ent-Labdane Diterpenoids from *Andrographis paniculata*[⊥]

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Six new *ent*-labdane diterpenoids, 3-*O*-*β*-D-glucopyranosyl-14,19-dideoxyandrographolide (**1**), 14-deoxy-17-hydroxyandrographolide (**2**), 19-*O*-[*β*-D-apiofuranosyl(1→2)-*β*-D-glucopyranoyl]-3,14-dideoxyandrographolide (**3**), 3-*O*-*β*-D-glucopyranosylandrographolide (**4**), 12*S*-hydroxyandrographolide (**5**), and andrographatoside (**6**), together with 17 known analogues, were isolated from the aerial parts of *Andrographis paniculata*. The structures of **1**−**6** were determined by spectroscopic data analysis. All compounds isolated were evaluated for their inhibitory activity against several bacterial and fungal strains.

Andrographis paniculata Nees (Acanthaceae), a well-known herbal medicine, is cultivated widely in southern mainland China and used as an effective antibacterial and antiphlogistic agent in Chinese folk medicine.[1] Extensive chemical and pharmaceutical investigations on this species have demonstrated that *ent*-labdane diterpenoids are the main bioactive components and have led to the clinical application of several antibacterial and anti-inflammatory drugs based on *A. paniculata* in the People's Republic of China.[2,3] More than 20 *ent*-labdane diterpenoids have been reported from *A. paniculata*.[4−10]

The present study on *A. paniculanta* has resulted in the isolation of six new *ent*-labdane diterpenoids (**1**−**6**), together with 17 known compounds of this series. The structures of compounds **1**−**6** were determined by extensive spectroscopic methods, including 1D and 2D NMR analysis. This paper reports the isolation, characterization, and antimicrobial screening of these diterpenoids. Moreover, compound **7** has been reported previously only as a product of solid-phase synthesis without the publication of complete NMR data.[11] Herein, we have assigned the NMR data of compound **7** through HMQC and HMBC experiments.

Results and Discussion

The 70% acetone−water extract of the aerial parts of *A. paniculata* was partitioned between ethyl acetate and water, and then between *n*-butanol and water. The ethyl acetate- and *n*-butanol-soluble extracts were chromatographed sequentially over MCI-gel CHP-20P and silica gel columns and further purified by high-performance liquid chromatography (HPLC), using a reversed-phase column, to yield 23 compounds.

Compound **1** was isolated as a white powder. The negative FABMS displayed a pseudomolecular ion $[M - 1]^-$ at *m*/*z* 479 and a fragment peak at *m*/*z* 317 $[M - 1 - 162]^-$. The HRESIMS (*m*/*z* 479.2655 $[M - 1]^-$), together with the NMR data, were used to determine the molecular formula of **1** as $C_{26}H_{40}O_8$. The ^{1}H and ^{13}C NMR spectra of **1** (Table 1) exhibited signals for three tertiary methyls, an exocyclic methylene group, a α,*β*-unsaturated-*γ*-lactone, and a glucose, suggesting that compound **1** is a glucose derivative of an andrographolide analogue. When compared to the ^{13}C NMR data of 14-deoxyandrographolide,[4] the aglycon moiety of **1** showed the absence of a resonance for a hydroxymethylene for C-19 and the presence of a signal for an additional tertiary methyl (δ_C 17.0) and implied that the hydroxymethylene of C-19 in 14-deoxyandrographolide was converted into a tertiary methyl in **1**. The glucose unit was linked to the C-3 position due to the observation of HMBC correlations between signals for the anomeric proton at δ_H 4.89 (1H, d, J = 7.7 Hz) and C-3 (δ_C 84.8). Accordingly, the structure of **1** was assigned as 3-*O*-*β*-D-glucopyranosyl-14,19-dideoxyandrographolide.

Table 1. ^{13}C NMR Spectroscopic Data of Compounds **1**−**7**

carbon	1	2	3	4	5	6	7
1	37.0 t	37.2 t	38.9 t	37.1 t	37.9 t	39.3 t	37.9 t
2	24.3 t	28.6 t	19.2 t	23.8 t	29.0 t	20.6 t	28.4 t
3	84.8 d	80.3 d	35.9 t	85.5 d	81.0 d	38.6 t	80.9 d
4	38.9 s	43.1 s	38.5 s	43.5 s	43.7 s	44.8 s	43.6 s
5	55.1 d	55.2 d	56.1 d	55.7 d	56.6 d	56.8 d	56.0 d
6	24.3 t	21.9 t	24.4 t	24.7 t	25.4 t	26.5 t	22.8 t
7	38.4 t	31.8 t	38.4 t	38.3 t	39.4 t	39.2 t	37.6 t
8	148.3 s	42.4 d	148.3 s	148.1 s	149.2 s	148.8 s	60.0 s
9	56.3 d	52.2 d	56.5 d	56.3 d	53.9 d	56.3 d	54.5 d
10	39.5 s	38.0 s	39.6 s	39.1 s	40.3 s	41.0 s	41.0 s
11	22.3 t	27.5 t	22.0 t	25.1 t	30.3 t	23.2 t	21.2 t
12	25.1 t	27.7 t	24.9 t	147.1 d	71.0 d	34.7 t	27.8 t
13	134.2 s	134.3 s	134.2 s	130.2 s	53.8 d	142.9 s	134.5 s
14	145.7 d	145.6 d	145.5 d	66.0 d	73.6 d	127.5 d	147.8 d
15	70.8 t	70.7 t	70.5 t	75.5 t	76.5 t	58.6 t	72.1 t
16	174.8 s	174.8 s	174.5 s	170.9 s	178.4 s	60.1 t	177.0 s
17	107.2 s	64.9 t	107.1 t	108.8 t	107.9 t	106.9 t	51.4 t
18	28.8 q	23.7 q	28.1 q	23.6 q	23.4 q	29.0 q	23.4 q
19	17.0 q	64.5 t	71.9 t	63.7 t	65.0 t	176.6 s	64.9 t
20	14.7 q	15.1 q	15.4 q	14.9 q	15.7 q	13.7 q	15.7 q
1′	102.5 d		103.8 d	101.3 d		95.8 d	
2′	75.2 d		78.7 d	75.2 d		74.1 d	
3′	78.7 d		78.8 d	78.7 d		79.1 d	
4′	72.1 d		72.0 d	71.9 d		71.1 d	
5′	78.5 d		78.2 d	78.7 d		79.4 d	
6′	63.3 t		62.8 t	63.1 t		62.3 t	
1″			110.9 d				
2″			78.0 d				
3″			80.7 s				
4″			75.7 t				
5″			66.3 t				

Compound **2** was isolated as a white powder. The HRESIMS (*m*/*z* 375.2155 $[M + Na]^+$) and the NMR data (Table 1) revealed the molecular formula as $C_{20}H_{32}O_5$. Characteristic signals for two tertiary methyl groups (δ_H 0.78, 1.48) and an α,*β*-unsaturated-*γ*-lactone (δ_C 134.3, 145.6, 174.8) implied that **2** is an analogue of 14-deoxyandrographolide. The absence of any signal for an exocyclic methylene, along with the observation of resonances for an additional methine (δ_C 42.4) and a hydroxymethylene (δ_C 64.9), suggested that the double bond at C-8(17) is hydrogenated and substituted by a hydroxyl at C-17. This was confirmed by HMBC correlations (Figure 1) of H_2-17 (δ_H 3.94 and 3.81) with C-7 (δ_C 31.8) and C-9 (δ_C 52.2). The relative configuration of the

[⊥] Dedicated to Dr. Norman R. Farnsworth of the University of Illinois at Chicago for his pioneering work on bioactive natural products.

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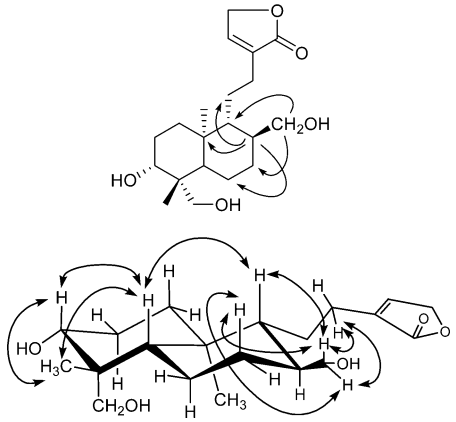


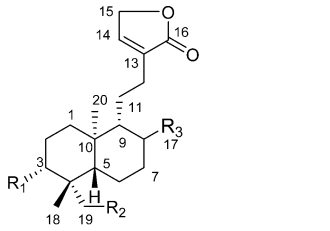
Figure 1. Key HMBC and ROESY correlations for compound **2**.

17-hydroxymethylene was assigned with a β-orientation, on the basis of ROESY correlations (Figure 1) of H_2-17 with H-9β (δ_H 0.95) and H-12a (δ_H 2.35). From these data, compound **2** was deduced to be 14-deoxy-17β-hydroxyandrographolide.

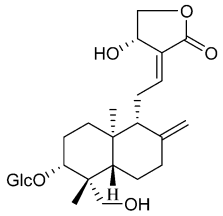
Compound **3**, a brown-yellow powder, exhibited a pseudomolecular ion at *m*/*z* 611 ([M − 1]$^-$) and a fragment peak at *m*/*z* 479 ([M − 1 − 132]$^-$) in the negative FABMS, consistent with the molecular formula, $C_{31}H_{48}O_{12}$, which was confirmed by negative HRESIMS (*m*/*z* 611.3074 [M − 1]$^-$). The ^{1}H NMR spectrum of **3** exhibited characteristic resonances similar to those of andrographolide, including two tertiary methyl signals (δ_H 0.60, 1.26) and three sp^2 proton signals (δ_H 4.69, 4.90, 7.18) for an exocyclic double bond and a trisubstituted double bond. The comparison of the ^{13}C and DEPT spectroscopic data (Table 1) of **3** with those of neoandrographolide revealed the presence of an apiofuranosyl unit, **3**.[4,12] An HMBC experiment of **3** showed a long-range correlation between the anomeric proton H-1″ of the apiose and the C-2′ (δ_C 78.7) of glucose, suggesting the apiosyl to be attached at C-2′ of the glucosyl moiety. Thus, compound **3** was determined as 19-*O*-[β-D-apiofuranosyl(1→2)-β-D-glucopyranoyl]-3,14-dideoxyandrographolide.

Compound **4** was obtained as a white powder. The molecular formula of $C_{26}H_{40}O_{10}$ was inferred from negative HRESIMS analysis ([M − 1]$^-$, *m*/*z* 511.2537) and NMR data. Careful comparison of the NMR data of **4** with those of andrographolide indicated that **4** has one more glucose than andrographolide. The HMBC correlation of anomeric proton of glucose at δ_H 4.95 with C-3 (δ_C 85.5) suggested that the glucose is linked to the C-3 position of andrographolide. From the above observations, compound **4** was concluded to be 3-*O*-β-D-glucopyranosyandrographolide.

The molecular formula of compound **5** was determined to be $C_{20}H_{32}O_6$ from its HRESIMS (*m*/*z* 391.2086 [M + Na]$^+$) and NMR data. The ^{1}H NMR spectrum of **5** exhibited two tertiary methyl signals at δ_H 0.69 and 1.99, a pair of doublets at δ_H 4.09 and 3.33 (each 1H, d, J = 10.8 Hz, H_2-19) for a hydroxymethylene, two proton signals for an exocyclic double bond at δ_H 4.88 and 4.68 (each 1H, s, H_2-17), and two doublets at 1.27 (1H, d, J = 12.7 Hz, H-5β) and 1.72 (1H, d, J = 11.2 Hz, H-9β), while the ^{13}C and DEPT NMR data (Table 1) showed 20 carbon signals, including two methyls, eight methylenes, six methines, and four quaternary carbon signals. The NMR spectroscopic data of **5** were closely similar to those of andrographolide,[4] except for the absence of signals for a trisubstituted double bond between C-12 and C-13 and the presence of resonances for two extra methines (δ_H 4.00, m and 2.43, t-like). The HMBC correlations (Figure 2) observed between the oxymethine signal at δ_H 4.00 and C-9 (δ_C 53.9), C-14 (δ_C 73.6), and C-16 (δ_C 178.4) implied a hydroxyl group at the C-12 position in **5**. The C-12 chirality could be determined from the chemical shifts of the vinyl protons at C-17. Due to the deshielding effect of the hydroxyl group at C-12, the H-17 protons



	R_1	R_2	R_3
1	OGlc	H	$=CH_2$
2	OH	OH	β-CH_2OH
3	H	OApi(1→2)Glc	$=CH_2$
7	OH	OH	
8	H	OH	$=CH_2$



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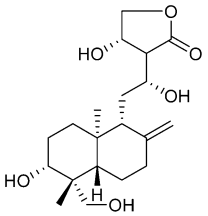

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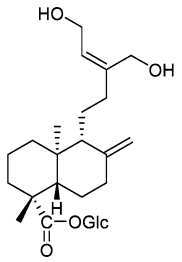

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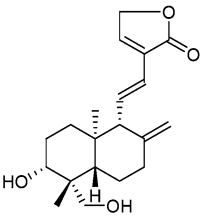

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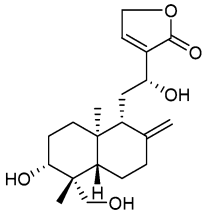

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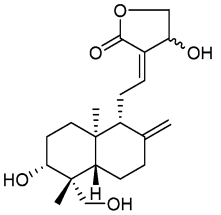

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in the 12*S*-isomer occur more downfield (near δ_H 4.7 ppm) than the corresponding protons (near δ_H 4.4 ppm) in the 12*R*-isomer.[13] Compound **5**, exhibiting two H-17 protons at δ_H 4.88 and 4.68, is considered to have 12*S*-chirality. Thus, compound **5** was determined as 12*S*-hydroxyandrographolide.

Compound **6** was obtained as a white powder, and the molecular formula $C_{26}H_{42}O_9$ was deduced from a pseudomolecular ion [M − 1]$^-$ at *m*/*z* 497 in the FABMS (negative) and from the NMR data and further confirmed by the negative HRESIMS (*m*/*z* 497.2764 [M − 1]$^-$). The ^{1}H NMR spectrum of **6** showed characteristic signals for two tertiary methyls (δ_H 0.92 and 1.28), one exocyclic double bond (δ_H 4.25 and 4.85), and one trisubstituted olefinic proton (δ_H 5.94, t, J = 6.6 Hz), similar to most andrographolide derivatives, suggesting an *ent*-labdane skeleton. Besides the signals for a glucose moiety, the ^{13}C and DEPT spectra of **6** (Table 1) also displayed a signal for an ester carbonyl (δ_C 176.6), ascribed to C-19, based on HMBC correlations (Figure 2) of H-3 (δ_H 2.40), H-5 (δ_H 1.32), and Me-18 with C-19. The HMBC correlation observed between the anomeric proton of glucose at

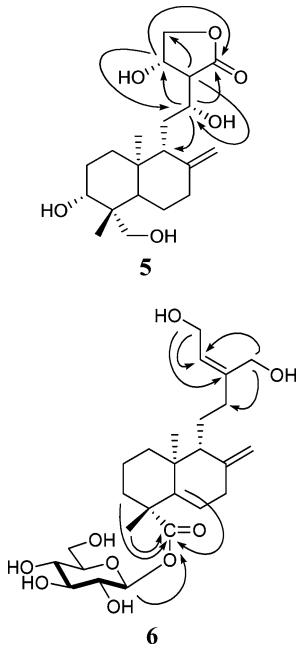

Figure 2. Key HMBC correlations for compounds **5** and **6**.

δ_H 6.25 (1H, d, J = 7.7 Hz) and C-19 indicated the linkage of glucose to C-19. The absence of an ester carbonyl at C-16 implied the opening of the γ-lactone ring and the formation of two hydroxymethylenes at C-15 and C-16, respectively. The geometrical configuration of the $\Delta^{13,14}$ double bond was determined to be *cis* on the basis of the ROESY correlations of H-14 with H-11 and H-12. Therefore, compound **6** was elucidated as 19-carboxylic acid-*O*-β-D-glucopyranosyl-8(17),13-*ent*-labdadien-15,16-diol and named andrographatoside.

The structures of the known compounds isolated were identified as 8,17-epoxy-14-deoxyandrographolide (**7**),[11] andrographolide,[4] andrographiside,[4] andrograpanin) (**8**),[5] neoandrographolide,[4] 14-deoxy-11,12-didehydroandrographolide (**9**),[4] 14-deoxy-11,12-dihydroandrographiside,[4] 14-deoxy-12-hydroxyandrographolide (**10**),[8] isoandrographolide (**11**),[4] 14-deoxyandrographolide,[4] andropanoside,[4] 12-*epi*-14-deoxy-12-methoxyandrographolide,[4] 14-deoxy-12-methoxyandrographolide,[4] 14-deoxy-11-oxoandrographolide,[5] 14-deoxy-11-hydroxyandrographolide,[4] bisandrogrpholide B,[4] and bisandrographolide C,[4] by comparison of their spectroscopic data with literature values.

All diterpenoids were qualitatively evaluated for inhibitory activity against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Micrococcus luteus*, *Sarcina lutea*, *Candida albicans*, *Candida sake*, and *Aspergillus niger*. Compounds **8**−**11** inhibited the growth of *Bacillus subtilis* and showed clear inhibition zones with a diameter of 7−8 mm at the minimal concentration (10 μg/mL) used. The other compounds were inactive against all organisms tested.

Experimental Section

General Experimental Procedures. Optical rotations were measured with a Horiba SEAP-300 spectropolarimeter. UV spectra were taken on a Shimadzu double-beam 210A spectrophotometer. ^{1}H NMR,^{13}C NMR, and 2D NMR spectra were recorded on a Bruker AM-400 and a Bruker DRX-500 spectrometer with TMS as internal standard. MS were obtained on a VG Autospec-3000 spectrometer. Semipreparative HPLC was performed on an Agilent 1100 liquid chromatograph with a Zorbax SB-C_{18}, 9.4 mm × 25 cm column. Preparative HPLC was performed on a Shimadzu LC-8A preparative liquid chromatograph with a Shimadzu PRC-ODS (K) column.

Plant Material. The aerial parts of *A. paniculata* were purchased in Juhuacun herbal market, Kunming, Yunnan Province, People's Republic of China, in October 2002, and were identified by one of the authors (Z.-W.L.). A voucher specimen (KIB-2002-10 Lin) was deposited at the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences.

Extraction and Isolation. The dried and powdered aerial parts of *A. paniculata* (9 kg) were extracted with 70% Me_2CO (3 × 25 L) at room temperature for 72 h and filtered. The filtrate was evaporated and partitioned, in turn, between H_2O and EtOAc, and H_2O and *n*-butanol.

The EtOAc extract (300 g) was applied to column chromatography over MCI-gel CHP-20P (MeOH−H_2O 9:1, 100% MeOH, 100% Me_2CO). The 90% MeOH fraction (201 g) was chromatographed sequentially over silica gel (200−300 mesh, 1.5 kg), eluting with $CHCl_3$−Me_2CO (1:0, 9:1, 8:2, 7:3, 6:4, 1:1, and 0:1) to afford fractions I−VII. Fraction I was chromatographed over silica gel (200−300 mesh) and Sephadex LH-20 and finally purified by semipreparative HPLC (MeOH−CH_3CN−H_2O, 10:35:55) to yield bisandrographolide B (122 mg) and bisandrographolide C (36 mg). Fraction II was repeatedly recrystallized with MeOH to afford 14-deoxy-11,12-didehydroandrographolide (7.746 g), and the mother liquor then was separated by semipreparative HPLC (MeOH−H_2O, 50:50) to obtain 14-deoxy-12-hydroxyandrographolide (21 mg). Fraction III was purified by recrystallization and repeated chromatography over silica gel, RP-18, and Sephadex LH-20 (MeOH), followed by semipreparative and preparative HPLC (MeOH−H_2O, 45:55, 50:50), to yield compounds **8** (34.087 g) and **10** (494 mg).

Fraction IV was repeatedly recrystallized with MeOH to afford compound **11** (18.452 g), and the mother liquor then was chromatographed sequentially over silica gel (eluting with $CHCl_3$−MeOH, 20:1, 9:1, 8:2, 7:3), RP-18 (MeOH−H_2O, 30:70, 35:65, 40:60, 45:55, 50:50, 60:40, 100:0), and Sephadex LH-20 (MeOH), followed by semipreparative and preparative HPLC (RP-18, MeOH−H_2O 45:55, 50:50), to yield **5** (48 mg), 12-*epi*-14-deoxy-12-methoxyandrographolide (142 mg), and 14-deoxy-12-methoxyandrographolide (36 mg). Fraction V was subjected to column chromatography over silica gel, eluted with $CHCl_3$−MeOH (9:1 and 8:2), to obtain 14-deoxy-11-hydroxyandrographolide (27 mg), and the mother liquor was purified by repeated chromatography over RP-18 (MeOH−H_2O, 30:70, 35:65, 40:60, 45:55, 50:50, 60:40, 100:0) and semipreparative and preparative HPLC (MeOH−H_2O, 45:55, 50:50) to yield **7** (8 mg), isoandrographolide (211 mg), and 14-deoxyandrographolide (21 mg). Fraction VI was respectively purified with similar chromatographic methods, by using silica gel ($CHCl_3$−MeOH 9:1) and Sephadex LH-20 (MeOH), followed by semipreparative and preparative HPLC (MeOH−CH_3CN−H_2O, 40:10:50), to yield **9** (57 mg), 14-deoxy-11,12-dihydroandrographiside (47 mg), andropanoside (124 mg), and 14-deoxy-11-oxoandrographolide (4 mg).

The *n*-butanol extract (161 g) was subjected to column chromatography over MCI-gel CHP 20P (90% MeOH−H_2O, 100% MeOH, 100% Me_2CO). The 90% MeOH fraction (130 g) was chromatographed over silica gel (200−300 mesh, 1.0 kg), eluting with a $CHCl_3$−Me_2CO (1:0−1:1) gradient system, to give fractions 1−5. Fraction 1 was chromatographed over RP-18 (MeOH−H_2O, 30:70, 35:65, 40:60, 45:55, 50:50, and 100:0) and further purified by preparative HPLC (MeOH−H_2O 35:65) to obtain **2** (72 mg). Fraction 2 was chromatographed sequentially over RP-18 (using MeOH−H_2O gradient system), Sephadex LH-20 (100% MeOH), and preparative HPLC (MeOH−H_2O, 35:65) to obtain **1** (25 mg) and **4** (33 mg). Fraction 4 was also chromatographed on a Sephadex LH-20 column ($CHCl_3$−MeOH, 1:1) and then subjected to preparative HPLC (MeOH−H_2O, 35:65) to afford **3** (13 mg) and **6** (21 mg).

3-*O*-β-D-Glucopyranosyl-14,19-dideoxyandrographo-lide (1): white powder; $[\alpha]_D^{25}$ −51.0° (*c* 0.28, MeOH); UV (MeOH) λ_{max} (log ϵ) 204 (4.11) nm; ^{1}H NMR (C_5D_5N, 500 MHz) δ_H 1.64 (1H, overlapped, H-1a), 0.98 (1H, overlapped, H-1b), 2.05 (1H, m, H-2a), 1.64 (1H, overlapped, H-2b), 3.51 (1H, dd, J = 4.4, 12.1 Hz, H-3β), 0.98 (1H, overlapped, H-5β), 1.48 (1H, m, H-6a), 1.30 (1H, m, H-6b), 2.36 (1H, m, H-7a),1.92 (1H, m, H-7b), 1.57 (1H, d, J = 11.5 Hz, H-9β), 1.71 (1H, m, H-11a), 1.64 (1H, overlapped, H-11b), 2.53 (1H, m, H-12a), 2.31 (1H, m, H-12b), 6.97 (1H, s, H-14), 4.78 (2H, s, H_2-15), 4.90 (1H, s, H-17a), 4.74 (1H, s, H-17b), 1.17 (3H, s, Me-18), 0.86 (3H, s, Me-19), 0.64 (3H, s, Me-20), 4.89 (1H, d, J = 7.7 Hz, H-1′), 3.99 (1H, overlapped, H-2′), 3.99 (1H, overlapped, H-3′), 4.21 (1H, t, J = 9.3 Hz, H-4′), 4.28 (1H, t, J = 9.5 Hz, H-5′), 4.58 (1H, d, J = 11.5 Hz, H-6′a), 4.37 (1H, dd, J = 5.5, 11.5 Hz, H-6′b); ^{13}C NMR (C_5D_5N, 125 MHz), see

Table 1; FABMS (negative) *m/z* 479 $[M - 1]^-$, 317 $[M - 1 - 162]^-$; HRESIMS (negative) *m/z* 479.2655 $[M - 1]^-$ (calcd 479.2644 for $C_{26}H_{39}O_8$).

14-Deoxy-17*β*-hydroxyandrographolide (2): white powder; $[\alpha]_D^{25}$ −9.4° (*c* 0.80, MeOH); UV (MeOH) λ_{max} (log ϵ) 208 (4.08) nm; ^{1}H NMR (C_5D_5N, 500 MHz) δ_H 1.82 (1H, m, H-1a), 1.02 (1H, dd, *J* = 3.7, 13.3 Hz, H-1b), 2.02 (1H, m, H-2a), 1.93 (1H, m, H-2b), 3.62 (1H, dd, *J* = 4.1, 11.0 Hz, H-3*β*), 0.98 (1H, d, *J* = 11.0 Hz, H-5*β*), 1.76 (1H, d, *J* = 12.8 Hz, H-6a), 1.32 (1H, m, H-6b), 2.19 (1H, m, H-7a), 1.45 (1H, overlapped, H-7b), 1.45 (1H, overlap, H-8α), 0.95 (1H, m, H-9*β*), 1.67 (1H, m, H-11a), 1.38 (1H, m, H-11b), 2.65 (1H, brt, H-12a), 2.35 (1H, m, H-12b), 7.21 (1H, brs, H-14), 4.75 (2H, s, H_2-15), 3.94 (1H, d, *J* = 10.6 Hz, H-17a), 3.81 (1H, dd, *J* = 4.1, 10.6 Hz, H-17b), 1.48 (3H, s, Me-18), 4.98 (1H, d, *J* = 10.6 Hz, H-19a), 3.64 (1H, d, *J* = 10.6 Hz, H-19b), 0.78 (3H, s, Me-20); ^{13}C NMR (C_5D_5N, 100 MHz), see Table 1; FABMS (positive) *m/z* 353 $[M + 1]^+$; HRESIMS (positive) *m/z* 375.2155 $[M + Na]^+$ (375.2147 for $C_{20}H_{32}O_5Na$).

19-*O*-[*β*-D-Apiofuranosyl(1→2)-*β*-D-glucopyranoyl]-3,14-dideoxyandrographolide (3): brown-yellow powder; $[\alpha]_D^{26}$ −57.1° (*c* 0.32, MeOH); UV (MeOH) λ_{max} (log ϵ) 204 (4.21) nm; ^{1}H NMR (C_5D_5N, 500 MHz) δ_H 1.60 (1H, overlapped, H-1a), 0.93 (1H, overlapped, H-1b), 1.60 (1H, overlapped, H-2a), 1.31 (1H, d, *J* = 14.3 Hz, H-2b), 2.33 (1H, d, *J* = 13.2 Hz, H-3a), 0.93 (1H, overlapped, H-3b), 1.13 (1H, d, *J* = 13.2 Hz, H-5*β*), 1.75 (1H, m, H-6a), 1.21 (1H, m, H-6b), 2.33 (1H, d, *J* = 7.6 Hz, H-7a), 1.88 (1H, m, H-7b), 1.60 (1H, overlapped, H-9*β*), 1.70 (1H, m, H-11a), 1.60 (1H, overlapped, H-11b), 2.49 (1H, m, H-12a), 2.17 (1H, m, H-12b), 7.18 (1H, s, H-14), 4.75 (2H, s, H_2-15), 4.90 (1H, overlapped, H-17a), 4.69 (1H, s, H-17b), 1.26 (3H, s, Me-18), 4.32 (1H, d, *J* = 9.9 Hz, H-19a), 3.41 (1H, d, *J* = 9.9 Hz, H-19b), 0.60 (3H, s, Me-20), 4.77 (1H, d, *J* = 7.7 Hz, H-1′), 4.15 (1H, t, *J* = 8.2 Hz, H-2′), 4.25 (1H, t, *J* = 8.2 Hz, H-3′), 4.12 (1H, t, *J* = 9.4 Hz, H-4′), 3.85 (1H, m, H-5′), 4.48 (1H, d, *J* = 11.5 Hz, H-6′a), 4.30 (1H, d, *J* = 11.5 Hz, H-6′b), 6.45 (1H, s, H-1″), 4.90 (1H, overlapped, H-2″), 4.71 (1H, d, *J* = 9.4 Hz, H-4″a), 4.40 (1H, d, *J* = 9.4 Hz, H-4″b), 4.23 (2H, s, H-5″); ^{13}C NMR (C_5D_5N, 125 MHz), see Table 1; FABMS (negative) *m/z* 611 $[M - 1]^-$, 479 $[M - 1 - 132]^-$; HRESIMS (negative) *m/z* 611.3074 $[M - 1]^-$ (calcd 611.3067 for $C_{31}H_{47}O_{12}$).

3-*O*-*β*-D-Glucopyranosyandrographolide (4): white powder; $[\alpha]_D^{25}$ −80.3° (*c* 0.48, MeOH); UV (MeOH) λ_{max} (log ϵ) 203 (4.05), 221 (4.03) nm; ^{1}H NMR (C_5D_5N, 400 MHz) δ_H 1.65 (1H, t, *J* = 12.3 Hz, H-1a), 1.05 (1H, t, *J* = 12.3 Hz, H-1b), 2.14 (2H, m, H_2-2), 3.81 (1H, dd, *J* = 3.8, 11.8 Hz, H-3*β*), 1.14 (1H, d, *J* = 12.1 Hz, H-5*β*), 1.79 (1H, m, H-6a), 1.39 (1H, m, H-6b), 2.28 (1H, m, H-7a), 1.87 (1H, m, H-7b), 1.79 (1H, overlapped, H-9*β*), 2.68 (2H, m, H_2-11), 7.16 (1H, d, *J* = 6.0 Hz, H-12), 5.38 (1H, brs, H-14), 4.63 (1H, d, *J* = 10.1 Hz, H-15a), 4.53 (1H, d, *J* = 10.1 Hz, H-15b), 4.85 (1H, brs, H-17a), 4.83 (1H, brs, H-17b), 1.45 (3H, s, Me-18), 4.26 (1H, d, *J* = 9.8 Hz, H-19a), 3.58 (1H, d, *J* = 9.8 Hz, H-19b), 0.63 (3H, s, Me-20), 4.95 (1H, d, *J* = 7.6 Hz, H-1′), 3.96 (1H, m, H-2′), 4.28 (1H, m, H-3′), 4.15 (1H, m, H-4′), 4.01 (1H, m, H-5′), 4.59 (1H, m, H-6′a), 4.38 (1H, m, H-6′b); ^{13}C NMR (C_5D_5N, 100 MHz), see Table 1; FABMS (negative) *m/z* 511 $[M - 1]^-$; HRESIMS (negative) *m/z* 511.2537 $[M - 1]^-$ (calcd 511.2543 for $C_{26}H_{39}O_{10}$).

12*S*-Hydroxyandrographolide (5): white powder; $[\alpha]_D^{25}$ −82.1° (*c* 0.32, MeOH); UV (MeOH) λ_{max} (log ϵ) 203 (3.83) nm; ^{1}H NMR (CD_3OD, 500 MHz) δ_H 1.87 (1H, m, H-1a), 1.36 (1H, m, H-1b), 1.77 (2H, m, H_2-2), 3.39 (1H, t, *J* = 8.3 Hz, H-3*β*), 1.27 (1H, d, *J* = 12.7 Hz, H-5*β*), 1.83 (1H, m, H-6a), 1.33 (1H, m, H-6b), 2.40 (1H, m, H-7a), 1.98 (1H, m, H-7b), 1.72 (1H, d, *J* = 11.2 Hz, H-9*β*), 2.21 (1H, m, H-11a), 1.62 (1H, m, H-11b), 4.00 (1H, m, H-12), 2.43 (1H, t-like, H-13), 4.38 (1H, m, H-14), 4.44 (1H, dd, *J* = 4.9, 9.4 Hz, H-15a), 4.05 (1H, dd, *J* = 2.0, 9.4 Hz, H-15b), 4.88 (1H, s, H-17a), 4.68 (1H, s, H-17b), 1.99 (3H, s, Me-18), 4.09 (1H, d, *J* = 10.8 Hz, H-19a), 3.33 (1H, d, *J* = 10.8 Hz, H-19b), 0.69 (3H, s, Me-20); ^{13}C NMR (CD_3OD, 100 MHz), see Table 1; EIMS (70 ev) *m/z* 368 (1) $[M]^+$, 350 (1), 332 (5), 314 (5), 309 (7), 296 (10), 291 (12), 284 (17), 219 (20), 201 (34), 189 (27), 173 (45), 159 (46), 145 (50), 133 (57), 121 (100), 105 (74), 93 (70), 81 (46); HRESIMS (positive) *m/z* 391.2086 $[M + Na]^+$ (calcd 391.2096 for $C_{20}H_{32}O_6Na$).

Andrographatoside (6): white powder; $[\alpha]_D^{26}$ −29.8° (*c* 0.35, MeOH); UV (MeOH) λ_{max} (log ϵ) 204 (4.03) nm; ^{1}H NMR (C_5D_5N, 500 MHz) δ_H 1.83 (1H, overlapped, H-1a), 1.02 (1H, m, H-1b), 2.13 (1H, overlapped, H-2a), 1.32 (1H, brd, H-2b), 2.40 (1H, overlapped, H-3*β*), 1.32 (1H, d, *J* = 12.5 Hz, H-5*β*), 2.40 (1H, overlapped, H-6a), 2.13 (1H, overlapped, H-6b), 1.95 (2H, m, H_2-7), 1.83 (1H, overlapped, H-9*β*), 1.83 (1H, overlapped, H-11a), 1.67 (1H, m, H-11b), 2.69 (1H, m, H-12a), 2.24 (1H, m, H-12b), 5.94 (1H, t, *J* = 6.6 Hz, H-14), 4.65 (2H, d, *J* = 6.6 Hz, H_2-15), 4.58 (1H, d, *J* = 12.7 Hz, H-16a), 4.53 (1H, d, *J* = 12.7 Hz, H-16b), 4.85 (1H, s, H-17a), 4.25 (1H, s, H-17b), 1.28 (3H, s, Me-18), 0.92 (3H, s, Me-20), 6.25 (1H, d, *J* = 7.7 Hz, H-1′), 4.15 (1H, t, *J* = 8.8 Hz, H-2′), 4.22 (1H, t, *J* = 8.8 Hz, H-3′), 4.29 (1H, d, *J* = 9.3 Hz, H-4′), 3.97 (1H, m, H-5′), 4.41 (1H, dd, *J* = 2.2, 12.1 Hz, H-6′a), 4.34 (1H, dd, *J* = 4.4, 12.1 Hz, H-6′b); ^{13}C NMR (C_5D_5N, 125 MHz), see Table 1; FABMS (negative) *m/z* 497 $[M - 1]^-$; HRESIMS (negative) *m/z* 497.2764 $[M - 1]^-$ (calcd 497.2750 for $C_{26}H_{41}O_9$).

8,17-Epoxy-14-deoxyandrographolide (7): white powder; $[\alpha]_D^{24}$ −5.7° (*c* 0.28, MeOH); UV (MeOH) λ_{max} (log ϵ) 208 (3.93) nm; ^{1}H NMR (CD_3OD, 400 MHz) δ_H 1.78 (1H, m, H-1a), 1.20 (1H, overlapped, H-1b), 1.73 (2H, m, H_2-2), 3.37 (1H, t-like, H-3*β*), 1.20 (1H, overlap, H-5*β*), 1.89 (1H, m, H-6a), 1.58 (1H, m, H-6b), 1.37 (2H, m, H_2-7), 1.45 (1H, overlap, H-9*β*), 1.45 (1H, overlapped, H-11a), 1.04 (1H, m, H-11b), 2.32 (1H, m, H-12a), 2.20 (1H, m, H-12b), 7.34 (1H, t, *J* = 1.4 Hz, H-14), 4.79 (1H, d, *J* = 1.4 Hz, H-15), 2.80 (1H, dd, *J* = 1.8, 4.0 Hz, H-17a), 2.50 (1H, d, *J* = 4.0 Hz, H-17b), 1.21 (3H, s, Me-18), 4.08 (1H, d, *J* = 10.1 Hz, H-19a), 3.39 (1H, d, *J* = 10.1 Hz, H-19b), 0.83 (3H, s, Me-20); ^{13}C NMR (CD_3OD, 100 MHz), see Table 1; FABMS (positive) *m/z* 351 $[M + 1]^+$; HRESIMS (positive) *m/z* 373.1989 $[M + Na]^+$ (373. 1990 for $C_{20}H_{30}O_5Na$).

Antimicrobial Testing. All diterpenoids were tested for inhibitory activity against eight microbial strains, namely, *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Micrococcus luteus*, *Sarcina lutea*, *Candida albicans*, *Candida sake*, and *Aspergillus niger* at a concentration 10 *μ*g/mL, using a disk diffusion assay on agar plates, as described previosly.[14]

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Supporting Information Available: This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Wu, C. Y. *Xinhua Herbal Outline*, 1st ed.; Shanghai Science and Technology Press: Shanghai, 1994; Vol. 2, pp 481−482.
(2) Committee of the National Pharmacopoeia. *The Pharmacopoeia of People*'s *Republic of China*; Chemical Industry Press: Beijing, 2000; Vol. 1, pp 541−542.
(3) Huang, T. K. *Handbook of Composition and Pharmacological Action of Commonly used Traditional Chinese Medicine*; China Medicine Science and Technology Press: Beijing, 1994; pp 1462−1467.
(4) Matsud, T.; Kuroyanagi, M.; Sugiyama, S.; Umehara, K.; Ueno, A.; Nishi, K. *Chem. Pharm. Bull.* **1994**, *42*, 1216−1225.
(5) Fujita, T.; Fujitani, R.; Takeda, Y.; Takaishi, Y.; Yamada, T.; Kido, M.; Miura, I. *Chem. Pharm. Bull.* **1984**, *32*, 2117−2125.
(6) Jantan, I.; Waterman, P. G. *Phytochemistry* **1994**, *37*, 1477−1479.
(7) Zhong, D. X.; Xuan, L. J.; Xu, Y. M.; Bai, D. L. *Acta Bot. Sin.* **2001**, *43*, 1077−1080.
(8) Zhang, S. J.; Ando, M. *Chin. J. Med. Chem.* **1997**, *7*, 270−273.
(9) Hu, C. Q.; Zhou, B. N. *Acta Pharm. Sin.* **1982**, *17*, 435−439.
(10) Reddy, M. K.; Reddy, M. V. B.; Gunasekar, D.; Murthy, M. M.; Caux, C.; Bodo, B. *Phytochemistry* **2003**, *62*, 1271−1275.
(11) Biabani, M. A. F.; Grover, R. K.; Singh, S. K.; Kumar, S.; Raj, K.; Roy, R.; Kundu, B. *Tetrahedron Lett.* **2001**, *42*, 7119−7121.
(12) Niu, X. M.; Li, S. H.; Wu, L. X.; Gao, L. H.; Sun, H. D. *Planta Med.* **2004**, *70*, 578−581.
(13) Shieh, Y. L.; Fang, J. M.; Cheng, Y. S. *Phytochemistry* **1998**, *47*, 845−850.
(14) Espinel-Ingroff, A.; White, T.; Pfaller, M. A. In *Antifungal Agents and Susceptibility Test Manual of Clinical Microbiology*, 7th ed.; Murray, P. R., Ed.; American Society for Microbiogy: Washington, D.C., 1999; pp 1640−1652.

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