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ILICIFOLIOSIDES A AND B, BIS-SECOIRIDOID GLYCOSIDES FROM *OSMANTHUS ILICIFOLIUS*

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Abstract – Two new bis-secoiridoid glycosides, named ilicifoliosides A and B, were isolated from the leaves of *Osmanthus ilicifolius*. Their structures have been determined by 1D and 2D NMR analysis.

INTRODUCTION

The Oleaceae is a family of medium size with 25 genera and about 600 species, and this family is a rich source of iridoid, secoiridoid, phenylpropanoid and lignan glycosides.[1] As part of our continued studies on the constituents of oleaceous plants.[2] We previously reported the isolation and identification of a new caffeoyl glycoside (*cis*-acteoside) and a new secoiridoid di-glycoside (hiragilide) along with 36 known constituents from the leaves of *Osmanthus ilicifolius* (Japanese name, hiiragi).[3] We have now re-examined on the constituents of the above plant. As a result two new bis-secoiridoid glycosides, named ilicifoliosides A (**1**) and B (**2**) have been isolated. This paper deals with the structural elucidation and identification of these compounds. The isolation procedure is described in detail in the Experimental section.

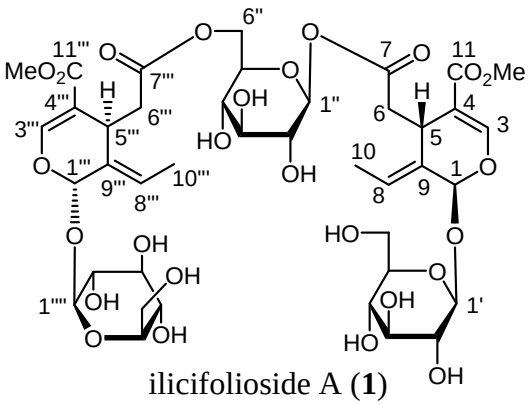
RESULTS AND DISCUSSION

Ilicifolioside A (**1**) was obtained as an amorphous powder, $[\alpha]_D$ -186.9 º (MeOH). The molecular formula of **1**, $C_{40}H_{56}O_{26}$, was confirmed by HR-FAB-MS. The ^{1}H NMR spectrum of **1** showed two sets of signals, one set similar to 7-(1-β-D-glucopyranosyl)oleoside 11-methy ester[4] and another to oleoside 11-methyl ester,[5] indicating a dimeric structure. This assumption was supported by alkaline hydrolysis of **1**, which afforded oleoside.[6] On acid hydrolysis with 1M HCl, **1** gave only D-glucose. The ^{13}C NMR spectrum of **1** contained a set of signals almost identical to those assigned to 7-(1-β-D-glucopyranosyl)oleoside 11-methy ester, except for the difference in chemical shifts at the C-5" (-2.1 ppm, δ_C 75.9) and C-6" (+2.5 ppm, δ_C 64.7). The other set of signals, corresponding to oleoside 11-methyl ester, were coincident, except for the chemical shift owing to C-7''' (-4.5 ppm, δ_C 173.0).

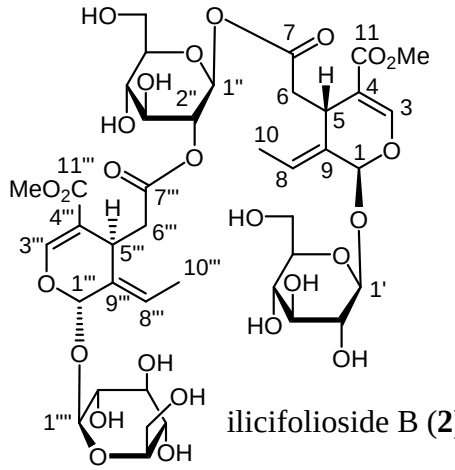
This paper is dedicated to Prof. Dr. Ekkehard Winterfeldt on the occasion of his 75th birthday.

These indicated that an oleoside 11-methyl ester unit was esterified with the C-6" hydroxyl of a 7-(1-β-D-glucopyranosyl)oleoside 11-methy ester unit. This deduction was supported by the ^{1}H-^{1}H COSY, NOE difference (NOED) and heteronuclear multiple bond correlation (HMBC) experiments as follows (Figure 1): C-7''' (δ_C 173.0) showed HMBC correlation with δ_H 4.28 (1H, dd, *J*=12.1, 1.8 Hz), which was evidently coupled with two protons at δ_H 3.56 (1H, ddd, *J*=9.5, 5.1, 1.8 Hz) and δ_H 4.20 (1H, dd, *J*=12.1, 5.1 Hz) in the ^{1}H-^{1}H COSY. Irradiation at δ_H 3.56 caused NOE enhancement in the signal of H-1" (δ_H 5.43. 14.3 %), which was correlated to δ_C 171.9 (C-7) in the HMBC experiment. Thus, the signals at δ_H 3.56 and δ_H 4.28 were assigned at H-5" and H-6"B, respectively. Therefore, the structure of **1** was esterified between the hydroxyl group at C-6" of 7-(1-β-D-glucopyranosyl)oleoside 11-methy ester and carboxylic acid group at C-7''' of oleoside 11-methyl ester. On the basis of the above data, the structure of ilicifolioside A (**1**) was established as depicted in the formula.

Ilicifolioside B (**2**) was obtained as an amorphous powder, $[\alpha]_D$ -189.4 º (MeOH). The molecular formula of **2**, $C_{40}H_{56}O_{26}$, was confirmed by HR-FAB-MS and was coincident with that of **1**. The ^{13}C NMR spectrum of **2** was similar to that of **1**, except for the chemical shifts owing to the 1-*O*-acyl-β-D-glucopyranosyl moiety of 7-(1-β-D-glucopyranosyl)oleoside 11-methy ester unit [δ_C 93.8 (C-1"), 75.8 (C-2"), 74.4 (C-3"), 71.4 (C-4"), 78.9 (C-5"), 62.2 (C-6")]. This indicated that an oleoside 11-methyl ester unit was esterified with the C-2" hydroxyl of a 7-(1-β-D-glucopyranosyl)oleoside 11-methy ester unit. This deduction was supported by the ^{1}H-^{1}H COSY and HMBC experiments as follows (Figure 1): C-7''' (δ_C 172.0) showed HMBC correlation with δ_H 4.79 (1H, dd, *J*=9.5, 8.3 Hz), which was evidently coupled with H-1" (δ_H 5.58) in the ^{1}H-^{1}H COSY. Thus, the signal at δ_H 4.79 was assigned at H-2". Therefore, the structure of **2** was esterified between the hydroxyl group at C-2" of 7-(1-β-D-glucopyranosyl)oleoside 11-methy ester and carboxylic acid group at C-7''' of oleoside 11-methyl ester. On the basis of the above data, the structure of ilicifolioside B (**2**) was established as depicted in the formula.



ilicifolioside A (**1**)



ilicifolioside B (**2**)

Table 1. ^{13}C NMR Chemical Shifts of **1** and **2**

	1	**2**		**1**	**2**
1	95.0	95.3	1'''	95.5	95.5
3	155.42	155.30	3'''	155.38	155.25
4	109.3	109.5	4'''	109.2	109.3
5	31.4	31.4	5'''	31.9	31.3
6	40.4	40.8	6'''	41.2	40.6
7	171.9	171.7	7'''	173.0	172.0
8	125.44	125.4	8'''	125.42	125.2
9	130.3	130.5	9'''	130.3	130.4
10	14.0	13.9	10'''	13.9	13.8
11	168.8	168.73	11'''	168.8	168.71
OMe	52.1	52.05	OMe	52.0	52.02
1'	100.5	100.9	1''''	101.1	101.0
2'	74.7	74.83	2''''	74.9	74.78
3'	78.4	78.5	3''''	78.6	78.4
4'	71.5	71.6	4''''	71.5	71.5
5'	77.8	78.0	5''''	78.00	78.0
6'	62.9	62.8	6''''	62.8	62.8
1''	95.8	93.8			
2''	73.8	75.8			
3''	78.02	74.4			
4''	71.0	71.1			
5''	75.9	78.9			
6''	64.7	62.2			

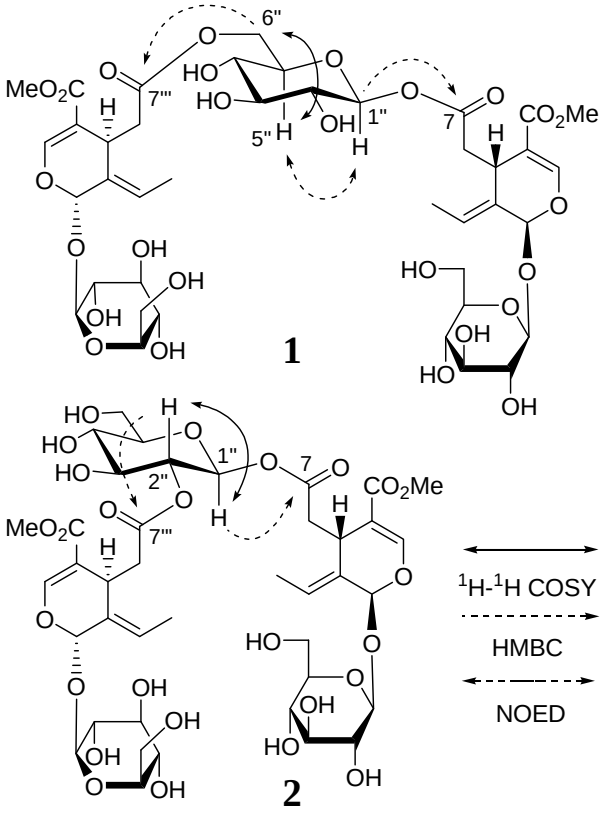


Figure 1. Diagnostic HMBC, ^{1}H-^{1}H COSY and NOED Correlations for **1** and **2**

EXPERIMENTAL

General Optical rotation were taken with a JASCO DIP-360 digital polarimeter. UV spectra were recorded with a Beckman DU-64 spectrometer. The ^{1}H and ^{13}C NMR spectra were recorded with JEOL JNM-LA 400 (400 MHz, 100 MHz, respectively) spectrometer. Chemical shifts are given in a δ (ppm) scale with tetramethylsilane (TMS) as an internal standard. FAB-MS were recorded on a JEOL JMS-DX 303 mass spectrometer. Column chromatography was carried out on Kieselgel 60 (Merck; 230－400 mesh) and Sephadex LH-20 (Pharmacia Fine Chemicals). HPLC was carried out on a Tosoh HPLC system [pump, CCPS; detector, UV-8020; column, TSK gel ODS 120T (7.8 mm i.d.×30 cm, Tosoh) and TSK gel Amide-80 (7.8 mm i.d.×30 cm, Tosoh).

Material The leaves of *O. ilicifolius* were collected in August, 2005 in Sendai, Miyagi prefecture, Japan, and identified by one of the authors (M. Kikuchi). A voucher specimen is held in the laboratory of M. Kikuchi.

Extraction and Isolation Fresh leaves of *O. ilicifolius* (2.6 kg) were extracted with MeOH at rt for eight months. The MeOH extract was concentrated under reduced pressure and the residue (466 g) was

suspended in water. This suspension was successively extracted with $CHCl_3$, AcOEt, *n*-BuOH and H_2O. The *n*-BuOH-soluble fraction was concentrated under reduced pressure to produce a residue (167 g). The extract (74 g) was chromatographed on a silica gel column using $CHCl_3$－MeOH－H_2O (50 : 10 : 1, 30 : 10 : 1, 10 : 10 : 1) and the eluate was separated into five fractions (frs. 1－5). Fr. 3 was chromatographed on a Sephadex LH-20 column using 50% MeOH and the eluate was separated into thirty fractions (frs. 3-1－3-30). Fraction 3-4 was subjected to preparative HPLC [column, TSK gel ODS 120T; mobile phase, MeOH－H_2O (2 : 5); UV detector, 205 nm; flow rate, 1.5 mL / min; column temperature, 40 ºC] to give eight peaks (peaks 1－8). Each of peaks 7 and 8 was purified by preparative HPLC [column, TSK gel Amide-80; mobile phase, CH_3CN－H_2O (5.5 : 1); UV detector, 205 nm; flow rate, 1.5 mL / min; column temperature, 40 º C] to give **1** (403.6 mg) and **2** (44.1 mg), respectively.

Ilicifolioside A (1) An amorphous powder, $[\alpha]_D^{27}$ -186.9 º (*c* 4.04, MeOH); UV λ_{max} (MeOH) nm (log ε): 236.0 (4.30); FAB-MS *m*/*z*: 975 $[M+Na]^+$; HR-FAB-MS *m*/*z*: 975.3012 ($C_{40}H_{56}O_{26}Na$, calcd for 975.2957). ^{1}H NMR (CD_3OD) δ: 1.72 (3H, dd, *J*=7.3, 1.5 Hz, H_3-10), 1.77 (3H, dd, *J*=7.3, 1.5 Hz, H_3-10'''), 2.50 (1H, dd, *J*=13.9, 8.8 Hz, H-6'''A), 2.65 (1H, dd, *J*=15.8, 9.5 Hz, H-6A), 2.76 (1H, dd, *J*=13.9, 4.8 Hz, H-6'''B), 2.81 (1H, dd, *J*=15.8, 3.3 Hz, H-6B), 3.56 (1H, ddd, *J*=9.5, 5.1, 1.8 Hz, H-5''), 3.60 (1H, dd, *J*=11.7, 6.2 Hz, H-6'A), 3.68 (1H, dd, *J*=11.9, 5.7 Hz, H-6''''A), 3.72, 3.73 (each 3H, s, 11-$COOCH_3$, 11'''-$COOCH_3$), 3.86 (1H, dd, *J*=11.7, 2.2 Hz, H-6'B), 3.88 (1H, dd, *J*=11.9, 1.8 Hz, H-6''''B), 3.99 (1H, br dd, *J*=8.8, 4.8 Hz, H-5'''), 4.02 (1H, br dd, *J*=9.5, 3.3 Hz, H-5), 4.20 (1H, dd, *J*=12.1, 5.1 Hz, H-6''A), 4.28 (1H, dd, *J*=12.1, 1.8 Hz, H-6''B), 4.80 (1H, d, *J*=7.7 Hz, H-1') 4.82 (1H, d, *J*=7.7 Hz, H-1''''), 5.43 (1H, d, *J*=8.4 Hz, H-1''), 5.89 (1H, br d , *J*=0.7 Hz, H-1'''), 5.96 (1H, br d, *J*=1.8 Hz, H-1), 6.10 (1H, dq, *J*=7.3, 1.1 Hz, H-8'''), 6.13 (1H, dq, *J*=7.3, 1.1 Hz, H-8), 7.52 (1H, s, H-3'''). 7.53 (1H, s, H-3). ^{13}C NMR (CD_3OD): Table 1.

Ilicifolioside B (2) An amorphous powder, $[\alpha]_D^{27}$ -189.4 º (*c* 0.40, MeOH); UV λ_{max} (MeOH) nm (log ε): 236.0 (4.39); FAB-MS *m*/*z*: 975 $[M+Na]^+$; HR-FAB-MS *m*/*z*: 975.2896 ($C_{40}H_{56}O_{26}Na$, calcd for 975.2957). ^{1}H NMR (CD_3OD) δ: 1.76 (6H, br d, *J*=7.1 Hz, H_3-10, H_3-10'''), 2.58 (1H, dd, *J*=15.4, 8.5 Hz, H-6A), 2.66 (1H, dd, *J*=15.0, 7.0 Hz, H-6'''A), 2.70 (1H, dd, *J*=15.4, 4.0 Hz, H-6B), 2.74 (1H, dd, *J*=15.0, 4.5 Hz, H-6'''B), 3.61 (1H, dd, *J*=9.5, 8.5 Hz, H-3''), 3.70 (3H, m, H-6'A, H-6''A, H-6''''A), 3.72, 3.73 (each 3H, s, 11-$COOCH_3$, 11'''-$COOCH_3$), 3.83 (1H, dd, *J*=12.0, 2.2 Hz, H-6''B), 3.90, 3.91 (each 1H, dd, *J*=12.2, 2.0 Hz, H-6'B, H-6''''B), 4.00 (2H, m, H-5, H-5'''), 4.79 (1H, dd, *J*=9.5, 8.3 Hz, H-2''), 4.81, 4.82 (each 1H, d, *J*=7.8 Hz, H-1', H-1''''), 5.58 (1H, d, *J*=8.3 Hz, H-1''), 5.91, 5.94 (each 1H, br s , H-1, H-1'''), 6.11 (2H, br q, *J*=7.1 Hz, H-8, 8'''), 7.51, 7.52 (each 1H, s, H-3, H-3'''). ^{13}C NMR (CD_3OD): Table 1.

Alkaline Hydrolysis of 1 and 2. Each solutions (*ca*. 8 mg) in 1.0 M NaOH (2 mL) was stirred for 1 h at rt. The reaction mixture was neutralized with Amberlite IR-120 (H^+ form) and concentrated *in vacuo*. The

resulting residue was purified by preparative HPLC [column, TSK gel ODS 120T; mobile phase, MeOH $-H_2O$ (1 : 4); UV detector, 205 nm; flow rate, 1.5 mL / min; column temperature, 40 ºC] to give **3** (5.0 mg). **3** was identified as oleoside by comparison of the spectroscopic data with reported values.[6]

3: $[\alpha]_D^{27}$ -138.1 º (*c* 1.52, MeOH); ^{1}H NMR (CD_3OD) δ: 1.76 (3H, dd, *J*=7.3, 1.5 Hz, H_3-10), 2.36 (1H, dd, *J*=14.1, 10.2 Hz, H-6A), 2.78 (1H, dd, *J*=14.1, 3.9 Hz, H-6B), 3.67 (1H, dd, *J*=12.2, 5.4 Hz, H-6'A), 3.88 (1H, dd, *J*=12.2, 1.5 Hz, H-6'B), 4.01 (1H, dd, *J*=10.2, 3.9 Hz, H-5), 4.81 (1H, d, *J*=7.8 Hz, H-1'), 5.93 (1H, br s, H-1), 6.12 (1H, br q, *J*=7.3 Hz, H-8), 7.51 (1H, s, H-3).

Acid Hydrolysis of 1 and 2. Each of compounds (*ca*. 2 mg) was refluxed with 1M HCl for 5 h. The reaction mixture was neutralized with Ag_2CO_3 and filtered. The solution was concentrated *in vacuo* and dried to give a sugar fraction. The sugar fraction was analyzed by HPLC under the following conditions: column, TSK gel Amide-80 (7.8 mm. i.d.×30 cm, Tosoh); column temperature, 45 ºC; mobile phase, CH_3CN-H_2O (4 : 1); flow rate, 1.0 mL / min; chiral detection (JASCO OR-2090). Identification of D-glucose present in the sugar fraction was carried out by the comparison of the retention time and optical rotation with that of authentic sample; t_R (min) 39.0 (D-glucose, positive optical rotation).

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