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AMINO ACID COMPOSITION OF GREEN GRAM (*PHASEOLUS RADIATUS* L. VAR. *TYPICUS* PRAIN)

Part IV. Studies on the Components in Basic Amino Acids Fraction

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INTRODUCTION

Green gram seeds have been utilized as raw material for production of bean-seedlings, bean-nodules and boiled bean. From the acidic amino acids fraction of green gram seeds, N-carboxymethyl- β -alanine and six γ -glutamyl peptides— γ -glutamylleucine, γ -glutamylmethionine, γ -glutamylmethioninesulfoxide, γ -glutamylphenylalanine, γ -glutamyltyrosine and γ -glutamyl- γ -glutamylmethionine—were isolated. Identification and quantitative analysis of these compounds were reported in previous papers of this series (16, 17, 18). Composition of free and protein amino acids were also described (17).

This paper deals with the isolation and identification of tyramine, adenine and ethanolamine from basic amino acids fraction.

MATERIALS AND METHODS

Separation of Amino Acids Fraction

Pulverised green gram seeds (50 kg) was extracted with 70% aq. ethanol. The extracts were passed through a column of Amberlite IR-120 (H^+), the column being washed with water. Amino acids fraction freed from sugars and salts was eluted with 2N ammonia water.

Separation of Neutral and Basic Amino Acids Fraction

The eluate with 2N ammonia water was concentrated under reduced pressure and adjusted to pH 4. After filtration, filtrate was put on a column of Dowex 1 \times 4 (AcO^-). Effluent with water from the column contained neutral and basic amino acids fraction. After washing with water, the

column was eluted with 2N acetic acid and eluate was used for studies on acidic amino acids fraction (16, 18).

Separation of Neutral Amino Acids Fraction

Neutral and basic amino acids fractions obtained as described above was put on a column of Dowex 50×4 (NH_4^+), and neutral amino acids fraction was washed out from the column with water.

Separation of Basic Amino Acids Fraction

After washing with water, the basic amino acids fraction was eluted with 2N ammonia water from the column. Paper chromatogram of the basic amino acids fraction showed two ninhydrin positive spots besides the normally occurring basic amino acids (Fig. 1). The one with higher Rf value was named BU-1 and the other with lower Rf value was BU-3, respectively.

Separation of BU-1, BU-2 and BU-3 from the Basic Amino Acids Fraction

The basic fraction was dissolved in small volume of n-butanol, acetic acid, water (4:1:2 v/v), placed on a cellulose column (4.5×53 cm) and developed with the same solvent system. By repeating the cellulose column chromatography BU-1 fraction and BU-3 fraction were obtained. BU-1 fraction was concentrated under reduced pressure and acidified with hydrochloric acid. After concentration, it was added with ethanol, and the resulting insoluble precipitates were named BU-2. BU-1·HCl was crystallized by adding ether to the ethanol solution. BU-2·HCl was crystallized from water and BU-3·HCl was from ethanol.

Identification of Tyramine (BU-1)

BU-1 gave positive reaction with ninhydrin and phenol reagent. Anal. Found: C, 54.81%; H, 6.97, N, 7.87. Calcd. for $\text{C}_8\text{H}_{11}\text{NO} \cdot \text{HCl}$: C, 55.30; H, 6.91; N, 8.06. IR (KBr) (Fig. 2): 3200 cm^{-1} (OH), ~ 2000 (NH_3^+), 1660 (NH_3^+), 1615 , 1595 , 1460 (C=C), 1230 (phenol-OH), 830 (p-substituted benzene). NMR ($\delta_{\text{H}_2\text{O}}^{\text{DSS}}$): 2.9 (t, 2H, $J=7$ Hz), 3.2 (t, 2H, $J=7$ Hz), 6.85 (d, 2H, $J=8$ Hz), 7.2 (d, 2H, $J=8$ Hz). MS (Fig. 3): m/e 137 (M^+), 107 ($\text{M}^+ - \text{CH}_2\text{NH}_2$), 91 (C_7H_7^+), 78 (C_6H_6^+), 77 (C_6H_5^+), 44 ($\text{C}_2\text{H}_6\text{N}^+$), 30 (CH_4N^+). UV ($\lambda_{\text{max}}^{\text{water}}$): $222 \text{ m}\mu$ ($\log \epsilon = 3.71$), 276 (3.08), 282 (sh) (3.00). From the color reactions and IR and NMR spec-

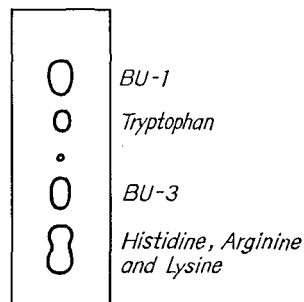


Fig. 1. Paper Chromatogram of Basic Amino Acids Fraction of Green Gram Seeds.

Solvent system: n-butanol, acetic acid, water (4:1:2 v/v) Color-producing reagent: ninhydrin.

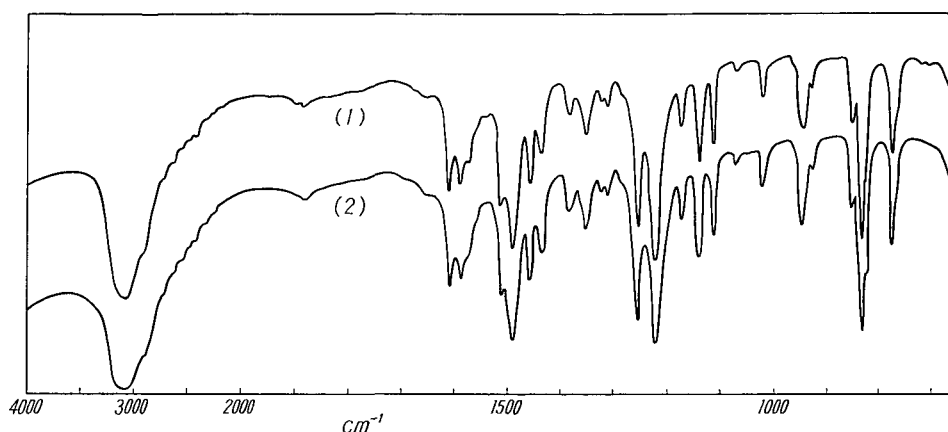


Fig. 2. IR-Spectra of BU-1·HCl (2) and Tyramine·HCl (1) (KBr).

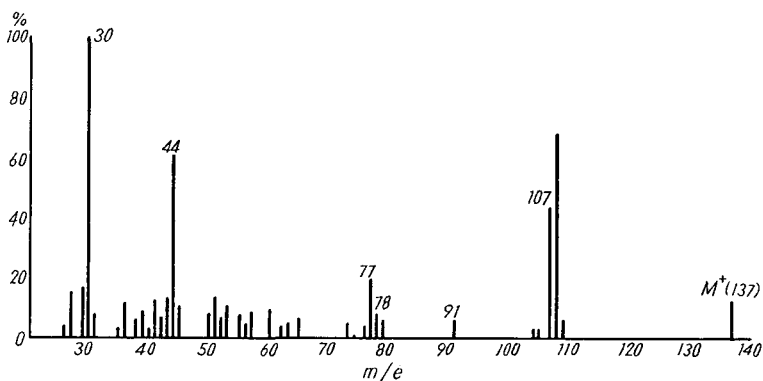


Fig. 3. Mass Spectrum of BU-1·HCl.

tra it was deduced that BU-1·HCl has the structure of $\text{HO}-\text{C}_6\text{H}_4-\text{CH}_2\text{CH}_2\text{NH}_2\cdot\text{HCl}$. Unequivocal evidences for the structure were given from mass and IR spectra. BU-1 has the same Rf values as tyramine with several solvent systems. Final proof of the structure was obtained by comparison of IR-spectrum of BU-1·HCl with that of authentic tyramine HCl (Fig. 2).

Identification of Adenine (BU-2)

BU-2 did not react with ninhydrin. Heat-treatment of BU-2 with 6N HCl at 120°C for 20 hours afforded a large amount of glycine, which was identified and determined by amino acid analyzer. Anal. Found: C, 33.14%; H, 3.83; N, 38.89. Calcd. for $\text{C}_5\text{H}_5\text{N}_5\cdot\text{HCl} \cdot 1/2\text{H}_2\text{O}$: C, 33.22; H, 3.88; N, 38.86. IR (KBr) (Fig. 4): 3300~2000 cm^{-1} (NH_3^+), 1695 ($\text{C}=\text{N}$), 1615, 1580

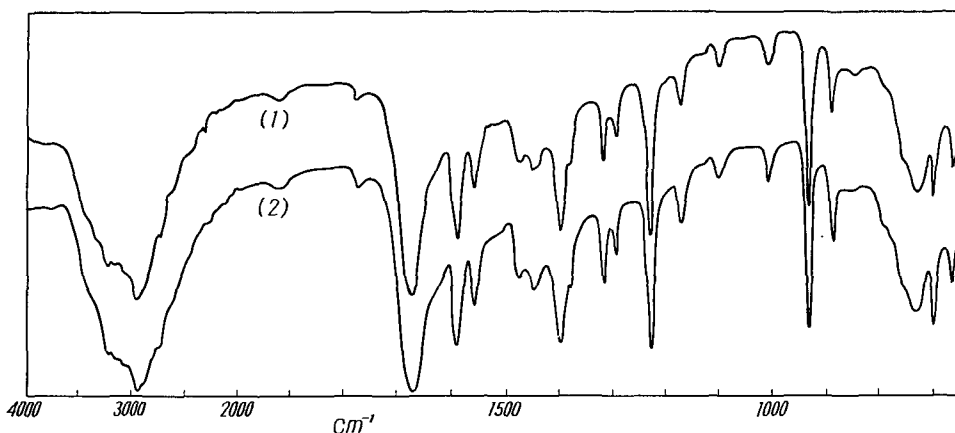


Fig. 4. IR-Spectra of BU-2·HCl (1) and Adenine·HCl (2) (KBr).

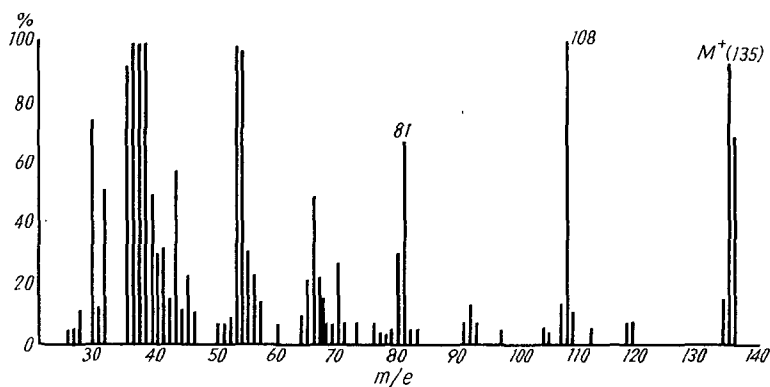


Fig. 5. Mass Spectrum of BU-2·HCl.

(C=C), 950 (ring-CH). NMR ($\delta_{D_2O}^{PSS}$): 8.45 (s), 8.52 (s). MS (Fig. 5): m/e 135 (M^+), 108 ($M^+ - HCN$), 81 (108-HCN). UV (λ_{max}^{water}): 262 $m\mu$ ($\log \epsilon = 4.14$). These data suggested the chemical structure of the BU-2 to be adenine (1, 2, 10, 21). Thus, IR spectrum of BU-2·HCl is completely consistent with that of authentic adenine HCl (Fig. 4).

Identification of Ethanolamine (BU-3)

BU-3 was liquid in free state and crystallized by forming the salt with hydrochloric acid. Anal. Found: C, 23.93%; H, 8.11; N, 14.02. Calcd. for $C_2H_7NO \cdot HCl$: C, 24.62; H, 8.21; N, 14.36. Low value of carbon content will be due to the very hygroscopic character of BU-3·HCl. IR (KBr) (Fig. 6): 3400 and 1070 (primary OH), ~ 2000 (NH_3^+), 1630, 1500 (NH_3^+).

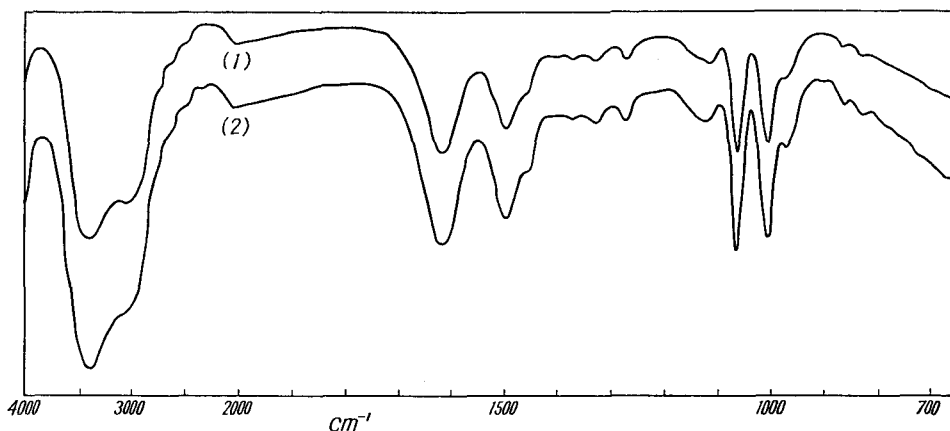


Fig. 6. IR-Spectra of BU-3·HCl (1) and Ethanolamine·HCl (KBr).

NMR ($\delta_{\text{DCI}}^{\text{DSS}}$): 3.22 (t, 2H, $J=6\text{Hz}$), 3.9 (t, 2H, $J=6\text{ Hz}$). MS (Fig. 7): m/e 61 (M^+), 31 ($M^+-\text{CH}_2\text{NH}_2$), 30 ($M^+-\text{CH}_2\text{OH}$). These spectra data suggested that BU-3·HCl has the structure of $\text{HOCH}_2\text{CH}_2\text{NH}_2\cdot\text{HCl}$. BU-3 revealed the same chromatographic behavior as ethanolamine. IR spectrum of BU-3·HCl was completely consistent with that of ethanolamine HCl (Fig. 6).

RESULTS AND DISCUSSION

Tyramine, adenine and ethanolamine were isolated from basic amino acids fraction of green gram seeds.

Tyramine, which has sympathomimetic and blood-pressure raising action, has been found from a variety of plants —*Hordeneum vulgare* (8, 12, 20, 22, 23), *Panicum miliaceum* (22), *Crinum* species (9), *Sarothamnus scoparius* (5, 6, 13, 25), *Chelidonium majus* (19), *Acacia berlandieri* (3), *A. greggii*, *A. roemeriana*, *A. texensis* (4), *A. caven*, *A. aroma* (11), *Gleditsia triacanthos*, *Prosopis glandulosa* (4), *Psittacanthus cuneifolius* (11), *Phoradendron wattii* (7), *P. argentinum*, *Bougainvillea stipitata*, *Acnistus breviflorus*, *Celtis spinosa* (11), *Silibum marianum* of Azerbaidzhan variety (15), banana, potato, red plum, avocado, tomato, spinach, orange, eggplant (27), citrus juice (26), scotch broom (14), sowa millet (24)— but its presence in green gram seeds has not been reported.

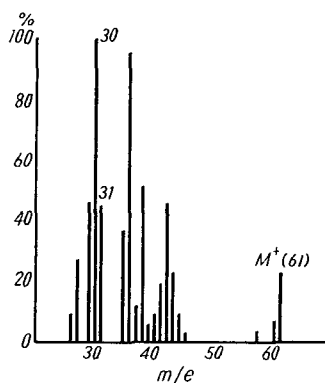


Fig. 7. Mass Spectrum of BU-3·HCl.

Tyramine content in green gram seed is relatively high: tyramine·HCl isolated from 50 kg of green gram seeds was 1.1 g, but fairly large amount of tyramine still remained in other fractions contaminated with additional basic components since the isolation work was only done on the fraction containing tyramine alone.

Adenine and ethanolamine were also first isolated from green gram seeds.

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