A New Sesquiterpene Lactone and Its Glucoside from the Pericarps of *Illicium majus*

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A novel sesquiterpene lactone, pseudomajucin (1) was isolated from the pericarps of *Illicium majus*, a Chinese *Illicium* plant, together with its glucoside (2). The structure elucidation of 1 was performed by the spectroscopic method, and finally by an X-ray crystallographic analysis. Interestingly, the structure of pseudomajucin resembled that of the lactone compound which was afforded by treatment of pseudoanisatin with a basic medium. The glucosidic linkage position in the structure of 2 was determined as C-7 in that of 1 by the spectroscopic analysis of 2 and its acetate.

Keywords *Illicium majus*; pseudomajucin; pseudoanisatin; anisatin; γ-lactone; X-ray analysis

During our investigation of the anisatin-like sesquiterpene lactones from the *Illicium* plants, we have previously reported pseudoanisatin[1] and 6-deoxymajucin[2] obtained from *Illicium anisatum* (Japanese star anise), 6-deoxypseudoanisatin, dunnianin, and 6-deoxydunnianin from *I. dunnianum*,[3] and majucin and neomajucin from *I. majus*.[4] Among these compounds, only neomajucin has been found to have toxicity like that of anisatin obtained from *I. anisatum* so far. We are making a further examination of the constituents of the pericarps of *I. majus*, which is distributed mainly in the southern part of China, and we now report on the structure elucidation of a novel sesquiterpene lactone and its glucoside from this plant.

The pericarps of *I. majus*, collected at Guangxi in China, were extracted with methanol, and defatted with *n*-hexane. The residual part was dissolved in water, and partitioned between water and AcOEt to give the AcOEt-soluble part. The isolation of pseudomajucin (**1**) from this part was achieved by a combination of counter-current distribution and chromatographic separation on silica gel.

Pseudomajucin (**1**), colorless prisms, mp 199—201 °C, $[\alpha]_D$ −89.6°, gave the molecular formula, $C_{15}H_{22}O_5$, from the electron impact-mass spectrum (EI-MS) (*m/z* 282) and the elemental analysis. In the proton nuclear magnetic resonance (^{1}H-NMR) spectrum of **1**, one quaternary and two secondary methyl signals and three sets of isolated methylene signals were observed. Those signals resembled the signals in the ^{1}H-NMR spectrum of 6-deoxypseudoanisatin (**4**). Together with this result, the proton–carbon (^{1}H–^{13}C) long-range two-dimensional shift-correlated spectrum of **1** suggested that **1** is a sesquiterpene which has a pseudoanisatin-like structure.[1] In the carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectrum of **1**, quaternary carbon signals at δ 100.3 and 106.9 were observed, which closely resembled the signals in the ^{13}C-NMR spectrum of compound **5**, obtained by the treatment of pseudoanisatin (**3**) with a basic medium, *i.e.*, sodium methoxide or potassium carbonate.[1] But the absorption maximum at 1725 cm^{-1} in the infrared (IR) spectrum of **1** did not correspond to that expected for the γ-lactone moiety (the absorption maximum for the γ-lactone of **5** was at 1760 cm^{-1}).[1]

To elucidate the whole structure including the stereostructure of **1**, an X-ray crystallographic analysis was carried out. The final *R*-value converged to 0.060 and a

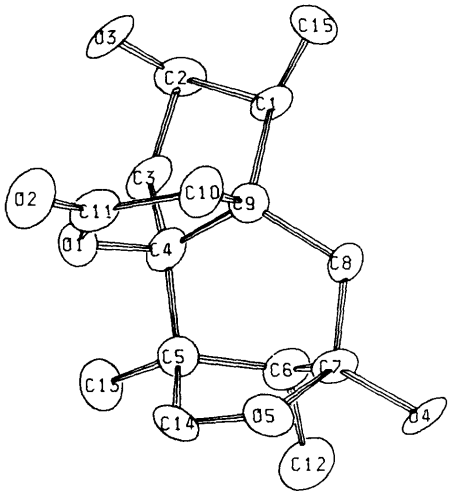
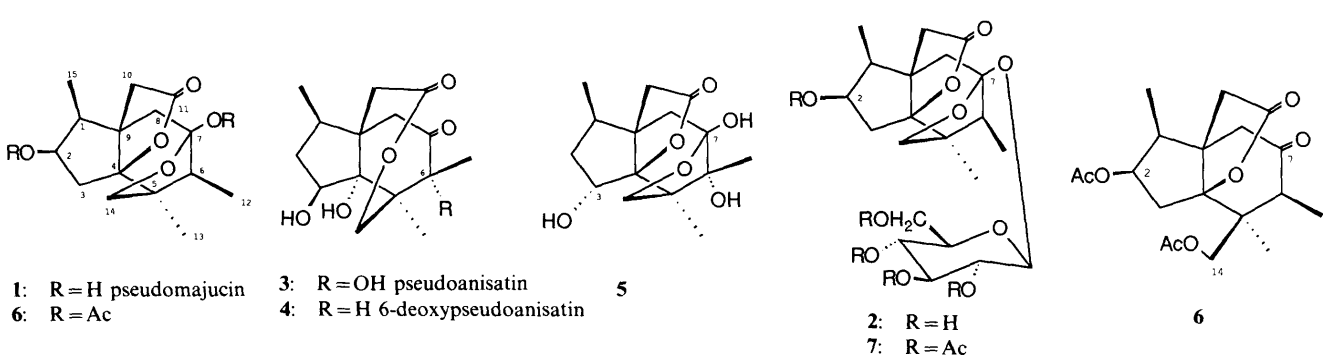


Fig. 1. ORTEP Drawing of **1**



1: R = H pseudomajucin
6: R = Ac

3: R = OH pseudoanisatin
4: R = H 6-deoxypseudoanisatin

5

2: R = H
7: R = Ac

6

Fig. 2

Table I. ^{1}H-NMR Chemical Shifts for Compounds **1**, **2**, and **7** in C_5D_5N (400 MHz)[a]

	1	**2**	**7**
H-1	1.98 dq (J=7.3, 3.3)	1.80 dq (J=7.3, 4.0)	1.95—2.10 m
H-2	4.42 ddd (J=4.4, 3.3, 1.8)	4.30 ddd (J=4.4, 4.0, 1.5)	5.24 br dd (J=4.0, 3.7)
H-3α	2.16 dd (J=14.3, 4.4)	2.04 dd (J=14.3, 4.4)	2.18 dd (J=15.0, 4.0)
H-3β	2.39 dd (J=14.3, 1.8)	2.31 dd (J=14.3, 1.5)	2.38 d (J=15.0)
H-6	1.94 q (J=7.3)	2.02 q (J=7.3)	1.97 q (J=7.0)
H-8β	2.14 d (J=13.6)	2.25 d (J=13.9)	2.14 d (J=13.9)
H-8α	2.55 d (J=13.6)	2.86 d (J=13.9)	2.68 d (J=13.9)
H-10a	2.94 d (J=18.3)	2.85 d (J=18.0)	2.82 d (J=18.3)
H-10b	3.18 d (J=18.3)	3.15 d (J=18.0)	3.02 d (J=18.3)
H-12	1.21 d (J=7.3)	1.19 d (J=7.3)	0.99 d (J=7.0)[b]
H-13	1.00 s	0.93 s	0.91 s
H-14a	3.76 d (J=9.2)	3.76 d (J=8.8)	3.74 d (J=9.2)
H-14b	4.18 d (J=9.2)	4.16 d (J=8.8)	4.09 d (J=9.2)
H-15	1.15 d (J=7.3)	1.09 d (J=7.3)	0.98 d (J=7.0)[b]
Glc			
H-1′		5.41 d (J=7.7)	5.45 d (J=8.1)
H-2′		4.08 dd (J=8.8, 7.7)	5.51 dd (J=9.5, 8.1)
H-3′		4.27 t (J=8.8)	5.79 t (J=9.5)
H-4′		4.22 t (J=8.8)	5.45 dd (J=9.9, 9.5)
H-5′		3.95 ddd (J=8.8, 5.5, 2.6)	4.17 ddd (J=9.9, 5.5, 2.6)
H-6′		4.32 dd (J=11.7, 5.5)	4.43 dd (J=12.1, 2.6)
		4.53 dd (J=11.7, 2.6)	4.51 dd (J=12.1, 5.5)
–OAc			2.02 (2 × Me), 2.06 (2 × Me)
(each s)			2.12

a) Chemical shifts are given on the δ (ppm) scale, and coupling constants are given in Hz (s, singlet; t, triplet; d, doublet; q, quartet; br, broad; m, multiplet). *b*) Assignments may be interchanged.

Table II. ^{13}C-NMR Data for Compounds **1**, **5**, **2**, and **7** in C_5D_5N[a]

	1	**5**	**2**	**7**
C-1	55.0	45.1	55.1	52.9
C-2	73.6	39.1	73.6	77.3
C-3	43.8	77.3	43.8	40.3
C-4	100.3	102.2	99.9	99.4
C-5	51.1	52.8	51.0	50.9
C-6	43.7	78.0	44.5	44.3
C-7	106.9	106.9	109.5	109.4
C-8	54.6	47.8	52.3	52.3
C-9	48.8	48.6	48.9	49.0
C-10	41.6	41.2	41.5	41.0
C-11	176.8	176.6	176.7	175.8
C-12	9.0	19.0	8.8	8.4
C-13	14.4	12.4[b]	14.0	13.6
C-14	71.1	72.2	72.2	72.4
C-15	10.0	13.9[b]	10.0	9.6
Glc				
C-1′			98.8	95.8
C-2′			75.4	71.7
C-3′			78.8	73.3
C-4′			71.8	69.4
C-5′			78.4	72.3
C-6′			62.9	62.7
OAc				
$-CH_3$				20.5
				20.5
				20.7
				20.7
				20.9
=O				170.3
				170.3
				170.2
				169.9
				169.6

a) Run at 22.5 MHz for **5**[1] and 100.4 MHz for compounds **1**, **2**, and **7**. Chemical shifts are given on the δ (ppm) scale. Assignments were made on the basis of 2D ^{1}H–^{13}C COSY spectra and 2D long-range ^{1}H–^{13}C COSY spectra for compounds **1** and **2**. *b*) Assignments may be interchanged.

Table III. Fractional Atomic Coordinates (× 10^4) and Thermal Parameters (Å), with Estimated Standard Deviations in Parentheses

Atom	x	y	z	B_{eq}[a]
O(1)	748 (6)	404 (3)	9504(5)	3.23
O(2)	3395 (6)	−575 (4)	10968 (6)	4.58
O(3)	187 (6)	892 (4)	12513 (5)	3.97
O(4)	2443 (6)	4173 (4)	7340 (5)	3.58
O(5)	2806 (6)	2289 (4)	7457 (5)	3.71
C(1)	962 (9)	2653 (5)	11711 (7)	3.10
C(2)	−718 (9)	1856 (6)	11636 (8)	3.77
C(3)	−1729 (8)	1645 (5)	9810 (8)	3.09
C(4)	57 (9)	1550 (5)	9208 (8)	2.99
C(5)	−477 (9)	1767 (5)	7410 (8)	3.35
C(6)	−466 (9)	3034 (5)	7212 (7)	3.39
C(7)	1863 (9)	3224 (6)	7927 (7)	3.36
C(8)	2634 (8)	3232 (5)	9741 (7)	3.07
C(9)	1890 (8)	2227 (5)	10455 (7)	2.84
C(10)	3610 (9)	1389 (5)	11125 (8)	3.52
C(11)	2627 (9)	300 (6)	10561 (8)	3.46
C(12)	−1496 (10)	3433 (7)	5478 (9)	4.75
C(13)	−2477 (10)	1189 (6)	6401 (8)	4.38
C(14)	1313 (10)	1448 (6)	6913 (8)	3.95
C(15)	2541 (10)	2898 (6)	13365 (8)	4.30

a) $B_{eq} = 4/3(\beta_{11}/a^{*2} + \beta_{22}/b^{*2} + \beta_{33}/c^{*2})$.

computergenerated drawing of the structure is shown in Fig. The atomic parameters of nonhydrogen atoms, bond lengths and bond angles are listed in Tables III, IV, and V, respectively. The crystal data are presented in the experimental section.

Acetylation of **1** with Ac_2O and pyridine afforded a diacetate (**6**). This compound was supposed to have been produced by the cleavage of the acetal bond of **1**, and its structure was elucidated as shown in the figure on the basis of the ^{1}H- and ^{13}C-NMR spectra of **6**.

The molecular structure of **1** indicated that a strong

TABLE IV. Bond Lengths (Å) of **1** with Estimated Standard Deviations in Parentheses

O(1)–C(4)	1.485 (7)	C(4)–C(5)	1.535 (9)
O(1)–C(11)	1.317 (6)	C(4)–C(9)	1.594 (8)
O(2)–C(11)	1.201 (8)	C(5)–C(6)	1.571 (9)
O(3)–C(2)	1.437 (8)	C(5)–C(13)	1.537 (8)
O(4)–C(7)	1.402 (8)	C(5)–C(14)	1.531 (6)
O(5)–C(7)	1.464 (7)	C(6)–C(7)	1.540 (6)
O(5)–C(14)	1.426 (7)	C(6)–C(12)	1.532 (10)
C(1)–C(2)	1.516 (7)	C(7)–C(8)	1.511 (9)
C(1)–C(9)	1.582 (7)	C(8)–C(9)	1.568 (8)
C(1)–C(15)	1.518 (9)	C(9)–C(10)	1.532 (8)
C(2)–C(3)	1.546 (9)	C(10)–C(11)	1.508 (9)
C(3)–C(4)	1.541 (5)		

TABLE V. Bond Angles (°) of **1** with Estimated Standard Deviations in Parentheses

C(4)–O(1)–C(11)	113.3 (3)	C(5)–C(6)–C(12)	114.4 (5)
C(7)–O(5)–C(14)	108.5 (3)	C(5)–C(6)–C(7)	98.8 (3)
C(2)–C(1)–C(9)	105.7 (4)	C(7)–C(6)–C(12)	113.9 (4)
C(2)–C(1)–C(15)	116.7 (5)	O(4)–C(7)–C(6)	112.4 (6)
C(9)–C(1)–C(15)	114.5 (4)	O(4)–C(7)–C(8)	111.0 (5)
C(1)–C(2)–C(3)	101.7 (4)	O(4)–C(7)–O(5)	108.9 (4)
O(3)–C(2)–C(3)	112.2 (5)	O(5)–C(7)–C(6)	106.5 (4)
O(3)–C(2)–C(1)	109.2 (5)	C(6)–C(7)–C(8)	110.7 (4)
C(2)–C(3)–C(4)	105.4 (4)	O(5)–C(7)–C(8)	107.0 (5)
O(1)–C(4)–C(5)	106.9 (4)	C(7)–C(8)–C(9)	113.0 (5)
O(1)–C(4)–C(9)	104.2 (4)	C(1)–C(9)–C(4)	103.5 (3)
C(3)–C(4)–C(5)	115.9 (5)	C(1)–C(9)–C(8)	108.3 (3)
C(3)–C(4)–C(9)	105.5 (4)	C(4)–C(9)–C(8)	115.6 (5)
O(1)–C(4)–C(3)	105.3 (3)	C(4)–C(9)–C(10)	103.8 (3)
C(5)–C(4)–C(9)	117.8 (4)	C(8)–C(9)–C(10)	111.0 (3)
C(4)–C(5)–C(6)	106.4 (4)	C(1)–C(9)–C(10)	114.6 (5)
C(4)–C(5)–C(13)	110.7 (4)	C(9)–C(10)–C(11)	105.7 (4)
C(4)–C(5)–C(14)	110.4 (5)	O(1)–C(11)–C(10)	111.6 (5)
C(6)–C(5)–C(13)	115.8 (5)	O(2)–C(11)–C(10)	126.7 (9)
C(6)–C(5)–C(14)	100.6 (3)	O(1)–C(11)–O(2)	121.6 (8)
C(13)–C(5)–C(14)	112.4 (5)	O(5)–C(14)–C(5)	107.0 (4)

hydrogen bond can be formed between the 2-hydroxy and the lactone carbonyl group. That is presumably why the anomalous absorption at 1725 cm^{-1} in the IR spectrum of **1** was observed.

Compound **2** was isolated from the water soluble part. A large amount of shikimic acid, contained in the water layer, was removed by Amberlite IRA-400 ion exchange column chromatography to give the acid-free aqueous solution, which was evaporated to give a brown gum. The isolation of **2** was achieved by repeated chromatographic separation on silica gel with the solvent system of $CHCl_3$–MeOH–H_2O.

Compound **2**, oily syrup, $[\alpha]_D$ −58.6°, gave the molecular formula $C_{21}H_{32}O_{10}$ from the fast-atom bombardment-mass spectrum (FAB-MS) (445 $[M^+ + H]$) and the proton and carbon counts in the ^{1}H- and ^{13}C-NMR spectra. In the ^{13}C-NMR spectrum of **2**, fifteen signals were similar to those of **1**, as shown in Table II, and the other six carbon signals suggested a glycosyl moiety. The hydrolysis of **2** with 1 N HCl afforded pseudomajucin (**1**) and glucose. The result of thin layer chromatography (TLC), and the ^{1}H- and ^{13}C-NMR spectra of the former compound were identical with those of authentic **1**, and the latter was confirmed by Avicel TLC with an authentic sample of glucose.

In the ^{13}C-NMR spectrum of **2**, the carbon signal of C-7 at δ 109.5 was shifted downfield (2.6 ppm, glycosylation shift) compared with that of **1**, suggesting that the glucosyl moiety was linked to the acetal hydroxy group at C-7 of pseudomajucin. This was supported by the following result; *i.e.*, compound **2** was acetylated with Ac_2O and pyridine to give the pentaacetate (**7**). In contrast to compound **1**, the normal acetylated compound was obtained in good yield in the case of compound **2**. The proton signal of H-2 at δ 4.30 in the ^{1}H-NMR spectrum of **2** was shifted downfield by 0.94 ppm in that of **7**, and the carbon signal of C-2 at δ 73.6 in the ^{13}C-NMR spectrum of **2** was shifted to δ 77.3 in that of **7**, indicating the linkage position of glucose to be at C-7 of pseudomajucin. Since the J value of the H-1′ signal of glucose at δ 5.41 in the ^{1}H-NMR spectrum of **2** was 7.7 Hz, the configuration at C-1′ was determined as β. Consequently, the structure of **2** was elucidated as 7-*O*-β-D-glucosyl pseudomajucin.

Experimental

The melting point of **1** was determined on a Yanagimoto micromelting point apparatus and is uncorrected. The IR spectra were recorded with a JASCO IR-810 spectrometer. MS were taken on a JEOL JMS-DX-303 mass spectrometer. The ^{1}H- and ^{13}C-NMR spectra were recorded on a JEOL GX-400 spectrometer operating at 399.5 MHz for ^{1}H and 100.40 MHz for ^{13}C nuclei using tetramethylsilane (TMS) as an internal standard. Kieselgel 60 (Merck) was used for column chromatography.

Isolation of the Constituents The pericarps (1.5 kg) of *I. majus*, collected at Guangxi in China, were extracted with methanol, then defatted with *n*-hexane. The residual part was dissolved in water, and partitioned with AcOEt and *n*-BuOH successively. The AcOEt soluble part was subjected to counter-current distribution between water and AcOEt to give 5 fractions. Of these fractions, fraction 2 was repeatedly chromatographed on silica gel, and finally purified on a Kusano Pre Packed Column Si 5 using $CHCl_3$–MeOH (7%) as the solvent, then recrystallized from AcOEt to give pseudomajucin (**1**) (121 mg) as colorless prisms.

The water soluble part was passed through an ion exchange column of Amberlite IR-400 several times, then evaporated to give a brown gum, which was chromatographed on silica gel using the solvent system of $CHCl_3$–MeOH–H_2O (85:15:1), then $CHCl_3$–acetone–MeOH (4:4:1). Further purification by chromatography on a Kusano Pre Packed Column C_{18}-20 using H_2O–MeOH (7:3) as the solvent afforded compound **2** (99 mg) as colorless oily syrup.

Pseudomajucin (1) Colorless prisms, mp 199—201 °C (AcOEt), $[\alpha]_D^{15}$ −89.6° (c=0.26, dioxane). IR ν_{max}^{KBr} cm^{-1}: 3295, 3420 (OH), 1725 (γ-lactone). MS m/z: 282 (M^+). *Anal.* Calcd for $C_{15}H_{22}O_5$: C, 63.81; H, 7.85. Found: C, 63.54; H, 7.71.

Crystal Data for 1 $C_{15}H_{22}O_5$, monoclinic, space group $P2_1$, a=7.019 (1), b=12.317 (2), c=8.949 (2) Å, β=111.56 (1)°, U=719.6 (2) Å^3, Z=2, D_c=1.30 g·cm^{-1}, λ (CuK_α)=1.5405 Å. A total of 1264 independent reflections (2θ<128°) were collected on a Rigaku AFC-5 diffractometer using graphite-monochromated CuK_α radiation with the θ–2θ scan technique. After correcting for Lorentz and polarization effects, 1234 reflections were considered as observed ($F_o > 2\sigma(F_o)$). The structure was solved by direct methods with the MULTAN84[5] series of programs, in which RATAN was used to solve the phase problem. Block-diagonal least-squares refinements with anisotropic nonhydrogen atoms and isotropic hydrogen atoms lowered the R value to 0.060.

Acetylation of 1 **1** (12 mg) was dissolved in a mixture of Ac_2O (0.5 ml) and anhydrous pyridine (0.5 ml) and the solution was allowed to stand for 20 h at room temperature. The reaction mixture was poured into water and extracted with $CHCl_3$. The $CHCl_3$ layer was dried over Na_2SO_4, then evaporated to give a residue, which was purified on a Kusano Pre Packed Si 5 column using *n*-hexane–AcOEt (1:1) as the solvent to give the diacetate (**6**) (8 mg, colorless oil). ^{1}H-NMR (C_5D_5N) δ: 2.11 (1H, dq, J=6.6, 2.6 Hz, 1-H), 5.29 (1H, dd, J=3.7, 2.6 Hz, 2-H), 2.50 (1H, dd, J=15.4, 3.7 Hz, 3-H$_\alpha$), 2.43 (1H, d, J=15.4 Hz, 3-H$_\beta$), 2.49 (1H, q, J=6.7 Hz, 6-H), 2.60 (1H, d, J=18.7 Hz, 8-H$_\beta$), 3.29 (1H, d, J=18.7 Hz, 8-H$_\alpha$), 2.81 (1H, d, J=19.1 Hz, 10-H$_a$), 2.90 (1H, d, J=19.1 Hz, 10-H$_b$), 1.16 (3H, d, J=6.7 Hz, 12-H), 1.01 (3H, s), 4.12 (1H, d, J=12.1 Hz, 14-H$_a$), 4.41 (1H, d, J=12.1 Hz, 14-H$_b$), 1.02 (3H, d, J=6.6 Hz, 15-H), 1.99 and 2.06 (each 3H, s). ^{13}C-NMR (C_5D_5N) δ: 8.7, 9.2, 19.6, 20.6, 20.9, 40.8, 42.1, 45.0, 47.1,

49.8, 50.4, 51.2, 64.4, 76.6, 96.9, 169.7, 170.2, 175.4, 208.3. EI-MS *m/z*: 366 [M^+].

Compound 2 Colorless oily syrup, $[\alpha]_D^{20}$ −58.6° (c = 1.09, dioxane). IR ν_{max}^{Nujol} cm^{-1}: 3350, 1745. FAB-MS *m/z*: 445 [$M^+ + H$].

Acid Hydrolysis of 2 A solution of **2** (21.5 mg) in 1 N HCl (2 ml) was heated at 75 °C for 1.5 h, then extracted with AcOEt. The AcOEt soluble part was washed with water. The washing was combined with the water layer, and dried over Na_2SO_4, then evaporated under reduced pressure to give the residue. This was purified by column chromatography on silica gel using $CHCl_3$–MeOH (9:1) as the solvent to give **1** (7.4 mg). This compound was identical with authentic pseudomajucin. The water layer was neutralized with silver carbonate, then concentrated. The sugar moiety in this solution was examined by Avicel TLC (Funakoshi Yakuhin Co., Ltd.) (developed with *n*-BuOH–pyridine–H_2O (6:4:3)) together with an authentic sample of glucose.

Acetylation of 2 A solution of **2** (14.2 mg) in a mixture of pyridine (1 ml) and Ac_2O (1 ml) was allowed to stand at room temperature overnight, then diluted with H_2O and extracted with $CHCl_3$. The $CHCl_3$ extract was washed with water, and dried over Na_2SO_4, then concentrated to dryness. The residue was purified by column chromatography on silica gel to give the pentaacetate (**7**) (12.4 mg) as a colorless oil. $C_{31}H_{42}O_{15}$ EI-MS *m/z*: 654 [M^+].

Acknowledgement We wish to thank Mr. Ohama for the measurement of 400 MHz NMR spectra.

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