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NEW NEOLIGNAN AND PHENYLPROPANOID GLYCOSIDES IN *JUNIPERUS COMMUNIS* VAR. *DEPRESSA*

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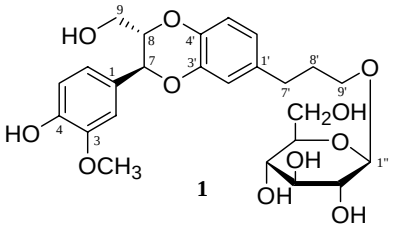
Abstract- Two new neolignan glucosides (junipercomnosides C and D) and two new phenypropanoid glycosides (junipercomnosides E and F) were isolated from aerial parts of *Juniperus communis* var. *depressa* along with seven known phenylpropanoid glycosides. The structures of the isolated compounds were determined by spectral analysis, in particular by the detailed analysis of 2D NMR and CD spectra.

In the previous phytochemical studies of North American useful plants, we had already identified four neolignan glycosides and seven flavonoid glycosides from aerial parts of *Juniperus communis* var. *depressa*.[1] Upon continued chemical investigation of the aerial parts of the same plant, we isolated two new neolignan glucosides (**1** and **2**) and nine phenylpropanoids (**3**-**11**) including two new phenylpropanoid glycosides (**8** and **10**). In this paper we describe the isolation and the structure determination of these compounds.

The n-BuOH soluble part obtained from the methanol extract was separated on silica gel and ODS column chromatography, followed by HPLC separation to isolate each compound.

RESULTS AND DISCUSSION

Compound (**1)** (junipercomnoside C), white powder, showed the $[M-H]^-$ ion peak at *m/z* 507.1864 in the negative ion HR FAB–MS, indicating the molecular formula to be $C_{25}H_{32}O_{11}$. The 1D ^1H NMR and 2D



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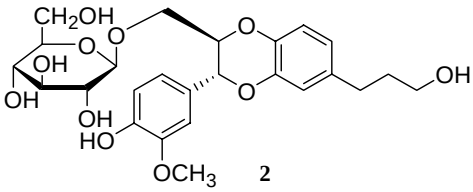

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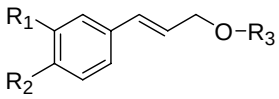
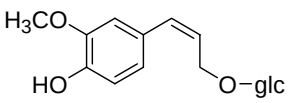
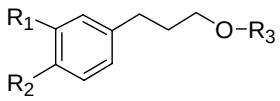


3: R_1=R_2=H
R_3=glc
4: R_1=R_2=H
R_3=glc-^{6}ara (*f*)
5: R_1=R_2=H
R_3=glc-^{6}ara (*p*)
6: R_1=R_2=H
R_3=glc-^{6}xyl
7: R_1=OMe, R_2=OH
R_3=glc
8: R_1=OMe, R_2=OH
R_3=glc-^{6}ara (*f*)



9



10: R_1=R_2=H
R_3=glc-^{6}ara (*f*)
11: R_1=OMe, R_2=OH
R_3=glc

Figure 1

^{1}H-^{1}H COSY spectra (Table 1) showed the presence of two 1, 2, 4-trisubstituted benzene rings [δ 7.00 (1H, d, *J* = 2.0 Hz, H-2), 6.83 (1H, d, *J* = 8.2 Hz, H-5), 6.89 (1H, dd, *J* = 8.2, 2.0 Hz, H-6) ; δ 6.85 (1H, d, *J* = 8.2 Hz, H-5'), 6.77 (1H, d, *J* = 2.0 Hz, H-2'), 6.71 (1H, dd, *J* = 8.2, 2.0 Hz, H-6')], a hydroxypropyl group [δ 2.63 (2H, t, *J* = 7.2 Hz, Ph-CH_2, H-7'), 1.88 (2H, m, CH_2, H-8'), 3.90, 3.54 (1H each, dt, *J* = 9.6, 6.6 Hz, OCH_2, H-9')], a sequence of methine-methine-methylene [Ph-CH(O)-CH(O)-CH_2(O)] successively coupled in this order [δ 4.86 (1H, d, *J* = 8.0 Hz, H-7), 4.00 (1H, m, H-8), 3.67 (1H, dd, *J* = 12.0, 2.7 Hz, H-9) and 3.47 (1H, dd, *J* = 12.0, 4.7 Hz, H-9)] and a methoxyl group (δ 3.82) (Table 1). In addition, characteristic signals due to a β-glucopyranosyl group were observed in both of the ^{1}H and ^{13}C NMR spectra [δ_H 4.24 ($J_{1'', 2''}$ = 7.8 Hz, anomeric H) : δ_C 104.5, 75.2, 78.2, 71.6, 78.0, 62.8]. Long range correlations through ^2J and ^3J observed between H-2/C-7, H-6/C-7 and H-8'/C-1' in the HMBC spectrum (Figure 2) indicated that **1** had two phenylpropanoid units. Furthermore significant HMBC correlation observed between H-7/C-3'

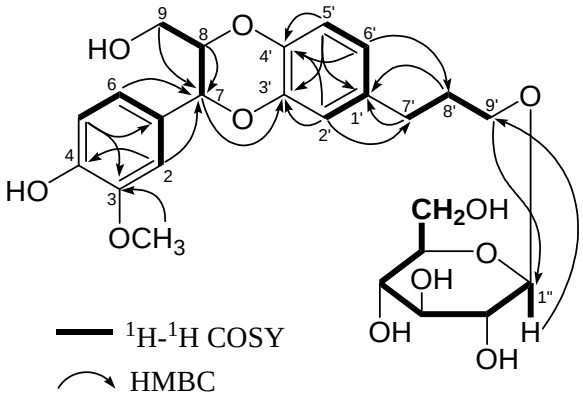


Figure 2. ^{1}H-^{1}H COSY and key HMBC correlations of **1**.

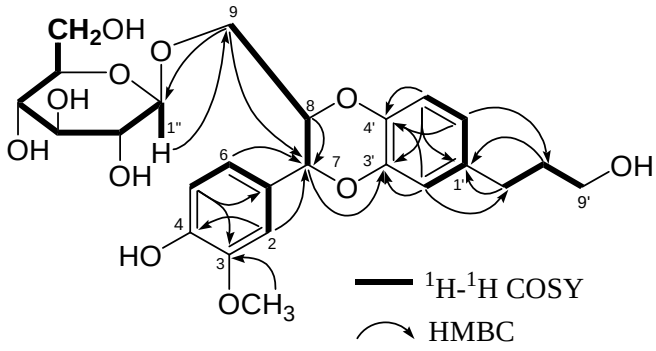


Figure 3. ^{1}H-^{1}H COSY and key HMBC correlations of **2**.

Table 1. ^{1}H and ^{13}C NMR spectral data of **1**, **1a** and **2**

	1		**1a**		**2**	
NO.	δ_H [a)]	δ_C [b)]	δ_H [c)]	δ_C [d)]	δ_H [a)]	δ_C [b)]
1		129.8		129.7		129.8
2	7.00 (d, 2.0)	112.2	6.99 (d, 2.0)	112.0	7.12 (d, 2.0)	112.5
3		149.3		149.2		149.1
4		148.4		148.3		148.2
5	6.83 (d, 8.2)	116.3	6.83 (d, 8.2)	116.3	6.81 (d, 8.3)	116.3
6	6.89 (dd, 8.2, 2.0)	121.7	6.89 (dd, 8.4, 2.0)	121.7	6.96 (dd, 8.3, 2.0)	121.6
7	4.86 (d, 8.0)	77.8	4.86 [e)]	77.7	5.09 (d, 8.0)	77.3
8	4.00 (m)	79.9	4.00 (ddd, 8.0, 4.4, 2.8)	79.9	4.15 (m)	78.8
9	3.67 (dd, 12.0, 2.7)	62.3	3.67 (dd, 12.0, 2.8)	62.2	4.14 (dd, 11.5, 2.3)	69.5
	3.47 (dd, 12.0, 4.7)		3.46 (dd, 12.0, 4.4)		3.37 (dd, 11.5, 3.8)	
1'		136.5		136.5		136.5
2'	6.77 (d, 2.0)	117.9	6.75 (d, 2.0)	117.8	6.75 (d, 2.0)	117.8
3'		145.1		145.1		145.1
4'		143.0		143.0		142.9
5'	6.85 (d, 8.2)	117.7	6.83 (d, 8.4)	117.8	6.83 (d, 8.0)	117.6
6'	6.71 (dd, 8.2, 2.0)	122.6	6.70 (dd, 8.4, 2.0)	122.4	6.69 (d, 8.0, 2.0)	122.4
7'	2.63 (t, 7.2)	32.5	2.58 (t, 7.6)	32.4	2.58 (t, 7.5)	32.4
8'	1.88 (m)	32.8	1.79 (m)	35.7	1.79 (m)	35.6
9'	3.90 (dt, 9.6, 6.6)	70.0	3.55 (t, 6.8)	62.2	3.55 (t, 6.5)	62.2
	3.54 (dt, 9.6, 6.6)					
-OCH_3	3.82 (s)	56.5	3.86 (s)	56.5	3.88 (s)	56.7
Glc 1"	4.24 (d, 7.8)	104.5			4.15 (d, 7.8)	105.0
2"	3.19 (dd, 9.0, 7.8)	75.2			3.25 (dd, 9.0, 7.8)	75.1
3"	3.35 (dd, 9.0, 9.0)	78.2			3.34 (dd, 9.0, 9.0)	78.0
4"	3.29 (dd, 9.0, 9.0)	71.6			3.30 (dd, 9.0, 9.0)	71.5
5"	3.24 (ddd, 9.0, 5.9, 2.4)	78.0			3.18 (ddd, 9.0, 5.9, 2.4)	77.9
6"	3.85 (dd, 12.0, 2.4)	62.8			3.79 (dd, 12.0, 2.4)	62.6
	3.66 (dd, 12.0, 5.9)				3.64 (dd, 12.0, 5.9)	

d_4-MeOH. a) Measured at 600 MHz b) Measured at 150 MHz c) Measured at 400 MHz d) Measured at 100 MHz
e) Overlapping with other signals.

suggested that the two phenylpropanoids formed a 1, 4-benzodioxane skeleton. The located position of methoxyl group was determined to be at C-3 on the basis of a NOESY correlation (H-2/OCH_3) and HMBC correlation drawn in Figure 2. The NOESY (H-1"/H_2-9') and HMBC (H-1"/C-9') correlations indicated that the β-glucosyl moiety was connected at C-9'. The absolute configuration of β-glucopyranose was determined to D.[2] Thus, the structure of **1** was determined as 3-methoxy-3',7-epoxy-8,4'-oxyneoligna-4,9-diol 9'-*O*-β-D-glucopyranoside except for the absolute structures of C-7 and C-8.

Compound (**2)** (junipercomnoside D), white powder, showed the $[M-H]^-$ ion peak at *m/z* 507.1873 in the

negative ion HR FAB–MS, corresponding to the molecular formula of $C_{25}H_{32}O_{11}$ which is the same as that of **1**. The ^{1}H and ^{13}C NMR spectral data (Table 1) were closely similar to those of **1** except for H-9 (C-9) and H-9' (C-9'). 2D NMR analysis [HMBC (Figure 3) and NOESY spectra] similar to that in **1** indicated that the planar structure of the aglycone part of **2** (= 3-methoxy-3', 7-epoxy-8, 4'-oxyneoligna-4, 9-diol) was the same as that of **1** and the located position of a β-glucose on the aglycone was at C-9 instead of C-9' in **1**. The absolute configuration of a β-glucopyranose in **2** was determined to D by the same manner in **1**.

The absolute structures of the aglycone part in both **1** and **2** were elucidated as follows. The coupling constant of H-7/H-8 (J = 8.0 Hz) and the NOESY correlations (H-7/H_2-9, H-2/H-8 and H-6/H-8) were observed in common in **1** and **2**. These data suggested that the relative configuration of H-7/H-8 was *trans* in both of **1** and **2**. In the CD spectral investigation, **1** gave a positive signed Cotton effect at 233 nm (Δε +0.73) and contrary to this, **2** showed a negative Cotton effect at 235 nm (Δε –3.58). According to the report,[3] the absolute stereochemistry of C-8 in **1** and **2** could be determined to *S* and *R*, respectively. Consequently, **1** and **2** are defined as (7*S*,8*S*)-3-methoxy-3',7-epoxy-8,4'-oxyneoligna-4,9-diol 9'-*O*-β-D-glucopyranoside and (7*R*,8*R*)-3-methoxy-3',7-epoxy-8,4'-oxyneoligna-4,9'-diol 9-*O*-β-D-glucopyranoside, respectively. 9'-*O*-Rhamnoside of aglycone in **1** was isolated from the leaves of *J. chinensis* var. *kaizuka*.[4]

On the way of the structural elucidation of **1**, the genuine aglycone (**1a**) (Table 1) was obtained by an enzymatic hydrolysis and it showed interesting chiroptical properties. That is, **1a** showed a specific rotation of ± 0^{o} (c = 0.37, MeOH) but the CD Cotton curve of **1a** in the region 200-250 nm was the same as that of **1** in the signs and the wavelengths of the maxima. This evidence demonstrated **1a** is obviously optically active and not racemate. On the other hand, some of 1,4-benzodioxane type neolignans with the *trans* 7, 8-configration which correspond to analogue of **1a** were recently isolated from a Euphorbiaceous plant but they were reported as racemic compounds.[5]

Compound (**8)** (junipercomnoside E), white powder, gave $[M-H]^{-}$ at *m/z* 473.1653 in the negative ion HR FAB–MS, indicative of the molecular formula $C_{21}H_{30}O_{12}$. The ^{1}H and ^{13}C NMR spectral data (Table 2) showed the presence of a β-glucosypyranosyl and an α-arabinofuranosyl moieties. The ^{1}H NMR and ^{1}H-^{1}H COSY spectra showed the presence of a CH=CH-CH_2(O) moiety [δ 6.54 (1H, br d, J = 16.1 Hz, H-7), 6.36 (1H, ddd, J = 16.1, 6.8, 6.3 Hz, H-8), 4.47 (1H, br dd, J = 12.9, 6.3 Hz, H-9), 4.28 (1H, br dd, J = 12.9, 6.8 Hz, H-9)] and a 1,2,4-trisubstituted benzene ring [δ 7.02 (1H, d, J = 2.0 Hz, H-2), 6.86 (1H, dd, J = 8.3, 2.0 Hz, H-6), 6.73 (1H, d, J = 8.3 Hz, H-5)]. These results indicated that **8** was a phenyl propanoid. The ^{1}H and ^{13}C NMR spectral data resembled those of *trans*-isoconiferin (**7**). In the ^{13}C NMR spectrum, signal due to C-6 of the glucose appeared in a lower filed (δ 68.1) compared with that of **7**

Table 2. ^{1}H and ^{13}C NMR spectral data of **4**, **7**, **8** and **10**

	4		**7**		**8**		**10**	
NO.	δ_H [a]	δ_C [b]	δ_H [a]	δ_C [b]	δ_H [a]	δ_C [b]	δ_H [a]	δ_C [b]
1		138.0		130.4		130.4		143.1
2	7.42 (br d, 7.3)	127.3	7.01 (d. 2.0)	110.6	7.02 (d, 2.0)	110.7	7.24 (br d, 7.2)	129.1
3	7.30 (br dd, 7.3, 7.3)	129.4		149.1		149.1	7.16 (br dd, 7.2, 6.8)	129.3
4	7.22 (br dd, 7.3, 7.3)	128.5		147.7		147.8	7.15 (br dd, 6.8, 6.8)	126.5
5	7.30 (br dd, 7.3, 7.3)	129.4	6.73 (d, 8.1)	116.2	6.73 (d, 8.3)	116.2	7.24 (br d, 7.2)	129.3
6	7.42 (br d, 7.3)	127.3	6.85 (dd, 8.1, 2.0)	121.2	6.86 (dd, 8.3, 2.0)	121.2	7.16 (br dd, 7.2, 6.8)	129.1
7	6.69 (br d, 15.9)	133.7	6.57 (br d, 15.8)	134.3	6.54 (br d, 16.1)	134.5	2.71 (t, 7.2)	32.1
8	6.39 (ddd, 15.9, 6.4, 5.9)	126.4	6.19 (ddd, 15.8, 6.8, 5.9)	123.7	6.36 (ddd, 16.1, 6.8, 6.3)	123.6	1.91 (m)	33.1
9	4.51 (ddd, 12.7, 5.9, 1.5)	70.8	4.49 (ddd, 12.5, 5.9, 1.0)	71.1	4.47 (br dd, 12.9, 6.3)	71.2	3.89 (dt, 9.2, 6.8)	70.1
	4.31 (ddd, 12.7, 6.4, 1.5)		4.29 (ddd, 12.5, 6.8, 1.0)		4.28 (br dd, 12.9, 6.8)		3.55 (dt, 9.2, 6.4)	
OCH_3			3.86 (s)	56.4	3.86 (s)	56.4		
Glc 1'	4.37 (d, 7.8)	103.2	4.36 (d, 7.8)	103.2	4.36 (d, 7.8)	103.2	4.25 (d, 7.8)	104.3
2'	3.22 (dd, 9.0, 7.8)	75.0	3.22 (dd, 9.0, 7.8)	75.2	3.21 (dd, 9.0, 7.8)	75.2	3.21 (dd, 9.0, 7.8)	75.0
3'	3.36 (dd, 9.0, 9.0)	77.9	3.36 (dd, 9.0, 9.0)	78.2	3.35 (dd, 9.0, 9.0)	78.1	3.35 (dd, 9.0, 9.0)	77.8
4'	3.31 (dd, 9.0, 9.0)	71.9	3.29 (dd, 9.0, 9.0)	71.7	3.31 (dd, 9.0, 9.0)	72.0	3.31 (dd, 9.0, 9.0)	71.8
5'	3.45 (ddd, 9.0, 6.1, 2.4)	76.7	3.29 [c]	78.0	3.45 (ddd, 9.0, 5.9, 2.4)	76.8	3.45 (ddd, 9.0, 5.9, 2.4)	76.6
6'	4.04 (dd, 11.0, 2.4)	68.0	3.88 (dd, 11.7, 2.0)	62.8	4.03 (dd, 11.2, 2.4)	68.1	4.03 (dd, 11.2, 2.4)	68.0
	3.63 (dd, 11.0, 6.1)		3.68 (dd, 11.7, 5.4)		3.63 (dd, 11.2, 5.9)		3.63 (dd, 11.2, 5.9)	
Ara(f) 1"	4.99 (d, 1.5)	109.8			4.99 (d, 1.5)	110.0	4.96 (d, 1.5)	109.8
2"	4.01 (dd, 3.4, 1.5)	83.2			4.01 (dd, 3.2, 1.5)	83.3	4.01 (dd, 3.2, 1.5)	83.1
3"	3.83 (dd, 5.7, 3.4)	78.8			3.83 (dd, 6.0, 3.2)	79.0	3.83 (dd, 6.0, 3.2)	78.8
4"	3.98 (ddd, 5.7, 5.7, 3.4)	85.8			3.99 (ddd, 6.0, 5.6, 3.4)	85.9	3.99 (ddd, 6.0, 5.6, 3.6)	85.7
5"	3.74 (dd, 12.0, 3.4)	63.0			3.74 (dd, 12.0, 3.4)	63.1	3.73 (dd, 12.0, 3.6)	62.9
	3.63 (dd, 12.0, 5.7)				3.63 [c]		3.63 (dd, 12.0, 5.6)	

d_4-MeOH a) Measured at 400 MHz b) Measured at 100 MHz c) Overlapping with other signals.

(δ 62.8), which indicated that the α-L-arabinofuranosyl moiety was linked at C-6 of the glucosyl group. The absolute configurations of a β-glucopyranose and an α-arabinofuranose were determined to D and L, respectively as described in the experimental part. Thus the structure of **8** was concluded to be *trans*-coniferyl alcohol 9-*O*-(6'-*O*-α-L-arabinofuranosyl)-β-D-glucopyranoside.

Compound (**10)** (junipercomnoside F), white powder, gave $[M-H]^-$ at *m/z* 429.1757 in the HR FAB–MS corresponding to the molecular formula $C_{20}H_{30}O_{10}$. The 1D ^{1}H and 2D ^{1}H-^{1}H COSY spectra showed the presence of a phenyl group and an oxypropyl group [$CH_2CH_2CH_2(O)$]. The ^{1}H and ^{13}C NMR spectral data also showed the presence of two sugars, a β-glucopyranose and an α-arabinofuranose. The ^{13}C NMR spectral data on the sugar moiety were superimposed on that of rosarin (**4**) and **8**. The absolute configurations of a β-glucopyranose and an α-arabinofuranose were confirmed to D and L, respectively as described in the experimental part. Thus the structure of **10** was determined as dihydrocinnamyl alcohol 9-*O*-(6'-*O*-α-L-arabinofuranosyl)-β-D-glucopyranoside (dihydrorosarin).

Compounds (**3)**-(**7)**, (**9)** and **(11)** were characterized as cinnamylalcohol 9-*O*-β-D-glucopyranoside (rosin) (**3**), cinnamylalcohol 9-*O*-(6'-*O*-α-L-arabinofuranosyl)-β-D-glucopyranoside (rosarin) (**4**), cinnamyl alchohol 9-*O*-(6'-*O*-α-L-arabinopyranosyl)-β-D-glucopyranoside (rosavin) (**5**), cinnamyl alcohol 9-*O*-(6'-*O*-β-D-xylopyranosyl)-β-D-glucopyranoside (**6**), *trans*-isoconiferin (**7**), *cis*-isoconiferin (**9**), and dihydroconiferyl alcohol 9-*O*-β-D-glucopyranoside (**11**) by comparison of the spectral data.

Although compounds (**3-5**) were isolated from *Rhodiola rosea*,[6] **3** and **5** are firstly isolated from genus *Juniperus* plants.

Other many phenolic glycosides are observed in the MeOH extract and the isolation of these compounds is now in progress.

EXPERIMENTAL

General Method

^{1}H and ^{13}C NMR spectra were measured on a GE-Omega 600 or JEOL JNM-ECA 600 (^{1}H at 600 MHz and ^{13}C at 150 MHz) spectrometers. Chemical shifts were given in δ values (ppm) relative to tetramethylsilane (TMS) as an internal reference. FAB- and HR FAB-MS spectra in a negative mode (matrix; triethanolamine), along with EI- and HR 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 column chromatography, Silica gel 60 (70-230 mesh, Merck), Chromatorex ODS DM1020T (Fuji Silysia), Sephadex LH-20 (Pharmacia Fine Chemicals) were used. Kiesel gel 60 F254 (Merck) was used for analytical TLC. Preparative HPLC was performed on a JAI LC-908 instrument (columns: JAIGEL GS-310, JAIGEL ODS, TSKgel ODS-80Ts).

Plant Material. Twigs with leaves of *Juniperus communis* var. *depressa* were collected in July 1997 at Oregon State, USA. The voucher specimen (No. 053) has been deposited in the Herbarium, Botanical Gardens, the University of Tokyo (TI), Japan.

Extraction and Isolation. The dried materials (2.4 kg) were extracted with MeOH (18 L x weekly, 3 times) at rt. The MeOH solution was evaporated *in vacuo* to give dark greenish extract (488 g). An aliquot of the extract (202 g) was partitioned with n-hexane and MeOH. The MeOH soluble part (130 g) was further partitioned between n-BuOH and water. The resulting n-BuOH extract (76 g) was chromatographed on silica gel eluted with $CHCl_3$-MeOH-H_2O (7 : 3 : 1, lower phase) to be divided into 10 fractions (A-J). The fraction F (6.2 g) was further purified by silica gel column chromatography [$CHCl_3$-MeOH-H_2O (9 : 3 : 1, lower phase)] followed by Sephadex LH-20 column chromatography (eluting with MeOH) and ODS column chromatography (eluting with 50 % MeOH) to be divided into three sub-fractions. The sub-fraction 1 was further purified by HPLC (JAIGEL-GS column; eluting with 50% MeOH) to give **11** (9.1 mg). The sub-fraction 2 was further purified by HPLC (JAIGEL-ODS column; eluting with 50% MeOH) to give **3** (8.4 mg). The fraction G (8.8 g) was applied onto Sephadex LH-20 column (eluting with MeOH) followed by ODS column chromatography (eluting successively with 50 % MeOH, 70% MeOH and MeOH) to be divided into 5 sub-fractions (G-1 to G-5). The sub-fraction G-3 was subjected to ODS column chromatography (eluting with 50% MeOH) and HPLC (JAIGEL-GS column ; eluting with 50% MeOH), followed by HPLC (TSKgel ODS-80Ts column ;

eluting with 50% MeOH) to afford **7** (34.6 mg) and **9** (4.2 mg) in that order. The sub-fraction G-5 was subjected to ODS column chromatography (eluting with 50 % MeOH) to be divided into three sub-fraction. The sub-fraction 1 was further purified by HPLC (JAIGEL-ODS column; eluting with 50% MeOH) to give **4** (56.0 mg) and **10** (25.9 mg), respectively. The sub-fraction 2 was repeatedly chromatographed on Sephadex LH-20 column chromatography (eluting with 50% MeOH), HPLC (JAIGEL-GS column; eluting with 50% MeOH) and HPLC (TSKgel ODS-80Ts column; eluting with 60% MeOH) to give **1** (33.2 mg) and **2** (6.8 mg), respectively. The fraction H (10.8 g) was divided to 13 sub-fractions (H-1 to H-13) by ODS column chromatography (eluting with 50 % MeOH). The sub-fraction H-6 was subjected to Sephadex LH-20 (eluting with 80 % MeOH) to be divided into the first and the second fractions. The second fraction was further fractionated by ODS column (eluting successively with 50% MeOH and 70% MeOH) to be divided into four sub-fractions, the sub-fraction 2 of which was further purified by ODS column chromatography (eluting with 50 % MeOH) to give **6** (16.4 mg) and other fraction, respectively. The other fraction purified by HPLC (JAIGEL-GS column; eluting with MeOH) to give **5** (23.3 mg). The sub-fraction H-8 was purified by ODS column chromatography (eluting with 50 % MeOH) to be divided into six sub-fraction. The sub-fraction 3 was further purified by HPLC (JAIGEL-GS column; eluting with 50% MeOH) to give **8** (8.1mg).

Compound (1) (junipercomnoside C). White powder ; $[\alpha]_D$ -5.0^o (c = 0.72, MeOH); CD (*c* = 7.2 x 10^{-5} mol/L, MeOH) $\Delta\varepsilon$ (nm): +1.24 (203), -3.84 (210), +0.73 (233) ; negative ion FAB–MS *m/z* : 507 $[M-H]^-$; negative ion HR FAB–MS *m/z* : 507.1864 (calcd. 507.1866 for $C_{25}H_{31}O_{11}$) ; the ^1H and ^{13}C NMR spetral data are shown in Table 1.

Enzymatic Hydrolysis of 1. Compound (**1**) (9.3 mg) was hydrolyzed with β-glucosidase (10 mg, Sigma Chemical Co.) in 2ml of H_2O at 37 ^oC for 14 hr. The reaction mixture was extracted with EtOAc. The EtOAc layer was washed with brine, dried over $MgSO_4$ and evaporated to give the corresponding aglycone (**1a**, 6.7 mg). Compound (**1a**) : White powder ; $[\alpha]_D \pm 0^o$ (*c* = 0.37, MeOH) ; CD (*c* = 1.15 x 10^{-4} mol/L, MeOH) $\Delta\varepsilon$ (nm) : +2.69 (203), –2.44 (209), +1.96 (235) ; EI-MS *m/z* : 346 $[M]^+$; HR EI–MS *m/z* : 346.1422 (calcd. 346.1406 for $C_{19}H_{22}O_6$) ; the ^1H and ^{13}C NMR spectral data are shown in Table 1.

Compound (2) (junipercomnoside D). White powder ; $[\alpha]_D$ $+16.9^o$ (*c* = 0.49, MeOH) ; CD (*c* = 7.80 x 10^{-5} mol/L, MeOH) $\Delta\varepsilon$ (nm) : –4.35 (203), +5.56 (209), –3.58 (235) ; negative ion FAB–MS *m/z* : 507 $[M-H]^-$; negative ion HR FAB-MS *m/z* : 507.1873 (calcd. 507.1866 for $C_{25}H_{31}O_{11}$) ; the ^1H and ^{13}C NMR spectral data are shown in Table 1.

Compound (8) (junipercomnoside E). White powder ; $[\alpha]_D$ -45.3^o (*c* = 0.47, MeOH) ; negative ion FAB-MS *m/z* : 473 $[M-H]^-$, 341 $[M-H-Ara]^-$, 179 $[M-H-Ara-Glc]^-$; negative ion HR FAB–MS *m/z* : 473.1653 (calcd. 473.1659 for $C_{21}H_{29}O_{12}$) ; the ^1H and ^{13}C NMR spectral data are listed in Table 2.

Compound (10) (junipercomnoside F). White powder ; $[\alpha]_D$ −40.2° (*c* = 0.47, MeOH) ; negative ion FAB-MS *m/z* : 429 $[M-H]^-$, 297 $[M-H-Ara]^-$; negative ion HR FAB–MS *m/z* : 429.1757 (calcd. 429.1761 for $C_{20}H_{29}O_{10}$) ; the ^1H and ^{13}C NMR spectral data are listed in Table 2.

Determination of Configurations of Component Sugars of 1, 2, 8 and 10. A solution of each of **1**, **2**, **8** and **10** (each 2.0 mg) in 2M HCl–EtOH (1 : 1 ; 0.5 mL) was refluxed with stirring for 2 h. The mixture solution was poured into water and extracted with EtOAc. The aqueous layer was neutralized with Amberlite IRA–93ZU and the resulting solution was evaporated and dried in *vacuo*. The residue was derived to corresponding thiazolidine derivative, followed by trimethylsilylation and GLC analysis.[2] The configurations of the glucose and the arabinose were confirmed to be D and L, respectively, by direct comparisons with D- and L- standards of both sugars.(t_R : D-Glc ; 13.25 min, L-Glc ; 13.90 min, D-Ara ; 8.22 min, L-Ara ; 7.65 min).

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REFERENCES

1. T. Nakanishi, N. Iida, Y. Inatomi, Y. Murata, A. Inada, J. Murata, F. A. Lang, M. Iinuma, and T. Tanaka, *Phytochemistry,* 2004, **65**, 207.
2. S. Hara, H. Okabe, and K. Mihashi, *Chem. Pharm. Bull.*, 1986, **34**, 1843.
3. M. Silva, J. Barbosa–Filho, M. Yoshida, and O. Gottlieb, *Phytochemistry,* 1989, **28**, 3477.
4. C.-K. Lee and Y.–S. Chen, *J. Chinese Chem. Soc*., 2001, **48**, 1077.
5. R. Waibel, G. Benirschke, M. Benirschke, and H. Achenbach, *Phytochemistry*, 2003, **62**, 805.
6. S. Y. Sokolov, V. M. Ivashin, G. G. Zapesochnaya, V. A. Kurkin, and A. N. Shchavlinskii, *Khimiko-Farmatsevticheskii Zhurnal*, 1985, **19**, 1367.