Determination of Contact Germicidal Efficiency (CGE). A modified serial dilution analysis was used to determine the time taken for various concentrations of the compounds to sterilize suspensions of the microorganisms. The organisms used and their concentrations in overnight broth culture were: *Staphylococcus aureus* (ATCC 6538), 6×10^6 to 8×10^6 cfu/mL; *Pseudomonas aeruginosa* (ATCC 9027), 12×10^6 to 13×10^6 organisms/mL; *Streptococcus pyogenes* (ATCC 19615), 5×10^6 cfu/mL (cfu/mL: colony forming units/milliliter). Concentrations were determined by standard dilution and plate count techniques.

Nutrient Broth Agar (BBL-11479). The broth contained 5 g of gelysate peptone, 3 g of beef extract, and 15 g of agar/1000 mL of distilled water.

Horse Serum T.C. A 10% horse serum solution in distilled water was freshly prepared and adjusted to pH 7, using carbon dioxide.

Method. A stock solution containing a known concentration of the quaternary salt in an appropriate buffer was prepared. Test solutions were left at room temeperature for 30 min prior to their use in the screen.

In the screen, 0.2 mL of an overnight broth culture of an organism was added to 5 mL of the test solution. At time intervals of 0.5, 1, 2, 3, 4, etc., min., a loop (Nicrom loop of 3-mm diameter, Scientific Products) of the suspension was subcultured into 5 mL of sterile nutrient broth. The subcultures were incubated at 37 °C for 7 days and observed daily for evidence of bacterial growth. The time reported for sterilization of a suspension of an organism corresponds to the smallest time interval in which a subculture that gave no growth during 7 days was prepared.

The following controls were conducted: (1) A 0.2-mL aliquot of an overnight culture was added to 5 mL of sterile 0.9% NaCl, and a loop of the suspension was subcultured into 5 mL of sterile nutrient broth. Bacterial growth in the subculture incubated at 37 °C indicated viable overnight culture. (2) At the same time intervals that subcultures were made of suspensions of microorganisms in test solutions, a loop of the suspension was subcultured on sterile nutrient agar plates. Following incubation of the plates at 37 °C, the morphology of the colonies was examined for contamination by foreign organisms. (3) Solutions identical with the test solutions, but without the quaternary compounds, were subjected to the same screen as the test solutions in order to ensure that the buffers were not bactericidal. Replicate experiments demonstrated that there were no significant differences in the results within and between experiments.

Toxicity Studies (LD_{50} Values and Intoxication Symptoms). White Swiss male mice (MCR-ICR) weighing 20–23 g were used. The number of animals used for a given dose varied from 10 to 20 (at levels closer to LD_{50}). For iv and ip administration, the quaternary salt was dissolved in isotonic NaCl solution, pH 7.0. The solution pH was adjusted to pH 7.0 if necessary, using saturated sodium bicarbonate. For oral administration, the compound was dissolved in 0.8% NaCl solution at the higher doses, and the pH was adjusted to 5.84 with saturated sodium bicarbonate. The animals were observed for 7 days following the administration. The LD_{50} and related confidence limits were calculated by the method of Litchfield and Wilcoxon.[8] The toxic symptoms at the higher levels included, in order of occurrence after injection: lack of movement, fur stringy and yellow, swelling of abdomen (ip), diarrhea (po), trembling, eyes irritated and shut, and darkening and paralysis of extremities.

(8) J. T. Litchfield, Jr., and F. W. Wilcoxon, *J. Pharmacol. Exp. Ther.*, 96, 99 (1949).

Soft Drugs. 3. A New Class of Anticholinergic Agents[1]

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A new class of antimuscarinic drugs was designed and synthesized. The compounds are "soft" quaternary ammonium esters in which there is only *one carbon atom* separating the ester oxygen and the quaternary head. The compounds are potent anticholinergics when derived from hindered "umbrella" acids and cholinergics when derivatives of simple aliphatic acids. The more potent anticholinergics have up to 10 times higher acetylcholine antagonist activity than atropine, but they have a much shorter duration of action. The compounds cleave hydrolytically with simultaneous destruction of the quaternary head. The compounds are promising as selective, local agents, particularly as inhibitors of eccrine sweating.

The present paper describes a new class of anticholinergic agents which are soft analogues[2] of some known anticholinergics, which were designed to have high local, but practically no systemic, activity.

It is well known that antimuscarinic compounds have three clinically useful effects: antispasmodic, antisecretory, and mydriatic. As antisecretory agents, one of the commonly known effects is inhibition of eccrine sweating and compensatory cutaneous flush. Systemically administered anticholinergics do generally decrease the secretion of the sweat glands, as well as saliva and other secretory glands. Based on these properties, it has been long investigated how one could safely use an antimuscarinic agent to inhibit local hyperhydration by topical application. Although a wide range of compounds have proved to be highly effective as antiperspirants when applied topically, it was concluded that none of them are really safe to use because of the well-known systemic effects of these drugs at a

(1) For paper 2 of this series, see N. Bodor and J. J. Kaminski, *J. Med. Chem.*, **23**, under Notes in this issue (1980).

(2) For paper 1 of this series, see N. Bodor, J. J. Kaminski, and S. Selk, *J. Med. Chem.*, **23**, preceding paper in this issue (1980).

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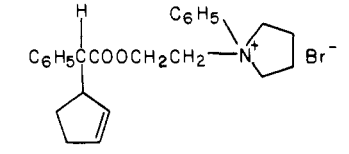
possible over-exposure. Side effects appear even at very low concentrations when highly potent anticholinergics are used. One important part of the side effects based on central action can be minimized by using quaternary ammonium type agents, since these ionic compounds cannot penetrate the blood–brain barrier. Consequently, the quaternaries are primary parasympathetic blocking agents which should be employed in selective inhibition of the peripheral receptors. A number of studies[3,4] have shown that various topically applied quaternary antimuscarinics do inhibit sweating. In the various orders of effectiveness established, a number of quaternary salts were very highly ranked, such as antrenyl (1), probanthine (2), homatropine methyl bromide (3) or compound AHR-483 (3-[(cyclohexylphenylacetyl)oxy]-1,1-dimethylpyrrolidinium bromide; 4) of Stoughton.[5] It was also found that some of the more potent tertiary amine type anticholinergics, such as scopolamine, have a very long lasting effect when applied topically. Thus, a 1% scopolamine solution inhibits sweating for six days, while an 18% solution has effect for over 30 days.

The most detailed systematic study on the effect of various anticholinergics on perspiration includes 95 anticholinergics,[6] of which 48 are amino alcohol esters. It was found that among the 11 most active compounds, three were quaternary salts or, interestingly enough, scopolamine methyl bromide was more effective on human subjects than scopolamine hydrobromide. Similarly to the case of scopolamine, quaternary anticholinergics,[6] such as **5** and **6**, were found to be more effective as topical antiperspirants than the corresponding tertiary amine type structures.



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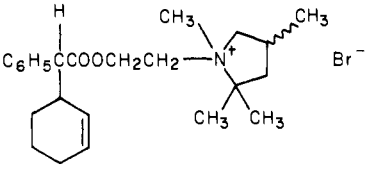

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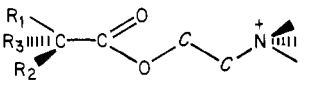
The basic approach described in the present work for developing an *effective and safe* topical antiperspirant differs in several major aspects from the solution based on derivatives of scopolamine.[6]

The development of the concept of "soft drugs"[2,7] and, specifically, the soft quaternary compounds provided a good theoretical basis for development of safe antimuscarinic agents of the soft analogue[2] type.

Hard quaternary salts of the amino alcohol ester type can be represented by structures **7** or **8**, where the ester oxygen and the quaternary head are separated by 2 or 3



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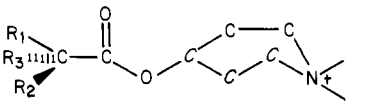

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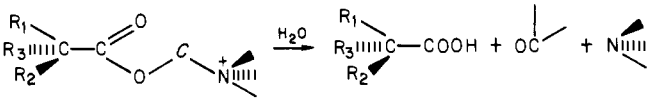
carbon atoms, respectively. A corresponding "soft analogue",[2,7] however, has only one carbon atom in the bridge (**9**), which results in destruction of the quaternary head during hydrolysis.



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It is generally believed that the muscarinic receptors require at least two carbon atoms separating the quaternary head and the ester oxygen in order to have significant binding and activity of the antagonists. Consequently, the question of the activity of the "soft" kind of quaternary salts (**9**) was the first important problem. It should be mentioned that it was found[8] that acetylcholine (**10**) is 5000 times more active that acetylnorcholine (**11**) on the guinea pig ileum test, while **11** is basically the typical soft analogue of acetylcholine.

The second important characteristic of the "soft" antimuscarinic agents should be their fast deactivation upon absorption, thus preventing any systemic effects. (It is known that atropine, for example, does not undergo hydrolytic cleavage, to any significant extent, in man.[9,10])

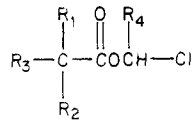
Chemistry. The soft quaternary salts[2,7] (**9**) generally are built up from three basic components: an acid, an aldehyde, and a tertiary amine.

In order to have close analogues of the known hard antimuscarinic agents, we have used hindered, "umbrella" acids (α-substituted phenylacetic acids, adamantanecarboxylic acid, etc.), simple tertiary amines (triethylamine, mono- and dialkylpyrrolidines, 1-methylimidazole, etc.), and simple aldehydes (formaldehyde, acetaldehyde, chloral). The synthesis first involved the preparation of the soft alkylating agents based on the corresponding acids and aldehydes, followed by their reaction with the tertiary amine. The new alkylating agents (**12a–p**) prepared and used in these studies are shown in Table I. Using seven different tertiary amines, selected quaternary salts were prepared which can be classified by the acid portion, as shown in Tables II–IV.

It is known that an α-OH or α-CH_2OH group enhances anticholinergic activity (for example, tropic acid). Thus, attempts were made to obtain compounds of these types. Soft quaternary salts are incompatible with free hydroxy groups (acylation will take place with concomitant liberation of the aldehyde—the rate is dependent on the structure); thus, the corresponding OH groups were protected via alkylation or nitration[11,12] (the nitrate esters can be removed reductively) but no stable soft alkylating agents could be prepared.

(3) R. Brun and N. Hunziker, *Dermatologica*, 110, 245 (1955).
(4) W. B. Shelley and P. M. Horvath, *J. Invest. Dermatol.*, 16, 267 (1957).
(5) R. B. Stoughton, F. Chiv, W. Fritsch and D. Nurse, *J. Invest. Dermatol.*, 42, 151 (1964).
(6) F. S. Kilmer McMillan, H. H. Reller, and F. H. Snyder, *J. Invest. Dermatol.*, 43, 363 (1964).
(7) N. Bodor, in "Design of Biopharmaceutical Properties by Prodrugs and Analogs", Acad. Pharm. Sci., 1977, Chapter 7, pp 98–135.
(8) W. B. Geiger and H. Alpers, *Arch. Int. Pharmacodyn.*, 148 352 (1964).
(9) R. Ammon, *Drug. Res.*, 27, 944 (1977).
(10) R. R. Scheline, "Mammalian Metabolism of Plant Xenobiotics", Academic Press, London, New York, and San Francisco, 1978, p 384.

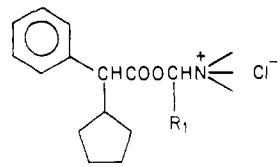
Table I. Soft Alkylating Agents[a] Prepared from the Corresponding Acyl Halides and Aldehydes

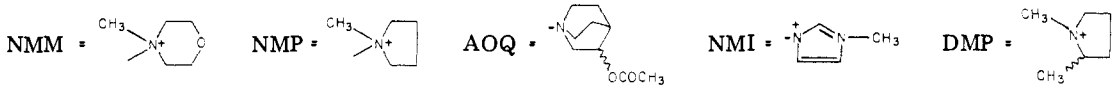


no.	R_1	R_2	R_3	R_4	formula	anal.	method of purifn	physical characteristics
12a	C_6H_5	cyclopentyl	H	H	$C_{14}H_{17}ClO_2$	C, H	*b*	*b*
12b	C_6H_5	cyclopentyl	H	CH_3	$C_{15}H_{19}ClO_2$	C, H	*b*	*b*
12c	C_6H_5	cyclohexyl	H	H	$C_{15}H_{19}ClO_2$	C, H	chromatography	mp 53-54 °C
12d	C_6H_5	H	H	H	$C_9H_9ClO_2$	C, H	chromat/dist	bp 132-134 °C (14 mm)
12e	C_6H_5	CH_3	H	H	$C_{10}H_{11}ClO_2$	C, H	chromat/dist	bp 101-105 °C (1.9 mm)
12f	C_6H_5	CH_2CH_3	H	H	$C_{11}H_{13}ClO_2$	C, H	chromat/dist	bp 103-105 °C (1.8 mm)
12g	C_6H_5	CH_2CH_3	H	CCl_3	$C_{12}H_{12}Cl_4O_2$	C, H	*b*	*b*
12h	C_6H_5	C_6H_5	CH_3	H	$C_{16}H_{15}ClO_2$	C, H	chromatography	colorless oil
12i	CH_3CH_2	CH_3	H	H	$C_6H_{11}ClO_2$	C, H	distillation	bp 34-37 °C (1 mm)
12j[c]		adamantyl		H	$C_{12}H_{17}ClO_2$	C, H	chromatography	colorless oil
12k	C_6H_5	1,1-cyclopentyl		H	$C_{13}H_{15}ClO_2$	C, H	cryst/pet. ether	mp 35-36 °C
12l	C_6H_5	1,1-cyclopropyl		H	$C_{11}H_{11}ClO_2$	C, H	chromatography	colorless oil
12m	C_6H_5	1,2-cyclopropyl		H	$C_{11}H_{11}ClO_2$	C, H	chromatography	colorless oil
12n	C_6H_5	C_6H_5	H	H	$C_{15}H_{13}ClO_2$	C, H	chromatography	mp 47-49 °C

[a] The general methods described under Experimental Section were applied. In addition, the available chloromethyl pivalate (12o) and the synthesized[15] chloromethyl acetate (12p) and bromophthalide (12r) were used. [b] See Experimental Section. [c] R_1, R_2, and R_3 together with the α carbon form the adamantyl residue.

Table II. Soft Quaternary Salts Based on α-Cyclopentylphenylacetic Acid

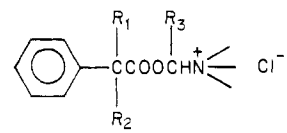




no.	R_1	N<	formula	synthetic conditions	mp, °C	anal.
13a	H	$-N(CH_2CH_3)_3$	$C_{20}H_{32}ClNO_2$	*a*	*a*	C, H, N
13b	H	NMM	$C_{19}H_{28}ClNO_3$	*b*	166-168	C, H, N
13c	H	NMP	$C_{19}H_{28}ClNO_2$	*b*	144-145	C, H, N
13d	H	AOQ	$C_{23}H_{32}ClNO_2$	*b*; 25 °C	171-174	C, H, N
13e	H	NMI	$C_{18}H_{23}ClN_2O_2$	*b*	151-160	C, H, N
13f	H	$-N(CH_3)(CH_2CH_2OCH_2CH_2CH_3)_2$	$C_{25}H_{42}ClNO_4$	*b*	64-66	C, H, N
13g	H	DMP	$C_{20}H_{30}ClNO_2$	*b*; 50 °C	35-40	C, H, N

[a] See Experimental Section. [b] See method described for 13a.

Table III. Soft Quaternary Salts Containing Substituted Phenylacetic Acid

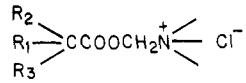


14

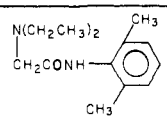
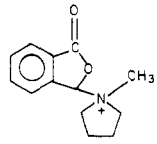
no.	R_1	R_2	R_3	N<	formula	synthetic conditions	mp, °C	anal.
14a	C_6H_{11}	H	H	$-N(CH_2CH_3)_3$	$C_{21}H_{34}ClNO_2$	*b*	128-129	C, H, N
14b	H	H	H	NMM[d]	$C_{14}H_{20}ClNO_3$	*b*; 80 °C	184-185	C, H, N
14c	CH_2CH_3	H	H	$-N(CH_2CH_3)_3$	$C_{17}H_{27}ClNO_2$	*b*	65-70	*c*
14d	CH_2CH_3	H	H	NMI[d]	$C_{15}H_{19}ClN_2O_2$	*b*	89-94	C, H, N
14e	CH_2CH_3	H	CCl_3	NMI[d]	$C_{16}H_{18}Cl_4N_2O_2$	*b*; 25 °C	48-52	C, H, N
14f	C_6H_5	CH_3	H	NMP[d]	$C_{21}H_{26}ClNO_2$	*b*	78-82	C, H, N
14g[a]	1,1-cyclopentyl		H	$-N(CH_2CH_3)_3$	$C_{19}H_{30}ClNO_2$	*b*	130-132	C, H, N
14h[a]	1,1-cyclopentyl		H	NMP[d]	$C_{18}H_{26}ClNO_2$	*b*; 25 °C	153-155	C, H, N
14i[a]	1,1-cyclopropyl		H	$-N(CH_2CH_3)_3$	$C_{17}H_{26}ClNO_2$	*b*	170-171	C, H, N
14j[a]	1,1-cyclopropyl		H	NMP[d]	$C_{16}H_{22}ClNO_2$	*b*	169-170	C, H, N
14k[a]	1,1-cyclopropyl		H	DMP[d]	$C_{17}H_{24}ClNO_2$	*b*	169-170	C, H, N

[a] R_1-C-R_2 together form the rings. [b] See method given for 13a. [c] Too hygroscopic; no analysis could be obtained. [d] Structures for NMM, NMP, AOQ, NMI, and DMP are in Table II.

Table IV. Soft Quaternary Salts Derived from Branched Aliphatic Carboxylic Acids



15

no.	R_1	R_2	R_3	N	formula	synthetic conditions; °C	mp, °C	anal.
15a	CH_3	CH_3	CH_3		$C_{20}H_{33}ClN_2O_3$	a	162.5-164	C, H, N
15b	CH_2CH_3	CH_3	H		$C_{13}H_{24}ClNO_2$	b; 25	134-137	C, H, N
15c	CH_2CH_3	CH_3	H	AOQ	$C_{15}H_{26}ClNO_4$	b	130-133	C, H, N
15d	CH_2CH_3	CH_3	H	NMP	$C_{11}H_{22}ClNO_2$	b; 90	76-78	C, H, N
15e	CH_2CH_3	CH_3	H	DMP	$C_{12}H_{24}ClNO_2$	b; 90	75-78	C, H, N
15f	H	H	H	$-N^+(CH_2CH_3)_3(Br^-)$		c		
15g	adamantyl			NMP	$C_{17}H_{28}ClNO_2$	b	181-182	C, H, N
15h	adamantyl			NMI	$C_{16}H_{23}ClN_2O_2$	b	158-164	C, H, N
15i[d]					$C_{13}H_{16}BrNO_2$	b; 90	162-165	C, H, N

[a] See Experimental Section. [b] See method for 15a. [c] See ref 15. [d] The intramolecular analogue 15i was arbitrarily included in this class. The counterion in this case was bromide. [e] For AOQ, NMP, DMP, and NMI, see Table II.

Similarly, the acid chloride of xanthene-9-carboxylic acid did decompose during the attempted reaction with paraformaldehyde.

In some other cases, the quaternization lead to byproducts, particularly if an α hydrogen is present in the acid portion. For example, the chloromethyl diphenylacetate did not give the corresponding soft quaternary salt with triethylamine but resulted in various byproducts, identified as 1,1-diphenylethylene and the expected methylenebis(diphenylacetate).

Highly activated soft alkylating agents, such as the chloral adduct **12g**, did decompose in the presence of strong tertiary amines, such as triethylamine, but the corresponding soft quaternary salt (**14g**) from the weaker base *N*-methylimidazole could be isolated.

Several typical soft quaternary agents were studied for their hydrolytic stability at various pH's. The disappearance of the soft quaternary salts and/or formation of the corresponding acid was followed by high-pressure LC. The results are given in Table V.

Based on the hydrolysis data, it is clear that the soft quaternary salts undergo much more facile cleavage than atropine,[9,10] for example. The rate is dependent mostly on the steric hindrance on the acid; thus, **14b** cleaves 300 times faster than **13c**. The nature of the amine has some effect on the rate; thus, derivatives of *N*-methylimidazole (weaker base) cleave slower than triethylamine, which is slower than 1,2-dimethylpyrrolidine and 1-methylpyrrolidine.

All data are consistent with the assumption that the intermediate *N*-hydroxymethylammonium salt cleaves very fast to the tertiary amine and the aldehyde. The ester cleavage is the rate-determining step. (The simple "formocholine" chloride, $[(CH_3)_3N^+CH_2OH]Cl^-$ (**31**), was found[13] to have the following half-lives (at pH's): 7.4 (2.97), 3.3 (3.36), 0.81 (3.95), and 0.49 min (4.16).) At physiological pH's the cleavage is probably too fast to be measurable.

Table V. Hydrolysis Kinetics of Selected Soft Quaternary Salts

no.	conditions	K_{obsd}, min^{-1}	$t_{1/2}$
13a	pH 7.4, 37 °C	3.61×10^{-4}	32 h[a]
13c	pH 9.4, 36 °C	1.39×10^{-1}	5 min[a]
	pH 7.4, 37 °C	5.78×10^{-4}	20 h[a]
	dog plasma, 36 °C	1.44×10^{-3}	8 h[a]
	dog plasma, 37 °C	1.68×10^{-3}	6.5 h[b]
14b	pH 7.4, 37 °C	8.66×10^{-2}	8 min[a]
	dog plasma, 37 °C	1.73×10^{-1}	4 min[a]
14c	pH 7.4, 37 °C	4.44×10^{-3}	2.6 h[a]
		4.68×10^{-3}	2.5 h[b]
	hog liver esterase, 37 °C	5.42×10^{-3}	2.1 h[a]
	dog plasma, 37 °C	8.15×10^{-3}	1.4 h[b]
14d	pH 7.4, 37 °C	2.03×10^{-3}	5.7 h[a]
		1.94×10^{-3}	6.0 h[b]
14f	pH 7.4, 37 °C	1.93×10^{-4}	60 h[a]
14h	pH 7.4, 37 °C	1.81×10^{-4}	64 h[a]
14i	pH 7.4, 37 °C	5.50×10^{-4}	21 h[a]
14j	pH 7.4, 37 °C	6.80×10^{-4}	17 h[a]
14k	pH 7.4, 37 °C	5.50×10^{-4}	21 h[a]

[a] From d[acid]/dt. [b] From d[quat]/dt.

Pharmacology. The new anticholinergic agents **13–15** were tested using two methods. First, the well-known guinea pig ileum test was used to determine the anticholinergic (acetylcholine antagonist) and antihistaminic pA_2 values. The value pA_2, the logarithm of the reciprocal of the concentration of antagonist which necessitates doubling the concentration of agonist in order to keep the effect constant, is equal to the logarithm of K, the affinity constant for the antagonist.[14] The results are listed in Table VI.

The duration of the acetylcholine antagonist effect of selected soft quaternary salts was then determined, as compared to atropine, using anesthetized cats. The method is described under the Experimental Section, and the results are given in Table VII. The values represent the

(11) O. Mantsch, N. Bodor, and F. Hodosan, *Rev. Roum. Chim.*, 13, 1435 (1968).
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Table VI. Acetylcholine Antagonist Potency of Selected Soft Quaternary Salts

no.	anti-cholinergic[a] pA_2	anti-histamine pA_2	cholinergic potency vs.[b] acetylcholine
13a	8.1	*c*	*c*
13c	8.4	*c*	*c*
13d	8.6	5.7	nt
13e	7.8	6.3	nt
13f	*c*	*c*	nt
13g	9.3	7.9	nt
14a	8.6	5.7	nt
14b	nonspecific depressant		
14c	6.6	6.5	nt
14d	5.9	5.9	nt
14f	8.4	*c*	nt
14g	7.1	*c*	nt
14h	7.3	*c*	nt
14i	*c*	nt	nt
14j	5.8	*c*	nt
14k	6.6	6.24	nt
15a	*c*	nt	1:10 Bl:atropine not hexamethonium
15b	*c*	nt	1:5 Bl:hexamethonium
15c	*c*	nt	1:10 Bl:atropine
15d	*c*	5.6	
15e	4.9	nt	*c*
15f	*c*	nt	$1:10^4$ Bl:atropine not hexamethonium
15g	6.9	*c*	*c*
15h	5.7	5.3	*c*
15i	*c*	nt	c
16[d]	8.5	7.3	

[a] pA_2 of atropine under the same experimental conditions was found to be 8.5. [b] The cholinergic potency was expressed in terms of relative activity vs. acetylcholine. In addition, it was tested if the cholinergic activity was blocked (Bl in the table) by atropine and/or hexamethonium. [c] No activity found at the doses used; nt = not tested. [d] The "hard" quaternary analogue (16) of 13a was included for comparison.

percent of the vasodepressor response to acetylcholine at various time intervals following administration of the test drugs. Atropine at the same concentrations eliminated the response completely for hours.

Discussion

It is clear that a number of the soft quaternary salts, designed to be active as anticholinergics, are indeed potent agents. The qualitative SAR indicates the need for an "umbrella", hindered acid component, but the activity is also dependent on the amine portion. In view of the generally accepted structural requirements, it is surprising that the soft agent **13a** is about as active as the corresponding hard analogue **16**. The influence of the amine can be seen in the series **13a–h**. While the tris(propyloxyethyl)amine derivative **13f** is completely inactive, **13g**, containing a 1,2-dimethylpyrrolidine, is almost an order of magnitude more potent than atropine. Thus, the order of activity in an analogous series is: 1,2-dimethylpyrrolidine > 3-(acetyloxy)quinuclidine > 1-methylpyrrolidine > triethylamine > *N*-methylimidazole ≫ tris(propyloxethyl)amine. As expected, the unhindered derivative **14b** is void of any activity. The less hindered (ethyl instead of cyclopentyl) derivatives **14c** and **14d** are about 100 times less active than the cyclopentyl. The more restricted **14i** is inactive compared to the open-chain analogue **14c**, but some of the activity can be gotten back by replacing triethylamine with the more active pyrrolidines (**14j** and **14k**). The situation is similar when **13a** and **13c** are compared to the corresponding **14i** and **14j** compounds.

When simple aliphatic acids are used, we jump to the other side of the scale. The simple pivalyloxymethyl derivative of lidocaine (**15a**) is cholinergic and so are derivatives of α-methylbutyric acid, **15b** and **15c**. Their cholinergic activity is surprisingly high, particularly in view of the very low activity of the acetylnorcholine analogue **15f**. In agreement with the qualitative SAR established in the **13a–g** series, the cholinergic activity of the α-methylbutyric acid derivatives disappear when the pyrrolidines are used; thus, **15d** is a weak antihistaminic, while **15e** is again anticholinergic. The more hindered adamantanecarboxylic acid derivatives **15g** and **15h** are again fairly active anticholinergics.

The cat blood-pressure studies indicate that the highly potent soft anticholinergics have a short duration of action. Thus, the activity of **13a** disappears completely after 9 min at a 5.7×10^{-8} mol/kg^{-1} dose, and over 50% of the acetylcholine response is back after 18 min at a 10 times higher dose level.

All these data indicate that the new type of anticholinergics are highly active, while "soft" in nature; consequently, they are promising as local agents. Preliminary studies indicate that **13c** is very effective in controlling eccrine sweating in man, using a modification of the well-known forearm test.[6] When a 1% ethanolic solution

Table VII. Acetylcholine Antagonist Effect of Selected Soft Quaternary Salts on Cat Blood Pressure

no.	concn, mol kg^{-1}	activity											
		1	3	5	7	9							time, min
13a	5.7×10^{-8}	0	18	55	82	100							% response[a]
		1	3	6	12	15	18	22	27	31	37		time, min
	5.7×10^{-7}	0	0	0	18	36	55	65	73	95	100		% response[a]
		1	3	4	5	9							time, min
13b	5.7×10^{-8}	0	45	82	90	100							% response[a]
		1	4	6	10	13	17	21	25	29			time, min
	5.7×10^{-7}	0	0	0	23	50	59	73	82	100			% response[a]
		1	2	4	7								time, min
13c	5.4×10^{-9}	0	0	67	100								% response[a]
		1	2	5	9	13	18	23	28	33	38	44	time, min
	5.4×10^{-8}	0	0	0	0	31	63	63	69	81	88	88	% response[a]
		1	2	4	6	8	10						time, min
14a	5.4×10^{-8}	0	0	50	67	83	100						% response[a]
		1	3	6	9	12	15	18	21	24			time, min
	5.4×10^{-7}	0	0	0	0	43	60	78	93	100			% response[a]

[a] Percent of the decrease in blood pressure after a dose of acetylcholine in the presence of antagonist, compared to the decrease after the same dose of acetylcholine in the absence of antagonist (100%).

of **13c** (50 μL) was applied to the forearm, it completely inhibited sweating for 24 h.

The effect of the optical isomerism of the α carbon in the acid portion was also considered, since it is known that the (−) isomers of a number of anticholinergics (for example, methylscopolamine salts) are more active than the (+) isomers. The highly active **13c** was selected for the studies. The starting α-cyclopentylphenylacetic acid was resolved using standard procedures, and the corresponding (−)- and (+)-**13c** derivatives were made. The relative pA_2 values [8.86 for the (−) and 8.57 for the (+)] do not seem to be sufficiently different to indicate any significant chiral dependence. Note also that the optically inactive **14h** is a highly potent compound.

Additional studies are currently undertaken to optimize the structures, determine the relative acute and chronic toxicities of selected compounds, and to evaluate their overall antisecretory properties.

It is conceivable to obtain practically exclusively local agents, if the rate of penetration–absorption and the rate of in vivo destruction are well balanced: if the latter is faster, no buildup and consequently no systemic effects due to the drug would be observed.

Experimental Section

Synthesis. The various α-chloroalkyl carboxylates were prepared according to the methods described,[2,15] by reacting the corresponding acyl halides with the aldehyde in the presence of a Lewis acid. The crude esters were purified by distillation or chromatography.

Chloromethyl (±)-α-Cyclopentylphenylacetate (12a). Redistilled thionyl chloride (7 mL, 0.17 mol) was allowed to react with (±)-α-cyclopentylphenylacetic acid (5 g, 0.024 mol) at room temperature overnight. The excess thionyl chloride was removed in vacuo to yield the crude acyl halide, which was not further purified: IR (neat) 2950, 2880, 1780, 1490, 1450, 1030, 980, 780, 730, 700, 620, 610 cm^{-1}. The crude product obtained with $(CH_2O)_n$ was chromatographed on Florisil (100–200 mesh) (chloroform): IR (neat) 2920, 2840, 1750, 1450, 1315, 1190, 1110, 1025, 760, 700 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.3 (s, 5 H), 5.7–5.2 (m, 2 H), 3.3 (d, 1 H), 3.0–0.8 (br m, 9 H). Anal. ($C_{14}H_{17}ClO_2$) C, H.

1-Chloroethyl (±)-α-Cyclopentylphenylacetate (12b). Paraldehyde (1.1 g, 0.008 mol) was added to the above acyl halide, and the mixture was heated in an oil bath at 75 °C for 10 min. Anhydrous zinc chloride (0.1 g) was then added and heating continued for 0.75 h. The solution was allowed to cool to room temperature and excess paraldehyde was removed in vacuo. The crude 1-chloroethyl (±)-α-cyclopentylphenylacetate was chromatographed on Florisil (100–200 mesh; chloroform) to yield a colorless liquid (5.5 g, 0.021 mol, 84%): IR (neat) 2940, 2860, 1740, 1490, 1445, 1270, 1200, 1120, 980, 700, 660 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.3 (s, 5 H), 6.7–6.3 (m, 1 H), 3.3 (d, 1 H), 2.9–0.8 (m, 12 H). Anal. ($C_{15}H_{19}ClO_2$) C, H.

(±)-1,2,2,2-Tetrachloroethyl (±)-2-Phenylbutyrate (12g). Redistilled thionyl chloride (7 mL, 0.17 mol) was allowed to react with (±)-2-phenylbutyric acid (5 g, 0.03 mol) at room temperature overnight. The excess thionyl chloride was removed in vacuo to yield the crude acyl halide, which was not further purified: IR (neat) 3060, 3020, 2960, 2920, 2880, 1790, 1595, 1580, 1490, 1450, 1380, 1260, 1115, 1110, 1030, 1005, 995, 970, 910, 900, 810, 790, 755, 730, 700 cm^{-1}. Redistilled trichloroacetaldehyde (2.98 mL, 0.03 mol) was added to the crude acyl halide, and the mixture was heated in an oil bath at 85 °C for 5 min. Anhydrous zinc chloride (0.1 g) was added and heating continued for 15 h. The solution was allowed to cool to room temperature and the crude 1,2,2,2-tetrachloroethyl (±)-2-phenylbutyrate was chromatographed on Florisil (100–200 mesh) (toluene) to yield a colorless liquid (6.84 g, 0.023 mol, 77%): IR (neat) 3060, 3020, 2970, 2930, 2880, 1765, 1600, 1570, 1495, 1460, 1380, 1360, 1320, 1260, 1180, 1130, 1100, 1080, 1030, 950, 920, 850, 830, 790, 760, 730, 700 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.3 (s, 5 H), 6.9–6.8 (m, 1 H), 3.6 (t, 1 H), 2.1 (octet, 2 H), 1.0 (t, 3 H). Anal. ($C_{12}H_{12}Cl_4O_2$) C, H.

Chloromethyl acetate (**12p**) was prepared according to the literature,[15] chloromethyl pivalate (**12o**) was obtained from Aldrich Chemical Co., and (±)-3-bromophthalide (**12r**) was prepared according to the literature.

The soft quaternary salts in the classes **13–15** were synthesized by direct quaternization of the corresponding amines.

(±)-[(α-Cyclopentylphenylacetoxy)methyl]triethylammonium Chloride (13a). Anhydrous chloroform was prepared by distillation from phosphorous pentoxide prior to use. Triethylamine (2.5 mL, 0.018 mol) was added to a stirred solution of chloromethyl (±)-α-cyclopentylphenylacetate (4.8 g, 0.019 mol) in anhydrous chloroform (5 mL). The reaction vessel was sealed, placed in an oil bath, and maintained at 75 °C overnight. On cooling to room temperature, anhydrous ether was added and the reaction triturated with ether until crystallization began. The solid was isolated by filtration under a nitrogen atmosphere and thoroughly washed with anhydrous ether. The product was dried in vacuo at 50 °C over calcium sulfate to yield a white, hygroscopic solid: mp 147–148 °C (5.3 g, 0.015 mol, 83%); IR (KBr) 1740 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.3 (s, 5 H), 5.6 (s, 2 H), 3.6–0.8 (m, 25 H). Anal. ($C_{20}H_{32}ClNO_2$) C, H, N.

[Diethyl[(pivaloyloxy)methyl]ammonio]-2′,6′-dimethylacetanilide Chloride (15a). A mixture of 5.0 g (0.02 mol) of ω-(diethylamino)-2,6-dimethylacetanilide (lidocaine) and 3.31 g (0.02 mol) of chloromethyl pivalate was heated at 50 °C for 48 h. Trituration in anhydrous ether and recrystallization from ethanol–ether gave 6.17 g (0.016 mol, 80%) of [diethyl[(pivaloyloxy)methyl]ammonio]-2′,6′-dimethylacetanilide chloride: mp 162.5–164 °C; ^1H NMR ($CDCl_3$) δ 1.3 (s, 9 H), 1.5 (t, 6 H), 2.2 (s, 6 H), 3.7 (q, 4 H), 5.0 (s, 2 H), 5.7 (s, 2 H), 7.0 (s, 3 H), 11.0 (s, 1 H). Anal. ($C_{20}H_{33}ClN_2O_3$) C, H, N.

***N,N,N*-Triethyl-2-[(±)-α-cyclopentylphenylacetoxy]ethanaminium Iodide (16).** Redistilled thionyl chloride (10 mL, 0.14 mol) was added to (±)-α-cyclopentylphenylacetic acid (3.0 g, 0.015 mol), and the solution was allowed to stand at room temperature overnight. Excess thionyl chloride was removed in vacuo to yield the crude acyl halide, which was not further purified.

Anhydrous chloroform was prepared by distillation from phosphorous pentoxide. The acyl halide was dissolved in the chloroform (50 mL), 2-(diethylamino)ethanol (1.9 mL, 0.01 mol) was added, and the solution was heated at reflux for 4 h. The solvent was removed in vacuo, and the residue was dissolved in 10% aqueous sodium hydroxide and extracted with diethyl ether. The organic phase was dried over magnesium sulfate, filtered, and evaporated to yield a colorless liquid. The liquid was dissolved in benzene (200 mL), ethyl iodide (5 mL, 0.06 mol) was added, and the solution heated at reflux for 10 h. After the solution cooled, the product crystallized from the reaction mixture and was filtered and dried in vacuo to yield *N,N,N*-triethyl-2-[(±)-α-cyclopentylphenylacetoxy]ethanaminium iodide as a white, hygroscopic solid. The product was recrystallized from chloroform–ether: mp 145–147 °C (1.2 g, 0.003 mol, 20%); IR (KBr) 1735 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.3 (s, 5 H), 4.7–4.4 (br m, 2 H), 3.8 (t, 2 H), 3.4 (q, 7 H), 3.0–2.3 (br m, 1 H), 2.3–1.0 (m, 17 H). Anal. ($C_{21}H_{34}INO_2$) C, H, N.

Preparation of the (−) and (+) Enantiomers of [(α-Cyclopentylphenylacetoxy)methyl]-1-methylpyrrolidinium Chloride (13ca and 13cb). 1. Resolution of the Acid. (±)-α-Cyclopentylphenylacetic acid (4.57 g, 2.24×10^{-2} mol) was dissolved in chloroform and added to a solution of strychnine (7.48 g, 2.24×10^{-2} mol) in chloroform. The solvent was removed in vacuo and the residue recrystallized repeatedly from chloroform–methanol. All of the mother liquors were stored.

The solid (3.46 g) was dissolved in water (200 mL), acidified, and extracted with ether (2×100 cm^{-3}). The ether extract was washed with 1 N hydrochloric acid (50 mL), followed by water (50 mL), dried over sodium sulfate, and evaporated in vacuo to yield a viscous oil which crystallized on standing. The product was recrystallized from *n*-hexane (1.03 g): mp 65–66 °C; $[\alpha]^{20}_D$ +60.3° (*c* 1, $CHCl_3$). The NMR of the product was identical with (±)-α-cyclopentylphenylacetic acid.

The chloroform–methanol mother liquors, containing the salts of the (−) isomer, were combined and evaporated in vacuo. The residue was extracted with excess ether. The ether was filtered, concentrated, and washed extensively with 1 N hydrochloric acid,

(15) L. H. Ulich and R. Adams, *J. Am. Chem. Soc.*, **43**, 660 (1921).

followed by water (50 mL). The organic phase was dried over sodium sulfate and evaporated. The residue was recrystallized from *n*-hexane to yield a white solid (0.88 g): mp 73–74 °C; $[\alpha]^{20}_D$ −52.1°. The NMR of this product was identical with (±)-α-cyclopentylphenylacetic acid.

2. Preparation of Chloromethyl Esters (12aa and 12ab). (+)-α-Cyclopentylphenylacetic acid (1.03 g, 5.0×10^{-3} mol) was treated with excess thionyl chloride, at room temperature overnight. The thionyl chloride was removed in vacuo to yield an oil which was not further purified: IR (neat) 1780 cm^{-1}. Paraformaldehyde (0.17 g, 5.6×10^{-3} mol) and zinc chloride (0.1 g) was added to the acyl halide, and the suspension was heated, with stirring, at 85 °C for 4 h.

The crude product was chromatographed on silica gel (Merck Kieselgel 60, 30–70 mesh) in toluene to yield a colorless liquid (**12ab**; 1.0 g, 4.0×10^{-3} mol, 79%): IR (neat) 2920, 2840, 1750, 1450, 1315, 1190, 1110, 1025, 760, 700 cm^{-1}; NMR ($CDCl_3$) δ 7.3 (s, 5 H), 5.7–5.2 (m, 2 H), 3.3 (d, 1 H), 3.0–0.8 (br m, 9 H); $[\alpha]^{20}_D$ +63.0° (*c* 1, $CHCl_3$).

Chloromethyl (−)-α-cyclopentylphenylacetate (**12aa**) was prepared from (−)-α-cyclopentylphenylacetic acid by the same procedure: IR (neat) 2920, 2840, 1750, 1450, 1315, 1190, 1110, 1025, 760, 700 cm^{-1}; NMR ($CDCl_3$) δ 7.3 (s, 5 H), 5.7–5.2 (m, 2 H), 3.3 (d, 1 H), 3.0–0.8 (br m, 9 H); $[\alpha]^{20}_D$ −56.3° (*c* 1, $CHCl_3$).

3. Preparation of the Quaternary Salts (13ca and 13cb). **(+)-[(α-Cyclopentylphenylacetoxy)methyl]-1-methylpyrrolidinium Chloride (13cb).** Anhydrous 1-methylpyrrolidine (0.37 mL, 3.6×10^{-3} mol) was added to a solution of chloromethyl (+)-α-cyclopentylphenylacetate (0.9 g, 3.6×10^{-3} mol) in chloroform (5 mL; freshly distilled from P_2O_5). The mixture was heated, with stirring, at 70 °C in a stoppered flask overnight. After the mixture cooled to room temperature, excess dry ether was added, and the precipitate was filtered, washed with dry ether, and dried in vacuo to yield a white solid (1.24 g, 3.6×10^{-4} mol, 100%): mp 152–153 °C; IR (KBr) 1740 cm^{-1}; NMR ($CDCl_3$) δ 7.3 (s, 5 H), 5.7 (s, 2 H), 4.0–0.7 (m, 21 H); $[\alpha]^{20}_D$ +39.2 (*c* 1, $CHCl_3$). Anal. ($C_{19}H_{28}ClNO_2$) C, H, N.

(−)-[(α-Cyclopentylphenylacetoxy)methyl]-1-methylpyrrolidinium chloride (**13ca**) was prepared by the same procedure. The product was recovered as a white solid: mp 145–147 °C; IR (KBr) 1740 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.3 (s, 5 H), 5.7 (s, 2 H), 4.0–0.7 (m, 21 H); $[\alpha]^{20}_D$ −27.2° (*c* 1, $CHCl_3$). Anal. ($C_{19}H_{28}ClNO_2$) C, H, N.

Hydrolysis Kinetics. The hydrolysis of the selected soft quaternary salts was followed by high-pressure LC. The relative concentrations of the quaternary salts and/or the acid produced by hydrolysis were determined against an appropriate internal standard. Where both components were sufficiently resolved, an estimation of the reaction half-life was obtained from both the rate of disappearance of the salt and the rate of appearance of the acid. When a quaternary salt containing some amine hydrochloride was used, such as **14c**, the initial concentration was calculated by measuring the final concentration of the 2-phenylbutyric acid formed after complete hydrolysis of **14c**.

For the chemical hydrolysis, two buffers were used: (1) 0.1 M sodium carbonate–bicarbonate, pH 9.4 (ionic strength 0.5 μ, NaCl added); (2) 0.5 M potassium phosphate, pH 7.4.

The enzymatic hydrolysis was carried out in 0.5 M potassium phosphate, pH 7.4, containing gentisic acid (potassium gentisate) as an internal standard. The enzyme used was an esterase (carboxylic ester hydrolase, EC 3.1.1.1) from hog liver (sigma E-9627). For the experiment, 2.4 units of enzyme was used for a substrate concentration of 5.6×10^{-3} mol L^{-1}.

Plasma samples were prepared using fresh dog blood. The blood was centrifuged and the upper layer filtered through 0.8- and 0.2-μm membranes. The hydrolysis studies were carried out in samples of 2 mL of plasma containing gentisic acid as internal standard. At time intervals, 5-μL aliquots were removed for assay.

For plasma hydrolysis, standard curves were obtained by dissolving the relevant acid in methanol containing gentisic acid as internal standard (0.14 mg/mL) and assaying 5-μL aliquots. The eluant was monitored at 254 nm.

The high-pressure LC analysis was carried out using a Constametric II supplied by Laboratory Data Control. Partisil PXS 10 25 or SAX column (Whatman Inc.) was used. Examples for the conditions are shown in Table VIII.

Table VIII

compound	system[a]	retention time, min
13c	A	
	B	
	C	3.3
α-cyclopentylphenylacetic acid	A	1.9
	B	2.7
	C	5.8
α-cyclohexylphenylacetic acid	A	2.2
	B	3.9
	C	1.8
phenylacetic acid	C	3.6
gentisic acid	C	6.3
14e	C	2.5
2-phenylbutyric acid	C	4.2
14f	C	2.0

[a] Column A = ODS; mobile phase, 10^{-3} M $NH_4H_2PO_4$, 65% MeOH; pH 5; rate 2.0 mL/min. Column B = ODS; mobile phase, 10^{-3} M $NH_4H_2PO_4$, 45% MeOH; pH 5; rate 2.0 mL/min. Column C = SAX; mobile phase, 10^{-2} M $NH_4H_2PO_4$, 10% MeOH; pH 5; rate 2.5 mL/min.

Pharmacology. Estimation of Acetylcholine Antagonist Potency. Strips of guinea pig whole ileum were set up in McEwan's solution, gassed with Carbogen (O_2/CO_2, 95:5), and maintained at 37 °C in a 10-mL organ bath. A tension of 1 g was applied to the tissue, and cumulative concentration–response curves recorded to acetylcholine until constant responses were obtained.

A concentration of the antagonist was then added to the bath, and after allowing 15–20 min for equilibration, further concentration–effect curves to acetylcholine were established. This procedure was then repeated using increasing concentrations of the antagonist.

Mean curves were plotted for the effects of acetylcholine alone and for acetylcholine in the presence of the various antagonist concentrations. Competitive antagonism was assessed from the dose ratios obtained.[16] Similar experiments were performed using histamine as an agonist instead of acetylcholine.

In Vivo Atropine-like Activity. Adult cats were anesthetized by the intraperitoneal injection of a mixture of α-chloralose (80 mg/kg) and sodium pentobarbitone (6 mg/kg). The animals were bilaterally vagotomized and artificially respired throughout the experiments. Arterial blood pressure was recorded from a cannulated carotid artery using a Statham PD23 pressure transducer coupled to a Grass 7C ink-writing polygraph. Heart rate was routinely measured using either the arterial pulse or lead II ECG's to trigger a cardiotachometer (Grass 7P4). In all experiments drugs were injected intravenously into a cannulated brachial vein.

Control responses were first obtained to a range of doses of acetylcholine. A dose producing 70–80% of the maximal vasodepressor response was then selected and used throughout the rest of the experiment (0.02–0.05 μkg/kg in different experiments).

After constant control responses to acetylcholine had been obtained, a dose of the test drug was injected and 1 min later a response to acetylcholine was monitored. Responses to acetylcholine were then assessed at approximately 2–3 min intervals. In this way a measure of both the activity and duration of action of the test drug could be assessed.

The effects of a number of doses of each test compound were monitored in every animal. After each test, responses to acetylcholine were allowed to return to control levels before the actions of higher doses were monitored.

For screening, each drug was made up in distilled water at a concentration of 10 mg/mL. All of the quaternary salts were freely soluble in water. The concentrations used are given in Table VII.

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