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Resveratrol oligomer C-glucosides and anti-viral resveratrol tetramers isolated from the stem bark of *Shorea uliginosa*

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ABSTRACT

A new resveratrol dimer C-glucoside [uliginosides D (**1**)] was isolated from the stem bark of *Shorea uliginosa*, along with 21 resveratrol derivatives (**2**–**22**). Eight of these compounds (**1**, **3**–**9**) comprised the building block, 4-*C*-glucosylresveratrol (**10**). The absolute configurations of six compounds (**1** and **3**–**7**) were determined using NMR and circular dichroism. The antiviral effects of the main oligostilbenoid compounds in this plant and representative oligostilbenoids from other Dipterocarpaceaeous plants were evaluated. Five resveratrol derivatives (**13**, **15**, **17**, and vaticanols B and G) were found to inhibit the replication of the herpes simplex virus types 1 and 2 with selectivity indices (SIs) ranging from 31 to 290. Two resveratrol tetramers (**13** and **15**) also showed an inhibitory effect on influenza A virus replication with SIs ranging from 21 to 66.

1. Introduction

Plants belonging to the family Dipterocarpaceae typically contain oligomers of resveratrol biosynthesized *via* oxidative coupling. The structural diversity exhibited by these oligomers stems from differing oligomer lengths, skeletal variations, stereoisomerism, and *O*- and *C*-glucosidation (Ito, 2011; Sotheeswaran and Pasupathy, 1993). The genus *Shorea* belongs to the Shoreae, one of the two major tribes of the subfamily Dipterocarpoideae, and consists of approximately 194 species distributed throughout southeast Asia, particularly in Indonesia, Malaysia, southern and eastern India, and Sri Lanka (Ashton and Arboretum, 1982). Despite a growing chemical library of compounds from the *Shorea*, the phytochemical aspects of this genus have not been comprehensively examined. Furthermore, our previous studies on *Shorea* (*S. hemsleyana*, *S. cordifolia*) dealt with a limited scope of resveratrol derivatives (Ito et al., 2000a,b; Tanaka et al., 2000a,b, 2001b; Ito et al., 2003, 2009, 2012, 2013b). In the current study, we investigated the highly polar chemical constituents of *S. uliginosa* to expand the chemical library of Shoreae. This was achieved with the isolation of a new *C*-glucosides of resveratrol dimer [uliginoside D (**1**)] along with uliginosides A–C (**3**, **5**, and **6**) and 18 known resveratrol derivatives (**2**, **4**, and **7**–**22**). The structures of these compounds were elucidated with extensive spectroscopic analysis, including 1D- and 2D-NMR, circular dichroism (CD), and mass spectroscopy, and clarified with computer-aided molecular modeling. The glucosides were all found to contain one or two building blocks of 4-*C*-glucosylresveratrol (4*C*-glc-Res; (*E*)-2-(β-glucopyranosyl)-5-(4-hydroxystyryl)benzene-1,3-diol).

Although the *Shorea* are known to be a good source of biologically active resveratrol oligomers with antibacterial (Nitta et al., 2002), cytotoxic (Dai et al., 1998; Saroyobudiono et al., 2008), and antifungal activities (Ge Hui et al., 2006) among others, only anti-HIV activity has been reported to date in terms of antiviral activity (Dai et al., 1998; Zhang et al., 2003). Oligostilbenoids are recognized as potential leads in the search for antiviral compounds. We, therefore, used the main oligostilbenoid compounds from *S. uliginosa* and representative oligostilbenoids from other Dipterocarpaceaeous plants to carry out *in vitro* antiviral assays against herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2), as well as influenza A virus (IAV).

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2. Results and discussion

2.1. Isolation

An acetone extract of the stem bark of *S. uliginosa* was subjected to open column chromatography on silica gel, ODS, and Sephadex LH-20. Further repeated purification by preparative TLC and HPLC achieved the isolation of 22 resveratrol derivatives (**1**–**22**).

2.2. Identification of known compounds

Structures of the known compounds were identified by comparing the spectroscopic data we obtained with those in the literature and our database. The compounds were identified as hemsleyanosides A (**8**), B (**4**), and C (**9**); (Ito et al., 2000a) shorealactone (**5**) (Ito et al., 2003; Tanaka et al., 2000a,c) (synonym: laevifonol (Hirano et al., 2003)); diptoindonesin A (**7**) (Aminah et al., 2002); 4-*C*-glucosylresveratrol (**10**) (Ito et al., 2000b); 2-*C*-glucopyranosyl resveratrol (**11**) (Tanaka et al., 2001a); piceid (**12**) (Tanaka et al., 2000b); (−)-hopeaphenol (**13**) (Ito et al., 2000b); hemsleyanols A (**18**) (Ito et al., 2000b), B (**16**) (Ito et al., 2000b), D (**14**) (Ito et al., 2000b), and E (**22**) (Tanaka et al., 2001b); (+)-α-viniferin (**17**) (Ito et al., 2000b); ampelopsins A (**19**) (Ito et al., 2000b) and F (**21**) (Oshima et al., 1993); and pauciflorol E (**20**) (Ito et al., 2004). Structural elucidation of shoreaketone (**15**), isolated from *S. uliginosa* as well as from *S. hemsleyana* and *Vateria indica*, was performed in our previous study (Ito et al., 2012). Structures of known compounds, **8**, **9**, **14**, **16**, and **18**–**22**, are included in Supporting Information (Fig. S1).

2.3. Absolute configuration of uliginoside D (1)

Uliginoside D (**1**) was obtained as pale yellow solid that showed a positive reaction to Gibbs reagent. HR-FAB-MS (*m/z* $[M-H]^-$ 789.2021) showed a molecular formula of $C_{40}H_{38}O_{17}$. ^1H NMR and ^{13}C NMR spectral data (Table 1) analyzed using ^1H,^1H-COSY, HMQC, and HMBC (Fig. 1: *a*) showed aromatic signals corresponding to two 4-oxygenated benzene rings (A_1 and B_1), one 3,5-dioxygenated benzene ring (A_2), one 1,2,3,4,5-pentasubstituted benzene ring (B_2), two mutually coupled benzylmethine sequences [CH(7a)–CH(8a) and CH (7b)–CH(8b)], and a mutually coupled aliphatic methine–methylene sequence successively coupled in order [CH(4′)–CH(5′)–CH_2(6′)]. The presence of an *C*-*β*-glucopyranosyl moiety was supported by NMR spectral data showing six carbon signals (δ_C: 76.9, 73.1, 79.9, 71.5, 82.4, and 62.6) and an anomeric proton [δ_H: 4.64 (d, J = 9.9 Hz)]. These results indicated that the aglycone of **1** has the composition $C_{34}H_{28}O_{12}$. The NMR data of the aglycone moiety showed close similarity with that of shorealactone (**2**) [Ito et al. 2000 and 2003 (Ito et al., 2003; Tanaka et al., 2000a,c)] {laevifonol [Hirano et al. 2003 (Hirano et al., 2003)]}. The HMBC and NOESY spectra (Fig. 1: *a*), *b*)) confirmed the structure of aglycone of **1** to be the same as **2**. The position of the *C*-glucosyl group was determined to be at C-12b by the ^3J cross peaks in the ^1H-^{13}C HMBC analysis, which displayed the cross peaks between the anomeric proton (δ_H 4.46 (H-Glc-1)) and the aromatic carbons at δ_C 160.2 (C-11b), δ_C 107.7 (C-12b), and δ_C 157.7 (C-13b). In our previous study, the absolute configuration of shorealactone (**2**) was determined using 2D-NMR technique, and X-ray crystal structure analysis of its 4-bromobenzoyl derivative was performed using anomalous scattering of the Br-atom. To determine the absolute configuration of **1**, the CD spectra of these two compounds were compared. The CD curve including the intensities of the Cotton effect for **1** was similar to those of **2** (**1**: Δ_{ext} = 217 nm, $\Delta\varepsilon$ = +31.1, and Δ_{ext} = 237 nm, $\Delta\varepsilon$ = −38.9; **2**: Δ_{ext} = 216 nm, $\Delta\varepsilon$ = +59.8, and Δ_{ext} = 238 nm, $\Delta\varepsilon$ = −44.9). Thus, the structure of uliginoside D (**1**) was elucidated to be shorealactone 12a-*C*-β-glucopyranoside.

Table 1
^1H and ^{13}C NMR Data for Uliginoside D (**6**).[a]

No.	δH	δC
1a		132.6
2a, 6a	6.76 (s)	129.1
3a, 5a	6.76 (s)	116.3
4a		158.5
7a	5.09 (d, J = 7.7)	94.9
8a	3.20 (br s)	57.2
9a		146.2
10a, 14a	5.90 (br d, J = 2.0)	107.7[b]
11a, 13a		159.9
12a	6.15 (t, J = 2.0)	102.7
1b		129.8
2b, 6b	6.95 (br d, J = 8.4)	128.5
3b, 5b	6.72 (br d, J = 8.4)	116.0
4b		159.0
7b	5.31 (d, J = 10.8)	90.7
8b	3.27 (d, J = 10.8)	56.7
9b		131.7
10b		123.8
11b		160.2
12b		107.7
13b		157.7
14b	7.18 (br s)	112.1
1'		173.7
2'		81.4
3'		119.2
4'	4.37 (br s)	89.5
5'	4.20 (m)	74.6
6'	3.98 (dd, J = 9.8, 4.4)	75.9
	4.10 (dd, J = 9.8, 2.4)	
Glc-1	4.64 (d, J = 9.9)	76.9
Glc-2	4.00 (m)	73.1
Glc-3	3.42 (m)	79.9
Glc-4	3.45 (m)	71.5
Glc-5	3.35 (m)	82.4
Glc-6	3.75, 3.87 (m)	62.6

[a] In CD3OD; at 300 (1 H) and 75 (13C) MHz, d in ppm, J in Hz.
[b] Overlapping.

2.4. Absolute configuration of uliginoside A (3), hemsleyanoside B (4), and diptoindonesin A (7)

The absolute configurations of uliginoside A (**3**) and hemsleyanoside B (**4**), as determined using NMR (in methanol-d_4) and CD spectroscopy, represent the first characterization of resveratrol dimers bearing an enantiomeric aglycone (Ito et al., 2009). Their relative structures were confirmed by combined analyses of ^1H,^1H-COSY; HMBC; ^{13}C,^1H-COSY; and NOESY spectra. The proposed relative structure of **3** was identical to the structure reported for **4**, and their NMR data showed strong resemblance. Clarification of their differences was achieved with CD spectroscopy, in which **3** and (−)-ε-viniferin displayed the same CD curves, whereas **3** and **4** displayed opposite curve patterns. In the current study, diptoindonesin A (**7**) was also isolated and the absolute configuration of the aglycone [(−)-ε-viniferin) was determined with NMR and CD (**7**: Δ_{ext} 236 nm, $\Delta\varepsilon$ −25.8; (−)-ε-viniferin: Δ_{ext} 236 nm, $\Delta\varepsilon$ −24.8 (Ito et al., 2009)]. An isomer bearing the enantiomeric aglycone of **7** was not isolated despite repeated purification attempts of the relevant fractions. The absence of ε-viniferin in the extract was finally confirmed with UPLC-DAD-ESIMS analysis.

2.5. Absolute configuration of uliginosides B (5) and C (6)

Uliginosides B (**5**) and C (**6**) are the first instances of diastereomeric resveratrol trimers with two *C*-glucopyranosyl units. ^1H and ^{13}C NMR data of **5** and **6** in methanol-d_4 were shown in a previous communication (Ito et al., 2009). Both compounds are resveratrol trimers with two *C*-glucopyranosyl units and bear two *trans*-oriented 1,2-