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SHORT COMMUNICATION

ISOLATION AND IDENTIFICATION OF L-CYSTATHIONINE AND L-SELENOCYSTATHIONINE FROM THE FOLIAGE OF *ASTRAGALUS PECTINATUS*

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Abstract—L-Cystathionine and L-selenocystathionine have been isolated from the foliage of *Astragalus pectinatus* In addition to these two amino acids, some *S*-methylcysteine and trace amounts of *Se*-methylselenocysteine were also detected in the foliage extracts The seeds of *A pectinatus* were found to contain significant amounts of all four of these amino acids plus the γ-glutamyl peptides of *S*-methylcysteine and *Se*-methylselenocysteine

INTRODUCTION

THE OCCURRENCE and metabolic functions of cystathionine in animals, bacteria and fungi are well known.[1] In contrast to this, very little is known about the metabolic role of cystathionine in plants, and recently, some doubt has been expressed regarding its occurrence in plants.[2,3] However, as early as 1941, Horn and Jones[4] isolated a crystalline material from the leaves of *Astragalus pectinatus* (narrow leaved milk vetch), which they identified as a mixture of selenocystathionine and cystathionine. This, to our knowledge, is still the best evidence for the natural occurrence of cystathionine in plants. The other component of the mixture, selenocystathionine, has more recently been isolated from the nuts of *Lecythis ollaria*[5] (monkey nut) Evidence for the presence of cystathionine in this nut is not available We have examined the foliage of *A. pectinatus*, and in the present paper report a one step ion-exchange column chromatographic procedure for the isolation of cystathionine and selenocystathionine The identification of these compounds as L-isomers is described.

RESULTS AND DISCUSSION

Proofs of Structure

L-*Cystathionine*. The material isolated from the plants analysed for $C_7H_{14}O_4N_2S$ (see Experimental) and had a specific rotation $[\alpha]_D^{24} + 24{\cdot}1°$ (N HCl, c = 1·95). The reported value for synthetic L-cystathionine is $[\alpha]_D + 23{\cdot}7°$, (N HCl) [6] The material eluted on the amino acid analyser at 432 ml, which is 8 ml before methionine. Authentic L-cystathionine also eluted at 432 ml. It was indistinguishable from authentic L-cystathionine on paper

[1] A MEISTER, *Biochemistry of the Amino Acids*, Vol II, p 757, Academic Press, New York (1965)
[2] A. SHRIFT, *Ann Rev Plant Physiol* **20**, 486 (1969)
[3] J L MARTIN and M L GERLACH, *Anal Biochem* **29**, 257 (1969)
[4] M J HORN and D B JONES, *J Biol Chem* **139**, 649 (1941)
[5] F KERDEL-VEGAS, F WAGNER, P B RUSSELL, N H GRANT, H E ALBURN, D E CLARK and J A MILLER, *Nature, Lond* **205**, 1186 (1965)
[6] J P GREENSTEIN and M WINITZ, *Chemistry of the Amino Acids*, Vol 3, p, 2684, Wiley, New York (1961)

chromatography in solvents I and II. Raney Nickel hydrogenolysis of the material yielded alanine and α-amino-*n*-butyric acid in the ratio of 1 0 95, identified by paper chromatography and by analyses on the amino acid analyser The specific rotations of alanine and α-amino-*n*-butyric acid were found to be $[\alpha]_D^{24}$ + 13 8° (5 N HCl, c = 1 3), and $[\alpha]_D^{25}$ + 21 5°, (5 N HCl, c = 1 07) respectively, reported values[7] for L-alanine and L-α-amino-*n*-butyric acid are + 14 6 (5 M HCl) and + 20 6 (5 M HCl) respectively Since, both amino acids formed on hydrogenolysis were L-amino acids, the cystathionine was assigned the L-configuration These data provide straightforward evidence for the configuration of natural cystathionine Hitherto, naturally occurring cystathionine from a number of sources has been assigned the L-configuration on considerations such as (a) its rotation,[8] which does not distinguish between L-cystathionine ($[\alpha]_D$ + 23 7°, N HCl) and D-allocystathionine ($[\alpha]_D$ + 24 5°, N HCl),[6] (b) comparison of the m p of the dibenzoyl derivative with that of synthetic dibenzoyl-L-cystathionine,[9] (c) paper chromatographic comparison with the synthetic isomers,[10] and (d) its complete oxidation by L-amino acid oxidase [11]

L-*Selenocystathionine* The isolated material analysed for $C_7H_{14}O_4N_2Se$ (see Experimental), and had a specific rotation $[\alpha]_D^{25}$ + 33 5 (N HCl, c = 1 91) Reported rotation for selenocystathionine isolated from *L. ollaria* is + 36 5° (N HCl, c = 1) [5] Its elution volume (439 ml) on the amino acid analyser was similar to that of methionine (440 ml) Authentic selenocystathionine eluted at 439 ml Its chromatographic behavior on paper with solvents I and II was the same as authentic cystathionine and selenocystathionine However, it could be distinguished from cystathionine by the starch-iodide test [12] On hydrogenolysis it gave alanine ($[\alpha]_D^{26}$ + 12 1°, 5 N HCl, c = 1 49) and α-amino-*n*-butyric acid ($[\alpha]_D^{24}$ + 21 1°, 5 N HCl, c = 1 5) in the ratio of 1 0 9 The isolated material is, therefore, L-selenocystathionine

TABLE 1 DISTRIBUTION OF SOME AMINO ACIDS IN *A. pectinatus* (μmoles/g DRY TISSUE WEIGHT)

Amino acid	Seeds	Seedlings*	Foliage†
S-Methylcysteine	16 0	6 9	4 7
γ-Glutamyl-*S*-methylcysteine	4 8	3 1	T
Se-Methylselenocysteine	7 8	2 1	T
γ-Glutamyl-*Se*-Methylselenocysteine	1 5	T	T
Cystathionine‡	1 6	5 2	5 0
Selenocystathionine§	18 2	10 0	4 5

* One-month-old seedlings, grown in nutrient medium containing 32 ppm of selenium as Na_2SeO_4 (C M CHOW, S N NIGAM and W B MCCONNELL, in press)

† Collected in the field before flowering

‡ In an earlier investigation of the seeds of *A. pectinatus*, Martin and Gerlach[3] did not detect cystathionine It is, therefore emphasized, that we have isolated and crystallized cystathionine only from the foliage Our proof of its occurrence in the seeds is based entirely on the chromatographic comparison of the seed and the foliage extracts

§ The peak includes methionine

[7] A MEISTER, *Biochemistry of the Amino Acids*, Vol 1, pp 141–142, Academic Press, New York (1965)
[8] H H TALLAN, S MOORE and W H STEIN, *J. Biol. Chem.* **230**, 707 (1958)
[9] N H HOROWITZ, *J. Biol. Chem.* **171**, 255 (1947)
[10] D B HOPE, *Biochem. J.* **66**, 486 (1957)
[11] D RAJGOPAL RAO, A H ENNOR and B THORPE, *Biochem.* **6**, 1208 (1967)
[12] T SCALA and H H WILLIAMS, *J. Chromatog.* **15**, 546 (1964)

Distribution

Table 1 gives the distribution of some sulphur and selenium amino acids in *A. pectinatus* seeds, seedlings, and field collected foliage before flowering. The table clearly shows, that the concentration of cystathionine was markedly higher in the seedlings than in the seeds, whereas the concentration of other compounds was higher in the seeds than in the seedlings. The foliage had measurable amounts of *S*-methylcysteine, cystathionine and selenocystathionine only The distribution of *S*-methylcysteine and *Se*-methylselenocysteine and their glutamyl peptides in *A pectinatus* is the same as that in the related species, *A bisulcatus*, in which the quantities of these amino acids are higher in the seeds than in the seedlings.[13] In *A pectinatus*, the principal selenium amino acid at all stages of development is selenocystathionine Large quantities of *Se*-methylselenocysteine are found only in the seeds, whereas in *A bisulcatus* this amino acid (free and peptide bound) appears to be the only selenium amino acid.[13] In the earlier study with *A. bisulcatus*, no indication of the presence of either cystathionine or of selenocystathionine was found.

DISCUSSION

The present report clearly establishes the occurrence of cystathionine in *A. pectinatus* leaves This observation is not surprising, since cystathionine in plants, like cystathionine in other living systems,[1] may be required for trans-sulphuration reactions. However, cystathionine, being an intermediate metabolite, probably does not accumulate in the tissues of most plants The fact that cystathionine accumulates in *A pectinatus*, a plant also accumulating selenocystathionine, probably indicates some unique features of sulphur and selenium metabolism in selenium accumulator plants Earlier, a similar observation was made with respect to the occurrence of large amounts of *S*-methylcysteine in two other related plants, i.e. *A. bisulcatus* and *A racemosus*, which accumulate selenium as *Se*-methylselenocysteine [13] However, in this case *S*-Methylcysteine is not a metabolic intermediate, and could be expected to accumulate in plants capable of its synthesis, it is indeed found in some other plants which are not selenium accumulators.[14]

EXPERIMENTAL

Materials and methods The foliage of *A pectinatus* was collected about 20 miles north of Regina on Highway 6 Selenocystathionine was kindly supplied by Dr P B Russell, and L-cystathionine was purchased from Calbiochem, Los Angeles, California, U S A

Amino acid analyses were performed on a Hitachi-Perkin Elmer amino acid analyser, as described before [13] Ascending paper chromatograms were run in *n*-BuOH–HOAc–H_2O (4 1 5) upper phase (solvent I), and in *n*-BuOH–pyridine–H_2O (1 1 1) (solvent II) In addition to the ninhydrin test, selenocystathionine was also visualized by starch–iodide test [12] Raney–Nickel hydrogenolysis followed the procedure of Mozingo *et al* [15] Rotations were taken in a Cenco–Kern full circle polarimeter

Isolation and purification 120 g of air dried foliage was extracted with 30% EtOH as described in an earlier paper [16] Amino acid analysis of an aliquot of the extract showed that it has 133 4 mg (0 11%) of cystathionine and 145 3 mg (1 2%) of selenocystathionine The value for selenocystathionine also includes any methionine present in the extract, as these two have similar elution volumes However, on the basis of the amount of pure selenocystathionine isolated, it appears that very little methionine was present in the extract After two trial separations on a column of Dowex-50 × 4 (1 × 50 cm) the following procedure was adopted for the preparative isolation

The dried extract from 112 g of foliage was taken up in 50 ml 1 N HCl and insoluble material was removed by centrifugation The clear supernatant was placed on a column of Dowex-50 × 4, H^+ form

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[14] A Meister, *Biochemistry of the Amino Acids*, Vol I, p 77, Academic Press, New York (1965)
[15] R Mozingo, D E Wolf, S A Harris and K Folkers, *J Am Chem Soc* **65**, 1013 (1943)

(5 × 40 cm) The column was eluted with 1 N HCl[16,17] (HCl was prepared by diluting constant boiling acid with boiled distilled water) and 17 ml fractions were collected The normality of HCl was increased to 2 N at tube No 320 (5 44 l) This roughly represents the position for the emergence of leucine Subsequent to this, one peak was found to emerge between tubes 422–445, and another peak between tubes 466–500 The tubes in each peak were pooled and evaporated to dryness in a rotary evaporator The first peak contained 300 mg of solid material and contained cystathionine The second peak yielded 310 mg and contained *Se*-cystathionine Two more similar isolations from 200 g of foliage were carried out giving a total of 600 mg of cystathionine (material from one isolation was lost) and 910 mg of selenocystathionine as hydrochloride salts Both were desalted on a column of Dowex-1 × 4 acetate form (1 5 × 15 cm), and the eluates decolorized with charcoal Subsequent evaporations gave 200 mg of cystathionine, and 360 mg of selenocystathionine

Cystathionine (200 mg) was crystallized three times from hot aqueous ethanol to yield 172 mg of pure crystalline material, m p 298–302° decomp Reported m p for synthetic L-cystathionine 312° [18] (Found N, 12 43, S, 14 27% $C_7H_{14}O_4N_2S$ requires N, 12 61, S, 14 41%) Selenocystathionine (360 mg) was also crystallized three times from hot aq EtOH to give 160 mg of pure crystalline material, m p 294–296°, decomp (Found N, 10 31% $C_7H_{14}O_4N_2Se$ requires N, 10 41%)

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[18] *Dictionary of Organic Compounds* Vol 2, p 808 Oxford University Press, Oxford (1965)

Key Word Index—Astragalus pectinatus, Leguminosae, cystathione, selenocystathione, non-protein amino acids