*Nuclear Magnetic Resonance Spectra of Amino Acids and their Derivatives. II. Analyses of NMR Spectra of Amino Acids**

By Shizuo FUJIWARA and Yoji ARATA

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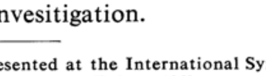
Investigations of proton resonance in amino acids have so far been restricted to those of the over-all features of the spectra[1-3]. This situation led us to make more detailed study of the spectra.

We will summarize here the results of precise analyses of the spectra of methylene and methine protons of amino acids of the type,



$$\underset{\displaystyle NH_2}{R-CH_2-\underset{|}{CH}-COOH}$$

where R has no protons interacting with those under invesitigation.

Experimental

Materials.—The amino acids studied were all commercial materials (Ajinomoto Co.). They were dissolved in heavy water, 2N HCl, and 2N NaOD at the concentration of 1 mol.%. 2N HCl was prepared by diluting concentrated hydrochloric acid with heavy water. 2N NaOD was prepared by adding sodium peroxide powder to heavy water under cooling.

Spectrometer.—A Varian Associates model V4301C NMR spectrometer operating at 60.00 Mc./sec. was employed for all measurements.

Results

Cysteine dissolved in diluted hydrochloric acid gives a simple spectrum as shown in Fig. 1d. The spectrum tells that the two protons attached to the beta-carbon atom are equivalent to each other. Serine and aspartic acid also

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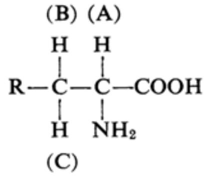
[1] M. Takeda and O. Jardetzky, *J. Chem. Phys.*, **26**, 1346 (1957).

[2] O. Jardetzky and C. D. Jardetzky, *J. Biol. Chem.*, **233**, 383 (1958).

[3] F. A. Bovey and G. V. D. Tiers, *J. Am. Chem. Soc.*, **81**, 2870 (1959).

Table I. Results of analyses in c. p. s. for the spectra measured at 60.00 Mc./sec.



$$R-\overset{\overset{H}{|}}{\underset{\underset{H}{|}}{C}}-\overset{\overset{H}{|}}{\underset{\underset{NH_2}{|}}{C}}-COOH$$

(B) (A) (C)

		$\nu_A-\nu_C$	$\nu_B-\nu_C$	J_{AC}	J_{BC}	J_{CA}
Phenylalanine	2N HCl	67.4	3.7	3.2	14.6	10.0
	2N NaOD	42.1	14.5	4.0	13.3	8.9
Cystine	2N HCl	68.3	4.3	2.0	15.1	9.8
Cysteine	2N HCl	74.2	0	5.0	—	5.0
	D_2O	55.5	0	4.9	—	4.9

give the spectra of three protons, two of which are equivalent.

Chemical shifts between alpha and beta protons vary from one amino acid to others. When the chemical shift is large compared to the spin coupling constant between these protons, the spectrum can easily be analyzed by means of the first-order perturbation theory. Cysteine in an acid medium shows a spectrum of this type.

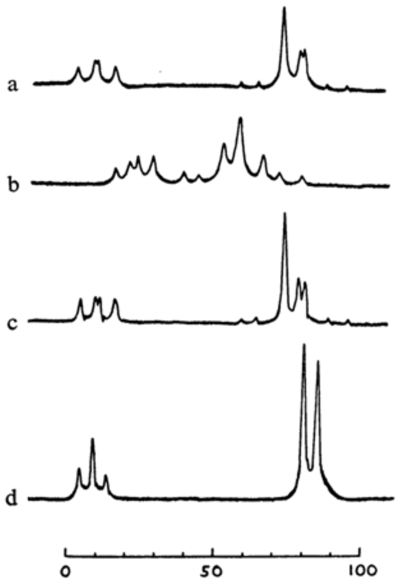


Fig. 1. NMR Spectra of amino acids measured at 60.00 Mc./sec.
(a) Phenylalanine in 2N HCl
(b) Phenylalanine in 2N NaOD
(c) Cystine in 2N HCl
(d) Cysteine in 2N HCl
In these spectra magnetic field increases from left to right.

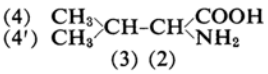
When cysteine is dissolved in diluted NaOD solution, the spectrum changes to a complex one of a non-equivalent three spin system.

When R is bulky as in the cases of phenylalanine, tryptophane, tyrosine, hystidine and cystine, the spectra take the features of a nonequivalent three spin system even in acid media as well as in basic media.

For the analyses of these spectra we used the method of the least-squares reported by Shimizu and the present authors⁴⁾. This method is useful in the present case, where we have relatively fewer than those theoretically expected. Some examples of the spectra are shown in Fig. 1a—1c and the results of the analyses are summarized in Table I.

The molecular constants shown in the table may be considered to be the weighted averages of chemical shifts and of spin-coupling constants over rotational isomers⁵⁾. The relative population of each rotational isomer depends upon the acidity of the solution. This situation is more clearly seen in the spectra of valine for several kinds of solutions with different pH. The results are summarized in Table II.

Table II. Chemical shifts and spin spin coupling constants in c. p. s. obtained from the spectra of valine at a series of pH values



$$\begin{matrix}(4)\ CH_3\\(4')\ CH_3\end{matrix}\!>\!CH\text{-}CH\!<\!\begin{matrix}COOH\\NH_2\end{matrix}$$

(3) (2)

pH	J_{23}	$\|\nu_4-\nu_{4'}\|$
14	5.1	4.2
13.0	5.1	4.2
7.0	4.2	3.0
2.6	4.4	3.0
1.0	4.3	1.9
1	4.3	2.1

The spectra were measured at 60.00 Mc./sec.

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Department of Chemistry
Faculty of Science
The University of Tokyo
Hongo, Tokyo (S. F.)
Univeresity of Electro-communication
Chofu, Tokyo (Y. A.)

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4) Y. Arata, H. Shimizu and S. Fujiwara, *J. Chem. Phys.*, **36**, 1951 (1962).

5) J. A. Pople, *Mol. Phys.*, **1**, 3 (1958).