Determination of isotope effects on acid–base equilibria by ^{13}C NMR spectroscopy

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New results from isotope effect measurements on acid–base equilibria by ^{13}C NMR spectroscopy show that in fatty acids deuterium isotope effects extend at least up to seven bonds and have unexpectedly small attenuation. In deuterium-substituted benzoic acids the isotope effect on pK_a is practically independent of the site of deuterium substitution. ^{2}H isotope effects on carboxy ionization in glycine and alanine are smaller than in the corresponding carboxylic acids. Different protonation shifts in unlabelled and labelled compounds are recorded. ^{18}O and ^{13}C isotope effects in acetic acid and ^{15}N effects in glycine isotopomers were measured. The application of auxiliary isotope substitution is demonstrated for the measurement of very small differences in pK_a values.

Introduction

A general method for measuring pK_a differences by ^{13}C NMR spectroscopy was elaborated in our previous publication.[1] In the present report, some additional aspects of the application of this method to isotope effect studies on acid–base equilibria are discussed. Pioneering work in this field[2] deserves more attention, since only a couple of research groups have used it mainly for ^{18}O isotope effect studies[3–5] and, in one case, for the measurement of the ^{15}N isotope effect in glycine.[6] The deuterium isotope effect has been determined by this method only in formic acid.[2] The conflicting results concerning the ΔpK_a value of [2,2,2-$^{2}H_3$]acetic acid, measured more than 30 years ago[7,8] have not found a reasonable explanation. Therefore a systematic study of deuterium isotope effects in carboxylic acids and in amino acids along with heavy atom isotope effects in these compounds was undertaken.

Experimental

Isotope effects on pK_a values were obtained from measurements of ^{13}C chemical shift differences on the atoms close to the ionisation site of labelled and unlabelled compounds. Such atoms generally behave as sensitive indicators during the titration of the mixtures. Ratios between corresponding dissociation constants were calculated from the plots of these chemical shift differences against the degree of protonation of the reference (unlabelled) compound. These plots give bell-shaped curves described by eqn. (1):

$$\delta - \delta^{a} = \delta_{d} - \delta^{a}_{d} - n(\delta_{d} - \delta_{p}) + Rn(\delta^{a}_{d} - \delta^{a}_{p})/[1 + (R - 1)n] \quad (1)$$

where δ is the chemical shift of the reference compound and δ_d and δ_p are the chemical shifts of the reference compound in the deprotonated and protonated states. The corresponding chemical shifts for the measured compound are given by δ^a, δ^a_d and δ^a_p; n is the degree of protonation of the reference compound, calculated from the formula $n = (\delta_d - \delta)/(\delta_d - \delta_p)$, and R is the ratio between the dissociation constants of reference and measured compound, calculated by the data fitting programs Origin (Microcal. Inc.) or Grafit (Erithacus Software Ltd.). NMR studies were performed on a Bruker AMX-500 spectrometer at 293 K. Details of the method and experimental procedure are reported in our previous publication.[1] For the study of higher fatty acids and benzoic acid isotopomers dioxane was required to increase the solubility of these acids in water.

^{18}O-labelled compounds were prepared by ^{18}O exchange under acidic conditions using ^{18}O-enriched water. The mixture of all isotopomers of deuteriated acetic acids was obtained by heating a sodium acetate–D_2O solution at 150 °C in a sealed glass tube for 24 h. [3,3,3-$^{2}H_3$]Propionic, [4,4,4-$^{2}H_3$]butyric, [6,6,6-$^{2}H_3$]-caproic and [2,3,4,5,6-$^{2}H_5$]benzoic acids originated from Cambridge Isotope Laboratories. The first of them was a gift from Larodan Fine Chemicals AB, Malmö, Sweden. Monodeuteriated benzoic acids and [2,3,5-$^{2}H_3$]benzoic acid were prepared from the corresponding monobromo or 2,3,5-triiodo derivative by reductive dehalogenation with Devarda's (Cu-Al-Zn) alloy in NaOD–D_2O solution.[9] In contrast to Raney Ni-Al the use of Cu-Al alloy results in high purity of deuteriated isotopomers. [^{15}N]Glycine was made from Boc-[^{15}N]glycine[10] by deprotection with formic acid[11]: 0.25–1 mmol of Boc-derivative was dissolved in formic acid (2–5 ml) in a small test tube in which all subsequent steps took place. After 3–6 h the acid was evaporated *in vacuo*. Upon addition of diethyl ether, a white, solid product was obtained which was carefully precipitated by centrifugation and the diethyl ether was decanted. Fresh diethyl ether was added twice followed by centrifugation. The yield of amino acid was 96–98% after drying.

[2-^{13}C,^{15}N]Glycine was made from Boc-[2-^{13}C,^{15}N]glycine[10] and L-[3,3,3-$^{2}H_3$]alanine from Boc-L-[3,3,3-$^{2}H_3$]alanine[12] using the same procedure.

Results and discussion

Long range (secondary) deuterium isotope effects

Long range deuterium isotope effects were studied in a series of fatty and benzoic acids with different numbers of intervening bonds between the label and the ionisation site. From the experimental point of view comparative measurements of deuteriated and undeuteriated compounds by ^{13}C NMR spectroscopy are facilitated by significant isotope shifts. A comparison of deuterium isotope effects on ^{13}C chemical shifts in some measured acids are presented in Table 1. Triple ^{2}H ω-substitution in fatty acids results in quite large long range effects, *e.g.* 76 ppb (9.6 Hz at 11.7 T) over three bonds in [4,4,4-$^{2}H_3$]butyric acid, which gives sufficient separation of signals. In

Table 1 ^{2}H isotope effects on chemical shifts[a] of deuteriated aliphatic carboxylic acids and deuteriobenzoic acids

	Aliphatic ω-[$^2H_3$] acids[b]			[^{2}H]Benzoic acids[c]		
Position	Propionic	Butyric	Caproic	*o*-	*m*-	*p*-
α	256	285	297	265	270	292
β	76	85	88	78/113	108/106	110
γ	—	25	28	<3/15	<3/4	<3
δ	—	—	−6	<3	<3	<3
ε	—	—	<1	—	—	—
COO	−10	−8	−2	<3	<3	<3

[a] In ppb, high field shifts positive, in aqueous solution. [b] Data reduced per single ^{2}H substitution. [c] Ring carbon with lower/higher number.

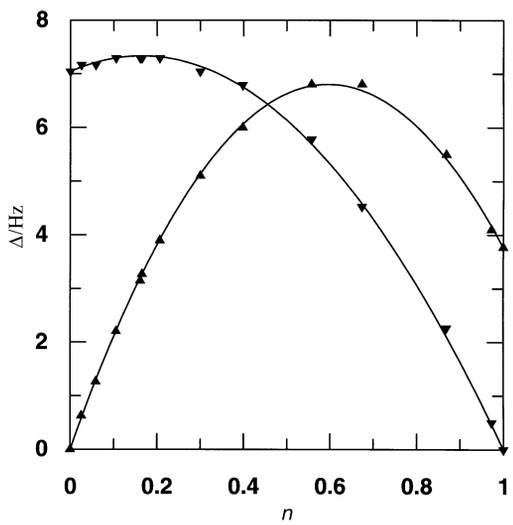

Fig. 1 Measured ^{13}C chemical shift differences on titration of a mixture of acetic and [2,2,2-$^2H_3$]acetic acid to determine the deuterium isotope effect on K_a: ▼-methyl carbons; ▲-carboxy carbons

the study of isotopic shifts in monodeuteriated benzoic acids at 22.6 MHz,[13] high field shifts were observed only on substituted carbon and its nearest neighbours. Our measurements at 125.7 MHz show some additional long range effects and lower the limit of measurable differential chemical shift values (3 ppb, in[13] 15 ppb). Nevertheless, long range isotopic shifts in benzoic acid are definitely smaller than in fatty acids. This is best illustrated by the absence of an isotopic shift on the carboxy carbon even in the [2-^{2}H] isotopomer.

Measurements of differential shieldings are somewhat complicated by the broadening of ^{13}C signals to several Hz from the coupling with deuterium nuclei over two and three bonds (in acetic acid $^2J_{CH}$ = 7.3 Hz,[14] in aromatic compounds $^3J_{CH}$ is *ca.* 8 Hz). This broadening is clearly seen on many signals and it lowers the spectral resolution.

The results of isotope effect measurements on acid–base equilibria in carboxylic acids are presented in Table 2 and in Figs. 1–5. The number of useful sites for the pK_a studies depends on the molecule studied. It can be up to three, as in propionic and butyric acid (Figs. 3 and 4), but in several cases only a single site gives the desired information. Carboxy carbon is ruled out for caproic and benzoic acid due to the very small isotope shifts and spectral broadening. It is interesting to note that in benzoic acid the carboxy carbon shift (4.8 ppm), unlike that in fatty acids, is not the most sensitive one for the measurement of the degree of protonation. The most sensitive site is instead C–1 (*ca.* 6.8 ppm, Table 2) and third place is taken by the *para*-position with a 2.65 ppm high field (!) protonation shift. The remaining *ortho*-(0.63 ppm) and *meta*-(0.48 ppm) positions are quite insensitive. The results based on various positions were practically the same (Table 2), although quite different curves

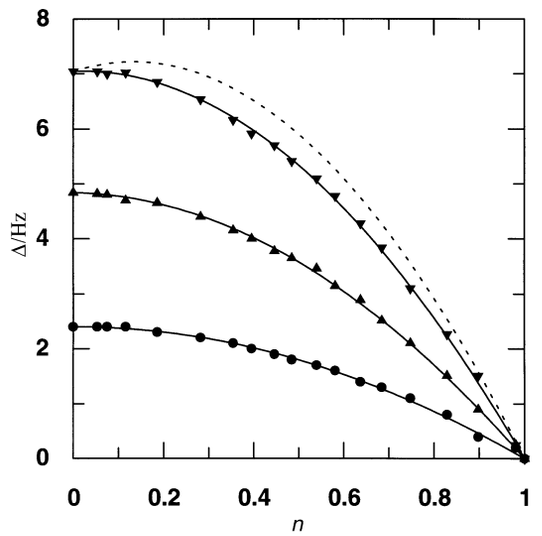

Fig. 2 Additivity of deuterium isotope effects on pK_a of acetic acid, measured on methyl carbon chemical shifts: ●-[2-^{2}H], ▲-[2,2-$^2H_2$], ▼-[2,2,2-$^2H_3$] isotopomers; --- curve for [2,2,2-$^2H_3$] from Fig. 1

describe the shift difference variation. Protonation shifts are different not only at different positions from the ionisation site, but also at the same sites in the labelled and unlabelled molecules. This last phenomenon has not been noticed in isotope effect studies.

Different deuterium isotope effects in trideuteriated acetic acid have previously been reported by Halevi *et al.*[7] (ΔpK_a = 0.026) and Streitwieser and Klein[8] (ΔpK_a = 0.014), who used potentiometric and conductometric methods, respectively. These authors were not able to find any reasonable explanation for the inconsistency of their results. The present data (Table 2, Fig. 1) are in good agreement with the latter,[8] which are also quoted by Ingold in his monograph.[15] Our binary mixture was about 0.1 M in each isotopomer, which is close to the upper concentration limit used previously.[8] At concentrations down to 0.004 M the isotope effect was practically the same and the authors concluded that cancellation of activity coefficient effects might occur.[8] Our experience with mixtures of carboxylic acids of higher concentration revealed a dependence of R on the total concentration of acids present in the sample.[1] Another sample with all deuteriated isotopomers (each *ca.* 0.25 M) was measured to check for high concentration effects, additivity of isotope substitution effects and differential shielding effects in the protonated and deprotonated states. The titration curves for the methyl carbons are presented in Fig. 2, which give for monodeuteriated acid R = 1.0074 (ΔpK_a = 0.0032), for dideuteriated acid R = 1.0144 (ΔpK_a = 0.0062) and for trideuteriated acid R = 1.0229 (ΔpK_a = 0.0098). Carboxy carbons cannot be used in this case because the four different broadened signals afford insufficient separation of the signals. The obtained data show good additivity in deuterium substitution effects and also in differential shielding between the protonated and deprotonated states. At the same time the R value is definitely smaller for the trideuteriated species at this higher concentration of acetic acid isotopomers as compared with the previous case (dashed curve in Fig. 2, Table 2).

The deuterium isotope effect measurements for propionic and butyric acid at three different sites (carboxy, α- and β-carbons) give consistent results (Figs. 3 and 4). In contrast to those for acetic acid the data reported for propionic acid[7] agree with our results, especially considering that a sample with only partial deuterium substitution in the methyl group was previously available.[7] In the mixture of [6,6,6-$^2H_3$]caproic acid and unlabelled isotopomer, carboxy carbons are separated at low and high pH values only by 0.8 Hz, the carboxy carbon of the deuteriated isotopomer being shifted to low field, as with all

Table 2 Deuterium isotope effects on pK_a in carboxylic acids

Acid	Measured site	δ_d–δ_p	R [a]	ΔpK_a	$\Delta\Delta G^{\ddagger}$ [b]
[^{2}H]Formic	COOH	6.51	1.082[c]	0.0342	46.5
[2,2,2-$^2H_3$]Acetic	COOH	4.60	1.0326 ± 0.0008	0.0139	18.9
	CH_3/CD_3	2.903/2.853	1.0298 ± 0.0008	0.0128	17.3
[3,3,3-$^2H_3$]Propionic	COOH	5.26	1.0191 ± 0.0006	0.0082	11.2
	CH_2	3.50	1.0188 ± 0.0005	0.0081	11.2
	CH_3/CD_3	1.856/1.820	1.0172 ± 0.0009	0.0074	10.1
[4,4,4-$^2H_3$]Butyric	COOH	4.84	1.0114 ± 0.0003	0.0049	6.7
	α-CH_2	3.81	1.0107 ± 0.0003	0.0046	6.3
	β-CH_2	1.38	1.0098 ± 0.0004	0.0042	5.8
[6,6,6-$^2H_3$]Caproic	β-CH_2	1.50	1.0012 ± 0.0003	0.0005	0.7
[2-^{2}H]Benzoic	C-1	6.783/6.779	1.0046 ± 0.0002	0.0020	2.7
[3-^{2}H]	C-4	−2.65	1.0045 ± 0.0002	0.0019	2.6
[4-^{2}H]	C-4	−2.627/−2.623	1.0042 ± 0.0005	0.0018	2.5
[2,3,5-$^2H_3$]	C-1	6.789/6.786	1.0137 ± 0.0002	0.0059	8.0
[2,3,4,5,6-$^2H_5$]	C-1	6.784/6.775	1.0230 ± 0.0002	0.0099	13.4

[a] With standard errors. [b] In cal mol^{-1}. [c] From ref. 2.

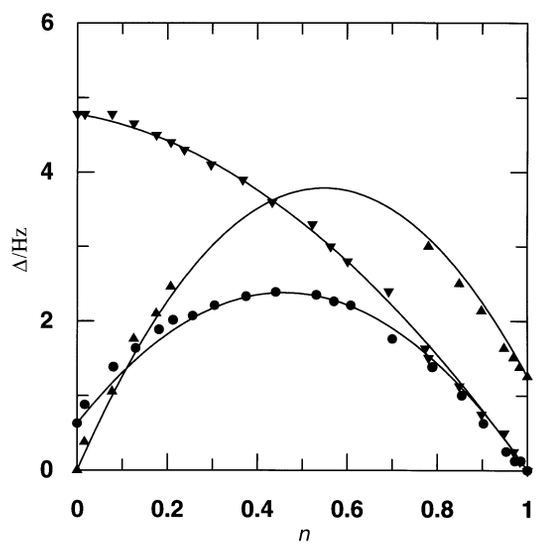

Fig. 3 ^{13}C NMR titration of a mixture of [3,3,3-$^2H_3$]propionic and unlabelled propionic acid: ▼-C–3; ●-C–2; ▲-C–1 (carboxy) carbons

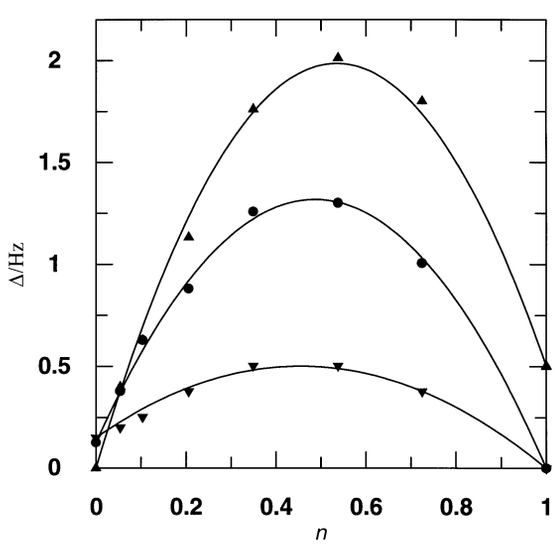

Fig. 4 ^{13}C NMR titration of a mixture of [4,4,4-$^2H_3$]butyric and unlabelled butyric acid: ▼-C–3; ●-C–2; ▲-C–1 (carboxy) carbons

other fatty acids. During the titration exchange broadening is observed and the carboxy site cannot be used for the measurement. The same broadening also influences α-carbon atoms to carboxy group without any measurable isotope shift. Well separated resonances from γ- and δ-positions cannot be used for the ΔpK_a measurement due to too small protonation shifts at these sites. The only suitable position in this case is the β-methylene carbon, which is shifted in the deuteriated isotopomer by 2.2 Hz to low (!) field, its protonation shift being only *ca.* 30% of that from the carboxy carbon. Nevertheless, this position can be exploited to determine the degree of protonation with the result showing that ω-trideuteriated caproic acid is weaker than caproic acid by 0.0005 units.

On the basis of the deuterium isotope effect in acetic acid, the corresponding values for higher fatty acids show unexpectedly small attenuation. In ref. 8 the authors have assumed that the magnitude of the deuterium isotope effects in fatty acids are consistent with the bond dipole changes and their attenuation obeys the regular fall-off of the inductive effect with distance by a factor of *ca.* 2.8 per carbon atom down the chain. These conclusions were based on the comparison of the ΔpK_a values of pivalic and acetic acid and their deuterio-isotopomers. On this basis, ΔpK_a for the propionic acid isotopomers should be 0.005, which is definitely lower than our measured value (0.008). The agreement is worse for the ω-trideuteriated butyric acid (calculated 0.0018, experimental 0.005). Finally, for the ω-trideuteriated caproic acid, the calculated value (ΔpK_a = 0.0002) is again less than the measured one (ΔpK_a = 0.0005).

The reason for this unexpected behaviour is most probably connected with the nonbonded interactions, which have been the object of earlier discussions of deuterium isotope effects.[8,16] More recently, isotopic perturbations of conformational equilibria have been observed in NMR spectra of various alicyclic compounds[17] and they are likely to be present also in aliphatic chains. This also explains the good separation of all corresponding signals in the butyric acid isotopomers and the unusual low field isotope shift of γ-carbon in the deuteriated caproic acid. The resulting variations of non-bonded interactions might also modify the pK_a values.

Deuteriated benzoic acids were studied to investigate the conclusions drawn only on the basis of [2,3,4,5,6-$^2H_5$] and [2,6-$^2H_2$] isotopomers of benzoic acid.[8] The reported ΔpK_a value for the pentadeuteriated isotopomer (0.010 ± 0.002) fits with the present result (Table 2). On the basis of additivity of isotope effects the reported value for the [2,6-$^2H_2$] isotopomer (0.003 ± 0.001) points to the possibility that in *meta*- or *para*-positions isotope effects can be even larger than in the *ortho*-position. Obviously, monodeuteriated isotopomers can clarify this situation. Measurement of the [2-^{2}H] isotopomer (Fig. 5) shows that the value obtained for the [2,6-$^2H_2$] isotopomer is a bit too small, although within the reported error limits.[8] Our results for the [3-^{2}H] and [4-^{2}H] (Fig. 5) give practically the same values as for the [2-^{2}H] isotopomer. Experimentally, the [4-^{2}H] isotopomer is the most difficult case because only C-4 can be exploited in this context, but C-4 gives a broadened triplet due to deuterium. The measurement of the [3-^{2}H] isotopomer, where C-4 is also

Table 3 Deuterium isotope effects on pK_a values of [2,2-$^2H_2$]glycine and [3,3,3-$^2H_3$]alanine in aqueous solutions

Acid	Protonation site	Measured site	Deprotonation shift (ppm)	R^a	ΔpK_a	$\Delta\Delta G^{\ddagger}$/cal mol^{-1}
Gly	COO	CH_2/CD_2	1.356/1.316	1.0056 ± 0.0002	0.0024	3.3
Ala	COO	CH	1.75	1.0142 ± 0.0003	0.006	8.3
Gly	NH_2	COO	7.66	1.054 ± 0.001	0.023	31.1
Gly	NH_2	CH_2/CD_2	2.683/2.558	1.051 ± 0.002	0.022	29.5
Ala	NH_2	CH_3/CD_3	4.177/4.090	1.0377 ± 0.0004	0.016	21.8

[a] With standard errors.

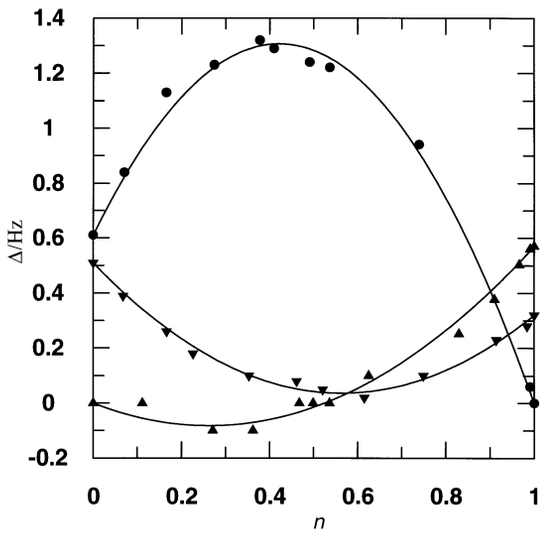

Fig. 5 ^{13}C NMR titration of binary mixtures of [2-^{2}H] (●, C-1), [3-^{2}H] (▼, C-4), [4-^{2}H] (▲, C-4) and undeuteriated benzoic acid. Concave curves for [3-^{2}H] and [4-^{2}H] isotopomers results from C-4 high field protonation shift

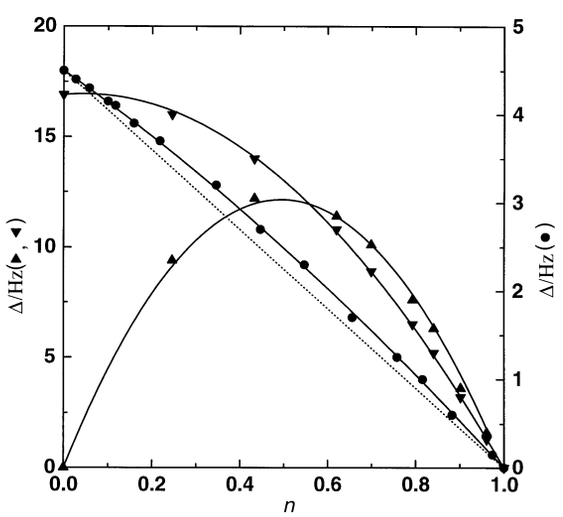

Fig. 6 ^{13}C NMR titration of a mixture of [2,2-$^2H_2$]glycine and glycine for the determination of K_a ratios for carboxy (right axis, ●-CH_2/CD_2; --- linear dependence) and amino (left axis, ▼-CH_2/CD_2, ▲-COO) groups

used, is more straightforward. Additional experiments with the [2,3,5-$^2H_3$] and [2,3,4,5,6-$^2H_5$] isotopomers were also performed. These are more convenient cases, because C-1 can be used and the results confirm the additivity of isotope effects within an aromatic nucleus. The results of our study on deuteriated isotopomers of benzoic acid are in full agreement with the earlier conclusions about the even distribution of deuterium isotope effects within the ring.[8] On the other hand, the predicted effect of one *meta*-deuterion on pK_a on the basis of the inductive effect model[8] 0.0012 is not in excellent agreement with the experiment.

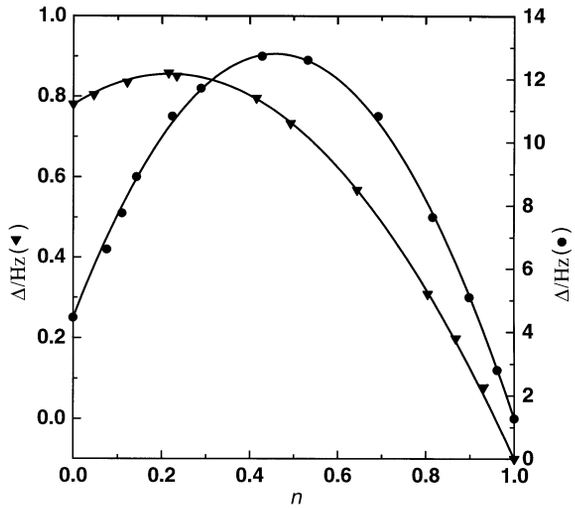

Fig. 7 ^{13}C NMR titration of a mixture of [3,3,3-$^2H_3$]alanine and alanine for the determination of K_a ratios for carboxy (right axis, ●-CH) and amino (left axis, ▼-CH_3/CD_3) groups

Secondary deuterium isotope effects in amino acids may be of practical importance and as first examples in this field [2,2-$^2H_2$]glycine and [3,3,3-$^2H_3$]alanine were measured in mixtures with the nondeuteriated counterparts. In these cases, the isotope effects on both pK_a values can be measured and the results are presented in Table 3 and Figs. 6 and 7. In the glycine experiment carboxyl protonation was monitored at the methylene carbon, whereas for the amino group the carboxy carbon was used. This is, in fact, the only possibility for determining the pK_a difference at the carboxy site because the carboxy carbons themselves are insufficiently resolved upon protonation, whereas the CH_2 and CD_2 signals are well separated due to the deuterium isotope effect on the shielding constant. For amino group protonation it is well known that the largest effect on chemical shifts are observed in the β-position and therefore the carboxy carbons are much more sensitive in this context. This does not rule out the possibility of using the methylene carbon for an additional check of the results. In the case of [3,3,3-$^2H_3$]alanine, the large protonation shifts of the carboxy carbon cannot be exploited because the deuterium isotope effect on the chemical shift is much smaller than in glycine and this results in overlapping signals. As noticed for acetic and propionic acid above, in alanine an unexpectedly large R value is observed for the carboxyl group protonation, more than twice the size of that for glycine. The isotope effect per deuterion on the pK_a value of the carboxyl group in glycine is less than one third of that in acetic acid. The deuterium isotope effect on the amino group pK_a in glycine is one order of magnitude higher as compared to that of the carboxy group. The deuterium isotope effect on the amino group pK_a in alanine is also remarkably high. Nevertheless, no straightforward explanation can be given for the significant effect on the carboxy group pK_a value in this amino acid.

Heavy atom isotope effects on pK_a

Among heavy nuclei studied in this context, ^{18}O has been used most frequently.[2–5] The relatively big change in atomic masses

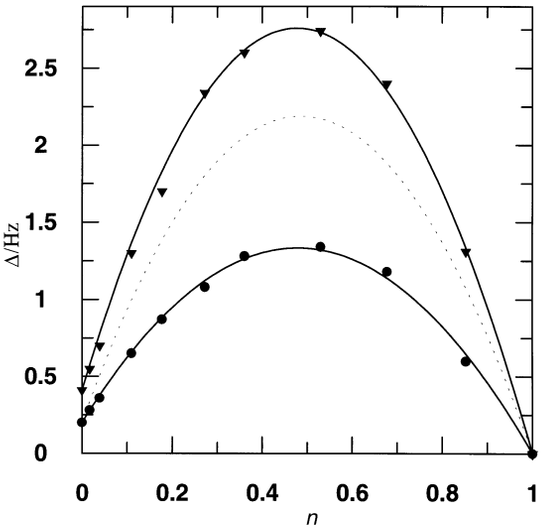

Fig. 8 Effects of one (●) and two (▼) ^{18}O atoms on the dissociation of acetic acid. The dashed curve corresponds to single ^{18}O substitution in formic acid.[2]

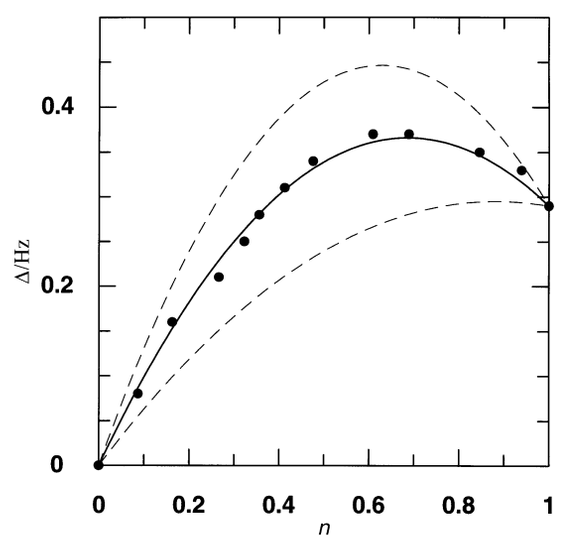

Fig. 9 Effect of ^{13}C substitution in acetic acid carboxy group on its dissociation constant: $R = 1.0021$; for the comparison calculated curves with $R = 1.003$ (upper curve) and $R = 1.001$ (lower curve) are also given

within the 'heavy atom scale' ($^{18}O/^{16}O = 1.125$), and being itself a protonation site, resulted in R values for formic acid of about 1.015 ($\Delta pK_a = 0.006$) for each isotopic substitution. We have now measured the acetic acid ^{18}O isotope effect and found that ΔpK_a per single isotope substitution is nearly twice as small as in formic acid, as shown in Fig. 8 ($R = 1.0086 \pm 0.0002$, $\Delta pK_a = 0.0037$), where the curve corresponding to single ^{18}O substitution in formic acid ($\Delta pK_a = 0.006\ 21$[2]) falls between the two curves for acetic acid. The ^{18}O isotope effect in glycine was also determined and the value obtained for a single substitution ($R = 1.0084 \pm 0.0001$) is the same as in acetic acid.

Recently, the $^{15}N/^{14}N$ isotope effect ($^{15}N/^{14}N = 1.07$) on the pK_a value of the amino group of glycine was determined by Rabenstein and Mariappan[6] and the R value 1.0224 ± 0.0003 was reported. From previous synthetic work on $^{13}C,^{15}N$ labelled glycines[18] we had access to all glycine isotopomers, including the ^{15}N labelled one. We have therefore been able to repeat the measurement and confirm the result. We obtained $R = 1.0227 \pm 0.0002$, giving further confidence in the method used.

The ^{15}N isotope effect on the pK_a value of the carboxy group

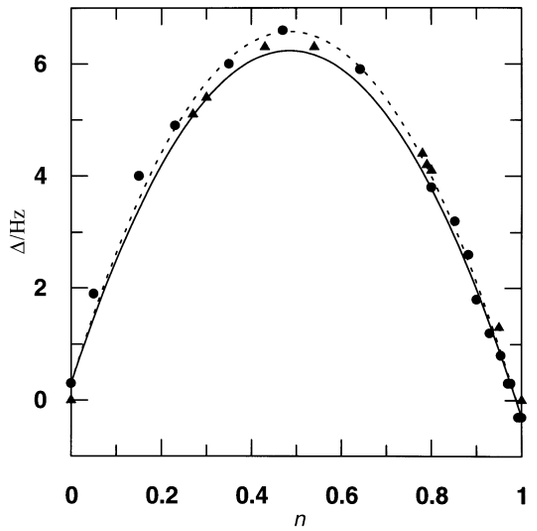

Fig. 10 Comparison of titration curves of [^{15}N] (▲) and [2-^{13}C,^{15}N]-glycine (●) in the mixtures with unlabelled glycine

is too small even for the present method. The introduction of a ^{13}C label into the carboxy group brings the heavy nucleus two additional bonds closer to the ionisation site. This also improves to some extent the prospect of observing the corresponding isotope effect, but raises the question of how to measure this effect using ^{13}C NMR spectroscopy. Proper species in the sample must be selected for the isotope effect measurements and not all molecules can be studied in this context, *e.g.* not formic acid with its single carbon atom. Therefore, carboxy-group-labelled acetic acid was chosen. Consequently, in this sample one exploits only the methyl ^{13}C signals of different multiplicity in the [2-^{13}C]CH_3COOH and [1,2-$^{13}C_2$]CH_3COOH isotopomers. In the proton decoupled spectrum one obtains a singlet from the first species and a doublet from the C-C coupled doubly labelled counterpart, which means that the ΔpK_a determination is carried out at natural abundance. The effect of protonation of ^{13}C labelled acetate is presented in Fig. 9. Only very small differences within 0.4 Hz were observed, which are again modified by different protonation shifts. The obtained R value 1.0021 ± 0.0001 gives a ΔpK_a value 0.0009 and free energy difference 1.2 cal mol^{-1}. For comparison two additional calculated curves with R values of 1.001 and 1.003 are also presented in Fig. 9 to show that they differ significantly from the observed dependence.

In contrast to the previous examples, multiple labels give additional possibilities for ΔpK_a studies. In Fig. 10 the results on protonation of the amino group in [2-^{13}C,^{15}N]-glycine are presented. In this experiment $R = 1.0239 \pm 0.0003$, $\Delta pK_a = 0.0103$, $\Delta\Delta G^{\ddagger} = 14$ cal mol^{-1}. The bell-shaped curve originating from this experiment is clearly shifted from that of [^{15}N]glycine and thus gives the possibility of estimating the ^{13}C isotope effect from the methylene carbon on the pK_a value of the amino group: $R = 1.0012 \pm 0.0005$, $\Delta pK_a = 0.0005$ and $\Delta\Delta G^{\ddagger} = 0.7$ cal mol(!). The consequence of the auxiliary ^{15}N labelling has, to our knowledge, not been exploited previously.

All of the above examples of isotope effects and also literature data show that replacement of a lighter nucleus by a heavier one always results in smaller dissociation constants (higher pK_a values). A summary of these data is presented in Table 4.

A reasonable explanation of isotope effects involves changes in vibrational states of molecules.[19] Finding quantitative relationships between these parameters is a problem which might be studied, together with the presented data, by the use of this very sensitive method based on ^{13}C NMR spectroscopy.

Table 4 Summary of long range isotope effects to pK_a values in organic compounds (per single isotope atom substitution)[a]

Effect	Compound	N^b	R	ΔpK_a	$\Delta\Delta G^{\ddagger c}$
$^2H/^1H$	Formic acid	2	1.082	0.034	46.5
$^2H/^1H$	Acetic acid	3	1.011	0.0047	6.5
$^2H/^1H$	Propionic acid	4	1.0062	0.0027	3.6
$^2H/^1H$	Butyric acid	5	1.0039	0.0017	2.3
$^2H/^1H$	Caproic acid	7	1.0002	0.0002	0.2
$^2H/^1H$	Benzoic acid	4,5,6	1.0045	0.0020	2.6
$^2H/^1H$	Glycine -COO	3	1.0028	0.0012	1.7
$^2H/^1H$	Glycine -NH_2	2	1.026	0.011	15.2
$^2H/^1H$	Alanine -COO	4	1.0047	0.002	2.8
$^2H/^1H$	Alanine -NH_2	3	1.0125	0.005	7.3
$^{18}O/^{16}O$	Formic acid	0	1.015	0.006	8.2
$^{18}O/^{16}O$	Acetic acid	0	1.0085	0.0037	5.0
$^{18}O/^{16}O$	Glycine -COO	0	1.0084	0.0036	4.9
$^{15}N/^{14}N$	Glycine -NH_2	0	1.0227	0.010	13.3
$^{13}C/^{12}C$	Acetic acid	1	1.0021	0.0009	1.2
$^{13}C/^{12}C$	Glycine -NH_2	1	1.0012	0.0005	0.7

[a] Results of present work except for formic acid.[2] [b] Number of bonds to the protonation site. [c] In cal mol^{-1}.

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