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Flavonoid Glycoside and Long Chain Ester from the Roots of *Vitex negundo*

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Vitexoside, new flavonoid glycoside and hexatetracontanoic acid derivative have been isolated from the roots of *Vitex negundo* and assigned structures sakuranetin 4′-*O*(6′′-*O*-α-L-rhamnopyranosyl)-β-D-glucopyranoside **1** and 3-(3-methoxy-4-hydroxyphenyl) propyl hexatetracontanoate **2** respectively. In addition agnoside **3,** 5-hydroxy-1,3-benzenodicarboxylic acid **4**, *R*-dalbergiphenol **5** and *R*-4-methoxy dalbergione **6** were also isolated for the first time from this species.

Key words: *Vitex negundo*, Verbenaceae, flavanone glycoside, hexatetracontanoic acid derivative

Vitex negundo Linn. (syn: *V. inesia* Lam.) a deciduous shrub belongs to family Verbenaceae which comprises 75 genera and nearly 2500 species, chiefly occurring in Pakistan, India and Ceylon [1,2]. Though almost all plant parts are used, the extract from leaves and roots is the most important in the field of medicine and is sold as drug [3]. The leaf extract is used in Ayurvedic and Unani system of medicine [4]. The decoction of leaves is considered as tonic, vermifuge and is given along with long pepper in catarrhal fever [3]. Water extract of mature fresh leaves exhibits anti-inflammatory, analgesic and antihistamine properties [5]. The methanol extract of roots possesses potent snake venom neutralizing capacity [6]. The acetone extract of *Vitex negundo* was found to possess insecticidal, ovicidal, feeding deterrence, growth inhibition and morphogenetic effects against various life stages of a noxious lepidoteron insect-pest, *Spilarctia obliqua* Walker [7]. Literature survey of *V. negundo* revealed the presence of volatile oil [8], triterpenes [9], diterpenes [10], sesquiterpenes [11], lignan [12], flavonoids [13], flavone glycosides [14], iridoid glycosides [15–17] and stilbene derivative [18]. In the present paper we report the isolation and structures of new flavanone glycoside **1** and hexatetracontanoic acid derivative **2**, respectively, besides agnoside **3,** 5-hydroxy-1,3-benzenodicarboxylic acid **4**, *R*-dalbergiphenol **5** and *R*-4-methoxy dalbergione **6,** reported for the first time from this species.

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RESULTS AND DISCUSSION

Compound **1** was isolated as amorphous white solid, with the molecular formula $C_{28}H_{34}O_{14}$ (HR-FAB-MAS: *m/z* 594.2567). The compound showed UV absorption maxima at 340, 313 and 273 nm, characteristic of flavanone-*O*-glycoside [19,20]. The IR spectrum exhibited absorption bands at 3200–3500, 2840, 1668, 1580, and 1370 cm^{-1} that revealed the presence of chelated hydroxyl, conjugated carbonyl and aromatic functionalities respectively. A signal at δ 12.50 for a chelated hydroxyl group and two *meta* coupled aromatic protons at δ 6.61 (d, J = 2.1 Hz) and δ 6.50 (d, J = 2.1 Hz) were diagnostic for a C-5 and C-7 oxygenated A ring. This was further supported by the mass fragment at *m/z* 167 representing ring A with one hydroxyl and one methoxyl group. For the B ring the ^{1}H-NMR spectrum showed four aromatic protons with AA′ BB′ system at δ 7.60 (2H, d, J = 8.8 Hz) and δ 7.04 (2H, d, J = 8.8 Hz) assigned to H-2′/H-6′ and H-3′/H-5′ respectively. The appearance of three doublets of doublet at δ 5.50 (J = 13, 3H3), δ 3.20 (J = 17, 13H3), and δ 2.70 (J = 17, 3H3) are characteristic for heterocyclic ring C. The presence of two sugar unit in β- and α-configurations was evident by the signals of anomeric protons at δ 5.64 (d, J = 7.2 Hz) and δ 5.40 (d, J = 1.5 Hz). The presence of methyl doublet at δ 1.55 (J = 6.8 Hz) identified one of the sugar unit as rhamnose. The ^{13}C-NMR spectrum (BB and DEPT) revealed the presence of one methyl, two methylene, one methoxy, seventeen methine and seven quaternary carbons in the molecule. The downfield signal at δ 196.9 was assigned to the carbonyl group, while signals of methoxyl and methyl groups were respectively observed at δ 55.2 and δ 18.5. The ^{1}H and ^{13}C-NMR data of flavanone skeleton was in complete agreement to those of sakuranetin. Vitexoside **1** is, therefore, glycoside of sakuranetin. Acid hydrolysis of **1** provided D-glucose and L-rhamnose. In the EIMS the peak at *m/z* 430 resulted from the loss of rhamnose moiety revealing its presence at the terminal position and the attachment of glucose moiety to the aglycone. The position of attachment of the rhamnose moiety was shown to be at the methylene carbon of the glucose unit as the latter showed downfield shift of ~ 7 ppm compared to glucose. The structure was fully supported by HMBC correlations. The anomeric proton of the glucose moiety at δ 5.64 showed ^3J correlations with signal at δ 161.4 (C-4′), δ 77.5 (C-3′′), δ 78.3 (C-5′′) and ^2J correlations at δ 74.5 (C-2′′). The anomeric proton of rhamnose moiety at δ 5.40 showed ^3J correlation with signals at δ 69.7 (C-6′′), δ 71.2 (C-3′′′) and δ 71.2 (C-5′′′) and ^2J correlation with signals at δ 72.7 (C-2′′′). The HMBC correlations of the aglycone moiety were in accordance to sakuranetin skeleton (Fig. 1). Thus vitexoside **1** was assigned the structure sakuranetin 4′-*O*(6′′-*O*-α-L-rhamnopyranosyl)-β-D-glucopyranoside.

Compound **2** was obtained as oil which showed the molecular ion peak at *m/z* 840 in FDMS. The molecular formula was determined as $C_{56}H_{104}O_4$ by positive mode high resolution FABHRMS measurement showing $[M + H]^+$ peak at *m/z* 841.7964 (calcd. for $C_{56}H_{105}O_4$, 841.8012). The ^{1}H NMR spectrum showed the signals for 1, 3, 4-trisubstituted benzene ring (δ 6.78, d, J = 8 Hz; δ 6.53, dd, J = 8, 1.6 Hz; δ 6.45, d,

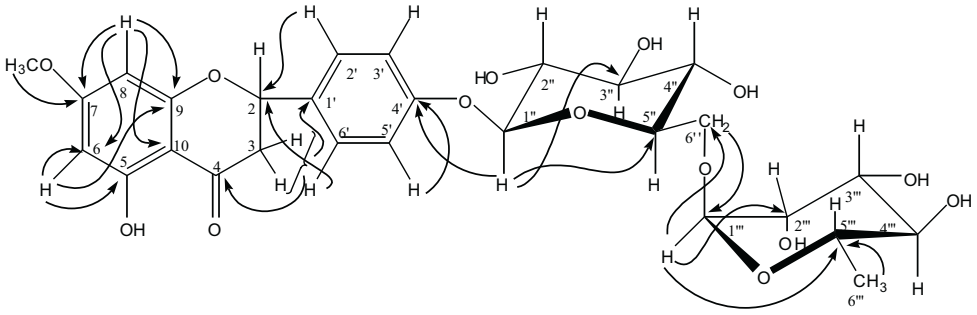

Figure 1. Structure of **1** and its important HMBC correlations.

J = 1.6 Hz). A methoxyl signal was also observed at δ 3.77. The FABMS showed characteristic peak of long chain ester at *m/z* 669 due to the loss of alkoxy group, and another peak at *m/z* 164 ($C_{10}H_{12}O_2$) was formed by McLafferty rearrangement. The alkoxy portions could be identified as 3-methoxy-4-hydroxy propyloxy by ^{1}H NMR spectrum. The methylene group adjacent to oxygen atom resonated as a triplet at δ 4.08 (J= 6.4 Hz) and the methylene group adjacent to the phenyl group gave a triplet at δ 2.57 (J= 7.5 Hz). Another methylene group was observed at δ 1.90 as doublet of triplets (J = 6.4, 7.5 Hz). The alkyl chain was found to be linear due to the absence of methine and quaternary carbons. It could further be confirmed by the presence of terminal ethyl group [triplet at δ 0.86 (J = 7.6 Hz) and multiplet at δ 1.28]. The methylene group adjacent to carbonyl moiety resonated at δ 2.29 as a triplet (J = 7.4 Hz) in agreement to the assigned structure. The important ^2J and ^3J correlations are illustrated in Fig. 2.

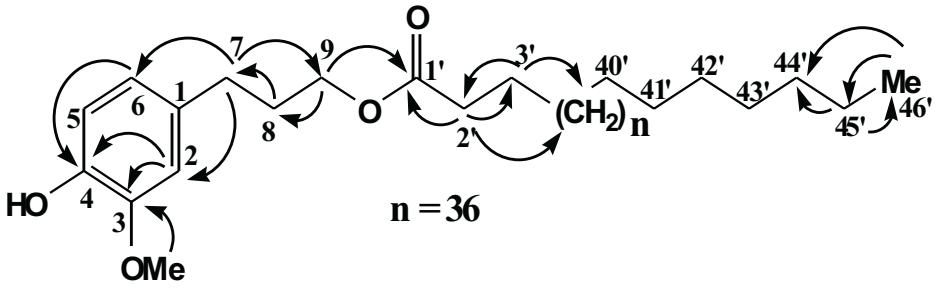

Figure 2. Structure of **2** and its important HMBC interactions.

EXPERIMENTAL

General. The ^{1}H- and ^{13}C-NMR, HMQC and HMBC spectra were recorded on Bruker spectrometers operating at 400 MHz for ^{1}H and 100 MHz for ^{13}C-NMR respectively. The chemical shift values are reported in ppm (δ) units and the coupling constants (J) are in Hz. MS and HR-MS were obtained on a JMS-HX-110 with a data system and on JMS-DA 500 mass spectrometers. Flash silica (230–400 mesh) was used in flash column chromatography. Visualization of the TLC plates was carried out under UV at 254 and 366 nm and by spraying with ceric sulphate reagent (with heating). The IR spectra were recorded on a 460 Shimadzu spectrometer.

Plant material. The roots of *Vitex negundo* Linn. were collected from Bannu district and identified by Prof. Abdur Rehman (Plant Taxonomist), Department of Botany, Govt. Post Graduate College Bannu, Pakistan. A voucher specimen (no. 318b) has been deposited at the herbarium of the Botany Department of Post Graduate College, Bannu, Pakistan.

Extraction and isolation. The shade dried roots (40 kg) of *Vitex negundo* was extracted three times, seven days each, with methanol. The combined methanolic extract was evaporated in vacuo. The resulting residue (1.5 kg) was suspended in water and extracted successively with n-hexane, chloroform, ethyl acetate and n-butanol. The hexane soluble fraction (80 g) was subjected to column chromatography (CC) over flash silica eluting with n-hexane and then n-hexane/chloroform gradient systems (20:1, 10:1, 8:1, 6:1, 5:1, 3:1, 2:1, 1:1). As a result fractions A-H were obtained. Fraction C was chromatographed over flash silica using solvent system hexane/ethyl acetate (20:1) to afford compound **5** (10 mg) and **6** (7 mg). Fraction D was submitted to CC (silica gel, hexane/ethyl acetate, 10:1) to give compound **2** (8 mg).

The n-butanol (200 gm) extract was subjected to column chromatography eluting with $CHCl_3$/MeOH (95:5, 90:10, 83:17, 70:30, 60:40) in increasing order of polarity. The fraction which eluted with $CHCl_3$/MeOH (95:5) was subjected to CC eluting with ethyl acetate/hexane (3:7) to afford compound **4**. The fractions, which were obtained from $CHCl_3$/MeOH (83:17), were combined and rechromatographed over flash silica eluting with $CHCl_3$/MeOH in increasing order of polarity. The fraction, which eluted with $CHCl_3$/MeOH (85:15), was then subjected to preparative TLC ($CHCl_3$/MeOH; 80:20) to afford compounds **1** (25 mg) and **3** (70 mg) respectively.

Sakuranetin 4′-*O*(6′′-*O*-α-L-rhamnopyranosyl)-β-D-glucopyranoside (**1**): amorphous white solid (25 mg): $C_{28}H_{34}O_{14}$; $[\alpha]_D^{25}$ –94.2 (c = 0.21, MeOH); UV (MeOH) λ_{max}: 340, 313, 273 nm; IR ν_{max} ($CHCl_3$): 3200–3500, 2840, 1668, 1580, 1370 cm^{-1}; (–) HRFABMS: found $[M–H]^-$ 593.2567, calculated for $C_{28}H_{33}O_{14}$; ^{1}H-NMR (in C_5D_5N): δ 5.50, dd, J = 3, 13 Hz, H-2, δ 3.20, dd, J = 13, 17 Hz, δ 2.70 dd, J = 3, 17 Hz, H_2-3, δ 6.61, d, J = 2.1 Hz, H-6, δ 6.50, d, J = 2.1, H-8, δ 7.60, d, J = 8.8 Hz, H-2′, 6′, δ 7.04 d, J = 8.8 Hz, H-3′, 5′, δ 5.64 d, J = 7.2 Hz, H-1′′, δ 5.40, d, J = 1.5 Hz, H-1′′′, δ 4.10 – 4.65 (H-2′′, H-2′′′ H-3′′, H-3′′′, H-4′′, H-4′′′, H-5′′, H-5′′′, H-6′′), δ 1.55, d, J = 6.8 Hz, H_3-6′′′; ^{13}C-NMR (C_5D_5N): see Table 1.

3-(3-Methoxy-4-hydroxyphenyl) propyl hexatetracontanoate (**2**): greenish oil (8 mg): $C_{56}H_{104}O_4$; UV (MeOH) λ_{max}: 203, 227, and 282 nm; IR ν_{max} ($CHCl_3$): 3394, 1240, 1736 cm^{-1}; (+) HRFABMS: found 841.7964, calculated for $C_{56}H_{105}O_4$; ^{1}H-NMR (in $CDCl_3$): δ 6.78, d, J = 8.0 Hz, H-5, δ 6.53, dd, J = 1.6, 8.0 Hz, H-6, δ 6.45, d, J = 1.6 Hz, H-2, δ 4.08, t, J = 6.4 Hz, 9-H_2, δ 3.77, s, 3-OMe, δ 2.57, t, J = 7.5, 7-H_2, δ 2.29, t, J = 7.4, 2′-H_2, δ 1.90, dt, J = 7.5, 6.4 Hz, 8-H_2, δ 1.60 quintet, J = 7.4 Hz, 3′-H_2, δ 1.23 brs, 4′-H_2–44′-H_2, δ 1.28 m, 45′-H_2, 0.86, t, J = 7.6, 46′-H_3; ^{13}C-NMR ($CDCl_3$): see Table 1.

Table 1. ^{13}C-NMR spectral data in δ value of compounds **1** and **2**.

Carbon number	**1** (δ)	**2** (δ)
C-1		131.6
C-2	79.2	111.2
C-3	43.0	146.4
C-4	196.9	143.9
C-5	164.4	114.0
C-6	97.8	121.7
C-7	166.0	35.1
C-8	96.4	39.7
C-9	163.4	64.0
C-10	104.2	
C-1′	131.1	173.0
C-2′	128.6	34.4
C-3′	114.5	25.0
C-4′	161.4	
C-5′	114.5	
C-6′	128.6	
C-7′–C-39′		29.6
C-40′		29.5
C-41′		29.4
C-42′		29.3
C-43′		29.2
C-44′		31.9
C-45′		22.6
C-46′		14.0
C-1′′	101.4	
C-2′′	74.5	
C-3′′	77.5	
C-4′′	72.0	
C-5′′	78.3	
C-6′′	69.7	
C-1′′′	102.4	
C-2′′′	72.7	
C-3′′′	71.2	
C-4′′′	74.0	
C-5′′′	67.2	
C-6′′′	18.5	
-OCH_3	55.2	55.6

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