Kalihinols, Multifunctional Diterpenoid Antibiotics from Marine Sponges *Acanthella* spp.[1]

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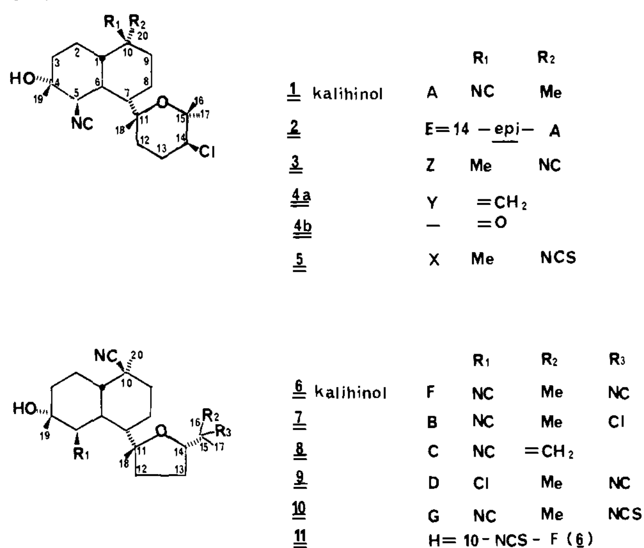
Abstract: Eleven highly functionalized diterpenes have been isolated from two Pacific marine sponges, *Acanthella* spp. Each bears two or three isocyano functions, a tertiary alcohol, a tetrahydropyranyl or tetrahydrofuranyl moiety, and occasionally chlorine or isothiocyano substituents. Two representative structures were determined by X-ray diffraction studies, and the others by spectral correlation. All kalihinols inhibit the growth of three test organisms, *Bacillus subtilis*, *Staphylococcus aureus*, and *Candida albicans*.

In two preliminary communications[3,4] we reported the structures of five isocyano diterpenoid antibiotics, kalihinols A, B, C, E, and F (Chart I), isolated from a Guam sponge, *Acanthella* sp. This paper provides detailed data for these compounds and for six additional kalihinols, the trace constituents D, G, and H from the Guam *Acanthella*, and kalihinols X, Y, and Z from a Fiji *Acanthella* sp. Common to all eleven kalihinols is a *trans*-decalin bearing a tertiary alcohol at C-4 and in all but three cases isocyano functions at C-5 and C-10. The kalihinols differ in their C_8 moiety, which is linked to C-7 of the decalin; in five compounds it is a tetrahydropyran bearing chlorine at C-14, exemplified by kalihinol A (**1**). In six kalihinols it is a tetrahydrofuranyl moiety that is functionalized at C-15 with NC, NCS, or Cl or where *gem*-dimethyl is replaced by isopropenyl. Kalihinol F (**6**) represents this series. X-ray diffraction techniques have secured the structures of A and F.[3,4,5]

Solvent extraction of the freeze-dried Guam *Acanthella* according to Kupchan's scheme[6] yielded carbon tetrachloride and chloroform extracts that inhibited the growth of *Bacillus subtilis*, *Staphylococcus aureus*, and *Candida albicans*. Purification by Sephadex LH-20, silica gel, and finally HPLC led to kalihinols A–H in a total yield of about 0.5% based on dry sponge weight. The separations were followed by TLC using a vanillin–sulfuric acid spray. IR spectra of the crude fractions revealed hydroxy and isocyano functions (3400, 2240 cm^{-1}). Lack of reactivity with acetic anhydride–pyridine indicated a tertiary hydroxy group. Broad triplets[7] in the 45–65 ppm region of ^{13}C spectra suggested multiple isocyano functions.

Kalihinol A (**1**) is the least polar of the Guam compounds. It is second in abundance (after F) and was the first to crystallize, mp 233 °C. In the ^1H NMR spectrum five methyls could be assigned unambiguously: a methyl at δ 1.40 geminal to the alcohol, a *gem*-dimethyl at δ 1.33 geminal to an ether oxygen, a broad triplet signal at δ 1.29 for a methyl on a carbon bearing isocyano,[7] and a singlet at δ 1.15 for an oxygen-linked methyl. The two low-field proton signals at δ 4.51 (br s) and a doublet of doublets at δ 3.72 were difficult to assign. Both chemical shifts are farther downfield than chemical shifts previously reported for methines functionalized by isocyano, e.g., in 2-isocyanopupukeanane, where the proton resonates at δ 3.03,[8] or at δ 3.27 as in acanthellin-1.[9]

Chart I



Only when X-ray analysis revealed the presence of chlorine, which had escaped detection in EI mass spectra, could the δ 3.72 signal be assigned to the Cl-bearing methine (H-14) and the δ 4.51 resonance to the methine bearing isocyano (H-5).

Kalihinol E (**2**) is the more polar C-14 epimer of kalihinol A and had to be separated from kalihinol F (**6**), which has nearly identical chromatographic mobility (see Experimental Section). Kalihinol E (**2**) was crystallized from hexanes/acetone, mp 197–199 °C. Its mass spectrum was virtually identical with that of A, differing from A in only one ^1H NMR resonance: the doublet of doublets at δ 3.72 (J = 5, 12 Hz) in kalihinol A and assigned to α-H-14 is replaced by a triplet at δ 3.95 (J = 3.5 Hz) in kalihinol E. Hence the two kalihinols are epimeric at C-14.[10]

The three remaining tetrahydropyranyl compounds were isolated from a Fiji *Acanthella* sp. The sponge was extracted like the Guam *Acanthella* sp. Its chloroform and carbon tetrachloride extracts inhibited the growth of the same microorganisms and stained similarly on TLC. The major and most polar constituent, kalihinol Z (**3**, 0.08% from dry sponge), crystallized from hexanes with a trace of acetone, mp 228–230 °C, and had a mass spectrum that appeared to be identical with that of kalihinol A. The two compounds differed in their NMR spectra. A Me-20 triplet in the ^1H NMR spectrum of kalihinol A at δ 1.29 is replaced by a signal at δ 1.39. The shift to lower field can be rationalized by an equatorial methyl at C-10, which is deshielded by comparison with the axial epimer. This interpretation was corroborated by INEPT experiments. Carbon chemical shifts for kalihinols A and Z were identical except for signals assigned to Me-20, which

(1) First reported at the International Chemical Congress of Pacific Basin Societies, Honolulu, HI, Dec. 16–21, 1984, Abstract ORGN 10E39.

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(5) Figure 1 in ref 3 depicts the enantiomer of kalihinol A.

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(10) In our preliminary communications[3,4] configuration at C-14 in kalihinol A and E is reversed. Structures **1** and **2** show the correct stereochemistry.

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Table I. ^{13}C NMR Chemical Shifts (δ, $CDCl_3$) of the THP Kalihinols[a]

carbon	kalihinol A	kalihinol E	kalihinol X	kalihinol Y	kalihinol Z
1	42.30 d	42.35 d	43.19 d	41.90 d	41.63 d
2	21.58 t[b]	21.72 t[b]	21.76 t[b]	24.01 t	21.35 t[b]
3	32.00 t	32.62 t	32.32 t	32.39 t	32.16 t
4	70.31 s	70.57 s	69.98 s	70.61 s	69.86 s
5	63.75[g]	63.75[g]	63.62[g]	63.95[g]	63.65[g]
6	35.90 d	36.05 d	36.38 d	37.74 d	35.99 d
7	48.42 d	48.35 d	48.55 d	49.48 d	48.51 d
8	21.87 t[b]	21.92 t[b]	22.15 t[b]	28.68 t	21.67 t[b]
9	39.70 t	39.77 t	39.26 t	35.63 t	38.33 t
10	59.73[g]	59.68[g]	75.88 s	150.91 s	60.82[g]
11	77.00 s[c]	76.17 s	77.00 s[c]	77.00 s[c]	77.00 s[c]
12	37.92 t	32.5 t	37.95 t	38.23 t	37.92 t
13	27.32 t	25.36 t	27.43 t	27.47 t	27.36 t
14	64.07 d	62.89 d	64.28 d	64.42 d	64.26 d
15	75.86 s[c]	73.93 s	76.57 s[c]	76.53 s[c]	75.78 s[c]
16	22.74 q	28.82 q[d]	22.74 q	22.72 q	22.67 q
17	30.46 s	30.27 q	30.56 q	30.57 q	30.52 q
18	19.10 q	20.48 q[f]	19.00 q	19.05 q	19.03 q
19	28.77 q	28.93 q[d]	28.65 q	28.73 q	28.53 q
20	20.66 q	20.76 q[f]	27.50 q	105.28 t	27.55 q

[a] Because of small sample size, insufficient pulsing, and the broadness of the signals, the isocyano and isothiocyano carbon resonances were usually not observed. [b-f] Interchangeable. [g] Resonance is a broad triplet ($J \sim 5$ Hz).

Table II. ^{13}C NMR Chemical Shifts (δ, $CDCl_3$) of the THF Kalihinols[a]

carbon	kalihinol B	kalihinol C	kalihinol D	kalihinol F	kalihinol G	kalihinol H
1	42.16 d	42.21 d	41.41 d	42.12 d	42.14 d	42.16 d
2	21.55 t	21.66 t	22.05 t	21.51 t	21.56 t	21.59 t
3	32.59 t	32.66 t	32.03 t	32.56 t	32.62 t	32.66 t
4	70.39 s	70.54 s	72.48 s	70.40 s	70.50 s	70.80 s
5	63.30[b]	63.20[b]	69.49 d	63.23[b]	63.22[b]	63.24[b]
6	35.93 d	36.07 d	37.75 d	35.89 d	35.99 d	36.04 d
7	46.38 d	46.33 d	46.01 d	46.28 d	46.22 d	46.30 d
8	24.14 t	24.30 t	24.17 t	24.14 t	24.15 t	24.31 t
9	39.86 t	39.95 t	39.91 t	39.79 t	39.87 t	39.90 t
10	59.83[b]	59.86[b]	59.90[b]	59.80[b]	59.81[b]	63.32 s
11	87.22 s	86.23 s	87.64 s	87.32 s	87.30 s	87.33 s
12	38.43 t	38.20 t	38.29 t	38.17 t	38.08 t	38.12 t
13	25.66 t	28.83 t	25.11 t	24.98 t	25.11 t	25.12 t
14	84.85 d	80.85 d	82.83 d	82.82 d	83.48 d	83.53 d
15	71.12 s	146.22 s	60.29[b]	59.90[b]	63.61 s	59.50[b]
16	29.94 q	109.97 t	26.31 q	25.90 q	25.50 q	25.51 q
17	26.06 q	18.24 q	24.03 q	23.85 q	24.33 q	24.23 q
18	17.79 q	18.17 q	17.92 q	17.75 q	17.74 q	17.17 q
19	28.67 q	28.70 q	29.71 q	28.65 q	28.79 q	28.83 q
20	20.70 q	20.72 q	20.78 q	20.70 q	20.74 q	20.75 q

[a] Because of small sample size, insufficient pulsing, and the broadness of the signals, the isocyano and isothiocyano carbon resonances were not generally observed. [b] Resonance is a broad triplet ($J \sim 5$ Hz).

resonate at δ 20.7 (ax) in kalihinol A (**1**) and at δ 27.6 (eq) in kalihinol Z (**3**). The two compounds, therefore, are epimers at C-10.

The remaining two kalihinols from the Fiji *Acanthella* sp. cocrystallized from hexanes with a trace of ethyl acetate as long needles but could eventually be separated by repeated HPLC runs through a Partisil-PAC column with hexanes/ethyl acetate (4:1) as the eluant. Kalihinol Y (**4a**) crystallized as long needles, mp 176–179 °C. Its molecular formula of $C_{21}H_{32}ClNO_2$ was secured by HREIMS. Unlike the other kalihinols, compound Y has a recognizable molecular ion (2.6%) at m/z 365, which loses chlorine and forms a prominent fragment, m/z 330 (43%). In its ^1H NMR spectrum the δ 1.29 triplet (Me-20) of kalihinol A (**1**) is replaced by two apparent singlets at δ 4.69 and 4.47. An IR band at 890 cm^{-1} confirmed that the Me-20 of kalihinol A has become an exo methylene in kalihinol Y, thereby making this a monoisocyano compound and providing an opportunity to degrade kalihinol Y to a decalone. Ozonolysis of kalihinol Y at −70 °C afforded kalihinone (**4b**), mp 207–210 °C. The IR spectrum showed the expected carbonyl frequency at 1720 cm^{-1} and lacked the exo methylene band at 890 cm^{-1}. The positive CD ellipticity of kalihinone measured at 282 nm and application of the octant rule[11] require an absolute configuration as shown in **1**. Since no adequate model could be found for a decalone with a large C-4 substituent similar to the C_8-trimethylpyran moiety at C-7 of kalihinone, the configurational assignment must be viewed with caution. Extrapolation of this assignment to the tetrahydrofuranyl series is not warranted (vide infra).

The least polar of the three metabolites from the Fiji sponge was kalihinol X (**5**), mp 199–200 °C. Evidence for an isothiocyano function was provided by a HREIMS peak at m/z 389.2276 (M^+ − Cl) for composition $C_{22}H_{33}N_2O_2S$, IR bands at 2150 and 2005 cm^{-1}, and a UV maximum at 251 nm (ϵ 1150). Placement of isothiocyano function at C-10 followed from examination of the ^1H NMR signals for the five methyl groups. The broad triplet at δ 1.29 assigned to Me-20 in kalihinol A has been replaced by a singlet at δ 1.38, accounting for the only ^1H NMR spectral difference of the two compounds. Kalihinol X is the C-10 isothiocyano analogue of kalihinol A.

Diagnostic characters for the five tetrahydropyranols, A, E, Z, Y, and X (**1–5**), are blue spots upon vanillin–sulfuric acid visualization on TLC, prominent peaks for fragment a at m/z 161/163 in the EIMS, and ^{13}C NMR resonances between 72 and 78 ppm which characterize α-carbons of pyrans. ^{13}C NMR data for these five compounds are summarized in Table I.

The most polar of the eight Guam *Acanthella* sp. constituents, kalihinol F (**6**), was difficult to separate from kalihinol E (**2**). Despite their structural dissimilarities the two compounds have almost identical chromatographic behavior. Once separated, kalihinol F—as all other tetrahydrofuranyl kalihinols—will stain purple-green rather than blue with vanillin–sulfuric acid. Kalihinol F served as the prototype of the tetrahydrofuranyl series. Our first clue that kalihinol F may be an unprecedented triisocyano compound was three ^{13}C NMR signals (δ 63.2, 59.9, and 59.8) in the region for carbons linked to isocyano. All three were multiplets due to coupling with ^{14}N.[7] Since the carbon signals assigned to the decalin part of kalihinol A and F were identical (Tables I and II), the third isocyano group had to be in the C_8 moiety. Further evidence came from two broad three-proton triplets (J = 1–1.5 Hz) at δ 1.39 and 1.32 assignable to methyls geminal to isocyano. ^{13}C NMR data also hinted that in kalihinol F the tetrahydropyranyl moiety (δ 77.0 and 75.9 for carbons α to pyran oxygen) was replaced by a tetrahydrofuranyl moiety (δ 87.3 and 82.8 for carbons α to furan oxygen). Eventually we succeeded in growing single crystals from acetone into which hexane was allowed to diffuse, mp 176–178 °C. The structure of kalihinol F (**6**) as determined by X-ray diffraction techniques was published.[4,12] While the conformation of the *trans*-decalin in crystalline kalihinol A (**1**) is described as two chairs,[3] kalihinol F (**6**) is described as two boats.[4] Photographs of Dreiding models (Figures 1 and 2) clearly show that kalihinol F and, by implication, the other five tetrahydrofuranyl compounds are highly strained decalins, and hence the octant rule is inapplicable for the prediction of absolute configuration of the tetrahydrofuranyls. The Dreiding models also show that the C-18 methyls have distinctly different environments in the two series, which provides the basis for their diagnostic ^1H NMR chemical shifts: δ 1.04–1.02 for the tetrahydrofuranyls vs. δ 1.23–1.13 for the tetrahydropyranyls.

Of the remaining five tetrahydrofuranyls, all of which are minor constituents, three, kalihinols B, C, and G, differ in the C_8 portion, while D and H differ by substitution at C-5 or C-10 in the decalin part of the molecule.

(11) Djerassi, C. *Optical Rotatory Dispersion*; McGraw-Hill: New York, 1960; p 178ff.

(12) Figure 1 in ref 4 represents the enantiomer of kalihinol F.

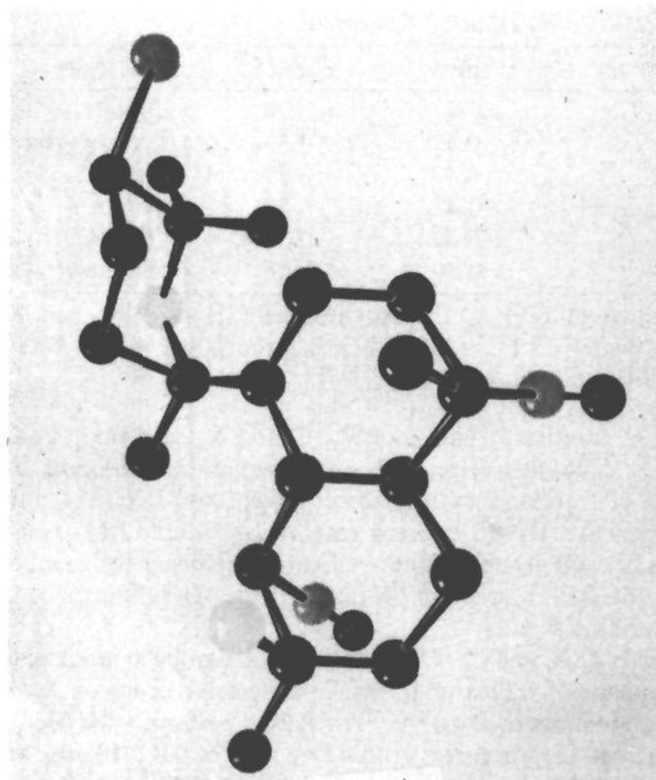


Figure 1. Dreiding model of kalihinol A.

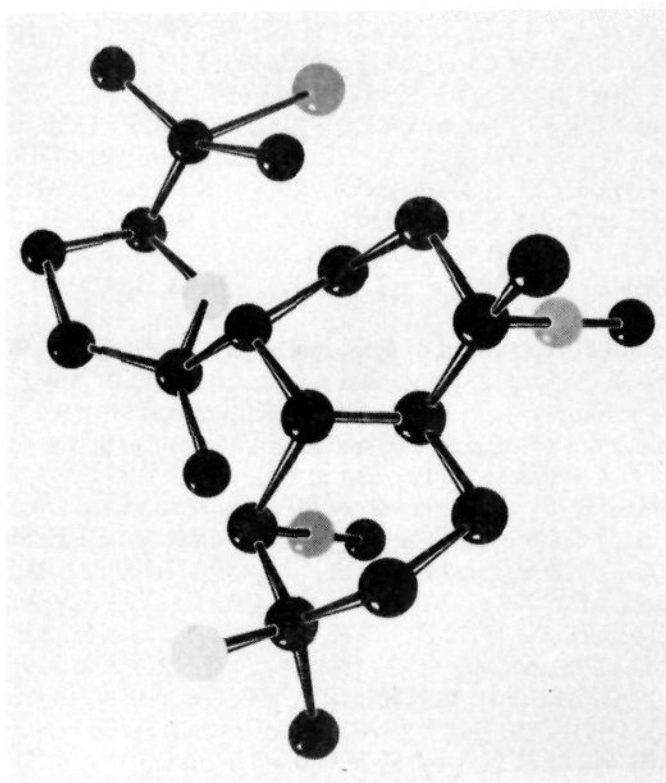


Figure 2. Dreiding model of kalihinol F.

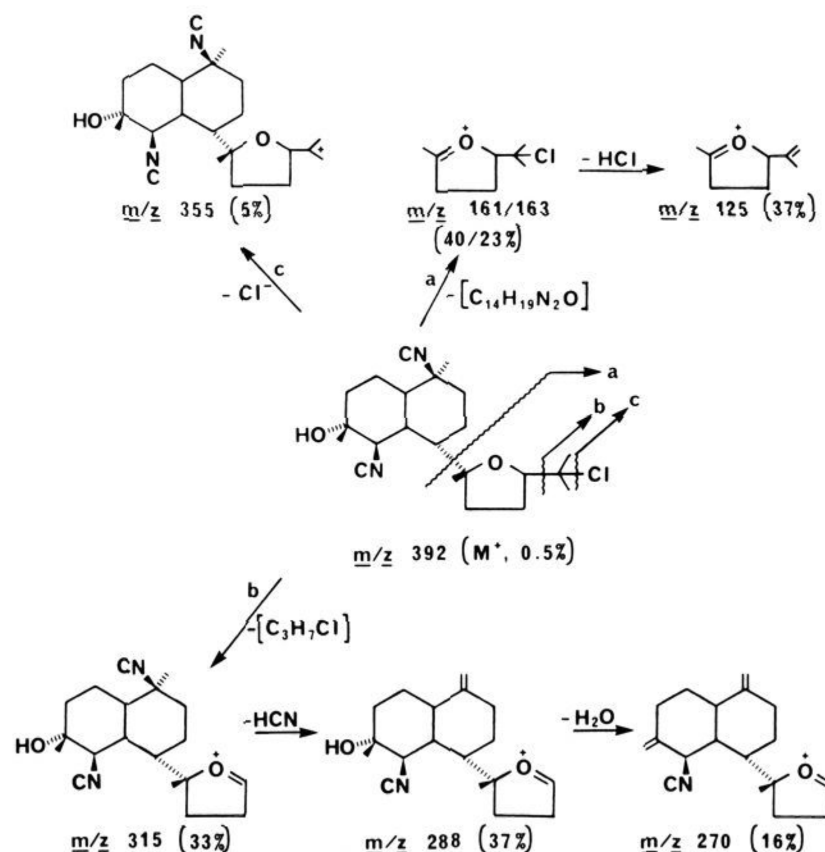
Kalihinol B (**7**) did not crystallize after its separation from A and C on reversed-phase HPLC. Placement of chlorine at C-15 followed from the downfield shift of the C-16,17 methyls (δ 1.58, 1.52), which typically resonate between δ 1.35 and 1.39, and from its MS fragmentation (Scheme I).

In the ^{1}H NMR spectrum of kalihinol C (**8**), which also was noncrystalline, two one-proton singlets at δ 5.05 and 4.78 replaced one of the *gem*-dimethyls (C-16,17) and shifted another downfield to δ 1.72. These changes coupled with the ^{13}C NMR data (Tables I and II) delineate the structure of kalihinol C (**8**).

The last three trace constituents, kalihinols D (**9**), G (**10**), and H (**11**), were separated with difficulty by two successive HPLC steps: reversed phase (methanol/water (3:1)) separated D and G from H, and normal phase (ethyl acetate/hexanes (2:3)) resolved D and G. During the repeated chromatographies it became apparent that kalihinols G (**10**) and H (**11**) had UV chromophores.

Our previous experience with kalihinol X (**5**) suggested that the isothiocyano group gave rise to the UV absorption of kalihinol G and H. Mass spectral data, molecular ions at m/z 415, which is 32 amu greater than the corresponding peak of kalihinol F (**6**), and infrared bands between 2150 and 2000 cm^{-1} reinforced this notion. Placement of the isothiocyano group in kalihinol G (**10**) at C-15 was based on NMR comparisons. The C-20 methyl signal at δ 1.32 was a triplet as one would expect from its locus geminal

Scheme I. EIMS of Kalihinol B (70 eV)



to an isocyano function. C-16,17 *gem*-dimethyls, by contrast, were sharp singlets at δ 1.37 and 1.35, as will be the case when the C-15 function is isothiocyano.

In kalihinol H (**11**), which also was not crystalline, on the other hand, the C-20 methyl gave rise to a sharp singlet at δ 1.32, while the C-16,17 *gem*-dimethyls generated a six-proton triplet, $J \sim$ 1.5 Hz. These data support assignment of a C-10 isothiocyano and a C-15 isocyano function.

The final tetrahydrofuranyl compound, and the second with a differently substituted decalin, is kalihinol D (**9**), which crystallized from heptane with a trace of acetone, mp 183–184 °C. Four of the ten kalihinols described above have varied substituents at C-10 of the decalin and all ten bear a secondary isocyano at C-5. The chemical shift of the C-5 methine varies from δ 4.51 to 4.59 in the tetrahydropyranyls and from δ 4.34 to 4.47 in the tetrahydrofuranyls. Kalihinol D (**9**) is a tetrahydrofuranyl on the basis of all diagnostic tests (TLC color stain, MS fragmentation, and ^{13}C NMR), yet H-5 resonates at δ 4.65, which is substantially downfield from the chemical shift of its congeners. The presence of chlorine in kalihinol D is revealed in the low-resolution mass spectrum by a weak parent ion at m/z 392 (0.1%) and fragments at m/z 377 (2%, loss of methyl) and m/z 357 (10%, loss of chlorine). Since the EIMS indicates that the C_8 moiety is devoid of chlorine, an observation that is supported by NMR spectral data, chlorine must be in the decalin system at C-5 as evidenced by ^{13}C NMR data (Table II).

The kalihinols, as has been the case with other isocyano terpenes, inhibited the growth of our test organisms, *Bacillus subtilis*, *Staphylococcus aureus*, and *Candida albicans*.

Experimental Section

Chromatography columns, packed with either Sephadex LH-20 or BioSil A (200–325 mesh), were interfaced with an ISCO UV monitor and fraction collector. Thin-layer chromatography involved both silica (EM silica gel 60 F_{254}) and reversed-phase (Whatman KC-18F) adsorbents. Detection of the heterocyclic isocyano compounds by TLC was accomplished by spraying a vanillin–sulfuric acid solution onto the plate. Warming the plate revealed the isocyano compounds as either blue, gray, yellow, or green spots. A Waters Associates (Millipore) HPLC system was used with Whatman Partisil-PAC, Knauer RP-18, or Knauer silica gel columns. The eluted fractions were detected by RI and UV (254 nm) monitors.

Collection. The first collection of the Guam sponge was made in Apra Harbor in May 1981 by Drs. C. Ireland and G. R. Schulte. Recollections by Drs. D. Roll and V. Paul were made at Family Beach, Apra Harbor, and Gun's Beach. The tuberculate sponge is orange-red, 7–10 cm in diameter; it turns brownish-red after lyophilization.

Two separate batches of *Acanthella* sp. were collected near Fish Patch, Vita Levu, Fiji, by Dr. D. Roll. The sponges were brownish-red.

Sponges were lyophilized prior to extraction. Voucher samples were treated with formalin and stored in EtOH/H_2O (7:3).

Bioactivity Assays. Crude extracts, fractions, or pure compounds were dissolved in an appropriate solvent and added to circular filter paper disks (Schleicher & Schuell no. 740E). After air-drying, these were tested against *Bacillus subtilis*, *Staphylococcus aureus*, *Candida albicans*, *Pseudomonas aeruginosa*, and *Escherichia coli*. Plates were examined after 24 h. Clear zones of inhibition around the disks constituted positive tests.

Extraction of Guam Sponge. The lyophilized sponge (30 g) was broken up and treated with 300 mL of MeOH in a blender for 2 min. The mash was transferred to a 1-L wide-mouth Erlenmeyer, and 200 mL of MeOH was added. After 5 h the mixture was filtered to yield a reddish-orange solution. The marc was transferred to the blender and reprocessed. After 12 h the mixture was filtered. The combined filtrates yielded 7.5 g of a gummy residue after rotary evaporation and lyophilization.

Partitioning of this residue was accomplished by successive extraction with hexanes, CCl_4, and $CHCl_3$ of an aqueous methanol solution with increasing proportions of water. The residue (7.5 g) was dissolved in 160 mL of MeOH/H_2O (9:1 (v/v)). The nearly homogeneous solution was extracted with hexanes (4 × 100 mL). The combined reddish-orange hexane fractions afforded 1.07 g of a reddish-black oil.

The aqueous phase, to which 22 mL of H_2O was added, was then extracted with CCl_4 (3 × 100 mL). The combined CCl_4 fractions yielded 0.47 g of a reddish solid.

Addition of 58 mL of water to the aqueous phase and two extractions of 100 mL each with chloroform produced after rotary evaporation 0.36 g of a yellowish brown gum.

The remaining aqueous methanolic solution was reduced to dryness in vacuo to give 3.43 g of a yellowish, hygroscopic material.

Bioassays of the hexane, CCl_4, $CHCl_3$, and MeOH fractions showed strong activity against *B. subtilis* and *S. aureus*. The CCl_4 and $CHCl_3$ fractions possessed strong activity against *C. albicans*, while the hexane and methanol fractions showed only slight activity. Retesting and TLC examination of the hexane and methanol fractions showed only traces of activity and of the active constituents.

CCl_4 Fraction. (1) Sephadex LH-20 Chromatography. A portion (100 mg) of the CCl_4 residue, triturated with 2 mL of $CHCl_3$, was filtered through glass wool and applied onto an LH-20 Sephadex column (2 × 90 cm). After 90 mL of bed volume was collected, UV-absorbing material appeared. Fractions were monitored and combined according to UV trace and TLC results: (1) 25 mL, 4.8 mg; (2) 22 mL, 25.4 mg; (3) 48 mL, 57.3 mg. Approximately 5 mg of undissolved material was retained by the glass wool. The fractions were assayed; the strongest activity was concentrated in fraction 3. TLC of this fraction revealed upon exposure by the spray reagent what appeared to be 2–4 compounds.

(2) Silica Gel Chromatography of Fraction 3. Fraction 3 (57 mg) from the previous column was dissolved in 0.5 mL of $CHCl_3$ and applied on a 1.5 × 70 cm column containing 25 g of BioSil A prepared with $CHCl_3$. On elution with $CHCl_3$ (31-mL bed volume), fractions A–C were obtained after smaller fractions were collected and combined on the basis of UV and TLC experiments. Fraction 3-A (18 mL, 9.9 mg) was chiefly pigmented material. Fraction 3-B (18 mL, 16 mg) contained compounds of high (~0.47) R_f, which were later resolved and designated kalihinols A, B, and C. Fraction 3-C (9 mL, 5.7 mg) yielded two compounds of low (~0.31) R_f, kalihinols E and F. These two groups of compounds, unresolved on normal-phase (silica gel) plates but resolvable on reversed-phase (KC 18F) plates, generated bluish spots, or violet spots when concentrated, after spraying with vanillin–H_2SO_4. The "D" fraction, R_f 0.41, was later separated into three compounds, kalihinols D (**9**), G (**10**), and H (**11**) by HPLC. Unlike kalihinols A, B, C, E, and F, "D" was UV active.

$CHCl_3$ Fraction. Chromatography. From 100 mg of the freeze-dried $CHCl_3$ residue by the above procedure, a bioactive fraction (28 mL, 61 mg) was obtained from Sephadex LH-20 after an initial 145 mL (including bed volume) of MeOH/$CHCl_3$ (1:1) was collected.

For the silica gel chromatography, $CHCl_3$/CCl_4 (3:1) provided good separation. Repeating the procedure used for the CCl_4 residue yielded 11.5 mg of the higher R_f kalihinols (A, B, and C) and 28.1 mg of the lower R_f kalihinols (E and F). The less polar fraction was mainly kalihinol A, approximately 8 mg, with traces of kalihinols B (1 mg) and C (1 mg). These and the more polar compounds, kalihinol E (5 mg), F (20 mg), and "D" (3 mg), were separated by HPLC. Final resolution of the "D" mixture into D, G, and H required repetitive HPLC runs.

HPLC Separations. The BioSil A fractions containing the kalihinols were first separated on a preparative silica column (EtOAc/hexanes (1:1)) into five groups. Fraction 1 contained compounds less polar than kalihinol A; fraction 2, kalihinols A, B, and C; fraction 3, kalihinol D; fraction 4, kalihinols G and H; and fraction 5, kalihinols E and F. Collection of fractions was monitored by RI and UV (254 nm).

Table III. TLC R_f Data of Kalihinols A–H

compd	silica[a]	RP-18[b]	color[c]
A	0.47	0.26	blue
B	0.47	0.34	yellow-green
C	0.47	0.29	green
D, G, H	0.41	0.30	violet
E	0.32	0.36	blue
F	0.30	0.48	violet-brown

[a] EM silica gel 60 F_{254}, EtOAc/hexanes (1:1). [b] Whatman KC-18F, methanol/water (3:1). [c] After heating the plate, which was sprayed with vanillin–sulfuric acid.

A preparative RP-18 column (methanol/water (3:1)) resolved the kalihinols in each group. Final purification involved rechromatography on either the RP-18 or silica column. Table III summarizes the TLC data of kalihinols A–H.

Kalihinols A, B, and C. While kalihinol A can be crystallized initially in the presence of traces of B and C, successive crops of A contained increasing amounts of B and C. An RP-18 column with MeOH/H_2O (3:1) separated the mixture. With a flow rate of 0.8 mL/min, kalihinols C, B, and A eluted in this order in a ratio of 5:5:90 (RI detector).

Kalihinol A (1): rectangular plates from hexanes, mp 233 °C, to a brown liquid; $[\alpha]_D$ +16° (*c* 1, $CHCl_3$); HREIMS, (M^+ – Cl) *m/z* 357.2561 (calcd for $C_{22}H_{33}N_2O_2$, 357.2542), (M^+ – Cl – HCN) *m/z* 330.2424 (calcd for $C_{21}H_{32}NO_2$, 330.2433); LREIMS, *m/z* 357 (4%), 330 (7), 216 (9), 202 (8), 163 (71), 162 (18), 161 (94), 125 (94), 107 (58), 105 (22), 93 (34), 81 (41), 79 (25), 71 (36), 67 (53), 59 (45), 55 (40), 53 (31), 43 (50), 41 (100); IR ($CHCl_3$) 3595 (free OH), 3390 br (assoc OH), 2135 2100 sh (NC), 1385, 1378 (*gem*-dimethyl), 1105 (C–O–C) cm^{-1}; ^1H NMR ($CDCl_3$) δ 4.51 (1 H, br s, H-5), 3.72 (1 H, dd, J = 12, 5 Hz, H-14), 1.40 (3 H, s, Me-19), 1.33 (6 H, s, Me-16 and Me-17), 1.29 (3 H, br t, J = 2, Me-20), 1.15 (3 H, s, Me-18), 2.0–0.8 (complex).

Attempted Acetylation of Kalihinol A (1). To kalihinol A (15 mg) dissolved in 0.45 mL of pyridine was added 0.25 mL of Ac_2O. After 24 h at room temperature, the brownish solution was stripped to dryness. Five milliliters of toluene was added, and the solvent was removed in vacuo. TLC indicated only starting material.

Kalihinol B (7). Kalihinol B could not be crystallized from a variety of solvents. Colorless oil, $[\alpha]_D$ +10° (*c* 1, $CHCl_3$); HREIMS, (M^+ – Me) *m/z* 377.2009 (calcd for $C_{21}H_{30}ClN_2O_2$, 377.1996); LREIMS, *m/z* 392 (0.4%, M^+), 377 (2, M^+ – Me), 357 (5, M^+ – Cl), 329 (4), 315 (33, M^+ – C_3H_6Cl), 288 (37, M^+ – C_3H_6Cl – HCN), 270 (16), 261 (12), 243 (18), 163 (23), 161 (40), 152 (15), 145 (17), 125 (37), 119 (18), 107 (27), 105 (22), 81 (34), 49 (100); IR ($CHCl_3$) ν_{max} 3600 (free OH), 3400 br (assoc OH), 2150 (NC), 1380 (*gem*-dimethyl), 1100 (C–O–C) cm^{-1}; ^1H NMR δ 4.40 (1 H, br s, H-5), 4.03 (1 H, dd, J = 9, 4 Hz), 1.58 (3 H, s, Me-16 or Me-17), 1.52 (3 H, s, Me-17 or Me-16), 1.4 (3 H, s, Me-19), 1.32 (3 H, br t, J = 1.5, Me-20), 1.02 (3 H, s, Me-18), 2.1–0.8 (complex).

Kalihinol C (8). Kalihinol C could not be crystallized. Colorless oil, $[\alpha]_D$ +6° (*c* 1, $CHCl_3$); HREIMS, (M^+ – Cl), *m/z* 356.2461 (calcd for $C_{22}H_{32}N_2O_2$, 356.2464); LREIMS, *m/z* 356 (14%, M^+), 341 (12, M^+ – Me), 329 (28, M^+ – HCN), 311 (14), 299 (10), 248 (29), 187 (25), 178 (35), 160 (51), 152 (40), 145 (49), 135 (41), 125 (100), 119 (49), 107 (58), 91 (51), 67 (54), 53 (48), 43 (70); IR ($CHCl_3$) ν_{max} 3600 (free OH), 3400 br (assoc OH), 2150 (NC), 1380 (*gem*-dimethyl), 1100 (C–O–C) cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.05 (1 H, s, H_A-16), 4.78 (1 H, s, H_B-16), 4.47 (1 H, m, H-14), 4.5 (1 H, br s, H-5), 1.72 (3 H, s, Me-17), 1.42 (3 H, s, Me-19), 1.33 (3 H, br t, J ~ 1.5, Me-20), 1.03 (3 H, s, Me-18), 2.4–0.8 (complex).

Kalihinols E (2) and F (6). Separation of kalihinol E from kalihinol F was achieved by silica gel chromatography with a $CHCl_3$/CCl_4 (3:1) solvent system. Intermediate fractions containing E and F were combined. These compounds can be resolved cleanly by using an RP-18 column with MeOH/H_2O (3:1) as the eluent.

Kalihinol E (2). Kalihinol E crystallized from hexanes/acetone: needles, mp 197–199 °C; $[\alpha]_D$ +4° (*c* 1, $CHCl_3$); HREIMS, (M^+ – Cl) *m/z* 357.2522 (calcd for $C_{22}H_{33}N_2O_2$, 357.2542); LREIMS, *m/z* 377 (2%, M^+ – Me), 357 (6, M^+ – Cl), 330 (4, M^+ – Cl – HCN), 202 (11), 164 (25), 163 (70), 162 (61), 161 (100), 125 (75), 107 (70), 105 (51), 93 (42), 91 (45), 81 (48), 79 (43), 71 (54), 67 (59), 55 (57), 43 (64), 41 (65); IR ($CHCl_3$) ν_{max} 3400 br (assoc OH), 2130 br (NC), 1380 (*gem*-dimethyl), 1100 (C–O–C) cm^{-1}; ^1H NMR ($CDCl_3$) δ 4.58 (1 H, br s, H-5), 3.95 (1 H, t, J = 3.5 Hz, H-14), 1.40 (3 H, s, Me-19), 1.34 (3 H, s, Me-16), 1.33 (3 H, s, Me-17), 1.30 (3 H, t, J = 1.2, Me-20),

Table IV. TLC R_f Data of Fiji Kalihinols

compd	silica[a]	RP-18[b]	color[c]
X	0.68	0.13	blue
Y	0.64	0.15	blue
Z	0.59	0.24	blue

[a] EM silica gel 60 F_{254}, hexanes/EtOAc (1:1). [b] Whatman KC-18F, methanol/water (3:1). [c] After heating the plate sprayed with vanillin-sulfuric acid.

1.13 (3 H, s, Me-18), 2.4–0.8 (complex).

Kalihinol F (6): mp 176–178 °C; $[\alpha]_D$ +8° (*c* 1, $CHCl_3$). The X-ray sample was obtained by dissolving in acetone and allowing hexane to diffuse. HREIMS, (M^+) *m/z* 383.2533 (calcd for $C_{23}H_{33}N_3O_2$, 383.2572); LREIMS, *m/z* 383 (0.1%, M^+) 368 (0.1), 356 (0.2), 341 (0.2), 329 (0.2), 315 (2), 288 (3), 199 (6), 152 (49), 125 (32), 85 (61), 83 (95), 43 (100); IR ($CHCl_3$) ν_{max} 3240–3540 (assoc OH), 2140 (NC), 1380 (*gem*-dimethyl), 1100 (C–O–C) cm^{-1}; ^1H NMR ($CDCl_3$) δ 4.35 (1 H, br s, H-5), 3.95 (1 H, dd, J = 8.6, 3.3 Hz, H-14, dd, distorted due to coupling with NC), 1.39 (6 H, s, Me-16, Me-17), 1.38 (3 H, s, Me-19), 1.32 (3 H, t, J = 1.5, Me-20), 1.03 (3 H, s, Me-18), 2.1–0.9 (16 H, complex).

Kalihinols D, G, and H. The trace mixture "D", which appeared to be homogeneous on both silica and ODS TLC plates, was resolved with difficulty into three compounds, D, G, and H, by HPLC by using normal [EtOAc/hexane (1:1)], reversed (MeOH/water (3:1)], and normal [EtOAc/hexanes (2:3)] phase chromatographies. Kalihinols D and G can be separated from H by a reversed-phase column. Kalihinols D and G, which are inseparable in reversed-phase chromatography under our conditions, were separated on a silica column with ethyl acetate/hexanes (2:3).

Kalihinol D (9). Kalihinol D crystallized from heptane/trace acetone: rectangular plates, mp 183–184 °C; $[\alpha]_D$ +8° (*c* 1.5, $CHCl_3$); LREIMS, *m/z* 392 (0.1%, M^+), 377 (2, M^+ – Me), 357 (10, M^+ – Cl), 330 (14, M^+ – Cl – HCN), 324 (3), 312 (8, M^+ – Cl – HCN – H_2O), 296 (3), 270 (17), 243 (26), 225 (14), 161 (14), 160 (16), 152 (100), 145 (15), 127 (22), 125 (40), 110 (23), 107 (18), 105 (20), 71 (50), 55 (26), 43 (79); IR ($CHCl_3$) ν_{max} 3600 (free OH), 3400 br (assoc OH), 2150 (C), 1380 (*gem*-dimethyl), 1100 (C–O–C) cm^{-1}; ^1H NMR ($CDCl_3$) δ 4.65 (1 H, s, H-5), 3.89 (1 H, dd, $J \sim$ 11, 4 Hz, distorted by coupling with C-15 NC), 1.37 (6 H, br t, $J \sim$ 1.5 Hz, Me-16, Me-1m), 1.36 (3 H, Me-19), 1.31 (3 H, br t, $J \sim$ 1.5 Hz, Me-20), 1.04 (3 H, s, Me-18), 2.1–1.1 (complex). Anal. Calcd for $C_{22}H_{33}ClN_2O_2$: C, 67.24; H, 8.46; N, 7.12; Cl, 9.02. Found: C, 67.24; H, 8.54; N, 7.08; Cl, 8.9.

Kalihinol G (10). Kalihinol G could not be crystallized. Colorless oil; $[\alpha]_D$ –12° (*c* 1, $CHCl_3$); HREIMS, (M^+) *m/z* 415.2267 (calcd for $C_{23}H_{33}N_3O_2S$, 415.2294); LREIMS, *m/z* 415 (3%, M^+), 388 (3, M^+ – HCN), 361 (1, M^+ – HNCS), 315 (15, M^+ – C_4H_6NS), 288 (40, M^+ – C_4H_6NS – HCN), 270 (14), 243 (15), 184 (31), 159 (26), 152 (24), 125 (65), 110 (17), 107 (24), 105 (23), 91 (20), 71 (36), 55 (30), 43 (100); IR ($CHCl_3$) ν_{max} 3600 (free OH), 3400 br (assoc OH), 2150 and 2125 br (NC, NCS), 1380 (*gem*-dimethyl), 1100 (C-O-C) cm^{-1}; UV λ_{max} (heptane) 247 nm (ϵ 1126); ^1H NMR ($CDCl_3$) δ 4.34 (1 H, s, H-5), 3.94 (1 H, dd, J = 9, 4 Hz), 1.43 (3 H, s, Me-19), 1.37 (3 H, s, Me-16), 1.35 (3 H, s, Me-17), 1.32 (3 H, t, J = 1.8 Hz, Me-20), 1.02 (3 H, s, Me-18), 2.2–0.8 (complex).

Kalihinol H (11). Kalihinol H could not be crystallized. Colorless oil; $[\alpha]_D$ +98° (*c* 1, $CHCl_3$); HREIMS, (M^+) *m/z* 415.2390 (calcd for $C_{23}H_{33}N_3O_2S$, 415.2294); LREIMS, *m/z* 415 (1%, M^+), 389 (2, M^+ – HCN), 357 (4), 330 (7), 329 (8), 288 (13), 161 (24), 152 (40), 125 (53), 107 (13), 71 (18), 43 (54), 28 (100); IR ($CHCl_3$) ν_{max} 3600 (free OH), 3400 br (assoc OH), 2150 and 2125 br (NC, NCS), 1380 (*gem*-dimethyl), 1100 (C–O–C) cm^{-1}; UV λ_{max} (heptane) 248 (ϵ 692); ^1H NMR ($CDCl_3$) δ 4.35 (1 H, s, H-5), 3.94 (1 H, dd, J = 9, 4 Hz, distorted by coupling with C-15 NC), 1.41 (3 H, s, Me-19), 1.39 (3 H, s, Me-19), 1.39 (6 H, t, $J \sim$ 1.5 Hz), 1.32 (3 H, s, Me-20), 1.03 (3 H, s, Me-18), 2.2–0.8 (complex).

Extraction of Fiji Sponge. Preliminary TLC examination of two sponge specimens indicated that one elaborated the characteristic bluish spots upon exposure to vanillin–sulfuric acid spray reagent while the other did not. Extraction of the isocyanide-containing sponge was the same as for the Guam *Acanthella* sp. Lyophilized sponge (30 g) was extracted as was the Guam sponge and yielded these residues: hexane, 0.74; CCl_4, 0.60; $CHCl_3$, 0.22; MeOH, 5.11 g.

Column Chromatographies of the CCl_4 and $CHCl_3$ Residues. These residues were processed as before. For example, the lyophilized $CHCl_3$ residue (100 mg), triturated with 3 mL of $CHCl_3$/MeOH (1:1), was filtered through Pyrex wool and applied onto the Sephadex column. After 81 mL of eluent had passed, fractions were collected and combined according to UV and TLC analyses. Combined fraction 1 (61 mL, 39 mg) contained mainly pigments. Fraction 2 (11 mL, 19 mg) was nearly homogeneous. The isolated compound was named kalihinol Z. Fraction 3 (43 mL, 37 mg) contained kalihinols Z (**3**), X (**5**), and Y (**4a**) in a ratio 4:1:1.

Separation by HPLC involved repeated injections onto the Partisil-PAC column (hexanes/EtOAc (4:1) as eluent, flow rate 0.5 mL/min).

The residue was purified as above. The concentration of kalihinols X and Y was greater in this than in the $CHCl_3$ residue. Table IV summarizes the TLC data of the Fiji *Acanthella* sp.

Kalihinol X (5): long needles from hexane, mp 199–200 °C; $[\alpha]_D$ – 22° (*c* 1.76, $CHCl_3$); HREIMS, (M^+ – Cl) *m/z* 389.2276 (calcd for $C_{22}H_{33}N_2O_2S$, 389.2263); LREIMS, *m/z* 424 (1.3%, M^+), 406 (7.7, M^+ – H_2O), 389 (26, M^+–Cl), 366 (8, M^+ – NCS), 331 (8), 228 (26), 216 (14), 201 (17), 163 (76), 161 (100), 145 (28), 125 (82), 107 (75), 107 (38), 93 (33), 91 (34), 81 (45), 71 (44), 59 (38), 43 (71); UV (hexanes) 251 nm (ϵ 1150); IR ($CHCl_3$) ν_{max} 3630 (free OH), 3470 br (assoc OH), 2150 and 2005 (NC, NCS), 1380 (*gem*-dimethyl), 1135 (C–O–C) cm^{-1}; ^1H NMR ($CDCl_3$) δ 4.56 (1 H, br s, H-5), 3.72 (1 H, dd, J = 17, 5 Hz, H-14), 1.382 (3 H, s, Me-19 or Me-20), 1.378 (3 H, s, Me-20 or Me-19), 1.34 (3 H, s, Me-16 or Me-17), 1.32 (3 H, s, Me-17 or Me-16), 1.23 (3 H, s, Me-18), 2.2–0.8 (complex).

Kalihinol Y (4a): long needles from hexanes/trace EtOAc, mp 176–179 °C; $[\alpha]_D$ –34° (*c* 1, $CHCl_3$); HREIMS, (M^+) *m/z* 365.2143 (calcd for $C_{21}H_{32}ClNO_2$, 365.2121); LREIMS, *m/z* 365 (2.6%, M^+), 330 (43, M^+ – Cl), 245 (10), 163 (78), 162 (52), 161 (100), 145 (21), 125 (79), 107 (66), 103 (20), 93 (32), 91 (45), 81 (37), 71 (42), 67 (48), 59 (32), 43 (76); IR ($CHCl_3$) ν_{max} 3615 (free OH), 3450 br (assoc OH), 2140 (NC), 1380 (*gem*-dimethyl), 1120, 1010 (C–O–C), 890 (–C=CH_2) cm^{-1}; ^1H NMR ($CDCl_3$) δ 4.69, 4.47 (1 H, br s, H_2C-20), 4.59 (1 H, br s, H-5), 3.74 (1 H, dd, J = 18, 5 Hz, H-14), 1.40 (3 H, s, Me-19), 1.34 (3 H, s, Me-16 or Me-17), 1.33 (3 H, s, Me-17 or Me-16), 1.13 (3 H, s, Me-18), 2.4–0.8 (complex).

Ozonolysis of Kalihinol Y (4b). Ozone from a microozonizer was bubbled through a solution of kalihinol Y (23 mg) in 5 mL of EtOAc at –70 °C for 5 min. The effluent was monitored with starch–iodide. After the reaction mixture was allowed to warm to room temperature, an additional 5 mL of EtOAc was added, and the ozonide was decomposed with 1% aqueous KI. Successive washes of the organic layer with 10% $Na_2S_2O_3$ and brine solutions, followed by drying (Na_2SO_4), filtration, and rotary evaporation, resulted in 23 mg of a colorless film.

HPLC separation (Partisil-PAC, hexanes/EtOAc (3:2), flow rate 0.7 mL/min) afforded 0.7 mg of starting material and 9.1 mg of ketone: ν_{max} 3620 (free OH), 3550 br (assoc OH), 2140 (NC), 1720 (C=O), 1385, 1370 sh (*gem*-dimethyl), 1125 (C–O–C) cm^{-1}. The 890-cm^{-1} band of the starting material was absent. Amorphous ketone from hexanes/trace acetone: mp 207–210 °C; CD at $[\Theta]_{282}$ +5053 (MeOH).

Kalihinol Z (3): rectangular plates from hexanes/trace acetone, mp 228–230 °C (brown melt); $[\alpha]_D$ –10° (*c* 1, $CHCl_3$); HREIMS, M^+ – Cl, *m/z* 357.2529 (calcd for $C_{22}H_{33}N_2O_2$, 357.2542); LREIMS, *m/z* 358 (3%), 330 (3), 216 (9), 202 (8), 163 (50), 162 (25), 161 (100), 125 (93), 107 (60), 86 (23), 81 (29), 71 (28), 67 (38), 43 (49), 41 (47); IR ($CHCl_3$) ν_{max} 3615 (free OH), 3450 br (assoc OH), 2140 (NC), 1385, 1370 (*gem*-dimethyl), 1130 (C–O–C) cm^{-1}; ^1H NMR ($CDCl_3$) δ 4.56 (1 H, br s, H-5), 3.72 (1 H, dd, J = 12, 1.5 Hz, H-14), 1.39 (3 H, t, $J \sim$ 1.5, Me-20), 1.38 (3 H, s, Me-19), 1.34 (3 H, d, Me-16 or Me-17), 1.32 (3 H, s, Me-17 or Me-16), 1.22 (3 H, s, Me-18), 2.2–1.1 (complex).

Acknowledgment. We thank Drs. Valerie Paul and Deborah Roll for *Acanthella* collections, and the National Science Foundation and the University of Hawaii Sea Grant College Program under Institutional Grant NA81AA-D-00070 from NOAA, Office of Sea Grant, U.S. Department of Commerce, for financial support. C.W.J.C. is grateful for an NSF Research Opportunity Award during Summer 1986.