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The Thermodynamics of Ionization of Amino Acids. V. The Ionization Constants of 3-Methoxy-DL-alanine (O-Methylserine) and Methoxyacetic Acid[1]

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RECEIVED DECEMBER 28, 1959

The thermodynamic functions for the ionization of methoxyacetic acid and 3-methoxyl-DL-alanine (O-methylserine) were obtained at ten temperatures from measurements on cells without liquid junction. The ionization constants were fitted to the equation $-\log K = (A/T) - B + CT$ where A, B and C are, respectively, 974.26, 3.6704 and 0.013325 for methoxyacetic acid; 1290.20, 6.0134 and 0.012490 for the ionization of the carboxyl group of the amino acid; and 2788.60, 2.0924 and 0.0064234 for the ionization of the ammonium group. The entropies of ionization of these acids lie between those of the analogous unsubstituted and hydroxyl substituted acids, for the methoxyl group is polar yet is less capable than hydroxyl of forming hydrogen bonds with solvent molecules. The heats of ionization are smaller (less positive or more negative) than those of the reference acids and this is reflected in the greater strength of the methoxy acids. The relative importance of polar effects and solvation in determining the heat of ionization is discussed.

The study of the effect of polar substituents on the ionization of weak acids has been a favorite field of work since the early days of the theory of ionization. Discussion of polar effects generally has been based on the electrostatic interaction between polar group and the ionizing proton or an inductive effect transmitted through the chain attached to the ionizing group. Few such discussions have been based on accurate thermodynamic ionization constants at several temperatures. As such data have gradually accumulated, attention has been drawn to other effects, particularly solvation,[2-6] which may be revealed not so much by variations in free energy of ionization or ionization constant as by large fluctuations in heat and entropy of ionization from one polar acid to another.

Two acids containing the methoxyl group were selected for investigation. The electron withdrawing power of this group is generally considered to be less than that of hydroxyl,[7] yet methoxyacetic acid, for example, is a stronger acid than glycolic acid. It has been suggested that the unexpected strength of the methoxy acid is a solvation effect.[8]

Measurements were made on three types of cells

$$(\mathrm{Pt})\mathrm{H_2} \mid \mathrm{CH_3OCH_2COOH}(m_1),\ \mathrm{CH_3OCH_2COONa}(m_2),\ \mathrm{NaCl}(m_3) \mid \mathrm{AgCl\text{-}Ag} \quad (\mathrm{I})$$

$$(\mathrm{Pt})\mathrm{H_2} \mid \mathrm{CH_3OCH_2CH(NH_3^+)COO^-}(m_1),\ \mathrm{CH_3OCH_2CH(NH_3^+)COOH},\ \mathrm{Cl^-}(m_2) \mid \mathrm{AgCl\text{-}Ag} \quad (\mathrm{II})$$

$$(\mathrm{Pt})\mathrm{H_2} \mid \mathrm{CH_3OCH_2CH(NH_3^+)COO^-}(m_1),\ \mathrm{CH_3OCH_2CH(NH_2)COO^-},\ \mathrm{Na^+}(m_2),\ \mathrm{NaCl}(m_3) \mid \mathrm{AgCl\text{-}Ag} \quad (\mathrm{III})$$

in which the ionization reactions were, respectively

$$\mathrm{CH_3OCH_2COOH + H_2O \rightleftharpoons CH_3OCH_2COO^- + H_3O^+}$$

$$\mathrm{CH_3OCH_2CH(NH_3^+)COOH + H_2O \rightleftharpoons CH_3OCH_2CH(NH_3^+)COO^- + H_3O^+}$$

$$\mathrm{CH_3OCH_2CH(NH_3^+)COO^- + H_2O \rightleftharpoons CH_3OCH_2CH(NH_2)COO^- + H_3O^+}$$

Measurements were made at ten equally spaced temperatures from 5 to 50° in order that the entropy and enthalpy changes associated with the ionization reactions in the standard state could be calculated. It was hoped that some deductions could be made from these thermodynamic properties about solvation effects on the ionization process.

Experimental

Two samples of methoxyacetic acid were prepared by repurifying a commercial product (Eastman Kodak Co.). One portion of crude material was distilled at a pressure of 13–14 mm. through a vacuum jacketed Vigreux column 20 cm. long. The middle fraction, b.p. 97°, was retained. A second sample was prepared by fractionally freezing the crude material four successive times and then fractionally distilling it. Stock solutions of the acid were made by dilution of the samples and were standardized against carbonate-free sodium hydroxide solution.

Two samples of O-methyl-DL-serine, A.p. grade, were obtained from the H. M. Chemical Company, Santa Monica, California and were used without further purification. They were subjected to a series of tests: assay by formol titration with standard sodium hydroxide solution, water content by the Karl Fischer method, and semiquantitative tests for chloride, iron, ammonia, phosphate and heavy metals.[9] For Lot No. 5.8.22 the results were: assay, 100.0%; water, 0.25%; and less than 0.004% of each of the five minor impurities. For Lot No. 114.65.37 they were: assay, 99.92%; water, 0.08%; Cl < 0.005%; the others, < 0.004% of each.

Other chemicals were prepared and standardized as in earlier work.[10,11] The apparatus and techniques have likewise been described before.[3,10,11] One standard cell was recalibrated by the National Bureau of Standards three months before measurements began. Buffer solutions were prepared by weight from appropriate combinations of acid, sodium chloride, hydrochloric acid and sodium hydroxide. For the acid buffers of Cells I and II a separate solution was prepared for each cell whereas for the alkaline buffers of Cell III the 18 cell solutions were made by dilution of six stock buffers. The results on the earliest six solutions in Cell III have been discarded because of possible contamination with carbon dioxide from the air. The values of pK derived from these six buffers were between 0.005 and 0.007 unit lower than those from the other twelve buffers from which carbon dioxide was excluded.

In determining the second ionization constants of amino acids a frequent source of difficulty is the solubility of silver chloride in the alkaline solutions. As a result the electromotive force at 25° measured at intervals during two days

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[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. A preliminary report was given at the American Chemical Society Meeting, New York, N. Y., in September, 1957.

[2] D. J. G. Ives and J. H. Pryor, *J. Chem. Soc.*, 2104 (1955).

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

[4] E. J. King, *ibid.*, **78**, 6020 (1956).

[5] F. S. Feates and D. J. G. Ives, *J. Chem. Soc.*, 2798 (1956).

[6] L. P. Fernandez and L. G. Hepler, THIS JOURNAL, **81**, 1783 (1959).

[7] See, for example, R. W. Taft, Jr., in "Steric Effects in Organic Chemistry," ed. by M. S. Newman, John Wiley and Sons, Inc., New York, N. Y., 1956, p. 619, and T. C. Bissot, R. W. Parry and D. H. Campbell, THIS JOURNAL, **79**, 796 (1957).

[8] L. A. Wiles, *Chem. Revs.*, **56**, 329 (1956).

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

[10] E. J. King, THIS JOURNAL, **73**, 155 (1951).

[11] E. J. King and G. W. King, *ibid.*, **74**, 1212 (1952).

usually decreases. This was not observed for Cell III. Only two cells out of the 18 containing alkaline buffers had electromotive forces at 25° at the end of the series of measurements that differed by more than 0.1 mv. from the original values. The average difference between initial and final electromotive forces of the other 16 cells was only 0.017 mv.

It is necessary to report smoothed values of electromotive forces to save space, but the original values have been used in the calculations that follow. The electromotive forces can be represented as functions of the temperature by

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

The parameters of this equation when the electromotive forces are in absolute volts, concentrations are in moles per kilogram of water, and the hydrogen pressure in one atmosphere are given in Table I. The standard deviations between observed and calculated values of the electromotive forces are ± 0.035 mv. for Cell I, ± 0.035 mv. for Cell II and ± 0.066 mv. for Cell III.

TABLE I

PARAMETERS OF EQUATION 1

Methoxyacetic acid

m_1	$m_2 = m_3$	E_{25}	10^6a	-10^8b
0.011791	0.010785	0.54958	590	−33
.01954	.01787	.53491	541	−25
.03446	.03152	.52179	495	−12
.05474	.05128	.50903	450	0
.07059	.06612	.50245	426	0
.10081	.09443	.49321	392	+10
.03050	.009629	.52665	506	38
.06188	.01954	.50714	443	25
.09398	.02967	.49593	406	25
.08393	.03964	.49825	415	10
.11138	.05260	.49086	389	10
.15615	.07374	.48206	358	15
.02382	.011205	.53202	526	20
.04149	.019515	.51696	478	10
.15396	.04876	.48273	361	12

3-Methoxylalanine, acid buffers

$m_1 = m_2$	E_{25}	10^6a	-10^8b
0.010013[a]	0.48807	196	132
.020045	.46455	107	115
.03000	.45197	60	108
.04012	.44329	·28	92
.05001	.43711	6	95
.07010	.42800	−28	90
$m_1 = 2m_2$			
0.02007	0.49645	208	95
.04008	.47558	128	88
.05015	.46929	105	82
.05922	.46487	89	82
.07405	.45888	66	76
.09039	.45386	48	72

3-Methoxylalanine, basic buffers

m_1	m_2	m_3	E_{25}	10^6a	-10^8b
0.02195	0.009955	0.010635	0.86194	+30	150
.04474	.02029	.02168	.84419	−31	165
.05450	.02472	.02640	.83940	−51	162
.06737	.03114	.03284	.83462	−61	210
.07519	.03476	.03665	.83187	−76	165
.08614	.03982	.04199	.82865	−89	168
.01835	.008908	.009086	.86791	+48	150
.02577	.012510	.012760	.85928	17	142
.02816	.01461	.01426	.85818	14	155
.03870	.01878	.01916	.84909	−17	160
.05623	.02730	.02784	.83994	−50	150
.09112	.04728	.04613	.82913	−90	160

[a] $m_2 = 0.010029$.

Calculations and Results

Since the method of calculating ionization constants from these data is well known,[12] discussion will be confined to the only unusual feature. For Cells I and II, which contain acidic buffers, the apparent hydrogen ion concentration is estimated with[13]

$$-\log m_{\mathrm{H}}' = (\mathfrak{F}/2.3026RT)(E - E_{\mathrm{w}}^0) + \log m_{\mathrm{Cl}} - 2\mathcal{S}\sqrt{\mu d_0}/(1 + A\mathring{a}\sqrt{\mu d_0}) \qquad (2)$$

The last term on the right is the Debye–Hückel approximation for the activity coefficient product of hydrochloric acid in the buffer solutions. The ion size parameter $\mathring{a}$ was taken to be 4.00 Å., an extrathermodynamic choice which has no significant effect on the ionization constant of an acid as weak as methoxyacetic acid ($pK \sim 3.5$) but does influence the value of the first ionization constant of the amino acid ($pK_1 \sim 2$).[11,14] The reason for choosing the value 4.00 Å. has been presented before.[15]

Values of the negative logarithms of the ionization constants, as obtained by extrapolation of the results of the calculations to zero ionic strength, are collected in Table II. The standard deviations given in the table indicate the errors in each set of intercepts (pK values) as obtained by the method of least squares. The constants A, B and C in Table II are the parameters of the Harned and Robinson equation[16]

$$pK = (A/T) - B + CT \qquad (3)$$

TABLE II

THE NEGATIVE LOGARITHMS OF THE IONIZATION CONSTANTS AND THE PARAMETERS OF EQUATION 3

Temp., °C.	Methoxyacetic acid pK	3-Methoxylalanine pK_1	3-Methoxylalanine pK_2
5	3.5380	2.0975	9.7182
10	3.5440	2.0814	9.5756
15	3.5505	2.0647	9.4363
20	3.5591	2.0474	9.3032
25	3.5704	2.0373	9.1756
30	3.5834	2.0298	9.0538
35	3.5970	2.0223	8.9356
40	3.6127	2.0172	8.8220
45	3.6314	2.0150	8.7167
50	3.6509	2.0160	8.6131
Std. dev.	±0.00057	±0.00136	±0.00138
A	974.26	1290.20	2788.60
B	3.6704	6.0134	2.0924
C	0.013325	0.012490	0.0064234

The thermodynamic functions associated with the ionization of the methoxy acids are presented in Tables III and IV together with those of related acids. They include the absolute temperature θ, at which K attains its maximum value, and the changes in free energy (ΔF^0), enthalpy (ΔH^0), entropy (ΔS^0) and heat capacity (ΔC_p^0) associated with the ionization reaction at 25° in the standard state. Free energy and enthalpy changes are given

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[12] H. S. Harned and B. B. Owen, "The Physical Chemistry of Electrolytic Solutions," 3rd Ed., Reinhold Publ. Corp., New York, N. Y., 1958, pp. 650–652, 658–660.

[13] See ref. 3 and 4 for the meaning of E_{w}^0.

[14] R. G. Bates, *J. Research Natl. Bur. Standards*, **47**, 127 (1951).

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

in calories per mole, entropy and heat capacity changes in calories per degree-mole, where one calorie is defined as 4.1840 joules.

TABLE III

IONIZATION FUNCTIONS OF METHOXYACETIC ACID AND RELATED ACIDS

Acid	$pK_{298.16}$	$\Delta F^0_{298.16}$	$\Delta H^0_{298.16}$	$\Delta S^0_{298.16}$	$\Delta C^0_{p298.16}$	θ
Acetic acid[17]	4.757	6486	−100	−22.11	−37	295
Methoxyacetic acid	3.570	4871	−960	−19.57	−36	270
Glycolic acid[18]	3.832	5225	+180	−16.94	−39	303

TABLE IV

IONIZATION FUNCTIONS OF 3-METHOXYALANINE AND RELATED ACIDS

Acid	Ionizing group	$pK_{298.16}$	$\Delta F^0_{298.16}$	$\Delta H^0_{298.16}$	$\Delta S^0_{298.16}$	$\Delta C^0_{p298.16}$	θ
Glycine[10]	–COOH	2.349	3205	950	−7.56	−34	324
3-Methoxyalanine	–COOH	2.037	2780	820	−6.56	−34	321
Serine[19]	–COOH	2.187	2984	1320	−5.57	−31	338
Glycine[10]	$-NH_3^+$	9.779	13340	10550	−9.05	−12	792
3-Methoxyalanine	$-NH_3^+$	9.176	12520	10150	−7.95	−18	659
Serine[19]	$-NH_3^+$	9.209	12560	10350	−7.41	−10	833

Discussion

The data of Tables III and IV show that the methoxy acids are stronger acids than the comparable unsubstituted and hydroxyl substituted compounds. The effect of a substituent can enter as a potential energy contribution to ΔF^0, *i.e.*, as a polar or inductive effect, and as a kinetic energy contribution dependent on the motions of solute and solvent molecules and ions. The polar effect can scarcely be all important, for the group moment of $-OCH_3$ is less than that of $-OH$, *viz.*, 1.3 as compared with 1.7 D.[20] Furthermore, the methoxyl group is generally considered to have less electron withdrawing power, at least in aromatic compounds.[7] Thus on the basis of a polar or inductive effect alone one would expect methoxy acids to be weaker than their hydroxy counterparts. Two possible interpretations of the data in Tables III and IV must be considered: either the methoxy acids are stronger than polar effects would lead one to expect or the hydroxy acids are weaker. For further clues it is necessary to examine the heats and entropies of ionization.

It is convenient to consider the entropy change first, for it is independent of potential energy contributions. Interaction of a substituent dipole with the ionizing proton occurs partly within the cavity of the acid molecule and partly through the solvent. Insofar as it depends on the dielectric constant of the solvent this interaction will contribute to the entropy change, but theoretical estimates indicate that such a contribution must be small.[21] This is also supported by the observation that acids as diverse in structure as formic acid, glycolic acid, bromoacetic acid and N-acetylglycine all have entropies of ionization at 25° in the range −16.9 to −17.3 cal. deg.$^{-1}$ mole^{-1}. Setting aside entropy effects associated with translational and vibrational motions and with the creation of electric fields about the ions as either being intrinsically small or not varying greatly from one acid to another, one is left with entropy effects associated with restricted internal rotations and with specific interactions of the ions and molecules of the acid with molecules of the solvent.[3]

It will be seen from Tables III and IV that the entropies of ionization of the methoxy acids are about half-way between those of the reference acids. This is most simply interpreted as a solvation effect. Water molecules about the methyl group in acetic acid are within 5 Å. of the carboxyl group and are thus subject to orientation by the negative charge produced by ionization with loss of entropy.[22] Water molecules about the hydroxyl group of glycolic acid molecules have their entropy lowered even before ionization by hydrogen bonding with the hydroxyl group. They are then less subject to perturbation by the field created by ionization than those around a methyl group. As a result, less entropy is lost in ionizing glycolic acid than in ionizing acetic acid. In methoxyacetic acid the methoxyl group contains an oxygen atom with two lone pairs of electrons and is still capable of forming hydrogen bonds[8] though to a much smaller degree than the hydroxyl group. Moreover, the terminal methyl group in methoxyacetic acid should have a structure-strengthening effect on water similar to that manifested in the behavior of acetic and propionic acids.[3] The entropy loss on ionization of methoxyacetic acid should therefore be larger than that for glycolic acid but smaller than that for acetic acid.

In methoxyalanine the methoxyl group is further from the carboxyl group than it is in methoxyacetic acid, yet it is still within 5 Å., the limit of influence of the carboxylate ion on water structure.[22] The order of the entropies of ionization of the acids in Table IV can thus be accounted for by the same reasoning as was used for those in Table III.

The greater strength of methoxy acids as compared with hydroxy acids cannot be an entropy effect, for if this were predominant the methoxy compounds would be the weaker acids. The entropy effect is more than offset by an enthalpy ef-

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[17] H. S. Harned and R. W. Ehlers, THIS JOURNAL, **55**, 652 (1933).

[18] L. F. Nims, *ibid.*, **58**, 987 (1936).

[19] P. K. Smith, A. T. Gorham and E. R. B. Smith, *J. Biol. Chem.*, **144**, 737 (1942); recalculated from the original electromotive force data using the constants recommended by F. D. Rossini, F. Gucker, Jr., H. Johnston, L. Pauling and G. Vinal, THIS JOURNAL, **74**, 2699 (1952). These are the constants on which the calculations of this paper are based.

[20] Landolt-Börnstein, "Zahlenwerte und Funktionen," ed. A. Eucken and K. H. Hellwege, Sechste Auflage, I. Band, 3. Teil, Springer Verlag, Berlin, 1951, pp. 507–508.

[21] F. Westheimer and J. Kirkwood, *Trans. Faraday Soc.*, **43**, 77 (1947).

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

fect. For methoxyacetic acid the heat of ionization is, at first glance, surprisingly large and negative. A second possibility must nevertheless be borne in mind: the anomalous heat of ionization may be that of the hydroxyl acid. A basis for deciding between the two possible anomalies can be obtained by examining the known heats of ionization of a wide variety of acids. The comparison will be made at 25° where ΔH^0 is known with the greatest precision. At higher temperatures all ΔH^0 values for carboxylic acids are less positive or more negative; because all are shifted to about the same extent, the conclusions of this discussion are not altered by the choice of a different temperature for the comparison.

Because it includes a potential energy contribution, ΔH^0 is a more complex quantity to interpret than ΔS^0. The large difference in the two heats of ionization of glycine (Table IV) is an illustration of the importance of the potential energy contribution, for it is a reflection of the difference in binding energy of the proton to the carboxylate and ammonium groups.

Uncharged carboxylic acids can be divided into several groups on the basis of their heats of ionization. The short chain fatty acids have small negative values of ΔH^0 at 25°. Acids which have large negative heats of ionization include the monohalogen derivatives of acetic acid, cyanoacetic acid and methoxyacetic acid. These are polar acids and the interaction of the polar substituent with the ionizing proton leads to a large negative potential energy contribution to ΔH^0. Glycolic, succinic and hydantoic acids, which contain good hydrogen bonding groups, namely: -OH, -COOH and $-NHCONH_2$, have positive heats of ionization. Hydrogen bonding between the molecules of these acids and water lowers the enthalpy of the un-ionized state and less enthalpy is lost on ionization. Other enthalpy effects are associated with changes in the alkyl chain, the position of the polar group, if any, being kept fixed. As the alkyl chain is lengthened or branched ΔH^0 becomes more negative. This effect is frequently coupled with a decrease in ΔS^0 with the result that ΔF^0 and pK remain almost unchanged.[3,4,15] Extension of the chain takes place in passing from glycolic to lactic acid, for example, and this is associated with a decrease in ΔH^0 from +180 to −120 cal./mole. Likewise, the heat of ionization of N-acetylglycine, $CH_3CONHCH_2COOH$, which has a terminal methyl group, is −150 cal./mole whereas that of hydantoic acid, $NH_2CONHCH_2COOH$, is +290 cal./mole. Groups that hydrogen bond to water and break its structure increase ΔH^0; those that repel water with the result that it draws away from them like a contractile skin and has its structure strengthened, decrease ΔH^0.

On the basis of this survey of heats of ionization it is now possible to come to some conclusion about the behavior of glycolic and methoxyacetic acids. It is to be expected that in passing from $HOCH_2COOH$ to CH_3OCH_2COOH a decrease in ΔH^0 should occur, for the hydrogen bonding power of the molecule decreases and a terminal methyl group is introduced. The large difference in heats of ionization of methoxyacetic acid (−960 cal./mole) and acetic acid (−100 cal./mole) is a reflection of the interaction between the polar methoxy group and the ionizing proton. Among polar acids iodoacetic acid, the least solvated of the monohalogenoacetic acids,[2] is most nearly comparable with methoxyacetic acid. These two acids have about the same entropy of ionization: −19.1 and −19.6 cal. deg.$^{-1}$ mole^{-1}. Iodoacetic acid has the more negative heat of ionization: −1370 cal./mole as compared with −960 for methoxyacetic acid, but this is reasonable in view of the larger group moment of iodine, 1.6 as against 1.3 D for methoxyl. For polar acids in general there is no correlation between dipole moment and heat of ionization,[2] because the heat of ionization includes a negative contribution from the polar effect and a positive one from solvation. Only when solvation is weak, as for iodoacetic and methoxyacetic acids, does an increase in dipole moment correspond to a more negative heat of ionization. For heavily solvated cyanoacetic acid ΔH^0 is only −840 cal./mole, much less negative than that for iodoacetic acid, despite the large polar effect to be expected of cyanide with a group moment of 3.4 D.[5] The polar effect for glycolic acid is smaller and is swamped by the strong solvation of the molecules; the heat of ionization is positive. It may thus be concluded that the enthalpy of solvation effect is responsible for the fact that hydroxy acids are weaker than methoxy acids.

Grateful acknowledgment is made to Dr. J. E. Prue for helpful discussions.