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Note

Lignan Glucosides from *Sinomenium acutum* Rhizomes

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The new lignan glucoside, acutumoside (1), was isolated from *Sinomenium acutum* rhizomes together with nine known compounds (2–10). The structure of 1 was elucidated on the basis of extensive spectroscopic analyses, including two-dimensional nuclear magnetic resonance and chemical reactions. Compounds 2, 7, 8, and 10 displayed potential antiproliferative activity against A549, SK-OV-3, SK-MEL-2, and HCT-15 cell lines, while compound 1 showed weak activity against these human tumor cells.

Key words: *Sinomenium acutum*; Menispermaceae; lignan; acutumoside; cytotoxicity

Sinomenium acutum (Thumb.) Rehd. et Wils. (Menispermaceae) is a deciduous shrub distributed in Asia, particularly in Japan, China, and Korea. The rhizome of *S. acutum*, known as qing-feng-teng, is a Chinese medicine commonly used for the treatment of rheumatism, neuralgia, arthritis, and edema.[1)] The characteristic constituents of this plant are such alkaloids as morphinane, hasubanane, bisbenzylisoquinoline, aporphine, and protoberberine alkaloids.[2–6)] Of these, the main component, sinomenine, displays marked biological activities including arthritis amelioration,[7)] immunomodulation,[8)] and vasodilation.[9)]

In our continuing search for new bioactive substances from Korean medicinal plants, we investigated the chemical constituents of the rhizomes of *S. acutum*. We isolated a new lignan glucoside, named acutumoside (**1**), together with nine known compounds (**2–10**) (Fig. 1A) from the rhizomes. Their structures were elucidated on the basis of extensive spectroscopic analyses, including two-dimensional nuclear magnetic resonance (2D NMR) and chemical reactions. This report describes the isolation and structural elucidation of new compound **1**, as well as the antiproliferative activities of the isolates (**1–10**) toward human cancer cell lines.

The dried rhizomes of *S. acutum* (3.5 kg) were chopped and extracted with 80% aqueous methanol (MeOH) three times daily at 60 °C and filtered. The filtrate was then concentrated under vacuum to afford a crude MeOH extract (390 g) which was suspended in H_2O and successively partitioned with *n*-hexane, chloroform ($CHCl_3$), and *n*-butanol (BuOH) to respectively yield 21, 21, and 65 g of a residue. The *n*-hexane-soluble fraction (20 g) was subjected to silica gel (500 g) column chromatography, eluting with *n*-hexane-ethyl acetate (EtOAc; 10/1 to 1/1, v/v), to yield 11 fractions (H1–H11) based on thin-layer chromatographic (TLC) analyses. Fraction H2 (2.7 g) was subjected to octadecyl silane (ODS, 100 g) column chromatography (MeOH–H_2O, 19:1) to give seven subfractions (H21–H27), of which H25 was further purified by semi-preparative high-performance liquid chromatography (HPLC), using an Alltech Apollo silica column (250 mm × 10 mm i.d., 5 μm; *n*-hexane-EtOAc = 18:1, v/v at a flow rate of 2 mL/min) equipped with a Shodex refractive index detector, to afford **7** (25 mg). Fraction H3 (5.0 g) was successively subjected to Sephadex LH-20 column chromatography (CH_2Cl_2–MeOH, 1:1, v/v) and silica gel (100 g) column chromatography to obtain four subfractions (H31–H34), of which H32 was further purified by semi-preparative HPLC, using an Alltech Econosil RP-18 column (250 mm × 10 mm i.d., 10 μm; 100% MeOH at a flow rate of 2 mL/min) equipped with a Shodex refractive index detector, to yield compounds **8** (6 mg) and **9** (5 mg). Compound **10** (26 mg) was isolated from fraction H4 (1.7 g) by passage through a Sephadex LH-20 column (CH_2Cl_2–MeOH, 1:1, v/v) and separation using the same HPLC system (*n*-hexane-EtOAc = 6:1). The *n*-BuOH-soluble fraction (20 g) was subjected to silica gel (500 g) column chromatography, eluting with $CHCl_3$-MeOH (20:1–1:1, v/v), to yield eight fractions (B1–B8) based on TLC analyses. Fraction B5 (2.4 g) was subjected to silica gel (100 g) column chromatography ($CHCl_3$–MeOH, 10:1) and then to ODS (100 g) column chromatography (MeOH–H_2O, 2:3) to give four subfractions (B51–B54), of which B53 was further purified by semi-preparative HPLC (MeOH–H_2O, 2:3) to yield compounds **1** (26 mg), **2** (15 mg), and **3** (66 mg). The purification of fraction B6 (5.0 g) by repeated column chromatography (7:1 $CHCl_3$–MeOH, and 2:3 MeOH–H_2O) and HPLC separation (3:7 MeOH–H_2O) resulted in the isolation of compounds **4** (13 mg), **5** (5 mg), and **6** (19 mg).

Compound **1** was isolated as a colorless gum with negative optical rotation ($[\alpha]_D^{25}$ −83.1, MeOH). The molecular formula of **1** was determined as $C_{25}H_{32}O_{10}$ from its positive mode high-resolution electron spray ionization mass spectroscopic data at m/z 515.1895 $[M + Na]^+$ (calcd. for $C_{25}H_{32}NaO_{10}$, 515.1893), which was compatible with the nuclear magnetic resonance

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Abbreviations: SRB, sulforhodamine B

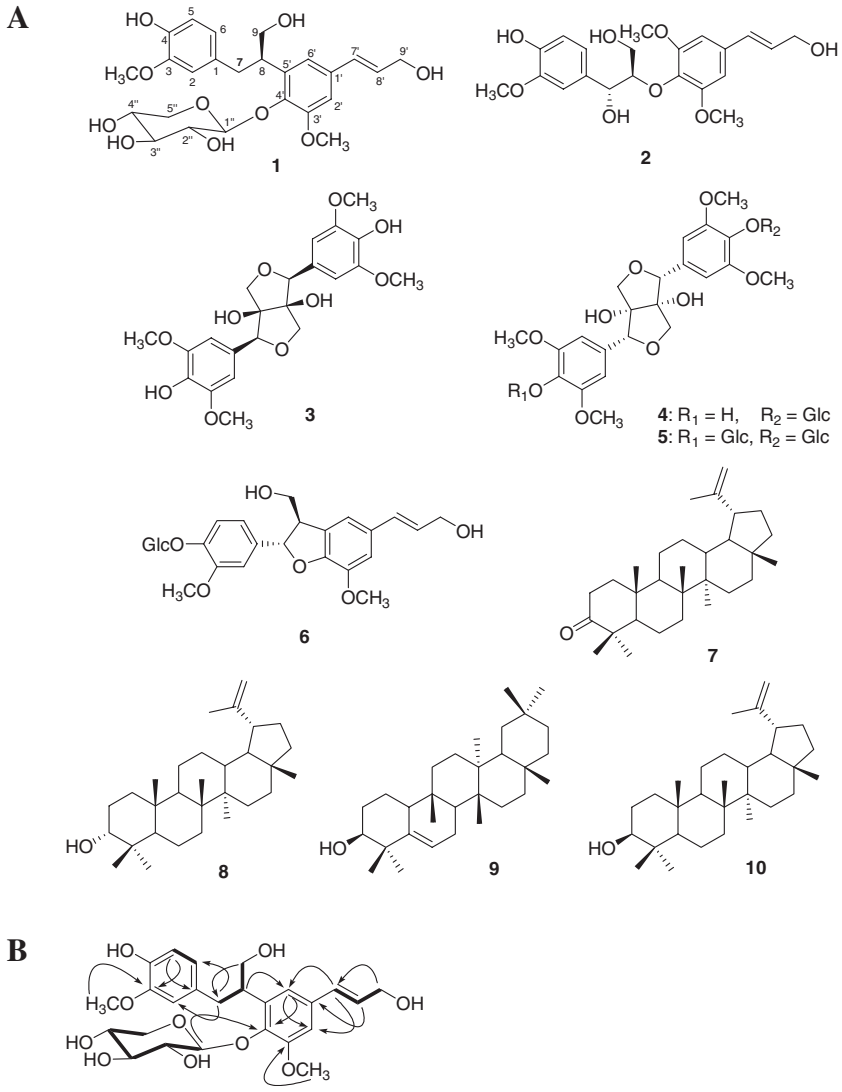

Fig. 1. Structures of Compounds **1**–**10**.
A, Structures of compounds **1**–**10**. B, Selected ^{1}H-^{1}H COSY (bold lines) and HMBC (arrow) correlations for **1**.

(NMR) data. The IR spectrum of **1** showed the presence of hydroxyl (3420 cm^{-1}) and aromatic groups (1615 and 1450 cm^{-1}). The ^{1}H- and ^{13}C-NMR spectral data (Table 1) of **1** resembled those of icariside E_5,[10] with the exception of resonances due to a sugar moiety [δ_H 4.98 (1H, d, $J = 8.0$), 3.84 (1H, m), 3.82 (1H, m), 3.32 (1H, m), 3.22 (1H, dd, $J = 8.5$, 7.5), and 3.17 (1H, dd, $J = 11.0$, 10.5); δ_C 103.3, 76.5, 73.5, 69.8, and 65.5] in **1**. Acid hydrolysis of **1** was conducted by heating a solution of **1** in 1 N HCl (dioxane/H_2O = 1:1, 5 mL) under reflux for 2 h, affording aglycone **1a** and a sugar moiety.[11] Aglycone **1a** was extracted with EtOAc and confirmed as isodehydrodiconiferyl alcohol on the basis of an analysis of its MS and ^{1}H-NMR data (Table 1) and by a comparison with reported spectroscopic data.[10] The sugar moiety obtained from the aqueous layer was analyzed as D-xylose by silica gel co-TLC in comparison with an authentic sample [$CHCl_3$/MeOH/H_2O solvent system (8:5:1); TLC (R_f 0.56)] and by comparing the sign of its specific rotation value ($[\alpha]_D^{25}$ +20.5, H_2O). The heteronuclear multiple-bond correlation (HMBC) spectrum showed that the signal for an anomeric proton at δ_H 4.98 had long-range correlation with the carbon resonance at δ_C 143.5 (C-4′), suggesting that the xylosyl group was located at the C-4′ position of the aglycone skeleton (Fig. 1B). The gross structure of **1** was determined by ^{1}H-^{1}H correlation spectroscopy (COSY), heteronuclear multiple-quantum coherence (HMQC), and HMBC experiments, the key ^{1}H-^{1}H COSY and HMBC correlations being given in Fig. 1B. The absolute configuration of C-8 was established by an analysis of the optical rotation of aglycone **1a** obtained from the acid hydrolysis. The optical rotation value ($[\alpha]_D^{25}$ −15.8, MeOH) of **1a** suggested it to have the 8*R* configuration by a comparison of the optical rotation of **1a** with that ($[\alpha]_D^{25}$ −18.4, MeOH) of the previously reported compound, (8*R*)-isodehydrodiconiferyl alcohol, in the literature.[10,12] On the basis of the foregoing data, the structure of **1** was determined to be (8*R*)-isodehydrodiconiferyl alcohol-4′-*β*-D-xylopyranoside and named acutumoside.[13]

The known compounds were identified as (1*R*)-(4-hydroxy-3-methoxyphenyl)-(2*R*)-[4-[(1*E*)-3-hydroxy-1-propen-1-yl]-2,6-dimethoxyphenoxy]-1,3-propanediol (**2**),[14] (−)-syringaresinol (**3**),[15] (+)-syringaresinol-*O*-*β*-D-glucopyranoside (**4**),[16,17] liriodendrin (**5**),[18] (7*S*,8*R*)-dehydrodiconiferyl alcohol 4-*O*-*β*-D-glucopyranoside (**6**),[19] lupenone (**7**),[20] 3-*epi*-lupeol (**8**),[21] 3*β*-hydroxy-

Table 1. ^{1}H-(500 MHz) and ^{13}C-(125 MHz) NMR Data for Compounds **1** and **1a** in CD_3OD (δ in ppm)

No.	**1**		**1a**
	δ_H	δ_C	δ_H
1		132.3	
2	6.74 br s	113.0	6.57 d (1.5)
3		147.3	
4		144.3	
5	6.65 br s	114.6	6.56 d (8.0)
6	6.65 br s	121.6	6.47 dd (8.0, 1.5)
7	2.93 dd (13.5, 6.5); 2.85 dd (13.5, 9.0)	37.0	2.95 dd (13.5, 6.0); 2.70 dd (13.5, 9.0)
8	3.81 m	42.3	3.95 m
9	3.78 m; 3.62 m	64.7	3.75 m; 3.68 m
1′		133.6	
2′	6.94 d (2.0)	108.5	6.91 d (2.0)
3′		152.0	
4′		143.5	
5′		137.4	
6′	6.89 d (2.0)	119.0	6.90 d (2.0)
7′	6.54 dd (15.5, 1.0)	130.3	6.53 dd (15.5, 1.0)
8′	6.27 dt (15.5, 6.0)	128.2	6.29 dt (15.5, 5.5)
9′	4.21 dd (6.0, 1.0)	62.5	4.21 dd (5.5, 1.0)
3-OCH_3	3.76 s	55.4	3.71 s
3′-OCH_3	3.85 s	55.3	3.85 s
1″	4.98 d (8.0)	103.3	
2″	3.22 dd (8.5, 7.5)	73.5	
3″	3.32 m	76.5	
4″	3.84 m	69.8	
5″	3.82 m; 3.17 dd (11.0, 10.5)	65.5	

*Assignments were based on ^{1}H-^{1}H COSY, HMQC, and HMBC experiments. Well-resolved couplings are expressed with coupling patterns and coupling constants in Hz in parentheses.

glutin-5-ene (**9**),[22] and lupeol (**10**)[23] by comparing the physicochemical and spectroscopic data with previously reported data. To the best of our knowledge, known compounds **2**, **4**, **6**, and **7**–**10** were isolated for the first time from this plant.

Isolated compounds **1**–**10** were evaluated for their antiproliferative activities against the four human tumor cell lines, A549 (non-small cell lung adenocarcinoma), SK-OV-3 (ovarian cancer cells), SK-MEL-2 (skin melanoma), and HCT15 (colon cancer cells) by using the SRB bioassay.[24] Among them, compounds **1**, **2**, **7**, **8**, and **10** had differing antiproliferative effects on these target cell lines, as summarized in Table 2. Compound **2** showed significant inhibitory activity against the proliferation of all the tested cell lines with IC_{50} values in the range of 7.38–18.83 μM. The isolated triterpenoids (**7**–**10**), except for compound **9**, were antiproliferative toward the tested cell lines, compound **8** particularly exhibiting significant antiproliferative activity against all the tested cell lines with IC_{50} values ranging from 9.73 to 12.16 μM. New compound **1** showed weak antiproliferative activity against the A549, SK-OV-3, and SK-MEL-2 cells, with IC_{50} values ranging from 25.87 to 29.10 μM.

In conclusion, we isolated and identified the new lignan glucoside, acutumoside (**1**), together with nine known compounds (**2**–**10**) from *S. acutum* rhizomes and evaluated their antiproliferative activities against four human tumor cell lines. Alkaloids have hitherto been studied with keen interest as the antitumor constituents of *S. acutum*. To our knowledge, this is the first report to investigate non-alkaloidal compounds from *S. acutum* suppressing the survival of cancer cells.

Table 2. Antiproliferative Activities of Compounds **1**–**10** against Four Cultured Human Tumor Cell Lines

Compound	IC_{50} (μM)[a]			
	A549	SK-OV-3	SK-MEL-2	HCT-15
1	25.87	29.10	26.53	>30.0
2	10.03	8.41	7.38	18.83
3	>30.0	>30.0	>30.0	>30.0
4	>30.0	>30.0	>30.0	>30.0
5	>30.0	>30.0	>30.0	>30.0
6	>30.0	>30.0	>30.0	>30.0
7	>30.0	>30.0	18.63	>30.0
8	12.16	11.84	9.73	11.93
9	>30.0	>30.0	>30.0	>30.0
10	>30.0	21.55	13.14	>30.0
Doxorubicin[b]	0.01	0.02	0.01	0.13

[a]IC_{50} value of compounds against each cancer cell line, which was defined as the concentration (μM) that caused 50% inhibition of cell growth *in vitro*. [b]Doxorubicin as a positive control.

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11) Acid hydrolysis of **1**. Compound **1** (9 mg) was hydrolyzed by 1 N HCl (dioxane/H_2O, 1:1, 5 mL) under the reflux condition for 2 h. After cooling, the reaction mixture was diluted with H_2O and then extracted with an equal amount of EtOAc (×3), the EtOAc layer being evaporated under reduced pressure. The resulting residue was dried and purified in a Waters silica gel Sep-Pak Vac 6 cc column ($CHCl_3$–MeOH, 15:1) to give aglycone **1a** (3.5 mg). This aglycone was identified as (8*R*)-isodehydrodiconiferyl alcohol by comparing its ^{1}H-NMR, MS, and specific rotation with the data reported in the literature. The aqueous fraction was neutralized by passage through an Amberlite IRA-67 column and was repeatedly evaporated under

reduced pressure to give a sugar fraction. This sugar fraction was subjected to column chromatography in a silica gel column, eluting with MeCN–H_2O (8:1) to yield D-xylose with a positive specific rotation ($[\alpha]_D^{25}$ +20.5, *c* 1.5 in H_2O). This D-xylose was analyzed by silica gel co-TLC in comparison with an authentic sample [$CHCl_3$/MeOH/H_2O solvent system (8:5:1); TLC (R_f 0.56)].

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13) Acutumoside (**1**): colorless gum; $[\alpha]_D^{25}$ −83.1 (*c* 1.2, MeOH); UV (MeOH) $\lambda_{max}(\log\varepsilon)$ nm: 281 (3.3), 267 (3.7), 217 (4.3); IR (KBr) ν_{max} cm^{-1}: 3420, 2945, 2830, 1661, 1615, 1450, 1353, 1032, 670; ^{1}H-(500 MHz) and ^{13}C-(125 MHz) NMR data, see Table 1; ESIMS (positive-ion mode) *m/z*: 515 $[M + Na]^+$; HR-ESIMS (positive-ion mode) *m/z*: 515.1895 $[M + Na]^+$ (calcd. for $C_{25}H_{32}NaO_{10}$, 515.1893). (8*R*)-Isodehydrodiconiferyl alcohol (**1a**): colorless gum; $[\alpha]_D^{25}$ −15.8 (*c* 0.16, MeOH); UV (MeOH) $\lambda_{max}(\log\varepsilon)$ nm: 225 (3.5); IR (KBr) ν_{max} cm^{-1}: 3451, 2945, 2830, 1680, 1615, 1450, 1354, 1030, 670; ^{1}H-(500 MHz) NMR data, see Table 1; ESIMS (positive-ion mode) *m/z*: 361 $[M + H]^+$.

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