A New Type of Monoaminodicarboxylic Acid, γ-Hydroxy-α-Aminopimelic Acid and Its Lactone in Green Plants

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Hydroxyl derivatives of aminodicarboxylic acids have not so far been found in the plant or animal kingdom. Dakin [1] believed to have isolated hydroxyglutamic acid, but his preparation has, on reinvestigation, proved to be a mixture of commonly occurring amino acids, mainly of glutamic acid and aspartic acid [2].

Virtanen and Berg [3] have recently found α-aminopimelic acid in *Asplenium septentrionale*, and also noticed two unknown spots on the two-dimensional paper chromatogram (solvents: butanol – acetic acid and phenol-NH_3) prepared from the alcohol extract of this plant.

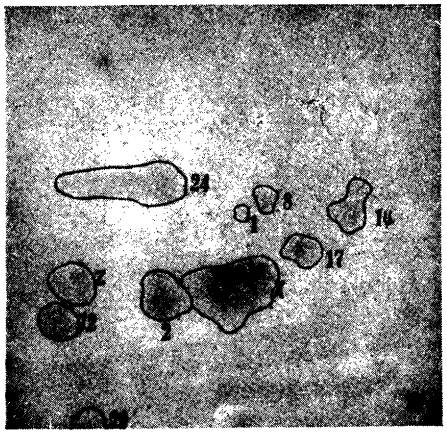


Fig. 1. Two-dimensional paper chromatogram of the unhydrolyzed 70 % alcohol extract of Asp. septentrionale. 1 = gly, 2 = ala, 16 = asp, 17 = glu, 24 = glu-NH_2 + some other amino acids, 72 = acetylornithine, X = unknown acidic amino acid which disappears on hydrolysis, Z = unknown neutral amino acid which becomes much more intense on hydrolysis.

We have closer investigated, and also succeeded to isolate, the amino acids which form these spots (X and Z in Fig. 1). Spot X moves somewhat faster with phenol than glutamic acid. The brownish colour of the spot distinguishes it from the spot of glutamic acid also in the case that the spots are not totally separated from each other. The colour of the other spot (Z) is in the beginning yellow but changes rapidly to reddish. It moves with phenol nearly with the same velocity as proline, but with butanol much slower.

When hydrolyzed with 1 *N* HCl at 108° C for 24 hours spot X disappears, and spot Z becomes much stronger. This observation already showed that there is a connection between the substances corresponding to spots X and Z on the paper chromatogram. It appeared very possible that the substance Z is the lactone of the amino acid X.

It could be shown by the paperelectrophoretic method that the amino acid X is acidic. It was separated in the same fraction as the other acidic amino acids (aspartic, glutamic, and α-aminopimelic acids) in the alcohol extract of *Asplenium septentrionale* when the extract was treated with Amberlite IR – 4B. In this way the unknown amino acid could be separated from neutral and basic amino acids.

The fraction of acidic amino acids was hydrolyzed with 1 *N* HCl at 108° C. The hydrolysate gave now the spots of aspartic, glutamic, and α-aminopimelic acids, and in addition a very strong spot Z. The spot was cut off before treatment with ninhydrin, and the piece of paper extracted with water. The extract contained practically only substance Z. Na_2CO_3 was added to the extract, and the 5 % Na_2CO_3-solution was boiled for 2 hours. It could be shown by the paper chromatographic method that spot Z to a great extent disappeared during this procedure, and that spot X reappeared. This result confirmed the opinion that the amino acid Z is the lactone of the hydroxy-α-aminodicarboxylic acid X. The properties of the lactone suggest that γ-lactone is in question.

The lactone was now isolated from the hydrolyzed fraction of acidic amino acids using again an Amberlite IR – 4B column. Lactone Z, which was formed through treatment with HCl, came from the column in the fractions 3 – 11. These were evaporated *in vacuo*. The paper chromatogram showed that the isolated substance contained practically only lactone Z and as im-

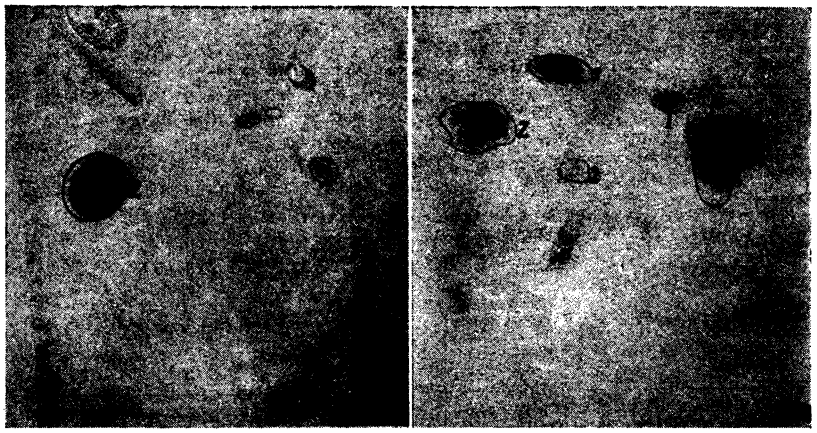


Fig. 2. A. Two-dimensional paper chromatogram from the isolated amino acid Z before treatment with Na_2CO_3. The preparation contained only traces of glycine, serine, and glutamic acid.

Fig. 2. B. Paper chromatogram of amino acid Z after boiling with 5 % Na_2CO_3. The amino acid X has been formed from Z in very large amounts. Traces of glycine (1) and serine (8) appear as impurities. A small amount of alanine and of an unknown substance (S) are formed during treatment with Na_2CO_3.

purities traces of glycine, serine, and glutamic acid (Fig. 2A). When boiled in a 5 % Na_2CO_3-solution for 2 hours large amounts of the amino acid X was formed, and small amounts of alanine and an unknown substance S (Fig. 2B).

The preparation of lactone Z was purified once more with Amberlite IR—4B.

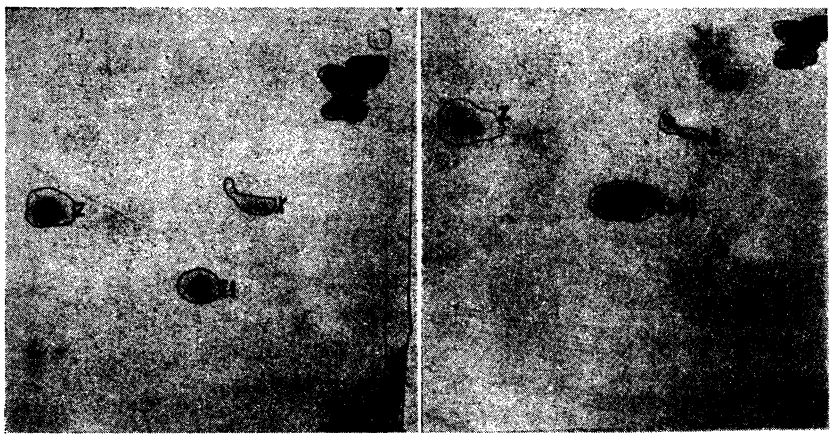


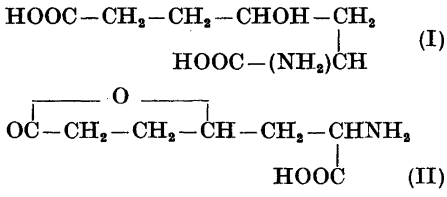
Fig. 3. A. Pure preparation of the amino acid Z after reduction with HJ and red P. α-Aminopimelic acid (71) is formed from Z through reduction. Part of Z has remained unchanged, and part is hydrolyzed to X.

Fig. 3. B. The same as A with the only difference that synt. α-aminopimelic acid was added to the solution (spot 71 + 71).

The solution was evaporated to a syrup. In the syrup appeared star-like clusters of needle-shaped crystals. The recrystallized substance which did not give other spots than Z on the paper chromatogram had a melting point 226–227° (decomposition). (Found: C 48.78; H 6.27; N 8.02. Calc. for $C_7H_{11}O_4N$: C 48.55; H 6.40; N 8.09.)

The substance was reduced with 66 % HJ (*d* 1.96) and red phosphorus at 136° C during 4 hours. α-Aminopimelic acid (Fig. 3) could be shown as the reduction product.

Our results show that *Asplenium septentrionale* contains γ-hydroxy-α-aminopimelic acid (I), and its lactone (II) as free amino acids. The γ-position of the OH-group was proved by oxidation of the deaminated lactone with permanganate in acid solution, whereby succinic acid was formed.



(I)

(II)

In the protein these amino acids could not be found. Also other *Asplenium* species seem to contain these amino acids. In addition to *Aspl. septentrionale* also *Aspl. nidus*, *Aspl. trichomonas*, and *Aspl. viviparum* have so far been investigated.

The amount of γ-hydroxy-α-aminopimelic acid in *Aspl. septentrionale* is relatively high (*cf.* Fig. 1). It is the most abundantly appearing free amino acid in this plant. The amount of its lactone is much smaller corresponding probably to the chemical equilibrium between the acid and the lactone.

We have not found in literature that any hydroxy-α-mono-aminopimelic acid should have been found earlier in living organisms Also synthetic products of this kind seem to be unknown.

Virtanen and Linko have found acetylornithine (Fig. 1) in *Asplenium* species, and in *Aspl. nidus* also free ornithin. They will communicate about the isolation of this acetyl compound.

We wish to thank Mr. R. E. Ruotsalo for his help in procuring *Asplenium septentrionale*, and Professor A. Kalela for *Asplenium* species obtained from the University Botanica Gardens.

1. Dakin, H. D. *Biochem. J. (London)* **12** (1918) 290; **13** (1919) 398.
2. Dent, C. E. and Fowler, D. I. *Biochem. J. (London)* **56** (1954) 54.
3. Virtanen, A. I. and Berg, A-M. *Acta Chem. Scand.* **8** (1954) 1085.

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Adsorptiochromism of 1,4-Naphthoquinones

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A number of organic compounds are adsorbed to inorganic salts with an accompanying color change [1-3]. We have observed that some 1,4-naphthoquinones (all of which are yellow) in petroleum ether solution were adsorbed to Al_2O_3 with a concomitant shift to deeper colors (Table 1). 1,4-Naphthoquinone and 1,2-naphtoquinone remained yellow.

Color intensity varied with the source of the aluminia. On Merck's *purissimo* colors were faint; they were more evident on Merck's Al_2O_3 *nach Brockmann*, and most intense on May and Baker's Al_2O_3

Table 1. Colors of some 1,4-naphthoquinones when adsorbed to aluminia.

Derivatives of 1,4-naphthoquinone	Color of compound on Al_2O_3
2-hydroxy-	orange
2-methyl-	deep violet
2-methyl-3-hydroxy-	orange
2,3,6-trimethyl-	pink
2,6,7-trimethyl-	pink
3,5,7-trimethyl-	pink
2-hydroxy-3-(2-methyl)octyl-	pink
2-methyl-3-phytyl-	violet
2,3-oxide of 2-methyl-3-phytyl	violet
2-methyl-3-difarnesyl-	violet

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