bons given previously were the mean of four determinations when using a specific gravity bottle. The present values differ from the former by 7 units in the fourth place for the *cis* and 1 unit for the *trans*. The present values are regarded as the more accurate of the two.

The surface tension data were plotted on the same large scale graph mentioned above (Fig. 1). The curves obtained were not quite straight lines. The regions of pronounced deviations from linearity have been indicated in Fig. 1 by the broken lines. These deviations may be due to errors as it was noticed that in certain regions it was much more difficult to check results than in others.

There appears to be a considerable change in slope for the *cis* compound at 50°. In this region it was possible to check the differential height readings to within 0.01 cm. of the two samples that were tested. Both samples were measured repeatedly at 60 and 70°, then cooled and measured at 20, 30, 40 and 50° in order to determine whether some change in the *cis* form did take place. Since it is felt the results are accurate to within 1% over the temperature range from 20 to 70°, some significance must be ascribed to the change in slope of the temperature–surface tension curve at 50°. Just what the nature of the change is cannot be explained at present. However, other measurements now in progress indicate that the *cis* form behaves in an abnormal manner in the temperature region about 50°.

Summary

1. The densities and surface tensions of *cis*- and *trans*-decahydronaphthalene have been measured from −30 to 180°.

2. The density–temperature relations are nearly linear, but the others deviate somewhat from linearity.

VANCOUVER, B. C. RECEIVED JUNE 3, 1941

[CONTRIBUTION FROM THE FRICK CHEMICAL LABORATORY OF PRINCETON UNIVERSITY]

The Rates of Ammonolysis of Some α-Halogen Acids and α-Halogen Acyl Peptides[1]

BY ALBERT F. CHADWICK[2] AND EUGENE PACSU

The most important method used for the synthesis of polypeptides is that which consists of first condensing an α-halogen acyl halide with an α-amino acid or with a di-, tri-, etc., peptide and then treating the condensation product with ammonia. This gives a polypeptide containing one more amino acid residue than the starting compound. Many amino acids themselves are synthesized from α-halogen acids and ammonia. Up to the present time the ammonolysis step in the synthesis has not given satisfactory yields as a rule. This is partly due to the hydrolysis which takes place if the polypeptide product is left longer in the concentrated ammonia solution than is necessary and partly due to the reaction between some of the amino compound as it is formed and the unreacted halogen compound, resulting in substituted secondary and tertiary amines.[3]

Emil Fischer, who has done the most work in this field, experimented with varying his reaction conditions but still selected them arbitrarily and made no quantitative study. He prepared diglycylglycine from chloroacetylglycylglycine by treating the latter compound with 25% aqueous ammonia for fifteen hours at room temperature.[4a] In the same paper, however, he describes the preparation of triglycyl- and tetraglycylglycine by boiling the corresponding chloroacetyl derivatives with concentrated ammonia for an hour. The same conditions were used in the preparation of leucylalanine from α-bromoisocaproylalanine,[4b] while leucylglycylglycine was prepared[4a] by boiling α-bromoisocaproylglycylglycine with ammonia for only half an hour. When several years later Abderhalden and Fodor[5] prepared diglycylglycine, they used more drastic conditions than did Fischer, treating the chloroacetyl compound with 25% ammonia for twenty-four hours at 37°. Their yield was no better than Fischer's. More recently, however, Abderhalden and co-workers[6] have made some quantitative measurements on

(1) This work was supported in part by a grant from the Rockefeller Foundation.

(2) Ethyl Gasoline Corporation Fellow in Chemistry.

(3) (a) Robertson, THIS JOURNAL, **49**, 2889 (1927); (b) Cheronis and Spitzmueller, *J. Org. Chem.*, **6**, 349 (1941).

(4) (a) Fischer, *Ber.*, **37**, 2486 (1904); (b) Fischer, *Ann.*, **340**, 123 (1905).

(5) Abderhalden and Fodor, *Ber.*, **49**, 561 (1916).

(6) (a) Abderhalden and Heumann, *Z. physiol. Chem.*, **205**, 271 (1932); (b) Abderhalden and Beckmann, *ibid.*, **207**, 93 (1932); (c) Abderhalden and Bahn, *Ber.*, **63**, 914 (1930).

similar reactions. Most of their work was done for the purpose of differentiation between various α-halogen acids by means of their rates of reaction with ammonia. They also measured the rates of ammonolysis of the α-bromopropionyl derivative of alanine, alanylalanine, dialanylalanine and trialanylalanine for preparative purposes. As they apparently made no accurate check on the concentration of the ammonia, one cannot conclude much about the relationship of rates which are so nearly alike.

The preparation of glycine from chloroacetic acid has been studied quite extensively but the yields obtained by treating it with aqueous ammonia have not been satisfactory. Robertson[3a] studied the effect on the yield of varying the ratio of ammonia to chloroacetic acid. With a 220 to 1 excess of ammonia he found that, after two days at room temperature, the primary amino content of the solution was 95% of the theoretical final value while all of the chlorine was present in the ionic form. The difference was due to the reaction of some of the glycine formed with chloroacetic acid yielding secondary and tertiary amino compounds. With a 60 to 1 excess of ammonia only 86% of the theoretical primary amino content was found. Orten and Hill[7] used Robertson's conditions (60 to 1 excess of ammonia) but improved the method of isolation. Cheronis and Spitzmueller[3b] modified the procedure by using a saturated solution of ammonium carbonate in aqueous ammonia at 60°. Their results with only a 4 to 1 excess of ammonium ion were as good as those of Orten and Hill with a 60 to 1 excess, probably because the lowering of the *p*H of the solution by the added ammonium ion prevented the formation of the secondary and tertiary amino compounds to a great degree.

With the starting compounds other than chloro- or bromoacetic acid these secondary reactions do not seem to be very important because yields as high as 85% have been obtained from several of them (see below) with only an 18 to 1 excess of ammonia. Fischer and Abderhalden usually employed an excess of from 10 or 15 to 1.

The purpose of the present investigation has been to determine the exact end-point of the reaction by measurement of the rates of ammonolysis of a group of nine compounds selected so as to give an indication of the effect of structure on the rates. In each case the measurement has been carried out at two or more temperatures so that the activation heats and entropies could be calculated.

(7) Orten and Hill, THIS JOURNAL, **53**, 2797 (1931).

Experimental

Chloroacetylglycine.—This compound was prepared from chloroacetyl chloride and glycine by the method of Landsteiner and Van der Scheer.[8] However, ethyl alcohol was used instead of ethyl acetate to recrystallize the product. The crystals obtained melted at 105–106° instead of 92–93° as reported by Landsteiner and Van der Scheer. By titration with standard alkali solution the neutralization equivalent of the acid was found to be 150.7 as compared with the theoretical value of 151.5.

Chloroacetylglycylglycine.—Glycine was converted into its ethyl ester hydrochloride by the method of Harries and Weiss.[9] Diketopiperazine was prepared from the glycine ethyl ester hydrochloride and, after hydrolysis with sodium hydroxide solution, was condensed with chloroacetyl chloride to give the desired chloroacetylglycylglycine. These reactions were carried out according to the directions of Fischer.[10]

Chloroacetyldiglycylglycine, *dl*-α-Bromoisocaproylglycine and *dl*-α-Bromoisocaproyl-*dl*-alanine.—All were prepared by Fischer's methods.[11]

Chloroacetic Acid, Bromoacetic Acid and *dl*-α-Bromopropionic Acid.—These were commercial products purified by vacuum distillation.

***dl*-α-Bromoisocaproic Acid.**—This was synthesized by standard methods and purified by vacuum distillation.

Method of Rate Measurement.—The method used for measuring the rates of ammonolysis was based on the Volhard titration for halide ion. A weighed quantity of the compound to be studied was placed in a volumetric flask, about 90% neutralized with dilute ammonia and then allowed to come to the temperature of the bath in which the flask was to be kept during the reaction. For this purpose a thermostat controlled bath was used which kept the temperature constant to within two or three hundredths of a degree. After the solution of the ammonium salt had reached the bath temperature, concentrated aqueous ammonia and water, both preheated to the bath temperature, were added up to the mark on the volumetric flask, which was then stoppered and immersed in the bath. The quantity of each constituent to be added was calculated so that the resulting solution would have as nearly as possible the desired concentration of ammonia, which was then quantitatively estimated on a sample of the solution. At convenient intervals of time samples were removed with a pipet and immediately run into an excess of ice-cold 3 *N* nitric acid solution. This, of course, stopped any further reaction. They were then titrated to determine the amount of ionized halide present. As one halogen ion is formed for every molecule of α-halogen compound which reacts, this indicates how far the reaction has gone when the sample is taken. In the titration the ionic chloride was first precipitated from the nitric acid solution with an excess of standard silver nitrate solution, and then the solu-

(8) Landsteiner and Van der Scheer, *J. Exptl. Med.*, **55**, 781 (1932).
(9) Harries and Weiss, *Ann.*, **327**, 365 (1903).
(10) Fischer, *Ber.*, **39**, 2893 (1906).
(11) Fischer, *ibid.*, **36**, 2983 (1903).

tion and precipitate were shaken with one cc. of nitrobenzene. The excess silver ion was then titrated with standard ammonium thiocyanate solution in the presence of ferric ion as an indicator. From a series of checks it was found necessary to add 2.5% to the value thus found to determine the true chloride concentration. In the estimation of the bromide ion the addition of nitrobenzene and correction by an empirical factor were found unnecessary.

The results for the various compounds are listed in Table I. In calculating the constants it was found necessary to use the first titration as the starting point because of two factors which made the true starting point indefinite. First, the ammonia cooled off slightly while it was being poured into the volumetric flask. This tended to make the constant low over the first interval. Second, some of the compounds were found to split off halide appreciably while their solutions, nearly neutralized with dilute ammonia, were being raised to the bath temperature prior to the addition of the concentrated aqueous ammonia. As a result the initial halide concentration was not zero. The reaction is bimolecular but gives a first order constant because the ammonia is present in great excess.

Methods of Preparation.—The rate measurements were made with 0.05 *N* solutions of the α-halogen compounds in order to eliminate errors due to the heat of the reaction. As this is too great a dilution to be practical for preparative purposes, the yield obtained by treating a more concentrated solution of chloroacetylglycylglycine under the same conditions was determined. It was found that a 0.5 *N* solution could be used without impairing the yield.

TABLE I

RATES OF AMMONOLYSIS OF SOME HALOGEN ACIDS AND HALOGEN ACYL PEPTIDES IN 0.05 *N* SOLUTIONS WITH CONCENTRATED AMMONIA AT DIFFERENT TEMPERATURES

Time, min.	*N* of Cl^-	$10^4 \times k_1$ sec.$^{-1}$	Time, min.	*N* of Cl^-	$10^4 \times k_1$ sec.$^{-1}$
Chloroacetic Acid					
(a) T = 39.9°; 9.2 *N* ammonia (P_{NH_3} = 0.537 atm.)			(b) T = 39.9°; 8.6 *N* ammonia (P_{NH_3} = 0.472 atm.)		
8	0.0032		8	0.0041	
15	.0064	1.67	15	.0069	1.49
30	.0124	1.64	30	.0123	1.48
60	.0221	1.64	60	.0214	1.50
90	.0294	1.65	90	.0285	1.53
120	.0351	1.68	120	.0340	1.51
180	.0420	1.67	180	.0409	1.52
360	.0490	1.67	278	.0462	1.50
1020			1140	.0503	
(c) T = 50.0°; 9.1 *N* ammonia (P_{NH_3} = 0.768 atm.)			(d) T = 50.0°; 9.4 *N* ammonia (P_{NH_3} = 0.812 atm.)		
5	0.0076		5	0.0076	
10	.0124	3.96	10	.0126	4.20
15	.0169	4.08	15	.0172	4.28
20	.0207	4.06	20	.0209	4.20
30	.0275	4.17	30	.0276	4.27
60	.0400	4.29	45	.0354	4.45
120	.0478	4.06	60	.0406	4.58
1320	.0504		120	.0481	4.58
			1320	.0499	

Compound	Temp., °C.	*N* Ammonia	P_{NH_3}, atm.	$10^4 \times k_1$ sec.$^{-1}$	Av. dev. (±)
Chloroacetylglycine	39.9	9.3	0.547	3.18	0.07
	39.9	8.6	.472	2.83	.05
	23.0	9.1	.254	0.51	.00
	20.4	9.3	.229	0.38	.01
Chloroacetylglycylglycine	39.9	9.6	.583	4.32	.30
	39.9	9.0	.514	3.68	.10
	39.9	2.3	.086	0.68	.01
	39.9	2.0	.074	0.58	.01
	19.1	9.4	.218	0.43	.00
	18.1	9.2	.203	0.36	.01
Chloroacetyldiglycylglycine	39.9	9.2	.537	4.03	.03
	39.9	8.6	.472	3.45	.07
	19.8	9.2	.221	0.48	.02
	19.8	9.2	.221	0.47	.03
Bromoacetic acid	39.9	9.2	.537	104.00	...
	24.6	9.0	.270	17.90	.50
	15.2	9.2	.174	6.40	.07
	13.6	9.2	.162	5.50	.20
	0.1	9.5	.086	1.33	.04
	0.1	9.0	.078	1.12	.03
dl-α-Bromopropionic acid	39.9	9.2	.537	4.00	.30
	39.9	8.9	.503	3.30	.10
	28.2	9.1	.328	1.06	.02
	28.2	9.0	.325	1.07	.01
	21.7	9.1	.244	0.53	.02
	21.0	9.1	.233	0.50	.02
dl-α-Bromoisocaproic acid	50.0	9.0	.755	2.43	.02
	50.0	8.6	.697	2.30	.03
	39.9	8.8	.491	0.85	.02
	39.9	8.3	.443	0.75	.02
dl-α-Bromoisocaproyl glycine	50.0	9.0	.755	2.12	.01
	39.9	9.1	.525	0.80	.03
dl-α-Bromoisocaproyl *dl*-alanine	50.8	8.9	.742	2.00	.05
	39.9	9.1	.525	0.72	.03
	39.9	8.9	.503	0.72	.02
	39.9	8.9	.503	0.70[a]	.03

[a] In 0.1 *N* solution.

In purifying glycine and the glycine peptides a modification of Fischer's general procedure was used. The reaction mixture was taken to dryness in a vacuum. To make the product absolutely dry a little absolute alcohol was added and the vacuum applied again. A large part of the ammonium chloride present was then removed by extraction with warm methyl alcohol. The product was finally purified by dissolving it twice in a minimum of warm water (50°) and precipitating it with ethyl alcohol. Some of the product may be recovered from the alcohol–water mother liquors. The yields obtained with 0.05 *N* solutions were: glycine 74%, glycylglycine 89%, diglycylglycine 86% and triglycylglycine 80%. With the 0.5 *N* solution of chloroacetylglycylglycine a yield of 86% of diglycylglycine was obtained.

To facilitate the synthesis of the corresponding peptides small samples of four halogen compounds, *dl*-α-bromopropionyl-*dl*-leucylglycine, *dl*-α-bromopropionylglycylglycine, *dl*-α-bromoisocaproylglycylglycine, and chloroacetyl-*dl*-leucyl-*dl*-alanine, were treated with 9 *N* ammonia at 50°. The end-points of the reactions were measured but not their rates. The α-bromopropionyl and chloro-

acetyl derivatives required about two and a half hours while the α-bromoisocaproylglycylglycine required ten hours. Using these data E. J. Wilson in this Laboratory obtained recrystallized yields of 80% of *dl*-alanylglycylglycine and *dl*-leucylglycylglycine, 83% of *dl*-alanyl-*dl*-leucylglycine and 70% of glycyl-*dl*-leucyl-*dl*-alanine. In all cases he used 0.5 *N* solutions of the halogen compounds and 9 *N* ammonia. The improvement in the yields is probably due to a combination of the two factors, the greater excess of ammonia and stopping the reaction at its exact end-point.

Theoretical

The reaction gives good first order constants as it should because the ammonia is in great excess. If it is actually a bimolecular reaction one will expect the first order constants to be proportional to the activity of the dissolved ammonia in each case. As the partial pressure of ammonia in equilibrium with the solution is a measure of the activity of the ammonia in the solution, the first order constants should be proportional to this partial pressure. For chloroacetylglycylglycine reacting with 2.0, 2.3, 9.0 and 9.6 *N* ammonia at 39.9° the quotients (k_1/P_{NH_3}) are 0.00078, 0.00080, 0.00075 and 0.00077 sec.$^{-1}$ atm.$^{-1}$, respectively. This constancy shows that the velocity of the reaction may be expressed by the equation $v = P_{NH_3}ck_2$ in which P_{NH_3} is the partial pressure of the ammonia, c is the concentration of the halogen compound, and k_2 is a second order rate constant. The pressure may be assumed to be constant during the course of the reaction where the ammonia is in large excess. Therefore, the second order constant may be found by dividing the first order constant by the partial pressure of the ammonia. The $k_2 = k_1/P_{NH_3}$ mean values for these bimolecular constants are given in Table II.

TABLE II

THE BIMOLECULAR CONSTANTS AT 39.9° AND THE HEATS AND ENTROPIES OF ACTIVATION OF THE AMMONOLYSIS OF SOME HALOGEN ACIDS AND HALOGEN ACYL PEPTIDES

Compound	k_2, atm.$^{-1}$ sec.$^{-1}$ (mean for 39.9°)	Heat $\Delta H^{\ddagger}$, kcal.	Entropy $\Delta S^{\ddagger}$, e. u.
Chloroacetic acid	0.00032	2.84	−65.5
Chloroacetylglycine	.00060	2.73	−64.5
Chloroacetylglycylglycine	.00077	2.84	−63.8
Chloroacetyldiglycylglycine	.00073	2.69	−64.4
Bromoacetic acid	.0165[a]	2.37	−59.2
dl-α-Bromopropionic acid	.00070	2.60	−65.0
dl-α-Bromoisocaproic acid	.00017	3.40	−65.0
dl-α-Bromoisocaproylglycine	.00015	3.20	−66.0
dl-α-Bromoisocaproyl-*dl*-alanine	.00014	3.60	−65.0

[a] This value was extrapolated from the rates at lower temperatures.

By the theory of absolute reaction rates[12] the velocity may be expressed as follows

$$v = P_{NH_3}\, c\frac{k_0T}{h}\, e^{-\Delta H^{\ddagger}/RT}\, e^{\Delta S^{\ddagger}/R}$$

$$\text{or } k_2 = \frac{k_0T}{h}\, e^{-\Delta H^{\ddagger}/RT}\, e^{\Delta S^{\ddagger}/R}$$

where $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ are the changes in heat and entropy during the formation of the activated complex, k_0 and h are the Boltzmann and Planck constants, respectively. $\Delta H^{\ddagger}$ is identical with the heat of activation of the reaction and may be calculated by plotting $k_2/(k_0T/h)$ against $1/T$ for the various compounds. The activation heats so found have been included in Table II. This equation may also be expressed in the form

$$\Delta S^{\ddagger} = \frac{\Delta H^{\ddagger}}{T} + R \ln\left(k_2\Big/\frac{k_0T}{h}\right)$$

which leads to the evaluation of the entropy of activation. These values have also been listed in Table II. Because the standard state for the ammonia is taken to be the gas at one atmosphere pressure, the large negative heat and entropy of solution are included in the values found from the equations above. This makes $\Delta H^{\ddagger}$ smaller than that for many organic reactions. The entropy change is correspondingly greater. The heats of activation are about the same for the four chloro compounds while the entropy change for the chloroacetic acid itself is a little greater than for the three condensed derivatives. In the case of the three bromo acids both the heat and entropy changes increase in the series: bromoacetic acid < α-bromopropionic acid < α-bromoisocaproic acid. The differences in rates among the three α-bromoisocaproyl derivatives are not great enough to show very well in the exponential variables.

As atomic models indicate that the steric effect of the longer side chains is probably negligible, all of these differences can best be explained by the theory of Ri, Magee and Eyring[13] in terms of the electronegativity of certain groups and the effect of increased size of the carbon chain upon distribution of the resulting charges in the mole-

(12) Wynne-Jones and Eyring, *J. Chem. Phys.*, **3**, 492 (1935).

(13) To be published shortly in the *J. Chem. Phys.* We are indebted to Drs. Ri, Magee and Eyring for allowing us to use this reference.

cule. In the activated complex of the reaction the amino group and the halide atom are most probably both weakly bound to the carbon on which the substitution takes place. During the formation of this complex the negatively charged NH_2 group must therefore approach this carbon. A positive charge on the carbon will naturally speed up the reaction by lowering the energy necessary for the formation of the activated state. With two compounds in which all factors but this are the same one will therefore expect the one with the greater positive charge on the reacting carbon to have the greater reaction velocity. The halogen atoms in the C–Cl and C–Br linkages are negative with respect to the carbon. The presence of this small positive charge on the carbon is shown by the dipole moments of compounds like methyl bromide and methyl chloride. When there is a side chain linked to this carbon, some of the charge is induced down the chain through the covalent bonds. This lowers the charge on the carbon atom which is linked to the halogen atom and slows the reaction. The nearer a carbon atom is to the one which carries the initial charge, the more of the charge it acquires. Therefore, a branched chain will slow the reaction more than a normal side chain of the same length.

The results described in the first parts of this paper as well as those of Abderhalden[6b,c] agree with this picture of the reaction mechanism. The rates of reaction for the normal α-bromo aliphatic acids decrease with increasing size of the acid molecule. The drop between the rates of bromoacetic and α-bromopropionic acids is large but the effect is smaller as one goes further in the series. Abderhalden's results show that, with the branched chain compounds, the effect is greater the nearer the branching is to the α-carbon. Thus the rates of ammonolysis of the various caproic acids decrease in the order: α-bromo-*n*-caproic acid > α-bromo-4-methylvaleric acid > α-bromo-3-methylvaleric acid. The effect of changing the free carboxyl into a peptide linkage is not definite from this work. Chloroacetylglycine reacts faster than chloroacetic acid, while α-bromoisocaproylglycine reacts slower than the corresponding acid.

Summary

The rates of reaction of nine different α-halogen acids and α-halogen acyl peptides with an excess of ammonia have been measured and the first order constants determined.

The reactions have been shown to be bimolecular because the first order constants are proportional to the partial pressures of ammonia in equilibrium with the solutions.

The heats and entropies of activation have been calculated for the reactions starting with gaseous ammonia at one atmosphere.

By means of the measurements the yields obtained from the ammonolysis reaction have been increased.

PRINCETON, NEW JERSEY RECEIVED JUNE 5, 1941

[CONTRIBUTION FROM THE CHEMISTRY LABORATORY OF THE OHIO STATE UNIVERSITY]

A New Method for the Esterification of Certain Sterically Hindered Acids

BY MELVIN S. NEWMAN

The fact that certain substituted benzoic acids are not appreciably esterified on refluxing with alcohols containing mineral acids has long been known. The method most commonly used for preparing methyl esters of such sterically hindered acids consists in treating the acid with diazomethane. Other methods involve treatment of the acid chloride with methanol, heating of the silver salt with methyl iodide, and pyrolysis of the tetramethylammonium salt.[1] With the exception of alcoholysis of the acid chloride all of these methods have obvious disadvantages if other than methyl esters are to be prepared. In this paper a new method for the esterification of certain sterically hindered acids is described. This method consists in dissolving the acid to be esterified in approximately 100% sulfuric acid and pouring the solution into the desired alcohol. In principle the new procedure is not limited with respect to the alcohol although we have been unable to prepare an ester from *t*-butyl alcohol in this way. The following esters have been prepared in excellent yield by this method: methyl, ethyl,

(1) Fuson, Corse and Horning, THIS JOURNAL, **61**, 1290 (1939).