6-GINGESULFONIC ACID, A NEW ANTI-ULCER PRINCIPLE, AND GINGERGLYCOLIPIDS A, B AND C, THREE NEW MONOACYLDIGALACTOSYLGLYCEROLS, FROM ZINGIBERIS RHIZOMA ORIGINATING IN TAIWAN

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By monitoring the effects on HCl / ethanol-induced gastric lesions in rats, a new anti-ulcer principle named 6-gingesulfonic acid was isolated from Zingiberis Rhizoma, the dried rhizome of *Zingiber officinale* ROSCOE (cultivated and processed in Taiwan) together with three new monoacyldigalactosylglycerols named gingerglycolipids A,B and C. Their chemical structures were elucidated on the basis of chemical and physicochemical evidence. 6-Gingesulfonic acid showed more potent anti-ulcer activity than 6-gingerol and 6-shogaol .

KEYWORDS Zingiberis Rhizoma ; *Zingiber officinale*; ginger ; 6-gingesulfonic acid; gingerglycolipid ; anti-ulcer effect ; Zingiberaceae

Zingiberis Rhizoma (Ginger ; Shoukyo in Japanese), the dried-rhizome of *Zingiber officinale* ROSCOE (Zingiberaceae), is one of the best known Chinese crude drugs and it has been investigated extensively in search of its bioactive principles.[1] In particular, the pungent constituents, which are the principal ingredients of Zingiberis Rhizoma, have been the subjects of many investigations, and various pungent constituents have been characterized.[1,2] During the course of our screening to find biologically active constituents contained in Zingiberis Rhizoma , we have found several bioactive compounds showing anticathartic, antiserotonergic, and gastrointestinal motility enhancing effect.[3] Furthermore, by monitoring with anti-ulcer effects in rats, we have isolated four sesquiterpenes (β–sesquiphellandrene, β-bisabolene, *ar*-curcumene and α-zingiberene), 6-gingerol and 6-shogaol as active compounds in stomachic medication from the lipophilic portion of Zingiberis Rhizoma originating in Taiwan.[4]

In continuing studies, we have examined the water-soluble portion of the Zingiberis Rhizoma and have isolated a new anti-ulcer principle named 6-gingesulfonic acid (**1**). This paper communicates the evidence which is consistent with the structure of **1**. In addition, three new monoacyldigalactosylglycerols, gingerglycolipids A (**5**), B (**6**) and C (**7**), were chemically elucidated.

The MeOH extract of Zingiberis Rhizoma was partitioned into an AcOEt-Water mixture. Repeated separation of the water-soluble portion by ordinary-phase and reversed-phase column chromatography furnished 6-gingesulfonic acid (**1**, 0.0013% from the crude drug) as anti-ulcer principle of the water-soluble portion together with (+)-angelicoidenol-2-*O*-β-D-glucopyranoside[5] (**4**, 0.0014%), gingerglycolipids A (**5**, 0.0013%) , B (**6**, 0.0014%) and C (**7**, 0.0014%).

6-Gingesulfonic acid (**1**) ,white powder, $[\alpha]_D$ - 1.0 ° (MeOH), $C_{17}H_{26}O_6S$,[6] UV(MeOH): 221.5 (1110), 281.5 (1000), was shown by its IR spectrum to have hydroxyl (3590 cm^{-1}), carbonyl (1710 cm^{-1}) and sulfonic acid (1209,1171,1046 cm^{-1}) functions. The negative FAB-MS of **1** showed the quasimolecular ion peak at *m/z* 357 $(M-H)^-$, while it showed the quasimolecular ion peaks at *m/z* 403 $(M-H+Na+Na)^+$ (glycerol matrix+NaCl) and *m/z* 371 $(M-H+Li+Li)^+$ (glycerol matrix+LiCl) in its positive FAB-MS. The ^{1}H- NMR spectrum[7] of **1** showed a sulfonic acid-bearing methine proton at δ 3.32 (dddd, *J*=2,6,6,7 Hz , 5-H) together with many other signals closely resembling those of 6-gingerol (**2**). Acetylation of **1** with Ac_2O-pyridine yielded the monoacetate (**1a**), $[\alpha]_D$ - 0.5 ° (MeOH), $C_{19}H_{28}O_7S$. Comparison of the ^{13}C-NMR spectra for **1** and **1a** with those of **2** let us to presume the structure of **1**. (Table I) Finally, the structure of 6-gingesulfonic acid (**1**) was determined by chemical correlation with 6-shogaol (**3**). Thus, treatment of **3** with $NaHSO_3$ in the presence of *t*-butyl perbenzoate in refluxing methanol furnished **1**, in 90 % yield.

Gingerglycolipid A (**5**) ,white powder, $[\alpha]_D$ +37.7° (MeOH), $C_{33}H_{56}O_{14}$, has hydroxyl (3569 cm^{-1}) and ester carbonyl

(1734 cm^{-1}) groups, as shown by its IR spectrum. The positive FAB-MS spectrum of **5** showed the quasimolecular ion peaks at *m/z* 699 $(M+Na)^+$ and *m/z* 683 $(M+Li)^+$. Treatment of **5** with 2% NaOMe in MeOH liberated a glyceryldigalactoside and methyl linolenate.

Table I. ^{13}C-NMR Data of **1** ,**1a** and **2**

	1	1a	2
C- 1	30.4	30.5	33.7
C- 2	44.7	44.6	47.2
C- 3	210.6	210.2	212.8
C- 4	45.9	45.6	52.1
C- 5	57.0	57.2	69.7
C- 6	32.9	33.0	39.1
C- 7	31.9	32.0	31.0
C- 8	27.8	28.0	27.1
C- 9	23.5	23.6	24.4
C-10	14.4	14.4	15.2
C- 1'	133.9	139.4	134.8
C- 2'	113.0	113.9	113.9
C- 3'	148.8	152.4	149.6
C- 4'	145.6	141.9	146.5
C- 5'	116.0	123.5	116.9
C- 6'	121.6	121.4	122.5
OMe	56.3	56.4	57.1

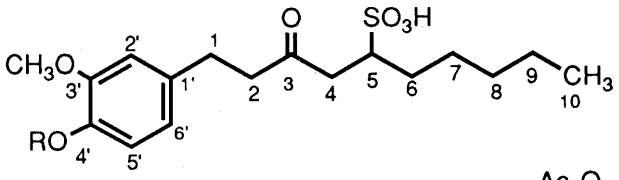
(δc at 67.5 MHz, in CD_3OD)

Table II.

Effect of 6-Gingesulfonic Acid (**1**) ,6-Gingerol (**2**) , 6-Shogaol (**3**) and Cetraxate[4] on HCl / ethanol Induced Gastric Ulcer in Rats

Treatment	Dose (mg / kg)	N	Total length (mm) (Mean ± S.E)	Inhibition (%)
Control		5	101.5 ± 19.5	-
6-Gingerol (2)	150	5	43.1 ± 9.2*	57.5
6-Shogaol (3)	150	5	30.2 ± 5.5*	70.2
6-Gingesulfonic acid (1)	150	5	7.4 ± 1.6**	92.7
6-Gingesulfonic acid (1)	300	6	0.5 ± 0.3**	99.6
Cetraxate	300	5	1.5 ± 1.1**	98.5

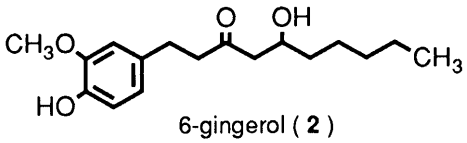
*P<0.05, **P<0.01.



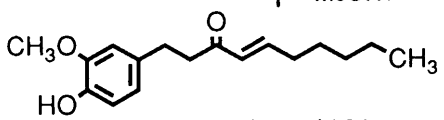
6-gingesulfonic acid (**1**) : R=H

Ac_2O → **1a** : R=Ac

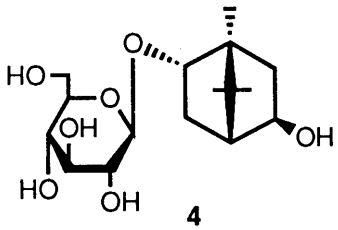
$NaHSO_3$
t-buty perbenzoate
MeOH / Δ



6-gingerol (**2**)



6-shogaol (**3**)



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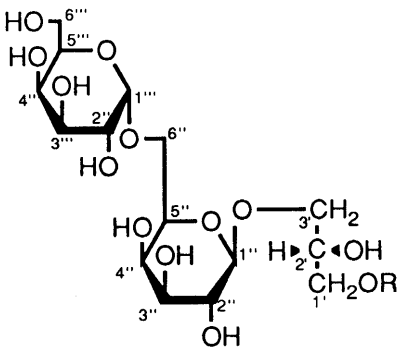
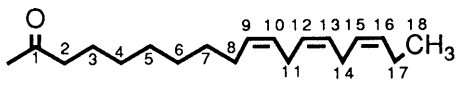


8 : R= H

gingerglycolipid A (**5**) : R =



gingerglycolipid B (**6**) : R =

gingerglycolipid C (**7**) : R =

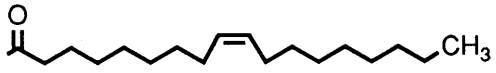


Chart 1

The glyceryldigalactoside, $[\alpha]_D$ + 80.0 ° (H_2O), was shown to be identical in all respects with 3-*O*-(α-D-galactopyranosyl-(1-6)-*O*-β–D-galactopyranosyl-*sn*- glycerol (**8**)[8]. Comparisons in detail of ^{1}H-NMR and ^{13}C-NMR data[9] for **5** with those for **8** have shown that the fatty acid residue in **5** attach to C-1' in the glycerol moiety. Consequently, the chemical structure of gingerglycolipid A (**5**) was determined as shown.

The structures of the other two gingerglycolipids have been elucidated in the same way. Gingerglycolipid B (**6**) ,white powder, $[\alpha]_D$ + 50.9 ° (MeOH), $C_{33}H_{58}O_{14}$, IR (KBr) : 3590,3569,1719 cm^{-1}, showed quasimolecular ion peaks at *m/z* 701 $(M+Na)^+$ and *m/z* 685 $(M+Li)^+$ in its positive FAB-MS. The ^{1}H-NMR and ^{13}C-NMR spectra[10] of **6** closely resembled those spectra of gingerglycolipid A (**5**) expected for signals due to the fatty acid moiety. Treatment of **6** with NaOMe as carried out for **5** furnished the same glyceryldigalactoside (**8**) and methyl linoleate. Finally, the ^{1}H-NMR and ^{13}C-NMR analysis of **6** in comparison with **5** have led to the formulation of gingerglycolipid B (**6**) as shown.

Alkaline treatment of gingerglycolipid C (**7**) ,white powder, $[\alpha]_D$ + 26.9 ° (MeOH), $C_{33}H_{60}O_{14}$, IR (KBr) : 3571,1736 cm^{-1}. FAB-MS : *m/z* 703 $(M+Na)^+$ and *m/z* 687 $(M+Li)^+$, liberated **8** and methyl oleate. These findings together with ^{1}H-NMR and ^{13}C-NMR data[11] for **7** led us to formulate gingerglycolipid C as **7**.

As given in Table II, 6-gingesulfonic acid (**1**) showed more potent anti-ulcer effect than 6-gingerol (**2**) and 6-shogaol (**3**). It is interesting to note that 6-gingesulfonic acid (**1**) exhibits weak pungency and higher water-solubility compared to **2** and **3**, and these property may be important for the use as a stomachic.

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6) The molecular composition of the compound given with the chemical formula was determined either by elemental analysis or by high resolution mass spectrometry.

7) ^{1}H-NMR (270MHz, CD_3OD, δ) of **1** : 0.78 (t, *J*=7Hz, 10-H), 0.97-1.26 (m, 7, 8, 9-H), {1.34 (m), 1.80 (td, *J*=7, 11Hz), 6-H}, 2.41, 2.94 (both dd, *J*=6, 18Hz, 4-H), 2.70 (br s, 1, 2-H), 3.32 (dddd, *J*=2, 6, 6, 7Hz, 5-H), 6.51 (dd, *J*=2, 8Hz, 6'-H), 6.59 (d, *J*=8Hz, 5'-H), 6.68 (d, *J*=2Hz, 2'-H).

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9) **5** : ^{1}H-NMR (CD_3OD, δ) : 0.97 (t, *J*=7Hz, 18-H), 2.35 (t, *J*=8Hz, 2-H), 4.14 (d, *J*=5Hz, 1'''-H), 4.24 (d, *J*=7Hz, 1"-H). ^{13}C-NMR (CD_3OD, δc) : 15.4 (C-18), 22.3, 26.7, 27.2, 27.3, 28.9, 31.0x2, 31.1, 31.5, 35.7 (methylene carbons), 63.5 (C-6'''), 67.4 (C-6"), 68.5 (C-3'), 70.4 (C-2"), 70.8x2 (C-2',4"'), 71.0 (C-4"), 71.8 (C-3"'), 72.2 (C-5"'), 72.9 (C-1'), 73.3 (C-2"), 75.3x2 (C-3",5"), 101.3 (C-1"'), 106.0 (C-1"), 129.0, 129.6, 130.0x2, 131.8, 133.5, 176.2 (C-1).

10) **6** : ^{1}H-NMR (CD_3OD, δ) : 0.89 (t, *J*=7Hz, 18-H), 2.45 (t, *J*=7Hz, 2-H), 2.86 (m, 11-H), 4.23 (d, *J*=5Hz, 1'''H), 4.34 (d, *J*=7Hz, 1"-H). ^{13}C-NMR (CD_3OD, δc) : 15.2 (C-18), 24.4, 26.8, 27.3, 29.0, 31.0x2, 31.1, 31.3, 31.5x2, 33.5, 35.7 (methylene carbons), 63.5 (C-6'''), 67.4 (C-6"), 68.5 (C-3'), 70.4 (C-2"'), 70.8x2 (C-2',4"'), 71.0 (C-4"), 71.8 (C-3"'), 72.2 (C-5"'), 72.9(C-1'), 73.3 (C-2"), 75.3x2 (C-3",5"), 101.3 (C-1"'), 106.0 (C-1"), 129.9x2, 131.7x2, 176.2 (C-1).

11) **7** : ^{1}H-NMR (CD_3OD, δ) : 0.90 (t, *J*=7Hz, 18-H), 2.36 (t, *J*=7Hz, 2-H), 4.15 (d, *J*=5Hz, 1'''-H), 4.25 (d, *J*=7Hz, 1"-H), 5.35 (td, *J*=11, 15Hz, 9, 10-H). ^{13}C-NMR (CD_3OD, δc) : 15.5 (C-18), 24.6, 27.0, 29.0x2, 30.8x2,31.0x2, 31.1, 31.3, 31.8x2, 34.0, 36.0 (methylene carbons), 63.5 (C-6'''), 67.4 (C-6"), 68.5 (C-3'), 70.4 (C-2"'), 70.8x2 (C-2',4"'), 71.0 (C-4"), 71.8 (C- 3"'), 72.2 (C-5"'), 72.9 (C-1'), 73.3 (C-2"), 75.3x2 (C-3",5"), 101.3 (C-1"'), 106.0 (C-1"), 131.5, 131.8, 176.4 (C-1).

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