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Flavonoids, triterpenoids and a lignan from *Vitex altissima* ☆

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Abstract

A new tetrahydrofuranoid lignan, altissinone (**1**) and a new acylated flavone *C*-glucoside, 2″-*O*-*p*-hydroxybenzoylorientin (**2**), were isolated in addition to several known triterpene acids and flavonoids from the ethyl acetate extractives of the leaves of *Vitex altissima*. The structures of the compounds were established based on interpretation of high resolution NMR (HMQC, HMBC and NOESY) spectral data. The ethyl acetate extract exhibited significant anti-inflammatory activity in rat paw edema model. The flavonoids and triterpene acids showed moderate antioxidant and 5-lipoxygenase enzyme inhibitory activities, respectively.

Keywords: *Vitex altissima*; Leaves; Lignan; Altissinone; Flavonoids; Triterpenoids; Anti-inflammatory

1. Introduction

Vitex altissima Linn. belongs to the family Verbenaceae. It is a moderate to large sized tree found in Eastern Ghats and Deccan plateau in India (Pullaiah and Sandhyarani, 1999). The leaves are reported to be useful in the treatment of rheumatism (Narayana Rao and Thammanna, 1990). We reported recently (Sridhar et al., 2004) the isolation and characterization of several new iridoids from the ethyl acetate extractives of the leaves of *V. altissima*. Further investigation on the ethyl acetate extractives of the same plant has led to the isolation of a new lignan, named altissinone (**1**) and a new flavonoid, 2″-*O*-*p*-hydroxybenzoylorientin (**2**), along with nine known triterpene acids and two flavonoids.

2. Results and discussion

Dried and powdered leaves of *V. altissima* were extracted with *n*-hexane, ethyl acetate, methanol and 70% methanol, successively. The ethyl acetate extractives which exhibited potent anti-inflammatory activity were subjected to silica gel column chromatography and reversed phase preparative HPLC to give a lignan (**1**), and a flavonoid (**2**) in addition to nine triterpene acids and two flavonoids. The triterpenoids have been identified as ursolic acid (Alves et al., 2000), corosolic acid (Murkami et al., 1993), epicorosolic acid (Bhandari et al., 1990), maslinic acid (Murkami et al., 1993), epimaslinic acid (Cheung and Yan, 1972), euscaphic acid (Kuang et al., 1989), euscaphic acid glucoside ester (Guang-Yl et al., 1989), 2α,3α,24-trihydroxyurs-12-en-28-oic acid (Jung et al., 2004), 2α,3α,24-trihydroxyurs-12,20(30)-dien-28-oic acid (Kojima et al., 1987) by comparison of spectral data with those reported in the literature. The flavonoids were identified as vitexin (Tomczyk et al., 2002), and luteolin 7-*O*-glucoside (Markham et al., 1978), by comparison with the literature data.

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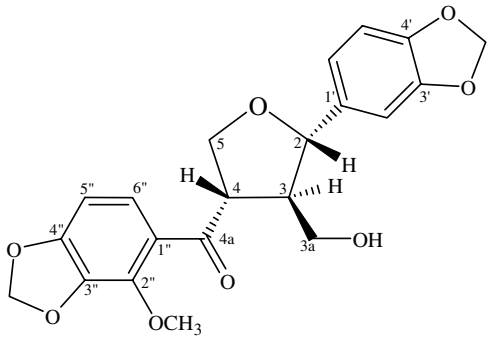

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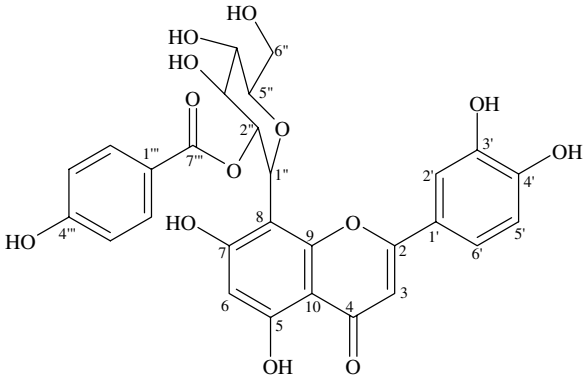

2

Table 1
NMR spectral data of compound **1** ($CDCl_3$, 500 MHz)[a]

Position	^1H	^{13}C	HMBC
2	4.57 *d* (9.0)	83.9	C-3, C-3a, C-1′, C-2′, C-6′
3	2.85 *m*	52.7	
3a	3.63 *dd* (11.0, 6.5) 3.73 *dd* (11.0, 5.0)	62.2	C-4, C-2
4	4.03 *m*	55.1	C-3, C-3a, C-5, C-4a
4a		200.3	
5	4.17 *dd* (9.0,6.0) 4.22 *t* (9.0)	70.8	C-3, C-2, C-4a
1′		134.7	
2′	6.97 *d* (1.5)	107.2	C-4′, C-6′
3′		147.3	
4′		147.8	
5′	6.77 *d* (8.0)	108.0	C-1′, C-3′,C-4′
6′	6.85 *dd* (8.0,1.5)	120.3	C-2, C-4′
1″		125.3	
2″		143.0	
3″		136.6	
4″		152.9	
5″	6.59 *d* (8.5)	103.3	C-3″, C-1″
6″	7.27 *d* (8.0)	125.6	C-2″, C-4″, C-4a
–OCH_3	4.11 *s* 3H	60.1	C-2″
–OCH_2O–	5.95 *s* 2H	101.0	C-3′, C-4′
–OCH_2O–	6.03 *s* 2H	101.8	C-3″, C-4″

[a] Chemical shifts (δ) are in ppm, and coupling constants (J in Hz) are given in parentheses.

Structures of the new compounds (**1** and **2**) have been deduced by the interpretation of high-resolution NMR data and the details are presented below.

(−)-Altissinone (**1**) was obtained as pale green flakes, m.p. 151–152 °C. The molecular formula $C_{21}H_{20}O_8$ was deduced from microanalytical and LC–MS [*m*/*z* 423, $(M + Na)^+$] data. IR spectrum of **1** showed bands at 3483 (hydroxyl), 1654 (carbonyl), 1592, 1485 (aromatic), 1041 and 942 cm^{-1} (methylenedioxy). The ^1H and ^{13}C NMR spectral data (Table 1) of **1** showed signals characteristic of a 2,3,4-trisubstituted furanoid lignan (Klemm, 1978; Banerji et al., 1984). The ^1H NMR data revealed the presence of an oxymethine proton at δ 4.57 (1H, *d*, J = 9.0 Hz) characteristic of H-2 of tetrahydrofuranoid lignans (Lin-Gen et al., 1983; Yu et al., 1998), two methine protons (1H, δ 2.85, *m*, H-3 and 1H, δ 4.03, *m*, H-4), and two oxymethylene groups [(1H, δ 3.63, *dd*, J = 11.0, 6.5 Hz, H_a-3a and 1H, δ 3.73, *dd*, J = 11.0, 5.0 Hz, H_b-3a) and (1H, δ 4.17 *dd*, J = 9.0, 6.0 Hz, H_a-5 and 1H, δ 4.22, *t*, J = 9.0 Hz, H_b-5)]. The ^1H NMR data also showed the presence of a piperonyl unit constituted by a 1,2,4-trisubstituted phenyl moiety [δ 6.77 (1H, *d*, J = 8.0 Hz), 6.85 (1H, *dd*, J = 8.0, 1.5 Hz) and 6.97 (1H, *d*, J = 1.5 Hz)] and a methylenedioxy group at δ 5.95 (2H, *s*). In addition, the ^1H NMR spectrum contained a methoxyl group (3H, δ 4.11, *s*), a methylenedioxy group (2H, δ 6.03, *s*) and two aromatic AB protons at δ 6.59 (1H, *d*, J = 8.5 Hz, H-5″) and 7.27 (1H, *d*, J = 8.0 Hz, H-6″), suggesting the presence of a 1,2,3,4-tetrasubstituted phenyl unit. From the above data, the structure of **1** was derived as a 2,3,4-trisubstituted tetrahydrofuranoid lignan (Jung et al., 1998) having a piperonyl and 2-methoxy-3,4-methylenedioxyphenyl moieties. The IR (1654 cm^{-1}) and ^{13}C NMR data (δ 200.3) suggested the presence of a ketone carbonyl. The HMBC data (Table 1) of **1** has shown correlations between H-6″, H-4, and H-5 protons and the ketone carbonyl (δ 200.3), suggesting the location of ketone on C-4a. Further, the H-3a, H-5 and H-6′ protons showed correlations with C-2 (δ 83.9). Based on the above, the structure of the lignan has been deduced as 2-(3′,4′-methylenedioxyphenyl)-3-hydroxymethyl-4-(2″-methoxy-3″,4″-methylenedioxybenzoyl)tetrahydrofuran, a new lignan named altissinone (**1**). The configuration of **1** at C-2, C-3 and C-4 was proposed to be identical with those of (−)-sesaminone (Maioli et al., 1997), based on the observed similarity in chemical shifts and optical rotation ($[\alpha]_D^{25} - 40.3°$) data. Compound **1** could have formed biosynthetically through an oxidative cleavage of a previously known 2-methoxysesamin (Jaensch et al., 1989).

Compound (**2**) was obtained as a pale yellow amorphous powder from methanol. The molecular formula $C_{28}H_{24}O_{13}$, was deduced from microanalytical and LC–MS [*m*/*z* 567 $(M - H)^-$] data. The absorption maxima at 267 and 342 nm in the UV spectrum are attributable to a flavonoid skeleton. IR spectrum of 2 showed