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New chalcones bearing a long-chain alkylphenol from the rhizomes of *Alpinia galanga*

Wan-Qiu Yang[a], Yuan Gao[b], Ming Li[b], De-Ren Miao[a]* and Fei Wang[b]*

[a]*Department of Chemical Science & Technology, Kunming University, Kunming 650214, China;* [b]*BioBioPha Co., Ltd., Kunming 650201, China*

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Three novel chalcones bearing a long-chain alkylphenol, galanganones A–C (**1**–**3**), were isolated from the rhizomes of *Alpinia galanga*. Their structures were elucidated by extensive spectroscopic analysis including 2D NMR experiments. Compounds **1**–**3** represent the first examples of long-chain alkylphenol-coupled chalcone.

Keywords: Zingiberaceae; *Alpinia galanga*; galanganone; chalcone; long-chain alkylphenol

1. Introduction

Alpinia galanga (commonly called greater galangal), a member of Zingiberaceae family, is used as a traditional medicine and a spice throughout Southeastern Asian countries, especially in Indonesian and Thai cuisines [1]. *Alpinia* species have been extensively investigated from both a phytochemical and a pharmacological point of view [2–7]. During the course of a program to discover novel bioactive compounds from the genus *Alpinia*, the chemical constituents of the rhizomes of *A. galanga* were studied, and three novel chalcones bearing a long-chain alkylphenol moiety, named galanganones A–C (**1**–**3**, Figure 1), have been isolated. Compounds **1**–**3** represent the first examples of long-chain alkylphenol-coupled chalcone, and their presence as markers may be helpful in chemotaxonomic classification. We report herein the isolation and structure elucidation of these intriguing natural products.

2. Results and discussion

Compound **1**, a light yellow amorphous solid, showed a quasi-molecular ion peak at *m/z* 517.2585 $[M + H]^+$ in the positive HR-ESI-MS, corresponding to an elemental formula $C_{32}H_{37}O_6$ (calcd for $C_{32}H_{37}O_6$, 517.2590). The absorption bands attributable to hydroxy groups at 3425 cm^{-1}, a conjugated carbonyl group at 1606 cm^{-1}, and aromatic ring groups at 1562, 1512, and 1436 cm^{-1} appeared in the IR spectrum. The ^1H NMR spectrum of **1** in methanol-d_4 (Table 1) exhibited signals for two 1,4-disubstituted benzene rings (H_2-2′ and 6′, δ_H 7.02, d, $J = 8.8$ Hz; H_2-3′ and 5′, δ_H 6.62, d, $J = 8.8$ Hz; H_2-11″ and 15″, δ_H 7.48, d, $J = 8.5$ Hz; H_2-12″ and 14″, δ_H 6.81, d, $J = 8.5$ Hz), a singlet aromatic proton (H-5″, δ_H 6.01), two *trans*-olefinic functionalities (H-2, δ_H 6.24, dd, $J = 15.3$, 8.8 Hz; H-3, δ_H 5.51, dt, $J = 15.3$, 7.0 Hz; H-8″, δ_H 7.79, d, $J = 15.5$ Hz; H-9″, δ_H 7.65, d, $J = 15.5$ Hz) as indicated by the large coupling constants, an aliphatic methine

*Corresponding authors. Emails: mdr31882@126.com; wangfei@mail.kib.ac.cn

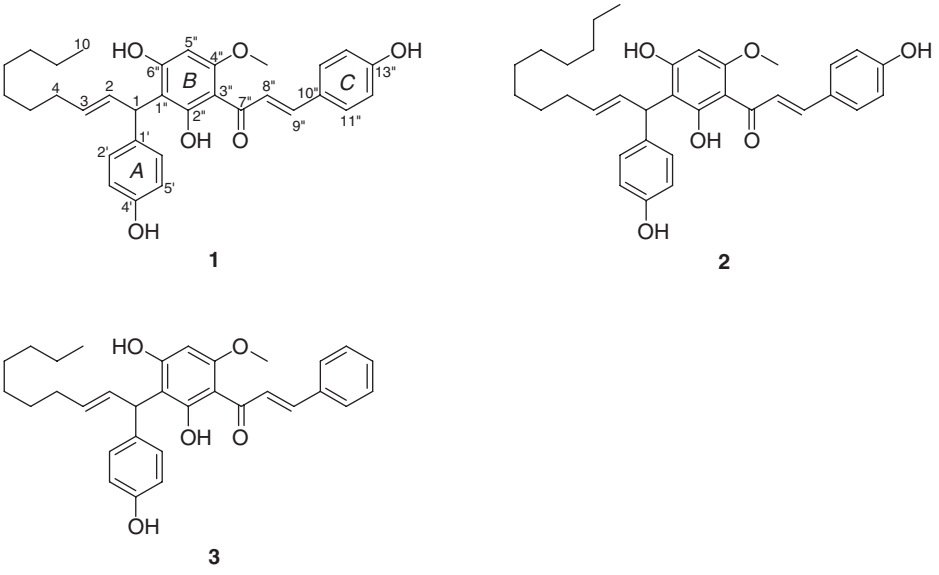

Figure 1. Structures of compounds **1**–**3**.

(H-1, δ_H 5.08, d, $J = 8.8$ Hz), a methoxy (δ_H 3.89, s), an aliphatic methylene (H_2-4, δ_H 2.06, q, $J = 7.0$ Hz), five high-field methylenes (H_2-5–H_2-9, δ_H 1.22–1.41, overlapped multiplets), and a triplet methyl (H_3-10, δ_H 0.87, t, $J = 7.4$ Hz). The ^{13}C NMR (DEPT) spectrum (Table 1) showed an aromatic ketone (C-7″, δ_C 194.1), 22 aromatic/olefinic carbon resonances corresponding to three benzene rings, and two olefin groups (including five oxygenated carbons: C-4′, C-2″, C-4″, C-6″, C-13″, δ_C 155.8, 166.3, 162.7, 163.8, 161.1, respectively), a methoxy (δ_C 56.2), an aliphatic methine (C-1, δ_C 43.2), six high-field methylenes (C-4, C-5, C-6, C-7, C-8, C-9, δ_C 33.5, 30.6, 30.3, 30.1, 33.1, 23.7, respectively), and a methyl group (C-10, δ_C 14.5).

In the HMBC spectrum of **1** (Figure 2), the cross-peaks from H-8″ to C-7″, C-9″, and C-10″, from H-9″ to C-7″, C-8″, and C-11″/15″, and from H-5″ to C-1″, C-3″, C-6″, and C-7″ (weak ^4J correlation) confirmed the existence of chalcone (1,3-diphenylprop-2-en-1-one) part (**1a**) [4]. In this moiety, the ketone carbonyl carbon (C-7″) was attached to C-3″ according to a weak four-bond correlation from the aromatic singlet (H-5″, δ_H 6.01) to C-7″. The methoxy group was assigned at C-4″ due to the HMBC correlations from H_3-OMe to C-4″ and C-5″ (weak ^4J correlation). In the ^{1}H NMR spectrum, the high-field signals at δ_H 0.85–2.10 revealed a saturated C_7 alkyl fragment connected to an olefinic group. The HMBC correlations from H-1 to C-2, C-1′, and C-2′/6′, from H-2 to C-1, C-4, and C-1′, and from H-3 to C-1, C-2, C-4, and C-5 indicated that **1** has a (*E*)-4-(dec-2-en-1-yl)phenol moiety (**1b**). Considering no oxygen atom was linked to C-1 (δ_C 43.2), the notable low-field shift of H-1 (δ_H 5.08) strongly indicated that the methine carbon (C-1) must be attached directly to three aromatic/olefinic groups. The HMBC correlations from H-1 to C-1″, C-2″, and C-6″, and from H-2 to C-1″ confirmed that **1b** was coupled with **1a** via a C-1—C-1″ bond. In addition, all these secondary metabolites **1**–**3** occur as racemates in view of the absence of optical activity and Cotton effects in the CD

Table 1. ^{1}H and ^{13}C NMR spectral data of compounds **1**–**3** (^{1}H: 600 MHz; ^{13}C: 125 MHz, in CD_3OD).

	1		**2**		**3**	
No.	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C
1	5.08 (d, 8.8)	43.2 (d)	5.08 (d, 8.8)	43.2 (d)	5.09 (d, 8.7)	43.2 (d)
2	6.24 (dd, 15.3, 8.8)	132.8 (d)	6.24 (dd, 15.2, 8.8)	132.8 (d)	6.24 (dd, 15.3, 8.7)	132.8 (d)
3	5.51 (dt, 15.3, 7.0)	132.1 (d)	5.51 (dt, 15.2, 7.1)	132.1 (d)	5.52 (dt, 15.3, 7.0)	132.1 (d)
4	2.06 (q, 7.0)	33.5 (t)	2.06 (q, 7.1)	33.5 (t)	2.06 (q, 7.0)	33.5 (t)
5	1.36–1.42 (m)	30.6 (t)	1.36–1.42 (m)	30.8 (t)	1.36–1.42 (m)	30.6 (t)
6	1.22–1.33 (m)	30.3 (t)[a]	1.22–1.33 (m)	30.6 (t)[a]	1.22–1.33 (m)	30.3 (t)[a]
7	1.22–1.33 (m)	30.1 (t)[a]	1.22–1.33 (m)	30.6 (t)[a]	1.22–1.33 (m)	30.1 (t)[a]
8	1.22–1.33 (m)	33.1 (t)	1.22–1.33 (m)	30.5 (t)[a]	1.22–1.33 (m)	33.1 (t)
9	1.22–1.33 (m)	23.7 (t)	1.22–1.33 (m)	30.2 (t)[a]	1.22–1.33 (m)	23.7 (t)
10	0.87 (t, 7.4)	14.5 (q)	1.22–1.33 (m)	33.1 (t)	0.87 (t, 6.9)	14.5 (q)
11			1.22–1.33 (m)	23.8 (t)		
12			0.87 (t, 7.0)	14.5 (q)		
1′		136.8 (s)		136.8 (s)		136.8 (s)
2′/6′	7.02 (d, 8.8)	129.4 (d)	7.02 (d, 8.6)	129.4 (d)	7.03 (d, 8.6)	129.4 (d)
3′/5′	6.62 (d, 8.8)	115.3 (d)	6.61 (d, 8.6)	115.3 (d)	6.62 (d, 8.6)	115.4 (d)
4′		155.8 (s)		155.8 (s)		155.8 (s)
1″		112.3 (s)		112.4 (s)		112.5 (s)
2″		166.3 (s)		166.3 (s)		166.3 (s)
3″		106.5 (s)		106.6 (s)		106.6 (s)
4″		162.7 (s)		162.7 (s)		162.8 (s)
5″	6.01 (s)	91.9 (d)	6.02 (s)	91.9 (d)	6.02 (s)	92.0 (d)
6″		163.8 (s)		163.8 (s)		164.2 (s)
7″		194.1 (s)		194.1 (s)		194.0 (s)
8″	7.79 (d, 15.5)	125.8 (d)	7.79 (d, 15.5)	125.9 (d)	7.92 (d, 15.6)	129.3 (d)
9″	7.65 (d, 15.5)	143.4 (d)	7.65 (d, 15.5)	143.4 (d)	7.66 (d, 15.6)	142.5 (d)
10″		128.4 (s)		128.4 (s)		137.0 (s)
11″/15″	7.48 (d, 8.5)	131.3 (d)	7.49 (d, 8.6)	131.3 (d)	7.61 (br d, 7.4)	129.3 (d)
12″/14″	6.81 (d, 8.5)	116.8 (d)	6.81 (d, 8.6)	116.8 (d)	7.34–7.42 (m)	130.0 (d)
13″		161.1 (s)		161.1 (s)	7.34–7.42 (m)	131.1 (d)
OMe	3.89 (s)	56.2 (q)	3.89 (s)	56.2 (q)	3.89 (s)	56.2 (q)

[a] Interchangeable.

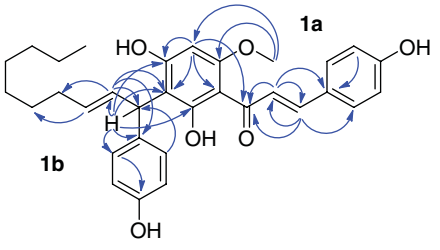

Figure 2. Key HMBC correlations of **1**.

spectra. Therefore, the structure of **1** was elucidated as (±)-(*E*)-1-{2,4-dihydroxy-3-[(*E*)-1-(4-hydroxyphenyl)dec-2-en-1-yl]-6-methoxyphenyl}-3-(4-hydroxyphenyl) prop-2-en-1-one, and given the trivial name galanganone A.

Compound **2**, a light yellow amorphous solid, had the molecular formula $C_{34}H_{40}O_6$ based on the HR-ESI-MS (pos.), showing a quasi-molecular ion peak at *m/z* 545.2900 (calcd for $C_{34}H_{41}O_6$, 545.2903) with 15 degrees of unsaturation. It gave almost the same IR spectrum as that of **1**. The ^{1}H and ^{13}C NMR spectra (Table 1) were very similar to those of **1**, except for two additional methylene resonances in

the alkyl chain in **2**. The NMR spectra revealed a saturated C_9 alkyl fragment connected to an olefinic group at δ_H 2.06 (H_2-4, q, $J = 7.1$ Hz)/δ_C 33.5, δ_H 1.22–1.41 (H_2-5–H_2-11, m)/δ_C 30.2, 30.5, 30.6, 30.6, 30.8, 33.1, 23.8, and δ_H 0.87 (H_3-12, t, $J = 7.0$ Hz)/δ_C 14.5, which was in agreement with the mass spectrum. Thereupon, the structure of **2** was established as shown in Figure 1 and named galanganone B.

Compound **3**, a light yellow amorphous solid, possessed the molecular formula $C_{32}H_{36}O_5$ as determined by the HR-ESI-MS (pos.), showing a quasi-molecular ion peak at *m/z* 523.2458 (calcd for $C_{32}H_{36}O_5Na$, 523.2460) with 15 degrees of unsaturation. The ^1H and ^{13}C NMR spectra (Table 1) were generally similar to those of **1**, except that a monosubstituted benzene ring at δ_H 7.61 (H-11″/15″, br d, $J = 7.4$ Hz)/δ_C 129.3, δ_H 7.34–7.42 (H-12″/14″, m)/δ_C 130.0, δ_H 7.34–7.42 (H-13″, m)/δ_C 131.1, and δ_C 137.0, replaced a *para*-disubstituted benzene ring of **1**. The monosubstituted benzene ring was assigned as ring C, according to the HMBC cross-peaks from H-11″/15″ to C-9″ and C-13″, and from H-8″ to C-10″. Accordingly, the structure of **3** was determined as shown in Figure 1 and named galanganone C.

3. Experimental

3.1 General experimental procedures

Optical rotations were measured on an SGW®-3 (INESA Instrument Co., Ltd, Shanghai, China) automatic polarimeter. UV data were obtained from online HPLC analysis. CD spectrum was recorded on a Chirascan (Applied Photophysics, Leatherhead, Surrey, UK). IR spectra were obtained using a Bruker Tensor 27 FT-IR (Bruker Optics GmbH, Ettlingen, Germany) spectrometer with KBr pellets. NMR spectra were acquired on a Bruker Avance III 600 MHz (Bruker BioSpin GmbH, Rheinstetten, Germany) spectrometer with the solvent signal (methanol-d_4: δ_H 3.30 ppm, δ_C 49.00 ppm) as an internal standard. Mass spectra were measured on Waters Xevo TQ-S and Agilent G6230 TOF mass spectrometers. Silica gel 200–300 mesh (Qingdao Marine Chemical, Inc., Qingdao, China), C18 (40–75 μm, Fuji Silysia Chemical Ltd, Kasugai, Aichi, Japan), and Sephadex LH-20 (Amersham Biosciences, Uppsala, Sweden) were used for column chromatography (CC). Medium pressure liquid chromatography (MPLC) was performed on a Büchi Sepacore System equipping with pump manager C-615, pump modules C-605, fraction collector C-660 (Büchi Labortechnik AG, Flawil, Switzerland), and C18 packed columns. Fractions were monitored and analyzed by TLC, in combination with Agilent 1200 series HPLC system equipped with an Extend-C18 column (5 μm, 4.6 × 150 mm).

3.2 Plant material

The rhizomes of *A. galanga* were collected in Jinping County of Yunnan Province, China, in May 2009, and identified by Mr Yu Chen of Kunming Institute of Botany, Chinese Academy of Sciences. A voucher specimen (No. BBP0128017AG) was deposited at BioBioPha Co., Ltd.

3.3 Extraction and isolation

Dried and powdered rhizomes (9.5 kg) of *A. galanga* were extracted with EtOH–H_2O (95:5, v/v; 3 × 15 l, each 5 days) at room temperature. The combined filtrates were concentrated under vacuum to produce a thick, dark extract (*ca.* 440 g), which was fractionated by silica gel CC successively eluted with a gradient of increasing acetone in petroleum ether (10:1, 8:1, 5:1, 3:1, 1:1, 0:1; v/v) to yield fractions A–F, respectively. Fraction C (*ca.* 12 g) was further separated on silica gel CC ($CHCl_3$/EtOAc; 10:1 → 5:1),

Sephadex LH-20 CC ($CHCl_3$/MeOH; 1:1), and normal pressure C18 CC (MeOH/H_2O; 9:1) to give **3** (5 mg). Fraction E (*ca.* 19 g) was subjected to preparative MPLC (70% → 80% MeOH in H_2O), followed by silica gel CC ($CHCl_3$/MeOH; 15:0 → 15:1) to successively afford subfractions E-1 and E-2. Subfraction E-1 (150 mg) was further purified by Sephadex LH-20 CC (MeOH) to afford **2** (42 mg) and **1** (108 mg) from subfraction E-2 (320 mg) by the same technique. The retention times (t_R) of **1**–**3** on an analytical HPLC Extend-C18 column (20% → 100% MeOH in H_2O over 8.0 min followed by 100% MeOH to 13 min, 1.0 ml/min, 25 °C) were 10.2, 10.6, and 10.9 min, respectively.

3.3.1 *Galanganone A (**1**)*

Light yellow amorphous solid; UV λ_{max} (MeOH): 370 nm; IR (KBr) ν_{max}: 3425, 2925, 2854, 1606, 1562, 1512, 1436, 1342, 1220, 1169, 1143, 1106 cm^{-1}; for ^1H and ^{13}C NMR spectroscopic data, see Table 1; ESI-MS (pos.) *m/z*: 517 $[M + H]^+$; HR-ESI-MS (pos.) *m/z*: 517.2585 $[M + H]^+$ (calcd for $C_{32}H_{37}O_6$, 517.2590).

3.3.2 *Galanganone B (**2**)*

Light yellow amorphous solid; UV λ_{max} (MeOH): 370 nm; IR (KBr) ν_{max}: 3424, 2925, 2853, 1606, 1561, 1512, 1436, 1342, 1219, 1169, 1143, 1106 cm^{-1}; for ^1H and ^{13}C NMR spectroscopic data, see Table 1; ESI-MS (pos.) *m/z*: 545 $[M + H]^+$; HR-ESI-MS (pos.) *m/z*: 545.2900 $[M + H]^+$ (calcd for $C_{34}H_{41}O_6$, 545.2903).

3.3.3 *Galanganone C (**3**)*

Light yellow amorphous solid; UV λ_{max} (MeOH): 350 nm; IR (KBr) ν_{max}: 3448, 2926, 2854, 1628, 1511, 1448, 1432, 1384, 1338, 1224 cm^{-1}; for ^1H and ^{13}C NMR spectroscopic data, see Table 1; ESI-MS (pos.) *m/z*: 501 $[M + H]^+$; HR-ESI-MS (pos.) *m/z*: 523.2458 $[M + Na]^+$ (calcd for $C_{32}H_{36}O_5Na$, 523.2460).

Disclosure statement

No potential conflict of interest was reported by the authors.

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