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# TWO PHENYLPROPANOID GLYCOSIDES FROM *SPARGANIUM STOLONIFERUM*

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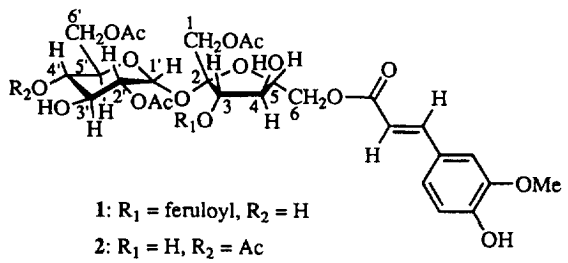
**Key Word Index**—*Sparganium stoloniferum*; Sparganiaceae; rhizome; phenylpropanoid glycosides.

**Abstract**—Two phenylpropanoid glycosides isolated from *Sparganium stoloniferum* were characterized as β-D-(1-*O*-acetyl-3,6-*O*-diferuloyl)fructofuranosyl-α-D-2′,6′-*O*-diacetylglucopyranoside and β-D-(1-*O*-acetyl-6-*O*-feruloyl)fructofuranosyl α-D-2′,4′,6′,-*O*-triacetylglucopyranoside. Copyright © 1997 Elsevier Science Ltd

## INTRODUCTION

'Sân Léng', a Chinese folk medicine, is used as an emmenagogue, a galactagogue, and an antispasmodic agent [1, 2], and originates from the rhizome of *Sparganium stoloniferum* Buch.–Hamil., *S. simplex* Huds., *S. stenophyllum* Maxima. (Sparganiaceae); *Scirpus flaviatilis* (Torr.) A. Gray. or *S. yagara* Ohwi (Cyperaceae) [1, 2]. Recently, we reported the isolation and the structure elucidation of three novel phenylpropanoid glycosides and three known phenylpropanoid glycerides from the rhizome of *S. stoloniferum* Buch.–Hamil. [3]. Further investigation of it led us to the isolation of two more novel phenylpropanoid glycosides. In this paper, structure elucidation of new isolates (**1** and **2**) by a combination of spectroscopic data including 2D NMR and chemical evidence is described.



**1**: $R_1$ = feruloyl, $R_2$ = H

**2**: $R_1$ = H, $R_2$ = Ac

## RESULTS AND DISCUSSION

Compound **1** was obtained as an amorphous solid with an elemental composition $C_{38}H_{44}O_{20}$, established by HR-mass spectrometry. In the $^1$H NMR spectrum (Table 1), it was suggested that **1** contained two feruloyl moieties and three acetyl groups. Fourteen oxymethine and methylene proton signals around $\delta_H$ 4.0–5.2, and 12 carbon signals around $\delta_C$ 65–103 suggested the presence of a disaccharide moiety. Alkaline and acid hydrolysis of **1** gave sucrose and a mixture of glucose and fructose, which were identified by direct comparison with authentic samples on TLC. A characteristic doublet signal with a smaller coupling constant at $\delta_H$ 6.26 (1H, *d*, $J$ = 3.7 Hz) appeared in the $^1$H which was ascribed to the anomeric proton in the α-glucopyranose unit [4, 5], also supported the presence of a sucrose moiety in **1**. The position of the linkage of the feruloyl and the acetyl groups on the sucrose was assigned by $^1$H-detected multiple-bond heteronuclear multiple quantum coherence spectroscopy (HMBC). That is to say, in this HMBC spectrum, the methine proton ($\delta_H$ 6.28) of position 3 and one *trans* olefinic proton ($\delta_H$ 8.05) of position 7″ on a feruloyl moiety gave cross-peaks with the same carbonyl carbon ($\delta_C$ 167.0), and one set of the methylene protons ($\delta_H$ 5.08 and 5.15) of position 6 and another *trans* olefinic proton ($\delta_H$ 7.98) of position 7″ with the same carbonyl carbon ($\delta_C$ 167.4). Also, the methine and methylene protons of position 2′ ($\delta_H$ 5.40) and 6′ ($\delta_H$ 4.66 and 5.10) on the glucose, and the methylene protons of position 1 ($\delta_H$ 4.57 and 4.78) on the fructose showed cross-peaks with respective acetyl carbonyl carbons ($\delta_C$ 170.9, 171.0 and 170.3). Furthermore, acetylation of **1** afforded a penta-acetate, which was identical with the tetraacetates of three phenylpropanoid glycosides that had already been isolated by us recently [3]. Based on these spectroscopic data and chemical evidence, **1** was determined to be β-D-(1-*O*-acetyl-3,6-*O*-diferuloyl)fructofuranosyl α-D-2′,6′-*O*-diacetylglucopyranoside.

Compound **2** obtained as an amorphous solid with an

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Table 1. $^1$H NMR chemical shifts (ppm number of protons, multiplicity and $J$/Hz) for compounds **1** and **2***

| Assignment | 1 | 2 |
|---|---|---|
| Fructose | | |
| 1-Ha | 4.57 1H *d* 11.6 | 4.15 2H *br s* |
| 1-Hb | 4.78 1H *d* 11.6 | |
| 3-H | 6.28 1H *d* 8.4 | 4.16 1H *br s* |
| 4-H | 5.21 1H *br m* 7.7 | 4.06 1H *t* 7.9 |
| 5-H | 4.88 1H *m* | 3.99 1H *br m* |
| 6-Ha | 5.08 1H *dd* 7.6, 12.0 | 4.45 1H *br d*, 12.0 |
| 6-Hb | 5.15 1H *dd* 2.7, 12.0 | 4.46 1H *br d*, 12.0 |
| 1-OAc | 2.02 3H *s* | 2.08[c] 3H *s* |
| Glucose | | |
| 1′-H | 6.26 1H *d* 3.7 | 5.60 1H *d* 3.6 |
| 2′-H | 5.40 1H *dd* 3.7, 10.0 | 4.81 1H *dd* 3.6, 10.0 |
| 3′-H | 4.70 1H *br* t 10.0 | 4.08 1H *t* 9.6 |
| 4′-H | 4.04 1H *br* t 9.5 | 4.87 1H *t* 9.6 |
| 5′-H | 4.90 1H *br d* 8.1 | 4.21 1H *br m* |
| 6′-Ha | 4.66 1H *dd* 6.6 11.6 | 4.15 2H *br s* |
| 6′-Hb | 5.10 1H *br d* 11.6 | |
| 2′-OAc | 2.08 3H *s* | 2.09[c] 3H *s* |
| 4′-OAc | | 2.11[c] 3H *s* |
| 6′-OAc | 2.12 3H *s* | 2.14[c] 3H *s* |
| 3-Feruloyl | | |
| 2″-H | 7.39 1H *br s* | |
| 5″-H | 7.15 1H *d* 8.3 | |
| 6″-H | 7.30 1H *dd* 1.9, 8.3 | |
| 7″-H | 8.05 1H *d* 16.0 | |
| 8″-H | 6.70 1H *d* 16.0 | |
| 3″-OMe | 3.80[a] 3H *s* | |
| 4″-OH | 7.85[b] 1H *br s* | |
| 6-Feruloyl | | |
| 2‴-H | 7.29 1H *br s* | 7.08 1H *d* 1.9 |
| 5‴-H | 7.17 1H *d* 8.0 | 6.93 1H *d* 8.3 |
| 6‴-H | 7.24 1H *br d* 8.0 | 7.09 1H *dd* 1.9, 8.3 |
| 7‴-H | 7.98 1H *d* 16.0 | 7.68 1H *d* 16.0 |
| 8‴-H | 6.79 1H *d* 16.0 | 6.35 1H *d* 16.0 |
| 3‴-OMe | 3.83[a] 3H *s* | 3.93 3H *s* |
| 4‴-OH | 8.21[b] 1H *br s* | |

*Measurements were performed in $C_5D_5N$ for **1** and $CDCl_3$ for **2** at 400 MHz 300 K.

[a,b,c]Assignments for values bearing the same superscript may be reversed.

elemental composition, $C_{30}H_{38}O_{18}$, was also a phenylpropanoid glycoside. The $^1$H NMR spectrum suggested that **2** had one feruloyl moiety and four acetyl groups linked to a sugar. Acid and alkaline hydrolysis followed by TLC indicated that **2** contained a sucrose moiety like **1**. The HMBC spectrum of **2** revealed long-range couplings between the oxygenated methylene protons at positions 6 on the fructose, as well as the *trans* olefinic protons at positions 8‴ on the feruloyl group, and their respective carbonyl carbons ($^1$H $^{13}$C $^1$H: 4.45 and 4.46/167.9/6.35 ppm), so the feruloyl group was located at C-6. Analogously, long range correlations between methine and methylene protons at 1, 2′, 4′ and 6′ on the sucrose, and acetyl carbonyl carbon ($^1$H $^{13}$C: 4.15/170.8 ppm for C-1 acetyl group; 4.81/171.0 ppm for C-2′ acetyl group; 4.87/171.1 ppm for C-4′ acetyl group; 4.15/171.3 ppm for C-6′ acetyl group) indicated that the four acetyl groups are located at C-1, C-2′, C-4′ and C-6′. Furthermore, significant downfield shift of the methine protons of position 3 ($\delta_H$ 4.16–5.47), 4 ($\delta_H$ 4.06–5.41) and 3′ ($\delta_H$ 4.08–5.45) in the tetraacetate of **2** clearly certified that the positions of nonesterified hydroxyl groups on **2** were attached at C-3, C-4 and C-3′. These data confirmed that **2** is β-D-(1-*O*-acetyl-6-*O*-feruloyl)fructofuranosyl α-D-2′,4′,6′-O-triacetylglucopyranoside.

Complete assignments of the $^1$H and $^{13}$C NMR signals of compounds **1** and **2**, elucidated by homo and hetero 2D NMR, are shown in Tables 1 and 2.

Up to now, analogous esters of the phenylpropanoid acids, such as ferulic, sinapic or coumaric acid, with sucrose have been reported from Liliaceae [6–12], Polygonaceae [13], Brassicaceae [5], Polygalaceae [14–16] and Celastraceae [17]. To our knowledge, our research is the first case of the isolation of such phenylpropanoid glycosides from the Sparganiaceae.

## EXPERIMENTAL

*General.* Mps uncorr. The $[\alpha]_D$ values are given in $10^{-1c}$ cm$^2$ g$^{-1}$. FAB-MS spectra were obtained on a JEOL AX-505H spectrometer. Medium-pressure liquid chromatography (MPLC) was performed with CIG column system (22 mm i.d. × 300 mm or 22 mm i.d. × 100 mm, Kusano Scientific Co., Tokyo) packed with 10 or 5 μm silica gel. TLC was conducted on precoated Kieselgel 60 F254 (Art. 5715; Merck) and the spots were detected by heating after spraying with 10% $H_2SO_4$ or with orcinol reagent (orcinol, $FeCl_3$ and $H_2SO_4$). 1D, 2D $^1$H and $^{13}$C NMR spectra were recorded on Varian spectrometers (Gemini 300 and Unity plus 400) at 298 K. The NMR coupling constants ($J$) are given in Hz.

*Plant materials.* Rhizomes of *S. stoloniferum* Buch.–Hamil. (3.7 kg) were purchased at Tianjin, China, in 1992. The botanical identification was made by Mr Zhang Tie-jin (Tianjin Institute of Pharmaceutical Research). A voucher specimen has been deposited in the herbarium of Tianjin Institute of Pharmaceutical Research, China and National Institute of Health Sciences, Japan.

*Extraction and isolation.* $CH_2Cl_2$ -soluble fr. (21.9 g) obtained from MeOH extract (364 g) of the rhizome (3.7 kg) of *S. stoloniferum* was subjected to silica gel column chromatography using a $CH_2Cl_2$–EtOAc gradient (1:0–0:1) followed by EtOAc–MeOH gradient (9:1–0:1) to give 17 frs (A–Q). Frs L and M (eluted by 50% EtOAc in $CH_2Cl_2$) were subjected to Sephadex LH-20 column chromatography with a *n*-hexane–$CH_2Cl_2$–MeOH (4:5:1) solvent system to get **1** and **2**. These compounds were further purified by silica gel MPLC with $CH_2Cl_2$–MeOH (19:1) and *n*-hexane–EtOAc–MeOH (5:3:2) solvent systems.

*Compound* **1**. β-D-(1-*O*-Acetyl-3,6-*O*-diferuloyl) fructofuranosyl-α-D-2′,6′-*O*-diacetylglucopyranoside (**1**) was obtained as an amorphous solid

Table 2. $^{13}C$ NMR chemical shifts (ppm) for compounds **1** and **2***

| Assignment | 1 | 2 |
|---|---|---|
| Fructose | | |
| C1 | 65.4 *t* | 63.9 *t* |
| C2 | 103.2 *s* | 103.4 *s* |
| C3 | 78.5 *d* | 77.6 *d* |
| C4 | 73.7 *d* | 75.3 *d* |
| C5 | 81.2 *d* | 79.4 *d* |
| C6 | 65.5 *t* | 63.9 *t* |
| 1-OAc; $CH_3$ | 20.6 *q* | 20.5[a] *q* |
| 1-OA; C=O | 170.3 *s* | 170.8 *s* |
| Glucose | | |
| C1′ | 90.5 *d* | 89.5 *d* |
| C2′ | 74.0 *d* | 72.6 *d* |
| C3′ | 71.9 *d* | 69.6 *d* |
| C4′ | 71.8 *d* | 70.9 *d* |
| C5′ | 72.1 *d* | 68.7 *d* |
| C6′ | 65.0 *t* | 62.5 *t* |
| 2′-OAc; $CH_3$ | 21.0 *q* | 20.6[a] *q* |
| 2′-OAc; C=O | 170.9 *s* | 171.0 *s* |
| 4′-OAc; $CH_3$ | | 20.6[a] *q* |
| 4′-OAc; C=O | | 171.1 *s* |
| 6′-OAc; $CH_3$ | 20.8 *q* | 20.6[a] *q* |
| 6′-OAc; C=O | 171.0 *s* | 171.3 *s* |
| 3-Feruloyl | | |
| C1″ | 126.2 *s* | |
| C2″ | 111.9 *d* | |
| C3″ | 149.0 *s* | |
| C4″ | 151.4 *s* | |
| C5″ | 116.8 *d* | |
| C6″ | 123.0 *d* | |
| C7″ | 147.1 *d* | |
| C8″ | 114.0 *d* | |
| C9″ | 167.0 *s* | |
| 3″-OMe | 55.9 *q* | |
| 6-Feruloyl | | |
| C1‴ | 126.4 *s* | 126.9 *s* |
| C2‴ | 111.4 *d* | 109.6 *d* |
| C3‴ | 149.0 *s* | 147.2 *s* |
| C4‴ | 151.2 *s* | 148.6 *s* |
| C5‴ | 116.8 *d* | 115.0 *d* |
| C6‴ | 123.5 *d* | 123.6 *d* |
| C7‴ | 146.1 *d* | 146.6 *d* |
| C8‴ | 114.8 *d* | 114.4 *d* |
| C9‴ | 167.4 *s* | 167.9 *s* |
| 3‴-OMe | 55.9 *q* | 55.9 *q* |

* Measurements were performed in $C_5D_5N$ for **1** and $CDCl_3$ for **2** at 100 MHz.

[a] Assignments for values bearing the same superscript may be reversed.

(16 mg): mp 94–101°; $[\alpha]_D^{25}+73.4°$(*c* 0.06, $CHCl_3$); UV $\lambda_{max}^{MeOH}$ nm (log $\varepsilon$): 218 (4.39), 237 (4.34), 302 (4.38), 329 (4.55); IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3407, 1746, 1632, 1603, 1516, 1431, 1375, 1240, 1159, 1032; $^1H$ NMR (400 MHz, $CDCl_3$) listed in Table 1; $^{13}C$ NMR (100 MHz, $CDCl_3$) listed in Table 2; positive FAB-MS *m/z* (rel. int.): $[M+Na]^+$ 843 (10), 820 (3), 557 (20), 177 (77); positive FAB-MS (added KI) *m/z* (rel. int.): $[M+K^+$ 859 (23), 557 (7), 177 (86); HRFAB-MS *m/z*: $[M+Na]^+$ 843.2305 (calcd for $C_{38}H_{44}O_{20}Na$, 843.2326), $[M]^+$ 820.2437 (calcd for $C_{38}H_{44}O_{20}$, 820.2423).

*Compound* **2**. β-D-(1-*O*-Acetyl-6-*O*-feruloyl)-fructofuranosyl-α-D-2′,4′,6′-*O*-triacetylglucopyranoside (**2**) was obtained as an amorphous solid (11 mg): mp 96–102°; $[\alpha]_D^{25}+45.1°$ (*c* 0.10, $CHCl_3$); UV $\lambda_{max}^{MeOH}$ nm (log $\varepsilon$): 216 (4.21), 233 (4.12), 296 (4.08), 326 (4.22); IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3455, 1746, 1632, 1601, 1516, 1431, 1373, 1240, 1157, 1034; $^1H$ NMR (400 MHz, $CDCl_3$) listed in Table 1; $^{13}C$ NMR (100 MHz, $CDCl_3$) listed in Table 2; positive FAB-MS *m/z* (rel. int.): $[M+Na]^+$ 709 (15), 686 (2.5), 667 (12), 381 (25), 177 (45); HRFAB-MS *m/z*: $[M+Na]^+$ 709.1949 (calcd for $C_{30}H_{38}O_{18}Na$, 709.1959), $[M]^+$686.2061 (clacd for $C_{30}H_{38}O_{18}$, 686.2058).

*Alkaline hydrolysis of* **1** *and* **2**. Each phenylpropanoid glycoside (*ca* 0.1 mg) was dissolved in 3% KOH/MeOH and kept at room temp for 2 hr. The reaction mixt. was neutralized with 1N HCl and was subjected to a Sephadex LH-20 column using MeOH as eluant. Frs containing sugar were compared with standard sugars on silica gel TLC developed with EtOAc–MeOH–$H_2O$–acetic acid (6:2:1:1) and $CHCl_3$–MeOH–$H_2O$ (7:3:0.5), and detected by spraying with orcinol reagent (orcinol, $FeCl_3$ and $H_2SO_4$).

*Acid hydrolysis of* **1** *and* **2**. Each phenylpropanoid glycoside (*ca* 0.1 mg) was dissolved in 1 N HCl and refluxed for 2 hr. The reaction mixt. was neutralized with 3% KOH/MeOH and was subjected to a Sephadex LH-20 column using MeOH as eluant. Sugar identification was performed as described under alkaline hydrolysis above.

*Acetylation of* **1**. Compound **1** (3 mg) was dissolved in pyridine (0.2 ml) and treated with excess $Ac_2O$ (0.2 ml) at room temp for 2 days. The product was subjected to silica gel MPLC using $CH_2Cl_2$–MeOH (98:2) to give the tetraacetate of **1** (1.6 mg) as an amorphous solid. By comparison of $^1H$ NMR spectra, purified derivative was identical with tetraacetyl derivatives of three pheylpropanoid glycosides that we had already isolated [3].

*Acetylation of* **2**. Compound **2** (3 mg) was dissolved in pyridine (0.2 ml) and treated with excess $Ac_2O$ (0.2 ml) at room temp. The product was purified as for **1** to give the tetraacetate (2 mg) as an amorphous solid: mp 61–68°; $^1H$ NMR (400 MHz, $CDCl_3$): $\delta$ 2.01 (3H, *s*) × 2, 2.09 (3H, *s*), 2.11 (3H, *s*), 2.12 (3H, *s*) × 2, 2.20 (3H, *s*), 2.33 (3H, *s*), 3.88 (3H, *s*), 4.16 (1H, *br d*, $J$ = 11.9 Hz, H-6′), 4.19 (2H, *br s*, H-1), 4.23 (1H, *br d*, $J$ = 11.9 Hz, H-6′), 4.28 (1H, *m*, H-5′), 4.32 (1H, *m*, H-5), 4.43 (1H, *dd*, $J$ = 7.1, 12.1 Hz, H-6), 4.51 (1H, *dd*, $J$ = 4.4, 12.1 Hz, H-6), 4.88 (1H, *dd*, $J$ = 3.7, 10.4 Hz, H-2′), 5.07 (1H, *t*, $J$ = 9.9 Hz, H-4′), 5.41 (1H, *t*, $J$ = 5.7 Hz, H-4), 5.45 (1H, *br t*, $J$ = 9.9 Hz, H-3′), 5.47 (1H, *d*, $J$ = 9.5 Hz, H-3), 5.69 (1H, *d*, $J$ = 3.7 Hz, H-1′), 6.43 (1H, *d*, $J$ = 15.9 Hz, H-8‴), 7.08 (1H, *d*, $J$ = 8.4 Hz, H-5‴), 7.14 (1H, *s*, H-2‴), 7.15 (1H, *br d*, $J$ = 8.0 Hz, H-6‴); positive FAB-MS

*m/z* (rel. int.): $[M+Na]^+$ 877 (1.5), $[M]^+$ 854 (0.75), 812 (12), 507 (66), 331 (28), 169 (100); positive FAB-MS (added KI) *m/z* (rel. int.): $[M+K]^+$ 893 (100), 507 (12), 331 (6), 169 (32).

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