Phytochemistry, 1971, Vol. 10, pp. 2693 to 2698. Pergamon Press. Printed in England.

BIOSYNTHESIS OF Se-METHYLSELENOCYSTEINE AND S-METHYLCYSTEINE IN *ASTRAGALUS BISULCATUS*: EFFECT OF SELENIUM AND SULPHUR CONCENTRATIONS IN THE GROWTH MEDIUM

C. M. CHOW, S. N. NIGAM and W. B. MCCONNELL

Department of Chemistry, University of Saskatchewan Regina Campus, Regina, Saskatchewan, Canada

(*Received* 11 *March* 1971)

Abstract—The effect of selenate and sulphate concentration in the growth medium on the quantities of Se-methylselenocysteine and S-methylcysteine in *Astragalus bisulcatus* has been investigated. Increasing concentrations of selenate at a constant sulphate concentration results in increasing quantities of Se-methylselenocysteine and decreasing quantities of S-methylcysteine. Similarly, increasing concentrations of sulphate at a constant selenate concentration results in increasing quantities of S-methylcysteine and decreasing quantities of Se-methylselenocysteine. Because of this interrelationship, it is suggested that a common enzyme system is involved in the synthesis of both S-methylcysteine and Se-methylselenocysteine. Plants grown in a medium containing 128 ppm of Se and no S developed red rings at stem nodes and a pink coloration of the roots. These phenomena, which were probably due to deposition of elemental Se, were not observed in the presence of sulphate even with higher selenate concentrations. This probably reflects reduced selenate uptake in the presence of sulphate.

INTRODUCTION

ROBINSON,[1] in 1933, showed that wheat from an area in South Dakota, where cattle suffered from alkali disease, contained Se. This observation prompted extensive investigation of Se in plants and resulted in the recognition of a number of native plants, including several species of *Astragalus*, as Se accumulating plants.[2,3] *A. bisulcatus*, *A. pectinatus*, and *A. racemosus* are the three widely distributed Se accumulating species. The first two of these are quite common in western Canada.[4] These species are known to accumulate large quantities (1000–10,000 ppm)[5] of Se in their tissues, and have been the subject of considerable study. Trelease and Trelease[6] observed that Se accumulation in *A. racemosus* depended upon both Se and S concentrations in the growth medium. It has also been shown that selenate uptake by *A. crotalariae* roots is an active transport process,[7–9] and that in barley root selenate competes with sulphate.[9]

[1] W. O. ROBINSON, *J. Assoc. Off. Agric. Chem.* **16,** 423 (1933).
[2] O. A. BEATH, J. H. DRAISE, H. F. EPPSON, C. S. GILBERT and O. C. MCCREARY, *J. Am. Pharm. Assoc.* **23,** 94 (1934).
[3] S. F. TRELEASE and A. L. MARTIN, *Botan. Rev.* **2,** 373 (1936).
[4] R. W. LODGE, A. MCLEAN and A. JOHNSTON, *Stock Poisoning Plants of Western Canada*, p. 20, Canada Department of Agriculture, publication 1361 (1968).
[5] I. ROSENFELD and O. A. BEATH, *Selenium*, p. 91, Academic Press, New York (1964).
[6] S. F. TRELEASE and H. M. TRELEASE, *Am. J. Bot.* **25,** 372 (1938).
[7] J. M. ULRICH and A. SHRIFT, *Plant Physiol.* **43,** 14 (1968).
[8] A. SHRIFT and J. M. ULRICH, *Plant Physiol.* **44,** 893 (1969).
[9] J. E. LEGGETT and E. EPSTEIN, *Plant Physiol.* **31,** 222 (1956).

Se-methylselenocysteine[10-12] has recently been identified as the principle seleno organic compound in the leaves of *A. bisulcatus*. The leaves of this plant also contain S-methylcysteine.[11-13] Significantly, S-methylcysteine is not found in non-accumulator species of *Astragalus*.[12,13] Because, Se-methylselenocysteine and S-methylcysteine occur together in the accumulator species of *Astragalus*, the present study was undertaken to determine the effects of selenium and sulphur concentrations in the growth medium upon the biosynthesis of these two amino acids.

RESULTS

The effect of Se concentration on the quantities of Se-methylselenocysteine and S-methylcysteine in *A. bisulcatus* is summarized in Table 1. The concentration of Se-methylselenocysteine increases from 3·9 μmole/g in the plants receiving no Se to 9·4 μmole/g in the plants receiving 256 ppm of Se. Furthermore, the quantity of glutamyl-Se-methylselenocysteine increases from trace amounts to 2·5 μmole/g. However, in the same series, the quantity of S-methylcysteine decreases from 19·2 μmole/g to 9·6 μmole/g.

TABLE 1. EFFECT OF SELENIUM CONCENTRATION IN THE NUTRIENT SOLUTION ON THE DISTRIBUTION OF Se-METHYLSELENOCYSTEINE, S-METHYLCYSTEINE AND THEIR GLUTAMYL PEPTIDES IN *A. bisulcatus* SEEDLINGS*

Compound	Concentration of Se in the nutrient solution† (ppm)				
	0	32	64	128	256
	(μmoles/g of the dry tissue weight)‡				
Se-Methylselenocysteine	3·9	5·1	10·7	7·9	9·4
Glu-Se-methylselenocysteine	T	T	1·0	1·9	2·5
Total Se-methylselenocysteine	3·9	5·1	11·7	9·8	11·9
S-Methylcysteine	19·2	10·1	15·1	8·6	9·6
Glu-S-methylcysteine	T	T	T	T	T

* Selenium given in the nutrient solution for 7 days.
† Concentration of sulphur in the medium 64 ppm.
‡ Extracts equivalent to 30–60 mg of the dry tissue weight were used in each analysis and the letter T represents the presence in trace amounts at these concentrations.

Table 2 gives the results of feeding nutrient solution containing 128 ppm of Se and various S concentrations to six groups of plants. The Se concentration of 128 ppm was selected, because the first set of experiments (Table 1) indicated that further increase in Se concentration in the growth medium did not appreciably increase the quantity of Se-methylselenocysteine produced. The maximum concentration of S (512 ppm) used was 4 times that of Se. Similarly, the maximum concentration of Se (256 ppm) in the first set of experiments was 4 times that of S (64 ppm). Increasing sulphur concentration from 0 to 512 ppm decreases the quantity of Se-methylselenocysteine in the plants from 11·3 μmole/g to 1·6 μmole/g. Significantly, there is little variation in the quantity of glutamyl-Se-methyl-

[10] S. F. TRELEASE, A. A. DISOMMA and A. L. JACOBS, *Science* **132**, 618 (1960).
[11] S. N. NIGAM and W. B. MCCONNELL, *Biochim. Biophys. Acta* **192**, 185 (1969).
[12] S. N. NIGAM, JAN-I. TU and W. B. MCCONNELL, *Phytochem.* **8**, 1161 (1969).
[13] P. M. DUNNILL and L. FOWDEN, *Phytochem.* **6**, 1659 (1967).

TABLE 2. EFFECT OF SULPHUR CONCENTRATION IN THE NUTRIENT SOLUTION ON THE DISTRIBUTION OF SE-METHYLSELENOCYSTEINE, S-METHYLCYSTEINE AND THEIR GLUTAMYL PEPTIDES IN *A. bisulcatus* SEEDLINGS*

Compound	Concentration of S in the nutrient solution† (ppm)					
	0	32	64	128	256	512
	(μmoles/g of the dry tissue weight)‡					
Se-Methylselenocysteine	11·3	9·2	6·3	1·9	1·8	1·6
Glu-Se-methylselenocysteine	1·2	1·0	1·4	1·3	1·3	1·4
Total Se-methylselenocysteine	12·5	10·2	7·4	3·2	3·1	3·0
S-Methylcysteine	1·9	2·0	25·9	23·6	23·5	24·0
Glu-S-methylcysteine	T	T	0·6	2·1	2·7	2·9
Total S-methylcysteine	1·9	2·0	26·5	25·7	26·2	26·9

* Sulphur given in nutrient solution for 7 days.
† Concentration of selenium in the nutrient solution 128 ppm.
‡ Extracts equivalent to 30–60 mg of dry tissue weight were used in each analysis and the letter T represents trace amounts at these concentrations.

selenocysteine produced. The quantity of S-methylcysteine, on the other hand, increases from 1·9 μmole/g to 24·0 μmole/g, this being accompanied by increases in the amounts of glutamyl-S-methylcysteine. The seeds used in this set of experiments and in the first set of experiments were collected in different years and therefore, in terms of absolute quantities, the results are not comparable. However, the purpose of these experiments was to study the effects of various concentrations of Se and S in the medium on the relative production of Se-methylselenocysteine and S-methylcysteine. In this respect the results are consistent and the trends are quite discernible.

TABLE 3. EFFECT OF SELENIUM CONCENTRATION IN THE NUTRIENT SOLUTION ON THE DISTRIBUTION OF SE-METHYLSELENOCYSTEINE, AND S-METHYLCYSTEINE IN *A. bisulcatus* SEEDLINGS*

Compound	Concentration of Se in the nutrient solution† (ppm)				
	0	32	64	128	256
	(μmoles/g of the dry tissue weight)‡				
Se-Methylselenocysteine	1·0	4·9	13·0	13·7	14·6
S-Methylcysteine	14·7	7·1	7·7	5·3	3·9

* Selenium given in nutrient solution for 30 days.
† Concentration of sulphur in the nutrient solution 64 ppm.
‡ Extracts equivalent to 30–60 mg of the dry tissue weight were used in each analysis.

Table 3 gives the results of experiments in which plants were given increasing concentrations of Se every other day for 30 days. Here, the quantity of Se-methylselenocysteine is seen to increase for the entire range of concentrations used. Furthermore, in these experiments, it was noted, that plants receiving 128 ppm of Se and no S (Table 2 first experiment) developed red rings around stem nodes and a pink coloration in the roots.

The quantities of some other neutral and acidic amino acids were also measured, and

were found to be not greatly influenced by changes in Se and S concentrations in the medium.

DISCUSSION

The results of selenium feeding to *A. bisulcatus* over periods of 7 days (Table 1), and 30 days (Table 3) clearly show that increasing concentrations of Se in the medium result in increasing quantity of Se-methylselenocysteine and decreasing quantity of S-methylcysteine in the plants. Similarly, increasing concentrations of S (Table 2) result in increasing quantity of S-methylcysteine and decreasing quantity of Se-methylselenocysteine. The close relationship between the quantities of Se- and S-methylcysteines produced and Se and S concentrations in the medium suggests that both these amino acids are synthesized by the same enzyme system. The observation, that Se-methylselenocysteine is found only in species of *Astragalus*[12,13] that also produce S-methylcysteine, is a further indication that a common enzyme system is involved.

The linear increase in the ratio of Se-methylselenocysteine to S-methylcysteine with the ratio of Se to S in the medium (Fig. 1, data from Table 1) with Se concentrations in the medium to 64 ppm suggests competition between Se and S containing intermediates involved in the biosynthesis of these two amino acids. Except for an initial lag, the results of the second set are similar. Here, (Fig. 2 data from Table 2) the ratio of S-methylcysteine to Se-methylselenocysteine increases linearly up to a S concentration of 128 ppm in the medium. Again competition between S and Se containing intermediates is indicated. Figures 1 and 2 also show, that Se concentrations higher than 64 ppm and S concentrations higher than 128 ppm produce little increase in the respective ratios. This marked levelling

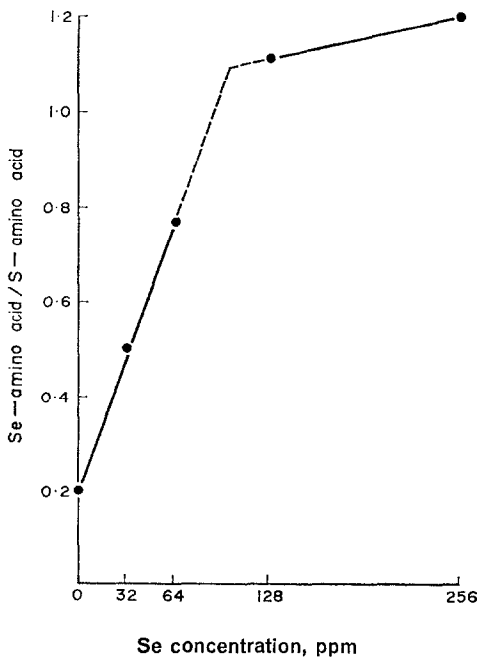

FIG. 1. VARIATION OF THE RATIO OF SE-METHYLSELENOCYSTEINE TO S-METHYLCYSTEINE WITH INCREASING CONCENTRATION OF SELENIUM IN THE NUTRIENT MEDIUM AT A CONSTANT SULPHUR CONCENTRATION OF 64 ppm. THE MEDIUM WAS SUPPLIED TO THE PLANTS EVERY DAY FOR 7 DAYS.

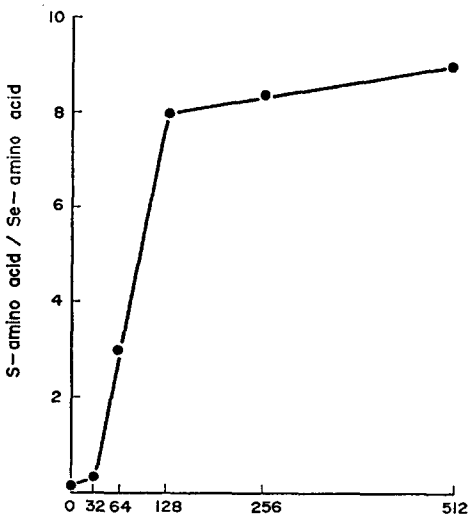

FIG. 2. VARIATION OF THE RATIO OF S-METHYLCYSTEINE TO *Se-METHYLSELENOCYSTEINE* WITH INCREASING CONCENTRATION OF SULPHUR IN THE NUTRIENT MEDIUM AT A CONSTANT SELENIUM CONCENTRATION OF 128 ppm. THE MEDIUM WAS SUPPLIED TO THE PLANTS EVERY DAY FOR 7 DAYS.

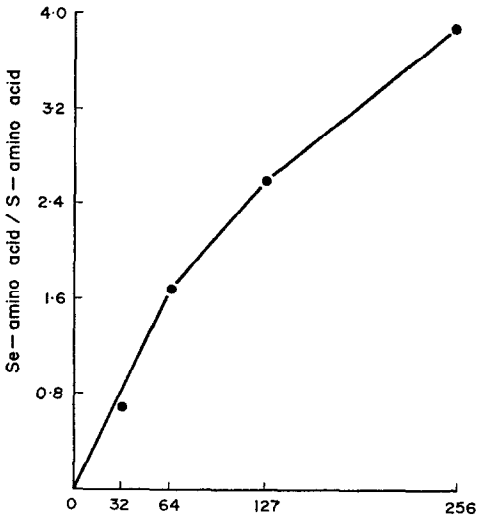

FIG. 3. VARIATION OF THE RATIO OF Se-METHYLSELENOCYSTEINE TO *S-METHYLCYSTEINE* WITH INCREASING CONCENTRATION OF SELENIUM IN THE NUTRIENT MEDIUM AT A CONSTANT SULPHUR CONCENTRATION OF 64 ppm. THE MEDIUM WAS SUPPLIED TO THE PLANTS EVERY OTHER DAY FOR 30 DAYS.

off of the rate of increase of the ratios at higher concentrations of Se or S in the medium is not consistent with the idea of simple competition. Even when a biosynthetic system is saturated with respect to the competing substrates any change in the ratio of the competing precursors should, in the absence of other complicating factors, be reflected by an appropriate change in the ratios of the products.

A similar examination of the data from Table 3 (Fig. 3) shows that, although the increase in ratios is not linear over the range of concentrations studied, the sharp levelling off at high Se concentrations was not observed. In the first experiments, (Table 1) the plants were given fresh nutrient solution each day, and it is possible that, at Se concentrations in the medium of 128 ppm and 256 ppm, the plants absorbed more Se than they were able to metabolize. Thus, sharp levelling off in the ratios may be related to the accumulation of unassimilated Se in the plant tissues, the probable fate of which is discussed below.

The present studies have shown that under certain conditions these plants can synthesize glutamyl-Se-methylselenocysteine. Although this dipeptide was earlier isolated[11] from the seeds of *A. bisulcatus*, it was not found in the field collected samples of the foliage. Earlier studies on the distribution of Se-methylselenocysteine and glutamyl-Se-methylselenocysteine during the life cycle of *A. bisulcatus*[12] has led us to suggest that the peptide is synthesized at the time of seed formation when Se-methylselenocysteine is translocated from the foliage to the seeds. The small and declining quantity of the peptide found in the young seedlings was assumed to have come from the seeds by translocation. However, in the present series of experiments (Table 1) glutamyl-Se-methylselenocysteine was found in the plants receiving high concentrations of Se (64 ppm and up). Thus, the absence of this peptide during the vegetative life of *A. bisulcatus* seems to be related to the concentration of Se rather than to the lack of biosynthetic capability.

The pink coloration of the roots and the red rings around stem nodes, that were observed in the plants receiving 128 ppm of Se and no S (Table 3, first experiment) are probably due to the deposition of elemental Se. These plants evidently had adsorbed more Se than they could metabolize. Neither of these phenomena are observed in plants receiving even higher concentrations of Se in presence of S (Tables 1 and 3). This may be due to the reduced uptake of Se in the presence of S because of competition. Pink coloration in the roots of selenite injured wheat had been observed by Hurd-Karrer,[14] and according to her it was due to the deposition of elemental Se. However, in the present study the plants did not appear to be visibly harmed in any way by the excessive amounts of selenium. Thus, it appears that Se is not toxic to *A. bisulcatus* even when present in greater than assimilable quantities. In this connection it may be noted that, in plants such as cereal grains which are poisoned by relatively small quantities of Se, this element is incorporated into protein.[15] It has also been suggested, that plants like *A. bisulcatus* can tolerate large quantities of Se, because formation of the non-protein amino acid Se-methylselenocysteine by methylation of Se-cysteine prevents the incorporation of latter amino acid into proteins.[16] Incorporation of Se into *bisulcatus* proteins was not investigated in the present study. However, since Se toxicity was not observed at any Se concentrations in the medium, it is most unlikely that there was general incorporation of Se into proteins. It seems that even at concentrations where elemental Se begins to be deposited in the plant tissues, the methylation of Se-cysteine continues efficiently. Saturation of this step would have resulted in the accumulation of Se-cysteine in the plants, which was not detected in any of the experiments. In view of this, it is suggested that in the series of biosynthetic reactions leading to Se-methylselenocysteine from selenate, the limiting step in the assimilation of selenate at high concentrations occurs before the formation of Se-cysteine.

EXPERIMENTAL

Materials. A. bisulcatus seeds were collected near Moose Jaw, Saskatchewan during the summers of 1968 and 1969.

Methods. The seeds were germinated in the dark after prior soaking in conc. H_2SO_4 for 30 min and washing with water.[17] After 2 days, the seedlings were transferred to pots of perlite and grown either in the green house or in an environmental chamber (Lab-Line Biotronette Mark III). Each pot received 30 ml of nutrient solution[18] every other day. This volume more than saturated the perlite in each pot, and since some solution drained from the bottom of the pots after watering, the procedure helped to prevent accumulation of salts in perlite. Experiments were started after 6 weeks growth as above by supplying the nutrient medium containing different concentrations of Se or S. In one series of 5 pots Se concentrations of 0, 32, 64, 128 and 256 ppm were used and the plants were watered every day for 7 days. A second series of 6 pots were all supplied with Se at the level of 128 ppm but the S was supplied to individual pots at levels of 0, 32, 64, 128, 256 and 512 ppm. This series was also watered every day for 7 days. In a third series of experiments the plants were given the same concentrations of Se as in the first series every other day for 30 days. The seedlings were harvested at the end of designated times, dried and extracted with 30% ethanol.[12] The quantities of Se-methylselenocysteine and S-methylcysteine were measured as described earlier[12] by analysis of the extracts on Hitachi Perkin–Elmer amino acid analyser, Model KLA-3B.

Acknowledgement—This research was supported by National Research Council of Canada, Grant No. A-3748.

[14] ANNIE M. HURD-KARRER, *Am. J. Bot.* **24,** 720 (1937).
[15] I. ROSENFELD and O. A. BEATH, *Selenium*, p. 128, Academic Press, New York (1964).
[16] T. K. VIRUPAKSHA and A. SHRIFT, *Biochim. Biophys. Acta* **107,** 79 (1965).
[17] I. ROSENFELD and O. A. BEATH, *Selenium*, p. 84, Academic Press, New York (1964).
[18] G. R. WALLER and L. M. HENDERSON, *J. Biol. Chem.* **236,** 1187 (1961).