

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

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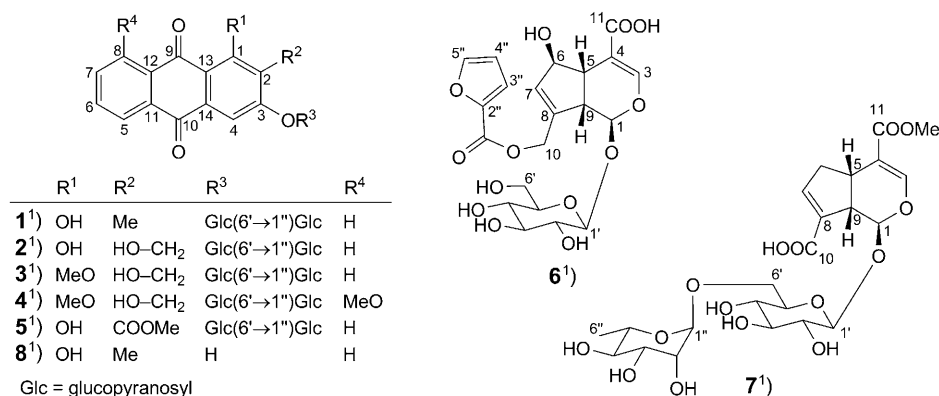
Five new anthraquinone glycosides, hedanthrosides A–E (**1–5**, resp.) and two new iridoid glycosides, hediridosides A and B (**6** and **7**, resp.), along with two known anthraquinones and four known iridoids, were isolated from the stems of *Hedyotis hedyotidea* (DC.) MERR. The structures of the new 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- κ B induced by the NF- κ B-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 CHCl₃ and H₂O. 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 H₂O fraction, and the CHCl₃-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



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 C₂₇H₃₀O₁₄ by means of HR-ESI-MS (*m/z* 601.1539 ([*M* + Na]⁺)). The aromatic region of the ¹H-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 unsubstituted and one trisubstituted ring. Other ¹H-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 ¹³C-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. hedyotideae*.

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 [*α*]_D²⁰ = +42.3 (*c* = 0.90, H₂O), 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-glucopyranoside¹⁾, named **hedanthroside A**.

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

Table 1. ^1H - and ^{13}C -NMR Data ((D₆)DMSO) for **1**, **2**, and **5**¹). δ in ppm, J in Hz.

1		2		5	
	$\delta(\text{H})$	$\delta(\text{C})$	$\delta(\text{H})$	$\delta(\text{C})$	$\delta(\text{H})$
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 (<i>s</i>)	105.9	7.47 (<i>s</i>)	106.4	7.50 (<i>s</i>)
H–C(5)	8.18 (<i>dd</i> , $J = 6.3, 2.1$)	126.8	8.17 (<i>dd</i> , $J = 6.7, 2.1$)	127.0	8.18–8.22 (<i>m</i>)
H–C(6)	7.90–7.95 (<i>m</i>)	134.7	7.90–7.95 (<i>m</i>)	134.8	7.92–7.99 (<i>m</i>)
H–C(7)	7.90–7.95 (<i>m</i>)	134.6	7.90–7.95 (<i>m</i>)	134.9	7.92–7.99 (<i>m</i>)
H–C(8)	8.22 (<i>dd</i> , $J = 7.1, 2.0$)	126.4	8.17 (<i>dd</i> , $J = 7.0, 2.0$)	126.6	8.22–8.27 (<i>m</i>)
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 HOCH ₂ –C(2)	2.19 (<i>s</i>)	8.4	4.65 (<i>d</i> , $J = 11.4$), 4.56 (<i>d</i> , $J = 11.4$)	50.9	
MeOOC–C(2)				3.86 (<i>s</i>)	52.6, 163.7
Glc'					
H–C(1')	5.18 (<i>d</i> , $J = 6.6$)	100.2	5.15 (<i>d</i> , $J = 6.8$)	100.8	5.30 (<i>d</i> , $J = 7.6$)
H–C(2')	3.36–3.38 (<i>m</i>)	76.0	3.77–3.82 (<i>m</i>)	75.6	3.36–3.38 (<i>m</i>)
H–C(3')	3.62–3.65 (<i>m</i>)	75.6	3.30–3.50 (<i>m</i>)	69.8	3.66–3.69 (<i>m</i>)
H–C(4')	3.36–3.38 (<i>m</i>)	73.1	3.00–3.20 (<i>m</i>)	73.5	3.22–3.27 (<i>m</i>)
H–C(5')	3.36–3.40 (<i>m</i>)	69.0	3.30–3.50 (<i>m</i>)	68.9	3.33–3.39 (<i>m</i>)
CH ₂ (6')	4.00 (<i>d</i> , $J = 9.6$), 3.65 (<i>d</i> , $J = 9.6$)	68.0	4.02 (<i>d</i> , $J = 9.8$), 3.79 (<i>d</i> , $J = 9.8$)	68.0	3.99 (<i>d</i> , $J = 9.5$), 3.65 (<i>d</i> , $J = 9.5$)
Glc''					
H–C(1'')	4.18 (<i>d</i> , $J = 7.5$)	103.4	4.19 (<i>d</i> , $J = 7.4$)	103.5	4.15 (<i>d</i> , $J = 7.6$)
H–C(2'')	2.98–3.00 (<i>m</i>)	76.8	3.00–3.20 (<i>m</i>)	76.9	3.07 (<i>m</i>)
H–C(3'')	3.03–3.07 (<i>m</i>)	76.6	3.00–3.20 (<i>m</i>)	73.4	3.07 (<i>m</i>)
H–C(4'')	2.94–2.98 (<i>m</i>)	73.4	3.00–3.20 (<i>m</i>)	76.5	2.98 (<i>m</i>)
H–C(5'')	3.02–3.06 (<i>m</i>)	69.9	3.30–3.50 (<i>m</i>)	73.2	3.08 (<i>m</i>)
CH ₂ (6'')	3.60 (<i>d</i> , $J = 10.7$), 3.42 (<i>d</i> , $J = 10.7$)	60.9	3.60 (<i>d</i> , $J = 11.6$), 3.38 (<i>d</i> , $J = 11.6$)	61.0	3.62 (<i>d</i> , $J = 10.3$), 3.44 (<i>d</i> , $J = 10.3$)

Compound **2**, an orange amorphous powder, displayed a sodiated molecular-ion peak at m/z 617.1482 ($[M + \text{Na}]^+$) in its HR-ESI-MS, corresponding to the molecular formula $\text{C}_{27}\text{H}_{30}\text{O}_{15}$, 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 CH₂ H-atoms represented by two *doublets* ($\delta(\text{H})$ 4.56 (*d*, $J = 11.4$), and $\delta(\text{H})$ 4.65 (*d*, $J = 11.4$)), with the disappearance of the *singlet* peak of Me H-atoms. Correlations of the CH₂ 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 \rightarrow 6)- β -D-glucopyranoside¹), named **hedanthroside B**.

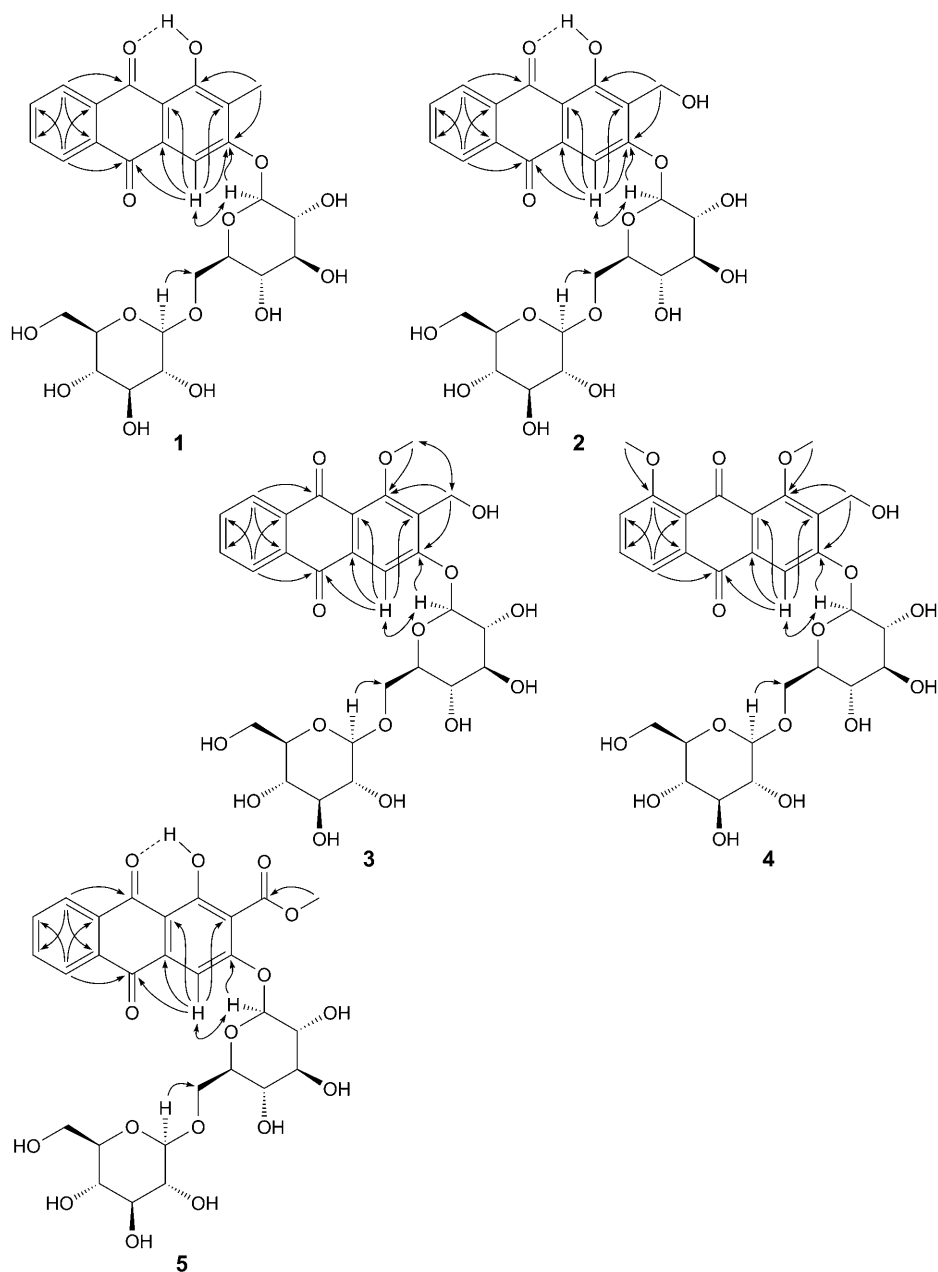


Fig. 1. Key *HMBC* ($H \rightarrow C$) and *ROESY* ($H \leftrightarrow H$) correlations of **1–5**

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 ^1H - and ^{13}C -NMR spectra (Table 2) displayed MeO signals at $\delta(\text{H})$ 3.68 (*s*) and $\delta(\text{C})$ 63.0. The 3J correlation (Fig. 1) observed between the MeO H-atoms ($\delta(\text{H})$ 3.68) and C(1) ($\delta(\text{C})$ 160.4) supported the location of the MeO moiety at C(1). Other HMBs 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 \rightarrow 6)- β -D-glucopyranoside¹), named **hedanthroside C**.

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

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

3 ^a)		4 ^b)	
	$\delta(\text{H})$	$\delta(\text{C})$	$\delta(\text{C})$
C(1)		160.4	161.7
C(2)		130.6	132.4
C(3)		160.4	161.5
H-C(4)	6.96 (<i>s</i>)	109.3	7.64 (<i>s</i>)
H-C(5)	7.20–7.30 (<i>m</i>)	126.4	7.62 (<i>d</i> , <i>J</i> = 6.8)
H-C(6)	7.20–7.30 (<i>m</i>)	134.2	7.66 (<i>dd</i> , <i>J</i> = 7.6, 6.8)
H-C(7)	7.28–7.33 (<i>m</i>)	135.4	7.42 (<i>d</i> , <i>J</i> = 7.6)
C(8)	7.38–7.48 (<i>m</i>)	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
MeO-C(1)	3.68 (<i>s</i>)	63.0	3.84 (<i>s</i>)
CH ₂ -C(2)	4.65 (<i>d</i> , <i>J</i> = 11.4), 4.56 (<i>d</i> , <i>J</i> = 11.4)	52.5	4.54 (<i>d</i> , <i>J</i> = 11.4), 4.56 (<i>d</i> , <i>J</i> = 11.4)
MeO-C(8)			3.82 (<i>s</i>)
Glc'			
H-C(1')	5.06 (<i>d</i> , <i>J</i> = 6.7)	100.1	5.28 (<i>d</i> , <i>J</i> = 6.7)
H-C(2')	3.28–3.32 (<i>m</i>)	76.3	3.38–3.44 (<i>m</i>)
H-C(3')	3.50–3.55 (<i>m</i>)	75.6	3.65–3.70 (<i>m</i>)
H-C(4')	3.50–3.55 (<i>m</i>)	73.5	3.64–3.68 (<i>m</i>)
H-C(5')	3.50–3.55 (<i>m</i>)	69.1	3.84–3.90 (<i>m</i>)
CH ₂ (6')	4.04 (<i>d</i> , <i>J</i> = 9.5), 4.32 (<i>d</i> , <i>J</i> = 9.5)	68.2	4.30 (<i>d</i> , <i>J</i> = 10.1), 3.97 (<i>d</i> , <i>J</i> = 10.1)
Glc''			
H-C(1'')	4.52 (<i>d</i> , <i>J</i> = 6.4)	103.2	4.46 (<i>d</i> , <i>J</i> = 7.6)
H-C(2'')	3.50–3.55 (<i>m</i>)	76.1	3.34–3.38 (<i>m</i>)
H-C(3'')	3.50–3.55 (<i>m</i>)	75.7	3.62–3.70 (<i>m</i>)
H-C(4'')	3.30–3.55 (<i>m</i>)	73.0	3.34–3.42 (<i>m</i>)
H-C(5'')	3.30–3.55 (<i>m</i>)	70.0	3.30–3.34 (<i>m</i>)
CH ₂ (6'')	3.75 (<i>d</i> , <i>J</i> = 11.3), 3.90 (<i>d</i> , <i>J</i> = 11.3)	61.2	3.92 (<i>d</i> , <i>J</i> = 10.5), 3.71 (<i>d</i> , <i>J</i> = 10.5)

^a) Recorded in D₂O. ^b) Recorded in CD₃OD and D₂O.

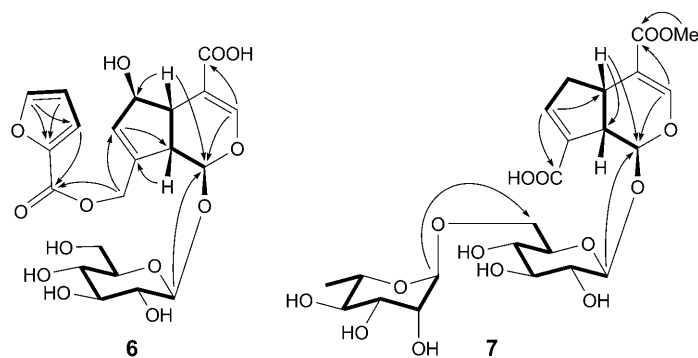
$J = 7.6, 6.8$), 7.62 ($d, J = 6.8$), and 7.42 ($d, J = 7.6$), and a 1-H *singlet* at $\delta(\text{H})$ 7.64, suggested that ring *A* is monosubstituted with a trisubstituted ring *C*. Presence of two MeO groups was evident from signals at $\delta(\text{H})$ 3.84 ($s, \text{H}-\text{C}(1a)$) and 3.82 ($s, \text{H}-\text{C}(8a)$). The HMBCs from $\text{H}-\text{C}(4)$ ($\delta(\text{H})$ 7.64) and $\text{H}-\text{C}(5)$ ($\delta(\text{H})$ 7.62) to the $\text{C}(10)=\text{O}$ group ($\delta(\text{C})$ 184.7), as well as the presence of the cross-peaks between both of the two MeO groups and the $\text{C}(9)=\text{O}$ group ($\delta(\text{C})$ 183.8) confirmed that two of the MeO groups were located at $\text{C}(1)$ and $\text{C}(8)$, 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 \rightarrow 6)- β -D-glucopyranoside¹, named **hedanthroside D**, for **4**.

Compound **5**, obtained as a yellow amorphous solid, was analyzed for $\text{C}_{28}\text{H}_{30}\text{O}_{16}$ by means of HR-ESI-MS (m/z 645.1443 ($[M + \text{Na}]^+$)). The aromatic region of the ^1H -NMR spectrum (*Table 1*) was similar to that of **1**, revealing a non-substituted ring *A* and a trisubstituted ring *C*. Comparison of the ^1H - and ^{13}C -NMR chemical shifts of **5** with those of **1** further revealed the presence of an ester CO group ($\delta(\text{C})$ 163.7) and a MeO group ($\delta(\text{H})$ 3.86) in **5** instead of a Me group in **1**. The HMBC spectrum showed correlations from the MeO H-atoms to ester CO ($\delta(\text{C})$ 163.7), and from $\text{H}-\text{C}(4)$ ($\delta(\text{C})$ 7.50) to $\text{C}(2)$ ($\delta(\text{C})$ 117.0), $\text{C}(13)$ ($\delta(\text{C})$ 111.1), $\text{C}(10)$ ($\delta(\text{C})$ 181.2), and $\text{C}(3)$ ($\delta(\text{C})$ 159.8). Comparing ^1H - 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 $\text{C}(2)$ 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 \rightarrow 6)- β -D-glucopyranoside¹, named **hedanthroside E**.

Optically active compound **6**, $[\alpha]_{\text{D}}^{20} = -12.7$ ($c = 0.15$, H_2O), was isolated as an almost white amorphous powder, with the molecular formula $\text{C}_{21}\text{H}_{24}\text{O}_{13}$ determined by means of HR-ESI-MS (m/z 507.1104 ($[M + \text{Na}]^+$)). 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 ^1H - and ^{13}C -NMR spectra of **6** (*Table 3*) showed signals of a furan-2-carboxy group ($\delta(\text{H})$ 6.74 ($dd, J = 3.5, 0.8$), 7.48 ($d, J = 3.5$), and 7.85 ($d, J = 0.8$); $\delta(\text{C})$ 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 $^1\text{H}, ^1\text{H}$ -COSY correlations (*Fig. 2*) from $\text{H}-\text{C}(5'')$ through $\text{H}-\text{C}(4'')$ to $\text{H}-\text{C}(3'')$, in combination

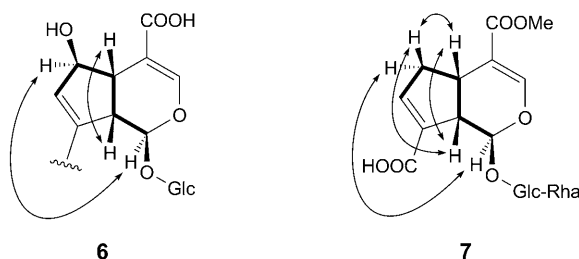
Table 3. ^1H - and ^{13}C -NMR Data (D_2O) of Compound **6**¹. δ in ppm, J in Hz.

	$\delta(\text{H})$	$\delta(\text{C})$		$\delta(\text{H})$	$\delta(\text{C})$
$\text{H}-\text{C}(1)$	5.29 ($d, J = 6.7$)	99.6	$\text{H}-\text{C}(1')$	4.85 ($d, J = 6.8$)	101.3
$\text{H}-\text{C}(3)$	7.28 (s)	150.9	$\text{H}-\text{C}(2')$	3.32–3.38 (m)	75.4
$\text{C}(4)$		117.4	$\text{H}-\text{C}(3')$	3.53–3.57 (m)	78.3
$\text{H}-\text{C}(5)$	3.14 ($dd, J = 4.7, 6.6$)	47.5	$\text{H}-\text{C}(4')$	3.42–3.46 (m)	72.2
$\text{H}-\text{C}(6)$	4.67 ($d, J = 4.6$)	83.5	$\text{H}-\text{C}(5')$	3.44–3.48 (m)	78.8
$\text{H}-\text{C}(7)$	6.09 (s)	134.7	$\text{CH}_2(6')$	3.89 ($dd, J = 12.0, 1.4$), 3.72 ($dd, J = 12.0, 5.6$)	63.4
$\text{C}(8)$		143.0	$\text{C}(2'')$		146.1
$\text{H}-\text{C}(9)$	3.23 ($dd, J = 7.1, 6.7$)	48.9	$\text{H}-\text{C}(3'')$	7.48 ($d, J = 3.5$)	122.2
$\text{CH}_2(10)$	5.13 (s)	65.6	$\text{H}-\text{C}(4'')$	6.74 ($dd, J = 3.5, 0.8$)	115.1
$\text{C}(11)$		178.3	$\text{H}-\text{C}(5'')$	7.85 ($d, J = 0.8$)	150.6
			$\text{C}=\text{O}$		162.8

Fig. 2. Key HMBC ($H \rightarrow C$) and $^1H,^1H$ -COSY (\rightarrow) 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 $C(1)$ ($\delta(C)$ 99.6). The furan-2-carboxy group was at $C(10)$, corroborated by means of the HMBC between $CH_2(10)$ ($\delta(H)$ 5.13) and $C=O$ ($\delta(C)$ 162.8). From these data, the structure of **6** was elucidated as *rel*-(1*R*,4*aR*,5*S*,7*aR*)-7-[(furan-2-yl-carboxy)methyl]-1-(β -D-glucopyranosyloxy)-5-hydroxy-1,4*a*,5,7*a*-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 1H - and ^{13}C -NMR spectra (Table 4) of **7** not only showed signals characteristic for an iridoid skeleton at $\delta(H)$ 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 $\delta(C)$ 98.0 ($C(1)$), 155.0 ($C(3)$), and 114.7 ($C(4)$), but also displayed additional glucopyranosyl and rhamnopyranosyl resonances ($\delta(H)$ 5.05 ($H-C(1')$), 4.85 ($H-C(1'')$), 1.36 (*d*, $J = 6.2$, $H-C(6'')$); $\delta(C)$ 101.0 ($C(1')$), 103.7 ($C(1'')$), 19.3 ($C(6'')$)). Different from most iridoid glycosides

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

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

obtained from the *Hedyotis* genus, compound **7** had a C(10)OOH ($\delta(\text{C})$ 176.1), and not a HO–CH₂(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 $[\alpha]_{\text{D}}^{20} = +42.3$ ($c = 0.90$, H_2O) and $[\alpha]_{\text{D}}^{20} = +6.3$ ($c = 1.10$, 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-rhamnopyranosyl- 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 TNF α -induced transcriptional

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

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Experimental Part

General. Column chromatography (CC): silica gel *H* (200–300 mesh; *Qingdao Marine Chemical Ltd.*, Qingdao, P. R. China), *Chromatorex C₁₈* and *C₈* (20–45 μ m; *Fuji Silysia Chemical Ltd.*), *NH-silica* gel (20–45 μ m; *Fuji Silysia Chemical Ltd.*), *MCI* gel *CHP-20P* (75–150 μ m; *Mitsubishi Chemical Industries Co., Ltd.*), *TSK* gel *Toyopearl HW-40F* (30–60 μ m; *Toso Co., Ltd.*), *Sephadex LH-20* (20–80 μ m; *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 \times 20 mm, S-5 μ m, 12 nm) column. TLC: Precoated *SiO₂ GF₂₅₄* 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 ¹H and 100 MHz for ¹³C. LR- and HR-ESI-MS: *Finnigan LCQ-DECA* and *Waters Micromass Q-TOF Ultima Globe* spectrometer, resp.

Plant Material. The stems of *Hedyotis hedyotideae* (Rubiaceae) were collected from Ningming, in Guangxi Province, P. R. 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 hedyotideae* (1 kg) were collected and powered. The material was extracted three times with aq. acetone (70%) at r.t. (10 l, 3 \times , each for 1 week). Solvent was removed under reduced pressure to obtain a crude extract (98 g), which was suspended in H₂O and partitioned with CHCl₃. The H₂O fraction (78 g) was subjected to CC (*MCI* gel; H₂O, 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/H₂O 0 to 20%) to afford two fractions, *Frs. A1–A2*. *Fr. A1* (0.9 g) was subjected to CC (*C₈*; MeOH/H₂O 5 to 10%) to give compound **6** (17 mg). *Fr. A2* (1.3 g) was subjected to CC (*HW-40F*; MeOH/H₂O 5 to 15%) to afford scandoside (26 mg). Isolation of *Fr. B* (13 g) by repeated CC (*C₈*; MeOH/H₂O 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/H₂O, 15 to 50%) finally was separated by *HW-40F* (MeOH/H₂O 10 to 40%) to afford two fractions, *Frs. C1–C2*. *Fr. C1* (1.1 g) was purified by CC (*C₈*; MeOH/H₂O 15 to 25%) to give methyl deacetylasperulosidate (34 mg). *Fr. C2* (0.9 g) was passed through a *C₁₈* column with MeOH/H₂O (30 to 45%) to afford asperulosidic acid methyl ester (35 mg). *Fr. D* (9.9 g) was submitted to CC (*C₈*; MeOH/H₂O 35 to 42%), followed by *HW-40F* CC (MeOH/H₂O 32%), and further separation by RP-HPLC (*YMC-Pack ODS-A* (250 \times 20 mm); MeOH/H₂O 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/H₂O 50%) to afford compound **2** (100 mg) and *Fr. E2*, which was purified by RP-HPLC (MeOH/H₂O 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 CHCl₃ fraction (18 g) was subjected to CC (*SiO₂*; petroleum ether (PE)/Me₂CO 35:1 \rightarrow 5:1) to give three fractions, *Frs. a–c*. *Fr. c* was concentrated and subjected to CC (*SiO₂*; CHCl₃/AcOEt 10:1) and repeated prep. TLC (CHCl₃/Me₂CO 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. $[\alpha]_D^{25} = -37.0$ ($c = 0.20$, MeOH). UV (MeOH): 208 (4.00), 268 (4.05), 353 (3.13). IR (KBr): 3363, 1668, 1631, 1591. ¹H- and ¹³C-NMR: see Table 1. ESI-MS (pos.): 601.2 ($[M + Na]^+$). HR-ESI-MS: 601.1539 ($[M + Na]^+$, C₂₇H₃₀NaO₁₄; 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. ^1H - and ^{13}C -NMR: see Table 1. ESI-MS (pos.): 617.3 ($[M + \text{Na}]^+$). HR-ESI-MS: 617.1482 ($[M + \text{Na}]^+$, $\text{C}_{27}\text{H}_{30}\text{NaO}_{15}$; 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. ^1H - and ^{13}C -NMR: see Table 2. ESI-MS (pos.): 631.2 ($[M + \text{Na}]^+$). HR-ESI-MS: 631.1637 ($[M + \text{Na}]^+$, $\text{C}_{28}\text{H}_{32}\text{NaO}_{15}$; 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. ^1H - and ^{13}C -NMR: see Table 2. ESI-MS (pos.): 661.2 ($[M + \text{Na}]^+$). HR-ESI-MS: 661.1749 ($[M + \text{Na}]^+$, $\text{C}_{29}\text{H}_{34}\text{NaO}_{16}$; 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. ^1H - and ^{13}C -NMR: see Table 1. ESI-MS (pos.): 645.2 ($[M + \text{Na}]^+$). ESI-MS (neg.): 621.9 ($[M - \text{H}]^-$). HR-ESI-MS: 645.1443 ($[M + \text{Na}]^+$, $\text{C}_{28}\text{H}_{30}\text{NaO}_{16}$; calc. 645.1432).

Hediridoside A (= rel-(1R,4aR,5S,7aR)-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. ^1H - and ^{13}C -NMR: see Table 3. ESI-MS (pos.): 507.1 ($[M + \text{Na}]^+$). ESI-MS (neg.): 483.5 ($[M - \text{H}]^-$). HR-ESI-MS: 507.1104 ($[M + \text{Na}]^+$, $\text{C}_{21}\text{H}_{24}\text{NaO}_{13}$; calc. 507.1115).

Hediridoside B (= rel-(1R,4aR,7aR)-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. ^1H - and ^{13}C -NMR: see Table 4. ESI-MS (pos.): 571.2 ($[M + \text{Na}]^+$). ESI-MS (neg.): 547.5 ($[M - \text{H}]^-$). HR-ESI-MS: 571.1660 ($[M + \text{Na}]^+$, $\text{C}_{23}\text{H}_{32}\text{NaO}_{15}$; 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% $\text{H}_2\text{SO}_4/\text{EtOH}$ 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 $\text{TNF-}\alpha$ (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).

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