1-N-acetyl-3-indolylmethylglucosinolate in Seedlings of Tovaria pendula Ruiz et Pav.

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Indole Glucosinolates, Tovaria pendula Ruiz et Pav., Tovariaceae, 1-N-Acetyl-3-indolylmethylglucosinolate

The existence of 1-N-acetyl-3-indolylmethylglucosinolate in young seedlings of Tovaria pendula Ruiz et Pav. has been proven by HPLC and mass-spectrometric methods. This compound is accompanied by 4-hydroxy-glucobrassicin, glucobrassicin, 4-methoxy-glucobrassicin and neoglucobrassicin (1-N-methoxy-glucobrassicin).

Introduction

The taxonomical order of Capparales is characterized by the ability of a manifold substitution of the indole ring system, leading to the biogenesis of a broad spectrum of indole glucosinolates (mustard oil glucosides; Fig. 1). The ubiquitous existence of the unsubstituted glucobrassicin (3-indolylmethylglucosinolate) is accompanied by a species-specific pattern of 1-N-methoxy-3-indolylmethylglucosinolate (neoglucobrassicin) [1], 1-N-sulfo-3-indolylmethylglucosinolate [2], 4-hydroxy-3-indolylmethylglucosinolate and 4-methoxy-3-indolylmethylglucosinolate [3—5]. Since in Tovaria pendula, the only species of Tovariaceae (Capparales), aside of glucobrassicin and neoglucobrassicin also serotonin (5-hydroxy-tryptamine) has been detected by chromatographic methods [7], the possibility of an existence of hydroxylated and/or methoxylated indole derivatives was re-investigated by using HPLC and mass spectrometry.

We here report on the isolation and identification of an 1-N-acetylated glucobrassicin.

Fig. 1. Structures of natural indole glucosinolates.

Results and Discussion

The existence of glucobrassicin and neoglucobrassicin in seedlings, shoots and leaves of T. pendula [6, 7] could be newly proven now by HPLC and mass spectrometry of their desulfo compounds (Table I).

In older plant parts (leaves and shoots) these indole glucosinolates are accompanied by two desulfo-glucosinolates which in retention time, TLC, UV spectrum and colour reactions show full correspondence with 4-hydroxy-indolylmethylglucosinolate and 4-methoxy-indolylmethylglucosinolate. Since extracts of young parts show additionally a main compound, identical with serotonin in its chromatographic and kataphoretic properties, the ratio of these hydroxylated indole derivatives is surprisingly low.

In seedlings as well as in lower, non pinnated leaves of young plants (up to node 6) a desulfoindoleglucosinolate, not observed yet in Capparidales, predominates in HPLC-separations (Fig. 2; Table II).

Desulfoglucosinolates with a corresponding retention-time in HPLC-separation have never been observed in any species of Caparaceae, Brassicaceae and Resedaceae yet. Its existence seems to be restricted to the family of Tovariaceae.

Since thermal instability prevented the formation of M'⁺ with the EI-mode, fragment ions have to be used for interpretation of its chemical structure [8, 9]. Mass spectra and intensities are summarized in Table I.

These masses correspond with those formerly reported for glucobrassicin, 4-methoxy-glucobrassicin and neoglucobrassicin [5].

Desulfoacetylg glucobrassicin is characterized by the existence of an acetyl group (mass shift of + 42
Table I. Electron impact induced mass spectra of indole glucosinolates. (The intensity relative to the major ion is given in parentheses.)

![Chemical structures and mass spectra](image)

<table>
<thead>
<tr>
<th>Glucosinolate</th>
<th>M⁺</th>
<th>a</th>
<th>b</th>
<th>c</th>
<th>a'</th>
<th>b'</th>
</tr>
</thead>
<tbody>
<tr>
<td>D-glucobrassicin</td>
<td>368</td>
<td>130</td>
<td>156</td>
<td>172</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>R₁ = R₂ = H</td>
<td></td>
<td>a</td>
<td>b</td>
<td>c</td>
<td>a'</td>
<td>b'</td>
</tr>
<tr>
<td>D-4-methoxy-glucobrassicin</td>
<td>398</td>
<td>160</td>
<td>156</td>
<td>172</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>R₁ = H R₂ = -OCH₃</td>
<td></td>
<td>b</td>
<td>c</td>
<td></td>
<td>a'</td>
<td>b'</td>
</tr>
<tr>
<td>D-‘N-acetyl-glucobrassicin</td>
<td>410</td>
<td>172</td>
<td>156</td>
<td>172</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>R₁ = -COCH₃ R₂ = H</td>
<td></td>
<td>c</td>
<td></td>
<td>a'</td>
<td>b'</td>
<td></td>
</tr>
<tr>
<td>D-‘N-methoxy-glucobrassicin</td>
<td>398</td>
<td>160</td>
<td>183</td>
<td>202</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>R₁ = -OCH₃ R₂ = H</td>
<td></td>
<td>b'</td>
<td></td>
<td>a'</td>
<td>b'</td>
<td></td>
</tr>
</tbody>
</table>

Fig. 2. HPLC elution profile of desulfoglucosinolates from young (A) and old (B) leaves of *T. pendula*. Peak numbers refer to indole glucosinolates listed in Table II. (Nucleosil 10 C₁₈; 20%–60% v/v MeOH; 2 ml min⁻¹.).

Table II. Contents and percental distribution of desulfoglucosinolates in leaves of *T. pendula* Ruiz et Pav. (µg desulfoglucobrassicin-equivalents/g fresh weight).

<table>
<thead>
<tr>
<th>Glucosinolate</th>
<th>Young leaves</th>
<th>Old leaves</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>[µg] [µg]</td>
<td>[%] [%]</td>
</tr>
<tr>
<td>(1) D-4-OH-glucobrassicin</td>
<td>4 (0.2)</td>
<td>79 (1.5)</td>
</tr>
<tr>
<td>(2) D-glucobrassicin</td>
<td>58 (3.4)</td>
<td>483 (9.3)</td>
</tr>
<tr>
<td>(3) D-4-methoxy-glucobrassicin</td>
<td>4 (0.2)</td>
<td>60 (1.2)</td>
</tr>
<tr>
<td>(4) D-‘N-acetyl-glucobrassicin</td>
<td>1577 (92.6)</td>
<td>92 (1.8)</td>
</tr>
<tr>
<td>(5) D-‘N-methoxy-glucobrassicin</td>
<td>59 (3.6)</td>
<td>4468 (86.2)</td>
</tr>
</tbody>
</table>

a.m.u), methyl fragmentation (*m/z* 157 [a — 15]⁺) and a keten split off. The poor CH₃-fragmentation (*m/z* 183 [b — 15]⁺) compared with the dominant keten fragmentation (*m/z* 156 [b—42]⁺) indicates an ‘N-acetylation.

The existence of this ‘N-acetyl glucobrassicin was obscured in former analysis of *Tovaria* extracts by the fact that its *Rₜ* corresponds to that of neogluco-brassicin in paper chromatographic systems formerly used [6, 7].

‘N-acetyl substituted indole compounds have been known so far only as some indole alkaloids. Especially among Strychnos-alkaloids acetylation of the...
heterocyclic nitrogen is known (e.g. Diabolin, Spermostrychnin). In the case of these secondary plant products a direct acetylation of the nitrogen is generally assumed, even if direct proof is missing. In the case of \(^{14}\)C-acetyl-glucosinolate all efforts to label the acetyl group by application of \(^{14}\)C-acetate to young leaves have failed so far, even if comparable to all other indoleglucosinolates, labelling of the indole system by addition of \(^{14}\)C D,L-tryptophane has been possible under conditions comparable to acetate feeding. The problem of the enzymology of indole substitution in glucosinolate producing plants is subject of running experiments.

**Experimental**

**Plant material**

Plants of *Tovaria pendula* have been grown in the greenhouse of the Abteilung für Allgemeine Botanik, Universität Ulm, under daylight conditions at 21 ± 2 °C. The seeds were a gift from the Botanischer Garten der Universität Frankfurt.

**Extraction and preparation of indoleglucosinolates**

Tissues were extracted in boiling methanol, and prepared for HPLC-separation according to the methods described by Götz and Schraudolf [5].

**HPLC**

The separation of the desulfglucosinolates were accomplished by a gradient elution: 20 min linear from 20 to 60% MeOH in water with a flow rate of 2 ml/min. The column (300 × 3.9 mm) was packed with Nucleosil 10 C\(_{18}\) (Machery Nagel & Co). Indole compounds were detected by UV-absorption (280 nm).

**Mass spectra**

Electron impact mass spectra were obtained with a VARIAN MAT 711 mass spectrometer using an emission current of 400 μA at 70 eV.

**Acknowledgements**

We thank Dr. G. Schmidtberg (Sektion für Massenspektrometrie, Universität Ulm) for recording and interpretation of MS and also Mr. K. Russ for the development of the HPLC separation system as well as for running the analysis. We also thank Mrs. C. Guha for skillful technical assistance and Mrs. K. Blankenberg for checking the English version of the manuscript.