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LETTERS

Dissociation Constants of Carboxylic Acids by ^{13}C-NMR in DMSO/Water

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Abstract: Measurements on eighteen ^{13}COOH-labeled acids, including hydrogen-bonded and non-hydrogen-bonded standards are self-consistent and suggest strongly that the aqueous pK_as of bilirubin are within the normal range for aliphatic carboxylic acids. Our empirical observations provide further evidence that the ^{13}C-NMR method is valid when suitable buffers, DMSO concentrations and extrapolations are used and refute recent suggestions to the contrary.

We recently used ^{13}C-NMR to estimate pK_as of simple mono- and dicarboxylic acids and several compounds related to bilirubin, a natural dicarboxylic acid. We made measurements in water and aqueous solutions containing not more than, and mostly very much less than, 31 mole% $(CD_3)_2SO$. With these methods we observed literature values for standard acids and were able to estimate the pK_as of bilirubin to be ~4.2–4.9, as expected for two non-interacting aliphatic COOH groups.[1] We undertook those studies because of our interest in applications of ^{13}C-NMR to ionization phenomena and our skepticism about recent reports[2] that had put the pK_as of bilirubin in the range 6.8–9.3, far from the typical aliphatic COOH value of ~5.

A subsequent Letter[3] has suggested that our estimates are unreliable. The criticisms appear to boil down to two main objections: (1) that we ignored effects of DMSO on COOH dissociation; and (2) that we failed to consider intramolecular interactions of carboxyl groups. Additionally, the Letter implied that our measurements of the pK_as of standard acids were inconsistent with accepted values. In the following we present additional validation of the ^{13}C-NMR method and show that it provides accurate estimates of the aqueous pK_as of aliphatic carboxylic acids, even those prone to intramolecular hydrogen bonding.

Fig. 1a shows ^{13}C-NMR titration data for [1-^{13}C]- phenylacetic acid in solutions containing from 0–31 mol% DMSO.[4] In these experiments, to avoid DMSO-buffer interactions, pH was adjusted only with HCl/NaOH and the pH plotted is the measured pH of the solution used for NMR runs. Textbook titration curves were obtained, even at 31 mole% DMSO, and apparent pK_a values for each solvent could be readily derived by standard methods. Plotting these apparent pK_as versus mole% DMSO (Fig. 1b) gives an acceptable linear relationship over the range of DMSO concentrations used and shows that apparent pK_as for DMSO-water solutions containing up to 31 mole% DMSO can be reliably extrapolated to give a good estimate of the value in DMSO-free solution (measured pK_a, 4.31; extrapolated, 4.35). For comparison, Fig. 1c shows ^{13}C-NMR titrations for ^{13}C-phenylacetic acid taken from an earlier paper.[1a] In this experiment, buffers were used to adjust pH and the pH used in plotting the data was

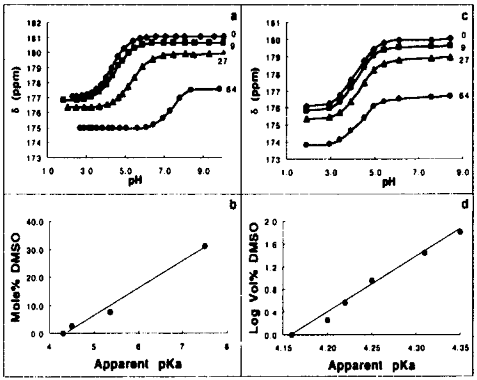

Figure 1. ^{13}C-NMR titrations of ~99% [1-^{13}C]- phenylacetic acid in DMSO/H_2O. Numbers beside the curves in (a) and (c) are vol% of DMSO. In (c) curves for 1.8 and 3.6 vol% DMSO have been omitted for clarity. For other details see the text.

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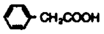
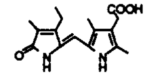
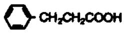
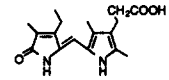
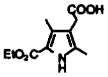
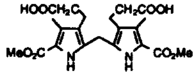
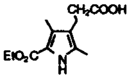
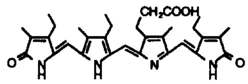
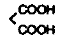
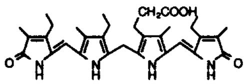
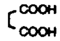
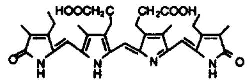
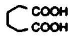
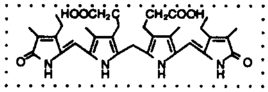
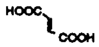
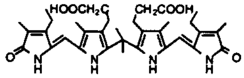
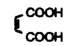
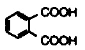
Table 1. Aqueous pK_a Values of Carboxylic Acids Obtained by Direct Measurement and by Extrapolation from Apparent pK_as Determined in DMSO/Water

	pK_a				pK_a
Compound	Direct	Lit.	Extrapolated	Compound	Extrapolated
	4.31[a] 4.16	4.31	4.35[a] 4.35		4.59
	4.59	4.66	4.56		4.68
	4.37		4.36		4.2 5.4
	4.68		4.68		4.3
	2.85 5.44	2.85 5.70			4.5
	4.30 5.48	4.21 5.71	4.4 5.2		3.9 5.3
	4.19 5.40	4.44 5.44	4.40 5.41		4.2 4.9
	3.2 4.4	3.02 4.38	3.2 4.4		4.2 5.6
	1.8 6.0	1.92 6.23	1.9 6.3		
	3.1 5.3	2.95 5.41	3.1 5.2		

[a] This work. Other measured values are taken from our previous papers.[1]
The dotted box surrounds mesobilirubin XIIIα.

the measured pH of the buffer before addition of phenylacetic acid in DMSO. Again, apparent pK_as can be readily derived from the curves.[5] In this case, plotting log vol% DMSO versus apparent pK_a gives a good linear relationship over the range of DMSO concentrations used and the pK_a for phenylacetic acid in water can be accurately estimated from the apparent values in DMSO-water (measured pK_a, 4.16; extrapolated, 4.16). This is the method that was used, for convenience, in our previous publications.[1] Excellent NMR titration curves and linear %DMSO/pK_a relationships were observed for phenylpropionic, adipic and several mono and dipyrrolic acids (Table 1). For standards, pK_as obtained by extrapolation of the DMSO–water data agreed well with values measured in DMSO-free water and with literature values. For the pyrrole acids, measured pK_a values were close to those of normal aliphatic COOH, as expected. These empirical observations provide strong support for the reliability of the ^{13}C-NMR titration method using low mole fractions ($\leq$ 0.31) of DMSO as cosolvent. However, to obtain satisfactory titration curves for sparingly soluble acids by this method, highly ^{13}C-enriched acids may be required. Careful choice of buffer is also necessary.[1]

Previously we used principally acetate and Tris buffers, but phosphate buffer was used in a few studies over a very narrow pH range and KCl/HCl, CH_3COOH/HCl or dilute HCl were used to attain low pH conditions. Figure 2a shows the effect of DMSO on the measured pH of several aqueous solvents. For HCl/

KCl, CH_3COOH/HCl, acetate and Tris buffers the presence of DMSO up to 8.6 mol% (27 vol%) has little effect. Above 8.6 mol%, deviations from linearity and from the line of equivalence become progressively more noticeable. Nevertheless, smooth titration curves can be obtained even at 31 mol% (64 vol%) DMSO by suitable choice of solvent (*e.g*. Fig. 1c). Apparent pK_as derived from these showed excellent linear correlation with apparent pK_as obtained at DMSO concentrations <8.6 mol% (Fig. 1d). In total we have observed smooth curves for some fifteen acids in buffers containing 31 mol% DMSO. However, our estimates for the aqueous pK_as of bilirubin do not solely depend on measurements using 31 mol% DMSO since we also obtained titration curves for the crucial model mesobilirubin XIIIα (Table 1) at 8.6 mol% DMSO.[1d,e]

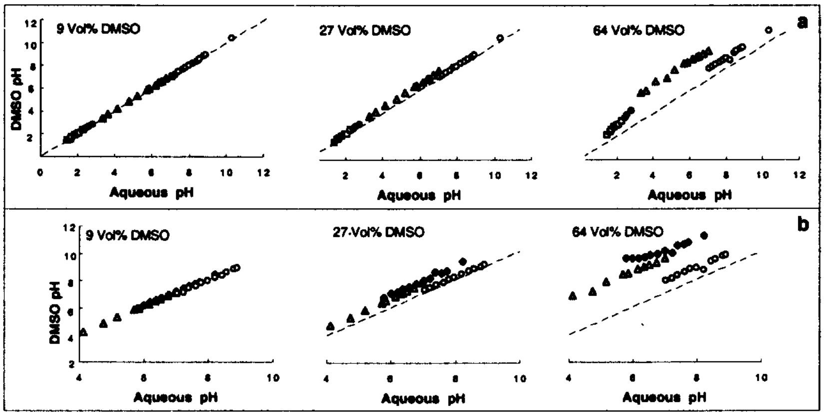


Figure 2. Plots of pH before (*abscissa*) and after (*ordinate*) adding DMSO to aqueous buffer solutions.
□ 0.05M KCl/HCl,
● 0.1M HOAc/HCl,
Δ 0.1M acetate,
◆ 0.05M phosphate,
o 0.05M Tris.
Dashed lines are lines of identity. Plots for 3.6 vol% DMSO fell on the line of identity and are omitted.

The effect of DMSO is more marked with phosphate than with acetate or Tris buffers (Fig. 2b). Thus, we used phosphate in only a few measurements over a narrow pH range (6.4–6.9), and not at 31 mol% DMSO.

Our previous studies were not aimed at measuring pK_a values of organic acids in DMSO-buffer mixtures or effects of DMSO on buffer pH.[3] Their purpose was to test the use of ^{13}C-NMR for estimating aqueous pK_as of bile pigments and related compounds. Because of the low water solubility of some of these, we used DMSO-d_6 as co-solvent when necessary. DMSO has a marked effect on COOH dissociation at high concentrations,[6] with apparent pK_as rising rapidly with increasing [DMSO] once it exceeds ~50 mol%, but there have been few studies on its effect at the relatively low concentrations that we used. For this reason we ran extensive controls on model compounds and acids of known pK_a to establish whether, and under what conditions, apparent pK_as measured in DMSO-water mixtures could be reliably extrapolated to give aqueous pK_a values. Some of our results are summarized in Table 1. For every acid at every concentration of DMSO that we used, we observed excellent $^{13}COOH$ chemical shift titration curves, from which apparent pK_a values could be readily derived. From these, pK_a values for water could be estimated by linear extrapolation from plots of apparent pK_a versus [DMSO]. As shown in this paper using phenylacetic acid as example, titration curves can be constructed by plotting ^{13}C chemical shift versus either the measured pH of the NMR solution or the measured pH of the buffer before addition of DMSO and acid. Either way, smooth curves are obtained (Fig. 1) from which apparent pK_as can be derived. For concentrations of DMSO up to 31 mole%, and with the buffers used, good linear correlations between apparent pK_a and either mole% DMSO (Fig. 1b) or log vol% DMSO (Fig. 1d) were obtained depending on the method used to construct the titration curves and derive a set of apparent pK_as.[7] From these, pK_a values for water could be estimated with excellent agreement with the literature for standards. Deviations from linearity in the pK_a/[DMSO] plots were observed at DMSO concentrations >31 mole%. For this reason, our measurements were done at DMSO mole fractions not >0.31 and in every case, except for two tetrapyrrolic monocarboxylic acids, were also determined at much lower concentrations (0.5–10 mole%). The weak effect of DMSO at *low* mole fractions is consistent with previous studies.[8] Most of the examples cited[3] showing large effects of added DMSO refer to solutions containing >50 mole% DMSO or even pure DMSO and have little bearing on our work.

The carboxyl groups in some of the compounds we studied, like those in bilirubin, can undergo internal hydrogen bonding. It has been suggested[3] that our methods may be invalid for estimating the aqueous pK_as of such compounds. However, we tested that very possibility by studying models,[9] such as maleic and phthalic acids, in which strong intramolecular hydrogen bonding is known to occur and by examining pairs of matched compounds in which hydrogen bonding occurs in only one member of the pair (*e.g.* maleic and fumaric acids; verdinoid and rubinoid propionic acids) (Table 1).[1d,e] Our results showed clearly that the ^{13}C-NMR method yields acceptable pK_a estimates for those compounds too and that hydrogen-bonding has only a very weak effect on the acid dissociation of mesobilirubin XIIIα.

Our detailed ^{13}C-NMR studies on eighteen mono and dicarboxylic acids, including several standards, are self-consistent and show that the method is reliable, provided that suitable aqueous solvents, model compounds, and standards are used, that DMSO concentrations are kept low, relative to the molar concentration of water, and that acid concentrations are not too high. From our data we estimate that the pK_as for bilirubin are in the range ~4.2–4.9,[1e] as most others have found.[1d] Published pK_as of 6.8 and 9.3[2b] and of 8.1 and 8.4[2a] cannot all be correct, and our data, taken *in toto*, provides strong evidence that none of them are. While our aqueous pK_as for bilirubin in water are only estimates, our data on model compounds makes it seem extremely unlikely that the use of DMSO as cosolvent, as we described, would have led to an underestimate of as much as 3 or 4 pK_a units.

Acknowledgements. We thank the National Institutes of Health (HD-17779, DK26307) for support.

References and Notes

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3. Mukerjee, P.; Ostrow, J. D. *Tetrahedron Lett.* **1998**, *39*, 423–426.
4. For general methods and synthesis of ^{13}COOH-labeled acids, see previous publications.[1] pH was measured with a glass combination electrode calibrated immediately before use with reference buffers and recorded only when constant. For NMR titrations of [1-^{13}C]- phenylacetic acid shown in Fig. 1a, 51 mg of acid was dissolved in 25 ml of solvent followed by 3-5 drops of 10% HCl. The pH was adjusted by addition of 0.1M NaOH in the same solvent. After each addition of base, pH was recorded and a sample transferred to a tube for ^{13}C-NMR measurements. In measurements of the effects of DMSO on buffer pH the following solvents were used: 0.2M KCl/HCl (0.2M KCl adjusted with 0.2M HCl to desired pH and diluted to 0.05M KCl), 0.1M HOAc/HCl (0.1M acetic acid adjusted with 0.1M HCl), 0.1M acetate buffer (0.1M acetic acid adjusted with 0.1 M sodium acetate), 0.1M phosphate buffer (0.1M KH_2PO_4 adjusted with 0.1M NaOH then diluted to 0.05M PO_4), 0.1M Tris buffer (0.1M Tris base adjusted with 0.1M HCl then diluted to 0.05M Tris); pH values were measured at 25 °C.
5. The pK_as for DMSO-buffer solutions obtained by this method are apparent, not true, pK_as that have no physical significance and are useful only for extrapolating to 0% DMSO. Comparing them with other reported pK_as[3] or using them in calculations[3] could be misleading.
6. (a) Ballash, N. M.; Robertson, E. B.; Sokolowski, M. D. *Trans. Faraday Soc.* **1970**, *66*, 2622–2628. (b) Baughman, E. H.; Krevoy, M. M. *J. Phys. Chem.* **1974**, *78*, 421–423. (c) Bordwell, F. G. *Acc. Chem. Res.* **1988**, *21*, 456–463.
7. As noted,[6a] there is no obvious reason for pK_a to be a linear function of [DMSO]. Where linear pK_a/[DMSO] curves have been reported, the slopes vary widely among different groups of acidic compounds.
8. (a) Rubino, J. T.; Berryhill, W. S. *J. Pharm. Sci.* **1986**, *75*, 182–186. (b) Siow, K.-S.; Ang, K.-P. *J. Solution Chem.* **1989**, *18*, 937–947. (c) Vesala, A.; Saloma, E. *Acta Chem. Scand. A* **1976**, *30*, 277–280.
9. As noted,[3] benzoic acid may not be a reliable model. We did not use benzoic acid.