Iridoid Glycosides from *Hedyotis corymbosa*

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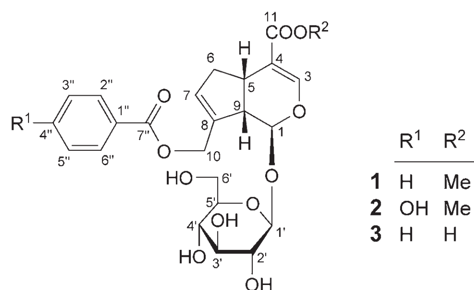
Three new iridoid glycosides, hedycorysides A–C (**1**–**3**, resp.), were isolated from the whole plant of *Hedyotis corymbosa* (LINN.) LAM., along with four known compounds. Their structures were elucidated by extensive 1D- and 2D-NMR analysis, as well as by HR-ESI-MS experiments. The three new compounds are the first benzoylated geniposide derivatives from *Hedyotis*.

Introduction. – Many *Hedyotis* species (Rubiaceae) are used in traditional Chinese medicine (TCM) for the treatment of appendicitis, tonsillitis, hepatitis, dysentery, snake bites, and bruising [1]. The chemical constituents of this genus include iridoid glycosides, triterpenoids, flavonoids, anthraquinones, coumarins, lignans, and alkaloids, some compounds exerting anti-inflammatory, neuroprotective, and cytotoxic effects [2]. *Hedyotis corymbosa* (LINN.) LAM. is an annual herb widely distributed in the southeast and southwest of China [3]. The whole plant is applied in clinic against malaria, intestinal abscess, boils, scald, and some kinds of tumors, such as gastric, esophageal, and colorectal carcinomas [3][4].

Some iridoid glycosides have been isolated from *H. corymbosa* previously [5][6], but the biological activities of these compounds were not investigated. Our phytochemical research on the BuOH-soluble fraction of the EtOH extract of the whole plant of *H. corymbosa* afforded three new iridoid glycosides, hedycorysides A–C (**1**–**3**, resp.), along with four known compounds, 10-*O*-benzoylscandoside methyl



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ester, 10-*O*-[(*E*)-*para*-coumaroyl]scandoside methyl ester, 10-*O*-(*para*-hydroxybenzoyl)scandoside methyl ester, and '10-*O*-benzoyl deacetyl asperulosidic acid methyl ester'. Herein, we report the structures of the new compounds, and the cytotoxicities of all seven isolates against various carcinoma cells.

Results and Discussion. – Hedycoryside A (**1**), an optically active compound ($[\alpha]_D^{24} = +13.2$), was isolated as a colorless, amorphous powder. Its molecular formula was deduced as $C_{24}H_{28}O_{11}$ by HR-ESI-MS (*m/z* 515.1522 ($[M+Na]^+$; calc. 515.1529)). The IR spectrum exhibited absorptions at 3371 cm^{-1} (OH), and at 1713 and 1633 cm^{-1} (α,β-unsaturated ester). The ^{1}H- and ^{13}C-NMR spectra of **1** (*Tables 1* and *2*, resp.) showed signals of a benzoyloxy (BzO) group [δ(H) 8.04 (*dd*, *J* = 1.3, 7.8 Hz, 2 H), 7.48 (*t*, *J* = 7.8 Hz, 2 H), 7.60 (br. *t*, *J* = 7.8 Hz, 1 H); δ(C) 131.8, 130.9, 129.9, 134.6, 168.2]. The anomeric signal at δ(H) 4.72 (*d*, *J* = 7.8 Hz, 1 H) and the signals in the region δ(H) 3.24 – 3.84, together with the relevant ^{13}C-NMR resonances, indicated the presence of a β-D-glucopyranosyl (Glc) unit. Furthermore, a MeO group at δ(C) 52.0, and the remaining ten ^{13}C-NMR signals, including C(1) and C(3) – C(11)[1]), were attributed to the iridoid skeleton of genipin [7].

Table 1. *^{1}H-NMR Data of* **1** – **3**. At 500 MHz; δ in ppm, *J* in Hz. Arbitrary atom numbering.

Atom	**1**[a])	**2**[b])	**3**[a])
H–C(1)	5.24 (*d*, *J* = 7.8)	5.25 (*d*, *J* = 7.5)	5.17 (*d*, *J* = 7.6)
H–C(3)	7.52 (br. *s*)	7.49 (br. *s*)	7.37 (br. *s*)
H–C(5)	3.24 (br. *t*, *J* = 8.0)	[c])	3.24 (br. *t*, *J* = 7.2)
H_α–C(6)	2.15 (*dd*, *J* = 8.0, 16.6)	2.14 (*dd*, *J* = 7.8, 16.5)	2.15 (*dd*, *J* = 7.2, 16.6)
H_β–C(6)	2.89 (*dd*, *J* = 8.0, 16.6)	2.87 (*dd*, *J* = 7.8, 16.5)	2.90 (*dd*, *J* = 7.2, 16.6)
H–C(7)	5.94 (br. *s*)	5.91 (br. *s*)	5.92 (br. *s*)
H–C(9)	2.84 (br. *t*, *J* = 7.8)	2.83 (br. *t*, *J* = 7.5)	2.80 (br. *t*, *J* = 7.6)
CH_2(10)	5.07 (br. *d*, *J* = 13.6), 5.01 (br. *d*, *J* = 13.6)	5.08 (br. *d*, *J* = 14.0), 4.93 (br. *d*, *J* = 14.0)	5.07 (br. *d*, *J* = 13.9), 5.01 (br. *d*, *J* = 13.9)
Me	3.71 (*s*)	3.69 (*s*)	–
H–C(1′)	4.72 (*d*, *J* = 7.8)	4.77 (*d*, *J* = 7.8)	4.73 (*d*, *J* = 7.8)
H–C(2′)	3.24 (*t*, *J* = 9.0)	3.29 (*t*, *J* = 8.6)	3.24 (*t*, *J* = 9.0)
H–C(3′)	3.38 – 3.35 (*m*)	3.47 (*t*, *J* = 8.6)	3.38 (*t*, *J* = 9.0)
H–C(4′)	[d])	3.40 (*t*, *J* = 8.6)	[d])
H–C(5′)	[d])	3.38 – 3.36 (*m*)	[d])
CH_2(6′)	3.84 (br. *d*, *J* = 11.5), 3.63 (*dd*, *J* = 5.3, 11.5)	3.82 (*dd*, *J* = 2.5, 11.9), 3.66 (*dd*, *J* = 5.8, 11.9)	3.84 (*dd*, *J* = 1.8, 12.0), 3.64 (*dd*, *J* = 5.3, 12.0)
H–C(2″,6″)	8.04 (*dd*, *J* = 1.3, 7.8)	7.94 (*d*, *J* = 8.8)	8.04 (*dd*, *J* = 1.2, 7.7)
H–C(3″,5″)	7.48 (*t*, *J* = 7.8)	6.93 (*d*, *J* = 8.8)	7.48 (*t*, *J* = 7.7)
H–C(4″)	7.60 (br. *t*, *J* = 7.8)	–	7.60 (*tt*, *J* = 1.2, 7.7)

[a]) In CD_3OD. [b]) In (D_6)acetone. [c]) Overlapped by the H_2O signal. [d]) Overlapped by the solvent signal.

Interpretation of the HMQC and HMBC spectra of **1** (*Fig. 1*) revealed the substitution pattern, and allowed us to fully assign all ^{1}H- and ^{13}C-NMR signals. The BzO group was located at C(10), as corroborated by HMBC correlations between

[1]) Arbitrary atom numbering.

Table 2. *^{13}C-NMR Data of* **1**–**3**. At 125 MHz; δ in ppm. Arbitrary atom numbering.

Atom	**1**[a])	**2**[b])	**3**[a])	Atom	**1**[a])	**2**[b])	**3**[a])
C(1)	98.7	97.9	98.4	C(1′)	100.9	100.5	100.8
C(3)	153.7	152.5	151.0	C(2′)	75.2	74.5	75.2
C(4)	112.7	112.1	116.3	C(3′)	78.3	77.6	78.2
C(5)	36.8	35.9	37.5	C(4′)	71.8	71.1	71.8
C(6)	40.3	39.5	40.5	C(5′)	78.7	77.8	78.6
C(7)	131.8	130.2	131.9	C(6′)	63.1	62.6	63.1
C(8)	139.9	139.7	140.0	C(1″)	131.8	122.1	131.8
C(9)	47.9	47.3	48.1	C(2″,6″)	130.9	132.6	130.9
C(10)	64.6	63.2	64.7	C(3″,5″)	129.9	116.1	129.9
C(11)	169.7	168.0	168.2	C(4″)	134.6	163.0	134.6
Me	52.0	51.4	–	C(7″)	168.2	166.7	168.2

[a]) In CD_3OD. [b]) In (D_6)acetone.

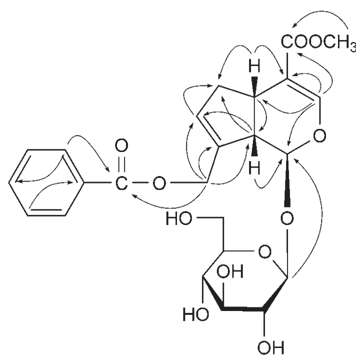

Fig. 1. *Selected HMBC correlations of* **1**

CH_2(10) (δ(H) 5.07, 5.01) and C(7″) (δ(C) 168.2). The Glc moiety was attached at C(1), as established by HMBC correlations of H–C(1′) (δ(H) 4.72) with C(1) (δ(C) 98.7), and of H–C(1) (δ(H) 5.24) with C(1′) (δ(C) 100.9). The presence of a COOMe group was confirmed by HMBC correlations between the Me group (δ(H) 3.71) and C(11) (δ(C) 169.7). From these data, the structure of **1** was, thus, elucidated as methyl (1*S*,4a*S*,7a*S*)-7-[(benzoyloxy)methyl]-1-(β-D-glucopyranosyloxy)-1,4a,5,7a-tetrahydrocyclopenta[*c*]pyran-4-carboxylate, and given the trivial name *hedycoryside A*.

The optically active compound **2** ($[\alpha]_D^{24} = +7.5$) was isolated as a colorless, amorphous powder. Its molecular formula was deduced as $C_{24}H_{28}O_{12}$ by HR-ESI-MS (*m/z* 531.1487 ($[M+Na]^+$; calc. 531.1478)). The UV and IR spectra suggested that **2** was an analogue of **1**. A comparison of their NMR data revealed that **2** contained a *para*-hydroxybenzoyloxy group, rather than a BzO moiety, which was supported by the ^{1}H-NMR signals of an aromatic *AA′BB′* spin system at δ(H) 7.94, 6.93 (2*d*, *J* = 8.8 Hz, 2 H each) and the ^{13}C-NMR signals at δ(C) 122.1 (C(1″)), 132.6 (C(2″,6″)), 116.1 (C(3″,5″)), 163.0 (C(4″)), and 166.7 (C(7″)). The *para*-hydroxybenzoyloxy group was attached at C(10), based on the HMBC correlations (*Fig. 2*) between CH_2(10) (δ(H) 5.08, 4.93) and C(7″) (δ(C) 166.7). Hence, the structure of **2** was elucidated as methyl

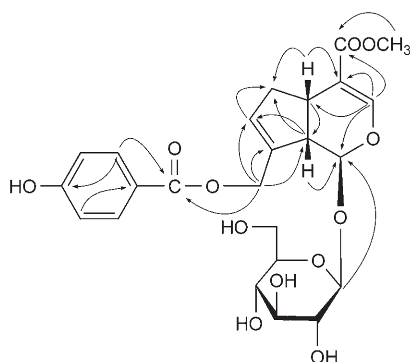

Fig. 2. *Selected HMBC correlations of* **2**

(1*S*,4a*S*,7a*S*)-1-(β-D-glucopyranosyloxy)-1,4a,5,7a-tetrahydro-7-{[(4-hydroxybenzoyl)-oxy]methyl}cyclopenta[*c*]pyran-4-carboxylate, and given the trivial name *hedycoryside B*.

The optically active compound **3** ($[\alpha]_D^{24} = +11.6$), was isolated as a colorless, amorphous powder. Its molecular formula was deduced as $C_{23}H_{26}O_{11}$ by HR-ESI-MS (*m/z* 501.1378 ($[M+Na]^+$; calc. 501.1372)). The UV, IR, and NMR data of **3** were very similar to those of **1**. The only difference was a COOH group in **3** instead of a COOMe group. In combination with HMQC and HMBC experiments, the structure of **3** was, thus, elucidated as (1*S*,4a*S*,7a*S*)-7-[(benzoyloxy)methyl]-1-(β-D-glucopyranosyloxy)-1,4a,5,7a-tetrahydrocyclopenta[*c*]pyran-4-carboxylic acid, and given the trivial name *hedycoryside C*.

The four known compounds were identified as 10-*O*-benzoylscandoside methyl ester, 10-*O*-[(*E*)-*para*-coumaroyl]scandoside methyl ester, 10-*O*-(*para*-hydroxybenzoyl)scandoside methyl ester, and '10-*O*-benzoyl deacetyl asperulosidic acid methyl ester' by comparing their physico-chemical and spectroscopic data with those reported in [5].

All of the above compounds were tested for their *in vitro* cytotoxicities against colon carcinoma (HCT-8, RKO, and LoVo) and gastric carcinoma (SGC-7901) cells, using a method reported previously [8]. However, none of them showed any activity in the concentration range 12.5 – 200 μM.

In the genus *Hedyotis*, most iridoid glycosides contain the skeletons of scandoside, deacetyl asperulosidic acid, and deacetyl asperuloside [1]. Although geniposide derivatives are quite common in iridoid glycosides, only geniposidic acid has been found in *Hedyotis* plants [6]. To our knowledge, hedycorysides A – C (**1** – **3**, resp.) are the first three benzoylated geniposide derivatives from *Hedyotis* species.

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Experimental Part

General. Column chromatography (CC): silica gel *H* (10 – 40 μm, 200 – 300 mesh; *Yantai Institute of Chemical Technology*, China), *Chromatorex RP-18* gel (20 – 45 μm; *Fuji Silysia Chemical, Ltd.*, Kasugai, Japan), *Diaion HP-20* (250 – 300 μm; *Mitsubishi Chemical Corporation*, Japan), *MCI gel CHP-20P* (75 –

150 µm; *Mitsubishi Chemical Corporation*, Japan), or *Sephadex LH-20* (*Amersham Biosciences*, *GE Health Care*). Prep. and anal. TLC: precoated silica-gel GF_{254} plates (10–40 µm; *Yantai Institute of Chemical Technology*, China). Optical rotation: *Jasco P1030* polarimeter. UV Spectra: *Shimadzu UV-2401PC* spectrophotometer; λ_{max} (log ε) in nm. IR Spectra: *Nicolet Avatar-360* spectrophotometer, with KBr pellets; in cm^{-1}. ^1H-, ^{13}C-, and 2D-NMR Spectra: *Bruker DRX-400* and *-500* instruments; chemical shifts δ in ppm rel. to residual solvent peaks (CD_3OD: δ(H) 3.30, δ(C) 49.3), or rel. to Me_4Si as internal standard, *J* in Hz. ESI-MS: *Applied Biosystems QSTAR Pulsar* mass spectrometer; in *m/z*.

Plant Material. The whole plant of *H. corymbosa* (LINN.) LAM. was bought from *Shanghai Medical Material Co.* in July 2003, and dried in air. The plant was identified by Prof. *Sheng-Li Pan* (Fudan University), and a voucher specimen (TCM 03-07-03 Hou) was deposited at the Herbarium of the Department of Pharmacognosy, School of Pharmacy, Fudan University, P. R. China.

Extraction and Isolation. The air-dried whole plant (3.6 kg) of *H. corymbosa* was percolated with 95% EtOH (30 l) at r.t for 4 d. The filtrate was concentrated *in vacuo* to afford a residue (386 g), which was suspended in H_2O (1 l), and extracted successively with petroleum ether (1 × 600 ml, 4 × 400 ml), AcOEt (1 × 600 ml, 4 × 400 ml), and BuOH (1 × 600 ml, 4 × 400 ml). The BuOH-soluble extract (36 g after evaporation) was subjected to CC (SiO_2; $CHCl_3$/MeOH 15 : 1 → 4 : 1): fractions *Fr. A–I*. *Fr. C* (5.5 g) was separated by CC (*Diaion HP-20*; MeOH/H_2O 1 : 4 → 4 : 1): *Fr. C1–C5*. *Fr. C3* was purified by CC (SiO_2; $CHCl_3$/i-PrOH 9 : 1 → 6 : 1): *Fr. C3.1–C3.7*. *Fr. C3.4* was first purified repeatedly by CC (1. SiO_2, Et_2O/i-PrOH 15 : 1 → 9 : 1; 2. *Sephadex LH-20*, MeOH/H_2O 1 : 1; 3. *RP-18*, MeOH/H_2O 2 : 3 → 3 : 2), and then by prep. TLC (SiO_2; $CHCl_3$/i-PrOH 3 : 1) to afford **1** (5 mg). *Fr. C3.5* was separated by CC (1. *CHP-20P*, MeOH/H_2O 1 : 1 → 3 : 2; 2. *RP-18*, MeOH/H_2O 2 : 3 → 1 : 1; 3. SiO_2, $CHCl_3$/MeOH 15 : 1 → 4 : 1) to afford 10-*O*-benzoylscandoside methyl ester (10 mg). *Fr. D* (8.5 g) was purified by CC (1. *Diaion HP-20*, MeOH/H_2O 1 : 4 → 4 : 1; 2. SiO_2, $CHCl_3$/i-PrOH 9 : 1 → 4 : 1; 3. SiO_2, $CHCl_3$/MeOH 15 : 1 → 9 : 1; 4. *Sephadex LH-20*, MeOH/H_2O 1 : 1; 5. *CHP-20P*, MeOH/H_2O 1 : 1 → 7 : 3), followed by prep. TLC (SiO_2; $CHCl_3$/MeOH 6 : 1), to afford 10-*O*-benzoyl deacetyl asperulosidic acid methyl ester (5 mg). *Fr. E* (4.6 g) was subjected to CC (*Diaion HP-20*; MeOH/H_2O 1 : 4 → 4 : 1): *Fr. E1–E7*. *Fr. E4* was separated by CC (SiO_2; $CHCl_3$/MeOH 9 : 1): *Fr. E4.1–E4.9*. *Fr. E4.6* was purified by CC (1. *CHP-20P*, MeOH/H_2O 1 : 1 → 3 : 2; 2. *RP-18*, MeOH/H_2O 3 : 2), followed by prep. TLC (SiO_2; $CHCl_3$/MeOH 4 : 1), to give 10-*O*-[(*E*)-*para*-coumaroyl]scandoside methyl ester (20 mg). *Fr. E4.8* was fractionated by CC (1. *RP-18*, MeOH/H_2O 2 : 3; 2. *Sephadex LH-20*, MeOH/H_2O 2 : 3), then by prep. TLC (SiO_2; $CHCl_3$/i-PrOH 2 : 1), to afford 10-*O*-(4-hydroxybenzoyl)scandoside methyl ester (8 mg). *Fr. E6* was purified by CC (1. *CHP-20P*, MeOH/H_2O 4 : 1; 2. SiO_2, AcOEt/i-PrOH 15 : 1 → 6 : 1): *Fr. E6.1–E6.8*. *Fr. E6.3* was separated by CC (*Sephadex LH-20*; MeOH/H_2O 2 : 3 → 1 : 1), followed by prep. TLC (SiO_2; $CHCl_3$/i-PrOH 4 : 1), to give **2** (14 mg). *Fr. E6.6* was fractionated by prep. TLC (SiO_2; $CHCl_3$/MeOH 3 : 1), then by CC (*RP-18*; MeOH/H_2O 1 : 4 → 3 : 7), to afford **3** (10 mg).

Hedycoryside A (= *Methyl (1*S*,4a*S*,7a*S*)-7-[(Benzoyloxy)methyl]-1-(β-*D*-glucopyranosyloxy)-1,4a,5,7a-tetrahydrocyclopenta[*c*]pyran-4-carboxylate*; **1**). Colorless, amorphous powder. $[\alpha]_D^{24} = +13.2$ ($c = 0.25$, MeOH). UV (MeOH): 231 (4.26). IR (KBr): 3371, 2921, 2851, 1713, 1633, 1452, 1385, 1273, 1158, 1073, 716. ^1H- and ^{13}C-NMR: see *Tables 1* and *2*, resp. ESI-MS (neg.): 491 ($[M-H]^-$). ESI-MS (pos.): 515 ($[M+Na]^+$). HR-ESI-MS (pos.): 515.1522 ($[M+Na]^+$, $C_{24}H_{28}O_{11}Na^+$; calc.: 515.1529).

Hedycoryside B (= *Methyl (1*S*,4a*S*,7a*S*)-1-(β-*D*-Glucopyranosyloxy)-1,4a,5,7a-tetrahydro-7-{[(4-hydroxybenzoyl)oxy]methyl}cyclopenta[*c*]pyran-4-carboxylate*; **2**). Colorless, amorphous powder. $[\alpha]_D^{24} = +7.5$ ($c = 0.65$, MeOH). UV (MeOH): 252 (4.20). IR (KBr): 3420, 2923, 1699, 1633, 1609, 1441, 1385, 1275, 1166, 1100, 1043, 758. ^1H- and ^{13}C-NMR: see *Tables 1* and *2*, resp. ESI-MS (neg.): 507 ($[M-H]^-$). ESI-MS (pos.): 531 ($[M+Na]^+$). HR-ESI-MS (pos.): 531.1487 ($[M+Na]^+$, $C_{24}H_{28}O_{12}Na^+$; calc.: 531.1478).

Hedycoryside C (= *(1*S*,4a*S*,7a*S*)-7-[(Benzoyloxy)methyl]-1-(β-*D*-glucopyranosyloxy)-1,4a,5,7a-tetrahydrocyclopenta[*c*]pyran-4-carboxylic Acid*; **3**). Colorless, amorphous powder. $[\alpha]_D^{24} = +11.6$ ($c = 0.50$, MeOH). UV (MeOH): 229 (4.13). IR (KBr): 3395, 2921, 2850, 1716, 1645, 1539, 1452, 1405, 1316, 1278, 1074, 903, 715. ^1H- and ^{13}C-NMR: see *Tables 1* and *2*, resp. ESI-MS (neg.): 477 ($[M-H]^-$). ESI-MS (pos.): 501 ($[M+Na]^+$). HR-ESI-MS (pos.): 501.1378 ($[M+Na]^+$, $C_{23}H_{26}O_{11}Na^+$; calc.: 501.1372).

REFERENCES

[1] Q. M. Yuan, H. W. Yang, F. G. Zhang, X. Wang, B. Z. Zheng, *J. Yunnan Univ.* **2004**, *26* (suppl.), 171.
[2] Y. H. Chen, F. R. Chang, C. C. Wu, M. H. Yen, C. C. Liaw, H. C. Huang, Y. H. Kuo, Y. C. Wu, *Planta Med.* **2006**, *72*, 75; J. F. Zhao, Q. M. Yuan, X. D. Yang, H. B. Zhang, L. Li, *Helv. Chim. Acta* **2005**, *88*, 2532; R. Ahmad, K. Shaari, N. H. Lajis, A. S. Hamzah, N. H. Ismail, M. Kitajima, *Phytochemistry* **2005**, *66*, 1141.
[3] Jiangsu College of New Medicine, in 'A Dictionary of the Traditional Chinese Medicines', Shanghai Scientific and Technical Publishers, Shanghai, 1977, p. 535.
[4] L. Wang, C. Zhou, *Lishizhen Medicine and Materia Medica Research* **2000**, *11*, 1139.
[5] H. Otsuka, K. Yoshimura, K. Yamasaki, M. C. Cantoria, *Chem. Pharm. Bull.* **1991**, *39*, 2049.
[6] S. Takagi, M. Yamaki, K. Masuda, Y. Nishihama, K. Sakina, *Yakugaku Zasshi* **1981**, *101*, 657.
[7] D. W. Cameron, G. I. Feutrill, P. Perlmutter, J. M. Sasse, *Phytochemistry* **1984**, *23*, 533; S. R. Jensen, *Phytochemistry* **1983**, *22*, 1761.
[8] Y. S. Zou, A. J. Hou, G. F. Zhu, *Chem. Biodiv.* **2005**, *2*, 131.

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