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# A New Lignan Glycoside from *Juniperus rigida*

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A new lignan glycoside, named juniperigiside (**1**) was isolated from the $CHCl_3$ soluble fraction of the MeOH extract of stems and leaves of *Juniperus rigida* S.*et* Z. Compound **1** was identified by 1D- and 2D-NMR spectroscopy as well as CD analysis as (2*R*,3*S*)-2,3-dihydro-7-methoxy-2-(4'-hydroxy-3'-methoxyphenyl)-3-hydroxymethyl-5-benzofuranpropanol 4'-*O*-(3-*O*-methyl)-α-L-rhamnopyranoside. Five known lignans, icariside E4 (**2**), desoxypodophyllotoxin (**3**), savinin (**4**), thujastandin (**5**), and (–)-nortrachelogenin (**6**) in addition to five known labdane diterpenes including *trans*-communic acid (**7**), 13-*epi*-torulosal (**8**), 13-*epi*-cupressic acid (**9**), imbricatoric acid (**10**), and isocupressic acid (**11**) were also isolated and their structures were characterized by comparing their spectroscopic data with those in the literature. All compounds were isolated for the first time from this plant, and **5** and **6** were first reported from the genus *Juniperus*. The isolated compounds were tested for cytotoxicity against four human tumor cell lines *in vitro* using a Sulforhodamin B bioassay. Compounds **3**, **4**, **7**, and **8** showed considerable cytotoxicity against four human cancer cell lines *in vitro*.

**Key words:** *Juniperus rigida*, Cupressaceae, Lignan glycoside, Labdane diterpene, Cytotoxicity

## INTRODUCTION

*Juniperus rigida* S.*et* Z. (Cupressaceae) is a deciduous conifer that is primarily distributed throughout the lime area of Korea (Lee, 2003). The fruits of *J. rigida*, known as 'Dusongsil' in Korea, have been used in Korean traditional medicine for the treatment of rheumatoid arthritis and dropsy (Lee et al., 2010). Diterpenes, such as ferruginol, cryptojaponol, sugiol and xanthoperol (Yanagawa and Hirose, 1971) as well as the sesquiterpenes, such as α-acoradiene, β-acoradiene, γ-acoradiene, δ-acoradiene, α-acorenol and β-acorenol (Tomita and Hirose, 1973) were found in the wood of *J. rigida*. Anti-lipase and lipolytic activities for the EtOH extract of *J. rigida* (Lee et al., 2010) have also been studied. As part of our continuing search for biologically active compounds from Korean medicinal plants, we investigated the $CHCl_3$ fraction of the MeOH extract of *J. rigida*, since the MeOH extract of this plant exhibited significant cytotoxic activity in our screening procedures. Indeed, we isolated a new lignan glycoside, juniperigiside (**1**), together with five known lignans (**2**-**6**) and five known labdane diterpenes (**7**-**11**). The isolated compounds were tested for their cytotoxic activities against four human cancer cell lines *in vitro* using an Sulforhodamin B bioassay (SRB).

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## MATERIALS AND METHODS

### General experimental procedures

Optical rotations were measured on a JASCO P-1020 Polarimeter. UV spectra were recorded with a Shimadzu UV-1601 UV-Visible spectrophotometer. Circular dichroism (CD) spectra were measured on a JASCOJ-810 spectropolarimeter. EI, ESI, and HRESI data were obtained on a JEOL JMS 700 and a Shimadzu LCMS-IT-TOF mass spectrometer. NMR spectra, including $^1$H-$^1$H COSY, HMQC, and HMBC were recorded on a Varian UNITY INOVA 500 NMR spectrometer operating at 500 MHZ ($^1$H) and 125 MHZ ($^{13}$C) using

TMS as an internal standard with chemical shifts given in ppm (δ). TLC was performed using Merck precoated Silica gel $F_{254}$ plates and RP-18 $F_{254}$s plates. Spots were detected on thin layer chromatography (TLC) under UV light or by heating after spraying with 10% $H_2SO_4$ in $C_2H_5OH$ (v/v). Packing material used for molecular sieve column chromatography was Sephadex LH-20 (Pharmacia Co.). Sep-Pak® (Waters, Vac 12cc) was also used for column chromatography. Low pressure liquid chromatography was carried out using a Merck LiChroprep Lobar®-A Si 60 (240 × 10 mm) column with a FMI QSY-0 pump (ISCO). Preparative high performance liquid chromatography (HPLC) HPLC was performed with a Gilson 306 pump and Knauer Dual Detector with Apollo Silica 5 μ (250 × 22 mm) or Econosil® RP-18 10 μ columns (250 × 22 mm). Silica gel 60 (Merck, 70~230 mesh and 230~400 mesh) was used for column chromatography.

## Plant material

*J. rigida* (6 kg) was collected in KyeongSan, Gyeongbuk Province, Korea in August, 2010 and was identified by one of the authors (K.R.L). A voucher specimen (SKKU-2010-3d) was deposited at the herbarium of the School of Pharmacy of Sungkyunkwan University, Korea.

## Extraction and isolation

The dried stems and leaves of *J. rigida* (6 kg) were extracted with 80% MeOH three times at 85°C and evaporated under reduced pressure to give a residue (290 g). The residue was dissolved in water (800 mL) and partitioned with solvents to give *n*-hexane (14.7 g), $CHCl_3$ (34 g), EtOAc (15 g), *n*-BuOH (70 g), and $H_2O$ (160 g) soluble portions. The chloroform fraction (14 g) was separated over a silica gel column with a *n*-hexane-$CHCl_3$-MeOH (2.5:25:0.01-0:1:1) solvent system as the eluent to give thirteen fractions (JC1-JC13). Fraction JC7 (1.7 g) was subjected to Sephadex LH-20 column chromatography ($CH_2Cl_2$-MeOH = 1:1) to give four fractions (JC71-JC74). JC73 (600 mg) was further separated over an RP-$C_{18}$ silica gel column (90% MeOH) to yield nine fractions (JC731-JC739). JC732 (125 mg) was separated over a silica Lobar A®-column (*n*-hexane-$CHCl_3$-MeOH = 5:35:0.09) and purified with RP-$C_{18}$ silica gel preparative HPLC (78% MeOH) to yield compound **3** (8 mg, $R_t$ = 9.5 min). JC733 (66 mg) was purified with silica gel preparative HPLC (*n*-hexane-$CHCl_3$-MeOH = 4:18:0.1) to yield compound **4** (8 mg, $R_t$ = 13.0 min). JC734 (60 mg) was purified silica gel preparative HPLC (*n*-hexane-$CHCl_3$-MeOH = 4:10:0.06) to yield compound **8** (5 mg, $R_t$ = 10.0 min). JC736 (30 mg) afforded compound **7** (13 mg, $R_t$ = 22.0 min) by RP-$C_{18}$ silica gel preparative HPLC (95% MeOH). The JC9 fraction (3 g) was separated over an RP-$C_{18}$ silica gel column (90% MeOH) to give twelve fractions (JC91-JC912). The JC91 fraction (83 mg) was purified with RP-$C_{18}$ silica gel preparative HPLC (50% MeOH) to yield compounds **5** (5 mg, $R_t$ = 16.0 min) and **6** (8 mg, $R_t$ = 18.5 min). The JC95 fraction (970 mg) was separated over an RP-$C_{18}$ silica gel column (90% MeOH) to yielded four fractions (JC951-JC954). The JC952 fraction (600 mg) was purified with RP-$C_{18}$ silica gel preparative HPLC (70% MeCN) to yielded compounds **10** (430 mg, $R_t$ = 15.0 min) and **11** (10 mg, $R_t$ = 14.0 min). The JC953 fraction (27 mg) afforded compound **9** (8 mg, $R_t$ = 12.5 min) by RP-$C_{18}$ silica gel preparative HPLC (95% MeOH). The JC11 fraction (1.6 g) was subjected to Sephadex LH-20 column chromatography (90% MeOH) and further separated over an RP-$C_{18}$ silica gel column with 50% MeOH as the eluant to yield nine fractions (JC111-JC119). JC111 (51 mg) was purified with RP-$C_{18}$ silica gel preparative HPLC (50% MeOH) to yield compound **1** (5 mg, $R_t$ = 12 min). JC12 (690 mg) was separated over an RP-$C_{18}$ silica gel column with 100% MeOH to yield two fractions, JC121 and JC122. JC121 (280 mg) fraction was subjected to Sephadex LH-20 column chromatography (90% MeOH) and further separated over a silica Lobar A®-column ($CHCl_3$-MeOH = 13:1) to furnish five fractions (JC1211-JC1215). JC1215 (34 mg) was purified with RP-$C_{18}$ silica gel preparative HPLC (50% MeOH) to yield compound **2** (3 mg, $R_t$ = 12 min).

### Juniperigiside (1)

Colorless gum, $[\alpha]_D^{25}$ –50.0° (*c* 0.25, MeOH); UV (MeOH) $\lambda_{max}$ (log ε): 289 (4.08) nm; HR-ESI-MS *m/z*: 543.2202 $[M+Na]^+$ (calculated for $C_{27}H_{36}O_{10}Na$, 543.2308); CD (MeOH) $\lambda_{max}$ nm (Δε) 293 (0.67), 271 (0.21), 251 (0.70), 220 (0.67); $^1$H-NMR and $^{13}$C-NMR data see: Table I.

### Icariside E4 (2)

Colorless gum, $[\alpha]_D^{25}$ –51.3° (*c* 0.15, MeOH); EI-MS *m/z*: 506 $[M]^+$; $^1$H-NMR (500 MHz, $CD_3OD$): δ 7.07 (1H, d, *J* = 7.5 Hz, H-5'), 7.02 (1H, d, *J* = 2.0 Hz, H-6'), 6.91 (1H, dd, *J* = 8.0, 2.0 Hz, H-2'), 6.73 (1H, s, H-6), 6.71 (1H, s, H-4), 5.55 (1H, d, *J* = 6.0 Hz, H-2), 5.33 (1H, d, *J* = 2.0 Hz, H-1''), 4.05 (1H, dd, *J* = 3.0, 2.0 Hz, H-2''), 3.87-3.83 (1H, m, H-3a, H-3''), 3.86 (3H, s, 7-$OCH_3$), 3.81-3.77 (1H, m, H-3a, H-5''), 3.80 (3H, s, 3'-$OCH_3$), 3.56 (2H, t, *J* = 6.5 Hz, H-5c), 3.48-3.42 (1H, m, H-3, H-4''), 2.62 (2H, t, *J* = 7.5 Hz, H-5a), 1.81 (2H, m, H-5b), 1.21 (3H, d, *J* = 6.5 Hz, H-6''); $^{13}$C-NMR (125 MHz, $CD_3OD$): δ 150.9 (C-5'), 146.3 (C-7a), 145.4 (C-4'), 144.0 (C-7), 137.6 (C-1'), 135.8 (C-5), 128.4 (C-4a), 118.4 (C-3'), 117.9 (C-2'), 116.7 (C-4), 113.0 (C-6), 110.1 (C-6'),

**Table I.** $^{1}$H- and $^{13}$C-NMR data for compound **1**

| Position | Chemical shifts (ppm) | | Position | Chemical shifts (ppm) | |
|---|---|---|---|---|---|
| | $\delta_C$[a] | $\delta_H$[b] | | $\delta_C$ | $\delta_H$ |
| 2 | 87.3 | 5.55 (1H, d, $J$ = 5.5 Hz)[c] | 1' | 137.7 | - |
| 3 | 54.4 | 3.46 (1H, m) | 2' | 110.0 | 7.04 (1H, d, $J$ = 2.0 Hz) |
| 3a | 63.9 | 3.84 (1H, m)<br>3.75 (1H, dd, $J$ = 11.0, 7.5 Hz) | 3' | 150.9 | |
| 4 | 116.7 | 6.72 (1H, s) | 4' | 145.2 | - |
| 4a | 128.4 | - | 5' | 118.5 | 7.08 (1H, d, $J$ = 8.5 Hz) |
| 5 | 135.9 | - | 6' | 117.9 | 6.92 (1H, dd, $J$ = 8.0, 1.5 Hz) |
| 5a | 31.7 | 2.62 (2H, t, $J$ = 6.5 Hz) | 1" | 100.2 | 5.36 (1H, d, $J$ = 2.0 Hz) |
| 5b | 34.6 | 1.81 (2H, m) | 2" | 66.8 | 4.26 (1H, t, $J$ = 2.0 Hz) |
| 5c | 61.0 | 3.56 (2H, t, $J$ = 6.5 Hz) | 3" | 80.7 | 3.52 (1H, dd, $J$ = 8.0, 3.0 Hz) |
| 6 | 113.0 | 6.73 (1H, s) | 4" | 71.5 | 3.46 (1H, m) |
| 7 | 144.0 | - | 5" | 69.6 | 3.82 (1H, m) |
| 7a | 146.3 | - | 6" | 16.7 | 1.21 (3H, d, $J$ = 6.0 Hz) |
| 7-$OCH_3$ | 55.6 | 3.86 (3H, s) | 3'-$OCH_3$ | 55.2 | 3.80 (3H, s) |
| | | | 3"-$OCH_3$ | 56.2 | 3.50 (3H, s) |

Spectra were recorded at [a]125 and [b]500 MHz in $CD_3OD$. [c]$J$ values (in Hz) are in parentheses.

100.2 (C-1"), 87.3 (C-2), 72.6 (C-4"), 71.0 (C-3"), 70.8 (C-2"), 69.6 (C-5"), 63.9 (C-3a), 61.0 (C-5c), 55.6 (3'-$OCH_3$), 55.2 (7-$OCH_3$), 54.4 (C-3), 34.6 (C-5b), 31.7 (C-5a), 16.7 (C-6").

**Desoxypodophyllotoxin (3)**

Colorless gum, $[\alpha]_D^{25}$ –66.0° (*c* 0.4, MeOH); EI-MS *m/z*: 398 $[M]^+$; $^1$H-NMR (500 MHz, $CD_3OD$): δ 6.71 (1H, s, H-2), 6.46 (1H, s, H-5), 6.39 (2H, s, H-2', 6'), 5.90 (2H, s, $OCH_2O$), 4.57 (1H, d, $J$ = 5.0 Hz, H-7'), 4.44 (2H, t, H-9), 3.71 (6H, s, 3', 5'-$OCH_3$), 3.70 (3H, s, 4'-$OCH_3$), 3.08 (1H, dd, $J$ = 15.0, 5.0 Hz, H-7a), 2.89 (1H, dd, $J$ = 13.5, 5.0 Hz, H-8'), 2.79 (1H, dd, $J$ = 12.5, 5.0 Hz, H-7b), 2.68 (1H, m, H-8); $^{13}$C-NMR (125 MHz, $CD_3OD$): δ 176.5 (C-9'), 152.5 (C-3', 5'), 147.2 (C-3, 5), 146.9 (C-4), 137.3 (C-1'), 136.7 (C-4'), 130.8 (C-6), 129.1 (C-1), 109.9 (C-5), 108.3 (C-2), 108.2 (C-6'), 101.2 ($OCH_2O$), 72.4 (C-9), 59.8 (4'-$OCH_3$), 55.3 (3', 5'-$OCH_3$), 47.1 (C-8'), 43.8 (C-7'), 33.2 (C-8), 32.4 (C-7).

**Savinin (4)**

Colorless gum, $[\alpha]_D^{25}$ –68.2° (*c* 0.4, MeOH); EI-MS *m/z*: 352 $[M]^+$; $^1$H-NMR (500 MHz, $CDCl_3$): δ 7.50 (1H, d, $J$ = 2.0 Hz, H-7), 7.08 (1H, dd, $J$ = 8.0, 2.0 Hz, H-6), 7.04 (1H, d, $J$ = 2.0 Hz, H-2), 6.88 (1H, d, $J$ = 8.0 Hz, H-5), 6.73 (1H, d, $J$ = 7.5 Hz, H-5'), 6.66 (1H, d, $J$ = 2.0 Hz, H-2'), 6.64 (H, dd, $J$ = 8.0, 2.0 Hz, H-6'), 6.04 (2H, s, 3-$OCH_2O$), 5.93 (2H, dd, $J$ = 4.0, 1.0 Hz, 3'-$OCH_2O$), 4.25 (2H, m, H-9'), 3.75 (1H, m, H-8'), 3.00 (2H, dd, $J$ = 14.0, 4.5 Hz, H-7'); $^{13}$C-NMR (125 MHz, $CDCl_3$): δ 172.8 (C-9), 149.4 (C-4), 148.5 (C-3), 148.1 (C-3'), 146.7 (C-4'), 137.5 (C-7), 131.7 (C-1'), 128.4 (C-1), 126.3 (C-6), 126.0 (C-8), 122.3 (C-6'), 109.4 (C-2'), 109.0 (C-5'), 108.8 (C-2), 108.7 (C-5), 101.9 (3-$OCH_2O$), 101.2 (3'-$OCH_2O$), 69.7 (C-9'), 40.1 (C-8'), 37.7 (C-7').

**Thujastandin (5)**

Colorless gum, $[\alpha]_D^{25}$ –11.6° (*c* 0.3, MeOH); EI-MS *m/z*: 390 $[M]^+$; $^1$H-NMR (500 MHz, $CD_3OD$): δ 7.03 (1H, d, $J$ = 1.5 Hz, H-5), 6.86 (1H, d, $J$ = 2.0 Hz, H-5'), 6.84 (1H, d, $J$ = 1.5 Hz, H-2), 6.73 (1H, d, $J$ = 2.0 Hz, H-2'), 6.70 (1H, dd, $J$ = 17.0, 4.5 Hz, H-6'), 6.57 (1H, dd, $J$ = 8.0, 2.0 Hz, H-6), 4.27 (2H, d, $J$ = 9.0 Hz, H-9'), 3.86 (3H, s, 3-$OCH_3$), 3.82 (3H, s, 3'-$OCH_3$), 3.64 (1H, d, $J$ = 9.0 Hz, H-7a), 3.05 (1H, d, $J$ = 3.0 Hz, H-7b), 2.67 (1H, d, $J$ = 13.5 Hz, H-7'a), 2.38 (1H, d, $J$ = 14.0 Hz, H-7'b); $^{13}$C-NMR (125 MHz, $CDCl_3$): δ 179.0 (C-9), 147.4 (C-3'), 147.1 (C-3), 145.2 (C-4), 145.1 (C-4'), 127.6 (C-1'), 127.0 (C-1), 124.0 (C-6), 122.6 (C-6'), 115.2 (C-5'), 114.7 (C-5), 114.3 (C-2), 113.9 (C-2'), 79.3 (C-8), 77.8 (C-8'), 74.6 (C-9'), 55.2 (3-$OCH_3$), 55.1 (3'-$OCH_3$), 37.4 (C-7'), 36.7 (C-7).

**(–)-Nortrachelogenin (6)**

Colorless gum, $[\alpha]_D^{25}$ –33.7° (*c* 0.4, MeOH); EI-MS *m/z*: 374 $[M]^+$; $^1$H-NMR (500 MHz, $CD_3OD$): δ 6.70 (1H, d, $J$ = 8.0 Hz, H-5), 6.69 (1H, d, $J$ = 8.0 Hz, H-5'), 6.68 (1H, d, $J$ = 2.0 Hz, H-2), 6.66 (1H, d, $J$ = 2.0 Hz, H-2'), 6.58 (1H, dd, $J$ = 8.5, 2.0 Hz, H-6'), 6.56 (1H, dd, $J$ = 8.0, 2.0 Hz, H-6), 3.97 (2H, d, $J$ = 7.5 Hz, H-9'), 3.80 (3H, s, 3-$OCH_3$), 3.77 (3H, s, 3'-$OCH_3$), 3.11 (1H, d, $J$ = 13.0 Hz, H-7a), 2.84 (1H, d, $J$ = 13.5 Hz, H-7b), 2.78 (1H, dd, $J$ = 13.5, 4.5 Hz, H-7'a), 2.46 (1H, dd, $J$ = 13.5, 4.5 Hz, H-7'b), 2.43 (1H, m, H-8'); $^{13}$C-NMR (125

MHz, $CD_3OD$): δ 179.4 (C-9), 147.8 (C-3'), 147.6 (C-3), 145.5 (C-4), 144.9 (C-4'), 130.7 (C-1'), 127.0 (C-1), 122.9 (C-6), 121.1 (C-6'), 115.0 (C-5'), 114.9 (C-5), 113.8 (C-2), 112.3 (C-2'), 76.2 (C-8), 70.6 (C-9'), 55.2 (3, 3'-$OCH_3$), 43.4 (C-8'), 40.6 (C-7), 31.0 (C-7').

***trans*-Communic acid (7)**

Colorless gum, $[\alpha]_D^{25}$ +30.5° (*c* 0.65, MeOH); EI-MS *m/z*: 302 $[M]^+$; $^1$H-NMR (500 MHz, $CDCl_3$): δ 6.32 (1H, dd, *J* = 17.5, 10.5 Hz, H-14), 5.41 (1H, t, *J* = 6.5 Hz, H-12), 5.03 (1H, d, *J* = 17.0 Hz, H-15a), 4.87 (1H, d, *J* = 11.0 Hz, H-15b), 4.84 (1H, s, H-17a), 4.47 (1H, s, H-17b), 1.75 (3H, s, H-16), 1.25 (3H, s, H-18), 0.65 (3H, s, H-20); $^{13}$C-NMR (125 MHz, $CDCl_3$): δ 183.9 (C-19), 148.1 (C-8), 141.8 (C-14), 134.1 (C-12), 133.6 (C-13), 110.1 (C-15), 107.9 (C-17), 56.8 (C-9), 56.6 (C-5), 44.4 (C-4), 40.5 (C-10), 39.4 (C-1), 38.6 (C-7), 38.1 (C-3), 29.2 (C-18), 26.0 (C-6), 23.5 (C-11), 20.1 (C-2), 13.0 (C-20), 12.0 (C-16).

**13-*epi*-Torulosal (8)**

Colorless gum, $[\alpha]_D^{25}$ +6.8° (*c* 0.25, MeOH); ESI-MS *m/z*: 329 $[M+Na]^+$; $^1$H-NMR (500 MHz, $CDCl_3$): δ 9.74 (1H, s, H-19), 5.90 (1H, dd, *J* = 17.0, 10.5 Hz, H-14), 5.20 (1H, dd, *J* = 17.5, 1.0 Hz, H-15a), 5.06 (1H, dd, *J* = 10.5, 1.0 Hz, H-15b), 4.86 (1H, s, H-17a), 4.52 (1H, s, H-17b), 1.27 (3H, s, H-16), 0.97 (3H, s, H-18), 0.56 (3H, s, H-20); $^{13}$C-NMR (125 MHz, $CDCl_3$): δ 206.0 (C-19), 147.7 (C-8), 145.2 (C-14), 111.9 (C-15), 107.6 (C-17), 73.8 (C-13), 56.3 (C-9), 56.0 (C-5), 48.9 (C-4), 41.4 (C-12), 40.4 (C-10), 38.6 (C-3), 34.6 (C-1), 28.3 (C-16), 24.6 (C-18), 24.2 (C-6), 19.4 (C-2), 18.1 (C-11), 13.7 (C-20).

**13-*epi*-Cupressic acid (9)**

Colorless gum, $[\alpha]_D^{25}$ +27.6° (*c* 0.3, MeOH); EI-MS *m/z*: 319 $[M]^+$; $^1$H-NMR (500 MHz, $CDCl_3$): δ 5.88 (1H, dd, *J* = 17.0, 10.5 Hz, H-14), 5.18 (1H, dd, *J* = 18.0, 2.0 Hz, H-15a), 5.02 (1H, dd, *J* = 10.5, 2.0 Hz, H-15b), 4.82 (1H, s, H-17a), 4.51 (1H, s, H-17b), 1.22 (3H, s, H-18), 1.18 (3H, s, H-16), 0.61 (3H, s, H-20); $^{13}$C-NMR (125 MHz, $CDCl_3$): δ 183.5 (C-19), 148.6 (C-8), 145.1 (C-14), 110.8 (C-15), 105.7 (C-17), 73.0 (C-13), 56.8 (C-9), 56.4 (C-5), 44.1 (C-4), 41.5 (C-12), 40.5 (C-10), 39.3 (C-1), 38.7 (C-7), 38.3 (C-3), 28.4 (C-18), 26.7 (C-16), 26.4 (C-6), 20.0 (C-2), 18.0 (C-11), 12.2 (C-20).

**Imbricatolic acid (10)**

Colorless gum, $[\alpha]_D^{25}$ +14.8° (*c* 2.4, MeOH); EI-MS *m/z*: 322 $[M]^+$; $^1$H-NMR (500 MHz, $CDCl_3$): δ 4.81 (1H, s, H-17a), 4.47 (1H, s, H-17b), 3.64 (2H, m, H-15), 1.21 (3H, s, H-18), 0.88 (3H, d, *J* = 6.5 Hz, H-16), 0.57 (3H, s, H-20); $^{13}$C-NMR (125 MHz, $CDCl_3$): δ 183.4 (C-19), 148.5 (C-8), 106.5 (C-17), 61.3 (C-15), 56.9 (C-5), 56.6 (C-9), 44.4 (C-4), 40.7 (C-10), 39.6 (C-12), 39.4 (C-1), 39.0 (C-14), 38.2 (C-7), 36.6 (C-3), 30.5 (C-13), 29.2 (C-18), 26.3 (C-6), 21.3 (C-11), 20.1 (C-2), 20.0 (C-16), 13.0 (C-20).

**Isocupressic acid (11)**

Colorless gum, $[\alpha]_D^{25}$ +56.6° (*c* 0.5, MeOH); EI-MS *m/z*: 320 $[M]^+$; $^1$H-NMR (500 MHz, $CDCl_3$): δ 5.37 (1H, t, *J* = 7.0 Hz, H-14), 4.84 (1H, s, H-17a), 4.52 (1H, s, H-17b), 4.15 (2H, d, *J* = 6.5 Hz, H-15), 1.66 (3H, s, H-16), 1.22 (3H, s, H-18), 0.59 (3H, s, H-20); $^{13}$C-NMR (125 MHz, $CDCl_3$): δ 183.5 (C-19), 148.1 (C-8), 140.7 (C-13), 123.2 (C-14), 106.6 (C-17), 59.6 (C-15), 56.5 (C-5), 55.8 (C-9), 44.5 (C-4), 40.6 (C-10), 39.3 (C-1), 38.9 (C-12), 38.6 (C-7), 38.2 (C-3), 29.2 (C-18), 26.3 (C-6), 22.2 (C-11), 20.1 (C-2), 16.5 (C-16), 13.0 (C-20).

**Test for cytotoxicity *in vitro***

SRB were used as cytotoxicity screening methods (Skehan et al., 1990). Cytotoxicity assays for each compound were performed *in vitro* against four cultured human tumor cell lines at the Korea Research Institute of Chemical Technology. The cell lines used were A549 (non-small cell lung adenocarcinoma), SK-OV-3 (ovarian cancer cells), SK-MEL-2 (skin melanoma), and HCT15 (colon cancer cells). Gemcitabine was used as a positive control.

## RESULTS AND DISCUSSION

Compound **1** was obtained as a colorless gum, $[\alpha]_D^{25}$ –50.0 (c 0.25, MeOH). The molecular formula was determined to be $C_{27}H_{36}O_{10}$ by HR-ESIMS *m/z* 543.2202 $[M+Na]^+$ (calcd 543.2308). The UV spectrum of **1** showed an absorption band at $\lambda_{max}$ 289 nm. The $^1$H-NMR spectrum showed the presence of five aromatic protons at $\delta_H$ 7.08 (1H, d, *J* = 8.5 Hz), 7.04 (1H, d, *J* = 2.0 Hz), 6.92 (1H, dd, *J* = 8.0, 1.5 Hz), 6.73 (1H, s), and 6.72 (1H, s), two methylenes at $\delta_H$ 2.62 (2H, t, *J* = 6.5 Hz), and 1.81 (2H, m), two hydroxymethylenes at $\delta_H$ 3.84 (1H, m), 3.75 (1H, dd, *J* = 11.0, 7.5 Hz), and 3.56 (2H, t, *J* = 6.5 Hz), two methine protons at $\delta_H$ 5.55 (1H, d, *J* = 5.5 Hz), and 3.46 (1H, m) and two methoxy groups at $\delta_H$ 3.86 (3H, s), and 3.80 (3H, s). The $^{13}$C-NMR spectrum also displayed 20 skeletal carbon resonances, which were composed of 7C, 7CH, $4CH_2$, and $2CH_3$ groups next to a six carbon sugar moiety and a methoxy group. The above NMR data implied that **1** was a benzofuran type lignan glycoside (Fukuyama et al., 1996; Yeo et al., 2004). These data were quite similar with data for **2**, which was isolated from *Epimedium diphyllum* (Miyase et al., 1989), except for the rham-

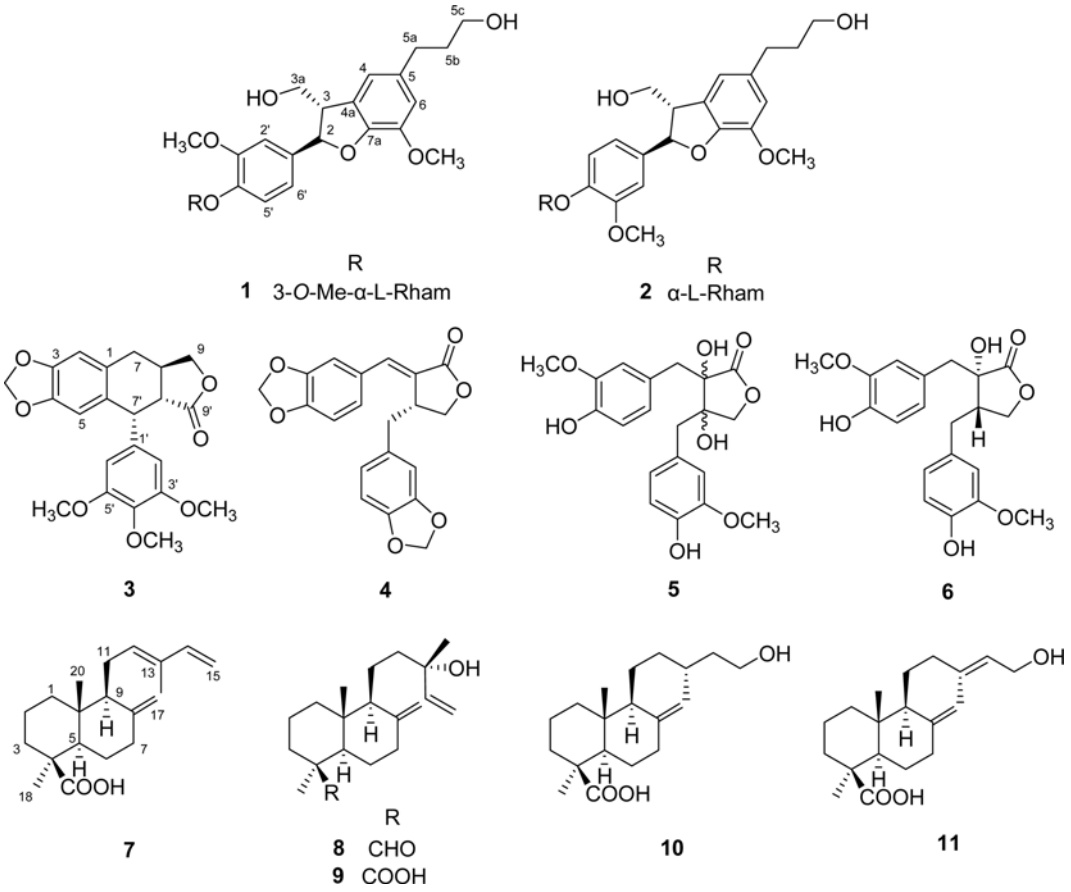


**Fig.1.** The structures of compounds **1**-**11**

nose moiety; the upfield shift of C-2" ($\delta_C$ 66.8) and C-4" ($\delta_C$ 71.5) with a downfield shift of C-3" ($\delta_C$ 66.8) in the $^{13}$C-NMR spectrum of **1** compared with **2** (C-2" - $\delta_C$ 70.8, C-4" - $\delta_C$ 72.6, C-3" - $\delta_C$ 71.0) plus the additional methoxy signal at $\delta_H$ 3.50 is shown. The position of the methoxy group was confirmed to be at Rham-C-3 by the HMBC spectrum (Fig. 2). The coupling constant ($J$ = 2.0 Hz) of the anomeric proton of L-rhamnose suggested that it was the α-form (Pan and Lundgren, 1995).

The *trans*-configuration at C-2 and C-3 were determined by coupling constants ($J$ = 5.5 Hz) (Matsuda et al., 1996; Li et al., 1997), and the absolute configuration at C-2 and C-3 were thought to be 2*R* and 3*S*, respectively, based on the CD spectrum showing a positive cotton effect at $\lambda_{max}$ 220 nm and a negative cotton effect at $\lambda_{max}$ 293 nm (Matsuda et al., 1996; Yuen et al., 1998). Thus, the structure of **1** was determined to be (2*R*,3*S*)-2,3-dihydro-7-methoxy-2-(4'-hydroxy-3'-methoxyphenyl)-3-hydroxymethyl-5-benzofuranpropanol 4'-*O*-(3-*O*-methyl)-α-L-rhamnopyranoside.

Compounds **2-6** and **7-11** were confirmed by comparing the $^1$H-, $^{13}$C-NMR and MS spectra with the

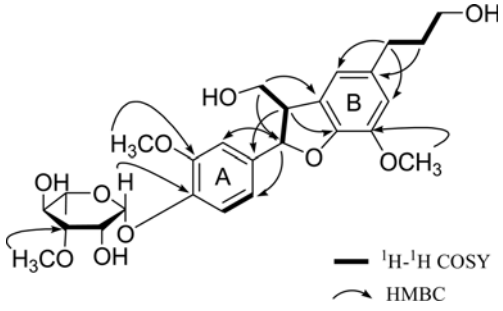


**Fig. 2.** Key ($^1$H-$^1$H COSY and HMBC) correlations of **1**

literature as icariside E4 (**2**) (Nakanishi et al., 2004), desoxypodophyllotoxin (**3**) (Ito et al., 1992), savinin (**4**) (Takaku et al., 2001), thujastandin (**5**) (Murakami, 1967; Nishibe et al., 1980), and (–)-nortrachelogenin (**6**) (Kato et al., 1979; Achenbach et al., 1983), *trans*-communic acid (**7**) (Gordien et al., 2009), 13-*epi*-torulosal (**8**) (Su et al., 1994; Hanari et al., 2002), 13-*epi*-cupressic acid (**9**) (Su et al., 1994; Liu et al., 2010), imbricatoric acid (**10**) (Kuo and Yu, 1996), and isocupressic aicd (**11**) (Gardner et al., 1994). All compounds were isolated for the first time from this plant and

**Table II.** Cytotoxic activities of compounds (**1-11**) from *J. rigida*

| Compound | $IC_{50}$ (μM)[a] | | | |
|---|---|---|---|---|
| | A549 | SK-OV-3 | SK-MEL-2 | HCT-15 |
| **1** | > 30 | > 30 | > 30 | > 30 |
| **2** | > 30 | > 30 | > 30 | > 30 |
| **3** | 0.0052 | 0.0041 | 0.0037 | 0.0035 |
| **4** | 5.75 | 4.16 | 4.37 | 4.82 |
| **5** | 26.47 | 15.62 | 17.03 | 28.96 |
| **6** | 25.12 | > 30 | > 30 | > 30 |
| **7** | 0.59 | 0.40 | 0.38 | 0.38 |
| **8** | 5.81 | 3.84 | 3.91 | 3.76 |
| **9** | > 30 | > 30 | > 30 | > 30 |
| **10** | 23.57 | > 30 | > 30 | 28.56 |
| **11** | > 30 | > 30 | > 30 | 23.42 |
| Gemcitabine[b] | 0.0007 | 0.104 | 0.196 | 0.263 |

[a]$IC_{50}$ value of compounds against each cancer cell line, which was defined as theconcentration (in moles per liter) that caused 50% inhibition of cell growth *in vitro*; [b]Gemcitabine as positive control.

compounds **5** and **6** were reported first from the genus *Juniperus* (Fig. 1).

The cytotoxic activities of the isolated compounds (**1-11**) were evaluated against A549, SK-OV-3, SK-MEL-2, and HCT15 human cell lines *in vitro* using the SRB (Table II).

The podophyllotoxin derivative, compound **3** had remarkable cytotoxicity against A549, SKOV-3, SK-MEL-2, and HCT15 cells lines: $IC_{50}$ = 0.0052, 0.0041, 0.0037, and 0.0035 μM, respectively. The significant cytotoxic activity to some cancer cell lines (DLD-1: human colorectal carcinoma cell, IMR-32: human neuroblastoma cell: $IC_{50}$ = 0.0057, 0.0035 μM, respectively) for compound **3** was already reported (Chen et al., 2011). Compound **4** also displayed considerable cytotoxic activity against the four tested human cell lines: $IC_{50}$ = 5.75, 4.16, 4.37, and 4.82 μM, respectively, although the cytotoxic activity against MCF-7 (breast adenocarcinoma cell: $ID_{50}$ = 0.5 μg/mL) cell was studied previously (Chang et al., 2000). The labdane type diterpenes, **7** and **8** also exhibited remarkable cytotoxicity against the tested four cell lines. Since significant cytotoxic activity for the compound **7**, its mechanism of action is to be further studied.

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