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

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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. 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 NaNO₃ 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 C₇H₁₂O₄N, hence the lactone of homoserine. (Found: C 47.22; H 6.83; N 13.94; equiv. wt. 101.9. Calc. for C₇H₁₂O₄N: 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

![Image of Fig. 1: Two-dimensional paper chromatogram (butanol-acetic acid and phenol-NH₃) 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-NH₂, 25 = asp-NH₂, 29 = γ-NH₂-butryric, 43 = ethanolamine, 60 = piperidine-2-carboxylic acid.]

![Image of 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.]

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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 beside 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 contains 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 acetalddehyde. Ninhydrin liberates neither COOH

\[
\begin{align*}
\text{CH}_3 & \text{NH} \text{CH} \text{CH}_3 \\
\text{CH}_3 & \text{CH}_4 \\
\text{HOOC} & \text{CH} \text{NH} \\
\text{NH} \text{CH}_4 & \text{CH}_4 \text{CH} \text{COOH} \\
\end{align*}
\]

I.

\[
\begin{align*}
\text{O} & \text{C} = \text{O} \\
\text{CH}_3 & \text{NH} \text{CH}_4 \text{CH}_3 \\
\text{CH}_3 & \text{CH}_4 \\
\text{O} & \text{C} \text{CH}_3 \text{NH} \text{O} \\
\text{O} & \text{NH} \text{CH}_4 \text{CH}_4 \text{CH}_4 \text{C}=\text{O} \\
\end{align*}
\]

II.

\[
\begin{align*}
\text{Homoserine} & \xleftrightarrow{\text{acid}} \text{Lactone of H-ser.} \\
\text{acid} & \text{or} \text{base} \\
\text{Unknown I} & \xrightarrow{\text{acid}} \text{base} \\
\text{Unknown II} & \text{acid} \text{or base} \\
\end{align*}
\]

CO₂ nor NH₃ 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 elsewhere. There is, however, now evidence for the structures proposed for I and II.

The following Rf-values were found:

<table>
<thead>
<tr>
<th>Lactone of</th>
<th>Phenol-H₂O-NH₃</th>
<th>BuOH-AcOH-H₂O</th>
</tr>
</thead>
<tbody>
<tr>
<td>homoserine</td>
<td>0.95</td>
<td>0.35</td>
</tr>
<tr>
<td>Unknown I</td>
<td>0.82</td>
<td>0.24</td>
</tr>
<tr>
<td>Unknown II</td>
<td>0.68</td>
<td>0.39</td>
</tr>
<tr>
<td>Alanine</td>
<td>0.62</td>
<td>0.27</td>
</tr>
<tr>
<td>Homoserine</td>
<td>0.57</td>
<td>0.23</td>
</tr>
</tbody>
</table>

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 bijolium, 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.

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

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In a paper by Virtanen and Alftan the appearance of two new α-keto acids and the corresponding α-amino acids in a fern (Phyllitis scolopendrium) was repor-

*Fig. 1. Two-dimensional paper chromatogram (butanol-acetic acid and phenol-NH₃) of acidic amino acids in Phyllitis scolopendrium. 16 = asp. V = unknown amino acid, 17 = glu, 79 = γ-methylene glutamic acid, P = unknown amino acid.*

*Fig. 2. One-dimensional paper chromatograms of P, synth. γ-methylene glutamic acid (77), and a mixture of both. A: butanol-acetic acid, B: phenol-NH₃.*

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