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SECOIRIDOID GLUCOSIDES FROM *FRAXINUS ORNUS* BARK

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Key Word Index—*Fraxinus ornus*; Oleaceae; secoiridoid glucosides; hydroxyframoside A; hydroxyframoside B; framoside; hydroxyornoside; ligstroside; oleuropein; l-hydroxypinoresinol glucoside.

Abstract—Two novel secoiridoid glucosides, hydroxyframoside A and hydroxyframoside B, were isolated as a mixture from the ethanolic extract of *Fraxinus ornus* bark, together with the known secoiridoids hydroxyornoside, ligstroside, framoside and oleuropein, and the lignan l-hydroxypinoresinol glucoside. The structures of the new compounds were elucidated on the basis of spectral data. © 1998 Elsevier Science Ltd. All rights reserved

INTRODUCTION

Our previous studies [1, 2] on the chemical composition of *Fraxinus ornus* bark resulted in the isolation of ligstroside (**1**) and the structurally new type macrocylic secoiridoids insularoside and hydroxyornoside (**2**). The occurrence of these interesting compounds prompted us to continue our investigations on the same plant species. Here, we describe the isolation and structure elucidation of two new secoiridoid glucosides named hydroxyframoside A (**3a**) and hydroxyframoside B (**3b**) together with the secoiridoids oleuropein (**4**) and framoside (**5**), and the lignan l-hydroxypinoresinol glucoside (**6**).

RESULTS AND DISCUSSION

A fraction of the ethanolic extract of *F. ornus* bark was worked up as described in the Experimental to give ligstroside (**1**), hydroxyornoside (**2**), the two new compounds hydroxyframoside A (**3a**) and hydroxyframoside B (**3b**), and oleuropein (**4**), framoside (**5**), and the l-hydroxypinoresinol glucoside (**6**) [3]. This is the first report for the presence of **4**–**6** in *F. ornus*.

The new secoiridoid glucosides hydroxyframoside A (**3a**) and hydroxyframoside B (**3b**) were isolated as a mixture **3a, b** in a ratio 2:1 as suggested by their ^{1}H and ^{13}C NMR spectra. The molecular formula $C_{32}H_{38}O_{14}$ was established for **3a** and **3b** on the basis of their ^{1}H and ^{13}C NMR data (Tables 1 and 2) and the negative ESIMS, where only one peak at m/z 645 was found for the $[M–H]^-$ ion in both compounds. The structure elucidation of **3a** and **3b**, and the complete assignment of all carbons and protons in their molecules were achieved by detailed 1D (^{1}H, TOCSY, ^{13}C) and 2D (COSY, HH-LR-COSY, GHSQC, HMBC, NOESY) NMR experiments (Tables 1 and 2, Fig. 1, and Experimental). The two compounds exhibited the same NMR pattern, almost identical coupling constants for all protons of the phenethoxy and oleoside moieties, but small differences for the corresponding chemical shifts. No differences for the glucosidic signals were observed. Except for the aromatic systems, the data were very similar to those of framoside (**5**) (Tables 1 and 2) [4] and jasmultiside (**7**) [5].

The ^{1}H NMR spectrum of **3a, b** revealed the presence of four methylene groups, one A_2B_2 and one AMX aromatic system for hydroxyframoside A as well as for hydroxyframoside B. This suggested that each of the isomeric compounds had one 4-hydroxyphenethoxy and one 3,4-dihydroxyphenethoxy unit. The exact position of their attachment to the oleoside nucleus of **3a** and **3b** was deduced on the basis of HMBC, HH-LR-COSY and NOESY spectra (see Experimental and Fig. 1). The heteronuclear long range correlations from the methylene protons at δ 4.10 (1H, *dt*, $J = 10.71$ and 7.07 Hz) and δ 4.21 (1H, *dt*, $J = 10.71$ and 7.07 Hz) of isomer **3a** to the carbon signal at δ 172.45 (assigned to C-7), and from the methylene protons at δ 4.09 (1H, *dt*, $J = 10.70$ and 7.17 Hz) and δ 4.22 (1H, *dt*, $J = 10.70$ and 7.17 Hz) of isomer **3b** to the carbon signal at δ 172.50 (C-7)

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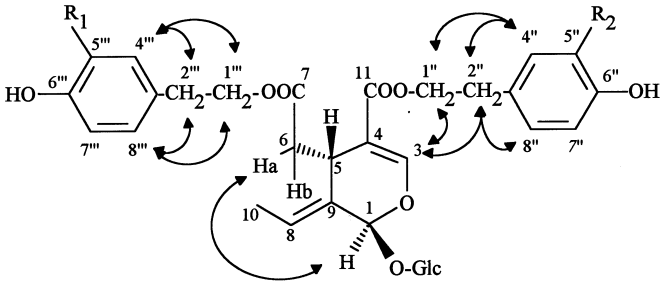


	R_1	R_2
3a	OH	H
3b	H	OH

Fig. 1. Important NOEs observed for **3a** and **3b**.

Table 1. ^{1}H NMR data of hydroxyframoside A (**3a**), hydroxyframoside B (**3b**) and framoside (**5**) in CD_3OD

H	3a	3b	5
1	5.90 *brs*	5.90 *brs*	5.90 *brs*
3	7.47 *s*	7.48 *s*	7.46 *s*
5	3.94 *dd* (9.40, 4.45)*	3.94 *dd* (9.40, 4.61)	3.94 *dd* (9.45, 4.20)
6a	2.39 *dd* (14.22, 9.40)	2.39 *dd* (14.22, 9.40)	2.38 *dd* (14.16, 9.45)
6b	2.64 *dd* (14.22, 4.45)	2.65 *dd* (14.22, 4.61)	2.63 *dd* (14.16, 4.20)
8	6.08 *qd* (7.10, 1.00)	6.07 *qd* (7.05, 1.00)	6.07 *qd* (6.95, 0.97)
10	1.66 *dd* (7.10, 1.48)	1.64 *dd* (7.05, 1.48)	1.65 *dd* (7.00, 1.35)
1′	4.80 *d* (7.82)	4.80 *d* (7.82)	4.80 *d* (7.81)
2′	3.2–3.4 obscured by MeOH	3.2–3.4 obscured by MeOH	3.2–3.4 obscured by Me
3′	3.41 *t* (8.83)	3.41 *t* (8.83)	3.42 *t* (8.91)
4′	3.2–3.4 obscured by MeOH	3.2–3.4 obscured by MeOH	3.2–3.4 obscured by Me
5′	3.2–3.4 obscured by MeOH	3.2–3.4 obscured by MeOH	3.2–3.4 obscured by Me
6′a	3.68 *dd* (11.87, 5.50)	3.68 *dd* (11.87, 5.50)	3.68 *dd* (12.09, 5.74)
6′b	3.89 *dd* (11.87, 1.70)	3.89 *dd* (11.87, 1.70)	3.89 *dd* (12.09, 1.83)
1″a	4.27 *dt* (10.80, 6.74)	4.27 *dt* (10.80, 6.74)	4.27 *dt* (10.82, 6.71)
1″b	4.31 *dt* (10.80, 6.74)	4.31 *dt* (10.80, 6.74)	4.30 *dt* (10.82, 6.71)
2″	2.87 2H, *t* (6.74)	2.81 2H, *t* (6.71)	2.87 2H, *t* (6.71)
4″	7.07 *d* (8.60)	6.68 *d* (2.02)	7.07 *d* (8.54)
5″	6.71 *d* (8.60)	—	6.71 *d* (8.54)
7″	6.71 *d* (8.60)	6.69 *d* (8.02)	6.71 *d* (8.54)
8″	7.07 *d* (8.60)	6.56 *dd* (8.02, 2.02)	7.07 *d* (8.54)
1‴a	4.10 *dt* (10.71, 7.07)	4.09 *dt* (10.70, 7.17)	4.10 *dt* (10.75, 7.08)
1‴b	4.21 *dt* (10.71, 7.07)	4.22 *dt* (10.70, 7.17)	4.23 *dt* (10.75, 7.08)
2‴	2.77 2H *t* (7.07)	2.82 2H *t* (7.17)	2.82 2H *t* (7.08)
4‴	6.67 *d* (2.09)	7.05 *d* (8.55)	7.05 *d* (8.55)
5‴	—	6.72 *d* (8.55)	6.72 *d* (8.55)
7‴	6.70 *d* (8.02)	6.72 *d* (8.55)	6.72 *d* (8.55)
8‴	6.55 *dd* (8.02, 2.09)	7.05 *d* (8.55)	7.05 *d* (8.55)

*(*J* in Hz)

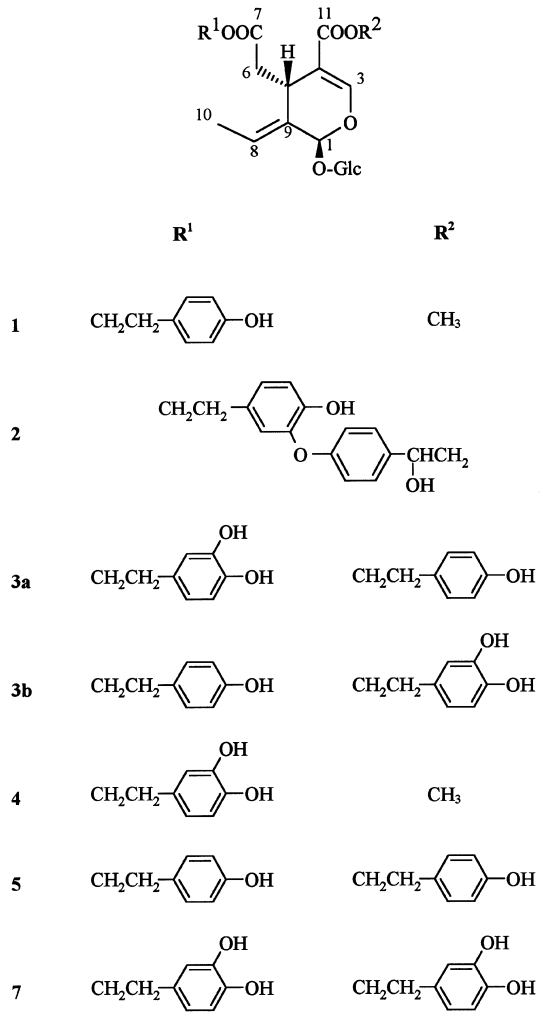
placed the corresponding CH_2 groups at position 1‴ in **3a** and **3b**, respectively.

In the COSY spectrum the CH_2-1‴ protons of **3a** and **3b** showed coupling cross peaks to the proton resonances of the methylene groups at δ 2.77 (2H, *t*, $J =$ 7.07 Hz) for **3a** and δ 2.82 (2H, *t*, $J =$ 7.17 Hz) for **3b**, assigned to the respective CH_2-2‴ in both compounds. The presence of the cross peaks δ 2.77 (CH_2-2‴)/δ 6.67 (1H, *d*, $J =$ 2.09 Hz) and δ 2.77 (CH_2-2‴)/δ 6.55 (1H, *dd*, $J =$ 8.02 and 2.09 Hz) for **3a** and δ 2.82 (CH_2-2‴)/δ 7.05 (2H, *d*, $J =$ 8.55 Hz) for **3b** in the HH-LR-COSY spectrum of **3a, b** indicated the linkage of one 3,4-dihydroxybenzene ring to CH_2-2‴ in **3a** and one 4-hydroxybenzene ring to the same

Table 2. ^{13}C NMR data of hydroxyframoside (**3a**) hydroxyframoside (**3b**) and framoside (**5**) in CD_3OD

C	3a	3b	5
1	94.51	94.45	95.14
3	154.39	154,36	155,10
4	108.84	108.86	109.53
5	31.04	31.07	31.75
6	40.47	40.47	41.16
7	172.45	172.50	173.15
8	124.14	124.14	124.85
9	130.02	130.02	130.42
10	12.88	12.86	13.58
11	167.47	167.47	168.14
1′	100.19	100.15	100.83
2′	74.06	74.06	74.78
3′	77.71	77.74	77.95
4′	70.77	70.80	71.50
5′	77.25	77.25	78.45
6′	62.03	62.06	62.77
1″	65.67	65.63	66.47*
2″	34.57	34.46*	35.27†
3″	129.72*	129.76†	130.21‡
4″	130.25	116.29	130.97§
5″	115.54	145.53	116.30
6″	156.34	144.22	157.17
7″	115.54	115.66	116.30
8″	130.25	120.58	130.97§
1‴	66.17	66.17	66.87*
2‴	34.70	34.82*	35.17†
3‴	129.52*	129.33†	130.01‡
4‴	116.36	130.29	131.00§
5‴	145.53	115.60	116.30
6‴	144.23	156.34	157.17
7‴	115.75	115.60	116.30
8‴	120.62	130.29	131.00§

*, †, ‡, § Values with the same superscript are interchangeable.



position in **3b**. These data unambiguously proved the substitution at C-7 in the two compounds: one 3,4-dihydroxyphenethoxy unit in **3a** and one 4-hydroxyphenethoxy unit in **3b**.

Furthermore, the HMBC, COSY and HH-LR-COSY spectra of **3a, b** gave evidence for the attachment of one 4-hydroxphenethoxy unit in **3a** and one 3,4-dihydroxyphenethoxy unit in **3b** to C-11 of their oleoside moieties.

The proposed arrangements of **3a** and **3b** were further confirmed by the following cross peaks in the NOESY spectrum of **3a, b**: for **3a**: H-3/H-2″ and H-2″/H-4″, 8″ (δ 7.07, 2H, *d*, $J = 8.60$ Hz), and for **3b**: H-3/H-2″ and H-2″/H-4″ (δ 6.68, 1H, *d*, $J = 2.02$ Hz), and H-2″/H-8″ (δ 6.56, 1H, *dd*, $J = 8.02$ and 2.02 Hz).

Therefore, the structures of the isomeric hydroxyframoside A and hydroxyframoside B have been confirmed to be 7-(3,4-dihydroxyphenethoxy)-11-(4-hydroxyphenethoxy)-oleoside (**3a**) and 7-(4-hydroxyphenethoxy) - 11 - (3,4 - dihydroxyphenethoxy) - oleoside (**3b**), respectively.

EXPERIMENTAL

General experimental procedures

^1H and ^{13}C NMR: 500 MHz (^1H NMR) and 125 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° or 25° with a 5 mm-PFG gradient inverse detection probe. GHSQC: 128 experiments, 2048 data points, FT size 8192×2048. HMBC: optimised for long-range couplings of 5 Hz and 12 Hz. 128 experiments, 2048 data points, FT size 8192×2048. NOESY: mixing time 1 s, 360 experiments, 2048 data points, FT size 4096×4096. HH-DQFCOSY: 330 experiments, 2048 data points, FT size 4096×4096. MS: ESI negative ion, Finnigan TSQ 700, 3.5 kV, flow 5 μl/min, solvent MeOH. CC: silica gel 60, Merck. LVC: silica gel LS 5–40 μ (Chemapol). Prep. HPLC: RP-18 (10 μm), LiChrospher 100 (250×16 mm), UV detector at 236 nm, flow 5.0 ml/min.

Plant material

A commercial sample of *F. 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 was deposited in the Herbarium of the Institute of Botany, BAS, Sofia.

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 to a small vol. After filtration of the deposited esculin (30.00 g), the mother liquor was concentrated under reduced pressure and subjected to a solvent-solvent partitioning using petrol and EtOAc to afford R-1 (20.01 g) and R-2 (50.02 g), respectively. R-2 (6.20 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 residues R-3 (0.84 g), R-4 (0.94 g) and R-5 (0.32 g).

Fraction R-4 was subjected to silica gel CC with $CHCl_3$–MeOH (9:1). The first fraction A-1 (0.28 g) was worked up on silica gel CC, with EtOAc–toluene–EtOH (4:1:1) to give one enriched in secoiridoids subfraction B-1 (0.18 g) and **6** (0.025 g). B-1 was subjected to prep. HPLC RP-18 using a MeOH–H_2O gradient (30 min 40% MeOH, 15 min to 60% MeOH) to obtain **4** (40 mg), **1** (9.8 mg), **2** (15.7 mg), **3a, b** (7 mg) and **5** (9 mg).

Mixture of hydroxyframoside A and hydroxyframoside B (**3a, b**). Powder; IR ν_{max}^{KBr} cm^{-1}: 3419, 1700, 1684, 1628, 1516; UV (MeOH) ν_{max}^{MeOH} nm: 240, 282, 326; ESI-MS: $m/z = 645\,[M-1]^-$; ^1H NMR: Table 1; ^{13}C NMR: Table 2. Significant HMBC data of **3a** and **3b** (δ in ppm)—for **3a**: C-11 (167.47)/H-1″a (4.27), H-1″b (4.31), H-3 (7.47); C-7 (172.45)/H-6a (2.39), H-6b (2.64), H-1‴a (4.10), H-1‴b (4.21); C-8‴ (120.62)/H-2‴ (2.77); for **3b**: C-11 (167.47)/H-1″a (4.27), H-1″b (4.31), H-3 (7.48); C-7 (172.50)/H-6a (2.39), H-6b (2.65), H-1‴a (4.09), H-1‴b (4.22); C-8″ (120.58)/H-2″ (2.81). Important proton long range correlations for **3a** and **3b** (δ in ppm)—for **3a**: H-2″ (2.87)/H-4″,8″ (7.07); H-2‴ (2.77)/H-4‴ (6.67), H-8‴ (6.55); for **3b**: H-2″ (2.81)/H-4″ (6.68), H-8″ (6.56); H-2‴ (2.82)/H-4‴,8‴ (7.05).

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