Phytochemistry 95 (2013) 333–340

Contents lists available at ScienceDirect

Phytochemistry

journal homepage: www.elsevier.com/locate/phytochem

Antioxidant lignoids from leaves of *Ribes nigrum*

Tatsunori Sasaki [a], Wei Li [a,*], Shinnosuke Zaike [a], Yoshihisa Asada [b], Qin Li [c], Fenghua Ma [d], Qingbo Zhang [d], Kazuo Koike [a]

[a] Faculty of Pharmaceutical Sciences, Toho University, Miyama 2-2-1, Funabashi, Chiba 274-8510, Japan
[b] Faculty of Pharmaceutical Sciences, Tokyo University of Science, 2641 Yamazaki, Noda, Chiba 278-8510, Japan
[c] Laboratory Center, The Fourth Affiliated Hospital, China Medical University, Shenyang 110032, People's Republic of China
[d] Heilongjang Institute for Food and Drug Control, Harbin 150001, People's Republic of China

ARTICLE INFO

Article history:
Received 21 September 2012
Received in revised form 15 February 2013
Available online 16 August 2013

Keywords:
Ribes nigrum
Grossulariaceae
Lignan
Sesquilignan
Dilignan
Antioxidant activity

ABSTRACT

Phytochemical investigation of the leaves of *Ribes nigrum* resulted in the isolation of fourteen compounds, including four 7,7′-epoxylignans, three tetrahydrofuran-type sesquilignans, and a spirocyclic dilignan. Their structures were elucidated by extensive spectroscopic analyses and by chemical transformations. The isolated compounds were evaluated for their antioxidant activities using superoxide anion scavenging assay and DPPH free radical scavenging assay. Ribesin D and ribesin G showed the most potent superoxide anion scavenging activity with EC_{50} values of 1.24 and 1.12 μM, respectively, and the structure–activity relationship was discussed.

1. Introduction

The genus *Ribes* belongs to the family of *Grossulariaceae* and has about 150 different species. *Ribes nigrum*, known as blackcurrant, is a perennial small shrub, which is widely distributed in Europe and North Asia, and is cultivated in many countries for its usage of the fruits in the food industry (Slimestad and Solheim, 2002; Castillo et al., 2004; Zheng et al., 2009). The leaves of *R. nigrum* have been used as a traditional medicine for treatment of rheumatic disease in Europe (Garbacki et al., 2004), and have been shown to exhibit antioxidant and anti-inflammatory effects (Garbacki et al., 2005, 2002). Although the finding of prodelphinidins and flavonoids has been previously reported (Tits et al., 1992; He et al., 2010; Tabart et al., 2011), knowledge on the chemical composition of their leaves is still quite limited. As a part of ongoing studies on bioactive compounds from traditional medicinal plants (Li et al., 2012; Bi et al., 2011), a phytochemical investigation was carried out herein on the leaves of *R. nigrum*. This work resulted in the isolation of fourteen compounds, including four novel 7,7′-epoxylignans (**1**–**4**), three novel tetrahydrofuran-type sesquilignans (**7**–**9**) and one novel spirocyclic dilignan (**10**) (Fig. 1). Their structures were elucidated by extensive spectroscopic analyses and chemical transformations. The antioxidant activities of the isolated compounds were evaluated by superoxide anion scavenging assay and DPPH free radical scavenging assay.

2. Results and discussion

The air-dried leaves of *R.nigrum* were extracted with 70% ethanol. The extract was separated by Diaion HP-20, silica gel, and ODS column chromatography, and further purification was carried out by reversed phase preparative HPLC to afford eight novel compounds (**1**–**4**, **7**–**10**) and six known compounds.

The known compounds were identified as (−)-larreatricin (**5**) (Moinuddin et al., 2003), 3,3′-didemethoxynectandrin B (**6**) (Xorge et al., 1990), roseoside (**11**) (Otsuka et al., 1995), icariside B1 (**12**) (Hisamoto et al., 2004), kaempferol (**13**) (Hadizadeh et al., 2003), and methyl *p*-coumarate (**14**) (Kwon and Kim, 2003), by detailed NMR, MS, optical rotation and CD spectroscopic analyses, as well as by comparison with literature data. The previously unknown lignoids (**1**–**4**, **7**–**10**) were isolated as colorless solids, and their structures were determined as follows.

The molecular formulas of ribesin A (**1**) and ribesin C (**3**) were $C_{18}H_{18}O_3$, and ribesin B (**2**) and ribesin D (**4**) were $C_{20}H_{22}O_5$, by analyses of the negative-ion HRESIMS data. In the ^{13}C NMR spectra of **1** and **2**, the number of carbon resonances was observed by half, suggesting their symmetrical nature.

As shown in Table 1, the ^{1}H and ^{13}C NMR spectroscopic data of **1** indicated characteristic A_2X_2-type aromatic proton resonances at δ_H 7.15 and 6.82, as well as carbon resonances at δ_C 133.9, 128.5,

* Corresponding author. Tel.: +81 47 4721161; fax: +81 47 4721404.
E-mail address: liwei@phar.toho-u.ac.jp (W. Li).

0031-9422/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.phytochem.2013.07.022

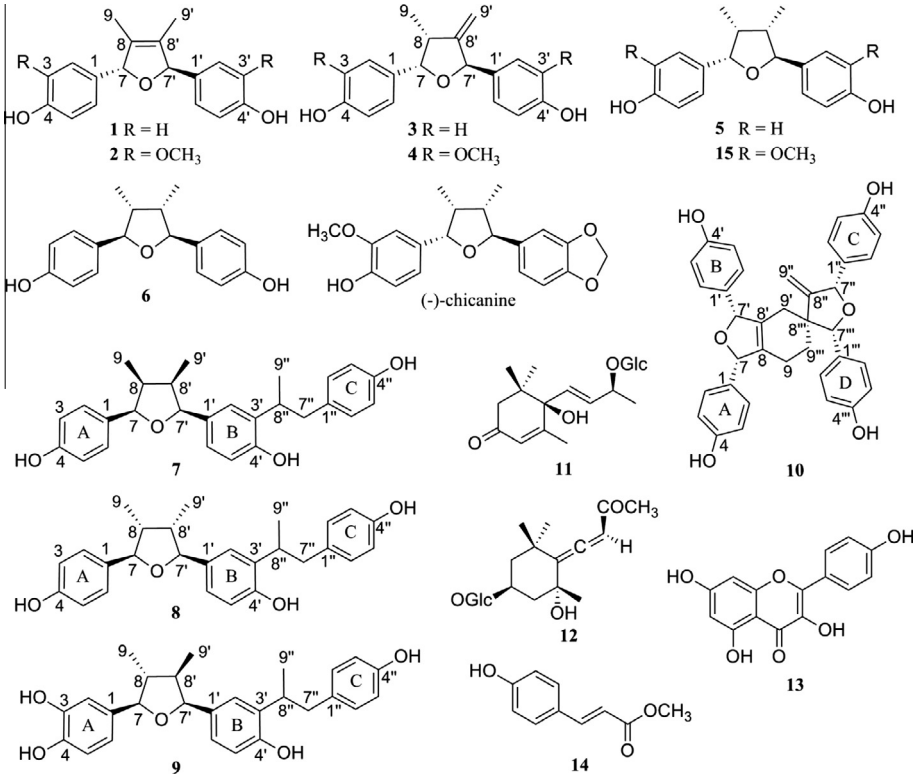

Fig. 1. Chemical structures.

Table 1
^{1}H and ^{13}C NMR spectroscopic data (δ) for compounds **1**–**4** in CD_3OD.

Position	**1**		**2**		**3**		**4**	
	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C
1		133.9		135.9		132.3		134.5
2	7.15 (d, 8.5)	128.5	6.74 (d, 2.0)	114.9	7.10 (d, 8.5)	129.2	6.76 (d, 2.3)	115.0
3	6.82 (d, 8.5)	115.2		149.1	6.75 (d, 8.5)	115.9		148.4
3-OCH_3			3.84 (s)	56.5			3.83 (s)	56.5
4		157.2		147.8		158.0		147.3
5	6.82 (d, 8.5)	115.2	6.89 (d, 8.5)	112.5	6.75 (d, 8.5)	115.9	6.86 (d, 8.3)	112.4
6	7.15 (d, 8.5)	128.5	6.76 (dd, 8.5, 2.0)	119.9	7.10 (d, 8.5)	129.2	6.71 (dd, 8.3, 2.3)	119.4
7	5.63 (s)	91.2	5.59 (s)	92.7	5.28 (d, 7.4)	85.3	5.23 (d, 7.6)	85.3
8		131.2		132.0	3.15 (m)	43.4	3.13 (m)	43.2
9	1.48 (s)	9.8	1.50 (s)	10.3	0.78 (d, 6.9)	14.6	0.77 (d, 7.1)	14.4
1′		133.9		135.9		135.0		137.1
2′	7.15 (d, 8.5)	128.5	6.74 (d, 2.0)	114.9	7.23 (d, 8.5)	129.0	6.88 (d, 1.8)	114.6
3′	6.82 (d, 8.5)	115.2		149.1	6.77 (d, 8.5)	116.2		148.7
3′-OCH_3			3.84 (s)	56.5		157.2	3.83 (s)	56.5
4′		157.2		147.8		158.3		147.6
5′	6.82 (d, 8.5)	115.2	6.89 (d, 8.5)	112.5	6.77 (d, 8.5)	116.2	6.89 (d, 8.0)	112.6
6′	7.15 (d, 8.5)	128.5	6.76 (dd, 8.5, 2.0)	119.9	7.23 (d, 8.5)	129.0	6.86 (dd, 8.0, 1.8)	119.0
7′	5.63 (s)	91.2	5.59 (s)	92.7	5.63 (br s)	84.4	5.60 (d, 2.1)	84.4
8′		131.2		132.0		157.9		157.5
9′a	1.48 (s)	9.8	1.50 (s)	10.3	4.92 (t, 2.1)	106.9	4.95 (t, 2.1)	106.9
9′b					4.99 (t, 2.1)		4.99 (t, 2.1)	

115.2 and 157.2, for a 4-hydroxyphenyl moiety. The ^{1}H NMR spectroscopic data also showed two singlet proton resonances, one for a quaternary methyl at δ_H 1.48, and the other for an oxymethine at δ_H 5.63, and their corresponding carbon resonances were assigned to δ_C 9.8 and 91.2, respectively, as indicated by the HMQC spectroscopic data. In the ^{13}C NMR spectrum, in addition to the aforementioned carbon resonances, the remaining resonance was an olefinic quaternary carbon at δ_C 131.2. The connection of these moieties in the sequence of 4-hydroxyphenyl, oxymethine, olefinic quaternary carbon, and quaternary methyl, was determined by detailed analysis of the HMBC spectroscopic data. Namely, in the HMBC spectrum, the $^3J_{CH}$ correlations were observed from δ_H 7.15 to δ_C 91.2, δ_H 5.63 to δ_C 9.8, and δ_H 1.48 to δ_C 91.2, and the $^2J_{CH}$ correlations were from δ_H 5.63 and 1.48 to δ_C 131.2 (Fig. 2). Taking account of the symmetrical structure and the chemical formula, the presence of 2,5-dihydrofuran ring moiety in **1** was elucidated.

Comparison of the ^{1}H and ^{13}C NMR spectroscopic data of **2** and **1**, suggested that **2** is only structurally different from **1** by its aromatic constituents. Namely, the presence of 4-hydroxy-3-methoxyphenyl moieties in **2** was indicated by the ABX-type aromatic proton reso-

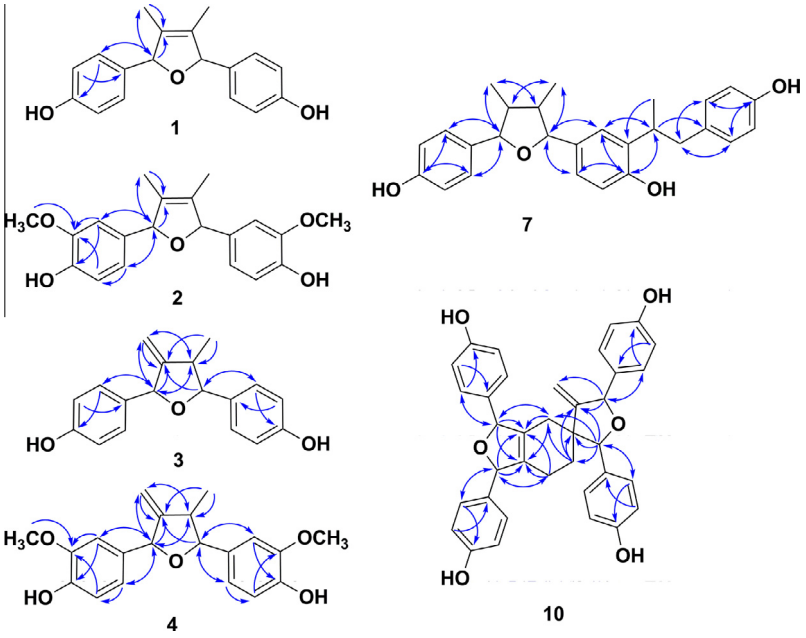

Fig. 2. Key HMBC correlations for compounds **1**–**4**, **7** and **10**.

nances at δ_H 6.89, 6.76 and 6.74, and the methoxy resonance at δ_H 3.84. The position of the methoxy group was confirmed by the NOESY correlation between δ_H 3.84 and 6.74.

The ^{1}H NMR spectroscopic data of **3** indicated the presence of two 4-hydroxyphenyl moieties by two groups of A_2X_2-type aromatic proton resonances at δ_H 6.77 and 7.23, and δ_H 6.75 and 7.10. Further resonances were observed for a methyl at δ_H 0.78 (H-9), a methine at δ_H 3.15 (H-8), a terminal methylene at δ_H 4.92 and 4.99 ($H_{a,b}$-9′), and two oxymethine protons at δ_H 5.28 and 5.63 (H-7, 7′). In the ^{13}C NMR spectrum, except for the carbon resonances assignable to the above-mentioned moieties, it also showed two olefinic quaternary carbon resonances at δ_C 157.9 and 106.9 (C-8′ and C-9′). The presence of a methyl–methine–oxymethine moiety (CH_3-9–CH-8–CH(O)-7) was deduced from the correlations between their protons in the ^{1}H–^{1}H COSY spectrum, and the $\Delta^{8',9'}$ double bond connecting to C-7′ was confirmed by the HMBC correlations from δ_H 4.92 and 4.99 ($H_{a,b}$-9′) to δ_C 84.4 (C-7′) and 157.9 (C-8′). Furthermore, the HMBC correlations from δ_H 0.78 (H_3-9) to δ_C 157.9 (C-8′), δ_H 4.92, 4.99 ($H_{a,b}$-9′) to δ_C 43.4 (C-8) and 157.9 (C-8′), δ_H 5.28 (H-7) to δ_C 157.9 (C-8′), and δ_H 5.63 (H-7′) to δ_C 43.4 (C-8) and 157.9 (C-8′) (Fig. 2), indicated the presence of a tetrahydrofuran ring in **3**. Two 4-hydroxyphenyl moieties connecting to C-7 and C-7′ oxymethines were deduced from the HMBC correlations from δ_H 7.10 (H-2, 6) to δ_C 85.3 (C-7), and δ_H 7.23 (H-2′, 6′) to δ_C 84.4 (C-7′) (Fig. 2). By these analyses, the gross structure of **3** was elucidated. The relative configurations in **3** were assigned by NOESY spectroscopic data analysis. The NOESY correlations between δ_H 7.10 (H-2,6)/0.78 (H_3-9), 7.10 (H-2,6)/5.63 (H-7′), 5.28 (H-7)/3.15 (H-8) and 5.28 (H-7)/7.23 (H-2′,6′), indicated that H-7 and H-7′ are *trans*-, and H-7 and H-8 are *cis*-relationship (Fig. 3).

Comparison of the ^{1}H and ^{13}C NMR spectroscopic data of **4** and **3** suggested that **4** is only structurally different from **3** by its aromatic constituents. Namely, the presence of two 4-hydroxy-3-methoxyphenyl moieties in **4** was indicated by two set of ABX-type aromatic proton resonances at δ_H 6.89, 6.86, 6.88, and δ_H 6.86, 6.71, 6.76, as well as from the methoxy proton resonance at δ_H 3.83. Furthermore, the relative configurations in **4** is also the same as **3** by their similar correlations in the NOESY spectra.

To determine the absolute configurations of compounds **1**–**4**, Pd/C catalyzed reductions were carried out. From **1** and **3**, the same product was obtained with identical spectroscopic data as for (−)-larreatricin (**5**), which was also isolated in this study. From **2** and **4**, was obtained the same product **15**, whose structure was indentified to be 3,3′-dimethoxylarreatricin by detailed spectroscopic analyses. Both optical rotation values of compounds **5** {$[\alpha]_D^{25}$: −20.3 (*c* 0.40, MeOH)} and **15** {$[\alpha]_D^{25}$: −78.0 (*c* 0.08, MeOH)} were similar to that, with previously reported natural compounds for (−)-larreatricin {$[\alpha]_D^{20}$: −19.3 (*c* 0.0145, MeOH)} from *Larrea tridentata* (Moinuddin et al., 2003) and 3,3′-dimethoxylarreatricin {$[\alpha]_D^{25}$: −85.7 (*c* 0.096, $CHCl_3$)} from *Castanea mollissima* (Long et al., 2008). However, their absolute configurations were not yet determined. Fortunately, the absolute configuration of (−)-chicanine with a very similar structure to that of **15** was recently established by its asymmetric total synthesis (Harada et al., 2011). Therefore, the absolute configuration of **15** was assigned by comparison of the CD data and optical rotation data with those of (−)-chicanine. Namely, the CD spectrum of **15** had the same negative Cotton effects at both 231 nm (K absorption band) and 286 nm (B absorption band) as (−)-chicanine due to π–π^* transitions, suggesting that they have same absolute configurations (*2S*,*3R*,*4S*,*5S*). Although the Cotton effects at B band (ca 280 nm) were too weak to be observed for **5**, the same negative Cotton effect at 232 nm in the CD spectrum, and the same negative value in optical rotation with those of **15**, indicated **15** had also the same absolute configurations.

Conclusively, ribesin A (**1**) was determined to be (7*R*,7′*R*)-8(8′)-ene-4,4′-dihydroxy-7,7′-epoxylignan, ribesin B (**2**) to be (7*R*,7′*R*)-8(8′)-ene-4,4′-dihydroxy-3,3′-dimethoxy-7,7′-epoxylignan, ribesin C (**3**) was identified to be (7*S*,7′*S*,8*R*)-8′(9′)-ene-4,4′-dihydroxy-7,7′-epoxylignan, ribesin D (**4**) was determined as (7*S*,7′*S*,8*R*)-8′(9′)-ene-4,4′-dihydroxy-3,3′-dimethoxy-7,7′-epoxylignan, and the known compounds of (−)-larreatricin (**5**) to be (7*S*,7′*S*,8*R*,8′*S*)-4,4′-dihydroxy-7,7′-epoxylignan, and 3,3′-dimethoxylarreatricin (**15**) to be (7*S*,7′*S*,8*R*,8′*S*)-4,4′-dihydroxy-3,3′-dimethoxy-7,7′-epoxyligan (Fig. 1).

Ribesin E (**7**) and ribesin F (**8**) were isomers with the same molecular formula of $C_{27}H_{30}O_4$ as determined by the positive-ion HRESIMS data. The ^{13}C NMR spectra of both **7** and **8** showed

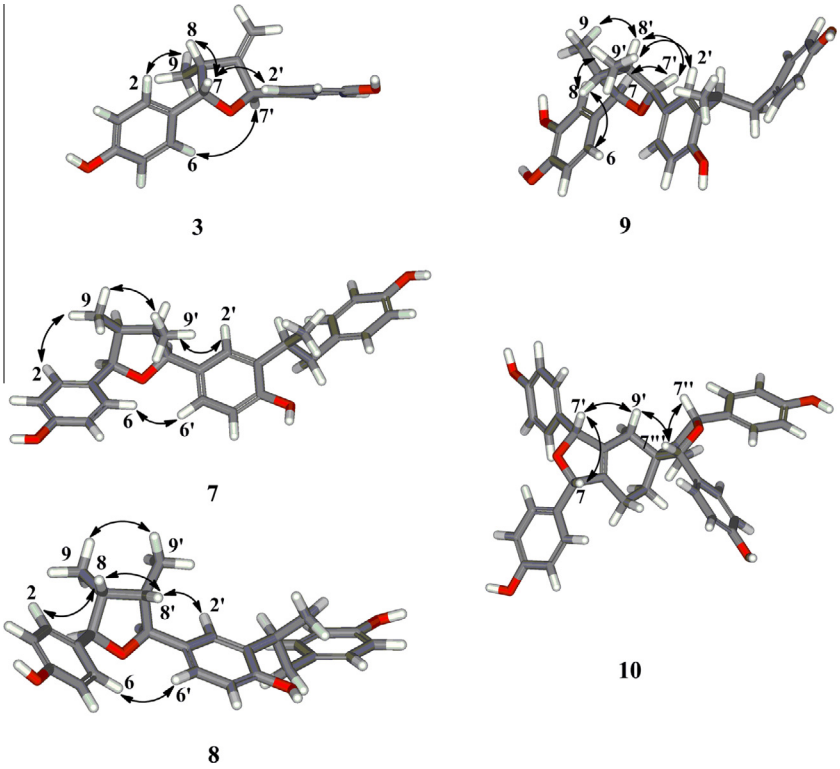

Fig. 3. Key NOESY correlations for compounds **3**, **7**–**9** and **10**.

resonances for 27 carbons, among which 18 were assignable to the aromatic carbons and 9 assignable to the aliphatic carbons, suggesting a sesquilignan structure (Table 2). Further comparison of the ^{1}H NMR spectroscopic data of **7** and **8** indicated quite similar resonances for the aromatic constituents, including two set of A_2X_2-type aromatic proton resonances and a set of ABX-type aromatic proton resonances assignable to three hydroxylated aromatic rings (A-, B- and C-ring) (Fig. 1).

In the ^{1}H NMR spectrum of **7**, the highfield resonances for two methine groups at δ_H 2.62 (H-8 and H-8′) were coupled with two oxymethine groups at δ_H 5.06 and 5.04 as well as with two nearly equivalent methyl groups at δ_H 0.55 and 0.50, suggesting the

Table 2
^{1}H and ^{13}C NMR spectroscopic data (δ) for compounds **7**–**9** in CD_3OD.

Position	**7**		**8**		**9**	
	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C
1		132.5		134.0		133.2
2	7.23 (d, 8.5)	128.7	7.23 (d, 8.5)	129.0	6.97 (d, 1.8)	115.4
3	6.79 (d, 8.5)	115.8	6.79 (d, 8.5)	116.2		146.5
4		157.5		158.2		146.3
5	6.79 (d, 8.5)	115.8	6.79 (d, 8.5)	116.2	6.78 (d, 8.2)	116.3
6	7.23 (d, 8.5)	128.7	7.23 (d, 8.5)	129.0	6.81 (dd, 8.2, 1.8)	119.7
7	5.06 (d, 6.6)	84.3	4.42 (d, 6.9)	88.8	4.26 (d, 9.4)	89.1
8	2.62 (m)	42.9	2.25 (m)	45.4	1.69 (m)	49.9
9	0.55 (d, 7.1)	12.2	0.98 (d, 7.1)	13.0	0.97 (d, 6.6)	14.8
1′		132.1		133.7		132.9
2′	7.10 (d, 2.0)	127.0	7.08 (d, 2.0)	126.7	7.02 (d, 2.1)	127.7
3′		133.8		134.2		133.7
4′		154.9		155.5		155.2
5′	6.73 (d, 8.4)	115.6	6.74 (d, 8.2)	115.7	6.72 (d, 8.6)	115.6
6′	7.03 (dd, 8.4, 2.0)	125.8	7.02 (dd, 8.2, 2.0)	125.8	6.98 (dd, 8.6, 2.1)	126.4
7′	5.04 (d, 7.1)	84.6	4.39 (d, 7.1)	89.2	5.03 (d, 8.6)	84.9
8′	2.62 (m)	42.8	2.00 (m)	45.9	2.17 (m)	47.2
9′	0.50 (d, 6.9)	12.4	0.94 (d, 6.9)	13.0	0.55 (d, 6.9)	15.3
1″		133.6		133.2		133.9
2″	6.93 (d, 8.5)	131.2	6.88 (d, 8.4)	131.3	6.93 (d, 8.5)	131.1
3″	6.61 (d, 8.5)	115.8	6.62 (d, 8.4)	115.6	6.60 (d, 8.5)	115.8
4″		156.3		156.3		156.2
5″	6.61 (d, 8.5)	115.8	6.62 (d, 8.4)	115.6	6.60 (d, 8.5)	115.8
6″	6.93 (d, 8.5)	131.2	6.88 (d, 8.4)	131.3	6.93 (d, 8.5)	131.1
7″	2.94 (dd, 13.5, 6.6)	43.2	2.89 (dd, 13.3, 5.9)	43.0	2.92 (dd, 13.5, 6.7)	43.2
	2.64 (m)		2.64 (dd, 13.3, 5.5)		2.62 (dd, 13.5, 8.0)	
8″	3.37 (m)	36.3	3.41 (m)	35.5	3.35 (m)	36.5
9″	1.19 (d, 7.1)	20.2	1.16 (d, 6.9)	19.8	1.18 (d, 7.1)	20.3

presence of a substituted tetrahydrofuran moiety (Toferna et al., 2000). Moreover, the methine proton resonance at δ_H 3.37 was coupled with the protons for a methylene at δ_H 2.94, 2.64 and a methyl at δ_H 1.19, suggesting the presence of an 1,2-diphenylpropyl group. The connection of these partial structures as shown in Fig. 2 was determined by analyses of the HMBC spectroscopic data. Namely, The HMBC correlations from δ_H 2.94, 2.64 ($H_{a,b}$-7″) to δ_C 131.2 (C-2″ and C-6″), δ_H 7.23 (H-2, 6) to δ_C 84.3 (C-7), δ_H 7.10 (H-2′), 7.03 (H-6′) to δ_C 84.6 (C-7′), δ_H 1.19 (H_3-9″) to δ_C 133.8 (C-3′), δ_H 3.37 (H-8″) to δ_C 127.0 (C-2′), 154.9 (C-4′), and δ_H 6.93 (H-2″, 6″) to δ_C 43.2 (C-7″), suggested that A-ring was connected at C-1 to C-7, B-ring was connected at C-1′ to C-7′ and at C-3′ to C-8″, and C-ring was connected at C-1″ to C-7″, respectively. The relative configurations in the tetrahydrofuran ring were determined by the NOESY spectroscopic data (Fig. 3). Namely, the NOESY correlations between δ_H 0.55 (H_3-9)/7.23 (H-2), 0.55 (H_3-9)/0.50 (H_3-9′), 0.50 (H_3-9′)/7.10 (H-2′) and 7.23 (H-6)/7.03 (H-6′), suggested the *cis*-relationship between H-7, H-8, H-7′ and H-8′. Thus, the structure of ribesin E (**7**) was determined as *rel*-(7*R*,8*S*,7′*S*,8′*R*)-4,4′-dihydroxy-3′-[1-methyl-2-(4-hydroxyphenyl)]-ethyl-7,7′-epoxylignan.

Detailed analyses of the 2D NMR spectroscopic data led to identical gross structures of **7** and **8**. When the tetrahydrofuran ring resonances of **8** were compared to those of **7**, the methyl protons at δ_H 0.98 (H_3-9) and 0.94 (H_3-9′) were shifted downfield and the oxymethine protons at δ_H 4.42 (H-7) and 4.39 (H-7′) were shifted upfield. These observations indicated that H-7 and H-7′ have *trans*-relationship with the adjacent protons of H-8 and H-8′ (Nguyen et al., 2010). The relative configurations in the tetrahydrofuran moiety were also supported by the NOESY spectroscopic data (Fig. 3). Namely, NOESY correlations were observed between δ_H 0.98 (H_3-9)/0.94 (H_3-9′), 2.25 (H-8)/2.00 (H-8′), 2.25 (H-8)/7.23 (H-2), 2.00 (H-8′)/7.08 (H-2′), and 7.23 (H-6)/7.02 (H-6′). Thus, the structure of ribesin F (**8**) was determined as *rel*-(7*R*,8*R*,7′*S*,8′*S*)-4,4′-dihydroxy-3′-[1-methyl-2-(4-hydroxyphenyl)]ethyl-7,7′-epoxylignan.

Ribesin G (**9**) was also a structurally related sesquilignan to **7** and **8**. Its molecular formula was $C_{27}H_{30}O_5$ as determined by the positive-ion HRESIMS data, which was one more oxygen atom than **7** and **8**. The presence of a 3,4-dihydroxyphenyl moiety in **9** was deduced from the characteristic ABX type aromatic proton resonances at δ_H 6.97, 6.78, 6.81 in the ^{1}H NMR spectrum, and *ortho*-dihydroxylated aromatic carbon resonances at δ_C 146.5 and 146.3 in the ^{13}C NMR spectrum. This 3,4-dihydroxyphenyl moiety was determined as A-ring at C-7 by the HMBC correlations from δ_H 6.97 (H-2) and δ_H 6.81 (H-6) to δ_C 89.1 (C-7), δ_H 4.26 (H-7) to δ_C 115.4 (C-2) and δ_C 119.7 (C-6), and δ_H 1.69 (H-8) to δ_C 133.2 (C-1). The relative configurations in the tetrahydrofuran moiety of **9** was determined by the NOESY correlations between δ_H 6.81 (H-6)/1.69 (H-8), 1.69 (H-8)/0.55 (H_3-9′), 0.55 (H_3-9′)/7.02 (H-2′), 4.26 (H-7)/5.03 (H-7′), 0.97 (H_3-9)/2.17 (H-8′) and 2.17 (H-8′)/5.03 (H-7′) (Fig. 3). Thus, the structure of ribesin G (**9**) was determined as *rel*-(7*R*,8*R*,7′*S*,8′*R*)-3,4,4′-trihydroxy-3′-[1-methyl-2-(4-hydroxyphenyl)]ethyl-7,7′-epoxylignan.

The molecular formula of ribesin H (**10**) was determined as $C_{36}H_{32}O_6$ by the negative-ion HRESIMS. The ^{13}C NMR spectrum showed resonances for 36 carbons, of which 24 assignable to the aromatic carbons, suggesting the dimeric lignan structure of **10** (Table 3). The presence of four 4-hydroxyphenyl moieties was deduced from the four sets of typical A_2X_2 type aromatic proton resonances in the ^{1}H NMR spectrum. The ^{13}C resonances corresponding to these aromatic rings were then assigned by analyzing the HMQC and HMBC correlations. The remaining ^{1}H and ^{13}C resonances were for one internal olefin [δ_C 135.0, 135.7]; one terminal olefin [δ_C 108.6, 159.4; δ_H 4.50 (d, 2.1), 4.81 (d, 2.1)], four oxymethines [δc 83.4, 89.4, 90.8, 91.0; δ_H 5.57 (s), 4.75 (s), 5.67 (s), 5.71 (s)], three methylenes [δc 19.8, 27.7, 30.6; δ_H 1.55 (d, 17.2), 1.94 (m), 0.95 (m), 1.74 (m), 1.68 (d, 17.2), 2.30 (d, 17.2)], and a quaternary carbon [δc 49.5]. Detailed analyses of the ^{1}H–^{1}H COSY and HMBC spectroscopic data deduced the connection of these structural moieties. The HMBC correlations from the oxymethine proton at δ_H 5.71 (H-7′) to δ_C 30.6 (C-9′), 135.0 (C-8), 135.7 (C-8′) and from the oxymethine proton at δ_H 5.67 (H-7) to δ_C 19.8 (C-9), 135.0 (C-8), 135.7 (C-8′) determined the presence of a tetrahydrofuran ring with the C8–C8′ double bond (Fig. 2). Furthermore, the ^{1}H–^{1}H COSY correlations between H-9 and H-9‴, as well as the HMBC correlations from methylene protons at δ_H 1.68, 2.30 ($H_{a,b}$-9′) and at δ_H 0.95, 1.74 ($H_{a,b}$-9‴) to δ_C 49.5 (C-8‴), suggested that the furano moiety was annulated. The HMBC correlations from the terminal olefinic protons at δ_H 4.50, 4.81 ($H_{a,b}$-9″) to δ_C 83.4 (C-7″), 159.4 (C-8″) indicated the $\Delta^{8',9'}$ double bond connecting to the oxymethine carbon C-7″, and the HMBC correlations from the methylene protons at δ_H 1.68, 2.30 ($H_{a,b}$-9′) to the nonprotonated olefinic carbon at δ_H 159.4 (C-8″) and the oxymethine carbon at δ_C 89.4 (C-7‴) enabled assembly of the spirocyclic portion in the molecule. Finally, the connections of four 4-hydroxyphenyl moieties (A, B, C and D-ring) to C-7 and C-7′ in the annulated furan moiety and C-7″ and C-7‴ in the spirocycle moiety, were determined by the HMBC correlations from four oxymethine protons (H-7, H-7′, H-7″ and H-7‴) to four aromatic carbons (C-2, C-2′, C-2″ and C-2‴), respectively. The NOESY correlations between δ_H 5.71 (H-7′)/5.67 (H-7), 1.68 (H-9′) and 4.75 (H-7‴)/1.68 (H-9′), 5.57 (H-7″), suggested *syn*-orientation for C-7, 7′, 7″, 7‴ aromatic substituent and β-orientation for C-9′ methylene (Fig. 3). On the basis of this evidence, the structure of ribesin H (**10**) was concluded to be as shown in Fig. 1.

Table 3
^{1}H and ^{13}C NMR spectroscopic data (δ) for compound **10** in CD_3OD.

Position	δ_H (J in Hz)	δ_C	Position	δ_H (J in Hz)	δ_C
1		133.5	1″		134.2
2	7.18 (d, 8.5)	129.1	2″	7.11 (d, 8.7)	130.3
3	6.80 (d, 8.5)	116.4	3″	6.73 (d, 8.7)	116.2
4		158.5	4″		158.5
5	6.80 (d, 8.5)	116.4	5″	6.73 (d, 8.7)	116.2
6	7.18 (d, 8.5)	129.1	6″	7.11 (d, 8.7)	130.3
7	5.67 (s)	90.8	7″	5.57 (s)	83.4
8		135.0	8″		159.4
9a	1.55 (m)	19.8	9″a	4.50 (d, 2.1)	108.6
9b	1.94 (m)		9″b	4.81 (d, 2.1)	
1′		133.9	1‴		129.6
2′	7.05 (d, 8.5)	129.5	2‴	7.15 (d, 8.7)	130.1
3′	6.76 (d, 8.5)	115.8	3‴	6.75 (d, 8.7)	116.4
4′		158.6	4‴		158.3
5′	6.76 (d, 8.5)	115.8	5‴	6.75 (d, 8.7)	116.4
6′	7.05 (d, 8.5)	129.5	6‴	7.15 (d, 8.7)	130.1
7′	5.71 (s)	91.0	7‴	4.75 (s)	89.4
8′		135.7	8‴		49.5
9′a	1.68 (d, 17.2)	30.6	9‴a	0.95 (m)	27.7
9′b	2.30 (d, 17.2)		9‴b	1.74 (m)	

Since the 70% ethanol extract had significant superoxide anion scavenging activity and DPPH free radical scavenging activity (EC_{50} = 1.84 and 34.07 μg/mL, respectively), the antioxidative effects of all isolated compounds were evaluated. Six lignoids (**2**, **3**, **4**, **6**, **9**, **10**) and a flavonol (**13**) showed potent superoxide anion scavenging activity with EC_{50} values ranging from 1.12 to 6.09 μM, and the lignan (**4**) and the flavonol (**13**) showed moderate DPPH free radical scavenging activity, which were comparable to those of the positive control, butylated hydroxyanisole (BHA) (Table 4). The effectiveness level of superoxide anion scavenging activity between test compounds could be due to their structural differences. Among the 7,7′-epoxylignans, compounds **3** and **4** have shown the most potent superoxide anion scavenging activity with EC_{50} values of 2.05 and 1.24 μM, indicating that the presence of $\Delta^{8',9'}$ double bond might be crucial for higher activity. A comparison

Table 4
Superoxide anion, DPPH radical scavenging activities of isolated compounds from the leaves of *Ribes nigrum* (mean value ± SD).

Sample	EC_{50}[a]	
	Superoxide anion	DPPH
70% EtOH extract	1.84 ± 0.27[b]	34.07 ± 0.60[b]
1	NA	NA
2	6.09 ± 0.68	NA
3	2.05 ± 0.17	NA
4	1.24 ± 0.33	32.33 ± 1.42
5	NA	NA
6	3.05 ± 0.50	NA
7	NA	NA
8	NA	NA
9	1.12 ± 0.11	NA
10	3.26 ± 0.13	NA
11	NA	NA
12	NA	NA
13	4.85 ± 0.92	31.52 ± 5.50
14	NA	NA
Butylated hydroxyanisole[c]	17.02 ± 0.95	26.71 ± 1.34

NA: No activity >50 μM.
[a] EC_{50} in μM.
[b] EC_{50} in μg/mL.
[c] Positive control.

of the structures between **4** and **3**, and **2** and **1**, indicated that the presence of 3- and 3′-methoxy moieties enhanced the activity. Among the closely structural related sesquilignans (**7**, **8**, **9**), only ribesin G (**9**) has shown potent superoxide anion scavenging activity with EC_{50} value of 1.12 μM, but the others (**7** and **8**) did not, suggesting that the ortho-hydroxyphenyl group or/and the stereo configurations in the tetrahydrofuran ring is important to exhibit the activity.

3. Conclusion

As a conclusion, fourteen compounds including eight novel compounds were isolated from leaves of *R. nigrum*. These compounds are classified into 7,7′-epoxylignans (**1**–**6**), tetrahydrofuran-type sesquilignans (**7**–**9**), spirocyclic dilignan (**10**), norsesquiterpene glucosides (**11** and **12**), flavonol-type flavonoid (**13**), and the phenylpropanoid (**14**). To the best of our knowledge, this is the first report of the occurrence of 7,7′-epoxylignans, sesquilignans, dilignan and norsesquiterpene glucosides in the plant family *Grossulariaceae*. The spirocyclic dilignan (**10**) has a unique furano-2-oxaspiro[4,5]decane core in its structure. This type of compounds have only been previously reported from *Zygophyllales* spp. (Zygophyllaceae), and were proposed to be biosynthesized from the corresponding monomeric 8(9),8′(9′)-diene-7,7′-epoxylignan through an inter [4 + 2] Diels–Alder reaction (Chavez et al., 2011). Seven compounds including four novel compounds showed potent superoxide anion scavenging activity. It is also worth noting that the present study demonstrated the superoxide anion scavenging activity of sesquilignans and dilignans for the first time. The leaves of*R. nigrum* may provide a beneficial effect for oxidative stress-induced diseases and have potential for the development of raw material of functional foods with antioxidant effects. Naturally, further investigations of the quality assessment were also demanded for promotion of utilization.

4. Experimental

4.1. General

Optical rotations were measured on a JASCO P-2200 polarimeter in a 0.5-dm cell. The CD spectra were measured on a JASCO J-720 W. The IR spectra were obtained on a JASCO FT/IR-4100 fourier transform infrared spectrometer by the KBr disk method. The UV spectra were acquired with a Shimadzu UV-160 spectrophotometer. The ^1H and ^{13}C NMR spectra were measured on a JEOL ECP-500 spectrometer with TMS as the internal reference, and the chemical shifts are expressed in δ (ppm). ESIMS and HRESIMS were conducted using a JEOL JMS-T100LP AccuTOF LC-plus mass spectrometer. For HPLC, a JASCO PU-2086 HPLC system, equipped with a JASCO RI-2301 Differential Refractometer detector, was used. RP-C18 silica gel column (YMC-Pack ODS-A, 20 × 150 mm, YMC CO., Ltd. Kyoto, Japan) was used for HPLC at a flow rate of 5.0 mL/min. Diaion HP-20 (Mitsubishi Chemical Corporation, Tokyo, Japan), silica gel (silica gel 60 N, Kanto Chemical Co., Inc., Tokyo, Japan), and ODS (100–200 mesh, Chromatorex DM1020T ODS, Fuji Silysia Chemical Co., Ltd., Aichi, Japan) were used for column chromatography (CC). TLC was conducted using silica gel 60 F254 plates (E. Merck). Absorbance was measured using ImmunoMini NJ-2300 microplate reader.

4.2. Plant material

Leaves of *R. nigrum* were collected at Shangzhi City, Heilongjiang Province, People′s Republic of China in September, 2006, and identified by one of the authors (FM). A voucher specimen (TH315) has been deposited at Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Toho University.

4.3. Extraction and isolation

Dried leaves of *R.nigrum* (3.0 kg) were extracted with EtOH–H_2O (7 L × 3, 70:30, v/v), and then the combined extracts were evaporated *in vacuo* to give an extract (695 g). This was then subjected to Diaion HP-20 CC, eluted sequentially with H_2O–MeOH, (100:0, 70:30, 30:70, 0:100, v/v) and acetone to give five fractions (1–5). Further separation was carried out by silica gel and ODS CC as well as preparative reversed-phase HPLC, from fraction 2 (9.9 g) to afford **11** (2 mg), **12** (6 mg) and **14** (340 mg), from fraction 3 (19.8 g) to afford **1** (41 mg), **2** (5 mg), **3** (16 mg), **4** (44 mg), **5** (115 mg), **6** (12 mg), **9** (3 mg), **13** (22 mg) and **10** (26 mg), and from fraction 4 (21.5 g) to afford **7** (6 mg) and **8** (10 mg), respectively.

4.4. Ribesin A (*1*): (7R,7′R)-8(8′)-ene-4,4′-dihydroxy-7,7′-epoxylignan

Colorless solid. $[\alpha]_D^{25}$: −88.0 (*c* 0.4, MeOH). UV (MeOH): λ_{max} nm (logε) 232 (4.39), 276 (3.59). IR (KBr): ν_{max} 3389, 3019, 2914, 2839, 2366, 2345, 1613, 1598, 1513, 1365, 1261, 1226, 1189, 1103, 1022, 1008 cm^{-1}. For ^1H NMR (CD_3OD, 500 MHz) and ^{13}C NMR (CD_3OD, 125 MHz) spectroscopic data, see Table 1. ESIMS (positive) *m/z*: 305.1 $[M+Na]^+$. HRESIMS (negative) *m/z*: 281.1177 $[M-H]^-$ (Calc. for $C_{18}H_{17}O_3$ 281.1178). CD (MeOH): $[\theta]^{25}$ (nm) −188,768 (234).

4.5. Ribesin B (*2*): (7R,7′R)-8(8′)-ene-4,4′-dihydroxy-3,3′-dimethoxy-7,7′-epoxylignan

Colorless solid. $[\alpha]_D^{25}$: −45.2 (*c* 0.25, MeOH). UV (MeOH): λ_{max} nm (logε) 232 (4.21), 282 (3.84). IR (KBr): ν_{max} 3427, 1595, 1509, 1441, 1383, 1364, 1270, 1238, 1218, 1129, 1176, 1160, 1028, 972 cm^{-1}. For ^1H NMR (CD_3OD, 500 MHz) and ^{13}C NMR (CD_3OD, 125 MHz) spectroscopic data, see Table 1. ESIMS (positive) *m/z*: 365.1 $[M+Na]^+$. HRESIMS (negative) *m/z*: 341.1384 $[M-H]^-$ (Calc. for $C_{20}H_{21}O_5$ 341.1390). CD (MeOH): $[\theta]^{25}$ (nm) −37,685 (236), −7877 (280).

*4.6. Ribesin C (**3**): (7S,7′S,8R)-8′(9′)-ene-4,4′-dihydroxy-7,7′-epoxylignan*

Colorless solid. $[\alpha]_D^{25}$:+33.0 (*c* 1.60, MeOH). UV (MeOH): λ_{max} nm (logε) 229 (4.41), 277 (3.60). IR (KBr): ν_{max} 3348, 3026, 2968, 2931, 2874, 1614, 1598, 1515, 1451, 1366, 1230, 1171, 1106, 1069 cm^{-1}. For ^1H NMR (CD_3OD, 500 MHz) and ^{13}C NMR (CD_3OD, 125 MHz) spectroscopic data, see Table 1. ESIMS (positive) *m/z*: 305.1 $[M+Na]^+$. HRESIMS (negative) *m/z*: 281.1177 $[M-H]^-$ (Calc. for $C_{18}H_{17}O_3$ 281.1178). CD (MeOH): $[\theta]^{25}$ (nm) −21,222 (235).

*4.7. Ribesin D (**4**): (7S,7′S,8R)-8′(9′)-ene-4,4′-dihydroxy-3,3′-dimethoxy-7,7′-epoxylignan*

Colorless solid. $[\alpha]_D^{25}$:+20.8 (*c* 0.05, MeOH). UV (MeOH): λ_{max} nm (logε) 229 (4.13), 280 (3.83). IR (KBr): ν_{max} 3434, 1593, 1510, 1442, 1377, 1274, 1128, 1028, 957 cm^{-1}. For ^1H NMR (CD_3OD, 500 MHz) and ^{13}C NMR (CD_3OD, 125 MHz) spectroscopic data, see Table 1. ESIMS (positive) *m/z*: 365.1 $[M+Na]^+$. HRESIMS (negative) *m/z*: 341.1394 $[M-H]^-$ (Calc. for $C_{20}H_{21}O_5$ 341.1390). CD (MeOH): $[\theta]^{25}$ (nm) −15,381 (237), −8905 (287).

*4.8. Ribesin E (**7**): rel-(7R,8S,7′S,8′R)-4,4′-dihydroxy-3′-[1-methyl-2-(4-hydroxyphenyl)]ethyl-7,7′-epoxylignan*

Colorless solid. $[\alpha]_D^{25}$: −35.0 (*c* 0.56, MeOH). UV (MeOH): λ_{max} nm (logε) 226 (4.50), 279 (3.87). IR (KBr): ν_{max} 3398, 3017, 2961, 2925, 2854, 1614, 1514, 1456, 1377, 1238, 1171, 1092, 1005 cm^{-1}. For ^1H NMR (CD_3OD, 500 MHz) and ^{13}C NMR (CD_3OD, 125 MHz) spectroscopic data, see Table 2. ESIMS (positive) *m/z*: 441.1 $[M+Na]^+$. HRESIMS (positive) *m/z*: 441.2044 $[M+Na]^+$ (Calc. for $C_{27}H_{30}O_4Na$ 441.2041). CD (MeOH): $[\theta]^{25}$ (nm) −9565 (221), 3267 (237).

*4.9. Ribesin F (**8**): rel-(7R, 8R, 7′S, 8′S)-4,4′-dihydroxy-3′-[1-methyl-2-(4-hydroxyphenyl)]ethyl-7,7′-epoxylignan*

Colorless solid. $[\alpha]_D^{25}$: −15.7 (*c* 0.40, MeOH). UV (MeOH): λ_{max} nm (logε) 226 (2.89), 278 (2.26). IR (KBr): ν_{max} 3387, 3025, 2959, 2325, 1613, 1514, 1450, 1377, 1238, 1171, 1120, 1101, 1028, 1005 cm^{-1}. For ^1H NMR (CD_3OD, 500 MHz) and ^{13}C NMR (CD_3OD, 125 MHz) spectroscopic data, see Table 2. ESIMS (positive) *m/z*: 441.1 $[M+Na]^+$. HRESIMS (positive) *m/z*: 441.2039 $[M+Na]^+$ (Calc. for $C_{27}H_{30}O_4Na$ 441.2041). CD (MeOH): $[\theta]^{25}$ (nm) 3185 (224), −4950 (236).

*4.10. Ribesin G (**9**): rel-(7R,8R,7′S,8′R)-3,4,4′-trihydroxy-3′-[1-methyl-2-(4-hydroxyphenyl)] ethyl-7,7′-epoxylignan*

Colorless solid. $[\alpha]_D^{25}$: −31.0 (*c* 0.30, MeOH). UV (MeOH): λ_{max} nm (logε) 226 (4.40), 280 (3.84). IR (KBr): ν_{max} 3422, 2955, 2924, 2854, 1610, 1513, 1459, 1377, 1250, 1173, 1106, 1022 cm^{-1}. For ^1H NMR (CD_3OD, 500 MHz) and ^{13}C NMR (CD_3OD, 125 MHz) spectroscopic data, see Table 2. ESIMS (positive) *m/z*: 456.7 $[M+Na]^+$. HRESIMS (positive) *m/z*: 457.1989 $[M+Na]^+$ (Calc. for $C_{27}H_{30}O_5Na$ 457.1991). CD (MeOH): $[\theta]^{25}$ (nm) −8276 (217), 1368 (236), −1318 (242), 1679 (266), −655 (277), 2426 (292).

*4.11. Ribesin H (**10**): rel-(2R,3S,5S,1′S,3′R)-2,5,1′,3′-tetra(4-hydroxyphenyl)-4-methylene-4,5,3′,4′,6′,7′-hexahydro-1′H,2H-spiro[furan-3,5′-isobenzofuran]*

Colorless solid. $[\alpha]_D^{25}$: −20.6 (*c* 0.52, MeOH). UV (MeOH): λ_{max} nm (logε) 230 (4.12), 277 (3.62). IR (KBr): ν_{max} 3413, 2925, 2859, 1613, 1518, 1452, 1375, 1235, 1169, 1106, 1023 cm^{-1}. For ^1H NMR (CD_3OD, 500 MHz) and ^{13}C NMR (CD_3OD, 125 MHz) spectroscopic data, see Table 3. ESIMS (positive) *m/z*: 583.3 $[M+Na]^+$. HRESIMS (negative) *m/z*: 559.2140 $[M-H]^-$ (Calc. for $C_{36}H_{31}O_6$ 559.2137). CD (MeOH): $[\theta]^{25}$ (nm) −64,824 (208), 5802 (223), −212,364 (235).

*4.12. Catalytic reduction using Pd/C of **1**–**4***

A solution of **1** (10.0 mg), **2** (1.0 mg), **3** (5.0 mg), and **4** (0.8 mg) in MeOH was separately treated with palladium-activated carbon (Pd 10%) (Wako, Japan, 1 mg) and then the reaction mixture was stirred at room temperature for overnight under H_2 atmosphere. The reaction mixture was passed through a Sep-pak C18 cartridge using H_2O and MeOH and further purified by reversed-phase HPLC with MeOH–H_2O (70:30, v/v) to give **5** (1.5 mg from **1**, and 4.0 mg from **3**), and **15** (0.5 mg from **2**, and 0.5 mg from **4**), respectively.

(7*S*,7′*S*,8*R*,8′*S*)-4,4′-Dihydroxy-7,7′-epoxylignan (**5**): colorless solid. $[\alpha]_D^{25}$: −20.3 (*c* 0.40, MeOH). ^1H NMR (CD_3OD, 500 MHz): δ 7.24 (2H, d, *J* = 8.0 Hz, H-2,6), 7.17 (2H, d, *J* = 8.0 Hz, H-2′,6′), 6.80–6.83 (4H, aromatic protons), 5.42 (1H, d, *J* = 4.6 Hz, H-7), 4.60 (1H, d, *J* = 9.3 Hz, H-7′), 2.42 (2H, m, H-8,8′), 0.97 (3H, d, *J* = 7.3 Hz, H-9′), 0.58 (3H, d, *J* = 6.7 Hz, H-9). ^{13}C NMR (CD_3OD, 125 MHz): δ 157.6 (C-4, 4′), 135.4 (C-1), 132.8 (C-1′), 128.3 (C-2, 6), 128.0 (C-2′, 6′), 115.8 (C-3,5), 115.5 (C-3′,5′), 86.2 (C-7), 85.2 (C-7′), 48.5 (C-8), 44.1 (C-8′), 12.1 (C-9), 9.8 (C-9′). CD (MeOH): $[\theta]^{25}$ (nm) −51,146 (232).

(7*S*,7′*S*,8*R*,8′*S*)-4,4′-Dihydroxy-3,3′-dimethoxy-7,7′-epoxylignan (**15**): colorless solid. $[\alpha]_D^{25}$: −78.0 (*c* 0.08, MeOH). ^1H NMR (CD_3OD, 500 MHz): δ 6.82–6.99 (6H, aromatic protons), 5.39 (1H, d, *J* = 7.8 Hz, H-7′), 4.59 (1H, d, *J* = 8.9 Hz, H-7), 3.88 (6H, s, OCH_3), 2.42 (2H, m, H-8,8′), 0.99 (3H, d, *J* = 5.7 Hz, H-9), 0.62 (3H, d, *J* = 6.4 Hz, H-9′). ^{13}C NMR (CD_3OD, 125 MHz): δ 146.0 (C-3′), 145.7 (C-3), 145.4 (C-4′), 145.3 (C-4), 136.6 (C-1′), 134.0 (C-1), 117.8 (C-6′), 117.6 (C-6), 112.5 (C-5′), 112.4 (C-5), 110.5 (C-2′), 110.2 (C-2), 85.4 (C-7), 84.5(C-7′), 56.1 (3-OCH_3), 56.0 (3′-OCH_3), 47.5 (C-8), 43.3 (C-8′), 11.9 (C-9), 9.4 (C-9′). CD (MeOH): $[\theta]^{25}$ (nm) −86,847 (231), −16,201 (286).

4.13. Superoxide anion scavenging assay and the DPPH radical scavenging assay

The assay was carried out as reported previously (Bi et al., 2011).

References

Bi, X., Li, W., Sasaki, T., Li, Q., Mitsuhata, N., Asada, Y., Zhang, Q., Koike, K., 2011. Secoiridoid glucosides and related compounds from *Syringa reticulate* and their antioxidant activities. Bioorg. Med. Chem. Lett. 21, 6426–6429.

Castillo, M.L.R.D., Dobson, G., Brennan, R., Gordon, S., 2004. Fatty acid content and juice characteristics in black currant (*Ribes nigrum* L.) genotypes. J. Agric. Food Chem. 52, 948–952.

Chavez, K.J., Feng, X., Flanders, J.A., Rodriguez, E., Schroeder, F.C., 2011. Spirocyclic lignans from *Guaiacum* (Zygophyllaceae) induce apoptosis in human breast cancer cell lines. J. Nat. Prod. 74, 1293–1297.

Garbacki, N., Angenot, L., Bassleer, C., Damas, J., Tits, M., 2002. Effects of prodelphinidins isolated from *Ribes nigrum* on chondrocyte metabolism and COX activity. Naunyn-Schmiedeberg's Arch. Pharmacol. 365, 434–441.

Garbacki, N., Tits, M., Angenot, L., Damas, J., 2004. Inhibitory effects of proanthocyanidins from *Ribes nigrum* leaves on carrageenin acute inflammatory reactions induced in rats. BMC Pharmacol. 4, 25. http://dx.doi.org/10.1186/1471-2210-4-25.

Garbacki, N., Kinet, M., Nusgens, B., Desmecht, D., Damas, J., 2005. Proanthocyanidins, from *Ribes nigrum* leaves, reduce endothelial adhesion molecules ICAM-1 and VCAM-1. J. Inflamm. 2, 2–9.

Hadizadeh, F., Khalili, N., Hosseinzadeh, H., Khair-Aldine, R., 2003. Kaempferol from *Saffron petals*. Iran. J. Pharm. Res. 2, 251–252.

Harada, K., Horiuchi, H., Tanabe, K., Carter, R.G., Esumi, T., Kubo, M., Hioki, H., Fukuyama, Y., 2011. Asymmetric synthesis of (−)-chicanine using a highly regioselective intramolecular Mitsunobu reaction and revision of its absolute configuration. Tetrahedron Lett. 52, 3005–3008.

He, D., Huang, Y., Ayupbek, A., Gu, D., Yang, Y., Aisa, H.A., Ito, Y., 2010. Separation and purification of flavonoids from black currant leaves by high-speed

countercurrent chromatography and preparative HPLC. J. Liq. Chromatogr. Related Technol. 33, 615–628.
Hisamoto, M., Kikuzaki, H., Nakatani, N., 2004. Constituents of the leaves of *Peucedanum japonicum* Thunb. and their biological activity. J. Agric. Food Chem. 52, 445–450.
Kwon, Y.S., Kim, C.M., 2003. Antioxidant constituents from the stem of *Sorghum bicolor*. Arch. Pharm. Res. 26 (7), 535–539.
Li, W., Huang, C., Li, S., Ma, F., Li, Q., Asada, Y., Koike, K., 2012. Clerodane diterpenoids from *Tinospora sagittata* (Oliv.) Gagnep. Planta Med. 78, 82–85.
Long, Z., Wu, L., Jiang, B., Sun, B., Huang, J., Gao, H., 2008. Chemical constituents from the seeds of *Castanea mollissima* Blume (III). J. Shenyang Pharm. Univ. 25, 883–885.
Moinuddin, S.G.A., Hishiyama, S., Cho, M.H., Davin, L.B., Lewis, N.G., 2003. Synthesis and chiral HPLC analysis of the dibenzyltetrahydrofruan lignans, larreatricins, 8′-epi-larreatricins, 3,3′-didemethoxyverrucosins and meso-3,3′-didemethoxynectandrin B in the creosote bush (*Larrea tridentate*): evidence of regiospecific control of coupling. Org. Biomol. Chem. 1, 2307–2313.
Nguyen, P.H., Le, T.V.T., Kang, H.W., Chae, J., Kim, S.K., Kwon, K.I., Seo, D.B., Lee, S.J., Oh, W.K., 2010. AMP-activated protein kinase (AMPK) activators from *Myristica fragrans* (nutmeg) and their anti-obesity effect. Bioorg. Med. Chem. Lett. 20, 4128–4131.
Otsuka, H., Yao, M., Kamada, K., Takeda, Y., 1995. Alangionosides G-M: glycoside of megastigmane derivatives from the leaves of *Alangium oremnifolium*. Chem. Pharm. Bull. 43, 754–759.
Slimestad, R., Solheim, H., 2002. Anthocyanins from black currants (*Ribes nigrum* L.). J. Agric. Food Chem. 50, 3228–3231.
Tabart, J., Kevers, C., Evers, D., Dommes, J., 2011. Ascorbic acid, phenolic acid, flavonoid, and carotenoid profiles of selected extracts from *Ribes nigrum*. J. Agric. Food Chem. 59, 4763–4770.
Tits, M., Angenot, L., Poukens, P., Warin, R., Dierckxsens, Y., 1992. Prodelphinidins from *Ribes nigrum*. Phytochemistry 31, 971–973.
Toferna, B., Jenett-Siemsa, K., Siemsb, K., Jakupovicb, J., Eich, E., 2000. Bonaspectins and neobonaspectins, first sesquilignans and sesquineolignans from a convolvulaceous species. Phytochemistry 53, 119–128.
Xorge, A., Dominguez, H., Sanchez, V., Griselda, C., Espinoza, B., Julia, V.S., Hans, A., Wolfgang, U., 1990. Lignans and nor-neolignans from *Krameria interior*. Phytochemistry 29, 2651–2653.
Zheng, J., Yang, B., Tuomasjukka, S., Ou, S., Kallio, H., 2009. Effects of latitude and weather conditions on contents of sugars, fruit acids, and ascorbic acid in black currant (*Ribes nigrum* L.) juice. J. Agric. Food Chem. 57, 2977–2987.