Nine New *ent*-Labdane Diterpenoids from the Aerial Parts of *Andrographis paniculata*

by **Li-Xia Chen**[a]), **Feng Qiu***[a]), **Hong Wei**[b]), **Ge-Xia Qu**[a]), and **Xin-Sheng Yao**[a])

[a]) Department of Natural Products Chemistry 81#, Shenyang Pharmaceutical University, 103 Wenhua Avenue, Shenyang, 110016, P. R. China
(phone: +86-24-23986463; fax: 86-24-23993994; e-mail: fengqiu2000@tom.com)
[b]) Guangxi Institute of Traditional Chinese Medical and Pharmaceutical Sciences, Nanning, Guangxi Province, P. R. China

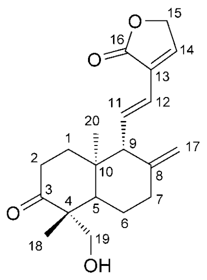
Nine new *ent*-labdane-type diterpenoids (**1**–**9**), mostly in the form of the corresponding 16,15-lactones, were isolated from the 85%-EtOH extract of the aerial parts of *Andrographis paniculata* NEES., together with nine known compounds (**10**–**18**). Their structures were deduced by in-depth NMR spectroscopy and high-resolution mass spectrometry.

Introduction. – *Andrographis paniculata* NEES. (Acanthaceae) is an erect herb widely distributed in Southeast China. The whole plant is used extensively as an anti-inflammatory and antipyretic drug for the treatment of fever, cold, laryngitis, diarrhea, and inflammation [1]. The extract of *A. paniculata* and its major *ent*-labdane diterpenoids have been shown to display antiviral [2], bacteriostatic [3], immunostimulatory [4], as well as hepatoprotective and hepatostimulating [5] activities. Phytochemical studies on the aerial parts of *A. paniculata* have led to the isolation of, so far, more than 20 *ent*-labdane diterpenoids [6–13].

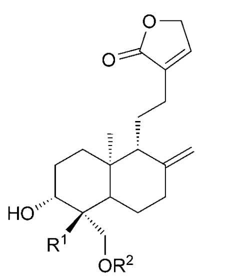
As a part of our ongoing research on the metabolism of *A. paniculata*, we have previously investigated the *in vivo* metabolism of andrographolide after oral administration in rats and humans [14–16]. To further explore the *in vivo* absorbed chemical constituents of the extract of this plant, we decided to systematically investigate the chemical constituents of the 85%-EtOH extract of the aerial parts of *A. paniculata*, which led to the isolation of nine new *ent*-labdane diterpenoid lactones or derivatives thereof: 19-hydroxy-3-oxo-*ent*-labda-8(17),11,13-trien-16,15-olide (**1**), 3,18,19-trihydroxy-*ent*-labda-8(17),13-dien-16,15-olide (**2**), 3,19-dihydroxy-*ent*-labda-8(17),12-dien-16,15-olide (**3**), 19-[(β-D-glucopyranosyl)oxy]-19-oxo-*ent*-labda-8(17),13-dien-16,15-olide (**4**), 3,19-dihydroxy-15-methoxy-*ent*-labda-8(17),11,13-trien-16,15-olide (**5**), *ent*-labda-8(17),13-diene-15,16,19-triol (**6**), 3,15,19-trihydroxy-*ent*-labda-8(17),13-dien-16-oic acid (**7**), 3,19-dihydroxy-14,15,16-trinor-*ent*-labda-8(17),11-dien-13-oic acid (**8**), and 13,14,15,16-tetranor-*ent*-labd-8(17)-ene-3,12,19-triol (**9**).

Also isolated were nine known constituents, which could be identified by comparison of their physico-chemical and spectroscopic properties with published data: neoandrographolide (**10**) [7][10][12], 3,14-dideoxyandrographolide (**11**) [10][12], andrographolide (**12**) [9][10], 14-deoxy-11,12-didehydroandrographolide (**13**) [8][10][12], 19-hydroxy-*ent*-labda-8(17),13-dien-15,16-olide (**14**) [17], 14-deoxyandrographolide

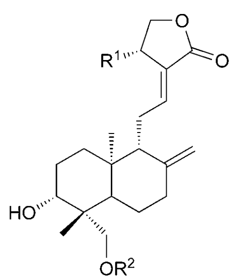
© 2006 Verlag Helvetica Chimica Acta AG, Zürich



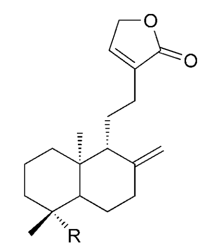
1



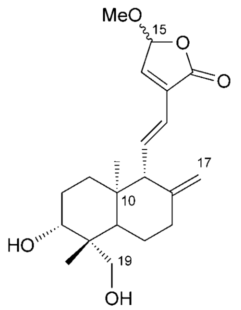
2 R^1 = $HOCH_2$, R^2 = H
15 R^1 = Me, R^2 = H
16 R^1 = Me, R^2 = Glc



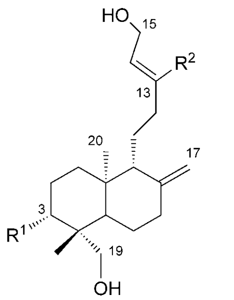
3 R^1 = H, R^2 = H
12 R^1 = OH, R^2 = H
18 R^1 = OH, R^2 = Glc



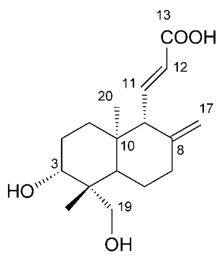
4 R = GlcOOC
10 R = $GlcOCH_2$
11 R = $HOCH_2$



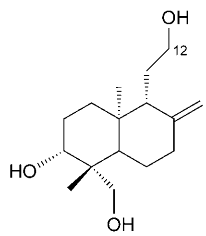
5



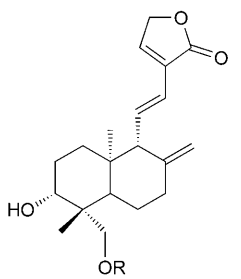
6 R^1 = H, R^2 = $HOCH_2$
7 R^1 = OH, R^2 = HOOC



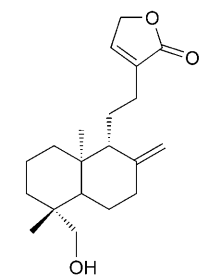
8



9



13 R = H
17 R = Glc



14

Glc = *β*-D-Glucopyranosyl

(**15**) [10] [12], deoxyandrographiside (**16**) [10] [12], 14-deoxy-11,12-didehydroandrographiside (**17**) [10], and andrographiside (**18**) [10].

Results and Discussion. – The aerial parts of *Andrographis paniculata* NEES. were extracted with 85% aq. EtOH. The residue of the EtOH extract was partitioned between AcOEt and H_2O, and the organic layer was subjected to column chromatography on silica gel, followed by repetitive reverse-phase HPLC to afford **1**–**18**.

HR-ESI-MS Analysis of **1** (*m*/*z* 353.1754 ([*M*+Na]$^+$)) indicated the molecular formula $C_{20}H_{26}O_4$, in combination with the ^{13}C- and ^{1}H-NMR spectroscopic data (*Tables 1* and *2*, resp.). The IR spectrum of **1** showed the presence of OH groups (3410), an α,β-unsaturated γ-lactone (1741, 1639), an *exo*-methylidene (889), and of a keto C=O group (1698 cm^{-1}). Positive *Legal* and *Kedde* color reactions [18] further confirmed the presence of an α,β-unsaturated γ-lactone. The characteristic ^{13}C-NMR data (*Table 1*) indicated that **1** was a labdane-type diterpene with an exocyclic CH_2 group (δ(C) 109.5, C(17)), a Me(18) group (δ(C) 21.2), a 19-CH_2OH group (δ(C) 64.5), and an angular Me(20) group (δ(C) 15.4).

The ^{1}H-NMR spectrum of **1** also indicated an α,β-unsaturated γ-lactone with signals at δ(H) 7.31 (*t*, *J*=2.0 Hz, H−C(14)) and 4.79 (br. *s*, CH_2(15)); the corresponding ^{13}C-NMR signals appeared at δ(C) 128.7 (C(13)), 145.4 (C(14)), 70.3 (C(15)), and 172.7 (C(16)). In the HMBC spectrum, correlations of H−C(11) (δ(H) 7.21) to C(13), and of H−C(12) (δ(H) 6.25) to C(14), C(16), and C(9) (δ(C) 61.1), were observed, indicating that the lactone moiety was attached to the labdane skeleton *via* a C=C bond between C(11) (δ(C) 135.1) and C(12) (δ(C) 122.4). Additionally, the C=O signal (δ(C) 213.7) could be assigned to C(3) based on its HMBC correlations to Me(18) (δ(C) 1.44), CH_2(19) (δ(H) 4.26, 3.79), CH_2(1) (δ(H) 1.79–1.76, 1.44), and CH_2(2). Furthermore, H−C(14) (δ(H) 7.31) showed a NOESY correlation with H−C(12) (δ(H)

Table 1. *^{13}C-NMR Chemical Shifts of* **1**–**9**. At 150 MHz in (D_5)pyridine.

Position	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
1	39.6	37.3	37.2	39.3	38.7	39.3	37.3	38.7	37.5
2	36.5	28.9	29.1	20.4	28.9	19.5	29.1	28.8	29.2
3	213.7	73.9	79.9	38.5	80.1	36.0	80.0	80.0	80.2
4	55.1	47.2	43.3	44.6	43.4	39.9	43.3	43.4	43.3
5	56.3	47.5	55.3	56.6	54.7	56.4	55.4	54.6	55.6
6	24.3	24.8	24.4	26.4	23.6	24.8	24.4	23.6	24.7
7	36.6	38.7	38.2	39.0	37.0	39.0	38.4	36.9	38.7
8	148.7	148.3	148.2	148.2	149.0	148.9	148.3	148.6	148.8
9	61.1	56.7	56.1	56.0	61.8	57.0	56.7	60.1	52.7
10	38.9	39.4	39.2	40.9	39.1	39.5	39.3	38.9	39.2
11	135.1	22.4	25.8	22.4	138.6	23.0	24.4	146.5	28.4
12	122.4	25.0	141.0	25.2	121.4	34.6	32.3	126.3	61.4
13	128.7	134.2	126.1	134.2	132.7	142.8	130.6	168.6	–
14	145.4	145.4	25.4	145.5	141.5	127.4	145.1	–	–
15	70.3	70.6	65.6	70.7	102.9	60.0	61.6	–	–
16	172.7	174.6	171.3	174.4	170.2	58.5	170.5	–	–
17	109.5	107.0	108.0	106.9	108.9	106.9	108.5	108.9	107.1
18	21.2	62.8	23.8	28.9	23.7	28.1	23.8	23.7	23.8
19	64.5	63.1	64.2	176.5	64.2	63.8	64.3	64.2	64.3
20	15.4	15.3	15.2	13.5	16.0	15.6	15.4	15.9	15.6
MeO	–	–	–	–	56.5	–	–	–	–

[a]) Sugar resonances for C(1′) to C(6′): δ(C) 95.7, 74.0, 79.4, 71.1, 79.2, and 62.2, resp.

Table 2. *^{1}H-NMR Spectroscopic Data of* **1**–**3**. At 600 MHz in (D_5)pyridine. Asterisks (*) denote overlapping signals.

Position	1	2	3
1	1.79–1.76 (*m*)	1.76–1.72 (*m*)	1.66 (br. *d*, *J* = 13.2)
	1.44 (*dt*, *J* = 14.0, 4.0)	1.21 (*dt*, *J* = 12.9, 4.2)	1.17 (*dt*, *J* = 13.2, 4.8)
2	2.84 (*dt*, *J* = 14.4, 4.0)	2.25–2.16 (*m*)	2.05–2.01 (*m*)
	2.44–2.40 (*m*)	2.13–2.09*	2.01–1.98 (*m*)
3	–	4.41 (br. *d*, *J* = 10.8)	3.68–3.63*
4	–	–	–
5	1.67–1.63*	2.05 (br. *d*, *J* = 13.6)	1.22 (*dd*, *J* = 12.6, 2.1)
6	1.75–1.72 (*m*)	2.13–2.09*	1.81–1.79 (*m*)
	1.67–1.63*	1.53–1.51 (*m*)	1.36–1.33 (*m*)
7	2.38–2.35 (*m*)	2.36 (br. *d*, *J* = 12.6)	2.32 (br. *d*, *J* = 12.6)
	2.05 (br. *t*, *J* = 13.6)	2.04–2.02 (*m*)	1.92 (*dt*, *J* = 12.6, 4.8)
8	–	–	–
9	2.45 (br. *d*, *J* = 10.0)	1.72 (br. *s*)	1.78 (br. *d*, *J* = 10.2)
10	–	–	–
11	7.21 (*dd*, *J* = 16.0, 10.0)	1.82–1.78 (*m*)	2.30–2.28 (*m*)
		1.66–1.63 (*m*)	2.23–2.20 (*m*)
12	6.25 (*d*, *J* = 16.0)	2.51 (br. *t*, *J* = 12.6)	6.86 (br. *t*, *J* = 6.6)
		2.16 (br. *d*, *J* = 12.6)	
14	7.31 (*t*, *J* = 2.0)	7.15 (br. *s*)	2..80 (br. *t*, *J* = 7.2, 2 H)
15	4.79 (br. *s*)	4.70 (br. *s*)	4.27 (*t*, *J* = 7.2, 2 H)
	–	–	–
16	4.91 (*d*, *J* = 2.0)	4.92 (br. *s*)	4.85 (br. *s*)
17	4.81 (*d*, *J* = 2.0)	4.74 (br. *s*)	4.51 (br. *s*)
18	1.44 (*s*)	4.83 (*d*, *J* = 10.9)	1.51 (*s*)
		4.17 (*d*, *J* = 10.9)	
19	4.26 (*d*, *J* = 10.8)	4.61 (*d*, *J* = 10.9)	4.47 (*d*, *J* = 10.8)
	3.79 (*d*, *J* = 10.8)	3.91 (*d*, *J* = 10.9)	3.68–3.63
20	1.19 (*s*)	0.79 (*s*)	0.71 (*s*)

6.25), but no correlation with H–C(11) (δ(H) 7.21), implying a predominantly transoid conformation of the C(12)–C(13) single bond.

On the basis of the above evidence, the structure of **1** was established as 19-hydroxy-3-oxo-*ent*-labda-8(17),11,13-trien-16,15-olide. The absolute configuration of **1** and of all other compounds described in this paper was tentatively assigned based on biogenetic grounds.

HR-ESI-MS Analysis of **2** (*m/z* 373.2002 ([M + Na]$^+$)) indicated the molecular formula $C_{20}H_{30}O_5$, in combination with NMR experiments. The IR spectrum of **2** showed the presence of OH groups (3492), an α,β-unsaturated γ-lactone (1753, 1652), and an *exo*-methylidene group (902 cm^{-1}). Positive *Legal* and *Kedde* color reactions [18] further confirmed the presence of an α,β-unsaturated γ-lactone. The characteristic NMR data of **2** indicated that it was also a labdane-type diterpene with an α,β-unsaturated γ-lactone (δ(C) 134.2, 145.4, 70.6, 174.6), very similar to the known 14-deoxyandrographolide (**15**) [10][12]. The significant difference between the two compounds was at C(18) of the *ent*-labdane skeleton, with signals at δ(H) 4.83 and 4.17 (2*d*, J = 10.9 Hz each, 2 × 1 H) attributable to an 18-CH_2OH function (δ(C) 62.8) in **2** instead of only one signal at δ(H) 1.49 (s, 3 H) for Me(18) (δ(C) 23.6) in **15**. This was confirmed by HMBC correlations of $CH_2(18)$ (δ(H) 4.83, 4.17) with C(3) (δ(C) 73.9), C(5) (δ(C) 47.5), and C(19) (δ(C) 63.1). NOESY Correlations of $CH_2(18)$ (δ(H) 4.83, 4.17) with H−C(3) (δ(H) 4.41) and H−C(5) (δ(H) 2.08), and of $CH_2(19)$ (δ(H) 4.61, 3.91) with Me(20) (δ(H) 0.79) further corroborated that $CH_2(18)OH$ was β-oriented, whereas $CH_2(19)OH$ was α-oriented. Based on the above evidence, the structure of **2** was established as 3,18,19-trihydroxy-*ent*-labda-8(17),13-dien-16,15-olide.

HR-ESI-MS Analysis of **3** (*m*/*z* 357.2054 ([*M*+Na]$^+$)) pointed to the molecular formula $C_{20}H_{30}O_4$, as further supported by NMR spectroscopy (*Tables 1* and *2*). The IR spectrum of **3** showed the presence of OH groups (3277), an α,β-unsaturated γ-lactone (1747, 1678), as confirmed by *Legal* and *Kedde* color reactions [18], and an *exo*-methylidene moiety (902 cm^{-1}). The NMR data of **3** indicated a labdane-type diterpene with an exocyclic CH_2 group (δ(C) 108.0, C(17)), a Me(18) group (δ(C) 23.8), a $CH_2(19)OH$ group (δ(C) 64.2), and an angular Me(20) group (δ(C) 15.2). The NMR data of **3** were very similar to those of andrographolide (**12**) [9][10], except for a signal at δ(H) 2.80 (br. *t*, *J*=7.2 Hz, 2 H) ascribable to a $CH_2(14)$ unit (δ(C) 23.6) in **3** instead of a signal at δ(H) 5.02 (*t*, *J*=2.4 Hz, 1 H) due to the hydroxymethine group at C(14) (δ(H) 66.0) in **12**. This was further substantiated by a HMBC experiment with **3**, showing long-range correlations of $CH_2(14)$ (δ(H) 2.80) to C(12) (δ(C) 141.0), C(13) (δ(C) 126.1), and C(16) (δ(C) 171.3). From the above evidence, the structure of **3** was established as 3,19-dihydroxy-*ent*-labda-8(17),12-dien-16,15-olide.

HR-ESI-MS Analysis of **4** (*m*/*z* 517.2410 ([*M*+Na]$^+$)) indicated the molecular formula $C_{26}H_{38}O_9$, in combination with NMR analyses (*Tables 1* and *3*). The IR spectrum of **4** showed the presence of OH groups (3421), an α,β-unsaturated γ-lactone (1747, 1644), as confirmed by *Legal* and *Kedde* color reactions [18], and an *exo*-methylidene (899 cm^{-1}) function.

The characteristic ^{13}C-NMR signals (*Table 1*) showed that **4** was a labdane with an exocyclic $CH_2(17)$ group (δ(C) 106.9), a Me(18) group (δ(C) 28.9), and an angular Me(20) group (δ(C) 13.5). The α,β-unsaturated γ-lactone moiety resonated at δ(H) 7.17 (*t*, *J*=0.7 Hz, H−C(14)) and 4.75 (br. *s*, $CH_2(15)$), with ^{13}C-NMR signals at δ(C) 134.2 (C(13)), 145.5 (C(14)), 70.7 (C(15)), and 174.4 (C(16)). The HMBC spectrum indicated that the lactone moiety was attached to the bicyclic skeleton *via* the aliphatic C(11)−C(12) chain (δ(C) 22.4, 25.2), which indicated that **4** was an analogue of neoandrographolide (**10**) [7][10][12]. However, the absence of a resonance for an oxymethylene for C(19), along with an additional C=O signal (δ(C) 176.5), suggested that the 19-CH_2OH group in neoandrographolide had been oxidized to a COOR group in **4**. This was confirmed by HMBC correlations between C(19) (δ 176.5) and $CH_2(3)$ (δ(H) 2.39–2.36, 1.05), H−C(5) (δ(H) 1.32), and Me(18) (δ(H) 1.28).

In addition, the ^{13}C- and ^{1}H-NMR signals of a β-D-glucopyranosyl (Glc) group were found at δ(C) 60–100 and δ(H) 3.9–4.5, resp. The β-configuration was assigned based on the coupling constant of the β-anomeric H-atom, H−C(1′), at δ(H) 6.28 (*d*, *J*=8.1 Hz), and was confirmed by specific hydrolysis of **4** with β-D-glucosidase [19]. The HMBC correlation between H−C(1′) and C(19) indicated the presence of an ester linkage between the glucopyranosyl residue and the COO group in position 19. In the NOESY spectrum, the correlation between Me(18) (δ(H) 1.28) and H−C(5) (δ(H) 1.32), and the absence of a correlation between Me(18) and Me(20) (δ(C) 0.97) dem-

Table 3. *^{1}H-NMR Spectroscopic Data of* **4**–**6**. At 600 MHz in (D_5)pyridine. Asterisks (*) denote overlapping signals.

Position	**4**[a])	**5**	**6**
1	1.80–1.77 (*m*)	1.43 (br. *d*, *J* = 13.2)	1.71–1.68 (*m*)
	1.03 (*dt*, *J* = 13.2, 3.6)	1.10 (*dt*, *J* = 13.2, 3.6)	1.03 (*dt*, *J* = 13.2, 3.6)
2	2.17–2.15 (*m*)	1.95 (br. *d*, *J* = 10.2)	1.59 (br. *d*, *J* = 12.0)
	1.46 (br. *d*, *J* = 13.2)	1.94–1.89 (*m*)	1.44–1.41 (*m*)
3	2.39–2.36 (*m*)	3.66–3.64*	2.21 (br. *d*, *J* = 13.2)
	1.05 (*dt*, *J* = 13.2, 3.6)		1.00 (*dt*, *J* = 13.2, 3.6)
4	–	–	–
5	1.32 (*dd*, *J* = 12.6, 2.4)	1.19 (br. *d*, *J* = 12.6)	1.23 (*dd*, *J* = 12.0, 2.1)
6	2.45–2.41 (*m*)	1.76 (br. *d*, *J* = 12.6)	1.83–1.80 (*m*)
	2.10 (br. *d*, *J* = 12.6)	1.40–1.37 (*m*)	1.41–1.38 (*m*)
7	2.40–2.36 (*m*)	2.39–2.36 (*m*)	2.37 (br. *d*, *J* = 12.6)
	1.93 (*dt*, *J* = 12.6, 3.6)	1.98 (*dt*, *J* = 13.8, 6.6)	1.96 (*dt*, *J* = 12.6, 4.8)
8	–	–	–
9	1.66 (br. *d*, *J* = 10.8)	2.36 (br. *d*, *J* = 10.5)	1.73 (br. *d*, *J* = 10.8)
10	–	–	–
11	1.77–1.75 (*m*)	7.19 (*d*, *J* = 15.6)	1.85–1.83 (*m*)
	1.65–1.61 (*m*)		1.70–1.66*
12	2.53 (br. *t*, *J* = 13.2)	6.27 (*d*, *J* = 15.6)	2.70 (br. *t*, *J* = 12.5)
	2.19 (br. *t*, *J* = 13.2)		2.27–2.24 (*m*)
14	7.17 (*t*, *J* = 0.7)	7.22 (br. *s*)	5.96 (*t*, *J* = 6.6)
15	4.75 (br. *s*)	6.01 (*s*)	4.59 (*d*, *J* = 6.3)
			4.55 (*d*, *J* = 6.3)
16	–	–	4.66 (br. *s*)
			4.65 (br. *s*)
17	4.86 (*s*)	4.84 (*s*)	4.91 (*s*)
	4.73 (*s*)	4.67 (*s*)	4.78 (*s*)
18	1.28 (*s*)	1.51 (*s*)	1.18 (*s*)
19	–	4.47 (*d*, *J* = 10.9)	3.98 (*d*, *J* = 10.2)
		3.66–3.64*	3.59 (*d*, *J* = 10.2)
20	0.92 (*s*)	0.86 (*s*)	0.71 (*s*)
MeO	–	3.46 (*s*)	–

[a]) Sugar resonances: 6.28 (*d*, *J* = 8.6, H–C(1′)); 4.16 (*t*, *J* = 8.6, H–C(2′)); 3.98 (*dt*, *J* = 8.6, 3.0, H–C(3′)); 4.33 (*t*, *J* = 8.6, H–C(4′)); 4.23 (*t*, *J* = 8.6, H–C(5′)); 4.43 (*dd*, *J* = 12.0, 1.7, 1 H of CH_2(6′)); 4.36 (*dd*, *J* = 12.0, 4.3, 1 H of CH_2(6′)).

onstrated that Me(18) was β-oriented; consequently, the GlcOOC(19) moiety was in α-position.

From the above data, compound **4** was identified as 19-[(β-D-glucopyranosyl)oxy]-19-oxo-*ent*-labda-8(17),13-dien-16,15-olide. Interestingly, its 4-epimeric analogue has been isolated before from the aquatic plant *Potamogeton lucens* [19].

The molecular formula of **5** was determined as $C_{21}H_{30}O_5$ by HR-ESI-MS (*m/z* 385.1986 ([M+Na]$^+$)) and NMR analyses (*Tables 1* and *3*). The IR spectrum of **5** showed the presence of OH groups (3419), an α,β-unsaturated γ-lactone (1762, 1644), and an *exo*-methylidene function (896 cm^{-1}). Again, positive *Legal* and *Kedde* color reactions [18] confirmed the unsaturated γ-lactone moiety. The NMR data of **5** showed characteristic signals similar to those of **13** [8][10][12], including an exocyclic $CH_2(17)$ group (δ(C) 108.9), a Me(18) group (δ(C) 23.7), an angular Me(20) group (δ(C) 16.0), and a 1,2-disubstituted (*E*)-configured C=C bond (δ(C) 138.6, 121.4). Comparison of the ^{1}H-NMR data of **5** with those of **13** revealed a MeO group at δ(H) 3.46 (*s*, 3 H) and a signal due to an acetal CH at δ(H) 6.01 (*s*, 1 H). The HSQC spectrum indicated that the signal at δ(H) 6.01 correlated to the acetal methine carbon signal at δ(C) 102.9. Long-range HMBC correlations of δ(H) 6.01 to C(13) (δ(C) 132.7), C(14) (δ(C) 141.5), and C(16) (δ(C) 170.2) suggested that the acetal H-atom was at C(15). The position of the MeO group at C(15) was determined from the HMBC correlation between the MeO H-atoms at δ(H) 3.46 and C(15) at δ(C) 102.9, and from the NOESY correlations between δ(H) 3.46 and both H−C(14) (δ(C) 7.22) and H−C(15) (δ(H) 6.01).

From the above data, the structure of compound **5** was assigned as 3,19-dihydroxy-15-methoxy-*ent*-labda-8(17),11,13-trien-16,15-olide. The absolute configuration at C(15) could not yet be established [19][20].

HR-ESI-MS Analysis of **6** (*m/z* 345.2412 ($[M+Na]^+$)), together with the NMR data (*Tables 1* and *3*), indicated the molecular formula $C_{20}H_{34}O_3$. The IR spectrum of **6** showed the presence of OH groups (3284) and an *exo*-methylidene (896 cm^{-1}), but no α,β-unsaturated γ-lactone, as corroborated by negative *Legal* and *Kedde* tests [18]. The ^{13}C-NMR signals indicated a labdane-type bicyclic skeleton with an exocyclic $CH_2(17)$ group (δ(C) 106.9), a Me(18) group (δ(C) 28.1), a $CH_2(19)OH$ group (δ(C) 63.8), and an angular Me(20) group (δ(C) 13.5), similar to most *ent*-labdane diterpenoids from *A. paniculata* [10]. However, the absence of the lactone C=O signal and the presence of two CH_2OH signals at δ(C) 60.0 and 58.5 implied that the α,β-unsaturated γ-lactone ring had been opened and transformed to CH_2OH groups in positions 15 and 16, respectively. In the HSQC spectrum, two signals at δ(H) 4.59 (*d*, $J=6.3$ Hz, 1 H) and 4.55 (*d*, $J=6..3$ Hz, 1 H), correlating with one CH_2OH (δ(C) 60.0), and two more signals at δ(H) 4.66 (*s*, 1 H) and 4.65 (*s*, 1 H), correlating with the second CH_2OH (δ(C) 58.5), were ascribed to $CH_2(15)$ and $CH_2(16)$, respectively. This was further substantiated by HMBC correlations between $CH_2(16)$ and C(11) (δ(C) 23.0), C(13) (δ(C) 142.8), and C(14) (δ(C) 127.4), and between $CH_2(15)$ and C(13) (δ 142.8) and C(14) (δ(C) 127.4), respectively.

The configuration of the C(13)=C(14) bond was determined to be (*Z*), based on NOESY correlations of H−C(14) with H−C(11) and H−C(12). From the above evidence, the structure of **6** was elucidated as *ent*-labda-8(17),13-diene-15,16,19-triol. Note that **6** is the enantiomer of a derivate of pinusolide isolated from *Biota orientalis* [17], based on the opposite absolute configurations at C(4), C(5), C(9), and C(10).

HR-ESI-MS Analysis of **7** (*m/z* 375.2135 ($[M+Na]^+$)) and NMR analyses (*Tables 1* and *4*) revealed the molecular formula $C_{20}H_{32}O_5$. The IR spectrum of **7** showed the presence of OH groups (3415), a COOH function (1677), as confirmed by reaction with Bromocresol Green, and an *exo*-methylidene group (902 cm^{-1}). Negative *Legal* and *Kedde* tests [18] confirmed the absence of an α,β-unsaturated γ-lactone ring. The NMR spectroscopic data of **7** were very similar to those of 14-deoxyandrographolide (**15**) [10][12], except that C(15) and C(16) were shifted upfield to δ(C) 61.6 and 170.5, respectively. Furthermore, the ^3J correlation between $CH_2(15)$ (δ(H) 4.24) and C(16) (δ(C) 170.5) could not be observed in the HMBC spectrum, confirming the open-

Table 4. *1H-NMR Spectroscopic Data of* **7**–**9**. At 600 MHz in (D_5)pyridine. Asterisks (*) denote overlapping signals.

Position	**7**	**8**	**9**
1	1.68 (br. *d*, *J* = 12.6)	1.37–1.41(*m*)	1.77–1.73 (*m*)
	1.18 (*dt*, *J* = 12.6, 3.6)	1.11 (*dt*, *J* = 13.2, 3.6)	1.21 (*dt*, *J* = 12.8, 4.0)
2	2.03–2.01 (*m*)	1.94 (*dd*, *J* = 12.0, 4.5)	2..08–2.04 (*m*)
	1.96 (br. *d*, *J* = 12.6)	1.91–1.87 (*m*)	1.89–1.95*
3	3.66–3.62*	3..66–3.62*	3.63–3.60*
4	–	–	–
5	1.21 (*dd*, *J* = 12.6, 2.4)	1.19 (br. *d*, *J* = 13.2)	1.24 (*dd*, *J* = 12.0, 4.4)
6	1.77–1.75 (*m*)	1.76 (br. *d*, *J* = 13.2)	1.80–1.77 (*m*)
	1.35–1.31 (*m*)	1.43–1.41 (*m*)	1.37–1.32 (*m*)
7	2.33 (br. *d*, *J* = 12.9)	2.37 (br. *d*, *J* = 13.5)	2.35 (br. *d*, *J* = 12.8, 4.0)
	1.93 (*dt*, *J* = 12.9, 3.6)	2.00 (*dt*, *J* = 13.5, 4.5)	1.98–1.94*
8	–	–	–
9	1.84 (br. *d*, *J* = 10.8)	2.51 (br. *d*, *J* = 10.2)	1.96 (br. *d*, *J* = 9.4)
10	–	–	–
11	2.65–2.61 (*m*)	7.35 (*dd*, *J* = 15.6, 10.2)	1.98–1.94*
	2.51–2.46 (*m*)		1.81–1.79 (*m*)
12	3.15 (br. *t*, *J* = 7.2)	6.25 (*d*, *J* = 15.6)	4.03–3.99 (*m*)
	3.15 (br. *t*, *J* = 7.2)		3.83–3.77 (*m*)
14	7.33 (*t*, *J* = 6.3)	–	–
15	4.26 (*t*, *J* = 6.3)	–	–
	4.23 (*t*, *J* = 6.3)		
16	–	–	–
17	4.91 (*s*)	4.83 (*s*)	4.89 (br.. *s*)
	4.72 (*s*)	4.64 (*s*)	4.69 (br. *s*)
18	1.49 (*s*)	1.19 (*s*)	1.48 (*s*)
19	4.46 (*d*, *J* = 10.9)	4.46 (*d*, *J* = 10.8)	4.47 (*d*, *J* = 10.8)
	3.60 (*d*, *J* = 10.9)	3.63*	3.63–3.60*
20	0.70 (*s*)	0.85 (*s*)	0.72 (*s*)

ing of the γ-lactone ring. The geometry of the C(13)=C(14) bond was determined to be (*Z*), on the basis of NOESY correlations between H–C(14) and both H–C(11) and H–C(12). From the above evidence, compound **7** was identified as 3,15,19-trihydroxy-*ent*-labda-8(17),13-dien-16-oic acid.

HR-ESI-MS Analysis of **8** (*m/z* 317.1721 ($[M+Na]^+$)), together with the NMR data (*Tables 1* and *4*), suggested the molecular formula $C_{17}H_{26}O_4$. The IR spectrum of **8** showed the presence of OH (3412), COOH (1689), and *exo*-methylidene (896 cm^{-1}) groups. Negative *Legal* and *Kedde* color reactions [18] confirmed the absence of an α,β-unsaturated γ-lactone ring. Reaction with Bromocresol Green gave a positive result, confirming the presence of a COOH group. The ^{13}C-NMR spectrum showed 17 C-atoms. A characteristic oxygenated CH in position 3 (δ(C) 80.0), an exocyclic methylidene at δ(C) 108.9 (C(17)), a Me(18) group (δ(C) 23.7), an oxygenated C(19) (δ(C) 64.2), and an angular Me(20) group (δ(C) 13.5), suggesting the presence of a nor-labdane skeleton similar to that of **7**. The HMBC correlations of both H–C(11)

(δ(H) 7.35) and H–C(12) (δ(H) 6.25) to the COOH group (δ(C) 168.6) confirmed that the 13-position was oxidized and attached to the bicyclic skeleton by a 1,2-disubstituted, (*E*)-configured C(11)=C(12) chain (δ(C) 138.6, 121.4, resp.). On the basis of the above evidence, the structure of **8** was established as '3,19-dihydroxy-14,15,16-trinor-*ent*-labda-8(17),11-dien-13-oic acid' (=(2*E*)-3-[(1*R*,5*R*,6*R*,8a*R*)-decahydro-6-hydroxy-5-(hydroxymethyl)-5,8a-dimethyl-2-methylidenenaphthalen-1-yl]prop-2-enoic acid).

The molecular formula of compound **9** was deduced as $C_{16}H_{28}O_3$ by HR-ESI-MS (*m*/*z* 291.1914 ([*M*+Na]$^+$)) and NMR analyses (*Tables 1* and *4*). The IR spectrum showed the presence of OH functions (3258) and an *exo*-methylidene (908 cm^{-1}), but no lactone (negative *Legal* and *Kedde* tests [18]). The ^{13}C-NMR spectrum showed 16 C-atoms, with an OH at C(3) (δ(C) 80.2), an exocyclic methylidene group (C(17), a Me(18) group (δ(C) 23.8), an oxygenated CH_2(15) (δ(C) 64.3), and an angular Me(20) group, implying that **9** was an analogue of **8**. In the HSQC spectrum, the two signals at δ(H) 4.03–3.99 (*m*, 1 H) and 3.83–3.77 (*m*, 1 H), correlating with δ(C) 61.4, were ascribed to CH_2(12). This was further substantiated by the HMBC correlations between CH_2(12) (δ(H) 4.03–3.99, 3.83–3.77) and both C(9) (δ(C) 52.7) and C(11) (δ(C) 28.4). Therefore, the structure of **9** was elucidated as '13,14,15,16-tetranor-*ent*-labd-8(17)-ene-3,12,19-triol' (=(1*R*,2*R*,4a*S*,5*R*)-decahydro-5-(2-hydroxyethyl)-1-(hydroxymethyl)-1,4a-dimethyl-6-methylidenenaphthalen-2-ol). Note that **9** is the enantiomer of the microbial-transformation product of communic acid [21], with opposite absolute configurations at C(3), C(4), C(5), C(9), and C(10).

Experimental Part

General. All reagents were of anal. grade and purchased from *Shenyang Chemical Company* (Shenyang, China). Prep. HPLC: *Waters-600* chromatograph with *ODS C_{18}* column (250×20 mm; *Inertsil Pak*) and *Waters-490* UV detector; as solvents, HPLC-grade MeOH and double-distilled H_2O were used. Column chromatography (CC) was performed on silica gel *60* (*Qingdao Haiyang Chemical Co., Ltd*, China), *Sephadex LH-20* (*Advanced Technology Industrial Co., Ltd*), and *ODS* (40–75 μm, *Fuji Silysia Chemical Ltd*, Japan). Thin-layer chromatography (TLC): silica gel *60*; visualization by spraying with *Kedde*'s reagent. IR Spectra: *Bruker IFS-55*; in cm^{-1}. NMR Spectra: *Bruker ARX-600* apparatus, at 600 (^{1}H) and 150 MHz (^{13}C) in (D_5)pyridine; δ in ppm rel. to Me_4Si, *J* in Hz. HR-ESI-MS: *Bruker APEX-II* mass spectrometer; in *m*/*z*.

Plant Material. The dried aerial parts of *Andrographis paniculata* NEES. were collected from Fujian Province, China. A voucher specimen was identified by Prof. *Qi-Shi Sun*, and deposited at the Department of Natural Products Chemistry, Shenyang Pharmaceutical University, China.

Extraction and Isolation. The plant material (10 kg) was cut into small pieces and heated at reflux with 85% aq. EtOH (3×). The resulting EtOH extract was concentrated *in vacuo*, suspended in H_2O, and partitioned between cyclohexane and AcOEt. The AcOEt layer (295 g) was concentrated and then subjected to CC (SiO_2, 10×120 cm; gradient of $CHCl_3$/MeOH 98:2, 97:3, 95:5, 9:1, 8:2): eight fractions (*Fr. 1–8*). *Fr. 1* was subjected to CC (*Sephadex LH-20*; $CHCl_3$/MeOH 1:1). The diterpenoid-containing fraction was re-subjected to CC (SiO_2; cyclohexane/AcOEt 9:1, 8:2, 7:3), followed by repeated prep. HPLC, to afford **11** (256.1 mg), **1** (17.6 mg), and **14** (32.8 mg). A part (10 g) of *Fr. 2* (60 g) was purified by CC (*Sephadex LH-20*; $CHCl_3$/MeOH 1:1), and the diterpenoid-containing fraction was subjected to MPLC (*ODS*; MeOH/H_2O 7:3) to afford **13** (34.2 mg) and **15** (102.3 mg). *Fr. 3* and *Fr. 4* were combined, evaporated, and subjected to CC (*Sephadex LH-20*; $CHCl_3$/MeOH 1:1), and the diterpenoid-containing fraction was re-subjected to CC (SiO_2; cyclohexane/acetone 9:1, 8:2, 7:3), followed by prep. HPLC, to afford **2** (46.2 mg), **5** (12.2 mg), **6** (28.2 mg), **8** (46.0 mg), and **9** (56.6 mg). *Fr.*

6 was recrystallized from MeOH to afford **12** (56 g). The mother liquor of **12** was purified by CC (*Sephadex LH-20*; $CHCl_3$/MeOH 1:1), and the diterpenoid-containing fraction was further purified by CC (SiO_2; $CHCl_3$/MeOH 97:3, 95:5, 9:1) to give **3** (22.2 mg) and **7** (36.5 mg). *Fr. 7* was purified by CC (*Sephadex LH-20*; $CHCl_3$/MeOH 1:1), and the diterpenoid-containing fraction was recrystallized from MeOH to provide **10** (15.7 g). The mother liquor of **10** was applied to MPLC (*ODS*; MeOH/H_2O 1:1) to give **4** (37.0 mg) and **16** (47.7 mg). *Fr. 8* was subjected to CC (*Sephadex LH-20*; $CHCl_3$/MeOH 1:1), and the diterpenoid-containing fraction was purified by CC (SiO_2; $CHCl_3$/MeOH 95:5, 9:1, 8:2) to afford, after purification by prep. HPLC, **17** (35.5 mg) and **18** (83.5 mg).

*19-Hydroxy-3-oxo-*ent*-labda-8(17),11,13-trien-16,15-olide* (**1**). Colorless needles. M.p. 155–156° (MeOH). $[\alpha]_D^{23} = -12.5$ ($c = 0.1$, MeOH). IR (KBr): 3410, 1741, 1698, 1639, 1101, 1050, 889. ^{1}H-NMR: see *Table 2*. ^{13}C-NMR: see *Table 1*. HR-ESI-MS: 353.1754 ([M+Na]$^+$, $C_{20}H_{26}NaO_4^+$; calc. for 353.1729).

*3,18,19-Trihydroxy-*ent*-labda-8(17),13-dien-16,15-olide* (**2**). Colorless needles. M.p. 153–154° (MeOH). $[\alpha]_D^{23} = -40.8$ ($c = 0.24$, MeOH). IR (KBr): 3492, 2925, 1753, 1652, 1637, 1059, 1014, 902. ^{1}H-NMR: see *Table 2*. ^{13}C-NMR: see *Table 1*. HR-ESI-MS: 373.2002 ([M+Na]$^+$, $C_{20}H_{30}NaO_5^+$; calc. 373.1991).

*3,19-Dihydroxy-*ent*-labda-8(17),12-dien-16,15-olide* (**3**). Colorless plates. M.p. 179–180° (MeOH). $[\alpha]_D^{23} = -2.2$ ($c = 0.22$, MeOH). IR (KBr): 3277, 2926, 1747, 1678, 1640, 1223, 1035, 1013, 902. ^{1}H-NMR: see *Table 2*. ^{13}C-NMR: see *Table 1*. HR-ESI-MS: 357.2054 ([M+Na]$^+$, $C_{20}H_{30}NaO_4^+$; calc. 357.2042).

*19-[(β-D-Glucopyranosyl)oxy]-19-oxo-*ent*-labda-8(17),13-dien-16,15-olide* (**4**). Pale-yellow powder (MeOH). $[\alpha]_D^{23} = -25.0$ ($c = 0.2$, MeOH). IR (KBr): 3421, 2932, 1747, 1644, 1448, 1073, 899. ^{1}H-NMR: see *Table 3*. ^{13}C-NMR: see *Table 1*. HR-ESI-MS: 517.2410 ([M+Na]$^+$, $C_{26}H_{38}NaO_9^+$; calc. 517.2408).

*3,19-Dihydroxy-15-methoxy-*ent*-labda-8(17),11,13-trien-16,15-olide* (**5**). Colorless powder (MeOH). $[\alpha]_D^{23} = +50.0$ ($c = 0.1$, MeOH). IR (KBr): 3419, 2936, 1762, 1644, 1449, 1090, 1031, 985, 896. ^{1}H-NMR: see *Table 3*. ^{13}C-NMR: see *Table 1*. HR-ESI-MS: 385.1986 ([M+Na]$^+$, $C_{21}H_{30}NaO_5^+$; calc. 385.1991).

ent*-Labda-8(17),13-diene-15,16,19-triol* (**6**). Colorless needles. M.p. 97–98° (MeOH). $[\alpha]_D^{23} = -25.7$ ($c = 0.17$, MeOH). IR (KBr): 3284, 2933, 1642, 1444, 1023, 980, 896. ^{1}H-NMR: see *Table 3*. ^{13}C-NMR: see *Table 1*. HR-ESI-MS: 345.2412 ([M+Na]$^+$, $C_{20}H_{34}NaO_3^+$; calc. 345.2405).

*3,15,19-Trihydroxy-*ent*-labda-8(17),13-dien-16-oic Acid* (**7**). Colorless needles. M.p. 186–187° (MeOH). $[\alpha]_D^{23} = +14.3$ ($c = 0.14$, MeOH). IR (KBr): 3415, 2927, 1747, 1678, 1223, 1078, 1013, 902. ^{1}H-NMR: see *Table 4*. ^{13}C-NMR: see *Table 1*. HR-ESI-MS: 375.2135 ([M+Na]$^+$, $C_{20}H_{32}NaO_5^+$; calc. 375.2147).

'*3,19-Dihydroxy-14,15,16-trinor-*ent*-labda-8(17),11-dien-13-oic Acid*' (=*(2*E*)-3-[(1*R*,5*R*,6*R*,8a*R*)-Decahydro-6-hydroxy-5-(hydroxymethyl)-5,8a-dimethyl-2-methylidenenaphthalen-1-yl]prop-2-enoic Acid*; **8**). Colorless plates. M.p. 240–241° (MeOH). $[\alpha]_D^{23} = -4.2$ ($c = 0.24$, MeOH). IR (KBr): 3412, 2935, 1689, 1657, 1304, 1047, 896. ^{1}H-NMR: see *Table 4*. ^{13}C-NMR: see *Table 1*. HR-ESI-MS: 317.1721 ([M+Na]$^+$, $C_{17}H_{26}NaO_4^+$; calc. 317.1729).

'*13,14,15,16-Tetranor-*ent*-labd-8(17)-ene-3,12,19-triol*' (=*(1*R*,2*R*,4a*S*,5*R*)-Decahydro-5-(2-hydroxyethyl)-1-(hydroxymethyl)-1,4a-dimethyl-6-methylidenenaphthalen-2-ol*; **9**). Colorless needles. M.p. 188–190° (MeOH). $[\alpha]_D^{23} = -27.3$ ($c = 0.22$, MeOH). IR (KBr): 3258, 2967, 2944, 1645, 1446, 1036, 972, 908. ^{1}H-NMR: see *Table 4*. ^{13}C-NMR: see *Table 1*. HR-ESI-MS: 291.1914 ([M+Na]$^+$, $C_{16}H_{28}NaO_3^+$; calc. 291.1936).

REFERENCES

[1] H. M. Chang, P. P. H. But, in 'Pharmacology and Application of Chinese Materia Medica', World Scientific, Singapore, 1987, Vol. 1, p. 918.
[2] X. J. Yao, M. A. Wainberg, M. A. Parniak, *Virology* **1992**, *187*, 56.
[3] M. George, K. M. Pandalai, *Indian J. Med. Res.* **1949**, *37*, 169.
[4] A. Puri, R. Saxena, R. P. Saxena, V. Srivastava, J. S. Tandon, *J. Nat. Prod.* **1993**, *56*, 995.
[5] P. T. Zeha, U. M. Rawal, *Indian J. Exper. Biol.* **2001**, *39*, 41.
[6] M. P. Cava, W. R. Chan, R. P. Stein, C. R. Willis, *Tetrahedron* **1965**, *21*, 2617.
[7] W. R. Chan, D. R. Taylor, C. R. Willis, R. L. Bodden, H. W. Fehlhaber, *Tetrahedron* **1971**, *27*, 5081.

[8] A. Balmain, J. D. Connolly, *J. Chem. Soc., Perkin Trans. 1* **1973**, 1247.
[9] T. Fujita, R. Fujitani, Y. Takeda, Y. Takaishi, T. Yamada, M. Kido, I. Miura, *Chem. Pharm. Bull.* **1984**, *32*, 2117.
[10] T. Matsuda, M. Kuroyanagi, S. Sugiyama, K. Umehara, A. Ueno, K. Nishi, *Chem. Pharm. Bull.* **1994**, *42*, 1216.
[11] K. R. Muntha, V. B. Mopuru, D. Gunasekar, M. M. Madugula, C. Caux, B. Bodo, *Phytochemistry* **2003**, *62*, 1271.
[12] Y. H. Shen, R. T. Li, W. L. Xiao, G. Xu, Z. W. Lin, Q. S. Zhao, H. D. Sun, *J. Nat. Prod.* **2006**, *69*, 319.
[13] S. Pramanick, S. Banerjee, B. Achari, B. Das, K. S. S. Ashi, S. Mukhopadhyay, A. Neuman, T. Prange, *J. Nat. Prod.* **2006**, *69*, 403.
[14] X. J. He, J. K. Li, H. Gao, F. Qiu, K. Hu, X. M. Cui, X. S.Yao, *Tetrahedron* **2003**, *59*, 6603.
[15] X. J. He, J. K. Li, G. Hao, F. Qiu, X. M. Cui, X. S. Yao, *Chem. Pharm. Bull.* **2003**, *51*, 586.
[16] L. Cui, F. Qiu, X. S. Yao, *Drug Metab. Dispos.* **2005**, *33*, 555.
[17] B. H. Han, H. O. Yang, Y. H. Kang, D.-Y. Suh, H. J. Go, W.-J. Song, Y. C. Kim, M. K. Park, *J. Med. Chem.* **1998**, *41*, 2626.
[18] 'Modern Methods in Plant Analysis', Eds. L. J. Haynes, K. I. Peach, M. V. Tracey, Springer-Verlag, Berlin, 1955, Vol. 2, p. 583.
[19] P. Waridel, J.-L. Wolfender, J.-B. Lachavanne, K. Hostettmann, *Phytochemistry* **2004**, *65*, 945.
[20] C. Zdero, F. Bohlmann, R. M. King, *Phytochemistry* **1991**, *30*, 1591.
[21] G. Aranda, R. Azerad, M. Maurs, G. Bertho, D. Jiménez-González, E. Cabrera-Torres, A. F. Barrero, *Phytochemistry* **2000**, *54*, 23.

Received May 24, 2006