Title	クサスギカズラ根の成分(第2報) : 遊離アミノ酸について
Sub Title	Constituents of the radix of asparagus cochinchinensis. II. : on the free amino acids
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Publisher	共立薬科大学
Publication year	1975
Jtitle	共立薬科大学研究年報 (The annual report of the Kyoritsu College of Pharmacy). No.20 (1975.) ,p.9- 13
JaLC DOI	
Abstract	
Notes	原報
Genre	Technical Report
URL	https://koara.lib.keio.ac.jp/xoonips/modules/xoonips/detail.php?koara_id=AN00062898-00000020-0009

〔共立薬科大学研究年報
No. 20, 9～13(1975)〕

クサスギカズラ根の成分（第2報）
遊離アミノ酸について

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Constituents of the Radix of *Asparagus cochinchinensis*. II.[1]
On the Free Amino Acids

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(Received September 4, 1975)

Nineteen free amino acids have been isolated from the tuberous roots of *Asparagus cochinchinensis* MERR. and examined. Citrulline, asparagine, serine, threonine, proline, glycine, alanine, valine, methionine, leucine, isoleucine, phenylalanine, tyrosine, aspartic acid, glutamic acid, arginine, hystidine and lysine were identified and determined. In addition, an unidentified acidic amino acid was obtained.

On the constituents of the tuberous roots of *Asparagus cochinchinensis* MERR., *neo*-kestose, and a tetrasaccharide, a pentasaccharide, a hexasaccharide, an octasac-

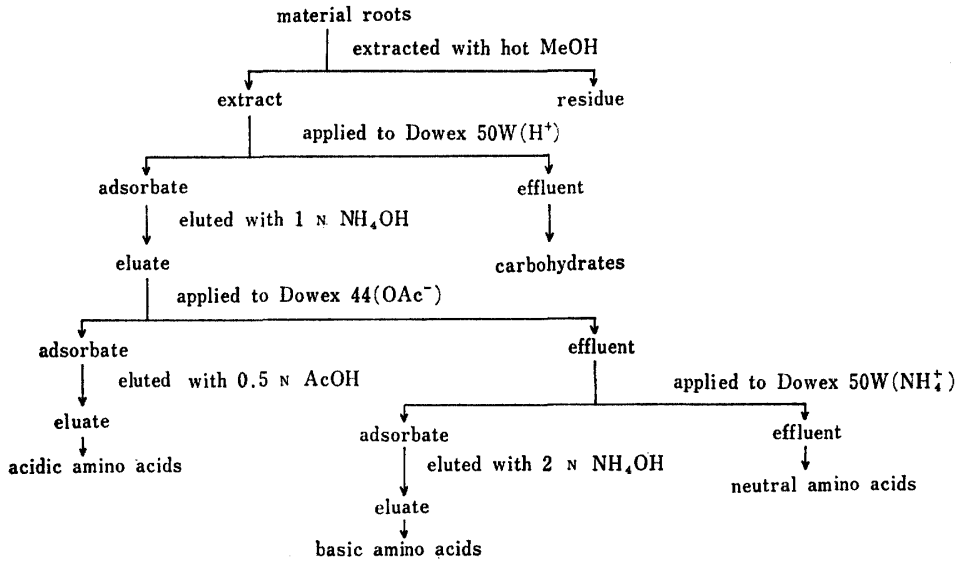

Chart 1. Extraction and Fractionation of Amino Acids

1) Part I : M. Tomoda and N. Satoh, *Chem. Pharm. Bull.* (Tokyo), **22**, 2306 (1974).
2) T. Kobayashi, T. Tomimori, T. Nakajima and N. Yahagi, *Yakugaku Kenkyu*, **30**, 477 (1958).

charide, a nonasaccharide and a decasaccharide which possess non-reducing linear structure made up of 2→1 linked β-D-fructofuranose residues having a *neo*-kestose unit on the end of the molecule have been reported from this laboratory[1] in addition to β-sitosterol, glucose and fructose.[2] As described previously in the preceding report,[1] we also found nineteen amino acids in the material, and the detail is described in this paper.

The material roots were extracted with hot methanol, and the extract was applied to a column of Dowex 50 W (H^+). The adsorbates were eluted with 1 N ammonium hydroxide followed by evaporation to dryness. Then it was applied to a column of Dowex 44 (OAc^-), and acidic amino acid fraction was obtained from the adsorbates after elution with 0.5 N acetic acid. On the other hand, the effluent from a column of Dowex 44 was applied to a column of Dowex 50 W (NH_4^+). Neutral amino acid fraction was obtained from the effluent, and basic amino acid fraction was obtained from the adsorbates after elution with 2 N ammonium hydroxide. These procedures are shown in Chart 1.

Each fraction was examined by two dimensional cellulose thin-layer chromatography (TLC), and three acidic amino acids, three basic amino acids and thirteen amino acids were detected. The analyses and determinations of amino acids were also performed by the use of an amino acid analyzer. The results are shown in Table I.

Table I. Contents of Amino Acids in the Total Fraction*

components	contents (%)	components	contents (%)
Aspartic acid	7.06	Proline	1.73
Glutamic acid	4.48	Glycine	0.33
Unidentified acidic amino acid	2.79	Alanine	0.89
Arginine	5.84	Valine	0.65
Histidine	0.43	Methionine	0.92
Lysine	0.93	Leucine	0.26
Citrulline	10.34	Isoleucine	0.16
Asparagine	5.63	Phenylalanine	0.16
Serine	2.36	Tyrosine	0.31
Threonine	0.66		

* This means the eluate obtained from a column of Dowex 50W (H^+) with NH_4OH.

Aspartic acid, glutamic acid, citrulline and asparagine were respectively isolated by preparative paper partition chromatography (PPC) and purified as crystals. Arginine was derived to benzylidene arginine. Each of them was identified by comparing with authentic sample.

An unidentified one in the acidic amino acid fraction was also isolated by preparative PPC and crystallized. The detail of its structure will be reported in the following paper. In addition to this substance, citrulline, arginine, aspartic acid, glutamic acid and asparagine were found as main amino acids. Compared with the presence of abundant asparagine, the absence of glutamine is interesting.

Experimental

Solutions were concentrated at or below 40° with rotary evaporators under reduced pressure. Melting points were measured on a micro melting point apparatus (an air-bath type) and are uncorrected. Optical rotation was measured with JASCO model DIP-SL automatic polarimeter. IR spectra were measured with Hitachi model EPI-G 3 infrared spectrophotometer. The determination of free amino acids was performed by the use of Hitachi model KLA-3 B amino acid analyzer.

Extraction and Fractionation of Amino Acids——The material was obtained in October of 1972 from the plants cultivated in Saitama prefecture. The fresh roots (245 g), which contain 80.5 % of water, were crushed, then extracted with hot methanol (1000 ml) for 30 min. After suction filtration, the extraction was similarly repeated. The extracts were combined and concentrated to 60 ml, then applied to a column (2 × 25 cm) of Dowex 50 W-X 8 (H^+, 50 to 100 mesh). After washing with water (600 ml), the effluents and washings were collected and concentrated, followed by lyophillization. This fraction contains carbohydrates, and the yield was 16.8 g. The total amino acid fraction was obtained by the elution with 1 N NH_4OH (500 ml) from the column of Dowex 50W (H^+). After removal of the solvent from the eluate by repeated evaporation, the yield was 7.6 g.

The total amino acid fraction was dissolved in water (50 ml) and applied to a column (2 × 25cm) of Dowex 44 (acetate, 20 to 50 mesh). After washing with water until the effluent showed no reaction with ninhydrin reagent, the effluents and washings were collected and concentrated to 50 ml. Then the solution was applied to a column (2 × 25cm) of Dowex 50W-X8 (NH_4^+, 50 to 100 mesh). After washing with water, the effluents and washings were collected and concentrated, followed by lyophillization. The yield of this neutral amino acid fraction was 2.9 g. Basic amino acid fraction was obtained by the elution with 2 N NH_4OH from the column of Dowex 50W (NH_4^+). After removal of the solvent from the eluate by repeated evaporation, the yield was 0.9 g. And acidic amino acid fraction was obtained by the elution with 0.5 N AcOH from the column of Dowex 44 (OAc^-). After removal of the solvent from the eluate by repeated evaporation, the yield was 1.2 g.

Thin-Layer Chromatography——Two dimensional cellulose TLC was carried

Table II. *Rf* Values of Amino Acids

compounds	Solvent A	Solvent B
Aspartic acid	0.21	0.12
Glutamic acid	0.27	0.24
Unidentified one	0.52	0.36
Arginine	0.33	0.62
Histidine	0.25	0.70
Lysine	0.18	0.48
Citrulline	0.30	0.64
Asparagine	0.18	0.42
Serine	0.22	0.33
Threonine	0.28	0.49
Proline	0.35	0.85
Glycine	0.24	0.40
Alanine	0.33	0.58
Valine	0.50	0.79
Methionine	0.43	0.84
Leucine	0.63	0.86
Isoleucine	0.61	0.86
Phenylalanine	0.57	0.88
Tyrosine	0.38	0.55

out for the qualitative analysis of amino acids. The primary solvent system (A) was BuOH : AcOH : H_2O (12 : 3 : 5) and the secondary (B) was C_6H_5OH : 0.3% NH_4OH (4 : 1). Ninhydrin reagent was used for detection. Table II gives the *Rf* values on TLC. The presences of leucine and isoleucine were confirmed by the use of an amino acid analyzer.

Separation of Acidic Amino Acids——Aspartic acid,- glutamic acid and an unidentified one were separated by PPC with Tôyô-Roshi No. 50 and solvent A. Aspartic acid, leaflets, mp 250—251°, and glutamic acid, rods, mp 229—231° (decomp.), were identified by comparing with the respective authentic sample by mixing mp, TLC and IR spectra. The unidentified one was obtained in colorless needles, mp 201.5—202°, $[\alpha]_D^{18}$ −57.8° (H_2O, c=0.15). It has not sulfur. Chromatographic and spectral data suggest that it belongs to an acidic amino acid.

Separation of Neutral Amino Acids——Neutral amino acids were separated into four fractions by PPC with Tôyô-Roshi No. 50 and solvent C, isoPrOH : HCOOH : H_2O (20 : 1 : 5). Fraction A gave asparagine in orthorhombs, mp 232—234° (decomp.). Fraction B was further separated into citrulline, needles, mp 220°, and serine by PPC with solvent D, BuOH : $(Me)_2CO$: $(Et)_2NH$: H_2O (10 : 10 : 2 :

5). Asparagine and citrulline were identified by comparing with the respective authentic sample by mixing mp, TLC and IR spectra. Fraction C contains proline and threonine. The other neutral amino acids were present in fraction D. But serine and they have not been crystallized.

Identification of Arginine[3]——The basic amino acid fraction (130 mg) was dissolved in water (1.4 ml). After addition of 40 % NaOH (0.14 ml), benzaldehyde (0.5 ml) was gradually added into the solution under vigorous stirring. Then the reaction mixture was kept at 0° overnight. Plates of benzylidenearginine separated out and it was washed with ice water and ether-methanol mixture (2 : 1), then dried. It showed mp 205°, and it was identified by comparing with the authentic sample by mixing mp and IR spectra.

Acknowledgement We are indebted to Mr. K. Asaoka, Department of Biochemistry and Biophysics, Faculty of Science, University of Tokyo, for the determination of amino acids.

3) M. Bergmann and L. Zervas, *Z. physiol. Chem.*, **152**, 282 (1926).