Taylor & Francis
Taylor & Francis Group

Natural Product Research
Formerly Natural Product Letters

ISSN: 1478-6419 (Print) 1478-6427 (Online) Journal homepage: http://www.tandfonline.com/loi/gnpl20

A new dihydrochalcone glycoside from the stems of Homalium stenophyllum

Shou-Yuan Wu, Yan-Hui Fu, Qi Zhou, Meng Bai, Guang-Ying Chen, Chang-Ri Han & Xiao-Ping Song

To cite this article: Shou-Yuan Wu, Yan-Hui Fu, Qi Zhou, Meng Bai, Guang-Ying Chen, Chang-Ri Han & Xiao-Ping Song (2017): A new dihydrochalcone glycoside from the stems of Homalium stenophyllum, Natural Product Research, DOI: 10.1080/14786419.2017.1374268

To link to this article: http://dx.doi.org/10.1080/14786419.2017.1374268

NATURAL PRODUCT RESEARCH, 2017
https://doi.org/10.1080/14786419.2017.1374268

Taylor & Francis
Taylor & Francis Group

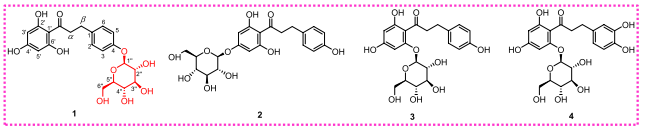
A new dihydrochalcone glycoside from the stems of *Homalium stenophyllum*

Shou-Yuan Wu[a1], Yan-Hui Fu[a1], Qi Zhou[a], Meng Bai[a], Guang-Ying Chen[a], Chang-Ri Han[a,b] and Xiao-Ping Song[a]

[a]Key Laboratory of Tropical Medicinal Plant Chemistry of Ministry of Education, Hainan Normal University, Haikou, P. R. China; [b]Hainan Institute of Science and Technology, Haikou, P. R. China

ABSTRACT

A new dihydrochalcone glycoside, phloretin-4-*O*-*β*-D-glucopyranoside (**1**), together with seven known flavonoids (**2–8**), were isolated from the stems of *Homalium stenophyllum*. The structure of **1** was elucidated by extensive spectroscopic methods and the known compounds were identified by comparisons with data reported in the literature. The known compounds (**2–8**) were isolated from the genus *Homalium* for the first time. All compounds were evaluated for their antibacterial activities against six pathogenic bacteria *in vitro*.



ARTICLE HISTORY
Received 1 July 2017
Accepted 27 August 2017

KEYWORDS
Homalium stenophyllum; dihydrochalcone glycoside; phloretin-4-*O*-*β*-D-glucopyranoside; antibacterial activities

1. Introduction

The genus *Homalium* (Flacourtiaceae) comprising about 130 species are mainly distributed in temperate and subtropical regions. There are about 12 species of this genus, growing in China from the south-west to Taiwan. Among them, *H. stenophyllum* Merr. et Chun is a Chinese endemic plant, only distributed in China's Hainan Island (Fan 1990). Previous chemical studies on the plants of this genus have led to the isolation of an array of compounds including phenolic glycosides (Ekabo et al. 1993a, 1993b; Shaari & Waterman 1995; Itoh et al. 2000), xanthones (Wu et al. 2015), iridoids (Johns & Lamberton 1969; Ekabo et al. 1993b; Shaari & Waterman 1996), coumarins (Charubala et al. 1974; Ekabo et al. 1993a), triterpenoids (Shaari & Waterman 1996) and alkaloids (Païs et al. 1973). These compounds show a range of biological activities such as antibacterial (Liu et al. 2013), antioxidant (Liu et al. 2013; Mahapatra et al. 2015), antiviral (Ekabo et al. 1993b; Ishikawa et al. 1998), antiplasmodial

CONTACT Chang-Ri Han hchr116@hainnu.edu.cn; Xiao-Ping Song sxp628@126.com
[1]Shou-Yuan Wu and Yan-Hui Fu are co-first authors.
Supplemental data for this article can be accessed at https://doi.org/10.1080/14786419.2017.1374268.

(Okokon et al. 2006), hypolipidemic (Okokon et al. 2007), hypoglycaemic activities (Okokon et al. 2007) and so on. As a Chinese endemic medicinal plant, up to now, there is only a preliminary investigation on the chemical composition of *H. stenophyllum* performed by us previously (Zhang et al. 2017). As a part of our ongoing research into structurally and biologically interesting natural products from tropical medicinal plants in China, a chemical investigation on *H. stenophyllum* was, thus, undertaken and had led to the isolation and characterisation of a new dihydrochalcone glycoside, phloretin-4-*O*-*β*-D-glucopyranoside (**1**), together with seven known flavonoids. Their structures were elucidated on the basis of extensive NMR and MS analyses. In addition, all compounds were evaluated for their antibacterial activities against six terrestrial pathogenic bacteria *in vitro*. Herein, we describe the isolation, structure elucidation and antibacterial activities of these compounds.

2. Results and discussion

The 95% EtOH extract of the stems of *H. stenophyllum* was suspended in water and extracted successively with petroleum ether and ethyl acetate. The ethyl acetate fraction was repeatedly subjected to silica gel, Sephadex LH-20, RP-18 column chromatography and semi-preparative HPLC, to yield a total of eight flavonoids (**1–8**), including a new dihydrochalcone glycoside, as shown in Figure 1.

Phloretin-4-*O*-*β*-D-glucopyranoside (**1**) was obtained as yellowish amorphous powder. Its molecular formula was determined as $C_{21}H_{24}O_{10}$ by HR-ESI-MS (*m/z* 437.1446 $[M + H]^+$, calcd 437.1448). Its IR spectrum showed the presence of hydroxyl groups (3290 cm^{-1}), a ketone carbonyl group (1628 cm^{-1}) and phenyl groups (1521, 1452 and 1367 cm^{-1}). The UV absorption band at 286 nm was characteristic of a dihydrochalcone skeleton. The ^{13}C NMR and DEPT data revealed the presence of 21 carbon atoms, including 13 sp^2 carbon atoms, five sp^3 methines and three sp^3 methylenes, which were attributable to one dihydrochalcone skeleton and one glucopyranosyl moiety. The above data revealed that the structure of **1** was similar to that of phloretin-4′-O-*β*-D-glucopyranoside (**2**) (Qin et al. 2015), except that the glucopyranosyl moiety was located at C-4′ in **2**, while the glucopyranosyl moiety was located at C-4 in **1**, which was further supported by the HMBC correlation of the anomeric proton resonating at δ_H 4.80 (1H, d, $J = 7.6$ Hz, H-1″) to C-4 (155.7). Furthermore, the coupling constant of the anomeric proton resonating at δ_H 4.80 (1H, d, $J = 7.6$ Hz, H-1″) suggested that the glucopyranosyl moiety was *β*-glucoside. In order to further confirm the structure

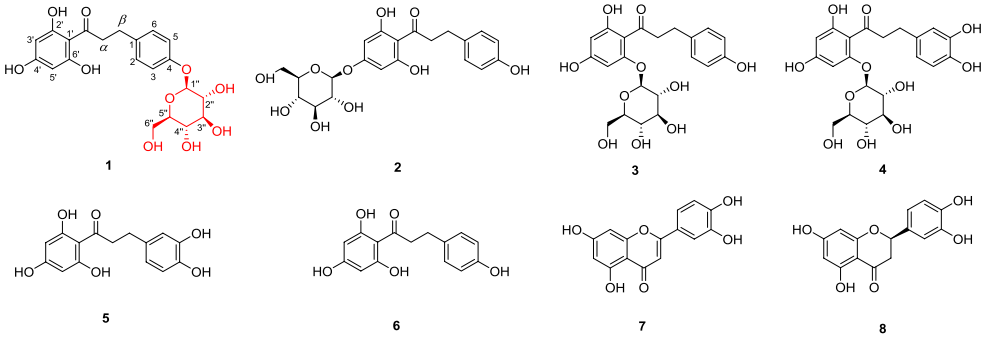


Figure 1. Chemical structures of compounds **1–8**.

of **1**, the acid hydrolysis reaction of **1** was carried out. As a result, a *β*-D-glucose was produced as the sole sugar identified on the basis of the same R_f value on co-TLC and the almost identical optical value by comparing with that of an authentic sugar sample. Detailed analysis of 2D NMR (HSQC, HMBC, ^{1}H-^{1}H COSY and ROESY) spectra confirmed the structure of **1**. Thus, **1** was determined as phloretin-4-*O*-*β*-D-glucopyranoside, as shown in Figure S1.

In addition to new dihydrochalcone glycoside **1**, seven other known flavonoids were isolated and identified as phloretin-4′-*O*-*β*-D-glucopyranoside (**2**) (Qin et al. 2015), phloridzosid (**3**) (Cuendet et al. 2000), 3-hydroxyphloridzin (**4**) (El-Naggar et al. 1980), eriodictyol dihydrochalcone (**5**) (Nakamura et al. 2003), phloretin (**6**) (Nakamura et al. 2003), luteolin (**7**) (Youssef & Frahm 1995) and (2*R*)-eriodictyol (**8**) (Pan et al. 2008), by comparing the experimental and reported physical data.

All isolated compounds were evaluated for their antibacterial activities against six pathogenic bacteria *in vitro*, including four terrestrial pathogenic bacteria and two marine pathogenic bacteria, with ciprofloxacin as a positive control. However, all compounds showed no antibacterial activity against these pathogenic bacteria in this assay (MIC values >20 μg/mL).

3. Experimental

3.1. General experimental procedures

Optical rotations were measured on a JASCO P-1020 digital polarimeter. UV spectra were recorded on a Beckman DU 640 spectrophotometer. IR spectra were obtained on a Nicolet 6700 spectrophotometer. NMR spectra were run on Bruker 400 MHz spectrometers using TMS as an internal standard. HRESIMS spectra were measured on a Q-TOF Ultima Global GAA076 LC mass spectrometer. Semi-preparative HPLC was performed on an Agilent 1260 LC series with a DAD detector using an Agilent Eclipse XDB-C_{18} column (250 × 9.4 mm, 5 μm). Silica gel (Qing Dao Hai Yang Chemical Group Co.; 200–300 mesh) and octadecylsilyl silica gel (YMC; 50 μm) were used for column chromatography (CC). Precoated silica gel plates (Yan Tai Zi Fu Chemical Group Co.; G60, F-254) were used for thin layer chromatography (TLC).

3.2. Plant material

The stems of *H. stenophyllum* were collected from Jianfengling Nature Reserve, Hainan Province China, in August 2015, and identified by Prof Qiong-Xin Zhong, College of Life Science, Hainan Normal University. A voucher specimen (No. SONG20150818) has been deposited at the Key Laboratory of Tropical Medicinal Plant Chemistry of Ministry of Education, Hainan Normal University.

3.3. Extraction and isolation

The powdered air-dried stems of *H. stenophyllum* (20.0 kg) were extracted with 95% EtOH at room temperature for three times, each for 5 days. The solvent was combined and condensed *in vacuum* to obtain a crude extract. After suspended in water, the crude extract was extracted successively with petroleum ether and ethyl acetate to obtain the petroleum ether extract and the ethyl acetate extract. The ethyl acetate extract (898.6 g) was subjected to

silica gel column chromatography, eluted with petroleum ether/ethyl acetate (from 1:0 to 1:1) to yield five fractions (Fr.1–Fr.5). Fr.5 (36.4 g) was subjected to RP-18 column chromatography, eluted with CH_3OH/H_2O (from 40% to 100%) to afford five fractions (Fr.5A–Fr.5F). Fraction 5A (2.8 g) was purified by Sephadex LH-20 column chromatography, eluted with CH_3OH, then separated by separated by semi-preparative HPLC (CH_3CN/H_2O, 20:80 *v/v*) to afford **1** (35.3 mg), **2** (168.5 mg), **3** (86.3 mg) and **4** (210.9 mg). Fraction 5B (4.5 g) was purified by Sephadex LH-20 column chromatography, eluted with CH_3OH, then separated by silica gel column chromatography, eluted with $CHCl_3/CH_3OH$ 9:1 to afford **5** (29.7 mg) and **9** (22.4 mg). Fraction 5C (894.8 mg) was further separated by semi-preparative HPLC (CH_3CN/H_2O, 50:50 *v/v*) to obtain **6** (89.6 mg) and **8** (131.3 mg).

Phloretin-4-*O*-*β*-D-glucopyranoside (**1**): Yellowish amorphous powder; $[\alpha]_D^{24}$+57.0 (c 0.12, CH_3OH); UV (CH_3OH) λ_{max} (log *ε*) 286 nm (3.92); IR (KBr) ν_{max} 3290, 1628, 1521, 1452, 1367, 1249 and 1203 cm^{-1}; ESI-MS *m/z* 437 $[M + H]^+$; HR-ESI-MS *m/z* 437.1446 (M + H; calcd for $C_{21}H_{24}O_{10}$, 437.1448); ^1H NMR (400 MHz, DMSO-d_6) *δ*: 7.14 (2H, d, *J* = 8.8 Hz, H-2, 6), 6.93 (2H, d, *J* = 8.8 Hz, H-3, 5), 5.79 (2H, s, H-3′, 5′), 4.80 (1H, d, *J* = 7.6 Hz, H-1″), 3.68 (1H, dd, *J* = 11.6, 5.2 Hz, H-6″*α*), 3.45 (1H, dd, *J* = 11.6, 6.0 Hz, H-6″*β*), 3.30 (2H, t, *J* = 7.6 Hz, H-*α*), 3.29 (1H, m, H-3″), 3.26 (1H, m, H-5″), 3.23 (1H, m, H-2″), 3.15 (1H, m, H-4″), 2.82 (2H, t, *J* = 7.6 Hz, H-*β*); ^{13}C NMR (100 MHz, DMSO-d_6) *δ*: 204.0 (C=O), 164.9 (C-4′), 164.5 (C-2′, 6′), 155.7 (C-4), 135.0 (C-1), 129.2 (C-2, 6), 116.2 (C-3, 5), 103.8 (C-1′), 100.6 (C-1″), 94.7 (C-3′, 5′), 77.0 (C-3″), 76.7 (C-5″), 73.3 (C-2″), 69.8 (C-4″), 60.7 (C-6″), 45.3 (C-*α*), 29.5 (C-*β*).

3.4. Acid hydrolysis of compound 1

Compound **1** (1.0 mg) was refluxed with 1 mL of 1 N HCl for 1 h at 100 °C. The reaction mixtures was extracted with ethyl acetate, and the aqueous phase was compared to an authentic sugar sample by co-TLC ($CH_3OH–H_2O–CH_3COOH$, 3:3:1, R_f = 0.46 for glucose). The identification of *β*-D-glucose in each aqueous layer was realised by comparing the optical rotation of the liberated glucose with that of an authentic sample of *β*-D-glucose ($[\alpha]_D^{24}$+55.0).

3.5. Antimicrobial assay

The antibacterial activities of all isolates were evaluated using the conventional broth dilution assay (Pierce et al. 2008). Six pathogenic bacteria were used, including four terrestrial pathogenic bacteria: *Escherichia coli*, *Staphylococcus aureus*, *Methicillin-resistant S. aureus* and *Bacillus cereus*, together with two marine pathogenic bacteria: *Vibrio parahaemolyticus* and *V. alginolyticus*.

4. Conclusions

A new dihydrochalcone glycoside, phloretin-4-*O*-*β*-D-glucopyranoside (**1**), together with seven known flavonoids (**2–8**), were isolated from the stems of *H. stenophyllum*. All known compounds (**2–8**) were isolated from the genus *Homalium* for the first time. The discovery of **1** is not only a further addition to diverse and complex array of dihydrochalcone glycoside, but also, the presence of **1–8** as characteristic marker may be helpful in chemotaxonomical classifications.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This work was financially supported by the National Natural Science Foundation of China [grant number 21362009], [grant number 81360478], [grant number 21302181]; the International S&T cooperation Program of China (ISTCP) [grant number 2014DFA40850]; the Special Project for TCM Modernization of Hainan Province [grant number 2015ZY19]; the Program for Innovative Research Team in University [grant number IRT-16R19].

References

Charubala R, Guggisberg A, Hesse M, Schmid H. 1974. Natural occurrence of 3-phenylisocoumarin. Helv Chim Acta. 57:1096–1097.

Cuendet M, Potterat O, Salvi A, Testa B, Hostettmann K. 2000. A stilbene and dihydrochalcones with radical scavenging activities from *Loiseleuria procumbens*. Phytochemistry. 54:871–874.

Ekabo OA, Farnsworth NR, Santisuk T, Reutrakul V. 1993a. A phytochemical investigation of *Homalium ceylanicum*. J Nat Prod. 56:699–707.

Ekabo OA, Farnsworth NR, Santisuk T, Reutrakul V. 1993b. Phenolic, iridoid and ionyl glycosides from *Homalium ceylanicum*. Phytochemistry. 32:747–754.

El-Naggar SAF, El-Feraly FS, Foos JS, Doskotch RW. 1980. Flavonoids from the leaves of *Kalmia latifolia*. J Nat Prod. 43:739–751.

Fan GS. 1990. A preliminary study on Flacourtiaceae from China. J Wuhan Bot Res. 8:131–141.

Ishikawa T, Nishigaya K, Uchikoshi H, Chen IS. 1998. Cochinolide, a new galkylidene bicyclic butenolide with antiviral activity, and its β-glucopyranoside from *Homalium cochinchinensis*. J Nat Prod. 61:534–537.

Itoh A, Tanahashi T, Ikejima S, Inoue M, Nagakura N, Inoue K, Kuwajima H, Wu HX. 2000. Five phenolic glycosides from *Alangium chinense*. J Nat Prod. 63:95–98.

Johns SR, Lamberton JA. 1969. Isolation of simple acid amides from *Allophylus cobbe* (Sapindaceae), *Homalium foetidum* (Flacourtiaceae), and from an *Aglaia* species (Meliaceae). Aust J Chem. 22:1315–1316.

Liu P, Xu Q, Wang C, Chen D, Sun Y. 2013. Antioxidant and antibacterial activities of crude extracts of *Homalium paniculiforum*. Asian J Chem. 25:4975–4978.

Mahapatra AK, Pani SS, Sahoo AK. 2015. Free radical-scavenging activities of *Homalium* species–An endangered medicinal plant of Eastern Ghats of India. Nat Prod Res. 29:2112–2116.

Nakamura Y, Watanabe S, Miyake N, Kohno H, Osawa T. 2003. Dihydrochalcones: evaluation as novel radical scavenging antioxidants. J Agric Food Chem. 51:3309–3312.

Okokon JE, Ita BN, Udokpoh AE. 2006. Antiplasmodial activity of *Homalium letestui*. Phytother Res. 20:949–951.

Okokon JO, Antia BS, Ita BN. 2007. Antidiabetic effects of *Homalium letestui* (Flacourtiaceae) in streptozotocin induced diabetic rats. Res J Med Plant. 1:134–138.

Païs M, Sarfati R, Jarreau FX, Goutarel R. 1973. Homalium alkaloids. Total synthesis of (2*S,* 2′*S*)-5, 5′-butylenedi(1-methyl-2-phenyl-1, 5-diazacyclooc-tane). Tetrahedron. 29:1001–1010.

Pan J, Zhang S, Yan L, Tai J, Xiao Q, Zou K, Wu J. 2008. Separation of flavanone enantiomers and flavanone glucoside diastereomers from *Balanophora involucrata* Hook. f. by capillary electrophoresis and reversed-phase high-performance liquid chromatography on a C_{18} column. J Chromatogr A. 1185:117–129.

Pierce CG, Uppuluri P, Tristan AR, Wormley FL, Mowat E, Ramage G, Lopez-Ribot JL. 2008. A simple and reproducible 96-well plate-based method for the formation of fungal biofilms and its application to antifungal susceptibility testing. Nat Protoc. 3:1494–1500.

Qin X, Xing YF, Zhou Z, Yao Y. 2015. Dihydrochalcone compounds isolated from crabapple leaves showed anticancer effects on human cancer cell lines. Molecules. 20:21193–21203.
Shaari K, Waterman PG. 1995. Glucosides of 2, 5-dihydroxybenzyl alcohol from *Homalium longifolium*. Phytochemistry. 39:1415–1421.
Shaari K, Waterman PG. 1996. D:A-friedo-oleanane triterpenes from the stems of *Homalium longifolium*. Phytochemistry. 41:867–869.
Wu SY, Fu YH, Chen GY, Li XB, Zhou Q, Han CR, Du XJ, Xie ML, Yao GG. 2015. Cytotoxic xanthene derivatives from *Homalium paniculiflorum*. Phytochemistry Lett. 11:236–239.
Youssef D, Frahm AW. 1995. Constituents of the egyptian *Centaurea scoparia*; III. Phenolic constituents of the aerial parts. Planta Med. 61:570–573.
Zhang ZQ, Zheng CJ, Bai M, Li XB, Song XP, Han CR. 2017. Cytotoxic constituents of the twigs of *Homalium stenophyllum*. Chem Nat Compd. 53:362–364.