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New Luffariellolide Derivatives from the Indonesian Sponge *Acanthodendrilla* sp.

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Investigation of the Indonesian sponge *Acanthodendrilla* sp. afforded five new luffariellolide-related sesterterpenes, acantholides A−E (**1**−**5**), in addition to luffariellolide and its 25-*O*-methyl and 25-*O*-ethyl derivatives. All structures were unambiguously established by 1D and 2D NMR and MS spectroscopy. Acantholide D and E are derivatives comprising the 1-acetylcyclopentan-5-ol moiety, which are new variants of the C_{14}−C_{20} segment for this type of linear sesterterpenes. Luffariellolide and its 25-*O*-methyl congener as well as acantholide E (**5**) were cytotoxic against the mouse lymphoma L5187Y cell line. Acantholide B (**2**), luffariellolide, and its 25-*O*-methyl congener were active against the Gram-positive bacteria *Staphylococcus aureus* and *Bacillus subtilis*, the Gram-negative bacterium *Escherichia coli*, the yeast *Candida albicans*, and the plant pathogenic fungus *Cladosporium herbarum*.

In the course of our search for biologically active compounds from Indo-Pacific marine sponges, a bioassay-guided fractionation of the crude alcoholic extract of the sponge *Acanthodendrilla* sp. led to the selection of the *n*-hexane-soluble portion of the extract for further isolation work. The *n*-hexane fraction showed 80% mortality in the brine shrimp assay at a concentration of 5 ppm and at a concentration of 10 μg exhibited inhibition zones at 10, 15, 10, and 12 mm against *S. aureus*, *B. subtilis*, *E. coli*, and *C. albicans*, respectively, in the agar plate diffusion assay.

A literature survey[1] showed that currently only one study on sponges of the genus *Acanthodendrilla* has been published. This latter sponge sample had been collected from the Sea of Japan and yielded sulfated sterols that exhibited strong biological activity against the yeast *Saccharomyces cerevisiae*.[2] The undescribed sponge *Acanthodendrilla* sp. collected in Indonesia, which was investigated in this study, yielded a series of linear sesterterpenes structurally related to luffariellolide. The compounds isolated include the known compound luffariellolide (**6**),[3] two luffariellolide derivatives (**7** and **8**), the latter being the 25-*O*-methyl- and 25-*O*-ethyl derivatives of **6**, and five new sesterterpene congeners for which we propose the names acantholides A (**1**), B (**2**), C (**3**), D (**4**), and E (**5**). Luffariellolide is a sesterterpene first isolated from the Palauan sponge *Luffariella* sp.[3] and has also been reported to be present in sponges belonging to the genus *Fascaplysinopsis*.[4] It possesses anti-inflammatory activity through reversible inhibition of phospholipase-A_2.[5] Besides the genus *Luffariella*,[6−10] linear sesterterpenoids structurally related to **6** have also been isolated from sponges belonging to other genera of the family Thorectidae, such as *Hyrtios*,[11−13] *Thorecta*,[14] *Thorectandra*,[15] *Fasciospongia*,[16] and *Cacospongia*,[17−19] and most recently from the genus

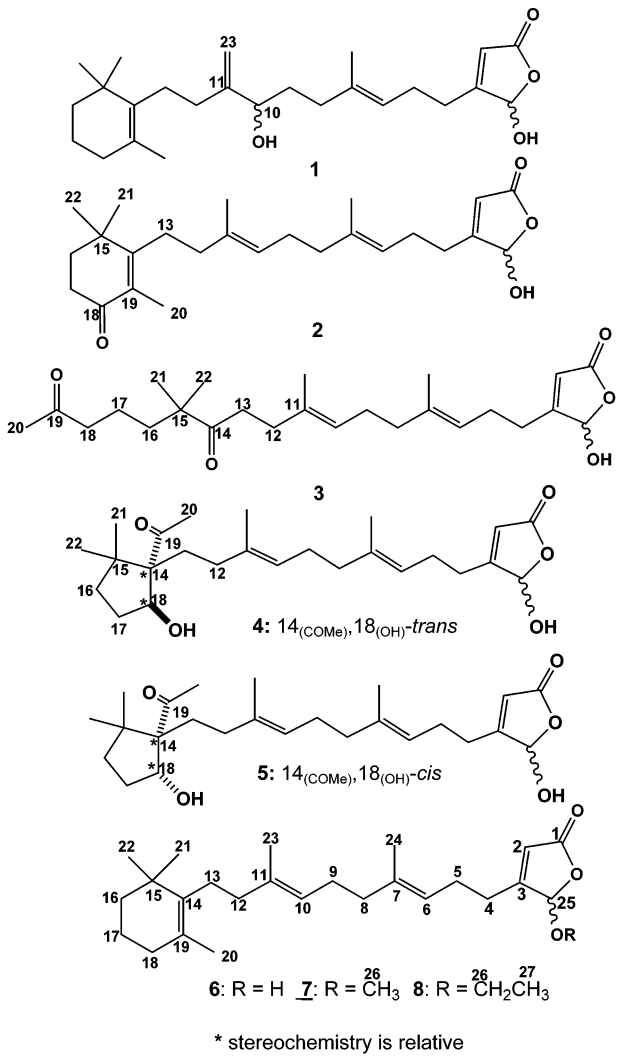

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Table 1. ^{1}H and ^{13}C NMR Data of Acantholide A (**1**) and B (**2**) Obtained in CD_3OD (δ in ppm)

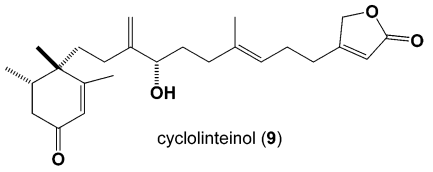
	1				**2**			
	δ_H (#H, m)	δ_C		HMBC (δ_H to δ_C)	δ_H (#H, m)	δ_C		HMBC (δ_H to δ_C)
1		173.6	qC			173.5	qC	
2	5.88 (1H, s)	117.9	CH	C-1, C-3, C-25	5.97 (1H, br s)	117.9	CH	C-1, C-4
3		172.1	qC			171.2	qC	
4	2.41 (1H, m) 2.52 (1H, m)	28.8	CH_2	C-3, C-5	2.40 (1H, m) 2.49 (1H, m)	28.7	CH_2	C-2, C-3, C-5
5	2.33 (2H, m)	26.1	CH_2	C-3, C-4	2.31−2.35 (2H, m)	26.2	CH_2	C-3, C-4
6	5.20 (1H, m)	124.1	CH	C-4, C-5, C-8, C-24	5.22 (1H, m)	124.2	CH	C-8, C-23
7		137.8	qC			137.7	qC	
8	2.03−2.10 (2H, m)	43.2	CH_2	C-6, C-10, C-11	2.05 (2H, m)	40.5	CH_2	C-6, C-7, C-9, C-24
9	2.07−2.15 (2H, m)	32.6	CH_2	C-7, C-11	2.14 (2H, m)	27.4	CH_2	C-8, C-11, C-10, C-23
10	4.11 (1H, m)	75.7	CH	C-8, C-11, C-12, C-23	5.17 (1H, m)	125.8	CH	C-9, C-12
11		153.8	qC			136.4	qC	
12	2.10−2.15 (2H, m)	36.8	CH_2	C-10, C-14	2.08−2.11 (2H, m)	39.7	CH_2	C-10, C-11, C-23
13	2.15−2.25 (2H, m)	28.7	CH_2	C-11, C-14	2.31−2.35 (2H, m)	31.4	CH_2	C-12, C-14, C-15, C-16,[a] C-19, C-20,[a] C-21[a]
14		138.2	qC			167.9	qC	
15		36.0	qC			36.7	qC	
16	1.48 (2H, m)	41.0	CH_2	C-18, C-15, C-20, C-21	2.46 (2H, t, J = 6.9 Hz)	38.4	CH_2	C-17, C-18, C-15
17	1.67 (2H, m)	20.6	CH_2	C-15, C-19, C-20, C-21	1.82 (2H, t, J = 6.9 Hz)	35.1	CH_2	C-16, C-18, C-19
18	1.98 (2H, m)	33.7	CH_2	C-14, C-16, C-17, C-19		201.4	qC	
19		128.4	qC			128.5	qC	
20	1.64 (3H, s)	20.1	CH_3	C-14, C-19	1.74 (3H, s)	11.8	CH_3	C-14, C-18, C-19
21	1.08 (3H, s)	29.1	CH_3	C-14, C-15, C-16, C-21	1.18 (3H, s)	27.2	CH_3	C-14, C-15, C-16
22	1.10 (3H, s)	29.1	CH_3	C-14, C-15, C-16, C-20	1.18 (3H, s)	27.2	CH_3	C-14, C-15, C-16
23	4.87 (1H, br s) 5.00 (1H, br s)	109.7	CH_2	C-10, C-11, C-12	1.65 (3H, s)	16.0	CH_3	C-10, C-11, C-12
24	1.61 (3H, s)	16.2	CH_3	C-6, C-7	1.68 (3H, s)	16.2	CH_3	C-6, C-7, C-8
25	5.98 (1H, br s)	101.0	CH	C-1, C-2, C-3	5.85 (1H, br s)	101.0	CH	C-1, C-2

[a] Four-bond coupling.

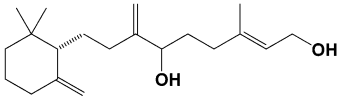
Sarcotragus[20] belonging to the family Irciniidae. The new sponge *Acanthodendrilla* sp. belongs to the family Dictyodendrillidae.

Acantholide A (**1**) was isolated as a yellow oily residue, which showed a (+)FABMS pseudomolecular ion peak at m/z 425 $[M + Na]^+$. Significant fragment ions at m/z 385 $[M + H - H_2O]^+$ and 367 $[M + H - 2H_2O]^+$ suggested the presence of two hydroxyl functions in the molecule. Compound **1** had a 16 mass unit difference from compound **6**. The base peak at m/z 137 suggested the presence of a cyclohexene moiety as in **6**. Through HRESIMS, **1** was established to have the molecular formula $C_{25}H_{38}O_4$. The ^{1}H and ^{13}C NMR spectra (Table 1) were comparable to those of **6** and implied that both compounds possessed identical terminal units, which included a cyclohexene unit and a γ-hydroxybutenolide moiety. The ^{13}C signals at δ_C 138.2 (C-14), 36.0 (C-15), 41.0 (C-16), 20.6 (C-17), 33.7 (C-18), 128.4 (C-19), 20.1 (Me-20), and 29.1 (Me-21/22) indicated the presence of a 2,6,6-trimethylcyclohexene moiety,[3,21,22] which coincided with the base peak at m/z 137, which is characteristic of the polyalkylated-cyclohexene moiety encountered in manoalide-related sesterterpenes.[23] The ^{13}C signals at δ_C 173.6 (C-1), 117.9 (C-2), 172.1 (C-3), and 101.0 (C-25), which corresponded to ^{1}H signals at δ_H 5.88 (1H, s, H-2) and 5.98 (1H, br s, H-25), indicated the presence of the γ-hydroxybutenolide moiety, which is also encountered in luffariellolide,[3] luffariolides,[6,9] manoalide,[24] luffariellins,[8,25] cacospongiolides,[18,19] fasciospongides,[16] and sarcotins.[20] The γ-hydroxybutenolide moiety was elucidated from HMBC data, including a correlation of the olefinic methine proton at δ_H 5.88 (C*H*-2) with the carbonyl at δ_C 173.6 (C-1) and the methine carbon at 101.0 ppm (C-25), as well as the correlation of the corresponding oxymethine proton (H-25) at 5.98 ppm with the carbonyl signal. The major differences from the signals in **6** were discernible in the segment of the methyl-substituted olefins. The signals for one such olefin were absent, and instead, additional signals were observed at δ_H 4.11 (1H, m), 4.87 (1H, br s), and 5.00 (1H, br s), which were in accordance with a new set of signals in the ^{13}C NMR spectrum. The ^{13}C NMR and DEPT data confirmed the presence of an exomethylene functionality in **1** from the characteristic methylene signal at 109.7 ppm (*C*H_2-23) and its respective quaternary carbon at 153.8 ppm (C-11). The HMQC correlations of the two signals at δ_H 4.87 and 5.00 to the carbon at 109.7 ppm also corroborated this assignment. The occurrence of an oxygenated methine carbon was deduced from the carbon resonance at 75.7 ppm (C-10). From the HMBC spectrum of **1**, it was shown that the oxymethine function was allylic to the exomethylene unit, as the olefin proton signals at δ_H 4.87 and 5.00 correlated with the methine carbon at 75.7 ppm. The location of this grouping adjacent to the cyclohexene moiety was confirmed through HMBC correlations between the exomethylene protons and the methylene carbon at 36.8 ppm (*C*H_2-12), which in turn correlated with a quaternary carbon signal at 138.2 ppm (C-14) of the cyclohexene system. In addition, the oxymethine proton signal at δ_H 4.11 (C*H*-10) showed correlations with the two methylenes at δ_C 36.8 and 43.2 for *C*H_2-12 and *C*H_2-8, respectively. Accordingly, the substructure for the C_8−C_{12} region was established through its ^{1}H−^{1}H COSY data. Comparison of the proton and carbon chemical shifts of the C_3−C_{12} region with those of cyclolinteinol (**9**)[17] and trixagodiol B (**10**)[26] afforded further confirmation for this substructure. Thus, the structure of **1** was unambiguously determined, and the name acantholide A is proposed for this new natural product. It was not possible to ascertain the absolute configuration of C-10 and C-25 from the very small amount of compound isolated.

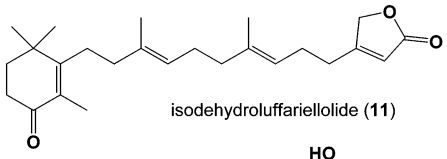
Acantholide B (**2**) was isolated as a yellow oily residue with UV_{max} absorbances at 202.5 and 252.1 nm, which signified the presence of an additional conjugated system when compared to those of the known luffariellolide derivatives. The (+)FABMS of **2** showed a pseudomolecular ion peak at m/z 423 $[M + Na]^+$. The significant fragment ions obtained were different from those of the luffari-



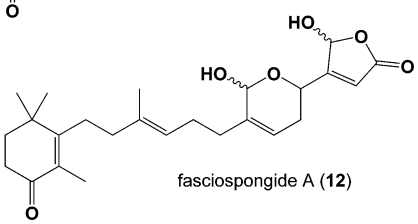
cyclolinteinol (**9**)



trixagodiol B (**10**)



isodehydroluffariellolide (**11**)



fasciospongide A (**12**)

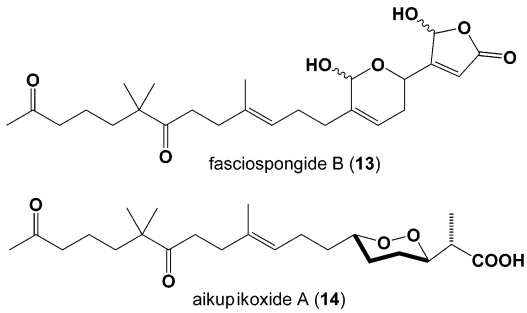
ellolides and the base peak at *m*/*z* 137 was not present, which attested the loss of the cyclohexene moiety. Through (+)HRESIMS, **2** had a molecular formula of $C_{25}H_{36}O_4$, which required 8 degrees of unsaturation instead of 7 in **1**. However, the ^{1}H and ^{13}C NMR spectra (Table 1) were comparable to those of luffariellolide (**6**). The occurrence of a γ-hydroxybutenolide moiety was also observable. Two sets of methyl monosubstituted olefinic carbons[21] were in agreement with the ^{13}C signals at δ_C 124.2 (*C*H-6), 137.7 (C-7), 16.2 (*C*H$_3$-24), 125.8 (*C*H-10), 136.4 (C-11), and 16.0 (*C*H$_3$-23) together with the ^{1}H signals at δ_H 5.22 (C*H*-6), 1.68 (C*H*$_3$-24), 5.17 (C*H*-10), and 1.65 (C*H*$_3$-23). From its ^{1}H−^{1}H COSY spectrum, typical allylic couplings of the olefinic methines at δ_H 5.22 and 5.17 with the respective methyl signals at δ_H 1.68 and 1.65 were discernible. Both the COSY and HMBC data indicated that the double bonds were not conjugated to each other. The COSY spectrum showed correlations of the olefinic methines with the adjacent methylenes resonating at δ_C 26.2 (C-5) and 27.4 (C-9), respectively. Similarly, the olefinic methyl protons and their corresponding olefinic methines correlated further with methylene signals at δ_C 40.5 (C-8) and 39.7 (C-12), respectively. The major difference was the absence of the 2,6,6-trimethylcyclohexene moiety in **2**. An additional aliphatic carbonyl signal at δ_C 201.4 (C-18) correlated with an olefinic methyl singlet at δ_H 1.74 (C*H*$_3$-20) and a methylene group at δ_H 1.82 (C*H*$_2$-16, t, J = 6.9 Hz) in its HMBC spectrum. The latter was part of an isolated CH$_2$−CH$_2$ spin system, as indicated by their signal multiplicities and as observed from the ^{1}H−^{1}H COSY spectrum. The HMBC spectrum of **2** also showed correlations of the geminal methyl groups at δ_H 1.18 (C*H*$_3$-21/22) with the quaternary carbons at δ_C 167.9 (C-14) and 36.7 (C-15) and the methylene carbon at δ_C 38.4 (C-16). The deshielding effect on C-14 was explained by the presence of an α,β-unsaturated carbonyl unit in which the carbonyl function could only be situated at C-18. This was confirmed from the correlation of the methyl signal at δ_H 1.74 with the quaternary carbons at δ_C 201.4 (C-18), 128.5 (C-19), and 167.9 (C-14). These resonances were characteristic of a trimethylcyclohexenone moiety and were comparable to those found in isodehydroluffariellolide (**11**)[4] and fasciospongide A (**12**).[16] The connectivity of the cyclohexenone moiety with that of the C_1−C_{13} region was established through the HMBC correlations of C*H*$_2$-13 with the quaternary carbons C-14, C-15, and C-19 and the four-bond couplings to C-16 and C-21/22. This unambiguously led to the elucidation of structure **2**, which was named acantholide B.

Acantholide C (**3**) was isolated as a pale yellowish oily residue that showed a (+)FABMS pseudomolecular ion peak at *m*/*z* 441 [M + Na]$^+$. The significant fragment ions were again different from those of **6**, and as in acantholide B, the base peak at *m*/*z* 137 was not detected, which suggested the absence of the cyclohexene moiety. The molecular formula of **3** was established as $C_{25}H_{38}O_5$ through HRESIMS. The ^{1}H and ^{13}C NMR data of **3** (Table 2) and **6** were similar for the C_1−C_{11} region. The signals associated with the cyclohexene or cyclohexenone moiety of **1** and **2** were not evident; instead new signals at δ_C 217.3 (s), 211.4 (s), and 49.5 (s) were observed. The presence of the two aliphatic carbonyl signals together with a signal for a methyl ketone residue (δ_C 29.9 and δ_H 2.16) suggested the presence of an open-chain dicarbonyl moiety,[16] which could be derived from oxidative opening of a cyclohexene ring[27] at the C-14−C-19 olefinic bond. This was also compatible with the 32 mass unit difference in the molecular weight of **3** compared to luffariellolide (**6**). The ^{1}H−^{1}H COSY and HMQC data allowed the unambiguous assignment of two spin systems which consisted of two and three methylene units, respectively. One methylene group of each unit was adjacent to a ketone function, as implied by their chemical shifts of δ_H 2.60 and 2.45 for C*H*$_2$-13 and C*H*$_2$-18, respectively. The first spin system consisted of the methylenes at δ_H 2.17 (C*H*$_2$-12) and 2.60 (C*H*$_2$-13). The HMBC spectra confirmed this system through correlations with the carbonyl at δ_C 217.3 (C-14). Moreover, the correlation of δ_H 1.65 (C*H*$_3$-23) with δ_C 34.6 (*C*H$_2$-12) and of C*H*$_2$-12 (δ_H 2.17) with the olefinic methine carbon at δ_C 124.7 (C-10) allowed the extension of the C_1−C_{11} skeleton to C_1−C_{13}. The second spin system consisted of the methylenes at δ_H 1.49 (C*H*$_2$-16), 1.38 (C*H*$_2$-17), and 2.45 (C*H*$_2$-18). An HMBC experiment also confirmed this system through correlations of C*H*$_2$-17 and C*H*$_2$-18 with the carbonyl signal at δ_C 211.4 (C-19). The same spectrum allowed the assignment and positioning of the remaining methyl groups. The geminal dimethyl groups (C*H*$_3$-21/22) at δ_H 1.10 (s, 6H) correlated with the carbonyl function at C-14 and C*H*$_2$-16, which suggested the attachment of C*H*$_3$-21/22 at C-15. The methyl singlet at δ_H 2.16 (C*H*$_3$-20) implied that this was the terminus, as it showed a cross-peak with *C*H$_2$-18 and the carbonyl at C-19. The ^{1}H and ^{13}C NMR resonances for the segment C_{11}−C_{20} were similar to those found in fasciospongide B (**13**)[16] and aikupikoxide A (**14**),[27] the latter being sesterterpenes isolated from *Fasciospongia* sp. and *Diacarnus erythraenus*, respectively. By comparison with the literature data together with the data obtained from the ^{1}H−^{1}H COSY, HMQC, and HMBC spectra, the structure of **3** was unambiguously elucidated and named acantholide C.

Acantholide D (**4**) was isolated as a colorless oil and showed a (+)FABMS pseudomolecular ion peak at *m*/*z* 441 [M + Na]$^+$. Diagnostic fragment ions at *m*/*z* 401 [M + H − H$_2$O]$^+$ and 383 [M + H − 2H$_2$O]$^+$ were observed, which implied the presence of two hydroxyl functions in the molecule. (+)HRESIMS established the molecular formula $C_{25}H_{38}O_5$ for **4**. The ^{1}H and ^{13}C NMR data (Table 2) were again comparable to **6** and **3**, which showed similar resonances for the region C_1−C_{12}. However, signals for the

Table 2. NMR Data of Acantholide C (**3**), D (**4**), and E (**5**) in CD_3OD (δ in ppm)

	3				**4**				**5**			
	δ_H (#H, m)	δ_C		HMBC (δ_H to δ_C)	δ_H (#H, m)	δ_C		HMBC (δ_H to δ_C)	δ_H (#H, m)	δ_C		HMBC (δ_H to δ_C)
1		173.6	qC			173.6	qC			173.6	qC	
2	5.87 (1H, br s)	117.9	CH	C-1, C-3, C-25	5.86 (1H, s)	117.9	CH	C-1, C-25	6.01 (1H, s)	117.4	CH	C-25
3		172.1	qC			171.7	qC			171.2	qC	
4	2.45 (1H, m) 2.55 (1H, m)	28.7	CH_2	C-3, C-5	2.49, 2.39 (2H, m)	28.7	CH_2	C-3	2.41 (1H, m)	28.7	CH_2	C-5
5	2.33 (2H, m)	26.2	CH_2	C-3, C-4, C-6	2.33 (2H, m)	26.2	CH_2	C-4, C-6, C-7	2.36 (2H, m)	26.2	CH_2	C-4, C-6
6	5.16 (1H, m)	124.1	CH	C-7, C-8, C-24	5.12 (1H, m)	124.0	CH	C-8, C-24	5.16 (1H, m)	124.1	CH	C-8, C-4, C-24
7		137.6	qC			137.8	qC			137.8	qC	
8	2.01 (2H, m)	40.5	CH_2	C-6, C-7, C-9, C-24	2.03 (2H, m)	40.7	CH_2	C-6, C-7	2.05 (2H, m)	40.6	CH_2	C-9, C-24
9	2.08 (2H, m)	27.4	CH_2	C-7, C-8, C-10, C-11	2.15 (2H, m)	27.4	CH_2	C-7, C-10, C-11	2.13 (2H, m)	27.4	CH_2	C-7, C-8
10	5.11 (1H, m)	124.7	CH	C-11, C-12, C-23	5.17 (1H, m)	124.9	CH	C-13, C-23	5.12 (1H, m)	125.4	CH	C-12, C-23
11		136.4	qC			137.3	qC			136.6	qC	
12	2.17 (2H, m)	34.6	CH_2	C-10, C-11, C-13, C-14, C-23	A 1.75 (m)	37.2	CH_2	C-11, C-13, C-21	A 1.88 (m)	36.7	CH_2	C-11, C-13
					B 2.40 (m)				B 2.18 (m)			
13	2.60 (2H, m)	36.7	CH_2	C-11, C-12, C-14	A 1.86 (m)	29.2	CH_2	C-12, C-14, C-16, C-19, C-22	A 1.62 (m)	32.2	CH_2	C-12, C-14
					B 2.49 (m)				B 1.98 (m)			
14		217.3	qC			67.8	qC			67.5	qC	
15		49.5	qC			44.8	qC			45.1	qC	
16	1.49 (2H, m)	40.3	CH_2	C-14, C-15, C-17, C-21, C-22	A 1.41 (m)	39.0	CH_2	C-14, C-15, C-17, C-21, C-22	A 1.56 (m)	39.8	CH_2	C-14, C-15, C-17, C-18
					B 1.68 (m)				B 1.81 (m)			
17	1.38 (2H, m)	20.0	CH_2	C-18	A 1.41 (m)	32.1	CH_2		A 1.70 (m)			
					B 2.20 (m)				B 2.15 (m)	33.8	CH_2	C-14, C-15
18	2.45 (2H, m)	44.3	CH_2	C-16, C-17, C-19	4.72 (1H,dd, J = 5.7, 8.2 Hz)	76.1	CH	C-15, C-17	4.31 (1H, dd, J = 5.0, 8.2 Hz)	81.5	CH	C-14, C-16, C-19
19		211.4	qC			215.5	qC				216.7	qC
20	2.16 (3H, s)	29.9	CH_3	C-18, C-19	2.24 (3H, s)	29.8	CH_3	C-14, C-19	2.25 (3H, s)	30.9	CH_3	C-15, C-19
21	1.10 (3H, s)		24.7	CH_3	C-14, C-15, C-16, C-22			0.93 (3H, s)		26.3	CH_3	C-14, C-15, C-16,
C-22	1.10 (3H, s)		26.9	CH_3 C-22	C-14, C-15, C-16,							
22	1.10 (3H, s)	24.7	CH_3	C-14, C-15, C-16, C-21	1.12 (3H, s)	27.6	CH_3	C-14, C-15, C-16, C-21	1.12 (3H, s)	27.5	CH_3	C-14, C-15, C-16, C-21
23	1.65 (3H, s)	16.2	CH_3	C-10, C-11, C-12,	1.61 (3H, s)	16.1	CH_3	C-10, C-11, C-13	1.65 (3H, s)	16.1	CH_3	C-10, C-11, C-12
24	1.68 (3H, s)	16.2	CH_3	C-6, C-7, C-8	1.63 (3H, s)	16.2	CH_3	C-6, C-7	1.69 (3H, s)	16.2	CH_3	C-6, C-7, C-8
25	5.99 (1H, br s)	101.0	CH	C-2, C-3	5.98 (1H, s)	101.5	CH	C-1, C-2	5.86 (1H, s)	101.5	CH	C-1, C-2



cyclohexene moiety were not detected for **4**. In its ^{1}H NMR spectrum, there was a new signal at δ_H 4.72 (1H, br s) that indicated the presence of an additional hydroxyl group, and this was compatible with the fragment ions at *m*/*z* 401 and 383. The ^{13}C NMR spectrum showed signals for 25 carbons as in **6** and **3** with the appearance of three new signals at δ_C 215.5 (s), 76.1 (d), and 67.8 (s). As in **3**, a methyl ketone signal was observed at δ_H 2.24 (CH_3-20) in accordance with the methyl carbon at δ_C 29.8. From its ^{1}H−^{1}H COSY spectrum, the new spin system replacing the cyclohexene unit in **6** could be unambiguously assigned. The hydroxymethine signal at δ_H 4.72 (C*H*-18) showed correlations with the signals at δ_H 1.41 and 2.20 for CH_2-17, and these correlated with the methylene signals at δ_H 1.68 and again at 1.41 for CH_2-16. This spin system was also validated by the correlations observed in the HMBC spectrum. The presence of a geminal dimethyl functionality was evident from the correlations of the methyl resonances at δ_H 0.93 and 1.12 with their corresponding carbons in addition to a similar set of correlations with the neighboring carbons. The position of the geminal dimethyl group was assigned at C-15 from the correlations to *C*H$_2$-16 and the quaternary carbons at δ_C 67.8 (C-14) and 44.8 (C-15). The deshielded CH$_3$-20 at δ_H 2.24 correlated with the carbonyl at δ_C 215.5 (C-19) and also with *C*-14, which further suggested that the keto methyl unit was adjacent to the geminal dimethyl group. The acetyl function at C-14 was attached with segment C_1−C_{13}, as shown from the correlation of CH_2-13 with *C*-14. The attachment of the hydroxyl group was at C-18, as the oxymethine signal at δ_H 4.31 showed cross-peaks with *C*H$_2$-16, *C*-14, and C-19, which indicated that the hydroxyl substituent was vicinal to the keto methyl function.

The presence of a substituted cyclopentane unit was inferred from correlations for C-14 to C-20. The coupling constants of 5.7 and 8.2 Hz of the oxymethine proton at δ_H 4.72 also implied the respective *trans* and *cis* couplings typically occurring in such a ring system.[28−30] Accordingly, the ^{13}C NMR resonances at δ_C 215.5 (s, C-19), 76.1 (d, C-18), 67.8 (s, C-14), and 29.8 (q, C-20) were compatible with those of a 1-acetylcyclopentan-5-ol moiety found in covilanone (**15**) with resonances at 213.9 (s), 72.6 (d), 70.0 (s), and 30.4 (q) ppm, respectively. Covilanone (**15**) is a rearranged labdane type of diterpene obtained from the aerial parts of *Halimium viscosum*.[31]

Since only a small quantity of **4** was isolated, it was not possible to determine the absolute stereochemistry at C-14 and C-18. However the relative stereochemistry in the 1-acetylcyclopentan-5-ol moiety was elucidated from a ROESY spectrum. This latter spectrum showed NOEs of C*H*-18 with CH_3-21 (δ_H 0.93), one of the methylene protons of CH$_2$-16 and CH$_2$-17 (δ_H 1.68 and 2.20), and the C-20 keto methyl signal (δ_H 2.24). The NOE of H-18 with the keto methyl group indicated that the acetyl function was *trans* to the hydroxyl substituent at C-18. In accordance, the methyl group at δ_H 1.12 (CH_3-22) exhibited an NOE with

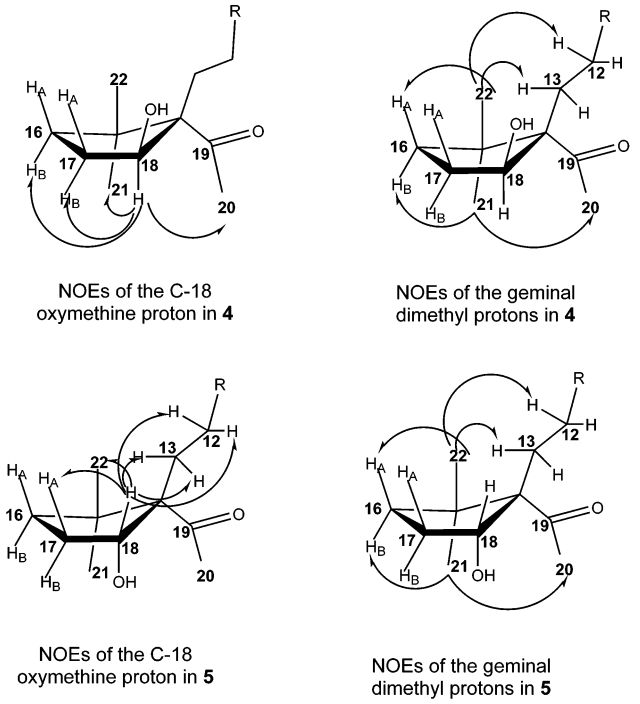


Figure 1. Important ROESY correlations in compounds **4** and **5**.

the methylene protons of C-13 and C-12. The "α" orientation of the oxymethine proton caused a deshielding effect to 4.72 ppm because of its *cis* orientation with the acetyl function. Consequently, the methyl group (CH_3-21) at C-15 was shielded to 0.93 ppm. The *trans* orientation of the hydroxyl and acetyl function in **5** was similar to that of **15**.[31]

Acantholide E (**5**) was also isolated as a colorless oily residue with a FABMS spectrum that was similar to **4**, as it showed a pseudomolecular ion peak at *m*/*z* 441 [M + Na]$^+$. Significant fragment ions were also observed at *m*/*z* 401 [M + H − H$_2$O]$^+$ and *m*/*z* 383 [M + H − 2H$_2$O]$^+$. The ^{1}H and ^{13}C specta were very similar to those of **4**. Inspection of the ^{1}H−^{1}H COSY and HMBC spectra indicated **5** had the same C_1−C_{12} skeleton (Table 2). The ^{13}C NMR resonances at δ_C 216.7, 81.5, 67.5, and 30.9 also indicated the presence of a 1-acetylcyclopentan-5-ol moiety as in **4**. When acantholide E (**5**) was compared to **4** and covilanone, a significant difference (Δ 5.4 and 8.9 ppm, respectively) in chemical shift was observed for C-18, which suggested a change in stereochemistry at this position. When compared with **4**, differences in ^{1}H and ^{13}C NMR chemical shifts were observable for the oxymethine as well as for the geminal dimethyl signals. One of these methyl groups was deshielded to δ_H 1.10, while the oxymethine proton was shielded to δ_H 4.31 (dd, J = 5.0, 8.2 Hz). This indicated that acantholide E is a diastereoisomer of **4**. H-18 showed NOE interactions with δ_H 1.88/2.18 for H-12A/B, δ_H 1.62/1.98 for H-13A/B, 1.70 (H-17A), and CH_3-22 at δ_H 1.12 (Figure 1). The NOE of the oxymethine proton to both of the methylene protons of C-12 and C-13 indicated that the C_1−C_{13} segment of the molecule was *cis* to H-18, which verified a change in stereochemistry at this position. This was also compatible with the observed NOE of CH_3-22 with CH_2-13 and of CH_3-21 at δ_H 1.10 with the keto methyl signal at δ_H 2.25.

Compound **7** was isolated as a yellowish oily residue with a UV_{max} absorbance at 214.3 nm, which was comparable to compound **6** with a UV_{max} absorbance at 214.0 nm. The FABMS of **7** showed a pseudomolecular ion peak at *m*/*z* 423 [M + Na]$^+$. A diagnostic fragment ion was observed at

m/*z* 369 [M − OCH_3]$^+$, and a base peak at *m*/*z* 137 representing the cyclohexene moiety was also present, as in **6**. Compound **7** had the molecular formula $C_{26}H_{40}O_3$ as established through (+)HRESIMS. The presence of four methines, nine methylenes, five methyl singlets, and a methoxy singlet was evident from the ^{1}H NMR spectrum, while its ^{13}C NMR spectrum showed the occurrence of 26 carbons. The ^{1}H NMR and ^{13}C NMR spectra of **7** (Table 3) were comparable to those of **6** and indicated that it had an identical carbon skeleton. The only difference between the two compounds was the appearance of an additional methoxyl group, which was positioned at C-25 from the respective correlations in the HMBC spectrum. Hence **7** was 25-*O*-methylluffariellolide.

Compound **8** was also isolated as a yellowish oily residue with a UV_{max} absorbance at 217.8 nm, which is comparable with that of **7**. The same elucidation techniques used for **7** showed that **8** was 25-*O*-ethylluffariellolide.

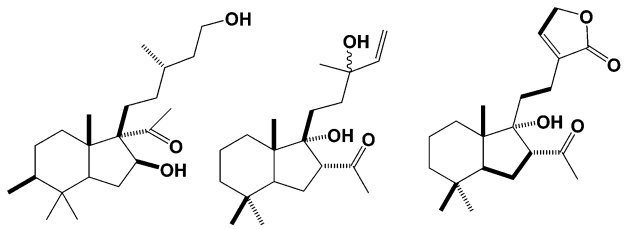
It is possible that compounds **7** and **8** were artifacts and not natural products since the sponge sample had been preserved in ethanol while the crude total extract was obtained with methanol. To examine this possibility, samples of **6** were dissolved in methanol or ethanol, respectively, and left at room temperature with stirring for 2 days. Another set of samples were incubated at 40 °C for 24 h. After treatment, the respective solvents were evaporated, the samples dried, and the ^{1}H NMR spectra recorded in DMSO-d_6. All treated samples yielded spectra that were identical with that of **6** and even showed signals for the OH function at δ_H 7.75, d, J = 7.7 Hz, together with its corresponding methine at δ_H 5.98, d, J = 7.7 Hz, at C-25. Their ^{1}H NMR spectra did not show any evidence of the 25-*O*-methyl or -ethyl congener. On the basis of these experiments, we predict that compounds **7** and **8** are probably natural products.

The sesterterpenes were tested in an agar plate diffusion assay for antimicrobial activity against *S. aureus*, *B. subtilis*, and *E. coli* at concentrations of 5 and 10 μg per disk (Table 4). Luffariellolide (**6**) was active against the Gram-positive bacteria *S. aureus* and *B. subtilis*, as well as the Gram-negative bacterium *E. coli*. Its 25-*O*-methyl congener (**7**) displayed only moderate antimicrobial activity against *S. aureus*, while 25-*O*-ethylluffariellolide (**8**) was selective toward *E. coli*. Acantholide B (**2**) was also found to be active against *S. aureus* and *B. subtilis*; however, it exhibited weak activity toward *E. coli*.

Luffariellolide (**6**) and acantholide B (**2**) were also active against the yeast *Candida albicans* at a concentration of 10 μg. Using the same concentration, 25-*O*-methylluffariellolide (**7**) and acantholide B (**2**) were found to be fungistatic toward the pathogenic plant fungus *Cladosporium herbarum*, as both compounds exhibited distinct but overcasted inhibition zones (Table 4). Luffariellolide was fungicidal toward *C. herbarum* with a clear inhibition zone of 20 mm at a disk concentration of 10 μg, while the same concentration of nystatin displayed a 30 mm inhibition zone. The MIC of luffariellolide (**6**) was 10.4 μg/mL, compared to 1.06 μg/mL for nystatin as estimated from a serial dilution assay.

The isolated sesterterpenes were assayed for their cytotoxicity toward mouse lymphoma (L5178Y), rat brain tumor (PC12), and human cervix carcinoma (Hela) cells. The compounds were not active toward the cell lines PC12 and Hela. However, only three of the congeners were found to be biologically active toward L5178Y cells (Table 4). 25-*O*-Methylluffariellolide (**7**) was more cytotoxic than luffariellolide (**6**), while its ethoxyl congener did not exhibit any cytotoxicity. Acantholide E (**5**) was also found to be active, while its diastereoisomer, acantholide D (**4**), was inactive, which suggested that the stereochemistry of 1-acetylcyclopentan-5-ol played an important role in the cytotoxicity of the compound.

Derivatives containing the 1-acetylcyclopentan-5-ol moiety, as found in acantholide D and E, are new variants for the C_{14}−C_{20} segment for this type of linear sesterterpenes. Their occurrence in nature is very rare and has never been found in any marine natural products. Hitherto, natural products with the 1-acetylcyclopentan-5-ol unit have been isolated only from terrestrial sources and include infuscadiol (**16**) from the liverwort *Jungermannia infusca*,[32] chapecoderin B (**17**) from the leaves of *Echinodorus macrophyllus*,[33] covilanone (**15**) from the aerial parts of *Halimium viscosum*,[31] and related compounds from *Gypothamnium pinifolium*[34] and *Galeopsis angustifolia*,[35] all of which were classified as labdane-derived diterpenoids.



covilanone (**15**) infuscadiol (**16**) chapecoderin B (**17**)

Experimental Section

General Experimental Procedures. Optical rotations were measured on a Perkin-Elmer model 341 LC polarimeter. UV spectra were measured in methanol on a Perkin-Elmer UV/vis lambda spectrophotometer. ^{1}H (1D, 2D COSY) and ^{13}C (1D, 2D HMBC) NMR spectra were recorded on Bruker AM 300, ARX 400, or DRX 500 NMR spectrometers. Mass spectra were recorded on a Finningan MAT TSQ-7000 mass spectrometer, while ESIMS measurements were performed on ThermoFinnigan LCQ Deca and HREIMS were obtained on a Finningan MAT 900 mass spectrometer. Solvents were distilled prior to use, and spectral grade solvents were used for spectroscopic measurements. TLC was performed on plates precoated with silica gel F_{254} (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. For semipreparative HPLC, a HPLC system (Merck, Darmstadt, Germany) coupled with UV detector L7400 (UV detection at 280 nm) was used. The separation column (250 × 8 mm, i.d.) was prepacked with Eurosphere 100 C_{18} (Knauer, Berlin, Germany). The compounds were eluted with mixtures of MeOH and H_2O at a flow rate of 5 mL/min.

Animal Material. The sponge *Acanthodendrilla* sp. belongs to the class Demospongiae, order Dendroceratida, family Dictyodendrillidae. It was collected near the coast of Kundingarengkeke Island, Indonesia, on August 1997 at a depth of 16−20 ft. It was a thick sponge with a dark amber color, and its texture was soft and to some extent compressible. A voucher specimen has been deposited in the Zoological Museum Amsterdam under the registration number ZMA POR. 16868.

Isolation. The sponge material was stored in ethanol upon collection. Prior to extraction, the sponge was freeze-dried and the ethanol macerate was concentrated. The lyophilized sponge (104.6 g) was extracted exhaustively with acetone and then with methanol. The total extract (12.4 g), which included the

Table 3. NMR Data for Luffariellolide (**6**), 25-*O*-Methoxy- (**7**), and 25-*O*-Ethoxyluffariellolide (**8**) in CD_3OD (δ in ppm)

	6			**7**				**8**			
	δ_H (#H, m)	δ_C		δ_H (#H, m)	δ_C		HMBC (δ_H to δ_C)	δ_H (#H, m)	δ_C		HMBC (δ_H to δ_C)
1		173.5	qC		172.0	qC			172.6	qC	
2	5.85 (1H, br s)	117.8	CH	5.91 (1H, br s)	118.8	CH	C-1, C-3, C-4, C-25	5.90 (1H, br s)	118.5	CH	C-1, C-4, C-3, C-25
3		171.7	qC		170.0	qC			170.3	qC	
4	2.39 (1H, m)	28.7	CH_2	2.39 (1H, m)	28.7	CH_2	C-3, C-5, C-6	2.39 (1H, m)	28.7	CH_2	C-3, C-5
	2.47 (1H, m)			2.45 (1H, m)				2.46 (1H, m)			
5	2.33 (2H, m)	26.2	CH_2	2.31 (2H, m)	26.1	CH_2	C-4, C-6, C-7	2.31 (2H, m)	26.2	CH_2	C-3, C-4, C-7
6	5.17 (1H, m)	124.0	CH	5.15 (1H, m)	124.0	CH	C-5, C-7, C-8, C-24	5.16 (1H, m)	124.0	CH	C-5, C-7, C-8, C-24
7		137.8	qC		138.2	qC			137.9	qC	
8	1.99 (2H, m)	40.6	CH_2	1.99 (1H, m)	40.7	CH_2	C-6, C-7, C-9, C-10, C-24	2.05 (2H, m)	40.7	CH_2	C-6, C-7, C-9, C-24
				2.07 (1H, m)							
9	2.11 (2H, m)	27.4	CH_2	2.11 (2H, m)	27.4	CH_2	C-7, C-10	2.11 (2H, m)	27.4	CH_2	C-7, C-10, C-11
10	5.12 (1H, m)	124.6	CH	5.12 (1H, m)	124.7	CH	C-8, C-12, C-23,	5.11 (1H, m)	124.7	CH	C-12, C-13, C-23
11		137.1	qC		137.2	qC			137.2	qC	
12	2.03 (2H, m)	41.5	CH_2	2.01−2.03 (2H, m)	41.7	CH_2	C-10, C-11, C-23	1.99−2.07 (2H, m)	41.5	CH_2	C-10, C-11, C-23
13	2.06 (2H, m)	29.5	CH_2	2.02, 2.05 (2H, m)	29.3	CH_2	C-11, C-14, C-19	2.08 (2H, m)	29.2	CH_2	C-11, C-12, C-14
14		138.2	qC		138.3	qC			138.3	qC	
15		35.9	qC		36.2	qC			35.9	qC	
16	1.42 (2H, m)	41.0	CH_2	1.43 (2H, m)	41.1	CH_2	C-14, C-17, C-21, C-22	1.48 (2H, m)	41.0	CH_2	C-15, C-18, C-21, C-22
17	1.57 (2H, m)	20.6	CH_2	1.57 (2H, m)	20.1	CH_2	C-15, C-18, C-19	1.67 (2H, m)	20.6	CH_2	C-15, C-18, C-19
18	1.91 (2H, m)	33.7	CH_2	1.91 (2H, m)	33.7	CH_2	C-14, C-19, C-20	1.90 (2H, m)	33.7	CH_2	C-14, C-16, C-20
19		128.1	qC		128.1	qC			128.1	qC	
21	0.99 (3H, s)	29.1	CH_3	0.99 (3H, s)	29.2	CH_3	C-14, C-15, C-16	0.99 (3H, s)	29.2	CH_3	C-14, C-15, C-16
22	0.99 (3H, s)	29.1	CH_3	0.99 (3H, s)	29.2	CH_3	C-14, C-15, C-16	0.99 (3H, s)	29.2	CH_3	C-14, C-15, C-16, C-21
20	1.60 (3H, s)	20.5	CH_3	1.60 (3H, s)	20.6	CH_3	C-14, C-15, C-16	1.60 (3H, s)	20.6	CH_3	C-14, C-15, C-16
23	1.63 (3H, s)	16.1	CH_3	1.63 (3H, s)	16.1	CH_3	C-10, C-11, C-12	1.63 (3H, s)	16.1	CH_3	C-10, C-11, C-12
24	1.65 (3H, s)	16.2	CH_3	1.65 (3H, s)	16.2	CH_3	C-6, C-7, C-8	1.65 (3H, s)	16.2	CH_3	C-6, C-7, C-8
25	5.98 (1H, br s)	101.0	CH	5.78 (1H, br s)	106.3	CH	C-2, C-26	5.85 (1H, br s)	105.6	CH	C-1, C-2, C-26
26				3.54 (3H, s)	57.3	CH_3	C-25	3.67 (1H, dq, J = 9.8, 7.2 Hz)	67.1	CH_2	C-27, C-25
								3.77 (1H, dq, J = 9.8, 7.2 Hz)			
27								1.26 (3H, t, J = 7.2 Hz)	15.5	CH_3	C-26

Table 4. Biological Activity of Some of the Isolated Luffariellolide Derivatives[a]

compound no.	observed zones of inhibition (in mm) from the agar plate diffusion assay										cytotoxicity assay with L5178Y mouse lymphoma cells (ED_{50})
	S. aureus		*B. subtilis*		*E. coli*		*C. albicans*		*C. herbarum*		
	5 μg	10 μg	5 μg	10 μg	5 μg	10 μg	5 μg	10 μg	5 μg	10 μg	
2	n.a.	10	n.a.	12	n.a.	9	n.a.	10	n.a.	10	>10 μg/mL
4	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	>10 μg/mL
5	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	7 μg/mL
6	7	11	7	12	7	10	n.a.	10	n.a.	20	3.3 μg/mL
7	n.a.	10	7	9	n.a.	n.a.	9	9	n.a.	15	0.7 μg/mL
8	n.a.	n.a.	n.a.	7	n.a.	12	n.a.	9	n.a.	n.a.	>100 μg/mL

[a] n.a. (not active), n.t. (not tested).

ethanol concentrate, was evaporated to dryness and partitioned between aqueous MeOH and the following organic solvents: hexane, EtOAc, and BuOH. The biological activities of the extracts were tested for brine shrimp lethality and antimicrobial activity. The hexane extract was chosen for further isolation work, as it displayed strong antimicrobial activity in the agar diffusion assay as well as being lethal in the brine shrimps assay. The *n*-hexane extract (4.2 g) was subjected to normal-phase silica gel column chromatography and eluted with *n*-hexane and EtoAc (7:3). The first nonpolar fraction afforded the methoxy- (**7**, 4 mg) and ethoxyluffariellolide (**8**, 3 mg), respectively. The second fraction yielded the known compound luffariellolide (**6**, 13 mg), which showed a single spot on TLC with an R_f of 0.56 (*n*-hexane/EtoAc, 7:3) as well as a pure peak in the HPLC chromatogram. Acantholides A (**1**, 3.4 mg), B (**2**, 2.7 mg), C (**3**, 3.1 mg), D (**4**, 2.3 mg), and E (**5**, 2.8) were isolated from the more polar fractions. Further purification of the compounds was accomplished by semipreparative HPLC.

Acantholide A (1): yellowish oily residue; $[\alpha]_D$ −20.8° (*c* 1.0, CH_3Cl); UV λ_{max} (MeOH) 222 nm; ^1H and ^{13}C NMR data, see Table 1; (+)FABMS *m/z* 425 $[M + Na]^+$ (15), 385 $[M + H - H_2O]^+$ (12), 203 (16), 137 (100) 95 (74), 55 (61); (+)HREIMS *m/z* 402.2675 $[M]^+$ (calcd for $C_{25}H_{38}O_4$, 402.2770); (+)HRESIMS *m/z* 420.2885 $[M + H_2O]^+$ (calcd for $C_{25}H_{40}O_5$, 420.2876), *m/z* 822.5652 $[2M + H_2O]^+$ (calcd for $C_{50}H_{78}O_9$, 822.5646).

Acantholide B (2): yellow oily residue; $[\alpha]_D$ −44.5°(*c* 1.0, CH_3Cl); UV λ_{max} (MeOH) 203 and 252 nm; ^1H and ^{13}C NMR data, see Table 1; (+)FABMS *m/z* 423 $[M + Na]^+$ (14), 383 $[M + H - H_2O]^+$ (8), 289 (12), 176 (36) 154 (100), 95 (78), 55 (52); (+)HRESIMS *m/z* 423.2518 $[M + Na]^+$ (calcd for $C_{25}H_{36}NaO_4$, 423.2511).

Acantholide C (3): faint yellow oily residue; $[\alpha]_D$ −58.2° (*c* 1.0, CH_3Cl); UV λ_{max} (MeOH) 210 nm; ^1H and ^{13}C NMR data, see Table 2; (+)FABMS *m/z* 441 $[M + Na]^+$ (100), 383 (11), 237 (15), 176 (47) 136 (83), 69 (82), 55 (53); (+)ESIMS *m/z* 419 $[M + H]^+$; (−)ESIMS *m/z* 417 $[M - H]^+$, *m/z* 835 $[2M - H]^+$; (+)HRESIMS *m/z* 441.2615 $[M + Na]^+$ (calcd for $C_{25}H_{38}NaO_5$, 441.2617); *m/z* 450.2982 $[M + CH_3OH]^+$ (calcd for $C_{26}H_{42}O_6$, 450.2981).

Acantholide D (4): colorless oil; $[\alpha]_D$ −21.6° (*c* 1.0, CH_3Cl); UV λ_{max} (MeOH) 213 nm; ^1H and ^{13}C NMR data, see Table 2; (+)FABMS *m/z* 441 $[M + Na]^+$ (27), 419 $[M + H]^+$ (15), 401 $[M + H - H_2O]^+$ (4), 383 $[M + H - 2H_2O]^+$ (5), 307 (8), 289 (9), 176 (38), 154 (100), 107 (54), 77 (53); (+)HRESIMS *m/z* 419.2789 $[M + H]^+$ (calcd for $C_{25}H_{39}O_5$, 419.2798); *m/z* 441.2622 $[M + Na]^+$ (calcd for $C_{25}H_{38}NaO_5$, 441.2617).

Acantholide E (5): colorless oil; $[\alpha]_D$ −41.2° (*c* 1.0, CH_3Cl); UV λ_{max} (MeOH) 212 nm; ^1H and ^{13}C NMR data, see Table 2; (+)FABMS *m/z* 441 $[M + Na]^+$ (100), 419 $[M + H]^+$ (32), 401 $[M + H - H_2O]^+$ (17), 383 $[M + H - 2H_2O]^+$ (23), 307 (33), 289 (30), 209 (50); (+)HRESIMS *m/z* 419.2793 $[M + H]^+$ (calcd for $C_{25}H_{39}O_5$, 419.2798); *m/z* 441.2620 $[M + Na]^+$ (calcd for $C_{25}H_{38}NaO_5$, 441.2617).

25-*O*-Methylluffariellolide (7): yellowish oily residue; $[\alpha]_D$ −55° (*c* 1.0, CH_3Cl); UV λ_{max} (MeOH) 214 nm; ^1H and ^{13}C NMR data, see Table 3; (+)FABMS *m/z* 423 $[M + Na]^+$ (18), 369 $[M - OCH_3]^+$ (9), 137 (100), 95 (58), 55 (29); (+)HRESIMS *m/z* 423.2879 $[M + Na]^+$ (calcd for $C_{26}H_{40}NaO_3$, 423.2875).

25-*O*-Ethylluffariellolide (8): yellowish oily residue; $[\alpha]_D$ −61° (*c* 1.0, CH_3Cl); UV λ_{max} (MeOH) 218 nm; ^1H and ^{13}C NMR data, see Table 3; (+)APCIMS *m/z* 415 $[M + H]^+$; (+)FABMS *m/z* 437 $[M + Na]^+$ (30), 369 $[M - OC_2H_5]^+$ (8), 137 (100), 95 (65), 55 (31); (+)HRESIMS *m/z* 437.3033 $[M + Na]^+$ (calcd for $C_{27}H_{42}NaO_3$, 437.3032), *m/z* 455.3135 $[M + Na + H_2O]^+$ (calcd for $C_{27}H_{44}NaO_4$, 455.3137), *m/z* 860.6518 $[2M + CH_3OH]^+$ (calcd for $C_{55}H_{88}O_7$, 860.6530).

Bioassays. Antimicrobial Assay. Sterile filter paper disks were impregnated with 20 μg of the samples using methanol as the carrier solvent. The impregnated disks were then placed on agar plates previously inoculated with *Bacillus subtilis* (DSM 2109), *Escherichia coli* (DSM 10290), *Staphylococcus aureus* (ATCC 25923), *Candida albicans*, and *Cladosporium herbarum* (DSM 63422). Solvent controls were run against each organism. After the plates were incubated at 37 °C for 24 h, antimicrobial activity was recorded as clear zones (in mm) of inhibition surrounding the disk. The test sample was considered active when the inhibition zone was greater than 7 mm.

Cytotoxicity Assay. Antiproliferative activity was examined against several cell lines and was determined through an MTT assay as described earlier.[36,37] Activity against brine shrimp, *Artemia salina*, was determined as previously outlined.[38]

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