Chemistry of Natural Compounds, Vol. 47, No. 6, January, 2012 [Russian original No. 6, November–December, 2011]

ANTITUMOR SESQUITERPENE POLYOL ESTERS FROM *Celastrus angulatus*

Shao-peng Wei,[1] Jie-yu Luan,[2] Zhi-qin Ji,[1] Bao-jun Shi,[1] Min Xu,[2] and Ji-wen Zhang[1]*

UDC 547.314 + 548.737

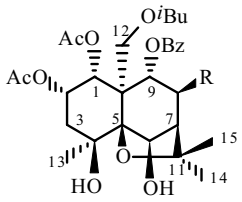
Two new antitumor sesquiterpene polyol esters with the β*-dihydroagarofuran skeleton,* ***1*** *and* ***2****, and three known compounds* ***3–5*** *were isolated from the high-polar MeOH extracts of the root bark of* Celastrus angulatus*. Their chemical structures were elucidated mainly by analyses of MS and NMR spectral data. Preliminary antitumor and insecticidal activities of these compounds were evaluated. Compounds* ***1*** *and* ***2*** *exhibited moderate antitumor activity against human breast cancer cell line (Bcap-37); the* IC_{50} *were 54.08 and 61.35* μ*M, respectively, and all the tested compounds were shown to possess minimal or no inhibitory activities against human colon (HT-29) and lung (NCI-H460) cancer cell lines. In addition, compounds* ***1–5*** *displayed mild insecticidal activities against the* 4^{th} *instar larvae* Mythimna separate*, the* KD_{50} *were 610.5, 308.8, 917.8, 510.6 and 1120.5, respectively.*

Keywords: *Celastrus angulatus*, *β*-dihydroagarofuran, antitumor activity, insecticidal activity.

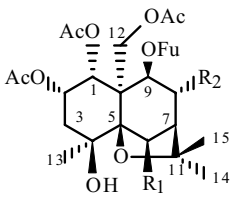
Natural products have been a valuable source of new compounds, as well as chemical entities in the agrochemical and pharmaceutical industries. Plant-derived products play important roles in natural sources. Over the last 30 years, a large variety of biologically-active secondary metabolites have been identified from the members of the Celastraceae [1].

Celastrus angulatus (Celastraceae) is widely distributed in China and has been used as a traditional medicinal herb and a plant insecticide [2–5] for many years. In previous studies, some antifeedant, narcotic, and insecticidal *β*-dihydroagarofuran sesquiterpene polyol esters and alkaloids were isolated from toluene extracts of the root bark of *C. angulatus* [6–13]. In an attempt to find novel bioactive compounds in the plant, we have restudied the constituents of its root barks. In the course of our continuing pursuit of the active principles of this plant, two new antitumor sesquiterpene polyol esters **1** and **2**, and three known compounds **3**, **4**, and **5** with the *β*-dihydroagarofuran skeleton, were isolated from the root bark of *C. angulatus*. In this article, the isolation, structure elucidation, and preliminary bioassay data for compounds **1–5** are presented.

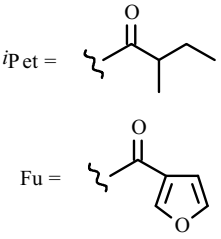
The air-dried root bark of *C. angulatus* was extracted with MeOH to obtain the crude extract. After evaporation, the crude extracts were subjected to macroporous resin column chromatography, repeated silica gel column chromatography, and RP-HPLC to yield **1–5**. Their structures were elucidated on the basis of UV, MS, and NMR spectroscopic evidence.



1, 2



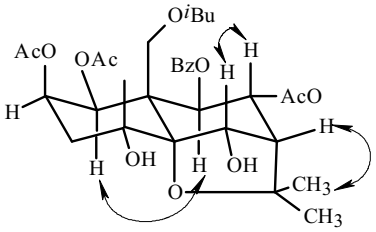
3 - 5



1: R = OAc; **2:** R = O*i*Pet; **3:** R_1 = OH, R_2 = OFu
4: R_1 = OAc, R_2 = OFu; **5:** R_1 = R_2 = OAc

1) Institute of Pesticide Science and College of Sciences Northwest Agricultural & Forestry University, Yangling, Shaanxi, 712100, P. R. China, e-mail: nwzjw@yahoo.com.cn; 2) Xi′an Modern Chemistry Research Institute, Xi′an, Shaanxi 710065, P. R. China. Published in Khimiya Prirodnykh Soedinenii, No. 6, pp. 796–799, November–December 2011. Original article submitted December 2, 2010.

 0009-3130/12/4706-0906



1

Fig. 1. Major NOESY correlations of compound **1**.

Compound **1**, molecular formula $C_{32}H_{42}O_{13}$, was analyzed by ESI-MS (positive mode), ^1H, and ^{13}C NMR spectroscopic data (Table 1). Its IR spectrum revealed characteristic ester absorption at 1742 cm^{-1} and a free hydroxyl absorption at 3463 cm^{-1}. The UV spectrum contained an aromatic moiety (λ_{max}, 240 nm). The ESI-MS/MS exhibited peaks attributable to the presence of acetate ($[M + Na - 60]^+$, *m/z* 597, CH_3COOH), benzoate ($[M + Na]^+ - 122$, *m/z* 535, C_6H_5COOH), and isobutanoate ($[M + Na]^+ - 88$, *m/z* 569, C_3H_7COOH) groups. This was confirmed by the NMR spectrum data, which also suggested the presence of signals for three acetate esters, δ_C 170.11, 169.69, 169.60, 21.27, 20.99, and 20.61, δ_H 2.11, 1.88 and 1.53 (each 3H, s); one isobutyrate ester, δ_C 176.89 (CO), 34.52 (CH) 19.29 (CH_3), and 19.22 (CH_3), δ_H 2.83 (1H, m), 1.34 and 1.33 (each 3H, dq, J = 7.5, 1.5 Hz), and one benzoate ester, δ_C 165.79 (CO), 133.63 (CH), 129.66 (2 × CH), 128.83 (2 × CH), and 129.46 (C), δ_H 7.86 (2H, d, J = 7.0 Hz), 7.56 (1H, t, J = 7.5 Hz), 7.42 (2H, t, J = 7.5 Hz). The ^1H NMR of compound **1** showed the presence of three tertiary methyl groups at δ 1.61, 1.77, and 1.71 (each 3H, s, H-13, 14, 15). Based on the published literature [15], the ^1H–^1H COSY spectrum signals at δ 5.47 (1H, d, J = 3.5 Hz, H-1), 5.38 (1H, m, H-2), 5.20 (1H, s, H-6), 5.60 (1H, dd, J = 3.5, 9.5 Hz, H-8), and 6.03 (1H, d, J = 9.5 Hz, H-9) can be assigned to five protons attached to carbon atoms bearing secondary ester groups, while signals at δ 4.85 (1H, d, J = 13.5 Hz, H-12α) and 4.65 (1H, d, J = 13.5 Hz, H-12β) can be assigned to the two protons attached to carbon atoms bearing primary ester groups.

The ^{13}C NMR and DEPT spectra of the parent skeleton of **1** revealed three methyls at δ 24.33, 26.44, and 30.23, two methylenes at δ 41.40 and 61.90, six methines at δ 53.65, 67.48, 74.33, 75.23, 75.50 and 77.07, and four quaternary carbons at δ 50.82, 72.32, 84.76, and 91.64. The chemical shifts were very similar to celangulin C, which was previously isolated from the same plant [8], suggesting that compound **1** contained a 1,2,4,6,8,9,12-heptasubstituted-β-dihydroagarofuran skeleton. We observed HMBC correlations H-C (9) with the carbonyl at δ 165.79 of the benzoate ester, H-C (12) with the carbonyl at δ 176.89 of the isobutyrate ester, and of H-C (1), H-C (2), and H-C (8) with the carbonyls at δ 170.11, 169.69, and 169.60 of three acetate esters. One of the two free hydroxyl groups was situated at C-4, and the second at C-6, as shown by the ^1H NMR chemical shift, because the normal value of H-6 is generally greater than or near to δ 6.00 when H-6 is esterified [7]. The relative stereochemistry of compound **1** was found to have the same stereochemistry for H-1, H-2, H-6, H-8, and H-9 as celangulin C because of the similar coupling patterns, coupling constants, and cross peaks in the NOESY spectrum, which also showed that the correlation between H-1 and H-9 indicated the presence of H-9eq and the correlation between H-6 and H-8 indicated the presence of H-8eq (Fig. 1). Thus, the chemical structure of compound **1** was identified as 1α,2α,8β-triacetoxy-9α-benzoyloxy-12-isobutanoyloxy-4β,6β-dihydroxy-β-dihydroagarofuran.

Compound **2** is an amorphous white powder, $C_{35}H_{48}O_{13}$. For ^1H and ^{13}C NMR spectroscopic data, see Table 1. Its IR spectrum revealed characteristic ester absorptions at 1738 cm^{-1} and a free hydroxyl absorption at 3493 cm^{-1}. The UV spectrum contained an aromatic moiety (λ_{max}, 241 nm). The ESI-MS/MS exhibited peaks attributable to the presence of acetate, α-methylbutanoate, benzoate, and isobutanoate groups, which were also confirmed by the NMR spectrum. The ^1H NMR of **2** showed the presence of three methyl groups at δ 1.61 (3H, s, H-13), 1.77 (3H, s, H-14), and 1.70 (3H, s, H-15). Based on the published literature [6–13], the ^1H–^1H COSY spectrum signals at δ 5.49 (1H, d, J = 3.5 Hz, H-1), 5.39 (1H, m, H-2), 5.21 (1H, s, H-6), 5.58 (1H, dd, J = 3.0, 9.5 Hz, H-8), and 6.07 (1H, d, J = 9.5 Hz, H-9) can be assigned to five protons attached to carbon atoms bearing secondary ester groups, while the signals at δ 4.93 (1H, d, J = 12.5 Hz, H-12α) and 4.58 (1H, d, J = 12.5 Hz, H-12β) can be assigned to the two protons attached to carbon atoms bearing primary ester groups.

TABLE 1. The ^{1}H, ^{13}C NMR Chemical Shifts of Compounds **1** and **2** (500 MHz, $CDCl_3$, δ, ppm, J/Hz)*

Position	**1**		**2**	
	δ_H	δ_C	δ_H	δ_C
1	5.47 (1H, d, J = 3.5)	75.23 (CH)	5.49 (1H, d, J = 3.5)	75.25 (CH)
2	5.38 (1H, m)	67.48 (CH)	5.39 (1H, m)	67.48 (CH)
3	2.02, 2.09 (2H, m)	41.40 (CH_2)	2.11, 2.03 (2H, m)	41.38 (CH_2)
4	–	72.32 (C)	–	72.29 (C)
5	–	91.64 (C)	–	91.66 (C)
6	5.20 (1H, s)	77.07 (CH)	5.21 (1H, s)	77.08 (CH)
7	2.60 (1H, d, J = 3.5)	53.65 CH)	2.59 (1H, d, J = 3.0)	53.70 (CH)
8	5.60 (1H, dd, J = 3.5, 9.5)	75.50 (CH)	5.58 (1H, dd, J = 3.0, 9.5)	75.39 (CH)
9	6.03 (1H, d, J = 9.5)	74.33 (CH)	6.07 (1H, d, J = 9.5)	73.96 (CH)
10	–	50.82 (C)	–	50.66 (C)
11	–	84.76 (C)	–	84.68 (C)
12	4.85 (1H, d, J = 13.5)	61.90 (CH_2)	4.93 (1H, d, J = 12.5)	
	4.65 (1H, d, J = 13.5)		4.58 (1H, d, J = 12.5)	61.83 (CH_2)
H-13	1.61 (3H, s)	24.33 (CH_3)	1.61 (3H, s)	24.22 (CH_3)
H-14	1.77 (3H, s)	30.23 (CH_3)	1.77 (3H, s)	30.23 (CH_3)
H-15	1.71 (3H, s)	26.44 (CH_3)	1.70 (3H, s)	26.46 (CH_3)
OH-4	3.10 (1H, s)		3.12 (1H, s)	

*Data are based on DEPT and ^{1}H–^{13}C (HSQC and HMBC) experiments.

The ^{13}C NMR and DEPT spectra of the parent skeleton of **2** revealed three methyls at δ 24.22, 26.46, and 30.23, two methylenes at δ 41.38 and 61.83, six methines at δ 53.70, 67.48, 73.96, 75.25, 75.39, and 77.08, and four quaternary carbons at δ 50.66, 72.29, 84.68, and 91.66. The chemical shifts were very similar to those of **1**, which suggested that compound **2** also contained a 1,2,4,6,8,9,12-heptasubstituted-*β*-dihydroagarofuran skeleton. We observed HMBC correlations of H-C (9) with the carbonyl at δ 165.82 of the benzoate ester, H-C (8) with the carbonyls at δ 175.84 of *α*-methylbutanoate ester, H-C (12) with the carbonyls at δ 176.32 of the isobutyrate ester, and H-C (1) and H-C (2) with the carbonyls at δ 169.67 and 169.58 of two acetate esters. One of the two free hydroxyl groups was situated at C-4, and the second at C-6, as shown by the ^{1}H NMR chemical shift. The relative stereochemistry of compound **2** was found to have the same stereochemistry for H-1, H-2, H-6, H-8, and H-9 as **1** because of the similar coupling patterns, coupling constants, and the cross peaks in the NOESY spectrum. Thus, the chemical structure of compound **2** was identified as 1*α*,2*α*-diacetyl-8*β*-(*α*-methyl)-butanoyloxy-9*α*-benzoyloxy-12-isobutanoyloxy-4*β*,6*β*-dihydroxy-*β*-dihydroagarofuran.

Compounds **3**, **4**, and **5** were the three known compounds 1*α*,2*α*,12-triacetoxy-8*α*,9*β*-difuroyloxy-4*β*,6*β*-dihydroxy-*β*-dihydroagarofuran (**3**), 1*α*,2*α*,6*β*,12-tetraacetoxy-8*α*,9*β*-difuroyloxy-4*β*-hydroxy-*β*-dihydroagarofuran (**4**), and 1*α*,2*α*,6*β*,8*α*,12-pentaacetoxy-9*β*-furoyloxy-4*β*-hydroxy-*β*-dihydroagarofuran (**5**), based on UV, IR, ESI-MS, and ^{1}H and ^{13}C NMR spectroscopic evidence [11, 14].

In vitro antitumor activities of compounds **1–5** against human colon (HT-29), breast (Bcap-37), and lung (NCI-H460) cancer cell lines were evaluated by the SRB method (for methodology, see [15]). The results showed that compounds **1–5** exhibited minimal or no antiproliferative activities against human colon (HT-29) and lung (NCI-H460) cancer cell lines. However, compounds **1–2** were shown to be moderately cytotoxic to Bcap-37. The IC_{50} were 54.08 and 61.35 μM, respectively. A comparison of the structural characteristic of these compounds shows that the compound substituted by benzoate ester at C-9 can have inhibitory activity against breast (Bcap-37) cancer cell. This result implied that the benzoate group at C-9 is crucial to the antitumor activity of these types of compounds.

Insecticidal activity results (for methodology, see [9, 10]) showed that the KD_{50} values (dose required to knock down 50% of the population of *Mythimna separate*) for compounds **1–5** were 610.5, 308.8, 917.8, 510.6, and 1120.5 $\mu g \cdot g^{-1}$, respectively, against the 4th instar larvae of *Mythimna separata*. It can be seen that the KD_{50} of compounds **2** and **4** are more than twice that of **1** and **5**. This suggested that the bulky groups at C-8 seem to contribute greatly to the insecticidal activity since these compounds only have an ester difference at C-8.

EXPERIMENTAL

General. HPLC-grade methanol was purchased from Tedia (Fairfield, USA); AR-grade acetone and methanol were obtained from Shaanxi Yangling (Shaanxi, China); water was purified by a Milli-Q Academic water purification system (Milford, MA, USA).

Melting points were measured on a Yanagimoto apparatus and are uncorrected. Optical rotations were measured on a Perkin–Elmer 341 polarimeter. Proton NMR spectra were recorded on a Bruker Avance 500 MHz Spectrometer with $CDCl_3$ as solvent and TMS as internal standard. Compounds were purified using a Shimadzu 6AD HPLC apparatus with a C_{18} preparative column (20 × 250 mm, 10 μm), MeOH–H_2O (5:5) as eluent, and a UV detector at 230 nm. Ionization was by positive ion electrospray using the ESI model, and the molecular scan range was 100–1000 amu using a Thermo Finnigan LCQ Advantage MAX LC/MS mass spectrometer.

Plant Material. The root bark of *C. angulatus* was collected in Qinling Mountain, Taibai County, Shaanxi Province, People′s Republic of China, in October 2006, and authenticated by Prof. Hua Yi of the College of Life Sciences, Northwest Agricultural & Forestry University. Voucher specimens (samples No. NWAU2006-A18) were deposited at the College of Life Sciences, Northwest Agricultural & Forestry University.

Extraction and Isolation. The MeOH crude extracts of the root bark of *Celastrus angulatus* (yellow semisolid residue, 100 g) were adsorbed onto a D101 macroporous resin column (8.0 × 120 cm) and gradient-eluted with methanol–water (2:8, 3:7, 4:6 and 5:5); 200 fractions of ca. 500 mL each were collected. After analysis with thin-layer chromatography, similar fractions were combined to afford 15 fractions. Then fractions 2 (13 g), 3 (7 g), and 7 (5 g) were successively chromatographed on a silica gel (200–300 mesh) column using increasing-polarity mixtures of acetone–methanol as eluent and on an RP-flash column using methanol–water. In this way, after several chromatographies on silica gel (acetone–methanol), RP-flash (methanol–water) column, and pre-HPLC, the new compounds **1** (21 mg) and **2** (35 mg) and three known compounds **3** (128 mg), **4** (160 mg), and **5** (113 mg) were isolated.

***In vitro* Antitumor Activity**. The test compounds were dissolved in DMSO (10 $mg \cdot mL^{-1}$; vincristine sulfate was used as control) and diluted with cell culture medium to six required concentrations (100, 50, 25, 12.5, 6.25, and 3.12 $\mu g \cdot mL^{-1}$). The final concentration of DMSO was less than 1% of the total volume. At this concentration, DMSO was found to be nontoxic to the cells tested. The cells were exposed to the drugs for 72 h. Cell growth was assayed using Sulforhodamine B (SRB). The optical density (OD) was read at 490 nm. All cytotoxicity tests were performed 3 times in quadruplicate. The IC_{50} values were calculated from curves constructed by plotting cell survival (%) versus compound concentration (μM). Vincristine sulfate was used as positive control [15].

Insecticidal Activity. Leaf discs of known area were treated with known amounts of the test samples dissolved in acetone (acetone was used as control). The 4th instar larvae of *Mythimna separate* were fed with the discs for 12 h (repeated 10 times for each sample). After 24 h, the numbers of knocked down larvae (symptoms: the larvae were narcotized, the bodies were very soft and immobilized, and response disappeared completely) were recorded, and the toxicity was ascertained by estimating the median knockdown dose (KD_{50} value) of the test sample [7–10].

Compound 1. $C_{32}H_{42}O_{13}$, amorphous white powder; mp 155–158°C; $[\alpha]_D$ +22.0° (*c* 0.50, CH_3COCH_3). UV/Vis (MeOH, λ_{max}, nm): 240. ^1H NMR ($CDCl_3$, δ, ppm, J/Hz): 1.61 (3H, s, Me-13), 1.77 (3H, s, Me-14), and 1.71 (3H, s, Me-15), OAc × 3 [δ 2.11, 1.88, and 1.53 (each 3H, s)], O*i*Bu [δ 2.83 (1H, m), 1.34 (3H, d, J = 7.5), and 1.33 (3H, d, J = 7.5)], OBz [δ 7.86 (2H, d, J = 7.0), 7.56 (1H, t, J = 7.5), 7.42 (2H, t, J = 7.5)]. ^{13}C NMR ($CDCl_3$, δ, ppm): OAc × 3 (170.11, 169.69, 169.60, 21.27, 20.99, and 20.61), O*i*Bu [176.89 (CO), 34.52 (CH), 19.29 (CH_3), and 19.22 (CH_3)], OBz [165.79 (CO), 133.63 (CH), 129.66 (2 × CH), 128.83 (2 × CH), and 129.22 (C)]. ESI-MS/MS *m/z* (%): 657 (3) $[M + Na]^+$, 597 (100) $[M + Na - AcOH]^+$, 569 (4) $[M + Na - i\text{BuOH}]^+$, 555 (39) $[M + Na - AcOH - Ac]^+$, 535 (51) $[M + Na - BzOH]^+$, 527 (22) $[M + Na - {}^i\text{BuOH} - Ac]^+$, 509 $[M + Na - {}^i\text{BuOH} - AcOH]^+$, 475 (18) $[M + Na - BzOH - AcOH]^+$.

Compound 2. $C_{35}H_{48}O_{13}$, amorphous white powder; mp 162–165°C; $[\alpha]_D$ +12.0° (*c* 0.50, CH_3COCH_3). UV/Vis (MeOH, λ_{max}, nm): 241. ^1H NMR ($CDCl_3$, δ, ppm, J/Hz): 1.61 (3H, s, Me-13), 1.77 (3H, s, Me-14), 1.70 (3H, s, Me-15), OAc × 2 [2.11 and 1.55 (each 3H, s)], O*i*Pet [2.36 (1H, m), 1.65, 1.55 (2H, m), 1.02 (3H, t, J = 7.5), and 0.93 (3H, d, J = 7.5)], O*i*Bu [2.59 (1H, m), 1.34 (3H, d, J = 7.5), and 0.96 (3H, d, J = 7.5)], OBz [7.87 (2H, d, J = 7.0), 7.55 (1H, t, J = 7.5), and 7.40 (2H, t, J = 7.5)]. ^{13}C NMR ($CDCl_3$, δ, ppm): OAc × 2 [169.67 (CO), 169.58 (CO), 21.26 (CH_3), and 20.62 (CH_3)], O*i*Pet [175.84 (CO), 41.71 (CH), 26.74 (CH_2), 17.05 (CH_3), and 11.83 (CH_3)], O*i*Bu [176.32 (CO), 34.25 (CH), 18.96 (CH_3), and 18.63 (CH_3)], OBz [165.82 (CO), 133.55 (CH), 129.64 (2 × CH), 128.75 (2 × CH), and 129.54 (C)]. ESI-MS/MS *m/z* (%): 699 (11) $[M + Na]^+$, 639 (100) $[M + Na - AcOH]^+$, 597 (55) $[M + Na - AcOH - Ac]^+$, and $[M + Na - i\text{PetOH}]^+$, 577 (57) $[M + Na - BzOH]^+$, 555 (22) $[M + Na - i\text{PetOH} - Ac]^+$, 517 (16) $[M + Na - BzOH - AcOH]^+$.

Compound 3. $C_{31}H_{36}O_{15}$, amorphous white powder; mp 122–125°C. UV/Vis (MeOH, λ_{max}, nm): 230. ^{1}H NMR ($CDCl_3$, δ, ppm, J/Hz): 1.61 (3H, s, Me-13), 1.73 (3H, s, Me-14), 1.66 (3H, s, Me-15), OAc × 3 [2.09, 2.04, and 1.60 (each 3H, s)], OFu × 2 [8.29 (1H, m), 8.08 (1H, m), 7.64 (1H, d, J = 1.5), 7.52 (1H, d, J = 1.5), 6.83 (1H, d, J = 1.0), and 6.72 (1H, d, J = 1.0)]. ^{13}C NMR ($CDCl_3$, δ, ppm): OAc × 3 [171.78 (CO), 171.51 (CO), 171.24 (CO), 21.12 × 2 (CH_3), and 20.76 (CH_3)], OFu × 2 [162.76 (CO), 162.23 (CO), 150.35 (CH), 149.93 (CH), 145.73 (CH), 145.61 (CH), 120.05 (C), and 110.62 (2 × CH)]. ESI-MS/MS *m/z* (%): 671 (1) $[M + Na]^+$, 611 (55) $[M + Na – AcOH]^+$, 569 (28) $[M + Na – AcOH – Ac]^+$, 559 (100) $[M + Na – FuOH]^+$, 499 (23) $[M + Na – FuOH – AcOH]^+$.

Compound 4. $C_{33}H_{38}O_{16}$, amorphous white powder; mp 152–154°C. UV/Vis (MeOH, λ_{max}, nm): 230. ^{1}H NMR ($CDCl_3$, δ, ppm, J/Hz): 1.49 (3H, s, Me-13), 1.70 (3H, s, Me-14), 1.57 (3H, s, Me-15), OAc × 4 [2.13, 2.10, 2.04, and 1.62 (each 3H, s)], OFu × 2 [8.32 (1H, m), 8.10 (1H, m), 7.63 (1H, d, J = 1.0), 7.60 (1H, d, J = 1.0), 6.88 (1H, d, J = 1.0), and 6.73 (1H, d, J = 1.0)]. ^{13}C NMR ($CDCl_3$, δ, ppm): OAc × 4 [172.01 (CO), 171.89 (CO), 171.54 (CO), 171.29 (CO), 21.42 (CH_3), 21.20 (CH_3), 21.09 (CH_3), and 20.79 (CH_3)], OFu × 2 [163.01 (CO), 162.20 (CO), 150.45 (CH), 150.28 (CH), 145.77 (CH), 145.73 (CH), 120.21 (C), 119.54, 110.84 (CH), and 110.68 (CH)]. ESI-MS/MS *m/z* (%): 713 (3) $[M + Na]^+$, 653 (60) $[M + Na – AcOH]^+$, 611 (35) $[M + Na – AcOH – Ac]^+$, 601 (100) $[M + Na – FuOH]^+$, 541 (13) $[M + Na – FuOH – AcOH]^+$.

Compound 5. $C_{30}H_{38}O_{15}$, amorphous white powder; mp 105–107°C. UV/Vis (MeOH, λ_{max}, nm): 230. ^{1}H NMR ($CDCl_3$, δ, ppm, J/Hz): 1.50 (3H, s, Me-13), 1.60 (3H, s, Me-14), 1.57 (3H, s, Me-15), OAc × 5 [2.25, 2.17, 2.11, 2.08, and 1.62 (each 3H, s)], OFu [8.08 (1H, m), 7.60 (1H, d, J = 1.0), and 6.71 (1H, d, J = 1.0)]. ^{13}C NMR ($CDCl_3$, δ, ppm): OAc × 5 [172.17 (CO), 171.88 (CO), 171.44 (CO), 171.19 (CO), 171.15 (CO), 21.41 (CH_3), 21.38 (CH_3), 21.24 (CH_3), 21.06 (CH_3), and 20.71 (CH_3)], OFu [162.39 (CO), 150.59 (CH), 149.79 (CH), 119.31 (C), and 110.64 (CH)]. ESI-MS/MS *m/z* (%): 661 (3) $[M + Na]^+$, 601 (100) $[M + Na – AcOH]^+$, 559 (25) $[M + Na – AcOH – Ac]^+$, 549 (7) $[M + Na – FuOH]^+$, 541 (10) $[M + Na – 2 \times AcOH]^+$.

ACKNOWLEDGMENT

This work was supported by the National Key S & T Research Foundation of China (2010CB126105) and the National Natural Science Foundation of China (30871663, 30800729).

REFERENCES

1. Jinming Gao, Wenjun Wu, Jiwen Zhang, and Yasuo Konishi, *Nat. Prod. Rep.*, **24**, 1153 (2007).
2. Weiping Yin, Tianzeng Zhao, Lingjie Gao, Dapeng Zou, Hongmin Liu, and Jianxun Kang, *Phytochemistry*, **52**, 1731 (1999).
3. Ming an Wang, Wenjun Wu, Jingbo Zhu, Zhiqin Ji, and Wenming Zhou, *Nat. Prod. Res*., **20**, 653 (2006).
4. Yongqiang Tu, Song, Qingbao Song, and Xiaoli Wu, *Acta Chim. Sin.*, **51**, 404 (1993).
5. N. Wakbayashi, Wenjun Wu, and R. Waters, *J. Nat. Prod.*, **51**, 537 (1988).
6. Zhiqin Ji, Zhaonong Hu, and Guoqiang Liu, *Acta Bot. Boreali-Occident. Sin.,* **24**, 748 (2004).
7. Wenjun Wu, Minan Wang, Jinbo Zhu, Wenming Zhou, Zhaonong Hu, and Zhiqin Ji, *J. Nat. Prod*., **64**, 364 (2001).
8. Wenjun Wu, Yongqiang Tu, Huixia Liu, and Jinbo Zhu, *J. Nat. Prod.*, **55**, 1294 (1992).
9. Shaopeng Wei, Minan Wang, Jiwen Zhang, Yong Qian, Zhiqin Ji, and Wenjun Wu, *Nat. Prod. Commun.*, **4**, 461 (2009).
10. Shaopeng Wei, Zhiqin Ji, and Jiwen Zhang, *Molecules*, **14**, 1396 (2009).
11. Zhiqin Ji, Wenjun Wu, Hua Yang, Baojun Shi, and Mingan Wang, *Nat. Prod. Res.*, **21**, 334 (2007).
12. Wenjun Wu, Minan Wang, Wenming Zhou, Jingbo Zhu, Zhiqin Ji, and Zhaonong Hu, *Phytochemistry*, **58**, 1183 (2001).
13. Jinbo Zhu, Minan Wang, Wenjun Wu, Zhiqin Ji, and Zhaonong Hu, *Phytochemistry*, **61**, 699 (2002).
14. Mingjian Wu, Tianzeng Zhao, and Yujun Shang, *Chin. Chem. Lett.*, **15**, 41 (2004).
15. Jinyung Kim, Hanmo Koo, and Darrick S. H. L. Kim, *Bioorg. Med. Chem. Lett.*, **11**, 2405 (2001).