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The Thermodynamics of Ionization of Amino Acids. III. The Ionization Constants of Some N-Carbamoylamino Acids[1]

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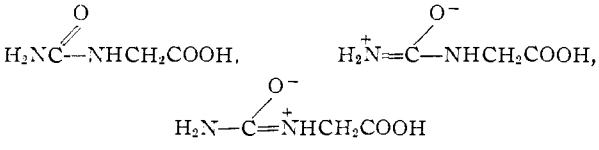
The thermodynamic ionization constants of the N-carbamoyl derivatives of glycine, DL-alanine, DL-α-amino-*n*-butyric acid, DL-α-aminoisobutyric acid, β-alanine and γ-aminobutyric acid were obtained at ten different temperatures from measurements on cells without liquid junction. Hydantoic acid (N-carbamoylglycine) is a slightly weaker acid than N-acetylglycine because the dipole moment of the terminal NH_2CONH- group of the former is slightly further away from the ionizing proton and is tilted at a larger angle than the moment of the CH_3CONH- group. The carbamoylamino group is more effective than the acetylamino group in orienting water molecules, so that the entropies of ionization of the carbamoyl amino acids are less negative than those of the acetylamino acids. The substitution of alkyl groups for an α-hydrogen atom is associated with decreases in the enthalpy and entropy of ionization that are generally similar to those previously reported.[2] The negative logarithm of the ionization constant varies linearly with the reciprocal of the number of carbon atoms that separate the polar and carboxyl groups. The entropy and enthalpy of ionization may vary in an analogous way if the behavior of the first acid in a series of ω-substituted acids is regarded as anomalous.

The important role played by water in the ionization of carboxylic acids has been emphasized in a recent paper.[2] Orientation of water molecules about the ions and molecules of acids is reflected more sensitively in the entropy of ionization than in the free energy of ionization or ionization constant. Attention was called in the earlier discussion to entropy effects associated with branching and lengthening of the alkyl chain attached to the carboxyl group and with shifts in the location of a polar group like the peptide linkage. These effects were illustrated by the behavior of some N-acylamino acids. The present paper is a report of an analogous study of some N-carbamoylamino acids. These acids differ from the acyl derivatives in having a polar, hydrogen-bonding amino group rather than an alkyl group at the end of the chain. From another point of view the carbamoylamino acids are substituted ureas. The charge distribution in the polar group will be somewhat different from that in the peptide linkage because the terminal amino group can participate in the resonance structures



It may be expected that the polar group on this account will have a greater order-producing effect on neighboring water molecules than the peptide linkage does but not as large an effect as the combination of peptide linkage and positively charged ammonium group found in the N-glycyl peptides.

The ionization constants and related thermodynamic properties of N-carbamoyl derivatives of glycine, DL-alanine, DL-α-amino-*n*-butyric acid, DL-α-aminoisobutyric acid, β-alanine and γ-aminobutyric acid have been obtained from measurements on the cell[3]

(Pt) H_2 |HA(m_1), NaA(m_2), NaCl(m_3) |AgCl-Ag (I)

where HA stands for any of the acids and m_1, m_2 and m_3 are the stoichiometric molalities. These acids have been chosen to illustrate the effects of chain branching at the α-carbon atom and of shifts in the position of the polar group. Measurements were made at 5° intervals from 5 to 50° in order that accurate values of the thermodynamic properties associated with the ionization reaction could be obtained.

Experimental

Apparatus.—This has been described before.[2,4] One of the unsaturated standard cells was recalibrated by the National Bureau of Standards in November, 1955. Its electromotive force had decreased 0.12 mv. since the previous calibration. The calculation of the ionization constants has been made with working standard electromotive forces, E_w^0, appropriate to the cells and technique used in this Laboratory.[2] The values of these given in the previous paper should be decreased by 0.06 mv. for use in the calculations that follow. The maximum error in the previously reported values of pK, the negative logarithm of the ionization constant, should not exceed 0.0002.

Materials.—The preparation and standardization of all of the chemicals except the acids themselves have been described before.[2,5] The acids were synthesized from the appropriate amino acid and potassium cyanate[6] and were recrystallized several times from conductivity water and dried in air. Their purity was tested by titration with standard sodium hydroxide solution, by determination of their water content with Karl Fischer reagent, and by various semi-quantitative tests.[7] The assays and water contents are, respectively: N-carbamoylglycine (hydantoic acid), 99.7% pure, 0.20% water; N-carbamoyl-DL-alanine, 99.6 and 0.38%; N-carbamoyl-DL-α-amino-*n*-butyric acid, 100.1 and 0.17%; N-carbamoyl-DL-α-aminoisobutyric acid, 99.8 and 0.18%; N-carbamoyl-β-alanine, 98.8 and 0.30%; and N-carbamoyl-γ-aminobutyric acid, 100.0 and 0.20%. All of the acids contained less than 0.004% iron, chloride, ammonia, phosphate and heavy metals except the derivative of α-amino-*n*-butyric acid which contained not more than 0.007% chloride. The derivative of β-alanine, which has a low assay, was examined by paper chromatography. Butanol-water-acetic acid was used as the solvent and two chromatograms were prepared by the ascending technique. One was developed with ninhydrin. No free β-alanine was found though 0.1% could have been detected under the conditions of the experiment. The second chromatogram was developed with brom cresol purple and only one acid spot was obtained.

Techniques.—These have been described before.[4,5] Almost all of the cells had electromotive forces at 25° after

(1) This investigation was supported by a research grant, H-1651, from the National Heart Institute of the National Institutes of Health, U. S. Public Health Service.

(2) E. J. King and G. W. King, THIS JOURNAL, **78**, 1089 (1956).

(3) H. S. Harned and B. B. Owen, "The Physical Chemistry of Electrolytic Solutions," 2nd. ed., Reinhold Publ. Corp., New York, N. Y. 1950, pp. 497–516.

(4) E. J. King, THIS JOURNAL, **73**, 155 (1951).

(5) E. J. King, *ibid.*, **76**, 1006 (1954).

(6) H. D. Dakin, *J. Chem. Soc.*, **107**, 434 (1915).

(7) M. P. Stoddard and M. S. Dunn, *J. Biol. Chem.*, **142**, 329 (1942).

two days that were within 0.10 mv. of the initial reading, but cells containing N-carbamoyl-α-aminoisobutyric acid showed a drop in electromotive force of 0.7 to 1 mv. over this period. Free α-aminoisobutyric acid was detected by paper chromatography in a typical cell solution after the measurements but not in the original acid. The rate of decrease of electromotive force at the higher temperatures was noted. The total changes in electromotive force at 25° that occurred overnight and over the first and second days were determined. Four of the cells were taken over the higher portion of the temperature range first, the other six over the lower. It has been possible on the basis of all of these observations to correct the electromotive forces to the time of preparation of the solutions with a maximum probable error of ±0.12 mv., the equivalent of ±0.0020 in pK.

The electromotive forces of cell I, corrected to a hydrogen pressure of one atmosphere, can be represented as a function of temperature by

$$E_t = E_{25} + a(t - 25) + b(t - 25)^2 \qquad (1)$$

The parameters of this equation for electromotive forces in absolute volts and concentrations in moles per kilogram of water are given in Table I together with the standard deviations between the observed and calculated electromotive forces of each set.

TABLE I

PARAMETERS OF EQUATION 1

N-CARBAMOYLGLYCINE (HYDANTOIC ACID)

m_2	m_3	E_{25}	10^6a	-10^8b
$m_1 = m_2$; stand. dev. = ±0.064 mv.				
0.01018	0.01018	0.57062	484	−15
.02025	.02027	.55254	423	−20
.03450	.03462	.53853	372	−28
.04775	.04784	.53020	344	−30
.06784	.06822	.52103	312	−20
.10005	.10072	.51103	276	−15
$m_1 = 2\ m_2$; stand. dev. = ±0.050 mv.				
0.01000	0.01011	0.55328	429	+6
.02021	.02907	.52553	424	−12
.03264	.03276	.52234	321	0
.05393	.05608	.50832	274	−9
.06791	.06827	.50307	254	−7
.1031	.1035	.49258	215	−7
$m_1 = 3\ m_2$; stand. dev. = ±0.041 mv.				
0.01028	0.01029	0.54337	397	+12
.02079	.02166	.52291	325	0
.03146	.03153	.51305	290	0
.04059	.04102	.50614	265	0
.05024	.05061	.50025	247	0
.07027	.07143	.49178	216	0

N-CARBAMOYL-DL-ALANINE

$m_1 = m_2$; stand. dev. = ±0.048 mv.				
0.01004	0.01004	0.57181	563	30
.01994	.01993	.55390	503	30
.02962	.02966	.54361	477	60
.04004	.04014	.53585	441	30
.05992	.06009	.52538	411	40
.07990	.07996	.51807	380	15
$m_1 = 2\ m_2$; stand. dev. = ±0.028 mv.				
0.01003	0.01004	0.55441	506	30
.02005	.02007	.53621	444	35
.02503	.02513	.53039	424	30
.03012	.03019	.52562	406	20
.04000	.04016	.51878	383	15
.04989	.04992	.51257	360	12

N-CARBAMOYL-DL-α-AMINO-n-BUTYRIC ACID

$m_1 = m_2$; stand. dev. = ±0.025 mv.				
0.01009	0.01008	0.57145	598	30
.01998	.02010	.55362	538	30
.02506	.02515	.54777	515	25
.03008	.03008	.54315	500	20
.04008	.04012	.53583	476	25
.05007	.05035	.53001	454	20
$m_1 = 0.5\ m_2$; stand. dev. = ±0.031 mv.				
0.01004	0.01008	0.58904	657	30
.02007	.02006	.57131	597	30
.02508	.02512	.56555	576	20
.03008	.03037	.56069	559	25
.04102	.04136	.55279	532	20
.05017	.05110	.54741	513	20

N-CARBAMOYL-DL-α-AMINOISOBUTYRIC ACID

$m_1 = m_2$; stand. dev. = ±0.080 mv.				
0.01003	0.01004	0.60513	608	65
.02505	.02513	.58169	529	65
.02757	.02760	.57853	519	60
.03007	.03012	.57694	513	60
$m_1 = 0.9467\ m_2$; stand. dev. = ±0.034 mv.				
0.00529	0.00541	0.61975	664	50
.01030	.01053	.60243	601	30
.01889	.01930	.58694	549	30
.02363	.02415	.58118	530	30
.02788	.02850	.57694	516	35
.03792	.03875	.56905	490	20

N-CARBAMOYL-β-ALANINE

$m_1 = m_2$; stand. dev. = ±0.026 mv.				
0.00998	0.01018	0.60617	617	40
.01989	.02027	.58864	555	25
.03493	.03499	.57487	503	25
.04064	.04064	.57105	490	20
.06018	.06047	.56117	452	20
.08064	.08119	.55390	423	20
$m_1 = 2\ m_2$; stand. dev. = ±0.031 mv.				
0.01004	0.01003	0.58888	558	30
.02002	.02001	.57129	496	35
.02503	.02604	.56468	469	45
.03005	.03005	.56105	458	30
.04008	.04009	.55383	430	35
.05000	.05001	.54831	409	30

N-CARBAMOYL-γ-AMINOBUTYRIC ACID

$m_1 = m_2$; stand. dev. = ±0.031 mv.				
0.01002	0.01007	0.61803	700	40
.01003	.01002	.61815	699	35
.01507	.01506	.60784	664	35
.02007	.02009	.60052	637	40
.02509	.02509	.59496	617	40
.03003	.03007	.59043	601	40
.04022	.04030	.58311	574	35
.05010	.05012	.57769	553	30

TABLE III

PARAMETERS OF EQUATION 2 AND THERMODYNAMIC PROPERTIES ASSOCIATED WITH THE IONIZATION OF CARBAMOYLAMINO ACIDS

Acid	A	B	C	$\Delta F^0_{298.16}$, cal. mole^{-1}	$\Delta H^0_{298.16}$, cal. mole^{-1}	$\Delta S^0_{298.16}$, cal. deg.$^{-1}$ mole^{-1}	$\Delta C^0_{p\,298.16}$, cal. deg.$^{-1}$ mole^{-1}	θ, °K.	pK_θ
Carbamoylglycine	1364.94	5.0675	0.014640	5287.3	290	−16.76	−40	305.3	3.8729
Carbamoylalanine	1088.10	3.5768	.012810	5309.8	−232	−18.59	−25	291.4	3.8901
Carbamoyl-α-amino-*n*-butyric acid	1018.65	3.3079	.012670	5302.0	−493	−19.43	−35	283.6	3.8772
Carbamoyl-α-aminoisobutyric acid	1125.63	2.9285	.012129	6089.0	+217	−19.69	−33	304.6	4.4614
Carbamoyl-β-alanine	1152.77	3.1036	.012490	6121.2	194	−19.88	−34	303.8	4.4854
Carbamoyl-γ-aminobutyric acid	1074.06	2.6066	.012364	6387.7	−115	−21.83	−34	294.7	4.6816

Calculations and Results

The method of calculating the ionization constants from electromotive force data is well known[3] and need not be described here. The negative logarithms of the thermodynamic ionization constants recorded in Table II are average values of the intercepts found by independent extrapolations of the results at different buffer ratios to zero ionic strength. The precision of the extrapolations can be judged from the standard deviations of each set of pK values.

TABLE II

THE NEGATIVE LOGARITHMS OF THE IONIZATION CONSTANTS OF CARBAMOYLAMINO ACIDS

Temp., °C.	Carbamoylglycine	Carbamoylalanine	Carbamoyl-α-amino-*n*-butyric acid	Carbamoyl-α-aminoisobutyric acid	Carbamoyl-β-alanine	Carbamoyl-γ-aminobutyric acid
5	3.9107	3.8979	3.8774	4.4904	4.5144	4.6938
10	3.8995	3.8935	3.8761	4.4805	4.5046	4.6873
15	3.8894	3.8906	3.8776	4.4726	4.4966	4.6838
20	3.8788	3.8900	3.8797	4.4653	4.4896	4.6820
25	3.8758	3.8924	3.8856	4.4627	4.4873	4.6831
30	3.8736	3.8963	3.8932	4.4612	4.4858	4.6848
35	3.8731	3.9018	3.9011	4.4603	4.4859	4.6887
40	3.8753	3.9084	3.9104	4.4627	4.4882	4.6941
45	3.8804	3.9186	3.9240	4.4667	4.4934	4.7028
50	3.8878	3.9305	3.9382	4.4742	4.5004	4.7134
Std. dev.	0.00090	0.00107	0.00099	0.00082	0.00086	0.00051

The variation of the ionization constants with temperature can be expressed by the Harned and Robinson equation[8]

$$pK = (A/T) - B + CT \qquad (2)$$

The parameters of this equation can be used to calculate the minimum pK value (pK_θ) at absolute temperature θ, and the changes in free energy (ΔF^0), enthalpy (ΔH^0), entropy (ΔS^0) and heat capacity (ΔC_p^0) associated with the ionization reactions in the standard state. The constants of eq. 2 and these various properties are given in Table III.

The minimum probable errors in the thermodynamic quantities resulting from random errors in preparation of the solutions and measurement of the electromotive forces should correspond to those in previous investigations.[2] Somewhat larger errors, but probably no more than twice as large, would be anticipated for carbamoyl-γ-aminobutyric acid because of the limited number of measurements and for carbamoyl-α-aminoisobutyric acid because of its decomposition at the higher temperatures. The uncertainty in the pK values for the β-alanine derivative caused by its low purity should not exceed 0.002 unless the impurities are acidic,[2] and the chromatographic experiments indicate that such contaminants probably are absent.

(8) H. S. Harned and R. A. Robinson, *Trans. Faraday Soc.*, **36**, 973 (1940).

Discussion

Comparison with Other Acids.—The N-carbamoylamino acids are slightly weaker than the corresponding N-acyl derivatives and have larger (less negative) entropies and enthalpies of ionization. This behavior is to be expected when the terminal alkyl group of the acyl amino acids is replaced by an amino group. The peptide linkage as found in acetylglycine has a dipole moment of 3.9 debyes inclined at about 40° to the axis of the C–N bond.[9] The terminal NH_2CONH–group in the carbamoylamino acids should have a moment of 3.9 to 4.1 debyes comparable with the moments of monoalkyl-substituted ureas,[10] and the angle between the moment and the C–N bond should be somewhat greater than 60°. The weaker acidic character of the present series of acids is thus a reflection both of the larger angle between the dipole and the line connecting its center to the proton and of the slightly greater distance from dipole to proton.[11]

Solvent orientation about polar groups in the neighborhood of the carboxyl group reduces the influence of the carboxylate ion on the solvent and leads to less negative entropies of ionization for polar acids than for the corresponding fatty acids.[2] The orienting power of the carbamoylamino group will surpass that of the acetylamino group partly because of the slightly positive terminal amino group but more particularly because of its more negative carbonyl oxygen.[12] Neither of these groups will have as strong an influence on the structure of water as does the N-glycylamino group in glycylglycine, for the latter contains not only the peptide linkage but also a charged ammonium group. Glycylglycine for that reason has the least negative entropy of ionization (−14.26 cal.deg.$^{-1}$mole^{-1}),[13]

(9) A. Kotera, S. Shibata and K. Sone, THIS JOURNAL, **77**, 6183 (1955).

(10) W. Kumler and G. Fohlen, *ibid.*, **64**, 1944 (1942).

(11) J. Kirkwood and F. Westheimer, *J. Chem. Phys.*, **6**, 506, 513 (1938).

(12) C. Cannon, *Mikrochem. Acta*, 555 (1955); *J. Chem. Phys.*, **24**, 491 (1956).

(13) Unpublished measurements made in this Laboratory.

then N-carbamoylglycine (−16.76), and finally N-acetylglycine (−17.24).

Chain Branching Effects.—The substitution of various alkyl groups for α-hydrogen atoms produces entropy effects in the present series of acids that closely resemble those shown by other carboxylic acids.[2] The introduction of a methyl group, for example, causes the entropy of ionization of carbamoyl-α-alanine to be 1.83 units more negative than that of carbamoylglycine at 25°. This is comparable with decreases of 1.83 units from acetylglycine to acetyl-α-alanine, 1.79 from propionic acid to isobutyric acid, and 0.99 from glycine hydrochloride to α-alanine hydrochloride. The substitution of a second methyl group and of ethyl in place of methyl cause further, smaller decreases in ΔS^0. These effects have been attributed to a combination of restricted rotation of the alkyl groups in the anion and of organization of high entropy water in the secondary hydration layer by alkyl groups.[2]

The entropy changes associated with substitution of methyl or ethyl for hydrogen on the α-carbon atom, like those with other carboxylic acids, are coupled with corresponding decreases in ΔH^0, so that ΔF^0 and pK are affected only slightly by chain branching. This coupling differentiates the heat and entropy changes caused by chain branching from those associated with a shift of the polar group. The data of Table III show that the change in ΔH^0 caused by substitution of a methyl group for an α-hydrogen atom is actually larger than that caused by moving the peptide linkage further from the carboxyl group. Studies of the relative apparent molal heat contents[14] and differential entropies of dilution[15] of amino acid solutions have also emphasized the importance of the size of the aliphatic radical and the relative unimportance of the location of the charged ammonium group in these acids for determining the thermochemical properties of their solutions.

The substitution of a second methyl group for hydrogen on the α-carbon atom causes the expected decrease in ΔS^0 but a considerable *increase* in ΔH^0 (−232 to +217 cal.mole^{-1}). As a result N-carbamoyl-α-aminoisobutyric acid is a considerably weaker acid than the other carbamoyl-α-amino acids. This anomalously high heat of ionization is not without precedent. A similar but smaller increase in ΔH^0 occurs from isobutyric acid to trimethylacetic acid (−799 to −724).[16] For the ionization of the carboxyl group in α-alanine and α-aminoisobutyric acid the entropies and enthalpies are coupled in the normal fashion, but the heat of ionization of the ammonium group in the latter is abnormally high (11,550 as compared with 10,890 for alanine).[17]

Chain branching or alkyl substitution effects are well known from the study of the kinetics of formation and hydrolysis of carboxylic acid esters.[18] There is indeed a fairly good correlation between the relative entropies of ionization of fatty acids with acetic acid as the standard and the relative entropies of activation with acetate esters as standards. This suggests that the solvation of the anion of the acid and of the activated complex are similar, but in view of the limited accuracy of the kinetic data and of the variety of solvents used it is hardly possible to push the correlation very far.

Polar Effects.—As the polar carbamoylamino group is shifted away from the carboxyl group an increase in ΔF^0 (or decrease in K) occurs which is similar to that in other series of ω-substituted acids, *e.g.*, the ω-amino acid hydrochlorides, the N-acyl-ω-amino acids and the ω-chloro-substituted fatty acids.[19] The values of pK or ΔF^0 are most simply represented by the relation of MacInnes[20]

$$pK = pK_\infty - (S/n) \qquad (3)$$

where n is the number of carbon atoms between the polar or charged substituent and the carboxyl group. For the four ω-amino acid hydrochlorides the standard deviation from this equation is ± 0.037 in pK and the intercept, $pK_\infty = 4.8606$, is very close to the pK value of n-hexoic acid, 4.8563 at 25°.[16] The intercept for the carbamoylamino acid series is somewhat larger, *viz.*, 5.083.

The theory of Kirkwood and Westheimer[11] requires that for polar acids account must be taken not only of the distance but also of the cosine of the angle of inclination of the group dipole to the line joining its mid-point to the ionizable proton. This angle is large in the present series and varies from one acid to the next. Small errors in estimating the angle will be magnified in taking its cosine. The uncertainties in the angle, in the location of the proton, and in the dimensions of the volume excluded by the molecules are too large to warrant a quantitative application of the theory to the N-carbamoylamino acids.

The heats and entropies of ionization of the N-carbamoyl-ω-amino acids decrease as the polar group is shifted away from the carboxyl group. The change in enthalpy of ionization between the first two members of the series is relatively small just as it is between glycine and β-alanine. The heats and entropies of ionization, unlike pK and ΔF^0, do not vary linearly with $(1/n)$. The data for the ω-amino acids, the only series for which more than three acids have been studied, indicate that the lack of linearity is perhaps entirely due to the anomalous behavior of the first member of the series. If glycine hydrochloride is omitted, the heats and entropies of ionization of the remaining three acids are linear in $(1/n)$ and the intercepts corresponding to infinite separation of ammonium and carboxyl groups match closely the heat and entropy of ionization of n-hexoic acid. The values of ΔH^0 and ΔS^0 for glycine hydrochloride are too low as compared with those of the other ω-amino acids. The entropy defect, for example, is about 6 cal. deg.$^{-1}$ mole^{-1}; $\Delta S^0 = -7.4$ at 25°[4] and it should be −1.2 to fit with the other acids. Since

(14) L. S. Mason, W. F. Offutt and A. L. Robinson, THIS JOURNAL, **71**, 1463 (1949).

(15) A. L. Robinson, *J. Chem. Phys.*, **14**, 588 (1946).

(16) D. H. Everett, D. A. Landsman and B. R. W. Pinsent, *Proc. Roy. Soc. (London)*, **215A**, 403 (1952).

(17) P. K. Smith, A. C. Taylor and E. R. B. Smith, *J. Biol. Chem.*, **122**, 109 (1937).

(18) R. Taft, Jr., THIS JOURNAL, **74**, 2729, 4231 (1952).

(19) See ref. 2, Fig. 1.

(20) D. MacInnes, THIS JOURNAL, **50**, 2587 (1928); J. Greenstein, *ibid.*, **58**, 1314 (1936).

the pK and ΔF^0 values of all of these acids are in line, the anomalous behavior of the first member must cancel in taking the difference between ΔH^0 and $T\Delta S^0$. This is reminiscent of the coupling of heat and effects due to chain branching and suggests that the anomalous behavior of glycine hydrochloride is caused by a solvation effect. The proximity of the two charged groups may cause a reinforcement of the orientation of water in the primary hydration layer thus making the entropy of the ionized form abnormally low as compared with that of β-alanine. From this point of view glycine in its dipolar form must have a structure-breaking effect on water. There is other evidence of this very behavior. Robinson has concluded from a study of the relative partial molal entropy of water in amino acid solutions[15] that glycine alone has a structure-breaking effect like that of the sodium and potassium halides and nitrates. The activity coefficient of glycine is also abnormally low in comparison with those of the other ω-amino acids.[21]

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(21) E. R. B. Smith and P. K. Smith, *J. Biol. Chem.*, **132**, 47 (1940); E. J. Cohn and J. T. Edsall, "Proteins, Amino Acids and Peptides," Reinhold Publ. Corp., New York, N. Y., 1943, pp. 217–221.