to establish what is the largest value of τ_G (slowest internal rotation) that we can measure. We must have

$$T_1^{\alpha}/N^{\beta}T_1^{\beta} \lesssim 0.8$$

Then eq 12 yields

$$\rho \gtrsim 0.6$$

Therefore, measurable τ_G values are restricted to

$$\tau_G \lesssim 2\tau_R \qquad (14)$$

It should also be noted that when the internal motion becomes very fast ($\tau_G \ll \tau_R$), then $N^{\beta}T_1^{\beta}$ approaches the limiting value $9T_1^{\alpha}$ and again becomes very insensitive to changes in τ_G.

In the lower half of Figure 2 we show NT_1 values of all resolved *protonated carbon* resonances of gramicidin S. With the exception of proline, all other residues show a measurable increase in NT_1 when going from the α carbons to the side chains. In the case of proline, no significant internal motion is expected, and thus C^{β}, C^{γ}, and C^{δ} should have the same NT_1 value as the α carbon. This is indeed the case for C^{δ}. The T_1 values of C^{β} and C^{γ} could not be measured, because of overlap of their resonances with those of C^{β} and C^{γ}, respectively, of ornithine (Figure 1).

The $N^{\beta}T_1^{\beta}$ values of the phenylalanine, leucine, and valine residues are all sufficiently longer than the α-carbon T_1 values to permit a determination of τ_G for the rotation about C^{α}–C^{β}. The resulting τ_G values for the phenylalanine and valine residues are $(3.3 \pm 0.7) \times 10^{-10}$ and $(5.1 \pm 1.2) \times 10^{-10}$ sec, respectively. The $N^{\beta}T_1^{\beta}$ value of the leucine residues is either 340 or 388 msec (Figure 2), because of the ambiguity in the assignments for C^{β} of leucine and C^{δ} of ornithine (Figure 1). If $N^{\beta}T_1^{\beta}$ is 340 msec, then τ_G for the rotation about C^{α}–C^{β} of leucine is $(6.9 \pm 0.9) \times 10^{-11}$ sec. If $N^{\beta}T_1^{\beta}$ is 388 msec, then τ_G is $(5.2 \pm 0.5) \times 10^{-11}$ sec.

The valine and leucine residues show further increases in NT_1 when going beyond C^{β}, with the largest increase occurring at the methyl groups. A quantitative interpretation of these NT_1 values must await further theoretical developments. The trend along the ornithine side chains cannot be observed in detail, because only C^{δ} yields a well resolved resonance whose T_1 can be measured accurately (see Figure 1).

In the case of phenylalanine, the axis of rotation about C^{β}–C^{γ} coincides with the C^{ζ}–H bond. Therefore, rotation about C^{β}–C^{γ} cannot affect the T_1 value of C^{ζ}. As a result, C^{ζ} should have the same NT_1 value as C^{β}. However, the difference in the observed values (Figure 2) is outside experimental error. This difference may be caused by a difference of C–H bond lengths of about 3%. Throughout our discussion we have assumed that all C–H bond lengths are 1.09 Å. A change of 3% in bond lengths would change the T_1 value of a protonated carbon by 19%. C–H bond lengths are not known with sufficient accuracy to establish if indeed a change in C–H bond length when going from an aliphatic to an aromatic carbon can account for the observed difference between NT_1^{β} and NT_1^{ζ} of the phenylalanine residues.

Internal rotation about C^{β}–C^{γ} of the phenylalanine residues should lengthen NT_1^{γ} and NT_1^{δ}. The experimental NT_1 values of C^{γ} and C^{δ} are indeed longer than NT_1^{ζ}.

Acknowledgment. This research was supported by the National Science Foundation (Grant GP-17966), by the donors of the Petroleum Research Fund, administered by the American Chemical Society, by the National Institutes of Health (Grant NS-10977), and by Eli Lilly and Co.

Thermodynamics of the Two Dissociation Steps of *N*-Tris(hydroxymethyl)methylglycine ("Tricine") in Water from 5 to 50° [1]

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Abstract: *N*-Tris(hydroxymethyl)methylglycine ("tricine"), a zwitterion, is a derivative of glycine containing a strongly hydrophilic substituent. Both of its acidic groups are stronger than those of the parent glycine, and consequently tricine is a useful buffer substance for the physiological region of pH 7.2 to 9. The equilibrium constants for the two dissociation steps of tricine have been determined at ten temperatures by measurement of the emf of cells without liquid junction containing hydrogen gas electrodes and silver–silver bromide electrodes. For the dissociation of protonated tricine, pK_1 is 2.023 at 25°, while pK_2 for the dissociation of tricine itself is 8.135 at 25°. The standard changes of Gibbs energy, enthalpy, entropy, and heat capacity have been derived from the change of the equilibrium constants for these two processes with temperature.

Considerable interest attaches to the acid–base behavior of glycine, the simplest amino acid. Inasmuch as glycine exists in a zwitterionic form, the thermodynamic constants for the dissociation equilibria are uncommonly informative. Furthermore, glycine buffer solutions have long found use for the control of pH in the range 8.5 to 10.5. Careful thermodynamic studies

(1) This work was supported in part by the National Science Foundation under Grant GP 14538 and by the donors of the Petroleum Research Fund, administered by the American Chemical Society.

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of the dissociation of glycine[3,4] and other amino acids[5] have contributed materially to an understanding of this important subject.

In their selection of buffer substances useful in the physiological region pH 6 to 8, Good and his associates[6] called attention to *N*-tris(hydroxymethyl)methylglycine ("tricine"), a compound whose deprotonation constant is about 10^{-8} at room temperature. Tricine is therefore better suited for pH control in the buffer region of greatest biochemical interest than is the parent compound glycine. The pH values of tricine buffers matching closely the pH of blood have recently been determined.[7]

In earlier studies, designed to elucidate the thermodynamic consequences of preferential solute–solvent interactions in mixed solvents, the effect of the tris(hydroxymethyl)methyl substituent in enhancing the hydrophilic character of an acid and its conjugate base has been utilized.[8] To this end, the solvent effects on ammonia and tris(hydroxymethyl)aminomethane ("tris," THAM) have been compared in methanol–water solvents[9,10] and contrasted with similar data for the hydrophobic nitroanilines.[6] A similar comparison of acids of neutral charge, namely acetic acid and tris(hydroxymethyl)acetic acid, was also made.[11] With a study of the thermodynamics of the two dissociation steps of tricine in water, an investigation of the solvent effect of the hydrophilic tris(hydroxymethyl)methyl substituent on the acid–base behavior of two other charge types, in which zwitterions are involved, is being initiated.

If the zwitterion $(CH_2OH)_3CN^+H_2CH_2COO^-$ is designated $T^{\pm}$, the first dissociation step is the equilibrium represented by the deprotonation of the carboxyl group of the species HT^+, that is

$$HT^+ \overset{K_1}{\rightleftharpoons} H^+ + T^{\pm} \qquad (1)$$

while the second dissociation step, the deprotonation of the substituted ammonium group of tricine itself, can be represented

$$T^{\pm} \overset{K_2}{\rightleftharpoons} H^+ + T^- \qquad (2)$$

where K_1 and K_2 are thermodynamic equilibrium constants.

Both pK_1 and pK_2 have been determined at ten temperatures from 5 to 50° by measuring the emf of cells without liquid junction of the two types

$$Pt|H_2\ (g,\ 1\ atm),\ THBr\ (m_1),\ T\ (m_2),\ AgBr|Ag \qquad (I)$$

and

$$Pt|H_2\ (g,\ 1\ atm),\ T\ (m_1),\ NaT\ (m_2),\ KBr\ (m_3),\ AgBr|Ag \qquad (II)$$

where m is molality. The standard changes of Gibbs energy, enthalpy, entropy, and heat capacity for the two acidic dissociation processes were derived by standard thermodynamic relationships from the least-squares constants of the equations representing the change of pK_1 and pK_2 as a function of the thermodynamic temperature, T.

Method

The method, essentially the same as that used by Owen[3] and King[4] in their earlier studies of the dissociation of glycine, was an adaptation of the precise emf procedure introduced by Harned and Ehlers.[12] As may be seen in the schematic outline of cells I and II, however, the silver–silver bromide electrode was employed instead of the silver–silver chloride electrode used for the earlier investigations. This electrode has been found to be highly reproducible and stable; furthermore, the lower solubility of silver bromide (as compared with silver chloride) makes this electrode more suitable for use in solutions of nitrogen bases.

First Dissociation Constant. The first dissociation constant (K_1) of tricine is related to the emf (E) of cell I by the expression

$$pK_1 = \frac{E - E^\circ}{(RT \ln 10)/F} + \log \frac{m_{HT} m_{Br}}{m_T} + \log \frac{\gamma_{HT}\gamma_{Br}}{\gamma_T} \qquad (3)$$

where E° is the standard emf of the cell. For simplicity, the charges have been omitted from the species written as subscripts; except for bromide ion, they are evident in eq 1.

The activity coefficient term of eq 3 is unknown; it is common practice in such situations to substitute a semiempirical representation (based usually on the Debye–Hückel formula) of such form that the "apparent" pK_1, designated pK_1', becomes a linear function of the ionic strength, I. The true pK_1 is then obtained by extrapolation of pK_1' to $I = 0$. First, however, the ratio m_{HT}/m_T must be obtained from the stoichiometric ratio m_1/m_2. Because of the appreciable dissociation of the species HT^+, $m_{HT} = m_1 - m_H$ and $m_T = m_2 + m_H$. The evaluation of the molality m_H of hydrogen ion with sufficient accuracy is difficult and imposes serious limitations on the accuracy with which the pK of an uncharged acid with pK less than 3 can be derived. The problem is somewhat less serious here, for the dissociation process (eq 1) produces no change in ionic strength, since the zwitterion $T^{\pm}$ is presumed to behave as an uncharged species.

The apparent pK was derived by the equation

$$pK_1' = \frac{E - E^\circ}{(RT \ln 10)/F} + \log \frac{m_1(m_1 - m_H')}{m_2 + m_H'} - \frac{2AI^{1/2}}{1 + B\mathring{a}I^{1/2}} \qquad (4)$$

and the "apparent" hydrogen ion molality (m_H') was calculated by

$$-\log m_H' = \frac{E - E^\circ}{(RT \ln 10)/F} + \log m_1 - \frac{2AI^{1/2}}{1 + B\mathring{a}I^{1/2}} \qquad (5)$$

(3) B. B. Owen, *J. Amer. Chem. Soc.*, **56**, 24 (1934).
(4) E. J. King, *J. Amer. Chem. Soc.*, **67**, 2178 (1945); **73**, 155 (1951).
(5) See the summary given by H. S. Harned and B. B. Owen, "The Physical Chemistry of Electrolytic Solutions," 3rd ed, Reinhold, New York, N. Y., 1958, pp 667 and 758.
(6) N. E. Good, G. D. Winget, W. Winter, T. N. Connolly, S. Izawa, and R. M. M. Singh, *Biochemistry*, **5**, 467 (1966).
(7) R. G. Bates, R. N. Roy, and R. A. Robinson, *Anal. Chem.*, **45**, 1663 (1973).
(8) R. G. Bates, *J. Electroanal. Chem.*, **29**, 1 (1971).
(9) M. Paabo, R. G. Bates, and R. A. Robinson, *J. Phys. Chem.*, **70**, 247 (1966).
(10) P. Schindler, R. A. Robinson, and R. G. Bates, *J. Res. Nat. Bur. Stand., Sect. A*, **72**, 141 (1968).
(11) S. Goldman, P. Sågner, and R. G. Bates, *J. Phys. Chem.*, **75**, 826 (1971).
(12) H. S. Harned and R. W. Ehlers, *J. Amer. Chem. Soc.*, **54**, 1350 (1932).

where $\mathring{a}$ is the ion-size parameter. The Debye–Hückel constants A and B and the Nernst slopes $(RT \ln 10)/F$ are listed elsewhere[13] for each of the temperatures of the present study. The standard emf was determined in earlier work.[14] The ionic strength is given by

$$I = m_1 \tag{6}$$

As I approaches 0, m_H' approaches m_H and pK_1' approaches pK_1.

Second Dissociation Constant. Similarly, pK_2 can be obtained from the emf of cell II by the extrapolation to $I = 0$ of "apparent" values pK_2' given by

$$pK_2' = \frac{E - E^\circ}{(RT \ln 10)/F} + \log \frac{m_1 m_3}{m_2} \tag{7}$$

The simple form of eq 7 results from (a) the expectation that the activity coefficient term $\log (\gamma_{T^\pm}\gamma_{Br^-}/\gamma_{T^-})$ will, because of the charge type of eq 2, be small and will vary linearly with I, and (b) the nearly neutral pH of the cell solutions, which makes hydrolysis corrections to m_1/m_2 unnecessary. The ionic strength of the solutions of cell II is given by

$$I = m_2 + m_3 \tag{8}$$

As expected, pK_2' varied linearly with I at each temperature; the intercept at $I = 0$ is the value of pK_2.

Experimental Section

A commercial sample of tris(hydroxymethyl)methylglycine was purified by crystallization from 70% ethanol. The sample was dried under vacuum at room temperature and then powdered and stored in a desiccator over Drierite until used. It assayed 99.97% (standard deviation 0.1%) when titrated under carbon dioxide-free conditions with a standard solution of sodium hydroxide, either to a theoretical equivalence point of pH 10.34 or to an end point read from a differential plot of $\Delta pH/\Delta V$ *vs.* volume (V) of the alkali solution. The glass electrode was used for the pH measurements.

Hydrobromic acid of reagent grade, diluted to the composition of the constant-boiling mixture, was distilled twice with the addition of a little red phosphorus. The middle third of the distillate was collected each time. An aqueous stock solution, approximately 0.3 M, was prepared from the redistilled acid and was standardized by a gravimetric determination of bromide as silver bromide. The average difference among triplicate determinations was 0.01%.

For the determination of pK_1, two different stock solutions containing an approximately 3:1 molar ratio of tricine to its hydrobromide were prepared by weight methods from tricine, the standard hydrobromic acid solution, and water. Twelve different buffer solutions were made by weight dilutions of the two stock solutions with distilled water. Vacuum corrections were applied to all weighings.

In the determination of pK_2, stock buffer solutions containing an approximately 1:1 molar ratio of tricine to its sodium salt, were prepared by mixing accurately weighed portions of tricine, standard carbonate-free sodium hydroxide solution, recrystallized potassium bromide, and carbon dioxide-free water. Eight cell solutions were made by diluting these stock solutions.

Purified hydrogen gas was bubbled through each solution to remove dissolved air before the cells were filled. The filling procedures were designed to exclude oxygen and carbon dioxide, insofar as possible, from the cells.

The design of the all-glass cells[15] and the methods by which the hydrogen electrodes[16] and the silver–silver bromide electrodes (thermal type)[17] were prepared have been described elsewhere. The emf measurements were made at intervals of 5° from 5 to 50°, each run extending over a period of 3 days. The emf at 25° was recorded three times, namely at the beginning, middle, and end of the temperature series. These readings agreed within 0.05 mV on the average, demonstrating the excellent stability of the cells. The emf values were measured by a Hewlett-Packard digital voltmeter and were recorded, at preset intervals, to the nearest 0.01 mV on a strip chart, together with the time and the temperature of the bath. Frequent checks of the readings were made with a Leeds & Northrup type K-3 potentiometer standardized with a group of temperature-controlled Weston cells. The two measurements agreed in all cases to 0.02 mV. The constant-temperature bath was controlled to 0.01°; its temperature was measured by means of a calibrated quartz thermometer.

Results

The emf of cell I was measured with 12 different buffer solutions ranging from $m_1 = 0.004725$ and $m_2 = 0.014170$ up to $m_1 = 0.09807$ and $m_2 = 0.2873$. For the eight solutions of cell II, approximate equality of m_1, m_2, and m_3 was maintained; this molality was varied in the range 0.0093 to 0.097 mol kg^{-1}. Duplicate cells of type II were prepared with each solution. On the average, duplicate measurements agreed to 0.04 mV at 5° and to 0.05 mV at 25 and 50°. The observed emf was corrected in the usual way[16] to a partial pressure of 1 atm of hydrogen. The corrected emf data are available elsewhere.[18] All of the calculations described below were performed with the aid of the IBM System/360 Model 65 computer at the University of Florida Computing Center.

First Dissociation Constant. "Apparent" molalities m_H' of hydrogen ion, derived by eq 5 for ionic strengths $I = m_1$, were substituted in eq 4 and used to calculate values of pK_1'. A linear extrapolation of pK_1' to $I = 0$ gave the values of pK_1 listed in Table I, second column. The standard deviation of the intercept is given in the third column. The experimental values of pK_1 at each of the ten temperatures were fitted by the method of least squares to an equation of the form suggested by Harned and Robinson.[19] The variation of pK_1 as a function of the thermodynamic temperature T (from $T = 278.15$ to 323.15°K) is expressed by

$$pK_1 = \frac{1800.3}{T} - 9.0217 + 0.016797T \tag{9}$$

Table I. Acidic Dissociation Constant (K_1) of Protonated Tricine from 5 to 50°

t, °C	pK_1(exptl)	S.d.[a]	pK_1(calcd)[b]
5	2.125	0.0015	2.1228
10	2.092	0.0008	2.0925
15	2.064	0.0007	2.0662
20	2.042	0.0005	2.0436
25	2.023	0.0006	2.0246
30	2.009	0.0009	2.0090
35	2.000	0.0010	1.9966
40	1.989	0.0013	1.9873
45	1.980	0.0008	1.9809
50	1.976	0.0008	1.9774

[a] Standard deviation of the intercept. [b] Calculated by eq 9.

(13) R. G. Bates, "Determination of pH," 2nd ed, Wiley, New York, N. Y., 1973, appendix, Tables 1 and 4.
(14) H. B. Hetzer, R. A. Robinson, and R. G. Bates, *J. Phys. Chem.*, **66**, 1423 (1962).
(15) R. Gary, R. G. Bates, and R. A. Robinson, *J. Phys. Chem.*, **68**, 1187 (1964).
(16) R. G. Bates, "Determination of pH," 2nd ed, Wiley, New York, N. Y., 1973, Chapter 10.
(17) R. N. Roy, R. A. Robinson, and R. G. Bates, *J. Chem. Thermodyn.*, **5**, 559 (1973).
(18) See paragraph at end of paper regarding supplementary material.
(19) H. S. Harned and R. A. Robinson, *Trans. Faraday Soc.*, **36**, 973 (1940).

The values of pK_1 calculated by eq 9 are given in the last column of Table I. The standard deviation for regression is 0.0020.

It is well known that the necessity of making an arbitrary choice of ion-size parameter $\mathring{a}$ is often a source of considerable uncertainty in the pK measured for moderately strong acids.[20,21] For example, the pK_1 found for phosphoric acid is shifted by 0.004 to 0.014 unit (depending on the buffer ratio of the cell solutions) when the assumed $\mathring{a}$ value is changed from 4 to 6 Å.[20] Consequently, it is desirable to reduce the hydrogen ion concentration somewhat by decreasing the ratio of free acid to conjugate base. Accordingly, the 3:1 ratio of tricine to tricine hydrobromide was chosen for the determination of pK_1. Although pK_1 for tricine is smaller than for phosphoric acid, the uncertainty in pK_1 caused by the uncertainty in $\mathring{a}$ is also smaller, inasmuch as a change in $\mathring{a}$ does not affect the ionic strength (see eq 6), as is the case with phosphoric acid.

In some instances, it is possible to judge the suitability of the values of the ion-size parameter chosen in terms of the standard deviation for regression of the experimental pK_1' from the best straight line drawn through the points. The calculation of pK_1 was therefore made with three values of $\mathring{a}$, namely 2, 4, and 6 Å. The results at 25°, which are typical of all the temperatures, were as follows in Chart I. Although some improvement in the linear fit of pK_1' to I is evident as $\mathring{a}$ is increased, the results do not provide a reliable guide to a choice of ion-size parameter. Values of $\mathring{a}$ greater than 6 Å were, however, considered unlikely. Consequently, $\mathring{a}$ was taken to be 6 Å at all temperatures and an uncertainty of 0.003 unit from this source estimated for the values of pK_1 given in Table I.

Chart I

$\mathring{a}$, Å	pK_1	S.d.
2	2.0181	0.0024
4	2.0203	0.0017
6	2.0230	0.0011

Second Dissociation Constant. Values of pK_2' derived from the emf data for cell II by eq 7 were fitted by the method of least squares to an equation linear in the ionic strength and the intercept (pK_2) at $I = 0$ determined at each temperature. The values of pK_2 and of the standard deviation of the intercept are given in the second and third columns, respectively, of Table II. The variation of pK_2 with the thermodynamic temperature T was found to be given by the expression

$$pK_2 = 2045.0/T - 0.07133 + 0.0045167T \quad (10)$$

when T lies between 278.15 and 323.15°K. The standard deviation for regression was 0.0008. Values of pK_2 calculated by eq 10 are given in the last column of Table II.

Table II. Acidic Dissociation Constant (K_2) of Tricine from 5 to 50°

t, °C	pK_2(exptl)	S.d.[a]	pK_2(calcd)[b]
5	8.537	0.0026	8.5371
10	8.430	0.0019	8.4299
15	8.326	0.0014	8.3272
20	8.228	0.0012	8.2287
25	8.135	0.0012	8.1343
30	8.044	0.0014	8.0437
35	7.957	0.0017	7.9569
40	7.873	0.0015	7.8735
45	7.794	0.0019	7.7934
50	7.717	0.0019	7.7166

[a] Standard deviation of the intercept. [b] Calculated by eq 10.

Discussion

The standard changes of Gibbs energy ($\Delta G°$), enthalpy ($\Delta H°$), entropy ($\Delta S°$), and heat capacity ($\Delta C_p°$) for the two acidic dissociation processes represented by eq 1 and 2 can be derived readily from the constants of eq 9 and 10 by simple thermodynamic relationships. Equations 9 and 10 are of the form[19]

$$pK = A_1/T - A_2 + A_3T \quad (11)$$

Consequently, one can write

$$\Delta G° = (R \ln 10)(A_1 - A_2T + A_3T^2) \quad (12)$$

$$\Delta H° = (R \ln 10)(A_1 - A_3T^2) \quad (13)$$

$$\Delta S° = (R \ln 10)(A_2 - 2A_3T) \quad (14)$$

and

$$\Delta C_p° = (R \ln 10)(-2A_3T) \quad (15)$$

These equations were used to obtain the thermodynamic constants for which the values at 10, 25, and 40° are summarized in Table III. The estimates of the standard deviations at 25°, calculated by the method of Please,[22] are as follows in Chart II.

Table III. Thermodynamic Functions for the Dissociation of Tricine at 10, 25, and 40°

t, °C	$\Delta G°$, cal mol^{-1}	$\Delta H°$, cal mol^{-1}	$\Delta S°$, cal K^{-1} mol^{-1}	$\Delta C_p°$, cal K^{-1} mol^{-1}
First Dissociation Step				
10	2711	2076	−2.2	−44
25	2762	1405	−4.6	−46
40	2847	701	−6.9	−48
Second Dissociation Step				
10	10922	7700	−11.4	−12
25	11097	7520	−12.0	−12
40	11282	7330	−12.7	−13

Chart II

	First step	Second step
$\Delta G°$, cal mol^{-1}	1.1	1.6
$\Delta H°$, cal mol^{-1}	16	23
$\Delta S°$, cal K^{-1} mol^{-1}	0.05	0.08
$\Delta C_p°$, cal K^{-1} mol^{-1}	2.3	3.5

The uncertainty in these thermodynamic quantities resulting from the arbitrary choice of $\mathring{a}$ in eq 4 and 5 was estimated by comparing the values derived from the pK_1 values for $\mathring{a} = 2$ with those for $\mathring{a} = 6$. At 25°, differences of 3 cal mol^{-1} in $\Delta H°$, 0.03 cal K^{-1} mol^{-1} in $\Delta S°$, and 0.1 cal K^{-1} mol^{-1} in $\Delta C_p°$ were found; hence, the effect of the choice of ion-size parameter on these thermodynamic functions is well within the experimental error of the determination.

Finally, it is of interest to compare the values of the thermodynamic functions for zwitterionic species of related structures. Such a comparison of values at 25° for the parent glycine and some substituted glycines is made in Table IV.

(20) R. G. Bates, *J. Res. Nat. Bur. Stand.*, **47**, 127 (1951).
(21) E. J. King and G. W. King, *J. Amer. Chem. Soc.*, **74**, 1212 (1952).
(22) N. W. Please, *Biochem. J.*, **56**, 196 (1954).

Table IV. Thermodynamic Quantities for the Dissociation of Glycine and Some Substituted Glycines at 25°

	pK	ΔH°, cal mol⁻¹	ΔS°, cal K⁻¹ mol⁻¹	ΔC_p°, cal K⁻¹ mol⁻¹
	First Dissociation Step			
Glycine[a]	2.350	953	−7.6	−34
Tricine	2.023	1405	−4.6	−46
	Second Dissociation Step			
Glycine[a]	9.780	10550	−9.4	−12
Bicine[b]	8.333	6279	−17.1	1
Tricine	8.135	7520	−12.0	−12
N-Methylglycine[c] (sarcosine)	10.200	9681	−14.2	−3
N,*N*-Dimethylglycine[c]	9.940	7654	−19.8	+14

[a] Reference 4. [b] Reference 24. [c] S. P. Datta and A. K. Grzybowski, *Trans. Faraday Soc.*, **54**, 1179, 1188 (1958).

The alterations in acidic strength for both dissociation steps brought about by N substitution in glycine are probably to be attributed both to inductive and steric effects.[23] The change in pK_2 is more pronounced than in pK_1, probably because the substituent is located adjacent to the protonated nitrogen but relatively remote from the carboxyl group. A similar enhancement of acidic strength is apparent in the pK_2 of "bicine," that is, *N*,*N*-bis(2-hydroxyethyl)glycine.[24] On the contrary, N-methyl substitution lowers the acidic strength of the protonated nitrogen group.

Hydroxymethyl or hydroxyethyl substitution usually lowers the value of $\Delta H°$ for isoelectric dissociation processes, in addition to lowering pK.[23,25] The first dissociation step of glycine corresponds closely with this charge type, provided the zwitterion behaves as an uncharged molecule. It appears, however, that $\Delta H°$ increases with substitution of the tris(hydroxymethyl)-methyl group into glycine. The changes of entropy likewise show no consistent pattern.

It is apparent that the value of $\Delta C_p°$ found for the second dissociation of tricine (−12 cal K⁻¹ mol⁻¹ at 25°) is very close to that (−11.7 cal K⁻¹ mol⁻¹) for glycine found by King[4] and to the average value (−14 cal K⁻¹ mol⁻¹) found for other amino acids,[5,26] but it differs by 13 cal K⁻¹ mol⁻¹ from that found for bicine.[24] Although charge type appears to be the primary factor determining the magnitude of $\Delta C_p°$ for a dissociation process,[27] there is mounting evidence[24] that nonelectrostatic effects involving changes in water structure often play an important role. These interactions are of such a complexity that any attempt to account for the thermodynamic quantities associated with the dissociation of these zwitterionic species is still necessarily speculative.

The authors thank Mr. T. K. Ghosh for assistance with the computer programming.

Supplementary Material Available. Tables of the emf of cells I and II will appear following these pages in the microfilm edition of this volume of the journal. Photocopies of the supplementary material from this paper only or microfiche (105 × 148 mm, 20 × reduction, negatives) containing all of the supplementary material for the papers in this issue may be obtained from the Journals Department, American Chemical Society, 1155 16th St., N.W., Washington, D. C. 20036. Remit check or money order for $3.00 for photocopy or $2.00 for microfiche, referring to code number JACS-73-8231.

(23) M. Paabo and R. G. Bates, *J. Phys. Chem.*, **74**, 702 (1970).
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(25) B. A. Timimi and D. H. Everett, *J. Chem. Soc. B*, 1380 (1968).
(26) E. J. Cohn and J. T. Edsall, "Proteins, Amino Acids, and Peptides," Reinhold, New York, N. Y., 1943, p 80.
(27) Reference 5, Chapter 15.

Consideration of the VSEPR Model by a Localized Molecular Orbital Study of the Geometry of H_2O

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Received March 12, 1973

Abstract: The H_2O molecule is studied as a function of HOH angle by the *ab initio* LCAO–SCF–MO method with extended basis sets. The symmetry adapted canonical MO's are transformed into a set of localized MO's (LMO's) by the minimum exchange energy criterion. A variety of energy interactions among these LMO's are considered with a view toward analyzing the VSEPR model for determining molecular geometry. In general, good agreement is obtained between the results and VSEPR. Other orbital characteristics such as charge density and orbital directionality are also considered, and an examination is made of alternate criteria which may be useful in determining molecular geometry.

One of the more useful concepts in predicting and understanding molecular shape is the localized electron pair, especially as formulated in the valence shell electron pair repulsion theory (VSEPR).[2] The fundamental principle of this theory is that electron pairs in the valence level of a central atom orient themselves about the nucleus and inner shell so that the net

(1) The Radiation Laboratory of the University of Notre Dame is operated under contract with the U. S. Atomic Energy Commission. This is AEC Document No. COO-38-881.

(2) (a) R. J. Gillespie, *Angew. Chem., Int. Ed. Engl.*, **6**, 819 (1967); (b) R. J. Gillespie, *J. Chem. Educ.*, **47**, 19 (1970).