New Phenylpropanoid Glycosides from the Fruits of *Illicium anisatum*

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Two new phenylpropanoid glycosides and three known sesquiterpene lactones were isolated from the fresh fruits of *Illicium anisatum* L. (Illiciaceae). Their structures were elucidated from spectroscopic and chemical data.

Key words *Illicium anisatum*; Illiciaceae; phenylpropanoid glycoside; sesquiterpene lactone

As part of a continuous investigation on the chemical constituents of *Illicium* plants,[1] we carried out a chemical examination of the fresh fruits of *I. anisatum* L., and isolated two new phenylpropanoid glycosides and three known sesquiterpenoids from the acetone extracts. This paper describes the isolation and structural elucidation of these components.

The acetone extract of the fresh fruits of *I. anisatum* L. was partitioned between H_2O and EtOAc, and the remaining H_2O layer was subjected to both Chromatorex ODS and silica gel chromatography to give compounds **1**—**5**. Compounds **1**, **2** and **3** were identified as pseudoanisatin,[2] pseudomajucin[3] and illicinolide A,[4] respectively, by comparing their ^{1}H-, ^{13}C-NMR spectral and physical data with reported values. Of these compounds, **4** and **5** are new, while **2** and **3** are isolated from this plant for the first time.

Compound **4**, $C_{21}H_{28}O_{12}$, a white amorphous powder, with a $[M+Na]^+$ ion peak at *m/z* 495 in positive FAB-MS. Its ^{1}H-NMR spectral data (Table 1) showed the presence of a 1, 2, 4, 5-tetrasubstituted benzene ring [δ 6.83 (s), 6.60 (s)], a propenyl group [δ 5.96 (ddt, J=6, 9, 17 Hz), 5.02 (dd, J=3, 17 Hz), 4.99 (dd, J=3, 9 Hz)] and two anomeric protons [δ 4.98 (d, J=2 Hz), 4.67 (d, J=8 Hz)], suggesting that **4** is a phenylpropanoid glycoside. The ^{1}H- and ^{13}C-NMR data (Tables 1 and 2) arising from the aglycone moiety are superimposable on those of compound **6**, previously isolated from the bark of *I. difengpi*,[5] indicating that **4** is a glycoside of 2-allyl-4, 5-methylenedioxyphenol. The ^{1}H- and ^{13}C-NMR data due to the sugar moieties showed the existence of a 6-substituted β-glucopyranosyl[5] and a β-apiofuranosyl moiety[6] in **4**. Furthermore, enzymatic hydrolysis of **4** by cellulase afforded aglycone 2-allyl-4,5-methylenedioxyphenol,[5] D-glucose ($[\alpha]_D$=+48.9°) and D-apiose ($[\alpha]_D$=+8.0°).[7] On the basis of above results, **4** was determined to be 1-allyl-4,5-methylenedioxyphenol-2-*O*-β-D-apiofuranosyl-(1→6)-*O*-β-D-glucopyranoside.

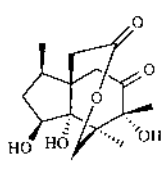
Compound **5** has the same formula as that of **4**, and its ^{1}H- and ^{13}C-NMR spectra (Tables 1 and 2) were very similar to those of **4**, except for the terminal sugar moiety. This suggests that **5** and **4** have the same aglycone with a 6-substi-

Table 1. ^{1}H-NMR Spectral Data of the Aglycones of **4**, **5**, and **6** (in CD_3OD)

No.	**4**	**5**	**6**
H-3	6.83 (s)	6.82 (s)	6.79 (s)
H-6	6.60 (s)	6.59 (s)	6.59 (s)
H-7	3.40 (m)	3.40 (m)	3.40 (m)
H-8	5.96 (ddt, 6, 9, 17 Hz)	5.95 (ddt, 7, 10, 17 Hz)	5.96 (ddt, 6, 9, 17 Hz)
H-9a	5.02 (dd, 3, 17 Hz)	5.03 (dd, 2, 17 Hz)	5.03 (dd, 2, 17 Hz)
H-9b	4.99 (dd, 3, 9 Hz)	4.98 (dd, 2, 10 Hz)	4.98 (dd, 2, 9 Hz)
H-10	5.88 (d, 1 Hz)	5.87 (d, 1 Hz)	5.87, 5.98 (d, 1 Hz)

Table 2. ^{13}C-NMR Data of **4**—**6**

No.	**4**	**5**	**6**
C-1	124.2	124.1	124.2
C-2	151.0	151.0	150.9
C-3	101.0	100.9	101.2
C-4	147.6	147.6	147.6
C-5	144.3	144.3	144.4
C-6	109.9	109.8	109.8
C-7	34.9	34.9	34.9
C-8	138.9	138.9	138.9
C-9	115.6	115.5	115.6
C-10	102.4	102.4	102.4
glc-1	104.3	104.3	104.4
2	75.0	75.0	75.0
3	78.2	78.2	78.2
4	71.6	71.8	71.6
5	76.8	76.7	76.8
6	69.0	68.3	68.1



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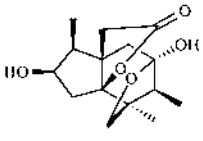

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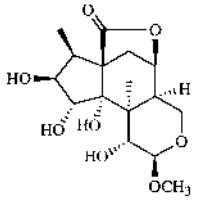

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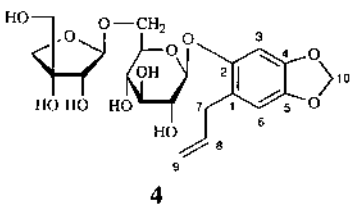

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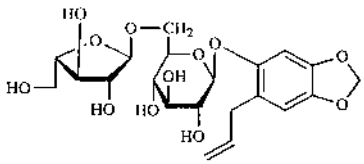

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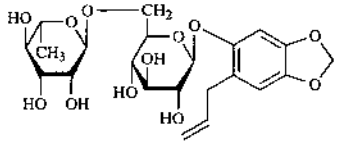

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tuted β-glucopyranosyl group positioned at C-2 of the aglycone. Detailed analysis of ^{1}H- and ^{13}C-NMR data indicated that the terminal sugar of **5** is an arabinofuranose, which was supported by a comparison of the ^{13}C-NMR data with that in the literature.[8] The arabinose and glucose obtained by acid hydrolysis of **5** were determined to be in the L and D form, respectively, by applying the method of Hara *et al*.[9] From the above evidence, **5** was concluded to be 1-allyl-4, 5-methylenedioxyphenol-2-*O*-α-L-arabinofuranosyl-(1→6)-*O*-β-D-glucopyranoside.

Experimental

General Optical rotations were measured with a JASCO DIP-370 digital polarimeter. ^{1}H- and ^{13}C-NMR spectra were recorded on Varian Gemini 300 spectrometers. Coupling constants (J) are expressed in Hz, and chemical shifts are given on a δ (ppm) scale with tetramethylsilane as internal standard. FAB-MS were recorded on a JEOL JMS DX-303 spectrometer with glycerol as a matrix. Gas chromatography analyses were performed on a Shimadzu GC-17A gas chromatograph equipped with a DB-1 (0.32×30 m) column. Analytical conditions: column temperature, 200 °C; injection temperature, 270 °C; carrier gas (He) flow rate, 30 ml/min. Column chromatography was performed on Kieselgel 60 (70—230 mesh, Merck), Bondapak C18/Porasil B (37—75 μm, Waters Associates Inc.) and Chromatorex ODS (100—200 mesh, Fuji Silysia Chemical Ltd.) columns. Thin-layer chromatography (TLC) was performed on precoated Kieselgel 60 F_{254} plates (0.2 mm thick, Merck), and spots were detected under ultraviolet (UV) light and by spraying with 10% sulfuric acid reagent.

Extraction and Isolation The acetone extracts (50 g) of the fresh fruits (1.73 kg) of *I. anisatum*, collected in July 1995 in Nagasaki, were partitioned between H_2O and EtOAc. The water layer (36.5 g) was subjected to chromatography on Chromatorex ODS (0—80% MeOH) to give fraction-1 (24.7 g, containing a large amount of shikimic acid), fraction-2 (3.2 g) and fraction-3 (4.1 g). Fraction-2 was applied to Bondapak ODS (30—50% MeOH) and silica gel [$CHCl_3$–MeOH (98 : 2—94 : 6)] columns to afford pseudoanisatin (**1**) (56 mg), pseudomajucin (**2**) (13 mg) and illicinolide A (**3**) (53 mg). Fraction-3 was subjected to Bondapak ODS (30—50% MeOH) column chromatography to give compounds **4** (7.5 mg) and **5** (22.2 mg).

Compound 4 A white amorphous powder, $C_{21}H_{28}O_{12}$, $[\alpha]_D^{15}$ −64.8° (c=0.6, MeOH). Positive FAB-MS m/z: 495 $(M+Na)^+$, 472 $(M)^+$. ^{1}H-NMR (300 MHz, CD_3OD): δ 4.98 (1H, d, J=2 Hz, api-1), 4.67 (1H, d, J=8 Hz, glc-1), 3.99 (1H, dd, J=2, 12 Hz, glc-6a), 3.98 (1H, d, J=11 Hz, api-4a), 3.90 (1H, d, J=2 Hz, api-2), 3.76 (1H, d, J=11 Hz, api-4b), 3.63 (1H, dd, J=6, 12 Hz, glc-6b), 3.58 (2H, s, api-5), 3.52 (1H, ddd, J=2, 6, 9 Hz, glc-5), 3.33—3.46 (3H, m, glc-2, 3, 4), aglycone see Table 1. ^{13}C-NMR (125 MHz, CD_3OD): aglycone and glucose moieties see Table 2. Apiose moiety: δ 111.0 (C-1), 80.5 (C-3), 78.2 (C-2), 75.0 (C-4), 65.8 (C-5).

Hydrolysis of 4 with Cellulase A solution of **4** (6.4 mg) and cellulase (Nagase Biochemical Ltd., 40 mg) in water (5 ml) was incubated with stirring at 37 °C for 15 d. The aqueous solution was extracted with Et_2O (3×10 ml) and the combined organic phase was evaporated to dryness under reduced pressure to furnish 2-allyl-4, 5-methylenedioxyphenol[5] (1.2 mg). ^{1}H-NMR (300 MHz, $CDCl_3$): δ 6.58, 6.43 (each 1H, s), 5.96 (1H, ddt, J=6, 9, 17 Hz), 5.89 (2H, s), 5.17 (1H, dd, J=2, 17 Hz), 5.15 (1H, dd, J=2, 9 Hz), 4.70 (1H, s), 3.31 (2H, m). The water layer was evaporated to dryness under reduced pressure and subjected to silica gel chromatography [$CHCl_3$–MeOH–H_2O (6 : 4 : 1)] to afford D-glucose (1.5 mg): $[\alpha]_D^{15}$=+48.9° (c=0.13, H_2O, measured 24 h later after dissolving in H_2O) and D-apiose (1.0 mg): $[\alpha]_D^{15}$=+8.0° (c=0.05, H_2O, measured 24 h later after dissolving in H_2O).[7]

Compound 5 A white amorphous powder, $C_{21}H_{28}O_{12}$, $[\alpha]_D^{15}$ −76.1° (c=1.7, MeOH). Positive FAB-MS m/z: 495 $(M+Na)^+$. ^{1}H-NMR (300 MHz, CD_3OD): δ 4.92 (1H, d, J=2 Hz, ara-1), 4.69 (1H, d, J=8 Hz, glc-1), 4.05 (1H, dd, J=2, 12 Hz, glc-6a), 4.02 (1H, dd, J=2, 3 Hz, ara-2), 3.84 (1H, dd, J=3, 6 Hz, ara-3), 3.72 (1H, dd, J=3, 11 Hz, ara-5a), 3.62 (1H, dd, J=6, 11 Hz, ara-5b), 3.61 (1H, dd, J=6, 12 Hz, glc-6b), 3.54 (1H, ddd, J=2, 6, 9 Hz, glc-5), 3.34—3.46 (4H, m, glc-2, 3, 4, ara-4), aglycone see Table 1. ^{13}C-NMR (125 MHz, CD_3OD): aglycone and glucose moieties see Table 2. Arabinose moiety: δ 111.0 (C-1), 85.8 (C-2), 83.2 (C-4), 78.8 (C-3), 62.9 (C-5).

Determination of Absolute Configurations of Component Sugars of 5 A solution of **5** (2 mg) in 2 N HCl/H_2O (2 ml) was heated at 80 °C for 2 h. After neutralizing with 1 N NaOH/H_2O, the solution was passed through an MCI-gel CHP 20P column and eluted with H_2O to afford the fraction containing the sugars which was then evaporated and dried *in vacuo*. The absolute configurations of the component sugars of **5** were determined by applying the method of Hara *et al*.[9] The retention times (t_R) of the peaks corresponding to the thiazolidine derivatives of glucose and arabinose of **5** are 27.28 min, 12.15 min, respectively, while those for D-glucose, L-glucose, D-arabinose and L-arabinose are 27.31 min, 25.38 min, 14.41 min, 12.32 min, respectively.

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