Iridoid Glucosides from *Wendlandia tinctoria* Roots

Biswanath DINDA,*[,a] Sudhan DEBNATH,[a] Shiho ARIMA,[b] Nariko SATO,[b] and Yoshihiro HARIGAYA[b]

[a] *Department of Chemistry, Tripura University; Suryamaninagar, Agartala 799 130, India: and* [b] *School of Pharmaceutical Sciences, Kitasato University; Minato-ku, Tokyo 108–8641, Japan.* Received July 25, 2005; accepted April 17, 2006

Four new iridoid glucosides, 5-dehydro-8-*epi*-adoxosidic acid, 5-dehydro-8-*epi*-mussaenoside, 10-*O*-dihydroferuloyldeacetyldaphylloside, and wendoside, together with one known iridoid glucoside, 8-*epi*-mussaenoside, β-D-glucose, D-mannitol and β-sitosterol have been isolated from the roots of *Wendlandia tinctoria*. On the basis of chemical and spectral analyses, the structures of new iridoid glucosides have been elucidated.

Key words *Wendlandia tinctoria*; Rubiaceae; iridoid glucoside; 5-dehydro-8-*epi*-adoxosidic acid; 5-dehydro-8-*epi*-mussaenoside; 10-*O*-dihydroferuloyldeacetyldaphylloside; wendoside

Wendlandia tinctoria (ROXB.) DC var. *normalis* HOOK (Rubiaceae) is a small tree, which grows mainly in the semi-forest area of Tripura and other northeastern states of India lying in sub-Himalayan areas.[1)] It is used by the local people as antidote of snake-bite.[1)] Earlier investigation on the stem of this plant reported the isolation of myricyl stearate, stearic acid, D-mannitol, β-sitosterol, stigmasterol and geniposidic acid.[2)] This paper describes the isolation and structure elucidation of four new iridoid glucosides and other known compounds from the roots of this plant.

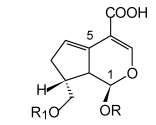
Results and Discussion

The iridoid glucosides, D-mannitol and β-D-glucose were isolated from the *n*-butanol fraction of methanol extract of the roots of *W. tinctoria* and β-sitosterol was isolated from the benzene fraction of the methanol extract. All the iridoid glucosides were isolated as their acetates from an acetylation mixture of oily fraction obtained from Diaion HP-20 column chromatography of the *n*-butanol fraction.

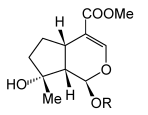
5-Dehydro-8-*epi*-adoxosidic acid **1** was obtained as a white amorphous powder of its pentaacetate **1a**. The molecular formula was established by HR-FAB-MS (*m*/*z* 607.1635 $[M+Na]^+$) as $C_{26}H_{32}O_{15}$ (MW 584). It showed UV absorption in MeOH at 234 (log ε, 4.05) and 280 nm (3.92) characteristic of conjugated diene system. Its IR spectrum in KBr showed the bands for acetate (1747, 1163, 1092 cm^{-1}), carboxyl (1705 cm^{-1}) and olefinic (1655, 1636 cm^{-1}) functions. The ^{1}H- and ^{13}C-NMR spectral data (Tables 1, 2) indicated its iridoid glucoside nature. The position of conjugated double bond at C-5 and C-6 was established by considering HMBC correlation of H-6 proton signal at δ 5.83 with C-5, C-7, C-8, C-9, carbons. Moreover, out of C-5 and C-6 carbons of the double bond, only C-6 is methine carbon as indicated by DEPT experiments. The NMR spectral data were very similar to those reported for 8α-dihydrogeniposide (8-*epi*-adoxoside) **6** by Inouye *et al.*[3)] The hydrolysis of the acetate **1a** with 1 N aqueous methanolic HCl gave an aglycone as semisolid mass, $C_{10}H_{12}O_5$ (MW 212) and D-glucose. The hydrolysis of **1a** with 0.1 N NaOH at room temperature gave an oily mass, $C_{16}H_{22}O_{10}$ (MW 374), which was identical with one of the spots on co-TLC of the parent oily mass used for acetylation. Therefore, the structure of **1** was elucidated as 5-dehydro-8-*epi*-adoxosidic acid.

8-*epi*-Mussaenoside **2** was isolated as tetraacetate **2a** in white amorphous powder, $C_{25}H_{34}O_{14}$ (MW 558). Comparison of its spectral (^{1}H-, ^{13}C-NMR) data with literature[4,5)] indicated that **2a** was 8-*epi*-mussaenoside tetraacetate and the parent glucoside was 8-*epi*-mussaenoside **2**.

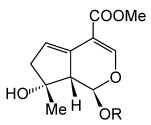
5-Dehydro-8-*epi*-mussaenoside **3** was also obtained as tetraacetate **3a** in white amorphous powder, $C_{25}H_{32}O_{14}$ (MW 556), negative FAB-MS *m*/*z* 555 $[M-H]^-$, $[\alpha]_D^{23}$ −18.3° ($CHCl_3$). It showed UV absorption in MeOH at 235 (log ε, 4.04) and 280.6 nm (3.81), suggesting the presence of conjugated diene system. The compound was presumed to be an iridoid glucoside from its ^{1}H- and ^{13}C-NMR spectral data (Tables 1, 2). The position of one olefinic double bond be-



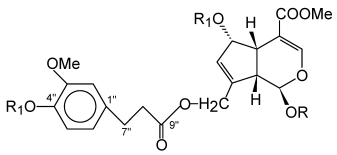
1 R = glc H_4, R_1 = H
1a R = glc $(Ac)_4$, R_1 = Ac



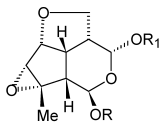
2 R = glc H_4
2a R = glc $(Ac)_4$



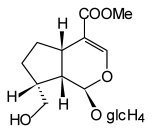
3 R = glc H_4
3a R = glc $(Ac)_4$



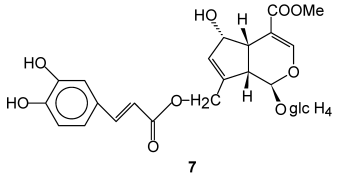
4 R = glc H_4, R_1 = H
4a R = glc $(Ac)_4$, R_1 = Ac



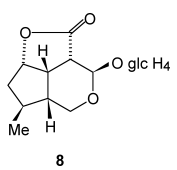
5 R = H, R_1 = glc H_4
5a R = Ac, R_1 = glc $(Ac)_4$
5b R = R_1 = H



6



7



8

* To whom correspondence should be addressed. e-mail: dindabtu@rediffmail.com

Table 1. ^{1}H-NMR Chemical Shifts of Glucoside Acetates **1a**, **2a**, **3a**, **4a**, **5a** (400 MHz, in $CDCl_3$)

H	**1a**	**2a**	**3a**	**4a**	**5a**
1	5.11 d (9.5)	5.32 d (3.0)	4.93 d (9.5)	4.82 d (8.0)	5.30 d (2.5)
3	7.52 d (1.0)	7.43 d (1.0)	7.43 d (1.0)	7.52 d (1.0)	6.54 br s
4	—	—	—	—	2.32 m
5	—	3.08 m	—	3.28 dd (8.0, 8.0)	3.06 m
6	5.83 br s	1.87 m	5.84 br s	5.56 dd (8.0, 2.0)	5.04 dd (7.5, 1.2)
7	2.19 m	1.70 m	1.95 d (18.0)	6.05 d (2.0)	3.82 br s
	2.83 dd (8.0, 2.0)		2.64 d (18.0)		
8	3.18 ddd (6.0, 6.0, 6.0)	—	—	—	—
9	2.86 dd (9.5, 6.0)	2.32 dd (9.5, 3.0)	3.42 d (9.5)	2.85 dd (8.0, 8.0)	2.62 m
10	4.66 dd (14.0, 2.0)	1.25 s	1.31 s	4.92 br s	1.23 s
11	—	—	—	—	3.84 dd (11.5, 4,5)
					3.92 dd (11.5, 2.5)
COOMe	—	3.71 s	3.74 s	3.73 s	—
Ar-OMe	—	—	—	3.82 s	—
Glucose moiety					
1′	4.87 d (8.0)	4.71 d (8.0)	4.82 d (8.0)	4.97 d (8.0)	4.73 d (7.5)
2′	5.01 dd (8.5, 8.0)	4.98 dd (9.5, 8.0)	4.98 dd (8.5, 8.0)	4.98 dd (8.5, 8.0)	5.22 dd (8.5, 7.5)
3′	5.22 dd (9.0, 8.5)	5.22 dd (9.5, 9.5)	5.23 dd (9.5, 8.5)	5.24 dd (9.0, 8.5)	5.24 dd (9.0, 8.5)
4′	5.08 dd (9.0, 8.5)	5.09 dd (9.5, 9.0)	5.09 dd (9.0, 8.5)	5.08 dd (9.0, 9.0)	5.08 dd (9.0, 9.0)
5′	3.72 m	3.72 m	3.75 ddd (9.5, 4.5, 2.5)	3.76 m	3.76 m
6′	4.16 dd (12.5, 2.5)	4.14 dd (12.5, 2.0)	4.13 dd (12.5, 2.5)	4.14 dd (12.5, 2.5)	4.12 dd (12.5, 2.5)
	4.24 dd (12.5, 4.5)	4.32 dd (12.5, 4.5)	4.33 dd (12.5, 4.5)	4.32 dd (12.5, 4.5)	4.24 dd (12.5, 4.5)
Dihydroferuloyl moiety					
1″	—	—	—	—	—
2″	—	—	—	7.50 d (2.0)	—
3″	—	—	—	—	—
4″	—	—	—	—	—
5″	—	—	—	7.02 d (8.0)	—
6″	—	—	—	7.56 dd (8.0, 2.0)	—
7″	—	—	—	2.84 t (7.0)	—
8″	—	—	—	2.34 t (7.0)	—
OAc	1.98, 2.00, 2.02,	1.95, 2.01,	1.95, 2.01, 2.03,	1.96, 1.99, 2.06,	2.02, 2.03, 2.04,
	2.07, 2.08	2.03, 2.07	2.10	2.07, 2.08, 2.26	2.05, 2.07

Assignments were confirmed by ^{1}H–^{1}H COSY experiments. Coupling constants (Hz) are given in parentheses.

tween C-5 and C-6 carbons was assigned on the basis of HMBC interaction of C-6 proton (δ 5.8 br s) with C-5, C-7, C-8 and C-9 carbons as well as downfield chemical shift of C-9 proton compared to that of 8-*epi*-mussaenoside tetraacetate **2a** (δ 2.32). The stereochemistry of C-8 carbon was assigned as β-methyl on the basis of C-10 chemical shift, which was very close to that of 8-*epi*-mussaenoside acetate **2a** as reported by Damtoft *et al.*[5] The acid hydrolysis of **3a** with 1 N aqueous methanolic HCl afforded D-glucose confirming its glucoside nature. The alkaline hydrolysis under mild condition with 0.1 N NaOH gave an oily mass which was identical in co-TLC with one of spots of parent oily mixture used for acetylation. Therefore, the structure of **3a** was assigned as 5-dehydro-8-*epi*-mussaenoside tetraacetate and hence of **3** as 5-dehydro-8-*epi*-mussaenoside.

10-*O*-Dihydroferuloyldeacetyldaphylloside **4** was isolated as hexaacetate **4a** in white amorphous powder. EI-MS of **4a** recorded as mass peak at *m/z* 834 $[M]^+$ corresponding the molecular formula, $C_{39}H_{46}O_{20}$. It showed UV absorption in MeOH at 236 ($\log\varepsilon$, 3.96), 280 (3.32), 302 (3.04) and 324 nm (3.20) suggesting the presence of enolic and aromatic chromophores. The IR spectrum in KBr also supported this fact by showing absorption bands at 1750 and 1095 (ester), 1640 (C=C), 1605, 1503, 1040 and 908 cm^{-1} (1,2,4-tri-substituted aromatic ring). Its iridoid glucoside nature was revealed from its ^{1}H- and ^{13}C-NMR spectral data (Tables 1, 2). The presence of one phenolic acetoxy group was indicated by appearance of an acetoxy methyl chemical shift at δ 2.26. The NMR spectral data of iridoid and aromatic moieties were very similar to those reported for 10-*O*-caffeoyldeacetyldaphylloside **7** by Sainty *et al.*[6] The attachment of dihydroferuloyl unit at C-10 was assigned by comparison of C-10 shift (δ_C 62.3), which appeared at the same value in compound **7**. Acid hydrolysis of **4a** afforded D-glucose and alkaline hydrolysis gave dihydroferulic acid. Mild alkaline hydrolysis with 0.1 N NaOH gave an oily mass which was identical in co-TLC with one of the spots of parent oily mixture used for acetylation. Based on all these facts, the structure of **4a** was established as 10-*O*-dihydroferuloyldeacetyldaphylloside hexaacetate and of **4** as 10-*O*-dihydroferuloyldeacetyldaphylloside.

Wendoside **5** was isolated as its pentaacetate **5a** in white amorphous powder. Positive FAB-MS recorded a mass peak at *m/z* 587 $[M+H]^+$ confirming the molecular formula, $C_{26}H_{34}O_{15}$. Positive FAB-MS also recorded a mass peak at *m/z* 331 for the oxonium ion of tetraacetyl hexose suggesting the presence of hexose moiety in the molecule. The IR spectrum in KBr showed the bands for ester (1755, 1160 cm^{-1}) and epoxy (2856, 1128 cm^{-1}) functions. The compound was supposed to be a C_{11}-iridoid glucoside from its ^{1}H- and ^{13}C-NMR data (Tables 1, 2). The C-6 proton signal at δ 5.04 was very similar to that of semperoside **8**, reported from *Gelsemium sempervirens* by Jensen *et al.*[7] The *cis* α-configurations of *O*-glucosyl unit at C-3 and oxymethylene group

Table 2. ^{13}C-NMR Chemical Shifts of Glucoside Acetates **1a**, **2a**, **3a**, **4a**, **5a** (100 MHz, in $CDCl_3$)

C	**1a**	**2a**	**3a**	**4a**	**5a**
1	96.2 d	95.9 d	93.0 d	95.8 d	101.3 d
3	152.9 d	151.7 d	153.3 d	154.0 d	103.1 d
4	111.3 s	111.0 s	111.1 s	106.9 s	31.8 d
5	136.9 s	30.1 d	137.1 s	42.5 d	54.3 d
6	131.2 d	29.7 t	132.2 d	75.7 d	85.7 d
7	38.4 t	40.7 t	38.3 t	131.5 d	56.4 d
8	33.5 d	79.5 s	78.3 s	150.0 s	77.5 s
9	46.3 d	51.0 d	46.2 d	46.7 d	53.8 d
10	61.8 t	24.4 q	24.7 q	62.3 t	29.2 q
11	172.1 s	170.6 s	168.6 s	169.3 s	72.0 t
COOMe	—	52.3 q	52.5 q	52.0 q	—
ArOMe	—	—	—	56.2 q	—
1′	96.9 d	99.3 d	96.3 d	97.5 d	103.1 d
2′	70.7 d	70.6 d	70.6 d	70.6 d	71.8 d
3′	72.4 d	72.1 d	72.5 d	72.5 d	73.0 d
4′	68.2 d	68.2 d	68.6 d	68.2 d	68.5 d
5′	72.1 d	72.5 d	74.3 d	72.1 d	72.0 d
6′	61.5 t	61.6 t	61.8 t	61.7 t	62.3 t
1″	—	—	—	136.9 s	—
2″	—	—	—	112.2 d	—
3″	—	—	—	149.8 s	—
4″	—	—	—	147.7 s	—
5″	—	—	—	116.3 d	—
6″	—	—	—	122.2 d	—
7″	—	—	—	29.6 t	—
8″	—	—	—	38.5 t	—
9″	—	—	—	168.6 s	—
OAc	169.1, 169.3×2, 170.2, 170.6 20.4, 20.5×2, 20.6, 20.8	169.1, 169.3, 170.2, 170.7 20.6×2, 20.7, 20.8	169.1, 169.4, 170.1, 170.6 20.5, 20.6, 20.7, 20.8	169.4, 169.5, 169.6, 170.7 171.3, 171.6, 20.6×2, 20.7, 20.8, 20.9, 21.1	169.3, 169.4×2, 170.4, 170.6 20.6×2, 20.7×3

Assignments were established by ^{13}C–^{1}H COSY experiments. Multiplicity of carbons was confirmed by DEPT spectra.

in C-4 were assigned on the basis of high chemical shifts of C-3 and C-6 protons. The position of the glucose unit was assigned at C-3 position because of high chemical shift value (δ_C 103.1) of the anomeric carbon, similar to that observed in semperoside **8**. The compound **5a** on acid hydrolysis gave an aglucone **5b**, $C_{10}H_{14}O_5$ (MW 214) and D-glucose confirming the presence of glucose unit in the molecule. The aglucone was possibly formed by deacetylation and hydrolysis of glucosidic linkage. Mild alkaline hydrolysis of **5a** furnished an oily deacetylated product which was identical on co-TLC with one of the components present in parent oily mass used for acetylation. Therefore, the structure of wendoside was assigned as **5**. This is the first report of an iridoid glucoside in which C-11 hydroxymethyl group is involved in the formation of tetrahydrofuran ring with C-6 carbon.

All the isolated five iridoid glucoside acetates (**1a**—**5a**) did not show significant antimicrobial activity against some Gram-positive and Gram-negative bacteria (*Staphylococcus aureus*, *Bacillus subtilis* and *Pseudomonas aeruginosa*), although the crude *n*-butanol fraction extract exhibited significant activity against *P. aeruginosa*. However, the isolation of those iridoid congeners **1a**—**5a** is interesting from chemotaxonomic viewpoint.

Experimental

All the melting points were recorded on a sulphuric acid bath in °C and are uncorrected. The UV spectra were recorded on a Chemito UV 2600 spectrometer; IR spectra on a Shimadzu FTIR-8100 spectrometer; ^{1}H-, ^{13}C-NMR and 2D-NMR on a Varian XL 400 spectrometer with TMS as an internal standard; and HR-FAB-MS, Positive and Negative FAB-MS and EI-MS on a JEOL JMS-AX505 HA spectrometer. In the NMR spectra, chemical shifts are expressed in δ values and coupling constants (J) in Hz. ^{1}H–^{13}C COSY, ^{1}H–^{1}H COSY and HMBC spectra were obtained with the usual pulse sequence. Diaion HP-20 and silica gel (60—120 mesh, Merck) were used for column chromatography (CC) and silica gel G (Merck) for thin layer chromatography (TLC).

Plant Material Fresh plant material (roots) in flowering stage of the plants was collected from the semi-forest hilly slope area of South Tripura, altitude of 12.8 m from sea level in April, 2003. The plant was identified by Dr. B. K. Dutta, Taxonomist, Department of Life Sciences, Tripura University. A voucher specimen (#BD-2/05) has been deposited in the Central National Herbarium, Botanical Survey of India, Shibpur, Howrah.

Extraction and Isolation The fresh roots of *W. tinctoria* were sliced and air-dried. Dry roots (6 kg) were extracted with MeOH (10 l×2). The MeOH extract after concentration to *ca.* 50 ml was diluted with *ca.* 200 ml of H_2O and fractionated into C_6H_6, $CHCl_3$ and *n*-BuOH soluble fractions by partition with the organic solvent.

β-Sitosterol: Benzene soluble fraction on CC over silica gel afforded *β*-sitosterol (30 mg), mp 138 °C, identified by comparison (mmp and co-TLC) with an authentic sample.

D-Mannitol: *n*-Butanol soluble fraction on concentration and standing overnight at room temperature gave crystals of D-mannitol, which on repeated crystallization from MeOH had mp 168 °C, $[\alpha]_D^{23}$ +12.5° (c=1.0, EtOH).

Concentrated *n*-BuOH fraction was subjected to CC over Diaion HP-20 and the column was eluted with H_2O, H_2O–MeOH (3 : 1), H_2O–MeOH (1 : 1) and MeOH. About 50 ml of eluates were collected in receivers and concentrated in a rotavapour. Concentrated eluates were examined on TLC. Some colorless fractions of H_2O and H_2O–MeOH (3 : 1) eluates were almost identical on TLC and were found to contain several compounds of close *Rf*. These fractions were mixed together and concentrated to an oily mass. The oily mass was dried in vacuum desiccator and a part of it was subjected to NMR spectral analysis in CD_3OD and no acetoxy group was detected in it.

A major part of this oily mass was acetylated under mild condition with Ac_2O–pyridine at room temperature for 48 h. Usual work up of the acetylated mixture gave a solid product which was subjected to CC over silica gel repeatedly to isolate the acetate derivatives.

D-Mannitol Hexaacetate Petrol–EtOAc (9 : 1) eluate on concentration gave colorless crystals of D-mannitol hexaacetate (300 mg), mp 115 °C, identified by comparison (mmp and co-TLC) with an authentic sample.

β-D-Glucose Pentaacetate Petrol–EtOAc (8 : 1) eluate on concentration afforded β-D-glucose pentaacetate in colorless needles (100 mg), mp 131—132 °C; $[\alpha]_D^{23}$ +4.2° (c=0.9, $CHCl_3$), identified by comparison of its spectral (^{1}H-, ^{13}C-NMR and MS) data with literature as well as by direct comparison (mmp and co-TLC) with an authentic sample.

5-Dehydro-8-*epi*-adoxosidic Acid Pentaacetate (1a) Petrol–EtOAc (4 : 1) eluate on concentration gave **1a** as white amorphous powder (70 mg), mp 124 °C; TLC: *Rf* 0.9 in petrol–EtOAc–HOAc (15 : 5 : 0.5); UV λ_{max} (MeOH) nm (log ε): 234 (4.05), 280 (3.92); IR (KBr) cm^{-1}: 2646, 1747, 1705, 1655, 1636; ^{1}H-NMR (400 MHz, $CDCl_3$): Table 1; ^{13}C-NMR (100 MHz, $CDCl_3$): Table 2; HMBC correlations: H-1/H-3, C-5, C-1′, C-2′; H-3/C-1, C-4, C-11; H-6/C-5, C-7, C-8, C-9; H-7/C-5, C-6, C-8, C-9; H-9/C-1, C-4, C-5, C-6, C-7, C-8; H-10/C-1, C-5, C-6, C-9. Positive-HR-FAB-MS *m/z*: 607.1635 $[M+Na]^+$ (Calcd for $C_{26}H_{32}O_{15}Na$: 607.1639); Positive FAB-MS *m/z* (rel. int.): 585 $[M+H]^+$ (5), 567 (2), 531 (4), 331 [oxonium ion of tetraacetyl glucose]$^+$ (87), 169 (100), 109 (65), 43 (93). *Anal.* Calcd for $C_{26}H_{32}O_{15}$: C, 53.43; H, 5.52. Found: C, 53.36; H, 5.44.

8-*epi*-Mussaenoside Tetraacetate (2a) Petrol–EtOAc (3 : 1) eluate on concentration afforded a solid residue, which on re-column chromatography gave **2a** as white amorphous powder (35 mg), mp 123 °C; TLC: *Rf* 0.33 in petrol–EtOAc (10 : 7); UV λ_{max} (MeOH) nm (log ε): 238 (3.84); IR (KBr) cm^{-1}: 3470, 1750, 1640; ^{1}H-NMR (400 MHz, $CDCl_3$): Table 1; ^{13}C-NMR (100 MHz, $CDCl_3$): Table 2.

5-Dehydo-8-*epi*-Mussaenoside Tetraacetate (3a) Petrol–EtOAc (3 : 2) eluate on concentration gave a residue of two components, which on re-column chromatography afforded **3a** as white amorphous powder (45 mg), mp 85 °C; TLC: *Rf* 0.14 in petrol–EtOAc (10 : 7); UV λ_{max} (MeOH) nm (log ε): 235 (4.04), 280.6 (3.81); IR (KBr) cm^{-1}: 3475, 1751, 1640; ^{1}H-NMR (400 MHz, $CDCl_3$): Table 1; ^{13}C-NMR (100 MHz, $CDCl_3$): Table 2; HMBC correlations: H-1/C-3, C-1′; H-3/C-1, C-4, C-11; H-6/C-5, C-7, C-8, C-9; H-7/C-6, C-8, C-9; H-9/C-4, C-5, C-7. Negative FAB-MS *m/z*: 555 $[M-H]^-$. *Anal.* Calcd for $C_{25}H_{32}O_{14}$: C, 53.96; H, 5.80. Found: C, 53.88; H, 5.71.

10-*O*-Dihydroferuloyldeacetyldaphylloside Hexaacetate (4a) Petrol–EtOAc (1 : 1) eluate on concentration gave a residue of three components, which on re-column chromatography afforded **4a** in white amorphous powder (30 mg), mp 116 °C; TLC: *Rf* 0.10 in petrol–EtOAc (10 : 7); UV λ_{max} (MeOH) nm (log ε): 236 (3.96), 280 (3.32), 302 (3.04), 324 (3.20); IR (KBr) cm^{-1}: 1750, 1640, 1605, 1503, 1040, 908; ^{1}H-NMR (400 MHz, $CDCl_3$): Table 1; ^{13}C-NMR (100 MHz, $CDCl_3$): Table 2; HMBC correlations: H-1/ C-3, C-1′; H-3/C-1, C-4, C-1′, C-11; H-5/C-3, C-4, C-9; H-6/C-5, C-7; H-9/C-1, C-3, C-5, C-8. EI-MS *m/z* (rel. int.): 834 $[M]^+$ (2), 792 (2), 375 (29), 331 (78), 169 (100), 139 (58). (*Anal.* Calcd for $C_{39}H_{46}O_{20}$: C, 56.11, H, 5.55. Found: C, 55.99; H, 5.48.

Wendoside Pentaacetate (5a) Petrol–EtOAc (1 : 2) eluate on concentration afforded a residue of two components. This residue on re-column chromatography gave **5a** as white amorphous powder (40 mg), mp 103 °C; TLC: *Rf* 0.54 in petrol–EtOAc (3 : 7); IR (KBr) cm^{-1}: 1755, 2856, 1128; ^{1}H-NMR (400 MHz, $CDCl_3$): Table 1; ^{13}C-NMR (100 MHz, $CDCl_3$): Table 2; HMBC correlations: H-1/C-3,C-1′; H-3/C-1, C-4; H-5/C-9; H-6/C-5, C-7, C-11; H-7/C-6, C-8; H-9/C-1, C-4, C-5, C-10; H-1′/C-3, C-4. EI-MS *m/z* (rel. int.): 586 $[M]^+$ (1), 418 $[M-4\times42]^+$ (13), 376 [418-42] (3), 331 (49), 169 (100), 109 (53). *Anal.* Calcd for $C_{26}H_{34}O_{15}$: C, 53.24; H, 5.84. Found: C, 53.18; H, 5.80.

Acid Hydrolysis of 1a A solution of **1a** (*ca.* 10 mg) in 1 N aqueous methanolic HCl (5 ml) was refluxed for 2 h. The reaction mixture was concentrated under a stream of N_2 to give a residue in which D-glucose was detected by TLC (*Rf* 0.48, *n*-BuOH–pyridine–H_2O, 6 : 4 : 3) and optical rotation (positive optical rotation) study. The residue on extraction with *n*-BuOH gave a semisolid mass of aglucone, $C_{10}H_{12}O_5$ (M^+ 212).

Deacetylation of 1a A solution of **1a** (*ca.* 5 mg) in 0.1 N aqueous methanolic NaOH was left at room temp. (35—40 °C) under a stream of N_2 for 3 h. The reaction mixture was acidified with dil H_2SO_4 at 0 °C and evaporated to a residue in which deacetylated product **1** was found to be identical on co-TLC with one of the components of the oily mixture used for acetylation.

Acid Hydrolysis of 3a Compound **3a** (*ca.* 8 mg) was hydrolysed in the same method as described in case of **1a**. In the residue of reaction mixture, D-glucose was detected by TLC and optical rotation study.

Deacetylation of 3a Compound **3a** (*ca.* 5 mg) was deacetylated in the same method as described above in case of **1a**. The deacetylated product **3** in the residue of the reaction mixture was identical on co-TLC with one of the components of the oily mixture used for acetylation.

Acid Hydrolysis of 4a Compound **4a** (*ca.* 6 mg) was hydrolysed in the same method as described above in case of **1a** and in the residue of the reaction mixture, D-glucose was detected by TLC and optical rotation study.

Alkaline Hydrolysis of 4a A solution of **4a** (*ca.* 12 mg) in 10 ml 1 M aqueous methanolic NaOH was refluxed for 2 h. The reaction mixture was concentrated under a stream of N_2 to a residue, which was dissolved in a little H_2O and acidified with dil H_2SO_4 to pH *ca.* 3. The resulting solution was extracted with $CHCl_3$ (15 ml×3). The combined $CHCl_3$ solution was evaporated to get a residue, in which dihydroferulic acid was detected by TLC comparison with an authentic sample.

Deacetylation of 4a Compound **4a** (*ca.* 5 mg) was deacetylated in the same method as described in case of **1a** by keeping at room temp. for 5 h. The deacetylated product **4** was found to be identical on co-TLC with one of the components of oily mixture used for acetylation.

Acid Hydrolysis of 5a Compound **5a** (*ca.* 10 mg) was hydrolysed in the same method as described above in case of **1a**. In the residue of the reaction mixture, D-glucose was detected by TLC and optical rotation study. The residue on extraction with THF gave a semisolid mass of the aglucone **5b**.

Deacetylation of 5a Compound **5a** (*ca.* 5 mg) was deacetylated in the same method as described in case of **1a**. In the residue of the reaction mixture, the deacetylated product **5** was found to be identical on co-TLC with one of the components of the oily mixture used for acetylation.

Bioassays Antibacterial activities of the crude *n*-butanol fraction and the compounds (**1a**—**5a**) isolated as acetates from this fraction were tested *in vitro* against (*Staphylococcus aureus*, *Bacillus subtilis* and *Pseudomonas aeruginosa*) by cup-plate method.[8] Ampicillin at a concentration of 2.5 mg/ml and gentamicin 1 mg/ml were used as references. All the compounds were tested at a concentration of 10 mg/ml. Except compound **5a**, the compounds showed an inhibition zone below 8 mm against all the microorganisms, whereas compound **5a** showed an inhibition zone of 20 mm against *P. aeruginosa*. The *n*-butanol fraction at a concentration of 250 mg/ml showed an inhibition zone of 15.5 mm, 5 mm and 7 mm against *P. aeruginosa*, *S. aureus* and *B. subtilis*, respectively.

Acknowledgements The authors wish to thank Prof. B. C. Ranu, IACS, Kolkata for IR spectra; CDRI, Lucknow for microanalyses and Dr. B. K. Dutta, Department of Life Sciences, Tripura University for identification of plant material. The work was supported by a grant (No. G-12/2002) from Department of Science and Technology, Government of India, New Delhi to BD.

References

1) Deb D. B., "The Flora of Tripura State," Vol. 2. Today & Tomorrow's Printers and Publisher's, New Delhi, 1983, p. 88.
2) Dinda B., Debnath S., Arima S., Sato N., Harigaya Y., *J. Indian Chem. Soc.*, **81**, 73—76 (2004).
3) Inouye H., Nishioka T., *Chem. Pharm. Bull.*, **21**, 497—499 (1973).
4) Takeda Y., Nishimura H., Inouye H., *Phytochemistry*, **16**, 1401—1404 (1977).
5) Damtoft S., Jensen S. R., Nielsen B. J., *Phytochemistry*, **32**, 885—889 (1993).
6) Sainty D., Delaveau P., Bailleul F., Moretti C., *J. Nat. Prod.*, **45**, 676—678 (1982).
7) Jensen S. R., Kirk O., Nielsen B. J., Norrestam R., *Phytochemistry*, **26**, 1725—1731 (1987).
8) Cruickshank R., Duguid J. P., Marmion B. P., Swain R. H. A., "The Practice of Medicinal Microbiology," 12th ed., Vol. II, Churchill Livingstone, London, 1975, p. 110.