THE OCCURRENCE OF SELENOCYSTATHIONINE IN *MORINDA RETICULATA* BENTH., A TOXIC SELENIFEROUS PLANT*

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Chronic selenosis in Australia has been described in horses and cattle (McCray and Hurwood 1963) from north-western Queensland and in horses (Knott, McCray, and Hall 1958) from the Cape York Peninsula. Ingestion of the seleniferous legume *Neptunia amplexicaulis* Domin. was implicated in the selenosis in the former instance and in earlier work from this laboratory Peterson and Butler (1967) isolated a seleno-amino acid which accounted for a considerable proportion of the selenium in the plant. The substance was characterized as selenocystathionine which had been shown by Aronow and Kerdel-Vegas (1965) to have pharmacological activity. However, *N. amplexicaulis* is apparently absent from the Cape York Peninsula, the selenosis being ascribed to ingestion of *Morinda reticulata*. The work reported in this paper was undertaken to identify the selenium-containing compound(s) in this latter plant species.

Materials and Methods

Seeds of *M. reticulata*, from plants growing near Cooktown, were germinated in a Petri dish of agar and a seedling selected for growth in a Hoagland and Arnon (1938) nutrient solution. After several weeks growth, two weekly additions of [^{75}Se]selenite (600 μCi) were made before the plant was harvested.

Soluble extracts were made following the method of Peterson and Butler (1962*b*) and subjected to high-voltage paper electrophoresis at pH 5·3, 2·0, and 9·2 (Peterson and Butler 1967) and chromatography in various solvent systems (Peterson and Butler 1962*a*). Radioactivity was measured with a scintillation detector assembly and the position of the radioactive compounds located by radioautography and rate-metering.

Hydrogenolysis with Raney nickel was also carried out by the method of Mozingo *et al.* (1943) and the products resolved by paper chromatography and electrophoresis.

Results and Discussion

At the end of the growth period in radioactive nutrient solution, the *M. reticulata* plant had accumulated $6{\cdot}1\times10^5$ disintegrations/min in the tops and $9{\cdot}7\times10^6$ disintegrations/min in the roots. Extracting the tissues with 80% aqueous ethanol followed by water solubilized 92·8% of the ^{75}Se from the tops and 54·4% of the ^{75}Se from the roots. In earlier work with another Australian selenium accumulator, *N. amplexicaulis*, the values for shoot and root solubles were 97% and 55% respectively (Peterson and Butler 1962*b*).

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Top and root extracts were concentrated, subjected to electrophoresis at pH 5·3, and rate-metered. Less than 2% of unmetabolized $^{75}SeO_3^{2-}$ was present in the extracts and nearly all of the radioactivity occurred in the neutral amino acid zone. Two-dimensional separation of the neutral amino acid zones from root or top extracts by high-voltage electrophoresis at pH 2, followed by chromatography in butan-1-ol–acetic acid–water solvent, showed that virtually all of the ^{75}Se occurred in a single compound. A radioautograph of the ^{75}Se area in both extracts exactly matched an amino acid area after spraying with ninhydrin. This radioactive area corresponded with the position of authentic selenocystathionine and indeed the two could not be separated when the marker substance was added to the extracts. Chromatography of the extracts and marker substance in butan-1-ol–pyridine–water and high-voltage electrophoresis at pH 5·3 or at 9·2 was consistent with the conclusion that they were one and the same compound.

Hydrogenolysis of the ^{75}Se-amino acid areas isolated from paper chromatograms of both extracts gave rise to two neutral amino acids of approximately equal proportions after two-dimensional separations. These amino acids behaved as alanine and α-aminobutyric acid in the various electrophoretic and chromatographic systems employed. Marker selenocystathionine behaved similarly throughout.

Selenocystathionine was the second most prominent amino acid in the soluble fraction of tops and represented 20% of the soluble amino acid nitrogen and 90% of the ^{75}Se-compounds present. On a quantitative basis the amount of selenocystathionine present at the stage of growth analysed was 650 μg/g fresh weight. In view of the known pharmacological property of this compound, the selenosis described in horses after ingestion of the plant can probably be ascribed to this compound.

The biosynthesis of selenocystathionine in appreciable amounts by *M. reticulata* is an interesting evolutionary trend, for its presence in this species adds yet another family to the list of plants capable of producing toxic quantities of the same selenoamino acid (refer Horn and Jones 1941; Virupaksha and Shrift 1963; Kerdel-Vegas *et al.* 1965; Peterson and Butler 1967). Various other selenium-accumulating plants toxic to livestock do not synthesize selenocystathionine; instead their principle selenoamino acid is Se-methylselenocysteine, the lower homologue of selenomethionine (Peterson and Butler 1967). There was no evidence for the occurrence of this selenoamino acid in *M. reticulata*.

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