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Isolation of Adenosine and Free Amino Acid Composition from the Leaves of *Allium tuberosum*

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Abstract

From the leaves of *Allium tuberosum* (Liliaceae), the purine nucleoside, adenosine was isolated and its structure was characterized on the basis of spectral data. Besides this nucleoside, the composition and relative content of free amino acids and related compounds, compared to standards determined under identical conditions was also investigated using automatic amino acid analyzer. Major free amino acids were alanine, glutamic acid, aspartic acid and valine.

Key words : *Allium tuberosum*, Liliaceae, purine nucleoside, adenosine, free amino acid, automatic amino acid analyzer, ^{13}C-NMR

INTRODUCTION

Allium tuberosum (Liliaceae) is a perennial herb which is cultivated widely and the leaves are used for food. According to the dictionary of Chinese drugs[1], it has been used for treatment of abdominal pain, diarrhea, hematemesis, snakebite and asthma. In the course of biological screening of Chinese drugs in our laboratory it was found that the methanol extract from the leaves of *Allium tuberosum* on repeated pretreatment of mice, caused a significant prolongation of hexobarbital-induced sleeping time[2]. And we reported the isolation of 1, 2, 3, 4-tetrahydro-β-carboline 3-carboxylic acid as one of the active principles of this plant[2,3]. The chemical study previously reported that the leaves contain sulfides[4], linalool[5], and flavonoid glycosides[6,7]. In this communication, we report the isolation of an additional compound, adenosine (**1**) as a minor component from the ethylacetate soluble fraction of the methanol extract, and the composition and relative content of free amino acids and related compounds from this plant part.

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MATERIALS AND METHODS

Instruments

The melting point (mp) was taken on a Thomas Hoover 6406-H apparatus and are uncorrected. The infrared (IR) was determined in KBr tablet on a Bomem MB-10 FT-IR spectrophotometer and the ultraviolet (UV) was run with CE 599 Universal automatic scanning spectrophotomter. The proton nuclear magnetic resonance (^{1}H-NMR) and carbon nuclear magnetic resonance (^{13}C-NMR) were recorded with a Brucker-AM 300 spectrometer (300MHz), and chemical shifts are given as δ (ppm). Electron impact mass spectrum (EIMS) was taken a Hewlett-Packard 5985B GC/MS spectrometer operating at 70eV. Optical rotation was measured on a Mitamura-Ricken polarimeter.

Analysis of free amino acids

The amino acid samples were analyzed quantitatively in an automatic amino acid analyzer (LKB 4150 ALPHA) using lithium buffer solutions. The Buffer formulations and operational conditions are

described in Table 1 and 2.

Preparation of free amino acid samples[8]

The leaves of *Allium tuberosum* obtained commercially were homogenized in cold 5% trichloroacetic acid (TCA) and the precipitated proteins were removed by centrifugation. TCA was then removed by shaking with Et_2O. The residual solution was then evaporated under reduced pressure and the amino acid compounds then dissolved in lithium buffer solution (pH 2. 2).

Extraction and fractionation

Commercially dried leaves of *A. tuberosum* (200g) were refluxed with hot MeOH. The MeOH extract (55g) was partitioned with hexane (17g), $CHCl_3$ (6g), EtOAc (3g), BuOH (20g) and H_2O (8g) successively.

Isolation

The EtOAc extract (3g) was subjected to SiO_2 column chromatography ($CHCl_3$: MeOH, gradient) to yield compound 1 (50mg, yield; 2.5×10^{-2} %) as colorless needles.

Compound 1 (adenosine)

Colorless needles from aqueous MeOH, mp 224 ~ 5° C, $[\alpha]_D^{25} = -59.5°$ C (c = 0.5, H_2O), UV λ_{max}^{MeOH} nm (log ε) ; 208 (4.03), 260 (3.83), IR (KBr, cm^{-1}) : 3350 (NH_2), 3100 (OH), 1684, 1607, 1575 (NH), 1106 (C-N), 1038 (C-O), 823 (NH_2), ^{1}H-NMR (300 MHz, DMSO-d_6 + D_2O) δ ; 8.32 (1H, s, H-8), 8.13 (1H, s, H-2), 5.85 (1H, d, J = 6.24, H-1′), 4.57 (1H, dd, J = 5.2 and 5.7Hz, H-2′), 4.13 (1H, dd, J = 3.1 and 5.0 Hz, H-3′), 3.97 (1H, q, J = 3.3Hz, H-4′), 3.59 (2H, ABq, J=3.6 and 12.3Hz, H-5′) ^{13}C-NMR (75.5MHz, DMSO-d_6) δ ; 156.11 (C-6), 152.30 (C-2), 149.04 (C-4), 139.84 (C-8), 119.32 (C-5), 87.87 (C-1′), 85.83 (C-4′), 73.39 (C-2′), 70.60 (C-3′), 61.62 (C-5′), MS (m/z, rel. int.); 267 (M^+, 1.2), 250 (1.0), 238 (3.4), 237 (9.7), 220 (1.4), 194 (1.7), 179 (10.1), 178 (35.1),

Table 2. Operational conditions of automatic amino acid analyzer

Column size	6 × 240mm (L)
Resin	Ultropac 11 resin (Li^+ form)
Mobile phase	1. 0.20M Lithium citrate buffer (pH 2.80)
	2. 0.30M Lithium citrate buffer (pH 3.00)
	3. 0.60M Lithium citrate buffer (pH 3.02)
	4. 1.00M Lithium citrate buffer (pH 3.45)
	5. 1.65M Lithium citrate buffer (pH 3.55)
	6. 0.30M LiOH
Analysis cycle time	200 min
Flow rate	Buffer 35 ml / hr, Ninhydrin 25 ml / hr
Pressure	Buffer 28 bar, Ninhydrin 16 bar
Column temperature	39° C, 61° C, 75° C
Chart speed	1 mm / min
Range of optical density	570 nm ; 0-1
	440 nm ; 0-1

Table 1. Lithium buffer formulations used in automatic acid analyzer

	Loading Buffer	Buffer 1	Buffer 2	Buffer 3	Buffer 4	Buffer 5	Lithium Hydroxide
pH	2.20	2.80	3.00	3.02	3.45	3.55	-
Li^+ ion concentration (M)	0.2	0.2	0.3	0.6	1.0	0.65	0.3
Citric acid (g)	48.00	48.00	48.00	48.00	48.00	105.05	-
Lithium hydroxide (g)	42.00	42.00	42.00	42.00	42.00	35.00	62.95
Lithium chloride (g)	-	-	21.25	84.75	170.00	314.35	-
Phenol (g)	5.00	5.00	5.00	5.00	5.00	5.00	-
Thiodiglycol 25% (ml)	400.00	40.00	40.00	40.00	40.00	40.00	-
Isopropanol (ml)	-	75.00	75.00	-	-	-	-
Conc. HCl (ml approx.)	80	77	74	73	55	-	-
Final volume (l)	5	5	5	5	5	5	5

165 (21.0), 164 (96.5), 137 (19.9), 136 (95.5), 135 (100), 134 (1.7), 121 (1.3), 119 (8.5), 109 (7.5), 108 (29.3), 81 (4.0), 73 (7.2).

RESULTS AND DISCUSSION

Free amino acids and related compounds

In the preceeding papers[2,3], we reported the isolation of L-tyrosine and other amino acid mixtures from the butanol soluble fraction of the methanol extract. In continuation of our chemical study, we now also report further amino acid composition of this plant extract using TCA precipitation method[8] by means of automatic amino acid analyzer.

The composition of free amino acids and related compounds of *Allium tuberosum*, compared to standard amino acids determined under identical conditions are presented in Figs. 1 and 2 and summarized in Table 3.

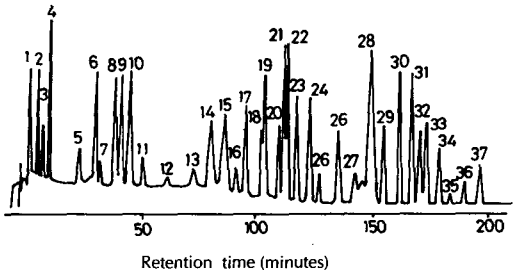

Fig. 1. Separation of standard amino acids and related compounds by automatic amino acid analyzer.

Peaks ;

1. phosphoserine	2. taurine
3. phosphoethanolamine	4. urea
5. unknown	6. aspartic acid
7. hydroxyproline	8. threonine
9. serine	10. glutamic acid
11. glutamine	12. α-aminoadipic acid
13. proline	14. glycine
15. alanine	16. α-aminobutyric acid
17. valine	18. cystine
19. methionine	20. cystathionine
21. isoleucine	22. leucine
23. tyrosine	24. phenylalanine
25. β-aminoisobutyric acid	26. γ-aminobutric acid
27. ethanolamine	28. ammonia
29. DL+allohydroxylysine	30. ornithine
31. lysine	32. 1-methylhistidine
33. histidine	34. 3-methylhistidine
35. anserine	36. carnosine
37. arginine	

The plant extract contained a number of proteineous, non-proteineous (taurine, phosphoserine and phosphoethanolamine) free amino acids and other related compounds. Among the proteineous free amino acids, alanine (9.90%) and glutamic acid (8.67%), representative of a delicious and sweet taste and aspartic acid (6.31%), and valine (7.44%), exhibiting a bitter taste, were predominantly present in the extract. Thus, it could be considered that these amino acids are attributed to the taste for this plant. In the case of non-proteineous amino acids such as phosphoserine, phosphoethanolamine and taurine, the chromatographic results studied in the present study satisfactorily separated and analyzed quantitatively, even though these may overlap with a number of other highly ionized or acidic compounds in the chromatographic methods available[9]. The relative contents of phosphoserine, phosphoethanolamine and taurine were 0.85%, 0.39% and 2.73%, respectively. It is of significance that the extract contained the highest concentration of taurine, an osmoregulator in some marine invertebrates, and possibly a neuronal transmitter or modulator in higher animals, but rarely in plant material[10].

Compound 1 (adenosine)

Column chromatography of the ethylacetate soluble fraction of the methanol extract yielded a compound 1. The compound 1, mp 224 ~ 5° C showed characteristic bands at 3100 ~ 3350 (OH and NH_2), 1684, 1607, 1575 (NH), 1106 (C-N), and 823 (NH_2)

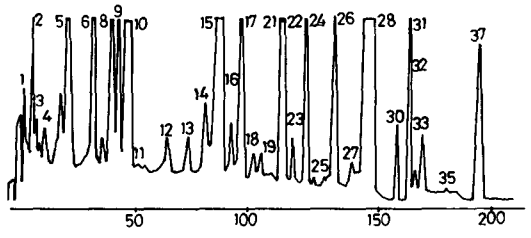

Fig. 2. Free amino acids and related compounds from *Allium tuberosum*.

Numerical name of peaks is the same as in Fig. 1.

Table 3. Composition and relative content of free amino acids and related compounds from *Allium tuberosum*

Composition	Rt*, %	Composition	Rt*, %
Phosphoserine	0.85	Cystine	0.47
Taurine	2.73	Methionine	0.66
Phosphoethanolamine	0.38	Isoleucine	4.33
Urea	0.66	Leucine	3.49
Unknown	7.35	Tyrosine	0.66
Aspartic acid	6.31	Phenylalanine	5.84
Threonine	5.66	β-Aminoisobutyric acid	0.38
Serine	4.71	γ-Aminobutyric acid	5.18
Glutamic acid	8.67	Ethanolamine	0.38
Glutamine	0.28	Ammonia	10.79
α-Aminoadipic acid	1.32	Ornithine	1.51
Proline	1.32	Lysine	4.81
Glycine	1.41	1-Methylhistidine	0.56
Alanine	9.90	Histidine	1.13
α-Aminobutyric acid	0.75	Anserine	0.09
Valine	7.44	Arginine	5.00

*Rt ; relative content

cm^{-1} in its IR spectrum. Its UV spectrum showed strong absorption peaks at 208 and 260nm, indicating that compound 1 was a purine nucleoside[11].

The MS spectrum of **1** showed a molecular ion at m/z 267 along with a base peak at m/z 135 corresponding to characteristic of adenosine and its analogs[12-14]. The ^{1}H-NMR spectrum of **1** showed two aromatic singlet signals at δ 8.13 and 8.32 and other signals (See experimental) assignable to the protons of ribofuranosyl moiety. These spectral data were in agreement with those for the structure of adenosine. Finally, the ^{13}C-NMR spectral data of **1** confirmed the structure of compound 1. It was further confirmed by the direct comparison with an authentic sample (mmp, co-TLC and ^{1}H-NMR).

The presence of this compound in the plants has previously been reported in *Verbena officinalis* L.[15], *Ganoderma lucidum*[16], *Angelica acutiloba*[17], *Polygonatum sibiricum*[18], *Panax ginseng*[19], and two Allium plants such as *A. bakeri* and *A. sativum*[20].

This is the first report of its occurence from this plant.

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부추 잎으로부터 Adenosine의 분리와 유리 아미노산 조성

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요 약

부추 잎으로부터 purine nucleoside인 adenosine을 분리하고 그 구조를 기기적인 분석방법에 의하여 동정하였으며 또한 유리 아미노산 관련 화합물들의 조성과 상대함량을 표준품과 동일 조건하에서 아미노산 자동 분석 기기로 비교 검토하였다. 가장 함량이 많은 유리 아미노산들은 alanine, glutamic acid, aspartic acid 그리고 valine이었다.