Fatty Acid Synthase Inhibitors from *Geum japonicum* THUNB. var. *chinense*

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Bioassay-guided fractionation of the MeOH extract of *Geum japonicum* THUNB. var. *chinense* using the fatty acid synthase inhibition assay led to the isolation of a new dimeric ellagitannin, gemin G (**1**), together with six known compounds, gemin A (**2**), casuarinin (**3**), pedunculagin (**4**), potentillin (**5**) , tellimagrandin II (**6**), and ellagic acid (**7**). Their structures were determined on the basis of spectroscopic analyses. Compounds **1**–**7** displayed strong inhibitory activities on fatty acid synthase with IC_{50} values in the range of 0.21–41.4 μM. Compounds **1**–**4** exhibited significant antioxidant activities higher than vitamin C in the ORAC assay. Compounds **1** and **2** also showed weak cytotoxic effects on BGC-823 cell.

Introduction. – Animal fatty acid synthase (E.C.2.3.1.85; FAS) is active as an homodimer, composed of two identical, 260–270-kDa subunits juxtaposed head-to-tail, each containing an acyl carrier protein and seven enzymatic active sites, including acetyl transacylase, malonyl transacylase, β-ketoacyl synthase, β-ketoacyl reductase, β-hydroxyacyl dehydratase, enoyl reductase, and thioesterase [1]. FAS catalyses the *de novo* synthesis of fatty acids from acetyl-CoA and malonyl-CoA in the presence of NADPH through the reaction which elongates the Ac group by C_2 units derived from malonyl-CoA in a stepwise manner [2][3]. FAS is an important lipogenic enzyme involved in energy metabolism in *vivo* and is related to various human diseases such as cancer and obesity [4]. Human cancer cells express high levels of FAS [5–10]. It has been reported that FAS inhibitors are selectively cytotoxic to many kinds of human cancer cells [11–14]. Inhibitors of FAS such as cerulenin, synthetic C75, and epigallocatechin gallate (EGCG) are known for their effects on some human cancers [15][16].

The whole plant of *Geum japonicum* THUNB. var. *chinense* F. Bolle (Rosaceae) has been used as diuretic and astringents in traditional Chinese medicine [17]. It is also used for the treatment of dizziness and headache in some regions of China [18]. In a previous study of this plant, we isolated two known ellagitannins, casuarinin, and pedunculagin [19]. The plants of the genus *Genum* are known to be rich in tannins. Several hydrolyzable tannins and triterpenoid acids have been isolated from *G.*

japonicum THUNB., a plant closely related to *G. japonicum* THUNB. var. *chinense* [20–22]. In the course of a continuing search for potential plant anti-FAS agents, the MeOH extract of the whole plant of *G. japonicum* THUNB. var. *chinense* was found to show significant inhibitory activity against FAS. In this article, we describe the isolation and structure elucidation of a new dimeric ellagitannin, gemin G (**1**), along with six known ellagitannins, gemin A (**2**), casuarinin (**3**), pedunculagin (**4**), potentillin (**5**), tellimagrandin II (**6**), and ellagic acid (**7**; *Fig. 1*), and their inhibitory activity against FAS, antioxidative activity, and cytotoxicity.

Results and Discussion. – The whole plant of *G. japonicum* THUNB. var. *chinense* was ground and macerated with MeOH at room temperature. The MeOH extract was suspended in H_2O and partitioned with AcOEt and H_2O-saturated BuOH, successively. The BuOH-soluble portion showing strong anti-FAS activity (IC_{50} 2.5 μg/ml) was subjected to *D-101* resin fractionation and further purified by *HW-40* gel to yield gemin G (**1**), gemin A (**2**), casuarinin (**3**), pedunculagin (**4**), potentillin (**5**), tellimagrandin II (**6**), and ellagic acid (**7**). The structures of compounds isolated were identified by NMR and MS analysis, and comparison with literature data. Compounds **1**–**6** were shown to be ellagitannins by the color reaction with the $NaNO_2$-AcOH reagent on cellulose TLC plates [20].

Gemin G (**1**) was isolated as amorphous powder ($[\alpha]_D + 71$). The HR-TOF-MS (*m*/*z* 1569.1626 ($[M-H]^-$)) and NMR analyses revealed the molecular formula as $C_{68}H_{50}O_{44}$. The presence of phenolic group(s) in the structure was indicated by its characteristic color reaction with $FeCl_3$ (violet). UV Absorption λ_{max} (MeOH) at 220 (log ε 4.82) and 273 nm (log ε 4.73) was indicative of a bibenzyl. The IR absorptions indicated the presence of OH (3425 cm^{-1}) and C=O groups (1716 cm^{-1}), and aromatic rings (1610, 1510, and 1445 cm^{-1}). The ^{1}H- and ^{13}C-NMR spectra of **1** (*Tables 1* and *2*) showed close similarity to those of the known ellagitannin gemin A that was also obtained in our study, indicating that compound **1** was dimeric ellagitannin. The dimeric nature of compound **1** was further substantiated by the pseudomolecular peak at *m*/*z* 1569 ($[M-H]^-$) and the corresponding fragment ion peak at *m*/*z* 785 in the negative-ion ESI-MS. The ^{1}H-NMR spectrum showed seven *singlet*s at δ 6.64, 6.50, 6.70, 6.83, 7.01 (2 H), 7.04 (2 H), and 7.33, two *meta*-coupled *doublet*s at δ 6.85 (J = 2.0), 7.30 (J = 2.0) in the aromatic region, in agreement with the presence of a dehydrodigalloyl, two hexahydroxydiphenoyl, and two galloyl groups in the molecule. Acid hydrolysis, followed by GLC analysis of the aldononitrile peracetate derivative, confirmed the presence of glucose units in the structure. The two anomeric H-atom signals at δ 6.18 (J = 8.0) and 6.31 (J = 4.0) indicated the β- and α-glucosidic linkages of the two glucose units of **1**. The presence of two glucose units in **1** was also indicated by two sets of the sequentially coupled H-atom signals at δ 6.31–3.60, which were fully assigned by ^{1}H,^{1}H-COSY experiment. The ^{13}C-NMR spectrum of **1** showed twelve distinctive glucose C-atom signals including those of β- and α-anomeric C-atoms at δ 92.9 and 92.2, eight C=O C-atom signals at δ 162.8–167.8 together with a group of aromatic C-atom signals at δ 107.1–147.1.

Based on these data, compound **1** is presumed to be a dimeric ellagitannin possessing β- and α-glucose units which are linked to each other through the dehydrodigalloyl group in a way analogous to gemin A. Allocation of each phenolic

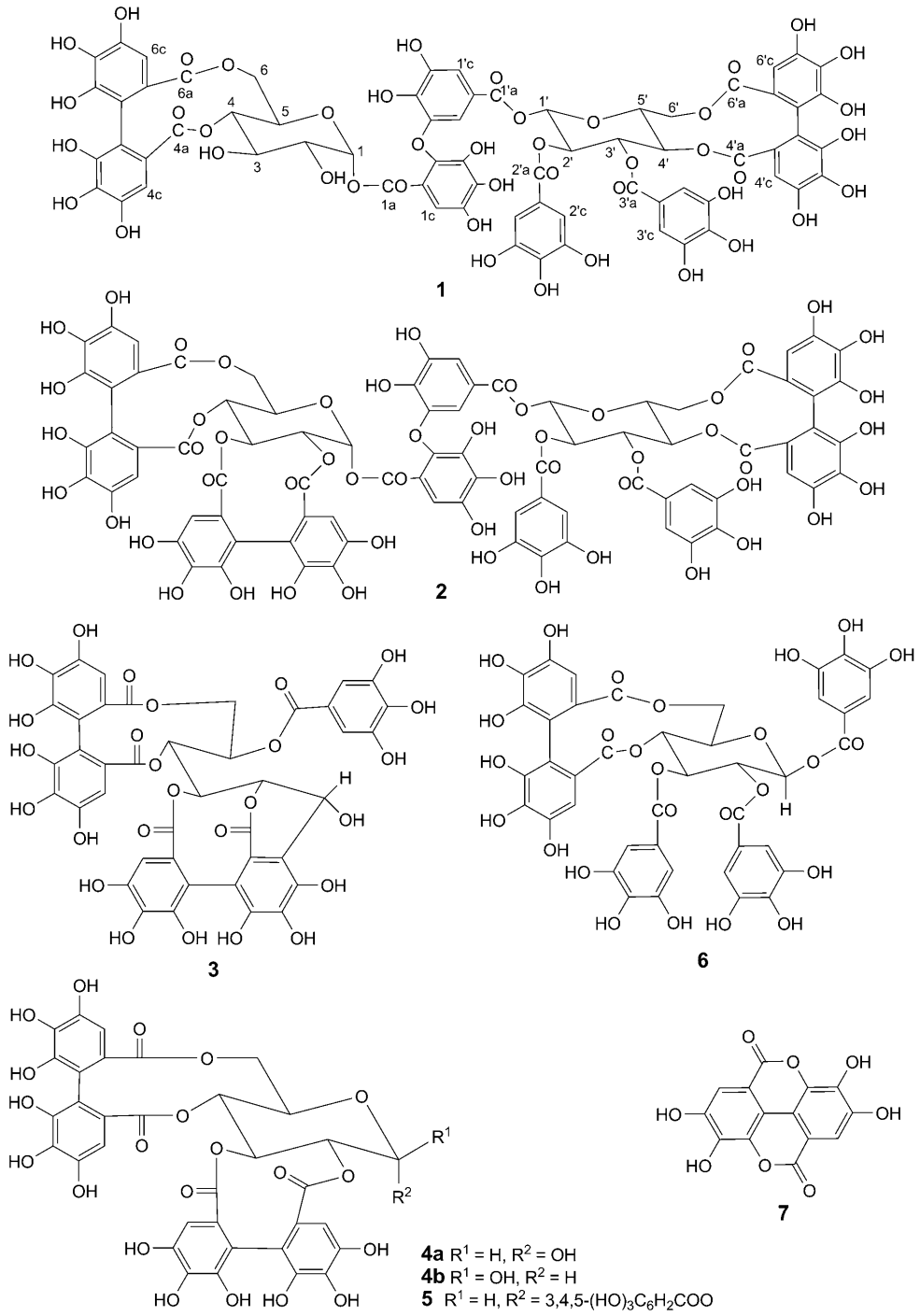

Fig. 1. *Structures of compounds* **1**–**7** *isolated from* Geum japonicum THUNB. *var.* chinense

Table 1. *^{1}H-NMR Data for Gemin G* (**1**) (500 MHz, (D_6)acetone; δ in ppm, J in Hz)

	δ(H)		δ(H)
β-Glucose		α-Glucose	
H–C(1′)	6.18 (*d*, *J* = 8.0)		6.31 (*d*, *J* = 4.0)
H–C(2′)	5.57 (*t*, *J* = 9.0)	H–C(2)	3.88 (*m*)
H–C(3′)	5.84 (*t*, *J* = 9.0)	H–C(3)	3.98 (*t*, *J* = 9.0)
H–C(4′)	5.18–5.21 (*m*)	H–C(4)	4.84 (*t*, *J* = 10.0)
H–C(5′)	4.51–4.53 (*m*)	H–C(5)	4.19 (*m*)
CH_2(6′)	5.35–5.37 (*m*),	CH_2(6)	5.05 (*dd*, *J* = 6.5, 13.0),
	3.87–3.89 (*m*)		3.60 (*d*, *J* = 13.0)
Ar–H		Ar–H	
H–C(1′c)	7.30 (*d*, *J* = 2.0)	H–C(1c)	7.33 (*s*)
H–C(1′g)	6.85 (*d*, *J* = 2.0)		
H–C(2′c,2′g)	7.04 (*s*)		
H–C(3′c,3′g)	7.01 (*s*)		
H–C(4′c)	6.50 (*s*)	H–C(4c)	6.64 (*s*)
H–C(6′c)	6.70 (*s*)	H–C(6c)	6.83 (*s*)

Table 2. *^{13}C-NMR Data for Gemin G* (**1**) (125 MHz, (D_6)acetone; δ in ppm)

	δ(C)		δ(C)
β-Glucose		α-Glucose	
C(1′)	92.9	C(1)	92.2
C(2′)	70.8	C(2)	72.1
C(3′)	72.3	C(3)	72.5
C(4′)	69.8	C(4)	71.9
C(5′)	72.3	C(5)	70.1
C(6′)	63.0	C(6)	62.3
Carbonyl C-atoms			
C(1′a)	164.1	C(1a)	162.8
C(2′a)	164.7		
C(3′a)	165.5		
C(4′a)	166.8	C(4a)	167.6
C(6′a)	167.3	C(6a)	167.8

ester groups on the glucopyranose ring was confirmed by HMBC correlations (*Fig. 2*). The H- and C-atom signals for two glucose moieties, and the phenolic ester C=O signals were assigned by ^{1}H,^{1}H-COSY, HSQC, and HMBC experiments.

The configuration of the hexahydroxybiphenyl groups in **1** was determined as (*S*) on the basis of CD-spectral measurements and the specific-rotation value. The strong positive *Cotton* effect at 237 nm (13.4×10^4) for **1** indicated that the hexahydroxybiphenyl groups in **1** have the (*S*)-configuration which is identical with that established for these groups in gemin A (**2**; 237 nm, 19.3×10^4; *Fig. 3*) [20]. This conclusion is also in accordance with the empirical rule in which the sign and amplitude of the *Cotton* effect around 235 nm reflect the configuration and the number of the hexahydroxybiphenyl groups in the molecules of ellagitannins [23]. Based on the above chemical and spectral interpretation, the structure for compound **1** was established. Compound **1** was designated as gemin G.

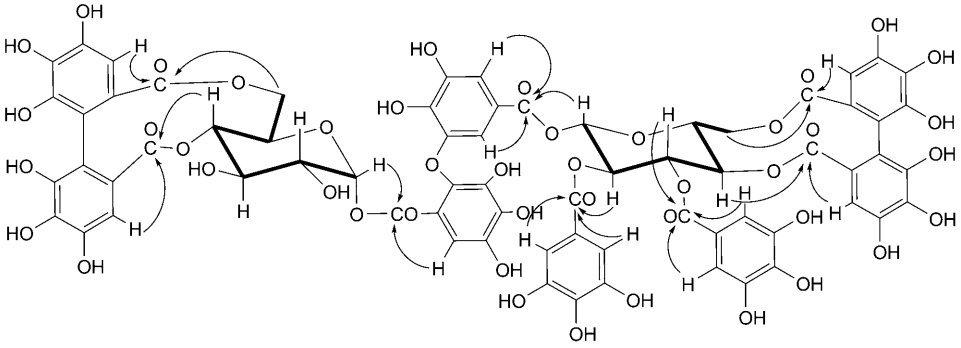

Fig. 2. *$^1H,^1H$-COSY* (—) *and HMBC Correlations* (H→C) *of compound* **1**

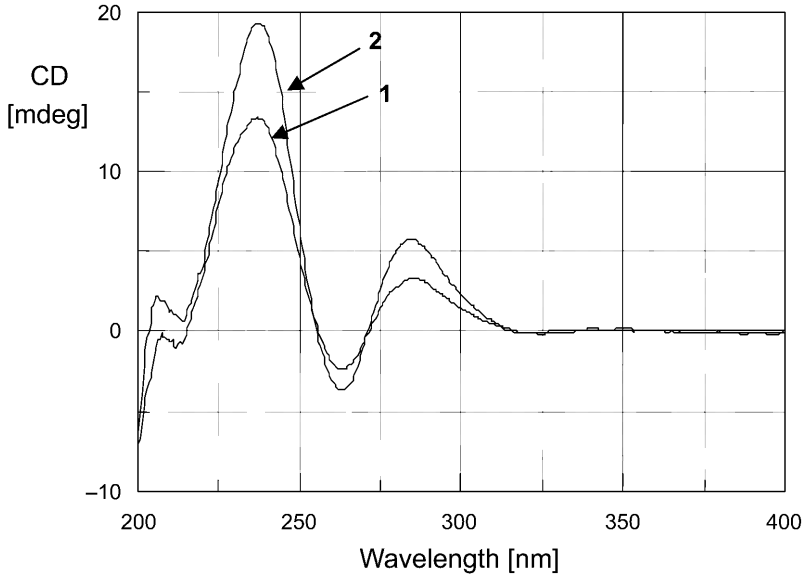

Fig. 3. *CD Spectra of compounds* **1** *and* **2** *in MeOH*

The structures for compounds **2**–**7** were determined by spectral analysis and comparison with published data [20–22].

Compounds **1**–**7** showed reversible fast-binding inhibition against FAS activity with the IC_{50} values of 0.79 ± 0.04, 0.21 ± 0.02, 8.01 ± 0.11, 3.19 ± 0.07, 2.51 ± 0.08, 2.40 ± 0.06, and 41.40 ± 0.45 μM, respectively. The epigallocatechin gallate (EGCG), which has been reported to be an inhibitor of FAS, exhibited an IC_{50} value of 52.03 ± 3.11 μM in our assay. The number of galloyl or hexahydroxybiphenyldicarbonyl moieties contributes most to the inhibition of fatty acid synthase. It has been shown that the galloyl moiety of green tea catechins is the critical structural feature to inhibit FAS [24]. The inhibitory activity of compound **3** is lower than that of compounds **4**–**6**, indicating that the intactness of the sugar ring also influences the activity. Since FAS inhibitors are

reported to be selectively cytotoxic to many kinds of human cancer cells, we tested the cytotoxicity of compounds **1**–**4** *in vitro*. Compounds **1** and **2** showed weak cytotoxic activity towards BGC-823 cells with IC_{50} values of 82.5 ± 2.2 and 75.6 ± 3.5 μg/ml, respectively.

The antioxidant activity of compounds **1**–**4** was evaluated by the Oxygen Radical Absorbance Capacity (ORAC) assay (*Table 3*). Vitamin C was used as positive control. Compounds **1**–**4** displayed potent peroxyl radical scavenging activity higher than that of vitamin C, which was in accordance with the strong antioxidant properties reported for galloyl groups. [25][26].

Table 3. *Antioxidative Values of Compounds* **1**–**4**[a])

Concentration [μM]	ORAC [μM trolox equiv.]				
	1	**2**	**3**	**4**	Vitamin C
100	99.2 ± 1.3	98.0 ± 1.3	96.8 ± 1.1	96.0 ± 0.8	62.8 ± 0.2
50	95.6 ± 1.8	84.3 ± 6.0	73.1 ± 2.0	58.3 ± 9.7	31.9 ± 0.1
25	40.8 ± 0.4	31.3 ± 6.2	38.3 ± 0.5	31.6 ± 3.6	15.8 ± 0.2
12.5	24.7 ± 2.6	20.1 ± 2.6	22.9 ± 1.1	15.6 ± 1.4	7.2 ± 0.5
6.25	14.3 ± 1.0	11.8 ± 2.2	16.5 ± 1.3	10.4 ± 0.7	3.8 ± 0.7
3.125	6.4 ± 1.2	7.5 ± 1.3	11.8 ± 0.5	6.2 ± 2.8	1.7 ± 0.4

[a]) ORAC Values are expressed as relative trolox equivalents. Data were expressed as mean ± SD. Vitamin C was used as positive control.

The chemistry and biology of ellagitannins have achieved great progress recently. Some dimeric ellagitannins promoted tumor necrosis in various mouse xenograft models at concentrations well below toxic levels. Biological mechanism-of-action studies led to a proposal that the ellagitannins were operating indirectly through endogenous effectors such as the cytokines interleukin 1β (IL-1β) and cytokine tumor necrosis factor alpha (TNFα) [27–29]. Our study is the first report of FAS inhibitory activity of ellagitannins, which may open new fields of application for these compounds, in particular in the treatment of obesity.

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Experimental Part

General. 2,2-Azobis (2-amidinopropane) dihydrochloride (AAPH), sodium fluorescein (FL), and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (*Trolox*) were purchased from *Wako Pure Chemical Industries, Ltd.* (Osaka, Japan). Acetyl-CoA and malonyl-CoA were purchased from *Sigma*, NADPH was from *Fluka*. All other reagents were local products of anal. grade. Column chromatography (CC): *D101* resin (*Anhui Sanxing Scientific Corporation*, China) and *HW-40F* (*Tosoh Corporation*, Japan) as packing materials. Anal. TLC: cellulose (funakoshi), with BuOH/AcOH/H_2O 4 : 1 : 5 (upper layer). GC: *HP-5890 SERIES II* spectrometer, with a *SE30* cap. column (12 m, 0.22 mm i.d.), hydrogen flame ionization detector (FID, 270°), the column temp. was 170–250° with the rate of 5°/min, and the carrier gas was N_2 (30 ml/min). Optical rotations: *Jasco P-1020* polarimeter. CD Spectra: *Jasco-810* spectropolarimeter. UV Spectra: *Shimadzu UV2401PC* UV/VIS spectrophotometer in MeOH. IR

Spectra: *Shimadzu FTIR 8400* spectrophotometer as KBr discs. NMR Spectra: *Bruker AVANCE 500* NMR spectrometer (500 MHz for ^{1}H, 125 MHz for ^{13}C) with Me_4Si as internal standard. ESI-MS: *Bruker Esquire 2000* mass spectrometer. HR-TOF-MS: *Waters Maldi Micro MX* instrument.

Plant Material. The whole plants of *Geum Japonicum* THUNB. var. *chinense* were collected in Guizhou Province in September 2005 and identified by *M. L.* A voucher specimen (YZXDN-2004) was deposited with the School of Chemical Biology and Pharmaceutical Science of Capital University of Medical Science, China.

Extraction and Isolation. The air-dried whole plants of *Geum Japonicum* THUNB. var. *chinense* (5 kg) were ground and macerated with 20 l of MeOH at r.t. for a week (3 times). After evaporation of solvent *in vacuo*, the residue (530 g; IC_{50} = 7.5 μg/ml) was suspended in H_2O (1000 ml), and partitioned with AcOEt (1000 ml) and BuOH (1000 ml) successively. The BuOH-soluble fraction exhibited significant FAS inhibitory activity (IC_{50} = 2.5 μg/ml). The active BuOH-soluble fraction (90 g) was first subjected to CC on *D101* resin (∅4 × 40 cm, 2000 ml) eluted with MeOH/H_2O (100 : 0 → 0 : 100) to afford nine fractions. *Fr. 4* was found to be active in FAS assay (IC_{50} = 1.5 μg/ml). *Fr. 4* (13 g) was further chromatographed on *HW-40* gel by gradient elution with MeOH/H_2O (30, 40, 50, 60, 70, 80, and 100%) and MeOH/H_2O/acetone (7 : 2 : 1 and 6 : 2 : 2), and the eluents were combined by cellulose TLC analysis to yield compounds **1** (MeOH/H_2O/acetone 6 : 2 : 2; 20 mg), **2** (MeOH/H_2O/acetone 7 : 2 : 1; 100 mg), **3** (70% MeOH/H_2O; 8 mg), **4** (60% MeOH/H_2O; 10 mg), **5** (80% MeOH/H_2O; 12 mg), **6** (80% MeOH/H_2O; 15 mg), **7** (50% MeOH/H_2O; 10 mg).

Gemin G (**1**): Amorphous powder. $[\alpha]_D^{27} = +71$ (c = 1.0, MeOH). UV (MeOH): λ_{max} (log ε) 220 (4.82), 273 (4.73) nm. CD (MeOH): $[\theta]_{237} + 13.4 \times 10^4$, $[\theta]_{263} - 2.4 \times 10^4$, $[\theta]_{285} + 3.4 \times 10^4$. IR (KBr): 3425, 1716, 1610, 1510, 1445, 1050. ^{1}H- and ^{13}C-NMR ((D$_6$)Acetone): see *Tables 1* and *2*. ESI-MS (neg.): 1569 ($[M-H]^-$), 785 ($[M-785]^-$). HR-TOF-MS: 1569.1626 ($[M-H]^-$, $C_{68}H_{49}O_{44}^-$; calc. 1569.1597).

Acid hydrolysis of **1** *and* **2**. A soln. of **1** and **2** (each 10 mg) in aq. 2N CF_3COOH was heated at 100° for 2 h in a sealed vial. The mixture was evaporated *in vacuo*. The obtained residue was dissolved in H_2O and extracted twice with AcOEt. The AcOEt extracts was purified by CC (silica gel; $CHCl_3$/MeOH 10 : 1, 9 : 1, 7 : 1, 5 : 1) to give gallic acid (1.5 mg) and ellagic acid (2.0 mg). Glucose was identified in the aq. layer by GLC as aldononitrile peracetate derivative, using standard aldononitrile peracetate of D-glucose as a reference sample [30].

Preparation and Activity Assay of FAS. The FAS from duck liver was purified according to an improved method described previously by *Tian et al.* [31]. The assay for FAS activity, which was described in [16], was performed with a *Shimadzu UV2550* double-wavelength/double-beam spectrophotometer at 37° by following the decrease of NADPH at 340 nm. The mixture contained 100 mM potassium phosphate buffer, pH 7.0, 1 mM EDTA, 3 mM acetyl-CoA, 10 mM malonyl-CoA, 35 mM NADPH, and 10 mg duck liver FAS in a total volume of 2.0 ml. Fast-binding inhibition was determined by adding the tested compounds to the reaction system before the reaction was initiated by the addition of FAS. After incubation at 37° for 5 min, the absorbance at 340 nm was measured. The remaining activity of FAS was assayed as A_i, and the control activity with solvent instead of test compounds was assayed as A_0. The fast-binding inhibition was calculated by the formula: $I = (1 - A_i/A_0) \times 100\%$. Inhibition of FAS was measured for tested compounds at different concentrations in triplicate, and IC_{50} values were obtained from the dose-response curves of inhibitors by software Graph 2.0. EGCG was used as positive control in tests.

Cytotoxicity Bioassay. The effects of compounds **1**–**4** on cell growth were investigated using gastric cancer cell line (BGC-823) purchased from Institute of Medicament Research of Chinese Academy of Medical Sciences and cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum, 100 units/ml of penicillin, and 100 μg/ml of streptomycin. Cells were maintained at 37° in a humidified atmosphere of 5% CO_2 in air. The effect on the cell viability was determined by MTT (= 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium hydrobromide) method in triplicate [32]. Fluorouracil was used as positive control with an IC_{50} value of 5.5 ± 0.3 μg/ml.

Oxygen Radical Absorbance Capacity (ORAC) Assay. The ORAC assay is based on free radical damage to a fluorescent probe. Evaluation of peroxyl-radical scavenging activity was based on the protocol described in [33–35]. In our assay, sodium fluorescein was used as a fluorescent probe, AAPH as a free radical initiator, and *Trolox* as a standard. The 180-μl test soln. contained 20 μl of sample soln.,

20 µl of phosphate buffer (7.5 mM), and 140 µl of AAPH soln. (12.8 mM). The reaction was started by the addition of 20 µl of fluorescein (63 nM) to the test soln. The fluorescence intensity was measured with *Genios* multi-detection microplate reader (*Tecan*; ex. 485 nm, em. 538 nm) every 2 min until the fluorescence was close to zero. The well-known antioxidant vitamin C and the solvent DMSO were used as the positive control and the blank, resp. The ORAC value is calculated by dividing the area under the sample curve by the area under the *Trolox* curve, with both areas being corrected by subtracting the area under the blank curve. One ORAC unit is defined as the net area of protection provided by *Trolox* at a final concentration 1 µmol l^{-1}. The area under the curve for the sample is compared to the area under the curve for *Trolox*, and the antioxidative value is expressed in µmol of *Trolox* equiv. per l. The experiments were carried out in triplicate and repeated for three times.

REFERENCES

[1] S. J. Wakil, J. K. Stoops, V. C. Joshi, *Annu. Rev. Biochem.* **1983**, *52*, 537.
[2] S. J. Wakil, *Biochemistry* **1989**, *28*, 4523.
[3] A. Jayakumar, M.-H. Tai, W.-Y. Huang, W. Al-Feel, M. Hsu, L. Abu-Elheiga, S. S. Chirala, S. J. Wakil, *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 8695.
[4] T. M. Loftus, D. E. Jaworsky, G. L. Frehywot, C. A. Townsend, G. V. Ronnett, M. D. Lane, P. K. Francis, *Science (Washington, DC)* **2000**, *288*, 2379.
[5] J. V. Swinnen, T. Roskams, S. Joniau, H. Van Poppel, R. Oyen, L. Baert, W. Heyns, G. Verhoeven, *Int. J. Cancer* **2002**, *98*, 19.
[6] Y. Wang, F. P. Kuhajda, J. N. Li, E. S. Pizer, W. F. Han, L. J. Sokoll, D. W. Chan, *Cancer Lett.* **2001**, *167*, 99.
[7] L. D. Vlad, C. A. Axiotis, M. J. Merino, W. Green, *Mod. Pathol*. **1999**, *12*, 70A.
[8] C. J. Piyathilake, A. R. Frost, U. Manne, W. C. Bell, H. Weiss, D. C. Heimburger, W. E. Grizzle, *Hum. Pathol.* **2000**, *31*, 1068.
[9] D. Innocenzi, P. L. Alò, A. Balzani, V. Sebastiani, V. Silipo, G. La Torre, G. Ricciardi, C. Bosman, S. Calvieri, *J. Cutan. Pathol.* **2003**, *30*, 23.
[10] P. Visca, V. Sebastiani, E. S. Pizer, C. Botti, P. De Carli, S. Filippi, S. Monaco, P. L. Alò, *Anticancer Res.* **2003**, *23*, 335.
[11] F. P. Kuhajda, E. S. Pizer, J. N. Li, N. S. Mani, G. L. Frehywot, C. A. Townsend, *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 3450.
[12] E. S. Pizer, F. D. Wood, H. S. Heine, F. E. Romantsev, G. R. Pasternack, F. P. Kuhajda, *Cancer Res.* **1996**, *56*, 1189.
[13] E. S. Pizer, F. J. Chrest, J. A. Digiuseppe, W. F. Han, *Cancer Res.* **1998**, *58*, 4611.
[14] F. P. Kuhajda, *Nutrition* **2000**, *16*, 202.
[15] E. W. Gabrielson, M. L. Pinn, J. R. Testa, F. P. Kuhajda, *Clin. Cancer Res.* **2001**, *7*, 153.
[16] X. Wang, W. Tian, *Biochem. Biophys. Res. Commun*. **2001**, *288*, 1200.
[17] Jiangsu New Medical College, 'Dictionary of Chinese Herb Medicines', Shanghai Scientific and Technologic Press, Shanghai, 1986, pp. 586.
[18] G. N. Li, 'Dictionary of Chinese Herb Medicines in Yunnan', Yunnan Scientific and Technologic Press, Yunnan, 1990, pp. 153.
[19] J. K. Li, H. W. Liu, N. L. Wang, M. Li, X. S. Yao, *J. Shenyang Pharm. Univ.* **2006**, *23*, 694.
[20] T. Yoshida, T. Okuda, *J. Chem. Soc., Perkin Trans. I* **1985**, 315.
[21] T. Okuda, T. Yoshida, M. Ashida, K. Yazaki, *J. Chem. Soc.*, *Perkin Trans. I* **1983**, 1765.
[22] S.-H. Lee, T. Tanaka, G.-i. Nonaka, I. Nishioka, *Phytochemistry* **1990**, *29*, 3621.
[23] T. Okuda, T. Yoshida, T. Hatano, T. Koga, N. Toh, K. Kuriyama, *Tetrahedron Lett*. **1982**, *23*, 3937.
[24] X. Wang, K.-S. Song, Q.-X. Guo, W.-X. Tian, *Biochem. Pharmacol.* **2003**, *66*, 2039.
[25] W. Bors, C. Michel, *Ann. N.Y. Acad. Sci.* **2002**, *957*, 57.
[26] S. Kinoshita, Y. Inoue, S. Nakama, T. Ichiba, Y. Aniya, *Phytomedicine* **2007**, *14*, 755.
[27] K. S. Feldman, *Phytochemistry* **2005**, *66*, 1984.

[28] K. Miyamoto, T. Murayama, M. Nomura, T. Hatano, T. Yoshida, T. Furukawa, R. Koshiura, T. Okuda, *Anticancer Res.* **1993**, *13*, 37.
[29] T. Murayama, N. Kishi, R. Koshiura, K. Takagi, T. Furukawa, K. Miyamoto, *Anticancer Res.* **1992**, *12*, 1471.
[30] H.-F. Tang, Y.-H. Yi, L. Li, P. Sun, S.-Q. Zhang, Y.-P. Zhao, *J. Nat. Prod*. **2005**, *68*, 337.
[31] W.-X. Tian, Y.-S. Wang, R. Y. Hsu, *Biochem. Biophys. Acta* **1989**, *998*, 310.
[32] S.-L. Wang, B. Cai, C.-B. Cui, H.-W. Liu, C.-F. Wu, X.-S. Yao, *J. Asian Nat. Prod. Res.* **2004**, *6*, 115.
[33] G. Zhong, R. T. Toledo, Z. D. Chen, *Agric. Sci. Chin.* **2003**, *2*, 1035.
[34] R. L. Prior, H. Hoang, L. Gu, X. Wu, M. Bacchiocca, L. Howard, M. Hampsch-Woodill, D. Huang, B. Ou, R. Jacob, *J. Agric. Food Chem*. **2003**, *51*, 3273.
[35] B. Ou, M. Hampsch-Woodill, R. L. Prior, *J. Agric. Food Chem*. **2001**, *49*, 4619.

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