New Iridoids from the Medicinal Plant *Barleria prionitis* with Potent Activity against Respiratory Syncytial Virus

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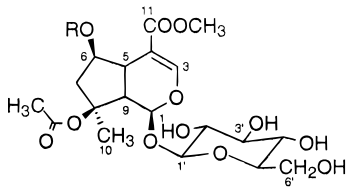
Two new iridoid glycosides (**1** and **2**), together with the known compounds barlerin (**3**) and verbascoside (**4**), were isolated from *Barleria prionitis*. The new iridoid glycosides were determined to be 6-*O*-*trans*-*p*-coumaroyl-8-*O*-acetylshanzhiside methyl ester (**1**) and its *cis* isomer (**2**) by using spectroscopic, especially 2D NMR, data. A 3:1 mixture of **1** and **2** was shown to have potent *in vitro* activity against respiratory syncytial virus (EC_{50} 2.46 μg/mL, IC_{50} 42.2 μg/mL).

Barleria prionitis L. (Acanthaceae) is a medicinal plant distributed throughout Africa, India, Sri Lanka, and tropical Asia.[1,2] Primary ethnobotanical field research by Shaman Pharmaceuticals in Thailand indicated that the leaves and roots of *B. prionitis* are taken orally to treat fever. Because a common cause of fever is a viral illness,[3] the use of this plant medicine may have been for antiviral activity. A decoction of leaves and flowers of *B. prionitis* is also used in India to treat fever,[4] while the green shoots of this plant are taken orally to treat whooping cough and asthma in infants and children.[5] One of the most common causes of "asthma-like" symptoms in infants is respiratory syncytial virus (RSV) infection of the lungs; some infants with RSV lung infections also cough excessively, giving symptoms resembling whooping cough.[6] A hot-water extraction of the dried leaves and roots of *B. cristata* is taken orally to treat bronchitis and coughs.[7]

Based on these ethnomedical uses, we evaluated a CH_2Cl_2−*i*-PrOH (1:1) extract of the whole plant of *B. prionitis* collected in Thailand for antiviral activity against RSV. The crude extract showed inhibitory activity against RSV strain A2 (EC_{50} 6.8 μg/mL) in the cytopathic effect (CPE) inhibition assay, but had limited cytotoxicity (IC_{50} 142 μg/mL). Guided by this *in vitro* antiviral activity, the extract was further fractionated into a mixture (EC_{50} 1.06 μg/mL, IC_{50} 20 μg/mL) enriched with iridoids and phenylpropanoid glycosides. Reversed-phase HPLC of the active fraction led to the isolation of two new iridoid glycosides, **1** and **2**. The known compounds barlerin (**3**) and verbascoside (**4**) were also isolated and identified by comparison of their NMR and MS with those reported in the literature.[8−11] The structures of **1** and **2** were determined by interpretation of their spectral data.

Compounds **1** and **2** were determined to be isomeric, with molecular formulas $C_{28}H_{34}O_{14}$, by HRFABMS. However, although a molecular ion for **1** was observed by positive ion FABMS, compound **2** only gave a molecular ion by negative ion FABMS. The similarity of their NMR spectra suggested that **1** and **2** were structurally related to the known iridoid glycoside barlerin (8-*O*-acetylshanzhiside methyl ester) (**3**). All of the ^{13}C NMR signals observed in **3** were also evident in both **1** and **2**, with the exception of the signals assigned to C-6. The presence of a glucose moiety was determined based on the ^1H and ^{13}C NMR signals assigned to the sugar and comparisons with known glucosides, including barlerin.[10,11] The β-configuration of the glucoside is consistent with the coupling constants observed for the anomeric protons (δ 4.68 ppm, J = 8.0 Hz for **1**; 4.66 ppm, J = 7.8 Hz for **2**). HMBC correlations for both **1** and **2** allowed assignment of the ^1H and ^{13}C NMR spectra and established that both compounds were *p*-coumaroyl ester derivatives of barlerin. Key HMBC correlations, observed in both **1** and **2**, required that the glucose be attached at C-1 (correlations from H-1 to C-1′, H-1′ to C-1) and the acetate at C-8 (H-8 to C=O). Both **1** and **2** also had NMR signals consistent with a *p*-coumaroyl ester; the UV absorbances at λ_{max} 229, 313 nm for **1** and 228, 309 nm for **2** were indicative of *p*-coumaroyl esters.



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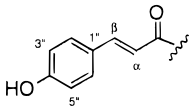
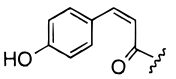
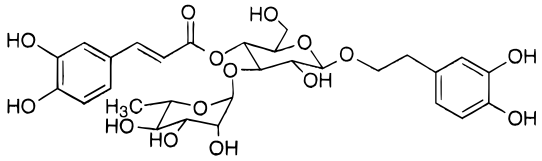
1 

2 

3 H



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The *p*-coumaroyl units were determined to be attached at C-6 on the iridoid group, based on the observed chemical shifts for C-6 (δ 78.86 for **1**, and 78.69 for **2**, as compared to δ 75.95 for **3**) and H-6 (δ 5.39 for **1** and 5.38 for **2**, as compared to δ 4.33 for **3**). The only difference between compounds **1** and **2** is the geometry of the double bonds in the coumaroyl unit, which were determined to be *trans* for **1** and *cis* for **2**, based on the observed chemical shifts and coupling constants (δ_α 6.34, δ_β 7.62, $J_{\alpha\beta} = 16.0$ Hz for **1**; δ_α 5.78, δ_β 6.90, $J_{\alpha\beta} = 12.7$ Hz for **2**). Hence, the structures of compounds **1** and **2** were determined to be 6-*O*-*trans*- and 6-*O*-*cis*-*p*-coumaroyl-8-*O*-acetylshanzhiside methyl ester, respectively. Although compounds **1**−**4** were all observed by HPLC analysis of the crude extract used for isolation, it is possible that compound **2** is formed from **1** during the extraction procedures.

A 3:1 mixture (as determined by ^{1}H NMR) of compounds **1** and **2** exhibited antiviral activity against RSV (A2 strain) in a cell culture-based CPE assay (EC_{50} 2.46 μg/mL). Under these assay conditions the sample was moderately cytotoxic (Hep-2 cells, IC_{50} 42 μg/mL) but had some selectivity for antiviral inhibition (selective index (SI) = $IC_{50}/EC_{50} = 17$). In comparison, verbascoside (**4**) was found to be more active (EC_{50} 0.80 μg/mL, IC_{50} 76.9 μg/mL, SI 85.4).[12] It is noteworthy that barlerin (**3**) was inactive in the same assay, implying that coumaroyl and/or caffeoyl moieties are important for anti-RSV activity. For comparison, ribavirin, an approved treatment for RSV infections, had EC_{50} 1.8 ± 0.2 μg/mL in the anti-RSV CPE assay. The quantities of pure compounds **1** and **2** isolated precluded their being evaluated individually for antiviral testing.

In conclusion, we have isolated three compounds with activity against RSV from the medicinal plant *B. prionitis*. The active constituents include the known compound verbascoside and two new iridoid glycosides, 6-*O*-*trans*-coumaroyl-8-*O*-acetylshanzhiside methyl ester and 6-*O*-*cis*-coumaroyl-8-*O*-acetylshanzhiside methyl ester. Although iridoid glycosides are commonly occurring natural products, iridoids with a coumaroyl group are rare.[11,13] The potent *in vitro* anti-RSV activity of compounds **1**, **2**, and **4**, together with the observed use of *B. prionitis* to treat symptoms of RSV, suggests that further investigations of the antiviral activity of these compounds are warranted.

Experimental Section

General Experimental Procedures. UV spectra were recorded on a Perkinf−Elmer Lambda 2 UV/vis spectrometer; and IR spectra, on a Perkin−Elmer 1600 Series FTIR spectrometer. All NMR spectra were recorded on a Varian Unity Plus 400 (400 MHz for ^{1}H and 100 MHz for ^{13}C), the chemical shifts (δ) are quoted in parts per million referenced to residual solvent signals, and coupling constants (J) are given in Hertz. MS were obtained on a Kratos MS-50 mass spectrometer. All solvents used for isolation were HPLC grade obtained from Fisher Scientific. Semipreparative HPLC separations were carried out using a Rainin Dynamax system.

Antiviral and Cytotoxicity Assays. The antiviral activities and cytotoxic effects of the described compounds and control antiviral compounds were determined using the viral CPE assay. The procedures used for the antiviral and cytotoxicity assays were previously described.[14,15] The antiviral activity of each sample was expressed in μg/mL as 50% effective concentration (EC_{50}), and cytotoxicity was expressed as 50% inhibitory concentration (IC_{50}).

Plant Material. The whole plant of *Barleria prionitis* L. (Acanthaceae) was collected on October 15, 1993, in Thailand by Michael Balick of the New York Botanical Garden and Rachan Pooma of the Royal Forest Department of Thailand. The plant was identified by Rachan Pooma and by Mary Merello of the Missouri Botanical Garden. Voucher specimens are deposited in the reference collection, Department of Ethnobotany and Conservation, Shaman Pharmaceuticals.

Extraction and Isolation. The whole plant of *B. prionitis* (125 g) was ground to a powder and extracted with CH_2Cl_2−*i*-PrOH (1:1) with gentle stirring at room temperature for ca. 24 h. After filtration, the extract was concentrated to dryness (2.4 g), suspended in MeOH (150 mL), and extracted successively with hexane (120, 100, and 80 mL). The MeOH-soluble fraction was evaporated (1.54 g), loaded onto a C18 Si gel column (4 × 20 cm), and eluted with 20, 50, and 100% aqueous MeOH (250 mL each). The antiviral activity was concentrated in a fraction eluting with 50% aqueous MeOH (250 mg). A portion of this fraction (50 mg) was purified by HPLC on C18 (Microsorb, 10 × 250 mm) using a MeCN−H_2O gradient (15−70% MeCN, 15 min; 70% MeCN, 10 min; 2.5 mL/min.) to give the mixture of **1** and **2** (t_R = 15 min., 12.0 mg), and known compounds **3** (t_R = 7 min., 16.5 mg, 0.066%) and **4** (t_R = 5 min., 4.0 mg, 0.016%). The mixture of **1** and **2** (5.2 mg) was purified by HPLC on C18 (Primesphere, 10 × 250 mm, 10% MeOH, 89% H_2O, 1% HOAc, 5 mL/min.) and the resulting fractions lyophilized to give **1** (3.8 mg, 0.035%) and **2** (1.0 mg, 0.009%).

6-*O*-*trans*-*p*-Coumaroyl-8-*O*-acetylshanzhiside methyl ester (1): isolated as an amorphous white solid; $[\alpha]_D$ −52.0° (MeOH, c 0.152); λ_{max} (log ϵ, MeOH) 229 (4.17), 313 (4.23) nm; IR (KBR) ν_{max} 3430 (br), 2952, 2918, 1714, 1634, 1606, 1516, 1442, 1373, 1279, 1169, 1082, 1056, 862, 835 cm^{-1}; ^{1}H NMR (CD_3OD, 400 MHz) δ 7.62 (1H, d, J = 16.0 Hz, H-β), 7.53 (1H, d, J = 1.4 Hz, H-3), 7.47 (2H, d, J = 8.6 Hz, H-2″,6″), 6.81 (2H, d, J = 8.6 Hz, H-3″,5″), 6.34 (1H, d, J = 16.0 Hz, H-α), 5.91 (1H, d, J = 3.3 Hz, H-1), 5.39 (1H, br dd, J = 5.4, 2.0 Hz, H-6), 4.68 (1H, d, J = 8.0 Hz, H-1′), 3.92 (1H, dd, J = 11.9, 2.0 Hz, H-6_a′), 3.70 (3H, s, 11-OCH_3), 3.67 (1H, dd, J = 11.9, 6.2 Hz, H-6_b′), 3.38 (1H, dd, J = 9.0, 8.8 Hz, H-3′), 3.35 (1H, ddd, J = 9.5, 6.2, 2.0 Hz, H-5′), 3.34 (1H, ddd, J = 8.6, 2.0, 1.4 Hz, H-5), 3.27 (1H, dd, J = 9.5, 8.8 Hz, H-4′), 3.20 (1H, dd, J = 9.0, 8.0 Hz, H-2′), 3.05 (1H, dd, J = 8.6, 3.3 Hz, H-9), 2.42 (1H, br d, J = 15.2 Hz, H-7_a), 2.13 (1H, dd, J = 15.2, 5.4 Hz, H-7_b), 1.98 (3H, s, 8-CH_3CO), 1.58 (3H, s, H-10); ^{13}C NMR (CD_3OD, 100 MHz) δ 172.85 (CO−acetate), 168.44 (2C, C-11, CO−coumaroyl), 161.35 (C-4″), 154.48 (C-3), 146.63 (C-β), 131.16 (2C, C-2″,C-6″), 127.12 (C-1″), 116.89 (2C, C-3″, C-5″), 115.42 (C-α), 108.62 (C-4), 100.38 (C-1′), 95.46 (C-1), 89.63 (C-8), 78.86 (C-6), 78.42 (C-5′), 78.00 (C-3′), 74.72 (C-2′), 71.70 (C-4′), 63.01 (C-6′), 51.86 (11-OCH_3), 50.44 (C-9), 45.19 (C-7), 40.04 (C-5), 22.22 ($COCH_3$), 21.84 (C-10); HMBC correlations H-1/C-3, C-5, C-1′; H-3/C-1, C-4, C-5, C-11; H-5/C-3, C-4, C-6, C-7, C-8; H-7a/C-5, C-6, C-8, C-9; H-9/C-1, C-4, C-5, C-8; H-10/C-7, C-8, C-9; 11-O*CH*$_3$/C-11; 8-CO*CH*$_3$/*C*OCH$_3$; H-1′/C-1; H-2′/C-1′, C-3′; H-3′/C-2′; H-4′/C-5′; H-5′/C-4′; H-2″,6″/C-2″,-4″, C-β; H-3″,5″/C-1″, C-3″,5″, C-4″; H-α/CO, C-1″; H-β/CO, C-α, C-2″,6″; positive HRFABMS m/z 595.1984 $[M + H]^+$, calcd for $C_{28}H_{35}O_{14}$ 595.2027, dev −7.2 ppm).

6-*O*-*cis*-*p*-Coumaroyl-8-*O*-acetylshanzhiside methyl ester (2): isolated as an amorphous solid; $[\alpha]_D$ −19.5° (MeOH, *c* 0.040); λ_{max} (log ϵ, MeOH) 228 (4.08), 309 (3.93) nm; IR (KBr) ν_{max} 3422 (br), 2924, 1715, 1634, 1605, 1514, 1440, 1372, 1161, 1084, 1010, 654 cm^{-1}; ^{1}H NMR (CD_3OD, 400 MHz) δ 7.63 (2H, d, J = 8.4 Hz, H-2″;6″), 7.52 (1H, d, J = 1.6 Hz, H-3), 6.90 (1H, d, J = 12.7 Hz, H-β), 6.76 (2H, d, J = 8.4 Hz, H-3″;5″), 5.87 (1H, d, J = 3.3 Hz, H-1), 5.78 (1H, d, J = 12.7 Hz, H-α), 5.37 (1H, ddd, J = 5.3, 2.0 Hz, H-6), 4.66 (1H, d, J = 7.8 Hz, H-1′), 3.91 (1H, dd, J = 12.1, 2.0 Hz, H-6_a′), 3.70 (3H, s, 11-OCH_3), 3.67 (1H, dd, J = 12.1, 6.2 Hz, H-6_b′), 3.37 (1H, dd, J = 9.0, 8.8 Hz, H-3′), 3.34 (1H, ddd, J = 9.5, 6.2, 2.0 Hz, H-5′), 3.28 (1H, dd, J = 9.5, 8.8 Hz, H-4′), 3.26 (1H, ddd, J = 8.0, 2.0, 1.6 Hz, H-5), 3.21 (1H, dd, J = 9.0, 7.8 Hz, H-2′), 2.92 (1H, dd, J = 8.0, 3.3 Hz, H-9), 2.40 (1H, br d, J = 15.0 Hz, H-7a), 2.10 (1H, dd, J = 15.0, 5.3 Hz, H-7b), 1.89 (3H, s, 8-CH_3-CO), 1.55 (3H, s, H-10); ^{13}C NMR (CD_3OD, 100 MHz) δ 172.88 (CO−acetate), 168.46 (C-11), 167.63 (CO−coumaroyl), 160.08 (C-4″), 154.55 (C-3), 144.94 (C-β), 133.59 (2C, C-2″,C-6″), 127.60 (C-1″), 116.92 (C-α), 115.91 (2C, C-3″, C-5″), 108.62 (C-4), 100.38 (C-1′), 95.43 (C-1), 89.55 (C-8), 78.69 (C-6), 78.35

(C-5′), 77.94 (C-3′), 74.64 (C-2′), 71.57 (C-4′), 62.92 (C-6′), 51.88 (11-OCH_3), 50.33 (C-9), 45.06 (C-7), 39.93 (C-5), 22.14 ($CO\mathit{C}H_3$), 21.88 (C-10); HMBC correlations H-1/C-3, C-5, C-1′; H-3/C-1, C-4, C-5, C-11; H-5/C-3, C-4, C-6, C-7, C-8; H-7a/C-5, C-6, C-8, C-9; H-9/C-1, C-4, C-5, C-8; H-10/C-7, C-8, C-9; 11-$OC\mathit{H}_3$/C-11; 8-$COC\mathit{H}_3$/$\mathit{C}OCH_3$; H-1′/C-1; H-2′/C-1′, C-3′; H-3′/C-2′; H-4′/C-5′; H-5′/C-4′; H-2″,6″/C-2″,-4″, C-*β*; H-3″,5″/C-1″, C-3″,5″, C-4″; H-α/CO, C-1″; H-*β*/CO, C-α, C-2″,6″; negative HRFABMS *m*/*z* 593.1817 $[M - H^+]^-$, calcd for $C_{28}H_{35}O_{14}$ 593.1870, dev −9.0 ppm).

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