75, IVb, R = *p*-NO_2; R_1 = 3,4,5-$(MeO)_3$**] was obtained from XVIIb by treating with *p*-nitrobenzoyl chloride as in method C.

1-(3,4,5-Trimethoxybenzoyl)-3-(*p*-aminobenzamido)piperidine [**74, IVb, R = *p*-NH_2; R_1 = 3,4,5-$(MeO)_3$**] was obtained from **75** in the same manner as **42** from **44**.

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Synthesis and Pharmacological Activity of Dihydrobenzofurans[1]

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The synthesis of the cis and trans isomers of 5- and 7-dimethylamino-3-hydroxy-2-methyl-2,3-dihydrobenzofuran methiodide (**11a–d**) started with nitration of 3-acetoxy-2-methyl-2,3-dihydrobenzofuran and subsequent separation of the isomers *cis*-5-, *trans*-5-, *cis*-7-, and *trans*-7-nitro-3-acetoxy-2-methyl-2,3-dihydrobenzofurans (**8a–d**). Catalytic reduction of the respective nitro compounds in the presence of CH_2O gave the corresponding dimethylamino compounds **9a–d**. Deacetylation to the alcohols **10a–d** and treatment with MeI yielded **11a–d**. Nitration of 2-methylcoumaran-3-one gave the 5- and 7-nitro ketones (**5a** and **5b**). Reduction and concurrent methylation with CH_2O followed by treatment of the separated isomers with MeI afforded 5-dimethylamino-2-methylcoumaran-3-one methiodide (**6a**) and the 7 isomer (**6b**). Using an excess of CH_2O in the same sequence with **5a** yielded the alcohol addition product, 5-dimethylamino-2-hydroxymethyl-2-methylcoumaran-3-one methiodide (**7**). Biological examination revealed muscarinic action (**6a**, 1/100 ACh) and nicotinic activity (**6a**, 1/20 nicotine, **11a**, 1/100 nicotine, **11b**, 1/200 nicotine). Both butyryl- and acetylcholinesterase were inhibited by **6a** and **6b**; the potency of **6a** ($K_i = 2.5 \times 10^{-8}$) was reflected in the LD_{50} (10 mg/kg). The remainder of the compounds displayed little or no activity and low toxicity (LD_{50} 50 to 200 mg/kg) with the exception of **11a** which was a weak muscarinic antagonist.

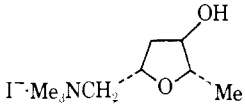
Acetylcholine (ACh) can assume an infinite number of conformations; based on this a great deal of research has been described that has restricted this freedom by the synthesis of rigid analogs of ACh.[2,3] Agents with a limited number of allowable conformations having both potent muscarinic and nicotinic effects are muscarine

(1) Supported by an NDEA Title IV Predoctoral Fellowship, 1966–1969 to L. J. P. and by Grant 1K3-CA-10739 from the National Cancer Institute of the National Institutes of Health. Abstracted in part from the thesis of L. J. P. submitted to the Graduate School, University of Kansas, Lawrence, Kan., in partial fulfillment of the requirements for the Doctor of Philosophy.

(2) Several recent examples of rigid acetylcholine analogs are noted. (a) J. B. Robinson, B. Belleau, and B. Cox, *J. Med. Chem.*, **12**, 848 (1969). (b) E. E. Smissman, W. L. Nelson, J. B. LaPidus, and J. Day, *ibid.*, **9**, 458 (1966). (c) E. E. Smissman and G. S. Chappell, *ibid.*, **12**, 429 (1969). (d) P. D. Armstrong, J. G. Cannon, and J. P. Long, *Nature (London)*, **220**, 65 (1968). (e) C. Y. Chiou, J. P. Long, J. G. Cannon, and P. D. Armstrong, *J. Pharmacol. Exp. Ther.*, **166**, 243 (1969).

(3) Several recent dioxolane analogs are cited. (a) M. May and D. J. Triggle, *J. Pharm. Sci.*, **57**, 511 (1968). (b) D. R. Garrison, M. May, H. F. Ridley, and D. J. Triggle, *J. Med. Chem.*, **12**, 130 (1969). (c) H. F. Ridley, S. S. Chatterjee, J. F. Morna, and D. J. Triggle, *ibid.*, **12**, 931 (1969).

(**1**) and the keto derivative, muscarone (**2**). The subtle differences in structure are difficult to explain, for example, Waser[4b] has referred to the freedom of rotation of the trimethylammoniummethyl group of muscarine (**1**) and muscarone (**2**) in differentiating muscarinic and nicotinic action.



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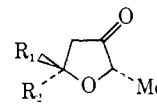
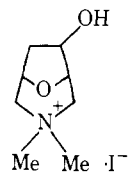


2a, L-(+)-muscarone, $R_1 = H$; $R_2 = N^+Me_3I^-$
b, 5R-allomuscarone, $R_1 = NMe_3^+I$; $R_2 = H$



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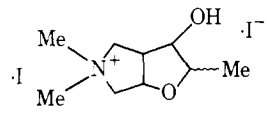

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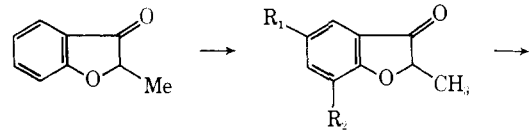
The unusual reversal of biological activity found in the muscarone analogs (**2a**) has prompted speculations on the nature of the ketone in linking to the receptor.[4,5] Analogy to ACh can be noted if the CO of muscarone is considered to bind at the same site as the AChCO, then the 3-atom chain, $CH_2C_5C_4$ of muscarone (**2a**) is comparable to the CH_2CH_2O chain spanning the quaternary N and the CO of ACh. Furthermore, C_4 of L-(+)-muscarone (**2**) and D(−)-(*R*)-acetyl-β-methylcholine have similar relative configurations and the same applies to the (5*R*)-allomuscarone (**2b**) and L-(+)-(*S*)-acetyl-β-methylcholine. Support for this analogy comes from the biological activity of **2a**, **2b**, and (+)- or (−)-acetyl-β- methylcholine which have pronounced muscarinic and nicotinic effects.[4,5] However, the cholinesterase activity of these isomers varies, the L-(+)-(*S*)-acetyl-β-methylcholine is a fair substrate for the enzyme while the D-(−) isomer inhibits the enzyme. L-(+)- and D-(−)-muscarone and (±)-allomuscarone are potent muscarinic agents (~4–10 times ACh). The inhibitory activity against cholinesterase is weak; both (±)-allomuscarone and (±)-muscarone have similar potency ($K_i \sim 10^{-4}$ *M*) which indicates the 2-Me group neither enhances nor inhibits binding. These and other correlations suggest the $OC_3C_4C_5CH_2N^+Me_3$ fragment of muscarones is interacting with the muscarinic receptor and esterase site.

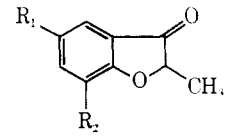
The synthesis of rigid analogs of muscarine has not been as successful as the synthesis of rigid analogs of ACh. Hardegger and Halder[6] reported the attempted synthesis of the bicyclic muscarine analogs **3** and **4** *via* an internal Mannich reaction on normuscarone. While neither bicyclic analog was obtained by this method, **4** was synthesized by an alternate route. In this work we wish to report the synthesis of *trans*-7-dimethylamino-3-hydroxy-2-methyl-2,3-dihydrobenzofuran methiodide (**11d**, benzomuscarine), the cis isomer **11c** (benzoepimuscarine) described earlier,[7a] and the keto derivative **6b** (benzomuscarone). In addition, the analogous 5 isomers **11a**, **11b**, and **6a** were also prepared.

Nitration of 2-methylcoumaran-3-one to give 5- and 7-nitro-2-methylcoumaran-3-one (**5a** and **5b**) has been described earlier.[7] Reductive alkylation of **5a** using excess CH_2O yielded the aldol addition product **7**.

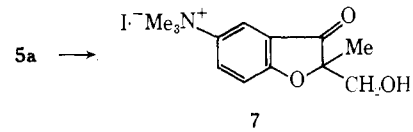
SCHEME I



5a, $R_1 = NO_2$; $R_2 = H$
b, $R_1 = H$; $R_2 = NO_2$



6a, $R_1 = \overset{+}{N}Me_3I^-$; $R_2 = H$
b, $R_1 = H$; $R_2 = \overset{+}{N}Me_3I^-$



7

However, if only 2 equiv of CH_2O were used, rather than the large excess, the condensation at C-2 could be prevented. Another problem which arose in the synthesis of **6** was the instability of the intermediate dimethylamino ketones; for this reason the reduction was performed in PhH. After 5 equiv of H_2 had been absorbed, the reaction mixture was filtered, the filtrate was dried, and an excess of MeI was added to give the methiodide salts of 5-dimethylamino-2-methylcoumaran-3-one (**6a**) and the 7 isomer (**6b**, benzomuscarone). The ppt which formed in the preparation of **6b** was difficult to dry completely. Attempts to recrystallize by the usual manner resulted in decomposition. An anal. sample was obtained by dissolving the ppt in MeOH at 25° and slowly adding Et_2O to give crys **6b**.

The synthesis of the isomeric amino alcohols (**11**, Scheme II) required a sequence that would give a reasonable means of separating the cis and trans isomers. As described earlier[7] the isomers of series **8** were prepared by reduction of the ketone, acetylation of the resulting alcohol, and separation of the isomers to give **8a–d**. Conversion into the respective dimethylaminoacetates **9a–d** was accomplished by reductive alkylation,[8] however, large amounts of catalyst were used to avoid long reaction times since the slow reduction of **8b** using a limited amount of catalyst gave a significant amount of the elimination product, 5-dimethylaminobenzofuran. Alkaline hydrolyses of the series **9a–d** to the alcohols **10a–d** was followed by treatment with MeI to give **11a–d**. The physical constants of **9a–d**, **10a–d**, and **11a–d** are reported in Table I.

The nmr spectra of the intermediates **9** and **10** showed the same characteristics as those of the nitro

(4) (a) For references see A. H. Beckett, N. J. Harper, and J. W. Clitherow, *J. Pharm. Pharmacol.*, **15**, 362 (1963). (b) P. G. Waser, *Pharmacol. Rev.*, **13**, 465 (1961).

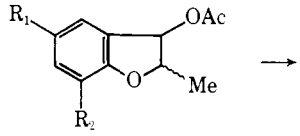
(5) A. H. Beckett, *Ann. N. Y. Acad. Sci.*, **144**, 675 (1967).

(6) E. Hardegger and N. Halder, *Helv. Chim. Acta.*, **50**, 1275 (1967).

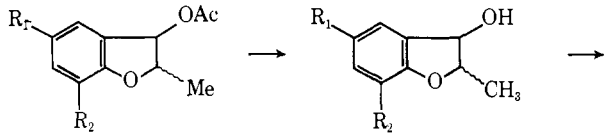
(7) (a) M. P. Mertes, Larry J. Powers, and Eli Shefter, *J. Org. Chem.*, in press. (b) L. J. Powers and M. P. Mertes, *J. Med. Chem.*, **13**, 1102 (1970).

(8) M. J. Martell, Jr., and J. H. Boothe, *ibid.*, **10**, 44 (1967).

SCHEME II

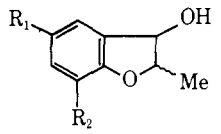


8a, *cis*-$R_1 = NO_2$; $R_2 = H$
b, *trans*-$R_1 = NO_2$; $R_2 = H$
c, *cis*-$R_1 = H$; $R_2 = NO_2$
d, *trans*-$R_1 = H$; $R_2 = NO_2$



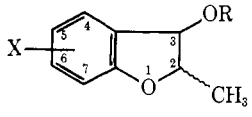
9a, *cis*-$R_1 = NMe_2$; $R_2 = H$
b, *trans*-$R_1 = NMe_2$; $R_2 = H$
c, *cis*-$R_1 = H$; $R_2 = NMe_2$
d, *trans*-$R_1 = H$; $R_2 = NMe_2$

10a, *cis*-$R_1 = NMe_2$; $R_2 = H$
b, *trans*-$R_1 = NMe_2$; $R_2 = H$
c, *cis*-$R_1 = H$; $R_2 = NMe_2$
d, *trans*-$R_1 = H$; $R_2 = NMe_2$



11a, *cis*-$R_1 = NMe_3I$; $R_2 = H$
b, *trans*-$R_1 = NMe_3I$; $R_2 = H$
c, *cis*-$R_1 = H$; $R_2 = NMe_3I$
d, *trans*-$R_1 = H$; $R_2 = NMe_3I$

TABLE I
PHYSICAL CONSTANTS OF SUBSTITUTED 2-METHYLDIHYDROBENZOFURANS



Compd	2,3 Isomer	X	R	Formula[a]	Mp, °C
9a	Cis	5-NMe_2	Ac	$C_{13}H_{18}ClNO_3$	157–158
9b	Trans	5-NMe_2	Ac	$C_{13}H_{18}ClNO_3$	144–145
9c	Cis	7-NMe_2	Ac	$C_{13}H_{18}ClNO_3$	144.5–146.5
9d	Trans	7-NMe_2	Ac	$C_{13}H_{18}ClNO_3$	132.5–133
10a	Cis	5-NMe_2	H	$C_{11}H_{15}NO_2$	90–91
10b	Trans	5-NMe_2	H	$C_{11}H_{16}ClNO_2$	144–145
10c	Cis	7-NMe_2	H	$C_{11}H_{16}ClNO_2$	142–143
10d	Trans	7-NMe_2	H	$C_{11}H_{16}ClNO_2$	129–130
11a	Cis	5-NMe_3I	H	$C_{12}H_{18}INO_2$	190.5–192
11b	Trans	5-NMe_3I	H	$C_{12}H_{18}INO_2$	171.5–172.5
11c	Cis	7-NMe_3I	H	$C_{12}H_{18}INO_2$	191–192
11d	Trans	7-NMe_3I	H	$C_{12}H_{18}INO_2$	182–183

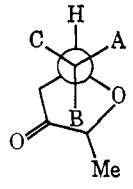
[a] Analysis for C, H, and N was found to be within ±0.4% of theoretical.

acetates **8** with regard to the protons at C-2 and C-3.[7] The coupling constants were always in the order $J_{cis\text{-}2,3} > J_{trans\text{-}2,3}$, and the C-3 proton of the cis isomers was always deshielded more than the C-3 proton of the trans isomers. The coupling constants were approximately 2 Hz in the trans isomers and 6 Hz in the cis isomers throughout the series.

Biological Results.—The muscarinic activity was tested on the guinea pig ileum by the cumulative dose–response method using ACh·Cl^- as the reference. Nicotinic activity was examined on the frog rectus abdominis muscle and the chicken biventer cervicis muscle and compared with nicotine. Toxicity in mice was examined by ip injection and results are reported as the orientation LD_{50}. The effects on cholinesterase were measured in several systems. Rat serum and the purified enzyme from horse serum (Type IV, Sigma) were the systems used to estimate inhibition results against pseudo- or butyrylcholinesterase. Erythrocyte preparations and the purified enzyme from the electric eel (Type III, Sigma) were used to estimate inhibition of "true" AChE. Activity was measured by the manometric method in rat serum and erythrocytes.

The results in Table II show that the 7-substituted compounds **11c**, **11d**, and **6b**, structurally related to the muscarine–muscarone series had little if any cholinergic action. However, the 5-substituted series did have both muscarinic and nicotinic like effects. The ketone **6a** was a muscarinic (1/100 ACh) and nicotinic agent (1/20 nicotine). The 5-substituted cis alcohol **11a** was a weak antagonist at 3×10^{-4} *M* and also had weak nicotinic effects (1/100 nicotine). The trans analog **11b** had only slight nicotinic activity (1/200 nicotine).

The absence of muscarinic activity in the 7-substituted compounds was unexpected when the high activity of (±)-4,5-dehydromuscarone is considered. As stated in the introduction one of the purposes of this research was to define the position of the quaternary N at the muscarinic receptor. The absence of activity could be construed as evidence that in the "receptor active form" the N of muscarine analogs is not in region A in projection **12** which views (+)-muscarone along



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the axis of the C_5–CH_2 bond. However, other reasons such as interference by the Ph ring or a change in the nature of the quaternary N in going from trimethylalkyl to trimethylaryl substitution could also prevent receptor interaction.

Potent anticholinesterase activity was observed in both the 5- and 7-substituted ketones **6a** and **6b**. The 5 isomer **6a** was a strong competitive inhibitor of both Ac and butyrylcholinesterase, with $K_i \cong 3 \times 10^{-8}$ *M* for both purified enzyme preparations. The 7-substituted analog **6b** was competitive but less inhibitory against butylrylcholinesterase ($K_i = 2.5 \times 10^{-5}$ *M*) and AChE ($K_i = 3.8 \times 10^{-5}$ *M*).

The high anticholinesterase activity of **6a** was reflected in the toxicity studies where the animals died with symptoms of cholinesterase blockade. The relatively lower toxicity of **11a** can be attributed to the associated atropine-like action observed in the muscarinic assay.

Recently Chothia and Pauling[9] proposed an active conformation for ACh that describes the interaction with AChE. Analysis of the conformation of the

(9) C. Chothia and P. Pauling, *Nature (London)*, **223**, 919 (1969).

TABLE II
BIOLOGICAL RESULTS

Compd	Isomer	Muscarinic activity,[a] ACh = 1.0	Nicotinic activity,[b] nicotine = 1.0	Butyrylcholinesterase inhibition		AChE inhibition,		ip LD_{50}, mg/kg
				Rat serum	Horse serum,[c] K_i	erythrocytes	Eel,[c] K_i	
Ach					$K_m = 4 \times 10^{-4}$ *M*		$K_m = 1 \times 10^{-4}$ *M*	
11a	*cis*-5	Weak antagonist	0.01	Weak[d]		Weak[d]		200
11b	*trans*-5		0.005	Weak[d]		Weak[d]		50
6a	5	0.01	0.05	100% at 2×10^{-5} *M*	2.5×10^{-8} *M*	97% at 2×10^{-5} *M*	3.0×10^{-8} *M*	10
11c	*cis*-7			Weak[d]		Weak[d]		100
11d	*trans*-7			Weak[d]		Weak[d]		100
6b	7			50% at 1×10^{-4} *M*	2.5×10^{-5} *M*	80% inhib at 10^{-4} *M*	3.8×10^{-5} *M*	100

[a] Measured on the guinea pig ileum. [b] Measured on the frog rectus abdominis muscle and the chicken biventer cervicis muscle. [c] K_i as determined by the titrimetric technique plotting $1/s$ *vs.* $1/v$. (Correlation coefficient > 0.96). [d] Weak inhibition (10–20%) was noted at 10^{-3} *M*.

esterase substrates, L-(−)-(*S*) and D-(+)-(*R*)-acetyl-α-methylcholine and L-(+)-(*S*)-acetyl-β- methylcholine suggested the substrate model exists in an antiplanar $NC_4C_5O_1$ (**13**, τ +150°) and antiplanar $C_4C_5O_1C_6$ structure (**14**, τ 180°). The authors noted that the con-

13, $\tau NC_4C_5O_1$ +150° **14**, $\tau C_4C_5O_1C_6$ +180°

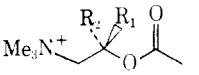
figuration of D-(+)-(*R*)-acetyl-β-methylcholine would prevent adoption of the τ of ≃150° for NC_4C_5O due to the steric hinderance in the partial eclipse of the β-Me group and N. If it can be assumed that the binding of inhibitors to the esterase requires the similar complimentation of the receptor site then D-(−)-(*R*)acetyl-β-methylcholine should have low affinity for the esterase site and should not be a substrate or an inhibitor of the enzyme. However, the D-(−)-(*R*)-β-Me isomer is known to inhibit the enzyme; the weak inhibition noted could be due to nonspecific binding, perhaps through the quaternary N since the alcohol choline also is a weak inhibitor ($K_i = 4 \times 10^{-4}$ *M*)[10] as are a variety of quaternary ammonium compounds such as phenyltrimethylammonium iodide ($K_i \sim 10^{-4}$ *M*).[11,12]

The strong inhibition of AChE by the 5-substituted ketone **6a** is presumed to be due to binding at CO and N. The inactivity of both the 5-substituted cis and trans alcohols (**11a** and **11b**) suggests that the ether O contributes little to binding. Furthermore, since the OH analogs are inactive whereas the ketone is strongly bound it is postulated that the binding to the enzyme is through an attraction of a nucleophilic site (imidazole or serine OH) of the enzyme for the electrophilic CO.[13] These modes of binding exclude H bonding with the CO of **6a** as the donor mainly because the slight difference in energy gained from bonding to a CO compared to a disubstituted (OH) O in **11a** or **11b** (about 1 kcal/mole) could not account for the vast difference in biological activity.[14]

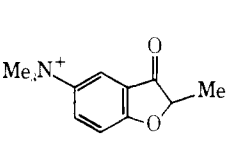
The planar structure of the active inhibitor **6a** can be accommodated in the model proposed by Chothia and Pauling[9] for the substrate of the esterase with a minor change. The optimal $NC_4C_5O_2$ torsional angle is proposed to be 150° whereas the analogous atoms $NC_5C_4C_{3a}$ in **6a** are antiplanar (180°).

Examination of models shows the 7-substituted ketone **6b** to have the same distance between the CO and the N as found in the most active isomer **6a**. This isomer (**6b**) was 1000-fold less active in inhibition of the esterase. Examination of this model reveals two obvious factors that may account for the difference. If the atoms analogous to the substrate ACh are examined, the $NC_7C_{7a}C_{3a}$ torsional angle is 180° and $C_7C_{7a}C_{3a}C_3$ is also 180°, a fully extended model between N and CO as seen in **6b** and the proposed ACh model. However, further study of the models reveals differences that can be related to the acetyl-β-methylcholines. If ACh interacts with the esterase in the extended form then the models can be oriented as shown in Scheme III. Both isomers of acetyl-α-methylcholine are

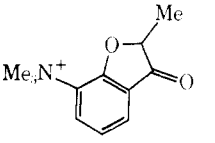
SCHEME III



15a, $R_1 = H$; $R_2 = Me$
b, $R_1 = Me$; $R_2 = H$



6a



6b

readily hydrolyzed by the enzyme. However, of the β-Me analogs, only the L-(+)-(*S*) isomer **15a** is attacked by the esterase and it is hydrolyzed at about half the rate of ACh; the D-(−)-(*R*) isomer **15b** is not hydrolyzed but inhibits the enzyme. The 7-substituted isomer **6b** has the ether O oriented in analogy to the Me group of acetyl-β-methylcholines. This compound should be

(10) (a) H. D. Baldridge, W. J. McCarville, and S. L. Friess, *J. Amer. Chem. Soc.*, **77**, 739 (1955). (b) S. L. Friess and H. D. Baldridge, *ibid.*, **78**, 2482 (1956).

(11) (a) F. Bergmann and R. Segal, *Biochem. J.*, **58**, 692 (1954). (b) R. M. Krupka, *Biochemistry*, **4**, 429 (1965).

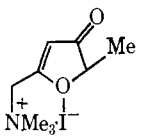
(12) J. P. Long in "Handbuch Der Experimentellea Pharmacologie," D. Eichler and A. Farah, Ed., G. B. Koelle, subeditor, Vol. 15, Springer-Verlag, Berlin, 1963, p 374.

(13) R. M. Krupka and K. J. Laidler, *J. Amer. Chem. Soc.*, **83**, 1458 (1961).

(14) (a) G. C. Pimentel and A. L. McClellan, "The Hydrogen Bond," W. H. Freeman & Co., San Francisco, Calif., 1960, pp 82–86. (b) K. F. Purcell, J. A. Stikeleather, and S. D. Brunk, *J. Amer. Chem. Soc.*, **91**, 4019 (1969).

less active because of a similar steric effect due to the additional substituent (O) on the central C between CO and N.

The fully extended antiplanar model for binding to the esterase requires 2 sites of binding, a Me_3N^+ group and CO. The low esterase inhibition by muscarine derivatives reported by Witkop and coworkers[15] and the dioxolanes ($K_i \sim 10^{-5}$ *M*) reported by Belleau and Lacasse[16] could be due to the inability to assume the fully antiplanar structure. The weak inhibition noted in these analogs suggests limited affinity for the esteratic site, perhaps at the quaternary nitrogen only. The poor affinity of the keto analogs of muscarine for the esterase[5] can be explained in terms of the fully extended antiplanar model wherein the required structure cannot be assumed in muscarones. The required planar structure is possible in (±)-dehydromuscarone (**16**) but re-



16

quires the improbable eclipsing of the quaternary ammonium group and the ether oxygen. The proposed model for esterase activity accommodates the classical carbamate cholinesterase inhibitors such as carbachol and neostigmine.[12]

Experimental Section[17]

5-Trimethylammonium-2-methylcoumaran-3-one Iodide (6a).—5-Nitro-2-methylcoumaran-3-one[7] (**5a**, 479 mg, 3 mmoles) was added to a suspension of 10% Pd/C (0.5 g) in dry C_6H_6 (40 ml). The suspension was stirred under H_2 until 220 ml of H_2 had been adsorbed. CH_2O (0.55 ml of a 37% H_2CO soln, 6 mmoles) was added. When an additional 150 ml of H_2 had been absorbed, the reaction mixt was filtered, and the filtrate was dried ($MgSO_4$) and concd to *ca.* 40 ml. MeI (5 ml) was added, and the soln was allowed to stand in a low actinic flask at 25° for 48 hr. The ppt (600 mg, 61%) was sepd and dried to give pure **6a**: mp 182–184 dec, ir (KBr) 1720 cm^{-1} (C=O); nmr (DMSO-d_6) δ 7.52 (q, 1, *J* = 9 Hz, aromatic H-7), and 8.2 (m, 2, aromatic H-4 and H-6), the remainder of the spectrum was as expected. *Anal.* ($C_{12}H_{16}INO_2$) C, H, N.

7-Trimethylammonium-2-methylcoumaran-3-one Iodide (6b).—7-Nitro-2-methylcoumaran-3-one[7] (**5b**, 479 mg, 3 mmoles) was reduced and alkylated in the same manner as **5a** in the synthesis of **6a**. The ppt from the MeI–C_6H_6 soln (400 mg, 40%) was sepd and dried to give **6b** as a light yellow powder. An aliquot of this material was dissolved in MeOH and Et_2O was added slowly to give pure **6b** as light yellow crystals: mp 163–164°; ir (KBr) 1725 cm^{-1} (C=O); nmr (DMSO-d_6) δ 7.2–7.5 (m, aromatic H-5 and C_6H_6), 7.87 (q, 1, *J* = 8 and 1.5 hz, aromatic H-4 or H-6), and 8.20 (q, 1, *J* = 8 and 1.5 Hz, aromatic H-4 or H-6), the remainder was as expected. *Anal.* ($C_{12}H_{16}INO_2$) C, H, N.

5-Trimethylammonium-2-hydroxymethyl-2-methylcoumaran-3-one Iodide (7).—5-Nitro-2-methylcoumaran-3-one[7] (**5a**, 0.5 g, 2.58 mmoles) in MeOH (10 ml) was added to a suspension of 10% Pd/C (0.5 g) and H_2CO (5 ml of a 37% solution) in MeOH (60 ml). The reaction mixt was stirred under 1 atm of H_2 until 318 ml of H_2 had been absorbed (3 hr). The reaction mixt was filtered, concd to *ca.* 20 ml, and poured into $CHCl_3$ (100 ml). The soln was washed with 5% $NaHCO_3$ and dried ($MgSO_4$), and the solvent was removed. The residual oil was dissolved in abs EtOH, and MeI (0.5 ml) was added. The mixt was refluxed for 30 min and filtered. The crystals (0.45 g, 48%) which sepd from the filtrate were pure: mp 209–211°; ir (KBr)3300 (OH),1725cm^{-1} (C=O); nmr (D_2O) as expected. *Anal.* ($C_{13}H_{18}INO_3$) C, H, N.

trans-**5-Dimethylamino-3-acetoxy-2-methyl-2,3-dihydrobenzofuran (9b).**—The nitro acetate **8b** (1.5 g, 6.3 mmoles) in a minimum amount (20 ml) of 2-methoxyethanol was added to a suspension of 10% Pd/C (1.0 g) and CH_2O (9 ml of a 37% aq soln) in MeOH (50 ml). Th reaction mixt was stirred at 25° under H_2 (1 atm) until 774 ml (31.5 mmoles) had been absorbed (3–9 hr). The reaction mixt was filtered, concd to *ca.* 30 ml, and poured into $CHCl_3$ (100 ml). The $CHCl_3$ soln was washed with 5% $NaHCO_3$ (3 × 40 ml) and dried ($MgSO_4$), and the solvent was removed to give a yellow residual oil. An aliquot (200 mg) of the oil was chromatographed on a preparative tlc plate (10% EtOAc–Skelly B). The major band (uv visualization, R_f *ca* 0.4–0.5) was removed and extd with Et_2O. The solvent was evapd, and the residual oil was dissolved in anhyd Et_2O and dried ($MgSO_4$). Dry HCl was passed into the soln to give a gum which solidified on standing. The solid was recrystd from Me_2CO–EtOAc: mp 144–145°. *Anal.* ($C_{13}H_{18}ClNO_3$), C, H, N.

trans-**5-Dimethylamino-3-hydroxy-2-methyl-2,3-dihydrobenzofuran Methiodide (11b).**—The amino acetate **9b** (1.5 g, 6.3 mmoles) was dissolved in MeOH (20 ml), and NH_4OH (10 ml) was added. The reaction mixt was stirred at 55° for 2 hr, cooled, and poured into H_2O (50 ml). The soln was extd with $CHCl_3$ (3 × 50 ml) and the combined exts were dried ($MgSO_4$). Evapn of the solvent gave a dark residual oil which was chromatographed over 100 g of Woelm Al_2O_3 (activity grade I, neutral, 0.5% MeOH—C_6H_6). The fractions contg the product (on the basis of tlc) were combined, and the solvent was removed to give a light yellow oil. The residue was converted into the HCl salt in Et_2O, and the salt of **10b** was recrystd from Me_2CO—EtOAc: mp 144–145°. *Anal.* ($C_{11}H_{16}ClNO_2$) C, H, N.

An additional product which was isolated from the synthesis of **10b** was identified as 5-dimethylamino-2-methylbenzofuran. It was converted into the HCl salt in Et_2O: mp 196–197°; nmr (free base, $CDCl_3$) as expected. *Anal.* ($C_{11}H_{14}ClNO$) C, H, N.

The dimethylamino alcohol **10b** (0.5–1.0 g) was dissolved in abs EtOH (5–10 ml), and CH_3I (0.5–1.0 ml) was added. The soln was refluxed for 30 min and then allowed to cool to room temp. The salt **11b** was collected and dried: mp 171.5–172.5°; nmr (D_2O) δ 7.07 (d, 1, *J* = 8 Hz, aromatic H-7), 7.6–8.0 (m, 2, aromatic H-4 and H-6), the remainder was as expected. *Anal.* ($C_{12}H_{18}INO_2$) C, H, N.

Cholinesterase Assays.—Electric eel Type III cholinesterase and horse serum Type IV cholinesterase (Sigma) were assayed by the standard titrimetric method[18] using a Radiometer pH Stat. The recorded titration was run in a constant temp (25°), stirred, anaerobic assay cell excluding CO_2. The assay soln contg either the horse serum enzyme (2.23 mg) or the eel enzyme (0.67 mg) in 10 ml of 0.1 *M* $MgCl_2$, 0.01 *M* NaCl, and inhibitor was adjusted to pH 7.2 and treated with concns of $ACh.Cl^-$ ranging from 0.025 to 10 μmoles/ml. The consumption of 0.01 *N* NaOH to maintain pH 7.2 was recorded against time and the data analyzed using plots of $1/v$ *vs.* $1/s$, s/v *vs.* s, and v *vs.* v/s. The K_m for acetylcholine in the horse serum enzyme was 4×10^{-4} *M* and the eel gave $K_m = 1 \times 10^{-4}$.

Acknowledgment.—The authors thank Dr. William Stephens and Mr. Cary Self for the data on the purified enzyme studies, and Professor J. Doul and Mr. D. Friesen for their assistance.

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(17) All melting points were determined on a Thomas-Hoover capillary melting point apparatus and are corrected. Elemental analyses were carried out either by Midwest Microlab, Inc., Indianapolis, Ind., or by Mrs. H. Kristiansen at the University of Kansas. Where analyses are indicated only by symbols of the elements analytical results obtained for those elements were within ±0.4% of the theoretical values. Spectra were recorded on a Beckman IR-10 or a Varian A-60A.

(18) D. Glick, *Biochem. J.*, **31**, 521 (1937).