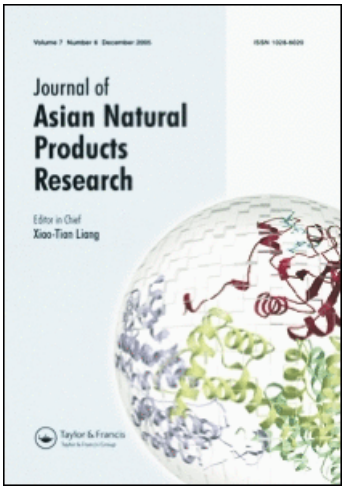

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A dipeptide and two glycosides from *Streptocaulon griffithii*

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A dipeptide and two glycosides from *Streptocaulon griffithii*

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Phytochemical investigation of the roots of *Streptocaulon griffithii* afforded a novel dipeptide, streptin (**1**), a new hemiterpenoid, (*R*)-3-ethyl-4-methylpentyl-β-rutinoside (**2**), and a new disaccharide, 1-methoxyl-4-*O*-β-glucopyronosyl-β-digitoxose (**3**), along with five known compounds. Their structures were identified by spectroscopic methods and comparison with literature values.

Keywords: *Streptocaulon griffithii*; Asclepiadaceae; dipeptide; hemiterpenoid; disaccharide; strepin

1. Introduction

Streptocaulon is a small genus of the Asclepiadaceae family with only five species. Two species (*Streptocaulon griffithii* and *Streptocaulon juventas*) are widely distributed in south China, Vietnam, Myanmar and neighboring countries. The roots of *S. griffithii* were used to treat malaria, diarrhea, nephritis, and influenza. The whole plant (especially the leaves) could be used for the treatment of snakebite [1]. In the previous phytochemical studies, cardenolides were shown to be the main chemical constituents [2,3], which exhibited significant antiproliferative activities against several human tumor cell lines [3]. Since hemiterpenoids, phenylpropanoids and a phenylethanoid were isolated from *S. juventas* besides cardenolides [4], further investigation on *S. griffithii* deserved our attention.

2,4-Diaminobutanoic acid (DABA) is a non-protein amino acid, which was first identified in *Polygonatum multiflorum* [5] and subsequently isolated from *Lathyrus latifolius* [6]. DABA is a homologue of ornithine [7]. It has been used as an important building block in medicinal chemistry exploration [8–10], and has been detected in several polypeptides [11,12]. But it has never been reported whether the unit 2,4-diguanidinobutanoic acid is derived from natural source or by chemical synthesis. Herein a novel dipeptide with the 2,4-diguanidinobutanoic acid unit, streptin (**1**), a new hemiterpenoid, (*R*)-3-ethyl-4-methylpentyl β-rutinoside (**2**), and a new disaccharide, 1-methoxyl-4-*O*-β-glucopyronosyl-β-digitoxose (**3**), were reported, along with five known compounds, 4,5-di-*O*-caffeoylquinic acid [13], 1,5-di-*O*-caffeoylquinic acid [14], caffeic acid [15], bergenin [16], and ciwujiatone [17].

2. Results and discussion

The air-dried roots of *S. griffithii* were extracted with H_2O/acetone (3:7). Subsequent bioassay-guided fractionation and separation resulted in the isolation and identification of the cardenolides [3]. Noncytotoxic fractions

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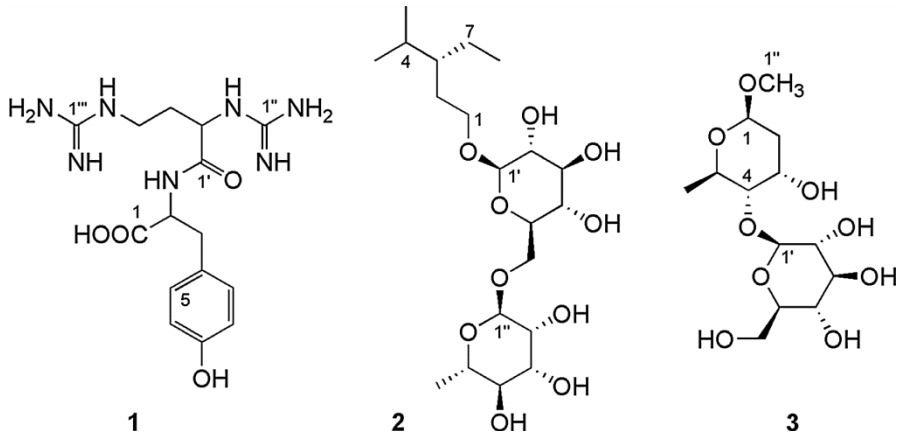

Figure 1. Structures of compounds (**1**–**3**).

contributed to all eight compounds by repetitive chromatography.

Compound **1** was obtained as yellow amorphous powder and was shown to possess a molecular formula of $C_{15}H_{23}N_7O_4$ by HRESIMS. Positive test to ninhydrin (purple) and guanidine-detection reagents (red) [18] could initially explain the high content of nitrogen in the molecular formula. IR bands at 3385 cm^{-1} (s, N—H), 1649 cm^{-1} (brs, C=N), 1385 cm^{-1} (m, C—N), and 1117 cm^{-1} (s, C—N) confirmed the existence of guanidine group. Two typical α-protons (δ_H 4.50, dd, $J = 4.0$, 9.8 Hz; δ_H 4.00, t, $J = 5.6$ Hz) in the ^1H NMR spectrum (Table 1) suggested **1** consists of two amino acid moieties. A pair of aromatic proton signals at δ_H 6.85 (2H, d, $J = 7.9$ Hz) and 7.20 (2H, d, $J = 7.9$ Hz), one methine at δ_H 4.50 (1H, dd, $J = 4.0$, 9.8 Hz) and one methene at δ_H 3.22 (1H, d, $J = 14.2$ Hz) and 2.82 (1H, dd, $J = 9.9$, 14.2 Hz) suggested one of the amino acids as tyrosine, which was also confirmed by ^{13}C NMR spectral data (δ_C 188.8, 59.3, 39.7, 132.4, 133.2, 133.2, 118.0, 118.0, 157.0) [19]. ^{13}C NMR spectrum (Table 2) implied DABA (δ_C 173.5, 51.8, 46.7, and 40.3) as another amino acid, and two remaining quaternary carbons (δ_C 159.8 and 159.4) as guanidine carbons [20]. HMBC correlation (Figure 2) from H-2 (δ_H 4.50, dd, $J = 4.0$, 9.8 Hz) to C-1′ (δ_C 173.5) connected two amino acid moieties. Also, HMBC correlations (Figure 2) from H-2′ (δ_H 4.00, t, $J = 5.6$ Hz) to C-1″ (δ_C 159.4), and from H-4′ [3.20 (1H, t, $J = 7.2$ Hz), 3.14 (1H, dd, $J = 3.0$, 14.1 Hz)] to C-1‴ (δ_C 159.8) connected two guanidine groups to C-2′ and C-4′, respectively. Therefore, the structure of compound **1** (streptin) was characterized as 2′,4′-diguanidinobutanol-tyrosine (Figure 1).

A molecular formula of $C_{20}H_{38}O_{10}$ was determined for compound **2** on the basis of the HRESIMS. The ^1H and ^{13}C NMR spectral data (Tables 1 and 2) for two anomeric signals ($\delta_{H/C}$ 4.23/105.0; $\delta_{H/C}$ 4.61/102.8) indicated **2** to be a diglycoside. Acid hydrolysis of **2** gave the glucose and rhamnose as the sugar moieties by co-TLC with authentic samples. The ^1H–^1H coupling constants ($J = 8.1$ Hz for glucose and $J = 1.6$ Hz for rhamnose) of the anomeric protons indicated the contrary glycosidic forms (β-form for glucose unit and α-form for rhamnose unit). The aglycone was determined to be (*R*)-3-ethyl-4-methylpentanol by comparing ^1H and ^{13}C NMR spectral data (Tables 1 and 2) and optical rotation values [$[\alpha]_D^{20} + 4$ ($c = 0.1$, $CHCl_3$)] with published data [21]. The HMBC correlations (Figure 2) revealed the presence of a β-rutinose (6-*O*-α-rhamnopyranosyl-β-glucopyranose) unit at C-1 (δ_C 70.6). Therefore, the structure of compound **2** was elucidated as (*R*)-3-ethyl-4-methylpentyl β-rutinoside.

The molecular formula of compound **3** was determined by HRESIMS as $C_{13}H_{24}O_9$.

The ^{1}H and ^{13}C NMR spectral data (Tables 1 and 2) for two anomeric signals ($\delta_{H/C}$ 4.61/100.5; $\delta_{H/C}$ 4.29/105.9) and a methoxy group signal ($\delta_{H/C}$ 3.34/56.9) indicated **3** to be a methoxy disaccharide. The methyl (δ_C 18.8) and a methene (δ_C 38.6) in the ^{13}C NMR spectrum inferred the presence of a deoxy-sugar. NMR assignments of the glucose and digitoxose units (Tables 1 and 2) were facilitated by comparison with the series NMR data of glycosides [4], and confirmed by $^{1}H-^{1}H$ COSY and HMBC experiments (Figure 2). The $^{1}H-^{1}H$ coupling constants ($J = 7.8$ Hz for glucose and $J = 1.9$, 9.5 Hz for digitoxose) of the anomeric protons indicated both sugar units to be in the β-glycosidic form. The HMBC correlations (Figure 2) between H-1′ (δ_H 4.29, 1H, d, $J = 7.8$) of the glucose unit and C-4 (δ_C 84.3) of the digitoxose unit, between protons of methoxyl group (δ_H 3.34, 3H, s) and C-1 (δ_C 100.5) of the digitoxose unit confirmed compound **3** as 1-methoxyl-4-*O*-β-glucopyronosyl-β-digitoxose.

Comparing the composition of compounds from *S. juventas* (Ueda *et al.* 2003), *S. griffithii* also offered cardenolides [2,3], hemiterpenoid and phenylpropanoids as the major constituents. But the minor constituents such as bergenin, ciwujiatone, and streptin (**1**) were only isolated from *S. griffithii*. Moreover, a peculiar constituent including a 2,4-diguanidinobutanoic acid unit, streptin (**1**), needs further chemical and pharmaceutical exploration.

3. Experimental

3.1 General experimental procedures

The optical rotations were obtained on a Perkin-Elmer 341 polarimeter, and the UV

Table 1. ^{1}H NMR spectral data of compounds **1**–**3** (400 Hz, δ-values)[a].

Atom	**1**[b]	Atom	**2**[b]	**3**[c]
2	4.50 (1H, dd, 4.0, 9.8)	1	3.98 (1H, dd, 1.9, 11.4) 3.53 (1H, d, 11.4)	4.61 (1H, dd, 1.9, 9.5)
3	3.22 (1H, d, 14.2) 2.82 (1H, dd, 9.9, 14.2)	2	1.59 (2H, m)	1.89 (1H, ddd, 2.1, 3.7, 13.5) 1.56 (1H, ddd, 2.9, 9.5, 13.5)
5, 9	6.85 (2H, d, 7.9)	3	1.20 (1H, m)	4.19 (1H, dd, 2.9, 6.2)
6, 8	7.20 (2H, d, 7.9)	4	1.73 (1H, m)	3.17 (1H, m)
2′	4.00 (1H, t, 5.6)	5	0.86 (3H, d, 7.2)	3.81 (1H, m)
3′	2.60 (1H, dd, 7.3, 14.3) 2.50 (1H, dd, 5.6, 14.3)	6	0.84 (3H, d, 7.2)	1.22 (3H, d, 6.3)
4′	3.20 (1H, t, 7.2) 3.14 (1H, dd, 3.0, 14.1)	7	1.25 (2H, m)	
		8	0.90 (3H, t, 6.9)	
		1′	4.23 (1H, d, 8.1)	4.29 (1H, d, 7.8)
		2′	3.15 (1H, dd, 7.8, 8.6)	3.14 (1H, t, 7.8)
		3′	3.32 (1H, m)	3.28 (1H, m)
		4′	3.24 (1H, m)	3.26 (1H, m)
		5′	3.39 (1H, m)	3.19 (1H, m)
		6′	3.96 (1H, dd, 1.9, 11.4) 3.59 (1H, m)	3.73 (1H, dd, 2.1, 11.9) 3.60 (1H, dd, 5.0, 11.9)
		1″	4.61 (1H, d, 1.6)	3.34 (3H, s)
		2″	3.87 (1H, m)	
		3″	3.62 (1H, m)	
		4″	3.36 (1H, m)	
		5″	3.67 (1H, m)	
		6″	1.30 (3H, d, 6.0)	

[a] Assignments are based on HMQC and HMBC spectra.
[b] Recorded in D_2O.
[c] Recorded in CD_3OD.

Table 2. ^{13}C NMR spectral data of compounds **1**–**3** (100 Hz, δ-values)[a].

Atom	**1**[b]	Atom	**2**[b]	**3**[c]
1	188.8 (s)	1	70.6 (t)	100.5 (d)
2	59.3 (d)	2	31.8 (t)	38.6 (t)
3	39.7 (t)	3	44.0 (d)	68.4 (d)
4	132.4 (s)	4	30.9 (d)	84.3 (d)
5, 9	133.2 (d)	5	20.3 (q)	70.0 (d)
6, 8	118.0 (d)	6	19.8 (q)	18.8 (q)
7	157.0 (s)	7	24.8 (t)	
1′	173.5 (s)	8	12.8 (q)	
2′	51.8 (d)	1′	105.0 (d)	105.9 (d)
3′	40.3 (t)	2′	75.6 (d)	75.3 (d)
4′	46.7 (t)	3′	78.7 (d)	78.1 (d)
1″	159.4 (s)	4′	72.2 (d)	71.4 (d)
1‴	159.8 (s)	5′	77.3 (d)	78.0 (d)
		6′	68.7 (t)	62.6 (t)
		1″	102.8 (d)	56.9 (q)
		2″	72.7 (d)	
		3″	72.9 (d)	
		4″	74.5 (d)	
		5″	70.3 (d)	
		6″	18.5 (q)	

[a] TMS was used as internal standard. Assignments are based on HMQC and HMBC spectra.
[b] Recorded in D_2O.
[c] Recorded in CD_3OD.

and IR spectra were recorded on a Shimadzu UV-2450 and a Perkin-Elmer 577 spectrometer, respectively. The NMR spectra were taken in D_2O and CD_3OD on a Varian mercury NMR spectrometer operating at 400 MHz for ^1H and 100 MHz for ^{13}C, with TMS as internal standard. ESI mass spectra were recorded on a Bruker Esquire-3000 mass spectrometer. Column chromatography was carried out on Diaion HP-20 (Mitsubishi Chemical Industries Co. Ltd, Tokyo, Japan), TSK gel Toyopearl HW-40F (30–60 μm; Toso Co. Ltd. Tokyo, Japan), MCI gel CHP-20P (75–150 μm; Mitsubishi Chemical Industries Co. Ltd), and Cosmosil 75 C_{18}-OPN (40–105 μm; Nacalai Tesque Inc., Tokyo, Japan). TLC was performed on $HSGF_{254}$ silica gel plates (Yantai Jiangyou Silica Exploration and Development Co., Ltd, Yantai, China).

3.2 Plant material

The roots of *S. griffithii* were collected from Guangxi Province, China, in August 2004, and identified by Professor He-Ming Yang. A voucher specimen (No. SG001) is deposited at the Herbarium of the Shanghai Institute of Materia Medica, Chinese Academy of Sciences.

3.3 Extraction and isolation

The air-dried and powdered roots (5 kg) were extracted with H_2O/acetone (3:7) at room temperature (15 l × 3). After concentration under vacuum to remove the organic solvent, the suspended residue was removed by centrifugation. The resulting aq. solution was submitted to column chromatography (Diaion HP-20; MeOH/H_2O 0:100, 50:50, 100:0) to afford three fractions (fractions A–C). Fraction A (sugar-containing fraction) was discarded, and fraction B was submitted to column chromatography over CHP-20P column and eluted with MeOH/H_2O (0:100, 50:50, 100:0) to give three subfractions B1–B3. Subfraction B1 was submitted to Toyopearl HW-40F column and eluted with H_2O to afford compounds **1** (21.2 mg) and

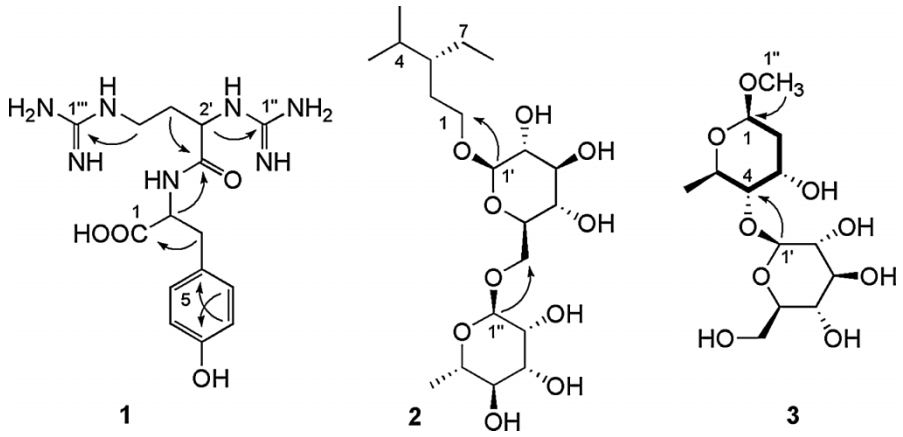

Figure 2. Key $^{1}H-^{1}H$ COSY (—) correlations of compound **3**, and key HMBC correlations ($^{1}H \rightarrow ^{13}C$) of compounds (**1**–**3**).

2 (9.5 mg). Subfraction B2 was submitted to Toyopearl HW-40F column and eluted with gradient MeOH/H_2O (10:90, 30:80, 50:50) to afford compound **3** (10.7 mg), caffeic acid (28.3 mg), 4,5-di-*O*-caffeoylquinic acid (11.1 mg), and 1,5-di-*O*-caffeoylquinic acid (7.6 mg). Subfraction B3 was submitted to Sephadex LH-20 column and eluted with MeOH to afford bergenin (108 mg). Fraction C was submitted to Cosmosil 75 C_{18}-OPN column and eluted with MeOH to afford ciwujiatone (5.6 mg).

*3.3.1 Streptin (**1**)*

Yellow amorphous powder; $[\alpha]_D^{20} + 1.4$ ($c = 0.25$; 0.5 N ammonia); UV (H_2O) λ_{max} (log ε) nm: 276 (2.81), 223 (3.78); IR (KBr) ν_{max}: 3385, 1649 (brs), 1516 (m), 1385 (m), 1117 (s) cm^{-1}; ^{1}H and ^{13}C NMR spectral data (Tables 1 and 2); ESIMS (positive-ion mode) *m/z* 366 $[M + H]^+$; ESIMS (negative-ion mode) *m/z* 364 $[M - H]^-$; HRESIMS *m/z* 366.1890 $[M + H]^+$ (calcd for $C_{15}H_{24}N_7O_4$, 366.1893).

*3.3.2 (*R*)-3-Ethyl-4-methylpentyl-β-rutinoside (**2**)*

Yellow amorphous powder; $[\alpha]_D^{20} + 3$ ($c = 0.2$; H_2O); IR (KBr) ν_{max}: 3423, 2958, 2933, 2875, 1601 (w), 1458, 1385, 1070 cm^{-1}; ^{1}H and ^{13}C NMR spectral data (Tables 1 and 2); ESIMS (positive-ion mode) *m/z* 461 $[M + Na]^+$; HRESIMS *m/z* 461.2384 $[M + Na]^+$ (calcd for $C_{20}H_{38}O_{10}Na$, 461.2363).

*3.3.3 Methoxyl-4-*O*-β-glucopyronosyl-β-digitoxose (**3**)*

Yellow amorphous powder; $[\alpha]_D^{20} - 2$ ($c = 0.15$; H_2O); IR (KBr) ν_{max}:3417, 2968, 1653, 1587 cm^{-1}; ^{1}H and ^{13}C NMR spectral data (Tables 1 and 2); ESIMS (positive-ion mode) *m/z* 347 $[M + Na]^+$; HRESIMS *m/z* 347.1332 $[M + Na]^+$ (calcd for $C_{13}H_{24}O_9Na$, 347.1318).

3.4 Acidic hydrolysis

A solution of **2** (5 mg) in 5% aq. HCl was heated in a boiling water bath for 5 h. After cooling, H_2O was added, and the solution was extracted with $CHCl_3$ (3 ×). The aq. layer was neutralized with 10% aq. Na_2CO_3 solution Glucose and rhamnose were identified by co-TLC of the aq. solution with authentic samples. Also, the organic layer was subjected to optical rotation experiment.

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