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Articles

Soft Drugs. 1. Labile Quaternary Ammonium Salts as Soft Antimicrobials[1]

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Strategies for the design of safer drugs are discussed. The various classes of "soft drugs" are designed to avoid undesired metabolic disposition (primarily various oxidative routes, occurring via possible toxic intermediates) and to be metabolized by a predictable manner with controlled rates. As a first example for the "soft analogue" type drugs, a new class of antimicrobial, surface-active quaternary salts of the type $RCOOCHR_1-N^+ < X^-$ was developed. These "soft" quaternary salts are isosteric analogues of known "hard" quaternary surfactants and are characterized by predictable and controllable cleavage (metabolism) to nontoxic components, while showing good activity against a wide range of bacteria. Due to their soft nature (low toxicity), the new antimicrobials are much safer than the conventional, hard analogues.

Successful predictions on a rational basis of the biological activity of compounds leading to new drugs are the main objective of drug designers. This is usually achieved by considering a known bioactive molecule as the basis for structural modifications, either by the group or biofunctional moieties approach or by altering overall physical–chemical properties of the molecule. Thus, the main aim is to design, synthesize, and test new compounds structurally analogous to the basic bioactive molecule which have, however, improved therapeutic and/or pharmacokinetic properties. Although "vulnerable" moieties have been identified as the ones whose role is the bioinactivation or metabolic elimination of the drug after it performed its role, little or no attention was paid in the drug-design process to the rational design of the metabolic disposition of the drugs. This is the case despite the fact that the toxicity of a number of bioactive molecules is due to their increased elimination half-life, stability, or other factors introduced during the design of increasing their activity. Drugs and particularly their metabolic processes contribute to the various toxic processes by formation of active metabolites. The phenomenon of metabolic activation to reactive intermediates which covalently bind to tissue macromolecules is the initial step in cell damage. It is also clear that the most toxic metabolites will not survive long enough to be excreted and identified; thus, studies of the stable metabolites may provide misleading information. The concept of a dose threshold[2] in explaining the toxicity of certain drugs at a higher dose level, due to depletion of glutathione, is a good example for the importance of the metabolic processes in drug toxicity.

It is clear that, in order to prevent and/or reduce toxicity problems related to drugs, the metabolic disposition of the drugs should be considered at an early stage of the drug-design process.

The basic idea of including metabolic considerations into the drug-design process is not entirely new. In one of his excellent reviews, Ariëns, has recently emphasized[3] the importance of the relationship between structure and drug metabolism. In order to avoid unwanted toxic side effects, Ariëns suggested[4] two main lines: the design of drugs which are *not metabolized* and design of drugs which can be metabolized along a pathway avoiding the highly reactive toxic intermediates (for example, it was suggested[4] to use alkyl chains as safe metabolic oxidizable handles). Ariëns suggests that designing drugs which do not metabolize is the more attractive route, since this would result in avoidance of unwanted toxicities, would simplify pharmacokinetics, and essentially it would give safe drugs. Although quite attractive, the route of the nonmetabolizable "hard" drugs is very unlikely to succeed. It is well recognized that the body can attack and alter chemically quite, stable structures and, even if a drug is 95% excreted unchanged, the unaccounted small portion can and most

(1) A preliminary account of this work was included in (a) N. Bodor, in "Design of Biopharmaceutical Properties by Prodrugs and Analogs", E. B. Roche, Ed., Academy of Pharmaceutical Sciences, Washington, D.C., 1977, Chapter 7, and (b) N. Bodor, U.S. Patent 3 989 711, Nov 2, 1976.

(2) S. D. Nelson, M. R. Boyd, and J. R. Mitchell, in "Drug Metabolism Concepts", P. M. Jerina, Ed., American Chemical Society, Washington, D.C., 1977, p 155.

(3) E. J. Ariëns, *Pharmacochem. Libr.*, **2**, 1 (1977), and references cited therein.

(4) E. J. Ariëns, *Med. Chem., Proc. Int. Symp., Main Sect., 4th*, 1974, p 303.

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likely will cause toxicity. In addition, in order to achieve nonmetabolizable quality, one has to go to pharmacokinetic extremes in the drug design: either highly lipophilic structures (for example, by steric "packing" of the metabolically sensitive parts) or very water-soluble, short half-life structures.

On the contrary, predicting metabolism of the drugs is not only possible, but it can be done on a more rational basis as more and more information on the various enzyme systems is obtained.

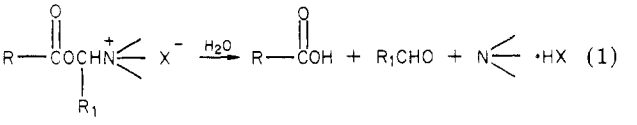
"Soft drugs" can be defined[1a] as biologically active chemical compounds (drugs) which might structurally resemble known active drugs (soft analogues) or could be entirely new types of structures, but which are all characterized by a predictable in vivo destruction (metabolism) to nontoxic moieties, after they achieve their therapeutic role. The metabolic disposition of the soft drugs takes place with a *controllable rate* in a *predictable manner*.

There are various possibilities how one can approach the rational design of soft drugs, and at this stage the soft drugs are classified in five basic types, among which the soft analogues represent a very important one. Compounds in this class are structural analogues of known active drugs, which, however, have a metabolically weak spot built in their structure, which provides their detoxification. These analogues are characterized by the following basic features: they are (1) *close structural analogues*, the whole molecule is isosteric and/or isoelectronic with the basic drug; (2) the metabolically *soft spot is built in a noncritical part* of the molecule, which results in little or no effect on the transport, affinity, and activity of the drug; (3) the built-in metabolism is the major or *preferentially the only metabolic route*; (4) *the rate* of the predicted metabolism *can be controlled* by molecular manipulations; (5) the resulting *products are nontoxic* or of very low toxicity (activity); and (6) the predicted metabolism *does not* require enzymatic processes leading to *highly active intermediates*.

Ideally, metabolism of this type of soft drugs results in profound structural changes leading to complete destruction of the binding, as well as of the active sites in the molecule. Such is the case of the soft antimicrobial agents described in the present paper.

Quaternary surface-active agents are known and widely used for numerous purposes in antimicrobial, cleansing, and cosmetic preparations, for example, cetylpyridinium chloride (1; 1-hexadecylpyridinium chloride) or benzalkonium chloride (2; a mixture of N-C_8H_{17} to N-$C_{18}H_{37}$ substituted dimethylbenzylammonium chlorides). These compounds are quite effective against both Gram-positive and Gram-negative bacteria, but they are also quite toxic (for example, LD_{50} of 1, iv (mouse), is 10 mg/kg; po, 108 mg/kg).[5] Their toxicity is related primarily to the various biological effects of the quaternary ammonium head and its metabolism (such as oxidative dealkylation), but it is also believed that the surfactant characteristics of the molecule, particularly in the liver, causes additional alterations in a number of chemical, biological, and transport phenomena.

In order to avoid systemic toxicity of the quaternary surface-active agents, we have designed a new class of soft quaternary agents, which cleave via chemical and/or enzymatic hydrolysis to nontoxic (nonquaternary and nonsurface active) moieties after they exert their antimicrobial effects. These new agents can be classified as soft analogues. The general structure of these soft antimicrobials is represented by 3. The main features of this compound are the close structural analogy to the corresponding hard quaternary salts (isosteric) and the hydrolytic sensitivity of the ester portion, leading to simultaneous destruction of both the quaternary head and the surfactant character, according to eq 1.

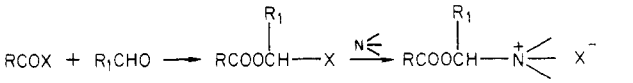


(1)

3

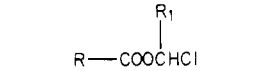
R = straight or branched long alkyl or aralkyl; R_1 = H, alkyl, aryl, etc.; ≯N = tertiary or unsaturated amine; X = Cl, Br, I, etc.

Chemistry. The soft quaternary salts (3) can easily be prepared by direct quaternization of the appropriate amines with a soft alkylating agent of the α-haloalkyl ester type 4. Compounds 4 can be obtained from the corresponding acyl halides and an aldehyde, for example, according to an early method described,[6] as shown by the following scheme:

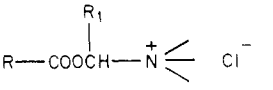


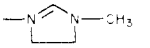
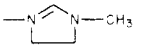
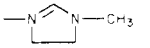
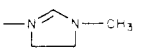
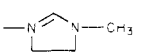
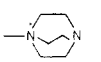
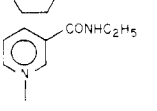
4 3

The alkylating agents (4) and the soft quaternary compounds (3) prepared and studied in this work were obtained in about 70% yield.



	R	R_1
4a	$CH_3(CH_2)_6$	H
b	$CH_3(CH_2)_{10}$	H
c	$CH_3(CH_2)_{12}$	H
d	$CH_3(CH_2)_{14}$	H
e	$CH_3(CH_2)_{10}$	CH_3
f	$CH_3(CH_2)_{14}$	CH_3



	R	R_1	N≯
3a	$CH_3(CH_2)_6$	H	pyridine
b	$CH_3(CH_2)_{10}$	H	pyridine
c	$CH_3(CH_2)_{12}$	H	pyridine
d	$CH_3(CH_2)_{14}$	H	pyridine
e	$CH_3(CH_2)_{10}$	H	
f	$CH_3(CH_2)_{12}$	H	
g	$CH_3(CH_2)_{14}$	H	
h	$CH_3(CH_2)_{10}$	CH_3	
i	$CH_3(CH_2)_{14}$	CH_3	
j	$CH_3(CH_2)_{10}$	H	
k	$CH_3(CH_2)_{10}$	H	
l	$CH_3(CH_2)_{10}$	H	

(5) (a) Toxic Substances List, ACS Edition, American Chemical Society, Washington, D.C., 1974; (b) M. R. Warren, *J. Pharmacol. Exp. Ther.*, **74**, 401 (1942).

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

Table I. Critical Micelle Concentration (cmc)[a] of Selected Soft Quaternary Salts[b]

no.	cmc, M
1[c]	1.3×10^{-4}
3b	6.5×10^{-4}
3c	1.7×10^{-4}
3g	6.0×10^{-4}

[a] Critical micelle concentration (cmc) determined in 0.1 M NaH_2PO_4 adjusted to pH 7.0; μ = 0.5, 25 ± 0.2 °C. [b] For compound structures, see text. [c] 1 = $CH_3(CH_2)_{15}Py^+Cl^-$.

Table II. Hydrolysis Kinetics for $CH_3(CH_2)_{10}COOCH_2Py^+Cl^-$ (3c)

conditions[a]	K_{obsd}, min^{-1}	$t_{1/2}$, min
pH 9.3, μ = 0.5, 25 ± 0.1 °C		
−(d[3c]/dt)	4.3×10^{-2}	16
+(d[Py]/dt)	4.3×10^{-2}	16
pH 7.0, 25 ± 0.1 °C		
μ = 0.01	1.5×10^{-3}	450
μ = 0.05	1.5×10^{-3}	450
μ = 0.10	1.5×10^{-3}	450
pH 7.0, μ = 0.1		
40 ± 0.1 °C	4.2×10^{-3}	166
45 ± 0.1 °C	5.6×10^{-3}	124
50 ± 0.1 °C	1.1×10^{-2}	64
pH 4.6, μ = 0.5		
50 ± 0.1 °C	5.2×10^{-4}	1325

[a] High-pressure LC analysis was performed on a Partisil SAX column (Whatman, Inc.). Mobile phase: 0.01 M $NH_4H_2PO_4$, 10% methanol.

Some additional physical–chemical characteristics of selected soft quaternary salts were determined in order to demonstrate their surface-active nature and their hydrolytic sensitivity. Thus, Table I shows that the critical micelle concentrations (cmc) of 1 and the isosteric soft analogue **3c** (both have 16 atoms in the straight chain) are extremely close.

As expected, the rate of chemical hydrolysis of **3c** is highly pH dependent, and it is catalyzed by OH^- ions. The results of Table II also indicate that any *N*-hydroxymethyl intermediate is highly unstable, since following the disappearance of **3c** gives the same rate constant as obtained by measuring the amount of pyridine formed.

Biology. A limited, but systematic, investigation was carried out to determine the relationship between the antimicrobial activity of the soft quaternary salts and the various structural and other factors. Thus, the alkyl chain length and the nature of the amine were varied first. The antimicrobial activity was examined first by determining the minimal inhibitory concentrations (MIC) against five selected bacteria, as compared to the structurally analogous hard quaternary salts cetylpyridinium chloride (1) and cetyltrimethylammonium bromide (6). The results are given in Table III.

The antimicrobial activities of the soft quaternary salts observed under these conditions can be considered as their equilibrium thermodynamic activity. In order to investigate the kinetic effect (time required for bacteriostatic action), the various soft quaternary salts were examined using the CGE procedure (contact germicidal efficiency). Details of the CGE method are given under Experimental Section. The shortest time interval for which growth of the microorganism is not observed is the sterilization time. The results are given in Table IV. It can be seen that at a relatively low concentration of about 0.1% all compounds, except the short chain **3a**, are quite effective.

Table III. Minimal Inhibitory Concentrations[a] of Selected Soft Quaternary Salts[b]

no.	*S. aureus*	*B. subtilis*	*S. typhimurium*	*P. aeruginosa*	*S. pyogenes*
1[d]	<2.0	<2.0	8.0	16.0	<2.0
3a	529.1	529.1	1058.2	1058.2	529.1
3b	8.9	143.1	35.8	511.5	71.5
3c	8.1	8.3	133.0	>1063.9	4.2
3d	16.7	1071.1	1071.1	1071.1	267.5
3e	4.1	16.3	65.3	261.2	<2.0
3f	<2.2	4.4	69.7	>1115.1	<2.2
3g	1.3	>42.4[c]	>42.4[c]	>42.4[c]	>42.4[c]
3l	130.4	32.6	521.7	1043.4	32.6
3j	65.5	32.8	524.2	1048.4	32.8
3k	16.4	32.8	262.1	1048.5	4.1
6[e]	<2.0	<2.0	64.3	128.5	<2.0

[a] Minimal inhibitory concentration determined in 0.1 M NaH_2PO_4 adjusted to pH 7.0 expressed in ppm. The decimal points indicate the concentrations of the actual solutions and are not to be interpreted as the accuracy of the MIC. [b] For compound structures, see text. [c] Approximately the saturated solubility. [d] 1 = $CH_3(CH_2)_{15}Py^+Cl^-$. [e] 6 (CTAB) = $CH_3(CH_2)_{14}CH_2N^+(CH_3)_3Br^-$.

Table IV. Contact Germicidal Efficiency (CGE) of Selected Soft Quaternary Salts[a]

		sterilization time,[c] min		
no.	concn, ppm[b]	*S. aureus*	*P. aeruginosa*	*S. pyogenes*
1[d]	1038.7	0.5	0.5	0.5
	936.0	0.5	0.5	0.5
3a	1035.9	>30	>30	>30
3b	1010.9	0.5	0.5	0.5
	896.0	0.5	0.5	0.5
3c	1042.1	1	1	0.5
3e	902	0.5	0.5	0.5
	1068	0.5	0.5	0.5
3f	1012.2	0.5	0.5	0.5
3l	1116.0	0.5	3	0.5
3j	1026	0.5	3	0.5
3k	1027	0.5	0.5	0.5
6[e]	1031	0.5	0.5	0.5

[a] In 0.1 M NaH_2PO_4 adjusted to pH 7.0. [b] The decimal points indicate the concentrations of the actual solutions and are not to be interpreted as the accuracy of the measurements. Amounts were weighed to obtain approximately 1000-ppm solutions; the deviations are due to differences in weighing. [c] Time intervals screened: 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, and 30 min. [d] 1 = $CH_3(CH_2)_{15}Py^+Cl^-$. [e] 6 = $CH_3(CH_2)_{15}N^+(CH_3)_3Br^-$.

It appears that both the MIC levels and CGE values are more affected by the chain length (i.e., surface activity) than the nature of the amine portion, although it is clear that derivatives of *N*-methylimidazole are the most effective. It was found that the pH does not affect substantially the activity as shown in Table V.

On the other hand, addition of horse serum to the soft quaternary salts solutions cuts their activity dramatically. The most active compounds **3e** or **3c** lose their CGE within 1 min after the addition of the horse serum, although the hard quaternary salts **1** and **6** are also affected. (Thus, at a concentration of 0.1%, **1** completely lost its activity against *P. aeruginosa* but not against *S. aureus*). The quinuclidine derivative **3k** maintains its activity the best in the presence of horse serum, as shown in Table VI.

A comparison between the sterilization times determined for the most active soft quaternary derivative **3e**, as a function of its concentration (Table VII), demonstrates that significant bactericidal activity is observed at concentrations near its MIC level.

Table V. Effect of pH on the Contact Germicidal Efficiency of Selected Soft Quaternary Salts

no.	pH	concn, ppm[b]	sterilization time, min[a] S. aureus	P. aeruginosa	S. pyogenes
1	6.0	1018	0.5	0.5	0.5
	7.0	1039	0.5	0.5	0.5
	8.0	1018	0.5	0.5	0.5
3b	6.0	1016	1	0.5	0.5
	7.0	1010	0.5	0.5	0.5
	8.0	1013	0.5	2	7
3e	6.0	1005	1	0.5	0.5
	7.0	1068	0.5	0.5	0.5
	8.0	1022	0.5	0.5	0.5
6	6.0	1007	0.5	0.5	0.5
	7.0	1031	0.5	0.5	0.5
	8.0	1042	0.5	0.5	0.5
3j	6.0	1009	7	0.5	0.5
	7.0	1026	0.5	3	0.5
	8.0	1008	0.5	4	3
3k	6.0	1016	1	0.5	0.5
	7.0	1027	0.5	3	0.5
	8.0	1022	0.5	0.5	2

[a] Time intervals screened: 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, and 30 min. [b] See footnote b in Table IV.

Table VI. Contact Germicidal Efficiency in the Presence of Horse Serum as a Function of Time[a]

no.	concn, ppm	diluent	contact time, min	sterilization time:[b] S. aureus
3k	1084	water		0.5
	1084	horse serum	$t = 0$	1
			$t = 5$	3
			$t = 10$	5
			$t = 15$	>30

[a] Contact germicidal efficiency determined in 0.1 M NaH_2PO_4, pH 7.0, diluted with horse serum, as described under Experimental Section. [b] Time intervals screened: 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, and 30 min.

Table VII. Contact Germicidal Efficiency as a Function of Concentration[a]

no.	concn, ppm	sterilization time, min[b] S. aureus	P. aeruginosa	S. pyogenes
3e	1068	0.5	0.5	0.5
	507	1	0.5	0.5
	258	2	0.5	0.5
	125	5	0.5	3
	63	$10 < t \leq 15$	1	6
	32	>30	>30	>30

[a] Contact germicidal efficiency determined in 0.1 M NaH_2PO_4, pH 7.0. [b] Time intervals screened: 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, and 30 min.

In order to verify the assumption as to the soft nature of the compounds studied, implying low toxicity due to their facile systemic metabolism, the acute toxicity of **3e** was studied. The results, as compared to the toxicity of the hard quaternary salt **1**, are shown in Table VIII.

Discussion

It is clear that it is possible to develop active, isosteric soft analogues of the hard antimicrobial quaternary salts. The new class of the surface-active soft quaternary salts is quite effective against a wide range of bacteria. The activity of **3e** is about 10 times less than that of the widely used **1**, but it is still highly effective at a relatively low, 0.1% (or even at 0.01%), concentration, which is less than

Table VIII. Relative Toxicity of $CH_3(CH_2)_{10}COOCH_2Im^+Cl^-$ (3e) vs. $CH_3(CH_2)_{15}Py^+Cl^-$ (1)

	LD_{50}, mg/kg (white swiss male mice) 3e	1[a]
iv	75-100	
ip	155 (140-160)[b]	10
po	4110 (2800-6000)[b]	108

[a] See ref 5. [b] The values in parentheses indicate 95% confidence limits.

the concentration of **1** generally used. On the other hand, **3e** is 15–40 times less toxic than **1**, which should more than compensate for the lower activity. When cleaved, compounds of type **3** lose both the surfactant and quaternary salt character, and thus any activity, as shown in Table IX.

Compounds of type **3** are easily deactivated by serum, due to their binding, on the one hand, and to their facile esterase cleavage, on the other hand. We have found that similar soft quaternary salts, such as *N*-[[(phenylacetyl)oxy]methyl]methylmorpholinium chloride (**7**), have only a 3.75-min half-life in dog serum.[7] On the other hand, compounds **3** are stable in the neat state and can easily be solubilized prior to use.

Experimental Section

Synthesis. General Procedure for 4a–f. Equimolecular amounts of the corresponding acyl chloride and paraformaldehyde (or paraldehyde, respectively) were mixed and heated between 90 and 100 °C for 3–6 h. The obtained chloromethyl and α-chloroethyl esters were purified by chromatography or crystallization (see Table X).

Chloromethyl *n*-Octanoate (4a): chromatographed on Florisil (petroleum ether, 30–60 °C); IR (neat) 2960, 2930, 1770, 1460, 1255, 1130, 1090, 1030, 700 cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.8 (s, 2 H), 2.4 (t, 2 H), 1.3 (br s, 10 H), 0.9 (br t, 3 H). Anal. ($C_9H_{17}ClO_2$) C, H.

1-[(*n*-Octanoyloxy)methyl]pyridinium Chloride (3a). A mixture of 1.93 g (0.01 mol) of chloromethyl *n*-octanoate and 0.79 g (0.01 mol) of pyridine was mixed and heated at 90 °C for 3 h. When the mixture cooled to room temperature, anhydrous ether was added and the mixture was triturated in anhydrous ether overnight. The solid was isolated by filtration under a nitrogen atmosphere and thoroughly washed with anhydrous ether. After drying in vacuo over calcium sulfate at room temperature, 1.90 g (0.007 mol, 70%) of **3a** was obtained as a white solid: mp 102–107 °C; IR (KBr) 3430, 3040, 2970, 1770, 1635, 1490, 1110, 760, 670 cm^{-1}; ^1H NMR ($CDCl_3$) δ 9.9 (d, 2 H), 8.8 (t, 1 H), 8.3 (t, 2 H), 7.0 (s, 2 H), 2.4 (t, 2 H), 1.3 (br s, 10 H), 0.9 (br t, 3 H). Anal. ($C_{14}H_{22}ClNO_2 \cdot H_2O$) C, H, N. Using the procedure described for the preparation of **3a**, the [(*n*-alkylcarboxy)methyl]pyridinium salts were prepared (Table XI).

1-[(*n*-Dodecanoyloxy)methyl]-3-methylimidazolium Chloride (3e). A mixture of 2.49 g (0.01 mol) of chloromethyl *n*-dodecanoate and 0.82 g (0.01 mol) of *N*-methylimidazole was mixed and heated at 90 °C for 3 h. When the mixture cooled to room temperature, anhydrous ether was added and the mixture was triturated in anhydrous ether overnight. The solid was isolated by filtration under a nitrogen atmosphere and thoroughly washed with anhydrous ether. After drying in vacuo over calcium sulfate at room temperature, 2.4 g (0.007 mol, 70%) of **3e** was obtained as a white solid: mp 60–63 °C; IR (KBr) 3400, 3110, 2960, 2920, 1750, 1470, 1140, 770 cm^{-1}; ^1H NMR ($CDCl_3$) δ 10.8 (s, 1 H), 8.0 (d, 2 H), 6.4 (s, 2 H), 4.2 (s, 3 H), 2.4 (t, 2 H), 1.4 (br s, 18 H), 0.9 (br t, 3 H). Anal. ($C_{16}H_{31}ClN_2O_2 \cdot H_2O$) C, H, N.

1-[(*n*-Dodecanoyloxy)methyl]-1,4-diazabicyclo[2.2.2]octane Chloride (3j). Chloromethyl *n*-dodecanoate (2.49 g, 0.01

(7) N. Bodor, R. Woods, C. Raper, P. Kearney, and J. J. Kaminski, *J. Med. Chem.*, **23**, following paper in this issue (1980).

Table IX. Soft Quaternary Contact Germicidal Efficiency[a] of 3e vs. Its Decomposition Products

compd	concn, ppm	sterilization time[b]		
		S. aureus	*P. aeruginosa*	*S. pyogenes*
3e	1000	0.5	0.5	0.5
$CH_3(CH_2)_{10}CO_2H$	610 ppm ≡ 1000[c]	>30	>30	>30
CH_2O	100 ppm ≡ 1000[c]	>30	>30	>30
	254 ppm ≡ 1000[c]	>30	>30	>30
mixture	610 + 100 + 254 ppm ≡ 1000	>30	>30	>30

[a] Contact germicidal efficiency determined using 0.1 M NaH_2PO_4, pH 7.0. [b] Time intervals screened: 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, and 30 min. [c] Equivalent to 1000 ppm of soft quaternary derivative.

Table X. Details for Previously Unreported α-Chloro Esters

no.	method of purifn[a]	formula	anal.	spectra[b]
4b	A	$C_{13}H_{25}ClO_2$	C, H	NMR, IR
4c	A	$C_{15}H_{29}ClO_2$	C, H	NMR, IR
4d	B	$C_{17}H_{33}ClO_2$	C, H	NMR, IR
4e	A	$C_{14}H_{27}ClO_2$	C, H	NMR, IR
4f	B	$C_{18}H_{35}ClO_2$	C, H	NMR, IR

[a] A, chromatographed on Florisil (petroleum ether, 30–60 °C); B, crystallized from petroleum ether. [b] NMR in $CDCl_3$; IR (neat), similar to 4a.

Table XI. Characteristics of Some Novel Surface Active Soft Quaternary Salts

no.	method	mp, °C	formula	anal.	spectra[c]
3b	a	120–124	$C_{18}H_{30}ClNO_2$	C, H, N	NMR, IR
3c	a	104–109	$C_{20}H_{34}ClNO_2 \cdot H_2O$	C, H, N	NMR, IR
3d	a	132–135	$C_{22}H_{38}ClNO_2$	C, H, N	NMR, IR
3f	b	68–74	$C_{19}H_{35}ClN_2O_2 \cdot H_2O$	C, H, N	NMR, IR
3g	b	80–84	$C_{21}H_{39}ClN_2O_2 \cdot H_2O$	C, H, N	NMR, IR
3h	b	54–59	$C_{18}H_{33}ClN_2O_2 \cdot H_2O$	C, H, N	NMR, IR
3i	b	68–71	$C_{22}H_{41}ClN_2O_2 \cdot H_2O$	C, H, N	NMR, IR

[a] See method for 3a. [b] See method for 3e. [c] NMR in D_2O; IR, KCl pellets.

mol) and 1,4-diazabicyclo[2.2.2]octane (1.12 g, 0.01 mol) were mixed and allowed to react together at room temperature for 72 h. Anhydrous ether was added to the mixture and the mixture was triturated in anhydrous ether overnight. The solid was isolated by filtration under a nitrogen atmosphere and thoroughly washed with anhydrous ether. After drying in vacuo over calcium sulfate at room temperature, 2.0 g (0.006 mol, 60%) of **3j** was obtained as a white solid: mp 106–110 °C; IR (KBr) 3400, 2960, 2920, 1760, 1460, 1120, 1080, 1050, 850 and 830 cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.8 (s, 2 H), 4.2–3.0 (mq, 12 H), 2.6 (t, 2 H), 1.3 (br s, 18 H), 0.9 (br t, 3 H). Anal. ($C_{19}H_{37}ClN_2O_2 \cdot H_2O$) C, H, N.

[(*n*-Dodecanoyloxy)methyl]quinuclidinium Chloride (3k). Chloromethyl *n*-dodecanoate (2.49 g, 0.01 mol) and quinuclidine (1.12 g, 0.01 mol) were mixed and allowed to react together at room temperature for 48 h. Anhydrous ether was added to the mixture, and the mixture was triturated in anhydrous ether overnight. The solid was isolated by filtration under a nitrogen atmosphere and thoroughly washed with anhydrous ether. After drying in vacuo over calcium sulfate at room temperature, 2.1 g (0.006 mol, 60%) of **3k** was obtained as a white solid: mp 170–172 °C; IR (KBr) 2960, 2930, 1765, 1470, 1120, 1090, 860, 830 cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.8 (s, 2 H), 3.9 (t, 6 H), 2.5 (t, 2 H), 2.2 (m, 7 H), 1.3 (br s, 18 H), 0.9 (t, 3 H). Anal. ($C_{20}H_{38}ClNO_2$) C, H, N.

1-[(*n*-Dodecanoyloxy)methyl]-*N*-ethylnicotinamide Chloride (3l). Chloromethyl *n*-dodecanoate (2.49 g, 0.01 mol) and *N*-ethylnicotinamide (1.50 g, 0.01 mol) were mixed and heated together at 90 °C for 1 h. When the mixture cooled to room temperature, anhydrous ether was added and the mixture was triturated in anhydrous ether overnight. The solid was isolated by filtration under a nitrogen atmosphere and thoroughly washed with anhydrous ether. After drying in vacuo over calcium sulfate at room temperature, 2.6 g (0.007 mol, 70%) of **3l** was obtained as a white solid: mp 131–135 °C; IR (KBr) 3220, 3060, 2965, 2930, 1770, 1680, 1640, 1470, 1110, 670 cm^{-1}; ^1H NMR ($CDCl_3$) δ 10.5 (s, 1 H), 9.8 (m, 3 H), 8.3 (t, 1 H), 6.8 (s, 2 H), 3.6 (q, 2 H), 2.5 (t, 2 H), 1.3 (br s, 21 H), 0.9 (br t, 3 H). Anal. ($C_{21}H_{35}ClN_2O_3$) C, H, N.

Physical–Chemical Studies. Critical Micelle Concentration (CMC). A concentrated solution of each compound was prepared in a 0.1 M NaH_2PO_4 solution (pH adjusted to 7 with NaOH; $\mu = 0.5$), assayed, and serially diluted to produce a range of concentrations. The solutions were equilibrated at 25 ± 0.2 °C. Using a Hitachi Perkin-Elmer MPF-2A spectrophotofluorimeter to excite the solutions at a wavelength of 410 nm, the intensity of the Raman peak of water occurring at 478 nm was recorded. The intensity was plotted against concentration and the inflection point in the curve determined the critical micelle concentration (cmc).

Chemical Kinetics. For each kinetic run, a stock solution of 2×10^{-3} M **3c** was prepared using the appropriate buffered solution previously equilibrated to the desired temperature. Sodium dihydrogen phosphate was used to prepare buffered solutions at pH 4.6 and 7.0, while sodium bicarbonate and sodium carbonate were used to prepare the buffer at pH 9.3. Ionic strength was adjusted with sodium chloride.

Periodically, samples (10 μL) were removed from the solution, and the concentration of **3c** and that of pyridine formed were determined by high-pressure liquid chromatography (LC). The decrease in the concentration of **3c** (at 254 nm) and the increase of pyridine concentration (at 380 nm) were monitored. Analysis as a first-order kinetic process gave the rate constants shown in Table II.

Biological Tests. Antibacterial Studies. Determination of the Minimum Inhibitory Concentrations (MIC). Prior to determination of the MIC, all bacteria were cultured for 18–20 h (37 °C) in tryptose phosphate broth, except *Streptococcus pyogenes* (ATCC 19615) which was cultured in brain heart infusion broth plus 10% normal horse serum. Immediately prior to testing, each culture was adjusted to an optical density of 0.01 (650 nm) and diluted subsequently into double-strength medium to approximately 2×10^5 viable organisms/mL. The other organisms used were *Staphylococcus aureus* (ATCC 6538), *Pseudomonas aeruginosa* (ATCC 6051), *Salmonella typhimurium* (ATCC 14028), and *Bacillus subtilis* (ATCC 6051).

The diluted cultures were aseptically distributed in 1 mL amounts of five sterile culture tubes for each organism. The test solution of the compound to be investigated was prepared using 0.1 M sodium dihydrogen phosphate buffered to pH 7.0 and subjected to twofold dilutions into sterile water. Then, 1 mL of each dilution was dispensed to separate tubes containing the organisms. The inoculated tubes were incubated at 37 °C for 18–20 h, and the MIC of the compound was determined. Controls were run to ensure viability of the organism, sterility, and lack or bactericidial effect from the buffer.

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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