Pyrrolizidine Alkaloids and Bisabolane Sesquiterpenes from the Roots of *Ligularia cymbulifera*

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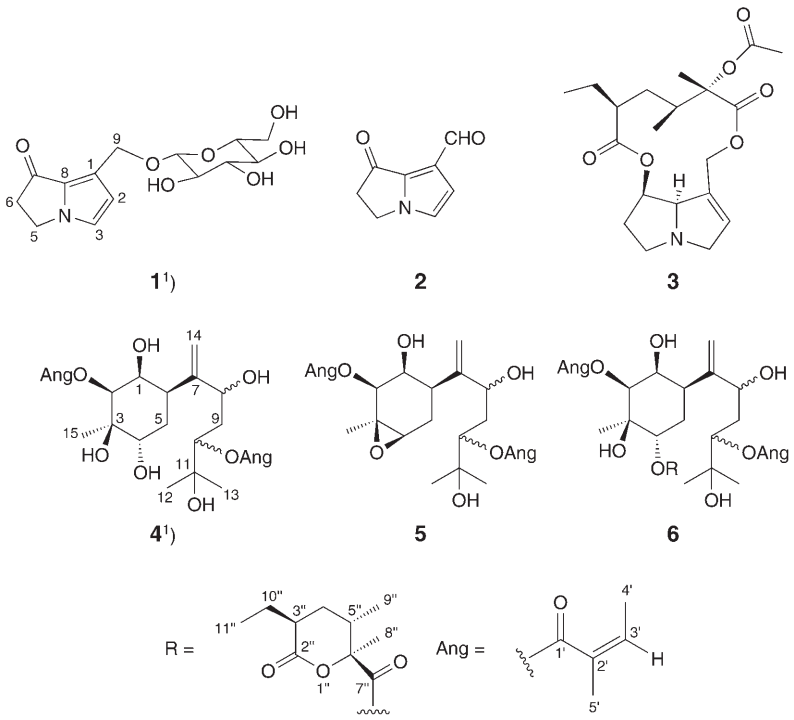
The new pyrrolizidine alkaloid glycoside **1**, and the three new highly oxygenated bisabolane sesquiterpenes **4**–**6**, together with the two known pyrrolizidine alkaloids **2** and **3**, were isolated from the roots of *Ligularia cymbulifera* (W. W. SMITH) HAND.-MAZZ. Their structures were established on the basis of spectroscopic analysis, especially 1D- and 2D-NMR data. The cytotoxic activities of compounds **1**, **2**, and **4**–**6** were evaluated against hepatoma (BEL-7402), human leukemia (HL-60), human ovarian carcinoma (HO-8910), and nasopharyngeal carcinoma (KB) cell lines (*Tables 1–3*). Compound **6** showed weak cell-growth inhibition of BEL-7402 cell.

Introduction. – Various types of sesquiterpenoids, such as eremophilane, guaiane, eudesmane, benzofurane, bisabolane (=1-(1,5-dimethylhexyl)-4-methylcyclohexane), and phenolic norsesquiterpene, have been isolated from the genus *Ligularia* belonging to the tribe Senecioneae of the Compositae [1–6]. Pyrrolizidine alkaloids are also widespread secondary metabolites in the genus *Ligularia* [7][8]. However, in recent years, little attention has been paid to pyrrolizidine alkaloids from *Ligularia*. It is reported that the pyrrolizidine alkaloids possess antitumor activity, and many of them are also highly toxic and can cause poisoning in livestock and in humans [9–13]. In the interests of public health and to systematically investigate the chemotaxonomic and bioactive components of pyrrolizidine alkaloids and sesquiterpenes of *Ligularia* plants, we continued to study the extract of *Ligularia cymbulifera*, which showed the presence of alkaloids due to a positive reaction to the *Dragendorff* reagent, and from which a new pyrrolizidine alkaloid **1** and the two known pyrrolizidine alkaloids **2** and **3** were obtained together with the three new highly oxygenated bisabolane sesquiterpenes **4**–**6**. The cytotoxicity of these compounds was screened against HL-60, BEL-7402, HO-8910, and KB cancer cell lines by using the MTT and SRB methods.

Results and Discussion. – Compound **1** was obtained as an amorphous solid which showed a quasi-molecular ion $[M + Na]^+$ at m/z 336.1058 in its HR-ESI-MS, consistent with the molecular formula $C_{14}H_{19}NO_7$ (six degrees of unsaturation). IR Absorption bands at 3417 and 1671 cm^{-1} indicated the presence of OH and conjugated carbonyl groups, and the bands at 3100, 1553, 1490, 1383, 1072, 983, and 759 cm^{-1} that of an aromatic skeleton, probably of pyrrole type.

The sugar obtained after acid hydrolysis of **1** was identified as D-glucose by comparing its specific rotation with that of an authentic sample of D-glucose. Further



spectroscopic data determined the structure of **1** as 1-[(β-D-glucopyranosyloxy)methyl]-5,6-dihydropyrrolizin-7-one[1]).

The characteristic low-field ^{1}H-NMR signals of **1** at δ(H) 7.14 (*d*, *J* = 2.0, H–C(3)) and 6.57 (*d*, *J* = 2.0, H–C(2)), together with the corresponding ^{13}C-NMR signals δ(C) 129.7 (C(8)), 123.9 (C(3)), 121.7 (C(1)), and 117.2 (C(2)), provided further evidence for an α,β-disubstituted pyrrole [14–16]. Two clear *t* at δ(H) 3.04 (*t*, *J* = 6.0, CH_2(6)) and 4.29 (*t*, *J* = 6.0, CH_2(5)) are assigned to an N–CH_2–CH_2–CO moiety by ^{1}H,^{1}H-COSY (CH_2(5)/CH_2(6)) and HMBC cross-peaks (CH_2(5), CH_2(6)/C(7)). The ^{13}C-NMR and DEPT spectra revealed six oxygenated C-atoms assignable to a glucose unit at δ(C) 102.1, 76.8, 76.8, 73.9, 70.4, and 61.6. The location of the glucoside linkage at C(9) was confirmed by the HMBC data (*Fig. 1*), which showed a correlation of the anomeric H–C(1′) (δ(H) 4.39) with C(9) (δ(C) 62.7). Similarly, the coupling constant (*J* = 8.0 Hz) of the anomeric-proton signal at δ(H) 4.39 indicated the glucosidic linkage to have a β-configuration.

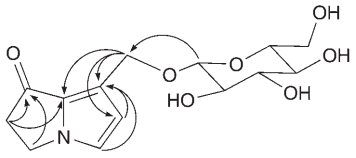


Fig. 1. *Selected HMBC correlations* (H → C) *of compound* **1**

[1]) Arbitrary atom numbering; for systematic names, see *Exper. Part.*

The ^{1}H- and ^{13}C-NMR data of **2** were very similar to those of **1**, except that the glucose unit was absent and an aldehyde group was present in **2**. Its molecular formula was deduced as $C_8H_7NO_2$ from the HR-ESI-MS (*m/z* 150.0552 ($[M + H]^+$)). The location of the aldehyde group at C(1) was confirmed by the ^{1}H,^{13}C-HMBC data (H–C(9) (δ(H) 10.23)/C(1) (δ(C) 123.0), C(2) (δ(C) 115.7), and C(8) (δ(C) 135.1)). The pyrrolizine derivative **2** is a known synthetic compound, but only the ^{1}H-NMR data have been reported [14]; here, **2** was identified for the first time as a natural product.

Compound **4** was obtained as colorless gum. Its molecular formula was determined as $C_{25}H_{40}O_9$ by the HR-ESI-MS (*m/z* 485.2743 ($[M + H]^+$)) with six degrees of unsaturation. The IR spectrum indicated the presence of OH groups (3489 cm^{-1}), a C=C bond (1647 cm^{-1}), and ester carbonyl groups (1715 cm^{-1}). Analysis of the ^{1}H- and ^{13}C-NMR, ^{1}H,^{1}H-COSY, and HMBC data, along with comparison of chemical shift values with those of known polysubstituted bisabolane sesquiterpenes, allowed us to elucidate compound **4** as a highly oxygenated bisabolane sesquiterpene [5][17][18]. Further data established the structure of **4** as (1β,2β,3β,4α,6β)-bisabol-7(14)-ene-1,2,3,4,8,10,11-heptol 2,10-diangelate[1]).

The presence of two angeloyloxy groups in the structure of **4** was indicated by the ^{1}H-NMR signals at δ(H) 6.14 (2*qq*, *J* = 7.2, 1.6, 1 H each), 2.05 (*dq*, *J* = 7.2, 1.4, 3 H), 1.98 (*dq*, *J* = 7.2, 1.4, 3 H), 1.94 (*dq*, *J* = 1.6, 1.4, 3 H), and 1.89 (*dq*, *J* = 1.6, 1.4, 3 H), in combination with the ^{13}C-NMR signals at δ(C) 168.1 and 167.6, 139.8 and 138.9, 127.4 and 127.3, 20.6 and 20.6, and 15.9 and 15.9 [19]. Apart from the two angeloyloxy groups, the ^{13}C-NMR and DEPT data revealed 15 skeletal C-atoms (three Me, three CH_2, and six CH groups, and three quaternary C-atoms), among which seven were O-bearing. The two angeloyloxy groups accounted for four degrees of unsaturation. The remaining two degrees of unsaturation required the presence of a monocyclic sesquiterpene skeleton with a terminal C=C bond (δ(H) 5.24 (br. *s*, 1 H) and 5.16 (br. *s*, 1 H); δ(C) 148.9 (C) and 114.8 (CH_2)). Two partial structures, –CH–CH_2–CH–CH–CH– and –CH–CH_2–CH–, were deduced from ^{1}H,^{1}H-COSY. The linkage of these two partial structures with quaternary C-atoms was achieved by the following long-range ^{1}H,^{13}C-correlations in the HMBC plot: Me(15)/C(2); H–C(2), H–C(4), Me(15)/C(3); H_α–C(5), Me(15)/C(4); H_α–C(5), H_a–C(14), H_b–C(14)/C(6); H–C(8), H_a–C(9), H_b–C(9), H_a–C(14), H_b–C(14)/C(7); H_a–C(9), H_b–C(9), H_a–C(14), H_b–C(14)/C(8); H–C(8), H–C(10)/C(9); H–C(8), H_a–C(9), H_b–C(9), Me(12), Me(13)/C(10); and Me(12), Me(13)/C(11).

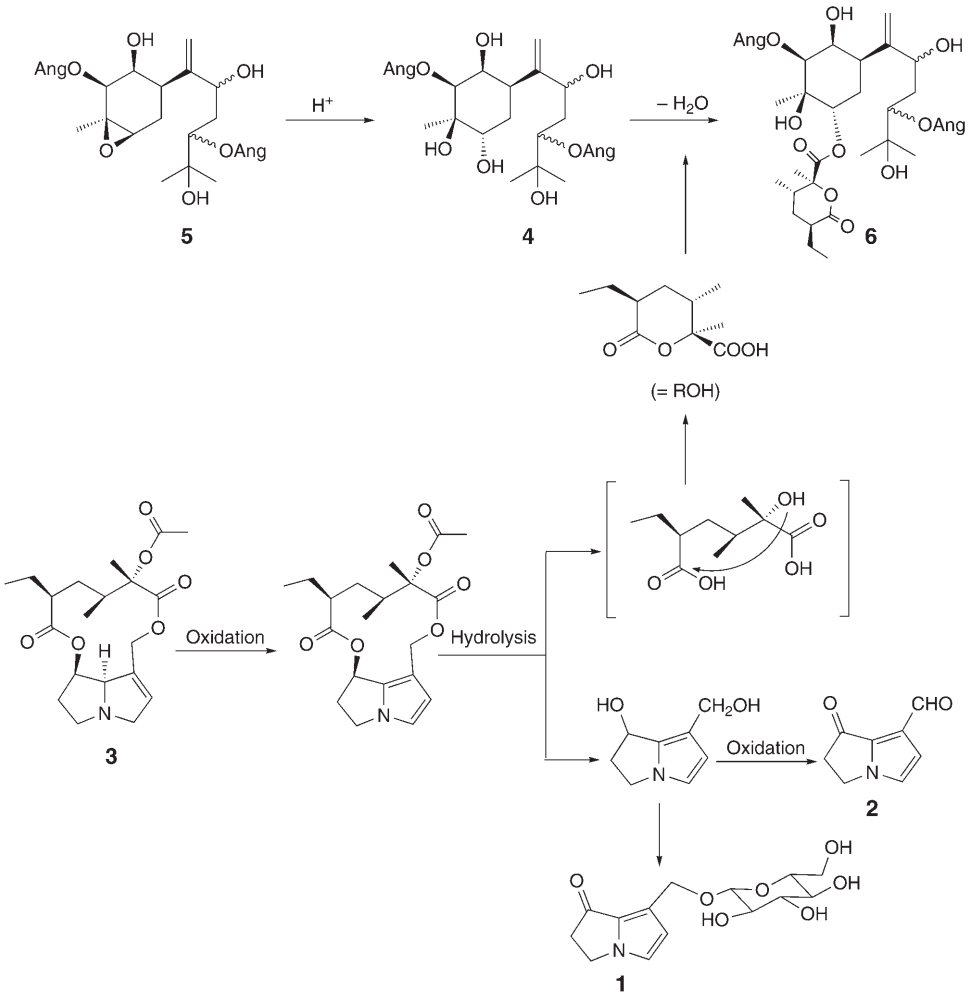
The HMBC plot showed also cross-peaks for the ester *C*=O atoms of the two angeloyloxy groups with H–C(2) and H–C(10), indicating that these two groups were attached to C(2) and C(10). Similarly, five OH groups (δ(H) 4.18 (*t*, *J* = 6.6, 1 H), 4.12 (br. *s*, 1 H), and 3.84 (br. *s*, 1 H); δ(C) 75.0 (C), 74.5 (CH), 72.9 (C), 72.4 (CH), and 69.9 (CH)) were located at C(1), C(3), C(4), C(8), and C(11) by ^{1}H,^{1}H-COSY and HMBC experiments. The relative configuration of **4** was established by the ^{1}H-NMR coupling constants and NOE experiments. If H–C(6) were α-oriented, H–C(1) should be α-oriented too because of the small coupling constant *J*(1,6). Similarly, H–C(4) should have a β-configuration because of the small coupling constant *J*(4,5). In an NOE experiment, irradiation of H–C(6) enhanced the signals of H–C(2) (3.25%) and H–C(1) (2.97%).

The molecular formula of **5** was determined as $C_{25}H_{38}O_8$ by the HR-ESI-MS (*m/z* 489.2456 ($[M + Na]^+$)). The NMR and IR spectra showed that **5** contained, like **4**, a bisabolane skeleton which carried the same side chain as **4**. However, further analysis of the NMR data of **5** indicated that an epoxy group (δ(H) 3.28 (br. *d*, *J* = 5.1, 1 H); δ(C) 61.5 (CH) and 60.9 (C)) was present between C(3) and C(4), which was confirmed by the cross-peaks in the HMBC (δ(H) 3.28 (H–C(4))/δ(C) 19.2 (C(15)), 25.0 (C(5)), and 60.9 (C(3))). Comparison of the coupling constants of **5** and **4** and the

NOE data revealed that **5** and **4** had same configuration. Compound **5** was therefore assigned as (1*β*,2*β*,3*β*,4*β*,6*β*)-3,4-epoxybisabol-7(14)-ene-1,2,8,10,11-pentol 2,10-di-angelate[1]).

The molecular formula of compound **6** was assigned as $C_{35}H_{54}O_{12}$ from its HR-ESI-MS (*m*/*z* 689.3501 ($M + Na]^+$)), indicating nine unsaturation degrees and revealing one $C_{10}H_{14}O_3$ unit more than **4**. The NMR spectra of **6** were similar to those of **4**, except for the signals arising from the extra $C_{10}H_{14}O_3$ unit, and established a bisabolane skeleton. The extra $C_{10}H_{14}O_3$ (+1 H) unit was elucidated as a tetrahydroclivonecoyl (= 5-ethyl-3,4,5,6-tetrahydro-2,3-dimethyl-6-oxo-2*H*-pyran-2-carbonyl) group by ^{1}H,^{1}H-COSY and HMBC [20–22], which biosynthetically was probably derived from compound **3** (*Scheme*) [23]. The structure of **6** was finally eluci-

Scheme. *Possible Biosynthetic Pathway for* **1**, **2**, *and* **6**



dated as (1*β*,2*β*,3*β*,4*α*,6*β*)-bisabol-7(14)-ene-1,2,3,4,8,10,11-heptol 2,10-diangelate 4-[(3*S*,5*S*,6*S*)-tetrahydroclivonecate][1]).

The ^{13}C-NMR and DEPT data of **6** showed the presence of ten Me, five CH_2, and ten CH groups, and ten quaternary C-atoms (three ester C=O), arising from two angeloyloxy groups (ten C-atoms), the bisabolane skeleton (fifteen C-atoms), and the extra $C_{10}H_{14}O_3$ (+1 H) unit. The latter unit gave rise to ^{1}H-NMR signals at δ(H) 2.40–2.36 (*m*, 1 H), 2.08–2.04 (*m*, 1 H), 2.06–2.04 (*m*, 1 H), 1.81–1.79 (*m*, 1 H), 1.64 (*s*, 3 H), 1.58–1.53 (*m*, 1 H), 1.31–1.28 (*m*, 1 H), 1.07 (*d*, *J* = 7.2, 3 H), and 0.99 (*t*, *J* = 7.6, 3 H) and the ^{13}C-NMR signals at δ(C) 173.7, 169.5, 87.5, 42.5, 37.6, 31.8, 24.1, 23.8, 16.5, and 11.4, which were compatible with a tetrahydroclivonecoyl group. This was confirmed by the ^{1}H,^{1}H-COSY and HMBC data. The following long-range correlations were observed in the HMBC (*Fig. 2*): H–C(8″)/C(5″), C(6″), and C(7″), H–C(9″)/C(4″), C(5″), and C(6″), H–C(10″)/C(2″), C(3″), C(4″), and C(11″), H–C(4″)/C(2″), C(3″), C(5″), C(6″), C(9″), and C(10″). The position of the ester groups was determined by 2D-NMR techniques (^{1}H,^{1}H-COSY and HMBC). In the HMBC experiment of **6**, the correlations δ(C) 168.2/H–C(10), δ(C) 167.0/H–C(2), and δ(C) 173.7/H–C(4) pointed to the tetrahydroclivonecoyloxy group at C(4) and the two angeloyloxy groups at C(2) and C(10), respectively. The relative configuration was established from the small coupling constants and NOE difference spectra: irradiation of H–C(6) enhanced the signals of H–C(1) (6.02%) and H–C(2) (6.21%).

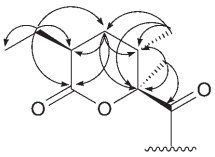


Fig. 2. *Selected HMBC correlations* (H → C) *of the* $C_{10}H_{15}O_3$ *unit of* **6**

The known compound **3** was characterized as 12-*O*-acetylyamataimine by comparison of its physical and spectroscopic data with published data [25].

The cytotoxic activities of compounds **1**, **2**, and **4**–**6** against BEL-7402, HL-60, HO-8910, and KB were tested by the MTT [26] and SRB methods [27]. The results are shown in *Tables 1–3*. Compound **6** showed weak cell-growth inhibition of BEL-7402 cell.

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Table 1. *Inhibitory Rates to the Growth of BEL-7402 of Compounds* **1**, **2**, *and* **4**–**6**

Compound	Concentration [mol/l]					Evaluation[a])
	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	
1	38.3	13.7	4.2	9.2	9.2	no effect
2	25.8	5.9	4.7	0.4	1.8	no effect
4	33.6	10.9	11.3	11.1	0	no effect
5	43.5	6.9	10.4	8.2	0	no effect
6	95.2	95.0	32.7	6.7	0	weak effect

[a]) Criteria: no effect: 10^{-5} mol/l < 85%; weak effect: 10^{-5} mol/l > 85%; strong effect: 10^{-6} mol/l > 85% or 10^{-7} mol/l > 50%.

Table 2. *Inhibitory Rates to the Growth of HL-60 of Compounds* **1**, **2**, *and* **4**–**6**

Compound	Concentration [mol/l]					Evaluation[a])
	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	
1	12.1	12.4	13.3	11.6	11.9	no effect
2	13.2	9.9	10.4	8.6	7.6	no effect
4	54.7	0	8.4	9.4	10.6	no effect
5	98.6	14.3	15.5	13.6	12.5	no effect
6	100	1.1	0.9	12.7	0	no effect

[a]) Criteria: no effect: 10^{-5} mol/l $< 85\%$; weak effect: 10^{-5} mol/l $> 85\%$; strong effect: 10^{-6} mol/l $> 85\%$ or 10^{-7} mol/l $> 50\%$.

Table 3. *Inhibitory Rates to the Growth of HO-8910 and KB of Compound* **6**

Cancer cell lines	Concentration [mol/l]					Evaluation[a])
	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	
HO-8910	12.1	12.3	8.9	17.6	24.7	no effect
KB	98.9	21.4	13.7	13.3	14.3	no effect

[a]) Criteria: no effect: 10^{-5} mol/l $< 85\%$; weak effect: 10^{-5} mol/l $> 85\%$; strong effect: 10^{-6} mol/l $> 85\%$ or 10^{-7} mol/l $> 50\%$.

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Experimental Part

General. Column chromatography (CC) and TLC: silica gel (200–300 mesh) and silica gel GF_{254} (10–40 μm), resp. both from *Qingdao Marine Chemical Factory*, Qingdao, People's Republic of China; TLC detection at 254 nm or by spraying with 5% H_2SO_4 in EtOH (*v*/*v*), followed by heating. Optical rotations: *Perkin-Elmer 341* polarimeter. IR Spectra: *Nicolet Nexus-670* FT-IR spectrometer; in cm^{-1}. UV Spectra: *Shimadzu UV-260* spectrometer; in λ_{max} (log ε). NMR Spectra: *Varian Mercury-400BB* NMR instrument; δ in ppm rel. to $SiMe_4$ as internal standard, *J* in Hz. MS: *VG ZAB-HS* (EI; 70 eV), *ZAB-HS* (FAB), and *Bruker APEX-II* mass spectrometer (HR-ESI) with glycerol as the matrix; in *m*/*z* (rel. %).

Plant Material. The roots of *Ligularia cymbulifera* were collected in Muli, Sichuan Province, People's Republic of China, in August 2004. The plant was identified by Prof. *Guo-Liang Zhang*, Department of Biology, Lanzhou University. A voucher specimen (No. 2004814) was deposited in the College of Chemistry and Chemical Engineering, Lanzhou University.

Extraction and Isolation. The extraction with petroleum ether/Et_2O/MeOH 1:1:1 and the CC of the crude extract were carried out similarly as described previously [5]. The CC fraction eluted with 100% AcOEt (*Fraction E*; 50 g) was further fractionated by CC (silica gel (450 g), $CHCl_3$/MeOH 10:1, 5:1, and 1:1): *Fr. E1–E3*. From *Fr. E1* (21 g), **2** (5 mg; R_f 0.55, $CHCl_3$/acetone 4:1), **4** (10 mg; R_f 0.55, $CHCl_3$/acetone 1:1), **5** (7 mg; R_f 0.55, petroleum ether/AcOEt 1:2), and **6** (5 mg; R_f 0.40, petroleum ether/AcOEt 1:2) were obtained by repeated CC (silica gel, petroleum ether/AcOEt (4:1 → 1:2). *Fr. E2* (5 g) was applied to CC (silica gel, $CHCl_3$/MeOH 9:1): **1** (5 mg; R_f 0.70, $CHCl_3$/MeOH 3:1). The dry material left after the extraction with petroleum ether/Et_2O/MeOH 1:1:1 was extracted with 95% EtOH

(3×) under reflux condition for 4 h. The resultant extract was concentrated and partitioned between Et_2O and 1M HCl. The aq. layer was basified to pH 9–10 with conc. NH_3 soln. and extracted exhaustively with $CHCl_3$ to give a crude alkaloid fraction. The crude alkaloid fraction (1.2 g) was subjected to CC (silica gel, $CHCl_3$/MeOH/Et_2NH): **3** (35 mg).

1-[(β-D-Glucopyranosyloxy)methyl]-5,6-dihydropyrrolizin-7-one (= *7-[(β-D-Glucopyranosyloxy)methyl]-2,3-dihydro-1H-pyrrolizin-1-one*; **1**): Amorphous solid. $[\alpha]_D^{21} = -20$ ($c = 1.30$, MeOH). UV (MeOH): 289.2 (1.30). IR (KBr): 3417, 3100, 1671, 1553, 1490, 1383, 1072, 983, 759. ^{1}H-NMR (CD_3OD, 400 MHz): 7.14 (*d*, *J* = 2.0, H–C(3)); 6.57 (*d*, *J* = 2.8, H–C(2)); 4.85 (*d*, *J* = 12.0, CH_2(9)); 4.39 (*d*, *J* = 8.0, H–C(1′)); 4.29 (*t*, *J* = 6.0, CH_2(5)); 3.89 (*dd*, *J* = 12.0, 1.2, 1 H–C(6′)); 3.67 (*dd*, *J* = 12.0, 5.2, 1 H–C(6′)); 3.38–3.35 (*m*, H–C(3′)); 3.34–3.29 (*m*, H–C(4′)); 3.31 (overlapped, H–C(5′)); 3.21 (*dd*, *J* = 9.2, 8.0, H–C(2′)); 3.04 (*t*, *J* = 6.0, CH_2(6)). ^{13}C-NMR (CD_3OD, 100 MHz): 191.4 (CO, C(7)); 129.7 (C(8)); 123.9 (CH(3)); 121.7 (C(1)); 117.2 (CH(2)); 102.1 (CH(1′)); 76.8 (CH(3′)); 76.8 (CH(5′)) 73.9 (CH(2′)); 70.4 (CH(4′)); 62.7 (CH(9)); 61.6 (CH_2(6′)); 42.1(CH_2(5)); 39.5 (CH_2(6)). HR-ESI-MS: 336.1058 ([*M* + Na]$^+$, $C_{14}H_{19}NNaO_7^+$; calc. 336.1054).

1-Formyl-5,6-dihydropyrrolizin-7-one (= *2,3-Dihydro-1-oxo-1H-pyrrolizidine-7-carboxaldehyde*; **2**): Amorphous solid. $[\alpha]_D^{21} = -7$ ($c = 0.70$, $CHCl_3$). UV ($CHCl_3$): 306.0 (1.60), 269.6 (1.28). IR (KBr): 3421, 2924, 2363, 2341, 1701, 1368. ^{1}H-NMR: in agreement with [14]. ^{13}C-NMR ($CDCl_3$, 100 MHz): 189.1 (C(7)=O); 183.9 (CHO); 135.1 (C(8)); 123.0 (C(1)); 123.0 (CH(3)); 115.7 (CH(2)); 43.2 (CH_2(5)); 39.3 (CH_2(6)). EI-MS (70 eV): 149 (92, M^+), 121 (93), 93 (100), 84 (66), 65 (59). HR-ESI-MS: 150.0552 ([*M* + H]$^+$, $C_8H_8NO_2^+$; calc. 150.0550).

(1β,2β,3β,4α,6β)-Bisabol-7(14)-ene-1,2,3,4,8,10,11-heptol 2,10-Diangelate (= *(2Z)-2-Methylbut-2-enoic Acid* rel-*(1R,2S,3R,5S,6R)-5-{2,5-Dihydroxy-5-methyl-1-methylene-4-{[(2Z)-2-methyl-1-oxobut-2-en-1-yl]oxy}hexyl}-2,3,6-trihydroxy-2-methylcyclohexyl Ester*; **4**): Colorless gum. $[\alpha]_D^{21} = -47$ (c = 0.60, acetone). IR (KBr): 3489, 1715, 1647. ^{1}H-NMR ($CDCl_3$, 400 MHz): 5.24 (br. *s*, 1 H–C(14)); 5.16 (br. *s*, 1 H–C(14)); 5.06 (*d*, *J* = 2.1, 1 H–C(2)); 4.85 (br. *d*, *J* = 4.5, H–C(10)); 4.18 (*t*, *J* = 6.6, H–C(8)); 4.12 (br. *s*, H–C(1)); 3.84 (br. *s*, H–C(4)); 3.00 (br. *d*, *J* = 14.1, H–C(6)); 2.41 (br. *t*, *J* = 14.1, H_β–C(5)); 2.28–2.20 (*m*, H_b–C(9)); 1.98 (overlapped, H_a–C(9)); 1.54 (br. *d*, *J* = 14.1, H_α–C(5)); 1.24 (*s*, Me(12)); 1.24 (*s*, Me(13)); 1.24 (*s*, Me(15)); angeloyloxy protons: 6.14 (*qq*, *J* = 7.2, 1.6, 2 H, H–C(3′)); 2.05 (*dq*, *J* = 7.2, 1.4, Me(4′)); 1.98 (*dq*, *J* = 7.2, 1.4, Me(4′)); 1.94 (*dq*, *J* = 1.6, 1.4, Me(5′)); 1.90 (*q*, *J* = 1.6, 1.4, Me(5′)). ^{13}C-NMR ($CDCl_3$, 100 MHz): 148.9 (C(7)); 114.8 (CH_2(14)); 76.7 (CH(10)); 75.0 (C(11)); 74.5(CH(4)); 72.9 (C(3)); 72.4 (CH(1)); 71.5 (CH(2)); 69.9 (CH(8)); 38.2 (CH(6)); 35.2 (CH_2(9)); 27.2 (CH_2(5)); 26.1 (Me(13)); 25.7 (Me(12)); 22.5 (Me(15)); 168.1 (C(1′)); 167.6 (C(1′)); 139.8 (CH(3′)); 138.9 (CH(3′)); 127.4 (C(2′)); 127.3 (C(2′)); 20.6 (Me(5′)); 20.6 (Me(5′)); 15.9 (Me(4′)); 15.9 (Me(4′)). HR-ESI-MS: 485.2743 ([*M* + H]$^+$, $C_{25}H_{41}O_7^+$; calc. 485.2745).

(1β,2β,3β,4β,6β)-3,4-Epoxybisabol-7(14)-ene-1,2,8,10,11-pentol 2,10-Diangelate (= *(2Z)-2-Methylbut-2-enoic Acid* rel-*(1R,2S,3S,4R,6R)-4-{2,5-Dihydroxy-5-methyl-1-methylene-4-{[(2Z)-2-methyl-1-oxobut-2-en-1-yl]oxy}hexyl}-3-hydroxy-1-methyl-7-oxabicyclo[4.1.0]hept-2-yl Ester*; **5**): Colorless gum. $[\alpha]_D^{21} = -63$ ($c = 0.40$, acetone). IR (KBr): 1715, 1647. ^{1}H-NMR ($CDCl_3$, 400 MHz): 5.22 (*d*, *J* = 4.8, H–C(2)); 5.19 (br. *s*, 1 H–C(14)); 5.17 (br. *s*, 1 H–C(14)); 4.90 (*dd*, *J* = 6.9, 3.6, H–C(10)); 4.24 (*t*, *J* = 6.6, H–C(8)); 4.01 (br. *s*, H–C(1)); 3.28 (br. *d*, *J* = 5.1, H–C(4)); 2.47 (br. *dd*, *J* = 12.3, 4.5, H–C(6)); 2.17 (overlapped, H_b–C(9)); 2.10 (overlapped, H_β–C(5)); 2.02 (overlapped, H_α–C(5)); 1.98 (overlapped, H_a–C(9)); 1.36 (*s*, Me(15)); 1.25 (*s*, Me(12), Me(13)); angeloyloxy protons: 6.15 (*qq*, *J* = 6.9, 1.5, 2 H, H–C(3′)); 2.06 (*dq*, *J* = 6.9, 1.2, Me(4′)); 2.02 (*dq*, *J* = 6.9, 1.2, Me(4′)); 1.97 (*dq*, *J* = 1.5, 1.2, Me(5′)); 1.92 (*dq*, *J* = 1.5, 1.2, H–C(5′)). ^{13}C-NMR ($CDCl_3$, 100 MHz): 149.5 (C(7)); 114.4 (CH(14)); 76.6 (CH(10)); 72.7 (CH(2)); 71.1 (CH(1)); 72.3 (C(11)); 69.9 (CH(8)); 61.5 (CH(4)); 60.9 (C(3)); 41.2 (CH(6)); 35.5 (CH_2(9)); 26.1 (Me(12)); 26.0 (Me(13)); 25.0 (CH_2(5)); 19.2 (Me(15)); 167.8 (C(1′)); 167.3 (C(1′)); 139.5 (CH(3′)); 139.0 (CH(3′)); 127.5 (C(2′)); 127.3 (C(2′)); 20.7 (Me(5′)); 20.6 (Me(5′)); 16.0 (Me(4′)); 15.9 (Me(4′)). FAB-MS: 467 ([*M* + H]$^+$), 449, 431. HR-ESI-MS: 489.2456 ([*M* + Na]$^+$, $C_{25}H_{38}NaO_8^+$; calc. for 489.2459).

(1β,2β,3β,4α,6β)-Bisabol-7(14)-ene-1,2,3,4,8,10,11-heptol 2,10-Diangelate 4-[(3S,5S,6S)-Tetrahydroclivonecate] (= rel-*(2R,3R,5R)-5-Ethyltetrahydro-2,3-dimethyl-6-oxo-2H-pyran-2-carboxylic Acid (1R,2S,3R,4R,5S)-5-{2,5-Dihydroxy-5-methyl-1-methylene-4-{[(2Z)-2-methyl-1-oxobut-2-en-1-yl]oxy}hexyl}-2,4-dihydroxy-2-methyl-3-{[(2Z)-2-methyl-1-oxobut-2-en-1-yl]oxy}cyclohexyl Ester*; **6**): Colorless

gum. ^{1}H-NMR ($CDCl_3$, 400 MHz): 5.24 (br. *s*, 1 H–C(14)); 5.16 (br. *d*, *J* = 2.4, H–C(4)); 5.11 (br. *s*, 1 H–C(14)); 4.90 (*d*, *J* = 2.4, H–C(2)); 4.88 (*dd*, *J* = 8.0, 3.2, H–C(10)); 4.14 (*t*, *J* = 6.8, H–C(8)); 4.12 (br. *s*, H–C(1)); 2.79 (br. *d*, *J* = 14.8, H–C(6)); 2.46 (br. *t*, *J* = 14.8, H_β–C(5)); 2.26–2.22 (*m*, H_b–C(9)); 2.01 (overlapped, H_a–C(9)); 1.58 (br. *d*, *J* = 14.8, H_α–C(5)); 1.25 (*s*, Me(12)); 1.25 (*s*, Me(13)); 1.15 (*s*, Me(15)); 2.40–2.36 (*m*, H–C(3″)); 2.08–2.04 (*m*, H_b–C(10″)); 2.06–2.04 (*m*, H–C(5″)); 1.81–1.79 (*m*, H_b–C(4″)); 1.64 (*s*, Me(8″)); 1.58–1.53 (*m*, H_a–C(10″)); 1.31–1.28 (*m*, H_a–C(4″)); 1.07 (*d*, *J* = 7.2, Me(9″)); 0.99 (*t*, *J* = 7.6, Me(11′)); angeloyloxy protons: 6.16 (*qq*, *J* = 7.3, 1.5, 2 H, H–C(3′)); 2.07 (*dq*, *J* = 7.3, 1.2, Me(4′)); 1.98 (*dq*, *J* = 7.3, 1.2, Me(4′)); 1.96 (*dq*, *J* = 1.5, 1.2, Me(5′)); 1.89 (*dq*, *J* = 1.5, 1.2, Me(5′)). ^{13}C-NMR ($CDCl_3$, 100 MHz): 148.2 (C(7)); 114.4 (CH_2(14)); 78.5 (CH(4)); 77.3 (CH(10)); 72.9 (C(3)); 72.6 (CH(1)); 72.3 (C(11)); 70.8 (CH(2)); 69.7 (CH(8)); 39.1 (CH(6)); 35.0 (CH_2(9)); 26.0 (Me(12)); 25.8 (Me(13)); 24.8 (CH_2(5)); 22.5 (Me(15)); 173.7 (C(2″)); 169.5 (C(7″)); 87.5 (C(6″)); 42.5 (CH(3″)); 37.6 (CH(5″)); 31.8 (CH_2(4″)); 24.1 (CH_2(10″)); 23.8 (Me(8″)); 16.5 (Me(9″)); 11.4 (Me(11″)); 167.2 (C(1′)); 167.0 (C(1′)); 140.0 (CH(3′)); 139.3 (CH(3′)); 127.5 (C(2′)); 127.2 (C(2′)); 20.5 (Me(5′)); 20.5 (Me(5′)); 15.9 (Me(4′)); 15.9 (Me(4′)). HR-ESI-MS: 689.3501 ($[M + Na]^+$, $C_{35}H_{54}NaO_{12}^+$; calc. 689.3507).

Acid Hydrolysis of **1**. A soln. of **1** (9 mg) in EtOH (10 ml) and 4% HCl soln. (6 ml) was heated at 90° in a water-bath for 5 h. After cooling, the mixture was diluted with H_2O and extracted with $CHCl_3$ (5 ×). The aq. layer was neutralized with $NaHCO_3$ and concentrated: D-glucose, as established by direct comparison with an authentic sample (TLC: AcOEt/PrOH/H_2O 65 :23 :12). $[\alpha]_D^{21} = +18$ ($c = 0.45$, H_2O).

Cytotoxicity Assays. Testing for *in vitro* cytotoxic activities of compounds **1**, **2**, and **4**–**6** against BEL-7402 (hepatoma cells), HL-60 (myeloid leukemia cells), HO-8910 (human ovarian carcinoma cells), and KB (nasopharyngeal carcinoma cells) was carried out by the *National Center for Drug Screening* in China, by means of the MTT [26] and SRB [27] methods, resp.

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