

[CONTRIBUTION FROM THE DEPARTMENTS OF CHEMISTRY AND CHEMICAL ENGINEERING, BRIGHAM YOUNG UNIVERSITY, PROVO, UTAH]

## Thermodynamics of Proton Dissociation in Aqueous Solution. III. L-Cysteine, S-Methyl-L-cysteine, and Mercaptoacetic Acid. Determination of Cysteine Microconstants from Calorimetric Data<sup>1a</sup>

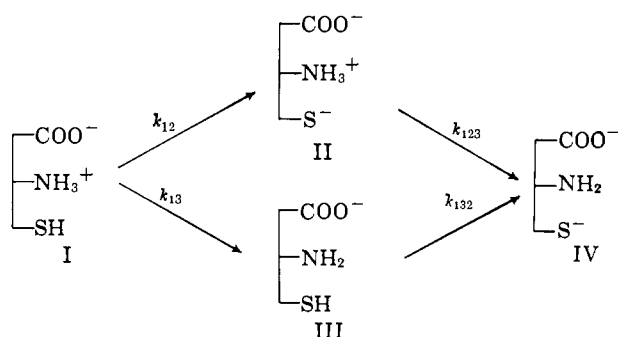
BY DONALD P. WRATHALL, REED M. IZATT,<sup>1b</sup> AND JAMES J. CHRISTENSEN<sup>1b</sup>

RECEIVED JUNE 13, 1964

Calorimetric  $\Delta H$  and  $pQ$  values are reported at 25° and over the ionic strength range 0.002 to 1 for the ionization of protons from (a) the -SH group of mercaptoacetic acid, (b) the -NH<sub>3</sub><sup>+</sup> group of S-methyl-L-cysteine, and (c) the -SH and -NH<sub>3</sub><sup>+</sup> groups of L-cysteine. A calorimetric method for microconstant determination is described and used to determine microconstant values in the case of cysteine through the ionic strength range 0.002 to 1.

### Introduction

Proton ionization from cysteine (CYS) has been investigated repeatedly for several decades with quite divergent results.<sup>2,3</sup> Cysteine is unusual in that proton ionization occurs simultaneously from both the sulfhydryl and the protonated amino groups in each of two pH regions. The microconstant scheme for cysteine<sup>2-4</sup> is



The relative concentrations of microspecies II and III are difficult to determine since pH measurements alone do not provide sufficient data to distinguish between them. At 25° the reactions represented by  $k_{12}$  and  $k_{13}$  occur in the pH region 8.3–8.8 while those represented by  $k_{123}$  and  $k_{132}$  occur in the pH region 9.5–10.5. The microconstants are related to the macroconstants,  $K_2$  and  $K_3$ , in the 8.3–8.8 ( $K_2$ ) and 9.5–10.5 ( $K_3$ ) pH regions by the expressions<sup>2,3</sup>

$$K_2 = k_{12}k_{13} \quad (1)$$

and

$$K_2K_3 = k_{12}k_{123} = k_{13}k_{132} \quad (2)$$

respectively.<sup>4</sup>

Many biologically important molecules contain cysteine or other groupings of atoms which may ionize to produce microspecies. The determination and correct interpretation of the thermodynamic data associated with proton ionization from such molecules is of vital importance to an understanding of their functioning in biological systems. In previous in-

vestigations of the cysteine system the necessary calculation of the ratio,  $R$ , of the concentrations of II and III has been made from spectral (Raman or absorption) data or has been estimated from  $pK$  data involving molecules in which an inert group (*e.g.*, CH<sub>3</sub>, C<sub>2</sub>H<sub>5</sub>, etc.) is substituted for the proton of an acidic group. Benesch and Benesch<sup>5</sup> and Elson and Edsall<sup>3</sup> have determined  $R$  to be approximately 2 by ultraviolet-absorption studies. However, the  $R$  values determined by these workers are uncertain since they are based on  $pK$  values which were not corrected to the  $pQ$  value corresponding to the ionic strength,  $\mu$ , of the solutions being studied. Gorin and Clary<sup>2</sup> measured the concentration change of II by following the ionization of the sulfhydryl group spectrophotometrically. Their conclusion that  $R$  does not vary significantly with  $\mu$  is probably not justified since they did not measure the concentration of III, but apparently assumed it to have the same  $\mu$  dependence as that found for II.

In the present study is described a calorimetric method for determining the relative concentrations of II and III. The method is based on the fact that the  $\Delta H$  values for proton ionization from the NH<sub>3</sub><sup>+</sup> group of S-methyl-L-cysteine (SMC) and the SH group of mercaptoacetic acid (MAA) differ by approximately 4 kcal./mole. This difference in  $\Delta H$  values, if maintained by the corresponding groups in the cysteine species, makes possible a rapid, calorimetric procedure for the determination of  $R$ . However, analysis of the calorimetric data requires accurate proton dissociation constant data. Although several proton dissociation studies have been reported<sup>2,6</sup> for cysteine, only one<sup>6a</sup> has been conducted as a function of  $\mu$ . It was necessary in the present study to know  $pQ$  as a function of  $\mu$  from  $\mu = 0$  to  $\mu = 1$  in order to make meaningful comparisons with other microconstant values<sup>2,3</sup> valid in high  $\mu$  regions. Since calorimetric  $\Delta H$  values have not been reported previously for proton ionization from MAA, SMC, or CYS, it was also necessary to determine these values as a function of  $\mu$ .

In the present study are reported  $pQ$ ,  $\Delta H$ , and  $\Delta S$  values determined at 25° and covering the range  $\mu = 0.002$  to  $\mu = 1.0$  for proton ionization from the -SH group of MAA, the NH<sub>3</sub><sup>+</sup> group of SMC, and the combined -SH and -NH<sub>3</sub><sup>+</sup> groups of CYS. CYS microconstant values valid at 25° and covering the  $\mu$  range

(5) R. Benesch and R. E. Benesch, *J. Am. Chem. Soc.*, **77**, 5877 (1955).(6) (a) H. Borsook, E. L. Ellis, and H. M. Huffman, *J. Biol. Chem.*, **117**, 281 (1934); (b) N. Bjerrum, *Z. Physik. Chem.*, **106**, 219 (1923); (c) R. C. Cannon and B. C. J. G. Knight, *Biochem. J.*, **21**, 1384 (1927); (d) M. A. Grafius and J. B. Neilands, *J. Am. Chem. Soc.*, **77**, 3389 (1955).

(1) (a) Supported by National Institutes of Health Grant No. RG-9430. Presented in part at the 145th National Meeting of the American Chemical Society, New York, N. Y., Sept., 1930. (b) To whom inquiries should be directed.

(2) For a critical review of previous work, see G. Gorin and C. W. Clary, *Arch. Biochem. Biophys.*, **90**, 40 (1960).(3) E. Elson and E. Edsall, *Biochemistry*, **1**, 1 (1962).(4) The symbols  $k_D$  and  $K_D$  are used to denote equilibrium constants valid at zero ionic strength. Corresponding equilibrium constants at finite ionic strength values are given the symbols  $k_D'$  and  $Q_D$ , respectively.

0.002 to 1.0 are calculated from the  $pQ$  and  $\Delta H$  data. Extrapolation of these data to  $\mu = 0$  gives the corresponding thermodynamic quantities in each case.

### Experimental

**Materials.**—CYS hydrochloride hydrate (California Biochem. Corp., A grade, Lot No. 503233), and SMC (California Biochem. Corp., A grade, Lot No. 671051) were used without further purification. An equivalent weight determination (carboxyl group) of CYS agreed to  $\pm 0.1\%$  of the theoretical value. MAA from two sources (Eastman White Label, Lot No. 2249, and Fisher Certified Reagent, 99.5%, Lot No. 733405) was used in the determinations with no distinguishable difference in the  $pQ$  and  $\Delta H$  results. The Eastman material was fractionally distilled, and the fraction boiling between 79.5 and 81.0° (1.0 mm.) was taken. The Fisher material was used without further purification. All water used in the solution preparations was recently boiled and doubly distilled. Special precautions were taken during the preparation of solutions to avoid oxygen contamination since such contamination caused oxidation of the CYS and MAA solutions. Other chemicals used (NaOH, HClO<sub>4</sub>, and NaCl) were of the highest available purity. Care was taken in the preparation of the NaOH solutions to exclude atmospheric CO<sub>2</sub>.

**Equilibrium Constant Determinations.**—CYS solutions were titrated with standard NaOH solutions at 25° in a water bath controlled to  $\pm 0.1^\circ$ . The solutions were allowed to equilibrate in the water bath approximately 30 min. before each titration and all titrations were carried out under a nitrogen atmosphere. The pH measurements were made using a Leeds and Northrup pH indicator (catalog no. 7401) equipped with a 1000-ohm helipot with a microdial setting making the precision of the readings approximately  $\pm 0.004$  pH unit. The Beckman E-2 glass and saturated calomel electrodes used in the determinations were calibrated against a pH 9.18 buffer solution which was prepared using borax (National Bureau of Standards sample no. 187a) and instructions supplied by the National Bureau of Standards. The electrodes were checked against the buffer solution before and after each run. Subsequent calculations were made using only data from runs in which the initial and final buffer readings agreed to within 0.02 pH unit. In these calculations the average of the initial and final buffer readings was taken as the reference buffer pH value.

**Heat Determinations.**—The thermometric titration apparatus used in the calorimetric determinations has been described previously.<sup>7</sup> Solutions were prepared for the calorimetric determinations using the same precautions that were used in preparing solutions for the  $pQ$  determinations. Sufficient NaOH was added in each case to form predominantly the ionized forms of MAA, SMC, and CYS. These solutions were then titrated with standard HClO<sub>4</sub> solution. Determinations were made using solutions having  $\mu$ -values comparable to those used in the  $pQ$  determinations.

**Calculations.**—The method used to calculate  $\Delta H$  and  $\Delta H^\circ$  values from the thermometric titration data has been described.<sup>7a,b</sup>

Values of  $pQ$  were obtained in the following manner from the pH titration data;  $\mu$  was calculated in the usual manner. A Debye-Hückel equation of the form<sup>8</sup>

$$-\log f_i = \frac{Az_i^2\mu^{0.5}}{1 + Ba_i\mu^{0.5}} \quad (3)$$

was used to convert pH to  $[H^+]$  and  $\bar{n}$  was calculated from electroneutrality and mass-balance expressions using titration readings within 0.1 unit of half-integral  $\bar{n}$  values. For high  $\mu$  values,  $f_i$  was taken as equal to the mean activity coefficient of hydrochloric acid in water.<sup>9</sup> All calculations, including a recycling process between  $\mu$  and  $f_i$  until the approximation attained the desired self-consistency, were done on IBM 650 and 7040 electronic computers.

$pK$  values were determined for each acid (1) by extrapolation of  $pQ$  vs.  $\mu^{0.5}$  plots to  $\mu = 0$ , and (2) using activity coefficients calculated from a Debye-Hückel expression of the form<sup>8</sup>

$$-\log f_i = \frac{Az_i^2\mu^{0.5}}{1 + Ba_i\mu^{0.5}} - \beta_i\mu \quad (4)$$

Data for the  $pQ$  vs.  $\mu^{0.5}$  plots were taken from the MAA, SMC, and CYS concentration ranges 0.0016 to 0.04 *F*.  $pQ$  values were also determined at  $\mu = 0.2$  and  $\mu = 1.0$  (using NaCl as inert electrolyte) in order to make comparisons with earlier work,<sup>2,3</sup> and to test the validity of (4) for estimating activity coefficients. It was found that  $pK$  values calculated using activity coefficients derived from (4) agreed well with those obtained by extrapolation in lower  $\mu$  regions.

Microconstants for proton ionization from the sulfhydryl and protonated amino groups of cysteine were calculated from  $pK$  and  $\Delta H$  data in the following manner.

If  $\alpha$  is the fraction of CYS molecules in which protons dissociate from the sulfhydryl group in the lower pH region ( $I = II + H^+$ ) then  $(1 - \alpha)$  is the fraction of CYS molecules in which protons dissociate from the protonated amino group in the same pH region ( $I = III + H^+$ ). The measured heats of proton dissociation in the two pH regions ( $\Delta H_2$  and  $\Delta H_3$ ) may be expressed by the relationships

$$\Delta H_2 = \alpha\Delta H_{12} + (1 - \alpha)\Delta H_{13} \quad (5)$$

$$\Delta H_3 = \alpha\Delta H_{123} + (1 - \alpha)\Delta H_{132} \quad (6)$$

Since  $\Delta H_{12}$ ,  $\Delta H_{13}$ ,  $\Delta H_{123}$ , and  $\Delta H_{132}$  cannot be measured directly, values are assigned to these quantities which are equal to the heats of proton dissociation from the sulfhydryl ( $\Delta H_s$ ) and protonated amino ( $\Delta H_a$ ) groups in MAA and SMC, respectively (*i.e.*,  $\Delta H_s$  is taken as equal to  $\Delta H_{12}$  or  $\Delta H_{132}$ , and  $\Delta H_a$  as equal to  $\Delta H_{13}$  or  $\Delta H_{123}$ ). Addition of  $\Delta H_3$  to both sides of (5),  $\Delta H_2$  to both sides of (6), and appropriate substitution of  $\Delta H_s$  and  $\Delta H_a$  leads to

$$\Delta H_2 + \Delta H_3 = \alpha(\Delta H_s + \Delta H_3) + (1 - \alpha)(\Delta H_a + \Delta H_3) \quad (7)$$

$$\Delta H_2 + \Delta H_3 = \alpha(\Delta H_a + \Delta H_2) + (1 - \alpha)(\Delta H_s + \Delta H_2) \quad (8)$$

Since the right hand members of (7) and (8) are equal, the ratio

$$R = \frac{\alpha}{1 - \alpha} = \frac{(\Delta H_2 - \Delta H_3) + (\Delta H_s - \Delta H_a)}{(\Delta H_3 - \Delta H_2) + (\Delta H_s - \Delta H_a)} \quad (9)$$

where the quantity  $\Delta H_2 - \Delta H_3$  is the measured difference between the heats of proton dissociation in the lower and higher pH regions, respectively, and  $\Delta H_s - \Delta H_a$  is the difference between the assigned heats of proton dissociation from the -SH and -NH<sub>3</sub><sup>+</sup> groups in CYS. It is seen from (9) that if  $\Delta H_s - \Delta H_a$  is large compared to  $|\Delta H_2 - \Delta H_3|$ , even relatively large errors in the assigned values of  $\Delta H_s$  and/or  $\Delta H_a$  will not change  $R$  significantly. For  $0.67 < R < 1.5$ , even an error in  $(\Delta H_s - \Delta H_a) = (\Delta H_{12} - \Delta H_{13}) = (\Delta H_{132} - \Delta H_{123})$  of  $\pm 1$  kcal. would cause an error in  $R$  of less than 10%. The respective microconstants are calculated from  $R$ ,  $K_2$ , and  $K_3$  using (1), (2), and the relationship

$$R = \alpha/(1 - \alpha) = k_{12}/k_{13} = k_{132}/k_{123} \quad (10)$$

### Results

In Table I are presented  $pQ$  values as a function of  $\mu$  and corresponding  $pK$  values for proton ionization from CYS, MAA, and SMC together with previous work. The estimated uncertainty of the  $pK$  values is given as the standard deviation. The pH of the buffer solutions are given by the National Bureau of Standards as accurate to  $\pm 0.005$  pH unit. This error is not included in the stated standard deviation. The  $pQ$  values are averages based on the indicated number of separate determinations in each case. The average  $pK$  value in each case was calculated from low ionic strength data using activity coefficient values estimated from (4).

In Fig. 1 are given the  $\Delta H$  values as a function of  $\mu^{0.5}$  for proton ionization from CYS, MAA, and SMC.

(7) (a) J. J. Christensen and R. M. Izatt, *J. Phys. Chem.*, **66**, 1030 (1962); (b) R. M. Izatt, *et al.*, *Inorg. Chem.*, **1**, 828 (1962).

(8) R. G. Bates, "Electrometric pH Determinations," John Wiley and Sons, Inc., New York, N. Y., 1954, p. 51.

(9) H. S. Harned and R. W. Ehlers, *J. Am. Chem. Soc.*, **55**, 2179 (1933).

TABLE I

pQ AND pK VALUES FOR THE INDICATED PROTON DISSOCIATION STEP OF CYS [(A) AND (B)], MAA (C), AND SMC (D)<sup>a</sup>

No. of detn.	$\mu \times 10^3$	pQ	pK
H <sub>2</sub> CYS (I) = II, III + H <sup>+</sup> ; $a_t = 4.5 \text{ \AA.}$ , $B_t = 0.1 \text{ (A)}$			
2	1000	8.071	8.389
4	160	8.142	8.386
	(~150) <sup>b</sup>	(8.14) <sup>b</sup>	
	(~160) <sup>c</sup>	(8.30) <sup>c</sup>	
	(~110) <sup>d</sup>	(8.17) <sup>d</sup>	
1	81.2	8.176	8.372
4	41.2	8.234	8.388
2	14.4	8.293	8.395
3	7.2	8.317	8.393
3	1.6	8.354	8.392
	(0) <sup>e</sup>		(8.33) <sup>e</sup>
Av. of all pK determinations in the $\mu$ range 0.0016-0.0412			
		8.392 ± 0.006	
II, III = CYS <sup>-2</sup> (IV) + H <sup>+</sup> ; $a_t = 5.5$ , $\beta_t = -0.1 \text{ (B)}$			
2	995	9.947	10.771
	(~320) <sup>b</sup>	(10.34) <sup>b</sup>	
4	176	10.213	10.747
	(~160) <sup>c</sup>	(10.40) <sup>c</sup>	
	(~110) <sup>d</sup>	(10.32) <sup>d</sup>	
3	79.4	10.382	10.769
2	29.3	10.504	10.770
3	13.0	10.557	10.751
3	2.7	10.664	10.761
	(0) <sup>e</sup>		(10.78) <sup>e</sup>
Av. of all pK determinations in the $\mu$ range 2.1-29.3			
		10.761 ± 0.010	
HMAA <sup>-</sup> = H <sup>+</sup> + MAA <sup>-2</sup> ; $a_t = 3$ , $\beta_t = 0.1 \text{ (C)}$			
3	1051	9.67	10.60
2	236	9.84	10.49
4	38	10.24	10.57
2	9.8	10.39	10.57
3	5.5	10.42	10.56
Av. of all pK determinations in the $\mu$ range 2.1-38			
		10.56 ± 0.02	
HSMC <sup>+2</sup> = H <sup>+</sup> + SMC <sup>-</sup> ; $a_t = 4$ , $\beta_t = 0.1 \text{ (D)}$			
3	998	8.65	8.99
3	194	8.69	8.95
	(~160) <sup>c</sup>	(8.75) <sup>c</sup>	
3	7.1	8.89	8.97
3	5.0	8.90	8.96
3	4.0	8.91	8.97
Av. of all pK determinations in the $\mu$ range 4.0-7.1			
		8.97 ± 0.01	

<sup>a</sup> Values are valid at 25 ± 0.1°. Uncertainties of the pK values are given as standard deviations. Values of  $a_t$  and  $\beta_t$  (see eq. 4) are given for each system. The reaction for which the data are valid is indicated in each case. Previous pQ and pK data are given in parentheses. <sup>b</sup> Ref. 6c, temperature = 30°. <sup>c</sup> Ref. 6d. <sup>d</sup> G. Gorin, *J. Am. Chem. Soc.*, **78**, 767 (1956); Gorin's values have been converted to concentration constants using the activity coefficient of HCl.<sup>9</sup> <sup>e</sup> Ref. 6a.

The brackets represent the range of the experimental  $\Delta H$  data. The  $\Delta H^\circ$  values obtained by extrapolation have the estimated uncertainties indicated by the arrows in each case. Considerable difficulty was experienced in obtaining consistent  $\Delta H$  values for MAA proton ionization. The  $\Delta H$  values reported in Fig. 1 were obtained using different calorimeters and different sources of MAA. It was never possible to obtain a set of determinations which had the precision of the SMC or CYS determinations. The reasons for the observed data scatter in the case of MAA are not known.

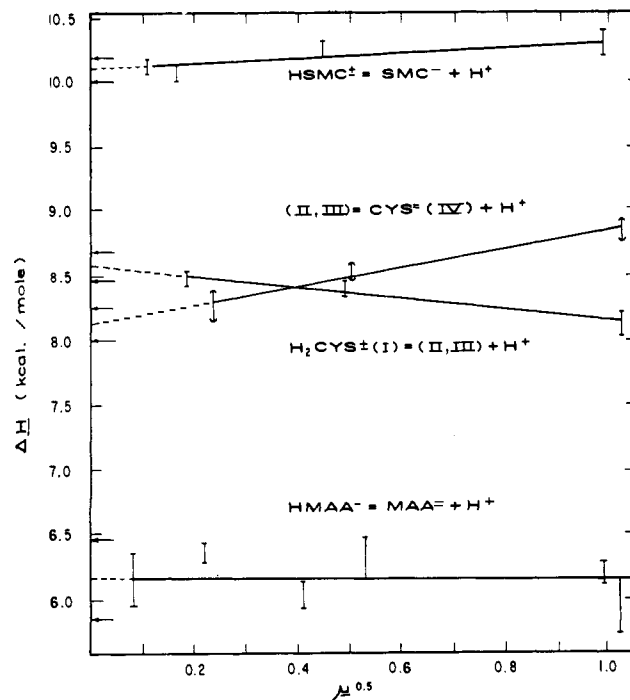


Fig. 1.—Plot of  $\Delta H$  (kcal./mole) vs.  $\mu^{0.5}$  for proton ionization from SMC, CYS, and MAA. Range of the experimental  $\Delta H$  and estimated uncertainty of the  $\Delta H^\circ$  values are indicated by brackets and arrows, respectively. Temperature = 25°.

In Fig. 2  $\mu^{0.5}$  is plotted vs. (a)  $R$ , (b)  $pK_{12}'$  and  $pK_{13}'$ , and (c)  $pK_{123}'$  and  $pK_{132}'$  for CYS ionization. The estimated uncertainty of the  $R$  and  $pK'$  values is indicated by the brackets. Previously reported  $R$  values are also given in Fig. 2a.

TABLE II

$\Delta S$  VALUES (E.U.) AS A FUNCTION OF  $\mu$  FOR PROTON IONIZATION FROM CYS, MAA, AND SMC<sup>a</sup>

Reaction	$\Delta S$ values			
	$\mu = 0$	$\mu = 0.04$	$\mu = 0.16$	$\mu = 1.0$
I = (II, III) + H <sup>+</sup>	-9.5	-9.2	-9.1	-9.6
(II, III) = IV + H <sup>+</sup>	-21.9	-19.9	-18.6	-15.8
HMAA <sup>-</sup> = MAA <sup>-2</sup> + H <sup>+</sup>	-27.6	-26.1	-25.1	-23.6
HSMC <sup>+2</sup> = SMC <sup>-</sup> + H <sup>+</sup>	-7.2	-6.4	-5.8	-5.0

<sup>a</sup> Values are valid at 25°, and are estimated to be accurate to 1 e.u.

In Table II are presented  $\Delta S$  values as a function of  $\mu$  for proton ionization from CYS, MAA, and SMC. The estimated uncertainty of these values is ±1 e.u.  $\Delta S$  values for proton ionization from the CYS microspecies cannot be calculated from the present data.

## Discussion

The pQ data in Table I are generally in good agreement with previous data where these were determined under the conditions used in the present study. A rough check can be made on the assumptions made in calculating the microconstants, *i.e.*,  $\Delta H_a = \Delta H_{12} = \Delta H_{132}$ , and  $\Delta H_s = \Delta H_{13} = \Delta H_{123}$ , since, if these assumptions are true,  $\Delta H_a + \Delta H_s = \Delta H_2 + \Delta H_3$ . At  $\mu = 0$ , the difference between these two sums (Fig. 1) is 0.45 kcal./mole, indicating the validity of the assumptions. It should be pointed out, however, that an alternate explanation is that the heats of proton ionization from the sulfhydryl and protonated amino groups in CYS differ in opposite directions (*i.e.*, one higher and one

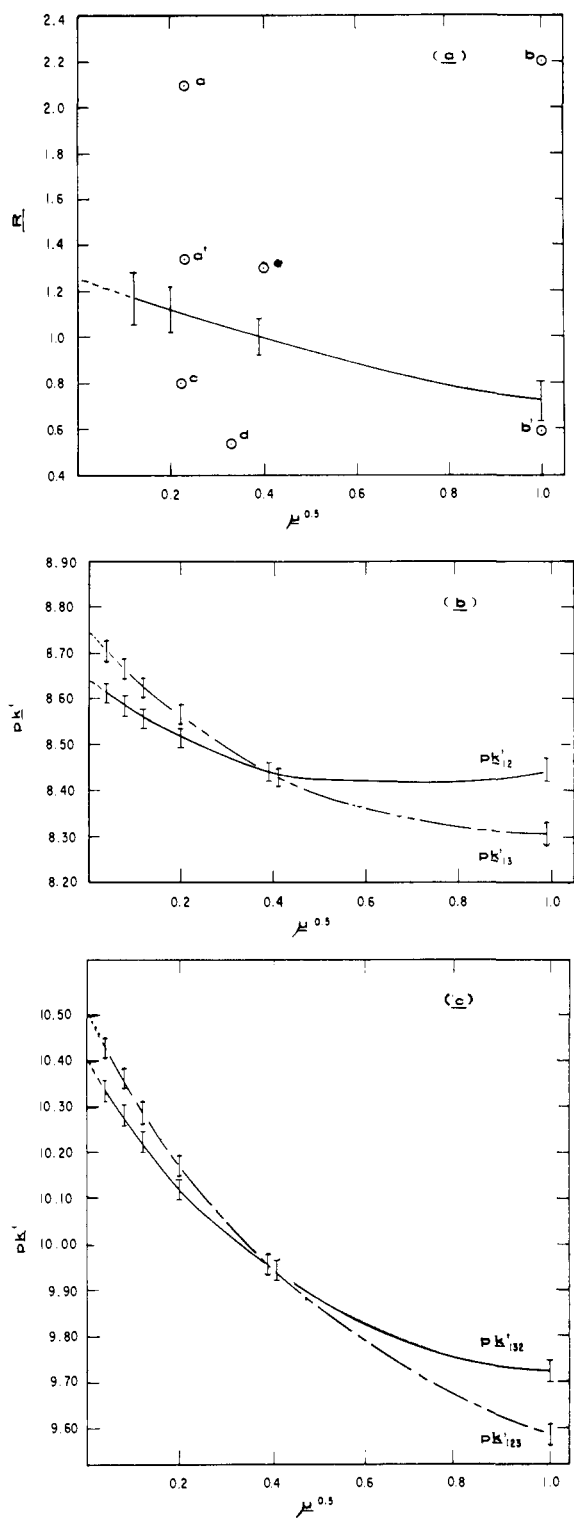


Fig. 2.—Plot of  $\mu^{0.5}$  vs. (a)  $R$ , (b)  $pK'_{12}$  and  $pK'_{13}$ , and (c)  $pK'_{132}$  and  $pK'_{133}$  for CYS ionization. The estimated uncertainty of the  $R$  and  $pK'$  values is indicated by brackets. Previously reported  $R$  values are given in (a), viz., a, ref. 5, b, ref. 3, c, R. L. Rykkan and C. L. A. Schmidt, *Arch. Biochem.*, **5**, 89 (1944), d, ref. c, Table I, and e, ref. 6d. Points a' and b' are calculated from points a and b, respectively, as explained in the text. Temperature = 25°.

lower) from their heats in MAA and SMC, respectively, in which case  $\Delta H_s + \Delta H_a$  could equal  $\Delta H_2 + \Delta H_3$ . Large  $\Delta H$  changes in opposite directions, however, seem unreasonable. A typical thermometric titration curve involving CYS, MAA, and SMC is shown in Fig. 3a. It is significant that the CYS

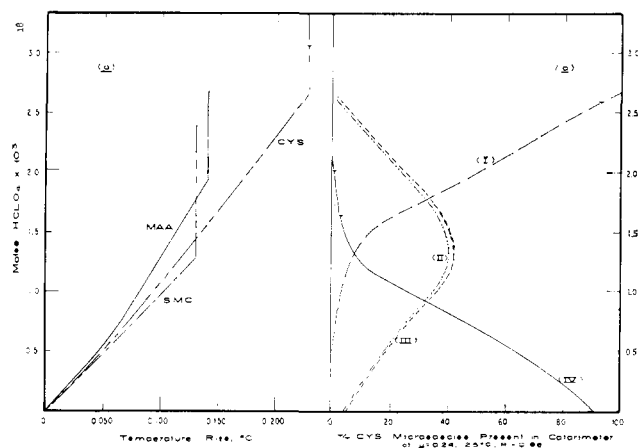


Fig. 3.—Correlation of (a) temperature rise with (b) per cent CYS species present in calorimeter during the titration of the CYS anion (IV) with 0.74  $F$   $HClO_4$ , i.e., (IV) = (II, III) = (I). Also given (a) are corresponding titration curves for the protonation of the  $-S^-$  and  $-\bar{N}H_2$  groups of MAA and SMC, respectively. Data are valid at  $\mu = 0.24$ ,  $R = 0.96$ , and temperature = 25°.

curve is intermediate between the other two. The data in Fig. 3a provide positive evidence for the simultaneous protonation of the  $-S^-$  and  $-NH_2$  groups of the CYS anion throughout both of the pH regions present. If such simultaneous protonation did not occur, heats of proton association for the two groups would appear consecutively as the distinctive slope due to protonation of the group in the upper pH region followed by the slope caused by protonation of the group in the lower pH region.<sup>10</sup>

Previously determined  $R$  values (Fig. 2a) differ widely. It was observed by us in two cases that when corrections were made for use of incorrect  $pQ$  values by earlier workers the resulting  $R$  values were in much better agreement with those obtained in the present study (see a' and b' in Fig. 2a). Point d in Fig. 2a was given by Gorin (see ref. c, Table I) as a lower limit. As stated by Gorin and Clary,<sup>2</sup> the data from which point c was taken were based on mixed  $pQ$  and  $pK$  values. The data from which point e was determined were consistent for the ionic strength used in the study. The data obtained in the present study (Fig. 2a) show  $R$  to vary regularly with  $\mu$ . This  $R$  trend is not apparent from the results of earlier studies. A CYS microspecies distribution plot is given in Fig. 3b for the case of  $\mu = 0.24$  ( $R = 0.96$ ).

Elson and Edsall,<sup>3</sup> using a procedure based on Raman spectroscopy, followed proton ionization from the  $-SH$  group of CYS as a function of pH and from these data calculated  $pK'_{12}$  and  $pK'_{132}$  values. The  $pK'_{12}$  value (8.50) calculated by these workers at  $\mu \sim 1$  is in excellent agreement with that determined in the present study at  $\mu = 1.0$  (Fig. 2b). The reason for the poor agreement of our  $pK'_{132}$  value with their  $pK'_{132}$  value (10.00) is not apparent although it is probably in part the result of the much larger  $\mu$ -value which would exist in their solutions in the pH range in which this constant was measured.

In the present study the effort has been made to be self-consistent in the use of  $pQ$  and  $pK$  values and to obtain data as a function of  $\mu$ . A definite dependence

(10) The thermometric titration curve for proton dissociation from protonated adenosine diphosphate provides such an example.<sup>7a</sup>

on  $\mu$  is observed for the  $pQ$  and  $pK'$  values. In the case of the  $pK_{13}'$  value (Fig. 2b) this dependence is similar to that observed previously for proton ionization from the  $-\text{NH}_3^+$  group of alanine.<sup>11</sup>

Gorin and Clary<sup>2</sup> measured the concentration change with  $\mu$  of the ionized sulfhydryl proton of CYS. They observed that the concentration of this species changed very little as a function of  $\mu$  except at low  $\mu$ -values, and concluded that  $R$  was likewise invariant with  $\mu$ . As seen in Fig. 2a, our results show that  $R$  does change appreciably with  $\mu$ . However, the results of Gorin and Clary can be understood by reference to the data in Fig. 2b. Gorin and Clary followed the  $pK_{12}'$  ionization and it is seen that over the  $\mu$  range used in their study ( $\mu^{0.5} \sim 0.03$  to  $\mu^{0.5} = 2.5$ ) there is little change in  $pK_{12}'$  except at low  $\mu$ -values. On this point our results and theirs agree. However,  $R$  depends upon the relative magnitudes of  $pK_{12}'$  and  $pK_{13}'$  and not upon that of  $pK_{12}'$  alone. It is seen that  $pK_{13}'$  has a considerably larger variation with  $\mu$  than has  $pK_{12}'$ . Thus,  $R$  is seen to have a considerable  $\mu$  variation. Furthermore, the data in Fig. 2c show both  $pK_{132}'$  and  $pK_{123}'$  to have much greater  $\mu$  dependencies than the corresponding dissociations in the lower pH region.

The  $\Delta S$  values reported in Table II for proton dissociation from MAA and SMC are of the expected magnitudes considering the ionic charges involved. The relative effects on  $pQ$  of the  $-T\Delta S$  and  $\Delta H$  changes between  $\mu = 0$  and  $\mu = 1$  are shown in Table III.

(11) A. C. Batchelder and C. L. A. Schmidt, *J. Phys. Chem.*, **44**, 880 (1940).

TABLE III

THE EFFECT OF CHANGING  $\mu$  ON  $T\Delta S$ ,  $\Delta H$ , AND  $pQ^a$ 

Reaction	$\frac{\partial(-T\Delta S)}{\partial\mu}\bigg _{\mu=0}$ $\bigg _{\mu=1}$	$\frac{\partial(\Delta H)}{\partial\mu}\bigg _{\mu=0}$ $\bigg _{\mu=1}$	$\frac{2.3RT\partial(pQ)}{\partial\mu}\bigg _{\mu=0}$ $\bigg _{\mu=1}$
H <sub>2</sub> CYS <sup>-</sup> (I) =			
(II, III) + H <sup>+</sup>	0	-0.44	-0.44
(II, III) = CYS <sup>-2</sup>			
(IV) + H <sup>+</sup>	-1.8	+0.72	-1.1
HMAA <sup>-</sup> =			
MAA <sup>-2</sup> + H <sup>+</sup>	-1.2	0	-1.2
HSMC <sup>+·-</sup> =			
SMC <sup>-</sup> + H <sup>+</sup>	-0.66	+0.22	-0.44

<sup>a</sup> Data in kcal./mole and taken from Tables I and II and Fig. 1; temperature = 25°.

The change in  $T\Delta S$  is seen to be the determining factor in the observed decrease of  $pQ$  with increasing  $\mu$  in all cases except the first. The data in Table III also show the large decrease of the  $pK_{123}'$  and  $pK_{132}'$  values compared to that of the  $pK_{12}'$  and  $pK_{13}'$  values with increasing  $\mu$  (Fig. 2c) to be the result of the much larger  $T\Delta S$  change with changing  $\mu$  upon proton ionization from the II, III species.

The method described here for microconstant determinations is more accurate than other reported methods when  $R$  is at or near unity and the heats of proton dissociation of the groups involved vary considerably.

**Acknowledgments.**—The authors gratefully acknowledge the valuable assistance in this study of Mr. Lee Hansen, Mr. Blaine Blad, and Mr. Nolan Edmunds.

[CONTRIBUTION OF THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF SOUTH CAROLINA, COLUMBIA, SOUTH CAROLINA]

## Ion-Solvent Interaction. The Interactions of Some Amines with Tertiary Ammonium Salts in Low Dielectric Solvents

BY EARL K. RALPH, III,<sup>1</sup> AND W. R. GILKERSON

RECEIVED MARCH 23, 1964

The conductances of solutions of tertiary ammonium salts have been studied at 25° in *o*-dichlorobenzene and chlorobenzene as a function of the concentration of added amines, such as pyridine and tri-*n*-butylamine. The results are consistent with the formation of a 1:1 cation-amine complex. The following order of increasing complex stability with tri-*n*-butylammonium ion in *o*-dichlorobenzene is observed: 4-cyanopyridine < acetonitrile < tri-*n*-butylamine < pyridine < 4-methylpyridine. The effect of solvent on the cation-amine complex equilibrium can be accounted for by postulating that the amine displaces one tightly bound solvent molecule in forming the complex.

Specific interaction between an electrophile and an anion or between a nucleophile and a cation, in solution, may be regarded<sup>2</sup> as a displacement of a solvating solvent molecule from the ion by the incoming electrophile or nucleophile. It would be of interest to study such interactions as a function of solvent, structure of anion and cation, and basicity (or acidity) and structure of the molecule displacing the solvent. It is hoped that some of the factors of importance in ion-solvent interaction may be elucidated in this manner. Fuoss and co-workers<sup>2,3</sup> are actively engaged in studying anion interactions with solvents and added electrophiles. We report here the results of a study of the conduct-

ances of several amine salt-nucleophile systems in the solvents *o*-dichlorobenzene (ODCB) and chlorobenzene (CB) at 25°. It was hoped that the results could be interpreted in terms of cation-nucleophile interaction. Incidental to the foregoing study, the conductances of tri-*n*-butylammonium bromide and iodide were studied in ethylene chloride (EC). Increasing conductivity with age of solutions of amines in this solvent, indicative of amine-solvent reaction, precluded studies of salt-amine systems in this solvent.

### Experimental

**Materials.**—ODCB (Allied Chemical Co., Solvay Process Division) was passed through alumina (Alcoa grade F-20) on a 30 × 2 cm. column and distilled on a 35 cm. column packed with glass helices. The middle fraction, b.p. 48° (5 mm.), was stored over alumina which had been fired to approximately 800° for 1 hr.

(1) Guy F. Lipscomb Fellow, 1962-1963.

(2) A. D'Aprano and R. M. Fuoss, *J. Phys. Chem.*, **67**, 1722 (1963).

(3) For recent leading references, see M. A. Coplan and R. M. Fuoss, *ibid.*, **68**, 1181 (1964).