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Phenylbutenoids from the rhizomes of *Alpinia flabellata*

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Abstract

A new phenylbutenoid dimer, (±)-*trans*-3-(2,4,5-trimethoxyphenyl)-4-[(*E*)-2,4,5-trimethoxystyryl]-cyclohexene has been isolated from the fresh rhizomes of *Alpinia flabellata* Ridley, along with alflabene and 2′,4′,5′-trimethoxyphenylbutadiene. Their structures were elucidated on the basis of spectroscopic and chemical methods. The natural occurrence of two racemic phenylbutenoid dimers in fresh rhizomes was confirmed by HPLC.

Keywords: *Alpinia flabellata*; Zingiberaceae; Rhizomes; Phenylbutenoid

1. Introduction

In the course of our investigations of chemical constituents in the Zingiberaceae which have been used for foodstuffs or traditional medicines in tropical and sub-tropical regions (Masuda, 1997), the constituents of *Alpinia flabellata* Ridley were studied. The plant is a rare species in Japan and grows only in the Iriomote and Ishigaki islands (Walker, 1976). This paper deals with the isolation and identification of a new dimeric phenylbutenoid along with two phenylbutenoids from the ethyl acetate soluble fraction of the acetone extract of the fresh rhizomes of *A. flabellata*.

2. Results and discussion

Compound **1** (m/z 220 $[M]^+$) was identified as 2′,4′,5′-trimethoxyphenylbutadiene, which was first isolated from this plant, but very recently isolated as an insecticide from *Zingiber cassumunar* (Nugroho, Schwarz, Wray & Proksh, 1996).

Compound **2** (alflabene) was isolated as colorless needles (m.p. 131°, lit. 130–131° (Mori et al., 1978)), which Mori and coworkers previously isolated from this plant (Mori et al., 1978).

Compound **3** was isolated as a colorless powder (m.p. 115–116°); its molecular formula was determined to be $C_{26}H_{32}O_6$ based on HRMS analysis. The ^1H and ^{13}C NMR spectral data of **3** were similar to those of **2**, indicating that **3** was an isomer of **2**. The COSY spectrum of **3** revealed the same H–H coupling connectivities to those of **2**; however, coupling patterns and chemical shifts of H-3, H-4 and H-1″ were considerably different, suggesting that **3** was a stereoisomer at the either positions of C-3 or C-4. Mori *et al.* determined that **2** had *cis* orientation at the 3 and 4-positions; thus, **3** is probably the *trans* isomer. This stereochemical relationship was confirmed by the proton coupling pattern of H-3 after hydrogenation of each compound. Fig. 1(A) shows the splitting pattern of the H-3 signal of the hydrogenated derivative **4**. Its coupling pattern (*d*, $J = 12.7$ and *t*, $J = 3.4$ Hz) reveals that H-3 was coupled with one *axial* oriented proton and two *equatorial* oriented protons on the cyclohexane ring. For compound **5**, unfortunately, the ^1H NMR spectrum of **5** gave only a broad H-3 signal under the same measurement conditions. To observe the actual coupling pattern, the ^1H NMR spectrum of **5** was measured again at an elevated temperature (70°) to remove any steric influence of 2′-OCH_3 on the hom-

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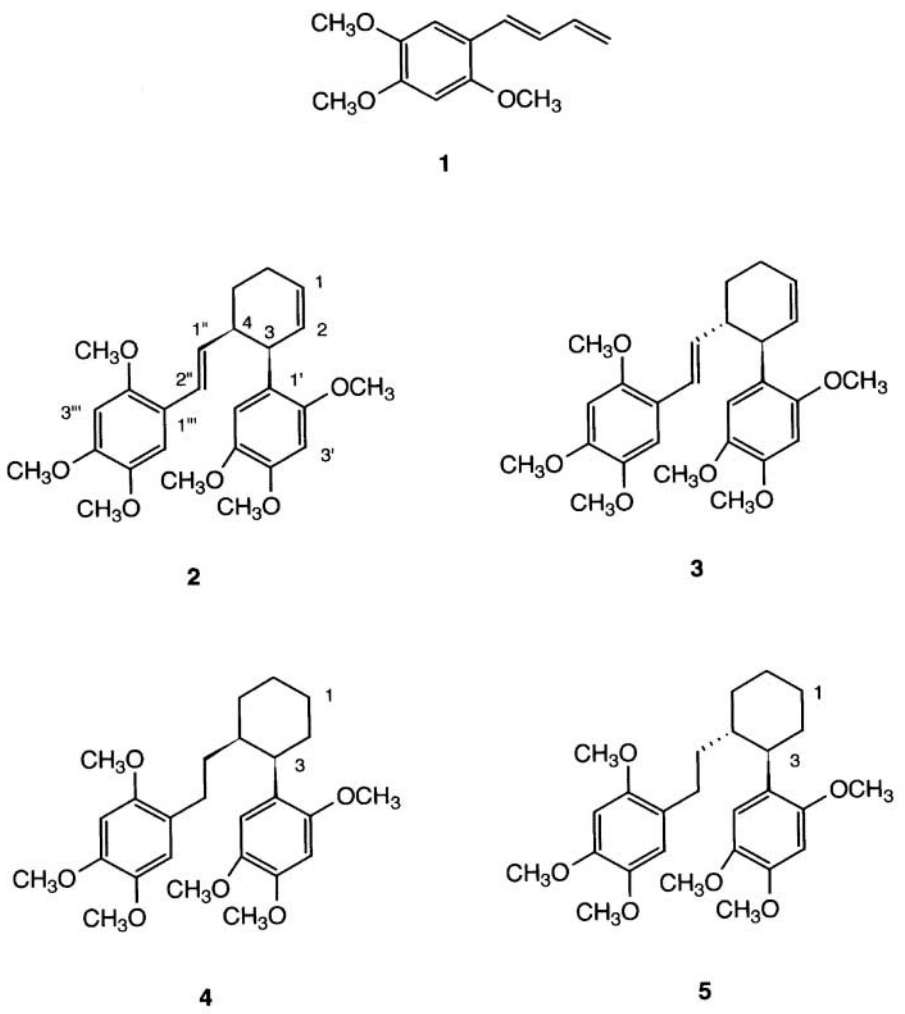

ogeneity of the compound (Jitoe, Masuda & Nakatani, 1993); the splitting pattern of the H-3 signal is shown in Fig. 1(B). The coupling pattern clearly shows that H-3 is coupled with two *axial* protons and one *equatorial* proton, revealing that **3** is the *trans* isomer.

Compounds **2** and **3** are structurally classified as Diels–Alder dimeric compounds from **1**. No optical rotation was observed in both compounds, indicating that **2** and **3** are present as racemates. Several Diels–Alder dimeric phenylbutenoids have been reported in another Zingiberaceae plant and all of them are reported to be racemates (Jitoe, Masuda & Nakatani, 1993; Amatayakul et al., 1979; Dinter & Hänsel, 1980; Kuroyanagi et al., 1980). These Diels–Alder dimers might be artifacts in purification process, because Diels–Alder dimerization is a simple thermal reaction. To attempt to confirm whether **2** and **3** were natural products (real plant constituents) or artifacts, the fresh rhizomes was re-collected and extracted again without any heating procedure and the extract was analyzed directly by HPLC. **2** and **3** were clearly observed at the retention times, 17.9 and 15.9 min, respectively, along with the diene **1** at 7.4 min. This result confirms that **2** and **3** exist naturally in the rhizomes.

3. Experimental

3.1. General

NMR: ^{1}H at 400 MHz, ^{13}C at 100 MHz (JEOL EX-400 spectrometer), TMS as int. standard. MS: EI and HR MS 70 eV (JEOL SX 102A spectrometer). CC, silica gel 60 (230–400 mesh, Merck) and LH-20 (Pharmacia). TLC and prep. TLC: precoated silica gel plates 60 F_{254} (0.25 and 0.5 mm).

3.2. Plant material

Fresh rhizomes of *A. flabellata* were collected in the forest of Iriomote island, Okinawa, Japan and identified by one of the authors (S. Y.). A voucher specimen (AF-9600-SY) was deposited in Tropical

Biosphere Research Center, University of Ryukyus, Japan.

3.3. Isolation

Fresh rhizomes (4.35 kg) of *A. flabellata* were chipped and extracted 3 times with acetone (15 l × 3) consecutive for 1 week interval at room temp. The combined Me_2CO sol was evapd *in vacuo*, giving 105 g of Me_2CO extract. The Me_2CO extract was partitioned with *n*-hexane and CH_3OH. The CH_3OH fraction was further partitioned with EtOAc and H_2O. The ethyl acetate fraction (11.25 g) was concd and subjected to SiO_2 CC with *n*-C_6H_{14}–Me_2CO as an eluent in a stepwise gradient mode[1:2 (1.5 l), 1:1 (1.8 l), 2:1 (1.8 l), 0:1 (1.8 l)] to separate 21 frs. Fr. 2 was evaporated (1.88 g) and subjected to SiO_2 CC with C_6H_6 and increasing amount of Me_2CO (1:0–0:1) to give 15 frs. Fr. 7 was crystallized with Et_2O–*n*-C_6H_{14} to give **1** (0.51 g). Fr. 9 was crystallized with CH_3OH to give **2** (0.05 g). Mother liquor of **2** was purified with Sephadex LH-20 eluted with CH_3OH and prep. SiO_2 TLC developed with CH_2Cl_2–*n*-C_6H_{14} = 2:1, subsequently, to give **3** (0.02 g).

3.4. 2′,4′,5′-Trimethoxyphenylbutadiene (**1**)

Colorless cubes from CH_3OH–H_2O. m.p. 56° (lit. oil as synthetic compound (Tuntiwachwuttikul, Pancharoen, Jaipetch & Reutrakul, 1981)). MS *m/z* (rel. int.) 220 $[M]^+$ (100), 189 (95). ^1H NMR $\delta(CDCl_3)$ 6.85 (1H, *d*, *J* = 16 Hz, H-1), 6.68 (1H, *dd*, *J* = 16 and 9 Hz, H-2), 6.52 (1H, *dt*, *J* = 16 and 9 Hz, H-3), 5.27 (1H, *brd*, *J* = 16 Hz, H-4a), 5.07 (1H, *brd*, *J* = 9 Hz, H-4b), 6.51 (1H, *s*, H-3′), 7.00 (1H, *s*, H-6′), 3.83 (3H, *s*, OCH_3), 3.88 (3H, *s*, OCH_3), 3.90 (3H, *s*, OCH_3).

3.5. (±)-Trans-3-(2,4,5-trimethoxyphenyl)-4-[(E)-2,4,5-trimethoxystyryl]-cyclohexene (**3**)

Colorless powder from ether–hexane. m.p. 115–116°. $[\alpha]_D^{30}$ ±0° ($CHCl_3$; *c* 1.0). MS *m/z* (rel. int.) 440 $[M]^+$ (13), 220 (100). HRMS Calcd for $C_{26}H_{32}O_6$: 440.2199; Found: 440.2195. ^1H NMR ($CDCl_3$) δ 1.70 (1H, *m*, H-5a), 1.90 (1H, *m*, H-5b), 2.21 (2H, *m*, H-6), 2.41 (1H, *br q*, *J* = 7.9 Hz, H-4), 3.73 (1H, overlapped, H-3), 3.73 (3H, *s*, OCH_3), 3.74 (3H, *s*, OCH_3), 3.84 (3H, *s*, OCH_3), 3.85 (6H, *br s*, OCH_3×2), 3.86 (3H, *s*, OCH_3), 5.60 (1H, *ddd*, *J* = 9.7, 3.5 and 2.0 Hz, H-2), 5.88 (1H, *ddd*, *J* = 9.7, 6.5 and 3.2 Hz, H-1), 6.03 (1H, *dd*, *J* = 16.2 and 7.9 Hz, H-1″), 6.42 (1H, *d*, *J* = 16.2 Hz, H-2″), 6.45 (2H, *s*, H-3′ and H-3″), 6.74 (1H, *s*, H-6′ or H-6″), 6.91 (1H, *s*, H-6′ or H-6″), ^{13}C NMR δ 127.4 (*d*, C-1), 130.5 (*d*, C-2), 39.8 (*d*, C-3), 45.2 (*d*, C-4), 28.1 (*t*, C-5), 24.4 (*t*, C-6) 125.0 (*s*, C-1′), 151.4 or 150.8 (*s*, C-2′), 98.3 (*d*, C-3′), 148.7 or 147.6 (*s*, C-4′), 143.3 or 143.0 (*s*, C-5′), 112.8 (*d*, C-6′) 132.9

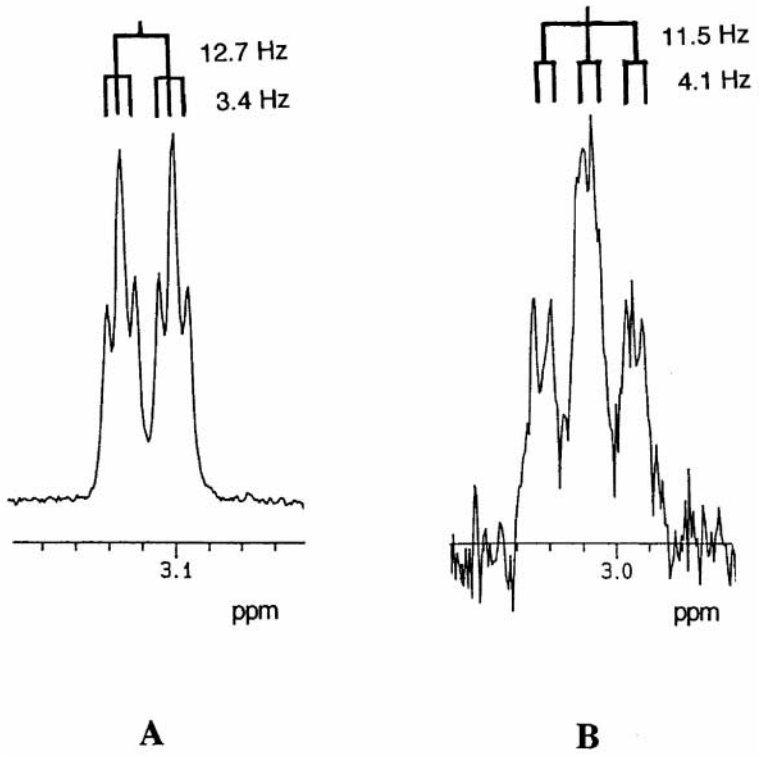

Fig. 1. Coupling pattern of H-3 in ^1H NMR spectrum. (A) H-3 of **4** in $CDCl_3$ at 24° and (B) H-3 of **5** in pyridine-d_5 at 70°.

(*d*, C-1″), 122.4 (*d*, C-2″), 119.2 (*s*, C-1‴), 151.4 or 150.8 (*s*, C-2‴), 97.7 (*d*, C-3‴), 148.7 or 147.6 (*s*, C-4‴), 143.4 or 143.0 (*s*, C-5‴) 109.6 (*d*, C-6‴), 56.9 (*q*, OCH_3), 56.7 (*q*, OCH_3), 56.6 (*q*, OCH_3), 56.5 (*q*, OCH_3), 56.1 (*q*, $OCH_3\times2$).

3.6. Catalytic hydrogenation of **2** and **3**

To a mixture of **2** (2 mg) and PtO_2 (2 mg) was added CH_3OH (0.5 ml). The mixture was stirred under hydrogen at room temp. for 30 min. The mixture was filtered and the filtrate was concentrated. The residue was purified with prep. SiO_2 TLC, giving hydrogenated compound **4** (2 mg). ^1H NMR ($CDCl_3$) δ 1.22–1.99 (11H, H-1, H-2, H-4, H-5, H-6 and H-1″), 2.09 (1H, *ddd*, J = 13.5, 9.3 and 7.3 Hz, H-2″a), 2.46 (1H, *ddd*, J = 13.5, 9.8 and 4.9 Hz, H-2″b), 3.12 (1H, *dt*, J = 12.7 and 3.4 Hz, H-3), 3.65 (3H, *s*, OCH_3), 3.70 (3H, *s*, OCH_3), 3.74 (3H, *s*, OCH_3), 3.80 (3H, *s*, OCH_3), 3.84 (3H, *s*, OCH_3), 3.87 (3H, *s*, OCH_3), 6.40 (2H, *s*, H-3′ and H-3‴), 6.43 (1H, *s*, H-6′ or H-6‴), 6.65 (1H, *s*, H-6′ or H-6‴). MS m/z (rel. int.) 444$[M]^+$ (100) 181 (85). In a similar manner, hydrogenated compound **5** (1 mg) was obtained from **3** (1 mg). ^1H NMR ($CDCl_3$) δ 1.03–1.87 and 2.11 (11H, H-1, H-2, H-4, H-5, H-6 and H-1″), 2.37 (1H, *ddd*, J = 13.5, 9.4 and 6.3 Hz, H-2″a), 2.51 (1H, *ddd*, J = 13.5, 9.7 and 4.8 Hz, H-2″b), 2.74 (1H, *br s*, H-3), 3.63 (3H, *s*, OCH_3), 3.75 (9H, *br s*, $OCH_3\times3$), 3.84 (3H, *s*, OCH_3), 3.85 (3H, *s*, OCH_3), 6.42 (1H, *s*, H-3′ or H-3‴), 6.49 (2H, *s*, H-3′, H-6′, H-3‴, or H-6‴), 6.57 (1H, *s*, H-6′ or H-6‴). MS m/z (rel. int.) 444 $[M]^+$(100), 181 (80).

3.7. Re-extraction of the fresh rhizomes and direct analysis by HPLC

The fresh rhizomes of the title plant was collected again in July 28, 1997 in Iriomote island and kept at −30° until analysis (*ca.* 2 weeks). The rhizomes (6.3 g) were chipped at 26° and immediately soaked in HPLC grade CH_3OH (40 ml). The extraction was carried out for 1 h at 26° with stirring. The suspension was filtered and the filtrate was concentrated to *ca.* 10 ml at 26° *in vacuo*. 10 μl of the concentrated extract was subjected to HPLC analysis. The analytical conditions were as follows: analytical column: DAISOPAK SP-120-5-ODS-AP (size: 250 × 4.6 mm I.D.); solvent: 70% CH_3CN in H_2O; flow rate: 1 ml/min; detection: 260 nm. Identification for each compound (**1**, **2** and **3**) was carried out by the co-injection method with injection of each authentic sample. Although the same extraction and analysis conditions were applied to the pure phenylbutadiene (**1**), no formation of **2** and **3** was observed.

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