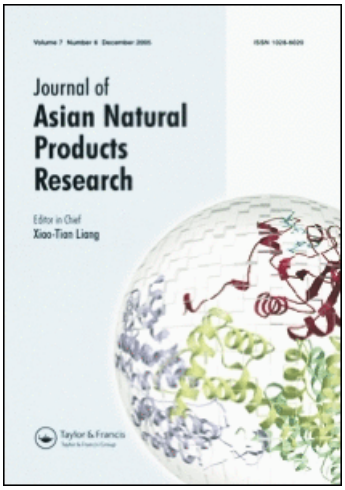

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Shegansu C, a Novel Phenylpropanoid Ester of Sucrose from *Belamcanda chinensis*

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SHEGANSU C, A NOVEL PHENYLPROPANOID ESTER OF SUCROSE FROM *BELAMCANDA CHINENSIS*

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A novel phenylpropanoid ester of sucrose, shegansu C (**1**), was isolated from the rhizome of *Belamcanda chinensis* (L.) DC. (**Iridaceae**) along with 18 known compounds. The structure of **1** was characterized as β-D-[3-*O*-(4-*O*-(3,4-,dimethoxycinnamoyl)-5-*O*-feruloyl)-caffeoyl]-fructofuranosyl(2-1′)-α-D-(3′-*O*-acetyl)-glucopyranoside on the basis of chemical and spectral evidence including 2DNMR studies.

Keywords: *Belamcanda chinensis*; Iridaceae; Phenylpropanoid sucrose shegansu C

INTRODUCTION

The rhizome of *Belamcanda chinensis* (L.) DC., a commonly used Chinese traditional medicine, has been prescribed as antiinfective, antitussive and expectorant. As part of our systematic studies on the chemical constituents of commonly used Chinese traditional medicine, we carried out the chemical work on *B. chinensis*. Various isoflavones and their glucosides [1,2], apocynin, sitosterol, *p*-hydroxybenzoic acid, resveratrol, isorhapontigenin and new compounds shegansu A [2] have been isolated from this drug. This paper describes the isolation and structure determination of the novel trimeric phenylpropanoid ester of sucrose, shegansu C (**1**). The structure differs from other phenylpropanoid sucrose ester discovered in many species of higher plant [4–7] in recent years.

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RESULTS AND DISCUSSION

Shegansu C (**1**) was isolated as yellowish amorphous powder with an optical rotation $[\alpha]_D^{31}$ +51.3 (c 0.075, EtOH). The FTMS of **1** showed a significant ion peak at *m/z* 911.2602 ($M^+ - 1$) which together with ^{1}HNMR of **1** and its acetyl derivative suggested that compound **1** had a molecular formula of $C_{44}H_{48}O_{21}$, bearing seven hydroxyl groups (one phenolic group) and one alcoholic acetyl group in the molecule. The UV spectrum of **1** exhibited typical absorptions of cinnamoyl moieties at λ_{max} 218, 236, 300, 326 nm. The IR spectrum of **1** showed the presence of hydroxyl groups (3300 cm^{-1}), a broad carbonyl band at 1700 cm^{-1} indicating the presence of at least two different types of carbonyl groups, double bond (1630 cm^{-1}) and aromatic ring (1600, 1520 cm^{-1}). The ^{1}HNMR spectrum of **1** (Table I) was complex in the region between δ 6.30–7.65 which integrated for 15 protons. Apparently in this region, the spectra were accommodated in three sets of coupled doublets at δ 6.45 and 7.61, δ 6.50 and 7.65 as well as at δ 6.30 and 7.47. The coupling constant for each set of doublets was 15.9 Hz, and each doublet integrated for one proton. These patterns suggested the presence of three sets of *trans* double bonds, each conjugated to a carbonyl group. The remaining nine protons could be attributed to three aromatic ABX systems of aromatic moieties to appear at δ 7.36, 7.31, 7.21 (each 1H, d, $J = 1.7$ Hz); δ 7.10, 6.94, 7.14 (each 1H, dd, $J = 8, 1.7$ Hz); δ 6.77, 6.75, 6.72 (each 1H, d, $J = 8$ Hz). The ^{1}HNMR spectrum also showed the presence of two overlapped doublet signals at δ 5.45 with coupling constants $J = 8.7$ and 3.6 respectively, two triplets at δ 5.21, 4.98 ($J = 9.7, 9.7$ Hz, each for 1H), multiple signals at δ 4.19–3.45 integrated for ten protons along with three methoxyl signals at δ 3.72, 3.73, 3.86 and one acetyl signal at δ 1.88. Examination of the signals between δ 5.45 and 3.45 in the ^{1}H-^{1}H COSY, ^{13}C-^{1}H COSY spectra for **1**, confirmed the presence of a sucrose moiety. One doublet ($J = 3.6$ Hz) at δ 5.45 was due to the anomeric proton of the glucosyl moiety. The doublet ($J = 8.7$ Hz) at δ 5.45 suggested the β configuration for H-3 of fructofuranosyl moiety. The ^{13}CNMR spectrum (Table II) further revealed 12 carbon signals arising from a disaccharide moiety, whose anomeric carbon signals at δ 101.5 and 91.3 were characteristic of sucrose. In addition, the ^{13}CNMR spectrum also displayed three pairs of olefinic carbons (δ 145.6, 114.0; 145.9, 113.8 and 145.4, 113.5), three ester carbonyls (δ 165.7, 166.0 and 165.9 ppm) and one acetyl group (δ 20.6, 169.7).

Thus, **1** consisted of three sets of phenylpropanoids and one sucrose acetyl derivative in the molecule (Fig. 1).

TABLE I ^{1}HNMR spectral data of compound **1** in DMSO-d_6, **1a** and **1b** in $CDCl_3$, 500 MHz

	1 (*J* in Hz)		**1a** (*J* in Hz)		**1b** (*J* in Hz)	
Fructose moiety						
1	a 4.16	d (12.0)	5.03	m		
	b 4.19	d (12.0)				
3	5.45	d (8.7)	5.71	d (6.5)		
4	4.21	t (8.7, 8.7)	5.56	d (6.5)		
5	3.86	m	4.21–4.25	m		
6	a 3.70		4.21–4.25	m		
	b 3.72					
Glucose moiety						
1′	5.45	d (3.6)	5.85	d (3.6)		
2′	3.70	dd (9.7, 3.6)	4.40	dd (9.7, 3.6)		
3′	5.21	t (9.7, 9.7)	5.27	dd (9.7, 9.7)		
4′	4.98	t (9.7, 9.7)	5.69	dd (9.7, 9.7)		
5′	4.19	ddd	4.30	m		
6′	a 3.70		4.49–4.31	m		
	b 3.45					
Caffeoyl moiety						
1″						
2″	7.21	dd (1.7)	7.09	dd (1.7, 8.0)		
3″						
4″						
5″	6.72	(8.0)	7.08	d (8.0)		
6″	7.14	dd (1.7, 8.0)	7.30	d (1.7)		
7″	7.65	d (15.8)	7.71	d (16.0)		
8″	6.50	d (15.8)	6.47	d (16.0)		
9″						
3,4-Dimethoycinnamoyl moiety						
1‴						
2‴	7.36	d (1.7)	7.09	dd (1.7, 8.0)	7.02	d (1.7)
3‴						
4‴						
5‴	6.77	d (8.0)	7.04	d (8.0)	6.91	d (8.0)
6‴	7.10	dd (1.7, 8.0)	7.31	d (1.7)	7.07	dd (1.7, 8.0)
7‴	7.47	d (15.8)	7.73	d (16.0)	7.62	d (15.8)
8‴	6.30	d (15.8)	6.29	d (16.0)	6.28	d (15.8)
9‴						
Feruloyl moiety						
1⁗						
2⁗	7.31	dd (1.7,)	7.16	dd (1.7, 8.0)		
3⁗						
4⁗						
5⁗	6.75	(8.0)	7.05	d (8.0)		
6⁗	6.94	dd (1.7, 8.0)	7.19	d (1.7)		
7⁗	7.61	d (15.8)	7.68	d (16.0)		
8⁗	6.45	d (15.8)	6.55	d (16.0)		
9⁗						
OCH_3	3.86	s (C-3⁗)			3.93 s	
OCH_3	3.73	s (C-3‴)	3.89	s (6H)	3.80 s	
OCH_3	3.72	s (C-4‴)	3.90	s (3H)		
OAc	1.88	s (C-3′)	1.98, 2.10, 2.12, 2.13, 2.15, 2.16, 2.17, 2.36			

TABLE II ^{13}CNMR spectral data of compound 1 in DMSO-d_6, 125 MHz

	δc		δc
Fructose moiety		3,4-Dimethoycinnamoyl moiety	
1	63.9	1‴	125.5
2	101.5	2‴	111.14
3	76.8	3‴	149.4
4	71.3	4‴	147.7
5	82.9	5‴	115.4
6	60.0	6‴	125.5
Glucose moiety		7‴	145.9
1′	91.3	8‴	113.8
2′	68.7	9‴	166.0
3′	72.9	Feruloyl moiety	
4′	68.7	1⁗	125.5
5′	70.5	2⁗	111.14
6′	61.6	3⁗	149.3
Caffeoyl moiety		4⁗	147.6
1″	125.3	5⁗	114.0
2″	111.14	6⁗	123.1
3″	149.4	7⁗	145.5
4″	147.7	8⁗	113.5
5″	115.4	9⁗	165.9
6″	123.3	OCH_3	55.7
7″	145.6	OCH_3	55.6
8″	114.0	OCH_3	55.5
9″	165.7	OAc	20.6
		C=O	169.7

^{13}CNMR of **1a** ($CDCl_3$, 125 MHz): 3 × MeO at δ 56.0–55.9, 12 signals for sucrose at δ 104.1–62.1, 27 signals for three cinnamic acid moieties at δ 165.7–111.5, eight OAc at δ 20.6 × 3, 20.7 × 3, 20.5 × 2; 170.6, 170.4, 170.3, 169.8 × 2, 169.7, 168.6, 168.5.

Acetylation of **1** with acetic anhydride in pyridine yielded the corresponding heptaacetate derivative (**1a**). The ^{1}HNMR spectrum of **1a** showed a new aromatic acetyl and six additional alcoholic acetyl signals. The FABMS spectrum of **1a** showed quasi-molecular ion peaks at *m/z* 1245 (M^+ + K) and *m/z* 1229 (M^+ + Na) suggesting a molecular weight of 1206, with typical cleavage indicated by fragment ion peaks at 640 [M-219-347]$^+$ and 331 (Fig. 2). The prominent fragment ion peaks appearing at *m/z* 219, 208, 194 and 177 were due to the acetyl feruloyl fragment (*m/z* 219), 3,4-dimethoxycinnamic acid (*m/z* 208), ferulic acid (*m/z* 194) and the feruloyl fragment (*m/z* 177) respectively. It was suggested that both of 3,4-dimethoxycinnamoyl and feruloyl residues were directly linked to the caffeoyl dihydroxy groups. It was also supported by the FABMS of **1** in which the fragment ion peaks showed at *m/z* 691 and 205 (Fig. 2) indicating the acetyl group to be located on the terminal sugar of **1**. The ^{1}HNMR spectrum of **1a** further corroborated the substitution pattern. The presence of six free

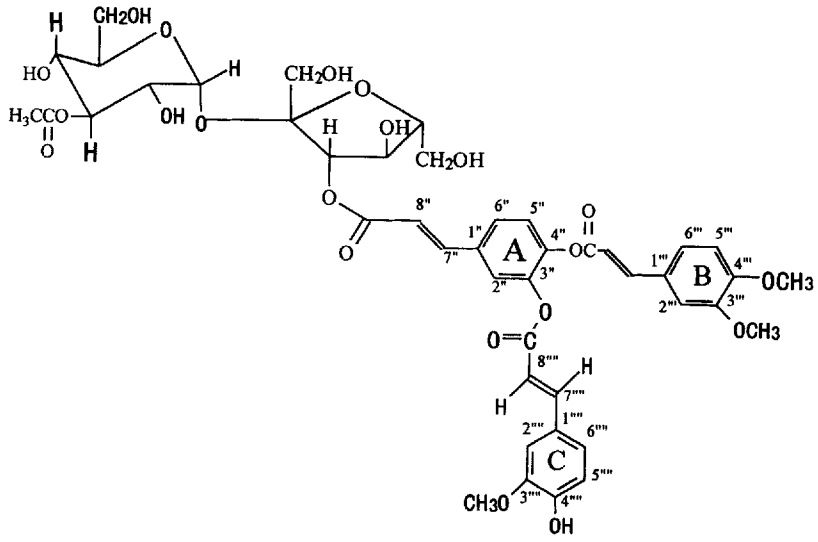

FIGURE 1 The structure of shegansu C.

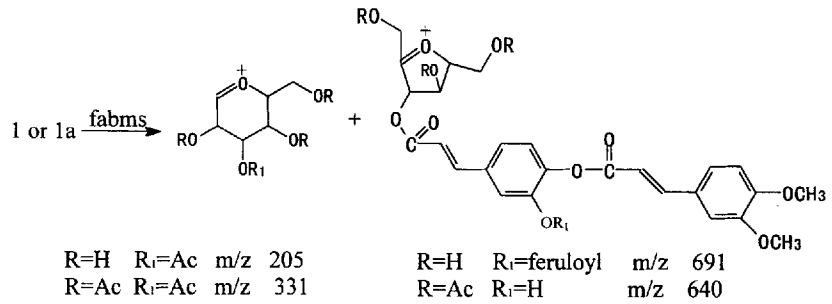

FIGURE 2 The major fragmentation ions of FABMS for shegansu C and its peracetate.

hydroxy groups at C-2′, C-4′ and C-6′ of glucose and C-1, C-4 and C-6 of fructose unit suggested 3′,3-disubstitution of the sucrose. In the ^{1}HNMR spectrum of **1a**, the chemical shifts of the protons H-1′, H-2′ and H-3 remained virtually unchanged when compared with those of **1**, whereas the signals of H-2′, H-4′, H-6′, H-1, H-4 and H-6 underwent significant downfield shifts of 0.4–1.5 ppm. The data for the sugar protons of

peracetate (**1a**) was almost similar to those for sucrose ester derivatives [3] and vanicosides A and B [6].

The connectivity of each residue were determined by HMBC and DIFNOE spectra of **1** and confirmed by the following cross peaks (Fig. 3): H-3 (δ 5.45) of fructose moiety/carbonyl carbon (δ 165.7) of caffeoyl; H-1′ (δ 5.45) of glucose moiety, H-3 (δ 5.45), H-1a, H-1b (δ 4.19, 4.16) of fructose/quaternary carbon (δ 101.5) C-2 of fructose; H-3′ (δ 5.21), H-4′ (δ 4.98) of glucose/acetyl carbonyl carbon (δ 169.7). Furthermore, application of DIFNOE made it possible to determine the linkages and the attachment position of three acid units (rings A, B and C, Fig. 3) in the molecule. Then rings C and B were shown to be attached to 3-OH and 4-OH of ring A respectively by the observation of NOEs by irradiating at the protons of three cinnamoyl moieties, and –OMe groups. Irradiation of –OMe groups at δ 3.86 and 3.73 gave NOEs to H-2′′′′ (δ 7.31) and 2′′′ (δ 7.36) respectively, illustrating the OMe of δ 3.86 and 3.73 substituted to rings C and B respectively. Thus three doublets δ 7.21, 7.36 and 7.31 were proved unambiguously to belong to rings A, B and C respectively (Table I). Irradiation of the olefinic proton at δ 6.45 (H-8′′′′) of feruloyl showed NOEs to

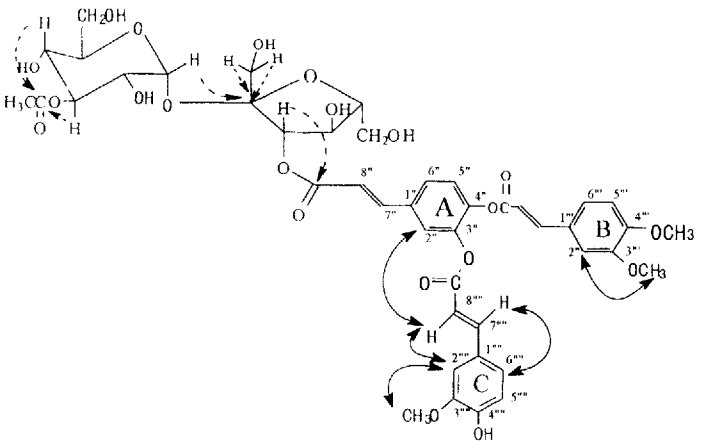

FIGURE 3 Important NOEs and selected HMBC correlations of shegansu C.

H-2'''' of ring C and H-2'' of ring A. Therefore caffeic acid was acylated with ferulic acid at 3-OH and acylated with 3,4-dimethoxycinnamoyl at 4-OH. After irradiating the protons of acetyl group, NOEs to the protons H-3' and H-4' of glucopyranose were also observed. It was indicated that the acetyl group was located on H-3' of glucopyranose. Thus fundamental structure of **1** was deduced to be a trimeric coumaroyl ester of sucrose (Fig. 3). Hydrolysis of **1** with 0.5 N NH_4OH solution afforded the crystals of 3,4-dimethoxycinnamic acid (**1b**), which was further verified by the MS and ^{1}HNMR spectra.

The mother liquor of **1b** consisted of caffeic acid, ferulic acid and 3,4-dimethoxycinnamic acid as well as sucrose by direct comparison with authentic samples on TLC. Consequently, the structure of **1** was established as β-D-[3-*O*-(4-*O*-(3,4-dimethoxycinnamoyl)-5-*O*-feruloyl)-caffeoyl]-fructofuranosyl(2-1')-α-D-(3'-*O*-acetyl)-glucopyranoside (Fig. 1). The structure as a direct linking to each other trimeric phenylpropanoid ester of sucrose was found in nature for the first time. Pharmacological test indicated that shegansu C has potent antagonism of lenkotriene D_4 receptor, with an IC_{50} of 10^{-5} mol L^{-1} [8]. Further pharmacological activity will be investigated in the future.

EXPERIMENTAL SECTION

General experimental procedures Melting point was determined on a micromelting point apparatus and uncorrected. UV spectrum was taken on a Shimadzu UV-300 spectrophotometer. IR spectra were run on Perkin Elmer 683 infrared spectrometer recorded in KBr pellets. The optical rotation was measured on Perkin-Elmer-241 spectrometer. ^{1}HNMR 500 MHz, ^{13}CNMR 125 MHz and 2DNMR were determined on Bruker AM 500 spectrometer using TMS as internal standard. The negative ion LSIMS spectrum was measured on Bruker APEX47e FTMS spectrometer, using NBA as matrix. FABMS was obtained with JMS-DX-300 mass spectrometer. Low pressure chromatography was performed on silica gel (10–40 μm). Plant material, rhizomes of *B. chinensis* were collected from Hebei province of China, identified by Professor W.Z. Song of our institute and a voucher specimen (00375) was deposited in the herbarium of the Institute of Materia Medica, Chinese Academy of Medical Sciences, Peking Union Medical College.

Extraction and isolation Powdered rhizomes (32 kg) were extracted with 95% EtOH at room temperature. The extract was concentrated *in vacuo* to

yield 3.1 kg of gum which was mixed with silica gel (60–100 mesh) eluted with petroleum ether, $CHCl_3$, Me_2CO and EtOH to give four fractions. The concentrated acetone fraction was yellowish solid which was processed on a silica gel column eluting with $CHCl_3$–MeOH–H_2O (8 : 2.5 : 1) to obtain iridin (550 mg), tectoridin (400 mg) and daucosterol (160 mg). After filtration of these crystals, the mother liquor was concentrated to afford 396 g gum which was further divided in five portions (I V) by silica gel column chromatography to elute with $CHCl_3$–MeOH (9 : 1). The portion II successively gave A–F six fractions, on silica gel column chromatography eluted with $CHCl_3$–MeOH (95 : 5). The fraction D (5.2 g) was separated on a low pressure silica gel column chromatography eluted with $CHCl_3$–MeOH–Me_2CO (94 : 5: 1) to collect 46 fractions. Fractions 30 34 yielded shegansu A [2] (20 mg). Further separation of fractions 25–29 with petrol–EtOAc–Me_2CO (1 : 6 : 1) on a low pressure column gave compound **1**. Fraction F (4.14 g) was chromatographed on silica gel to yield isorhapontigenin (61 mg). Fraction E (3.80 g) was treated on low pressure silica gel column chromatography to elute with $CHCl_3$–MeOH–EtOAc (9 : 0.5 : 0.5) to obtain resveratrol (400 mg) and shegansu B (100 mg). Portion III (23 g) was separated on a silica gel column chromatography eluted with C_6H_{14} : $CHCl_3$: EtOAc : MeOH : H_2O (1 : 7.5 : 0.5 : 1 : 0.1) to obtain the crystals of shegansu C (200 mg).

Shegansu C (**1**) It is a yellowish amorphous powder, $[\alpha]_D^{31}$ + 51.3 (C 0.075, EtOH), UV (EtOH) λ_{max}: 218, 236, 300, 326 nm; IR (KBr) ν_{max}: 3300, 1700(br), 1630, 1600, 1520, 1250, 1020, 820 cm^{-1}; for ^{1}HNMR data see Table I; for ^{13}CNMR data see Table II; FABMS *m/z* 913 (M^+ + 1), 895, 691, 205; negative ion LSIMS spectrum *m/z*: 933.2423 [M – 2H + Na]$^-$, 911.2604 [M – H]$^-$.

Acetylation of **1** Ac_2O (2 ml) was added to a pyridine (2 ml) solution of compound **1** (22 mg). The mixture was kept at room temperature for 36 h. The solution was poured into ice-H_2O and the crude peracetate was recrystallized with MeOH to obtain **1a** (27 mg). For the data of **1a** ^{1}HNMR see Table I. FABMS of **1a** *m/z*: 640, 598, 556, 514, 507, 465, 362, 331, 288, 219, 208, 194(80), 177(70), 159, 126(85), 60(82), 43, 42.

Alkaline hydrolysis Compound **1** (10 mg) was added to a 0.5 N NH_4OH solution (3 ml) at room temperature, and stirred for 4 h. The water solution was adjusted to pH 7 with 0.1 M HCl and extracted with Et_2O. After concentration the crystals of **1b** were collected by filtration. For the data of ^{1}HNMR (500 MHz, $CDCl_3$) see Table I. EIMS of **1b**: *m/z* 208 (M^+, 100), 177(98). The mother liquor consisted of caffenic acid, ferulic acid and 3,4-dimethoxycinnamic acid by direct comparison with authentic samples

on TLC silica gel with $CHCl_3$–MeOH–EtOAc–HOAc (9 : 0.5 : 0.5 : 0.1). The mother liquor was concentrated *in vacuo* to dryness and treated with EtOH to develop on TLC silica gel (the plate prepared with 0.1 N $NaHCO_3$) with EtOAc : HOAc : MeOH : H_2O (65 : 15 : 15 : 10) by spraying with naphtharesorcinol and comparison with an authentic sample of sucrose.

Acknowledgements

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The negative ion LSIMS was measured at The Chinese University of Hong Kong.

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