

deuteration of the carboxyl group. This would suggest that it must correspond to a vibrational mode that does not involve the hydrogen atoms in any significant way, and the proposal of Tsuboi, *et al.*,<sup>4</sup> might well be correct.

Since this paper was submitted we have seen the important paper of Suzuki, Shimanouchi, and Tsuboi,<sup>17</sup> who studied the infrared spectra of crystals of  $^+H_3NCH_2COO^-$ ,  $^+D_3NCH_2COO^-$ , and  $^+D_3NCD_2COO^-$ . They studied these molecules only in the isoelectric form and were not concerned, as we are here, with the effects of ionization. Their data and ours are generally in good agreement, especially if one allows for the fact that they studied the infrared spectra of crystals, whereas we have studied the Raman spectra of solutions. There is one rather strong vibrational frequency in the infrared spectra, which lies between 330 and 360  $cm^{-1}$ , to which we have observed nothing corresponding in the Raman spectra. Suzuki, *et al.*,<sup>17</sup> assign this to a CCN bending motion. They have calculated the frequencies for a seven-body model of isoelectric glycine with  $C_s$  symmetry, assuming a Urey-Bradley type of force field. The  $-NH_3^+$  group was taken as a single atom in the model. Their assignments of observed frequencies to modes of vibration are much

(17) S. Suzuki, T. Shimanouchi, and M. Tsuboi, *Spectrochim. Acta*, **19**, 1195 (1963).

more detailed than ours; in general, our more limited attempts at such assignments are in harmony with theirs. They conclude that, in the compounds containing  $-CD_2-$  groups, the skeletal stretching vibrations couple heavily with the  $CD_2$  scissoring and wagging modes. Thus no one of the vibrations in the 800–1200  $cm^{-1}$  region can be uniquely assigned to a  $CD_2$  scissoring motion, a conclusion which gives a rational basis for our failure to identify such a vibrational mode in the Raman spectra.

Our studies on the effects of ionization do lead us to question their assignment of the 1334  $cm^{-1}$  frequency in  $^+H_3NCH_2COO^-$  to a  $CH_2$  wagging motion. In the Raman spectrum of the cation  $^+H_3NCH_2COOH$  this frequency lies at 1308 (Table I). It is relatively weak, with  $\rho = 0.63$ . In the dipolar ion the observed Raman band is at 1327; it is very intense, and  $\rho$  is only 0.27. This suggests that two different modes of vibration are involved, and that the one which is most intense and characteristic in the dipolar ion is somehow associated with the ionized carboxyl group. Apart from this one point, our conclusions appear to be almost entirely in harmony with those of Suzuki, *et al.*, insofar as the two investigations overlap.

**Acknowledgments.**—We are greatly indebted to Mrs. Turid Alsos Eskelund for extremely capable assistance in much of the work described in this paper.

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, CORNELL UNIVERSITY, ITHACA, NEW YORK]

## The Influence of Hydrogen and Hydrophobic Bonds on the Stability of the Carboxylic Acid Dimers in Aqueous Solution<sup>1</sup>

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The temperature dependence of the molality quotient of dimerization for formic acid has been determined in a 3 *m* NaCl ionic medium by potentiometric titrations at 10, 25, 40, and 55°. The enthalpy of dimerization,  $\Delta H_D = 0 \pm 1$  kcal./mole, is considered to be equivalent to  $\Delta H_D^\circ$ , giving the strength of a typical hydrogen bond between polar groups in water solution. If it is assumed that cyclization is not a mechanism for the increasing stabilization of the carboxylic acid dimers with chain length, the increase of the dimerization constant may be attributed to the formation of pairwise hydrophobic bonds between the carboxylic acid side chains. Experimental values of the standard free energy of formation for methyl-methyl, ethyl-ethyl, propyl-propyl, and benzyl-benzyl hydrophobic bonds are in quantitative agreement with the values for the same side-chain interactions calculated from the theory of Némethy and Scheraga.<sup>3</sup>

### Introduction

The contribution of hydrophobic bonds to the stability of protein conformations in aqueous solution has recently been considered by several investigators. Némethy and Scheraga<sup>3</sup> developed a theoretical treatment of these interactions which predicts, among other things, the thermodynamic parameters for pairwise hydrophobic bond formation between nonpolar side chains. In order to verify and extend the conclusions of this theory, experimental results are required on an adequate model system. Dimers of small hydrocarbon molecules in aqueous solution would be the most satisfactory model but such dimers will not form because of the low solubility of hydrocarbons in aqueous

solution. This necessitates the use of model compounds containing polar groups.

The carboxylic acids represent a useful series of such model compounds. It has been shown by several groups of investigators<sup>4–8</sup> that the carboxylic acids dimerize in aqueous solution. Rossotti and co-workers<sup>8</sup> have carried out the most extensive series of measurements and have shown that, while higher oligomers form in solutions of the acids after acetic, dimerization is the major process occurring over most of the low concentration range for all the acids investigated.

(4) A. Katchalsky, H. Eisenberg, and S. Lifson, *J. Am. Chem. Soc.*, **73**, 5889 (1951).

(5) (a) M. Davies and D. M. L. Griffiths, *Z. physik. Chem. (Frankfurt)*, **2**, 353 (1954); (b) *ibid.*, **6**, 143 (1956).

(6) D. R. Cartwright and C. B. Monk, *J. Chem. Soc.*, 2500 (1955).

(7) G. R. Nash and C. B. Monk, *ibid.*, 4274 (1957).

(8) (a) D. L. Martin and F. J. C. Rossotti, *Proc. Chem. Soc.*, 60 (1959); (b) *ibid.*, 73 (1961); (c) K. Schlyter and D. L. Martin, *Kgl. Tek. Hogskol. Handl.*, No. 175 (1961); (d) J. D. E. Carson and F. J. C. Rossotti, in "Advances in the Chemistry of the Coordination Compounds." The Macmillan Co., New York, N. Y., 1961, p. 180.

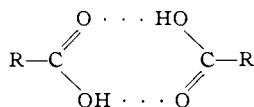
(1) This work was supported by a research grant (AI-01473) from the National Institute of Allergy and Infectious Diseases of the National Institutes of Health, U. S. Public Health Service, and by a research grant (GB-75) from the National Science Foundation.

(2) National Institutes of Health Postdoctoral Fellow, 1962–1963.

(3) G. Némethy and H. A. Scheraga, *J. Phys. Chem.*, **66**, 1773 (1962).

All of these investigations are open to criticism on various experimental or theoretical grounds. Most recently, the work of Rossotti, *et al.*,<sup>8</sup> has been attacked by Danielsson and Suominen,<sup>9</sup> who showed that changes in the nature of the ionic medium in which the potentiometric titrations were carried out led to most of the deviations in the titration curves which Rossotti, *et al.*,<sup>8</sup> had interpreted as being due to polynuclear complex formation. In a re-analysis of the data, Farrer and Rossotti<sup>10</sup> have shown that, even with these objections, a molarity quotient for the dimerization of acetic acid molecules in a 3 *M* Na(ClO<sub>4</sub>) medium can be obtained which is in fair accord with what had been originally presented by Rossotti, *et al.*<sup>8</sup> Presumably, the molarity quotients for the other acids show similar small deviations from their original values.

Even with the objections which can be raised, there appears to be order-of-magnitude agreement between the results of the various investigators. What is much more important is that all the data of the different investigators show the same trend, *i.e.*, the dimerization constants increase as a function of the chain length of the carboxylic acid. Such a chain length dependence does not appear in the dimerization constants measured in nonaqueous solvents<sup>11</sup> where the dimers are assumed to be primarily cyclic, *i.e.*, of the form



However, if increasing tendency toward cyclization with increasing chain length were to occur in *aqueous* solution, to account for the increasing stability of the dimers, a large  $\Delta H^\circ$  for hydrogen bond formation would be required to overcome the loss of entropy on cyclization.

It will be shown that the temperature coefficient of the molality quotient of the dimerization of formic acid is zero, suggesting that the  $\Delta H^\circ$  for C=O $\cdots$ HO hydrogen bond formation is near zero in aqueous solution. It is assumed, on the basis of this result, that a greater tendency towards cyclization with increasing chain length is not responsible for increasing dimer stability. An alternative hypothesis, that of increased stabilization due to hydrophobic bonding, can be proposed; and it is shown here that this explanation appears to have quantitative validity because of the excellent agreement of standard free energies for hydrophobic bond formation obtained from the data of all investigators with the values calculated from the theory of Némethy and Scheraga.<sup>3</sup>

### Experimental

The determination of the dimerization constant of formic acid was carried out at 10, 25, 40, and 55° by the potentiometric method described by Rossotti and Rossotti.<sup>12</sup> A glass electrode was employed in conjunction with a calomel reference electrode. The salt used to provide a constant ionic medium was NaCl. Some experimental details pertinent to this investigation are described below.

(9) I. Danielsson and T. Suominen, *Acta Chem. Scand.*, **17**, 979 (1963).

(10) H. N. Farrer and F. J. C. Rossotti, *ibid.*, in press.

(11) J. Wenograd and R. A. Spurr, *J. Am. Chem. Soc.*, **79**, 5844 (1957).

(12) F. J. C. Rossotti and H. Rossotti, "The Determination of Stability Constants," McGraw-Hill Book Co., New York, N. Y., 1961.

**Description of Measuring Instrument.**—The instrument used for the potentiometric titrations was designed and built in this laboratory by Gary Davenport.<sup>13</sup> The measuring circuit consisted of a Fluke precision decade potentiometer, Model 60A, supplied with 1 v. from a Zener diode-controlled power supply which was standardized against a calibrated standard cell, a null indicator consisting of a Radiometer vibrator, a storage capacitor, a tuned a.c. amplifier, and an oscilloscope.

The difference between the outputs of the potentiometer and the electrode system forms a null voltage source of very high impedance. This is switched across the capacitor by the vibrator, and the capacitor is allowed to charge for 10 msec.<sup>14</sup> The charged capacitor is then switched across the relatively low input impedance of the amplifier and allowed to discharge, forming a pulse of about 10- $\mu$ sec. duration. This is amplified and viewed on the oscilloscope, the pulse generation being repeated at a 60-cycle repetition rate. The impedance of the null source is thus lowered by about 1000 times to accommodate the lower impedance (500,000 ohms) of the amplifier.

The potentiometer is adjusted until no signal is discernible on the oscilloscope and the voltage across the electrodes is read in millivolts from the dial. The accuracy and resolution of the instrument alone, exclusive of electrode uncertainties, are estimated to be  $\pm 0.05$  mv. (0.001 pH) at 500 megohms electrode resistance.

**Electrodes and Electrode Calibration.**—A Beckman general purpose glass electrode (No. 40498) and a Radiometer calomel electrode were used in the measurements. These were calibrated before and after titration using buffers made up to give the molal ionization,  $K = (m_{\text{H}^+})(m_{\text{A}^-})/(m_{\text{HA}})$ , of acetic acid and of glycine, respectively, in a 3 *m* (*i.e.*, 3 molal) Na(Cl) medium. For this purpose, the data of Harned and Hickey<sup>15</sup> were used to provide values of the ionization for acetic acid at each temperature while those of King<sup>16</sup> were used for glycine. The buffers thus prepared were shown to be mutually consistent at every temperature except 55° within  $\pm 0.25$  mv., which is the experimental uncertainty in each value of the ionization in our experiments. At 55°, the experimental value of the ionization of acetic acid differed by 1 mv. from the value extrapolated from the data of Harned and Hickey<sup>15</sup> obtained at lower temperature and was used instead of the Harned-Hickey value.

**Method of Procedure.**—Reagent grade chemicals were used throughout this study. Solutions were made up by weight. All solutions were 3 *m* in sodium ion, the remainder of the ionic strength being made up by chloride or formate. Titrations were carried out on a weight basis and were done at each temperature at 12 different total formate concentrations from 0.01 to 1.0 *m*. A full titration curve at any one total formate concentration was a composite of two titration curves, one in which formic acid was titrated with sodium formate and the other, the reverse of this. Sufficient overlap existed in the middle region of the curve so that the reversibility of the titration curve could be established. Temperature was controlled to  $\pm 0.05^\circ$  and e.m.f. measurements were made with an over-all uncertainty of  $\pm 0.15$  mv. Preliminary data treatment and computations related to the construction of appropriate theoretical curves were carried out with the aid of a Control Data Corporation 1604 computer at the Cornell Computing Center.

### Results

The data resulting from the titration experiments were the weights of titrant added to known weights of sample, together with a measured e.m.f., for each weight of titrant added to a given sample. The e.m.f. for each point could be converted to  $-\log h$ , where  $h$  is the free hydrogen ion molality, by the use of the buffer values mentioned previously. The weight of titrant, the original sample weight, and the hydrogen ion molality were used to calculate the degree of formation,  $\bar{n}$

$$\bar{n} = \frac{(H) - h}{(F_m)_T} \quad (1)$$

(13) A circuit diagram and other details relating to the construction of this instrument may be obtained by writing to Mr. Davenport.

(14) J. I. Carasso and R. W. Pittman, *J. Chem. Soc.*, 1084 (1956).

(15) H. S. Harned and F. C. Hickey, *J. Am. Chem. Soc.*, **59**, 1284 (1937).

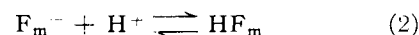
(16) E. J. King, *ibid.*, **67**, 2178 (1945).

TABLE I

| $\bar{n} (-\log h)$ FOR VARIOUS TOTAL FORMATE MOLALITIES, $(F_m)_T$ , AT 25 AND 40° |           |           |           |           |           |                                    |           |           |           |           |           |
|---|-----------|-----------|-----------|-----------|-----------|------------------------------------|-----------|-----------|-----------|-----------|-----------|
| $\bar{n}$   | $-\log h$ | $\bar{n}$ | $-\log h$ | $\bar{n}$ | $-\log h$ | $\bar{n}$                          | $-\log h$ | $\bar{n}$ | $-\log h$ | $\bar{n}$ | $-\log h$ |
| $T = 25.0^\circ (F_m)_T = 0.010 m$  |           |           |           |           |           | $T = 25.0^\circ (F_m)_T = 0.80 m$  |           |           |           |           |           |
| 0.053   | 4.870     | 0.436     | 3.794     | 0.545     | 3.607     | 0.044                              | 5.128     | 0.443     | 3.822     | 0.611     | 3.494     |
| .123  | 4.512     | .445      | 3.782     | .575      | 3.555     | .104                               | 4.717     | .465      | 3.778     | .631      | 3.452     |
| .162  | 4.365     | .464      | 3.746     | .604      | 3.500     | .155                               | 4.510     | .471      | 3.768     | .654      | 3.403     |
| .226  | 4.213     | .465      | 3.746     | .642      | 3.429     | .207                               | 4.349     | .488      | 3.736     | .692      | 3.322     |
| .274  | 4.098     | .487      | 3.708     | .678      | 3.360     | .246                               | 4.241     | .493      | 3.726     | .728      | 3.238     |
| .313  | 4.021     | .489      | 3.704     | .716      | 3.279     | .292                               | 4.134     | .514      | 3.684     | .772      | 3.126     |
| .350  | 3.948     | .508      | 3.672     | .757      | 3.189     | .325                               | 4.060     | .517      | 3.680     | .820      | 2.988     |
| .377  | 3.899     | .512      | 3.664     | .795      | 3.091     | .354                               | 3.999     | .539      | 3.637     | .874      | 2.793     |
| .400  | 3.858     | .528      | 3.636     | .832      | 2.985     | .381                               | 3.944     | .549      | 3.617     | .931      | 2.493     |
| .409  | 3.845     | .542      | 3.613     | .865      | 2.868     | .405                               | 3.897     | .558      | 3.601     | .982      | 1.841     |
| .428  | 3.812     |           |           |           |           | .425                               | 3.858     | .583      | 3.549     |           |           |
|   |           |           |           |           |           | .440                               | 3.827     |           |           |           |           |
| $T = 25.0^\circ (F_m)_T = 0.050 m$  |           |           |           |           |           | $T = 25.0^\circ (F_m)_T = 1.00 m$  |           |           |           |           |           |
| 0.047   | 5.001     | 0.428     | 3.812     | 0.589     | 3.529     | 0.050                              | 5.108     | 0.441     | 3.838     | 0.577     | 3.568     |
| .099  | 4.651     | .449      | 3.774     | .618      | 3.476     | .110                               | 4.724     | .462      | 3.796     | .604      | 3.513     |
| .145  | 4.461     | .458      | 3.762     | .641      | 3.432     | .174                               | 4.478     | .464      | 3.797     | .627      | 3.465     |
| .188  | 4.328     | .471      | 3.736     | .670      | 3.376     | .227                               | 4.323     | .486      | 3.750     | .656      | 3.403     |
| .220  | 4.239     | .475      | 3.731     | .705      | 3.306     | .263                               | 4.228     | .491      | 3.743     | .692      | 3.324     |
| .257  | 4.151     | .494      | 3.696     | .742      | 3.228     | .299                               | 4.143     | .508      | 3.705     | .729      | 3.235     |
| .290  | 4.079     | .502      | 3.682     | .777      | 3.140     | .343                               | 4.047     | .514      | 3.696     | .771      | 3.128     |
| .320  | 4.015     | .514      | 3.661     | .824      | 3.011     | .377                               | 3.972     | .535      | 3.656     | .825      | 2.967     |
| .347  | 3.963     | .519      | 3.652     | .868      | 2.865     | .403                               | 3.919     | .539      | 3.645     | .882      | 2.756     |
| .375  | 3.910     | .543      | 3.611     | .904      | 2.704     | .424                               | 3.874     | .551      | 3.624     | .944      | 2.385     |
| .402  | 3.858     | .568      | 3.567     | .937      | 2.502     | .434                               | 3.857     |           |           | .984      | 1.787     |
| $T = 25.0^\circ (F_m)_T = 0.10 m$   |           |           |           |           |           | $T = 40.0^\circ (F_m)_T = 0.010 m$ |           |           |           |           |           |
| 0.062   | 4.885     | 0.461     | 3.756     | 0.627     | 3.462     | 0.040                              | 5.076     | 0.480     | 3.699     | 0.623     | 3.446     |
| .117  | 4.577     | .466      | 3.748     | .662      | 3.396     | .093                               | 4.670     | .483      | 3.694     | .659      | 3.379     |
| .174  | 4.374     | .491      | 3.706     | .691      | 3.338     | .166                               | 4.375     | .499      | 3.667     | .690      | 3.315     |
| .215  | 4.258     | .498      | 3.693     | .721      | 3.272     | .234                               | 4.186     | .509      | 3.649     | .719      | 3.256     |
| .258  | 4.155     | .514      | 3.664     | .755      | 3.199     | .287                               | 4.063     | .515      | 3.639     | .750      | 3.186     |
| .294  | 4.075     | .520      | 3.654     | .786      | 3.120     | .326                               | 3.981     | .532      | 3.607     | .791      | 3.084     |
| .324  | 4.014     | .531      | 3.635     | .828      | 3.001     | .370                               | 3.897     | .539      | 3.596     | .820      | 3.005     |
| .356  | 3.951     | .536      | 3.625     | .870      | 2.858     | .396                               | 3.849     | .554      | 3.569     | .842      | 2.940     |
| .389  | 3.887     | .546      | 3.607     | .917      | 2.639     | .423                               | 3.800     | .565      | 3.549     | .864      | 2.865     |
| .420  | 3.831     | .571      | 3.564     | .955      | 2.350     | .452                               | 3.747     | .590      | 3.505     |           |           |
| .445  | 3.786     | .598      | 3.514     |           |           |                                    |           |           |           |           |           |
| $T = 25.0^\circ (F_m)_T = 0.50 m$   |           |           |           |           |           | $T = 40.0^\circ (F_m)_T = 0.050 m$ |           |           |           |           |           |
| 0.065   | 4.915     | 0.431     | 3.832     | 0.582     | 3.549     | 0.034                              | 5.131     | 0.459     | 3.733     | 0.625     | 3.435     |
| .116  | 4.633     | .448      | 3.801     | .607      | 3.497     | .085                               | 4.706     | .474      | 3.707     | .665      | 3.361     |
| .168  | 4.439     | .456      | 3.783     | .634      | 3.450     | .126                               | 4.512     | .475      | 3.707     | .714      | 3.259     |
| .219  | 4.290     | .469      | 3.760     | .670      | 3.376     | .167                               | 4.370     | .503      | 3.654     | .758      | 3.159     |
| .251  | 4.208     | .485      | 3.732     | .699      | 3.311     | .219                               | 4.221     | .504      | 3.653     | .787      | 3.086     |
| .292  | 4.115     | .493      | 3.717     | .737      | 3.226     | .252                               | 4.140     | .530      | 3.610     | .831      | 2.961     |
| .323  | 4.047     | .508      | 3.688     | .773      | 3.138     | .298                               | 4.038     | .532      | 3.602     | .863      | 2.853     |
| .354  | 3.983     | .518      | 3.670     | .818      | 3.009     | .346                               | 3.941     | .552      | 3.567     | .892      | 2.733     |
| .379  | 3.932     | .531      | 3.643     | .874      | 2.777     | .373                               | 3.888     | .557      | 3.561     | .917      | 2.602     |
| .404  | 3.883     | .546      | 3.617     | .915      | 2.537     | .408                               | 3.826     | .570      | 3.534     | .935      | 2.490     |
| .430  | 3.834     | .549      | 3.610     |           |           | .447                               | 3.753     | .591      | 3.500     |           |           |

for each experimental point. Here  $(H)$  is the total molal concentration of all hydrogen ions, free and combined, and  $(F_m)_T$  that of all formate, as  $HF_m$  and  $F_m^-$ . These data,  $\bar{n}$  and  $-\log h$ , are given for a number of total formate concentrations at 25 and 40° in Table I. The data given represent about one-fourth of the total data taken in this investigation. If each set of data for a particular total formate concentration above 0.05  $m$  is plotted as  $\bar{n}$  vs.  $-\log h$ , these plots deviate in a regular fashion from that<sup>17</sup> of the same function for formic acid at concentrations less than 0.05  $m$  where the state of the acid in the system can be assumed to be completely described by the single equilibrium shown in eq. 2.

(17) Ref. 12, p. 87.



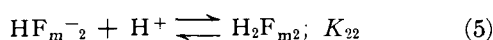
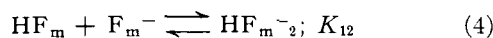
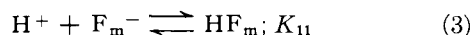
In the very low concentration region, therefore,  $\bar{n}(-\log h)$  is independent of  $(F_m)_T$ . At molalities greater than 0.05  $m$   $(F_m)_T$ , dependence of  $\bar{n}(-\log h)$  on  $(F_m)_T$  is assumed to indicate, among other things, that polynuclear association occurs. As mentioned above, medium effects have been shown to be significant here as well.<sup>9,10</sup> They will not be considered in the present treatment. It has been shown<sup>10</sup> that the value of the association constant of interest,  $K_D$ , is only slightly changed in the case of the closely similar acetic acid system when medium effects are taken into account.

The dependence of  $\bar{n}(-\log h)$  on  $(F_m)_T$  and the fact that an isohydric point,  $\bar{n}^*$ , i.e., a point where all curves  $\bar{n}(-\log h)$  intersect, exists in this system,

TABLE I (continued)

| $\bar{n}$                                       | $-\log h$ | $\bar{n}$ | $-\log h$ | $\bar{n}$ | $-\log h$ |
|---|-----------|-----------|-----------|-----------|-----------|
| $T = 40.0^\circ \quad (F_m)_T = 0.10 \text{ m}$ |           |           |           |           |           |
| 0.038   | 5.084     | 0.432     | 3.780     | 0.587     | 3.504     |
| .081  | 4.734     | .452      | 3.745     | .629      | 3.427     |
| .113  | 4.752     | .456      | 3.739     | .670      | 3.346     |
| .153  | 4.417     | .475      | 3.705     | .716      | 3.250     |
| .201  | 4.270     | .489      | 3.680     | .767      | 3.132     |
| .247  | 4.156     | .498      | 3.664     | .815      | 3.003     |
| .290  | 4.057     | .515      | 3.634     | .879      | 2.784     |
| .326  | 3.981     | .525      | 3.615     | .921      | 2.572     |
| .360  | 3.916     | .538      | 3.592     | .943      | 2.421     |
| .393  | 3.854     | .556      | 3.561     | .953      | 2.328     |
| .426  | 3.792     | .558      | 3.556     |           |           |
| $T = 40.0^\circ \quad (F_m)_T = 0.30 \text{ m}$ |           |           |           |           |           |
| 0.033   | 5.184     | 0.460     | 3.743     | 0.602     | 3.481     |
| .079  | 4.777     | .461      | 3.742     | .639      | 3.410     |
| .125  | 4.551     | .481      | 3.704     | .679      | 3.332     |
| .178  | 4.363     | .489      | 3.687     | .723      | 3.235     |
| .240  | 4.196     | .505      | 3.661     | .780      | 3.096     |
| .294  | 4.069     | .533      | 3.610     | .846      | 2.899     |
| .344  | 3.964     | .536      | 3.606     | .909      | 2.632     |
| .390  | 3.874     | .561      | 3.560     | .948      | 2.361     |
| .424  | 3.811     | .564      | 3.553     | .972      | 2.074     |
| .436  | 3.786     |           |           |           |           |
| $T = 40.0^\circ \quad (F_m)_T = 0.50 \text{ m}$ |           |           |           |           |           |
| 0.042   | 5.098     | 0.445     | 3.779     | 0.600     | 3.487     |
| .103  | 4.664     | .445      | 3.778     | .633      | 3.423     |
| .170  | 4.406     | .467      | 3.738     | .665      | 3.359     |
| .232  | 4.229     | .480      | 3.712     | .696      | 3.291     |
| .274  | 4.128     | .504      | 3.671     | .751      | 3.163     |
| .318  | 4.030     | .505      | 3.666     | .814      | 2.993     |
| .346  | 3.971     | .527      | 3.625     | .890      | 2.715     |
| .387  | 3.889     | .529      | 3.623     | .951      | 2.327     |
| .415  | 3.835     | .553      | 3.576     | .978      | 1.951     |
| .424  | 3.818     | .560      | 3.564     |           |           |
| $T = 40.0^\circ \quad (F_m)_T = 0.70 \text{ m}$ |           |           |           |           |           |
| 0.023   | 5.392     | 0.449     | 3.784     | 0.597     | 3.499     |
| .058  | 4.972     | .461      | 3.760     | .638      | 3.416     |
| .123  | 4.605     | .468      | 3.747     | .681      | 3.325     |
| .204  | 4.330     | .489      | 3.709     | .736      | 3.201     |
| .261  | 4.180     | .493      | 3.701     | .786      | 3.070     |
| .306  | 4.075     | .513      | 3.660     | .857      | 2.846     |
| .349  | 3.982     | .521      | 3.646     | .936      | 2.441     |
| .398  | 3.882     | .536      | 3.616     | .970      | 2.088     |
| .427  | 3.825     | .550      | 3.591     | .981      | 1.869     |
| .429  | 3.826     | .569      | 3.553     |           |           |
| $T = 40.0^\circ \quad (F_m)_T = 0.90 \text{ m}$ |           |           |           |           |           |
| 0.018   | 5.549     | 0.425     | 3.841     | 0.567     | 3.562     |
| .063  | 4.963     | .439      | 3.812     | .605      | 3.487     |
| .099  | 4.739     | .442      | 3.806     | .644      | 3.404     |
| .168  | 4.461     | .470      | 3.753     | .692      | 3.301     |
| .230  | 4.277     | .471      | 3.751     | .738      | 3.192     |
| .289  | 4.131     | .499      | 3.699     | .815      | 2.979     |
| .338  | 4.021     | .500      | 3.693     | .906      | 2.621     |
| .367  | 3.960     | .526      | 3.643     | .953      | 2.282     |
| .401  | 3.889     | .564      | 3.569     |           |           |

may be used to compute the stability constants for the various species assumed to be present.<sup>12</sup> Let us consider the sequence of stepwise equilibria,<sup>18</sup> assumed to be operative in this system



(18) The treatment presented here follows closely that given in ref. 8d.

Material balance requires that

$$(\text{F}_m)_T \bar{n} = [\text{HF}_m] + [\text{HF}_m^-] + 2[\text{H}_2\text{F}_m] = K_{11} h f_m + K_{11} K_{12} h f_m^2 + 2K_{11} K_{12} K_{22} h^2 f_m^2 \quad (6)$$

and

$$(\text{F}_m)_T = f_m + [\text{HF}_m] + 2[\text{HF}_m^-] + 2[\text{H}_2\text{F}_m] = f_m + K_{11} h f_m + 2K_{11} K_{12} h f_m^2 + 2K_{11} K_{12} K_{22} h^2 f_m^2 \quad (7)$$

where  $f_m$  is the concentration of free formate ions. A transformation of the data,  $\bar{n} (\log h)_{(\text{F}_m)_T}$ , to the form  $\log (\text{F}_m)_T (\log h)_{\bar{n}}$  and comparison with the normalized curves,<sup>19</sup>  $\log (\text{F}_m)_T (\log h)_{\bar{n},R}$ , provide a method of confirming that the experimental data conform to the mass balance eq. 6 and 7 and allow the three stability constants to be determined. The normalized variables are defined as

$$\log (\text{F}_m)_T = \log (\text{F}_m)_T + \log K_{12} \quad (8)$$

$$\log h = \log h + \log K_{11} \quad (9)$$

and the parameter  $R$  is defined by

$$\log R = \log K_{22} - \log K_{11} \quad (10)$$

The normalized curves were calculated using the relationship

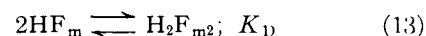
$$(\text{F}_m)_T = \frac{[h - \bar{n}(1 + h)][(1 - 2R)h - 1]}{h[2\bar{n}(1 + Rh) - (1 + 2Rh)]^2} \quad (11)$$

which is obtained by manipulation of eq. 6-10. The value of  $R$  was calculated from the relationship

$$R = \frac{2\bar{n}^* - 1}{2\bar{n}^*} \quad (12)$$

where  $\bar{n}^*$  is the value of  $\bar{n}$  at the isohydric point. The value of  $\bar{n}^*$  at a given temperature was obtained by averaging a set of preliminary  $\bar{n}^*$  values, compiled by reading all the curves,  $\bar{n} (-\log h)_{(\text{F}_m)_T}$ , in pairs. After a value of  $R$  was obtained from eq. 12, the appropriate normalized curve could be generated from eq. 11.

The fit of the experimental data at all temperatures to the normalized curves supported the hypothetical equilibria, eq. 3-5, and allowed calculation of the relevant stability constants. The constant,  $K_D$ , for the process



could be calculated from  $K_D = RK_{12}$ . The set of stability constants resulting from this treatment is given in Table II. These results were obtained before the publication of the paper by Danielsson and Suominen<sup>9</sup>; by analogy with Farrer and Rossotti's<sup>10</sup> recalculation of their data for acetic acid, the values of  $\log K_{11}$  given here do not require correction while those for  $\log K_D$  should be more negative, perhaps by 0.2 at each temperature.  $\log K_{22}$  should equal  $\log K_{11}$  and  $\log K_{12}$  is approximately equal to  $\log K_D$  if it is assumed that the data here can be revised along the lines of the changes made for acetic acid by Farrer and Rossotti.<sup>10</sup>

(19) Ref. 12, p. 372, *et seq.*

TABLE II  
MOLALITY QUOTIENTS FOR PROTON-FORMATE ASSOCIATION IN A 3 *m* SODIUM (CHLORIDE) IONIC MEDIUM IN THE TEMPERATURE RANGE 10–55°

| <i>T</i> , °C. | log <i>K</i> <sub>11</sub> | log <i>K</i> <sub>12</sub> | log <i>K</i> <sub>21</sub> | log <i>K</i> <sub>D</sub> | log <i>R</i> |
|----------------|----------------------------|----------------------------|----------------------------|---------------------------|--------------|
| 10.0           | 3.743 ± 0.005              | -0.35 ± 0.04               | 3.07 ± 0.12                | -1.02 ± 0.15              | -0.67 ± 0.11 |
| 25.0           | 3.685 ± .005               | - .32 ± .04                | 3.04 ± .07                 | -0.96 ± .10               | - .64 ± .06  |
| 40.0           | 3.656 ± .005               | - .30 ± .02                | 3.03 ± .08                 | -0.93 ± .09               | - .63 ± .07  |
| 55.0           | 3.652 ± .005               | - .36 ± .04                | 2.98 ± .10                 | -1.03 ± .13               | - .67 ± .09  |

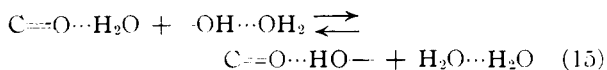
The difference between the value obtained here at 25° for  $-\log K_D$ , 1.00 on a *molarity* scale, as compared with Martin and Rossotti's<sup>8</sup> value of 1.24, may be explained by differences in the ionic medium used in each case, NaCl as against NaClO<sub>4</sub>. The thermodynamic stability constants  $-\log {}^T K_D$ , may be calculated from

$$-\log {}^T K_D = -\log K_D - \log f_{H_2A_2} + 2 \log f_{HA} \quad (14)$$

where  $f_{HA}$  and  $f_{H_2A_2}$  are the activity coefficients of the monomer and dimer, respectively, in the salt solution. Calculation of these activity coefficients based on data available in the literature, together with an appropriate interpolation formula, *i.e.*, the Long-McDevit equation,<sup>20</sup> enabled us to calculate  $-\log {}^T K_D$ . The value of  $\log f_{H_2A_2}$  was approximated by using the value of the activity coefficient of the appropriate dicarboxylic acid (oxalic acid, in this case) in the salt solution. The value of  $-\log {}^T K_D$  for formic acid dimerization turned out to be 1.54 from this work and 1.40 from the result of Martin and Rossotti.<sup>8</sup> The relative agreement of these values is good and the agreement with the other values<sup>4,6</sup> for this quantity is fair (see Table II). As mentioned previously, recalculation based on the Farrer-Rossotti scheme<sup>10</sup> would make the absolute agreement of these values with those of Cartwright and Monk<sup>6</sup> even better.

The temperature coefficient of  $\log K_D$ , *i.e.*,  $\Delta H$  of dimerization of formic acid, appears to be equal to  $0 \pm 1$  kcal./mole within experimental error. The fact that the experimental points obtained in this work follow the shape of the theoretical curves of Martin and Rossotti<sup>8</sup> closely at each temperature indicates that a recalculation of the results by Farrer and Rossotti's method<sup>10</sup> would yield substantially the same temperature coefficient.

The relationship between this  $\Delta H$  and the standard enthalpy of formation of the hydrogen bond in water ( $\Delta H_D^\circ$ ) deserves some comment. We assume that the temperature variation of the activity coefficient of the uncharged species appearing in eq. 13 is negligible. Such an assumption is in keeping with the results of Morrison<sup>21</sup> on the salting-out of phenylacetic and benzoic acids by NaCl over an extensive temperature range. We conclude, therefore that  $\Delta H_D$  obtained in a 3 *m* Na(Cl) medium is equivalent to  $\Delta H_D^\circ$ . An intuitive argument supports this contention. While it is true that the enthalpies of formation of bonds such as  $C=O \cdots H_2O$  and  $-OH \cdots OH_2$  may be different at high salt concentrations from their values in water, the reaction to form the hydrogen bond of any dimer



(20) W. F. McDevit and F. A. Long, *J. Am. Chem. Soc.*, **74**, 1773 (1952).

(21) T. J. Morrison, *Trans. Faraday Soc.*, **40**, 43 (1944).

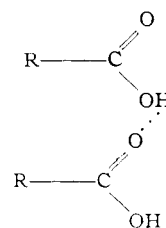
is a transfer reaction involving the formation of one set of hydrogen bonds with the concomitant destruction of another set. If this concept of hydrogen bond formation as a transfer reaction is not drastically modified by the presence of salt, it is reasonable to identify this enthalpy change with a standard enthalpy of formation.

A value of  $\Delta H_D^\circ$  near zero for formic acid dimerization is supported by the similar results of Martin and Rossotti<sup>8b</sup> and of Nash and Monk<sup>7</sup> for the other carboxylic acids. Martin and Rossotti<sup>8b</sup> obtained a value of  $\Delta H_D = +0.4$  kcal./mole from calorimetric measurements in the acetic acid system while the temperature coefficients for the dimerization of acetic, propionic, and butyric acids obtained by Nash and Monk<sup>7</sup> lead to values for  $\Delta H_D^\circ = 0 \pm 1$  kcal./mole. Small positive contributions to  $\Delta H_D^\circ$  from hydrophobic bonding could tend to cancel a negative  $\Delta H^\circ$  from hydrogen bond formation in these higher acids but the magnitudes of the  $\Delta H^\circ$  (hydrogen bond) which could be cancelled in this manner are probably less than  $-1.5$  kcal./mole.<sup>3</sup>

## Discussion

If it is assumed, as is suggested by the above results, that cyclization is not a factor in the increasing stability of these dimers with chain length, an alternative explanation may be attempted. Table III presents a compilation of all the available data and indicates the striking trend which we will attempt to account for here. Columns 6 and 7 contain values of  $-\log {}^T K_D$  which have been obtained from the original  $K_D$  values by the application of activity coefficient terms as mentioned in the previous section.

It appears possible to explain this trend in the data by the application of the hydrophobic bonding theory of Némethy and Scheraga.<sup>3</sup> The dimers are assumed to be in the extended form



with the nonpolar portions in maximum contact with each other, thereby minimizing their (unfavorable) contact with water. The hydrogen bond of the dimer is formed in the direction of one of the lone-pair orbitals of the carbonyl group allowing maximum interaction between the side chains and, at the same time, permitting the carbonyl oxygen to form a second hydrogen bond with water. The carbonyl oxygen and OH not involved in the formation of the dimer hydrogen bond remain in hydrogen-bond equilibrium with

TABLE III  
DIMERIZATION CONSTANTS OF THE CARBOXYLIC ACIDS AT  
25° OBTAINED IN VARIOUS INVESTIGATIONS

| Acid <sup>a</sup> | 1 <sup>b</sup> | 2 <sup>b</sup> | 3 <sup>b</sup> | 4 <sup>b</sup> | 5 <sup>c</sup> | 6 <sup>b</sup> | 7 <sup>b</sup> | 8 <sup>c</sup> |
|-------------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|
| Formic            | 1.40           | 2.08           |                |                | 1.24           | 1.40           | 1.54           |                |
| Acetic            | 0.82           | 1.30           | 1.45           | 1.25           | 0.73           | 0.81           |                | 0.96           |
| Propionic         | .64            | 1.30           |                | 1.00           | .50            | .60            |                |                |
| Butyric           | .44            | 1.00           | 1.00           | 0.82           | .26            | .37            |                |                |
| Phenyl-<br>acetic |                |                |                |                | .25            | .25            |                |                |

<sup>a</sup> Column head numbers refer to the work from which the data were obtained: (1) Katchalsky, Eisenberg, and Lifson<sup>4</sup>; (2) Cartwright and Monk<sup>5</sup>; (3) Davies and Griffiths<sup>6</sup>; (4) Nash and Monk<sup>7</sup>; (5) Rossotti, *et al.*<sup>8</sup>; (6) Rossotti, *et al.*,<sup>8</sup> corrected for salt effects; (7) this work, corrected for salt effects; (8) Farrer and Rossotti.<sup>10</sup> <sup>b</sup>  $-\log K_D = -\log [a_{H_2A_2}]/[a_{HA}]^2$ . <sup>c</sup>  $-\log K_D = -\log [C_{H_2A_2}]/[C_{HA}]^2$ .

water. Courtauld's molecular models indicate that such structures can form with no strain.

The data given in Table III can readily be converted to standard free energies of dimerization by the equation

$$\Delta F_D^\circ = -2.3RT \log K_D \quad (16)$$

Experimental values of the standard free energies of hydrophobic bond formation may then be obtained by subtracting, in each set of data, the free energy of formation of the dimer of formic acid for that set from the other values, *i.e.*

$$\Delta F_{H\phi}^\circ = \Delta F_D^\circ (HA) - \Delta F_D^\circ (\text{formic acid}) \quad (17)$$

This subtraction corrects for losses in translational and rotational entropy<sup>22</sup> when the dimers are formed since these contributions should depend only slightly, if at all, on chain length. This subtraction may also eliminate some of the sources of disagreement in the results of different investigators if the same systematic error is present throughout a particular set of data. Table IV shows the results of these calculations. Experimental hydrophobic bond energies are recorded there for the data of Katchalsky, *et al.*,<sup>4</sup> the results of Rossotti, *et al.*,<sup>8</sup> with the activity coefficient terms included which were mentioned previously, and the averages of the data of Cartwright and Monk<sup>5</sup> and Davies and Griffiths<sup>6</sup> which appear to agree closely. The hydrophobic bond energies calculated from the theory of Némethy and Scheraga<sup>3</sup> are given in the last two columns. The numbers given in the column headed  $\Delta Y$  are the total number of water molecules removed from contact with the nonpolar portions of both monomer molecules when the hydrophobic bond is formed in the dimer.<sup>3</sup> The values calculated from the

(22) Since no hydrophobic bonding is assumed to exist in the formic acid dimer, the hydrophobic contributions to the stability of the other dimers are unaltered by this subtraction.

theory are given in the column numbered 4, while column 5 gives the values of the hydrophobic bond free energies if a correction for the loss of rotational free energy of the side chains on forming the hydrophobic bond is omitted, *i.e.*, the  $\Sigma \Delta F_{rot}$  term in eq. 5 of ref. 3. This correction was not part of the general statistical mechanical development of the theory<sup>3</sup> and has yet to be experimentally verified. In any case, the agreement between the calculated and experimental values is very good. This agreement suggests that hydrophobic bonds may be responsible for the increasing stabilization with chain length of the carboxylic acid dimers.

TABLE IV  
COMPARISON OF EXPERIMENTAL AND CALCULATED STANDARD  
FREE ENERGIES OF HYDROPHOBIC BOND FORMATION,  
 $\Delta F_{H\phi}^\circ$ , AT 25°

| Side chain <sup>a</sup>                           | Experimental $\Delta F_{H\phi}^\circ$ ,<br>kcal./mole |       |       | $\Delta Y^b$ | Calculated $\Delta F_{H\phi}^\circ$ ,<br>kcal./mole |       |
|---|---|-------|-------|--------------|---|-------|
|   | 1   | 2     | 3     |              | 4   | 5     |
| CH <sub>3</sub> -                                 | -0.79   | -0.95 | -0.80 | 4            | -0.70   | -0.70 |
| CH <sub>3</sub> CH <sub>2</sub> -                 | -1.03   | -1.06 | -1.09 | 6            | -0.90   | -1.00 |
| CH <sub>3</sub> CH <sub>2</sub> CH <sub>2</sub> - | -1.31   | -1.47 | -1.41 | 8            | -1.15   | -1.35 |
| C <sub>6</sub> H <sub>5</sub> CH <sub>2</sub> -   |   |       | -1.57 | 12           | -1.45   | -1.63 |

<sup>a</sup> Column head numbers indicate the work from which the data were obtained: (1) Katchalsky, Eisenberg, and Lifson<sup>4</sup>; (2) average of the results of Cartwright and Monk<sup>5</sup> and Davies and Griffiths<sup>6</sup>; (3) Martin and Rossotti,<sup>8</sup> corrected for salt effects; (4) calculated from the model of Némethy and Scheraga<sup>3</sup>; (5) calculated from the model of Némethy and Scheraga<sup>3</sup> omitting the correction for the loss of rotational free energy of the side chains on forming the hydrophobic bond. <sup>b</sup>  $\Delta Y$  is the total number of water molecules removed from contact with the nonpolar portions of both molecules when the hydrophobic bond is formed.<sup>3</sup>

The effect of polar groups on hydrophobic bonds may also be considered. The proximity of polar groups to nonpolar residues in the acids has been shown to have little or no effect on hydrophobic bond energies in the carboxylic acids. It may well be, as Frank and Wen<sup>23</sup> point out, that polar groups fit easily into existing water clusters without affecting the distribution of water molecules around the nonpolar sites.

It has been demonstrated in this work that hydrophobic bonds may have a significant part to play in the stabilization of model species in aqueous solutions and, by inference, in stabilizing the conformations of natural macromolecules as well. The compounds studied here provide an experimental probe into the study of these important secondary interactions. A variety of experiments can be conceived using these model systems which may help to test various structural concepts which have been applied to biological macromolecules.

(23) H. S. Frank and W.-Y. Wen, *Discussions Faraday Soc.*, **24**, 133 (1957).