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New Triterpenoids and Other Constituents from a Special Microbial-Fermented Tea—Fuzhuan Brick Tea

Tie-Jun Ling,[†,‡] Xiao-Chun Wan,*[,†,‡] Wei-Wei Ling,[‡] Zheng-Zhu Zhang,[‡] Tao Xia,[‡] Da-Xiang Li,[‡] and Ru-Yan Hou[‡]

[†]Postdoctoral Research Station in Horticulture and [‡]Key Laboratory of Tea Biochemistry and Biotechnology of Ministry of Education and Ministry of Agriculture, Anhui Agricultural University, 130 West Changjiang Road, Hefei 230036, People's Republic of China

Fuzhuan brick tea, a special microbial-fermented tea prepared from the leaves of *Camellia sinensis* var. *sinensis*, is a traditional beverage used in China throughout history. Phytochemical investigation of this material led to the identification of three new triterpenoids, 3β,6α,13β-trihydroxyolean-7-one (**1**), 3β-acetoxy-6α,13β-dihydroxyolean-7-one (**2**), and 3β-*O*-(8-hydroxyoctanoyl)-12-oleanene (**3**), together with 11 known compounds, friedelin (**4**), β-amyrone (**5**), β-amyrin (**6**), α-spinasterone (**7**), α-spinasterol (**8**), 22,23-dihydro-α-spinasterone (**9**), 22,23-dihydro-α-spinasterol (**10**), α-phytol (**11**), α-tocopherol (**12**), α-tocoquinone (**13**), and caffeine (**14**). The structures of **1**−**13** were determined by spectroscopic and chemical methods. Compounds **1** and **2** are the first two examples of triterpenoids possessing a 6-hydroxy-7-one function. All of the compounds, except **6**, **8**, **10**, **11**, and **14**, were isolated from tea and *Camellia* spp. for the first time. The antibacterial activities of **1** were assessed against some enteric pathogenic microbes. Compound **1** showed no cytotoxic activity against A-549, Bel-7402, and HCT-8 cell lines.

KEYWORDS: Fuzhuan brick tea; *Camellia sinensis* var. *sinensis*; triterpenoids; antibacterial activities; cytotoxic activity

INTRODUCTION

Tea is a popular beverage with a long history. On the basis of different manufacturing processes, Chinese commercial teas are usually classified into six categories, namely, green tea, oolong tea, black tea, white tea, yellow tea, and dark tea. Among them, dark tea is the common name for several microbial-fermented teas, with Puer (Pu-er or Pu-erh) tea and Fuzhuan brick tea (**Figure 1**) being the major brands (*1*, *2*). The healthy benefits of green tea, oolong tea, and black tea have been investigated thoroughly (*3*−*5*). Several new anticancer, antioxidant, or anti-allergic flavonoids in oolong tea and two new oxidation products of epigallocatechin gallate in black tea have been reported in recent years (*6*−*11*). However, few phytochemical works on dark tea have been carried out, although it has recently received some attention for its special beneficial health functions (*12*−*16*).

Fuzhuan brick tea, a popular beverage within ethnic groups in the border regions of southern/western China, has been produced exclusively in the Hunan province of China since the 1860s. This material is prepared from the leaves of *Camellia sinensis* var. *sinensis* and is unique because of a "fungal fermentation" stage in its manufacturing process. In this stage, many yellow fungi, which are called "golden flora", grow within the tea leaves under controlled temperature and moisture. The fungus growing during the fermentation has been identified as a mixture of several microorganisms, with *Eurotium* spp. as the dominant one (*12*).

*To whom correspondence should be addressed. Tel: +86-551-5786002. Fax: +86-551-5786765. E-mail: xcwan@ahau.edu.cn.

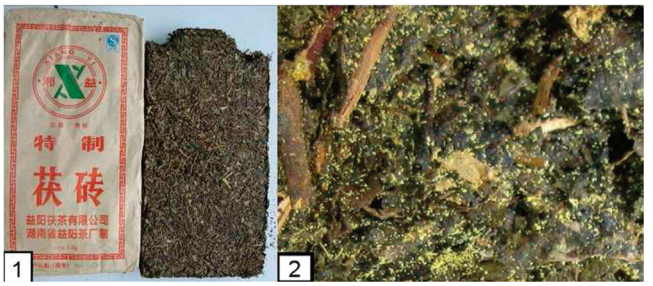

Figure 1. Fuzhuan brick tea (**1**) and the "golden flora" (the yellow dots) in its leaves (**2**).

Fungal growth is considered to be the key stage in the manufacture of Fuzhuan brick tea, as complex biochemical changes take place to produce the characteristic aroma and flavor.

Fuzhuan brick tea is not only a beverage but also a folk medicine for its remarkable antidysentery activity. It is known that this activity is increased with the course of the microbial fermentation, implying that certain metabolites of the fungi growing in tea leaves have the function of inhibiting enteric pathogenic microbes (*12*). In this investigation, we isolated a number of triterpenoids and other constituents from Fuzhuan brick tea and determined their structures by spectroscopic and chemical methods. The antibacterial and cytotoxic activities of a novel triterpenoid in this material were assessed against some enteric pathogenic microbes and some cancer cell lines.

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MATERIALS AND METHODS

General. The melting point was uncorrected. Optical rotations were measured on a P-1020 Polarimeter (Jasco, Tokyo, Japan). IR spectra were measured on a Nicolet 8700 FT-IR spectrophotometer (Thermo, United States). The ^{1}H NMR (400 MHz), ^{13}C NMR (100 MHz), distortionless enhancement by polarization transfer (DEPT), and 2D NMR spectra such as ^{1}H−^{1}H correlation spectroscopy (COSY), HXCO, heteronuclear single quantum coherence (HSQC), heteronuclear multiple bond correlation (HMBC), and nuclear Overhauser effect spectroscopy (NOESY) were recorded on an Avance-400 instrument (Bruker, United States). Chemical shifts were expressed in ppm (δ). The high-resolution time-of-flight−mass spectrometry (HRTOF-MS) were recorded on a GCT-MS instrument (Micromass Ltd., United Kingdom) by direct inlet. The electrospray ionization−mass spectrometry (ESI-MS) data were obtained with a Finnigan LTQ LC/MS system (Thermo) by direct inlet (ESI-MS conditions: sheath gas flow rate, 14 arb; aux/sweep gas flow rate, 0 arb; spray voltage, 4.20 kV; capillary temperature, 300 °C; capillary voltage, 4.30 V; and tube lens offset, 30.0 V). The gas chromatography−mass spectrometry (GC-MS) analyses were performed with a Shimadzu GCMS-QP2010S instrument [GC conditions: DB-5 capillary column (30 m × 0.25 mm); column temperature, 50 °C (hold time, 2 min), 50→250 °C (rate of temperature increase, 8 °C/min), 250→300 °C (rate of temperature increase, 3 °C/min), 300 °C (hold time, 18 min); injector temperature, 250 °C; and He at 1.0 mL/min]. Silica gel 60 (200−300 mesh, Qingdao Marine Chemical Co. Ltd., Qingdao, People's Republic of China) and YMC GEL ODS-A (50 μm, YMC Co. Ltd., Japan; column dimension: 500 mm × 25 mm i.d.; flow rate, 7 mL/min) were used for column chromatography (CC). The column eluant was monitored by thin-layer chromatography (TLC), which was performed on precoated silica gel plates (GF254, Liangchen Chemical Co. Ltd., Huoshan, Anhui province, People's Republic of China) or on precoated RP-18 silica gel plates (F254, Merck, Germany), with detection by spraying in sequence with 2% ethanolic $FeCl_3$ and 1% methanolic *p*-anisaldehyde H_2SO_4 reagent followed by heating.

Materials. Fuzhuan brick tea (produced in December 2006) was purchased from Yiyang Fu Cha Industry Development Co. Ltd. (Yiyang, Hunan province, People's Republic of China). Butyl *p*-hydroxybenzoate was purchased from Beijing Chemical Co. Ltd. (Beijing, People's Republic of China). Noroxin was purchased from Shanghai Yan-an Pharmaceutical Co. Ltd. (Shanghai, People's Republic of China). Berberine hydrochloride was purchased from Yabaoguangtai Pharmaceutical Co. Ltd. (Pengzhou, Sichuan province, People's Republic of China). Beef extract and peptone were purchased from Beijing Aobo-xing Biotechnology Co. Ltd. (Beijing, People's Republic of China). Agar powder was purchased form Hangzhou Microbial Reagent Co. Ltd. (Hangzhou, Zhejiang province, People's Republic of China). Sodium chloride and sodium hydroxide were purchased from Xuzhou Chemical Co. Ltd. (Xuzhou, Jiangsu province, People's Republic of China). Caffeine was purchased from Sigma Chemical Co. Ltd. (United States).

Extraction and Isolation. Fuzhuan brick tea (3.6 kg) was ground and extracted with 70% aqueous acetone at room temperature three times (10, 6, and 6 L). After acetone was removed under reduced pressure, the aqueous solution was extracted with petroleum ether and chloroform, respectively. The petroleum ether solution (fraction A) was concentrated under reduced pressure to afford a residue (50 g). This residue was applied to a silica gel CC, eluting with a petroleum ether−acetone mixture of increasing polarity (20:1 to 5:1), yielding five fractions (**Figure 2**).

Fraction 1 (4 g) was fractionated by silica gel CC using petroleum ether−acetone (99: 1) as the eluant, resulting in three subfractions (1A−1C). Compound **4** (65 mg) was obtained from subfraction 1A by recrystallization. Subfraction 1B was subjected to a silica gel CC using petroleum ether−$CHCl_3$ (5: 1) as the eluant, yielding **5** (70 mg). Subfraction 1C was subjected to repeated ODS CC eluted with methanol to yield **7** (30 mg) and **9** (45 mg). Fraction 2 (5 g) was subjected to a silica gel CC, eluted with petroleum ether−acetone (99: 1) and an ODS CC eluted with methanol to yield **12** (50 mg). Fraction 3 (2 g) was subjected to an ODS CC using methanol as the eluant to yield **6** (62 mg), **11** (106 mg), and **13** (11 mg). Fraction 4 (5 g) was sequentially chromatographed on a silica gel column eluted with $CHCl_3$−MeOH (99: 1) mixture and repeated ODS CC using methanol as the eluant, yielding **8** (7 mg) and **10** (9 mg). Fraction 5 (6 g) was fractionated by silica gel CC employing a gradient of $CHCl_3$ to $CHCl_3$−MeOH (98: 2) resulting in three subfractions (5A−5C). Subfractions 5A and 5B were purified by ODS CC eluting with methanol to afford **2** (5 mg) and **3** (11 mg), respectively. Subfraction 5C was sequentially chromatographed on a silica gel column eluted with petroleum ether−acetone (19: 1) mixture and an ODS CC using methanol−H_2O (90: 10) as the eluant to afford **1** (20 mg).

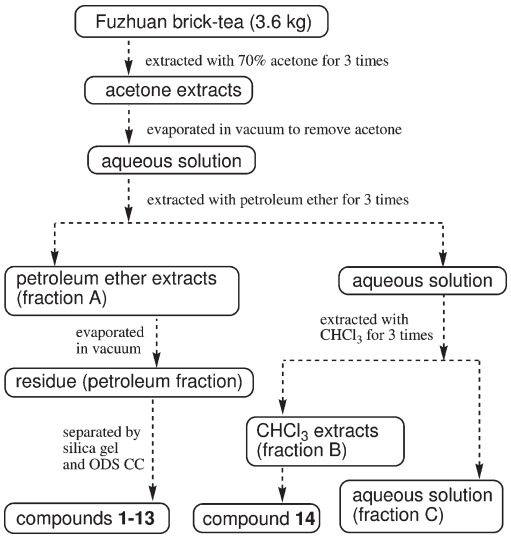

Figure 2. Flowchart of extraction and isolation of **1**−**14** from Fuzhuan brick tea.

The $CHCl_3$ extract (fraction B) was concentrated to give the crude compound **14** (40 g). The remaining aqueous solution was named as fraction C.

3*β*,6α,13*β*-Trihydroxyolean-7-one (1). Colorless gum, $[\alpha]_D^{25}$ + 15 (*c* 0.002, MeOH). IR (KBr) ν_{max} (cm^{-1}): 3435, 2919, 2850, 1707, 1464, 1395, 1364, 1294, 1101, 1031, 989. ESI-MS: *m*/*z* 473 $[M - H]^-$ in negative mode, 497 $[M + Na]^+$ in positive mode. HRTOF-MS: *m*/*z* 474.3712 (calcd for $C_{30}H_{50}O_4$, 474.3709). GC-MS: GC t_R 48.71 min. EI-MS (70 eV): *m*/*z* 456 (45), 441 (79), 405 (17), 355 (9), 303 (21), 281 (14), 263 (16), 219 (100), 175 (27), 151 (28), 135 (36), 121 (52), 109 (39), 95 (64), 83 (67), 69 (68), 55 (71), 41 (74). ^{1}H and ^{13}C NMR data, see **Table 1**.

3*β*-Acetoxy-6α,13*β*-dihydroxyolean-7-one (2). Colorless needles, mp 285−289 °C, $[\alpha]_D^{25}$ + 14 (*c* 0.001, MeOH). IR (KBr) ν_{max} (cm^{-1}): 3485, 3434, 3003, 2923, 2854, 1716, 1463, 1398, 1377, 1269, 1098, 1026. ESI-MS: *m*/*z* 515 $[M - H]^-$ in negative mode, 539 $[M + Na]^+$ in positive mode. HRTOF-MS: *m*/*z* 516.3812 (calcd for $C_{32}H_{52}O_5$, 516.3815). GC-MS: GC t_R 51.02 min. EI-MS (70 eV): *m*/*z* 498 (14), 483 (38), 355 (3), 302 (7), 219 (61), 175 (17), 151 (15), 136 (15), 121 (23), 107 (20), 95 (32), 83 (22), 69 (35), 55 (24), 43 (100, CH_3CO). ^{1}H NMR ($CDCl_3$) δ: 1.11 (1H, overlapped, H-1α), 2.589 (1H, ddd, *J* = 14.0, 3.6, 3.6 Hz, H-1*β*), 1.61 (2H, overlapped, H-2), 4.491 (1H, dd, *J* = 10.4, 6.0 Hz, H-3α), 1.53 (1H, overlapped, H-5), 4.907 (1H, d, *J* = 12.0 Hz, H-6*β*), 0.811 (1H, m, H-9), 1.58 (2H, overlapped, H-11), 1.19 (2H, overlapped, H-12), 1.08 (1H, overlapped, H-15α), 2.097 (1H, ddd, *J* = 13.6, 13.6, 4 Hz, H-15*β*), 1.823 (1H, ddd, *J* = 13.6, 13.6, 4.0 Hz, H-16α), 1.18 (1H, overlapped, H-16*β*), 1.51 (1H, overlapped, H-18), 1.989 (1H, dd, *J* = 12.8, 2.0 Hz, H-19α), 1.26 (1H, overlapped, H-19*β*), 1.47 (1H, overlapped, H-21a), 1.32 (1H, overlapped, H-21b), 1.38 (1H, overlapped, H-22a), 1.11 (1H, overlapped, H-22b), 0.872 (3H, s, CH_3-23), 0.895 (3H, s, CH_3-24), 1.146 (3H, s, CH_3-25), 1.373 (3H, s, CH_3-26), 0.923 (3H, s, CH_3-27), 1.233 (3H, s, CH_3-28), 0.955 (3H, s, CH_3-29), 0.903 (3H, s, CH_3-30), 2.041 (3H, s, C**H**$_3$CO). ^{13}C NMR ($CDCl_3$) δ: 39.95 (C-1), 23.83 (C-2), 80.52 (C-3), 39.42 (C-4), 56.87 (C-5), 72.35 (C-6), 211.17 (C-7), 43.39 (C-8), 55.46 (C-9), 39.04 (C-10), 17.57 (C-11), 34.04 (C-12), 82.39 (C-13), 45.19 (C-14), 22.58 (C-15), 30.57 (C-16), 33.59 (C-17), 49.19 (C-18), 38.33 (C-19), 31.39 (C-20), 33.99 (C-21), 38.53 (C-22), 28.20 (C-23), 16.47 (C-24), 16.05 (C-25), 20.51 (C-26), 18.44 (C-27), 31.27 (C-28), 31.86 (C-29), 25.04 (C-30), 170.97 (CH_3**C**O), 21.32 (CH_3CO).

3*β*-*O*-(8-Hydroxyoctanoyl)-12-oleanene (3). White amorphous powder, $[\alpha]_D^{25}$ + 74.5 (*c* 0.001, MeOH). HRTOF-MS: *m*/*z* 568.4861 (calcd. for $C_{38}H_{64}O_3$, 568.4855). ^{1}H NMR ($CDCl_3$) δ: 5.140 (1H, t, *J* = 3.6 Hz,

Table 1. NMR Spectroscopic Data of **1** (in $CDCl_3$)

position	δ_C	δ_H (J in Hz)	HMBC (^{1}H to ^{13}C)
1α	40.26 t	1.05[a]	
1β		2.569 ddd (14.0, 3.6, 3.6)	C-2, 3, 9, 10, 25
2	27.57 t	1.59[a]	C-3, 4
3	78.52 d	3.190 dd (10.4, 6.0)	C-4, 23, 24
4	39.54 s		
5	56.97 d	1.512 br d (12.0)[a]	C-4, 6, 7, 9, 25
6	72.39 d	4.900 d (12.0)	C-4, 5, 7, 8
7	211.32 s		
8	43.36 s		
9	55.35 d	0.706 dd (10.6, 3.8)	C-5, 10, 11
10	39.39 s		
11	17.68 t	1.60[a]	C-9, 10, 12
12	34.03 t	1.19[a]	C-13, 18
13	82.36 s		
14	45.18 s		
15α	22.59 t	1.1[a]	
15β		2.10 ddd (13.6, 13.6, 4.0)	C-8, 13, 14, 16, 17, 27
16α	30.55 t	1.822 ddd (13.6, 13.6, 4.0)	C-14, 15, 17, 18, 22, 28
16β		1.20[a]	
17	33.58 s		
18	49.12 d	1.512 br d (12.0)[a]	C-12, 13, 14, 19, 28
19α	38.54 t	1.979 dd (12.8, 2.0)	C-13, 17, 18, 20, 21, 29, 30
19β		1.28[a]	
20	31.38 s		
21	34.03 t	1.36[a]	C-19, 20
22	39.03 t	1.22[a], 1.39[a]	
23	28.21 q	0.995 s	C-3, 4, 24
24	15.32 q	0.817 s	C-3, 4, 23
25	16.02 q	1.120 s	C-5, 9, 10
26	20.52 q	1.369 s	C-8, 14
27	18.47 q	0.922 s	C-8, 13, 14, 15
28	31.28 q	1.230 s	C-16, 17, 18, 22
29	31.88 q	0.895 s	C-19, 20, 21, 29
30	25.02 q	0.951 s	C-19, 20, 21, 30
OH		3.673 br s	

[a] Signals were overlapped.

H-12), 4.463 (1H, dd, J = 10.0, 6.4 Hz, H-3), 3.598 (2H, t, J = 6.8 Hz, H-8′), 2.258 (2H, t, J = 7.2 Hz, H-2′), 1.130 (3H, s, CH_3-27), 0.963 (6H, s, CH_3-25 and 26), 0.866 (12H, s, CH_3-23, 24, 29, 30), 0.828 (3H, s, CH_3-28). ^{13}C NMR ($CDCl_3$) δ: 38.30 (C-1), 23.70 (C-2), 80.68 (C-3), 38.78 (C-4), 55.31 (C-5), 18.29 (C-6), 32.64 (C-7), 39.85 (C-8), 47.60 (C-9), 36.89 (C-10), 23.56 (C-11), 121.68 (C-12), 145.23 (C-13), 41.76 (C-14), 28.40 (C-15), 26.17 (C-16), 32.51 (C-17), 47.29 (C-18), 46.83 (C-19), 31.08 (C-20), 34.77[a] (C-21), 37.17 (C-22), 28.08 (C-23), 16.78 (C-24), 15.55 (C-25), 16.84 (C-26), 25.96 (C-27), 26.97 (C-28), 33.32 (C-29), 23.63 (C-30), 173.59 (C-1′), 34.79[a] (C-2′), 25.06 (C-3′), 29.03[b] (C-4′), 29.11[b] (C-5′), 29.69 (C-6′), 32.71 (C-7′), 63.00 (C-8′). Superscript *a* and *b* indicate that signals could be interexchanged. The assignments were confirmed by a combination of the 1D NMR as well as HSQC and HMBC spectra.

Methanolysis of 3. Compound **3** (0.7 mg) was refluxed with 0.9 N HCl in 82% aqueous MeOH (10 mL) for 18 h. The resulting solution was extracted three times with chloroform. The chloroform solution was dried over anhydrous Na_2SO_4 and then concentrated to yield a white amorphous powder. GC-MS: GC t_{R1} 15.86 min (8-hydroxyoctanic acid methyl ester). EI-MS (70 eV): m/z 144 (8), 124 (9), 101 (19), 87 (47), 74 (100), 59 (26), 55 (74), 43 (46), 41 (47). GC t_{R2} 43.87 min (β-amyrin). EI-MS (70 eV): m/z 426 (2), 257 (1), 218 (100), 203 (50), 189 (18), 175 (9), 161 (8), 147 (9), 135 (12), 119 (15), 107 (20), 95 (25), 81 (21), 69 (25), 55 (23), 41 (20).

Antibacterial Assays. Antibacterial activities were evaluated by the hole plate diffusion method (*17*). The test microorganisms were enteropathogenic *Escherichia coli* (EPEC), *E. coli*, *Staphyloccocus aureus*, *Shigella dysenteriae*, and *Salmonella typhi*, which were obtained from the School of Basic Medical Sciences, Anhui Medical University (Hefei, People's Republic of China). Compound **1**, noroxin (positive control 1), berberine hydrochloride (positive control 2, a moderate antidysentery drug with few side effect), and butyl *p*-hydroxybenzoate (positive control 3, a standard preservative) were individually dissolved and diluted with dimethyl sulfoxide (DMSO) to obtain serial concentrations of 800, 400, 200, 100, 50, and 25 μg/mL (for noroxin, the solutions were serially diluted from

Table 2. Antibacterial Activities of **1**

	MIC (μg/mL)[a]				
compd	EPEC	*E. coli*	*S. aureus*	*S. dysenteriae*	*S. typhi*
1	400	>800	>800	100	800
noroxin	25	12.5	1.56	6.25	0.78
BHC[b]	50	800	400	100	800
BHB[c]	50	800	800	400	>800

[a] The results were the average of three readings. [b] Berberine hydrochloride. [c] Butyl *p*-hydroxybenzoate.

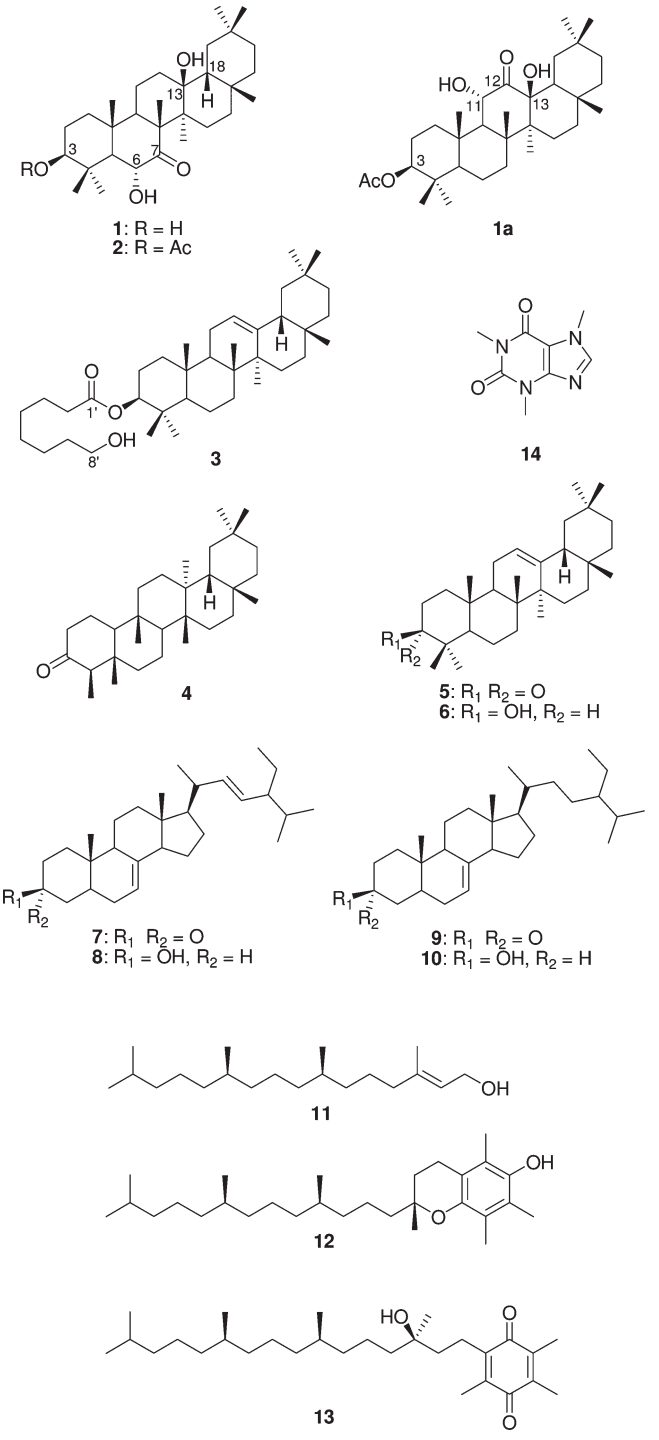

Figure 3. Structures of the compounds **1**−**14**.

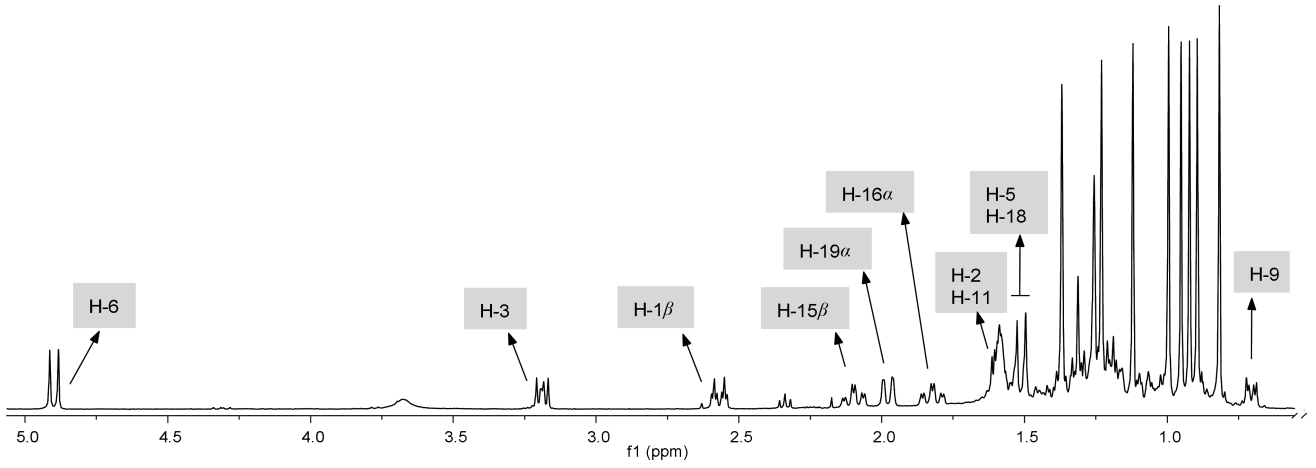

Figure 4. ^{1}H NMR spectrum of **1**.

800 to 0.39 μg/mL). Five 6 mm wide holes were bored with a sterilized steel borer into the nutrient agar media (3 g of beef extract, 10 g of peptone, 17 g of agar, 5 g of NaCl, and 1000 mL of H_2O, pH 7.2) in each Petri dish inoculated with the test microorganism. The solution of the compound (60 μL) at a specific concentration was added into each of the holes. DMSO was used as the negative control. The plates were then incubated at 37 °C for 24 h. The inhibition zones around the holes were measured, and the minimal inhibitory concentration (MIC), which was defined as the lowest concentration able to inhibit any visible bacterial growth, was recorded. The assays were performed three times to guarantee reproducibility of the results (see **Table 2**).

Cytotoxic Assays. A-549, Bel-7402, and HCT-8 cells were obtained from the National Center for Drug Screening of Institute of Materia Medica, Chinese Academy of Medical Sciences & Peking Union Medical College. Cytotoxicity assays of **1** were carried out by MTT colorimetric method.

Cells were seeded in 96-well microplates (500 cells/well) and routinely cultured in a humidified incubator (37 °C in 5% CO_2) for 24 h. Fluorouracil (positive control, Sigma Chemical Co. Ltd.) and **1** (10 μL each) were added in serial concentrations (0.005, 0.05, 0.5, and 5 μg/mL) with an additional 190 μL of RPMI1640 cell culture medium. The cells were reincubated for 72 h. Then, the medium was discarded, and 100 μL of tetrazolium dye (MTT) solution (0.5 mg/mL in serum free medium) was added to each well, and the solution was incubated for an additional 4 h. Then, the medium was discarded again, and 200 μL of DMSO was added to dissolve the formazan crystals. The plate was read on a microplate reader at 544 nm. MTT solution with DMSO (without cells and medium) acted as a blank control in microplate reading. Compound **1** showed no cytotoxic activity against the three cell lines.

RESULTS AND DISCUSSION

The 70% aqueous acetone extract of commercial Fuzhuan brick tea was separated successively by partitioning with petroleum ether and $CHCl_3$ and by repeated silica gel CC and ODS CC to afford three new triterpenoids, **1**−**3** (**Figure 3**), as well as 11 other known compounds, which were identified as friedelin (**4**) (*18*), β-amyrone (**5**) (*19*), β-amyrin (**6**) (*20*), α-spinasterone (**7**) (*21*), α-spinasterol (**8**) (*22*), 22,23-dihydro-α-spinasterone (**9**) (*22*), 22,23-dihydro-α-spinasterol (**10**) (*22*), α-phytol (**11**) (*23*), α-tocopherol (**12**) (*24*), α-tocoquinone (**13**) (*24*, *25*), and caffeine (**14**), respectively, on the basis of their spectroscopic data and by comparison with the reference values and by authentic samples.

Compound **1** was obtained as a colorless gum. It could be dissolved in DMSO, acetone, methanol, or chloroform very well. The molecular formula of **1** was suggested as $C_{30}H_{50}O_4$ by the HRTOF-MS at *m*/*z* 474.3712. The presence of hydroxyl and carbonyl groups was indicated by its IR spectrum (3435 and 1707 cm^{-1}). The ^{1}H NMR spectrum of **1** (**Figure 4**) showed a dd

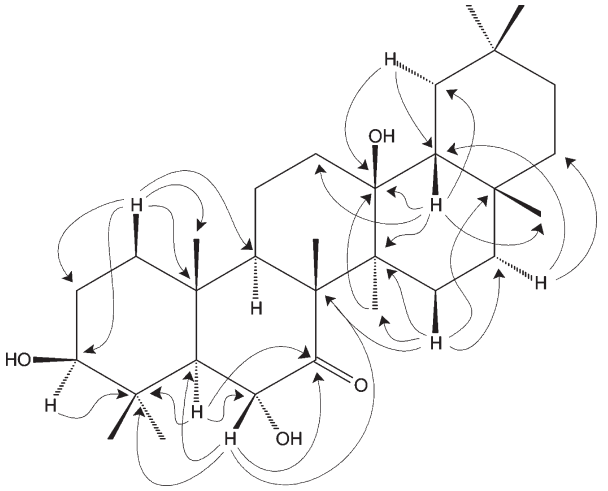

Figure 5. Key HMBC correlations of **1** (^{1}H to ^{13}C).

signal at δ 3.190 (*J* = 10.4, 6.0 Hz), as well as eight tertiary methyl group signals at δ 1.369, 1.230, 1.120, 0.995, 0.951, 0.922, 0.895, and 0.817. The ^{13}C NMR spectrum showed 30 carbon signals, which were assigned by DEPT as eight methyl, nine methylene, four methine, and nine quaternary (including a carbonyl) carbons. The above evidence revealed an oleanane skeleton with a keto and 3β-hydroxyl groups. The ^{1}H and ^{13}C NMR spectra of **1** were similar to those of 3β-acetoxy-11α,13β-dihydroxyolean-12-one (**1a**, **Figure 3**) (*26*, *27*). However, the different chemical shifts of the carbonyl carbon between **1** (δ 211.32) and **1a** (δ 201.2) indicated that the ketone in **1** had only one hydroxyl group at its α position (*28*). The HMBC (**Figure 5**) spectrum of **1** showed correlations from δ 3.190 (H-3) and δ 1.512 (br d) to δ 39.54 (C-4). By combination of the HXCO spectrum, the signals of δ 1.512 (br d) and δ 56.97 (CH) were assigned to H-5 and C-5, respectively. The ^{1}H−^{1}H COSY coupling between the signals of δ 1.512 (H-5) and δ 4.900, as well as the HXCO correlation from δ 4.900 to δ 72.39 (CH), together with the HMBC correlations from δ 1.512 (H-5) and δ 4.900 to δ 211.32, suggested that the ketone was positioned at C-7, and C-6 was oxygenated. The assignment of a hydroxyl group at C-13 was deduced from the HMBC correlations from CH_3-27 (δ 0.922), H-18 (δ 1.512), and H-19α (δ 1.979) to C-13 (δ 82.36). It should be noted that the ^{1}H NMR signals of H-5 and H-18 were overlapped with each other (**Table 1** and **Figure 4**). However, the identification of these two proton signals was not so hard with analysis of the HMBC

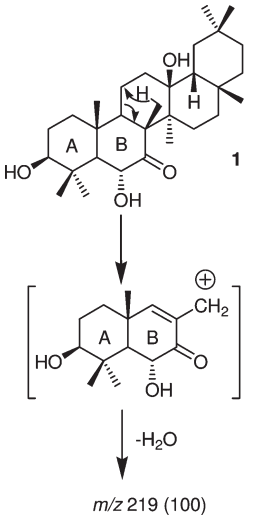

Figure 6. Significant EI-MS fragmentation pattern of **1**.

spectrum. Furthermore, the structure of the A/B rings was supported by the EI-MS signal of 219 (100), which was caused by migration of H-26 followed by loss of a H_2O (**Figure 6**) (*29*). The α configuration of 6-OH was indicated by the trans diaxial coupling constant of 12.0 Hz for H-5 and H-6 and by analysis of the NOESY spectrum, which showed cross-peaks between H-6 and CH_3-24, -25, and -26, respectively. The hydroxyl group at C-13 should automatically be axial to minimize the ring strain (*26*, *27*). The NOESY spectrum of **1** did not show any valuable signals for H-18. However, the chemical shift of C-28 (δ 31.28) supported the 18βH configuration very well, as the carbon signal of C-28 should be shifted upfield into δ 15−17 ppm region in the case of 18αH-oleanane, due to the shield of two γ-gauche interactions with the axial protons at C-19 and C-21 (*30*, *31*). Therefore, the structure of **1** was determined as 3β,6α,13β-trihydroxyolean-7-one.

Compound **2** was isolated as colorless needles. Its molecular formula was found to be $C_{32}H_{52}O_5$ as evidenced by HRTOF-MS at *m*/*z* 516.3812. The NMR spectra of **2** illustrated similar features to **1**, with few variations. The extra 3H singlet at δ 2.041, and the downfield shift of the H-3 double doublet from δ 3.190 to 4.491 in the ^{1}H NMR spectrum, together with the extra ^{13}C NMR signals of δ 170.97 (s) and 21.32 (q), indicated that the 3-OH of **2** was acetylated. The structure was confirmed by combined analysis of the HSQC and HMBC spectra. Similar to those of **1**, the β-configurations of 3-OH, H-6, and H-18 were determined by analysis of the ^{1}H and ^{13}C NMR data, and the orientation of 13-OH should also be β to have the stable skeleton. On the base of these evidence, **2** was identified as 3β-acetoxy-6α,13β-dihydroxy-olean-7-one.

Compound **3** was found to possess a molecular formula of $C_{38}H_{64}O_3$ as the HRTOF-MS gave the signal of *m*/*z* 568.4861. The NMR spectra of **3** were similar to those of β-amyrin (**6**). The ^{1}H NMR spectrum of **3** showed two extra 2H triplets at δ 3.598 (J = 6.8 Hz) and 2.258 (J = 7.2 Hz), respectively, together with downfield shift of H-3 double doublet (J = 10.0, 6.4 Hz, axial) to δ 4.463. Correspondingly, eight extra carbon signals at δ 173.59 (s), 63.00 (t), 34.79 (t), 32.71 (t), 29.69 (t), 29.11 (t), 29.03 (t), and 25.06 (t) were observed in its ^{13}C NMR and DEPT spectra. The above information indicated that the 3-OH of **3** was acylated by a terminal hydroxylized octanoic acid. To prove this supposition, methanolysis of **3** was achieved to afford a fatty acid methyl ester and a triterpenoid moiety, which were identified as 8-hydroxyoctanic acid methyl ester and β-amyrin, respectively, by GC-MS analysis. In conclusion, compound **3** was identified as 3β-*O*-(8-hydroxyoctanoyl)-12-oleanene.

The antibacterial activities of **1** against EPEC, *E. coli*, *S. aureus*, *S. dysenteriae*, and *S. typhi* were evaluated by the hole plate diffusion method (*17*). Compound **1** exhibited weak activities against EPEC and *S. typhi*, with MIC values of 400 and 800 μg/mL, respectively. All were less potent than noroxin. However, the inhibition activity of **1** against *S. dysenteriae* was as valuable as that of berberine hydrochloride with a moderate MIC value of 100 μg/mL. The results of the present assays may imply **1** as a potential safe antidysentery drug or a lead compound, for it showed very low antibacterial activity against *E. coli*, which plays an important role in human intestinal functions.

It is interesting that we did not find any evidence for the presence of triterpenoid saponins in fraction C, by very careful TLC surveys of color reactions (*32*, *33*). This situation is not compatible with the previous chemical reports on the leaves of *C. sinensis* var. *sinensis* (*34*−*36*). Furthermore, the structures of the triterpenoids in this material are rather unique, as compounds **1** and **2** are the first two examples of triterpenoids possessing 6-hydroxy-7-one function, and triterpenoids with saturated skeletons (**1**, **2**, and **4**) have never been reported from *Camellia* spp. before. These information may suggest that the triterpenoids and their saponins in tea leaves are chemically modified by fungal growth in Fuzhuan brick tea.

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Supporting Information Available: Graphs, data, and photographs. This material is available free of charge via the Internet at http://pubs.acs.org.

LITERATURE CITED

(1) Wan, X. C. *Tea Biochemistry*, 3rd ed.; China Agriculture Press: Beijing, People's Republic of China, 2003; pp 268−275 (in Chinese).
(2) Jin, X. Y.; Chen, J. B.; Ji, K. W. *Tea Process Engineering*, 1st ed.; China Agriculture Press: Beijing, People's Republic of China, 2003; pp 45−125 (in Chinese).
(3) Yang, C. S.; Wang, X.; Lu, G.; Picinich, S. C. Cancer prevention by tea: animal studies, molecular mechanisms and human relevance. *Nat. Rev. Cancer* **2009**, *9*, 429–439.
(4) Chopade, V. V.; Phatak, A. A.; Upaganlawar, A. B.; Tankar, A. A. Green tea (*Camellia sinensis*): Chemistry, traditional, medicinal uses and its pharmacological activities—A review. *Pharmacogn. Rev.* **2008**, *2*, 157–162.
(5) Das, S.; Thakuria, N.; Kanodia, L. Biological actions and medicinal applications of tea (*Camellia sinensis*). *J. Clin. Diagn. Res.* **2008**, *2*, 1215–1225.
(6) Ishida, H.; Wakimoto, T.; Kitao, Y.; Tanaka, S.; Miyase, T.; Nukaya, H. Quantitation of chafurosides A and B in tea leaves and isolation of prechafurosides A and B from oolong tea leaves. *J. Agric. Food Chem.* **2009**, *57*, 6779–6786.
(7) Lee, V. S. Y.; Chen, C. R.; Liao, Y. W.; Tzen, J. T. C.; Chang, C. I. Structural determination and DPPH radical-scavenging activity of two acylated flavonoid tetraglycosides in oolong tea (*Camellia sinensis*). *Chem. Pharm. Bull.* **2008**, *56*, 851–853.
(8) Niho, N.; Mutoh, M.; Sakano, K.; Takahashi, M.; Hirano, S.; Nukaya, H.; Sugimura, T.; Wakabayashi, K. Inhibition of intestinal carcinogenesis by a new flavone derivative, chafuroside, in oolong tea. *Cancer Sci.* **2006**, *97*, 248–251.

(9) Mihara, R.; Mitsunaga, T.; Fukui, Y.; Nakai, M.; Yamaji, N.; Shibata, H. A novel acylated quercetin tetraglycoside from oolong tea (*Camellia sinensis*) extracts. *Tetrahedron Lett*. **2004**, *45*, 5077–5080.
(10) Sano, M.; Suzuki, M.; Miyase, T.; Yoshino, K.; Yamamoto, M. M. Novel antiallergic catechin derivatives isolated from oolong tea. *J. Agric. Food Chem*. **1999**, *47*, 1906–1910.
(11) Tanaka, T.; Matsuo, Y.; Kouno, I. A novel black tea pigment and two new oxidation products of epigallocatechin-3-O-gallate. *J. Agric. Food Chem*. **2005**, *53*, 7571–7578.
(12) Mo, H. Z.; Zhu, Y.; Chen, Z. M. Microbial fermented tea—A potential source of natural food preservatives. *Trends Food Sci. Technol*. **2008**, *19*, 124–130.
(13) Xu, X. Q.; Mo, H. Z.; Yan, M. C.; Zhu, Y. Analysis of characteristic aroma of fungal fermented Fuzhuan brick-tea by gas chromatography/mass spectrophotometry. *J. Sci. Food Agric*. **2007**, *87*, 1502–1504.
(14) Kuo, K. L.; Weng, M. S.; Chang, C. T.; Tsai, Y. J.; Lin-Shiau, S. Y.; Lin, J. K. Comparative studies on the hypolipidemic and growth suppressive effects of oolong, black, Pu-erh, and green tea leaves in rats. *J. Agric. Food Chem*. **2005**, *53*, 480–489.
(15) Duh, P. D.; Yen, G. C.; Yen, W. J.; Wang, B. S.; Chang, L. W. Effects of Pu-erh tea on oxidative damage and nitric oxide scavenging. *J. Agric. Food Chem*. **2004**, *52*, 8169–8176.
(16) Zhou, Z. H.; Zhang, Y. J.; Xu, M.; Yang, C. R. Puerins A and B, two new 8-C substituted flavan-3-ols from Pu-er tea. *J. Agric. Food Chem*. **2005**, *53*, 8614–8617.
(17) Feng, N.; Ye, W. H.; Wu, P.; Huang, Y. C.; Xie, H. H.; Wei, X. Y. Two new antifungal alkaloids produced by *Streptoverticillium morookaense*. *J. Antibiot*. **2007**, *60*, 179–183.
(18) Ageta, H.; Arai, Y.; Suzuki, H.; Kiyotani, T.; Kitabayashi, M. NMR spectra of triterpenoids. III. oleanens and migrated oleanens. *Chem. Pharm. Bull*. **1995**, *43*, 198–203.
(19) Lin, J. F.; Zhang, C. F.; Zhang, M.; Wang, Z. T. Study on the chemical constituents in roots and rhizomes of *Ligularia duciforms*. *China J. Chin. Mat. Med*. **2005**, *30*, 1927–1929 (in Chinese).
(20) Seo, S.; Tomita, Y.; Tori, K. Carbon-13 NMR spectra of urs-12-enes and application to structural assignments of components of *Isodon Japonicus* Hara tissue cultures. *Tetrahedron Lett*. **1975**, *16*, 7–10.
(21) Akihisa, T.; Kimura, Y.; Tai, T.; Arai, K.; Astertarone, B. a hydroxy-triterpenoid ketone from the roots of *Aster tataricus* L. *Chem. Pharm. Bull*. **1999**, *47*, 1161–1163.
(22) Ding, L. S.; Chen, Y. Z.; Wu, F. E. A study on the constituents of the root of *Berneuxia thibetica* Decne. *China J. Chin. Mat. Med*. **1991**, *16*, 289–290 (in Chinese).
(23) Brown, G. D. Phytene-1,2-diol from *Artemisia annua*. *Phytochemistry* **1994**, *36*, 1553–1554.
(24) Du, G. S.; Cai, X. H.; Shang, J. H.; Luo, X. D. Non-alkaline constituents from the leaf of *Alstonia scholaris*. *Chin. J. Nat. Med*. **2007**, *5*, 259–262 (in Chinese).
(25) Rasool, N.; Khan, A. Q.; Ahmad, V. U.; Malik, A. A benzoquinone and a coumestan from *Psoralea plicata*. *Phytochemistry* **1991**, *30*, 2800–2803.
(26) Herath, H. M. T. B.; Athukoralage, P. S.; Jamie, J. F. Two oleanane triterpenoids from *Gordonia ceylanica* and their conversions to taraxarane triterpenoids. *Phytochemistry* **2000**, *54*, 823–827.
(27) Herath, H. M. T. B.; Athukoralage, P. S. Triterpenoids of *Gordonia dassanayakei*. *Nat. Prod. Sci*. **2000**, *6*, 102–105.
(28) Lee, C.; Hong, S. S.; Han, X. H.; Jin, Q. H.; Li, D. Y.; Kim, T. O.; Kim, H. K.; Lee, J.; Kwon, S. H.; Kim, Y. B.; Lee, M. K.; Hwang, B. Y. A new abietane diterpenoid from *Isodon inflexus*. *Arch. Pharm. Res*. **2008**, *31*, 1381–1384.
(29) Budzikiewicz, H.; Wilson, J. M.; Djerassi, C. Mass spectrometry in structural and stereochemical problems. XXXII.[1] Pentacyclic triterpenes. *J. Am. Chem. Soc*. **1963**, *85*, 3688–3699.
(30) Duddeck, H.; Elgamal, M. H. A.; Ricca, G. S.; Danieli, B.; Palmisano, G. Carbon-13 nuclear magnetic resonance spectra VIII-18α-and 18β-glycyrrhetic acid derivatives. *Org. Magn. Reson*. **1978**, *11*, 130–132.
(31) Ricca, G. S.; Danieli, B.; Palmisano, G.; Duddeck, H.; Elgamal, M. H. A. Carbon-13 NMR spectra of some pentacyclic triterpenoids with the olean-12-ene and 18α-olean-12-ene skeleton. *Org. Magn. Reson*. **1978**, *11*, 163–166.
(32) Kreutzer, S.; Schimmer, O.; Waibel, R. Triterpenoid sapogenins in the genus *Grindelia*. *Planta Med*. **1990**, *56*, 392–394 (in German).
(33) Lee, T. M.; Marderosian, A. D. Two-dimensional TLC analysis of ginsenosides from root of dwarf ginseng (*Panax trifolius* L.) araliaceae. *J. Pharm. Sci*. **1981**, *70*, 89–91.
(34) Morikawa, T.; Nakamura, S.; Kato, Y.; Muraoka, O.; Matsuda, H.; Yoshikawa, M. Bioactive saponins and glycosides. XXVIII.[1)] New triterpene saponins, foliatheasaponins I, II, III, IV, and V, from Tencha (the leaves of *Camellia sinensis*). *Chem. Pharm. Bull*. **2007**, *55*, 293–298.
(35) Kobayashi, K.; Teruya, T.; Suenaga, K.; Matsui, Y; Masuda, H.; Kigoshi, H. Isotheasaponins B_1–B_3 from *Camellia sinensis* var. *sinensis* tea leaves. *Phytochemistry* **2006**, *67*, 1385–1389.
(36) Sagesaka, Y. K.; Uemura, T.; Watanabe, N.; Sakata, K.; Uzawa, J. A new glucuronide saponin from tea leaves (*Camellia sinensis* var. *sinensis*). *Biosci., Biotechnol., Biochem*. **1994**, *58*, 2036–2040.

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