The Isolation of $\gamma$-Glutamyl-$\beta$-aminoisobutyric Acid from Iris Bulbs

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The natural occurrence of $\beta$-aminoisobutyric acid was first established in 1951 when it was isolated from urine (1, 2). The first evidence for the existence of this amino acid in plants was reported in 1959 with the isolation of $\beta$-aminoisobutyric acid from iris bulbs (Iris tingitana var. Wedgewood) (3). This paper records the evidence for a bound form of $\beta$-aminoisobutyric acid in the iris bulb.

The compound was discovered in the nonprotein acidic amino acid fraction as an acid-labile substance which occupied an unusual position on a two directional paper chromatogram ($R_f$ in butanol-acetic acid-water, 9:1:2.5, = 0.40 and $R_f$ in phenol-water, 8:3, = 0.75). The compound was isolated by ion exchange techniques. Chemical and physical characteristics of both the isolated substance and its hydrolytic products established the structure as a dipeptide, $\gamma$-glutamyl-$\beta$-aminoisobutyric acid.

EXPERIMENTAL PROCEDURE

Isolation—Macerated bulbs (45 kg) of Wedgewood iris were extracted with 80% ethanol at room temperature. The amino acids in the extract were separated as a group by absorption on Dowex 50 in the hydrogen form and elution with ammonia at 1° (4). A water solution of half the amino acid fraction was put on a 3.4- by 115-cm column of Dowex 1 (acetate form, 200 to 400 mesh) at room temperature. The neutral and basic amino acids were washed through with water, and the acidic amino acids were fractionated by elution with 0.05 N acetic acid. After collection of 500 ml, 15-ml fractions were collected every 7 minutes. Individual fractions were tested for ninhydrin activity and examined by two directional paper chromatography. Fractions 220 to 350 were combined with the comparable fractions from a second run with the other half of the amino acids.

The total dry weight from the combined fractions was 5.6 g. The material contained an impurity which was responsible for about 25% of the ninhydrin activity. After four recrystallizations from water, 2.4 g of an uncontaminated crystalline material was obtained.

Properties—The elemental analysis of the isolated compound was 46.97% C, 6.94% H, and 11.82% N. Theoretical values for C$_6$H$_9$N$_2$O$_4$ are 46.6% C, 6.95% H, and 12.06% N. The compound was unstable to acid and alkaline hydrolysis. $R_f$ values in butanol-acetic acid-water and phenol-water were 0.40 and 0.75, respectively. In the quantitative amino acid analysis (5), the color obtained was only 42.5% of that obtained for leucine on the basis of the nitrogen content. Prolonged heating did not increase the color. A pyridoxal test (6) gave a positive test for an $\alpha$-amino acid. The mobility of the isolated material in the chromatographic system of Moore et al. (7) was close to that of serine and asparagine. Under these conditions, the molar color yield was 86% of that of leucine (8).

Identification—After the purified material was hydrolyzed by heating it in 6 N HCl for 3 hours at 120°, two ninhydrin-active compounds were observed on two directional paper chromatograms. The two products were separated by passage of a neutral solution through a column of Dowex 1 in the acetate form. One amino acid was acidic, as evidenced by its retention on the resin. After elution with acetic acid, this compound was chromatographed on paper with glutamic acid.

The other amino acid which was not retained by the resin reacted like a non-$\alpha$-amino acid in the pyridoxal test (9). This acid had the same $R_f$ values in butanol-acetic acid-water and phenol-water as $\beta$-aminoisobutyric acid. Examination of the hydrolysis mixture with the automatic amino acid analyzer (7, 8) showed that the products had the same mobility as glutamic acid and $\beta$-aminoisobutyric acid, and were present in a molar ratio of 0.94. The amount of these two acids recovered accounted for 98% of the peptide hydrolyzed.

The 2,4-dinitrophenyl derivative of the peptide was prepared (9) and hydrolyzed with 4 N HCl for 3 hours at 120°. The dinitrophenyl moiety chromatographed with the dinitrophenyl derivative of glutamic acid in Levy's solvent (9), and separated from dinitrophenyl-$\beta$-aminoisobutyric acid.

The work of Sachs and Brand (10) has demonstrated that $\gamma$-glutamyl peptides react with nitrous acid under the usual conditions of the Van Slyke reaction to give approximately 2 moles of nitrogen per mole of peptide. $\gamma$-Glutamyl peptides with a substituent on either the $\alpha$-carboxyl group or the $\alpha$-amino group, and $\alpha$-glutamyl peptides do not yield more than 1 mole of nitrogen per mole. The isolated peptide produced 1.85 moles of nitrogen per mole, showing that the $\beta$-aminoisobutyric acid was attached through the $\gamma$-carboxyl group of the glutamic acid and that the amino group of the glutamic acid moiety was unsubstituted.

Proof of Identity of Hydrolytic Products—The peptide (600 mg) was hydrolyzed with HCl and the excess of acid removed by evaporation. A neutral solution of the products of hydrolysis was hydrolyzed with $\mathrm{H}_2\mathrm{O}$ and the excess of acid removed by evaporation. A neutral solution of the products of hydrolysis was then turned into a neutral solution through a column of Dowex 1 in the acetate form. The molar color yield was 86% of that of leucine (8).

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was passed through a column of Dowex 1 in the acetate form to separate glutamic acid from the β-aminoisobutyric acid (see above). The β-aminoisobutyric acid was recrystallized from ethanol water several times to yield 180 mg of crystalline material. The elemental analysis was 46.78% C, 8.67% H, and 13.66% N. Theoretical values for β-aminoisobutyric acid are 46.60% C, 8.80% H, and 13.59% N. Specific rotation was \([\alpha]_D^2 -17\) (c = 1%)

The β-aminoisobutyric acid was racemized with 5 N NaOH (16 hours at 120°C), separated from the alkali by passage through Amberlite IRC-50 in the hydrogen form, and recrystallized from 95% ethanol. There was no difference between the infrared absorption patterns of this racemized acid and of commercially available β-aminoisobutyric acid.

The glutamic acid which was eluted from the resin with 2 N acetic acid was recrystallized twice from water (yield, 280 mg). The elemental analysis of the glutamic acid fragment showed the following percentage composition:

Calculated: C 40.81, H 6.17, N 9.52

Found: C 41.05, H 6.46, N 9.58

The infrared absorption spectra of the isolated glutamic acid and authentic L-glutamic acid were identical. The specific rotation of the isolated glutamic acid in 5 N HCl was \([\alpha]_D^2 +32\) (c = 1 To) as compared with a published value of \([\alpha]_D^2 +31.8\) (11).

The combined evidence proves that the isolated compound could be only the dipeptide, γ-glu-tamyl-β-aminoisobutyric acid.

**DISCUSSION**

A comparable lot of bulbs from a later harvest was examined with the automatic amino acid analyzer (7, 8). The contents of γ-glutamyl-β-aminoisobutyric acid and of β-aminoisobutyric acid were, respectively, 2.71 and 0.02 pmoles per gram fresh weight. The peptide accounted for over 15% of the free amino groups as indicated by the ninhydrin color. This is analogous to the situation in the kidney bean in which S-methylcysteine occurs in much larger quantities as a dipeptide (12) than as the uncombined acid (13), and in which the peptide represents a third of the free amino groups. The presence of γ-glutamyl peptides in storage organs in relatively large amounts is an interesting but unexplained phenomenon. This is the first report of β-aminoisobutyric acid occurring in peptide form, although β-alanine is present in several peptides (14).

**SUMMARY**

A previously unknown naturally occurring peptide has been isolated from the bulbs of *Iris tingitana* (var. Wedgewood) by chromatography on ion exchange resins. This compound has been shown to be γ-L-glutamyl-β-aminoisobutyric acid.

The proof is based on elemental analysis, hydrolysis, infrared spectra, and the reactions with nitrous acid and fluoro-dinitrobenzene of the peptide. The separated hydrolytic products had the same infrared spectra, elemental analysis, and behavior in an automatic amino acid analyzer as authentic L-glutamic acid and (−)-β-aminoisobutyric acid.

The peptide is responsible for about 15% of the nonprotein amino nitrogen present in extracts of the tissue.

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