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α-GLUCOSIDASE INHIBITORS FROM *Barleria prionitis* LINN.

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Abstract. Phytochemical studies on the methanolic extract of *Barleria prionitis* Linn. collected in Luang Namtha province, Laos have led to the isolation and structural elucidation of four secondary metabolites, including two iridoid glycosides: shanzhiside methyl ester (**1**), barlerin (**2**); one sterol: stigmasterol (**3**); and a sterol glycoside: β-sitosterol glycoside (**4**). Their structures were identified by spectroscopic analyses such as 1D and 2D NMR spectra. In addition, two iridoid glycosides (**1**, **2**) showed weak inhibition of α-glucosidase.

Keywords: *Barleria prionitis*, iridoid glycoside, sterol, barlerin, α-glucosidase.

1. Introduction

Barleria is the third largest genus in the family of *Acanthaceae*. Many of these species have been used in traditional of many cultures for the treatment of various ailments. Evidence derived from several studies has demonstrated the antioxidant, antibacterial, antifungal, anti-inflammatory, anticancer, antidiabetic, and antiviral properties and toxicity of extracts of some *Barleria* species [1- 5]. Also, studies have reported that bioactive compounds such as flavonoids, quinones, quercetin, iridoids, iridoid glycoside, and phenylethanoid glycosides are responsible for the above biological activities. Therefore, plants of this genus have been studied for their chemical composition and biological activity such as *B. prionitis, B. cristata, B. grandiflora*, and *B. lupulina*. The compounds isolated from plants of this genus can be divided into six groups including iridoid, phenolic, phenylethanoid, flavonoid, terpenoid, quinone, and other compounds [6]. *Barleria prionitis* Linn. (Acanthaceae) is widely distributed throughout Africa, India, Sri Lanka, and tropical Asia such as China, Vietnam, Thailand, and Laos. This plant has been used extensively in folk medicines for the treatment of many common diseases such as cough, cold, sore throat, making sweat,

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detoxify, tooth decay, hemorrhoids, dermatitis, inflammation of the lymph nodes, antiviral, snake bite, stomach disorders, urinary affections, catarrh, fever in children, and cancer treatment [7-9]. The plant has also been reported for its biological activity against respiratory syncytial virus, anti-inflammatory, antimicrobial, antioxidant, antidiabetic, and anticancer, and showed hepatoprotective, antistress, and immunorestorative properties [10-15]. However, only a few natural compounds isolated from this plant have been tested for their biological activity. Furthermore, there is no report about the chemical constituents and biological activity of this plant from Laos. In the course of our investigation on the biologically active compounds from Laotian medicinal plants, we have collected *Baleria prionitis* Linn. in Luang Namtha province, Laos which allowed us to study its chemical constituents and the result is reported in this paper.

2. Content

2.1. Material and methods

2.1.1. Plant material

The whole plant of *Barleria prionitis* Linn. was collected in Luang Namtha province, Laos from July to December 2021 and identified by Bounnam XANGYAORN, Luangnamtha Teacher Training College, Luangnamtha, Laos. The voucher specimen (BP2021) has been deposited at the Faculty of Chemistry, Hanoi University of Education, Vietnam.

2.1.2. General procedure

TLC was carried out on precoated Si gel GF_{254} (Merck). TLC spots were viewed at 254, 302, and 366 nm and visualized by spraying with 10% H_2SO_4 in methanol followed by heating until the spots appeared. Column chromatography was carried out on silica gel 60 (60 - 100 µM, Merck) and Sephadex LH-20 (Amersham Pharmacia Biotech). Preparative medium-pressure liquid chromatography (MPLC) was performed with a Work-21 pump (Lab-Quatec Co., Ltd, Japan) and a Lobar column (Merck), with a flow rate of 1.0 mL/min. 1D and 2D NMR (^1H, ^{13}C NMR, HSQC, HMBC) spectra were recorded on a Bruker Avance 600 MHz Instrument. The mass spectra were obtained from a UPLC-ESI S4SH8000 (Water).

2.1.3. Extraction and Isolation

The whole plant of *Barleria prionitis* Linn. (10.0 kg) was dried and powdered. Then, it was extracted with methanol to afford the crude methanol extract (471 g), which was partitioned between *n*-hexane, chloroform, EtOAc, butanol, and water. The EtOAc extract (64 g) was isolated by silica gel column, using *n*-hexane/EtOAc gradient (from 2/1 to 1/1, v/v) and EtOAc/MeOH (from 98:2 to 80:20 v/v) to give 10 fractions (BPE1-10). Fraction PBE9 (6.8 g) was subjected to Sephadex LH-20 column, using MeOH/$CHCl_3$ (7/3, v/v), followed by MPLC with Lobar column, using $CHCl_3$/MeOH/H_2O (25/2.5/0.1, v/v/v) to afford compound **1** (17.5 mg) and compound **2** (23 mg). In addition, the *n*-hexane fraction (127 g) was chromatographed on a silica gel column, eluting with *n*-hexane/EtOAc gradient (from 20/1 to 1/5, v/v) and

EtOAc/MeOH (from 90:10 to 80:20 v/v) to give 23 sub-fractions (BPH1-23). Sub-fraction BPH7 was recrystallized and washed by MeOH several times to yield compound **3** (2.3 g). Finally, compound **4** (3.8 g) was collected by recrystallization and washing the precipitate of the sub-fraction BPH20D in the MeOH several times.

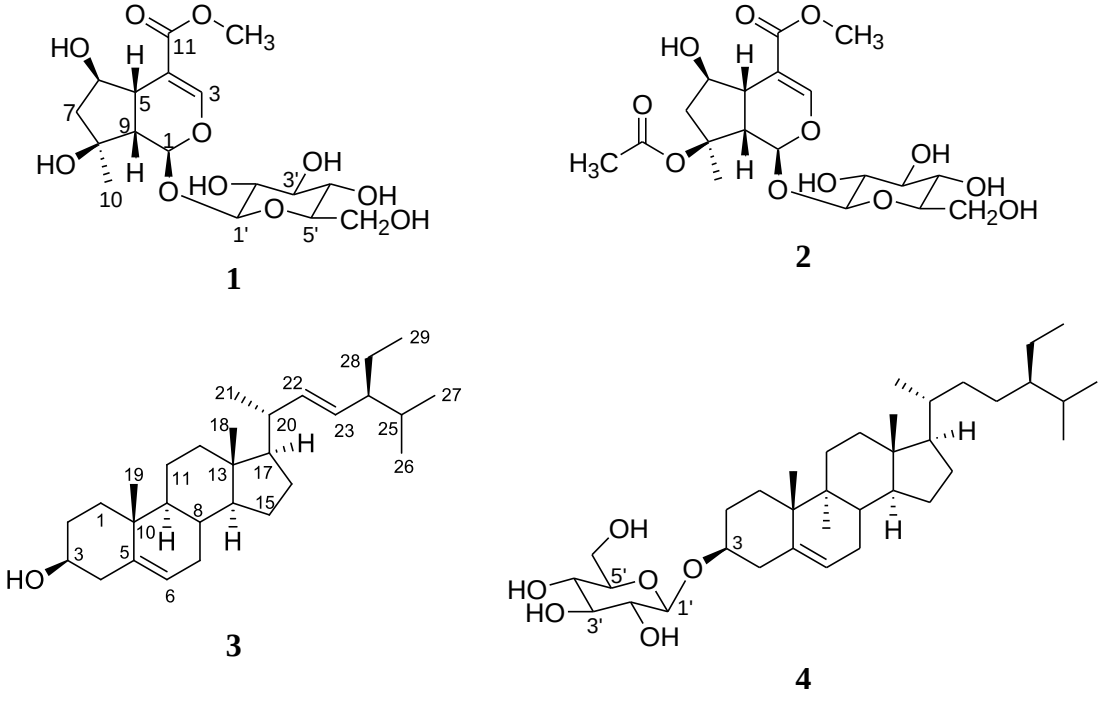

Figure 1. Structures of compounds 1-4

2.1.4. Spectral data for 1-4

Shanzhiside methyl ester (1): ^{1}H NMR (600 MHz, CH_3OD): δ_H 7.42 (d, *J* = 1.2 Hz, H-3), 5.59 (d, *J* = 2.4 Hz. H-1), 4.65 (d, *J* = 7.8 Hz, H-1'), 4.06 (m, H-6), 3.92 (dd, *J* = 1.8, 12.0 Hz, H-6'), 3.75 (3H, s, –OMe), 3.67 (dd, *J* = 6.0, 12.0 Hz, H-6'), 3.36 (t, *J* = 9.0 Hz, H-3'), 3.24 (m, H-5'), 3.27 (t, *J* = 9.0 Hz, H-4'), 3.19 (dd, *J* = 7.8, 9.0 Hz, H-2'), 3.02 (dd, *J* = 3, 10.2 Hz, H-5), 2.64 (dd, *J* = 2.4, 10.2 Hz, H-9), 2.03 (dd, *J* = 6.6, 13.2 Hz, H-7a), 1.85 (dd, *J* = 6, 13.2 Hz, H-7b), 1.28 (3H, s, H-10). ^{13}C NMR (150 MHz, CH_3OD): δ_C 169.73 (C-11), 152.80 (C-3), 111.43 (C-4), 99.83 (C-1'), 94.85 (C-1), 79.02 (C-8), 78.37 (C-5'), 78.00 (C-3'), 77.48 (C-6), 74.65 (C-2'), 71.65 (C-4'), 62.87 (C-6'), 51.88 (C-9), 51.78 (-OMe), 49.14 (C-7), 41.44 (C-5), 24.68 (C-10). ESI-MS: *m/z* 406.16 $[M]^+$.

Barlerin (2): ^{1}H NMR (600 MHz, CH_3OD): δ_H 7.46 (d, *J* = 1.2 Hz, H-3), 5.93 (d, *J* = 2.4 Hz, H-1), 4.66 (d, *J* = 8.4 Hz, H-1'), 4.05 (m, H-6), 3.92 (dd, *J* = 2.4, 12.0 Hz, H-6'), 3.74 (3H, s, –OMe), 3.69 (dd, *J* = 6.6, 12.0 Hz, H-6'), 3.38 (t, *J* = 9 Hz, H-3'), 3.33 (dd, *J* = 1.2, 4.8 Hz, H-9), 3.28 (t, *J* = 9 Hz, H-5'), 3.19 (dd, *J* = 7.8, 9.0 Hz, H-4'), 3.09 (dd, *J* = 1.8, 9.0 Hz, H-5), 3.02 (dd, *J* = 2.4, 9.0 Hz, H-2'), 2.22 (brs d, *J* = 14.4 Hz, H-7a), 2.06 (dd, *J* = 5.4, 15.0 Hz, H-7b), 2.03 (3H, s, –OCOMe), 1.53 (3H, s, H-10). ^{13}C NMR (125 MHz, CH_3OD): δ_C 173.14 ($\underline{C}OCH_3$), 169.05 (C-11), 153.67 (C-3), 109.85 (C-4),

100.39 (C-1'), 95.73 (C-1), 89.76 (C-8), 78.33 (C-5'), 78.03 (C-3'), 76.00 (C-6), 74.69 (C-2'), 71.65 (C-4'), 62.98 (C-6'), 51.79 (OCH_3), 49.98 (C-9), 47.64 (C-7), 42.30 (C-5), 22.21 ($COCH_3$), 22.20 (C-10). ESI-MS: *m/z* 449.06 $[M+H]^+$.

Stigmasterol (3): ^{1}H NMR (600 MHz, $CDCl_3$): δ_H 5.35 (m, H-6), 5.16 (dd, *J* = 8.4, 15.0 Hz, H-23), 5.02 (dd, *J* = 8.7, 15.0 Hz, H-22), 3.51 (m, H-3), 1.03 (3H, d, *J* = 6.6 Hz, H-21), 0.70 (s, H-19), 0.92 (d, *J* = 6.6 Hz, H-26), 0.85 (m, H-29), 0.83 (d, *J* = 6.6 Hz, H-27), 1.02 (s, H-18). ^{13}C NMR (125 MHz, $CDCl_3$): δ_C 140.79 (C-5), 138.32 (C-22), 129.32 (C-23), 121.73 (C-6), 71.84 (C-3), 56.81 (C-14), 56.11 (C-17), 45.89 (C-24), 50.21 (C-9), 42.34 (C-13), 42.34 (C-4), 40.49 (C-20), 39.81 (C-12), 37.29 (C-1), 36.54 (C-10), 31.94 (C-7, C-8), 31.90 (C-2), 31.70 (C-25), 29.21 (C-16), 25.42 (C-28), 24.39 (C-15), 21.23 (C-21), 21.11 (C-11, C-26), 19.83 (C-27), 19.00 (C-19), 12.25 (C-18), 12.07 (C-29).

β-sitosterol glycoside (4): ^{1}H NMR (600 MHz, DMSO): δ_H 5.33 (t, *J* = 2.4 Hz, H-6), 4.22 (d, *J* = 7.9 Hz, H-1'), 3.46 (m, H-3), 0.96 (s, H-19), 0.90 (d, *J* = 6.6 Hz, H-21), 0.82 (t, *J* = 7.2 Hz, H-29), 0.80 (d, *J* = 6.6 Hz, H-27), 0.77 (d, *J* = 6.6 Hz, H-26), 0.65 (s, H-18). ^{13}C NMR (125 MHz, DMSO): δ_C 140.44 (C-5), 121.15 (C-6), 100.78 (C-1'), 76.93 (C-3), 76.75 (C-3'), 76.70 (C-5'), 73.44 (C-2'), 70.10 (C-4'), 61.08 (C-6'), 56.24 (C-17), 56.15 (C-14), 49.59 (C-9), 45.13 (C-24), 41.83 (C-13), 39.16 (C-12), 38.29 (C-4), 36.81 (C-1), 36.19 (C-10), 35.45 (C-20), 33.33 (C-22), 31.40 (C-8), 31.34 (C-7), 29.24 (C-2), 28.70 (C-25), 27.75 (C-16), 25.46 (C-23), 23.83 (C-15), 22.59 (C-28), 20.57 (C-11), 19.67 (C-27), 19.06 (C-19), 18.82 (C-26), 18.59 (C-21), 11.75 (C-29), 11.64 (C-18).

2.2. Results and discussion

Compound **1** was isolated as an amorphous powder. Its ESI-MS has a molecular ion peak at *m/z* 406.16 $[M]^+$. The ^{1}H-NMR spectrum showed two singlet signals at δ_H 1.28 (s) and 3.75 (s) attributable to methyl (H-10) and methoxy group (-OMe) respectively. Also, it showed methine signals at δ_H 5.59 (d, *J* = 2.4 Hz), 7.42 (d, *J* = 1.2 Hz), 3.02 (dd, *J* = 3, 10.2 Hz), 4.06 (m), 2.64 (dd, *J* = 2.4, 10.2 Hz) assignable to H-1, H-3, H-5, H-6, H-9 respectively. Furthermore, an AB type of methylene signal was observed at δ_H 2.03 (dd, *J* = 6.6, 13.2 Hz) and 1.85 (dd, *J* = 6.6, 13.2 Hz) attributable to H-7a and H-7b. Analysis of ^{13}C NMR spectral data indicated that this compound contained 19 carbon atoms including six in a glucopyranosyl, one carbonyl carbon (δ_C 169.73, C-11), two olefinic carbon (δ_C 152.80 and 111.43, C-3 and 4), a methoxy group (δ_C 51.78, -OMe), a methyl group (δ_C 24.68, C-10), and others as shown in the experimental section. Inspection ^{1}H and ^{13}C NMR spectral data of compound **1** revealed the presence of one β-glucosyl and displayed signals characteristic of an iridoid glycoside. The glucopyranosyl was attached at C-1 due to HMBC correlations between i) H-1 and C-1', C-3, C-5, C-8; ii) H-1'and C-1, C-3', C-5'. Moreover, the double bond was deduced at C-3 and C-4 since H-3 was coupled to C-5, C-1, C-4, and C-11 in its HMBC spectrum. Furthermore, the hydroxy group was located at C-6 and C-8 due to HMBC correlations between H-6 and C-4, C-8, C-9; and C-8 and H-1, H-5, H-7, H-9, H-10 (Figure 2). The coupling constant between H-1 and H-9 (*J*=2.4 Hz), H-5 and H-9 (*J*=10.2 Hz) led to the conclusion that the position of the protons at C-1, C-5, and C-9 was in *α*, *β* and *β*-orientations, respectively. Finally, the structure of compound **1** was determined as shown in Figure 1, and named shanhiside methyl ester [16].

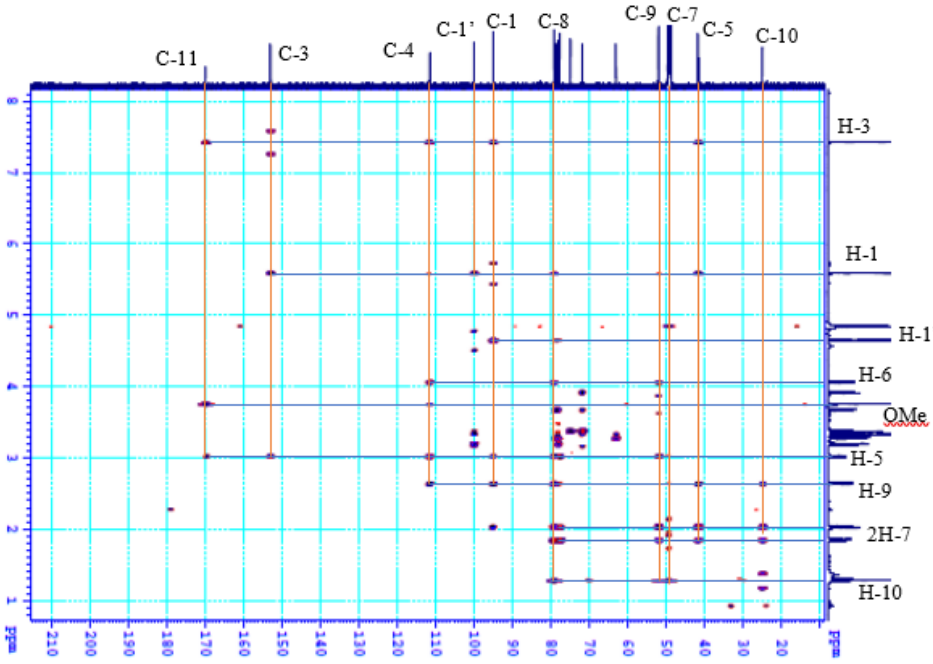

Figure 2. Important HMBC correlations of compound 1

Compound **2** was also isolated as an amorphous powder. The ESI-MS of compound **2** shows a *quasi*-molecular ion peak at *m/z* 449.06 $[M+H]^+$. The ^1H and ^{13}C NMR spectra indicated an iridoid glycoside structure. The chemical shifts were very similar to those of compound **1**, shanhiside methyl ester except that the acetyl group was observed in the spectra. The attachment of the acetyl group was assigned to C-8 (δ_C 98.76 ppm), the carbon signal of which was shifted to downfield by 10.74 ppm (compared with compound **1,** δ_C 79.02 ppm). Therefore, the structure of compound **2** was identified as barlerin [16].

Compound **3** was isolated as a white powder. Analysis of its ^1H and ^{13}C NMR spectra indicated that it contained two double bonds, including the first double bond (δ_H 5.33 ppm and δ_C 140.79, 121.73 ppm) and the second double bond (δ_H 5.16, dd, J = 9.0, 15.0 Hz and δ_H 5.02, dd, J = 9.0, 15.0 Hz; δ_C 129.32 and δ_C 138.32). The second double bond was established as *trans*-configuration due to their big coupling constants (J = 15.0 Hz). One carbinol group (δ_H 3.52 ppm and δ_C 71.8 ppm) together with six methyl groups, including two methyl singlets, three methyl doublets, and one methyl triplet were also observed. These NMR spectral data were identical to those of stigmasterol. Thus, compound **3** is stigmasterol [17].

Compound **4** was also obtained as a white powder. The NMR spectral data of compound **4** were quite similar to those of compound **3** except for two points. The first point is the absence of two olefinic signals at H-22 and H-23 in its ^1H NMR spectrum. Another point is the presence of β-D-glucose, an anomeric proton and carbon were resonance at 4.22 (d, J = 7.9 Hz) in its ^1H NMR spectrum and at 100.78 ppm in its ^{13}C NMR spectrum, respectively. Therefore, compound **4** was identified as β-sitosterol glycoside [18].

Previously, iridoid glycosides showed several interesting biological activities, such as anti-inflammatory [19, 20], and antiviral [21]. Thus, the antidiabetic of shanzhiside methyl ester (**1**) and barlerin (**2**) against α-glucosidase was investigated by the method described in [22]. The result showed that compounds **1** and **2** showed weak inhibition of α-glucosidase. At 256 µg/mL, compounds (**1**, **2**) suppressed α-glucosidase, with inhibition percentages of 25% and 36%, respectively.

3. Conclusions

Four secondary metabolites, shanzhiside methyl ester (**1**), barlerin (**2**), stigmasterol (**3**), and β-sitosterol glycoside (**4**) were isolated from the Laotian medicinal plant *Barleria prionitis* Linn. Their structures were determined by 2D NMR and MS spectroscopic methods. This is the first report on the chemical constituents and antidiabetic of the Laotian *Barleria prionitis* Linn.

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