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Neolignan and flavonoid glycosides in *Juniperus communis* var. *depressa*

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Abstract

Two neolignan glycosides (junipercomnosides A and B) were isolated from aerial parts of *Juniperus communis* var. *depressa* along with two known neolignan glycosides and seven flavonoid glycosides. The structures of the isolated compounds were determined by spectral analysis, in particular by 2D-NMR analysis. The significance of distribution of flavonoids in the chemotaxonomy of genus *Juniperus* was also discussed.

Keywords: *Juniperus communis* var. *depressa*; Cupressaceae; Neolignan glycoside; Flavonoid glycoside; Junipercomnoside A; Junipercomnoside B; Isoscutellarein 7-*O*-β-xylopyranoside; Hypolaetin 7-*O*-β-xylopyranoside

1. Introduction

Genus *Juniperus* belongs to Family Cupressaceae, which comprises about 70 species distributed in the Northern Hemisphere. Among them, *Juniperus communis* is a familiar species with the characteristic flavor of distilled liquor "gin" (Bailey et al., 1976). Biflavones (De Pascual et al., 1980; Ilyas and Ilyas, 1990) and diterpenes (Kagawa et al., 1993; San Felicianao et al., 1991) have previously been isolated from *J. communis*. Recently, we reported the characterization of two novel phenol glucosides together with known biflavones and diterpene in leaves of *J. occidentalis*. In continuation of this study, we now report the isolation and structure determination of the constituents of *J. communis* var. *depressa*.

Chromatographical separation and purification with silica gel, ODS and HPLC of the *n*-BuOH soluble part (see Section 3) resulted in isolation of eleven phenolic compounds; four neolignan glycosides (**1–4**) and seven flavonoid glycosides (**5–11**) including two new neolignan glycosides, named junipercomnosides A (**1**) and B (**2**). In the present paper, we wish to describe the structure determination of the isolated compounds. In addition, the chemotaxonomical significance of flavonoids such as isoscutellarein and hypolaetin glycosides is discussed.

2. Results and discussion

Compound **1** (junipercomnoside A), a colorless amorphous powder, showed the $[M–H]^-$ ion peak at m/z 477.1759 in the negative ion HRFAB-MS, indicating the molecular formula $C_{24}H_{30}O_{10}$. The 1D ^1H NMR and 2D ^1H–^1H COSY spectra (Fig. 1) showed the presence of a 1,2,4-trisubstituted benzene ring (ring A) [δ 7.06 (1H, *d*, $J = 1.8$ Hz, H-2′), 7.05 (1H, *d*, $J = 8.4$ Hz, H-5′) and 6.94 (1H, *dd*, $J = 8.4$, 1.8 Hz, H-6′), a 1,2,3,5-tetrasubstituted benzene ring (ring B) [δ 6.59 (1H, *br s*, H-4) and 6.57 (1H, *br s*, H-6)], hydroxy propyl group [δ 2.55 (*t*, $J = 7.7$ Hz, Ph-C*H*$_2$, H-5a), 1.78 (*tt*, $J = 7.7$, 6.5 Hz, C*H*$_2$, H-5b) and 3.55 (*t*, $J = 6.5$ Hz, OC*H*$_2$, H-5c)]

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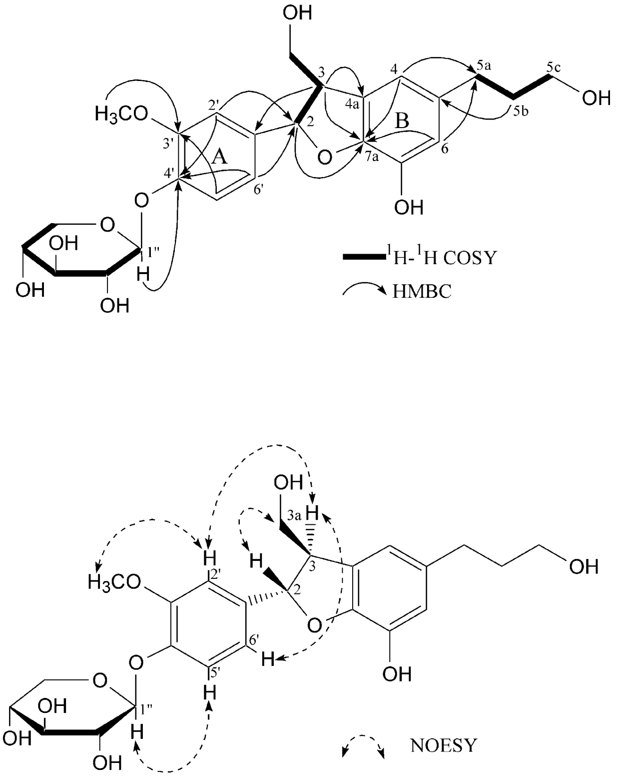


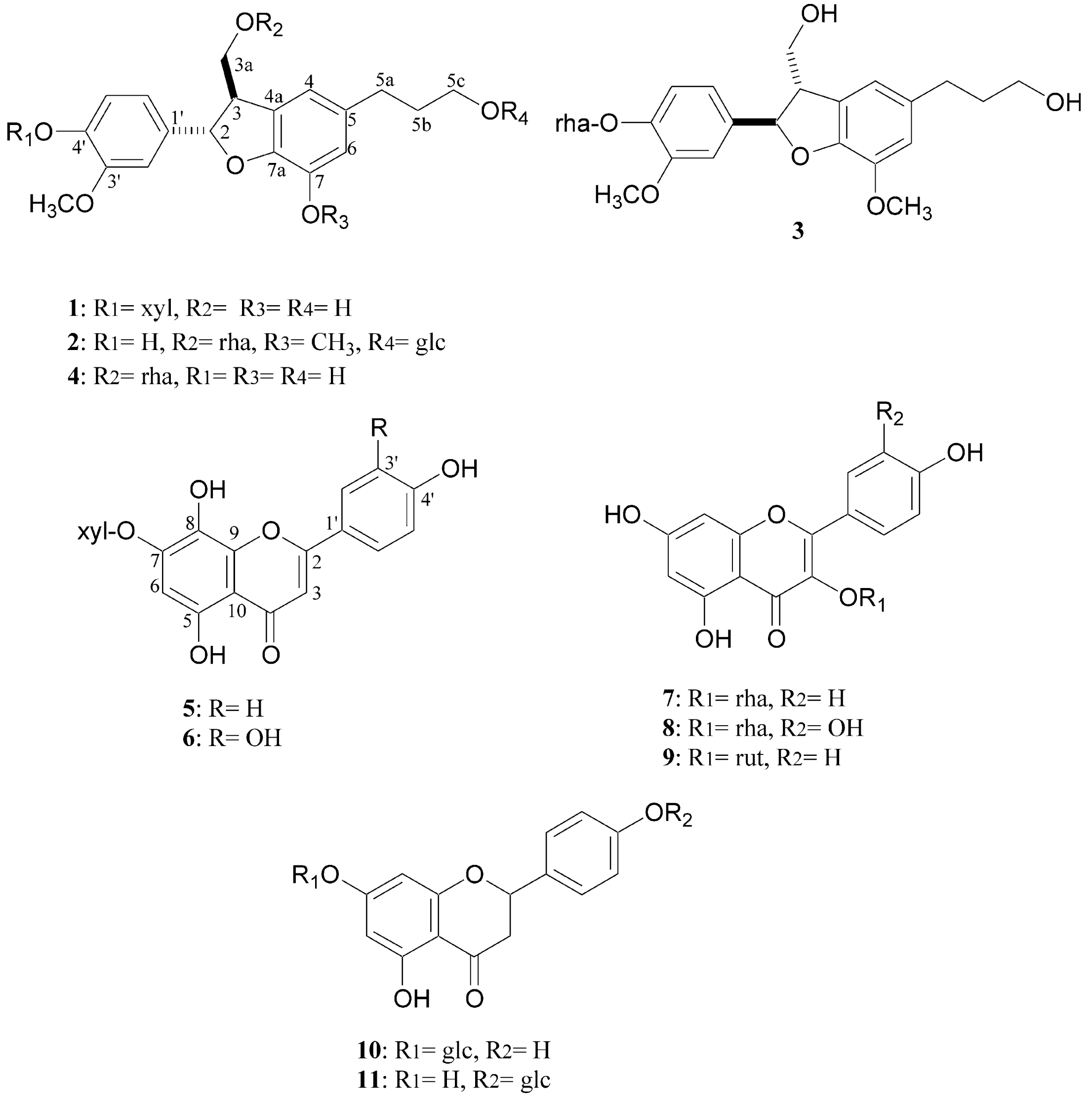
Fig. 1. Selected ^{1}H–^{1}H COSY, HMBC and NOESY correlations of **1**.

and a sequence of methine-methine-methylene [C*H*(O)-C*H*(Ph)-C*H*$_2$O] successively coupled in this order [δ 5.55 (*d*, J = 5.8 Hz, H-2), 3.44 (*br q*, J = 5.4 Hz, H-3), 3.84 (1H, *dd*, J = 11.4, 5.4 Hz, H-3a) and 3.74 (1H, *dd*, J = 11.4, 7.8 Hz, H-3a)] in addition to a methoxyl group (δ 3.82). Furthermore, the ^{1}H and ^{13}C NMR spectral data (Tables 1 and 2) exhibited the presence of a β-xylopyranosyl moiety ($J_{1'',2''}$ = 7.8 Hz). Long-range correlations through ^3J in the HMBC spectrum (Fig. 1) were observed between each of H-2′ and H-6′/C-2 and between each of H-4a and H6/C-5a. These results indicated that **1** had two phenylpropanoid units. Significant HMBC correlations were also observed between H-2/C-7a and H-3/C-4a. Therefore, it could be concluded that two phenylpropanoids formed a 2,3-dihydro-3-hydroxymethyl-2-phenyl-5-benzofuranpropanol skeleton. The position of the methoxyl group was determined to be at C-3′ by the consideration of an NOE and HMBC correlations as drawn in Fig. 1. The NOESY (H-1″/H-5′) and HMBC correlation (H-1″/C-4′) indicated that the xylosyl moiety was connected at C-4′. The relative configuration of H-2 and H-3 was *trans* from the NOE correlations between H-2 and H-3a protons and between H-3 and H-2′, H-6′ protons (Fig. 1). The CD spectrum of **1** showed a negative Cotton effect at 227 nm ($\Delta\varepsilon$−2.53), which indicated the absolute configurations of **1** to be 2*S*, 3*R*-configurations (Lemier et al., 1995; Matsuda et al., 1996). Therefore, the structure of **1** was determined to (2*S*, 3*R*)-2,3-dihydro-7-hydroxy-3-hydroxymethyl-2-(4′-hydroxy-3′-methoxyphenyl)-5-benzofuran propanol 4′-*O*-β-xylopyronoside as drawn in Fig. 2.

Compound **2** (junipercomnoside B) obtained as a colorless amorphous powder gave an [M–H]$^-$ ion peak at *m*/*z* 667.2609 in the negative ion HRFAB-MS, corresponding to the molecular formula $C_{32}H_{44}O_{15}$. The ^{1}H NMR spectrum including ^{1}H–^{1}H COSY (Table 1) showed a partial structure of Ph-C$\underline{H}_2$C$\underline{H}_2$C$\underline{H}_2$O [δ 2.68 (2H, *m*, H-5a), 1.91 (2H, *m*, H-5b), 3.92 (2H, *dt*, J = 9.6, 6.2 Hz, H-5c)], C$\underline{H}$(O)C$\underline{H}$(Ph)C$\underline{H}_2$O [δ 5.46 (1H, *d*, J = 6.7 Hz, H-2), 3.62 (1H, H-3), 3.88 (1H, *dd*, J = 9.6, 7.2 Hz, H-3a) and 3.73 (1H, *dd*, J = 9.6, 5.4 Hz, H-3a)]. The presence of a 1,2,4-trisubstituted benzene ring [δ 6.94 (1H, *d*, J = 1.8 Hz, H-2′), 6.78 (1H, *d*, J = 8.4 Hz, H-5′) and 6.83 (1H, *dd*, J = 8.4, 1.8 Hz, H-6′)] and a 1,2,3,5-tetrasubstituted benzene ring [δ 6.73 (1H, *br s*, H-4) and 6.76 (1H, *br s*, H-6)] was suggested in addition to two methoxyl groups (δ 3.83 and 3.86) in the spectrum. The signals for an α-rhamnopyranosyl ($J_{1'',2''}$ = 1.8 Hz) and a β-glucopyranosyl ($J_{1''',2'''}$ = 7.8 Hz) moieties were also observed in the ^{1}H and ^{13}C NMR spectrum as listed in the Tables 1 and 2. The HMBC correlations (Fig. 3) indicated that **2** was a neolignan with a 2,3-dihydro-3-hydroxymethyl-2-phenyl-5-benzofuranpropanol skeleton similary as **1**. The NOESY correlations in **2** (Fig. 3) indicated that the two methoxyl groups were substituted at C-3′ and C-7, and the configuration of dihydrofuran ring was *trans*. In the comparison with **1**, the signal of C-5c in the ^{13}C NMR spectrum was downfield shifted. This result suggested that one of glycosyl moieties was linked by glycosidic linkage at C-5c. Finally, the β-glucosyl and the α-rhamnosyl moieties were connected at C-5c and C-3a, respectively, by the analysis of the HMBC and NOESY spectra drawn in Fig. 3. By means of the CD spectra, the configuration of dihydrofuran ring was deduced as *2S,3R*. Consequently, **2** is (*2S,3R*) 2,3-dihydro-3-hydroxymethyl-7-methoxy-2-(4′-hydroxy-3′-methoxy-phenyl)-5-benzofuranpropanol 5c-*O*-β-glucopyranoside-3c-*O*-α-rhamnopyranoside.

A dihydrobenzofuran-type neolignan such as the aglycone of **1** (carrying 3′-OMe) or **2** (carrying 7,3′-OMe) rarely occur as its xyloside in nature. About 10 years ago, a neolignan xyloside (carrying 7,3′-di-OMe and 3a-O-xyloside) has been isolated from bark of *Ilicium difengpi* (Kouno et al., 1993) as the first example and the isolation of **1** should be the second one.

Compound **3**, a colorless amorphous powder, showed the [M–H]$^-$ ion peak at *m*/*z* 505.2072 in the negative ion HR FAB-MS, indicating the molecular formula $C_{26}H_{34}O_{10}$. Furthermore, the CD cotton curve of **3** was entirely opposite to that of **2**, suggesting that the aglycone of **3** is the enantiomer of **2**. On the basis of spectral analyses in a similar manner as in **1** and **2**, the structure of **3** was determined to (2*R*, 3*S*) 2,3-dihydro-3-hydroxymethyl-7-methoxy-2-(4′-hydroxyphenyl-3′-methoxy)-5-benzofur-



1: R1= xyl, R2= R3= R4= H
2: R1= H, R2= rha, R3= CH_3, R4= glc
4: R2= rha, R1= R3= R4= H

5: R= H
6: R= OH

7: R1= rha, R2= H
8: R1= rha, R2= OH
9: R1= rut, R2= H

10: R1= glc, R2= H
11: R1= H, R2= glc

Fig. 2. Structures of neolignan and flavonoid glycosides.

anpropanol 4′-*O*-α-rhamnopyranoside (icariside E_4) which had been first obtained from *Epimedium diphyllum* (Miyase et al., 1989). In the present paper, the complete NMR signal assignments are shown in Tables 1 and 2.

Compound **4** was obtained as a colorless amorphous powder and gave the molecular formula $C_{25}H_{32}O_{10}$ based on the HR FAB-MS study. The NMR spectroscopic data using the 2D techniques indicated that the planar structure of the aglycone is the same as that of **1**. Additionally, the NMR and FAB-MS data showed the presence of an α-rhamnopyranosyl moiety in the molecule. The NOESY correlation between H-1″ (anomeric proton)/H-3a indicated that the α-rhamnosyl moiety could be connected at C-3a in **4**. The CD data indicated that the configuration of dihydrofuran ring was *2S, 3R*. Therefore, the structure of **4** was concluded to be (2*S*, 3*R*)-2,3-dihydro-7-hydroxy-3-hydroxymethyl-2-(4′-hydroxy-3′-methoxyphenyl)-5-benzofuranpropanol 3a-*O*-α-rhamnopyranoside. The isolation of this neolignan has been originally reported from two conifers, *Larix leptoleps* (Miki et al., 1979) and *Pinus massoniana* (Lundgren et al., 1985) However, in these papers, the stereochemistry on the dihydrofuran ring has not been determined and chemical shifts without their assignment were only reported as the ^1H and ^{13}C NMR data. The complete assignments of every protons and carbons of **4** are first presented in Tables 1 and 2.

The occurrence of neolignans such as **1–4** in the genus *Juniperus* plants is unusual and this paper the second example is presented. In addition, 4′-*O*-neolignan glycosides are common but 3a- and/or 5c-*O*-glycosides such as **2** and **4** are very rare in nature. Neolignan diglycosides are also rare, because only two diglycosides have so far isolated from *Stachys riedri* (Ikeda et al., 1994) and *Alangium platanifolium* var. *trilobum* (Otsuka et al., 1996) to the best of our knowledge.

Compound **5**, a pale yellow amorphous powder, showed the [M–H]⁻ ion peak at *m*/*z* 417.0818 in negative

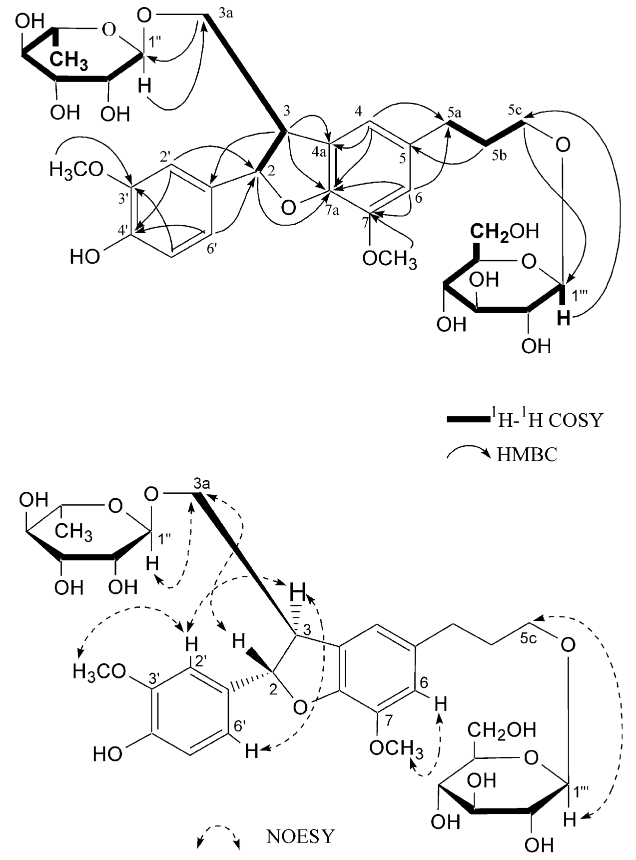


Fig. 3. Selected ^{1}H–^{1}H COSY, HMBC and NOESY correlations of **2**.

ion HRFAB-MS, indicating the molecular formula, $C_{20}H_{18}O_{10}$. The UV spectrum (278, 308 and 327 nm) showed that **5** had a flavone skeleton. The ^{1}H NMR spectrum exhibited the signals of a set of *ortho*-coupled protons in A_2X_2 system assignable to a *p*-substituted phenyl moiety [δ 6.96 (2H, *d*, J = 8.9 Hz, H-3′, 5′) and 8.00 (2H, *d*, J = 8.9 Hz, H-2′, 6′)] and two aromatic singlets (δ 6.58 and 6.83). The spectrum also showed the presence of three phenolic hydroxyl groups [δ 8.72, 10.40 and 12.40 (chelated)]. The presence of a β-xylopyranosyl moiety was confirmed by the ^{1}H and ^{13}C NMR spectrum (Table 3). A significant fragment peak *m*/*z* 285 in the negative ion FAB-MS and *m*/*z* 286 in the EI MS, which corresponded to the aglycone, indicated that **5** was a tetrahydroxyflavone xyloside. In the HMBC spectrum, the chelated hydroxyl group caused a cross peak with C-6 carbon signal which was correlated with the aromatic singlet at δ 6.58 in the C–H COSY. As a fragment peak derived from A-ring was observed at *m*/*z* 168 ($A_1^{+}\bullet$) in the EI-MS, the A-ring was characterized as 5,7,8-trihydroxyl substitution. Thus the aglycone was determined to be 5,7,8,4′-tetrahydroxyflavone (isoscutellarein). The β-xylopyranosyl moiety was connected at C-7 of isoscutellarein as evidenced by an NOE (H-6/H-1″) and a HMBC correlation (H-1″/C-7) (Fig. 4). Finally the structure of **5** was proposed as isoscutellarein 7-*O*-β-xylopyranoside.

Compound **6**, a pale yellow amorphous, showed the $[M–H]^-$ ion peak at *m*/*z* 433.0769 in the negative ion HRFAB-MS, indicating the molecular formula, $C_{20}H_{18}O_{11}$. The spectral data (UV, ^{1}H and ^{13}C NMR: Table 3) indicated that **6** was a flavone xylopyranoside similar to **5**. Although the A-ring moiety of **6** was superimposed to that of **5**, the B-ring of **6** was observed as an ABX system in the ^{1}H NMR spectrum, which indicated that the B ring substitution was a 3′,4′-dihydroxyl pattern. Accordingly, the structure of **6** was determined to be hypolaetin (5,7,8,3′,4′-pentahydroxyflavone) 7-*O*-β-xylopyranoside. These two xylosides have been isolated from a New Zealand conifer, *Libocedrus bedwilli* (Markham et al., 1990). The occurrence of scutellarein (5,6,7,4′-tetrahydroxyflavone) 7-*O*-β-xylopyranoside, an isomer of **5**, was reported in *J. communis*. (Lamer-Zarawska, 1977). Our attempt to isolate the scutellarein xyloside was unsuccessful. It is possible that scutellarein 7-*O*-β-xylopyranoside reported in *J. communis* was actually **5** since the structure of scutellarein xyloside has been determined only by the result of shift reagents and UV spectra.

The flavonoids **7–11** were identified as kaempferol 3-*O*-αrhamnopyranoside (**7**), quercitrin (**8**), nicotiflorin (**9**), naringenin 7-*O*-β-glucopyranoside (**10**) and naringenin 4′-*O*-β-glucopyranoside (**11**), respectively by the analysis of spectral data.

Biflavones are well known as important chemotaxonomic markers for the classification of gymnosperms. Markham et al. (1990) pointed out that the occurrence of 8-hydroxyflavone glycoside such as **5** and **6** were very useful chemotaxonomic markers. Lamer-Zarawska (1980) isolated hypolaetin 7-*O*-glucoside from *J. communis*. In our idea, the presence of polyhydroxyflavones such as **5** and **6** was estimated as clue to the classification of genus *Juniperus*.

The isolation of other glycosides from the MeOH extract of *J. communis* var. *depressa* and the biological activities of the isolated compounds are now investigated. Attempts to isolate neolignans and flavonoids from other *Juniperus* plants are also in progress from the viewpoint of chemotaxonomic interest.

3. Experimental

3.1. General

^{1}H and ^{13}C NMR spectra were measured on a GE-Omega 600 (^{1}H at 600 MHz and ^{13}C at 150 MHz) or JEOL JNM-GX 400 (^{1}H at 400 MHz and ^{13}C at 100 MHz) spectrometers. Chemical shifts were given in δ values (ppm) relative to trimethylsilane (TMS) as an internal reference. FAB and HR-FAB-MS spectra in a

Table 1
^{1}H NMR spectral data of **1–4**[a]

	1	**2**	**3**	**4**
2	5.55 (*d*, 5.8)	5.46 (*d*, 6.7)	5.55 (*d*, 5.9)	5.44 (*d*, 6.5)
3	3.44 (*br q*, 5.4)	3.62[b]	3.46 (*br q*, 5.4)	3.61[b]
3a	3.84 (*dd*, 11.4, 5.4)	3.88 (*dd*, 9.6, 7.2)	3.85 (*dd*, 11.4, 5.4)	3.88 (*dd*, 10.2, 7.8)
	3.74 (*dd*, 11.4, 7.8)	3.73 (*dd*, 9.6, 5.4)	3.75 (*dd*, 11.4, 7.8)	3.71 (*dd*, 10.2, 5.1)
4	6.59 (*br s*)	6.73 (*br s*)	6.72 (*br s*)	6.54 (*br s*)
5a	2.55 (*t*, 7.7)	2.68 (*m*)	2.62 (*t*, 7.7)	2.56 (*t*, 7.4)
5b	1.78 (*tt*, 7.7, 6.5)	1.91 (*m*)	1.81 (*tt*, 7.7, 6.5)	1.80 (*tt*, 7.4, 6.3)
5c	3.55 (*t*, 6.5)	3.92 (*dt*, 9.6, 6.2)	3.56 (*t*, 6.5)	3.55 (*t*, 6.3)
6	6.57 (*br s*)	6.76 (*br s*)	6.73 (*br s*)	6.55 (*br s*)
2′	7.06 (*d*, 1.8)	6.94 (*d*, 1.8)	7.03 (*d*, 1.8)	6.96 (*d*, 1.8)
5′	7.05 (*d*, 8.4)	6.78 (*d*, 8.4)	7.08 (*d*, 8.4)	6.76 (*d*, 8.4)
6′	6.94 (*dd*, 8.4, 1.8)	6.83 (*dd*, 8.4, 1.8)	6.91 (*dd*, 8.4, 1.8)	6.84 (*d*, 8.4, 1.8)
7-OCH_3		3.86 (*s*)	3.86 (*s*)	
3′-OCH_3	3.82 (*s*)	3.83 (*s*)	3.80 (*s*)	3.64 (*s*)
Xyl 1	4.85 (*d*, 7.8)			
2	3.48 (*dd*, 7.8, 9.0)			
3	3.42 (*dd*, 9.0, 9.0)			
4	3.56 (*ddd*, 9.0, 10.2, 5.2)			
5	3.89 (*dd*, 11.1, 5.2)			
	3.29 (*dd*, 11.1, 10.2)			
Glc 1		4.26 (*d*, 7.8)		
2		3.20 (*dd*, 7.8, 9.0)		
3		3.36 (*dd*, 9.0, 9.0)		
4		3.29 (*dd*, 9.0, 9.0)		
5		3.20 (ddd, 9.0, 5.4, 2.4)		
6		3.86 (*dd*, 12.0, 2.4)		
		3.67 (*dd*, 12.0, 5.4)		
Rha 1		4.73 (*d*, 1.8)	5.34 (*d*, 1.8)	4.73 (*d*, 1.8)
2		3.81 (*dd*, 1.8, 3.6)	4.06 (*dd*, 1.8, 3.6)	3.81 (*dd*, 1.8, 3.6)
3		3.60 (*dd*, 3.6, 9.6)	3.87 (*dd*, 3.6, 9.6)	3.62 (*dd*, 3.6, 9.6)
4		3.37 (*dd*, 9.6, 9.6)	3.45 (*dd*, 9.6, 9.6)	3.37 (*dd*, 9.6, 9.6)
5		3.52 (*dd*, 9.6, 6.0)	3.80[b]	3.56[b]
6		1.24 (*d*, 6.0)	1.21 (*d*, 6.0)	1.25 (*d*, 6.0)

Measured in MeOH-d_4.
[a] 600 MHz.
[b] Overlapping with other signals.

negative mode (matrix; triethanolamine except for compound **4**; compound **4** matrix: glycerine), along with EI-MS spectra, were obtained with a JEOL JMS-700T spectrometer. UV spectra were recorded on a UV-2200 spectrophotometer (Shimadzu) and optical rotations were measured on JASCO DIP-140 polarimeter. CD spectra were recorded on a JASCO J-820 spectropolarimeter. For cc, Silica gel 60 (70-230 mesh, Merck), Chromatorex ODS DM1020T (Fuji Silysia), and Sephadex LH-20 (Pharmacia Fine Chemicals) were used. Kiesel gel 60 F_{254} (Merck) was used for analytical TLC. Preparative HPLC was performed on a JAI LC-908 instrument (columns: JAIGEL GS-310, JAIGEL ODS).

3.2. Plant material

Twigs with leaves of *J. comnis* var. *depressa* were collected in July 1997, in Oregon State, USA. Voucher specimens (No. 053) were deposited in the Herbarium, Botanical Gardens, the University of Tokyo (TI), Japan.

3.3. Extraction and isolation

The dried materials (2.4 kg) were extracted with MeOH (3×18 l, weekly) at room temp. The MeOH solution was evaporated *in vacuo* to give a dark greenish extract (488 g). An aliquot of the extract (202 g) was partitioned with *n*-hexane and MeOH. The MeOH soluble part was further partitioned between *n*-BuOH and water. The resulting *n*-BuOH extract (75.7 g) was applied to silica gel eluted with $CHCl_3$–MeOH–H_2O (7:3:1, lower phase) to give 10 fractions (A–J). Fraction F (6.2 g) was further purified by silica gel cc [$CHCl_3$–MeOH–H_2O (9:3:1, lower phase)] followed by Sephadex LH 20 cc (MeOH) to afford two fractions. The fraction containing flavonoids was further purified by preparative HPLC separation (JAIGEL-GS column; eluting with MeOH) to afford **5** (8.2 mg) and **7** (25.2 mg).

Table 2
^{13}C NMR spectral data of **1–4**

No.	**1**[a]	**2**[a]	**3**[a]	**4**[b]
2	88.3	89.5	88.6	89.2
3	56.0	52.8	55.7	53.0
3a	65.2	70.5	65.1	70.2
4	116.7	117.9	118.0	116.4
4a	129.6	129.6	129.7	129.4
5	137.0	137.0	137.1	134.8
5a	32.7	32.9	32.9	32.7
5b	35.8	33.0	35.8	35.7
5c	62.4	70.0	62.3	62.2
6	117.1	114.5	114.3	116.1
7	142.0	145.3	145.3	141.9
7a	146.5	147.7	147.6	147.5
1′	139.0	134.6	138.9	136.8
2′	111.3	110.6	111.4	110.4
3′	151.3	149.2	152.2	149.1
4′	147.2	147.5	146.6	146.4
5′	118.6	116.2	119.7	117.1
6′	119.3	119.9	119.2	119.8
7-OCH$_3$		56.5	56.5	
3′-OCH$_3$	56.7	56.9	56.9	56.4
Xyl 1	103.6			
2	74.7			
3	77.4			
4	71.1			
5	66.9			
Glc 1		104.5		
2		75.2		
3		78.2		
4		71.8		
5		78.0		
6		62.9		
Rha 1		101.8	101.5	101.7
2		72.2	72.1	72.2
3		72.5	72.3	72.5
4		73.9	73.9	73.8
5		70.3	70.9	70.4
6		18.1	18.0	18.0

Measured in MeOH-d_4.
[a] 150 MHz.
[b] 100 MHz.

Table 3
^{1}H and ^{13}C NMR spectral data of **5** and **6**

No.	**5**		**6**	
	δ_{H}[a]	δ_{C}[b]	δ_{H}[c]	δ_{C}[d]
2		164.0		164.1
3	6.83 (*s*)	102.6	6.72 (*s*)	102.6
4		182.3		182.1
5	12.40 (*s*, OH)	152.2	12.40 (*s* OH)	152.2
6	6.58 (*s*)	98.4	6.56 (*s*)	98.3
7		150.9		150.8
8	8.72 (*s*, OH)	127.0		126.9
9		144.5		144.4
10		105.1		105.1
1′		121.2		121.5
2′	8.00 (*d*, 8.9)	128.5	7.49 (*br s*)	115.9
3′	6.96 (*d*, 8.9)	115.9		145.6
4′	10.40 (*s*, OH)	161.2		149.8
5′	6.96 (*d*, 8.9)	115.9	6.91 (*br d*, 8.3)	113.5
6′	8.00 (*d*, 8.9)	128.5	7.48 (*br d*, 8.3)	119.1
Xyl 1″	5.00 (*d*, 7.8)	101.5	4.96 (*d*, 7.8)	101.5
2″	3.40 (*m*) 5.42 (*d*, 4.2, OH)	72.9	3.35–3.37[e]	72.9
3″	3.29 (*ddd*, 8.7, 8.7, 4.2) 5.16 (*d*, 4.2, OH)	75.6	3.28–3.35[e]	75.5
4″	3.42 (*m*) 5.12 (*d*, 4.2, OH)	69.2	3.37–3.39[e]	69.2
5″	3.81 (*dd*, 10.8, 4.0) 3.41 (*m*)	65.7	3.80 (*br d*, 10.8) 3.37–3.35[e]	65.7

Measured in DMSO-d_6.
[a] 600 MHz.
[b] 150 MHz.
[c] 400 MHz.
[d] 100 MHz.
[e] Overlapping with other signals.

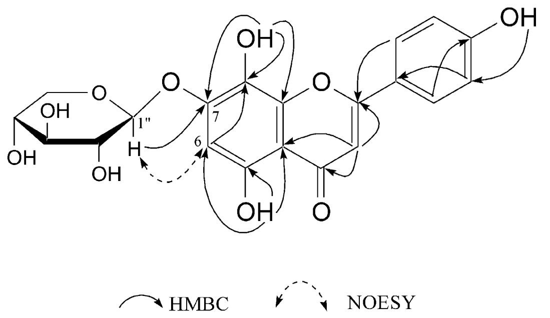


Fig. 4. Selected ^{1}H–^{1}H COSY, HMBC and NOESY correlations of **5**.

The other fraction was further purified by ODS cc (50% MeOH) and subsequent HPLC (JAIGEL-ODS column; eluting with 50% MeOH) to give **3** (135.3 mg) and **4** (3.9 mg), respectively. Fraction G (8.8 g) was repeatedly applied onto a Sephadex LH-20 column (eluting with MeOH) followed by HPLC separation (JAIGEL-GS column; eluting with MeOH) to give **8** (49.4 mg), **10** (1.5 mg) and **11** (1.5 mg), respectively. Fraction H (10.8 g) was divided into 13 sub-fractions (H-1 to H-13) by ODS cc (eluting with 50% MeOH). The sub-fraction H-6 was applied onto a Sephadex LH-20 column (eluting with 80% MeOH) to yield two fractions. The first fraction containing a flavonoid was further purified by HPLC (JAIGEL-GS column; eluting with MeOH) to give **6** (7.2 mg). The other fraction was further fractionated by ODS column (eluting successively with 50% MeOH→70% MeOH) to afford four sub-fractions. The sub-fraction 1 was further purified by HPLC (JAIGEL-ODS column; eluting with 50% MeOH) to **1** (24.1 mg) and sub-fraction 3 was purified using a Sephadex LH-20 column (eluting with MeOH) and then HPLC (JAIGEL-ODS column; eluting with 50% MeOH) to afford **2** (2.3 mg). Sub-fraction H-8 was purified by ODS cc (eluting with 50% MeOH) followed by HPLC (JAIGEL-ODS column; eluting with 50% MeOH) to give **9** (8.7 mg).

3.4. Compound **1** (junipercomnoside A)

A colorless amorphous powder; $[\alpha]_D$ −26.3° (c 0.80, MeOH); CD ($c = 7.95 \times 10^{-5}$ mol/l, MeOH) $\Delta\varepsilon$ (nm); −2.53 (227), +0.34 (246), +0.66 (292); negative ion FAB-MS m/z: 477 $[M–H]^-$, 345 $[M–H–Xyl]^-$; negative ion HRFAB-MS m/z: 477.1759 (calc. 477.1760 for $C_{24}H_{29}O_{10}$); the ^1H and ^{13}C NMR spectral data are shown in Tables 1 and 2.

3.5. Compound **2** (junipercomnoside B)

A colorless amorphous powder; $[\alpha]_D$ −14.8° (c 0.23, MeOH); CD ($c = 6.88 \times 10^{-5}$ mol/l, MeOH) $\Delta\varepsilon$ (nm); −0.69 (224), +2.31 (240), +0.98 (292); negative ion FAB-MS m/z: 667 $[M–H]^-$, 505 $[M–H–Glc]^-$; negative ion HRFAB-MS m/z: 667.2609 (calc. 667.2602 for $C_{32}H_{43}O_{15}$); the ^1H and ^{13}C NMR spectral data are shown in Tables 1 and 2.

3.6. Compound **3** (icariside E$_4$)

A colorless amorphous powder; $[\alpha]_D$ −59.2° (c 1.01, MeOH); CD ($c = 7.11 \times 10^{-5}$ mol/l, MeOH) $\Delta\varepsilon$ (nm); +0.93 (224), −1.54 (241), −0.72 (284); negative ion FAB-MS m/z: 505 $[M–H]^-$, 359 $[M–H–Rha]^-$; negative ion HRFAB-MS m/z: 505.2072 (calc. 505.2074 for $C_{26}H_{33}O_{10}$); the ^1H and ^{13}C NMR spectral data are listed in Tables 1 and 2.

3.7. Compound **4**

A colorless amorphous powder; $[\alpha]_D$ −8.0° (c 0.39, MeOH); CD ($c = 7.93 \times 10^{-5}$ mol/l, MeOH) $\Delta\varepsilon$ (nm); −0.47 (223), +0.91 (240), +1.00 (293); negative ion FAB-MS m/z: 491 $[M–H]^-$, 345 $[M–H–Rha]^-$; negative ion HRFAB-MS m/z: 491.1911 (calc. 491.1917 for $C_{25}H_{31}O_{10}$); the ^1H and ^{13}C NMR spectral data are shown in Tables 1 and 2.

3.8. Compound **5** (isoscutellarein 7-O-β-xylopyranoside)

A pale yellow amorphous powder; $[\alpha]_D$ −32.8° (c 0.32, pyridine); UV (nm, MeOH): 278, 308, 327. EIMS m/z: 286, 168; negative ion FAB-MS m/z: 417 $[M–H]^-$, 285 $[M–H–Xyl]^-$; negative ion HRFAB-MS m/z: 417.0818 (calc. 417.0822 for $C_{20}H_{17}O_{10}$); the ^1H and ^{13}C NMR spectral data are shown in Table 3.

3.9. Compound **6** (hypolaetin 7-O-β-xylopyranoside)

A pale yellow amorphous powder; $[\alpha]_D$ −33.8° ($c = 0.15$, pyridine); UV (nm, MeOH): 273, 303, 342; EIMS m/z: 302, 168; negative ion FAB-MS m/z: 433 $[M–H]–$, 301 $[M–H–Xyl]^-$; negative ion HRFAB-MS m/z: 433.0769 (calc. 433.0771 for $C_{20}H_{17}O_{11}$); the ^1H and ^{13}C NMR spectral data are shown in Table 3.

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