Woorenol, a Novel Sesquineolignan with a Unique Spiro Skeleton, from the Rhizomes of *Coptis japonica* var. *dissecta*

Kazuko Yoshikawa,* Hiroshi Kinoshita, and Shigenobu Arihara

Faculty of Pharmaceutical Sciences, Tokushima Bunri University, Tokushima 770, Japan

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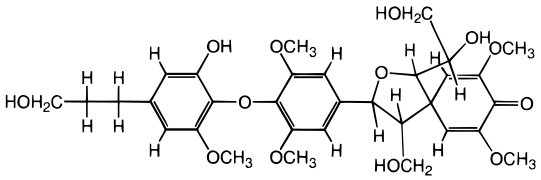
Woorenol (**1**) has been isolated from the rhizomes of *Coptis japonica* var. *dissecta*. The structure of **1** was proposed on the basis of extensive NMR experiments. Woorenol is the first sesquineolignan with a spiro skeleton.

The rhizomes of *Coptis* species are used in China as a crude drug for digestive−system problems.[1] In previous contributions, we reported the isolation and structure determination of six neolignans, four hemiterpenoid glucosides, two phenylpropanoid glucosides, and a flavonoid glycoside[2,3] from the rhizomes of *Coptis japonica* var. *dissecta* Nakai (Ranunclaceae). Further investigation of the phenylpropanoid constituents of this plant has revealed a new sesquineolignan with a spiro skeleton, woorenol (**1**). The structure of **1** was elucidated by chemical and spectroscopic methods, 2D-NMR techniques being especially helpful. This paper deals with the isolation and structure elucidation of **1**.



1 R_1=H
2 R_2=CH_3

The EtOH extract obtained from the fresh rhizomes of *Coptis japonica* var. *dissecta* was passed through an Amberlist 15 column to give a non-alkaloid fraction. Repeated separation of this fraction by ordinary-phase (SiO_2) and reversed-phase (ODS) column chromatography furnished a new neolignan named woorenol (**1**). $^1H-^1H$ COSY, $^1H-^{13}C$ COSY, HMBC, and ROESY experiments provided sufficient information to determine the complete structure of **1**. Woorenol (**1**), $[\alpha]_D$ +93.6° (*c* 1.6, MeOH), was obtained as a pale yellow solid. The molecular formula of **1** was established as $C_{32}H_{40}O_{13}$ on the basis of HRFABMS and corresponds to 13 degrees of unsaturation. The IR spectrum of **1** showed the presence of hydroxy (3420 cm^{-1}, br), enone carbonyl (1660 and 1620 cm^{-1}), and aromatic groups (1600, 1460 cm^{-1}). The ^{13}C-NMR spectrum in CD_3OD revealed 32 signals; these were sorted, by DEPT experiments, into MeO × 5, OCH_2 × 3, O-CH × 3, CH_2 × 2, CH × 1, C × 1, =CH × 6, =C × 10 and C=O × 1 (Table 1). Expansion of the formula to $C_{27}H_{25}O_8\cdot(OMe)_5$ suggested that **1** was a sesquilignan. One of the C_6-C_3 moieties was assigned as a hydroxydihydroconiferyl alcohol (fragment A in the formula), since the MS indicated cleavage of the molecular ion into a fragment of *m*/*z*

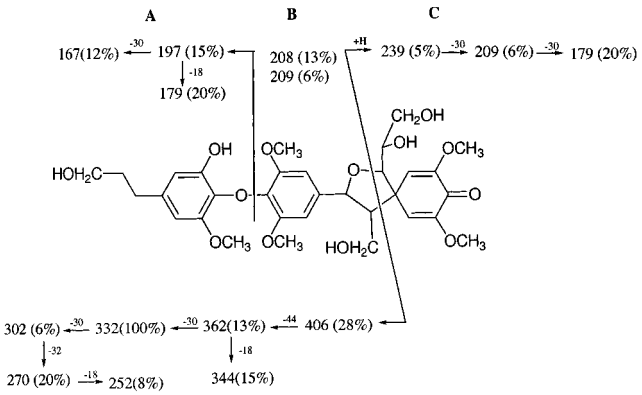


Figure 1. Diagnostic EIMS fragments ion (*m*/*z*) of **1**.

197 (15%). Thus, in the $^1H-^1H$ COSY spectrum of **1**, coupling between a 2H triplet at δ 2.52 (H_2-7) and a 2H multiplet at δ 1.76−1.77 (H_2-8) was apparent, as was a correlation with another 2H triplet located at δ 3.54 (H_2-9). These proton resonances corresponded to carbon peaks at δ 33.1 (C-7), 35.3 (C-8), and 62.2 (C-9), respectively, in the $^1H-^{13}C$ COSY spectrum of **1**, revealing the propyl alcohol portion of a hydroxydihydroconiferyl alcohol moiety. The remaining signals for an aromatic moiety indicated two sets of *meta*-coupled doublets at δ 6.29 (H-2) and 6.32 (H-6) and a 3H singlet due to one methoxy group at δ 3.65 (3-*O*-Me) and the NOE between H-2/H_2-7 and the methoxy group.

The second C_6-C_3 moiety was deduced from the observation of the fragment ion at *m*/*z* 406 (28%) formed by the cleavage b that **1** has a hydroxysinapyl alcohol moiety. The existence of fragment B in **1** was revealed by a 1H double triplet at δ 2.80 (J = 9.1, 6.0 Hz), two sets of geminal-coupled double doublets at δ 3.49 and 3.53 (J = 11.8, 6.0 Hz), a 6H singlet at δ 3.89, a 1H doubleat at δ 5.15, and a 2H singlet at δ 6.88. The COSY data showed a correlation between the resonance at δ 5.15 (H-7′) and the methine proton at δ 2.80 (H-8′). The H-8′ proton was correlated to the unequivalent methylene pair at δ 3.49 and 3.53 (H_2-9′), establishing a C-7′−C-9′ network. The remaining 6H singlet at δ 3.89 (3-O-Me and 5-O-Me) and 2H singlet at δ 6.88 (H-2′ and H-6′) indicated a symmetrical structure for fragment B. The HMBC experiment revealed long-range couplings from H-2′ and H-6′ to C-7′, indicating that the positions of two methoxy groups were at C-3′ and C-5′.

The last C_6-C_3 moiety was assigned to the fragment C (Figure 1) observed at *m*/*z* 239 in the MS. The UV spectrum showed λ_{max} (MeOH) 277.5 nm (log $\Delta\epsilon$ 4.14), characteristic of a *para*-blocked α,α-dimethoxy cyclo-

* To whom correspondence should be addressed. Phone: 0886-22-9611. FAX: 0886-55-3051.
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Table 1. ^{1}H- and ^{13}C-NMR Data (150 and 600 MHz), including CH Correlations for Woorenol (**1**) in CD_3OD[a]

carbon	^{13}C	mult[b]	^{1}H	mult	HMBC (H to C)
1	140.0	s			H-2, H-6, H-8
2	105.0	d	6.29	(1H, d, J = 1.9 Hz)	3-*O*-Me, H-6, H-7
3	154.5	s			H-2, 3-*O*-Me
4	131.3	s			H-2, H-6
5	152.2	s			
6	109.9	d	6.32	(1H, d, J = 1.9 Hz)	H-2, H-6
7	33.1	t	2.52	(1H, dd, J = 1.9 Hz)	H-2, H-7, H-8, H-9
8	35.3	t	ca 1.76	(1H, m)	H-7, H-9
			ca 1.77	(1H, m)	H-7, H-9
9	62.2	t	3.54	(2H, t, J = 6.3 Hz)	H-7, H-8
3-*O*-Me	56.1	q	3.65	(3H, s)	
1′	134.9	s			H-2′, H-6′, H-7′, H-8′
2′	104.1	d	6.88	(1H, s)	3′-*O*-Me, H-6′, H-7′
3′	149.4	s			H-2′, 3′-*O*-Me
4′	135.9	s			H-2′, H-6′
5′	149.4	s			5′-*O*-Me, H-6′
6′	104.1	d	6.88	(1H, s)	H-2′, 5′-*O*-Me, H-7′
7′	83.2	d	5.15	(1H, d, J = 9.1 Hz)	H-2′, H-6′, H-8′, H-9′
8′	62.7	t	2.80	(1H, dt, J = 9.1, 6.0 Hz)	H-7′, H-9′, H-2″, H-7″
9′	60.4	t	3.49	(1H, dd, J = 11.8, 6.0 Hz)	H-7′, H-8′
			3.53	(1H, dd, J = 11.8, 6.0 Hz)	H-7′, H-8′
3′,5′-*O*-Me	56.8	q	3.89	(6H, s)	
1″	54.2	s			H-8′, H-9′, H-8″
2″	115.7	d	6.02	(1H, d, J = 2.5 Hz)	H-8′, H-6″, H-7′, 4″-*O*-Me
3″	153.2	s			H-2″, 3″-*O*-Me
4″	179.5	s			H-2″, H-6″
5″	152.5	s			5″-*O*-Me, H-6″
6″	121.1	d	6.38	(1H, d, J = 2.5 Hz)	H-8′, H-2″, H-7″, 5″-*O*-Me
7″	86.2	d	4.87	(1H, d, J = 7.1 Hz)	H-7′, H-2″, H-6″, H-8″, H-9″
8″	81.1	d	4.09	(1H, dt, J = 7.1, 2.5 Hz)	H-7″, H-9″
9″	61.1	t	3.62	(1H, dd, J = 12.1, 2.5 Hz)	H-7″
			3.83	(1H, dd, J = 12.1, 2.5 Hz)	H-7″
3″-*O*-Me	55.8	q	3.56	(3H, s)	
5″-*O*-Me	55.8	q	3.82	(3H, s)	

[a] δ in ppm. [b] Multiplity in DEPT.

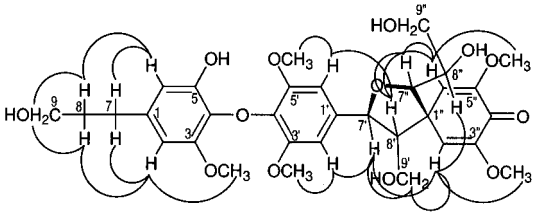

Figure 2. NOESY correlations for **1**.

hexadienone.[4] The *W*-type long-range coupling displayed between 1H doublet at δ 6.02 (H-2″) and 1H doublet δ 6.38 (H-6″), which were coupled to carbon peaks at δ 115.7 (C-2″) and 121.0 (C-6″), respectively, in ^{1}H−^{13}C COSY spectrum of **1**, confirmed that the cyclohexadiene was an approximately planar form. Moreover, the ^{1}H-NMR spectrum due to cyclohexadiene moiety showed two 3H singlet protons at δ 3.56 and 3.82. The C−H long-range correlations between the H-2″/C-8′, C-3″ and C-4″, H-6″/C-8′, C-4″ and C-5″, O-Me at δ 3.56″/C-3″, and O-Me at δ 3.82/C-5″, confirmed the existence of a *para*-blocked α,α′-dimethoxy cyclohexadienone in fragment C. The COSY signals for the remaining C-3 units (propane part) showed a correlation between a 1H doublet at δ 4.87 (H-7″) and a 1H doublet at δ 4.09 (H-8″), which was further correlated to a pair of double doublets at δ 3.62 and 3.83 (H_2-9″), indicating the presence of glycerol-like portion of fragment C.

The HMBC experiment revealed long-range couplings from the C-8′ proton to C-2″ and C-6″, from the C-2″ proton to C-8′ and C-7″, from C-6″ proton to C-8′ and C-7″, and from C-7″ proton to C-1″, C-2″ and C-6″, indicating a linkage between C-8′ and C-1″. Additional HMBC correlations between H-7′ and C-7″ established the connectivity between C-7′ and C-7″ via an ether linkage, confirming the spiro linkage between fragments B and C. The fragment ion at *m*/*z* 406 showed that the connection between C-4′ and C-4 or C-5 was via an ether linkage. To elucidate the location of the oxygen bridge in structure A, a methyl ether (**2**) was prepared from **1** by treatment with diazomethane. The ^{1}H-NMR spectrum of **2** showed signals due to two aromatic and two methoxy protons on ring A at the same position, indicating that the junction between the two units of **1** was at C-4 and C-4′ via an ether bridge. Thus, the planar structure of **1** was determined. The relative stereochemistry of woorenol was established by NOESY experiments (Figure 1). The NOEs between H-7′/H-2′ and H-2″, H-8′/H-6′, H-6″ and H-7″, H-2″/H-9′ and H-8″ and H-6″/H-7″ established the C-7′*R**, C-8′*S**, and C-7″*S**. Thus, the structure of woorenol was suggested to be **1**. To the best of our knowledge, **1** is the first sesquineolignan with a spiro skeleton.

Experimental Section

General Experimental Procedures. The following instruments were used: JASCO FT/IR-5300 (IR), Shimadzu UV-160 (UV), JASCO DIP-1000 polarimeter (optical rotation), JEOL JMS-HX-100 mass spectrometer (HRMS), and Varian UNITY 600 spectrometer or JEOL JNM-GX-400FT NMR (^{1}H and ^{13}C NMR).

Plant Material. The rhizomes of *Coptis japonica* var. *dissecta* cultivated in Sannan-cho, Hyougo Prefecture, were collected in December 1993. A voucher specimen is deposited in the herbarium of the Department of Pharmacognosy, Tokushima Bunri University, Tokushima, Japan.

Extraction and Isolation. The fresh rhizomes (5.0 kg) of *C. japonica* var. *dissecta* were extracted with absolute EtOH at room temperature for 3 weeks. The 20% EtOH solution obtained by adding H_2O was passed through an Amberlist 15 column to give the non-alkaloid fraction and then an Amberlite XAD-2 column to include H_2O-soluble substances. The Amberlite XAD-2 column was eluted with MeOH to give a neutral and phenolic eluate. The MeOH eluate (23 g) was subjected to Si gel column chromatography with CH_2Cl_2−MeOH−H_2O (25:10:0.1) to give four fractions (1−4). Fraction 2 (8 g) was subjected to HPLC on ODS (12% CH_3CN) to give woorenol (**1**, 3 mg).

Woorenol (1): pale yellow solid; $[\alpha]^{25}_D$ +93.6° (*c* 1.6, MeOH); FT-IR (dry film) 3420 (br), 1660, 1620, 1600, 1515, 1460, 1270, 1230, 1120 cm^{-1}; UV (MeOH) λ_{max} (log ϵ) 207 (5.01), 230 (4.52), 277.5 (4.14); CD $\Delta\epsilon$ +6.28 (279 nm) (*c* 0.0248, MeOH); HRFABMS, obsd *m*/*z* 632.2460, $C_{32}H_{40}O_{13}$ calcd *m*/*z* 632.2466, ^{1}H and ^{13}C NMR, see Table 1.

***O*-Methylwoorenol (2).** Woorenol (10 mg) in 1.0 of MeOH was treated with CH_2N_2 in Et_2O for one day at 4 °C. The residue after solvent removal was purified by preparative HPLC on C18 to give compound (**2**): pale yellow solid; $[\alpha]^{25}_D$ +108° (*c* 0.52, MeOH); FT-IR (dry film) 3400 (br), 1660, 1625, 1600, 1515, 1460, 1270, 1230, 1120, cm^{-1}; UV (MeOH) λ_{max} (log ϵ) 206 (4.95), 232 (4.50), 278 (4.00); HRFABMS, obsd *m*/*z* 646.2615, $C_{33}H_{42}O_{13}$ calcd *m*/*z* 646.2623; ^{1}H NMR (CD_3OD) δ ca. 1.79 (1H, m, H-8), ca. 1.81 (1H, m, H-8), 2.62 (2H, t, *J* = 7.7 Hz, H_2-6), 2.78 (1H, dt, *J* = 8.7, 5.8 Hz, H-8′), 3.50 (1H, dd, *J* = 11.8, 5.8 Hz, H-9′), 3.55 (2H, t, *J* = 7.3 Hz, H_2-9), 3.57 (1H, dd, *J* = 11.8, 5.8 Hz, H-9′), 3.57 (3H, s, 3″-*O*-Me), 3.62 (1H, dd, *J* = 12.0, 2.5 Hz, H-9″), 3.73 (6H, s, 3 and 5-*O*-Me), 3.83 (1H, dd, *J* = 12.0, 2.5 Hz, H-9″), 3.84 (3H, s, 5″-*O*-Me), 3.89 (6H, s, 3′ and 5′-*O*-Me), 4.09 (1H, dt, *J* = 7.7, 2.5 Hz, H-8″), 4.87 (1H, d, *J* = 7.7 Hz, H-7″), 5.20 (1H, d, *J* = 8.7 Hz, H-7′), 6.09 (1H, d, *J* = 2.5 Hz, H-6″), 6.40 (1H, d, *J* = 2.5 Hz, H-2″), 6.49 (2H, s, H-2 and H-6), 6.94 (2H, s, H-2′ and H-6′); ^{13}C NMR (CD_3OD) δ 33.4 (C-7), 35.4 (C-8), 54.4 (C-1″), 55.9 (3″ or 5″-*O*-Me), 56.0 (5″ or 3″-*O*-Me), 56.7 (3 and 5-*O*-Me), 56.8 (3′ and 5′-*O*-Me), 60.6 (C-9′), 61.4 (C-9″), 62.2 (C-9), 62.7 (C-8′), 80.9 (C-8″), 83.0 (C-7′), 85.9 (C-7″), 104.1 (C-2′ and C-6′), 106.8 (C-2 and C-6), 115.2 (C-2″), 121.3 (C-6″), 132.2 (C-4), 138.2 (C-4′), 140.0 (C-1′), 140.3 (C-1), 152.6 (C-5″), 153.3 (C-3″), 154.7 (C-3, 5, 3′ and C-5′), 179.6 (C-4″).

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References and Notes

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