Nuclear Magnetic Resonance Spectra of Amines III. Identification of *N*-Substituted Amino Acids

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Spectra of *N*-substituted amino acids examined in alkaline deuterium oxide and in trifluoroacetic acid show chemical shifts and first-order spin-spin splitting patterns that are useful for identification of *N*-methyl, *N*-methylene, *N*-phenyl, and other groups attached to the nitrogen of an amino acid.

STRUCTURE-SPECTRA correlations of amino acids have been a favorite research subject for many years now, so it is not surprising that considerable work has been done correlating amino acid structures with NMR spectra. Among the more interesting studies that have been reported in this field are the correlation of NMR spectra with L- and *m*-structures of cystine (1), and a study of the effect of optical activity of amino acids on their NMR spectra (2). There have also been a significant number of comprehensive analyses of specific amino acid spectra using NMR (3–6). The spectra reported in this paper were needed for the identification of amino acid side chains on a drug metabolite. The authors are following the technique initiated by Silverstein (7, 8), using the simplest compounds available to illustrate the NMR spectrum due to a particular functional group.

The authors have examined NMR spectra of glycine and *N*-substituted glycine in alkaline D_2O and in trifluoroacetic acid as solvents to illustrate the spectral effects of conversion of *N*-substituted amino acid anions into cations.

EXPERIMENTAL

All spectra were obtained with a Varian model A-60 NMR spectrometer using Varian sample tubes. One normal KOD in deuterium oxide was used as the solvent for anion spectra and trifluoroacetic acid as solvent for cation spectra. The probe temperature was 38°. The concentration of amino acid was 50 mg./ml. The sample of glycine was A grade (Calbiochem) purchased from the California Corp. for Biochemical Research. The *N*-substituted glycines were used as purchased from the K & K Laboratories, Inc., Plainview, N. Y.

RESULTS AND DISCUSSION

Figures 1–4 show the effect of conversion of the anions to cations on the NMR spectra of *N*-substituted glycines. The first-order splitting pattern of the methylene or methyl(s) in the cations is related to the number of protons on the nitrogen (9). The number of lines in a multiplet is one more than the number of protons on the nitrogen (cation), a quartet for a primary amine ion, $-NH_3^+$ (Fig. 1, B), a triplet for a secondary amine ion, $=NH_2^+$ (Fig. 2, B) and a doublet for a tertiary amine ion, $\equiv NH^+$ (Fig. 3, B).

The spectrum for *N*-phenylglycine as anion (Fig. 4, A) shows a complex AB_2X_2 pattern for the phenyl protons in the δ = 6.6–7.6 p.p.m. region. When converted to the cation (Fig. 4, B) the AB_2X_2 pattern collapses into a single peak at 7.6 p.p.m. A possible cause of this signal coalescence was postulated in a previous note (10).

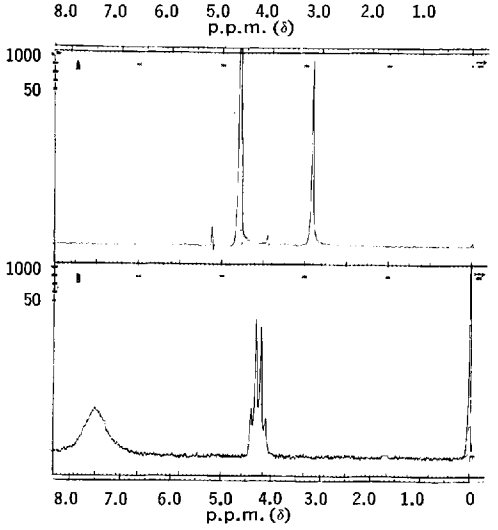

Fig. 1.—NMR spectra for glycine. Key: A, 1 *N* KOD in D_2O as solvent; B, CF_3COOH as solvent.

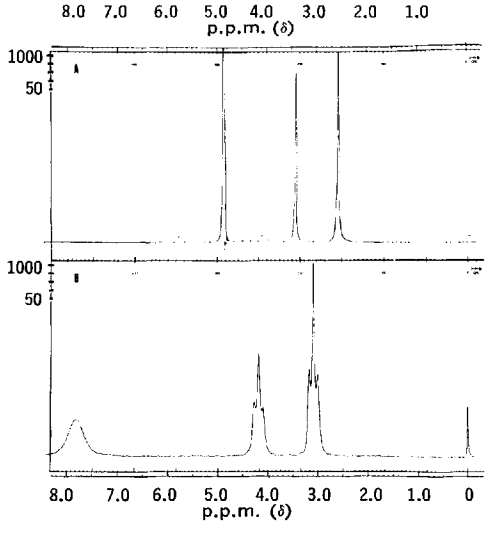

Fig. 2.—NMR spectra for sarcosine (*N*-methylglycine). Key: A, 1 *N* KOD in D_2O as solvent; B, CF_3COOH as solvent.

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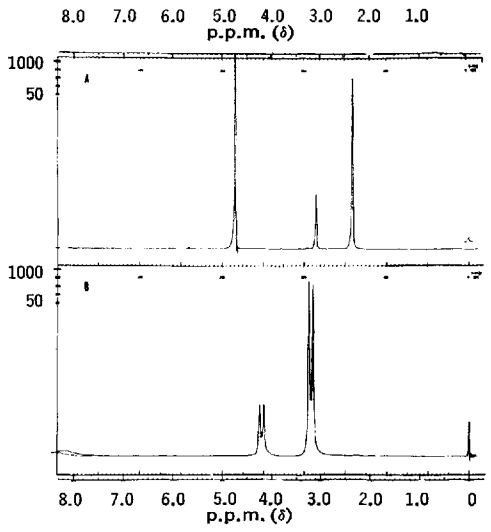

Fig. 3.—NMR spectra for *N,N*-dimethylglycine. Key: A, 1 *N* KOD in D_2O as solvent; B, CF_3COOH as solvent.

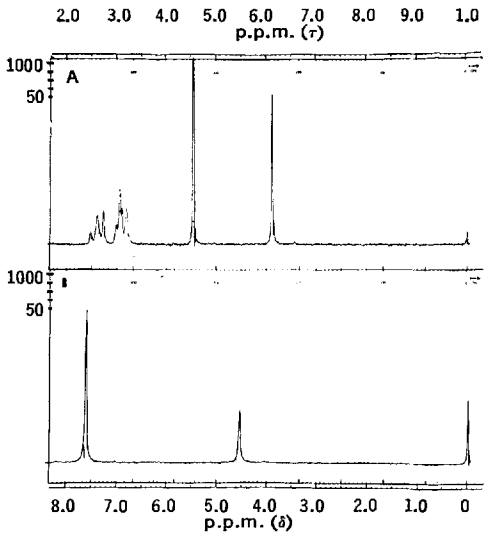

Fig. 4.—NMR spectra for *N*-phenylglycine. Key: A, 1 *N* KOD in D_2O as solvent; B, CF_3COOH as solvent.

TABLE I.—NMR CHEMICAL SHIFTS FOR *N*-SUBSTITUTED GLYCINES

Compd.	Chemical Shifts as Anion in 1 *N* KOD/D_2O	Chemical Shifts as Cation in CF_3COOH
Glycine	$D_2NCH_2COO^-$	$NH_3{}^+CH_2COOH$
	3.18	4.25
	s	*q*
Sarcosine	$CH_3NDCH_2COO^-$	$CH_3NH_2{}^+CH_2COOH$
	2.57 3.40	3.07 4.17
	s *s*	*t* *t*
N,N-Dimethylglycine	$(CH_3)_2NCH_2COO^-$	$(CH_3)_2NH^+CH_2COOH$
	2.35 3.02	3.22 4.23
	s *s*	*d* *d*
N-Phenylglycine	$C_6H_5NDCH_2COO^-$	$C_6H_5NH_2{}^+CH_2COOH$
	6.6–7.6 3.82	7.60 4.53
	AB_2X_2 *s*	*s* *s*

The methylene absorption at 4.53 p.p.m. in Fig. 4, B, remains a single peak in trifluoroacetic acid since this acid is not strong enough to protonate the nitrogen irreversibly. The rapid exchange of protons prevents observation of spin-spin splitting in this solvent at 38°.

Chemical shift data for these *N*-substituted glycines are listed in Table I.

Numbers below hydrogen attached to carbon are chemical shift values in parts per million downfield relative to 3-(trimethylsilyl)-1-propanesulfonate ion for D_2O solutions and relative to tetramethyl silane for trifluoroacetic acid solutions. The abbreviations for splitting patterns are: *s* = single peak, *d* = doublet, *t* = triplet, *q* = quartet, and AB_2X_2 = 5 proton pattern of up to 110 lines.

The spin-spin splitting constant for the doublets, triplets, and quartet was 5 c.p.s.

The chemical shifts for all nonexchangeable protons are downfield on conversion of the anion to cation. These changes in magnetic shielding range from 0.5 p.p.m. downfield for the methyl in sarcosine to 1.21 p.p.m. downfield for the methylene in *N,N*-dimethylglycine. The shielding values for nonexchangeable protons in CF_3COOH solutions seem more consistent than the shielding values in KOH–D_2O. The values for sarcosine in water indicate considerable solvent interaction.

CONCLUSIONS

The spectral changes for an *N*-substituted amino acid observed between alkaline D_2O and CF_3COOH as solvents are useful for locating *N*-methyl, *N*-methylene, *N*-phenyl, and other adjacent structures attached to the nitrogen of an amino acid.

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