Norlanostane Triterpenoidal Saponins from the Marine Sponge *Melophlus sarassinorum*

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Along with five known 30-norlanostane-type saponins, sarasinosides A_1 (**5A**), A_3 (**6A**), I_1 (**7**), I_2 (**8**), and H_2 (**9**), four new triterpenoidal saponin congeners, sarasinosides J (**1**), K (**2**), L (**3**), and M (**4**), were isolated from the Indonesian sponge *Melophlus sarassinorum*. Sarasinosides J (**1**) and K (**2**) are the 24,25-hydrogenated congeners of the previously described sarasinosides A_1 and H_2, respectively. The carbon skeleton of sarasinoside M (**4**) possesses a rearranged 8α,9α-epoxy-8,9-seconorlanosta-8(14),9(11),24-triene system, which is novel and unprecedented in nature. The structures of the new compounds were confirmed by spectral analyses, chemical derivatization, and GC analyses. Compounds **1** and **5A** exhibited antimicrobial activity toward *Bacillus subtilis* and *Saccharomyces cerevisiae*.

Steroidal and triterpenoidal saponins are commonly found to occur in echinoderms,[1] especially among sea stars (family Asteroidea)[2] and holothurians (family Holothuroidea).[3a−e] This group of compounds was even considered to be a chemotaxonomic marker for these families.[1b] Saponins have been reported to possess antitumor,[3f−g,5,8,13] antifungal,[3h−j] and molluscidal[4] properties, whereas some of the glycosides are known for their pharmacological efficacy to inhibit Na^+, K^+-ATPase[3k,5] and for their neuraminidase activity.[6] A number of saponins have also been isolated from sponges[6−24] belonging to various genera. Among these is the genus *Melophlus*, for which three chemical studies have been published.[23−25] *Melophlus isis* collected from Guam has been reported to yield norlanostane triterpenoid oligoglycosides,[23] while *Melophlus sarassinorum* from Japan[24] and Sulawesi, Indonesia,[25] afforded tetramic acids.

Investigation of the water-soluble extract of the sponge *M. sarassinorum*, which was also collected from Sulawesi, Indonesia, led to the isolation of five known 30-norlanostane-type saponins, sarasinosides A_1 (**5A**), A_3 (**6A**), I_1 (**7**), I_2 (**8**), and H_2 (**9**), and four new congeners, sarasinosides J (**1**), K (**2**), L (**3**), and M (**4**) (Figure 1). This paper reports on the isolation and structure elucidation of the isolated triterpenoidal saponin compounds. Their structures were unambiguously elucidated by extensive spectroscopic analysis (2D-NMR experiments and ESIMS), through chemical derivatization and GC analysis. Bioassays showed that compounds **1** and **5A** possessed strong antimicrobial activity.

Results and Discussion

The sponge *M. sarassinorum* was collected by scuba near the shores of Makassar, Sulawesi Island, and was kept frozen until it was extracted. The lyophilized specimens were macerated and successively extracted with acetone and MeOH. The combined extracts were concentrated to dryness and partitioned between EtOAc and H_2O. Further isolation was performed on the antimicrobial aqueous extract, which also showed an interesting chemical profile as exhibited by its HPLC-ESIMS chromatogram. The aqueous extract was fractionated over Dionin HP20, which afforded five known sarasinoside derivatives, A_1 (**5A**),[7,8] A_3 (**6A**),[9] I_1 (**7**),[23] I_2 (**8**),[23] and H_2 (**9**),[23] and four new congeners, sarasinosides J (**1**), K (**2**), L (**3**), and M (**4**).

Sarasinoside J (**1**) was obtained as a yellow amorphous solid. A pseudomolecular ion was detected at *m/z* 1329 [M + Na]$^+$ in ESI-LC/MS, which was compatible with the molecular formula $C_{62}H_{102}N_2O_{27}$ as established by HRESIMS. Compound **1** had a 18 mass unit difference from sarasinoside A_1 (**5A**). The NMR spectra of **1** showed the presence of seven methyl groups, two acetoxy units, five anomeric protons, one olefinic bond, and one carbonyl group (Tables 1 and 2). The ^{1}H and ^{13}C NMR data were quite similar to those of sarasinoside A_1, indicating the occurrence of almost the same triterpenoidal saponin skeleton in both compounds. The ^{13}C NMR chemical shifts of the methyl groups at C-18 and C-19 were in agreement with those of axially oriented substituents, which consequently followed the *trans*−*trans*−*trans* configuration for the ABCD ring junctions. The configuration of H-3 was assigned as α on the basis of its vicinal coupling constants of 11.2 and 4.2 Hz for $J_{2\alpha,3\alpha}$ and $J_{2\beta,3\alpha}$, respectively.[15] The major differences in the spectral data of **5A** compared to those of **1** were the presence of an additional quaternary carbon signal at δ_C 68.6 in **1** and the loss of an olefinic peak at ca. 6.0 ppm previously assigned for the methine proton at C-24, which was discernible in **2**. These differences hinted at changes occurring in the side chain that indicated the presence of a hydroxyl substituent at C-25 and simultaneously the loss of the olefinic bond at position 24(25). Consequently, the methylene group at position 24 showed a typical AB resonance at δ 2.49 and 2.40 with a geminal coupling constant of 13.8 Hz. The absence of the conjugated system in the side chain was supported by the downfield shift (Δ 7 ppm) of the carbonyl function on C-23 at δ 210.3 and a shift in the absorption maximum from 240 nm (for sarasinoside A_1) to 202 nm (for compound **1**) and is in agreement with the molecular mass difference of 18. In addition, the observed fragment at *m/z* 1271 [M + Na^+ − 58]$^+$ could be explained by the loss of the corresponding 2-propanol moiety in the side chain of the aglycon.

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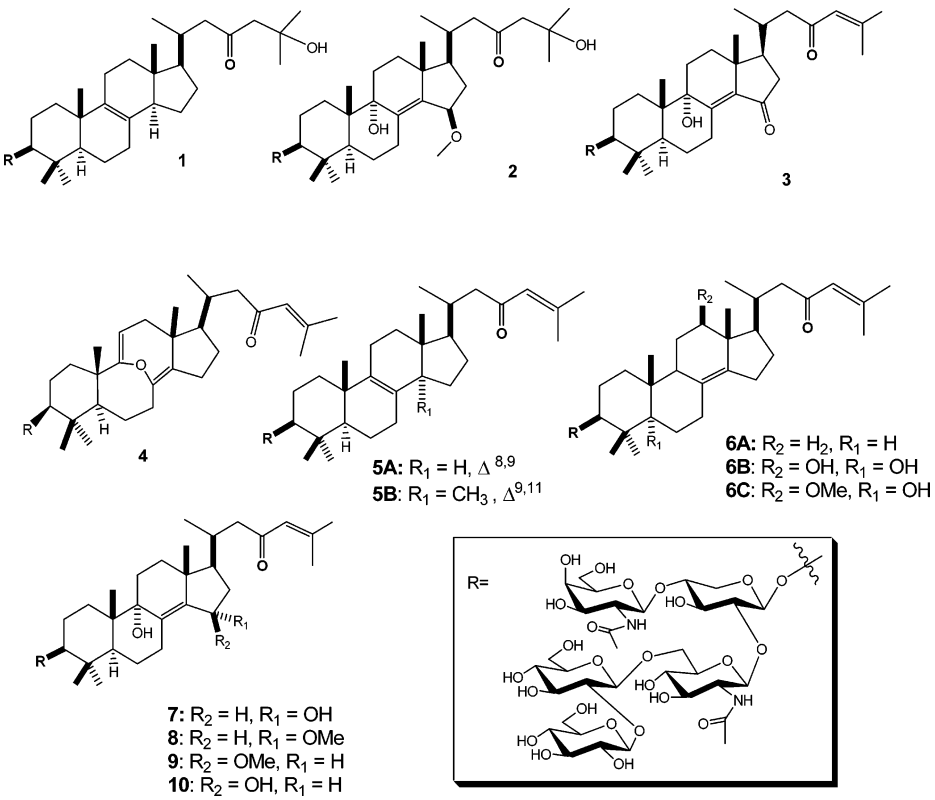

Figure 1. Chemical structures of sarasinoside derivatives isolated from *Melophlus sarassinorum* and some related congeners from *Asteropus sarassinorum*.

Table 1. ^{13}C NMR Data in CD_3OD for the Aglycon Moiety of **1**, **2**, **3**, and **4**, Referenced to the CD_3OD Solvent Signals at δ 49.00

	δ_C							
position	**1**[a]		**2**		**3**		**4**	
1	35.3	CH_2	31.5	CH_2	31.3	CH_2	39.8	CH_2
2	26.5	CH_2	28.4	CH_2	28.2	CH_2	28.2	CH_2
3	88.7	CH	91.8	CH	91.8	CH	91.7	CH
4	39.3	qC	40.6	s	40.6	s	38.5	s
5	50.5	CH	45.6	CH	45.2	CH	53.7	CH
6	17.9	CH_2	23.1	CH_2	24.5	CH_2	24.6	CH_2
7	28.4	CH_2	27.6	CH_2	27.6	CH_2	29.0	CH_2
8	127.0	qC	135.1	qC	141.7	qC	149.8	qC
9	135.7	qC	76.2	qC	76.5	qC	172.7	qC
10	36.2	qC	42.5	qC	43.6	qC	42.0	qC
11	21.6	CH_2	29.1	CH_2	29.1	CH_2	111.0	CH
12	36.2	CH_2	35.2	CH_2	35.2	CH_2	35.6	CH_2
13	41.7	qC	44.0	qC	44.4	qC	47.8	qC
14	51.3	CH	143.0	qC	152.0	qC	132.4	qC
15	23.3	CH_2	77.8	CH	213.0	qC	28.7	CH_2
16	28.0	CH_2	39.5	CH_2	34.0	CH_2	33.5	CH_2
17	54.1	CH	54.6	CH	54.6	CH	54.6	CH
18	11.2	CH_3	17.9	CH_3	17.8	CH_3	18.5	CH_3
19	19.6	CH_3	18.5	CH_3	18.0	CH_3	22.5	CH_3
20	32.1	CH	32.1	CH	32.9	CH	33.3	CH
21	19.7	CH_3	20.7	CH_3	20.7	CH_3	20.9	CH_3
22	51.2	CH_2	54.6	CH_2	51.7	CH_2	51.8	CH_2
23	210.3	qC	213.0	qC	203.1	qC	203.8	qC
24	55.4	CH_2	55.2	CH_2	127.2	CH	125.3	CH
25	68.6	qC	68.7	qC	157.3	qC	157.0	qC
26	29.4[b]	CH_3	20.7	CH_3	20.9	CH_3	20.9	CH_3
27	29.8[b]	CH_3	27.9	CH_3	27.6	CH_3	27.6	CH_3
28	16.0	CH_3	17.4	CH_3	17.4	CH_3	17.3	CH_3
29	27.4	CH_3	30.7	CH_3	29.1	CH_3	29.2	CH_3
30			54.1	CH_3				

[a] NMR data for the aglycon moiety of **1** in CD_3SOCD_3. [b] Interchangeable signals.

The HMBC correlations (Figure 2) of the seven methyl protons with their neighboring carbons unequivocally defined their positions along with the other substituents in the norlanostane framework. The long-range correlations of the methyl proton signals at δ 0.98 (CH_3-28) and 0.80 (CH_3-29) with the methine carbon signals at δ_{C-5} 50.5 and δ_{C-3} 88.7 identified the position of the ether oxygen at C-3 of ring A (Table 1). Similarly, the placement of the other oxygenated substituent at C-25 was supported by the long-range correlations of CH_3-26 and CH_3-27 at δ 1.13 (6H) with the quaternary carbon at δ_{C-25} 68.6. The HMBC spectrum revealed that the olefinic carbon signal at δ_{C-9} 135.7 had a long-range correlation with the singlet signal of CH_3-19 at δ 0.92, which further exhibited cross-peaks with the quaternary carbon at δ_{C-10} 36.2, the methine carbon at δ_{C-5} 50.5, and the methylene carbon at δ_{C-1} 35.3. Since no olefinic proton resonances were observed in the ^1H NMR spectrum, the double bond was assigned between C-8 and C-9 accordingly. The tetrasubstituted double bond was also compatible to that of (5α)-cholest-8(9)-en-3β-ol with carbon shifts at δ 127.0 and 134.8 for C-8 and C-9, respectively.[53] This was further confirmed by the HMBC correlations of the quaternary carbon at δ_{C-8} 127.0 with the overlapping methine and methylene protons at δ 1.61 for CH-14 and CH_2-7, respectively. The location of the carbonyl group was established at C-23 (δ 210.3) through its long-range correlations with the methylene protons at δ 2.50/2.25 (CH_2-22) and δ 2.40/2.49 (CH_2-24). From these data, the aglycon was identified as 3β,25-dihydroxy-30-norlanosta-8(9)-en-23-one. Table 1 shows the assignments of ^1H and ^{13}C NMR data on the basis of the 2D NMR experiment.

Sarasinoside J (**1**) contained five sugar residues at C-3, which is identical to sarasinoside A_1. Its ^1H and ^{13}C NMR spectra revealed five anomeric protons at δ_H 4.77 (d, J = 8.3 Hz), 4.69 (d, J = 7.7 Hz), 4.74 (d, J = 7.6 Hz), 4.39 (d, J = 8.3 Hz), and 4.22 (d, J = 7.4 Hz), which correlated with the anomeric carbon signals at δ_C 100.2, 101.2, 101.3, 104.2, and 104.2. The TOCSY spectrum indicated that the

Table 2. ^{1}H NMR Data in CD_3OD for the Aglycon Moiety of **1**, **2**, **3**, and **4**, Referenced to the Residual Solvent Signal at δ 3.35

	δ_H			
position	**1**[a]	**2**	**3**	**4**
1	1.13 m	1.71 m	1.78 m	1.78 m
		1.52 m	1.53 m	1.57 m
2	1.72 m	1.89 m	1.91 m	1.89 m
	1.57 m	1.72 m	1.72 m	1.79 m
3	2.97 dd (11.2, 4.2)	3.16 dd (11.2, 3.9)	3.18 dd (11.2, 3.9)	3.18 dd (11.2, 3.9)
5	1.03 t (11.0)	1.95 m	1.90 dd (12.7, 3.3)	1.98 m
6	1.42 m	1.68 m	1.62 m	1.65 m
		1.36 m	1.34 m	1.45 m
7	1.61 m	2.66 m	2.65 brd (14.2)	2.70 brd (14.2)
		2.34 m	2.29 m	2.18 m
11	1.53 m	1.95 m	2.02 m	5.21 t (6.9)
		1.49 m	1.55 m	Δ 9,11
12	1.23 m	1.76 brd (13.2)	1.76 brd (13.2)	3.00 dd (13.2, 6.9)
		1.41 m	1.41 m	2.12 dd (13.2, 6.9)
14	1.61 m	Δ 8,14	Δ 8,14	Δ 8,14
15	1.85 m	4.20 dd (7.8, 6.4)	keto unit	1.50 m
16	1.98 m	2.30 ddd (13.2, 7.8, 8.3)	2.38 dd (13.2, 8.3)	2.00 m
		1.48 m	2.14 dd (13.2, 7.8)	
17	1.33 m	1.22 m	1.60 m	1.19 m
18	0.58 s	0.98 s	0.92 s	0.90 s
19	0.92 s	1.02 s	1.18 s	1.25 s
20	1.87 m	2.18 m	2.14 m	2.09 m
21	0.85 d (6.5)	1.00 d (6.5)	1.15 d (5.9)	1.00 d (5.9)
22	2.50 (overlapped)	2.59 m	2.58 dd (14.7, 2.9)	2.58 dd (14.7, 2.9)
	2.25 dd (16.6, 9.9)	2.18 m	2.14 m	2.18 m
24	2.49 d (13.8)	2.52 brs	6.18 brs	6.18 brs
	2.40 d (13.8)			
26	1.12[b] s	1.28 s	2.15 brs	2.11 brs
27	1.13[b] s	1.28 s	1.91 brs	1.91 brs
28	0.98 s	1.01 s	1.01 s	0.96 s
29	0.80 s	1.17 s	1.19 s	1.12 s
30		3.32 s		

[a] NMR data for the aglycon moiety of **1** in CD_3SOCD_3. [b] Interchangeable signals.

oligosaccharide moiety of **1** comprised two glucose units, one xylose unit, one NAc-glucosamine, and one NAc-galactosamine. Its ESI positive MS/MS spectrum provided information on the sugar sequence of the molecule. Fragments at *m/z* 1109 $[M + Na^+ - 58 - 162]^+$ and 1068 $[M + Na^+ - 58 - 203]^+$ showed the loss of two terminal sugars, which were glucose and NAc-galactosamine, respectively. Further fragmentation afforded pseudomolecular ion peaks at *m/z* 947 $[M + Na^+ - 58 - 162 - 162]^+$, 744 $[M + Na^+ - 58 - 162 - 162 - 203]^+$, 541 $[M + Na^+ - 58 - 162 - 162 - 203 - 203]^+$, and 408 $[M + Na^+ - 58 - 162 - 162 - 203 - 203 - 132 - H]^+$, which corresponded to the successive loss of 2-propanol + glucose + glucose + NAc-glucosamine + NAc-galactosamine + xylose + H and accordingly indicated the sugar sequence in the oligosaccharide moiety.

The attachment of the sugar moieties was established from the HMBC spectrum (Figure 2). A long-range correlation of H′-1 (δ 4.22) with C-3 (δ 88.7) revealed the linkage between C-1′ of xylose and C-3 of the aglycon. Similarly, the long-range correlation of H-1″ (δ 4.77) with C-2′ (δ 77.6) assigned the connectivity between 2-NAc-glucosamine and the xylose unit, a long-range correlation of H-1‴ (δ 4.69) with C-6″ (δ 68.6) determined the attachment between the glucose unit and 2-NAc-glucosamine, while the linkage between the two glucose units was revealed through the long-range correlation of H-1⁗ (δ 4.42) with C-2‴ (δ 82.5). Finally, the connectivity between 2-NAc-galactosamine and xylose was resolved by the correlation of H-6⁗′ (δ 4.39) with C-4′ (δ 78.3). The chemical shifts and the large *J* values (ca. 7.5−8.5 Hz) were indicative of the β-glycoside linkages. The absolute stereochemistry of the sugar moieties was confirmed by hydrolysis and subsequent butanolysis of the sugar residues with (−)-2-butanol.[72] GC analysis of the monosaccharide residues by comparison with reference standards proved that the sugar moieties of **1** were identical to those of sarasinoside A_1, which were assigned as D-xylose, D-NAc-galactosamine, and D-NAc-glucosamine, and with two units of D-glucose. Thus, the structure of compound **1** 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-aminogalactopyranosyl]-25-hydroxy-30-norlanosta-8(9)-en-23-one and named sarasinoside J.

Sarasinoside K (**2**) was obtained as a yellow amorphous solid. A pseudomolecular ion was detected at *m/z* 1375 $[M + Na]^+$ in the ESI-LC/MS and was compatible with the molecular formula $C_{63}H_{104}N_2O_{29}$ as established by HRES-IMS. The ^{1}H and ^{13}C NMR data were quite comparable to those of sarasinoside H_2 (**9**), which also indicated the occurrence of a similar triterpenoidal saponin skeleton in both compounds. As in sarasinoside H_2,[23] the NMR spectra for compound **2** were also measured in MeOD. Compound **2** had an 18 mass unit difference compared to compound **9**. Inspection of the ^{1}H and ^{13}C NMR spectra of **2** showed the presence of seven methyl groups, two acetoxy units, one methoxyl group, five anomeric protons, one olefinic bond, and one carbonyl group (Tables 1 and 2) similar to sarasinoside H_2 (**9**). The ^{1}H and ^{13}C NMR data of sarasinoside K (**2**) for the oligosaccharide moiety were superimposable with those of sarasinoside H_2, which implied that **2** also contained a sugar unit identical to that in **9**. The major differences in the spectral data of **2** compared to **9** were the appearance of an additional tertiary hydroxyl carbon signal at δ_C 68.7 in **2** and the loss of an olefinic peak at ca. 6.0 ppm previously assigned to the methine proton at C-24 which was found in **9**. Since a similar case has been observed in the structure elucidation of sarasinoside J, these differences again hinted at the presence of a hydroxyl

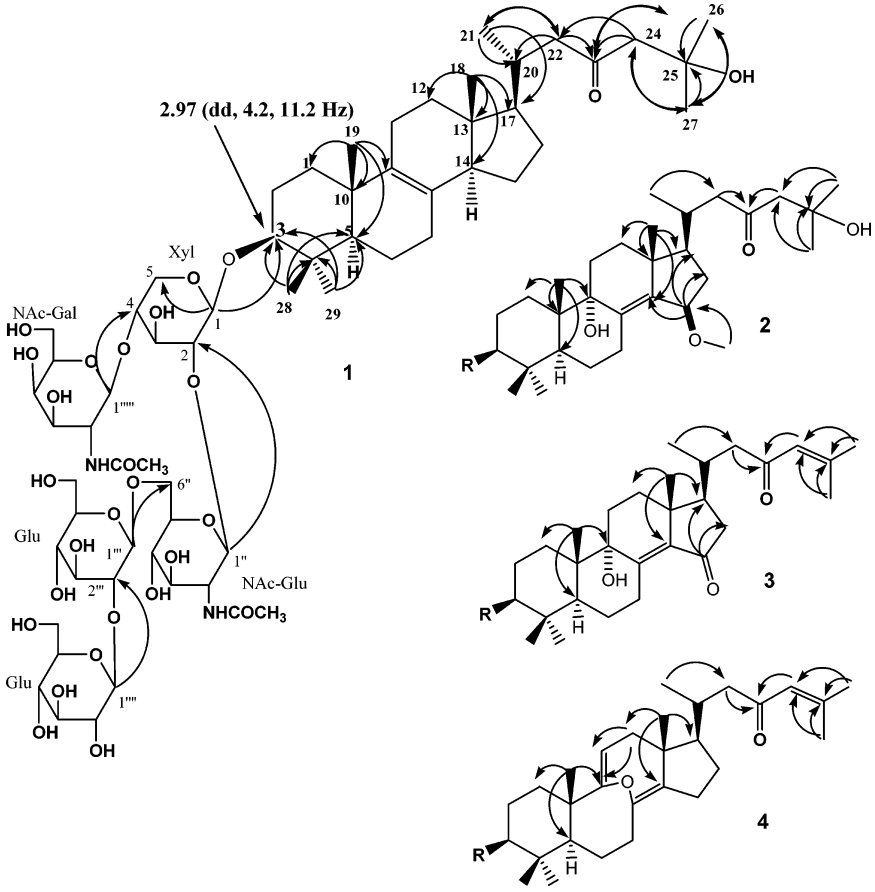

Figure 2. Key long-range correlations of **1**, **2**, **3**, and **4**.

substituent at C-25 and simultaneously the loss of the olefinic bond at position 24(25). As in **1**, the absence of the conjugated system in the side chain was again supported by the downfield shift (Δ 10 ppm) of the carbonyl function on C-23 to δ 213.0, and a shift in the absorption maximum from 238 nm (sarasinoside H_2) to 204 nm (in **2**) was also observed. In addition, this could also be further explained by the loss of the corresponding 2-propanol moiety in the side chain of the aglycon as detected through the fragment ion at *m/z* 1317 $[M + Na^+ - 58]^+$. These data were in accordance with the molecular mass difference of 18 mass units between sarasinoside H_2 and compound **2**. From these data, the aglycon was identified as 3β,9α,25-trihydroxy-15β-methoxy 30-norlanosta-8(14)-en-23-one, and the new compound was named sarasinoside K.

Sarasinoside L (**3**) was obtained as a yellow amorphous solid. A pseudomolecular ion was detected at *m/z* 1341 $[M + Na]^+$ in the ESI-LC/MS and was compatible with the molecular formula $C_{63}H_{98}N_2O_{28}$ as established by HRES-IMS. Inspection of the ^{1}H and ^{13}C NMR spectra of **3** showed the presence of seven methyl groups, two acetoxy units, five anomeric protons, two olefinic bonds, and two carbonyl groups (Tables 1 and 2). The ^{1}H and ^{13}C NMR data were similar to those of sarasinosides H_1 (**10**) and I_1 (**7**).[23] Compared to sarasinosides H_1 and I_1, **3** was two mass units smaller, suggesting the presence of an additional double bond in the molecule. When the spectral data of **3** were compared to those of **7** and **10**, the appearance of an additional carbonyl peak at δ 213.0 in the ^{13}C NMR spectrum of **3** and the absence of the proton resonance at δ 4.20, previously assigned to H-15 in the corresponding known homologues, suggested the presence of a keto unit at C-15. Accordingly, changes in chemical shifts were also observed for the neighboring methylene protons CH_2-16, which were shifted downfield to δ 2.38 and 2.14, as well as the downfield shift (Δ 9 ppm) of C-14 to δ_C 152.0. The keto substituent on C-15 was further confirmed by its HMBC correlations (Figure 2) with CH_2-16 and the methine proton at C-17 (δ 1.60), which further showed a cross-peak with CH_3-18 at δ 0.92. Likewise, the HMBC correlations (Figure 2) of the methyl protons with their neighboring carbons again unambiguously afforded their positions along with the other substituents in the norlanostane framework (Tables 1 and 2). From these spectral data, the aglycon was identified as 3β,9α-dihydroxy-30-norlanosta-8(14),24-dien-15,23-dione, and the new compound was named sarasinoside K.

Sarasinoside M (**4**) was obtained as a yellow amorphous solid. A pseudomolecular ion was detected at *m/z* 1303 $[M + H]^+$ in the ESI-LC/MS and was compatible with the molecular formula $C_{62}H_{98}N_2O_{27}$ as established by HRES-IMS. It differed from sarasinoside L (**3**) by the loss of 16 mass units, which indicated that **4** has one oxygen atom less than the latter congener. Inspection of the ^{1}H and ^{13}C NMR spectra of **4** revealed the presence of seven methyl groups, two acetoxy units, five anomeric protons, three olefinic bonds, and only one carbonyl group (Tables 1 and 2). The loss of one carbonyl signal at δ 213.0 as found in sarasinoside L for C-15 suggested hydrogenation of the ketone function at this position. The ^{1}H and ^{13}C NMR data of **4** for the oligosaccharide moiety were again identical to those of the latter derivatives. Since the ^{1}H and ^{13}C NMR signals assigned to the side chain (C-17 through C-27) were virtually identical to the respective substructure present in sarasinoside L (**3**), possible differences could only occur in the triterpenoid core in **4**. In the ^{1}H NMR spectrum (see Table 1), two methine protons at δ 6.18 (H-24, bs) and 5.21 (H-11, t) were observed in the olefinic region. The ^{13}C NMR

Table 3. ^{13}C NMR Data for Sugar Moiety of **1** (in CD_3SOCD_3), **2**, **3**, and **4** (in CD_3OD)

carbon	in CD_3SOCD_3	in CD_3OD	carbon	in CD_3SOCD_3	in CD_3OD
Xyl-1′	104.2	106.7	Glc^2-1‴′	104.2	105.8
Xyl-2′	77.6	78.2	Glc^2-2‴′	74.8	75.9
Xyl-3′	76.1	77.6	Glc^2-3‴′	75.9	77.9
Xyl-4′	78.3	79.9	Glc^2-4‴′	67.6	71.5
Xyl-5′	62.8	64.2	Glc^2-5‴′	75.5	78.2
NAcglc-1″	100.2	102.0	Glc^2-6‴′	60.7	62.7
NAcglc-2″	56.5	58.0	NAcgal-1⁗′	101.3	102.6
NAcglc-3″	75.1	76.9	NAcgal-2⁗′	52.4	52.2
NAcglc-4″	69.6	72.4	NAcgal-3⁗′	71.3	72.8
NAcglc-5″	75.1	78.2	NAcgal-4⁗′	70.9	69.9
NAcglc-6″	68.6	70.0	NAcgal-5⁗′	76.1	77.2
NAcglc-Ac	23.1, 169.7	23.0, 174.2	NAcgal-6⁗′	61.0	63.1
Glc^1-1‴	101.2	102.6	NAcgal-Ac	27.1, 169.9	23.0, 173.9
Glc^1-2‴	82.5	83.6			
Glc^1-3‴	75.3	78.0			
Glc^1-4‴	69.8	71.5			
Glc^1-5‴	75.9	77.2			
Glc^1-6‴	60.7	62.7			

and DEPT spectra displayed six signals for sp^2-hybridized carbon atoms, which included four quaternary carbons at δ 149.8 (C-8), 172.7 (C-9), 132.4 (C-14), and 157.0 (H-24) and two protonated carbons at δ 111.0 (C-11) and 125.3 (C-24). Compared with **3**, the additional olefinic proton triplet at δ 5.21 (H-11, 5.9 Hz) showed coupling with the methylene pair of a doublet of doublets (CH_2-12) at δ 3.00 and 2.12, which was compatible with those found in sarasinoside G (**5B**).[10] The downfield shifts of the quaternary carbons observed at δ 149.8 (C-8) and 172.7 (C-9) implied the presence of an oxygen-bearing carbon. Since **4** has only three oxygen atoms in its aglycon moiety from the HRMS, there must be a bridging oxygen atom between C-8 and C-9. As with the other congeners above, the HMBC correlation data (Figure 2) of the methyl protons were used to explicitly define the positions of the substituents in the norlanostane core (Tables 1 and 2). Accordingly, a cross-peak was detected between the methyl proton signal at δ 1.25 (CH_3-19) and the carbon resonance at δ 172.7 (C-9), which further correlated with the methylene proton signals of CH_2-12. On the other hand, a correlation was observed between the methyl singlet at δ 0.90 (CH_3-18) and the methylene signals of CH_2-12, which confirmed the double bond between C-9 and C-11, while the olefinic bond at C-8 and C-14 was established from the cross-peak between CH_3-18 and the carbon signal at δ 132.4 (C-14). It is also noteworthy that C-14 is 20 ppm upfield of that in sarasinoside L (**3**), which is further evidence for the loss of the keto function at C-15. However, when **4** was compared with sarasinoside K (**2**), C-14 has moved 11 ppm upfield, while C-8 was shifted 14 ppm downfield to δ 149.8. These changes in chemical shifts in the C ring of the lanostane core are compatible with the 8,9-secosteroid framework found in the structurally related compound eryloside L, an 8α,9α-epoxy-4α-methyl-8,9-secocholesta-7,9(11),14-triene that has been isolated from the sponge *Erylus lendenfeldi* (Geodiidae) collected in the Red Sea.[15] In eryloside L, the carbon shifts for C-8, C-9, C-11, and C-14 were δ 153.0, 169.8, 107.4, and 151.5, respectively. The aglycon was thus identified as 3β-hydroxy-8α,9α-epoxy-8,9-seconorlanosta-8(14),9(11),-24-trien-23-one, and the new compound was named sarasinoside M.

The ^{13}C NMR (Table 3) and ^1H NMR data for the oligosaccharide moiety in **1**, **2**, **3**, and **4** agreed appropriately with those found in **5A**, **6A**, **7**, **8**, and **9**. The new derivatives contained the same sugar units as the known congeners: sarasinosides A_1, A_2, A_3, B_1, B_2, B_3, C_1, C_2, C_3, and D−G. In terms of both the sugar moieties and the 3β-hydroxy-30-norlanosta-24-en-23-one triterpenoid core, the novel sarasinosides J (**1**), K (2), L (**3**), and M (**4**) are closely related to the earlier isolated derivatives. While sarasinosides J (**1**) and K (**2**) are the 24,25-hydrogenated congeners of **5A** and **9**, respectively, the carbon skeleton of sarasinoside M (**4**) is rearranged to an unusual 8α,9α-epoxy-8,9-seconorlanosta-8(14),9(11),24-triene system. The 8,9-epoxy-steroid core is commonly found in nature, but there are only a few natural products with the similar oxygen-bridged 8,9-secosteroid framework. From the marine environment, besides eryloside L, there are only two known natural products that belong to this family of compounds. Jereisterol A, which has been described from the New Caledonian sponge *Jereicopsis graphidiophora*,[27] is an 8α,9α-epoxy-8,9-secocholesta-7,9(11)-diene congener, featuring a 3-methoxyl function and a 24-methyl substituent in its nonhydroxylated side chain. A corresponding 24-ethyl derivative of jereisterol A was also isolated from the Senegalese sponge *Microscleroderma spirophora*.[28] From the terrestrial environment, tylopiol A is the only representative compound known to date. Tylopiol A is a structurally related ergostane-derived 8,9-epoxy-8,9-secosteroid, which was obtained from the basidiomycete fungus *Tylopilus plumbeoviolaceus*.[29] To this relatively new group of secosteroids,[30] sarasinoside M is considered to be unique and unprecedented in nature. Sarasinoside M is the only compound to possess the 8(14),9(11)-diene assemblage, while all earlier reported natural products have the 7,9-(11)-diene system.

An antimicrobial assay showed that sarasinoside A_1 (**5A**) was selectively and strongly active against the yeast *Saccharomyces cerevisiae*. Sarasinoside A_1 (**5A**) was inactive against *B. subtilis* and *E. coli*. Sarasinoside J (**1**) was strongly active against *S. cerevisiae* and showed moderate antibacterial activity toward *B. subtilis*. In an agar diffusion assay, using a loading concentration of 10 μg, sarasinoside J (**1**) exhibited zones of inhibition of 9 and 13 mm toward *B. subtilis* and *S. cerevisiae*, respectively. On the other hand, sarasinoside A_1 (**5A**) gave a zone of inhibition of 10 mm against *S. cerevisiae*. Sarasinoside A_1 (**5A**) has also been reported to possess mild cytotoxicity to the P388 lymphocytic leukemia cell line.[8]

Experimental Section

General Experimental Procedures. Optical rotations were measured on a Perkin-Elmer Model 341 LC polarimeter. ^1H NMR and ^{13}C NMR spectra were recorded on Bruker ARX400 and DMX600 NMR spectrometers. ESI mass spectra were obtained on a Thermofinnigan LCQ DECA mass spectrometer coupled to an Agilent 1100 HPLC system equipped

with a photodiode array detector. HRESI-MS were determined on a Micromass Qtof 2 mass spectrometer. GC analysis was performed on an Agilent 6850 series gas chromatograph using an HP-5 column (cross-linked 5% PHM/Siloxan, Macherey und Nagel, Düren, Germany) and a flame ionization detector. Column chromatography was performed on silica gel 60 (0.040−0.063 mm) or RP-18 Lobar columns (40−63 μm, 310 mm × 15 mm i.d.), and TLC analyses were carried out using aluminum sheets precoated with silica gel 60 F_{254} (all purchased from Merck, Darmstadt, Germany). For HPLC analysis, samples were injected into a HPLC system equipped with a photodiode-array detector (Dionex, München, Germany). Routine detection was at 235 and 254 nm. The separation column (125 × 4 mm, i.d.) was prefilled with Eurospher 100-C_{18}, 5 μm (Knauer, Berlin, Germany). Separation was achieved by applying a linear gradient from 90% H_2O (pH 2.0) to 100% MeOH over 40 min. TLC plates were detected by UV absorption at 254 nm. All solvents were distilled prior to use. Spectral grade solvents were utilized for chromatographic analysis.

Animal Material. The sponge *Melophlus sarassinorum* was collected by scuba near the shores of Makassar, Sulawesi Island, Indonesia. The sponge sample was immediately immersed in methanol after collection and transported to the University of Düsseldorf, Germany, for further isolation work. A voucher fragment is kept in 70% methanol under the registration number ZMA POR. 17544 in the Zoological Museum in Amsterdam.

Extraction and Isolation. Sponge samples of *M. sarassinorum* (ca. 1200 g, wet weight) were lyophilized to give a dry weight of 73.0 g. The freeze-dried sponge sample was macerated and repeatedly extracted with acetone (1 L × 2) and MeOH (1 L × 3) successively. The combined crude extract (12.0 g) was partitioned between EtOAc and H_2O. The aqueous extract was concentrated and loaded onto a column of Dionin HP20, which was successively eluted with 3 bed volumes of water and 2 bed volumes of methanol. The methanol eluate was dried under reduced pressure at room temperature, which gave a 10.0 g residue. The concentrated methanol eluate was chromatographed over a RP-18 column by gradient elution utilizing MeOH and H_2O as solvent system. This gave nine fractions, and the last fraction afforded pure sarasinoside A_1 (**5A**, 4.0 g) as the major compound of this sponge. The fourth fraction yielded the known congener sarasinoside I_1 (**7**, 7 mg), while the fifth fraction yielded new compounds, sarasinosides J (**1**, 40 mg) and K (**2**, 20 mg), together with the known derivative I_2 (**8**, 17 mg). The sixth fraction provided the known derivatives A_3 (**6A**, 20 mg) and H_2 (**9**, 59 mg) together with the new congeners sarasinosides L (**3**, 29 mg) and M (**4**, 12 mg). The compounds were further purified over a silica gel column using CH_2Cl_2−MeOH−H_2O (80:30:0.55) as eluent.

Sarasinoside J (1): yellow amorphous solid; $[\alpha]_D^{20}$ −9.8 (*c* 0.5, MeOH); UV (MeOH) λ_{max} 202; ^{1}H and ^{13}C NMR, see Table 1 and Table 2; HRESIMS *m/z* calcd for $C_{62}H_{102}N_2O_{27}Na$, $[M + Na]^+$ 1329.6569, found 1329.6568.

Sarasinoside K (2): yellow amorphous solid; $[\alpha]_D^{20}$ −6.8 (*c* 0.5, MeOH); UV (MeOH) λ_{max} 204; ^{1}H and ^{13}C NMR, see Table 1 and Table 2; HRESIMS *m/z* calcd for $C_{63}H_{104}N_2O_{29}Na$, $[M + Na]^+$ 1375.6622, found 1375.6560.

Sarasinoside L (3): yellow amorphous solid; $[\alpha]_D^{20}$ −10.2 (*c* 0.5, MeOH); UV (MeOH) λ_{max} 249; ^{1}H and ^{13}C NMR, see Table 1 and Table 2; HRESIMS *m/z* calcd for $C_{62}H_{98}N_2O_{28}Na$, $[M + Na]^+$ 1341.6200, found 1341.6170; $C_{63}H_{99}N_2O_{28}$, $[M + H]^+$ 1319.6380, found 1319.6370.

Sarasinoside M (4): yellow amorphous solid; $[\alpha]_D^{20}$ −7.6 (*c* 0.5, MeOH); UV (MeOH) λ_{max} 240; ^{1}H and ^{13}C NMR, see Table 1 and Table 2; HRESIMS *m/z* calcd for $C_{62}H_{99}N_2O_{27}$, $[M + H]^+$ 1303.6435, found 1303.6430.

Acid Hydrolysis and GC Analysis.[23,24] A solution of **1** (3.0 mg) was hydrolyzed by treatment with 3 N HCl and stirred at 80 °C for about 5 h. The solution was concentrated using N_2. Upon drying with a flow of nitrogen, the residue was redissolved in (−)-2-butanol (0.5 mL) and one drop of trifluoroacetic acid. The reaction solution was transferred to an ampule, which was sealed and heated at 130 °C in an oven overnight until butanolysis was complete. After the reaction was complete, the solution was taken to dryness and the resulting residue was further reacted with hexamethyldisilazane−chlorotrimethylsilane−pyridine (0.1 mL, 1:1:5) for 30 min at room temperature. The solution was then centrifuged, and the supernatant (1 μL) was analyzed by GC using a HP-5 column. The injection port and detector temperatures were set at 200 and 220 °C, respectively. A temperature gradient from 135 to 200 °C at 1 °C/min was applied. The retention times of the hydrolysate were detected and were in agreement with those of the authentic standards: D-xylose (27.00 and 31.69 min), D-NAc-galactosamine (37.99 and 41.85 min), D-NAc-glucosamine (32.72 and 44.09 min), and D-glucose (41.91 and 47.57 min). Authentic standards were prepared in a similar manner from commercially available D- and L-isomers. For the hydrolysate, the following peaks were detected: 26.95 and 31.67 min for D-xylose; 37.98 and 41.84 min for D-NAc-galactosamine; 32.73 and 44.08 min for D-NAc-glucosamine; and 41.84 and 47.52 min for D-glucose.

Agar Diffusion Assays. Susceptibility disks (5 mm in diameter) were impregnated with 10 μg of the isolated compounds dissolved in MeOH and placed on LB agar plates inoculated with the test bacteria *B. subtilis* DSM2109 and *E. coli* DSM10290. The plates were observed for zones of inhibition after 24 h of incubation at 37 °C. The compounds were also assayed using *S. cerevisiae* as test organism, which was inoculated on YPD agar plates, and zones of inhibition were recorded after 24 h of incubation at 27 °C. In all cases, for the controls containing only the respective amount of solvent, no zones of inhibition were observed.

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