

Sesinoside, a New Iridoid Glucoside from Sesame (*Sesamum indicum*) Seedlings

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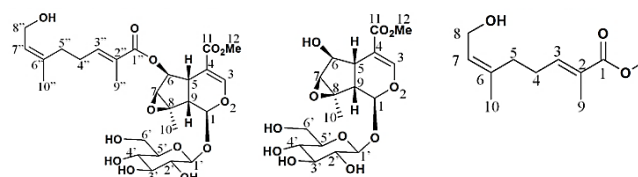
A new iridoid glucoside, sesinoside (**1**), was isolated from the seedlings of *Sesamum indicum*. The structure of **1** was elucidated by spectroscopic analyses and by methanolysis of **1**, which produced the known compounds, phlorigidosides C (**2**) and (6*Z*)-foliamenthic acid methyl ester (**3**). This is the first report of an iridoid glucoside with **3**.

Keywords: Sesinoside, Iridoid glucosides, *Sesamum indicum*, Seedling, Photostress.

Iridoids are secondary metabolites of terrestrial and marine flora and fauna and are found in a large number of plant families, usually as glucosides. Currently, a thousand or more iridoid compounds have been reported [1]. They have a great variety of bioactivity, for example, antibacterial [2], antiviral [3], antiprotozoal [4], anticancer [5], anti-inflammatory [6], and melanogenesis inhibitory [7]. In addition, there is a report that drought stress increases iridoid glucoside biosynthesis in the roots of *Scrophularia ningpoensis* seedlings [8]. In this paper, we report the new iridoid glucoside, sesinoside (**1**), isolated from the illuminated seedlings of sesame (*Sesamum indicum*).

Sesinoside (**1**) was obtained as an amorphous powder. The molecular formula of **1** was established as C₂₇H₃₈O₁₃ by high-resolution electrospray ionization mass spectrometry (HRESI-MS, *m/z* 593.2217 [M + Na]⁺, Δ+0.7 mmu). The IR spectrum exhibited absorption bands at 3398 cm⁻¹ for the hydroxyl group, and 1705 cm⁻¹ and 1635 cm⁻¹ for α,β-unsaturated ester groups. The ¹³C NMR spectrum (Table 1) of **1** indicated the presence of 27 carbons, which were assigned to two ester carbonyl carbons at δ_c 170.2 and 169.7; six olefinic carbons at δ_c 154.4, 144.5, 139.5, 129.8, 127.4, and 108.6; nine oxygenated methine carbons at δ_c 100.7, 96.9, 81.8, 79.6, 78.7, 75.8, 72.7, 63.8, and 63.5; two oxygenated methylene carbons at δ_c 63.9 and 59.7; a methoxy carbon at δ_c 52.7; two methine carbons at δ_c 45.0 and 35.8; two methylene carbons at δ_c 32.4 and 29.2; and three methyl carbons at δ_c 24.3, 18.6, and 13.3. The ¹H NMR spectrum of **1** showed three olefinic protons at δ_H 7.52, 6.89, and 5.46; eight oxygenated methine protons at δ_H 5.48, 5.21, 4.85, 3.47, 3.45, 3.39, 3.30, and 3.26; two oxygenated methylene protons at δ_H 4.13 and 3.98/3.65; a methoxy proton at δ_H 3.62; two methine protons at δ_H 3.02 and 2.42; two methylene protons at δ_H 2.38 and 2.29; and three methyl protons at δ_H 1.90, 1.82, and 1.61.

The ¹H-¹H COSY data of **1** implied connectivities of H-1'/H-9/H-5/H-6/H-7; H-1'/H-2'/H-3'/H-4'/H-5'/H-6'; H-3''/H-4''/H-5'' and H-7''/H-8'' (Figure 2). The HMBC spectrum (Figure 2) showed the correlations of H-1'/C-1'; H-3'/C-1, C-4 and C-11; H-5'/C-3, C-4 and C-11; H-6'/H-1''; H-7'/C-10; H-9'/C-10; H-10'/C-8 and C-9; H-12'/C-11; H-3''/C-1'', C-2'' and C-9''; H-7''/C-5'' and C-10''; H-9''/C-1'' and C-2''; and H-10''/C-5'' and C-6''. According to the molecular formula of **1**, there were nine degrees of unsaturation. Two carbonyl



Sesinoside (**1**) Phlorigidoside C (**2**) Foliamenthic acid methyl ester (**3**)

Figure 1: Structures of sesinoside (**1**), phlorigidoside C (**2**) and foliamenthic acid methyl ester (**3**)

Table 1: ¹³C and ¹H NMR data of sesinoside (**1**) and phlorigidoside C (**2**) in CD₃OD^a.

1				2			
	¹³ C, δ _c		¹ H, δ _H (mult, J in Hz)		¹³ C, δ _c		¹ H, δ _H (mult, J in Hz)
1	96.9 ^a	CH	5.48 ^a d (9.7)	97.3 ^a	CH	5.31 ^a	d (9.7)
3	154.4	CH	7.52 br s	155.0	CH	7.59	br s
4	108.6	C		109.4	C		
5	35.8	CH	3.02 dd (8.3,7.4)	39.3	CH	2.71	td (7.9,1.2)
6	81.1	CH	5.21 dd (8.3,1.2)	80.3	CH	4.04	dd (7.9,1.2)
7	63.8	CH	br s	65.7	CH		br s
8	63.5	C		63.9	C		
9	45.0	CH	2.42 dd (9.7,7.4)	46.0	CH	2.45	dd (9.7,7.6)
10	18.6	CH ₃	1.61 s	18.8	CH ₃	1.58	s
11	169.7	C		171.7	C		
12	52.7	CH ₃	3.62 s	53.1	CH ₃	3.78	s
1'	100.7	CH	4.85 dd (7.9)	100.7	CH	4.83	dd (7.9)
2'	75.8	CH	3.30 dd (9.3,7.9)	75.7	CH	3.30	dd (9.3,7.9)
3'	78.7	CH	3.45 dd (9.3,9.0)	78.7	CH	3.26	dd (9.3,9.0)
4'	72.7	CH	3.26 t (9.0)	72.6	CH	3.45	t (9.0)
5'	79.6	CH	3.39 m	79.6	CH	3.39	m
6'	63.9	CH ₂	3.98 dd (11.9,2.1)	63.9	CH ₂	3.97	dd (11.9,2.1) dd (11.9,7.0)
			3.65			3.65	
1''	170.2	C					
2''	129.8	C					
3''	144.5	CH	6.89 tq (7.4,1.5)				
4''	29.2	CH ₂	2.38 t (7.4)				
5''	32.4	CH ₂	2.29 t (7.4)				
6''	139.5	C					
7''	127.4	CH	5.46 t (6.8)				
8''	59.7	CH ₂	4.13 d (6.8)				
9''	13.3	CH ₃	1.90 br s				
10''	24.3	CH ₃	1.82 s				

^aδ in ppm.

groups, three olefins, an iridoid skeleton, and one β-glucose unit accounted for eight of those. The remaining degree of unsaturation was assumed to be due to an epoxide ring formed between C-7 and C-8, as inferred from the ¹³C NMR data (δ_c 63.8 and 63.5). Therefore, the constitution of **1** was deduced. The relative structure of **1** was deduced from a nuclear Overhauser enhancement spectroscopy (NOESY) spectrum of **1**. The representative correlations are shown in Figure 2. In the NOESY spectrum, the

correlations of H-5''/H-8'' and H-7''/H-10'' were observed. Thus, the configuration of the C-6''/C-7'' double bond was denoted as *Z*. The correlations of H-1/H-10, H-6/H-7, and H-7/H-10 suggested that the relative structure of sesinoside (**1**) was as in Figure 1.

To determine the configuration of **1**, a methanolysis reaction was applied to **1** (Figure 3). Sodium methoxide solution was added to a solution of **1** in anhydrous methanol at room temperature under N₂ for 6 h. Then, phlorigidoside C (**2**) and foliamenthic acid methyl ester (**3**) were derived in 34% and 46% yield, respectively. The ¹H and ¹³C NMR spectroscopic (Table) and ESI-MS data of **2** were identical with those of phlorigidoside C [9].

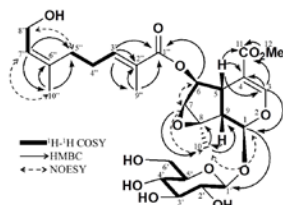


Figure 2: 2D NMR correlations of **1**.

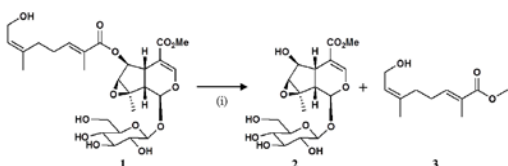


Figure 3: Methanolysis reaction of **1** [(i) NaOMe, MeOH, rt, 6 h, 34% (**2**), 46% (**3**)]

Experimental

General experimental procedure: Optical rotations, JASCO DIP-370 spectrometer; UV, HITACHI U-2000A spectrometer; IR, JASCO FT/IR-300 spectrometer; NMR, BRUKER Avance 500 spectrometer; ESI-MS, WATERS SYNAPT G2 mass spectrometer.

Plant materials: Sesame seeds were purchased from Fukutane K. K. (Fukui, Japan). Seeds were spread evenly on moist vermiculite in trays and incubated at 25°C in the dark for 3-4 days. The seedlings (hypocotyls length, ca. 3~4 cm) were then illuminated by white light (30-50 μmol m⁻² s⁻¹; FL 20SS/ W/ 18; Panasonic Electric Works Co., Osaka, Japan) for 7 days. After a week, they were harvested, collected, and frozen at -80°C until use.

Extraction and isolation: All parts of the illuminated seedlings (73 g FW) were homogenized in acetone (250 mL×2). The homogenate was filtered and allowed to dry *in vacuo* at 40°C. The residue (0.95 g) was subjected to ODS CC (φ 1.1 × 30 cm) with a MeOH/H₂O (2:8/4:6/6:4/8:2/10:0) stepwise gradient and EtOAc, and separated into 5 fractions (SIA1-5). SIA4 (58.3 mg) was purified by reversed-

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phase HPLC (TSK-gel ODS-80Ts, TOSOH, φ 7.8 × 300 mm, flow rate 2.0 mL/min, 30% MeCN isocratic) to yield sesinoside (**1**, 5.6 mg).

Methanolysis reaction of sesinoside: Sodium methoxide solution (0.5 N, 0.1 mL) was added to a solution of sesinoside (**1**, 5.0 mg, 8.8 × 10⁻⁶ mol) in anhydrous methanol (0.5 mL) and the mixture was stirred at room temperature under N₂ for 6 h. After 6 h, HCl (1 N, 0.12 mL) was added to stop the reaction. The reaction mixture was partition with EtOAc (10 mL) and H₂O (10 mL). The EtOAc layer was dried with MgSO₄, and both layers were evaporated. Water layer was purified by reversed-phase HPLC (TSK-gel ODS-80Ts, TOSOH, φ 7.8 × 300 mm, flow rate 2.0 mL/min, MeCN:H₂O, 1:9 (0 min)→2:8 (30 min)) to afford phlorigidoside C (**2**, 1.2 mg) in 34% yield. The EtOAc layer was purified by reversed-phase HPLC [TSK-gel ODS-80Ts, TOSOH, φ 7.8 × 300 mm, flow rate 2.0 mL/min, MeCN:H₂O, 1:9 (0 min)→5:5 (30 min)] to afford (6*Z*)-foliamenthic acid methyl ester (**3**, 0.8 mg) in 46% yield.

Sesinoside (1)

Amorphous powder.

[α]_D²⁵: -65.6 (c 1.00, CH₃OH).

IR (KBr): 3398, 1705, 1635 cm⁻¹.

UV λ_{max} (MeOH) nm (log ε): 230 (4.10).

¹H (500 MHz) and ¹³C NMR (125 MHz): Table 1.

ESI-MS (positive ion): *m/z* 593 [M + Na⁺].

HRESI-MS: *m/z* 593.2217 [M + Na⁺] calcd for C₂₇H₃₈O₁₃Na: 593.2210.

Phlorigidoside C (2)

¹H (500 MHz) and ¹³C NMR (125 MHz): Table 1.

ESI-MS (positive ion): *m/z* 427 [M + Na⁺].

HRESI-MS: *m/z* 427.1226 [M + Na⁺] calcd for C₁₇H₂₄O₁₁Na: 427.1216.

(6Z)-Foliamenthic acid methyl ester (3)

¹H NMR (500 MHz, CD₃OD): δ_H 6.79 (1H, tq, *J*=7.4 and 1.5 Hz, H-3), 5.45 (1H, t, *J*=6.5 Hz, H-7), 4.10, (2H, dd, *J*=6.5 and 1.0 Hz, H-8), 3.76, (3H, s, OMe), 2.37, (2H, td, *J*=7.5 and 7.4 Hz, H-4), 2.26, (2H, t, *J*=7.5 Hz, H-5), 1.87, (3H, br s, H-9), 1.80(3H, s, H-10)

ESI-MS (positive ion): *m/z* 221 [M + Na⁺].

HRESI-MS: *m/z* 221.1156 [M + Na⁺] calcd for C₁₁H₁₈O₅Na: 221.1154.

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