Cardenolides from *Saussurea stella* with Cytotoxicity toward Cancer Cells

Tian-Min Wang,[†] Toshinari Hojo,[‡] Fu-Xiang Ran,[†] Ru-Feng Wang,[†] Rui-Qing Wang,[†] Hu-Biao Chen,*[,†,§] Jing-Rong Cui,[†] Ming-Ying Shang,[†] and Shao-Qing Cai*[,†]

The State Key Laboratory of Natural and Biomimetic Drugs, School of Pharmaceutical Sciences, Peking University Health Science Center, Beijing 100083, People's Republic of China, ARSOA Honsha Corporation ARSOA R&D Center, Yamanashi, Japan, and School of Chinese Medicine, Hong Kong Baptist University, Hong Kong, People's Republic of China

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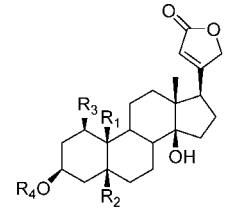
Three new cardenolides, 3-*O*-*β*-D-fucopyranosylstrophanthidin (**1**), 3-*O*-*β*-D-quinovopyranosylperiplogenin (**2**), and 3-*O*-*β*-D-glucopyranosyl-(1→4)-α-L-rhamnopyranosylcannogenin (**3**), together with seven known cardenolides (**4**–**10**), were isolated from a cytotoxic ethanol extract of the whole dried plants of *Saussurea stella*. The structures of these compounds were established by spectroscopic and chemical methods. When the cytotoxicity of compounds **2**–**10** toward Bel-7402 human hepatoma cells and BGC-823 human gastric cancer cells was evaluated, all compounds showed IC_{50} values of <1 μM for both cell lines. This is the first report of cardenolides occurring in a species of the family Asteraceae.

Saussurea stella Maxim. of the family Asteraceae is distributed in the southwest of mainland China; about 30 species of this genus are used as folk medicines.[1] As recorded in the "Zhonghua Bencao", *S. stella* has "heat-evil clearing", "wind-evil dispelling", and "dampness eliminating" effects and can be used for the treatment of rheumatism.[2] In the present study, an EtOH extract of *S. stella* strongly inhibited the growth of several human cancer cell lines including Bel-7402 hepatoma cells and BGC-823 gastric cancer cells. There are only two published reports on the chemistry of *S. stella*;[3,4] while these studies mainly concerned flavonoid constituents, some of these compounds were shown to have antioxidant properties.[5] The lack of chemical studies together with the potent biological activity stimulated us to investigate the chemical constituents of *S. stella* further.

Cardenolides, also known as cardiotonic steroids, occur in members of the families Apocynaceae, Asclepiadaceae, Cruciferae, Liliaceae, and Scrophulariaceae. Asteraceae, one of the biggest families of flowering plants, comprises many species distributed all over the world, and research on the chemical constituents of its members is extensive. However, there have been no reports of cardenolides having been isolated from Asteraceae. In this study, 10 cardenolides (**1**–**10**), including three new compounds (**1**–**3**), were isolated from *S. stella* and evaluated against two cancer cell lines for their cytotoxic effects.

Results and Discussion

A cytotoxic EtOH extract of the entire herb of *S. stella* was suspended in H_2O and partitioned with petroleum ether, EtOAc, and *n*-BuOH, successively. The EtOAc and *n*-BuOH extracts were separated and purified by chromatographic methods. Three new cardenolides, **1**–**3**, together with seven known cardenolides, 3-*O*-*β*-D-xylopyranosylperiplogenin (**4**), 3-*O*-*β*-D-quinovopyranosylstrophanthidin (**5**), 3-*O*-*β*-D-xylopyranosylstrophanthidin (**6**), 3-*O*-*β*-D-fucopyranosylperiplogenin (**7**), 3-*O*-α-L-rhamnopyranosylcannogenol (**8**), 3-*O*-α-L-rhamnopyranosylstrophanthidin (convallatoxin, **9**), and 3-*O*-α-L-rhamnopyranosylacovenosigenin A (**10**), were isolated from the EtOAc and *n*-BuOH extracts. The structures of compounds **1**–**3** were elucidated through detailed 1D and 2D NMR analysis, and the NMR data for the known compounds **4**–**7** are being reported for the first time. Compounds **8**–**10** were identified by comparing their spectroscopic data with those reported in the literature.[6,7]



1 R_1 = CHO, R_2 = OH, R_3 = H, R_4 = Fuc

2 R_1 = CH_3, R_2 = OH, R_3 = H, R_4 = Qui

3 R_1 = CHO, R_2 = H, R_3 = H, R_4 = Rha (4→1) Glc

4 R_1 = CH_3, R_2 = OH, R_3 = H, R_4 = Xyl

5 R_1 = CHO, R_2 = OH, R_3 = H, R_4 = Qui

6 R_1 = CHO, R_2 = OH, R_3 = H, R_4 = Xyl

7 R_1 = CH_3, R_2 = OH, R_3 = H, R_4 = Fuc

8 R_1 = CH_2OH, R_2 = H, R_3 = H, R_4 = Rha

9 R_1 = CHO, R_2 = OH, R_3 = H, R_4 = Rha

10 R_1 = CH_3, R_2 = H, R_3 = OH, R_4 = Rha

(Fuc = *β*-D-fucopyranosyl; Qui = *β*-D-quinovopyranosyl; Rha = *α*-L-rhamnopyranosyl; Xyl = *β*-D-xylopyranosyl)

Compound **1** was isolated as an amorphous powder. It was assigned the molecular formula $C_{29}H_{42}O_{10}$, which was deduced from HRESIMS (*m/z* 573.2650, $[M + Na]^+$). This substance was determined to be a cardenolide possessing one sugar moiety, according to the UV, IR, and 1D NMR spectra. The ^1H (Table 1) and ^{13}C NMR data (Table 2) for the aglycon were similar to those reported for 3-*O*-substituted strophanthidins.[6,8] Thus, the aglycon of **1** was identified as strophanthidin. An anomeric proton (*δ* 4.32, m, H-1′), a methyl group as a doublet (*δ* 1.25, $J = 6.5$ Hz, H-6′), and four additional protons between *δ* 3.44 and 3.63 in the ^1H NMR spectrum suggested that **1** bears a 6-deoxyhexose unit. The correlation of H-1′ (*δ* 4.32) with C-3 (*δ* 75.4) in the HMBC spectrum demonstrated that the sugar moiety is substituted at C-3. The presence of a D-fucosyl unit was established by acid hydrolysis and by comparing the ^{13}C NMR data for the sugar moiety of **1** with those reported for the D-fucosyl group.[9] A ^1H NMR spectrum measured in D_2O was performed to determine the configuration of the glycosidic bond in **1**. A doublet for the anomeric proton (*δ* 4.48, $J = 8.0$ Hz) in the ^1H NMR spectrum of **1** showed that the

* Corresponding authors. S.-Q.C., Tel/Fax: 86 10 82801693. E-mail: sqcai@hsc.pku.edu.cn. H.-B.C., Tel: 852 34112060. Fax: 852 34112461. E-mail: hbchen@hkbu.edu.hk.

[†] Peking University Health Science Center.

[‡] ARSOA Honsha Corporation.

[§] Hong Kong Baptist University.

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Table 1. ^{1}H NMR Spectroscopic Data (500 MHz, CD_3OD) for **1**–**7** (δ values, J in Hz)

position	**1**	**2**	**3**	**4**	**5**	**6**	**7**
1	1.32, m	1.36, m	1.41, m	1.39, m	1.32, m	1.32, m	1.35, m
	2.15, m	1.81, m	1.59, m	1.81, m	2.15, m	2.14, m	1.83, m
2	1.65, m	1.70, m	1.64, m	1.71, m	1.65, m	1.66, m	1.71, m
	1.89, m	1.70, m,	1.70, m	1.71, m	1.89, m	1.82, m	1.71, m
3	4.17, brs	4.15, brs	3.98, brs	4.17, brs	4.16, brs	4.14, brs	4.16, brs
4	1.72, m	1.69, m	1.79, m	1.73, m	1.72, m	1.72, m	1.69, m
	2.10, m	2.12, m	1.89, m	2.15, m	2.10, m	2.12, m	2.12, m
5			2.18, m				
6	1.67, m	1.38, m	1.61, m	1.41, m	1.67, m	1.65, m	1.37, m
	2.09, m	1.73, m	1.65, m	1.75, m	2.09, m	2.10, m	1.73, m
7	1.69, m	1.20, m	1.27, m	1.25, m	1.69, m	1.68, m	1.19, m
	2.20, m	1.92, m	1.85, m	1.94, m	2.20, m	2.16, m	1.92, m
8	1.95, m	1.64, m	1.84, m	1.67, m	1.95, m	1.95, m	1.64, m
9	1.71, m	1.61, m	1.88, m	1.65, m	1.71, m	1.70, m	1.61, m
11	1.21, m	1.33, m	1.51, m	1.36, m	1.21, m	1.20, m	1.32, m
	1.53, m	1.45, m	1.51, m	1.49, m	1.53, m	1.51, m	1.45, m
12	1.44, m	1.48, m	1.49, m	1.51, m	1.44, m	1.44, m	1.48, m
	1.48, m	1.51, m	1.53, m	1.54, m	1.48, m	1.48, m	1.52, m
15	1.68, m	1.70, m	1.68, m	1.74, m	1.68, m	1.68, m	1.70, m
	2.13, m	2.14, m	2.18, m	2.15, m	2.13, m	2.13, m	2.14, m
16	1.85, m	1.87, m	1.90, m	1.88, m	1.85, m	1.87, m	1.88, m
	2.16, m	2.18, m	2.16, m	2.19, m	2.16, m	2.18, m	2.18, m
17	2.82, dd	2.83, dd	2.83, dd	2.86, dd	2.82, dd	2.82, dd	2.83, dd
	(9.0, 6.0)	(8.0, 5.5)	(8.5, 6.0)	(8.5, 5.5)	(9.0, 5.0)	(9.0, 5.5)	(8.0, 6.0)
18	0.85, s	0.88, s	0.94, s	0.91, s	0.85, s	0.84, s	0.88, s
19	10.03, s	0.93, s	9.57, s	0.96, s	10.04, s	10.04, s	0.93, s
21	4.90, dd	4.91, dd	4.91, dd	4.94, dd	4.90, dd	4.90, dd	4.91, s
	(18.5, 1.5)	(18.5, 1.5)	(18.5, 1.0)	(18.5, 1.5)	(18.5, 2.0)	(18.5, 1.5)	
	5.02, dd	5.02, dd	5.03, dd	5.06, dd	5.02, dd	5.07, dd	5.02, s
	(18.5, 1.5)	(18.5, 1.5)	(18.5, 1.0)	(18.5, 1.5)	(18.5, 1.5)	(18.5, 1.5)	
22	5.89, brs	5.89, t (1.5)	5.90, brs	5.92, brs	5.89, brs	5.89, brs	5.89, s
sugar moieties	Fuc	Qui	Rha	Xyl	Qui	Xyl	Fuc
1′	4.32, m	4.36, d (7.5)	4.79, d (1.5)	4.36, d (7.5)	4.37, d (7.5)	4.33, d (7.5)	4.32, m
2′	3.46[a]	3.16, dd	3.81, m	3.18, dd	3.15, dd	3.14, dd	3.46[a]
		(7.5, 9.0)		(7.5, 9.0)	(7.5, 9.0)	(7.5, 9.0)	
3′	3.46[a]	3.31[a]	3.91, dd	3.35[a]	3.31[a]	3.31[a]	3.46[a]
			(9.5, 3.0)				
4′	3.58, brs	2.98, t (9.0)	3.61, t (9.5)	3.50, ddd	2.99, t (9.0)	3.47, ddd	3.58, d (1.0)
				(5.5, 10.5, 9.0)		(5.5, 9.0, 10.5)	
5′	3.63, q (6.5)	3.31[a]	3.69[a]	3.86, dd	3.31[a]	3.84, dd	3.62, dq
				(5.5, 11.5)		(5.5, 11.5)	(6.5, 1.0)
				3.22, dd		3.19, dd	
				(10.5, 11.5)		(10.5, 11.5)	
6′	1.25, d (6.5)	1.25, d (6.0)	1.3, d (6.0)		1.26, d (6.0)		1.25, d (6.5)
			Glc				
1″			4.58, d (7.5)				
2″			3.20, dd				
			(7.5, 8.5)				
3″			3.36, t				
			(8.5, 8.5)				
4″			3.28[a]				
5″			3.28[a]				
6″			3.84, dd				
			(12.0, 2.0)				
			3.69[a]				

[a] Overlapped signals.

glycosidic bond is in a β-configuration. Furthermore, the coupling constants of H-1′ with H-2′ (J = 8.0 Hz), H-2′ with H-3′ (J = 10.0 Hz), and H-3′ with H-4′ (J = 3.5 Hz) in the ^{1}H NMR spectrum measured in D_2O gave support for the identification of the β-D-fucosyl unit in compound **1**. Thus, the structure of **1** was elucidated as 3-*O*-β-D-fucopyranosylstrophanthidin.

The molecular formula of compound **2** was determined to be $C_{29}H_{44}O_9$ from its HRESIMS (*m/z* 559.2901, $[M + Na]^+$). The ^{1}H NMR (Table 1) and ^{13}C NMR (Table 2) spectra indicated that compound **2** is also a cardenolide bearing a hexose unit. The ^{13}C NMR data for the aglycon of **2** were identical with those for 3-*O*-substituted periplogenins reported in the literature.[10] Accordingly, the aglycon of **2** was assigned as periplogenin. The sugar moiety of **2** is also a 6-deoxyhexose, as deduced from a methyl doublet (δ 1.25, J = 6.0 Hz), which was assigned to H-6′, in the ^{1}H NMR spectrum. The 3-*O*-linkage of the sugar moiety was ascertained by the correlations of H-1′ (δ 4.36) with C-3 (δ 76.2) and H-3 (δ 4.15) with C-1′ (δ 101.9) in the HMBC spectrum of **2**. Acid hydrolysis of **2** yielded D-quinovose. The coupling constants of H-1′ with H-2′ (J = 7.5 Hz), H-2′ with H-3′ (J = 9.0 Hz), H-3′ with H-4′ (J = 9.0 Hz), and H-4′ with H-5′ (J = 9.0 Hz) supported the conclusions that the glycosyl of **2** is a D-quinovosyl and that the glycosidic bond is in a β-configuration. Consequently, the structure of **2** was determined as 3-*O*-β-D-quinovopyranosylperiplogenin.

Compound **3** was obtained as an amorphous powder. As deduced from its HRESIMS (*m/z* 719.3247, $[M + Na]^+$), its molecular formula was determined to be $C_{35}H_{52}O_{14}$. The similarities of the ^{1}H NMR (Table 1) and ^{13}C NMR (Table 2) spectra of compound **3** to those of compounds **1** and **2** revealed that **3** is also a cardenolide glycoside. One main difference between the ^{13}C NMR data for the aglycons of **3** and **1** was the absence of the quaternary carbon signal (δ 75.3) associated with the presence of a methine signal (δ 31.7,

Table 2. ^{13}C NMR Spectroscopic Data (CD_3OD, 125 MHz) for **1–7** (δ values)

position	1	2	3	4	5	6	7
1	25.9	26.5	29.8	26.5	25.8	25.4	26.5
2	26.5	27.0	26.5	26.9	26.6	26.1	26.9
3	75.4	76.2	73.1	76.4	75.4	75.2	76.1
4	36.1	34.8	30.4	34.9	36.1	35.7	34.8
5	75.3	75.1	31.7	75.1	75.3	74.8	75.1
6	38.3	36.2	23.6	36.2	38.3	37.8	36.2
7	19.5	25.0	23.0	25.0	19.5	19.0	25.0
8	43.1	41.6	43.6	41.6	43.1	42.6	41.6
9	40.8	40.1	36.4	40.1	40.8	40.3	40.1
10	56.7	41.8	52.7	41.8	56.7	56.2	41.8
11	23.7	22.6	22.5	22.6	23.7	23.2	22.6
12	41.0	40.9	41.3	40.9	41.0	40.5	40.9
13	51.2	50.9	51.5	50.8	51.2	50.7	50.9
14	86.5	86.3	86.5	86.3	86.4	85.9	86.3
15	33.0	33.4	33.3	33.4	33.0	32.5	33.4
16	28.4	28.0	28.5	28.0	28.4	27.9	28.0
17	52.3	51.9	52.5	51.9	52.3	51.7	52.0
18	16.7	16.3	16.7	16.3	16.7	16.2	16.3
19	210.6	17.2	208.6	17.2	210.5	210.0	17.2
20	178.7	178.3	178.8	178.4	178.7	178.2	178.4
21	75.8	75.3	75.8	75.3	75.8	75.3	75.3
22	118.4	117.8	118.4	117.8	118.4	117.9	117.8
23	177.7	177.2	177.7	177.2	177.7	177.2	177.2
1′	102.9	101.9	100.3	102.9	102.3	102.8	102.6
2′	72.8	75.4	73.2	75.0	75.8	74.9	72.3
3′	75.8	78.0	73.1	78.0	78.5	78.0	75.3
4′	73.6	77.0	84.0	71.2	77.5	71.2	73.1
5′	72.6	73.4	69.3	67.1	73.9	67.1	72.1
6′	17.3	18.1	18.6		18.6		16.8
1″			106.2				
2″			76.6				
3″			78.7				
4″			72.0				
5″			78.6				
6″			63.3				

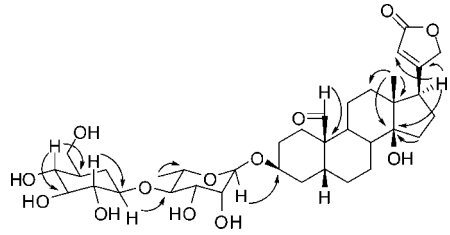

Figure 1. Key HMBC correlations for compound **3**.

C-5) in the ^{13}C NMR spectrum of **3**. This difference suggested that the hydroxyl group located at C-5 in **1** is missing from **3**. The β-orientation of H-5 was confirmed by comparing the chemical shift of C-5 with that reported for 5α-H and 5β-H cardenolides.[11–13] Accordingly, the aglycon of **3** was characterized as cannogenin.[11] Acid hydrolysis of **3** produced D-glucose and L-rhamnose. The glycosyl moiety of **3** was found to be the same as that of 5α-oleandrigenin 3-[*O*-β-D-glucopyranosyl-(1→4)-α-L-rhamnopyranoside],[13] as deduced from the similarities in the ^{13}C NMR data for their sugar moieties. Therefore, the structure of **3** was assigned as 3-*O*-β-D-glucopyranosyl-(1→4)-α-L-rhamnopyranosylcannogenin. This identification was also supported by the COSY, HMQC, and HMBC (Figure 1) spectra.

Compounds **4–7** have been reported earlier.[14–18] Their ^1H (Table 1) and ^{13}C NMR (Table 2) data are presented herein because they have not appeared in the literature previously. The aglycons of compounds **4** and **7** are the same as that of **2**, while the aglycons of compounds **5** and **6** are identical with that of **1**. Acid hydrolysis of **4–7** furnished D-xylose, D-quinovose, D-xylose, and D-fucose, respectively. Thus, the structures of compounds **4–7** were ascertained, in turn, as 3-*O*-β-D-xylopyranosylperiplogenin, 3-*O*-β-D-quinovopyranosylstrophanthidin, 3-*O*-β-D-xylopyranosylstrophanthidin, and 3-*O*-β-D-fucopyranosylperiplogenin.

Table 3. IC_{50} Values of Cardenolides **2–10** toward Two Cancer Cell Lines (μM)

	cell lines[a]	
compound	BGC-823	Bel-7402
2	0.070	0.31
3	0.29	0.87
4	0.23	0.48
5	0.10	0.020
6	0.016	0.13
7	0.12	0.40
8	0.077	0.028
9	0.016	0.050
10	0.056	0.24

[a] Cell lines: BGC-823, human gastric cancer; Bel-7402, human hepotama.

The known compounds **4–7**, on which there are only a few published reports, are all rare natural products. Compound **4** has been isolated only from the secretions of a chrysomelid beetle, *Chrysolina coerulans* L. G. Scriba.[14] With the exception of compound **3**, which has a two-sugar chain including a terminal glucopyranosyl moiety, compounds **1–10** are linked to a single 6-deoxyhexose moiety. While the aglycons of these cardenolides isolated from *S. stella* are common, the common 6-deoxyhexoses including quinovose,[19,20] fucose,[21–23] and xylose[14,24–27] are not frequently encountered among cardenolides. Moreover, the cardenolides reported herein have been isolated from a plant in the Asteraceae family for the first time.

Compounds **2–10**, isolated from the cytotoxic EtOH extract of *S. stella*, showed potent inhibitory effects on the growth of two cell lines tested: BGC-823 human gastric cancer cells and Bel-7402 human hepatoma cells (Table 3). The IC_{50} values of compounds **2–10** were all <1 μM. Previously, compound **9** has been shown to inhibit the growth of the Eagle KB strain of human

epidermoid carcinoma,[28] and compound **10** was found to be cytotoxic to mouse L1210 leukemia cells, human HeLa cervical cancer cells, human A549 lung cancer cells, and human HL-60 leukemia cells.[7] Of the nine compounds tested here, compound **3**, which has a two-sugar side chain, showed the least potent inhibitory effects toward the cancer cell lines tested. Compounds with an aldehyde group at C-10 (compounds **5**, **6**, and **9**) were more potent than those with a methyl group at C-10 (compounds **2**, **4**, and **7**) in inhibiting the growth of these cancer cell lines. The BGC-823 cell line seems to be a little more sensitive than the Bel-7402 cell line to the compounds tested.

Experimental Section

General Experimental Procedures. Melting points were measured on an XT-4A micromelting point apparatus without correction. Optical rotations were determined on a Perkin-Elmer 243 B polarimeter. UV spectra were measured with a Cary 300 UV–vis spectrophotometer, while IR spectra were collected from a Nicolet NEXUS-470 FTIR spectrophotometer. Varian INOVA-500 and Bruker DRX-500 spectrometers were used to obtain the NMR spectra. The chemical shifts are expressed as δ values using solvent as internal standard. HRESIMS and ESIMS were detected with Bruker APEX IV FT and ABI Q-STAR mass spectrometers, respectively. Column chromatography was performed with silica gel (200–300 mesh, Qingdao Marine Chemical Co., Ltd.) and macroporous resin D101 (Tianjin Resin Co., Ltd.). Semipreparative HPLC was conducted on an Alltima C_{18} column (10 mm i.d. × 250 mm, 10 μm) equipped with an Alltech 426 HPLC pump and an Alltech single-wavelength UV detector. Analytical HPLC was carried out on an Agilent 1100 liquid chromatography system with a DAD UV detector using a Zobax SB-C_{18} column (4.6 mm i.d. × 250 mm, 5 μm). D-Fucose, D-quinovose, D-xylose, L-(–)-α-methylbenzylamine, and $NaBH_3CN$ were purchased from Sigma (St. Louis, MO). D-glucose and L-rhamnose were obtained from Beijing Chemical Reagent Company. All other chemical solvents used for isolation were of analytical grade (Beijing Beihua Fine Chemicals Co., Ltd.).

Plant Material. The entire plants of *Saussurea stella* Maxim. were collected from suburbs of Shiqu County, Sichuan Province, People's Republic of China, in August 2003 and May 2004. The plant material was identified by Prof. Hu-Biao Chen. Voucher specimens (030803 and 040501) were deposited in the herbarium of the School of Pharmaceutical Sciences, Peking University Health Science Center.

Extraction and Isolation. The air-dried and powdered *S. stella* (2.5 kg, 030803) was extracted with 95% EtOH at room temperature (25 L, 23 L, 18 L; 3 × 7 days). The resulting EtOH extract was concentrated under reduced pressure to obtain a crude extract (100 g). After suspension in water, the crude extract was partitioned with petroleum ether, EtOAc, and *n*-BuOH, successively.

The EtOAc extract (20 g) was subjected to silica gel column chromatography eluted with gradient mixtures of petroleum–EtOAc–MeOH (1:0:0 → 0:0:1) to yield fractions E1–E55. Fractions E26–28 (1.2 g) were subjected to silica gel column chromatography using $CHCl_3$–MeOH mixtures (50:1 → 1:1) for elution and gave subfractions EC1–EC16. Compounds **10** (6 mg) and **9** (8 mg) were obtained by purifying EC7 (0.09 g) and EC8 (0.1 g) with semipreparative HPLC eluted with MeOH–H_2O (50:50), respectively.

The *n*-BuOH extract (20 g) was chromatographed over macroporous resin D101 eluted with EtOH–H_2O (0% → 95%) to give fractions Bu1–Bu8. Fraction Bu4 (2.5 g) was subjected to silica gel column chromatography using a $CHCl_3$–MeOH mixture (7:1 → 0:1) as eluants to afford subfractions BuC1–BuC45. Compound **3** (16 mg) was isolated from subfraction BuC1 (0.1 g) by semipreparative HPLC (MeOH–H_2O, 45:55). Subfraction BuC2 (0.3 g) was subjected to semipreparative HPLC eluted with MeOH–H_2O (45:55) and yielded compounds **1** (3 mg), **5** (14 mg), and **8** (7 mg).

The air-dried and powdered larger re-collected sample of *S. stella* (9 kg, 040501) was extracted with 95% EtOH under reflux (120 L, 100 L; 2 × 2 h). The extract was filtrated, and the residue was extracted with 50% EtOH under reflux (100 L, 100 L; 2 × 2 h). All resulting EtOH extracts were concentrated under reduced pressure to obtain a crude extract (1.2 kg). About 1.1 kg of the crude extract was subjected to silica gel column chromatography and eluted with petroleum and gradient $CHCl_3$–MeOH mixtures to yield 80 fractions. Fractions 43–49 (30 g) were repeatedly chromatographed on silica gel columns eluted with gradient mixtures of $CHCl_3$–MeOH (1:0 → 0:1) to produce 14 subfractions. Further separations of the subfractions were conducted on semipreparative HPLC using different conditions to furnish compounds **2** (60 mg), **4** (10 mg), **6** (12 mg), and **7** (14 mg). The final solvents used to isolate compounds **2**, **4**, and **6** were mixtures of MeOH–H_2O (60:40), while the final solvents used for purification of compound **7** were mixtures of 50% MeOH and 50% H_2O.

3-*O*-β-D-Fucopyranosylstrophanthidin (1): white, amorphous powder; mp 178–180 °C; $[\alpha]^{20}_D$ +12.5 (*c* 0.4, MeOH); UV (MeOH) λ_{max} (log ε) 216 (4.42) nm; IR (KBr) ν_{max} 3456, 2933, 1741, 1621, 1071 cm^{-1}; ^1H NMR (CD_3OD, 500 MHz), see Table 1; ^1H NMR (D_2O, 500 MHz) δ 10.14 (1H, s, H-19), 5.97 (1H, s, H-22), 5.05 (1H, dd, J = 1.0, 19.0 Hz, H-21a), 5.00 (1H, dd, J = 1.0, 19.0 Hz, H-21b), 4.22 (1H, brs, H-3), 4.48 (1H, d, J = 8.0 Hz, H-1′), 3.78 (1H, q, J = 6.5 Hz, H-5′), 3.74 (1H, d, J = 3.5 Hz, H-4′), 3.64 (1H, dd, J = 10.0, 3.5 Hz, H-3′), 3.46 (1H, dd, J = 8.0, 10.0 Hz, H-2′), 2.88 (1H, m, H-17), 1.24 (3H, d, J = 6.5 Hz, H-6′), 0.83 (3H, s, H-18); ^{13}C NMR (CD_3OD, 125 MHz), see Table 2; HRESIMS $[M + Na]^+$ *m/z* 573.2650 (calcd for $C_{29}H_{42}O_{10}Na$, 573.2670).

3-*O*-β-D-Quinovopyranosylperiplogenin (2): white, amorphous powder; mp 187–188 °C; $[\alpha]^{20}_D$ −10.0 (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ε) 212 (4.23) nm; IR (KBr) ν_{max} 3414, 2938, 1740, 1623, 1062 cm^{-1}; ^1H NMR (CD_3OD, 500 MHz), see Table 1; ^{13}C NMR (CD_3OD, 125 MHz), see Table 2; HRESIMS $[M + Na]^+$ *m/z* 559.2901 (calcd for $C_{29}H_{44}O_9Na$, 559.2878).

3-*O*-β-D-Glucopyranosyl-(1→4)-α-L-rhamnopyranosylcannogenin (3): white, amorphous powder; mp 208–210 °C; $[\alpha]^{20}_D$ −35.0 (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ε) 212 (4.29) nm; IR (KBr) ν_{max} 3420, 2932, 1740, 1624, 1067, 1030 cm^{-1}; ^1H NMR (CD_3OD, 500 MHz), see Table 1; ^{13}C NMR (CD_3OD, 125 MHz), see Table 2; HRESIMS $[M + Na]^+$ *m/z* 719.3247 (calcd for $C_{35}H_{52}O_{14}Na$, 719.3249).

3-*O*-β-D-Xylopyranosylperiplogenin (4): white, amorphous powder; mp 174–176 °C; ^1H NMR (CD_3OD, 500 MHz), see Table 1; ^{13}C NMR (CD_3OD, 125 MHz), see Table 2; ESIMS $[M + Na]^+$ *m/z* 545.2224.

3-*O*-β-D-Quinovopyranosylstrophanthidin (5): white, amorphous powder; mp 169–171 °C; ^1H NMR (CD_3OD, 500 MHz), see Table 1; ^{13}C NMR (CD_3OD, 125 MHz), see Table 2; ESIMS $[M + Na]^+$ *m/z* 573.2149.

3-*O*-β-D-Xylopyranosylstrophanthidin (6): white, amorphous powder; mp 182–183 °C; ^1H NMR (CD_3OD, 500 MHz), see Table 1; ^{13}C NMR (CD_3OD, 125 MHz), see Table 2; ESIMS $[M + Na]^+$ *m/z* 559.2020.

3-*O*-β-D-Fucopyranosylperiplogenin (7): white, amorphous powder; mp 166–168 °C; ^1H NMR (CD_3OD, 500 MHz), see Table 1; ^1H NMR (D_2O, 500 MHz) δ 5.97 (1H, s, H-22), 5.07 (1H, dd, J = 1.5, 18.5 Hz, H-21a), 5.01 (1H, dd, J = 1.5, 18.5 Hz, H-21b), 4.21 (1H, brs, H-3), 4.49 (1H, d, J = 8.0 Hz, H-1′), 3.78 (1H, q, J = 6.5 Hz, H-5′), 3.74 (1H, d, J = 3.5 Hz, H-4′), 3.65 (1H, dd, J = 10.0, 3.5 Hz, H-3′), 3.48 (1H, dd, J = 8.0, 10.0 Hz, H-2′), 2.89 (1H, dd, J = 9.0, 5.0 Hz, H-17), 1.24 (3H, d, J = 6.5 Hz, H-6′), 0.90 (3H, s, H-19), 0.87 (3H, s, H-18); ^{13}C NMR (CD_3OD, 125 MHz), see Table 2; ESIMS $[M + Na]^+$ *m/z* 559.2385.

Acid Hydrolysis of 1–7. Compounds **1–7** (each 2 mg) were dissolved individually in methanol (0.2 mL) and refluxed with 1 N HCl for 1 h. Each reaction mixture was evaporated to dryness under vacuum and then partitioned between $CHCl_3$ and H_2O. The H_2O layer was concentrated to yield a sugar residue hydrolyzed from the corresponding compound. The residue was dissolved in H_2O (0.1 mL), to which a solution of L-(–)-α-methylbenzylamine (2 mg) and $NaBH_3CN$ (1 mg) in 0.1 mL of methanol was added. After warming at 60 °C for 1 h, the mixture was acidified to pH 4 by addition of glacial acetic acid (0.05 mL) and evaporated to dryness. The resultant product was treated with acetic anhydride (0.4 mL) and anhydrous pyridine (0.4 mL) at 100 °C for 1 h and then concentrated to remove the solvent. The residue was partitioned between $CHCl_3$ and H_2O. The $CHCl_3$ layer, in which the derivates of the hydrolyzed sugars were present, was concentrated and redissolved in methanol (0.5 mL) for HPLC analysis: Zobax SB-C_{18} column (4.6 mm i.d. × 250 mm, 5 μm); detection, UV 230 nm; mobile phase, CH_3CN–H_2O (2:3); flow rate, 0.8 mL/min; column temperature, 25 °C. Identification of the sugars hydrolyzed from compounds **1–7** was carried out by comparing the retention times of the derivative products of the hydrolyzed sugars with those of the authentic known sugars: D-fucose (t_R = 19.51 min) was

detected from **1** and **7**; D-quinovose (t_R = 23.80 min) was detected from **2** and **5**; D-xylose (t_R = 17.11 min) was detected from **4** and **6**; D-glucose (t_R = 22.87 min) and L-rhamnose (t_R = 26.33 min) were detected from **3**.

Cytotoxicity Assay. The IC_{50} values of the inhibitory effects of the isolated compounds on the growth of two cancer cell lines (BGC-823 human gastric cancer cells and Bel-7402 human hepatoma cells) were evaluated. The test samples were dissolved in DMSO to get a stock solution. Different dilutions of the test samples were added to the cell cultures (4×10^3 cells/well). After incubation at 37 °C in 5% CO_2 for 48 h, the growth inhibition ratios of the cancer cells were evaluated by the sulforhodamine B (SRB) method.[29] Data points were taken in triplicate. The IC_{50} values were calculated by probit analysis.

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