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NMR Spectra of α- and γ-L-Glutamyl-α-aminoisobutyric Acid and Some Related Compounds

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Two methyl groups of α-L-glutamyl-α-aminoisobutyric acid which were equivalent in the acidic solution became unequivalent in the aqueous and basic solutions. Such an unequivalence of two methyl groups was not manifested in the cases of γ-L-glutamyl-α-aminoisobutyric acid, α- and γ-L-glutamylisopropylamide, N-glutaryl-α-aminoisobutyric acid and N-glutarylisopropylamine.

In previous papers, we described that the sequence of a dipeptide containing glutamic acid could be determined by measuring the NMR spectra of the dipeptide in acidic, aqueous and basic solutions[1,2] and also reported about the difference in NMR spectra between some α- and γ-glutamyl dipeptides.[3] This paper deals with the NMR spectra of α- and γ-L-glutamyl-α-aminoisobutyric acid and some related compounds.

For measuring NMR spectra, sample solutions were prepared as follows; 0.1 mmole each of glutamyl-α-aminoisobutyric acid, N-glutaryl-α-aminoisobutyric acid and glutamylglycine was dissolved in 1 ml of 0.2 N NaOD, and 0.1 mmole each of glutamylisopropylamide and N-glutarylisopropylamine was dissolved in 1 ml of 0.1 N NaOD yielding an anionic solution. One-tenth mmole each of glutamyl-α-aminoisobutyric acid, glutamylisopropylamide and glutamylglycine was dissolved in 1 ml of 0.1 N DCl yielding a cationic solution. Chemical shifts were expressed relative to DSS as an internal standard.

As shown in Table I, two methyl groups of α-L-glutamyl-α-aminoisobutyric acid which were equivalent in the acidic solution became unequivalent in the aqueous and basic solutions. But two methyl groups of α-L-glutamylisopropylamide corresponding to the compound which lost the C-terminal carboxyl group of α-L-glutamyl-α-aminoisobutyric acid were equivalent in cationic, zwitter ionic, and anionic forms. Two methyl groups of N-glutaryl-α-aminoisobutyric acid corresponding to the compound which lost the N-terminal amino group of α-L-glutamyl-α-aminoisobutyric acid were also equivalent in the aqueous and basic solutions. The unequivalence of two methyl groups was also not recognized in the case of N-glutarylisopropylamine which does not have both N-terminal amino group and C-terminal carboxyl group. Two methyl groups of γ-L-glutamyl-α-aminoisobutyric acid and γ-L-glutamylisopropylamide were equivalent. Consequently the presence of α-amino group and ionized C-terminal carboxyl group seems to be required for the appearance of the unequivalence of two methyl groups of α-L-glutamyl-α-aminoisobutyric acid.

The glycyl methylene group of α-L-glutamylglycine which was equivalent in the acidic solution became unequivalent in the aqueous and basic solutions (Table II). The same conditions as in the case of α-L-glutamyl-α-aminoisobutyric acid may be required for the appearance of the unequivalence of the glycyl methylene group of α-L-glutamylglycine; namely, α-amino and ionized carboxyl groups are essential.

The unequivalence of glycyl methylene protons has been observed in a many kind of dipeptides containing glycine as C-terminal constituent.[4~8] The observed unequivalence

TABLE I. CHEMICAL SHIFTS OF METHYL GROUPS OF α- AND γ-L-GLUTAMYL-α-AMINOISOBUTYRIC ACID AND SOME RELATED COMPOUNDS (Hz from DSS as an Internal Standard. 90 MHz).

$HOOC\underset{R_1}{CH}CH_2\underset{R_2}{CH}CONHC\underset{R_3}{(CH_3)_2}$ (1); R_1=H, R_2=NH_2, R_3=COOH. (2); R_1=NH_2, R_2=H, R_3=COOH. (3); R_1=H, R_2=NH_2, R_3=H. (4); R_1=NH_2, R_2=H, R_3=H. (5); R_1=R_2=H, R_3=COOH. (6); R_1=R_2=R_3=H.

	In DCl Solution[a]	In D_2O Solution	In NaOD Solution[a]
α-L-Glutamyl-α-aminoisobutyric acid (1)	131(s, 6H)	126(s, 3H), 125(s, 3H)	125(s, 3H), 123(s, 3H)
γ-L-Glutamyl-α-aminoisobutyric acid(2)	128(s, 6H)	130(s, 6H)	121(s, 6H)
α-L-Glutamylisopropylamide (3)	102(d, J=7Hz, 6H)	102(d, J=7Hz, 6H)	100(d, J=7Hz, 6H)
γ-L-Glutamylisopropylamide (4)	98(d, J=7Hz, 6H)	98(d, J=7Hz, 6H)	98(d, J=7Hz, 6H)
N-Glutaryl-α-aminoisobutyric aicd (5)	128(s, 6H)	128(s, 6H)[b]	123(s, 6H)
N-Glutarylisopropylamine (6)	98(d, J=7Hz, 6H)	98(d, J=7Hz, 6H)[b]	99(d, J=7Hz, 6H)

[a] See the text for concentrations of sample, DCl and NaOD.
[b] Carboxyl group does not dissociate as in DCl solution.

TABLE II. CHEMICAL SHIFTS OF GLYCYL METHYLENE PROTONS OF α- AND γ-L-GLUTAMYLGLYCINE (Hz from DSS as an Internal Standard. 90 MHz).

	In DCl Solution[a]	In D_2O Solution	In NaOD Solution[a]
α-L-Glutamylglycine	365(s, 2H)	352(d, J=18Hz, 1H)	341(d, J=17Hz, 1H)
		342(d, J=18Hz, 1H)	332(d, J=17Hz, 1H)
γ-L-Glutamylglycine	359(s, 2H)	354(s, 2H)	334(s, 2H)

[a] See the text for concentrations of Sample, DCl and NaOD.

should reflect the restriction of rotation in molecules by interaction between α-amino group and ionized C-terminal carboxyl group.[5] It seems unlikely that the unequivalence of two methyl groups of α-L-glutamyl-α-aminoisobutyric acid and that of the glycyl methylene group of α-L-glutamylglycine are due to the influence of the *cis-trans* isomerism of the peptide bond,[9] because such an unequivalence is not manifested in α- and γ-L-glutamylisopropylamide, γ-L-glutamyl-α-aminoisobutyric acid, N-glutaryl-α-aminoisobutyric acid, N-glutarylisopropylamine and γ-L-glutamylglycine. It is also not interpreted by the *cis-trans* isomerism of the peptide bond that the glycyl methylene protons of α-L-glutamylglycine give four lines of AB pattern and that the peak areas of unequivalent two methyl groups of α-L-glutamyl-α-aminoisobutyric acid are equal. If the unequivalence of two methyl groups of α-L-glutamyl-α-aminoisobutyric acid was due to the *cis-trans* isomerism of the peptide bond, the amount of *cis*-form might be equal to that of *trans*-form, because the peak areas of these unequivalent two methyl groups are equal. But LaPlanche *et al.* have described that eighty-eight percent of N-isopropylformamide exists in *trans*-form and N-isopropylacetamide and N-isopropylisobutylamide are present only in *trans*-form.[10] According to them, it is not conceivable that a half of α-L-glutamyl-α-aminoisobutyric acid exists in *cis*-form.

EXPERIMENTAL

NMR spectra were obtained with a Hitachi R-22 (90 MHz) Spectrometer. Chemical shifts were expressed relative to DSS as an internal standard.

Preparation of α- and γ-L-glutamyl-α-aminoisobutyric acid. A mixture of α- and γ-L-glutamyl-α-aminoisobutyric acid obtained by the reaction of N-carbobenzoxy-L-glutamic acid anhydride with α-aminoisobutyric acid followed by removal of the protective group was separated to each component with the use of Dowex 1×4 ($HCOO^-$).[11] *Anal.* Found for α-: C, 46.30; H, 7.04; N, 12.18. Found for γ-: C, 46.52; H, 6.99; N, 11.93. Calcd. for $C_9H_{16}N_2O_5$: C, 46.59; H, 6.95; N, 12.08.

Preparation of α- and γ-L-glutamylisopropylamide. The reaction of a mixture of α- and γ- benzylester of N-carbobenzoxy-L-glutamic acid with isopropylamine

by the mixed anhydride method[12,13] and removal of the protective group gave a mixture of α- and γ-L-glutamylisopropylamide. They separated to each component with the use of Dowex 1×4 ($HCOO^-$). *Anal.* Found for α-: C, 50.59; H, 8.47; N, 14.64. Found for γ-: C, 50.88; H, 8.66; N, 14.85. Calcd. for $C_8H_{16}N_2O_3$: C, 51.06; H, 8.51; N, 14.89.

Preparation of N-glutaryl-α-aminoisobutyric acid. The reaction of glutaric anhydride with α-aminoisobutyric acid gave the desired product. *Anal.* Found: C, 49.53; H, 6.92; N, 6.46. Calcd. for $C_9H_{15}NO_5$: C, 49.81; H, 6.97; N, 6.46.

Preparation of N-glutarylisopropylamine. The compound was obtained by the reaction of glutaric anhydride with isopropylamine. *Anal.* Found: C, 55.52; H, 8.88; N, 8.13. Calcd. for $C_8H_{15}NO_3$: C, 55.54; H, 8.74; N, 8.10.

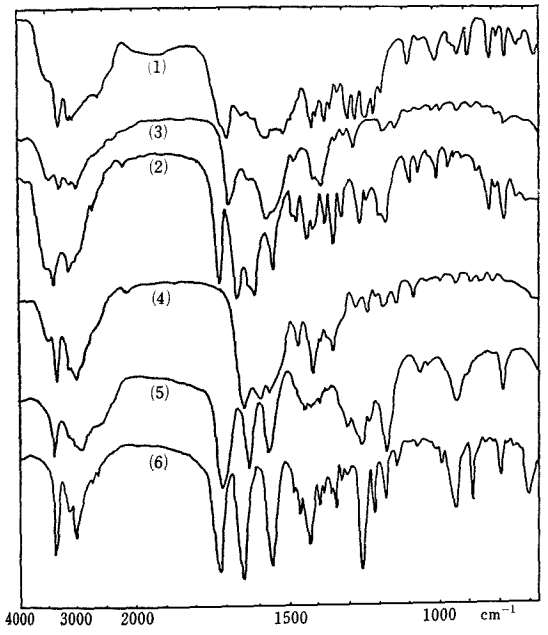

FIG. 1. IR Spectra of α-L-Glutamyl-α-aminoisobutyric acid (1), α-L-Glutamylisopropylamide (3), γ-L-Glutamyl-α-aminoisobutyric acid (2), γ-L-Glutamylisopropylamide (4), N-Glutaryl-α-aminoisobutyric acid (5) and N-Glutarylisopropylamine (6) (KBr disks).

Preparation of α- and γ-L-glutamylglycine. These compounds were prepared from N-carbobenzoxy-L-glutamic acid anhydride and glycine by the same method as for the preparation of α- and γ-L-glutamyl-α-aminoisobutyric acid. *Anal.* Found for α-: C, 41.15; H, 5.80; N, 13.79. Found for γ-: C, 41.07; H, 5.82; N, 13.80. Calcd. for $C_7H_{12}N_2O_5$: C, 41.21; H, 5.93; N, 13.73.

Infrared spectra of glutamyl-α-aminoisobutyric acid and its derivatives are shown in Fig. 1. Infrared spectra of many α- and γ-glutamyl dipeptides will be described elsewhere.[14]

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