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5-Lipoxygenase Inhibitors Isolated from Gardeniae Fructus

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Five 5-lipoxygenase inhibitors, chlorogenic acid (**1**), 6''-*p*-coumaroyl genipin gentiobioside (**2**), 3,4-di-*O*-caffeoylquinic acid (**3**), 3-*O*-caffeoyl-4-*O*-sinapoylquinic acid (**4**) and 3,5-di-*O*-caffeoyl-4-*O*-(3-hydroxy-3-methyl)glutaroylquinic acid (**5**), were isolated from Gardeniae Fructus. The structures of the new compounds (**2**, **4** and **5**) were elucidated on the basis of spectral data and chemical evidence. These hydroxycinnamic acid derivatives inhibit 5-lipoxygenase activity, and **3** was the most potent inhibitor. The inhibitory effects were enhanced on methylation of the carboxyl group(s) of **1**, **3**, **4** and **5**, and the ID_{50} values of the methyl esters of **3**, **4** and **5** were of the order of 10^{-8} M.

Keywords——Gardeniae Fructus; *Gardenia jasminoides*; Rubiaceae; lipoxygenase inhibitor; chlorogenic acid; 6''-*O*-*p*-coumaroyl genipin gentiobioside; 3,4-di-*O*-caffeoylquinic acid; 3-*O*-caffeoyl-4-*O*-sinapoylquinic acid; 3,5-di-*O*-caffeoyl-4-*O*-(3-hydroxy-3-methyl)glutaroylquinic acid; long-range selective proton decoupling (LSPD)

Gardeniae Fructus is a crude drug made of ripe fruits of *Gardenia jasminoides* ELLIS (Rubiaceae), and has been used in China and Japan. It has been also used as a yellow dye, and crocin and crocetin[1] were isolated as yellow pigments. Recently, geniposide,[2] genipin gentiobioside[3] and other iridoid glycosides[4] were isolated from this crude drug. Among these iridoid glycosides, geniposide, the main constituent of Gardeniae Fructus, was shown to have diuretic[5] and cholagogic activities.[6] In the Kanpo prescription (traditional Chinese medicine), however, Gardeniae Fructus has also been used as an anti-inflammatory. Moreover, it has been traditionally used to treat contusions in Japan.

The 5-lipoxygenase is the first enzyme in the conversion of arachidonic acid to leukotrienes, which are believed to take part in inflammatory reactions. Thus, we have investigated the presence of 5-lipoxygenase inhibitor in Gardeniae Fructus, and have isolated new hydroxycinnamic acid derivatives.[7,8] In this paper, we report the isolation and structural elucidation of hydroxycinnamic acid derivatives in Gardeniae Fructus, and their inhibitory effects on 5-lipoxygenase.

The *n*-butanol soluble portion of the aqueous acetone extract of Gardeniae Fructus was fractionated on a column of Diaion HP-20 using a stepwise gradient of water and methanol with increasing methanol content. The 20%, 60% and 80% methanol eluates were further chromatographed repeatedly on columns of Diaion HP-40 and octadecyl silica (ODS). Compound **1** was obtained from the 20% methanol eluate, and compounds **2**, **3**, **4** and **5** were isolated from the 60% and 80% methanol eluates.

Compound **1** was obtained as colorless crystals from water. The ^{1}H-nuclear magnetic resonance (^{1}H-NMR) spectrum indicated that **1** was chlorogenic acid (Table I). The compound was identified by comparing the spectral data of **1** and its trimethyl derivative **1b**

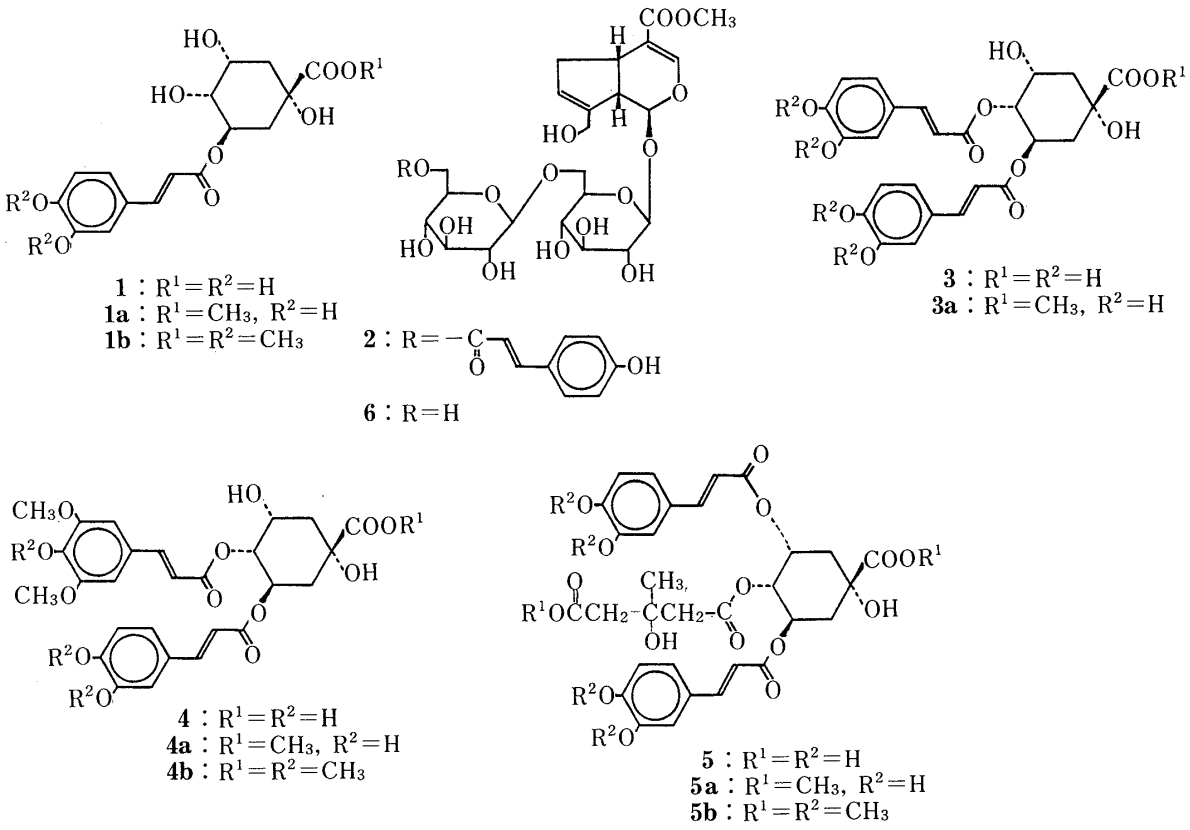

Chart 1

with those of authentic samples.

Compound **2** was obtained as an amorphous powder, which showed the $(M+H)^+$ ion peak at *m/z* 697 in its field-desorption mass spectrum (FD-MS). The ^{1}H- and ^{13}C-NMR spectra indicated that **2** is a *p*-coumaroyl ester of genipin gentiobioside (**6**). On hydrolysis with 1 N NaOH, **2** gave *p*-coumaric acid and **6** (the later was obtained after treatment of the hydrolysate with diazomethane).

The *p*-coumaroyl group in **2** was determined to be located at the 6-hydroxy group of the terminal glucose in the gentiobiose moiety, because the protons at the 6′′-position of **2** were observed at about 0.6 ppm lower field compared with those of **6** in the ^{1}H-NMR spectra (Table II). Therefore, **2** was elucidated to be 6′′-*O*-*p*-coumaroyl genipin gentiobioside, a new *p*-coumaroyl ester of an iridoid glycoside.

Compound **3** was obtained as an amorphous powder, and showed the $(M+H)^+$ ion peak at *m/z* 517 in its FD-MS. The ^{1}H-NMR spectrum of **3** indicated that **3** is 3,4-di-*O*-caffeoylquinic acid (Table I). The compound was identified by comparing its spectral and physical data with those of an authentic sample isolated from unroasted coffee beans.[9]

Compound **4** was obtained as a pale yellow amorphous powder. The FD-MS of **4** showed the M^+ ion peak at *m/z* 560, which is 44 mass units higher than that of **3**. The ^{1}H-NMR spectrum indicated that **4** is a 3,4-di-*O*-acylated quinic acid (see Table I), and showed signals assignable to a caffeoyl moiety and a sinapoyl moiety [δ 7.64 (d, J=15.9 Hz), 6.98 (s), 6.42 (d, J=15.9 Hz), and 3.89 (s)].

On methylation, **4** gave a tetramethyl derivative (**4b**), which showed the M^+ ion peak at *m/z* 616 in its electron impact-mass spectrum (EI-MS). The ^{1}H-NMR spectrum of **4b** showed the signals of five aromatic methoxy groups and one carboxymethyl group. On partial methanolysis with anhydrous methanolic HCl, **4b** gave 4-*O*-methylsinapic acid methyl ester and di-*O*-methylchlorogenic acid methyl ester (**1b**) as main products. These were identified by

TABLE I. ^{1}H-NMR Data for **1**, **3**, **4** and **5**

	1		**3**		**4**		**5**	
Quinic acid moiety								
C_2-H	2.01 dd	(10.3, 12.8)	2.18 dd	(11.0, 13.2)	2.18 dd	(10.6, 13.2)	2.23 dd	(6.2, 13.9)
	2.26 ddd	(1.8, 4.4, 12.8)	2.39 ddd	(2.5, 4.8, 13.2)	2.39 ddd	(2.5, 4.8, 13.2)	2.39 dd	(3.7, 13.9)
C_3-H	5.38 ddd	(4.4, 9.2, 10.3)	5.70 ddd	(4.8, 9.7, 11.0)	5.70 ddd	(4.8, 9.7, 10.6)	5.65 m	
C_4-H	3.76 dd	(2.9, 9.2)	5.16 d	(2.9, 9.7)	5.18 dd	(2.9, 9.7)	5.33 dd	(3.3, 7.0)
C_5-H	4.22 m		4.45 br d	(2.9)	4.45 br d	(2.9)	5.58 m	
C_6-H	2.12 m[a]		2.29 m[a]		2.29 m[a]		2.34 m[a]	
Caffeoyl moiety								
C_2-H	7.18 d	(1.8)	7.13 d	(2.2)	7.13 d	(2.0)	7.15 d	(1.8)
			7.14 d	(2.2)			7.18 d	(1.8)
C_5-H	6.87 d	(8.1)	6.98 dd	(2.2, 8.1)	7.01 d	(2.0, 8.3)	7.04 d	(1.8, 8.1)
			6.99 dd	(2.2, 8.1)			7.07 d	(1.8, 8.1)
C_6-H	7.02 d	(1.8, 8.1)	6.83 d	(8.1)	6.84 d	(8.3)	8.88 d	(8.1)[a]
			6.84 d	(8.1)				
Olefin α-H	6.29 d	(16.1)	6.22 d	(16.1)	6.24 d	(15.9)	6.24 d	(15.8)
			6.27 d	(16.1)			6.32 d	(15.8)
Olefin β-H	7.57 d	(16.1)	7.54 d	(16.1)	7.54 d	(15.9)		
			7.56 d	(16.1)				
Sinapoyl moiety								
C_2,C_6-H					6.98 s			
Olefin α-H					6.42 d	(15.9)		
Olefin β-H					7.64 d	(15.9)		
OMe					3.89 s			
HMG[c] moiety								
CH_2							2.71 m[b]	
CH_3							1.43 s	

The spectra were measured in acetone-d_6 + D_2O and the values in parentheses showed the coupling constant(s) in Hz. *a*) Two protons. *b*) Four protons. *c*) 3-Hydroxy-3-methylglutaroyl.

comparing their spectral data with those of authentic samples. These results indicated that the caffeoyl and sinapoyl groups are attached to the C_3- and C_4-hydroxy groups of quinic acid, respectively.[7]

This conclusion was further supported by the NMR spectroscopic studies on **4**. In the two dimensional shift correlation spectrum (2D-COSY) of **4**, cross peaks were observed between the olefin protons at δ 6.42 and 7.64, and δ 6.24 and 7.54. There was a cross peak between the signals at δ 7.64 and 6.98 (Fig. 1). Therefore, the signal at δ 6.42 was assigned to the olefin α-proton of the sinapoyl moiety, and the signal at δ 6.24 to that of the caffeoyl moiety. The results of the long-range selective proton decoupling (LSPD) experiments on **4** are shown in Fig. 2. In the LSPD spectrum irradiated at δ 6.42 (α-H of the sinapoyl moiety, (D)), the signal due to the ester carbonyl carbon at δ 167.7 was doublet-like. This signal was also observed as a doublet on irradiation at δ 5.18 (C_4-H proton of quinic acid, (E)). Another ester carbonyl carbon signal at δ 167.4 was changed when the protons at δ 5.70 (C_3-H of quinic acid moiety, (B)) or δ 6.24 (α-H of caffeoyl moiety, (A)) were irradiated. Therefore, **4** was determined to be 3-*O*-caffeoyl-4-*O*-sinapoylquinic acid.

Sinapic acid is a hydroxycinnamic acid widely distributed in the plant kingdom, and many reports on the sinapoyl esters of D-glucose,[10] flavonoids[11] and anthocyans[12] have been published. However, only 3-sinapolyquinic acid[13] has been reported as a sinapoyl ester of quinic acid, and thus **4** is the second such compound. Recently, 3-*O*-caffeoyl-4-*O*-feruloyl-quinic acid[14] and 3-*O*-feruloyl-4-*O*-caffeoylquinic acid[15] have been found in unroasted coffee beans as quinic acid derivatives bearing two different hydroxycinnamic acid moieties.

TABLE II. ^{1}H-NMR Data for **2** and **6**

	2 (Acetone-d_6 + D_2O)	**6** (D_2O)
Aglycone		
C_1-H	5.21 d (7.3)	5.27 d (7.0)
C_3-H	7.46 d (1.0)	7.56 br s
C_5-H	3.14 dd (7.7, 14.5)	3.23 dd (7.7, 15.4)
C_6-H	2.76 m	2.85 m
	2.21 m	2.16 m
C_7-H	5.81 br s	5.88 br s
C_8-H	2.76 t (7.7)	2.85 t (7.7)
C_{10}-H	4.19 d (14.0)	4.23 d (14.0)
	4.31 d (14.0)	4.30 d (14.0)
$COOCH_3$	3.69 s	3.74 s
Gentiobiose moiety		
$C_{1'}$-H	4.77 d (8.1)	4.84 d (8.1)
$C_{2'}$-H, $C_{3'}$-H, $C_{4'}$-H, $C_{5'}$-H	3.27—3.55	3.27—3.55
$C_{6'}$-H	4.14 dd (1.8, 11.7)	4.16 dd (1.8, 12.5)
	3.88 m	3.86 dd (6.6, 12.5)
$C_{1''}$-H	4.48 d (7.7)	4.47 d (8.1)
$C_{2''}$-H, $C_{3''}$-H, $C_{4''}$-H, $C_{5''}$-H	3.27—3.55	3.27—3.55
$C_{6''}$-H	4.53 dd (1.8, 12.1)	3.70 dd (1.8, 11.7)
	4.31 dd (5.9, 12.1)	3.91 dd (7.0, 11.7)
p-Coumaroyl moiety		
Olefin α-H	6.40 d (16.1)	
Olefin β-H	7.66 d (16.1)	
$C_{2,6}$-H	6.90 d (8.8)	
$C_{3,5}$-H	7.56 d (8.8)	

The values in parentheses are coupling constant(s) in Hz. The chemical shifts of **2** were assigned on the basis of decoupling experiments.

Compound **4** is the first example of a sinapoyl and caffeoyl ester of quinic acid.

Compound **5** was obtained as an amorphous powder, and showed the $(M+H)^+$ ion peak at *m/z* 661 in its FD-MS. In the ^{1}H-NMR spectrum of **5**, the signals of two methylene protons and protons of a methyl group on a quaternary carbon bearing an oxygen function were observed in addition to those assignable to two caffeoyl and one 3,4,5-tri-*O*-acylated quinic acid moieties. The ^{13}C-NMR spectrum of **5** exhibited the signals due to one carboxyl carbon (δ 173.7), one ester carbonyl carbon (δ 171.4), two methylene carbons (δ 38.5 and 36.7), one methyl carbon (δ 28.1) and one quaternary carbon (δ 74.5) bearing an oxygen function in addition to those due to di-*O*-caffeoylquinic acid.

On methylation, **5** gave a hexamethyl derivative (**5b**), which showed the M^+ ion peak at *m/z* 744 in its EI-MS. The ^{1}H- and ^{13}C-NMR spectra of **5b** showed the signals due to four aromatic methoxy groups and two carboxymethyl groups.

On partial methanolysis in anhydrous methanolic HCl, **5b** gave di-*O*-methylcaffeic acid methyl ester, di-*O*-methylchlorogenic acid methyl ester (**1b**), and 3-hydroxy-3-methylglutaric acid (HMG) dimethyl ester. These were identified by comparing their spectral data with those of authentic samples. Along with these products, a 3,5-di-*O*-acylated quinic acid derivative

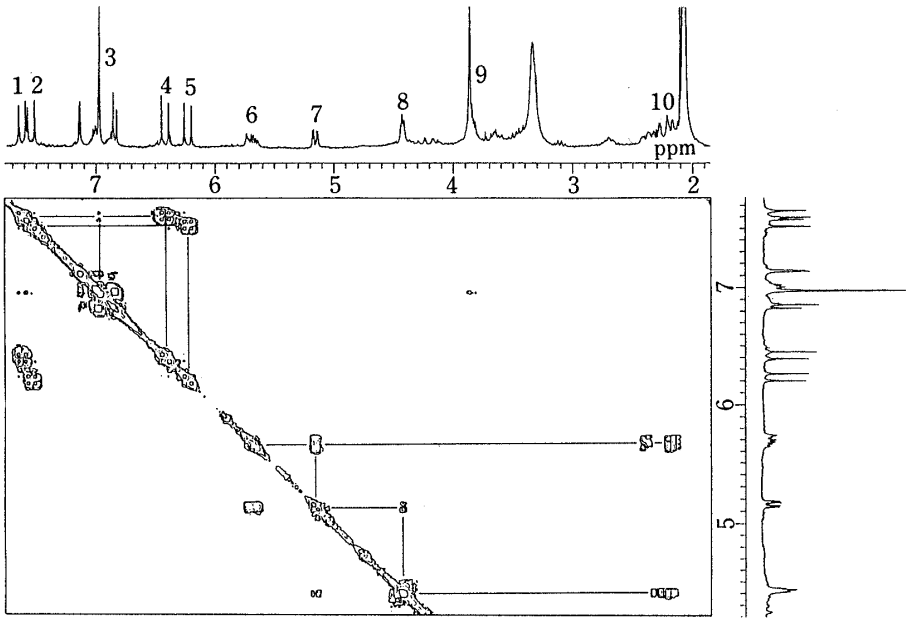

Fig. 1. The COSY Spectrum of **4**

1, olefin β-proton of caffeoyl moiety (CA); 2, olefin β-proton of sinapoyl moiety (SI); 3, C_2, C_6-H of SI; 4, olefin α-proton of SI; 5, olefin α-proton of CA; 6, C_3-H of quinic acid (QA); 7, C_4-H of QA; 8, C_5-H of QA; 9, OCH_3 of SI; 10, methylene proton of QA.

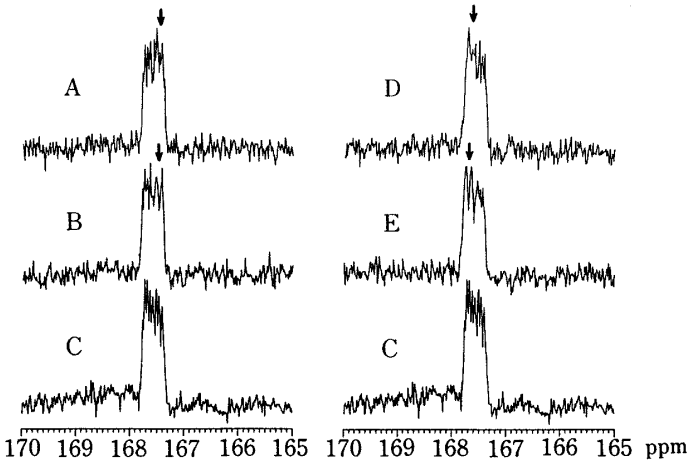

Fig. 2. The LSPD Spectra of **4**

(A) Irradiated at δ 6.24 (olefin α-proton of CA). (B) Irradiated at δ 5.70 (C_3-H of QA). (C) Nondecoupled spectra. (D) Irradiated at δ 6.42 (olefin α-proton of SI). (E) Irradiated at δ 5.18 (C_4-H of QA).

which had a caffeoyl moiety and an HMG moiety was obtained. These results implied that **5** is 3,4-di-*O*-caffeoyl-5-*O*-(3-hydroxy-3-methyl)glutaroylquinic acid.[8)]

However, the results of LSPD experiments on **5** were not compatible with those of the partial methanolysis. As shown in Fig. 3, irradiation at δ 2.71 (methylene protons of HMG) affected the signals of carbonyl carbons at δ 173.7 and 171.4 (D). This indicated that the signal at δ 171.4 should be assigned to the ester carbonyl carbon of HMG, and this signal was affected when the signal at δ 5.33 (C_4-H proton of the quinic acid moiety) was irradiated (B). The conflict is presumably the result of acyl migration under the conditions of methanolysis. Therefore, the structure of **5** was concluded to be 3,5-di-*O*-caffeoyl-4-*O*-(3-hydroxy-3-methyl)glutaroylquinic acid.

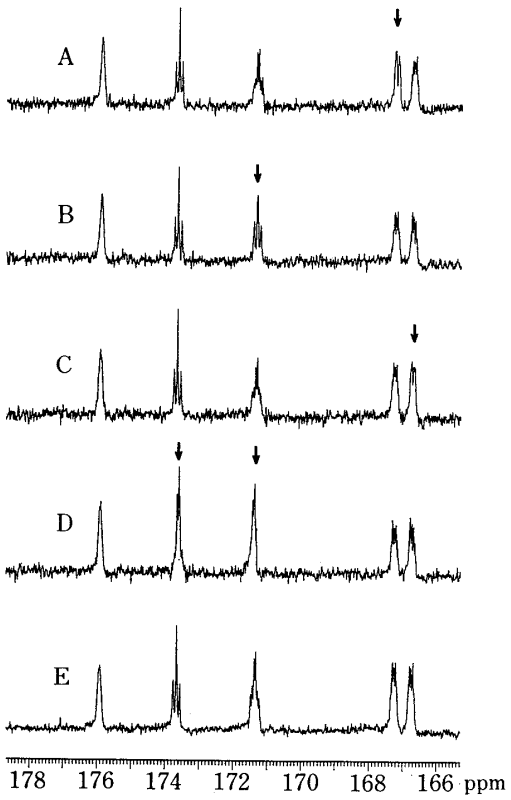

Fig. 3. The LSPD Spectra of **5**

(A) Irradiated at δ5.67 (C_3-H of QA). (B) Irradiated at δ5.33 (C_4-H of QA). (C) Irradiated at δ5.18 (C_5-H of QA). (D) Irradiated at δ2.71 (methylene of 3-hydroxy-3-methylglutaroyl moiety). (E) Nondecoupled spectrum.

TABLE III. Inhibitory Effects of **1**, **2**, **3**, **4**, **5** and Their Methyl Esters on 5-Lipoxygenase

Compound	Inhibition (%)					Approximate ID_{50} (M)
	10^{-4} M	10^{-5} M	10^{-6} M	10^{-7} M	10^{-8} M	
1	62	14	0	—[a]	—	5.6×10^{-5}
1a	89	76	47	0	—	1.5×10^{-6}
2	24	11	10	—	—	—[b]
3	90	88	69	35	14	2.8×10^{-7}
3a	—	91	83	65	21	6.2×10^{-8}
4	79	45	0	—	—	1.5×10^{-5}
4a	—	92	85	64	23	6.2×10^{-6}
5	92	63	23	—	—	5.1×10^{-6}
5a	—	92	89	80	48	1.0×10^{-8}

a) Not tested. *b*) $>10^{-4}$ M.

There have been only three reports on naturally occurring HMG esters,[16] and **5** is the first example of an HMG ester of quinic acid.

As described above, Gardeniae Fructus has been shown to contain many hydroxycinnamic acid derivatives, especially esters of quinic acid. We have already reported that caffeic acid inhibits 5-lipoxygenase activity,[17] so the inhibitory effects of these compounds on 5-lipoxygenase were tested. As shown in Table III, **1**, **2**, **3**, **4** and **5** inhibited the enzyme 14, 11, 88, 45 and 63%, respectively, at the concentration of 10^{-5} M. Compound **3** was shown to be the most potent inhibitor of 5-lipoxygenase among the constituents of Gardeniae Fructus tested in this study, and the 50% inhibitory concentration (ID_{50}) was about 2.8×10^{-7} M.

From the comparison of the inhibitory effects of **3** and **4**, a compound having caffeoyl group(s) may be a more potent inhibitor than one having sinapoyl group(s).

Table III also showed the inhibitory effects of methyl esters (**1a**, **3a**, **4a** and **5a**) of four caffeoylquinic acid derivatives isolated from Gardeniae Fructus. The inhibitory effects of the caffeoylquinic acid derivatives were enhanced by the methylation of carboxyl group(s), and **1a**, **3a**, **4a** and **5a** caused 47, 83, 85 and 89% inhibition, respectively, at 10^{-6} M. The approximate ID_{50} values of **3a**, **4a** and **5a** were 6.2×10^{-8}, 6.2×10^{-8} and 1.0×10^{-8} M, respectively. These methyl esters rank among the most potent 5-lipoxygenase inhibitors ever reported.[18]

Experimental

Melting points were determined on a Kofler hot stage apparatus and are uncorrected. Optical rotations were measured with a Jasco DIP-4 digital polarimeter, and ultraviolet (UV) and infrared (IR) spectra were obtained with Shimazdu UV-220 and Jasco A-102 spectrometers, respectively. ^{1}H-NMR spectra were obtained with JEOL GX-400 (400 MHz), GX-270 (270 MHz) and FX-100 (100 MHz) spectrometers, and ^{13}C-NMR spectra with an FX-90Q (22.5 MHz) instrument using tetramethylsilane as an internal standard. EI-MS and FD-MS were measured with a JEOL LMS-D-300 and a JMS-01SG-2, respectively. Thin layer chromatography (TLC) was performed on Merck precoated plate (Kieselgel 60 F_{254}) with the following solvent systems: solvent 1, $CHCl_3$–MeOH (50 : 1, v/v); solvent 2, $CHCl_3$–MeOH (3 : 1); solvent 3, $CHCl_3$–EtOAc–HCOOH (5 : 4 : 1); solvent 4, MeOH–$CHCl_3$–EtOAc–HCOOH (25 : 50 : 40 : 10). Column chromatography was carried out on Wakogel C-200 (200 mesh), an RQ-2 column (Fujigel Co., Ltd.) and an ODS-5251-SH column (Senshu Co., Ltd.). High performance liquid chromatography (HPLC) was carried out on an apparatus consisting of an APS-7L pump (Gasukuro Kogyo Co., Ltd.), a JASCO UVIDEC-100-II UV detector (operated at 330 nm) and a Rheodyne 7125 sample injector using a TSK-ODS 80 TM column (4.6 i.d. × 250 mm, Toyo Soda Co., Ltd.) and the following solvent systems (flow rate, 1.2 ml/min) : solvent A, CH_3CN–H_2O–HCOOH (20 : 80 : 1); solvent B, CH_3CN–H_2O–HCOOH (30 : 70 : 1).

Isolation——Gardeniae Fructus (obtained from Dr. Hong-Yen Hsu, Brion Research Institute of Taiwan, 3.73 kg) was extracted with 50% aqueous acetone three times (5.5 l × 3). The combined extracts were concentrated to about 1 l, and extracted with hexane (500 ml × 3), ethyl acetate (500 ml × 3) and *n*-butanol (500 ml × 3). After evaporation, the *n*-butanol extracts (182 g) were dissolved in 200 ml of water and applied to a column of Diaion HP-20 (12 i.d. × 40 cm). The column was washed with water, and then eluted with a water–methanol solvent system with increasing methanol content. The 20% methanol eluates (13.3 g) was fractionated on ODS columns (RQ-2 and ODS-5251-SH) using solvent systems of acetonitrile–water–acetic acid or acetonitrile–water–formic acid, and compound **1** (245 mg) was obtained. The 60% and 80% methanol eluates (24.3 and 16.8 g, respectively) were each fractionated by the same method; compounds **2** (192 mg), **3** (52 mg), **4** (120 mg) and **5** (177 mg) were obtained from the 60% methanol eluate, and compounds **2** (420 mg), **4** (250 mg) and **5** (980 mg) from the 80% methanol eluate.

Chlorogenic Acid (1)——Colorless needles from water. mp 192.5—195 °C. $[\alpha]_D^{20}$ −38° (c=0.2, MeOH). TLC *Rf* (solvent 3): 0.08, *Rf* (solvent 4): 0.14. HPLC t_R (solvent A) min: 3.6. ^{1}H-NMR (acetone-d_6 + D_2O, 270 MHz): see Table I. ^{13}C-NMR (acetone-d_6 + D_2O, 22.5 MHz) δ: 176.4, 168.2, 149.3, 146.6 (2C), 127.5, 122.9, 116.6, 115.6, 115.3, 76.2, 73.5, 71.7, 71.4, 39.1, 38.0.

Methyl Ester of 1 (1a)——Compound **1** (20 mg) was treated with 5% anhydrous methanolic HCl for 1 h at room temperature and 18 mg of **1a** was obtained. $[\alpha]_D^{20}$ −25° (c=0.71, MeOH). FD-MS m/z: 368 (M^+, base peak). TLC *Rf* (solvent 2): 0.20. ^{1}H-NMR (acetone-d_6, 100 MHz) δ: 7.54 (d, J=15.6 Hz, 1H), 7.16 (d, J=2.0 Hz, 1H), 7.05 (dd, J=2.0, 8.3 Hz, 1H), 6.86 (d, J=8.3 Hz, 1H), 6.24 (d, J=15.6 Hz, 1H), 5.34 (dt, J=4.4, 8.8 Hz, 1H), 4.17 (m, 1H), 3.73 (dd, J=2.9, 9.3 Hz, 1H), 3.68 (s, 3H), 2.10—2.34 (m, 4H).

Dimethyl Ether of 1a (1b)——Compound **1a** (25 mg) was treated with Na_2CO_3 and $(CH_3)_2SO_4$ in dry acetone, and 22 mg of **1b** was obtained. $[\alpha]_D^{20}$ −40° (c=0.58, $CHCl_3$). MS m/z: 376 (M^+), 191 (dimethyl caffeoyl). TLC *Rf* (solvent 2): 0.28. ^{1}H-NMR ($CDCl_3$, 100 MHz) δ: 7.60 (d, J=15.9 Hz, 1H), 7.04 (dd, J=2.0, 8.3 Hz, 1H), 6.99 (d, J=2.0 Hz, 1H), 6.80 (d, J=8.3 Hz, 1H), 6.28 (d, J=15.9 Hz, 1H), 5.32 (ddd, J=4.8, 9.8, 10.0 Hz, 1H), 4.16 (br dd, J=3.4, 7.3 Hz, 1H), 3.87 (dd, J=3.8, 9.8 Hz, 1H), 2.06—2.50 (m, 4H).

6″-*p*-Coumaroyl Genipin Gentiobioside (2)——An amorphous powder. $[\alpha]_D^{20}$ +3.9° (c=1.0, MeOH). FD-MS m/z: 734 $(M+K)^+$, 718 $(M+Na)^+$, 697 $(M+H)^+$. IR ν_{max}^{Nujol} cm^{-1}: 3300—3350 (OH), 1700, 1680 (C=O). UV λ_{max}^{MeOH} nm (ε): 230 (20000), 300 sh, 313 (21100). TLC *Rf* (solvent 4): 0.32. HPLC t_R (solvent A) min: 6.8. ^{1}H-NMR (acetone-d_6 + D_2O): see Table II. ^{13}C-NMR (acetone-d_6 + D_2O, 22.5 MHz) δ: 169.5, 168.9, 153.1, 148.9, 146.6, 143.6, 131.2, 128.9, 126.7, 116.8, 114.8, 112.2, 104.1, 100.0, 98.0, 76.9 (2C), 76.3, 74.6, 74.2, 73.9, 70.9, 70.4, 69.5, 60.7, 64.5, 52.1, 46.5, 39.2, 35.6.

Hydrolysis of 2——A solution of **2** (55 mg) in 1 N NaOH (5 ml) and EtOH (5 ml) was kept for 20 h at 35 °C. The solution was then neutralized with 1 N HCl, and concentrated under reduced pressure to remove EtOH. The aqueous

solution was applied to a column of Diaion HP-20 (1.5 i.d. × 6 cm). The column was washed with 100 ml of water, 50 ml of 50% MeOH and 50 ml of MeOH successively. Genipin gentiobioside was obtained from the 50% MeOH eluate after treatment with CH_2N_2, and *p*-coumaric acid was obtained from the MeOH eluate. These products were identified by comparing their ^{1}H-NMR spectra, *Rf* values in TLC and t_R in HPLC with those of authentic samples.

3,4-Di-*O*-caffeoylquinic Acid (3)——An amorphous powder. $[\alpha]_D^{20}$ −213° (*c*=0.69, MeOH). FD-MS *m/z*: 539 $(M+Na)^+$, 517 $(M+H)^+$, 498, 180, 163. TLC *Rf* (solvent 4): 0.23. HPLC t_R (solvent B) min: 5.6. ^{1}H-NMR (acetone-d_6+D_2O, 270 MHz): see Table I. ^{13}C-NMR (acetone-d_6+D_2O, 22.5 MHz) δ: 176.0, 167.7, 167.6, 149.3 (2C), 147.0 (2C), 146.6 (2C), 127. 4 (2C), 123.0 (2C), 116.6 (2C), 115.2 (2C), 76.2, 75.9, 69.5, 68.6, 39.8, 38.3. *Anal.* Calcd for $C_{25}H_{24}O_{12}\cdot 1/2H_2O$: C, 57.14; H, 4.80. Found: C, 57.27; H, 4.62.

Methyl Ester of 3 (3a)——Compound **3** (25 mg) was treated as described above and 21 mg of the methyl ester (**3b**) was obtained. $[\alpha]_D^{20}$ −239° (*c*=0.91, MeOH). FD-MS *m/z*: 530 (M^+), 368 (base peak), 180, 163. TLC *Rf* (solvent 3): 0.31. ^{1}H-NMR (acetone-d_6, 100 MHz) δ: 7.58 (d, *J*=16.1 Hz, 1H), 7.54 (d, *J*=16.1 Hz, 1H), 7.12 (d, *J*=2.0 Hz, 2H), 7.02 (dd, *J*=2.0, 8.3 Hz, 2H), 6.83 (d, *J*=2.0 Hz, 2H), 6.26 (d, *J*=16.1 Hz, 1H), 6.20 (d, *J*=16.1 Hz, 1H), 5.65 (dt, *J*=4.9, 9.3 Hz, 1H), 5.10 (dd, *J*=2.9, 9.3 Hz, 1H), 4.41 (br dd, *J*=2.9, 5.8 Hz, 1H), 3.72 (s, 3H), 2.14—2.31 (m, 4H).

3-*O*-Caffeoyl-4-*O*-sinapoylquinic Acid (4)——A pale yellow amorphous powder. $[\alpha]_D^{20}$ −252° (*c*=0.54, MeOH). IR ν_{max}^{Nujol} cm^{-1}: 3380 (OH), 1690 (br, COO). UV λ_{max}^{MeOH} nm (ε): 328 (40300), 239 (28800), 220 (31800). FD-MS *m/z*: 560 (M^+), 542, 224, 180. TLC *Rf* (solvent 4): 0.30. HPLC t_R (solvent B) min: 7.5. ^{1}H-NMR (acetone-d_6+D_2O, 270 MHz): see Table I. ^{13}C-NMR (acetone-d_6+D_2O) δ: 175.8, 167.7, 167.4, 149.3 (2C), 147.3, 147.0, 146.7, 127.6, 126.4, 123.1, 116.2, 116.1, 115.5 (2C), 107.4 (2C), 78.1, 76.1, 69.7, 68.8, 57.2 (2C), 40.3, 38.5. *Anal.* Calcd for $C_{27}H_{28}O_{13}\cdot 1/2H_2O$: C, 56.94; H, 5.10. Found: C, 56.54; H, 5.36.

Methyl Ester of 4 (4a)——Compound **4** (23 mg) was treated as described above and 20 mg of the methyl ester (**4b**) was obtained. $[\alpha]_D^{20}$ −188° (*c*=0.94, MeOH). FD-MS *m/z*: 574 (M^+, base peak). TLC *Rf* (solvent 3): 0.37. ^{1}H-NMR (acetone-d_6, 100 MHz) δ: 7.62 (d, *J*=15.6 Hz, 1H), 7.54 (d, *J*=15.6 Hz, 1H), 7.05 (d, *J*=2.0 Hz, 1H), 7.01 (dd, *J*=2.0, 8.3 Hz, 1H), 6.99 (s, 2H), 6.84 (d, *J*=8.3 Hz, 2H), 6.41 (d, *J*=15.6 Hz, 1H), 6.21 (d, *J*=15.6 Hz, 1H), 5.64 (dt, *J*=4.9, 9.3 Hz, 1H), 5.13 (dd, *J*=3.4, 9.3 Hz, 1H), 4.41 (m, 1H), 3.87 (s, 6H), 3.72 (s, 3H), 2.16—2.34 (m, 4H).

Trimethyl Ether of 4a (4b)——Compound **4a** (35 mg) was treated as described above and 31 mg of **4b** was obtained. IR ν_{max}^{Nujol} cm^{-1}: 3500 (OH), 1725 sh, 1700 (COO). EI-MS *m/z*: 616 (M^+), 221 (methyl sinapoyl), 191 (dimethyl caffeoyl). ^{1}H-NMR ($CDCl_3$, 270 MHz) δ: 7.63 (d, *J*=15.8 Hz, 1H), 7.59 (d, *J*=15.8 Hz, 1H), 7.03 (dd, *J*=1.8, 8.4 Hz, 1H), 7.00 (d, *J*=1.8 Hz, 1H), 6.84 (d, *J*=8.4 Hz, 1H), 6.72 (s, 6H), 6.41 (d, *J*=15.8 Hz, 1H), 6.24 (d, *J*=15.8 Hz, 1H), 5.76 (ddd, *J*=4.8, 10.3, 11.3 Hz, 1H), 5.21 (dd, *J*=3.1, 10.3 Hz, 1H), 4.41 (br d, *J*=3.1 Hz, 1H), 3.895 (s, 3H), 3.886 (s, 3H), 3.86 (s, 3H), 3.85 (s, 6H), 3.84 (s, 3H), 2.47 (dd, *J*=4.8, 13.0 Hz, 1H), 2.24 (m, 2H), 2.08 (dd, *J*=11.3, 13.0 Hz, 1H). ^{13}C-NMR ($CDCl_3$, 22.5 MHz) δ: 174.5, 166.5, 166.4, 153.5 (2C), 151.5, 149.4, 145.8, 145.6, 130.4, 129.8, 127.3, 122.9, 116.9, 115.3, 111.2, 109.9, 105.6 (2C), 77.3, 75.7, 69.1, 61.0, 56.2 (2C), 56.0 (2C), 53.5, 39.5, 37.4.

Partial Methanolysis of 4b——A solution of 26 mg of **4b** in 5% methanolic HCl (3 ml) was refluxed for 3 h. The reaction mixture was diluted with water and extracted with $CHCl_3$ (5 ml) three times. The combined extracts were washed with water, dried over Na_2SO_4 and concentrated under reduced pressure. Preparative TLC (solvent 1) yielded 4-*O*-methyl-sinapic acid methyl ester [1.8 mg; TLC *Rf* (solvent 1): 0.68; EI-MS *m/z*: 252 (M^+, base peak), 237; ^{1}H-NMR ($CDCl_3$, 100 MHz) δ: 7.62 (d, *J*=16.1 Hz, 1H), 6.76 (s, 2H), 6.35 (d, *J*=16.1 Hz), 3.89 (s, 9H), 3.81 (s, 3H)], 3,4-di-*O*-methylcaffeic acid methyl ester (1.9 mg) and dimethylether of chlorogenic acid methyl ester [4.0 mg; $[\alpha]_D^{20}$ −38° (*c*=0.26, $CHCl_3$); other spectral data were identical with those of **1b**] along with unreacted **4b** (14 mg).

3,5-Di-*O*-caffeoyl-4-*O*-(3-hydroxy-3-methyl)glutaroylquinic Acid (5)——An amorphous powder. $[\alpha]_D^{20}$ −170° (*c*=0.95, MeOH). IR ν_{max}^{Nujol} cm^{-1}: 3300—3400 (OH), 1700—1720 (COO). UV λ_{max}^{MeOH} nm (ε): 329 (30800), 304 sh, 242 (17600), 216 (26600). FD-MS *m/z*: 683 $(M+Na)^+$, 661 $(M+H)^+$. TLC *Rf* (solvent 4): 0.25. HPLC t_R (solvent B) min: 5.9. ^{1}H-NMR (acetone-d_6+D_2O, 400 MHz): see Table I. ^{13}C-NMR (acetone-d_6+D_2O, 22.5 MHz) δ: 175.9, 173.7, 173.7, 171.4, 167.3, 166.8, 149.1, 149.0, 147.0, 146.9, 146.7, 146.5, 128.1, 128.0, 123.2, 123.1, 116.8 (2C), 116.1, 115.8 (2C), 115.6, 74.5, 72.2, 70.5, 69.5, 68.9, 46.6, 45.5, 38.5, 36.7, 28.1. *Anal.* Calcd for $C_{31}H_{32}O_{16}\cdot 1/2H_2O$: C, 55.60; H, 4.97. Found: C, 55.49; H, 5.14.

Dimethyl Ester of 5 (5a)——Compound **5** (23 mg) was treated as described above and 20 mg of **5a** was obtained. $[\alpha]_D^{20}$ −158° (*c*=0.88, MeOH). FD-MS *m/z*: 688 (M^+, base peak). TLC *Rf* (solvent 3): 0.37. ^{1}H-NMR (acetone-d_6, 100 MHz) δ: 7.61 (d, *J*=16.1 Hz, 1H), 7.55 (d, *J*=15.6 Hz, 1H), 7.17 (d, *J*=2.0 Hz, 2H), 7.07 (dd, *J*=2.0, 8.3 Hz, 2H), 6.87 (d, *J*=8.3 Hz, 2H), 6.29 (d, *J*=15.6 Hz, 1H), 6.23 (d, *J*=16.1 Hz, 1H), 5.55 (m, 2H), 5.29 (dd, *J*=2.9, 7.3 Hz, 1H), 3.70 (s, 3H), 3.57 (s, 3H), 2.69 (d, *J*=4.9 Hz, 4H), 2.22—2.42 (m, 4H), 1.34 (s, 3H).

Tetramethyl Ether of 5a (5b)——Compound **5a** (40 mg) was treated as described above and 35 mg of **5b** was obtained. IR ν_{max}^{Nujol} cm^{-1}: 3500 (OH), 1725 sh, 1700 (COO). EI-MS *m/z*: 744 (M^+), 191 (dimethyl caffeoyl). ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 7.63 (d, *J*=15.8, 1 Hz), 7.59 (d, *J*=15.8 Hz, 1H), 7.03 (dd, *J*=1.8, 8.4 Hz, 1H), 7.00 (d, *J*=1.8 Hz, 1H), 6.84 (d, *J*=8.4 Hz, 1H), 6.72 (s, 6H), 6.41 (d, *J*=15.8 Hz, 1H), 6.24 (d, *J*=15.8 Hz, 1H), 5.76 (ddd, *J*=4.8, 10.3, 11.3 Hz, 1H), 5.21 (dd, *J*=3.1, 10.3 Hz, 1H), 4.41 (br d, *J*=3.1 Hz, 1H), 3.895 (s, 3H), 3.886 (s, 3H), 3.86 (s, 3H), 3.85 (s, 6H), 3.84 (s, 3H), 2.47 (dd, *J*=4.8, 13.0 Hz, 1H), 2.24 (m, 2H), 2.08 (dd, *J*=11.3, 13.0 Hz, 1H). ^{13}C-NMR ($CDCl_3$, 22.5 MHz) δ: 174.5, 166.5, 166.4, 153. 5 (2C), 151.5, 149.4, 145.8, 145.6, 130.4, 129.8, 127.3, 122.9,

116.9, 115.3, 111.2, 109.9, 105.6 (2C), 77.3, 75.7, 69.1, 61.0, 56.2 (2C), 56.0 (2C), 53.5, 39.5, 37.4.

Partial Methanolysis of 5b——Compound **5b** (30 mg) was treated with 5% methanolic HCl (3 ml) as described above. Two major products were obtained along with unreacted **5b** (11 mg), 3.4-di-*O*-methylcaffeic acid methyl ester (2.5 mg) and HMG dimethyl ester [2.0 mg; ^{1}H-NMR ($CDCl_3$, 100 MHz) δ: 3.71 (s, 6H), 2.67 (m, 4H), 1.36 (s, 3H); EI-MS *m/z*: 43 (base peak), 85, 117, 143, 175]. One was identified as di-*O*-methylchlorogenic acid methyl ester (**1b**, 6.5 mg) by TLC and ^{1}H-NMR, and the other was characterized as a 3,5-di-*O*-acylquinic acid bearing caffeoyl and HMG moieties by ^{1}H-NMR [($CDCl_3$, 400 MHz) δ: 7.65 (d, J=15.9 Hz, 1H), 7.11 (dd, J=1.7, 8.3 Hz, 1H), 7.05 (d, J=1.7 Hz, 1H), 6.87 (d, J=8.3 Hz, 1H), 6.32 (d, J=15.9 Hz, 1H), 5.52 (m, 1H), 5.47 (m, 1H), 3.80 (m, 1H), 3.921 (s, 3H), 3.917 (s, 3H), 3.79 (s, 3H), 2.66—2.78 (m, 4H), 2.31 (m, 1H), 2.23 (m, 2H), 2.03 (dd, J=11.0, 12.2 Hz, 1H), 1.42 (s, 3H)].

Assay of 5-Lipoxygenase Activity——The assays were based on the method of Koshihara *et al.*[19] using the enzyme obtained from cloned mastocytoma P-815, 2-E-6 cells which had been treated with 1 mM *n*-butryate for 40 h. The enzyme was incubated with 0.2 μCi of ^{14}C-arachidonic acid, 1.0 mM $CaCl_2$ and 2×10^{-5} M indomethacin at 37 °C for 7 min. The synthesized HETEs were extracted with 8 volumes of ethyl acetate and subjected to TLC. Labeled products thus separated were scraped off the plate for the measurement of radioactivity. The activity of 5-lipoxygenase was expressed as the sum of radioactivity due to 5-HETE and 5,12-diHETE. Inhibitors were dissolved in ethyl alcohol of reagent grade.

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