New Anthraquinone and Iridoid Glycosides from the Stems of *Hedyotis hedyotidea*

by **Xiao-Ping Hu**, **Shu-Wei Zhang**, **Shan-Shan Liu**, and **Li-Jiang Xuan***

State Key Laboratory of Drug Research, Shanghai Institute of Materia Medica, Shanghai Institute for Biological Sciences, Chinese Academy of Sciences, 501 Haike Road, Zhangjiang Hi-Tech Park, Shanghai 201203, P. R. China (phone: +86-21-20231968; e-mail: ljxuan@mail.shcnc.ac.cn)

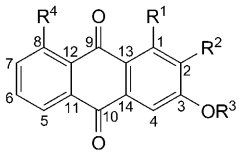
Five new anthraquinone glycosides, hedanthrosides A–E (**1**–**5**, resp.) and two new iridoid glycosides, hediridosides A and B (**6** and **7**, resp.), along with two known anthraquinones and four known iridoids, were isolated from the stems of *Hedyotis hedyotidea* (DC.) MERR. The structures of the new compounds were elucidated on the basis of 1D- and 2D-NMR, and HR-MS analysis and chemical methods.

Introduction. – The genus *Hedyotis* (Rubiaceae family) contains 69 species distributed in China [1], some of which have been used as Traditional Chinese Medicines (TCM), such as *Hedyotis diffusa* for treating cancer, and *Hedyotis chrysotricha* for treating enteritis [2]. The characteristic chemical constituents of this genus are anthraquinones, flavonoids, iridoids, and triterpenoids [3]. Plants of this genus have been found to exhibit anti-inflammatory and anticancer activities [3].

Hedyotis hedyotidea (DC.) MERR. [4], a 3–5-m tall shrub growing in the south of China [1], has been traditionally used for the treatment of cold, cough, gastro-enteritis, heatstroke, *Herpes zoster*, rheumatoid arthritis, *etc.* [5]. Previously, some iridoids have been identified from *H. hedyotidea*, and meanwhile the activity of the crude extract of this herb against stress ulcer in mice was mentioned [6], but the bioactive components were unknown. As part of our ongoing effort to discover new bioactive agents from Chinese medicinal plants, the stems of *H. hedyotidea* were investigated. We report here on the isolation and characterization of seven anthraquinones, including five new glycosides, **1**–**5**, together with six iridoids, which comprise two new glycosides, **6** and **7**. Inhibitory activities of compounds obtained to NF-κB induced by the NF-κB-activating cytokine TNF-α (tumor necrosis factor α) was investigated, but all turned out to be inactive.

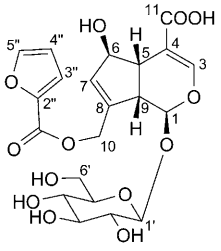
Results and Discussion. – The stems of *Hedyotis hedyotidea* collected from Guangxi were percolated with 70% aqueous acetone. After removal of the organic solvent under reduced pressure, the extract was partitioned between $CHCl_3$ and H_2O. Compounds **1**–**7**, along with 1,3-dihydroxy-2-(hydroxymethyl)-9,10-anthraquinone 3-*O*-β-D-glucopyranoside [7], scandoside [8], borreriagenin [9], methyl deacetylasperulosidate [10], and asperulosidic acid methyl ester [11] were obtained from the H_2O fraction, and the $CHCl_3$-soluble fraction afforded rubiadin (**8**) [12].

The UV spectra of compounds **1**–**5** with the common absorbance maxima at 260 and 355 nm were typical of anthraquinone-type compounds. The IR spectra of them

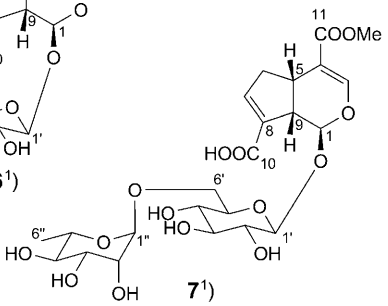


	R^1	R^2	R^3	R^4
1[1])	OH	Me	Glc(6'→1")Glc	H
2[1])	OH	HO–CH_2	Glc(6'→1")Glc	H
3[1])	MeO	HO–CH_2	Glc(6'→1")Glc	H
4[1])	MeO	HO–CH_2	Glc(6'→1")Glc	MeO
5[1])	OH	COOMe	Glc(6'→1")Glc	H
8[1])	OH	Me	H	H

Glc = glucopyranosyl



6[1])



7[1])

also showed characteristic absorption bands for free OH groups in the range of 3363–3423 cm^{-1}, CO peaks at 1670 cm^{-1}, and aromatic group bands in the range of 1570 and 1670 cm^{-1}.

Compound **1**, was obtained as a yellow amorphous powder, and analyzed for $C_{27}H_{30}O_{14}$ by means of HR-ESI-MS (*m/z* 601.1539 ($[M+Na]^+$)). The aromatic region of the ^{1}H-NMR spectrum (*Table 1*) indicated the presence of five aromatic H-atoms. Their multiplicity, an *AABB* system at δ(H) 8.22 (*dd*, *J* = 7.1, 2.0), δ(H) 8.18 (*dd*, *J* = 6.3, 2.1), δ(H) 7.90–7.95 (*m*, 2 H), and a 1-H *singlet* at δ(H) 7.48, were suggestive of an anthraquinone with one nonsubstituted and one trisubstituted ring. Other ^{1}H-NMR signals were a *singlet* of a Me group at δ(H) 2.19, and characteristic signals of two β-glucopyranosyl units with the anomeric H-atoms at δ(H) 4.18 (*d*, *J* = 7.5), and 5.18 (*d*, *J* = 6.6). The signals corresponding to a chelated CO group, a non-chelated CO group, two O-bearing C-atoms, a Me group, along with signals of two sugar units in the ^{13}C-NMR spectrum, supported the earlier assignments of the H-atoms. In the HMBC spectrum, correlations from both of H–C(5) (δ(H) 8.18) and the *singlet* aromatic H-atom (δ(H) 7.48) to the non-chelated C(10)=O group (δ(C) 181.6), confirmed that C(4) is unsubstituted. Consequently, a free OH group should be located at C(1) to chelate with C(9)=O. Cross-peak from the Me (δ(H) 2.19) to C(1) (δ(C) 161.4) in the HMBC supported the assignment of the Me group at C(2). Accordingly, the aglycone of **1** was confirmed to be rubiadin (**8**), which was also found in *H. hedyotidea*.

The linkage sites of the two glucopyranosyl units were deduced from the HMBC correlations H–C(1′) (δ(H) 5.18)/C(3) (δ(C) 161.2), and H–C(1″) (δ(H) 4.18)/C(6′) (δ(C) 68.0). Furthermore, in the ROESY spectrum (*Fig. 1*), correlation H–C(1′)/H–C(4) confirmed that the glucopyranosyl units were located at C(3). Enzymatic hydrolysis of **1** afforded glucose, which was identified by TLC comparison with authentic sample. The glucose isolated from the hydrolysate gave optical rotation $[\alpha]_D^{20} = +42.3$ (*c* = 0.90, H_2O), indicating it as D-glucose. Therefore, **1** was determined to be 1,3-dihydroxy-2-methyl-9,10-anthraquinone 3-*O*-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranoside[1]), named hedanthroside A.

---

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

Table 1. *^{1}H- and ^{13}C-NMR Data* ((D_6)DMSO) *for* **1**, **2**, *and* **5**[1]). δ in ppm, J in Hz.

	1		**2**		**5**	
	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)
C(1)		161.4		161.2		159.6
C(2)		120.7		123.6		117.0
C(3)		161.2		162.0		159.8
H–C(4)	7.48 (*s*)	105.9	7.47 (*s*)	106.4	7.50 (*s*)	105.6
H–C(5)	8.18 (*dd*, *J* = 6.3, 2.1)	126.8	8.17 (*dd*, *J* = 6.7, 2.1)	127.0	8.18–8.22 (*m*)	127.0
H–C(6)	7.90–7.95 (*m*)	134.7	7.90–7.95 (*m*)	134.8	7.92–7.99 (*m*)	134.9
H–C(7)	7.90–7.95 (*m*)	134.6	7.90–7.95 (*m*)	134.9	7.92–7.99 (*m*)	134.7
H–C(8)	8.22 (*dd*, *J* = 7.1, 2.0)	126.4	8.17 (*dd*, *J* = 7.0, 2.0)	126.6	8.22–8.27 (*m*)	126.5
C(9)		187.1		187.1		186.8
C(10)		181.6		181.5		181.2
C(11)		132.4		132.9		132.9
C(12)		133.0		132.8		132.7
C(13)		110.9		111.4		111.1
C(14)		131.9		133.8		135.3
Me–C(2) or HO*C*H_2–C(2)	2.19 (*s*)	8.4	4.65 (*d*, *J* = 11.4), 4.56 (*d*, *J* = 11.4)	50.9		
*Me*OO*C*–C(2)					3.86 (*s*)	52.6, 163.7
Glc′						
H–C(1′)	5.18 (*d*, *J* = 6.6)	100.2	5.15 (*d*, *J* = 6.8)	100.8	5.30 (*d*, *J* = 7.6)	99.9
H–C(2′)	3.36–3.38 (*m*)	76.0	3.77–3.82 (*m*)	75.6	3.36–3.38 (*m*)	76.2
H–C(3′)	3.62–3.65 (*m*)	75.6	3.30–3.50 (*m*)	69.8	3.66–3.69 (*m*)	75.6
H–C(4′)	3.36–3.38 (*m*)	73.1	3.00–3.20 (*m*)	73.5	3.22–3.27 (*m*)	72.9
H–C(5′)	3.36–3.40 (*m*)	69.0	3.30–3.50 (*m*)	68.9	3.33–3.39 (*m*)	68.9
CH_2(6′)	4.00 (*d*, *J* = 9.6), 3.65 (*d*, *J* = 9.6)	68.0	4.02 (*d*, *J* = 9.8), 3.79 (*d*, *J* = 9.8)	68.0	3.99 (*d*, *J* = 9.5), 3.65 (*d*, *J* = 9.5)	67.9
Glc″						
H–C(1″)	4.18 (*d*, *J* = 7.5)	103.4	4.19 (*d*, *J* = 7.4)	103.5	4.15 (*d*, *J* = 7.6)	103.4
H–C(2″)	2.98–3.00 (*m*)	76.8	3.00–3.20 (*m*)	76.9	3.07 (*m*)	76.8
H–C(3″)	3.03–3.07 (*m*)	76.6	3.00–3.20 (*m*)	73.4	3.07 (*m*)	76.5
H–C(4″)	2.94–2.98 (*m*)	73.4	3.00–3.20 (*m*)	76.5	2.98 (*m*)	73.4
H–C(5″)	3.02–3.06 (*m*)	69.9	3.30–3.50 (*m*)	73.2	3.08 (*m*)	69.9
CH_2(6″)	3.60 (*d*, *J* = 10.7), 3.42 (*d*, *J* = 10.7)	60.9	3.60 (*d*, *J* = 11.6), 3.38 (*d*, *J* = 11.6)	61.0	3.62 (*d*, *J* = 10.3), 3.44 (*d*, *J* = 10.3)	60.9

Compound **2**, an orange amorphous powder, displayed a sodiated molecular-ion peak at *m*/*z* 617.1482 ($[M + Na]^+$) in its HR-ESI-MS, corresponding to the molecular formula $C_{27}H_{30}O_{15}$, which was suggestive of an oxidized form of **1**. Comparison of the NMR data (*Table 1*) of **2** with those of **1** indicated the presence of the CH_2 H-atoms represented by two *doublet*s (δ(H) 4.56 (*d*, *J* = 11.4), and δ(H) 4.65 (*d*, *J* = 11.4)), with the disappearance of the *singlet* peak of Me H-atoms. Correlations of the CH_2 to C(1) and C(3), confirmed that the Me at C(2) in compound **1** was hydroxylated in compound **2**. The configuration of other functional groups were determined by HMBC experiment (*Fig. 1*), which revealed that compound **2** exhibited the same diglucosyl unit as **1**, including the location and the interglycosidic linkage. From the above results, compound **2** was characterized as 1,3-dihydroxy-2-(hydroxymethyl)-9,10-anthraquinone 3-*O*-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranoside[1]), named hedanthroside B.

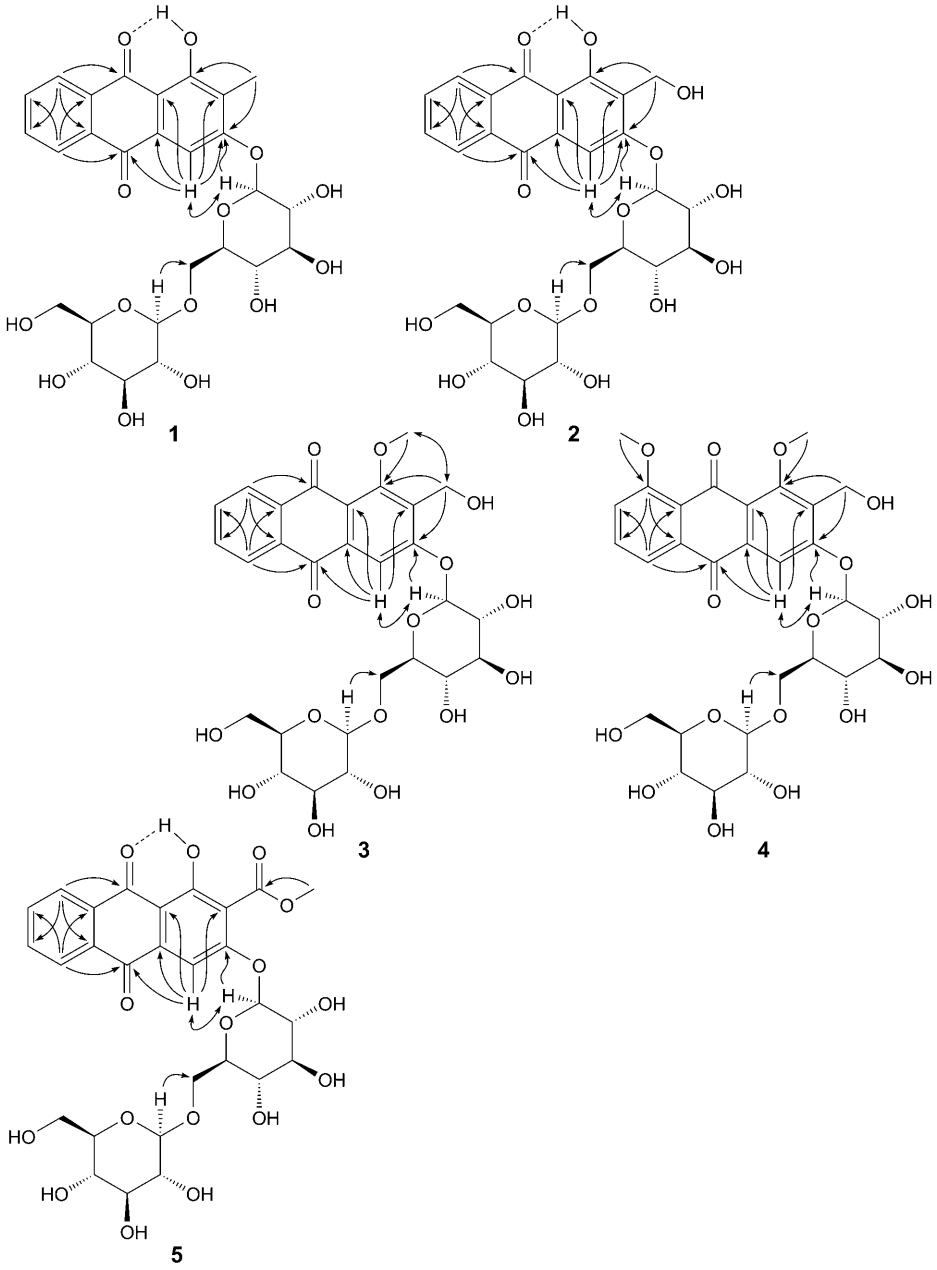


Fig. 1. *Key HMBC* (H → C) *and ROESY* (H ↔ H) *correlations of* **1**–**5**

Compound **3**, isolated as a yellow amorphous powder, had the molecular formula $C_{28}H_{32}O_{15}$ on the basis of the *pseudo*-molecular ion at *m*/*z* 631.1637 ($[M + Na]^+$) in the

HR-ESI-MS. Together with the MS analysis, NMR data led to the conclusion that **3** was an 1-*O*-Me derivative of **2**. The ^{1}H- and ^{13}C-NMR spectra (*Table 2*) displayed MeO signals at δ(H) 3.68 (*s*) and δ(C) 63.0. The ^3J correlation (*Fig. 1*) observed between the MeO H-atoms (δ(H) 3.68) and C(1) (δ(C) 160.4) supported the location of the MeO moiety at C(1). Other HMBCs similar to those of **2** as shown in *Fig. 1* established **3** as 3-hydroxy-2-(hydroxymethyl)-1-methoxy-9,10-anthraquinone 3-*O*-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranoside[1]), named hedanthroside C.

Compound **4** was obtained also as a yellow amorphous powder. The molecular formula, $C_{29}H_{34}O_{16}$, was determined from the HR-ESI-MS signal at *m*/*z* 661.1749 ($[M + Na]^+$). The aromatic region of the ^{1}H-NMR spectrum (*Table 2*) indicated the presence of four aromatic H-atoms. Their multiplicity, an *ABC* system at δ(H) 7.66 (*dd*,

Table 2. *^{1}H- and ^{13}C-NMR Data for Compounds* **3** *and* **4**[1]). δ in ppm, *J* in Hz.

	3[a])		**4**[b])	
	δ(H)	δ(C)	δ(H)	δ(C)
C(1)		160.4		161.7
C(2)		130.6		132.4
C(3)		160.4		161.5
H–C(4)	6.96 (*s*)	109.3	7.64 (*s*)	110.0
H–C(5)	7.20–7.30 (*m*)	126.4	7.62 (*d*, *J* = 6.8)	120.6
H–C(6)	7.20–7.30 (*m*)	134.2	7.66 (*dd*, *J* = 7.6, 6.8)	136.6
H–C(7)	7.28–7.33 (*m*)	135.4	7.42 (*d*, *J* = 7.6)	120.6
C(8)	7.38–7.48 (*m*)	126.9		160.9
C(9)		181.4		183.8
C(10)		182.3		184.7
C(11)		130.9		135.2
C(12)		133.0		123.2
C(13)		120.1		124.0
C(14)		135.2		136.5
*Me*O–C(1)	3.68 (*s*)	63.0	3.84 (*s*)	64.6
CH_2–C(2)	4.65 (*d*, *J* = 11.4), 4.56 (*d*, *J* = 11.4)	52.5	4.54 (*d*, *J* = 11.4), 4.56 (*d*, *J* = 11.4)	54.0
*Me*O–C(8)			3.82 (*s*)	57.5
Glc′				
H–C(1′)	5.06 (*d*, *J* = 6.7)	100.1	5.28 (*d*, *J* = 6.7)	101.9
H–C(2′)	3.28–3.32 (*m*)	76.3	3.38–3.44 (*m*)	77.6
H–C(3′)	3.50–3.55 (*m*)	75.6	3.65–3.70 (*m*)	74.6
H–C(4′)	3.50–3.55 (*m*)	73.5	3.64–3.68 (*m*)	70.7
H–C(5′)	3.50–3.55 (*m*)	69.1	3.84–3.90 (*m*)	77.1
CH_2(6′)	4.04 (*d*, *J* = 9.5), 4.32 (*d*, *J* = 9.5)	68.2	4.30 (*d*, *J* = 10.1), 3.97 (*d*, *J* = 10.1)	69.7
Glc″				
H–C(1″)	4.52 (*d*, *J* = 6.4)	103.2	4.46 (*d*, *J* = 7.6)	104.6
H–C(2″)	3.50–3.55 (*m*)	76.1	3.34–3.38 (*m*)	77.8
H–C(3″)	3.50–3.55 (*m*)	75.7	3.62–3.70 (*m*)	77.3
H–C(4″)	3.30–3.55 (*m*)	73.0	3.34–3.42 (*m*)	71.4
H–C(5″)	3.30–3.55 (*m*)	70.0	3.30–3.34 (*m*)	75.0
CH_2(6″)	3.75 (*d*, *J* = 11.3), 3.90 (*d*, *J* = 11.3)	61.2	3.92 (*d*, *J* = 10.5), 3.71 (*d*, *J* = 10.5)	62.5

[a]) Recorded in D_2O. [b]) Recorded in CD_3OD and D_2O.

$J = 7.6, 6.8$), 7.62 (*d*, $J = 6.8$), and 7.42 (*d*, $J = 7.6$), and a 1-H *singlet* at δ(H) 7.64, suggested that ring *A* is monosubstituted with a trisubstituted ring *C*. Presence of two MeO groups was evident from signals at δ(H) 3.84 (*s*, H–C(1a)) and 3.82 (*s*, H–C(8a)). The HMBCs from H–C(4) (δ(H) 7.64) and H–C(5) (δ(H) 7.62) to the C(10)=O group (δ(C) 184.7), as well as the presence of the cross-peaks between both of the two MeO groups and the C(9)=O group (δ(C) 183.8) confirmed that two of the MeO groups were located at C(1) and C(8), respectively. Other HMBCs as shown in *Fig. 1* led to the structure 3-hydroxy-2-(hydroxymethyl)-1,8-dimethoxy-9,10-anthraquinone 3-*O*-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranoside[1]), named hedanthroside D, for **4**.

Compound **5**, obtained as a yellow amorphous solid, was analyzed for $C_{28}H_{30}O_{16}$ by means of HR-ESI-MS (*m*/*z* 645.1443 ($[M + Na]^+$)). The aromatic region of the ^{1}H-NMR spectrum (*Table 1*) was similar to that of **1**, revealing a non-substituted ring *A* and a trisubstituted ring *C*. Comparison of the ^{1}H- and ^{13}C-NMR chemical shifts of **5** with those of **1** further revealed the presence of an ester CO group (δ(C) 163.7) and a MeO group (δ(H) 3.86) in **5** instead of a Me group in **1**. The HMBC spectrum showed correlations from the the MeO H-atoms to ester CO (δ(C) 163.7), and from H–C(4) (δ(C) 7.50) to C(2) (δ(C) 117.0), C(13) (δ(C) 111.1), C(10) (δ(C) 181.2), and C(3) (δ(C) 159.8). Comparing ^{1}H- and ^{13}C-NMR data (*Table 1*) and key HMBCs of compound **5** with those of **1**, the only difference was that the substituent at C(2) was a COOMe group instead of a Me group in **1**. Thus, compound **5** was determined as 1,3-dihydroxy-2-(methoxycarbonyl)-9,10-anthraquinone 3-*O*-β-D-glucopyranosyl(1 → 6)-β-D-glucopyranoside[1]), named hedanthroside E.

Optically active compound **6**, $[\alpha]_D^{20} = -12.7$ ($c = 0.15$, H_2O), was isolated as an almost white amorphous powder, with the molecular formula $C_{21}H_{24}O_{13}$ determined by means of HR-ESI-MS (*m*/*z* 507.1104 ($[M + Na]^+$)). The IR spectrum exhibited absorption bands due to OH (3444 cm^{-1}), ester CO (1641 cm^{-1}), and alkene groups (1384 and 1078 cm^{-1}). The ^{1}H- and ^{13}C-NMR spectra of **6** (*Table 3*) showed signals of a furan-2-carboxy group (δ(H) 6.74 (*dd*, $J = 3.5, 0.8$), 7.48 (*d*, $J = 3.5$), and 7.85 (*d*, $J = 0.8$); δ(C) 146.1, 122.2, 115.1, 150.6, and 162.8), which were similar to those of synthetic compounds with a furan-2-carboxy group reported in [13]. The ^{1}H,^{1}H-COSY correlations (*Fig. 2*) from H–C(5″) through H–C(4″) to H–C(3″), in combination

Table 3. *^{1}H- and ^{13}C-NMR Data* (D_2O) *of Compound* **6**[1]). δ in ppm, *J* in Hz.

	δ(H)	δ(C)		δ(H)	δ(C)
H–C(1)	5.29 (*d*, $J = 6.7$)	99.6	H–C(1′)	4.85 (*d*, $J = 6.8$)	101.3
H–C(3)	7.28 (*s*)	150.9	H–C(2′)	3.32–3.38 (*m*)	75.4
C(4)		117.4	H–C(3′)	3.53–3.57 (*m*)	78.3
H–C(5)	3.14 (*dd*, $J = 4.7, 6.6$)	47.5	H–C(4′)	3.42–3.46 (*m*)	72.2
H–C(6)	4.67 (*d*, $J = 4.6$)	83.5	H–C(5′)	3.44–3.48 (*m*)	78.8
H–C(7)	6.09 (*s*)	134.7	CH_2(6′)	3.89 (*dd*, $J = 12.0, 1.4$), 3.72 (*dd*, $J = 12.0, 5.6$)	63.4
C(8)		143.0	C(2″)		146.1
H–C(9)	3.23 (*dd*, $J = 7.1, 6.7$)	48.9	H–C(3″)	7.48 (*d*, $J = 3.5$)	122.2
CH_2(10)	5.13 (*s*)	65.6	H–C(4″)	6.74 (*dd*, $J = 3.5, 0.8$)	115.1
C(11)		178.3	H–C(5″)	7.85 (*d*, $J = 0.8$)	150.6
			C=O		162.8

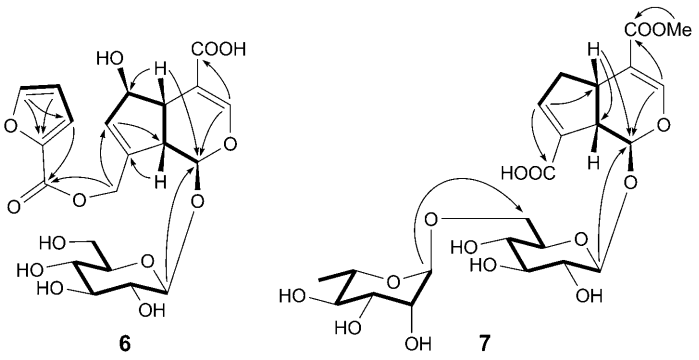


Fig. 2. *Key HMBC* (H → C) *and* $^1H,^1H$*-COSY* (—) *correlations of* **6** *and* **7**

with HMBCs (*Fig. 2*) from H–C(5″) to C(3″) and C(2″), and from H–C(4″) to C(2″), further confirmed the presence of a furan-2-carboxy moiety in **6**.

The remaining 16 ^{13}C-NMR signals were attributed to the iridoid glycoside skeleton of scandoside [8]. The scandoside skeleton was further confirmed by the NOESY spectrum (*Fig. 3*) in which correlations were observed between H–C(5) and H–C(9), and between H–C(6) and H–C(1). The glucopyranosyl moiety was at C(1), established by means of HMBC between H–C(1′) and C(1) (δ(C) 99.6). The furan-2-carboxy group was at C(10), corroborated by means of the HMBC between CH_2(10) (δ(H) 5.13) and C=O (δ(C) 162.8). From these data, the structure of **6** was elucidated as *rel*-(1*R*,4a*R*,5*S*,7a*R*)-7-[(furan-2-yl-carboxy)methyl]-1-(β-D-glucopyranosyloxy)-5-hydroxy-1,4a,5,7a-tetrahydrocyclopenta[*c*]pyran-4-carboxylic acid, named hediridoside A.

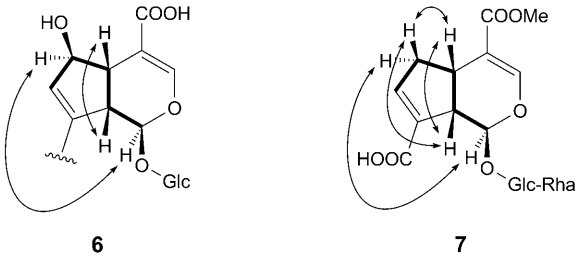


Fig. 3. *Selected ROESY correlations of* **6** *and* **7**

Compound **7** was obtained as a white, amorphous powder, and its molecular formula was deduced as $C_{23}H_{32}O_{15}$ by HR-ESI-MS (*m*/*z* 571.1660 ([M + Na]$^+$)). The IR spectrum exhibited absorption bands for OH (3446 cm^{-1}) and CO (1633 cm^{-1}) groups. The ^{1}H- and ^{13}C-NMR spectra (*Table 4*) of **7** not only showed signals characteristic for an iridoid skeleton at δ(H) 5.74 (*d*, J = 3.6, H–C(1)), 7.59 (*s*, H–C(3)), and 6.55 (*d*, J = 1.5, H–C(7)); and at δ(C) 98.0 (C(1)), 155.0 (C(3)), and 114.7 (C(4)), but also displayed additional glucopyranosyl and rhamnopyanosyl resonances (δ(H) 5.05 (H–C(1′)), 4.85 (H–C(1″)), 1.36 (*d*, J = 6.2, H–C(6″)); δ(C) 101.0 (C(1′)), 103.7 (C(1″)), 19.3 (C(6″))). Different from most iridoid glycosides

Table 4. *^{1}H- and ^{13}C-NMR Data* (D_2O) *of Compound* **7**[1]). δ in ppm, J in Hz.

	δ(H)	δ(C)		δ(H)	δ(C)
H–C(1)	5.74 (*d*, *J* = 3.6)	98.0	H–C(1′)	5.05 (overlapped)	101.0
H–C(3)	7.59 (*s*)	155.0	H–C(2′)	3.36 (*dd*, *J* = 9.2, 8.4)	75.1
C(4)		114.7	H–C(3′)	3.53 (*dd*, *J* = 9.2, 9.2)	78.1
H–C(5)	3.35 – 3.39 (*m*)	35.1	H–C(4′)	3.84 (*dd*, *J* = 9.2, 9.2)	72.8
CH_2(6)	2.48 (*ddd*, *J* = 18.2, 7.5, 2.2, H_α), 2.88 (*ddd*, *J* = 18.2, 7.5, 2.2, H_β)	40.8	H–C(5′)	3.40 – 3.44 (*m*)	72.6
H–C(7)	6.55 (*d*, *J* = 1.5)	142.8	CH_2(6′)	4.06 (br. *d*, *J* = 10.8), 3.78 (*dd*, *J* = 10.8, 5.6)	70.0
C(8)		142.0	H–C(1″)	4.85 (overlapped)	103.7
H–C(9)	3.30 – 3.34 (*m*)	49.4	H–C(2″)	4.00 – 4.04 (*m*)	72.7
C(10)		176.1	H–C(3″)	3.62 – 3.68 (*m*)	77.8
C(11)		172.7	H–C(4″)	3.44 – 3.50 (*m*)	74.7
MeO	3.80 (*s*)	54.4	H–C(5″)	3.76 – 3.80 (*m*)	71.4
			Me(6″)	1.36 (*d*, *J* = 6.2)	19.3

obtained from the *Hedyotis* genus, compound **7** had a C(10)OOH (δ(C) 176.1), and not a HO–CH_2(10) group.

The HMBC spectrum showed cross-peaks between the anomeric H-atoms H–C(1′) with C(1), and H–C(1″) with C(6′). These interactions supported the presence of a sugar moiety rha-(1 → 6)-glc, and its linkage to C(1) of the iridoid aglycon. Acid hydrolysis of **7** afforded glucose and rhamnose, which were identified by TLC comparison with authentic samples. The glucose and rhamnose isolated from the hydrolysate gave optical rotations $[\alpha]_D^{20} = +42.3$ ($c = 0.90$, H_2O) and $[\alpha]_D^{20} = +6.3$ ($c = 1.10$, H_2O), respectively, indicating that they were D-glucose and L-rhamnose.

In the ROESY NMR spectrum (*Fig. 3*) of **7**, cross-peaks from H–C(5) to H_β–C(6), from H–C(5) to H–C(9), from H–C(9) to H_β–C(6), and from H_α–C(6) to H–C(1) indicated the β-, β-, and α-orientations of H–C(5), H–C(9) and H–C(1), respectively. Accordingly, the structure of compound **7** was determined as 1,4a,5,7a-tetrahydro-4-(methoxycarbonyl)-1-[(6-*O*-α-L-rhamnopyanosyl-β-D-glucopyranosyl)oxy]cyclopenta-[*c*]pyran-7-carboxylic acid, named hediridoside B.

Anthraquinones and iridoids are the characteristic chemical constituents of the genus *Hedyotis*. The anthraquinone glycosides we obtained are common anthraquinones with a substituent at C(2), which is biogenetically reasonable. Most iridoid glycosides obtained from the *Hedyotis* genus contain the skeleton of scandoside, geniposide, deacetyl asperulosidic acid, and deacetyl asperuloside [2], and several derivatives have been isolated from the genus, such as the benzoylated geniposide derivatives [14], 10-acetylscandoside methyl ester [2], *etc*. Furan-2-carboxy moiety is a usual functional group in synthetic compounds, but rarely occurred in the plant secondary metabolites. Hediridoside A (**6**) is the first iridoid glycoside with a furan-2-carboxy moiety. Furthermore, hediridoside B (**7**) is the first iridoid glycoside with a carboxy group at C(10), found in the genus *Hedyotis*.

Since the plants from the genus *Hedyotis* were reported to possess anti-inflammatory and anticancer activities, we evaluated the effects of compounds **1** – **7** as well as of the six isolated known compounds on TNFα-induced transcriptional

activity of NF-κB, using a luciferase reporter gene assay [15]. However, none of the compounds exhibited any significant effect on basal NF-κB transcription.

We are grateful to the *National Science and Technology Major Protect* '*Key New Drug Creation and Manufacturing Program*', P. R. China (Number: 2009ZX09301-001), the *State Key Laboratory* (SIMM0907 KF-04), and the *National Natural Science Foundation of China* (No. 3090185).

Experimental Part

General. Column chromatography (CC): silica gel *H* (200–300 mesh; *Qingdao Marine Chemical Ltd.,* Qingdao, P. R. China), *Chromatorex* C_{18} and C_8 (20–45 μm; *Fuji Silysia Chemical Ltd.*), *NH-silica* gel (20–45 μm; *Fuji Silysia Chemical Ltd.*), *MCI* gel *CHP-20P* (75–150 μm; *Mitsubishi Chemical Industries Co., Ltd.*), *TSK* gel *Toyopearl HW-40F* (30–60 μm; *Toso Co., Ltd.*), *Sephadex LH-20* (20–80 μm; *Amersham Pharmacia Biotech AB*), and *Diaion HP20* (*Mitsubishi Chemical Industries Co., Ltd.*). Reversed-phase (RP) HPLC: *Agilent 1100* series system equipped with *YMC-Pack ODS-A* (250 × 20 mm, *S-5* μm, 12 nm) column. TLC: Precoated SiO_2 GF_{254} plates (*Yantai Huiyou Inc.*, Yantai, P. R. China). Optical rotations: *Perkin-Elmer 341* polarimeter. UV and IR spectra: *Shimadzu UV-2450 and Perkin-Elmer 577* spectrophotometer, resp. NMR Spectra: *Varian Mercury* NMR spectrometer, at 400 MHz for ^{1}H and 100 MHz for ^{13}C. LR- and HR-ESI-MS: *Finnigan LCQ-DECA* and *Waters Micromass Q-TOF Ultima Globe* spectrometer, resp.

Plant Material. The stems of *Hedyotis hedyotidea* (Rubiaceae) were collected from Ningming, in Guangxi Province, R. P. China, in September 2006, and authenticated by Prof. *Heming Yang*. A voucher specimen (No. SIMMGX51) is deposited with the Herbarium of Shanghai Institute of Materia Medica, Chinese Academy of Sciences, P. R. China.

Extraction and Isolation. Air-dried stems of *Hedyotis hedyotidea* (1 kg) were collected and powered. The material was extracted three times with aq. acetone (70%) at r.t. (10 l, 3 ×, each for 1 week). Solvent was removed under reduced pressure to obtain a crude extract (98 g), which was suspended in H_2O and partitioned with $CHCl_3$. The H_2O fraction (78 g) was subjected to CC (*MCI* gel; H_2O, 10, 50, 70, and 100% MeOH gradiently) to give five fractions, *Frs. A–E*. *Fr. A* (27 g) was further submitted to CC (*Sephadex LH-20*; MeOH/H_2O 0 to 20%) to afford two fractions, *Frs. A1–A2*. *Fr. A1* (0.9 g) was subjected to CC (C_8; MeOH/H_2O 5 to 10%) to give compound **6** (17 mg). *Fr. A2* (1.3 g) was subjected to CC (*HW-40F*; MeOH/H_2O 5 to 15%) to afford scandoside (26 mg). Isolation of *Fr. B* (13 g) by repeated CC (C_8; MeOH/H_2O 8 to 40%) afforded compound **7** (27 mg) and borreriagenin (18 mg). *Fr. C* (7.0 g) which was loaded on a *MCI* gel column (MeOH/H_2O, 15 to 50%) finally was separated by *HW-40F* (MeOH/H_2O 10 to 40%) to afford two fractions, *Frs. C1–C2*. *Fr. C1* (1.1 g) was purified by CC (C_8; MeOH/H_2O 15 to 25%) to give methyl deacetylasperulosidate (34 mg). *Fr. C2* (0.9 g) was passed through a C_{18} column with MeOH/H_2O (30 to 45%) to afford asperulosidic acid methyl ester (35 mg). *Fr. D* (9.9 g) was submitted to CC (C_8; MeOH/H_2O 35 to 42%), followed by *HW-40F* CC (MeOH/H_2O 32%), and further separation by RP-HPLC (*YMC-Pack ODS-A* (250 × 20 mm); MeOH/H_2O 30%; flow rate of 2.0 ml/min) to yield compound **3** (15 mg; t_R 12.0–14.0 min) and **4** (20 mg; t_R 15.3–17.2 min). *Fr. E* (13.0 g) was loaded on *MCI* gel column (MeOH/H_2O 50%) to afford compound **2** (100 mg) and *Fr. E2*, which was purified by RP-HPLC (MeOH/H_2O 50%; flow rate of 2.0 ml/min) to yield compound **1** (27 mg; t_R 4.1–5.9 min), 1,3-dihydroxy-2-(hydroxymethyl)-9,10-anthraquinone 3-*O*-β-D-glucopyranoside (13 mg; t_R 9.0–11.0 min) and compound **5** (4 mg; t_R 15.3–16.8 min). The $CHCl_3$ fraction (18 g) was subjected to CC (SiO_2; petroleum ether (PE)/Me_2CO 35 : 1 → 5 : 1) to give three fractions, *Frs. a–c*). *Fr. c* was concentrated and subjected to CC (SiO_2; $CHCl_3$/AcOEt 10 : 1) and repeated prep. TLC ($CHCl_3$/Me_2CO 50 : 1) to yield rubiadin (**8**; 12 mg).

Hedanthroside A (= *9,10-Dihydro-4-hydroxy-3-methyl-9,10-dioxoanthracen-2-yl 6-*O*-β-D-Glucopyranosyl-β-D-glucopyranoside*; **1**). Yellow amorphous powder. $[\alpha]_D^{23} = -37.0$ ($c = 0.20$, MeOH). UV (MeOH): 208 (4.00), 268 (4.05), 353 (3.13). IR (KBr): 3363, 1668, 1631, 1591. ^{1}H- and ^{13}C-NMR: see *Table 1*. ESI-MS (pos.): 601.2 ($[M+Na]^+$). HR-ESI-MS: 601.1539 ($[M+Na]^+$, $C_{27}H_{30}NaO_{14}^+$; calc. 601.1533).

Hedanthroside B (=*9,10-Dihydro-4-hydroxy-3-(hydroxymethyl)-9,10-dioxoanthracen-2-yl 6-O-β-*D-*Glucopyranosyl-β-*D-*glucopyranoside*; **2**). Orange amorphous powder. $[\alpha]_D^{23} = -33.0$ ($c = 0.20$, MeOH). UV (MeOH): 203 (4.04), 256 (3.92), 357 (3.01). IR (KBr): 3403, 1660, 1631, 1591. ^{1}H- and ^{13}C-NMR: see *Table 1*. ESI-MS (pos.): 617.3 ($[M + Na]^+$). HR-ESI-MS: 617.1482 ($[M+Na]^+$, $C_{27}H_{30}NaO_{15}^+$; calc. 617.1482).

Hedanthroside C (=*9,10-Dihydro-3-(hydroxymethyl)-4-methoxy-9,10-dioxoanthracen-2-yl 6-O-β-*D-*Glucopyranosyl-β-*D-*glucopyranoside*; **3**). Yellow amorphous powder. $[\alpha]_D^{23} = -35.0$ ($c = 0.25$, MeOH). UV (MeOH): 210 (3.99), 269 (4.15), 360 (3.00). IR (KBr): 3403, 1672, 1577. ^{1}H- and ^{13}C-NMR: see *Table 2*. ESI-MS (pos.): 631.2 ($[M + Na]^+$). HR-ESI-MS: 631.1637 ($[M+Na]^+$, $C_{28}H_{32}NaO_{15}^+$; calc. 631.1639).

Hedanthroside D (=*9,10-Dihydro-3-(hydroxymethyl)-4,5-dimethoxy-9,10-dioxoanthracen-2-yl 6-O-β-*D-*Glucopyranosyl-β-*D-*glucopyranoside*; **4**). Yellow amorphous powder. $[\alpha]_D^{23} = -34.0$ ($c = 0.17$, MeOH). UV (MeOH): 206 (4.08), 258 (4.12), 357 (2.87). IR (KBr): 3423, 1627, 1690, 1585. ^{1}H- and ^{13}C-NMR: see *Table 2*. ESI-MS (pos.): 661.2 ($[M + Na]^+$). HR-ESI-MS: 661.1749 ($[M+Na]^+$, $C_{29}H_{34}NaO_{16}^+$; calc. 661.1745).

Hedanthroside E (= *Methyl 3-[(6-*O*-β-*D*-Glucopyranosyl-β-*D*-glucopyranosyl)oxy]-9,10-dihydro-1-hydroxy-9,10-dioxoanthracene-2-carboxylate*; **5**). Yellow amorphous solid. $[\alpha]_D^{23} = -32.0$ ($c = 0.20$, MeOH). UV (MeOH): 207 (3.83), 265 (4.15), 354 (2.69). IR (KBr): 3372, 1731, 1673, 1633, 1591. ^{1}H- and ^{13}C-NMR: see *Table 1*. ESI-MS (pos.): 645.2 ($[M + Na]^+$). ESI-MS (neg.): 621.9 ($[M - H]^-$). HR-ESI-MS: 645.1443 ($[M+Na]^+$, $C_{28}H_{30}NaO_{16}^+$; calc. 645.1432).

Hediridoside A (= rel-*(1*R*,4a*R*,5*S*,7a*R*)-7-[[(Furan-2-ylcarbonyl)oxy]methyl]-1-(β-*D*-glucopyranosyloxy)-1,4a,5,7a-tetrahydro-5-hydroxycyclopenta[*c*]pyran-4-carboxylic Acid*; **6**). White amorphous powder. $[\alpha]_D^{20} = -12.7$ ($c = 0.15$, H_2O). UV (MeOH): 259 (3.91). IR (KBr): 3444, 1641, 1384, 1078. ^{1}H- and ^{13}C-NMR: see *Table 3*. ESI-MS (pos.): 507.1 ($[M + Na]^+$). ESI-MS (neg.): 483.5 ($[M - H]^-$). HR-ESI-MS: 507.1104 ($[M+Na]^+$, $C_{21}H_{24}NaO_{13}^+$; calc. 507.1115).

Hediridoside B (= rel-*(1*R*,4a*R*,7a*R*)-1,4a,5,7a-Tetrahydro-4-(methoxycarbonyl)-1-[(6-*O*-α-*L*-rhamnopyranosyl-β-*D*-glucopyranosyl)oxy]cyclopenta[*c*]pyran-7-carboxylic Acid*; **7**). White amorphous powder. $[\alpha]_D^{20} = -4.0$ ($c = 0.125$, MeOH), UV (MeOH): 238 (3.62). IR (KBr): 3446, 1633. ^{1}H- and ^{13}C-NMR: see *Table 4*. ESI-MS (pos.): 571.2 ($[M + Na]^+$). ESI-MS (neg.): 547.5 ($[M - H]^-$). HR-ESI-MS: 571.1660 ($[M+Na]^+$, $C_{23}H_{32}NaO_{15}^+$; calc. 571.1639).

Enzymatic Hydrolysis of **1**. Compound **1** (15 mg) was dissolved in H_2O (10 ml), β-cellulase (15 mg) was added, and the soln. was kept at 37° for 2 d. The mixture was extracted with AcOEt, and the aq. phase was compared with an authentic sugar sample by co-TLC (AcOEt/MeOH/H_2O/AcOH 13 : 3 : 3 : 4; R_f 0.40 for glucose). Identification of D-glucose was carried out by comparing the optical rotation of the liberated glucose with that of an authentic sample of D-glucose ($[\alpha]_D^{20} = +52$).

Acidic Hydrolysis of **7**. A soln. of compound **7** (8 mg) in 5% H_2SO_4/EtOH was refluxed for 3 h. The mixture was neutralized and concentrated *in vacuo* to remove the alcohol, and extracted with AcOEt. The aq. layer was evaporated and separated over a C_8 and a *NH*-SiO_2 column. Glucose and rhamnose were separated, and compared with authentic samples by co-TLC (AcOEt/MeOH/H_2O/AcOH 13 : 3 : 3 : 4, R_f 0.44 for glucose and 0.62 for rhamnose). Identification of D-glucose and L-rhamnose was carried out by comparing the optical rotations of the liberated glucose and rhamnose with those of authentic samples of D-glucose ($[\alpha]_D^{20} = +52$) and L-rhamnose ($[\alpha]_D^{20} = +7.2$).

Bioassay. A HEK293 cell line was stably transfected with a κB-luciferase reporter gene. For the luciferase assay, the cells were seeded into 24-well plate at *ca.* 90% confluency and were pretreated with the tested compounds (10 μm) for 1 h. After stimulation of the cells by TNF-α (25 IU/ml) for 5 h, equal cell numbers were collected for the assay, and the luciferase activity was measured by a luminometer using a luciferase assay system (*Promega*, Shanghai, P. R. China).

REFERENCES

[1] R. J. Wang, N. X. Zhao, *J. Trop. Subtrop. Bot.* **2001**, *9*, 219.
[2] Q. M. Yuan, G. F. Zhang, X. Wang, B. Z. Zheng, *J. Yunnan Univ.* **2004**, *26* (suppl.), 166.

[3] J. Y. Si, D. H. Chen, R. L. Pan, X. H. Zhao, *Nat. Prod. Res. Dev.* **2007**, *19*, 517.
[4] Delectis Florae Reipublicae Popularis Sinicae, Agendae Academiae Sinicae Edita, Flora Reipublicae Popularis Sinicae, Beijing, Science Press, 1999, vol. 71, pp. 6–86.
[5] J. N. Peng, X. Z. Feng, X. T. Liang, *Chin. Tradit. Herbal Drugs* **1997**, *28(A10)*, 45.
[6] J.-N. Peng, X.-Z. Feng, X.-T. Liang, *Phytochemistry* **1998**, *47*, 1657.
[7] G. C. H. Derksen, H. A. G. Niederländer, T. A. van Beek, *J. Chromatogr., A* **2002**, *978*, 119.
[8] D.-H. Kim, H.-J. Lee, Y.-J. Oh, M.-J. Kim, S.-H. Kim, T.-S. Jeong, N.-I. Beek, *Arch. Pharmacal Res.* **2005**, *28*, 1156.
[9] K. Kamiya, Y. Tanaka, H. Endang, M. Umar, T. Satake, *Chem. Pharm. Bull.* **2005**, *53*, 1597.
[10] C. Li, X. Xue, D. Zhou, F. Zhang, Q. Xu, L. Ren, X. Liang, *J. Pharm. Biomed. Anal.* **2008**, *48*, 205.
[11] Z. Guvenalp, N. Kilic, C. Kazaz, Y. Kaya, L. Ö. Demirezer, *Turk. J. Chem.* **2006**, *30*, 515.
[12] K. Likhitwitayawuid, S. Dej-adisai, V. Jonbunprasert, J. Krungkrai, *Planta Med.* **1999**, *65*, 754.
[13] B. M. Santoyo, R. Herrera, R. Aguilar, A. Fuentes-Benites, F. Jiménez, J. Tamariz, *J. Mex. Chem. Soc.* **2007**, *51*, 198.
[14] W. Jiang, L.-S. Kuang, A.-J. Hou, M. Qian, J.-Z. Li, *Helv. Chim. Acta* **2007**, *90*, 1296.
[15] F. Folmer, W. T. A. Harrison, J. N. Tabudravu, M. Jaspars, W. Aalbersberg, K. Feussner, A. D. Wright, M. Dicato, M. Diederich, *J. Nat. Prod.* **2008**, *71*, 106.

Received July 19, 2010