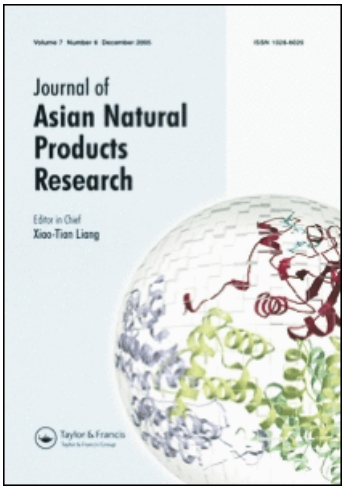

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A new acylated flavone glycoside from the fruits of *Stocksia brauhica*

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Phytochemical investigations of the fruits of *Stocksia brauhica* (Sapindaceae) resulted in the isolation of a new acylated flavone glycoside. Its structure of the new compound brauhenefloroside D (**1**) was established as 3-*O*-[(α-L-rhamnopyranosyl)oxy]-7-*O*-[(acetyl)-β-D-glucopyranosyl (1 → 4)]-[6-*O*-(4-hydroxy-*E*-cinnamoyl)-β-D-glucopyranosyl-(1 → 2)-α-L-rhamnopyranosyl)-oxy]-kaempferol. The structure elucidation of the new compound was based primarily on 1D and 2D NMR analysis, including COSY, HMBC and HMQC correlations.

Keywords: *Stocksia brauhica*; Sapindaceae; Acylated flavone glycosides; Brauhenefloroside D

1. Introduction

Stocksia brauhica (Sapindaceae) is a monotypic genus found in Iran, Afghanistan and Pakistan. It is a shrub up to 4 m tall and found in upper Baluchistan (Pakistan) [1]. The interesting metabolites from various plants of the family Sapindaceae [2–5] attracted our attention to investigate *Stocksia brauhica.* Plant species of Sapindaceae are known for their traditional medicinal uses as diuretic, stimulant, expectorant, natural selective vermifuge and against stomach ache and dermatitis in many parts of the world [5–8]. Some of its species, such as *Paullina cupania* is well known for its stimulant properties [9–10]. *Cupania vernalis* has antifungal activity. *Dodonaea viscosa* Linn. possesses hypoglycemic activity. Leaves and young stems of *Allophyllus edulis* are used as refresher, digestant, to a lesser extent in the treatment of hepatitis [11]. In this paper we wish to describe the isolation and characterisation of a new acylated flavone glycoside.

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2. Results and discussion

The concentrated MeOH extract of the fruits of *Stocksia brauhica* was partitioned between *n*-butanol and water. The BuOH soluble extract was subjected to silica gel and *Sephadex LH-20* column chromatography and recycling HPLC yielded flavone glycoside, brauhenefloroside **1** (figure 1).

Brauhenefloroside D **1** was obtained as yellow gummy material, which had the molecular formula $C_{50}H_{58}O_{27}$ as supported by HR-MS 1090.3368 and ^{13}C NMR analysis. The UV spectrum combined with the ^{1}H and ^{13}C NMR data indicated the flavone skeleton with oxy-substituents at C-3 [12], C-7 and free OH groups at C-5, C-4′. Its IR spectrum (KBr) exhibited absorptions at 3440 (OH), 2925 (CH), 2859 (C═C), 1722 (C═O), 1027–1130 (*O*-glycosidic linkage) and 825 cm.$^{-1}$ The EI-MS showed the aglycone peak at *m*/*z* 286. The positive-ion FAB mass spectrum exhibited the protonated molecular ion peak at *m*/*z* 1091 and negative ion FAB mass spectrum exhibited the $(M - H)^-$ peak at *m*/*z* 1089, which confirms the mass of **1** as 1090.

The ^{13}C NMR spectrum of **1** (see table 1) showed the presence of 50 carbons, which were resolved through DEPT experiment as three methyl, two methylene, thirty two methine and thirteen quaternary carbons.

An aglycone was recognized as kaempferol [13] with the help of ^{1}H and ^{13}C NMR, HMQC, HMBC and COSY correlations (figure 2). The ^{1}H NMR spectrum showed a pair of doublets at δ 7.68 (d, $J = 8.6$ Hz) and 6.92 (d, $J = 8.6$ Hz), which were assigned to δ 131.9 (C-2′) and 116.6 (C-3′) respectively (assigned via HMQC). The same value of coupling constant and mutual interaction of these protons in the COSY 45° experiment confirmed that both were *ortho* to each other which was further supported by strong HMBC interactions of these protons with δ 162.0 (C-2), 122.4 (C-1′) and 161.2 (C-4′) and confirmed their positions in ring B of flavonoid. In the same spectrum a doublet at δ 6.34 ($J = 1.8$ Hz) and a broad singlet at δ 6.54 each with one proton integration and were correlated to carbons at δ 100.3 and 95.5 respectively (assigned via HMQC). These chemical shifts are characteristic values for C-6 and C-8 [14] in ring A of the flavonoid and further supported by strong HMBC interactions of these protons with δ 157.7 (C-5) and 163.7 (C-7) indicated that both these were *meta* coupled.

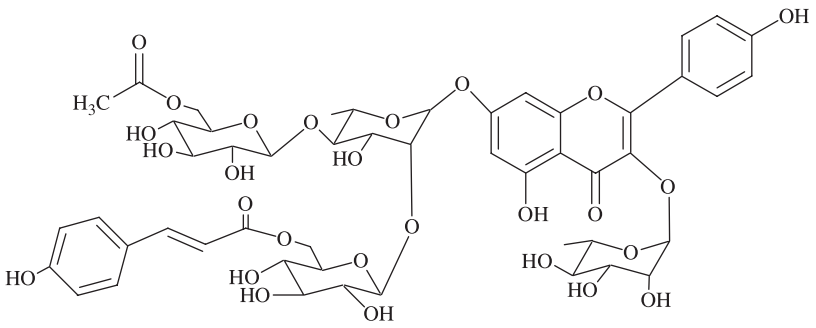

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Figure 1. Acylated flavone glycoside **1** from *Stocksia brauhica*.

Table 1. NMR data of compound **1** in CD_3OD-d_6 (500 MHz for ^1H, 125 for ^{13}C, δ in ppm).

Positions	$C^{\dagger}$	δ_C	δ_H (*J in Hz*)
Aglycone			
2	C	159.0	
3	C	135.3	
4	C	180.2	
5	C	157.7	
6	CH	100.3	6.25 (d, $J = 1.8$)
7	C	163.4	
8	CH	95.5	6.54 (br, s)
9	C	162.0	
10	C	107.4	
1′	C	122.4	
2′	CH	131.9	7.68 (d, $J = 8.6$)
3′	CH	116.6	6.92 (d, $J = 8.6$)
4′	C	161.2	
5′	CH	116.6	6.92 (d, $J = 8.6$)
6′	CH	131.9	7.68 (d, $J = 8.6$)
Rham I			
1	CH	101.2	5.72 (br, s)
2	CH	71.1	4.04 (br, s)
3	CH	71.7	3.82 (t, $J = 3.1$)
4	CH	73.2	3.49 (t, $J = 9.4$)
5	CH	71.1	3.61 (m)
6	CH_3	17.9	1.32 (d, $J = 6.1$)
Rham II			
1	CH	99.9	5.55 (br, s)
2	CH	81.0	4.44 (t, $J = 3.5$)
3	CH	71.9	3.33 (dd, $J = 3.5$, 9.0)
4	CH	78.7	3.41 (t, $J = 9.0$)
5	CH	70.2	3.63 (m)
6	CH_3	18.1	0.98 (d, $J = 6.1$)
Glc I			
1	CH	104.0	4.68 (d, $J = 7.8$)
2	CH	75.4	3.20 (t, $J = 8.5$)
3	CH	78.7	3.36 (t, $J = 8.3$)
4	CH	72.7	3.25 (t, $J = 8.5$)
5	CH	77.9	3.42 (m)
6	CH_2	64.3	4.40 (dd, $J = 5.1$, 12.0)
			4.60 (dd, $J = 3.5$, 12.0)
Glc II			
1	CH	105.4	4.72 (d, $J = 7.4$)
2	CH	75.3	3.22 (t, $J = 8.0$)
3	CH	78.2	3.39 (t, $J = 8.4$)
4	CH	72.1	3.82 (t, $J = 8.5$)
5	CH	77.6	3.30 (m)
6	CH_2	65.0	4.16 (dd, $J = 4.5$ 12.3)
			4.30 (dd, $J = 5.0$, 12.0)
p-Coumaroyl moiety			
1	C	169.5	
2	CH	114.9	6.15 (d, $J = 15.9$)
3	CH	146.3	7.49 (d, $J = 15.9$)
4	C	126.2	
5	CH	131.0	7.27 (d, $J = 8.5$)
6	CH	116.6	6.69 (d, $J = 8.5$)
7	C	160.7	
8	CH	116.6	6.69 (d, $J = 8.5$)
9	CH	131.0	7.27 (d, $J = 8.5$)
Ac			
1	C	169.9	
2	CH_3	24.4	1.92 (s)

† Assignment based on HMQC experiments.

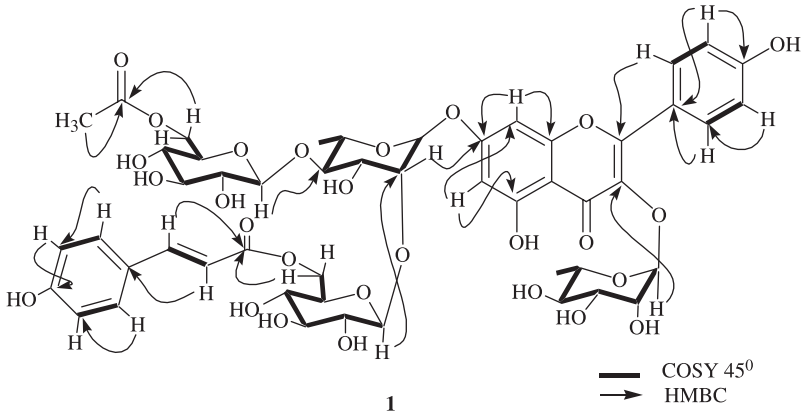

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Figure 2. Important HMBC and $^{1}H-^{1}H$ COSY correlations of **1**.

The ^{1}H and ^{13}C NMR spectra of **1** indicated the presence of four monosaccharide units including two 6-deoxy sugars, suggested by the four anomeric protons at δ 5.72 (s), 5.55 (s), 4.72 (d, $J = 7.4$ Hz) and 4.68 (d, $J = 7.8$ Hz) which correlated in the HMQC spectrum with carbons at δ 101.2, 99.9, 105.4 and 104.0 respectively. The ring protons of the monosaccharide residues were assigned starting from the anomeric protons by means of the COSY, HMQC and HMBC NMR plots and their coupling constant by 2D *J*-resolved (table 1). Evaluation of spin–spin couplings and chemical shifts allowed the identification of two α-L-rhamnopyranosyl (Rha), two β-D glucopyranosyl (Glc), acetate and a *p*-coumaroyl moiety.

The β-orientation of anomeric centers of glucose was supported by considering the *J* values of anomeric protons (7.4 and 7.8 Hz) whereas the α-orientation of rhamnopyranosyl residues was supported by their ^{13}C NMR shifts [15,16]. The downfield chemical shifts of C-2 at δ 81.0 and C-4 at δ 78.7 of Rha II, provided evidence for the sites of attachment of Rha II to Glc I and Glc II respectively.

The position of attachment of glycoside moieties with the aglycone was confirmed by the $^{3}J_{CH}$ interactions of δ 5.5 (H-1 of Rha II) to 163.4 (C-7 of aglycone) and 5.72 (H-1 of Rha I) to 135.4 (C-3 of aglycone).

The ^{1}H and ^{13}C NMR spectra of **1** were in full agreement with the proposed structure. The data were consistent with the presence of two rhamnose, two glucose, acetate and a *p*-coumaroyl unit. The location of a *p*-coumaroyl and acetate moiety was determined to be at C-6 hydroxyl group of the glucose I and glucose II. All sugar protons and carbons assigned by COSY and HMQC experiment. The signal of H-5 of glucose I at δ 3.42 correlated to downfield signals δ 4.40 and 4.60 assigned to H-6a and H-6b, further correlated to carbonyl carbon at δ 169.5 in the HMBC experiment. The correlation of *trans* olefinic proton at δ 7.49 to 169.5 indicated that this is the part of *p*-coumaroyl moiety attached to the glucose I (1 → 2) α-L-rhamnose. In the same way the location of acetyl group was determined at H-6 of glucose II in which δ 4.16 and 4.30 (H-6a and H-6b) correlated to 169.9, a carbonyl of an acetate showed the correlation with δ 1.29 (CH_3) in HMBC experiment.

p-Coumaroyl moiety was also confirmed by the ^{1}H NMR spectra. A spectrum showed the typical pairs of doublets, one with a coupling constant of 15.9 Hz (C-2 and C-3), identifying

the *trans* olefinic protons and the other caused by the four aromatic ring protons which were *ortho* coupled giving the coupling constant $J = 8.5$ Hz.

Acid hydrolysis of **1** yielded aglycone and two sugar moieties, which were identified as glucose and rhamnose by co-TLC with authentic samples, while the absolute configuration of sugars were identified by subjecting them on GC as a thiazolidine derivative.

All these cumulative results confirmed the structure of **1** as 3-*O*-[(α-L-rhamnopyranosyl)oxy]-7-*O*-[6-*O*-(acetyl)-β-D-glucopyranosyl(1 → 4)]-[6-*O*-(4-hydroxy-*E*-cinnamoyl)- β-D-glucopyranosyl-(1 → 2)-α-L-rhamnopyranosyl)-oxy]-kaempferol.

3. Experimental

3.1 General experimental procedures

Column chromatography (cc) was carried out on silica gel, 70–230 mesh, 230–400 mesh, Sephadex LH-20. HPLC was performed on recycling preperative HPLC from Japan Analytical Industry Co., Ltd. TLC was carried out on E. Merck silica gel plates using the indicated solvents: BAW = 12:3:5 butanol/AcOH/water; EtOAc/MeOH/HOAc/H_2O, 11:2:2:2 (for sugars), detected at 254 nm. TLC sprayed with ceric sulphate reagent and aniline phthalate (for sugars). The IR- and UV-spectra were recorded on a Jasco-320-A and Hitachi-UV-240, respectively. Optical rotation was recorded on Schmidt and Haensch polatronic-D polarimeter. GC of sugars derivative was carried out on a Shimadzu GC-17 A (capillary column, SPB5 15 m × 0.53 mm × 0.50 μm). Fast atomic bombardment mass spectra (FAB-MS) were recorded on a double focusing Varian MAT-312 spectrometer. ^{1}H NMR and ^{13}C NMR, COSY, HMQC, HMBC and 2D *J*-resolved spectra in CD_3OD for **1** at 500 and 125 MHz, respectively, using an AM 500 Bruker Spectrometers. Chemical shifts δ in ppm and coupling constants in Hz. EI: JMS-HX-110 with a data system.

3.2 Plant material

The plant of *Stocksia 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.

3.3 Extraction and isolation

The fruits of plant (2.25 kg) were crushed and first extracted three times with hexane and then with methanol (15 L each, three times) at room temperature. The resulting methanol extract (196.2 g) was suspended in water and partitioned with ethyl acetate, provided suspension which was then soluble in *n*-butanol and successively partitioned with water gave *n*-butanol (34.3 g) fractions. The butanolic extract was subjected to column chromatography on silica gel using a gradient of methanol in $CHCl_3$. The fraction eluted with 15–20% (A) methanol in $CHCl_3$ was then subjected to repeated column chromatography on silica column provided fraction A-1. This fraction was then subjected to Sephadex LH-20 and eluted A-2 with 10–15% methanol in water, which were free from impurities. Compound **1** was purified on

recycling HPLC using a reverse phase semi preparative (M-80 column) from fraction A-2. Elution was carried out at a flow rate of 4 ml/min under isocratic condition with MeOH/H_2O (50:50). The peaks were detected by UV (254 nm) and RI detector. The peaks of compound **1** (8 mg) was obtained at retention time of 48 min.

3.3.1 Compound 1. Yellowish gummy material (8 mg). $[\alpha]_D^{24} = -17.5$ ($c = 0.019$) $R_f = 0.54$ (BuOH/AcOH/H_2O, 12:3:5). UV λ_{max}(MeOH): 193; 205, 268, 315 nm. Its IR spectrum (KBr) exhibited absorptions at ν_{max}3460 (OH), 2925 (CH), 2854 (C═C), 1721 (C═O), 1027–1130 (*O*-glycosidic linkage) and 825 cm^{-1}; EI-MS; *m/z* 286, Positive FAB MS: *m/z* 1091 $[M + H]^+$, Negative FAB MS *m/z* 1089$[M - H]^-$, HR-MS *m/z* 1090.3368 $[M]^+$ (calcd for $C_{50}H_{58}O_{27}$, 1090.3170), ^1H (MeOH, 500 MHz) and ^{13}C (MeOH, 125 MHz) NMR spectral data.

3.4 Acid hydrolysis of 1

Compound **1** (3 mg) in MeOH (5 ml) was hydrolyzed with 10% aq HCl for 3 h at 100°C. On cooling, the aglycone was extracted by 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).

3.5 Synthesis of L-cysteine methyl ester hydrochloride

L-Cysteine was converted into the corresponding methyl ester hydrochloride salt as follows: to a −10°C solution (3.6 M) of thionyl chloride (3.6 equiv.) in methanol was added the α-amino acid (1 equiv.). After being stirred for 24 h at room temperature, the reaction mixture was concentrated under reduced pressure. Subsequent treatment of the crude material with ether led to the precipitation of the desired α-amino ester hydrochloride salt [17].

3.6 Determination of absolute configuration of sugars

The concentrated residue of the hydrolyzed sugar in pyridine (100 μl) and L-cysteine methyl ester hydrochloride (0.06 mol/L) were mixed, and the solution was warmed at 60°C for 1 h. Acetic anhydride (150 μl) was then added, the mixture was warmed at 90°C for another 1 h. After evaporation of pyridine and acetic anhydride *in vacuo*, each residue was dissolved in acetone (350 μl) and the solution (1 μl) was subjected to GC under the following conditions capillary column, SPB5 (15 m × 0.53 mm × 0.50 μm); column temperature, 220°C; injection temperature, 270°C; carrier N_2 gas. A peak for a peracetylated thiazolidine derivative with retention time at 6.4 and 3.5 min was observed for **1**, which was identical to the derivative of authentic D-glucose and L-rhamnose prepared in the same manner [18].

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