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New iridoid glucoside from *Wendlandia tinctoria* roots

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Abstract

A new iridoid glucoside, 10-*O*-veratroyleranthemoside (**1**) was isolated from the roots of *Wendlandia tinctoria*. The structure was established by spectroscopic (including 2D NMR) and chemical methods.

Keywords: *Wendlandia tinctoria*; Rubiaceae; Iridoid glucoside; 10-*O*-Veratroyleranthemoside

Wendlandia tinctoria (Roxb) DC var. *normalis* Hook (Rubiaceae) is a small tree found scatterly in the semi-forest hills of the north eastern states of India. The root juice of the plant has been used by the native communities in traditional medicines including antidote of snake-bite [1]. Our previous studies on roots and stem have resulted in the isolation of some iridoids and steroids [2]. Our continued studies on the roots of the same plant have resulted in the isolation of one new iridoid glucoside (**1**).

1. Experimental

IR, Shimadzu FIIR-8100 spectrometer; NMR, Varian XL-400 spectrometer; FAB-MS, JEOL JMS-AX 505 HA spectrometer; Diaion HP-20 (Mitsubishi Chemical, Japan) and silica gel (60–120 mesh, Merck) were used for CC and silica gel G (Merck) for TLC.

Fresh roots of the plant, at the flowering stage, were collected from hilly area of South Tripura in April, 2003. The plant was identified by Dr. B.K. Datta, Tripura University. A voucher specimen ($^{\#}$BD-2/05) has been deposited at the Herbarium of Botanical Survey of India, Shibpur, Howrah.

Fresh dried semi-powdered roots (6 kg) were extracted with MeOH (10 L $\times$2). The concentrated semisolid extract (385 g) was suspended in H_2O (Ca. 50 mL) and successively partitioned with hexane, chloroform and *n*-butanol. The butanol extract (55 g) was column chromatographed over Diaion HP-20 using H_2O and H_2O with increasing MeOH

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(25–100%) to get five fractions. Fractions of H_2O–MeOH (1:1 and 1:3) exhibiting identical TLC pattern were mixed and concentrated to a semisolid mass. A part of this mass (8.5 g) was subjected to column chromatography (CC) over silica gel using $CHCl_3$, $CHCl_3$ with increasing MeOH (5–80%). The fractions from $CHCl_3$–MeOH (60:40 and 50:50) were almost similar in composition on TLC. These fractions were mixed and were further purified through silica gel CC to get compound **1** (70 mg) in yellow amorphous powder.

10-O-Veratroyleranthemoside (**1**): yellow amorphous powder; $[\alpha]_D^{25}$ – 41.2° (*c* 0.15, MeOH); UV λ_{max} (MeOH) nm: 275.8, 300 sh, 330 sh; IR (KBr): 3400, 1710, 1641, 1605, 1508, 1373, 1270, 1080, 648 cm^{-1}; ^1H and ^{13}C NMR (CD_3OD) are listed in Table 1; FAB-MS: (rel.int.%): 533 $[M+Na]^+$ (7), 511 $[M+H]^+$ (10), 347 $[M-veratroyl+2H]^+$ (13), 183 [veratric acid+H]$^+$ (100); HR-FAB-MS: *m/z* $[M+H]^+$ calcd. for $C_{24}H_{31}O_{12}$: 511.1810; found 511.1808.

Acetylation of **1**: compound **1** (20 mg) was suspended in a mixture of pyridine (4 drops), and acetic anhydride (3 mL) and was kept at room temperature (20 °C) for overnight. Usual workup gave light yellow amorphous solid of **1a** (15 mg).

10-O-Veratroyleranthemoside tetraacetate (**1a**): light yellow amorphous solid. IR (KBr): 3429, 1743, 1710, 1636, 1609, 1508, 1235, 1055 cm^{-1}; ^1H and ^{13}C NMR ($CDCl_3$) are shown in Table 1. FAB-MS *m/z* (rel.int.%): 701 $[M+Na]^+$ (4), 331 (13), 183 (21), 169 (79), 109 (36), 43 (100).

Alkaline hydrolysis of **1**: compound **1** (5 mg) was stirred with 1 mol/L NaOH (2 mL) for 2 h under N_2 at room temperature. The reaction mixture was extracted with ether (×2) after acidification by 2 mol/L HCl. From the ether extract, veratric acid was detected by co-TLC and super imposable IR spectra with an authentic sample.

Acid hydrolysis of **1**: compound **1** (5 mg) was stirred with 2 mol/L aq-methanolic HCl for 1 h. After neutralization with Amberlite IRA-400 (OH^- form), the reaction mixture was concentrated. D-Glucose was detected by co-TLC with

Table 1
NMR spectral data of **1** (CD_3OD) and **1a** ($CDCl_3$) (400 MHz for ^1H NMR and 100 MHz for ^{13}C NMR).

Position	**1**		**1a**	
	δ_C	δ_H	δ_C	δ_H
1	95.0 d	5.35 d (2.0)	93.3 d	5.33 d (2.0)
3	139.6 d	6.11 dd (6.0, 2.0)	138.8 d	6.33 dd (6.0, 2.0)
4	106.5 d	4.93 dd (6.0, 3.0)	107.2 d	4.98 dd (6.0, 2.0)
5	38.6 d	3.07 br m	34.7 d	3.42[a]
6	138.0 d	6.01 dd (5.5, 2.5)	138.3 d	5.95 dd (5.5, 2.5)
7	133.1 d	5.86 dd (5.5, 1.0)	133.9 d	5.86 dd (5.5, 2.5)
8	85.8 s	–	85.8 s	–
9	45.9 s	2.53 dd (8.5, 2.0)	46.0 d	2.51 dd (8.5, 2.0)
10	71.2 t	4.23 d (11.5), 4.12 d (11.5)	70.5 t	4.22 d (11.5), 4.11 d (11.5)
Gln				
1′	100.8 d	4.72 d (7.0)	101.3 d	4.98 d (8.0)
2′	74.2 d	3.21–3.34[a]	71.1 d	4.95 dd (8.0, 9.0)
3′	76.4 d		72.5 d	5.26 dd (9.0, 9.5)
4′	71.2 d		68.4 d	5.05 dd (9.5, 9.5)
5′	78.6 d		72.6 d	3.76 m
6′	62.3 t	3.83 dd (12.5, 4.5) 3.68 dd (12.5, 2.0)	61.9 t	4.28[a] 4.12 dd (12.5, 2.0)
Veratroyl moiety				
1″	123.3 s	–	123.2 s	–
2″	113.6 d	7.53 d (2.0)	113.4 d	7.51 d (2.0)
3″	150.2 s	–	152.8 s	–
4″	153.2 s	–	153.3 s	–
5″	112.0 d	7.00 d (8.5)	110.3 d	7.02 d (8.5)
6″	125.9 d	7.63 dd (8.5, 2.0)	126.6 d	7.62 dd (8.5, 2.0)
7″	168.2 s	–	166.8 s	–
3″-OMe	56.4 q	3.87 s	56.2 q	3.85 s
4″-OMe	56.1 q	3.82 s	56.0 q	3.82 s
Ac			169.4, 170.2, 170.5, 170.6 (each s), 20.5, 20.6, 20.7, 21.0 (each q)	2.02, 2.04, 2.10, 2.12

[a] Signal pattern is unclear due to overlapping.

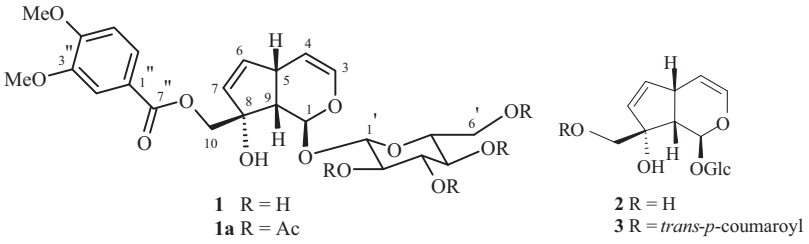

Fig. 1.

an authentic sample as well as GLC analysis of its TMS derivative obtained on treatment of the dry residue with 5 drops of TMS-imidazole for 15 min at 60 °C followed extraction with hexane and analysis in OV-1 column at 180 °C.

2. Results and discussion

The positive ion FAB-MS of compound **1** (Fig. 1) displayed quasi-molecular ions $[M+H]^+$ and $[M+Na]^+$ at *m/z* 511 and 533 compatible with molecular formula $C_{24}H_{30}O_{12}$. It exhibited UV spectrum in MeOH at λ_{max} 275.8, 300 sh and 330 sh nm, characteristic of aromatic chromophore. The IR spectrum showed absorptions at 3400 (OH), 1710 (ester), 1641 and 1270 (enol ether) and 1605 and 1508 (aromatic) cm^{-1}. The ^1H NMR spectrum (Table 1) revealed the presence of an C-4-unsubstituted iridoid moiety [δ 6.11 (dd, J = 6.0, 2.0 Hz, H-3) and 4.93 (dd, J = 6.0, 3.0 Hz, H-4), 5.35 (d, J = 2.0 Hz, H-1)] along with a glucose moiety [δ 4.72 (d, J = 7.0 Hz, H-1′), 3.68 (dd, J = 12.5, 2.0 Hz, H-6′) and 3.83 (dd, J = 12.5, 4.5 Hz, H-6′)] and veratroyl moiety [δ 7.53 (d, J = 2.0 Hz, H-2″), 7.00 (d, J = 8.5 Hz, H-5″), 7.63 (dd, J = 8.5, 2.0 Hz, H-6″), 3.87 (s, MeO-3″) and 3.82 (s, MeO-4″)] [3a]. Both the ^1H and ^{13}C NMR (including DEPT) data (Table 1) also suggested the presence of one olefine group (δ_H 6.01, δ_C 138.0 and δ_H 5.86, δ_C 133.1), one tertiary hydroxyl group (δ_C 85.8) and one oxymethylene group (δ_H 4.12 and 4.23, δ_C 71.2). The point of attachment of a β-glucopyranosyl unit at C-1 was confirmed by long range HMBC correlations between H-1 (δ_H 5.35) and C-1′ (δ_C 100.8) and between H-1′ (δ_H 4.72) and C-1 (δ_C 95.0). The C-10 side chain was linked to veratroyl moiety forming an ester linkage, based on the downfield shift value of C-10 (δ_C 85.8) and the existence of HMBC coupling between C-10 protons with C-7″ carbonyl (δ_C 168.2). The stereochemistry of the C-8 CH_2O-group was determined to be β on the basis of the shielding chemical shifts of C-9 and C-7 (δ_C 45.9 and 133.1, respectively) as well as from ROESY cross peaks between H-6″ and H-5 and between H-2″ and H-10. The small coupling of $J_{1,9}$ (2.0 Hz) indicated their dihedral angle of nearly 90°. The coupling (8.5 Hz) between H-5 and H-9 indicated a small dihedral angle demonstrating the *cis*-configuration at the ring fusion. ROSEY correlations between H-6″ and H-5 and between H-2″ and H-10 confirmed the attachment of veratroyl unit at C-10 CH_2O-group. Moreover, ROSEY cross peaks between H-10, H-9 and H-5 revealed their *cis*-orientations. The compound on mild acid hydrolysis with 2 mol/L-methanolic HCl afforded D-glucose and on alkaline hydrolysis with 1 mol/L-methanolic NaOH gave veratric acid. The compound on acetylation with Ac_2O and pyridine at room temperature (20 °C) for 24 h afforded tetraacetate (**1a**), $C_{32}H_{38}O_{16}$ (MW 678) (FAB-MS, *m/z* 701 $[M+Na]^+$). The EI-MS of the tetraacetate showed a strong mass peak at *m/z* 331 supporting no ester linkage at the glucose moiety. The NMR data of the compound **1** were very similar to that of eranthemoside (**2**) isolated from *Eranthemum pulchellum* (Acanthaceae) and 10-*O*-*trans*-coumaroyleranthemoside (**3**) isolated from *Barleria strigosa* (Acanthaceae) except of aryl moiety [3b] and [3c]. Based on these evidence the structure of compound **1** (Fig. 1) was elucidated as 10-*O*-veratroyl-eranthemoside. Isolation of this compound from the family Rubiaceae may be interesting from biogenetic and chemotaxonomic point for study of phylogeny of the angiosperms.

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