Sesquiterpenes from the Sponge *Axinyssa isabela*

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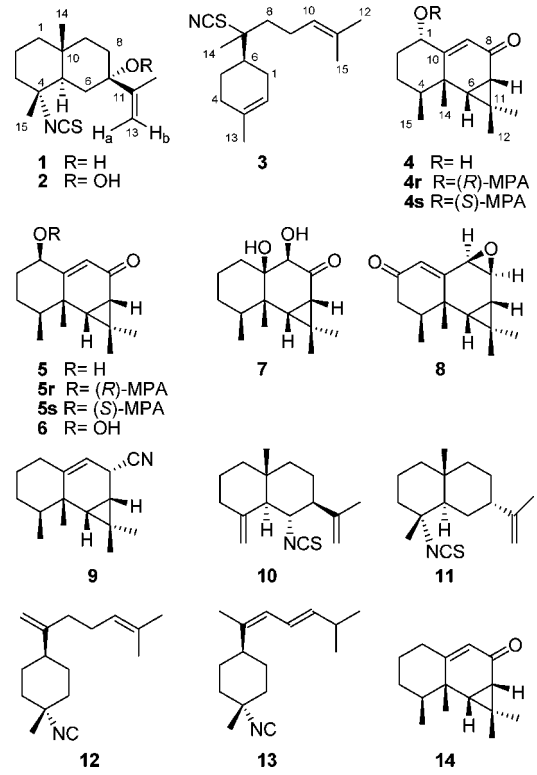
Further research on the constituents of the sponge *Axinyssa isabela* collected in the Gulf of California has led to the isolation of nine new sesquiterpenes, the eudesmanes axinisothiocyanates M and N (**1**, **2**), the bisabolane axinythiocyanate A (**3**), and the aristolane derivatives axinysones A−E (**4**−**8**) and axinynitrile A (**9**), together with four known sesquiterpenoids (**10**−**13**). The structures of the new metabolites have been established by spectroscopic techniques. The absolute configuration of axinysones A (**4**) and B (**5**) has been assigned after esterification with (*R*)- and (*S*)-MPA acids. In addition, the unusual nitrile-containing sesquiterpene **9** has been synthesized from (+)-aristolone (**14**). The cytotoxic activity of the compounds isolated has been tested against three human tumor cell lines.

Chemical studies on sponges of the genus *Axinyssa* (order Halichondrida, family Halichondriidae) have shown that the secondary metabolism of these organisms is dominated by the presence of sesquiterpenes exhibiting a wide range of carbon skeletons and a nitrogenous functionality such as an isocyano, isothiocyanate, thiocyanate, or formamide group.[1,2] In a few instances, these nitrogen-containing metabolites have been described to be accompanied by sesqui- and diterpenes without nitrogenous functionality.[3] In addition, a new family of compounds displaying a sesquiterpene residue linked to a nitrogen-containing fragment, likely of amino acid origin, has recently been described from *A. aplysinoides*.[4] On the other hand, terpenoids from *Axinyssa* species have shown a wide range of biological activities, which include antimicrobial, antifouling, antihelmintic, antimalarial, and cytotoxic properties.[1,2a−c,3d,5]

In a previous account we described the isolation of new cadinane-related metabolites possessing an isothiocyanate group and various oxygenated functions from a sponge of the genus *Axinyssa* collected in the Gulf of California.[5] Meanwhile, the sponge has been classified as the new species *Axinyssa isabela*.[6] Further research on the minor constituents of this sponge has yielded the new sesquiterpenes axinisothiocyanates M (**1**) and N (**2**), axinythiocyanate A (**3**), axinysones A−E (**4**−**8**), and axinynitrile A (**9**), together with the known compounds acanthene B (**10**),[7] **11**,[8] **12**,[9] and 3-isocyanotheonellin (**13**).[10]



Results and Discussion

Freeze-dried specimens of *A. isabela* 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 eluting with hexanes/Et_2O mixtures of increasing polarity, then $CHCl_3$/MeOH, and finally MeOH. Repeated separation of fractions eluted with hexanes/Et_2O mixtures afforded the new sesquiterpenoids **1**−**9** and the known compounds **10**−**13**.

Axinisothiocyanate M (**1**) possessed the molecular formula $C_{16}H_{25}NOS$, determined by HRCIMS. The presence of hydroxyl and isothiocyanate functions was established from the IR absorptions at 3466 and 2082 (broad) cm^{-1}, respectively. The ^1H NMR spectrum displayed the resonances of an isopropenyl unit [δ_H 5.07 (br s, H-13a), 4.85 (dq, J = 1.3, 1.3 Hz, H-13b), 1.85 (br s, Me-12)] and two methyl groups [δ_H 1.31 (s, Me-15) and 0.90 (s, Me-14)] that suggested a sesquiterpene framework. The most distinctive signals of the ^{13}C NMR spectrum were those of the double bond of the isopropenyl group [δ 151.8 (C-11), δ 109.4 (C-13)] and two resonances at δ 74.4 (C, C-7) and 64.9 (C, C-4) that were assigned to two fully substituted sp^3 carbons linked to the hydroxyl and the isothiocyanate groups, respectively. The COSY and HMBC correlations indicated that **1** was a eudesmane sesquiterpene bearing the isothiocyanate at C-4 and the hydroxyl group at C-7. Thus, the carbon linked to the isothiocyanate function (δ_C 64.9, C-4) showed HMBC correlations with the methyl group at δ_H 1.31 (Me-15) and with the methine at δ_H 2.08 (H-5), the carbon of which at δ_C 47.4 (C-5) showed correlations with the methyl group at δ_H 0.90 (Me-14). On the other hand, the location of the hydroxyl function at C-7 was supported by HMBC correlations of the hydroxylated carbon (δ_C 74.4, C-7) with the olefinic protons and the methyl group of the isopropenyl substituent. The relative configuration of compound **1** (4*R**,5*R**,7*S**,10*R**) was deduced from the NOESY spectrum and modeled using MM2 for energy minimization (Figure 1). The NOE interactions of Me-15 with Me-14 and H-6ax indicated the 1,3-diaxial relationship between Me-14 and Me-15 and the

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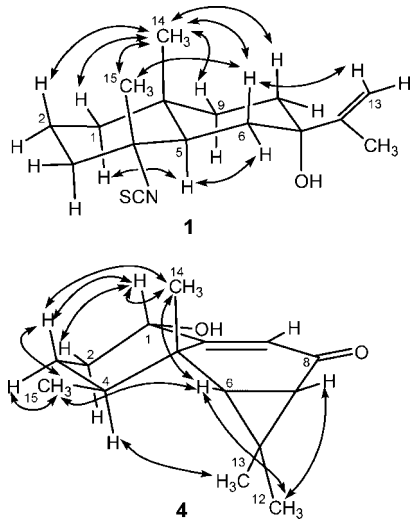


Figure 1. Selected NOESY correlations for compounds **1** and **4**.

trans-fusion of rings. This assignment was further supported by the NOESY correlations Me-14/H-1eq, H-2ax, H-6ax, H-8ax, H-9eq and H-5/H-1ax, H-6eq. Finally, the NOESY correlation of the olefinic proton H-13a with H-6ax indicated the β-equatorial orientation of the isopropenyl group at C-7 and, therefore, the α-axial orientation of the hydroxyl function.

The molecular formula of axinisothiocyanate N (**2**), $C_{16}H_{25}NO_2S$, together with the broad IR absorption at 2090 cm^{-1} indicated that it was also an isothiocyanate sesquiterpene. The NMR spectra of **2** were similar to those of **1** except for the downfield shift of the oxygenated carbon at δ_C 85.2 and the presence of a proton resonance at δ_H 7.32, devoid of correlation in the HSQC spectrum. These data indicated that compound **2** differed from **1** by the presence of a hydroperoxy function. Furthermore, the HMBC correlations of the carbon attached to the isothiocyanate group [δ_C 64.9 (C-4)] with Me-15 [δ_H 1.31 (s)] and H-5 [δ_H 1.97 (dd, J = 13.1, 2.5 Hz)] and those of the oxygenated carbon [δ_C 85.2 (C-7)] with the protons of the isopropenyl group confirmed the location of the isothiocyanate and hydroperoxy functions at C-4 and C-7, respectively. A series of 1D-NOESY experiments revealed the NOE interactions Me-14/Me-15, H-1eq, H-2ax, H-6ax, H-8ax, H-9eq, H-5/H-1ax, H-6eq, and H-13a/H-6ax, H-6eq, which indicated that compound **2** possessed the same relative configuration as **1**.

The molecular formula $C_{16}H_{25}NS$ of axinythiocyanate A (**3**) was determined by HRCIMS analysis. The presence of a thiocyanate function was deduced from the sharp IR absorption at 2148 cm^{-1} and the ^{13}C NMR resonance at δ_C 112.2. The remaining 15 resonances of the ^{13}C NMR spectrum, together with four methyl groups in the ^1H NMR spectrum at δ 1.69 (d, J = 0.8 Hz, Me-12), 1.65 (br s, Me-13), 1.63 (br s, Me-15), and 1.51 (s, Me-14), were attributable to a sesquiterpene framework. The NMR spectra included the signals of two trisubstituted double bonds [δ_C 134.1 (C-3) and 119.7 (C-2)/δ_H 5.37 (br s, H-2); δ_C 132.8 (C-11) and 122.7 (C-10)/δ_H 5.09 (tsept, J = 7.2, 1.4 Hz, H-10)], whereas the resonance at δ_C 63.7 (C, C-7) was assigned to the carbon bearing the thiocyanate group. As these functional groups accounted for four of the five unsaturations deduced from the molecular formula, compound **3** had to be monocyclic. The COSY and HMBC correlations indicated that **3** possessed a bisabolane framework and defined the positions of the functional groups mentioned above. One of the double bonds was located at C-10 based on the allylic coupling of the olefinic proton at δ 5.09 (H-10) with two methyl groups [δ 1.69 (Me-12) and 1.63 (Me-15)]. The HMBC correlations of the carbon bearing the thiocyanate group [δ_C 63.7 (C-7)] with the methyl group at δ_H 1.51 (Me-14) and the methine at δ_H 1.88 (H-6) defined the attachment of the thiocyanate function to C-7 of the bisabolane skeleton. The location of the remaining double bond in the molecule at C-2 was supported by the HMBC correlations of the olefinic proton at δ_H 5.37 (H-2) with the methyl group at δ_C 23.1 (Me-13) and the methine at δ_C 42.5 (C-6). The 12.1 Hz coupling between H-5ax (δ_H 1.38) and H-6 indicated the axial orientation of H-6, while the configuration at C-7 remains undetermined.

Axinysone A (**4**) possessed the molecular formula $C_{15}H_{22}O_2$ determined by HRCIMS. The NMR data (Table 1) were related to those of (+)-aristolone (**14**),[11] which was the major metabolite of the sponge.[5] Thus, the NMR spectra of **4** exhibited the signals of an enone [δ_C 196.5 (C, C-8) and 120.5 (CH, C-9)/δ_H 6.20 (dd, J = 2.1, 1.2 Hz, H-9) and δ_C 168.5 (C, C-10)], two methine protons of a cyclopropane ring [δ_H 1.75 (dd, J = 8.0, 1.2 Hz, H-7) and 1.37 (d, J = 8.0 Hz, H-6)], and four methyl groups [δ_H 1.23 (s, Me-13), 1.21 (s, Me-12), 1.17 (s, Me-14), and 1.07 (d, J = 6.8 Hz, Me-15)]. The most significant difference between the NMR spectra of **4** and (+)-aristolone (**14**)[11] was the absence in **4** of the resonances due to the allylic methylene at C-1, showing those of an oxymethine at δ_C 69.0/δ_H 4.38 (br d, J = 12.0 Hz) instead. Moreover, the oxygenated function was identified as a hydroxyl group from the IR absorption at 3404 cm^{-1}. The location of the hydroxyl group at C-1 was further supported by the HMBC correlations of the oxymethine carbon (δ_C 69.0, C-1) with the olefinic proton H-9 (δ_H 6.20) and the methylene protons at C-2 and C-3 [δ_H 2.15 (H-2eq), 1.39 (H-2ax), 1.62 (H-3eq), and 1.48 (H-3ax)]. The NOESY correlations Me-15/H-3eq, H-3ax defined the β-equatorial orientation of Me-15, while the β-axial orientation of Me-14 was supported by the correlation Me-14/H-3ax (Figure 1, energy minimized using MM2). The α-orientation of the cyclopropane ring was deduced from the NOE interactions H-6/Me-14 and Me-13/H-4. Finally, the NOESY correlations of H-1 with H-2eq, H-3ax, and Me-14 established the β-axial orientation of H-1 and, therefore, the α-equatorial orientation of the hydroxyl group. On the basis of biogenetic grounds axinysone A (**4**) was expected to belong to the same enantiomeric series as the co-occurring (+)-aristolone (**14**).[11] This proposal was confirmed by assignment of the absolute configuration of **4**. Treatment of **4** with (*R*)- and (*S*)-α-methoxy-α-phenylacetic (MPA) acids yielded the diastereomeric esters **4r** and **4s**, respectively. Positive chemical shift differences ($\Delta\delta = \delta_R - \delta_S$) were observed for H-9, H-7, H-6, and Me-14 (+0.37, +0.06, +0.05, and +0.04 ppm, respectively), whereas negative $\Delta\delta$ values were obtained for H-2eq, H-2ax, H-3eq, and H-3ax (−0.26, −0.23, −0.07, and −0.07 ppm, respectively). These data indicated an *S* configuration[12] for C-1 and, therefore, an absolute configuration 1*S*,4*S*,5*S*,6*R*,7*S* for axinysone A (**4**).

The HRCIMS analysis of axinysone B (**5**) indicated that it was an isomer of **4**. Furthermore, the COSY and HMBC correlations defined for compound **5** a planar structure identical to that of **4**. The NOESY correlations observed for Me-15, Me-14, H-4, and H-6 indicated that compound **5** also possessed the same relative configuration as **4** at C-4, C-5, C-6, and C-7. However, the 3.0 Hz coupling of the oxymethine proton H-1 with H-2eq and H-2ax in **5**, together with the NOESY correlations of H-1 with H-2eq, H-2ax, and H-9 established the α-equatorial orientation of H-1 and, therefore, the β-axial position of the hydroxyl group. The absolute configuration of **5** was secured through ^1H NMR analysis of the MPA esters **5r** and **5s**. Positive chemical shift differences ($\Delta\delta = \delta_R - \delta_S$) were observed for H-2eq and H-2ax (+0.15 and +0.11, respectively), whereas negative $\Delta\delta$ values were obtained for H-9, H-7, H-6, and Me-14 (−0.03, −0.07, −0.08, and −0.30 ppm, respectively). These data indicated an *R* configuration[12] for C-1 and, therefore, an absolute configuration 1*R*,4*S*,5*S*,6*R*,7*S* for axinysone B (**5**). A compound exhibiting the same structure and relative configuration as **5** has been previously described from the terrestrial plant *Aristolochia debilis*.[13] Although the optical rotation

Table 1. NMR Spectroscopic Data ($CDCl_3$) for Compounds **4**, **7**, **8**, and **9**[a]

	4[b]		**7**[b]		**8**[c]		**9**[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	69.0	4.38 br d (12.0)	32.2	1.98 m eq 1.43 ddd (13.7, 13.7, 4.1) ax	129.9	6.09 s	32.6	2.19 ddddd (14.0, 14.0,4.8, 4.2, 2.3) ax 2.09 dddd (14.0,4.2, 2.2,1.8) eq
2	35.4	2.15 m eq 1.39 dddd (13.5, 12.0, 12.0,3.2) ax	22.9	1.66 m eq 1.34 m ax	198.3		26.3	1.70 m eq 1.30 m ax
3	28.4	1.62 dddd (13.5, 3.8,3.2, 3.2) eq 1.48 dddd (13.5, 13.5,12.4, 3.2) ax	29.4	1.49 m eq 1.30 m ax	42.4	2.35 m	30.9	1.50 m eq 1.35 m ax
4	38.5	1.84 dqd (12.4, 6.8, 3.8)	34.3	1.95 m	38.3	2.25 m	37.7	1.66 m
5	39.6		42.3		37.7		36.8	
6	39.8	1.37 d (8.0)	41.6	1.19 d (8.4)	32.1	0.81 d (9.0)	31.5	0.80 d (9.2)
7	35.2	1.75 dd (8.0, 1.2)	36.4	1.97 d (8.4)	22.8	1.41 dd (9.0, 2.1)	20.8	1.13 ddd (9.2, 6.9, 1.3)
8	196.5		208.8		56.1	3.65 ddd (4.2, 2.1, 1.0)	25.4	3.68 ddd (6.9, 4.2, 2.5)
9	120.5	6.20 dd (2.1, 1.2)	74.6	4.23 d (2.6)	56.7	3.46 d (4.2)	112.2	5.11 br s
10	168.5		82.7		164.6		146.1	
11	24.4		30.4		21.3		19.3	
12	29.7	1.21 s	32.0	1.22 s	29.2	1.11 s	29.8	1.11 s
13	16.5	1.23 s	18.7	1.41 s	16.2	1.11 s	16.1	1.33 s
14	23.3	1.17 s	17.9	1.22 s	21.5	1.30 s	21.4	1.03 s
15	15.9	1.07 d (6.8)	17.0	1.03 d (6.7)	14.8	1.03 d (6.6)	15.9	0.96 d (7.0)
-OH		2.22 br s		4.02 d (2.6), 1.72 br s				
-CN							121.7	

[a] Assignments aided by COSY, HSQC, HMBC, and NOESY experiments. [b] Recorded at 600 MHz. [c] Recorded at 400 MHz.

for that compound was not reported, it likely belongs to the enantiomeric series of (−)-aristolone, also isolated from the plant.

The molecular formula of axinysone C (**6**), $C_{15}H_{22}O_3$, was determined by HRCIMS and indicated that **6** possessed the same degree of unsaturation as axinysones A (**4**) and B (**5**) but with an additional oxygen atom. The NMR spectra of **6** were related to those of **5** except for the significant downfield shift of the oxymethine carbon at δ_C 85.9 and the presence of a proton resonance at δ_H 7.93 (br s). These data suggested that compound **6** was the hydroperoxy analogue of **5**. This assignment was supported by the HMBC correlation of the oxymethine carbon (δ_C 85.9, C-1) with the olefinic proton H-9 (δ_H 5.96) and the correlation of the oxymethine proton (δ_H 4.47, H-1) with C-5 (δ_C 39.0). The 2.7 Hz coupling of H-1 with both H-2eq and H-2ax supported the α-equatorial orientation of H-1 and, therefore, the β-axial orientation of the hydroperoxy group.

Axinysone D (**7**) possessed the molecular formula $C_{15}H_{24}O_3$, determined by HRCIMS. The presence of hydroxyl and ketone functions was defined from the IR absorptions at 3436 and 1690 cm^{-1}, respectively. The ^1H NMR spectrum displayed the resonances of four methyl groups [δ 1.41 (s, Me-13), 1.22 (s, Me-12), 1.22 (s, Me-14), and 1.03 (d, $J = 6.7$ Hz, Me-15)] and those of two methine protons at δ 1.97 (d, $J = 8.4$ Hz, H-7) and 1.19 (d, $J = 8.4$ Hz, H-6) attributable to the protons of the cyclopropane ring of an aristolane sesquiterpene. The NMR spectra also included the signals of a ketone carbonyl at δ_C 208.8 (C-8), an oxymethine at δ_C 74.6 (C-9)/δ_H 4.23 (d, $J = 2.6$ Hz, H-9), and a fully substituted carbon linked to a hydroxyl at δ_C 82.7 (C-10). The HMBC correlations of the carbonyl carbon with the protons of the cyclopropane ring [δ 1.97 (H-7), 1.19 (H-6)] and with the oxymethine proton (δ 4.23) defined the location of the carbonyl group at C-8 and the secondary hydroxyl group at C-9 of the aristolane framework. The location of remaining hydroxyl group at C-10 was deduced from the HMBC correlations of the oxygenated carbon at δ_C 82.7 (C-10) with the methylene protons at δ_H 1.98 (H-1eq)/1.43 (H-1ax) and the methine at δ_H 1.19 (H-6). The relative configuration of compound **7** was defined from NOESY and 1D-NOESY data and modeled using MM2 for energy minimization, as shown in Figure 2. Thus, the

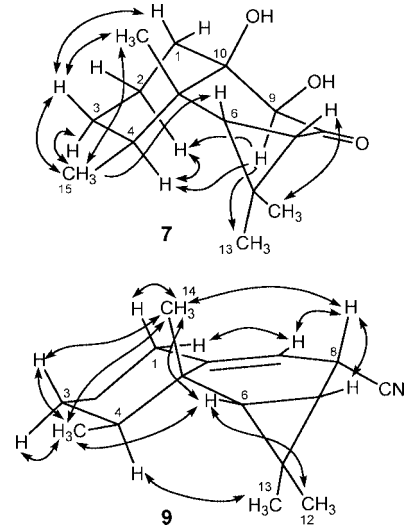
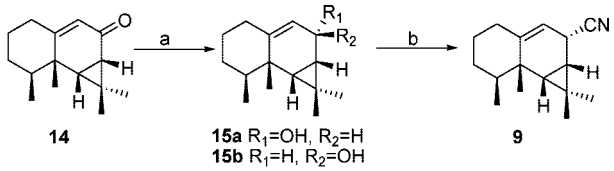


Figure 2. Selected NOESY (↔) and NOESY-1D (→) correlations for compounds **7** and **9**.

NOESY correlations of Me-15 with H-3ax and H-3eq defined the β-equatorial orientation of Me-15, whereas the NOESY cross-peak between Me-14 and H-3ax supported the β-axial orientation of Me-14. On the other hand, the irradiation of H-9 caused NOEs on H-2ax, H-4, and Me-13. These data indicated the α-orientation of H-9, the *cis*-fusion of the six-membered rings, and the α-orientation of the cyclopropane ring (Figure 2).

Axinysone E (**8**) possessed the molecular formula $C_{15}H_{20}O_2$, determined by HRCIMS measurement. The ^1H NMR spectrum exhibited the signals of four methyl groups [δ 1.30 (3H, s, Me-14), 1.11 (6H, s, Me-12 and Me-13), 1.03 (3H, d, $J = 6.6$ Hz, Me-15)] and two methine protons [δ 1.41 (dd, $J = 9.0$, 2.1 Hz, H-7) and 0.81 (d, $J = 9.0$ Hz, H-6)] indicative of an aristolane sesquiterpene. The NMR signal of a carbonyl at δ_C 198.3 (C-2) together with those of a trisubstituted double bond at δ_C 129.9 (C-

Scheme 1. Synthesis of Axinynitrile A (**9**) from (+)-Aristolone (**14**)



1)/δ_H 6.09 (s, H-1) and δ_C 164.6 (C-10) established the presence of an α,β-unsaturated ketone. Two methines at δ_C 56.1 (C-8)/δ_H 3.65 (ddd, J = 4.2, 2.1, 1.0 Hz, H-8) and δ_C 56.7 (C-9)/δ_H 3.46 (d, J = 4.2 Hz, H-9), together with the remaining oxygen atom of the molecular formula, were accommodated in an oxirane ring. In the HMBC spectrum, the carbonyl carbon (δ_C 198.3, C-2) was correlated with the methylene protons at δ_H 2.35 (m, H_2-3), the carbon of which at δ_C 42.4 (C-3) showed a correlation with the methyl group at δ_H 1.03 (d, J = 6.6 Hz, Me-15). These data indicated the location of the ketone at C-2 and, consequently, of the conjugated double bond at C-1,C-10 of the aristolane skeleton. On the other hand, the location of the epoxy function at C-8,C-9 was supported by the COSY correlation between the oxymethine proton at δ 3.65 (H-8) and the bridgehead proton H-7 (δ 1.41). The NOESY correlations indicated that compound **8** possessed the same relative configuration as compounds previously described at C-4, C-5, C-6, and C-7, while the β-orientation of the oxirane ring was proposed from the NOESY correlations of H-8 and H-9 with Me-13.

The molecular formula of axinynitrile A (**9**), $C_{16}H_{23}N$, was determined by HRCIMS. The NMR spectra featured resonances attributable to an aristolane sesquiterpene containing a trisubstituted double bond [δ_C 146.1 and 112.2/δ_H 5.11 (br s)]. This unsaturation was located at C-9 from the HMBC correlations of the olefinic carbons at δ_C 112.2 (C-9) and 146.1 (C-10) with the bridgehead protons at δ_H 1.13 [(ddd, J = 9.2, 6.9, 1.3 Hz, H-7)] and 0.80 [(d, J = 9.2 Hz, H-6)], respectively. In addition to the 15 resonances of the sesquiterpene skeleton, the ^{13}C NMR spectrum exhibited a signal at δ 121.7 (C) that together with the weak IR absorption at 2236 cm^{-1} and the nitrogen atom of the molecular formula was assigned to a nitrile group. This function had to be linked to the methine which gave rise to the signals at δ_C 25.4/δ_H 3.68 (ddd, J = 6.9, 4.2, 2.5 Hz). This methine was identified as C-8 of the aristolane skeleton from the COSY correlations of the proton at δ 3.68 (H-8) with the cyclopropyl proton H-7 and the HMBC correlation of the carbon at δ_C 25.4 (C-8) with the cyclopropyl proton H-6. The NOESY correlations Me-15/H-3eq, H-3ax and Me-14/H-1ax, H-3ax supported the β-equatorial orientation of Me-15 and the β-axial orientation of Me-14, respectively (Figure 2, energy minimized using MM2). The NOESY correlation H-6/Me-14 defined the β-orientation of the cyclopropyl protons. Finally a weak NOESY correlation between H-8 and Me-14 suggested the β-orientation of H-8 and, therefore, the α-orientation of the nitrile group. In order to confirm the unusual presence of the nitrile functionality and to assign the absolute configuration of the molecule, compound **9** was synthesized from (+)-aristolone (**14**), also isolated from the sponge (Scheme 1). Luche reduction of **14** led to a 3:1 mixture of the epimeric alcohols **15a** and **15b**. Attempts to separate both isomers by chromatography were unfruitful since the allylic alcohols were readily transformed into 1(10),8-aristoladiene[14] through an acid-catalyzed 1,4-elimination. Nonetheless, the analysis of the NMR spectra of the mixture allowed the full assignment of the NMR data of **15a** and partial assignment of those corresponding to the minor isomer **15b**. In particular, the configuration at C-8 for each isomer was assigned from the NOESY spectrum. In the major isomer **15a** the proton geminal to the hydroxyl [δ 4.51 (ddd, J = 7.1, 3.8, 2.5 Hz, H-8)] exhibited a weak NOE interaction with Me-14 [δ 0.92 (s)], whereas in **15b** the proton H-8 [δ 4.13 (d, J = 4.4 Hz)] showed a NOESY correlation with Me-13 [δ 1.08 (s)]. The substitution of the hydroxyl group by a nitrile function was achieved by treatment of the alcohols **15a/15b** with TMSCN and $BF_3 \cdot Et_2O$[15] to yield the cyano derivative **9** (77.9% yield) together with the elimination product 1(10),8-aristoladiene[14] (11.8% yield). Compound **9** exhibited optical rotation and spectroscopic data identical to those of the natural axinynitrile A.

The new sesquiterpenes **1**−**9** and the known compounds **10**, **11**, **13**, and **14** isolated from *A. isabela* 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**, **8**, and **13** were mildly active. In particular, compound **2** inhibited the growth of MDA-MB-231 and A-549 cell lines with GI_{50} values of 33.3 and 32.6 μM, respectively. Compound **8** was active against A-549 and HT-29 with GI_{50} values of 38.7 and 38.3 μM, respectively. Finally, compound **13** exhibited growth inhibitory activity with GI_{50} values of 27.0, 36.4, and 33.4 μM against MDA-MB-231, A-549, and HT-29, respectively. The remaining compounds were inactive at the highest concentration tested (10 μg/mL).

This study, taken together with our preceding results,[5] has shown that the sponge *A. isabela* is a prolific source of sesquiterpenoids, which exhibit diverse skeletal types and comprise derivatives with and without nitrogenous functionalities. Among the nitrogen-containing metabolites herein described, compounds **3** and **9** exhibit unusual features. Compound **3** adds to the uncommon class of thiocyano-substituted marine terpenoids, for which only seven representatives have been previously reported.[1c,2a] Moreover, axinythiocyanate A (**3**) is the first account of a bisabolane sesquiterpene bearing a thiocyanate group. On the other hand, the nitrile functionality has been found in a small number of terrestrial and marine natural products exhibiting very diverse structures.[16] Among these metabolites, cyanopuupehenol and cyanopuupehenone from a Verongid sponge[17] together with compound **9** herein described from a Halichondrid sponge are, to the best of our knowledge, the only examples of natural terpenoids bearing a cyano group. Moreover, the presence of the cyano substituent in axinynitrile A (**9**) represents a departure from the wide array of nitrogen-containing terpenoids so far described from sponges of the order Halichondrida, usually containing isocyano, isothiocyanate, or formamide groups and less frequently thiocyanate or isocyanate functionalities.[1,2] From a biosynthetic point of view, the nitrile function in **9** could arise from a cyanide ion, which has been demonstrated to be a precursor of the isocyano, isothiocyanate, and thiocyanate substituents present in terpenoids of sponges.[1c] With regard to the non-nitrogenous sesquiterpenes herein described from *A. isabela*, all of them fall in the aristolane class. In spite of the vast array of sesquiterpenes so far described from marine organisms, only a few compounds feature the aristolane skeleton.[18] Compounds **4**−**9** and **14** from *A. isabela*, together with (+)-9-aristolene from *Acanthella cavernosa*,[18e] appear to be the only aristolane sesquiterpenoids described from sponges.

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 $CDCl_3$ or C_6D_6 as solvents. Chemical shifts were referenced using the corresponding solvent signals [δ_H 7.26 and δ_C 77.0 for $CDCl_3$, 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 mass 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 differential refractometer RI-71. All solvents were spectroscopic grade or were distilled prior to use.

Collection and Identification. Specimens of *Axinyssa isabela*[6] (order Halichondrida, family Halichondriidae) were collected by hand using scuba in Isla Isabel (Gulf of California, Mexico) and immediately frozen. A voucher specimen is deposited in the Sponge Collection of the UNAM under the code LEB-ICML-UNAM-56.

Extraction and Isolation. The extraction of the sponge and the column chromatography of the resulting extract has been previously described.[5] The fraction of the general chromatography eluted with hexanes/Et_2O (95:5) was chromatographed over a silica gel column using hexanes and hexanes/Et_2O mixtures (99:1 to 80:20) as eluants. Repeated purifications of selected fractions by normal-phase HPLC using hexanes or hexanes/EtOAc (99:1) yielded acanthene B (**10**)[7] (5.3 mg, 1.9 × 10^{-3} % dry wt), **11**[8] (3.6 mg, 1.3 × 10^{-3} % dry wt), **3** (9.7 mg, 3.5 × 10^{-3} % dry wt), **12**[9] (5.0 mg, 1.8 × 10^{-3} dry wt), **13**[10] (5.5 mg, 2.0 × 10^{-3} % dry wt), and **9** (4.5 mg, 1.6 × 10^{-3} % 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, 94:6 or 93:7) afforded compounds **1** (1.2 mg, 4.3 × 10^{-4} % dry wt) and **2** (3.8 mg, 1.3 × 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). Separations of selected fractions by normal-phase HPLC (hexanes/EtOAc, 84:16) afforded compound **8** (2.5 mg, 8.9 × 10^{-4} % 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 compound **7** (3.5 mg, 1.2 × 10^{-3} % dry wt). The fractions of the general chromatography eluted with hexanes/Et_2O (30:70, 20:80) 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 compound **6** (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 (MeOH/H_2O, 65:35) to obtain compounds **5** (16.2 mg, 5.8 × 10^{-3} % dry wt) and **4** (16.4 mg, 5.8 × 10^{-3} % dry wt).

Axinisothiocyanate M (1): colorless oil; $[\alpha]^{25}_D$ −40.8 (*c* 0.1, CCl_4); UV (MeOH) λ_{max} (log ε) 243 (3.09) nm; IR (film) ν_{max} 3466, 2082 cm^{-1}; ^1H NMR ($CDCl_3$, 600 MHz) δ 5.07 (1H, br s, H-13a), 4.85 (1H, dq, *J* = 1.3, 1.3 Hz, H-13b), 2.08 (1H, dd, *J* = 13.0, 2.9 Hz, H-5), 2.03 (1H, dddd, *J* = 13.2, 3.3, 3.3, 1.5 Hz, H-3eq), 1.85 (3H, br s, Me-12), 1.82 (1H, ddd, *J* = 13.2, 13.2, 4.8 Hz, H-3ax), 1.76 (1H, ddd, *J* = 14.0, 13.8, 4.2 Hz, H-8ax), 1.73 (1H, dd, *J* = 13.4, 13.0 Hz, H-6ax), 1.64 (1H, m, H-9ax), 1.61 (1H, m, H-6eq), 1.55 (2H, m, H_2-2), 1.48 (1H, dddd, *J* = 14.0, 3.8, 2.7, 2.7 Hz, H-8eq), 1.43 (1H, m, H-1eq), 1.31 (3H, s, Me-15), 1.23 (2H, m, H-1ax, H-9eq), 0.90 (3H, s, Me-14); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 151.8 (C, C-11), 109.4 (CH_2, C-13), 74.4 (C, C-7), 64.9 (C, C-4), 47.4 (CH, C-5), 41.9 (CH_2, C-3), 40.0* (CH_2, C-1), 39.9* (CH_2, C-9), 34.4 (C, C-10), 32.9 (CH_2, C-6), 31.8 (CH_2, C-8), 22.1 (CH_3, Me-15), 19.0 (CH_3, Me-12), 18.9 (CH_2, C-2), 18.1 (CH_3, Me-14), signals marked with an asterisk may be interchanged; CIMS *m/ z* 279 (6) $[M]^+$, 221 (36), 220 (20), 203 (100); HRCIMS(+) *m/z* 279.1635 (calcd for $C_{16}H_{25}NOS$, 279.1657).

Axinisothiocyanate N (2): colorless oil; $[\alpha]^{25}_D$ −75.7 (*c* 0.05, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 244 (3.21) nm; IR (film) ν_{max} 3416, 2090 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.32 (br s, −OOH), 5.07 (1H, br s, H-13a), 5.03 (1H, br s, H-13b), 2.18 (1H, ddd, *J* = 13.7, 2.5, 2.5 Hz, H-6eq), 2.03 (1H, dddd, *J* = 13.1, 3.2, 3.2, 1.5 Hz, H-3eq), 1.97 (1H, dd, *J* = 13.1, 2.5 Hz, H-5), 1.85 (1H, m, H-8eq), 1.85 (3H, br s, Me-12), 1.81 (1H, ddd, *J* = 13.1, 13.1, 4.6 Hz, H-3ax), 1.64 (1H, ddd, *J* = 13.9, 13.9, 4.2 Hz, H-8ax), 1.62 (1H, dd, *J* = 13.7, 13.1 Hz, H-6ax),1.57 (2H, m, H_2-2), 1.52 (1H, m, H-9ax), 1.44 (1H, m, H-1eq), 1.31 (3H, s, Me-15), 1.21 (2H, m, H-1ax, H-9eq), 0.91 (3H, s, Me-14); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 148.0 (C, C-11), 112.1 (CH_2, C-13), 85.2 (C, C-7), 64.9 (C, C-4), 47.5 (CH, C-5), 41.9 (CH_2, C-3), 39.9* (CH_2, C-1), 39.8* (CH_2, C-9), 34.5 (C, C-10), 28.1 (CH_2, C-8), 27.3 (CH_2, C-6), 21.7 (CH_3, Me-15), 18.8 (CH_2, C-2), 18.7 (CH_3, Me-12), 18.2 (CH_3, Me-14), signals marked with an asterisk may be interchanged; HRCIMS(+) *m/z* 295.1597 (calcd for $C_{16}H_{25}NO_2S$, 295.1606).

Axinythiocyanate A (3): colorless oil; $[\alpha]^{25}_D$ +39.7 (*c* 0.1, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 233 (3.23) nm; IR (film) ν_{max} 2148 cm^{-1}; ^1H NMR ($CDCl_3$, 600 MHz) δ 5.37 (1H, br s, H-2), 5.09 (1H, tsept, *J* = 7.2, 1.4 Hz, H-10), 2.10 (3H, m, H-1, H_2-9), 2.02 (2H, m, H_2-4), 1.98 (1H, m, H-1), 1.93 (1H, m, H-5eq), 1.88 (1H, m, H-6), 1.81 (1H, m, H-8), 1.77 (1H, m, H-8), 1.69 (3H, d, *J* = 0.8 Hz, Me-12), 1.65 (3H, br s, Me-13), 1.63 (3H, br s, Me-15), 1.51 (3H, s, Me-14), 1.38 (1H, dddd, *J* = 12.1, 12.1, 12.1, 5.6 Hz, H-5ax); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 134.1 (C, C-3), 132.8 (C, C-11), 122.7 (CH, C-10), 119.7 (CH, C-2), 112.2 (C, −SCN), 63.7 (C, C-7), 42.5 (CH, C-6), 38.9 (CH_2, C-8), 30.9 (CH_2, C-4), 27.2 (CH_2, C-1), 25.7 (CH_3, Me-12), 24.6 (CH_2, C-5), 23.8 (CH_3, Me-14), 23.1 (CH_2, C-9), 23.1 (CH_3, Me-13), 17.7 (CH_3, Me-15); CIMS *m/ z* 263 (4) $[M]^+$, 205 (78), 204 (27), 189 (9), 69 (100); HRCIMS(+) *m/z* 263.1694 (calcd for $C_{16}H_{25}NS$, 263.1708).

Axinysone A (4): colorless oil; $[\alpha]^{25}_D$ +254.0 (*c* 0.1, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 232 (3.93) nm; IR (film) ν_{max} 3404, 1639 cm^{-1}; ^1H NMR ($CDCl_3$, 600 MHz) see Table 1; ^{13}C NMR ($CDCl_3$, 150 MHz) see Table 1; EIMS *m/ z* 234 (50) $[M]^+$, 219 (21), 216 (88), 201 (100); HRCIMS(+) *m/z* 235.1696 $[M + H]^+$ (calcd for $C_{15}H_{23}O_2$, 235.1698).

Axinysone B (5): colorless oil; $[\alpha]^{25}_D$ +108.0 (*c* 0.1, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 232 (3.96) nm; IR (film) ν_{max} 3400, 1641 cm^{-1}; ^1H NMR ($CDCl_3$, 600 MHz) δ 5.86 (1H, d, *J* = 1.3 Hz, H-9), 4.38 (1H, dd, *J* = 3.0, 3.0 Hz, H-1), 1.99 (1H, dddd, *J* = 14.2, 3.0, 3.0, 3.0 Hz, H-2eq), 1.84 (1H, dddd, *J* = 13.2, 12.9, 12.9, 3.0 Hz, H-3ax), 1.79 (1H, dd, *J* = 7.9, 1.3 Hz, H-7), 1.77 (1H, m, H-4), 1.58 (1H, dddd, *J* = 14.2, 13.2, 3.6, 3.0 Hz, H-2ax), 1.42 (1H, d, *J* = 7.9 Hz, H-6), 1.40 (1H, m, H-3eq), 1.36 (3H, s, Me-14), 1.21 (3H, s, Me-12), 1.19 (3H, s, Me-13), 1.09 (3H, d, *J* = 6.6 Hz, Me-15); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 197.2 (C, C-8), 165.2 (C, C-10), 127.3 (CH, C-9), 73.2 (CH, C-1), 40.3 (CH, C-6), 39.0 (C, C-5), 38.8 (CH, C-4), 36.4 (CH, C-7), 32.7 (CH_2, C-2), 29.8 (CH_3, Me-12), 25.4 (C, C-11), 24.9 (CH_2, C-3), 24.6 (CH_3, Me-14),16.2 (CH_3, Me-15), 16.1 (CH_3, Me-13); EIMS *m/z* 234 (16) $[M]^+$, 219 (14), 216 (40), 201 (72), 173 (36), 149 (57), 105 (80), 91 (100); HRCIMS(+) *m/z* 235.1685 $[M + H]^+$ (calcd for $C_{15}H_{23}O_2$, 235.1698).

Axinysone C (6): colorless oil; $[\alpha]^{25}_D$ +139.0 (*c* 0.1, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 232 (3.94) nm; IR (film) ν_{max} 3382, 1643 cm^{-1}; ^1H NMR ($CDCl_3$, 600 MHz) δ 7.93 (1H, br s, OOH), 5.96 (1H, d, *J* = 1.3 Hz, H-9), 4.47 (1H, dd, *J* = 2.7, 2.7 Hz, H-1), 2.16 (1H, m, H-2eq), 1.83 (1H, dd, *J* = 7.9, 1.3 Hz, H-7), 1.80 (1H, m, H-4), 1.62 (2H, m, H-2ax, H-3ax), 1.44 (1H, d, *J* = 7.9 Hz, H-6), 1.40 (1H, m, H-3eq), 1.31 (3H, s, Me-14), 1.22 (3H, s, Me-12), 1.22 (3H, s, Me-13), 1.08 (3H, d, *J* = 6.6 Hz, Me-15); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 196.7 (C, C-8), 159.8 (C, C-10), 130.6 (CH, C-9), 85.9 (CH, C-1), 40.1 (CH, C-6), 39.0 (C, C-5), 38.6 (CH, C-4), 36.3 (CH, C-7), 29.8 (CH_3, Me-12), 29.4 (CH_2, C-2), 25.4 (C, C-11), 23.4 (CH_2, C-3), 23.3 (CH_3, Me-14), 16.2 (CH_3, Me-13), 16.2 (CH_3, Me-15); EIMS *m/z* 251 (1) $[M + H]^+$, 217 (3), 109 (40), 59 (100); HRCIMS(+) *m/z* 251.1644 $[M + H]^+$ (calcd for $C_{15}H_{23}O_3$, 251.1647).

Axinysone D (7): white solid; $[\alpha]^{25}_D$ −39.5 (*c* 0.1, $CHCl_3$); IR (film) ν_{max} 3436, 1690 cm^{-1}; ^1H NMR ($CDCl_3$, 600 MHz) see Table 1; ^{13}C NMR ($CDCl_3$, 150 MHz) see Table 1; EIMS *m/z* 252 (3) $[M]^+$, 237 (2), 234 (2), 219 (2), 139 (13), 127 (49), 126 (88), 111 (100); HRCIMS(+) *m/z* 252.1719 (calcd for $C_{15}H_{24}O_3$, 252.1725).

Axinysone E (8): colorless oil; $[\alpha]^{25}_D$ +84.3 (*c* 0.07, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 242 (3.87) nm; IR (film) ν_{max} 1670 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) see Table 1; ^{13}C NMR ($CDCl_3$, 100 MHz) see Table 1; CIMS *m/z* 233 (89) $[M + H]^+$, 217 (47), 203 (45), 161 (84), 91 (100); HRCIMS *m/z* 233.1529 $[M + H]^+$ (calcd for $C_{15}H_{21}O_2$, 233.1541).

Axinynitrile A (9): white solid; $[\alpha]^{25}_D$ +70.0 (*c* 0.1, $CHCl_3$); IR (film) ν_{max} 2236 cm^{-1}; ^1H NMR ($CDCl_3$, 600 MHz) see Table 1; ^{13}C NMR ($CDCl_3$, 150 MHz) see Table 1; EIMS *m/ z* 229 (36), 214 (47), 186 (95), 130 (100); HRCIMS(+) *m/z* 229.1825 (calcd for $C_{16}H_{23}N$, 229.1830).

Synthesis of the (*R*)-MPA Ester 4r. A solution of **4** (0.8 mg, 3.4 × 10^{-3} mmol) in 0.25 mL of CH_2Cl_2 was treated with CH_2Cl_2 solutions of *N*,*N*′-dicyclohexylcarbodiimide (2.2 mg, 0.011 mmol in 0.25 mL), *N*,*N*-dimethylaminopyridine (0.9 mg, 7.4 × 10^{-3} mmol in 0.25 mL), and (*R*)-α-methoxy-α-phenylacetic acid (1.8 mg, 0.011 mmol in 0.25 mL) and stirred at room temperature for 5 h. The reaction mixture was purified over a preparative TLC (hexanes/EtOAc, 1:1) to obtain 1.2 mg of the (*R*)-MPA ester **4r**: ^1H NMR ($CDCl_3$, 600 MHz; selected

data, assignments aided by COSY and NOESY experiments) δ 5.94 (1H, dd, $J = 1.9$, 1.2 Hz, H-9), 5.57 (1H, ddd, $J = 12.5$, 5.6, 2.1 Hz, H-1), 1.87 (1H, dddd, $J = 12.3$, 5.6, 3.3, 3.3 Hz, H-2eq), 1.81 (1H, dqd, 12.5, 6.9, 4.0, H-4), 1.77 (1H, dd, $J = 8.1$, 1.2 Hz, H-7), 1.55 (1H, m, H-3eq), 1.46 (1H, m, H-3ax), 1.38 (1H, d, $J = 8.1$, H-6), 1.23 (3H, s, Me-13), 1.22 (1H, m, H-2ax), 1.22 (3H, s, Me-14), 1.21 (3H, s, Me-12), 1.05 (3H, d, $J = 6.9$ Hz, Me-15).

Synthesis of the (*S*)-MPA Ester 4s. A solution of **4** (1.2 mg, 5.1×10^{-3} mmol) in 0.25 mL of CH_2Cl_2 was treated with CH_2Cl_2 solutions of *N*,*N*′-dicyclohexylcarbodiimide (2.0 mg, 9.7×10^{-3} mmol in 0.25 mL), *N*,*N*-dimethylaminopyridine (1.0 mg, 8.2×10^{-3} mmol in 0.25 mL), and (*S*)-α-methoxy-α-phenylacetic acid (1.5 mg, 9.0×10^{-3} mmol in 0.25 mL) and stirred at room temperature for 3 h. The reaction mixture was purified over preparative TLC (hexanes/EtOAc, 1:1) to obtain 1.8 mg of the (*S*)-MPA ester **4s**: ^{1}H NMR ($CDCl_3$, 600 MHz; selected data, assignments aided by COSY and NOESY experiments) δ 5.57 (1H, dd, $J = 2.0$, 1.3 Hz, H-9), 5.52 (1H, ddd, $J = 12.1$, 5.6, 2.1 Hz, H-1), 2.13 (1H, m, H-2eq), 1.85 (1H, m, H-4), 1.71 (1H, dd, $J = 8.1$, 1.2 Hz, H-7), 1.62 (1H, m, H-3eq), 1.53 (1H, m, H-3ax), 1.45 (1H, m, H-2ax), 1.33 (1H, d, $J = 8.1$, H-6), 1.23 (3H, s, Me-13), 1.20 (3H, s, Me-12), 1.18 (3H, s, Me-14), 1.06 (3H, d, $J = 6.7$ Hz, Me-15).

Synthesis of the (*R*)-MPA Ester 5r. A solution of **5** (3.0 mg, 0.013 mmol) in 0.3 mL of CH_2Cl_2 was treated with CH_2Cl_2 solutions of *N*,*N*′-dicyclohexylcarbodiimide (8.0 mg, 0.039 mmol in 0.3 mL), *N*,*N*-dimethylaminopyridine (3.0 mg, 0.025 mmol in 0.3 mL), and (*R*)-α-methoxy-α-phenylacetic acid (6.0 mg, 0.036 mmol in 0.3 mL) and stirred at room temperature for 24 h. The reaction mixture was purified over a preparative TLC (hexanes/EtOAc, 6:4) to obtain 3.0 mg of the (*R*)-MPA ester **5r**: ^{1}H NMR ($CDCl_3$, 600 MHz; selected data, assignments aided by COSY and NOESY experiments) δ 5.94 (1H, d, $J = 1.2$ Hz, H-9), 5.54 (1H, dd, $J = 2.5$, 2.5 Hz, H-1), 1.95 (1H, m, H-2eq), 1.73 (1H, m, H-4), 1.72 (1H, dd, $J = 7.8$, 1.2 Hz, H-7), 1.60 (2H, m, H-2ax, H-3ax), 1.42 (1H, m, H-3eq), 1.31 (1H, d, $J = 7.8$, H-6), 1.17 (3H, s, Me-12), 1.15 (3H, s, Me-13), 1.03 (3H, d, $J = 6.9$ Hz, Me-15), 0.84 (3H, s, Me-14).

Synthesis of the (*S*)-MPA Ester 5s. A solution of **5** (3.0 mg, 0.013 mmol) in 0.3 mL of CH_2Cl_2 was treated with CH_2Cl_2 solutions of *N*,*N*′-dicyclohexylcarbodiimide (8.0 mg, 0.039 mmol in 0.3 mL), *N*,*N*-dimethylaminopyridine (3.0 mg, 0.025 mmol in 0.3 mL), and (*S*)-α-methoxy-α-phenylacetic acid (6.0 mg, 0.036 mmol in 0.3 mL) and stirred at room temperature for 24 h. The reaction mixture was purified over preparative TLC (hexanes/EtOAc, 6:4) to obtain 2.9 mg of the (*S*)-MPA ester **5s**: ^{1}H NMR ($CDCl_3$, 600 MHz; selected data, assignments aided by COSY and NOESY experiments) δ 5.97 (1H, d, $J = 1.2$ Hz, H-9), 5.46 (1H, dd, $J = 2.8$, 2.8 Hz, H-1), 1.80 (1H, dddd, $J = 14.8$, 3.0, 3.0, 3.0 Hz H-2eq), 1.79 (1H, dd, $J = 7.8$, 1.2 Hz, H-7), 1.71 (1H, m, H-4), 1.49 (1H, m, H-2ax), 1.39 (1H, d, $J = 7.8$, H-6), 1.19 (3H, s, Me-12), 1.17 (3H, s, Me-13), 1.14 (3H, s, Me-14), 1.00 (3H, d, $J = 6.9$ Hz, Me-15).

Reduction of (+)-Aristolone (14). To a cooled (−78 °C) solution of **4** (42.0 mg, 0.19 mmol) in CH_2Cl_2 (1.5 mL) was added a solution of $CeCl_3 \cdot 7H_2O$ (212.0 mg, 0.57 mmol) in MeOH (4 mL). After stirring the mixture for 10 min, $NaBH_4$ (36.0 mg, 0.95 mmol) was added. The reaction mixture was further stirred at −78 °C for 30 min and then left to recover to room temperature. The reaction was quenched with H_2O (15 mL), vigorously stirred, and extracted with Et_2O (3 × 15 mL). The organic layer was washed with brine and dried over $MgSO_4$. After filtration, the solvent was evaporated under reduced pressure to yield a 3:1 mixture of alcohols **15a** and **15b** (40.0 mg, 0.18 mmol, 94.7% yield). Compound **15a**: ^{1}H NMR (C_6D_6, 600 MHz) δ 5.30 (1H, br s, H-9), 4.51 (1H, ddd, $J = 7.1$, 3.8, 2.5 Hz, H-8), 2.09 (1H, m, H-1ax), 1.97 (1H, m, H-1eq), 1.57 (1H, m, H-4), 1.54 (1H, m, H-2eq), 1.33 (1H, m, H-3eq), 1.27 (3H, s, Me-12), 1.20 (2H, m, H-2ax, H-3ax), 1.13 (1H, ddd, $J = 9.3$, 7.1, 1.2 Hz, H-7), 1.02 (3H, s, Me-13), 0.92 (3H, s, Me-14), 0.91 (3H, d, $J = 6.9$ Hz, Me-15), 0.74 (1H, d, $J = 9.3$ Hz, H-6); ^{13}C NMR (C_6D_6, 150 MHz) δ 143.3 (C, C-10), 123.6 (CH, C-9), 65.9 (CH, C-8), 38.6 (CH, C-4), 37.5 (C, C-5), 35.0 (CH, C-6), 32.9 (CH_2, C-1), 31.5 (CH_2, C-3), 30.8 (CH_3, Me-12), 28.4 (CH, C-7), 26.9 (CH_2, C-2), 21.6 (CH_3, Me-14), 19.6 (C, C-11), 17.8 (CH_3, Me-13), 16.5 (CH_3, Me-15). Compound **15b**: ^{1}H NMR (C_6D_6, 600 MHz) δ 5.25 (1H, ddd, $J = 4.4$, 1.6, 1.6 Hz, H-9), 4.13 (1H, d, $J = 4.4$ Hz, H-8), 1.08 (3H, s, Me-13), 1.07 (3H, s, Me-14), 1.00 (1H, m, H-7), 0.97 (3H, s, Me-12), 0.88 (3H, d, $J = 6.9$ Hz, Me-15), 0.62 (1H, d, $J = 9.0$ Hz, H-6); ^{13}C NMR (C_6D_6, 150 MHz) δ 145.3 (C, C-10), 122.3 (CH, C-9), 63.2 (CH, C-8), 32.0 (CH, C-6), 29.9 (CH_3, Me-12), 28.2 (CH, C-7), 22.7 (CH_3, Me-14), 16.1 (CH_3, Me-15), 15.9 (CH_3, Me-13).

Reaction of Alcohols 15a and 15b with TMSCN. To a cooled (−10 °C) solution of **15a/15b** (15 mg, 0.068 mmol) in CH_2Cl_2 (1.5 mL) was added TMSCN (60 μL, 0.48 mmol). Then 700 μL of a 0.1 M solution of $BF_3 \cdot Et_2O$ in CH_2Cl_2 was added in small portions during 1 h. The mixture was further stirred at −10 °C for 30 min and then at 0 °C for 30 min. The reaction was quenched with saturated aqueous $NaHCO_3$ solution (12 mL), stirred for 20 min, and extracted with CH_2Cl_2 (3 × 15 mL). The organic layer was washed with brine and dried over $MgSO_4$. After filtration and solvent evaporation under reduced pressure, the residue was chromatographed over a SiO_2 column eluted with hexanes and hexanes/Et_2O (95:5) to obtain 1(10),8-aristoladiene[17] (1.6 mg, 0.008 mmol, 11.8% yield) and axinynitrile A (**9**, 12.2 mg, 0.053 mmol, 77.9% yield).

Cytotoxicity Assays. Compounds **1**−**11**, **13**, and **14** were tested against the human tumor cell lines MDA-MB-231 (breast adenocarcinoma), A-549 (lung adenocarcinoma), and HT-29 (colon adenocarcinoma). Doxorubicin was used as positive control ($GI_{50} = 0.1$, 0.1, and 0.1 μM against MDA-MB-231, A-549, and HT-29, respectively). A colorimetric assay using sulforhodamine B (SRB) has been adapted for quantitative measurement of cell growth and viability as described in the literature.[19]

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Supporting Information Available: ^{1}H and ^{13}C NMR spectra of compounds **1**−**9**. This information is available free of charge via the Internet at http://pubs.acs.org.

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