New Triterpenoid Saponins from the Sponge *Melophlus isis*

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Four new triterpenoid saponins were isolated, along with two known 30-norlanostane-type saponins—sarasinosides A_1 (**1**) and A_3 (**2**)—from the sponge *Melophlus isis* collected from Guam. The structures of these new compounds (**3**−**6**) proved to be 30-norlanosta-8(14),24-dien-23-ones bearing two hydroxyl groups or the corresponding methoxy groups at the 9α,15α- and 9α,15β-positions and the pentasaccharide portion identical with those of **1** and **2** at the 3β position, by spectral, chemical, and GC analyses.

Triterpenoid saponins are widely recognized as a representative group of echinoderm metabolites. Several compounds of this structural class have exhibited cytotoxic, antifungal, antiviral, and hemolytic activities.[1] Despite their great abundance in echinoderms, however, triterpenoid saponins have been found in relatively few other marine organisms. Sponges, the most extensively investigated marine organisms, produce mainly steroids and triterpenoids, but also some related saponins.[1,2] Since 1987, a number of saponins, some possessing potent bioactivities, have been isolated from sponges.[3−14]

As a part of our continuing search for novel secondary metabolites of biomedical and ecological importance from tropical marine animals, we collected the sponge *Melophlus isis* de Laubenfels, 1954 (Ancorinidae) from Guam.[15,16] The crude extract of these specimens exhibited moderate cytotoxicity (LC_{50} 197 μg/mL) against the human leukemia cell-line K562. Herein we report the structure and bioactivity of six saponins, including four novel compounds of the 30-norlanostane nortriterpenoid pentaglycoside (sarasinoside) class. The new compounds, designated as sarasinosides H_1, H_2, I_1, and I_2, possessed an unusual 9,15-dihydroxy-8(14),24-dien-23-one or the corresponding 15-methoxy functionality in their norlanosterol moiety.[17]

Results and Discussion

The sponge was collected by hand at a site known as Sponge Mound in Apra Harbor, Guam. The lyophilized specimens were macerated and sequentially extracted with CH_2Cl_2, MeOH, and acetone. Guided by the results of cytotoxicity and ^{1}H NMR analysis, the combined crude extracts were separated by solvent-partitioning and C_{18} vacuum flash chromatography. The fractions (30−10% aqueous MeOH) rich with secondary metabolites were repeatedly separated by reversed-phase HPLC to afford six pure saponins.

By combined spectroscopic analyses, the structures of compounds **1** and **2**, the least polar of all the saponins, were defined as sarasinosides A_1 and A_3, respectively. These saponins were previously isolated from the tropical sponges *Asteropus sarasinosum* and an unidentified *Asteropus* sp.[3,4,7] The spectral data of these compounds were in good agreement with those reported previously.

Sarasinoside H_1 (**3**) was analyzed for $C_{62}H_{100}N_2O_{28}$ by combined HRFABMS and ^{13}C NMR analyses. The ^{13}C NMR spectra of this compound showed signals characteristic of a carbonyl and two double bonds at δ 203.7 (C), 157.2 (C), 146.6 (C), 135.2 (C), and 125.2 (CH). The chemical shift of the carbonyl carbon indicated the presence of an α,β-unsaturated ketone functionality. This interpretation was supported by an absorption maximum at 239 nm. In addition, the presence of two amide groups was recognized by carbon signals at δ 174.2 and 173.9 in the ^{13}C NMR spectrum. With the aid of this information, comparison of the ^{1}H and ^{13}C NMR data of this compound with those of **1**, **2**, and previously reported saponins suggested that **3** possessed the same 30-norlanostane framework as the other sarasinosides.[4,7]

The structure of the aglycon of **3** was determined by a combination of ^{1}H COSY, TOCSY, gradient HSQC, and gradient HMBC experiments. The gradient HSQC data precisely matched all of the proton-bearing carbons and their protons. By combined ^{1}H COSY and TOCSY data, several proton spin systems were readily recognized. The HMBC correlations between the signals of seven upfield methyl protons and those of neighboring carbons were particularly helpful to define all of the functional groups and to locate them in the norlanostane framework. That is, long-range correlations of the methyl proton signals at δ 1.12 and 0.97 with the methine carbon signal at δ 91.9 placed a carbinol (or an ether) oxygen at C-3 of ring A (Table 1). Similarly, the placement of another oxygen at C-9 was supported by an observation of a long-range correlation of H-19 at δ 0.94 with the carbon resonance at δ 76.2. Examination of the gradient HMBC data revealed that the carbon signal at δ 146.6 had long-range couplings with the signals of H-18 at δ 1.05 and a downfield proton at δ 4.54 (1H, dd, J = 7.3, 6.9 Hz). The latter proton was additionally coupled with another olefinic carbon at δ 135.2 and the C-13 quaternary carbon at δ 44.1. Accordingly, the double bond and carbinol carbon were assigned at C-8(14) and C-15, respectively. The assignment of an oxygen-bearing carbon at C-15 was supported by the ^{1}H COSY cross-peaks of the proton signal at δ 4.54 with H-16 at δ 2.27 (1H, ddd, J = 12.7, 7.8, and 7.3 Hz), which also had long-range correlations with C-13 and C-14. Finally, the location of an α,β-unsaturated carbonyl group was established at C-23 by long-range correlations of the olefinic carbons with H-26 and H-27 and a TOCSY correlation containing both H-21 and H-23. The aglycon of **3** had three carbinol (or ether) carbons at C-3, C-9, and C-15. The

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Table 1. ^{1}H and ^{13}C NMR Data of the Aglycon Moieties of Compounds **3**−**6** in CD_3OD

	3		**4**		**5**		**6**	
carbon	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1	1.69 m	31.5	1.68 m	31.5	1.69 m	31.5	1.69 m	31.4
	1.38 m		1.35 m		1.42 m		1.40 m	
2	1.84 m	27.9	1.84 m	27.9	1.85 m	27.8	1.83 m	27.9
	1.69 m		1.68 m		1.69 m		1.70 m	
3	3.08 dd (11.2, 3.9)	91.9	3.08 dd (11.2, 3.9)	91.9	3.09 dd (11.2, 3.9)	91.8	3.08 dd (10.7, 3.9)	91.8
4		40.6		40.6		40.5		40.5
5	1.89 dd (12.7, 2.9)	45.6	1.90 dd (12.7, 3.3)	45.5	1.95 dd (12.7, 2.9)	45.5	1.94 dd (11.7, 2.4)	45.4
6	1.62 br d (13.3)	22.9	1.62 m	22.9	1.61 m	22.7	1.59 m	22.8
	1.41 m		1.34 dddd (13.7, 13.2, 12.7, 4.4)		1.32 dddd (14.2, 13.2, 12.7, 4.4)		1.31 m	
7	2.65 br d (12.2)	26.1	2.53 br d (14.2)	26.3	2.51 br d (14.7)	29.1	2.28 br dd (8.3, 3.4, 2H)	29.1
	2.11 m		2.14 m		2.30 ddd (14.7, 14.2, 4.9)			
8		135.2		136.5		136.8		138.1
9		76.2		76.2		76.6		76.3
10		42.5		42.4		42.1		42.1
11	1.94 m	28.4	1.94 m	28.3	2.02 m	28.6	2.02 m	28.7
	1.47 m		1.45 m		1.49 br dd (13.7, 2.9)		1.49 m	
12	1.76 br d (12.7)	35.3	1.76 br d (13.2)	35.2	1.85 m	35.2	1.82 br d (12.7)	34.9
	1.41 m		1.41 m		1.56 m		1.49 m	
13		44.1		44.0		44.7		44.4
14		146.6		144.1		149.6		147.2
15	4.54 dd (7.3, 6.9)	70.2	4.22 dd (7.8, 6.4)	79.6	4.62 dd (5.3, 2.0)	70.2	4.21 br d (5.4)	80.5
16	2.27 ddd (12.7, 7.8, 7.3)	39.5	2.27 ddd (13.2, 7.8, 7.3)	36.4	1.73 m (2H)	40.1	1.92 br d (12.7)	34.6
	1.41 m		1.41 m				1.55 m	
17	1.12 m	55.6	1.16 ddd (12.7, 8.3, 8.3)	55.5	1.56 m	55.0	1.43 m	55.3
18	1.05 s	18.2	0.99 s	17.9	0.89 s	18.8	0.88 s	18.7
19	0.94 s	18.5	0.92 s	18.5	0.95 s	18.7	0.93 s	18.7
20	2.10 m	33.0	2.09 m	33.2	2.09 m	32.0	2.08 m	32.2
21	0.94 d (5.9)	20.6	0.94 d (5.9)	20.5	0.97 d (6.3)	20.6	0.95 d (6.3)	20.6
22	2.56 br d (12.7)	52.1	2.58 dd (14.7, 2.9)	52.0	2.59 dd (15.1, 3.9)	52.3	2.59 dd (15.1, 3.9)	52.2
	2.15 m		2.14 m		2.19 dd (15.1, 9.3)		2.20 dd (15.1, 9.3)	
23		203.7		203.8		203.6		203.6
24	6.17 hep (1.0)	125.2	6.18 br s	125.2	6.19 hep (1.5)	125.2	6.19 hep (1.5)	125.3
25		157.2		157.2		157.1		157.1
26	2.11 d (1.0)	20.9	2.11 d (1.0)	20.9	2.13 d (1.5)	20.9	2.12 d (1.5)	20.9
27	1.90 d (1.0)	27.7	1.91 d (1.0)	27.7	1.92 d (1.5)	27.7	1.91 d (1.5)	27.7
28	0.97 s	17.5	0.96 s	17.4	0.97 s	17.4	0.96 s	17.4
29	1.12 s	29.1	1.12 s	29.1	1.13 s	29.1	1.12 s	29.1
OMe			3.28 s	56.1			3.29 s	56.4

gradient HMBC data revealed that only C-3 had a long-range correlation with a sugar proton at δ 4.29 (1H, d, J = 7.8 Hz). Therefore, the oligosaccharide portion of the molecule was connected at C-3 through a glycosidic linkage, while free hydroxyl groups were attached at other carbons.

In addition to the trans A/B and C/D ring junctions assigned by the upfield ^{13}C NMR shift of the C-18 and C-19 carbons, the aglycon of **3** possessed asymmetric carbon centers at C-3, C-9, and C-15. The stereochemistry at these oxygen-bearing centers was determined by combined proton−proton coupling constants and ROESY analysis. The configuration of H-3 was assigned as α on the basis of its vicinal coupling constants ($J_{2,3}$ = 11.2 and 3.9 Hz) and the ROESY correlations with H-5 and H-29. Similarly, the ROESY correlation of H-19 with H-11β, which, in turn, strongly correlated with H-18, revealed an α orientation for the hydroxyl group at C-9. Because H-15 and H-18 showed spatial proximity with H-16α and H-16β, respectively, by the ROESY analysis, the orientation of the C-15 hydroxyl group was assigned as β. Supporting this interpretation was another ROESY correlation between H-15 and H-17. Thus, the aglycon of sarasinoside H_1 (**3**) was determined as 3β,9α,15β-trihydroxy-30-norlanosta-8(14),24-dien-23-one. A literature survey revealed that the functionality of the aglycon of **3** is unprecedented among saponins and steroids from marine organisms.[1]

Sarasinoside H_1 (**3**) contained five sugar residues. The identification of each sugar unit as well as the arrangement of them was established by chemical degradation, GC analysis, and extensive NMR experiments. After the hydrolysis of **3** with HCl, the hydrolysate was trimethylsilated and GC retention times of each sugar were compared with those of the authentic samples prepared by the same manner. In this way, the sugar moieties of **3** were determined to be identical to those of **1** and **2**: one β-D-xylose, two β-D-glucoses (peak area, 1.89 times of xylose), one β-D-2-NAc-glucosamine, and one β-D-2-NAc-galactosamine. The result of GC analysis was confirmed by combined 1D TOCSY and HSQC data in which the chemical shifts and splitting patterns of most of the proton signals were accurately measured (Table 2).

The ^{13}C NMR chemical shifts (δ > 100 ppm) of the anomeric methines suggested that all the sugars were connected through β-glycosidic linkages.[18] The arrangement of these residues was established by a combination of the gradient HMBC and ROESY experiments. A long-range correlation of H-1′ with C-3 revealed a linkage between C-1′ of the xylose and C-3 of the aglycon. This interpretation was confirmed by a strong ROESY cross-peak between H-3 and H-1′. Similarly, long-range correlations between H-2′ and C-1″ and also between H-1″ and C-2′ assigned the linkage between the 2-NAc-glucosamine and the xylose. The linkage between two glucose units was defined by a HMBC correlation of the anomeric proton of a glucose at δ 4.61 with the methine carbon of another at δ 83.3. The linkage between the xylose and 2-NAc-galactosamine was assigned by a HMBC correlation between C-4′ at δ 79.7 and the anomeric proton of the amino sugar

Table 2. ^{1}H and ^{13}C NMR Data of the Sugar Moiety of Compound **3** in CD_3OD

position		δ_H[a]	δ_C
Xyl	1′	4.29 d (7.8)	106.7
	2′	3.91 dd (8.8, 7.8)	78.1
	3′	3.53 dd (8.8, 8.8)	77.6
	4′	3.68	79.7
	5′	3.86; 3.16 dd (10.7, 10.3)	64.2
2-NAc-Glc	1″	4.83 d (8.3)	101.9
	2″	3.67 dd (9.3, 8.3)	57.9
	3″	3.41 dd (9.3, 8.8)	76.9
	4″	3.15 dd (8.8, 8.8)	72.4
	5″	3.67	78.1
	6″	4.10 br d (11.7); 3.77 dd (11.7, 6.9)	70.0
	Ac	1.99 s[b]	23.0
			174.2[c]
Glc-I	1‴	5.08 d (7.8)	102.5
	2‴	3.38 dd (8.8, 7.8)	83.3
	3‴	3.70 dd (9.3, 8.8)	78.0
	4‴	3.32 dd (9.3, 9.3)	71.5
	5‴	3.45 ddd (9.3, 4.9, 2.4)	77.1
	6‴	3.81 br d (10.7); 3.72 dd (10.7, 4.9)	62.5
Glc-II	1⁗	4.61 d (7.3)	105.7
	2⁗	3.41	75.8
	3⁗	3.41	77.8
	4⁗	3.32 dd (9.8, 7.3)	71.5
	5⁗	3.27 ddd (9.8, 5.4, 2.0)	78.2
	6⁗	3.89 br d(12.2); 3.71 br d (12.2, 5.4)	62.6
2-NAc-Gal	1⁗′	4.49 d (8.3)	102.5
	2⁗′	3.88 dd (10.7, 8.3)	54.5
	3⁗′	3.64 dd (10.7, 3.9)	72.8
	4⁗′	3.80	69.8
	5⁗′	3.63	77.2
	6⁗′	3.90; 3.71	63.1
	Ac	1.98 s[b]	23.0
			173.9[c]

[a] Splitting patterns and coupling constants were measured by combined 1D TOCSY and gradient HSQC experiments. [b,c] Interchangeable signals.

and also a ROESY correlation of the latter with H-5′ax at δ 3.16. Finally, the linkage between the terminal methylene of the 2-NAc-glucosamine and the anomeric methine of a glucose was revealed by a correlation of C-6″ with H-1‴ of the amino sugar and was supported by a ROESY correlation between the latter and one of H-6″ at δ 4.10. Thus, the structure of sarasinoside H_1 (**3**) was determined as 3β-O-[β-D-glucopyranosyl (1 → 2) β-D-glucopyranosyl (1 → 6) β-D-*N*-acetyl-2-amino-glucopyranosyl (1 → 2) β-D-xylopyranosyl (4 → 1) β-D-*N*-acetyl-2-amino-galactopyranosyl]-9α,15β-dihydroxy-30-norlanosta-8(14),24-dien-23-one. This compound had a sugar chain identical with those of sarasinosides A_1 and A_3 but differed in the structure of the aglycon moiety.

A closely related metabolite, sarasinoside H_2 (**4**), was isolated as a white amorphous solid, and the molecular formula $C_{63}H_{102}N_2O_{28}$ was deduced by combined HRFABMS and ^{13}C NMR analysis. The NMR data for this compound were very similar to those obtained for **3**, with the appearance of signals for a methoxy group [^{1}H δ 3.28 (3H, s), ^{13}C δ 56.1(CH_3)] as the only noticeable difference. Therefore, **4** was thought to be a methoxy analogue of **3**. A combination of 2D NMR experiments showed that **4** possessed the same pentasaccharide portion as **3**. In addition, the same analyses showed that the aglycons of these compounds were also almost identical to each other (Table 1). However, gradient HMBC data revealed an additional long-range correlation between signals of the methoxy proton and C-15 at δ 79.6. The configuration of the methoxy group was assigned as β on the basis of the ROESY correlations of this proton with H-16β and H-18. The same experiment also showed spatial proximity of H-15 with H-16α and H-17. Thus, sarasinoside H_2 (**4**) was structurally defined as the 15-methoxy analogue of sarasinoside H_1 (**3**).

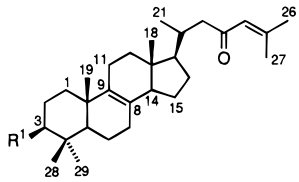
The molecular formula of sarasinoside I_1 (**5**) was determined as $C_{62}H_{100}N_2O_{28}$, identical to that of sarasinoside H_1 (**3**), by HRFABMS and ^{13}C NMR spectroscopic methods. The spectral data for this compound were highly compatible with those of **3**. Careful examination of the NMR spectra, however, showed that the chemical shifts of several protons and carbons corresponding to the D ring of the aglycon differed significantly from those of **3** (Table 1). A combination of the ^{1}H COSY, TOCSY, HSQC, and HMBC experiments revealed that the planar structure of the aglycons of these compounds were identical with each other. In addition, both compounds were found to possess the same pentasaccharide moiety. Comparison of the NMR assignments for **3** and **5** showed that the most significant difference occurred for the chemical shifts of protons and carbons at C-14−C-16. Therefore, the structural difference was thought to exist only at the configuration of the C-15 asymmetric center. The ROESY experiment showed a strong 1,3-diaxial correlation between H-15 and H-18 instead of the correlation between H-16β and H-18 in **3**. Thus, the structure of sarasinoside I_1 (**5**) was represented as 3β-O-[β-D-glucopyranosyl (1 → 2) β-D-glucopyranosyl (1 → 6) β-D-*N*-acetyl-2-amino-glucopyranosyl (1 → 2) β-D-xylopyranosyl (4 → 1) β-D-*N*-acetyl-2-amino-galactopyranosyl]-9α,15α-dihydroxy-30-norlanosta-8(14),24-dien-23-one.

Sarasinoside I_2 (**6**) was analyzed for $C_{63}H_{102}N_2O_{28}$ by combined HRFABMS and ^{13}C NMR spectrometry. The spectral data for this compound were very similar to those obtained for **5**. As observed for **3** and **4**, the only noticeable difference in the spectral data of **5** and **6** was the appearance of a new methoxy resonance [^{1}H δ 3.29 (3H, s), ^{13}C δ 56.4 (CH_3)] in the NMR spectra of **6**. A combination of 2D NMR experiments revealed that the C-15 hydroxyl group of **5** was replaced by a methoxy group in **6**. The 15α configuration was assigned on the basis of the ROESY correlation between H-15 and H-18 and between H-16α and the methoxy protons. Thus, the structure of sarasinoside I_2 (**6**) was determined as the 15-methoxy derivative of sarasinoside I_1 (**5**).

The literature survey revealed that saponins isolated from sponges exhibit diverse bioactivities. For examples, sarasinosides A_1 and B_1 exhibited potent piscicidal activity and inhibitory effect against the cell division of fertilized starfish eggs.[7] In addition, the crude extract containing sarasinosides D−G showed cytotoxicity against human tumor cells and inhibition against the action of protein kinase C.[11] Ectyoplasides, recently isolated norlanostane oligosaccharides, also exhibited cytotoxicity against cancer cells.[14] In our measurement, sarasinosides A_1 (**1**) and A_3 (**2**) showed cytotoxicity against the human leukemia cell-line K562 with LC_{50} values of 6.5 and 17.1 μg/mL, respectively. However, the new metabolites, sarasinosides H_1 (**3**), H_2 (**4**), I_1 (**5**), and I_2 (**6**) were not active (LC_{50} > 100 μg/mL). To check a possible ecological role of these metabolites, antimicrobial tests against marine bacteria using the paper-disk method revealed that sarasinosides were not active against the marine bacteria *Photobacterium leiognathi*, *Photobacterium phosphoreum*, or *Vibrio harveyi* at the concentration of 100 μg/mL.

Experimental Section

General Experimental Procedures. Melting points were measured on a Fisher-Johns apparatus and are reported uncorrected. Optical rotations were measured on a JASCO digital polarimeter using a 5-cm cell. IR spectra were recorded



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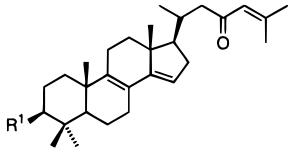

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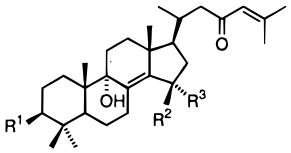
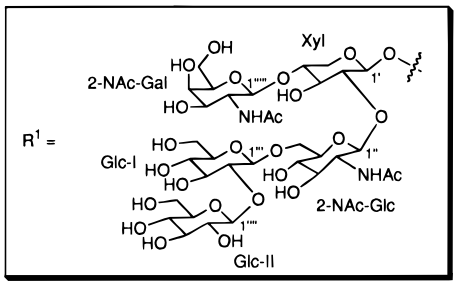


3 R^2 = OH, R^3 = H

4 R^2 = OMe, R^3 = H

5 R^2 = H, R^3 = OH

6 R^2 = H, R^3 = OMe



on a Mattson GALAXY spectrophotometer. UV spectra were obtained in methanol using a Milton-Roy spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded in CD_3OD solution containing TMS as internal standard on a Varian Unity 500 spectrometer with standard sequences operating at 500 and 125 MHz, respectively. Mass spectra were provided by the Mass Spectrometry Facility, Department of Chemistry, University of California, Riverside. All solvents used were spectral grade or were distilled from glass prior to use.

Animal Material. The sponge *M. isis* (BMNH 1995.6.22.5) were collected by hand using scuba at 20−35 m depth at the Sponge Mound, in Apra Bay, Guam, on August 24, 1996. This same sponge had previously been called *Stellettinopsis isis* and *Asteropus sarasinosum* in Guam (G. Paulay, personal communication). The sponge is large, massive, tan-brown, barrel-shaped, and typically anchored to the bottom by numerous leg-like growths. The sponge has a single well-developed osculum on top and numerous incurrent ostia on the sides. A photograph of this sponge taken at the site of collection appears in *Tropical Pacific Invertebrates* (plate 19, p 16).[19]

Extraction and Isolation. The fresh sponge was immediately frozen and kept at −25 °C until investigated chemically. The specimens were lyophilized (dry wt 75.7 g), macerated, and repeatedly extracted with CH_2Cl_2 (1 L × 2), MeOH (1 L × 3), and acetone (1 L × 2). The combined crude extract (24.9 g) was partitioned between *i*-BuOH and H_2O. The *i*-BuOH layer was evaporated in vacuo, and the residue (10.96 g) was re-partitioned between *n*-hexane (1.40 g) and 15% aqueous MeOH (7.74 g). An aliquot (1.98 g) of the material from the latter phase was subjected to C_{18} reversed-phase vacuum flash chromatography using mixtures of MeOH and H_2O as eluents (elution order: 50%, 40%, 30%, 20%, 10% aqueous MeOH, 100% MeOH), and finally acetone. The fraction eluted with 30% aqueous MeOH was dried and the residue (132 mg) separated by reversed-phase HPLC (YMC ODS-A column, 1 cm × 25 cm, 40% aqueous MeOH) to yield 47.1 and 29.5 mg of pure sarasinosides H_1 (**3**) and I_1 (**5**), as white amorphous solids, respectively. The fractions successively eluted with 20 and 10% aqueous MeOH (363 and 490 mg, respectively) were combined and separated by reversed-phase HPLC (25% aqueous MeOH) to afford sarasinosides H_1 (**3**), I_1 (**5**), H_2 (**4**), I_2 (**6**), A_3 (**2**), and A_1 (**1**), in order of elution. Final purification was accomplished by reversed-phase HPLC (YMC ODS-A column, water−MeCN−MeOH, 60:35:5) to yield 193.9, 32.7, 26.1, 29.3, 49.2, and 17.8 mg of pure sarasinosides A_1, A_3, H_1, H_2, I_1, and I_2, respectively.

Sarasinoside A_1 (1): white amorphous solid, mp 209−212 °C (lit. 207−210 °C[7]); $[\alpha]^{25}_D$ −15.5° (*c* 0.1, MeOH) [lit. −7.4° (*c* 0.3, MeOH)[4] and −14° (*c* 1, MeOH)[7]]; UV (MeOH) λ_{max} (log ϵ) 237 (4.15) nm [lit. 237 (4.13) nm[7]]; IR (KBr) ν_{max} 3400 (br), 2930, 1640 (br), 1565, 1380, 1270, 1120, 1075 cm^{-1}; HRFABMS *m/z* 1311.6447 [M + Na]$^+$ (calcd for $C_{62}H_{100}N_2O_{26}Na$, 1311.6462, Δ 1.5 mmu).

Sarasinoside A_3 (2): white amorphous solid, mp 207−211 °C (lit. 205−208 °C[7]); $[\alpha]^{25}_D$ −8.7° (*c* 0.12, MeOH) [lit. −5.5° (*c* 0.5, MeOH)[7]]; UV (MeOH) λ_{max} (log ϵ) 246 (4.37) nm [lit. 243 (4.37) nm[7]]; IR (KBr) ν_{max} 3400 (br), 2935, 1650, 1630, 1540, 1375, 1115, 1075 cm^{-1}; HRFABMS *m/z* 1309.6282 [M + Na]$^+$ (calcd for $C_{62}H_{98}N_2O_{26}Na$, 1309.6305, Δ 2.4 mmu).

Sarasinoside H_1 (3): white amorphous solid, mp 204−207 °C; $[\alpha]^{25}_D$ −9.0° (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ϵ) 239 (4.18) nm; IR (KBr) ν_{max} 3400 (br), 2930, 1640 (br), 1560, 1450, 1375, 1270, 1120, 1075 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; HMBC correlations H-5/C-4, C-10; H-15/C-8, C-13, C-14; H-16 (δ 2.27)/C-13, C-14; H-18/C-12, C-13, C-14, C-17; H-19/C-1, C-5, C-9, C-10; H-21/C-17, C-20, C-22; H-22 (δ 2.56)/C-21, C-23; H-24/C-23, C-26, C-27; H-26/C-24, C-25, C-27; H-27/C-24, C-25, C-26; H-28/C-3, C-4, C-5; H-29/C-3, C-4, C-5; H-1′/C-3, C-2′; H-2′/C-1′, C-1″; H-3′/C-2′; H-1″/C-2′; H-6″ (δ 4.10)/C-1‴; H-1‴/C-6″; H-1⁗/C-2‴; H-2⁗/C-1⁗; H-1⁗/C-4′; ROESY correlations H-1α/H-3, H-1α/H-5, H-1β/H-19, H-2α/H-3, H-2β/H-19, H-2β/H-28, H-3/H-5, H-3/H-29, H-3/H-1′, H-5/H-29, H-6α/H-29, H-7β/H-15, H-11β/H-18, H-11β/H-19, H-12β/H-18, H-15/H-16α, H-15/H-17, H-16β/H-18, H-16β/H-22, H-28/H-1‴, H-29/H-1′, H-1′/H-3′, H-1′/H-5ax′, H-2′/H-1″, H-3′/H-1⁗, H-5ax′/H-1″, H-5ax′/H-1⁗, H-6″ (δ 4.10)/H-1‴, H-1‴/H-5‴, H-5‴/H-3⁗, H-1⁗/H-5⁗; HRFABMS *m/z* 1343.6302 [M + Na]$^+$ (calcd for $C_{62}H_{100}N_2O_{28}Na$, 1343.6360, Δ 5.8 mmu).

Sarasinoside H_2 (4): white amorphous solid, mp 201−203 °C; $[\alpha]^{25}_D$ −8.9° (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ϵ) 238 (4.04) nm; IR (KBr) ν_{max} 3400 (br), 2930, 1730, 1645 (br), 1555, 1375, 1270, 1115, 1070 cm^{-1}; ^{1}H and ^{13}C NMR data of the aglycon, see Table 1; ^{1}H NMR data of the sugar portion (CD_3OD) Xyl δ 4.29 (1H, d, *J* = 7.8 Hz, H-1′), 3.91 (H-2′), 3.87 (H-5′), 3.68 (H-4′), 3.52 (1H, dd, *J* = 9.3, 8.8 Hz, H-3′), 3.15 (1H, dd, *J* = 10.7, 9.8 Hz, H-5′); 2-NAc-Glc δ 4.83 (1H, d, *J* = 8.3 Hz, H-1″), 4.10 (1H, br d, *J* = 12.2 Hz, H-6″), 3.78 (1H, dd, *J* = 12.2, 6.9 Hz, H-6″), 3.69 (H-5″), 3.68 (H-2″), 3.42 (H-3″), 3.16 (1H, dd, *J* = 10.3, 8.8 Hz, H-4″), 1.99 (3H, s, NHAc); Glc-I: δ 5.08 (1H, d, *J* = 7.9 Hz, H-1‴), 3.81 (H-6‴), 3.70 (H-3‴), 3.69 (H-6‴), 3.45 (1H, ddd, *J* = 9.3, 4.9, 2.4 Hz, H-5‴), 3.40 (H-2‴), 3.32 (H-4‴); Glc-II δ 4.62 (1H, d, *J* = 7.3 Hz, H-1⁗), 3.88 (H-6⁗), 3.71 (H-6⁗), 3.41 (H-2⁗, H-3⁗), 3.33 (H-4⁗), 3.29 (H-5⁗); 2-NAc-Gal δ 4.48 (1H, d, *J* = 8.8 Hz, H-1⁗), 3.90 (H-6⁗), 3.88 (H-2⁗), 3.80 (H-4⁗), 3.71 (H-6⁗), 3.64 (H-5⁗), 3.63 (1H, dd, *J* = 10.7, 3.9 Hz, H-3⁗), 1.98 (3H, s, NHAc); ^{13}C NMR data of the sugar portion (CD_3OD) Xyl δ 106.7 (C-1′), 79.6 (C-4′), 78.1 (C-2′), 77.6 (C-3′), 64.2 (C-5′); 2-NAc-Glc δ 174.2 (NHAc), 101.9 (C-1″), 78.1 (C-5″), 76.9 (C-3″), 72.3 (C-4″), 70.0 (C-6″), 57.9 (C-2″), 23.0 (NHAc); Glc-I δ 102.5 (C-1‴), 83.2 (C-2‴), 78.0 (C-3‴), 77.2 (C-5‴), 71.5 (C-4‴), 62.4 (C-6‴); Glc-II δ 105.6 (C-1⁗), 78.2 (C-5⁗), 77.8 (C-3⁗), 75.8 (C-2⁗), 71.5 (C-4⁗), 62.6 (C-6⁗); 2-NAc-Gal δ 173.9 (NHAc), 102.5 (C-1⁗), 77.2 (C-5⁗), 72.8 (C-3⁗), 69.8 (C-4⁗), 63.1 (C-6⁗), 54.5 (C-2⁗), 23.0 (NHAc); HMBC correlations of the aglycon, H-15/C-13; H-16 (δ 2.27)/C-13; H-18/C-12, C-13, C-14, C-17; H-19/C-1, C-5, C-9, C-10; H-21/C-17, C-20, C-22; H-22 (δ 2.58)/C-23; H-24/C-23, C-26, C-27; H-26/C-24, C-25, C-27; H-27/C-24, C-25, C-26; H-28/C-3, C-4, C-5, C-29; H-29/C-3, C-4, C-5, C-28; OMe/C-15; H-1′/C-3; ROESY correlations of the aglycon, H-1α/H-3, H-2β/H-19, H-2β/H-28, H-2α/H-3, H-3/H-5, H-3/H-29, H-3/H-1′, H-5/H-29, H-6α/H-29, H-6β/H-19, H-6β/H-28, H-7β/H-15, H-11β/H-18, H-11β/H-19, H-12β/H-18, H-12β/H-21, H-15/H-16α, H-15/H-17, H-15/OMe,

H-16α/H-17, H-16β/OMe, H-18/OMe, H-28/H-1‴, H-29/H-1′; HRFABMS *m/z* 1357.6520 $[M + Na]^+$ (calcd for $C_{63}H_{102}N_2O_{28}$-Na, 1357.6516, Δ −0.3 mmu).

Sarasinoside I_1 (5): white amorphous solid, mp 219−222 °C; $[\alpha]^{25}_D$ −5.3° (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ϵ) 238 (4.14) nm; IR (KBr) ν_{max} 3400 (br), 2930, 1640 (br), 1560, 1375, 1270, 1120, 1075 cm^{-1}; ^1H and ^{13}C NMR data of the aglycon see Table 1; ^1H NMR data of the sugar portion (CD_3OD) Xyl δ 4.31 (1H, d, *J* = 7.8 Hz, H-1′), 3.91 (H-2′), 3.88 (H-5′), 3.68 (H-4′), 3.53 (1H, dd, *J* = 8.8, 8.8 Hz, H-3′), 3.16 (1H, dd, *J* = 10.7, 10.3 Hz, H-5′); 2-NAc-Glc δ 4.85 (1H, d, *J* = 8.8 Hz, H-1″), 4.09 (1H, br d, *J* = 12.7 Hz, H-6″), 3.78 (1H, dd, *J* = 12.7, 6.4 Hz, H-6″), 3.68 (H-2″, H-5″), 3.42 (H-3″), 3.17 (1H, dd, *J* = 9.3, 9.3 Hz, H-4″), 2.00 (3H, s, NHAc); Glc-I δ 5.08 (1H, d, *J* = 7.8 Hz, H-1‴), 3.81 (H-6‴), 3.72 (H-6‴), 3.70 (H-3‴), 3.47 (1H, ddd, *J* = 9.8, 4.9, 2.4 Hz, H-5‴), 3.42 (H-2‴), 3.33 (H-4‴); Glc-II δ 4.63 (1H, d, *J* = 7.3 Hz, H-1⁗), 3.89 (H-6⁗), 3.72 (H-6⁗), 3.40 (H-2⁗, H-3⁗), 3.32 (H-4⁗), 3.28 (1H, ddd, *J* = 9.8, 5.4, 2.0 Hz, H-5⁗); 2-NAc-Gal δ 4.50 (1H, d, *J* = 8.3 Hz, H-1⁗⁗), 3.90 (H-6⁗⁗), 3.88 (H-2⁗⁗), 3.81 (H-4⁗⁗), 3.73 (H-6⁗⁗), 3.66 (H-5⁗⁗), 3.64 (1H, dd, *J* = 11.3, 3.9 Hz, H-3⁗⁗), 2.00 (3H, s, NHAc); ^{13}C NMR data of the sugar portion (CD_3OD) Xyl δ 106.7 (C-1′), 79.7 (C-4′), 78.1 (C-2′), 77.6 (C-3′), 64.2 (C-5′); 2-NAc-Glc δ 174.2 (NHAc), 101.9 (C-1″), 78.1 (C-5″), 76.9 (C-3″), 72.3 (C-4″), 70.0 (C-6″), 57.9 (C-2″), 23.1 (NHAc); Glc-I δ 102.5 (C-1‴), 83.3 (C-2‴), 78.0 (C-3‴), 77.2 (C-5‴), 71.5 (C-4‴), 62.5 (C-6‴); Glc-II δ 105.7 (C-1⁗), 78.2 (C-5⁗), 77.8 (C-3⁗), 75.8 (C-2⁗), 71.5 (C-4⁗), 62.6 (C-6⁗); 2-NAc-Gal δ 173.9 (NHAc), 102.5 (C-1⁗⁗), 77.2 (C-5⁗⁗), 72.8 (C-3⁗⁗), 69.8 (C-4⁗⁗), 63.1 (C-6⁗⁗), 54.5 (C-2⁗⁗), 23.1 (NHAc); HMBC correlations of the aglycon H-7 (δ 2.30)/C-8, C-14; H-16/C-13; H-18/C-12, C-13, C-14, C-17; H-19/C-1, C-5, C-9, C-10; H-21/C-17, C-20, C-22; H-22 (δ 2.19)/C-20, C-21, C-23; H-24/C-23, C-26, C-27; H-26/C-24, C-25, C-27; H-27/C-24, C-25, C-26; H-28/C-3, C-4, C-5, C-29; H-29/C-3, C-4, C-5, C-28; H-1′/C-3; ROESY correlations of the aglycon H-1α/H-5, H-2α/H-3, H-2β/H-28, H-3/H-5, H-3/H-29, H-3/H-1′, H-5/H-7α, H-5/H-29, H-6α/H-29, H-6β/H-19, H-7β/H-15, H-11β/H-18, H-11β/H-19, H-12β/H-18, H-15/H-18, H-18/H-22 (δ 2.19), H-28/H-29, H-29/H-1′; HRFABMS *m/z* 1343.6394 $[M + Na]^+$ (calcd for $C_{62}H_{100}N_2O_{28}Na$, 1343.6360, Δ −3.4 mmu).

Sarasinoside I_2 (6): white amorphous solid, mp 216−219 °C; $[\alpha]^{25}_D$ −6.3° (*c* 0.12, MeOH); UV (MeOH) λ_{max} (log ϵ) 238 (4.06) nm; IR (KBr) ν_{max} 3400 (br), 2930, 1640 (br), 1565, 1375, 1115, 1075 cm^{-1}; ^1H and ^{13}C NMR data of the aglycon see Table 1; ^1H NMR of the sugar portion (CD_3OD) Xyl δ 4.29 (1H, d, *J* = 7.8 Hz, H-1′), 3.91 (H-2′), 3.88 (H-5′), 3.68 (H-4′), 3.52 (1H, dd, *J* = 8.8, 8.8 Hz, H-3′), 3.16 (1H, dd, *J* = 10.7, 10.3 Hz, H-5′); 2-NAc-Glc δ 4.84 (1H, d, *J* = 8.3 Hz, H-1″), 4.10 (1H, br d, *J* = 11.7 Hz, H-6″), 3.78 (1H, dd, *J* = 11.7, 7.3 Hz, H-6″), 3.70 (H-5″), 3.68 (H-2″), 3.42 (H-3″), 3.15 (1H, dd, *J* = 10.3, 8.8 Hz, H-4″), 1.99 (3H, s, NHAc); Glc-I δ 5.07 (1H, d, *J* = 7.8 Hz, H-1‴), 3.81 (H-6‴), 3.70 (H-3‴), 3.69 (H-6‴), 3.46 (1H, ddd, *J* = 9.8, 5.4, 2.4 Hz, H-5‴), 3.39 (H-2‴), 3.32 (H-4‴); Glc-II δ 4.61 (1H, d, *J* = 7.8 Hz, H-1⁗), 3.89 (H-6⁗), 3.71 (H-6⁗), 3.41 (H-2⁗, H-3⁗), 3.32 (H-4⁗), 3.27 (1H, ddd, *J* = 9.8, 5.4, 2.4 Hz, H-5⁗); 2-NAc-Gal δ 4.49 (1H, d, *J* = 8.3 Hz, H-1⁗⁗), 3.91 (H-6⁗⁗), 3.88 (H-2⁗⁗), 3.80 (H-4⁗⁗), 3.71 (H-6⁗⁗), 3.64 (1H, dd, *J* = 10.7, 3.9 Hz, H-3⁗⁗), 3.62 (H-5⁗⁗), 1.99 (3H, s, NHAc); ^{13}C NMR data of the sugar portion (CD_3OD) Xyl δ 106.8 (C-1′), 79.6 (C-4′), 78.1 (C-2′), 77.6 (C-3′), 64.2 (C-5′); 2-NAc-Glc δ 174.2 (NHAc), 101.9 (C-1″), 78.1 (C-5″), 76.9 (C-3″), 72.4 (C-4″), 70.0 (C-6″), 57.9 (C-2″), 23.0 (NHAc); Glc-I δ 102.5 (C-1‴), 83.3 (C-2‴), 78.0 (C-3‴), 77.2 (C-5‴), 71.5 (C-4‴), 62.5 (C-6‴); Glc-II δ 105.7 (C-1⁗), 78.2 (C-5⁗), 77.8 (C-3⁗), 75.8 (C-2⁗), 71.5 (C-4⁗), 62.7 (C-6⁗); 2-NAc-Gal δ 173.9 (NHAc), 102.5 (C-1⁗⁗), 77.2 (C-5⁗⁗), 72.8 (C-3⁗⁗), 69.9 (C-4⁗⁗), 63.1 (C-6⁗⁗), 54.5 (C-2⁗⁗), 23.0 (NHAc); HMBC correlations of the aglycon H-5/C-28; H-12 (δ 1.49)/C-13; H-15/C-13, C-14, C-17; H-16 (δ 1.92)/C-14, C-15; H-16 (δ 1.55)/C-17, C-20; H-18/C-12, C-13, C-14, C-17; H-19/C-1, C-5, C-9, C-10; H-21/C-17, C-20, C-22; H-22(δ 2.20)/C-20, C-21, C-23; H-24/C-23, C-26, C-27; H-26/C-24, C-25, C-27; H-27/C-24, C-25, C-26; H-28/C-3, C-4, C-5, C-29; H-29/C-3, C-4, C-5, C-28; OMe/C-15; ROESY correlations of the aglycon H-1α/H-3, H-1α/H-5, H-1β/H-19, H-2α/H-3, H-2β/H-19, H-2β/H-28, H-3/H-5, H-3/H-29, H-3/H-1′, H-6β/H-19, H-7/H-15, H-11β/H-18, H-11β/H-19, H-12β/H-18, H-15/H-18, H-15/H-16β, H-15/OMe, H-16α/H-17, H-16α/H-22 (δ 2.59), H-16α/OMe, H-16β/H-18, H-18/H-22 (δ 2.20), H-20/H-22 (δ 2.20); HRFABMS *m/z* 1357.6511 $[M + Na]^+$ (calcd for $C_{63}H_{102}N_2O_{28}Na$, 1357.6516, Δ 0.6 mmu).

Acid Hydrolysis and GC Analysis of 3. A solution of **3** (1.4 mg) in 1 N HCl (0.5 mL) was stirred at 60 °C for 4 h. After cooling, the solution was concentrated by blowing with N_2. The residue was dissolved in Trisil-Z (0.2 mL), and the solution was stirred at 60 °C for 5 min. After drying the solution with a stream of N_2, the residue was separated by water and CH_2Cl_2 (1 mL, v:v = 1:1). The CH_2Cl_2 layer was analyzed by GC using a Chirasil-Val column (0.32 mm × 25 m). Temperatures of injector and detector were 200 °C for both. A temperature gradient system was used for the oven; the initial temperature was maintained at 100 °C for 1 min and then raised to 200 °C at the rate of 5 °C/min. Peaks of the hydrolysate were detected at 12.04, 15.63, 21.83, and 22.28 min. The ratio of the areas of peaks observed at 15.63 to 12.04 min was 1.89. Retent times for authentic samples after being treated simultaneously with Trisil-Z were 12.09 (D-xylose), 12.17 (L-xylose), 15.60 (D-glucose), 15.74 (L-glucose), 21.75 (2-*N*-acetyl-D-galactosamine), and 22.25 (2-*N*-acetyl-D-glucosamine), respectively. Co-injection of the hydrolysate with the authentic silyated D-xylose, D-glucose, 2-*N*-acetyl-D-galactosamine, and 2-*N*-acetyl-D-glucosamine gave single peaks at 12.09, 15.57, 21.73, and 22.25 min, respectively.

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