Isothiocyanate Sesquiterpenes from a Sponge of the Genus *Axinyssa*

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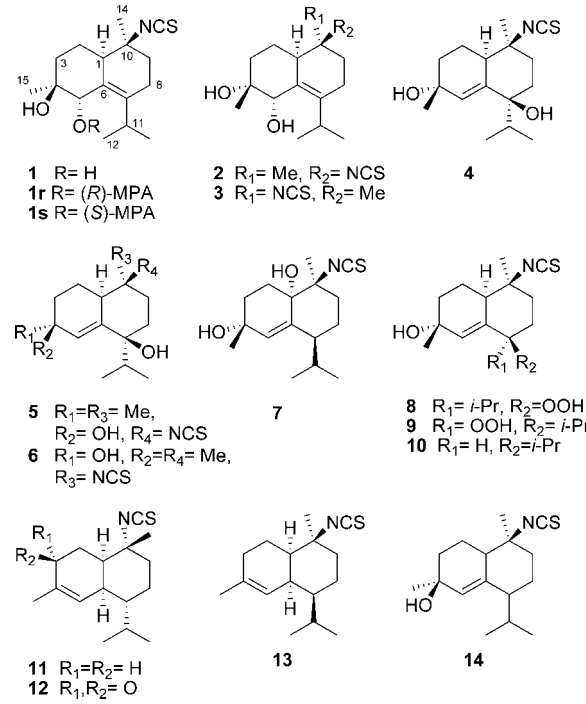
The chemical study of a sponge of the genus *Axinyssa* collected in the Gulf of California has led to the isolation of the new bicyclic sesquiterpenes axinisothiocyanates A−L (**1**−**12**) together with the known compounds (1*R*,6*S*,7*S*,10*S*)-10-isothiocyanato-4-amorphene (**13**), (4*R**,5*R**,7*S**,10*R**)-4-isocyanoeudesm-11-ene, (−)-epipolasin A, and (+)-aristolone. The structures of the new metabolites have been established by spectroscopic techniques, including the analysis of pyridine-induced ^{1}H NMR chemical shifts. The cytotoxic activity has been tested against three human tumor cell lines.

Sesquiterpenes containing isocyano, isothiocyanate, or formamide functionalities are secondary metabolites frequently found in sponges of the orders Axinellida and Halichondrida and their associated opisthobranch mollusks.[1−4] From a structural point of view, these sesquiterpenes exhibit an array of skeletal types, and with a few exceptions, the nitrogenous-containing group is the only hetero function present in the molecule. Active research on the biosynthesis of terpene isocyanides and related compounds in sponges has demonstrated the incorporation of cyanide and thiocyanate ions into various isocyano, isothiocyanate, and thiocyanate metabolites, although the biochemical source of the cyanide/thiocyanate employed by sponges has not yet been disclosed.[3] Biological activities such as antihelmintic,[5] antimicrobial,[5,6] and cytotoxic[7,8] properties have been ascribed to some of these nitrogen-containing sesquiterpenes, although the most significant results have been described in the antifouling[9,10] and antimalarial[11] areas.

As a part of our project aimed on searching for new bioactive metabolites from sponges, we have investigated specimens of a sponge of the genus *Axinyssa* collected in the Gulf of California (Mexico). Herein we report the isolation, structure determination, and cytotoxic activity of the new cadinane-type sesquiterpenes axinisothiocyanates A−L (**1**−**12**). These metabolites, characterized as possessing an isothiocyanate group and various oxygenated functions, extend the uncommon group of nitrogen-containing sesquiterpenes that exhibit additional hetero functions.[5,10,12,13]



Results and Discussion

Freeze-dried specimens of *Axinyssa* sp. were extracted with acetone/MeOH (1:1), and the resulting residue was partitioned between H_2O and Et_2O. The organic extract was subjected to column chromatography eluted with hexanes/Et_2O mixtures of increasing polarities, then $CHCl_3$/MeOH mixtures, and finally MeOH. Repeated separation of fractions eluted with hexanes/Et_2O mixtures afforded the new sesquiterpenoids **1**−**12** along with the known compounds (1*R*,6*S*,7*S*,10*S*)-10-isothiocyanato-4-amorphene (**13**),[5] (4*R**,5*R**,7*S**,10*R**)-4-isocyanoeudesm-11-ene,[7] (−)-epipolasin A,[14] and (+)-aristolone.[15]

Axinisothiocyanate A (**1**) possessed the molecular formula $C_{16}H_{25}NO_2S$, determined by HRCIMS. The presence of an isothiocyanate function was deduced from a strong IR absorption at 2103 cm^{-1} and a broad signal in the ^{13}C NMR spectrum at δ 131.5 (Table 1). The remaining 15 resonances of the ^{13}C NMR spectrum together with the ^{1}H NMR signals of two methyl groups [δ 1.43 (s, Me-14), δ 1.31 (s, Me-15)] and an isopropyl group [δ 1.04 (3H, d, J = 6.8 Hz, Me-13), 1.01 (3H, d, J = 6.8 Hz, Me-12), 3.07 (1H, sept, J = 6.8 Hz, H-11)] suggested a sesquiterpenoid framework. The NMR spectra included signals corresponding to a tetrasubstituted double bond [δ_C 142.5 (C, C-7) and 127.9 (C, C-6)] and two carbons linked to oxygenated functions [δ_C 72.5 (CH, C-5)/δ_H 4.47 (1H, s, H-5) and δ_C 71.6 (C, C-4)], whereas the resonance at δ_C 62.9 (C, C-10) was assigned to a fully substituted carbon bearing the isothiocyanate group. Diagnostic HMBC correlations were those of the oxymethine proton (δ 4.47, H-5) with the methylene at δ_C 32.0 (C-3), the methyl at δ_C 26.0 (Me-15), the oxygenated carbon at δ_C 71.6 (C-4), the methine at δ_C 41.7 (C-1), and the olefinic carbon at δ_C142.5 (C-7), which in turn correlated with the methyls of the isopropyl group [δ_H 1.04 (Me-13) and 1.01 (Me-12)]. These data defined the presence of the moiety −CH_2−C(CH_3)(OH)−CH(OH)−C(CH)=C(i-Pr)− in the molecule. The olefinic carbon bearing the isopropyl group was further connected to a −CH_2−CH_2−C(CH_3)(NCS)− moiety based on the HMBC correlations of the carbon at δ 142.5 (C-7) with two methylene protons at δ 2.08 (H-8) and 1.99 (H-9); the latter one also correlated with the carbon bearing the isothiocyanate group (δ_C 62.9, C-10) and the remaining methyl of the molecule (δ_C 27.3, Me-14). All these

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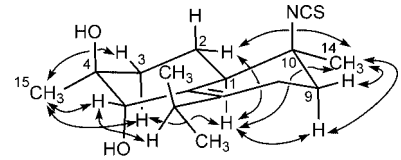
Table 1. NMR Spectroscopic Data (600 MHz, $CDCl_3$) for Compound **1**[a]

position	δ_C	δ_H (J in Hz)	HMBC
1	41.7	2.39 br d (12.5)	
2	23.5	1.87 dddd (13.4, 4.5, 4.5, 2.7)eq	C-3
		1.50 m ax	C-1, C-3, C-10
3	32.0	1.92 ddd (13.7, 13.4, 4.5)ax	C-1, C-2
		1.60 m eq	C-4
4	71.6		
5	72.5	4.47 s	C-1, C-3, C-4, C-7, C-15
6	127.9		
7	142.5		
8	20.9	2.24 dddd (18.1, 9.4, 5.3, 2.7)ax	
		2.08 dddd (18.1, 5.3, 5.1, 1.5)eq	C-6, C-7, C-9, C-10
9	34.7	1.99 ddd (13.1, 5.3, 5.1)eq	C-1, C-7, C-8, C-10, C-14
		1.63 ddd (13.1, 9.4, 5.3)ax	C-1, C-7, C-8,
10	62.9		
11	28.7	3.07 sept (6.8)	C-6, C-7, C-12, C-13
12	21.6	1.01 d (6.8)	C-7, C-11, C-13
13	21.0	1.04 d (6.8)	C-7, C-11, C-12
14	27.3	1.43 s	C-1, C-9, C-10
15	26.0	1.31 s	C-3, C-5
−NCS	131.5		

[a] Assignments aided by COSY, HSQC, HMBC, and NOESY experiments.

data indicated that axinisothiocyanate A (**1**) was a cadinane-type sesquiterpene containing hydroxyl groups at C-4 and C-5, a double bond at C-6, C-7, and the isothiocyanate group at C-10. The NOESY correlations of Me-15 with H-3eq and H-3ax and those of Me-14 with H-9eq and H-9ax indicated an equatorial orientation of both Me-15 and Me-14, whereas the correlations of H-1 with H-3ax and H-9ax indicated an axial orientation of H-1 (Figure 1, energy minimized using MM2 calculations). The proton H-5 exhibited NOEs only with Me-15 and the isopropyl group, suggesting an equatorial orientation of H-5 and, therefore, an axial orientation of the hydroxyl group at C-5. This assignment was confirmed from the pyridine-induced solvent shifts in the ^{1}H NMR spectrum. Pyridine interaction with hydroxyl groups through hydrogen bonding and collision complex associations causes a significant deshielding of protons occupying positions geminal, vicinal, and 1,3-diaxial to a hydroxyl function.[16,17] In particular, protons 1,3-diaxial to a hydroxyl group have been described to experience deshielding effects on the order 0.2–0.4 ppm relative to chloroform.[16] The ^{1}H NMR spectrum of **1** in C_5D_5N exhibited the H-1 resonance at δ 2.76, that is, 0.37 ppm downfield shifted with respect to the H-1 resonance in $CDCl_3$. These data indicated that the hydroxyl at C-5 was 1,3-diaxial to H-1. Similarly, the H-2ax resonance was 0.58 ppm downfield shifted in C_5D_5N relative to $CDCl_3$, in agreement with the axial orientation of the hydroxyl group at C-4. The absolute configuration of **1** was assigned by derivatization with (*R*)- and (*S*)-α-methoxy-α-phenylacetic (MPA) acids to yield the diastereomeric esters **1r** and **1s**, respectively.[18] Positive chemical shift differences ($\Delta\delta = \delta_R - \delta_S$) were observed for Me-15, H-3eq, and H-3ax (+0.37, +0.11, and +0.07 ppm, respectively), whereas negative $\Delta\delta$ values were obtained for H-1, Me-14, H-9ax, H-9eq, H-8ax, and H-8eq (−0.55, −0.30, −0.34, −0.15, −0.10, and −0.12 ppm, respectively). These data indicated an *S* configuration[18] for C-5 and therefore an absolute configuration 1*R*,4*S*,5*S*,10*S* for axinisothiocyanate A (**1**).

The molecular formula $C_{16}H_{25}NO_2S$ determined by HRCIMS analysis of axinisothiocyanates B (**2**) and C (**3**), together with the similarities of their NMR spectra with those of **1**, suggested that **2** and **3** were stereoisomers of **1**. The correlations observed for H-1, H-5, and Me-14 in the NOESY spectrum of **2** were similar to those



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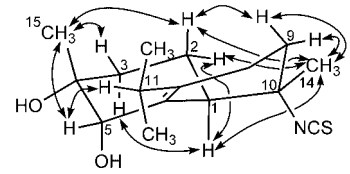

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Figure 1. Selected NOESY correlations for compounds **1** and **3**.

described for **1**, indicating that both compounds had the same relative configuration at C-1, C-5, and C-10. However, the Me-15 in **2** showed NOESY correlations with H-2ax and H-3eq, which indicated the β-axial orientation of Me-15. Further support for the relative configuration of **2** was obtained from the comparison of the ^{1}H NMR data recorded in $CDCl_3$ and C_5D_5N. In particular, H-1 was shifted by 0.30 ppm in C_5D_5N relative to $CDCl_3$, in agreement with a 1,3-diaxial relationship between H-1 and the hydroxyl at C-5. On the contrary, there was not a significant difference between the chemical shifts of H-2ax in $CDCl_3$ and C_5D_5N, as expected from the equatorial orientation of the hydroxyl group at C-4. All these data defined that axynisothiocyanate B (**2**) was the C-4 epimer of compound **1**. With regard to compound **3**, the NOESY spectrum exhibited the correlations H-1/H-2eq, H-3ax, Me-15/H-2ax, H-3eq, and H-5/Me-15, H-11, which defined the α-axial orientation of H-1, the β-axial orientation of Me-15, and the β-equatorial orientation of H-5, respectively (Figure 1). Therefore, compound **3** displayed the same relative configuration as **2** at C-1, C-4, and C-5. Consequently, both compounds had to differ by their configuration at C-10. The upfield shift of H-2ax (δ 1.02) and the downfield shift of H-1 (δ 2.66) in **3** with respect to **2** [δ(H-2ax) 1.37, δ(H-1) 2.42] were consistent with an α-orientation of the isothiocyanate group. The NOESY correlations Me-14/H-1, H-2ax, H-2eq, H-9ax, H-9eq and H-2ax/H-9ax supported this proposal and also indicated that ring B in **3** adopts a conformation different from that of **1** and **2**, which allows the β-oriented Me-14 to be equatorial (Figure 1).

Axinisothiocyanate D (**4**) also possessed the molecular formula $C_{16}H_{25}NO_2S$, determined by HRCIMS. The IR spectrum showed the presence of hydroxyl (3416 cm^{-1}) and isothiocyanate (2120 cm^{-1}) functions. The NMR data (Table 2) were consistent with a cadinane-related sesquiterpene containing a trisubstituted double bond [δ_C 139.0 (C) and δ_C 131.2 (CH)/δ_H 5.94 (dd, J = 1.5, 1.5 Hz)] and three fully substituted sp^3 carbons, two of them linked to hydroxyl groups [δ_C 74.5 (C) and 69.0 (C)] and the remaining one bearing the isothiocyanate function [δ_C 65.5 (C)]. In the HMBC spectrum, the olefinic proton (δ 5.94, H-5) exhibited correlations with the methyl group at δ_C 28.2 (Me-15), with the methine at δ_C 43.5 (C-1), and with the hydroxylated carbon at δ_C 74.5 (C, C-7), which in turn was correlated with the methyl groups of the isopropyl unit [δ_H 0.96 (Me-13) and 0.77 (Me-12)]. On the other hand, the olefinic methine carbon (δ 131.2, C-5) exhibited HMBC correlations with the methine proton at δ 2.08 (m, H-1), which in turn was correlated with the carbon bearing the isothiocyanate group (δ 65.5, C-10). These and the remaining correlations defined the location of the double bond at C-5,C-6, the hydroxyl groups at C-4 and C-7, and the isothiocyanate function at C-10 of the bicyclic framework. The overlap of signals in the ^{1}H NMR spectrum in $CDCl_3$, and in particular of those corresponding to Me-14 and Me-15, complicated unambiguous assignment of the NOESY correla-

Table 2. NMR Spectroscopic Data for Compounds **4**, **7**, **8**, and **11**[a]

	4[b]		**7**[c]		**8**[d]		**11**[b]	
position	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)
1	43.5	2.08 m	72.6		45.1	2.08 ddd (9.2, 6.6, 2.0)	45.4	1.85 br d (12.8)
2	21.7	1.99 dddd (13.4, 5.7, 5.7, 3.4)eq 1.72 m ax	31.1	2.19 ddd (14.0, 14.0, 3.4)ax 2.09 ddd (14.0, 4.4, 3.8)eq	22.2	2.02 m eq 1.72 dddd (13.5, 13.5, 9.2, 3.3)ax	19.8	1.57 m eq 1.50 m ax
3	36.0	1.92 m eq 1.51 ddd (12.3, 12.3, 3.4)ax	34.6	2.37 m ax 2.00 dddd (12.3, 4.4, 3.4, 1.5)eq	36.5	1.97 m eq 1.56 m ax	30.9	2.00 m
4	69.0		69.1[e]		69.2		133.5	
5	131.2	5.94 dd (1.5, 1.5)	134.2	5.95 dd (1.8, 1.5)	133.1	5.87 dd (2.0, 1.7)	124.2	5.57 dq (5.6, 1.3)
6	139.0		137.9		133.6		35.2	2.26 ddd (11.0, 5.6, 5.6)
7	74.5		41.9	2.45 dddd (12.9, 4.5, 3.4, 1.8)	86.1		43.5	1.27 dddd (11.3, 11.0, 2.8, 2.8)
8	33.2	2.08 m eq 1.63 ddd (14.0, 14.0, 3.4)ax	22.2	1.59 dddd, (13.1, 4.5, 4.0, 3.0)eq 1.48 dddd (13.1, 13.1, 12.9, 3.5)ax	25.0	2.51 ddd (14.2, 14.2, 4.3)ax 1.95 m eq	20.3	1.50 m eq 1.37 m ax
9	36.7	1.92 m eq 1.69 m ax	35.4	2.35 ddd (13.5, 13.1, 4.0)ax 1.72 ddd (13.5, 3.5, 3.0)eq	36.2	2.02 m eq 1.68 ddd (14.2, 14.2, 3.6)ax	34.3	1.77 br d (13.0)eq 1.48 m ax
10	65.5		69.2[e]		64.5		65.7	
11	29.7	1.90 m	26.9	1.97 sept d (6.7, 3.4)	31.8	1.95 sept (6.9)	26.6	2.00 m
12	16.3	0.77 d (6.9)	22.1	0.80 d (6.7)	16.6	0.86 d (6.9)	21.5	0.90 d (6.9)
13	15.7	0.96 d (6.9)	17.6	0.86 d (6.7)	16.3	1.01 d (6.9)	15.3	0.87 d (6.9)
14	26.8	1.41 s	22.1	1.46 s	26.5	1.39 s	27.5	1.39 s
15	28.2	1.41 s	28.3	1.71 s	28.2	1.46 s	23.4	1.65 br s
−NCS	130.5		129.8		nd		130.0	
−OH				6.88 (s)				
−OOH						7.21 s		

[a] Assignments aided by COSY, HSQC, HMBC, and NOESY experiments. [b] Recorded in $CDCl_3$ at 400 MHz. [c] Recorded in C_5D_5N at 600 MHz. [d] Recorded in $CDCl_3$ at 600 MHz. [e] Signals marked with the same letter in the same column may be interchanged.

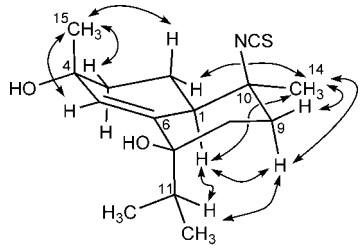

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Figure 2. Selected NOESY correlations for compound **4**.

tions. However, the ^1H NMR spectrum recorded in C_5D_5N exhibited a better dispersion of signals, and the NOESY spectrum in this solvent was analyzed to define the relative configuration of **4**. The correlations H-11/H-1, H-9ax and H-1/H-9ax indicated 1,3-diaxial relationships among the isopropyl group, H-1, and H-9ax (Figure 2, energy minimized using MM2 calculations). The α-equatorial orientation of Me-14 was defined from the NOESY correlations Me-14/H-1, H-2eq, H-9eq, H-9ax, whereas the correlations Me-15/H-2ax, H-3eq indicated the *β*-axial orientation of Me-15. All these data defined the relative configuration 1*R**,4*R**,7*S**,10*S** of axinisothiocyanate D (**4**).

The HRCIMS analysis of axinisothiocyanates E (**5**) and F (**6**) established that both compounds were isomeric with those above-described. In particular, the COSY and HMBC correlations indicated that **5** and **6** possessed a planar structure identical to that of compound **4**. Similar to **4**, the NOESY spectrum of **5** in C_5D_5N showed correlations consistent with an α-axial orientation of H-1 and the isopropyl group (H-11/H-1, H-9ax and H-1/H-9ax) and with an α-equatorial orientation of Me-14 (Me-14/H-1, H-2eq, H-9ax, H-9ec). Therefore, compound **5** had to differ from **4** by the configuration at C-4 and possesses the Me-15 α-equatorially oriented. Unfortunately the close chemical shifts of Me-15 and H-3ax in C_5D_5N, and also in $CDCl_3$, precluded the observation of the expected NOE between Me-15 and H-3ax in the NOESY and 1D-NOESY experiments. Nevertheless, the higher chemical shift of H-2ax in C_5D_5N relative to $CDCl_3$ by 0.29 ppm fully supported the axial orientation of the hydroxyl at C-4 and therefore the equatorial position of Me-15. On the other hand, the NOESY spectrum of **6** indicated that it was the C-10 epimer of **4** exhibiting the correlations Me-14/H-2ax, H-8ax, H-9eq that supported the *β*-axial orientation of Me-14.

Axinisothiocyanate G (**7**) was the seventh isomer of this series of sesquiterpenes of molecular formula $C_{16}H_{25}NO_2S$. The NMR spectra of **7** recorded in $CDCl_3$ and also in C_5D_5N (Table 2) were related to those of compounds **4**−**6**, displaying the resonances of a trisubstituted double bond [δ_C 137.9 (C) and δ_C 134.2 (CH)/δ_H 5.95 (dd, J = 1.8, 1.5 Hz) in C_5D_5N], and those attributable to two hydroxylated carbons and one linked to the isothiocyanate group [δ_C 72.6 (C), 69.2 (C), and 69.1 (C)]. The location of the double bond at C-5,C-6 of the cadinane framework was inferred from the allylic coupling of the olefinic proton [δ_H 5.95 (dd, J = 1.8, 1.5 Hz)] with a methine proton at δ 2.45 (dddd, J = 12.9, 4.5, 3.4, 1.8 Hz, H-7) whose corresponding carbon [δ_C 41.9 (CH, C-7)] exhibited HMBC correlations with the methyl groups of the isopropyl unit [δ 0.86 (Me-13) and 0.80 (Me-12)]. On the other hand, the hydroxylated carbon at δ 72.6 (C, C-1) showed HMBC correlations with the olefinic proton H-5 and with a methyl group at δ_H 1.46 (s, Me-14), which additionally exhibited a weak four-bond correlation with the carbon atom of the isothiocyanate group (δ_C 129.8). These data unambiguously defined the location of the isothiocyanate group at C-10 and of the hydroxyl group at C-1. The remaining hydroxyl function of the molecule had therefore to be placed at C-4, geminal to the methyl at δ_H 1.71 (s, Me-15). The large coupling constant

(12.9 Hz) between H-7 and H-8ax indicated the α-axial orientation of H-7 and, therefore, a *β*-equatorial orientation of the isopropyl group. This assignment was further supported by NOESY correlations H-11/H-5, Me-12/H-8eq, and Me-13/H-5, H-8ax. The NOESY correlations Me-14/H-2eq, H-9ax, H-9eq and Me-15/H-2ax, H-3eq defined the α-equatorial orientation of Me-14 and the *β*-axial orientation of Me-15, respectively. The relative configuration at C-1 was deduced from the NOESY correlation of the proton of the hydroxyl group at C-1 [*δ* 6.88 (s)] with H-9ax and H-2eq. This assignment was further supported by the pyridine-induced shifts of protons 1,3 with respect to the hydroxyl at C-1. Thus, H-7 and H-9ax were deshielded in C_5D_5N relative to $CDCl_3$ by 0.31 and 0.24 ppm, respectively, in agreement with the α-axial orientation of the hydroxyl at C-1. All these data defined the configuration 1*S**,4*R**,7*S**,10*S** for axinisothiocyanate G (**7**).

The molecular formula of axinisothiocyanate H (**8**), $C_{16}H_{25}NO_3S$, indicated that **8** possessed the same degree of unsaturation as the compounds described above but with an additional oxygen atom. These data together with the ^{1}H NMR resonance at δ_H 7.21 (1H, s), devoid of correlations in the HSQC spectrum, indicated that **8** contained a hydroperoxide function. Furthermore, the ^{13}C NMR resonance at δ_C 86.1 (C) was attributed to a fully substituted carbon bearing the hydroperoxy group. The COSY and HMBC correlations defined for **8** a planar structure identical to that of compounds **4**−**6**, except for the presence of the hydroperoxy group at C-7. In particular, this assignment was supported by the HMBC correlations of the carbon at *δ* 86.1 (C, C-7) with the olefinic proton (*δ* 5.87, H-5) and the methyl groups of the isopropyl unit [*δ* 1.01 (Me-13) and 0.86 (Me-12)]. The NOESY correlations of H-1 with Me-14 and H-11, of Me-15 with H-2ax and H-3eq, and of H-5 with Me-15 and the hydroperoxide proton indicated that **8** possessed the same relative configuration as compound **4**.

The NMR spectra of axinisothiocyanate I (**9**) also exhibited the resonances of a hydroperoxide function [δ_H 7.05 (1H, br s)] linked to a fully substituted carbon [δ_C 85.6 (C, C-7)] as well as those of a hydroxylated carbon [δ_C 70.0 (C, C-4)], a carbon bearing the isothiocyanate group [δ_C 60.1 (C, C-10)], and a trisubstituted double bond [δ_C 138.2 (CH, C-5)/δ_H 5.92 (br s, H-5) and δ_C 132.0 (C, C-6)]. The COSY and HMBC correlations defined for **9** the same planar structure as **8**. Furthermore, the NOESY correlations H-1/H-2eq, H-9ax, Me-15/H-2ax, H-3eq, and Me-14/H-1, H-2eq, H-9ax, H-9eq confirmed that **9** possessed the same relative configuration as **8** at C-1, C-4, and C-10, respectively. Therefore, the stereochemical difference between **9** and **8** had to be at C-7. In fact, the NOESY correlation between the olefinic proton H-5 and H-11 supported the *β*-equatorial orientation of the isopropyl group.

The molecular formula of axinisothiocyanate J (**10**), $C_{16}H_{25}NOS$, was determined by HRCIMS. The most distinctive difference between the NMR spectra of **10** and those of compounds **4**−**6** described above was the absence of the signal due to the hydroxylated carbon C-7, showing the signals of a methine at δ_C 47.4 (CH)/δ_H 1.69 (m) instead. Further confirmation was obtained from the HMBC correlations of this methine carbon with the olefinic proton H-5 [*δ* 5.57 (d, *J* = 1.0 Hz)] and the methyl groups of the isopropyl unit [*δ* 0.98 (d, *J* = 6.8 Hz) and 0.92 (d, *J* = 6.8 Hz)]. The relative configuration 1*R**,4*R**,7*S**,10*S** of compound **10** was assigned from NOESY data. Due to the overlapping of signals, the NOESY spectra in $CDCl_3$ and in C_5D_5N were analyzed. The NOESY spectrum in C_5D_5N exhibited correlations of H-1 with H-7 and H-9ax, which indicated 1,3-diaxial relationships among H-1, H-7, and H-9ax. The *β*-equatorial orientation of the isopropyl group was further supported from the correlations H-11/H-5 and Me-13/H-8ax observed in the NOESY in $CDCl_3$. The correlations Me-14/H-1, H-2eq, H-9eq, H-9ax in C_5D_5N defined the α-equatorial orientation of Me-14, whereas the *β*-axial orientation of Me-15 was deduced from the correlation Me-15/H-2ax observed in the NOESY spectrum in $CDCl_3$. The sesquiterpene **14**, closely related to **10**, had been previously described from the sponge *Axinyssa fenestratus*.[5] Compound **14** was proposed to possess Me-14 and Me-15 α-equatorial on the basis of ^{13}C NMR chemical shifts, while the configuration at C-1 and C-7 was not determined. The ^{13}C NMR data reported for **14** (in $CDCl_3$) are almost identical to those of **10** (except for signals assigned in ref 5 to C-11 and Me-15, C-1 and C-7, which could be interchanged), suggesting that **14** should be revised to **10**, for which the relative configuration has been unambiguously defined. Surprisingly, the partial ^{1}H NMR data (C_6D_6) reported for **14** are significantly different from those recorded for **10** in C_6D_6, and we therefore cannot conclude if compound **14** isolated from *A. fenestratus* is identical or not to axinisothiocyanate J (**10**).

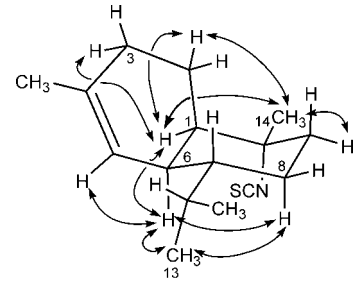

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Figure 3. Selected NOESY correlations for compound **11**.

Axinisothiocyanate K (**11**) possessed the molecular formula $C_{16}H_{25}NS$, determined by HRCIMS. The IR absorption at 2096 cm^{-1} and a broad ^{13}C NMR signal at *δ* 130.0 confirmed the presence of an isothiocyanate group. The remaining 15 resonances of the ^{13}C NMR spectrum (Table 2) were attributable to a cadinane-type sesquiterpene containing a trisubstituted double bond [*δ* 133.5 (C) and 124.2 (CH)] and a fully substituted carbon linked to the isothiocyanate function [*δ* 65.7 (C)]. In particular, the analysis of the COSY and HMBC correlations indicated that **11** possessed the same planar structure as compound **13** from *A. fenestratus*[5] and two cadinanes from *Stylissa* sp.[4c] and *Acanthella cavernosa*,[19] respectively. The relative configuration 1*R**,6*S**,7*R**,10*R** of compound **11** was determined from the analysis of the coupling constants and the NOESY data. Thus, the NOESY correlation H-1/H-6 together with the $J_{H\text{-}1,H\text{-}6}$ coupling constant of 5.6 Hz indicated the *cis* (equatorial-axial) orientation of these protons (Figure 3, energy minimized using MM2). The 11.0 Hz coupling between H-6 and H-7 defined the *trans*-1,2-diaxial relationship between these protons and, therefore, the equatorial orientation of the isopropyl group at C-7. These assignments were also supported by the NOESY correlations H-6/H-8ax and H-6/Me-13. Furthermore, the spatial arrangement deduced for the *cis*-fused bicycle of **11** (Figure 3) exhibits a dihedral angle between H-5 and H-6 of 45°, which is consistent with the observed coupling constant $J_{H\text{-}5,H\text{-}6}$ of 5.6 Hz. It is worth noting that this spatial arrangement is different from that of the *cis*-fused rings of compound **13** (H-6 1,3-diaxial to H-2ax and H-1 1,3-diaxial to H-9ax), where the dihedral angle H-5,H-6 is close to 90° and, consequently, the coupling constant $J_{H\text{-}5,H\text{-}6}$ is almost zero. Finally, the NOESY correlations of Me-14 with H-1, H-2eq, and H-9eq defined the *β*-equatorial orientation of Me-14.

The molecular formula of axinisothiocyanate L (**12**), $C_{16}H_{23}NOS$, together with the IR absorption at 2088 cm^{-1} suggested that **12** was also an isothiocyanate sesquiterpene. In addition to a fully substituted carbon bearing the nitrogenous function [δ_C 64.3 (C, C-10)], the NMR spectra showed the resonances of a carbonyl [δ_C 197.6 (C)] and a trisubstituted double bond at δ_C 135.2 (C) and δ_C 149.3 (CH)/δ_H 6.96 (dq, *J* = 6.4, 1.4 Hz), that together with a vinylic methyl group at δ_H 1.81 (dd, *J* = 1.4, 1.0 Hz) indicated the presence of an α-methyl-α,*β*-unsaturated ketone moiety. The olefinic proton showed a COSY correlation with the methine proton

at δ 2.62 (H-6) and an HMBC correlation with a methine at δ_C 44.8 (C-1), whose corresponding proton (δ 2.41, H-1) was correlated with the carbonyl carbon. These data supported the presence of the cyclohexenone ring depicted in formula **12**. The remaining NMR data were accommodated in a six-membered ring bearing the isothiocyanate function and the isopropyl group at the same positions as in the previously described compounds. The coupling constants of H-6 with H-1, H-5, and H-7 (4.3, 6.4, and 10.2 Hz, respectively) together with the NOESY correlations Me-14/H-1, H-2eq, H-9eq indicated that **12** possessed the same relative configuration as **11**. Among the different stereochemical classes of the cadinane-related sesquiterpenoids, compounds **11** and **12** belong to the muurolane class, characterized by possessing H-1, H-6, and the isopropyl group *cis*-oriented.

Compounds **1**−**8** and **10**–**13** isolated from *Axinyssa* sp. were tested in cytotoxicity assays against the human tumor cell lines MDA-MB-231 (breast adenocarcinoma), A-549 (lung adenocarcinoma), and HT-29 (colon adenocarcinoma). Compounds **2** and **10**−**13** showed moderate activity, whereas compounds **1** and **3**−**8** were not active at the highest concentration tested (10 μg/mL). In particular, the most active compound was axinisothiocyanate J (**10**), which exhibited GI_{50} values of 10.4, 11.8, and 11.8 μM and TGI values of 14.3, 19.0, and 14.0 μM against MDA-MB-231, A-549, and HT-29 cell lines, respectively. Compounds **2**, **11**, and **12** exhibited growth inhibitory activity of A-549 and HT-29 cells with GI_{50} values ranging from 21.0 to 34.5 μM [GI_{50} (μM) for **2**: 25.7 (A-549) and 21.0 (HT-29); for **11**: 34.5 (A-549) and 22.8 (HT-29); for **12**: 31.4 (A-549) and 31.4 (HT-29)]. Finally, compound **13** inhibited the growth of the A-549 cell line with a GI_{50} value of 33.4 μM.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a Perkin-Elmer 241 polarimeter. UV spectra were recorded on a GBC Cintra-101 spectrometer. IR spectra were recorded on a Perkin-Elmer FT-IR System Spectrum BX spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded on a Varian INOVA 600 or on a Varian INOVA 400 spectrometer using $CDCl_3$, C_5D_5N, or C_6D_6 as solvent. ^{1}H and ^{13}C chemical shifts were referenced using the corresponding solvent signals [δ_H 7.26 and δ_C 77.0 for $CDCl_3$, δ_H 7.55 and δ_C 135.5 for C_5D_5N, and δ_H 7.15 and δ_C 128.0 for C_6D_6]. COSY, HSQC, HMBC, and NOESY experiments were performed using standard Varian pulse sequences. Low-resolution mass spectra were recorded on a Finnigan Voyager GC8000^{top} spectrometer. High-resolution mass spectra (HRMS) were obtained on a Autospec-Q spectrometer. Column chromatography was carried out on Merck silica gel 60 (70–230 mesh). HPLC separations were performed on a LaChrom-Hitachi apparatus equipped with LiChrospher Si-60 (Merck) columns in normal phase and LiChrosorb RP-18 columns in reversed phase, using a RI-71 differential refractometer. All solvents were spectral grade or were distilled prior to use.

Collection and Identification. Specimens of *Axinyssa* sp. (family Halichondriidae, order Halichondrida) were collected by hand using scuba at the Gulf of California and immediately frozen. The sponge is an undescribed species whose morphological features are typical of the genus *Axinyssa*. It is an incrusting to massive globular amorphous, sometimes lobate, yellow sponge, with a fleshy and compressible consistency in life, but crumbly when dry. The largest specimen measured 17 cm long by 12 cm wide and 5 cm high. The surface in some parts is smooth, but generally is conulose-microconulose or hispid due to the terminal part of the choanosomal fibers that form brushes of spicules on the surface. Oscules are from 1 to 5 mm in diameter and scattered irregularly on the surface. Aquiferous canals are visible around some oscula. The ectosome is thin, membranous, partly detachable, and without a specialized skeleton, but with some spicules arranged criss-cross or paratangentially to the surface. The choanosomal skeleton is largely confused, with some vague ascending single spicules or tracts ending at the surface in bouquets. The spicules are curved oxeas, stylotes, and some strongyles in a wide range of sizes but difficult to divide into categories. The oxeas are 320-(619.5)-720 × 5-(10)-15 μm, strongyles measure 475-(526)−625 × 9-(9.6)-10 μm, and stylotes measure 480-(520)-590 × 6-(8.8)-10 μm. The species is relatively common in vertical walls, small caves, and overhangs at depths between 4 and 25 m from Isabel Island (Nayarit, Mexico, 21°50′33″ N 105°53′10″ W), the only locality where the species has been found. A voucher has been deposited in the Sponge Collection of the UNAM under the code LEB-ICML-UNAM-56.

Extraction and Isolation. Freeze-dried specimens of the sponge *Axinyssa* sp. (280.5 g) were repeatedly extracted with acetone/MeOH (1:1, 5 × 3 L) at room temperature. After filtration, the solution was evaporated under reduced pressure to obtain a residue that was partitioned between H_2O and Et_2O. The organic layer was evaporated to dryness to give an extract (18.1 g), which was chromatographed on a SiO_2 column using solvents of increasing polarities from hexanes/Et_2O (95:5) to Et_2O, then $CHCl_3$/MeOH (80:20 and 50:50), and finally MeOH. The fraction eluted with hexanes/Et_2O (95:5) was chromatographed over a silica gel column using hexane and hexanes/Et_2O mixtures (99:1 to 80:20) as eluants. Repeated purifications of selected fractions by normal-phase HPLC using hexanes and hexanes/EtOAc (99:1) yielded (−)-epipolasin A[14] (110.5 mg, 3.9 × 10^{-2} % dry wt), compound **11** (30.0 mg, 1.1 × 10^{-2} % dry wt), compound **13** (85.0 mg, 3.0 × 10^{-2} % dry wt), and 4-isocyanoeudesm-11-ene[7] (70.0 mg, 2.5 × 10^{-2} % dry wt). The fraction of the general chromatography eluted with hexanes/Et_2O (90:10) was further separated over a silica gel column using hexanes/Et_2O mixtures (92:8 to 80:20) as eluants. Repeated separations of selected fractions by normal-phase HPLC (hexanes/EtOAc, 92:8) afforded compound **12** (27.0 mg, 9.6 × 10^{-3} % dry wt). The fraction of the general chromatography eluted with hexanes/Et_2O (80:20) was subjected to column chromatography eluted with hexanes/Et_2O mixtures (90:10 to 50:50) to yield (+)-aristolone[15] (440 mg, 0.157% dry wt). The fraction of the general chromatography eluted with hexanes/Et_2O (70:30) was chromatographed over a silica gel column eluted with hexanes/Et_2O mixtures (85:15 to 60:40). Subsequent purification of selected fractions by normal-phase HPLC (hexanes/EtOAc 70:30) yielded compounds **5** (11.0 mg, 3.9 × 10^{-3} % dry wt) and **10** (21.0 mg, 7.4 × 10^{-3} % dry wt). The fractions of the general chromatography eluted with hexanes/Et_2O (50:50 and 30:70) and Et_2O were joined and chromatographed over a silica gel column eluted with hexanes/Et_2O mixtures (30:70 and 20:80) and then Et_2O. The fraction eluted with hexanes/Et_2O (30:70) was further separated by HPLC in normal (hexanes/EtOAc, 70:30) and reversed phase (MeOH/H_2O, 80:20) to yield compounds **1** (8.0 mg, 2.9 × 10^{-3} % dry wt) and **3** (2.0 mg, 7.1 × 10^{-4} % dry wt). The fraction eluted with hexanes/Et_2O (20:80) was subjected to repeated HPLC separations in normal phase (hexanes/EtOAc, 60:40) and reversed phase (acetone/H_2O, 75:25, MeOH/H_2O, 80:20 or 65:35) to obtain compounds **8** (11.0 mg, 3.9 × 10^{-3} % dry wt), **4** (27.0 mg, 9.6 × 10^{-3} % dry wt), **2** (15.5 mg, 5.5 × 10^{-3} % dry wt), **7** (1.5 mg, 5.3 × 10^{-4} % dry wt), **6** (2.5 mg, 8.9 × 10^{-4} % dry wt), and **9** (1.0 mg, 3.6 × 10^{-4} % dry wt).

Axinisothiocyanate A (1): white solid; $[\alpha]^{25}_D$ −33.0 (*c* 0.1, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 243 (3.13) nm; IR (film) ν_{max} 3416, 2103, 1656 cm^{-1}; ^{1}H NMR ($CDCl_3$, 600 MHz) see Table 1; ^{1}H NMR (C_5D_5N, 600 MHz) δ 6.23 (br s, OH), 4.89 (1H, s, H-5), 3.22 (1H, sept, J = 6.9 Hz, H-11), 2.76 (1H, br d J = 12.5 Hz, H-1), 2.27 (1H, ddd, J = 13.5, 13.5, 4.3 Hz, H-3ax), 2.15 (1H, m, H-8), 2.08 (1H, dddd, J = 13.5, 12.5, 12.5, 3.7 Hz, H-2ax), 1.89 (2H, m, H-8, H-9), 1.87 (1H, m, H-2eq), 1.80 (1H, br ddd, J = 13.5, 3.7, 2.8 Hz, H-3eq), 1.69 (3H, s, Me-15), 1.48 (1H, m, H-9), 1.29 (3H, s, Me-14), 1.00 (3H, d, J = 6.9 Hz, Me-12), 0.90 (3H, d, J = 6.9 Hz, Me-13); ^{13}C NMR ($CDCl_3$, 150 MHz) see Table 1; EIMS *m/z* 295 (6) $[M]^+$, 280 (3), 252 (42), 236 (36), 218 (20), 179 (44), 175 (78), 107 (75), 79 (74), 55 (100); HRCIMS(+) *m/z* 295.1602 (calcd for $C_{16}H_{25}NO_2S$, 295.1606).

Axinisothiocyanate B (2): white solid; $[\alpha]^{25}_D$ −2.0 (*c* 0.1, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 238 (3.03) nm; IR (film) ν_{max} 3392, 2097, 1660 cm^{-1}; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 4.52 (1H, s, H-5), 3.04 (1H, sept, J = 6.7 Hz, H-11), 2.42 (br d, J = 12.5 Hz, H-1), 2.22 (1H, dddd, J = 18.3, 10.3, 5.3, 3.0 Hz, H-8ax), 2.07 (1H, dddd, J = 18.3, 5.3, 3.8, 1.6 Hz, H-8eq), 1.98 (1H, ddd, J = 13.1, 13.1, 4.4, H-3ax), 1.98 (1H, m, H-9eq), 1.92 (1H, dddd, J = 13.1, 4.4, 4.4, 2.8 Hz, H-2eq), 1.63 (1H, m, H-3eq), 1.63 (1H, ddd, J = 13.0, 10.3, 5.3 Hz, H-9ax), 1.42 (3H, s, Me-14), 1.37 (1H, m, H-2ax), 1.20 (3H, s, Me-15), 1.02 (3H, d, J = 6.7 Hz, Me-12), 1.02 (3H, d, J = 6.7 Hz, Me-13); ^{1}H NMR (C_5D_5N, 600 MHz) δ 4.81 (1H, s, H-5), 3.07 (1H, sept, J = 6.9 Hz, H-11), 2.72 (1H, br d, J = 12.5 Hz, H-1), 2.38 (1H, ddd, J = 13.4, 13.4, 4.3 Hz, H-3ax), 2.12 (1H, dddd, J = 17.9, 9.7, 5.2, 2.8 Hz, H-8ax), 1.89 (2H, m, H-2eq, H-8eq), 1.79 (1H, ddd, J = 12.8, 5.2, 5.0 Hz, H-9eq), 1.75 (1H, m, H-3eq), 1.46 (2H, m, H-2ax, H-9ax), 1.37

(3H, s, Me-15), 1.22 (3H, s, Me-14), 0.93 (3H, d, $J = 6.9$ Hz, Me-13), 0.87 (3H, d, $J = 6.9$ Hz, Me-12); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 140.8 (C, C-7), 130.6 (C, NCS), 127.7 (C, C-6), 72.7 (C, C-4), 72.6 (CH, C-5), 62.8 (C, C-10), 42.1 (CH, C-1), 35.2 (CH_2, C-9), 32.8 (CH_2, C-3), 28.8 (CH, C-11), 27.3 (CH_3, Me-14), 24.7 (CH_2, C-2), 23.7 (CH_3, Me-15), 21.5 (CH_3, Me-12), 20.6 (CH_2, C-8), 20.6 (CH_3, Me-13); EIMS *m/z* 295 (15) $[M]^+$, 280 (11), 277 (12), 262 (11), 252 (72), 236 (83), 234 (27), 219 (25), 218 (51), 179 (79), 175 (100); HRCIMS(+) *m/z* 295.1604 (calcd for $C_{16}H_{25}NO_2S$, 295.1606).

Axinisothiocyanate C (3): white solid; $[\alpha]^{25}_D$ +57.0 (*c* 0.1, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 239 (3.05) nm; IR (film) ν_{max} 3406, 2091 cm^{-1}; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 4.55 (1H, s, H-5), 3.00 (1H, sept, $J = 6.8$ Hz, H-11), 2.66 (1H, br d, $J = 12.7$ Hz, H-1), 2.22 (1H, dddd, $J = 18.1$, 10.1, 5.8, 2.0 Hz, H-8ax), 2.08 (1H, dddd, 18.1, 5.5, 4.1, 1.3 Hz, H-8eq), 1.97 (1H, ddd, $J = 13.3$, 13.3, 4.4 Hz, H-3ax), 1.80 (1H, dddd, $J = 13.1$, 5.8, 4.1, 1.0 Hz, H-9eq), 1.75 (1H, dddd, $J = 12.4$, 4.4, 4.4, 2.9 Hz, H-2eq), 1.64 (1H, dddd, $J = 13.3$, 4.0, 2.9, 1.0 Hz, H-3eq), 1.58 (1H, ddd, $J = 13.1$, 10.1, 5.5 Hz, H-9ax), 1.34 (3H, s, Me-14), 1.12 (3H, s, Me-15), 1.06 (3H, d, $J = 6.8$ Hz, Me-12), 1.02 (1H, m, H-2ax), 0.99 (3H, d, $J = 6.8$ Hz, Me-13); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 139.3 (C, C-7), 128.5 (C, C-6), 73.0 (CH, C-5), 72.7 (C, C-4), 62.5 (C, C-10), 42.9 (CH, C-1), 34.6 (CH_2, C-3), 32.2 (CH_2, C-9), 28.8 (CH, C-11), 25.9 (CH_2, C-2), 25.5 (CH_3, Me-14), 23.7 (CH_3, Me-15), 21.4 (CH_3, Me-12), 21.0 (CH_2, C-8), 21.0 (CH_3, Me-13); HRCIMS(+) *m/z* 295.1592 (calcd for $C_{16}H_{25}NO_2S$, 295.1606).

Axinisothiocyanate D (4): colorless oil; $[\alpha]^{25}_D$ +90.0 (*c* 0.1, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 242 (3.19) nm; IR (film) ν_{max} 3416, 2120 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) see Table 2; ^{1}H NMR (C_5D_5N, 600 MHz) δ 6.67 (1H, dd, $J = 1.8$, 1.2 Hz, H-5), 5.89 (s, OH), 2.16 (1H, ddd, $J = 13.7$, 3.5, 3.2 Hz, H-8eq), 2.14 (1H, m, H-1), 2.04 (1H, dddd, $J = 12.6$, 4.9, 3.6, 1.2 Hz, H-3eq), 1.94 (1H, m, H-2eq), 1.94 (1H, ddd, $J = 14.2$, 13.7, 3.8 Hz, H-8ax), 1.89 (1H, sept, $J = 6.9$ Hz, H-11), 1.84 (1H, ddd, $J = 12.6$, 12.6, 3.6 Hz, H-3ax), 1.74 (1H, ddd, $J = 14.2$, 3.8, 3.2 Hz, H-9eq), 1.72 (1H, m, H-2ax), 1.64 (1H, ddd, $J = 14.2$, 14.2, 3.5 Hz, H-9ax), 1.63 (3H, s, Me-15), 1.32 (3H, s, Me-14), 1.09 (3H, d, $J = 6.9$ Hz, Me-13), 0.97 (3H, d, $J = 6.9$ Hz, Me-12); ^{13}C NMR ($CDCl_3$, 125 MHz) see Table 2; EIMS *m/z* 295 (3) $[M]^+$, 252 (22), 234 (28), 218 (27), 203 (26), 202 (49), 175 (100); HRCIMS(+) *m/z* 295.1600 (calcd for $C_{16}H_{25}NO_2S$, 295.1606).

Axinisothiocyanate E (5): colorless oil; $[\alpha]^{25}_D$ +30.0 (*c* 0.1, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 237 (3.50) nm; IR (film) ν_{max} 3407, 2092 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.05 (1H, dd, $J = 1.4$, 1.4 Hz, H-5), 2.11 (1H, m, H-8), 2.08 (1H, m, H-1), 1.94 (3H, m, H_2-2, H-9), 1.89 (2H, m, H-3, H-11), 1.68 (2H, m, H-8, H-9), 1.41 (3H, s, Me-14), 1.39 (1H, m, H-3), 1.32 (3H, s, Me-15), 0.98 (3H, d, $J = 6.8$ Hz, Me-13), 0.71 (3H, d, $J = 6.8$ Hz, Me-12); ^{1}H NMR (C_5D_5N, 600 MHz) δ 6.60 (1H, dd, $J = 1.6$, 1.4 Hz, H-5), 2.23 (1H, dddd, $J = 13.0$, 12.6, 9.1, 3.5, H-2ax), 2.16 (1H, ddd, $J = 13.4$, 3.3, 3.3 Hz, H-8eq), 2.06 (1H, m, H-3eq), 2.04 (1H, ddd, $J = 9.1$, 6.4, 1.6 Hz, H-1), 1.98 (1H, ddd, $J = 14.0$, 13.4, 3.3 Hz, H-8ax), 1.89 (1H, sept, $J = 6.8$ Hz, H-11), 1.80 (1H, dddd, $J = 13.0$, 6.4, 5.5, 3.5, H-2eq), 1.74 (1H, ddd, $J = 14.0$, 3.8, 3.3 Hz, H-9eq), 1.66 (1H, ddd, $J = 14.0$, 14.0, 3.5 Hz, H-9ax), 1.47 (3H, s, Me-15), 1.46 (1H, ddd, $J = 13.0$, 12.6, 3.5 Hz, H-3ax), 1.35 (3H, s, Me-14), 1.11 (3H, d, $J = 6.8$ Hz, Me-13), 0.94 (3H, d, $J = 6.8$ Hz, Me-12); ^{13}C NMR (C_5D_5N, 150 MHz) δ 141.1 (C, C-6), 131.5 (CH, C-5), 130.9 (C, NCS), 74.0 (C, C-7), 66.6 (C, C-10), 66.2 (C, C-4), 44.0 (CH, C-1), 36.7 (CH_2, C-9), 36.5 (CH_2, C-3), 33.2 (CH_2, C-8), 31.5 (CH_3, Me-15), 30.4 (CH, C-11), 27.1 (CH_3, Me-14), 21.0 (CH_2, C-2), 17.0 (CH_3, Me-12), 16.3 (CH_3, Me-13); EIMS *m/z* 277 (3) $[M - H_2O]^+$, 234 (49), 175 (100); HRCIMS(+) *m/z* 295.1597 (calcd for $C_{16}H_{25}NO_2S$, 295.1606).

Axinisothiocyanate F (6): colorless oil; $[\alpha]^{25}_D$ −73.0 (*c* 0.1, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 240 (3.05) nm; IR (film) ν_{max} 3390, 2080, 1668 cm^{-1}; ^{1}H NMR (C_5D_5N, 600 MHz) δ 6.56 (1H, d, $J = 1.8$ Hz, H-5), 5.60 (s, OH), 2.49 (1H, ddd, $J = 7.0$, 7.0, 1.8 Hz, H-1), 2.14 (1H, ddd, $J = 13.9$, 3.6, 3.6 Hz, H-8eq), 2.09 (1H, dddd, $J = 13.9$, 7.0, 7.0, 4.4 Hz, H-2eq), 2.00 (1H, sept, $J = 6.8$ Hz, H-11), 1.94 (1H, ddd, $J = 13.9$, 13.3, 3.6 Hz, H-9ax), 1.85 (1H, m, H-3), 1.82 (1H, m, H-3), 1.76 (1H, ddd, $J = 13.3$, 3.6, 3.6 Hz, H-9eq), 1.69 (1H, dddd, $J = 13.9$, 9.6, 7.0, 4.4 Hz, H-2ax), 1.56 (1H, ddd, $J = 13.9$, 13.9, 3.6 Hz, H-8ax), 1.43 (3H, s, Me-15), 1.25 (3H, s, Me-14), 1.09 (3H, d, $J = 6.8$ Hz, Me-13), 0.96 (3H, d, $J = 6.8$ Hz, Me-12); ^{13}C NMR (C_5D_5N, 150 MHz) δ 138.8 (C, C-6), 133.1 (CH, C-5), 74.1 (C, C-7), 67.5 (C, C-4), 66.0 (C, C-10), 43.9 (CH, C-1), 37.8 (CH_2, C-9), 36.9 (CH_2, C-3), 33.0 (CH_2, C-8), 30.3 (CH, C-11), 29.5 (CH_3, Me-15), 21.6 (CH_2, C-2), 21.4 (CH_3, Me-14), 17.1 (CH_3, Me-12), 16.3 (CH_3, Me-13); CIMS *m/z* 295 (1) $[M]^+$, 277 (2), 252 (100), 236 (47), 219 (31), 204 (59), 193 (96), 175 (97); HRCIMS *m/z* 295.1601 (calcd for $C_{16}H_{25}NO_2$, 295.1606).

Axinisothiocyanate G (7): colorless oil; $[\alpha]^{25}_D$ +69.0 (*c* 0.1, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 240 (3.66) nm; IR (film) ν_{max} 3390, 2101, 1660 cm^{-1}; ^{1}H NMR (C_5D_5N, 600 MHz) see Table 2; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 5.61 (1H, br s, H-5), 2.14 (1H, m, H-7), 2.11 (2H, m, H-2, H-9ax), 2.08 (1H, m, H-11), 1.87 (2H, m, H-2, H-3eq), 1.80 (1H, ddd, $J = 13.8$, 3.5, 3.3 Hz, H-9eq), 1.71 (1H, m, H-3ax), 1.69 (1H, m, H-8eq), 1.45 (1H, dddd, $J = 13.2$, 13.2, 13.2, 3.8 Hz, H-8ax), 1.40 (3H, s, Me-15), 1.39 (3H, s, Me-14), 0.99 (3H, d, $J = 6.8$ Hz, Me-12), 0.91 (3H, d, $J = 6.8$ Hz, Me-13); ^{13}C NMR (C_5D_5N, 150 MHz) see Table 2; EIMS *m/z* 295 (3) $[M]^+$, 280 (4), 252 (19), 236 (89), 234 (34), 219 (15), 201 (15), 193 (56), 183 (56), 175 (48), 165 (100); HRCIMS(+) *m/z* 296.1668 [M + H] (calcd for $C_{16}H_{26}NO_2S$, 296.1684).

Axinisothiocyanate H (8): colorless oil; $[\alpha]^{25}_D$ +42.0 (*c* 0.1, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 241 (3.64) nm; IR (film) ν_{max} 3386, 2102 cm^{-1}; ^{1}H NMR ($CDCl_3$, 600 MHz) see Table 2; ^{13}C NMR ($CDCl_3$, 150 MHz) see Table 2; HRCIMS(+) *m/z* 311.1564 (calcd for $C_{16}H_{25}NO_3S$, 311.1555).

Axinisothiocyanate I (9): colorless oil; $[\alpha]^{25}_D$ +34.0 (*c* 0.1, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 238 (3.07) nm; IR (film) ν_{max} 3376, 2084 cm^{-1}; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.05 (1H, br s, OOH), 5.92 (1H, br s, H-5), 2.64 (1H, sept, $J = 6.9$ Hz), 2.42 (1H, ddd, $J = 9.5$, 6.3, 2.1 Hz, H-1), 2.05 (1H, dddd, $J = 13.5$, 6.3, 5.0, 3.5 Hz, H-2eq), 1.96 (dddd, $J = 12.9$, 5.0, 3.2, 1.2 Hz, H-3eq), 1.85 (2H, m, H-8, H-9), 1.74 (2H, m, H-8, H-9), 1.70 (1H, dddd, $J = 13.5$, 12.9, 9.5, 3.2 Hz, H-2ax), 1.60 (1H, ddd, $J = 12.9$, 12.9, 3.5 Hz, H-3ax), 1.44 (3H, s, Me-15), 1.37 (3H, s, Me-14), 1.00 (3H, d, $J = 6.9$ Hz, Me-13), 0.99 (3H, d, $J = 6.9$ Hz, Me-12); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 138.2 (CH, C-5), 132.0 (C, C-6), 85.6 (C, C-7), 70.0 (C, C-4), 60.1 (C, C-10), 43.1 (CH, C-1), 36.3 (CH_2, C-3), 34.4 (CH_2, C-9), 27.9 (CH_3, Me-15), 27.7 (CH, C-11), 26.4 (CH_3, Me-14), 23.2 (CH_2, C-8), 21.9 (CH_2, C-2), 18.9 (CH_3, Me-12), 16.2 (CH_3, Me-13); EIMS *m/z* 311 (1) $[M]^+$, 295 (3), 253 (5), 183 (14), 85 (100); HRCIMS(+) *m/z* 278.1559 $[M + H - H_2O_2]^+$ (calcd for $C_{16}H_{24}NOS$, 278.1579).

Axinisothiocyanate J (10): colorless oil; $[\alpha]^{25}_D$ +118.0 (*c* 0.1, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 241 (3.46) nm; IR (film) ν_{max} 3384, 2090, 1659 cm^{-1}; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 5.57 (1H, d, $J = 1.0$ Hz, H-5), 2.15 (1H, sept d, $J = 6.8$, 3.3 Hz, H-11), 2.05 (1H, ddd, $J = 13.5$, 3.2, 3.2 Hz, H-9eq), 2.00 (1H, m, H-1), 1.97 (1H, m, H-2eq), 1.92 (1H, dddd, $J = 12.4$, 5.5, 3.4, 1.0 Hz, H-3eq), 1.73 (1H, m, H-8eq), 1.69 (2H, m, H-2ax, H-7), 1.61 (1H, ddd, $J = 13.5$, 13.2, 3.7 Hz, H-9ax), 1.53 (1H, ddd, $J = 12.4$, 12.4, 3.2 Hz, H-3ax), 1.44 (1H, m, H-8ax), 1.41 (3H, s, Me-15), 1.38 (3H, s, Me-14), 0.98 (3H, d, $J = 6.8$ Hz, Me-12), 0.92 (3H, d, $J = 6.8$ Hz, Me-13); ^{1}H NMR (C_5D_5N, 600 MHz) δ 5.92 (s, OH), 5.87 (br s, H-5), 2.03 (1H, m, H-3eq), 2.00 (1H, m, H-11), 1.90 (1H, m, H-1), 1.87 (1H, m, H-2eq), 1.82 (2H, m, H-3ax, H-9eq), 1.68 (3H, s, Me-15), 1.63 (1H, m, H-2ax), 1.56 (1H, br d, $J = 11.4$ Hz, H-7), 1.50 (1H, m, H-8eq), 1.42 (1H, m, H-9ax), 1.37 (1H, m, H-8ax), 1.18 (3H, s, Me-14), 0.84 (3H, d, $J = 6.9$ Hz, Me-12), 0.83 (3H, d, $J = 6.9$ Hz, Me-13); ^{1}H NMR (C_6D_6, 600 MHz) δ 5.49 (1H, br s, H-5), 1.98 (1H, sept d, $J = 6.8$, 2.5 Hz, H-11), 1.72 (1H, dddd, $J = 12.3$, 5.5, 3.0, 1.1 Hz, H-3eq), 1.51 (1H, m, H-9eq), 1.46 (1H, m, H-2), 1.42 (1H, m, H-2), 1.42 (3H, s, Me-15), 1.35 (1H, m, H-1), 1.38 (1H, m, H-3ax), 1. 32 (3H, m, H-7, H_2-8), 0.91 (1H, m, H-9ax), 0.87 (3H, d, $J = 6.8$ Hz, Me-12), 0.86 (3H, d, $J = 6.8$ Hz, Me-13), 0.80 (3H, s, Me-14); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 137.2 (C, C-6), 130.2 (CH, C-5), 69.3 (C, C-4), 66.0 (C, C-10), 47.4 (CH, C-7), 46.9 (CH, C-1), 40.4 (CH_2, C-9), 36.2 (CH_2, C-3), 28.2 (CH_3, Me-15), 26.8 (CH, C-11), 26.7 (CH_3, Me-14), 22.5 (CH_2, C-8), 22.1 (CH_3, Me-12), 21.6 (CH_2, C-2), 17.5 (CH_3, Me-13); EIMS *m/z* 279 (5) $[M]^+$, 264 (21), 261 (3), 236 (5), 220 (8), 149 (38), 55 (100); HRCIMS(+) *m/z* 279.1658 $[M]^+$ (calcd for $C_{16}H_{25}NOS$, 279.1657).

Axinisothiocyanate K (11): colorless oil; $[\alpha]^{25}_D$ +26.3 (*c* 0.1, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 242 (3.38) nm; IR (film) ν_{max} 2096 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) see Table 2; ^{13}C NMR ($CDCl_3$, 125 MHz) see Table 2; EIMS *m/z* 263 (36) $[M]^+$, 248 (8), 205 (94), 161 (82), 149 (58), 121 (97), 95 (100); HRCIMS (+) *m/z* 263.1703 (calcd for $C_{16}H_{25}NS$, 263.1707).

Axinisothiocyanate L (12): colorless oil; $[\alpha]^{25}_D$ −14.7 (*c* 0.1, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 240 (4.05) nm; IR (film) ν_{max} 2088, 1673 cm^{-1}; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 6.96 (1H, dq, $J = 6.4$, 1.4 Hz, H-5), 2.62 (1H, ddd, $J = 10.2$, 6.4, 4.3 Hz, H-6), 2.41 (1H, dddd, $J = 15.1$, 4.3, 3.9, 1.0 Hz, H-1), 2.40 (1H, dd, $J = 16.4$, 3.9 Hz, H-2eq),

2.34 (1H, dd, $J = 16.4$, 15.1 Hz, H-2ax), 1.92 (1H, hept d, $J = 7.2$, 2.6 Hz, H-11), 1.87 (1H, m, H-9eq), 1.81 (3H, dd, $J = 1.4$, 1.0 Hz, Me-15), 1.59 (2H, m, H-8eq, H-9ax), 1.50 (2H, m, H-7, H-8ax), 1.36 (3H, s, Me-14), 0.96 (3H, d, $J = 7.2$ Hz, Me-13), 0.94 (3H, d, $J = 7.2$ Hz, Me-12); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 197.6 (C, C-3), 149.3 (CH, C-5), 135.2 (C, C-4), 132.0 (C, NCS), 64.3 (C, C-10), 44.8 (CH, C-1), 42.7 (CH, C-7), 36.3 (CH, C-6), 35.8 (CH_2, C-2), 33.8 (CH_2, C-9), 27.7 (CH, C-11), 26.8 (CH_3, Me-14), 21.2 (CH_3, Me-12), 20.2 (CH_2, C-8), 15.9 (CH_3, Me-15), 15.6 (CH_3, Me-13); CIMS *m/z* 277 (7) $[M]^+$, 219 (100); HRCIMS *m/z* 277.1496 $[M]^+$ (calcd for $C_{16}H_{23}NOS$, 277.1500).

Synthesis of the (*R*)-MPA Ester 1r. A solution of **1** (1.5 mg, 5.1 $\times$ 10^{-3} mmol) in 0.3 mL of CH_2Cl_2 was treated with CH_2Cl_2 solutions of *N*,*N*′-dicyclohexylcarbodiimide (5.0 mg, 0.024 mmol in 0.4 mL), *N*,*N*-dimethylaminopyridine (1.0 mg, 8.2 $\times$ 10^{-3} mmol in 0.3 mL), and (*R*)-α-methoxy-α-phenylacetic acid (4.0 mg, 0.024 mmol in 0.4 mL) and stirred at room temperature for 24 h. The reaction mixture was purified using preparative TLC (hexane/EtOAc, 7:3) to obtain 1.3 mg of the (*R*)-MPA ester **1r**: ^{1}H NMR ($CDCl_3$, 600 MHz; selected data, assignments aided by COSY and NOESY experiments) δ 5.58 (1H, s, H-5), 3.18 (1H, sept, $J = 6.9$ Hz, H-11), 2.08 (1H, dddd, $J =$ 18.1, 7.9, 5.3, 2.0 Hz, H-8ax), 1.89 (1H, dddd, $J = 18.1$, 6.6, 5.2, 1.4 Hz, H-8eq), 1.74 (3H, m, H-2eq, H-3ax, H-9eq), 1.64 (m, H-3eq), 1.50 (1H, m, H-1) 1.38 (1H, m, H-2ax), 1.20 (3H, s, Me-15), 1.19 (1H, m, H-9ax), 1.00 (3H, s, Me-14), 0.97 (3H, d, $J = 6.9$ Hz, Me-12), 0.91 (3H, d, $J = 6.9$ Hz, Me-13).

Synthesis of the (*S*)-MPA Ester 1s. A solution of **1** (1.5 mg, 5.1 $\times$ 10^{-3} mmol) in 0.3 mL of CH_2Cl_2 was treated with CH_2Cl_2 solutions of *N*,*N*′-dicyclohexylcarbodiimide (5.0 mg, 0.024 mmol in 0.4 mL), *N*,*N*-dimethylaminopyridine (1.0 mg, 8.2 $\times$ 10^{-3} mmol in 0.3 mL), and (*S*)-α-methoxy-α-phenylacetic acid (4.0 mg, 0.024 mmol in 0.4 mL) and stirred at room temperature for 24 h. The reaction mixture was purified over preparative TLC (hexane/EtOAc, 7:3) to obtain 1.4 mg of the (*S*)-MPA ester **1s**: ^{1}H NMR ($CDCl_3$, 600 MHz; selected data, assignments aided by COSY and NOESY experiments) δ 5.59 (1H, s, H-5), 3.20 (1H, sept, $J = 6.8$ Hz, H-11), 2.18 (1H, dddd, $J = 18.1$, 7.7, 5.5, 2.1 Hz, H-8ax), 2.05 (1H, m, H-1), 2.01 (1H, dddd, $J = 18.1$, 6.6, 5.3, 1.4 Hz, H-8eq), 1.89 (1H, ddd, $J = 12.9$, 6.6, 5.5 Hz, H-9eq), 1.85 (1H, dddd, $J = 13.1$, 4.4, 4.2, 2.6 Hz, H-2eq), 1.67 (1H, ddd, $J =$ 13.9, 13.9, 4.4 Hz, H-3ax), 1.53 (2H, m, H-3eq, H-9ax), 1.46 (1H, m, H-2ax), 1.30 (3H, s, Me-14), 1.00 (3H, d, $J = 6.8$ Hz, Me-12), 0.94 (3H, d, $J = 6.8$ Hz, Me-13), 0.83 (3H, s, Me-15).

Cytotoxicity Assays. Compounds **1**−**8** and **10**−**13** were tested against the human tumor cell lines MDA-MB-231 (breast adenocarcinoma), A-549 (lung adenocarcinoma), and HT-29 (colon adenocarcinoma). A colorimetric assay using sulforhodamine B (SRB) was adapted for quantitative measurement of cell growth and viability as described in the literature.[20]

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Supporting Information Available: ^{1}H and ^{13}C NMR spectra of compounds **1**–**12** and a photograph of the sponge *Axinyssa* sp. This information is available free of charge via the Internet at http://pubs.acs.org.

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