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Isofraxisecoside, a new coumarin-secoiridoid from the stem bark of Fraxinus xanthoxyloides

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A new coumarin-secoiridoid diglucoside, named Isofraxisecoside, was isolated from the stem bark of Fraxinus xanthoxyloides (G. Don) Wall. ex A. DC. along with nine known compounds. The structure of new compound has been determined on the basis of 1H, 13C, 2D NMR and HRMS methods.

1. Introduction

The genus Fraxinus L. belongs to the Oleaceae family and comprises about 43 species. In the flora of Algeria the genus is represented by two species Fraxinus xanthoxyloides and Fraxinus angustifolia (Wallender 2008; Quezel and Santa 1963). Fraxinus species have been used in folk medicine in different parts of the world for their diuretic and mild purgative effects as well as for the treatment of constipation, dropsy, arthritis, rheumatic pain, cystitis and itching scalp (Kostova and Iossifova 2007; Si et al. 2015). The occurrence of coumarins, secoiridoids and phenylethanoids is a characteristic feature of this Fraxinus species (Kostova...
However, no phytochemical investigation has been carried out on the Algerian *Fraxinus* species to date. In the present study, we describe the isolation and structure elucidation of Isofraxisecoside, a new natural compound consisting of a coumarin glucoside unit bound via an ester function to a secoiridoid glucoside unit together with nine known glucosides.

2. Results and discussion

In our investigation of the stem bark of *F. xanthoxyloides*, a novel coumarin secoiridoid diglucoside named Isofraxisecoside (1), together with nine known compounds were isolated. The known compounds were identified by comparison of their spectroscopic data with those reported in the literature as fraxisecoside (2) (Xiao et al. 2008), fraxin (3) (Yu et al. 2014), cichoriin (4) (Kuwajima et al. 1992), osmanthuside H (5) (Varughese et al. 2009), syringin (6) (Gohari et al. 2009), coniferin (7) (Sugiyama et al. 1993), calcelarioside B (8) (Ersoz et al. 2002), 8-hydroxypinoresinol-4′-O-β-glucoside (9) (Piccinelli et al. 2004), 1-acetoxyipinoresinol-4′-β-glucoside (10) (Tsukamoto et al. 1984).

Compound (1) was obtained as a yellow amorphous powder. The HR-ESI-MS showed deprotonated molecular ion [M − H]− at *m/z* 755.20404 pointing out molecular formula C_{33}H_{40}O_{20} which was confirmed by 1H and 13C NMR data. The UV spectrum showed absorption maxima at 229.5, 328.5, 335.5, 345.0 suggesting a conjugated aromatic system. Detailed analysis of its 1H, 13C, HSQC, HMBC and ROESY spectra indicated the presence of two structural units: a secoiridoid glucoside and a coumarin glucoside. The signals in 1H NMR spectrum of three protons at δH 6.04 (1H, d, J = 9.3 Hz, H-3″), δH 7.83 (1H, d, J = 9.3 Hz, H-4″) and δH 6.84 (1H, s, H-5″) as well as the anomeric proton at δH 4.85 (1H, d, J = 7.8 Hz) indicated a 6-, 7-, 8-substituted coumarin glucoside structure. A singlet at δH 3.84 suggested the presence of OCH3 group and the observed cross-peak in HMBC spectrum between methoxyl protons and C-6″ (δC 150.09) and correlation in ROESY experiment between H-5″ and the same protons confirmed the attachment of OCH3 at C-6″. The spectroscopic data of the coumarin part were consistent with those of the uncommon coumarin isofraxoside (fraxetin–7-O–β–glucoside) (Godecke et al. 2005). Another set of signals at δH 7.50 (1H, s, H-3), δH 5.87 (1H, s, H-1), δH 6.05 (1H, overlapped, H-8), δH 1.61 (3H, dd, J = 1.1, 7.2 Hz, Me-10) and δH 4.85 (1H, d, J = 7.8 Hz, H-1″) and the corresponding carbon signals at δC 155.38, 95.39, 125.32, 13.63 and 100.69 were attributed to the secoiridoid glucoside moiety. The presence of another methoxy group was observed (δH 3.68, s; δC 52.53) and the position of methylester was determined at C-11 by correlation between the signal of OCH3 and C=O (δC 169.32, C-11) in the HMBC spectrum. The signals of the secoiridoid part corresponded well to the previously reported oleoside-11-methylester (Damtoft et al. 1992). Further correlations between H-1″ (δH 4.85) and C-7″ (δC 133.49) and between H_{a}-6″ (δH 4.33), H_{b}-6″ (δH 4.21) and C-7 (δC 173.74) confirmed glycosylation of coumarin part at C-7″ and ester linkage between OH group of C-6″ and carboxyl group of oleoside at C-7. The presence of D-glucose was authenticated by acid hydrolysis and synthesis of its tolylthiocabamoyl-thiazolidine derivative (Tanaka et al. 2007). On the basis of these evidences, compound 1 was identified as fraxetin–7″-O-[11-methyloleosidyl-(7-6″)]-β-β-glucopyranoside, named isoaxisecoside and its structure is shown in Figure 1.

Isofraxisecoside (1) is the third example of a natural compound consisting of one coumarin glucoside unit linked to a secoiridoid moiety of oleoside type after previously described
escuside (Iossifova et al. 2002) and fraxissecoside (Xiao et al. 2008) isolated from the same genus.

3. Experimental

3.1. General experimental procedures

Optical rotation was recorded in MeOH using a Galan-Taylor Prism polarimeter. NMR spectra were acquired on Bruker AVII + 600 spectrometer (Bruker, Karlsruhe, Germany), $^1$H NMR (600.11 MHz), $^{13}$C NMR (150.91 MHz) in CD$_3$OD, purchased from Deutero-GmbH (Kastellaun, Germany), with TMS as internal standard. UV spectra: Helios Gamma UV spectrophotometer (Thermo Scientific, Bremen, Germany) in MeOH. HR-ESI-MS analysis was performed on a Thermo Scientific Q Exactive Plus (Bremen, Germany) in negative mode.

Column chromatography (CC) on Polyamide 6 (Fluka, Germany), Sephadex LH-20 and Silica gel 60, particle size 0.063–0.200, 70–230 mesh ASTM (Merck, Darmstadt, Germany) as well as Lobar chromatography (Lobar RP-8 and RP-18, Merck, Darmstadt, Germany) were used for separation and purification of the individual compounds. Preparative thin layer chromatography (PTLC) was performed on pre-coated plates 60 F$_{254}$ 0.5 × 0.25 mm and thin layer chromatography (TLC) was performed on 60 F$_{254}$ plates (Merck, Darmstadt, Germany). Separation was visualized by spraying with 20% H$_2$SO$_4$ in ethanol (v/v) solution. All solvents used for chromatographic purposes were of analytical grade.

3.2. Plant material

The stem bark of *F. xanthoxyloides* was collected in June 2014 from the region of Khenchela, Algeria. The plant was kindly identified by Prof Mohamed Kaabache, Setif University, Algeria. A voucher specimen, with the identification number 06/2014/KFX was deposited in the Herbarium of the Department of Biochemistry, Setif University.
3.3. Extraction and isolation

The stem bark material of *F. xanthoxyloides* was air dried and powdered by electric mill. The plant material was subjected to exhaustive extraction with MeOH at room temperature (3 × 48 h). The methanol extract was filtered and evaporated under reduced pressure to yield a residue (22.11 g). Part of methanolic extract (10 g) was dissolved in water and applied on polyamide 6 column chromatography with H₂O–MeOH gradient system (100:0 to 0:100). Using TLC analysis on silica gel (CHCl₃:MeOH:H₂O, 60:22:4) fractions having similar profiles were combined to give nine main fractions. A part of fraction F1 (1.8 g) was dissolved in water and chromatographed on Lobar column (RP-8, size B), using H₂O-MeOH (5% to 55%) gradient to afford seven sub-fractions. Purification of sub-fraction F1-1 (23.9 mg) by PTLC using CHCl₃:MeOH:H₂O (60:22:4/v:v:v) eluent yielded 3.3 mg of compound 9. F1-2 (144.8 mg) was subjected to Sephadex LH-20 CC using MeOH:H₂O (2:1/v:v) as mobile phase to give two fractions and a fraction was then purified by PTLC with CHCl₃:MeOH:H₂O (60:22:4/v:v:v) eluent to afford compound 3 (8.9 mg) and compound 5 (28.7 mg). Sub-fraction F1-6 (51.3 mg) was subjected to Sephadex LH-20 column chromatography, using MeOH:H₂O (2:1/v:v) as eluent, to give compound 1 (27.8 mg) and compound 2 (15.6 mg). Fraction F3 was dissolved in water and applied on Lobar column (RP-8, size B), using a gradient of H₂O:MeOH (5% to 55%) to obtain eight sub-fractions. Sub-fraction F3-6 was subjected to Sephadex LH-20 CC and eluted with MeOH:H₂O (2:1/v:v) to provide compound 7 (14.3 mg). Compound 6 (12 mg) was isolated from fraction F3-8 (39 mg), which was submitted to CC over Sephadex LH-20 and eluted with MeOH:H₂O (2:1/v:v). Fraction F4 (550 mg) was dissolved in water and applied on Lobar column (RP-8, size B) eluting with H₂O:MeOH (5% to 55%) gradient to obtain thirteen sub-fractions. Sub-fractions F4-3 (43 mg) and F4-13 (33.3 mg), were separately purified by preparative TLC, developed with CHCl₃:MeOH:H₂O (60:22:4/v:v:v), to afford compound 4 (5.1 mg) and compound 10 (23.1 mg), respectively. Fraction F7 (470 mg) was applied on Lobar column (RP-8, size B), using H₂O:MeOH (20% to 50%) gradient to yield compound 8 (111 mg).

3.4. Acid hydrolysis of compound 1

The absolute configuration of the sugar was established using the method of Tanaka et al. (2007) with some modifications (Kokanova-Nedialkova et al. 2015). Briefly, compound 1 (5 mg) was refluxed with 2 mL of 2 N HCl-MeOH (1:1) for 2 h. The reaction mixture was filtered through Diaion HP-20SS followed by subsequent elution with H₂O and MeOH. The water portion was filtered through Amberlite IRC 86 resin and then evaporated to dryness. The dry water eluate was treated with a solution (0.1 mL) of L-cysteine methyl ester in pyridine (5 mg/mL) at 60 °C for 1 h. A solution (0.1 mL) of o-tolylisothiocyanate in pyridine (5 mg/mL) was added to the mixture and heated at 60 °C for 1 h. The resulting solution was analyzed using HPLC [Purospher STAR RP-18 5 μm column (Merck; 4.6 × 250 mm) with 25% ACN in 50 mM H₃PO₄, flow rate 1 mL/min, UV detection at 250 nm]. The presence of D-glucose (tₘ value of the tolylthiocarbamoyl-thiazolidine derivative was 18.7 min) was found in the residue.

**Isofraxisecoside (1):** Yellow amorphous powder, [α]D²⁰ = −135.8 (c = 0.095, MeOH); UV max (MeOH): (log ε) 229.5 (2.59), 328.5 (0.86), 335.5 (0.93), 345.0 (0.98) nm; ¹H (CD₃OD, 600.11 MHz) δ: 5.87 (H-1, s, 1H), 7.50 (H-3, s, 1H), 3.87 (H-5, overlapped, 1H), 2.66 (H₆-6, dd,
5.2, 14.1, 1H), 2.43 (Hb-6, dd, 8.6, 14.1, 1H), 6.05 (H-8, overlapped, 1H), 1.61 (H-10, dd, 1.1, 7.2, 3H), 3.68 (OMe-11, s, 3H), 4.85 (H-1’, d, 7.8, 1H), 3.28 – 3.59 (H-2’, H-3’, H-4’, H-5’, m, 4H), 3.87 (H-6’, d, 11.5, 1H), 3.70 (Hb-6’, d, 11.6, 1H), 6.04 (H-3”, d, 9.3, 1H), 7.83 (H-4”, d, 9.3, 1H), 6.84 (H-5”, s, 1H), 3.84 (OMe-6”, s, 3H), 4.85 (H-1’”, d, 7.8, 1H), 3.57 (H-2”’, m, 1H), 3.48 (H-3”’, t, 9.0, 1H), 3.42 (H-4””, t, 9.0, 1H), 3.53 (H-5””, m, 1H), 4.33 (Ha-6””, dd, 1.6, 11.8, 1H), 4.21 (Hb-6””, dd, 6.3, 11.8, 1H); 13C (CD3OD, 150.91 MHz) δ: 95.39 (C-1), 155.38 (C-3), 109.26 (C-4), 31.45 (C-5), 41.33 (C-6), 173.74 (C-7), 125.32 (C-8), 129.96 (C-9), 13.63 (C-10), 169.32 (C-11), 52.53 (OMe-11), 100.69 (C-1’), 74.28 (C-2’), 77.35 (C-3’), 71.14 (C-4’), 77.89 (C-5’), 62.12 (C-6’), 165.34 (C-2”), 108.11 (C-3”), 147.49 (C-4”), 105.20 (C-5”), 150.09 (C-6”), 56.77 (OMe-6”), 133.49 (C-7”), 155.39 (C-8”), 145.77 (C-9”), 108.28 (C-10”), 106.30 (C-1”), 74.82 (C-2”), 77.41 (C-3”), 70.95 (C-4”), 75.35 (C-5”), 65.01 (C-6””; HR-ESI-MS: m/z found: 755.20404 for [M − H]−; calcd. for C33H39O20: 755.2038 [M − H]−.

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ORCID
Aldjia Hadroug http://orcid.org/0000-0003-0274-7362

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