PN 1253

Biosynthesis of *Se*-methylselenocysteine from selenite in selenium-accumulating plants

The genus Astragalus L. (Leguminosae) represents an unusual biochemical dichotomy that has evolved in higher plants; it contains species that can accumulate selenium and non-accumulating species that are poisoned by the element. The accumulators, some of which actually require selenium for normal growth[1], absorb selenite or selenate, and convert them largely into a water-soluble organic form. In 1941, HORN AND JONES[2] obtained a crystalline substance from *Astragalus pectinatus* which had the empirical formula, $C_{21}H_{42}N_6Se_2SO_{12}$. The authors inferred that their material consisted of a combination of cystathionine and its selenium analogue in a ratio of 1:2. However, HORN AND JONES could account for only 2 % of the selenium present in the initial plant material. Recently, TRELEASE, DISOMMA AND JACOBS[3] isolated *Se*-methylselenocysteine from an extract of dried leaves of field-grown *A. bisulcatus*. In other accumulators, however, the chemical form of the organic selenium is unknown. We have undertaken a survey of these plants in order to characterize the organic selenium compounds and to study their biosynthesis. This communication will describe experiments in which [^{75}Se]selenite, when supplied to excised leaves of *A. crotalariae*, was converted to *Se*-methylselenocysteine. *Oonopsis condensata*, another selenium accumulator, also synthesized the compound.

Seeds of *A. crotalariae* were collected at Indio, Calif., and refrigerated at 4° until used. Preparation of Astragalus seeds for germination has been described by TRELEASE AND BEATH[1]. After germination on moist filter paper in sterile petri dishes, the seeds were transferred to beakers of sterile vermiculite and kept moistened with a nutrient solution (*cf.* ref. 4 for major salts and ref. 5 for trace elements) at half concentration. The seedlings were grown at 20–22° under a bank of fluorescent lights of about 100 ft-candles.

Plants were harvested after two months. The leaves were severed at the base of the petiole and immediately placed in small vials that contained full strength, sulfur-less nutrient solution, to which was added carrier-free $H_2{}^{75}SeO_3$ in dil. HCl (obtained from Oak Ridge National Laboratory). At the end of 24 h or sooner, most of the radioactive solution had been absorbed, and the sulfur-less medium was then replenished periodically. At about 72 h the petioles of the leaves were rinsed with water, blotted dry, cut into small segments and frozen.

For analysis, the frozen leaves were extracted with cold 5 % trichloroacetic acid in a Potter–Elvejhem hand homogenizer. The homogenate was centrifuged and the residue reextracted thrice with cold 5 % trichloroacetic acid. The trichloroacetic acid in the pooled supernatant was extracted with ether and residual ether was removed under a stream of N_2 gas. The extract was then flash evaporated to about 3 ml at 35–40°, and an aliquot was withdrawn for radioactivity measurements. All radioactivity measurements were made in a Packard Auto-Gamma Spectrometer, model 410A at 405 keV and 1 % window width.

Another aliquot was placed on a column of Dowex-50, H^+ form, and eluted successively with water, 1.5 N HCl and 4 N HCl. A typical elution pattern is shown in Fig. 1. Effluent fractions corresponding to a volume of 80–105 ml were pooled and flash evaporated at about 35°. The residue, in a small volume of water, was applied as a band to Whatman No. 1 paper for descending chromatography in

Solvent 1*. A reference strip was sprayed with 1 % ninhydrin in 95 % ethanol. Successive 0.5-in pieces were cut from the reference strip and were placed in 16-mm test tubes for radioactivity counts. Most of the radioactivity was located in one region with the same R_F as that of synthetic *Se*-methylselenocysteine. The radioactive band

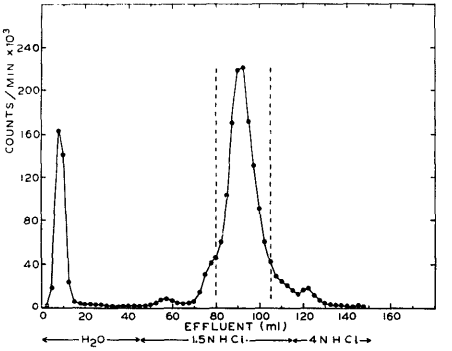

Fig. 1. Column chromatography of trichloroacetic acid extract from *A. crotalariae* leaves supplied with [^{75}Se]selenite; on Dowex-50 X8, H^+ form, 200–400 mesh, 1 × 14-cm column; 2.5-ml fractions collected.

from the main chromatogram was eluted with water for 24 h at room temperature and evaporated to a small volume. Aliquots were subjected to the following identification procedures:

1. High-voltage paper electrophoresis on Whatman No. 1 paper at pH 3.5 (pyridine–acetic acid–water; 1:10:90, v/v) and pH 6.4 (pyridine–acetic acid–water; 10:0.4:90, v/v). A potential of 40 V/cm was applied for 1 h. Both at pH 3.5 and pH 6.4, the seleno-amino acid had the same mobility as that of other neutral amino acids.

2. Two-dimensional chromatography on Whatman No. 1 paper with Solvent 1 in the first direction and Solvent 2* in the second direction. The chromatogram was sprayed with ninhydrin reagent, and a single ninhydrin spot appeared which had the mobility of *Se*-methylselenocysteine in the two solvent systems. On the radioautograph a single radioactive spot could be detected which coincided with the ninhydrin spot**.

3. The seleno-amino acid was co-chromatographed with synthetic *Se*-methylselenocysteine on DEAE-cellulose paper (Whatman DE 20). The developing solvent was 0.01 M acetate buffer (pH 4.7) with 0.001 M EDTA. A single ninhydrin spot could

* Solvents for paper chromatography: Solvent 1, *n*-butanol–acetic acid–water (60:15:25, v/v); Solvent 2, *n*-butanol–pyridine–water (1:1:1, v/v).

** Occasionally, on some two-dimensional chromatograms, a second radioactive spot could be detected which had the R_F of marker *Se*-methylselenocysteine in Solvent 1, but which had the R_F of marker *S*-methylcysteine sulfoxide in Solvent 2. This artifact is probably the selenoxide which arose during the drying of the seleno-amino acid spot after chromatography in the first solvent.

be detected which had the brown-gray color characteristic of this class of amino acids on DEAE-cellulose paper[6]. All the radioactivity was located in this spot.

4. A sample of the seleno-amino acid was treated with 3% (v/v) H_2O_2 (ref. 6) on Whatman No. 1 paper and chromatographed in Solvent 1 or in Solvent 2. Only a small portion of the applied radioactivity could be located in the region corresponding to marker *S*-methylcysteine sulfoxide. Most of the radioactivity moved in the same manner as selenate, and another ninhydrin-negative component could be detected which had an R_F of 0.65 in Solvent 1. When a synthetic sample of *Se*-methylselenocysteine was treated in the same manner with 3% H_2O_2, most of it also was destroyed. By contrast, the sulfur analogue, *S*-methylcysteine, when treated in this way, was converted to the sulfoxide almost entirely.

5. *S*-methyl-L-Cysteine was added to an aliquot of the seleno-amino acid, and methanol was added until a slight turbidity developed. When the solution was cooled, crystals of *S*-methyl-L-cysteine separated, and they were washed several times with anhyd. methanol. Nearly 80% of the radioactivity was associated with the crystals.

From these results it appears that the major soluble, organic selenium compound formed in *A. crotalariae* under our experimental conditions is *Se*-methylselenocysteine. We have also found that *O. condensata*, another selenium accumulator[1], but from the family Compositae, also synthesized the compound. However, preliminary experiments with extracts of a non-accumulator, *A. canadensis*, show that the elution pattern is different from that of Fig. 1. The occurrence of *Se*-methylselenocysteine in three accumulators, *A. crotalariae*, *A. bisulcatus*[3], and *O. condensata*, therefore, may indicate that accumulators and non-accumulators differ in their ability to synthesize this seleno-amino acid. The related compound, *S*-methylcysteine, is found in legumes[7], but its metabolic role has not been clarified. The formation of *Se*-methylselenocysteine by accumulators may be a mechanism whereby excess selenium is rendered innocuous, but it is equally possible that this amino acid may play an essential role in selenium-requiring species.

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