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SECOIRIDOID GLUCOSIDES FROM *FRAXINUS OXYCARBA*

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Key Word Index—*Fraxinus oxycarba*; *Oleaceae*; fraxicarbosides A, B and C; acyl secoiridoid glucosides; lignans; caffeoyl esters; flavonol glycosides.

Abstract—Three secoiridoid glucosides, fraxicarboside A, 6″-*O*-*trans*-*p*-coumaroyl-10-hydroxyoleuropein, fraxicarboside B, 6″-*O*-*trans*-caffeoyl-10-hydroxyoleuropein, and fraxicarboside C, 3″-*O*-acetyl-6″-*O*-*trans*-caffeoyl-10-hydroxyoleuropein have been isolated for the first time from the leaves of *Fraxinus oxycarba* Willd. together with four known secoiridoids; oleuropein, ligstroside, 10-hydroxyoleuropein and 10-hydroxyligstroside, three known lignans; (+)-pinoresenol-4′-*O*-β-D-glucopyranoside (+)-fraxiresinol-1-*O*-β-D-glucopyranoside and (+)-1-hydroxypinoresinol-4′-*O*-β-D-glucopyranoside, two known phenylpropanoid glycosides; verbascoside (= acteoside), and 6-*O*-caffeoyl-β-D-glucopyranoside, and three known flavonol glycosides; kaempferol-3-*O*-β-D-glucopyranoside, kaempferol-3-*O*-α-L-rhamnopyranosyl-(1 → 6)-β-D-glucopyranoside and quercetin-3-*O*-α-L-rhamnopyranosyl-(1 → 6)-β-D-glucopyranoside. The complete ^{1}H and ^{13}C NMR spectral assignments of the new compounds were confirmed by the conventional 1D NMR methods and 2D shift-correlated techniques: COSY, HMBC and HMQC. © 1998 Elsevier Science Ltd. All rights reserved

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INTRODUCTION

In recent years, an increased interest in the phytochemistry of the *Oleaceae*, particularly of the genus *Fraxinus* (tribe Fraxineae, subfamily Oleoideae) [1], has been motivated by the discovery of the secoiridoids that constitute the major secondary metabolites [2–11] and shown an interesting spectrum of biological activities [12–15]. *Fraxinus oxycarba* Willd. (*Oleaceae*) is an evergreen shrub that is cultivated as an ornamental plant in Egypt [16, 17]. Previous reports on *F. oxycarba* revealed the presence of coumarin and flavonol in the fruits, flowers, leaves and root bark [18–22]. As part of a chemotaxonomic study of the genus *Fraxinus* we previously reported the isolation of several new secoiridoid glucosides of the oleoside type, such as angustifoluosides A, B, C, and others, from the leaves of *Fraxinus angustifolia* [14, 15]. Continuing our studies on the constituents of the genus *Fraxinus*, and in order to obtain more detailed chemical information on *F. oxycarba*, we describe in this paper the isolation and structure elucidation of seven secoiridoid glucosides including three new acylated secoiridoid glucosides (**A–C**), together with the identification of several known lignan, caffeoyl and flavonol glycosides from the leaves of *F. oxycarba*.

RESULTS AND DISCUSSION

The methanol extract of the leaves of *F. oxycarba* was partitioned between water and *n*-butanol. The *n*-butanol soluble portion was subjected to successive column chromatographic separations using polyamide CC, silica gel for flash CC, reversed-phase silica gel (RP-18) and Sephadex LH-20 to afford compounds **1–15**. Identification of compounds **4–15** were performed by comparison of spectroscopic data (UV, IR, FAB mass spectrum, ^{1}H and ^{13}C NMR) with reported data in the literature as oleuropein (**4**) [7, 13], ligstroside (**5**) [10, 13], 10-hydroxyoleuropein (**6**) [23], 10-hydroxyligstroside (**7**) [13, 24] (+)-pinoresenol-4′-*O*-β-D-glucopyranoside (**8**) [25] (+)-fraxiresinol-1-*O*-β-D-glucopyranoside (**9**) [26] (+)-1-hydroxypinoresinol-4′-*O*-β-D-glucopyra-noside (**10**) [25], verbascoside (= acteoside) (**11**) [27], 6-*O*-*trans*-caffeoyl-β-D-glucopyranoside (**12**) [28], kaempferol-3-*O*-β-D-glucopyranoside (**13**) [29, 30], kaempferol-3-*O*-α-L-rhamnopyranosyl-(1 → 6)-β-D-glucopyranoside (**14**) [29, 30] and quercetin-3-*O*-α-L-rhamnopyranosyl-(1 → 6)-β-D-glucopyranoside (**15**) [29, 30]. Compounds **1–3** were new acylated secoiridoid glucosides and their structures were elucidated as described below.

Fraxicarboside A (**1**) was obtained as a white amorphous powder. The positive HRFAB mass spectrum of **1** showed an intense peak at *m/z* 725 corresponding to the [M+Na]$^{+}$ quasimolecular ion of

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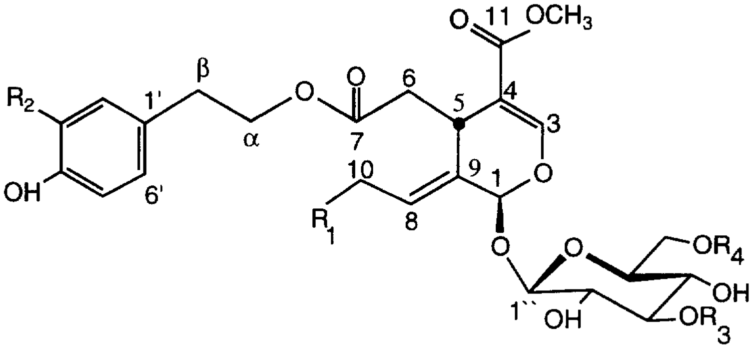
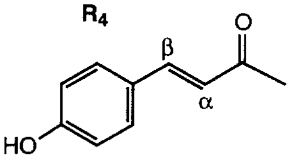
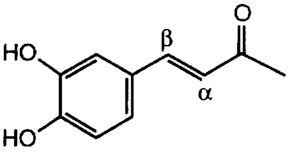
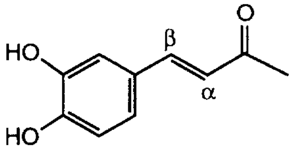
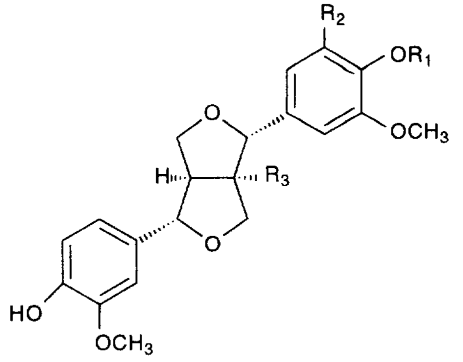
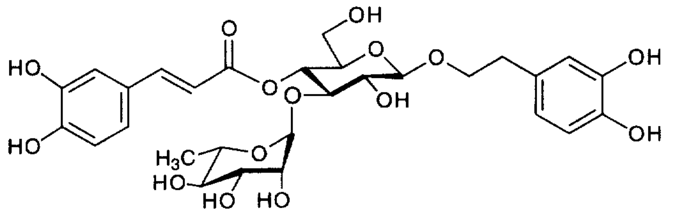


	R1	R2	R3	R4
1	OH	OH	H	
2	OH	OH	H	
3	OH	OH	Ac	
4	H	OH	H	H
5	H	H	H	H
6	OH	OH	H	H
7	OH	H	H	H

1. Weak ions at *m/z* 703 and 741 corresponding to $[M+H]^+$ and $[M+K]^+$, respectively, were also observed, indicating the M_r of **1** was 702 which corresponds to the molecular formula $C_{34}H_{38}O_{16}$. The UV and IR spectra indicated the presence of hydroxyl groups and a conjugated carbonyl group, which are characteristics of many iridoid and secoiridoid glucosides. Alkaline methanolysis of **1** yielded 10-hydroxyoleuropein and *p*-coumaric acid methyl ester which were identified on TLC. Complete ^{1}H and ^{13}C NMR resonance assignments were carried out by 1D and 2D shift-correlated NMR techniques. COSY, HMQC and HMBC showed a signal pattern very similar to that of 10-hydroxyoleuropein (**6**) except for the signals of the sugar moiety and accompanied with aromatic and olefinic protons signals. The data are summarized in Table 1 and Experimental and was in agreement with the proposed formula. The ^{13}C NMR spectrum of **1** (Table 1) contained 24 signals. Comparison with the spectrum of 10-hydroxyoleuropein (**6**) allowed eleven signals to be assigned to an iridoid aglycone moiety and eight signals to a dihydroxyphenylethyl moiety identical to those found in **6** (except for a constant deviation of ca 1 ppm). Furthermore, six signals could be assigned to a *β*-glucopyranosyl moiety acylated at the 6-position as seen by a downfield shift (ca 4 ppm) for the C-6″ signal and an upfield shift (ca 3 ppm) for the C-5″ signal in **1** as compared to the usual position of these shifts. This established the structure of **1** to be an acylated deriva-



	R1	R2	R3
8	Glc	H	H
9	H	OCH3	O-Glc
10	Glc	H	OH



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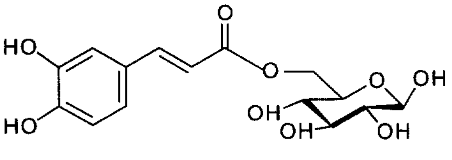

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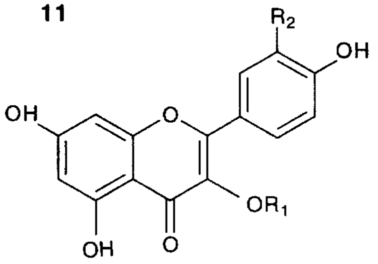

	R1	R2
13	Glc	H
14	Rha (1→6) Glc	H
15	Rha (1→6) Glc	OH

tive of **6**. The remaining nine signals in the spectrum fitted well with a *p*-*E*-coumaroyl unit, consistent with the signals in the ^{1}H NMR spectrum. Of these, the AB-system (J_{AB} = 16 Hz) at δ 6.44 and 7.68 proved the *E*-geometry of the double bond. Additionally, the low field position of the H-6″ signals (δ 4.48 and 4.69) further proved the point of attachment for the *p*-coumaroyl moiety. Linkage of the ester moiety to H-6″ signals was also confirmed by the intense cross-peak in the HMBC spectrum between H-6″ and the carbonyl carbon at (δ 169.82) of the *trans*-*p*-coumaroyl unit clearly indicated the location of the acyl unit at the 6″-position of the glucosyl moiety.

In order to complete the structure deductions concerning **1**, it was acetylated. The product obtained from the acetylation yielded the fully acetylated (**1a**). As expected, the FAB mass spectrum contained a $[M+H]^+$ ion at m/z 851 corresponding to the molec-

Table 1. ^{13}C NMR spectral data (ppm) of fraxicarbosides A, (**1**), B (**2**), C (**3**) and 10-hydroxyoleuropein (**6**). (90.15 MHz, CD_3OD, TMS as internal standard)

C-Atom	**1**	**2**	**3**	**6**
Secoiridoid moiety				
1	94.7 *d*	95.1 *d*	94.7 *d*	95.8 *d*
3	155.1 *d*	154.5 *d*	154.8 *d*	156.1 *d*
4	108.9 *s*	109.1 *s*	109.3 *s*	109.3 *s*
5	31.8 *d*	30.6 *d*	30.8 *d*	31.4 *d*
6	41.9 *t*	40.8 *t*	41.1 *t*	40.8 *t*
7	173.3 *s*	171.8 *s*	173.2 *s*	172.7 *s*
8	128.1 *d*	128.9 *d*	129.3 *d*	129.0 *d*
9	130.2 *d*	131.3 *d*	131.9 *d*	131.1 *d*
10	60.6 *t*	59.1 *t*	59.4 *t*	60.2 *t*
11	168.1 *s*	168.7 *s*	167.6 *s*	168.3 *s*
OCH_3	51.8 *q*	52.1 *q*	52.4 *q*	51.9 *q*
Phenylethyl moiety				
1′	131.1 *s*	130.7 *s*	130.8 *s*	132.1 *s*
2′	115.8 *d*	116.2 *d*	116.9 *d*	116.5 *d*
3′	146.5 *s*	146.6 *s*	146.2 *s*	147.1 *s*
4′	145.1 *s*	145.9 *s*	144.8 *s*	144.9 *s*
5′	116.8 *d*	117.1 *d*	117.5 *d*	117.5 *d*
6′	122.1 *d*	121.3 *d*	121.8 *d*	121.6 *d*
C-α	71.1 *t*	72.1 *t*	72.6 *t*	70.3 *t*
C-β	35.9 *t*	35.4 *t*	36.1 *t*	36.7 *t*
Glucosyl moiety				
1″	99.2 *d*	98.7 *d*	97.8 *d*	100.4 *d*
2″	74.5 *d*	75.3 *d*	74.9 *d*	75.1 *d*
3″	77.4 *d*	77.1 *d*	79.5 *d*	76.9 *d*
4″	71.1 *d*	70.5 *d*	68.6 *d*	71.2 *d*
5″	74.1 *d*	75.1 *d*	74.6 *d*	78.1 *d*
6″	66.1 *t*	66.2 *t*	65.1 *t*	62.4 *t*
Ester moiety	*p*-*E*-coumaroyl	*trans*-caffeoyl	*trans*-caffeoyl	
C-1‴	126.8 *s*	127.8 *s*	127.1 *s*	—
C-2‴	130.6 *d*	115.1 *d*	115.2 *d*	—
C-3‴	116.4 *d*	148.7 *s*	148.6 *s*	—
C-4‴	160.3 *s*	146.9 *s*	147.3 *s*	—
C-5‴	116.4 *d*	117.3 *d*	116.7 *d*	—
C-6‴	130.6 *d*	122.7 *d*	123.4 *d*	—
Cα′	115.8 *d*	115.9 *d*	116.5 *d*	—
C-β′	144.6 *d*	145.6 *d*	147.8 *d*	—
C=O	169.8 *s*	168.6 *s*	169.6 *s*	—
$\underline{C}OCH_3$	—	—	171.0 *s*	—
$CO\underline{C}H_3$	—	—	21.3 *q*	—

ular formula $C_{39}H_{46}O_{21}$. The main fragment peaks recorded were at *m*/*z* 873 $[M+Na]^+$ and *m*/*z* 478 for the loss of a [tetra-*O*-acetyl glucose-courmaroyl ion]$^+$. The ^{1}H NMR spectrum of **1**-acetate revealed the presence of seven acetyl signals belonging to four aliphatic [δ 1.88, 1.97, 2.10, 2.12] and three aromatic [δ 2.21, 2.28, 2.32] (each 3H, *s*) hydrogens, thus confirming the presence of a substituted glucosyl moiety in **1**. No downfield shift was observed for the signal of H-6″ of the glucosyl moiety (δ 4.56, *d*, $J = 12.3$ Hz, H-6$''_a$, and δ 4.48, *d*, $J = 12.3$ Hz, H-6$''_b$), (see Experimental) indicating this to be the site of acylation. The 2D-^{1}H-^{1}H COSY and ^{1}H-^{13}C HMBC NMR spectra of **1**-acetate revealed all the connectivities and confirmed also no downfield shifts upon acetylation for H-6″. Based on these findings, the chemical structure of fraxicarboside A (**1**) has been elucidated to be 6″-*O*-*trans*-*p*-coumaroyl-10-hydroxyoleuropein.

Fraxicarboside B (**2**) was obtained as an amorphous powder with the molecular formula $C_{34}H_{38}O_{17}$ (positive HRFAB mass spectrum: $m/z = 719$ $[M+H]^+$, 741 $[M+Na]^+$), suggesting that in **2** a $C_9H_6O_3$ moiety was present in addition to the skeleton present in **6**. Its UV and IR spectra exhibited absorption bands similar to **1**. Alkaline methanolysis of **2** yielded 10-hydroxyoleuropein and caffeic acid methyl ester which chromatographically identified on TLC. The ^{1}H NMR integration was in agreement with the proposed formula, showing the existence of 34 protons in the molecule. The proton signals of **2** present in its 1D and 2D ^1H-^1H-homonuclear COSY and NOSEY NMR spectra, which permitted the identification of all cross peaks between adjacent coupled protons in the sugar ring, secoiridoid, phenylethyl and aromatic acyl moieties, were found to be closely related to those of **1** except for the acyl moiety. This latter signal clearly demonstrated that the *trans-p*-coumaroyl group in **1** was replaced by a *trans*-caffeoyl group in the structure of **2** which was consistent with the signals in the ^1H NMR spectrum. Of these, the AB-system ($J_{AB} = 15.7$ Hz) at δ 6.29 and 7.68 proved the *E*-geometry of the double bond. Additionally, nine carbon signals which can be attributed to a *trans*-caffeoyl moiety were reasonably assigned as shown in the ^{13}C NMR and DEPT spectra of this ester moiety (Table 1). The HMBC allowed a complete assignment of all protons and carbons including those of the glucose residue. In this way the caffeoyl carbonyl-carbon signal at δ 168.65 was correlated with the proton signals at the site of esterification, H-$6''_a$ (δ 4.53, *dd*, $J = 12.2/5.5$ Hz) and H-$6''_b$ (δ 4.76, *dd*, $J = 12.2/2.0$). Based on the aforementioned evidence, the chemical structure of fraxicarboside B (**2**) was elucidated to be 6″-*O*-*trans*-caffeoyl-10-hydroxyoleuropein.

Fraxicarboside C (**3**) was obtained as an amorphous powder. The HRFAB mass spectrum showed fragments at *m/z* 783 $[M+Na]^+$ and 761 $[M+H]^+$, consistent with the molecular formula of $C_{36}H_{40}O_{18}$. Compound **3** was also presumed to be a 10-hydroxyoleuropein with caffeic acid ester at C-6 of glucose, from the similarity of its UV, IR and NMR spectral data to those of **2**. The concerted use of 1D (^1H and ^{13}C NMR) and 2D (COSY, HMQC, and HMBC) data in **3** showed the existence of an acetyl group linked to position C-3 of the glucose, as well as a *trans*-caffeoyl moiety which was found to be linked to position C-6 of glucose. The 3H singlet at δ 1.99 of aliphatic acetyl group found in ^1H NMR, which was also deduced from the signals at δ 21.23 (CH_3) and 171.74 (C=O) in the ^{13}C NMR spectrum (Table 1) were in agreement with the resonance of an acetyl group. The strong fragment ion at *m/z* 367 in the FAB mass spectrum produced presumably by elimination of glucosyl moiety, esterified with caffeic and acetic acids also confirmed these results. To assign the points of attachment of the two acyl groups, their respective carbonyl signals were used as entry points in the HMBC spectrum, since the methine proton (δ 5.13) of position C-3″ and an acetyl proton (δ 1.99) gave cross-peaks with the same carbonyl carbon (δ 171.74, aliphatic CO group), while the carbonyl carbon in the *E*-caffeoyl moiety at δ 169.61 showed correlation with four protons; H-α' (δ 6.33), H-β' (δ 7.62), H-$6''_a$ (δ 4.41) and H-$6''_b$ (δ 4.64) revealing the C-3 and C-6 positions of the glucosyl unit as the linkage points of the acetyl and caffeoyl moieties, respectively. Furthermore, significant upfield shifts of the methine protons of positions C-10, C-2″ and C-4″ in the acetate derivative of **3** (see Experimental) clearly certified that the positions of nonesterified hydroxyl groups in **3** were C-10, C-2″ and C-4″. On the basis of these spectroscopic data and chemical evidence the full structure of **3** was thus determined to be 3″-*O*-acetyl-6″-*O*-*trans*-caffeoyl-10-hydroxyoleuropein.

EXPERIMENTAL

General

UV spectra (λ_{max}) were determined on a Hitachi 340 spectrophotometer. IR spectra (cm^{-1}) were obtained using a Nicolet 205 FT-IR spectrometer connected to a Hewlett-Packard ColorPro plotter. All NMR spectra were recorded in a Bruker NM 360 spectrometer operating at (360.134, for ^1H-) and (90.15 MHz, for ^{13}C NMR) respectively. ^{13}C Multiplicities were determined by the DEPT pulse sequence. All spectra were obtained in CD_3OD and $CDCl_3$ using TMS as internal standard, with the chemical shifts quoted in δ (ppm) and the coupling constants (*J*) in Hz. The one-bond ^1H-^1H and ^1H-^{13}C chemical shift correlated spectra (HMQC) and the multiple bond ^1H-^{13}C chemical shift correlated spectra (HMBC) were acquired and processed with a Varian AMX-600 high-field spectrometer equipped with an IBM Aspect-2000 processor and with software VNMR version 4.1, using TMS as internal standard in appropriate solvents. HRFAB mass spectra were performed on a VG-ZAB-HF reversed geometry (BE configuration, where B is a magnetic sector and E is an electrostatic analyzer) mass spectrometer (MS) (VG Analytical, Inc.). TLC was performed on precoated Merck Aluminum plates (Kieselgel 60 F_{254}) and RP-18 F_{254} spots were visualized by spraying with 1% Vanillin-H_2SO_4, followed by heating at 100° for 5 min. CC stationary phases were Polyamide CC (ICN Biomedicals), Sephadex LH-20 (Pharmacia). Flash CC was performed using J.T. Baker glassware on various column sizes charged with 40 μm silica gel (Baker) and sepralyte C18, 40 μm; as the stationary adsorbent phases at a flow rate of 5 ml min^{-1} under N_2 pressure.

Plant material

The plant material (leaves) were collected from Zoological Garden, Guiza, Egypt in April 1996. The plant was kindly authenticated by Prof. Dr M. N. El-Hadidy, Faculty of Science, Cairo University. A voucher specimen has been deposited in the Pharmacognosy Department, Faculty of Pharmacy, Al-Azhar University, Cairo, Egypt as (FOO, No. 1202).

Extraction and isolation

The air-dried plant material (650 g) was ground into powder and extracted exhaustively with MeOH (3 × 5 l). The combined methanolic extracts were concn *in vacuo* at 40° to dryness (148 g). The residue was dissolved in H_2O (100 ml) and filtered through Celite. The filtrate and washings were combined and defatted with petrol. The concn defatted crude extract (59 g) was partitioned with H_2O and *n*-BuOH. The *n*-BuOH extract (34 g) was coarsely fractionated over a polyamide column (120 × 5 cm), and was eluted initially with H_2O, followed by addition of MeOH in 10% increments. Frs. were collected in 100 ml aliquots and pooled according to their TLC profile in $CHCl_3$–MeOH–H_2O (70:30:10) (organic phase) and $CHCl_3$–MeOH–H_2O (80:20:2) to yield seven main fractions; **I** (5.65 g), **II** (4.18 g), **III** (3.05 g), **IV** (3.12 g), **V** (2.75 g), **VI** (2.55 g) and **VII** (1.98 g). Frs. **I**, **II** and **III** were separately subjected to a series of flash CC at 5 ml min^{-1} under N_2 pressure [stationary phase: silica gel for flash CC, 40 μm, eluting successively with CH_2Cl_2 and a gradient of CH_2Cl_2–MeOH (9:1 → 3:7), CH_2Cl_2–MeOH–H_2O (80:10:1 → 70:30:3), or EtOAc– MeOH–H_2O (30:2:1 → 20:3:2)]; flash CC at a flow rate 2 ml min^{-1} under N_2 pressure [stationary phase: sepralyte C-18, 40 μm using an H_2O-MeOH gradient solvent system (5 → 45%)]; and final purification by gel filtration CC over Sephadex LH-20 eluted either with MeOH–$CHCl_3$ (1:1) or with MeOH to give compounds **1** (33 mg), **2** (25 mg), **3** (29 mg), **4** (58 mg), **5** (36 mg), **6** (18 mg), **7** (12 mg), **8** (9 mg), **9** (14 mg), **10** (17 mg), **11** (329 mg), **12** (61 mg). Subsequent flash CC (90 × 3 cm) of frs. **V** (2.75 g) and **VI** (2.55 g), separately on silica gel for flash CC, 40 μm, eluting with mixtures of EtOAc–MeOH–H_2O (40:2:1 → 30:3:1) which were further applied to a series of chromatographic methods including; flash CC (Sepralyte-C18, 40 μm), using H_2O–MeOH mixtures (10 → 40%) and gel filtration (Sephadex LH-20) eluted with MeOH, to give pure compounds **13** (27 mg), **14** (22 mg) and **15** (32 mg).

Fraxicarboside A (**1**). A white amorphous powder; UV λ_{max}^{MeOH} nm: 233, 329; IR ν_{max}^{KBr} cm^{-1}: 3450–3350 (OH), 2950 (CH), 1715 (C═O), 1646 (C═C), 1570 (Ar), 1490, 1470, 1300, 1070, 975 (C—O and *trans*-CH═CH) and 776 (sust. Ar). HRFAB mass spectrum: 702.778, *m/z* 725 $[M+Na]^+$, 741 $[M+K]^+$, 703 $[M+H]^+$ (calc. for $C_{34}H_{38}O_{16}$); *m/z* 309 [6-*O*-*p*-*E*-coumaroyl-β-D-glucopyranoside]$^+$ and *m/z* 147 [*p*-coumaroyl moiety]$^+$. ^1H NMR (CD_3OD, 360 and 600 MHz): secoiridoid moiety: δ 5.92 (*brs*, H-1), 7.63 (*s*, H-3), 3.78 (*dd*, *J* = 9.5/4.5 Hz, H-5), 2.47 (*dd*, *J* = 14.5/8.5 Hz, H-6_a), 2.59 (*dd*, *J* = 14.5/4.3 Hz, H-6_b), 6.21 (*t*, *J* = 7.3, H-8), 3.96 (*dd*, *J* = 9.2/7.5 Hz, H-10_a), 4.38 (*dd*, *J* = 12.5/7.5 Hz, H-10_b) 3.71 (*s*, COOMe). Phenylethyl moiety: δ 6.67 (*d*, *J* = 2.0 Hz, H-2′), 6.63 (*d*, *J* = 8.0 Hz, H-5′), 6.51 (*dd*, *J* = 8.0/2.0 Hz, H-6′), 4.13 (*t*, *J* = 7.3 Hz, H-α), 2.80 (*t*, *J* = 7.3 Hz, H-β), glucosyl moiety: δ 4.76 (*d*, *J* = 8.0 Hz, H-1″), 3.13 (*dd*, *J* = 8.5/8.0 Hz, H-2″), 3.29 (*dd*, *J* = 9.2/8.5 Hz, H-3″), 3.26 (*dd*, *J* = 9.8/8.2 Hz, H-4″), 3.34 (*ddd*, *J* = 9.8/5.3/2.1 Hz, H-5″), 4.48 (*dd*, *J* = 11.5/5.3 Hz, H-$6''_a$), 4.69 (*dd*, *J* = 11.5/2.1 Hz, H-$6''_b$), *p*-*E*-coumaroyl moiety: δ 7.36 (*d*, *J* = 8.6 Hz, H-2‴, H-6‴), 6.87 (*d*, *J* = 8.6 Hz, H-3‴, H-5‴), 6.44 (*d*, *J* = 16.0 Hz, H-α′), 7.68 (*d*, *J* = 16.0 Hz, H-β′).

Fraxicarboside B (**2**). A white amorphous powder; UV λ_{max}^{MeOH} nm: 230, 335; IR ν_{max}^{KBr} cm^{-1}: 3400–3370 (OH), 2955 (CH), 1700 (C═O), 1630 (C═C), 1580 (Ar), 1500, 1440, 1270, 1160, 1070, 975, 860 (C—O and *trans*-CH═CH) and 785 (sust. Ar). HRFAB mass spectrum: 718.723, *m/z* 741 $[M+Na]^+$, 757 $[M+K]^+$, 719 $[M+H]^+$, 325 [6-*O*-*E*-caffeoyl-β-D-glucopyranoside]$^+$ and *m/z* 163 [*trans*-caffeoyl moiety]$^+$ (calc. for $C_{34}H_{38}O_{17}$). ^1H NMR (CD_3OD, 360 and 600 MHz): secoiridoid moiety: δ 5.83 (*brs*, H-1), 7.75 (*s*, H-3), 3.76 (*dd*, *J* = 9.2/4.5 Hz, H-5), 2.36 (*dd*, *J* = 14.2/9.0 Hz, H-6_a), 2.63 (*dd*, *J* = 14.2/4.5 Hz, H-6_b), 6.32 (*brt*, *J* = 7.0, H-8), 3.88 (*dd*, *J* = 9.2/7.5 Hz, H-10_a), 4.25 (*d*, *J* = 12.5/7.5 Hz, H-10_b) 3.68 (*s*, COOMe). Phenylethyl moiety: δ 6.71 (*d*, *J* = 2.3 Hz, H-2′), 6.67 (*d*, *J* = 8.0 Hz, H-5′), 6.58 (*dd*, *J* = 8.0/2.5 Hz, H-6′), 4.10 (*t*, *J* = 7.5 Hz, H-α), 2.79 (*t*, *J* = 7.5 Hz, H-β), glucosyl moiety: δ 4.86 (*d*, *J* = 7.8 Hz, H-1″), 3.41 (*dd*, *J* = 8.2/7.8 Hz, H-2″), 3.48 (*dd*, *J* = 9.0/8.2 Hz, H-3″), 3.33 (*dd*, *J* = 10.0/8.5 Hz, H-4″), 3.39 (*ddd*, *J* = 9.8/5.3/2.1, Hz, H-5″), 4.53 (*dd*, *J* = 12.2/5.5 Hz, H-$6''_a$), 4.76 (*dd*, *J* = 12.2/2.0 Hz, H-$6''_b$), *trans*-caffeoyl moiety: δ7.11 (*d*, *J* = 2.2 Hz, H-2‴), 6.79 (*d*, *J* = 8.2 Hz, H-5‴), 7.05 (*dd*, *J* = 8.2/2.2 Hz, H-6‴), 6.29 (*d*, *J* = 15.7 Hz, H-α′), 7.58 (*d*, *J* = 15.7 Hz, H-β′).

Fraxicarboside C (**3**). A white amorphous powder; UV λ_{max}^{MeOH} nm: 222, 245, 330; IR ν_{max}^{KBr} cm^{-1}: 3410–3350 (OH), 2948 (CH), 1688 (C═O), 1635 (C═C), 1590 (Ar), 1514, 1444, 1265, 1115, 1112, 1060, 915, 830 (C—O and *trans*-CH═CH) and 780 (sust. Ar). HRFAB mass spectrum 760.877: *m/z* 783 $[M+Na]^+$, 761 $[M+H]^+$ (calc. for $C_{36}H_{40}O_{18}$). ^1H NMR (CD_3OD, 360 and 600 MHz): secoiridoid moiety: δ 5.90 (*brs*, H-1), 7.54 (*s*, H-3), 3.85 (*dd*, *J* = 9.0/4.5 Hz, H-5), 2.39 (*dd*, *J* = 14.5/9.0 Hz, H-6_a), 2.48 (*dd*, *J* = 14.5/4.5 Hz, H-6_b), 6.28 (*t*, *J* = 7.5, H-8), 3.83 (*dd*, *J* = 9.2/7.5 Hz, H-10_a), 4.19 (*dd*, *J* = 12.5/7.5 Hz, H-10_b) 3.72 (*s*, COOMe). Phenylethyl moiety: δ 6.65 (*d*, *J* = 1.9 Hz, H-2′), 6.70 (*d*, *J* = 8.0 Hz, H-5′), 6.53 (*dd*, *J* = 8.0/2.0 Hz, H-6′), 4.10 (*t*, *J* = 7.5 Hz, H-α), 2.89 (*t*, *J* = 7.5 Hz, H-β), glucosyl moiety: δ 5.02 (*d*, *J* = 7.9 Hz, H-1″), 3.47 (*t*, *J* = 9.5 Hz, H-2″), 5.13 (*dd*, *J* = 9.5/9.0 Hz, H-3″), 3.43 (*dd*, *J* = 10.0/8.5 Hz, H-4″), 3.89 (*ddd*, *J* = 9.8/5.3/2.1, Hz, H-5″), 4.41 (*dd*, *J* = 12.5/5.7 Hz, H-$6''_a$), 4.64 (*dd*, *J* = 12.5/2.5, Hz, H-$6''_b$), *trans*-caffeoyl moiety: δ 7.07 (*d*, *J* = 2.0 Hz, H-2‴), 6.83 (*d*, *J* = 8.5 Hz, H-5‴), 6.98 (*dd*, *J* = 8.5/2.0 Hz, H-6‴), 6.33 (*d*, *J* = 16.1 Hz, H-α′), 7.62 (*d*, *J* = 16.1 Hz, H-β′), 1.99 (*s*, $COCH_3$).

10-*hydroxyoleuropein* (**6**). A white amorphous powder; UV λ_{max}^{MeOH} nm: 237, 288; IR ν_{max}^{KBr} cm^{-1}: 3575–3250 (OH), 2935 (CH), 1720 (C═O), 1645 (C═C), 1510 (Ar), 1495, 1450, 1300, 1050, 975 (C—O) and 770 (sust. Ar). FAB mass spectrum: *m/z* 579 $[M+Na]^+$, 557 $[M+H]^+$ 395 (M + H − glu) (calc. for $C_{25}H_{32}O_{14}$). ^1H NMR (CD_3OD 360 and 600 MHz) Secoiridoid moiety: δ 5.79 (*brs*, H-1), 7.58 (*s*, H-3), 3.82 (*dd*,

J = 9.5/4.5 Hz, H-5), 2.57 (*dd*, J = 14.5/9.0 Hz, H-6$_a$), 2.67 (*dd*, J = 14.5/4.5 Hz, H-6$_b$), 6.19 (*t*, J = 7.0, H-8), 3.98 (*dd*, J = 10.0/7.5 Hz, H-10$_a$), 4.16 (*dd*, J = 13.5/7.5 Hz, H-10$_b$). 3.66 (*s*, COOMe). Phenylethyl moiety: δ 6.95 (*d*, J = 2.0 Hz, H-2′), 6.63 (*d*, J = 8.0 Hz, H-5′), 6.51 (*dd*, J = 8.0/2.0 Hz, H-6′), 4.24 (*t*, J = 7.0 Hz, H-α), 2.87 (*t*, J = 7.0 Hz, H-β), glucosyl moiety: δ 4.98 (*d*, J = 7.8 Hz, H-1″), 3.18 (*dd*, J = 9.5/8.0 Hz, H-2″), 3.25 (*dd*, J = 9.5/9.0 Hz, H-3″), 3.36 (*dd*, J = 10.0/9.5 Hz, H-4″), 3.52 (*m*, H-5″), 3.66 (*dd*, J = 12.0/5.7 Hz, H-6$''_a$), 3.82 (*dd*, J = 12.0/1.8 Hz, H-6$''_b$).

Acetylation of compounds **1** *and* **3**

Compounds **1** and **3** were separately treated (10 mg) with Ac_2O (1 ml) and pyridine (1 ml) overnight at room temp. The reaction mixture was added to cold water was extracted with $CHCl_3$. The $CHCl_3$ extract was subjected to CC over silica gel using C_6H_6–Me_2CO (8:2–7:3) gave the fraxicarboside A heptaacetate (**1**-acetate), and fraxicarboside C heptaacetate (**3**-acetate), respectively.

Fraxicarboside heptaacetate (**1a**). A white amorphous powder; FAB mass spectrum: m/z 1019 $[M+Na]^+$, 997 $[M+H]^-$ (calc. for $C_{48}H_{52}O_{23}$). ^1H NMR ($CDCl_3$, 600 MHz): δ 1.88, 1.97, 2.10 (each 3H, *s*, 3 × aliph. acetoxyl) 2.21, 2.25, 2.28, 2.32 (each 3H, *s*, 4 × arom. acetoxyl). Secoiridoid moiety: δ 5.77 (*brs*, H-1), 7.53 (*s*, H-3), 3.61 (*dd*, J = 9.0/4.5 Hz, H-5), 2.38 (*dd*, J = 14.2/9.0 Hz, H-6$_a$), 2.49 (*dd*, J = 14.2/4.8 Hz, H-6$_b$), 6.12 (*q*, J = 7.0, H-8), 4.52 (*dd*, J = 9.5/7.5 Hz, H-10$_a$), 4.67 (*dd*, J = 12.5/7.8 Hz, H-10$_b$). Phenylethyl moiety: δ 6.59 (*d*, J = 1.8 Hz, H-2′), 6.62 (*d*, J = 8.0 Hz, H-5′), 7.18 (*dd*, J = 8.0/2.0 Hz, H-6′), 4.24, (*m*, H-α), 2.91 (*t*, J = 7.0 Hz, H-β), glucosyl moiety: δ 4.87 (*d*, J = 8.0 Hz, H-1′), 5.10 (*dd*, J = 9.5/8.0 Hz, H-2′), 5.26 (*dd*, J = 9.5/9.5 Hz, H-3′), 5.16 (*t*, J = 9.5 Hz, H-4′), 3.82 (*m*, H-5′), 4.38 (*dd*, J = 12.0/6.5 Hz, H-6$'_a$), 4.56 (*dd*, J = 12.0/2.2 Hz, H-6$'_b$), *p-O-E*-coumaroyl moiety: δ 7.56 (*d*, J = 8.5 Hz, H-2‴), 6.93 (*d*, J = 8.5 Hz, H-3‴, H-5‴), 7.60 (*d*, J = 8.5 Hz, H-6‴), 6.49 (*d*, J = 16.0 Hz, H-α'), 7.72 (*d*, J = 16.0 Hz, H-β').

Fraxicarboside heptaacetate (**3a**). A white amorphous powder; FAB mass spectrum: m/z 1119 $[M+Na]^+$, 1096 $[M+H]^+$ (calc. for $C_{52}H_{56}O_{26}$). ^1H NMR ($CDCl_3$, 600 MHz): δ 1.99, 2.07, 2.11 (each 3H, *s*, 3 × aliph. acetoxyl) 2.21, 2.24, 2.27, 2.30, 2.33 (each 3H, *s*, 5 × arom. acetoxyl). Secoiridoid moiety: δ 5.93 (*brs*, H-1), 7.41 (*s*, H-3), 3.59 (*dd*, J = 9.3/5.0 Hz, H-5), 2.41 (*dd*, J = 14.5/9.5 Hz, H-6$_a$), 2.57 (*dd*, J = 14.5/9.5 Hz, H-6$_a$), 2.64 (*dd*, J = 14.5/4.5 Hz, H-6$_b$), 6.31 (*t*, J = 7.7 Hz, H-8), 4.65 (*dd*, J = 9.5/8.0 Hz, H-10$_a$), 4.78 (*dd*, J = 12.9/8.0 Hz, H-10$_b$), Phenylethyl moiety: δ 6.67 (*d*, J = 2.0 Hz, H-2′), 6.73 (*d*, J = 8.5 Hz, H-5′), 7.08 (*dd*, J = 8.0/2.0 Hz, H-6′), 4.33, (*m*, H-α), 2.94 (*t*, J = 7.5 Hz, H-β), glucosyl moiety: δ 5.01 (*d*, J = 7.7 Hz, H-1″), 5.38 (*dd*, J = 9.3/7.9 Hz, H-2″), 5.21 (*t*, J = 9.3 Hz, H-3″), 4.63 (*t*, J = 9.3 Hz, H-4′), 4.14 (*m*, H-5′), 4.43 (*dd*, J = 12.5/6.8 Hz, H-6$'_a$), 4.80 (*dd*, J = 12.5/2.0 Hz, H-6$'_b$), *trans*-caffeoyl moiety: δ 7.52 (*m*, H-2‴, H-6‴), 6.88 (*d*, J = 8.5 Hz, H-3‴, H-5‴), 7.40 (*m*, H-4‴), 6.35 (*d*, J = 16.1 Hz, H-α), 7.76 (*d*, J = 16.1 Hz, H-β).

Methanolysis of compounds **1** *and* **2**

A methanolic soln (5 ml) of each glucoside (5 mg) was treated with Na_2CO_3 (5 mg) at 40°C for 3 h. The reaction mixture was neutralized with Dowex 50 w × 8 (H^+ form). The ion exchange resin was removed by filtration. The filtrate was evapd *in vacuo* to give a crude product. Purification of the product by flash CC [stationary phase: silica gel for flash CC, 40 μm, eluting with (CH_2Cl_2–MeOH–H_2O, 80:10:1 → 8:20:2) under N_2 pressure, afforded 10-hydroxyoleuropein identified by [(^1H NMR, and co-chromatography with **6** on TLC silica gel, $CHCl_3$–MeOH–H_2O (61:32:7) and EtOAc–MeOH–H_2O (100:16.5:13.5)] and *p-E*-coumaric acid methyl ester for **1** and *trans*-caffeic acid methyl ester for **2** which were shown to be identical with authentic samples on TLC silica gel, $CHCl_3$–MeOH (8:2).

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