Stable Carbonium Ions. LXXXIII.[1] Protonation of Amino Acids, Simple Peptides, and Insulin in Superacid Solutions

GEORGE A. OLAH, DONALD L. BRYDON,[2a] AND RICHARD D. PORTER[2b]

Department of Chemistry, Case Western Reserve University, Cleveland, Ohio 44106

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The protonation of α-, β-, γ-, and δ-amino acids, protein-occurring α-amino acids, some simple peptides, and porcine insulin has been studied by nmr spectroscopy in FSO_3H–SbF_5–SO_2 and FSO_3H–SbF_5 solution. For comparison, protonation of some lactones was also studied in the same solvent systems. In the case of the amino acids, protonation of the amino and carboxyl groups, as well as of other available basic sites, was observed. No dehydration of the protonated α- and β-amino acids to oxocarbonium ions was observed, but some cleavage of protonated γ- and almost complete cleavage of δ-amino acids took place. Protonation on carboxyl oxygen of peptides was observed, besides protonation of other basic sites.

Earlier papers in this series have reported the nmr observation of the protonation of carboxylic and dicarboxylic acids and their subsequent dehydration to the respective oxocarbonium ions in the strong acid system FSO_3H–SbF_5.[3] The nmr spectra of amino acids and peptides have been investigated in basic, acidic, and neutral solvent systems.[4-7] Recently, the spectra of 20 amino acids in CF_3CO_2H and CF_3CO_2D solutions have been obtained at 220 MHz and used in an investigation of protein structure.[8] The conformation of polypeptides has been investigated by nmr and optical methods.[9]

Protonation of the carboxyl group in amino acids is not observed in trifluoroacetic acid, but Thomas and Niemann[10] interpreted their cryoscopic studies of L-leucine in 100% sulfuric acid in terms of the presence of small amounts of L-leucine protonated on both the amino and carboxyl groups. Subsequent cryoscopic studies in 100% sulfuric acid[11] indicated that, as the amino group is further removed from the carboxyl group in amino acids, the carboxyl group is protonated to a greater extent (i = 2.3 for L-leucine, i = 2.7 for β-alanine, and i = 3.0 for aminocaproic acid).

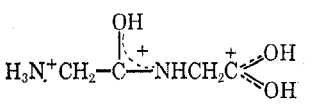
In our continued studies, the protonation and thermal stability of protein-occurring α-amino acids and of a range of α-, β-, γ-, and δ-amino acids in the strong acid system FSO_3H–SbF_5 was investigated by nmr spectroscopy. In the same acid system, the protonation of some simple peptides and lactams was investigated, as well as that of porcine insulin.

Results and Discussion

All the α-amino acids naturally occurring in proteins,[12] as well as δ-aminovaleric acid, α-, β-, and γ-aminobutyric acids, some simple peptides, and insulin, were dissolved in the strong acid system FSO_3H–SbF_5–SO_2 at −76°, and the nmr spectra of the solutions were examined over a range of temperatures from −90 to −20°. Generally, protonation of the amino function was observed (3 H) as a broad resonance at *ca.* δ 7.0 with the protonated carboxyl group (2 H) appearing at *ca.* δ 14.5. The protonated amino group could usually be observed over the whole range of temperatures studied, while the protons of the carboxylic group generally exchanged too rapidly to be observed at temperatures higher than −40°. The additional deshielding of the protonated carboxylic group by the protonated amino group on the α-carbon atom is illustrated by the fact that the protons of the protonated carboxylic groups of aliphatic carboxylic acids are observed at *ca.* δ 12.6.[3] To study the possible cleavage of protonated amino acids, representative samples of the α-amino acids, glycine, L-alanine, L-valine, L-phenylalanine, L-proline, L-lysine, and L-glutamic acid were heated for 2 hr at −40° in the FSO_3H–SbF_5 solution. Apart from L-phenylalanine, whose aromatic group reacted with the acid system at temperatures above −30°, the diprotonated amino acids were observed to be stable at this temperature. There was no dehydration to the corresponding oxocarbonium ion, as is the case with the protonated aliphatic carboxylic acids.[3]

$$RCO_2H_2^+ \xrightarrow[0 \text{ to } -30^\circ]{FSO_3H\text{–}SbF_5\text{–}SO_2} RCO^+ + H_3O^+$$

At temperatures below −20° in FSO_3H–SbF_5–SO_2 solution, the simple peptides examined were observed to be chemically stable, and to be protonated on the terminal amino and carboxyl groups and on the carbonyl oxygen of the peptide bonds.



Diprotonated α- and β-aminobutanoic acids in FSO_3H–SBF_5 solution underwent no decomposition after being maintained at 45° for 4 hr. γ-Aminobutanoic acid underwent some side reactions but was observed to undergo about 50% dehydration. Diprotonated δ-aminovaleric acid dehydrated slowly at −20°, and after 4 hr at 45°, 97% dehydration had occurred to yield the oxocarbonium ion $H_3N^+(CH_2)_3CO^+$. The facility with which diprotonated amino carboxylic acids dehydrated in the acid system studied increased with the separation of the protonated amino group from

(1) Part LXXXII: G. A. Olah and D. L. Brydon, *J. Org. Chem.*, **35**, 313 (1970).

(2) (a) National Institutes of Health Postdoctoral Research Investigator, 1967–1968; (b) National Institutes of Health Predoctoral Research Investigator, 1968–1969.

(3) G. A. Olah and A. M. White, *J. Amer. Chem. Soc.*, **89**, 3591, 4752, 7072 (1967).

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(7) S. Fujiwara and Y. Arata, *Bull. Chem. Soc. Jap.*, **36**, 578 (1963); **37**, 344 (1964).

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(11) J. L. O'Brien and C. Niemann, *J. Amer. Chem. Soc.*, **73**, 4264 (1951).

(12) J. P. Greenstein and M. Winitz, "Chemistry of the Amino Acids," Vol. 1, John Wiley & Sons, Inc., New York, N. Y., 1961, p 3.

TABLE I

NMR CHEMICAL SHIFTS (PARTS PER MILLION) AND COUPLING CONSTANTS[a] (HERTZ) OF PROTONATED MONOAMINO MONOCARBOXYLIC ALIPHATIC ACIDS AT −60° IN FSO_3H–SbF_5–SO_2 SOLUTION

Species	Registry no.	H_1	H_2	H_3	H_4	H_5	H_6	H_7
$CH_2(\overset{+}{N}H_3)CO_2H_2^+$[b]	22493-25-0	14.43 ... 14.2[d] 14.43[d]	7.13 br	5.25[c] q ($J_{2,3}$ = 6)				
$CH_3CH(\overset{+}{N}H_3)CO_2H_2^+$[b]	22493-26-1	14.37	7.00 br	5.40[c] m	2.30[c] d ($J_{3,4}$ = 7.5) 2.40[c] d ($J_{3,4}$ = 7.5)			
$(CH_3)_2CHCH(\overset{+}{N}H_3)CO_2H_2^+$[b]	22493-27-2	14.45	7.03 br	5.13[c] m	3.07[c] m	1.50[c] d ($J_{4,5}$ = 6.8) 1.54[c] d ($J_{4,5}$ = 6.8) 1.60[c] d ($J_{4,5}$ = 6.8)		
$(CH_3)_2CHCH_2CH(\overset{+}{N}H_3)CO_2H_2^+$	22493-28-3	14.40 (14.50)	7.03 br (7.00)	5.20 m (5.20)	2.43[c] m (2.37)	2.43[c] m (2.37)	1.43[c] d ($J_{5,6}$ = 5) (1.40)	
$CH_3CH_2CH(CH_3)CH(\overset{+}{N}H_3)CO_2H_2^+$	22493-29-4	14.50	7.03 br	5.20[c] m	1.45[c] d ($J_{4,5}$ = 6)	2.8[c] m	1.90[c] m	1.62[c] d ($J_{6,7}$ = 7)
$[-SCH_2CH(\overset{+}{N}H_3)CO_2H_2]_2^+$	22493-30-7	14.93[e]	7.27[e] br	5.17[e] m	3.93[e] m			
$CH_3\overset{+}{S}(H)CH_2CH_2CH(\overset{+}{N}H_3)CO_2H_2^+$	22493-31-8	15.17[e]	7.20 br	5.30 m	3.23 m	3.93 m	6.93 m	3.3 d ($J_{6,7}$ = 8)

[a] Coupling constant are in parenthesis. Multiplicity is indicated as follows: d, doublet; q, quartet; m, multiplet; br, broad. [b] Spectra may be obtained from ASIS National Auxiliary Publications Service, % CCM Information Corp., 909 3rd Ave., New York, N. Y. 10022. Order Document No. 00602. [c] At −20°. [d] At −90°. [e] At −80°.

the protonated carboxyl group. This is most probably due to an electrostatic repulsion, since there is a greater positive charge on the carboxylic carbon atom in the protonated carboxylic acid than on the oxocarbonium carbon atom in the respective oxocarbonium ion (as evidenced by the comparative deshielding of the α-hydrogen atoms by *ca.* δ 1.0 in the case of δ-aminovaleric acid). Similarly, it has been found that the ease with which diprotonated dicarboxylic acids can be dehydrated increases with the increasing separation of the acid functions.[3]

Monoamino Monocarboxylic Aliphatic Acids.—The nmr spectral data of the diprotonated species obtained by dissolving glycine, L-alanine, L-valine, L-leucine, and L-isoleucine in FSO_3H–SbF_5–SO_2 are summarized in Table I, as are the data for protonated L-cystine and L-methionine (Figure 1).

The simplest amino acid studied was glycine, in which, at −60°, the protons on nitrogen (3 H) were observed as a broad peak at δ 7.13, while the carboxylic protons (2 H) appeared as a sharp singlet at δ 14.43 and as two lines at δ 14.2 and 14.43 at −90°. At −20°, the methylene protons (2 H) were resolved into a quartet at δ 5.25 (J_{CH-NH} = 6 Hz).

The methyl resonances of diprotonated L-alanine and L-valine are interesting in that, with alanine, a very low intensity doublet at δ 2.30 was observed as well as the strong doublet at δ 2.40, while with valine, two intense doublets were observed at δ 1.60 and 1.54 and a very low intensity doublet was discernible at δ 1.50.

L-serine and L-threonine underwent chemical change in the strong acid system, as evidenced by the absence of low-field resonance absorptions. A singlet at δ 5.77 and a broader singlet of equal area at δ 7.50 was observed from −20 to −80° in the case of serine. With threonine, a doublet (3 H) appeared at δ 2.35 (J = 6 Hz), a broad singlet (3 H) at δ 7.47, and broad multiplets at δ 5.57 (1 H) and 6.17 (1 H). The reaction of serine and threonine in trifluoroacetic acid solution has been discussed previously.[6,8]

The carboxylic protons of L-cystine appear as a broad exchanging resonance at δ 14.93. Even at −80°, no protonation of the disulfide system was detected and the chemical shifts of the protonated amino group at δ 7.27 (3 H), the methine proton at δ 5.17 (1 H), and the methylene protons at δ 3.93 (2 H) in FSO_3H–SbF_5–SO_2 are similar to the chemical shifts of δ 7.75, 4.80, and 3.61, respectively, determined in trifluoroacetic acid.[6] L-Cysteine underwent chemical reaction in the strong acid system in contrast to its behavior in trifluoroacetic acid.[6,8] Broad peaks were observed at δ 7.33 (NH_3) and 5.43 (CH), no low-field resonances were seen, and after a few hours a white precipitate was deposited.

A very characteristic spectrum was obtained with L-methionine (Figure 1). The terminal group (3 H) appeared at δ 3.3 as an extremely sharp doublet (J_{CH_3-SH} = 8 Hz) as a result of the sulfur protonation. These values compare with protonated dimethyl sulfide, where the methyl protons appear as a doublet at δ 3.08 and the proton on sulfur as a septuplet at δ 6.52 (J_{CH_3-SH} = 8 Hz).[13] Again, the carboxyl protons

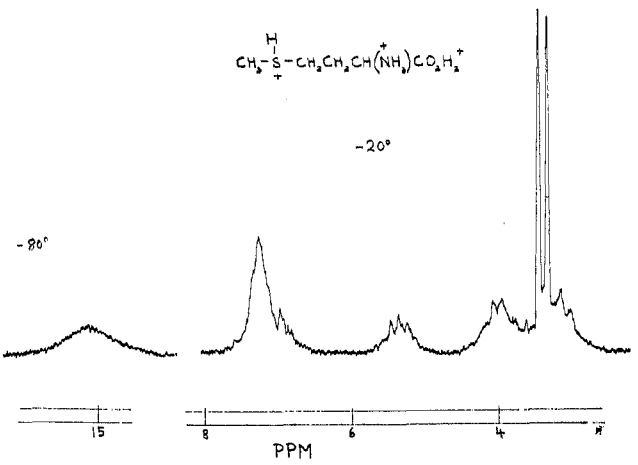


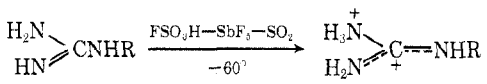
Figure 1.—60-MHz nmr spectrum of L-methionine in $HFSO_3$–SbF_5–SO_2.

could only be observed at −80°, and then as a broad exchanging resonance at δ 15.17.

Diamino Monocarboxylic and Monoamino Dicarboxylic Aliphatic Acids.—The nmr parameters obtained upon protonation of diamino monocarboxylic and amino dicarboxylic acids in FSO_3H–SbF_5–SO_2 solutions are summarized in Table II. L-Lysine was observed to be triprotonated, with the α-amino protons (3 H) and the amino protons (3 H) appearing at δ 7.06 and 6.03, respectively. All the methylene and methine protons appeared as broad peaks at −20°, and only at −80° are the carboxylic protons observed as a sharp singlet at δ 14.53.

With δ-hydroxy lysine, poorly resolved spectra were obtained. Only at −80 and −90° could the protons of the carboxylic group be observed as a very broad resonance at δ 14.8, and no resonances could be observed for the hydroxy group. It is conceivable that the δ-hydroxy lysine underwent ring closure to form a lactone. If this were the case, the observed species might have been either the protonated lactone or an equilibrium mixture of protonated acid and protonated lactone.

Arginine and its nonprotein-occurring homolog, homoarginine, are basic α-amino acids which possess a guanidine end group. Olah and White[14] have shown that guanidines are diprotonated in FSO_3H–SbF_5–SO_2 in the following manner.



The α-amino group (3 H) in arginine appeared at δ 7.07, the protonated amino function (3 H) of the guanidine group appeared at δ 8.9 as a sharper peak than that owing to the protonated amino group (2 H), and the substituted amino group (1 H) appeared at δ 8.13. Homoarginine exhibited a very similar spectrum to arginine at −60°, with the difference that in the case of arginine the carboxylic protons exchanged too rapidly to be seen, while in the case of homoarginine the carboxylic protons (2 H) were observed as a sharp singlet.

Aspartic acid is triprotonated in the strong acid solution. The carboxylic protons (4 H) were observed

(13) G. A. Olah, D. H. O'Brien, and C. U. Pittman, *J. Amer. Chem. Soc.*, **89**, 2996 (1967).

(14) G. A. Olah and A. M. White, *ibid.*, **90**, 6087 (1968).

TABLE II

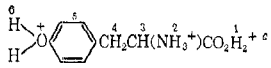
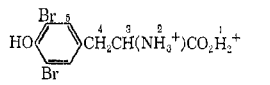
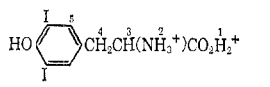
NMR CHEMICAL SHIFTS (PARTS PER MILLION) AND COUPLING CONSTANTS[a] (HERTZ) OF PROTONATED DIAMINO MONOCARBOXYLIC AND MONOAMINO DICARBOXYLIC ALIPHATIC ACIDS AT −60° IN FSO_3H–SbF_5–SO_2 SOLUTION

Species	Registry no.	H_1	H_2	H_3	H_4	H_5	H_6	H_7	H_8
$CH_2(NH_3^+)(CH_2)_2CH_2CH(NH_3^+)CO_2H_2^+$ [b]	22493-32-9	14.53[c]	7.06 br	5.2 br	2.63 br	2.06 br	6.03 br	3.57 br	
$CH_2(NH_3^+)CH(OH)(CH_2)_2CH(NH_3^+)CO_2H_2^+$	22533-99-9	14.8[c]	7.23[d] br	5.33 br	2.87 br	5.60 br	6.70 br	4.17 br	
$C(NH_3^+)(NH_2^+)=NHCH_2(CH_2)_2CH(NH_3^+)CO_2H_2^+$	22493-33-0	14.87[c] br	7.07 br	5.2 m	2.6 br	4.06 br	8.13	8.13	8.9
$C(NH_3^+)(NH_2^+)=NHCH_2(CH_2)_3CH(NH_3^+)CO_2H_2^+$ [b]	22493-34-1	14.53	7.10 br	5.23 m	2.20 br 2.60 br	4.0 br	8.10	8.10	8.87
$H_2O_2CCH_2CH(NH_3^+)CO_2H_2^+$ [b]	22493-35-2	14.40[c] m	7.50[d] br	5.93[d] m	4.60[d] d ($J_{3,4}$ = 5)	14.40[c] m			
$^+H_2O_2CCH_2CH_2CH(NH_3^+)CO_2H_2^+$ [b]	22493-36-3	15.07[c] br	7.27 br m	5.37[d] m	3.23[d] m	3.97[d] t ($J_{4,5}$ = 6)	13.38		
$HO(=^+CCH_2CH(NH_3^+)CO_2H_2^+)(=NH_2^+)$	22493-37-4	15.48[e]	7.43[d] br	5.8[d] m	4.30[d] br	8.87[d] 9.23[d]	11.53		
$HO(=^+CCH_2CH_2CH(NH_3^+)CO_2H_2^+)(=NH_2^+)$ [b]	22493-38-5	15.06[c] br	7.2[d] br	5.30[d] m	3.17[d] m	3.53[d] m	10.63 d (J = 3)	8.43 8.80	

[a] Coupling constants are in parenthesis. Multiplicity is indicated as follows: d, doublet; t, triplet; m, multiplet; br, broad. [b] See footnote *b*, Table I. [c] At −80°. [d] At −20°. [e] At −90°.

TABLE III

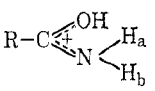
NMR PARAMETERS[a] (δ, PARTS PER MILLION) OF PROTONATED AROMATIC AMINO ACIDS AT $-60°$ IN FSO_3H–SbF_5–SO_2[b] SOLUTION

Species	Registry no.	H_1	H_2	H_3	H_4	H_5	H_6
$C_6H_5CH_2CH(NH_3^+)CO_2H_2^+$[c]	22493-39-6	14.47 br	6.83 br	5.30 br	3.87 br	7.77 br	
	22493-40-9	14.77[d]	7.03 br	5.43 br	4.07 br	7.97	13.0
	22493-41-0	14.73[d] br	7.00 br	5.33 br	3.87 br	8.03	
	22493-42-1	14.73[e]	6.96[e] br	5.33[e] br	3.8[e] br	8.23[e]	

[a] Multiplicity is indicated as follows: br, broad. [b] Phenylalanine in 9:1 FSO_3H–SbF_5. [c] See footnote *b*, Table I. [d] At $-80°$. [e] At $-90°$.

at $-80°$ as a broad peak at δ 14.40. The methylene group (2 H) appeared at δ 4.60 and resolved at $-20°$ into a doublet (J_{CH_2OH} = 5 Hz). In the spectrum of glutamic acid, the carboxylic proton resonances are observed at δ 13.38 and 15.07 (the latter only at $-80°$). The methylene (2 H) group furthest from the amino group appears at $-20°$ as a poorly resolved triplet at δ 3.97 ($J_{CH_2-CH_2}$ = 6 Hz). Compared with the spectra of diprotonated dicarboxylic acids,[3] a considerable deshielding effect is observed owing to the protonated amino group.

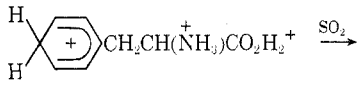
In the spectra of asparagine and glutamine, the carboxylic protons can only be observed as broad peaks at δ 15.48 ($-90°$) and 15.06 ($-80°$), respectively. O-Protonation of the amide group is observed in both cases. With asparagine, the proton is observed as a singlet at δ 11.53 and the amide protons (2 H) on nitrogen as two resonances at δ 8.87 and 9.23. With glutamine, the proton on amide oxygen appears as a doublet (J = 3 Hz) at δ 10.63 and the amide protons (2 H) on nitrogen appear at δ 8.43 and 8.80. The amide protons on nitrogen are nonequivalent owing to the partial double-bond character of the C–N bond. The protonation of amides has

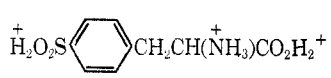


been discussed by Katritzky and Jones[15] and, in the case of fluorosulfuric acid, by Gillespie and Birchall.[16] For protonated acetamide ($-80°$), the latter workers observed the proton on oxygen at δ 10.72 and the protons on nitrogen at δ 8.24 and 8.36.

Aromatic Amino Acids.—The nmr data obtained for protonated aromatic amino acids in FSO_3H–SbF_5 are summarized in Table III.

When L-phenylalanine was dissolved in the usual manner with a sixfold excess of 1:1 FSO_3H–SbF_5 and diluted with sulfur dioxide (15 *M*), sulfonylation occurred[17] to give a mixture of diprotonated phenylalanines. The *para*-protonated sulfinic acid of diprotonated phenylalanine was formed by ring protonation and reaction with sulfur dioxide.





The presence of the sulfinic acid derivative was evidenced by the appearance of an AA′BB′ quartet at δ 8.2 ($J_{A\text{-}B(A'\text{-}B')}$ = 8 Hz) and a resonance attributable to the protonated sulfinic group at δ 9.83. When the temperature of the solution was raised to $-30°$, an irreversible reaction of the aromatic nucleus with the acid system took place. When FSO_3H alone was used as the solvent, protonation of only the amino group was observed. With 1:1 FSO_3H–SbF_5 diluted with sulfuryl chloride fluoride, the solution was bright red, and a reaction was observed with the aromatic nucleus. Similarly, 12.5:1 HF–SbF_5 reacted with the phenylalanine, and after 1 hr at $-60°$ a yellow oil separated. However, 9:1 FSO_3H–SbF_5 was found to be the best strong acid system, and a spectrum of diprotonated phenylalanine [$C_6H_5CH_2CH(NH_3^+)CO_2H_2^+$] could be obtained at temperatures below $-50°$. At temperatures above $-50°$, the acid system reacted with the aromatic nucleus. At $-60°$, the carboxylic protons (2 H) appeared as a broad singlet at δ 14.47, the aromatic protons (5 H) and the ammonium protons (3 H) as broad singlets at δ 7.77 and 6.83, respectively, and the methylene (2 H) and methine (1 H) protons as broad resonances at δ 3.87 and 5.30.

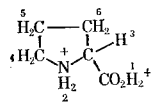
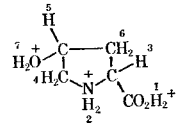
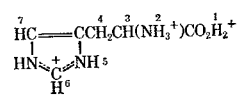
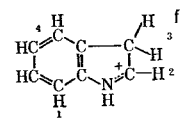
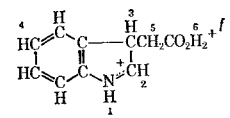
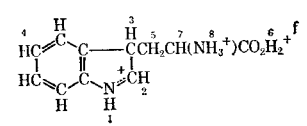
L-Tyrosine underwent no chemical transformation in 1:1 FSO_3H–SbF_5 diluted with SO_2, even at $-20°$; consequently, this strong acid system was used to study its protonation. The aromatic protons (4 H) appeared as a sharp singlet at δ 7.97, and at $-60°$ a broad singlet was observed at δ 13.0. This latter resonance is assigned to the two protons on phenolic oxygen, resulting from its protonation. The fact that the aromatic resonance appeared as a singlet indicates that the positive charge from this protonation resided on the phenolic oxygen and was not delocalized to any appreciable extent over the aromatic ring. The carboxylic protons appeared as a broad peak at $-60°$, and at $-70°$ as a singlet (2 H) at δ 14.77.

Spectral parameters were obtained for L-3,5-dibromotyrosine at -60 and $-80°$, where the strong acid solution was of pale brown color. However, when the temperature of the solution was raised, the color rapidly darkened, and diffuse spectra were observed indicating an irreversible process. An exchanging resonance absorption was observed for the carboxylic protons (1.5 H) at -80 and $-90°$ and, at these temperatures

(15) A. R. Katritzky and R. A. Y. Jones, *Chem. Ind.* (London), **722** (1961).
(16) R. J. Gillespie and T. Birchall, *Can. J. Chem.*, **41**, 148 (1962).
(17) G. A. Olah and T. E. Kiovsky, *J. Amer. Chem. Soc.*, **89**, 5692 (1967).

TABLE IV

NMR CHEMICAL SHIFTS (PARTS PER MILLION) AND COUPLING CONSTANTS[a] (HERTZ) OF PROTONATED HETEROCYCLIC AMINO ACIDS AND RELATED COMPOUNDS AT −60° IN FSO_3H–SbF_5–SO_2 SOLUTION

Species	Registry no.	H_1	H_2	H_3	H_4	H_5	H_6	H_7
	22493-43-2	14.30 14.30[b] 14.50[b]	7.30 br	5.73 m	4.07 m	2.80 br m	2.80 br m	
	22493-44-3	15.30[c] br	8.17[d] br	5.97[d] br	4.87[d] br	6.5[d]	3.83[d] br m	11.51[d] br
	22534-00-5	15.4[b] br	7.27 br	5.53 m	4.23 br m	11.33 11.43	9.07	8.03
	22493-45-4	7.97 m	9.48 d ($J_{2,3}$ = 6)	4.73	7.97 m			
	22493-46-5	8.10[e] m	9.58[e] d ($J_{1,2}$ = 6)	5.22[e] t ($J_{3,5}$ = 7)	8.10[e] m	4.40[e] 4 lines	13.15[c] 13.50[c]	
	22493-47-6		9.53[e] d ($J_{1,2}$ = 5.6) 9.63 d ($J_{1,2}$ = 5.6)	5.30[e] br m	8.15[e] m	3.50[e] br m (width = 100 Hz)	15.3[b] br	5.10[e] m (H_8, 7.3 br)

[a] Coupling constants are in parenthesis. Multiplicity is indicated as follows: d, doublet; t, triplet; m, multiplet; br, broad. [b] At −90°. [c] At −70°. [d] At −35°. [e] At −20°. [f] See footnote *b*, Table I.

and at −60°, although the aromatic resonance was quite sharp, the other resonances appeared as broad peaks. Only at −90° could a spectrum of diprotonated L-3,5-diiodotyrosine be obtained. With rise of temperature, chemical reaction was observed, giving a deeply colored solution at −50°. No resonance attributable to phenolic protons was observed with either the dibromo or diiodo tyrosines.

Spectra of protonated L-3,5,3′-triiodotyrosine and L-thyroxine could not be obtained in solutions of 1:1 FSO_3H–SbF_5 and SO_2 or 9:1 FSO_3H–SbF_5.

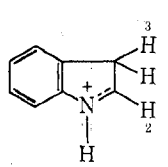
Heterocyclic Amino Acids.—The nmr data obtained in FSO_3H–SbF_5–SO_2 for protonated heterocyclic amino acids as well as related protonated model compounds such as indole and indoleacetic acid are summarized in Table IV.

L-Proline (Figure 2) is diprotonated and chemically stable at −40°. At −90°, the protons on oxygen split into two peaks at δ 14.30 and 14.50. In the case of L-hydroxyproline, triprotonation occurs. The carboxyl protons only appear at −80° as a broad resonance at δ 15.30, while the protons on nitrogen (2 H) appear at δ 8.17 (δ 0.87 deshielded compared to the corresponding protons in diprotonated proline), and the hydroxyl protons appear at δ 11.51 as an unresolved peak adjacent to the solvent peak at −35°.

L-Histidine (Figure 3) is triprotonated. The temperature of the solution had to be lowered to −90° to slow the rate of exchange of the carboxyl protons before they could be observed at δ 15.4. The protons on the nitrogen atoms of the imidazole ring were observed at δ 11.33 and 11.43 as shoulders of the solvent peak at −60°. The other aromatic protons, at positions 2 and 5 of the imidazole ring, appeared at −60° as singlets at δ 9.07 and 8.03. The protons on the imidazole nitrogen atoms are better resolved from the solvent peak in 9:1 FSO_3H–SbF_5 and appear (−20°) as a broad singlet at δ 11.3.

Indole and indoleacetic acid were used as model compounds to study the protonation of tryptophan.

Indole was found to protonate on the carbon atom of the pyrrole ring β to the nitrogen atom, with consequent formation of a methylene group and delocalization of the positive charge over the appropriate C–N system, giving the C–N bond some ethylenic character.



The methylene group was not coupled to the vicinal proton because of their angular relationship, but this latter proton exhibited a cisoid coupling to the proton on nitrogen. Thus the methylene protons appeared as a singlet (2 H) at δ 4.73, the proton 2 appeared as a sharp doublet (1 H) at δ 9.48 ($J_{CH\text{-}NH}$ = 6 Hz), and the proton on nitrogen (1 H) was under the aromatic resonance (4 H) at δ 7.97.

The indole system of indoleacetic acid was protonated similarly to indole itself, in that protonation occurred at the 3 position. The carboxylic group was also found to protonate, and at −80° two peaks appeared at δ 13.15 and 13.50 (2 H). The aromatic protons (4 H)

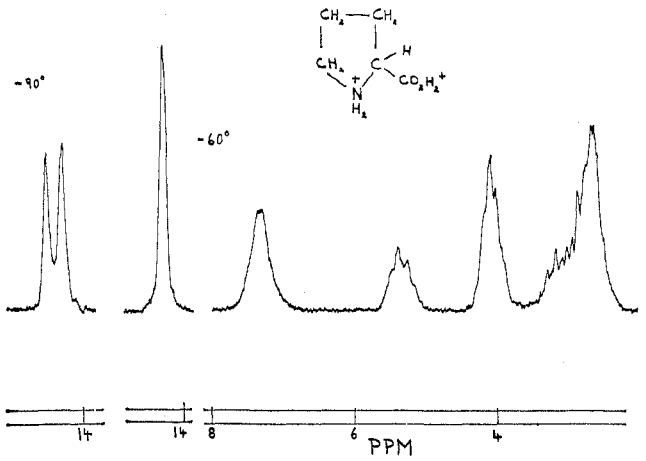


Figure 2.—60-MHz nmr spectrum of L-proline in $HFSO_3$–SbF_5–SO_2.

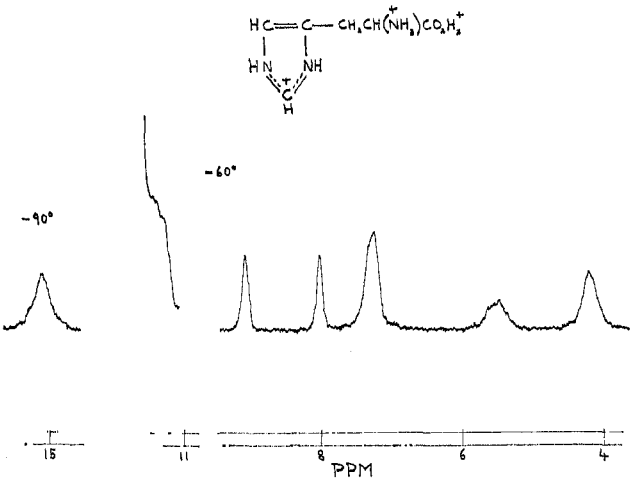


Figure 3.—60-MHz nmr spectrum of L-histidine in $HFSO_3$–SbF_5–SO_2.

and the proton on nitrogen (1 H) appeared at δ 8.10 as an intense multiplet, while the proton adjacent to nitrogen in position 2 of the indole system appeared as a doublet ($J_{CH\text{-}NH}$ = 6 Hz) at δ 9.58 (1 H). As before, this multiplicity was due to a cisoid coupling with the proton on nitrogen and absence of coupling with the proton in the 3 position. The proton in the 3 position (1 H) appeared as a triplet ($J_{CH\text{-}CH_2}$ = 5.7 Hz) at δ 5.22 and the adjacent methylene group appeared at δ 4.40 (2 H) as an asymmetric resonance of four lines. An artifact appeared (0.4 H) at δ 4.9.

As in the cases of indole and indoleacetic acid, L-tryptophan was protonated at the 3 position of indole and, also, the δ-amino group and the carboxylic group were observed to be protonated. The indole nitrogen proton could not be observed, and was probably masked by either the aromatic or ammonium peaks. The proton at the 2 position of the indole residue appeared as two overlapping doublets (1 H) at δ 9.53 and 9.63 ($J_{CH\text{-}NH}$ = 5.6 Hz), probably owing to the existence of two conformers with different magnetic environments at the 2 position. It is unlikely that there was coupling

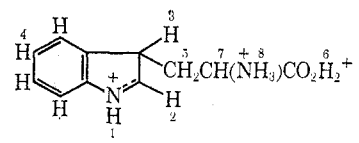
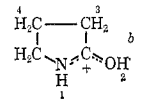
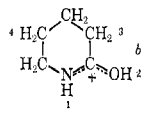
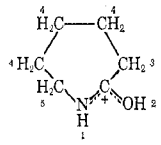


TABLE V

NMR PARAMETERS[a] (δ, PARTS PER MILLION) OF PROTONATED LACTAMS AT −60° IN FSO_3H–SbF_5–SO_2 SOLUTION

Species	Registry no.	H_1	H_2	H_3	H_4	H_5
	22483-24-5	8.55[c]	10.00[c]	3.37 t	2.77 m	4.20 t
	22483-25-6	8.70[c]	9.43[c] br	3.07 m	2.17 m	3.90 m
	22483-26-7	8.77[c]	9.60[c] m	3.20 m	2.13	3.95 m

[a] Multiplicity is indicated as follows: t, triplet; m, multiplet; br, broad. [b] See footnote *b*, Table I. [c] At −90°.

TABLE VI

NMR CHEMICAL SHIFTS (PARTS PER MILLION) AND COUPLING CONSTANTS[a] (HERTZ) OF PROTONATED α-, β-, γ-, AND δ-MONOAMINO MONOCARBOXYLIC ALIPHATIC ACIDS AND δ-AMINO *n*-BUTYLOXOCARBONIUM ION AT −60° IN FSO_3H–SbF_5–SO_2 SOLUTION

Species	Registry no.	H_1	H_2	H_3	H_4	H_5	H_6
$CH_3CH_2CH(NH_3^+)CO_2H_2^+$	22493-48-7	14.37	7.0 br	5.2[b] m 6 lines	2.70[b] m 5 lines	1.57[b] t ($J_{4,5}$ = 7.5)	
$CH_3CH(NH_3^+)CH_2CO_2H_2^+$	22493-49-8	13.57	6.43 br	3.95[b] d ($J_{3,4}$ = 6.0)	4.66[b] m	2.03[b] d ($J_{4,5}$ = 6.5)	
$CH_2(NH_3^+)CH_2CH_2CO_2H_2^+$	22493-50-1	12.80 12.97	6.17	3.57[b] t ($J_{3,4}$ = 7.0)	2.66[b] m	4.0[b] m	
$CH_2(NH_3^+)CH_2CH_2CH_2CO_2H_2^+$ [c]	22493-51-2	12.57 12.80	6.07 br	3.63[b] m	2.37[b] m	2.37[b] m	3.63[b] m
$CH_2(NH_3^+)CH_2CH_2CH_2C{=}O^+$ [c]	22493-52-3		6.13 br	4.63[b] t ($J_{3,4}$ = 7.0)	2.60[b] m	2.60[b] m	3.73[b] m

[a] Coupling constants are in parenthesis. Multiplicity is indicated as follows: d, doublet; t, triplet; m, multiplet; br, broad. [b] At −20°. [c] See footnote *b*, Table I.

between the protons at position 2 and position 3, since this was not observed for protonated indole or protonated indoleacetic acid. Protons 3 and 7 appeared as overlapping multiplets (2 H) centered at δ 5.30 and 5.10, while the methylene protons (5) were a broad multiplet (width = 100 Hz) centered at δ 3.50. The carboxylic protons could only be observed as a broad exchanging resonance at δ 15.3.

Lactams.—For comparison, nmr data were also obtained for protonated 2-pyrrolidinone, δ-valerolactam, and ε-caprolactam in FSO_3H–SbF_5–SO_2. These are summarized in Table V.

All three lactams were O-protonated and were quite stable even when maintained at 40° for 4 hr.

The OH and NH protons are best observed at −90°. Even at −60°, they appear as broad exchanging peaks. The assignment of the methylene groups is based on the assumption that most of the positive charge resides on the nitrogen atom.

2-Pyrrolidinone gave a particularly well-resolved spectrum. No coupling of the OH and NH protons was observed and the methylene resonance resolved into a complex pattern at 20°.

α-, β-, γ-, and δ-Amino Acids.—The nmr data for the behavior of solutions of α-, β-, and γ-aminobutyric acids and δ-valeric acid in FSO_3H–SbF_5–SO_2 are summarized in Table VI.

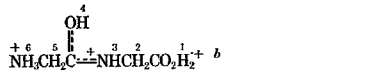
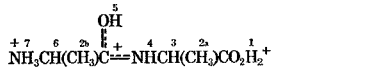
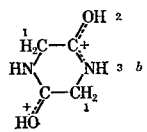
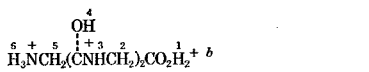
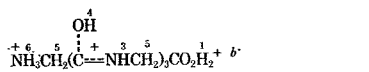
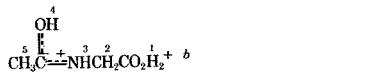
No changes were observed in the spectra of diprotonated α- and β-aminobutyric acids after they had been maintained for 4 hr at 45° and then left at room temperature for 12 hr. A complex spectrum was obtained with γ-aminobutyric acid. Apart from the assigned peaks given in Table VI, unassigned peaks were observed at δ 5.5, 7.2, 8.0, and 9.0. At −20°, a weak triplet was observed (J = 7.0 Hz) at δ 4.62. When the solution was left at −20° for several hours, this peak grew in intensity, and after the solution had been maintained at 45° for 4 hr, this triplet and the triplet at δ 3.57 were of about equal intensity. This, coupled with the decrease in intensity of the carboxyl resonance and the appearance of a new multiplet at *ca.* δ 0.5 downfield from the multiplet at δ 2.66 is interpreted in terms of dehydration of the protonated acid[3] to the extent of about 50% to yield the γ-aminopropyloxocarbonium ion.

$$H_3N^+(CH_2)_3CO_2\overset{+}{H}_2 \xrightarrow{-H_2O} H_3\overset{+}{N}(CH_2)_3CO^+$$

Diprotonated δ-aminovaleric acid formed the δ-aminobutyloxocarbonium ion slowly at −20° ($t_{1/2} \cong$ 1.5

TABLE VII

NMR CHEMICAL SHIFTS (PARTS PER MILLION) AND COUPLING CONSTANTS[a] (HERTZ) OF PROTONATED PEPTIDES AND N-ACETYLGLYCINE AT −60° IN FSO_3H–SbF_5–SO_2 SOLUTION

Species	Registry no.	H_1	H_2	H_3	H_4	H_5	H_6	H_7
	22493-53-4	14.33	5.71[c] d ($J_{2,3}$ = 5.5)	9.82[c] t ($J_{2,3}$ = 5.5)	12.90	5.15[c] q ($J_{5,6}$ = 6.0)	7.15[c] br	
	22493-54-5	14.40[d]	2.32[c] d 2.38[c] d ($J_{2a,3}$ = $J_{2b,6}$ = 7)	5.90[c] m	9.73[c] d ($J_{3,4}$ = 8)	12.83	5.23[c] m	7.07 br
	22479-35-2	5.43[c]	11.27[c]	9.47[c]				
		14.18	5.73[c] br	9.77[c] br	12.80[c] br 13.47[c] br	5.2[c] q ($J_{5,6}$ = 6)	7.23[c] br	
	22493-55-6	14.17	5.75[c] br	9.77[c] br	12.70[c] br 13.27[c] br 13.43[c] br	5.25[c] q ($J_{5,6}$ = 6)	7.27[c] br	
	22479-36-3	13.66[c]	5.45[c] d ($J_{2,3}$ = 6)	8.97[c] br	11.4[c] d ($J_{3,4}$ = 2.5)	3.07[c]		

[a] Coupling constants are in parenthesis. Multiplicity is indicated as follows: d, doublet; t, triplet; q, quadruplet; m, multiplet; br, broad. [b] See footnote *b*, Table I. [c] At −20°. [d] At −80°. [e] At −70°.

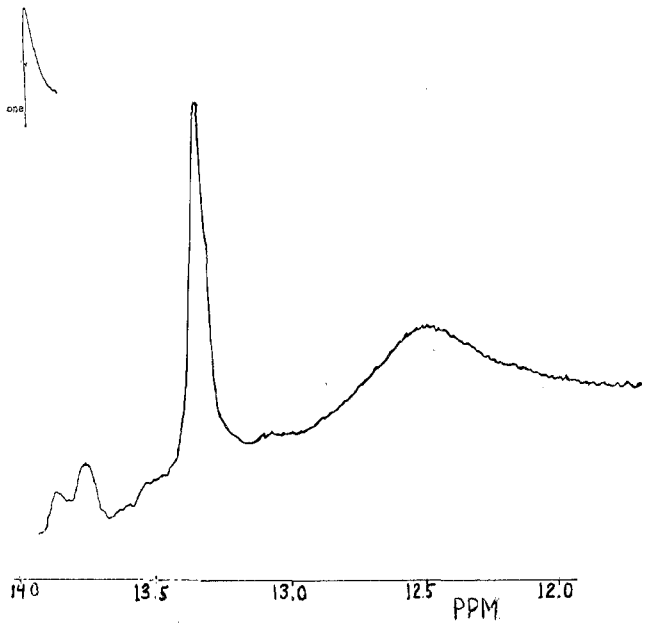


Figure 4.—Low-field portion of time-averaged 100-MHz nmr spectrum of porcine insulin in $HFSO_3$–SbF_5–SO_2 at −80°.

hr), and, after 4 hr at 45°, 97% formation of the oxocarbonium ion had taken place.

$$H_3N^+(CH_2)_4CO_2H_2^+ \xrightarrow[-H_2O]{FSO_3H-SbF_5} H_3N^+(CH_2)_4CO^+$$

In the δ-amino-*n*-butyloxocarbonium ion, there is a downfield shift of δ 1 for the methylene protons α to the oxocarbonium center, and a downfield shift of *ca.* δ 0.5 for the β-methylene protons compared with the corresponding protons in the protonated acid. These shifts are very similar to the shifts found in the dehydration of aliphatic mono- and dicarboxylic acids to oxocarbonium and dioxocarbonium ions.[3] Also, the ease of dehydration increased with increasing separation of the positively charged centers, for both of the diprotonated amino acids and the dicarboxylic acids. In none of the amino acids studied in FSO_3H–SbF_5 was there any evidence of dehydration to form the appropriate protonated lactam.

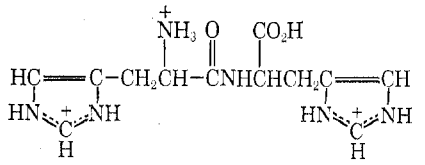
Simple Peptides.—The nmr data, obtained from solutions of N-acetylglycine and the simple peptides diglycine, triglycine, tetraglycine, dialanine, and diketopiperazine in FSO_3H–SbF_5–SO_2, are summarized in Table VII. At temperatures below −20°, diglycine was observed to exist in the strong acid solution as a stable triprotonated species. The peptide linkage was

$$H_3N^+CH_2C(OH)=NHCH_2CO_2H_2^+$$

O-protonated, the proton on oxygen at −60° appears at δ 12.90 as a sharp singlet (1 H), and at −20° the proton on nitrogen (1 H) appears at δ 9.82 as a poorly resolved triplet coupled to the adjacent methylene group. No allylic NH–OH coupling is observed. The terminal protonated amino and carboxyl (2 H) groups appear at the commonly observed amino acid shifts of δ 7.15 and 14.33. At −90°, the carboxylic resonance is a broad doublet. The methylene protons were coupled to the protons on adjacent nitrogen atoms and at −20° resolve into a doublet and a quartet, respectively. After the acid solution was heated at 40° for 2 hr, the peptide was seen to have undergone chemical change, since at −60° only peaks at δ 5.30 (4 H), 7.1 (3 H), and 10.57 (*ca.* 1 H) had been observed.

The cyclic dipeptide diketopiperazine was di-O-protonated, and at −20° the protons on oxygen (2 H) appear as a sharp singlet at δ 11.27, the protons on nitrogen (2 H) as a broad singlet at δ 9.47, and the methylene protons as a sharp singlet at δ 5.43. No allylic OH–NH coupling or vicinal NH–CH coupling are observed because of the dihedral relationship of the protons. A solution of diketopiperazine in FSO_3H–SbF_5 was not affected on being maintained for 2 hr at 40°.

The imidazole rings in histidylhistidine were observed to be protonated as in histidine itself, but, over the temperature range −20 to −90°, protonated carboxyl group, protonated peptide linkage (OH and NH), and imidazole ring NH protons could not be detected, probably because of their rapid chemical exchange.



At −60° broad lines were observed at δ 4.00 (4 H) for the methylene protons, δ 5.37 (1 H) and 5.9 (1 H) for the aliphatic methine protons, δ 7.20 (3 H) for the ammonium group, and δ 7.97 (2 H) and 9:00 (2 H) for the imidazole ring CH protons.

Triglycine was tetraprotonated. The carboxylic protons could be observed at −60°, but the two peptide linkage OH protons were best observed at −70°, when they appear at δ 12.80 and 13.47. The resonance at δ 13.47 broadened more rapidly than that at δ 12.80 with temperature rise.

Tetraglycine behaved similarly to triglycine. Pentaprotonation was observed, with the carboxyl protons being observed as a sharp peak at −60°, and the three peptide OH protons being observed at −70° as three broad exchanging peaks.

As in diglycine and triglycine, the methylene group protons adjacent to the terminal ammonium group were observed as a quartet at higher field (*ca.* δ 0.5) than the other methylene protons.

The data obtained from diprotonated N-acetylglycine provide an interesting comparison with the data from the simple peptides. At somewhat higher field than for the corresponding protons in peptides, the carboxylic protons (2 H) and the NH proton (1 H) appear at −20°, at δ 13.66 and 8.97, respectively. As in the case of the peptide linkage, O-protonation occurs and the proton on oxygen appears at −20° as a doublet as a result of allylic coupling with the NH proton (J_{OH-NH} = 2.5 Hz). The methylene protons (2 H) appear at δ 5.45 as a doublet (J_{CH_2-NH} = 6 Hz), and the methyl protons as a singlet at δ 3.07. As in the case of protonated carbamates and carbamic acids[18] and protonated formylglycine, restricted rotation about the C–N bond is assumed and the spectrum of protonated N-acetylglycine is explained on the basis of the observation of one geometrical isomer.

(18) G. A. Olah and M. Calin, *J. Amer. Chem. Soc.*, **90**, 401 (1968).

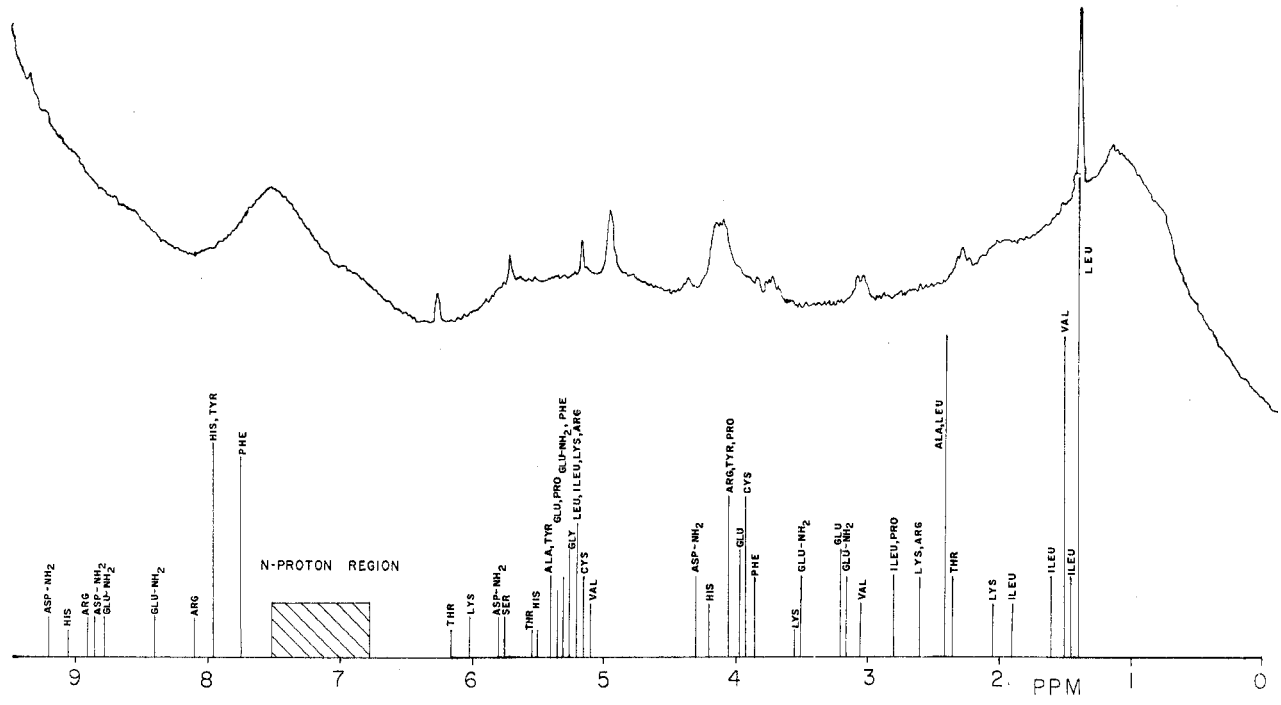


Figure 5.—100-MHz spectrum of porcine insulin in $HFSO_3$–SbF_5–SO_2 time averaged from δ 0 to 10 at −70°.

After having been maintained at 40° for 4 hr, a solution of N-acetylglycine in FSO_3H–SbF_5 was observed to have undergone some chemical change, as evidenced by the appearance of new peaks at δ 5.17 and 10.13.[19]

Porcine insulin was examined in FSO_3H–SbF_5–SO_2 solution at 100 MHz. The low-field portion (δ 11.20–16.20) of the spectrum obtained in low concentration at −80° (Figure 4) was enhanced by computer averaging (525 passes). No improvement was seen using a necessarily more dilute solution at −96.5°. The heavy absorption at δ 13.33 is assigned to the carboxylic acid protons of glutamic acid moieties and protonated hydroxyl groups of tyrosine. These resonances are at δ 13.37 and 13.00, respectively, in the corresponding amino acids in this solvent system. The two peaks at δ 13.76 and 13.87 can be assigned to the terminal carboxylic acid groups for the A and B chains. As amino acids in this medium, asparagine and alanine show absorptions at δ 15.4 and 14.37. In this case it is reasonable to expect a relative shielding effect to be observed, because of the obviously smaller charge density in this region in the protonated peptide than in the protonated amino acid. The broad signal at δ 12.50 is due to O-protonation of various peptide linkages.

The higher field portion of the spectrum (Figure 5) was time averaged at −70° for 185 passes. Figure 5 also shows schematically the absorption signals observed for the protonated amino acids in the proportions in which they comprise porcine insulin. Assignments cannot be made unequivocally by simple comparison with absorptions of the related protonated amino acids. Certain major characteristics, however, can be derived. The broad shielded absorptions as well as the sharp peak at δ 1.38 are mainly due to methyl proton absorptions of leucine, isoleucine, and valine, which comprise 72 of the 294 nonexchanging protons. The broad absorption observed at δ 5–6, is due to the respective methine protons, and that at δ 7.50 to N–H and overlapping aromatic protons.

We had hoped that extensive protonation, not only on nitrogen but also on carbonyl oxygen, would cause sufficiently varying deshielding effects in the superacid media to allow more rigorous characterization of high molecular weight peptides. Work is now in progress on some lower molecular weight peptides to determine characteristics of protonation of the peptide bond and the effect of a wide range of neighboring amino acids.

(19) As our paper was submitted for publication, a brief communication [J. L. Sudmeier and K. E. Schwartz, *Chem. Commun.*, 1646 (1968)] indicated that, in FSO_3H–SbF_5–SO_2 solution at −90°, di-, tri-, and tetraglycines, as well as L-alanylglycine, showed two low-field absorptions for the nonequivalent terminal carboxylic acid protons. Freezing out the C–O bond rotations involved in protonated carboxylic acids was known from previous work. In the present work, carried out generally at −60°, temperatures were not low enough to observe the two isomers of the protonated acids. The nonequivalencies of the carboxylic acid protons were observed only in glycine and proline. In serine, threonine, and cysteine, exchange at the temperatures studied was so rapid that no carboxylic acid proton absorption was observed.

Experimental Section

Materials.—All the compounds used were commercially available in high purity. All asymmetric compounds were of the L configuration apart from DL-glutamic acid, δ-DL-(+)-allohydroxylysine, α- and β-aminobutanoic acid, D-thyroxine, DL-histidyl-DL-histidine, and DL-alanyl-DL-alanine. The monohydrochloride of L-lysine and the monohydrochloride monohydrate of L-homoarginine, L-cysteine, and L-histidine were used.

Nmr Spectra.—A Varian Associates Model A-56/60A nmr spectrometer with a variable-temperature probe was used for all spectra except those for porcine insulin. Coupling constants are believed accurate to ±0.1 Hz. The reference standard used was capillary TMS.

The porcine insulin was examined in a Varian Associates Model HA-100 nmr spectrometer. Signal enhancement was accomplished with a Varian Model C-1024 time-averaging computer. As internal lock, the main acid peak was used, the chemical shift of which was subsequently determined relative to capillary TMS. Owing to limited space, all spectra cannot be published with this article. Spectra, however, may be obtained by ordering from ASIS National Auxiliary Publications Service, % CCM Information Corp., 909 3rd Ave., New York, N. Y. 10022, remitting $1.00 for microfiche or $3.00 for photocopies, Document No. 00602.

Preparation of Strong Acid Solutions.—Solutions were made up with at least 3 mol of 1:1 FSO_3H–SbF_5 for each site available for protonation in the solute, and the solutions were further diluted with *ca.* 3 mol of sulfur dioxide for each mol of 1:1 FSO_3H–SbF_5. The aromatic amino acids were diluted in a solution of 9:1 FSO_3H–SbF_5.

Few of the starting compounds investigated dissolved in sulfur dioxide, and the solutions were made up slowly, at low temperature, by vigorous agitation of the suspensions with the acid solusolutions, resulting in homogeneous solutions of the protonated substrates.

The presence of a large excess of acid was ensured by observing the characteristic acid peak at *ca.* δ 11.

Registry No.—Protonated histidylhistidine, 22493-22-7; protonated triglycine, 22493-23-8.

Acknowledgment.—Professor M. Bodansky is thanked for samples of porcine insulin and stimulating discussions. The research was possible through a grant from the National Institutes of Health.

Stable Carbonium Ions. LXXXIV.[1] Diprotonation of Dialkyl Hydrazodiformates and Their Cleavage to Diprotonated Hydrazodiformic Acid and Alkylcarbonium Ions

GEORGE A. OLAH, JUDITH A. OLAH, AND RICHARD H. SCHLOSBERG[2]

Department of Chemistry, Case Western Reserve University, Cleveland, Ohio 44106

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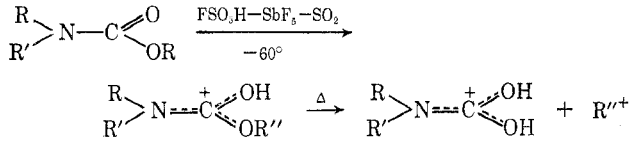
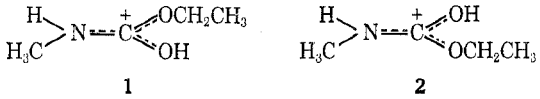
A series of dialkyl hydrazodiformates [$(RO_2CNH)_2$] has been investigated in FSO_3H–SbF_5–SO_2 and/or HF–SbF_5–SO_2 solution. Carbonyl oxygen protonation was observed in all cases, regardless of substituents, by means of low-temperature pmr spectroscopy. With certain of the diprotonated dialkyl hydrazodiformates, cleavage occurred in the extremely strong acid systems at higher temperatures to give stable alkylcarbonium ions and diprotonated hydrazodiformic acid [$(NHCO_2H_2^+)_2$]. Diprotonation of the related azodicarbonamide in strong acid media was also observed by low-temperature pmr spectroscopy.

In an earlier study we reported the observation of protonated alkyl carbamates.[3]



R, R′ = alkyl or hydrogen; R″ = alkyl

For example, in the pmr spectrum of protonated ethyl N-methyl carbamate, the proton on oxygen appears at δ 9.71 as an overlapping doublet on top of a singlet. The OH proton is expected to show two absorptions owing to *cis* and *trans* isomers (**1** and **2**) if on



1 **2**

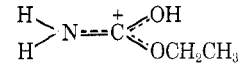
the pmr time scale rotation about the C–N bond is slow. That one of the OH resonances is a doublet (J = 2.8 Hz) is presumably due to long-range coupling in **1** with the NH proton.

Olah and Calin[3] observed that at temperatures as low as −60° protonated alkyl carbamates undergo alkyl-oxygen cleavage to give carbonium ions and protonated carbamic acids and that the rate of cleavage is tertiary alkyl carbamates > secondary > primary > methyl.

Dialkyl hydrazodiformates are bisalkyl carbamates. In an extension of our studies of protonation of weak organic bases, we investigated the chemistry of these compounds in superacid media. Protonation of the following dialkyl hydrazodiformates was examined in FSO_3H(HF)–SbF_5–SO_2 solution: dimethyl, diethyl, di-*n*-propyl, di-*n*-butyl, and di-isobutyl hydrazodiformate.

As in the case with alkyl carbamates,[3] imides,[4] amides,[5] and simple dipeptides,[6] protonation in the extremely strong acid media is observed exclusively at the carbonyl oxygen atoms for all dialkyl hydrazodiformates studied. The protonated species give well-resolved low-temperature pmr spectra in the superacid media. The spectral parameters for the diprotonated dialkyl hydrazodiformates are summarized in Table I.

As a representative case, the pmr spectrum of diprotonated diethyl hydrazodiformate (Figure 1) can be compared with that of protonated ethyl carbamate.[3] The OH proton of protonated ethyl carbamate (**3**)



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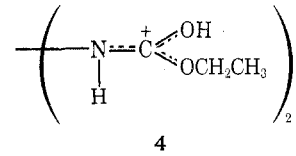
shows a doublet (J = 2 Hz) at δ 9.86 caused by coupling to the protons on nitrogen, which show two broad singlets at δ 7.40 and 7.33. The methyl protons show a triplet at δ 1.60 and the methylene protons show a quartet at δ 4.86. In the case of diprotonated diethyl hydrazodiformate (**4**), the chemical shifts of both the



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NH and OH protons are deshielded by about 1 ppm from those in the carbamate, as would be expected for a doubly charged species such as **4**. On closer inspection the spectrum very clearly shows the presence of two virtually identical (Δ *ca.* 2 Hz) ethyl groups. Second-order splitting is ruled out, as Δ is the same for

(1) Part LXXXIII: G. A. Olah, D. L. Brydon, and R. D. Porter, *J. Org. Chem.*, **35**, 317 (1970).
(2) Postdoctoral Research Investigator, 1968–1969.
(3) G. A. Olah and M. Calin, *J. Amer. Chem. Soc.*, **90**, 401 (1968).
(4) G. A. Olah and R. H. Schlosberg, *ibid.*, **90**, 6464 (1968).
(5) T. Birchall and R. J. Gillespie, *Can. J. Chem.*, **41**, 148 (1963).
(6) G. A. Olah, D. L. Brydon, and R. D. Porter, *J. Org. Chem.*, in press.