^{13}C NMR Determination of Acid-Base Tautomerization Equilibria

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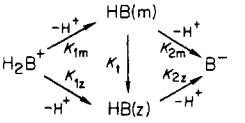
The acid-base tautomeric partitioning ratios of *p*- and *m*-aminobenzoic acids are determined by a novel ^{13}C NMR method that combines the chemical shift displacements of model compounds on protonation with experimentally derived intrinsic chemical shifts of neutral, cationic, and anionic *p*- and *m*-aminobenzoic acids. A statistical analysis yields estimates of the reliabilities of the partitioning ratios and the validities of the model compounds. At 30 °C *p*-aminobenzoic acid is shown to exist almost exclusively as the "molecular" species while the meta isomer is a mixture of 41% "molecular" and 59% "zwitterion" species.

It has long been recognized that partially neutralized polyfunctional acids or bases may exist as a mixture of tautomers.[1] In cases where such species feature nonequivalent functional groups, the relative proportions of tautomers in solution may be ambiguous. For example, if acidic glycinium ion $^+NH_3CH_2CO_2H$ is partially neutralized with 1 equiv of base, the remaining acidic proton might reside either on the amine group forming the "zwitterion" $^+NH_3CH_2CO_2^-$ or on the carboxylate forming the "molecular" $NH_2CH_2CO_2H$. In this case it is well established[1] that the zwitterion is the dominant form. Past studies of partitioning between acid-base tautomers have employed a variety of methods. The simplest of these involves direct comparison of acidic dissociation constants of the substance in question with those of similarly structured systems which can serve as chemical models.[2] More sophisticated analyses rely on comparison of acid dissociation $\Delta H°$ or $\Delta S°$ values with those of closely related compounds whose structures are unambiguous. This approach has been used in several studies of nicotinic and isonicotinic acids[3] and the aminobenzoic acids.[4] Other studies utilize measurements of UV absorption[5,6] or some other physical property[7] of model compounds. While all these methods have yielded estimates of partitioning between tautomers, they provide no internal means of determining the accuracies of the results. The only accuracy checks are to compare results found by independent methods. Indeed different determinations have sometimes led to conflicting results. For example, the monoprotic structures of nicotinic and isonicotinic acids have not been clearly assigned by classical modeling methods.[8]

In recent years NMR spectrometric measurements have been employed to study acid dissociation equilibria. ^1H NMR chemical shift displacements of neighboring groups lead to qualitative discrimination between dissociation reactions at various sites in polyfunctional acids[9] including nicotinic acid.[10] Chemical shift displacements of other NMR-active nuclei have also served to distinguish proton addition or loss in molecular or macromolecular systems. For example, Schaal et al. employed ^{13}C NMR to investigate the zwitterionic vs. molecular structure of nicotinic acid in H_2O/Me_2SO solvents.[10] Bachovchin and Roberts used ^{15}N NMR spectrometry to locate the tautomeric histidyl proton in the catalytic triad of α-lytic protease.[11] We now report a novel application of ^{13}C NMR spectrometry to quantitative determination of proton tautomerization under conditions of fast exchange. The method is based on the phenomenon of additivity of substituent effects.[12] It has the unique advantage of providing simultaneous independent estimates of partitioning between tautomeric protic species, and these multiple estimates serve as an internal check on accuracy.

Calculational Strategy

Consider the set of acid-base equilibria described by the reaction scheme



In this scheme HB(m) and HB(z) represent tautomeric molecular and zwitterionic monoprotonated HB species, respectively, while H_2B^+ and B^- refer to the diprotonated and unprotonated species, respectively. The microscopic equilibrium constants K_{1m}, K_{2m}, K_{1z}, and K_{2z} may not be measured directly. Instead, any measurement of the overall acid-base properties results in values of K_1 and K_2, the composite acidic dissociation constants defined by eq 1 and 2, where the activity coefficient γ_{HB} refers to both

$$K_1 = \frac{[H^+]\{[HB(m)] + [HB(z)]\}\gamma_{H^+}\gamma_{HB}}{[H_2B^+]\gamma_{H_2B^+}} \quad (1)$$

$$K_2 = \frac{[H^+][B^-]\gamma_{H^+}\gamma_{B^-}}{\{[HB(m)]+ [HB(z)]\}\gamma_{HB}} \quad (2)$$

HB(m) and HB(z) species. Comparing these expressions with the definitions of the microscopic equilibrium constants yields a simple set of relationships (eq 3a-d), where

$$K_{1m} = K_1/(1 + K_t) \quad (3a)$$

$$K_{1z} = K_1K_t/(1 + K_t) \quad (3b)$$

$$K_{2m} = K_2(1 + K_t) \quad (3c)$$

$$K_{2z} = K_2(1 + K_t)/K_t \quad (3d)$$

$$K_t \equiv [HB(z)]/[HB(m)] = (1 - X_m)/X_m$$

X_m is the mole fraction of the molecular tautomer in the HB mixture. This set of equations yields values for the

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Table I. Intrinsic ^{13}C Chemical Shifts[a] Determined for *m*-Aminobenzoic and *p*-Aminobenzoic Acid Species

	C1	C2	C3	C4	C5	C6	Cα
				m-Aminobenzoic Acid Species			
HB	136.5 ± 0.1[b]	121.8 ± 0.1	137.6 ± 0.1	124.8 ± 0.1	131.0 ± 0.1	126.9 ± 0.1	173.54 ± 0.1
H_2B^+	132.80 ± 0.08	125.27 ± 0.05	131.65 ± 0.11	129.13 ± 0.05	131.69 ± 0.05	131.43 ± 0.04	169.89 ± 0.05
B^-	138.73 ± 0.07	117.59 ± 0.05	147.35 ± 0.10	120.02 ± 0.05	130.32 ± 0.05	120.90 ± 0.05	176.63 ± 0.05
				p-Aminobenzoic Acid Species			
HB	120.7 ± 0.1	132.8 ± 0.1	116.3 ± 0.1	152.2 ± 0.1			172.0 ± 0.1
H_2B^+	131.8 ± 0.13	132.48 ± 0.06	124.52 ± 0.06	135.51 ± 0.12			170.25 ± 0.06
B^-	127.34 ± 0.10	131.90 ± 0.05	116.10 ± 0.05	150.82 ± 0.09			176.53 ± 0.05

[a] Downfield from external Me_4Si at 30 °C. [b] Uncertainties are standard error estimates provided by the multiple regression analysis and based on ±0.02-ppm resonance line uncertainty plus ±0.03-ppm uncertainty due to sample tube variability.

four microconstants from experimental determinations of K_1, K_2 and X_m.

To illustrate the ^{13}C NMR method of finding X_m, we shall refer to two specific systems whose tautomeric partitioning has been studied by other methods, i.e. *m*- and *p*-aminobenzoic acids. Thus, in the following exposition the symbol B^- will denote $NH_2C_6H_4CO_2^-$. Previous investigators have reported various X_m values of *m*-aminobenzoic acid[4,7,13-15] in the range between 0.3 and 0.5 and of *p*-aminobenzoic acid[4,5,7,13,14,16-18] between 0.83 and 0.99. First we must measure intrinsic chemical shifts for the species H_2B^+, HB, and B^-. It is hypothesized that because of rapid proton exchange, the chemical shifts for HB are mole-weighted averages of the intrinsic shifts of HB(m) and HB(z). In the *m*-aminobenzoic acid system we observe seven nonequivalent carbon resonances and in *p*-aminobenzoic acid we observe five. Each such resonance in principle may be used as an independent measure of the partitioning of HB, and the self-consistency of these multiple determinations serves as a validation of the method. The overall intrinsic chemical shifts are obtained from ^{13}C NMR spectrometric measurements of aminobenzoic acid solutions treated with portions of HCl or NaOH at 30 °C in 5% (v/v) D_2O. For each carbon the experimentally observed resonances δ_{obsd} are again regarded as mole-weighted averages of intrinsic chemical shifts of the three amino benzoic acid species in solution. Thus, for each carbon this averaging is expressed as eq 4,

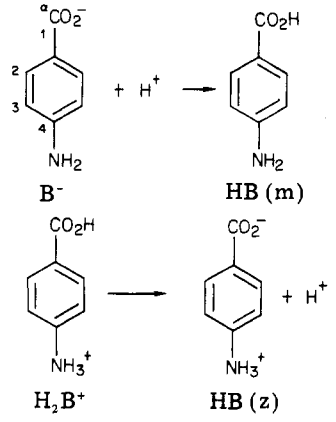
$$F\delta_{obsd} = [H_2B^+]\delta_{H_2B^+} + [HB]\delta_{HB} + [B^-]\delta_{B^-} \quad (4)$$

where F is the analytical concentration of aminobenzoic acid, brackets denote molar concentrations of species, and δ are intrinsic resonances of subscripted species. Typically we obtain spectra of five or six aminobenzoic solutions containing varying proportions of H_2B^+, HB, and B^-. Line assignments are made with due consideration of symmetry factors, relaxation rates, and approximate additivity of substituent effects.[19] We calculate species concentrations for use in eq 4 from solution composition and known values of pK_{a1} and pK_{a2}.[4] Ionic activity coefficients are estimated by using the Debye–Hückel correlation[20] with ion-size parameters of 0.9, 0.35, and 0.6 nm for H_3O^+, OH^-, and aminobenzoic ionic species, respectively. To obtain intrinsic chemical shifts and their uncertainties, we analyze the concentration and chemical shift data by a multiple regression procedure.[21] The results of these calculations appear in Table I.

Next, we hypothesize the principle of additivity of substituent displacements of ^{13}C NMR resonances to estimate intrinsic chemical shifts of the tautomers HB(m) and HB(z). Although there are many ways of making these estimates, we have selected four to apply to each of the aminobenzoic systems. Each such estimate will be called a "modeling" method in the following discussion. For example, consider the estimation of the intrinsic chemical shifts of the five nonequivalent carbons of *p*-aminobenzoic acid species HB(m) and HB(z). One modeling method is to assume that the ^{13}C NMR resonance displacements due to proton transfer at each carbon in *p*-aminobenzoic acid is the same as those observed at the corresponding carbon for proton transfer in the benzoic acid/benzoate system. Observed resonances of modeling system compounds are given in Table II. Thus, the resonance displacement at carbon j due to protonation of benzoate ion is

$$\Delta_j(C_6H_5CO_2H) \equiv \delta_{C_6H_5CO_2H} - \delta_{C_6H_5CO_2^-}$$

with one such displacement for each nonequivalent carbon of benzoic acid. We now regard HB(m) as the product of the carboxylate protonation of B^- and HB(z) as the product of the carboxylic acid deprotonation of H_2B^+.



Using the benzoic acid model for protonation displacements of the ^{13}C NMR resonances, we assume that at each carbon in HB(m) or HB(z), we can estimate the resonance by eq 5a or 5b. For example, at C4 of B^- we find δ_{B^-} =

$$\delta_m = \delta_{B^-} + \Delta_j(C_6H_5CO_2H) \quad (5a)$$

$$\delta_z = \delta_{H_2B^+} - \Delta_j(C_6H_5CO_2H) \quad (5b)$$

150.82 ppm. The corresponding carbon of benzoic acid is at the para position where we observe $\delta_{C_6H_5CO_2H}$ = 134.72 ppm and $\delta_{C_6H_5CO_2^-}$ = 132.24 ppm. Thus $\Delta(C_6H_5CO_2H)$ =

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Table II. Intrinsic ^{13}C NMR Chemical Shifts[a] of Four Modeling Systems

modeling system	carbon position[b] ipso	ortho	meta	para	Cα
		Benzoic Acid[d]			
$\delta_{C_6H_5CO_2H}$	130.91 ± 0.06[c]	130.64 ± 0.06	129.74 ± 0.06	134.72 ± 0.06	171.97 ± 0.06
$\delta_{C_6H_5CO_2^-}$	137.33 ± 0.05	129.84 ± 0.05	129.34 ± 0.05	132.24 ± 0.05	176.62 ± 0.05
		p-Hydroxybenzoic Acid[d]			
$\delta_{HOC_6H_4CO_2H}$	122.54 ± 0.06	133.21 ± 0.06	116.48 ± 0.06	161.81 ± 0.06	171.55 ± 0.06
$\delta_{HOC_6H_4CO_2^-}$	129.28 ± 0.05	132.28 ± 0.05	116.05 ± 0.05	159.69 ± 0.05	176.24 ± 0.05
		Aniline			
$\delta_{C_6H_5NH_3^+}$	131.33 ± 0.21	123.95 ± 0.11	131.23 ± 0.06	130.13 ± 0.15	
$\delta_{C_6H_5NH_2}$	147.35 ± 0.21	117.51 ± 0.11	130.59 ± 0.06	120.51 ± 0.15	
		Sulfanilic Acid			
$\delta_{^-O_3SC_6H_4NH_3^+}$	144.21 ± 0.17	128.43 ± 0.26	124.75 ± 0.16	134.06 ± 0.25	
$\delta_{^-O_3SC_6H_4NH_2}$	133.32 ± 0.16	128.14 ± 0.24	116.35 ± 0.15	150.97 ± 0.23	

[a] In 5% D_2O/H_2O at 30 °C, ppm downfield from external Me_4Si. [b] Relative to the carboxylate or amino group. [c] Uncertainties are standard error estimates based on ±0.02-ppm line uncertainty plus ±0.03 ppm due to sample tube variability. [d] Reference 17.

Table III. Calculated Molecular/Zwitterionic Partitioning[a] of Monoprotic *m*-Aminobenzoic and *p*-Aminobenzoic Acids

carbon index	protonation/deprotonation displacement model: benzoic acid $X_m \pm \sigma_{X_m}$[b]	*p*-hydroxybenzoic acid $X_m \pm \sigma_{X_m}$	aniline $X_m \pm \sigma_{X_m}$	sulfanilic acid $X_m \pm \sigma_{X_m}$
		m-Aminobenzoic Acid		
1	0.394 ± 0.017	0.403 ± 0.015	0.397 ± 0.017	0.387 ± 0.025
2	0.439 ± 0.018	0.436 ± 0.018	0.428 ± 0.021	0.459 ± 0.012
3	0.385 ± 0.008	0.385 ± 0.008	0.384 ± 0.008	0.395 ± 0.009
4	0.446 ± 0.026	0.454 ± 0.022	0.439 ± 0.029	0.471 ± 0.014
5	0.509 ± 0.186	0.510 ± 0.208	0.474 ± 0.561	0.494 ± 0.135
6	0.418 ± 0.012	0.415 ± 0.012	0.415 ± 0.013	0.435 ± 0.010
α	0.391 ± 0.043	0.394 ± 0.041		
mean, $\bar{X}_m$[c]	0.401 ± 0.006	0.402 ± 0.005	0.399 ± 0.006	0.429 ± 0.005
$\Delta\bar{X}_m$[d]	0.020 (4.9%)	0.020 (4.9%)	0.016 (4.0%)	0.028 (6.5%)
		p-Aminobenzoic Acid		
1	1.013 ± 0.010	0.994 ± 0.009	1.104 ± 0.019	1.012 ± 0.020
2	1.098 ± 0.171	0.977 ± 0.120	*e*	*e*
3	1.026 ± 0.025	1.080 ± 0.025	1.420 ± 0.074	0.978 ± 0.038
4	0.946 ± 0.007	0.962 ± 0.008	1.037 ± 0.018	0.988 ± 0.026
α	0.960 ± 0.051	0.948 ± 0.049		
mean, $\bar{X}_m$	0.972 ± 0.006	0.978 ± 0.006	1.080 ± 0.013	1.000 ± 0.014
ΔX_m	0.030 (3.1%)	0.018 (1.8%)	0.070 (6.5%)	0.04 (1.4%)

[a] Partitioning in terms of X_m = mole fraction of molecular HB at 30 °C. [b] X_m uncertainties are standard error estimates from propagation of variance calculations. [c] Weighted means for each modeling method. Uncertainties are standard error estimates of the weighted means. [d] Weighted rms deviation of X_m values from $\bar{X}_m$; see eq 11. [e] δ_m and δ_z are essentially equal. No partitioning estimate is possible.

2.48 ppm and δ_m = 153.30 ppm. Using this same displacement model for C4 of H_2B^+ where $\delta_{H_2B^+}$ = 135.51 ppm, we estimate δ_z = 133.03 ppm in HB(z). By applying the same procedure to the other nonequivalent carbons of *p*-aminobenzoic acid, we obtain five independent pairs of intrinsic resonances for HB(z) and HB(m) from which we can make five independent estimates of the partitioning ratio from the relationship $\delta_{HB} = X_m\delta_m + (1 - X_m)\delta_z$ or

$$X_m = \frac{\delta_{HB} - \delta_z}{\delta_m - \delta_z} \quad (6)$$

At carbon C4 we note from Table I that δ_{HB} = 152.2 ppm and so calculate X_m = 0.946 from eq 6. The other independent estimates of X_m from the benzoic acid model are given in Table III.

The choice of benzoic acid to estimate protonation displacements is arbitrary. We can use any carboxylic acid system that provides a set of ^{13}C NMR lines in correspondence to the lines of *p*-aminobenzoic acid. Any member of the large set of ring-substituted derivatives of benzoic acid satisfies this requirement. We are limited only by the practical consideration that the acid must have sufficient aqueous solubility to enable measurement of the ^{13}C NMR spectrum. Also, not all model systems will be equally successful, but the consistency of the multiple independent determinations of HB partitioning serves as an indication of the model's degree of success. In this paper we report the results of a second carboxylic acid modeling system, *p*-hydroxybenzoic acid/*p*-hydroxybenzoate.[22] Table II shows the intrinsic ^{13}C NMR chemical shifts, and the five independent estimates of X_m derived from these are given in Table III. In addition to modeling the carboxylate protonation or carboxylic acid deprotonation, there are other methods of estimating the intrinsic shifts of the HB tautomers. We shall consider here only two of the other several models, both involving protonation or deprotonation at the amine site. Now we regard the neutral tautomer as the product of the deprotonation of the ammonium group of H_2B^+ and the zwit-

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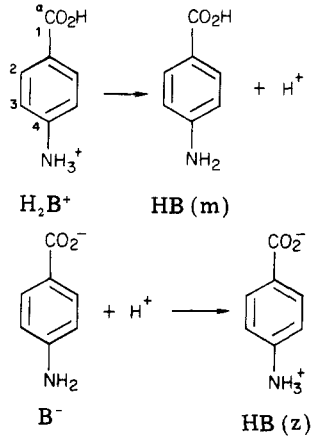
terion as the product of the amine protonation of B^-. The



displacements of ^{13}C NMR resonances upon protonation or deprotonation of the amine group are now assumed to be the same as those observed at corresponding carbons when aniline or some aniline derivative undergoes the same proton transfer. We will use aniline and sulfanilic acid in this manner as our third and fourth modeling systems. Table II shows the intrinsic chemical shifts for conjugate acids and bases for these. When modeling protonation of the amine group of the aminobenzoic acid, the estimated chemical shifts of the HB tautomers are given by eq 7a or 7b rather than by the analogous eq 5a and 5b. The number

$$\delta_m = \delta_{H_2B^+} - \Delta_j(C_6H_5NH_2) \quad (7a)$$

$$\delta_z = \delta_{B^-} + \Delta_j(C_6H_5NH_2) \quad (7b)$$

of independent estimates of the partitioning ratio using amine protonation is less by one that that using carboxylate protonation because the aniline models provide no displacement estimate for the carboxylate carbon. Nevertheless, the amine models provide six and four independent estimates for the partitioning ratio in *m*- and *p*-aminobenzoic acid HB species, respectively. The results calculated by using all four models with both aminobenzoic systems are given in Table III.

Comparative Error Analysis

Using four modeling methods each with four to seven independent estimates of the same quantity (X_m) provides us with an array of data requiring statistical analysis. This analysis is designed to address several questions. (a) Within each modeling method, to what extent are the multiple X_m estimates consistent?; (b) what is the "best" value of X_m provided by each modeling system?; (c) what are the comparative reliabilities of the "best" X_m values?; and (d) what single overall X_m summarizes the results of all four modeling methods? The error analysis that must be done to answer these questions requires an understanding of the error structure of the problem. We are aware of both random and systematic errors in these procedures. The sources of random error are the statistical uncertainties (standard deviations) in the observed ^{13}C NMR chemical shifts of both aminobenzoic acid and modeling compound solutions. These random errors propagate to the X_m results by well-known statistical procedures. Systematic errors arise whenever hypothesized equations fail to represent physicochemical behavior accurately. We consider two such possible sources of error. One is the adequacy of the equations and parameters used to calculate concentrations of species H_2B^+, HB, and B^- from solution compositions. The use of experimentally found pK_{a1} and pK_{a2} values for the aminobenzoic acids and the use of the Debye–Hückel correlation to estimate ionic activity coefficients both carry a degree of uncertainty into the calculation of intrinsic chemical shifts of H_2B^+, HB, and B^-. But because these pK_a values are believed to be quite accurate and because our solution ionic strengths never exceeded ~0.1 M, we are convinced that these sources of systematic error are small relative to one other. This other source of major error is the assumption of substituent effect additivity in applying protonation/deprotonation resonance displacements from model compounds to the aminobenzoic acid systems. This additivity assumption can never be perfectly accurate because of various second-order interactions that always exist between carbons and substituents in the different ring systems. These systematic errors can be written explicitly into the resonance line displacement equations as follows. Equations 5a and 5b become

$$\delta_m = \delta_{B^-} + \Delta_j(C_6H_5CO_2H) + \epsilon_{mj,C_6H_5CO_2H} \quad (5c)$$

$$\delta_z = \delta_{H_2B^+} - \Delta_j(C_6H_5CO_2H) + \epsilon_{zj,C_6H_5CO_2H} \quad (5d)$$

and eq 7a and 7b become

$$\delta_m = \delta_{H_2B^+} - \Delta_j(C_6H_5NH_2) + \epsilon_{mj,C_6H_5NH_2} \quad (7c)$$

$$\delta_z = \delta_{B^-} + \Delta_j(C_6H_5NH_2) + \epsilon_{zj,C_6H_5NH_2} \quad (7d)$$

Here the nonadditivities of substituent displacements are denoted by ϵ, and these appear with elaborate designators in order to convey the very specific character of each error. The additivity depends on the tautomer (m or z), on the particular carbon (j), on the model from which the Δ is derived, and, of course, on the aminobenzoic acid system (meta or para). When these δ_m and δ_z values are substituted into eq 6, the ϵ errors propagate as systematic errors in the X_m values. We have no external quantitative information about these errors to make a priori estimate of these systematic errors in the X_m. Chemical reasoning can aid in the selection of a model compound similar in structure to aminobenzoic acid in question, and the success of this selection, hopefu leads to small ϵ errors. When this occurs, we regard the modeling method as successful and we monitor the relative success of different methods by observing the degree of discrepancy between the several independent X_m values predicted by each method.

We begin by analyzing the random errors alone. To do this, we disregard the ϵ terms in eq 5c, 5d, 7c, and 7d and proceed to estimate statistical uncertainties in the partitioning ratios. The standard error estimates are the square roots of variance estimates for the X_m as calculated from the ^{13}C NMR resonance line uncertainties by using propagation-of-variance procedures. These procedures are valid in cases when the input data uncertainties are small relative to the corresponding data magnitudes. This is clearly true here since all resonance lines are greater than 120 ppm in magnitude and no line uncertainty exceeds 0.3 ppm. The variances of the resonance lines propagate to X_m by combining eq 6 and, say, for example, 5a and 5b to form

$$X_m = \frac{\delta_{HB} - \delta_{H_2B^+} + \Delta}{\delta_{B^-} - \delta_{H_2B^+} + 2\Delta} \equiv \frac{T}{U}$$

The variance in X_m is related to the variances of the resonances by

$$\sigma_{X_m}^2 = \left(\frac{\partial X_m}{\partial \delta_{HB}}\right)^2 (\text{var } \delta_{HB}) + \left(\frac{\partial X_m}{\partial \delta_{B^-}}\right)^2 (\text{var } \delta_{B^-}) + \left(\frac{\partial X_m}{\partial \delta_{H_2B^+}}\right)^2 (\text{var } \delta_{H_2B^+}) + \left(\frac{\partial X_m}{\partial \Delta}\right)^2 (\text{var } \Delta)$$

which upon substitution of the partial derivatives becomes eq 8. For each X_m value estimated by carboxylate protonation/deprotonation modeling, eq 8 is solved for the standard error estimate s_{X_m}. The variances of intrinsic resonances var δ are the squares of the uncertainties shown in Table I and the variances var Δ are the sums of the two squares of the modeling compound resonances from table II. For each X_m estimated by amine protonation/deprotonation modeling eq 9 is used rather than eq 8.

$$U^4\sigma_{X_m}{}^2 = U^2(\text{var } \delta_{HB}) + T^2(\text{var } \delta_{H_iB^+}) + (U - T)^2(\text{var } \eta_{,B^2}) + (U - 2T)^2(\text{var } \Delta) \quad (8)$$

$$U^4\sigma_{X_m}{}^2 = U^2(\text{var } \delta_{HB}) + T^2(\text{var } \delta_{B^-}) + (U - T)^2(\text{var } \delta_{H_2B^+}) + (U - 2T)^2(\text{var } \Delta) \quad (9)$$

The standard error estimates calculated in this manner are given in Table III accompanying each X_m entry. We observe a wide variation in magnitudes of these errors within each modeling method. These variations reflect both the different uncertainties in the chemical shift data and the relative displacements at the different carbons observed on protonation. This latter effect is particularly dramatic and important. For example, consider the *m*-aminobenzoic acid system with benzoic acid protonation modeling and compare carbon C5, which features a total displacement of 1.37 ppm between H_2B^+ and B^- (see Table I), to carbon C6, which features a corresponding 10.53 ppm total displacement. When these values are combined with $\Delta(C_6H_5CO_2H)$ values, the comparison becomes a $|\delta_m - \delta_z|$ difference of 0.57 ppm at C5 and 8.93 ppm at C6. Because X_m is determined by the position of δ_{HB} within the $|\delta_m - \delta_z|$ spread, the precision of the estimate using the larger spread at C6 is much greater than the precision using C5. This behavior is reflected in eq 8 where the standard error estimate σ_X is shown to be approximately inversely proportional to the square of $(\delta_m - \delta_z)$ difference, here denoted by U. Thus the various independent X_m values calculated with any particular modeling method cannot be regarded as equally precise and any averaging must take due cognizance of their precisions as measured by the σ_{X_m} values.

We will here represent the "best" estimate of X_m provided by a modeling method as the weighted mean $\bar{X}_m$ of the independent estimates at each carbon. The formula is [23]

$$\bar{X}_m = \frac{\sum_j W_i X_{mj}}{\sum_j W_j} \quad (10)$$

where the weighting factors are the reciprocals of the variances var X_{mj}, that is $W_j = \sigma_{X_{mj}}{}^{-2}$. When the mean is constructed in this way, the variance estimate of the mean is[23] var$(\bar{X}_m) = 1/\sum W_j$ and the standard error estimate of the mean is the square root of this variance. The weighted mean $\bar{X}_m$ values and their standard errors are given in Table III for each modeling method. These standard error estimates are representations of precision in the sense that replicate ^{13}C NMR spectroscopic measurements would be expected to yield X_m values whose replicate standard deviations would be approximately σ_{X_m}.

We next turn our attention to the uncertainties in X_m generated by the systematic nonadditivity errors ϵ in eq 5c, 5d, 7c, and 7d. Lacking any a priori estimates of the magnitudes of these errors, we can only observe their effect on the calculated X_m values. If these errors were all negligibly small for any particular modeling methods, we would expect the several independent X_m values to agree with one another to within the statistical uncertainties of their standard errors. One method of detecting this agreement is to perform t tests on each pair of X_m, σ_X to verify the null hypothesis that the two X_m values have the same population mean. The test is done for two entries X_{m1} and X_{m2} by comparing the ratio $|X_{m1} - X_{m2}|/(\sigma_{X_{m1}}{}^2 + \sigma_{X_{m2}}{}^2)^{1/2}$ with the critical Student's t for some arbitrary confidence level. If we choose the 95% level, this critical t is 1.96, which corresponds to the large number of degrees of freedom inherent in the $\sigma_{X_m}{}^2$ variances. The following results are obtained from these t tests. For *m*-aminobenzoic acid, of the 21 pairs of X_m values tested by using the benzoic acid model, only 3 pairs have systematic errors large enough to reject the null hypothesis and these involve X_m at C3 with those at C2, C4, and C_6. Identical results are found with the *p*-hydroxybenzoic acid model. Using the aniline model, of 15 pairs tested only 2 reject the null nypothesis, C3 with C2 and C6. Using sulfanilic acid, tests of 5 of 15 pairs reject the hypothesis. These summaries reflect the relative successes of the substituent effect modeling methods. The more successful models generate smaller additivity systematic errors, which result in a greater proportion of "successful" t tests among the pairs of independent X_m estimates. From this point of view all four modeling methods applied to *m*-aminobenzoic acid are rather successful, with sulfanilic acid being somewhat less so than the other three. Turning to *p*-aminobenzoic acid, we find that with both benzoic and *p*-hydroxybenzoic acid models only two of ten pairs tested reject the null hypothesis. These are C4 vs. C1 and C3 with both models. With aniline, all three of three possible tests reject the null hypothesis, whereas with sulfanilic acid all three tests accept the null hypothesis. At the C2 carbon in both of these models we observe a combination of low precision and severe error such that meaningful X_m could not be calculated. Nevertheless, we can conclude that while aniline is an unsatisfactory model for *p*-aminobenzoic acid, the other three are satisfactory.

We will conclude this error analysis by deriving a quantitative measure of the accuracy or success of a given modeling method. This accuracy estimate is calculated by an ad hoc procedure designed to neglect the average effect of the additivity errors on the X_m values and also to take into account the relative precisions of these X_m. To this end, we will define for each model a quantity

$$\Delta X_m = \left[\frac{\sum_j W_j (X_{mj} - \bar{X}_m)^2}{\sum_j W_j} \right]^{1/2} \quad (11)$$

where W_j are the same weighting factors used in calculating the means $\bar{X}_m$. This ΔX_m quantity is thus a weighted root-mean-square average of the scatter of the several X_m values from their mean. Values calculated from this formula are shown in Table III. Both ΔX_m and $(\Delta X_m/\bar{X}_m)$ are listed. We observe that this measure of reliability leads to conclusions similar to those derived from t testing pairs of X_m values. All four additivity models for *m*-aminobenzoic acid are quite successful, with accuracy estimates ranging from 4.0% for aniline to 6.5% for sulfanilic acid. Furthermore, all four models yield consistent results in the sense that the weighted grand mean of the four $\bar{X}_m$ values, which is 0.408, is well within the accuracy ranges of all four models. In the case of *p*-aminobenzoic acid, we see that aniline is somewhat less satisfactory as a model than the other three. Here the weighted grand mean of 0.982 is in agreement with benzoic and *p*-hydroxybenzoic acid models and near agreement with the

(23) Bevington, P. R. "Data Reduction and Error Analysis for the Physical Sciences"; McGraw-Hill: New York, 1969; Chapter 5.

sulfanilic acid model but not with the aniline model.

Discussion

On the basis of the foregoing results it appears that the ^{13}C NMR methodology we have described here provides an experimentally simple yet reliable technique for precise determination of tautomer partitioning. While the method relies on chemical model systems as do earlier approaches, the criteria of internal consistency available with ^{13}C NMR analysis provides a level of reliability not previously available. This is a direct consequence of the essentially independent partitioning estimates provided by each carbon resonance and by independent modeling of functional groups. We note in this connection that the choice of chemical models seems to have little influence on the precision or reliability of partitioning coefficients based on carboxylic acid dissociation models. Thus, benzoate and hydroxybenzoate models provided adequate modeling for both *m*- and *p*-aminobenzoic acids. Apparently, resonance interaction of the electron-donating hydroxyl substituent with both the protonated ionized carboxyl substituents is not reflected in the ^{13}C NMR protonation displacements. However, it does seem likely that the converse effect, withdrawal of electron density from the ring by CO_2H and CO_2^- substituents, is responsible for the failure of aniline as a model for amine protonation in monoprotic *p*-aminobenzoic acid. In that case the electron-withdrawing properties of the sulfonate group in sulfanilic acid seemed to adequately model the NMR data. While it is difficult to generalize from these few examples, it does seem likely that large differences in resonance interactions rather than in electric field effects play a dominant role in ^{13}C protonation displacements of aniline derivatives.

Experimental Section

Stock solutions of commercial samples (Aldrich Chemical Co., 0.05 F) of anilinium chloride, *m*- and *p*-aminobenzoic acids, and 4-sufanilic acids were prepared by diluting weighed portions of the solid samples to 25 or 100 mL with 5% D_2O/H_2O (v/v). In a typical run, a 5-mL aliquot of the stock solution was pipetted into a 10-mm NMR sample tube and the ^{13}C NMR spectrum was recorded initially and following the addition of each of 2 half-equiv of 0.95 F NaOH or HCl solution (5% D_2O) with a 200-μL micropipet.

^{13}C NMR data were acquired at 30 ± 2 °C on a Bruker HX-270 spectrometer operating at 67.89 MHz for ^{13}C detection. Typical instrument settings were 7-μs pulse width (~30° tip angle), 14-kHz spectral width, 0.3-s acquisition time, 1.7-s pulse delay time, and 4-8K transients.

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Registry No. *m*-Aminobenzoic acid, 99-05-8; *p*-aminobenzoic acid, 150-13-0.

Cyclic Imides. 13.[1] An Analysis of the Experimental Dipole Moments of Five-Membered Cyclic Imides in 1,4-Dioxane Solution[2]

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The dipole moments of six unsubstituted cyclic imides and three *N*-methylimides have been determined by the Guggenheim–Smith method for dioxane solution at 20 °C. The results have been compared with values derived from published data for the Halverstadt–Kumler method. The Guggenheim–Smith method gave lower values than the Halverstadt–Kumler method. The difference between the orientation polarizations, calculated by the two methods, is a measure of atomic polarization. The H–N bond moment is 0.6 D for measurements of the dipole moments taken in dioxane solution. This value, with standard bond and group moments, gives good estimates for the dipole moments of saturated and aromatic cyclic imides in dioxane solution but not for imides containing an olefinic double bond in the imide ring.

The solubilities of succinimide and of phthalimide in benzene are too low for measurements of the dipole moments of these compounds to be made with benzene solutions. Cowley and Partington[4] used 1,4-dioxane as the solvent for the earliest of these measurements. Most other cyclic imides are also insoluble in hydrocarbon solvents. Dioxane has served as solvent in all subsequent measurements of the dipole moments of cyclic imides. This use of dioxane in dipole moment measurements creates two problems. The first of these problems is experimental in nature. The second problem lies in the interpretation of the data in terms of molecular structure.

The experimental problem lies in the instability of dioxane toward atmospheric moisture and oxygen. It has been suggested that purified dioxane should be kept and used under an atmosphere of dry nitrogen.[5] There is no evidence, however, that this precaution has been taken in any of the studies of the dipole moments of imides.

Comparison of experimental results is complicated by the use of different experimental approaches by different authors. These are the Halverstadt–Kumler method[6] and

(1) Paper 12: Caswell, L. R.; Campbell, J. A. B.; Cecil, R. *J. Heterocycl. Chem.* **1979**, *16*, 225.

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(3) Faculty Research Associate of Texas Woman's University, Spring, 1983.

(4) Cowley, E. G.; Partington, J. R. *J. Chem. Soc.* **1936**, *47*.

(5) Hess, K.; Frahm, H. *Ber. Dtsch. Chem. Ges. B* **1938**, *71*, 2627.

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