Secoiridoid Constituents from the Fruits of *Ligustrum lucidum*

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Four new secoiridiod glucosides, *p*-hydroxyphenethyl 7-*β*-D-glucosideelenolic acid ester (**1**), 6′-elenolylnicotiflorine (**2**), 6‴-acetylnicotiflorine (**3**), and oleoside 7-ethyl 11-methyl ester (**4**), as well as six known glucosides, nuezhenide (**5**), Gl-3 (**6**), nicotiflorine (**7**), isonuezhenide (**8**), neonuezhenide (**9**), and oleoside 11-methyl ester (**10**) were isolated from the fruits of *Ligustrum lucidum*. Their structures were elucidated by spectroscopic methods. Compound **4** was an artifact produced during extraction.

Introduction. – The fruits of *Ligustrum lucidum* AIT. (Oleaceae) are known as Nuzhenzi, and they are commonly used for their tonic effects in Chinese medicine [1]. Previous studies have found volatile components, triterpenes, flavonoids, secoiridoid glucosides, and phenolic compounds from this plant [2–4]. In our serial research on chemical constituents of the fruits of *Ligustrum lucidum*, we have reported before five dammarane triterpenes from its petroleum ether extract [5], and seven flavonoids from its AcOEt extract [6]. In this article, we describe the isolation and structural elucidation of ten secoiridiod glucosides from the BuOH extract. They were characterized as *p*-hydroxyphenethyl 7-*β*-D-glucosideelenolic ester (**1**), 6′-elenolylnicotiflorine (**2**), 6‴-acetylnicotiflorine (**3**), oleoside 7-ethyl 11-methyl ester (**4**), nuezhenide (**5**), Gl-3 (**6**), nicotiflorine (**7**), isonuezhenide (**8**), neonuezhenide (**9**), and oleoside 11-methyl ester (**10**) (*Fig. 1*). Amongst these, compounds **1**, **2**, **3**, and **4** are new secoiridoid glucosides, while compounds **6** and **7** are isolated from this plant for the first time. Compound **4** was proven to be an artifact produced during extraction.

Results and Discussion. – The air-dried fruits of *Ligustrum lucidum* were extracted with 80% EtOH, the resulting extract was then suspended in H_2O and extracted successively with petroleum ether, AcOEt, and BuOH. The BuOH extract was chromatographed on macroporous resin, silica gel, and *ODS* to give ten secoiridiod glucosides. The six known glucosides **5**–**10** were identified by comparing their spectral data with those reported in the literature [7–9].

Compound **1** was obtained as a colorless amorphous powder; the molecular formula was determined to be $C_{25}H_{32}O_{12}$ from the HR-ESI-MS (*m*/*z* 547.1786, $[M+Na]^+$). The UV and IR spectra suggested the presence of an enol-ether system conjugated with a

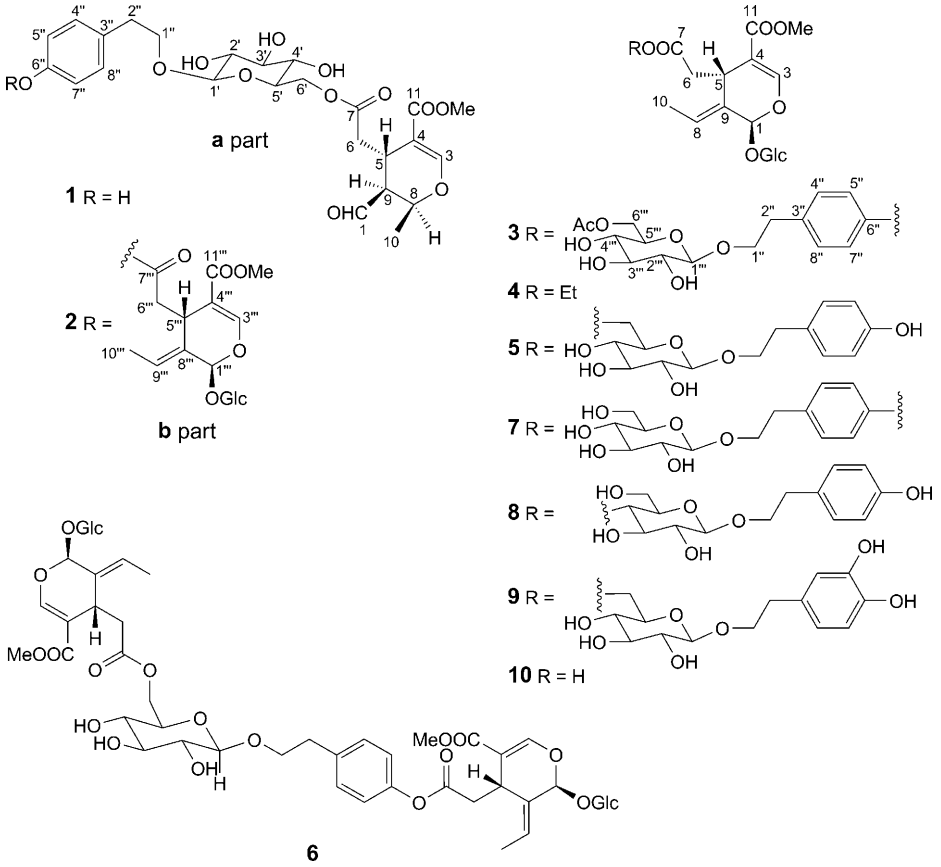

Fig. 1. *Compounds from the fruits of* Ligustrum lucidum[1])

CO group (230 nm; 1715, 1700, and 1630 cm^{-1}), which is typical for many iridoid and secoiridoid skeletons [10]. In the UV and IR spectra, absorptions due to a phenol chromophore (281 nm, 1510 and 1460 cm^{-1}) were also observed. The ^{1}H-NMR spectrum (*Table 1*) showed the following signals due to an elenolic acid moiety [11]: an aldehyde H-atom δ(H) 9.64 (*s*, H–C(1)[1])), a Me group at δ(H) 1.56 (*d*, $J = 6.7$, Me(10)), an olefinic H-atom δ(H) 7.56 (*s*, H–C(3)). The ^{1}H-NMR spectrum also indicated the presence of a *p*-substituted aromatic ring and a glucose moiety. The signal of the anomeric H-atom at δ(H) 4.31 (*d*, $J = 7.8$) revealed the β-configuration of the glucose residue.

The HMBC spectrum showed a correlation between H–C(1′) (δ(H) 4.31) of the glucose unit and C(1″) (δ(C) 72.4) of the 2-phenylethyl moiety, which suggested that the 2-phenylethyl group was located at C(1′). In addition, the correlation between CH_2(6′) (δ(H) 4.20–4.23, 4.47–4.50) of the glucose unit and C(7) (δ(C) 173.8) of the elenolic acid moiety indicated that the glucosyl residue was located at C(7). In the

[1]) Arbitrary atom numbering. For systematic names of **1**–**4**, see *Exper. Part.*

Table 1. *^{1}H-NMR Data of Compounds* **1** *and* **2** *in CD_3OD,* **3** *and* **7** *in (D_6)DMSO* (500 MHz). δ in ppm, J in Hz.

	1	**2**		**3**		**7**	
		a part	**b** part				
1,1‴	9.64 (*s*)	9.58 (*dd*, $J = 0.8, 2.7$)	6.02 (*s*)	5.97 (*s*)		6.02 (*s*)	
3,3‴	7.56 (*s*)	7.53 (*s*)	7.59 (*s*)	7.57 (*s*)		7.56 (*s*)	
5,5‴	3.35–3.39 (*m*)	3.32–3.37 (*m*)	4.11 –4.13 (*m*)	3.96–4.00 (*m*)		4.11 (*dd*, $J = 9.5, 4.5$)	
6,6‴	2.38 (*dd*, $J = 15.1, 8.5$), 2.73 (*dd*, $J = 15.1, 5.2$)	2.37 (*dd*, $J = 15.5, 8.5$), 2.73 (*dd*, $J = 15.5, 5.5$)	2.73 (*dd*, $J = 15.5, 8.5$), 2.96 (*dd*, $J = 15.5, 5.5$)	2.73 (*dd*, $J = 15.5, 8.5$), 2.96 (*dd*, $J = 15.5, 4.9$)		2.73 (*dd*, $J = 15.0, 9.5$), 2.95 (*dd*, $J = 15.0, 4.5$)	
8,8‴	4.19–4.21 (*m*)	4.20–4.24 (*m*)	6.17 (*q*, $J = 7.0$)	6.05 (*q*, $J = 6.3$)		6.18 (*qd*, $J = 7.0, 1.2$)	
9,9‴	2.61–2.65 (*m*)	2.73–2.77 (*m*)	1.70 (*d*, $J = 7.0$)				
10,10‴	1.56 (*d*, $J = 6.7$)	1.50 (*d*, $J = 6.7$)	4.82 (*d*, $J = 7.7$)	1.70 (*d*, $J = 6.3$)		1.76 (*dd*, $J = 7.0, 1.5$)	
MeO	3.62 (*s*)	3.65 (*s*)	3.69 (*s*)	3.66 (*s*)		3.64 (*s*)	
1′,1‴′/1‴	4.31 (*d*, $J = 7.8$)	4.32 (*d*, $J = 7.9$)	4.82 (*d*, $J = 7.7$)	4.68 (*d*, $J = 7.6$)	4.32 (*d*, $J = 7.7$)	4.81 (*d*, $J = 8.0$)	4.30 (*d*, $J = 8.0$)
2′,2‴′/2‴	3.23–3.27 (*m*)	3.23–3.27 (*m*)	3.23–3.27 (*m*)	3.22–3.26 (*m*)	3.22–3.26 (*m*)	3.23–3.29 (*m*)	3.23–3.29 (*m*)
3′,3‴′/3‴	3.48–3.52 (*m*)	3.49–3.53 (*m*)	3.49–3.53 (*m*)	3.46–3.50 (*m*)	3.46–3.50 (*m*)	3.47–3.51 (*m*)	3.47–3.51 (*m*)
4′,4‴′/4‴	3.40–3.43 (*m*)	3.39–3.43 (*m*)	3.39–3.43 (*m*)	3.38–3.41 (*m*)	3.38–3.41 (*m*)	3.40–3.43 (*m*)	3.40–3.43 (*m*)
5′,5‴′/5‴	3.44–3.47 (*m*)	3.45–3.47 (*m*)	3.45–3.47 (*m*)	3.42–3.46 (*m*)	3.42–3.46 (*m*)	3.44–3.47 (*m*)	3.44–3.47 (*m*)
6′,6‴′/6‴	4.20–4.23 (*m*), 4.47–4.50 (*m*)	4.24–4.28 (*m*), 4.42–4.46 (*m*)	3.70–3.73 (*m*), 3.89–3.92 (*m*)	3.69–3.72 (*m*), 3.90–3.93 (*m*)	4.04–4.08 (*m*), 4.23–4.26 (*m*)	3.70–3.74 (*m*), 3.91–3.94 (*m*)	3.70–3.74 (*m*), 3.91–3.94 (*m*)
1″	3.74–3.76 (*m*), 3.96–4.00 (*m*)	3.77–3.82 (*m*), 4.10 (*dt*, $J = 8.9, 4.6$)		3.71–3.76 (*m*), 3.86 (*dd*, $J = 9.5, 6.5$)		3.76 (*dt*, $J = 10.0, 7.0$), 4.10 (*dt*, $J = 10.0, 7.0$)	
2″	2.81–2.84 (*m*)	2.92–2.96 (*m*)		2.85 (*t*, $J = 7.2$)		2.94 (*t*, $J = 7.0$)	
4″	7.01 (*d*, $J = 8.3$)	7.28 (*d*, $J = 8.5$)		7.30 (*d*, $J = 8.5$)		7.28 (*d*, $J = 8.5$)	
5″	6.63 (*d*, $J = 8.3$)	6.99 (*d*, $J = 8.5$)		6.99 (*d*, $J = 8.5$)		6.99 (*d*, $J = 8.5$)	
7″	6.63 (*d*, $J = 8.3$)	6.99 (*d*, $J = 8.5$)		6.99 (*d*, $J = 8.5$)		6.99 (*d*, $J = 8.5$)	
8″	7.01 (*d*, $J = 8.3$)	7.28 (*d*, $J = 8.5$)		7.30 (*d*, $J = 8.5$)		7.28 (*d*, $J = 8.5$)	
Ac				2.00 (*s*)			

NOESY spectrum (*Fig. 2*), H–C(1) correlated with Me(10) and H–C(5), and H–C(3) correlated with H–C(9), which confirmed the relative configuration of the elenolic acid moiety [12]. Other 2D-NMR correlations confirmed the proposed structure which is shown in *Fig. 1*. Thus, the structure of compound **1** was established as *p*-hydroxyphenethyl 7-*β*-D-glucosideelenolic acid ester.

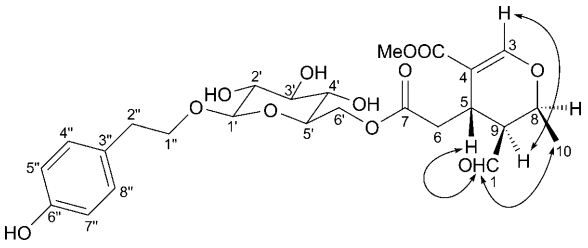

Fig. 2. *Key NOESY correlations for* **1**[1])

Compound **2** was obtained as a colorless amorphous powder; the molecular formula was determined to be $C_{42}H_{54}O_{22}$ from the HR-ESI-MS (*m*/*z* 933.2986, $[M+Na]^+$). The ^{1}H- and ^{13}C-NMR spectra (*Tables 1* and *2*) showed that compound **2** differed from **7** (nicotiflorine) by the presence of an additional elenolic acid moiety. Comparison of the ^{1}H- and ^{13}C-NMR spectral data of **2** with those of **7** showed that the $CH_2(6')$[1]) chemical shift of **2** was shifted downfield by 0.54 and 0.51 ppm and the C(6′) chemical shift of **2** was shifted downfield by 4.0 ppm. The HMBC spectrum showed the correlation between $CH_2(6')$ and C(7) (δ(C) 173.2), which confirmed that the C(7) carboxy group of the elenolic acid moiety was linked to C(6′) of the glucose unit. 2D-NMR including HMQC and HMBC experiments allowed us to assign all the other H- and C-atom signals for **2**, so the structure of **2** was established as 6′-elenolylnicotiflorine.

Compound **3** was obtained as a colorless amorphous powder; the molecular formula was determined to be $C_{33}H_{44}O_{18}$ from the HR-ESI-MS (*m*/*z* 751.2421, $[M+Na]^+$). The ^{1}H- and ^{13}C-NMR spectral data (*Tables 1* and *2*) were almost consistent with those of **7** except that the chemical shift of C(6‴)[1]) of **3** was shifted downfield by 3.0 ppm, and an additional AcO group with signals at δ(C) 20.8 and 170.4 appeared in the ^{13}C-NMR spectrum of **3**, which indicated the presence of an AcO group at C(6‴) of glucose. In the HMBC spectrum, the correlation between $CH_2(6''')$ and the CO group at (δ(C) 170.4) of the AcO unit clearly indicated the location of AcO unit at C(6‴). Furthermore, the ESI-MS spectrum (positive-ion mode) showed a *quasi*-molecular ion peak ($[M+Na]^+$ at *m*/*z* 751, indicating an excess of 42 mass units in comparison with that of **7**. So the structure of **3** was established as 6‴-acetylnicotiflorine.

Compound **4** was obtained as a colorless amorphous powder; the molecular formula was determined to be $C_{19}H_{28}O_{11}$ from the HR-ESI-MS (*m*/*z* 455.1513, $[M+Na]^+$). The ^{1}H- and ^{13}C-NMR data (*Tables 1* and *2*) were almost consistent with those of oleoside-11-methyl ester (**10**) except for the signal of an additional Et moiety. Linkage of the Et moiety to C(7)[1]) was confirmed by the intense cross-peak in the HMBC spectrum between a H-atom (δ(H) 3.30) of the CH_2 group and the C(7)=O group (δ(C) 173.6). Therefore, the structure of **4** was concluded to be oleoside 7-ethyl 11-methyl ester.

Table 2. *^{13}C-NMR Spectral Data of Compounds* **1** *and* **2** *in CD_3OD,* **3** *and* **7** *in (D_6)DMSO* (125 MHz). δ in ppm.

	1	**2**		**3**		**7**	
		a part	**b** part				
1,1‴	201.3	202.1	95.8	93.3		93.7	
3,3‴	158.4	155.7	158.4	153.7		153.6	
4,4‴	109.0	108.5	109.8	107.7		107.7	
5,5‴	29.7	29.2	32.2	30.2		30.1	
6,6‴	41.9	40.0	41.5	40.5		39.4	
7,7‴	173.8	173.2	172.1	169.6		170.0	
8,8‴	72.1	72.0	125.6	123.3		123.4	
9,9‴	53.1	52.4	131.3	129.4		129.0	
10,10‴	17.4	18.5	14.3	13.3		12.1	
11,11‴	170.3	169.9	169.1	166.3		167.0	
MeO	52.0	52.4	52.8	51.4		50.3	
1′,1⁗/1‴	105.1	104.9	101.5	99.3	103.1	99.4	102.7
2′,2⁗/2‴	75.8	75.6	75.4	73.4	73.7	73.1	73.4
3′,3⁗/3‴	78.3	78.3	78.3	76.6	76.6	76.3	76.4
4′,4⁗/4‴	72.5	71.9	71.6	69.6	70.1	69.7	70.0
5′,5⁗/5‴	75.5	75.2	78.8	77.4	77.3	76.7	76.3
6′,6⁗/6‴	65.3	65.0	63.1	61.2	63.7	61.0	60.7
1″	72.4	72.1		70.1		69.8	
2″	37.1	37.0		35.1		34.8	
3″	131.8	138.3		136.5		136.3	
4″	132.0	131.4		129.9		129.3	
5″	116.4	122.9		121.4		120.8	
6″	157.2	151.0		148.7		148.9	
7″	116.4	122.9		121.4		120.8	
8″	132.0	131.4		131.4		129.3	
Me*C*O	–	–		170.4		–	
*Me*CO	–	–		20.8		–	

Compound **4** was an artifact produced during extraction, since it was not detected by LC/MS analysis in an extract prepared with MeOH instead of 80% EtOH.

This work was supported by the *Natural Science Foundation* of *Jiangsu Science and Technology Department*, which are gratefully acknowledged.

Experimental Part

General. Column chromatography (CC): silica gel (SiO_2; 200–300 mesh, *Qingdao Haiyang Chemical Group Co. Ltd.*, P. R. China), macroporous resin *D-101* (*Tianjin Agricultural Chemical Co. Ltd.*, P. R. China), *Sephadex LH-20* (*Pharmacia Fine Chemical Co. Ltd.*), and *Chromatorex ODS* (200–300 mesh, *Fuji Silysia Chemical Ltd.*, Japan). Optical rotations *JASCO DIP-370* digital polarimeter. UV Spectra: *Shimadzu UV-3100PC* spectrophotometer. IR Spectra: *Perkin-Elmer 8900* FT-IR instrument, as pressed KBr disks. 1D- and 2D-NMR spectra: in CD_3OD on a *Varian 500 Unity Plus* NMR spectrometer. ESI-MS: *Finnigan TSQ 7000*. HR-ESI-MS Spectra: *Bruker APEX-II* mass spectrometer.

Plant Material. The fruits of *Ligustrum lucidum* were collected from Nanjing city, Jiangsu Province, P. R. China in November 2004 and identified by Prof. *Qian Shihui* of Jiangsu Institute of Traditional

Chinese Medicine. A voucher specimen (No. S-04-00020) is deposited in the Herbarium of Jiangsu Institute of Traditional Chinese Medicine.

Extraction and Isolation. Air-dried fruits (8 kg) were extracted with 80% EtOH (2 × 50 l) for 2 h under reflux, and the combined extracts were concentrated *in vacuo*. The resulting extract (1.835 kg) was then suspended in H_2O (2000 ml) and extracted successively with petroleum ether (PE), AcOEt, and BuOH. The combined BuOH layers were concentrated under vacuum to leave the residue (1366 g), which was absorbed on *D-101* macroporous resin (2 kg) and then eluted with H_2O, 20% EtOH, 40% EtOH, and EtOH, to afford four fractions *A* (455 g), *B* (173 g), *C* (502 g), and *D* (42 g). *Fr. C* (100 g) was chromatographed on SiO_2 (1 kg) eluting with $CHCl_3$/MeOH, step-wise gradient (98 : 2 → 7 : 3), and 11 subfractions were collected. *Subfr. 2* (4 g) was purified by SiO_2 CC ($CHCl_3$/MeOH 95 : 5) and *ODS* CC (MeOH/H_2O 43 : 57) to give compounds **1** (253 mg) and **4** (14 mg). *Subfr. 3* (6 g) was purified by SiO_2 CC ($CHCl_3$/MeOH 10 : 1) and *ODS* CC (MeOH/H_2O 45 : 55 → 50 : 50) to afford compounds **10** (20 mg), **3** (22 mg), and **2** (49 mg). *Subfr. 4* (5 g) was purified by *ODS* CC (MeOH/H_2O 40 : 60) to obtain compound **5** (1.258 g). *Subfr. 5* (2 g) was purified by *ODS* CC (MeOH/H_2O 43 : 57) to obtain compound **7** (33 mg). *Subfr. 7* (2 g) was purified by *ODS* CC (MeOH/H_2O 45 : 55) to give compound **9** (8 mg). *Subfr. 8* (1.5 g) was purified by *ODS* CC (MeOH/H_2O 40 : 60) to afford compound **8** (11 mg). *Subfr. 11* (3 g) was purified by *ODS* CC (MeOH/H_2O 40 : 60) to obtain compound **6** (649 mg).

p-*Hydroxyphenethyl 7-β-*D*-glucosideelenolic Acid Ester* (=*2-(4-Hydroxyphenyl)ethyl 6*-O-*{[(2*R**,3*R**,4*R**)-3-Formyl-3,4-dihydro-5-(methoxycarbonyl)-2-methyl-2*H*-pyran-4-yl]acetyl}-β-*D*-glucopyranoside*; **1**). Colorless amorphous powder. $[\alpha]_D^{20} = -83$ ($c = 0.34$, MeOH). UV (EtOH): 230 (4.10), 281 (3.42). IR (KBr): 3400, 1715, 1700, 1630, 1510, 1460, 1070. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. ESI-MS: 523 ($[M-H]^-$). HR-ESI-MS: 547.1786 ($[M+Na]^+$, $C_{25}H_{32}NaO_{12}^+$; calc. 547.1791).

6′-Elenolylnicotiflorine (=*2-[4-([[(2*R**,3*E*,4*R**)-3-Ethylidene-2-(β-*D*-glucopyranosyloxy)-3,4-dihydro-5-(methoxycarbonyl)-2*H*-pyran-4-yl]acetyl}oxy)phenyl]ethyl 6*-O-*{[(2*R**,3*R**,4*R**)-3-Formyl-3,4-dihydro-5-(methoxycarbonyl)-2-methyl-2*H*-pyran-4-yl]acetyl}-β-*D*-glucopyranoside*; **2**). Colorless amorphous powder. $[\alpha]_D^{20} = -148$ ($c = 0.25$, MeOH). UV (EtOH): 228 (4.30), 279 (3.56). IR (KBr): 3400, 1745, 1704, 1630, 1510, 1069. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. ESI-MS: 911 ($[M-H]^-$). HR-ESI-MS: 933.2986 ($[M+Na]^+$, $C_{42}H_{54}NaO_{22}^+$; calc. 933.3004).

6‴-Acetylnicotiflorine (=*Methyl (2*R**,3*E*,4*R**)-4-[2-(4-{2-[(6-*O*-Acetyl-β-*D*-glucopyranosyl)oxy]-ethyl}phenoxy)-2-oxoethyl]-3-ethylidene-2-(β-*D*-glucopyranosyloxy)-3,4-dihydro-2*H*-pyran-5-carboxylate*; **3**). Colorless amorphous powder. $[\alpha]_D^{20} = -104$ ($c = 0.47$, MeOH). UV (EtOH): 230 (4.15), 280 (3.38). IR (KBr): 3410, 1750, 1706, 1630, 1515, 1070. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. ESI-MS: 727 ($[M-H]^-$). HR-ESI-MS: 751.2421 ($[M+Na]^+$, $C_{33}H_{44}NaO_{18}^+$; calc. 751.2425).

Oleoside-7-ethyl-11-methyl Ester (=*Methyl (2*R**,3*E*,4*R**)-4-(2-Ethoxy-2-oxoethyl)-3-ethylidene-2-(β-*D*-glucopyranosyloxy)-3,4-dihydro-2*H*-pyran-5-carboxylate*; **4**). Colorless amorphous powder. $[\alpha]_D^{20} = -114$ ($c = 0.26$, MeOH). UV (EtOH): 228 (4.10). IR (KBr): 3407, 1750, 1706, 1629, 1503, 1072. ^{1}H-NMR[1]) (500 MHz, CD_3OD): 7.47 (*s*, H–C(3)); 6.03 (*q*, *J* = 6.9, H–C(8)); 5.86 (*s*, H–C(1)); 4.75 (*d*, *J* = 7.5, H–C(1′)); 3.66 (*s*, Me); 3.30 (*q*, *J* = 7.3, Me*CH*$_2$O); 1.67 (*d*, *J* = 6.9, Me(10)); 1.17 (*t*, *J* = 7.30, *Me*CH$_2$O). ^{13}C-NMR[1]) (125 Hz, CD_3OD): 173.6 (C(7)); 169.1 (C(11)); 155.6 (C(3)); 130.8 (C(9)); 125.3 (C(8)); 109.7 (C(4)); 101.3 (C(1′)); 95.6 (C(1)); 78.6 (C(5′)); 78.2 (C(3′)); 75.1 (C(2′)); 71.8 (C(4′)); 63.1 (C(6′)); 62.3 (Me*C*H$_2$O); 52.5 (MeO); 41.7 (C(6)); 32.2 (C(5)); 14.9 (C(10)); 14.1 (*Me*CH$_2$O). ESI-MS: 431 ($[M-H]^-$). HR-ESI-MS: 455.1513 ($[M+Na]^+$, $C_{19}H_{28}NaO_{11}^+$; calc. 455.1529).

MeOH Extraction and Analysis of **4** *by HPLC-DAD-MS*. Air-dried fruits (1 g) were extracted with MeOH (25 ml) for 2 h under reflux, and then combined extracts were concentrated *in vacuo*. The resulting extract was dissolved in 2 ml MeOH and was filtrated by 0.45 μm micropore and a volume of 10 μl sample was injected for LC analysis. The same experiments were done for compound **4**. The HPLC system consisted of an *Agilent 1100* series HPLC with a Diode Array Detector. The column was *LiChrospher 100RP-18* (250 × 4.6 mm, 5.0 μm, *Merck*, Germany) maintained at 30°. The eluents were MeOH (*A*) and H_2O (*B*) at a flow rate of 1 ml/min. The linear gradient was from 20 to 100% MeOH in H_2O in 40 min. The chromatographic profiles were recorded at 230 nm for qualitative analysis. The above HPLC system was interfaced with an *Agilent a HP 1100* Mass Selective Detector with electrospray ionization (*Agilent Technologies*, MA, USA). The ESI-MS spectra were acquired in negative ionization modes recorded on a mass range of *m*/*z* 100–700. Capillary voltage was 3500 V. Drying gas temp. was set

at 300° with a flow rate of 8.0 l/min. In the reference sample, compound **4** (15.6 min) was detected by the *quasi*-molecular ion peak $[M-H]^-$ at *m*/*z* 431. No corresponding ion was observed in the MeOH extract. Therefore, compound **4** was likely to be an artifact.

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Received April 27, 2009