Gingerol Derivatives from the Rhizomes of *Zingiber Officinale*

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Two new gingerol derivatives, [8]-gingesulfonic acid (**1**) and [10]-gingesulfonic acid (**2**), were isolated from the rhizomes of *Zingiber officinale*, along with nine known gingerol derivatives, [6]-gingesulfonic acid (**3**), 1-dehydro-[6]-gingerdione (**4**), 1-dehydro-[8]-gingerdione (**5**), 1-dehydro-[10]-gingerdione (**6**), [6]-gingerdione (**7**), [10]-gingerdione (**8**), [6]-gingerol (**9**), methyl [6]-gingerdiol (**10**), and 6-hydroxyl-[6]-shogaol (**11**). Their structures were elucidated on the basis of spectral evidence and comparisons with literature data.

Key words: Zingiber officinale, Zingiberaceae, Gingerol Derivatives, [8]-Gingesulfonic Acid, [10]-Gingesulfonic Acid

Introduction

The rhizome of *Zingiber officinale* also known as ginger is one of the most popular spices and is a traditional medicine in China and also in India. Gingerols, the so-called "pungent principles", are the constituents responsible for the taste of ginger [1]. Phytochemical and pharmacological reports have shown that the major bioactive constituents of ginger, the gingerols, possess the following activities: anti-platelet aggregation [2, 3], anticancer [4 – 6], anti-oxidation and anti-inflammation [7, 8], inhibition of COX-2 expression [9], antifungal [10] and others. Therefore the isolation and purification of the gingerols and their derivatives from ginger are of great interest.

In this study, we report the isolation of two new sulfonated gingerol derivatives, [8]-gingesulfonic acid (**1**) and [10]-gingesulfonic acid (**2**), along with nine known gingerol derivatives, [6]-gingesulfonic acid (**3**) [11], 1-dehydro-[6]-gingerdione (**4**) [1], 1-dehydro-[8]-gingerdione (**5**) [1, 12], 1-dehydro-[10]-gingerdione (**6**) [12], [6]-gingerdione (**7**) [13], [10]-gingerdione (**8**) [13], [6]-gingerol (**9**) [14, 15], methyl [6]-gingerdiol (**10**) [16], and 6-hydroxyl-[6]-shogaol (**11**) [17] (Fig. 1). Their structures were established by mass-spectrometric and spectroscopic analyses, especially 2D-NMR techniques (^{1}H, ^{1}H-COSY, HSQC, HMBC), and comparisons with literature data. Herein, the isolation and structural elucidation of compounds **1** and **2** are described.

Results and Discussion

[8]-gingesulfonic acid (**1**) was obtained as colorless powder. Its molecular formula of $C_{19}H_{30}O_6S$ was established from HRMS ((–)-TOF) ([M–H]$^-$, m/z = 385.1686, calcd. 385.1684) and ^{13}C NMR (DEPT) data, indicating 5 degrees of unsaturation. The IR spectrum showed absorption bands of hydroxyl (3432 cm^{-1}), carbonyl (1713 cm^{-1}), and sulfonic acid (1207, 1127, 1055 cm^{-1}) functions. Typical benzenoid absorption was seen at 204, 224, and 282 nm in the UV spectrum. The ^{1}H NMR spectrum (Table 1) exhibited separate signals for one methoxy group [δ_H = 3.87 (s)], one methyl group [δ_H = 0.94 (t, J = 6.5 Hz, H-12)], one methine group [δ_H = 3.37 (m, H-5)], and four methylene groups [δ_H = 2.84 (m, H-1), 2.85 (m, H-2), 2.57 (dd, J = 13.4, 6.4 Hz, H-4a), 3.08 (dd, J = 13.4, 5.7 Hz, H-4b), 1.48 (m, H-6a), 1.95 (m, H-6b)], and overlapping resonances of five methylene groups [δ_H = 1.29 – 1.40 (m, H-7→H-11)]. Signals of three aromatic protons were assigned to H-2′ [δ_H = 6.82 (d, J = 1.4 Hz)], H-5′ [δ_H = 6.72 (d, J = 8.0 Hz)], and H-6′ [δ_H = 6.66 (dd, J = 8.0, 1.4 Hz)], which suggested the

Table 1. ^{1}H NMR (400.13 MHz) and ^{13}C NMR (100.62 MHz) data of **1** and **2** in CD_3OD[a].

	1		**2**	
Position	δ(C)	δ(H)	δ(C)	δ(H)
1	30.56 t	2.84 (m, overlap)	30.57 t	2.83 (m, overlap)
2	46.11 t	2.85 (m, overlap)	46.09 t	2.84 (m, overlap)
3	210.78 s		210.71 s	
4	44.84 t	2.57 (dd, 13.4, 6.4)	44.74 t	2.59 (dd, 17.5, 6.4)
		3.08 (dd, 13.4, 5.7)		3.07 (dd, 17.5, 5.7)
5	57.17 d	3.37 (m)	57.12 d	3.37 (m)
6	32.11 t	1.48 (m), 1.95 (m)	32.03 t	1.47 (m), 194 (m)
7	28.34 t	1.29 – 1.40 (m)	28.29 t	1.29 – 1.39 (m)
8	30.83 t[b]	1.29 – 1.40 (m)	30.86 t[b]	1.29 – 1.39 (m)
9	30.37 t[b]	1.29 – 1.40 (m)	30.83 t[b]	1.29 – 1.39 (m)
10	33.13 t	1.29 – 1.40 (m)	30.69 t[b]	1.29 – 1.39 (m)
11	23.83 t	1.29 – 1.40 (m)	30.57 t[b]	1.29 – 1.39 (m)
12	14.58 q	0.94 (t, 6.5)	33.20 t	1.29 – 1.39 (m)
13			23.87 t	1.29 – 1.39 (m)
14			14.59 q	0.92 (t, 7.0)
1′	134.14 s		134.06 s	
2′	113.24 d	6.701 (overlap)	113.26 d	6.81 (d, 2.1)
3′	148.99 s		149.00 s	
4′	145.84 s		145.90 s	
5′	116.24 d	6.78 (br. s)	116.26 d	6.62 (d, 8.3)
6′	121.84 d	6.656 (overlap)	121.84 d	6.67 (overlap)
OCH_3	56.51 q	3.87 (s)	56.49 q	3.85 (s)

[a] Chemical shift values δ in ppm, coupling constants J in Hz; [b] values may be interchangeable in the same column.

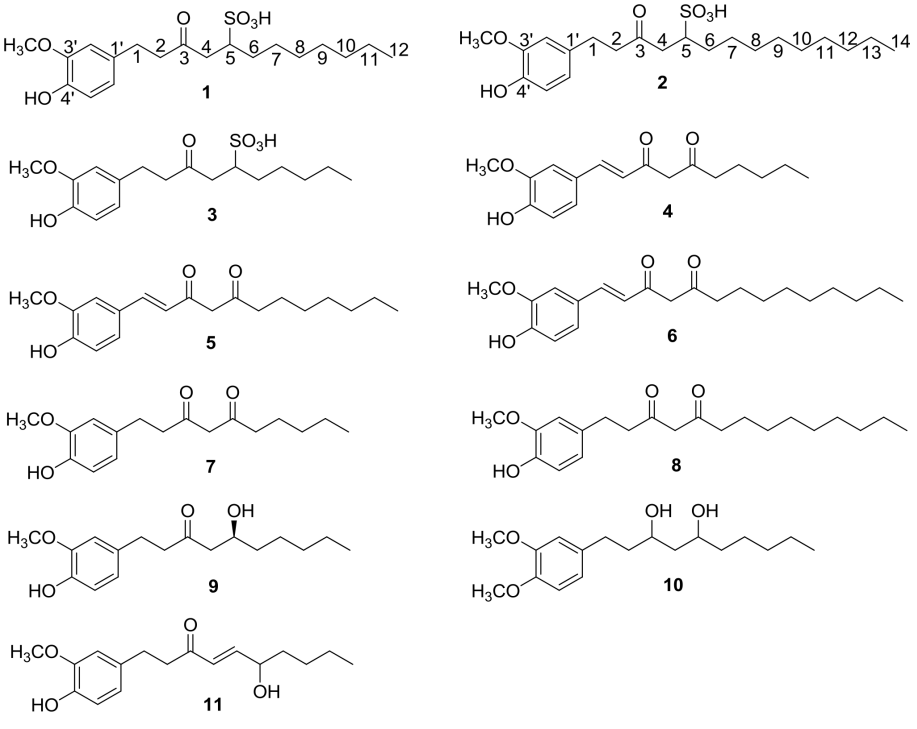

Fig. 1. Structures of compounds **1** – **11**.

existence of one 1,3,4-trisubstituted benzene ring. The ^{13}C NMR (DEPT) spectrum (Table 1) showed signals of one methoxyl carbon [δ_C = 56.51], one methyl carbon [δ_C = 14.58 (C-12)], nine methylene carbons [δ_C = 23.83 (C-11), 28.34 (C-7), 30.37 (C-9), 30.56 (C-1), 30.83 (C-8), 32.11 (C-6), 33.13 (C-10), 44.84 (C-4), 46.11 (C-2)], one methine carbon [δ_C = 57.17 (C-5)], one carbonyl carbon [δ_C = 210.78 (C-3)], as well as of one aromatic ring with two oxygen-bearing carbons [δ_C = 148.99 (C-3′), 145.84 (C-4’)], three CH carbons

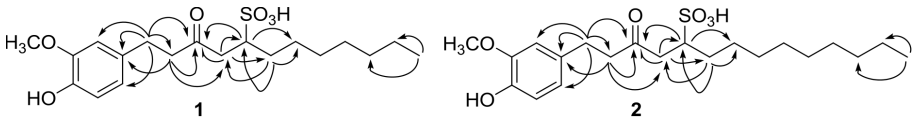

Fig. 2. Key HMBC correlations for compounds **1** and **2**.

[δ_C = 113.24 (C-2′), 116.24 (C-5′), 121.84 (C-6′)], and one C-bound carbon [δ_C = 134.14 (C-1′)].

The negative HRMS (TOF) of **1** revealed the [M–H]$^-$ ion peak at m/z = 385.1686, 28 mass units (C_2H_4) more than that of [6]-gingesulfonic acid [18]. The ^{1}H and ^{13}C NMR (Table 1) spectroscopic features were similar to those of [6]-gingesulfonic acid [18], except for additional signals due to two methylene groups present in the alkyl chain. In addition, the signals at δ_H =3.37/δ_C = 57.17 due to H-5/C-5 resembled those of [6]-gingesulfonic acid [18]. Hence, **1** was characterized as a 5-dehydroxy 5-sulfonated derivative of [8]-gingerol, and named [8]-gingesulfonic acid.

The structure of **1** was further confirmed by 2D-NMR techniques (^{1}H, ^{1}H-COSY, HSQC, HMBC), especially the HMBC correlations as shown in Fig. 2. The ^{1}H, ^{1}H-COSY correlations of H-5/H-4 and H-5/H-6 showed the connectivity C(4)-C(5)-C(6), which was further confirmed by HMBC correlations (Fig. 2) of H-4/C-5, H-4/C-6, H-5/C-4, H-5/C-6, H-6/C-4, and H-6/C-5. The HMBC correlations of H-1/C-2, H-1/C-3, H-2/C-1, H-2/C-3, H-2/C-4, H-4/C-3, and H-5/C-3 showed the linkage of C(1)-C(2)-C(3)-C(4)-C(5). The connectivity C(10)-C(11)-C(12) was established by the HMBC correlations of H-12/C-10 and H-12/C-11. The HMBC experiment also showed the long-range couplings H-1/C-1′, H-1/C-2′, H-1/C-6′, and H-2/C-1′, which indicated that the alkyl chain is linked to the aromatic ring. Therefore, the structure of **1** was again deduced as a sulfonated gingerol derivative, named [8]-gingesulfonic acid (Fig. 1).

[10]-gingesulfonic acid (**2**), isolated as a colorless powder, showed an [M–H]$^-$ ion peak at m/z = 413.1991 ($C_{21}H_{33}O_6S$), 28 mass units (C_2H_4) more than that of **1**, in the HRMS ((–)-TOF). The IR spectrum showed absorption bands of hydroxyl (3431 cm^{-1}), carbonyl (1712 cm^{-1}), and sulfonic acid (1207, 1126, 1054 cm^{-1}) functions. A typical benzenoid absorption was seen at 204, 225, and 282 nm in the UV spectrum. The ^{1}H and ^{13}C NMR (Table 1) spectroscopic features were similar to those of **1**, except for additional signals due to two methylene groups present in the alkyl chain. In addition, the signals at δ_H = 3.37/δ_C = 57.12 due to H-5/C-5 resembled those of **1**. The structure of **2** was also further confirmed by 2D-NMR techniques, especially the HMBC correlations as shown in Fig. 2. Hence, the structure of **2** was established as a 5-dehydroxy 5-sulfonated derivative of [10]-gingerol, and named [10]-gingesulfonic acid (Fig. 1).

Comparison of the spectroscopic and physical data with those published allowed us to establish the structures of nine known gingerol derivatives, [6]-gingesulfonic acid (**3**) [11], 1-dehydro-[6]-gingerdione (**4**) [1], 1-dehydro-[8]-gingerdione (**5**) [1, 12], 1-dehydro-[10]-gingerdione (**6**) [12], [6]-gingerdione (**7**) [13], [10]-gingerdione (**8**) [13], [6]-gingerol (**9**) [14, 15], methyl [6]-gingerdiol (**10**) [16], and 6-hydroxyl-[6]-shogaol (**11**) [17], respectively.

As mentioned above, three sulfonated derivatives of gingerol were isolated from *Zingiber officinale*, and their structures were established as [8]-gingesulfonic acid (**1**), [10]-gingesulfonic acid (**2**), and [6]-gingesulfonic acid (**3**). However, the stereochemistry at C-5 of **1** – **3** is still under investigation. In order to determine the 5*S* or 5*R* configuration of **1** – **3**, we tried to grow single crystals but the structure determination failed due to the small size of the needle-shaped crystals obtained. We also hoped to compare their optical rotations with those of the known analogous compounds, [6]-gingesulfonic acid and [4]-gingesulfonic acid, to determine the absolute stereochemistry of **1** – **3**, but the stereochemistry at C-5 has not been reported in the literature [11]. Trying to grow single crystals suitable for X-ray structure determinations of compounds **1** – **3** as well as of **10** and **11** is clearly necessary in further work.

Experimental Section

General experimental procedures

Optical rotation was measured on a Horiba SEPA-300 polarimeter. A UV-2401PC spectrometer was used to obtain the UV spectrum in methanol (MeOH). IR spectra were taken on a Bruker Tensor 27 FTIR spectrometer from KBr pellets. NMR spectra were measured on a Bruker AM-400 spectrometer with TMS as an internal standard. MS-TOF and HRMS-TOF spectra were obtained on a API-QSTAR-Pulsar-1 spectrometer. Column chromatography was carried out on Sephadex LH-20 gel (25 – 100 μm, Pharmacia Fine Chem-

ical Co. Ltd.), MCI gel CHP-20P (75 – 150 μm, Mitsubishi Chemical Co.), Chromatorex ODS (30 – 50 μm, Fuji Silysia Chemical Co. Ltd.), and silica gel (SiO_2; 200 – 300 mesh, Qingdao Haiyang Chemical Co. Ltd., P. R. China). Thin layer chromatography (TLC) was carried out on silica gel G precoated plates (Qingdao Haiyang Chemical Co. Ltd.), and spots were detected by spraying with 5 % H_2SO_4 in EtOH followed by heating.

Plant material

The dry rhizomes of *Zingiber officinale* Roscoe (Zingiberaceae) were purchased from a local drug store in March 2010 and identified by Prof. Dr. Kai-Jin Wang from the School of Life Sciences, Anhui University, where a voucher specimen (No. 20100302) was deposited.

Extraction and isolation

The dry rhizomes of *Zingiber officinale* (8.0 kg) were extracted with 90 % EtOH at room temperature (3 × 20 L, each 4 d). The extracts were combined and concentrated under vacuum to give a residue (2.4 kg). The residue was suspended in H_2O and extracted with petroleum ether (3 × 2 L), ethyl acetate (3 × 2 L) and *n*-butanol (3 × 2 L), successively. The organic solvents were evaporated under reduced pressure to provide extracts of petroleum ether (418.4 g), ethyl acetate (981.6 g), and *n*-butanol (28.8 g). The ethyl acetate part (981.6 g) was chromatographed on a silica gel column eluted with $CHCl_3$-MeOH (from 100 : 0 to 0 : 100, v/v) to furnish 6 fractions A – F. Fraction A (88.5 g) was subjected to a Sephadex LH-20 (EtOH-H_2O, 0 : 1 – 1 : 0) to yield fractions A_1 – A_4. Fraction A_1 was purified by MCI (EtOH-H_2O, 0 : 1 – 1 : 0) and then Sephadex LH-20 (EtOH) to afford **7** (10 mg) and **8** (46 mg). Fraction A_2 was subjected to a Sephadex LH-20 column eluting with EtOH-H_2O (0 : 1 – 1 : 0) to obtain compound **6** (55 mg). Compound **4** (42 mg) was obtained from fraction A_3 by column chromatography on MCI (EtOH-H_2O, 0 : 1 – 1 : 0). Fraction B (72.4 g) was purified by Sephadex LH-20 (EtOH-H_2O, 0 : 1 – 1 : 0) to yield fractions B_1 – B_3. Fraction B_1 was subjected to Sephadex LH-20 (EtOH) and then MCI (EtOH-H_2O, 0 : 1 – 1 : 0) repeatedly to afford **11** (23 mg). Fraction B_2 was further subjected to MCI (EtOH-H_2O, 0 : 1 – 1 : 0) and then Sephadex LH-20 (EtOH) to yield **10** (16 mg). Compounds **5** (36 mg) and **9** (24 mg) were obtained from fraction B_3 by column chromatography on Sephadex LH-20 (EtOH) and then MCI (EtOH-H_2O, 0 : 1 – 1 : 0). Fraction F (45.0 g) was purified by Sephadex LH-20 chromatography (EtOH-H_2O, 0 : 1 – 1 : 0) to yield three fractions (F_1 – F_3). Fraction F_1 was subjected to a Sephadex LH-20 column (EtOH-H_2O, 0 : 1 – 1 : 0; then EtOH) to afford compound **2** (45 mg). Compound **1** (36 mg) was obtained from fraction F_2 by column chromatography on MCI (EtOH-H_2O, 0 : 1 – 1 : 0) and then Sephadex LH-20 (EtOH). Compound **3** (123 mg) was obtained from fraction F_3 by column chromatography on MCI (EtOH-H_2O, 0 : 1 – 1 : 0).

Physical and spectroscopic data

*[8]-Gingesulfonic acid (**1**)*

Colorless powder. – UV/Vis (MeOH): $\lambda_{max}(\lg \varepsilon_{max})$ = 204 (4.11), 224 (3.75), 282 (3.40) nm. – $[\alpha]_D^{16} = -5.6$ (c = 0.44, MeOH). – IR (KBr): ν = 3432 (OH), 2954, 2927, 2856, 1713 (C=O), 1639, 1610, 1518, 1465, 1433, 1374, 1269, 1207, 1127, 1055, 814, 722, 607, 556 cm^{-1}. – ^1H (400.13 MHz, CD_3OD, TMS) and ^{13}C NMR (100.62 MHz, CD_3OD): see Table 1. – MS ((–)-TOF): m/z (%) = 385 (100). – HRMS ((–)-TOF): m/z = 385.1686 (calcd. 385.1684 for $C_{19}H_{29}O_6S$, $[M–H]^-$).

*[10]-Gingesulfonic acid (**2**)*

Colorless powder. – UV (MeOH): $\lambda_{max}(\lg \varepsilon_{max})$ = 204 (4.10), 225 (3.75), 282 (3.42) nm. – $[\alpha]_D^{16} = -5.9$ (c = 0.33, MeOH). – IR (KBr): ν = 3431 (OH), 2926, 2854, 1712 (C=O), 1629, 1612, 1518, 1465, 1371, 1270, 1207, 1126, 1054, 814, 722, 606, 557 cm^{-1}. – ^1H (400.13 MHz, CD_3OD, TMS) and ^{13}C NMR (100.62 MHz, CD_3OD): see Table 1. – MS ((–)-TOF): m/z (%) = 413 (100). – HRMS ((–)-TOF): m/z = 413.1991 (calcd. 413.1997 for $C_{21}H_{33}O_6S$, $[M–H]^-$).

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