Camellisins A—C, Three New Triterpenoids from the Roots of *Camellia sinensis*

Chun LEI,[a,#] Zhuo HU,[c,#] Jian-Xin PU,*[,a] Yuan-Yuan WANG,[a] Wei-Lie XIAO,[a] Ning-Bo GONG,[b] Yan LI,[a] Yang LU,[b] Qi-Tai ZHENG,[b] and Han-Dong SUN*[,a]

[a] *State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences; Kunming 650204, P.R. China:* [b] *Institute of Materia Medica, Chinese Academy of Medical Sciences & Peking Union Medical College; Peking 100050, P.R. China: and* [c] *College of Chemistry and Biotechnology, Yunnan Nationalities University; Kunming 650204, P.R. China.*

Received March 18, 2010; accepted April 23, 2010; published online April 26, 2010

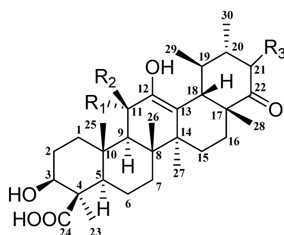
Eighteen compounds, including three new triterpenoids, camellisins A—C (1—3), were isolated from the roots of *Camellia sinensis*. Their structures were determined on the basis of detailed spectroscopic analysis.

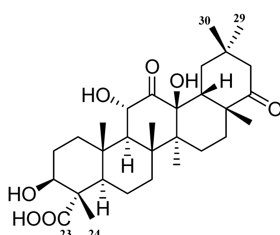
Key words *Camellia sinensis*; triterpenoid; camellisin

Camellia sinensis is an evergreen shrub plant of the *Theaceae* family. Their leaves and leaf buds are used to produce tea. Since the tea was discovered for the first time in China[1] in 2737 BC, it is at present, only next to water, the most popular beverage, and is currently grown and cultivated in at least 30 countries around the world.[2] Phytochemical and pharmacological studies of tea have provided convincing evidence that the polyphenolic antioxidants present in tea are capable of affording chemoprevention in cancer.[2,3] Root of *C. sinensis* is a common traditional Chinese medicine, which has been used to cure rheumatic and hypertensive cardiopathy, coronary heart disease and arrhythmia. Previous phytochemical studies of the roots have led to the identification of some sugars, phenolic components, steroids, and triterpene saponins.[4,5] With the aim of searching new natural compounds with interesting biological activities, we carried out phytochemical investigations on the roots of this plant collected in China. Three new triterpenoids, camellisins A—C (**1**—**3**), together with 15 known compounds have been isolated. Here, we report the structure elucidation of these new triterpenoids on the basis of their spectroscopic data and the bioassay of their cytotoxicity against five kinds of the human tumor cell lines, including HL-60, SMMC-7721, A-549, PANC-1 and SK-BR-3.

Results and Discussion

Phytochemical studies on the 70% aqueous acetone extract of the roots of *C. sinensis* led to the isolation of three new triterpenoids, camellisins A—C (**1**—**3**), and 15 known compounds including one triterpenoid, ursolic acid,[6] two steroids, chondrillasterol[7] and α-spinasterone,[8] six phenolic compounds, lariciresinol,[9] pinoresinol,[10] 4-*O*-methylcedrusin,[11] (+)-balanophonin,[12] ω-hydroxypropioguaiacone,[13] (*E*)-ferulaldehyde,[14] and six other constituents, 5-megastigmene-3,9-diol,[15] 4,5-dihydroblumen-ol A,[16] (6*R*,9*R*)-9-hydroxy-4-megastigmen-3-one,[17] blumenol B,[18] glycerol 1-hexadecanoate,[19] and hexadecanoic acid.[19] The known compounds were determined by comparing their mass spectra and NMR data with those of literatures. The new compounds were characterized on the basis of comprehensive spectroscopic analysis.





1 R_1 = OH, R_2 = H, R_3 = β-OH
2 R_1 = R_2 = O, R_3 = H
3

Camellisin A (**1**) was obtained as colorless needle crystals from MeOH. The high-resolution-electrospray mass spectrometry (HR-ESI-MS) exhibited a pseudo-molecular ion peak $[M-H]^-$ at *m/z* 517.3166 (Calcd. 517.3165) corresponding to the molecular formula $C_{30}H_{46}O_7$, indicating eight degrees of unsaturation. The ^{1}H- and ^{13}C-NMR spectra displayed 30 carbon resonances comprising seven methyl groups (including five tertiary ones and two secondary ones), six sp^3 methylenes, eight sp^3 methines (three oxygenated at δ_C 78.9, 70.6, 77.7), one carbonic carbon (δ_C 180.9), one carbonyl carbon (δ_C 216.7), a pair of quaternary olefinic bond (δ_C 148.6, 112.8), and five quaternary sp^3 carbons (Table 1). This information, coupled with the molecular formula indicated that compound **1** was a triterpenoid with five rings and four hydroxyls.

The ^{1}H–^{1}H shift correlation spectroscopy (COSY) spectrum revealed five groups of correlations including H_2-1/H_2-2/H-3, H-5/H_2-6/H_2-7, H-9/H-11, H_2-15/H_2-16, H-18/H-19(H_3-29)/H-20 (H_3-30)/H-21 (Fig. 1). The heteronuclear multiple bond correlation (HMBC) spectrum displayed distinct correlations from five singlet methyl groups: from H_3-24 (δ_H 1.40) to C-3, C-4, C-5; from H_3-25 (δ_H 1.10) to C-1, C-5, C-9, C-10; from H_3-26 (δ_H 1.09) to C-7, C-8, C-9, C-14; from H_3-27 (δ_H 1.32) to C-13, C-14, C-15; and from H_3-28 (δ_H 1.03) to C-16, C-17, C-18, and C-22 (Fig. 1). The COSY correlations above and the key HMBC cross peaks built up the five rings framework as shown and assigned three of the four hydroxyls to be located at C-3 (δ_C 78.9), C-11 (δ_C 70.6), and C-21 (δ_C 77.7), respectively. The remained hydroxyl could only constructure an enol system with the olefinic bond of C-12 and C-13, which can be deduced by the HMBC correlations from H-9 and H-18 to C-12, H_3-27 and H-18 to C-13, respectively (Fig. 1). The carbonic acid was

* To whom correspondence should be addressed. e-mail: pujianxin@mail.kib.ac.cn; hdsun@mail.kib.ac.cn
© 2010 Pharmaceutical Society of Japan
[#] These authors contributed equally to this work.

Table 1. ^{1}H- and ^{13}C-NMR Data of Compounds **1**—**3** (400, 100 MHz, in C_5D_5N, δ in ppm, J in Hz)

Position	**1**		**2**		**3**	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1α	1.28[a]	42.3 t	1.21[a]	40.1 t	1.39 (m)	42.0 t
1β	2.41 (m)		3.13 (m)		3.41[a]	
2α	1.67[a]	29.3 t	2.63 (m)	29.0 t	2.54[a]	29.7 t
2β	2.10[a]		1.98 (m)		2.00[a]	
3	3.15 (dd, 4.5, 12.1)	78.9 d	3.41 (dd, 4.5, 12.0)	80.0 d	3.44 (dd, 4.4, 13.2)	78.1 d
4		49.4 s		49.4 s		50.0 s
5	0.98[a]	57.8 d	1.13[a]	56.5 d	1.19 (dd, 4.5, 12.6)	57.1 d
6α	1.85[a]	21.1 t	2.23 (m)	20.0 t	2.58 (m)	20.1 t
6β	1.84[a]		2.12 (m)		2.07 (m)	
7α	1.50 (m)	35.3 t	1.67 (dd, 4.0, 13.0)	33.5 t	1.53 (m)	34.7 t
7β	1.39[a]		1.44[a]		1.47 (m)	
8		44.1 s		42.0 s		43.5 s
9	1.72 (d, 9.3)	54.0 d	2.75 (s)	59.6 d	1.92 (d, 12.1)	57.4 d
10		39.6 s		38.2 s		40.6 s
11	4.07 (d, 9.3)	70.6 d		195.4 s	5.51 (d, 12.1)	73.4 d
12		148.6 s		146.6 s		212.8 s
13		112.8 s		129.9 s		81.8 s
14		42.3 s		45.1 s		45.8 s
15α	1.12[a]	26.7 t	1.15[a]	26.6 t	1.24[a]	22.0 t
15β	1.78[a]		1.78[a]		2.32 (dd, 4.4, 12.1)	
16α	2.18[a]	29.3 t	2.17 (dd, 4.5, 10.0)	27.2 t	2.10[a]	25.9 t
16β	1.20[a]		1.28[a]		2.04[a]	
17		49.4 s		48.4 s		49.0 s
18	2.67 (d, 11.7)	48.6 d	3.28 (d, 11.0)	48.4 d	2.69 (d, 12.3)	47.5 d
19α	2.00 (m)	40.1 d	1.89 (m)	41.0 d	1.28 (dd, 4.4, 12.3)	41.7 t
19β					2.39[a]	
20	1.24 (m)	48.8 d	1.50 (m)	39.0 d		33.8 s
21α	4.10 (d, 10.6)	77.7 d	2.43 (dd, 12.6, 14.5)	46.3 t	2.51 (d, 12.6)	49.0 t
21β			2.48 (dd, 5.5, 14.5)		1.96 (d, 12.6)	
22		216.7 s		214.4 s		217.5 s
23	1.40 (s)	24.7 q	1.74 (s)	24.7 q		180.8 s
24		180.9 s		180.4 s	1.78 (s)	24.9 q
25	1.10 (s)	15.3 q	1.58 (s)	14.6 q	1.57 (s)	14.7 q
26	1.09 (s)	18.5 q	1.19 (s)	18.5 q	1.77 (s)	20.5 q
27	1.32 (s)	24.0 q	1.48 (s)	20.7 q	1.04 (s)	17.0 q
28	1.03 (s)	21.0 q	1.17 (s)	21.6 q	1.66 (s)	26.7 q
29	1.04 (d, 6.6)	16.6 q	0.92 (d, 6.5)	20.9 q	0.96 (s)	31.5 q
30	1.16 (d, 6.6)	17.0 q	1.05 (d, 6.5)	16.4 q	1.13 (s)	28.6 q
HO-13					7.08 (s)	

a) Overlapped signals.

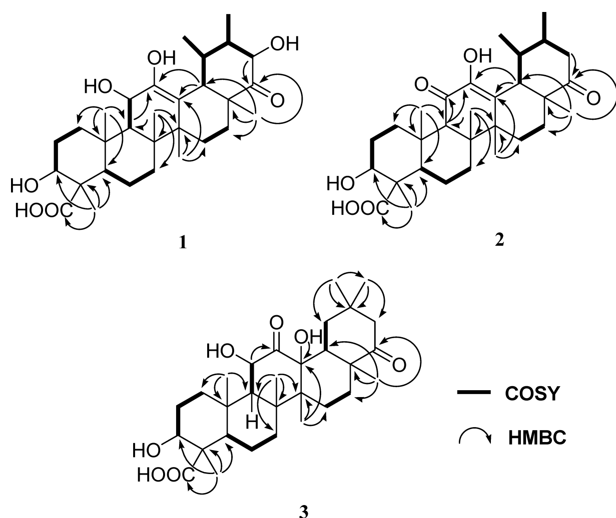


Fig. 1. ^{1}H–^{1}H COSY and Selected HMBC Correlations of **1**—**3**

assigned at C-23 from the distinct HMBC correlations from H-3, H-5, and H_3-24 to signal at δ_C 180.9 (Fig. 1). And the carbonyl carbon (δ_C 216.7) was confirmed to be C-22, since the HMBC correlations existed in both H-21 and H_3-28 with δ_C 216.7 (Fig. 1). Thus, the planar structure of compound **1** was established.

The relative stereochemistry of **1** was ascertained by the nuclear Overhauser effects (NOEs) and X-ray diffraction. From the biosynthetic point of view, H-5 and CH_3-27 were α-orientations, while H-18, CH_3-25, CH_3-26, and CH_3-28 were β-orientations.[20] Thus, the rotating frame Overhauser enhancement (ROE) correlations of H-5 with CH_3-23 and of H-11 with CH_3-25 indicated both CH_3-23 and HO-11 were α-directed. Correlations of both H-18 and CH_3-28 with CH_3-29 suggested that CH_3-29 was β-orientation, while H-19 with CH_3-30 and H-21 indicated the α-orientations of both CH_3-30 and H-21 (Fig. 2). The X-ray diffraction analysis of compound **1** further confirmed its relative stereochemistry (Fig. 3). The circular dichroism (CD) spectrum of **1** showed the relatively strong $\pi\rightarrow\pi^*$ transition around 208 nm for the olefin group are positive, while the weak $n\rightarrow\pi^*$ transition

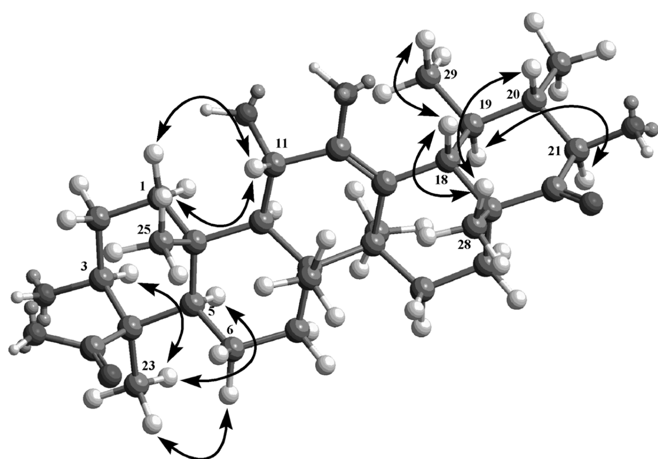


Fig. 2. Partial ROE Correlations of **1**

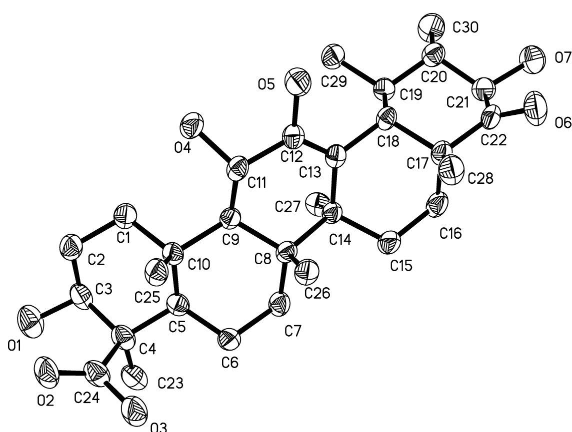


Fig. 3. X-Ray Crystal Structure of **1** Showing the Relative Configuration

around 285 nm for the cyclohexanone group is negative. Analysis of the Cotton effect according to the olefin octant rule[21] and the cyclohexanone octant rule[22] indicated the rings B/C is *trans* while the D/E system is *cis* in **1**. Thus, the absolute configuration of **1** was established as shown. Therefore, compound **1** was elucidated to be an ursane triterpenoid analogy, 3β, 11α, 12, 21β-tetrahydroxy-22-oxo-urs-12-en-24-oic acid, named camellisin A.

Camellisin B (**2**) was isolated as colorless needle crystals in MeOH. Its molecular formula, $C_{30}H_{44}O_6$, was deduced from the positive HR-ESI-MS ion peak $[M+Na]^+$ *m/z* 523.3037 (Calcd 523.3036), requiring nine degrees of unsaturation. The ^{13}C-NMR spectrum displayed 30 carbon signals, most of which were similar to those of compound **1**. The main differences were restricted to the signals of rings C and E. Among them, two oxygenated carbon signals disappeared while a carbonyl carbon one appeared. The C-9 was slightly downfield shifted (Table 1) which indicated that the adjacent oxygenated C-11 in **1** was further oxygenated into the carbonyl carbon in **2**. The slightly up shift of C-12 and dramatically down shift of C-13 indicated that the carbonyl carbon and the olefinic bond formed as an α,β-unsaturated ketone moiety on ring C (Table 1). This assumption was finally confirmed by the key HMBC correlations from H-9 to C-11 (δ_C 195.4) and C-12 (δ_C 146.6), from H-18 to C-12 and C-13 (δ_C 129.9), and from H_3-27 to C-13 and C-14 (δ_C 45.1) (Fig. 1). Thus, the oxygenated methine C-21 in **1** was changed into a methylene in **2**, which can be confirmed from the ^{1}H–^{1}H COSY correlations of H-18/H-19/H-20/H_2-21 (Fig. 1). ROE experiment of compound **2** showed correlations of CH_3-23 with H-3 and H-5, which indicated the *R** configuration of C-4. The other chiral centers had the same stereochemistry as that of **1**. Thus, compound **2** was established to be another ursane triterpenoid, 3β,12-dihydroxy-11,22-dioxo-urs-12-en-24-oic acid.

Camellisin C (**3**), colorless needle crystals in MeOH, possessed a molecular formula of $C_{30}H_{46}O_7$ as derived from its HR-ESI-MS (*m/z* 541.3148, $[M+Na]^+$, Calcd 541.3141). The ^{1}H-NMR spectrum displayed seven quaternary methyl groups at δ_H 0.96, 1.04, 1.13, 1.57, 1.66, 1.77, 1.78, and a hydroxyl singlet signal at δ_H 7.08 (Table 1). The ^{13}C-NMR data revealed 30 carbon signals including seven methyls, eight methylenes, five methines (two oxygenated), seven sp^3 quaternary carbon (one oxygenated), two carbonyl groups, and one carboxyl carbon. The 1D-NMR information, together with the eight degrees of unsaturation, indicated that compound **3** was a five ring triterpenoid with three hydroxyls. Detailed comparison of its 1D-NMR data with those of compounds **1** and **2** showed that they possessed the same rings A and B. This can be further confirmed by the ^{1}H–^{1}H COSY correlations of H_2-1/H_2-2/H-3 and H-5/H_2-6/H_2-7, along with the key HMBC cross peaks of H_3-24 with C-3 and C-5, H_3-25 with C-1, C-5, C-9, C-10, and H_3-26 with C-7, C-8, C-9, and C-14 (Fig. 1). The hydroxyl at δ_H 7.08 (s) was assigned to be located at C-13 by the obvious HMBC correlation of δ_H 7.08 with C-13. The third hydroxyl group was placed at C-11, as H-9 showed obvious COSY correlation with a doublet proton at δ_H 5.51 (J=12.1 Hz, H-11) (Fig. 1). The distinct HMBC correlations of H-11 with δ_C 212.8 (s) assigned C-12 to be a carbonyl group (Fig. 1). The 6/6 membered rings D and E were established by ^{1}H–^{1}H COSY correlations of H_2-15/H_2-16 and H-18/H_2-19, along with three groups of HMBC correlations from H_3-27 to C-13, C-14, C-15, from H_3-28 to C-16, C-17, C-18, and from H_3-29 to C-19, C-20, C-21, C-30 (Fig. 1). The other carbonyl group was located at C-22, which can be deduced from the key HMBC correlations of δ_C 217.5 (s) with both H_3-28 and H-21 (Fig. 1).

The relative stereochemistry of **3** was established by the ROE correlations. As an oleanane triterpenoid, H-5, H-9, and CH_3-27 were biogenetically α-orientations, while CH_3-25, CH_3-26, and CH_3-28 were biogenetically β-orientations, just as those of compound **1**.[20] Thus, CH_3-24 showed ROE correlation with H-2β, H-6β, and CH_3-26, indicated the β-orientation of CH_3-24 and the *S** configuration of C-4. H-11, presenting correlation with both CH_3-25 and CH_3-26, and HO-13, exhibiting correlations with H-11 and H-18, suggested that all of them were β-orientations (Fig. 4). Thus, compound **3** was elucidated to be a new oleanane triterpenoid, 3β, 11α, 3β-trihydroxy-12,22-dioxo-olean-23-oic acid.

Compounds **1**—**3** were tested for cytotoxicity against HL-60 (human myeloid leukemia cell line), SMMC-7721 (human hepatocarcinoma cell line), A-549 (lung cancer cell line), PANC-1 (human pancreatic carcinoma) and SK-BR-3 (breast cancer cell line) cell lines. All compounds were inactive with IC_{50} values greater than 40 μM, while the positive control *cis*-platin showed IC_{50} values of 1.7, 19.4, 29.7, 38.0, and

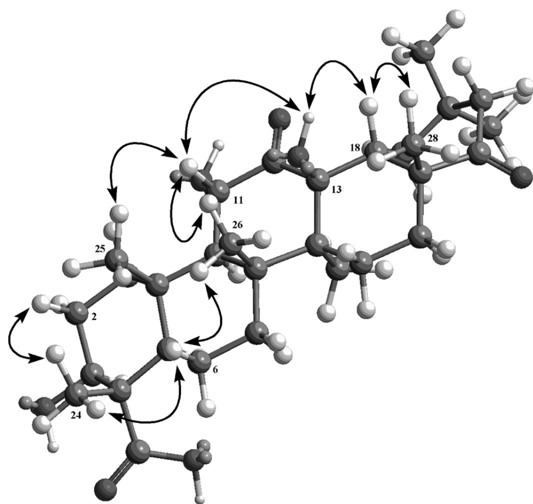


Fig. 4. Key ROE Correlations of **3**

17.4 μM, respectively.

Experimental

General Procedure Petroleum ether (PE, 60—90 °C), EtOAc, $CHCl_3$, Acetone, MeOH, EtOH, and *i*-PrOH were analytical grade and produced by Sinopharm Chemical Reagent Co., Ltd., China. Column chromatography (CC) was performed on silica gel (200—300 mesh; Qingdao Marine Chemical Inc., Qingdao, People's Republic of China), Lichroprep RP-18 gel (40—63 μm, Merck, Darmstadt, Germany), and Sephadex LH-20 (Pharmacia). Fractions were monitored by TLC, and spots were visualized by spraying with 10% H_2SO_4 in EtOH, followed by heating. Semi-preparative HPLC was performed on an Agilent 1100 liquid chromatograph with a Zorbax SB-C_{18}, 9.4 mm×25 cm column. Melting point was obtained on an XRC-1 apparatus and was uncorrected. Optical rotations were measured on a JASCO DIP-370 digital polarimeter. CD spectra were measured on a JASCO J-810 spectropolarimeter. UV data were obtained using a UV-210A spectrometer. IR spectra were obtained on a Bio-Rad FtS-135 spectrophotometer with KBr pellets. MS were recorded on a VG Auto Spec-3000 spectrometer. NMR spectra were obtained on a Bruker DRX-400 instrument with TMS as an internal standard.

Plant Material The roots of *C. sinensis* were collected in Zhejiang province, China, in July 2004, and identified by Prof. Xi-Wen Li, Kunming Institute of Botany. A voucher specimen (No. 20040910) has been deposited in the Herbarium of the Kunming Institute of Botany, Chinese Academy of Sciences.

Extraction and Isolation The air-dried and powdered roots (12 kg) of *C. sinensis* were extracted with 70% aqueous Acetone (3×30 l) at room temperature to yield an extract, which was successively extracted with petroleum ether and EtOAc. The EtOAc extract was evaporated to dryness under reduced pressure to give an extract (227 g) that was separated by Si gel CC (2 kg, 100—200 mesh) and eluted with a petroleum ether/ethyl acetate gradient system (9 : 1, 8 : 2, 7 : 3, 6 : 4, 5 : 5) to give fractions 1—5. Fraction 1 (30 g), 2 (20 g) and 3 (15 g) were subjected to CC (200—300 mesh) with petroleum ether/ethyl acetate (40 : 1), petroleum ether/ethyl acetate (20 : 1), and petroleum ether/*i*-PrOH, respectively, affording ursolic acid (16 mg), chondrillasterol (254 mg), α-spinasterone (10 mg), glycerol 1-hexadecanoate (36 mg), and hexadecanoic acid (13 mg). Fraction 4 (10 g) was subjected to CC (200—300 mesh) with $CHCl_3$/acetone (20 : 1) to afford 3 fractions, which were further purified by semipreparative HPLC (MeOH/H_2O) to give compounds pinoresinol (62 mg), 5-megastigmene-3,9-diol (27 mg), and (6*R*,9*R*)-9-hydroxy-4-megastigmen-3-one (3 mg). Fraction 5 (110 g) was subjected to CC, Sephadex LH-20 (MeOH), RP-18, preparative and semi-preparative HPLC (MeOH–H_2O) to give compound camellisin A (**1**, 56 mg), camellisin B (**2**,160 mg), camellisin C (**3**, 33 mg), lariciresinol (10 mg), 4-*O*-methylcedrusin (8 mg), (+)-balanophonin (12 mg), ω-hydroxypropioguaiacone (15 mg), (*E*)-ferulaldehyde (4 mg), 4,5-dihydroblumen-ol A (14 mg), and blumenol B (8 mg).

Camellisin A (**1**): Colorless needle crystals in MeOH; mp 187—188 °C; $[\alpha]_D^{24.4}$ +18.1 (c=0.09, C_5H_5N); CD λ_{max} (c=0.28, MeOH) nm ($\Delta\varepsilon$) 203.5 (+53.8), 208 (+52.4), 212 (+47.0), 217 (+35.6), 241 (−13.9), 245 (−15.8), 285 (−7.9), 301 (−9.8); UV λ_{max} (MeOH) nm (log ε): 207 (4.15), 360 (1.90), 365 (1.88), 390 (1.87); IR (KBr) ν_{max} cm^{-1}: 3421, 2980, 2934, 2872, 1697, 1640, 1459, 1379, 1311, 1275, 1247, 1194, 1089, 1063, 1029, 1002, 985; ^{1}H- and ^{13}C-NMR: listed in Table 1; ESI-MS (neg.): *m/z* 1036 $[2M]^-$ (6), 517 $[M-H]^-$ (100), 501 (7); HR-ESI-MS (neg.): *m/z* 517.3166 ($[M-H]^-$, $C_{30}H_{45}O_7$, Calcd 517.3165).

X-Ray Crystal Structure Analysis of **1**: $(C_{30}H_{46}O_7)_2\cdot(CH_3OH)_3\cdot H_2O$ (M=518.68), triclinic P_1: a=11.1828 (12) Å, b=12.0281 (13) Å, c=13.6749 (15) Å, α=110.2639 (10)°, β=90.0150 (20)°, γ=114.4298 (10)°, Z=2, V=1548.7 (3) Å^3, D_{calcd}=1.235 g/cm^3, R=0.069, Rw=0.168. From a crystal sized 0.38×0.13×0.10 mm, 11727 independent reflections were measured on a Bruker Nonius area detector with Mo$K\alpha$ radiation, of which 6946 were observed ($|F|^2 \geq 2\sigma|F|^2$). The structure was solved by direct methods (SHELXS-97) and expanded by Fourier techniques, refined by the program NOMCSDP and full-matrix least-squares calculations. Drawing of the molecule was achieved with ORTEP. Crystallographic data for the structure of **1** have been deposited in the Cambridge Crystallographic Data Centre (deposition number CCDC 769198). Copies of the data can be obtained free of charge *via* www.ccdc.acm.ac.Uk/conts/retrieving.html [or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB21EZ, U.K.; FAX (+44) 1223–336–033; or deposit@ ccdc.cam.ac.uk].

Camellisin B (**2**): Colorless needle crystals in MeOH; mp 189—190 °C; $[\alpha]_D^{24.3}$ +131.3 (c=0.15, C_5H_5N); UV λ_{max}(MeOH) nm (log ε): 202 (3.88), 287 (4.20), 374 (2.70); IR (KBr) ν_{max} cm^{-1}: 3426, 2974, 2933, 2874, 1694, 1665, 1637, 1460, 1388, 1379, 1363, 1307, 1284, 1252, 1191, 1166, 1040; ^{1}H- and ^{13}C-NMR: listed in Table 1; electron ionization-mass spectrometry (EI-MS): *m/z* (%): 500 $[M]^+$ (83), 485 $[M-CH_3]^+$ (100), 471 (20), 317 (56), 303 (62), 233 (80); HR-ESI-MS (pos.): *m/z* 523.3037 ($[M+Na]^+$, $C_{30}H_{44}O_6Na$, Calcd 523.3036).

Camellisin C (**3**): Colorless needle crystals in MeOH; mp 206—208 °C; $[\alpha]_D^{24.4}$ +10.0 (c=0.10, C_5H_5N); UV λ_{max} (MeOH) nm (log ε): 197 (3.40), 206 (3.67), 352 (0.67); IR (KBr) ν_{max} cm^{-1}: 3430, 3004, 2977, 2955, 2875, 1738, 1709, 1673, 1637, 1475, 1461, 1401, 1369, 1301, 1276, 1229, 1184, 1152, 1033; ^{1}H- and ^{13}C-NMR: listed in Table 1; ESI-MS (pos.): *m/z* (%): 1059 $[2M+Na]^+$ (5), 557 $[M+K]^+$ (6), 541 $[M+Na]^+$ (100); HR-ESI-MS (pos.): *m/z* 541.3148 ($[M+Na]^+$, $C_{30}H_{46}O_7Na$, Calcd 541.3141).

Cytotoxicity Assay The following human tumor cell lines were used: HL-60 (human myeloid leukemia cell line), SMMC-7721 (human hepatocarcinoma cell line), A-549 (lung cancer cell line), PANC-1 (human pancreatic carcinoma) and SK-BR-3 (breast cancer cell line). All the cells were cultured in RPMI-1640 or Dulbecco's modified Eagle's medium (DMEM) (Hyclone, Logan, UT, U.S.A.), supplemented with 10% fetal bovine serum (Hyclone, U.S.A.) at 37 °C in a humidified atmosphere with 5% CO_2. Cell viability was assessed by conducting colorimetric measurements of the amount of insoluble formazan formed in living cells based on the reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (Sigma, St. Louis, MO, U.S.A.). Briefly, 100 μl adherent cells were seeded into each well of a 96-well cell culture plate and allowed to adhere for 12 h before drug addition, while suspended cells were seeded just before drug addition, both with initial density of 1×10^5 cells/ml in 100 μl medium. Each tumor cell line was exposed to the tested compound at various concentrations in triplicates for 48 h, with *cis*-platin (Sigma, U.S.A.) as positive control. After the incubation, MTT (100 μg) was added to each well, and the incubation continued for 4 h at 37 °C. The cells were lysed with 100 μl 20% sodium dodecyl sulfate (SDS)–50% *N*,*N*-dimethylformamide (DMF) after removal of 100 μl medium. The optical density of the lysate was measured at 595 nm in a 96-well microtiter plate reader (Bio-Rad 680, U.S.A.). The IC_{50} value of each compound was calculated by the Reed and Muench's method.

Acknowledgment This work was supported financially by the NSFC (No. 20902093 to J.-X. Pu), State Key Laboratory of Phytochemistry and Plant Resources in West China (No. P2008-ZZ13), the Major State Basic Research Development Program of China (No. 2009CB522300), the Western Doctoral Foundation of Chinese Academy of Sciences (J.-X. Pu).

References

1) Harbowy M. E., Balentine E. A., *Crit. Rev. Plant Sci.*, **16**, 415—480 (1997).
2) Katiyar S. K., Mukhtar H., *Int. J. Oncol.*, **8**, 221—238 (1996).
3) Ahmad N., Katiiyar S. K., Mukhtar H., "Nutrition and Chemical Toxicity," ed. by Ioannides C., John Wiley & Sons, West Sussex, England, 1998, pp, 301—343.
4) Lu Y., Umeda T., Yagi A., Sakata K., Chaudhuri T., Ganguly D. K., Sarma S., *Phytochemistry*, **53**, 941—946 (2000).

5) Chaudhuri T., Das S. K., Vedasiromoni J. R., Ganguly D. K., *J. Indian Chem. Soc.*, **74**, 166 (1997).
6) Kikuchi T., Matsuda S., Kadota S., Sakai Y., Namba T., Watanabe K., Dissanayake D. M. R. B., *Chem. Pharm. Bull.*, **32**, 3906—3911 (1984).
7) Yu D. L., Zhang Q., Zhang H. Y., Pan J. X., *Nat. Prod. Res. Dev.*, **9**, 14—18 (1997).
8) Zhou S. M., Zhou K., Xiao D. J., *Nat. Prod. Res. Dev.*, **17**, 428—430 (2005).
9) Erdemoglu N., Sahin E., Sener B., Ide S., *J. Mol. Struct.*, **692**, 57—62 (2004).
10) Antonio G. G., Rafael E. R., Carmen M., *J. Nat. Prod.*, **52**, 1139—1142 (1989).
11) Luc P., Tess D. B., Magda C., Arnold V., *J. Nat. Prod.*, **56**, 899—906 (1993).
12) Tsutomu W., Yoshimi N., Tadataka N., *Phytochemistry*, **66**, 589—597 (2005).
13) Hans A., Markus S., Manuel A. C., *Phytochemistry*, **27**, 1835—1841 (1988).
14) Heba H. B., Mahmoud A. M. N., Joachim B., Michael L., *Phytochemistry*, **26**, 1837—1838 (1987).
15) Hideaki O., Zhong X. N., Hirata E., Shinzato T., Takeda Y., *Chem. Pharm. Bull.*, **49**, 1093—1097 (2001).
16) Antonio G. G., Jose A. G., Angel G. R., Ignacio A. J., *J. Nat. Prod.*, **57**, 400—402 (1994).
17) Brigida D. A., Marina D. G., Antonio F., Pietro M., Palma O., Fabio T., *Phytochemistry*, **65**, 497—505 (2004).
18) Mark A. S., Francis I. L., Williams P. J., *J. Agric. Food Chem.*, **38**, 2045—2049 (1990).
19) Rajib K. M., Yoshinori F., Katsumi K., *Phytochemistry*, **37**, 1641—1643 (1994).
20) Xu R., Fazio G. C., Matsuda S. P. T., *Phytochemistry*, **65**, 261—291 (2004).
21) Scott A. I., Wrixon A. D., *Tetrahedron*, **26**, 3695—3715 (1970).
22) Moffitt W., Woodward R. B., Moscowitz A., Klyne W., Djerassi C., *J. Am. Chem. Soc.*, **83**, 4013—4018 (1961).