

## Squadinorlignoside: A Novel 7,9'-Dinorlignan from the Stems of *Annona squamosa*

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Two new polar lignans, *i.e.*, squadinorlignoside (= 4-[(1*E*)-1-(hydroxymethyl)-3-(4-hydroxyphenyl)prop-1-en-1-yl]phenyl  $\beta$ -D-glucopyranoside; **1**) and (6*R*,7*R*,8*S*)-7a-[( $\beta$ -D-glucopyranosyl)oxy]-1-methoxyisolariciresinol (**2**) were isolated from the stems of *Annona squamosa*, together with eight known lignans and five known neolignans (compounds **3–15**; Fig. 1). All of these constituents are reported for the first time from the genus *Annona*. The structures, absolute configurations, and selected conformational aspects of the new compounds were elucidated spectroscopically. Compound **1** is the first example of a 7,9'-dinorlignan natural product.

**Introduction.** – In previous studies, a number of bioactive phytochemicals, including *ent*-kaurane diterpenoids, alkaloids, annonaceous acetogenins, cyclic peptides, *etc.*, were isolated from *Annona squamosa* [1–3]. In the present work, we report a series of constituents isolated from the *polar* fractions and the aqueous layer of the MeOH-soluble extracts of *A. squamosa*. The following 15 lignans and/or neolignans were isolated (Fig. 1): squadinorlignoside (**1**)<sup>1</sup>, (6*R*,7*S*,8*S*)-7a-[( $\beta$ -D-glucopyranosyl)oxy]-1-methoxyisolariciresinol (**2**), (6*R*,7*R*,8*S*)-1-methoxyisolariciresinol (**3**) [4], (6*S*,7*S*,8*R*)-7a-[( $\beta$ -D-glucopyranosyl)oxy]isolariciresinol (**4**) [5], (6*R*,7*R*,8*S*)-isolariciresinol (**5**) [6], (6*R*,7*S*,8*S*)-7a-[( $\beta$ -D-glucopyranosyl)oxy]lyoniresinol (**6**) [7], (6*R*,7*R*,8*R*)-7a-[( $\beta$ -D-glucopyranosyl)oxy]lyoniresinol (**7**) [7], [(2*R*\*,2'*R*\*)-secoisolariciresin-4-yl]  $\beta$ -D-glucoside (**8**) [8], (2*R*\*,2'*R*\*)-secoisolariciresinol (**9**) [9], (7*S*,8*R*,8'*R*)-5,5'-dimethoxyariciresinol (**10**) [10], (7*S*,8*R*)-7,9,9'-trihydroxy-3,3'-dimethoxy-8-*O*-4'-neolignan-4-*O*- $\beta$ -D-glucopyranoside (**11**) [11], (7*S*,8*R*)-4,7,9,9'-tetrahydroxy-3,3'-dimethoxy-8-*O*-4'-neolignan (**12**) [11], (7*S*,8*R*)-urolignoside (**13**) [12], (7*S*,8*R*)-dihydrodehydrodiconiferylalcohol (**14**) [12], and (7*S*,8*R*)-5-methoxydihydrodehydrodiconiferylalcohol (**15**) [13]. All of these compounds were obtained from *Annona* species for the first time, lignans **1** and **2** being new compounds.

**Results and Discussion.** – Compound **1**, obtained as syrup, had the molecular formula C<sub>22</sub>H<sub>26</sub>O<sub>8</sub> based on its HR-FAB-MS data. In the <sup>1</sup>H-NMR spectrum, the resonances of two 1,4-disubstituted Ph groups were observed ( $\delta$ (H) 6.67, 6.93 (*2d*, *J* = 8.6 Hz each, 2  $\times$  2 H); 7.11, 7.19 (*2d*, *J* = 8.8 Hz each, 2  $\times$  2 H)). By analyzing the chemical shifts and coupling constants, one olefinic H-atom at  $\delta$ (H) 5.83 (*td*, *J* = 7.6, 1.2), and two sets of CH<sub>2</sub> resonances at 3.21 (*d*, *J* = 7.6) and 4.24 (*d*, *J* = 1.2 Hz) indicated a trisubstituted

<sup>1</sup>) For systematic names of the new compounds **1** and **2**, see the *Exper. Part*.

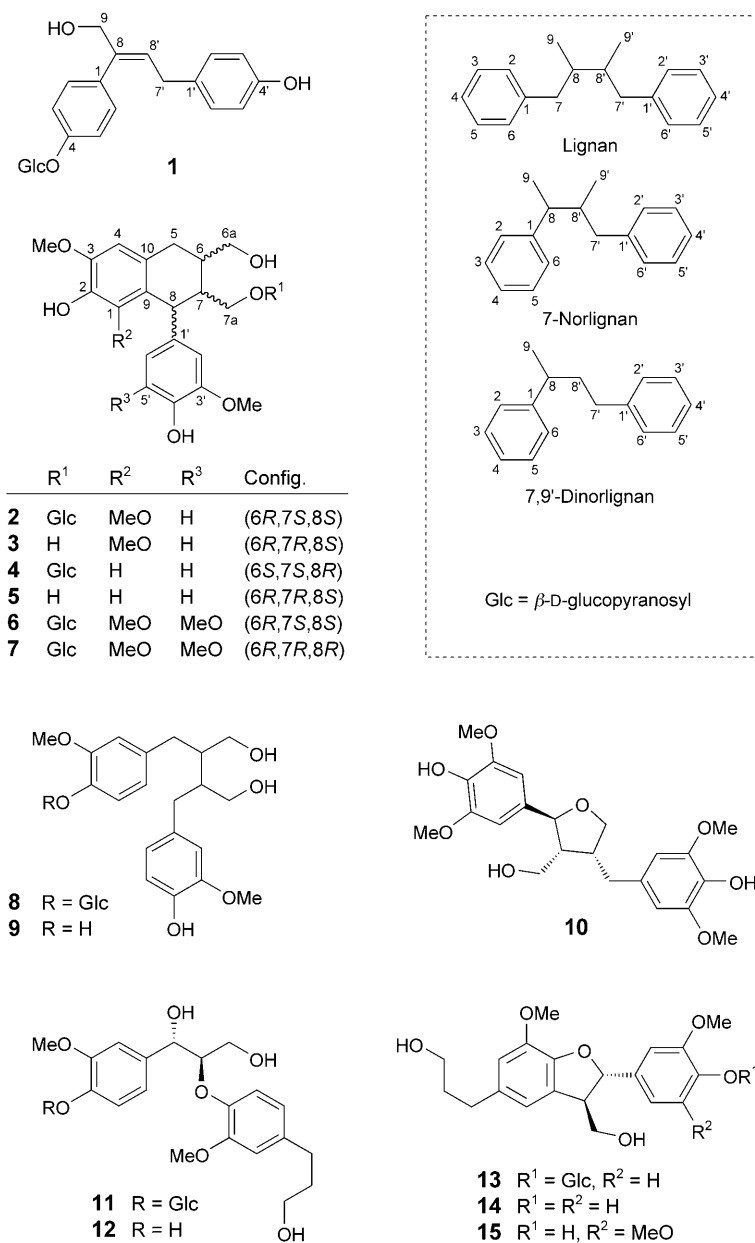


Fig. 1. Structures of compounds 1–15. Basic lignan frameworks and IUPAC atom numbering [14] are shown in the box.

olefinic group flanked by two CH<sub>2</sub> groups, one of which was oxygenated ( $\delta$ (H) 4.24,  $\delta$ (C) 67.9). The structure of the aglycone was fully established by 2D-NMR experiments, *i.e.*, <sup>1</sup>H,<sup>1</sup>H-COSY, TOCSY, HMQC, and HMBC spectra, and the configuration

of the olefinic group was assigned by NOESY (Fig. 2). The key NOE correlations of H–C(9)/H–C(8') and H–C(6)/H–C(7') established the (*E*)-configuration.

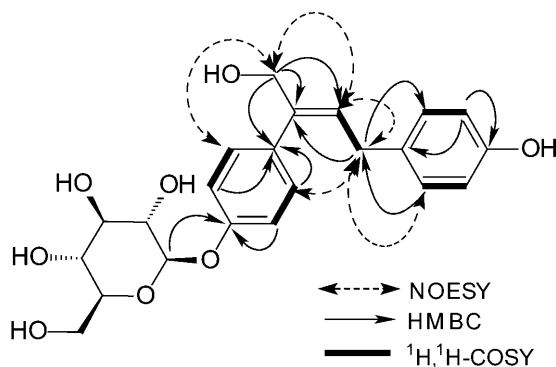


Fig. 2. Selected NOESY, HMBC, and  $^1\text{H}, ^1\text{H}$ -COSY correlations of **1**

The sugar moiety of **1** was found to correspond to a  $\beta$ -D-glucopyranosyloxy (GlcO) residue attached at C(4) of the 7,9'-norlignan skeleton (see Fig. 1), as deduced from the HMBC spectrum and from the corresponding EI-MS fragments (Fig. 3). Thus, from the above data, the structure of compound **1** was identified as 4-[(*E*)-1-(hydroxymethyl)-3-(4-hydroxyphenyl)prop-1-en-1-yl]phenyl  $\beta$ -D-glucopyranoside, and the compound was named *squadinorlignoside*. According to the IUPAC nomenclature of norlignans [14], this compound has an unprecedented 7,9'-dinorlignan skeleton (Fig. 1).

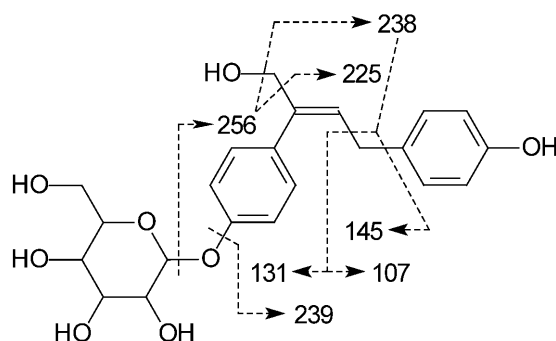
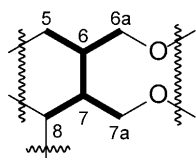


Fig. 3. EI-MS Fragments of **1**

Compound **2** was optically active,  $[\alpha]_{\text{D}}^{22} = +130.8$  ( $c=0.026$ , MeCN), and had the molecular formula  $\text{C}_{27}\text{H}_{36}\text{O}_{12}$ , as determined by HR-FAB-MS. The  $^{13}\text{C}$ -NMR spectrum of **2** (see the Table in the *Exper. Part*) was very similar to those of the known isolariciresinol-type lignan glycosides **4**, **6**, and **7** [5][7]. The  $^1\text{H}$ -NMR spectrum of **2** exhibited signals for one set of *ABX*-type aromatic H-atoms, indicating 1,3,4-trisubstitution ( $\delta(\text{H})$  6.76 (*d*,  $J=2.0$ , 1 H); 6.64 (*d*,  $J=8.4$ , 1 H); 6.50 (*dd*,  $J=8.4$ , 2.0 Hz, 1 H)), as well as an aromatic *singlet* at  $\delta(\text{H})$  6.57 (1 H). In the  $^1\text{H}, ^1\text{H}$ -COSY and TOCSY spectra, the partial structure **A** was revealed (Fig. 4), and three MeO groups ( $\delta(\text{H})$  3.31, 3.77, 3.85) at C(1), C(3), and C(3'), respectively, were identified from the NOE cross-peaks of 1-MeO/H–C(8), 3-MeO/H–C(4), and 3'-MeO/H–C(2') (Fig. 5).



—  $^1\text{H}, ^1\text{H}$ -COSY

Fig. 4.  $^1\text{H}, ^1\text{H}$ -COSY and TOCSY Correlations for the partial structure **A** of **2**

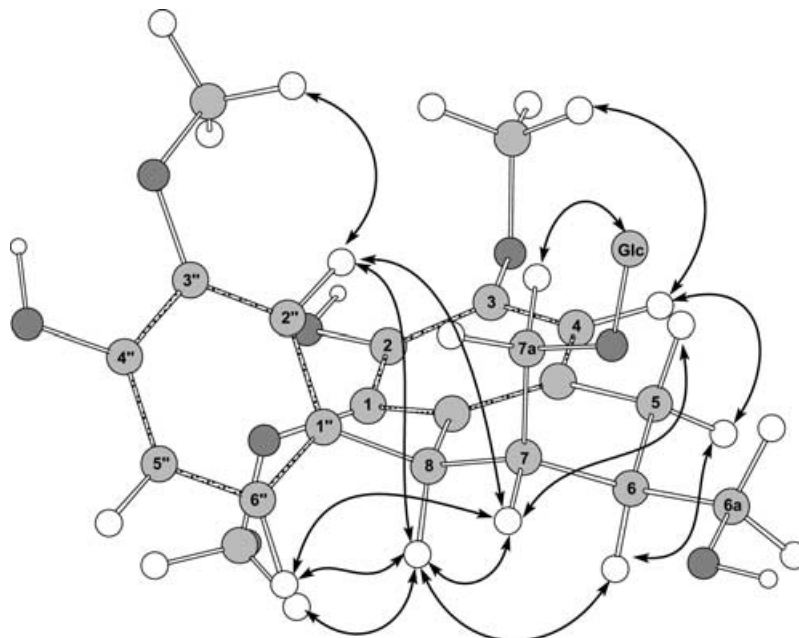
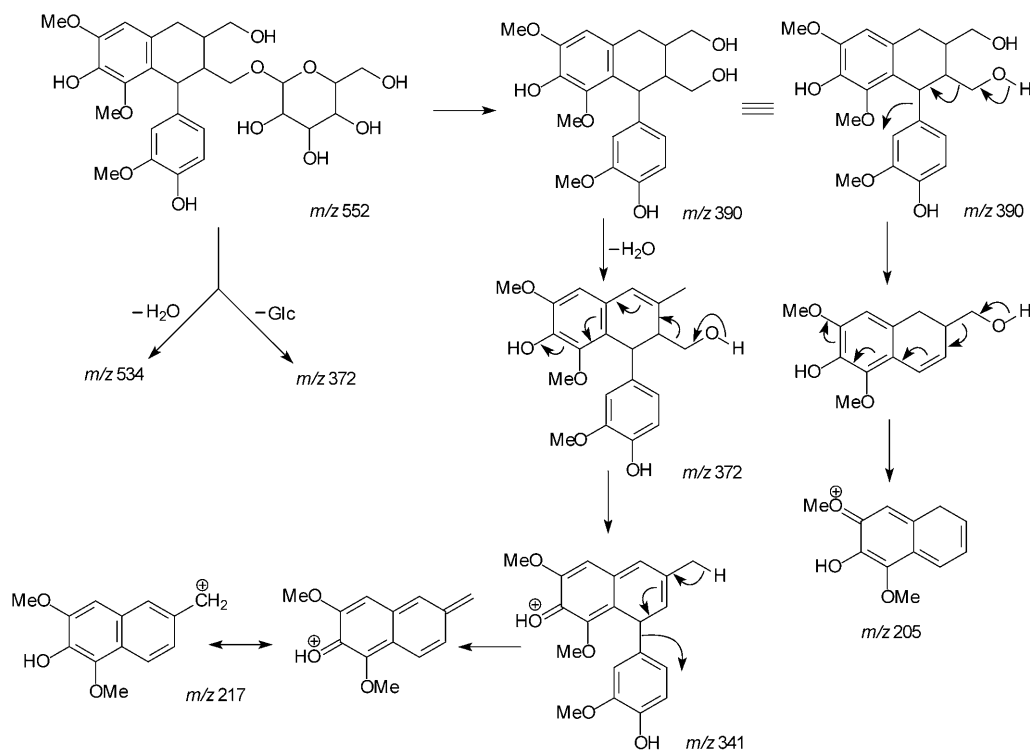


Fig. 5. Selected NOESY correlations of **2**. The cross-peak between H-C(7a) and Glc refers to the anomeric H-atom H-C(1'').

The presence of a Glc group in **2** was inferred from its  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR spectra. The sugar moiety was attached at C(7a), as deduced from the NOE cross-peaks between the anomeric Glc H-atom ( $\delta(\text{H})$  4.27 (*d*,  $J=7.6$  Hz)) and  $\text{CH}_2(7a)$  ( $\delta(\text{H})$  3.45 (*dd*,  $J=9.8, 4.0$ ), 3.89 (*dd*,  $J=9.8, 5.6$  Hz)).

The above results, in combination with a detailed analysis of the EI-MS fragments of **2** (Fig. 6), indicated that the compound was a  $\beta$ -D-glucoside of 1-methoxyisolaricirensinol. The relative configurations at C(6) to C(8) were determined by a NOESY experiment (Fig. 5), and corroborated by inspection of  $^1\text{H}, ^1\text{H}$ -coupling constants. The NOE correlations between H-C(2',6') and H-C(7), together with a  $J(7,8)$  value of 6.4 Hz, indicated that H-C(7) and H-C(8) are in an axial/equatorial (ax/eq) relation. The coupling constants for H-C(5) [ $\text{H}_{\text{ax}}\text{-C}(5)$  at  $\delta(\text{H})$  2.59 (*dd*,  $J=14.8, 11.6$  Hz);  $\text{H}_{\text{eq}}\text{-C}(5)$  at  $\delta(\text{H})$  2.71 (*dd*,  $J=14.8, 4.8$  Hz)] evidenced that H-C(6) is in an axial orientation. The NOESY cross-peaks of H-C(7) and H-C(8) with H-C(6), and of  $\text{H}_{\text{ax}}\text{-C}(5)$  with H-C(7), and the absence of a cross-peak between H-C(5) and H-C(8), and H-C(5) and

Fig. 6. EI-MS Fragments of **2**

H–C(2',6'), indicated that the cyclohexane ring of **2** is in a half-envelope conformation (Fig. 5), with the relative (6*R*\*,7*S*\*,8*S*\*)-configuration. From circular-dichroism (CD) experiments, the absolute (6*R*,7*S*,8*S*)-configuration was established, based on  $\Delta\epsilon$  values of  $-0.06$  and  $+0.11$  at 299 and 275 nm, respectively [15]. From all these data, compound **2** was identified as [(1*S*,2*S*,3*R*)-1,2,3,4-tetrahydro-7-hydroxy-1-(4-hydroxy-3-methoxyphenyl)-3-(hydroxymethyl)-6,8-dimethoxynaphthalen-2-yl]methyl  $\beta$ -D-glucopyranoside.

The other isolated lignanoids **3–15** were structurally elucidated by spectroscopic analysis and comparison with literature data. In previous studies, only furofuran lignans with a 7,9':7',9-diepoxylic lignan skeleton have been reported from *Annona* species [16][17]. In the present study, three different lignan skeletons were identified: the 2,7'-cyclolignans **2–7**, the diarylbutanelignans **8** and **9**, and the 7',9'-epoxylic lignan **10**, all of which are biogenetically derived from the furofuran lignans. In addition, five neolignans, **11–15**, were isolated for the first time from *Annona*. So far, only one neolignan analogue, named grossamide, has been reported from *Annona* [18].

#### Experimental Part

*General.* Silica gel 60 (230–400 mesh; Merck) was used for column chromatography (CC). Prep. HPLC: Develosil ODS and C30-UG-5 columns (250 × 20 mm) on a JASCO PU-1580 apparatus with a UV-1575 detector.

TLC: Spots were detected by spraying with 50% H<sub>2</sub>SO<sub>4</sub>, and then heated on a hot plate. UV Spectra: JASCO V-530 spectrophotometer;  $\lambda_{\max}$  in nm. Optical rotations: JASCO P-1020 digital polarimeter. CD Spectra: JASCO J-720 spectropolarimeter;  $\lambda$  ( $\Delta\epsilon$ ) in nm. IR Spectra: a Mattson Genesis-II spectrophotometer; in cm<sup>-1</sup>. <sup>1</sup>H-NMR: at 400 or 500 MHz in (D<sub>6</sub>)acetone or CD<sub>3</sub>OD;  $\delta$  in ppm, *J* in Hz. <sup>13</sup>C-NMR, DEPT, <sup>1</sup>H,<sup>1</sup>H-COSY, TOCSY, HMBC, HMQC, and NOESY Spectra: Varian Unity Plus-400 and Unity INOVA-500. EI-MS: Finnigan POLARISQ mass spectrometer, with direct-insert probe; HR-FAB-MS: Jeol JMS-HX-110 mass spectrometer; in *m/z* (rel. %).

**Plant Material.** Fresh stems of *A. squamosa* were collected from Shueimen, Pingtung County, Taiwan, in May 2000. The plant was identified by Dr. Hsin-Fu Yen, National Museum of Natural Science, Taichung, Taiwan. A voucher specimen (Annona 6) was deposited at the Graduate Institute of Natural Products, Kaohsiung Medical University, Kaohsiung, Taiwan.

**Extraction and Isolation.** Fresh stems of *A. squamosa* (15 kg) were extracted repeatedly with MeOH at r.t. The combined extracts were evaporated under reduced pressure to yield a dark-brown syrup (550 g), which was partitioned between CHCl<sub>3</sub> and H<sub>2</sub>O. Both layers were further processed separately. *a*) The CHCl<sub>3</sub> layer was extracted with 3% aq. HCl to remove alkaloids. The 'neutral' CHCl<sub>3</sub> soln. was dried and evaporated to leave

Table. NMR Data of the 2,7-Cyclolignans **2–4**, **6**, and **7**. At 500/125 MHz, resp., in CD<sub>3</sub>OD;  $\delta$  in ppm, *J* in Hz.

Position	<b>2</b>		<b>3</b>	<b>4</b>	<b>6</b>	<b>7</b>
	$\delta$ (H)	$\delta$ (C)	$\delta$ (C)	$\delta$ (C)	$\delta$ (C)	$\delta$ (C)
1	–	147.5	147.6	117.4	147.6	147.6
2	–	138.9	138.9	145.9	138.9	138.9
3	–	148.6	148.5	147.3	148.6	148.8
4	6.57 ( <i>s</i> )	107.9	107.8	112.3	107.9	107.9
5	2.59 ( <i>dd</i> , <i>J</i> = 14.8, 11.6) 2.71 ( <i>dd</i> , <i>J</i> = 14.8, 4.8)	33.9	33.6	33.6	33.8	33.8
6	1.68–1.74 ( <i>m</i> )	40.6	40.9	41.1	40.6	41.2
6a	3.52 ( <i>dd</i> , <i>J</i> = 10.8, 6.4) 3.60–3.66 ( <i>m</i> )	66.3	66.8	65.5	66.2	66.2
7	2.04–2.10 ( <i>m</i> )	46.8	49.6	45.3	46.7	46.6
7a	3.45 ( <i>dd</i> , <i>J</i> = 9.8, 4.0) 3.89 ( <i>dd</i> , <i>J</i> = 9.8, 5.6)	71.5	64.1	70.7	71.5	71.6
8	4.40 ( <i>d</i> , <i>J</i> = 6.4)	42.4	42.0	48.3 <sup>a</sup> )	42.7	43.2
9	–	126.6	126.4	129.3	126.4	126.2
10	–	130.2	130.1	138.8	130.2	130.2
1'	–	140.1	140.1	133.7	139.3	139.4
2'	6.76 ( <i>d</i> , <i>J</i> = 2.0)	113.6	113.4	113.9	106.9	107.1
3'	–	148.7	148.6	149.0	149.0	149.0
4'	–	145.3	145.3	145.2	134.5	134.5
5'	6.64 ( <i>d</i> , <i>J</i> = 8.4)	115.7	115.7	116.0	149.0	149.0
6'	6.50 ( <i>dd</i> , <i>J</i> = 8.4, 2.0)	121.7	121.7	123.5	106.9	107.1
1''	4.27 ( <i>d</i> , <i>J</i> = 7.6)	104.8	–	103.8	104.8	104.2
2''	3.20–3.70	75.2	–	75.0	75.2	75.1
3''	3.20–3.70	78.2	–	78.2	78.2	78.2
4''	3.20–3.70	71.7	–	71.4	71.7	72.0
5''	3.20–3.70	77.9	–	77.8	77.9	78.0
6''	3.63–3.69 ( <i>m</i> ) 3.81 ( <i>dd</i> , <i>J</i> = 10.0, 2.0)	62.8	–	62.4	62.8	62.7
1-MeO	3.31 ( <i>s</i> )	60.1	60.1	–	60.2	60.1
3-MeO	3.85 ( <i>s</i> )	56.6	56.6	56.5	56.6	56.6
3'-MeO	3.77 ( <i>s</i> )	56.5	56.3	56.4	56.8	56.8
5'-MeO	–	–	–	–	56.8	56.8

<sup>a</sup>) Overlapping with solvent peak.

a brownish, viscous residue (160 g), which was subjected to CC (SiO<sub>2</sub>; CHCl<sub>3</sub>/MeOH mixtures of increasing polarity): 22 fractions (Fr.) on the basis of TLC. Fr. 20 was subjected to HPLC to afford 15 subfractions: Fr. 20.1–20.15. Compounds **12** (13 mg), **15** (19 mg), and **14** (18 mg) were isolated by PR-HPLC (C18; H<sub>2</sub>O/MeCN 80:20) from Fr. 20.6, Fr. 20.14, and Fr. 20.15, resp. Compounds **3** (5 mg), **5** (6 mg), and **10** (7 mg) were obtained by RP-HPLC (C30; H<sub>2</sub>O/MeCN 80:20) from Fr. 20.7, Fr. 20.8, and Fr. 20.13, resp. Further purification of Fr. 21 by RP-HPLC (C30; H<sub>2</sub>O/MeCN 80:20) yielded compound **9** (10 mg).

b) The original aq. extract (see above) was subjected to CC (Diaion HP-20; H<sub>2</sub>O/MeOH): Fr. A1–A5. Fr. A3 (eluted with H<sub>2</sub>O/MeOH 1:1) was partitioned between CHCl<sub>3</sub> and H<sub>2</sub>O, and the aq. layer was re-extracted with AcOEt. The resulting AcOEt layer was subjected to CC (SiO<sub>2</sub>; AcOEt/MeOH 10:1): Fr. A3.1–A3.14. Fr. A3.10 was further separated into nine subfractions: Fr. A3.10-1–A3.10-9. Recyclic RP-HPLC (C30, MeCN/H<sub>2</sub>O 30:70) of Fr. A3.10-4 afforded **6** (4 mg). Compounds **1** (3 mg), **2** (4 mg), **4** (4 mg), **7** (5 mg), **8** (4 mg), **11** (5 mg), and **13** (16 mg) were obtained by recyclic RP-HPLC (C30; MeCN/H<sub>2</sub>O 15:85) from the subfractions -2, -3, -5, -6, -7, -8, and -9, resp., of Fr. A3.10.

*Squadinorlignoside* (= 4-[(1E)-1-(Hydroxymethyl)-3-(4-hydroxyphenyl)prop-1-en-1-yl]phenyl β-D-glucopyranoside; **1**). Syrup. UV (MeCN): 195, 225 (sh), 274. IR (neat): 3415, 1618, 1520. <sup>1</sup>H-NMR (400 MHz; CD<sub>3</sub>OD)<sup>2</sup>: 3.21 (d, J = 7.6, CH<sub>2</sub>(7'')); 3.40 (m, H-C(4'')); 3.43 (m, H-C(5'')); 3.47 (m, H-C(2'',3'')); 3.70 (dd, J = 12.0, 5.6, H<sub>a</sub>-C(6'')); 3.90 (dd, J = 12.0, 2.4, H<sub>b</sub>-C(6'')); 4.24 (d, J = 1.2, CH<sub>2</sub>(9)); 4.90 (overlapping, H-C(1'')); 5.83 (td, J = 7.6, 1.2, H-C(8'')); 6.67 (d, J = 8.6, H-C(3',5'')); 6.93 (d, J = 8.6, H-C(2',6'')); 7.11 (d, J = 8.8, H-C(3,5)); 7.19 (d, J = 8.8, H-C(2,6)). <sup>13</sup>C-NMR (125 MHz, CD<sub>3</sub>OD): 34.8 (C(7'')); 62.5 (C(6'')); 67.9 (C(9)); 71.4 (C(4'')); 74.9 (C(2'')); 78.0 (C(3'')); 78.1 (C(5'')); 102.3 (C(1'')); 116.2 (C(3',5'')); 117.5 (C(3,5)); 127.9 (C(8'')); 130.2 (C(2',6'')); 130.9 (C(2,6)); 133.1 (C(1'')); 134.1 (C(1)); 141.7 (C(8)); 156.5 (C(4'')); 158.2 (C(4)). EI-MS: 256 (10), 239 (57), 238 (100), 225 (40), 145 (22), 131 (47), 107 (73). HR-ESI-MS: 441.1540 ([M + Na]<sup>+</sup>; C<sub>27</sub>H<sub>36</sub>NaO<sub>12</sub><sup>+</sup>; calc. 441.1525).

(6R,7R,8S)-7a-[(β-D-Glucopyranosyl)oxy]-1-methoxyisolariciresinol (= [(1S,2S,3R)-1,2,3,4-Tetrahydro-7-hydroxy-1-(4-hydroxy-3-methoxyphenyl)-3-(hydroxymethyl)-6,8-dimethoxynaphthalen-2-yl]methyl β-D-glucopyranoside; **2**). Syrup. UV (MeCN): 201, 232 (sh), 282. [α]<sub>D</sub><sup>25</sup> = +130.8 (c = 0.026, MeCN). CD (MeCN): 299 (−0.06), 275 (+0.11), 249 (+0.28). IR (neat): 3400, 1620, 1515. <sup>1</sup>H- and <sup>13</sup>C-NMR: see the Table. EI-MS: 552 (5, M<sup>+</sup>), 390 (35), 389 (29), 372 (91), 371 (100), 340 (50), 218 (21), 210 (23). HR-FAB-MS: 575.2108 ([M + Na]<sup>+</sup>; C<sub>27</sub>H<sub>36</sub>NaO<sub>12</sub><sup>+</sup>; calc. 575.2105).

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<sup>2</sup>) For atom numbering, see Fig. 1. Doubly primed atoms refer to the Glc moiety, the 1''-position corresponding to the anomeric center.