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SHORT COMMUNICATION

A new trisaccharide derivative from *Prenanthes purpurea*

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Abstract: A methanolic extract of *Prenanthes purpurea* L. leaves yielded 1,6”-di-*O*-cinnamoyl-*β*-D-glucopyranosyl-(1→3)-*O*-*α*-L-rhamnopyranosyl-(1→6)-*O*-*β*-D-glucopyranoside. The NMR and physical data of this new natural compound are reported.

Keywords: Asteraceae; Cichorieae; Hypochaeridinae; phenolic acids; *Prenanthes purpurea* L.; trisaccharides.

INTRODUCTION

Prenanthes purpurea L. is distributed over Central and Southern Europe and the Caucasus.[1] Recent molecular results revealed that the genus *Prenanthes* is monotypic and a member of the Hypochaeridinae subtribe within the Cichorieae tribe of the Asteraceae family.[2] The present communication deals with the isolation and structure elucidation of 1,6”-di-*O*-cinnamoyl-*β*-D-glucopyranosyl-(1→3)-*O*-*α*-L-rhamnopyranosyl-(1→6)-*O*-*β*-D-glucopyranoside, a new di-*O*-cinnamoyl-trisaccharide derivative from a methanolic extract of leaves of *P. purpurea* of Austrian origin. The structure elucidation was based on extensive NMR studies as well as HR-MS data.

RESULTS AND DISCUSSION

Compound **1** was isolated from the ethyl acetate layer of the methanolic extract of *P. purpurea* leaves employing silica gel column chromatography (CC), repeated Sephadex LH-20 CC and semi-preparative RP-18 HPLC.

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Physical data. Amorphous white compound, glass transition above 141 °C; FTIR (ZnSe, cm^{-1}): 3420 (br), 2920, 1710, 1636, 1578, 1529, 1496, 1450, 1332, 1312, 1283, 1204, 1174, 1074, 914, 865, 840, 807, 769, 713, 685. HRMS (*m*/*z*): 771.2520 $[M+Na]^+$, calculated for $C_{36}H_{44}O_{17}Na^+$: 771.2471. UV (MeOH) (λ_{max} / / nm (log ε)) 278 (4.22); $[a]_D^{20}$ –10° (MeOH; *c* 0.0267 g ml^{-1}).

The ESI mass spectrum of compound **1** displayed signals at *m*/*z* = 771 $[M+Na]^+$, 641 $[M–cinnamoyl+Na]^+$, and 511 $[M–2\text{-}cinnamoyl+Na]^+$ in the positive mode. Together with the ^{1}H-NMR and ^{13}C-NMR data, which showed signals of three sugar moieties and two cinnamoyl moieties, these major mass signals were indicative of a molecular formula of $C_{36}H_{44}O_{17}$. Based on one- and two-dimensional NMR experiments (Table I), the three sugar moieties were identified as two glucose and one rhamnose moiety. Linkage from the anomeric carbon of the rhamnose moiety to *O*-6 of the first glucose moiety was revealed by an HMBC crosspeak from H-1' to C-6. Likewise, an HMBC crosspeak from the anomeric proton of the second glucose moiety (H-1") to C-3' of the glucose moiety indicated linkage of the second glucose moiety to the rhamnose moiety in this position. Based on their ^{1}H-NMR coupling constants, the anomeric protons were identified as β-configured for the two glucose moieties and α-configured for the rhamnose moiety. Thus, the sugar backbone of the structure was identified as a β--D-glucopyranosyl-(1→3)-*O*-α-L-rhamnopyranosyl-(1→6)-*O*-β-D-glucopyranoside.

TABLE I. NMR data, δ / ppm, of 1,6"-di-*O*-cinnamoyl-β-glucopyranosyl-(1→3)-*O*-α-rhamnopyranosyl-(1→6)-*O*-β-D-glucopyranoside (**1**) isolated from *P. purpurea* (measured in DMSO-d_6 at 600 and 150 MHz for ^{1}H and ^{13}C, respectively; referenced to solvent residual signals and solvent signals of DMSO-d_6, ^{1}H-NMR: 2.50 ppm and ^{13}C-NMR: 39.50 ppm, respectively; coupling constants in Hz)

Position	^{1}H-NMR	^{13}C-NMR	Position	^{1}H-NMR	^{13}C-NMR
	Glucose **1**			Glucose **2**	
1	5.48 1H, *d* (7.5)	94.1	1"	4.46 1H, *d* (7.5)	104.2
2	3.24 1H, *m*[a]	72.1	2"	3.10 1H, *br t* (8.0)	73.5
3	3.30 1H, *m*[a]	75.9	3"	3.24 1H, *m*[a]	75.6
4	3.06 1H, *br t* (9.0)	69.6	4"	3.19 1H, *m*[a]	69.5
5	3.13 1H, *m*[a]	76.5	5"	3.47 1H, *m*[a]	76.1
6	3.80 1H, *m*[a]	66.9	6"	4.33 1H, *dd* (12.0, 2.0)	63.4
	3.40 1H, *m*[a]			4.25 1H, *dd* (12.0, 6.0)	
	Cinnamoyl **1**			Cinnamoyl **2**	
1"'	–	133.8	1""	–	133.9
2"'/6"'	7.41 2H, AA'BB'C	128.9	2""/6""	7.41 2H, AA'BB'C	128.9
3"'/5"'	7.70 2H, AA'BB'C	128.4	3""/5""	7.70 2H, AA'BB'C	128.4
4"'[b]	7.43 1H, AA'BB'C	130.4	4""[b]	7.43 1H, AA'BB'C	130.6
7"'	7.70 1H, *d* (16.0)	145.6	7""	7.62 1H, *d* (16.0)	144.6
8"'	6.57 1H, *d* (16.0)	117.5	8""	6.61 1H, *d* (16.0)	117.9
9"'	–	164.8	9""	–	166.2

TABLE I. Continued

Position	^{1}H-NMR	^{13}C-NMR
	Rhamnose	
1’	4.49 1H, *d* (1.5)	100.3
2’	3.83 1H, *m*[a]	69.3
3’	3.54 1H, *m*[a]	81.6
4’	3.40 1H, *m*[a]	70.5
5’	3.51 1H, *m*[a]	67.7
6’	1.14 3H, *d* (6.0)	17.8

[a]Overlapping signals; [b]signals might be exchangeable

This trisaccharide is known as a constituent of other natural products, *e.g.*, flavonoids found in tea (*Camellia sinensis* (L.) Kuntze).[3,4] Esterification of the anomeric C of the first glucose moiety was also proven by an HMBC experiment, which revealed a crosspeak from H-1 to the carbonyl moiety (C-9”’) of one of the two cinnamoyl moieties of the molecule. HMBC crosspeaks from the two protons in position 6’ of the second glucose moiety to the carbonyl (C-9””) of the second cinnamoyl moiety revealed that the second cinnamoyl moiety was attached *via* an ester linkage in this position. Conclusively, compound **1** was identified as 1,6”-di-*O*-cinnamoyl-*β*-glucopyranosyl-(1→3)-*O*-*α*-rhamnopyranosyl--(1→6)-*O*-*β*-glucopyranoside (Fig. 1). This compound represents a new natural product.

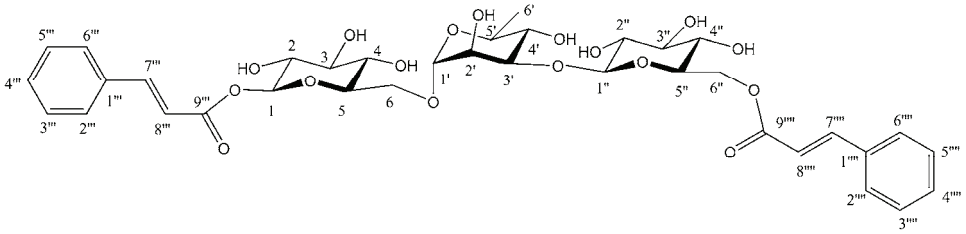

Fig. 1. 1,6”-Di-*O*-cinnamoyl-*β*-D-glucopyranosyl-(1→3)-*O*-*α*-L-rhamnopyranosyl-(1→6)-*O*-*β*-D-glucopyranoside (**1**) isolated from the leaves of *Prenanthes purpurea* L.

Additionally, extracts of flowering heads and leaves were analyzed separately for the occurrence of known phenolic acids using established protocols.[5,6] In the course of these investigations, in extracts of both leaves and flowering heads, the following caffeic acid derivatives were detected by HPLC/DAD and HPLC/MS: caffeoyltartaric acid, cichoric acid, chlorogenic acid, 3,5-dicaffeoylquinic acid, and 4,5-dicaffeoylquinic acid.

Induction of apoptosis was measured by flow cytometry in human CCRF-CEM and in human NCI-H929 cells.[7] Both after 24 h and after 48 h, compound **1** showed no cytotoxicity up to the highest concentration tested (100 μM).

EXPERIMENTAL

Plant material

Leaves of *Prenanthes purpurea* L. were collected in August 1996 NW Wieserberg, Zell/Salzburg/Austria at 1200 m above mean sea level (coordinates (WGS84): N 47°27'; E 12°46'). Voucher specimens were deposited in the herbarium of the Institut für Pharmazie (CZ-960930i) and the private herbarium of CZ.

Extraction and isolation

Air-dried, ground leaves (468 g) of *P. purpurea* were exhaustively macerated with MeOH to yield 64.1 g of crude extract after evaporation of the solvent *in vacuo*. The crude extract was re-dissolved in a mixture of MeOH and H_2O (1/2, v/v) and successively partitioned with petroleum ether 40–60 °C , EtOAc, and *n*-BuOH. The EtOAc layer was brought to dryness *in vacuo* to yield 7.95 g of residue. This residue was first fractionated by silica gel column (150 cm×2.0 cm) chromatography using a gradient of CH_2Cl_2 and MeOH. Fractions containing **1** were successively (three times) fractionated on Sephadex LH-20 using a mixture of methanol, acetone and water (3/1/1, v/v/v) as the mobile phase. Impure compound **1** (98.5 mg) was finally purified using semi-preparative RP-18 HPLC (Dionex-P580 pump, ASI-100 autosampler, UVD170U UV-detector, and Gilson-206 fraction collector; Waters (7.8 mm×100 mm) XTerra-Prep-MS-C18 column (5 μm)) using a gradient of H_2O and CH_3CN to yield 44.5 mg of **1**.

Characterization

Melting point/glass transition: Kofler hot-stage microscope, uncorrected. FTIR: Bruker IFS 25; samples were applied to a ZnSe disk and measured in the transmission mode. UV: Shimadzu U-2000 UV–Vis photometer. Optical rotation: Perkin Elmer Polarimeter 341. ESIMS and HRMS: Daltronics-Esquire-3000 (ion trap) and Finnigan-SSQ-7000 (quadrupole) mass spectrometers, respectively. NMR: Bruker Ultrashield 600 Plus.

Bioactivity

Induction of apoptosis was measured in human CCRF-CEM (T-acute lymphocytic leukemia cell line) and in human NCI-H929 (multiple myeloma cell line) cells by flow cytometry using established protocols.[7] Briefly, 0.5×10^6 cells ml^{-1} were incubated for 24 and 48 h with or without compound (1, 10, 50 or 100 μM) dissolved in DMSO. Analyses were performed in quadruplicate and appropriate solvent controls were included. The extent of apoptosis was calculated as percentage of AnnexinV/PI negative cells compared to the controls.

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ИЗВОД

НОВИ ТРИСАХАРИДИ ИЗОЛОВАНИ ИЗ *Prenanthes purpurea*

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Из метанолног екстракта лишћа биљке *Prenanthes purpurea* L. Изоловани су 1,6”-ди-*O*-цинамоил-*β*-D-глукопиранозил-(1→3)-*O*-*α*-L-рамнопиранозил-(1→6)-*O*-*β*-D-глукопиранозиди. Приказани су НМР спектри и аналитички подаци нових природних једињења.

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