mation of PLP semicarbazone is as would be expected from its pK_a value in spite of the experimental activation energy of the reaction.

The specific reaction rate for the formation of pyridoxal carbohydrazone is markedly less than for the formation of PLPCH (Table I). Similar qualitative results also were obtained with semicarbazide and thiosemicarbazide. The experimental activation energy for the formation of pyridoxal carbohydrazone (PLCH) is almost double that for the formation of the phosphorylated complex. Both the rate of reaction and the experimental activation energy indicate more favorable conditions for the formation of PLPCH than PLCH.

Davison[3] presented evidence that PLP isonicotinyl hydrazone is the initial complex formed in an *in vitro* homogenate preparation, with an experimental activation energy of approximately 16 kcal./mole for the reaction. Davison's value for the energy of activation for the formation of PLP-INH obtained with only PLP and INH present is 14 kcal./mole. This latter value does not correspond very closely to the value of 9.9 kcal./mole presented in Table I. The activation energy for the reaction between pyridoxal and INH has not been determined. However, with pyridoxal and carbohydrazide the activation energy is 17.2 kcal./mole. Reference to Table I shows that the values reported by Davison more closely approximate the value for the experimental activation energy of pyridoxal carbohydrazone than any of the PLP hydrazones.

The urinary excretion of pyridoxal semicarbazone by dogs treated with semicarbazide reported by Williams[6] may be due to dephosphorylation of the initial complex, since high phosphatase activity for PLPSC has been demonstrated in the kidney.[12]

It is suggested that the higher specific reaction rates and lower experimental activation energies favor the formation of hydrazones of pyridoxal 5-phosphate rather than of pyridoxal. Direct demonstration of the pyridoxal 5-phosphate hydrazone complexes *in vivo* will be the subject of a further communication.

(12) R. G. Wiegand, Ph.D. Thesis, Emory University, 1956.

EMORY UNIVERSITY, GA.

[CONTRIBUTION FROM THE MALLINCKRODT CHEMICAL LABORATORIES OF HARVARD UNIVERSITY]

The Quantitative Evaluation of the Effect of Hydrogen Bonding on the Strength of Dibasic Acids

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A quantitative measure of the effect of hydrogen bonding on the ionization constants of a dibasic acid can be obtained by comparing the first ionization constant of the dibasic acid with the ionization constant of the corresponding methyl ester. It can thus be established that, for most dibasic acids, the effect of hydrogen bonding on the K_1/K_2 ratio is negligible relative to the electrostatic effect; for maleic acid and for some highly alkylated aliphatic acids, the hydrogen bonding effect is appreciable but not dominant. The conjugate acids of bipyridyl, cited by McDaniel and Brown, do not show evidence of an important hydrogen bonding effect.

Introduction

In a recent article Hunter[2] pointed out that internal hydrogen bonding will increase the ratio of the first to the second ionization constants of maleic and of some other dicarboxylic acids. Subsequently, Brown and his collaborators[3] extended Hunter's qualitative treatment to a large number of additional dibasic acids and suggested that, for certain acids, the hydrogen-bonding effect may be an important one. A method for the quantitative evaluation of the effect of hydrogen bonding on the K_1/K_2 ratio in dibasic acids is here formulated and applied to the examples previously[3] cited; in general, the effect of hydrogen bonding is found to be small. Salicylic acid[4–6] and similar acids where the hydrogen bond is in a ring of six atoms including two double bonds,[7] and some highly alkylated aliphatic acids,[3] are exceptional cases where the effect of hydrogen bonding may be important. For most dibasic acids, a statistical factor of 4 and an electrostatic effect[8–10] are primarily responsible for the values of the K_1/K_2 ratios.

The first ionization constant of phthalic acid is 1.2×10^{-3}, the ionization constant, K_E, of monomethyl phthalate is 0.6×10^{-3}. On statistical grounds alone, the first ionization constant of a dibasic acid should be twice the ionization constant of the corresponding monoester.[11] Since for phthalic acid such is almost exactly the fact, there is very little room for an effect of hydrogen bonding. Presumably the fact that the ring for hydrogen-bonded phthalic acid contains seven members, and the fact that internal hydrogen bonding must compete with external (solvent) hydrogen bonding, are responsible for the experimental finding above.

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[1] On leave of absence from Haverford College, Haverford, Penna.

[2] L. Hunter, *Chemistry & Industry*, 155 (1953); *cf.* I. Jones and F. G. Soper, *J. Chem. Soc.*, 133 (1936).

[3] (a) D. H. McDaniel and H. C. Brown, *Science*, **118**, 370 (1953); (b) H. C. Brown, D. H. McDaniel and O. Häfliger in Braude and Nachod, "Determination of Organic Structures by Physical Methods," Academic Press, Inc., New York, N. Y., 1955, p. 628.

[4] G. E. K. Branch and D. L. Yabroff, THIS JOURNAL, **56**, 2568 (1934).

[5] W. Baker, *Nature*, **137**, 236 (1936).

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[8] N. Bjerrum, *Z. physik. Chem.*, **106**, 219 (1923).

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Theoretical

An upper limit to the effect of hydrogen bonding can be based on the ratio K_1/K_E. In the equations below, H_2A, HE and HA^- refer to the free diacid, ester and monoanion (without hydrogen bonding), whereas $\overline{H_2A}$, $\overline{HE}$ and $\overline{HA^-}$ refer to the corresponding hydrogen bonded species.

Let

$$(\overline{H_2A})/(H_2A) = K_H;\ (\overline{HA^-})/(HA^-) = K_H' \quad (1)$$

and

$$(\overline{HE})/(HE) = K_H''$$

Then

$$\frac{(H^+)[(HA^-) + (\overline{HA^-})]}{(H_2A) + (\overline{H_2A})} = K_1 \quad (2)$$

and

$$\frac{(H^+)(A^=)}{(HA^-) + (\overline{HA^-})} = K_2 \quad (3)$$

Therefore

$$K_1 = \frac{K_{H_2A}\,(1 + K_H')}{1 + K_H} \quad (4)$$

and

$$K_2 = \frac{K_{HA^-}}{1 + K_H'} \quad (5)$$

where K_{H_2A} and K_{HA^-} are the ionization constants for their respective species which would obtain in the absence of hydrogen bonding. Therefore

$$\frac{K_1}{K_2} = \frac{K_{H_2A}}{K_{HA^-}} \times \frac{(1 + K_H')^2}{1 + K_H} \quad (6)$$

Furthermore

$$K_E = \frac{(H^+)(E^-)}{(HE) + (\overline{HE})} = \frac{K_{HE}}{1 + K_H''} \quad (7)$$

where K_{HE} is the ionization constant for the monoester which would obtain in the absence of hydrogen bonding. From 4 and 7

$$\frac{K_1}{K_E} = \frac{K_{H_2A}}{K_{HE}} \times \frac{(1 + K_H')(1 + K_H'')}{(1 + K_H)} \quad (8)$$

But from purely statistical considerations (and assuming the electrical equivalence of the carboxyl and carbalkoxyl groups)

$$K_{H_2A} = 2K_{HE} \text{ and } K_H = 2K_H'' \quad (9)$$

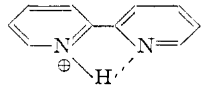
So

$$K_H' = \frac{K_1}{K_E}\left[\frac{1 + 2K_H''}{2 + 2K_H''}\right] - 1 \quad (10)$$

Unless the hydrogen bonding in the ester is very strong, the factor in brackets in equation 10 approaches $^1/_2$; in any event, the factor is restricted to the range from $^1/_2$ to 1.

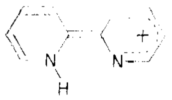
Dicarboxylic Acids.—The best available data on the ionization constants for the monoesters of dibasic acids are those of Walker[12]; they have been tabulated by Brown, *et al.*[3b] Most of the values of the K_1/K_E ratio lie close to 2. Since the values of K_H'' must be small compared with those of K_H', the application of equation 10 shows that the equilibrium constants for hydrogen bonding, even in the monoanions, are usually very small. However, for maleic acid, tetramethylsuccinic acid and diethylmalonic acid, the ratios K_1/K_E are 10.6, 27 and 32, respectively. The *maximum* effect of hydrogen bonding upon acidity will occur if there is no hydrogen bonding in the diacid (or, by analogy, in the half-ester); if this is so, then hydrogen bonding can increase the strengths of the acids listed above by the factors 28, 182 and 256, respectively. Since the ionization constant ratios, K_1/K_2, for these acids are about 16,000, 6,000 and 100,000, respectively, hydrogen bonding may contribute, for these acids, a significant fraction of the total observed effect. If K_H, although smaller than K_H', is not negligible with respect to it, the effect of hydrogen bonding on the K_1/K_2 ratio is smaller than is indicated by the figures shown above.

Diamines.—Brown and McDaniel[13a] quote Baxendale and George[13] to the effect that 2,2'-bipyridyl is a monoacid base, and assume that



hydrogen bonding stabilizes the cation so that a second proton is not added to the molecule. Recently, Krumholz[14] has shown that the first ionization constant of doubly protonated bipyridyl is in the neighborhood of unity, and hence the K_1/K_2 ratio is about 100,000. In the present work, this ionization constant of bipyridyl and the ionization constant of the protonated mono-N-methylbipyridyl cation were determined spectroscopically by Hammett's method.[15] The ratio of the ionization constants for the di-cation to that of the protonated monomethyl cation (*i.e.*, K_1/K_E) is 6.7. Statistical considerations account for a factor of 2 in this ratio.

The K_1/K_2 ratio for the conjugate acids of bipyridyl is surprisingly large. However, even bipyridyl may not be quite planar,[16] and the spectra (see Experimental) of diprotonated bipyridyl and of the protonated methochloride suggest that both of these molecules are twisted. The positive charges in the di-cations are therefore close and the electrostatic effects correspondingly large. Some differences may exist in the conformation of the various cations, and the exact electrostatic calculation will depend to some extent on the angle between the two rings. However, the small K_1/K_E ratio suggests that neither hydrogen bonding nor resonance contributions from such structures as



make large contributions to the K_1/K_2 ratio.

Experimental

2,2'-Bipyridyl.—Eastman Kodak Co. 2,2'-bipyridyl was recrystallized to constant melting point from aqueous alcohol; m.p. 69–69.5°.

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[12] J. Walker, *J. Chem. Soc.*, **61**, 696 (1892); *cf.* P. Mastagli and G. Bardinet, *Compt. rend.*, **223**, 667 (1946); G. Bardinet, *ibid.*, **225**, 736 (1947); **227**, 1358 (1948).

[13] J. H. Baxendale and P. George (a) *Nature*, **162**, 777 (1948); (b) *Trans. Faraday Soc.*, **46**, 55 (1950).

[14] P. Krumholz, *J. Phys. Chem.*, **60**, 87 (1956).

[15] L. A. Flexser, L. P. Hammett and A. Dingwall, THIS JOURNAL, **57**, 2103 (1935).

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2,2'-Bipyridyl Monomethiodide.—2,2'-Bipyridyl (5 g.) and methyl iodide (4.33 g.) in 10 ml. of absolute methanol were heated in a sealed tube for two hours on a steam-bath. A yellow residue (the dimethiodide) was removed by filtration, and the filtrate evaporated to dryness in a stream of air. Bipyridyl was recovered from the product by extraction with boiling petroleum ether (0.87 g. recovered). The monomethiodide was recrystallized from absolute alcohol to constant melting point, m.p. 145–146°, yield 4.75 g. or 60%. *Anal.* Calcd. for $C_{11}H_{11}N_2I$: C, 44.3; H, 3.72. Found: C, 43.9; H, 3.78. Its ultraviolet spectrum in H_2O shows a peak at 225 mμ due to iodide ion and a peak at 277 mμ with a log ε of 3.97.

Preparation of Test Solution.—In order to obtain a spectrum of only the methylbipyridyl cation, an aqueous solution of the methiodide (0.1 g. in 100 ml.) was shaken for 15-minute intervals with successive amounts of freshly prepared silver chloride, until further addition of silver chloride did not depress the spectrum further in the 225 mμ region. This procedure quantitatively removed the iodide peak[17] (log ε of iodide ion = 4.1) while leaving the remaining spectrum (260–350 mμ) entirely unchanged. The methylbipyridyl cation therefore has a single maximum at 277 mμ.

Determination of Ionization Constant of 2,2'-Bipyridyl.—In Fig. 1 are shown the spectra of 2,2'-bipyridyl (7.87×10^{-5} *M*) in 0.0125 *N* HCl, 1.29 *M* H_2SO_4 and 7.0 *M* H_2SO_4, determined on a Beckman DU spectrophotometer at 25 ± 1°. Spectrum A is that of the monoprotonated amine, C that of the diprotonated amine, and B that of a mixture of the two. The monoprotonated bipyridyl has peaks at 241 and 302 mμ with molar extinction coefficients of 7.24×10^3 and 14.7×10^3, respectively. The diacid has a peak at 290 mμ; ε 15.2×10^3.

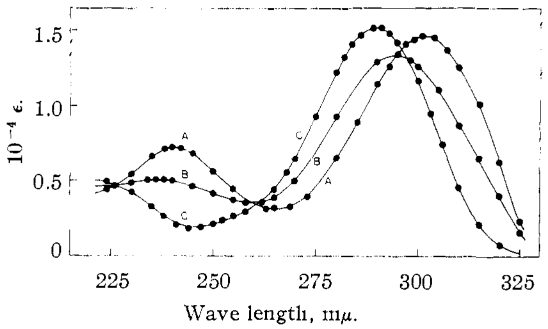

Fig. 1.—The molar extinction coefficients (m.cm./l.) plotted against wave length (mμ) for 2,2'-bipyridyl: A, in 0.0125 *M* HCl; B, in 1.29 *M* H_2SO_4; C, in 7.0 *M* H_2SO_4.

Figure 1 shows that the isosbestic region[15] is large in the neighborhood of 295 mμ, smaller in the 260 mμ region and reduces to a point at 226 mμ. In the 230 to 250 mμ region where the change in optical density is largest, no correction for the effect of medium is thus necessary. Taking $H_0 = -0.44$ for solution B from the recalculated values of Paul and Long,[18] pK_1 for bipyridyl is found to be -0.52 ± 0.02 ($K_1 = 3.3$). Krumholz[14] obtained a value for K_1 of 1.4 ± 0.3 from the 300 mμ region in HCl–LiCl mixtures. But in this region the solvent effect on the spectrum is large and the salt strongly enhances the acidity of the medium.[19] There is an error in our value corresponding to the factor log $(f_{B^+}f_{BH^+}/f_{BH^+}{}^{+}f_B)$ because the H_0 values are based on the protonation of an uncharged base. This factor is equal to $H_0 - H_+$ and is probably small since it is only 0.28 in 100% sulfuric acid[20] and zero in dilute aqueous solution.

Bipyridyl and its monoacid have two peaks in their ultraviolet spectrum, whereas the diacid and the methyl cation (protonated and unprotonated) have only one peak. These facts agree with Krumholz' suggestion[21] that the second peak is lost when the molecule is no longer coplanar. The second protonation of *o*-phenanthroline in strongly acid solution does not lead to the loss of one of the two peaks.[22]

Determination of pK_E of the Protonated Monomethyl Cation of 2,2'-Bipyridyl.—Figure 2 shows the molar extinction coefficients of the methylbipyridyl cation at 270 mμ at different values of the acidity function. The coefficient for the pure base was taken from the value at *p*H 3, that for the acid from sulfuric acid solutions with $H_0 = -3$ and -5, the latter value being corrected for a slight lateral medium shift. Determination of pK_E for points in the intermediate region gives a value of $+0.31 \pm 0.08$ ($K_E = 0.49$). The curve shown on the figure is the theoretical curve calculated from the experimental value of pK_E.

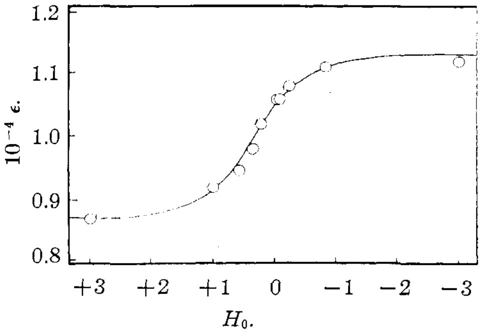

Fig. 2.—The molar extinction coefficients (m.cm./l.) at 270 mμ plotted against H_0 for N-methylbipyridyl cation and its conjugate acid.

Solutions were prepared by diluting 1 ml. of test solution to 100 ml. with the different solvents, correcting the acid strength for the dilution. The protonated methylbipyridyl cation has a maximum log ε of 4.06 at 271 mμ, shifting to 270 mμ at higher acidities.

Acknowledgment.—The authors are indebted to Mr. Edmund Jacobson who carried out preliminary measurements on the spectrum of the conjugate acids of bipyridyl.

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[17] Landolt-Börnstein, "Physikalisch-chemische Tabellen," 6th Edn., Vol. I (3), p. 232.

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[20] J. C. D. Brand, W. C. Horning and M. B. Thornley, *J. Chem. Soc.*, 1374 (1952).

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