A New Type of Nitrogen Compound in Green Plants. A Cyclic Homoserine Derivative in Some *Liliaceae* Plants

ARTTURI I. VIRTANEN and P. LINKO

Laboratory of the Foundation for Chemical Research, Biochemical Institute, Helsinki, Finland

Species of *Convallaria* and related plants have been widely investigated because of the high content of many important alkaloids. We have, however, found no report in the literature confirming the free amino acid composition of these plants.

It was found by paper chromatography that *Polygonatum officinale* contained large amounts of an unknown ninhydrin-positive substance (I; spot 0 on Fig. 1). After ninhydrin spraying the color of the spot was brownish violet. A strong red color developed by treatment with sodium 1,2-naphthoquinone-4-sulfonate and alkali [1]. *p*-Dimethylaminobenzaldehyde gave a positive reaction, too, somewhat weaker only than with citrulline. When hydrolysed with 1 *N* HCl, substance I either disappeared totally or at least diminished very much and two other spots were formed. One of them (spot 51 on Fig. 2) had a typical violet color reaction with ninhydrin and was identified as homoserine. The other one (II; spot 00 on Fig. 2) appeared as a very intensive deep-yellow spot on chromatograms sprayed with ninhydrin. The color of this spot changed, however, rather rapidly to violet. Both I and II were quite stable against deamination with nitrogen oxides (from $NaNO_2$ and HCl). As will appear from the following, I is built up exclusively from homoserine though, in acid hydrolysis, it also gives substance II.

The unknown substance I was isolated by using Dowex 50 ion exchange resin, and HCl from the hydrochloride was removed with Amberlite IR-120. 4 g of a white crystalline product was obtained from 2.9 kg material (1.6 kg roots and 1.3 kg leaves; fresh wt.). Its elementary composition corresponded to the formula $C_4H_7O_2N$, hence the lactone of homoserine. (Found: C 47.22; H 6.83; N 13.94; equiv. wt. 101.9. Calc. for $C_4H_7O_2N$: C 47.51; H 6.98; N 13.87; Equiv. wt. 101.1). However, no lactone reaction could be detected. In the infrared spectra no typical absorptions either for lactones or for dioxopiperazines could be found. By

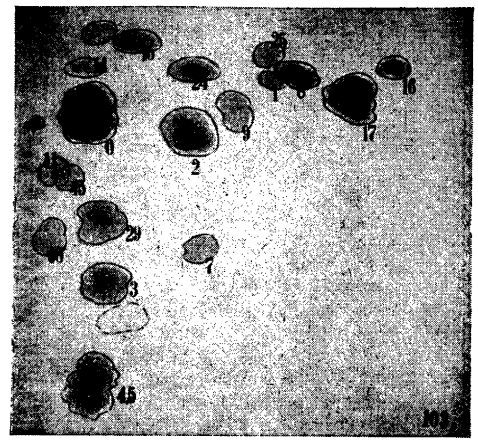


Fig. 1. Two-dimensional paper chromatogram (butanol-acetic acid and phenol-NH_3) of the free amino acids of Polygonatum officinale. O = unknown I, 1 = gly, 2 = ala, 3 = val, 4 = ileu, 5 = leu, 7 = tyr, 8 = ser, 9 = threo, 11 = pro, 14 = arg, 15 = lys, 16 = asp, 17 = glu, 24 = glu-NH_2, 25 = asp-NH_2, 29 = γ-NH_2-butyric, 45 = ethanolamine, 60 = piperidine-2-carboxylic acid.

Fig. 2. Partial hydrolysis (with HCl) of the unknown I. Alanine is added to the chromatogram. 00 = unknown II, 51 = homoserine, 81 = lactone of homoserine.

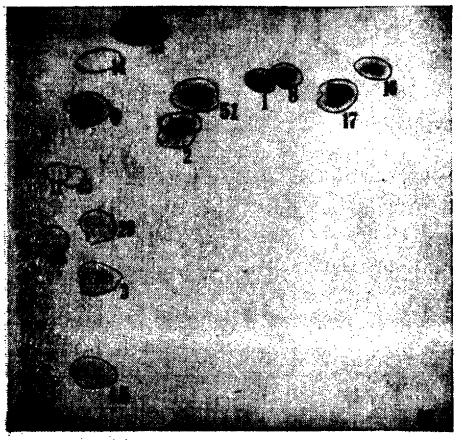


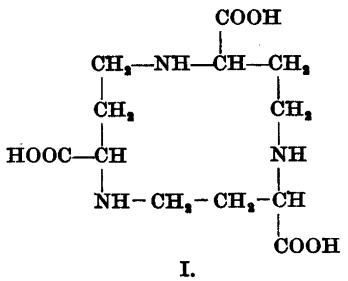
Fig. 3. Treatment of the free amino acid fraction of P. officinale with 4 N NaOH.

using the method of Wendt [2] a molecular weight approximately = 303 was found (Calc. for $(C_4H_7O_3N)_3$: 303.32).

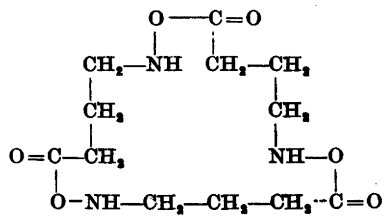
Substance II was prepared and purified in the following way. 926 mg of I were hydrolysed with 6 *N* HCl in a sealed tube for 20.5 hours at 95° C. The reaction products were fractionated on a Dowex 50 column (2 × 42 cm) and the fractions containing mainly II were again hydrolysed to remove traces of I and then deaminated to remove small amounts of homoserine and its lactone. After purification with Amberlite IR-120 some conc. HCl was added and the solution was evaporated to a small volume on a water bath. On precipitation with absolute ethanol 27.8 mg pure hydrochloride of II was obtained. (Found: C 34.23; H 5.84; N 9.93; equiv. wt. 136.0. Calc. for $C_4H_7O_3N$. HCl: C 34.92; H 5.86; N 10.18; equiv. wt. 137.6). Accordingly, II had the same empirical formula as I.

When treated with a saturated solution of $Ba(OH)_2$ II can be changed to I, which then is decomposed to homoserine. Substance I thus gives only homoserine on treatment with alkalies (Fig. 3). It is therefore probable that II, which is formed by acid hydrolysis of I as a byproduct besides the main hydrolysis product homoserine, is some rearrangement product of I. Hence the relationships between I, II and homoserine and its lactone can be shown as depicted below.

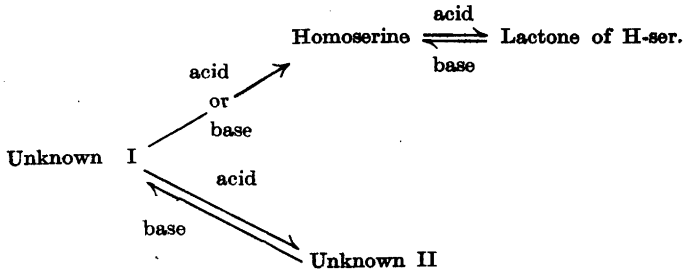
Unknown I has all its carboxyl groups free, but containes no primary or tertiary amino groups. With ninhydrin it liberates the theoretical amount of CO_2 and 55 % of total N as ammonia. It gives a strongly positive reaction for the secondary amino group with sodium nitroprusside and acetaldehyde. Ninhydrin liberates neither



I.



II.



CO_2 nor NH_3 from II, the latter being ninhydrin-positive only on paper, which may be due to some kind of decomposition. However, substance II gives no positive reaction for the secondary amino group as does I. Both I and II are neutral on paper electrophoresis.

The study of these compounds is continued and the experiments and results will be published in detail elsewere. There is, however, now evidence for the structures proposed for I and II.

The following R_F-values were found:

	Phenol-H_2O-NH_3	BuOH-AcOH-H_2O
Lactone of homoserine	0.95	0.35
Unknown I	0.82	0.24
Unknown II	0.66	0.33
Alanine	0.62	0.27
Homoserine	0.57	0.23

When investigating other specimens of the family *Liliaceae*, substance I could be found only in *Convallaria majalis*, *Polygonatum giganteum*, *P. multiflorum*, *Smilacina stellata* and *Majanthemum bifolium*, but not *e. g.* in *Paris quadrifolia*. From a taxonomical point of view this result is very interesting.

We wish to express our deeply felt gratitude to Mr. Andreas Rosenberg, Uppsala for taking the infrared spectra.

1. Giri, K. V. and Nagabhushanan, A. *Naturwiss.* **39** (1952) 548.
2. Wendt, G. *Ber.* **75** (1942) 425.

Received March 7, 1955.

New Aminodicarboxylic Acids and Corresponding α-Keto Acids in *Phyllitis scolopendrium*

ARTTURI I. VIRTANEN and ANN-MARIE BERG

Laboratory of the Foundation for Chemical Research, Biochemical Institute, Helsinki, Finland

In a paper by Virtanen and Alfthan [1] the appearance of two new α-keto acids and the corresponding α-amino acids in a fern (*Phyllitis scolopendrium*) was reported. These amino acids formed the spots V and P on a two-dimensional paper chromatogram (butanol-acetic acid and phenol-NH_3). Both acids were found to be acidic. We have isolated these amino acids and established their chemical nature. At

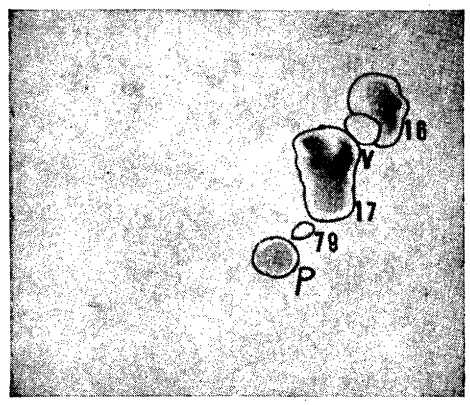


Fig. 1. Two-dimensional paper chromatogram (butanol-acetic acid and phenol-NH_3) of acidic amino acids in Phyllitis scolopendrium. 16 = asp. V = unknown amino acid, 17 = glu, 79 = γ-methyleneglutamic acid, P = unknown amino acid.

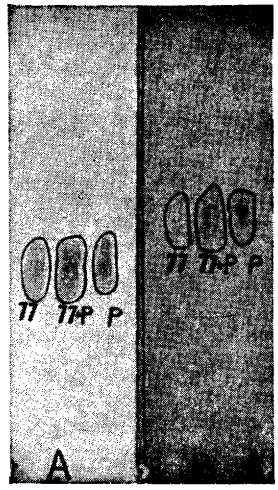


Fig. 2. One-dimensional paper chromatograms of P, synth. γ-methylglutamic acid (77), and a mixture of both. A: butanol-acetic acid, B: phenol-NH_3.