### Title

Stereochemical challenges in characterizing nitrogenous spiro-axane sesquiterpenes from the Indo-Pacific sponges Amorphinopsis and Axinyssa




### Journal

Tetrahedron, 62(44)

### ISSN

0040-4020



### Authors

Wegerski, C J
Sonnenschein, R N
Cabriales, F
et al.



### Publication Date

2006-10-01

Peer reviewed

# Stereochemical Challenges in Characterizing Nitrogenous Spiro-Axane Sesquiterpenes from the Indo-Pacific Sponges *Amorphinopsis* and *Axinyssa*.

[Terpene_v 5.0 Revised]

Christopher J. Wegerski,[†] Rachel N. Sonnenschein,[†] Freddy Cabriales,[†] Frederick A. Valeriote,[‡] and Phillip Crews[*†]

[†]*Department of Chemistry and Biochemistry & Institute of Marine Sciences, University of California, Santa Cruz, California 95064 USA,* [‡]*Henery Ford Health System, Department of Internal Medicine, Division of Hematology and Oncology, Detroit, MI 48202, USA*

*RECEIVED DATE (will be automatically inserted after manuscript is accepted).*

---

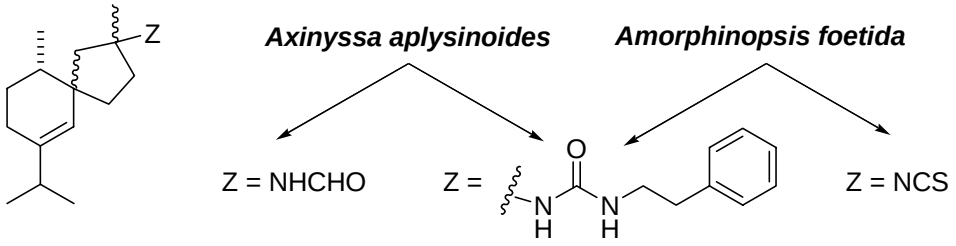
*To whom correspondence should be addressed: Tel: 831-459-2603; Email: phil@chemistry.ucsc.edu

**Abstract**

An investigation was conducted to identify the structures and bioactive properties of five compounds isolated from the Halichondrida sponges *Amorphinopsis foetida* and *Axinyssa aplysinoides*. All compounds possessed the spiro-axane sesquiterepene core and all were substituted at C-2 with nitrogen containing functionality. The stereochemistry of one known compound has been revised to (2*R*,5*R*,10*S*)-2-formamido-6-axene (**3**). It exhibited mild selective solid tumor and mild antibacterial activity and was found from *Axinyssa*. A second known substance whose stereochemistry has also been revised, (2*R*,5*R*,10*S*)-2-isothiocyanato-6-axene (**4**) plus its undescribed diastereomer (**5**) were isolated from *Amorphinopsis*. Both sponges were the source of two new *N*-phenethyl-2-formamido-6-axene diastereomeric compounds (**6**) and (**7**). No solid tumor or antibacterial activity was found for **4** – **7**.

**Keywords**: Terpene, Marine Natural Products, *Amorphinopsis*, *Axinyssa*.

**Graphical Abstract**

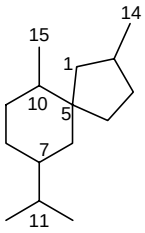


## Introduction

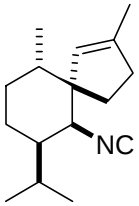
The strategy of using an in vitro cell-based assay to identify solid tumor selectivity[1] continues to be a robust tool in our quest to investigate unusual constituents of Indo-Pacific sponges.[2] The stimulus for this work was the in vitro solid tumor selectivity observed for extracts of two Indo-Pacific sponges belonging to different genera but in the same family consisting of *Amorphinopsis foetida*[3] (Order Halichondrida, family Halichondriidae) obtained from Papua New Guinea and *Axinyssa aplysinoides*[4] collected from Vanuatu.[5] The parallel bioactivity pattern we observed suggested that similar metabolites might be present in both sponges. Nitrogen containing terpenes, which often possess many structural variants, are commonly isolated from sponges of the Order Halichondrida and are exemplified by compounds containing isocyanide, isothiocyanate, thiocyanate, and formamide moieties.[6]

Our pursuit to pinpoint the major constituents of *A. foetida* and *A. aplysinoides* became of priority when preliminary data was obtained indicating that nitrogenous spiro-axane sesquiterpene metabolites were to be isolated. The current literature shows fifteen sponge or nudibranch derived spiro-axane compounds of general structure **A** divided between fourteen substituted with *N*-functionality and one with an OH group.[6,7] While the dereplication of a spiro-axane skeleton is now relatively straightforward, defining the configuration at each of the possible chiral centers (C-2, C-5, C-7 and C-10) as well as the sites for nitrogen attachement (C-1, C-2 or C-6) can be more difficult. There are two sponge derived compounds that provide important stereostructural templates for the members of this series and these are (+)-axisonitrile-3 (**1**)[8] and (+)-exiguamide (**2**),[9] whose defined absolute configurations were established after extensive experimentation.

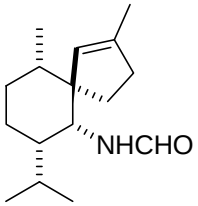
An important feature for both is the 10*S* configuration and the variation in geometry from 5*R* in **1** to 5*S* in **2**, which illustrates the biosynthetic similarities and differences that are possible for this series. Thus, the major challenge we faced was to determine the substitution pattern and absolute configurations for each of the compounds isolated. Described below are properties of five compounds obtained including the known substances, 2-formamido-6-axene (**3**), and 2-isothiocyanato-6-axene (**4**),[10] its undescribed diastereomer **5**, and a set of new diastereomeric compounds, *N*-phenethyl-2-formamido-6-axenes **6** and **7**.



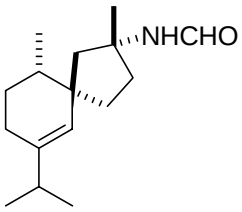

A



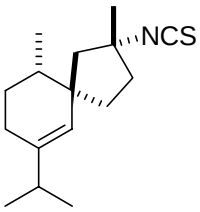

(**1**) (+)-axisonitrile-3



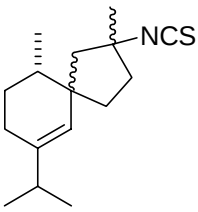

(**2**) (+)-exiguamide



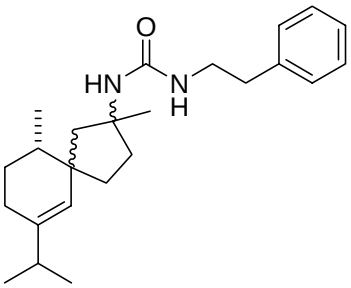

(**3**) 2*R*, 5*R*, 10*S*



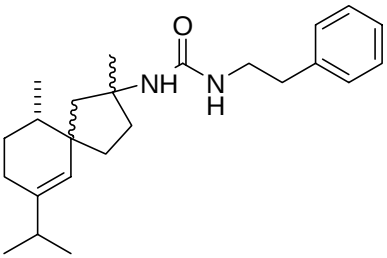

(**4**) 2*R*, 5*R*, 10*S*




(**5**) 2*S*, 5*R*, 10*S* or 2*R*, 5*S*, 10*S*




(**6**) 2*S*, 5*S*, 10*S* or 2*R*, 5*R*, 10*S*




(**7**) 2*S*, 5*R*, 10*S* or 2*R*, 5*S*, 10*S*

**Results and Discussion**

The extracts of *A*. *aplysinoides* (coll. no. 03411, Vanuatu) were investigated first as the preliminary data from the disk diffusion solid tumor whole cell assays fit the profile for C38 selectivity. Two semi-pure fractions (see Figure S1 in Supporting Information) were selected for the isolation work and these include the hexane soluble fraction (coded as XFH) and the dichloromethane crude extract (coded as XFD). Reversed-phase HPLC on the XFD afforded known **3**[10] whose NMR spectra contained doubled peaks ascribed to a mixture of amide rotamers in ratio of 6:4 as observed by $^1$H NMR ($CDCl_3$). LCMS evaluation also revealed **3** (*m/z* = 250.2) as a major component of the XFH fraction. Further fractionation on the XFD guided by LCMS screening revealed a subfraction containing two isomeric compounds exhibiting a *m/z* of 369.3 $[M+H]^+$. Further HPLC purification afforded small quantities of **6** and **7** (~ 0.5 mg each) presumed to have a spiro-axane core but, by NMR, without multiple rotameric forms. Their structures were elucidated after re-isolation of more material as described below.

The sponge *A*. *foetida* (coll. no. 00381, Papua New Guinea) was examined next and the focus was on the butanol partition fraction of the crude extract, coded as WB (see

Figure S2 in Supporting Information). The preliminary data from the disk diffusion solid tumor whole cell assay on WB also exhibited a profile indicative of C38 selectivity. HPLC purification afforded fifteen fractions (coded as H1 to H15), which were then screened in the solid tumor assay. The H4 - H6 fractions exhibited the largest selectivity for solid tumors. Further LC screening of the HPLC fractions using evaporative light scattering (ELSD), UV, and MS detection was used to identify possible spiro-axane containing samples. Attention was given to fractions H7 and H12 with *m/z* peaks of 369.3 amu and 264.2 amu respectively. Repeated HPLC of H12 yielded the previously reported compound 2-isothiocyanato-6-axene (**4**)[10] and its undescribed diastereomer 2-isothiocyanato-6-axene (**5**). Similarly, HPLC of H7 yielded additional samples of **6** (5 mg) and **7** (6 mg).

The structures of **3** (HRESIMS m/z 250.2093 $[M+H]^+$ requiring molecular formula $C_{16}H_{27}NO$) and **4** (HRESIMS *m/z* 264.1706 $[M+H]^+$ requiring molecular formula $C_{16}H_{25}NS$) were confirmed by comparing their properties to those in the literature. Their NMR data also provided an important reference to evaluate the new compounds that were isolated. Compounds **4** and **5** possessed identical molecular formulas and their $^1H$ and $^{13}C$ NMR data, shown in Table 1, were also extremely similar. Two-dimensional NMR data for **5** including gCOSY, gHMQC, and gHMBC (Table 1) indicated it had the same atom connectivity as in **4**. Alternatively, their optical rotations were of different overall sign and magnitude, **4** $[\alpha]^{25}_D = -22.4^\circ$ vs. **5** $[\alpha]^{25}_D = +44.1^\circ$, indicating that one or more stereocenters were different between this pair.

**Table 1**. NMR Data[a] for Compounds **4** and **5** in $CDCl_3$

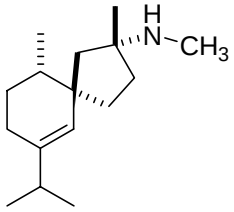
| | **4** | | | **5** | | | |
|---|---|---|---|---|---|---|---|
| position | $\delta_C$ | $\delta_H$ (*J* in Hz) | NOE | $\delta_C$ | $\delta_H$ (*J* in Hz) | gHMBC | NOE |
| 1 | 55.5 | 2.09 dd (1.5, 14.7) | | 54.2 | 1.98 dd (14.1, 2.0) | 2, 5, 14 | $1_b$, 6 |
| | | 1.68 d (14.7) | $1_a$, 6, 14 | | 1.74 d (14.1) | | $1_a$, $3_b$, 10, 14, 15 |
| 2 | 68.0 | | | 68.5 | | | |
| 3 | 41.2 | 2.01 dddd (1.6, 4.2, 6.4, 12.7) | | 42.2 | 2.05 m | 1, 2, 4, 5 | $3_b$, $4_a$, 6 |
| | | 1.81 m | | | 1.60 m | | |
| 4 | 35.1 | 1.46 m | 6, 15 | 34.0 | 1.77 m | 1, 2, 3, 10 | 15 |
| | | 1.43 m | 6, 15 | | 1.63 m | | |
| 5 | 46.4 | | | 46.5 | | | |
| 6 | 127.3 | 5.14 s | $1_b$, $3_b$, $4_b$, 11, 12, 13, 14 | 128.6 | 5.45 s | 1, 5, 8, 10, 11 | $1_a$, $4_b$, 11, 12, 13 |
| 7 | 140.5 | | | 140.1 | | | |
| 8 | 22.5 | 1.92 m | | 24.4 | 1.90 ddd (1.5, 5.9, 7.2) | 9, 10 | $9_a$, $9_b$, 10, 11, 12, 13, 15 |
| | | 1.82 m | | | | | |
| 9 | 27.8 | 1.93 m | | 28.6 | 1.58 m | 5, 7, 8, 15 | |
| | | 1.63 m | | | 1.36 dddd (7.2, 7.2, 9.3, 13.3) | | $4_a$, 15 |
| 10 | 36.9 | 1.70 ddq (2.9, 6.8, 6.8) | | 37.4 | 1.45 ddq (2.4, 9.3, 6.8) | 4, 5, 9 | $1_b$, 8, 15 |
| 11 | 35.0 | 2.11 sept (6.8) | | 34.9 | 2.15 sept (6.8) | 6, 7, 8, 12, 13 | $4_a$, 6, 8, 12, 13 |
| 12 | 21.7 | 0.96 d (6.8) | 6, $8_a$, $8_b$, 11 | 21.7 | 0.99 d (6.8) | 7, 11 | 6, 8, 11 |
| 13 | 21.5 | 0.96 d (6.8) | 6, $8_a$, $8_b$, 11 | 21.6 | 0.99 d (6.8) | 7, 11 | 6, 8, 11 |
| 14 | 28.5 | 1.51 s | | 27.8 | 1.49 s | 1, 2, 3 | $1_b$ |
| 15 | 15.2 | 0.90 d (6.8) | $1_a$, $4_a$, $8_a$, 10 | 15.8 | 0.85 d (6.8) | 5, 9, 10 | $1_b$, $4_a$, $9_a$, $9_b$, 10 |
| 16 | 129.2 | | | 129.3 | | | |

[a] Measured at 500 MHz ($^1H$) and 125 MHz ($^{13}C$). $H_a$ = downfield proton, $H_b$ = upfield proton.

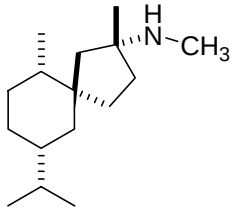
There were two issues considered as a prelude to elucidating the stereochemistry of **5**. First, a reasonable assumption of 10*S* chirality was assigned for both **4** and **5** based on biosynthetic analogy to this absolute stereochemistry rigorously assigned to both (+)-axisonitrile 3 (**1**)[8] and (+)-exiguamide (**2**).[9] Second, on reexamining the argument for the previous assignment of the 2*R**,5*R**,10*R** configuration proposed for **4**,[10] based primarily on NOE data, it was clear that other plausible arrangements had not been ruled out. The four possibilities we envisioned consisted of 2*S*, 5*S*, 10*S*; 2*R*, 5*R*, 10*S*; 2*R*, 5*S*, 10*S*; or 2*S*, 5*R*, 10*S*. An NOE correlation we observed for **4** from H-6 to $H_3$-14, shown in Figure 1, can be used to rule out the latter two. Consistent with the literature,[10] we observed other key NOE enhancements (Figure 1) from H-6 (δ 5.45) to H-$1_b$ (δ 1.68), H-$3_b$ (δ 1.81), H-$4_b$ (δ 1.43),[11] and $H_3$-14 (δ 1.51), which indicates these atoms are on the same side of the molecule, but this does not differentiate between the remaining two possibilities. The interpretation of these results is further complicated by the presence of two conformers for the cyclohexene ring of **4** in which $H_3$-15 can be pseudo-axial or pseudo-equatorial. Modeling experiments for all four conformers (Figure 1) provided two sets of predicted *J* values: (a) $H_3$-15(eq) $^3J_{9\text{-}10}$ = 7.2 and 5.7 Hz, vs. (b) $H_3$-15(ax) $^3J_{9\text{-}10}$ = 2.5 and 2.1 Hz. Comparison of these data to that observed experimentally, $^3J_{9\text{-}10}$ = 6.8 and 2.9 Hz, indicated significant populations were present of both conformations. Thus, either possibility of 2*S*, 5*S*, 10*S* or 2*R*, 5*R*, 10*S* are consistent with the NOE and *J* data.

The results of semi-synthesis provided additional information to resolve this uncertainty. This process began with the LAH reduction of the isothiocyanate **4**, yielding **8**, which was then hydrogenated to afford **9**. The $^1$H NMR data collected supported the

*cis* arrangement of the equatorial isopropyl at C-7 and the axial methyl at C-10 in **9**. A complex $^1$H NMR multiplet was observed for H-10 (δ 1.69) with a *J* value sum of 27 Hz (see Figure S3). Simulation of the ddq patterns expected for H-10 as a function of variation in its geometry gave a *J* value sum of 33.1 Hz for axial ($^3J$ = 9.4, 2.4, 7.1 Hz) versus a *J* value sum of 26.5 Hz for equatorial ($^3J$ = 3.7, 1.5, 7.1 Hz). The next step was to obtain and interpret NOE data for **9**. The key result consisted of a strong enhancement from H-$4_a$ to $H_3$-15 (axial) which requires that C-4 is equatorial. Finally, these data indicate the configuration of **9** as 2*R*,5*R*,10*S*, which translates into reversal of assignments at C-2 and C-5 as was previousy reported for **4**.[10] In addition, the stereochemistry of **3** can provisionally be revised to 2*R*,5*R*,10*S* since both **3** and **4** have previoulsly been determined to have the same stereochemistry when isolated from the same sponge.[10]

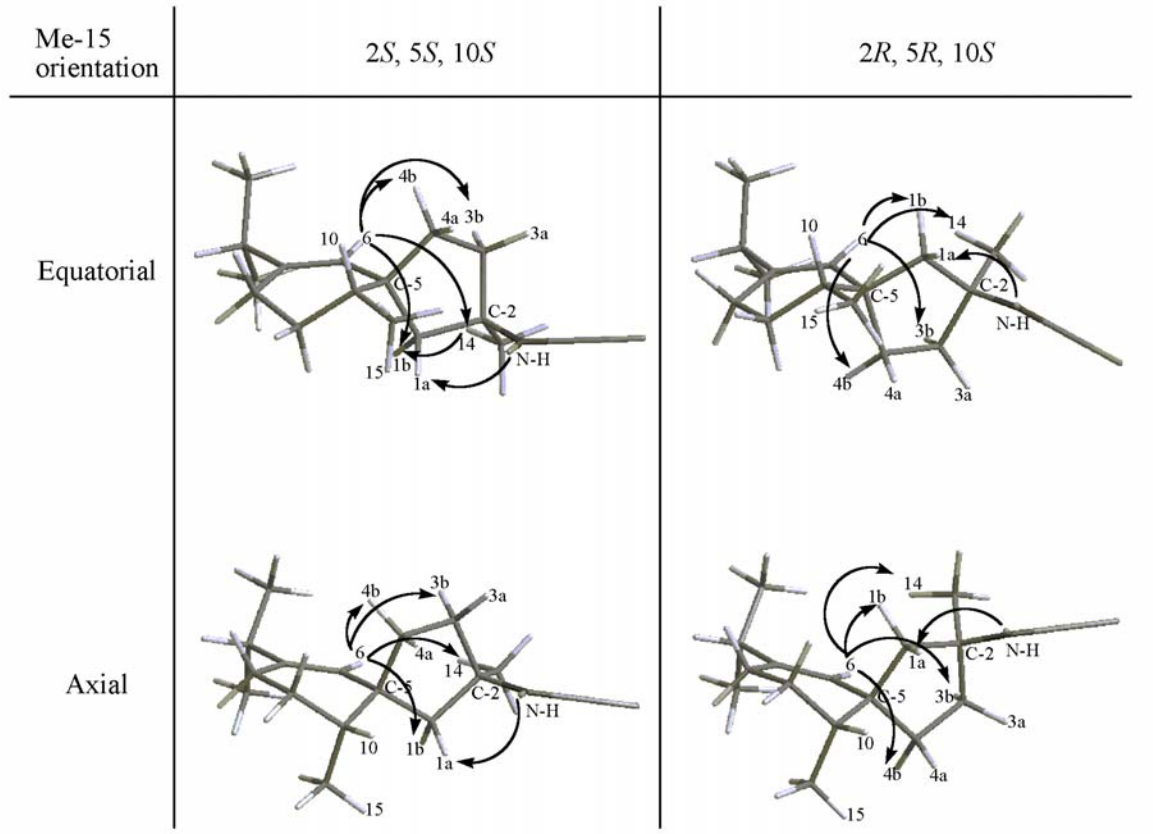



(**8**) 2*R*, 5*R*, 10*S*




(**9**) 2*R*, 5*R*, 10*S*

**Figure 1**. NOE correlations for candidate structures of **4**.

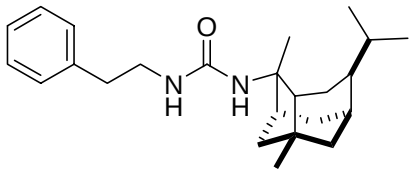



With the stereochemistry of **4** defined above, there were now three possibilities to be considered for **5**: 2*S*, 5*S*, 10*S*; 2*S*, 5*R*, 10*S*; or 2*R*, 5*S*, 10*S*. The important NOE correlation observed in **4** from H-6 to $H_3$-14 was not in the data set for **5**, which allowed elimination of the first possibility listed above. In parallel to the situation with **4**, all of the additional NOE data collected for **5** was consistent with the two remaining possibilities. These data included NOE enhancements from H-6 to H-$1_a$ (δ 1.98) and H-$4_b$ (δ 1.63), indicating these atoms were on the same molecular face. Also important to note is in **4** the NOE from H-6 was to H-$1_b$ (δ 1.68), indicating that the isothiocyanate and methyl groups at C-2 are on opposite sides of the five-membered ring than was

determined for **4**. Subsequently, the changes in the relative shifts of the diastereotopic protons at C-1 and the differences in their NOE correlations to H-6 in **4** vs. **5** gave indications to which side of the molecule these atoms were on, however, they were not of real diagnostic value in determining *R* or *S* stereochemistry for the C-2/C-5 positions. Identical synthetic modifications performed on **4** were carried out on **5**, however, overlapping signals and low yields precluded the necessary and extensive NOE analysis of the product.

The structures of **6** and **7** were deduced as outlined below. A molecular formula of $C_{24}H_{36}N_2O$ (HRESIMS *m/z* 369.2881 $[M+H]^+$), requiring seven degrees of unsaturation, was established for **6**. Dereplication using this formula as a search seed gave 200 compounds as hits, but only one compound *N*-phenethyl-*N′*-2-trachyopsanylurea (**10**),[12] was a natural product and this nitrogenous sesquiterpene was also isolated from an Indo Pacific *A*. *aplysinoides*. Comparison of the $^1H$ and $^{13}C$ NMR data between of **6** (shown in Table 2) and **10** indicated that the phenethyl urea side chain was also present in the former. The remaining NMR signals were also consistent with the presence of a spiro-axane sesquiterpene residue with the nitrogenous group attached at C-2, as seen in **3 - 5**. The elements of the gross structure shown were further confirmed from gCOSY and gHMBC correlations. The formula of $C_{24}H_{36}N_2O$ (HRESIMS *m/z* 369.2879 $[M+H]^+$) was established for **7**, which is identical to that of **6**. The NMR properties of **7** including 2D correlations and $^{13}C$ shifts were identical to those observed for **6** (Table 2). Minor but significant differences were observed for the $^1H$ NMR shifts of **7** vs. **6** for H-3, H-4, H-8 and H-9. Thus, it was clear that **6** and **7** were diastereomers probably differing in the configuration at C-2 and/or C-5. The results of 1D NOE

experiments were used to provisionally rule out two of the four stereoiosmer possibilities. For example, an NOE enhancement at $H_3$-14 was found when irradiating the vinylic H-6 for **7**, indicating $H_3$-14 and H-6 were on the same side of the molecule. This was consistent with 2*S*, 5*S*, 10*S* or 2*R*, 5*R*, 10*S* configuration. Conversely, irradiation of H-6 on **6** showed no enhancement to $H_3$-14, which by analogy to the arguments above for **5** was also consistent both the preceding assignments.




**10**

**Table 2**. NMR Data[a] for Compounds **6** and **7** in MeOH-d4

| | **6** | | | | | | **7** | |
|---|---|---|---|---|---|---|---|---|
| position | $\delta_C$ | $\delta_H$ (*J* in Hz) | gCOSY | gHMBC | TOCSY | NOE | $\delta_C$ | $\delta_H$ (*J* in Hz) |
| 1 | 53.9 | 2.04 d (14.2) | | | | $1_b$ | 52.4 | 2.05 dd (1.0, 14.2) |
| | | 1.58 d (14.2) | | 2, 5, 14 | | $1_a$ | | 1.60 d (14.2) |
| 2 | 59.7 | | | | | | 60.2 | |
| 3 | 39.7 | 1.84 m | | 1, 2, 4 | 4 | | 40.4 | 1.92 m |
| | | 1.71 m | | | | | | 1.56 ddd (7.3, 8.7, 12.7) |
| 4 | 34.0 | 1.76 m | | 2, 3, 5, 10 | 3 | | 33.3 | 1.70 ddd (5.3, 7.4, 12.9) |
| | | 1.3 m | | | | | | 1.42 m |
| 5 | 45.8 | | | | | | 45.8 | |
| 6 | 128.5 | 5.26 s | | 1, 5, 8, 10, 11 | | 14, 12, 13, 11 | 129.7 | 5.40 s |
| 7 | 139.2 | | | | | | 138.4 | |
| 8 | 22.4 | 1.92 m | | 9, 10 | | | 23.3 | 1.89 m |
| | | 1.85 m | | | | | | |
| 9 | 27.5 | 1.66 m | | 5, 7, 8, 15 | | | 28.1 | 1.62 m |
| | | 1.42 m | | | | | | |
| 10 | 36.8 | 1.6 m | 15 | 4, 5, 9 | 15 | | 37.3 | 1.46 m |
| 11 | 34.7 | 2.12 sept (6.5) | 12, 13 | 6, 7, 8, 12, 13 | 12, 13 | 6 | 34.7 | 2.11 sept (6.6) |
| 12 | 20.5 | 0.98 d (7.0) | 11 | 7, 11 | 11 | 6 | 20.5 | 0.95 d (7.0) |
| 13 | 20.4 | 0.98 d (7.0) | 11 | 7, 11 | 11 | 6 | 20.4 | 0.95 d (7.0) |
| 14 | 26.7 | 1.38 s | | 1, 2, 3 | | 6 | 25.9 | 1.36 s |
| 15 | 14.3 | 0.87 d (7.0) | 10 | 5, 9, 10 | 10 | | 14.5 | 0.87 d (7.0) |
| 16 | 158.9 | | | | | | 159.0 | |
| 17 | 40.7 | 3.30 m | 18 | 16, 18, 19 | | | 40.8 | 3.30 m |
| 18 | 36.1 | 2.73 t (7.2) | 17 | 17, 19 | | | 36.2 | 2.75 dt (2.5, 7.2) |
| 19 | 139.4 | | | | | | 139.5 | |
| 20 | 128.0 | 7.26 m | | 19, 21, 22 | 21, 22 | | 128.0 | 7.26 m |
| 21 | 128.4 | 7.19 m | | 19, 20, 22 | 20, 22 | | 128.4 | 7.20 m |
| 22 | 125.7 | 7.17 m | | 20, 21 | 20, 21 | | 125.7 | 7.17 m |

[a] Measured at 500 MHz ($^1$H) and 125 MHz ($^{13}$C).

The combination of bioactivity and LCMS screening was the key factor that allowed us to interrelate the parallel chemistry from two distantly related Indo Pacific sponges. This came through the isolation of sesquiterpenes **3**, **6**, and **7** from *A*. *aplysinoides* vs. **4**, **5**, **6**, and **7** obtained from *A*. *foetida*. The observation of diastereomeric pairs (**4**/**5** and **6**/**7**) adds another element of distinctiveness to this study. Others have noted the difficulties we encountered in defining the compound(s) responsible for the solid tumor selectivity of the initial extract, which eventually yielded nitrogenous sesquiterpenes.

This was the circumstance in the attempt to isolate bioactive consitituents from *Axinyssa aplysinoides* active in an assay against DNA-repair deficient yeast mutants,[12] and was also problematic in the study of antimitotic compounds from another collection of this same species.[13] Our search for the potent cytotoxins from these sponges is continuing, and it is clear that **3** was only mildly solid tumor selective while it also exhibited mild antibacterial activity against *Staphylococcus epidermitis* (ATTC no. 12228), and *Enterococcus durans* (ATTC no. 11576) with MIC's of 0.1 mg/mL, and 0.1 mg/mL respectively. Finally, no antibacterial activity was found for **4** - **7**.

**Experimental Section**

**Animal Material.** The sponge *Amorphinopsis foetida* (coll. no. 00381) was collected in December 2000 using SCUBA at a depth of 30 ft in the Madang region of Papua New Guinea. Voucher samples have been deposited at the Zoological Museum of Amsterdam (ZMA POR. 17553). An abovewater photograph of the sponge is available from the Crews laboratory. The sponge *Axinyssa aplysinoides* (coll. no. 03411) was collected in November 2003 by hand using SCUBA at depths of 30-60 ft in the Mele Bay region of Vanuatu. Voucher samples have been deposited at the Zoological Museum of Amsterdam (ZMA POR. 17767). Above-water and underwater photographs of the sponge are available from the Crews laboratory.

**Extraction and Isolation.** Both sponges was preserved by soaking in 1:1 ethanol:seawater for 24 hours, decanting, and vaccuum sealing with transport back to the laboratory at ambient temperature. The Vanuatu sponge (coll. no. 03411, 1.5 kg wet wt)

was extracted using the ASE to give three fractions. The procedure for extraction with the ASE is as follows. The preserved sponge allowed to air dry (~ 48 h). After this, the sponge was dissected into small pieces and partitioned using the ASE by first extracting with hexanes (3×), then with $CH_2Cl_2$ (3×) and finally with MeOH (3×). The $CH_2Cl_2$ fraction (XFD) (780 mg) was subjected to preparative reversed-phase HPLC (49:50:1 acetonitrile:water:isopropanol to 99:1 acetonitrile:isopropanol) to yield 11 fractions. Fraction 6 yielded compound **3** (78 mg). Fraction 8 (32 mg) was further purified using reversed-phase semi-preparative HPLC (isocratic 75:35 acetonitrile:water both with 0.1% formic acid) to yield 20 fractions. H7 contained compound **3** (24 mg), H10 contained **6** (0.6 mg), and H11 contained **7** (1.0 mg).

The Papua New Guinean sponge (coll. no. 00381, 1.5 kg wet wt) was extracted using a Kupchan style solvent partition method. The field preserved sponge was first extracted with MeOH (3×). The resulting oil was then partitioned between water and $CH_2Cl_2$. The aqueous layer was extracted with butanol and the butanol layer was evaporated in vacuo to yield a brown gum. The gum was subjected to reversed-phase HPLC using a gradient solvent system of 10:90 methanol:water to 100% methanol over 60 min to afford fifteen fractions (H1 – H15). Fraction H7 (24.7 mg) was run on HPLC resulting in five fractions. The fourth fraction (H7H4) contained a mixture of both **6** and **7**. Fraction H7H4 was subjected to a shallow gradient HPLC run resulting in **6** (5.2 mg) and **7** (6.3 mg) in pure form. Fraction H12 from the crude WB was run on HPLC to yield **4** (7.0 mg) and **5** (5.5 mg).

**Antibacterial Assay.** Three different bacterial strains were employed including *Escherichia coli*, *Staphylococcus epidermidis* (ATTC no. 12228), and *Enterococcus durans* (ATTC no. 11576). Minimum inhibitory concentrations (MIC) against these three bacteria were measured using a micro broth dilution test in 96-well microtiter plates with 0.2 mL per well. The maximum concentration of **3** used was 400 μg/mL, and this was serially diluted down to 6.25 μg/mL. The microtiter plates were inoculated with 0.1 mL of overnight cultures that were diluted and adjusted to give concentrations of $10^5$-$10^6$ CFU/mL (per well) and a final volume of 0.2 mL. The 96-well microtiter plates were then incubated at 37 °C overnight for 24 h. A growth control was included to demonstrate the viability of the inoculum in each assay plate. Penicillin G and vancomycin were included as positive controls and DMSO was used as a negative control. The MIC values were determined by visual inspection as the minimum concentration of compound that gives 100% inhibition of bacterial growth.

**(2*R*,5*R*,10*S*)-2-Formamido-6-axene (3):** colorless solid, $[\alpha]^{25}_D$ +17.4 ° (*c* 1.5 $CHCl_3$); HRESIMS *m/z* 250.2089 $[M + H]^+$ (calcd. for $C_{16}H_{27}NO$ + H 250.2093). NMR data in accordance with literature values.[10]

**(2*R*,5*R*,10*S*)-2-Isothiocyanato-6-axene (4):** colorless oil, $[\alpha]^{25}_D$ –22.4 ° (*c* 1.0 $CHCl_3$); UV ($CHCl_3$) $\lambda_{max}$ (log ε) 244 (3.19) nm; $^1H$ and $^{13}C$ NMR data, see Table 1; HRESIMS *m/z* 264.1706 $[M + H]^+$ (calcd for $C_{16}H_{25}NS$ + H 264.1702).

**2-Isothiocyanato-6-axene (5):** colorless oil, $[\alpha]^{25}_D$ +44.1 ° (*c* 1.0 $CHCl_3$); UV ($CHCl_3$) $\lambda_{max}$ (log ε) 244 (3.19) nm; $^1H$ and $^{13}C$ NMR data, see Table 1; HRESIMS *m/z* 264.1706 $[M + H]^+$ (calcd for $C_{16}H_{25}NS$ + H 264.1702).

***N*-Phenethyl-2-formamido-6-axene (6):** colorless solid, $[\alpha]^{25}_{D}$ –18.6° (*c* 0.1, MeOH); UV (MeOH) $\lambda_{max}$ (log ε) 354 (3.81) nm; $^{1}$H and $^{13}$C NMR data, see Table 2; HRESIMS *m/z* 369.2881 $[M + H]^{+}$ (calcd for $C_{24}H_{36}N_2O$ + H 369.2900).

***N*-Phenethyl-2-formamido-6-axene (7):** colorless solid, $[\alpha]^{25}_{D}$ +38.6° (*c* 0.1, MeOH); UV (MeOH) $\lambda_{max}$ (log ε) 354 (3.81) nm; $^{1}$H and $^{13}$C NMR data, see Table 2; HRESIMS *m/z* 369.2879 $[M + H]^{+}$ (calcd for $C_{24}H_{36}N_2O$ + H 369.2900).

**(2*R*,5*R*,10*S*)-2-*N*-Methyl-6-axene (8):** To a suspension of 10 mg LAH in 20 mL dry ether was added dropwise **4** (3.0 mg) in 250 μL dry ether. This was then allowed to reflux overnight. Workup consisted of cooling to 0 °C then adding 0.25 mL 10% NaOH followed by 0.5 mL $H_2O$. Vacuum filtration followed by HPLC resulted in **8** (1.0 mg) colorless oil; $^{1}$H NMR ($CDCl_3$) δ 9.20 (N-H), 5.31 (H-6), 2.62 (H-16), 2.15 (H-11), 1.78 (H-$1_b$), 1.43 (H-14), 0.98 (H-12, H-13), 0.92 (H-15); $^{13}$C NMR ($CDCl_3$, 125 MHz) δ 141.8 (C-7), 126.9 (C-6), 65.7 , 50.9, 46.3, 37.3, 36.5, 35.0, 33.7, 27.9, 27.6, 23.7, 23.4, 21.6 (C-12 or C-13), 21.5 (C-12 or C13), 15.2 (C-15); HRESIMS *m/z* 236.2369 $[M + H]^{+}$ (calcd for $C_{16}H_{29}N$ + H 236.2373).

**(2*R*,5*R*,10*S*)-2-*N*-Methyl axane (9):** A solution of **8** (1.0 mg) in MeOH (0.5 mL) along with 2.0 mg of 10% wt. Pd/C was placed under 3 atm of $H_2$ and shaken overnight to yield **9** (0.5 mg). Colorless oil; $^{1}$H NMR ($CDCl_3$) δ 9.19 (N-H), 2.57 (H-16), 2.08 (H-$3_a$), 1.98 (H-$1_a$), 1.92 (H-$4_a$), 1.79 (H-$1_b$), 1.78 (H-$3_b$), 1.69 (H-10), 1.40 (H-$4_b$, H-$8_a$, H-11), 1.27 (H-$6_a$, H-$9_a$), 1.13 (H-$6_b$, H-$8_b$, H-$9_b$), 0.89 (H-15), 0.86 (H-12, H-13); 13C NMR ($CDCl_3$, 125 MHz) δ 66.3 (C-2), 48.2 (C-1), 46.6 (C-5), 40.4 (C-7), 38.0 (C-10), 37.4 (C-4), 35.3 (C-3), 34.6 (C-6), 33.0 (C-11), 30.0 (C-9), 27.4 (C-16), 25.0 (C-14), 23.0 (C-8), 20.0 (C-

12 or C-13), 19.8 (C-12 or C-13), 15.0 (C-15); HRESIMS *m/z* 238.2533 $[M + H]^+$ (calcd for $C_{16}H_{31}N + H$ 238.2529).

**Acknowledgements.** This research was supported by NIH grant 2 RO1 CA047135 and NMR equipment grants from NSF BIR-94-19409 and the Elsa U. Pardee Foundation. Additional support was provided by the GAANN fellowship and the MBRS program. We thank Dr. van Soest for the expert assistance in the taxonomic identification of both sponges. Special thanks to L. Matainaho, University of Papua New Guinea, and PNG Bionet for assistance in obtaining Papua New Guinea collection permits and the crew and Captain (C. DeWitt) of the *M/V Golden Dawn* for their assistance. We would also like to thank Moses Amos, Director of Fisheries, for assistance in obtaining Vanuatu collection permits.

**Supporting Information Available**: General experimental proceedures, compound isolation diagrams, comparison of simulated and experimental H-10 multiplets for **9**, and proton NMR spectra of **4** - **7**. This material is available free of charge via the internet at http://pubs.acs.org.

**References and Notes**

(1) Bioassay procedures are described in Valeriote, F.; Grieshaber, C. K.; Media, J.; Pietraszkewicz, H.; Hoffmann, J.; Pan, M.; McLaughlin, S. *J. Exp. Ther. Oncol.* **2002**, *2*, 228-236.

(2) (a) Cichewicz, R. H.; Valeriote, F. A.; Crews, P. *Org. Lett.*, **2004**, *6*, 1951-1954. (b) Amagata, T; Amagata, A.; Tenney, K.; Valeriote, F. A.; Lobkovsky, E.; Clardy, J.; Crews, P. *Org. Let.* **2003**, *5*, 4393-4396. (c) Amagata, T.; Rath, C.; Rigot, J. F.; Tarlov, N.; Tenney, K.; Valeriote, F. A.; Crews, P. *J. Med. Chem.* **2003**, *46*, 4342-4350.

(3) Hooper, J. N. A.; Van Soest, R. W. M. In *Systema Porifera*; Kluwer Academic/Plenum Publishers: New York, 2002; pp 790.

(4) Hooper, J. N. A.; Van Soest, R. W. M. In *Systema Porifera*; Kluwer Academic/Plenum Publishers: New York, 2002; pp 792.

(5) (a) Casapullo, A.; Cutignano, A.; Bruno, I.; Bifulco, G.; Debitus, C.; Gomez-Paloma, L.; Riccio, R. *J. Nat. Prod.* **2001**, *64*, 1354-1356. (b) Ciasullo, L.; Cutignano, A.; Casapullo, A.; Puliti, R.; Mattia, C. A.; Debitus, C.; Riccio, R.; Gomez-Paloma, L. *J. Nat. Prod.* **2002**, *65*, 1210-1212. (c) Ciasullo, L.; Casapullo, A.; Cutignano, A.; Bifulco, G.; Debitus, C.; Hooper, J.; Gomez-Paloma, L.; Riccio, R. *J. Nat. Prod.* **2002**, *65*, 407-410. (d) Crews, P.; Kakou, Y.; Quinoa, E. *J. Am. Chem. Soc.* **1988**, *110*, 4365-4368. (e) Quinoa, E.; Kakou, Y.; Crews, P. *J. Org. Chem.* **1988**, *53*, 3642-3644. (f) Quinoa, E.; Crews, P. *Tetrahedron Lett.* **1988**, *29*, 2037-2040.

(6) (a) Garson, M. J.; Simpson, J. S. *Nat. Prod. Rep.* **2004**, *21*, 164-179. (b) Chang, C. W. J.; Scheuer, P. J. in *Topics in Current Chemistry,* ed. P. J. Scheuer, Springer-Verlag, Berlin, 1993, p. 33. (c) Chang, C.W. J. in *Prog. Chem. Org. Nat. Prod*. ed. W. Herz, H. Falk, G. W. Kirby, R. E. Moore, Springer, New York, 2000.

(7) Fontana, A.; Tramice, A.; Cutignano, A.; d'Ippolito, G.; Gavagnin, M.; Cimino, G. *J. Org. Chem*. **2002**, *68*, 2405-2409.

(8) (a) Di Blasio, B.; Fattorusso, E.; Magno, S.; Mayol, L.; Pedone, C.; Santacroce, C.; Sica, D. *Tetrahedron* **1976**, *32*, 473-478. (b) The structure of (-)-epi-axisonitrile-3 has been determined to have 10*S* chirality. Uy, M. M.; Ohta, S.; Yanai, M.; Ohta, E.; Hirata, T.; Ikegami, S. *Tetrahedron* **2003**, *59,* 731-736.

(9) Uy, M. M.; Ohta, S.; Yanai, M.; Ohta, E.; Hirata, T.; Ikegami, S. *Bioorg. Med. Chem. Lett*. **2002**, *12*, 3037-3039.

(10) He, H.; Faulkner, D. J.; Shumsky, J. S.; Clardy, J. *J. Org. Chem.* **1989**, *54*, 2511-2514.

(11) $H_a$ = downfield proton, $H_b$ = upfield proton

(12) Patil, A. D.; Freyer, A. J.; Reichwein, R.; Bean, M. F.; Faucette, L.; Johnson, R. K.; Haltiwanger, R. C.; Eggleston, D. S. *J. Nat. Prod.*, **1997**, *60*, 507-510.

(13) Williams, D. E.; Patrick, B. O.; Tahir, A.; Van Soest, R.; Roberge, M.; Andersen, R. J. *J. Nat. Prod.* **2004**, *67*, 1752-1754.