Pergamon

Tetrahedron Vol. 50, No. 38, pp. 11079-11090, 1994

0040-4020/94 $7.00+0.00

0040-4020(94)00674-1

Variation Among Known Kalihinol and New Kalihinene Diterpenes from the Sponge *Acanthella Cavernosa*

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Abstract: Seven new diterpene constituents were isolated from the sponge *Acanthella cavernosa* investigated from Fijian locations. Their structures were established based on a combination of spectroscopic data, x-ray crystallographic data and biogenetic arguments. These new diterpenes, of the kalihinane family, can be divided into the kalihinene (compounds **4** - **6**) or the 6-hydroxy-kalihinene (compounds **7** - **10**) frameworks. Also present were three known compounds, kalihinol A (**1**), isokalihinol F (**2**) and kalihinene (**3**). It is proposed that the kalihinane type diterpenes are a chemotaxonomic marker for *A. cavernosa*. Its diterpenes can be clustered into three biogenetic families based on the stereochemistry of the decalin ring forming process. The presence of different members of these biogenetic types might be used to chemically distinguish various populations of *A. cavernosa*. The compounds, **1**, **3** and **7** were major constituents in both the bulk collection extracted after collection and in 15 specimens held in a controlled aquaculture system for seven months before extraction. A subtle chemical difference could be discerned among these samples based on the content of compounds **1**, **3** - **5** and **7**.

INTRODUCTION

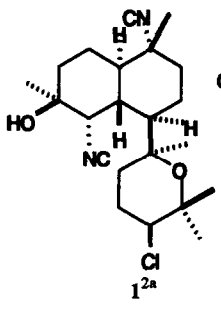
Many of the orange colored tropical sponges of the family Dictyonellidae are a rich source of unusual, highly functionalized terpenoids.[1,2] Several years ago we discovered that one of these sponges, *Acanthella cavernosa*, was both extremely hardy and persistent in its exclusive biogenesis of diterpenes. This species can be found in every Indo-Pacific region we have visited, and it has consistently yielded closely related diterpenes richly substituted with isonitrile, isothiocyanate, or formamide moieties.[3] It turns out that *A. cavernosa* has been studied by several research groups using samples collected from Guam,[2a,b,c,e] Thailand,[2d] Fiji,[2e,f] the Nansei Islands of Japan[2g] and Seychelles[2h].

Several years ago we were able to dislodge this sponge from its coral reef environment, transplant it to an aquarium in our laboratory (UCSC) and after one year continue to observe isonitrile diterpenes in extracts prepared from a few individual sponges. These positive developments prompted a larger effort designed to measure the variations of the secondary metabolites of *A. cavernosa* kept under different conditions in a closed aquaculture system. The first step of our investigation consisted of establishing base line chemical data which could then be used to estimate the extent of metabolite change as a function of time. Initially we did not know whether or not the diterpenoid content would be invariant for *A. cavernosa* obtained at different depths or at different collection sites. An important precedent came from the findings of Thompson who showed that the

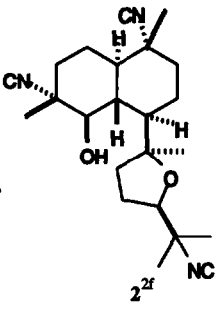
diterpene content of *Rhophaloeides odorabile* was constant but the composition among four compounds varied according to the environment.[4] The NMR spectra of crude extracts from Fijian organisms, obtained for this new study at four sites within close proximity of one another, revealed that diterpenoids were the major constituents. HPLC analysis, guided by known standards, showed the presence of three major constituents kalihinol A (**1**)[2a], isokalihinol F (**2**)[2f] and kalihinene (**3**)[2g]. Much to our surprise these compounds were accompanied by new metabolites that were divided into two groups based on their structural relationship either to kalihinene (**3**) or to a new 6-hydroxy-kalihinene framework. Described in this report are the properties of these new diterpenoids **4** - **6** (related to kalihinene) and compounds **7** - **10** (possessing the new 6-hydroxy *cis* decalin framework). We also outline how the compositions of the most abundant diterpenes vary and might be provisionally used to divide *A. cavernosa* into different groups.[5] Data will also be presented to show that these diterpenes persist in sponges which have been kept alive in a closed aquaculture system for seven months.

RESULTS AND DISCUSSION

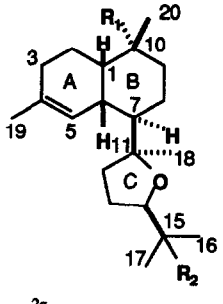
Sponges were collected for this study in November 1992 from the Fiji Islands at sites near Pacific Harbor and the nearby reefs of the Benga (=Beqa) Lagoon. One batch consisted of a bulk collection (0.4 kg) which was preserved by brief soaking in *n*PrOH:H_2O (1:1). Both the solvent and the damp organisms were transported back to UCSC for further MeOH extraction. The combined MeOH and *n*PrOH extracts were further processed according to our standard procedure[6] affording a CH_2Cl_2 solvent partition fraction that was purified by HPLC yielding compounds **1** - **10**. Individual specimens were also collected and they were transported alive to the aquaculture facility at CalBioMarine Technologies in Carlsbad, CA and held for seven months before MeOH extraction.



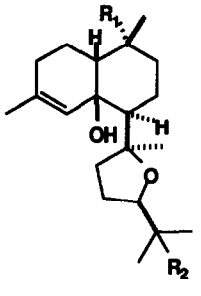
1[2a]



2[2f]



3[2g] R_1=R_2=NC
4 R_1=NHCHO, R_2=NC
5 R_1=NC, R_2=NHCHO
6 R_1=R_2=NHCHO



7 R_1=R_2=NC
8 R_1=NC, R_2=NHCHO
9 R_1=NHCHO, R_2=NC
10 R_1=NHCHO, R_2=NCS

Work on the new compounds began with the kalihinene derivatives **4** - **6**. A known compound, kalihinene (**3**),[2g] was a major component and the ^{13}C NMR properties we measured are shown in Table 1. These data established that compounds **4** - **5** had A/B/C rings analogous to that of **3** ($C_{22}H_{32}N_2O$). In particular, the ^{13}C NMR shifts at carbons 1 - 9, 11 - 14 and Me-19 of **4** and **5** were almost identical to those of **3**. That one of the -NC groups of **3** had been replaced by formamide residue in **4** and **5** was indicated by the molecular formula of **4**, $C_{22}H_{34}N_2O_2$ (HRFABMS *m/z* 359.2693 $[M+H]^+$, Δ 0.5 mmu of calcd) and of **5** $C_{22}H_{34}N_2O_2$ (HRFABMS $[M+H]^+$ 359.2690, Δ 0.8 mmu of calcd). It was the presence of the formamide groups which added additional complication to the appearance of the NMR spectra. For example, the ^1H NMR spectrum of **4** included downfield doubled signals at δ 8.23 (d, J = 12.0 Hz) and 8.06 (d, J = 2.0 Hz) each of which exhibited HMQC

Table 1. ^{13}C NMR ($CDCl_3$) Data for the Kalihinene Derivatives.

Atom	3	4*t*	4*c*	5*t*	5*c*	7	8*c*	8*t*
1	43.8	41.5	45.8	43.8	43.8	50.3	50.3	50.6
2	20.4	19.0	19.4	20.4	20.4	21.7	22.1	22.1
3	30.7	30.7	30.9	30.7	30.7	30.3	30.3	30.3
4	131.0	130.4	130.6	131.6	131.3	131.9	131.6	131.6
5	126.3	127.1	126.9	126.7	126.8	131.4	132.1	132.8
6	34.7	34.9	35.4	34.7	34.7	75.4	75.6	75.6
7	48.1	48.5#	48.3#	47.6	48.2	51.6	50.8	52.1
8	24.7	24.6	24.5	24.9	24.9	23.6	23.6	23.6
9	33.9	33.3*	32.6*	33.9	34.0	35.7	35.8	35.8
10	60.4	56.7	55.3	60.9	60.9	61.8	63.0	63.0
11	86.5	86.6	86.8	86.2	86.1	87.3	86.9	86.9
12	37.2	37.2	37.2	37.7	37.8	38.9	39.8	39.7
13	25.7	25.7	25.7	25.1	25.1	25.6	25.0	25.0
14	82.9	82.8	82.7	84.2	84.2	83.1	84.5	84.3
15	60.9	60.5	60.4	54.5	55.7	60.2	54.6	-
16	26.3	26.3	26.4	27.2	24.7	26.3	27.2	24.8
17	25.1	25.1	25.1	23.9	21.7	25.5	24.3	21.8
18	19.7	19.6	19.6	19.5	20.0	22.9	22.6	22.6
19	23.3	23.5	23.3	23.3	23.3	23.6	23.0	23.0
20	26.8	27.2	23.4	26.8	26.8	30.2	30.3	30.0
	153.2	153.9	153.9	154.6	-	154.6	-	-
	153.4	162.7	160.1	163.2	160.8	153.5	163.3	160.9

#, * Interchangeable signals.

correlations respectively to doubled carbons at δ 162.7 and 160.1. Almost all of the carbon NMR signals of **4** (Table 1) were doubled and the ratio of isomers, calculated from ^1H NMR integrations, differed from **4***c* (*cis*)/**4***t* (*trans*) = 52:48 in $CDCl_3$ to = 77:23 in C_6D_6. (Although in $CDCl_3$ the ratio of *cis*/*trans* isomers was almost 1:1, the ^{13}C spectrum was assigned using HMQC data with the CHO proton of **4***t* as anchor point). The formamide group present in **4** was placed at C10 based on observing Me16 and Me17 as triplets (δ 1.31 and 1.34, J = 1.6 Hz) and Me20 as sharp singlets (δ 1.53 *trans*, 1.43 *cis*). Data from a NOESY experiment (C_6D_6), summarized in Figure 1, afforded correlations between Me20/H6 and Me20/H1 analogous to those reported for **3**.[2g] Such results required a mutual *cis* geometry between these proton sets. In addition, the NOESY correlation between Me18 and H6 in **4** indicated a close proximity between these atoms as reported in the x-ray crystallographic and NOE examination of **3**.[2g] The additional stereochemistry shown for **4** is based on the remaining similarities in the NMR parameters between **3** and **4** including comparison of the ^{13}C NMR shifts at C11 and C14 and the J = 10.3 Hz between H6 an H7 measured for **4***c* in the ^1H NMR (C_6D_6, 300 MHz) spectrum.

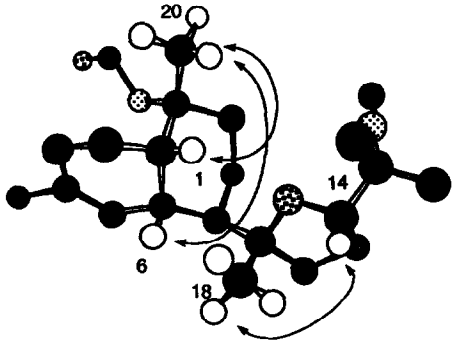


Figure 1. NOESY Correlations of **4** as shown in an energy minimized conformation. Some hydrogens are omitted for clarity.

Additional comparisons between the NMR

data for **3** and **4** to that of **5** were used to complete the structure elucidation of the latter compound. The split proton signals for Me16 (δ 1.20/1.33), Me17 (δ 1.31/1.38) and for H14 (δ 3.77 and 3.85 dd each) in **5** indicated that the NHCHO group was now at C15 and this carbon also appeared as doubled signals at δ 54.5 (s) and 55.7 (s). A 71:29 ratio of *trans/cis* rotamers **5t/5c** was deduced by ^{1}H NMR in $CDCl_3$. The stereochemistry proposed for **5** was justified by the parallel carbon shifts for **3** - **5** at atoms 1, 6, 7, 11, 14, and Me20 as is evident in Table 1.

Compound **6** was isolated in small amounts as a colorless solid. The additional O and H atoms present in its molecular formula of $C_{22}H_{36}N_2O_3$ (*m/z* 377.2794, $[M+H]^+$, Δ 1.0 mmu), versus that of **3**, indicated that both of the -NC groups of **3** were replaced by -NHCHO residues. Resonances for both formamides were observed as a plethora of signals between δ 8.0 and 8.3. These included eight signals arising from the four isomeric arrangements possible for the two formamides at C10 and C15. Other doubled signals were also observed for Me16 and Me17. The paucity of sample prevented extensive NMR work so the structure and stereochemistry shown in **6** must be considered as provisional as it is based on analogies to compounds **3** - **5**.

The second series of new compounds was headed by 6-hydroxy-kalihinene (**7**). Compound **7** was obtained as colorless long needles from EtOH and the molecular formula of $C_{22}H_{32}N_2O_2$ was established by HRFABMS (*m/z* 511.2678, $[M+Magic\ Bullet+H]^+$, $C_{26}H_{43}N_2O_4S_2$, Δ 1.4 mmu). Key ^{13}C NMR resonances pinpointed the A/B/C rings and these included δ 50.3 (d) C1, 51.6 (d) C7, 87.3 (s) C11, and 83.1 (s) C14. There were two broad triplets ($J \approx 5$ Hz) at δ 61.8 and 60.2 for quaternary carbons, C10 and C15, each bearing isocyano groups. The δ 75.4 (s) signal was unusual and comparison to shifts of **3** indicated that it was due to an oxygenated decalin bridgehead carbon. Comparison of the remaining ^{13}C resonances between **7** and **3** indicated that most of the other structural features, except at the ring junction positions, were similar. We could not envision a direct NMR method to establish the ring junction stereochemistry between the H and OH, so a single crystal x-ray diffraction of **7** was undertaken. The computer generated drawing of the x-ray results appears in Figure 2 and shows that the A/B ring junction is *cis*. Additional conformational features evident in the crystal structure are that ring B adopts a regular chair conformation, ring A is in a half-chair, and the tetrahydrofuran ring sits in an approximately C_2 conformation with C14 on the two-fold axis.

A minor component, compound **8**, possessing the A/B/C ring core of **7**, was isolated as a colorless white solid. Mass spectrometry established a molecular formula of $C_{22}H_{34}N_2O_3$ (*m/z* 529.2778, [M +Magic Bullet

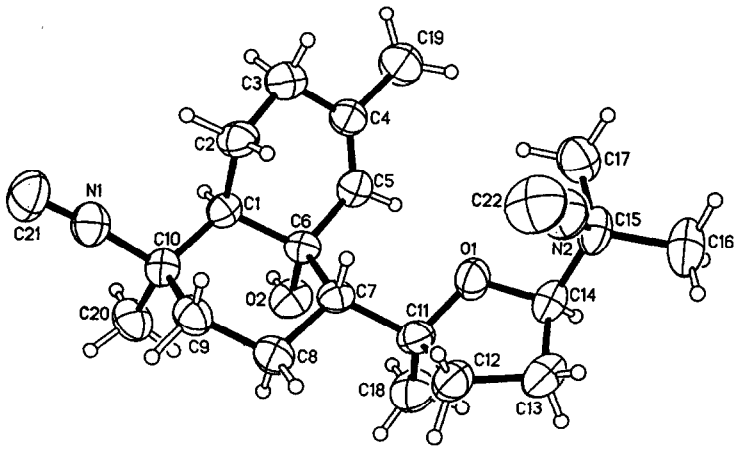


Figure 2. Computer generated perspective drawing of the final x-ray model of 6-hydroxy-kalihinene (**7**). No absolute stereochemistry is implied.

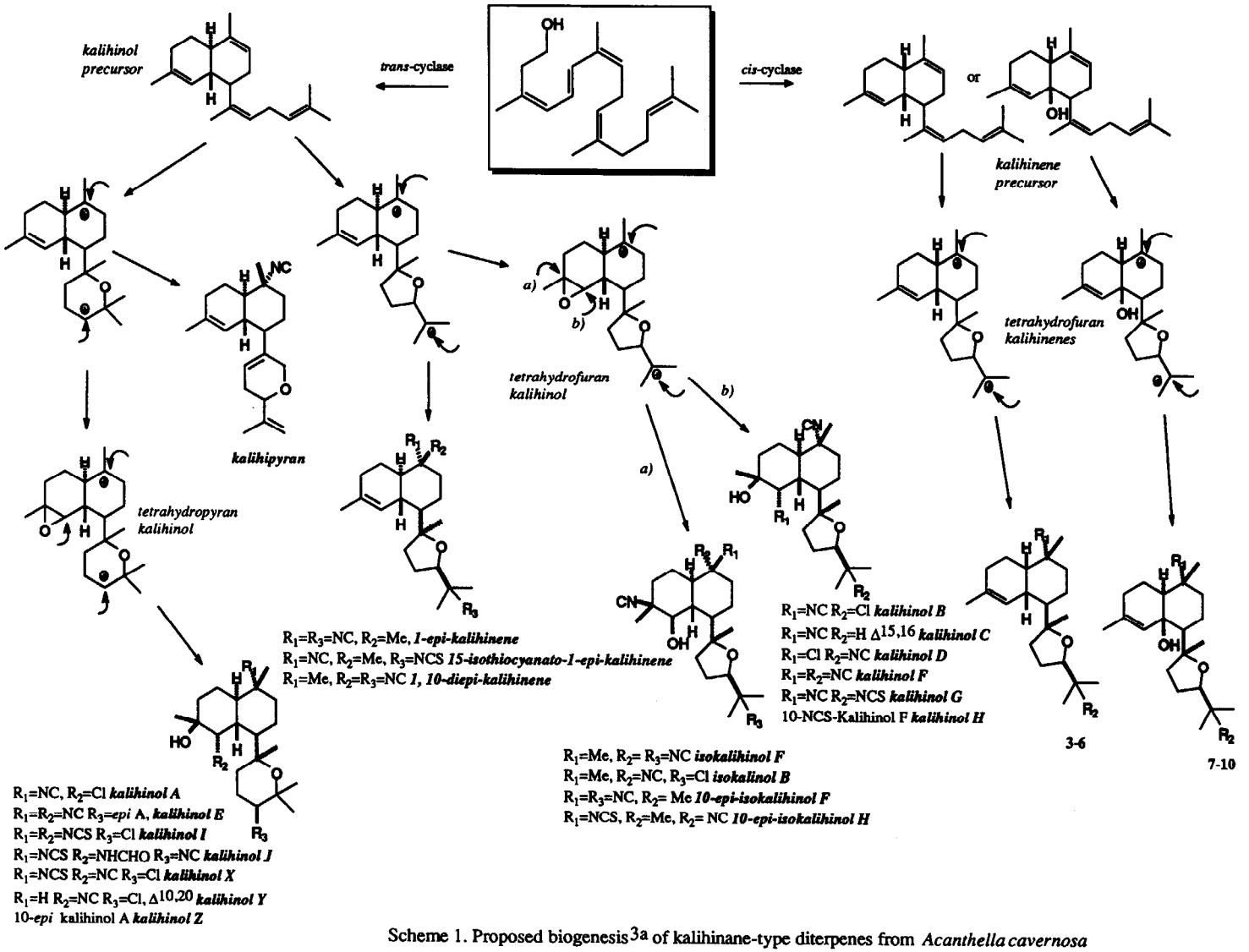
Table 2. ^{1}H NMR ($CDCl_3$) Data for the Kalihinene Derivatives.

Proton	**6**	**9**	**10**
H5	5.75 bs	5.65 bd	5.63 s
H14	3.75-3.85 m	3.82 m	3.82 dd, J = 8.5 and 4.3 Hz
Me16	1.20 s/1.32 s	1.27 bs	1.35 bs
Me17	1.37 s/1.42 s	1.31bs	1.41 bs
Me18	1.20 s	1.21 s	1.27 s
Me19	1.60 bs	1.61 bs	1.60 bs
Me20	1.47 s/1.55 s	1.70 s/1.82 s	1.61 s
NH	4.55 bs/5.23 bs 5.70 bd/5.89 bd	5.70 bd (*trans*)/5.21 bs (*cis*)	5.70 bd (*trans*)/5.21 bs (*cis*)
CHO	8.3-8.0	8.21 d, J = 12.0 Hz (*cis*) 8.03 d, J = 2.0 Hz (*trans*)	8.22 d, J = 12.5 Hz (*cis*) 8.05 d, J = 1.8 Hz (*trans*)

+H]$^+$, $C_{26}H_{45}N_2O_5S_2$, Δ 0.8 mmu) and it was evident that one -NC of **7** had been replaced by an NHCHO in **8**. Key NMR features which established the similarity between this pair of compounds included resonances in **8** for the hydroxylated C6 at δ 75.6 (s), for the formamide group proton (in $CDCl_3$) δ 8.16 d J = 12.6 Hz / 8.06 d J = 1.8 Hz (ratio of *trans/cis* = 69:31), and doubled signals for the carbons of Me16 and Me17 (Table 1). The Me20 peaks appeared as narrow triplets in the ^{1}H NMR spectrum which justified the assignment of the NC at C10. The similarities in ^{13}C NMR data between **7** and **8** were the basis for the parallel stereochemistry assigned for the latter.

Two additional components, **9** and **10**, each possessing the 6-hydroxy-decalin framework, were isolated in minute quantities. Their structures and stereochemical features are based on HRFABMS data, ^{1}H NMR spectra, and analogy to **7** and **8**. That **9** had one formamide group was shown by the *m/z* peak at 375.2635 ([M+H]$^+$ $C_{22}H_{35}N_2O_3$, Δ 1.2 mmu of calcd). Its ^{1}H NMR also contained doubled signals and especially diagnostic were those of the formamide group at δ 8.21, J = 12.0 Hz (**9*t***, CHO), δ 8.03, J = 2.0 Hz (**9*c***, CHO), δ 5.70 bd (**9*t***, NH), δ 5.21 bs (**9*c***, NH). The molecular formula $C_{22}H_{34}N_2O_3S$ of **10**, isolated as a white solid, with HRFABMS *m/z* 407.2362 [M+H]$^+$ (Δ 0.6 mmu of calcd) suggested the functional groups as hydroxy, formamide and isothiocyanate. The lowest field ^{1}H NMR ($CDCl_3$) resonances at δ 8.22 (d J = 12.5 Hz) and 8.05 (d J = 1.8 Hz) were ascribed to a *trans/cis* ratio = 42:58 of the formamide moiety. Drawing on an analogy to the structures of **7** - **9**, the remaining hetero atoms were assigned as an OH at C6, NHCHO at C10 and NCS at C15 (H14 was a sharp dd as in kalihinol G[2e], so the NCS was placed at C15) and the stereochemistry is provisionally assigned for **10** based on the comparison of the ^{1}H NMR data to **7**.

Adding the new compounds introduced above to those already in the literature[2] gives a tally of some 28 kalihinane type diterpenes. We believe that this class of diterpenes is a good chemotaxonomic marker for *Acanthella carvenosa*, which is described in the Experimental and also shown in Figure 5. However, there is confusion about both its identification and production of secondary metabolites which can now be resolved. We have repeatedly found only kalihinane type diterpenes from *A. cavernosa*. The specimens studied by Scheuer, which yielded the various kalihinanes, were identified as *Acanthella* sp.,[2a-c,e] but an underwater photograph, kindly provided by Prof. Scheuer, indicates it to be *A. cavernosa*. Similarly, material studied by Fusetani named as *A. klethra*[2g,7] is actually *A. cavernosa* as shown by our taxonomic study of a voucher specimen generously supplied by Prof. Fusetani.



Scheme 1. Proposed biogenesis[3a] of kalihinane-type diterpenes from *Acanthella cavernosa*

The members of the kalihinane family can be clustered into three biogenetic families based on the stereochemistry of the A/B ring forming process. These divisions are shown in Scheme 1 and are based on an extension of ideas previously proposed by Scheuer.[3a] A different initial cyclization is envisioned to account for the variation in the A/B ring junction among the various compounds that are shown. For example, the twenty compounds, headed by kalihinols A and B, isokalihinol F and *epi*-kalihinene, are all related by their *trans* A/B ring fusion possessing two ring junction H's (and they can be further subdivided into five or six membered ether rings). There are four compounds, headed by kalihinene (**3**) which have a *cis* A/B ring fusion with two ring junction H's, and there are four compounds, headed by 6-hydroxy-kalihinene (**7**), which have a *cis* A/B ring fusion with a ring junction H and OH. These biogenetic differences might provide the basis to chemically distinguish the populations of *A. cavernosa,* by analogy to suggestions made in the past for seaweeds, gorgonians and sponges.[5,8] The previous studies of *A. cavernosa* by our group[2d,f] by the Scheuer group,[2a-c,e] and by the Faulkner group,[2h] reported exclusively *trans* A/B ring fusion kalihinanes, such as **1** and **2**. This exemplifies one distinct situation where only these types of compounds are present as major isolation products. A contrast is provided by the isolation work from the Fusetani[2g] laboratory where compounds having both A/B *trans* and *cis* ring fusion with two ring junction H's were obtained but no compounds of the third ring junction series were reported. A third case is represented by the results of our own work in which compounds of all three ring junction types were major components of the isolation work.

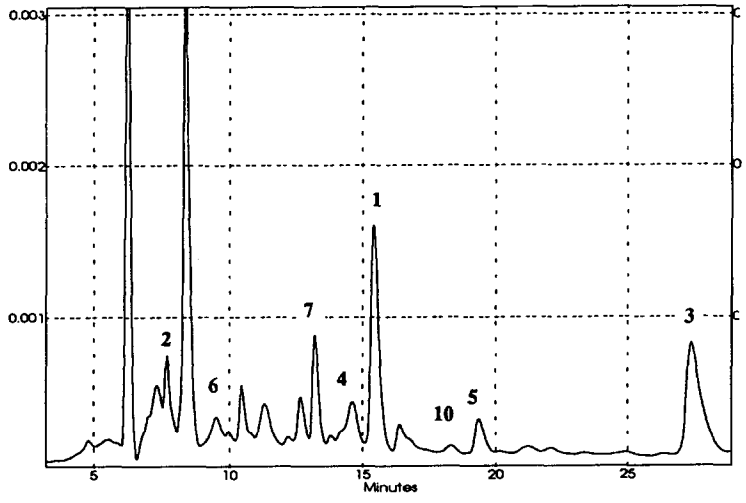


Figure 3. HPLC chromatogram of the crude CH_2Cl_2 partition fraction of sponge 148A.

With all of the major and many of the minor constituents of the bulk sample of *A. cavernosa* characterized we began to examine the extent to which **1** - **10** were present among the 15 individual specimens maintained in a tropical aquarium for seven months. The analytical method we developed consisted of isocratic reversed phase chromatography on a spherical silica C18 analytical column (250 x 4.6 mm; 300 Å pore size, 5μ particle size) with 25% aqueous MeOH. Each individual sponge was worked-up separately and the methanol extract was subjected to solvent partitioning and the subsequent crude CH_2Cl_2 partition fraction was analyzed further. A typical HPLC plot is shown in Figure 3 and it was analyzed by a calibration curve based on the standard RI detector responses to pure samples of **1** - **10** injected at known concentrations. Interestingly, the major constituents of the collected samples not subjected to aquaculture (= bulk samples) were kalihinol A (**1**), kalihinene (**3**) and 6-hydroxy-kalihinene (**7**) and these were also major constituents of the individual sponges held in aquaculture. Clearly the collected

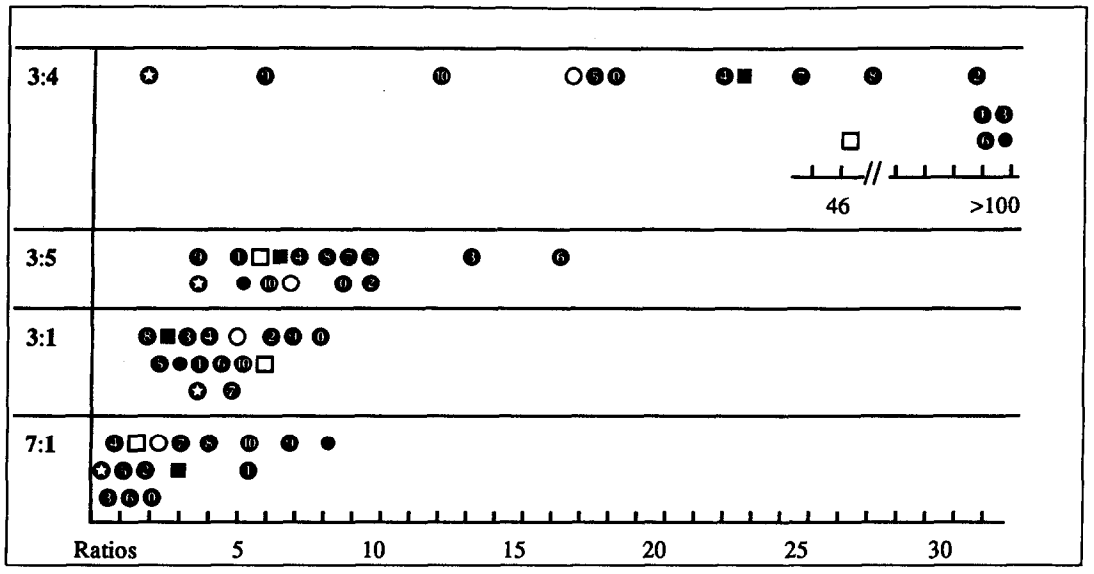


Figure 4. Ratios of kalihinane-diterpenes from *A. cavernosa* extracted before and after being held in aquaculture. Ocean sample: ✪ Bulk. Aquaculture samples: ❶ 90A, ❶ 232A, ❶ 155A, ❶ 140A, ❶ 48A, ❶ 36A, ❶ 78A, ❶ 136A, ❶ 124A, ❶ 9A, ❶ 127A, ○ 113A, ■ 148A, □156A, ● 43A.

and the aquaculture samples were comparable chemically as they both had major constituents from each biogenetic pathway shown in Scheme 1. We recognize that our sample size is small but even at this preliminary stage we feel that a subtle yet significant chemical difference could be discerned among the individual cultured sponges based on the content of compounds **3** - **5** as illustrated in Figure 4. In this limited assemblage the ratios of both **3**:**1** and **7**:**1** are comparable, the ratios of **3**:**5** are also close but it is the wide range in the ratios of **3**:**4** which can be used to tentatively divide the specimens into two groups – based on the values of **3**:**4** < 50 or > 100. It should be noted that these differences are independent of the collection sites (see experimental). It is tempting to consider that the shift in content of **3** to **4** (Figure 4) is significant because it represents the conversion of -NC to formamide, which has been shown by Scheuer[2c,3a,c] to be a metabolic event carried out by the sponge.

It is possible that the phenomenon of observing chemical differences between different collections of the same genus of sponges is widespread. That, as we have shown above, consistent distributions of secondary metabolites can be observed between wild and cultured specimens from the same lot is important. We believe that the results of this study will help in the future design of experiments to explore chemical differences among bulk populations of the same sponge species or between specimens which grow side-by-side.

EXPERIMENTAL

General Experimental Procedures. The NMR spectra were recorded at 250, 300 or 500 MHz for ^{1}H and 62.9 or 125.7 MHz for ^{13}C. Multiplicities of ^{13}C NMR resonances were determined from APT data, DEPT data, or ^{1}H-^{13}C COSY experiments (300 MHz) or HMQC (500 MHz). Low and high resolution

electron impact mass spectrometry data were obtained on VG 70-VSE spectrometer. Some FAB spectra were done in a Magic Bullet matrix consisting of a mixture of dithiothreito:dithioerythritol (3:1). High performance liquid chromatography (HPLC) was done using columns that included 10μ ODS (semiprep) or spherical silica C18 analytical column 5μ ODS, 300 Å pore size. The analysis of individual sponges was done on a Waters 501 HPLC equipped with a refractive index detector which was interfaced to a computer data software package, EZChrom™. The calibrations of the compounds were determined using an external method.

Collection and Identification. A representative voucher from Fiji collections was subjected to careful taxonomic analysis. The sample (coll. no. 92002) was identified as *Acanthella cavernosa*, Dendy 1922, (order Halichondrida, family Dictyonellidae[9]). The morphology of this voucher specimen was massive semi-globular to rounded in shape. Its cavernous body is formed by intertwined thick (1-3 mm) planar spicule tracts (trabeculae) that surround rounded cavities, and end in stout conules at surface. The color of the sponge alive is bright orange to reddish, both inside and out. The sponge is compressible and very cavernous. The spicules are straight styles (500-800 x 10-20 μm) and flexous strongyles (600-1000 x 3-10 mm). The skeleton consists of thick spicule tracts dominated by axially compacted flexous strongyles and fewer styles, and styles echinating them. There is not an ectonosomal skeletal specialization. In addition to these features the appearance of the sponge shown in Figure 5 matches the descriptions and drawings published by van Soest.[10] The underwater photograph of material studied by Prof. Scheuer matches that shown in Figure 5. The voucher supplied by Prof. Fusetani presented the same external morphology, skeletal arrangement and overall spiculation as compared to our material. All of the sponges used in this study were obtained at a depth-range of 10-40 ft at Pacific Harbor (PH): 18°16'15", 178°03'15"; Pratt Reefs (PR): 18°19'44", 178°08'28": the Benga

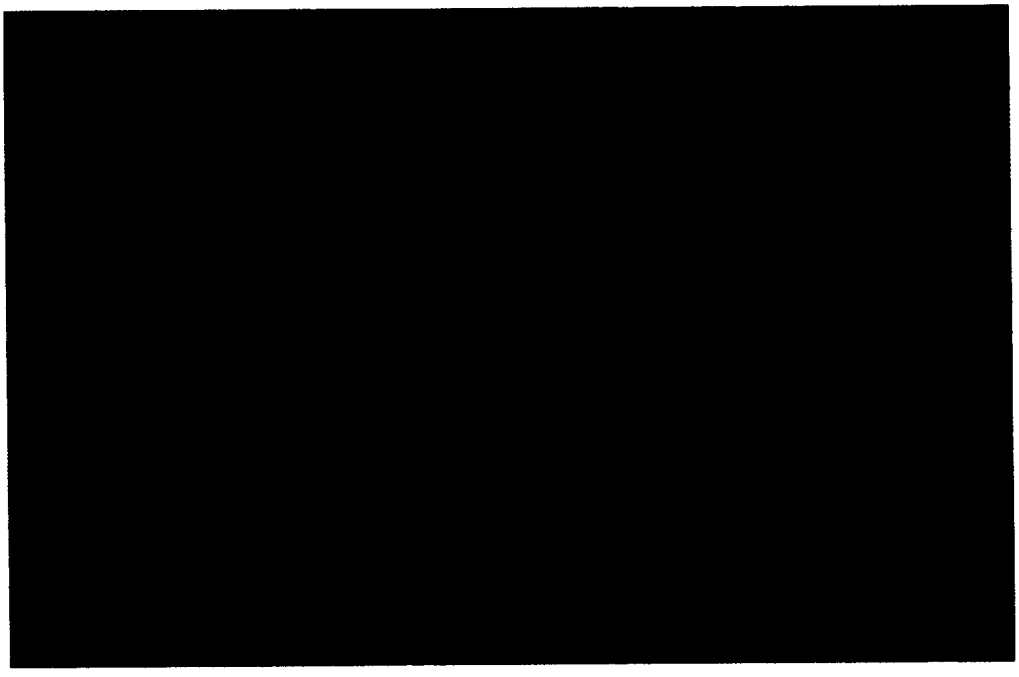


Figure 5. Underwater photograph of *Acanthella cavernosa.*

Lagoon (BL): 18°22'42", 178°5'40"; and Sulfur Passage (SP): 18°21'33", 178°11'17". The sites of collection for the individual samples are: PH - 232A, 148A; PR - 90A, 155A, 48A, 36A, 124A, 9A, 127A, 113A, 156A, 43A; BL - 140A, 136A; SP - 78A.

Extraction and Isolation. The bulk collection from the four sites (16 specimens comprising the bulk collection, 0.4 kg wet/103.5 g dried) were preserved by being immersed in a 50:50 *n*PrOH:H_2O solution. After decanting this solution the damp organisms were placed in separate nalgene bottles and shipped back to the home lab at ambient temperature. Next, 100% MeOH was added and the organisms were soaked for 24 hr. This procedure was repeated two more times. The combined organics afforded a crude oil (49.31 g) which was then successively partitioned between equal volumes of water and CH_2Cl_2. The latter extract (1.08 g) was dissolved in aqueous MeOH and percent adjusted to produce a biphasic solution, then a solvent series of hexanes and CH_2Cl_2. Its CH_2Cl_2 fraction (853.8 mg) was subjected to a C18 silica HPLC column (25% aqueous MeOH) yielding kalihinol A (**1**) 12.4 mg, isokalihinol F (**2**) 15.8 mg, kalihinene (**3**) 30.0 mg, 10-formamido-kalihinene (**4**) 29.9 mg, 15-formamido-kalihinene (**5**) 12.1 mg, 10,5 bis-formamido-kalihinene (**6**) 1.2 mg, 6-hydroxy-kalihinene (**7**) 12.6 mg, 6-hydroxy-15-formamido-kalihinene (**8**) 12.0 mg, 6-hydroxy-10-formamido-kalihinene (**9**) 2.0 mg, 6-hydroxy-10-formamido-15-isothycyano-kalihinene (**10**) 1.3 mg.

The individual live specimens were held for 7 months in a controlled-environment saltwater aquarium and then each was individually extracted with MeOH. The respective sponge dry wt data in g and CH_2Cl_2 extract yield in mg is as follows: ✪ bulk (103.5/853.8) 0.8%, ⓿ 90A (0.65/22.2) 3.4%, ❶ 232A (0.52/11.9) 2.3%, ❷ 155A (0.99/21.3) 2.1%, ❸ 140A (0.62/15.5) 2.5%, ❹ 48A (1.18/27.2) 2.3%, ❺ 36A (0.47/19.9) 4.2%, ❻ 78A (1.11/33.8) 3.0%, ❼ 136A (0.54/23.3) 4.3%, ❽ 124A (1.06/21.5) 2.0%, ❾ 9A (1.13/22.2) 1.9%, ❿ 127A (0.31/10.0) 3.2%, ○ 113A (0.87/22.0) 2.5%, ■ 148A (0.48/11.8) 2.4%, □ 156A (1.22/39.5) 3.2%, ● 43A (1.43/53.5) 3.7%.

10-Formamido-kalihinene (4c-t): ^{1}H NMR ($CDCl_3$) **4c**: δ 1.60 m (H1), 1.57 m/1.72 m (H2), 1.96 m (H3), 5.66 bs (H5), 2.28 m (H6), 1.60 m (H7), 1.32 m/1.54 m (H8), 1.54 m (H9), 1.76 m/1.71 m (H12), 2.06 m/1.87 m (H13), 3.80 m (H14), 1.31 t J = 1.6 Hz (Me16), 1.34 t J = 1.6 Hz (Me17), 1.13 s (Me18), 1.61 bs (Me19), 1.53 s (Me20), 8.06 d J = 2.0 Hz (CHO), 5.18 d J = 2 Hz (NH). **4t**: δ 2.30 m (H1), 1.57 m (H2), 1.96 m (H3), 5.66 bs (H5), 2.23 m (H6), 1.60 m (H7), 1.32 m/1.54 m (H8), 1.54 m (H9), 1.76 m/1.71 m (H12), 2.06 m/1.87 m (H13), 3.80 m (H14), 1.31 t J = 1.6 Hz (Me16), 1.34 t J = 1.6 Hz (Me17), 1.13 s (Me18), 1.59 bs (Me19), 1.43 s (Me20), 8.23 d J = 12.0 Hz (CHO), 5.75 d J = 12.0 Hz (NH). (C_6D_6) **4t**: δ 5.80 bs (H5), 2.24 m (H6), 1.83 m/1.49 m (H13), 3.62 m (H14), 1.35 t J = 1.8 Hz (Me16), 1.13 t J = 1.8 Hz (Me17), 1.04 s (Me18), 1.87 bs (Me19), 1.17 s (Me20), 8.44 d J = 12.2 Hz (CHO), 5.99 d J = 12 Hz (NH). **4c**: δ 2.76 dt J = 4.1, 11.5 Hz (H1), 1.71 m/1.61 m (H2), 5.80 bs (H5), 2.38 ddd J = 5.8, 4.1, 10.3 Hz (H6), 1.82 m (H7), 1.83 m/1.49 m (H13), 3.62 m (H14), 1.36 t J = 1.8 Hz (Me16), 1.13 t J = 1.8 Hz (Me17), 1.11 s (Me18), 1.90 bs (Me19), 1.70 s (Me20), 7.93 d J = 1.7 Hz (CHO), 4.20 bs (NH). IR (neat) cm^{-1}: 3350, 2968, 2127, 1679, 1530.

15-Formamido-kalihinene (5c-t): ^{1}H NMR ($CDCl_3$) **5t**: δ 5.71 bd (H5), 3.77 dd J = 7.2 and 6.8 Hz (H14), 1.20 s (Me16), 1.31 s (Me17), 1.12 s (Me18), 1.65 bs (Me19), 1.53 bs (Me20), 8.16 d J = 12.3 Hz (CHO), 5.82 d J = 12.3 Hz, (NH). **5c**: δ 5.71 bd (H5), 3.85 dd J = 7.2 and 6.8 Hz (H14), 1.33 s (Me16), 1.38 s (Me17), 1.14 s (Me18), 1.63 bs (Me19), 1.53 bs (Me20), 8.03 d J = 1.8 Hz (CHO), 4.12 bs (NH). IR (neat) cm^{-1}; 3330, 2970, 2129, 1677.

10,15-Bis formamido-kalihinene (6): ^{1}H NMR ($CDCl_3$): see Table 2. IR (neat) cm^{-1}: 3350, 2130, 2965, 1677, 1532.

6-Hydroxy-kalihinene (7): ^{1}H NMR ($CDCl_3$) δ 2.00 m (H3), 5.62 q *J* = 1.2 Hz (H5), 3.82 m (H14), 1.33 t *J*=1.6 Hz (Me16), 1.38 t *J*=1.6 Hz (Me17), 1.24 s (Me18), 1.65 t *J*=1.6 Hz (Me19), 1.76 bs (Me20). IR (neat) cm^{-1}: 3350, 2965, 2128, 1660, 1532.

Single crystal x-ray analysis of 6-hydroxy-kalihinene (7). A colorless flat needle crystal of **7** was mounted on a glass fiber in a random orientation. Preliminary examination and data collection were performed with monochromated Cu Kα radiation (λ = 1.5418 Å) on a Siemens R3m diffractometer. Preliminary experiments showed orthorhombic symmetry and unit cell dimensions of a = 12.595(1), b = 12.790(2), c = 13.369(2) Å. The cell volume is 2153.6(5) Å^3, with a calculated density of 1.12 g/mm^3, Z = 4. Intensities of 1912 diffraction maxima (2θ<110°) were measured using ω/2θ-scans with a variable scan speed (2.0-29.3 °/min) at room temperature. Three check reflections were measured every 97 reflections, and no significant variation in intensities were found. Lorentz and polarization corrections were applied to a data set of 1785 unique reflections. The structure was solved by direct methods (SHELXTL), and first map showed 20 out of 24 atoms. The remaining atoms were located in subsequent difference maps. Full matrix least-squares refinements on F^2 (SHELX-93) of anisotropic nonhydrogen atoms and isotropic riding hydrogen atoms converged to R_1 = 5.2 %, wR_2 = 13.6 %, GOF = 1.02 for 1458 reflections with I>2σ(I). A final difference Fourier map revealed no peaks greater than 0.31 e/Å^3. The x-ray experiment defined only the relative stereochemistry.

6-Hydroxy-15-formamido-kalihinene (8c-t): ^{1}H NMR ($CDCl_3$) **8*t***: δ 5.64 s (H5), 3.80 t *J* = 7.2 Hz (H14), 1.26 s (Me16), 1.34 s (Me17), 1.21 s (Me18), 1.59 bs (Me19), 1.72 bs (Me20), 8.16 d *J* = 12.6 Hz (CHO), 5.84 d *J* = 12.3 Hz, (NH). **8c**: δ 5.61 s (H5), 3.91 dd *J* = 7.2 Hz (H14), 1.36 s (Me16), 1.39 s (Me17), 1.30 s (Me18), 1.59 bs (Me19), 1.76 bs (Me20), 8.06 d *J* = 1.8 Hz (CHO), 5.87 bs (NH). IR (neat) cm^{-1}: 3347, 2980, 2129, 1680, 1532.

6-Hydroxy-10-formamido-kalihinene (9): ^{1}H NMR ($CDCl_3$): see Table 2. IR (neat) cm^{-1}; 3350, 2965, 2131, 1676, 1529.

6-Hydroxy-10-formamido-15-thyocyano-kalihinene (10):^{1}H NMR ($CDCl_3$): see Table 2. IR (neat) cm^{-1}: 3340, 2965, 2131, 2120, 1676, 1529.

Acknowledgment. Financial support at UCSC was from NIH grants CA52955 and CA47135 and from NSF Phase II SBIR grant no. III-9214477 (to CBMT). JR acknowledges a fellowship from Ministerio de Educación y Ciencia (Spain). The Cornell group was partially supported by NIH CA24487 and the New York State Sea Grant. Thanks to Dr. R. W. M. van Soest (Institute of Taxonomic Zoology, University of Amsterdam) for conferring with us on the taxonomic identifications. Mr. M Varoglu (UCSC) assisted in the collection of the sponges. Investigators at CalBioMarine Technologies, Inc., Mr. D. Mendola and Dr. J. Thompson, participated in the collection of the sponges, maintained the live sponges in their facility, were the PI's in the NSF SBIR grant, and they provided valuable comments about this paper. We are also grateful for the cooperation with local formalities from Prof. B. Aalbersberg (U. of South Pacific) and the staff of the Fisheries Department.

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(*Received in USA* 6 *June* 1994; *revised* 25 *July* 1994; *accepted* 26 *July* 1994)