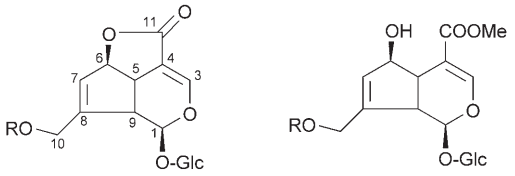
Two New Iridoid Glycosides from *Hedyotis tenelliflora* BLUME

by **Jing-Feng Zhao**, **Qin-Mei Yuan**, **Xiao-Dong Yang**, **Hong-Bing Zhang**, and **Liang Li***

School of Pharmacy, Center for Advanced Studies of Medicinal and Organic Chemistry, Yunnan University, Kunming 650091, Yunnan, P. R. China
(phone: +86-871-5033644; fax: +86-871-5035538; e-mail: liliang5758@sina.com)

Two new iridoid glycosides, teneoside A (=(2a*R*,5*S*)-5-[(β-D-glucopyranosyl)oxy]-2a,4a,5,7b-tetrahydro-4-{[(α-L-rhamnopyranosyl)oxy]methyl}-1*H*-2,6-dioxacyclopenta[*cd*]inden-1-one; **1**) and teneoside B (=methyl (1*S*,5*R*)-1-[(β-D-glucopyranosyl)oxy]-1,4a,5,7a-tetrahydro-5-hydroxy-7-{[(α-L-rhamnopyranosyl)oxy]methyl}-cyclopenta[*c*]pyran-4-carboxylate; **2**), were isolated from the roots of *Hedyotis tenelliflora* BLUME, along with two known compounds, deacetylasperuloside (**3**) and scandoside methyl ester (**4**). Their structures were elucidated by chemical methods (acid hydrolysis) and spectroscopic analyses.

1. Introduction. – Many species of the genus *Hedyotis* (Rubiaceae) are used in Chinese folk medicine [1]. Iridoid glycosides, triterpenoids [2], lignan glycosides, flavonids, and anthraquinones [3] have been reported from several *Hedyotis* genera [4]. *Hedyotis tenelliflora* BLUME is a medicinal herb called '*xiazicao*' by the Dai people living in Lincang, Yunnan Province. This plant has been used for the treatment of snake wounds, nephritis, hepatitis, rheumatic arthritis, and inflammations [5]. The plant, although commonly found in China, has not been examined with regard to chemical constituents. In this paper, we report two new iridoid glycosides from *H. tenelliflora*, teneoside A (**1**) and teneoside B (**2**), which were isolated together with two known iridoid glycosides, deacetylasperuloside (**3**) and scandoside methyl ester (**4**).



1 R = Rha
3 R = H

2 R = Rha
4 R = H

Glc = β-D-glucopyranosyl, Rha = α-L-rhamnopyranosyl

2. Results and Discussion. – Compound **3**, an amorphous powder, had the molecular formula $C_{16}H_{20}O_{10}$, as established by HR-FAB-MS (*m/z* 372.1054 ($[M+H]^+$, calc. 372.1057)). The IR spectrum indicated a OH (3429), an α,β-unsaturated ester (1709), and C=C groups (1635 cm^{-1}). The ^{1}H- and ^{13}C-NMR spectra of **3** (*Tables 1* and *2*, resp.) displayed signals typical of a dimeric iridoid glycoside [6]. ^{1}H- and ^{13}C-NMR assignments were made with the help of ^{1}H,^{1}H-COSY and HSQC experiments, starting with the easily distinguishable acetal H–C(1) atom at δ(H) 5.78 (δ(C) 96.5), H–C(9)

at δ(H) 3.21 (δ(C) 45.6), and H–C(5) at δ(H) 3.52 (δ(C) 39.5), and further correlated with the HMBC spectrum. By comparison of the ^{1}H- and ^{13}C-NMR spectroscopic data of **3** with literature values, this compound was identified as deacetylasperuloside, which had previously been isolated from *H. chrysotricha* [7][8].

Table 1. *^{1}H-NMR Data for Compounds* **1**–**4**. At 500 MHz in D_2O; δ in ppm, J in Hz. Primed (′) and doubly primed (″) numbers refer to Glc and Rha atoms, resp. Arbitrary atom numbering[1]).

Position	**1**	**2**	**3**	**4**
1	5.82 (*d*, *J* = 1.8)	5.38 (*d*, *J* = 5.1)	5.78 (*d*, *J* = 1.4)	5.42 (*d*, *J* = 5.0)
3	7.31 (*d*, *J* = 2.8)	7.48 (*d*, *J* = 0.86)	7.23 (*d*, *J* = 2.8)	7.51 (*d*, *J* = 0.92)
5	3.55 (*d*, *J* = 3.6)	3.21 (*t*, *J* = 6.3)	3.52 (*d*, *J* = 3.8)	3.24 (*t*, *J* = 6.5)
6	5.60 (*m*)	4.58 (*t*, *J* = 1.9)	5.53 (*m*)	4.60 (*t*, *J* = 1.7)
7	5.68 (*m*)	5.81 (*t*, *J* = 1.8)	5.56 (*m*)	5.84 (*t*, *J* = 1.7)
9	3.33 (*m*)	3.05 (*m*)	3.21 (*m*)	3.09 (*m*)
10	4.56 (*s*)	4.88 (*s*)	4.07 (*s*)	4.33 (*dd*, *J* = 0.5, 15.4)
				4.26 (*dd*, *J* = 0.5, 15.2)
MeO	–	3.75 (*s*)	–	3.76 (*s*)
1′	4.76 (*d*, *J* = 8.2)	4.80 (*d*, *J* = 8.0)	4.79 (*d*, *J* = 8.2)	4.79 (*d*, *J* = 7.9)
2′	3.27 (*dd*, *J* = 7.9, 9.3)	3.27 (*dd*, *J* = 7.9, 9.1)	3.27 (*dd*, *J* = 8.2, 9.5)	3.271 (*dd*, *J* = 8.2, 9.4)
3′	3.40 (*t*, *J* = 9.1)	3.36 (*t*, *J* = 9.1)	3.38 (*t*, *J* = 9.5)	3.39 (*t*, *J* = 9.5)
4′	3.25 (*t*, *J* = 9.1)	3.23 (*t*, *J* = 9.1)	3.24 (*t*, *J* = 9.9)	3.49 (*t*, *J* = 9.2)
5′	3.34 (*m*)	3.33 (*m*)	3.46 (*ddd*, *J* = 2.4, 6.4, 8.1)	3.44 (*m*)
6′	3.67 (*dd*, *J* = 11.9, 6.7)	3.65 (*dd*, *J* = 6.7, 12.0)	3.78 (*dd*, *J* = 2.1, 12.6)	3.89 (*dd*, *J* = 2.2, 12.4)
	3.94 (*dd*, *J* = 11.9, 2.1)	3.94 (*dd*, *J* = 2.2, 12.4)	3.56 (*dd*, *J* = 5.8, 12.4)	3.72 (*dd*, *J* = 5.8, 12.4)
1″	5.10 (*d*, *J* = 1.8)	5.10 (*d*, *J* = 1.7)	–	–
2″	3.93 (*dd*, *J* = 3.5, 1.8)	3.86 (*dd*, *J* = 3.7, 2.0)	–	–
3″	3.67 (*dd*, *J* = 3.3, 9.5)	3.81 (*dd*, *J* = 3.7, 9.3)	–	–
4″	3.64 (*t*, *J* = 9.8)	3.52 (*t*, *J* = 9.5)	–	–
5″	3.81 (*dd*, *J* = 10.5, 6.2)	3.91 (*dd*, *J* = 10.0, 6.2)	–	–
6″	1.21 (*d*, *J* = 6.2)	1.21 (*d*, *J* = 6.2)	–	–

Compound **1**, an amorphous powder, had the molecular formula $C_{22}H_{30}O_{14}$, established on the basis of HR-FAB-MS (*m*/*z* 518.1640 ([*M* + H]$^+$, calc. 518.1636)). The IR, ^{1}H-, and ^{13}C-NMR spectra displayed signals typical of a dimeric iridoid glycoside like **3**. However, **1** displayed signals for *two* anomeric H-atoms at δ(H) 4.82 (*d*, *J* = 58.0 Hz; δ(C) 100.29) and 5.12 (*d*, *J* = 51.5 Hz; δ(C) 98.13), which indicated two sugar moieties. By comparison with NMR chemical-shift values and coupling constants [9][10], as well as by acid hydrolysis, followed by TLC and GC/MS analyses, one β-D-glucopyranosyl (Glc) and one α-L-rhamnpyranosyl (Rha) moiety (δ(H) 1.21 (*d*, *J* = 6.2 Hz; δ(C) 18.0) were identified. Regarding the aglycone of **1**, H–C(1) exhibited HMBC long-range couplings with C(1′) of Glc, and H–C(10) correlated with C(1″) of the Rha moiety, which indicated that Glc and Rha were connected to the aglycone *via* glycoside linkages at positions 1 and 10, respectively. From these data, the structure of **1** was identified as 10-*O*-(α-L-rhamno)deacetylasperuloside, for which we proposed the trivial name *teneoside A*[1]).

Compound **4**, an amorphous powder, had the molecular formula $C_{17}H_{24}O_{11}$, as established on the basis of HR-FAB-MS (*m*/*z* 404.1322 ([*M* + H]$^+$, calc. 404.1319)).

[1]) For systematic names, see the *Exper. Part*.

Table 2. *^{13}C-NMR Data for Compounds* **1**–**4**. At 125 MHz in D_2O; δ in ppm. Primed (′) and doubly primed (″) numbers refer to Glc and Rha atoms, resp. Arbitrary atom numbering[1]).

Position	**1**	**2**	**3**	**4**
H–C(1)	95.7	98.7	96.5	99.7
H–C(3)	152.3	153.8	153.0	154.4
C(4)	107.4	112.5	108.0	114.0
H–C(5)	38.6	43.5	39.5	45.8
H–C(6)	88.5	81.8	89.5	83.4
H–C(7)	127.0	133.5	128.1	131.5
C(8)	149.1	147.8	150.2	148.7
H–C(9)	45.1	48.5	45.6	48.3
CH_2(10)	70.9	71.8	62.1	62.4
C(11)	174.5	172.1	174.1	172.2
MeO	–	54.4	–	54.6
H–C(1′)	102.5	102.2	102.5	101.7
H–C(2′)	75.8	75.4	75.8	75.3
H–C(3′)	79.5	79.6	79.3	79.5
H–C(4′)	71.9	72.4	72.5	72.6
H–C(5′)	78.5	78.8	78.5	78.8
CH_2(6′)	63.6	63.5	63.8	63.8
H–C(1″)	102.0	102.1	–	–
H–C(2″)	72.1	72.3	–	–
H–C(3″)	72.1	72.3	–	–
H–C(4″)	73.9	73.8	–	–
H–C(5″)	70.3	70.2	–	–
Me(6″)	18.0	18.0	–	–

The IR spectrum indicated OH (3429)), ester C=O (1738), and C=C groups (1635 cm^{-1}). The ^{1}H- and ^{13}C-NMR spectra displayed signals typical of an iridoid glycoside. Sequential ^{1}H- and ^{13}C-NMR assignments were made with the help of ^{1}H,^{1}H-COSY, HSQC, and HMBC spectra. By comparison with literature values, compound **4** was identified as scandoside methyl ester, which had previously been isolated from *H. chrysotricha* [7][8].

Compound **2**, an amorphous powder, had the molecular formula $C_{23}H_{34}O_{15}$, as established on the basis of HR-FAB-MS (*m/z* 550.1892 ($[M+H]^+$, calc. 550.1898)). The ^{1}H- and ^{13}C-NMR spectra of **2** were similar to those of **4**, except for signals arising from the sugar moieties. The ^{1}H-NMR spectrum exhibited signals for two anomeric H-atoms at δ(H) 4.80 (*d*, $J = 8.0$ Hz; δ(C) 102.2) and δ(H) 5.10 (*d*, $J = 1.7$ Hz; δ(C) 102.1). By comparison with literature NMR data [9][10], the two sugar moieties were identified as Glc and Rha. This was further confirmed by acid hydrolysis, followed by TLC and GC/MS analyses. The H–C(1) resonance of the aglycone of **2** exhibited HMBC long-range couplings with C(1′) of Glc, and H–C(10) correlated with C(1″) of Rha, which indicated glycoside linkages at C(1) and C(10), respectively. From all these data, the structure of **2** was identified as 10-*O*-(α-L-rhamno)scandoside methyl ester, which was named *teneoside B*.

Experimental Part

General. Column chromatography (CC): silica gel (100–200 or 200–300 mesh; *Quingdao*) or *Sephadex LH-20* gel (*Amersham Pharmacia*). Thin-layer chromatography (TLC): silica gel GF_{254} plates (*Qingdao*). All solvents were industrial products, and redistilled before using. M.p.: *Kofler* apparatus, uncorrected. UV Spectra: *Shimadzu UV-210A* apparatus; λ_{max} in nm ($\log \varepsilon$). IR Spectra: *Shimadzu IR-450* spectrophotometer, KBr pellets; in cm^{-1}. ^{1}H- (500 MHz), ^{13}C- and DEPT 90- and 135-NMR (125 MHz), and two dimensional (2D)-NMR (COSY, HMBC, HMQC, NOESY) spectra were recorded on a *Bruker AV300* spectrometer in D_2O; δ in ppm rel. to Me_4Si, J in Hz. HR-FAB-MS (pos. mode): *VG Auto Spec-3000* spectrometer; in m/z. GC/MS: *Thermo Finnigan Trace* apparatus, *Rtx-5 MS* column (15 m × 0.25 mm; 0.25 μm; *Thamek Restek UK, Ltd.*).

Plant Material. The plants were collected from LinCang, Yunnan Province, P. R. China, and identified by Prof. *Hu Zhihao*, Department of Biology, Yunnan University, P. R. China. A voucher specimen was deposited at the Phytochemistry Department, School of Pharmacy, Yunnan University.

Extraction and Isolation. Air-dried, finely sliced roots of *Hedyotis tenelliflora* BLUME (5.2 kg) were extracted repeatedly with 95% EtOH. The extracts were combined, and concentrated *in vacuo*. The resulting residue was dissolved in H_2O, filtered, and the filtrate was purified with the aid of a macro-reticular resin column, eluting successively with H_2O, 50% aq. EtOH, and 95% aq. EtOH: fractions *Fr. 1*, *2*, and *3*. *Fr. 3* (25 g) was separated by vacuum CC (SiO_2; $CHCl_3$/MeOH mixtures of increasing polarity). The fraction eluted with $CHCl_3$/MeOH 85:15 was re-chromatographed (1. *Sephadex LH-20*, MeOH; 2. SiO_2) to afford **4** (80 mg) and **1** (20 mg). *Fr. 2* (15 g) was suspended in H_2O, and extracted with $CHCl_3$. The aq. layer (47 g) was subjected to CC (*Sephadex LH-20*; MeOH). The iridoid fractions were re-chromatographed (SiO_2; $CHCl_3$/MeOH) to afford **3** (80 mg) and **2** (20 mg).

Teneoside A (= *(2a*R,*5*S*)-5-[(β-*D*-Glucopyranosyl)oxy]-2a,4a,5,7b-tetrahydro-4-[[(α-*L*-rhamnopyranosyl)oxy]methyl]-1*H*-2,6-dioxacyclopenta[cd]inden-1-one*; **1**). Amorphous powder. M.p. 193–194°. UV (MeOH): 233 (4.23). $[\alpha]_D^{25} = -156.5$ ($c = 0.023$, MeOH). IR (KBr): 3430, 2930, 1755, 1650, 1070. ^{1}H-NMR: see *Table 1*. ^{13}C-NMR: see *Table 2*. FAB-MS: 518 ($[M+H]^+$), 337 ($[M-\text{Rha}-\text{OH}]^+$), 321 ($[M-\text{Glc}-\text{OH}]^+$). HR-FAB-MS: 518.1640 ($[M+H]^+$, $C_{22}H_{31}O_{14}^+$; calc. 518.1636).

Teneoside B (= *Methyl (1*S,*5*R*)-1-[(β-*D*-Glucopyranosyl)oxy]-1,4a,5,7a-tetrahydro-5-hydroxy-7-[[(α-*L*-rhamnopyranosyl)oxy]methyl]cyclopenta[c]pyran-4-carboxylate*; **2**). Amorphous powder. M.p. 182–184°. UV (MeOH): 233 (4.480). $[\alpha]_D^{25} = -132.5$ ($c = 0.068$, MeOH). IR (KBr): 3430, 1685, 1630, 1307, 1020. ^{1}H-NMR: see *Table 1*. ^{13}C-NMR: see *Table 2*. FAB-MS: 550 ($[M+H]^+$), 369 ($[M-\text{Rha}-\text{OH}]^+$), 354 $[M-\text{Glc}-\text{OH}]^+$). HR-FAB-MS: 550.1892 ($[M+H]^+$, $C_{23}H_{35}O_{15}^+$; calc. 550.1898).

Deacetylasperuloside (= *(2a*R,*5*S*)-5-[(β-*D*-Glucopyranosyl)oxy]-2a,4a,5,7b-tetrahydro-4-(hydroxymethyl)-1*H*-2,6-dioxacyclopenta[cd]inden-1-one*; **3**). Amorphous powder. M.p. 156–157° (MeOH). UV (MeOH): 234 (4.28). $[\alpha]_D^{25} = -132.5$ ($c = 0.068$, MeOH). IR (KBr): 3430, 2924, 1745, 1650, 1070, 1020. ^{1}H-NMR: see *Table 1*. ^{13}C-NMR: see *Table 2*. FAB-MS: 372 ($[M+H]^+$), 354 ($[M+H-H_2O]^+$), 175 $[M-\text{Glc}-\text{OH}]^+$). HR-FAB-MS: 372.1054 ($[M+H]^+$, $C_{16}H_{21}O_{10}^+$; calc. 372.1057).

Scandoside Methyl Ester (= *Methyl (1*S,*5*R*)-1-[(β-*D*-Glucopyranosyl)oxy]-1,4a,5,7a-tetrahydro-5-hydroxy-7-(hydroxymethyl)cyclopenta[c]pyran-4-carboxylate*; **4**). Amorphous powder. M.p. 167–168°. UV (MeOH): 234 (4.36). $[\alpha]_D^{25} = -23.5$ ($c = 0.078$, MeOH). IR (KBr): 3425, 1689, 1632, 1650, 1307. ^{1}H-NMR: see *Table 1*. ^{13}C-NMR: see *Table 2*. FAB-MS: 404 ($[M+H]^+$), 207 ($[M-\text{Glc}-\text{OH}]^+$). HR-FAB-MS: 404.1322 ($[M+H]^+$, $C_{17}H_{25}O_{11}^+$; calc. 404.1319).

Acid Hydrolysis. The appropriate compound (10 mg) was heated in a mixture of 0.5N aq. HCl (0.5 ml) and EtOH (0.5 ml) at 100° for 90 min. The precipitated aglycone was collected by filtration, and the filtrate was concentrated *in vacuo* below 40°. The resulting residue was dissolved in EtOH (2 ml), and subjected to GC/MS; and the TLC R_f values were compared with those of authentic Glc and Rha samples.

REFERENCES

[1] 'Zhong Guo Zhi Wu Zhi', Science Press, Beijing, a, 1999, Vol. 71, p. 32.
[2] Q.-M. Yuan , J.-F. Zhao, J.-H. Yang, L. Li, *Zhong Cao Yao* **2001**, *32*, 754.
[3] D. Permana, N. H. Lajis, A. G. Othman, A. M. Ali, N. Aimi, M. Kitajima, H. Takayama *J. Nat. Prod.* **1999**, *62*, 1430.
[4] Q.-M. Yuan, J.-F. Zhao, L. Li, *World Phytomed.* **2001**, *16*, 148.
[5] G.-N. Li, 'Yun Nan Zhong Yao Zhi', Yunnan Science & Technique Press, Kunming, 1990, p. 222.
[6] S. Damtoft, S. Rosendal Jensen, J. Nielsen, B. J. Nielsen, *Phytochemistry* **1981**, *20*, 2717.

[7] J.-N. Peng, X.-Z. Feng, X.-T. Liang, *J. Nat. Prod.* **1999**, *62*, 611.
[8] J. N. Peng, X. Z. Feng, X. T. Liang, *Yaoxue Xuebao* **1997**, *32*, 908.
[9] B. Ahmed, A. J. Al-Rehaily, T. A. Al-Howiriny, *Biol. Pharm. Bull.* **2003**, *26*, 462.
[10] M. Hosny, J. P. N. Rosazza, *J. Nat. Prod.* **1998**, *61*, 734.

Received April 18, 2005