

# Isolation of $\beta$ -Aminoisobutyric Acid from Bulbs of *Iris tingitana* var. Wedgewood

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In the course of paper chromatographic analysis of the free amino acids in bulbs of Wedgewood iris (*Iris tingitana*) a ninhydrin-reacting compound which occupied a spot corresponding to that of  $\beta$ -aminoisobutyric acid (Spot 11, Fig. 1) was observed. The compound was resistant to acid hydrolysis and did not form a copper complex, because it moved through a barrier of copper carbonate (1) on paper chromatograms. This indicated that it was not an  $\alpha$ -amino acid. The  $R_F$  values in several solvent systems corresponded with those for authentic  $\beta$ -aminoisobutyric acid.

$\beta$ -Aminoisobutyric acid was first synthesized by Clemo and Melrose (2) in 1942 as the ethyl ester and as the free acid by Pollack (3) in the following year. Subsequently, it was isolated from normal human urine by Crumpler *et al.* (4) and also independently established by Fink *et al.* (5) as a urinary constituent in man both normally and in patients with neoplastic disease. In a review of the nitrogenous compounds in plants, Steward *et al.* (6) had indicated that the occurrence of  $\beta$ -aminoisobutyric acid in plants had not been established. The present report describes the isolation and identification of this substance from the bulbs of Wedgewood iris.

## EXPERIMENTAL

45 kg. of Wedgewood iris bulbs<sup>1</sup> were extracted by grinding the bulbs with 80 per cent ethanol in a "King-size" blender and emulsifier. The ethanol extract was filtered through Hyflo Super-Cel and stored at  $-5^\circ$  for 3 days. The free amino acids in the supernatant solution, approximately 50 l., were concentrated and freed of salts by passage through Dowex 50-X8 (50 to 100 mesh) resin in the hydrogen form. The amino acids were displaced from the resin with 7.5 N ammonium hydroxide, and the ammonium hydroxide was removed from the effluent in a vacuum at  $30^\circ$ . The final volume of the ammonium hydroxide-free effluent was reduced to 500 ml. Total amino nitrogen was determined by reaction with ninhydrin (7) after removal of ammonia by treatment with alkaline buffer (8) and found to be 8.90 gm., or approximately 0.02 per cent of the fresh weight

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of the iris bulbs. By visual comparison of chromatograms the total concentration of  $\beta$ -aminoisobutyric acid was estimated to be 600 to 700 mg., or approximately 1 per cent of the total non-protein amino nitrogen.

Two-dimensional descending chromatograms of the desalted extract of Wedgewood iris bulbs were prepared (9, 10).

The amino acid solution was put through a  $7.5 \times 50$ -cm. column of Dowex 1-X4 (200 to 400 mesh) resin in the chloride form to remove the acidic amino acids which interfere with displacement chromatography on Dowex 50 resin. The basic and neutral amino acids were washed through the column with deionized water and were absorbed on a  $5.8 \times 95$ -cm. column of the acid form of Dowex 50-X4 (200 to 400 mesh) resin. The amino acids were eluted from the resin with 0.1 N ammonium hydroxide and 20-ml. fractions collected (11). The amino acids in the fractions were detected by two-dimensional paper chromatography (12). Fractions were numbered from the time the first amino acid appeared in the effluent.  $\beta$ -Aminoisobutyric acid was found in Fractions 140 to 180 and was contaminated mainly with  $\gamma$ -aminobutyric acid,  $\beta$ -alanine, leucine, isoleucine, and the aromatic amino acids. Fractions 140 to 180 were concentrated by distillation in a vacuum and were added to the top of a  $5.8 \times 200$ -cm. column of Dowex 1-X4 (200 to 400 mesh) resin in the chloride form. This column was packed in approximately 7-cm. bands by pouring a suitable volume of a suspension of the resin into a 25-cm. column of water and allowing the resin to settle completely before pouring the next portion. The amino acids were washed through the resin column with deionized water,<sup>2</sup> and 15-ml. fractions were collected. Fractions 190 to 240 contained  $\beta$ -aminoisobutyric acid and  $\beta$ -alanine. Fractions 190 to 200 contained a small amount of  $\gamma$ -amino butyric acid, and the  $\alpha$ -amino acids contaminated Fractions 217 to 240. Fractions 217 to 240 were concentrated by distillation in a vacuum, and the amino acids were again fractionated on the same Dowex 1 column. The results were similar to the first fractionation on Dowex 1 except that the fractions containing both  $\beta$ -aminoisobutyric acid and  $\alpha$ -amino acids had a smaller proportion of the former substance. Fractions 190 to 216 from the first run and the corresponding fractions from the second run were evaporated to dryness. The dry material was dissolved in 25 ml. of a mixture of *n*-butyl alcohol:methyl alcohol:water (2:2:1, volume for volume) and was placed on a paper roll (13) which had been previously

<sup>2</sup> J. F. Thompson and C. J. Morris, unpublished data.



Fig. 1. Two-dimensional descending paper chromatogram of the free amino acids in Wedgewood iris bulbs developed with 0.25 per cent ninhydrin in acetone: (1) leucine, (2) phenylalanine, (3) isoleucine, (4) tyrosine, (5) methionine, (6) valine, (7) ethanolamine, (8) proline, (9) threonine, (10) alanine, (11)  $\beta$ -aminoisobutyric acid, (12) histidine, (13) serine, (14) glycine, (15)  $\beta$ -alanine, (16)  $\gamma$ -aminobutyric acid, (17) glutamine, (18) unknown, (19) asparagine, (20) arginine, (21) aspartic acid, (22) glutamic acid, (23)  $\alpha$ -amino adipic acid, (24) lysine.

TABLE I

*R<sub>F</sub> values of authentic  $\beta$ -aminoisobutyric acid and ninhydrin-reactive substance from Wedgewood iris bulbs*

| Solvent composition  | <i>R<sub>F</sub> values</i> |                               |
|--|-----------------------------|-------------------------------|
|  | Authentic substance         | Substance from Wedgewood iris |
| tert-Butyl alcohol:formic acid:water (70:15:15, volume for volume) . . . . .           | .63                         | .63                           |
| tert-Amyl alcohol:2,4-lutidine (1:1, volume for volume saturated with water) . . . . . | .10                         | .10                           |
| <i>n</i> -Butyl alcohol:methyl alcohol:water (2:2:1, volume for volume) . . . . .      | .52                         | .52                           |
| <i>n</i> -Butyl alcohol:acetic acid:water (6:1:2, volume for volume) . . . . .         | .46                         | .46                           |
| Phenol:water (73:27, weight for weight) . . . . .                                      | .82                         | .81                           |
| Ethyl alcohol:water (70:30, volume for volume) . . . . .                               | .55                         | .55                           |

equilibrated with this solvent. The amino acids were washed through the paper roll with the *n*-butyl alcohol:methyl alcohol:water solvent, and the effluent was collected in 15-ml. fractions. The  $\gamma$ -aminobutyric acid,  $\beta$ -aminoisobutyric acid, and  $\beta$ -alanine

were eluted in that order in separate bands. Fractions 270 to 330 containing  $\beta$ -aminoisobutyric acid were evaporated to dryness, dissolved in water, and decolorized by boiling the solution with activated charcoal. The solution was filtered and evaporated to dryness in a vacuum. The last traces of water were removed by the addition of absolute ethanol and evaporation to dryness. The residue was dissolved in hot absolute ethanol and colorless crystals separated on cooling. After a second crystallization, 72 mg. of dry crystals were obtained.

## RESULTS

The isolated compound from Wedgewood iris bulbs was found to be indistinguishable from authentic  $\beta$ -aminoisobutyric acid when cochromatographed on paper in several solvents (Table I). Elemental analysis showed that it contained 46.7 per cent C, 8.85 per cent H, and 13.3 per cent N, whereas the theoretical values for  $C_4H_9O_2N$  are 46.6 per cent C, 8.80 per cent H, and 13.6 per cent N. The isolated compound melted at 183° (uncorrected) and gave an optical rotation of  $[\alpha]_D^{25} -21^\circ$  (C = 0.43 per cent in water).

Since none of the natural  $\beta$ -amino acid isomer was available, 30 mg. of the isolated crystals were racemized by heating them at 100° for 5 hours in 5 *N* sodium hydroxide (4). The sodium hydroxide was removed by passing the solution through a column

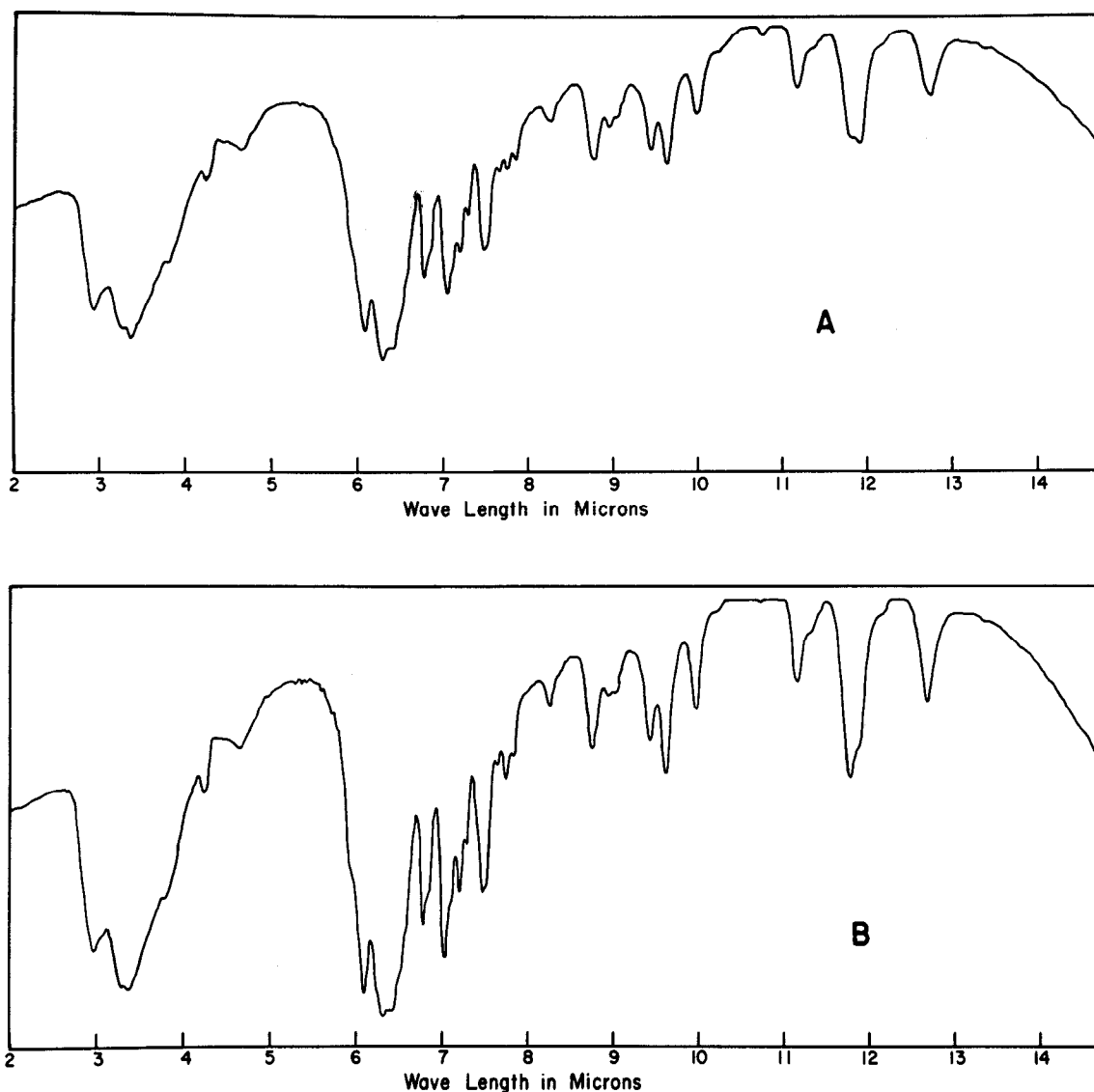


FIG. 2. Infrared spectra of authentic  $\beta$ -aminoisobutyric acid (A), and the ninhydrin-reactive substance from Wedgewood iris bulbs (B). These were prepared with KBr discs containing approximately 2 mg. of the respective compounds.

of IRC-50 resin. After drying and crystallization from absolute ethanol, the crystals were compared with authentic  $\beta$ -aminoisobutyric acid in an infrared spectrophotometer (Fig. 2). The melting point of both the synthetic and the racemized isolated substance was  $179^\circ$  (uncorrected).

#### DISCUSSION

Part of the purification procedure used for the isolation of  $\beta$ -aminoisobutyric acid involved the separation of the  $\beta$ -amino acids on a column of Dowex 1 in the salt form.<sup>2</sup> This procedure was derived from the ion exclusion technique of Wheaton and Bauman (14) and by the use of it  $\alpha$ -,  $\beta$ -, and  $\gamma$ -amino acids could be separated from one another.

The use of butanol (5) for crystallization caused breakdown, possibly by deamination to methacrylic acid. Absolute ethanol was a suitable solvent for crystallization and apparently caused no breakdown. Traces of HCl can prevent crystallization (5).

The melting point of the isolated material agrees well with that reported by Crumpler *et al.* (4). The optical rotation is in the same direction but somewhat higher ( $-21^\circ$  as compared

with  $-13^\circ$ ),<sup>3</sup> although the value obtained may be in error because of the low concentration of the solution.

The infrared spectra (Fig. 2) for authentic  $\beta$ -aminoisobutyric acid and the racemized isolated material show the same absorption bands. The relative intensities are not all identical probably because of differences in the amounts of material actually present in the KBr disks.

The cumulative evidence of paper and column chromatography, elemental analysis, melting point, and infrared absorption spectra indicate that the compound isolated from the bulbs of Wedgewood iris is identical with  $\beta$ -aminoisobutyric acid.

#### SUMMARY

$\beta$ -Aminoisobutyric acid has been isolated from the bulbs of Wedgewood iris in pure form by a series of fractionations on ion exchange resins and on a paper roll. Its identity was established by chromatographic behavior, elemental analysis, melting point, and infrared absorption spectra.

<sup>3</sup> Personal communication from R. G. Westall.

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