Novel Diterpenoids and Diterpenoid Glycosides from *Siegesbeckia orientalis*

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Eight new *ent*-pimarane diterpenoids and diterpenoid glycosides, namely, *ent*-12α,16-epoxy-2*β*,15α,19-trihydroxypimar-8-ene (**1**), *ent*-12α,16-epoxy-2*β*,15α,19-trihydroxypimar-8(14)-ene (**2**), *ent*-2α,15,16,19-tetrahydroxypimar-8(14)-ene (**3**), *ent*-15-oxo-2*β*,16,19-trihydroxypimar-8(14)-ene (**4**), *ent*-2-oxo-15,16-dihydroxypimar-8(14)-en-16-*O*-*β*-glucopyranoside (**5**), *ent*-2-oxo-15,16,19-trihydroxypimar-8(14)-ene (**6**), *ent*-2-oxo-3*β*,15,16-trihydroxypimar-8(14)-en-3-*O*-*β*-glucopyranoside (**7**), and *ent*-2*β*,15,16,19-tetrahydroxypimar-8(14)-en-19-*O*-*β*-glucopyranoside (**9**), together with seven known diterpenes (**8**, **10**−**15**) were isolated from *Siegesbeckia orientalis*. Compounds **1** and **2** are novel *ent*-pimarane diterpenoids with an unprecedented 12α,16-epoxy group. Their structures were established by spectral methods, especially 1D and 2D NMR spectral methods.

The plants of the genus *Siegesbeckia* (Compositae) are annual herbs widely distributed in tropical, subtropical, and temperate parts of the world. Three species of *Siegesbeckia* grow in China, and the aerial parts have been used as a traditional Chinese medicine, "Xi-Xian", to treat rheumatic arthritis, hypertension, malaria, neurasthenia, and snakebite.[1] Extracts and some chemical constituents of *Siegebeckia* exhibited antioxidative,[2] antiallergic,[3] infertile,[4] and other bioactivities. A series of *ent*-kaurane[1,5] and *ent*-pimarane[1,5−10] diterpenoids and sesquiterpenoids[11,12] from *Siegebeckia* species have been reported. The current investigation has led to the isolation of 15 *ent*-pimarane diterpenoids and diterpenoid glycosides from the aerial parts of *Siegesbeckia orientalis* L. (Compositae). These compounds include eight new compounds (**1**−**7** and **9**) and seven known compounds (**8**, **10**−**15**). Compounds **1** and **2** are novel *ent*-pimarane diterpenoids with an unprecedented 12α,16-epoxy group. This paper deals with the isolation and structural elucidation of these compounds.

Results and Discussion

Compound **1** was obtained as a white amorphous powder, $[\alpha]_D^{20}$ +13.8° (*c* 0.63, MeOH). The IR spectrum showed the presence of hydroxyls (3406 cm^{-1}) and a double bond (1657 cm^{-1}). The positive ion ESIMS showed a quasimolecular ion at *m/z* 359 $[M + Na]^+$. The molecular formula $C_{20}H_{32}O_4$ was deduced from its HREIMS, inferring the presence of five degrees of unsaturation. The ^1H NMR spectrum showed the existence of three methyl groups on quaternary carbons (*δ* 0.92, 1.02, 1.02, each 3H, s), two oxygenated methylenes at *δ* 3.36 and 3.66 (each 1H, both d, $J = 11.1$ Hz) and *δ* 3.57 (1H, dd, $J = 10.0$, 1.6 Hz) and 4.18 (1H, dd, $J = 10.0$, 4.6 Hz), and three oxygenated methines at *δ* 3.56 (1H, br s), 3.78 (1H, dd, $J = 4.6$, 1.6 Hz), and 3.85 (1H, dddd, $J = 11.6$, 11.2, 4.0, 3.6 Hz). The ^{13}C NMR spectrum (with DEPT experiments) showed 20 carbon signals attributable to three methyls, eight methylenes (two oxygenated, at *δ* 66.3 and 75.1), four methines (three oxygenated, at *δ* 65.9, 81.0, and 84.7), and five quaternary carbons (two olefinic, at *δ* 126.0 and 143.4). The EIMS ion at *m/z* 275 $[M - 61]^+$ is indicative of the loss of $-CH(OH)CH_2OH$ like most of the *ent*-pimarane diterpenoids isolated from this plant. The two quaternary olefinic carbon signals could only be assignable to a persubstituted double bond at C-8. The remaining four degrees of unsaturation were consistent with a tetracyclic ring system in **1**. The known pimarane type diterpenoids possess a tricyclic carbon skeleton, suggesting that **1** possibly contained an epoxy group. This conclusion was supported by the downfield shifted secondary carbon signal at *δ* 75.1 (C-16) and the tertiary carbon signal at *δ* 84.7 (C-12) in the ^{13}C NMR spectrum by comparison with the known compound **10**. Analysis of 2D NMR spectra ($^1H-^1H$ COSY, HMQC, and HMBC; spectra S1−S5, Supporting Information) established the planar structure of **1**. The relative stereochemistry of **1** was established by a NOESY experiment (spectrum S6, Supporting Information). The structure of compound **1** was therefore established as *ent*-12α,16-epoxy-2*β*,15α,19-trihydroxypimar-8-ene.

Compound **2** was obtained as a white amorphous powder, $[\alpha]_D^{20}$ +6.5° (*c* 1.40, MeOH). The IR spectrum showed absorption bands at 3356 cm^{-1} for hydroxyls and 1635 cm^{-1} for a double bond. The positive ion ESIMS showed a quasimolecular ion at *m/z* 359 $[M + Na]^+$. The EIMS gave the molecular ion at *m/z* 336 $[M]^+$ and important fragment ions at 318 $[M - H_2O]^+$ and 257 $[M - H_2O - CH(OH)CH_2OH]^+$. The molecular formula was also established as $C_{20}H_{32}O_4$ from its HREIMS spectrum. The ^1H and ^{13}C NMR spectra of **2** were very similar to those of compound **1** except for the location of the double bond: a trisubstituted double bond in **2** instead of the persubstituted double bond in **1**. Spectral evidence implied that **2** possessed a double bond between C-8 and C-14, which was confirmed by the comparison of the data with those of **10**. The ^{13}C NMR data of both compounds **2** and **10** were very similar, except that the signals of C-12 (*δ* 87.0) and C-16 (*δ* 72.7) in **2** were shifted downfield, indicating that an epoxy was formed between C-12 and C-16. The coupling constant of H-15 (*δ* 3.88, 1H, dd, $J = 6.5$, 2.6 Hz) suggested that the 15-OH was also α-oriented. Thus, the structure of **2** was elucidated as *ent*-12α,16-epoxy-2*β*,15α,19-trihydroxypimar-8(14)-ene.

Compound **3** was obtained as a white amorphous powder, $[\alpha]_D^{20}$ −18.4° (*c* 0.50, MeOH). The IR spectrum exhibited absorption bands at 3404 cm^{-1} for hydroxyls and 1649 cm^{-1} for a double bond. The EIMS also showed typical fragment ions at *m/z* 277 $[M - CH(OH)CH_2OH]^+$, 259 $[M - CH(OH)CH_2OH - H_2O]^+$, and 241 $[M - CH(OH)CH_2OH - 2H_2O]^+$.

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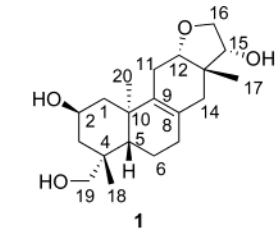

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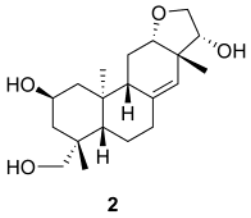

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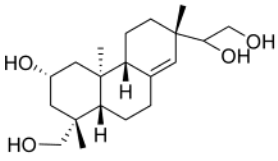

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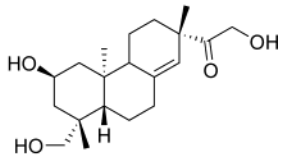

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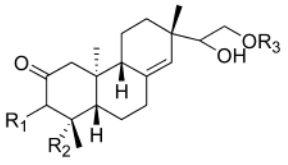
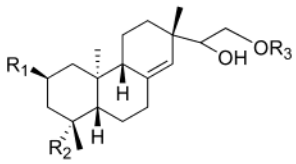
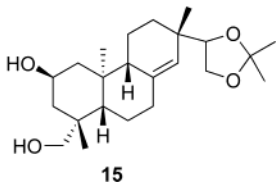


5 R_1=H R_2=CH_3 R_3=Glc
6 R_1=H R_2=CH_2OH R_3=H
7 R_1=OGlc R_2=CH_3 R_3=H
8 R_1=H R_2=CH_2OGlc R_3=H



9 R_1=OH R_2=CH_2OGlc R_3=H
10 R_1=OH R_2=CH_2OH R_3=H
11 R_1=H R_2=CH_2OGlc R_3=H
12 R_1=OGlc R_2=CH_3 R_3=H
13 R_1=H R_2=COOGlc R_3=H
14 R_1=OH R_2=CH_2OH R_3=Ac



15

The molecular formula $C_{20}H_{34}O_4$ was determined by HREIMS and ^{13}C NMR. The EIMS and ^1H and ^{13}C NMR spectra inferred that **3** was an isomer of **10**, and the B- and C-rings in both compounds were the same. The coupling constant of H-2 was obviously different from that of **10**, suggesting that **3** had a 2α-OH instead of the 2*β*-OH in **10**. The 2D NMR experiments (HMQC and HMBC) indeed confirmed the planar structure of compound **3**. Analysis of the NOESY spectrum confirmed the 2-OH α-orientation. The structure of **3** was elucidated as *ent*-2α,15,16,19-tetrahydroxypimar-8(14)-ene.

Compound **4** was obtained as a white amorphous powder, $[\alpha]_D^{20}$ −15.1° (*c* 0.55, MeOH). The IR spectrum showed the presence of hydroxyls (3406 cm^{-1}), a ketone carbonyl (1714 cm^{-1}), and a double bond (1649 cm^{-1}). The molecular formula $C_{20}H_{32}O_4$ was deduced from its HRESIMS spectrum at *m/z* 359.2195 $[M + Na]^+$ (calcd 359.2198). The NMR spectrum was very similar to that of compound **10** except for the chemical shifts of the side chain. Compared with compound **10**, the signals of H_2-16 (*δ* 4.33 and *δ* 4.41) were strongly downfield shifted, suggesting that the ketone group was located at C-15 (*δ* 215.8), which was confirmed by the coupling patterns of the two H_2-16 protons (only ^2J was observed). In the HMBC spectrum of **4**, the correlations of C-15 with H_2-16 and CH_3-17 definitely indicated a C-15 ketone group. The structure of **4** was thus established to be *ent*-15-oxo-2*β*,16,19-trihydroxypimar-8(14)-ene.

Compound **5** was obtained as a white amorphous powder, $[\alpha]_D^{20}$ −51.7° (*c* 1.58, MeOH). The IR spectrum revealed the presence of hydroxyls (3413 cm^{-1}), a carbonyl (1701 cm^{-1}), and a double bond (1660 cm^{-1}). EIMS showed a weak molecular ion at *m/z* 482 and major fragment ions at *m/z* 464 $[M - 18]^+$ and 259 [M − 162 − 61], suggesting that **5** was a diterpenoid glycoside. The molecular formula $C_{26}H_{42}O_8$ was determined by HREIMS. The ^1H NMR spectrum clearly showed four methyl groups on quaternary carbons (*δ* 0.84, 0.92, 0.92, 1.13, each 3H, s), a trisubstituted olefinic proton at *δ* 5.31 (1H, s), and an anomeric proton at *δ* 4.28 (1H, d, J = 7.9 Hz) for a sugar moiety. The ^{13}C NMR spectrum showed carbon signals for a diterpenoid aglycone and a glucopyranose; four methyls, eight methylenes (two oxygenated, at *δ* 63.2 and 72.9), nine methines (five oxygenated, at *δ* 72.1, 75.4, 75.5, 78.4, 78.5, one hemiacetal (*δ* 104.5), and one olefinic (*δ* 130.9)), and five quaternary carbons (one carbonyl, at *δ* 215.4 and one olefinic, at *δ* 139.7). Compared with **10**, the downfield shifted carbon signals of C-2 (*δ* 215.4), C-1 (*δ* 54.9) and C-3 (*δ* 57.5) suggested that the ketone group was at C-2; the downfield shifted C-16 signal (*δ* 72.9) and slightly upfield shifted C-15 (*δ* 75.5) were considered to be caused by glycosylation, suggesting that the sugar moiety was at C-16. The structural assignment of **5** was further supported by 2D NMR spectra (HMQC and HMBC). In the HMBC, C-2 (*δ* 215.4) correlated with H_2-1 (*δ* 2.09 and 2.55), H_2-3 (*δ* 2.27 and 2.44), and CH_3-20 (*δ* 0.84), confirming the C-2 ketone group; the correlations between H-1′ (*δ* 4.28) and C-16 (*δ* 72.9) and between H_2-16 (*δ* 3.83) and C-1′ (*δ* 104.5) verified the linkage between the aglycone and sugar moiety. The coupling constant of the anomeric proton at *δ* 4.28 (d, J = 7.9 Hz) and the anomeric carbon at *δ* 104.5 indicated that the sugar moiety was a *β*-glucose. The structure of compound **5** was therefore established as *ent*-2-oxo-15,16-dihydroxypimar-8(14)-en-16-*O*-*β*-glucopyranoside.

Compound **6** was obtained as a white amorphous powder, $[\alpha]_D^{20}$ −29.9° (*c* 1.55, MeOH). The IR spectrum indicated the presence of hydroxyls (3415 cm^{-1}), a carbonyl (1697 cm^{-1}), and a double bond (1670 cm^{-1}). The EIMS gave the fragment peaks at 275 $[M - CH(OH)CH_2OH]^+$. The molecular formula of **6** was deduced as $C_{20}H_{32}O_4$ from HREIMS and ^{13}C NMR. Comparison of the spectral data of compounds **4** and **6** suggested they were structural isomers, the difference being the location of the ketone group. The ^1H NMR spectrum of **6** showed the presence of three methyl groups on quaternary carbons (*δ* 0.71, 0.75, 1.02, each 3H, s) and one olefinic proton (*δ* 5.15, 1H, s). The ^{13}C NMR of **6** showed the presence of three methyls, eight methylenes (two oxygenated, at *δ* 64.8 and 66.3), four methines (one oxygenated, at *δ* 78.1, and one olefinic, at *δ* 131.2), and five quaternary carbons (one olefinic, at *δ* 139.2, and one carbonyl, at *δ* 215.5). Comparing the ^{13}C NMR of **6** with that of known compound **10**, the compounds were similar, except for the carbon signals of C-1, C-2, and C-3 in the A-ring, suggesting that the ketone group was most likely located at C-2 in **6**. In the ^{13}C NMR of **6**, the signals of C-1, C-2, and C-3 resonated at *δ* 55.0, 215.5, and 51.4, respectively, confirming the C-2 ketone group. The 2D NMR spectra of **6** (HMQC and HMBC) confirmed the

structural assignment. In the HMBC of **6**, C-2 correlated with H_2-1 and CH_3-20. Thus, the structure of compound **6** was elucidated as *ent*-2-oxo-15,16,19-trihydroxypimar-8(14)-ene.

Compound **7** was obtained as pale gum, $[\alpha]_D^{20}$ −22.2° (*c* 1.35, MeOH). The IR spectrum displayed absorptions for hydroxyls (3404 cm^{-1}), a carbonyl (1707 cm^{-1}), and a double bond (1637 cm^{-1}). The EIMS exhibited major fragment ions at *m/z* 480 $[M - H_2O]^+$, 437 $[M - CH(OH)CH_2OH]^+$, and 275 $[M - CH(OH)CH_2OH - 162]^+$, suggesting that **7** was also a diterpenoid glycoside. The molecular formula $C_{26}H_{42}O_9$ was deduced from its HREIMS and ^{13}C NMR. The ^1H NMR spectrum showed the presence of four methyl groups on quaternary carbons (δ 0.77, 0.82, 0.84, 1.15), one anomeric proton at δ 4.26 (1H, d, $J = 7.5$ Hz), and one olefinic proton at δ 5.22 (1H, s). The ^{13}C NMR spectrum showed carbon signals consistent with a diterpenoid aglycone and a glucopyranose. Except for the glucopyranose, the ^{13}C NMR spectrum also revealed the signals of one ketone group (δ 216.0), one trisubsitituted double bond (δ 130.9 and 139.4), two oxygenated methines (δ 77.8 and 93.6), and one oxygenated methylene (δ 64.6). The ^{13}C NMR data of B- and C-rings and the sugar moiety in both compounds **7** and **12** were similar, and the ^{13}C NMR data of the A-ring were very different. The planar structure of **7** was further supported by 2D NMR spectra (HMQC and HMBC). In the HMBC, C-2 (δ 216.0) correlated with H-1 (δ 3.09) and H-3 (δ 3.23), indicating that the ketone group was located at C-2, and the correlations of H-3 with C-1, C-2, C-4, C-18, C-19, and C-1′ suggested that the sugar moiety was linked to C-3. The H-3 equatorial proton correlated with both the 18- and 19-Me groups and was assigned by the NOESY spectrum. The coupling of anomeric proton H-1′ (δ 4.26, 1H, d, $J = 7.5$ Hz) and the carbon signal C-1′ (δ 106.2) proved a β-glucopyranose. The structure of **7** was thus elucidated as *ent*-2-oxo-3β,15,16-trihydroxypimar-8(14)-en-3-*O*-β-glucopyranoside.

Compound **9** was obtained as a pale gum, $[\alpha]_D^{20}$ −35.9° (*c* 1.32, MeOH). The IR spectrum displayed absorptions due to hydroxyls (3386 cm^{-1}) and a double bond (1647 cm^{-1}). The EIMS exhibited fragment ions at *m/z* 439 $[M - CH(OH)CH_2OH]^+$, 277 $[M - CH(OH)CH_2OH - 162]^+$, and 259 $[M - CH(OH)CH_2OH - 162 - H_2O]^+$, features typical of a diterpenoid glycoside. The molecular formula $C_{26}H_{44}O_9$ was established using HREIMS and ^{13}C NMR. The ^1H NMR spectrum of **9** clearly displayed the presence of three methyl groups on quaternary carbons (δ 0.83, 0.83, 1.07), one olefinic proton (δ 5.18, 1H, s), and one anomeric proton (δ 4.19, 1H, d, $J = 7.8$ Hz). The ^{13}C NMR spectrum of **9** showed the presence of three methyls, nine methylenes, ten methines, and four quaternary carbons. The ^{13}C NMR spectrum also revealed that **9** possessed one trisubstituted double bond (δ 130.4 and 139.6), two oxygenated methylenes (δ 64.7 and 74.5), and two oxygenated methines (δ 65.7 and 77.9) in the aglycone, and glucopyranose. The spectral data indicated that compound **10** was the aglycone of compound **9** and that the sugar moiety was linked to C-19 as judged from the downfield shifted C-19 signal at δ 74.5 (ca. $\Delta\delta$ 8.4) resulting from glycosylation. The coupling constant of the anomeric proton at δ 4.19 (d, $J = 7.8$ Hz) and C-1′ signal at δ 105.2 indicated a β-glucopyranose. The structure of **9** was thus elucidated as *ent*-2β,15,16,19-tetrahydroxypimar-8(14)-en-19-*O*-β-glucopyranoside.

The known compounds were identified as kirenol (**10**),[8] 16-acetylkirenol (**14**),[8] isopropylidenkirenol (**15**),[8] pubeside A (**12**),[9] pubeside B (**11**),[9] pubeside C (**13**),[9] and pubeside D (**8**)[9] by comparison of the ^1H and ^{13}C NMR data with those reported in the literature.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a Perkin-Elmer 341 polarimeter (Na filter, $\lambda = 589$ nm). IR spectra were recorded on a Perkin-Elmer 577 spectrometer. NMR spectra were recorded on a Bruker AM-400 (400 MHz) or Bruker AM-500 (500 MHz) spectrometer with TMS as internal standard. EIMS spectra including high-resolution mass spectra were recorded on a Finnigan MAT 95 mass spectrometer. ESIMS was recorded on a Finnigan LCQ^{DECA} mass spectrometer. All solvents used were of analytical grade (Shanghai Chemical Plant). Silica gel (200−300 mesh), silica gel H60, and Sephadex LH-20 were used for column chromatography, and precoated silica gel GF254 plates (Qingdao Marine Chemical Plant) were used for TLC. C-18 reversed-phase silica gel (150−200 mesh, Merck) and MCI GEL CHP20P (75−150 μm) (Mitsubishi Chemical Industry LTD) were also used for column chromatography.

Plant Material. The aerial part of *Siegesbeckia orientalis* L. was purchased from Hua Yu Pharmaceutical Co. Ltd in Shanghai in May 2002 and was authenticated by one of the authors, Y.X., of Shanghai Institute of Materia Medica, the Chinese Academy of Sciences, where a voucher specimen (accession number Sieg-2002-1Y) was deposited.

Extraction and Isolation. The air-dried powder of *S. orientalis* (3 kg) was extracted with 95% EtOH at ambient temperature. The crude extract was mixed with H_2O (2 L) to form a suspension and extracted with petroleum ether, EtOAc, and *n*-BuOH successively. The EtOAc-soluble part was subjected to silica gel column chromatography (CC) eluted with petroleum ether−EtOAc (5:1, 4:1, 3:1, 2:1, and EtOAc) and CH_3OH to give five fractions, 1−5. Fraction 5, composed of mainly diterpenoids, was subjected to MCI gel CC eluted with 70% CH_3OH in H_2O to give five subfractions, 5A−5E. Fraction 5B was separated by silica gel CC eluted with $CHCl_3$−MeOH (15:1, 10:1, 5:1, and MeOH), and each major fraction was then purified by C-18 reversed-phase silica gel CC to yield compounds **1** (10 mg), **2** (25 mg), **4** (11 mg), and **10** (5.5 g). Fraction 5C was subjected to silica gel CC eluted with $CHCl_3$−MeOH (10:1, 5:1, and MeOH), and each major fraction was further separated by C-18 reversed-phase silica gel column chromatography to give **3** (6 mg), **5** (80 mg), **6** (21 mg), **11** (10 mg), **12** (10 mg), and **13** (10 mg), respectively. Fraction 5D was subjected to MCI gel CC eluted with 60% MeOH in H_2O, and the major component was then purified by silica gel CC eluted with $CHCl_3$−MeOH (50:1) to give **14** (9 mg) and **15** (24 mg). The *n*-BuOH part was subjected to MCI gel CC to remove the pigments and then separated by silica gel CC eluted with $CHCl_3$−MeOH (20:1, 10:1, 5:1, 4:1, 3:1, and 2:1) to afford five fractions (Bu 1−5). Bu 2 was subjected to C-18 reversed-phase silica gel CC eluted with 60% CH_3OH in H_2O to give a major fraction, which was further purified on Sephadex LH-20 to yield **7** (22 mg). Bu 5 was subjected to C-18 reversed-phase silica gel CC eluted with 60% CH_3OH in H_2O to give two major fractions, which were respectively purified by silica gel CC eluted with $CHCl_3$−MeOH−H_2O (4:1:0.1) to give **8** (14 mg) and **9** (32 mg).

***Ent*-12α,16-epoxy-2β,15α,19-trihydroxypimar-8-ene (1):** white amorphous powder, $[\alpha]_D^{20}$ +13.8° (*c* 0.63, MeOH); IR (KBr) ν_{max} 3406, 2933, 1657, 1460, 1381, 1032, 615 cm^{-1}; ^1H NMR data (Table 1); ^{13}C NMR data (Table 3); positive ion ESIMS *m/z* 359 $[M + Na]^+$; EIMS *m/z* 336 $[M]^+$ (29), 287 (47), 275 (60), 257 (46), 239 (27), 227 (87), 180 (5); HREIMS *m/z*: 336.2302 (calcd for $C_{20}H_{32}O_4$, 336.2301).

***Ent*-12α,16-epoxy-2β,15α,19-trihydroxypimar-8(14)-ene (2):** white amorphous powder, $[\alpha]_D^{20}$ +6.5° (*c* 1.40, MeOH); IR (KBr) ν_{max} 3356, 2939, 1635, 1456, 1383, 1101, 471 cm^{-1}; ^1H NMR data (Table 1); ^{13}C NMR data (Table 3); positive ion ESIMS *m/z* 359 $[M + Na]^+$; EIMS *m/z* 336 $[M]^+$ (4), 318 (3), 305 (3), 257 (6), 245 (5), 227 (7), 180 (6), 167 (100), 159 (13); HREIMS *m/z* 336.2300 (calcd for $C_{20}H_{32}O_4$, 336.2301).

Table 1. ^{1}H NMR Data (δ) of Compounds **1**−**4** (CD_3OD)

	1[a] (mult, *J* (Hz))	**2**[b] (mult, *J* (Hz))	**3**[a] (mult, *J* (Hz))	**4**[a] (mult, *J* (Hz))
1	0.97 (1H, t, 11.2)	0.87 (1H, dd, 11.8, 11.5)	1.41 (1H, d, 14.1)	1.01 (1H, dd, 12.1, 11.3)
	2.11 (1H, br d, 11.2)	2.00 (1H, br d, 11.8)	1.80 (1H, d, 14.1)	1.90 (1H, br d, 12.1)
2	3.85 (1H, dddd, 11.6, 11.2, 4.0, 3.6)	3.70 (1H, dddd, 11.6, 11.5, 3.9, 3.8)	4.04 (1H, br s)	3.73 (1H, dddd, 11.5, 11.3, 3.9, 3.7)
3	0.88 (1H, dd, 12.6, 11.6)	0.77 (1H, dd, 12.2, 11.6)	1.24 (1H, m)	0.88 (1H, t, 12.1)
	2.16 (1H, br d, 12.6)	2.11 (1H, m)	1.91 (1H, d, 14.2)	2.15 (1H, br d, 12.1)
5	1.27 (1H, m)	1.26 (1H, d, 12.3)	1.26 (1H, d, 11.9)	1.22 (1H, m)
6	2.04 (2H, d, 4.6)	1.26 (1H, dd, 12.3, 9.5)	1.38 (1H, m)	1.33 (1H, dd, 12.6, 4.5)
		1.43 (1H, m)	1.67 (1H, m)	1.75 (1H, br d, 12.6)
7	1.49 (1H, m)	1.84 (1H, br t, 14.7)	1.98 (1H, d, 14.1)	2.07 (1H, m)
	1.86 (1H, m)	2.08 (1H, m)	2.23 (1H, d, 14.1)	2.38 (1H, br d, 14.2)
9		1.73 (1H, br s)	1.69 (1H, m)	1.85 (1H, m)
11	1.87 (1H, m)	1.28 (1H, dd, 9.5, 3.5)	1.55 (2H, m)	1.64 (2H, m)
	2.38 (1H, m)	1.54 (1H, dd, 9.5, 3.4)		
12	3.56 (1H, br s)	3.89 (1H, br s)	0.81 (1H, m)	1.09 (1H, dd, 12.7, 2.6)
			1.93 (1H, m)	2.29 (1H, br d, 12.7)
14	1.25 (1H, d, 12.6)	5.69 (1H, d, 1.9)	5.13 (1H, s)	5.50 (1H, s)
	1.33 (1H, d, 12.6)			
15	3.78 (1H, dd, 4.6, 1.6)	3.88 (1H, dd, 6.5, 2.6)	3.52 (1H, m)	
16	3.57 (1H, dd, 10.0, 1.6)	3.48 (1H, dd, 11.2, 6.5)	3.42 (1H, d, 10.9)	4.33 (1H, d, 18.8)
	4.18 (1H, dd, 10.0, 4.6)	3.87 (1H, dd, 11.2, 2.6)	3.64 (1H, d, 10.9)	4.41 (1H, d, 18.8)
17	0.92 (3H, s)	0.94 (3H, s)	0.79 (3H, s)	1.09 (3H, s)
18	1.02 (3H, s)	0.91 (3H, s)	0.93 (3H, s)	1.01 (3H, s)
19	3.36 (1H, d, 11.1)	3.33 (1H, d, 11.0)	3.36 (1H, d, 10.7)	3.32 (1H, d, 11.0)
	3.66 (1H, d, 11.1)	3.61 (1H, d, 11.0)	3.93 (1H, d, 10.7)	3.64 (1H, d, 11.0)
20	1.02 (3H, s)	0.73 (3H, s)	0.98 (3H, s)	0.69 (3H, s)

[a] Measured at 500 MHz. [b] Measured at 400 MHz.

Table 2. ^{1}H NMR Data (δ) of Compounds **5**−**7** and **9** (CD_3OD)

	5[a] (mult, *J* (Hz))	**6**[a] (mult, *J* (Hz))	**7**[a] (mult, *J* (Hz))	**9**[b] (mult, *J* (Hz))
1	2.09 (1H, d, 12.6)	2.18 (1H, dd, 12.8, 2.1)	3.09 (1H, d, 11.8)	1.01 (1H, m)
	2.55 (1H, d, 12.6)	2.31 (1H, d, 12.8)	2.06 (1H, d, 11.8)	1.99 (1H, m)
2				3.76 (1H, m)
3	2.27 (1H, br d, 12.3)	2.15 (1H, br d, 13.3)	3.23 (1H, s)	0.89 (1H, dd, 16.3, 4.3)
	2.44 (1H, d, 12.3)	2.42 (1H, dd, 13.3, 2.1)		3.25 (1H, m)
5	1.79 (1H, m)	1.77 (1H, dd, 13.2, 2.3)	2.04 (1H, dd, 9.9, 2.3)	1.19 (1H, m)
6	1.46 (1H, dd, 13.0, 4.2)	1.36 (1H, dd, 13.2, 4.5)	1.38 (1H, dd, 12.9, 4.4)	1.38 (1H, dd, 12.7, 4.2)
	1.82 (1H, m)	1.73 (1H, m)	1.61 (1H, br d, 12.9)	1.73 (1H, br d, 12.7)
7	2.20 (1H, br d, 14.3)	2.01 (1H, m)	2.11 (1H, dd, 14.3, 8.3)	1.99 (1H, m)
	2.39 (1H, dd, 14.3, 2.7)	2.24 (1H, dd, 13.3, 2.5)	2.31 (1H, dd, 14.3, 2.8)	2.29 (1H, m)
9	2.13 (1H, dd, 8.5, 8.1)	1.99 (1H, m)	2.07 (1H, m)	1.81 (1H, dd, 8.3, 8.1)
11	1.56 (2H, m)	1.43 (2H, br d, 8.0)	1.53 (2H, m)	1.56 (2H, m)
12	1.04 (1H, dd, 12.4, 4.7)	0.84 (1H, dd, 12.6, 4.7)	0.95 (1H, m)	0.90 (1H, m)
	2.08 (1H, d, 12.4)	1.89 (1H, ddd, 12.6, 4.5, 3.3)	1.99 (1H, br d, 13.3)	1.99 (1H, m)
14	5.31 (1H, s)	5.15 (1H, s)	5.22 (1H, s)	5.18 (1H, s)
15	3.74 (1H, dd, 7.7, 2.1)	3.43 (1H, dd, 8.8, 2.0)	3.53 (1H, dd, 10.6, 1.8)	3.57 (1H, dd, 9.0, 1.6)
16	3.83 (2H, m)	3.35 (1H, m)	3.44 (1H, dd, 10.8, 10.6)	3.45 (1H, dd, 10.7, 9.0)
		3.57 (1H, dd, 10.8, 2.0)	3.66 (1H, dd, 10.8, 1.8)	3.68 (1H, m)
17	0.92 (3H, s)	0.75 (3H, s)	0.84 (3H, s)	0.83 (3H, s)
18	1.13 (3H, s)	1.02 (3H, s)	0.82 (3H, s)	1.07 (3H, s)
19	0.92 (3H, s)	3.22 (1H, m)	1.15 (3H, s)	3.32 (1H, m)
		3.39 (1H, m)		3.86 (1H, dd, 12.0, 1.5)
20	0.84 (3H, s)	0.71 (3H, s)	0.77 (3H, s)	0.83 (3H, s)
1′	4.28 (1H, d, 7.9)		4.26 (1H, d, 7.5)	4.19 (1H, d, 7.8)
2′	3.25 (1H, t, 8.4)		3.29 (1H, t, 7.8)	3.17 (1H, t, 8.2)
3′	3.31 (1H, m)		3.33 (1H, m)	3.25 (1H, m)
4′	3.32 (1H, m)		3.31 (1H, m)	3.29 (1H, m)
5′	3.37 (1H, dd, 13.8, 5.6)		3.18 (1H, m)	3.68 (1H, m)
6′	3.70 (1H, dd, 11.8, 5.6)		3.61 (1H, dd, 11.8, 5.3)	3.69 (1H, m)
	3.90 (1H, dd, 11.8, 1.2)		3.68 (1H, dd, 11.8, 2.3)	4.02 (1H, d, 9.6)

[a] Measured at 500 MHz. [b] Measured at 400 MHz.

***Ent*-2α,15,16,19-tetrahydroxypimar-8(14)-ene (3):** white amorphous powder, $[\alpha]_D^{20}$ −18.4° (*c* 0.50, MeOH); IR (KBr) ν_{max} 3404, 2937, 1649, 1452, 1385, 1078, 1032, 879 cm^{-1}; ^{1}H NMR data (Table 1); ^{13}C NMR data (Table 3); EIMS *m*/*z* 338 $[M]^+$ (1), 320 $[M - H_2O]^+$ (1), 277 $[M - CH(OH)CH_2OH]^+$ (36), 259 (49), 241 (32), 229 (8), 201 (7), 185 (7), 173 (6), 159 (8), 151 (14), 133 (15), 121 (100), 107 (31), 95 (32); HREIMS *m*/*z* 277.2168 (calcd for $C_{18}H_{29}O_2$, 277.2167).

***Ent*-15-oxo-2*β*,16,19-trihydroxypimar-8(14)-ene (4):** white amorphous powder, $[\alpha]_D^{20}$ −15.1° (*c* 0.55, MeOH); IR (KBr) ν_{max} 3406, 2937, 1714, 1649, 1454, 1385, 1038, 569 cm^{-1}; ^{1}H NMR data (Table 1); ^{13}C NMR data (Table 3); positive ion ESIMS *m*/*z* 359 $[M + Na]^+$, 695 $[2M + Na]^+$, positive ion HRESIMS *m*/*z* 359.2195 $[M + Na]^+$ for $C_{20}H_{32}O_4Na$ (calcd 359.2198).

***Ent*-2-oxo-15,16-dihydroxypimar-8(14)-en-16-*O*-*β*-glucopyranoside (5):** white amorphous powder, $[\alpha]_D^{20}$ −51.7° (*c* 1.58, MeOH); IR (KBr) ν_{max} 3413, 2953, 1701, 1660, 1456, 1371, 1078, 1034, 631 cm^{-1}; ^{1}H NMR data (Table 2); ^{13}C NMR data (Table 3); EIMS *m*/*z* 482 $[M]^+$ (1), 464 (3), 422 (13), 285 (6), 260 (94), 259 $[M - 163 - 60]^+$ (100), 245 (16), 203 (6), 175 (17), 151 (31); HREIMS *m*/*z* 482.2890 (calcd for $C_{26}H_{42}O_8$, 482.2889).

Table 3. ^{13}C NMR Data (δ) of Compounds **1**−**7** and **9** (CD_3OD)

	1[a]	**2**[b]	**3**[a]	**4**[a]	**5**[a]	**6**[a]	**7**[a]	**9**[b]
1	46.7	49.6	46.2	49.2	54.9	55.0	50.9	49.3
2	65.9	65.3	68.6	65.4	215.4	215.5	216.0	65.7
3	45.7	45.5	42.4	45.3	57.5	51.4	93.6	46.0
4	41.6	41.2	39.6	41.7	41.1	46.0	44.0	41.3
5	53.4	52.1	56.4	56.5	55.8	56.4	50.4	57.0
6	22.6	21.1	24.1	23.4	24.3	24.2	23.4	23.8
7	20.2	24.6	38.1	37.3	37.1	37.5	36.9	37.9
8	143.4	135.8	140.2	142.8	139.7	139.2	139.4	139.6
9	126.0	52.9	53.7	52.8	52.2	52.4	52.4	53.0
10	40.7	38.2	39.05	41.2	46.0	45.4	46.1	41.1
11	32.6	34.0	20.1	21.9	19.9	20.0	19.6	20.2
12	84.7	87.0	33.8	34.0	33.4	33.4	33.3	33.7
13	44.3	45.0	39.13	48.5	38.9	39.0	38.9	39.0
14	30.9	126.3	130.5	126.0	130.9	131.2	130.9	130.4
15	81.0	78.5	78.2	215.8	75.5	78.1	77.8	77.9
16	75.1	72.7	64.9	66.9	72.9	64.8	64.6	64.7
17	15.1	21.7	23.6	28.0	23.5	23.5	23.3	23.6
18	28.2	28.0	29. 1	28.3	34.1	28.1	28.4	29.0
19	66.3	65.8	68.2	66.0	24.2	66.3	21.5	74.5
20	21.8	16.8	19.1	17.1	16.3	17.1	16.0	17.9
1′					104.5		106.2	105.2
2′					75.4		75.5	75.6
3′					78.4		78.2	78.1
4′					72.1		71.7	72.0
5′					78.5		78.5	78.5
6′					63.2		62.9	63.1

[a]Measured at 125 MHz. [b]Measured at 100 MHz.

***Ent*-2-oxo-15,16,19-trihydroxypimar-8(14)-ene (6):** white amorphous powder, $[\alpha]_D^{20}$ −29.9° (*c* 1.55, MeOH); IR (KBr) ν_{max} 3415, 2941, 2874, 1697, 1670, 1456, 1286, 1034, 966 cm^{-1}; ^1H NMR data (Table 2); ^{13}C NMR data (Table 3); EIMS *m*/*z* 275 $[M - CH(OH)CH_2OH]^+$ (100), 259 (23), 241 (8), 229 (6), 217 (12), 199 (5), 173 (9), 167 (20), 149 (7), 135 (10), 121 (77), 109 (46), 107 (41), 91 (28); HREIMS *m*/*z* 275.2009 (calcd for $C_{18}H_{27}O_2$, 275.2011).

***Ent*-2-oxo-3*β*,15,16-trihydroxypimar-8(14)-en-3-*O*-*β*-glucopyranoside (7):** pale gum; $[\alpha]_D^{20}$ −22.2° (*c* 1.35, MeOH); IR (KBr) ν_{max} 3404, 2939, 1707, 1637, 1458, 1392, 1076, 1036, 554 cm^{-1}; ^1H NMR data (Table 2); ^{13}C NMR data (Table 3); EIMS *m*/*z* 480 $[M - H_2O]^+$ (5), 437 (14), 337 (5), 287 (4), 276 (29), 275 (100), 257 (49), 229 (14), 185 (10), 175 (23), 149 (23), 145 (25), 121 (61); HREIMS *m*/*z* 480.2720 (calcd for $C_{26}H_{40}O_8$, 480.2723).

***Ent*-2*β*,15,16,19-tetrahydroxypimar-8(14)-en-19-*O*-*β*-glucopyranoside (9):** pale gum; $[\alpha]_D^{20}$ −35.9° (*c* 1.32, MeOH); IR (KBr) ν_{max} 3386, 2937, 1647, 1456, 1371, 1078, 1034, 630 cm^{-1}; ^1H NMR data (Table 2; ^{13}C NMR data (Table 3); EIMS *m*/*z* 439 $[M - CH(OH)CH_2OH]^+$ (7), 277 (53), 259 (100), 241 (35), 229 (8), 173 (6), 159 (13), 151 (32), 121 (75); HREIMS *m*/*z* 439.2702 (calcd for $C_{24}H_{39}O_7$, 439.2696).

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Supporting Information Available: Selected HMBC correlations of compounds **1** and **3**−**7**, NOESY correlations of compounds **1**, **3**, and **7**, and NMR spectra (^1H, ^{13}C, ^1H−^1H COSY, HMQC, HMBC, and NOESY) of **1**. This material is available free of charge at http:/pubs.acs.org.

References and Notes

(1) Xiong, J.; Ma, Y. B.; Xu, Y. L. *Phytochemistry* **1992**, *31*, 917−921.
(2) Su, J. D.; Osawa, T.; Namiki, M. *Agric. Biol. Chem.* **1986**, *50*, 199−203.
(3) Kim, H. M.; Kim, C. Y.; Kwon, M. H.; Shin, T. Y.; Lee, E. J. *Arch. Pharmacol. Res.* **1997**, *20*, 122−127.
(4) Dong, X. Y.; Chen, M.; Jin, W.; Huang, D. X.; Shen, S. M.; Li, H. T. *Acta Pharm. Sin.* **1989**, *24*, 833−836.
(5) Murakami, T.; Isa, T.; Satake, T. *Tetrahedron Lett.* **1973**, *14*, 4991−4994.
(6) Canonica, L.; Rindone, B.; Scolastico, C.; Han, K. D.; Kim, J. H. *Tetrahedron Lett.* **1969**, *10*, 4801−4804.
(7) Kim, J. H.; Han, K. D.; Yamasaki, K.; Tanaka, O. *Phytochemistry* **1979**, *18*, 894−895.
(8) Liu, K.; Röder, E. *Planta Med.* **1991**, *57*, 395−396.
(9) Xiong, J.; Jin, Q. D.; Xu, Y. L. *Chin. Chem. Lett.* **2001**, *12*, 51−54.
(10) Fu, H. Z.; Feng, R.; Du, Z. H.; Miu, Z. C.; Yan, X. X.; Li, Y. G. *Zhongcaoyao* **1997**, *28*, 327−329.
(11) Baruah, R. N.; Sharma, R. P.; Madhusudanan, K. P.; Thyagarajan, G.; Herz, W.; Murari, R. *Phytochemistry* **1979**, *18*, 991−994.
(12) Zdero, C.; Bohlmann, F.; King, R. M.; Robinson, H. *Phytochemistry* **1991**, *30*, 15791584.

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