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Brauhenefloroside E and F; acylated flavonol glycosides from *Stocksia brauhica* Linn

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Two new acylated flavonol glycosides, 3-*O*-{[2-*O*-β-D-glucopyranosyl]-3-[*O*-β-D-glucopyranosyl]-4-[(6-*O*-*p*-coumaroyl)-*O*-β-D-glucopyranosyl]}-α-L-rhamnopyranosyl-kaempferol 7-*O*-α-L-rhamnopyranoside and 3-*O*-{2-[(6-*O*-*p*-coumaroyl)-*O*-β-D-glucopyranosyl]-3-[*O*-β-D-glucopyranosyl]-4-[(6-*O*-*p*-coumaroyl)-*O*-β-D-glucopyranosyl]}-α-L-rhamnopyranosyl-kaempferol 7-*O*-α-L-rhamnopyranoside, trivially named as brauhenefloroside E (1) and F (2), respectively, were isolated from the fruits of *Stocksia brauhica* and their structures were elucidated using spectroscopic methods, including 2D NMR experiments. Copyright © 2010 John Wiley & Sons, Ltd.

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Keywords: NMR; ^{1}H NMR; ^{13}C NMR; 2D NMR; acylated kaempferol glycosides; *Stocksia brauhica*

Introduction

Stocksia brauhica (Sapindaceae) is a monotypic genus found in Iran, Afghanistan, and Pakistan.[1] Our previous studies on the chemical constituents of this genus have led to the isolation of benzoic acid derivatives,[2] diphenyl acetic acid and an isoflavone derivative,[3] flavonol glycosides,[4] and saponins.[5] The current report describes the isolation and structure elucidation of two new acylated flavonol glycosides, brauhenefloroside E (**1**) and F (**2**) (Fig. 1) from the title plant.

Experimental

Methods

CC was done using silica gel, 70–230 mesh, 230–400 mesh, and Sephadex LH-20. Final purification was done on LC-908 W recycling HPLC (JAI Co. Ltd., Japan) attached with ODS-M-80 column (YMC). Thin layer chromatography (TLC) was carried out on E. Merck silica gel plates using the indicated solvents, B : A : W = 12 : 3 : 5 (*n*-butanol-AcOH-water), and detected at 254 nm and by ceric sulfate reagent. The IR and UV spectra were recorded on Jasco-320-A and Hitachi UV-240 spectrophotometers, respectively. Optical rotations were taken on Polartronic-D polarimeter using a 5-cm cell-tube. FAB-MS were recorded on a double focusing Varian MAT-312 spectrometer. The ^{1}H NMR spectra were recorded on either Bruker AC-300 or Bruker Avance 500 spectrometer in CD_3OD. The ^{13}C NMR spectra were recorded on either Bruker Avance 300 or Bruker Avance 500 spectrometer in CD_3OD. Chemical shifts in parts per million (δ), relative to the solvent peaks (δ_H 3.31 and δ_C 49.0 from CD_3OD) and scalar couplings were reported in Hertz. The 2D NMR spectra were recorded on either Bruker Avance 500 or 600 spectrometer. The pulse conditions were as follows: for the ^{1}H NMR spectra of compound **1**, spectrometer frequency (SF) 500.13 MHz, acquisition time (AQ) 1.586 s, number of transients (NS) 128, receiver gain (RG) 128, temperature (TE) 300 K, dummy scans (DS) 0, F_1 4896.17 Hz; for the ^{1}H NMR spectra of compound **2**, SF 300.13 MHz, AQ 2.916 s, NS 128, RG 8, TE 297 K, F_1 2642.64 Hz; for the ^{13}C NMR spectra of compound **1**, SF 75.467 MHz, AQ 1.769 s, NS 28011, RG 16384, TE 298 K, DS 2, F_1 17569.62 Hz; for the ^{13}C NMR spectra of compound **2**, SF 125.757 MHz, AQ 0.521 s, NS 37017, RG 16384, TE 300 K, DS 2, F_1 29554.32 Hz; for the COSY 45° spectra, SF01 600.232 MHz, NS 16, DS 4, pulse width (PW) 7.30 µs, TE 299 K, RG 71.8; for the NOESY experiments, SF 500.332 MHz, NS 16, PW 6.50 µs, F1L0 3980.35 Hz, F2L0 3992.09 Hz; for the HMQC Spectra, SF01 600.232 MHz, AQ 0.1066 s, NS 64, DS 16, RG 29193, TE 300 K, F1L0 22763.57 Hz, F2L0 4758.86 Hz; for the HMBC Spectra, SF01 600.232 MHz, SF02 150.945 MHz, AQ 0.4261 s, RG 32768, NS 64, DS 16, TE 300 K, F1L0 28689.21 Hz, F2L0 4878.58 Hz; for the 1D TOCSY spectra, SF01 600.232 MHz, AQ 0.213 s, F_1 5264.30 Hz, SW 4807.85 Hz, mixing time 120 ms.

Plant material

The plant *S. brauhica* (Sapindaceae) (4.75 kg) was collected from Quetta, Baluchistan, Pakistan, in 2002, and was identified by one of us (RBT). A voucher specimen (no. 535) has been deposited at the herbarium of the Botany Department, Baluchistan University, Quetta, Pakistan.

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Extraction and isolation

The air-dried fruits (2.25 kg) were separated manually from fruiting part (4.75 kg) of *S. brauhica* and were defatted with hexane and then extracted with methanol at room temperature (7 days × 3). The combined methanol extract (196.2 g) was suspended in water and partitioned with EtOAc, provided suspension which was then soluble in *n*-BuOH. The *n*-BuOH soluble portion (34.3 g) was subjected to column chromatography on silica gel using $CHCl_3$–MeOH gradient. The fraction designated as A eluted with 20–25% MeOH in $CHCl_3$ was subjected to Sephadex LH-20 (100% MeOH) and then to reverse phase flash chromatography using Lichrosphere RP-18 (H_2O : MeOH, 1 : 1), which yielded a semi-pure fraction of flavone glycosides. The semi-pure fraction was further purified on recycling HPLC (ODS-M80 column, 1 : 1 MeOH/H_2O, flow rate 4 ml/min) to yield compounds **1** (19 mg) and **2** (14 mg).

*Brauhenefloroside E (**1**)*

Yellow gum (19 mg); $C_{54}H_{66}O_{31}$. $[\alpha]_D^{24} = +7.8^\circ$ ($c = 0.024$, MeOH). UV λ_{max} (MeOH) nm: 268, 315. IR ν_{max} (KBr) cm^{-1}: 3460 (OH), 2925 (CH stretch), 2854 (CH stretch), 1721 (C═O), 1027–1130 (C–O–C asymmetric stretch), 825 (aromatic hydrogens on adjacent carbons). HR-FAB-MS (positive mode) *m/z*: 1233.2350 (calculated for $C_{54}H_{66}O_{31}$ + Na, 1233.3456). ^{1}H NMR (CD_3OD, 500.13 MHz) and ^{13}C NMR (CD_3OD, 75.467 MHz) (Table 1).

*Brauhenefloroside F (**2**)*

Yellow gum (14 mg); $C_{63}H_{72}O_{33}$. $[\alpha]_D^{24} = -12.0^\circ$ ($c = 0.019$, MeOH). UV λ_{max} (MeOH) nm: 269, 314. IR ν_{max} (KBr) cm^{-1}: 3420 (OH), 2962 (CH stretch), 2854 (CH stretch), 1721 (C═O), 1027–1130 (C–O–C asymmetric stretch), 803 (aromatic hydrogens on adjacent carbons). HR-FAB-MS (positive mode) *m/z*: 1379.2332. (calculated for $C_{63}H_{72}O_{33}$ + Na, 1379.2341). ^{1}H NMR (CD_3OD, 300.13 MHz) and ^{13}C NMR data (CD_3OD, 125.757 MHz) (Table1).

Acid hydrolysis of 1 and 2

Compounds **1** and**2** (3 mg each) in MeOH (5 ml) were hydrolyzed with 10% aq. HCl for 3 h at 100 °C. On cooling, the aglycone was extracted with EtOAc. The aqueous hydrolysate was neutralized with silver carbonate and concentrated; the sugars were found to be glucose and rhamnose by co-TLC with the standard solvent system EtOAc : MeOH : HOAc : H_2O (11 : 2 : 2 : 2).

Determination of absolute configuration of sugars

The absolute configuration of sugars was determined as follows.[5] The concentrated residue of the hydrolyzed sugars in pyridine and L-cysteine methyl ester hydrochloride was mixed, and the solution was warmed at 60 °C for 1 h. Acetic anhydride was then added and the mixture was then warmed at 90 °C for another 1 h. After evaporation of pyridine and acetic anhydride *in vacuo*, each residue was dissolved in acetone and the solution was subjected to GC under the following conditions.

Capillary column: SBP 5 (0.5 μm; 15 m × 0.53 mm); column temperature: 220 °C; injection temperature: 270 °C; carrier gas: N_2. The retention times for β-D-glucose and α-L-rhamnose were found to be 6.4 and 3.5 min, respectively.

Results and Discussion

Brauhenefloroside E (**1**) was obtained as a yellow gummy material and its molecular formula, $C_{54}H_{66}O_{31}$, was established from a pseudo-molecular ion peak at *m/z* 1233.2350 $[M + Na]^+$ in a high resolution mass spectrum (HR-FAB-MS). The UV spectrum of **1** in methanol had absorption maxima at 268 and 315 nm, which were typical for flavonol glycosides.[6] The bathochromic shift (47 nm) in band I with $AlCl_3$-HCl and no shift in band II with NaOAc suggested that **1** was a 3,7-disubstituted flavonol glycoside.[7,8]

The aglycone was confirmed as kaempferol by comparison of spectroscopic data with the literature[7] (Supplementary figures have been deposited). Besides the signals of glycone and aglycone, the ^{1}H NMR showed high frequency signals as doublets at δ 7.47 ($J = 15.9$ Hz, 1H) and 6.18 ($J = 15.9$ Hz, 1H) due to *E* configured olefinic protons while the two doublets at δ 7.02 ($J = 8.5$ Hz, 2H) and 6.29 ($J = 8.5$ Hz, 2H) were assigned to H-5^g/9^g and H-6^g/8^g of the *p*-coumaroyl moiety, respectively.

The ^{13}C NMR showed 50 signals of which 13 were attributed to kaempferol skeleton, 30 to the sugar units and 7 to the coumaroyl moiety.

The ^{1}H NMR signals of the sugar moieties indicated the presence of three D-glucopyranosyl and two L-rhamnopyranosyl units, further supported by 1D-TOCSY experiment. Based on the anomeric proton coupling constants ($^3J_{H1,H2}$), glucopyranosyl (7.8–8.0 Hz), and rhamnopyranosyl (0–0.9 Hz) residues were depicted to have β and α configurations, respectively. In ^{13}C NMR spectrum of **1** (Table 1) the C-2^c, C-3^c, and C-4^c of the central Rha moiety shifted to higher frequencies (δ_C 80.5, 80.9, 78.7, respectively) helping directly to establish the location of glycosylation.[9] Furthermore, the interglycosidic linkages in **1** were also supported by the HMBC studies (Fig. 2). Thus cross peaks between H-1^d of Glc I (δ_H 4.55) and C-2^c of Rha II (δ_C 80.5), H-1^e of Glc II (δ_H 4.70) and C-3^c of Rha II (δ_C 80.9), and H-1^f of Glc III (δ_H 4.68) and C-4^c of Rha II (δ_C 78.7) indicated that the three β-D-glucopyranosyl moieties (Glc I, II and III) were linked to C-2^c, C-3^c and C-4^c of Rha II, respectively. Similarly, the cross peaks between H-1^c of Rha II residue (δ_H 5.70) and C-3 of the kaempferol (δ_C 135.6) indicated that the tetrasaccharide chain was attached to C-3 of the kaempferol *via* C-1^c of Rha II. The higher frequency chemical shift at δ_C 65.3 of hydroxymethylene of Glc III (C-6^f) in the ^{13}C NMR spectrum indicated its attachment with coumaroyl moiety. This was also supported by the HMBC correlation of H-6^f (δ 4.60) with carbonyl carbon of coumaroyl moiety (δ 169.0). The anomeric proton of Rha I at δ_H 5.54 showed a long-range correlation in HMBC with δ_C 163.5 showing its connectivity at C-7 of the kaempferol moiety. On the basis of these observations, the structure of **1** was deduced as 3-*O*-{[2-*O*-β-D-glucopyranosyl]-3-[*O*-β-D-glucopyranosyl]-4-[(6-*O*-*p*-coumaroyl)-*O*-β-D-glucopyranosyl]}-α-L-rhamnopyranosyl-kaempferol 7-*O*-α-L-rhamnopyranoside.

Acid hydrolysis of **1** yielded kaempferol and two sugar moieties, which were identified as glucose and rhamnose by co-TLC with authentic samples, while the absolute configuration of sugars was determined by subjecting them to GC as thiazolidine derivatives (see Section on Experimental).

Brauhenefloroside F (**2**), a yellow gummy material, displayed a pseudo-molecular ion peak in HR-FAB-MS at *m/z* 1379.2332 $[M + Na]^+$ corresponding to a molecular formula $C_{63}H_{72}O_{31}$. Its

Table 1. ^{1}H and ^{13}C NMR data of compounds **1** and **2** in CD_3OD

	1		**2**	
Position	δ_H (J = Hz)	δ_C (ppm)	δ_H (J = Hz)	δ_C (ppm)
2	–	158.6	–	158.1
3	–	135.6	–	136.1
4	–	179.2	–	179.2
5	–	163.9	–	162.8
6	6.36 d (1.8)	100.6	6.32 d (2.0)	100.5
7	–	163.5	–	163.2
8	6.52 br s	95.3	6.37 br s	95.4
9	–	160.7	–	161.1
10	–	107.4	–	107.6
1[a]	–	122.3	–	122.1
2[a]	7.74 d (8.6)	132.0	7.68 d (8.7)	131.9
3[a]	6.93 d (8.6)	117.4	6.89 d (8.7)	116.7
4[a]	–	161.8	–	162.6
5[a]	6.93 d (8.6)	117.4	6.89 d (8.7)	116.7
6[a]	7.74 d (8.6)	132.0	7.68 d (8.7)	131.9
Rha I				
1[b]	5.54 s	99.3	5.55 s	99.4
2[b]	4.02 dd (1.7, 3.2)	71.9	4.02 dd (1.6, 3.3)	71.5
3[b]	3.84 t (3.4)	72.0	3.85 dd (3.5, 9.5)	71.5
4[b]	3.50 t (9.5)	73.6	3.52 t (9.4)	73.7
5[b]	3.61 m	70.8	3.82 m	71.1
6[b]	1.27 d (6.1)	18.1	1.28 d (6.1)	17.9
Rha II				
1[c]	5.70 d (0.9)	101.5	5.68 d (0.9)	101.5
2[c]	4.42 br s	80.5	4.47 br s	80.9
3[c]	4.05 dd (3.5, 9.0)	80.9	4.15 dd (3.0, 9.0)	81.8
4[c]	3.42 t (9.0)	78.7	3.75 t (10.0)	78.6
5[c]	3.20 m	71.1	3.49 m	70.9
6[c]	0.95 d (6.2)	17.9	1.03 d (6.1)	18.1
Glc I				
1[d]	4.55 d (7.9)	105.9	4.51 d (7.8)	106.1
2[d]	3.22 t (8.5)	77.9	3.72 t (9.5)	77.2
3[d]	3.02 t (8.5)	75.8	3.28 t (9.0)	75.2
4[d]	3.44 t (8.0)	72.1	3.58 t (9.0)	72.5
5[d]	3.71 t (8.5)	78.2	3.42 t (9.0)	78.3
6[d]	3.81 dd (12.0, 3.5) 3.61 dd (12.6, 5.4)	62.9	4.60 dd (11.5, 3.5) 4.50 dd (11.5, 4.0)	64.5
Glc II				
1[e]	4.70 d (8.0)	105.4	4.72 d (7.9)	105.4
2[e]	3.40 t (9.5)	78.2	3.08 t (8.0)	75.4
3[e]	3.70 t (9.5)	76.3	3.24 t (8.0)	76.0
4[e]	3.21 t (9.5)	71.6	3.67 t (9.5)	71.7
5[e]	3.26 t (10.0)	78.4	3.26 t (10.0)	77.7
6[e]	3.80 dd (12.5, 4.5) 3.71 dd (12.0, 5.1)	62.5	3.81 dd (11.0, 3.5) 3.61 dd (10.5, 3.0)	62.8
Glc III				
1[f]	4.68 d (7.9)	104.0	4.70 d (7.9)	104.0
2[f]	3.05 t (8.5)	76.4	3.05 t (8.8)	75.8
3[f]	3.29 t (9.5)	77.6	3.43 t (9.5)	77.6
4[f]	3.25 t (9.0)	71.7	3.65 t (8.5)	72.1
5[f]	3.32 t (9.0)	77.6	3.70 t (8.5)	78.2
6[f]	4.60 dd (12.5, 4.0) 4.40 dd (12.5, 5.4)	65.3	4.47 dd (12.5, 5.0) 4.16 dd (12.0, 4.0)	65.3
p-coumaroyl I				
1[g]	–	169.0	–	169.2
2[g]	6.18 d (15.9)	114.9	6.12 d (16.0)	114.9
3[g]	7.47 d (15.9)	146.6	7.48 d (16.0)	146.5
4[g]	–	126.3	–	126.3

Table 1. (*Continued*)

Position	1		2	
	δ_H (J = Hz)	δ_C (ppm)	δ_H (J = Hz)	δ_C (ppm)
5[g]	7.02 d (8.5)	130.4	7.24 d (8.5)	130.4
6[g]	6.29 d (8.5)	116.3	6.64 d (8.5)	116.3
7[g]	–	160.7	–	160.7
8[g]	6.29 d (8.5)	116.3	6.64 d (8.5)	116.3
9[g]	7.02 d (8.5)	130.4	7.24 d (8.5)	130.4
p-coumaroyl II				
1[h]	–	–	–	169.2
2[h]	–	–	6.18 d (15.9)	114.4
3[h]	–	–	7.42 d (15.9)	146.8
4[h]	–	–	–	126.5
5[h]	–	–	6.94 d (8.5)	131.4
6[h]	–	–	6.67 d (8.5)	116.6
7[h]	–	–	–	160.9
8[h]	–	–	6.67 d (8.5)	116.6
9[h]	–	–	6.94 d (8.5)	131.4

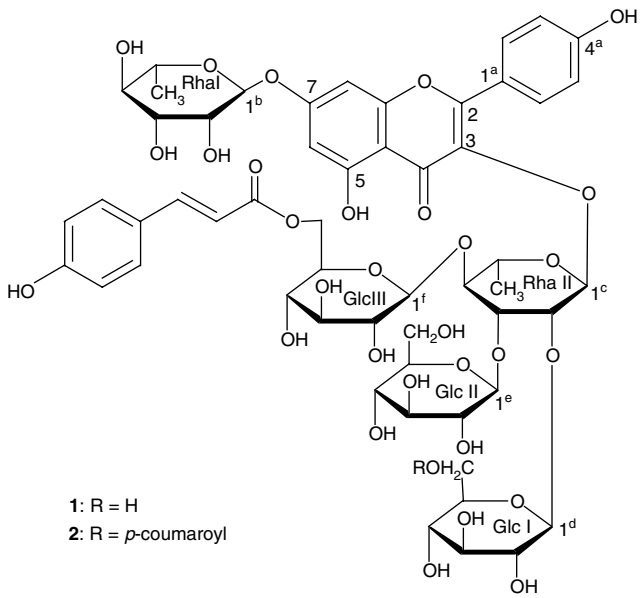

Figure 1. Structures of **1** and **2**.

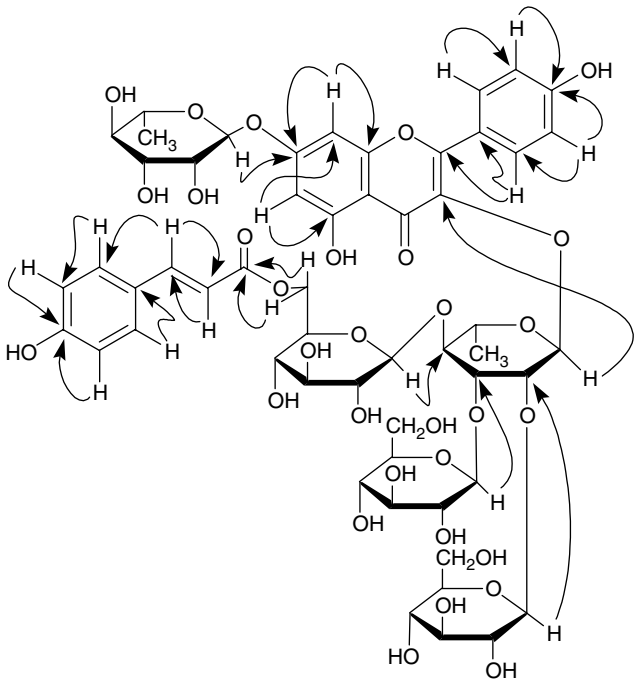

Figure 2. Important HMBC correlations of **1**.

spectroscopic data consisting of ^{1}H- and ^{13}C NMR, ^{1}H–^{1}H COSY, HMQC, and HMBC were very similar to those of **1** suggesting it to be an analogue of **1**. The increment of 146 units in FAB-MS and higher frequency signals in ^{1}H NMR at δ 7.42 (d, J = 15.9 Hz, 1H) and 6.18 (d, J = 15.9 Hz, 1H), and two doublets at δ 6.94 (J = 8.5 Hz, 2H) and 6.67 (J = 8.5 Hz, 2H) indicated the presence of an additional *p*-coumaroyl moiety in **2**. The higher frequency chemical shift of hydroxymethylene (C-6[d]) at δ_C 64.5 revealed the attachment of a coumaroyl moiety at Glc I. The cross peaks due to long-range correlations (Fig. 3) between H-6[d] (δ 4.60) and carbonyl carbon of coumaroyl at δ_C 169.2 confirmed the linkage of second coumaroyl residue to C-6[d] of Glc I. Thus, compound **2** was characterized as 3-*O*-{2-[(6-*O*-*p*-coumaroyl)-*O*-β-D-glucopyranosyl]-3-[*O*-β-D-glucopyranosyl]-4-[(6-*O*-*p*-coumaroyl)-*O*-β-D-glucopyranosyl]}-α-L-rhamnopyranosyl-kaempferol 7-*O*-α-L-rhamnopyranoside.

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Supporting information

Supporting information may be found in the online version of this article.

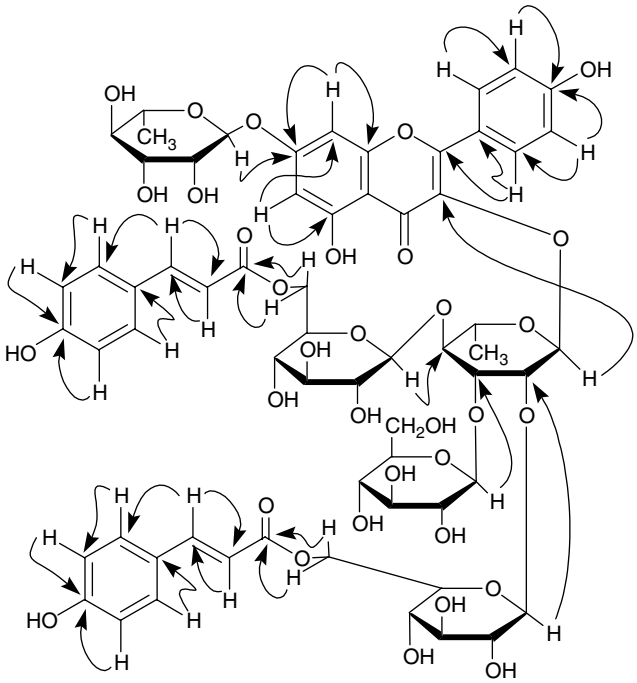

Figure 3. Important HMBC correlations of **2**.

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