

synthesis. We tried to use puromycin for confirmation of these results, but the synergistic effects of ethionine and puromycin did not allow the test animals to survive long enough.

We have also done similar experiments with guinea-pigs as test animals. This species has markedly low biotinidase activity both in liver and serum.<sup>9</sup> Ethionine feeding had no effect on the biotinidase activities in these animals.

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1. Wright, L. D., Driscoll, C. A. and Boger, W. P. *Proc. Soc. Exptl. Biol. Med.* **86** (1954) 335.
2. Thoma, R. W. and Peterson, W. H. *J. Biol. Chem.* **210** (1954) 569.
3. Koivusalo, M., Elorriaga, C., Kaziro, Y. and Ochoa, S. *J. Biol. Chem.* **238** (1963) 1038.
4. Knappe, J., Brümmer, W. and Biederbick, K. *Biochem. Z.* **338** (1963) 599.
5. Pispä, J. *Ann. Med. Exptl. Biol. Fenniae (Helsinki)* **43** (1965) *Suppl.* **5**.
6. Koivusalo, M. and Pispä, J. *Acta Physiol. Scand.* **58** (1963) 13.
7. Stekol, J. A., Mody, U., Bedrock, E., Keller, S. and Perry, J. *Federation Proc.* **19** (1960) 37.
8. Villa-Trevino, S., Shull, K. H. and Farber, E. *J. Biol. Chem.* **238** (1963) 1757.
9. Raina, A., Jänne, J. and Siimes, M. *Acta Chem. Scand.* **18** (1964) 1804.
10. Farber, E., Lombardi, B. and Castillo, A. E. *Lab. Invest.* **12** (1963) 873.
11. Hsu, J. M. and Geller, S. *Arch. Biochem. Biophys.* **118** (1967) 85.
12. Sie, H.-G. and Hablanian, A. *Biochem. J.* **97** (1965) 32.
13. Lyman, R. L., Thenen, S. and Tucker, R. *Can. J. Biochem.* **44** (1966) 1345.
14. Chatagner, F. and Trautman, O. *Nature* **200** (1963) 75.
15. McLean, P. *Biochem. J.* **99** (1966) 776.
16. Kolenbrander, H. M. *Can. J. Biochem.* **47** (1969) 437.

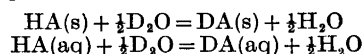
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## Solubility of Picric Acid in Light and Heavy Water and Isotopic Fractionation of Hydrogen between Water and Solid Picric Acid

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Standard free energies of transfer of sparingly soluble substances from H<sub>2</sub>O to D<sub>2</sub>O are usually calculated from the solubilities of the substances in the two waters.<sup>1</sup> However, if the solute in question contains hydrogen atoms that are exchangeable with those of the solvent, the "transfer free energy" obtained is actually the sum of several free energy changes. For example, when we compare the solubility of a solid compound HA in H<sub>2</sub>O with that of DA in D<sub>2</sub>O, the calculated free energy change includes the standard free energy changes of the isotope exchange reactions



as well as the genuine transfer free energy change when the solute is transferred from light to heavy water.

The above point is illustrated by the solubilities of proto- and deuteriopicroic acids shown in Table 1. The solubility, 0.0569 M, of protopicroic acid in light water containing no perchloric acid is in excellent agreement with the value, 0.05684 ± 0.00010 M, reported by Halban and Kortschak.<sup>2</sup>

The solubility products given in Table 1 were calculated from

$$K_s^\circ = \gamma^2 c_{\text{L}^+} c_{\text{Pi}^-}, \quad (\text{L}^+ = \text{H}^+, \text{D}^+; \text{Pi}^- = \text{picrate anion}) \quad (1)$$

in which the mean molar ionic activity coefficients  $\gamma$  were estimated from a semiempirical Debye-Hückel approximation with parameter values given earlier.<sup>3</sup> A correction for the presence of undissociated picric acids was applied when calculating the ionic strengths and the molar concentrations of L<sup>+</sup> and Pi<sup>-</sup>. If the activity coefficients of the undissociated acids remain unaltered in the solutions studied, their molar concentrations would be given by

$$(\text{LPi})_{\text{undiss.}} = K_s^\circ / K_{\text{LPi}}^\circ \quad (2)$$

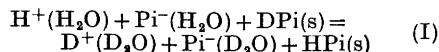
where  $K_{\text{LPi}}^\circ$  are the dissociation constants of the picric acids. The value  $0.60 \text{ mol l}^{-1}$ , determined by Kortüm and Wilski,<sup>4</sup> was used for  $K_{\text{HPi}}^\circ$ . The value  $0.23 \text{ mol l}^{-1}$  was obtained for deuteriopiepic acid from the above value and from the ratio  $K_{\text{HPi}}^\circ / K_{\text{DPi}}^\circ$  determined earlier.<sup>3</sup> The values of  $K_s^\circ$  were then calculated from eqns. (1) and (2) by the method of successive approximations, until the values of  $(\text{LPi})_{\text{undiss.}}$  remained unaltered. The molar concentrations thus obtained for the undissociated acids were  $(\text{HPi}) = 0.0032 \pm 0.0001$  and  $(\text{DPi}) = 0.0017 \pm 0.0001$ .

From the ratio of the solubility products presented in Table I one obtains a value

*Table I.* Solubility of protopicric acid in light water and that of deuteriopiepic acid in heavy water and in perchloric acid solutions at 25°C.  $c_{\text{HPi}}$  and  $c_{\text{DPi}}$  are the stoichiometric concentrations of the dissolved picric acids in mol per liter, and  $c_{\text{HClO}_4}$  and  $c_{\text{DClO}_4}$  the concentrations of added perchloric acids. The values of  $I$  are the ionic strengths (calculated as explained in the text) and the  $K_s^\circ$ 's are the solubility products in  $\text{mol}^2 \text{ l}^{-2}$ .

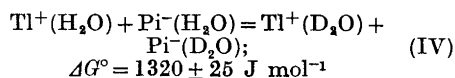
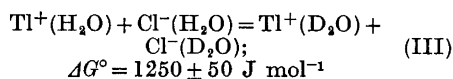
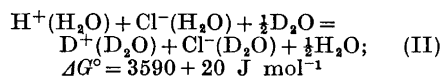
$\text{H}_2\text{O}$			
$c_{\text{HClO}_4}$	$c_{\text{HPi}}$	$I$	$10^3 \times K_s^\circ$
—	0.0569	0.0537	2.05
0.0111	0.0513	0.0592	2.01
0.0235	0.0452	0.0655	1.92
0.0334	0.0418	0.0720	1.92
0.0445	0.0390	0.0803	1.96
0.0556	0.0356	0.0880	1.92
0.0667	0.0337	0.0972	1.98
0.0778	0.0301	0.1047	1.86
0.0889	0.0288	0.1145	1.92
0.1001	0.0275	0.1244	1.96
<i>Av.</i> $K_s^\circ = (1.95 \pm 0.02) \times 10^{-3}$			
$\text{D}_2\text{O}$			
$c_{\text{DClO}_4}$	$c_{\text{DPi}}$	$I$	$10^4 \times K_s^\circ$
—	0.0239	0.0222	3.77
0.0115	0.0192	0.0290	3.79
0.0229	0.0162	0.0374	3.94
0.0354	0.0131	0.0468	3.78
0.0458	0.0122	0.0563	4.11
<i>Av.</i> $K_s^\circ = (3.88 \pm 0.07) \times 10^{-4}$			

of  $4000 \pm 80 \text{ J mol}^{-1}$  for the standard free energy change of the process



If the standard states of the solutes are converted to the mol fraction scale,<sup>5</sup> the free energy change will be  $3980 \pm 80 \text{ J mol}^{-1}$ . This value is much higher in magnitude than those calculated earlier<sup>3</sup> for the transfer of undissociated picric acid and its ions from  $\text{H}_2\text{O}$  to  $\text{D}_2\text{O}$ , which were, respectively,  $470 \text{ J mol}^{-1}$  and  $880 \text{ J mol}^{-1}$ . This shows that the isotope exchange processes, in particular those involving isotopically different hydronium ions and waters,<sup>3,6</sup> are the main contributors to the overall isotope effect on solubility.

When the above free energy value for process (I) is combined with the free energy values for processes (II), (III), and (IV),<sup>5,7</sup>



the value  $-320 \pm 100 \text{ J mol}^{-1}$  is obtained for the standard free energy change of the reaction



A consistent value,  $-340 \pm 105 \text{ J mol}^{-1}$ , is obtained when the transfer free energy values determined for potassium chloride<sup>8</sup> and potassium picrate<sup>5</sup> are used instead of the values for the corresponding thallium(I) salts (reactions III and IV).

It is of interest to note that the standard free energy of reaction (V) is negative, whereas it is positive for the similar reaction of dissolved aqueous picric acid.<sup>3</sup> This implies a small enrichment of the heavier hydrogen isotope in the solid form of picric acid. This is probably due to relatively strong hydrogen bonding in solid picric acid and the fact that deuterium forms stronger hydrogen bonds than protium.<sup>1,6</sup>

The materials and the experimental techniques used were those described in connection with a study of isotope effects in the dissociation of picric acid and related reactions.<sup>3</sup>

1. Arnett, E. M. and McKelvey, D. R. In Coetzee, J. F. and Ritchie, C. D. *Solute-Solvent Interactions*, Marcel Dekker, New York 1969, Chapter 6.
2. Halban, H. and Kortschak, H. *Helv. Chim. Acta* **21** (1938) 392.
3. Salomaa, P. *Suomen Kemistilehti* **B 45** (1972) 149.
4. Kortüm, G. and Wilski, H. *Z. physik. Chem. (Frankfurt)* **2** (1954) 256.
5. Salomaa, P. and Mattsén, M. *Acta Chem. Scand.* **25** (1971) 361.
6. Gold, V. *Advan. Phys. Org. Chem.* **7** (1969) 259.
7. Salomaa, P. *Acta Chem. Scand.* **25** (1971) 365.
8. Salomaa, P. and Aalto, V. *Acta Chem. Scand.* **20** (1966) 2035.

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## Solvent Isotope Effects in Chemical Potentials of Some Solutes in H<sub>2</sub>O-D<sub>2</sub>O Mixtures

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In theoretical treatments of acid-base reactions in H<sub>2</sub>O-D<sub>2</sub>O mixtures,<sup>1-3</sup> it has been frequently assumed that the standard free energy of transfer of a solute from one water to another is linearly dependent on the change in the deuterium atom fraction of the solvent. An equivalent statement is that the "transfer activity coefficients",<sup>3</sup> which are incorporated in the relative equilibrium or rate coefficients in these solvents, can be represented by  $(Y)^n$ , where  $n$  is the deuterium atom fraction and  $Y$  is a constant characteristic of the reaction in question.

We report here results of solubility measurements which were made to test the validity of the above hypothesis. When suitable model solutes were chosen, several points were kept in mind. The first requirement was that the solutes did not contain hydrogens exchangeable with those of the solvent, so that complications arising from isotope exchange reactions<sup>4</sup> could be avoided. Second, particularly in the case of electrolytes, the solubilities had to be sufficiently low to permit satisfactory estimation of the ionic activity coefficients (or, at least, the contribution of the ionic strength corrections had to be so small that any errors in them did not affect the conclusions). Third, the solutes had to have transfer free energies of widely varying magnitudes.

The results for four solutes are collected in Table 1. The measured solubilities are expressed in "aquamolal" units (mol of solute in 55.51 mol of water)<sup>5</sup> and are averages of at least six replicate determinations.

If the above postulate about the "transfer activity coefficients" is valid, the standard free energies of solution should be linear functions of the deuterium atom fraction  $n$ . For a sparingly soluble non-electrolyte, such as 1,3,5-trinitrobenzene, this free energy is

$$\Delta G_{\text{soln}}^{\circ}(n) = -RT \ln(m)_n \quad (1)$$

when the standard state of the solid form is the pure substance (mol fraction = 1) and the standard state of the dissolved form a hypothetical one aquamolal infinitely dilute solution. For a 1-1 electrolyte, the corresponding free energy change is

$$\Delta G_{\text{soln}}^{\circ}(n) = -2RT \ln(m\gamma)_n \quad (2)$$

where  $\gamma$  is the mean ionic activity coefficient at the saturation aquamolality  $m$ . For the present purpose, the activity coefficients could be satisfactorily estimated from the Debye-Hückel approximation

$$-\ln(\gamma)_n = A_n \sqrt{m}/(1 + a\beta_n \sqrt{m}) \quad (3)$$

The parameters  $A_n$  and  $\beta_n$  were calculated from the densities of H<sub>2</sub>O-D<sub>2</sub>O mixtures<sup>6</sup> and from the dielectric constants of light and heavy water.<sup>7</sup> For isotopically mixed waters, it was assumed that the dielectric constant is a linear function of  $n$ . The empirical values of Kielland<sup>8</sup> were used for the ion-size parameters  $a$ .

The calculated values of  $\Delta G_{\text{soln}}^{\circ}$  are shown in Fig. 1. Although the estimated