^{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 ubiquiteous 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 glucosinolates was re-investigated by using HPLC and mass spectrometry.

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

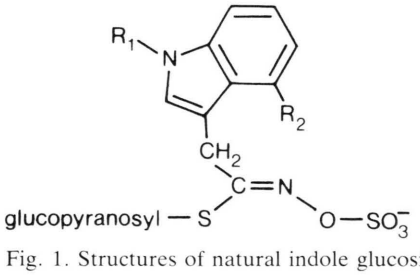

Fig. 1. Structures of natural indole glucosinolates.

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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 desulfoglucosinolates 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 serotonine 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 $M^{+\cdot}$ 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].

Desulfoacetylglucobrassicin is characterized by the existence of an acetyl group (mass shift of + 42

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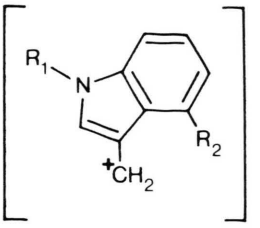
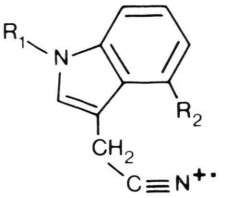
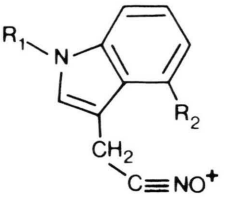
Table I. Electron impact induced mass spectra of indole glucosinolates. (The intensity relative to the major ion is given in parentheses.)

a = 

b = 

c = 

	M$^+$	a	b	c	a′	b′
D-glucobrassicin $R_1 = R_2 = H$	368 (2)	130 (93)	156 (70)	172 (1)		
D-4-methoxy-glucobrassicin $R_1 = H$ $R_2 = -OCH_3$	398 (–)	160 (80)	186 (90)	202 (5)	130 (40)	156 (3) 155 (11) 145 (23) 171 (100)
D-^{1}N-acetyl-glucobrassicin $R_1 = -COCH_3$ $R_2 = H$	410 (–)	172 (5)	198 (38)	214 (1)	130 (74)	156 (100) 155 (73) 157 (13) 183 (–)
D-^{1}N-methoxy-glucobrassicin $R_1 = -OCH_3$ $R_2 = H$	398 (–)	160 (30)	186 (75)	202 (1)	130 (39)	156 (30) 155 (100) 145 (13) 171 (30)

a′ = **a** − (CH_2CO) or (CH_2O) **b′** = **b** − (CH_2CO) or (CH_2O)

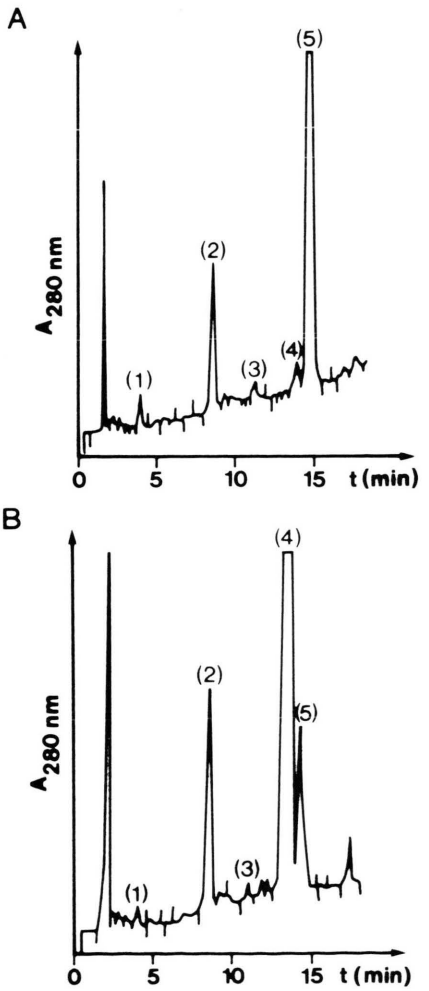


Table II. Contents and percental distribution of desulfoindoleglucosinolates in leaves of *T. pendula* Ruiz et Pav. (µg desulfoglucobrassicin-equivalents/g fresh weight).

	Young leaves [µg]	[%]	Old leaves [µg]	[%]
(1) D-4-OH-glucobrassicin	4	(0.2)	79	(1.5)
(2) D-glucobrassicin	58	(3.4)	483	(9.3)
(3) D-4-methoxy-glucobrassisin	4	(0.2)	60	(1.2)
(4) D-^{1}N-acetyl-glucobrassicin	1577	(92.6)	92	(1.8)
(5) D-^{1}N-methoxy-glucobrassicin	59	(3.6)	4468	(86.2)

a.m.u), methyl fragmentation (*m/z* 157 $[a-15]^+$) and a keten splitt off. The poor CH_3-fragmentation (*m/z* 183 $[b-15]^+$) compared with the dominant keten fragmentation (*m/z* 156 $[b-42]^+$) indicates an ^{1}N-acetylation.

The existence of this ^{1}N-acetyl glucobrassicin was obscured in former analysis of *Tovaria* extracts by the fact that its R_f corresponds to that of neoglucobrassicin in paper chromatographic systems formerly used [6, 7].

^{1}N-acetyl substituted indole compounds have been known so far only as some indole alkaloids. Especially among Strychnos-alkaloids acetylation of the

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 C_{18}; 20%–60% v/v. MeOH; 2 ml min^{-1}).

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 ^{1}N-acetyl-glucobrassicin 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 additon 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 desulfoglucosinolates 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.

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