New Flavonol−Phenylbutadiene Adducts from the Leaves of *Alpinia flabellata*

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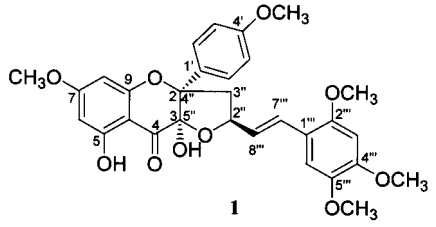
Two new flavonol−phenylbutadiene adducts, *rel*-5-hydroxy-7,4′-dimethoxy-2″*S*-(2,4,5-trimethoxy-*E*-styryl)-tetrahydrofuro[4″*R*,5″*R*:2,3]flavanonol (**1**) and *rel*-5-hydroxy-7,4′-dimethoxy-3″*S*-(2,4,5-trimethoxy-*E*-styryl)tetrahydrofuro[4″*R*,5″*R*:2,3]flavanonol (**2**), were isolated from the leaves of *Alpinia flabellata*, along with three known compounds, 2,4,5-trimethoxybenzoic acid, 2,4,5-trimethoxycinnamic acid, and 5-hydroxy-3,7,4′-trimethoxyflavone. The structures of **1** and **2** were determined by spectroscopic interpretation.

During the course of our investigation on the bioactive chemical components of the plants belonging to the family Zingiberaceae,[1−6] we have focused on *Alpinia flabellata* Ridley, which is a rare species and grows only in the southwestern islands of Iriomote and Ishigaki, Okinawa, Japan. Previous studies on this plant have reported phenylbutanoid dimers (alflabene and its isomer) from the rhizomes[7,8] and phenylbutanoid dimers,[4] a blanched phenylbutenal,[5] and a labdane diterpene adducted by a phenylbutenoid[5] from the leaves. Each of these isolated compounds possesses a 2,4,5-trimethoxyphenyl moiety in the molecule. The latter rare type of labdane diterpene seems to be formed by a Diels−Alder reaction between a labdane diterpene and a 2,4,5-trimethoxyphenylbutenoid. We now describe the isolation and characterization of two new flavonol−phenylbutadiene adducts (**1** and **2**).



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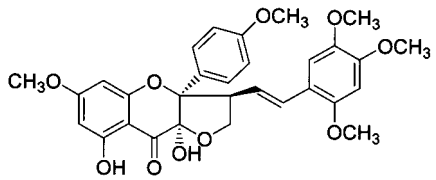

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Compound **1** was assigned a molecular formula of $C_{30}H_{30}O_{10}$ from the HREIMS. The ^{13}C NMR and HMQC spectra revealed a carbonyl, 20 aromatic and olefinic carbons, two oxygenated quaternary carbons, one oxygenated methine, one methylene, and five aromatic methoxyl groups (Table 1). The aromatic singlets at δ_H 6.47 and 6.95 and three methoxyl groups at δ_H 3.80, 3.83, and 3.89 suggested the presence of a 2,4,5-trimethoxyphenyl unit, which was supported by the HMBC correlations (Table 1). Furthermore, the ^1H NMR spectrum showed signals characteristic of an *E*-double bond [δ_H 6.25 (1H, dd, J = 8.5, 15.6 Hz) and 6.88 (1H, br d, J = 15.6 Hz)]. In the HMBC spectrum, correlations were observed from H-8‴ to C-1‴ and from H-6‴ to C-7‴, suggesting the presence of a 2,4,5-trimethoxy-*E*-styryl moiety (Table 1).

Table 1. NMR Data for Compound **1**[a]

position	δ_C	δ_H	HMBC (C→H)
2(4″)	93.3		2′, 6′, 3″b, OH-3(5″)
3(5″)	98.3		3″b, OH-3(5″)
4	188.7		OH-3(5″)
5	164.5		6, OH-5
6	95.0	6.08 (d, 2.2)	8, OH-5
7	169.6		6, OMe-7
8	94.9	6.07 (d, 2.2)	6
9	161.1		8
10	100.1		6, 8, OH-5
OMe-7	55.8	3.82 (3H, s)	
OH-3(5″)		4.69 (br s)	
OH-5		11.07 (s)	
1′	127.3		3′, 5′
2′	128.6	7.40 (d, 9.0)	6′
3′	113.5	6.82 (d, 9.0)	5′
4′	159.5		2′, 3′, 5′, 6′, OMe-4′
5′	113.5	6.82 (d, 9.0)	3′
6′	128.6	7.40 (d, 9.0)	2′
OMe-4′	55.2	3.77 (3H, s)	
2″	80.3	5.19 (br ddd, 2.9, 8.5, 9.3)	3″a, 7‴
3″a	44.9	3.14 (dd, 9.3, 13.7)	
3″b		2.72 (dd, 2.9, 13.7)	
1‴	117.0		3‴, 6‴, 7‴, 8‴
2‴	151.7		6‴, 7‴, OMe-2‴
3‴	97.4	6.47 (s)	
4‴	149.8		3‴, 6‴, OMe-4‴
5‴	143.2		3‴, 6‴, OMe-5‴
6‴	110.3	6.95 (s)	7‴
7‴	127.5	6.88 (br d, 15.6)	2″, 6‴
8‴	127.2	6.25 (dd, 8.5, 15.6)	3″a, 3″b
OMe-2‴	56.6	3.80 (3H, s)	
OMe-4‴	56.0	3.89 (3H, s)	
OMe-5‴	56.6	3.83 (3H, s)	

[a] Coupling constants (J in Hz). Assignments based on 2D experiments (COSY, HMQC, and HMBC).

In addition to above-mentioned signals, the ^1H NMR spectrum of **1** showed the presence of six aromatic protons at δ_H 6.07 (1H, d, J = 2.2 Hz, H-8), 6.08 (1H, d, J = 2.2 Hz, H-6), 6.82 (2H, d, J = 9.0 Hz, H-3′, 5′), and 7.40 (2H, d, J = 9.0 Hz, H-2′, 6′), two methoxyl groups at δ_H 3.77 and 3.82, a hydroxyl group at δ_H 4.69, and a chelating hydroxyl group at δ_H 11.07. Furthermore, the IR spectrum showed absorption bands at 3416 and 1643 cm^{-1}, consistent with the presence of a hydroxyl group and a conjugated carbonyl group in the molecule. The UV spectrum exhibited characteristic absorption peaks of the flavanone moiety at 298.6 and 321.6 (sh) nm.[9] All of these data were consistent with the presence of a dihydroxydimethoxyflavanone moi-

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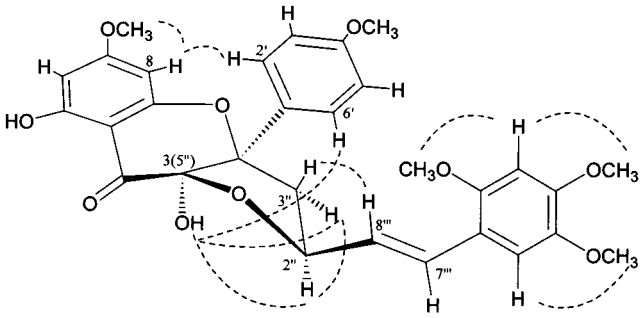

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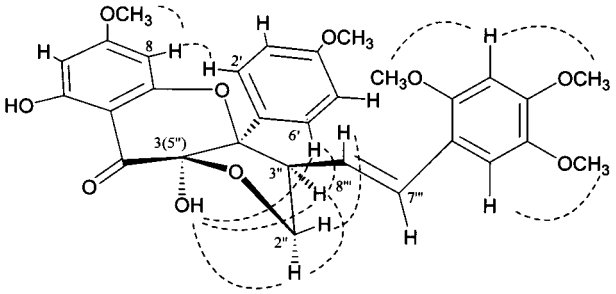

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Figure 1. Key NOESY correlations for compounds **1** and **2**.

ety in the molecule of **1**. A fragment ion peak at *m*/*z* 314 in the EIMS corresponded to dihydroxydimethoxyflavone, and the substitution of the B-ring by a methoxyl group was supported by a fragment ion peak at *m*/*z* 135.

The $^1H-^1H$ COSY spectrum of **1** showed an oxymethine proton at δ_H 5.19 coupled with both an olefinic proton of H-8‴ and methylene protons (δ_H 2.72 and 3.14). Furthermore, the methylene proton at δ_H 2.72 was correlated with quaternary carbons (δ_C 93.3 and 98.3) attributable to C-2 and C-3, which indicated that C-2 and C-3 were constitutive members of the tetrahydrofuran ring. The HMBC correlations of the hydroxyl proton at δ_H 4.69 with C-2(4″), C-3(5″), and C-4 indicated that the hydroxyl group was located at the C-3 position (Table 1). This substitution was supported by the downfield shifted C-3 at δ_C 98.3 comparable to the chemical shifts of C-3 of other flavanonols bearing an oxygen atom at C-3.[10,11] The NOESY correlation (Figure 1) between δ_H 7.40 (H-2′ and 6′) and the hydroxyl proton at C-3 revealed that the B-ring and OH-3 were arranged in a *cis* orientation. Furthermore OH-3 correlated to H-2″, indicating that OH-3 and the 2,4,5-trimethoxystyryl moiety were situated in a *trans*-configuration relative to one another. In addition, the correlation between H-2′ and 6′ and H-8 (δ_H 6.07) confirmed the quasi-axial orientation of the B-ring. Thus, compound **1** was determined as *rel*-5-hydroxy-7,4′-dimethoxy-2″*S*-(2,4,5-trimethoxy-*E*-styryl)-tetrahydrofuro[4″*R*,5″*R*:2,3]flavanonol. It is likely that compound **1** is a racemate because it exhibited no discernible optical rotation.

Compound **2** showed the same molecular formula of $C_{30}H_{30}O_{10}$ and fragment ion peaks at *m*/*z* 314 and 135 in its HREIMS and EIMS as those of compound **1**. The NMR (Table 2), UV, and IR data of **2** were also very similar to those of **1**, suggesting that **2** was an isomer of **1**. A pair of doublets of doublets attributed to oxymethylene protons at δ_H 4.16 (J = 8.2, 9.6 Hz) and 4.48 (J = 7.8, 8.2 Hz) and a broad doublet of doublets of doublets at δ_H 3.99 (1H, J = 7.8, 8.8, 9.6 Hz, H-3″) were observed for **2**. In the $^1H-^1H$ COSY spectrum of **2**, H-3″ correlated to both H-2″ and H-8‴. These correlations in combination with the observed HMBC correlations from H-2″a and H-3″ to C-8‴, and H-7‴ to C-3″ (Table 2), suggested that the 2,4,5-trimethoxystyryl moiety is attached to C-3″ in compound **2**. The relative configuration at C-2 (4″), C-3 (5″), and C-3″ was determined on the basis of NOESY experiments (Figure 1). The NOESY correlation between δ_H 7.43 (H-2′ and 6′) and H-3″ indicated that the B-ring and the 2,4,5-trimethoxystyryl moiety were arranged in a *trans* orientation. Furthermore, the correlation between H-2′ and 6′ and OH-3 at δ_H 4.81 showed that the B-ring and OH-3 were situated in a *cis* configuration relative to one another. Thus, compound **2** was determined as *rel*-5-hydroxy-7,4′-dimethoxy-3″*S*-(2,4,5-trimethoxy-*E*-styryl)tetrahydrofuro-[4″*R*,5″*R*:2,3]flavanonol.

Table 2. NMR Data for Compound **2**[a]

position	δ_C	δ_H	HMBC (C→H)
2(4″)	93.5		2′, 6′, 2″b
3(5″)	98.5		2″a, 2″b
4	188.4		
5	164.4		6, OH-5
6	95.2	6.09 (d, 2.2)	8, OH-5
7	169.6		6, 8, OMe-7
8	94.8	6.18 (d, 2.2)	6
9	161.5		8
10	99.8		6, 8, OH-5
OMe-7	55.9	3.87 (3H, s)	
OH-3(5″)		4.81 (br s)	
OH-5		10.98 (s)	
1′	126.2		3′, 5′
2′	128.9	7.43 (d, 9.0)	6′
3′	113.4	6.79 (d, 9.0)	5′
4′	159.3		2′, 3′, 5′, 6′, OMe-4′
5′	113.4	6.79 (d, 9.0)	3′
6′	128.9	7.43 (d, 9.0)	2′
OMe-4′	55.1	3.738 (3H, s)	
2″a	71.9	4.16 (dd, 8.2, 9.6)	3″, 8‴
2″b		4.48 (dd, 7.8, 8.2)	
3″	53.1	3.99 (br ddd, 7.8, 8.8, 9.6)	2″a, 7‴, 8‴
1‴	117.8		3‴, 6‴, 7‴, 8‴
2‴	151.4		3‴, 6‴, 7‴, OMe-2‴
3‴	98.0	6.46 (s)	
4‴	149.8		3‴, 6‴, OMe-4‴
5‴	143.4		3‴, 6‴, OMe-5‴
6‴	110.3	6.96 (s)	7‴
7‴	129.4	6.72 (br d, 15.6)	3″, 6‴
8‴	119.6	6.16 (dd, 8.8, 15.6)	2″a, 3″, 7‴
OMe-2‴	56.9	3.743 (3H, s)	
OMe-4‴	56.1	3.88 (3H, s)	
OMe-5‴	56.9	3.88 (3H, s)	

[a] Coupling constants (J in Hz). Assignments based on 2D experiments (COSY, HMQC, and HMBC).

2,4,5-Trimethoxyphenylbutadiene has been reported from the rhizomes of *A. flabellata*.[7] We also reported 2,4,5-trimethoxybenzene-related compounds[4,5] and flavonol[4] from the leaves of *A. flabellata*. However, the present report on the isolation of **1** and **2** constituted, to our knowledge, the first study of conjugated metabolites involving 2,4,5-trimethoxyphenylbutene and flavonol units.

The structures of 2,4,5-trimethoxybenzoic acid, 2,4,5-trimethoxycinnamic acid, and 5-hydroxy-3,7,4′-trimethoxyflavone were determined on the basis of their NMR spectral data and by comparison of these data with those reported in the literature.[12] This is the first isolation of these compounds from *A. flabellata*.

Experimental Section

General Experimental Procedures. Optical rotations were measured using a JASCO P-1030 automatic digital polarimeter. UV spectra were recorded on a Shimadzu UV-2500PC UV−vis spectrophotometer. IR spectra were run on a Perkin-Elmer 1800 instrument. NMR spectra were recorded on a Varian Unity Plus 500 spectrometer at 500 MHz (^{1}H) and 125 MHz (^{13}C) in $CDCl_3$ using TMS as internal standard. EIMS and HREIMS were performed on a Hitachi M-2000 instrument. Si gel 60 (70−230 mesh, Merck), Sephadex LH-20 (Pharmacia), and octadecyl silica gel (ODS, Fuji Silysia) (100−200 mesh) were used for column chromatography. Thin-layer chromatography (TLC) was performed on Si gel GF-254 (Merck) and RP-18 F_{2545} (Merck), and spots were detected by ultraviolet (UV) illumination. Analytical HPLC was performed using an ODS-HG-5 Develosil packed column (4.6 × 250 mm, Nomura Chemicals) and a UV detector (280 nm). Elution was performed with H_2O−MeOH (10:90) at 0.5 mL/min at an ambient temperature.

Plant Material. Leaves of *Alpinia flabellata* were collected in the forest on Iriomote Island, Okinawa, Japan, in October 1997 and identified by Professor Sigetomo Yonemori (University of the Ryukyus). A voucher specimen has been deposited at the Tropical Biosphere Research Center, University of the Ryukyus, Okinawa, Japan.

Extraction and Isolation. Dried leaves of *A. flabellata* (800 g) were chopped, pulverized, and extracted five times each with 1 L of CH_2Cl_2 at room temperature for 16 h. The combined CH_2Cl_2 extract was concentrated under reduced pressure to give 27.2 g of a greenish residue. A portion of this extract (25.8 g) was subjected to column chromatography over Si gel (5.5 × 40 cm) eluting with *n*-hexane−Me_2CO (3:1) to yield 90 fractions of 100 mL each. Fractions were monitored by Si gel TLC (*n*-hexane−Me_2CO, 2:1), and similar fractions were combined to give seven fractions. Fractions 6 (4 g) and 7 (6.8 g) were independently subjected to gel filtration on Sephadex LH-20 (3 × 20 cm) using *i*-PrOH as an eluting solvent to give 12 fractions (fractions F6-1−F6-l2 and F7-1−F7-12). Rechromatography of fraction F6-7 (24.6 mg) over Si gel (1 × 22 cm) eluting with C_6H_6−Me_2CO (98:2) afforded 2.5 mg of **2**. Fraction F6-8 (17.2 mg) was also subjected to column chromatography under the same conditions to afforded 3.3 mg of **1**. Fraction F7-5 (2.3 g) was chromatographed using a Si gel column (1.5 × 30 cm) eluting with C_6H_6−Me_2CO (9:1) to give 10 fractions (fractions F7-5-1−F7-5-10). Fraction F7-5-7 (200 mg) was rechromatographed over ODS gel (0.5 × 20 cm), and stepwise elution was performed using H_2O−MeOH. Fractions were combined on the basis of analytical HPLC profiles to afford 10.9 mg of 2,4,5-trimethoxybenzoic acid. Fraction F7-5-9 (14 mg) was purified under the same manner to give 1.9 mg of 2,4,5-trimethoxycinnamic acid. Fraction 4 was subjected to column chromatography over Si gel (2 × 30 cm) with CH_2Cl_2−MeOH (99:1) as an eluting solvent to give nine fractions (fractions F4-1−F4-9). Fraction F4-2 (43.8 mg) was purified by chromatography on Sephadex LH-20 (1.5 × 24 cm, eluted with *i*-PrOH) and Si gel (1.5 × 19 cm, eluted with *n*-hexene−Me_2CO, 86:14) to give 5.5 mg of 5-hydroxy-3,7,4′-trimethoxyflavone.

***rel*-5-Hydroxy-7,4′-dimethoxy-2″*S*-(2,4,5-trimethoxy-*E*-styryl)tetrahydrofuro[4″*R*,5″*R*:2,3]flavanonol (1):** amorphous yellow powder; $[\alpha]^{21}_D$ 0° (*c* 0.3, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 269.0 (3.23), 298.6 (3.28), 321.6 (sh, 3.07) nm; IR (film) ν_{max} 3416, 1643, 1576, 1514, 1267, 1211, 1160, 1100, 1039 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 1; EIMS *m*/*z* 550 $[M]^+$ (62), 314 (15), 220 (55), 205 (57), 189 (52), 166 (100), 135 (55); HREIMS *m*/*z* 550.1827 [calcd for $C_{30}H_{30}O_{10}$, 550.1837 $(M)^+$].

***rel*-5-Hydroxy-7,4′-dimethoxy-3″*S*-(2,4,5-trimethoxy-*E*-styryl)tetrahydrofuro[4″*R*,5″*R*:2,3]flavanonol (2):** amorphous yellow powder; $[\alpha]^{21}_D$ +5.1° (*c* 0.2, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 271.8 (3.19), 297.2 (3.27), 325.0 (sh, 2.98) nm; IR (film) ν_{max} 3384, 1642, 1572, 1514, 1266, 1208, 1160, 1101, 1037 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 2; EIMS *m*/*z* 550 $[M]^+$ (4), 314 (100), 271 (10), 236 (27), 207 (20), 176 (16), 135 (16); HREIMS *m*/*z* 550.1843 [calcd for $C_{30}H_{30}O_{10}$, 550.1837 $(M)^+$].

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