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 β-D-glucopyranoside; **1**) and (6*R*,7*R*,8*S*)-7a-[(β-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**)[1]), (6*R*,7*S*,8*S*)-7a-[(β-D-glucopyranosyl)-oxy]-1-methoxyisolariciresinol (**2**), (6*R*,7*R*,8*S*)-1-methoxyisolariciresinol (**3**) [4], (6*S*,7*S*,8*R*)-7a-[β-D-glucopyranosyl)oxy]isolariciresinol (**4**) [5], (6*R*,7*R*,8*S*)-isolariciresinol (**5**) [6], (6*R*,7*S*,8*S*)-7a-[(β-D-glucopyranosyl)oxy]lyoniresinol (**6**) [7], (6*R*,7*R*,8*R*)-7a-[(β-D-glucopryanosyl)oxy]lyoniresinol (**7**) [7], [(2*R**,2′*R**)-secoisolariciresin-4-yl] β-D-glucoside (**8**) [8], (2*R**,2′*R**)-secoisolariciresinol (**9**) [9], (7*S*,8*R*,8′*R*)-5,5′-dimethoxylariciresinol (**10**) [10], (7*S*,8*R*)-7,9,9′-trihydroxy-3,3′-dimethoxy-8-*O*-4′-neolignan-4-*O*-β-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 $C_{22}H_{26}O_8$ based on its HR-FAB-MS data. In the ^{1}H-NMR spectrum, the resonances of two 1,4-disubstituted Ph groups were observed (δ(H) 6.67, 6.93 (2*d*, *J*=8.6 Hz each, 2×2 H); 7.11, 7.19 (2*d*, *J*=8.8 Hz each, 2×2 H)). By analyzing the chemical shifts and coupling constants, one olefinic H-atom at δ(H) 5.83 (*td*, *J*=7.6, 1.2), and two sets of CH_2 resonances at 3.21 (*d*, *J*=7.6) and 4.24 (*d*, *J*=1.2 Hz) indicated a trisubstituted

[1]) For systematic names of the new compounds **1** and **2**, see the *Exper. Part*.

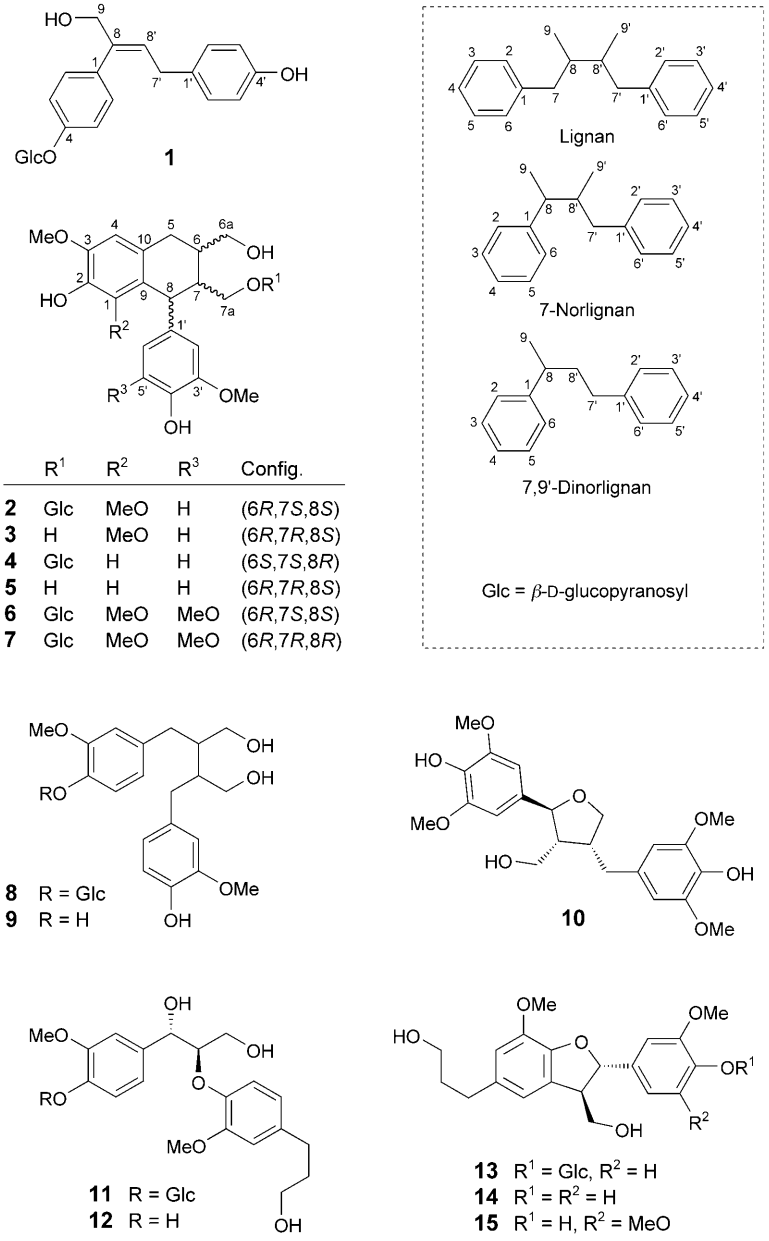


Fig. 1. *Structures of compounds* **1**–**15**. Basic lignan frameworks and *IUPAC* atom numbering [14] are shown in the box.

olefinic group flanked by two CH_2 groups, one of which was oxygenated (δ(H) 4.24, δ(C) 67.9). The structure of the aglycone was fully established by 2D-NMR experiments, *i.e.*, ^{1}H,^{1}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.

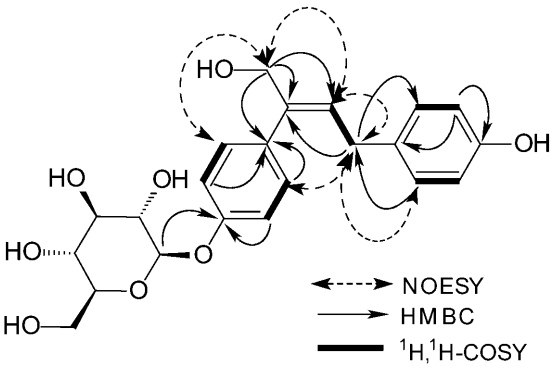


Fig. 2. *Selected NOESY, HMBC, and ^{1}H,^{1}H-COSY correlations of* **1**

The sugar moiety of **1** was found to correspond to a β-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-[(1*E*)-1-(hydroxymethyl)-3-(4-hydroxyphenyl)prop-1-en-1-yl]phenyl β-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*).

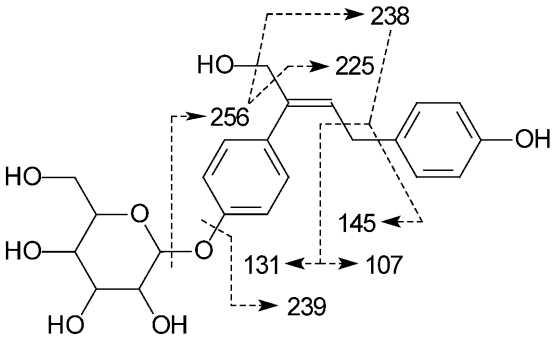
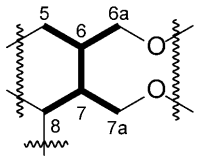


Fig. 3. *EI-MS Fragments of* **1**

Compound **2** was optically active, $[\alpha]_D^{22} = +130.8$ ($c = 0.026$, MeCN), and had the molecular formula $C_{27}H_{36}O_{12}$, as determined by HR-FAB-MS. The ^{13}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}H-NMR spectrum of **2** exhibited signals for one set of *ABX*-type aromatic H-atoms, indicating 1,3,4-trisubstitution (δ(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 δ(H) 6.57 (1 H). In the ^{1}H,^{1}H-COSY and TOCSY spectra, the partial structure **A** was revealed (*Fig. 4*), and three MeO groups (δ(H) 3.31, 3.77, 3.85) at C(1), C(3), and C(3′), respectively, were identified form the NOE cross-peaks of 1-MeO/H–C(8), 3-MeO/H–C(4), and 3′-MeO/H–C(2′) (*Fig. 5*).



$^1H,^1H$-COSY

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

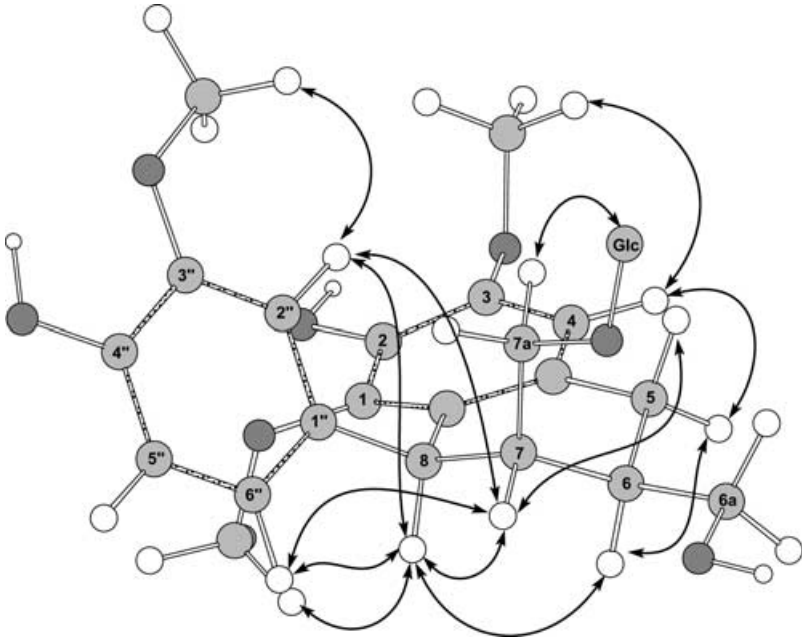


Fig. 5. *Selected NOESY correlations of* **2**. The cross-peak between H–C(7a) and Glc refers to the anomeric H-atom H–C(1″).

The presence of a Glc group in **2** was inferred from its ^1H- and ^{13}C-NMR spectra. The sugar moiety was attached at C(7a), as deduced from the NOE cross-peaks between the anomeric Glc H-atom (δ(H) 4.27 (*d*, $J=7.6$ Hz)) and CH_2(7a) (δ(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 β-D-glucoside of 1-methoxyisolariciresinol. The relative configurations at C(6) to C(8) were determined by a NOESY experiment (*Fig. 5*), and corroborated by inspection of $^1H,^1H$-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) [H_{ax}–C(5) at δ(H) 2.59 (*dd*, $J=14.8$, 11.6 Hz); H_{eq}–C(5) at δ(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 H_{ax}–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

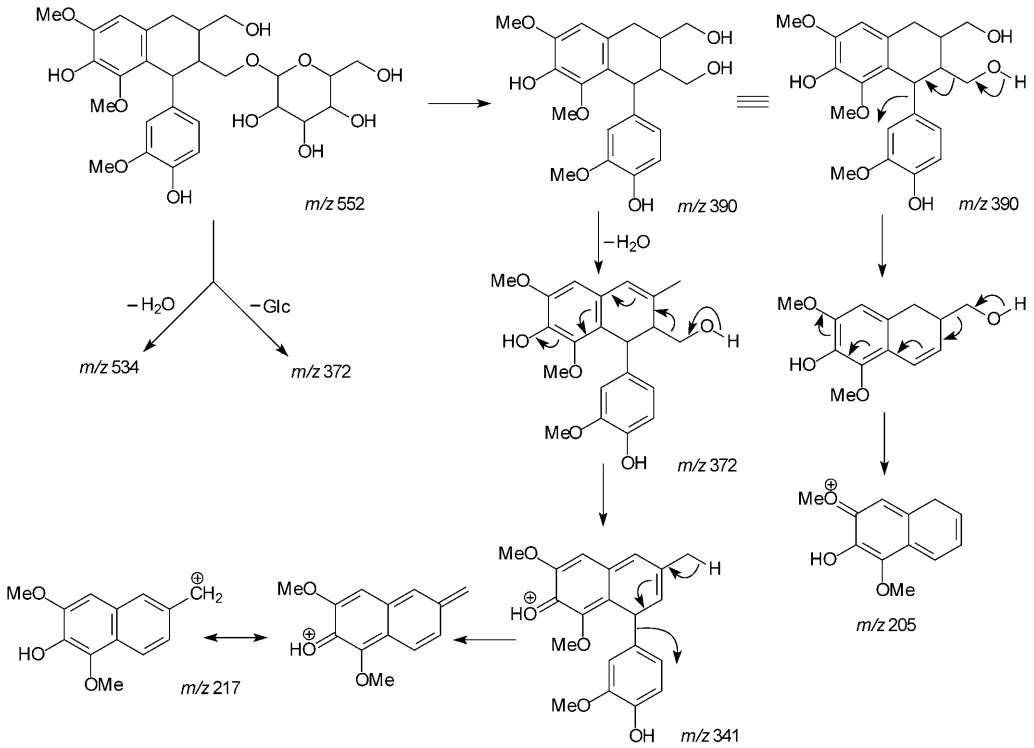


Fig. 6. *EI-MS Fragments of* **2**

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 $\Delta\varepsilon$ 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 β-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-diepoxylignan 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′-epoxylignan **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_2SO_4, and then heated on a hot plate. UV Spectra: *JASCO V-530* spectrophotometer; λ_{max} in nm. Optical rotations: *JASCO P-1020* digital polarimeter. CD Spectra: *JASCO J-720* spectropolarimeter; λ ($\Delta\varepsilon$) in nm. IR Spectra: a *Mattson Genesis-II* spectrophotometer; in cm^{-1}. ^{1}H-NMR: at 400 or 500 MHz in (D_6)acetone or CD_3OD; δ in ppm, J in Hz. ^{13}C-NMR, DEPT, ^{1}H,^{1}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 $CHCl_3$ and H_2O. Both layers were further processed separately. *a*) The $CHCl_3$ layer was extracted with 3% aq. HCl to remove alkaloids. The 'neutral' $CHCl_3$ 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_3OD; δ in ppm, J in Hz.

	2		**3**	**4**	**6**	**7**
Position	δ(H)	δ(C)	δ(C)	δ(C)	δ(C)	δ(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 (*s*)	107.9	107.8	112.3	107.9	107.9
5	2.59 (*dd*, *J* = 14.8, 11.6) 2.71 (*dd*, *J* = 14.8, 4.8)	33.9	33.6	33.6	33.8	33.8
6	1.68–1.74 (*m*)	40.6	40.9	41.1	40.6	41.2
6a	3.52 (*dd*, *J* = 10.8, 6.4) 3.60–3.66 (*m*)	66.3	66.8	65.5	66.2	66.2
7	2.04–2.10 (*m*)	46.8	49.6	45.3	46.7	46.6
7a	3.45 (*dd*, *J* = 9.8, 4.0) 3.89 (*dd*, *J* = 9.8, 5.6)	71.5	64.1	70.7	71.5	71.6
8	4.40 (*d*, *J* = 6.4)	42.4	42.0	48.3[a])	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 (*d*, *J* = 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 (*d*, *J* = 8.4)	115.7	115.7	116.0	149.0	149.0
6′	6.50 (*dd*, *J* = 8.4, 2.0)	121.7	121.7	123.5	106.9	107.1
1″	4.27 (*d*, *J* = 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 (*m*) 3.81 (*dd*, *J* = 10.0, 2.0)	62.8	–	62.4	62.8	62.7
1-MeO	3.31 (*s*)	60.1	60.1	–	60.2	60.1
3-MeO	3.85 (*s*)	56.6	56.6	56.5	56.6	56.6
3′-MeO	3.77 (*s*)	56.5	56.3	56.4	56.8	56.8
5′-MeO	–	–	–	–	56.8	56.8

[a]) Overlapping with solvent peak.

a brownish, viscous residue (160 g), which was subjected to CC (SiO_2; $CHCl_3$/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_2O/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_2O/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_2O/MeCN 80:20) yielded compound **9** (10 mg).

b) The original aq. extract (see above) was subjected to CC (*Diaion HP-20*; H_2O/MeOH): *Fr. A1–A5*. *Fr. A3* (eluted with H_2O/MeOH 1:1) was partitioned between $CHCl_3$ and H_2O, and the aq. layer was re-extracted with AcOEt. The resulting AcOEt layer was subjected to CC (SiO_2; 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_2O 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_2O 15:85) from the subfractions *-2*, *-3*, *-5*, *-6*, *-7*, *-8*, and *-9*, resp., of *Fr. A3.10*.

Squadinorlignoside (=*4-[(1*E*)-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. ^{1}H-NMR (400 MHz; CD_3OD)[2]): 3.21 (*d*, *J* = 7.6, CH_2(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_a–C(6″)); 3.90 (*dd*, *J* = 12.0, 2.4, H_b–C(6″)); 4.24 (*d*, *J* = 1.2, CH_2(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)). ^{13}C-NMR (125 MHz, CD_3OD): 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 ($[M+Na]^+$, $C_{22}H_{26}NaO_8^+$; calc. 441.1525).

*(6*R*,7*R*,8*S*)-7a-[(β-*D*-Glucopyranosyl)oxy]-1-methoxyisolariciresinol* (=*[(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 β-*D*-glucopyranoside*; **2**). Syrup. UV (MeCN): 201, 232 (sh), 282. $[\alpha]_D^{22} = +130.8$ (*c* = 0.026, MeCN). CD (MeCN): 299 (−0.06), 275 (+0.11), 249 (+0.28). IR (neat): 3400, 1620, 1515. ^{1}H- and ^{13}C-NMR: see the *Table*. EI-MS: 552 (5, M^+), 390 (35), 389 (29), 372 (91), 371 (100), 340 (50), 218 (21), 210 (23). HR-FAB-MS: 575.2108 ($[M+Na]^+$; $C_{27}H_{36}NaO_{12}^+$; calc. 575.2105).

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Received May 3, 2005

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[2]) For atom numbering, see *Fig. 1*. Doubly primed atoms refer to the Glc moiety, the 1″-postion corresponding to the anomeric center.