Bioactive Triterpene Glycosides from the Sea Cucumber *Holothuria fuscocinerea*

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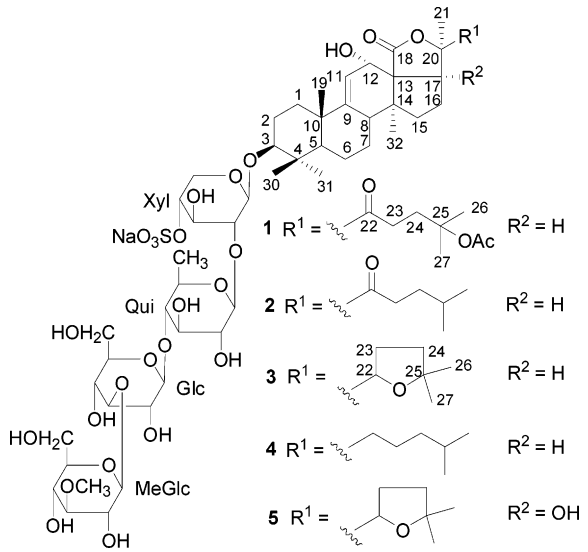
Bioassay-guided fractionation of the active *n*-BuOH extract of the sea cucumber *Holothuria fuscocinerea* resulted in the isolation of three new triterpene glycosides, fuscocinerosides A (**1**), B (**2**), and C (**3**), along with two known glycosides, pervicoside C (**4**) and holothurin A (**5**), as active compounds causing morphological abnormality of *Pyricularia oryzae* mycelia. Compounds **1**−**5** possess the same tetrasaccharide moiety, 3-*O*-methyl-*β*-D-glucopyranosyl-(1→3)-*β*-D-glucopyranosyl-(1→4)-*β*-D-quinovopyranosyl-(1→2)-4-*O*-sodiumsulfato-*β*-D-xylopyranosyl, linked to C-3 of holostane triterpene aglycones that differ in their side chains and 17-substituents. Their structures were elucidated by extensive spectral studies as well as chemical evidence. All the glycosides showed in vitro cytotoxicity against two human tumor cell lines.

Triterpene glycosides are the predominant secondary metabolites of holothurians and are responsible for their general toxicity. These glycosides have been reported to have a wide spectrum of biological effects, including cytotoxic, antifungal, hemolytic, and immunomodulatory activities.[1,2] More than 100 of these glycosides have been described, and the majority are usually lanosterol type triterpenes with an 18(20) lactone and a sugar chain linked to the C-3 of the aglycone.[3] *Holothuria fuscocinerea* Jaeger (family Holothuridae) is an abundant sea cucumber distributed in the South China Sea and used as a tonic in China;[4] however, no chemical or pharmacological studies have been reported to date on this species. *Pyricularia oryzae* P-2b, a plant pathogenic fungus, has been used as a test microorganism for the primary screening of antineoplastic and antifungal agents.[5−7] In the course of our search for new bioactive compounds from echinoderms,[7−11] we found that an *n*-BuOH extract prepared from *H. fuscocinerea* showed significant deforming effect [minimum morphological deformation concentration (MMDC) = 32 *μ*g/mL, 5-FU as positive control with MMDC = 4 *μ*g/mL] against *P. oryzae*. Bioassay-guided fractionation led to the isolation of five triterpene glycosides, **1**−**5**. We report herein the isolation, structural elucidation, and biological activity of three new glycosides, fuscocinerosides A (**1**), B (**2**), and C (**3**), together with two known glycosides, which were identified as pervicoside C (**4**), originally isolated from the sea cucumber *H. pervicax*, and holothurin A (**5**), originally isolated from the sea cucumber *H. leucospilota*, by comparison of physical and spectroscopic data (MS and 2D NMR) with literature values.[12,13]

An ethanolic extract of *H. fuscocinerea* was suspended in H_2O and partitioned successively with petroleum ether, $CHCl_3$, and *n*-BuOH. The *n*-BuOH extract was subjected to several chromatographic purification steps to afford **1**−**5**.

Fuscocineroside A (**1**), obtained as colorless crystals, was positive to Liebermann-Burchard and Molish tests. The molecular formula was established as $C_{56}H_{87}O_{28}SNa$ from the $[M + Na]^+$ ion at *m*/*z* 1285.4910 in the positive ion mode HRESIMS and $[M - Na]^-$ ion at *m*/*z* 1239 in the negative ion mode ESIMS. A fragment ion peak at *m*/*z* 1165 $[M + Na - NaHSO_4]^+$ indicated the presence of a sulfate group in **1**, which was confirmed by the IR spectrum with absorption bands at 1258 and 1210 cm^{-1}. The IR spectrum also showed an absorption band due to a *γ*-lactone moiety (1768 cm^{-1}) and strong broad absorptions (3423, 1070 cm^{-1}) reminiscent of a glycosidic structure.



The ^1H NMR, ^{13}C NMR, and DEPT spectra displayed resonances due to seven tertiary methyl groups, one olefinic bond (δ_H 5.60; δ_C 153.2, 115.9), one lactone carbonyl group (δ_C 176.5), and one doublet (δ_H 4.42; δ_C 67.7) that was ascribed to a methine proton linked to a carbon bearing a hydroxy group and suggested that the aglycone of **1** had a holostane triterpenoid skeleton with a 9(11)-en-12-ol moiety.[14] The 12α configuration of the hydroxy group was confirmed by a cross-peak at δ 4.42/1.68 (H-12/H_3-21) in the NOESY spectrum and from the coupling constant for H-12 with H-11 (4.4 Hz).[15] The assignments of the NMR signals associated with the aglycone moiety (Tables 1 and 2) were derived from DQCOSY, TOCSY, HMQC, and HMBC experiments. These data were closely similar to those observed for glycoside **4**, except for the signals due to the side chain. The NMR spectrum of the side chain in the aglycone moiety of **1** showed resonances due to two methyl groups (CH_3-26 and CH_3-27: δ_H 1.39, 1.38; δ_C 25.9, 25.8), an acetoxy group (δ_H 1.84; δ_C 170.1, 22.1), one oxygen-bearing quaternary carbon (C-25: δ_C 81.2), two methylenes (CH_2-23 and CH_2-24: δ_H 2.18, 2.84; δ_C 34.3, 34.4), and a ketone (C-22: δ_C 210.5). The HMBC spectrum showed cross-peaks H_3-26/C-25, H_3-26/C-24, H_3-27/C-25, H_3-27/C-24, H_3-26/C-27, and H_3-27/C-26, which, together with the chemical shift of C-25, supported the presence of the two methyl groups attached to C-25 bearing the acetoxy group. This was confirmed by a fragment ion at *m*/*z* 1225

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Table 1. ^{1}H NMR Spectroscopic Data[a] (400 MHz, C_5D_5N) for Fuscocinerosides A (**1**), B (**2**), and C (**3**)

position	1	2	3	position	1	2	3
1	1.77, brt (6.0), 1.29, m	1.75, 1.30, m	1.75, 1.34, m	Xyl			
2	2.01, 1.81, m	2.02, 1.80, m	2.00, 1.87, m	1	4.57, d (6.8)	4.56, d (6.8)	4.56, d (6.4)
3	3.05, brd (8.8)	3.06, brd (8.8)	3.03, m	2	3.91, m	3.93, m	3.93, m
4				3	4.16, m	4.16, m	4.16, m
5	0.89, brd (11.2)	0.91, m	0.88, m	4	4.98, m	4.96, m	4.99, m
6	1.64, 1.47, m	1.63, 1.47, m	1.65, 1.54, m	5	4.54, t-like, 3.59, m	4.55, 3.59, m	4.56, 3.58, m
7	1.67, 1.34, m	1.65, 1.34, m	1.69, 1.35, m	Qui			
8	3.23, dd (12.4, 4.0)	3.23, brd (12.4)	3.29, m	1	4.90, d (7.6)	4.87, d (8.0)	4.90, d (7.6)
9				2	3.85, m	3.88, m	3.90, m
10				3	3.99, m	3.99, m	4.00, m
11	5.60, d (4.4)	5.61, brs	5.61, d (4.0)	4	3.54, m	3.56, m	3.55, m
12	4.42, d (4.4)	4.44, brs	4.45, brs	5	3.62, m	3.61, m	3.59, m
13				6	1.59, d (6.0)	1.59, d (6.0)	1.58, d (5.6)
14				Glc			
15	1.51, 1.32, m	1.53, 1.32, m	1.56, 1.32, m	1	4.85, d (7.6)	4.85, d (6.8)	4.86, d (7.2)
16	2.11, 1.87, m	2.09, 1.82, m	2.25, 1.94, m	2	3.97, m	3.98, m	3.99, m
17	3.51, m	3.53, m	3.27, m	3	4.10, m	4.11, m	4.13, m
18				4	4.00, m	4.00, m	3.98, m
19	1.28, s	1.29, s	1.29, s	5	3.88, m	3.91, m	3.88, m
20				6	4.31, dd (12.4, 1.6), 4.13, m	4.31, brd (13.2), 4.13, m	4.31, brd (11.4), 4.12, m
21	1.68, s	1.67, s	1.50, s	MeGlc			
22			4.14, m	1	5.17, d (7.6)	5.18, d (7.2)	5.20, d (6.0)
23	2.18, m	2.65, m	1.88, 1.80, m	2	3.94, m	3.95, m	3.96, m
24	2.84, m	1.48, m	1.57, m	3	3.57, m	3.58, m	3.56, m
25		1.46, m		4	3.96, m	3.97, m	4.01, m
26	1.39, s	0.75, d (6.0)	1.15, s	5	3.87, m	3.90, m	3.92, m
27	1.38, s	0.75, d (6.0)	1.11, s	6	4.34, dd (11.6, 1.6), 4.15, m	4.35, brd (11.2), 4.15, m	4.36, brd (11.2), 4.15,m
30	0.94, s	0.94, s	0.93, s	OCH_3	3.74, s	3.74, s	3.74, s
31	1.13, s	1.13, s	1.10, s				
32	1.12, s	1.12, s	1.17, s				
$COCH_3$	1.84, s						

[a] Assignments aided by DQCOSY, TOCSY, and NOESY experiments. Coupling constants (in Hz) are given in parentheses.

Table 2. ^{13}C NMR Spectroscopic Data[a] (100 MHz, C_5D_5N) for Fuscocinerosides A (**1**), B (**2**), and C (**3**)

position	1	2	3	position	1	2	3
1	36.5	36.5	36.6	Xyl			
2	27.2	27.2	27.1	1	105.1	105.1	105.1
3	88.8	88.8	88.9	2	83.3	83.3	83.3
4	40.0	40.0	40.0	3	75.2	75.2	75.2
5	52.8	52.8	52.9	4	76.2	76.2	76.3
6	21.2	21.2	21.3	5	64.2	64.2	64.2
7	28.8	28.8	28.9	Qui			
8	40.2	40.2	40.2	1	105.4	105.3	105.3
9	153.2	153.2	153.3	2	75.4	75.4	75.4
10	39.7	39.7	39.7	3	75.8	75.8	75.9
11	115.9	115.9	116.2	4	86.6	86.6	86.6
12	67.7	67.8	68.4	5	71.9	71.9	71.9
13	63.8	63.8	63.9	6	18.0	18.0	18.1
14	46.5	46.6	46.3	Glc			
15	37.0	37.0	37.4	1	104.7	104.7	104.7
16	27.0	27.0	23.8	2	74.0	74.0	74.0
17	46.9	46.9	47.8	3	88.0	88.0	88.0
18	176.5	176.6	177.5	4	69.5	69.5	69.6
19	22.5	22.6	22.5	5	77.6	77.6	77.6
20	90.2	90.2	83.7	6	61.8	61.8	61.9
21	24.8	24.9	21.0	MeGlc			
22	210.5	211.0	80.4	1	105.8	105.8	105.8
23	34.3	37.4	27.4	2	74.9	74.9	75.0
24	34.4	32.1	38.7	3	87.9	87.9	87.9
25	81.2	27.7	81.2	4	70.7	70.7	70.7
26	25.9	22.5	28.7	5	78.2	78.2	78.2
27	25.8	22.4	27.4	6	62.1	62.1	62.2
30	16.7	16.7	16.7	OMe	60.7	60.7	60.7
31	28.1	28.1	28.2				
32	21.8	22.0	22.1				
$COCH_3$	170.1						
$COCH_3$	22.1						

[a] Assignments aided by HMQC and HMBC experiments.

$[M + Na - CH_3COOH]^+$ in the positive ion mode ESIMS. The TOCSY spectrum indicated that the two methylenes comprised a four-spin system. The location of the ketone carbonyl group at C-22 was deduced from the chemical shift of the C-22 signal, which showed correlations with the H_2-24 protons at δ 2.84 and the H_3-21 protons at δ 1.68 in the HMBC spectrum. Thus, the aglycone of **1** was determined to be 25-acetoxy-22-oxo-9(11)-holostene-3β,12α-diol, whose relative stereochemistry was confirmed from the NOESY spectrum as shown in Figure S1, Supporting Information.

The sugar moieties of **1** were determined to be D-xylose (Xyl), D-quinovose (Qui), D-glucose (Glc), and 3-*O*-methyl-D-glucose (MeGlc) in the ratio 1:1:1:1 by acidic hydrolysis with aqueous 2 mol/L trifluoroacetic acid and preparation of the corresponding aldononitrile peracetates, which were analyzed by GC-MS. The common D-configuration for the four carbohydrate units was assumed also according to those most often encountered among the sea cucumber glycosides.[16,17] The ^{1}H NMR spectrum showed signals for four anomeric protons at δ 4.57, 4.85, 4.90, and 5.17, each a doublet with $^3J_{H-1/H-2} = 6.8-7.6$ Hz, which were correlated in the HMQC experiment with the corresponding carbons at δ 105.1, 104.7, 105.4, and 105.8, respectively. The large vicinal coupling constants of each anomeric proton indicated a *trans*-diaxial orientation with respect to their coupling partners (β-configuration).

The DQCOSY experiment allowed the sequential assignment of most of the resonances for each sugar ring, starting from the easily distinguished signals due to anomeric protons. Complete assignment was achieved by combination of DQCOSY and TOCSY results. The HMQC experiment correlated all proton resonances with those of their corresponding carbons. Data from the above experiments (Table 1 and 2) indicated that four sugar residues are in their pyranose forms, and the location of the interglycosidic linkages was deduced from the chemical shifts of Xyl C-2 (δ 83.3), Qui C-4 (δ 86.6), and Glc C-3 (δ 88.0), which were downfield relative to shifts expected for the corresponding methyl glycopyranosides.[18] The data for the carbohydrate moiety of **1** were identical to those of **4**, which has a sulfate attached to C-4 of the xylose residue. The structure of the carbohydrate chain of **1** was corroborated by the HMBC spectrum, which showed a cross-peak between C-3 of

Table 3. Cytotoxicity of Glycosides **1**−**5** against Two Cancer Cell Lines in Vitro (IC_{50}, μmol/L)[a]

cell line	**1**	**2**	**3**	**4**	**5**	HCP[b]
HL-60	6.21 ± 1.22	3.75 ± 0.78	0.88 ± 0.32	4.48 ± 0.97	4.42 ± 1.06	0.41 ± 0.13
BEL-7402	5.58 ± 0.36	2.64 ± 0.34	0.58 ± 0.05	0.66 ± 0.08	0.76 ± 0.11	0.28 ± 0.03

[a] IC_{50} values are means from three independent experiments (average ± SD). [b] 10-Hydroxycamptothecine (HCP) as positive control.

the aglycone and H-1 of xylose, indicating that Xyl was connected to C-3 of the aglycone, a cross-peak Qui H-1/Xyl C-2, which showed the linkage of the quinovose at C-2 of Xyl, and the linkages of the terminal 3-*O*-methylglucose at the C-3 of Glc in turn linked to C-4 of Qui by cross-peaks MeGlc H-1/Glc C-3 and Glc H-1/Qui C-4. The exact sequence of the sugars and their points of attachment were also confirmed by the NOESY spectrum as shown in Figure S1, Supporting Information. The site of the sulfate linkage was determined by comparing the ^{13}C NMR data of **1** with those of **4** and DS-pervicoside C, the desulfated derivative of **4**.[12] A downfield esterification shift was observed for the signal of Xyl C-4 (from δ 71.0 to 76.2), indicating that the sulfate group was located at C-4 of the xylose unit.

Hence, the structure of fuscocineroside A (**1**) was elucidated as 3-*O*-[3-*O*-methyl-β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl-(1→4)-β-D-quinovopyranosyl-(1→2)-4-*O*-sodiumsulfato-β-D-xylopyranosyl]-25-acetoxy-22-oxo-9(11)-holostene-3β,12α-diol. More than 10 sea cucumber glycosides bearing a 23-oxo functionality in the side chain have been reported; however, compound **1** is the first example of a sea cucumber glycoside with a ketone at C-22, which is a very uncommon structural feature and previously found in only three asterosaponins from the starfish *Goniopecten demonstrans*.[19]

Fuscocineroside B (**2**) was obtained as colorless crystals. The molecular fomula was determined as $C_{54}H_{85}O_{26}SNa$ by ^{13}C NMR and HRESIMS. The positive ion mode ESIMS showed an $[M + Na]^+$ ion peak at *m*/*z* 1227 shifted by 58 mass units relative to **1** (*m*/*z* 1285), thus indicating the absence of an acetoxy group in the side chain of **2**. A fragment ion peak at *m*/*z* 1107 $[M + Na - NaHSO_4]^+$ confirmed the presence of a sulfate group. The presence of D-xylose, D-quinovose, D-glucose, and 3-*O*-Me-D-glucose in a 1:1:1:1 ratio was established by acid hydrolysis and preparation of the corresponding aldononitrile peracetates, which were analyzed by GC-MS. The ^{13}C NMR data for the aglycone and sugar portion of **2** bore a close resemblance to those of **1**, from which **2** differed only by the replacement of the quaternary carbon (C-25) bearing the acetoxy group by the signal of a methine (δ 27.7) and downfield or upfield shifts of the neighboring signals due to C-23, C-24, C-26, and C-27. The absence of the acetoxy group linked to C-25 in **2** was confirmed on the basis of analysis of 2D NMR spectra, especially the TOCSY cross-peaks displayed by H-25 at δ 1.46 and the H-23, H-24, H_3-26, and H_3-27 protons, as well as the HMBC correlations between the methine carbon C-25 (δ 27.7) and the H-23, H-24, H_3-26, and H_3-27 protons. The presence of a ketone at C-22 in **2** was also deduced from correlations between C-22 (δ 211.0) and the H_2-24 (δ 1.48) and H_3-21 (δ 1.67) protons in the HMBC spectrum. Thus, the aglycone of **2** was determined to be 22-oxo-9(11)-holostene-3β,12α-diol. This is the second example of the aglycone of a sea cucumber glycoside bearing a ketone at C-22. Therefore, **2** was elucidated as 3-*O*-[3-*O*-methyl-β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl-(1→4)-β-D-quinovopyranosyl-(1→2)-4-*O*-sodiumsulfato-β-D-xylopyranosyl]-22-oxo-9(11)-holostene-3β,12α-diol.

Fuscocineroside C (**3**), colorless crystals, had the molecular formula $C_{54}H_{85}O_{26}SNa$, as deduced from combined HRESIMS and ^{13}C NMR analyses. Ion peaks at *m*/*z* 1227 $[M + Na]^+$ and 1107 $[M + Na - NaHSO_4]^+$ confirmed the presence of a sulfate group. Comparison of the NMR spectra of **3** with those of **1** and extensive 2D NMR studies indicated that **3** and **1** possess the same tetrasaccharide chain and holotane skeleton, but differ in their side chains. The side chain in the aglycone moiety of **3** was shown to be identical to that of glycoside **5**, by comparison of the NMR spectra of their corresponding side chain parts, i.e., the 22,25-epoxy side chain. The ^1H and ^{13}C NMR signals attributable to the aglycone moiety were assigned by the 2D NMR experiments, and the data confirmed the presence of 22,25-epoxy-9(11)-holostene-3β,12α-diol as the aglycone of **3**, identical to that of holothurinoside C previously isolated from the sea cucumber *H. forskalii*.[20] A cross-peak at δ 4.14/1.11 (H-22/H_3-27) in the NOESY spectrum indicated that H-22 and CH_3-27 were on the same side of the furan ring; however, the absolute stereochemistry at C-22 remains to be determined. Thus, fuscocineroside C (**3**) was defined as 3-*O*-[3-*O*-methyl-β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl-(1→4)-β-D-quinovopyranosyl-(1→2)-4-*O*-sodiumsulfato-β-D-xylopyranosyl]-22,25-epoxy-9(11)-holostene-3β,12α-diol.

When tested for bioactivity of inducing morphological deformation of *P. oryzae* mycelia, glycosides **1**−**5** exhibited significant activity, with MMDC values of 16, 16, 4, 4, and 8 μg/mL, respectively. The in vitro cytotoxicity of **1**−**5** against human leukemia HL-60 and human hepatoma BEL-7402 cells was also evaluated. The IC_{50} of each glycoside tested was measured on the basis of cell viability, after 72 h treatment. The results are listed in Table 3 and showed that all the glycosides exhibited cytotoxicity against the two cell lines, while **3** was more potent in the HL-60 cell line and **3**−**5** possessed more significant cytotoxicity in the BEL-7402 cell line.

Membranolytic activity of triterpene glycosides probably determines the defense of the sea cucumbers against predators and correlates with their cytotoxicity to tumor cells.[21,22] Earlier studies on the structure−activity relationship for sea cucumber glycosides revealed that the structural generality, i.e., the lanostane triterpene with an 18(20) lactone and a sugar chain linked to the C-3 of the aglycone, is responsible for the membranolytic activity, which may be the reason for the remarkable cytotoxic activity of these glycosides. The differences in the cytotoxicity against BEL-7402 cancer cells between glycosides **1**, **2** and **3**, **4**, **5** were ascribed to the difference of the side chains. Glycosides **3** and **5** share the same tetrasaccharide chain and holostane skeleton, but differ only in the 17-substituents. Such a minor difference led to the large difference in cytotoxicity against HL-60 cells. However, there were not significant differences between the cytotoxic effects of **3** and **5** against BEL-7402 cells. It was envisioned that, on the basis of the data available, the cytotoxicity of sea cucumber glycosides is very sensitive to their precise functionalization, and perhaps they show different sensitivities against different cancer cell lines. Therefore, more extensive studies are needed before a clear structure−activity relationship can be reached. Meanwhile, it is worthy of mention that the *P. oryzae* bioassay has a predictive value in screening anticancer agents.

Experimental Section

General Experimental Procedures. Melting points were determined on an XT5-XMT apparatus. Optical rotations were measured with a Perkin-Elmer 341 polarimeter. IR spectra were recorded on a Bruker Vector 22 infrared spectrometer. NMR spectra were recorded in C_5D_5N on a Varian Inova-400 spectrometer, and the 2D NMR spectra were obtained using standard pulse sequences. ESIMS and HRESIMS were recorded on a Micromass Quattro mass spectrometer. GC-MS were performed on a Finnigan Voyager apparatus using a DB-5 column (30 m × 0.25 mm i.d., 0.25 μm) with an initial temperature of 150 °C for 2 min and then temperature programming to 300 °C at a rate of 15 °C/min. Semipreparative HPLC was carried out on an Agilent 1100

liquid chromatograph equipped with a refractive index detector using a Zorbax 300 SB-C_{18} column (25 cm × 9.4 mm i.d.). Column chromatographies were performed on Si gel H (10−40 μm, Qingdao Marine Chemical Inc.), Sephadex LH-20 (Pharmacia), and reversed-phase Si gel (Lichroprep RP-18, 40−63 μm). Fractions were monitored by TLC, and spots were visualized by heating Si gel plates sprayed with 10% H_2SO_4 in EtOH.

Animal Material. Specimens of *H. fuscocinerea* were collected at a depth of 2−30 m by a fishery bottom trawler from offshore waters of Leizhou Bay (Zhanjiang, Guangdong Province) in the South China Sea in November 2004 and dried in the sun. The organisms were identified by Professor Rui-Zao Yi of the Third State Marine Research Bureau, China. A voucher specimen was deposited in the Research Center for Marine Drugs, School of Pharmacy, Second Military Medical University, Shanghai, China, under the registration code number HF-2004-11.

Extraction and Isolation. The sea cucumbers (4.4 kg, dry weight) were crumbled and extracted three times with 60% EtOH. The combined extracts were concentrated to leave a rufous residue, which was suspended in H_2O (4 L) and then partitioned successively using petroleum ether (4 L × 3), $CHCl_3$ (4 L × 3), and *n*-BuOH (4 L × 4). The *n*-BuOH fraction (15 g) was chromatographed on Si gel eluting with a $CHCl_3$/MeOH/H_2O (8:2:1 to 6.5:3.5:1) (lower phase) gradient to give four major fractions (A−D) based on TLC analysis. Fractions C (1.9 g) and D (2.0 g) mainly contained triterpene glycosides and proved to be active against *P. oryzae* (MMDC = 16 and 32 μg/mL, respectively). Fraction C was subjected to MPLC on a Lobar column (Lichroprep RP-18) eluting with MeOH/H_2O (49:51) to give three major fractions (C1, C2, and C3). The crude glycoside fraction C2 (600 mg) exhibited the strongest activity against *P. oryzae* (MMDC = 8 μg/mL) and was further purified by repeated semipreparative HPLC to afford pure glycosides **1** (208 mg, t_R = 19.2 min), **2** (89 mg, t_R = 21.4 min), **3** (50 mg, t_R = 16.1 min), and **4** (30 mg, t_R = 26.4 min) using MeOH/H_2O (49:51) as the mobile phase and a flow rate of 2 mL/min. Fraction D was subjected to size exclusion chromatography on a Sephadex LH-20 column equilibrated with MeOH/H_2O (4:6) and finally purified by means of HPLC eluting with MeOH/H_2O (48:52) at a flow rate of 1.5 mL/min to yield pure glycoside **5** (29 mg) in 25.5 min.

Fuscocineroside A (1): colorless crystals; mp 249−250 °C; $[\alpha]^{20}_D$ +6.3 (*c* 0.76, pyridine); IR (KBr) ν_{max} 3423, 1768, 1642, 1258, 1210, 1070 cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 2; ESIMS (positive ion mode) *m/z* 1301 $[M + K]^+$, 1285 $[M + Na]^+$, 1245 $[M - OH]^+$, 1225 $[M + Na - CH_3COOH]^+$, 1213 $[1245 - OMe - H]^+$, 1183 $[M + Na - SO_3Na + H]^+$, 1165 $[M + Na - NaHSO_4]^+$, 1105 $[1165 - CH_3COOH]^+$, 545 [aglycone + H]$^+$; ESIMS (negative ion mode) *m/z* 1239 $[M - Na]^-$; HRESIMS (positive ion mode) *m/z* 1285.4910 $[M + Na]^+$ (calcd for $C_{56}H_{87}O_{28}SNa_2$, 1285.4900).

Fuscocineroside B (2): colorless crystals; mp 248−250 °C; $[\alpha]^{20}_D$ +7.4 (*c* 0.82, pyridine); IR (KBr) ν_{max} 3422, 1766, 1640, 1257, 1210 cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 2; ESIMS (positive ion mode) *m/z* 1243 $[M + K]^+$, 1227 $[M + Na]^+$, 1107 $[M + Na - NaHSO_4]^+$; ESIMS (negative ion mode) *m/z* 1181 $[M - Na]^-$; HRESIMS (positive ion mode) *m/z* 1227.4851 $[M + Na]^+$ (calcd for $C_{54}H_{85}O_{26}SNa_2$, 1227.4845).

Fuscocineroside C (3): colorless crystals; mp 250−251 °C; $[\alpha]^{20}_D$ −3.5 (*c* 0.82, pyridine); IR (KBr) ν_{max} 3433, 1756, 1673, 1264, 1217 cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 2; ESIMS (positive ion mode) *m/z* 1227 $[M + Na]^+$, 1107 $[M + Na - NaHSO_4]^+$; ESIMS (negative ion mode) *m/z* 1181 $[M - Na]^-$; HRESIMS (positive ion mode) *m/z* 1227.4853 $[M + Na]^+$ (calcd for $C_{54}H_{85}O_{26}SNa_2$, 1227.4845).

Acid Hydrolysis of 1−5. Each glycoside (1 mg) was heated with 2 mol/L trifluoroacetic acid (1 mL) and heated at 120 °C for 2 h. The reaction mixture was evaporated to dryness, and the residue was partitioned between CH_2Cl_2 and H_2O. The aqueous phase was concentrated under reduced pressure. Then, pyridine (1 mL) and NH_2-OH·HCl (2 mg) were added to the dried residue, and the mixture was heated at 90 °C for 30 min. After that time, 1 mL of Ac_2O was added and heating at 90 °C was continued for a further 1 h. The solution was concentrated, and the resulting aldononitrile peracetates were analyzed by GC-MS using standard aldononitrile peracetates as reference samples. Xylose, quinovose, glucose, and 3-*O*-methylglucose were identified in a 1:1:1:1 ratio for all five glycosides.

Bioassays. The *P. oryzae* bioassay was performed following a reported method.[5] The bioactivity against *P. oryzae* was expressed as MMDC, and an MMDC value of <512 μg/mL was considered active. The cytotoxicity against human leukemia HL-60 cells was determined using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) colorimetric assay on 96-well microplates as previously reported.[23] The cytotoxicity data against human hepatoma BEL-7402 cells was evaluated by the sulforhodamine B (SRB) protein assay described in a previous paper.[24] Dose−response curves were plotted for the samples, and the IC_{50} values were calculated as the concentrations of the test glycosides resulting in 50% reduction of absorption compared to the control cells. The data represented the means of three independent experiments in which each compound concentration was tested in three replicate wells. The anticancer agent 10-hydroxycamptothecine (HCP) was used as the positive control.

Supporting Information Available: Spectroscopic details of **4** and **5** (Tables S1 and S2), key HMBC correlations of **1**−**3** (Table S3), key NOESY correlations of **1** (Figure S1), and key DQCOSY and HMBC correlations of **1** (Figure S2). This material is available free of charge via the Internet at http://pubs.acs.org.

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