0.5 hr. Methylcyclopropyl ketone (8.4 g.) in 25 ml. of ether was then added and stirring was continued for 1 hr. more. The reaction was mixed with 150 ml. of water and the ether layer was then separated and dried. Distillation yielded 1.5 g. of XIII, b.p. 50° (43 mm.). The infrared spectrum showed a carbon–carbon stretching band at 6.22 μ and a cyclopropyl band at 9.51 μ. Other bands were at 3.25, 3.41, 3.72, 4.77, 5.23, 7.01, 7.48, 8.28, 8.64, 9.51, and 12.50 μ. Compound XIII was separated into its *cis* and *trans* isomers using preparative v.p.c. (Ucon oil column). The n.m.r. spectrum of one isomer consisted of a multiplet for cyclopropyl CH_2 centered at τ 9.37 (relative area 4), a methyl singlet at τ 8.60 (relative area 3), and a broad peak for the vinyl proton at τ 4.28 (relative area 1). The n.m.r. spectrum of the other isomer likewise consisted of a multiplet centered at τ 9.49, a singlet at τ 8.36, and a broad peak at τ 4.25 (relative areas 4:3:1). No attempt was made to assign which isomer was *cis* and which was *trans*. The infrared spectrum of each isomer was indistinguishable from the mixture of the two.

Anal. Calcd. for C_6H_9Cl: C, 61.78; H, 7.73; Cl, 30.49. Found: C, 61.71; H, 7.87; Cl, 30.26.

7,7-Dichlorobicyclo[4.1.0]hept-2-ene (XXI).—Potassium *t*-butoxide (33.7 g.) was slurried in 500 ml. of dry *n*-pentane containing 46.2 g. of cyclohexa-1,3-diene. The mixture was cooled in an ice–salt bath, and chloroform (34.6 g.) was added dropwise over a 2-hr. period, the temperature being kept below 0°. The reaction mixture was then warmed to room temperature and washed with dilute hydrochloric acid. The organic layer was separated, washed with water, dried, and distilled. Compound XXI (18.5 g.) was obtained as a colorless, sweet-smelling liquid, b.p. 65° (5.5 mm.). The infrared spectrum of XXI showed bands at 6.00 (C=C stretch) and 9.53 μ (cyclopropyl). Other bands were at 3.42, 6.20, 7.05, 7.22, 7.71, 8.18, 10.25, 11.89, 12.20, and 13.65 μ. The n.m.r. spectrum of this material consists of multiplets centered at τ 8.78 (cyclopropyl CH), 7.82 (methylene CH_2), 7.02 (allylic CH_2), and 3.98 (vinyl). All four groups of signals have approximately the same areas.

Anal. Calcd. for $C_7H_8Cl_2$: C, 51.53; H, 4.91; Cl, 43.56. Found: C, 51.53; H, 4.88; Cl, 43.58.

4-Dichloromethylenecyclohexene (XXII).—Compound XXI was passed through a 1-m. Vycor tube packed with glass wool and held at 275°, 18 times at a rate of 30 ml./hr. The product showed only two major peaks in the v.p.c. These two materials were separated by preparative v.p.c. on a Ucon oil column. One was shown, by comparison with an authentic sample, to be benzyl chloride. The other was shown to be compound XXII. The infrared spectrum of XXII consisted of a carbon–carbon stretching absorption at 6.32 μ and other bands at 3.41, 6.33, 7.14, 7.42, 7.68, 8.01, 8.24, 9.53, 10.18, and 12.20 μ. The n.m.r. spectrum of XXII consisted of a multiplet for methylene protons centered at τ 8.15 (relative area 3) and an olefinic proton signal centered at τ 4.33 (relative area 1).

Anal. Calcd. for $C_7H_8Cl_2$: C, 51.53; H, 4.91; Cl, 43.56. Found: C, 51.47; H, 4.90; Cl, 43.64

Kinetic Measurements.—Samples (0.5 ml.) of compounds I or V were placed in 25-ml. Pyrex ampoules and thoroughly degassed on a vacuum line at $<10^{-6}$ mm. They were then sealed at $<10^{-6}$ mm. and placed in a furnace held at $\pm 1°$ of the reaction temperature by an electronic controller. Sample tubes were removed at 15-min. intervals and quenched in a stream of compressed air after which they were analyzed by v.p.c. Products obtained in this way were shown to be identical with those obtained by pyrolysis in open tubes.

Analogs of Neuroeffectors. V. Neighboring-Group Effects in the Reactions of Esters, Thiolesters, and Selenolesters. The Hydrolysis and Aminolysis of Benzoylcholine, Benzoylthiolcholine, Benzoylselenolcholine, and of Their Dimethylamino Analogs[1]

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To obtain information about the relative importance of neighboring-group effects in the reactions of isologous esters, thiolesters, and selenolesters, a study of the aminolysis and hydrolysis of 2-dimethylaminoethyl benzoate, 2-dimethylaminoethyl thiolbenzoate, 2-dimethylaminoethyl selenolbenzoate, benzoyl choline, benzoyl thiolcholine, and benzoyl selenolcholine was carried out. Aminolysis rates increased sharply from ester to thiolester to selenolester. Tertiary and quaternary analogs reacted with *n*-butylamine at essentially identical rates. The three choline isologs underwent hydrolysis at rather similar rates, the order of reactivity being selenolester > ester > thiolester. Below a pH of 8 the ability of the selenolester and, to a lesser extent, of the thiolester to interact with phosphate became kinetically significant. For the tertiary amino compounds, plots of pH against log k_{obsd} yielded sigmoid curves from which the dissociation constants of the dimethylammonium groups could be derived. Anchimeric assistance by the dimethylamino group accelerated the reaction rates of all the tertiary amino compounds above those of the choline analogs below a pH of 11; this effect was more pronounced for the thiolester and selenolester than for the ester. Nucleophilic catalysis rather than general acid–specific base catalysis appears to be involved as suggested by the observation that the relative order of hydrolysis, selenolester faster than thiolester faster than ester, is that previously noted for the aminolysis and different from that normally noted for the hydrolysis of such isologs. At pH values above 11, the tertiary amino compounds underwent hydrolysis more slowly than their quaternary analogs.

Since the recognition of the crucial metabolic roles played by thioacyl derivatives of coezyme A[2] in biological systems, a great deal of attention has been centered on the comparative mechanisms of the reactions of thiolesters[3–11] and of esters. It was noted that, while hydrolysis rates were affected relatively little when the oxygen of esters was replaced by sulfur,

(1) This work was supported, in part, by grants from the National Science Foundation (GB-1626) and the National Cancer Institute of the U. S. Public Health Service (CA-3937-07).

(2) L. Jaenicke and F. Lynen, "The Enzymes," Vol. 3B, 2nd Ed., Academic Press Inc., New York, N. Y., p. 3 ff.

(3) L. H. Noda, S. A. Kuby, and H. A. Lardy, *J. Am. Chem. Soc.*, **75**, 913 (1953).

(4) R. Schwyzer, *Helv. Chim. Acta*, **36**, 414 (1953).

(5) (a) P. J. Hawkins and D. S. Tarbell, *J. Am. Chem. Soc.*, **75**, 2982 (1953); (b) J. T. G. Overbeek and V. V. Koningsberger, *Proc. Koninkl. Ned. Akad. Wetenschap.*, **B57**, 464 (1954); (c) R. Benesch and R. E. Benesch, *ibid.*, **78**, 1597 (1956).

(6) T. C. Bruice, *ibid.*, **81**, 544 (1959).

(7) W. P. Jencks, S. Cordes, and J. Carriuolo, *J. Biol. Chem.*, **235**, 3608 (1960).

(8) K. A. Connors and M. L. Bender, *J. Org. Chem.*, **26**, 2498 (1961).

(9) T. C. Bruice, J. J. Bruno, and W. S. Chou, *J. Am. Chem. Soc.*, **85**, 1659 (1963).

(10) L. R. Fedor and T. C. Bruice, *ibid.*, **86**, 4117 (1964).

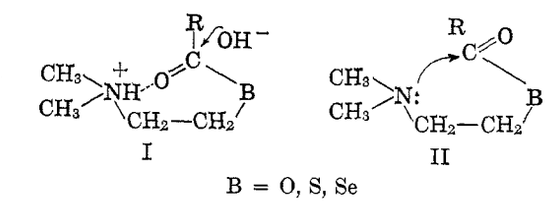
(11) T. C. Bruice and L. R. Fedor, *ibid.*, **86**, 4886 (1964).

thiolesters underwent aminolysis more readily than their oxygen isologs. For instance,[8] ethyl *p*-nitrothiolbenzoate, but not ethyl *p*-nitrobenzoate, will undergo nucleophilic attack by *n*-butylamine at 25°. Recently, Bruice and co-workers[9-11] investigated the relative abilities of δ-thiovalerolactone and of *p*-nitrophenyl acetate to react with numerous bases. They postulated[9] that, for those nucleophiles that have nearly identical reaction rates with the ester and the thiolactone, the determined rate constants referred to attack by the nucleophile on the carbonyl carbon, while, for those nucleophiles reacting at appreciably different rates with ester and thiolactone, partitioning of the tetrahedral transition intermediate was of kinetic significance. While this postulate is reasonable, it should be noted that, in comparing structurally dissimilar acyl and thioacyl compounds, steric differences may complicate interpretations of observed differences in reaction rates. This problem can be minimized by comparing isologous thiolesters and selenolesters, such compounds being sterically, if not electronically, very similar, in their abilities to react with various nucleophiles. A comparative study of the reactions of esters, thiolesters, and selenolesters would have the further advantage of yielding three-point relationships.

During the course of work aimed at the synthesis of selenocoenzyme A,[12] studies with model compounds indicated that selenolesters undergo aminolysis much more readily than isologous thiolesters.[13,14] The differences in reactivity were ascribed to the relatively favorable entropy of activation in the case of the selenoacyl as compared to the thioacyl compound and related to the high leaving tendency from the tetrahedral transition state of the selenomercaptide as compared to the mercaptide group.[14] The ability of selenolesters to undergo aminolysis under very mild conditions has recently found a useful application in the synthesis of peptides.[15]

The model compounds used in our original studies,[13,14] N,S-dibenzoylcysteamine and N,Se-dibenzoylselenocysteamine, had the disadvantage of being only slightly soluble in water so that kinetic measurements had to be made in ethanol solution. Furthermore, the reactions of the oxygen analog could not be followed spectrophotometrically because of interference by the ultraviolet absorption peak of the benzamido group. During a search for more suitable isologous esters, thiolesters, and selenolesters, the study of benzoylcholine, benzoylthiocholine, and benzoylselenocholine[16] as well as of their dimethylamino analogs was undertaken. This work gained added interest when it was noted[17] that the ability of acetylcholine to act as a neutrotransmittor was modified greatly when the oxygen of the ester was replaced by sulfur and selenium.

It has been known for some time that the hydrolysis rates of esters of choline or of cholinethiol differed from the hydrolysis rates of the corresponding 2-dimethylaminoethyl compounds. The latter compounds underwent hydrolysis considerably more rapidly than their trimethylammonium analogs[18-21] at a pH below 10. This effect was ascribed either to intramolecular general acid catalysis by the proton of the dimethylammonium group[19,21] (I), or to intramolecular nucleophilic catalysis by the dimethylamino group[22] (II). The



B = O, S, Se

finding that, near neutrality, choline esters undergo hydrolysis more slowly than their dimethylamino analogs is of interest, since, in the presence of acetylcholinesterase, the opposite relative order of hydrolysis rates for choline esters and their dimethylamino analogs is observed.[23] In order to study the relative importance of anchimeric assistance in the reactions of esters, thiolesters, and selenolesters, a comparison of the hydrolysis rates of benzoylcholine, benzoylthiocholine, benzoylselenocholine, 2-dimethylaminoethyl benzoate, 2-dimethylaminoethyl thiolbenzoate, and 2-dimethylaminoethyl selenolbenzoate was carried out over a 7–13 pH range. The aminolysis rates of these compounds were compared as well.

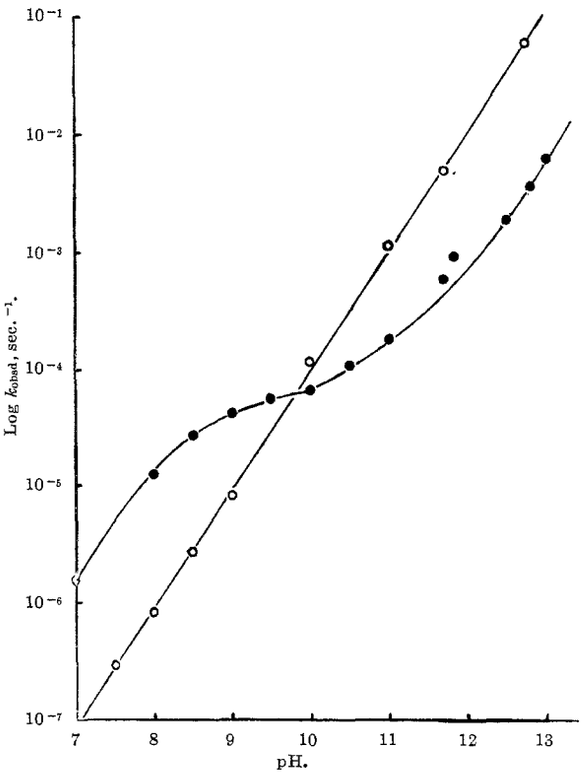


Figure 1.—Hydrolysis of benzoylcholine [$(CH_3)_3N^+CH_2CH_2OOCC_6H_5$, ○] and 2-dimethylaminoethyl benzoate [$(CH_3)_2NCH_2CH_2OOCC_6H_5$, ●].

(12) W. H. H. Günther and H. G. Mautner, *J. Am. Chem. Soc.*, **87**, 2708 (1965).
(13) H. G. Mautner and W. H. H. Günther, *ibid.*, **83**, 3342 (1961).
(14) H. G. Mautner, S. H. Chu, and W. H. H. Günther, *ibid.*, **85**, 3458 (1963).
(15) H. D. Jakubke, *Chem. Ber.*, **97**, 2816 (1964).
(16) W. H. H. Günther and H. G. Mautner, *J. Med. Chem.*, **7**, 229 (1964).
(17) K. A. Scott and H. G. Mautner, *Biochem. Pharmacol.*, **13**, 907 (1964).
(18) T. Wieland and H. Hornig, *Ann.*, **600**, 12 (1956).
(19) B. Hansen, *Acta Chem. Scand.*, **12**, 324 (1958).
(20) A. Ågren, U. Hedsten, and B. Jonsson, *ibid.*, **15**, 1532 (1961).
(21) E. Schätzle, M. Rottenberg, and M. Thürkauf, *Helv. Chim. Acta*, **42**, 1708 (1959).
(22) W. P. Jencks, *Ann. Rev. Biochem.*, **32**, 641 (1963).
(23) I. B. Wilson and E. Cabib, *J. Am. Chem. Soc.*, **78**, 202 (1956).

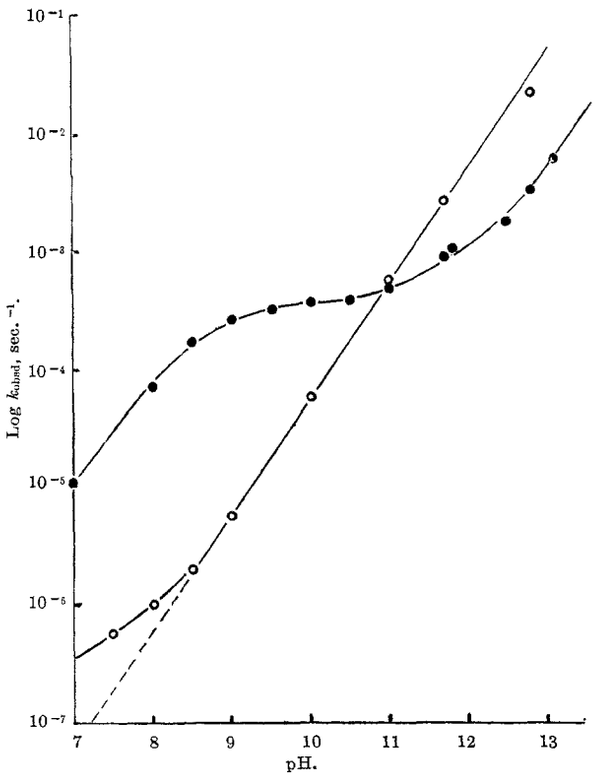


Figure 2.—Hydrolysis of benzoylthiolcholine [$(CH_3)_3N^+CH_2CH_2SC(=O)C_6H_5$, O] and 2-dimethylaminoethyl thiolbenzoate [$(CH_2)_2NCH_2CH_2SC(=O)C_6H_5$, ●].

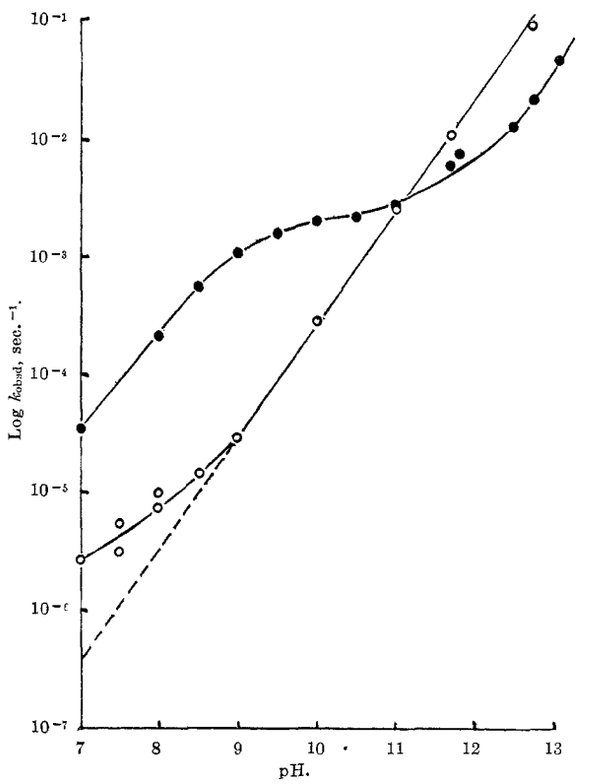


Figure 3.—Hydrolysis of benzolyselenolcholine [$(CH_3)_3N^+CH_2CH_2SeC(=O)C_6H_5$, O] and 2-dimethylaminoethyl selenobenzoate [$(CH_3)_2NCH_2CH_2SeC(=O)C_6H_5$), ●].

Experimental Section

Materials.—Benzoylcholine,[24] benzoylthiolcholine,[25] benzoylselenolcholine,[16] 2-dimethylaminoethyl benzoate,[21] 2-dimethylaminoethyl thiolbenzoate,[25] and 2-dimethylaminoethyl selenolbenzoate[16] were synthesized according to literature methods. Each compound was recrystallized at least three times. *n*-Butylamine was purified by distillation from solid potassium hydroxide.

Kinetic Measurements.—For hydrolysis experiments, 1.0-ml. aliquots of ester solution (5.44×10^{-4} *M*) were mixed with 2.0-ml. aliquots of phosphate buffer of the desired pH adjusted to constant ionic strength (0.66) with sodium chloride, and placed in thermostated, stoppered quartz cuvettes. For the aminolysis experiments 1.0-ml. solutions of the acyl derivatives (5.44×10^{-4} *M*) were mixed with 2.0-ml. aliquots of *n*-butylamine (0.161 *M*) in phosphate solution. Reaction rates were followed by observing the disappearance of ultraviolet absorption at 230 mμ for the benzoates, at 275 mμ for the thiolbenzoates, and at 285 mμ for the selenolbenzoates. Either a Cary Model 15 recording spectrophotometer or a Beckman Model DU spectrophotometer with a Gilford optical density converter equipped with a Leeds and Northrup recorder was used. Temperatures inside the cell compartment were determined with a telethermometer (YSI). Plots of log ($O.D._{obsd} - O.D._{\infty}$) against time gave straight lines the slopes of which, when multiplied by 2.303, yielded the first-order rate constants.

For very slow reactions, 5-ml. samples of reaction mixture were introduced into ampoules, under nitrogen, with a hypodermic syringe equipped with a Millipore filter. The progress of the reactions was followed spectrophotometrically by analyzing the contents of individual ampoules. All determinations were carried out at least in duplicate.

Hydrolysis kinetics, in the absence of buffer, were measured by means of a Radiometer (Copenhagen) titrator (TTTlc) and Titrigraph (SBR2c). Aliquots of 10.0 ml. were placed in a thermostated vessel under nitrogen and the consumption of base was recorded. Corrections had to be made for the release of cholinethiol and cholineselenol, these compounds having pK_a values of 7.7 and 4.7, respectively.

Isolation of Products.—For every reaction studied, isolation of the products in yields ranging from 60 to 98% was carried out using solutions of similar concentration as those used in kinetic experiments.

For hydrolysis studies, the solutions were kept at room temperature until the reaction had essentially reached completion; then they were concentrated, acidified with hydrochloric acid, and extracted exhaustively with ether to remove benzoic acid. Treatment of the aqueous layer with sodium hydroxide solution was followed by ether extraction. The bubbling of hydrogen chloride gas into the dried ether extract resulted in the separation of bis(2-dimethylaminoethyl) disulfide or diselenide in the form of the hydrochloride. Choline disulfide and choline diselenide were isolated as the Reinecke salts.[26] The progress of the aminolysis reactions was followed as described in a previous publication.[14]

Discussion of Results

The results of the studies dealing with the hydrolysis of the benzoyl esters of choline, cholinethiol, and cholineselenol are summarized in Table I and in Figures 1–3. Plots of the observed rate constants against pH, above a pH of 8.5, yielded straight lines with a slope of 0.96 for benzoylcholine, 1.00 for benzoylthiocholine, and 1.02 for benzoylselenocholine, indicating specific base catalysis for all three choline esters over the pH range studied. The following second-order rate constants were obtained: benzoylcholine, 1.14 sec.$^{-1}$ M^{-1}; benzoylthiocholine, 0.63 sec.$^{-1}$ M^{-1}; and benzoylselenocholine, 2.85 sec.$^{-1}$ M^{-1}. It can be seen that the three chalcogenic esters underwent hydrolysis at similar rates. The order of reactivity, selenolester greater than ester greater than thiolester, is the

(24) J. M. Gulland, M. W. Partridge, and S. S. Randall, *J. Chem. Soc.*, 419 (1940).

(25) R. R. Renshaw, P. F. Dreisbach, M. Ziff, and D. Green, *J. Am. Chem. Soc.*, **60**, 1765 (1938).

(26) A. D. Marenzi and C. E. Cardini, *J. Biol. Chem.*, **147**, 363 (1943).

TABLE I

KINETICS OF HYDROLYSIS OF BENZOYLCHOLINE, BENZOYLTHIOLCHOLINE, AND BENZOYLSELENOLCHOLINE OVER A 7.0–12.8 pH RANGE[a]

pH	10^{-5} k_{obsd}, sec.$^{-1}$ M^{-1}		
	Selenolester	Thiolester	Ester
7.0	0.255	*b*	*b*
7.5	0.587	0.0573	0.0288
8.0	0.725	0.100	0.0854
8.5	1.47	0.199	0.271
9.0	2.98	0.547	0.817
10.0	29.40	5.98	12.10
11.0	280.0	64.5	113.0
11.7	1200	265	480
12.8	9590	2380	5590

[a] Initial concentration of ester, 1.81×10^{-4} M[3]; ionic strength, 0.666 at 25°. A phosphate concentration of 0.066 M was employed over the pH range 7.0–11.7; the desired ionic strength was achieved with sodium chloride. At pH above 11.7 no phosphate, only sodium chloride and sodium hydroxide, was used for this purpose. [b] Rates too slow for convenient measurement.

same as that reported recently by Gosselck and his co-workers.[27]

While the pH profile for the hydrolysis of benzoylcholine was linear over the whole pH range studied, hydrolysis of benzoylselenolcholine, and, to a lesser extent, benzoylthiolcholine, deviated from linearity below a pH of 8.5. The increase in rate, when the pH is near neutrality, appears to be due to the ability of these compounds to interact with phosphate to a kinetically significant extent. It can be seen in Figure 4 that, when a plot of the log of the observed rate constant for the hydrolysis of benzoylselenolcholine *vs.* phosphate concentration at a pH of 7.5 is extrapolated to zero buffer concentration, the rate constant thus obtained is in good agreement with the rate constant thus obtained is in good agreement with the rate constant expected at this pH in the absence of phosphate (Figure 3). The importance of salt effects in the hydrolysis of onium esters has been noted.[28]

The pH profiles for the hydrolysis of the tertiary amine analogs of the choline esters are summarized in Table II and in Figures 1–3. The sigmoid shapes of the curves obtained when pH is plotted against the observed rate constants are consistent with a mechanism assuming intramolecular nucleophilic attack by the unprotonated dimethylamino group,[21,22,29] or, as already noted, with intramolecular general acid–specific base catalysis. In either case, the observed rate constant can be expressed as

$$k_{obsd} = k_1 \frac{K_1}{K_1 + H^+} + k_2(OH^-)$$

where K_1 refers to the dissociation constant of the dimethylammonium group.

At pH values where specific base catalysis compared to intramolecular nucleophilic or intramolecular general acid–specific base catalysis is relatively insignificant, the following relationships may be used. It follows

$$k_{obsd} = k_1 \frac{K_1}{K_1 + H^+}$$

$$k_{obsd} = k_1 - \frac{K_1}{k_{obsd}H^+}$$

(27) J. Gosselck, H. Barth, and L. Béres, *Ann.*, **671**, 1 (1964).
(28) G. Aksnes and J. E. Prue, *J. Chem. Soc.*, 103 (1959).
(29) T. C. Bruice and S. J. Benkovic, *J. Am. Chem. Soc.*, **85**, 1 (1963).

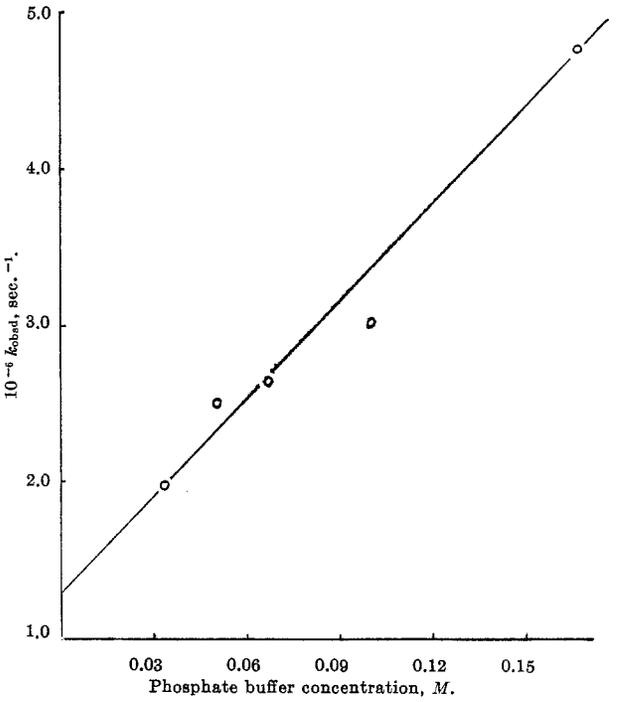


Figure 4.—Dependence of hydrolysis of benzoylselenolcholine at pH 7.5 on phosphate concentration at constant ionic strength (0.666).

TABLE II

KINETICS OF HYDROLYSIS OF 2-DIMETHYLAMINOETHYL BENZOATE, THIOLBENZOATE, AND SELENOLBENZOATE OVER A 7.0–13.1 pH RANGE[a]

pH	10^{-5} k_{obsd}, sec.$^{-1}$ M^{-1}		
	Selenolester	Thiolester	Ester
7.0	3.49	1.09	0.16
8.0	22.3	7.15	1.30
8.5	57.2	17.2	2.75
9.0	113.5	26.3	4.16
9.5	157.0	32.6	5.55
10.0	204	36.8	6.44
10.5	225	37.0	10.4
11.0	277	54.4	17.4
11.7	612	90.9	61.6
11.8	783	116.0	80.6
12.5	1270	177.0	174.0
12.8	2350	322.0	353.0
13.1	4850	610.0	650.0

[a] Initial concentration of ester, 1.81×10^{-4} M; ionic strength, 0.666 at 25.0°. A phosphate concentration of 0.066 M was employed over the pH range 7.0–11.7; the desired ionic strength was achieved with sodium chloride. At pH above 11.7, no phosphate, only sodium chloride and sodium hydroxide, was used for this purpose.

that a plot of k_{obsd} *vs.* $k_{obsd}H^+$ will yield a line with a slope of $1/K_1$ and an intercept of k_1. A representative plot is shown in Figure 5. Table III shows

TABLE III

DISSOCIATION CONSTANTS (K_1) OF DIMETHYLAMMONIUM GROUPS AND FIRST-ORDER RATE CONSTANTS (k_1) FOR INTRAMOLECULAR NUCLEOPHILIC ATTACK BY DIMETHYLAMINO GROUP FOR HYDROLYSIS OF 2-DIMETHYLAMINOETHYL ESTERS

2-Dimethylamino Ester	pK_1		k_1, sec.$^{-1}$ M^{-1}
	Titrimetric	From kinetic data	
Benzoate	8.47	8.51	5.4×10^{-5}
Thiolbenzoate	8.47	8.60	4.0×10^{-4}
Selenolbenzoate	8.74	8.71	1.9×10^{-3}

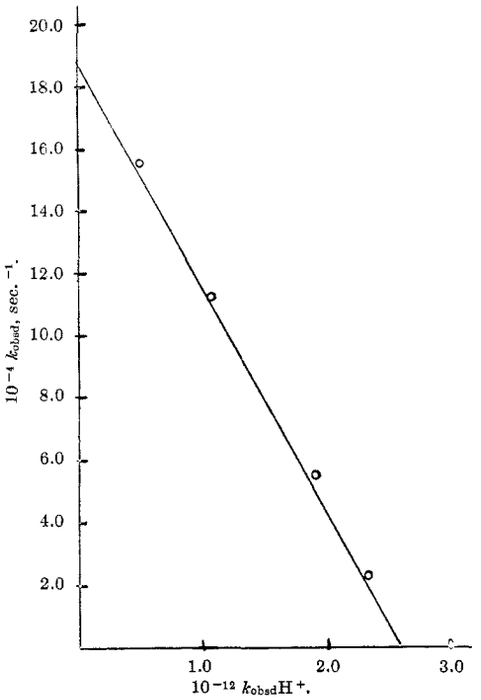


Figure 5.—Derivation of dissociation constant (K_1) for the dimethylammonium group of 2-dimethylaminoethyl selenolbenzoate from kinetic data. Slope = $-1/K_1$; K_1 = 8.71. Intercept = k_1 = 1.88 × 10^{-3} sec.$^{-1}$.

values for k_1 and K_1 obtained by this approach. It can be seen that values for the dissociation constants of the dimethylammonium groups of the three esters calculated from kinetic data are in good agreement with dissociation constants obtained by potentiometric titrations.

In contrast to the hydrolysis of the choline esters, hydrolysis of the 2-dimethylamino compounds was not sensitive to buffer effects. Table IV indicates

TABLE IV

RATES OF HYDROLYSIS OF 2-DIMETHYLAMINOETHYL ESTERS AT pH 10 IN THE PRESENCE AND ABSENCE OF SALT

2-Dimethylamino amino ester	10^{-4} k_{obsd}, sec.$^{-1}$ M^{-1} Distilled water	Sodium chloride	Lithium chloride	Sodium phosphate
Benzoate	0.68	0.65		0.64
Thiolbenzoate	3.18	3.45		3.68
Selenolbenzoate	17.0	17.0	17.0	20.0

that hydrolysis proceeded at very similar rates in phosphate, sodium chloride, and lithium chloride solutions, as well as in distilled water. It should be noted that sensitivity to ionic strength has been postulated to be useful for distinguishing between nucleophilic and general acid–specific base catalysis.[30] That the former rather than the latter mechanism is taking place is suggested by the observation that the order of the hydrolysis of the tertiary amino compounds below a pH of 11, selenolester > thiolester > ester as evidenced by the values for k_1 in Table III, is that previously noted for the aminolysis[13,14] and different from that normally noted for the hydrolysis of such isologs. Anchimeric assistance accelerates the reaction rates of the 2-dimethylaminoethyl thiolester and selenolester more than that of their oxygen isolog. At high pH values, a crossover in the relative hydrolysis rates of the choline isologs and their dimethylamino analogs occurs, with the former becoming more reactive than the latter. It is interesting to note that at high pH values the order of the hydrolysis of dimethylamino compounds, as in the case of their trimethylammonium analogs, becomes selenolester > ester > thiolester.

The kinetics for the reaction of the above compounds with *n*-butylamine (Table V) are rather different from the kinetics for hydrolysis. At a pH of 8 the aminolysis rates for the selenolesters are much faster than those for the corresponding thiolesters, while the esters do not appear to react with butylamine under these conditions. Aminolysis was found to proceed at very similar rates for tertiary and quaternary analogs, as shown in Table V. Thus, neighboring-group participation of the dimethylamino group is of negligible importance when *n*-butylamine, rather than hydroxide, is the attacking nucleophile.

TABLE V

REACTIONS WITH *n*-BUTYLAMINE AT pH 8

Compound	10^{-3} k_{obsd}, sec.$^{-1}$ M^{-1}
Benzoylcholine	No aminolysis observed
Benzoylthiolcholine	1.88
Benzoylselenolcholine	140.0
2-Dimethylaminoethyl benzoate	No aminolysis observed
2-Dimethylaminoethyl thiolbenzoate	1.74
2-Dimethylaminoethyl selenolbenzoate	157.0

[a] Initial concentrations of esters, 1.81 × 10^{-4} M, and *n*-butylamine, 1.08 10^{-1} M; ionic strength, 0.666; temperature, 25.0°.

The finding that esters, thiolesters, and selenolesters undergo hydrolysis at fairly similar and aminolysis at widely different rates should have useful applications in utilizing acyl residues as protective groups for sulfur and selenium. Thus, the separation of selenocoenzyme A from isoselenocoenzyme A was greatly facilitated when these compounds were converted to the selenobenzoyl derivatives, which proved resistant to hydrolysis during chromatography. Once separation of the two compounds had been achieved, the acyl residues could be removed by aminolysis under mild conditions.[12] These studies also suggest that aminolysis should be a convenient method for removing S-acyl groups introduced as cysteine blocking groups[31] and should prove useful in studying the applications of selenoacyl compounds as disulfide acylating agents.[32,14]

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(30) T. C. Bruice and S. J. Benkovic, "Topics in Bio-Organic Mechanisms," W. A. Benjamin Inc., New York, N. Y., *in press*, Chapter I.

(31) L. Zervas, I. Photaki, and N. Ghelis, *J. Am. Chem. Soc.*, **85**, 1337 (1963).

(32) W. H. H. Günther and H. G. Mautner, Abstracts, 150th National Meeting of the American Chemical Society, Atlantic City, N. J., Sept. 1965, p. 118C.