Thalictricoside, a New Phenolic Compound from *Thalictrum orientale*

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From the underground parts of *Thalictrum orientale* Boiss., a new phenolic compound **1** was isolated in addition to one known cyanoglycoside, lithospermoside (**2**). For the structure elucidation of all compounds, 1D- and 2D-NMR techniques (DEPT, COSY, HMBC, HSQC) and MS (HR-MALDI) were used. The structure of the new compound was established as 2-(4′-hydroxyphenyl)-nitroethane-4′-*O*-[β-xylopyranosyl-(1→6)-β-glucopyranoside] (**1**).

Key words: Thalictrum orientale, Ranunculaceae Family, Thalictricoside, Lithospermoside

Introduction

The genus *Thalictrum* belongs to the Ranunculaceae family and is commonly known as 'meadow rue' (Schiff and Doskotch, 1970). The *Thalictrum* species are perennial herbaceous plants which are distributed in the temperate and tropical regions of the world (Bahadur and Shukla, 1983). Nine species of *Thalictrum* are known to grow in Turkey, namely; *T. aquilegifolium* L., *T. flavum* L., *T. foetidum* L., *T. isopyroides* C. A.Meyer, *T. lucidum* L., *T. minus* L., *T. orientale* Boiss., *T. simplex* L., *T. sultanabadense* Stapf, and three varieties of *T. minus* L. var. *majus* (Crantz) Crepin, var. *minus* L., and var. *microphyllum* Boiss. (Davis, 1965). Throughout the world, *Thalictrum* species have been used as stomachic, tonic, bitter, aperient, and for the treatment of snake bite, jaundice, rheumatism, etc. (Schiff and Doskotch, 1970). *Thalictrum* species have been extensively studied for their alkaloidal content. Several *Thalictrum* alkaloids have been reported to exhibit antitumor, antimicrobial, antitussive and hypotensive effects (Schiff and Doskotch, 1970). On the other hand, chemical studies on *Thalictrum* species have resulted in the isolation of cyanogenic glycosides, cycloartane glycosides, oleanane glycosides, flavone-C and O-glycosides (Sharples *et al.*, 1972; Yoshimitsu *et al.*, 1992; Yoshimitsu *et al.*, 1994; Gromova *et al.*, 1998; Wagner *et al.*, 1971).

The first study in Turkey on the alkaloids of *Thalictrum* was achieved with *T. lucidum* in Turkey by Baytop and Berghmans (1975). Baser *et al.* have studied the alkaloids of several *Thalictrum* species (Baser, 1986; Baser and Kirimer, 1987; 1988; Baser and Ertan, 1990; Ertan and Baser 1997; Kirimer and Baser, 1991). Fifty eight alkaloids including twelve new compounds have been isolated and characterized by chromatographic and spectral studies (Erdemgil *et al.*, 2001a). Recently, berberine, fuzitine and fangchinoline were isolated from *Thalictrum orientale* (Erdemgil *et al.*, 2000; 2001b). Here we report on the isolation and characterization a new phenolic compound, thalictricoside (**2**) from *T. orientale* which is known as 'Kayaotu' in Nigde, Turkey. No pharmacological study and ethnomedical use of this plant has been reported (Erdemgil, 1999).

Materials and Methods

General experimental procedures

Optical rotation was measured on a Perkin-Elmer 141 polarimeter using a sodium lamp operating at 589 nm. UV spectra were performed on a Shimadzu UV-160A spectrophotometer. NMR measurements in pyridine-d_5 and CD_3OD were performed on Bruker AMX 300 and DRX 500 spectrometers operating at 300 and 500 MHz for

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^{1}H and 75.5 MHz for ^{13}C, respectively, using the XWIN NMR software package for the data acquisition and processing. HR-MALDI mass spectra were obtained on an IonSpec Ultima FTMS spectrometer (IonSpec, California, USA) by using 2,5-dihydroxybenzoic acid (DHB) as matrix.

Plant material

T. orientale was collected from Nigde, Ulukisla, Horoz village, in Turkey in June 1996, at an altitude of a *ca.* 1000 m. Voucher specimens have been deposited at the Herbarium of Faculty of Pharmacy, Anadolu University, Eskisehir, Turkey (ESSE 13296).

Extraction and isolation

The air-dried, coarsely powdered roots and rhizomes (1 kg) of *T. orientale* were extracted with 80 % MeOH in H_2O (6 l × 2) under reflux for 8 h at 50 °C and filtered. The filtrate was concentrated to dryness *in vacuo* (260 g, yield 26 %). The extract was dissolved in H_2O (500 ml) and partitioned with $CHCl_3$ (150 ml × 4) and BuOH (750 ml × 3), respectively. The chloroform phase was evaporated to dryness *in vacuo* (1.59 g) and the butanol phase was concentrated by rotary evaporation yielding 17.0 g of butanolic extract. The butanol soluble part of the methanolic extract (17.0 g) was subjected to VLC using silica gel (Kieselgel 60, 30 g), employing $CHCl_3$, $CHCl_3$-MeOH (90:10) and $CHCl_3$-MeOH-H_2O (90:10:1→50:50:5) and MeOH as the eluents. 12 main fractions were collected (fraction A–L); A (26 mg), B (1.233 g), C (28 mg), D (148 mg), E (155 mg), F (146 mg), G (771 mg), H (948 mg), I (416 mg), J (1.554 g), K (4.142 g) and L (995 mg). Fraction I (416 mg) was applied to MPLC (column dimensions: 45 × 2.5 cm) using Lichroprep RP-18 as a stationary phase and eluted with MeOH-H_2O mixtures (10–100 %) to give eighteen fractions (fraction volume; 100 ml). Fraction G (82 mg) was subjected to a Si gel (30 g) column using (EtOAc-MeOH-H_2O) (100:10:5; 200 ml; 100:15:5, 100 ml; 100:15:10, 100 ml; 100:20:10, 100 ml; 100:30:20, 100 ml) to yield compound **1** (frs. 19–20, 8.3 mg). Fraction K (4.142 g) was subjected to MPLC (column dimensions: 460 × 26 mm) using Lichroprep RP-18 as a stationary phase and eluted with MeOH-AcN-H_20 mixtures with increasing polarity (20:5:75→80:10:10) and MeOH to yield twenty four fractions (fraction volume; 250 ml). Fraction B and C were combined (2.332 g) and was subjected to normal phase silica gel (100 g) column chromatography using $CHCl_3$-MeOH-H_2O-NH_4OH mixture (61:32:5:1, v/v/v/v) yielding compound **2** (frs. 1–6; 57.79 mg).

Thalictricoside (**1**): Amorphous colorless powder; $[\alpha]_D^{20}$ – 57.9° (*c* = 0.1, MeOH); HR-MALDIMS *m/z*: 484 $[M+Na]^+$, 413 $[M-NO_2]^+$; UV λ_{max} (MeOH, nm): 224 (2.51), 272 (2.00); ^{1}H-NMR (500 MHz, CD_3OD) and ^{13}C-NMR (CD_3OD, 75.5 MHz): Table I.

Lithospermoside (**2**): Amorphous colorless powder; $[\alpha]_D^{20}$ – 41° (*c* = 0.1, MeOH); HR-MALDIMS *m/z*: 313.1041 $[M+Na]^+$; UV λ_{max} (MeOH, nm): 207 (sh), 229, 275; IR ν_{max} (KBr, cm^{-1}) 3346 (OH), 1722 (ester C=O), 1457 (aromatic ring); ^{1}H-NMR (300 MHz, CD_3OD) and ^{13}C-NMR (CD_3OD, 75.5 MHz): Table II.

Results and Discussion

The butanol-soluble part of the methanolic extract of the underground parts of *T. orientale* was separately fractionated by vacuum liquid chromatography (VLC). After repeated chromatography (medium-pressure liquid chromatography = MPLC) of these fractions, a new compound (**1**) and the known compound lithospermoside (**2**) were isolated.

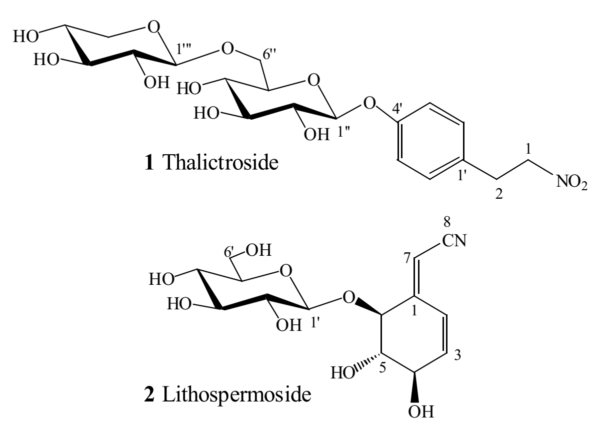

Fig. 1. N-containing glycosides (**1**, **2**) from *Thalictrum orientale.*

The positive-ion HR-MALDI of compound **1** exhibited a pseudomolecular ion peak for $[M + Na]^+$ at *m/z* 484 which is compatible with the molecular formulae $C_{19}H_{27}O_{12}N$. The UV spectrum of **1** (λ_{max} 224 and 272 nm) was characteristic for phenolic compounds. Analysis of the ^{1}H-NMR spectrum of **1** (Table I) revealed the presence of a diglycosidic structure with a phenylethyl moiety. The anomeric proton resonances at δ_H 4.32 (d, J = 7.5 Hz) and 4.87 (d, J = 7.7 Hz) suggested the presence of two sugar units in **1**. The complete assignments of all proton and carbon resonances were based on the DQF-COSY, HSQC and HMBC experiments. The DEPT and ^{13}C-NMR spectra showed a total of 19 carbons consisting of four methylene, thirteen methine and two quaternary carbon resonances. Of the carbon signals eleven were attributed to the sugar moieties. This observation led us to consider the presence of hexose and pentose units. The signals belonging to the sugar units (Table I) were assigned by the help of COSY and HSQC experiments, indicating the presence of β-glucose and β-xylose units. In the HMBC experiment, the long-range correlation between the C-6″ (δ_C 69.7) of glucose unit and the anomeric proton of xylose (H-1‴; δ_H 4.32) indicated the presence of β-xylopyranosyl-(1→6)-β-glucopyranoside. The remaining carbon and proton signals suggested the presence of a *p*-substituted phenylethyl moiety. Two aromatic signals observed as an AA′BB′ system at δ_H 7.07 and 7.19 (J_{AB}= 8.7 Hz) confirmed this assumption. Additionally, an isolated spin system observed at δ_H 3.24 and 4.67 (each t, J = 6.9 Hz) was attributed to an ethylene bridge-taking place in between a phenyl ring and a heteroatom. The connectivities between two molecular fragments were resolved by an HMBC experiment. The long-range correlations between C-4 (δ_C 158) of phenyl moiety and the anomeric proton of glucose (δ_H 4.87, H-1″) unit revealed the site of glycosidation; while C-1′ (δ_C 131.6) of the phenyl moiety showed the long-range correlations to the methylene protons (δ_H 4.67 and 3.24; H_2-1 and H_2-2, respectively) of the ethylenic fragment. Positive ion HR-MALDI mass spectrum of **1** exhibited $[M + Na]^+$ peak at

Table I. ^{1}H and ^{13}C NMR spectroscopic data and HMBC correlations for thalictricoside (**1**), (^{1}H NMR, 500 MHz; ^{13}C NMR, 75.5 MHz).*

C/H Atom	δ_C (ppm)	DEPT-135	δ_H (ppm), J [Hz]	HMBC (from C to H)
Aglycone				
1	77.5	CH_2	4.67 t (6.9)	H-2′, H-6′, H_2-2
2	33.6	CH_2	3.24 t (6.9)	H_2-1
1′	131.6	C	–	H-2′, H-6′, H_2-1 H_2-2
2′	130.8	CH	7.19 d (8.7)	
3′	118.0	CH	7.07 d (8.7)	
4′	158.1	C	–	H-1″, H-2′, H-3′, H-5′, H-6′
5′	118.0	CH	7.07 d (8.7)	
6′	130.8	CH	7.19 d (8.7)	
Glucose				
1″	102.1	CH	4.87 d (7.7)	
2″	74.8	CH	3.47[a]	
3″	77.6	CH	3.45[a]	
4″	71.4	CH	3.37 t (9.5)	
5″	77.4	CH	3.36 ddd (9.5/6.4/1.8)	
6″	69.7	CH_2	4.10 dd (11.7/1.8) 3.76 dd (11.7/6.4)	
Xylose				
1‴	105.3	CH	4.32 d (7.5)	H_2-6″$_{a,b}$, H-5‴$_{a,b}$
2‴	75.0	CH	3.19 dd (7.5/9.0)	
3‴	77.5	CH	3.27 t (9.0)	
4‴	71.2	CH	3.47 m	
5‴	66.9	CH_2	3.82 dd (11.4/5.3) 3.10 dd (11.4/10.3)	

* All proton and carbon assignment are based on 2D NMR (DQF-COSY, HSQC and HMBC).
[a] Signal patterns are unclear due to overlapping.

m/z 484.141. The fragment ion appearing at *m/z* 413.3 $[M-NO_2]^+$ suggested the presence of a NO_2 group on the ethylenic side chain. This assumption was also confirmed by the chemical shift value of one of the methylene signals observed at δ_H 4.67 (H_2-2). This chemical shift value assigned to the methylene signal next to the NO_2 group was in good agreement with those reported for aliphatic nitro derivatives (Pretsch *et al.*, 1981). Based on these results, the structure of **1** was established as 2-(4′-hydroxyphenyl)-nitroethane-4′-*O*-[*β*-xylopyranosyl-(1→6)-*β*-glucopyranoside]. For this novel structure thalictricoside was proposed as trivial name.

The spectroscopic data (Table II) for compound **2** were found to be identical with those reported for lithospermoside, a cyanoglucoside isolated previously from *T. rugosum* (Wu *et al.*, 1979).

Table II. ^1H and ^{13}C NMR spectroscopic data and HMBC correlations for lithospermoside (**2**) (^1H NMR, 500 MHz; ^{13}C NMR, 75.5 MHz).*

C/H Atom	δ_C (ppm)	DEPT-135	δ_H (ppm), *J* [Hz]	HMBC (from C to H)
Aglycone				
1	156.4	C	–	H-2, H-3, H-5, H-6, H-7,
2	126.6	CH	6.22 br d (9.9)	H-3, H-6, H-7
3	139.7	CH	6.35 dd (9.9/2.3)	H-2
4	71.7	CH	4.72 br s	H-2
5	76.1	CH	4.49[a]	H-6
6	77.4	CH	5.28 d (8.0)	H-1′, H-2, H-7
7	97.1	CH	5.60 br s	H-2, H-6
8	117.9	C	–	H-2, H-6, H-7
Glucose				
1′	104.4	CH	5.67 d (7.7)	H-6
2′	75.2	CH	4.38 dd	
3′	78.5	CH	4.24[a]	
4′	71.5	CH	4.23[a]	
5′	78.6	CH	3.98 m	
6′	63.1	CH_2	4.55 m 4.35 m	

* All proton and carbon assignment are based on 2D NMR (DQF-COSY, HSQC and HMBC).
[a] Signal patterns are unclear due to overlapping.

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