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A NEW TRITERPENE AND A NEW PHLOROGLUCINOL DERIVATIVE FROM THE AERIAL PARTS OF *Pogostemon auricularius*

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*A new triterpene, pogostem (**1**), and a new phloroglucinol derivative, pogostemon D (**2**), were isolated from the aerial parts of* Pogostemon auricularius *(L.) Hassk. Their structures were determined from a combination of 1D NMR, 2D NMR, and HR-ESI-MS. Furthermore, pogostem (**1**) inhibited the proliferation of five human cancer cell lines, KB, SW-480, AGS, Hep-G2, and LU-1, with IC_{50} values ranging from 45.92 ± 5.65 to 65.83 ± 7.05 μg/mL.*

Keywords: *Pogostemon auricularius*, Lamiaceae, pogostemon, pogostem, cytotoxicity.

Pogostemon auricularius (L.) Hassk., a member of the Lamiaceae family, is distributed in many tropical and subtropical countries such as India, Sri Lanka, Bangladesh, China, and Southeast Asia and has been widely used in traditional folk medicine [1, 2]. *P. auricularius* leaf is given orally to treat tetanus and to relieve stomachache and urinary problems in children [3]. All of its parts have long been used to cure diarrhea and rheumatism [4, 5]. Furthermore, according to traditional Chinese medicine, *P. auricularius* is used to reduce fever and treat snake bites [2, 6]. Some extracts of *P. auricularius* were reported to have anti-inflammatory, antidiarrhea, and antimicrobial activities [7, 8]. Despite these reported activities, the number of studies on the chemical composition of *P. auricularius* is limited. Just four diterpenes have been isolated from *P. auricularius* [9, 10], and therefore further studies are warranted to clarify its phytochemical composition and bioactivities. In this study, the chemical structures and cytotoxic activities of a new triterpene, pogostem (**1**), and a new phloroglucinol derivative, pogostemon D (**2**), isolated from the aerial parts of *P. auricularius* collected in Vietnam are reported.

Compound **1** was obtained as a white amorphous powder. Its molecular formula was established as $C_{30}H_{48}O_5$ from the negative mode HR-ESI-MS quasimolecular ion at *m/z* 523.3201 $[M + Cl]^-$ (calcd for $C_{30}H_{48}O_5Cl$, 523.3190). The ^1H NMR spectrum of **1** exhibited five tertiary methyl groups at δ 0.90–1.09, ten methylenes including an oxygenated methylene group at δ 3.68 (1H, d, J = 10.0 Hz, H-23a) and 3.44 (1H, d, J = 10.0 Hz, H-23b), seven sp^3 methines including three oxymethines at δ 3.76 (1H, ddd, J = 10.5, 10.0, 4.0 Hz, H-2), 3.40 (1H, d, J = 10.0 Hz, H-3), and 4.08 (1H, d, J = 2.5 Hz, H-18), and one clearly distinguishable dioxygenated methine at δ 4.18 (1H, s, H-29) (Table 1). The signal at δ 5.77 (1H, quin, J = 2.5 Hz, H-12) was attributed to an olefinic proton. Additionally, a singlet signal at δ 3.36 (3H, s, H-28) was assigned to a methoxy group. The ^{13}C NMR and HSQC spectra gave 30 signals that were assigned to an olean-12-ene nor-triterpene with an unusual side chain. The chemical shifts at δ 69.0 (C-2), 80.9 (C-3), 49.7 (C-5), 47.6 (C-9), 118.6 (C-12), and 74.6 (C-18) and a hemi-acetal signal at δ 106.9 (C-29) were attributed to seven sp^3 methine carbons. A total of 10 methylenes including one hydroxyl methylene at δ 71.1 (C-23), one methoxy at δ 54.9 (C-28), and seven quaternary carbons were also clearly observed.

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TABLE 1. ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR Spectral Data for Compound **1** ($CDCl_3$, δ, ppm, J/Hz)

C atom	δ_C	δ_H	C atom	δ_C	δ_H
1	46.8	2.06 (dd, J = 4.5, 12.5); 0.94*	16	31.1	1.68*; 1.23 (t, J = 3.5)
2	69.0	3.76 (ddd, J = 10.5, 10.0, 4.0)	17	46.1	–
3	80.9	3.40 (d, J = 10.0)	18	74.6	4.08 (d, J = 2.5)
4	42.6	–	19	45.0	1.78 (br.d, J = 11.0); 1.08*
5	49.7	1.05*	20	44.1	–
6	18.5	1.40*	21	36.0	1.68*; 1.29 (dd, J = 4.5, 12.5)
7	33.7	1.51*; 1.40*	22	28.7	1.66*; 1.01*
8	39.6	–	23	71.1	3.68 (d, J = 10.0); 3.44 (d, J = 10.0)
9	47.6	1.55*	24	12.8	0.90 (s)
10	38.4	–	25	17.6	1.09 (s)
11	23.1	1.96 m	26	17.6	0.95 (s)
12	118.6	5.77 (quin, J = 2.5)	27	23.0	1.08 (s)
13	138.6	–	28	54.9	3.36 (s)
14	43.6	–	29	106.9	4.18 (s)
15	27.3	1.59*; 1.03*	30	21.6	0.96 (s)

*Overlapping signals.

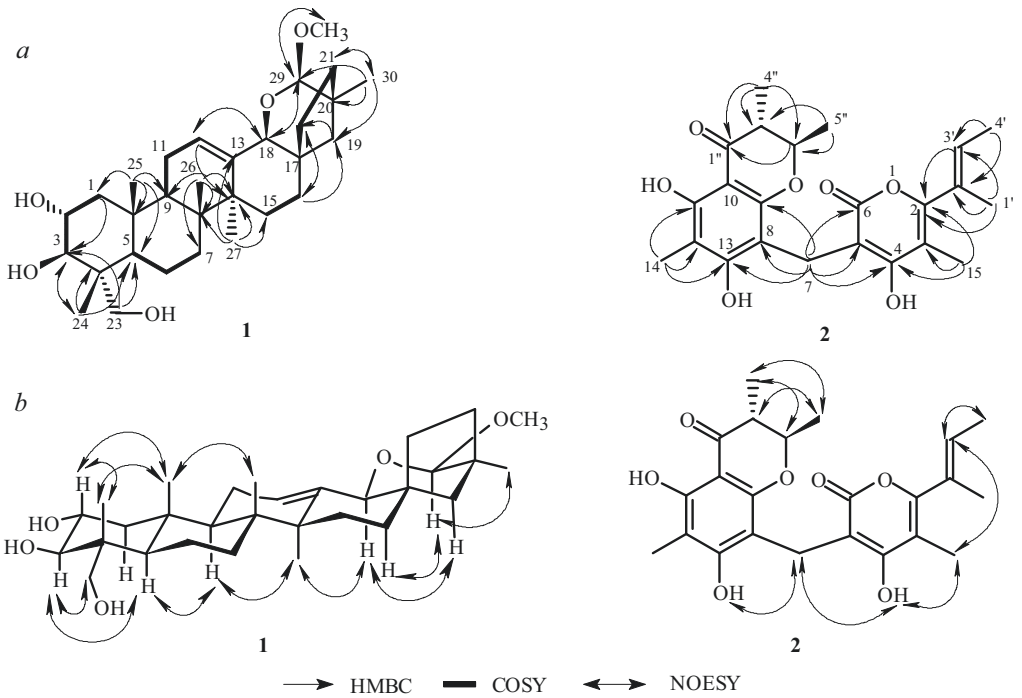

Fig. 1. Key HMBC, COSY (*a*), and NOESY (*b*) correlations of compounds **1** and **2**.

By comparing the NMR data of **1** with those of gomphoparvin B isolated from *Gomphostemma parviflorum* [11], the hemi-acetal group in both of these compounds was clearly recognized at C-29, as well as the F-ring that was formed by the linkage between C-18 and C-29 through an oxygen bridge [11]. This linkage was confirmed by the HMBC cross peak between H-29 (δ 4.18, s) and C-18 (δ 74.6) (Fig. 1). Additionally, the main difference between these compounds is the one methoxy group at C-29 (δ 106.9) in **1** instead of a hydroxyl in the same position in gomphoparvin B, which was supported by the HMBC correlations between δ_H 3.36 (3H, s) and C-29 (δ 106.9) and C-30 (δ 21.6). Compound **1** was confirmed to have a planar structure from analysis of its NOESY properties (Fig. 1). NOESY correlations were observed for H-2 (δ 3.76), H-24 (δ 0.90), and H-25 (δ 1.09) indicating that these protons were in the same β-orientation. By comparison, the NOE signals between H-3 and H-23, two pairs of H-27 and H-18, H-18 and H-29 and H-30 indicated they were all positioned in a similar α-orientation (Fig. 1). On the basis of the foregoing data, the structure of compound **1** was proposed to be (2α,3β,17*R*)-(18,29(methoxy))-epoxy-19(18→17)-abeo-28-norolean-12-ene-2,3,23-triol, and this compound was named pogostem.

TABLE 2. ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR Spectral Data for Compound **2** ($CDCl_3$, δ, ppm, J/Hz)

C atom	δ_C	δ_H	C atom	δ_C	δ_H
2	160.5	–	14	7.4	2.06 (s)
3	107.8	–	15	11.4	2.00 (s)
4	167.3	–	1′	14.5	1.88 (s)
5	102.1	–	2′	128.2	–
6	168.5	–	3′	132.0	5.78 (dq, J = 1.5, 7.0)
7	17.6	3.65 (s)	4′	13.9	1.79 (dd, J = 0.5, 7.0)
8	103.6	–	1″	197.3	–
9	154.6	–	2″	45.7	2.65 (dq, J = 12.0, 7.0)
10	101.1	–	3″	80.4	4.36 (dq, J = 12.0, 6.5)
11	160.5	–	4″	10.1	1.25 (d, J = 7.0)
12	107.4	–	5″	19.4	1.66 (d, J = 6.5)
13	163.1	–			

Compound **2** was obtained as a white amorphous powder. The molecular formula of **2** was established as $C_{23}H_{26}O_7$ on the basis of HR-ESI-MS that showed a molecular ion at $[M + H]^+$ *m/z* 415.1753 (calcd for $C_{23}H_{27}O_7$, 415.1757). In the ^{1}H NMR spectrum, an olefin proton was assigned to δ 5.78 (1H, dq, J = 1.5, 7.0 Hz, H-3′). In the high field, the signals of six methyl groups were identified including three singlet signals at δ 2.06 (3H, s, H-14), 2.00 (3H, s, H-15), and 1.88 (3H, s, H-1′), a double doublet signal at δ 1.79 (3H, dd, J = 0.5, 7.0 Hz, H-4′), and two double resonances at δ 1.25 (3H, d, J = 7.0 Hz, H-4″) and 1.66 (3H, d, J = 6.5 Hz, H-5″) (Table 2). Additionally, the signal of one methylene group was observed at δ 3.65 (2H, s, H-7). Interestingly, the other signals in the ^{1}H NMR spectrum were quite unique, including a proton signal at δ 12.27 attributed to a chelated hydroxyl group and two sp^3 proton signals at δ 2.65 (1H, dq, J = 12.0, 7.0 Hz, H-2″) and 4.36 (1H, dq, J = 12.0, 6.5 Hz, H-3″). The ^{13}C NMR data and HSQC experiment indicated the presence of 23 carbon atoms including six methyls, one methylene, three methines, and 13 quaternary carbons. Among them, two carbonyl carbons were identified at δ 197.3 (C-1″) and 168.5 (C-6), and two methyl carbons attached to the aromatic ring were observed at δ 7.4 (C-14) and 11.4 (C-15). The methylene carbon signal at δ 17.6 and its corresponding proton signals at δ 3.65 (2H, s, H-7) from the HSQC spectrum are typical for a methylene bridge of an A and B moiety and support the proposal that the main skeleton of compound **2** is a derivative of phloroglucinol [12]. Among the carbon signals of **2**, 10 of them were assigned to the α-pyrone in the B unit of the phloroglucinol derivative [12].

Compared with the A unit of phloroglucinol [12], compound **2** contained an oxygenated methine at δ_C 80.4, which suggested that the ring was closed at C-3″ (Fig. 1). This was confirmed by the coupling constants between H-2″ (2.65, 1H, dq, J = 12.0, 7.0 Hz) and H-3″ (4.36, 1H, dq, J = 12.0, 6.5 Hz) and H-4″ (1.25, 3H, d, J = 7.0 Hz), and H-5″ (1.66, 3H, d, J = 6.5 Hz), and the downfield shift of δ_C 154.6 (C-9) in **2**. The correlations between δ_H 1.25 (3H, H-4″) and δ_C 197.3 (C-1″), 45.7 (C-2″), and 80.4 (C-3″), between δ_H 1.66 (3H, H-5″) and δ_C 45.7 (C-2″) and 80.4 (C-3″), as well as between H-2″ (δ 2.65), H-3″ (δ 4.36) and C-1″ (δ 197.3), were observed in the HMBC spectrum (Fig. 1). The above NMR data suggested that there was a 2,3-dimethyl-4-chromanone skeleton in unit A of compound **2** [13]. The relative stereochemistry of the proton signals at C-2″ and C-3″ was deduced to be *trans* from their large coupling constant of 12.0 and 7.0 Hz and 12.0 and 6.5 Hz, respectively, and indicated a diaxial configuration between H-2″ and H-3″. Therefore, the absolute configuration of **2** was assigned as 2″*R*,3″*R* from the already established configuration of papuanic acid isolated from *Calophyllum papuanum* [14]. This was further supported by the analysis of the NOESY spectrum (Fig. 1). Comparison of the CD spectra of compound **2** with that of *trans*-(2*R*,3*R*)-5,7-dihydroxy-2,3-dimethyl-4-chromanone isolated from *Helichrysum paronychioides* [13] indicated the similarity in the curves, hence the absolute configuration of compound **2** was assigned as 2*R*,3*R*. Based on the above NMR data, the chemical structure of **2** is shown in Fig. 1 and it was given the common name pogostemon D.

The cytotoxicity of the isolated compounds against the growth of KB, SW-480, AGS, Hep-G2, and LU-1 cancer cell lines was tested using a sulforhodamine B assay [15], and the results are described in Table 3. The new triterpene pogostem (**1**) exhibited moderate growth inhibition with IC_{50} values ranging from 45.92 ± 5.65 to 65.83 ± 7.05 μg/mL. However, pogostemon D (**2**) did not show significant growth inhibitory activities (IC_{50} > 100 μg/mL) against the tested cancer cell lines.

TABLE 3. Cytotoxicity of Compound **1** against Five Human Cancer Cell Lines (IC_{50}[a], μg/mL)

Cell lines	**1**	Ellipticine[b]	Cell lines	**1**	Ellipticine[b]
KB	45.92 ± 5.65	0.37 ± 0.04	AGS	52.63 ± 4.72	0.41 ± 0.06
LU-1	65.83 ± 7.05	0.42 ± 0.06	SW-480	46.61 ± 4.23	0.38 ± 0.04
Hep-G2	54.23 ± 5.23	0.39 ± 0.03			

[a]IC_{50} (concentration of compound that inhibits of 50% of cell growth); [b]positive control.

EXPERIMENTAL

General Experimental Procedures. Optical rotations were measured using a JASCO P-2100 polarimeter (Hachioji, Tokyo, Japan). The 1D and 2D NMR were performed on a Bruker Avance 500 spectrometer (Billerica, Massachusetts) with TMS as internal reference. HR-ESI-MS data were measured an Agilent 6530 Accurate-Mass spectrometer (Agilent). The Agilent 1260 Infinity II system (Agilent Technologies) was used for analytical and preparative HPLC (250 × 9.4 mm). Open column chromatography was performed using silica gel (60 N, spherical, neutral, 40–50 μm, Kanto Chemical Co., Inc., Tokyo, Japan) and YMC RP-18 (Fuji Silysia Chemical Ltd, Kasugai, Aichi, Japan). Analytical TLC was performed on pre-coated silica gel $60F_{254}$ and RP-18 F_{254S} plates (0.25 or 0.50 mm thickness, Merck KGaA, Darmstadt, Germany). The cell lines KB (epidermoid carcinoma), LU-1 (lung adenocarcinoma), Hep-G2 (hepatocellular carcinoma), AGS (stomach gastric adenocarcinoma), and SW-480 (colon adenocarcinoma) were used for the cytotoxic activity determinations. Cell culture flasks and 96-well plates were from Corning Inc. (Corning, NY, USA). An ELISA plate reader (Bio-Rad, California) was used to measure the absorbance of the cells in the cytotoxicity assay.

Plant Material. The aerial parts of *Pogostemon auricularius* (L.) Hassk. were collected from Quang Tri Province, Vietnam (N16°44′38.9″ E107°14′51.1″) in May 2017 and were identified by Dr. Cuong The Nguyen, Institute of Ecology and Biological Resources, VAST, Vietnam. A voucher specimen (PA01) was deposited at the Faculty of Pharmacy, Hue University of Medicine and Pharmacy, Vietnam.

Extraction and Isolation. The dried leaves of *P. auricularius* (2.5 kg) were extracted with MeOH (10.0 L × 3 times) at room temperature to yield 207 g of a dark solid extract. The extract was then suspended in H_2O and successively partitioned with *n*-hexane, CH_2Cl_2, and EtOAc (each 5.0 L × 3 times) to obtain the *n*-hexane (H, 65 g), CH_2Cl_2 (D, 51 g), EtOAc (E, 47 g), and H_2O (W, 44 g) layers after removal of the solvents *in vacuo*.

The EtOAc extract was chromatographed on a silica gel column eluted with an *n*-hexane–EtOAc gradient system (100:0, 95:5, 90:10, 50:10, 10:10, 0:100, each 1.0 L) to obtain six corresponding fractions (E1–E6). Fraction E3 (7.9 g) was applied to a silica gel column and eluted with CH_2Cl_2–MeOH (15:1) to obtain seven subfractions (E3A–E3G). Fraction E3D (1.2 g) was partitioned on a Sephadex LH-20 column by elution with MeOH to give four smaller fractions (E3D1–E3D4). Fraction E3D3 (310 mg) was applied to an YMC RP-18 column and eluted with acetone–H_2O (10:1) to obtain five subfractions (E3D3A–E3D3E). Finally, fraction E3D3C (62 mg) was purified by preparative reversed phase HPLC (Zorbax SB – C_{18} 5 μm, 9.4 × 250 mm) using MeCN–H_2O (90:10, flow rate 2 mL/min) as the eluent to afford **1** (11.8 mg). Fraction E4 (8.3 g) was separated on a silica gel column eluted with CH_2Cl_2–acetone–MeOH (10:5:1) to give six smaller fractions (E4A–E4F). Fraction E4B (1.6 g) was applied to an YMC RP-18 column and eluted with MeOH–H_2O (10:1) to obtain seven subfractions (E4B1–E4B7). Fraction E4B2 (250 mg) was chromatographed on a silica gel column eluted with CH_2Cl_2–MeOH–H_2O (5:1:0.1) to obtain five subfractions (E4B2A–E4B2E). Fraction E4B2C (75 mg) was then partitioned on a Sephadex LH-20 column eluted with MeOH to yield **2** (7.9 mg).

Compound 1, white amorphous powder; $[\alpha]_D^{22}$ +103° (*c* 0.1, $CHCl_3$). For ^1H (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$), see Table 1. HR-ESI-MS *m/z* 523.3201 $[M + Cl]^-$ (calcd for $C_{30}H_{48}O_5Cl$, 523.3190).

Compound 2, white amorphous powder; $[\alpha]_D^{22}$ +192° (*c* 0.1, $CHCl_3$). For ^1H (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$), see Table 2. HR-ESI-MS *m/z* 415.1753 $[M + H]^+$ (calcd for $C_{23}H_{27}O_7$, 415.1757).

SRB Assay for Evaluating Cytotoxic Activity. The sulforhodamine B assay was used to examine the cytotoxic activities of the compounds isolated from the aerial parts of *P. auricularius* against the growth of five monolayer human cancer cell lines. The cell lines tested included oral epidermoid carcinoma (KB), colon adenocarcinoma (SW-480), gastric adenocarcinoma (AGS), liver hepatocellular carcinoma (Hep-G2), and lung adenocarcinoma (LU-1). Stock cultures were

grown in T-75 flasks containing 50 mL of Dulbecco′s modified Eagle medium with 2 mM L-glutamine, 1.5 g/L sodium bicarbonate, and 10% fetal bovine serum. The full methodology was described in our previous paper [16].

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