*Nuclear Magnetic Resonance Spectra of Amino Acids and their Derivatives. I. DNP Amino Acids**

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The molecular structure of amino acids has already been investigated by a variety of physical and chemical techniques. High-resolution NMR spectroscopy has also been used for this purpose. Using it, Takeda and Jardetzky[1], Jardetzky and Jardetzky[2], and Bovey and Tiers[3] have measured the proton chemical shift of NH and CH groups in amino acids.

Our aim is to study the NMR spectra of amino acids in more detail in order to compile fundamental data for the investigation of the structure of polypeptides and proteins by the NMR method.

The signal due to the NH proton of an amino acid cannot usually be observed in a water solution, since the NH proton of the amino group is subject to rapid exchange between neighboring molecules. However, in an *N*-(2, 4-dinitrophenyl) amino acid (DNP amino acid), when the sample is dissolved in dehydrated dioxane, the exchange of the NH proton is slow enough to permit the observation of the signal.

The patterns of the spectra arising from the protons on the NH and DNP groups are found to be useful for the identification of DNP amino acids.

Experimental

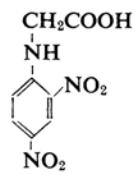
Materials. — All of the samples were purchased from Wako Chemicals & Co. Solutions (10%, w/v) of each DNP amino acid were prepared in dehydrated dioxane, and 2,4-dinitroaniline was dissolved in dehydrated acetone. The solutions were sealed in 5 mm. o. d. sample tubes. Sixteen kinds of DNP amino acids were used in this experiment: *N*-(2,4-dinitrophenyl) derivatives of glycine (Gly), L-alanine (Ala), L-serine (Ser), L-methionine (Met), L-threonine (Thr), L-allothreonine (Althr), L-leucine (Leu), L-isoleucine (Ileu), L-valine (Val), L-lysine (Lys), L-aspartic acid (Asp), L-glutamic acid (Glu), L-phenylalanine (Phe), L-tryptophan (Try), L-proline (Pro) and L-hydroxyproline (Hyp).

Spectrometer.—A Varian Associates model V4300C NMR spectrometer, operating at 56.44 Mc./sec., was employed for all measurements.

Calibration of the Spectra. — The frequency separations of the spectra with reference to the dioxane signal were determined by the usual side-band technique. The chemical shift data, expressed in p. p. m., are summarized in Fig. 3.

Results

The signals of the three protons of the 2, 4-dinitrophenyl group are easily identified, as is shown in the spectrum of DNP-glycine (Fig. 2). The assignment is found to be unambiguous if the spectrum is compared with that of



2, 4-dinitroaniline (Fig. 2d). In these spectra the signals due to $H_{(3)}$ and $H_{(6)}$ are both doublet; the spin-spin coupling constant between these protons (J_{36}) is estimated to be smaller than 1 c. p. s. J_{35} and J_{56} are found to be 2.8 and 9.2 c. p. s. respectively.

The signal due to the NH proton of DNP-glycine is observed as a slightly broad triplet at the high field side of the $H_{(3)}$ signal (Fig. 2a). The splitting can be attributed to the spin-spin coupling with CH_2 protons. The spin-spin coupling constant is 5.6 c. p. s. When a small amount of water is added, the signal is further broadened. This can be interpreted in terms of an increasing exchange rate of the NH proton (Fig. 2b, 2c). A detailed analysis of the broadening of the signal will be the subject of a forthcoming paper.

In all the other DNP amino acids except DNP-lysine, the NH proton signals are doublet, and in some cases they are overlapped by the $H_{(3)}$ signals. DNP-lysine gives rise to two signals of the NH protons; one is due to the α-NH proton, and the other is due to the ε-NH proton. These signals are split into doublet and triplet, respectively, by interaction with $C_{(2)}$-H and $C_{(6)}$-H protons.

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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).

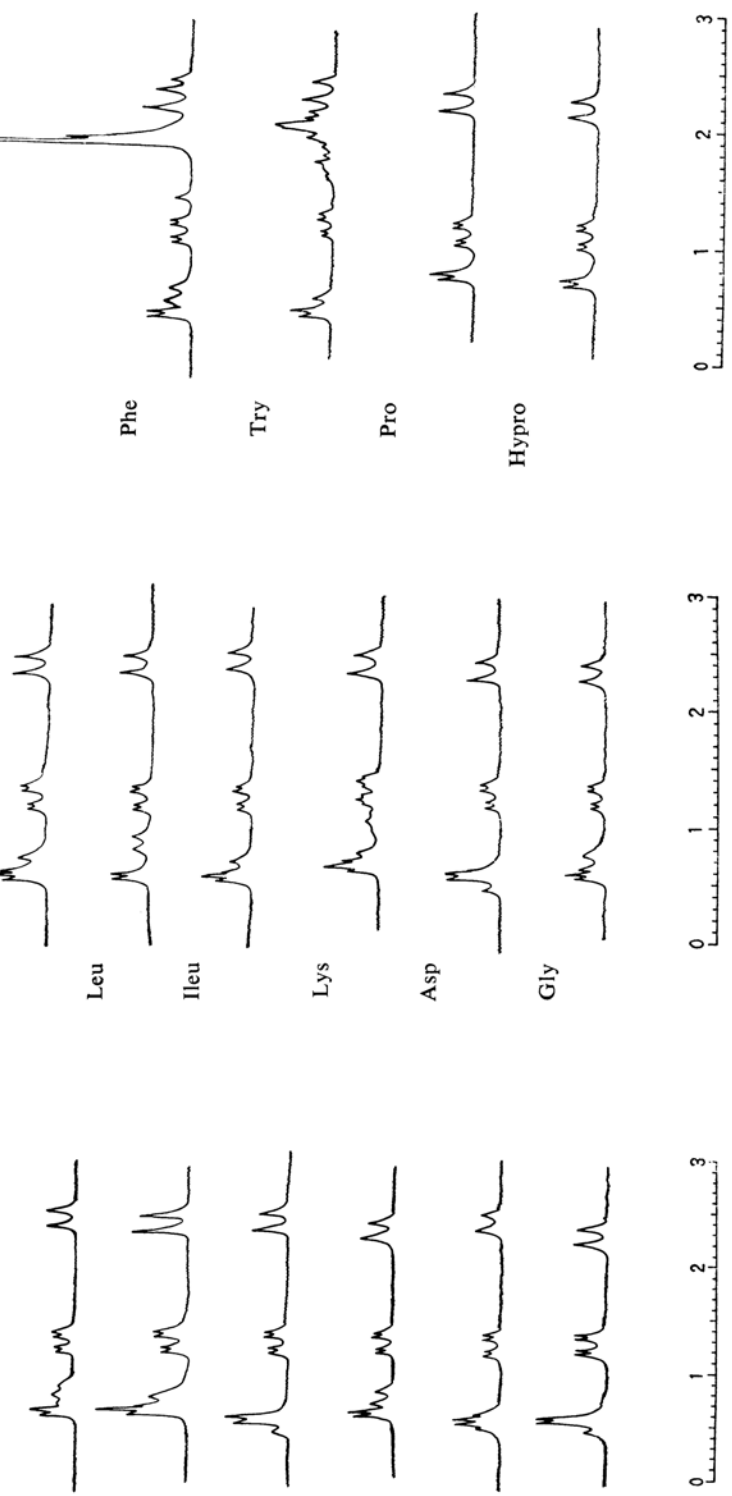

Fig. 1-1.

Fig. 1-2.

Fig. 1-3.

Fig. 1. The proton resonance spectra of the NH and 2,4-dinitrophenyl groups in DNP amino acids. Magnetic field increases from left to right.

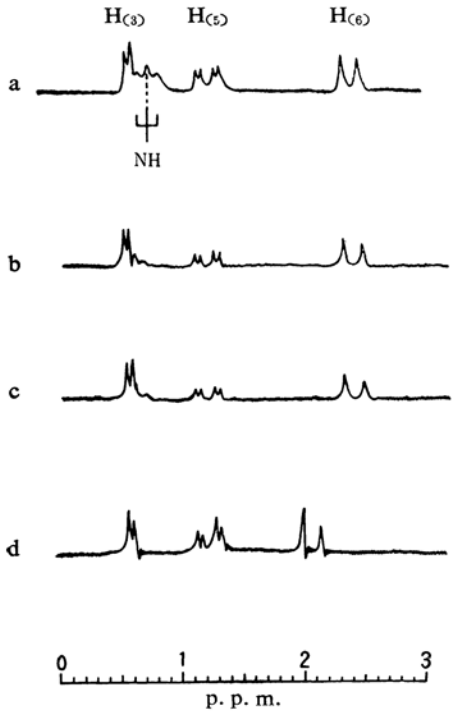

Fig. 2. The proton resonance spectra of DNP-glycine (a), (b) and (c) and of 2,4-dinitroaniline (d).
(a) Assignment of the signals. (b) Distortion of the NH proton signal caused by addition of a small amount of water. (c) The distortion by further addition of water.

In the spectra of DNP-proline and DNP-hydroxyproline, which have no NH proton, signals corresponding to those mentioned above are not observed. This also confirms the assignment of the NH proton signal.

The patterns of the spectra arising from the protons of the NH and DNP groups are sensitive to a slight change in the molecular structure. Thus, DNP-threonine and DNP-allothreonine can easily be discriminated. The same is true for DNP-leucine, DNP-isoleucine, and DNP-valine, and also for DNP-aspartic acid and DNP-glutamic acid.

In DNP-proline and DNP-hydroxyproline, the chemical shift between $H_{(3)}$ and $H_{(5)}$ is small compared with that in the other DNP

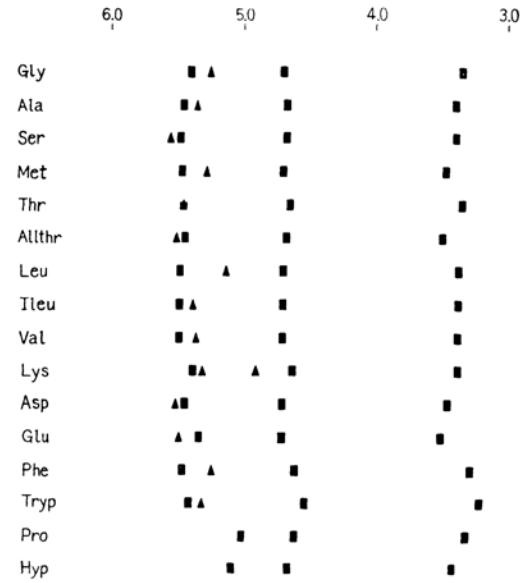

Fig. 3. Proton chemical shifts in p. p. m. of the NH and 2,4-dinitrophenyl groups referred to dioxane. Magnetic field increases from left to right. In the figure ▲ and ■ represent the center of the NH and CH proton signals, respectively.

amino acids. This may be interpreted in terms of a twisting of the NO_2 group at carbon 2 of the DNP group caused by the presence of the bulky 2-carboxy-1-pyrrolidyl group.

Signals due to alkyl chain protons cannot be identified in a dioxane solution. Therefore, an investigation of the signals of free amino acids in a D_2O solution is now in progress. The results will be discussed in the next paper.

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