

## 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 axinytrile 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.

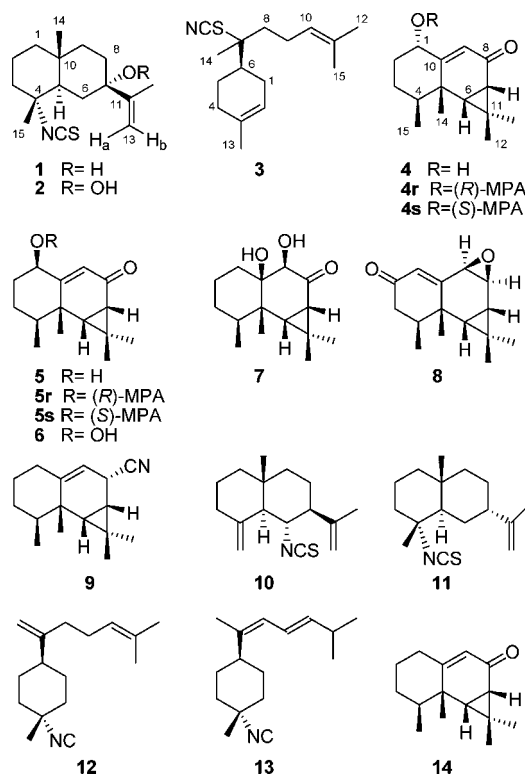
Chemical studies on sponges of the genus *Axinyssa* (order Halichondrida, family Halichondriidae) have shown that the secondary metabolism of these organisms is dominated by the presence of sesquiterpenes exhibiting a wide range of carbon skeletons and a nitrogenous functionality such as an isocyano, isothiocyanate, thiocyanate, or formamide group.<sup>1,2</sup> In a few instances, these nitrogen-containing metabolites have been described to be accompanied by sesqui- and diterpenes without nitrogenous functionality.<sup>3</sup> 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*.<sup>4</sup> 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.<sup>1,2a–c,3d,5</sup>

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.<sup>5</sup> Meanwhile, the sponge has been classified as the new species *Axinyssa isabela*.<sup>6</sup> 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 axinytrile A (**9**), together with the known compounds acanthenone B (**10**),<sup>7</sup> **11**,<sup>8</sup> **12**,<sup>9</sup> and 3-isocyanotheonellin (**13**).<sup>10</sup>

### Results and Discussion

Freeze-dried specimens of *A. isabela* were extracted with acetone/MeOH (1:1), and the resulting residue was partitioned between H<sub>2</sub>O and Et<sub>2</sub>O. The organic extract was subjected to column chromatography eluting with hexanes/Et<sub>2</sub>O mixtures of increasing polarity, then CHCl<sub>3</sub>/MeOH, and finally MeOH. Repeated separation of fractions eluted with hexanes/Et<sub>2</sub>O mixtures afforded the new sesquiterpenoids **1**–**9** and the known compounds **10**–**13**.

Axinisothiocyanate M (**1**) possessed the molecular formula C<sub>16</sub>H<sub>25</sub>NOS, determined by HRCIMS. The presence of hydroxyl and isothiocyanate functions was established from the IR absorptions at 3466 and 2082 (broad) cm<sup>-1</sup>, respectively. The <sup>1</sup>H NMR spectrum displayed the resonances of an isopropenyl unit [ $\delta_{\text{H}}$  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 [ $\delta_{\text{H}}$  1.31 (s, Me-15) and 0.90 (s, Me-14)] that suggested a sesquiterpene framework. The most distinctive

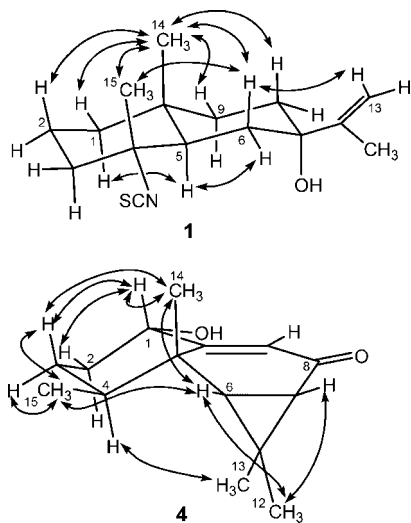


signals of the <sup>13</sup>C NMR spectrum were those of the double bond of the isopropenyl group [ $\delta$  151.8 (C-11),  $\delta$  109.4 (C-13)] and two resonances at  $\delta$  74.4 (C, C-7) and 64.9 (C, C-4) that were assigned to two fully substituted sp<sup>3</sup> carbons linked to the hydroxyl 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 ( $\delta_{\text{C}}$  64.9, C-4) showed HMBC correlations with the methyl group at  $\delta_{\text{H}}$  1.31 (Me-15) and with the methine at  $\delta_{\text{H}}$  2.08 (H-5), the carbon of which at  $\delta_{\text{C}}$  47.4 (C-5) showed correlations with the methyl group at  $\delta_{\text{H}}$  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 ( $\delta_{\text{C}}$  74.4, C-7) with the olefinic protons and the methyl group of the isopropenyl substituent. The relative configuration of compound **1** (*4R*\*,*5R*\*,*7S*\*,*10R*\*) 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  $\beta$ -equatorial orientation of the isopropenyl group at C-7 and, therefore, the  $\alpha$ -axial orientation of the hydroxyl function.

The molecular formula of axinisothiocyante N (**2**),  $C_{16}H_{25}NO_2S$ , together with the broad IR absorption at  $2090\text{ cm}^{-1}$  indicated that it was also an isothiocyante sesquiterpene. The NMR spectra of **2** were similar to those of **1** except for the downfield shift of the oxygenated carbon at  $\delta_C$  85.2 and the presence of a proton resonance at  $\delta_H$  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 isothiocyante group [ $\delta_C$  64.9 (C-4)] with Me-15 [ $\delta_H$  1.31 (s)] and H-5 [ $\delta_H$  1.97 (dd,  $J = 13.1, 2.5\text{ Hz}$ )] and those of the oxygenated carbon [ $\delta_C$  85.2 (C-7)] with the protons of the isopropenyl group confirmed the location of the isothiocyante 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 axinythiocyante A (**3**) was determined by HRCIMS analysis. The presence of a thiocyante function was deduced from the sharp IR absorption at  $2148\text{ cm}^{-1}$  and the  $^{13}C$  NMR resonance at  $\delta_C$  112.2. The remaining 15 resonances of the  $^{13}C$  NMR spectrum, together with four methyl groups in the  $^1H$  NMR spectrum at  $\delta$  1.69 (d,  $J = 0.8\text{ Hz}$ , 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 [ $\delta_C$  134.1 (C-3) and 119.7 (C-2)/ $\delta_H$  5.37 (br s, H-2);  $\delta_C$  132.8 (C-11) and 122.7 (C-10)/ $\delta_H$  5.09 (tsept,  $J = 7.2, 1.4\text{ Hz}$ , H-10)], whereas the resonance at  $\delta_C$  63.7 (C, C-7) was assigned to the carbon bearing the thiocyante 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  $\delta$  5.09 (H-10) with two methyl groups [ $\delta$  1.69 (Me-12) and 1.63 (Me-15)]. The HMBC correlations of the carbon bearing the thiocyante group [ $\delta_C$  63.7 (C-7)] with the methyl group at  $\delta_H$  1.51 (Me-14) and the methine at  $\delta_H$  1.88 (H-6) defined the attachment of the thiocyante 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  $\delta_H$  5.37 (H-2) with the methyl group at  $\delta_C$  23.1 (Me-13) and the methine at  $\delta_C$  42.5 (C-6). The 12.1 Hz coupling between H-5ax ( $\delta_H$  1.38) and H-6 indicated the axial orientation of H-6, while the configuration at C-7 remains undetermined.

Axinysonone 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**),<sup>11</sup> which was the major metabolite of the sponge.<sup>5</sup> Thus, the NMR spectra of **4** exhibited the signals of an enone [ $\delta_C$  196.5 (C, C-8) and 120.5 (CH, C-9)/ $\delta_H$  6.20 (dd,  $J = 2.1, 1.2\text{ Hz}$ , H-9) and  $\delta_C$  168.5 (C, C-10)], two methine protons of a cyclopropane ring [ $\delta_H$  1.75 (dd,  $J = 8.0, 1.2\text{ Hz}$ , H-7) and 1.37 (d,  $J = 8.0\text{ Hz}$ , H-6)], and four methyl groups [ $\delta_H$  1.23 (s, Me-13), 1.21 (s, Me-12), 1.17 (s, Me-14), and 1.07 (d,  $J = 6.8\text{ Hz}$ , Me-15)]. The most significant difference between the NMR spectra of **4** and (+)-aristolone (**14**)<sup>11</sup> was the absence in **4** of the resonances due to the allylic methylene at C-1, showing those of an oxymethine at  $\delta_C$  69.0/ $\delta_H$  4.38 (br d,  $J = 12.0\text{ Hz}$ ) instead. Moreover, the oxygenated function was identified as a hydroxyl group from the IR absorption at  $3404\text{ cm}^{-1}$ . The location of the hydroxyl group at C-1 was further supported by the HMBC correlations of the oxymethine carbon ( $\delta_C$  69.0, C-1) with the olefinic proton H-9 ( $\delta_H$  6.20) and the methylene protons at C-2 and C-3 [ $\delta_H$  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  $\beta$ -equatorial orientation of Me-15, while the  $\beta$ -axial orientation of Me-14 was supported by the correlation Me-14/H-3ax (Figure 1, energy minimized using MM2). The  $\alpha$ -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  $\beta$ -axial orientation of H-1 and, therefore, the  $\alpha$ -equatorial orientation of the hydroxyl group. On the basis of biogenetic grounds axinysonone A (**4**) was expected to belong to the same enantiomeric series as the co-occurring (+)-aristolone (**14**).<sup>11</sup> This proposal was confirmed by assignment of the absolute configuration of **4**. Treatment of **4** with (*R*)- and (*S*)- $\alpha$ -methoxy- $\alpha$ -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<sup>12</sup> for C-1 and, therefore, an absolute configuration 1*S*,4*S*,5*S*,6*R*,7*S* for axinysonone A (**4**).

The HRCIMS analysis of axinysonone 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  $\alpha$ -equatorial orientation of H-1 and, therefore, the  $\beta$ -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<sup>12</sup> for C-1 and, therefore, an absolute configuration 1*R*,4*S*,5*S*,6*R*,7*S* for axinysonone B (**5**). A compound exhibiting the same structure and relative configuration as **5** has been previously described from the terrestrial plant *Aristolochia debilis*.<sup>13</sup> Although the optical rotation

**Table 1.** NMR Spectroscopic Data (CDCl<sub>3</sub>) for Compounds **4**, **7**, **8**, and **9**<sup>a</sup>

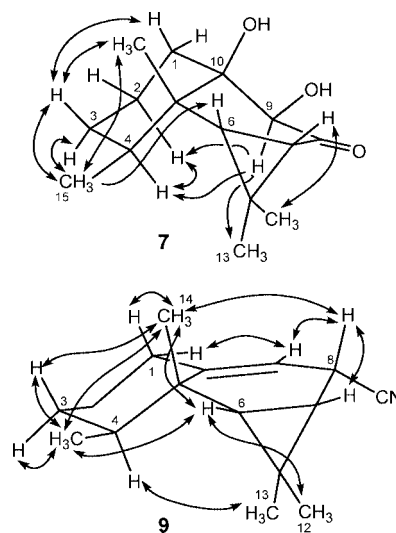
position	<b>4</b> <sup>b</sup>		<b>7</b> <sup>b</sup>		<b>8</b> <sup>c</sup>		<b>9</b> <sup>b</sup>	
	$\delta_C$	$\delta_H$ ( <i>J</i> in Hz)	$\delta_C$	$\delta_H$ ( <i>J</i> in Hz)	$\delta_C$	$\delta_H$ ( <i>J</i> in Hz)	$\delta_C$	$\delta_H$ ( <i>J</i> in Hz)
1	69.0	4.38 br d (12.0)	32.2	1.98 m eq 1.43 ddd (13.7, 13.7, 4.1) ax	129.9	6.09 s	32.6	2.19 dddd (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	

<sup>a</sup> Assignments aided by COSY, HSQC, HMBC, and NOESY experiments. <sup>b</sup> Recorded at 600 MHz. <sup>c</sup> 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 axinysonone C (**6**), C<sub>15</sub>H<sub>22</sub>O<sub>3</sub>, 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  $\delta_C$  85.9 and the presence of a proton resonance at  $\delta_H$  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 ( $\delta_C$  85.9, C-1) with the olefinic proton H-9 ( $\delta_H$  5.96) and the correlation of the oxymethine proton ( $\delta_H$  4.47, H-1) with C-5 ( $\delta_C$  39.0). The 2.7 Hz coupling of H-1 with both H-2eq and H-2ax supported the  $\alpha$ -equatorial orientation of H-1 and, therefore, the  $\beta$ -axial orientation of the hydroperoxy group.

Axinysonone D (**7**) possessed the molecular formula C<sub>15</sub>H<sub>24</sub>O<sub>3</sub>, determined by HRCIMS. The presence of hydroxyl and ketone functions was defined from the IR absorptions at 3436 and 1690 cm<sup>-1</sup>, respectively. The <sup>1</sup>H NMR spectrum displayed the resonances of four methyl groups [ $\delta$  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  $\delta$  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  $\delta_C$  208.8 (C-8), an oxymethine at  $\delta_C$  74.6 (C-9)/ $\delta_H$  4.23 (d, *J* = 2.6 Hz, H-9), and a fully substituted carbon linked to a hydroxyl at  $\delta_C$  82.7 (C-10). The HMBC correlations of the carbonyl carbon with the protons of the cyclopropane ring [ $\delta$  1.97 (H-7), 1.19 (H-6)] and with the oxymethine proton ( $\delta$  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  $\delta_C$  82.7 (C-10) with the methylene protons at  $\delta_H$  1.98 (H-1eq)/1.43 (H-1ax) and the methine at  $\delta_H$  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

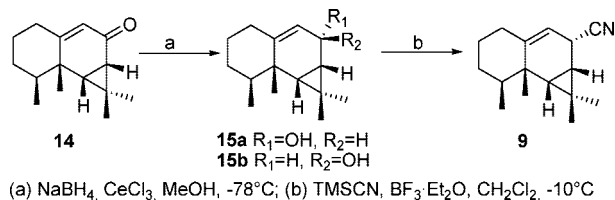


**Figure 2.** Selected NOESY ( $\leftrightarrow$ ) and NOESY-1D ( $\rightarrow$ ) correlations for compounds **7** and **9**.

NOESY correlations of Me-15 with H-3ax and H-3eq defined the  $\beta$ -equatorial orientation of Me-15, whereas the NOESY cross-peak between Me-14 and H-3ax supported the  $\beta$ -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  $\alpha$ -orientation of H-9, the *cis*-fusion of the six-membered rings, and the  $\alpha$ -orientation of the cyclopropane ring (Figure 2).

Axinysonone E (**8**) possessed the molecular formula C<sub>15</sub>H<sub>20</sub>O<sub>2</sub>, determined by HRCIMS measurement. The <sup>1</sup>H NMR spectrum exhibited the signals of four methyl groups [ $\delta$  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 [ $\delta$  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  $\delta_C$  198.3 (C-2) together with those of a trisubstituted double bond at  $\delta_C$  129.9 (C-



**Scheme 1.** Synthesis of Axinyitrile A (**9**) from (+)-Aristolone (**14**)(a) NaBH<sub>4</sub>, CeCl<sub>3</sub>, MeOH, -78°C; (b) TMSCN, BF<sub>3</sub>·Et<sub>2</sub>O, CH<sub>2</sub>Cl<sub>2</sub>, -10°C

1)/ $\delta_{\text{H}}$  6.09 (s, H-1) and  $\delta_{\text{C}}$  164.6 (C-10) established the presence of an  $\alpha,\beta$ -unsaturated ketone. Two methines at  $\delta_{\text{C}}$  56.1 (C-8)/ $\delta_{\text{H}}$  3.65 (ddd,  $J = 4.2, 2.1, 1.0$  Hz, H-8) and  $\delta_{\text{C}}$  56.7 (C-9)/ $\delta_{\text{H}}$  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 ( $\delta_{\text{C}}$  198.3, C-2) was correlated with the methylene protons at  $\delta_{\text{H}}$  2.35 (m, H<sub>2</sub>-3), the carbon of which at  $\delta_{\text{C}}$  42.4 (C-3) showed a correlation with the methyl group at  $\delta_{\text{H}}$  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  $\delta$  3.65 (H-8) and the bridgehead proton H-7 ( $\delta$  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  $\beta$ -orientation of the oxirane ring was proposed from the NOESY correlations of H-8 and H-9 with Me-13.

The molecular formula of axinyitrile A (**9**), C<sub>16</sub>H<sub>23</sub>N, was determined by HRCIMS. The NMR spectra featured resonances attributable to an aristolane sesquiterpene containing a trisubstituted double bond [ $\delta_{\text{C}}$  146.1 and 112.2/ $\delta_{\text{H}}$  5.11 (br s)]. This unsaturation was located at C-9 from the HMBC correlations of the olefinic carbons at  $\delta_{\text{C}}$  112.2 (C-9) and 146.1 (C-10) with the bridgehead protons at  $\delta_{\text{H}}$  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 <sup>13</sup>C NMR spectrum exhibited a signal at  $\delta$  121.7 (C) that together with the weak IR absorption at 2236 cm<sup>-1</sup> 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  $\delta_{\text{C}}$  25.4/ $\delta_{\text{H}}$  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  $\delta$  3.68 (H-8) with the cyclopropyl proton H-7 and the HMBC correlation of the carbon at  $\delta_{\text{C}}$  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  $\beta$ -equatorial orientation of Me-15 and the  $\beta$ -axial orientation of Me-14, respectively (Figure 2, energy minimized using MM2). The NOESY correlation H-6/Me-14 defined the  $\beta$ -orientation of the cyclopropyl protons. Finally a weak NOESY correlation between H-8 and Me-14 suggested the  $\beta$ -orientation of H-8 and, therefore, the  $\alpha$ -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<sup>14</sup> 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 [ $\delta$  4.51 (ddd,  $J = 7.1, 3.8, 2.5$  Hz, H-8)] exhibited a weak NOE interaction with Me-14 [ $\delta$  0.92 (s)], whereas in **15b** the proton H-8 [ $\delta$  4.13 (d,  $J = 4.4$

Hz)] showed a NOESY correlation with Me-13 [ $\delta$  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<sub>3</sub>·Et<sub>2</sub>O<sup>15</sup> to yield the cyano derivative **9** (77.9% yield) together with the elimination product 1(10),8-aristoladiene<sup>14</sup> (11.8% yield). Compound **9** exhibited optical rotation and spectroscopic data identical to those of the natural axinyitrile 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<sub>50</sub> values of 33.3 and 32.6  $\mu\text{M}$ , respectively. Compound **8** was active against A-549 and HT-29 with GI<sub>50</sub> values of 38.7 and 38.3  $\mu\text{M}$ , respectively. Finally, compound **13** exhibited growth inhibitory activity with GI<sub>50</sub> values of 27.0, 36.4, and 33.4  $\mu\text{M}$  against MDA-MB-231, A-549, and HT-29, respectively. The remaining compounds were inactive at the highest concentration tested (10  $\mu\text{g/mL}$ ).

This study, taken together with our preceding results,<sup>5</sup> 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 thiocyno-substituted marine terpenoids, for which only seven representatives have been previously reported.<sup>1c,2a</sup> 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.<sup>16</sup> Among these metabolites, cyanopuupehenol and cyanopuupehenone from a Verongid sponge<sup>17</sup> 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 axinyitrile 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.<sup>1,2</sup> 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.<sup>1c</sup> 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.<sup>18</sup> Compounds **4–9** and **14** from *A. isabela*, together with (+)-9-aristolene from *Acanthella cavernosa*,<sup>18e</sup> 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. <sup>1</sup>H and <sup>13</sup>C NMR spectra were recorded on a Varian INOVA 600 or on a Varian INOVA 400 spectrometer using CDCl<sub>3</sub> or C<sub>6</sub>D<sub>6</sub> as solvents. Chemical shifts were referenced using the corresponding solvent signals [ $\delta_{\text{H}}$  7.26 and  $\delta_{\text{C}}$  77.0 for CDCl<sub>3</sub>,  $\delta_{\text{H}}$  7.15 and  $\delta_{\text{C}}$  128.0 for C<sub>6</sub>D<sub>6</sub>]. COSY, HSQC, HMBC, and NOESY experiments were performed using standard Varian pulse sequences. Low-resolution mass spectra were recorded on a Finnigan Voyager GC8000<sup>0P</sup> 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 *Axinysa isabela*<sup>6</sup> (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.<sup>5</sup> The fraction of the general chromatography eluted with hexanes/Et<sub>2</sub>O (95:5) was chromatographed over a silica gel column using hexanes and hexanes/Et<sub>2</sub>O 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**)<sup>7</sup> (5.3 mg,  $1.9 \times 10^{-3}$  % dry wt), **11**<sup>8</sup> (3.6 mg,  $1.3 \times 10^{-3}$  % dry wt), **3** (9.7 mg,  $3.5 \times 10^{-3}$  % dry wt), **12**<sup>9</sup> (5.0 mg,  $1.8 \times 10^{-3}$  % dry wt), **13**<sup>10</sup> (5.5 mg,  $2.0 \times 10^{-3}$  % dry wt), and **9** (4.5 mg,  $1.6 \times 10^{-3}$  % dry wt). The fraction of the general chromatography eluted with hexanes/Et<sub>2</sub>O (90:10) was further separated over a silica gel column using hexanes/Et<sub>2</sub>O 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,  $4.3 \times 10^{-4}$  % dry wt) and **2** (3.8 mg,  $1.3 \times 10^{-3}$  % dry wt). The fraction of the general chromatography eluted with hexanes/Et<sub>2</sub>O (80:20) was subjected to column chromatography eluted with hexanes/Et<sub>2</sub>O mixtures (90:10 to 50:50). Separations of selected fractions by normal-phase HPLC (hexanes/EtOAc, 84:16) afforded compound **8** (2.5 mg,  $8.9 \times 10^{-4}$  % dry wt). The fraction of the general chromatography eluted with hexanes/Et<sub>2</sub>O (70:30) was chromatographed over a silica gel column eluted with hexanes/Et<sub>2</sub>O 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,  $1.2 \times 10^{-3}$  % dry wt). The fractions of the general chromatography eluted with hexanes/Et<sub>2</sub>O (30:70, 20:80) and Et<sub>2</sub>O were joined and chromatographed over a silica gel column eluted with hexanes/Et<sub>2</sub>O mixtures (30:70 and 20:80) and then Et<sub>2</sub>O. The fraction eluted with hexanes/Et<sub>2</sub>O (30:70) was further separated by HPLC in normal (hexanes/EtOAc, 70:30) and reversed phase (MeOH/H<sub>2</sub>O, 80:20) to yield compound **6** (2.0 mg,  $7.1 \times 10^{-4}$  % dry wt). The fraction eluted with hexanes/Et<sub>2</sub>O (20:80) was subjected to repeated HPLC separations in normal-phase (hexanes/EtOAc, 60:40) and reversed-phase (MeOH/H<sub>2</sub>O, 65:35) to obtain compounds **5** (16.2 mg,  $5.8 \times 10^{-3}$  % dry wt) and **4** (16.4 mg,  $5.8 \times 10^{-3}$  % dry wt).

**Axinisothiocyanate M (1):** colorless oil;  $[\alpha]_D^{25} -40.8$  (c 0.1, CCl<sub>4</sub>); UV (MeOH)  $\lambda_{max}$  (log  $\epsilon$ ) 243 (3.09) nm; IR (film)  $\nu_{max}$  3466, 2082 cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 600 MHz)  $\delta$  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<sub>2</sub>-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); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 150 MHz)  $\delta$  151.8 (C, C-11), 109.4 (CH<sub>2</sub>, C-13), 74.4 (C, C-7), 64.9 (C, C-4), 47.4 (CH, C-5), 41.9 (CH<sub>2</sub>, C-3), 40.0\* (CH<sub>2</sub>, C-1), 39.9\* (CH<sub>2</sub>, C-9), 34.4 (C, C-10), 32.9 (CH<sub>2</sub>, C-6), 31.8 (CH<sub>2</sub>, C-8), 22.1 (CH<sub>3</sub>, Me-15), 19.0 (CH<sub>3</sub>, Me-12), 18.9 (CH<sub>2</sub>, C-2), 18.1 (CH<sub>3</sub>, Me-14), signals marked with an asterisk may be interchanged; CIMS  $m/z$  279 (6) [M]<sup>+</sup>, 221 (36), 220 (20), 203 (100); HRCIMS(+)  $m/z$  279.1635 (calcd for C<sub>16</sub>H<sub>25</sub>NOS, 279.1657).

**Axinisothiocyanate N (2):** colorless oil;  $[\alpha]_D^{25} -75.7$  (c 0.05, CHCl<sub>3</sub>); UV (MeOH)  $\lambda_{max}$  (log  $\epsilon$ ) 244 (3.21) nm; IR (film)  $\nu_{max}$  3416, 2090 cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz)  $\delta$  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<sub>2</sub>-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); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz)  $\delta$  148.0 (C, C-11), 112.1 (CH<sub>2</sub>, C-13), 85.2 (C, C-7), 64.9 (C, C-4), 47.5 (CH, C-5), 41.9 (CH<sub>2</sub>, C-3), 39.9\* (CH<sub>2</sub>, C-1), 39.8\* (CH<sub>2</sub>, C-9), 34.5 (C, C-10), 28.1 (CH<sub>2</sub>, C-8), 27.3 (CH<sub>2</sub>, C-6), 21.7 (CH<sub>3</sub>, Me-15), 18.8 (CH<sub>2</sub>, C-2), 18.7 (CH<sub>3</sub>, Me-12),

18.2 (CH<sub>3</sub>, Me-14), signals marked with an asterisk may be interchanged; HRCIMS(+)  $m/z$  295.1597 (calcd for C<sub>16</sub>H<sub>25</sub>NO<sub>2</sub>S, 295.1606).

**Axinisothiocyanate A (3):** colorless oil;  $[\alpha]_D^{25} +39.7$  (c 0.1, CHCl<sub>3</sub>); UV (MeOH)  $\lambda_{max}$  (log  $\epsilon$ ) 233 (3.23) nm; IR (film)  $\nu_{max}$  2148 cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 600 MHz)  $\delta$  5.37 (1H, br s, H-2), 5.09 (1H, sept,  $J = 7.2, 1.4$  Hz, H-10), 2.10 (3H, m, H-1, H<sub>2</sub>-9), 2.02 (2H, m, H<sub>2</sub>-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); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 150 MHz)  $\delta$  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<sub>2</sub>, C-8), 30.9 (CH<sub>2</sub>, C-4), 27.2 (CH<sub>2</sub>, C-1), 25.7 (CH<sub>3</sub>, Me-12), 24.6 (CH<sub>2</sub>, C-5), 23.8 (CH<sub>3</sub>, Me-14), 23.1 (CH<sub>2</sub>, C-9), 23.1 (CH<sub>3</sub>, Me-13), 17.7 (CH<sub>3</sub>, Me-15); CIMS  $m/z$  263 (4) [M]<sup>+</sup>, 205 (78), 204 (27), 189 (9), 69 (100); HRCIMS(+)  $m/z$  263.1694 (calcd for C<sub>16</sub>H<sub>25</sub>NS, 263.1708).

**Axinysone A (4):** colorless oil;  $[\alpha]_D^{25} +254.0$  (c 0.1, CHCl<sub>3</sub>); UV (MeOH)  $\lambda_{max}$  (log  $\epsilon$ ) 232 (3.93) nm; IR (film)  $\nu_{max}$  3404, 1639 cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 600 MHz) see Table 1; <sup>13</sup>C NMR (CDCl<sub>3</sub>, 150 MHz) see Table 1; EIMS  $m/z$  234 (50) [M]<sup>+</sup>, 219 (21), 216 (88), 201 (100); HRCIMS(+)  $m/z$  235.1696 [M + H]<sup>+</sup> (calcd for C<sub>15</sub>H<sub>23</sub>O<sub>2</sub>, 235.1698).

**Axinysone B (5):** colorless oil;  $[\alpha]_D^{25} +108.0$  (c 0.1, CHCl<sub>3</sub>); UV (MeOH)  $\lambda_{max}$  (log  $\epsilon$ ) 232 (3.96) nm; IR (film)  $\nu_{max}$  3400, 1641 cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 600 MHz)  $\delta$  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); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 150 MHz)  $\delta$  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<sub>2</sub>, C-2), 29.8 (CH<sub>3</sub>, Me-12), 25.4 (C, C-11), 24.9 (CH<sub>2</sub>, C-3), 24.6 (CH<sub>3</sub>, Me-14), 16.2 (CH<sub>3</sub>, Me-15), 16.1 (CH<sub>3</sub>, Me-13); EIMS  $m/z$  234 (16) [M]<sup>+</sup>, 219 (14), 216 (40), 201 (72), 173 (36), 149 (57), 105 (80), 91 (100); HRCIMS(+)  $m/z$  235.1685 [M + H]<sup>+</sup> (calcd for C<sub>15</sub>H<sub>23</sub>O<sub>2</sub>, 235.1698).

**Axinysone C (6):** colorless oil;  $[\alpha]_D^{25} +139.0$  (c 0.1, CHCl<sub>3</sub>); UV (MeOH)  $\lambda_{max}$  (log  $\epsilon$ ) 232 (3.94) nm; IR (film)  $\nu_{max}$  3382, 1643 cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 600 MHz)  $\delta$  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); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 150 MHz)  $\delta$  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<sub>3</sub>, Me-12), 29.4 (CH<sub>2</sub>, C-2), 25.4 (C, C-11), 23.4 (CH<sub>2</sub>, C-3), 23.3 (CH<sub>3</sub>, Me-14), 16.2 (CH<sub>3</sub>, Me-13), 16.2 (CH<sub>3</sub>, Me-15); EIMS  $m/z$  251 (1) [M + H]<sup>+</sup>, 217 (3), 109 (40), 59 (100); HRCIMS(+)  $m/z$  251.1644 [M + H]<sup>+</sup> (calcd for C<sub>15</sub>H<sub>23</sub>O<sub>3</sub>, 251.1647).

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

**Axinysone E (8):** colorless oil;  $[\alpha]_D^{25} +84.3$  (c 0.07, CHCl<sub>3</sub>); UV (MeOH)  $\lambda_{max}$  (log  $\epsilon$ ) 242 (3.87) nm; IR (film)  $\nu_{max}$  1670 cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) see Table 1; <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz) see Table 1; CIMS  $m/z$  233 (89) [M + H]<sup>+</sup>, 217 (47), 203 (45), 161 (84), 91 (100); HRCIMS  $m/z$  233.1529 [M + H]<sup>+</sup> (calcd for C<sub>15</sub>H<sub>21</sub>O<sub>2</sub>, 233.1541).

**Axinynitrile A (9):** white solid;  $[\alpha]_D^{25} +70.0$  (c 0.1, CHCl<sub>3</sub>); IR (film)  $\nu_{max}$  2236 cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 600 MHz) see Table 1; <sup>13</sup>C NMR (CDCl<sub>3</sub>, 150 MHz) see Table 1; EIMS  $m/z$  229 (36), 214 (47), 186 (95), 130 (100); HRCIMS(+)  $m/z$  229.1825 (calcd for C<sub>16</sub>H<sub>23</sub>N, 229.1830).

**Synthesis of the (R)-MPA Ester 4r.** A solution of **4** (0.8 mg,  $3.4 \times 10^{-3}$  mmol) in 0.25 mL of CH<sub>2</sub>Cl<sub>2</sub> was treated with CH<sub>2</sub>Cl<sub>2</sub> solutions of *N,N'*-dicyclohexylcarbodiimide (2.2 mg, 0.011 mmol in 0.25 mL), *N,N*-dimethylaminopyridine (0.9 mg,  $7.4 \times 10^{-3}$  mmol in 0.25 mL), and (*R*)- $\alpha$ -methoxy- $\alpha$ -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**: <sup>1</sup>H NMR (CDCl<sub>3</sub>, 600 MHz; selected



data, assignments aided by COSY and NOESY experiments)  $\delta$  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,  $5.1 \times 10^{-3}$  mmol) in 0.25 mL of  $\text{CH}_2\text{Cl}_2$  was treated with  $\text{CH}_2\text{Cl}_2$  solutions of *N,N'*-dicyclohexylcarbodiimide (2.0 mg,  $9.7 \times 10^{-3}$  mmol in 0.25 mL), *N,N*-dimethylaminopyridine (1.0 mg,  $8.2 \times 10^{-3}$  mmol in 0.25 mL), and (S)- $\alpha$ -methoxy- $\alpha$ -phenylacetic acid (1.5 mg,  $9.0 \times 10^{-3}$  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**:  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 600 MHz; selected data, assignments aided by COSY and NOESY experiments)  $\delta$  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  $\text{CH}_2\text{Cl}_2$  was treated with  $\text{CH}_2\text{Cl}_2$  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)- $\alpha$ -methoxy- $\alpha$ -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**:  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 600 MHz; selected data, assignments aided by COSY and NOESY experiments)  $\delta$  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  $\text{CH}_2\text{Cl}_2$  was treated with  $\text{CH}_2\text{Cl}_2$  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)- $\alpha$ -methoxy- $\alpha$ -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**:  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 600 MHz; selected data, assignments aided by COSY and NOESY experiments)  $\delta$  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 (1H, 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  $\text{CH}_2\text{Cl}_2$  (1.5 mL) was added a solution of  $\text{CeCl}_3 \cdot 7\text{H}_2\text{O}$  (212.0 mg, 0.57 mmol) in MeOH (4 mL). After stirring the mixture for 10 min,  $\text{NaBH}_4$  (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  $\text{H}_2\text{O}$  (15 mL), vigorously stirred, and extracted with  $\text{Et}_2\text{O}$  ( $3 \times 15$  mL). The organic layer was washed with brine and dried over  $\text{MgSO}_4$ . 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**:  $^1\text{H}$  NMR ( $\text{C}_6\text{D}_6$ , 600 MHz)  $\delta$  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);  $^{13}\text{C}$  NMR ( $\text{C}_6\text{D}_6$ , 150 MHz)  $\delta$  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 ( $\text{CH}_2$ , C-1), 31.5 ( $\text{CH}_2$ , C-3), 30.8 ( $\text{CH}_3$ , Me-12), 28.4 (CH, C-7), 26.9 ( $\text{CH}_2$ , C-2), 21.6 ( $\text{CH}_3$ , Me-14), 19.6 (C, C-11), 17.8 ( $\text{CH}_3$ , Me-13), 16.5 ( $\text{CH}_3$ , Me-15). Compound **15b**:  $^1\text{H}$  NMR ( $\text{C}_6\text{D}_6$ , 600 MHz)  $\delta$  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);  $^{13}\text{C}$  NMR ( $\text{C}_6\text{D}_6$ , 150 MHz)  $\delta$  145.3 (C, C-10), 122.3

(CH, C-9), 63.2 (CH, C-8), 32.0 (CH, C-6), 29.9 ( $\text{CH}_3$ , Me-12), 28.2 (CH, C-7), 22.7 ( $\text{CH}_3$ , Me-14), 16.1 ( $\text{CH}_3$ , Me-15), 15.9 ( $\text{CH}_3$ , 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  $\text{CH}_2\text{Cl}_2$  (1.5 mL) was added TMSCN (60  $\mu\text{L}$ , 0.48 mmol). Then 700  $\mu\text{L}$  of a 0.1 M solution of  $\text{BF}_3 \cdot \text{Et}_2\text{O}$  in  $\text{CH}_2\text{Cl}_2$  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  $\text{NaHCO}_3$  solution (12 mL), stirred for 20 min, and extracted with  $\text{CH}_2\text{Cl}_2$  ( $3 \times 15$  mL). The organic layer was washed with brine and dried over  $\text{MgSO}_4$ . After filtration and solvent evaporation under reduced pressure, the residue was chromatographed over a  $\text{SiO}_2$  column eluted with hexanes and hexanes/ $\text{Et}_2\text{O}$  (95:5) to obtain 1(10),8-aristoladiene<sup>17</sup> (1.6 mg, 0.008 mmol, 11.8% yield) and axinyntitrile 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 ( $\text{GI}_{50} = 0.1, 0.1$ , and 0.1  $\mu\text{M}$  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.<sup>19</sup>

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**Supporting Information Available:**  $^1\text{H}$  and  $^{13}\text{C}$  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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