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DISTRIBUTION OF SELENOMETHYLSELENOCYSTEINE AND SOME OTHER AMINO ACIDS IN SPECIES OF *ASTRAGALUS*, WITH SPECIAL REFERENCE TO THEIR DISTRIBUTION DURING THE GROWTH OF *A. BISULCATUS*

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Abstract—Studies of the distribution of selenomethylselenocysteine and glutamylselenomethylselenocysteine in *Astragalus bisulcatus* indicate that the free amino acid is the principal seleno amino acid in vegetative parts, whereas the seeds are richer in the glutamyl peptide. Examination of the plants at different stages of growth has shown that during germination the peptide is probably hydrolyzed to free *Se*-methylselenocysteine which is present in 1-week-old seedlings in larger quantities than the peptide. The quantity of the peptide further declines as the seedlings grow so that in 1-month-old plants the amino acid bound selenium is present primarily as free *Se*-methylselenocysteine. The dipeptide reappears only in maturing pods, and it is suggested that this may be the time in the life of *A. bisulcatus* when it is synthesized. Sulphur analogues of the above compounds are also present in this plant and have a remarkably similar pattern of distribution. All four compounds are absent from the seeds of selenium non-indicator species of *Astragalus*.

INTRODUCTION

Astragalus is one of the largest and most widely distributed genera of flowering plants,[1] and in North America it is represented by about 300 species. Twenty-one of these species and three varieties[2] are known to be selenium indicator (sometimes called selenium accumulating) species. The selenium indicator species are toxic to cattle and other animals, the toxicity being attributed mainly to the selenium present.[3] We have succeeded in isolating and purifying two selenium-containing compounds[4] from the seeds and the leaves of *Astragalus bisulcatus*, one of the selenium indicator species native to the plains area of Saskatchewan. One compound was identified as glutamylselenomethylselenocysteine and the other as selenomethylselenocysteine. The latter contaminated with *S*-methylcysteine had previously been obtained from the leaves of *A. bisulcatus* by Trelease *et al.*[5] Because the toxic property of these plants has been associated with their selenium content, we undertook the present investigation of the distribution of the seleno compounds in *A. bisulcatus* during its growth.

One interesting feature of the genus *Astragalus* is the fact that the species found in the Americas (New World species) have a haploid chromosome number of 11, 12 or 13, whereas the species found in the Eurasian and the African regions (Old World species) have a haploid

[1] R. GOOD, *The Geography of the Flowering Plants*, Longmans Green, London (1953).
[2] IRENE ROSENFELD and O. A. BEATH, *Selenium*, p. 63, Academic Press, New York (1964).
[3] S. F. TRELEASE and A. L. MARTIN, *Botan. Rev.* 2, 380 (1936).
[4] To be published elsewhere.
[5] S. F. TRELEASE, A. A. DI SOMMA and A. L. JACOBS, *Science* 132, 618 (1960).

number of 8.[6] Ledingham, after a survey of the chromosome numbers of a large number of species, has suggested that the New World species evolved in America.[7] In view of the above differences between the two geographic populations of *Astragalus*, this investigation was extended to a comparative survey of the amino acid composition of a few representative species of each group.

RESULTS AND DISCUSSION

Table 1 gives the distribution of some amino acids at different stages of growth of *Astragalus bisulcatus*. Seedlings I and II both yield larger quantities of all of the common amino acids, except glycine and proline, than do the seeds. Compared to seedlings I, seedlings II

TABLE 1. DISTRIBUTION OF Se-METHYLSELENOCYSTEINE, ITS GLUTAMYL PEPTIDE AND A FEW OTHER AMINO ACIDS IN *Astragalus bisulcatus*

Compound	Seeds	Seedlings I*	Seedlings II†	Seedlings III‡	Leaves I§	Leaves II‖	Flowers	Pods
	(μmoles per g of the dry tissue weight)							
S-methylcysteine	44·0	36	77·2	13·5	87·5	29·8	40·0	45·9
Glu-*S*-methylcysteine	78·2	24·0	51·5	1·1	3·3	T	0·7	9·0
Se-methylselenocysteine	3·6	T	10·1	5·0	22·4	T	—	T
Glu-*Se*-methylselenocysteine	17·6	5·3	6·5	T	T	T	—	0·6
Alanine	6·2	16·8	34·3	7·0	35·0	5·6	10·0	15·7
Asparagine + glutamine¶	21·6	864	366	53	350	20	80	73
Aspartic acid	1·3	34·8	46·8	7·5	8·7	2·8	2·8	3·9
Glutamic acid	10·8		96·7	7·3		3·1	5·6	11·9
Glycine	3·2	T	1·8	T	9·8	T	0·4	4·9
Proline	4·6	T	T	8·1	92·9	37·8	46·0	58·1
Serine	2·7	93·6	46·8	3·9	28·4	5·9	18·8	14·9
Threonine	2·9	15·6	21·0	1·7	16·4	2·8	2·6	3·0
Valine	6·6	9·9	36·6	T	27·3	5·3	4·2	11·9

* Seeds germinated and grown in dark for 1 week. † Seeds germinated and grown in light for 1 week. ‡ Seeds germinated and grown in soil in the greenhouse for 1 month. § Leaves I from very young plants 4–6 in. high collected in the third week of May. ‖ Leaves II from plants after seed formation. ¶ Calculated from the constant for asparagine. Extracts equivalent to about 10–60 mg of the dry tissue weight used in each analysis and the symbol — refers to the absence, and the letter T to the presence in trace amounts at these concentrations.

contained larger amounts of the common amino acid except asparagine and serine. The presence of an exceptionally large quantity of asparagine in etiolated seedlings (seedlings I) is well known.[8] However, the higher amounts of serine may be related to the metabolism of the selenium and sulphur amino acids in this plant. This point is discussed below.

Se-methylselenocysteine and *S*-methylcysteine are non-protein amino acids and they as well as their glutamyl peptides have a pattern of distribution quite different from that of other

[6] H. A. SENN, *Bibliographia Genet.* **12**, 175, (1938); C. D. DARLINGTON and A. P. WYLIE, *Chromosome Atlas of Flowering Plants*, Allen and Unwin, London (1955); M. M. ANDERSON, M.A. Thesis, University of Saskatchewan, Saskatoon, Saskatchewan (1940); H. VILKOMERSON, *Bull. Torrey Botan. Club* **70**, 430 (1943); L. E. JAMES, *Contr. Dudley Herb.* **4**, 63 (1951); B. L. TURNER, *Am. J. Botany* **43**, 577 (1956); S. C. HEAD, *Madrono* **14**, 95 (1957).

[7] G. F. LEDINGHAM, *Can. J. Genet. Cytol.* **2**, 124 (1960).

[8] E. BALDWIN, *Dynamic Aspects of Biochemistry*, p. 267, Cambridge University Press (1963).

amino acids. The quantities of the two glutamyl peptides fall considerably from the seeds to 1-week-old seedlings and continue to decline as the seedlings grow. In 1-month-old seedlings (seedlings III) only a trace of the selenium peptide remains (compare to 17·6 μmoles in seeds), and the amount of the sulphur peptide has declined to 1·1 μmoles from 78·2 μmoles in the seeds. Similar disappearance of glutamyl peptides at the time of sprouting of onion and garlic bulbs has been observed by Virtanen.[9] Although germination results in a decline in the quantity of the selenium and sulphur peptides in seedling II, there is an increase in the quantity of free *Se*-methylselenocysteine and *S*-methylcysteine. Thus, during germination in light, there does not appear to be a significant change in the total amounts (free and peptide bound) of these two amino acids. This may be due to their slow metabolism under these conditions, or it may be due to their formation at a rate sufficient to compensate for their metabolism. However, in etiolated plants (seedlings I) there is a net loss in the total quantity of both the selenium and sulphur amino acids. It is not possible to determine, on the basis of the present data, whether the difference results from more extensive metabolism in etiolated seedlings or to a compensating synthesis in illuminated plants. It is significant that although seedlings I compared to seedlings II have only trace amounts of *Se*-methylselenocysteine, they have, as mentioned before, twice the amount of serine. In view of this relationship in the distribution of these two amino acids on the one hand and of serine on the other, and because of structural similarity of the three, it appears likely that they are metabolically related. This possibility is currently being investigated.

Seedlings III have smaller amounts of soluble nitrogen than do seedlings I and II and, as has been pointed out above, the glutamyl peptides are present only in trace amounts. Most of the amino acid bound selenium and sulphur is present as free *Se*-methylselenocysteine and *S*-methylcysteine. A similar relative distribution of these four compounds is observed in the leaves (leaves I, Table 1) collected from young *A. bisulcatus* plants growing in the field, in which there was 22·4 μmoles of *Se*-methylselenocysteine and 92·9 μmoles of *S*-methylcysteine compared to trace amounts of the Se peptide and 3·3 μmoles of the S peptide per gram of the dry weight.

The glutamyl peptides make their appearance in the pods (Table 1) and in these there is more bound *Se*-methylselenocysteine than free amino acid. Taking into consideration the amounts of both these substances in ripe seeds (Table 1) it is concluded that from the initial stage of pod formation to the ripening of seeds, the quantities of both substances in this tissue increase and that the bound *Se*-methylselenocysteine predominates. It is suggested that the synthesis of glu-*Se*-methylselenocysteine takes place at this time in the life of the plant. Leaves II (Table 1) were taken from plants with pods of differing maturity. These, when compared to leaves from young plants (leaves I), have only trace amounts of *Se*-methylselenocysteine, and less than half the quantity of *S*-methylcysteine. The decline in the quantity of *Se*- and *S*-methylcysteines in the leaves during the stages of maturation may be the result of their translocation to pods.

It is interesting to note that the distribution of total *Se*-methylselenocysteine (free and peptide bound) roughly corresponds to the distribution of total selenium in this plant.[10] Thus the ripe seeds and the foliage of young plants that were found to be rich in total *Se*-methylselenocysteine are also known to be rich in total selenium. Similarly, the low selenium content of immature pods and the foliage after seed formation is paralled by the low *Se*-methylselenocysteine content of these two tissues. In view of the reasonably large quantities

[9] A. I. Virtanen, *Angew. Chem. Internat. Edit.* **1**, 299 (1962).
[10] Irene Rosenfeld and O. A. Beath, *Selenium*, p. 97, Academic Press, New York (1964).

in which *Se*-methylselenocysteine and its glutamyl peptide are present in the foliage and the seeds, and the similarity of their distribution with that of total selenium, it is probable that they are the principal seleno compounds in *A. bisulcatus*. Very recently dimethyldiselenide[11] and a few other volatile seleno compounds have been shown to occur in exceedingly small amounts in *A. racemosus*, and it is possible that these occur in *A. bisulcatus* as well.

TABLE 2. DISTRIBUTION OF SOME AMINO ACIDS IN THE SEEDS OF SPECIES OF *Astragalus*

	Old World species					New World species				
						Se non-indicator				Se indicator
Compound	Canadensis	Schelichovii	Alopecuroides	Chlorostachys	Sieversianus	Neglectus	Beckivithii	Drummondii	Mollissimus	Racemosus
	(% of total)					(μmoles)				
S-Methylcysteine	—	—	—	—	—	—	—	—	—	11·2
Glu-*S*-methylcysteine	—	—	—	—	—	—	—	—	—	40
Se-Methylselenocysteine	—	—	—	—	—	—	—	—	—	0·8
Glu-*Se*-methylselenocysteine	—	—	—	—	—	—	—	—	—	4·7
Alanine	5·0	5·8	1·5	2·8	1·3	5·2	5·6	3·8	3·4	2·4
Asparagine + glutamine*	26·4	15·7	83·0	56·2	80·0	26·1	53·3	43·5	70·8	13·4
Aspartic acid	3·2	14·2	3·4	4·9	2·6	4·0	6·4	7·5	2·0	3·2
Glutamic acid	14·1	28·0	5·1	8·0	7·5	10·0	13·1	12·2	7·3	14·3
Glycine	3·5	3·1	1·3	1·2	0·8	4·6	3·4	2·8	3·2	0·9
Isoleucine	3·0	T	2·0	T	T	2·5	1·3	1·7	0·7	0·8
Leucine	3·2	T	T	T	T	3·4	1·6	1·5	0·6	1·2
Methionine	T	T	T	T	T	T	T	T	0·2	0·7
Proline	T	T	T	T	T	T	T	T	T	1·5
Serine	7·1	11·2	T	5·8	2·2	20·7	T	5·4	4·3	1·8
Threonine	2·0	2·2	0·6	1·1	0·5	1·5	2·3	1·9	1·0	1·2
Valine	6·4	T	T	T	T	2·5	2·8	2·3	1·8	0·7
X_{422}†	26·1	19·8	3·1	20·0	5·1	19·5	10·2	17·4	4·7	1·2
	100	100	100	100	100	100	100	100	100	100

The quantities are expressed as percentages of the total of all the above amino acids in μmoles as determined on the amino acid analyser. Extracts, equivalent to 60–75 mg of the seed powders, were used for each analysis, and the symbol — refers to the absence, and the letter T to the presence in trace amounts at these concentrations. * Calculated by using the asparagine constant. † Not identified. Amounts calculated by using an average constant of common amino acids.

Table 2 gives the distribution of some amino acids in five Old World and five New World species of *Astragalus*. Of the latter, *A. racemosus* like *A. bisulcatus* is a Se indicator species, and is the only one which contains *Se*-methylselenocysteine, *S*-methylcysteine and their glutamyl peptides. It is noteworthy that *S*-methylcysteine and its glutamyl peptide are present only in selenium indicator species. The presence of these two sulphur compounds in selenium indicator species was recently reported by Dunnill and Fowden.[12] However,

[11] C. S. EVANS, C. J. ASHER and C. M. JOHNSON, *Australian J. Biol. Sci.* **21**, 13 (1968).
[12] P. M. DUNNILL and L. FOWDEN, *Phytochem.* **6**, 1659 (1967).

because of the chromatographic procedure used, they were not able to observe the presence of the Se analogues. There are some quantitative difference in the distribution of common amino acids from species to species but these could not be related to the geographic distribution.

EXPERIMENTAL

Material

The seed samples were supplied by Dr. G. F. Ledingham, and the other plant tissues were collected in Moose Jaw areas of Saskatchewan.

Germination of Seeds

All seeds, prior to germination, were soaked in conc. H_2SO_4 for 30 min,[13] and then washed thoroughly with distilled water. One group of seeds were germinated in the dark in Petri dishes over moist filter papers. After a period of 7 days, the seedlings were washed and then dried in a vacuum desiccator over P_2O_5. These are referred to as seedlings I. Another group of seeds was similarly germinated in light (seedlings II). Seedlings III were grown in a greenhouse in pots of soil containing 100 mg of Na_2SeO_4 per 1000 g of soil. After a period of 1 month the seedlings were carefully removed and then dried as before.

Analyses of Amino Acids

Dried plant material was pulverized and then stirred with hexane for 4 hr at room temperature. Hexane was removed by centrifugation and the residue stirred with 30% ethanol at 5° for a period of 16 hr. Ethanol was removed by centrifugation and the residue extracted once more with 30% ethanol by stirring for an additional period of 4 hr. The combined 30% ethanol extracts were evaporated to dryness at room temperature and the residue dissolved in a known volume of water. Aliquots of these solutions were analyzed on a Hitachi Perkin-Elmer amino acid analyzer, Model KLA-3B, by the physiological fluid analysis procedure of Spackman, Stein, and Moore,[14] except that the temperatures chosen were 34 and 55° instead of 30 and 50°. The higher temperatures resulted in clear separation of *S*-methylcysteine from glutamic acid. However, at these temperatures, the separation of serine from asparagine, and of proline from glutamic acid was not always satisfactory and in these cases another analysis of 30° was required.

Glu-Se-methylselenocysteine has an elution volume similar to serine and therefore its quantity was determined by first isolating a ninhydrin-reacting acidic fraction from a portion of the extract by electrophoresis as a band on Whatman 3 MM paper in pyridine acetate buffer, pH 5·3, for 3 hr at 8 V/cm and subsequent analysis of the acidic fraction on the amino acid analyser. The electrophoretic mobility of glu-*Se*-methylselenocysteine at pH 5·3 is very similar to that of glu-*S*-methylcysteine and therefore an identical loss in the quantity of both S and Se peptides was assumed in this step and a correction made in the amount of the Se peptide.

The presence of selenium compounds in the extracts was also detected on paper after electrophoresis by spraying the strips with starch iodide after oxidation with H_2O_2.[15] Depending upon the tissue being examined only two spots, one in the acidic and the other in the neutral region, were observed. In no case was a positive reaction observed in the basic region, and therefore the extracts were not analyzed for basic amino acids.

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[13] IRENE ROSENFELD and O. A. BEATH, *Selenium*, p. 84, Academic Press, New York (1964).
[14] D. H. SPACKMAN, W. H. STEIN and S. MOORE, *Anal. Chem.* **30**, 1190 (1958).
[15] T. SCALA and H. H. WILLIAMS, *J. Chromatog.* **15**, 546 (1964).