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Plants which Accumulate Metals. Part IV. A Possible Copper–Proline Complex from the Roots of *Armeria Maritima*

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Metal complexes from plants have been identified in a number of cases. Recently iron has been found as the citrate [1], nickel in malic and malonic acid complexes [2], and zinc as the galacturonate [3]. This paper presents a study of the copper complex of a ninhydrin positive ligand in the water extract from the roots of *Armeria maritima*. The *Armeria* plants were collected from a mineralised bog near Dolgellau, Wales, and are highly copper tolerant [4].

Experimental and Results

Detection Reagents for Copper

The water extracts from *Armeria maritima* contain both copper and zinc. The location agent PAN (α-pyridylazol β-naphthol) was used since it is very sensitive for copper and distinguishes between copper and zinc (violet for copper and pink for zinc).

Extract Preparation

Root stocks of *Armeria maritima* were cleaned, dried and powdered as before [4]. 2 g of the powdered material was shaken for 6 hours at room temperature with 200 cm^3 of demineralised water. The solid material was filtered off using a millipore filter, and re-extracted twice. The three extracts were combined, freeze dried and stored in a sealed container in the refrigerator until used. A minimum volume of demineralised water was added before the chromatographic experiments.

Chromatography

Preliminary experiments showed that a complex of copper was present and that in both strongly acid (methylethylketone–conc. HCl–water, 25:3:2 by volume) and in basic (butanol–pyridine–water, 1:1:1 by volume) media, the complex undergoes decomposition. However, in phenol–water (4:1 weight: volume) decomposition did not appear to take place. The solvent system n-propanol–water (1:1 by volume) was chosen as the second solvent (Table I). Other combinations of solvent and papers or thin layer sheets produce dissociation.

TABLE I. R_f Values of Copper in Water Extract of *Armeria maritima*, Detected by PAN.

Spots Applied	Whatman No. 1 phenol–water	MN 300 cellulose thin layer n-propanol–water
Extract	93	37
Cu^{2+} Ions	0	Streaks

TABLE II. Amino Acid Analysis of Root Extract of Copper-tolerant *Armeria maritima* Expressed as Percentage of Total of Ninhydrin Positive Material.

Alanine, 1.08; Argenine, 6.50; Asparagine, 10.80; Aspartic acid, 2.60; Glutamic acid, 7.70; Glutamine, 7.70; Histidine, 0.94; Isoleucine, 0.47; Leucine, 0.42; Lysine, 1.96; β-Phenylalanine, 1.35; Proline, 40.45; Serine, 1.16; Threonine, 2.54; Tyrosine, 0.58; Valine, 1.57 (also ammonia, 7.42; five unknowns, 4.74).

TABLE III. Comparison of the Chromatographic Behaviour of the Root Extract with that of Copper Proline/Proline Mixtures on Whatman No. 1 Paper.

Spot Applied	Phenol–Water	n-Propanol–Water	
	R_f with PAN	R_f with PAN	R_f with isatin
Cu^{2+} ions	0	Streaks	–
Proline	–	–	61
Extract	94	64	64
Cu-proline	96		
Proline/Cu-proline 2.5:1	96	71	71
Proline/Cu-proline 5:1	95	70	70
Proline/Cu-proline 10:1	94	62	62

The preliminary investigations thus indicated that a ninhydrin positive ligand was present. The uncomplexed ninhydrin positive compounds were removed as follows. Streaks of the concentrated extract were placed on five sheets of Whatman 3MM chromatography paper. These were developed in the ascending mode with phenol–water solvent. Thin strips were cut from the sides of the papers and sprayed with PAN. Three bands were identified: Band 3, $R_f = 93 \pm 5$; Band 2, $R_f = 63 \pm 5$; Band 1, $R_f = 46 \pm 1$. Bands 1 and 2 were due to zinc complexes, the ratio of copper to zinc being 10:4 (metal analyses by AAS). The three bands were cut from the papers and were eluted with demineralised water,

using descending chromatography. The extracts were concentrated and rechromatographed.

The copper containing extract was further purified by Sephadex G25 gel filtration using water as eluent.

Sequential extraction [4] has already indicated that the copper was not associated with water soluble proteins. These were therefore removed [5] from a sample of the crude extract, and the remaining water soluble amino acids were analysed. The results are shown in Table II.

Solutions of marker complexes of the ten most plentiful amino acids in the extract were chromatographed. Complexes were made by mixing 0.01 *M* amino acid solution and 0.01 *M* copper chloride solution 2:1 by volume. Only the copper proline, copper valine and copper β-phenylalanine complexes had high enough R_f values to be considered. Chromatography of the purified extract indicated that the complex was copper proline, however the R_f value was below that of the marker complex. It was found that the R_f value of the copper proline complex depended on the amount of excess amino acid present (Table III).

Discussion

The results show that the sample of *Armeria maritima* investigated had very high concentrations of proline in the tissues of the roots. The results also indicate that the water soluble copper is associated with this proline. Reilly [6] has suggested that copper amino acid complexes exist in the water soluble extracts of the copper accumulator, *Becium homblei*, but the explanation of the copper tolerance of the plant must be looked for elsewhere. In the roots of *Armeria maritima* about 20% of the total copper appears in the water extract. In the leaves the figure is 12%. It has been suggested [7] that in human serum there is an equilibrium between copper bound to albumin and copper bound to amino acids, and that the biological role of this system is the transport of copper through membranes. It seems possible that copper amino acid complexes in plant systems may also play a part in copper transport. Most of the insoluble copper in *Armeria maritima* is in association with carbohydrates, and this possibly constitutes the storage mechanism. Work is at present being undertaken to find out if non-tolerant *Armeria maritima* from an unmineralised area (containing little copper) also has high concentrations of proline in the root tissues.

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