# Anthelmintic Polyfunctional Nitrogen-Containing Terpenoids from Marine Sponges

K. A. Alvi, Laura Tenenbaum, and Phillip Crews

*J. Nat. Prod.*, **1991**, 54 (1), 71-78• DOI:
10.1021/np50073a002 • Publication Date (Web): 01 July 2004

*Journal of Natural Products*
*Vol. 54, No. 1, pp. 71-78, Jan-Feb 1991*



# ANTHELMINTIC POLYFUNCTIONAL NITROGEN-CONTAINING TERPENOIDS FROM MARINE SPONGES

K.A. ALVI, LAURA TENENBAUM, and PHILLIP CREWS*

*Department of Chemistry and Institute for Marine Sciences, University of California, Santa Cruz, California 95064*

ABSTRACT.—The study of the anthelmintic terpenoid components from the Fiji sponge *Axinyssa fenestratus* (senior synonym of *Leucophloeus fenestratus*) and two Thailand sponges, *Acanthella cavernosa* and *Topsentia* sp., has yielded several new nitrogen-containing sesqui- and diterpenes of known carbon skeletons. Amorphane sesquiterpenes from *A. fenestratus* included (1*R*, 6*S*, 7*S*, 10*S*)-10-isothiocyanato-4-amorphene [(+)-**1**] and new metabolites (1*R**, 4*S**, 6*R**, 7*S**)-4-isothiocyanato-9-amorphene [**2**], 10-isothiocyanato-4,6-amorphadiene [**3**], and (4*S**, 10*S**)-10-isothiocyanato-5-amorphen-4-ol [**4**]. The amorphene (+)-**1** of this study may be antipodal to (−)-**1** previously isolated from a Hawaiian sponge. A similar relationship may exist at C-1, C-6, C-7 between (+)-**2** of this study and (−)-**11** isolated from a Palauian sponge. Another known sesquiterpene, axisonitrile 3 [**5**] was obtained from *Topsentia* sp. The diterpenes obtained from *A. cavernosa* included known kalihinols X [**6**] and Y [**8**] and new kalihinols J [**7**] and I [**9**]. Those terpenoids with potent antiparasite activity include **1**, **2**, **3–5**, and **7–9**.

Marine sponges, especially of the orders Dictyoceratida and Dendroceratida (1), are a dependable source of a variety of oxygenated terpenoids. We are conducting a systematic study of soft-bodied sponges which might be sources of novel nitrogen-containing compounds (2). Thus, the orders Axinellida, Halichondrida, and Lithistida were of interest because, in the past, they have been a source of unique sulfur- and/or nitrogen-containing terpenoids (1–3). A search of our marine sponge data base shows that >85% of nitrogen-containing terpenes have been reported from sponges within these three orders: Axinellida (*Axinella, Acanthella, Pseudaxinella*, and *Agelas*), Halichondrida (*Halichondria* and *Ciocalypta*), Lithistida (*Theonella*). It is important to note that the genus *Axinyssa* (Fam. Halichondriidae, Order Halichondrida) has been redefined to include a series of species previously located in *Axinyssa, Pseudaxinyssa, Leucophloeus*, and *Raphisia* (21,22). Thus *Leucophloeus fenestratus* (Ridley) and *Axinyssa fenestratus* (Ridley) are synonyms (21,22).

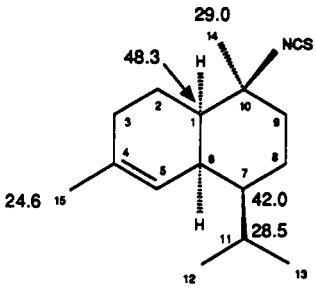
A parallel study was begun on members of the first two of these orders whose crude extracts showed potency in anthelmintic prescreens.[1] The isolation efforts were bioassay-guided and resulted in the purification of a series of terpenes functionalized with isonitrile, isothiocyanate, or formamide substituents. Amorphane sesquiterpenes **1**, **2**, **3**, and **4** were obtained from the Fiji sponge *Ax. fenestratus* while a spirocyclic sesquiterpene axisonitrile 3 [**5**] (4,5) was isolated from a Thailand *Topsentia* sp. (Halichondrida). Diterpenes including kalihinols X [**6**] (6), J [**7**], Y [**8**] (6), and I [**9**] were obtained from a Thailand collection of *Acanthella cavernosa* (Dendy) (Axinellida). The new compounds **2**, **3**, **4**, **7**, and **9** are described below along with their anti-parasitic properties.

## RESULTS AND DISCUSSION

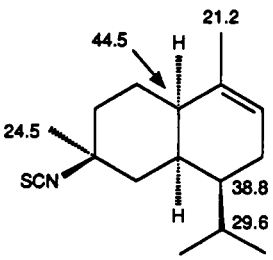
Mixtures of bioactive sesquiterpenes were present in *Ax. fenestratus*; their composition varied from **1** and **2** to **3** and **4** in two different collections. A well known sesquiterpene, axisonitrile 3 [**5**] (4,5), was observed in *Topsentia* sp. as a single component.

The characterization of (+)-**1** ($C_{16}H_{25}NS$) was facilitated when we noted that the physical properties [$^1$H nmr, ir, ms] of (+)-**1** matched those reported for (1*S*, 6*R*, 7*R*,

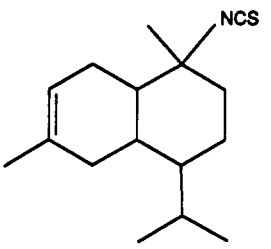
[1]We thank Dr. Tom Matthews (Syntex Research, Palo Alto, CA) and his staff for these assay data using the parasitic stages of *Nippostrongylus brasiliensis*.



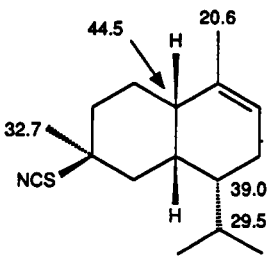

(+)-**1**



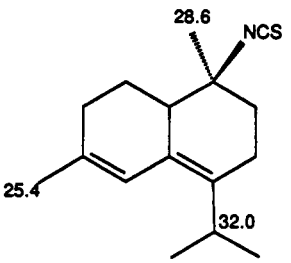

**2***



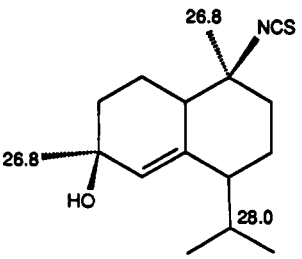

**10**



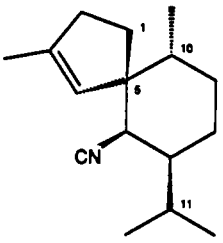

**11***




**3***



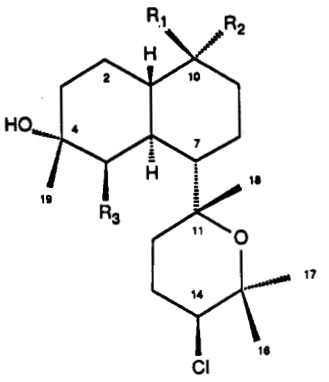

**4***




**5***

*Absolute stereochemistry is not implied here.

10*R*)-10-isothiocyanato-4-amorphene [(−)-**1**]. This compound was previously described by Burreson *et al.* (7) from a deep-water *Halichondria* sp. collected off Oahu Island, Hawaii. The earlier characterization of (−)-**1** included a description of its absolute stereochemistry that was based on $^1$H-nmr *J* values and degradation chemistry results in which 5-oxoamorphane was obtained and examined by cd analysis. We were able to completely assign the $^{13}$C-nmr spectrum of (+)-**1** (see Table 1) based on $^1$H-$^{13}$C and $^1$H-$^1$H COSY nmr data. Consistent with the structure and relative stereochemistry shown in **1** were the $^3J$ of 12.3 Hz between H-7 and H-8, indicating equatorial stereochemistry of the isopropyl group, and the $^{13}$C-nmr shifts of the two remaining methyls in **1** suggestive of, respectively, a *Z* vinylic Me-15 (δ 24.6) and an equatorial Me-14 (δ 29.0) arrangement (8–11). [The relative chemical shift values of the six-membered-ring methyls are diagnostic of their geometry. The cases here involve methyls with a geminal substituent but no additional γ-substituent. Inspection of literature data for a variety of cyclic sesquiterpenes with ring methyls in this exact environment indicates characteristic shifts as a function of methyl stereochemistry as follows: axial δ ≈20 and equatorial δ ≈30. These expected ranges are based on data from the literature (8–11).] Burreson *et al.* (7) have pointed out previously that *J* values to H-




| | $R_1$ | $R_2$ | $R_3$ |
|---|---|---|---|
| **6** | Me | NCS | NC |
| **7** | Me | NCS | NHCHO |
| **8** | | $=CH_2$ | NC |
| **9** | Me | NCS | NCS |

6 are diagnostic of the amorphane ring stereochemistry at C-1, C-6, and C-7; the resonances at δ 2.62 (H-6) and 5.30 (H-5) match the profile noted by Burreson *et al.* (7) for the C-1, C-6, and C-7 bicyclic stereochemistry shown in **1**. These nmr patterns provided important model data which was later applied in the characterizations of the three other related metabolites. Comparison of the optical rotations of the two samples of **1**, whose data in $CCl_4$ are respectively −63° (7) and +100°, suggests they are antipodal, in spite of the large disagreement in their absolute values.

A second amorphene, (+)-**2**, $C_{16}H_{25}NS$, was isolated; it was isomeric to **1**. Its $^1$H-

TABLE 1. $^{13}$C-nmr Data of Compounds **1–4** ($CDCl_3$, at 75 MHz) and **11**.

| Carbon | Compound | | | | |
|---|---|---|---|---|---|
| | **1** | **2** | **11**[a] | **3** | **4** |
| | δ mult | δ mult | δ mult | δ mult | δ mult |
| C-1 | 48.3, d | 44.5, d | 44.5, d | 46.7, d | 47.5, d |
| C-2 | 24.0, t | 24.1, t[b] | 24.3, t[b] | 25.4, t | 22.6, t |
| C-3 | 27.5, t | 33.9, t[c] | 32.7, t[c] | 29.5, t | 36.0, t |
| C-4 | 137.0, s | 61.4, s | 58.5, s | 137.0, s | 69.0, s |
| C-5 | 118.0, d | 35.0, t[c] | 33.8, t[c] | 121.5, d | 130.0, d |
| C-6 | 36.0, d | 32.8, d | 32.4, d | 125.8, s | 137.0, s |
| C-7 | 42.0, d | 38.8, d | 39.0, d | 135.6, s | 47.0, d |
| C-8 | 23.0, t | 27.7, t[b] | 27.3, t[b] | 22.2, t | 22.0, t |
| C-9 | 42.7, t | 124.2, d | 124.0, d | 37.7, t | 40.5, t |
| C-10 | 61.3, s | 132.3, s | 132.6, s | 63.5, s | 66.0, s |
| C-11 | 28.5, d | 29.6, d | 29.5, d | 32.0, d | 28.0, d |
| C-12 | 21.6, q[b] | 20.7, q | 20.6, q | 21.9, q | 17.6, q |
| C-13 | 20.5, q[b] | 20.6, q | 21.2, q | 21.9, q | 22.0, q |
| C-14 | 29.0, q | 21.2, q | 20.6, q | 28.6, q | 26.8, q |
| C-15 | 24.6, q | 24.5, q | 32.7, q | 25.4, q | 26.8, q |
| NCS | 126.0, s | — | 111.8, s | 131.4, s | 126.0, s |

[a]Data are from He *et al.* (12).

[b,c]Sets of signals may be interchanged.

nmr spectrum revealed similar substructures of a singlet vinylic methyl, a singlet methyl attached to an $sp^3$ quaternary carbon, and a secondary isopropyl group. A very different trisubstituted double bond was evident because a long range $^1H$-$^{13}C$ COSY nmr correlation was observed between the vinylic Me and the vinylic H and this proton in turn exhibited a regular $^1H$-$^1H$ COSY correlation to a $CH_2$ (δ 1.96). The gross structure **10** was initially proposed on biogenetic grounds based on analogy to **1**, but alternative **2** was equally resonable. This ambiguity could not be resolved by $^1H$ nmr because too many signals were overlapping. The X-ray-determined structure of the amorphene thiocyanate (−)-**11** (12) provided important reference $^{13}C$-nmr data because **2** and **11** exhibited nearly identical $^{13}C$-nmr shifts (Table 1) with the exception of those at C-4, Me-15, and at the NCS substituent (not observed). The difference in $^{13}C$-nmr shifts at C-4 in **11** (δ 58.5) as compared to that in **2** (δ 61.4) suggested that the NCS substitutent in **2** was the more common isothiocyanate rather than the rare thiocyanate. The upfield shift of Me-15 in **2** (δ 24.5) versus that in **11** (δ 32.7) was indicative of a change in the methyl geometry from axial to equatorial. The differing rotations between **2** (+112°) and **11** (−14°) hint at enantiomeric-like stereochemistry at C-1, C-6, and C-7 for this pair, but at this juncture only relative stereochemistry has been assigned for **11** and only the relative stereochemistry 1*R**, 4*S**, 6*R**, 7*S** can be designated for **2**.

A more unsaturated compound (+)-**3**, $C_{16}H_{23}NS$, $[M]^+$ 261, contained a conjugated diene chromophore as defined by uv (λ max 231.8 nm, ε = 10,700 vs. calcd 229 nm) and $^{13}C$ nmr [δ 137.0 (s, C-4), 121.5 (d, C-5), 125.8 (s, C-6), 135.6 (s, C-7)]. An amorphane framework with four units of unsaturation in addition to an -NCS substitution was consistent with this and the additional data in Table 1. Such a situation is rare and is analogous to only one other case, that of axisothiocyanate 4 (13). Underscoring this point is the recent comment by Capon and MacLeod (14) who noted that all sesquiterpene isothiocyanates and isonitriles possess the same $C_{15}H_{25}X$ (X = NC or NCS) molecular formula which means all have three units of unsaturation in the carbon framework. A search of our data base indicates the count of such compounds has grown to 20 and 24 examples, respectively. The isothiocyanate function of (+)-**3** was confirmed by a $^{13}C$ resonance at δ 131.4 (bs). The attachment of a methyl [δ 1.77 (s, Me-15)] and an isopropyl group [δ 3.07 (H-11) as a sharp septet] to the diene chromophore was reinforced by $^1H$-nmr data as the vinylic proton [δ 6.22 (brs, H-5)] showed long range $^1H$-$^1H$ COSY nmr correlations with the δ 1.77 Me peak and δ 2.12 H-3 resonance. The Me-14 equatorial stereochemistry was assigned from a signal in the $^{13}C$ nmr at δ 28.6 along with the chemical relationship of **3** with **4** noted below.

The last of the amorphane derivatives was an isothiocyanato alcohol **4**. It was isolated from the more polar solvent partition fractions and was a colorless oil with a molecular formula of $C_{16}H_{25}NSO$, $[M]^+$ 279. The presence of the double bond [δ 130.0 (d, C-5) and 137.0 (s, C-6)] was supported by the sharp vinyl singlet at δ 6.24 (H-5). The equatorial stereochemistry of both Me's is consistent with their $^{13}C$-nmr shifts, δ 26.8 (C-14) and 26.8 (C-15) in comparison to the trends noted previously. Quaternary sites C-4/C-10 were the attachment points for the OH and isothiocyanate, but their exact connection order could not be resolved. A handy solution was provided when **4** was quantitatively transformed in to diene **3** on standing. Confirmation that **3** was not an artifact was obtained by observing the vinyl carbons in a $^{13}C$ nmr of the crude extract.

The next phase of this work involved pursuit of anthelmintic compounds from *Ac. cavernosa*. Previous work (6,11) on this species has involved tropical Pacific specimens, and a total of 12 kalihinols, which are profusely functionalized isonitrile and isothiocyanate sesquiterpenes, have been reported. Structurally, the kalihinols fall into

two main groups based on the nature of the tetrahydropyran or tetrahydrofuran which is attached to a *trans*-decalin frame. Our examination of a Thailand collection offered an opportunity to discover new patterns of kalihinols. Its oil (10.0 g) afforded derivatives from the $CCl_4$ and $CH_2Cl_2$ fractions which included known compounds kalihinols X [**6**] (6) and Y [**8**] (6), and two new compounds, kalihinols J [**7**] and I [**9**].

The molecular formula of **7**, $C_{22}H_{35}N_2SO_3Cl$, was established by lrcims, $[M+H]^+$ peak cluster at *m/z* 445/443 (ratio 1:3), and by the CH count from an APT $^{13}$C-nmr spectrum. The formamide and isothiocyanate groups were recognized by ir (2103, 1680 $cm^{-1}$), $^1$H nmr [δ 8.12 (d, $J$ = 10.5 Hz, NC(=O)H), 6.63 (br t, $J$ = 10.5 Hz, NH)] (15), and $^{13}$C nmr [δ 166.9 (d), 131.0 (s)]. The remaining $^{13}$C-nmr shifts were similar to those of **6**, allowing **7** to be assigned as its formamide derivative; however, a switch of the *N*-formyl and isothiocyano between C-5 (δ 58.9, s) and C-10 (δ 76.5, s) might not be detected by a $^{13}$C shift analysis. This ambiguity was easily settled by $^1$H-$^1$H COSY nmr data, which showed a correlation from the NH (δ 6.63) to H-5 (δ 4.25) and by the hydrolysis of **6** to yield **7**.

Another crystalline compound, kalihinol I [**9**], exhibited a molecular formula of $C_{22}H_{33}N_2O_2S_2Cl$ established from lrcims data, $[M+H]^+$ peak cluster at *m/z* 459/457 (ratio 1:4), and by the CH count from an APT $^{13}$C-nmr spectrum. The isothiocyanate was recognized by the ir (broad band at 2103 $cm^{-1}$), uv (λ max 245 nm), and $^{13}$C-nmr [δ 129 (two C's)] data. The chlorotetrahydropyranyl ring was pinpointed by a key lrms fragment cluster [*m/z* 161 (100%) and 163 (35%)] and the $^1$H-nmr data [δ 3.74 (dd, $J$ = 11.7, 4.8, H-14)] (6). The remaining nmr data (Table 2) were essentially identical to the assignments previously reported for **6** (6,11) and supported the complete structure shown for **9**.

TABLE 2. $^{13}$C-nmr Data of Compounds **6–9** ($CDCl_3$, at 75 MHz).

| Carbon | Compound | | | |
|---|---|---|---|---|
| | **6** | **7** | **8** | **9** |
| | δ mult | δ mult | δ mult | δ mult |
| C-1 | 43.2, d | 43.1, d | 41.9, d | 44.0, d |
| C-2 | 21.8, t | 22.4, t | 24.0, t | 21.9, t |
| C-3 | 32.3, t | 33.3, t | 32.4, t | 33.0, t |
| C-4 | 69.9, s | 71.1, s | 70.6, s | 70.6, s |
| C-5 | 63.6, d | 58.9, d | 63.9, d | 65.9, d |
| C-6 | 36.4, d | 36.3, d | 37.7, d | 38.3, d |
| C-7 | 48.5, d | 45.9, d | 49.5, d | 49.3, d |
| C-8 | 22.2, t | 22.4, t | 28.7, t | 22.4, t |
| C-9 | 39.3, t | 38.8, t | 35.6, t | 39.2, t |
| C-10 | 75.8, s | 76.5, s | 150.9, s | 76.0, s |
| C-11 | 77.0, s | 79.1, s | 77.0, s | 77.7, s |
| C-12 | 37.9, t | 38.0, t | 38.2, t | 38.1, t |
| C-13 | 27.4, t | 27.6, t | 27.5, t | 27.5, t |
| C-14 | 64.3, d | 64.4, d | 64.4, d | 64.4, d |
| C-15 | 76.6, s | 71.1, s | 76.5, s | 77.3, s |
| C-16 | 22.7, q | 23.4, q | 22.7, q | 23.0, q |
| C-17 | 30.6, q | 31.3, q | 30.6, q | 30.9, q |
| C-18 | 19.0, q | 19.7, q | 19.1, q | 19.4, q |
| C-19 | 28.6, q | 28.7, q | 28.7, q | 29.1, q |
| C-20 | 27.5, q | 28.8, q | 105.3, t | 27.6, q |
| NCS | — | 131.0, s | — | 129.9, s |
| NHCHO | — | 166.9 | — | — |

Seven of the above terpenes, **1–3** and **5–8**, were evaluated against *N. brasiliensis*, and all but compound **6** were active. In addition, both **5** and **6** showed pronounced antimicrobial activity against *Staphylococcus aureus, Candida albicans,* and *Trichophyton mentagrophytes*. An interesting breadth of chemistry is now evident for *Ax. fenestratus* when the novel cyclic peptides, the fenestins (20), also isolated from the Fiji specimens, are taken into account. Most terpene isocyanides from sponges occur along with parallel isothiocyanates, formamides, and amines. Fookes *et al.* (16) have demonstrated that cyanide is important in the biosynthesis of the diterpene diisocyanoadociane, while Karuso and Scheuer (17) have also proved that cyanide is a specific precursor for the isocyano function in selected sesqui- and diterpenes. In an additional study, Hagadone *et al.* (18) have shown the interconversion of RNC to RNHCHO in sponges, but Tada *et al.* (19) have also shown this same interconversion can occur during chromatography of an isocyanate. At this time little is known about the origin of the isothiocyanate group.

## EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Nmr spectra were recorded at 100 MHz for $^1$H and 25.0 MHz for $^{13}$C or at 300 MHz for $^1$H and 75 MHz for $^{13}$C. Multiplicities of $^{13}$C-nmr peaks were determined from APT data and 2D COSY nmr experiments. Ms data were obtained on a quadrupole MS apparatus. Hplc was done using a Regis 10μ-ODS or 10μ Si gel column (25 × 1.0 cm). All solvents were distilled and dried for hplc and were spectral grade for spectroscopy. Rotations were measured on a Perkin-Elmer 141 polarimeter.

TWO-DIMENSIONAL NMR PROCEDURES.—Standard pulse sequences were used for the homo COSY and the hetero COSY experiments.

TAXONOMY.—All sponges were identified by C. Diaz, UC Santa Cruz Institute of Marine Sciences. They included: *L. fenestratus* (vouchers available from PC) (20) which is a junior synonym of *Ax. fenestratus* (21,22), *Ac. cavernosa*, and *Topsentia* sp., which is an unknown species. The *Topsentia* sp. (collection no. 88017) was characterized based on the following properties. It is yellow-tan in color with a massive-amorphous shape, hard consistency, smooth to sight, and rough to touch; an ectosomal skeleton was absent and the organic skin had some spicules tangentially arranged. The choanosome possessed a radial arrangement of spicules which were most evident toward the epidermus. The spicules were long fusiform oxeas (700–100) × (8–20) μm. The spicule arrangement was typical of the genus *Topsentia* (22). Other key characteristics consisted of no spongin visible in the choanosome, non-ectosomal skeleton specialization, and a simple skeleton of fusiform oxeas. The tendency of the spicules to a radial arrangement towards the surface has been reported before for Atlantic *Topsentia* species (22). A species description fitting the specimen studied has not been located, suggesting the possibility that our specimen is a new species.

EXTRACTION.—*Ax. fenestratus* was collected from Fiji in the summer of 1986 (1.40 kg wet wt) and 1987 (3.80 kg wet wt). Both *Topsentia* sp. (2.865 kg wet wt) and *Ac. cavernosa* were collected from Thailand in the winter of 1988. All sponges were preserved and returned to the University of California, Santa Cruz, for workup consisting of soaking (48 h, room temperature) in MeOH three times. Each of the dark viscous oils was examined by $^{13}$C-nmr spectroscopy, which revealed a mixture of lipids and terpenes in each of the extracts, but the relative percentage of lipids increased to a maximum in the third extract. The combined extracts were worked up by the following representative procedure. The combined MeOH extracts of *Ax. fenestratus* yielded a dark viscous oil (23.96 g). Solvent partitioning of the oil (aqueous MeOH against hexane, $CCl_4$, $CH_2Cl_2$, and percent $H_2O$ adjusted to produce a biphase solution) afforded: hexane (18.78 g), $CCl_4$ (1.48 g), and $CH_2Cl_2$ (1.20 g). The partitioned fractions of each sponge were bioassayed, and the greatest bioactivity was observed in the $CCl_4$ and $CH_2Cl_2$ partition fraction for *Ac. cavernosa* and in the hexane fraction for *Ax. fenestratus* and *Topsentia* sp. The bioactive fractions were then chromatographed (flash column) and further purified via preparative normal-phase (10 μm Si gel column) or reversed-phase (10 μm ODS) hplc to yield **1–9** as described below.

10-ISOTHIOCYANATO-4-AMORPHENE [**1**].—The hexane partition fraction was subjected to repeated regular and reversed-phase hplc. A single hplc (reversed-phase) peak was eventually obtained [C-18 ODS with MeOH-$H_2O$ (9:1)] as a dark oil (54.7 mg): $[\alpha]_D$ + 100.45 ($c$ = 5.47, $CCl_4$); lreims *m/z* (percent) $[M]^+$ 263 (52), 230 (23), 205 (61), 161 (100) ($C_{16}H_{25}NS$ requires 263); ir 2098 $cm^{-1}$ (NCS); $^1$H nmr (300 MHz, $CDcl_3$) [assignments based on $^1$H-$^1$H and $^1$H-$^{13}$C COSY data] 0.95 (m, H-1), 1.8 (m, H-2 ax), 2.13 (ddd, $J$ = 13.2, 6.9, 2.1, H-2 eq), 1.95 (m, H-3 ax), 2.30 (m, H-3 eq), 5.30 (s, H-5), 2.62 (bs, $w_{1/2}$ = 15, H-6), 1.45 (m, H-7), 1.32 (dq, $J$ = 12.3, 12.3, 12.3, 2.4, H-8 ax), 1.68 (m, H-8 eq), 1.44

(dt, $J$ = 10.0, 10.0, 1.0, H-9 ax), 1.95 (m, H-9 eq), 1.70 (m, H-11), 0.96 and 0.90 (each d, $J$ = 6.6, Me-12, Me-13), 1.75 (s, Me-14), 1.42 (s, Me-15).

4-ISOTHIOCYANATO-9-AMORPHENE [**2**].—An oil (14.8 mg) was obtained as above: $[\alpha]_D$ +111.69 ($c$ = 2.5, $CHCl_3$); lreims *m/z* (percent) $[M]^+$ 263 (60), 230 (-SH, 10), 205 (-NCS, 100), 204 (-HNCS, 72), 161 (-HNCS -isopropyl, 60) ($C_{16}H_{25}NS$ requires 263); ir 2103 $cm^{-1}$ (NCS); $^1H$ nmr (300 MHz, $CDCl_3$) δ 2.2 (m), 1.96 (m), 1.04 (m), 1.90 (m), 5.47 (m, H-9), 1.43 (m, H-11), 0.87 (d, $J$ = 6, Me-12 and Me-13), 1.63 (s, Me-14), 1.47 (s, Me-15).

10-ISOTHIOCYANATO-4,6-AMORPHADIENE [**3**].—The inactive $CH_2Cl_2$ fraction was purified by reversed-phase hplc [MeOH-$H_2O$ (9:1)], and an oil (16.4 mg) was obtained: $[\alpha]_D$ +74.42 ($c$ = 9.8, $CHCl_3$); lreims *m/z* (percent) $[M]^+$ 261 (11), 85 (70), 83 (100) ($C_{16}H_{23}NS$ requires 261); ir 2104 (NCS), 1673 $cm^{-1}$ (conjugated diene); $^1H$ nmr (300 MHz, $CDCl_3$) 2.12 (bd, $J$ = 5.1, H-3), 6.22 (brs, H-5), 2.04 (bm, H-8 ax and H-9 eq), 2.20 (m, H-8 eq), 1.60 (dt, $J$ = 13.0, 12.9, 6.0, H-9 ax), 3.07 (h, $J$ = 6.9, H-11), 0.97 and 1.02 (each d, $J$ = 6.6, Me-12, Me-13), 1.44 (s, Me-14), 1.77 (s, Me-15).

10-ISOTHIOCYANATO-5-AMORPHEN-4-OL [**4**].—An oil (11.1 mg) was obtained from the inactive fraction as above: lreims *m/z* (percent) $[M]^+$ 279 (2), 278 (5), 262 (23), 261 (100) ($C_{16}H_{25}NSO$ requires 279); ir 3561 (br OH), 2100 $cm^{-1}$ (NCS); $^1H$ nmr (300 MHz, $C_6D_6$) 6.24 (s, H-5), 3.01 (s, H-7), 3.01 (h, $J$ = 6.9, H-11), 0.96 and 1.01 (each d, $J$ = 6.6, Me-12, Me-13), 1.63 (s, Me-14), 1.63 (s, Me-15), 2.21–0.83 (complex).

AXISONITRILE 3 [**5**].—Crystals were obtained from the bioactive hexane fraction, and purified by recrystallization from $Et_2O$ (mp 100–102°). Compound **5** displayed properties identical to those in the literature (4,5). $^{13}C$ nmr (75 MHz, $CDCl_3$, δ in ppm, multiplicity) 35.0 (t, C-1), 35.9 (t, C-2), 144.8 (s, C-3), 123.6 (d, C-4), 57.1 (s, C-5), 64.5 (d, C-6), 43.8 (d, C-7), 24.9 (t, C-8), 31.2 (t, C-9), 34.4 (d, C-10), 29.7 (d, C-11), 20.3 (q, C-12), 20.8 (q, C-13), 17.0 (q, C-14), 16.1 (q, C-15), 155.7 (s, NC).

KALIHINOLS X [**6**], J [**7**], and Y [**8**].—These were concentrated in the bioactive fractions and were purified by regular phase followed by reversed-phase hplc (10μ-ODS, 25 × 1.0 cm, 98% aqueous MeOH). Compounds **6** and **8** displayed properties identical to those previously described (6). Kalihinol J [**7**], $C_{22}H_{35}N_2O_3SCl$, exhibited the following properties: lreims *m/z* (percent) $[M]^+$ 445 (30), $[M]^+$ 443 (80), 425 (60), 389 (20), 384 (100), 366 (20), 163 (10), 161 (32); uv λ max (MeOH) 242 nm (ε = 652); ir 3579 (br OH), 3440 (NH), 2103 (NCS), 1680 (NHCO), 1140 (-C-O-C-) $cm^{-1}$; $^1H$ nmr (300 MHz, $CDCl_3$) 4.25 (brd, $J$ = 10.5, H-5), 2.41 (dt, $J$ = 11.4, 1.8 Hz, H-6), 1.47–1.67 (m, many H's including H-1 and H-7), 3.66 (dd, $J$ = 12.0, 4.5, H-14), 1.33 (s, Me-16), 1.38 (s, Me-17), 1.20 (s, Me-18), 1.34 (s, Me-19), 1.31 (s, Me-10), 6.63 (brt, $J$ = 10.5, NH), 8.12 (d, $J$ = 10.5, CHO).

KALIHINOL I [**9**].—Long colorless needles: mp 180° ($Et_2O$); $C_{22}H_{33}N_2O_2S_2Cl$; lrcims *m/z* (percent) 459 (0.5), $[M+H]^+$ 457 (2), 439 (10), 398 (20), 163 (35), 161 (100); uv λ max (MeOH) 245 nm (ε = 673); ir 3581 (br, OH), 2103 (NCS), 1382 (gem dimethyl), 1138 (-C-O-C-) $cm^{-1}$; $^1H$ nmr (300 MHz, $CDCl_3$) 3.74 (dd, $J$ = 11.7, 4.8, H-14eq), 1.33 (s, Me-16), 1.39 (s, Me-17), 1.26 (s, Me-18), 1.34 (s, Me-19), 1.35 (s, Me-20), 2.25–1.33 (complex).

## ACKNOWLEDGMENTS

Partial research support was from NOAA, National Sea Grant College Program, Department of Commerce, grant number NA85AA-D-SG140, project number R/MP-41, through the California Sea Grant College Program. The U.S. Government is authorized to produce and distribute reprints for governmental purposes. Other financial support was from Syntex Inc. Our field work in Fiji was partially supported through the UREP program. We are also grateful to the Fiji Government for their cooperation. Thanks are due to Mr. Jim Loo (UCSC) for assistance with nmr measurements and to Cristina Dias for help with all the taxonomic information contained in this paper. We note assistance in the collections of sponges in Thailand from Drs. Bob Clemens, Hank Chaney, Tom Matthews, Ms. Lisa Hunter, Ms. Teresa Matthews, and Mr. Cal Ponzini.

## LITERATURE CITED

1. P.R. Bergquist and R.J. Wells, in: "Marine Natural Products, Chemical and Biological Perspectives." Ed. by P.J. Scheuer, Academic Press, New York, Vol. 5. pp. 1–50 (1983).
2. W.D. Inman, M. O'Neil-Johnson, and P. Crews, *J. Am. Chem. Soc.*, **112**, 1 (1990).
3. L.V. Manes, P. Crews, M.R. Kernan, D.J. Faulkner, F.R. Fronczek, and R.D. Gandour, *J. Org. Chem.*, **53**, 570 (1988).
4. J.C. Braekman, D. Daloze, F. Deneubourg, J. Huysecom, and G. Vandevyver, *Bull. Soc. Chem. Belg.*, **96**, 539 (1987).

5. B. Di Blasio, E. Fattorusso, S. Magno, L. Mayol, C. Pedone, C. Santacroce, and D. Sica, *Tetrahedron*, **32**, 473 (1976).
6. C.W.J. Chang, A. Patra, J.A. Baker, and P.J. Scheuer, *J. Am. Chem. Soc.*, **109**, 6119 (1987).
7. B.J. Burreson, C. Christophersen, and P.J. Scheuer, *Tetrahedron*, **31**, 2015 (1975).
8. P. Crews and P. Bescansa, *J. Nat. Prod.*, **49**, 1041 (1986).
9. J.E. Thompson, R.P. Walker, S.J. Wratten, and D.J. Faulkner, *Tetrahedron*, **38**, 1865 (1982).
10. P. Ciminiello, E. Fattorusso, S. Magno, and L. Mayol, *Can. J. Chem.*, **65**, 518 (1987).
11. S. Omar, C. Albert, T. Fanni, and P. Crews, *J. Org. Chem.*, **53**, 5971 (1988).
12. H.y. He, D.J. Faulkner, J.S. Shumsky, K. Hong, and J. Clardy, *J. Org. Chem.*, **54**, 2511 (1989).
13. M. Adinolfi, L. De Napoli, B. Di Blasio, A. Iengo, C. Pedone, and C. Santacroce, *Tetrahedron Lett.*, **32**, 2815 (1977).
14. R.J. Capon and J.K. MacLeod, *Aust. J. Chem.*, **41**, 979 (1988).
15. H. Tada, T. Tozyo, and M. Shiro, *J. Org. Chem.*, **53**, 3366 (1988).
16. C.J. Fookes, M.J. Garson, J.K. MacLeod, B.W. Skelton, and A.H. White, *J. Chem. Soc., Perkin Trans. 1*, 1003 (1988).
17. P. Karuso and P.J. Scheuer, *J. Org. Chem.*, **54**, 2092 (1989).
18. M.R. Hagadone, P.J. Scheuer, and A. Holm, *J. Am. Chem. Soc.*, **106**, 2447 (1984).
19. H. Tada, T. Tozyo, and M. Shiro, *J. Org. Chem.*, **53**, 3366 (1988).
20. S. Omar, L. Tenenbaum, L.V. Manes, and P. Crews, *Tetrahedron Lett.*, **43**, 5489 (1988).
21. M.C. Diaz, S.A. Pomponi, and R.W.M. van Soest, in: "Fossil and Recent Sponges." Ed. by H. Keupp and J.E. Reitner, Springer-Verlag, New York, 1990, in press.
22. R.W.M. van Soest, M.C. Diaz, and S.A. Pomponi, *Beaufortia*, **40** (no. 2), 15 (1990).

*Received 15 December 1989*