Cytotoxic Pentacyclic Triterpenoids from the Rhizome of *Astilbe chinensis*

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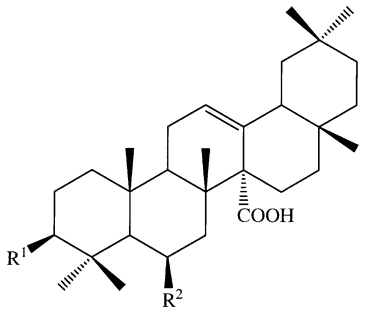
Guided by *in vitro* antineoplastic tests, four cytotoxic pentacyclic triterpenoids, 3β-hydroxyolean-12-en-27-oic acid (**1**), 3β-hydroxyurs-12-en-27-oic acid (**2**), 3β,6β-dihydroxyolean-12-en-27-oic acid (**3**), and 3β-acetoxyolean-12-en-27-oic acid (**4**) were isolated from the rhizome of *Astilbe chinensis*. Their structures were determined on the basis of chemical evidence and extensive spectroscopic methods including one-dimensional and two-dimensional NMR. These compounds showed cytotoxic activities against HO-8910, Hela and HL60 cell lines. In addition, β-sitosterol palmitate, daucosterol, and β-sitosterol have also been isolated.

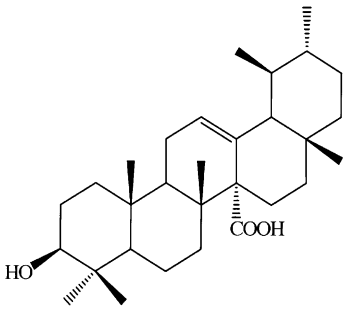
Introduction. – *Astilbe chinensis* (MAXIM.) FRANCH. et SAVAT. (Saxiffagaceae) is a perennial herbaceous plant growing at an altitude of 390–3600 m in China, Russia, Japan, and Korea. Its rhizome, known as '*Luo Xinfu*' (Chinese name) and '*Aka-shouma*' (Japanese name), has been used for headache, arthragia, chronic bronchitis and stomachalgia in traditional Chinese medicine [1–3]. *Jin et al.* reported the isolation of gallic acid, (+)-catechin, (+)-gallocatechin, bergenin, and 11-*o*-gallobergenin from the rhizome of *A. chinensis* var. *davidii* [4]. Pharmacological experiments indicated that the extracts from *A. chinensis* had antineoplastic and immunopotentiating activities [5][6]. In this paper, we report chemical studies of the petroleum-ether extract from the rhizome of *Astilbe chinensis* by screening with antineoplastic tests *in vitro*. We have obtained four cytotoxic pentacyclic triterpenoids: 3β-hydroxyolean-12-en-27-oic acid (**1**), 3β-hydroxyurs-12-en-27β-oic acid (**2**), 3β,6β-dihydroxyolean-12-en-27-oic acid (**3**), and 3β-acetoxyolean-12-en-27-oic acid (**4**). In addition, β-sitosterol palmitate (**5**) [7], β-sitosterol (**6**) [8], and daucosterol (**7**) [8] have been also isolated.

Results and Discussion. – The EtOH extract of the rhizome of *Astilbe chinensis* was partitioned into petroleum-ether-, AcOEt-, BuOH-, and H_2O-soluble fractions. From the petroleum-ether extract, four components, **1**–**4**, were obtained and purified by repeated chromatography on silica gel. Each compound was subjected to detailed spectroscopic analysis to elucidate their chemical structures.

Compound **1** was isolated as colorless crystals of m.p. 240.5–242.5°, and showed positive *Lieberman–Buchard* reaction. High-resolution EI-MS showed the molecular ion at *m*/*z* 456.3594 in agreement with the molecular formula $C_{30}H_{48}O_3$ (calc. 456.3603). EI-MS displayed the base peak fragment at *m*/*z* 248 ($C_{16}H_{24}O_2$), and other prominent



1 R^1=OH, R^2=H
3 R^1=OH, R^2=OH
4 R^1=AcO, R^2=H



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fragments at *m/z* 207 ($C_{14}H_{23}O$), 206 ($C_{14}H_{22}O$), and 190 ($C_{14}H_{22}$). Its IR spectrum showed OH (3460 cm^{-1}), COOH (3420–2650, 1710 cm^{-1}), and olefinic (1630 cm^{-1}) groups. The assignments of all the ^{1}H- and ^{13}C-NMR signals of **1** were successfully carried out with ^{1}H,^{1}H-COSY, HMQC, and HMBC experiments (*Table 1*). Thus, compound **1** was identified as 3β-hydroxyolean-12-en-27-oic acid.

The ^{13}C-NMR and DEPT spectra (125 MHz, CD_3OD) of compound **1** allowed the assignment of 30 ^{13}C signals to seven Me, ten CH_2, 5 CH groups, and eight quaternary C-atoms. The ^{1}H-NMR spectrum (500 MHz) showed seven tertiary Me signals at δ 0.78 (Me(24)), 0.82 (Me(29)), 0.84 (Me(30)), 0.85 (Me(28)), 0.96 (Me(25)), 0.97 (Me(23)), and 1.02 (Me(26)). The signal at δ 3.20 (*dd*, $J=14$, 5, H–C(3)) corresponded to a H-atom geminal to the OH function and correlated to the C-atom at δ 78.4 (C(3)). A *triplet*, appearing at δ 5.66 (*t*, $J=4.5$, H–C(12)), corresponded to the olefinic H-atom present and correlated to the C-atom at δ 126.2 (C(12)). The olefinic C-atom C(13) was quaternary and appeared at 138.2. These data and the molecular formula suggested that **1** is an oleanane-type triterpene with a OH group in ring A or B, a C(12)=C(13) bond, and a COOH group in ring D or E.

Assuming an oleane skeleton for compound **1**, the only point remaining to be established is the position of the COOH group. The most-significant differences between the ^{13}C-NMR spectrum of compound **1** and that of oleanolic acid are the resonances of the olefinic C-atoms. Except for those cases with substituents in close proximity to a C(12)=C(13) bond, the chemical shifts of C(12) and C(13) of olean-12-enes are at δ *ca.* 122 and 145 ppm, respectively. The presence of a COOH group at C(14) (γ and δ to C(13) and C(12), resp.) has a pronounced effect on the olefinic C-atom resonance [9]. The chemical shifts of C(12) and C(13) in olean-12-enes such as cincholic acid appear at δ 125.9 and 138.1, respectively, *i.e.*, C(12) is deshielded by 3.8 ppm and C(13) is shielded by 5.3 ppm [10]. The chemical shifts of C(12) and C(13) in compound **1** appear at δ 126.2 and 138.2, respectively. Thus, the COOH group was placed at C(14). *Dawidar et al.* reported ^{13}C-NMR data of the methyl ester derivative prepared from manevalic acid (3β,6α-dihydroxyolean-12-en-27-oic acid) and azidic acid (3β,6α-dihydroxyolean-12-en-27,28-dioic acid) containing a MeOCO group at C(14) [11]. The chemical shifts of C(12) and C(13), however, have normal values of the

Table 1. *^{1}H- and ^{13}C-NMR Data of Compounds **1**, **3** and **4**, as well as* ^{1}H,^{1}H-COSY and HMBC Data of Compound **1** (δ in ppm, J in Hz)[a])

Position	**1** (CD_3OD)				**3** (C_5ND_5)		**4** (CD_3OD)	
	^{13}C-NMR	^{1}H-NMR	H,H-COSY	HMBC	^{13}C-NMR	^{1}H-NMR	^{13}C-NMR	^{1}H-NMR
1	38.6 (*t*)	1.65, 1.69		C(3),C(5), C(10), C(25)	41.5 (*t*)	1.24, 1.75	38.0 (*t*)	1.65, 1.69
2	27.5 (*t*)	1.68, 2.03	H–C(3)	C(1), C(3)	28.5 (*t*)	0.97, 1.86	23.5 (*t*)	1.67, 1.98
3	79.4 (*d*)	3.20 (*dd*, J = 5, 14)	H–C(2)	C(2), C(4), C(24)	78.4 (*d*)	3.30 (*dd*, J = 5.5, 14.5)	80.8 (*d*)	4.51 (*dd*, J = 6.3, 14.3)
4	38.67 (*s*)				40.4 (*s*)		37.6 (*s*)	
5	55.2 (*d*)	0.69 (*d*, J = 13)	H–C(6)	C(4), C(6), C(10), C(25)	56.1 (*d*)	1.09 (*m*)	55.1 (*d*)	0.79 (*t*, J = 2.5)
6	18.2 (*t*)	1.50, 1.53	H–C(5)	C(5), C(7), C(8), C(10)	67.5 (*d*)	4.84 (*m*)	18.2 (*t*)	1.30, 1.47
7	36.3 (*t*)	1.65, 1.22		C(5), C(6), C(8), C(9), C(14), C(26)	45.1 (*t*)	2.37, 2.04	36.3 (*t*)	1.22, 1.70
8	39.74 (*s*)				39.6 (*s*)		39.9 (*s*)	
9	47.2 (*d*)	2.05 (*m*)		C(8), C(10), C(11), C(12), C(13), C(25), C(26)	48.1 (*d*)	2.91 (*dd*, J = 9, 12)	46.9 (*d*)	2.12 (*dd*, J = 6.0, 14.5)
10	37.1 (*s*)				37.3 (*s*)		36.9 (*s*)	
11	22.8 (*t*)	1.95, 1.08	H–C(12)	C(9), C(10), C(12), C(13), C(25), C(26)	23.6 (*t*)	1.80, 2.25	22.7 (*t*)	1.08, 1.89
12	126.2 (*d*)	5.66 (*t*, J = 4.5)	H–C(11)	C(9), C(11), C(14), C(18)	125.9 (*d*)	5.85 (*t*, J = 4.5)	126.2 (*d*)	5.70 (*dd*, J = 3, 11.5)
13	138.2 (*s*)				138.1 (*s*)		137.4 (*s*)	
14	55.8 (*s*)				56.9 (*s*)		55.7 (*s*)	
15	22.2 (*t*)	1.73, 2.03		C(13), C(27)	23.1 (*t*)	2.04, 2.47	22.2 (*t*)	1.71, 2.00
16	26.9 (*t*)	1.66 (*m*)		C(15), C(17), C(28)	28.5 (*t*)	2.08, 2.47	27.5 (*t*)	1.71, 2.10
17	32.8 (*s*)				33.5 (*s*)		32.9 (*s*)	
18	49.0 (*d*)	2.03 (*m*)		C(12), C(13), C(19)	49.9 (*d*)	2.19 (*dd*, J = 4.5, 14.0)	49.2 (*d*)	2.00 (*dd*, J = 6.5, 12.5)
19	43.9 (*t*)	1.33, 0.99		C(13), C(17), C(18), C(20), C(21), C(30)	44.5 (*t*)	1.36, 1.79	43.8 (*t*)	1.04, 1.30
20	31.1 (*s*)				31.2 (*s*)		31.0 (*s*)	
21	36.5 (*t*)	1.14, 1.22		C(17), C(19), C(20), C(22)	34.8 (*t*)	1.05, 1.30	34.3 (*t*)	0.99, 1.16
22	34.3 (*t*)	0.99, 1.08		C(16), C(17), C(20), C(2)	37.1 (*t*)	1.20, 1.38	36.5 (*t*)	1.20, 1.34
23	28.1 (*q*)	0.97 (*s*)		C(3), C(4), C(5), C(24)	28.7 (*q*)	1.257 (*s*)	28.1 (*q*)	0.84 (*s*)
24	16.0 (*q*)	0.78 (*s*)		C(3), C(4), C(5), C(23)	18.1 (*q*)	1.72 (*s*)	16.5 (*q*)	0.85 (*s*)
25	16.4 (*q*)	0.96 (*s*)		C(1), C(5), C(9), C(10)	18.0 (*q*)	1.70 (*s*)	16.8 (*q*)	0.99 (*s*)
26	18.0 (*q*)	1.02 (*s*)		C(7), C(8), C(9), C(14)	20.4 (*q*)	1.68 (*s*)	18.1 (*q*)	1.03 (*s*)
27	179.4 (*s*)				178.8 (*s*)		180.7 (*s*)	
28	28.2 (*q*)	0.85 (*s*)		C(16), C(17), C(18), C(22)	28.5 (*q*)	1.01 (*s*)	28.5 (*q*)	0.84 (*s*)
29	33.5 (*q*)	0.82 (*s*)		C(19), C(20), C(21), C(30)	33.5 (*q*)	0.72 (*s*)	33.4 (*q*)	0.82 (*s*)
30	23.6 (*q*)	0.84 (*s*)		C(19), C(20), C(21), C(29)	23.8 (*q*)	0.88 (*s*)	23.6 (*q*)	0.84 (*s*)
1′							21.4 (*q*)	2.06 (*s*)
2′							171.3 (*s*)	

[a]) ^{1}H-NMR, ^{1}H,^{1}H-COSY, HMBC, and ^{13}C-NMR spectra were obtained at 500 and 125 MHz at room temperature, respectively. Multiplicities by DEPT experiments in parentheses: *s:* quaternary; *d:* CH; *t:* CH_2, and *q:* Me C-atoms.

oleanolic-acid type and show no shielding effect of a COOH group. Evidently, a re-investigation of the structures of these two triterpene acids appears to be necessary.

The C=C bond in compound **1** was established to be located between C(12) and C(13) due to HMBC correlations from H–C(9), H–C(11), and H–C(18) to C(12), correlations from H–C(9), H–C(11), H–C(15), and H–C(18) to C(13), and correlations from H–C(12) to C(9), C(11), C(14), and C(18), and by H,H-COSY correlation between H–C(11) and H–C(12). The OH group was assigned to be at C(3) based on the HMBC correlations from H–C(3) to C(2), C(4), C(23), and C(24). We observed that H–C(2), H–C(23), and H–C(24) showed correlations to C(3); and H–C(3) had correlation with H–C(2). The β configuration of the OH group at C(3) was evident from the chemical-shift values and coupling constants [12]. Further ^{13}C-NMR spectral evidence for this assignment was obtained. *Crews* and *Kho-Wiseman* have shown that the ^{13}C resonance of Me groups at C(4) is strongly affected by the configuration of the OH group at C(3) [13]. Conversion of the OH group from axial to equatorial position results in an upfield shift of *ca.* 5 ppm for an axial Me group at C(4), while the equatorial Me group at C(4) is essentially unaffected by this transformation. The COOH group at C(14) was confirmed by HMBC correlation observed from H–C(15) to C(27).

Compound **2** was isolated as colorless crystals of m.p. 239–241°, and showed positive *Lieberman–Buchard* reaction. High-resolution EI-MS showed the molecular ion at *m/z* 456.3606 in agreement with the molecular formula $C_{30}H_{48}O_3$ (calc. 456.3604). EI-MS indicated the base peak fragment at *m/z* 248 ($C_{16}H_{24}O_2$), and other prominent fragments at *m/z* 208 ($C_{14}H_{24}O$), 207 ($C_{14}H_{24}O$), and 190 ($[C_{16}H_{26}O_2 - MeCOOH]^+$). Its IR spectrum showed OH (3460 cm^{-1}), COOH (3420–2650, 1710 cm^{-1}), and olefinic (1630 cm^{-1}) groups. The assignments of all the ^{1}H- and ^{13}C-NMR signals of **2** were successfully carried out with ^{1}H,^{1}H COSY, HMQC, and HMBC experiments (*Table 2*). Thus, the structure of compound **2** was established to be 3β-hydroxyurs-12-en-27-oic acid.

The ^{13}C-NMR and DEPT spectra (125 MHz, CD_3OD) allowed the attribution of 30 ^{13}C signals to seven Me, ten CH_2, six CH groups, and seven quaternary C-atoms. The ^{1}H-NMR spectrum (500 MHz) demonstrated five tertiary Me signals at δ 0.78 (Me(24)), 0.82 (Me(28)), 0.96 (Me(25)), 0.96 (Me(23)), and 1.02 (Me(26)), and two secondary Me signals at δ 0.80 and 0.86. The signal at δ 3.22 (*dd*, $J = 6.5, 14$, H–C(3)) corresponded to a H-atom geminal to a OH function and correlated to the C-atom at δ 79.1 (C(3)). A broad *doublet*, appearing at δ 5.55, corresponded to the only olefinic H-atom present and correlated to the C-atom at δ 128.5 (C(12)). The olefinic C-atom C(13) was quaternary and appeared at 133.2. These data and molecular formula suggested that **2** is an ursane-type triterpene with a OH group in ring A or B, a C(12)=C(13) bond, and a COOH group in ring D or E.

The most-significant differences between the ^{13}C-NMR spectrum of compound **2** and that of ursolic acid involve the resonance of the olefinic C-atoms. Except for those cases with substituents in close proximity to a C(12)=C(13) bond, the chemical shifts of C(12) and C(13) of urs-12-enes are at δ *ca.* 125 and 138, respectively. The presence of a COOH group at C(14) (γ and δ to C(13) and C(12), resp.) has a pronounced effect on the olefinic C-atom resonance. The chemical shifts of C(12) and C(13) in urs-12-enes such as quinovic acid appear at δ 129.1 and 134.2, respectively, C(12) is deshielded by

Table 2. *^{1}H- and ^{13}C-NMR, ^{1}H,^{1}H-COSY, and HMBC Data of Compound* **2** *in CD_3OD* (δ in ppm, J in Hz)[a])

Position	^{13}C-NMR	^{1}H-NMR	H,H-COSY	HMBC
1	38.7 (*t*)	1.07, 1.70		C(2), C(3), C(5), C(9), C(10), C(25)
2	27.0 (*t*)	1.65, 2.03	H–C(3)	C(1), C(3)
3	79.1 (*d*)	3.21 (*dd*, *J* = 6.5, 14)	H–C(2)	C(2), C(4), C(24)
4	38.6 (*s*)			
5	55.1 (*d*)	0.71 (*d*, *J* = 14.5)	H–C(6)	C(4), C(7), C(10), C(25)
6	18.2 (*t*)	1.56 (*m*)	C(5)	C(5), C(7), C(8)
7	36.6 (*t*)	1.15, 1.67		C(5), C(6), C(8), C(9), C(14), C(26)
8	39.8 (*s*)			
9	46.8 (*d*)	2.21 (*dd*, *J* = 6.5, 14.5)	H–C(11)	C(8), C(10), C(11), C(25), C(26)
10	36.9 (*s*)			
11	22.7 (*t*)	1.92, 2.03	H–C(9), H–C(12)	C(8), C(9), C(10), C(12), C(13)
12	128.5 (*d*)	5.55 (br. *s*)	C(11)	C(9), C(11), C(14), C(18)
13	133.2 (*s*)			
14	55.9 (*s*)			
15	22.4 (*t*)	1.76, 1.98	H–C(16)	C(8), C(16), C(27)
16	28.9 (*t*)	0.90, 2.08	H–C(15)	C(15), C(17), C(18), C(28)
17	33.7 (*s*)			
18	60.2 (*d*)	1.34 (*m*)		C(12), C(13), C(14), C(19), C(28)
19	39.8 (*d*)	0.86 (*m*)		C(18), C(20), C(21), C(29)
20	37.5 (*d*)	0.85 (*m*)		C(19), C(21), C(30)
21	30.4 (*t*)	1.24, 1.34		C(17), C(19), C(20), C(22), C(30)
22	40.9 (*t*)	1.18, 1.41		C(17), C(20), C(21), C(28)
23	28.1 (*q*)	0.96 (*s*)		C(3), C(4), C(5), C(24)
24	15.7 (*q*)	0.78 (*s*)		C(3), C(4), C(5), C(23)
25	16.5 (*q*)	0.96 (*s*)		C(1), C(5), C(9), C(10)
26	18.2 (*q*)	1.02 (*s*)		C(7), C(8), C(9), C(14)
27	179.8 (*s*)			
28	29.0 (*q*)	0.80 (*s*)		C(16), C(17), C(18), C(22)
29	17.8 (*q*)	0.79 (*s*)		C(18), C(19), C(20)
30	21.3 (*q*)	0.86 (*s*)		C(19), C(20), C(21)

[a]) ^{1}H-NMR, ^{1}H,^{1}H-COSY, HMBC, and ^{13}C-NMR spectra were obtained at 500 and 125 MHz at room temperature, respectively. Multiplicity by DEPT experiments in parentheses; *s:* quaternary, *d:* CH, *t:* CH_2, and *q:* Me C-atoms.

3.6 ppm, and C(13) is shielded by 3.8 ppm. The chemical shifts of C(12) and C(13) in compound **2** appear at δ 128.5 and 133.2, respectively. Thus, the COOH group was located at C(14).

The C=C bond in compound **2** was established to be located between C(12) and C(13) due to HMBC correlations from H–C(9), H–C(11), and H–C(18) to C(12), correlations from H–C(11), H–C(15), and H–C(18) to C(13), and correlations from H–C(12) to C(9), C(11), C(14), and C(18), and ^{1}H,^{1}H-COSY correlation between H–C(11) and H–C(12). The OH group was determined to be at C(3) based on the HMBC correlations from H–C(3) to C(2), C(4), C(23), and C(24), correlations from H–C(2), H–C(23), and H–C(24) to C(3), and H,H-COSY correlation between H–C(2) and H–C(3). The β of the OH group at C(3) was evident from the chemical-shift values and coupling constant [10], and the ^{13}C resonance of Me groups at C(4)

[11]. The COOH group at C(14) was confirmed by HMBC correlation observed from H–C(15) to H–C(27).

Compound **3** was isolated as a white crystalline powder of m.p. 229–232°, and showed positive *Lieberman–Buchard* reaction. High-resolution EI-MS showed the molecular ion at *m/z* 472.3553 in agreement with the molecular formula $C_{30}H_{48}O_4$ (calc. 472.3553). EI-MS indicated the base-peak fragment at *m/z* 248 ($C_{16}H_{24}O_2$), and other prominent fragments at *m/z* 224 ($C_{14}H_{24}O_2$), 223 ($C_{14}H_{23}O_2$), and 190 ($C_{14}H_{242}$). The assignments of all the ^{1}H- and ^{13}C-NMR signals of **3** were successfully carried out with ^{1}H,^{1}H-COSY, HMQC, and HMBC experiments (*Table 1*). On the basis of its spectroscopic data and comparison with those of compound **1**, compound **3** was assigned to be 3β,6β-dihydroxyolean-12-en-27-oic acid.

The ^{13}C-NMR and DEPT spectra (125 MHz, C_5ND_6) exhibited 30 ^{13}C signals (seven Me, nine CH_2, six CH groups, and eight quaternary C-atoms). The ^{1}H- (500 MHz) and ^{13}C-NMR spectral data for compound **3** were similar to those of **1** with the exception that a signal for an additional OH group was present. The OH group was observed as a *multiplet* at δ 4.84, corresponding to the C-atom signal at δ 67.5 (C(6)) in the HMQC experiment. The only other difference in the ^{13}C-NMR spectral data between **1** and **3** occurred for C-atoms C(1)–C(7); C(1), C(2), and C(4)–C(7) were shifted downfield, and C(3) was shifted upfield (same positions in **1**), with the most marked differences observed for C(1), C(6), and C(7). The secondary OH group was assigned to the C(6) position based on an HMBC correlation from H–C(6) to C(8), a correlation from H–C(7) to C(6), and a ^{1}H,^{1}H-COSY correlation of H–C(6) with H–C(5) and H–C(7). The configuration of the OH group at C(6) was determined as β due to the coupling constants in the ^{1}H-NMR spectrum. The coupling pattern is similar to that of other 6β-OH in contrast to 6α-OH triterpenes, such as missourin, which shows a *doublet* of a *triplet* ($J = 11.4$, 7.2) pattern [14][15].

Compound **4** was isolated as colorless crystals of m.p. 235–237° and showed positive *Lieberman–Buchard* reaction. High-resolution EI-MS displayed the molecular ion at *m/z* 498.3705 in agreement with the molecular formula $C_{32}H_{50}O_4$ (calc. 498.3709). EI-MS indicated the base peak fragment at *m/z* 190 ($C_{16}H_{26}O_2$ – MeCOOH), and other prominent fragments at *m/z* 454 ($[M - CO_2]^+$), 438 ([M – MeCOOH]), 250 ($C_{16}H_{26}O_2$), 248 ($C_{16}H_{24}O_2$), and 203 ($[C_{16}H_{24}O_2 - COOH]^+$). The assignments of all the ^{1}H- and ^{13}C-NMR signals of **4** were successfully carried out with ^{1}H,^{1}H-COSY, HMQC, and HMBC experiments (*Table 1*). On the basis of its spectroscopic data and comparison with those of compound **1**, compound **4** was identified as 3β-acetoxyolean-12-en-27-oic acid.

The ^{13}C-NMR and DEPT spectra (125 MHz, $CDCl_3$) exhibited 32 ^{13}C signals (eight Me, ten CH_2, five CH groups, and nine quaternary C-atoms). The ^{1}H- (500 MHz) and ^{13}C-NMR spectral data for compound **4** were similar to those of **1** with the major difference being the absence of signals for a OH group and the presence of signals for the AcO group. An AcO Me signal was observed as a *singlet* at δ 2.06, corresponding to the C-atom at δ 21.4 in the HMQC experiment, with the AcO C=O signal appearing at δ 171.3. The signal at δ 4.51 (*dd*, $J = 6.3$, 14.3, H–C(3)) corresponded to a H-atom geminal to the AcO group and correlated to the C-atom at δ 80.8 (C(3)). The only other difference in the ^{13}C-NMR spectral data between **1** and **4** occurred for C-atoms C(1)–C(4), and C(24). C(3) and C(24) were shifted downfield, and C(1), C(2), and

C(4) were shifted upfield in comparison with the same positions in **1**, with the most marked differences occurring for C(2) and C(3), indicating that the AcO group in **4** has replaced the OH group in **1** at C(3). The AcO group at C(3) was corroborated by HMBC correlations from H–C(3) to C(2), C(4), C(23), and C(24), correlations from H–C(1), H–C(2), H–C(23), and H–C(24) to C(3), and ^{1}H,^{1}H-COSY correlation between H–C(2) and H–C(3).

Compound **5** was obtained as a white amorphous powder of m.p. 85–87° and showed positive *Lieberman–Buchard* reaction. High-resolution EI-MS showed the molecular ion at *m/z* 652.6140 in agreement with the molecular formula $C_{45}H_{80}O_2$ (calc. 652.6158). EI-MS indicated the base peak fragment at *m/z* 396. The assignments of all the ^{1}H- and ^{13}C-NMR signals of **5** were successfully carried out with ^{1}H,^{1}H-COSY, HMQC, and HMBC experiments (*Table 3*). Thus, compound **5** was assumed to be β-sitosterol palmitate [7].

The base peak at *m/z* 396 could stem from elimination of palmitic acid, leading to a conjugated diene system in the sterol moiety. The IR absorption maximum at 1740 cm^{-1} together with ^{1}H- and ^{13}C-NMR spectral data of **5** indicated a steroidal ester. The ^{1}H-NMR spectrum showed a *multiplet* between δ 0.64 and 2.1 from which a number of Me groups emerged. A *triplet* at δ 2.28 corresponding to the CH_2COO moiety was overlayed with another *multiplet*, but still discernible. *Multiplets* at δ 4.61 and 5.37 originated from H–C(3) and H–C(6), respectively. The ^{1}H- and ^{13}C-NMR spectral data for compound **5** were identical to those of β-sitosterol, with the major difference being that **5** showed an additional group of CH_2 signals from δ 29.2 to 29.7. This cluster of CH_2 groups is typical for a fatty acid chain. The only other differences were observed in the ^{13}C-NMR spectral data for C(2)–C(6) and C(24), indicating that the ester group had replaced the OH group in **5** at C(3). This position was confirmed by HMBC correlation observed from H–C(2), H–C(4) to C–C(3), and ^{1}H,^{1}H-COSY correlation observed for H–C(3) with H–C(2) and H–C(4). To identify the fatty acid, compound **5** was saponified, and palmitic acid was identified in the acid fraction by comparison of its EI-MS with a computer reference database.

The cytotoxicities of compounds **1**–**5** have not been reported previously. Therefore, the antineoplastic activity of **1**–**5** was determined by using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) colorimetric assay with three tumor cell lines: human ovarian carcinoma cell (HO-8910), human cervical squamous carcinoma cell (Hela), and human leukemia cell (HL60) [16]. The compounds **1**–**4** exhibited strong cytotoxic activity against these tumor cells *in vitro*. The inhibitory rates (%) of compound **1** were 87.8+5.5, 93.7 ± 1.5, and 96.4 ± 0.9 at 20 mg/l against HO-8910, Hela, and HL60, respectively. Those of compound **2** were 91.9 ± 5.6, 92.7 ± 2.1, and 90.0 ± 3.0 at 20 mg/l, respectively. Those of compound **3** were 85.2 ± 1.8, 93.1 ± 4.5, and 79.7 ± 13.5 at 40 mg/l, respectively, and those of compound **4** were 76.3 ± 6.6, 94.6 ± 1.1, and 94.4 ± 1.1 mg/l, respectively. With the inhibitory rates against three tumor cells being much bigger than 85% at 20 mg/l, compounds **1** and **2** should be effective tumor inhibitors.

Table 3. *^{1}H- and ^{13}C-NMR, ^{1}H,^{1}H-COSY, and HMBC Data of β-Sitosterol Palmitate* (**5**) *in $CDCl_3$* (δ in ppm, J in Hz)[a])

Position	^{13}C-NMR	^{1}H-NMR	^{1}H,^{1}H-COSY	HMBC
1	37.0 (*t*)	1.15, 1.86		C(3)
2	27.8 (*t*)	1.84 (*m*)	H–C(3)	C(1), C(3)
3	73.7 (*d*)	4.62 (*t*, J = 5.5)	H–C(2), H–C(4)	C(1′)
4	38.1 (*t*)	2.30 (*dd*, J = 10, 16.5)	H–C(3), H–C(6)	C(2), C(3), C(5), C(6), C(10)
5	139.7 (*s*)			
6	122.6 (*d*)	5.38 (*d*, J = 4.5 Hz)	H–C(4), H–C(7)	C(4), C(7), C(8), C(10)
7	31.9 (*t*)	1.20 (*m*)	H–C(6)	
8	31.8 (*d*)			
9	50.0 (*d*)			
10	36.6 (*s*)			
11	21.0 (*t*)			
12	39.7 (*t*)	1.20, 2.02		
13	42.3 (*s*)			
14	56.7 (*d*)	1.07 (*d*, J = 7.0)		C(13), C(18)
15	24.3 (*t*)			
16	28.2 (*t*)			
17	56.0 (*d*)	1.11 (*m*)		C(13), C(18)
18	11.8 (*q*)	0.68 (*s*)		C(12), C(13), C(14), C(17)
19	19.3 (*q*)	1.02 (*s*)		C(10), C(9), C(5)
20	36.1 (*d*)			
21	18.8 (*q*)	0.90 (*m*)		C(17), C(22), C(20)
22	33.9 (*t*)			
23	26.0 (*t*)			
24	45.8 (*d*)	0.93 (*d*, J = 8.5)		C(22), C(25)
25	29.1 (*d*)			
26	19.8 (*q*)	0.84 (*m*)		C(24), C(27), C(29)
27	19.0 (*q*)	0.81 (*m*)		C(24), C(26), C(29)
28	23.0 (*t*)			
29	12.0 (*q*)	0.82 (*m*)		C(24), C(28)
1′	173.4 (*s*)			
2′	34.7 (*t*)	2.28 (*t*, J = 9.5)	H–C(3′)	C(1′), C(3′), C(4′)
3′	25.1 (*t*)	1.26, 1.62	H–C(2′)	C(1′), C(2′), C(4′)
4′	29.3 (*t*)			
5′	29.4 (*t*)			
6′	29.5 (*t*)			
7′	29.6 (*t*)			
8′–13′	29.7 (*t*)			
14′	31.9 (*t*)			
15′	22.7 (*t*)			
16′	14.1 (*q*)	0.88 (*m*)		C(15′), C(14′)

[a]) ^{1}H-NMR, ^{1}H,^{1}H-COSY, HMBC, and ^{13}C-NMR spectra were obtained at 500 and 125 MHz at room temperature, respectively. Multiplicity by DEPT experiments in parentheses: *s:* quaternary, *d:* CH, *t:* CH_2, and *q:* Me C-atoms.

Experimental Part

General. TLC: silica-gel plates (*Kieselgel 60 F_{254}, E. Merck AG*, Germany); detection by spraying with 10% H_2SO_4 in EtOH, followed by beating (100°). Column chromatography (CC): silica gel (200–300 mesh). M.p.: *Shimadzu LIBROR AEC-200* instrument. IR Spectra: KBr pellets; *Perkin-Elmer 577* spectrometer. ^{1}H- and ^{13}C-NMR, DEPT, ^{1}H,^{1}H-COSY, HMQC, and HMBC spectra: at 500 MHz for ^{1}H, and 125 MHz for ^{13}C with a

Bruker DRX-500 NMR spectrometer; TMS as an internal standard. HR-EI-MS and EI-MS: *VG AUTOSPEC 800* spectrometer with glycerol as matrix.

Plant Material. The rhizomes of *Astilbe chinensis* were collected in Anji county, Zhejiang province, China, in August 2001. A voucher specimen (No. 200120) was kept in Laboratory of Nature and Biochemistry, College of Science, Zhejing university, Hangzhou, China, and identified by Prof. *Xue-xiang Ji* (Deparment of pharmacognosy, College of Pharmacy, Zhejiang University, Hangzhou, China).

Extraction and Isolation Procedures. The rhizomes of *Astilbe chinensis* were dried at 40° in the dark, in a ventilated hood, and ground. The material (5.0 kg) was extracted at r.t. three times with petroleum ether, with occasional stirring and filtered. The extracts were evaporated *in vacuo* to give 41.2 g of a gelatinous material. The extract (42.0 g) was absorbed onto silica gel (60 g) and chromatographed on a silica-gel (600 g) column with petroleum ether/AcOEt 50 : 1, 30 : 1, 15 : 1, 5 : 1, 3 : 1, 2 : 1 gradients. The eluted fractions were evaluated by TLC and combined to give 19 main fractions. *Fr. 3* (2 g) was rechromatographed on a silica-gel (40 g) column with petroleum ether to give pure *β*-sitosterol (**6**). From *Fr. 6*, crude crystals of *β*-sitosterol palmitate (**5**) were obtained. Recrystallization from AcOEt/MeOH 1 : 1 gave **5**. *Fr. 10* (1.2 g) was separated on a silica-gel (24 g) column with CH_3Cl to afford pure compounds **1**–**4**. *Fr. 17* was recrystallized from MeOH to give dancosterol (**7**).

3β-Hydroxyolean-12-en-27-oic Acid (**1**). Colorless crystals. M.p. 240.5–242.5°. IR (KBr): 3460, 3420–2650, 1710, 1630. ^{1}H- and ^{13}C-NMR, ^{1}H,^{1}H-COSY, and HMBC: see *Table 1*. HR-EI-MS: 456.3594 ($C_{30}H_{48}O_3^+$, M^+; calc. 456.3603). EI-MS: 456 (M^+), 438 ($[M-H_2O]^+$), 423 ($[M-H_2O-Me]^+$), 412 ($[M-CO_2]^+$), 397 ($[M-COOH-Me]^+$), 248 ($C_{16}H_{24}O_2^+$), 207 ($C_{14}H_{23}O^+$), 190 ($C_{14}H_{22}^+$), 175 ($C_{13}H_{19}^+$).

3β-Hydroxyurs-12-en-27-oic Acid (**2**). Colorless crystals. M.p. 239–241°. IR (KBr): 3420–2650, 1710, 1630. HR-EI-MS: 456.3606 ($C_{30}H_{48}O_3^+$, M^+; calc. 456.3604). ^{1}H- and ^{13}C-NMR, ^{1}H,^{1}H-COSY, and HMBC: see *Table 2*. EI-MS: 456 (M^+), 438 ($[M-H_2O]^+$), 423 ($[M-H_2O-Me]^+$), 411 ($[M-COOH]^+$), 395 ($[M-COOH-OH]^+$), 248 ($C_{16}H_{24}O_2^+$), 207 ($C_{14}H_{23}O^+$), 190 ($C_{14}H_{22}^+$), 175 ($C_{13}H_{19}^+$).

3β,6β-Dihydroxyolean-12-en-27-oic Acid (**3**). White crystalline powder. M.p. 229–232°. IR (KBr): 3460, 3420–2650, 1710, 1630. ^{1}H- and ^{13}C-NMR: see *Table 1*. HR-EI-MS: 472.3553 ($C_{30}H_{48}O_4^+$, M^+; calc. 472.3553). EI-MS: 472 (M^+), 454 ($[M-H_2O]^+$), 436 ($[M-2\,H_2O]^+$), 428 ($[M-CO_2]^+$), 413 ($[M-H_2O-CO_2]^+$), 248 ($C_{16}H_{24}O_2^+$), 224 ($C_{14}H_{24}O_2^+$), 223 ($C_{14}H_{23}O_2^+$), 206 ($C_{14}H_{22}O^+$), 191 ($C_{13}H_{21}O^+$), 180, 123.

3β-Acetoxyolean-12-en-27-oic Acid (**4**). Colorless crystals: M.p. 235–237°. IR (KBr): 3420–2650, 1710, 1630. ^{1}H- and ^{13}C-NMR: see *Table 1*. HR-EI-MS: 498.3705 ($C_{32}H_{50}O_4^+$, M^+; calc. 498.3709). EI-MS: 498 (M^+), 480 ($[M-H_2O]^+$), 454 ($[M-CO_2]^+$), 438 ($[M-MeCOOH]^+$), 423 ($[M-MeCOOH-Me]^+$), 395 ($[M-MeCOOH-Me-CO_2]^+$), 250 ($C_{16}H_{26}O_2^+$), 248 ($C_{16}H_{24}O_2^+$), 203 ($[C_{16}H_{24}O_2-COOH]^+$), 190 ($[C_{16}H_{26}O_2-MeCOOH]^+$), 175 ($[C_{16}H_{26}O_2-MeCOOH-Me]^+$).

β-Sitosterol Palmitate (**5**). White amorphous powder. M.p. 85–87°. IR (KBr): 1740 cm^{-1}. ^{1}H- and ^{13}C-NMR, ^{1}H,^{1}H-COSY, and HMBC: see *Table 3*. HR-EI-MS: 652.6140 ($C_{45}H_{30}O_2^+$, M^+; calc. 652.6158). EI-MS: 653 (M^+), 396 ($[M-Me(CH_2)_{14}COOH]^+$), 257 ($Me(CH_2)_{14}COOH^+$), 213 ($Me(CH_2)_{14}^+$).

Cytotoxicity Assays. The cytotoxicity of compounds **1**–**5** was tested in three cell lines: human ovarian carcinoma cell (HO8910), human cervical squamous carcinoma cell (Hela), and human leukemic cell (HL60). Cells were cultured at 37° under a humidified atmosphere of 5% CO_2 in RPMI 1640 medium supplemented with 10% fetal calf serum and dispersed in replicate 96-well plates with 5×10^4 cells/well for 48 h. Compounds **1**–**5** (1.25–200 μg/ml) or vincristine (used as a positive control) were then added. After 48 h of exposure to the toxins, cell viability was determined by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) colorimetric assay [14] by measuring the absorbance at 570 nm with an ELISA reader. Each test was performed in triplicate.

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REFERENCES

[1] J. T. Pan, *Acta Phytotaxonomica Sinica* **1985,** *23*, 432.
[2] J. T. Pan, *Acta Phytotaxonomica Sinica* **1995,** *33*, 390.
[3] M. Kubo, Y. Kimura, T. Namba, *Yakushigaku Zasshi* **1975**, *10*, 34.
[4] O. K. Jin, C. Y. Seuk, C. J. Shik, *Yakhak Hoechi* **1992**, *36*, 474.

[5] P. F. Lai, P. F. Chen, L. L. Chen, P. Zhang, *Journal of Zhejiang College of TCM* **1996**, *20*, 49.
[6] P. F. Chen, P. F. Lai, P. Zhang, L. L. Chen, *China Journal of Chinese Materia Medica* **1996**, *21*, 302.
[7] H. X. Sun, Y. P. Ye, K. Yang, *China Journal of Chinese Materia Medica* **2002**, *27*, 751.
[8] V. S. Parmar, S. C. Jain, S. Gupta, S. T. War, V. K. Rajwanshi, R. Kumar, *Phytochemistry* **1998**, *49*, 1069.
[9] B. M. Shashi, P. K. Asish, *Phytochemistry* **1994**, *37*, 1517.
[10] A. Rumbero-Sanchez, P. Vazquez, *Phytochemistry* **1991,** *30*, 623.
[11] A. A. Dawidar, J. Reisch, M. Amer, *Chem. Pharm. Bull*. **1979**, *27*, 2938.
[12] J. St. Pyrek, *Pol. J. Chem.* **1979**, *53*, 1071.
[13] P. Crews, E. Kho-Wiseman, *Tetrahedron Lett*. **1978**, 2487.
[14] R. Aquino, F. De Simeone, F. F. Vincieri, C. Pizza, *J. Nat. Prod.* **1990**, *53*, 559.
[15] S. M. Wong, Y. Oshima, J. P. Pezzuto, H. H. S. Fong, N. R. Farnsworth, *J. Pharm. Sci.* **1986**, *75*, 317.
[16] P. Price, T. J. McMillan, *Cancer Res.* **1990**, *50*, 1392.

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