

Dibenzocyclooctene lignan compounds isolated from the fruits of *Schisandra chinensis* Baill

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Abstract – *Schisandra chinensis* Baill. (Schisandraceae) is used commonly in traditional Korean herbal medicine. One new dibenzocyclooctene lignan compound, benzoylgomisin Q, was found, for the first time, in the fruits of *Schisandra chinensis* Baill., together with five known lignan compounds; schisandrin, gomisin A, gomisin G, deoxyschisandrin, and gomisin N, using an ESI-positive MS-preparative HPLC auto-purification system. The structures of these compounds were determined via UV, MS, and NMR spectral studies.

Keywords – *Schisandra chinensis* Baill, Schisandraceae, dibenzocyclooctene, lignan

Introduction

Schisandra chinensis Baill. (Schisandraceae) grows wild in Russia, China, Japan, and Korea (Lebedev, 1971). The seeds and fruits of this plant are used in medical applications. The plant, in Chinese, is called, “Wuweizi” (five taste fruit), in Japanese “Gomishi”, and in Korean, “Omicha”. The fruits of *Schisandra* fall into two categories: *Schisandra chinensis* Baill. (Northern *Schisandra*) and *Schisandra sphenanthera* Rehd. et. Wils. (Southern *Schisandra*). The fruits of *Schisandra* is listed officially in the Korean pharmacopoeia (eighth edition, KP VIII, KFDA), and is indexed as an antioxidant, anticancer, tonic, and anti-aging drug. The fruits of *Schisandra* have been utilized in traditional Korean herbal medicine for thousands of years. The chemistry of this drug has been studied fairly extensively. A host of dibenzocyclooctene lignan compounds have been isolated from the fruits of *Schisandra* (Taguchi and Ikeya, 1975, 1977; Ikeya *et al.*, 1979a, 1979b, 1980, 1988a, 1988b, 1990a, 1990b). In this study, we attempted to determine the structures of the chemical constituents of *Schisandra chinensis* Baill..

Here, we have elucidated the structure of several dibenzocyclooctene lignan compounds; schisandrin, gomisin A, benzoylgomisin Q, gomisin G, deoxyschisandrin, and gomisin N, all of which were isolated simultaneously from *Schisandra chinensis* Baill. (Schisandraceae), using an ESI-positive MS-preparative HPLC auto-purification

system. Benzoylgomisin Q, one of these compounds isolated from the fruits of *Schisandra chinensis* Baill., was, in this study, reported for the first time, to the best of our knowledge.

Material and Methods

Plant materials – The fruits of *Schisandra chinensis* Baill. (Schisandraceae) were purchased from the Kyong-dong market, in Korea.

Instrumentation – ¹H-NMR and ¹³C-NMR spectra were determined with a Varian Unity Inova 500 MHz NMR spectrometer (Varian, Palo Alto, California, USA) in CDCl₃, using tetramethylsilane as an internal standard. Chemical shifts were expressed as δ values. We also utilized a Waters HPLC, a photodiode array detector, a fraction collector II, (Milford, MA, USA), and a Micromass ZQ-4000 mass spectrometer (Manchester, UK) in this study.

Solvents and reagents – All solvents used HPLC-grade in this experiment. The acetonitrile and methanol were purchased from J. T. Baker (USA). The trifluoroacetic acid was purchased from Sigma-Aldrich (USA). A membrane filter (pore size 0.25 μm) was used to filter each of the samples.

ESI-Mass spectrometry – All ESI-MS spectra were obtained with a Micromass ZQ-4000 mass spectrometer (Manchester, UK) which had been equipped with an ESI probe Assay Phoenix/Solo-2 (Waters, USA, P/N M955 015DC6). The spectra were acquired in positive mode.

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The mass ranges were measured at m/z values of 400-600. Mass spectrometer conditions were optimized, in order to ensure maximum sensitivity. These conditions were as follows: Capillary voltage, +3.50 KV; Cone voltage, +50.00 V; Extractor voltage, 5.00 V; RF Lens voltage, 0.50 V; Source temperature, 120°C; Desolvation temperature, 250°C; Cone gas flow, 50 L/hr; Desolvation gas flow, 250 L/hr; Nubulizing N_2 , 100 p.s.i..

Isolation with the ESI-positive MS-preparative HPLC auto-purification system – In this study, we used a Waters ESI-positive MS-Preparative HPLC auto-purification system, equipped with a Waters photodiode-array detector (Milford, MA, USA), a Micromass ZQ-4000 mass spectrometer (Manchester, UK) fitted with an ESI probe, a Waters 2700 sample manager, a Waters Delta 600 gradient pump with a Waters 600 controller, a Waters reagent manager (make up pump), a Waters switching valve (1 or 2), a Waters stream splitter (1000 : 1), and a Waters fraction collector II. All data acquisition and analysis were conducted using Waters MassLynx 3.5 and FractionLynx 3.5 software. The dried fruits of *Schisandra chinensis* Baill. (100 g) were extracted with methanol (300 ml) three times under reflux, for 3 hours, and then filtered. The extract solvents were combined and concentrated *in vacuo*. The concentrated methanol extract (5.8 g) was then suspended in water (50 ml), and partitioned with chloroform in order to obtain the chloroform extract (1.2 g). The aqueous layer was concentrated, and the residue dissolved in methanol, then subjected to the Waters XTerra Preparative MS C_{18} column (7.8×100 mm; 5 μ m, Waters, USA, Part No. 186001156) using a Waters ESI-positive MS-Preparative HPLC auto-purification system.

The gradient conditions for solvent A (water), solvent B (MeCN), and solvent C (0.1% TFA), were programmed as follows: 0.00 min, 25% (B) and 10% (C); 1.00 min, 25% (B) and 10% (C); 30.00 min, 50% (B) and 10% (C); 35.00 min, 25% (B) and 10% (C); 40.00 min, 25% (B) and 10% (C); (Table 1.) The flow rate was set to 10.00

ml/min, and the column temperature was set to 40°C. The eluent was monitored by absorption at wavelengths of 254 nm and 365 nm, and the fractions corresponding to every peak time were collected. An aliquot of each of the fractions was transferred to a 13×100 mm (10.00 ml) glass test tube, and the solvent was evaporated using a Genevac EZ-2 evaporation system (CM Corporation, UK).

The results for the lignan compounds are summarized in Table 2. The results for the lignan compounds were as follows: schisandrin (**1**) (6.89 min, $[M]^+$ m/z 432), gomisin A (**2**) (8.95 min, $[M]^+$ m/z 416), benzoylgomisin Q (**3**) (11.19 min, $[M]^+$ m/z 552), gomisin G (**4**) (13.59 mg, $[M]^+$ m/z 536), deoxyschisandrin (**5**) (17.53 min, $[M]^+$ m/z 416), and gomisin N (**6**) (19.96 min, $[M]^+$ m/z 400), respectively.

Isolation of compound (1)–(6) – Fraction 1–6 were isolated using a Preparative MS C_{18} column (7.8×100 mm; 5 μ m) with a water-MeCN-0.1% TFA eluent. Compound **1** (9.2 mg), **2** (18.8 mg), **3** (12.3 mg), **4** (12.1 mg), **5** (9.6 mg), **6** (10.7 mg) were obtained.

Compound 1 – Colorless prisms, $C_{24}H_{32}O_7$, UV λ_{max} nm 218, 254sh, 280sh; ESI positive Mass: m/z 455 ($[M+Na]^+$, 100), 433 ($[M+H]^+$, 25). 1H -NMR (500 MHz, $CDCl_3$): δ 0.82 (3H, *d*, C-8- CH_3 , J = 6.0 Hz), 1.26 (3H, *s*, C-7- CH_3), 1.79 (H, *s*, C-7- OH), 1.88 (H, *t*, C-8-H, J = 7.0 Hz), 2.36 (1H, *t*, H-6 β , J = 3.5 Hz), 2.38 (1H, *t*, H-9a, J = 3.0 Hz), 2.65 (1H, *t*, H-9 β , J = 2.0 Hz), 2.67 (1H, *t*, H-6a, J = 1.5 Hz), 3.58, 3.59, 3.87, 3.88, 3.89, 3.90 (18H, *s*, 1, 2, 3, 12, 13, 14- OCH_3), 6.53 (H, *s*, H-4), 6.61 (H, *s*, H-11). ^{13}C -NMR (125 MHz, $CDCl_3$): Table 3.

Table 1. Solvent gradient condition for ESI-positive MS-Preparative HPLC auto-purification system

Time (min)	Flow rate (ml min ⁻¹)	Water	MeCN	0.1% TFA
0.00	10.00	65	25	10
1.00	10.00	65	25	10
30.00	10.00	40	50	10
35.00	10.00	65	25	10
40.00	10.00	65	25	10

Table 2. Summary of dibenzocyclooctene lignans of isolated from the fruits of the *Schisandra chinensis* Baill. by ESI-positive MS-Preparative HPLC auto-purification system

Isolation of compound HPLC retention time (min)	ESI positive MS (m/z)
Compound 1	6.89 455 $[M+Na]^+$, 433 $[M+H]^+$
Compound 2	8.95 439 $[M+Na]^+$, 417 $[M+H]^+$
Compound 3	11.19 575 $[M+Na]^+$, 553 $[M+H]^+$, 453 $[M+Na]^+ -C_6H_5COOH$, 431 $[M+H]^+ -C_6H_5COOH$
Compound 4	13.59 559 $[M+Na]^+$, 537 $[M+H]^+$, 437 $[M+Na]^+ -C_6H_5COOH$, 415 $[M+H]^+ -C_6H_5COOH$
Compound 5	17.53 439 $[M+Na]^+$, 417 $[M+H]^+$
Compound 6	19.96 423 $[M+Na]^+$, 401 $[M+H]^+$

Table 3. ^{13}C -NMR chemical shift of isolated dibenzocyclooctene lignans (CDCl_3 , 125 MHz)

Carbon No.	Schisandrin (1)	Gomisin A (2)	Benzoyl Gomisin Q (3)	Gomisin G (4)	Deoxyschisandrin (5)	Gomisin N (6)
1	152.12	152.38	152.39	141.44	151.84	151.87
2	141.06	141.02	141.03	135.39	140.31	140.30
3	152.66	152.57	152.19	148.98	153.11	151.80
4	110.71	110.57	110.32	106.39	107.43	110.90
5	132.03	132.24	130.42	133.46	139.37	134.32
6	41.09	40.77	84.47	84.58	35.84	39.36
7	72.17	71.93	72.65	72.76	41.01	33.78
8	42.07	42.27	42.34	42.43	33.99	40.95
9	34.53	33.99	36.96	36.99	39.38	35.65
10	134.09	132.74	136.66	135.39	133.94	138.05
11	110.23	106.18	107.53	107.54	110.75	103.18
12	152.32	148.14	153.41	153.41	151.74	148.88
13	140.48	135.19	140.12	141.04	139.97	134.79
14	151.82	141.47	151.95	151.45	151.54	141.32
15	123.04	122.13	122.91	121.96	123.64	121.60
16	124.46	124.41	122.15	121.16	122.59	123.55
17	16.09	16.02	19.26	19.16	12.90	21.74
18	30.00	30.29	28.33	28.49	21.99	13.07
OCH ₃ -1, 14	60.87, 60.84	60.83, 59.88	60.76, 60.41	60.36, 60.11	61.16, 61.15	60.76, 59.85
OCH ₃ -2, 13	61.18, 61.17	61.25	61.89, 60.06	60.76	60.75, 60.74	61.22
OCH ₃ -3, 12	56.24, 56.16	56.21	56.30, 56.15	56.15	56.16, 56.13	56.12
OCH ₂ O		101.07		101.64		100.95
CO-C ₆ H ₅ C-1			129.76	129.74		
CO-C ₆ H ₅ C-2, 6'			130.44, 130.42	130.51, 129.86		
CO-C ₆ H ₅ C-3, 5'			127.91, 127.15	128.43, 127.34		
CO-C ₆ H ₅ C-4			133.31	133.42		
C=O			166.06	165.02		

Compound 2 – Colorless needles, $\text{C}_{23}\text{H}_{28}\text{O}_7$, UV λ_{max} nm 222, 255sh, 282sh; ESI positive MS: m/z 439 ($[\text{M}+\text{Na}]^+$, 100), 417 ($[\text{M}+\text{H}]^+$, 28). ^1H -NMR (500 MHz, CDCl_3): δ 0.82 (3H, *d*, C-8-CH₃, $J=7.0$ Hz), 1.25 (3H, *s*, C-7-CH₃), 1.86 (H, *t*, C-8-H, $J=7.5$ Hz), 1.79 (H, *s*, C-7-OH), 2.34 (1H, *d*, H-9a, $J=4.0$ Hz), 2.35 (1H, *t*, H-6 β , $J=5.0$ Hz), 2.60 (1H, *dd*, H-9 β , $J=1.5$ Hz, $J=1.0$ Hz), 2.69 (1H, *d*, H-6a, $J=13.5$ Hz), 3.52, 3.83, 3.89, 3.90 (12H, *s*, 1, 2, 3, 14-OCH₃), 5.96 (*s*, OCH₂O), 6.48 (H, *s*, H-11), 6.62 (H, *s*, H-4). ^{13}C -NMR (125 MHz, CDCl_3): Table 3.

Compound 3 – A white amorphous powder. $\text{C}_{31}\text{H}_{36}\text{O}_9$, UV λ_{max} nm: 224, 255sh, 289sh; ESI positive MS: m/z 575 ($[\text{M}+\text{Na}]^+$, 14), 553 ($[\text{M}+\text{H}]^+$, 100), 453 ($[[\text{M}+\text{Na}]^+ - \text{C}_6\text{H}_5\text{COOH}]$, 56), 431 ($[[\text{M}+\text{H}]^+ - \text{C}_6\text{H}_5\text{COOH}]$, 61). ^1H -

NMR (500 MHz, CDCl_3): δ 1.22 (3H, *d*, C-8-CH₃, $J=7.5$ Hz), 1.36 (3H, *s*, C-7-CH₃), 1.60 (H, *brs*, C-7-OH), 2.18 (H, *m*, C-8-H), 2.29 (1H, *d*, H-9 β , $J=8.5$ Hz), 2.43 (1H, *d*, H-9a, $J=6$ Hz), 3.17, 3.39, 3.59, 3.89, 3.93, 3.98 (18H, *s*, 1, 2, 3, 12, 13, 14-OCH₃), 5.90 (1H, *s*, H-6), 6.69 (H, *s*, H-11), 6.82 (H, *s*, H-4), 7.20-7.50 (5H, *m*, C-6-OCO C₆H₅); ^{13}C -NMR (125 MHz, CDCl_3): Table 3.

Compound 4 – Colorless prisms, $\text{C}_{30}\text{H}_{32}\text{O}_9$, UV λ_{max} nm: 224, 255sh, 290sh; ESI (positive)-MS: m/z 559 ($[\text{M}+\text{Na}]^+$, 33), 537 ($[\text{M}+\text{H}]^+$, 91), 437 ($[[\text{M}+\text{Na}]^+ - \text{C}_6\text{H}_5\text{COOH}]$, 49), 415 ($[[\text{M}+\text{H}]^+ - \text{C}_6\text{H}_5\text{COOH}]$, 62). ^1H -NMR (500 MHz, CDCl_3): δ 1.17 (3H, *d*, C-8-CH₃, $J=4.5$ Hz), 1.33 (3H, *s*, C-7-CH₃), 1.57 (H, *brs*, C-7-OH), 2.09 (H, *m*, C-8-H), 2.00-2.60 (2H, *m*, C-9-H), 3.16, 3.41, 3.82, 3.98 (12H, *s*, 1, 12, 13, 14-OCH₃), 5.89 (1H, *s*, H-

6), 6.00 (2H, s, OCH₂O), 6.70 (H, s, H-11), 6.77 (H, s, H-4), 7.20-7.50 (5H, m, C₆-OCO C₆H₅); ¹³C-NMR (125 MHz, CDCl₃): Table 3.

Compound 5 – Colorless prisms, C₂₄H₃₂O₆, UV λ_{max} nm: 220, 255sh, 280sh; ESI positive MS: *m/z* 439 ([M+Na]⁺, 100), 417 ([M+H]⁺, 20). ¹H-NMR (500 MHz, CDCl₃): δ 0.74 (3H, *d*, C-8-CH₃, *J*=7.0 Hz), 1.00 (3H, *s*, C-7-CH₃), 1.89 (H, *m*, C-7-H), 1.89 (H, *m*, C-8-H), 2.01 (1H, *d*, H-9β, *J*=13.5 Hz), 2.06 (1H, *d*, H-9α, *J*=12.5 Hz), 3.58, 3.58, 3.88, 3.88, 3.89, 3.89 (18H, *s*, 1, 2, 3, 12, 13, 14-OCH₃), 6.53 (H, *s*, H-11), 6.54 (H, *s*, H-4); ¹³C-NMR (125 MHz, CDCl₃): Table 3.

Compound 6 – Colorless prisms, C₂₃H₂₈O₆, mp 106-108°. UV λ_{max} nm 222, 255sh, 275sh; ESI positive MS: *m/z* 423 ([M+Na]⁺, 100), 401 ([M+H]⁺, 15). ¹H-NMR (500 MHz, CDCl₃): δ 0.73 (3H, *d*, C-8-CH₃, *J*=7.0 Hz), 0.97 (3H, *s*, C-7-CH₃), 1.88 (H, *m*, C-7-H), 1.89 (H, *m*, C-8-H), 2.00 (1H, *d*, H-9β, *J*=13.5 Hz), 2.25 (1H, *d*, H-9α, *J*=13.5 Hz), 2.57 (2H, *m*, C-6-H), 3.55, 3.81, 3.90, 3.90 (12H, *s*, 1, 2, 3, 14-OCH₃), 5.94 (s, OCH₂O), 6.47 (H, *s*, H-11), 6.55 (H, *s*, H-4); ¹³C-NMR (125 MHz, CDCl₃): Table 3.

Results and Discussion

Each of the fractions was isolated as a single peak using the ESI-positive MS and preparative HPLC auto-

purification systems. The preparative HPLC eluent was fractionated at every single peak, at 6.89 min (fraction 1), 8.95 min (fraction 2), 11.19 min (fraction 3), 13.59 min (fraction 4), 17.53 min (fraction 5), and 19.96 min (fraction 6). Fractions 1, 2, 3, 4, 5 and 6 were obtained at yields of 9.2, 18.8, 12.1, 12.3, 10.7, 9.6 mg, respectively. Six lignan compounds, including one new compound, 3, were isolated from the chloroform fractions of the fruit of *Schisandra chinensis* Baill. These compounds were structurally identified via spectroscopic methods, including UV, ESI-MS, and ¹H- and ¹³C-NMR spectroscopy.

The structure of compound 1~2, and 4~6 were elucidated as schisandrin, gomisin A, gomisin G, deoxy-schisandrin, and gomisin N, respectively, by comparing its spectral data with those in literature (Ikeya *et al.*, 1979a, 1979b, 1980, 1988a, 1988b, 1990a, Taguchi and Ikeya, 1977). The structure and the NMR data are presented in Fig. 1 and Table 3, respectively.

Compound 3 was isolated as a white amorphous powder, C₃₁H₃₆O₉, and exhibited a UV spectrum (λ_{max} nm 224, 255sh, 289sh) characteristic of a dibenzocyclooctene lignan compound. The ESI-positive MS spectrum (Table 2), with ion peaks at *m/z* 575 [M+Na]⁺, 553 [M+H]⁺, 453 [[M+Na]⁺-C₆H₅COOH] and 431 [[M+H]⁺-C₆H₅COOH] verified the presence of a benzoyloxyl group in compound 3, and also supported the conclusion that the molecular weight of 3 was 552. ¹H-NMR spectral

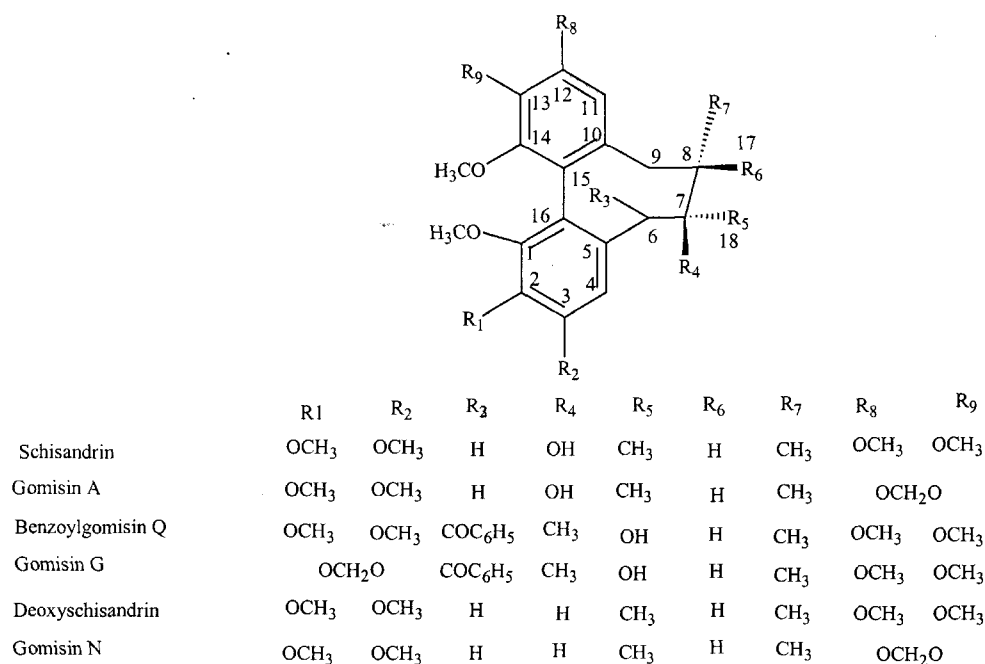


Fig. 1. Structure of dibenzocyclooctene lignans isolated from the fruits of *Schisandra chinensis* Baill..

analyses of compound **3** revealed the presence of a benzoyloxyl (δ 7.20-7.50 ppm) group. ^{13}C -NMR spectral (Table 3.) analyses of compound **3** indicated the presence of a benzoyloxyl group (δ 129.76, 130.44, 130.42, 127.91, 127.15, 133.31, 166.06 ppm). The ^1H -NMR spectral analyses of compound **3** indicated the presence of six methoxy (δ 3.17, 3.39, 3.59, 3.89, 3.93, 3.98 ppm) groups in its aromatic rings, and a secondary methyl (δ 1.22 ppm) group and tertiary methyl (δ 1.36 ppm) group attached to a carbon that carried a hydroxyl (δ 1.60 ppm) group and a benzoyloxyl group on the cyclooctene ring. The ^{13}C -NMR spectral (Table 3.) analyses of compound **3** revealed the presence of C-17 axial secondary methyl (δ 19.26 ppm) and C-18 equatorial methyl carbons (δ 28.33 ppm), benzylic methylene (C-6 and C-9) and methine (C-7 and C-8) carbons (δ 84.47, 36.96; δ 72.65, 42.34 ppm), methoxy (OCH_3 -1, 2, 3, 12, 13, 14) carbons (δ 60.76, 61.89, 56.30, 56.15, 60.06, 60.41 ppm), and aromatic (C-1, 2, 3, 4, 5, 10, 11, 12, 13, 14, 15, 16) carbons (δ 152.39, 141.03, 152.19, 110.32, 130.42, 136.66, 107.53, 153.41, 140.12, 151.95, 122.91, 122.15 ppm). Based on these spectral data, compound **3** was identified as benzoylgomisin Q. Compound **3** has never before been reported to exist in the fruits of *Schisandra chinensis* Baill. However, it has been isolated from the fruit of *Schisandra sphenanthera* Rehd. et wils. (Ikeya *et al.*, 1990b).

In conclusion, the ESI-positive MS-preparative HPLC auto-purification system has proven to be a fast and effective method for the structural investigation of the dibenzocyclooctene lignan compounds in the fruit of *Schisandra chinensis* Baill.. The considerable fragmentation information acquired via ESI-positive MS was extremely useful in the structural elucidation of both known and unknown compounds. We simultaneously isolated six lignan compounds; schisandrin, gomisin A, benzoylgomisin Q, gomisin G, deoxyschisandrin, and gomisin N, from the fruits of *Schisandra chinensis* Baill., and elucidated their structures via UV, MS, and NMR spectral analyses. Compound **3**, benzoylgomisin Q, was isolated from *Schisandra sphenanthera* Rehd. et Wils. (Ikeya *et al.*, 1990b), but there is no previous report of this compound being isolated from *Schisandra chinensis* Baill..

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