

601. *Aryl-2-halogenoalkylamines. Part IX.* A Comparison of the Stability of Esters derived from Some Aryl-2-halogenoalkylamines towards Acid Hydrolysis with Those obtained from Other Radiomimetic Compounds.*

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The rates of hydrolysis of acetates derived from some aryl-2-halogenoalkylamines under acid conditions and in 80% aqueous acetone have been measured and compared with those of esters derived from sulphur and nitrogen "mustards" and also from ethylene oxide. Under these conditions the stability of the various esters is comparable with that of ethyl and *iso*-propyl acetates.

In Part VIII * it was shown that acetates and benzoates derived from so-called "radiomimetic" alkylating agents such as sulphur and nitrogen "mustards" and also certain epoxides were more rapidly hydrolysed under alkaline conditions than simple alkyl esters. There was a wide variation in the velocity constants for the hydrolysis of the different esters, those derived from epoxides being by far the most alkali-labile. It was realised that the rates of hydrolysis under these conditions did not necessarily bear any direct relation to the ease of hydrolysis of the various esters under physiological conditions. In order to obtain more complete information regarding the relative stability of esters derived from the various cytotoxic agents the acid hydrolysis of a series of these esters has now been studied. The relative stabilities to acid hydrolysis were of particular interest since it is later hoped to treat proteins with the different alkylating agents and then by degradation of the products by acid to attempt to decide which amino-acid residues had combined with the reagent.

In the present study the acetates derived from the following compounds have been examined: 2-chloroethyldimethylamine, di-2-chloroethylmethylamine, 2-chloroethyl ethyl sulphide, di-2-chloroethyl sulphide (mustard gas), *N*-2-chloroethyl-*N*-ethylaniline, *NN*-di-2-chloroethylaniline, *NN*-di-2-chloroethyl-*p*-anisidine, di-2-chloroethyl- β -naphthylamine, and di-2-chloro-*n*-propyl- β -naphthylamine. Since the rates of hydrolysis of acetates and benzoates under alkaline conditions were previously found to be in the same relative order it was not deemed necessary to examine the benzoates in the present study. The acetates were prepared by the methods described in Part VIII.

METHODS.

On account of the very low aqueous solubility of the esters derived from the sulphur and nitrogen "mustards" it was necessary to carry out the determinations of the rates of hydrolysis in 80% acetone. The hydrolyses were made in sealed Pyrex bulbs, essentially by the method of Timm and Hinshelwood (*J.*, 1938, 864). Table I shows the details for the hydrolysis of di-2-acetoxyethyl sulphide. The fact

TABLE I.

Hydrolysis of di-2-acetoxyethyl sulphide in 80% aqueous acetone.

Ester used for each determination, 51.8 mg. 0.025N-Hydrochloric acid used in each run, 20 ml. Total reaction solution, 100 ml. Equivalent vol. (*a*) of 0.025N-acid which would be liberated at complete hydrolysis, 20.1 ml. Temp., 50°. pH of solution, 1.9.

Time (hours).	0.025N-NaOH for titration (ml.).	0.025N-acid liberated (ml.), <i>x</i> .	$\log_{10} a/(a - x)$.
0	21.50	—	—
42.5	24.75	3.25	0.077
66.75	26.50	5.00	0.126
90.25	27.95	6.45	0.169
118.5	29.40	7.90	0.217

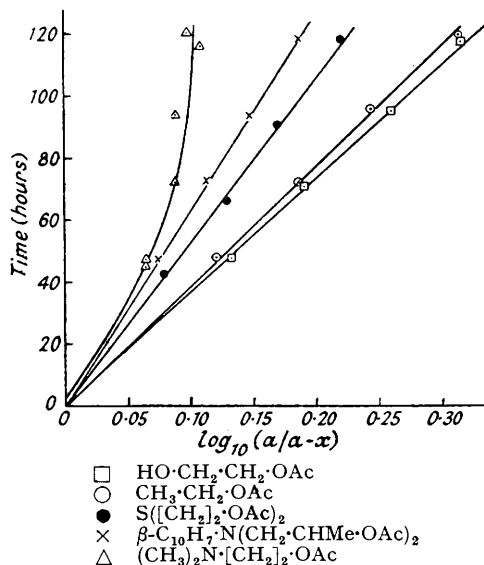
From the figure the slope of the line $2.303/k[H^+] = 120 \times 3600/0.2225$. Since $[H^+] = 0.005$, $k = 2.36 \times 10^{-4}$.

that a plot of *t* against $\log a/(a - x)$ (see figure)—the slope of this line is $2.303/k[H^+]$ —is generally linear over the first 40% of the reaction shows that the re-esterification reaction can be disregarded. Other data are recorded in the figure. The value of the hydrogen-ion concentration used in the calculation of the rate constant *k* was taken as 0.005 equiv. per l., it being assumed that dissociation of the hydrochloric

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acid was complete. The pH of a solution containing this concentration of acid in 80% acetone was found to be 1.9 by means of a glass electrode system with a Cambridge portable electrometer valve pH meter—a value of 2.3 would have been obtained in purely aqueous solution. It is well known that anomalous values are obtained when pH measurements are made with glass electrodes in non-aqueous solutions (Dole, "The Glass Electrode," New York, 1941). In the present studies the glass electrode is simply being used to ensure that the hydrogen-ion concentration is the same in all the hydrolyses.

Addition of most of the esters now examined to the 80% acetone solution of hydrochloric acid did not affect the pH reading determined as above and this reading remained constant throughout the reaction. Di-2-acetoxyethylmethylamine, 2-acetoxyethylmethylamine, and *N*-2-acetoxyethyl-*N*-ethylaniline, which have pK_a values in 80% acetone of 5.7, 7.3 (Davis and Ross, *loc. cit.*), and 2.4 respectively, were sufficiently basic to reduce the hydrogen-ion concentration by salt formation. The pH of these solutions



was adjusted by addition of hydrochloric acid to give a meter reading of 1.9 at the start of the reaction. It is realised that the value of the hydrogen-ion concentration used in the calculations may not be strictly correct but since in all reaction mixtures the meter reading was the same the values of k given in Table II represent the relative stability of the esters towards acid hydrolysis.

The pK_a of the di-2-acetoxyethylarylamines investigated was lower than 0.5 in 80% acetone solution.

Hydrolysis of 2-acetoxyethylmethylamine gave anomalous results, for although the pH of the reaction solution remained constant throughout the experiment the velocity constant fell steadily from 1.76×10^{-4} at 48 hours to 1.04×10^{-4} at 120 hours.

DISCUSSION.

The results given in Table II show that generally esters derived from the cytotoxic chloroethylamines and chloroethyl sulphides are somewhat more stable to acid hydrolysis than is ethyl acetate. This contrasts sharply with the greater alkali-lability of the same esters. The slowest rates were observed for di-2-acetoxyethylmethylamine and 2-acetoxyethylmethylamine, which are sufficiently basic to be present in the cationic form under the chosen reaction conditions; however, the rate constant for *N*-2-acetoxyethyl-*N*-ethylaniline, some 60–70% of which will be present in the cationic form, is not especially low. The rate constants for the aliphatic esters are about one-half of that for ethyl acetate. There is no significant difference

TABLE II.

Second-order velocity constants for acid hydrolysis at 50° in 80% acetone.

Compound.	10^4k .	Compound.	10^4k .
$\text{CH}_3\cdot\text{CH}_2\cdot\text{OAc}$	3.28	$\text{Ph}\cdot\text{N}(\text{CH}_2\cdot\text{CH}_3)\cdot[(\text{CH}_2)_2\cdot\text{OAc}]$	2.33
$(\text{CH}_3)_2\text{CH}\cdot\text{OAc}$	1.96	$\text{Ph}\cdot\text{N}[(\text{CH}_2)_2\cdot\text{OAc}]_2$	2.18
$(\text{CH}_3)_2\text{N}\cdot[(\text{CH}_2)_2\cdot\text{OAc}]$	1.76–1.04	<i>p</i> -MeO·C ₆ H ₄ ·N[(CH ₂) ₂ ·OAc] ₂	2.06
$\text{CH}_3\cdot\text{N}[(\text{CH}_2)_2\cdot\text{OAc}]_2$	1.54	$\beta\text{-C}_{10}\text{H}_7\cdot\text{N}[(\text{CH}_2)_2\cdot\text{OAc}]_2$	2.06
$\text{CH}_3\cdot\text{CH}_2\cdot\text{S}\cdot[(\text{CH}_2)_2\cdot\text{OAc}]$	2.38	$\beta\text{-C}_{10}\text{H}_7\cdot\text{N}(\text{CH}_2\cdot\text{CHMe}\cdot\text{OAc})_2$	1.96
$\text{S}[(\text{CH}_2)_2\cdot\text{OAc}]_2$	2.36	$\text{HO}\cdot\text{CH}_2\cdot\text{CH}_2\cdot\text{OAc}$	3.46

between the rates of hydrolysis of *isopropyl acetate* and the ester of di-2-hydroxy-*n*-propyl- β -naphthylamine which has a comparable structure.

The variation of the alcohol component of the esters thus has much less effect on the rate of acid hydrolysis that it has on alkaline hydrolysis (cf. Hammett, "Physical Organic Chemistry," New York, 1940, p. 213), in particular, the monoester of ethylene glycol which is hydrolysed approximately ten times as fast as ethyl acetate by alkali is hydrolysed by acid at practically the same rate as the latter. This might have been expected since in acid solution the hydroxy-ester could not assume the ionic form which was regarded as responsible for the high rate of hydrolysis in alkali (Davis and Ross, *loc. cit.*).

A comparison of the second-order velocity constants in 80% acetone for the acid- and base-catalysed hydrolyses now reported—the values are 2.36×10^{-4} and 8100×10^{-4} respectively for di-2-acetoxyethyl sulphide—suggests that the hydroxyl-ion-catalysed reaction will be far more significant at physiological pH.

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