PERGAMON

Phytochemistry 50 (1999) 297–301

PHYTOCHEMISTRY

Caffeic acid esters of phenylethanoid glycosides from *Fraxinus ornus* bark

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Revised 17 July 1998

Abstract

A new phenylethanoid glucoside, 2-(3,4-dihydroxyphenyl)-ethyl-*O*-β-D-glucopyranosyl-(1 → 6)-3-*O*-*trans*-caffeoyl-β-D-glucopyranoside, named isolugrandoside, was isolated from *Fraxinus ornus* bark, together with the five known phenylethanoid glycosides 2-(4-hydroxyphenyl)-ethyl-(6-*O*-caffeoyl)-β-D-glucopyranoside, calceolarioside B, verbascoside, isoacteoside and lugrandoside. Isomerization of lugrandoside to isolugrandoside was not found under the employed conditions of isolation and purification.

Keywords: Fraxinus ornus L; Oleaceae; Phenylethanoid glycosides; 2-(4-Hydroxyphenyl)-ethyl-(6-*O*-caffeoyl)-β-D-glucopyranoside; Calceolarioside B; Verbascoside; Isoacteoside; Lugrandoside; Isolugrandoside

1. Introduction

It is known that the Oleaceae family is a rich source of secoiridoids and phenylethanoid glycosides (PhGs). Many cinnamic esters of PhGs of diverse structures have been isolated from the genera *Syringa*, *Forsythia*, *Ligustrum*, *Jasminum* and *Osmanthus* (Jimenez & Riguera, 1994). However, only the occurrence of verbascoside and calceolarioside B in *Fraxinus* species has been reported so far (Damtoft, Franzyk, & Jensen, 1992; Shen, Chen, & Lee, 1993; Kostova, Yossifova, Vassileva, & Mikhova, 1993). In a previous work we reported on the isolation of calceolarioside B from *F. ornus* leaves (Kostova et al., 1993). In this paper we describe the isolation of six caffeic acid esters of PhGs **1**–**6** from *Fraxinus ornus* bark and the structure elucidation of isolugrandoside **6**, which is a novel compound.

2. Results and discussion

Six caffeic acid esters of PhGs were isolated from the polar part of the EtOH extract of *F. ornus* bark. Five of them were identified as the known 2-(4-hydroxyphenyl)-ethyl-(6-*O*-caffeoyl)-β-D-glucopyranoside (**1**), calceolarioside B (**2**), verbascoside (**3**), isoacteoside (**4**) and lugrandoside (**5**) on the basis of their NMR and APCI-MS spectra, and comparison with literature data (Shimomura, Sashida, & Adachi, 1987; Andary, Wylde, Laffite, Privat, & Winternitz, 1982; Mayase et al., 1982; Baudouin, Skaltsounis, Tillequin, & Koch, 1988). The occurrence of **1** and **5** in Oleaceae has not been reported so far.

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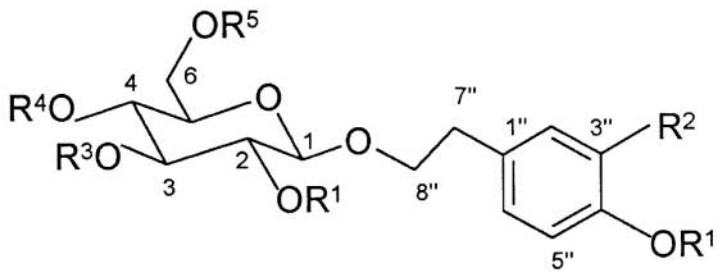
0031-9422/98/$ - see front matter
PII: S0031-9422(98)00538-X



	R^1	R^2	R^3	R^4	R^5
1	H	H	H	H	Caff
2	H	OH	H	H	Caff
3	H	OH	Rha	Caff	H
4	H	OH	Rha	H	Caff
5	H	OH	H	Caff	Glc
6	H	OH	Caff	H	Glc
6a	Ac	OAc	Caff.2Ac	Ac	Glc.4Ac

Rha = α-L-rhamnopyranosyl

Glc = β-D-glucopyranosyl

Caff = caffeoyl

The structure of the novel PhG-ester **6**, named isolugrandoside, was deduced by concerted application of 1D and 2D NMR methods and MS studies.

A molecular formula of $C_{29}H_{36}O_{16}$ was established for **6** on the basis of its negative HRFAB-MS ($[M-H]^-$ at m/z 639.1950) and the ^1H, ^{13}C, and DEPT spectra. The UV spectrum (EtOH) exhibited absorption maxima at 222, 255 (sh), 292 and 328 nm. The IR (KBr) spectrum showed bands at 3398 (hydroxy groups), 1700 (C=O in conjugated esters), 1628 (C=C in α,β-unsaturated acid derivatives) and 1604, 1522 cm^{-1} (aromatic ring).

The ^1H-NMR spectrum of **6** revealed the signals typical of a *trans*-caffeoyl ester unit, a 3,4-dihydroxyphenethyl unit and two β-glucose units (anomeric protons at δ 4.51 (d, $J = 7.8$ Hz) and δ 4.43 (d, $J = 7.8$ Hz)). The detailed analysis of ^1H-NMR, COSY, 1D-TOCSY, ^{13}C-NMR, GHSQC and HMBC spectra of **6** fixed the positions of the ester and glucosidic linkages to one central β-glucopyranosyl moiety (Glc-1). The HMBC correlation from H-1 (δ 4.51) to C-8″ (δ 72.9) gave evidence for the attachment of the 3,4-dihydroxyphenethoxy moiety to C-1 of the central glucose unit, as usual in the PhGs (Jimenez & Riguera, 1994).

The position of linkage of the terminal glucopyranosyl moiety (Glc-2) at C-6 of the central glucose unit was deduced from the ^{13}C NMR spectrum where the resonance of C-6 and C-5 were observed at δ 69.9 and 76.9, respectively. These chemical shifts were in line with literature data for C-6 glycosylated sugars

(Jimenez & Riguera, 1994). The HMBC correlation from the anomeric proton 1‴ (δ 4.43) to C-6 (δ 69.9) indicated that the interglucosidic linkage is between C-1‴ of Glc-2 and C-6 of Glc-1.

The downfield triplet at δ 5.06 (1H, $J = 9.0$ Hz), assigned to H-3 on the basis of 1D-TOCSY and COSY spectra, suggested that the position of the *trans*-caffeoyl unit is at C-3 of the central glucose unit. As expected, the acylated carbon C-3 was also deshielded and appeared at δ 79.7 (Jimenez & Riguera, 1994). The position of this ester linkage was further confirmed by the HMBC correlation from H-3 to C-9′.

Acid hydrolysis of a mixture of **5** and **6** produced caffeic acid, 3,4-dihydroxyphenethyl alcohol and D-glucose. The identification of D-glucose including its absolute configuration was conducted according to the procedure of Oshima, Yamauchi and Kumanotani (1982).

Based on these data the structure of isolugrandoside **6** was unambiguously established as 2-(3,4-dihydroxyphenyl)-ethyl-*O*-β-D-glucopyranosyl-(1 → 6)-3-*O*-*trans*-caffeoyl-β-D-glucopyranoside.

The proposed structure of **6** was in full agreement with the ^{1}H NMR spectrum of its decaacetate **6a** which exhibited in particular the singlets of four aromatic and six aliphatic acetyl groups (Table 1).

Our studies showed that the only difference between compounds **5** and **6** is in the position of the caffeoyl ester units i.e. isolugrandoside (**6**) is a positional isomer of lugrandoside (**5**) (Baudouin et al., 1988). However, our experimental data excluded the isomerization of **5** to **6** during the isolation and purification procedure and confirmed the natural occurrence of isolugrandoside (**6**).

Table 1
^{1}H and ^{13}C NMR data of isolugrandoside **6** and isolugrandoside acetate **6a**

Moiety	Position	**6**[a]		**6a**[b], δ_H (ppm) (J (Hz))
		δ_H (ppm) (J (Hz))	δ_C (ppm)	
Glc-1	1	4.51 d (7.8)	104.5	4.50 d (8.57)
	2	3.44 t-like (9.0)	73.6	5.08 t (9.0)
	3	5.06 t (9.0)	79.7	5.30 t (9.0)
	4	3.64 m	69.8	4.97 t (9.0)
	5	3.62 m	76.9	3.72 m
	6a	3.86 dd (11.3, 4.6)	69.9	3.64 m
	6b	4.18 br d (11.3)	–	3.89 br d (10.9)
Caffeoyl	1′	–	128.3	–
	2′	7.12 d (1.9)	115.9	7.34 d (2.0)
	3′	–	146.8	–
	4′	–	149.6	–
	5′	6.85 d (8.2)	117.9	7.22 d (8.5)
	6′	7.02 dd (8.2, 1.9)	123.7	7.38 d (8.5, 2.0)
	7′	7.62 d (15.9)	147.7	7.58 d (16.0)
	8′	6.37 d (15.9)	115.8	6.28 d (16.0)
	9′	–	170.0	–
Phenethyl alcohol	1″	–	132.2	–
	2″	6.76 (2.0)	117.3	7.08 br s
	3″	–	144.6	–
	4″	–	146.1	–
	5″	6.75 d (8.1)	117.2	7.06 d (8.0)
	6″	6.64 dd (8.1, 2.0)	122.2	7.06 br d (8.0)
	7″	2.82 t, 2H, (7.1)	36.6	2.90 m
	8″a	3.79 dt (9.7, 7.1)	72.9	3.65 m
	8″b	4.05 dt (9.7, 7.1)		4.12 m
Glc-2	1‴	4.43 d (7.8)	104.9	4.58 d (8.14)
	2‴	3.28 t-like (9.0)	75.2	5.00 m
	3‴	3.42 t (9.0)	78.1	5.21 t (9.0)
	4‴	3.35 m	71.7	5.08 m
	5‴	3.30 m	78.0	3.67 m
	6‴a	3.70 dd (11.6, 5.2)	62.8	4.14 br d (12.6)
	6‴b	3.89 dd (11.9, 1.9)	–	4.28 dd (12.6, 4.8)
	CH_3COO–Ph	–	–	2.32, 2.31, 2.29, 2.28
	CH_3COO–Glc	–	–	2.10, 2.04, 2.02, 2.00, 1.98, 1.91

[a]Measured in CD_3OD.[b]Measured in $CDCl_3$.

The *trans–cis* isomerization known for caffeoyl esters was also observed for compounds **1**–**6** leading to an equilibrium at 70% for the *trans* form (Jimenez & Riguera, 1994).

3. Experimental

3.1. General experimental procedures

^{1}H and ^{13}C spectra were obtained on a Varian Unity Inova spectrometer operating at 500 (^{1}H NMR) and 75 MHz (^{13}C NMR). All experiments, HH-DQFCOSY, HH-LR-COSY, GHSQC, HMBC, NOESY, were implemented using the standard Varian pulse library. Spectra were obtained under temperature control at 24.5°C or 25°C with a 5 mm PFG gradient inverse detection probe (500 MHz).

Negative APCI-MS was carried out on a Finnigan TSQ 700. Negative HRFAB-MS was obtained on Jeol MStation JMS-700, NBA as a matrix. Liquid vacuum chromatography (LVC): silica gel LS 5–40 μ (Chemapol). Anal. HPLC: Spherisorb ODS2 (2 μm, 125 × 4.6 mm). Prep. HPLC: LiChrospher 100, RP-18 (10 μm, 250 × 16 mm), UV detector at 236 nm.

3.2. Plant material

A commercial sample of *Fraxinus ornus* L. bark collected in 1991 in the region of Dragoman, Bulgaria, was investigated. The plant material was authenticated by Dr. A. Mitrev and a voucher specimen (No. SOM 153320) is deposited in the Herbarium of the Institute of Botany, BAS, Sofia.

3.3. Extraction and isolation

Dried and well-ground bark (1 kg) was extracted with hot EtOH (3 × 7 l). The insoluble material was removed by filtration and the extract was concentrated under reduced pressure at temperature 40°C to a small volume (200 ml). After filtration of the deposited esculin (30.0 g), the mother liquor was concentrated (40°C, reduced pressure) and subjected to a solvent–solvent partitioning using petroleum ether and EtOAc to afford R-1 (20.0 g) and R-2 (50.0 g), respectively. R-2 (6.2 g) was further worked up by LVC over 70 g silica gel, using dichloroethane (DCE) and DCE–MeOH with increasing polarity (10:1, 5:1, 3:1). Fractions eluted with DCE:MeOH (5:1) were combined and concentrated under reduced pressure to give subfractions R-3 (840 mg), R-4 (940 mg) and R-5 (320 mg). Fractions eluted with DCE:MeOH (3:1) were combined to give subfractions R-6 (130 mg) and R-7 (90 mg). R-5 (160 mg) was subjected to prep. HPLC using a gradient MeOH–H_2O (20 min 30% MeOH, 10 min to 40% MeOH, 15 min 40% MeOH) to give esculin (47.0 mg), fraxin (10.0 mg), 2-(4-hydroxyphenyl)-ethyl-(6-*O*-caffeoyl)-β-D-glucopyranoside (**1**, 4.5 mg) and calceolarioside B (**2**, 45.0 mg). R-6 subjected to prep. HPLC with 40% MeOH gave verbascoside (**3**, 25.0 mg) and isoacteoside (**4**, 18.0 mg). R-7 was subjected to prep. HPLC with 28% MeOH to give isolugrandoside (**6**, 5.6 mg) and lugrandoside (**5**, 4.8 mg).

3.4. Isolugrandoside (*6*)

Amorphous powder, $[\alpha]_D^{25^\circ C}$ −16.6° (*c* 0.06, MeOH). IR ν_{max}^{KBr} (cm^{-1}): 3398, 1700, 1628, 1604, 1522, UV: λ_{max}^{MeOH} (log ε) (nm): 222 (4.44), 255 (sh, 4.00), 292 (4.01), 328 (4.05); negative ion HRFAB-MS: *m*/*z* 639.1950 $[M-H]^-$; $C_{29}H_{35}O_{16}$ requires 639.1914. ^{1}H and ^{13}C NMR see Table 1.

Lugrandoside (**5**) was subjected to the above described procedure used for isolation of **5** and **6**. No isomerization from **5** to **6** was found at any experimental step – boiling with EtOH, solvent–solvent partitioning, treatment with silica gel and HPLC using RP18 column.

3.5. Isolugrandoside acetate (*6a*)

Isolugrandoside (**6**) (2 mg) was acetylated with pyridine–Ac_2O (1:1) at room temperature for 24 h. The product was purified on silica gel using Et_2O–toluene (2:1) to give **6a** (1.2 mg). ^{1}H NMR see Table 1.

3.6. Acid hydrolysis of mixture of *5* and *6*

A soln of a mixture of **5** and **6** (8 mg) in 3 ml 2 N TFA was refluxed at 100°C for 3 h. The reaction mixture was extracted with EtOAc. The EtOAc extract was proven to contain caffeic acid and 3,4-dihydroxyphenethyl alcohol by direct TLC comparison with authentic samples. D-Glucose was found as the only sugar in the water part following the procedure of Oshima, Yamauchi and Kumanotani (1982).

Acknowledgements

T. Iossifova thanks Deutscher Akademischer Austauschdienst (DAAD) for a scholarship. We are obliged to Ms. S. Reeb, University of Hohenheim, Stuttgart, Germany, for the NMR measurements and

to Mr. Norbert Nieth, University of Heidelberg, Germany, for the HR-MS.

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