Bioactive Asterosaponins from the Starfish *Culcita novaeguineae*

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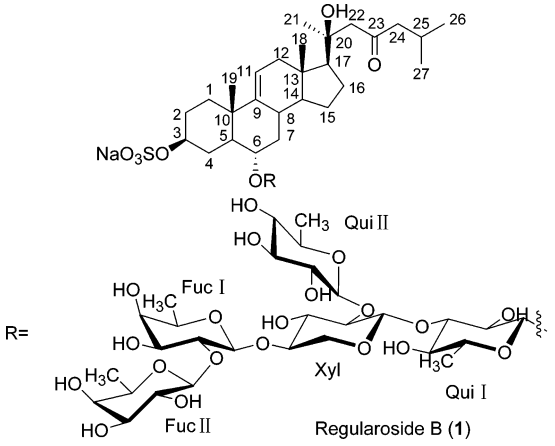
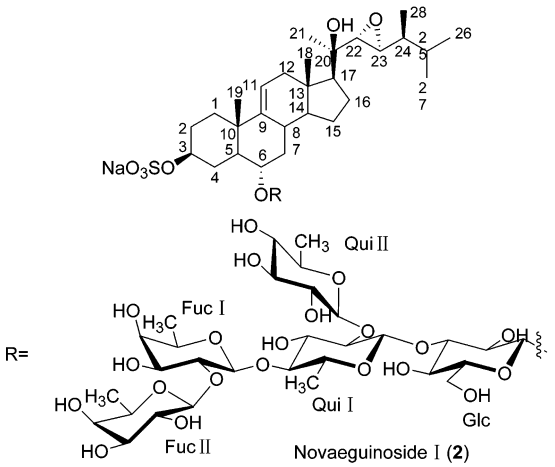
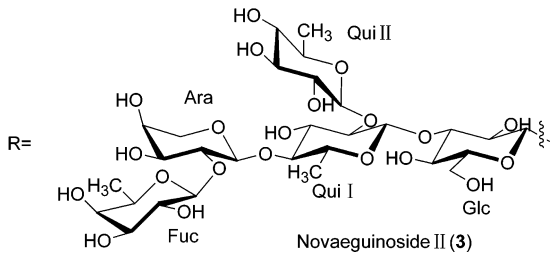
Two new sulfated steroidal pentaglycosides (asterosaponins), novaeguinosides I (**2**) and II (**3**), along with the known regularoside B (**1**) were isolated from the starfish *Culcita novaeguineae*. Their structures were elucidated by extensive NMR techniques as well as chemical evidence. The new asterosaponins showed marginal in vitro cytotoxicity against two human tumor cell lines.

Steroidal glycosides are the predominant metabolites of starfish and are responsible for their general toxicity.[1,2] According to the chemical structures, they have been subdivided into three main groups: asterosaponins, cyclic steroidal glycosides, and glycosides of polyhydroxylated steroids. Members of the first group are usually sulfated steroidal penta- or hexaglycosides based on a $\Delta^{9(11)}$-3β,6α-dioxysteroidal aglycone with a sulfate group attached at C-3 and the oligosaccharide moiety at C-6.[3] These saponins have been reported to exhibit various biological activities, including cytotoxic, hemolytic, antibacterial, antiviral, and antifungal effects.[4] Cushion stars (*Culcita novaeguineae* Müller et Troschel, order Valvatida, family Oreasteridae) are abundant starfish distributed in the South China Sea. Iorizzi and Kicha have reported the isolation of 13 polyhydroxysteroid glycosides and five polyhydroxysteroids from the starfish.[5−7] However, no asterosaponins have been reported to date. As part of our search for new bioactive compounds from echinoderms,[8,9] we have investigated the ethanolic extract of *C. novaeguineae*, which showed a significant deforming effect [minimum morphological deformation concentration (MMDC) = 64 μg/mL, 5-FU as positive control with MMDC = 5 μg/mL] against *Pyricularia oryzae* P-2b.[10] We report herein the isolation, structural elucidation, and biological activity of two new asterosaponins, novaeguinosides I (**2**) and II (**3**), together with a known saponin, which was deduced as regularoside B (**1**) originally isolated from the starfish *Halityle regularis* and *Luidia clathrata*, by comparison of physical and spectral data (MS and 2D NMR) with literature values.[11,12]







Results and Discussion

An ethanolic extract of *C. novaeguineae* was suspended in H_2O and partitioned successively with petroleum ether and *n*-BuOH. The *n*-BuOH layer was dried and subjected to several chromatographic purification steps to afford **1**−**3**.

Novaeguinoside I (**2**), colorless crystals, was positive to Liebermann−Burchard and Molish tests. The molecular formula was established as $C_{58}H_{95}O_{28}SNa$ from the $[M + Na]^+$ ion at *m/z* 1317.5535 (calcd for $C_{58}H_{95}O_{28}SNa_2$, 1317.5526) in the positive ion mode HRESIMS and the $[M - Na]^-$ ion at *m/z* 1271 in the negative ion mode ESIMS. The fragment ion peak at *m/z* 1197 $[M + Na - NaHSO_4]^+$ in the positive ion mode ESIMS/MS indicated the presence of a sulfate group in the glycoside. This was confirmed by the IR spectrum with absorption bands at 1242 and 1212 cm^{-1}.

The ^1H, ^{13}C NMR and DEPT spectra displayed resonances due to three tertiary methyl groups (δ_H 0.99, 0.92,

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Table 1. ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR Data[a] and Key HMBC and NOESY Correlations of Novaeguinoside I (**2**) in C_5D_5N

position	δ_H mult[b]	δ_C mult[c]	position	δ_H mult[b]	δ_C	HMBC	NOESY
1	α = 1.38 m, β = 1.63 m	36.1 t	Glc				
2	α = 2.75 m, β = 1.84 m	29.5 t	1	4.90d(7.6)/4.95d(7.6)[d]	105.5	C-6	H-6
3	4.87 m	77.7 d	2	3.95 m	73.9	Glc C-1, -3	
4	α = 3.48 m, β = 1.67 m	30.9 t	3	3.81 m	91.9	QuiI C-1	QuiI H-1
5	1.49 m	49.4 d	4	4.05 m	69.8	Glc C-3	
6	3.79 m[e]	81.1 d	5	3.82 m	77.6		
7	α = 1.24 m, β = 2.70 m	41.7 t	6	4.44 d (10.8), 4.27 brt (6.0)	62.3		
8	2.03 m	35.3 d	QuiI				
9		145.7 s	1	4.89 d (7.2)/4.89 d (7.2)[d]	103.9	Glc C-3	Glc H-3
10		38.4 s	2	4.06 m	82.2	QuiII C-1, QuiI C-1	QuiII H-1
11	5.23 brd (4.9)	116.7 d	3	4.01 m	75.2	QuiI C-2, -4	
12	α = 2.05 m, β = 2.29 dd (16.2, 5.4)	42.4 t	4	3.50 t (8.4)	86.0	FucI C-1, QuiI C-3, -5, -6	FucI H-1
13		41.9 s	5	3.83 m	71.8	QuiI C-1, -3, -4, -6	
14	1.16m	53.8 d	6	1.69 d (5.4)	18.3	QuiI C-5	
15	α = 1.81 m, β = 2.12 m	23.1 t	QuiII				
16	α = 1.61 m, β = 1.16 m	25.3 t	1	5.25d(6.2)/5.02d(7.6)[d]	104.8	QuiI C-2	QuiI H-2
17	1.66 m	59.6 d	2	4.02 m	76.3	QuiII C-1	
18	0.99 s	13.5 q	3	4.03 m	76.7		
19	0.92 s	19.3 q	4	4.04 m	75.5		
20		71.4 s	5	3.62 m	73.9		
21	1.41 s	23.6 q	6	1.72 d (6.0)	17.9	QuiII C-4, -5	
22	2.85 d (1.8)	64.5 d	FucI				
23	2.93 d d(7.8, 1.8)	57.5 d	1	4.74 d (7.5)/4.76 d (7.6)[d]	102.9	QuiI C-4	QuiI H-4
24	1.19 m	42.0 d	2	4.37 t (8.4)	82.6	FucII C-1, FucI C-1, -3	FucII H-1
25	1.69 m	31.8 d	3	4.10 m	75.2		
26	0.95 d (7.2)	20.5 q	4	3.99 m	71.6		
27	0.88 d (6.6)	19.2 q	5	3.80 m	71.8	FucI C-1, -4, -6	
28	0.87 d (8.4)	13.0 q	6	1.43 d (6.0)	17.1	FucI C-5	
			FucII				
			1	4.88 d (8.1)/4.87 d (8.0)[d]	107.2	FucI C-2	FucI H-2
			2	4.38 t (8.4)	73.5	FucII C-1, -3	
			3	3.97 m	75.4		
			4	3.93 m	72.5		
			5	3.61 m	71.9	FucII C-1, -4, -6	
			6	1.41 d (6.0)	16.8	FucII C-5	

[a] Assignments aided by DQCOSY, TOCSY, HMQC, and HMBC experiments. [b] Coupling constants (in Hz) are given in parentheses. [c] Multiplicity by DEPT. [d] Recorded in $C_5D_5N-D_2O$. [e] Cross-peak was detected in the HMBC spectrum for H-6 of aglycone with Glc C-1.

1.41; δ_C 13.5, 19.3, 23.6), one olefinic proton (δ_H 5.23; δ_C 145.7, 116.7), and one multiplet (δ_H 4.87; δ_C 77.7) that was ascribed to a methine proton linked to a carbon bearing a sulfate group, and suggested that the aglycone of **2** has a $\Delta^{9(11)}$-3β,6α,20-trihydroxysteroidal nucleus.[13] Glycosidation at C-6 was supported by the downfield shift of the C-6 signal in the ^{13}C NMR spectrum with respect to the corresponding value in synthetic asterosaponin aglycones containing the 3β,6α-dihydroxy oxidation pattern.[14] The assignments of the NMR signals associated with the aglycone moiety (Table 1) were derived from DQCOSY, TOCSY, HMQC, and HMBC experiments. These data were identical with those observed in regularoside A,[11] pectinioside B,[15] and patirioside A,[16] the asterosaponins containing the same (20*R*,22*R*,23*S*,24*S*)-22,23-epoxy-24-methyl-5α-cholest-9(11)-ene-3β,6α,20-triol 3β-sulfated aglycone, derived from the starfishes *Halityle regularis*, *Asteina pectinifera*, and *Patiria miniata*, respectively. Particularly diagnostic for the 22,23-epoxy-24-methyl side chain are the two one-proton signals at δ 2.85 (d, J = 1.8 Hz) and 2.93 (dd, J = 7.8, 1.8 Hz) for the epoxide methine protons that correlated in the HMQC experiment with carbon signals at δ 64.5 and 57.5, and those at δ 0.87 (3H, d, J = 8.4 Hz) for the C-28 methyl protons.[16] The determination of the stereochemistry at C-20, -22, -23, and -24 in regularoside A has been achieved by comparison of the ^{1}H and ^{13}C NMR spectra with those of model stereoisomers, i.e., (22*S*,23*S*,-24*R*; 22*R*,23*R*,24*R*; 22*S*,23*S*,24*S*; and 22*R*,23*R*,24*S*) 24-methyl-22,23-epoxy-3α,5-cyclo-5α-cholestan-6β-yl acetate, since the NMR signals due to the side chain of all the model stereoisomers were more or less different from each other.[11] In the NMR spectra of **2** the proton and carbon signals were in good agreement with those of regularoside A, and thus **2** was presumed to have the same stereochemistry for the asymmetric carbons in the side chain.

The ^{1}H NMR spectrum of **2** exhibited five doublets in the downfield region, ascribable to the anomeric protons (δ 4.74, 4.88, 4.89, 4.90, and 5.25) that correlated in the HMQC experiment with carbons at δ 102.9, 107.2, 103.9, 105.5, and 104.8, respectively. The large vicinal coupling constants ($^3J_{H-1/H-2}$ = 6.2−8.1 Hz) of each anomeric proton indicated a *trans*-diaxial orientation with respect to their coupling partners (β-configuration). The methyl doublets at δ 1.41, 1.43, 1.69, and 1.72 in the ^{1}H NMR spectrum indicated the presence of four 6-deoxy sugar moieties. The presence of D-quinovose, D-fucose, and D-glucose in a 2:2:1 ratio was confirmed by acidic hydrolysis with aqueous 2 mol/L trifluoroacetic acid and preparation of the corresponding aldononitrile peracetates, which were analyzed by GC-MS.[8,13] The common D-configuration for the five carbohydrate units was assumed also according to those most often encountered among the starfish saponins.[17]

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 or methyl groups of 6-deoxy sugars. Complete assignment was achieved by combination of DQCOSY and TOCSY results. Indeed the TOCSY experiment clearly showed correlations for the H-1 to H-5 spin system of quinovose and for the H-1 to H_2-6 spin system of glucose. The coherence transfer to H-5 of fucose was not obtained because of the small H-4/H-5 coupling. The HMQC experi-

Table 2. ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR Data[a] and Key HMBC Correlations of Novaeguinoside II (**3**) in C_5D_5N

position	δ_H mult[b]	δ_C mult[c]	position	δ_H mult[b]	δ_C	HMBC
1	α = 1.24 m, β = 1.50 m	36.0 t	Glc			
2	α = 2.64 m, β = 1.71 m	29.5 t	1	4.783 d (6.3)	105.4	C-6
3	4.74 m	77.7 d	2	3.85 m	73.8	Glc C-1
4	α = 3.37 d (10.6), β = 1.55m	30.8 t	3	3.69 m	91.8	QuiI C-1
5	1.37 m	49.4 d	4	3.88 m	69.7	
6	3.67 m[d]	80.9 d	5	3.71 m	77.5	
7	α = 1.13 brd (11.9), β = 2.57 brd (11.6)	41.6 t	6	4.32 m, 4.15 m	62.3	
8	1.92 m	35.3 d	QuiI			
9		145.7 s	1	4.773 d (8.0)	103.9	Glc C-3
10		38.3 s	2	3.92 m	82.6	QuiII C-1
						QuiI C-1
11	5.11 m	116.6 d	3	3.94 m	75.2	QuiI C-2
12	α = 1.98 m, β = 2.16 dd (15.6, 3.9)	42.3 t	4	3.44 t (8.0)	84.7	QuiI C-3, Ara C-1
13		41.8 s	5	3.67 m	71.8	QuiI C-1
14	1.03 m	53.7 d	6	1.50 d (5.7)	18.3	QuiI C-4, -5
15	α = 1.70 m, β = 2.01 m	23.1 t	QuiII			
16	α = 1.47 m, β = 1.01 m	25.2 t	1	5.11 d (7.1)	105.0	QuiI C-2
17	1.53 m	59.6 d	2	3.91 m	76.2	QuiII C-1
18	0.87 s	13.4 q	3	3.92 m	76.7	
19	0.80 s	19.3 q	4	3.93 m	75.4	
20		71.3 s	5	3.52 m	73.6	
21	1.29 s	23.5 q	6	1.61 d (5.9)	17.8	QuiII C-4, -5
22	2.72 brs	64.4 d	Ara			
23	2.81 brd (8.2)	57.4 d	1	4.761 d (7.3)	102.6	QuiI C-4
24	1.08 m	41.9 d	2	4.31 m	81.8	Fuc C-1, Ara C-1, -3
25	1.57 m	31.7 d	3	4.13 m	73.5	
26	0.82 d (6.8)	20.4 q	4	4.16 m	68.1	
27	0.75 d (6.2)	19.2 q	5	3.59d (11.4), 4.20 d (12.3)	66.0	Ara C-1
28	0.74 d (6.3)	12.9 q	Fuc			
			1	4.745 d (8.0)	106.8	Ara C-2
			2	4.25 t (8.2)	73.4	Fuc C-1, -3
			3	3.86 m	75.1	
			4	3.83 m	72.5	
			5	3.53 m	71.8	Fuc C-1, -4, -6
			6	1.30 d (6.1)	17.0	Fuc C-4, -5

[a] Assignments aided by ^{1}H−^{1}H COSY, TOCSY, HSQC, and HMBC experiments. [b] Coupling constants (in Hz) are given in parentheses. [c] Multiplicity by DEPT. [d] Cross-peak was detected in HMBC spectrum for H-6 of aglycone with Glc C-1.

ment correlated all proton resonances with those of their corresponding carbons. The locations of the interglycosidic linkages in the pentasaccharide chain were deduced from the chemical shifts of Glc C-3 (δ 91.9), Qui I C-2 (δ 82.2), Qui I C-4 (δ 86.0), and Fuc I C-2 (δ 82.6), which were downfield relative to shifts expected for the corresponding methyl glycopyranosides.[18] The exact sequence of the sugars and their points of attachment were solved by means of HMBC and NOESY experiments. In the HMBC spectrum, a cross-peak between C-6 of the aglycone and H-1 of glucose indicated that glucose (Glc) was connected to C-6 of the aglycone. The linkage of quinovose (Qui I) at C-3 of Glc was indicated by the cross-peaks Qui I H-1/Glc C-3. Similarly, the linkages of the terminal fucose (Fuc II) at the C-2 of the other fucose (Fuc I), in turn linked to C-4 of Qui I, were indicated by the cross-peaks Fuc II H-1/Fuc I C-2 and Fuc I H-1/Qui I C-4. Finally, the location of the terminal quinovose (Qui II) at C-2 of Qui I was clearly indicated by the cross-peak Qui II H-1/Qui I C-2. This conclusion was confirmed by the correlations between H-6 of the aglycone and Glc H-1, between Qui I H-1 and Glc H-3, between Qui II H-1 and Qui I H-2, between Fuc I H-1 and Qui I H-4, and between Fuc II H-1 and Fuc I H-2 in the NOESY spectrum.

Hence, the structure of novaeguinoside I (**2**) was elucidated as sodium (20*R*,22*R*,23*S*,24*S*)-6α-*O*-{β-D-fucopyranosyl-(1→2)-β-D-fucopyranosyl-(1→4)-[β- D-quinovopyranosyl-(1→2)]-β-D-quinovopyranosyl-(1→3)-β-D-glucopyranosyl}-22,23-epoxy-20-hydroxy-24-methyl-5α-cholest-9(11)-en-3β-ylsulfate.

Novaeguinoside II (**3**) was obtained as colorless crystals. An examination of its ^{1}H and ^{13}C NMR spectra revealed signals due to aglycone protons and carbons identical with those observed in novaeguinoside I (**2**), containing the same typical $\Delta^{9(11)}$-3β,6α-dihydroxysteroidal nucleus with a 20-hydroxy-22,23-epoxide-24-methyl side chain. The four asymmetric carbons in the side chain were presumed to have the same stereochemistry as those of **2**, because in the NMR spectrum of **3** the aglycone proton and carbon signals were in good agreement with those of **2**.

The molecular formula of **3** was determined as $C_{57}H_{93}O_{28}SNa$ by ^{13}C NMR as well as from HRESIMS (positive ion mode). Ion peaks at *m/z* 1303 $[M + Na]^+$ and 1183 $[M + Na - NaHSO_4]^+$ confirmed the presence of a sulfate group. The negative ion mode ESIMS showed a series of fragmentations with the following sugar losses: *m/z* 1257 $[M - Na]^-$; *m/z* 1111 $[M - 146]^-$ loss of a deoxyhexose; *m/z* 979 $[1111 - 132]^-$ loss of arabinose; *m/z* 965 $[1111 - 146]^-$ loss of a deoxyhexose; *m/z* 833 $[979 - 146$ or $965 - 132]^-$ loss of a deoxyhexose or arabinose; *m/z* 687 $[833 - 146]^-$ loss of a deoxyhexose; *m/z* 522 $[687 - 162]^-$ loss of glucose unit.

The sugar moieties of **3** were determined to be D-quinovose, D-fucose, L-arabinose, and D-glucose in the ratio 2:1:1:1 by acidic hydrolysis with aqueous 2 mol/L trifluoroacetic acid and preparation of the corresponding aldononitrile peracetates, which were analyzed by GC-MS. We hypothesize the D-configuration for quinovose, fucose, and glucose and the L-configuration for arabinose by analogy with all reported asterosaponins containing those sugar units, in which these monosaccharides all occurred in the above fixed D- or L-configurations.[1,19] The ^{1}H NMR spectrum showed signals for five anomeric protons at δ 4.745, 4.761, 4.773, 4.783, and 5.11, each a doublet with $^3J_{H-1/H-2}$ = 6.3−8.0 Hz, which were correlated in the HSQC experi-

ment with the corresponding carbons at δ 106.8, 102.6, 103.9, 105.4, and 105.0, respectively. The ^{1}H and ^{13}C NMR signals attributable to the various sugar units were assigned by the application of 2D NMR experiments including ^{1}H−^{1}H COSY, TOCSY, HSQC, and HMBC (Table 2). The β-configuration of the sugars (α for arabinose unit) in the pyranose form was fully defined from the chemical shift and coupling constant of each anomeric proton. By analysis of the ^{13}C NMR, a 2,4-glycosidated quinovopyranosyl unit, a 3-glycosidated glucopyranosyl unit, a 2-glycosidated arabinopyranosyl unit, and a quinovopyranose and a fucopyranose as terminal units were identified. The linkage positions for the sugar units were confirmed by methylation of **3** followed by acid hydrolysis and GC-MS analysis of the partially methylated alditol acetates derived from 2,4-linked quinovopyranose, 3-linked glucopyranose, and 2-linked arabinopyranose.[13] Finally, direct support of the sequence of the sugars and binding sites came from the results of an HMBC experiment, which showed the following cross-peaks: δ_H 4.783 (Glc H-1) with δ_C 80.9 (C-6 of aglycone), δ_H 4.773 (Qui I H-1) with δ_C 91.8 (Glc C-3), δ_H 5.11 (Qui II H-1) with δ_C 82.6 (Qui I C-2), δ_H 4.761 (Ara H-1) with δ_C 84.7 (Qui I C-4), and δ_H 4.745 (Fuc H-1) with δ_C 81.8 (Ara C-2). Therefore, novaeguinoside II (**3**) was defined as sodium (20*R*,22*R*,23*S*,24*S*)-6α-*O*-{β-D-fucopyranosyl-(1→2)-α-L-arabinopyranosyl-(1→4)-[β-D-quinovopyranosyl-(1→2)]-β-D-quinovopyranosyl-(1→3)-β-D-glucopyranosyl}-22,23-epoxy-20-hydroxy-24-methyl-5α-cholest-9(11)-en-3β-ylsulfate. L-Arabinose is a less common monosaccharide to be encountered in asterosaponins,[1,19] and the carbohydrate moiety of **3** is reported in asterosaponins for the first time.

Asterosaponins **1**, **2**, and **3** were tested for in vitro cytotoxicity against human leukemia K-562 cells and human hepatoma BEL-7402 cells. The IC_{50} of each saponin tested was measured on the basis of cell viability, after 72 h treatment. Our results indicated that **1**, **2**, and **3** were marginally cytotoxic against K-562 cells (IC_{50}'s 17.4, 8.6, and 4.9 μg/mL, respectively) and BEL-7402 cells (IC_{50}'s 14.5, 9.2, and 4.1 μg/mL, respectively).

Experimental Section

General Experimental Procedures. Melting points were determined on a 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 of **1**, **2**, and **3** were recorded in C_5D_5N on Varian Inova-400, Inova-600, and Bruker AMX-500 spectrometers, respectively. The 2D NMR spectra were obtained using standard pulse sequences. ESIMS, ESIMS/MS, 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 silica gel H (10−40 μm, Qingdao Marine Chemical Inc.), Sephadex LH-20 (Pharmacia), and reversed-phase silica gel (Lichroprep RP-18, 40−63 μm). Fractions were monitored by TLC, and spots were visualized by heating silica gel plates sprayed with 20% H_2SO_4 in EtOH.

Animal Material. Specimens of *C. novaeguineae* were collected at a depth 2−15 m by hand using scuba or by trawl from offshore waters of the Sanya Bay in the South China Sea in May 2003 and stored in ethanol at room temperature. The organisms were identified by Dr. Hans Uwe Dahms of the Oldenburg University, Germany. 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 HNHX-02.

Extraction and Isolation. The starfish (68 kg, wet wt) were cut into pieces and extracted three times with refluxing ethanol. The combined extracts were concentrated to leave a rufous residue, which was suspended in H_2O (10 L) and then partitioned successively using petroleum ether (10 L × 3) and *n*-BuOH (10 L × 5). The *n*-BuOH fraction (115 g) was chromatographed on Si gel eluting with a $CHCl_3$/*n*-BuOH (saturated with H_2O)/MeOH (2:1:0 to 0:6:1) (lower phase) gradient to give 23 fractions (1−23). Fraction 20 (5.9 g) mainly contained the asterosaponins. The crude asterosaponins fraction was subjected to size exclusion chromatography on a Sephadex LH-20 column equilibrated with MeOH/H_2O (2:1) to give five major fractions (I−V). Fraction IV (240 mg) proved to be active against *P. oryzae* (MMDC = 32 μg/mL), was further purified by MPLC with a Lobar column (Lichroprep RP-18), and was finally subjected to repeated semipreparative HPLC to afford pure saponins **1** (13.8 mg, t_R = 42.05 min), **2** (21.6 mg, t_R = 38.82 min), and **3** (9.1 mg, t_R = 37.26 min) using MeOH/H_2O (46:54) as the mobile phase and a flow rate of 1.9 mL/min.

Novaeguinoside I (2): colorless crystals; mp 217−219 °C; $[\alpha]^{20}_D$ +10° (*c* 0.204, MeOH); IR (KBr) ν_{max} 3427, 1643, 1242, 1212, 1064 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 1; ESIMS (positive ion mode) *m/z* 1317 $[M + Na]^+$, 769 $[4 \times 146 + 162 + Na]^+$, 623 $[3 \times 146 + 162 + Na]^+$, 453 $[2 \times 146 + 162 - H]^+$, 453 $[3 \times 146 - H]^+$, 315 $[2 \times 146 + Na]^+$; ESIMS/MS (positive ion mode, *m/z* 1317) *m/z* 1197 $[M + Na - NaHSO_4]^+$, 1051 $[1197 - 146]^+$, 905 $[1197 - 2 \times 146]^+$, 787 $[4 \times 146 + 162 + Na + H_2O]^+$, 607 $[4 \times 146 + Na]^+$; ESIMS (negative ion mode) *m/z* 1271 $[M - Na]^-$; ESIMS/MS (negative ion mode, *m/z* 1271) *m/z* 1125 $[1271 - 146]^-$, 979 $[1271 - 2 \times 146]^-$, 833 $[1271 - 3 \times 146]^-$, 687 $[1271 - 4 \times 146]^-$, 525 $[1271 - 4 \times 146 - 162]^-$, 507 $[525 - H_2O]^-$; HRESIMS (positive ion mode) *m/z* 1317.5535 $[M + Na]^+$ (calcd for $C_{58}H_{96}O_{28}SNa_2$, 1317.5526).

Novaeguinoside II (3): colorless crystals; mp 215−217 °C; $[\alpha]^{20}_D$ +3° (*c* 0.132, MeOH); IR (KBr) ν_{max} 3441, 1641, 1242, 1213, 1063 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 2; ESIMS (positive ion mode) *m/z* 1303 $[M + Na]^+$, 755 $[3 \times 146 + 132 + 162 + Na]^+$; ESIMS/MS (positive ion mode, *m/z* 1303) *m/z* 1183 $[M + Na - NaHSO_4]^+$, 1038 $[1183 - 147]^+$, 889 $[1183 - 2 \times 146]^+$, 593 $[3 \times 146 + 132 + Na]^+$, 447 $[2 \times 146 + 132 + Na]^+$; ESIMS (negative ion mode) *m/z* 1257 $[M - Na]^-$; ESIMS/MS (negative ion mode, *m/z* 1257) *m/z* 1157 $[1257 - 100]^-$ (cleavage of C_{22}−C_{23} bond), 1111 $[1257 - 146]^-$, 979 $[1257 - 146 - 132]^-$, 965 $[1257 - 2 \times 146]^-$, 865 $[1157 - 2 \times 146]^-$, 833 $[1257 - 2 \times 146 - 132]^-$, 687 $[1257 - 3 \times 146 - 132]^-$, 525 $[1257 - 3 \times 146 - 132 - 162]^-$, 507 $[525 - H_2O]^-$; HRESIMS (positive ion mode) *m/z* 1303.5353 $[M + Na]^+$ (calcd for $C_{57}H_{93}O_{28}SNa_2$, 1303.5369).

Acid Hydrolysis of 1−3. Each saponin (1 mg) was heated in an ampule with 2 mol/L trifluoroacetic acid (1 mL) at 120 °C for 2 h. The reaction mixture was evaporated to dryness, and the residue was partitioned between $CHCl_3$ and H_2O. The aqueous phase was concentrated under reduced pressure. Then, pyridine (1 mL) and $NH_2OH \cdot HCl$ (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 the 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.

Methylation of Saponin 3 Followed by Hydrolysis. A solution of **3** (2 mg) in anhydrous DMSO (1 mL) was treated with NaOH (40 mg) and sonicated for 20 min at 25 °C. The reaction mixture was treated with CH_3I (0.3 mL) and sonicated for a further 30 min. After addition of H_2O (4 mL), the mixture was extracted with $CHCl_3$ (5 mL). The $CHCl_3$ phase was washed with H_2O (3 mL × 3) and evaporated to dryness under nitrogen. The residue was heated in an ampule with 2 mol/L trifluoroacetic acid (0.75 mL) at 120 °C for 2 h. After extraction with $CHCl_3$, the aqueous residue was evaporated and further coevaporated with H_2O (0.75 mL × 2) and MeOH (1 mL). The

sugar mixture was treated with 0.5 mol/L NH_4OH (0.5 mL) and $NaBH_4$ (4 mg) at 25 °C for 4 h. After acidification with 1 mol/L AcOH, the solution was evaporated to dryness. H_3BO_3 in the residue was removed by three cycles of co-distillation with MeOH. The resulting methylated alditol mixture was acetylated with Ac_2O (0.5 mL) and pyridine (0.5 mL) at 100 °C for 45 min. The reaction mixture was partitioned between $CHCl_3$ (1.5 mL) and H_2O (1.5 mL). The chloroform phase was washed with H_2O (1 mL), saturated $NaHCO_3$ solution (1 mL), and H_2O (1 mL) and evaporated to dryness under reduced pressure. The mixture thus obtained was subjected to GC-MS and the following carbohydrates could be assigned: 3-linked glucose (1,3,5-tri-*O*-acetyl-2, 4,6-tri-*O*-methylglucitol; $t_R = 7.43$ min; *m/z* 233, 189, 161, 129, 117, 101, 87, 43); 2-linked arabinose (1,2,5-tri-*O*-acetyl-3,4-di-*O*-methylarabinitol; $t_R =$ 6.98 min; *m/z* 189, 129, 117, 101, 87, 43); 2,4-linked quinovose (1,2,4,5-tetra-*O*-acetyl-3-*O*-methylquinovitol; $t_R = 6.32$ min; *m/z* 203, 189, 143, 129, 117, 101, 87, 43); terminal fucose (1,5-di-*O*-acetyl-2,3,4-tri-*O*-methylfucitol; $t_R = 5.74$ min; *m/z* 175, 161, 131, 117, 115, 101, 89, 72, 43); terminal quinovose (1,5-di-*O*-acetyl-2,3,4-tri-*O*-methylquinovitol; $t_R = 5.48$ min; *m/z* 175, 161, 131, 117, 115, 101, 89, 72, 43).

Cytotoxicity Assay. The cytotoxicity against human leukemia K-562 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.[20] The cytotoxicity data against human hepatoma BEL-7402 cells was evaluated by the sulforhodamine B (SRB) protein assay described in a previous paper[21] using 10-hydroxycamptothecine as the positive control. Dose−response curves were plotted for the samples, and the IC_{50}'s were calculated as the concentrations of the tested saponins resulting in 50% reduction of absorption compared to the control cells.

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Supporting Information Available: Spectroscopic details of **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

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