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Thesinine-4′-*O*-β-D-glucoside the first glycosylated plant pyrrolizidine alkaloid from *Borago officinalis*

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Abstract

The glycosylated pyrrolizidine alkaloid, thesinine-4′-*O*-β-D-glucoside, has been isolated from the aqueous methanol extract of dried, defatted seeds of *Borago officinalis* (Boraginaceae). The structure was established by means of spectroscopic and chemical analysis. © 2002 Elsevier Science Ltd. All rights reserved.

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1. Introduction

Borage (*Borago officinalis* L., Boraginaceae) is an annual herb originating from the Mediterranean area, now cultivated not only in the northern hemisphere but also in Australia, New Zealand, South Africa and South America for the production of its seed oil. Borage seed oil is a rich source of the essential fatty acid γ-linolenic acid (18–25%) and is, therefore, used in nutritional, cosmetical and medical applications (Roeder et al., 2001).

However, members of the Boraginaceae family are also known for their content of pyrrolizidine alkaloids (PAs). Especially unsaturated PAs have been shown to be hepatotoxic and carcinogenic. So far seven PAs were identified in leaves, flowers and seeds of borage with thesinine, a saturated and therefore probably non-toxic necine base described as a major alkaloid. Besides thesinine six unsaturated PAs, amabiline, supinine, lycopsamine, intermedine, acetyllycopsamine and acetylintermedine, were identified as minor constituents (Langer and Franz, 1997). The total alkaloid amount of the plant was determined as less than 0.001% (Roeder, 1995) whereas mature seeds yielded about 0.03% crude alkaloids (Dodson and Stermitz, 1986).

In the present study we report on the isolation and structure elucidation of the first glycosylated pyrrolizidine alkaloid, thesinine-4′-*O*-β-D-glucoside (**1**), obtained from the aqueous methanol extract of borage seeds.

2. Results and discussion

Compound **1** was isolated as a colourless, amorphous solid from the aqueous methanol extract of defatted, dried borage seeds after partitioning between aqueous HCl and ethyl acetate followed by RP-18 chromatography of the concentrated aqueous phase. The molecular formula of **1** was determined as $C_{23}H_{31}NO_8$ by HR–DCIMS, which exhibited a $[M+H]^+$ ion peak at *m*/*z* 450.2143 (calc. 450.2128). Three structure units could be identified by spectroscopic means. From the UV spectral data with absorption maxima at 305, 225 and 204 nm the presence of a *p*-hydroxy cinnamoyl group was deduced (Lee, 2000), which was supported by a set of AA′XX′ doublets at δ 7.51 (2 H, *d*, $J=8.6$ Hz) and 7.08 (2 H, *d*, $J=8.6$ Hz) and two *trans*-configurated olefinic protons at δ 7.66 (1 H, *d*, $J=16.0$ Hz) and 6.34 (1H, *d*, $J=16.0$ Hz) in the ^{1}H NMR spectrum. A further unit was established from the chemical shifts of the six methylene and two methine groups C-1 to C-9 in the ^{1}H and ^{13}C NMR spectra and their correlations in the ^{1}H–^{1}H COSY NMR spectrum as 1-hydroxymethyl pyrrolizidine residue (Table 1). Both units together indicate the structural relation to the known thesinine.

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Unfortunately, no confirming HMBC correlation was observed between H-9 and the carbonyl carbon C-9′, even when HMBC data were recorded under a variety of different experimental conditions. Further signals in the ^{1}H and ^{13}C NMR spectra of **1** could be assigned to a sugar unit, as evident from an anomeric proton signal at δ 4.99 (1H, *d*, $J = 6.8$ Hz) and carbon signals at δ 100.6, 76.7 (2 carbon atoms), 73.5, 70.1 and 61.7. The connectivity of the sugar unit was established from the HMBC spectrum showing a correlation between H-1″ and C-4′.

Full structure elucidation was achieved by acidic and alkaline hydrolysis of **1** (Fig. 1). Acidic hydrolysis gave the hexopyranose and the PA aglycone. The latter was confirmed to be thesinine (**2**) by LC–MS (ESI) spectroscopy, giving the expected pseudomolecular ion peak at *m*/*z* 288 for $[M+H]^+$, and by comparison of the ^{1}H and ^{13}C NMR data with those published by Dodson and Stermitz (1986). The complete assignments of the proton and carbon shifts of **1** and **2** aided by DEPT, ^{1}H–^{1}H COSY and C,H correlated 2D NMR spectra are given in Table 1 and the experimental section, respectively. The sugar residue was determined as glucose by TLC and HPLC comparison with an authentic sample. From the large coupling constant of the anomeric proton H-1″ ($J = 6.8$ Hz) the β-configuration of the hexopyranose was deduced. Alkaline hydrolysis yielded *p*-coumaric acid-4-*O*-β-D-glucoside (**3**), which was identified by NMR spectroscopy (Foo et al., 2000). Comparison of the optical rotation data of **3** with those published in literature (Chapman & Hall Chemical Databases, 2001) established the D-configuration of C-1″ of the glucose moiety. Thus, the structure of compound **1** was elucidated as thesinine-4′-*O*-β-D-glucoside.

In 1999 a PA glycoside, most likely senecionine-*O*-glucoside, was identified by LC-MS as detoxification product in PA sequestering chrysomelid leaf beetles (Hartmann et al., 1999). However, to our knowledge thesinine-4′-*O*-β-D-glucoside (**1**) is the first glycosylated plant PA described in literature. With a content of about 0.5% in dried borage seeds and 0.02% in fresh aerials, **1** is occurring in much higher amounts than the non glycosylated PAs earlier described for *Borago officinalis*. Presumably because of its high polarity it has not been isolated before. Applying the usual alkaloid extracting procedure including acidic-basic liquid-liquid partition (e. g. Parvais et al., 1994), **1** is not found in the organic alkaloid phase but remains completely in the aqueous layer.

Structurally related to **1** are several lupin alkaloid glycosides, e. g. (*trans*-4′-glucosyloxycinnamyl)lupinine (**4**), isolated from *Lupinus luteus* (Leguminosae) seed-

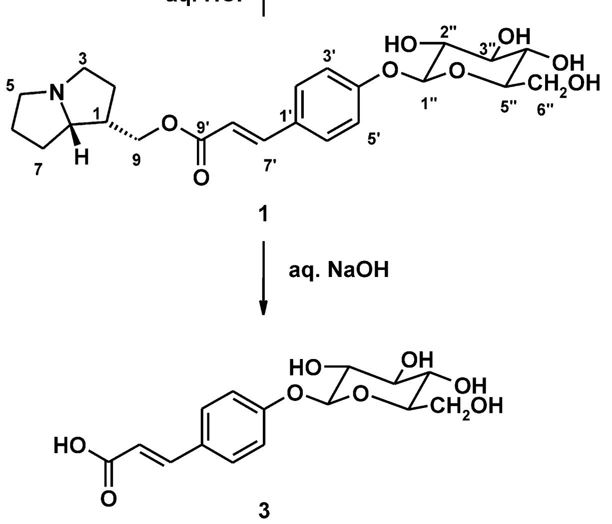

Fig. 1. Thesinine-4′-*O*-β-D-glucoside (**1**) and reaction products obtained from acidic and alkaline hydrolysis.

Table 1
^{1}H and ^{13}C NMR spectral data of thesinine-4′-*O*-β-D-glucoside (**1**)[a]

Position	δ_C	δ_H	^{1}H–^{1}H COSY
1	44.5	2.40 (*m*)	H-2, 8, 9
2	29.5	2.23 (*m*)	H-1, 3
		1.91 (*m*)	H-1, 3
3	54.1	3.90 (*m*)	H-2
		2.88 (*ddd*, $J = 6.0$, 10.9 and 10.9 Hz)	H-2
5	54.3	3.52 (*m*)	H-6
		2.99 (*ddd*, $J = 5.8$, 5.8 and 11.5 Hz)	H-6
6	25.1	2.10 (*m*)	H-5, 6
7	30.7	2.23 (*m*)	H-6, 8
		1.91 (*m*)	H-6, 8
8	69.3	4.01 (*m*)	H-1, 7
9	64.7	4.33 (*dd*, $J = 5.9$ and 11.4 Hz)	H-1
		4.23 (*dd*, $J = 7.1$ and 11.4 Hz)	H-1
1′	128.6		
2′/6′	130.1	7.51 (*d*, $J = 8.6$ Hz)	H-3′, 5′
3′/5′	117.0	7.08 (*d*, $J = 8.6$ Hz)	H-2′, 6′
4′	159.5		
7′	145.6	7.65 (*d*, $J = 16.0$ Hz)	H-8′
8′	115.4	6.34 (*d*, $J = 16.0$ Hz)	H-7′
9′	167.4		
1″	100.6	4.99 (*d*, $J = 6.8$ Hz)	H-2″
2″	73.5	3.52 (*m*)	
3″	76.7	3.52 (*m*)	
4″	70.1	3.52 (*m*)	
5″	76.7	3.52 (*m*)	
6″	61.7	3.79 (*m*)	H-5″

[a] Measured in $CDCl_3$ with CD_3OD added to improve solubility at 300 (^{1}H NMR) and 75 MHz (^{13}C NMR).

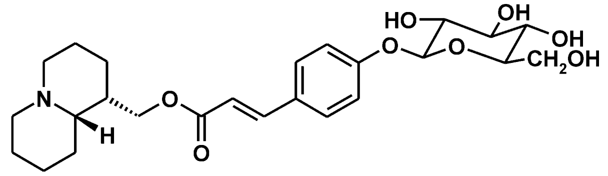

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Fig. 2. (*trans*-4′-Glucosyloxycinnamyl)lupinine (**4**).

lings by Murakoshi et al. (1977, 1979). In contrast to **1** they consist of quinolizidine 6-rings instead of the pyrrolizidine 5-rings (Fig. 2).

3. Experimental

3.1. General

The following instruments were used: OR: polarimeter 241 (Perkin-Elmer), IR: IFS25 FTIR- spectrometer (Bruker), UV: U3300 spectrophotometer (Hitachi), ^{1}H and ^{13}C NMR: Varian VXR-300 (300 and 75.5 MHz, respectively with TMS as internal standard), MS: MAT-95 mass spectrometer (Finnigan). Analytical HPLC: HP1100, column YMC ODS-AQ, 5 µm, 150×3 mm with 10×3 mm guard column, flow rate 0.6 ml/min, λ 330 nm, temp. 40 °C, gradient: A = water + 0.1% HCO_2H, B = CH_3CN, 0–10 min/2.5–25% B, 10–15 min/25–40% B, HPLC–MS: HP1100 with Bruker Esquire LC, ion source: ESI, nebulizer gas 30 PSI, dry gas 10 l/min, dry temp. 300 °C.

3.2. Plant material

Borage seeds (Boraginis sem. tot.) were purchased from Alfred Galke GmbH, D-37534 Gittelde, Germany. Locally grown borage (1999) was used for the extraction of fresh aerials.

3.3. Extraction of borage seeds and isolation of *1*

Fifty grams of milled borage seeds were defatted by hexane extraction (2×100 g, 1 h under reflux each). The residue was refluxed with 200 g of $MeOH/H_2O$ 4:1 (w/w) for 1 h. After evaporation, the $MeOH/H_2O$ extract (1.8 g) was dissolved in aq. HCl (pH 2–3) and extracted twice with EtOAc. The aqueous phase was neutralized with Na_2CO_3, concentrated to dryness and separated by RP18-MPLC (YMC ODS-AQ, 10/20 µm, 120 C, 470 × 40 mm, $MeOH/H_2O$ 20:80 + 0.5 ml HCO_2H/l, λ 220 nm, flow rate 15 ml/min) to give 85 mg of **1**.

3.3.1. Thesinine-4′-O-β-D-glucoside (*1*)

A Colourless amorphous solid; analytical HPLC: R_t = 9.5 min; $[\alpha]_D^{20}$−22.8° (DMSO, *c* 1.99); UV (MeOH) λ_{max} nm (log ε): 305 (4.29), 297 (4.28), 225 (4.06), 204 (4.15); IR (KBr) ν_{max} cm^{-1}: 3410, 1711, 1604, 1510, 1240, 1171, 1073; ^{1}H NMR (see Table 1); ^{13}C NMR (see Table 1); DCIMS (NH_3), 120 eV, *m*/*z* = 450 $[M+H]^+$, HR–DCIMS (NH_3), 120 eV, calc. for $C_{23}H_{32}NO_8$ $[M+H]^+$ 450.2128, found: 450.2143.

3.4. Acidic hydrolysis of *1*

Forty-five milligrams of **1** were dissolved in 6 ml of MeOH and 25 ml of 3% aq. HCl and hydrolysed under reflux (1 h). After concentration in vacuo, aq. $NaHCO_3$ was added and the alkaline solution was extracted with EtOAc. The organic phase was evaporated to dryness and separated by preparative RP18-HPLC (column: Eurospher 80 C18, 10 µm, 250×16 mm; eluent: MeOH/H_2O 50:50 + 0.5 ml HOAc/l, flow rate: 10 ml/min, λ: 330 nm) to give 5 mg of thesinine (**2**).

The aqueous layer was concentrated in vacuo and glucose was identified by Co-TLC [aluminium sheets silica gel 60 F254, eluent: $CHCl_3/Me_2CO/MeOH/H_2O$ 3:3:3:1, detection: anisaldehyde/H_2SO_4, 120 °C, R_f (glucose) 0.42] and HPLC [column: Phenomenex REZEX RPM Monosaccharide, 300×7 mm, eluent: H_2O, temp. 80 °C, flow rate 0.6 ml/min, R_t (glucose) 12.1 min] with MS detection (ESI, positive ion mode, *m*/*z* 203 for $[M+Na]^+$) with an authentic sample.

3.4.1. Thesinine (*2*)

Analytical HPLC: R_t = 12.6 min; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.56 (1H, *d*, *J* = 16.0 Hz, H-7′), 7.35 (2 H, *d*, *J* = 8.5 Hz, H-2′ and H-6′), 6.89 (2 H, *d*, *J* = 8.5 Hz, H-3′ and H-5′), 6.13 (1 H, *d*, *J* = 16.0 Hz, H-8′), 4.32 (1 H, *dd*, *J* = 5.3 and 11.3 Hz, H-9_A), 4.13 (1 H, *dd*, *J* = 7.4 and 11.3 Hz, H-9_B), 4.07 (1 H, *m*, H-8), 3.81 (1H, *m*, H-3_A), 3.53 (1 H, *m*, H-5_A), 2.88 (1 H, *ddd*, *J* = 6.1, 6.1 and 12.0 Hz, H-5_B), 2.78 (1 H, *ddd*, *J* = 6.4, 10.9 and 10.9 Hz, H-3_B), 2.33 (1H, *m*, H-1), 2.18 (2 H, *m*, H-2_A and H-7_A), 1.98 (3 H, *m*, H-2_B and H-6), 1.81 (1 H, *m*, H-7_B); ^{13}C NMR ($CDCl_3$) δ 167.0 (*s*, C-9′), 160.4 (*s*, C-4′), 146.0 (*d*, C-7′), 130.2 (*d*, C-2′ and 6′), 125.4 (*s*, C-1′), 116.2 (*d*, C-3′ and C-5′), 113.1 (*d*, C-8′), 68.7 (*d*, C-8), 63.9 (*t*, C-9), 54.2 (*t*, C-5), 54.0 (*t*, C-3), 44.3 (*d*, C-1), 30.7 (*t*, C-7), 29.3 (*t*, C-2), 25.2 (*t*, C-6); HPLC–MS (ESI, positive ion mode) *m*/*z* 288 for $[M+H]^+$.

3.5. Alkaline hydrolysis of *1*

Twenty milligrams of **1** were dissolved in 5 ml 2.5% aq. NaOH and stirred for 10 min at room temp. H_2SO_4 was added and the acidic solution was extracted twice with EtOAc. The organic phase was evaporated to dryness and separated by preparative RP18-HPLC (column: Eurospher 80 C18, 10 µm, 250×16 mm; eluent: $MeOH/H_2O$ 50:50 + 0.5 ml HOAc/l, flow rate: 10 ml/min, λ: 330 nm) to give 6 mg of **3**.

3.5.1. *trans-p-Coumaric acid 4-O-β-D-glucoside (3)*

Analytical HPLC: $R_t = 7.4$ min; $[\alpha]_D^{24}$ −69.5° (MeOH, *c* 0.7) Lit. $[\alpha]_D^{24}$ −72.6° (Chapman & Hall Chemical Databases, 2001); HPLC–MS (ESI, negative ion mode) *m/z* 325 for $[M–H]^-$.

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