ORGANIC AND BIOLOGICAL CHEMISTRY

[CONTRIBUTION FROM THE RESEARCH LABORATORIES OF THE UPJOHN CO.]

The Kinetics of Hydrolysis of Scopolamine Derivatives with an Unusual Elimination on Alkaline Hydrolysis[1]

BY EDWARD R. GARRETT

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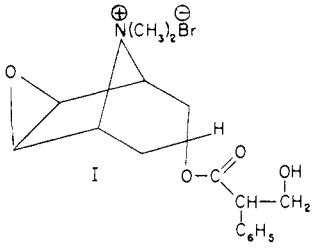
Acylscopolamine methyl halides and acylscopolamines are dehydrated to the corresponding aposcopolamines in the first step of their alkaline hydrolysis. This is proven by spectrophotometric and titrimetic kinetic studies and by isolation and identification of products. Dehydration of scopolamine methyl bromide, scopolamine and sodium acetyltropate and their hydrolytic products does not occur on alkaline hydrolysis. Acid-catalyzed hydrolysis does not dehydrate any acylscopolamine or scopolamine derivatives. A cyclic mechanism is proposed which can account for those scopolamine derivatives that do and do not eliminate on hydrolysis. The effect of the positive charge in quaternary scopolamine derivatives and the effect of various substituents on the possible ester hydrolysis steps are evaluated.

The excellent gastric antisecretory and visceral antispasmodic properties of scopolamine methyl bromide (I)[2] have led to the synthesis of a number of derivatives of scopolamine.[3] The discovery of the excellent anticholinergic properties of acetylscopolamine methyl bromide[3] raised the question as to whether its pharmacological action was unique to the compound or due to ready hydrolysis to scopolamine methyl bromide. This motivated these kinetic studies on the hydrolysis of scopolamine derivatives.

Scopolamine methyl bromide (I) is readily hydrolyzed by alkali to scopine methyl bromide (II) and tropic acid (III).[4]



I

TABLE I

BIMOLECULAR RATE CONSTANTS[a] FOR ALKALINE HYDROLYSIS OF VARIOUS SCOPOLAMINE METHYL BROMIDES

	Scopolamine methyl bromide	Trimethylacetylscopolamine methyl bromide	Acetylscopolamine methyl bromide
	Most readily saponifiable ester		
15°	0.467	1.50	2.28
20°	.670	2.52	3.25
25°	.931	3.78	4.03
30°	1.31	5.94	5.31
	Trimethylacetylscopolamine methyl bromide	Acetylscopolamine methyl bromide	Aposcopolamine methyl bromide
	Least readily saponifiable ester		
15°	0.0397	0.0519	...
20°	.0681	.0745	...
25°	.0928	.104	...
30°	.120	.142, 0.125	0.125

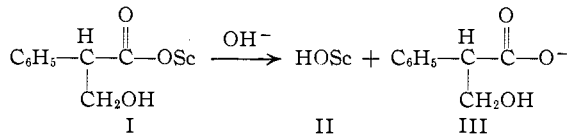
[a] k in l./mole/sec.

(1) Presented in part at the 130th Meeting of the American Chemical Society, Atlantic City, N. J., September, 1956.

(2) Pamine bromide, The Upjohn Co. brand of methscopolamine bromide.

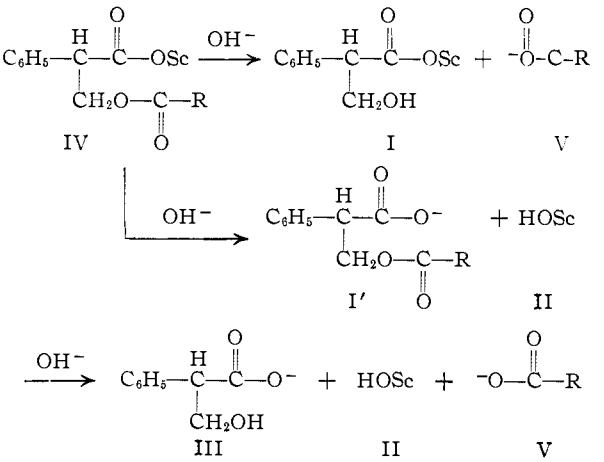
(3) R. B. Moffett and B. D. Aspergren, THIS JOURNAL, **78**, 3448 (1956).

(4) R. B. Moffett and E. R. Garrett, *ibid.*, **77**, 1245 (1955).



I II III

Acylation of the hydroxyl group of the tropic acid moiety in I would provide two ester groups available for alkaline hydrolysis and the simplest hypotheses for the sequence of hydrolyses of acylscopolamine methyl bromide (IV) are



IV I V I′ II III II V

This scheme proposes two possible routes of hydrolysis, through I or I′.

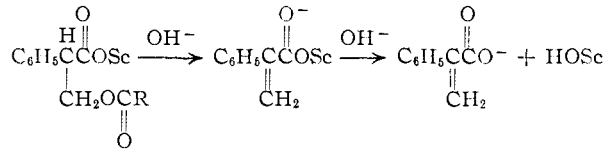
The results of the kinetic studies (Table I) on acylated scopolamine methyl bromides (IV, R = CH_3–, $(CH_3)_3C$–) show two widely separated hydrolysis rates for the two ester groups; the rate constant of one is of the magnitude of fifty times greater than that of the other.

On the basis of the above simple hypothesis, if the first hydrolysis is to the intermediate I, it follows that the rate constants for the least readily saponifiable ester in Table I would be the same as the rate constant for the alkaline hydrolysis of scopolamine methyl bromide (I). This is not so. Scopolamine methyl bromide hydrolysis is of a magnitude ten times greater than the least readily saponifiable group of acylscopolamine methyl bromides. The alternative hypothesis of fast hydrolysis to I′, the anion of acyltropic acid, and the subsequent slow hydrolysis to acetic and tropic acids

is denied by the fact that the latter rate is not similar to the rate of alkaline hydrolysis of acetyltropic acid.

The non-ether extractable product of acidified, equimolar alkaline hydrolyzed acetylscopolamine methyl bromide was a crystalline material whose infrared spectrum was coincident with that of aposcopolamine methyl bromide. Also, the rate constant for the alkaline hydrolysis of aposcopolamine methyl bromide was similar to those determined for the least readily saponifiable ester of the acylscopolamine methyl bromides (Table I).

Thus the mode of alkaline hydrolysis of an acylscopolamine methyl bromide (IV) was determined to be, first, the hydrolysis of the acetate grouping with concomitant dehydration to the apo derivative and, second, the slower hydrolysis of the resultant aposcopolamine methyl bromide (VI) to scopine methyl bromide,[4] (II) and dehydrotropic acid (VII).



IV VI VII II

Further confirmation was obtained by following the appearance of a strong ultraviolet chromophore at 247 mμ on alkaline hydrolysis of the acylscopolamine methyl bromides. Acylscopolamine methyl bromides (IV) and scopolamine methyl bromide (I) have small absorptivities at this wave length whereas the absorptivity of aposcopolamine methyl bromide (VI) is high due to conjugation with both the ester carbonyl and the phenyl ring. The rate of appearance of the chromophore was comparable to the rate of fast hydrolysis as estimated by titration techniques.

This proven unusual concomitance of deacylation and dehydration suggested the investigation of the study of the alkaline hydrolysis of non-quaternary amine analogs, the acylscopolamines.

The alkaline hydrolysis rate constants of various compounds of this type are given in Table II as determined at 30.3° and in 48% ethanol. The two possible hydrolyses of the acylscopolamines are widely different in magnitude although the more slowly hydrolyzed esters of acetylscopolamine and trimethylacetylscopolamine are similar in rate. Neither the faster nor the slower are comparable to the hydrolysis rate of scopolamine or acetyltropic acid. However, the slower is similar to the rate of alkaline hydrolysis of aposcopolamine.

TABLE II

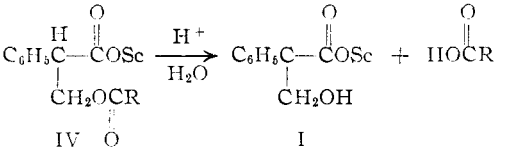
BIMOLECULAR RATE CONSTANTS (k IN L./MOLE/SEC.) FOR ALKALINE HYDROLYSIS OF VARIOUS SCOPOLAMINES AT 30.3° IN 48% ETHANOL

	Most readily saponifiable ester	Least readily saponifiable ester
Acetylscopolamine	1.23	0.000287
Trimethylacetylscopolamine	1.42	.000260
Aposcopolamine		.000255
Scopolamine		.0080, 0.0086, 0.0091
Sodium acetyltropate		.026, 0.024

The non-ether-extractable product of acidified, equimolar alkaline hydrolyzed acetylscopolamine was a crystalline material whose infrared spectrum was consistent with that of aposcopolamine nitrate. A similar equimolar alkaline hydrolysis without acidification yielded an ether extract which on evaporation produced a material whose infrared spectrum was coincident with that of aposcopolamine. The aqueous fraction on evaporation contained only one type of crystal, sodium acetate trihydrate.

As before, further confirmation was obtained by following the appearance on alkaline hydrolysis of a strong chromophore with ultraviolet absorption at 247 mμ. This could only be attributed to the appearance of aposcopolamine. The rate of appearance of this chromophore for both acetylscopolamine and trimethylacetylscopolamine was comparable to the rate of the faster hydrolysis of these compounds as estimated by titration techniques.

Peculiarly, acid hydrolysis of acetylscopolamine methyl bromide (IV) shows no enhancement of ultraviolet absorption at 247 mμ so that acid-catalyzed deacylation is not concomitant with dehydration.



IV I

The classical acid hydrolysis of the acetate group of acetylscopolamine methyl bromide (IV) is summarized in Table III as based on equation 1 where the slope of the plots of log [IV] *vs.* time is taken as $k/2.303$ in Fig. 1.

$$\log [IV] = -(k/2.303)t + \log [IV]_0 \qquad (1)$$

TABLE III

RATE CONSTANTS FOR THE ACID HYDROLYSIS OF ACETYLSCOPOLAMINE METHYL BROMIDE

0.0200 M at 37.5 ± 0.1°

[HCl]	k, hr.$^{-1}$	$k' = k/[HCl]$, l./mole/hr.
0.0100	0.0055	0.55
.0200	.0101	.51
.0300	.0163	.57
.0400	.0214	.53
	Average	0.54

An analogous study of 0.02 M scopolamine methyl bromide in 0.0400 M HCl at 37.5° had shown no appearance of additional acid over a period of three days (a random variation of ±0.010 ml. of 0.1000 M NaOH titer for 10-ml. aliquots). A previous study in 0.100 M HCl has also shown no apparent acidic hydrolysis.[4]

Experimental

The scopolamine derivatives used in these studies were synthesized and supplied by R. B. Moffett and B. D. Aspergren who have recently reported on their preparations and their characterization.[3]

Alkaline Hydrolysis as Studied by Titration.—The kinetics of alkaline hydrolysis of the ester groups of acetylscopolamine, trimethylacetylscopolamine, scopolamine and apo-

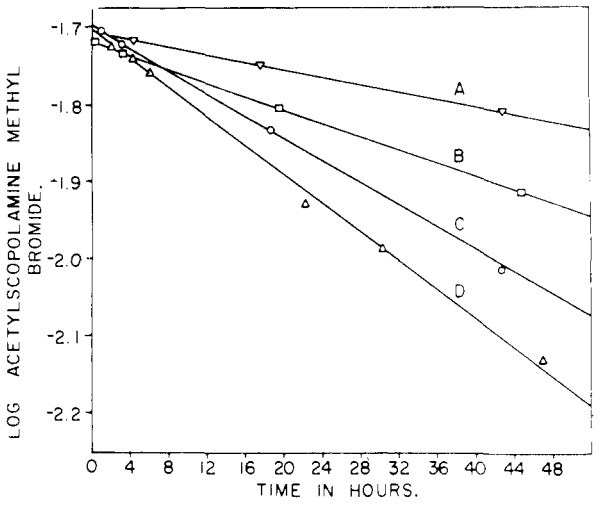


Fig. 1.—Pseudo-first-order rate plots for the acid hydrolyses of acetylscopolamine methyl bromide: A, 0.0100 *M* HCl; B, 0.0200 *M* HCl; C, 0.0300 *M* HCl; D, 0.0400 *M* HCl.

scopolamine, as well as the corresponding quaternary amines, the methyl bromides, were determined by estimation of the slopes appropriate to the bimolecular rate expressions

$$1/[OH^-] = kt + \text{constant} \quad (2)$$

$$\log([\text{alkaloid}]/[\text{NaOH}]) = kt\left(\frac{([\text{alkaloid}]_0 - [\text{NaOH}]_0)}{2.303}\right) + \text{constant} \quad (3)$$

Thus 1/[NaOH] was plotted against time when $[NaOH]_0 = [alkaloid]_0$, and log ([alkaloid]/[NaOH]) was plotted against time when $[NaOH]_0 \neq [alkaloid]_0$ where $[alkaloid]_0$ and $[NaOH]_0$ are the initial concentrations of the reactants. The bimolecular rate constants (k) are given in l./mole./sec. in Tables I and II.

There was sufficient difference between the hydrolysis rates of the two ester groups in the acylscopolamines and their methyl bromides for differentiation by studying 1:1 and 1:2 acylscopolamine derivative–sodium hydroxide mixtures. This, of course, assumes prior neutralization of the non-quaternary scopolamine halides. Typical examples of such rate plots are given in Figs. 2 and 3. Scopolamine, aposcopolamine and their methyl bromides were studied in solutions 0.0100 *M* in alkaloid and NaOH. Acetyl- and trimethylacetylscopolamine and their methyl bromides were studied in solutions 0.0100 *M* in alkaloid and 0.0100 *M* and 0.0200 *M* in NaOH. The procedure of study was to pipet ten 10-ml. aliquots, each into 2 ml. of 0.1000 *N* HCl and back-titrate with 0.1000 *N* NaOH to the appropriate end-point using a micro-buret. Phenolphthalein was used as the indicator for the methyl bromides and thymolphthalein for the others. Blowout pipets were used for the fast hydrolyzing ester groups and the times of sampling were recorded at the mid-point of the blowout. The kinetic studies were conducted for time in excess of the half-life of the esters.

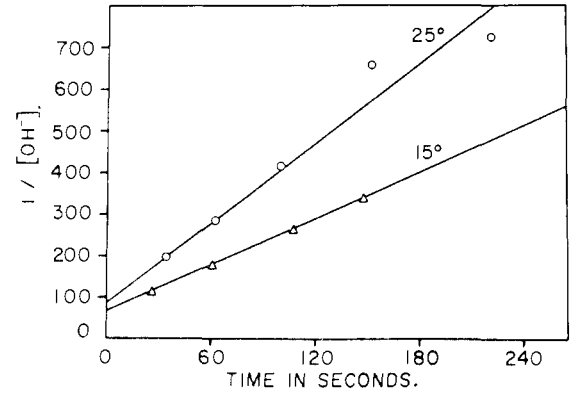


Fig. 2.—Typical bimolecular rate plots for the alkaline hydrolysis of the faster hydrolyzing group of acetylscopolamine methyl bromide.

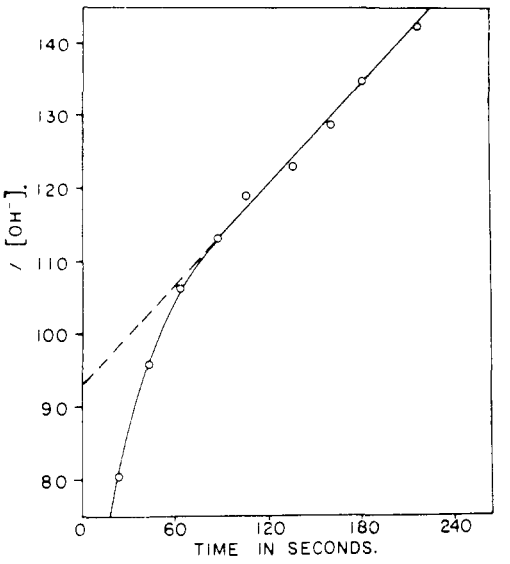


Fig. 3.—A typical bimolecular rate plot for the alkaline hydrolysis of the slower hydrolyzing group of acetylscopolamine methyl bromide at 25°.

In addition to the data given in Tables I and II, rate constants for the alkaline hydrolysis of acetylscopolamine methyl bromide were determined in 48% ethanol at 30.3° and were 0.541 and 0.0429 l./mole./sec. The rate constant at 30.3° for scopolamine in water was 0.0132 l./mole./sec.

Alkaline Hydrolysis as Studied by Spectrophotometry.—The much greater absorptivity in the ultraviolet region of 247 mμ of aposcopolamine methyl bromide and aposcopolamine over their corresponding scopolamine and acylscopolamine analogs permitted following the appearance of absorbance at this wave length as a measure of hydrolysis rates.

The molar absorptivities (a_m) of pertinent compounds at this wave length, in water unless specified, are: aposcopolamine in 0.07 *M* NaOH and 0.04 *M* HCl in 48% ethanol and water, a_m = 3,870; aposcopolamine methyl bromide, a_m = 3,500; scopolamine and acetylscopolamine, their hydrobromides and methyl bromides, a_m ~150; *dl*-tropic acid in 0.1 *M* NaOH, a_m = 180 and in 0.1 *M* HCl, a_m = 130; dehydrotropic acid in 0.1 *M* NaOH, a_m = 6,100 (6,300 in 48% ethanol) and in 0.1 *M* HCl, a_m = 3,700 (4,100 in 48% ethanol).

The absorbance at 247 mμ of a solution 3.145×10^{-4} *M* in acetylscopolamine and 2.855×10^{-4} *M* in NaOH in 48% ethanol was recorded as it changed with time. The concentration of aposcopolamine was calculated from the knowledge of the molar absorptivity and these absorbances. The alkali concentration was calculated on the postulate of 1:1 reaction of hydroxide ion with alkaloid on application of equation 3. A similar procedure was used for a solution 3.204×10^{-4} *M* in trimethylacetylscopolamine and 3.000×10^{-4} *M* in NaOH in 48% ethanol.

The determined rate constants (based on recorded absorbances that exceeded 80% of the total absorbance expected for the postulated transformation to aposcopolamine) were 1.28 l./mole./sec. for acetylscopolamine and 1.43 l./mole./sec. for trimethylacetylscopolamine at 30°. These were the same values obtained by titration techniques for the fast hydrolyzing ester groups as given in Table II. The plots conformed to bimolecular kinetics.

No increase in absorbance at 247 mμ with time was noted for scopolamine in 48% ethanol, 0.1 *M* in NaOH.

A similar procedure was used for the study of the monoester group alkaline hydrolysis in aqueous solution 3.0×10^{-4}

M in NaOH and acetylscopolamine methyl bromide. A slight deviation was noted from true bimolecular kinetics in that the plot of calculated $1/[OH^-]$ against time as per equation 2 was not entirely linear. This indicated that this hydrolysis may not give total conversion to aposcopolamine methyl bromide.

An estimate of the rate constant was 3.2 l./mole./sec. which is of the same order of magnitude as the value determined by titration techniques for the fast hydrolyzing ester and which is given in Table I.

No increase in absorbance at 247 mμ with time was noted for the alkaline hydrolysis of sodium acetyltropate in 48% ethanol.

Identification of Products of Alkaline Hydrolysis.—Two millimoles of acetylscopolamine methyl bromide were treated with equimolar alkali in 45 ml. of water for ten minutes, acidified with 50 ml. of 0.1 *M* HCl and extracted with five 10-ml. washings of ether. The combined ether extracts were evaporated to dryness and the slight amount of oil that resulted gave an infrared spectrum similar to, but of less purity than, that of prepared acetyltropic acid. The acetyltropic acid had been synthesized by the method of Wolffenstein and Mamlock.[5]

The aqueous fraction was evaporated to dryness and the resultant material had the same infrared spectrum as aposcopolamine methyl bromide.[3] A hydrated molecule was indicated with both isolated and reference materials.

The above evidence added to the results of the ultraviolet spectrophotometric studies clearly indicates that acetylscopolamine methyl bromide is most readily hydrolyzed by alkali primarily to aposcopolamine methyl bromide, acetate and water with a small amount of simultaneous hydrolysis to acetyltropate and scopine methyl bromide.

Two millimoles of acetylscopolamine hydrobromide was neutralized with base, treated with equimolar alkali in 190 ml. of water for 15 minutes, acidified with 100 ml. of 0.1 *M* HCl and extracted with 5–25 ml. washings of ether. The combined ether extracts were evaporated to dryness with negligible non-volatile residue. The aqueous fraction was evaporated to dryness and the resultant product had an infrared spectrum consistent with that of aposcopolamine nitrate.[3] A hydrated molecule was indicated with both isolated and reference materials.

The same procedure was used on another two millimoles of acetylscopolamine hydrobromide except that after hydrolysis by equimolar alkali, the solution was immediately extracted with five 50-ml. washings of ether. The combined ether extracts were evaporated to dryness and the infrared spectrum of this material was the same as aposcopolamine. The reference aposcopolamine was obtained by the evaporation of an ether extract of an aqueous solution of aposcopolamine nitrate[3] adjusted to *p*H 10. No free hydroxyl was observed in either spectrum.

The aqueous fraction of the equimolar alkaline-hydrolyzed acetylscopolamine was evaporated. The crystalline residue was observed to be of one kind and by microscopic examination and infrared was sodium acetate trihydrate.

The above evidence added to the results of the ultraviolet spectrophotometric studies clearly indicates that acetylscopolamine is most readily hydrolyzed to aposcopolamine, acetate and water.

Potentiometric Investigations.—Additional evidence was obtained by potentiometric titration using a Cambridge *p*H meter, model R, equipped with glass-saturated calomel electrodes. By the half-neutralization techniques, the pK_a's were determined for scopoline nitrate, 8.20; aposcopolamine nitrate, 7.72; acetylscopolamine hydrobromide, 7.35; scopolamine hydrobromide, 7.55; and tropic acid, 4.23 (5.90 in 65% ethanol).

The product of equimolar alkaline hydrolysis of acetyl scopolamine had a pK_a of 7.7, consistent with aposcopolamine and not scopoline. The product of equimolar alkaline hydrolysis of scopolamine had a pK_a of 8.3, consistent with scopoline.

Acid Hydrolysis.—Two millimoles of acetylscopolamine methyl bromide was dissolved in each of 100 ml. of 0.0100, 0.0200, 0.0300 and 0.0400 *M* HCl and maintained at 37.5°. The respective solvents were also so maintained. Ten-ml. pipetted aliquots were titrated at intervals with 0.1000 *M* NaOH to phenolphthalein end-point. Controls were run on the corresponding HCl solutions. Two millimoles of scopolamine methyl bromide were also dissolved in 100 ml. of 0.0400 *M* HCl and 10-ml. aliquots also titrated at intervals. No change in titer was noted in the latter case.

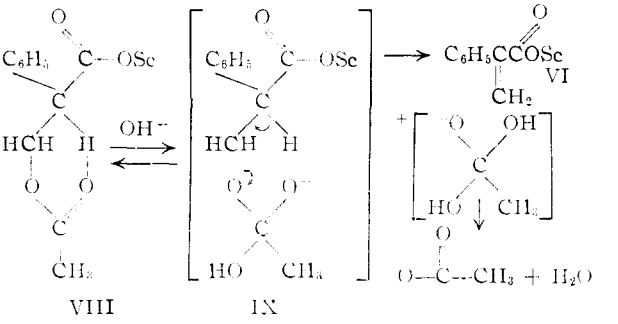
Solutions that were 3×10^{-4} *M* in scopolamine methyl bromide, acetylscopolamine hydrobromide and scopolamine hydrobromide and 0.04 *M* in HCl reacted at 30° for 21 days. The absorbance at 247 mμ was low and did not change throughout the entire time of study. Acid-catalyzed dehydration could not be concluded. Also, dehydration is not concomitant with the possible acid-catalyzed hydrolysis of such esters.

(5) R. Wolffenstein and L. Mamlock, *Ber.*, **41**, 723 (1908).

Discussion

The concomitant dehydration of an alcohol with the alkaline hydrolysis of its ester is an unusual phenomenon and, since it occurs with both acetylscopolamine methyl bromide and acetylscopolamine and with comparable rates, it cannot be directly attributed to the presence of a positive charge in the molecule. A postulated mechanism must follow the observed bimolecular kinetics. It is likely that it conforms to the accepted intermediates[6] with the rate-determining step of hydroxyl ion attack and with dehydration being non-rate determining.

A mechanism consistent with these facts could accept a chelate ring structure,[7,8] VIII, which carries over into the reaction intermediate,[6] or transition state, IX. The observed high rates of acetate ester alkaline hydrolysis in acylscopolamines and their methyl halides could be attributed to the greater activation of the carbonyl carbon by the hydrogen bonding of the carbonyl oxygen with the tertiary hydrogen. The resultant dehydro product aposcopolamine (VI) could be obtained easily by the simultaneous shift of electrons as in IX.[9]



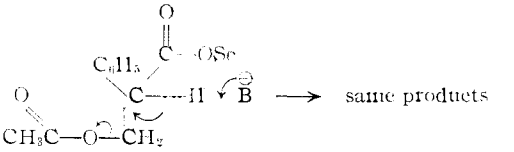
The effect of such a mechanism on the rate of hydrolysis would be enhanced with positively charged molecules (acylscopolamine methyl halides), would be somewhat less effective in the acylscopolamines and would be repressed in the acyltropates. In the latter case, X, the carboxylate anion would inhibit hydrogen bonding and even repel the acetate ester. The lack of increase in ab-

(6) M. L. Bender, THIS JOURNAL, **73**, 1626 (1951).

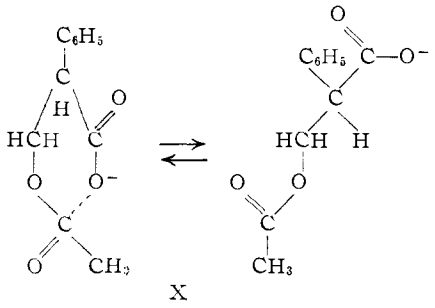
(7) H. A. Smith and J. P. McReynolds, *ibid.*, **61**, 1963 (1939).

(8) J. F. J. Dippy, *Chem. Revs.*, **25**, 151 (1939).

(9) An alternate mechanism suggested by the referee is an ordinary bimolecular elimination. This would have base abstracting an enolic proton as



sorbance at 247 mμ on alkaline hydrolysis of acetyltropate is indicative.



X

The increased heat of activation (Table IV) for the first step in the alkaline hydrolysis of trimethylacetylscopolamine methyl bromide over the acetyl derivative may be attributed to the greater steric hindrance of the trimethyl groups in the formation of the cyclic intermediate prior to hydrolysis. The Arrhenius plots of the bimolecular rate constants are given in Fig. 4.

TABLE IV

TABULATION OF CONSTANTS OF THE LOGARITHMIC ARRHENIUS EQUATION[a]

Compound	S	E, kcal./mole	log A
Most readily saponifiable ester			
Scopolamine methyl bromide	2570	11.8	8.61
Acetylscopolamine methyl bromide	2010	9.2	7.35
Trimethylacetylscopolamine methyl bromide	3370	15.4	11.88
Least readily saponifiable ester			
Acetylscopolamine methyl bromide	2580	11.8	7.65
Trimethylacetylscopolamine methyl bromide	2590	11.8	7.63

[a] Log $k = S/T + \log A$ where $E = 2.303\ RS$ for alkaline hydrolysis as estimated from rate constants determined at 15, 20, 25 and 30° where k is in l./mole./sec.

No such chelated intermediate as IX could occur with scopolamine or scopolamine methyl bromide so that the analogous aposcopolamines (VI) could not be, and were not, products.

Inspection of models[10] of the acylated alkaloids shows that the nitrogen is spatially removed from the more readily hydrolyzed ester group (see I). Thus, the enhancement of rate (Tables I and II) by the positive charge in the quaternary analogs must be by field effects and not by direct involvement in the mechanism. There are relatively small differences in rates of alkaline hydrolysis for the first step between the acylscopolamines and their corresponding methyl bromides (*ca.* 5-fold). However, the large differences in rates between aposcopolamine methyl halide and aposcopolamine, scopolamine methyl halide and scopolamine (*ca.* 200-fold), may be attributed to the greater proximity of this ester group to the nitrogen.

In most cases an unsaturated carbon-to-carbon linkage near the carbonyl carbon in carboxylic esters speeds the rate of hydrolysis.[8,11,12] However, this is not so with aposcopolamine and its methyl bromide compared to scopolamine and its methyl bromide, respectively (Tables I and II). This cannot be blamed entirely on the conjugation of the unsaturated bond and the carboxyl carbonyl which only slightly depresses the rate of alkaline hydrolysis of acrylic ester compared to propionic ester.[12] The conjugation effect must be greatly enhanced by the phenyl group to sufficiently depress the apo hydrolysis rates to less than one-tenth that of their saturated analogs. An alternative rationale is that an α-carbinol substituent elevates hydrolysis rates.

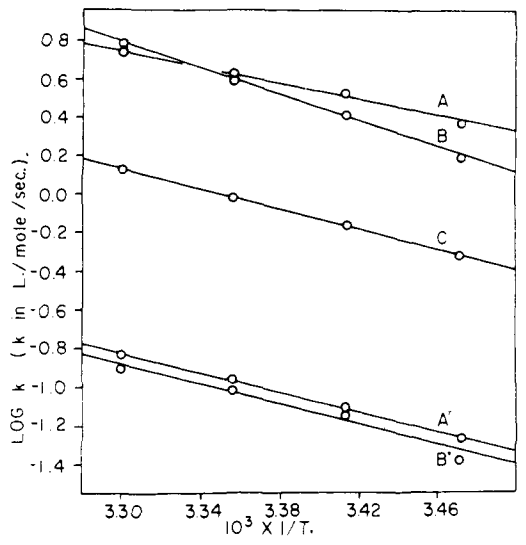


Fig. 4.—Arrhenius plots of the bimolecular rate constants for the alkaline hydrolysis of various scopolamine methyl bromides: A, the faster hydrolyzing group in acetylscopolamine methyl bromide; B, the faster hydrolyzing group in trimethylacetylscopolamine methyl bromide; C, scopolamine methyl bromide; A′ and B′, the corresponding slower hydrolyzing groups in acetyl- and trimethylacetylscopolamine methyl bromides.

The postulated intermediate IX for the alkaline hydrolyses of acylscopolamines and their methyl halides also explains why dehydration does not as readily accompany mild acid hydrolysis since introduction of an anion is a prerequisite. Again, in acid hydrolysis, the diminished field effect of the ester further removed from the positively charged quaternary nitrogen permits its hydrolysis. However, the proximity of the positive charge to the closer ester group makes the acid hydrolysis of scopolamine methyl bromide extremely difficult.

The slow acid hydrolysis of acetylscopolamine methyl bromide should not be of any consequence in the gastric system (minimum *p*H of 1.5). Alkaline hydrolysis does not produce scopolamine methyl bromide. If the possibility of specific enzymatic transformations are excluded, it is most probable that acetylscopolamine methyl bromide reaches the site of absorption or physiological action in the gastrointestinal system substantially

(10) A. Nickon and L. F. Fieser, THIS JOURNAL, **74**, 5566 (1952); P. F. Smith and W. H. Hartung, *ibid.*, **75**, 3859 (1953); F. C. Nachod and A. M. Lands, *Trans. N. Y. Acad. Sci.*, **16**, 2 (1953); A. Heusner, *Arzneim.-Forsch.*, **6**, 105 (1956).

(11) R. T. Myers, A. R. Collett and C. L. Lazzell, *J. Phys. Chem.*, **56**, 461 (1952).

(12) E. A. Halonen, *Acta Chem. Scand.*, **9**, 1492 (1955).

unchanged into scopolamine methyl bromide, that the anticholinergic action of acetylscopolamine methyl bromide is unique unto itself and not due to change prior to arrival at this site.

Acknowledgment.—The author is indebted to Miss Kathryn G. Stimson for excellent technical assistance, to Mrs. Anne Fonken for infrared analyses, to Dr. R. B. Moffett and B. D. Aspergren for the many scopolamine derivatives, to Dr. John Shell for the microscopic identifications and to Dr. George Slomp for very helpful discussion.

KALAMAZOO, MICHIGAN

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, OREGON STATE COLLEGE]

Kinetics of the Alcoholysis of *o*- and *p*-Nitroacetanilides[1]

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The kinetics of the base-catalyzed alcoholysis of *p*-nitroacetanilide in methanol and ethanol and of *o*-nitroacetanilide in methanol have been studied with the aid of ultraviolet spectrometry. The results are in accord with the normal acyl-oxygen ester hydrolysis mechanism.

Of the common carbonyl reactions of esters and amides susceptible to basic catalysis only the alcoholysis of amides has failed to receive careful kinetic investigation. This is, of course, not particularly surprising in view of the unfavorable equilibrium constant for that reaction in most cases.[2] The interesting discovery of Verkade,[3] that *o*- and *p*-nitroanilides react readily with methanol under catalysis by methoxide ion to give high yields of the corresponding free amine and a methyl ester provided the opening for kinetic examination of this reaction. Since acetylation of the nitroanilines has been shown to cause a marked hypsochromic shift in the ultraviolet spectral band nearest the visible,[4] a suitable method of analysis was thus available. The work reported here was initiated in early 1948 and completed in 1951, but publication was held up in the hope that further work might be accomplished on this interesting reaction. Despite the fact that Verkade[5] reported some semi-quantitative kinetic measurements shortly after this work was commenced, a report of our somewhat more accurate study seems in order.

Experimental

Spectra.—All spectral measurements were carried out using a Beckman model DU spectrometer with matched quartz cells.

Materials.—Methanol was distilled through a bubble-cap column rated at twelve plates and a cut boiling from 64.7–64.9° was treated with furfural and potassium hydroxide.[6] Product boiling at 64.8–64.9° was dried according to Lund and Bjerrum,[7] and the product distilled directly into a storage vessel, fitted with an all-glass automatic syphon and protected from both moisture and carbon dioxide by Drierite and soda lime. Ethanol was prepared in similar manner (excepting the furfural treatment) and a cut boiling over 0.1° was used.

Solutions of the sodium alkoxides were prepared under a dry oxygen-free nitrogen atmosphere by adding sodium freshly cut under dry ether to the cold alcohol in an apparatus carefully protected from atmospheric moisture and carbon dioxide. These solutions were stored in an inert atmosphere and in the dark. Though the solutions were reasonably stable when dilute, freshly prepared solutions were used in all cases. Their concentration was determined by titration with aqueous hydrochloric acid using phenolphthalein. Solutions of hydrogen chloride in methanol and ethanol were prepared by passing the dried gas into cold alcohol. Their concentration was determined by titration against aqueous sodium hydroxide using phenolphthalein.

p-Nitroaniline was Eastman Kodak Co. white label grade crystallized from ethanol to a constant melting point of 150.5–151.0°. *p*-Nitroacetanilide was prepared by acylation of the above product using acetic anhydride and was crystallized from glacial acetic acid. The nearly white crystals melted at 212–213.5°. *o*-Nitroaniline, of similar grade, was recrystallized from 50% aqueous ethanol, m.p. 72.5–73.0°. The corresponding anilide was prepared by direct acylation and crystallized from glacial acetic acid and finally acetone, m.p. 93.0–94.0°.

Kinetic Procedure.—Stock solutions of the anilide in the various alcohols were prepared by adding accurately weighed amounts of the anilide to the proper solvent in a volumetric flask. Initial concentrations were determined from the known concentration of the stock solution. Solutions of both anilide and alkoxide were thermostated prior to each run. Ten-milliliter samples of anilide solution were placed in test-tubes fitted with ground glass stoppers. To initiate a run, tubes were removed from the thermostat at one-minute intervals and appropriate amounts of alkoxide solution added. The bath was maintained at 30.00 ± 0.01° checked against a thermometer calibrated by the National Bureau of Standards. At appropriate intervals tubes were withdrawn from the bath, the base neutralized with alcoholic hydrogen chloride and the optical density determined.

Analytical Method.—Solutions of the amines in the various alcohols were found to obey Beer's law, and though neither anilide nor acid at low concentration caused interference, the presence of anilide and base caused a notable interference. Thus the rate could not be followed directly, and analysis was carried out after neutralization with alcoholic hydrogen chloride. Analysis for *p*-nitroaniline was made at 450 mμ and for *o*-nitroaniline at 500 mμ. Solutions of the amines in the alcohols were stable, being unchanged within experimental error in 148 hr.

Results

The ultraviolet absorption spectra for *o*- and *p*-nitroaniline and the corresponding anilides were determined under a variety of conditions. For

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