was converted to DL-tryptophan-β-H^3 diacetate by the procedure of Snyder and Smith.[14]

Assay of the Radioactive Samples.—Samples containing only C^{14} and those containing both C^{14} and H^3 were assayed for C^{14} using a Nuclear-Chicago Model C-115 low background Q gas flow counter. Determinations were carried out on samples of finite thickness making corrections for efficiency and self-absorption. Compounds containing C^{14} and H^3 were assayed for both isotopes in a Tri-Carb liquid scintillation counter, Model 314 E (Packard Instrument Co.).[22] The solvent used for assay of the tryptophan-β-H^3 consisted of 70% toluene and 30% ethanol and contained 0.5% 2,5-diphenyloxazole (PPO) as a scintillator. Gramine and its degradation products were assayed in toluene containing 0.5% PPO as a primary scintillator and 0.05% 1,4-bis-2-(4-methyl-5-phenyloxazolyl)-benzene as a secondary scintillator.

(22) We thank Dr. Frank Ungar of the Department of Physiological Chemistry for the use of his scintillation counter. We are also indebted to Drs. Maura E. Beary and Robert L. Conner for invaluable help in the preparation of samples and use of the counter.

Administration of the Tracers to the Plants and Isolation of the Gramine.—Charlottetown No. 80 barley (*Hordeum distichum*)[23] (720 g.) was germinated by placing in distilled water in Pyrex dishes as previously described.[4] It was found advantageous to spread out the barley on a piece of cotton cloth, almost complete germination thus being obtained. An aqueous solution of the labeled tryptophans was added to the roots of the barley seedlings 5 days after germination. The shoots (wet wt. 1065 g.) were cut off 6 days later and extracted as previously described[4] yielding gramine (201 mg.). The incorporation of tracers was 0.27%.

Degradation of Gramine.—A sample of the synthetic gramine labeled with H^3 on the methylene group was treated with ethyl iodide and sodium ethoxide yielding 3-ethoxymethylindole[4] which had the same specific activity as the gramine. Fusion of the tritium labeled gramine with potassium hydroxide yielded inactive indole-3-carboxylic acid. The radioactive gramine isolated from the barley was subjected to the same degradations and the activities of the degradation products are recorded in Table I.

(23) We thank Mr. R. B. MacLaren of the Experiment Station, Charlottetown, Prince Edward Island, Canada, for a generous supply of barley.

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, POLYTECHNIC INSTITUTE OF BROOKLYN, BROOKLYN 1, N. Y.]

Kinetics and Equilibria of Amide Formation in Aqueous Media[1,2]

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Rates of amide formation were measured in aqueous solutions containing various combinations of a 0.2 *N* carboxylic acid (formic, acetic, propionic, butyric, isobutyric and succinic) and 1 *N* amine (ammonia, methylamine, ethylamine, isopropylamine, ethanolamine and dimethylamine) at one or several temperatures. The rate is first order in the anion of the acid and the basic form of the amine, the second-order rate constant being independent of the acidity of the medium. At 44.4°, formate reacts with methylamine 140 times more rapidly than acetate; for the higher fatty acids the rate decreases only slightly. Methylamine reacts with propionate and isobutyrate at 75.8° eleven and six times, respectively, as fast as with ammonia; the activation energy for the methylamine reaction is on the average lower by 5 kcal./mole. Equilibrium constants for amide formation (formulated as $R_1COO^- + R_2R_3NH \rightleftarrows R_1CONR_2R_3 + OH^-$) in dilute aqueous systems were calculated at one temperature for six systems and at a number of temperatures for seven systems from rate constants for amide formation and hydrolysis. Values for N-methylpropionamide and N-methylisobutyramide at 75.8° were larger by factors of about one hundred than those for the primary amides; the value for N,N-dimethylpropionamide was intermediate. For primary amides, ΔH of amide formation is 6–9 kcal./mole, for N-methylamides it is slightly negative. While N-methylformamide formation has ΔF^0 more negative than N-methylacetamide, the order is reversed with N-isopropylformamide and N-isopropylacetamide due, possibly, to an interaction of the hydrophobic residues in N-isopropylacetamide. Thermodynamic data for primary amide formation obtained in this study are similar to those reported for enzymatically catalyzed systems, but the formation of N-methylamides has ΔF^0 more negative by 4–5 kcal./mole and ΔH more negative by 8 kcal./mole than for the formation of peptide bonds. Amide formation from propionate and ammonia or methylamine is accelerated by addition of 1-propanol, the maximum rate (three times as high as in water) occurring at 70 weight % of the alcohol. No acceleration is observed on addition of 2-propanol.

Introduction

Very little is known about equilibria of organic reactions in dilute aqueous systems. This is particularly unfortunate in view of the fact that the life process proceeds in a medium of very high water concentration. The living cell is a dynamic system in which the concentrations of reagents and reaction products are generally not related by the conditions of chemical equilibrium, but a knowledge of the free energy relations of important biochemical processes is important to an understanding of the energy balance of the cell. In the absence of the required free energy data, biochemists have frequently used reaction heats as a rough measure of the driving force, but such a procedure has obvious shortcomings.

One of the most important biochemical processes is the synthesis of the amide bond, required for the production of protein molecules. It has long been known that this process is characterized, under physiological conditions, by a positive free energy change and that it must, therefore, be coupled to an exergonic reaction. Lipmann was first to suggest[3] that the energy required for the synthesis of an amide bond is derived from the splitting of the "high energy phosphate bond" of adenosine triphosphate and this mechanism has been demonstrated convincingly in the enzymatically catalyzed syntheses of peptides and proteins.[4]

The magnitude of the free energy requirement in the synthesis of a dipeptide from two amino acids was estimated by Huffman[5] for processes formulated as:

$$H_3\overset{+}{N}CHR_1COO^-(s) + H_3\overset{+}{N}CHR_2COO^-(s) \rightleftarrows H_3\overset{+}{N}CHR_1CONHCHR_2COO^-(s) + H_2O(l) \quad (1)$$

The ΔF_{293}^0 varied from +1.4 to +3.7 kcal./mole. Attempts to obtain similar data for reactions proceeding in aqueous solution were first made by Borsook and Dubnoff,[6] who estimated ΔF^0 for the condensation of benzoate and glycine to hippurate from heats of combustion, heat capacity and solubility data. Later studies of the condensation of benzoyltyrosine with glycinamide[7a] and of glutamate or aspartate with ammonia[7b] utilized enzymatic catalysis to attain rapid chemical equilibrium. Since very little amide was

(1) Based in part on a Ph.D. thesis to be submitted by P. S. Otaki to the Graduate School, Polytechnic Institute of Brooklyn, in June, 1963.

(2) This investigation was supported by a grant of the National Institutes of Health.

(3) F. Lipmann, *Advan. Enzymology*, **1**, 154 (1941); *Federation Proc.*, **8**, 597 (1949).

(4) H. Chantrenne, "The Biosynthesis of Proteins," Pergamon Press, New York, N. Y., 1961, pp. 92-112.

(5) H. M. Huffman, *J. Phys. Chem.*, **46**, 885 (1942).

(6) H. Borsook and J. W. Dubnoff, *J. Biol. Chem.*, **132**, 307 (1942).

(7) (a) A. Dobry, J. S. Fruton and J. M. Sturtevant, *ibid.*, **195**, 149 (1952); (b) T. Benziger, C. Kitzinger, R. Hems and K. Burton, *Biochem. J.*, **71**, 400 (1959).

present at equilibrium under their experimental conditions, the determination of the equilibrium required the use of tracer techniques[7a] or of very high reagent concentrations.[7b]

In the present study conditions were established under which carboxylic acids in dilute aqueous solutions are converted to amides without enzymatic catalysis at analytically detectable rates. This appears to be the first such study of the kinetics of amide formation except for a brief report on the condensation of benzocaine or aniline with citric acid.[8]

It was desired to obtain data at relatively low temperatures so as to allow valid extrapolations to temperatures characteristic of life processes. Since reaction equilibrium could not be reached under these conditions in practicable times, the equilibrium constants were estimated as the ratios of the rate constants for amide formation and amide hydrolysis. The method was applied to systems containing a variety of carboxylic acids with ammonia, primary amines and one secondary amine.

Results and Discussion

The Formulation of the Reaction.—The equilibrium of amide formation is usually represented by

$$R_1COO^- + H_3\overset{+}{N}R_2 \rightleftharpoons R_1CONHR_2 + H_2O \quad (2)$$

This formulation has the advantage that it represents the reagents in their ionized form, which is the form in which most of the reagent molecules are present under physiological conditions. However, when an attempt is made to interpret the experimental data, this formulation introduces the undesirable complication that the amide formation is accompanied by the disappearance of two ionic charges. On the other hand, if we use the representation, as recommended by Carpenter[9]

$$R_1COOH + R_2NH_2 \rightleftharpoons R_1CONHR_2 + H_2O \quad (3)$$

then an estimate of the concentration of the two unionized reagent species requires a knowledge of the ionization equilibrium constant, which is inconvenient if elevated temperatures and mixed solvent media are to be included in the study. In addition, data interpreted on the basis of 2 or 3 suffer from an inconsistency in the choice of the standard state, since the activity of all the species are taken as equal to the molarity in dilute solution, except for water where the activity is taken as unity for the pure liquid. We found it, therefore, most desirable to use the formulation

$$R_1COO^- + HN(R_2)(R_3) \underset{k_2}{\overset{k_1}{\rightleftharpoons}} R_1CON(R_2)(R_3) + OH^- \quad (4)$$

It is immediately apparent that the extent to which the carboxylate ion is converted to amide will increase with the ratio of the concentrations of amine and hydroxide ion. This ratio may be made very high in buffer systems containing the carboxylic acid with an excess of amine and under such conditions an appreciable fraction of the acid may be converted to the amide.

The Kinetic Order of Amide Formation.—The kinetic order of amide formation from a carboxylic acid anion and the basic form of an amine was studied in detail for the propionate–methylamine system. The results are listed in Table I and they show that amide formation is first order in both propionate and unionized methylamine. In particular, a threefold increase in the $[CH_3NH_2]/[CH_3NH_3^+]$ ratio does not lead to a significant shift of the rate constant, proving that hydrogen and hydroxyl ion concentrations affect the rate of amide formation only insofar as they determine the state of ionization of the reagents.

(8) T. Higuchi and T. Miki, *J. Am. Chem. Soc.*, **83**, 3899 (1961).
(9) F. H. Carpenter, *ibid.*, **82**, 1111 (1960).

TABLE I

THE KINETIC ORDER OF AMIDE FORMATION FROM PROPIONIC ACID AND METHYLAMINE AT 75.8°

$[C_2H_5COO^-]$, moles/l.	$[CH_3NH_2] + [CH_3NH_3^+]$, moles/liter	$[CH_3NH_2]/[CH_3NH_3^+]$	10^5k_1, l. mole^{-1}-sec.$^{-1}$
1.0	3.0	2	3.83
2.0	6.0	2	3.01
0.5	2.5	4	3.25
1.0	5.0	4	3.25
0.5	3.5	6	2.92

Effect of Reactant Concentrations on the Equilibrium Constant.—In spite of the high sensitivity of the analytical methods used in this study, detectable reaction rates could be obtained at moderate temperatures only with fairly high reagent concentrations. Thus, the reaction medium was significantly different from pure water and the question arose what changes in the rate constant might be introduced by such changes in the solvent medium. To answer this question, the reaction of propionate with ammonia and methylamine, respectively, was studied at a varying concentration of the reagents, keeping the ratio of their stoichiometric concentrations fixed at 1:5. The results in Table II indicate a possible trend for the rate constants to increase at low reagent concentrations, but the effect is obviously rather small.

TABLE II

AMIDE FORMATION FROM PROPIONIC ACID AND AMMONIA OR METHYLAMINE. EFFECT OF REAGENT CONCENTRATION ON THE RATE CONSTANT

Stoichiometric ratio of acid and amine concentration 1:5

$[C_2H_5COO^-]$, mole/l.	10^5k_1, l.-mole^{-1}-sec.$^{-1}$	
	NH_3 at 94.4°	CH_3NH_2 at 75.8°
0.10	2.0	5.0
.15	1.7	4.2
.20	1.7	4.4
.25	1.8	4.5

Rates of Amide Formation from Various Reagents.—A summary of the rate constants obtained for the amide formation from fifteen pairs of reagents, mostly over a range of reaction temperatures, is given in Table III. All these data were obtained with initial stoichiometric concentrations of 1 *N* amine and 0.2 *N* carboxylic acid. The following comparisons are worth noting: (1) Formate is very much more reactive than acetate for the reaction with methylamine at 44.4°; the ratio of their rate constants is 140. The difference between the reactivities of acetate, propionate, butyrate and isobutyrate is much less pronounced. The reactivity of succinate is very similar to that of propionate, showing that a neighboring carboxylate does not participate in the mechanism of amide formation.

(2) The reaction rate of methylamine is considerably above that of ammonia; the ratio of the reaction rates at 75.8° is 11 with propionic acid and 6 with isobutyric acid. The reaction rate of dimethylamine is slightly above that of ammonia. The increase in the reactivity in passing from NH_3 to $MeNH_2$ reflects undoubtedly the increased basicity of the nitrogen; with Me_2NH the further increase in basicity appears to be nullified by a steric hindrance effect. It is surprising that the rate constant drops by a factor of four in passing from the propionate–methylamine system to the propionate–ethylamine system. Isopropylamine is still more inert, but the ratio of its reaction rate to that of methylamine depends to a surprising extent on the acid,

TABLE III

RATE CONSTANTS FOR AMIDE FORMATION

Amine	Acid	10^3k_1, l.-mole^{-1}-sec.$^{-1}$ 44.4°	60.0°	75.8°	85.7°	94.4°	104°	ΔE^*, kcal./mole
NH_3	Propionic			3.6	8.1	16.3		20.6
NH_3	Butyric			2.4	5.9	9.7		18.8
NH_3	Isobutyric			2.2	5.2	11.8		22.9
NH_3	Succinic			3.9	8.7	19	40	21.5
CH_3NH_2	Formic	830						
CH_3NH_2	Acetic	5.9						
CH_3NH_2	Propionic			44	77	113		12.9
CH_3NH_2	Isobutyric		5.2	13	23			13.7
CH_3NH_2	Succinic		10	38	86	180		20.4
$C_2H_5NH_2$	Propionic						55	
$C_2H_5NH_2$	Isobutyric						8.6	
$HOC_2H_4NH_2$	Propionic		5.0					
$(CH_3)_2CHNH_2$	Formic	6.7	21					
$(CH_3)_2CHNH_2$	Acetic	0.3						
$(CH_3)_2CHNH_2$	Propionic					2.5	4.4	
$(CH_3)_2NH$	Propionic			5.7	10.5	17.4		15.2

TABLE IV

RATE CONSTANTS FOR HYDROXIDE ION CATALYZED AMIDE HYDROLYSIS

Amide	10^4k_2, l.-mole^{-1}-sec.$^{-1}$ 25°	44.4°	50°	60°	75.8°	85.7°	94.4°	104°	ΔE^*, kcal./mole
Propionamide[a]					10.5				14.5
Butyramide[b]					4.53				14.7
Isobutyramide[b]					4.15				14.2
Succinamic acid[c]	0.29								13.9
N-Methylformamide		15.0							
N-Methylacetamide		0.32							
N-Methylpropionamide				0.51	1.44	2.73			15.5
N-Methylisobutyramide					0.23	0.42	0.75		16.1
N-Methylsuccinamic acid			0.176						
N-Ethylpropionamide			0.146						
N-Isopropylformamide		8.3							
N-Isopropylacetamide		0.18							
N-Isopropylpropionamide								0.52	
N,N-Dimethylpropionamide				1.11	2.85	4.72			13.3

[a] Interpolated from data in ref. 10. [b] Interpolated from data in ref. 11. [c] Data from ref. 12.

being 1:120 for the reaction with formate, but only 1:20 for the reaction with acetate. Finally, it may be noted that ethanolamine reacts with propionate only half as fast as methylamine, so that the β-hydroxyl group does not seem to participate in the mechanism of amide formation.

In comparing the activation energies, we find that the values for the ammonia reaction with the monocarboxylic acid anions (19–23 kcal./mole) are substantially higher than those for the corresponding reactions with methylamine (13–14 kcal./mole). The rates differ by a smaller factor than would correspond to this difference in activation energies, indicating that it is partially compensated by a larger entropy of activation of the ammonia reaction. With the doubly charged succinate ion, the activation energies for the ammonia and the methylamine reaction were found to be similar (21.5 and 20.4 kcal./mole, respectively).

Amide Hydrolysis.—Second-order rate constants k_2 for the hydroxyl ion-catalyzed amide hydrolyses are listed in Table IV. The data for unsubstituted amides are from Bruylants'[10–12] laboratory, while those for secondary and tertiary amides were obtained in the present investigation. While we used initial concentrations of 0.01 M for the amide and hydroxide ion, Bruylants, *et al.*, used 0.05 M. This difference is considered insignificant and the results of both sets of data may be assumed to approximate closely the rate constants characterizing the limit of infinite dilution. We may note the sharp almost fiftyfold decrease in rate in passing from N-methylformamide to N-methylacetamide. The generally lower reactivity of secondary as compared with primary amides may be due largely to steric factors, but the fact that N,N-dimethylpropionamide reacts faster than N-methylpropionamide is unexpected. Another peculiarity concerns a comparison of the rates of N-methyl- and N-isopropylamides. With the formic and acetic acid derivatives, the N-methyl- and N-isopropylamides hydrolyze at fairly similar rates, while N-isopropylpropionamide has to be heated to 104° to react at a rate similar to that of N-methylpropionamide at 60°.

Equilibrium Constants for Amide Formation.—The equilibrium constants for amide formation K as formulated in eq. 4 were obtained from the ratios k_1/k_2 and are listed in Table V. The procedure used for obtaining equilibrium constants was checked by an experiment in which the equilibrium for the reaction

$$C_2H_5COO^- + CH_3NH_2 \rightleftharpoons C_2H_5CONHCH_3 + OH^- \quad (5)$$

was approached from both sides under comparable conditions (see Fig. 1). From the kinetic curves it was estimated that 31% of the propionate is converted into amide at equilibrium under the conditions employed. This estimate led to an equilibrium constant within 10% of that obtained from the ratio of the rate constants of the forward and the backward reaction. The following comparisons are of interest: (1) At 75.8°, the equilibrium constants for N-methylpropionamide

(10) M. Willems and A. Bruylants, *Bull. soc. chim. Belges*, **60**, 191 (1951).
(11) Mlle. DeKoo and A. Bruylants, *ibid.*, **63**, 140 (1954).
(12) F. Kezdy and A. Bruylants, *ibid.*, **68**, 225 (1959).

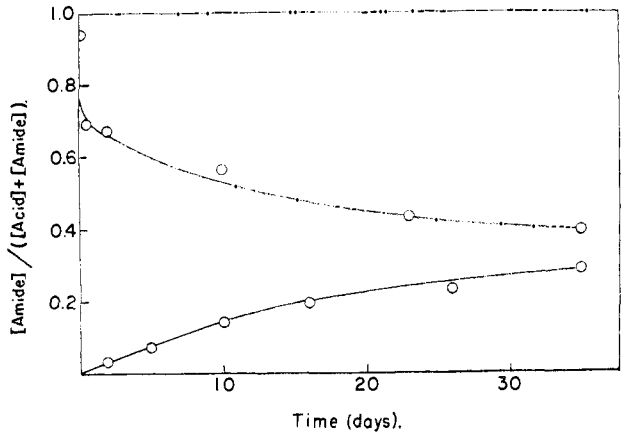


Fig. 1.—Formation and hydrolysis of N-methylpropionamide at 75.8°; initial condition for amide formation: 1.33 *M* acid, 6.66 *M* amine; initial condition for amide hydrolysis: 1.33 *M* amide, 5.33 *M* amine.

and N-methylisobutyramide are larger by factors of about a hundred than those for the corresponding primary amides. There is a large difference in the enthalpy of the reaction, the formation of primary amides being endothermic to the extent of 6–9 kcal./mole while the formation of N-methylpropionamide and N-methylisobutyramide are slightly exothermic.

TABLE V

EQUILIBRIUM CONSTANTS FOR AMIDE FORMATION IN AQUEOUS SOLUTION

Amine	Acid	Temp., °C.	10^4K	ΔF^0_{298}, kcal./mole	ΔH, kcal./mole
NH_3	Propionic	75.8	3.3	8.4	6.1
NH_3	Butyric	75.8	5.3	7.8	4.1
NH_3	Isobutyric	75.8	5.3	8.5	8.7
NH_3	Succinic	75.8	4.7	8.4	7.6
CH_3NH_2	Formic	44.4	550		
CH_3NH_2	Acetic	44.4	220		
CH_3NH_2	Propionic	75.8	310	5.6	−3.2
CH_3NH_2	Isobutyric	75.8	570	4.0	−2.4
CH_3NH_2	Succinic	50.0	180		
$(CH_3)_2CHNH_2$	Formic	44.4	8.1		
$(CH_3)_2CHNH_2$	Acetic	44.4	15[a]		
$(CH_3)_2CHNH_2$	Propionic	104	84		
$(CH_3)_2NH$	Propionic	75.8	20	6.7	1.9

[a] Subject to a relatively large error because of the very slow rate of amide formation.

(2) In view of the very large differences in the *rates* of formation and hydrolysis of formamides and amides of higher fatty acids, it is striking that the equilibrium constants for amide formation are nearly the same. However, there is an interesting reversal in the relative stabilities of secondary formamides and acetamides depending on the alkyl substituent on the nitrogen. With N-methyl substitution the equilibrium constant is three times larger for the formamide formation; with N-isopropyl substitution the equilibrium constant for the formamide is smaller by a factor of two. We shall return below to a consideration of possible causes of this effect.

(3) The equilibrium constant for the formation of one tertiary amide which we have studied (N,N-dimethylpropionamide) is intermediate between values for the unsubstituted and the N-methyl substituted amide.

(4) It is of interest to compare our data with various results obtained in the past with the use of different techniques. These results are generally given in terms of eq. 2, but we have recalculated them to conform to the formulation of eq. 4 and the values are listed in Table VI. We may note that ΔF^0_{298} values for asparagine and glutamine formation are a little less than 2 kcal./mole above our value for succinamic acid, which would appear to be most nearly comparable in polarity. The data obtained for the secondary amides of hippurate and benzoyltyrosylglycinamide have ΔF^0_{298} values about 5 kcal./mole above those we found for the N-methylamides of propionic and isobutyric acids.

A recent publication from Sturtevant's laboratory[13] contains a listing of a large number of ΔH values for enzymatically catalyzed hydrolyses of amide and peptide bonds. Using for the heats of dissociation of ammonium ion and the substituted ammonium group of a typical amino acid $\Delta H = +12.8$ and $+10.8$ kcal./mole, respectively,[14] and $\Delta H = 13.6$ kcal./mole for the self-ionization of water, these data lead to $\Delta H = 5$ kcal./mole for the formation of a peptide and $\Delta H = 7$ kcal./mole for the formation of a primary amide bond when the reactions are formulated according to eq. 4. The value for the primary amide is quite similar to that obtained in the present study. However, the formation of peptide bonds is apparently 3–7 kcal./mole more endothermic than that of the N-methylamides investigated in the present study. This difference, which accounts for the corresponding difference in the ΔF^0_{298} values, may be a reflection of the mutual interaction of the polar groups in hippurate and benzoyltyrosylglycinamide, accompanied by an endothermic release of bound water molecules.

TABLE VI

LITERATURE DATA OR THE FREE ENERGY OF AMIDE FORMATION IN AQUEOUS SOLUTIONS

Reaction	ΔF^0_{298}, kcal./mole	Ref.
Benzoate + glycinate → hippurate + OH^-	8.7	6
Benzoyl tyrosinate + glycinamide → benzoyltyrosylglycinamide + OH^-	8.8	7a
Alanine + glycinate → alanylglycine + OH^-	10.1	6
Aspartate + ammonia → asparagine + OH^-	10.2	7b
Glutamate + ammonia → glutamine + OH^-	10.0	7b

TABLE VII

RATE CONSTANTS FOR AMIDE FORMATION FROM PROPIONIC ACID IN MIXED SOLVENTS (AT 60°)

Cosolvent	Reagent	Wt. % H_2O	10^5k_1
1-Propanol	NH_3	10	2.1
1-Propanol	NH_3	20	2.1
1-Propanol	NH_3	30	2.8
1-Propanol	NH_3	50	2.6
1-Propanol	NH_3	75	1.2
1-Propanol	NH_3	100	0.8
1-Propanol	CH_3NH_2	5	13
1-Propanol	CH_3NH_2	30	27
1-Propanol	CH_3NH_2	50	22
1-Propanol	CH_3NH_2	75	16
1-Propanol	CH_3NH_2	100	10
2-Propanol	NH_3	15	1.0
2-Propanol	NH_3	30	0.8
2-Propanol	NH_3	50	1.0
2-Propanol	NH_3	75	0.8
2-Propanol	NH_3	100	0.8
2-Propanol	CH_3NH_2	10	6.2
2-Propanol	CH_3NH_2	20	6.0
2-Propanol	CH_3NH_2	30	5.8
2-Propanol	CH_3NH_2	50	5.6
2-Propanol	CH_3NH_2	75	6.1
2-Propanol	CH_3NH_2	100	10

(13) M. Rawitscher, I. Wadsö and J. M. Sturtevant, *J. Am. Chem. Soc.*, 83, 3180 (1961).

(14) E. J. Cohn and J. T. Edsall, "Proteins, Amino Acids and Peptides," Reinhold Publ. Corp., New York, N. Y., 1943, pp. 80, 82.

TABLE VIII

RATE CONSTANTS FOR HYDROXIDE ION CATALYZED HYDROLYSIS OF AMIDES IN MIXED SOLVENTS AT 60°

Amide	Cosolvent	Wt. % H_2O	10^5k_2
Propionamide	1-Propanol	100	38[a]
Propionamide	1-Propanol	95	36
Propionamide	1-Propanol	80	21
Propionamide	1-Propanol	50	13
Propionamide	1-Propanol	20	1.7
N-Methylpropionamide	1-Propanol	100	5.1
N-Methylpropionamide	1-Propanol	95	3.0
N-Methylpropionamide	1-Propanol	75	2.1
N-Methylpropionamide	1-Propanol	50	1.4
N-Methylpropionamide	1-Propanol	30	0.69
N-Methylpropionamide	1-Propanol	20	0.27
N-Methylpropionamide	2-Propanol	75	2.5
N-Methylpropionamide	2-Propanol	50	1.6
N-Methylpropionamide	2-Propanol	30	0.18
N-Methylpropionamide	2-Propanol	20	0.043

[a] Extrapolated from data in ref. 10.

Interactions of Non-polar Substituents.—When both the carboxylic acid and the amine reagent carry non-polar substituents, these substituents will be brought close to one another in the formation of the amide bond and their interaction might make a contribution to the free energy change characterizing the reaction. Frank and Evans[15] have first drawn attention to the fact that the introduction of non-polar residues into an aqueous medium is exothermic and produces a striking decrease of entropy and they have interpreted these findings as due to the formation of a highly ordered water structure ("iceberg") in the neighborhood of non-polar groups. Kauzman[16] has later introduced the concept of a "hydrophobic bond" where the increase in entropy due to "iceberg melting," when two non-polar residues in aqueous solution are brought into contact with each other, is considered as the main contribution to the driving force in such association processes. In applying these ideas to the formation of secondary amides, we have to take into account the geometry of such molecules. The partial double bond character of the C–N bond ensures coplanarity of the O=C–N–H structure[17] and the crystal structure of acetylglycine[18] as well as the dipole moment of N-acetylacetamide in carbon tetrachloride solution[19] show that the amide has the *trans* structure. If the *trans* structure is also exclusively present in aqueous media, effects due to interactions of the nitrogen and carbonyl carbon substituents would be expected to be small, but it seems possible that the *cis* structure is present in significant concentration due to the operation of hydrophobic bonds. This would manifest itself in an increased stability of amides derived from carboxylic acids and amines with interacting paraffinic residues. The data in Table V give some evidence of such an effect since the equilibrium constant for N-isopropylacetamide formation is twice as high as for N-isopropylformamide, while that for N-methylacetamide is only one-third of the N-methylformamide value. Although the equilibrium constant for N-isopropylacetamide is based on a rate of amide formation which is extremely low and therefore subject to a larger relative error than the rest of the data, its unexpectedly high value seems to be a consequence of an interaction between the non-polar residues of the acid and the amine moiety.

Amide Formation and Hydrolysis in Mixed Solvents.—Since perturbations of the water structure will have an important effect on the kinetics and equilibria of amide formation, some interesting variations in these quantities would be expected when the solvent medium is altered. Table VII lists the rate constants for amide formation from propionate and ammonia or methylamine in the water–1-propanol and the water–2-propanol system. It was found that the two mixed solvent systems behaved surprisingly differently. When 1-propanol was added to water, the rate of amide formation increased, reaching a maximum (about three times as high as the rate in pure water) in a medium containing about 70 weight % of the alcohol. This effect was totally absent with 2-propanol, which left the propionate–ammonia rate very nearly unchanged and tended to repress the reaction rate of propionate with methylamine. On the other hand, the amide hydrolysis rates were reduced by both 1-propanol and by 2-propanol addition (see Table VIII).

The interpretation of solvent effects on the rates of amide formation or hydrolysis is quite complex. On electrostatic grounds, one would expect reaction rates involving an approach of a charged species to a strongly polarized functional group to be favored by a reduction of the dielectric constant of the medium.[20] Nevertheless, hydrolyses of esters and amides are commonly slowed down by additions of organic solvents to the aqueous reaction medium. Tommila and his collaborators found that in alkaline ester hydrolyses, the activation energy typically passes through a minimum when organic solvents are added to the aqueous solvent medium.[21–23] As a result, the reaction rate frequently passes through a maximum in mixed solvent media, particularly if the reaction is studied at low temperature. The effect was interpreted by assuming that the solvation of the transition state is optimized in the mixed solvent medium.

A more detailed consideration of mixed solvent effects has been given more recently by Hyne and his collaborators.[24] They pointed out that charge localization on a solute species should lead to a local accumulation of the more polar constituent of a binary solvent mixture so that the local effective dielectric constant will remain virtually unchanged for moderate additions of less polar cosolvent to the system. This will then tend to result in a decrease in the activation energy if the charge is more localized in the transition state than in the reagents. The effect was illustrated on the hydrolysis of *p*-substituted benzyl chlorides in the water–ethanol system, where the decrease in the activation energy below the value observed in pure water became larger with an increasing tendency of the substituent to stabilize a carbonium ion.

Bender and Ginger have recently pointed out that a proper interpretation of the effect of the solvent medium on the rate of hydroxide ion catalyzed amide hydrolysis requires a determination of the effect of the medium on the rates of the various steps in the reaction mechanism.[25] Considering the tetrahedral reaction

(15) H. S. Frank and M. W. Evans, *J. Chem. Phys.*, **13**, 507 (1945).

(16) W. Kauzman, *Advan. Protein Chem.*, **14**, 1 (1959).

(17) L. Pauling, "The Nature of the Chemical Bond," third edition, Cornell University Press, Ithaca, N. Y., 1960, pp. 281–282.

(18) G. B. Carpenter and J. Donohue, *J. Am. Chem. Soc.*, **72**, 2315 (1950).

(19) S. Mizushima, T. Shimanouchi, S. Nagakura, K. Kuratani, M. Tsuboi; H. Baba and O. Fujioka, *ibid.*, **72**, 3490 (1950).

(20) E. S. Amis and G. Jaffé, *J. Chem. Phys.*, **10**, 598 (1942).

(21) (a) E. Tommila, A. Koivisto, J. P. Lyyra, K. Antell and S. Heimo, *Ann. Acad. Sci. Fennicae, Ser. A, Chem.*, No. 47 (1952); (b) E. Tommila and A. Hella, *ibid.*, No. 53 (1953).

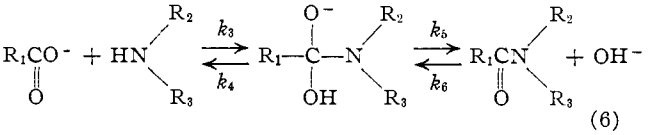
(22) E. Tommila, *Suomen Kem.*, **B25**, 37 (1952).

(23) (a) E. Tommila and M. P. O. Ilomaki, *Acta Chem. Scand.*, **6**, 1249 (1952); (b) E. Tommila and S. Hietala, *ibid.*, **8**, 257 (1954).

(24) J. B. Hyne, *J. Am. Chem. Soc.*, **82**, 5129 (1960); J. B. Hyne, R. Willis and R. E. Wonkka, *ibid.*, **84**, 2914 (1962).

(25) M. L. Bender and R. D. Ginger, *ibid.*, **77**, 348 (1955).

intermediate, we have to modify eq. 4 to



(6)

With the usual steady state assumptions, the observed rate constant for amide formation is $k_1 = k_3/[1 + (k_4/k_5)]$ while the observed rate constant for amide hydrolysis is $k_2 = k_6/[1 + (k_5/k_4)]$. Bender and Ginger found for the hydrolysis of benzamide at 109° that a substitution of two-thirds of the water with dioxane reduced k_2 by a factor of 6 while k_5/k_4 was increased from 10 to 34. The reduction of the hydrolysis rate is therefore due both to a decrease in k_6 and an increase in the ratio k_5/k_4. As for amide formation, the data of Bender and Ginger show that $k_4/k_5 << 1$ for amide hydrolysis both in the presence and the absence of an organic cosolvent and we are, therefore, justified in approximating $k_1 \approx k_3$. It is then the rate of formation of the tetrahedral intermediate from propionate and ammonia or methylamine which is being observed in studies of the rate of amide formation. The striking difference in the result obtained with the water–1-propanol and the water–2-propanol system suggests that the solvation of the transition state complex is surprisingly sensitive to the details of the structure of the cosolvent.

Experimental

Reagents.—Reagent grade ammonia (Allied Chemical Co.), methylamine (Eastman Organic Chemicals) and ethylamine (Matheson) were received as aqueous solutions. Reagent grade anhydrous dimethylamine, isopropylamine, ethanolamine, formic acid and acetic acid (Eastman) were used without purification. Reagent grade propionic, butyric and isobutyric acids (Eastman), 1-propanol and 2-propanol (Brothers Chem. Co.) were purified by fractional distillation. Succinic acid (Eastman, reagent grade) melted at 186.8°. Reagent grade (Eastman) N-methylformamide, N-methylacetamide and N-methylpropionamide were fractionally distilled; propionamide was recrystallized from acetone–water. N-Isopropylformamide was prepared from an equimolar mixture of methyl formate and isopropylamine which was allowed to stand at room temperature for 3 hours. The fraction boiling at 220° (lit.[26] 220°) was collected. The other amides used (N-methylisobutyramide, m.p. 20°, lit.[27a] 20°, N-isopropylacetamide, b.p. 89–90°, lit.[27b] 89–90°, N,N-dimethylpropionamide, 81–82°,[28] N-ethylpropionamide 105–110°,[29] N-isopropylpropionamide (hygroscopic crystals)) were made by a reaction of the appropriate acid chloride and amine. N-Methylsuccinamic acid (m.p. 111°) was made from succinic anhydride and methylamine; its acid number was 96.5% of theory, the amine released on base hydrolysis 101% of theory.

Determination of Hydrolysis Rates.—Evacuated sealed tubes containing 30 ml. of solutions 0.01 *M* in both NaOH and amide were placed into a thermostated bath. Sample tubes were removed at intervals and the ammonia or amine liberated was distilled under vacuum from a water-bath at 5–8° into a known amount of dilute H_2SO_4 in water–1-propanol at −15°. When about one-third of the reaction solution had distilled over, the excess H_2SO_4 was titrated with 0.01 *N* NaOH using either phenolphthalein indicator or conductometry. This method was not applicable to the amides of formic and acetic acids, since they reacted at appreciable rates even at the low temperature of the distillation step. In these cases the partially hydrolyzed amide solutions were passed through an Amberlite IR 120 cation exchange column and the carboxylic acid was determined conductometrically in the eluate.

Determination of the Rate of Amide Formation.—Unless otherwise specified, the formation of amide was followed in aqueous solutions containing initially 1 *N* amine and 0.2 *N* carboxylic acid. Sealed evacuated tubes containing 20 ml. of the solution were placed into a thermostated bath. At specified times, a sample tube was cooled to room temperature and most of the excess amine was distilled off under a pressure of 10–20 mm. The solution was then diluted and passed through a cation exchange column which was subsequently washed with 200 ml. of distilled water. The eluate was made basic with 20 g. of NaOH and was heated. The amine liberated was distilled into a H_2SO_4 solution of known concentration adjusted so as to represent a moderate excess over the expected amount of amine. The acid was then back-titrated conductometrically with NaOH.

In the case of amide formation from the non-volatile ethanolamine, a different procedure had to be employed. The unreacted acids and amine were removed by successive passage through columns of the anion exchanger Amberlite IR 400 and the cation exchanger Amberlite IR 120 followed by a second passage over the anion exchanger. A quantity of NaOH equivalent to the initial amount of carboxylic acid was added to the eluate, which was heated to hydrolyze the amide. After removal of sodium ions and amine by passage through a cation exchanger, the carboxylic acid was determined conductometrically in the effluent.

Evaluation of Analytical Methods.—A solution containing 1 *M* ammonia, 0.2 *M* ammonium propionate and 0.002 *M* propionamide in 50 ml. was subjected to the analysis for amide used in following rates of amide formation. The results of the analysis carried out in triplicate was 95%, 96% and 105% of theory. A solution containing 0.0075 *M* N-methylpropionamide, 0.0075 *M* sodium hydroxide and 0.00375 *M* N-methylammonium propionate was analyzed for methylamine by the procedure used in following rates of amide hydrolysis. The results of a triplicate analysis were 96%, 98% and 99% of theory.

Characteristics of Kinetic Runs.—The amide formation was followed to conversions which were in most cases below 1% but never more than 4%. In this range the amide hydrolysis is not yet significant and the amide content is linear in time. Analyses carried out at zero time gave apparent amide concentrations corresponding to apparent conversions of 0.02–0.09%. Amide hydrolyses were first order in amide and first order in hydroxide ion. In one case, that of propionamide hydrolysis at 50°, our second-order rate constant of 2.57×10^{-4} l.-mole^{-1}-sec.$^{-1}$ could be compared with the value of 1.97×10^{-4} obtained by extrapolation of the data obtained by Willems and Bruylants[10] in the temperature range of 65–85°. The discrepancy may be due to the much lower ionic strength used in our study.

(26) J. Parker, A. L. Thomson and J. Waughan, *J. Chem. Soc.*, 2601 (1955).

(27) (a) A. P. N. Franchimont, *Chem. Zentr.*, **84, II**, 1960 (1913); (b) K. G. Wyness, *J. Chem. Soc.*, 2934 (1958).

(28) W. P. Ratchford and C. H. Fisher, *J. Org. Chem.*, **15**, 317 (1950).

(29) H. Meerwein, *et al.*, *Ber.*, **89**, 209 (1956).

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The Preparation of Cyclic Cyclopropylcarbinols[1]

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The reaction of cyclic allyl alcohols or related methyl ethers with methylene iodide and zinc-copper couple has been shown to give cyclopropylcarbinols (or methyl ethers) in high yield. The reaction has been found to be stereospecific, yielding only the *cis* isomer. The mechanism of the reaction is discussed.

The synthesis of the cyclopropylcarbinyl system has been widely studied since such a system occurs in various natural products and in many materials produced by photochemical reactions and since reactions of such a system permit evaluation of the conjugative ability of a cyclopropyl ring. Many methods have been utilized for the preparation of such a system, but most of the methods either demanded complex starting materials or were not general in scope.[4] In

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