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Structure-Activity Studies of Carbamate and Other Esters: Agonists and Antagonists to Nicotine

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ABOOD, L. G., K. SHAHID SALLES AND A. MAITI. *Structure-activity studies of carbamate and other esters: Agonists and antagonists to nicotine*. PHARMACOL BIOCHEM BEHAV 30(2) 403–408, 1988.—A number of aromatic, cycloalkyl, and heterocyclic carbamic acid esters, thiocarbamic acid esters, and carboxylic acid esters of di- and trialkylaminoalkyl and heterocyclic amino alcohols have been synthesized and tested for their pharmacologic and receptor binding characteristics at the nicotine receptor. Receptor binding was measured in rat brain membranes using (−)-^{3}H-nicotine or ^{3}H-methylcarbamylcholine as radioligands. The compounds were tested for their ability to produce seizures and prostration and to antagonize the nicotine-induced prostration and seizures as well as the hypertensive action of nicotine in rats. Among the potent agonists were the N-methylcarbamyl and N-methylthiocarbamyl esters of choline (trimethylaminoethanol), with the tertiary amino derivatives between considerably less potent than the quaternary. Potent antagonists included trimethylaminoethyl benzoate, 3-quinuclidinyl benzoate, and trimethylaminoethyl esters of phenyl and phenylthiocarbamic acids. One of the most potent antagonists to nicotine was α-lobeline.

Nicotine agonists Nicotine antagonists Carbamate esters ^{3}H-Nicotine binding

IN the course of investigating the structure-activity relationships of ^{3}H-nicotine binding sites to rat brain membranes [1,2], it was noted that a number of carbamate esters of choline exhibited a high affinity for such sites [3]. The most potent of this series was methylcarbamylcholine, and ^{3}H-methylcarbamylcholine (^{3}H-MCC) was found comparable to ^{3}H-nicotine in its binding characteristics and pharmacologic profile [3]. Of particular interest was the observation that an N-methyl substituent on carbamylchloride increased the affinity for the ^{3}H-nicotine binding site over 10-fold, while abolishing affinity for the muscarinic cholinergic site [3]. In the light of this observation, a study was undertaken to examine the effect of other N-substituted carbamates and related compounds for their binding and pharmacologic properties. The present study describes the synthesis and pharmacology of a number of carbamates, cycloalkyl, and aryl esters of choline and other aminoalkyl alcohols with high affinity for the nicotinic binding site and which are effective antagonists to the psychotropic and other pharmacologic effects of nicotine.

METHOD

Measurement of ^{3}H-Nicotine and ^{3}H-MCC Binding

The procedure for preparation of rat brain membranes and for measuring specific ^{3}H-nicotine and ^{3}H-MCC binding is described elsewhere [1]. Membranes were obtained from whole rat brain after homogenization in 30 volumes of 0.05 M $NaPO_4$, pH 7.5, and centrifugation at 50,000 × g for 30 min. To a 2 ml polypropylene tube was added 2 mg membrane protein along with 1×10^{-9} M ^{3}H-MCC (specific activity=80 Ci/mmole) or (−)-^{3}H-nicotine (New England Nuclear, specific activity=75 Ci/mmole) with or without various concentrations of unlabeled nicotine, nicotine analogues, carbamate esters, and other agents, in a final volume of 1.2 ml 0.05 M $NaPO_4$ buffer containing 0.1 M NaCl at pH 7.5. All assays were performed in triplicate. After incubating in an ice bath (0–4°) for 30 min, the tubes were centrifuged in an Eppendorf centrifuge for 2 min and the pellet washed twice by filling the tubes with buffer and aspirating. The bottom of the tubes was then cut off (animal nail clipper) and counted by liquid scintillation.

Pharmacologic Measurements

The psychotropic action of the agents was determined by assessing their ability, when administered into the fourth ventricle (ICV), to produce prostration (agonists) or prevent the nicotine-induced prostration (antagonists) by a procedure described elsewhere [1]. The agents were administered into cannulae chronically implanted into the lateral ventricles of Sprague-Dawley male rats (200–250 g), in 1 μl volumes. A typical prostration response, occurring within 1–3 sec following the injection of 2 nmoles of nicotine, generally involved all four limbs with body and neck muscles. Antagonism was determined by administering the test agent 1 min prior to giving 2 nmoles of nicotine. Antagonism was also

TABLE 1

^{3}H-NICOTINE AND ^{3}H-MCC BINDING OF VARIOUS AGENTS AND THEIR ABILITY TO PRODUCE PROSTRATION

	^{3}H-Nicotine Binding IC_{50} M	^{3}H-MCC Binding IC_{50} M	Prostration ED_{50} nmoles
s-(−)-nicotine	2×10^{-9}	8×10^{-9}	2
N′-methyl nicotinium	7×10^{-6}	4×10^{-6}	100
N′ethyl nornicotine	3×10^{-8}	1×10^{-7}	20
N′-propyl nornicotine	6×10^{-7}	1×10^{-6}	80
DMAE carbamate	1×10^{-5}	1×10^{-5}	400
TMAE carbamate	4×10^{-7}	1×10^{-6}	100
DMAE methylcarbamate	5×10^{-7}	8×10^{-7}	300
TMAE methylcarbamate	8×10^{-9}	6×10^{-9}	10
DMAP methylcarbamate	2×10^{-7}	5×10^{-8}	100
DMAE acetate	5×10^{-5}	5×10^{-5}	>1000
TMAE acetate (acetylcholine)	6×10^{-5}	5×10^{-6}	300
DMAE succinate	5×10^{-5}	6×10^{-5}	1000
TMAE succinate butyrylthiocholine	8×10^{-7}	7×10^{-7}	50
cytisine	6×10^{-9}	1×10^{-10}	2

DMAE = 2-dimethylaminoethyl; TMAE = 2-trimethylaminoethyl; DMAP = 3-dimethylaminopropyl.

determined by measuring an agent's ability to prevent nicotine-induced seizures. The test agent was administered intraperitoneally (IP) 7 min prior to the administration of 1.5 mg/kg nicotine IP. A third method was to determine a test agent's ability to prevent lethality produced by 3 mg/kg nicotine IP.

Arterial blood pressure was determined by means of a Gould Statham P23ID pressure transducer attached to the right femoral artery of rats anesthetized with 60 mg/kg sodium pentothal IP and connected to a polygraph. Various doses of test agent were administered into the left femoral vein (cannulated) 1 min prior to the administration of a dose of nicotine (0.05 mg/kg) which resulted in a 42±20 mmHg elevation of systolic blood pressure. Before a new drug was injected, the previous injection was flushed out with 0.3 ml of isotonic saline followed by a control injection of saline. The mean dose of a test agent necessary to completely block the hypertensive action of nicotine was determined. In a typical experiment 5 injections of nicotine and the test drug was administered over a period of 90 minutes.

RESULTS

Comparison of Various Ester Agonists With ^{3}H-Nicotine and ^{3}H-MCC Binding and Their Psychotropic Potency

The various esters were compared for their ability to compete with ^{3}H-nicotine and ^{3}H-MCC binding to rat brain membranes, utilizing a concentration of 1×10^{-9} M of each radioligand, which yielded an IC_{50} value for nicotine of 2×10^{-9} M and 6×10^{-9} M for ^{3}H-MCC (Table 1). TMAE carbamate (carbamylcholine) had an IC_{50} of 4×10^{-7} M and 1×10^{-6} for ^{3}H-nicotine and ^{3}H-MCC, respectively, whereas the corresponding DMAE derivative had a value of 1×10^{-5} M with either radioligand. The most potent agent was TMAE methylcarbamate (methylcarbamylcholine) with an IC_{50} value of 8×10^{-9} M and 6×10^{-9}, compared to a value of 5×10^{-7} M and 8×10^{-7} for the two radioligands, respectively, for the DMAE analogue. No difference was noted in the IC_{50} values between TMAE acetate (acetylcholine), TMAE succinate (succinylcholine), and their corresponding DMAE analogues, all having values of about 5×10^{-5} M with either radioligand. The one exception was acetylcholine which had a 10-fold greater affinity with ^{3}H-MCC than with ^{3}H-nicotine.

The pyschotropic potency of the various agents administered into the fourth ventricle was assessed by means of the prostration syndrome [1]. When 2 nmoles of nicotine (1 μl) was administered ICV, there immediately ensued a prostration involving all four limbs and lasting 2–4 min (Table 1). At 50 times this dose, carbamylcholine produced weakness in all limbs and head muscles, while DMAE carbamate had a similar effect at 100 times the dose of nicotine. The most potent of the esters was TMAE methylcarbamatecholine which produced a prostration lasting over 20 min; but unlike the response to nicotine, the rats seemed somewhat paralyzed and unable to move their heads or limbs. At a dose of 500 nmoles, DMAE carbamate produced moderate weakness in the hindlimbs and head. With the exception of acetylcholine, which had an ED_{50} of 300 nmols, the remainder of the compounds tested were either inactive or had only a slight effect. All of the active compounds including nicotine produced deep diaphragmatic breathing and a decreased respiratory rate.

Comparison of Various Ester Antagonists for Binding and Blockade of Prostration

Among the various esters, TMAE benzoate was the most effective compound in blocking nicotine-induced prostration

TABLE 2

^{3}H-NICOTINE AND ^{3}H-MCC BINDING OF VARIOUS AGENTS AND THEIR EFFICACY IN BLOCKING NICOTINE-INDUCED PROSTRATION

	^{3}H-Nicotine Binding IC_{50} M	^{3}H-MCC Binding IC_{50} M	Blockade of Prostration ED_{50} nmoles
DMAE benzoate	1×10^{-6}		100
TMAE benzoate	8×10^{-8}	4×10^{-8}	10
DMAE cyclohexyl-carboxylate	2×10^{-6}	4×10^{-6}	100
TMAE cyclohexyl-carboxylate	8×10^{-8}	5×10^{-8}	10
DMAE phenyl-carbamate	1×10^{-4}	1×10^{-4}	IA
TMAE phenyl-carbamate	6×10^{-6}	8×10^{-7}	200
DMAE phenyl-thiocarbamate	7×10^{-5}	2×10^{-5}	
TMAE phenyl-thiocarbamate	5×10^{-7}	5×10^{-7}	50
DMAE nicotinate	2×10^{-7}	2×10^{-7}	50
TMAE nicotinate			
3-Quinuclidinyl benzoate	7×10^{-6}	6×10^{-6}	200
3-Quinuclidinyl methylcarbamate	3×10^{-6}	2×10^{-6}	100
DMAE phenylacetate	7×10^{-5}	7×10^{-6}	
TMAE phenylacetate	2×10^{-6}	3×10^{-7}	100
DMAE napththoate	8×10^{-5}	7×10^{-5}	
TMAE napththoate	9×10^{-6}	5×10^{-6}	
N-methyl-3-piperdyl-benzoate	3×10^{-5}	2×10^{-5}	300
N-benzylpiperidyl methylcarbamate	IA	IA	
N-benzyl-4-piperdyl methylcarbamate	IA	IA	IA
N-methyl-3-piperidyl-diphenylacetate	IA	IA	IA
atropine	IA	IA	IA
N-methyltetra-hydropapavarine	7×10^{-5}	8×10^{-5}	
9-aminotetra-hydroacridine	2×10^{-5}	1×10^{-5}	200
procaine	7×10^{-5}	3×10^{-5}	
cocaine	$>1\times10^{-4}$	$>1\times10^{-4}$	IA
α-lobeline	5×10^{-9}	7×10^{-10}	10
lobelanine	1×10^{-8}	5×10^{-9}	25
lobelanidine	2×10^{-8}	5×10^{-9}	25

DMAE = 2-dimethylaminoethyl; TMAE = 3-trimethylaminoethyl; IA = inactive; IC_{50} = concentration inhibiting binding 50%; ED_{50} = dose in nmoles producing 50% efficacy.

when both compounds were administered into the lateral ventricles of rats; next in effectiveness and of comparable activity were TMAE phenylcarbamate, TMAE phenylthiocarbamate, TMAE phenylacetate, and DMAE nicotinate (Table 2). The quaternary esters were generally 10-fold more potent than the tertiary analogues, both with respect to their binding affinity and psychotropic action. The most potent of the compounds tested was the natural alkaloid, α-lobeline, which exhibited an even greater affinity than (−)-nicotine for the receptor and blocked the nicotine-induced prostration with a dose of 1 nmoles ICV. Other agents exhibiting some binding affinity were procaine and 9-aminotetrahydroacridine. The other agents listed, including cocaine and atropine, were inactive.

TABLE 3

ANTAGONISM OF VARIOUS AGENTS TO NICOTINE-INDUCED SEIZURES IN RATS

Agent	Seizures	% Incidence of Seizures
Nicotine	10/10	100
+ 5 mg/kg α-lobeline	1/8	12
+ 25 mg/kg TMAE benzoate	1/8	12
+ 50 mg/kg DMAE benzoate	5/9	56
+ 50 mg/kg DMAE cyclohexyl-carboxylate	4/8	50
+ 25 mg/kg TMAE cyclohexyl-carboxylate	1/8	12
+ 25 mg/kg TMAE phenylcarbamate	1/9	11
+ 5 mg/kg 3-quinuclidinyl benzoate	1/7	14
+ 25 mg/kg atropine	6/6	100

The test agents were administered intraperitoneally 5 min prior to 1.5 mg/kg nicotine. Data present as ratio of number of rats responding over total.

TABLE 4

PROTECTION AGAINST NICOTINE MORTALITY IN RATS BY α-LOBELINE AND VARIOUS ESTERS

Agent	Mortality	% Mortality
(−)-nicotine 3 mg/kg	8/10	80
+ 5 mg/kg α-lobeline	2/10	20
+ 25 mg/kg TMAE benzoate	3/10	30
+ 25 mg/kg TMAE cyclohexyl-carbamate	3/9	33
+ 25 mg/kg TMAE phenylcarbamate	4/10	40
+ 25 mg/kg TMAE phenylthio-carbamate	3/10	30
+ 5 mg/kg 3-quinuclidinyl benzoate	4/10	50
+ 25 mg/kg atropine	8/10	100

Data are based on 10 rats given test agent followed by 3 mg/kg nicotine (neutralized with HCl) 5 min later; both agents given IP.

TABLE 5

ANTAGONISM OF HYPERTENSIVE ACTION OF NICOTINE BY VARIOUS AGENTS

Agent	Antagonistic Dose
α-lobeline	0.2 ± 0.05
TMAE benzoate	1.5 ± 0.3
DMAE benzoate	10.0 ± 25
TMAE phenylcarbamate	5.0 ± 1.0
TMAE cyclohexylcarboxylate	5.0 ± 1.5
TMAE phenylthiocarbamate	4.0 ± 0.8
3-quinuclidinyl benzoate	5.0 ± 1.2

The dose (±s.d.) refers to that needed to completely block a 42 ± 20 mmHg elevation in systolic blood pressure following the administration of 0.05 mg/kg nicotine IV. A total of 4 rats was used for each drug.

Antagonism of Various Esters to Nicotine-Induced Seizures

At a dose of 1.5 mg/kg nicotine intraperitoneally, rats exhibited prostration followed by tremors, fasciculations, cyanosis and labored breathing, and myoclonic seizures (Table 3). When the rats were given 20 mg/kg TMAE benzoate or TMAE cyclohexylcarboxylate 7 min prior to nicotine, there was a 78% blockade of seizure activity. At a dose of 25 mg/kg DMAE benzoate or DMAE cyclohexylcarboxylate, about half of the rats exhibited seizures. Both TMAE phenylcarbamate and 3-quinuclidinyl benzoate afforded almost complete protection. It should be noted that muscle weakness, some tremors, and a slower respiratory rate persisted in all animals despite the antagonist. At a dose of 5 mg/kg, α-lobeline afforded complete protection against seizures. Procaine and cocaine, which are local anesthetics, both effectively block nicotine-induced prostration. No significant behavioral effects were observed with any of the esters alone.

Protection by Various Agents Against Nicotine Mortality in Rats

At a dose of 5 mg/kg IP, α-lobeline resulted in a 75% reduction of the mortality produced by 3 mg/kg nicotine IP in rats. A dose of 25 mg/kg TMAE benzoate, TMAE cyclohexylcarboxylate. TMAE phenylcarbamate, TMAE phenylthiocarbamate, and 3-quinuclidinyl benzoate resulted in a 50–70% reduction in mortality (Table 4). Atropine was ineffective.

Antagonism of Hypertensive Action of Nicotine

The agents were tested for their ability to completely reverse a 25% increase in systolic blood pressure following a dose of 0.05 mg of nicotine given intravenously to anesthetized rats (Table 5). The most effective agent was α-lobeline at a dose of 0.2 mg/kg followed by TMAE benzoate and TMAE cyclohexylcarboxylate at 1.5 mg/kg. At higher doses, ranging from 5–10 mg/kg, all the other esters tested were also effective.

DISCUSSION

The present study has demonstrated that (1) a number of newly synthesized and other esters of aminoalkyl and aminocycloalkyl alcohols and aromatic acids, and (2) α-lobeline are effective antagonists to the psychotropic and

peripheral actions of nicotine and other nicotinic agonists. The most effective antagonist in blocking the prostration, seizures, and mortality following nicotine administration in rats was α-lobeline. Among the various esters of various alkylaminoalcohols, TMAE benzoate was the most effective antagonist. Both antagonists also exhibited a very high affinity for brain nicotine receptors using either (−)-^{3}H-nicotine or ^{3}H-MCC as ligands. When the phenyl group of TMAE benzoate was substituted with phenylalkyl, naphthoyl or phenylcarbamyl, the antagonistic potency was diminished. Replacement of the choline moiety by quinuclidinyl, piperidyl, or pyrrolidyl diminished antagonistic potency. It is of interest that although acetylcholine has a relatively low affinity for the nicotine receptor [1,2] as compared to TMAE methylcarbamate [3], TMAE benzoate is a more potent antagonist than TMAE phenylcarbamate to the pharmacologic actions of nicotine. It remains to be seen whether such differences can be attributed to differential affinity of the compounds for the multiple binding sites for nicotine in brain [3,13].

As described elsewhere [3] for a series of carbamyl and alkyl esters of tertiary amino alcohols serving as agonists to the nicotine receptor, quaternization of N resulted in about a 20-fold increase in receptor affinity and a marked increase in pharmacologic potency. A similar increase in receptor affinity and antagonist potency was observed with the present series of esters acting as antagonists.

A number of miscellaneous alkaloids and esters were found to be antagonists, including procaine, a local anesthetic, and 9-aminotetrahydroacridine, an anticholinesterase. Other local anesthetics, such as cocaine and lidocaine (not shown) and anticholinesterases, such as physostigmine and prostigmine (not shown) were inactive.

A comparison of the chemical structures of the nicotine-like compounds with the carbamates with respect to their action on prostration reveals significant differences in the two classes of compounds. Both nicotine and 3-dimethylaminopyridine, which are arylamines, are agonists; whereas aryl esters of amino alcohols, such as DMAE nicotinate and TMAE benzoate, are antagonistic to the nicotine-induced prostration; while alkyl esters of amino alcohols are agonists. Another striking difference is that within the series of arylamines related to nicotine, quarternization of the N′-nitrogen decreases binding affinity by 3 orders of magnitude and a virtual loss of psychotropic action, whereas in the series of alkyl esters quaternization of the amino alcohol results in about a 50-fold increase in affinity for the nicotine receptor and a corresponding increase in psychotropic potency.

Although α-lobeline has been reported to suppress the desire for tobacco [14], the pharmacologic relationship of this natural alkaloid to nicotine is obscure. Included among the pharmacologic effects of α-lobeline are its hypertensive action resulting from the stimulation of the carotid body [10], bronchio-construction [6], and bronchoarrythmia [11]. It has been suggested that the cardiovascular effects are both parasympathetic (muscarinic) and sympathetic [11]. It has been generally assumed that α-lobeline is a ganglionic stimulant resembling nicotine [15]. The present study would indicate that the alkaloid is also acting as an antagonist at nicotine receptors both centrally and peripherally. It would appear, therefore, that α-lobeline is acting as a mixed agonist-antagonist at the nicotinic receptor.

A number of di- and trialkyl N-aryl carbamates have been synthesized and shown to have local anesthetic [4,5], herbicidal [8], and anthelminthic [9] as well as antileukemic and hypotensive properties [7].

ADDENDUM

CHEMICAL SYNTHESIS OF COMPOUNDS

DMAP Methylcarbamate

To 0.05 moles of 2-dimethylaminopropanol in 100 ml of dry toluene was added 0.07 moles of methylisocyanate, and the mixture was refluxed for 16 hr. After removal of the solvent in vacuo, the viscous liquid was taken up in 25 ml of $CHCl_3$ and extracted with H_2O. Upon removal of the $CHCl_3$, a white oily product was obtained and the final product was recovered after distillation in vacuo at 5 mm and a temperature of 84°C. The yield was 60%.

Analytic: i.r.: 2990, 1730, 1560
m.s.: 160, 91, 70, 58

3-Quinuclidinyl Methylcarbamate

To 0.05 moles of 3-quinuclidinyl dissolved in 100 ml of dry toluene was added 0.07 moles of methylisocyanate and the mixture was refluxed for 8 hr. After removal of the solvent in vacuo, the mixture was taken up in 50 ml of H_2O and extracted twice with H_2O. After removal by $CHCl_3$, the yellow, oily liquid was distilled in vacuo at 5 mmHg and 130°C. The yield was 60%.

Analytic: i.r.: 2985, 1730, 1530, 1265
m.s.: 184, 156, 58

DMAE and TMAE Phenylcarbamate

To 0.05 moles of dry dimethylaminoethanol in 100 ml of dry toluene was added 0.07 moles of phenylisocyanate, and the mixture was refluxed for 16 hr. After removal of the solvent in vacuo, the viscous liquid was taken up in 25 ml of $CHCl_3$ and extracted with H_2O. Upon removal of the $CHCl_3$, a white, oily product was obtained and the final product was recovered after distillation in vacuo at 5 mm at 145°C. The yield was 65%.

Analytic: i.r.: 1730, 1600
m.s.: 212, 164, 119

TMAE phenylcarbamate was prepared by adding 0.013 moles of methyl iodide to 0.010 moles of DMAE phenylcarbamate in 50 ml of acetone and allowing the reaction to proceed overnight at room temperature. The white crystalline material was filtered, washed twice with 50 ml of ethyl ether and dried. The yield was 90%.

DMAE and TMAE Phenylthiocarbamate

Same conditions as for DMAE and TMAE phenylcarbamate except that reflux was 8 hr. DMAE phenylthiocarba-

mate was recovered by distillation in vacuo at 5 mm and at 117°C. The yield was 58%.

Analytic: i.r.: 2990, 1725, 1550, 1225
m.s.: 219, 194, 93

DMAE and TMAE Naphthoate

To 0.05 mole of dry dimethylaminoethanol in 100 ml of dry methylene chloride at room temperature was added slowly with stirring over a period of 15 min 0.05 ml of napthoyl chloride in 50 ml of methylene chloride. After another 30 min, the contents were extracted with H_2O and the organic phase concentration in vacuo. The final product was purified by distillation at 5 mmHg and 176°C. The yield was 55%.

Analytic: i.r.: 2980, 1750, 1595, 1240
m.s.: 199. 155. 127

TMAE naphthoate: same as for TMAE phenylcarbamate.

DMAE and TMAE Phenylacetate

Procedure same as for DMAE napththoate except that phenylacetyl chloride replaced naphthoyl chloride. DMAE phenylacetate was purified by distillation at 5 mmHg and 160°C. The yield was 55%.

Analytic: i.r.: 2960, 1730, 1450, 1380
m.s.: 207, 163, 142

3-Quinuclidinyl Benzoate and N-Methyl-3-Quinuclidinyl Benzilate

To a mixture of 0.05 mole of 3-quinuclidinyl in 100 ml of dry methylene chloride at room temperature was added slowly with stirring over a period of 15 min 0.05 moles of benzoyl chloride in 50 ml of methylene chloride. After the reaction had proceeded an additional 30 min, the contents were extracted with H_2O and the organic phase removed and concentrated in vacuo. 3-Quinuclidinyl benzoate was purified by distillation at 5 mmHg and 176°C. The yield was 40%.

Analytic: i.r.: 2950, 1730, 1600
m.s.: 227, 100, 137

DMAE and TMAE Cyclohexycarboxylate

To 0.05 mole of dry dimethylaminoethanol in 100 ml of dry methylene chloride at room temperature was added slowly with stirring over a period of 15 min 0.05 ml of cyclohexycarboxyl chloride in 50 ml of methylene chloride. After another 30 min, the contents were extracted with H_2O and the organic phase concentrated in vacuo. The final product was purified by distillation at 5 mmHg and 176°C. The yield was 55%.

Analytic: i.r.: 2940, 1730, 1450, 1250, 1175
m.s.: 224, 170, 143, 97

N-methyl-3-quinuclidinyl benzoate was prepared by the addition of 0.013 moles of methyl iodide to 0.01 moles of 3-quinuclidinyl benzoate.

TMAE cyclohexylcarboxylate: same as for TMAE phenylcarbamate.

DMAE and TMAE Cyclopentylcarboxylate

Same as for DMAE cyclohexylcarboxylate except that cyclopentylcarboxyl chloride replaced cyclohexylcarboxyl chloride.

Analytic: i.r.: 2940, 1730, 1450, 1170
m.s.: 197, 143, 140, 127

TMAE cyclopentylcarboxylate: same as for TMAE phenylcarbamate.

DMAE Nicotinate

This compound was prepared according to the procedure of Blicke and Jenner [10]. TMAE nicotinate was prepared by reacting DMAE nicotinate with methyl iodide.

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