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Short Communications

The Occurrence of Free Ornithine and its N-Acetyl Derivative in Plants

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When investigating the free amino acids in plants it was found in this laboratory that *Asplenium* species surprisingly contained many unknown ninhydrin-positive substances. Virtanen *et al.*[1,2] have recently isolated α-aminopimelic acid, γ-hydroxy-α-aminopimelic acid and its lactone from *Asplenium septentrionale*. We noticed that on hydrolysis of the free amino acid fraction of *Aspl. nidus*, even with 1 *N* HCl a large amount of ornithine was formed while the unknown spot 72 disappeared entirely (Fig. 1). A smaller amount of free ornithine was also present before hydrolysis. This was identified both chromatographically and by vanillin[3] spraying.

Ornithine is known to be a part of some cyclic peptides, tyrocidin[4], gramicidin[5] and bacitracin[6]. The free amino acid has only twice been chromatographically identified in plants[7,8]. Manske[9] found, however, in 1937, that the roots of *Corydalis ochotensis* contained 10 % of mono-N-acetylornithine based on the weight of air-dried material. We therefore examined the Finnish specimen *Corydalis bulbosa* and found a spot on the chromatograms, which was identical with that of our unknown substance. We have now been able to isolate this substance from the leaves of *Asplenium nidus*.

The 70 % ethanol extract (380 g fresh wt., 55 g dry wt.) was first purified with Amberlite IR-120 resin. However, only the basic amino acids, arginine, ornithine and lysine remained on the column. This was presumably due to the high content of slimy material, which filled the column, because the second treatment with the same resin gave only a mixture of neutral and acidic amino acids. Acidic amino acids were removed with Amberlite IR-4 B resin and the remaining solution of neutral amino acids was then fractionated by means of Dowex 50 resin (column 26 × 520 mm). 100 fractions of 20 ml each with 1.5 *N* HCl and 120 fractions af 15 ml each with 2.5 *N* HCl were taken. Fractions 25—60 contained serine and threonine, 40—45 glycin and alanine, 40—60 the lactone of γ-hydroxy-α-aminopimelic acid, 60—65 valine *etc.* and 160—190 only the unknown amino acid in question. The eluate was evaporated *in vacuo* at 33° C to a syrup (126.2 mg). After drying in a vacuum desiccator a crystalline mass (105.4 mg) was obtained on the addition of a small amount of absolute ethanol. It was found chromatographically that during concentration 3—5 % of the compound was decomposed to ornithine, but crystallization from ethanol-ether gave 20.8 mg of pure product. The substance decomposed at 200° C. (Found: C 34.62; H 6.54. Calc. for $C_7H_{14}N_2O_3 \cdot 2$ HCl: C 34.02; H 6.53. On titration 0.919 mg required 0.80 ml 0.00917 *N* NaOH in water and 1.19 ml in 80 % ethanolic solution. Found equivalent weights 125.14 and 84.13. Calc. for $C_7H_{14}N_2O_3 \cdot 2$ HCl: 123.57 and 82.38, respectively). By the method of Virtanen and Pulkki[10] 6.22 mg acetic acid was found from 26.2 mg substance (97.0 % of theor.). Acetate was qualitatively identified by means of $FeCl_3$, iodine and $La(NO_3)_3$, by As_2O_3 as cacodyl oxide and dry distillation with $Ca(CO_2)_2$ to acetone, which reacts with *o*-nitrobenzaldehyde to form indigo.

The unknown amino compound was chromatographically identical with acetylornithine isolated by Manske. It was unstable in the presence of nitrogen oxides (from $NaNO_2$ and HCl) and gave negative results with *p*-dimethyl-aminobenzaldehyde, isatin, iodine, vanillin and $KMnO_4$ when the

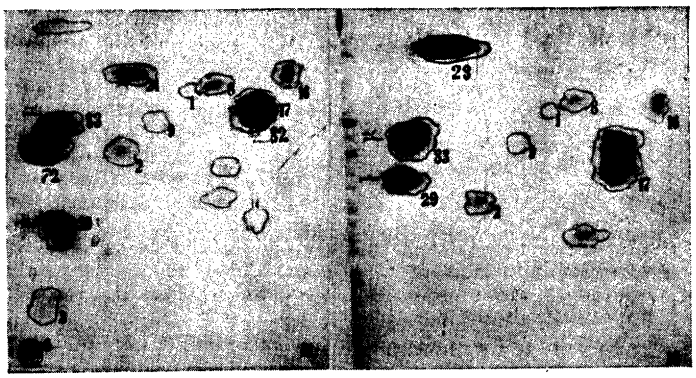

Fig. 1. Two-dimensional paper chromatograms of purified 70 % ethanol extract containing free neutral and acidic amino acids of Asplenium nidus; before hydrolysis (left) and after hydrolysis (right). 1 = gly, 2 = ala, 3 = val, 6 = phen.ala, 8 = ser, 9 = threo, 16 = asp, 17 = glu, 23 = orn, 24 = glutamine, 29 = γ-aminobutyric, 72 = acetylorn, 82 = γ-hydroxy-α-aminopim, 83 = lactone of 82.

reagents were sprayed on the chromatograms. Negative results using the methods of Hanes and Isherwood [11] and Chargaff, Levine and Green [12] indicated that the substance did not contain any phosphorus or sulphur. On paper electrophoresis it proved to be neutral, but after hydrolysis strongly basic ornithine appeared. From all these data it is clear that the substance in question is mono-N-acetyl-ornithine.

No chromatographical data about this substance are to be found in the literature. We obtained the following R_F-values:

	Butanol-acetic acid-water	Phenol-NH_3-water
Proline	0.32	0.91
Alanine	0.27	0.62
Acetylornithine	0.28	0.84
Ornithine	0.07	

In the protein hydrolysate no ornithine could be found. In addition to *Aspl. nidus* and *Corydalis bulbosa*, *Aspl. septentrionale*, *Aspl. viviparum* and *Aspl. trichomanes* also contained acetylornithine. *Dryopteris linnaeana*, *D. filix mas*, *Woodsia ilvensis*, *Eupteris aquilina*, *Struthiopteris filicastrum* and *Athyrium filix femina* were investigated, but no acetylornithine could be found.

Acetylornithine-N in *Aspl. nidus* was about 10 % of the free amino acid nitrogen and 1.14 % of the total nitrogen.

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