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SHORT COMMUNICATION

THE AMINO ACIDS OF THE GENUS *ASTRAGALUS*

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Abstract—The distribution of certain non-protein amino acids and other ninhydrin-reacting compounds in seed extracts of 120 *Astragalus* species was determined. No decisive chemotaxonomic conclusions emerged from the survey beyond those reached in earlier studies concerned with selenium accumulation by species of the genus.

INTRODUCTION

IN AN earlier study, Brown and Fowden[1] showed that some *Astragalus* species, in common with many other legumes, contained δ-acetylornithine. Brown's[2] detailed survey established that other non-protein amino acids and small peptides, such as canavanine, γ-glutamylphenylalanine and γ-glutamyltyrosine, also occurred in seed of *Astragalus* species. Now the pattern of distribution of these and other amino compounds within many more representative species of the complex *Astragalus* genus has been studied to determine whether this group of compounds can provide the type of useful chemotaxonomic information already derived from similar distribution studies within the *Lathyrus* and *Vicia* genera (see Bell[3]).

RESULTS AND DISCUSSION

Extracts of seeds of about 120 *Astragalus* species were examined by two-dimensional paper chromatography. Typical chromatograms showed the presence of several ninhydrin-positive spots superimposed upon the regular pattern given by unbound forms of the protein amino acids (see Fig. 1). The distribution of some of these additional amino compounds in certain *Astragalus* extracts are shown in Table 1. The table includes only species found in North America, and each species is assigned to the section of the genus as originally listed by Jones.[4] Other species examined included North American representatives not listed by Jones, as well as species growing in Europe and Asia: many were obtained from Botanical Institutes in the U.S.S.R.

An inspection of the table suggests that few distinctive differences of amino acid composition exist between species. This conclusion is further emphasized by analysis of more than eighty other *Astragalus* species not included in Table 1. Therefore the present amino acid survey adds little of critical taxonomic value to the conclusions reached previously by Rosenfeld and Beath[5] upon the basis of selenium accumulation by *Astragalus* species, or by Ledingham[6] using chromosome numbers.

[1] D. H. BROWN and L. FOWDEN, *Phytochem.* **5**, 881 (1966).
[2] D. H. BROWN, Ph.D. Thesis, Univ. of London (1964).
[3] E. A. BELL, In *Comparative Phytochemistry* (edited by T. SWAIN), p. 195. Academic Press, New York (1966).
[4] M. E. JONES, *Revision of North American Species of Astragalus*, published by author. Salt Lake City (1923).
[5] I. ROSENFELD and O. A. BEATH, *Selenium: Geobotany, Biochemistry, Toxicity and Nutrition*. Academic Press, New York (1964).
[6] G. F. LEDINGHAM, *Proc. Gen. Soc. Can.* **3**, 15 (1958).

TABLE 1. THE DISTRIBUTION OF CERTAIN NON-PROTEIN AMINO ACIDS AND OTHER NINHYDRIN-REACTIVE COMPOUNDS IN SEED OF *Astragalus* SPECIES

Species*	Compound									
	Canavanine	δ-Acetyl-ornithine/T††	Homoserine	Spot B†	γ-Hydroxy-norvaline	δ-Hydroxy-norvaline	γ-Glutamyl-phenylalanine	γ-Glutamyl-tyrosine	*S*-Methylcysteine	γ-Glutamyl-*S*-methylcysteine
1. Homalobi										
A. tenellus	M	W	M	T	0	T	T	M	0	0
4. Inflati										
A. miser	M	W	M	T	W	T	T	W	0	0
A. vaseyi	M	S	W	0	W	T	T	M	0	0
A. vestitus	S	S	M	M	W	T	M	S	0	0
A. crotalariae	0	M	M	W	W	W	M	S	M	M
A. macrodon	S‡	M	M	M	W	W	T	M	0	0
A. douglasii	S	M	M	W	W	W	W	M	0	0
A. allochrous	S‡	S	M	M	M	T	M	S	0	0
A. oxyphysus	S‡	M	M	W	W	W	T	M	0	0
A. leucophyllus	S‡	T	M	M	T	W	T	W	0	0
A. leucopsis	S‡	M	M	W	W	T	W	S	0	0
A. lentiginosus	S‡	S	M	W	T	T	T	M	0	0
6. Alpini										
A. aboriginum	0	W	W	T	M	W	W	S	0	0
A. alpinus	S‡	T	M	T	T	W	W	M	0	0
7. Collini										
A. collinus	S‡	W	M	M	T	T	T	M	0	0
8. Podo-sclerocarpi										
A. pachypus	S‡	M	M	W	T	T	T	M	0	0
A. pectinatus	W	W	W	W	W	W	0	0	S‡	W
9. Preussii										
A. preussii	W	M	M	T	M	T	W	M	M	M
A. sabulosus	0	W	W	T	M	T	T	W	S‡	M
A. pattersoni	W	M	T	T	W	T	T	0	S‡	S
11. Uliginosi										
A. canadensis	S	T	M	W	W	W	W	W	0	0
12. Hypoglottides										
A. nitidus	S‡	W	M	W	T	W	W	W	0	0
16. Strigulosi										
A. rubyi	S‡	W	M	W	T	T	M	W	0	0
17. Flexuosi										
A. flexuosus	S	M	M	W	W	W	W	W	0	0
20. Mollissimi										
A. mollissimus	0	S‡	W	T	T	0	T	T	0	0
A. giganteus	S	M	M	T	W	0	T	T	0	0
21. Sarcocarpi										
A. crassicarpus	S‡	S	M	W	0	0	T	W	0	0
A. mexicanus	S‡	M	M	W	0	W	M	W	0	0
23. Bisulcati										
A. haydenianus	T	W	W	T	M	M	T	T	S‡	M
A. bisulcatus	0	0	0	M	0	0	W	M	S	S‡
A. diholcos	T	W	W	W	M	W	T	W	S‡	M
24. Galegiformis										
A. racemosus	0	0	T	T	M	M	T	T	S	S‡
25. Lonchocarpi										
A. osterhouti	0	W	T	W	W	W	W	W	S‡	M
26. Hamosi										
A. arizonicus	M	S	W	W	T	0	W	W	0	0
27. Leptocarpi										
A. nuttallianus	S	M	M	T	W	T	M	T	0	0
29. Didymocarpi										
A. brazoensis	S‡	M	M	W	0	0	T	W	0	0

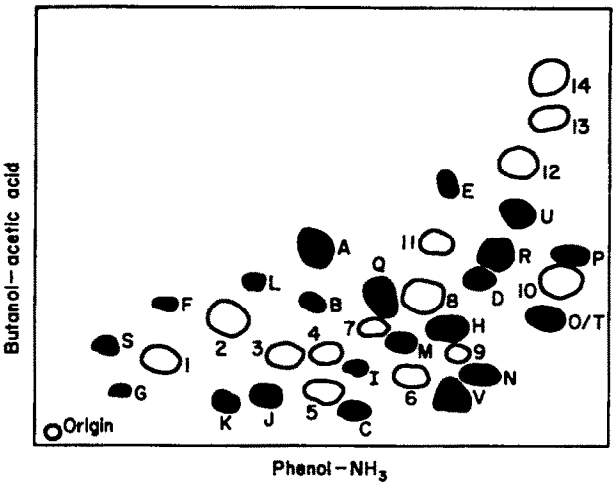

FIG. 1. SHOWS THE POSITIONS ON TWO-DIMENSIONAL CHROMATOGRAMS OF SOME PROTEIN AND NON-PROTEIN AMINO ACIDS FROM *Astragalus* SEEDS.

Key to spots: 1, aspartic acid; 2, glutamic acid; 3, serine; 4, glycine; 5, asparagine; 6, glutamine; 7, threonine; 8, alanine; 9, hydroxyproline; 10, proline; 11, tyrosine; 12, valine; 13, phenylalanine; 14, leucine; A, γ-glutamyltyrosine; C, γ-glutamyl-*S*-methylcysteine sulphoxide; D, γ-hydroxynorvaline; E, γ-glutamylphenylalanine; H, δ-hydroxynorvaline; L, α-aminoadipic acid; M, homoserine; N, *S*-methylcysteine sulphoxide; O, δ-acetylornithine; P, ethanolamine; Q, γ-glutamyl-*S*-methylcysteine; R, *S*-methylcysteine; U, γ-aminobutyric acid; V, canavanine. B, F, G, I, J, K, and T represent unidentified ninhydrin-positive spots encountered frequently in *Astragalus* species.

The main differences we found involved certain sulphur-containing compounds. *S*-Methylcysteine (isolated earlier from *A. bisulcatus*[7]) and γ-glutamyl-*S*-methylcysteine were absent from most species, but were invariably present in relatively high concentrations in species known to be selenium accumulators. (The corresponding selenoamino acids are not separated from the sulphur-containing compounds by normal paper chromatographic procedures.) In addition, *S*-methylcysteine sulphoxide and γ-glutamyl-*S*-methylcysteine sulphoxide (at position C in Fig. 1) were frequently present in the accumulator species: a ninhydrin-positive substance occurring at position C in other species was chemically distinct from the γ-glutamyl peptide. Species characterized by high concentrations of these sulphur amino acids in their seeds usually contained little or no canavanine, in contrast with most other species where canavanine represented the major component of the soluble-nitrogen fraction of the seeds. Among the species we have examined, high sulphur amino acid–low canavanine-containing species are confined to the sections Podo-sclerocarpi, Preussii, Bisulcati, Galegiformes and Lonchocarpi. The single exception is *A. crotalariae*, which Jones[4] included among the Inflati, a section otherwise containing only species having high

[7] S. F. TRELEASE, A. A. DI SOMMA and A. L. JACOBS, *Science* **132**, 618 (1960).

Footnotes to Table 1

* Species are arranged in the sections to which they were assigned by Jones.[4]

Key to concentrations as judged by ninhydrin reaction: S, strong; M, moderate; W, weak; T, trace component; 0, not detected.

† See Fig. 1.

‡ Indicates that the substance formed the major constituent of the seed's free amino acid pool.

canavanine–negative *S*-substituted cysteine derivatives. On the basis of selenium accumulation, Rosenfeld and Beath[5] later transferred *A. crotalariae* to the section Preussii which contains several other accumulator species; our analyses do not contradict this assignment.

Canavanine occurs widely in the sub-family Papilionoideae,[8] and without further evidence it would be unwise to conclude that particular *Astragalus* species completely lack the enzyme complement necessary for its biosynthesis. The reciprocal relationship encountered between *S*-substituted amino acid–canavanine concentrations may have a nutritional basis. When plants accumulate high concentrations of sulphur/selenium, much of the seeds' available soluble-nitrogen will be required for the synthesis of the sulphur amino acids, leaving little nitrogen for the formation of nitrogen-rich canavanine molecules. In non-accumulator plants, the canavanine biosynthetic enzymes will not be subject to similar competition for available nitrogen. Occasionally the inverse concentration relationship did not hold and species lacking both detectable canavanine and substituted cysteines were encountered, e.g. *A. mollissimus* and *A. aboriginum*.

A spot coincident with δ-acetylornithine was present in all species listed in the Table except the two having the highest observed concentrations of *S*-methylcysteine and particularly γ-glutamyl-*S*-methylcysteine, i.e. *A. racemosus* and *A. bisulcatus*. Frequently, this spot was among the strongest observed on chromatograms from individual seed extracts. Although Brown and Fowden[1] established that δ-acetylornithine occurs in seed of certain *Astragalus* species, the amino acid present in this position has not been established with certainty as δ-acetylornithine except in a few species of those now examined. Other naturally occurring amino acids such as *O*-acetylhomoserine and γ-acetyl-α,γ-diaminobutyric acid are known to co-chromatograph with δ-acetylornithine in the phenol–butanol–acetate acid solvent combination used for our two-dimensional chromatograms. Now, we have established that some species, i.e. *A. mollissimus* and *A. alopecuroides*, contain an additional amino compound that runs exactly with δ-acetylornithine. This latter substance (Spot T, Fig. 1) could be separated from δ-acetylornithine on paper chromatograms developed in a pyridine–ethyl acetate solvent, and it was shown to be acid-labile yielding homoserine as the sole ninhydrin-reactive hydrolysis product: however T was not identical with *O*-acetylhomoserine because the two substances could be separated chromatographically in the pyridine–ethyl acetate solvent. Spot B, which generally formed a minor constituent of the *Astragalus* extracts, also yielded homoserine after acid hydrolysis. The isolation of larger quantities for chemical identification of substances T and B awaits an adequate supply of seed of an appropriate species. Two further hydroxyamino acids, γ- and δ-hydroxynorvalines, formed minor components of most seed extracts. Previous isolations of these substances also have been from legume seeds, e.g. δ-hydroxynorvaline from soybean,[9] and γ-hydroxynorvaline from *Lathyrus odoratus*.[10]

METHODS

Pulverized seed material was extracted by shaking with 70% ethanol for several days at 25°. The free amino acid fraction was separated from each extract using small cation-exchange resin columns (as Dunnill and Fowden[11]), and an aliquot representing 0·2 g seed material was applied to two-dimensional chromatograms. These were developed first in 75% (w/w) phenol-NH_3, followed by butan-1-ol–acetic acid–water (90:10:29, by vol.). Amino compounds were revealed using 0·1% ninhydrin in ethanol as a chromogenic reagent.

[8] B. L. TURNER and J. B. HARBORNE, *Phytochem.* **6**, 863 (1967).
[9] J. F. THOMPSON, C. J. MORRIS and G. E. HUNT, *J. Biol. Chem.* **239**, 1122 (1964).
[10] L. FOWDEN, *Nature* **209**, 807 (1966).
[11] P. M. DUNNILL and L. FOWDEN, *Phytochem.* **4**, 933 (1965).

For purposes of identification, small quantities of material were eluted from appropriate areas of chromatograms and were co-chromatographed with authentic compounds on chromatograms developed in the above two solvent systems, and in pyridine–ethyl acetate–water (1:2:2 by vol.) and *tert*-amyl alcohol–acetic acid–water (10:1:10 by vol.). Acid hydrolyses were performed by heating the eluted materials with 6 N-HCl at 100° for 18 hr.

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