Protonation of Carbonyl Groups in Peptides and Amino-acids

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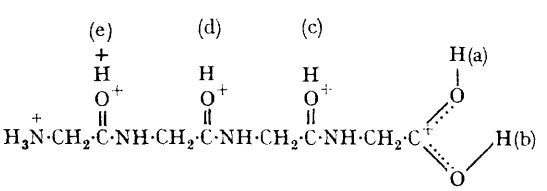
THERE has been considerable study of protonation of carbonyl groups in aliphatic ketones,[1-6] aldehydes,[4,7,8] carboxylic acids,[3,4,9,10,11] esters,[3,12] anhydrides,[13] and simple amides.[14] Now we report that peptides and amino-acids are protonated in FSO_3H–SbF_5–SO_2 at $-70°$ without decomposition and that the C=O–H protons are readily observed by n.m.r. The C=O–H proton chemical shifts are spread over a wide range (*ca.* 1·8 p.p.m.), which facilitates identification of *C*-terminal ($\delta \approx 13{\cdot}9$ p.p.m.), *N*-terminal ($\delta \approx 12{\cdot}3$ p.p.m.), and central C=O–H protons ($\delta \approx 12{\cdot}9$ p.p.m.).

In the n.m.r. spectrum of tetraglycine at $-100°$, even the two central C=O–H protons are resolved, leading to the five-line spectrum shown in the Figure. The assignment of the highest field resonance (12·27 p.p.m.) to proton (*e*) in the structural formula is based upon analogous chemical shifts for *N*-terminal C=O–H protons in di- and triglycine (*cf.* Table). The two peaks at $\delta \approx 13{\cdot}7$ p.p.m. are known to arise from carboxyl-group protons (*a*) and (*b*) because they coalesce at $-90°$ as the rate of rotation about the C–O



bond increases. Proton (*a*) is tentatively assigned to the lower field peak by analogy with other carboxylic acids.[4,9,10,11] Protons (*c*) and (*d*) are assigned to the remaining two peaks, although it is

Chemical shifts of C=O–H *protons at* −95°[a] (δ, *p.p.m. with reference to tetramethylsilane*)[b]

	C-terminal	Central	*N*-terminal
Tetraglycine	(*a*) 13·75 (*b*) 13·65	(*c*) 13·03 (*d*) 12·79 or *vice versa*	(*e*) 12·27
Triglycine	13·80 13·70	12·93	12·36
Diglycine	13·97 13·79		12·42
Glycine	14·03 13·85		
L-Alanylglycine	14·10 13·86		12·49
Glycyl-L-alanine	13·93		12·28
Glycyl-D-valine	14·07		12·36
Diglycine ethyl ester	13·70		12·38
L-Alanyl-L-alanine	14·02		12·38
L-Alanyl-D-alanine	14·01		12·42
DL-Alanine	14·03		
DL-Valine	14·07		

[a] Due to variations in temperature calibration, systematic errors of as much as 10° may be expected. The temperature coefficient of the C=O–H proton chemical shifts indicates that systematic errors of as much as 0·05 p.p.m. may be expected.

[b] CH_2Cl_2, 5·30 p.p.m. from tetramethylsilane, used as internal standard.

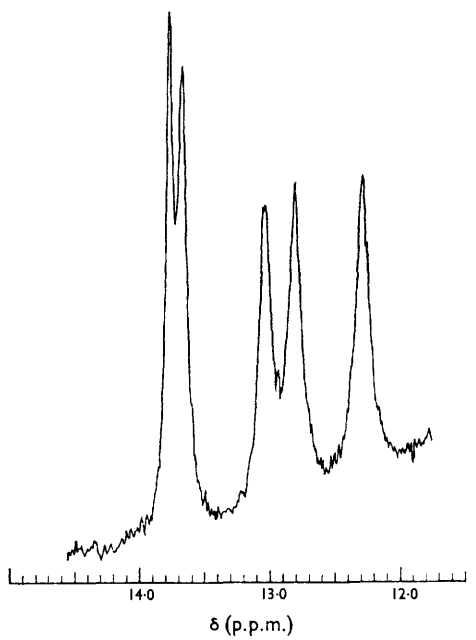


FIGURE. *Partial* 100 MHz *n.m.r. spectrum of tetraglycine in* FSO_3H–SbF_5–SO_2 −100°.

uncertain which of the two possible combinations is correct. Little is known about *cis–trans* isomerism of C=O–H protons in amides, and no evidence for the existence of such isomers was found in the present work.

The ease of differentiating various C=O–H protons is in sharp contrast to the situation with the amide N–H protons, which all appear at 9·30 p.p.m. in the protonated peptides, and with the $-CH_2-$ protons which, with the exception of the *C*-terminal residue (δ 4·75 p.p.m.), all appear at δ 5·30 p.p.m. for the glycine peptides. Terminal $-NH_3$ protons appear at δ 6·75 p.p.m. in all peptides and amino-acids reported herein.

Above −80°, the two central C=O–H proton peaks in the Figure broaden, merge, and continue to broaden. At −80°, the two low-field peaks assigned to carboxyl-group protons (*a*) and (*b*) have coalesced into a sharp singlet. At −75° this peak has begun to broaden, while the *N*-terminal C=O–H proton resonance remains very sharp. These observations, and similar observations for dipeptides, indicate that the rates of proton exchange with solvent are in the order: central > *C*-terminal > *N*-terminal. The coalescence temperature for rotation about C–O bonds in glycine and in all peptides bearing *C*-terminal glycines is in the range −80 to −90°. No evidence has been found for slow rotation about C–O bonds in alanines and valines at temperatures as low as −100°.

The data in the Table reveal the high degree of similarity among *C*-terminal, *N*-terminal, and central C=O–H proton chemical shifts, irrespective of the amino-acid. Several aspects of these data are surprising, *e.g.* the fact that C=O–H proton on *N*-terminal carbonyl groups are more shielded than those on central carbonyl groups.

As an extension of the present work to larger peptides, preliminary experiments with bovine

serum albumin (M = 69,000) and with poly-L-glutamic acid (average M = 105,000) have been carried out, yielding no significant C=O–H resonances.

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