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Flacourside, a new 4-oxo-2-cyclopentenylmethyl glucoside from the fruit juice of *Flacourtia indica*

Nilupa R. Amarasinghe [a], Lalith Jayasinghe [a,*], Noriyuki Hara [b], Yoshinori Fujimoto [b]

[a] *Institute of Fundamental Studies, Natural Products Project, Hantana Road, Kandy, Sri Lanka*
[b] *Department of Chemistry and Materials Science, Tokyo Institute of Technology, Meguro, Tokyo 152-8551, Japan*

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Abstract

A new glucoside ester, named flacourside, has been isolated together with known methyl 6-*O*-(*E*)-*p*-coumaroyl glucopyranoside and 6-*O*-(*E*)-*p*-coumaroyl glucopyranose from the *n*-butanol extract of fruit juice of the *Flacourtia indica*. The structure of flacourside has been determined to be 4-oxo-2-cyclopentenylmethyl 6-*O*-(*E*)-*p*-coumaroyl-β-D-glucopyranoside on the basis of spectroscopic studies.

Keywords: *Flacourtia indica*; Flacourside; Flacourtiaceae; 4-Oxo-2-cyclopentenylmethanol

1. Introduction

Flacourtiaceae is a family of 93 genera and over 1000 species (Trease & Evans, 1983). There are two *Flacourtia* species *Flacourtia indica* (Burm. F.) Merr. and *F. inermis* Roxb. in Sri Lanka. *F. indica* is a moderate size tree growing in Sri Lanka. It is used as an ornamental plant. Its fruits are edible and very popular in Sri Lanka (Ashton et al., 1997). Flacourtin (3-hydroxy-4-hydroxymethylphenyl-6-*O*-benzoyl-β-D-glucopyranoside) (Bhaumik, Guha, Biswas, & Mukherjee, 1987) and sitosterol(6′-*O*-fattyacyl)-β-D-glucopyranosides (Dehmlow, Guntenhoner, & Van, 2000) were reported from the bark and leaves respectively, of the plant. No chemical work has been reported on the fruits of this plant. In this paper, we report the isolation and structure elucidation of a new 6-*O*-(*E*)-*p*-coumaroyl glucoside, named flacourside (**1**), from the fruit juice of the plant together with known methyl 6-*O*-(*E*)-*p*-coumaroyl glucopyranoside (**2**) (Lu, Sun, Foo, McNabb, & Molan, 2000) and 6-*O*-(*E*)-*p*-coumaroyl glucopyranose (**3**) (Shimomura, Sashida, & Adachi, 1988). Compound **3** was reported to show OH free radical scavenging activities (Wang, Pan, Gao, & Jia, 2000) and repair effects on the radical cation of deoxynucleotides (Shi et al., 1999).

2. Materials and methods

2.1. General

Optical rotations were measured on a JASCO DIP-360 polarimeter. UV spectra were recorded on a Shimadzu UV-1600PC spectrometer. ^{1}HNMR and ^{13}CNMR spectra were recorded on a Bruker DRX500 (500 MHz for ^{1}H and 125 MHz for ^{13}C) spectrometer in CD_3OD solution. The residual protium and carbon signals were used as references for ^{1}H (δ 3.30) and ^{13}C (δ 49.0) chemical shifts, respectively. FABMS(−) were obtained on a JEOL JMS-700 spectrometer with glycerol as matrix. HPLC separations were carried out on a Shimadzu LC-6A apparatus equipped with a UV detector under a reverse phase C_{18} column and isocratic solvent condition.

2.2. Plant material

Fruits of *F. indica* were collected from the Central Province of Sri Lanka in May 2004. A voucher specimen of the

* Corresponding author. Tel.: +94 812 232 002; fax: +94 812 232 131.
E-mail addresses: ulbj2003@yahoo.com, lalith@ifs.ac.lk (L. Jayasinghe).

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plant was deposited at the Institute of Fundamental Studies, Kandy.

2.3. Extraction and isolation

Fresh ripe fruits (330 g) of *F. indica* were blended using a mechanical blender. The red colored juice was filtered using a Büchner funnel. Filtrate was partitioned with *n*-butanol and water. Evaporation of the *n*-butanol gave a dark brown solid (5.5 g). A portion (5 g) was chromatographed over silica (*n*-hexane–EtOAc–MeOH), followed by Sephadex LH-20 (MeOH) and reverse phase HPLC (STR Prep-ODS 20×250 mm column; 65% H_2O–MeOH, 5 ml/min; UV detection 254 nm) to furnish **2** (5 mg), **1** (18 mg), an anomer of **3** (26 mg) and the other anomer of **3** (33 mg) at 53, 60, 62, 66 min in the order.

4-Oxo-2-cyclopentenylmethyl 6-O-(E)-p-coumaroyl-β-D-*glucopyranoside* (***1***): light brown colored amorphous solid; $[\alpha]_D^{25} + 37.1^\circ$ (*c* 5.3, MeOH); UV λ_{max}^{MeOH} nm (log ε): 313 (4.50), 300 sh (4.25), 222 (4.27), 214 (4.26) nm; ^{1}H NMR (500 MHz, CD_3OD): δ 2.18 (1H, *dd*, 19.2, 2.2 Hz, H_a-6″), 2.44 (1H, *dd*, 19.2, 6.4 Hz, H_b-6″), 3.20 (1H, *t*, 8.4 Hz, H-2′), 3.30 (1H, *m*, H-2″), 3.33 (1H, *t*, 6.9 Hz, H-4′), 3.35 (1H, *t*, 6.9 Hz, H-3′), 3.52 (1H, *m*, H-5′), 3.63 (1H, *dd*, 9.9, 6.8 Hz, H_a-1″), 3.90 (1H, *dd*, 9.9, 6.4 Hz, H_b-1″), 4.31 (1H, *d*, 7.8 Hz, H-1′), 4.34 (1H, *dd*, 12.1, 6.2 Hz, H_a-6′), 4.49 (1H, *dd*, 12.0, 2.2 Hz, H_b-6′), 6.14 (1H, *dd*, 5.6, 2.2 Hz, H-4″), 6.35 (1H, *d*, 16.1 Hz, H-8), 6.80 (2H, *d*, 6.8 Hz, H-3, H-5), 7.45 (2H, *d*, 8.8 Hz, H-2, H-6), 7.61 (1H, *d*, 16.1 Hz, H-7), 7.84 (1H, *dd*, 5.9, 2.4 Hz, H-3″); ^{13}C NMR (125 MHz, CD_3OD): δ 38.8 (C-6″), 43.7 (C-2″), 64.5 (C-6′), 71.7 (C-4′), 72.7 (C-1″), 75.0 (C-2′), 75.5 (C-5′), 77.9 (C-3′), 104.7 (C-1′), 114.9 (C-8), 116.8 (C-3, C-5), 127.1 (C-1), 131.2 (C-2, C-6), 135.3 (C-4″), 146.8 (C-7), 161.4 (C-4), 168.8 (C-3″), 169.1 (C-9), 212.5 (C-5″); HRFABMS(−): *m*/*z* 419.1350 $[M–H]^-$, $C_{21}H_{23}O_9$ requires 419. 1342.

3. Results and discussion

Red colored fruit juice of *F. indica* was partitioned with *n*-butanol and water. Chromatographic separation of *n*-butanol extract over silica gel, Sephadex LH-20 and reverse phase HPLC yielded compound **1**, **2** and **3** (Fig. 1). Structures of **1**–**3** were established by analysis of spectral data including 2D NMR and FABMS.

Compound **1** showed a pseudomolecular ion peak at *m*/*z* 419 $[M–H]^-$ in the negative FABMS, indicating the molecular formula $C_{21}H_{24}O_9$. The ^{13}CNMR spectrum displayed 21 signals, which were classified into 14 methine, three methylene and four quaternary carbons by DEPT experiments. Further analysis of the NMR data indicated that compound **1** is an glycoside derivative of 6-*O*-(*E*)-*p*-coumaroyl glucose (**3**) (Shimomura et al., 1988). *Ortho*-coupled protons at δ 7.45 (2H, *d*, 8.8 Hz), 6.80 (2H, *d*, 8.8 Hz) and *trans*-olefinic protons at δ 7.61, 6.35 (each 1H, *d*, $J = 16.1$ Hz) evidenced the presence of (*E*)-*p*-coumaroyl moiety. The H–H COSY spectrum showed a coupling network from H-1′ to H_2-6′ [δ 4.31 (*d*, H-1′), 3.20 (*t*, H-2′), 3.33 (*t*, H-3′), 3.35 (*t*, H-4′), 3.52 (*m*, H-5′), 4.34 (*dd*, H_a-6′)/4.49 (*dd*, H_b-6′)]. These data, together with the coupling constants for the protons listed in experimental part revealed the sugar moiety to be glucopyranoside. HMBC correlation (Fig. 2) from the C-6′ methylene protons of the sugar to the coumaroyl carbonyl (δ 169.1) evidenced the ester linkage through C-6 of the sugar moiety.

The remaining six-carbon fragment was supposed to be an alcohol unit to form the glycosidic linkage. The alcohol unit should have the formula $C_6H_8O_2$ and three degrees of unsaturation. The H–H COSY spectrum of **1** showed that oxymethylene protons at δ 3.63 and 3.90 were correlated with an methine proton at δ 3.30, which was in turn coupled to two olefinic protons at δ 7.84 and 6.14 as well as another methylene protons at δ 2.18 (H_a-6″) and 2.44 (H_b-6″). Analysis of the ^{13}C NMR data showed the signals

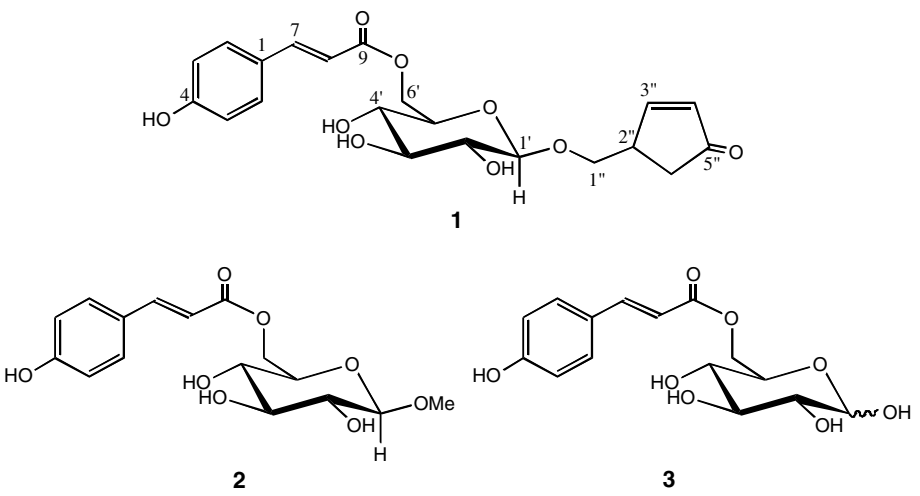

Fig. 1. Structures of compounds **1**–**3**.

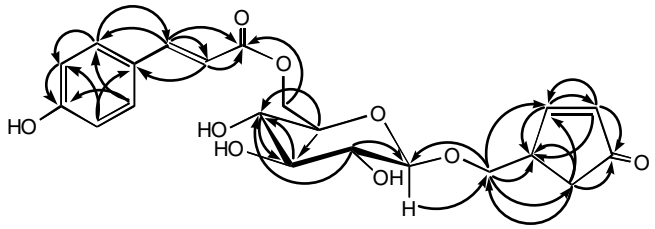

Fig. 2. HMBC correlations from H to C for flacourside (**1**).

for an oxymethylene (δ 72.7), an aliphatic methine (δ 43.7), an aliphatic methylene (δ 38.8), a carbonyl (δ 212.5) and two olefinic (δ 168.8 and 135.3) carbons for this moiety. These data and HMBC correlations shown in Fig. 2 unambiguously established the structure of the alcohol unit as 4-oxo-2-cyclopentenylmethanol. Finally, HMBC correlations as depicted in Fig. 2 evidenced the glycosidic linkage of the glucopyranoside and the alcohol unit. Hence, the structure of flacourside (**1**) was determined to be 4-oxo-2-cyclopentenylmethyl 6-*O*-(*E*)-*p*-coumaroyl-β-D-glucopyranoside. MS fragmentation pattern observed in the negative FABMS fully supported the assigned structure, showing intense fragment ions at *m*/*z* 339, 325 and 163 (Fig. 3).

The two known compounds, methyl 6-*O*-(*E*)-*p*-coumaroyl glucopyranoside (**2**) (Lu et al., 2000) and 6-*O*-(*E*)-*p*-coumaroyl glucopyranose (**3**) (Shimomura et al., 1988) were identified by comparing their ^{1}H and ^{13}C NMR data with the reported values. The former compound is rare and this paper is the second to isolate this compound from plants. Compound **3**, obtained as a 2:1 mixture of the β- and α-anomers, has been isolated from several plants such as *Prunus buergeriana* (Shimomura et al., 1988). We experienced that both anomers of **3** were separable in HPLC, but concentration of the separated sample-containing eluate gave a mixture of the same 2:1 ratio.

4-Oxo-2-cyclopentenemethanol has not been encountered in nature, although the chemical synthesis of both enantiomers were reported (Cortez, Tennyson, & Romo, 2001, Zanoni et al., 2001). Flacourside (**1**) is, therefore, the first natural product, which contains 4-oxo-2-cyclopentenemethanol unit. Literature survey revealed that the seed

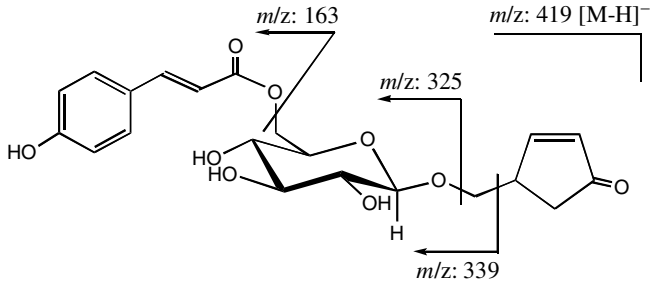

Fig. 3. FABMS(−) fragmentation pattern of flacourside (**1**).

oils of certain Flacourtiaceae plants contain 2-cyclopentenyl fatty acids (Francois & Pelt, 1961,Spener & Mangold, 1974) which are reportedly biosynthesized from aleprolic acid (2-cyclopentenecarboxylic acid) (Rehfeldt, Schulte, & Spener, 1980). It is, therefore, reasonable to assume that the biogenesis of 4-oxo-2-cyclopentenemethanol and aleprolic acid is linked each other. Oxidation at C-4 and reduction of the carboxylic function of aleprolic acid could yield the cyclopentenemethanol. Since the stereochemistry of natural chaulmoogric acid, one of 2-cyclopentenyl fatty acids obtained from Flacourtiaceae plants, was determined to be *S* (Mislow & Steinberg, 1955), it can be assumed that the configuration at the stereogenic center of the 4-oxo-2-cyclopentenemethanol moiety in **1** is *S*.

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