Stereochem. **1994**, *21*, 1; g) E. Vedejs, M. J. Peterson, *Adv. Carbanion Chem.* **1996**, *2*, 1.
[9] S. Trippett, D. M. Walker, *J. Chem. Soc.* **1961**, 1266.
[10] General procedures for the reaction with ester ylides and the cyano ylide together with preparations and characterization for neopentyl (triphenylphosphoranylidene) acetate, neopentyl dihydrocinnamate and pyrene-1-ylpropionitrile are contained in the Supporting Information.
[11] E. von Rudloff, *Can. J. Chem.* **1956**, *34*, 1413.
[12] P. C. Bulman Page, M. J. McKenzie, S. M. Allin, D. R. Buckle, *Tetrahedron* **2000**, *56*, 9683.

A Straightforward Detection of Deprotonated Conformers of Malonic Acid by Solid-State ^{13}C NMR Spectroscopy

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There is wide interest in the study of dicarboxylic acids, which are known to be substrates of a large number of enzymes.[1] Very recently, it has also been reported that malonic acid based inhibitors of matrix metalloproteinases involved in tissue remodeling, and thus in various disease processes such as tumor development and joint destruction, reveal a unique mode of binding to the enzymes.[2] In view of the role of malonic acid in biological metabolism, and of the resulting chemistry, the detection of its deprotonated forms during various processes is of prime importance.

Herein we demonstrate, for the first time, that solid-state ^{13}C NMR spectroscopy permits information to be obtained in a straightforward manner about the presence and the nature of various deprotonated forms of malonic acid in lyophilizates prepared from parent solutions at different pH values. The clear advantage of solid-state over liquid-state NMR spectroscopy arises from the existence of well-separated, isotropic ^{13}C signals for protonated and deprotonated species in the lyophilizate as a result of a dramatic slowing down of inter- and intramolecular proton exchanges on the NMR time scale.[3–4] Another benefit of solid-state measurements results from easy access to the principal values of carbon chemical shift anisotropy (CSA) tensors, which, by virtue of their nature, are much more sensitive to the changes in the ionization state and the hydrogen-bonding interactions than the isotropic chemical shift.[5]

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Figure 1 shows the changes in the isotropic positions in high-resolution ^{13}C cross polarization magic-angle-spinning (CP/MAS) NMR spectra of malonic acid lyophilizates prepared from solutions at different pH values. The principal elements δ_{ii} of protonated and deprotonated carboxy CSA

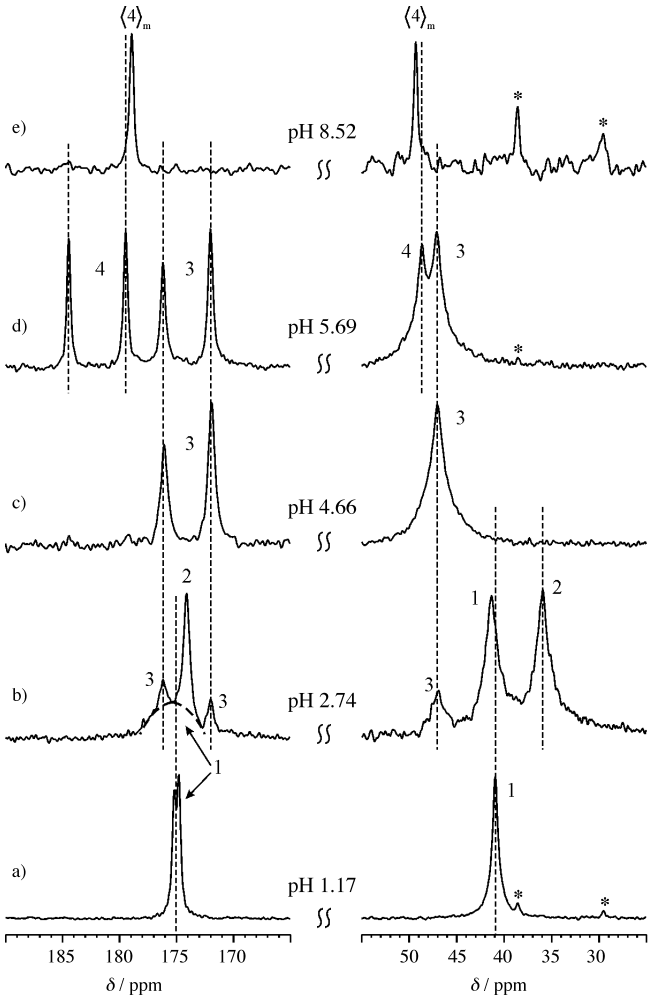

Figure 1. Evolution of isotropic positions in low-speed MAS ($\nu_r = 2.0$ kHz) ^{13}C NMR spectra of malonic acid lyophilized from solutions at pH 1.17 (a), 2.74 (b), 4.66 (c), 5.69 (d), and 8.52 (e). The numbered isotropic peaks refer to malonic acid (1), monoanion conformers (2) and (3), and double deprotonated rigid (4) as well as the motionally averaged ($\langle 4 \rangle_m$) form. For better visualization, the signals in (c) and (e) have been presented with equal height. The signals labeled as * are the isotropic positions of adamantane placed at the bottom of the rotor for the purpose of chemical shift calibration.

tensors were derived from spinning sideband manifolds (Figure 2). The isotropic chemical shifts δ_{iso} and the principal CSA elements δ_{ii} are reported in Table 1. The isotropic chemical shifts of hydroxy protons involved in hydrogen bonding, as revealed from high-speed ^{1}H MAS spectra (not shown), are also included.

The high-resolution ^{13}C spectra show that the successive steps of deprotonation are manifested by sharp changes in the δ_{iso}, δ_{11}, and δ_{22} values, which are significantly different for each species and for individual carboxy groups. We ascribe the two closely placed isotropic resonance peaks at pH 1.17 to

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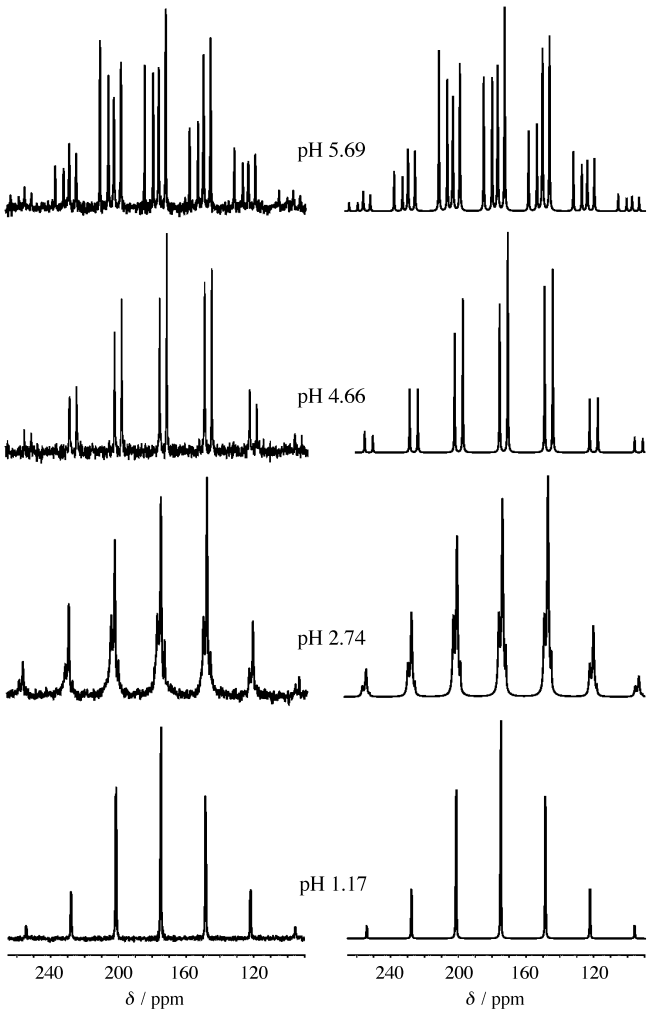


Figure 2. Experimental (left) and calculated (right) low spinning speed ($\nu_r = 2.0$ kHz) chemical shift anisotropy sideband manifolds of carboxy groups of malonic acid lyophilized from solutions at pH 1.17 (a), 2.74 (b), 4.66 (c), and 5.69 (d).

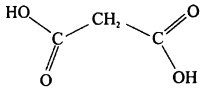
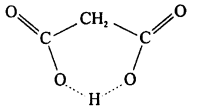
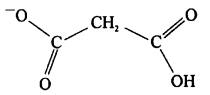
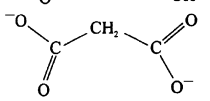
slightly magnetically inequivalent carboxy carbon atoms in the lowest energy conformer of malonic acid in which two carboxylic groups are in a nearly orthogonal arrangement, with one of them being coplanar with the carbon atom backbone.[6] The δ_{ii} values for this fully protonated malonic acid are close to those reported in alkanedicarboxylic acids.[7]

New isotropic signals are visible at pH 2.74. This clearly indicates that two different hydrogen malonate acid species are present in addition to the fully protonated form. Both types of deprotonated species show a more shielded δ_{22} value compared to the fully protonated form. We attribute these two deprotonated forms to different conformers of the hydrogen malonate ion, namely its planar and nonplanar form. A symmetrical C_{2v}, intramolecularly hydrogen-bonded planar structure was indeed found to be the most stable conformer in the case of the monoanion by ab initio calculations.[8] This conformer has to be responsible for the high-field resonance signal of the CH_2 group at $\delta = 35.9$ ppm and a single resonance signal of the carboxy groups at 174.1 ppm with δ_{11} and δ_{22} values equal to $\delta = 252$ and 165 ppm, respectively. The existence of this strongly intramolecularly hydrogen-bonded conformer at such a pH value corroborates with the appearance of a new isotropic peak corresponding to hydroxy groups at $\delta = 15.6$ ppm in the ^{1}H MAS spectrum.

The second, singly deprotonated form, is responsible for the resonance signal of the CH_2 group at $\delta = 47.1$ ppm and two signals at $\delta = 172$ and 176.8 ppm. We ascribe the higher and lower field peaks to the carboxy and carboxylate carbon atoms, respectively on the basis of different values of the δ_{11} element. We attribute this form to the most stable nonplanar conformer with carboxylate and carboxylic groups in a nearly orthogonal arrangement.[9] Somewhat surprisingly, this is a unique conformational form of the hydrogen malonate acid present in the lyophilizate at pH 4.66 in which a single isotropic chemical shift of hydroxy protons is seen at $\delta = 14.4$ ppm.

Very recently we have shown that solid-state, natural-abundance ^{13}C NMR determinations of acid:base ratios of lyophilized L-histidine can be used to measure its three pK values in parent solutions, without recourse to full titration curves and subsequent curve-fitting procedures.[4] In the

Table 1. ^{13}C and ^{1}H NMR data of malonic acid and its deprotonated species.

Entry	pH range	^{13}C CSA [ppm][a]				^{13}C [ppm]	^{1}H [ppm][b]	Form/conformer	pK[c]
		δ_{11}[d]	δ_{22}[d]	δ_{33}[d]	δ_{iso}	$\delta_{iso}^{CH_2}$	$\delta_{iso}^{hydroxyl}$		
1	1–3	235	180	109	174.8	40.3	13.2		
		242	175	109	175.2				
2	2–3	252	165	105	174.1	35.9	15.6		2.57
3	2.5–5.7	242	166	107	172	47.1	14.4		
		254	167	108	176.8				
4	5.7–8.5	242	191	105	179.4	48.6			5.52
		244	204	104	184.4				

[a] Carboxy and carboxylate groups. [b] Hydrogen-bonded hydroxy protons. [c] Calculated from solid-state acid:base ratios. [d] Average standard deviations of ± 2 ppm.

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present case, a pK_1 value of 2.57, relative to the first ionization of malonic acid, can be obtained from the ratio of the integrated intensities of the deconvoluted, well-separated signals of CH_2 groups at pH 2.74. This pK_1 value is indeed remarkably close to the average pK_1 value of 2.66 ± 0.05 calculated from a set of 22 values measured in aqueous solutions and reported in the IUPAC Stability Constants Database.[8]

A second step of the deprotonation of the carboxylic end is clearly visible in the ^{13}C spectra of lyophilizates prepared at pH values above 5.0. Compared to the spectrum recorded at pH 4.66, a second resonance signal of CH_2 and two new resonance signals of carboxylate groups appear at pH 5.69. These two carboxylate groups, as also revealed by different NMR relaxation techniques, do not become more involved in hydrogen bonding with remaining monoanions. We ascribe the presence of two isotropic peaks (instead of a single one), both with large deshielded δ_{22} values, to two different conformations of the carboxylate groups, and is reminiscent of the orthogonal orientation of these groups in the nonplanar conformer of the monoanion. This result indicates that the variation in the middle tensor element δ_{22} of the carboxy groups not only arises because of the change in the ionization state and the presence of hydrogen bonding,[5] but may also depend strongly (as in the present case) on the conformation of the relevant molecular fragment. Somewhat unexpectedly, the second deprotonation step leads to a backward shift of the δ_{11} value for the previously deprotonated site, while the newly deprotonated site maintains its δ_{11} value. We explain the observed backward shift in the value corresponding to the carboxylate end by the disappearance of hydrogen-bonding interactions in the doubly deprotonated form, and the unchanged δ_{11} value of the newly deprotonated end by the opposite, and consequently, canceling effects of deprotonation and disappearance of hydrogen-bonding interactions. Finally, as expected, the most shielded δ_{33} element, which is orthogonal to the plane of carboxy groups,[10] is rather insensitive to any changes, whatever their origin.

From the ratio of the integrated intensity of the resonance peaks of both forms, and taking into account the $T_{1\rho}(^1H)$ relaxation decay of the deprotonated, somewhat more mobile form on the kHz frequency scale, the calculated pK_2 value is 5.52, which is only slightly higher in value than the average pK_2 value of 5.30 ± 0.06 calculated from a set of 22 values measured in aqueous solutions.[8] As in the case of L-histidine,[4] we can assign such a difference to temperature effects, since the freezing out of aqueous parent solutions begins at temperatures close to 0 °C, whereas most of the liquid-state titrations were performed at 25 °C.

Only a single resonance signal is observed for both carboxylate groups in a lyophilizate prepared from a parent solution at pH 8.52. This is a consequence of the final disappearance of the three-dimensional network of hydrogen-bonded monoanions and consequently to a substantial molecular mobility of the doubly deprotonated form. This situation leads in turn to a greatly averaged CSA tensor of carboxylate groups with a single isotropic resonance position at 178.9 ppm, which is identical to that observed in the liquid state. Table 1 summarizes the presence of different ionic forms/conformers of malonic acid together with the calculated pK values.

In conclusion, we have shown that the solid-state ^{13}C NMR study of malonic acid lyophilizates allows a straightforward detection of its deprotonated conformers present in the parent solution at different pH values. Calculated pK values from solid-state acid:base ratios r are found to be equal to those classically measured in the liquid state. Numerous applications of such an approach using readily available ^{13}C-enriched dicarboxylic acid substrates can be envisaged in order to obtain a deeper insight into the chemistry of many enzymatic processes. We believe this study also to be the first experimental observation of the planar intramolecularly hydrogen-bonded monoanion present at pH values close to the pK_1 value of the parent solution. Futher investigations will be necessary to check if the equilibrium between the two conformers of the monoanionic species can be affected by the nature of the counterions.

Experimental Section

Lyophilization: A sample of malonic acid (104 mg, Aldrich, 99 %) was dissolved in bidistilled water, and the volume adjusted to 10 mL (0.1M solution). The pH value was adjusted with NaOH at 25 °C using an Orion 901 pH meter equipped with an Orion 91-03 electrode which was previously standardized with appropriate buffer solutions. Samples were frozen out in a 50-mL rotating flask immersed in liquid nitrogen to obtain a thin solid layer with a large surface area. The flask was then detached from the rotating system (Büchi Rotavapor) and fitted to a lyophilizator (CHRIS Alpha 1–4) for 6 h at −80 °C and 300 Pa.

NMR spectroscopy: ^{13}C spectra of lyophilizates were recorded on a Bruker DSX 300 spectrometer at 75.47 MHz equipped with a 4-mm CP/MAS[11] probe and using a cross-polarization contact time of 2 ms. Chemical shifts were referenced to TMS using the CH_2 signal of adamantane ($\delta_{iso} =$ 38.56 ppm) as an internal reference. Generally, 200 transients with a 30 s recycle delay were accumulated.

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[1] C. Walsh, *Enzymatic Reaction Mechanisms*, Freeman, San Francisco, **1979**.
[2] E. Graf von Roedern, F. Grams, H. Brandstetter, L. Moroder, *J. Med. Chem.* **1998**, *41*, 339 – 345.
[3] M. H. Frey, S. J. Opella, *J. Magn. Reson.* **1986**, *66*, 144 – 147.
[4] B. Henry, P. Tekely, J. J. Delpuech, *J. Am. Chem. Soc.* **2002**, *124*, 2025 – 2034.
[5] Z. Gu, A. J. McDermott, *J. Am. Chem. Soc.* **1993**, *115*, 4282 – 4285; Z. Gu, R. Zambrano, A. J. McDermott, *J. Am. Chem. Soc.* **1994**, *116*, 6368 – 6372; Y. Wei, A. C. de Dios, A. J. McDermott, *J. Am. Chem. Soc.* **1999**, *121*, 10389 – 10394.
[6] E. M. S Maçôas, R. Fausto, J. Lundell, M. Pettersson, L. Khriachtchev, M. Räsänen, *J. Phys. Chem.* A **2000**, *104*, 11725 – 11732.
[7] N. R. Jagannathan, *Magn. Reson. Chem.* **1989**, *27*, 941 – 946.
[8] J. F. Powell, The IUPAC Stability Constants Database, Academic Software and IUPAC, **1992**–**2000**.
[9] M. Merchán, F. Tomás, I. Nebot-Gil, *J. Mol. Struct.* **1984**, *109*, 51 – 60.
[10] F. Separovic, R. Smith, C. S. Yannoni, B. A. Cornell, *J. Am. Chem. Soc.* **1990**, *112*, 8324 – 8328.
[11] C. A. Fyfe, *Solid State NMR for Chemists*, C.F.C. Press, Guelph, ON, Canada, **1983**.

  0044-8249/02/4124-4745 $ 20.00+.50/0