Structures and Radical-Scavenging Activities of Phenolic Constituents from the Bark of *Picea jezoensis* var. *jezoensis*

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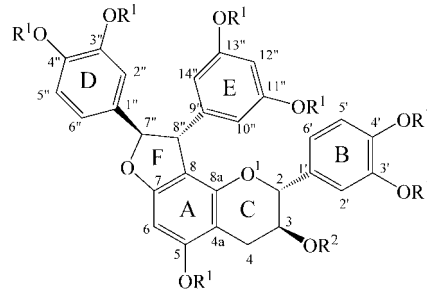
The MeOH extract of the bark of *Picea jezoensis* var. *jezoensis* was found to show scavenging activity for the DPPH radical. Bioassay-guided fractionation led to the isolation of two new flavonostilbenes named jezonocinols A (**1**) and B (**2**) and one nor-flavonostilbene named jezonocinol C (**3**) together with six known phenolic compounds. Their structures were elucidated by spectroscopic methods, and the absolute configurations of jezonocinols A (**1**) and B (**2**) were determined by Mosher's method, CD, and NOESY data.

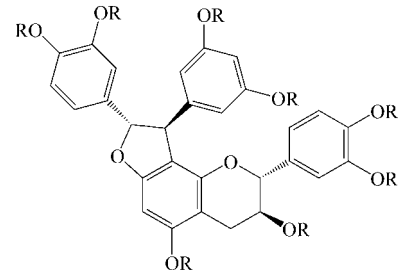
We have searched for biologically active constituents from the leaves and bark of coniferous trees that have been treated as waste in the forestry industry.[1,2] As part of this study, we have isolated triterpenoids from the $CHCl_3$ extract of the bark of *Picea jezoensis* var. *jezoensis.*[3–5] It was reported that one of these triterpenoids, 13α,14α-epoxy-3β-methoxyserratan-21β-ol, has potent antitumor-promoting activity.[6] Recently, radical scavengers have attracted special interest because they can protect the human body from damage by free radicals, which may cause diseases.[7,8] The MeOH extract from the bark of *P. jezoensis* var. *jezoensis* showed strong radical-scavenging activity on the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical. To search for the active principles in the bark of *P. jezoensis* var. *jezoensis*, we examined the chemical constituents of the MeOH extract. Bioassay-guided fractionation of the MeOH extract led to the isolation of two new flavonostilbenoids,[9] named jezonocinols A (**1**) and B (**2**), and the nor-flavonostilbene jezonocinol C (**3**), together with six known compounds. We describe in this paper the isolation and structure elucidation of **1**, **2**, and **3** and their radical-scavenging activities.



	R^1	R^2
1	H	H
1a	$COCH_3$	$COCH_3$
1b	CH_3	H
1c	CH_3	*R*-MTPA
1d	CH_3	*S*-MTPA

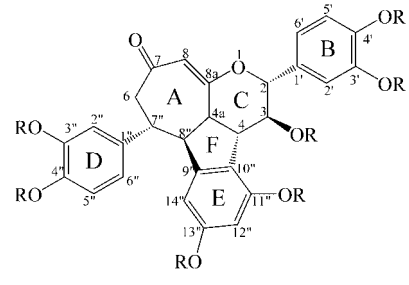
Results and Discussion

The air-dried stem bark of *P. jezoensis* var. *jezoensis* was extracted successively with $CHCl_3$ and MeOH. The MeOH extract, which had potent scavenging activity toward DPPH radicals [50% scavenging concentration (SC_{50}): ca. 20 μg/mL], was separated by a combination of normal-phase Si gel and Sephadex LH-20 gel permeation chromatography, medium-pressure liquid chromatography (MPLC), and preparative TLC followed by HPLC to afford three new flavonostilbenoids[9] (**1**, **2**, and **3**) and six known phenolic compounds. The known compounds were identified as 3,4-dihydroxybenzaldehyde,[10] 4-hydroxycinnamic acid,[11] dihydrodehydrodiconiferyl alcohol,[12] isolariciresinol,[13] protocatechuic acid,[11] and dihydroquercetin.[14]



	R
2	H
2a	$COCH_3$

Jezonocinol A (**1**) was obtained as a brown, amorphous powder and showed a protonated molecular ion peak at *m/z* 533 $[M + H]^+$ in the secondary ion MS (SIMS). Its molecular formula was determined to be $C_{29}H_{24}O_{10}$ by high-resolution SIMS analysis. The UV and IR spectra of **1** showed absorption bands at 235 and 283 nm, and 3400 br, 1621, 1522, and 1459 cm^{-1}, respectively, indicative of the presence of aromatic rings and hydroxy groups. Acetylation of **1** with acetic anhydride and pyridine afforded the octaacetate **1a**, whose *O*-acetyl resonances were observed at δ_H 2.294 (3H, s), 2.279 (3H, s), 2.278 (3H, s), 2.256 (9H, s), 2.253 3H, s), and 1.979 (3H, s) in the ^1H NMR spectrum. Thus, **1** was deduced to possess eight hydroxy groups. The aromatic region of



	R
3	H
3a	$COCH_3$

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Table 1. ^{1}H NMR Data for Jezonocinols A (**1**), B (**2**), and C (**3**) (500 MHz)[a,b]

position	**1**[c]	**2**[c]	**3**[d]
2	4.65 (d, J = 7.0)	4.48 (d, J = 8.0)	4.76 (d, J = 9.5)
3	3.89 (ddd, J = 7.5, 7.0, 5.0)	3.95 (ddd, J = 8.5, 8.0, 5.5)	3.69 (dd, J = 10.4, 9.5)
4	2.58 (dd, J = 16.3, 7.5)	2.57 (dd, J = 16.0, 8.5)	3.51 (dd, J = 10.4, 10.2)
	2.79 (dd, J = 16.3, 5.0)	2.96 (dd, J = 16.0, 5.5)	
4a			4.16 (ddd, J = 10.2, 10.2, 1.7)
6	6.11 (s)	6.13 (s)	2.28 (brd, J = 13.3, 1.7)
			3.28 (dd, J = 13.3, 8.0)
8			5.49 (t, J = 1.7)
2′	6.67 (d, J = 2.0)	6.81 (d, J = 1.9)	6.83 (d, J = 2.0)
5′	6.62 (d, J = 8.3)	6.70 (d, J = 8.4)	6.74 (d, J = 8.5)
6′	6.37 (dd, J = 8.3, 2.0)	6.63 (dd, J = 8.4, 1.9)	6.72 (dd, J = 8.5, 2.0)
2″	6.83 (d, J = 2.0)	6.83 (d, J = 2.3)	6.62 (d, J = 2.0)
5″	6.80 (d, J = 8.0)	6.81 (d, J = 8.0)	6.70 (d, J = 8.0)
6″	6.70 (dd, J = 8.0, 2.0)	6.70 (dd, J = 8.0, 2.3)	6.52 (dd, J = 8.0, 2.0)
7″	5.34 (d, J = 4.8)	5.26 (d, J = 4.0)	3.13 (ddd, J = 10.3, 8.0, 1.5)
8″	4.23 (d, J = 4.8)	4.25 (d, J = 4.0)	3.74 (dd, J = 10.2, 10.3)
10″	6.21 (d, J = 2.0)	6.11 (d, J = 2.0)	
12″	6.26 (t, J = 2.0)	6.15 (t, J = 2.0)	6.06 (d, J = 1.5)
14″	6.21 (d, J = 2.0)	6.11 (d, J = 2.0)	5.73 (d, J = 1.5)

[a] Assignments were confirmed by ^{1}H–H COSY, NOESY, and HMBC spectra. [b] J values are given in Hz. [c] Acetone-d_6. [d] DMSO-d_6.

the ^{1}H NMR spectrum of **1** showed the presence of three sets of aromatic protons (Table 1). One set corresponded to a 3,5-dihydroxyphenyl group and appeared at δ_H 6.26 (1H, t, J = 2.0 Hz, H-12′) and 6.21 [2H, d, J = 2.0 Hz, H-10′(14′)], and two sets corresponded to 3,4-dihydroxyphenyl groups at δ_H 6.67 (1H, d, J = 2.0 Hz, H-2′), 6.62 (1H, d, J = 8.3 Hz, H-5′), and 6.37 (1H, dd, J = 8.3, 2.0 Hz, H-6′), and at δ_H 6.83 (1H, d, J = 2.0 Hz, H-2″), 6.80 (1H, d, J = 8.0 Hz, H-5″), and 6.70 (1H, dd, J = 8.0, 2.0 Hz, H-6″), respectively. The spectrum further exhibited a set of mutually coupled methine protons [δ_H 5.34 (1H, d, J = 4.8 Hz, H-7″), 4.23 (1H, d, J = 4.8 Hz, H-8″)] and a 3-hydroxy-2,8 (or 2,6)-disubstituted 5,7-dioxy-3,4-dihydrobenzopyran [δ_H 6.11 (1H, s, H-6), 4.65 (1H, d, J = 7.0 Hz, H-2), 3.89 (1H, ddd, J = 7.5, 7.0, 5.0 Hz, H-3), 2.79 (1H, dd, J = 16.3, 5.0 Hz, H-4 eq), 2.58 (1H, dd, J = 16.3, 7.5 Hz, H-4 *ax*)]. The ^{13}C NMR spectrum showed 29 carbon resonances and supported the above assignments (Table 2). From these data, **1** was suspected to be composed of a flavan-3-ol unit and a piceatannol unit and to be formed by oxidative coupling of these units. Analysis of the HMBC spectrum of **1** (Figure 1) gave the cross-peak correlations H-2/C-2′, -6′, and -8a, H-4/C-5, H-6/C-4a, -5, and -7, H-7″/C-2″ and -6″, and H-8″/C-8, -8a, and -10″ (14″). These data revealed the linkage of C-2/C-1′ and C-8/C-8″. Therefore, the structure of **1** was confirmed as shown in Figure 1.

Jezonocinol B (**2**) was obtained as a brown, amorphous powder and was assigned the molecular formula $C_{29}H_{24}O_{10}$, the same as **1**, by HRSIMS analysis. The UV and IR spectra of **2** showed absorption bands similar to those of **1** (UV: λ_{max} 234, 283 nm; IR ν_{max}: 3400 br, 1622, 1522, 1457 cm^{-1}). The ^{1}H NMR resonances of **2** were closely related to those of **1**, and the ^{13}C NMR spectrum of **2** was very similar to that of **1** (Tables 1 and 2). Furthermore, analysis of the 2D NMR spectra of **2** suggested the same structure as **1** (Figure 1). The difference between **1** and **2** in the specific rotation and the HPLC retention time indicated that **2** is a diastereoisomer of **1**.

The relative configurations of C-2 and C-3 in **1** and **2** were concluded to be of the catechin type from the characteristic feature of the H-2 resonance in the ^{1}H NMR spectrum [**1**: δ_H 4.65 (1H, d, J = 7.0 Hz); **2**: δ_H 4.48 (1H, d, J = 8.0 Hz)]. The spatial relationships were deduced from the NOESY spectra (Figure 2). The *trans* orientations of the C-7″/8″ protons were established by the H-7″/H-10″(14″) and H-8″/H-2″ and -6″ correlations. In the NOESY spectrum of **1** (but not of **2**), additional correlations were observed for H-2′/H-10″(14″) and H-6′/H-10″(14″). From these data, the relative configurations of C-2, C-3, C-7″, and C-8″ in **1** and **2** could be deduced as shown in Figure 2. The absolute

Table 2. ^{13}C NMR Data for Jezonocinols A (**1**), B (**2**), and C (**3**) (125 MHz)[a]

position	**1**[b]	**2**[b]	**3**[c]
2	81.8 (d)	82.3 (d)	82.7 (d)
3	68.2 (d)	68.2 (d)	72.0 (d)
4	28.1 (t)	29.1 (t)	45.3 (d)
4a	101.3 (s)	101.7 (s)	44.9 (d)
5	157.3 (s)	157.4 (s)	
6	90.1 (d)	90.4 (d)	50.4 (t)
7	160.7 (s)	161.1 (s)	196.8 (s)
8	107.3 (s)	106.8 (s)	110.2 (d)
8a	152.4 (s)	153.0 (s)	176.6 (s)
1′	132.0 (s)	132.0 (s)	128.2 (s)
2′	114.7 (d)	114.9 (d)	115.4 (d)
3′	144.9 (s)	145.5 (s)	144.9 (s)
4′	145.1 (s)	145.8 (s)	145.6 (s)
5′	115.8 (d)	115.6 (d)	115.1 (d)
6′	119.1 (d)	120.0 (d)	119.4 (d)
1″	135.3 (s)	135.5 (s)	135.5 (s)
2″	113.3 (d)	113.2 (d)	114.8 (d)
3″	145.7 (s)	145.4 (s)	143.7 (s)
4″	145.9 (s)	146.0 (s)	145.2 (s)
5″	116.0 (d)	116.1 (d)	115.6 (d)
6″	118.0 (d)	117.9 (d)	118.2 (d)
7″	93.7 (d)	93.8 (d)	44.1 (d)
8″	56.4 (d)	56.0 (d)	55.7 (d)
9″	147.3 (s)	147.4 (s)	118.6 (s)
10″	107.0 (d)	106.4 (d)	147.2 (s)
11″	159.3 (s)	159.4 (s)	158.4 (s)
12″	101.8 (d)	101.7 (d)	101.3 (d)
13″	159.3 (s)	159.4 (s)	153.7 (s)
14″	107.0 (d)	106.4 (d)	102.1 (d)

[a] Assignments were confirmed by DEPT, ^{1}H–^{1}H COSY, NOESY, and HMBC spectra. [b] Acetone-d_6. [c] DMSO-d_6.

configuration of C-3 in **1** was determined by Mosher's method on the *R*-(+)-α-methoxy-α-(trifluoromethyl)phenylacetic acid (MTPA) ester (**1c**) of the heptamethyl ether (**1b**) of **1** and the *S*-(–)-MTPA ester (**1d**) (Figure 3).[15] In the ^{1}H NMR spectrum of **1c**, H-2 and H-2′, -5′, and -6′ in the B ring appeared upfield, whereas H-4 and H-6 were downfield in comparison to the same protons of **1d**, indicating that in the *R*-MTPA ester, H-2 and H-2′, -5′, and -6′ were affected by the phenyl ring of the MTPA moiety. Considering these and the aforementioned NOESY data, the absolute configurations at C-2, C-3, C-7″, and C-8″ in **1** were concluded to be *R*, *S*, *R*, and *R*, respectively. The absolute configuration of **2** was determined by comparing the CD data with that of **1**. The CD spectra of **1a** and **2a** showed Cotton effects of opposite signs at 249 nm (**1a**, Δε −5.3; **2a**, Δε +2.7) and 284 nm (**1a**, Δε −3.2; **2a**,

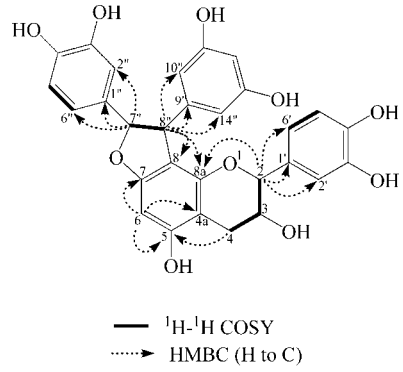


Figure 1. Diagnostic HMBC and ^{1}H–^{1}H COSY correlations of jezonocinols A (**1**) and B (**2**).

$\Delta\varepsilon$ +1.8). Considering these data and the NOESY data, the absolute configurations at C-2, C-3, C-7″, and C-8″ in **2** are *R*, *S*, *S*, and *S*, respectively.

Jezonocinol C (**3**) was isolated as a brown, amorphous powder and showed a protonated molecular ion peak at *m/z* 505 $[M + H]^+$. Acetylation of **3** with acetic anhydride and pyridine afforded the heptaacetate **3a**, whose *O*-acetyl resonances were observed at δ_H 2.328 (3H, s), 2.295 (6H, s), 2.293 (3H, s), 2.287 (3H, s), 2.222 (3H, s), and 1.863 (3H, s) in the ^{1}H NMR spectrum; its SIMS spectrum showed a protonated molecular ion at *m/z* 799 $[M + H]^+$. These data indicated that **3** has seven hydroxy groups and the molecular formula $C_{28}H_{24}O_9$. The UV spectrum of **3** showed absorption maxima at 237 and 292 nm. The IR spectrum showed absorption bands at 3269 br, 1626, 1617, 1592, 1521, and 1468 cm^{-1}, indicating the presence of hydroxy and α,β-unsaturated carbonyl groups and of aromatic rings. The ^{1}H and ^{13}C NMR spectra (Tables 1 and 2) of **3** showed resonances assignable to two sets of 3,4-dihydroxyphenyl groups [δ_H 6.83 (1H, *d*, *J* = 2.0 Hz, H-2′), 6.74 (1H, d, *J* = 8.5 Hz, H-5′), 6.72 (1H, dd, *J* = 8.5, 2.0 Hz, H-6′) and δ_H 6.70 (1H, d, *J* = 8.0 Hz, H-5″, 6.62 (1H, d, *J* = 2.0 Hz, H-2″), 6.52 (1H, dd, *J* = 8.0, 2.0 Hz, H-6″), respectively] and a 1,2,3,5-tetrasubstituted benzene ring [δ_H 6.06 (1H, d, *J* = 1.5 Hz, H-12″), 5.73 (1H, d, *J* = 1.5 Hz, H-14″); δ_C 101.3 (d, C-12″), 102.1 (d, C-14″), 118.6 (s, C-9″), 147.2 (s, C-10″), 153.7 (s, C-13″), 158.4 (s, C-11″)], a trisubstituted olefin [δ_H 5.49 (1H, t, *J* = 1.7 Hz, H-8); δ_C 110.2 (d, C-8), 176.6 (s, C-8a)], a carbonyl group [δ_C 196.8 (s, C-7)], and eight aliphatic protons coupled successively in the order CH(O)–CH(OH)–CH–CH–CH–CH–CH_2(O)– [δ_H 4.76 (1H, d, *J* = 9.5 Hz, H-2), 4.16 (1H, ddd, *J* = 10.2, 10.2, 1.7 Hz, H-4a), 3.74 (1H, dd, *J* = 10.3, 10.2 Hz, H-8″), 3.69 (1H, dd, *J* = 10.4, 9.5 Hz, H-3), 3.51 (1H, dd, *J* = 10.4, 10.2 Hz, H-4), 3.28 (1H, dd, *J* = 13.3, 8.0 Hz, H-6A), 3.13 (1H, ddd,*J* = 10.3, 8.0, 1.5 Hz, H-7″), 2.28 (1H, dd, *J* = 13.3, 1.5 Hz, H-6B)]. The aliphatic protons were also assigned by analysis of the ^{1}H–^{1}H COSY spectrum of **3** (Figure 4a). In the HMBC spectrum of **3** (Figure 4a), the correlations observed between H-2/C-2′(6′) and -8a, H-4/C-10″, H-4a/C-8a, H-14″/C-8″, H-2″(6″)/C-7″, H-6/C-7, -1″, -7″, and -8″, and H-8/C-4a and -8a revealed the respective connections C-2/C-1′, C-2-O-C-8a, C-4/C-10″, C-4a/C-8a, C-8″/C-9″, C-1″/C-7″, and C-6/C-7. These connections allowed the structure of **3** to be drawn as in Figure 4. The *trans* configuration of H-2/3 was also deduced from the large H-2/3 coupling constant in the ^{1}H NMR spectrum [δ_H 4.76 (1H, d, *J* = 9.5 Hz)]. In the NOESY spectrum of **3** (Figure 4b), the cross-peaks implicating H-2/H-4 and -4a, H-4a/H-4 and -7″, and H-7″/H-14″ enhancements indicated that these protons were cofacial, while the H-3/H-8″ and H-8″/H-2″ and -6″ correlations established that these protons were on opposite sides of the molecule. Thus the relative stereostructure of jezonocinol C was drawn as **3**. Its absolute configuration has not been determined. Figure 5 shows a possible mechanism for the biogenetic formation of **3** via oxidative coupling of (+)-catechin and piceatannol with decarboxylation. Two similar tropilene structures, longusone A and vitisinol C, were recently isolated from *Cyperus longu*[16] and *Vitis thunbergii*,[17] respectively. Jezonocinol C represents the first reported isolation of a nor-flavonostilbene.

The DPPH radical-scavenging effects of all compounds except **5** and **6** were comparable to those of (+)-catechin and gallic acid used as positive controls (Table 3). The inhibition of superoxide anion radical ($^{\bullet}O_2^-$) generation was strongest for gallic acid and for the flavonoids **1**, **2**, **9**, and catechin. It could not be estimated for **3** because its reaction with a component of the assay buffer produced a colored complex, which interfered with the measurement of absorbance at 560 nm.

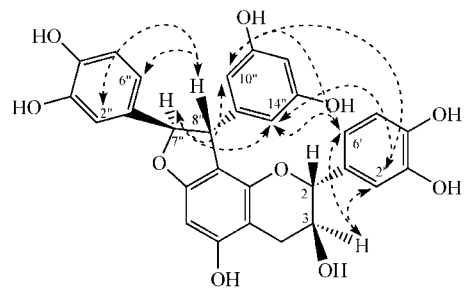
Experimental Section

General Experimental Procedures. Optical rotations were measured using a JASCO DIP-1000 digital polarimeter. IR spectra were run on a JASCO FT/IR-680 Plus spectrophotometer, UV spectra on a Hitachi U-2000 spectrophotometer, and CD spectra on a JASCO J-500 spectropolarimeter at room temperature. ^{1}H and ^{13}C NMR spectra were obtained on a Varian INOVA 500 spectrometer with standard pulse sequences, operating at 500 and 125 MHz, respectively. Acetone-d_6, DMSO-d_6, and $CDCl_3$ were used as solvents, and TMS was used as internal standard. EIMS and SIMS data were recorded on a Hitachi 4000H double-focusing mass spectrometer, using Ce^+ as primary ion for SIMS. Column chromatography was carried out over Si gel (70–230 mesh) and MPLC with Si gel (230–400 mesh, Merck). Chromatography fractions were monitored by TLC (Si gel 60 HF_{254}) and ^{1}H NMR. Preparative TLC was carried out on Merck Si gel PF_{254} plates (20 × 20 cm, 0.5 mm thick). HPLC was performed on a Waters Delta 600. Acetylation (ca. 1 mg sample) was carried out as usual (Ac_2O/pyridine, each 1 mL).

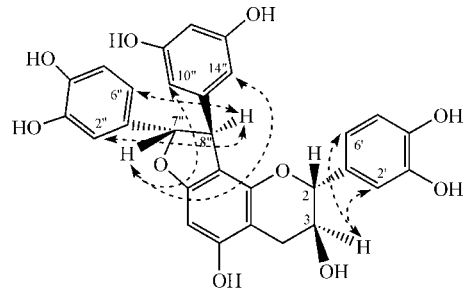
Plant Material. The stem bark of *P. jezoensis var. jezoensis* was collected in the mountainous terrain under the control of National Hokkaido Bureau, Hidaka-cho, Japan, in August 2004. A voucher specimen (PJ-0408-1) is deposited at the Herbarium of the Laboratory of Medicinal Chemistry, Osaka University of Pharmaceutical Sciences. The extraction was carried out in October 2004.

Extraction and Isolation. The chopped bark (18 kg) of *P. jezoensis* var. *jezoensis* was extracted successively with $CHCl_3$ and MeOH, and the solvents were evaporated to give the $CHCl_3$ (1.34 kg) and the MeOH (325 g) extracts. Throughout the isolation procedure all fractions were tested for DPPH radical-scavenging activity. The MeOH extract was chromatographed on Si gel by gradient elution using $CHCl_3$ with increasing concentrations of MeOH to give eight fractions [fr. I (208.8 g), $CHCl_3$–MeOH (9:1, v/v); fr. II (129.1 g), $CHCl_3$–MeOH (9:1); fr. III (135.6 g), $CHCl_3$–MeOH (5:1); fr. IV (35.5 g), $CHCl_3$-MeOH (2:1); fr. V (18.6 g), $CHCl_3$–MeOH (1:1); fr. VI (9.4 g), $CHCl_3$–MeOH (1:2); fr. VII, (1.9 g), $CHCl_3$–MeOH (1:5); fr. VIII (2.3 g), MeOH]. All these fractions had potent activities on the DPPH radical. Rechromatography with Si gel of frs. II and III using $CHCl_3$–MeOH (20:1) and $CHCl_3$–MeOH (10:1) afforded four and 11 fractions, II-A–IV-D and III-A–III-K, respectively. Further fractionation of II-B (11.1 g) by a combination of MPLC ($CHCl_3$–MeOH, 10:1), Sephadex LH-20 gel permeation chromatography (MeOH–$CHCl_3$, 1:1), and preparative TLC (*n*-hexane–AcOEt, 8:2) gave four known compounds, 3,4-dihydroxybenzaldehyde (44.6 mg),[10] 4-hydroxycinnamic acid (18.7 mg),[11] dihydrodehydrodiconiferyl alcohol (16.1 mg),[12] and isolariciresinol (13.0 mg).[13] Fr. II-C (15.8 g) was fractionated on MPLC using $CHCl_3$–MeOH, (10:1) followed by preparative TLC ($CHCl_3$–MeOH, 10:1) to give protocatechuic acid (20.5 mg)[11] and dihydroquercetin (17.2 mg).[14] Frs. III-G (6.2 g) and III-H (1.1 g) were respectively separated by Sephadex LH-20 (MeOH), followed by HPLC [column TSKgel ODS-80Ts (TOSOH, 21.5 mm × 300 mm); solvent MeOH–H_2O (40:60); flow rate 6 mL/min; detection UV 280 nm] to afford jezonocinol C (**3**, 3.5 mg) from fr. III-G and jezonocinols A (**1**, 7.8 mg) and B (**2**, 2.8 mg) from fr. III-H.

Jezonocinol A (1): brown, amorphous powder; $[\alpha]_D^{22}$ −167.8 (*c* 0.75, MeOH); UV (EtOH) λ_{max} (log ε) 206 (4.12), 235 (3.58), 283 (3.15) nm; IR ν_{max} (KBr) 3400 br, 1621, 1522, 1459, 1284, 1155, 1108, 1006, 815, 688 cm^{-1}; ^{1}H NMR and ^{13}C NMR, see Tables 1 and 2, respectively; SIMS *m/z* (rel int) 533 (3), 381 (3), 315 (5), 223 (28), 207 (61), 131 (10), 115 (100); HRSIMS $C_{29}H_{25}O_{10}$ (*m/z* 533.1443 [M + H]$^+$, calcd 533.1446).

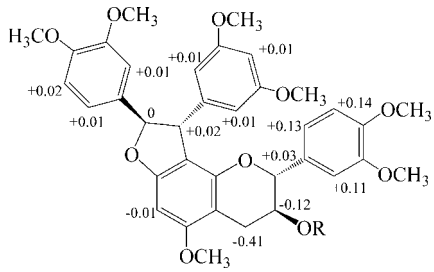


Jezonocinol A (**1**)



Jezonocinol B (**2**)

Figure 2. NOESY correlations of jezonocinols A (**1**) and B (**2**).



1c R = *R*-MTPA

1d R= *S*-MTPA

Figure 3. ^{1}H chemical shift differences ($\Delta\delta = \delta_S - \delta_R$) between the *R*- (**1c**) and *S*-MTPA ester (**1d**) of **1b**.

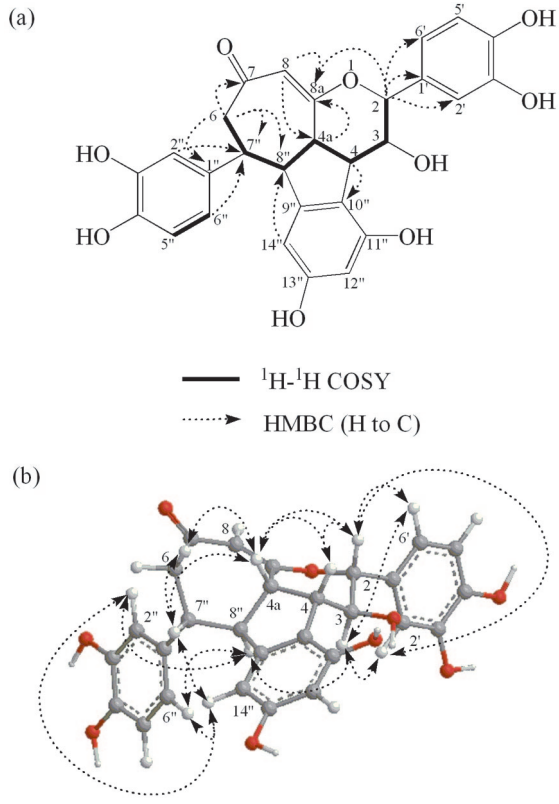


Figure 4. (a) Diagnostic HMBC and ^{1}H–^{1}H COSY correlations and (b) NOESY correlations of jezonocinol C (**3**).

Octa-*O*-acetyljezonocinol A (1a): brown, amorphous powder; $[\alpha]_D^{22}$ −3.1 (*c* 0.28, $CHCl_3$); ^{1}H NMR δ ($CDCl_3$) 2.294 (3H, s), 2.279 (3H, s), 2.278 (3H, s), 2.256 (9H, s), 2.253 (3H, s), 1.979 (3H, s); EIMS *m/z* (rel int) 868 (4) $[M]^+$; CD (*c* 2.65 × 10^{-5} M, EtOH) $\Delta\varepsilon$ (nm) −33.0 (208), −5.3 (249), −3.2 (284).

Jezonocinol B (2): brown, amorphous powder; $[\alpha]_D^{22}$ +11.6 (*c* 0.6, MeOH); UV (EtOH) λ_{max} (log ε) 205 (3.71), 234 (3.15), 283 (2.70) nm; IR ν_{max} (KBr) 3400 br, 1622, 1522, 1457, 1284, 1154, 1107, 1008, 817, 688 cm^{-1}; ^{1}H NMR and ^{13}C NMR, see Tables 1 and 2, respectively; SIMS *m/z* (rel int) 533 (3), 381 (4), 315 (2), 223 (19), 207 (49), 131 (12), 115 (100), HRSIMS $C_{29}H_{25}O_{10}$ (*m/z* 533.1444 $[M + H]^+$, calcd 533.1446).

Octa-*O*-acetyljezonocinol B (2a): brown, amorphous powder; $[\alpha]_D^{22}$ −23.9 (*c* 0.77, $CHCl_3$); ^{1}H NMR δ ($CDCl_3$) 2.292 (3H, s), 2.290 (6H, s), 2.260 (3H, s), 2.258 (3H, s), 2.226 (6H, s), 1.958 (3H, s); EIMS *m/z* (rel int) 868 (2) $[M]^+$; CD (*c* 4.38 × 10^{-5} M, EtOH) $\Delta\varepsilon$ (nm) −14.4 (218), +2.7 (249), +1.8 (284).

Jezonocinol C (3): brown, amorphous powder; $[\alpha]_D^{22}$ −133.4 (*c* 0.74, MeOH); UV (EtOH) λ_{max} (log ε) 237.0 (3.71), 291.5 (3.65) nm; IR ν_{max} (KBr) 3269 br, 1626, 1617, 1592, 1521, 1468, 1373, 1284, 1184, 1142, 983, 816 cm^{-1}; ^{1}H NMR and ^{13}C NMR, see Tables 1 and 2, respectively; SIMS *m/z* (rel int) 505 (14), 419 (32), 391 (38), 369 (10), 353 (11), 312 (10), 176 (93), 149 (100); HRSIMS $C_{28}H_{25}O_9$ (*m/z* 505.1516 $[M + H]^+$, calcd 505.1497).

Hepta-*O*-acetyljezonocinol C (3a): brown, amorphous powder; $[\alpha]_D^{22}$ −15.9 (*c* 0.23, $CHCl_3$); ^{1}H NMR δ ($CDCl_3$) 7.29 (1H, dd, *J* = 9.9 2.0 Hz, H-6′), 7.24 (1H, d, *J* = 9.6 Hz, H-5′), 7.22 (1H, d, *J* = 9.9 Hz, H-5″), 7.21 (1H, d, *J* = 2.0 Hz, H-2′), 7.15 (1H, dd, *J* = 8.5, 2.0 Hz, H-6″), 7.10 (1H, d, *J* = 2.0 Hz, H-2″), 6.70 (1H, d, *J* = 1.5 Hz, H-12″), 6.53 (1H, d, *J* = 1.5 Hz, H-14″), 6.00 (1H, t, *J* = 1.5 Hz, H-8), 5.04 (1H, d, *J* = 10.0 Hz, H-2), 5.04 (1H, dd, *J* = 10.0, 9.5 Hz, H-3), 4.15 (1H, ddd, *J* = 9.3, 9.3, 1.5 Hz, H-4a), 3.99 (1H, dd, *J* = 10.0, 9.3 Hz, H-8″), 3.78 (1H, dd, *J* = 9.5, 9.3 Hz, H-4), 3.41 (1H, ddd, *J* = 10.0, 7.3, 1.5 Hz, H-7″), 3.25 (1H, dd, *J* = 13.5, 7.3 Hz, H-6A), 2.78 (1H, brd,*J* = 13.5 Hz, H-6B), 2.328 (3H, s, $\underline{CH_3}CO$–), 2.295 (6H, s, $\underline{CH_3}CO$–), 2.293 (3H, s, $\underline{CH_3}CO$–), 2.287 (3H, s, $\underline{CH_3}CO$–), 2.222 (3H, s, $\underline{CH_3}CO$–), 1.863 (3H, s, $\underline{CH_3}CO$–); ^{13}C NMR δ ($CDCl_3$) 197.2 (s, C-7), 172.7 (s, C-8a), 168.9 (s, $CH_3\underline{CO}$), 168.7 (s, $CH_3\underline{CO}$), 168.2 (s, $CH_3\underline{CO}$), 168.0 (s, $CH_3\underline{CO}$), 167.95 (s, 2 × $CH_3\underline{CO}$), 167.90 (s, $CH_3\underline{CO}$), 151.6 (s, C-13″), 147.1 (s, C-11″), 147.0 (s, C-9″), 142.6 (s, C-3′), 142.2 (s, C-4″), 141.9 (s, C-4′), 141.7 (s, C-1″), 141.3 (s, C-3″), 134.5 (s, C-1′), 129.5 (s, C-10″), 125.6 (d, C-6″), 125.1 (d, C-6′), 124.3 (d, C-5′), 123.6 (d, C-5″), 123.4 (d, C-2″), 122.9 (d, C-2′), 115.6 (d, C-14″), 115.2 (d, C-12″), 80.5 (d, C-8), 80.4 (d, C-2), 71.9 (d, C-3), 56.7 (d, C-8″), 49.0 (t, C-6), 46.5 (d, C-4a), 45.3 (d, C-7″), 44.5 (d, C-4), 21.1 (q, $\underline{CH_3}CO$), 21.0 (q, $\underline{CH_3}CO$), 20.7 (q, 2 × $\underline{CH_3}CO$), 20.64 (q, $\underline{CH_3}CO$), 20.61 (q, $\underline{CH_3}CO$), 20.5 (q, $\underline{CH_3}CO$); SIMS *m/z* (rel int) 799 (2) $[M + H]^+$; CD (*c* 3.38 × 10^{-5} M, EtOH) $\Delta\varepsilon$ (nm) −19.6 (210), +6.6 (261), +2.0 (307).

Synthesis of MTPA Esters. A mixture of **1** (2.0 mg) and diazomethane in MeOH–Et_2O (1:1, 10 mL) was stirred for 24 h. The reaction mixture was concentrated to give jezonocinol A hepta-*O*-methyl ether (**1b**, 2.1 mg). To a stirred solution of **1b** (1.0 mg) in dry pyridine (1 mL) was added *R*-(+)-MTPA chloride or *S*-(+)-MTPA chloride (20 μL). The mixture was stirred overnight at 80 °C and subjected to preparative TLC with $CHCl_3$–MeOH (10:1) to afford the *R*-(+)-MTPA ester (**1c**, 1.9 mg) or the *S*-(+)-MTPA ester (**1d**, 2.0 mg).

***R*-(+)-MTPA ester (1c):** colorless, amorphous solid; EIMS *m/z* (rel int) 846 (100) $[M]^+$; ^{1}H NMR δ ($CDCl_3$) 6.83 (1H, dd, *J* = 8.0, 2.0 Hz, H-6″), 6.81 (1H, d, *J* = 2.0 Hz, H-2″), 6.79 (1H, d, *J* = 8.0 Hz, H-5″), 6.52 (1H, d, *J* = 8.0 Hz, H-5′), 6.46 (1H, d, *J* = 1.5 Hz, H-2′), 6.42 (1H, dd, *J* = 8.0, 1.5 Hz, H-6′), 6.34 (2H, brs, H-10″, 14″), 6.34 (1H, brs, H-12″), 6.24 (1H, s, H-6), 5.53 (1H, d, *J* = 5.5 Hz,

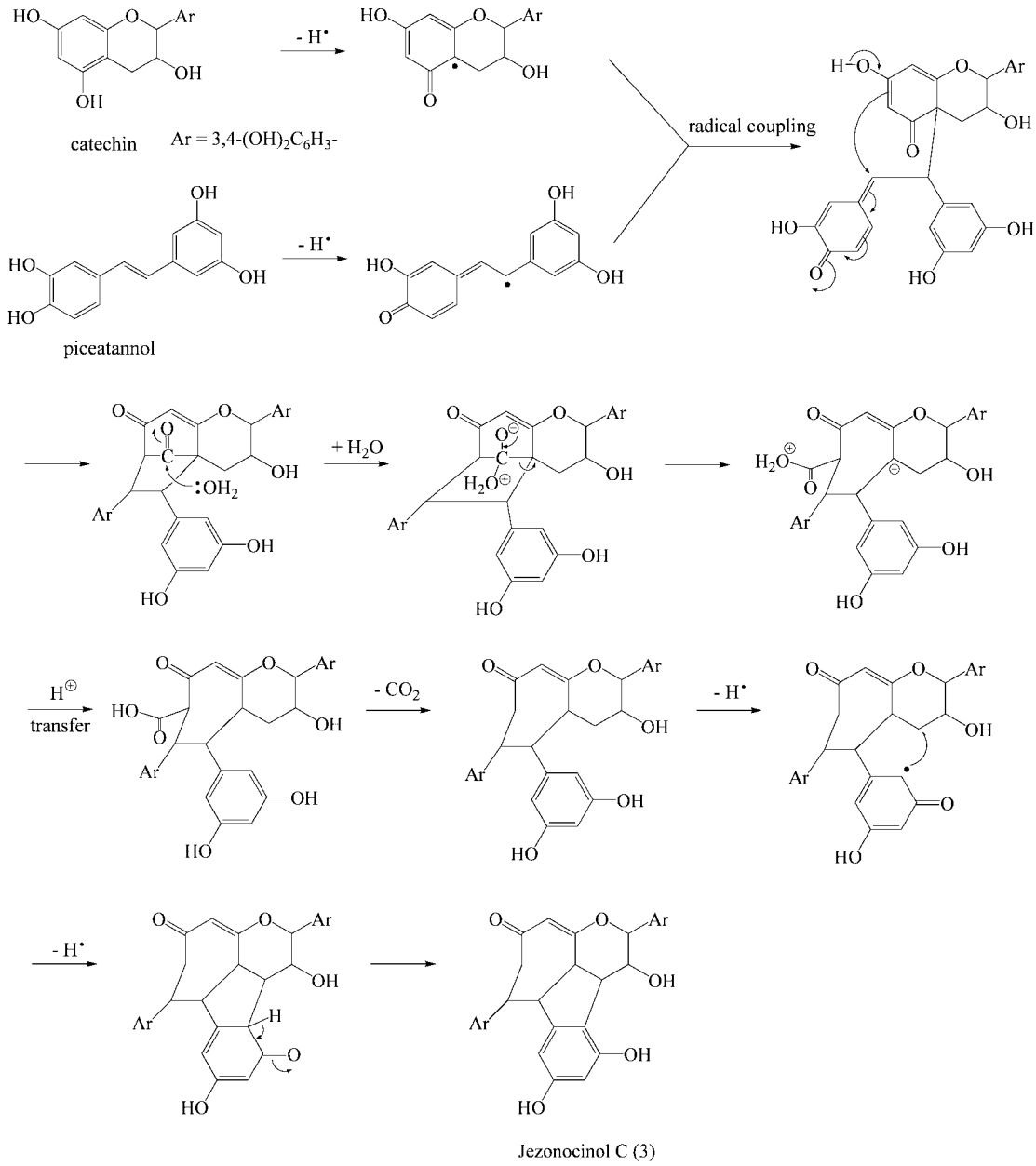


Figure 5. Proposed biogenesis of jezonocinol C (**3**).

Table 3. Scavenging of DPPH Radical and Inhibition of Superoxide Anion Radical Generation by Phenolic Compounds

compound	SC_{50} (μM)[a] DPPH	IC_{50} (μM)[b] $\cdot O_2^-$
1	9.92	2.03
2	11.8	1.80
3	3.83	n.d.[c]
4	12.2	25.3
5	117	104
6	69.1	344
7	13.7	70.7
8	17.3	3.31
9	10.4	1.41
(+)-catechin	11.4	2.31
gallic acid	8.08	0.53

[a] SC_{50}: 50% scavenging concentration. [b] IC_{50}: 50% inhibitiory concentration. [c] n.d.: not determined.

H-7″), 5.33 (1H, ddd, J = 8.5, 8.0, 5.5 Hz, H-3), 4.89 (1H, d, J = 8.0 Hz, H-2), 4.41 (1H, d, J = 5.5 Hz, H-8″), 2.80 (2H, d, J = 8.5 Hz, H-4).

***S*-(+)-MTPA ester (1e):** colorless, amorphous solid; EIMS *m/z* (rel int) 846 (100) $[M]^+$; ^{1}H NMR δ ($CDCl_3$) 6.85 (1H, dd, J = 8.5, 2.0 Hz, H-6″), 6.83 (1H, d, J = 2.0 Hz, H-2″), 6.81 (1H, d, J = 8.5 Hz, H-5″), 6.66 (1H, d, J = 8.0 Hz, H-5′), 6.57 (1H, d, J = 1.9 Hz, H-2′), 6.55 (1H, dd, J = 8.0, 1.9 Hz, H-6′), 6.35 (2H, brs, H-10″, 14″), 6.35 (1H, brs, H-12″), 6.24 (1H, s, H-6), 5.53 (1H, d, J = 5.5 Hz, H-7″), 5.21 (1H, ddd, J = 8.5, 8.2, 5.5 Hz, H-3), 4.92 (1H, d, J = 8.2 Hz, H-2), 4.42 (1H, d, J = 5.5 Hz, H-8″), 2.65 (2H, d, J = 8.5 Hz, H-4).

DPPH Radical Scavenging Effect. The scavenging effects of the isolated compounds were determined by measuring the intensities of DPPH radical quenching, as described by Hatano et al.[18] An aliquot (120 μL) of EtOH solution of sample was transferred to the well of a 96-well microplate. A mixture composed of 156 μL of 0.1 M HOAc–NaOAc buffer (pH 5.5) and 24 μL of 800 μM DPPH in EtOH was added to each well and allowed to react for 30 min at room temperature. Then the absorbance was measured at 517 nm using a BIO-RAD model 680 microplate reader.

Effects on Superoxide Anion Radical Generation. The production of superoxide anions in the xanthine–xanthine oxidase system was determined by the method of Imanari et al.[19] A mixture composed of 2.4 mL of 0.05 M Na_2CO_3 (pH 10.2) and 0.1 mL each of 3 mM

xanthine, 3 mM EDTA, 1.5 mg/mL BSA, 0.75 mM nitroblue tetrazolium dichloride, and test sample was added to 0.1 mL of 0.1 mg/mL xanthine oxidase to start the reaction. After incubation at 25 °C for 20 min, the reaction was stopped with 0.1 mL of 6 mM $CuCl_2$ and the absorbance was measured at 560 nm.

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Supporting Information Available: CD spectra for compounds **1a**, **2a**, and **3a**. ^1H and ^{13}C NMR spectra and 2D NMR spectra (^1H–^1H COSY, HMBC, and NOESY) for compound **3**. This material is available free of charge via the Internet at http://pubs.acs.org.

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