Cytotoxic Germacrane-Type Sesquiterpenes, Pimarane-Type Diterpenes, and a Naphthalene Derivative from *Wollastonia biflora*

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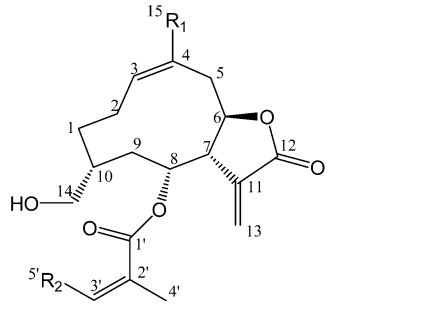
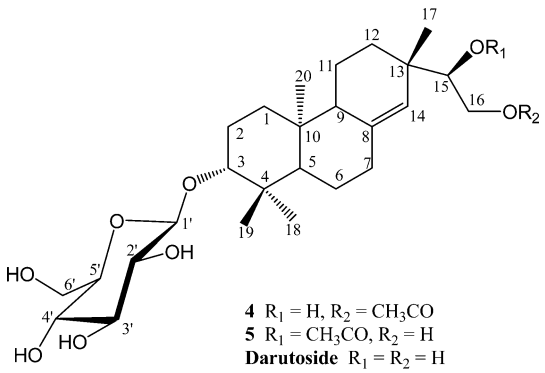
Phytochemical investigation of the whole plants of *Wollastonia biflora* led to the isolation and identification of three new germacrane-type sesquiterpenes (**1**−**3**), two new pimarane-type diterpenes (**4**, **5**), and a new naphthalene glycoside (**6**), along with 11 known compounds. Their structures were characterized on the basis of spectroscopic analyses and chemical methods. Compounds **1**, **2**, and **3** showed significant cytotoxic activity against the growth of hepatocellular carcinoma BEL-7402 cells *in vitro*.

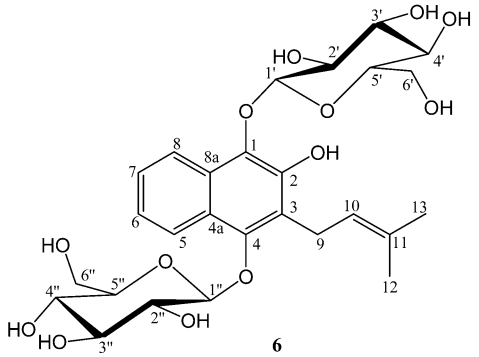
The family Asteraceae, comprising about 1000 genera and over 25 000 species, ranks first among the dicotyledons and second among the angiosperms. About 200 genera and 2000 species are found in China. Many Asteraceae species have been used for medicinal purposes since ancient times,[1] and many bioactive compounds with diverse skeletons have been identified.[2] *Wollastonia biflora* (L.) DC. [syn. *Melanthera biflora* (L.) Wild. or *Wedelia biflora* (L.) DC.], an Asteraceae species (tribe Heliantheae) widely distributed in the south of Asia and Oceania, has been used as a folk medicine for the treatment of rheumatism, bone aches, sore galls, and injuries in some provinces of China.[3,4] Previous phytochemical studies of this species have yielded phenols,[5] steroids,[6] and kaurane-type diterpenoids.[6,7] In our continuing efforts to find new bioactive compounds from plants used in Chinese traditional and folk medicine,[8,9] a systematic investigation into the chemical constituents of *W. biflora* (L.) DC. was undertaken. As a result, six new compounds (**1**−**6**), together with 11 known compounds, darutoside,[10,11] spathulenol,[12] darutigenol,[10] 3,3′,4′,7′-tetramethoxy-5-hyhroxyflavone,[13] 3,7-dimethoxy-3′,4′,5-trihydroxyflavone,[13] 3-methoxy-3′,4′,5,7-tetrahydroxyflavone,[14] pubeside B,[15] 2-carbomethoxy-3-prenyl-1,4-naphthohydroquinone diglucoside,[16] scandoside,[17] 6-*O*-acetylscandoside,[18] and asperuloside,[19] have been isolated from the whole plants of *W. biflora* (L.) DC. The new compounds (**1**−**3**) showed significant cytoxicity against carcinoma BEL-7402 cells.



1 $R_1 = CH_2OH$, $R_2 = CH_3$
2 $R_1 = CHO$, $R_2 = H$
3 $R_1 = CH_2OH$, $R_2 = H$



4 $R_1 = H$, $R_2 = CH_3CO$
5 $R_1 = CH_3CO$, $R_2 = H$
Darutoside $R_1 = R_2 = H$



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Results and Discussion

The whole plants of *W. biflora* (L.) DC. collected in Wenshan County of Yunnan Province and Jinxiu County of Guangxi Province, People's Republic of China, (each 2.5 kg), were respectively percolated at room temperature with 95% ethanol three times. After evaporation of ethanol *in vacuo* and filtration of the precipitated chlorophyll in 20% ethanol, the aqueous residue was extracted with chloroform and *n*-butanol, successively, yielding chloroform and *n*-butanol fractions, respectively. These fractions were subjected to chromatography, including silica gel and Sephadex LH-20 columns, HPLC, and PTLC to afford six new compounds (**1**−**6**) and 11 known compounds.

Compound **1** was obtained as a colorless gum with the molecular formula $C_{20}H_{28}O_6$ as deduced by HRESIMS and NMR analyses. Its ^{1}H NMR spectrum revealed the existence of two terminal olefinic protons (δ_H 6.25, 1H, s and δ_H 5.66, 1H, s), one doublet methyl (δ_H 1.94, d, 7.2 Hz), and one singlet methyl at δ_H 1.78. The ^{13}C NMR spectrum displayed 20 signals separated by DEPT experiments into two methyls, seven methylenes (one for sp^2 methylene and two for oxygenated methylenes), six methines (two for sp^2 methines and two for oxygenated methines), and five quaternary carbons (two for carboxylate carbons and three for sp^2 carbons). Analysis of its HSQC and ^{1}H−^{1}H COSY spectra led to the deduction of the fragment C-3−C-2−C-1−C-10−(C-14)−C-9−C-8−C-7−C-6−C-5. The NMR data suggested that **1** was similar

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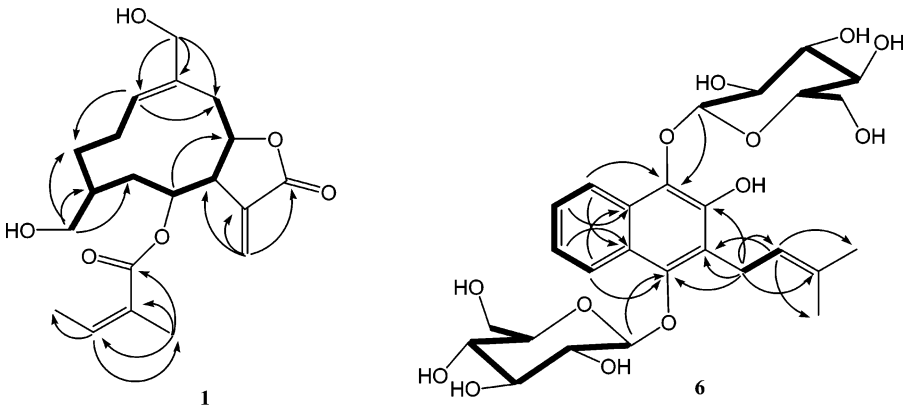

Figure 1. $^1H-^1H$ COSY (—) and key $^1H-^{13}C$ long-range correlation signals ($^1H \curvearrowright {}^{13}C$) in the HMBC spectra of **1** and **6**.

in structure to orientin,[20] a compound that differs from **1** only by its acyl moiety and the position of the double bond. The planar structure of **1** was confirmed by its HMBC spectrum (Figure 1). In the NOESY spectrum of **1**, NOE correlation signals were found at H-3/H-15, H-15/H-5α, H-5α/H-6, H-5β/H-8, H-5β/H-2, H-7/H-8, H-7/H-2, H-8/H-10, H-14/H-1α, H-14/H-9α, H-14/H-10, H-1α/H-3, H-3′/H-4′, and H-3′/H-5′. Therefore, **1** was characterized as 8α-angeloxy-14,15-dihydroxy-3(4),11(13)-germacradien-6,12-olide.

Compound **2** had the molecular formula $C_{19}H_{24}O_6$ as deduced by HRESIMS and NMR analyses. Its ^1H and ^{13}C NMR spectra were similar to those of **1**, except for the emergence of an aldehyde group (δ_H 9.44, 1H, s; δ_C 194.3) and two additional terminal olefinic protons (δ_H 5.98, 1H, s and δ_H 5.52, 1H, s) and the disappearance of the oxygenated methylene (δ_H 4.14, 2H, s; δ_C 67.6, CH_2-15), the olefinic hydrogen (δ_H 6.05, 1H, dd, $J = 1.5$ and 7.2 Hz, H-3′), and the doublet methyl (CH_3-5′). Analysis of the HSQC, $^1H-^1H$ COSY, HMBC, and NOESY spectra of **2** enabled the establishment of its structure. Therefore, compound **2** was characterized as 8α-methylacryloxy-14-hydroxy-15-al-3(4),11(13)-germacradien-6,12-olide.

The NMR spectra of **3** were nearly superimposable with those of **2**, except for the emergence of an oxygenated methylene (δ_H 4.19, s; δ_C 67.4) and the disappearance of the aldehyde group (δ_H 9.44, s; δ_C 194.3, C-15) in **2**. Meanwhile, the NMR spectra of **3** indicated that it differed from **1** only by its acyl moiety. Therefore, compound **3** was characterized as 8α-methylacryloxy-14,15-dihydroxy-3(4),11(13)-germacradien-6,12-olide.

Compound **4** possessed the elemental composition $C_{28}H_{46}O_9$ as determined by HRESIMS and NMR analyses. Its ^1H and ^{13}C NMR spectra were nearly superimposable with those of darutoside except for an additional acetyl moiety. The acetyl group was unambiguously attached to 16-OH, which was concluded from the variances of the chemical shifts of C-16 (+3.1 ppm) and C-15 (−3.4 ppm), with the chemical shifts of other carbons almost unchanged in the ^{13}C NMR spectrum compared to those of darutoside.[10,11] Therefore, compound **4** is 16-*O*-acetyldarutoside.

Compound **5** showed the same molecular formula as **4**. Its ^1H and ^{13}C NMR spectra were again similar to those of **4** and darutoside. The ^{13}C NMR spectrum showed chemical shifts of C-15 (+2.1 ppm) and C-16 (−2.0 ppm) compared to darutoside,[10,11] which suggested attachment of the acetyl group to the −OH at C-15. Therefore, compound **5** was determined to be 15-*O*-acetyldarutoside. The structures of **4** and **5** were confirmed by their HMBC spectra and the following chemical transformation: acetylation of the aglycones of **4**, **5**, and darutoside led to the same product, 3,-15,16-tri-*O*-acetyldarutigenol. The coexistence of **4** and **5**, and their similar content found in the plant, suggested that they may be equilibrated by molecular transesterification.

Compound **6** was obtained as a pale yellow powder with the molecular formula $C_{27}H_{36}O_{13}$. Its ^1H NMR spectrum displayed a pattern typical of one *ortho*-substituted phenyl group and two singlet methyls. The ^{13}C NMR spectrum of **6** exhibited 27 signals constituted by 12 aromatic/olefinic signals (five for sp^2 methines) in the low-field region, 12 signals typical of sugar moieties, and two methyls and one methylene in the high-field region. The NMR data were similar to those of 2-carbomethoxy-3-prenyl-1,4-naphthohydroquinone diglucoside,[16] a known compound isolated with **6**, except that signals for the carbomethoxy group of the former compound were missing in **6**, which, in addition to the variance of their molecular weights, suggested that **6** had the carbomethoxy group displaced by −OH at C-2. The hypothesis was confirmed by the HMBC spectrum (Figure 1). To determine its sugar moieties, enzymatic and acid hydrolysis experiments were performed and glucose was identified by co-TLC of the hydrolyzed samples with authentic sugar samples. However, the "aglycons" were characterized as lapachol (due to the instability of its aglycon)[21] and α- and β-lapachone[22] (due to the instability of its chain under acidic conditions), respectively. The two glucose moieties were both determined as β-D-configured on the basis of the ^1H NMR and optical rotation values. Thus, **6** was determined to be lapachol-1,4-di-*O*-β-D-glucopyranoside.

Although the two collections of plant material from Wenshan County of Yunnan Province and Jinxiu County of Guangxi Province shared the same name and common appearance, there were significant differences in the chemical constituents found in their *n*-butanol extracts. The main components of the former were darutoside and its derivatives, while the latter contained mainly scandoside, 6-*O*-acetylscandoside, and asperuloside. However, their chloroform extracts showed very similar patterns on the co-TLC plates. New compounds **1**−**5** were isolated from the former collection of plant material, while **6** was isolated from the latter collection.

In preliminary pharmacological tests, all of the isolated compounds were evaluated for inhibitory activity against the growth of hepatocellular carcinoma BEL-7402 cells *in vitro*. The three new germacrane-type sesquiterpenes **1**, **2**, and **3** exhibited significant inhibitory activity against the growth of hepatocellular carcinoma BEL-7402 cells *in vitro*, with IC_{50} values of 3.00 ± 0.84, 1.50 ± 0.14, and 1.72 ± 0.24 μM, respectively. Germacrane-type sesquiterpenes having the α-methylene-δ-butanolide moiety have been reported previously as cytotoxic components.[23,24]

Experimental Section

General Experimental Procedures. Optical rotations were measured with a Perkin-Elmer 241MC polarimeter or Perkin-Elmer 341 polarimeters. UV spectra were recorded with a Beckman DU-7 spectrometer. IR spectra were recorded using a Perkin-Elmer 577 spectrometer. LR-ESIMS were measured using a Finnigan LCQ-DECA instrument, and HR-ESIMS data were obtained on a Mariner spectrometer. LR-EIMS were obtained on a MAT-95 spectrometer, and HR-EIMS were obtained on a Kratos 1H spectrometer. NMR spectra were run on a Bruker AM 400 or INOVA-600 spectrometer with TMS as

internal standard. Preparative HPLC was carried out using a Varian SD-1 instrument, equipped with NW25 C_{18} column (10 μm, 20 mm × 250 mm, Merck) and a Prostar 320 UV/vis detector. Column chromatographic separations were carried out using a LiChroprep RP-18 Lobar column (40−63 μm, Merck) and using silica gel H60 (300−400 mesh) (Qingdao Haiyang Chemical Group Corporation, Qingdao, People's Republic of China) and Sephadex LH-20 (Pharmcia Biotech AB, Uppsala, Sweden) as packing materials. HSGF254 silica gel TLC plates (Yantai Chemical Industrial Institute, Yantai, People's Republic of China) and RP-18 WF_{254} TLC plates (Merck) were used for analytical TLC. β-Cellulase was manufactured by Lizhu Dongfeng BioTech Co. Ltd., Shanghai, People's Republic of China.

Plant Material. Whole plants of *Wollastonia biflora* (L.) DC. were collected in Wenshan County of Yunnan Province and Jinxiu County of Guangxi Province, People's Republic of China (each 2.5 kg), in May 2005. They were identified by Professor Jingui Shen of Shanghai Institute of Materia Medica, Chinese Academy of Sciences. Voucher specimens (No. 050015SIMM and No. 050016SIMM, respectively) are deposited in the herbarium of Shanghai Institute of Materia Medica.

Extraction and Isolation. Powdered air-dried whole plants of *W. biflora* collected in Wenshan County of Yunnan Province, People's Republic of China (2.5 kg) were percolated at room temperature with 95% ethanol (15 L × 3). The filtrate was concentrated to dryness *in vacuo* and then suspended in 20% ethanol overnight. After filtration of the precipitated chlorophyll and evaporation of ethanol in the filtrate, the aqueous residue (1 L) was extracted with chloroform and *n*-butanol (500 mL × 3 each), successively, yielding chloroform (5 g) and *n*-butanol fractions (40 g), respectively. The chloroform fraction was chromatographed on silica gel eluted with a gradient of petroleum−acetone (6:1 to 0:1) to yield fractions C1 (2.1 g), C2 (130 mg), C3 (1.2 g), C4 (302 mg), and C5 (1.1 g). Fraction C2 was subjected to a silica gel column eluted with petroleum−acetone (20:1) to afford spathulenol (18.6 mg) and darutigenol (25.3 mg). Fraction C3 was separated by HPLC eluted with a methanol−water gradient (40% to 100% methanol) to give fractions C3A (540 mg), C3B (240 mg), C3C (50 mg), and C3D (320 mg). Fraction C3B was subjected to a silica gel column eluted with chloroform−methanol (30:1), affording **3** (83.3 mg) and **2** (18.7 mg). Fraction C3C was purified by a silica gel column eluted with chloroform−methanol (30:1) to give **1** (15.1 mg). Fraction C4 was separated by a silica gel column eluted with chloroform−methanol (20:1) to afford fractions C4A (130 mg), C4B (65 mg), and C4C (80 mg), which were further purified by a Sephadex LH20 column eluted with 95% ethanol to yield 3,3′,4′,7′-tetramethoxy-5-hyhroxyflavone (10.1 mg), 3,7-dimethoxy-3′,4′,5-trihydroxyflavone (7.5 mg), and 3-methoxy-3′,4′,5,7-tetrahydroxyflavone (8.4 mg), respectively. The *n*-butanol fraction was separated by a silica gel column eluted with chloroform−methanol (10:1 to 1:1), affording fractions B1 (708 mg), B2 (5.4 g), and B3 (32 g). Fraction B1 was subjected to HPLC eluted with methanol−water (30% to 100% methanol) to give fractions B1A (360 mg), B1B (102 mg), and B1C (300 mg). Fraction B1B was separated by PTLC (developed with chloroform−methanol 7:1) to afford **4** (30 mg) and **5** (40 mg). Fraction B2 was subjected to HPLC eluted with methanol−water (20% to 100% methanol within 60 min) to give fraction B2A (2.2 g), darutoside (2.5 g), and pubeside B (50 mg).

Powdered air-dried whole plants of *W. biflora* collected in Jinxiu County of Guangxi Province, People's Republic of China (2.5 kg), were extracted using the same procedure as mentioned above to give chloroform (3 g) and *n*-butanol fractions (34 g). The *n*-butanol fraction was separated by a silica gel column eluted with chloroform−methanol (10:1 to 1:1), affording fractions B′1 (1.2 g), B′2 (2.5 g), B′3 (4.4 g), and B′4 (25 g). Fraction B′2 was subjected to HPLC eluted with methanol−water (20% to 100% methanol) to give fractions B′2A (1.8 g), B′2B (407 mg), and B′2C (250 mg). Fraction B′2B was separated by PTLC (developed with chloroform−methanol−water, 7:3:0.1) to afford **6** (230 mg) and 2-carbomethoxy-3-prenyl-1,4-naphthohydroquinone diglucoside (150 mg). A portion of B′3 (500 mg) was separated by PTLC to give scandoside (30 mg) and 6-*O*-acetylscandoside (17 mg) (developed with chloroform−methanol−water 3:2:0.1) and asperuloside (14 mg) (developed with chloroform−methanol−water, 7:3:0.1), respectively.

Compound 1: colorless gum; $[\alpha]^{25}_D$ −84.8 (*c* 0.11, $CHCl_3$); UV (methanol) λ_{max} 220 nm; IR (KBr) ν_{max} 3419, 2928, 2872, 1755, 1716, 1647, 1456, 1385, 1269, 1229, 1151, 1043, 997, 754 cm^{-1}; ^1H NMR (in $CDCl_3$, 600 MHz) δ_H 1.15 (1H, m, H-1α), 2.00 (1H, m, H-1*β*), 2.30 (2H, m, H-2), 5.64 (1H, dd, *J* = 6.2 and 10.8 Hz, H-3), 2.58 (1H, dd, *J* = 5.3 and 13.5 Hz, H-5α), 2.70 (1H, dd, *J* = 10.3 and 13.5 Hz, H-5*β*), 5.38 (1H, m, H-6), 3.15 (1H, br s, H-7), 4.85 (1H, m, H-8), 1.97 (1H, m, H-9α), 1.54 (1H, m, H-9*β*), 1.88 (1H, m, H-10), 5.66 and 6.25 (each 1H, s, H-13), 3.41 (2H, ddd, *J* = 6.3, 10.4 and 16.9 Hz, H-14), 4.14 (2H, s, H-15), 6.05 (1H, q, *J* = 7.2 Hz, H-3′), 1.78 (3H, s, H-4′), 1.94 (3H, d, *J* = 7.2 Hz, H-5′); ^{13}C NMR (in $CDCl_3$, 100 MHz) δ_C 30.2 (t, C-1), 26.5 (t, C-2), 131.4 (d, C-3), 133.3 (s, C-4), 30.9 (t, C-5), 76.2 (d, C-6), 47.7 (d, C-7), 79.2 (d, C-8), 40.4 (t, C-9), 42.2 (d, C-10), 136.8 (s, C-11), 169.8 (s, C-12), 123.7 (t, C-13), 67.8 (t, C-14), 67.6 (t, C-15), 167.0 (s, C-1′), 127.6 (s, C-2′), 139.8 (d, C-3′), 20.3 (q, C-4′), 15.7 (q, C-5′); HRESIMS *m/z* 387.1790 $[M + Na]^+$ (calcd for $C_{20}H_{28}O_6Na$, 387.1784).

Compound 2: colorless gum; $[\alpha]^{23}_D$ −129.0 (*c* 0.23, DMSO); UV (methanol) λ_{max} 220 nm; IR (KBr) ν_{max} 3435, 2928, 1759, 1720, 1682, 1637, 1454, 1406, 1275, 1163, 1014, 818, 731 cm^{-1}; ^1H NMR (in $CDCl_3$, 600 MHz) δ_H 1.38 (1H, m, H-1α) 2.19 (1H, m, H-1*β*), 2.64 (2H, m, H-2), 6.70 (1H, t, *J* = 8.1 Hz, H-3), 2.64 (1H, dd, *J* = 5.1 and 13.6 Hz, H-5α), 2.94 (1H, dd, *J* = 10.5 and 13.6 Hz, H-5*β*), 5.50 (1H, m, H-6), 2.78 (1H, br s, H-7), 4.75 (1H, m, H-8), 1.96 (1H, m, H-9α), 1.42 (1H, m, H-9*β*), 1.94 (1H, m, H-10), 5.65 and 6.24 (each 1H, s, H-13), 3.48 (2H, ddd, *J* = 6.5, 10.7 and 16.6 Hz, H-14), 9.44 (1H. s, H-15), 5.52 and 5.98 (each 1H, s, H-3′), 1.80 (3H, s, H-4′); ^{13}C NMR (in $CDCl_3$, 100 MHz) δ_C 30.1 (t, C-1), 27.8 (t, C-2), 157.1 (d, C-3), 138.7 (s, C-4), 27.3 (t, C-5), 74.9 (d, C-6), 48.5 (d, C-7), 79.0 (d, C-8), 40.4 (t, C-9), 41.9 (d, C-10), 135.9 (s, C-11), 169.4 (s, C-12), 124.3 (t, C-13), 67.5 (t, C-14), 194.3 (d, C-15), 166.0 (s, C-1′), 135.6 (s, C-2′), 126.4 (t, C-3′), 18.1 (q, C-4′); HRESIMS *m/z* 371.1494 $[M + Na]^+$ (calcd for $C_{19}H_{26}O_6Na$, 371.1471).

Compound 3: colorless gum; $[\alpha]^{23}_D$ −94.0 (*c* 0.26, DMSO); UV (methanol) λ_{max} 220 nm; IR (KBr) ν_{max} 3398, 2928, 2872, 1757, 1716, 1664, 1456, 1387, 1296, 1163, 1013, 820 cm^{-1}; ^1H NMR (in $CDCl_3$, 600 MHz) δ_H 1.20 (1H, m, H-1α), 2.06 (1H, m, H-1*β*), 2.36 (2H, m, H-2), 5.74 (1H, dd, *J* = 5.4 and 10.7 Hz, H-3), 2.66 (1H, dd, *J* = 4.9 and 13.7 Hz, H-5α), 2.79 (1H, dd, *J* = 10.7 and 13.7 Hz, H-5*β*), 5.39 (1H, m, H-6), 3.22 (1H, br s, H-7), 5.00 (1H, m, H-8), 2.06 (1H, m, H-9α), 1.60 (1H, m, H-9*β*), 1.95 (1H, m, H-10), 5.99 and 6.34 (each 1H, s, H-13), 3.44 (1H, dd, *J* = 6.4 and 10.3 Hz, H-14a), 3.51 (1H, dd, *J* = 6.0 and 10.3 Hz, H-14b), 4.19 (2H, s, H-15), 5.26 and 6.10 (each 1H, s, H-3′), 1.93 (3H, s, C-4′); ^{13}C NMR (in $CDCl_3$, 100 MHz) δ_C 30.1 (t, C-1), 26.4 (t, C-2), 131.3 (d, C-3), 133.2 (s, C-4), 30.7 (t, C-5), 77.4 (d, C-6), 47.6 (d, C-7), 79.2 (d, C-8), 40.3 (t, C-9), 41.9 (d, C-10), 136.8 (s, C-11), 170.0 (s, C-12), 123.9 (t, C-13), 67.6 (t, C-14), 67.4 (t, C-15), 166.7 (s, C-1′), 135.5 (s, C-2′), 126.8 (t, C-3′), 18.0 (q, C-4′); HRESIMS *m/z* 373.1614 $[M + Na]^+$ (calcd for $C_{19}H_{26}O_6Na$, 373.1627).

Compound 4: colorless gum; $[\alpha]^{25}_D$ −23.6 (*c* 0.23, MeOH); IR (KBr) ν_{max} 3396, 2941, 2877, 1720, 1647, 1456, 1369, 1259, 1086, 1041, 883 cm^{-1}; ^{13}C NMR (in CD_3OD, 100 MHz) δ_C 38.5 (t, C-1), 24.8 (t, C-2), 86.4 (d, C-3), 39.8 (s, C-4), 56.6 (d, C-5), 23.9 (t, C-6), 37.6 (t, C-7), 140.9 (s, C-8), 52.4 (d, C-9), 39.4 (s, C-10), 19.8 (t, C-11), 33.6 (t, C-12), 39.0 (s, C-13), 129.5 (d, C-14), 74.6 (d, C-15), 67.9 (t, C-16), 23.3 (q, C-17), 29.7 (q, C-18), 15.7 (q, C-19), 17.8 (q, C-20), 102.3 (d, C-1′), 75.5 (d, C-2′), 78.1 (d, C-3′), 72.3 (d, C-4′), 78.6 (d, C-5′), 63.4 (t, C-6′), 21.4 (q, 16-OCO*C*H_3), 173.5 (s, 16-O*C*OCH$_3$); HRESIMS *m/z* 549.3052 $[M + Na]^+$ (calcd for $C_{28}H_{46}O_9$-Na, 549.3040).

Compound 5: colorless gum; $[\alpha]^{23}_D$ −28.0 (*c* 0.07, DMSO); IR (KBr) ν_{max} 3415, 2941, 2874, 1720, 1647, 1454, 1371, 1238, 1078, 1027 cm^{-1}; ^{13}C NMR (in CD_3OD, 100 MHz) δ_C 38.5 (t, C-1), 24.9 (t, C-2), 86.6 (d, C-3), 39.9 (s, C-4), 56.6 (d, C-5), 24.2 (t, C-6), 37.6 (t, C-7), 141.8 (s, C-8), 52.4 (d, C-9), 39.6 (s, C-10), 19.9 (t, C-11), 34.1 (t, C-12), 38.5 (s, C-13), 128.5 (d, C-14), 80.1 (d, C-15), 62.8 (t, C-16), 24.2 (q, C-17), 29.7 (q, C-18), 15.8 (q, C-19), 17.8 (q, C-20), 102.4 (d, C-1′), 75.6 (d, C-2′), 78.2 (d, C-3′), 72.4 (d, C-4′), 78.7 (d, C-5′), 63.5 (t, C-6′), 21.6 (q, 15-OCO*C*H_3), 173.5 (s, 15-OCOCH$_3$); HRESIMS *m/z* 549.3041 $[M + Na]^+$ (calcd for $C_{28}H_{46}O_9Na$, 549.3040).

Compound 6: pale yellow powder; $[\alpha]^{23}_D$ −1.0 (*c* 0.08, MeOH); UV (methanol) λ_{max} 235 nm, 285 nm, 295 nm, 333 nm; IR (KBr) ν_{max} 3415, 2918, 1630, 1605, 1452, 1402, 1365, 1068, 1026, 771 cm^{-1}; ^1H NMR (in DMSO-d_6, 300 MHz) δ_H 8.40 (1H, d, *J* = 8.3 Hz, H-5), 7.26 (1H, t, *J* = 8.3 Hz, H-6), 7.36 (1H, t, *J* = 8.3 Hz, H-7), 8.30 (1H, d, *J* = 8.3 Hz, H-8), 3.90 (1H, dd, *J* = 8.0 and 14.1 Hz, H-9a), 3.30 (1H, m, H-9b), 5.20 (1H, dd, *J* = 7.6 and 6.5 Hz, H-10), 1.59 (3H, s, H-12),

1.73 (3H, s, H-13), 4.53 (1H, d, $J = 7.8$ Hz, H-1′), 3.43 (1H, m, H-2′), 3.29 (1H, m, H-3′), 3.26 (1H, m, H-4′), 3.20 (1H, m, H-5′), 3.65 and 3.52 (each 1H, m, H-6′), 5.69 (1H, d, $J = 7.6$ Hz, H-1″), 3.43 (1H, m, H-2″), 3.25 (1H, m, H-3″), 3.15 (1H, m, H-4″), 2.95 (1H, m, H-5″), 3.61 and 3.41 (each 1H, m, H-6″); ^{13}C NMR (in DMSO-d_6, 100 MHz) δ_C 135.4 (s, C-1), 145.3 (s, C-2), 124.7 (s, C-3), 147.1 (s, C-4), 122.2 (s, C-4a), 122.5 (d, C-5), 123.3 (d, C-6), 125.8 (d, C-7), 121.8 (d, C-8), 127.9 (s, C-8a), 24.3 (t, C-9), 123.4 (d, C-10), 130.4 (s, C-11), 25.7 (q, C-12), 18.2 (q, C-13), 107.0 (d, C-1′), 72.4 (d, C-2′), 76.1 (d, C-3′), 69.6 (d, C-4′), 77.3 (1H, C-5′), 60.7 (t, C-6′), 105.0 (d, C-1″), 74.3 (d, C-2″), 76.5 (d, C-3″), 70.0 (d, C-4″), 77.1 (d, C-5″), 61.2 (t, C-6″); HRESIMS *m*/*z* 591.2051 $[M + Na]^+$ (calcd for $C_{27}H_{36}O_{13}Na$, 591.2054).

Acidic Hydrolysis of 6. Compound **6** (100 mg) dissolved in 50% MeOH (10 mL) containing 7% HCl was heated in boiling water for 2 h. After cooling, the reaction mixture was neutralized and extracted with chloroform. Glucose was identified as the sugar moiety by co-TLC of the aqueous solution compared with an authentic glucose sample. The organic residue was subjected to PTLC (developed with chloroform−acetone, 20:1) to afford α-lapachone (16.7 mg) and *β*-lapachone (22.3 mg). The aqueous layer was subjected to HPLC eluted with water to afford glucose {36 mg, $[\alpha]^{21}_D$ +50 (*c* 0.2, H_2O)}.

Enzymatic Hydrolysis of Compounds 4, 5, Darutoside, and 6. Compounds **4**, **5**, darutoside, and **6** (2 mg for both **4** and **5**, 100 mg for both darutoside and **6**) and *β*-cellulase (2 mg for both **4** and **5**, 100 mg for both darutoside and **6**) were dissolved in H_2O (2 mL for **4** and **5**, 40 mL for darutoside and **6**), respectively, and kept at 37 °C for 2 days. The aqueous solutions were then extracted with chloroform, and the aglycons of **4**, **5**, darutoside, and lapachol (0.9, 1.1, 34.3, and 25.2 mg, respectively) were obtained from the organic layer.

3,15,16-Tri-*O*-acetyldarutigenol. The aglycons of **4**, **5**, and darutoside (obtained from the above enzymatic hydrolysis) were dissolved in pyridine (0.5 mL for the former two compounds and 3 mL for the last one), each followed by the addition of acetyl chloride (one drop for the former two and three drops for the last one). These mixtures were then stirred at 80 °C for 2 h, and a common spot was observed on their co-TLC plate. After evaporation of pyridine, the residues were subjected to PTLC (developed by petroleum−acetone, 15:1) to give 3,15,16-tri-*O*-acetyldarutigenol (21 mg): $[\alpha]^{23}_D$ −29.5 (*c* 0.2, $CHCl_3$); ESIMS *m*/*z* 471 $[M + Na]^+$; ^{1}H NMR (in $CDCl_3$, 300 MHz) δ_H 5.12 (1H, dd, $J = 2.4$ and 9.2 Hz, H-15), 5.10 (1H, s, H-14), 4.50 (1H, dd, $J = 4.5$ and 11.4 Hz, H-3), 4.36 (1H, dd, $J = 2.4$ and 11.5 Hz, H-16), 4.03 (1H, dd, $J = 9.2$ and 11.5 Hz, H-16), 2.00, 2.01, and 2.03 (each 3H, s, 3 × $-COCH_3$), 0.82, 0.83, 0.84, and 0.87 (each 3H, s, 4 × methyl).

In Vitro Cytotoxicity Assay. The cytotoxicity of compounds **1**−**3** against human hepatoma BEL-7402 cells was evaluated by the sulforhodamine B (SRB) assay as described previously.[25] Briefly, BEL-7402 cells were incubated with different concentrations of compounds **1**−**3** (5, 0.5, and 0.05 μM, respectively) at 37 °C for 72 h. At the end of incubation, SRB was added and the absorbance of the SRB solution was measured at 560 nm. The IC_{50} values were calculated on the basis of percentage inhibition using the Logit linear regression method.

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