New Constituents of the Leaves of *Alpinia flabellata*

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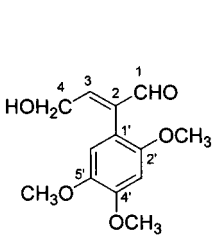
Two new compounds were isolated from the leaves of *Alpinia flabellata*. The structures of these compounds were determined by a combination of NMR techniques and HREIMS as 4-hydroxy-2-(2,4,5-trimethoxyphenyl)-2*E*-butenal (**1**) and *rel*-labd-12-en-15(16)-olid-7-one-8*R*-spiro-1′-[2*S*-(2,4,5-trimethoxyphenyl)-3-cyclohexene] (**2**).

As a part of our studies on the constituents of plants belonging to the family Zingiberaceae,[1−5] we have focused on *Alpinia flabellata* Ridley, which is a rare species in Japan. The leaves of this plant have been used as a wrapping and flavoring material for foods on the islands of Iriomote and Ishigaki, Okinawa, which is the southernmost part of Japan. Only two studies on the isolation of several phenylbutenoids from the rhizomes of *A. flabellata* have been reported.[6,7] We have reported previously the isolation and structural elucidation of three phenylbutanoid dimers from the leaves of *A. flabellata*[8] and now describe the isolation and characterization of a new phenylbutenal (**1**) and a novel labdane diterpene (**2**) adducted by a phenylbutenoid.



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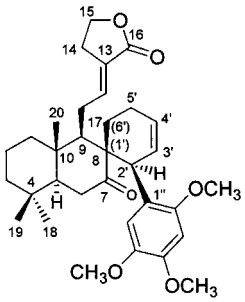

2

Compound **1** had a molecular formula of $C_{13}H_{16}O_5$ from HREIMS. The IR spectrum showed absorption bands at 3450 and 1700 cm^{-1}, consistent with the presence of a hydroxyl and a carbonyl group in the molecule. The presence of a formyl group was supported by a proton signal at δ 9.65 in the ^{1}H NMR spectrum and a fragment ion peak at *m*/*z* 223 $[M - CHO]^+$ in the EIMS. The ^{13}C NMR spectrum revealed 13 carbon signals, indicating a carbonyl, six aromatic carbons, two olefinic carbons, one oxygenated methine, and three aromatic methoxyl groups. In the ^{1}H NMR spectrum, two aromatic and three aromatic methoxyl proton signals as singlets were observed, suggesting the presence of a 2,4,5-trimethoxyphenyl group. This was also supported by the HMBC and NOESY correlations (Figures 1 and 2). The ^{1}H NMR spectrum showed that an olefinic proton at δ 6.87 was coupled with two oxymethylene protons at δ 4.31. In the HMBC spectrum, correlation contours were observed between a formyl proton and C-2 and C-3, between the olefinic proton and C-2 and C-4, and between the oxymethylene protons and

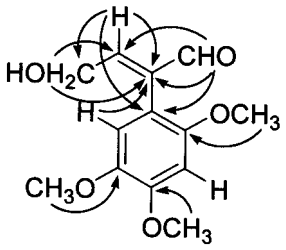

Figure 1. HMBC correlations for **1**.

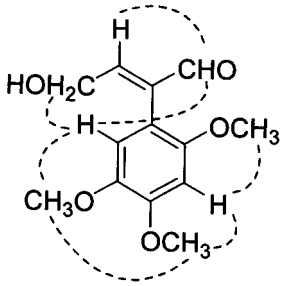

Figure 2. NOESY correlations for **1**.

C-2 and C-3. These results suggested the presence of a 4-hydroxy-2-butenal moiety (Figure 1). The bonding of C-1′ to C-2 was indicated from the HMBC spectrum, wherein both H-1 and H-3 showed a correlation with the aromatic carbon (C-1′), and the aromatic proton (H-6′) showed a correlation with the olefinic carbon (C-2). The *E*-configuration of the double bond was supported by the NOESY correlations between H-1 and H-3 and between H_2-4 and H-6′ (Figure 2). Thus, compound **1** was determined as 4-hydroxy-2-(2,4,5-trimethoxyphenyl)-2*E*-butenal.

Compound **2** exhibited a $[M]^+$ peak which was in good agreement with a molecular formula of $C_{33}H_{44}O_6$. The presence of a 2,4,5-trimethoxyphenyl group was supported by two 1H singlets at δ 6.53 and 6.80 and three 3H singlets at δ 3.79, 3.82, and 3.90 in the ^{1}H NMR spectrum and six characteristic aromatic carbon signals in the ^{13}C NMR spectrum. The remaining 24 carbon signals were assigned to three quaternary methyls, nine methylenes, three methines, four olefinic carbons, two carbonyls, and three quaternary carbons based on a HMQC (one-bond CH-correlation) measurement. The ^{1}H−^{1}H COSY spectrum indicated the presence of four fragments, $-CH_2CH_2CH_2-$ (δ 0.84 and 1.38, 1.37 and 1.46, and 0.99 and 1.32), $-CHCH_2-$ (δ 0.81, and 2.02 and 2.25), $-CHCH_2CH{=}C-$ (δ 2.11, 2.36 and 2.45, and 6.93), and $-CH_2CH_2O-$ (δ 2.90 and 4.42). The HMBC NMR experiment enabled the connection of these moieties and three quaternary methyl groups to be determined. The observed key HMBC correlation contours were as follows: H-1eq/C-5, H_3-18, -19, and

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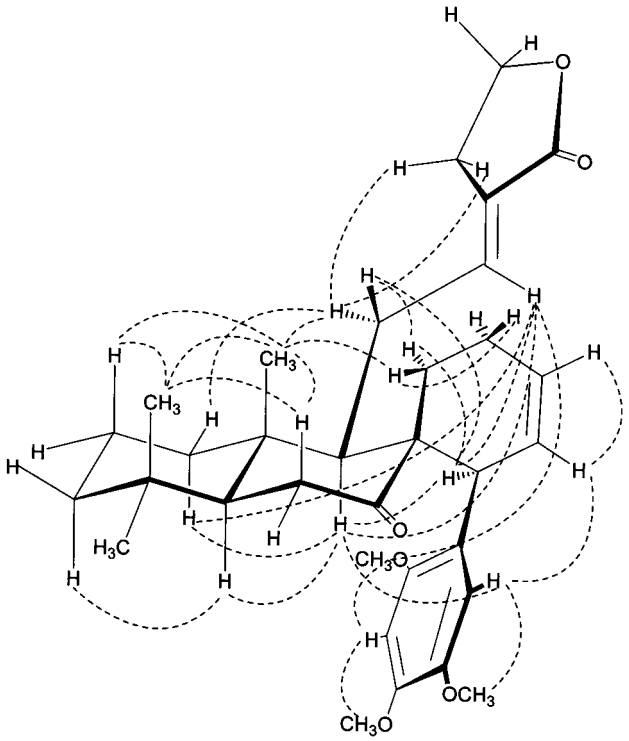

Figure 3. Key NOESY correlations for **2**.

-20/C-5, H-5/C-4, C-9, and C-10, H_3-18 and -19/C-3 and C-4, H_3-20/C-1 and C-10, and H-9/C-8 and C-20. The C-6 methylene protons were observed at δ 2.02 and 2.25 as doublets of doublets, and both methylene protons showed correlations with the carbonyl carbon at δ 214.1, which suggested that C-7 was a carbonyl carbon. The *trans* diaxial configuration of the C-20 methyl with respect to H-5 was confirmed on the basis of a shielding methyl carbon signal of C-20 at δ 15.8 and the NOESY correlations between H_3-20 and both H-2ax and H_3-19. Furthermore, a NOESY experiment confirmed the axial orientation of H-9 based on the correlation with both H-1ax and H-5. A long-range coupling was observed between the olefinic proton at δ 6.93 and the methylene protons at δ 2.90, and the HMBC correlations were observed between this methylene and an olefinic carbon at C-13. Therefore, the methylene signal at δ 2.90 was assigned to H_2-14, an allylic methylene. Furthermore, the oxymethylene at δ 4.42 attributed to H_2-15 was coupled with an ester carbonyl carbon (δ 171.5). These results and the IR absorption band at 1752 cm^{-1} showed the presence of a five-membered lactone ring conjugated with an exo-olefinic bond in the molecule. The downfield shift of the olefinic proton of H-12 (δ 6.93) and the NOESY correlation of a methylene proton at δ 2.36 (H-11a) with H_2-14 indicated the conjugation of the lactone carbonyl with *E* configuration. Thus, compound **2** was established as a labdane diterpene-related compound.

The $^1H-^1H$ COSY spectrum revealed another isolated partial structure in the molecule of **2**, namely, $-CH_2CH_2-CH{=}CHCH-$ (δ 1.84 and 1.89, 2.06 and 2.39, 5.79, 5.52, and 3.96). In the HMBC spectrum, a methylene proton at δ 1.89 (H-6′b) was correlated with C-8 and a methine carbon at δ 39.2 (C-2′). Furthermore, a methylene proton at δ 2.39 (H-5′b) and an olefinic proton at δ 5.52 (H-3′) showed correlations with C-8, indicating a spiro-bicyclohexane unit at C-8. Finally, the HMBC correlation of the aromatic proton of H-6″ with C-2′ indicated that the 2,4,5-trimethoxyphenyl group was attached to C-2′. This proposed structure of **2** was supported by a major fragment ion peak at *m*/*z* 220.1148 in the HREIMS, corresponding to a trimethoxyphenylbutadiene, which may have occurred due to a retro-Diels−Alder reaction. The NOESY correlations between H-6′ (17)b and H_3-20 and between H-2′ and H-9 suggested that C-2′ was α-oriented at C-8, while C-6′ was β-oriented (top surfaced). The latter NOESY correlation also suggested the β-orientation of the 2,4,5-trimethoxybenzene unit, which was supported by the higher shielded methyl protons at δ 0.60 (H_3-18) and H-5 (δ 0.81) compared with other labdanes reported.[5,9] Consequently, compound **2** was determined as *rel*-labd-12-en-15(16)-olid-7-one-8*R*-spiro-1′-[2*S*-(2,4,5-trimethoxyphenyl)-3-cyclohexene].

Generally, labdanes possess an exomethylene group at C-8. The unique structure of compound **2** might be formed by a Diels−Alder reaction between an exomethylene at C-8 and a 1,3-butadienoid unit. To our knowledge, this is the first report of such a labdane diterpene coupled with phenylbutenoid from a natural source.

Labdane-type diterpenes and phenylbutenoids are quite characteristic of the genus *Alpinia* and have been isolated from *A. flabellata*,[6] *A. formosana*,[10] *A. galanga*,[11] *A. javanica*,[12] *A. katsumadai*,[13] and *A. speciosa*.[14]

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 (^1H) and 125 MHz (^{13}C) in $CDCl_3$ using TMS as an internal standard. EIMS and HREIMS were performed on a Hitachi M-2000 instrument. Merck Si gel 60 (70−230 mesh), Pharmacia Sephadex LH-20, and Fuji Silysia octadecyl silica (reversed phase) (ODS) (100−200 mesh) were used for column chromatography. Thin-layer chromatography (TLC) was performed on Merck Si gel GF-254 and Merck RP-18 F_{2545}, and spots were detected by ultraviolet (UV) illumination. HPLC analysis was carried out with a pump and a system controller (Hitachi) connected to a UV detector (Hitachi) operating at 280 nm. Analytical and preparative HPLC were carried out on an ODS-HG-5 Develosil pack column (4.6 × 250 mm, Nomura Chemicals) and an ODS-5 Develosil pack column (20 × 250 mm, Nomura Chemicals), respectively.

Plant Material. Leaves of *Alpinia flabellata* were collected in the forest on Iriomote Island, Okinawa, Japan, in October 1997, and identified by one of the authors (S.Y.). 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 each. The combined CH_2Cl_2 extracts were concentrated under reduced pressure to give 27.2 g of a greenish residue. This extract (25.8 g) was subjected to column chromatography over Si gel (650 g) 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 a total of 11 fractions. Fraction 5 (3.2 g) was chromatographed on a Si gel (250 g) column and eluted with C_6H_6−MeOH (99:1). Fractions were combined according to their TLC patterns (CH_2Cl_2) to yield 15 fractions. Fractions 5−8 (1.1 g) were combined and chromatographed using Si gel (44 g) with CH_2Cl_2 as eluting solvent to give 10 fractions (fractions A−J) based on Si gel TLC (C_6H_6−Me_2CO, 90:5) monitoring. The *i*-PrOH-soluble part of fraction H (222 mg) was successively purified by chromatography on Sephadex LH-20 (6 g, eluted with *i*-PrOH), Si gel (10 g, eluted with C_6H_6−Me_2CO, 90:5), and ODS gel (5 g, eluted with H_2O−MeOH, 25:75) columns. Final purification was employed using C_{18} reversed-phase

Table 1. NMR Data for Compound **2**[a,b] (in $CDCl_3$)

position	δ_C	δ_H	HMBC
1ax	40.2	0.84 (ddd, 3.9, 12.9, 12.9)	20
1eq		1.38 (br d, 12.9)	
2ax	18.2	1.46 (ddddd, 3.7, 3.9, 12.9, 13.5, 13.5)	
2eq		1.37 (m)	
3ax	41.1	0.99 (ddd, 3.7, 13.5, 13.5)	18, 19
3eq		1.32 (br d, 13.5)	
4	32.9		5, 6ax, 18, 19
5	50.5	0.81 (dd, 5.4, 13.4)	1eq, 6ax, 6eq, 18, 19, 20
6ax	39.0	2.25 (dd, 13.4, 17.8)	
6eq		2.02 (dd, 5.4, 17.8)	
7	214.1		6ax, 6eq
8 (1′)	55.2		3′, 5′b, 6′a, 6′b, 9
9	55.1	2.11 (dd, 2.9, 7.9)	5, 11a, 20
10	38.5		1eq, 5, 6eq, 11a, 20
11a	28.3	2.36 (ddd, 5.4, 7.9, 17.6)	
11b		2.45 (br dddd, 2.7, 2.9, 8.1, 17.6)	
12	145.3	6.93 (ddd, 2.7, 5.4, 8.1)	9, 11a
13	122.2		11a, 14, 15
14	25.3	2.90 (2H, m)	15
15	65.3	4.42 (2H, t, 7.3)	
16	171.5		15
18	32.4	0.60 (s)	19
19	20.8	0.75 (s)	5, 18
20	15.8	0.93 (s)	1ax, 5, 9
2′	39.2	3.96 (m)	3′, 6′b, 6″
3′	131.0	5.52 (ddd, 2.3, 3.8, 10.0)	
4′	126.0	5.79 (dddd, 2.3, 2.3, 4.6, 10.0)	6′b
5′a	23.5	2.06 (br d, 17.0)	3′, 6′b
5′b		2.39 (m)	
6′ (17)a	27.8	1.84 (ddd, 5.4, 10.5, 13.7)	
6′ (17)b		1.89 (br ddd, 2.2, 6.1, 13.7)	
1″	120.9		3″, 6″
2″	151.7		3″, 6″, OMe-2″
3″	96.7	6.53 (s)	
4″	148.0		3″, 6″, OMe-4″
5″	142.7		3″, 6″, OMe-5″
6″	115.2	6.80 (s)	
OMe-2″	56.3	3.82 (3H, s)	
OMe-4″	56.0	3.90 (3H, s)	
OMe-5″	56.4	3.79 (3H, s)	

[a] Chemical shifts (δ) are relative to TMS; coupling constants J (Hz). [b] Assignments based on 2D experiments (COSY, HMQC, and HMBC).

preparative HPLC at a flow rate of 5 mL/min with H_2O-CH_3CN (30:70) as eluting solvent to give 1.3 mg of **2**. Fractions 10 and 11 (6.8 g) were combined and subjected to gel filtration on Sephadex LH-20 (35 g) using *i*-PrOH as eluting solvent to give 12 fractions (fractions a−l) according to their TLC patterns ($C_6H_6-Me_2CO$, 80:20). Further rechromatography of fraction e (2.3 g) over Si gel (20 g) eluting with $C_6H_6-Me_2CO$ (90:10) afforded 21 fractions. Finally, fraction 13 (30 mg) was purified by ODS column chromatography (7 g) eluting with $H_2O-MeOH$ (70:30), collecting fractions of 5 mL. Fractions were combined on the basis of analytical HPLC at a flow rate of 0.5 mL/min eluting with $H_2O-MeOH$ (40:60) to give 9.4 mg of **1** (t_R 7.4 min).

4-Hydroxy-2-(2,4,5-trimethoxyphenyl)-2*E*-butenal (1): yellow oil; UV (EtOH) λ_{max} (log ϵ) 227 (4.30), 292 (3.61) nm; IR (film) ν_{max} 3450, 1700, 1650, 1610, 1519, 1216, 1040, 935 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 9.65 (1H, s, H-1), 6.87 (1H, t, J = 6.1 Hz, H-3), 6.58 (1H, s, H-3′), 6.56 (1H, s, H-6′), 4.31 (2H, d, J = 6.1 Hz, H-4), 3.91 (3H, s, OMe-2′), 3.85 (3H, s, OMe-5′), 3.74 (3H, s, OMe-4′); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 193.4 (C-1), 152.3 (C-3), 151.2 (C-4′), 150.2 (C-2′), 143.1 (C-5′), 139.7 (C-2), 114.1 (C-6′), 112.0 (C-1′), 97.9 (C-3′), 60.5 (C-4), 56.8 (OMe-4′), 56.5 (OMe-5′), 56.1 (OMe-2′); EIMS m/z 252 $[M]^+$ (100), 234 (23) $[M - H_2O]^+$, 223 (60) $[M - CHO]^+$, 207 (7), 195 (47), 193 (19), 177 (14), 162 (19), 149 (17), 133 (9), 121 (15), 105 (11); HREIMS m/z 252.1010 [calcd for $C_{13}H_{16}O_5$, 252.0997 $(M)^+$].

***rel*-Labd-12-en-15(16)-olid-7-one-8*R*-spiro-1′-[2*S*-(2,4,5-trimethoxyphenyl)-3-cyclohexene] (2):** colorless oil; $[\alpha]^{25}_D$ −107.1° (*c* 0.10, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 234 (4.03), 292 (3.69) nm; IR (film) ν_{max} 1752, 1682, 1609, 1516, 1210, 1038 cm^{-1}; ^1H NMR and ^{13}C NMR, see Table 1; EIMS m/z 536 $[M]^+$ (52), 290 (2), 233 (27), 220 (100), 189 (26), 180 (27), 167 (8), 123 (5), 91 (5), 81 (2); HREIMS m/z 536.3128 [calcd for $C_{33}H_{44}O_6$, 536.3135 $(M)^+$], 220.1148 (calcd for $C_{13}H_{16}O_3$, 220.1099).

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