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Phenylpropanoid Glycosides from *Linum olympicum* (Linaceae)

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The lignan content of *Linum olympicum* Boiss. was investigated by HPLC and GC-MS in comparison with standards, and 2 phenylpropanoid glycosides, syringin **(1)** and coniferin **(2)**, were isolated from the aerial parts. Identification of the 2 compounds was achieved by comparison with standards using TLC and HPLC and the structures of the isolated compounds were established by UV and ^{1}H-NMR analysis.

Key Words: Coniferin, Linaceae, *Linum olympicum*, Phenylpropanoid glycosides, Syringin

Introduction

The genus *Linum* belongs to the family Linaceae and comprises about 200 species mainly distributed in the Mediterranean region. This genus is represented by 39 species in Turkey[1,2]. To date, lignans, especially aryltetralin group lignans, have been isolated from Turkish *Linum* species. Syringin **(1)** and coniferin **(2)** are phenylpropanoid glycosides and in previous studies coniferin **(2)** was isolated from *L. flavum* and *L. capitatum*[3], and syringin **(1)** and coniferin **(2)** were isolated from *L. flavum* var. *compactum*[4]. In this report we describe the isolation and structural identification of the phenylpropanoid glycosides syringin **(1)** and coniferin **(2)** from *L. olympicum* Boiss., which is an endemic species. The lignan content of this species was investigated by HPLC and GC-MS in comparison with standards. This is the first phytochemical study on *L. olympicum*.

Experimental

General Experimental Procedures: NMR measurements in methanol-*d* were recorded on a Bruker DRX 500 spectrometer operating at 500 MHz for ^{1}H. UV (λ_{max}) spectra were recorded on a Shimadzu UV-160 spectrometer. GC-MS analyses were performed on a Unicam 610 GC-MS (column: WCOT fused-silica CP-Sil 5 CB (15 mm x 0.31 mm i.d., film thickness, 0.25 μm; Chrompack; Middelburg; the Netherlands)). HP 1100 was used for HPLC analyses (column: C-18-RP 5 μm, 25 cm x 4.6 mm i.d.). Silica gel 60 GF_{254}

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(Merck) was used for separation on preparative TLC and plates were coated with 0.5 mm thickness silica. TLC analyses were carried out on pre-coated silica gel 60 F_{254} aluminium sheets (Merck). Compounds were detected by UV and by spraying HNO_3: acetic acid (3:10) followed by heating.

Plant Materials: *L. olympicum* was collected from Uludağ, Bursa, 2245 m, in Turkey in September 2002 and identified by Prof. Hayri Duman from the Department of Biology (Gazi University). A voucher specimen (AEF no: 22951) has been deposited in the herbarium of the Department of Pharmaceutical Botany, Faculty of Pharmacy (University of Ankara).

Extraction and Isolation

The dried aerial parts (284.73 g) of *L. olympicum* were powdered and extracted 3 times with MeOH (1.5 L x 3) at 70 °C. The combined MeOH extract was evaporated under reduced pressure to give a crude extract (43.76 g). The crude MeOH extract (11.94 g) was chromatographed by preparative TLC on silica gel layers using CH_2Cl_2:MeOH (85:15) as the solvent system to give a glycoside band. The glycoside band was eluted with Me_2CO:MeOH (1:1) and was then rechromatographed by preparative TLC on silica gel layers with $CHCl_3$:MeOH (5:1)[5]. Further purification of each compound was carried out by preparative TLC using $CHCl_3$:MeOH (5:1), EtOAc:MeOH:H_2O (100:16.5:13.5) as solvent systems to yield syringin **(1)** (42 mg) and coniferin **(2)** (35 mg).

GC-MS Analysis

The lignan content of *L. olympicum* was investigated by HPLC and GC-MS. Samples for GC-MS were prepared from air-dried parts of *L. olympicum* as reported in the literature[6]. Polygamain, hinokinin, isojusticidin, morelensin, bursehernin, podophyllotoxin, 6-methoxypodophyllotoxin, β-peltatin and β-peltatin-A-methylether were used as standards. The oven temperature program was 150 to 320 °C at 15 °C/min and 320 °C maintained for 5 min. The injector temperature was 260 °C. The carrier gas used was helium with a split ratio of 20:1. The injected volume was 4 μL. To obtain mass spectra, electron impact ionization (70eV) was used with an ion source temperature of 250 °C, interface temperature of 280 °C, a scan speed of 2 scans/s and mass range of 34-600 u. The acquired data were stored and afterwards analysed with Lucy Display V2.70 software[6].

None of these compounds were detected in *L. olympicum*.

HPLC Analysis

Three different samples were prepared from *L. olympicum* as reported in the literature for podophyllotoxin and 6-methoxypodophyllotoxin analyses with HPLC. For elution we used gradient system acetonitrile-water[7−10].

This study shows that these compounds were not detected in *L. olympicum*.

Results

The known lignans, used as standards for HPLC and GC-MS analysis, were not detected in *L. olympicum*. However, 2 phenylpropanoid glycosides, syringin **(1)** and coniferin **(2)** (Figure), were isolated from this

plant. Identification of the 2 compounds was achieved by comparison with standards on TLC and HPLC and structural elucidation based on ^{1}H-NMR analysis.

Syringin **(1)**: $\lambda_{max.}$(MeOH) nm 236, 281; ^{1}H NMR (500 MHz, methanol-d): Table 1

Coniferin **(2)**: $\lambda_{max.}$(MeOH) nm 228, 273; ^{1}H NMR (500 MHz, methanol-d): Table 2

Table 1. ^{1}H NMR data of syringin.

Proton	δ (ppm) J (Hz)
2, 6 (2H)	6.66 s
1′	6.42 ddd(dt) (1.5, 1.5, 16)
2′	6.25 ddd(dt) (5, 5, 16)
1″	4.66 d (7)
3′(2H)	4.10 br dd
Ome at C-3 and C-5(6H)	3.74 s
6″a	3.65 br dd (2, 11)
6″b	3.53 br dd (6, 11)
2″-5″	3.1-3.4 m.

Table 2. ^{1}H NMR data of coniferin.

Proton	δ (ppm) J (Hz)
5	7.12 d (8)
2	7.11 d (2)
6	6.93 dd (2, 8)
1′	6.55 ddd(dt) (1.5, 1.5, 16)
2′	6.32 ddd(dt) (5, 5, 16)
1″	4.93 d (7)
3′ (2H)	4.22 dd (1.5, 5)
Ome at C-3	3.88 s
6″a	3.875 dd (2, 12)
6″b	3.70 dd (5, 12)
2″-5″	3.3-3.6 m.

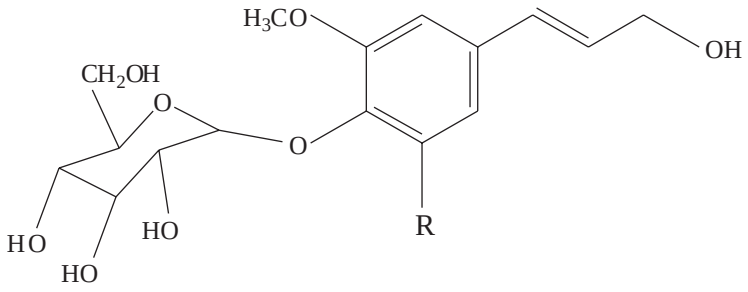

Figure. Phenypropanoid glycosides from *L. olympicum*.

Discussion

In previous studies on Turkish *Linum* species, lignans, especially aryltetralin group lignans, and phenylpropanoids were isolated. Phytochemical studies are continuing on this genus. This is the first phytochemical study on *L. olympicum*. The known lignan compounds, which were used as standards, were not detected in *L. olympicum* but 2 phenylpropanoid glycosides were isolated. Syringin is the methoxy analogue of

coniferin. Coniferyl alcohol, which is the aglycone of coniferin, is known to be a biosynthetic precursor of lignans[3]. Two coniferyl alcohol moieties dimerise in early steps in lignan biosynthesis[11,12]. Further studies on *L. olympicum* will be performed.

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