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Terpenoids with Antifouling Activity against Barnacle Larvae from the Marine Sponge *Acanthella cavernosa*

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Abstract: Fourteen terpenoids, including six new compounds, have been isolated from the marine sponge *Acanthella cavernosa* collected off Hachijo-jima Island. They inhibit of larval attachment and metamorphosis of the barnacle *Balanus amphitrite*. Their structures were determined mainly by spectroscopic methods.

INTRODUCTION

Sessile marine organisms possess various defense systems against predators, against settling larvae of other sessile organisms, and against pathogenic microbes. Hence chemical defense is significant in these organisms and their secondary metabolites are potential antifouling agents.[1]

During our screening for antifouling compounds from Japanese marine invertebrates,[2] the marine sponge *Acanthella cavernosa* collected off Hachijo-jima Island (300 km south of Tokyo), inhibited settlement of cyprid larvae of the barnacle *Balanus amphitrite*. Bioassay-guided isolation afforded 14 active terpenoids, six of which are new. This paper describes the isolation, structural elucidation, and antifouling activity of these terpenoids.

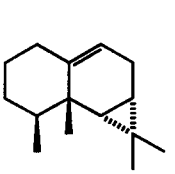
RESULTS AND DISCUSSION

Isolation of Antifouling Compounds.

Both EtOH and MeOH extracts of the frozen marine sponge *Acanthella cavernosa* were separately partitioned between hexane and water. Both hexane layers showed antifouling activity against barnacle larvae. They were combined and subjected to silica gel column chromatography with a $CHCl_3$/MeOH system. The fraction eluted with $CHCl_3$ was chromatographed on a silica gel column using gradient elution from hexane to MeOH. The fraction eluted with hexane was purified by normal-phase HPLC to afford eight bioactive compounds, **1** - **8**. From the more polar fractions (hexane-MeOH eluents), two bioactive compounds, **9** and **10**, were obtained after purification by reverse-phase HPLC. The fractions eluted with 3-10 % MeOH-$CHCl_3$ from the first column were separated by reverse-phase HPLC to yield five bioactive compounds, **10** - **14**.

*Structures of Hydrocarbons **1**, **2**, and **3**.*

Sesquiterpene hydrocarbons **1** and **2** were identified as (+)-9-aristolene[3] and (+)-α-muurolene,[4] respectively, from spectral data including complete assignments of ^1H and ^{13}C NMR signals. The absolute



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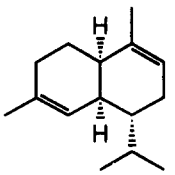

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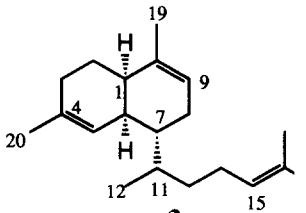

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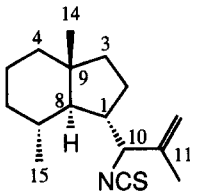

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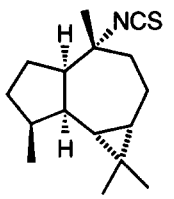

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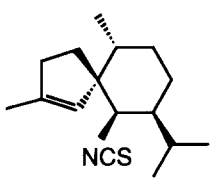

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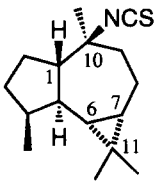

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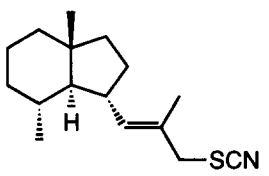

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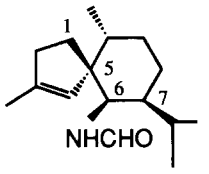

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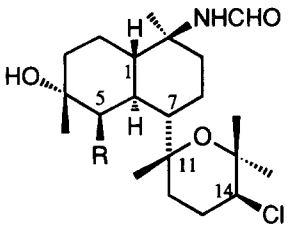
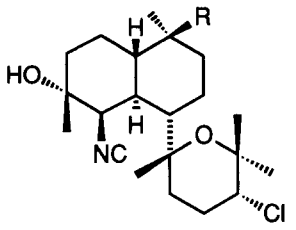


10 : R = NC

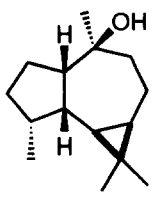
13 : R = NCO

14 : R = NCS



11 : R = NC

12 : R = NHCHO



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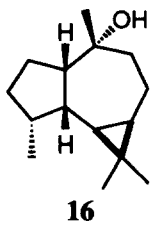

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configuration of these compounds was determined by comparing $[\alpha]_D$ values with those reported in the literature.[3,4]

Compound **3** had a molecular formula of $C_{20}H_{32}$ as determined by HREIMS. The presence of three double bonds was straightforward from the ^1H and ^{13}C NMR spectra which included three olefinic proton signals (δ 5.07, 5.38, and 5.47) without any correlation to each other and six olefinic carbon signals (δ 121.5, 123.9, 125.0, 131.0, 134.4, and 136.6). Five degrees of unsaturation indicated that **3** had two rings. From comparison of NMR spectra of **3** with those of **2** it was obvious that **3** had the same ring system as **2**. Extensive 2D NMR experiments disclosed that **3** was an isoprenoid unit homologue of **2** in which a methyl hydrogen of the isopropyl group of **2** was replaced by a prenyl group. Thus, **3** is biflora-4,9,15-triene, a rare biflorane diterpene.[5,6] Stereochemistry at C-11 and absolute stereochemistry of **3** remained to be elucidated.

*Structures of Axane Sesquiterpenes **4** and **8**.*

Compound **8** was identified as (-)-cavernothiocyanate from complete assignment of ^1H and ^{13}C NMR data, which were identical with those previously determined.[7]

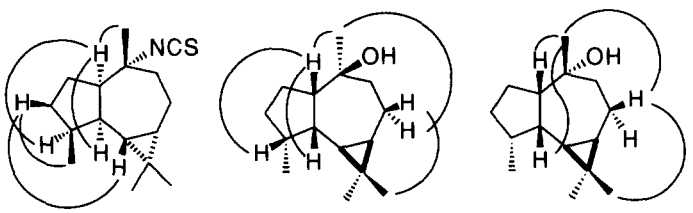
Compounds **4** and **8** had an identical molecular formula of $C_{16}H_{25}NS$ as established by HREIMS. The presence of an isothiocyanate group in **4** was deduced from a prominent IR absorption at 2085 cm^{-1} and a signal at δ 131.3 for the isothiocyanate carbon. From extensive NMR experiments including COSY, HMQC, and HMBC, planar structure of **4** was deduced to be 10-isothiocyanato-11-axene; all ^1H and ^{13}C signals could be assigned by comparison with those of cavernothiocyanate (**8**). Stereochemistry of **4** was confirmed by interpretation of the NOESY spectrum. Me-14 protons (δ 0.75) showed correlation peaks with 3β-H (δ 1.40), 7β-H (δ 1.55), 4β-H (δ 1.61), and 1β-H (δ 2.9), and the angular proton (δ 0.99) at C-8 correlated with 4α-H (δ 1.18) and 3α-H (δ 1.25), thus revealing that the relative stereochemical assignment of **4** was 1β-H, 7α-Me, 8α-H, and 9β-Me. C-10 stereochemistry and the absolute configuration of **4** remain to be elucidated.

*Structures of Aromadendrane Sesquiterpenes **5** and **7**.*

Compound **7** was identified as (+)-axiothiocyanate-2[8-11] from spectral data including complete ^1H and ^{13}C NMR spectral assignments.

The molecular formula ($C_{16}H_{25}NS$), IR absorption (2110 cm^{-1}), and NMR data implied that compound **5** had the same planar structure as **7**. Interpretation of the NOESY spectrum of **5** led to the determination of its stereochemistry, except for that at C-10. Compound **5** was one of 10-isothiocyanatoalloaromadendranes, both diastereomers of which are known.[8,9] ^{13}C NMR chemical shifts of **5** were quite different from those of 10-isothiocyanatoalloaromadendrane isolated from the marine sponge *Axinella cannabina*,[9] but almost identical with those of 10-isothiocyanatoalloaromadendrane prepared by treatment of alloaromadendrene with HSCN.[8] However, NOE correlations reported in the literature,[8] which was the only positive data to support the stereochemistry of the product by an unexpected stereoselective HSCN addition reaction, was not observed for our compound. To confirm the stereochemistry of **5** at C-10 independently from the literature,[8,9] we prepared both diastereomers of 10-hydroxyalloaromadendrane at C-10 non-stereospecifically from alloaromadendrene by almost the same method as reported in the literature.[12] Treatment of (-)-alloaromadendrene with *m*-chloroperbenzoic acid followed by lithium aluminum hydride reduction afforded both diastereoisomers of 10-hydroxyalloaromadendrane at C-10 in approximately a 4 : 1 ratio. Comparison of their ^1H and ^{13}C NMR chemical shifts and NOE data (**Fig. 1**) showed the major product was a 10*S* isomer **15** (viridiflorol) and the

minor one (10*R*) **16** was ledol. From comparison of ^{13}C NMR chemical shifts and NOE data of **5** with those of alcohols **15** and **16**, **5** was deduced to have the same stereochemistry as the 10*S* isomer **15**, that is, **5** had the structure reported by da Silva et al.[8] The literature reported that 1 % NOE enhancement of 6-H had been observed on irradiation of 10-Me in a 1D NOE difference experiment. Judging from our NOESY experiments, however, 1 % NOE of 6-H might have resulted not from irradiation at 10-Me (δ 1.32) but that of 3α-H (δ 1.29), as the multiplet signals are very close to the 10-Me signal. As the $[\alpha]_D$ value of **5** is $+8.0^o$, which is the opposite sign of that in the literature,[8] **5** was (+)-10(*R*)-isothiocyanatoalloaromadendrane, the enantiomer of da Silva's compound. Hence this is the first report of compound **5** as a natural product.



5 **15** **16**

Fig. 1. NOESY correlations in **5**, **15**, and **16**

*Structures of Spiroaxane Sesquiterpenes **6** and **9**.*

Compound **6** was identified as (+)-axisothiocyanate-3[13] from complete assignment of ^1H and ^{13}C NMR spectral data.

From the spectral data, compound **9** was determined as axamide-3.[13] Though the ^1H and ^{13}C NMR spectra of compound **9** were observed as an equilibrium mixture of two rotational isomers (s-*cis* and s-*trans* forms) at the formamide group,[14] ^{13}C NMR data of both s-*cis* and s-*trans* forms and ^1H NMR signals of the major s-*trans* isomer could be completely assigned from comparison of these data with those of axisothiocyanate-3 (**6**).

*Structures of Kalihinane Diterpenes **10** - **14**.*

Compound **11** was identified as kalihinol-E by comparison of spectral data with those reported in the literature.[15]

Compound **12** had a molecular formula of $C_{22}H_{35}ClN_2O_3$ which was established by HRFABMS. The presence of a formamide group was deduced by IR (2800 and 1670 cm^{-1}), ^1H NMR [ca. 2:3 mixture of δ 8.06 (J = 1.9 Hz) and 8.25 (12.2 Hz)], and ^{13}C NMR spectra (ca. 2:3 mixture of δ 160.5 and 162.6). This was also supported by the fact that most ^1H and ^{13}C NMR signals were doubled due to the presence of the equilibrium mixture (ca. 2:3) of s-*cis* and s-*trans* forms. Intense IR bands at 3300 and 2100 cm^{-1} were reminiscent of hydroxyl and isocyanide groups, respectively. Assignments of NMR signals for each isomer (Table 1) could be accomplished by interpretation of 2D NMR spectra including HMQC and HMBC, which led to the framework of **12**, identical with that of **11** (kalihinol-E). The formamide group was placed at C-10 replacing an isocyanide group in **11** from the chemical shift of C-10 (δ 59.7 for **11**, and δ 55.1 and 56.4 for **12**) and an HMBC correlation peak between the amide protons and C-10 for the *cis*-isomer. The presence of an

Table 1. ^{13}C NMR Data for **10, 11, 12, 13,** and **14** (in $CDCl_3$)

	10		**11**	**12**		**13**		**14**	
Assignment	s-*trans*	s-*cis*		s-*trans*	s-*cis*	s-*trans*	s-*cis*	s-*trans*	s-*cis*
1	43.5	41.5	42.3	43.5	41.7	43.7	41.0	44.3	42.1
2	20.9	21.3	21.7	21.0	21.4	21.2	21.6	21.0	21.0
3	32.7	32.8	32.6	32.8	32.8	33.0	33.1	33.4	33.4
4	70.4	70.4	70.6	70.5	70.5	71.1	71.1	71.0	71.0
5	64.0	64.1	63.8	64.0	64.0	63.4	63.5	66.2	66.3
6	36.7	36.5	36.1	36.7	36.5	38.4	38.2	38.6	38.1
7	48.7	48.8	48.3	48.6	48.6	49.0	49.0	49.3	49.3
8	22.6	22.5	21.9	22.5	22.4	22.8	22.8	23.0	22.7
9	40.9	37.2	39.8	40.9	37.3	41.2	37.1	41.2	37.3
10	55.0	56.4	59.8	55.1	56.4	55.1	56.6	55.1	56.5
11	77.0	77.0	76.6	76.2	76.2	77.0	77.0	77.0	77.0
12	38.1	38.1	28.8	28.8	28.8	38.2	38.2	38.1	38.1
13	27.4	27.5	25.3	25.4	25.4	27.5	27.6	27.4	27.5
14	64.2	64.4	62.9	63.0	63.0	64.3	64.5	64.2	64.4
15	75.9	75.8	73.9	73.9	73.8	76.2	76.1	76.0	75.9
16	22.8	22.8	28.8	28.8	28.8	23.0	23.0	23.0	23.0
17	30.6	30.6	30.3	30.3	30.2	30.7	30.7	30.8	30.9
18	19.2	19.1	20.5	20.5	20.5	19.4	19.3	19.4	19.3
19	28.9	28.9	29.0	28.9	28.9	29.4	29.4	29.3	29.3
20	19.5	19.2	20.7	19.6	19.0	19.6	19.1	19.5	19.1
NHCHO	162.6	160.6		162.6	160.5	162.5	160.4	162.5	160.4
NC	157.1	156.9	157.4	157.1	157.4				
NC			153.0						
NCO						122.7	120.4		
NCS								128.8	130.9

Table 2. Percentages of Metamorphosed and Dead Larvae*1

% of larvae	c*2	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**
metamorphosed	92	8	37	29	38	4	8	33	42	0	0	0	0	0	0
dead	0	0	13	0	0	0	0	0	0	0	0	0	0	0	0

*1 : larvae exposed to compounds at a concentration of 5 μg/mL for 48 h

*2 : control

Table 3. Percentages of Inhibition of Larval Settlement and Metamorphosis

Concentration (μg/mL)	**10**	**11**	**12**	**13**	**14**
5	100	100	100	100	100
0.5	69	61	49	44	67
0.05	24	24	17	50	42

isocyanide group at C-5 was supported from the small splitting of the ^{13}C signal at C-5 into a triplet by ^{13}C-^{15}N spin-spin coupling. Finally, comparison of J_{HH} values and NOESY spectra between **11** and **12** disclosed that **12** was 10β-formamidokalihinol-E.

Spectral data of compound **10** were consistent with the same planar structure as **12** containing a formamide group. Significant differences between **10** and **12** were found in coupling constants of H-14 (δ 3.73, J = 12.3 and 4.6 Hz for the major isomer of **10**; δ 3.96, J = 5.7 and 3.3 Hz for the major isomer of **12**) and ^{13}C chemical shifts around C-14 (see Table 1). These values for **10** were quite similar to those of kalihinol-A.[15] Extensive NMR experiments in combination with comparison with those of kalihinol-A allowed assignment of **10** as a 10-formamide analog of kalihinol-A, that is, 10β-formamidokalihinol-A.

Compound **13** had a molecular formula of $C_{22}H_{35}ClN_2O_4$, as determined by HRFABMS, containing one more oxygen atom than **10** or **12**. An intense IR absorption at 2250 cm^{-1} and a set of ^{13}C signals at δ 122.7 and 120.4 due to two rotamers suggested the presence of an isocyanate group. Most ^{13}C NMR signals of **13** were superimposable on those of **10**, except for chemical shifts at C-4, C-5, and C-6 which were remarkably different from those of **10**, suggesting the isocyanide group of **10** was replaced by the isocyanate group in **13**. This was confirmed by 2D NMR spectra. Thus **13** was 10β-formamido-5-isocyanatokalihinol-A.

Compound **14** was deduced to be the isothiocyanate analog of **13** from the molecular formula of $C_{22}H_{35}ClN_2O_3S$ (HRFABMS), IR band (2100 cm^{-1}), and ^{13}C NMR signals (δ 128.8 and 130.9; a set of signals resulted from two rotamers). Finally, extensive NMR experiments allowed assignment of **14** as 10β-formamido-5β-isothiocyanatokalihinol-A.

Antifouling Activities against Barnacle Larvae.

Antifouling activities of isolated terpenoids against cyprid larvae of the barnacle *Balanus amphitrite* are shown in Tables 2 and 3. Table 2 summarizes percentages of metamorphosed and dead larvae within 48 hr when exposed to terpenoids **1** - **14** at a concentration of 5 μg/mL. Kalihinane derivatives **10** -**14** and axamide-3 (**9**) inhibited larval metamorphosis completely. Table 3 shows percentages of inhibition at three concentrations for kalihinanes (**10** - **14**). Isocyanate and isothiocyanate derivatives **13** and **14** were highly antifouling (EC_{50} ca. 0.05 μg/mL).

Conclusion.

A variety of terpenoids have been isolated from the marine sponge *Acanthella cavernosa* collected off Hachijo-jima Island as potential antifouling agents against cyprid larvae of the barnacle *Balanus amphitrite*. Most of them contained either isocyanate, isocyanide, or formamide group(s).

Terpenoids embracing isocyanate are very rare as natural products, especially from marine organisms.[16] It is noteworthy that kalihinol-A derivatives (**10**, **13**, and **14**) are highly antifouling.

EXPERIMENTAL

General.

IR spectra were recorded on a JASCO IR-700 spectrometer. Measurements of mass spectra were performed with a JEOL JMS-SX102A mass spectrometer. ^1H and ^{13}C NMR spectra were recorded on a BRUKER ARX 500 spectrometer in $CDCl_3$ at 500.14 MHz and 125.77 MHz at 300 K. Chemical shifts were

reported using residual $CHCl_3$ (δ 7.24), $CDCl_3$ (δ 77.0), C_6HD_5 (δ 7.15), and C_6D_6 (δ 128.0) as internal standards. Optical rotations were measured with a JASCO DIP-140 or DIP-1000 polarimeter.

Bioassay.

Samples were dissolved in MeOH; aliquots of the solution were applied to wells of polystyrene multi-well plates and air-dried. To each well were added 2 mL of 80 % filtered sea water and six one-day-old cyprid larvae of the barnacle *Balanus amphitrite* . After incubation for 48 h in the dark at 25 °C, the numbers of larvae which attached, metamorphosed, or died were counted under a microscope.

Sponge, Extraction and Isolation of Antifoulants.

The marine sponge was collected by scuba diving at depths of 10～15 m off Hachijo-jima Island and identified as *Acanthella cavernosa* Dendy, 1922 (Class Demospongiae, Order Halichondrida, Family Dictyonellidae) by Dr. Rob van Soest; a voucher specimen (ZMA POR. 6128) was deposited at the Institute for Systematics and Population Biology, University of Amsterdam. The frozen specimens (910 g) were extracted with EtOH (2×3 L) and then with MeOH (1×1 L). The EtOH extract was concentrated and partitioned between water and hexane to obtain the hexane extract (4.67 g). The MeOH extract was processed in the same way to furnish the hexane soluble material (0.48 g). Two hexane solubles were combined, and a 3.3 g portion was fractionated by silica gel column chromatography with $CHCl_3$, 3 % MeOH/$CHCl_3$, and 10 % MeOH/$CHCl_3$.

The fraction eluted with $CHCl_3$ (2.22 g) was chromatographed on silica gel with hexane, 50 % hexane/benzene, benzene, 50 % benzene/$CHCl_3$, $CHCl_3$, 10 % MeOH/$CHCl_3$, 50 % MeOH/AcOEt, and MeOH. The fraction eluted with hexane (466 mg) which showed antifouling activity was separated by normal phase HPLC with hexane to afford eight bioactive compounds (**1** - **8**; **1**: 6.0 mg, 9.3×10^{-4} % yield from wet sponge; **2**: 4.4 mg, 6.8×10^{-4} %; **3**: 4.0 mg, 6.2×10^{-4} %; **4**: 4.8 mg, 7.5×10^{-4} %; **5**: 3.9 mg, 6.1×10^{-4} %; **6**: 0.6 mg, 9.3×10^{-5} %; **7**: 6.1 mg, 9.5×10^{-4} %; **8**: 3.8 mg, 5.9×10^{-4} %) in order of increasing polarity. Combined fraction eluted with $CHCl_3$ (176 mg) and 10 % MeOH/$CHCl_3$ (194 mg) was purified by ODS-HPLC (80 % MeOH/H_2O) to afford a bioactive formamide (**9**: 6.2 mg, 9.6×10^{-4} %), while 50 % MeOH/AcOEt (194.0 mg) was repeatedly purified by ODS-HPLC (MeOH and 80 % MeOH/H_2O) to afford a kalihinane compound (**10**: 7.0 mg, 1.09×10^{-3} %).

The fraction eluted with 3 % MeOH/$CHCl_3$ (102.8 mg) from the first column was purified by ODS-HPLC (80 % MeOH/H_2O) to yield kalihinol-E (**11**: 3.1 mg, 4.8×10^{-4} %).

The MeOH-soluble part of the 10 % MeOH/$CHCl_3$ fraction (167.0 mg) from the first column was repeatedly separated by ODS-HPLC MeOH and 80 % MeOH/H_2O) to afford four kalihinane derivatives: **12** (5.8 mg, 9.0×10^{-4} %), **10** (10.0 mg, 1.6×10^{-3} %), **13** (1.9 mg, 3.0×10^{-4} %), and **14** (2.2 mg, 3.4×10^{-4} %).

1: $[\alpha]_D$ +40.0° (*c* 0.1, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 0.60 (6-H), 0.72 (7-H), 0.91 (4-Me), 1.00 (11β-Me), 1.02 (5-Me), 1.05 (11α-Me), 1.22 (2α–H), 1.30 (3β–H), 1.42 (3α–H), 1.58 (4-H), 1.63 (2β-H), 1.95 (1α-H), 2.03 (8α-H), 2.15 (1β-H), 2.27 (8β-H), and 5.07 (9-H); ^{13}C NMR ($CDCl_3$) δ 15.7 (11α-Me), 16.0 (4-Me), 17.9 (C-11), 19.2 (C-7), 21.4 (5-Me), 21.8 (C-8), 27.2 (C-2), 29.9 (11β-Me), 31.4 (C-3), 32.1 (C-6), 33.0 (C-1), 36.8 (C-5), 37.8 (C-4), 118.3 (C-9), and 141.6 (C-10).

2: $[\alpha]_D$ +17.5° (*c* 0.04, $CHCl_3$); ^1H NMR (C_6D_6) δ 0.83 (11-Me), 0.87 (11-Me), 1.48 (2-H), 1.51 (7-H), 1.66 (4-Me), 1.69 (10-Me), 1.78 (2-H and 8-H), 1.83 (3-H_2), 1.87 (8-H), 1.99 (1-H), 2.05 (11-H), 2.15 (6-H), 5.44 (9-H), and 5.56 (5-H); ^{13}C NMR (C_6D_6) δ 16.1 (11-Me), 21.5 (11-Me), 21.8 (10-Me), 24.1

(4-Me), 24.9 (C-8), 25.0 (C-2), 26.9 (C-11), 30.6 (C-3), 37.1 (C-6), 39.4 (C-1), 41.5 (C-7), 122.0 (C-9), 124.7 (C-5), 134.3 (C-4), and 136.0 (C-10).

3: $[\alpha]_D$ +64° (*c* 0.05, $CHCl_3$); IR (neat) ν 1675 and 800 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.80 (12-H_3), ~1.2 (13-H_2), 1.35 (2-H), 1.51 (7-H), 1.57 (16Z-Me), 1.65 (16E-Me), 1.67 (10-Me), 1.68 (4-Me), 1.78 (11-H), ~1.8 (2-H and 8-H_2), ~1.9 (3-H and 14-H), 1.92 (1-H), ~2.0 (3-H and 14-H), 2.02 (6-H), 5.07 (15-H), 5.38 (9-H), and 5.47 (5-H); ^{13}C NMR ($CDCl_3$) δ 13.3 (C-12), 17.6 (16Z-Me), 21.7 (10-Me), 23.9 (4-Me), 24.6 (C-2 and C-8), 25.7 (16E-Me), 26.2 (C-14), 30.8 (C-3), 31.4 (C-11), 35.7 (C-13), 36.4 (C-6), 38.9 (C-7), 39.6 (C-1), 121.5 (C-9), 123.9 (C-5), 125.0 (C-15), 131.0 (C-16), 134.4 (C-4), and 136.6 (C-10); EILRMS *m/z* 272 (M^+; 22), 257 (2), and 159 (100); EIHRMS *m/z* 272.2510 (Calcd for $C_{20}H_{32}$, Δ +0.6 mmu).

4: $[\alpha]_D$ -52.0° (*c* 0.1, $CHCl_3$); IR (neat) ν 2085 and 900 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.75 (14-H_3), 0.95 (6-H), 0.97 (15-H_3), 0.99 (8-H), 1.18 (4α-H), 1.25 (3α-H), 1.40 (3β-H), 1.48 (2-H), 1.52 (5-H_2), 1.55 (7-H), 1.61 (4β-H), 1.62 (6-H), 1.63 (2-H), 1.73 (11-Me), 2.11 (1-H), 4.47 (10-H), 4.91 and 5.06 (12-H_2); ^{13}C NMR ($CDCl_3$) δ 18.3 (C-14), 20.2 (11-Me), 21.3 (C-15), 21.9 (C-5), 22.7 (C-2), 32.1 (C-7), 36.9 (C-6), 38.9 (C-4), 39.5 (C-3), 42.8 (C-1), 43.1 (C-9), 55.7 (C-8), 66.3 (C-10), 111.8 (C-12), 131.3 (-NCS), and 141.7 (C-11); EILRMS *m/z* 263 (M^+; 14), 248 (10), 205 (50), 189 (39), and 151 (100); EIHRMS *m/z* 263.1697 (Calcd for $C_{16}H_{25}NS$, Δ -1.1 mmu).

5: $[\alpha]_D$ +8.0° (*c* 0.1, $CHCl_3$); IR (neat) ν 2110 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.11 (6-H), 0.63 (7-H), 0.92 (4-Me), 1.017 (11α-Me), 1.022 (11β-Me), 1.29 (3β-H), 1.32 (10-Me), 1.34 (8α-H), 1.54 (2β-H), 1.61 (2α-H), 1.72 (9-H_2), 1.80 (8β-H), 1.81 (3α-H), 1.87 (5-H), 2.02 (4-H), and 2.10 (1-H); ^{13}C NMR ($CDCl_3$) δ 16.1 (4-Me and 11α-Me), 18.7 (C-11), 20.6 (C-8), 22.2 (C-6), 24.4 (C-2), 28.2 (C-7), 28.6 (11β-Me), 28.9 (C-3), 30.4 (10-Me), 37.2 (C-9), 38.1 (C-4), 40.6 (C-5), 57.3 (C-1), and 68.4 (C-10); EILRMS *m/z* 263 (M^+; 100), 248 (21), 205 (3), and 121 (58); EIHRMS *m/z* 263.1721 (Calcd for $C_{16}H_{25}NS$, Δ +1.3 mmu).

6: $[\alpha]_D$ +52.9° (*c* 0.07, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 0.75 (10-Me), 0.89 (11-Me), 0.91 (11-Me), 1.08 (9α-H), 1.22 (7-H), 1.25 (8β-H), 1.49 (11-H), 1.52 (9β-H), 1.67 (10-H), 1.72 (3-Me), 1.78 (8α-H), 1.87 (1β-H), 1.93 (1α-H), 2.22 (2-H_2), 3.67 (6-H), and 5.12 (4-H); ^{13}C NMR ($CDCl_3$) δ 16.1 (10-Me), 16.9 (3-Me), 20.4 (11-Me), 20.8 (11-Me), 25.4 (C-8), 30.2 (C-11), 31.3 (C-9), 35.0 (C-10), 35.1 (C-1), 35.9 (C-2), 45.4 (C-7), 59.0 (C-5), 67.4 (C-6), 124.0 (C-4), and 144.8 (C-3).

7: $[\alpha]_D$ +75.0° (*c* 0.1, $CHCl_3$); IR (neat) ν 2085 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.53 (6-H), 0.59 (7-H), 0.90 (4-Me), 0.94 (8α-H), 0.95 (11α-Me), 0.99 (11β-H), 1.25 (10-Me), 1.26 (3β-H), 1.26 (5-H), 1.42 (2α-H), 1.68 (3α-H), 1.80 (8β-H), 1.82 (9β-H), 1.87 (2β-H), 1.92 (9α-H), 2.02 (4-H), and 2.18 (1-H); ^{13}C NMR ($CDCl_3$) δ 15.8 (11α-Me), 16.0 (4-Me), 19.3 (10-Me), 19.8 (C-8), 20.4 (C-11), 26.6 (C-7), 27.5 (C-2), 28.4 (C-6), 28.5 (11β-Me), 34.3 (C-3), 36.7 (C-4), 39.2 (C-5), 43.3 (C-9), 56.6 (C-1), 66.6 (C-10), and 129.3 (-NCS).

8: $[\alpha]_D$ -6.0° (*c* 0.1, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 0.75 (8-H), 0.77 (15-H_3), 0.78 (14-H_3), 0.81 (6-H), 1.09 (4-H), 1.24 (3-H), 1.25 (2-H), 1.42 (3-H), 1.44 (7-H), 1.48 (5-H_2), 1.61 (6-H), 1.62 (4-H), 1.75 (11-Me), 2.01 (2-H), 2.51 (1-H), 3.47 and 3.55 (12-H_2), and 5.57 (11-H); ^{13}C NMR ($CDCl_3$) δ 14.9 (11-Me), 18.5 (C-14), 21.3 (C-15), 22.2 (C-5), 29.8 (C-2), 32.3 (C-7), 36.8 (C-6), 39.4 (C-4), 39.7 (C-1), 39.8 (C-3), 42.8 (C-9), 44.5 (C-12), 60.7 (C-8), 124.5 (C-11), and 141.9 (C-10).

9: $[\alpha]_D$ -15° (*c* 0.06, $CHCl_3$); ^1H NMR of major s-*trans* isomer ($CDCl_3$) δ 0.75 (10-Me), 0.86 (11-Me), 0.90 (11-Me), 0.95 (8-H), ~1.1 (9-H), 1.25 (11-H), 1.28 (7-H), 1.38 (10-H), ~1.5 (9-H), ~1.6 (1-H), ~1.7

(3-Me), ~1.8 (8-H), 1.83 (1-H), 2.05~2.35 (2-H_2), 4.24 (6-H), 5.23 (4-H), and 8.24 (-NHCHO); ^{13}C NMR of major s-*trans* isomer ($CDCl_3$) δ 16.2 (10-Me), 17.0 (3-Me), 20.9 (11-Me), 21.2 (11-Me), 25.3 (C-8), 29.5 (C-11), 31.6 (C-9), 33.6 (C-1), 35.6 (C-10), 36.0 (C-2), 44.2 (C-7), 58.1 (C-5), 53.9 (C-6), 125.0 (C-4), 143.6 (C-3), and 160.9 (-NHCHO).

10: $[\alpha]_D$ +11.0° (*c* 0.145, $CHCl_3$); IR (neat) ν 3300, 2150, and 1670 cm^{-1}; ^1H NMR of major s-*trans* isomer ($CDCl_3$) δ 1.09 (8-H), 1.17 (11-Me), 1.21 (10-Me), 1.32 (15α-Me), 1.33 (15β-Me), 1.38 (4-Me), 1.44 (1-H), 1.45 (2-H), 1.52 (12-H), 1.54 (3-H), 1.60 (9-H and 12-H), 1.61 (2-H), 1.65 (7-H and 8-H), 1.76 (3-H), 1.81 (9-H), 2.00 (13-H), 2.06 (13-H), 2.10 (6-H), 3.73 (dd, 12.3 and 4.6 Hz, 14-H), 4.54 (5-H), 5.52 (NH), and 8.25 (CHO); ^{13}C NMR see Table 1; FABLRMS (glycerol, positive) *m/z* 503, 505 $(M+glycerol+H)^+$, 411, 413 $(M+H)^+$, and 366; FABHRMS *m/z* 411.2426 (Calcd for $C_{22}H_{35}{}^{35}ClNO_3+H$, Δ +1.2 mmu).

11: $[\alpha]_D$ +4.2° (*c* 0.09, $CHCl_3$); ^{13}C NMR see Table 1.

12: $[\alpha]_D$ -2.8° (*c* 0.145, $CHCl_3$); IR (neat) ν 3300, 2800, 2100, 1670, and 750 cm^{-1}; ^1H NMR of major s-*trans* isomer ($CDCl_3$) δ 1.08 (8-H), 1.17 (11-Me), 1.22 (10-Me), 1.31 (12-H), 1.33 (15α-Me), 1.34 (15β-Me), 1.39 (4-Me), 1.48 (1-H), 1.53 (3-H), 1.63 (9-H), 1.77 (8-H), 1.78 (3-H and 7-H), 1.81 (2-H), 1.82 (9-H), 1.94 (13-H), 1.95 (12-H), 2.10 (6-H), 2.32 (13-H), 3.96 (dd, 5.7 and 3.3 Hz, 14-H), 4.60 (5-H), 5.62 (NH), and 8.25 (CHO); ^{13}C NMR see Table 1; FABLRMS (glycerol, positive) *m/z* 503, 505 $(M+glycerol+H)^+$, 411, 413 $(M+H)^+$, and 366; FABHRMS *m/z* 411.2417 (Calcd for $C_{22}H_{35}{}^{35}ClNO_3+H$, Δ +0.3 mmu).

13: $[\alpha]_D$ -25° (*c* 0.02, $CHCl_3$); IR (neat) ν 3300, 2250, 1670, and 700 cm^{-1}; ^1H NMR of major s-*trans* isomer ($CDCl_3$) δ 1.09 (8-H), 1.20 (10-Me), 1.23 (11-Me), 1.27 (4-Me), 1.33 (15α-Me), 1.36 (15β-Me), 1.39 (1-H), 1.45 (3-H), 1.46 (9-H), 1.50 (7-H), 1.53 (12-H), 1.59 (12-H), 1.64 (3-H), 1.67 (8-H), 1.81 (9-H), 2.00 (13-H), 2.06 (13-H), 2.13 (6-H), 3.74 (14-H), 4.47 (5-H), 5.50 (NH), and 8.24 (CHO); ^{13}C NMR see Table 1; FABLRMS (glycerol, positive) *m/z* 519, 521 $(M+glycerol+H)^+$, 427, and 429 $(M+H)^+$; FABHRMS *m/z* 427.2361 (Calcd for $C_{22}H_{35}{}^{35}ClNO_4+H$, Δ -0.3 mmu).

14: $[\alpha]_D$ +24° (*c* 0.05, $CHCl_3$); IR (neat) ν 3300, 2100, 1630, and 770 cm^{-1}; ^1H NMR of major s-*trans* isomer ($CDCl_3$) δ 1.07 (8-H), 1.20 (11-Me), 1.21 (10-Me), 1.33 (4-Me), 1.34 (15-Me x 2), 1.35 (1-H), 1.54 (12-H), 1.55 (3-H), 1.59 (7-H), 1.58 (9-H), 1.61 (12-H), 1.65 (3-H), 1.67 (8-H), 1.81 (9-H), 2.01 (13-H), 2.06 (13-H), 2.14 (6-H), 3.75 (14-H), 4.67 (5-H), 5.50 (NH), and 8.26 (CHO); ^{13}C NMR see Table 1; FABLRMS (glycerol, positive) *m/z* 443, 445 $(M+H)^+$, 398, 400, 380, and 382; FABHRMS *m/z* 443.2138 (Calcd for $C_{22}H_{35}{}^{35}ClNO_3S+H$, Δ +0.3 mmu).

*Preparation of 10-hydroxyalloaromadendranes (**15** and **16**).*

To a solution of (-)-alloaromadendrane (43 mg; commercially available from Fluka) in chloroform (4 mL) was added *m*-chloroperbenzoic acid (70 %; 63 mg), and the mixture was stirred at rt for 30 min. After addition of aqueous sodium bisulfite solution followed by sodium bicarbonate solution, the mixture was extracted with chloroform and washed with H_2O. After evaporation of chloroform, the residue (48 mg) was dissolved in ether (5 mL), to which was added a large excess of lithium aluminum hydride (10 mg), and the mixture was stirred at rt overnight. After addition of 2 M HCl, the reaction mixture was extracted with ether; the ether extract was successively washed with brine, sodium bicarbonate solution, and 2 times with brine; then dried over sodium sulfate, affording 49 mg of reaction product mixture after evaporation. The mixture was passed through a silica gel (13 g) column with chloroform to yield a mixture of 10-hydroxyalloaromadendrane (**15** and **16**; ca. 30 mg),

which was purified by HPLC (SiO_2; $CHCl_3$) to afford (+)-10(*S*)-hydroxyalloaromadendrane (**15**; 15 mg) and (-)-10(*R*)-hydroxyalloaromadendrane (**16**; 4 mg).

15: $[\alpha]_D$ +2.1° (*c* 0.1, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 0.09 (6-H), 0.59 (7-H), 0.90 (4-Me), 0.97 (11β-Me), 1.02 (11α-Me), 1.13 (10-Me), 1.24 (3α-H), 1.37 (8β-H), 1.54 (9α-H), 1.53 (2α-H), 1.62 (8α-H), 1.63 (2β-H), 1.69 (9β-H), 1.78 (3β-H), 1.80 (1-H), 1.81 (5-H), and 1.95 (4-H); ^{13}C NMR ($CDCl_3$) δ 16.1 (11β-Me), 16.3 (4-Me), 18.4 (C-11), 18.8 (C-8), 22.3 (C-6), 25.8 (C-2), 28.6 (C-7), 28.7 (11α-Me), 29.1 (C-3), 32.1 (10-Me), 37.8 (C-9), 38.5 (C-4), 39.7 (C-5), 58.2 (C-1), and 74.6 (C-10); EILRMS *m/z* 222 (M^+; 20), 204 (100), and 189 (70); EIHRMS *m/z* 222.1968 (Calcd for $C_{15}H_{26}O$, Δ -1.6 mmu).

16: $[\alpha]_D$ -10.5° (*c* 0.1, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 0.31 (6-H), 0.70 (7-H), 0.91 (4-Me), 0.98 (11β-Me), 1.02 (11α-Me), 1.12 (10-Me), 1.19 (8β-H), 1.28 (3α-H), 1.66 (2β-H), 1.67 (9-H), 1.68 (3β-H), 1.76 (5-H), 1.80 (8α-H), 1.83 (9-H), 1.87 (2α-H), 1.97 (4-H), and 2.07 (1-H); ^{13}C NMR ($CDCl_3$) δ 15.4 (11β-Me), 16.0 (4-Me), 19.2 (C-11), 20.3 (C-8), 23.4 (C-6), 24.6 (C-2), 25.0 (C-7), 28.6 (11α-Me), 30.5 (10-Me), 30.8 (C-3), 38.4 (C-4), 39.2 (C-9), 40.8 (C-5), 53.8 (C-1), and 74.6 (C-10); EILRMS *m/z* 222 (M^+; 78), 204 (100), and 189 (92); EIHRMS *m/z* 222.2000 (Calcd for $C_{15}H_{26}O$, Δ +1.7 mmu).

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