Glycosides of *Atractylodes ovata*

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A new coumarin glycoside and a new glycoside of an acetylene derivative were isolated from the water-soluble portion of the methanolic extract of *Atractylodes ovata* rhizome together with eight known compounds. Their structures were characterized as scopoletin β-D-xylopyranosyl-(1→6)-β-D-glucopyranoside and (2*E*)-2-decene-4,6-diyne-1,8-diol 8-*O*-β-D-apiofuranosyl-(1→6)-β-D-glucopyranoside, respectively, based on chemical and spectroscopic investigations. A comparison of the polar constituents among *Atractylodes japonica*, *Atractylodes lancea*, and *A. ovata* is led to the conclusion that *A. ovata* is distinguishable from *A. lancea* and *A. japonica*, as also shown by phylogenetic analysis.

Key words *Atractylodes ovata*; Atractylodes Rhizome; chemotaxonomy; scopoletin glycoside; 2-decene-4,6-diyne-1,8-diol glycoside

In the previous paper, we reported the isolation and characterization of eight sesquiterpenoid glycosides, including atractyloside A—E, G, and a secoguaiane derivative, a monoterpenoid glucoside, seven aromatic compound glycosides, and L-phenylalanine from the water-soluble portion of the rhizome of *Atractylodes japonica*.[1] In addition, 16 sesquiterpenoid glycosides, four monoterpenoid glucosides, two hemiterpenoid glycosides, an alkyl glycoside, five aromatic compound glycosides, an acetylene derivative compound glucoside, two nucleosides, and L-tryptophan were isolated from the water-soluble portion of the rhizome of *Atractylodes lancea*.[2,3] The rhizomes of *Atractylodes* plants are classified into two groups that contain β-eudesmol and hinesol as the main constituents of the essential oil (*A. lancea* and *Atractylodes chinensis*; *so-jutsu*), and atractylon as the main constituent of the essential oil (*A. japonica* and *Atractylodes ovata*; *byaku-jutsu*) in the Japanese Pharmacopoeia.[4] Sometimes, however, the rhizomes are prescribed in traditional medicine indistinguishably. In addition, *A. ovata* is distinguishable from other *Atractylodes* plants using RAPD analysis,[5] and the phylogenetic relationship between *A. japonica* and *A. lancea* is suggested to be closer than that between *A. japonica* and *A. ovata*. We then undertook examination of the water-soluble portion of *A. ovata* to determine the chemotaxonomic relationships among *A. ovata*, *A. japonica*, and *A. lancea*.

The dried rhizome of *A. ovata*, which was cultivated in the Tokyo Metropolitan Medical Plants Garden, was extracted with 70% methanol, and the methanolic extract was suspended in water and successively extracted with ether and ethyl acetate. The aqueous layer was chromatographed on Amberlite XAD-II to give water and methanol eluate fractions. The methanol eluate fraction was chromatographed on Sephadex LH-20 followed by silica gel, Lobar RP-8 column chromatography, and HPLC, affording a coumarin glycoside (**1**), an acetylene derivative compound glycoside (**2**), four aromatic compound glycosides (**3**—**6**), three guaiane-type sesquiterpenoid glucosides (**7**—**9**), and L-tryptophan (**10**). Among them, **1** and **2** are new, and their structures were characterized as follows. Their molecular formulae were suggested from the accurate mass number of the $[M+H]^+$ ion peak in the high-resolution positive FAB-MS.

The major coumarin glycoside **1**, $C_{21}H_{26}O_{13}$, showed $[M+K]^+$ and $[M+H]^+$ ion peaks at *m/z* 525 and 487, respectively, in the positive FAB-MS. Enzymatic hydrolysis of **1** gave an aglycone (**1a**), which was identified as scopoletin (7-hydroxy-6-methoxycoumarin), and D-glucose and D-xylose as the sugar components. The ^{13}C-NMR data of **1** were similar to those of scopoletin β-D-glucopyranoside (**1b**),[6] except for the signals due to a β-D-xylopyranosyl group. Cross-peaks between the C-7/glucosyl H-1 and glucosyl C-6/xylosyl H-1 were observed in the heteronuclear multiple bond connectivity (HMBC) spectrum of **1** (see Experimental), suggesting that the xylosyl group was located at C-6 of the glucose in **1b**. This was also supported by the cross-peaks observed between H-5/–OCH_3 and between H-8/glucosyl H-1 in its nuclear Overhauser and exchange spectroscopy (NOESY) spectrum. Therefore **1** was characterized as scopoletin β-D-xylopyranosyl-(1→6)-β-D-glucopyranoside.

Acetylene derivative compound glycoside **2**, $C_{21}H_{30}O_{11}$, showed $[M+H]^+$ and $[M-C_{11}H_{20}O_{10}+H]^+$ ion peaks at *m/z* 459 and 183, respectively, in the positive FAB-MS, and $[M-H]^-$ and $[M-C_{11}H_{20}O_{10}-H]^-$ ion peaks at *m/z* 457 and 181, respectively, in the negative FAB-MS. The NMR data showed the presence of two triple bonds, one disubstituted double bond, one *prim*-methyl, one hydroxymethyl, one methylene, and one oxygenated methine in addition to the β-D-apiofuranosyl-(1→6)-β-D-glucopyranosyl group.[1] Comparison of its NMR data with those of (2*E*,8*E*)-decadiene-4,6-diyne-1,10-diol 1-*O*-β-D-glucopyranoside, which was isolated from the rhizome of *A. lancea*,[3] and the results of an HMBC experiment (Fig. 1) showed that the aglycone of **2** was 2-decene-4,6-diyne-1,8-diol, and the glycosyl group was located at C-8. Therefore **2** was concluded to be (2*E*)-decene-4,6-diyne-1,8-diol 8-*O*-β-D-apiofuranosyl-(1→6)-β-D-glucopyranoside.

Aromatic compound glycosides **3** to **6** and guaiane-type sesquiterpenoid glucosides **7** to **9** were identified as icariside F_2,[7] icariside D_1,[7] syringin,[7] dihydrosyringin,[8] atractyloside A,[2] 10-*epi*-atractyloside A,[3] and atractyloside B,[2] re-

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spectively (Fig. 2).

While the commom guaiane-type sesquiterpenoid glucosides were found in the rhizomes of *A. japonica*, *A. lancea*, and *A. ovata*, we were able to isolate the new characteristic coumarin glycoside **1** as the main glycoside of the rhizome of *A. ovata*. A comparison of the polar constituents among *A. japonica*, *A. lancea*, and *A. ovata* (Table 1) showed that *A. ovata* is distinguishable from *A. lancea* and *A. japonica*, as did the phylogenetic analysis.

Experimental

The instruments used and the experimental conditions for spectral mesurements and chromatography were the same as those reported in the previous papers.[1,3] Symmetryprep C18 7 μm (Waters; column size, 7.8× 300 mm; ODS) and Carbohydrate analysis (Waters; column size, 3.9× 300 mm; CHA) were used as columns for HPLC separations.

Extraction and Separation The dried rhizome of *A. ovata* (1.5 kg), which was cultivated in the Tokyo Metropolitan Medical Plant Garden (Kodaira, Tokyo, Japan), was extracted with 70% methanol (5 l×3) for 2 weeks, and the extract (573.5 g) was partitioned between ether/water and then ethyl acetate/water. The aqueous portion (555.3 g) was chromatographed over Amberlite XAD-II (H_2O→MeOH) to give a water eluate (521.0 g) and a methanol eluate (34.3 g).

The methanol eluate was subjected to Sephadex LH-20 [MeOH–H_2O (9 : 1)] to give six fractions (frs. A—F). Fraction C (14.72 g) was chromatographed on silica gel [$CHCl_3$–MeOH–H_2O (4 : 1 : 0.1→7 : 3 : 0.5→ 6 : 4 : 0.5)→MeOH] to give 15 fractions (frs. C_1—C_{15}). Fraction C_4 (0.13 g) was passed through a Lobar RP-8 column [MeCN–H_2O (3 : 17)] to give 11 fractions (frs. $C_{4\text{-}1}$—$C_{4\text{-}11}$), and fr. $C_{4\text{-}3}$ was subjected to HPLC [ODS, MeCN–H_2O (3 : 17)] to give **5** (6 mg) and **6** (5 mg). Fraction C_6 (0.13 g) was passed through a Lobar RP-8 column [MeCN–H_2O (3 : 17)] to give 11 fractions (frs. $C_{6\text{-}1}$—$C_{6\text{-}11}$), fr. $C_{6\text{-}7}$ was subjected to HPLC [CHA, MeCN–H_2O (19 : 1)] to give **3** (65 mg), and fr. $C_{6\text{-}10}$ was subjected to HPLC [ODS, MeCN–H_2O (3 : 17)] to give **4** (20 mg). Fraction C_7 (0.50 g) was passed through a Lobar RP-8 column [MeCN–H_2O (3 : 17)] to give nine fractions (frs. $C_{7\text{-}1}$—$C_{7\text{-}9}$). Fraction $C_{7\text{-}4}$ was recrystallized from methanol to give **1** (200 mg), and fr. $C_{7\text{-}8}$ was subjected to HPLC [ODS, MeCN–H_2O (3 : 17) and CHA, MeCN–H_2O (24 : 1)] to give **2** (2 mg). Fraction C_9 (0.33 g) was passed through a Lobar RP-8 column [MeCN–H_2O (3 : 17)] to give seven fractions (frs. $C_{9\text{-}1}$—$C_{9\text{-}7}$), and fr. $C_{9\text{-}3}$ was subjected to HPLC [ODS, MeCN–H_2O (3 : 37)] to give **8** (7 mg) and **7** (40 mg). Fraction C_{11} (1.31 g) was passed through a Lobar RP-8 column [MeCN–H_2O (3 : 17)] to give seven fractions (frs. $C_{11\text{-}1}$—$C_{11\text{-}7}$). Fraction $C_{11\text{-}3}$ was subjected to HPLC [CHA, MeCN–H_2O (14 : 1)] to give **9** (16 mg), and fr. $C_{11\text{-}4}$ was subjected to Sephadex LH-20 (MeOH) to give **10** (15 mg).

The following compounds were identified by comparison with authentic compounds or published physical and spectral data: icariside F_2 (**3**), icariside D_1 (**4**), syringin (**5**), dihydrosyrindine (**6**), atractyloside A (**7**), 10-*epi*-atractyloside A (**8**), atractyloside B (**9**), and L-tryptophan (**10**).

Scopoletin 7-*O*-β-D-Xylopyranosyl-(1→6)-β-D-glucopyranoside (**1**): Colorless needles (MeOH), mp 243—245 °C, $[\alpha]_D^{23}$ −148° (c=0.5, H_2O). Positive FAB-MS m/z: 525.1011 $[M+K]^+$ (Calcd for $C_{21}H_{26}KO_{13}$, 525.1011), 487.1465 $[M+H]^+$ (base, Calcd for $C_{21}H_{27}O_{13}$, 487.1452). ^{1}H-NMR (DMSO-d_6, 500 MHz) δ: 6.33 (1H, d, J=9.5 Hz, H-3), 7.96 (1H, d, J=9.5 Hz, H-4), 7.30 (1H, s, H-5), 7.20 (1H. s. H-8), 3.82 (3H, s, 6-OCH_3), 5.10 (1H, d, J=7.5 Hz, Glc H-1), 4.12 (1H, d, J=7.5 Hz, Xyl H-1). ^{13}C-NMR (DMSO-d_6, 125 MHz) δ: 160.50 (C-2), 113.34 (C-3), 144.14 (C-4), 109.72 (C-5), 145.96 (C-6), 149.76 (C-7), 103.01 (C-8), 148.92 (C-9), 112.31 (C-10), 56.01 (6-OCH_3), 99.50 (Glc C-1), 72.99 (Glc C-2), 76.52 (Glc C-3), 69.19 (Glc C-4), 75.33 (Glc C-5), 68.17 (Glc C-6), 104.02 (Xyl C-1), 73.25 (Xyl C-2), 76.54 (Xyl C-3), 69.42 (Xyl C-4), 65.60 (Xyl C-5). HMBC correlations: H-3/C-2, C-10; H-4/C-2, C-5, C-9, C-10; H-5/C-4, C-6, C-7, C-9, C-10; H-8/C-6, C-7, C-9, C-10; –OCH_3/C-6; Glc H-1/C-7; Xyl H-1/Glc C-6.

Enzymatic Hydrolysis of 1 A mixture of **1** (25 mg) and β-glucosidase (5 mg; Toyobo Co. Ltd., Lot 93240) in water (5 ml) was shaken in a water bath at 37 °C for 5 d. The mixture was concentrated *in vacuo* to dryness and the residue was chromatographed over silica gel [$CHCl_3$–MeOH (4 : 1 to 1 : 1)] to afford an aglycone (**1a**; 10 mg) and a sugar fraction. The sugar fraction was passed through Sephadex LH-20 (MeOH) to give a syrup, and HPLC [carbohydrate analysis (Waters); detector, JASCO RI-930 detector and JASCO OR-990 chiral detector; solv., MeCN–H_2O (9 : 1), 2 ml/min; t_R 4.20 min (same location as that of D-xylose) and t_R 6.80 min (same location as that of D-glucose)] showed the presence of D-glucose and D-xylose.

Scopoletin (**1a**): Colorless needles (EtOH), mp 205—207 °C, ^{1}H-NMR (DMSO-d_6, 270 MHz) δ: 6.21 (1H, d, J=9.5 Hz, H-3), 7.91 (1H, d, J=9.5 Hz, H-4), 7.21 (1H, s, H-5), 6.77 (1H. s. H-8), 3.81 (3H, s, 6-OCH_3).

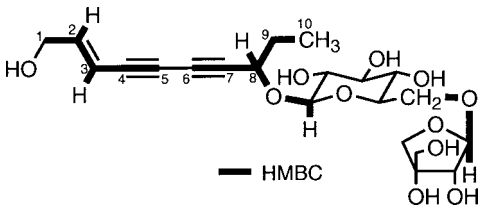

Fig. 1. Structure and HMBC Correlations of **2**

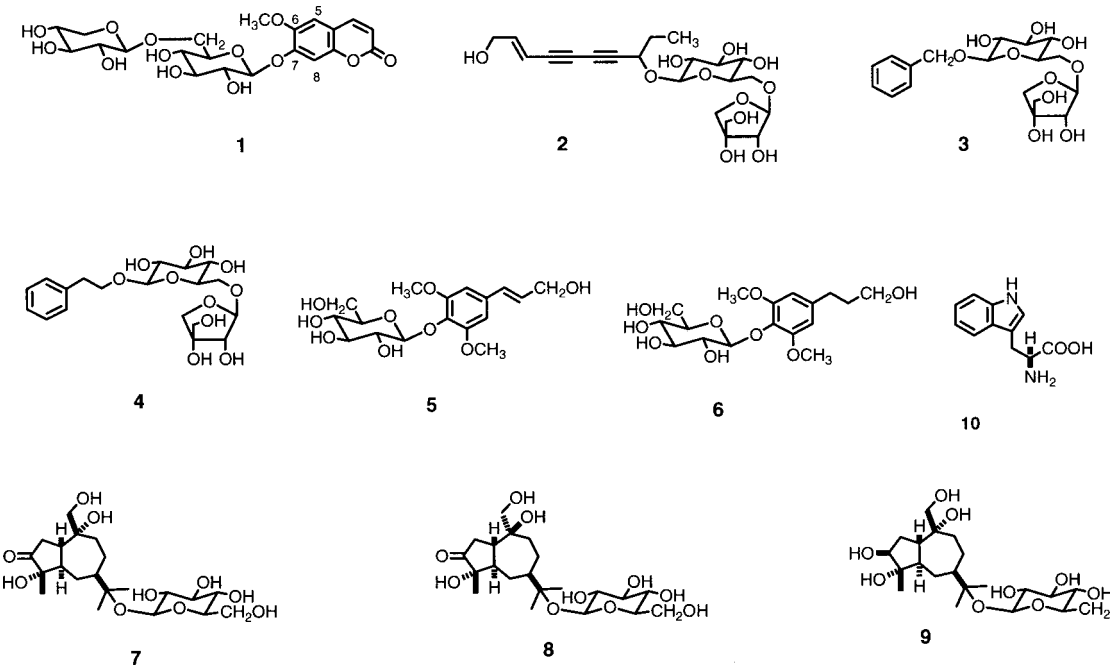

Fig. 2. Structures of **1**—**10**

Table 1. Polar Constituents of *A. japonica*, *A. lancea*, and *A. ovata* Rhizome

	Type	*A. japonica*	*A. lancea*	*A. ovata*
Sesquiterpenoid alcohol glycosides				
Guaiane-type	**7**	+++++	+++++	+++
	8	++	+++	+
	9	++	++	++
	11	+++	+++++	
	12	+	+++	
	14		+++	
	15		++	
Secoatractylolactone-type	**13**	++	+++	
Eudesmane-type	**35**	+++		
	16		++++	
	17		++++	
	18		+	
	19		++	
	20		++	
	21		+	
	22		+++	
	23		+++	
Hemiterpenoid alcohol glycosides				
	28		+	
	29		+	
Monoterpenoid alcohol glucosides				
1,8-Cineole-type	**24**	+	+	
	25		+	
p-Menthane-type	**26**		+	
	27		+	
Aromatic glycosides				
C_6 type	**31**	+	+	
	32	+	++	
	33	+	++	
	36	+		
C_6—C_1 type	**3**	++	++	++++
C_6—C_2 type	**4**	+		++
	37	++		
C_6—C_3 type	**5**		++	+
	1			++++++
	6			+
Acetylene glycoside	**34**		+	
	2			+
Alkyl glycoside	**30**		+	
Amino acids	**38**	+		
	10		++++	++

1—9 mg, +; 10—19 mg, ++; 20—39 mg, +++; 40—89 mg, ++++; 90—199 mg, +++++; ≧200 mg, ++++++ (from 1.4 kg of *A. japonica*, 1.5 kg of *A. lancea*, and 1.5 kg of *A. ovata*). **11**: (1*S*,4*S*,5*S*,7*R*,10*R*)-10,11,14-trihydroxyguai-3-one 11-*O*-*β*-D-glucopyranoside, **12**: (1*S*,4*S*,5*R*,7*R*,10*R*)-10,11,14-trihydroxyguai-3-one 11-*O*-*β*-D-glucopyranoside, **13**: (1*S*,5*R*,7*R*,10*R*)-secoatractylolactone 11-*O*-*β*-D-glucopyranoside, **14**: atractyloside A 14-*O*-*β*-D-fructofuranoside, **15**: (1*S*,4*S*,5*S*,7*R*,10*S*)-10,11,14-trihydroxyguai-3-one 11-*O*-*β*-D-glucopyranoside, **16**: (5*R*,7*R*,10*S*)-isopterocarpolone *β*-D-glucopyranoside, **17**: atractyloside I, **18**: *cis*-atractyloside I, **19**: atractyloside C, **20**: atractyloside D, **21**: atractyloside E, **22**: atractyloside G, **23**: (2*R*,3*R*,5*R*,7*R*,10*S*)-atractyloside G 2-*O*-*β*-D-glucopyranoside, **24**: (1*R*,2*R*,4*S*)-2-hydroxy-1,8-cineole *β*-D-glucopyranoside, **25**: (1*S*,2*S*,4*R*)-2-hydroxy-1,8-cineole *β*-D-glucopyranoside, **26**: (4*S*)-*p*-menth-1-ene-7,8-diol 8-*O*-*β*-D-glucopyranoside, **27**: (1*S*,2*R*,4*S*)-*p*-menthane-1,2,8-triol 8-*O*-*β*-D-glucopyranoside, **28**: 3-methyl-3-butenyl *β*-D-apiofuranosyl-(1→6)-*β*-D-glucopyranoside, **29**: 3-methyl-2-butenyl *β*-D-apiofuranosyl-(1→6)-*β*-D-gluco-pyranoside, **30**: isopropyl *β*-D-apiofuranosyl-(1→6)-*β*-D-glucopyranoside, **31**: 4-hydroxy-3-methoxyphenyl *β*-D-glucopyranoside, **32**: 4-hydroxy-3-methoxyphenyl *β*-D-apiofuranosyl-(1→6)-*β*-D-glucopyranoside, **33**: 4-hydroxy-3-methoxyphenyl *β*-D-xylopyranosyl-(1→6)-*β*-D-glucopyranoside, **34**: (2*E*,8*E*)-2,8-decadiene-4,6-diyne-1,10-diol 11-*O*-*β*-D-glucopyranoside, **35**: (3*R*,5*S*,8*S*,10*S*)-3-hydroxyatractylerolide III 3-*O*-*β*-D-glucopyranoside, **36**: seguinoside B, **37**: phenethyl *α*-L-rhamnopyranosyl-(1→6)-*β*-D-glucopyranoside, **38**: L-phenyalanine.

^{13}C-NMR (DMSO-d_6, 67.5 MHz) δ: 160.72 (C-2), 111.51 (C-3), 144.52 (C-4), 109.56 (C-5), 145.36 (C-6), 151.49 (C-7), 102.77 (C-8), 149.61 (C-9), 110.40 (C-10), 56.01 (6-OCH_3).

(2*E*)-2-Decene-4,6-diyne-1,8-diol 8-*O*-*β*-D-Apiofuranosyl-(1→6)-*β*-D-glucopyranoside (**2**): An amorphous powder, $[\alpha]_D^{23}$ −144° (*c*=0.1, MeOH). Positive FAB-MS *m/z*: 459.1870 $[M+H]^+$ (Calcd for $C_{21}H_{31}O_{11}$, 459.1867), 183 $[M-C_{11}H_{20}O_{10}+H]^+$ (base). Negative FAB-MS *m/z*: 457 $[M-H]^-$, 181 $[M-C_{11}H_{20}O_{10}-H]^-$ (base). ^{1}H-NMR (pyridine-d_5, 500 MHz) δ: 4.41 (2H, br s, H_2-1), 6.67 (1H, td, *J*=4.0, 16.0 Hz, H-2), 6.25 (1H, d, *J*=16.0 Hz, H-3), 5.11 (1H, dd, *J*=6.5, 6.5 Hz, H-8), 1.84 (2H, m, H_2-9), 0.99 (3H, t, *J*=7.5 Hz, H_3-10), 5.25 (1H, d, *J*=7.5 Hz, Glc H-1), 5.81 (1H, d, *J*=2.5 Hz, Api H-1). ^{13}C-NMR (pyridine-d_5, 125 MHz) δ: 61.89 (C-1), 149.91 (C-2), 106.92 (C-3), 77.92 (C-4), 74.33 (C-5), 71.20 (C-6), 82.26 (C-7), 69.14 (C-8), 29.34 (C-9), 9.76 (C-10), 101.91 (Glc C-1), 75.02 (Glc C-2), 78.53 (Glc C-3), 71.79 (Glc C-4), 77.34 (Glc C-5), 68.89 (Glc C-6), 111.31 (Api C-1), 77.80 (Api C-2), 80.45 (Api C-3), 75.03 (Api C-4), 65.57 (Api C-5). HMBC correlations: H-2/C-1, C-3,C-4; H-3/C-5; H-8/C-6, C-7, C-9, C-10, Glc C-1; H2-9/C-7, C-8, C-10; H_3-10/C-8, C-9; Glc H-1/C-8; Api H-1/Glc C-6.

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