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 $3-O-\beta$ -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

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

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 ¹H-NMR spectrum (*Table 1*) indicated the presence of five aromatic H-atoms. Their multiplicity, an AABB system at $\delta(H)$ 8.22 (dd, J=7.1, 2.0), $\delta(H)$ 8.18 (dd, J= $(6.3, 2.1), \delta(H)$ 7.90 – 7.95 (m, 2 H), and a 1-H singlet at $\delta(H)$ 7.48, were suggestive of an anthraquinone with one nonsubstituted and one trisubstituted ring. Other ¹H-NMR signals were a *singlet* of a Me group at $\delta(H)$ 2.19, and characteristic signals of two β glucopyranosyl units with the anomeric H-atoms at $\delta(H)$ 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 Hatom ($\delta(H)$ 7.48) to the non-chelated C(10)=O group ($\delta(C)$ 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, 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 \rightarrow 6)- β -D-glucopyranoside¹), named hedanthroside A.

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

	1		2		5	
	$\delta(H)$	$\delta(C)$	$\delta(H)$	$\delta(C)$	$\delta(H)$	$\delta(C)$
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 (<i>m</i>)	127.0
H–C(6)	7.90 - 7.95(m)	134.7	7.90–7.95 (<i>m</i>)	134.8	7.92–7.99 (<i>m</i>)	134.9
H-C(7)	7.90 - 7.95(m)	134.6	7.90–7.95 (<i>m</i>)	134.9	7.92–7.99 (<i>m</i>)	134.7
H–C(8)	8.22 (dd, J = 7.1, 2.0)	126.4	8.17 (<i>dd</i> , <i>J</i> = 7.0, 2.0)	126.6	8.22-8.27 (<i>m</i>)	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	2.19 (s)	8.4	4.65 (d, J = 11.4),	50.9		
$HOCH_2-C(2)$			4.56 (d, J = 11.4)			
MeOOC-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 (<i>m</i>)	76.0	3.77 - 3.82 (m)	75.6	3.36–3.38 (<i>m</i>)	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 (<i>m</i>)	73.1	3.00 - 3.20 (m)	73.5	3.22-3.27 (<i>m</i>)	72.9
H–C(5′)	3.36 - 3.40(m)	69.0	3.30 - 3.50(m)	68.9	3.33–3.39 (<i>m</i>)	68.9
$CH_{2}(6')$	4.00 (d, J = 9.6),	68.0	4.02 (d, J = 9.8),	68.0	3.99(d, J = 9.5),	67.9
	3.65 (d, J = 9.6)		3.79 (d, J = 9.8)		3.65 (d, J = 9.5)	
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 (<i>m</i>)	76.8
H–C(3")	3.03 - 3.07(m)	76.6	3.00 - 3.20 (m)	73.4	3.07 (<i>m</i>)	76.5
H–C(4")	2.94 - 2.98(m)	73.4	3.00 - 3.20 (m)	76.5	2.98 (<i>m</i>)	73.4
H–C(5")	3.02 - 3.06(m)	69.9	3.30 - 3.50(m)	73.2	3.08 (<i>m</i>)	69.9
CH ₂ (6")	3.60 (d, J = 10.7),	60.9	3.60 (d, J = 11.6),	61.0	3.62(d, J = 10.3),	60.9
	3.42 (d, J = 10.7)		3.38 (d, J = 11.6)		3.44 (d, J = 10.3)	

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

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 CH₂ H-atoms represented by two *doublets* ($\delta(H)$ 4.56 (d, J = 11.4), and $\delta(H)$ 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- $O-\beta$ -D-glucopyranosyl-($1 \rightarrow 6$)- β -D-glucopyranoside¹), named hedanthroside B.



Fig. 1. Key HMBC $(\mathrm{H}\,{\rightarrow}\,\mathrm{C})$ and ROESY $(\mathrm{H}\,{\leftrightarrow}\,\mathrm{H})$ 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 ¹³C-NMR spectra (*Table 2*) displayed MeO signals at $\delta(H)$ 3.68 (*s*) and $\delta(C)$ 63.0. The ³J correlation (*Fig. 1*) observed between the MeO H-atoms ($\delta(H)$ 3.68) and C(1) ($\delta(C)$ 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-glucopyrano-syl-($1 \rightarrow 6$)- β -D-glucopyranoside¹), 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 ¹H-NMR spectrum (*Table 2*) indicated the presence of four aromatic H-atoms. Their multiplicity, an *ABC* system at $\delta(H)$ 7.66 (*dd*,

	3 ^a)	4 ^b)		
	$\delta(H)$	$\delta(C)$	$\delta(H)$	$\delta(C)$
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
MeO-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
MeO-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 <i>(m)</i>	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 <i>(m)</i>	73.0	3.34–3.42 <i>(m)</i>	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 ₂ O. ^b) Recorded in CD ₃ OD and	$D_2O.$		

Table 2. ¹H- and ¹³C-NMR Data for Compounds 3 and 4¹). δ in ppm, J in Hz.

J=7.6, 6.8), 7.62 (d, J=6.8), and 7.42 (d, J=7.6), and a 1-H singlet at $\delta(H)$ 7.64, suggested that ring A is monosubstituted with a trisubstituted ring C. Presence of two MeO groups was evident from signals at $\delta(H)$ 3.84 (s, H–C(1a)) and 3.82 (s, H–C(8a))). The HMBCs from H–C(4) ($\delta(H)$ 7.64) and H–C(5) ($\delta(H)$ 7.62) to the C(10)=O group ($\delta(C)$ 184.7), as well as the presence of the cross-peaks between both of the two MeO groups and the C(9)=O group ($\delta(C)$ 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- $O-\beta$ -D-glucopyranoside¹), 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 ¹H-NMR spectrum (*Table 1*) was similar to that of **1**, revealing a non-substituted ring Aand a trisubstituted ring C. Comparison of the ¹H- and ¹³C-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 ¹H- and ¹³C-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 1,3dihydroxy-2-(methoxycarbonyl)-9,10-anthraquinone 3-*O*- β -D-glucopyranosyl(1 \rightarrow 6)- β -D-glucopyranoside¹), named hedanthroside E.

Optically active compound **6**, $[a]_{D}^{20} = -12.7$ (c = 0.15, H₂O), was isolated as an almost white amorphous powder, with the molecular formula C₂₁H₂₄O₁₃ 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 ¹H- and ¹³C-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 ¹H, ¹H-COSY correlations (*Fig. 2*) from H–C(5'') through H–C(4'') to H–C(3''), in combination

	$\delta(H)$	$\delta(C)$		$\delta(H)$	$\delta(C)$
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 <i>(m)</i>	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 <i>(m)</i>	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

Table 3. ¹H- and ¹³C-NMR Data (D₂O) of Compound 6¹). δ in ppm, J in Hz.



Fig. 2. Key HMBC ($H \rightarrow C$) and ${}^{1}H, {}^{1}H-COSY$ (—) 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 ¹³C-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 CH₂(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*,4*aR*,5*S*,7*aR*)-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 ¹H- and ¹³C-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''))).

	$\delta(\mathrm{H})$	$\delta(C)$		$\delta(H)$	$\delta(C)$
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 , $J = 18.2, 7.5, 2.2, H_a$),	40.8	H-C(5')	3.40 - 3.44(m)	72.6
	2.88 (ddd , $J = 18.2, 7.5, 2.2, H_{\beta}$)				
H-C(7)	6.55 (d, J = 1.5)	142.8	$CH_{2}(6')$	4.06 (br. $d, J = 10.8$),	70.0
				3.78 (dd, J = 10.8, 5.6)	
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

Table 4. ¹*H*- and ¹³*C*-*NMR* Data (D₂O) of Compound **7**¹). δ in ppm, J in Hz.

obtained from the *Hedyotis* genus, compound **7** had a C(10)OOH (δ (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- $(1 \rightarrow 6)$ -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, H₂O) and $[\alpha]_{D}^{20} = +6.3$ (c = 1.10, H₂O), 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_a–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 group at C(10), found in the genus *Hedyotis*.

Since the plants from the genus *Hedyotis* were reported to possess antiinflammatory and anticancer activities, we evaluated the effects of compounds 1-7as 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 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 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 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. ($10 l, 3 \times$, 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 (C_8 ; 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 (C_8 ; 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 (C_8 ; MeOH/H₂O 15 to 25%) to give methyl deacetylasperulosidate (34 mg). Fr. C2 (0.9 g) was passed through a C_{18} column with MeOH/H₂O (30 to 45%) to afford asperulosidic acid methyl ester (35 mg). Fr. D (9.9 g) was submitted to CC (C_8 ; MeOH/H₂O 35 to 42%), followed by HW-40F CC (MeOH/H₂O 32%), and further separation by RP-HPLC (YMC-Pack ODS-A (250 × 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; $t_{\rm R}$ 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/ $Me_2CO 50:1$) to yield rubiadin (8; 12 mg).

Hedanthroside A (=9,10-*Dihydro-4-hydroxy-3-methyl-9*,10-*dioxoanthracen-2-yl* 6-O- β -D-*Glucopy-ranosyl-\beta-D-glucopyranoside*; **1**). Yellow amorphous powder. [a]_D²³ = -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-\beta*-D-*glucopyranoside*; **2**). Orange amorphous powder. [a]_D²³ = -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 ¹³C-NMR: see *Table 1*. ESI-MS (pos.): 617.3 ([M + Na]⁺). HR-ESI-MS: 617.1482 ([M + Na]⁺, C₂₇H₃₀NaO₁₅; calc. 617.1482).

Hedanthroside C (= 9,10-*Dihydro-3-(hydroxymethyl)-4-methoxy-9,10-dioxoanthracen-2-yl* 6-O- β -D-*Glucopyranosyl-\beta-D-glucopyranoside*; **3**). Yellow amorphous powder. [a]₂₀²² = -35.0 (c = 0.25, MeOH). UV (MeOH): 210 (3.99), 269 (4.15), 360 (3.00). IR (KBr): 3403, 1672, 1577. ¹H- and ¹³C-NMR: see *Table 2*. ESI-MS (pos.): 631.2 ([M + Na]⁺). HR-ESI-MS: 631.1637 ([M + Na]⁺, C₂₈H₃₂NaO₁₅; calc. 631.1639).

Hedanthroside D (=9,10-Dihydro-3-(hydroxymethyl)-4,5-dimethoxy-9,10-dioxoanthracen-2-yl 6β-D-Glucopyranosyl-β-D-glucopyranoside; **4**). Yellow amorphous powder. $[a]_{D}^{25} = -34.0$ (c = 0.17, MeOH). UV (MeOH): 206 (4.08), 258 (4.12), 357 (2.87). IR (KBr): 3423, 1627, 1690, 1585. ¹H- and ¹³C-NMR: see *Table 2*. ESI-MS (pos.): 661.2 ($[M + Na]^+$). HR-ESI-MS: 661.1749 ($[M + Na]^+$, C₂₉H₃₄NaO₁₆; 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. $[a]_{D}^{23} = -32.0$ (*c* = 0.20, MeOH). UV (MeOH): 207 (3.83), 265 (4.15), 354 (2.69). IR (KBr): 3372, 1731, 1673, 1633, 1591. ¹H- and ¹³C-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-(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. [α]^D_D = -12.7 (c = 0.15, H₂O). UV (MeOH): 259 (3.91). IR (KBr): 3444, 1641, 1384, 1078. ¹H- and ¹³C-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]⁺, C₂₁H₂₄NaO⁺₁₅; calc. 507.1115).

Hediridoside B (=rel-(*I*R,4*a*R,7*a*R)-*1*,4*a*,5,7*a*-*Tetrahydro-4*-(*methoxycarbonyl*)-*1*-[(6-O- α -L-*rhamnopyranosyl*- β -D-glucopyranosyl)oxy]cyclopenta[c]pyran-7-carboxylic Acid; **7**). White amorphous powder. [a]_D²⁰ = -4.0 (c = 0.125, MeOH), UV (MeOH): 238 (3.62). IR (KBr): 3446, 1633. ¹H- and ¹³C-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]⁺, C₂₃H₃₂NaO⁺₁₅; calc. 571.1639).

Enzymatic Hydrolysis of **1**. Compound **1** (15 mg) was dissolved in H₂O (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/H₂O/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 ($[a]_D^{20} = +52$).

Acidic Hydrolysis of 7 A soln. of compound 7 (8 mg) in 5% H₂SO₄/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-SiO₂ column. Glucose and rhamnose were separated, and compared with authentic samples by co-TLC (AcOEt/MeOH/H₂O/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 ($[\alpha]_D^{20} = +72$).

Bioassay. A HEK293 cell line was stably transfected with a κ B-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).

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