A New Hydrolyzable Tannin from *Balanophora harlandii* with Radical-Scavenging Activity

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One new hydrolyzable tannin, 1-*O*-[(*E*)-*p*-coumaroyl]-3-*O*-galloyl-β-D-glucopyranose (**1**), was isolated from the rhizome of *Balanophora harlandii*, together with 18 known phenolic compounds. Their structures were determined by detailed spectroscopic analysis. Of the known compounds, 3-*O*-caffeoyl-D-glucopyranose (**6**) was obtained as a natural product for the first time, and compounds **2**–**6** and **8**–**19** were identified for the first time from this plant. The radical-scavenging activity of the isolated compounds was tested by a DPPH assay.

Introduction. – The genus *Balanophora* (Balanophoraceae) comprising about 80 species is mainly distributed in the tropical and subtropical areas of Asia and Oceania. Among them, about 19 species are growing in P. R. China, particularly in the southwestern part. All of these species are parasitic and normally growing on the roots of the evergreen broadleaf trees of the Leguminosae, Ericaceae, Urticaceae, and Fagaceae families in particular. Some caffeoyl-, coumaroyl-, galloyl-, and hexahydroxydiphenoyl-substituted glucoses were reported as characteristic components from this genus [1–5] (caffeic acid = (2*E*)-3-(3,4-dihydroxyphenyl)prop-2-enoic acid; *p*-coumaric acid = (2*E*)-3-(4-hydroxyphenyl)prop-2-enoic acid; gallic acid = 3,4,5-trihydroxybenzoic acid; hexahydroxydiphenic acid = 4,4′,5,5′,6,6′-hexahydroxy[1,1′-biphenyl]-2,2′-dicarboxylic acid).

Balanophora harlandii HOOK. F. is mainly distributed in Guangdong, Guangxi, and Yunnan provinces of China, growing in the humid soil humus of the forest shade at an altitude of 600–2100 m. The whole plant is a folk medicine used as a tonic and for the treatment of hemorrhoids, stomachache, and hemoptys. Several triterpenoids and phenolics were isolated from the aerial part of this herb [1]. We have now investigated the fresh rhizome of this plant growing on *Debregeasia orientalis* C. J. CHEN (Urticaceae). *D. orientalis* is an evergreen shrub found commonly in shady and moist places which constitute the typical habitat of *Balanophora spp*. In the course of this study, one new hydrolyzable tannin, 1-*O*-[(*E*)-*p*-coumaroyl]-3-*O*-galloyl-β-D-glucopyranose[1]) (**1**), together with the 18 known phenolic compounds **2**–**19** were isolated (*Fig. 1*). The isolated compounds were examined for their 1,1-diphenyl-2-picrylhydra-

[1]) Trivial atom numbering; see *Exper. Part* for the systematic name.

zyl (=2,2-diphenyl-1-(2,4,6-trinitrophenyl)hydrazinyl = DPPH) radical-scavenging activities. This article presents the details of this study.

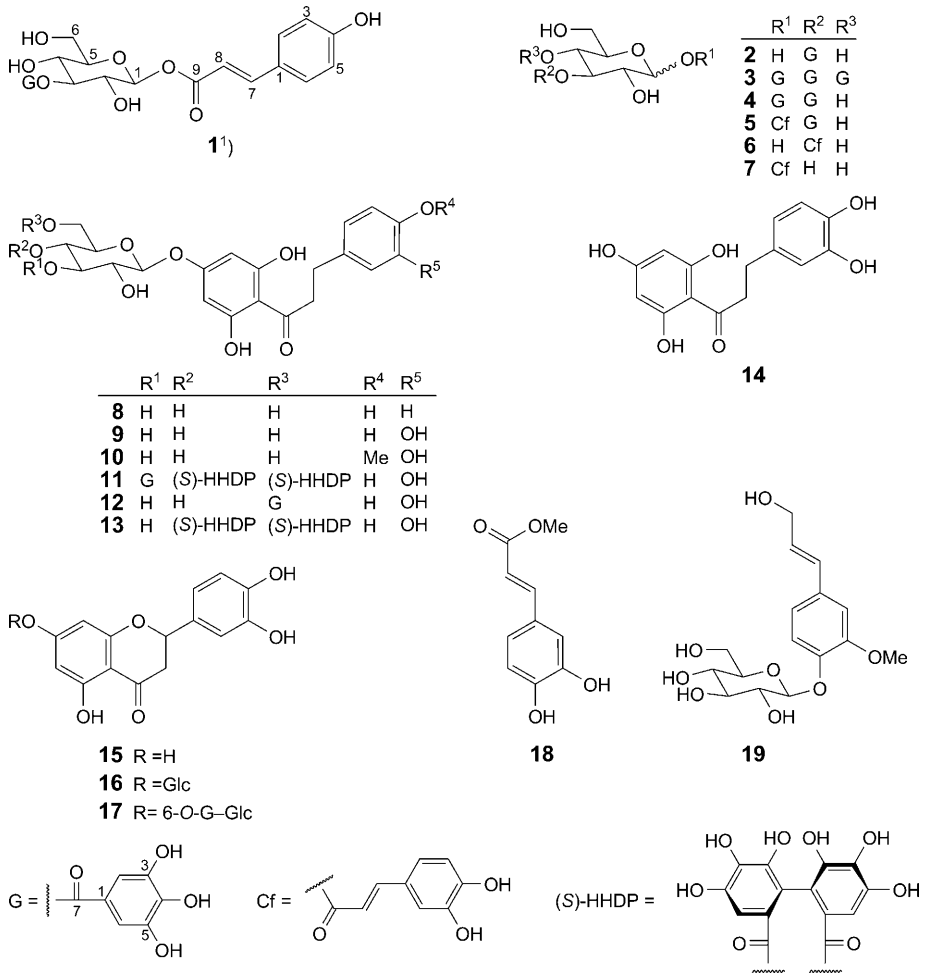

Fig. 1. *Compounds* **1**–**19** *isolated from* Balanophora harlandii

Results and Discussion. – 1. *Chemistry.* The 80% aqueous acetone extract of the fresh rhizome of *B. harlandii* was partitioned between AcOEt and H_2O. The AcOEt fraction was further chromatographed on *Diaion-HP20SS*, *Sephadex-LH-20*, *MCI-CHP-20P*, and *Chromatorex-ODS* columns to afford one new hydrolyzable tannin, *i.e.*, **1**. In addition, 18 known compounds, including the six hydrolyzable tannins **2**–**7**, the seven dihydrochalcones (=1,3-diphenylpropan-1-ones) **8**–**14**, the three flavanones (=2,3-dihydro-2-phenyl-4*H*-1-benzopyran-4-ones **15**–**17**, and the two simple phenolic compounds **18** and **19** were obtained. The known compounds were identified as 3-*O*-galloyl-D-glucopyranose (**2**) [6], 1,3,4-tri-*O*-galloyl-*β*-D-glucopyranose (**3**) [7], 1,3-di-

O-galloyl-β-D-glucopyranose (**4**) [3], 1-*O*-caffeoyl-3-*O*-galloyl-β-D-glucopyranose (**5**) [3], 3-*O*-caffeoyl-D-glucopyranose (**6**) [3], 1-*O*-caffeoyl-β-D-glucopyranose (**7**) [1], phloretin 4′-β-D-glucoside (**8**) [8] (phloretin = 3-(4-hydroxyphenyl)-1-(2,4,6-trihydroxyphenyl)propan-1-one), 3-hydroxyphloretin 4′-β-D-glucoside (**9**) [9], hesperetin dihydrochalcone 4′-β-D-glucoside (**10**) [10], 3-hydroxyphloretin 4′-[3″-*O*-galloyl-4″,6″-di-*O*-(*S*)-HHDP-β-D-glucoside] (**11**) [5], 3-hydroxyphloretin 4′-(6″-*O*-galloyl-β-D-glucoside) (**12**) [5], 3-hydroxyphloretin 4′-[4″,6″-di-*O*-(*S*)-HHDP-β-D-glucoside] (**13**) [5], 3-hydroxyphloretin (**14**) [9], eriodictyol ((=2-(3,4-dihydroxyphenyl)-2,3-dihydro-5,7-dihydroxy-4*H*-1-benzopyran-4-one; **15**) [11], eriodictyol 7-β-D-glucoside (**16**) [12], eriodictyol 7-(6″-*O*-galloyl-β-D-glucoside) (**17**) [12], methyl caffeate (**18**) [13], and coniferin (=4-(3-hydroxyprop-1-en-1-yl)-2-methoxyphenyl β-D-glucopyranoside; **19**) [14] by direct comparison with authentic samples or comparison of spectral data with those reported in the literature. Except for **7**, all these compounds were isolated from this plant for the first time. Though 3-*O*-caffeoyl-D-glucopyranose (**6**) was obtained as a hydrolytic product by tannase from *Balanophora japonica* [3], this is the first time that compound **6** was reported as a natural product.

The β-D-glucopyranose **1** was obtained as a yellow amorphous powder and had a molecular formula $C_{22}H_{22}O_{12}$, as derived from the negative-ion-mode HR-ESI-MS (*m/z* 477.1063 ($[M-H]^-$)) and ^{13}C-NMR (DEPT) spectra (*Table 1*). On the basis of IR, 1D- and 2D-NMR spectral data, the structure of **1** could be established. The IR spectrum of **1** indicated the presence of OH (3412 cm^{-1}) and aromatic acyl (1604 cm^{-1}) groups, as well as of a benzene ring (1514, 1447, 1346, and 1226 cm^{-1}). The ^{1}H-NMR spectrum (*Table 1*) displayed signals arising from a galloyl (δ(H) 7.13 (*s*, 2 H)), an (*E*)-*p*-coumaroyl (δ(H) 7.52 and 6.84 (*d*, *J* = 8.5 Hz, each 2 H) from an 1,4-disubstituted benzene ring and δ(H) 7.69 and 6.35 (*d*, *J* = 15.9 Hz, each 1 H) from *trans* C=C bond), and a β-glucosyl (anomeric H-atom at δ(H) 5.68 (*d*, *J* = 8.2 Hz)) unit. The downfield chemical shifts of H–C(1) (δ(H) 5.68) and H–C(3) (δ(H) 5.23 (*t*, *J* = 9.5 Hz)) of the glucosyl unit suggested that these two positions were acylated with galloyl and (*E*)-*p*-coumaroyl groups, respectively. The acylated positions were further determined by ^{1}H,^{1}H-COSY and HMBC experiments. In the HMBC spectrum of **1** (*Fig. 2*), the cross-peaks glucosyl H–C(3) (δ(H) 5.23)/galloyl C=O (δ(C) 167.0) and glucosyl H–C(1) (δ(H) 5.68)/(*E*)-*p*-coumaroyl C=O (δ(C) 166.5) were observed. Other HMBCs are shown in *Fig. 2*. Based on the above evidence, the structure of compound **1** was deduced to be 1-*O*-[(*E*)-*p*-coumaroyl]-3-*O*-galloyl-β-D-glucopyranose[2]).

In this study, one new hydrolyzable tannin, 1-*O*-[(*E*)-*p*-coumaroyl]-3-*O*-galloyl-β-D-glucopyranose (**1**), and one new natural hydrolyzable tannin, 3-*O*-caffeoyl-D-glucopyranose (**6**), were isolated from the fresh rhizomes of *B. harlandii*, in addition to seven dihydrochalcones **8**–**14**, five galloyl- and caffeoyl-substituted glucoses **2**–**5**, and **7**, and some other phenolic compounds. So far, the dihydrochalcones were only found from *B. tobiracola* [5][9].

2. *Radical-Scavenging Activity.* The isolated compounds could be divided into four groups, hydrolyzable tannins **1**–**7**, dihydrochalcones **8**–**14**, flavanones **15**–**17**, and simple phenolic compounds **18** and **19**. Their DPPH-radical-scavenging activities were tested by a reported method, with ascorbic acid as a positive control [15], and the

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[2]) The absolute configuration of glucose was supposed to be D from biogenetic considerations.

Table 1. *^1H- and ^{13}C-NMR Data* (500 and 125 MHz, resp., (D_6)acetone) *of Compound* **1**[1]). δ in ppm, J in Hz.

		δ(H)	δ(C)
Glucose:	H–C(1)	5.68 (*d*, *J* = 8.2)	95.0
	H–C(2)	3.25–3.47 (*m*)	71.7
	H–C(3)	5.23 (*t*, *J* = 9.5)	78.3
	H–C(4)	3.25–3.47 (*m*)	68.7
	H–C(5)	3.25–3.47 (*m*)	78.0
	H–C(6)	3.25–3.47 (*m*)	61.5
Coumaroyl:	C(1)		126.0
	H–C(2,6)	7.52 (*d*, *J* = 8.5)	131.1
	H–C(3,5)	6.84 (*d*, *J* = 8.5)	116.5
	C(4)		161.0
	H–C(7)	7.69 (*d*, *J* = 15.9)	147.2
	H–C(8)	6.35 (*d*, *J* = 15.9)	113.9
	C(9)		166.5
Galloyl:	C(1)		120.8
	H–C(2,6)	7.13 (*s*, 2 H)	109.8
	C(3,5)		145.9
	C(4)		138.0
	C(7)		167.0

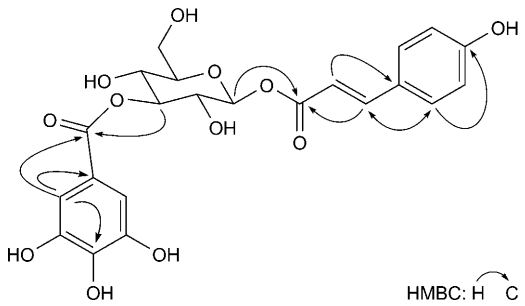

Fig. 2. *Key HMBCs of compound* **1**

results are shown in *Table 2*. Most of the isolated compounds showed an obvious scavenging activity on the DPPH radical. Among the isolated constituents from *B. harlandii*, the hydrolyzable tannins **1**–**5** with a galloyl group in the molecule and the dihydrochalcones **9** and **11**–**14** with a catechol (= benzene-1,2-diol) moiety as ring *B* exhibited higher activities than ascorbic acid. The flavanone **17** acylated with a galloyl moiety also displayed stronger activity. Incorporating a 3-*O*-galloylglucosyl structure, compound **5** with an additional caffeoyl group in the molecule showed a higher activity than **1**, in which an additional *p*-coumaroyl group was present. Compared to compound **2** with one galloyl group, compounds **6** and **7** with one caffeoyl group showed less radical-scavenging activities due to one less phenolic OH group in the caffeoyl moiety. In addition, the opening of ring *C* such as in compound **14** led to a much stronger radical-scavenging activity as compared to the ring-closed **15**, owing to the presence of

one more phenolic OH group in **14**. In the case of the simple phenolics **18** and **19**, the number of phenolic OH groups was important for their radical-scavenging activity. Because of the lack of a free phenolic OH group, compound **19** showed no activity compared with compound **18**. Therefore, the more phenolic OH groups are present in a compound, the higher is the DPPH-radical scavenging-activity. These results were in accord with the trend reported previously that compounds with more adjacent phenolic OH groups (galloyl, pyrogallol, or catechol group) had higher radical-scavenging activities on DPPH [16]. The here isolated phenolic compounds may play an important role for the folk-medicinal uses of the herb *B. harlandii*.

Table 2. *DPPH-Radical-Scavenging Activities of Compounds* **1**–**19**

	SC_{50} [μM][a])[b])		SC_{50} [μM][a])[b])
Ascorbic acid	39.5 ± 0.1	**10**	73.8 ± 0.8
Hydrolyzable tannins:		**11**	8.2 ± 0.1
1	29.6 ± 0.2	**12**	9.2 ± 0.1
2	20.8 ± 0.3	**13**	10.3 ± 0.1
3	16.2 ± 0.3	**14**	18.0 ± 0.2
4	11.8 ± 0.4	Flavanones:	
5	17.3 ± 0.3	**15**	75.2 ± 0.3
6	45.1 ± 0.3	**16**	100 ± 0.2
7	32.0 ± 0.3	**17**	11.9 ± 0.1
Dihydrochalcones:		Simple phenolics:	
8	364 ± 1	**18**	42.7 ± 0.2
9	23.6 ± 0.1	**19**	–[c])

[a]) SC_{50} = Radical-scavenging activity (concentration in μM necessary for 50% reduction of DPPH radicals). [b]) Values represent means ± s.d. ($n = 3$). [c]) No activity.

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Experimental Part

General. Thin layer chromatography (TLC): precoated silica-gel *H* plates (*Qingdao Haiyang Chemical Plant*). Column chromatography (CC): *MCI CHP20P* (75–150 μm; *Mitsubishi Chemical Industry*), *Diaion HP20SS* (*Mitsubishi Chemical Industry*), *Chromatorex ODS* (100–200 mesh; *Fuji Silysia Chemical Co., Ltd.*), *Sephadex LH-20* (25–100 μm, *Pharmacia Fine Chemical Co., Ltd.*). Optical rotation: *Jasco-20* polarimeter. UV Spectra: *Shimadzu-UV-210A* spectrometer in MeOH; λ_{max} (ε) in nm. IR Spectra: *Bio-Rad FTS-135*; $\tilde{\nu}$ in cm^{-1}. NMR Spectra: *Bruker AV-400* and *DRX-500*; δ in ppm rel. to Me_4Si as internal standard, *J* in Hz. MS: *VG Autospec-3000* mass spectrometer; in *m*/*z* (rel. %).

Plant Material. The whole plants of *B. harlandii*, growing on the roots of *Debregeasia orientalis* C. J. CHEN (Urticaceae), were collected at the western suburbs of Kunming City, Yunnan Province, P. R. China, in December 2006, and identified by Prof. *Xi-Wen Li* from the Key Laboratory of Biodiversity and Biogeography, Kunming Institute of Botany, Chinese Academy of Sciences. A voucher specimen (KUN No. 0840094) was deposited with the Herbarium of the Kunming Institute of Botany, the Chinese Academy of Sciences.

Extraction and Isolation. The fresh rhizomes of *B. harlandii* (1.29 kg) were extracted with 80% aq. acetone (3 × 3 l) at r.t. for a week. After evaporation of the org. solvent, the extract was concentrated to a small volume (1 l) and partitioned with AcOEt (3 × 3 l). The AcOEt fraction (62 g) was applied to CC (*Diaion HP20SS*; step gradient H_2O/MeOH 1:0 → 0:1, each gradient 500 ml, followed by 50% aq. acetone): *Fractions 1–10*. *Fr. 2* (2.45 g) was subjected to CC (*Sephadex LH-20*, 0–100% MeOH; then *MCI* gel *CHP20P*, 0–100% MeOH, and *Chromatorex ODS*, 40–100% MeOH): **2** (97 mg), **4** (7 mg), **5** (142 mg), **7** (328 mg), **9** (241 mg), and **19** (52 mg). *Fr. 4* (1.35 g) was subjected to CC (*Sephadex LH-20*, 0–100% MeOH; then *MCI* gel *CHP20P*, 0–100% MeOH, and *Chromatorex ODS*, 40–100% MeOH): **3** (104 mg) and **16** (84 mg). Repeated CC (*Sephadex LH-20*, 0–100% MeOH; then *MCI* gel *CHP20P*, 0–100% MeOH, and *Chromatorex ODS*, 40–100% MeOH) yielded **6** (54 mg), **12** (60 mg), **17** (187 mg), and **18** (38 mg) from *Fr. 5* (16.2 g), and **1** (15 mg), **8** (29 mg), **10** (14 mg), **11** (1087 mg), **13** (151 mg), and **14** (79 mg) from *Fr. 7* (5.8 g), and **15** (204 mg) from *Fr. 9* (2.5 g), resp.

1-O-*[(*E*)*-p-*Coumaroyl]-3*-O-*galloyl-β*-D-*glucopyranose* (=*β*-D-*Glucopyranose 1-[(2*E*)-3-(4-Hydroxyphenyl)prop-2-enoate] 3-(3,4,5-Trihydroxybenzoate*; **1**): Yellow amorphous powder. $[\alpha]_D^{25} = 22.5$ ($c = 3.78$, MeOH). UV: 316 (17097), 201 (23409), 193 (17784). IR (KBr): 3412, 1706, 1604, 1514, 1447, 1346, 1226, 1069. ^{1}H- and ^{13}C-NMR: *Table 1*. HR-ESI-MS: 477.1063 ($[M-H]^-$, $C_{22}H_{21}O_{12}^-$; calc. 477.1033). ESI-MS: 477 ($[M-H]^-$).

DPPH-Radical-Scavenging Assay. The DPPH assay was performed as described in our previous publication [16], and ascorbic acid was used as a positive control. Scavenging activity was determined by the following equation: % scavenging activity = $100 \times (A_{control} - A_{sample})/A_{control}$. The SC_{50} value was obtained through extrapolation from linear regression analysis and denoted the concentration of sample required to scavenge 50% of DPPH radicals.

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