A New Coumarin Glucoside from *Daphne arisanensis*

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A new coumarin glucoside named daphneside was isolated along with two known coumarin glucosides, daphnin and daphnetin-8-glucoside, and two known phenylpropanoid glucosides, syringin and syringinoside, from a water-soluble fraction of *Daphne arisanensis* HAYATA (Thymelaeaceae) collected in Taiwan. The structure of daphneside was elucidated by spectroscopic and chemical methods.

Keywords Thymelaeaceae; *Daphne arisanensis*; coumarin; phenylpropanoid; glucoside; daphneside; HPLC; CD

Our continuous study on the constituents of the Thymelaeaceae plants[2)] resulted in the isolation of a new coumarin glucoside named daphneside along with four known compounds (daphnin, daphnetin-8-glucoside, syringin, and syringinoside) from a water-soluble fraction of *Daphne arisanensis* HAYATA collected in Taiwan. This paper deals with the isolation and structure elucidation of daphneside by means of spectroscopic and chemical methods.

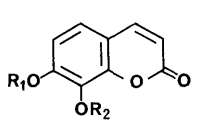
Results and Discussion

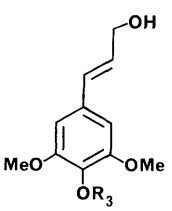
The methanol extract of the bark and root of the plant was subjected successively to partition to afford the fractions soluble in *n*-hexane, benzene, ethyl acetate, *n*-butanol and water. The water-soluble fraction was charged on an Amberlite XAD-2 resin column. Furthermore, the fraction eluted with 70% aqueous ethanol was separated by a combination of reversed-phase medium pressure and high-performance liquid chromatographies (MPLC and HPLC) to give five cmpounds (**1**—**5**).

Compound **1**, named daphneside, was obtained as colorless needles, mp 237 °C (dec.), $[\alpha]_D$ −17.1°. The molecular formula was determined as $C_{21}H_{26}O_{14}$ from the high-resolution fast atom bombardment mass spectrum (HR-FAB-MS). In the proton and carbon-13 nuclear magnetic resonance (^{1}H- and ^{13}C-NMR) spectra, the signals at δ_H 5.30 (1H, d, J=7.6 Hz), 5.34 (1H, d, J=7.6 Hz), and δ_C 102.6 (d), 105.4 (d) suggested the presence of two sugar moieties in the molecule. Furthermore, the signals at δ_H 6.47 (1H, d, J=9.5 Hz), 7.34 (1H, d, J=8.8 Hz), 7.44 (1H, d, J=8.8 Hz), 8.02 (1H, d, J=9.5 Hz), and δ_C 115.3 (d), 117.7 (d), 127.3 (d), 147.9 (d), 165.6 (s), coupled with the infrared (IR) absorptions at ν 1736, 1610 cm^{-1} suggested the presence of a coumarin skeleton. In fact the hydrolysis of daphneside (**1**) with 5% hydrochloric acid afforded daphnetin (**6**).[3)] This result suggested that the structure of daphneside may be daphnetin-7,8-di-*O*-saccharide (**1**), daphnetin-7-*O*-disaccharide (**7**) or daphnetin-8-*O*-disaccharide (**8**).

Recently, Nakanishi *et al.*[4)] reported the microscale structure determination of oligosaccharides using circular dichroism (CD) measurements for the identification of the component monosaccharides, linkage pattern, and absolute configuration.

Daphneside was esterified with *p*-bromobenzoyl chloride in the presence of silver trifluoromethanesulfonate (AgOTf) and 4-dimethylaminopyridine (DMAP) in pyridine to give daphneside octakis (*p*-bromobenzoate) (**9**) whose structure was confirmed by FAB-MS (*m/z* 1966.4 $(M+H)^+$). The benzoate (**9**) was subjected to a series of operations [i) HBr cleavage in $BrCH_2COOH$, ii) glycosidation with silver acetate and methanol, iii) deprotection with thiourea, iv) *p*-methoxycinnamoylation]. The structure **1** gives only a per-*p*-bromobenzoylated monosaccharide as a degradation product. On the other hand, the structure **2** or **3** gives a *p*-methoxycinnamoylated monosaccharide *p*-bromobenzoate as well as a per-*p*-bromobenzoated monosaccharide. The HPLC of the degradation product showed only one peak,





1 : $R_1=R_2=\beta$-D-glucopyranosyl
2 : R_3=6-*O*-β-D-glucopyranosyl-β-D-glucopyranosyl
3 : $R_1=\beta$-D-glucopyranosyl, R_2=H
4 : $R_3=\beta$-D-glucopyranosyl
5 : R_1=H, $R_2=\beta$-D-glucopyranosyl
6 : $R_1=R_2$=H
7 : R_1=sugar-sugar, R_2=H
8 : R_1=H, R_2=sugar-sugar

Chart 1

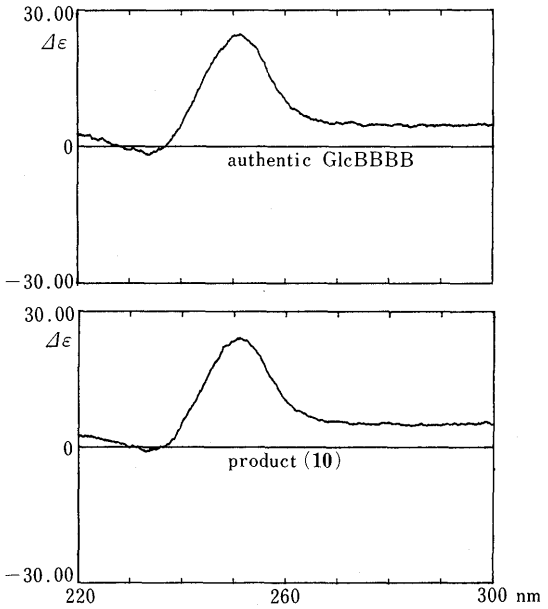


Fig. 1. CD Spectra of Methyl β-D-Glucopyranoside Tetrakis(*p*-bromobenzoate) (GlcBBBB) (**10**) in Acetonitrile

which was identified as methyl β-glucopyranoside tetrakis (*p*-bromobenzoate) (**10**) by comparison of the retention time (t_R 5.95) and FAB-MS (*m/z* 927 $(M+H)^+$), and no other peak corresponding to *p*-methoxycinnamoylated monosaccharide. Furthermore, the CD spectrum of this product after HPLC purification was in excellent agreement with one of the synthetic standard from D-glucose as shown in Fig. 1.[4] As discussed above, the structure of daphneside should be represented as daphnetin-7,8-di-*O*-β-D-glucopyranoside (**1**).

The mass and ^{1}H-NMR spectra of compounds **3** and **5** were very similar to each other and both compounds gave the same products, daphnetin (**6**) and D-glucose, on hydrolysis with 5% hydrochloric acid. These results suggested **3** and **5** to be daphnetin monoglucosides. The linkage position of glucose was determined by a nuclear Overhauser effect (NOE) experiment. On irradiation of the anomeric proton (δ_H 4.92) in **3**, NOE (6.6%) was observed at H-6 (δ_H 7.03). Therefore, compound **3** and **5** were characterized as daphnetin-7-*O*-β-D-glucopyranoside (daphnin) and daphnetin-8-*O*-β-D-glucopyranoside, respectively.[5,6]

Compounds **2** and **4** have a common structure, the dimethoxyphenyl propenol moiety. Compound **2** has two sugar moieties (δ_H 4.24 (1H, d, J=7.8 Hz) and 4.87 (1H, d, J=7.6 Hz)) and compound **4** has one sugar moiety (δ_H 4.87 (1H, d, J=7.6 Hz)). These data are consistent with those of syringinoside (**2**) and syringin (**4**), respectively.[2]

Recently Chinese chemists reported that daphnin (**3**) inhibited rabbit platelet agrregation, prolonged the clotting time of blood, and reduced rat platelet adhesion.[7] Daphnetin (**6**) is slightly soluble in water, and daphnetin monoglucosides (**3** and **5**) are poorly soluble in water, but daphneside (**1**) is freely soluble in water. So we examined **1** for biological activity.

Experimental

General Procedures IR spectra were recorded on a JASCO FT-IR 5000 infrared spectrophotometer in KBr disk, ultraviolet (UV) spectra on a JASCO UVIDEC-610, optical rotations on a JASCO DIP-181 polarimeter at 25 °C, CD spectra on a JASCO J-600 spectropolarimeter in MeCN solution with $c=1\times10^{-5}$ M, and ^{1}H- and ^{13}C-NMR spectra on a JEOL GX-400 NMR spectrometer. MS (electron impact (EI) and FAB) spectra were measured on a Hitachi M-80 or JEOL HX-110 mass spectrometer. HPLC was carried out with a JASCO BIP-I (detector: JASCO UVIDEC-100-V, 254 nm).

Extraction The bark and root of the plant (1.0 kg) collected in the south of Taiwan in 1986 were immersed in MeOH (5 l) at room temperature for 2 weeks. Evaporation of MeOH under reduced pressure afforded a brownish residue (152 g), which was partitioned 3 times between hexane (each 400 ml) and 50% aqueous MeOH (1500 ml). The aqueous MeOH solution was concentrated to half the initial volume under reduced pressure, followed by dilution with water (500 ml). The aqueous solution was extracted 3 times successively with benzene (each 400 ml), AcOEt (each 400 ml), and BuOH (each 300 ml). The yields of the hexane-, benzene-, AcOEt-, BuOH-, and water-soluble fractions were 8.4, 1.0, 14.8, 15.9, 107.2 g, respectively.

Isolation A part of the above water-soluble fraction (60 g) was chromatographed on Amberlite XAD-2 (500 ml) with H_2O and then 70% aqueous EtOH to give fractions (53.3, 4.1 g). The second fraction (4.1 g) eluted with aqueous EtOH was repeatedly chromatographed on reversed-phase silica gel (Develosil C8-30/50, i.d. 25×250 mm), using MeOH–H_2O (30 : 70 and 15 : 85) to give the crude cmpounds. Each crude cmpound was purified by HPLC (Develosil C8-5, i.d. 10×250 mm), MeOH–H_2O (20 : 80), to give pure **1** (18 mg), **2** (15 mg), **3** (18 mg), **4** (66 mg), and **5** (12 mg).

Compound **1**: Colorless needles, mp 237 °C (dec.). $[\alpha]_D$ −17.1° (c=0.29, H_2O). HR FAB-MS *m/z*: Found 525.1205, $(C_{21}H_{26}O_{14}+Na)^+$ requires 525.1221. UV λ_{max} (MeOH) nm (ε): 304 (11200), 253 (3700), 205 (33800). IR ν (KBr): 3300 br., 1736, 1610 cm^{-1}. ^{1}H-NMR (D_2O): 3.45—4.05 (12H, complex), 5.30 (1H, d, J=7.6 Hz), 5.34 (1H, d, J=7.6 Hz), 6.47 (1H, d, J=9.5 Hz), 7.34 (1H, d, J=8.8 Hz), 7.44 (1H, d, J=8.8 Hz), 8.02 (1H, d, J=9.5 Hz). ^{13}C-NMR (D_2O): 62.8 (t), 62.8 (t), 71.57 (d), 71.61 (d), 75.1 (d), 76.0 (d), 77.8 (d), 77.9 (d), 78.6 (d), 78.6 (d), 102.6 (d), 105.4 (d), 115.3 (d), 115.7 (s), 117.7 (d), 127.3 (d), 134.4 (s), 147.9 (d), 149.5 (s), 154.2 (s), 165.6 (s).

Compound **3**: $[\alpha]_D$ −92.4° (c=0.13, CH_3OH). FAB-MS *m/z*: 341 $(M+H)^+$. ^{1}H-NMR (CD_3OD): 3.35—3.60 (4H, complex), 3.71 (1H, dd, J=12.0, 5.1 Hz), 3.90 (1H, dd, J=12.0, 2.0 Hz), 4.92 (1H, d, J=7.6 Hz), 6.28 (1H, d, J=9.5 Hz), 7.03 (1H, d, J=8.8 Hz), 7.21 (1H, d, J=8.8 Hz), 7.86 (1H, d, J=9.5 Hz). This was identical with an authentic sample of daphnetin-7-glucopyranoside.[5]

Compound **5**: $[\alpha]_D$ +31.0° (c=0.14, CH_3OH). FAB-MS *m/z*: 341 $(M+H)^+$. ^{1}H-NMR (CD_3OD): 3.40—3.60 (4H, complex), 3.72 (1H, dd, J=12.0, 4.6 Hz), 3.80 (1H, dd, J=12.0, 2.4 Hz), 4.87 (1H, d, J=8.1 Hz), 6.22 (1H, d, J=9.5 Hz), 6.55 (1H, d, J=8.6 Hz), 7.29 (1H, d, J=8.6 Hz), 7.86 (1H, d, J=9.5 Hz). This was identical with an authentic sample of daphnetin-8-glucopyranoside.[6]

Compound **2**: $[\alpha]_D$ −38.1° (c=0.17, H_2O). FAB-MS *m/z*: 535 $(M+H)^+$. ^{1}H-NMR (CD_3OD): 3.86 (6H, s), 4.22 (2H, dd, J=5.6, 1.5 Hz), 4.24 (1H, d, J=7.8 Hz), 4.87 (1H, d, J=7.6 Hz), 6.33 (1H, dt, J=15.9, 5.6 Hz), 6.55 (1H, dt, J=15.9, 1.5 Hz), 6.75 (2H, s). This was identical with an authentic sample of syringinoside.[2]

Compound **4**: $[\alpha]_D$ −16.1° (c=0.11, H_2O). FAB-MS *m/z*: 373 $(M+H)^+$. ^{1}H-NMR (CD_3OD): 3.86 (6H, s), 4.22 (2H, dd, J=5.6, 1.7 Hz), 4.87 (1H, d, J=7.6 Hz), 6.32 (1H, dt, J=15.9, 5.6 Hz), 6.54 (1H, dt, J=15.9, 1.7 Hz), 6.75 (2H, s). This was identical with an authentic sample of syringin.[2]

Bromobenzoylation of Daphneside (1) A mixture of daphneside (**1**) (2.2 mg, 4.38 μmol), *p*-bromobenzoyl chloride (9.2 mg, 42.0 μmol), AgOTf (10.8 mg, 42.0 μmol), and DMAP (1 crystal) in pyridine (500 μl) was stirred overnight at room temperature. After dilution with $CHCl_3$ the reaction mixture was passed through a SiO_2 pipet column and eluted with $CHCl_3$. The eluate was concentrated and the residue was separated by preparative thin layer chromatography (TLC) (Merck 5744, $CHCl_3$–benzene; 2 : 1) to give the desired compound (**9**) (6.1 mg). FAB-MS *m/z*: 1966.4 $(C_{77}H_{51}O_{22}Br_8+H)^+$. The molecular ion distribution pattern was in complete agreement with the theoretical pattern.

Preparation of Methyl β-Glucopyranoside Tetrakis(*p*-bromobenzoate) (10) A mixture of methyl β-D-glucopyranoside (Nacalai Tesque, 15.9 mg, 81.9 μmol), *p*-bromobenzoyl chloride (86.2 mg, 393 μmol), AgOTf (101 mg, 393 μmol), and DMAP (2 mg) in pyridine (0.1 ml) was stirred overnight at room temperature. After dilution with $CHCl_3$, the reaction mixture was separated by SiO_2 column chromatography and preparative TLC (Merck 13895, benzene) to give the desired compound (**10**) (41 mg). FAB-MS *m/z*: 927 $(C_{37}H_{26}O_{10}Br_4+H)^+$. The molecular ion distribution pattern was in excellent agreement with the theoretical pattern. ^{1}H-NMR ($CDCl_3$): 3.54 (3H, s), 4.14 (1H, complex), 4.50 (1H, dd, J=4.9, 12.2 Hz), 4.64 (1H, dd, J=3.4, 12.2 Hz), 4.75 (1H, d, J=8.1 Hz), 5.47 (1H, dd, J=8.1, 9.8 Hz), 5.63 (1H, dd, J=9.8, 9.8 Hz), 5.82 (1H, dd, J=9.5, 9.78 Hz), 7.43 (2H, d, J=8.5 Hz), 7.48 (2H, d, J=8.5 Hz), 7.52 (2H, d, J=8.5 Hz), 7.54 (2H, d, J=8.5 Hz), 7.67 (2H, d, J=8.5 Hz), 7.72 (2H, d, J=8.5 Hz), 7.80 (2H, d, J=8.5 Hz), 7.85 (2H, d, J=8.5 Hz).

Cleavage Reaction of Daphneside Octakis(*p*-bromobenzoate) (9) and Glycosidation Reaction of the Products Under an argon atmosphere, bromoacetyl bromide (250 μl, 2.87 mmol) and water (50 μl, 2.77 mmol) were added to daphneside octakis (*p*-bromobenzoate) (**9**) (2.2 mg, 1.1 μmol) in a special glass tube[4] at −78 °C. The vessel was sealed with a Teflon cap, and the mixture was stirred at room temperature for 12 h, then cooled again to −78 °C and the Teflon valve was carefully opened (HBr gas is released when the seal is broken). HBr gas was removed *in vacuo* under aspirator pressure for 5 min and then under high vacuum for 30 min. The resulting solid was treated with AgOAc (10 mg) in MeOH (0.2 ml) at room temperature for 1 h in the dark under an argon atmosphere. Silver salts were removed by filtration and the filtrate was concentrated. The resulting solid residue was suspended in hexane–EtOAc (2 : 1, 1 ml) and passed through a Pasteur pipett filled with a slurry of neutral Al_2O_3 in hexane–EtOAc (2 : 1). The Al_2O_3 column was washed with EtOAc (5 ml), and the eluate and washings were combined and concentrated to give a residue, which was lyophilized with benzene (0.2 ml).

Deprotection and Cinnamoylation Reactions Thiourea (3 mg) was added to a solution of the product mixture in CH_2Cl_2–MeOH (2 : 1, 0.3 ml), and

the mixture was stirred at room temperature for 2 h. $AgNO_3$ (10 mg) in CH_3CN (0.5 ml) was then added with stirring for an additional 5 min. The mixture was diluted with CH_2Cl_2 (3 ml) and passed through a Pasteur pipet filled with SiO_2 (0.5 g). The SiO_2 column was washed with CH_2Cl_2–MeOH (9 : 1, 10 ml), and the eluate and washings were concentrated to dryness, then lyophilized with benzene (0.2 ml) to give an amorphous powder.

p-Methoxycinnamoyl chloride (5 mg), AgOTf (5 mg) and DMAP (1 crystal) were added to a solution of the product in pyridine (0.2 ml) under an argon atmosphere. The reaction was allowed to proceed at room temperature for 12 h in the dark, then water (1 drop) was added, and the mixture was stirred for an additional 1 h. The reaction mixture was concentrated to dryness, suspended in hexane–EtOAc (2 : 1, 1 ml), then passed through a Pasteur pipet filled with 1 g of a neutral Al_2O_3 slurry in hexane–EtOAc (2 : 1). The Al_2O_3 column was washed with EtOAc (5 ml), and the eluate and washings were concentrated to afford a residue, which was separated by HPLC (column, Cosmosil 5SL, i.d. 4.6 × 150 mm; solvent, hexane–EtOAc (80 : 20); flow rate, 1.0 ml/min). t_R 5.95 min. FAB-MS *m/z*: 927 $(M+H)^+$. The molecular ion distribution pattern was identical with that of **10**. UV λ_{max} (CH_3CN): 243 nm. This product was concluded to be methyl β-glucopyranoside tetrakis(*p*-bromobenzoate) (**10**).

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References and Notes

1) Present address: *School of Pharmacy, Kaosiung Medical College, Kaosiung 80731, Taiwan, R. O. C.*
2) M. Niwa, Y. Iwadare, Y. -C. Wu, and Y. Hirata, *Chem. Pharm. Bull.*, **36**, 1158 (1988).
3) Daphnetin (**6**) is commercially available.
4) W. T. Wiesler, N. Berova, M. Ojika, H. V. Meyers, M. Chang, P. Zhou, L. -C. Lo, M. Niwa, R. Takeda, and K. Nakanishi, *Helv. Chim. Acta*, **73**, 509 (1990).
5) An authentic sample of daphnin (**3**) was isolated from *Daphne odora* THUNB.
6) An authentic sample of daphnetin-8-glucoside was kindly provided by Professor F. Murai (Aichi Medical University).
7) S. -Y. Qu, X. -L. Jiang, X. -H. Zhao, D. -B. Pan, X. -R. Wang, Y. -L. Chen, G. Pan, and Y. -J. Wu, *Acta Pharmaceutica Sinica*, **21**, 498 (1986).