Identification of Sucrose Diesters of Aryldihydronaphthalene-Type Lignans from *Trigonotis peduncularis* and the Nature of Their Fluorescence

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Phytochemical investigation of *Trigonotis peduncularis* resulted in the isolation of three aryldihydronaphthalene-type lignan sucrose diesters named trigonotins A−C (**1**−**3**). These lignans showed a strong yellow-green fluorescence emission under basic conditions. The structures of the new compounds were elucidated by means of spectroscopic methods, and the nature of their fluorescence was examined.

Trigonotis peduncularis Benth. ex S. Moore & Baker (Boraginaceae) is a biennial herb of about 15 to 30 cm in height distributed widely in the temperate zone of Asia. The Boraginaceae includes many medicinal plants, such as *Alkanna tinctoria, Lithospermum erythrorhizon*, and *Macrotomia euchroma*. However, no chemical investigation of *T. peduncularis* has been conducted previously, except for analyses of its essential oils[1] and fatty acids.[2] From the whole parts of *T. peducularis*, three lignan sucrose diesters, trigonotins A−C (**1**−**3**), were isolated along with rosmarinic acid[3] and (6*R*,9*R*)-3-oxo-α-ionol-*O*-*β*-D-glucopyranoside.[4,5] This paper deals with the structural elucidation of the new compounds and discusses the nature of the fluorescence emission of these trigonotins.

Results and Discussion

Trigonotin A (**1**), $[\alpha]_D$ +491, was isolated as an amorphous, yellow powder, and its elemental composition was determined to be $C_{35}H_{40}O_{19}$ by negative-ion HRFABMS. The IR spectrum showed that compound **1** has a glycosidic unit (3403 and 1037 cm^{-1}) and contained absorption bands for esters (1717 cm^{-1}) and aromatic rings (1629, 1574, 1513, and 1502 cm^{-1}). The UV spectrum also indicated the presence of aromatic rings (253 and 355 nm). A total of 35 carbon signals was observed in the ^{13}C NMR spectrum and were assigned for one tri- and one pentasubstituted benzene ring, a trisubstituted double bond, two methines, and two carboxyl functional groups, along with three methoxyl moieties and an acetyl group (δ_C 172.8 and 20.4, and δ_H 1.64) (Tables 1 and 2). From this evidence, the structure of the core portion of **1** was assigned an aryldihydronaphthalene-type lignan with two carboxyl functional groups. In the sugar signal region of the ^{13}C NMR spectrum (δ_C 60−80), 11 carbon signals, three of which were primary alcohols, were observed. Although this implied that two hexose moieties were present in the molecule, only one anomeric proton (δ_H 5.41) and carbon (δ_C 94.6), which showed a cross-peak in the HSQC spectrum, were assigned. The coupling constant of the anomeric proton was too small (J = 3.7 Hz) to expect a D-sugar series having a β-linkage. From these data, the sugar moiety was assigned as a sucrose, with an α-glucopyranoside linked to fructopyranose. The degree of unsaturation indicated one more cyclic system in the molecule. This was confirmed by the information obtained in the HMBC experiment (Figure 1), and one of the carboxyl carbon signals (δ_C 175.0) showed a cross-peak with a proton at δ_H 4.85 (H-3f), and the other (δ_C 168.9) with the protons at δ_H 4.91 and 4.07 (H_2-6f). Since HMBC correlations were observed from δ_H 6.89 (H-8) to δ_C 142.4 (C-1) and δ_H 7.74 (H-1) to δ_C 168.9 (C-2a), and from δ_H 6.66 (H-6′) to δ_C 40.0 (C-4) and δ_H 4.77 (H-4) to δ_C 175.0 (C-3a), the dihydronaphthalene benzene ring was assigned as pentasubstituted with three deshielded carbon atoms and an aryl benzene ring ABX-type trisubstituted with two deshielded carbon atoms. Therefore, the structure of trigonotin A (**1**) was proposed as a sucrose diester of an aryldihydronaphthalene-type lignan. This was confirmed by hydrolysis of trigonotin A (**1**) in 0.1 N CH_3ONa to give a hydrolysis product (**1a**) and sucrose (**1b**). NMR spectra of the sucrose were identical with those of an authentic sample. The small coupling value (J = 1.3 Hz) between H-3 and H-4 was consistent with the substituents on C-3 and C-4 having a *cis* relationship. The first positive [$\Delta\varepsilon$ +7.18 (360 nm)] and second negative [$\Delta\varepsilon$ −3.60 (312 nm)] Cotton effects in the CD spectrum

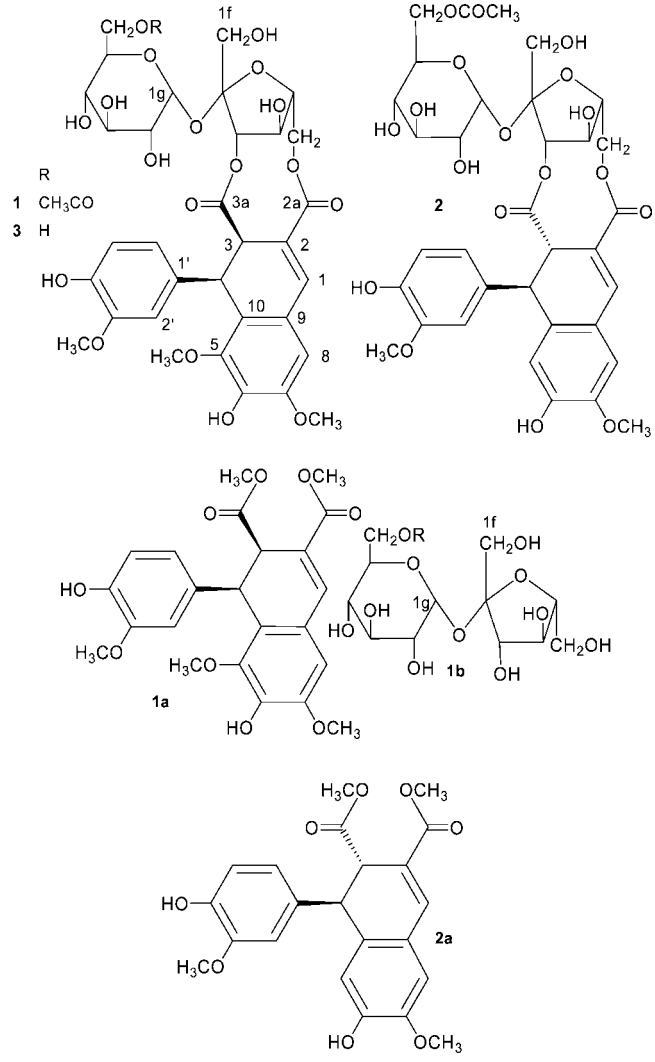

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Table 1. ^{1}H NMR Spectroscopic Data for Trigonotins A (**1**), B (**2**), and C (**3**)

position	**1**	**2**	**3**
1	7.74 (brd, 0.9)	7.55 (d, 2.5)	7.70 (brd, 0.9)
3	4.09 (brd, 1.3)	4.20 (dd, 15.3, 2.5)	3.95 (dd, 2.1, 0.9)
4	4.77 (d, 1.3)	4.34 (dd, 15.3, 1.1)	4.76 (d, 2.1)
5		6.14 (d, 1.1)	
8	6.89 (brs)	6.94 (s)	6.87 (brs)
2′	6.61 (brs)	6.88 (d, 1.8)	6.65 (d, 2.0)
5′	6.60 (d, 7.7)	6.87 (d, 8.0)	6.69 (d, 8.1)
6′	6.66 (dd, 7.7, 0.9)	6.82 (d, 8.0, 1.8)	6.61 (dd, 8.1, 2.0)
OCH_3-5	3.47 (s)		3.49 (s)
OCH_3-7	3.90 (s)	3.86 (s)	3.90 (s)
OCH_3-3′	3.72 (s)	3.85 (s)	3.74 (s)
1f	3.94 (d, 12.4)	3.88 (d, 12.5)	3.91 (d, 12.0)
	3.73 (d, 12.4)	3.72 (d, 12.5)	3.78 (d, 12.0)
3f	4.85 (s)	4.61 (s)	4.85 (s)
4f	3.87 (s)	4.68 (s)	3.91 (s)
5f	4.16 (brd, 2.9)	4.24 (brd, 2.4)	4.16 (brd, 2.8)
6f	4.91 (dd, 13.0, 2.9)	4.64 (dd, 12.0, 2.4)	4.88 (dd, 12.8, 2.8)
	4.07 (brd, 13.0)	4.06 (brd, 12.0)	4.08 (brd, 12.8)
1g	5.41 (d, 3.6)	5.31 (d, 3.7)	5.40 (d, 3.7)
2g	3.48 (dd, 9.5, 3.6)	3.41 (dd, 9.4, 3.7)	3.48 (dd, 9.2, 3.7)
3g	3.76 (d, dd, 9.5, 9.5)	3.63 (dd, 9.4, 9.2)	3.74 (dd, 9.2, 9.2)
4g	3.23 (dd, 9.5, 9.1)	3.30 (m)	3.43 (dd, 10.1, 9.2)
5g	4.30 (ddd, 9.1, 7.3, 1.7)	4.27 (m)	4.03 (br dt, 10.1, 3.5)
6g	4.17 (dd, 11.8, 1.7)	4.22 (brd, 11.8)	3.58 (dd, 11.9, 4.0)
	4.01 (dd, 11.8, 7.3)	4.09 (dd, 11.8, 5.6)	3.54 (dd, 11.9, 3.1)
$COCH_3$	1.64 (s)	1.91 (s)	

of **1a** indicated that the absolute configuration at C-4 is *R* and that C-3 is also *R*. An acetyl group was placed on the hydroxyl group of C-6g by the HMBC experiment (Figure 1) and the observed low-field shifts of the C-6g and H_2-6g signals in the NMR spectra, when compared with the corresponding signals of **1** and sucrose. Accordingly, the structure of trigonotin A was elucidated to be **1**, as shown.

Trigonotin B (**2**), $[\alpha]_D$ +135, was isolated as an amorphous, yellow powder, and its elemental composition was determined to be $C_{34}H_{38}O_{18}$ by negative-ion HRFABMS. The IR and UV spectra showed similar absorption maxima to those of **1**. The ^{1}H and ^{13}C NMR spectra also indicated that in **2** one of the methoxyl groups at C-5 (δ_C 60.7) in **1** was replaced by an aromatic proton, which appeared at δ_H 6.14 and was coupled with H-4 (J = 1.1 Hz). The relative configuration of H-3 and H-4 was assigned as *trans* from their large coupling constant (J = 15.3 Hz), and the absolute configuration of C-4 was deduced as *R* from the first positive [$\Delta\varepsilon$ +5.88 (345 nm)] and second negative [$\Delta\varepsilon$ −2.15 (312 nm)] Cotton effects in the CD spectrum of the hydrolysis product, **2a**. Therefore, the structure of trigonotin B was elucidated to be **2**, as shown.

Trigonotin C (**3**), $[\alpha]_D$ +394, was isolated as an amorphous, yellow powder, and its elemental composition was determined to be $C_{33}H_{38}O_{18}$ by negative-ion HRFABMS. All physicochemical data were similar to those of **1**, except for the absence of the acetyl group. The ^{13}C NMR chemical shifts of C-4g, C-5g, and C-6g were essentially the same as those of sucrose itself. Thus, **3** was elucidated to be a deacetyl derivative of **1**, as shown.

As far as we know, only two compounds have been isolated as a sucrose diester, namely, 4,4′-dihydroxy-3,3′-dimethoxy-β-truxinic acid from*Avena sativa* (oat)[6] and 4,4′-dihydroxy-β-truxinic acid from *Bidens parviflora*.[7]

During alkaline hydrolysis of trigonotin A (**1**), a strong deep green-yellow luminescence emission was observed. Thus, fluorescence emission was analyzed in buffer solutions of various pH values. In the 20 mM acetate buffer at pH 5.0, the excitation maximum for trigonotin A (**1**) was 360 nm, but only slight emission was observed at the wavelength of 526 nm with an arbitrary value of 11.1 (Figure 2a). Under basic conditions at pH 8.0, the maximum excitation wavelength shifted down to 435 nm and the magnitude of the fluorescence was drastically enhanced at the wavelength of 526 nm with the arbitrary value of 685. At the same pH values, the fluorescence strengths were the same and not influenced by the different buffer solutions. Anionic forms of the phenolic hydroxyl groups and proper torsion angles of the two aromatic systems in the molecule might be important, since in the related compound triogonotin B (**2**) the strength of the fluorescence was decreased (Figure 2c). The methanolysis product (**1a**) of trigonotin A retained its fluorescence propensity, but the strength was lower than that of

Table 2. ^{13}C NMR Spectroscopic Data for Trigonotins A (**1**), B (**2**), and C (**3**)

position	**1**	**2**	**3**
1	142.4	140.4	142.0
2	123.2	126.2	123.3
2a	168.9	168.0	168.6
3	50.6	51.7	50.8
3a	175.0	176.2	174.9
4	40.0	48.2	40.2
5	147.0	116.4	147.1
6	144.5	148.0	144.5
7	149.2	150.4	149.2
8	110.2	113.9	110.1
9	123.2	135.0	123.1
10	125.6	124.3	125.7
1′	137.5	133.6	133.7
2′	112.7	114.6	112.7
3′	148.9	149.2	148.9
4′	146.6	147.0	146.4
5′	116.6	117.0	116.6
6′	121.5	123.6	121.5
OCH_3-5	60.7		60.7
OCH_3-7	57.0	56.9	57.0
OCH_3-3′	56.8	56.8	56.7
1f	63.6	63.4	63.5
2f	109.8	110.3	110.2
3f	80.4	82.3	80.5
4f	75.3	73.5	75.6
5f	88.2	87.6	88.1
6f	66.4	66.6	66.6
1g	94.6	94.6	95.0
2g	73.6	73.6	73.7
3g	75.3	75.2	75.2
4g	72.1	71.2	71.2
5g	72.2	71.2	74.2
6g	66.0	65.4	62.2
$COCH_3$	172.8	173.0	
$COCH_3$	20.4	20.7	

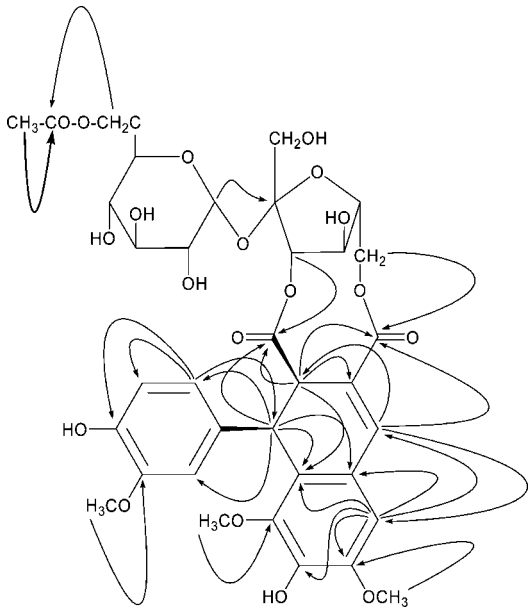

Figure 1. Selected HMBC correlations of trigonotin A (**1**).

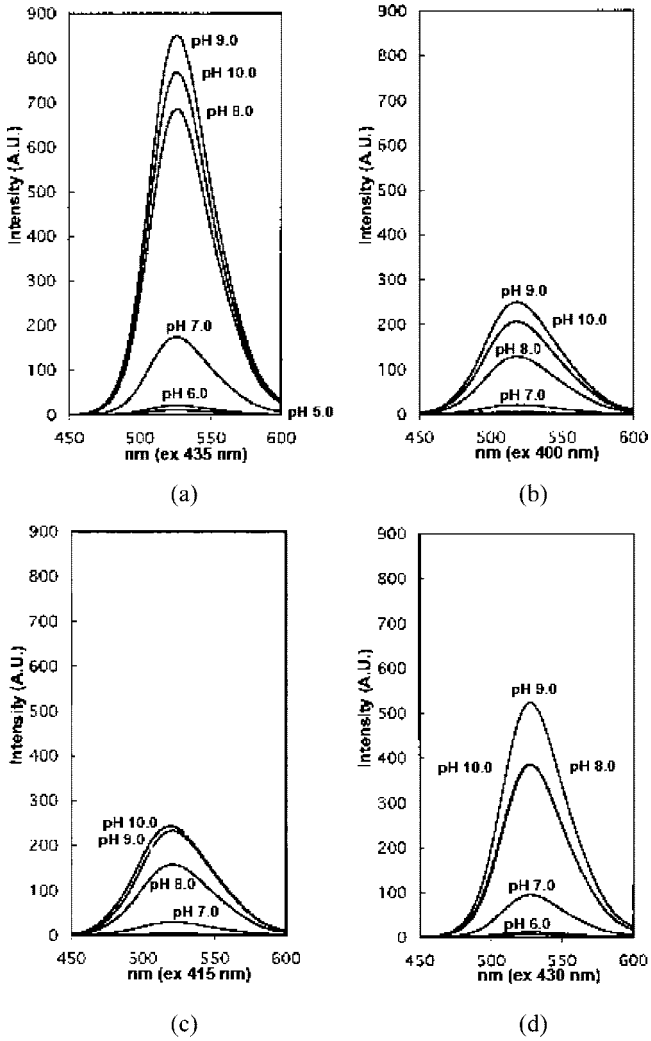

Figure 2. Emission spectra of (a) trigonotin A (**1**), (b) the trigonotin A hydrolysis product (**1a**), (c) trigonotin B (**2**), and (d) trigonotin C (**3**).

1 (Figure 2b). A simple 1:1 mixture of **1a** and sucrose (**1b**) did not restore the fluorescence ability (data not shown). In an aqueous alkaline solution, the fluorescence emission decreased daily and finally ceased completely. Thus, an ester of a carboxylic acid and anionic forms of the phenolic hydroxyl groups were essential for the emission of fluorescence, and interaction of the solvent molecules with the lignan may affect the strength of the fluorescence. As far as we know, this is the first report of aryldihydronaphthalene-type lignans that show strong fluorescent luminescence.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a JASCO P-1030 digital polarimeter. FT-IR and UV spectra were recorded on a Horiba FT-710 and a JASCO V-520 UV/vis spectrophotometer, respectively. CD and fluorescence spectra were measured with a JASCO J-720 polarimeter and a FP-6500 spectrophotometer, respectively. ^{1}H and ^{13}C NMR spectra were taken on a JEOL α-400 spectrometer (400 and 100 MHz, respectively) with TMS as the internal standard. HRFABMS were carried out on a JEOL SX-102 mass spectrometer using PEG-400 as the calibration matrix. Silica gel column chromatography (CC) and reversed-phase (ODS) gel open CC were performed on silica gel 60 (Merck, 70−230 mesh) and Cosmosil 75C_{18}-OPN (Nacalai Tesque Co., Ltd., Kyoto, Japan) [∅ 50 mm,L = 25 cm, linear gradient: MeOH−H_2O (1:9, 1 L) → (1:1, 1 L)], respectively, with fractions of 10 g being collected. Droplet countercurrent chromatography (DCCC) was conducted on a Tokyo Rikakikai (Tokyo, Japan) instrument, equipped with 500 glass columns (∅ = 2 mm, L = 40 cm), and the lower and upper layers of a solvent mixture of $CHCl_3$−MeOH−H_2O−*n*-PrOH (9:12:8:2) were used as the stationary and mobile phase, respectively. Five-gram fractions were collected and numbered according to the order of elution of the mobile phase. Preparative HPLC was performed using ODS (YMC-Pack, ∅ = 20 mm, L = 15 cm, YMC, Kyoto, Japan) or ODS (Inertsil, ∅ = 6 mm, L = 25 cm, GL Science, Tokyo, Japan) columns.

Plant Material. *Trigonotis peduncularis* was collected in Hiroshima City, Japan, in May 2000. The plant was identified by Prof. Takakazu Shinzato of University of the Ryukyus, and a voucher specimen was deposited in the Herbarium of Pharmaceutical Sciences, Graduate School of Biomedical Sciences, Hiroshima University (00-TP-Hiroshima-0507).

Extraction and Isolation. Air-dried leaves of *T. peduncularis* (3.04 kg) were extracted with MeOH (15 L) three times. The MeOH extract was concentrated to 1.5 L, and then 75 mL of H_2O was added to make a 95% aqueous solution. This solution was washed with 1.5 L of *n*-hexane, and the methanolic layer was concentrated to a viscous gum. The gummy residue was suspended in 1.5 L of H_2O and then extracted with 1.5 L each of EtOAc and 1-BuOH, successively, to give 26.2 g of an EtOAc-soluble extract and 35.6 g of a 1-BuOH-soluble extract. The 1-BuOH extract fraction was subjected to highly porous synthetic resin CC (Diaion HP-20, Mitsubishi Chemical Co., Ltd., ∅ = 55 mm, L = 36 cm) using H_2O−MeOH (4:1, 3 L; 2:3, 2 L; 3:2, 2 L; and 1:4, 2 L) and MeOH (2 L), with 500 mL fractions being collected. The residue (11.3 g in fractions 12−15) of the 40% MeOH eluent was subjected to silica gel (250 g) CC, with elution by $CHCl_3$ (2 L) and $CHCl_3$−MeOH [(99:1, 2 L), (49:1, 2 L), (24:1, 2 L), (47:3, 2 L), (23:2, 2 L), (9:1, 2 L), (7:1, 2 L), (17:3, 2 L), (33:7, 2 L), (4:1, 2 L), (3:1, 2 L), and (7:3, 2 L)], with again 500 mL fractions being collected. Combined fractions 21−30 (2.76 g) were separated by reversed-phase (ODS) gel open CC. The residue (884 mg) of fractions 114−126 was subjected to DCCC to give 388 mg of **1** in fractions 88−97 and 33 mg of (6*R*,9*R*)-3-oxo-α-ionol-*O*-*β*-D-glucopyranoside in fractions 121−140. The residue (164 mg) of fractions 140−147 was subjected to DCCC to give 62.3 mg of **2** in fractions 88−97 and 33.0 mg of (6*R*,9*R*)-3-oxo-α-ionol-*O*-*β*-D-glucopyranoside in fractions 120−139. The residue (3.11 g in fractions 31−40) obtained on silica gel CC (12.5−17.5% MeOH eluent) was subjected to open reversed-phase (ODS) gel CC, and the residue (81 mg in fractions 98−105) was then separated by DCCC to give 32.4 mg of **3** in fractions 41−51.The residue (1.12 g in fractions 48−54) obtained on silica gel CC was subjected to reversed-phase (ODS) gel open CC to give 197 mg of rosemarinic acid in fractions 51−76.

Trigonotin A (1): amorphous, yellow powder; $[\alpha]_D^{25}$ +491 (*c* 1.03, MeOH); IR ν_{max} (film) 3403, 2942, 1717, 1629, 1574, 1513, 1502, 1457, 1349, 1272, 1204, 1107, 1037 cm^{-1}; UV (log ε) λ_{max} (MeOH) 253 (4.25), 355 (4.09) nm; CD (*c* 5.36 × 10^{-5} M, MeOH) −8.87 (227), −21.7 (256), +4.76 (293), +19.0 (360) Δε (nm); ^{1}H and ^{13}C NMR

(CD_3OD), Tables 1 and 2, respectively; HRFABMS (negative-ion mode) *m/z* 763.2065 $[M - H]^-$ (calcd for $C_{35}H_{39}O_{19}$, 763.2086).

Trigonotin B (2): amorphous, yellow powder; $[\alpha]_D^{25}$ +135 (*c* 1.19, MeOH); IR ν_{max} (film) 3396, 2942, 1725, 1626, 1569, 1515, 1451, 1370, 1249, 1025 cm^{-1}; UV (log ε) λ_{max} (MeOH) 249 (4.23), 340 (4.03) nm; CD (*c* 6.53 × 10^{-5} M, MeOH) +3.66 (230), +4.81 (257), +6.04 (293), +0.85 (368) $\Delta\varepsilon$ (nm); ^1H and ^{13}C NMR (CD_3OD), Tables 1 and 2, respectively; HRFABMS (negative-ion mode) *m/z* 733.2005 $[M - H]^-$ (calcd for $C_{34}H_{37}O_{18}$, 733.1980).

Trigonotin C (3): amorphous, yellow powder; $[\alpha]_D^{25}$ +394 (*c* 1.65, MeOH); IR ν_{max} (film) 3396, 2940, 17157, 1629, 1574, 1513, 1459, 1270, 1204 cm^{-1}; UV (log ε) λ_{max} (MeOH) 252 (4.17), 350 (4.02) nm; CD (*c* 9.22 × 10^{-5} M, MeOH) −8.54 (228), −19.1 (256), +5.21 (291), +17.2 (359) $\Delta\varepsilon$ (nm); ^1H and ^{13}C NMR (CD_3OD), Tables 1 and 2; respectively; HRFABMS (negative-ion mode) *m/z* 721.1952 $[M - H]^-$ (calcd for $C_{33}H_{37}O_{18}$, 721.1980).

Mild Alkaline Hydrolysis of Trigonotin A (1). Trigonotin A (**1**) (52.6 mg) was treated with 4 mL of 0.1 N CH_3ONa (4.5 mL) for 24 h at 50 °C under a N_2 stream. The reaction mixture was neutralized with Amerlite IR-120B (H^+) (Organo) and then filtered and evaporated to dryness. The residue was purified by silica gel column chromatography (∅ = 15 mm, *L* = 20 cm) with $CHCl_3$ (150 mL), $CHCl_3$−MeOH (9:1) (200 mL), and $CHCl_3$−MeOH−H_2O (35:15:2) (300 mL), with 10 g fractions being collected. The hydrolysis product (**1a**) (10.3 mg) was obtained in fractions 20−22. The $CHCl_3$−MeOH−H_2O eluate was evaporated to afford 2.6 mg of sucrose (**1b**).

Trigonotin A Hydrolysis Product (1a): amorphous, pale yellow powder; $[\alpha]_D^{25}$ +138 (*c* 0.69, MeOH); IR ν_{max} (film) 3417, 2950, 1700, 1632, 1605, 1575, 1513, 1461, 1269, 1208 cm^{-1}; UV (log ε) λ_{max} (MeOH) 249 (4.24), 291sh (3.82), 332 (4.10) nm; CD (*c* 6.19 × 10^{-5} M, MeOH) −10.5 (230), −4.87 (253), +1.71 (293), −3.60 (312), +7.18 (360) $\Delta\varepsilon$ (nm); ^1H (CD_3OD) δ 3.54 (3H, s, C-5-OCH_3), 3.61 (3H, s, C-3a-OCH_3), 3.71 (3H, s, C-3′-OCH_3), 3.72 (3H, s, C-2a-OCH_3), 3.89 (3H, s, C-7-OCH_3), 3.93 (1H, d,*J* = 1.2 Hz, H-3), 4.95 (1H, brs, H-4), 6.14 (1H, d,*J* = 1.1 Hz, H-5), 6.53 (1H, dd,*J* = 2.0, 8.3 Hz, H-6′), 6.59 (1H, d,*J* = 8.3 Hz, H-5′), 6.63 (1H, d,*J* = 2.0 Hz, H-2′), 6.87 (1H, s, H-8), 7.68 (1H, s, H-1); ^{13}C NMR (CD_3OD) δ 40.7 (C4), 48.2 (C-3), 52.4 (C-2a-OCH_3), 52.9 (C-3a-OCH_3), 56.3 (C-3′-OCH_3), 56.8 (C-7OCH_3), 60.7 (C-5-OCH_3), 112.2 (C-2′), 116.0 (C-5′), 119.5 (C-8), 120.9 (C-6′), 123.0 (C-9), 125.1 (C-10), 124.3 (C-2), 135.4 (C-1′), 139.8 (C-1), 143.6 (C-6), 148.8 (C-3′), 146.3 (C-4′), 146.8 (C-5), 149.4 (C-7), 169.0 (C-2a), 174.3 (C-3a); HRFABMS (negative-ion mode) *m/z* 443.1377 $[M - H]^-$ (calcd for $C_{23}H_{23}O_9$: 443.1342).

Sucrose (1b): amorphous, white powder; $[\alpha]_D^{25}$ +60 (*c* 0.17, H_2O); ^1H NMR (CD_3OD) δ 5.39 (1H, d, *J* = 3.7 Hz, H-1g); ^{13}C NMR (CD_3OD) δ 62.4 (C-6g), 63.4 (C-1f), 64.2 (C-6f), 71.5 (C-4g), 73.3 (C-2g), 74.5 (C-5g), 74.9 (C-3g), 75.9 (C-4f), 79.6 (C-3f), 83.9 (C-5f), 93.7 (C-1g), 105.4 (C-2f).

Mild Alkaline Hydrolysis of Trigonotin B (2). Similar treatment of trigonotin B (**2**) (24.3 mg) to **1** gave 9.9 mg of **2a**. Trigonotin B hydrolysis product (**2a**): amorphous, pale yellow powder; $[\alpha]_D^{25}$ +147 (*c* 0.66, MeOH); IR ν_{max} (film) 3406, 2951, 1701, 1601, 1576, 1513, 1270, 1240, 1210 cm^{-1}; UV (log ε) λ_{max} (MeOH) 249 (4.22), 289 sh (3.81), 313 sh (3.94), 340 (4.06) nm; CD (*c* 3.99 × 10^{-5} M, MeOH) +1.36 (233), −8.41 (252), +2.38 (290), −2.15 (312), +5.88 (345) $\Delta\varepsilon$ (nm); ^1H NMR (CD_3OD) δ 3.59 (3H, s, C-3a-OCH_3), 3.731 (3H, s, C-2a-OCH_3 or C-3′-OCH_3), 3.733 (3H, s, C-3′-OCH_3 or C-2a-OCH_3), 3.89 (3H, s, C-7-OCH_3), 3.92 (1H, d, *J* = 3.8 Hz, H-3), 4.46 (1H, brd, *J* = 3.8 Hz, H-4), 6.41 (1H, ddd, *J* = 0.5, 2.2, 8.2 Hz, H-6′), 6.55 (1H, s, H-5), 6.63 (1H, d, *J* = 2.2 Hz, H-2′), 6.64 (1H, d, *J* = 8.2 Hz, H-5′), 7.00 (1H, s, H-1), 7.68 (1H, s, H-1); ^{13}C NMR (CD_3OD) δ 47.2 (C-4), 49.1 (C-3), 52.4 (C-2a-OCH_3), 52.8 (C-3a-OCH_3), 56.5 (C-7-OCH_3), 56.7 (C-3′-OCH_3), 112.5 (C-8), 113.8 (C-4), 116.2 (C-5), 117.2 (C-5′), 121.4 (C-6′), 123.1 (C-6′), 124.9(C-10), 132.7 (C-1′), 135.4 (C-9), 139.7 (C-1), 146.6 (C-4′), 148.5, 149.0 (C-3′), 150.4 (C-8), 169.0 (C-2a), 175.0 (C-3a); HRFABMS (negative-ion mode) *m/z* 413.1253 $[M - H]^-$ (calcd for $C_{22}H_{21}O_8$, 413.1236).

Measurement of Fluorescence. The buffer solutions used were as follows: 20 mM acetate buffer for pH 5.0, 20 mM phosphate buffer for pH 6.0, 7.0, and 8.0, 20 mM borate buffer for pH 8.0 and 9.0, and carbonate buffer for pH 9.0 and 10.0. The concentrations were adjusted to be 1 μM, and fluorescence was measured at 25 °C. Maximum excitation wavelengths were determined by measurement of emission spectra.

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