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LONG-RANGE SHIELDING OF PROTONS IN CARBOXYLIC COMPOUNDS

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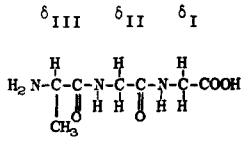
Relatively few studies of the dependence of proton chemical shifts on ionisation state have been published. Communications related to this work report the influence of pH variation for methyl amines (1), for several naturally occurring amino acids (2-6), for some glycyl peptides (7-10), and for a series of poly-functional carboxylic acids (11-13).

With the aim to distinguish long-range shielding, which is dependent on anisotropy and dipole effects and therefore on spatial parameters and temperature, from inductive effects, we undertook the recording of PMR spectra of a series of peptides and a series of saturated and unsaturated carboxylic acids in their different ionisation states.

All compounds studied were commercially available and their purity was checked by means of GLC, paper chromatography, refractive index, IR or NMR spectroscopy. If necessary, they were further purified.

For each substance spectra were run at about 30 different pH-values on degassed 0.25 M solutions at 38°C. The pH of these solutions was adjusted with DCl or NaOD and measured with an Electrofact pH-meter, equipped with a micro-electrode.

Chemical shifts (δ) are quoted in c/s downfield from internal trimethylsilylpropanesulfonic acid, and were measured at 60 Mc with a Varian A-60. In the tables δ_I, δ_{II} and δ_{III} represent the chemical shifts of the carbon-bound proton(s), numbered according to their distance from the carbonyl group. In the same manner δ_{IM} is the δ-value of the protons of the methylgroup, attached to carbon atom I. This code is exemplified by alanylglycylglycine:



The fast exchanging protons in HDO, carboxyl, amide, and amino groups produce a single line in the PMR spectra at an averaged δ-value of about 280. Glycyl-methylene protons produce singlets. A tertiary alanyl proton exhibits a quartet with $|J| \sim 7$ c/s and a 1:3:3:1 intensity distribution. Alanyl-methyl groups invariably produce symmetrical doublets with $|J| \sim 7$ c/s. For compounds which are only sparingly soluble in water a convenient solvent is a 60% dioxane/D_2O mixture. Table I shows that valeric acid, dissolved in D_2O or in this mixture produces almost identical spectra.

RESULTS AND DISCUSSION

Only some striking results are discussed here.

1. Influence of dissociation on chemical shift

Dissociation of a -COOH or $-NH_3^+$ group, caused by raising the pH, results in increased shielding (smaller δ-values) of the protons in the ion or molecule in all cases, because of charge density and magnetic anisotropy differences between the conjugated acid and base groups.

Fast proton exchange averages the chemical shifts in mixtures of incompletely dissociated species which makes the curve of δ as a function of pH similar to a titration curve. Generally the inflection point in this curve coincides with the p_K-value of the acid within 0.2 pH-units. The amino acids and oligopeptides studied exhibit two inflection points in the pH-range 1.0 to 13.0 and lack evidence of amide-oxygen protonation (14). This was confirmed by potentiometric titrations.

TABLE I

Proton Chemical Shifts of Some Carboxylic Acids in D_2O-Solution (see Experimental)

Acid	pH	α-protons δ	α-protons Δδ	CH_3* δ	CH_3* Δδ	C=CH* δ	C=CH* Δδ
Formic acid	< 1.5 > 6.0	498.5 512.5	-14.0	-		-	
Acetic acid	< 2.5 > 6.5	126.5 115.0	11.5**	-		-	
Valeric acid	< 3.0 > 6.5	145.0 131.5	13.5	54.0 52.8	1.2	-	
Valeric acid***	Acid Basic	138.5 126±1.0	12.5±1	54.0 52.6	1.4	-	
4cis-Decenoic acid***	Acid Basic	138.6 128.6	10.0	-		322.3 320.6	1.7

*Distorted triplet; the middle peak has been measured.
**Ref. 12 quotes 0.190 p.p.m. = 11.4 c/s.
***In 60% dioxane/D_2O mixture.

The only exception to the rule that a higher pH-value leads to smaller δ-values is formic acid (see Table I), whose non-exchangeable proton resonates at lower field on formation of the magnetically anisotropic $-[C\overset{O}{\leq}_{O}]^-$ group. If the electronegativity of $-[COO]^-$ is smaller than that of the carboxylic group (5) the influence of the anisotropy difference in the formate ion and formic acid presumably causes the observed negative dissociation shift. This influence can differ from that in all other acids by the exceptional location of the measured proton.

Also a π-electron charge density on the carbon atom in the ion, which is smaller than in the molecule may contribute to the reversed shift.

From Table II it can be inferred that

$$\overset{\oplus}{NH_3}CH_2COOH \text{ and } \overset{\oplus}{NH_3}-CH_2-\underset{\underset{O}{\|}}{C}-\underset{\underset{H}{|}}{N}-X$$

have equal δ-values for the methylene protons, viz. 235.5±2.5. The tertiary proton in alanyl residues in the analogous two ions has a δ-value of 251.0±2.0. Going from

$$NH_2-\underset{\underset{CH_3}{|}}{\overset{\overset{H}{|}}{C}}-COO^{\ominus} \quad \text{to} \quad X-\underset{\underset{O}{\|}}{C}-\underset{\underset{H}{|}}{N}-\overset{\overset{H}{|}}{\underset{\underset{CH_3}{|}}{C}}-COO^{\ominus}$$

yields a paramagnetic shift of δ of the tertiary proton (197.5→250±2.0), which is larger than in the similar glycyl ions (190.5→227±2). Also the dissociation shift of the tertiary proton in $\overset{\oplus}{NH_3}-\underset{\underset{CH_3}{|}}{CH}-\underset{\underset{O}{\|}}{C}-\underset{\underset{H}{|}}{N}-X$

(Δδ = 37.0±0.5) is larger than for the methylene protons in a comparable N-terminal glycyl unit (Δδ = 30±1).

Another unexpected feature is shown by alanylglycine in the pH-range where it exists as zwitterion. The absorption of its glycyl-methylene proton exhibits a splitting of 0.6 c/s, which is not due to cis-trans isomerism about the amide link, but to magnetic non-equivalence, caused by the asymmetry of the alanyl residue (15). For, under high gain, the glycyl doublet turns out to be part of an AB-quartet with $|J|$ = 16.0 c/s. This non-equivalence is temperature-dependent and therefore related to conformer populations.

In other peptides and especially in the structurally related glycylalanine and alanylglycylglycine the absence of observable analogous non-equivalence is remarkable.

Diastereoisomeric peptides with two or three alanyl units, however, produce identical spectra.

TABLE II

Proton Chemical Shifts of Glycine, L-Alanine and some of their Oligopeptides in D_2O-Solution (see Experimental) (δ-values without a decimal have an uncertainty of $\pm$1.0 c/s)

Substance	pH	δ_I	δ_{II}	δ_{III}	δ_{IM}	δ_{IIM}	δ_{IIIM}
Glycine*,**	< 1	235.5					
	4-8	214.0					
	> 12	190.5					
Glycyl-	< 3.5	247.5	235				
glycine*	5	233	232.5				
	> 10	228.5	202.5				
Diglycyl-	< 2.5	244.0	245.5	236.5			
glycine*	5-6	228.0	243.5	236.1			
	> 9.5	227.5	241.0	205.0			
L-Alanine*,**	< 0.5	251.5			95.0		
	4-6.5	228.0			88.5		
	> 12	197.5			72.5		
Alanyl-	< 2	268.5	250.0		88.0	94.5	
alanine	5-6.5	251	247		82	93	
	> 11	251.0	210.5		80	75.5	
Dialanyl-	< 2	267	267	251	87.5	86.5	94.6
alanine	5-7	250	263	249.5	81	85.5	94.1
	> 9	249	261.5	212.5	80	83.5	76.5
Glycyl-	< 2	268	233		87.0		
alanine	5-7	251.5	230.5		81.0		
	> 11	251.5	200.0		80.5		
Alanyl-	< 2	243.5	250			93.5	
glycine	4.5-5.5	228.0	247			92.5	
	> 10	226	210			75.3	
Glycyl-	< 2	266.5	243.5	236.2	86.5		
glycyl-	4.5-7	252.5	242.0	235.5	80.6		
alanine	> 10	252	239.5	204.5	80.2		
Alanyl-	< 2	244.5	244.5	252.5			94.6
glycyl-	4.5-7	228.5	243.0	251.5			94.1
glycine	> 10	228.0	240.5	214.5			77.0
Glycyl-	< 1.5	264.0	264.0	232.2	86.5	84.5	
alanyl-	5.5-6	248.4	262.5	231.5	79.8	84.0	
alanine	> 11.5	247.9	261.5	201.0	79.5	83.0	

*In agreement with values deduced from ref. 7 or 10 to within 3.0 c/s.

**In agreement with values deduced from ref. 4, to within 4.0 c/s.

2. Long-range shieldings

Reproducible shifts in the δ-values of protons separated by as many as 11 bonds from the acid proton, whose dissociation is the primary origin of these "dissociation shifts", can be observed by careful control of the experimental procedure and by averaging several measurements at each of as many pH-values as is practical.

Long-range effects over 11 bonds were observed in dialanylalanine as well as in alanylglycylglycine. For instance, the latter produces δ_{IIIM}-values of 94.6±0.2 (for 5 different pH-values) between pH = 0.0 and 2.0 and 94.1±0.1 (for 4 pH-values) between pH = 4.7 and 6.0. In the pH-range 3.0 to 4.5 the -COOH group ionises, as is shown clearly by the shift in δ_I of 16.0 c/s. In 7 cases a similar effect has been observed for a separation by 10 bonds, as can be deduced from Table II. Table III shows the correlation between the average magnitude of the dissociation shifts of protons and their separation by n bonds from the dissociating proton as measured in 9 di- and tripeptides. The same correlation holds for a single peptide, which indicates that head-to-tail association of the charged terminal groups of the zwitterions occurs to a non-observable extent, if at all.

TABLE III

"Dissociation Shifts", Δδ, in Di- and Tripeptides of Glycine and Alanine as a Function of the Number of Bonds (n) Between the Acid Proton and the Resonating Proton

n	Number of peptide links	Δδ in c/s at 60 Mc	Number of cases
3	-	34.1	8
4	-	17.3	12
5	-	5.7	4
6	1	2.17	7
7	1	1.99	11
8	1	0.93	3
9	2	0.75	4
10	2	0.56	7
11	2	0.5	2

An attempt to find a long-range shielding difference over 13 bonds in triglycylglycine failed because of limited solubility (Table IV).

TABLE IV

Proton Chemical Shifts of Triglycylglycine and Norvaline
(see Experimental)

Substance	pH	δ_I	δ_{II}	δ_{III}	δ_{IV}	δ_{CH_3}
Triglycylglycine	< 1	244.0	244.0	246.0	237.0	
	3-9	Insufficiently soluble				
	> 10.5	228.5	241.0	243.5	205.0	
Norvaline	< 1	250±1				57.5
	5-7.5	225.0				56.0
	> 11.5	194.0				54.0

We expect a third-power relation between relevant through-space distances and the magnitude of long-range differential shieldings, derivable from Table II and from spectra of simple saturated and unsaturated carboxylic acids, if differences in conformer populations do not interfere.

In norvaline (Table IV) and valeric acid (Table I) the dissociation shifts of the terminal methyl groups (n = 7) are larger than 1.0 c/s. Compare ref. 16 for a shielding difference of 1.0 c/s (at 100 Mc) in the cis and trans isomers of a phenyl-substituted double bond, separated from the measured methylene protons by 8 bonds.

The results of an approximate M.O. (molecular orbital) calculation of the σ-electron charge densities in some oligopeptides, following del Re et al. (17) only show the influence of ion charge over very short distances.

3. Potential applications

Except for their bearing on magnetic anisotropies, and on statistical conformations of chain molecules, the observed smoothly pH-dependent chemical shifts are important for the unravelling of overlapping multiplets in any molecule which possesses some group which can dissociate or be protonated. In several of the peptides studied, assignment of resonance lines was possible, merely by following their pH dependence.

A second analytical application is the use of the magnitude of the dissociation shift of a vinyl proton as a measure of its distance in a carbon chain from an acid group (Table I, last entry). Recording of the spectrum of two ionisation states permits localisation of unsaturated centres, many kinds of functional groups, and methyl groups over far longer distance than can be deduced from one spectrum. A suitable solvent or solvent mixture has to allow for adjustment of the pH till complete dissociation or protonation is reached, while overlap by solvent absorption should be absent.

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