New Nitrogenous Eudesmane-Type Compounds Isolated from the Caribbean Sponge *Axinyssa ambrosia*

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Fractionation of an acetone−methanol (1:1) extract of the Caribbean marine sponge *Axinyssa ambrosia* yielded three new sesquiterpenes whose structures were established by spectroscopic methods as (4*R**,5*R**,7*S**,10*R**)-eudesm-11-en-4-ylamine hydrochloride (**1**), axinyssamine hydrochloride, (4*R**,5*R**,7*S**,-10*R**)-4-isocyanatoeudesm-11-ene (**3**), and (4*R**,5*R**,7*S**,10*R**)-formamidoeudesm-11-ene (**4**). Compound **1** exhibited significant cytotoxic activity against cancer cells and was also active in a lethality test using polyps of the scleractinian coral *Madracis mirabilis*.

During the last 10 years, marine sponges of the genus *Axinyssa* (order Halichondrida, family Halichondriidae) have attracted considerable research interest mainly due to the presence of the unusual sesquiterpene isocyanides accompanied by the corresponding isothiocyanates and formamide derivatives.[1] Recently, sesquiterpene dichlorimines and a guaianae-type sesquiterpene peroxide[2] have also been reported. Biological activities of some of these nitrogen-containing terpenes have demonstrated potency in antihelmintic,[1c] antimalarial,[1g] and antifouling[2,3] screenings. A 5α,8α-epidioxy sterol, axinysterol,[4] has also been isolated from sponges of this genus. Epidioxysterols have also shown potent antifouling activity.[5]

In the course of our continuing search for biologically active secondary metabolites and the possible role that these compounds play in the chemical defense of marine sponges,[6,7] we have examined a methanolic extract of the Caribbean sponge *Axinyssa ambrosia* (De Laubenfels, 1936). The study led to the isolation of a new sesquiterpene amine hydrochloride (compound **1**), which showed cytotoxic and lethal activities against tumor cell lines and polyps of the coral *Madracis mirabilis*, respectively. Further fractionation of the above-mentioned extract permitted the isolation of two additional new eudesmane-type compounds, the isocyanide **3** and the formamide derivative **4**, together with the known nitrogenous sesquiterpenes **5**−**10**.

Results and Discussion

Compound **1** was isolated as white crystals, and the molecular formula $C_{15}H_{27}N{\cdot}HCl$ was determined from its MS data. The positive-ion FABMS displayed an intense ion peak at *m*/*z* 222 for which the formula $C_{15}H_{28}N$ was assigned on the basis of HRFABMS data. The negative-ion FABMS showed peaks at *m*/*z* 35 and 37 (ca. 3:1 ratio) as well as 127 and 129 (ca. 3:1 ratio, [Cl + glycerine matrix]$^-$). The IR spectrum of **1** exhibited a broad band at 3383 cm^{-1}, supporting the contention that compound **1** is an amine salt. Further, alkaline treatment of **1** gave the free amine **2**, which was different from **1** when analyzed by NMR and TLC. The EIMS of **2** exhibited a molecular ion peak at *m*/*z* 221, accompanied by a peak at *m*/*z* 204 ($M^+ - NH_3$). Furthermore, treatment of the amine with diluted HCl regenerated the hydrochloride **1**.

In the ^1H NMR spectrum, compound **1** displayed signals of two singlet methyls at δ 0.97 and 1.31, an olefinic methyl at δ 1.78, and terminal methylene protons at δ 4.87 and 4.92, among others. The ^{13}C NMR spectrum displayed 15 carbon signals, which were classified into three CH_3, seven CH_2, two CH, and three quaternary carbons by DEPT experiments. Further NMR studies including $^1H-^1H$ COSY, HMQC, and HMBC experiments allowed the determination of a partial structure as shown in Figure 1. Long-range correlations observed are also shown in Figure 1. The absence of any further sp^2 carbon resonances other than the peaks at δ 111.59 and 146.16 required two carbocycles in **1** to account for the three degrees of unsaturation in the molecule. Due to the overlapping of five proton signals in the region δ 1.54−1.58 and the coincidental overlapping of the two carbon signals at δ 23.2, it was not straightforward to connect the unidentified linkages depicted in Figure 2. However, some proton signals, e.g., δ 1.39 (td, J = 13.2, 3.0 Hz), 1.72 (tt, J = 13.8, 3.0 Hz), 1.35 (br d, J = 13.2 Hz), and 2.15 (br d, J = 9.0 Hz), were detected as those of axial and equatorial representatives typical of a chair form of a six-membered ring, thus strongly suggesting a decalin structure for this compound. Furthermore, when the NMR data were compared with those for other compounds containing similar structural elements,[8] it was clear that **1** had an eudesmane carbon skeleton as shown in Figure 2.

Careful and systematic analysis of HMBC cross-peaks with moderate and weak intensity revealed long-range connectivities of H-3 (δ 2.15) to C-1, H-6 (δ 2.08) to C-10, and H-6 (δ 2.08) to C-8. The absence of cross-peaks between H-3 (δ 2.15) and H-1 (δ 1.13 and 1.35) in the $^1H-^1H$ COSY spectrum meant that C-1 and C-3 are not linked directly, but the unsettled methylene carbon could be located between the two carbons. Finally C-5 and C-9 were connected to C-6 and C-8, respectively, since the reverse connection would give rise to cross-peaks between H-6 (δ 2.08) and H-9 (δ 1.11 and 1.39), which were not detected in the $^1H-^1H$ COSY spectrum. The foregoing data permitted the formulation of the complete structure of **1** as shown

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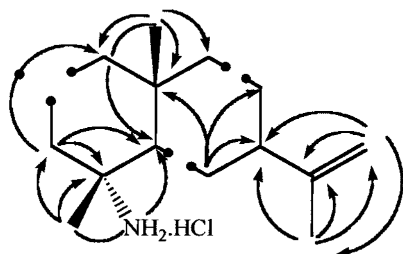

Figure 1. Partial structure of compound **1**, with HMBC correlations. A methylene carbon (δ 18.4) remains to be incorporated into the structure.

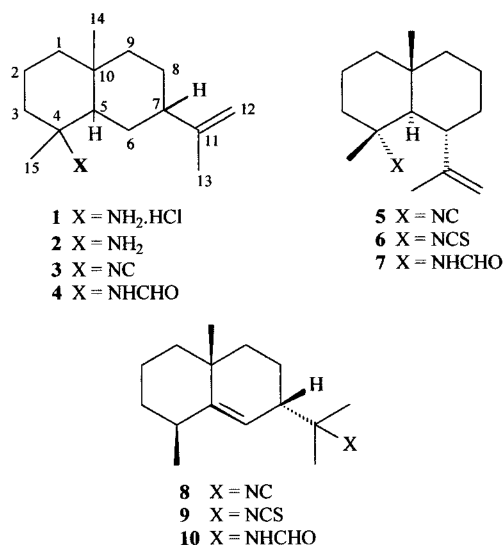

Figure 2. Chemical structures of the compounds isolated from the marine sponge *Axinyssa ambrosia*.

in Figure 2. The ^{1}H and ^{13}C NMR assignments of **1** are listed in Table 1. The ^{13}C NMR data for the free amine **2** are also listed in this table. Carbon resonances for C-3, C-4, C-5, and C-15 of **2** exhibited significant differences in their chemical shifts from the respective signals of **1**, while the other signals resonated nearly at the same positions. In addition, comparative studies of the NMR data of **1** and a closely related eudesmane isocyanate isolated from the marine sponge *Acanthella klethra*[8] supported the assignment made for **1**.

The relative stereochemistry of the four chiral centers in **1** was determined as follows. Irradiation of H_3-14 (δ 0.97) caused an NOE enhancement of the signal of H_3-15 (δ 1.31), thus establishing 1,3-diaxial relationships of the two methyl groups as well as the trans-linkage of the ring junction (Figure 3). The stereochemistry at the C-7 position was deduced from a small $W_{1/2}$ value (12.5 Hz) of H-7, which requires this hydrogen to be located in an equatorial orientation (β configuration) and from NMR comparisons of H-7 and C-7 resonances in eudesmanes containing a β proton at C-7.[8] No NOE enhancements were observed between the H-5 and H-7 hydrogens. Thus, the structure of **1** was established as (4*R**,5*R**,7*S**,10*R**)-eudesm-11-en-4-ylamine hydrochloride (4-aminoeudesm-11-ene HCl salt). The absolute stereochemistry of **1** was not determined.

Compound **3** was obtained as a colorless oil. Its molecular formula was established as $C_{16}H_{25}N$ on the basis of HRFABMS. The molecular ion peak at *m/z* 231 in the EIMS of **3** was typical of a sesquiterpene isonitrile. The isocyano funcionality was confirmed by the presence of the ions at *m/z* 204 (M − HCN) and 189 (M − HCN − CH_3) and by the IR band at 2125 cm^{-1}. The ^{1}H NMR spectrum of **3** showed, among others, signals for two singlet methyls at δ 0.94 and 1.31, an olefinic methyl at δ 1.80, and terminal methylene protons at δ 4.91 and 4.97. In the ^{13}C NMR spectrum, 15 signals were displayed (the −NC signal was obscure), which included an olefinic quaternary carbon at δ 146.1, an olefinic methylene carbon at δ 111.3 (δ_H 4.91 and 4.97), and one methyl group at δ 22.7 (δ_H 1.80), forming an isopropylene unit as revealed by the ^{1}H−^{1}H COSY experiment. Additionally, two methyls, six aliphatic methylenes, two methines, and two quaternary carbons, with one of these at δ 61.1 assigned to the carbon bearing the isonitrile group, were observed in the spectrum. Comparison of the NMR data with those of **1** and **2** also suggested the eudesmane-type skeleton for compound **3**. The relative stereochemistry of the chiral centers in **3** was determined in the same manner as described for compound **1**. Irradiation of H_3-15 (δ 1.31) caused a NOE enhancement on the signal of H_3-14 (δ 0.97) and H-7 appeared at δ 2.49 with a small $W_{1/2}$ value (12 Hz). These analyses led us to determine the structure of **3** as (4*R**,5*R**,7*S**,10*R**)-4-isocyanatoeudesm-11-ene (Figure 2). The assignments of ^{13}C NMR signals, together with additional ^{1}H assignments revealed by the ^{1}H−^{1}H COSY spectrum, are presented in Table 1.

Compound **4**, isolated as white crystals, has the molecular formula $C_{16}H_{27}NO$, as deduced from HRFABMS. The molecular ion peak at *m/z* 249 observed in the EIMS was shifted 18 mass units when compared with that of **3**. The IR spectrum indicated the presence of a formamide group (3317, 1670, and 1540 cm^{-1}), which was also confirmed by the fragment ions at *m/z* 204 (M − $HCONH_2$) and 189 (M − $HCONH_2$ − CH_3) in the EIMS of **4**. The ^{1}H NMR spectrum, as is often the case for sesquiterpene formamides, showed pairs of signals due to *E* and *Z*-isomers of the formamide moiety: at δ 8.23 (d, *J* = 12.6 Hz, (*E*)-NHC*H*O) and δ 8.04 (d, *J* = 2 Hz, (*Z*)-NHC*H*O), and at δ 5.43 (br d, *J* = 12.6 Hz, (*E*)-N*H*CHO) and 4.93 (br d, (*Z*)-N*H*CHO). These signals were observed in a 4:1 ratio, with the *E*-isomer favored. The olefinic methylene protons and the H_3-13, H_3-14, and H_3-15 signals were also detected as pairs, confirming the presence of the two above-mentioned isomers. The signal of the characteristic equatorially oriented H-7 (m, 1H unit) was observed at δ 2.44 with a small $W_{1/2}$ value (13.3 Hz). In the ^{13}C NMR spectrum of **4**, most of the signals appeared as pairs. Interpretation of the NMR data and the results of NOE experiments established that compound **4** has the structure (4*R**,5*R**,7*S**,10*R**)-4-formamidoeudesm-11-ene (Figure 2). The ^{13}C NMR assignments for the major *E*-isomer of **4**, which were achieved with the aid of DEPT experiments, are listed in Table 1.

Compounds **5**−**7** were identified as 4α-isocyanogorgon-11-ene, 4α-isothiocyanogorgon-11-ene, and 4α-formamidogorgon-11-ene, respectively, by comparing their NMR and MS data with reported values.[9] In a similar way compounds **8**−**10** were identified as 11-isocyano-7β-H-eudesm-5-ene, 11-isothiocyano-7β-H-eudesm-5-ene, and 11-formamido-7β-H-eudesm-5-ene, respectively.[10] The ^{13}C NMR data for compound **10** are reported herein for the first time (Experimental Section).

Compound **1** showed cytotoxic effects in vitro against P-388 (mouse lymphoma), A-549 (human lung carcinoma), and HT-29 (human colon carcinoma) cells with IC_{50} values of 2, 2, and 2 μg/mL, respectively. The effect of compound **1** against polyps of the coral *Madracis mirabilis* was also examined (Figure 4). At a concentration of 10 μg/mL, equivalent to that of the sponge tissues, all polyps were dead when exposed for 2 h to the substance. In contrast,

Table 1. NMR Data for Compounds **1**–**4** ($CDCl_3$)

	1[a,b]		**2**[a,b]	**3**[a,b]		**4**[a–c]
position	δ^{13}C	δ^{1}H	δ^{13}C	δ^{13}C	δ^{1}H	δ^{13}C
1	40.4 (CH_2)	1.13 (td, *J* = 13.2, 3.0) 1.35 (brd, *J* = 13.2)	40.8 (CH_2)	40.5 (CH_2)	1.13 (td, *J* = 12.9, 3.3) 1.37 (m)	40.8 (CH_2)
2	18.4 (CH_2)	1.50–1.62 (m)	19.8 (CH_2)	18.4 (CH_2)	1.50–1.62 (m)	18.8 (CH_2)
3	38.7 (CH_2)	2.15 (brd, *J* = 9.0) 1.50–1.62 (m)	44.6 (CH_2)	41.6 (CH_2)	2.02 (brd, *J* = 12.8) 1.50–1.62 (m)	42.7 (CH_2)
4	57.8 (C)		51.1 (C)	61.1 (C)		55.8 (C)
5	45.7 (CH)	1.50–1.62 (m)	49.8 (CH)	46.8 (CH)	obscured	48.3 (CH)
6	23.2 (CH_2)	1.50–1.62 (m) 2.08 (brd, *J* = 13.2)	22.9 (CH_2)	23.1 (CH_2)	1.60 (m) 2.10 (brd, *J* = 12.5)	22.9 (CH_2)
7	38.8 (CH)	2.47 (brs)	39.6 (CH)	39.2 (CH)	2.49 (brs)	39.3 (CH)
8	23.2 (CH_2)	1.72 (tt, *J* = 13.8, 3.0) 1.77 (brd, *J* = 13.8)	23.6 (CH_2)	23.3 (CH_2)	1.69–1.80 (m) 1.69–1.80 (m)	23.5 (CH_2)
9	40.4 (CH_2)	1.11 (dt, *J* = 13.2, 3.0) 1.39 (td, *J* = 13.2, 3.0)	41.8 (CH_2)	40.5 (CH_2)	obscured	40.9 (CH_2)
10	35.0 (C)		35.2 (C)	35.0 (C)		35.4 (C)
11	146.2 (C)		147.0 (C)	146.1 (C)		146.3 (C)
12	111.6 (CH_2)	4.87 (brs) 4.92 (brs)	110.7 (CH_2)	111.3 (CH_2)	4.91 (brs) 4.97 (brs)	111.2 (CH_2)
13	23.4 (CH_3)	1.77 (brs)	22.3 (CH_3)	22.7 (CH_3)	1.80 (brs)	22.8 (CH_3)
14	19.0 (CH_3)	0.97 (s)	18.7 (CH_3)	18.8 (CH_3)	0.94 (s)	19.2 (CH_3)
15	19.1 CH_3)	1.31 (s)	22.9 (CH_3)	21.9 (CH_3)	1.31 (s)	20.7 (CH_3)

[a] Coupling constants (*J* value) are given in Hz. [b] The results of DEPT experiments are given in parentheses. [c] Data for the more abundant *E*-isomer.

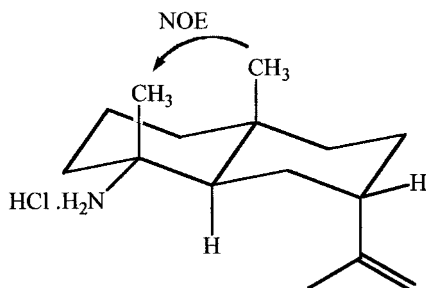

Figure 3. Stereostructure of compound **1**.

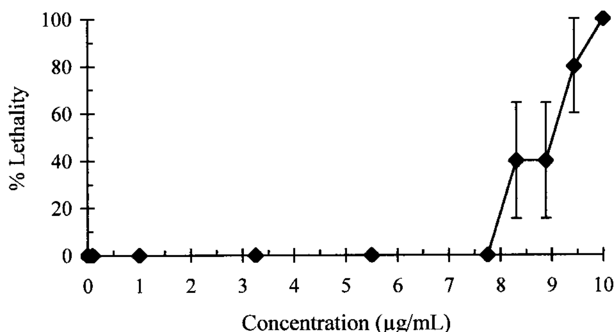

Figure 4. Lethality of compound **1** against polyps of the coral *Madracis mirabilis* in the laboratory. Coral branches were exposed to various concentrations of compound **1** for 2 h. The number of polyps in a 1 cm^2 area of each branch, which were still contracted 48 h after changing the compound solutions for CFW, were counted. Data are mean (±1 standard error) percent of dead polyps on five coral branches.

at a concentration of 7.75 μg/mL there was no death; the LD_{50} was estimated as 9.4 μg/mL.

Toxicity against corals could represent a preliminary indicator of the role of **1** in repelling predators.[11] Detailed studies on a possible use of compound **1** by the sponge in allelopathic interactions against corals in nature are underway in our laboratory.

In contrast to compound **1**, coral polyps exposed to the free amine **2**, all retracted after 24 h, but 40%–70% recovered after 48 h at a concentration of 10 μg/mL (data not shown). These results may indicate that the sponge could be using a mechanism of salt formation (incorporation of chloride) in order to increase its chemical defense ability. In general, compounds having halogen atoms seem to show greater toxicities than compounds lacking them.[12]

Experimental Section

General Experimental Procedures. The optical rotations were measured on a JASCO DIP-360 polarimeter. IR spectra were recorded on a Perkin-Elmer FT-IR Poragon 500 spectrophotometer. NMR spectra were measured in $CDCl_3$ on a Bruker DRX-600 or DRX-500 instrument using TMS as internal standard. FABMS and EIMS were obtained on JEOL JMS-AX505H and Shimadzu QP-5050 spectrometers, respectively. The following adsorbents were used for purification: column chromatography under vacuum, Merck Kieselgel 60 HF 254; column chromatography, Merck Kieselgel 60 (70–230 mesh); analytical TLC, Merck Kieselgel 60 F_{254}. TLC chromatograms were visualized by spraying with 5% phosphomolybdic acid in EtOH followed by heating.

Sponge Material. Several individuals of the marine sponge *Axinyssa ambrosia* were collected by scuba at Santa Marta Bay on the Caribbean Coast of Colombia, at depths of 20–26 m, in November 1999, and were kept frozen until used. Voucher specimens have been deposited at the Instituto de Ciencias Naturales, Museo de Historia Natural of the Universidad Nacional de Colombia at Bogotá [ICN-MHN(Po) 0173], and at the Instituto de Investigaciones Marinas y Costeras-INVEMAR at Santa Marta (INV.POR 0522). A detailed taxonomic description will be reported elsewhere.[13]

Extraction and Isolation. The sponge (1.0 kg, wet wt) was extracted with acetone–methanol (1:1) at room temperature for 24 h. The extract was concentrated and the resulting residue was subjected to silica gel chromatography under vacuum using 250 mL each of hexane, hexanes–ethyl acetate (11:1), hexanes–ethyl acetate (5:3), and methanol in a discontinuous gradient for elution. The fraction that eluted with methanol was repeatedly fractionated on silica gel using $CHCl_3$–MeOH (85:15) to yield 15 mg of pure compound **1**.

The fraction that eluted with hexane containing isothiocyanate compounds as monitored by HRGC–MS was subjected to low-pressure column chromatography with hexane as mobile phase. One hundred fractions (one mL each) were collected. Combined fractions 25–30 were further purified by preparative HPLC with a Ultrasphere ODS column (3 μm, 4.6 × 75 mm) using CH_3CN–H_2O (85:15) as mobile phase, at a flow rate of 0.5 mL/min, to give 3 mg of pure compound **9** and 10 mg of compound **6**.

The fraction that eluted with hexane−ethyl acetate (11:1) was further fractionated on silica gel using a discontinuous gradient of 1000 mL of hexane−ethyl acetate (25:1) and 250 mL of ethyl acetate. One hundred fractions (10 mL each) of hexanes−ethyl acetate were collected. Combined fractions 55−85 and 86−106 contained isonitriles, and the fraction eluted with ethyl acetate contained formamides as monitored by HRGC−MS. One part of the isonitrile mixture (combined fractions 55−85) was subjected first to low-pressure silica gel (30−60 μm) column chromatography with hexane−ethyl acetate (25:1) and finally to preparative TLC using the solvent system mentioned above to yield 3.8 mg of pure compound **3**. The other part of the combined fractions 55−85 was subjected to a silica gel column using a gradient system hexane to hexane−ethyl acetate (25:1) and further purification by TLC with hexane−ethyl acetate (25:1) as solvent system to obtain 5 mg of pure compound **8**. On the other hand, combined fractions 86−106 were first purified by silica gel column chromatography with 100 mL of hexane−ethyl acetate (25:1). Fractions 21−26 were found to be enriched in compound **5**, which was finally purified by TLC using the solvent system just mentioned above to give 10 mg of pure isonitrile **5**. The formamide mixture was rechromatographed on silica gel (30−60 μm) with hexane−ethyl acetate (1:5). Five hundred fractions were collected (10 mL each). The combined fractions 207−213 were finally separated by preparative HPLC using an ALTEX Ultrasphere ODS column (3 μm, 4.6 × 75 mm) with CH_3CN-H_2O (85:15) as mobile phase, at a flow rate of 0.7 mL/min, to give 3, 3, and 15 mg of pure compounds **10**, **4**, and **7**, respectively.

(4*R**,5*R****,7*S****,10*R****)-Eudesm-11-en-4-ylamine hydrochloride (1):** mp 210−220 °C (partially turned brown about 195 °C); $[\alpha]_D$ −17.2° (*c* 1.0, $CHCl_3$); IR (KBr) ν_{max} 3383, 2932, 2048, 1614, 1515 cm^{-1}; NMR data, see Table 1; EIMS *m*/*z* 221 $[M - HCl]^+$ (4), 206 (10), 204 (2), 189 (1), 178 (8), 149 (1), 136 (1), 110 (2), 84 (18), 70 (100), 57 (60), 41 (18); positive FABMS *m*/*z* 222 $[MH - HCl]^+$ (100), 205 $[MH - HCl - NH3]^+$ (80); HRFABMS *m*/*z* 222.2176 (calcd for $C_{15}H_{28}N$ 222.2222); negative FABMS *m*/*z* 127, 129 $[Cl + \text{glycerine matrix}]^-$ (3:1), 35, 37 $[Cl]^-$ (3:1).

(4*R**,5*R****,7*S****,10*R****)-4-Aminoeudesm-11-ene (2).** A solution of **1** (10 mg) in $CHCl_3$ (5 mL) was washed with saturated aqueous $NaHCO_3$ twice. The separated $CHCl_3$ layer was dried over Na_2SO_4 and then concentrated to give an oily free amine **2** (7 mg): $[\alpha]_D$ −17.5° (*c* 0.9, $CHCl_3$); IR (KBr) ν_{max} 3376, 2928, 2847, 1640 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.95, 0.96 (3H, each s, H_3-14 and H_3-15), 1.75 (3H, s, H_3-13), 2.00 (1H, dq, J = 14.0, 2.2 Hz, Heq-6), 2.44 (m, H-7), 4.85, 4.91 (1H, each s, H_2-12); ^{13}C NMR data (125 MHz) are listed in Table 1; EIMS *m*/*z* 222 $[MH]^+$ (6), 221 $[M]^+$ (3), 206 (4), 204 (7), 194 (5), 178 (7), 166 (3), 149 (7), 136 (6), 123 (7), 109 (5), 95 (5), 84 (18), 70 (100), 57 (50), 41 (24); HREIMS *m*/*z* 221.2112 (calcd for $C_{15}H_{27}N$, 221.2143). A part of compound **2** was dissolved in $CHCl_3$ and treated with 2 N HCl in a separator funnel. The separated $CHCl_3$ layer was dried over Na_2SO_4 and concentrated to give a HCl salt, which was identical to compound **1**.

(4*R**,5*R****,7*S****,10*R****)-4-Isocyanatoeudesm-11-ene (3):** colorless oil; $[\alpha]_D$ −15.0° (*c* 0.20, $CHCl_3$); IR ($CHCl_3$) ν_{max} 3070, 2933, 2125, 1642, 1447, 1387 cm^{-1}; NMR data, see Table 1; EIMS *m*/*z* 231 $[M]^+$ (1), 216 (52), 204 (12), 189 (30), 175 (10), 161 (37), 147 (21), 143 (11), 133 (31), 123 (67), 109 (68), 107 (60), 105 (48), 81 (94), 67 (73), 55 (67), 41 (100); HRFABMS *m*/*z* 232.2029 (calcd for $C_{16}H_{26}N$, 232.2065).

(4*R**,5*R****,7*S****,10*R****)-4-Formamidoeudesm-11-ene (4):** white crystals; mp 104−107 °C (from MeOH); $[\alpha]_D$ −10.0° (*c* 0.22, $CHCL_3$); IR ($CHCl_3$) ν_{max} 3317, 2929, 1670, 1540, 1458, 1387 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) (major trans isomer) δ 1.00 (3H, s, H-14), 1.22 (3H, s, H-15), 1.70 (3H, s, H-12), 2.44 (1H, br s, H-7), 4.78, 4.92 (2H, each, br s, H-13), 5.46 (1H, br d, J = 12.5 Hz, NH), 8.21 (1H, d, J = 12.4 Hz, CHO); (minor cis isomer) δ 0.99 (3H, s, H-14), 1.27 (3H, s, H-15), 1.72 (3H, s, H-12), 2.44 (1H, br s, H-7), 4.83, 4.93 (2H, each, br s, H-13), 5.60 (1H, br s, NH), 8.04 (1H, s, CHO); for ^{13}C NMR data (125 MHz), see Table 1; EIMS *m*/*z* 249 $[M]^+$ (2), 234 (1), 220 (1), 204 (100), 189 (88), 175 (12), 161 (74), 147 (25), 135 (20), 133 (38), 122 (32), 121 (22), 107 (43), 105 (43), 98 (77), 93 (42), 81 (42), 70 (53), 55 (52), 41 (81); HRFABMS *m*/*z* 250.2193 (calcd for $C_{16}H_{28}NO$ 250.2171).

11-Formamido-7*β*-H-eudesm-5-ene (10): ^{13}C NMR ($CDCl_3$, 125 MHz) (major trans isomer) δ 40.95 (C-1), 17.62 (C-2), 33.45 (C-3), 38.99 (C-4), 152.12 (C-5), 119.18 (C-6), 44.93 (C-7), 20.06 (C-8), 39.17 (C-9), 34.43 (C-10), 55.80 (C-11), 27.16 (C-12), 26.73 (C-13), 25.11 (C-14), 22.29 (C-15), 162.99 (C-16).

Cytotoxicity Evaluation Against Cancer Cells. The cytotoxicity tests were performed in vitro by Biomar S. A. (León, Spain), using P-388 (mouse lymphoma), A-549 (human lung carcinoma), and HT-29 (human colon carcinoma) cells and methanol-DMSO (9:1) as solvent for compound **1**. Compounds **2**−**10** were not tested.

Toxicity Against Coral. Branches of the Scleractinarian coral *Madracis mirabilis* were collected at Nenguange Bay at depths of 1−3 m and kept in the laboratory in aquaria with aerated, filtered seawater (FSW). The animals were acclimatized for 2−3 days and fed daily with brine shrimp (*Artemia salina*) nauplii.[11] A partitioning of the sponge crude extract with methanol and its major component axinyssamine hydrochloride **1** were initially evaluated against the coral at a volumetric equivalent of the sponge natural concentration (the sponge volume was measured by displacement, to calculate the volumetric concentration of the extract and pure substances in the body of the sponge). Individual coral branches were placed in separate containers with 300 mL of FSW in which fractions and pure compounds had been dissolved with the aid of a small amount of 96% EtOH; controls included FSW with no additions and FSW with solvent only with three replicates being used for each treatment. After 2 h of exposure, the water was replaced by fresh FSW and the behavior of the polyps followed up to 24 h. Polyps were counted on 1 cm^2 areas of the sides and tips, and the percentage of retracted polyps was calculated. Since lethality of all polyps was obtained at both fraction and pure compound in their natural concentrations (all polyps on controls were expanded after 24 h), a further series of experiments with pure compound **1** were carried out at 0.10, 1.00, 3.25, 5.50, 7.75, 8.31, 8.88, 9.43, and 10.0 μg/mL. These were carried out as above, but placing five coral branches in a single container with 200 mL of FSW for each concentration and for solvent and pure FSW controls. Polyps were followed for up to 48 h, and the mean percentage of retracted polyps was calculated from the five subsample branches. An assay with the free amine compound **2** was also carried out but only at 10.0 μg/mL concentration.

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(13) The taxonomy of *Axinyssa ambrosia* is being reviewed by S. Zea, and R. W. M. van Soest and will be published elsewhere.

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