New Diterpene Isonitriles from the Sponge *Phakellia pulcherrima*

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Seven new diterpene isonitriles and isothiocyanates were isolated from the sponge *Phakellia pulcherrima* along with eight known ones. Six of the new compounds, **9**−**14**, and the eight known ones, **1**−**8**, belong to the kalihinol family of diterpenes. Structures were determined from spectroscopic data. This is the first report of diterpenes from sponges of this genus. All of the other kalihinol diterpenoids have been isolated from the sponge *Acanthella cavernosa* collected in diverse locations.

Since the discovery of the highly functionalized diisocyanate diterpenoid kalihinol A (**1**) from the sponge *Acanthella* sp. (*cavernosa*) by Scheuer et al.,[1] 34 similar kalihinols with a broad spectrum of biological activities, including antibiotic,[1,2] anthelmintic,[3] cytotoxic,[4] and antifouling[5] activity, have been identified. In general, these diterpenes can be divided into three structurally different groups: those with a *trans*-decalin and a tetrahydropyranyl group, ones with a *trans*-decalin and a tetrahydrofuranyl group, and those with a *cis*-decalin and a tetrahydrofuranyl group. These are represented, respectively, by kalihinol A (**1**),[1,2] kalihinol B (**2**),[1,2] and kalihinene (**3**).[4]

The kalihinol family of compounds has a biflorane carbon skeleton. Outside the kalihinol family the biflorane skeleton appears rarely in marine metabolites, two examples being the isocyanate-substituted bifloradienes isolated from the sponge *Cymbastela hooperi*[6] and a sponge of the family *Adocidae*.[7] The proposed biosynthesis of the kalihinols/kalihinenes envisages cyclization of geranylgeraniol to the cis and trans forms of the biflorane skeleton followed by further oxidative cyclizations and addition of the isocyanate group.[8,9]

Recently, we undertook a chemical investigation of the sponge *Phakellia pulcherrima* Ridley and Dendy 1886 (family Axinellidae), collected in the Philippines, because its extracts were toxic to brine shrimp. This has led to the isolation of six new kalihinols, **9**−**14**, a new diisocyanatobiflorin, **15**, and eight known members of the kalihinol family: kalihinols A (**1**), B (**2**), C (**4**), X (**5**), Y (**6**), Z (**7**), kalihenene (**3**), and 1-*epi*-kalihinene (**8**).[10] Sponges of the genus *Phakellia* have been reported to yield peptides,[11] acetylenic acids,[12] sterols,[13] and alkaloids.[14] This is the first report of diterpenoids from a sponge of this genus.

Results and Discussion

Methanol and methanol−methylene chloride extracts of *P. pulcherrima* were subjected to solvent partitioning (see Experimental Section), and the methylene chloride solubles were fractionated further by Si gel chromatographies. Selected fractions from the Si gel columns were processed by reversed-phase HPLC using different solvent combinations to give **1**−**7** in pure form, while 1-*epi*-kalihinene (**8**) was identified in a 3:1 mixture of **8** and **3**. Compounds **1**−**8** were identified by comparison of their spectroscopic data (MS, ^{1}H and ^{13}C NMR, IR) with literature values, which were limited in some cases.[1,2,4,10] Complete ^{1}H and ^{13}C NMR assignments were made for kalihinols B (**2**), C (**4**), and Y (**6**) using data from HMQC and HMBC experiments, and these data, in combination with reported values, facilitated the elucidation of the structures of the new kalihinols.

Particularly helpful in assigning structures in this series of compounds are several diagnostic spectroscopic features established by previous work.[15] Thus, the presence of isonitrile groups is revealed not only by very sharp IR absorption at ca. 2135 cm^{-1}, but also by coupling of the nitrogen of the isonitrile with adjacent carbons and geminal and vicinal protons. Tetrahydrofuranyl groups are indicated by lower field ^{13}C NMR shifts for their ether carbons (δ 82−87) compared to the corresponding shifts in the tetrahydropyranyl analogues (δ 75−77). The relative stereochemical relationship of H-1, H-6, H-5, and H-7, as found in kalihinol A (**1**) and other kalihinols having a *trans*-decalin skeleton, can often be confirmed from the coupling pattern of H-6 (one small and three large *J*s). In cases where the H-6 signal is not resolved, the coupling patterns of H-5, H-7, and H-1 can provide the stereochemical information.

The molecular formula of **9**, $C_{21}H_{32}ClN_2O$ by HRFABMS, was identical with that of kalihinol Y (**6**), and ^{1}H and ^{13}C NMR data for **9**, which were assigned with the aid of COSY, HMQC, and HMBC experiments (see Tables 1 and 2, respectively), showed extensive correspondence to those of kalihinol Y (**6**). The ^{13}C NMR shifts of C-11 and C-15 (δ 77.1 and 75.7) confirmed the presence of the tetrahydropyranyl ring, and the broad ^{13}C NMR signal at δ 63.6 was consistent with the ^{14}N coupling due to an isonitrile function attached to C-5. The principal difference in the NMR spectra of **9** and kalihinol Y (**6**) was the presence of signals for a methyl-substituted double bond in the spectrum of **9** (δ 1.61, s, H-20; δ 5.30, br d, H-9) instead of the exocyclic double bond as observed for kalihinol Y (**6**). The location of the double bond at C-9,10 was confirmed by COSY and HMBC experiments. The *trans*-decalin arrangement was supported by the coupling patterns of H-1$_{ax}$ [δ 2.16 (br t, J = 9.5 Hz)] and H-7$_{ax}$ [δ 1.75 (ddd, J = 11.2, 11.2, 6.0 Hz)] and the similarity of the ^{13}C data of **9** and kalihinol Y (**6**). The relative stereochemistry shown for **9** was further supported by the observation of NOEs between the C-4 methyl group and H-5, and the C-11 methyl group and H-13$_{ax}$, the latter being identified by its large coupling with H-14. Compound **9** was thus confirmed as Δ^9-kalihinol Y.

Kahilinols K (**10**) ($C_{22}H_{32}N_2O_2$) and L (**11**) ($C_{22}H_{32}N_2O_2S$) differed in formula only by the presence of sulfur (**11**), but the compounds exhibited nearly identical ^{1}H and ^{13}C NMR spectral data (see Tables 1 and 2). These data resembled

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Table 1. ^{1}H NMR Data for **2**, **9**−**15**

H#	**2**[a] ($CDCl_3$)	**9**[a] ($CDCl_3$)	**10** (C_6D_6)	**11** (C_6D_6)	**12**[a] (C_6D_6)	**13** (C_6D_6)	**14**[a] (C_6D_6)	**15**[c] ($CDCl_3$)
1	1.79	2.16 br t 9.5	1.52 m	1.50 m	1.64 td 11.7, 2.8	1.62 td 11, 4.1		
5	4.40 br s 6[d]	4.30 br s 6[d]	4.28 br s 7[d]	4.32 s 4[d]	4.16 br s 7[d]	4.33 br s 6[d]	4.43 br s 6[d]	3.58 br s 6[d]
6	2.00 m	1.99 m	2.08 br t 11	1.90 m	1.73 td 11.7, 3.3	2.21 tq 11, 3	1.82 tq 11.8, 3.6	1.75 m
7	1.74 m	1.75 td 11.2, 6	1.83 m	1.71 ddd 13, 11, 4	1.55 m	1.20 m		1.56 m
8	1.64 dt 13, 3.7 1.17 qd 13, 3	1.91 m 2.00 m	1.43 dq 13.1, 2.8 0.90 qd 12.2, 3.8	1.41 dq 13, 4 0.87 qd 13, 3.4	0.98 m 0.51 qd 13.3, 3.3	1.36 dt 13.4, 3.5 0.80 qd 13.4, 3.5	0.59 qd 12.7, 3.8	1.56 m 1.14 qd 12, 2.9
14	4.04 dd 9.3, 4.4	5.30 br d 4.4	3.64 t 6.6	3.60 t 6.9	3.52 dd 8.9, 4.5	3.52 dd 8.1, 4.7	4.29 dd 8.8, 5.1	5.10 br t 6.0 (1H)
16	1.51 s	1.35 s	1.24 br s	1.20 br s	1.09 s	1.21 br s	5.26 br s 4.87 br s	1.68 br s
17	1.57 s	1.36 s	1.21 br s	1.20 br s	1.11 s	1.12 br s	1.75 br s	1.59 br s
18	1.02 s	1.18 s	0.68 s	0.67 s	0.61 s	0.87 s	0.75 s	0.73 d 6.8
19	1.40 s	1.41 s	1.14 s	1.03 s	1.07 s	1.09 s	0.91*	1.44 s
20	1.31 s	1.61 br s (3H)	4.77 d 2.0 4.66 s	4.76 s 4.64 s	0.87 s	0.84 s	0.88 s*	1.32 br s

[a] Assignments aided by HMQC data. [b] Assigned by comparison with **4**.[2b] [c] Assigned by comparison with **4**[2b] and 10-isothiocyanatobiflora-4,14-diene.[6] [d] $W_{1/2}$ in Hz. *Asterisked values may be interchanged.

Table 2. ^{13}C NMR Data of **2**, **9**−**15**

	2[a] ($CDCl_3$)	**9**[b] ($CDCl_3$)	**10**[c] (C_6C_6)	**11**[c] (C_6C_6)	**12**[b] ($CDCl_3$)	**13**[b] ($CDCl_3$)	**14**[d] ($CDCl_3$)	**15**[e] ($CDCl_3$)
1	42.2 d	37.1 d	42.1 d	43.8 d	42.6 d	43.0 d	42.8	42.6
2	21.5 t	25.5 t	24.3 t[g]	24.3 t[g]	21.9 t	21.7 t	22.0	21.0
3	32.6 t	33.9 t	32.8 t	33.2 t	32.6 t	32.4 t	32.7	32.6
4	70.4 s[h]	71.0 s	70.1 s	71.0 s	70.5 s	70.0 s	70.6	70.4
5	63.3 br d	63.6 br d	63.2 br d	65.7 d	63.2 br d	63.2 br d	63.3 br d	60.1 br d[h]
6	35.9 d	38.7 d	38.0 d	38.6 d	36.4 d	36.4 d	36.5	35.7
7	46.4 d	45.4 d	47.6 d	48.0 d	46.3 d	46.5 d	46.4	40.5
8	24.1 t	26.8 t	31.3 t	31.3 t	24.6 t	24.5 t	24.8	20.8
9	39.9 t	120.4 t	38.3 t	38.2 t	40.0 t	39.4 t	40.1	40.2
10	59.8 br s	134.6 s	151.3 s	151.3 s	64.61 s[h]	64.3 s	63.9	60.6 br[h]
11	87.2 s	77.1 s	87.7 s	87.6 s	87.3 s	87.7 s	86.2	30.3
12	38.4 t	37.6 t	36.0 t	36.1 t	38.0 t	38.2 t	38.2	35.4
13	25.6 t	27.3 t	25.0 [g]	24.9 t [g]	25.1 t	25.1 t	28.8	26.0
14	84.8 d	64.7 d	83.0 d	82.9 d	83.4 d	82.8 d	80.8	124.1
15	71.1 s[h]	75.8 s	60.0 br s	59.7 br s	64.58 s[h]	60.2 br s	146.3	131.8
16	26.0 q	22.9 q	23.3 q	23.6 q	24.3 q	24.0 q	18.25[h]	17.7
17	29.9 q	30.8 q	26.1 q	26.0 q	25.5 q	26.1 q	110.0	25.7
18	17.8 q	17.9 q	17.5 q	17.5 q	17.7 q	17.8 q	18.16[h]	13.2
19	28.7 q	28.7 q	28.7 q	28.7 q	28.8 q	28.5 q	28.7	28.7
20	20.7 q	20.7 q	105.7 t	105.6 t	20.8 q	27.5 q	20.9	19.3
NC	153.3, 153.0	156.4	160.4, 157.9	158.0	158.1	157.8[f]	*f*	*f*
NCS				130.5	130.7	132.2	*f*	

[a] Assignments aided by HMQC and HMBC experiments. [b] Assignments aided by HMQC experiments. [c] Multiplicities assigned by DEPT experiment. [d] Assignments by comparison with kalihinol C.[2b] [e] Assignments by comparison with 10-Isothiocyanatobiflora-4,14-diene.[6] [f] Not observed. [g,h] Exchangeable.

closely those of kalihinol B (**2**, furanyl ring) and also kalihinol Y (**6**, exocyclic double bond). Hence, structures **10** and **11** were indicated as likely structures for kalihinols K and L. The IR spectrum of **11** exhibited overlapping absorptions expected for isocyanate (2135 cm^{-1}, sharp) and isothiocyanate (2115 cm^{-1}, br) groups, while **10** showed only an intense, sharp peak at 2137 cm^{-1} for the isocyanate functionality. The location of these functional groups was assigned from ^{1}H and ^{13}C NMR data. The downfield proton signal at δ 4.31 assigned to H-5 was significantly broader in the spectrum of **10** than in **11** ($W_{1/2}$ ca. 7 vs. 4 Hz) indicating an isonitrile substituent at C-5 in **10** and an isothiocyanate group in **11**. Likewise, the weak carbon resonance assigned to C-5 in **10** (δ 63.2) was broad due to ^{14}N coupling, while the corresponding signal for C-5 of **11** was sharp. An isonitrile group was located at C-15 in **10** and **11** based on the ^{13}C NMR chemical shift and peak shape (^{14}N coupled) of C-15, cf. 1-*epi*-kalihinene (**8**) data.

The ^{1}H NMR signal of H-6 in the spectrum of **10** was resolved in C_6D_6 (δ 2.08), and its coupling pattern (br t, J = 11 Hz) confirmed the *trans*-decalin structure with axial and equatorial substituents, respectively, at C-5 and C-7. The same relative stereochemistry was established for **11** from the appearance of the H-6 ^{1}H NMR signal in pyridine-d_5 (δ 2.34, td, J = 11.2, 2.6 Hz). The pyridine-induced shift[16] of the H-6 signal ($\Delta\ \delta$ 0.4) further supported the configuration assigned at C-4 by comparison of the ^{1}H and ^{13}C NMR shifts of **10** and **11** with kalihinol B (**2**). Consistent with structures **10** and **11**, the following NOEs were observed: H-18 with H-14, H-5, and H-6; H-19 with H-5; H-5 with H-6. Of all the kalihinols reported, only two others have an isothiocyanate group at C-5: kalihinol I[3b] and 10β-formamide-5β-isothiocyanatokalihinol A.[5b]

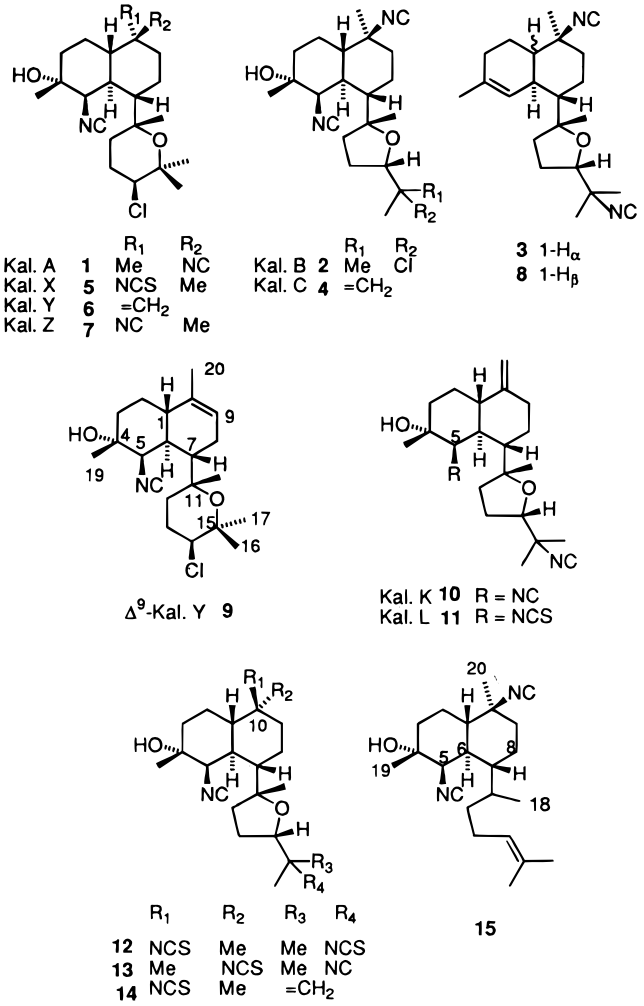
The ^{1}H and ^{13}C NMR data for **12**, $C_{23}H_{33}N_3O_2S_2$ by HRFABMS, matched closely those reported for kalihinol G, as well as our own data for kalihinol B (**2**).[2] Thus, the same *trans*-decalin skeleton with an isonitrile at C-5, a tertiary hydroxyl at C-4, and a tetrahydrofuranyl group was inferred. The H-6 signal multiplicity, crucial in confirming the *trans*-decalin and C-5 and C-7 stereochemistry, was evident in C_6D_6 (δ 1.73, dt, 3.3, 11.7 Hz). The only significant difference in the ^{13}C NMR spectra of **12** and kalihinol G, was in the resonance for C-10: a sharp

signal at δ 63.61 for **12** vs. a broad triplet at δ 59.81 for kalihinol G. This is consistent with the presence of an isothiocyanate group at C-10 in **12**. The methyl group at C-10 was assigned the α (axial) configuration based on its ^{13}C NMR shift, δ 20.8, which is the same as that in kalihinol G. NOESY correlations ($CDCl_3$) were observed between H-19 and H-5, as well as between H-18 and H-14. Compound **12** was thus confirmed to be 10-isothiocyanatokalihinol G.

10-*epi*-kalihinol H (**13**) has the same molecular formula, $C_{23}H_{33}N_3O_2S$, as kalihinol H.[2b] The limited number of ^1H NMR signals reported for kalihinol H matched the corresponding signals for **13**. The ^{13}C NMR signals for the two compounds were virtually identical except for C-20, which occurred at δ 27.5 in the spectrum of **13** vs. δ 20.75 for the kalihinol H spectrum. This indicates that the methyl at C-10 is β (equatorial) in **13**, and hence, this metabolite is 10-*epi*-kalihinol H. In the proton spectrum of **13** measured in C_6D_6 the signals for the geminal dimethyls at C-15 are resolved, and both are broad singlets indicative of ^{14}N coupling with the isonitrile group. The coupling constants of H-6 (dt, 3, 11.1) confirmed the *trans*-decalin and C-5,C-7 stereochemistry, and NOESY correlations between H-14 and H-18 supported the tetrahydrofuran ring stereochemistry.

The ^1H and ^{13}C NMR data for compound **14**, $C_{22}H_{32}N_2O_2S$ by HRFABMS, match closely the reported values for kalihinol C (**4**).[2] The only significant difference in the ^{13}C NMR spectra of the two compounds was in the C-10 resonance: a sharp signal at δ 63.9 in **14** and a broad singlet at δ 59.9 in kalihinol C (**4**). This corresponds to the presence of an isothiocyanate group at C-10 in **14**. The stereochemistry at C-10 in **14** and kalihinol C (**4**) must be the same because the ^{13}C NMR chemical shift of C-20 is the same in both compounds. The following NOESY correlations were observed: H-6_{ax} (α), H-18, and H-14; and H-19 with H-5. Compound **14** can thus be designated as 10-isothiocyanatokalihinol C.

A trace amount (0.3 mg) of pulcherrimol (**15**) was obtained. The highest mass ion observed in the HRFABMS corresponded to $(M - HCN)^+$, and in combination with ^1H and ^{13}C NMR data the formula $C_{22}H_{32}N_2O$ was deduced. IR data provided evidence for the presence of hydroxyl and isonitrile groups. The ^{13}C NMR spectrum possessed broad peaks at δ 60.1 and 60.2 characteristic of isonitrile-bearing carbons, but lacked the downfield signals indicative of the ether rings found in the kalihinols. The ^1H NMR spectrum showed one sharp methyl singlet signal at δ 1.44 and a broad methyl singlet at δ 1.32, just as found in kalihinol C (**4**), with the broadening of the latter signal being due to ^{14}N coupling of an isonitrile substituent. The ^1H NMR spectrum also showed signals indicative of an isopropenyl group [δ 5.10 (1 H), 1.53 (3 H), 1.68 (3H)] and a secondary methyl group (δ 0.73, d, 6.8 Hz). Comparison of the ^{13}C NMR data for the entire decalin portion of kalihinol C (**4**) with the shift data for **15** revealed an excellent fit between the two and strongly suggested equivalence in overall structure and relative stereochemistry for this portion of these two compounds. The remaining signals in the ^{13}C NMR spectrum of **15** matched almost exactly those in the side chain of 10-isothiocyanatobiflora-4,14-diene.[6] ^1H NMR shift data and $^1H-^1H$ COSY data also support this structure. NOEs between H-20 and H-6/H-8_{ax} (δ 1.14, dq, 2.9, 12 Hz) were observed in accordance with expectations for structure **15**. Other NOEs observed were H-5_{eq} with H-19, H-6, and the C-11 methyl. The three large couplings in the H-8 signal reveal that H-7 must be axial, and thus the C-7 side chain must be equatorial. Pulcherrimol is thus assigned structure **15** with the relative stereochemistry at C-11 undetermined.



The new kalihinols **1**−**6** and pulcherrimol (**7**) constitute additions to a well-known family of diterpenes. To our knowledge this is the first report of kalihinols from a sponge other than *Acanthella cavernosa*. *P. pulcherrima*, however, is in the same family as *A. cavernosa*.

Experimental Section

General Experimental Procedures. All solvents were redistilled. Chromatography was performed with Merck Si gel 60 (230−240 mesh) for vacuum flash chromatography. Reversed-phase HPLC was conducted using a RI detector and a Phenomenex C_{18} column (250 × 10 mm). IR spectra were obtained on a Bio-Rad 3240-SPC FT instrument. MS were measured with either a Hewlett−Packard 5985B or a VG ZAB E mass spectrometer. NMR experiments were conducted with a Varian VXR-500 instrument equipped with a 3-mm $^1H/^{13}C$ switchable gradient microprobe (MDG-500-3) and a pulsed-field gradient driver; signals are reported in parts per million (ppm).

Animal Material. The sponge *Phakellia pulcherrima* Ridley and Dendy 1886 (family Axinellidae) was collected in April 1996, at Davao, Philippines, and frozen shortly after collection. A voucher specimen is maintained at the University of Oklahoma (24-PH-96).

Extraction and Isolation. Freshly thawed specimens of the sponge (1.02 kg wet wt, 154 g dry wt after extraction) were minced and soaked in MeOH (2 × 1 L) followed by MeOH−CH_2Cl_2 (1:1, 2 × 1 L). All extracts were combined, the solvents

removed in vacuo, and the residue subjected to solvent partitioning involving MeOH with varying amounts of H_2O vs. organic solvents as described previously[16] to afford hexane- (1.6 g), CH_2Cl_2- (2.2 g), and *n*-BuOH- (2.1 g) soluble fractions. The CH_2Cl_2 solubles were fractionated by vacuum flash chromatography over Si gel (SiO_2) using increasing amounts of MeOH in $CHCl_3$ as eluent (5% MeOH to 100% MeOH) to yield fractions A−F. Further rechromatography of fraction A by flash chromatography over SiO_2 [increasing amounts of EtOAc in hexane as eluent (10% EtOAc to 100%)] afforded 7 fractions, A1−A7. Reversed-phase HPLC (12% H_2O−MeOH) of fraction A2 afforded kalihinene (**3**)/1-*epi*-kalihinene (**8**) (1:3 mixture) (11.7 mg), kalihinol X (**5**) (5.7 mg), kalihinol Y (**6**) (5.5 mg), and a mixture (2.2 mg) of **9** (80%) and **6** (20%), which was further purified by reversed-phase HPLC using 20% H_2O−MeOH to afford the pure Δ^9-kalihinol Y (**9**) (0.5 mg). Fraction A4 (48.7 mg) was fractionated by reversed-phase HPLC (20% H_2O−MeOH) to give three fractions: A4a−A4c. Fraction A4a (10.4 mg) contained a 1:1 mixture of **10** and **13**; fraction A4b consisted of kalihinol Z (**7**) (0.5 mg); and fraction A4c (4.3 mg) contained compounds **14**, **11**, and **15**. Reversed-phase HPLC of fraction A4a using 30% H_2O−MeOH as eluent resulted in a clear separation of **10** (2.0 mg) and **13** (1.8 mg). Rechromatography of fraction A4c on a reversed-phase column using 17% H_2O in MeOH effected the separation of **15** (0.3 mg), **14** (0.7 mg), and **11** (1.5 mg). The known kalihinols C (**4**, 5.0 mg), B (**2**, 3.1 mg), and A (**1**, 22.2 mg) and **12** (6.5 mg) were purified by reversed-phase HPLC using 23% H_2O−MeOH from fraction E.

Kalihinol A (1): $[\alpha]^{20}_D$ +12° (*c* 0.9, $CHCl_3$) [lit.[1,2b] $[\alpha]^{20}_D$ +16° (*c* 1.0, $CHCl_3$)]; ^1H and ^{13}C NMR ($CDCl_3$) data same as literature.[1]

Kalihinol B (2): $[\alpha]^{20}_D$ +5° (*c* 0.25, $CHCl_3$) [lit.[2b] $[\alpha]^{20}_D$ +10° (*c* 1.0, $CHCl_3$)]; ^1H and ^{13}C NMR ($CDCl_3$), see Tables 1 and 3 and literature data.[2b]

Kalihinol C (4): $[\alpha]^{20}_D$ +11° (*c* 0.47, $CHCl_3$) [lit.[2b] $[\alpha]^{20}_D$ + 6 (*c* = 1.0, $CHCl_3$)]; ^1H and ^{13}C NMR data, see literature data.[2b]

Kalihinol X (5): $[\alpha]^{20}_D$ −15° (*c* 0.3, $CHCl_3$) [lit.[2b] $[\alpha]^{20}_D$ −22° (*c* 1.76, $CHCl_3$)]; ^1H and ^{13}C NMR, same as literature data.[2b]

Kalihinol Y (6): $[\alpha]^{20}_D$ −20° (*c* 0.31, $CHCl_3$) [lit.[2b] $[\alpha]^{20}_D$ −34° (*c* 1.0, $CHCl_3$)]; ^1H and ^{13}C NMR, same as literature data.[2b]

Kalihinol Z (7): $[\alpha]^{20}_D$ −7° (*c* 0.17, $CHCl_3$) [lit.[2b] $[\alpha]^{20}_D$ −10° (*c* 1.0, $CHCl_3$)]; ^1H and ^{13}C NMR, same as literature.[2b]

Δ9-Kalihinol Y (9): $[\alpha]^{20}_D$ +9° (*c* 0.05, $CHCl_3$); IR (film) ν_{max} 3411 (br, OH), 2141 (s, NC), 1381 (s, *gem*-dimethyl) cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 2; HRFABMS *m/z* 366.2208 $[M + H]^+$ (calcd for $C_{21}H_{33}NO_2{}^{35}Cl$, 366.2200).

Kalihinol K (10): $[\alpha]^{20}_D$ −9° (*c* 0.7, $CHCl_3$); IR (film) ν_{max} 3420 (br, OH), 2137 (s, NC) cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 2; ^1H NMR ($CDCl_3$) δ 4.71 (d, J = 2.0 Hz, H-20), 4.61 (s, H-20′), 4.33 (br s, H-5), 3.94 (dd, J = 9.1, 4.0 Hz, H-14), 2.32 (ddd, J = 13.1, 2 × 3.0 Hz, H-9_{eq}), 2.07 (br dd, J = 2 × 13.1 Hz, H-9_{ax}), 2.04 (br dd, J = 14.1, 9.1 Hz, H-13), 1.90 (m, H-6_{ax}), 1.89 (m, H-7), 1.85 (ddm, J = 14.1, 4.0, H-13), 1.80 (dddd, J = 12.9, 3 × 4.0 Hz, H-8_{eq}), 1.73, 1.62 (m, H-2, 2′), 1.6−1.8 (m, H-12), 1.41 (s, H-15, -16), 1.40 (s, H-19), 1.16 (dddd, J = 3 × 12.9, 4.0 Hz), 1.00 (s, H-18); HRFABMS *m/z* 357.2542 $[M + H]^+$ (calcd for $C_{22}H_{33}N_2O_2$, 357.2571).

Kalihinol L (11): $[\alpha]^{20}_D$ −5° (*c* 0.19, $CHCl_3$); IR (film) ν_{max} 3440 (br, OH), 2135 (s, NC), 2115 (br, NCS) cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 2; ($CDCl_3$) δ 4.71 (s, H-20), 4.61 (s, H-20′), 4.39 (s, H-5), 3.93 (dd, J = 9.5, 4.7 Hz, H-14), 2.32 (ddd, J = 13.3, 2 × 3.8, H-9_{eq}), 2.07 (dm, J = 13.3 Hz, H-9_{ax}), 2.04 (m, H-13), 1.98 (m, H-6), 1.86 (m, H-13), 1.81 (m, H-7, H-8_{eq}), 1.76 (m, H-2_{eq}), 1.64 (m, H-2_{eq}), 1.40 (s, H-16,17), 1.35 (s, H-19), 1.15 (dddd, J = 3 × 13.3, 3.8 Hz), 1.01 (s, H-18); FABMS *m/z* 389 $[M + H]^+$, HRFABMS *m/z* 362.2172 $[M - HCN]^+$ (calcd for $C_{21}H_{32}NO_2S$, 362.2154).

10-Isothiocyanatokalihinol G (12): $[\alpha]^{20}_D$ −26° (*c* 0.38, $CHCl_3$); IR (film) ν_{max} 3422 (br, OH), 2136 (s, NC), 2091 (br, NCS) cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 2; ^1H NMR ($CDCl_3$) δ 4.33 [br s, 6 ($W_{1/2}$), H-5], 3.93 (dd, J = 9.5, 4.2 Hz, H-14), 2.01 (m, H-6), 1.78 (m, H-7), 1.76 (m, H-1), 1.42 (s, H-19), 1.36 (s, H-17), 1.34 (s, H-16), 1.31 (s, H-20), 1.02 (s, H-18); HRFABMS *m/z* 448.2073 $[M + H]^+$ (calcd for $C_{23}H_{34}N_3O_2S_2$, 448.2092).

10-*epi*-kalihinol H (13): $[\alpha]^{20}_D$ −30° (*c* 0.18, $CHCl_3$); IR (film) ν_{max} 3420 (br, OH), 2137 (s, NC), 2100 (br, NCS) cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 2; ^1H NMR ($CDCl_3$) δ 4.39 [(br s, 6 ($W_{1/2}$), H-5], 3.93 (dd, J = 9.1, 3 Hz, H-14), 2.19 (tq, J = 11.1, 1.3 Hz, H-6), 1.74 (m, H-7), 1.39 (s, H-16, -17, -19, -20) 1.10 (s, H-18); HRFABMS *m/z* 416.2348 $[M + H]^+$ (calcd for $C_{23}H_{34}N_3O_2S$, 416.2372).

10-Isothiocyanatokalihinol C (14): $[\alpha]^{20}_D$ −11° (*c* 0.06, $CHCl_3$); IR (film) ν_{max} 3425 (br, OH), 2139 (s, NC), 2078 (br, NCS) cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 2; ^1H NMR ($CDCl_3$) δ 5.01 (br s, H-16), 4.75 (br s, H-16′), 4.47 [br s, 6 ($W_{1/2}$), H-5], 4.39 (dd, J = 8.9, 3.8 Hz, H-14), 2.03 (m, H-6), 1.71 (br s, H-17), 1.40 (s, H-19), 1.31 (s, H-20), 1.03 (s, H-18); HR-FABMS *m/z* 389.2277 $[M + H]^+$, (calcd for $C_{22}H_{32}N_2O_2S_2$, 389.2263).

Pulcherrimol (15): $[\alpha]^{20}_D$ +18° (*c* 0.03, $CHCl_3$); IR (film) ν_{max} 3421 (br, OH), 2132 (s, NC) cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 2; HRFABMS *m/z* $[M^+]$ not observed, 316.2654 $[M - HCN]^+$ (calcd for $C_{21}H_{34}NO$, 316.2640).

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References and Notes

(1) Chang, C. W. J.; Patra, A.; Roll, D. M.; Scheuer, P. J. *J. Am. Chem. Soc.* **1984**, *106*, 4644−4646.
(2) (a) Patra, A.; Chang, C. W. J.; Scheuer, P. J.; Van Duyne, D. G.; Matsumoto, G. K.; Clardy, J. *J. Am. Chem. Soc.* **1984**, *106*, 7981−7983. (b) Chang, C. W. J.; Patra, A.; Baker, J. A.; Scheuer, P. J. *J. Am. Chem. Soc.* **1987**, *109*, 6119−6123.
(3) Omar, S.; Albert, C.; Fanni, T.; Crews, P. *J. Org. Chem.* **1988**, *53*, 5971−5972. (b) Alvi, K. A.; Tenenbaum, L.; Crews, P. *J. Nat. Prod.* **1991**, *54*, 71−78.
(4) Fusetani, N.; Yasumura, K.; Kawai, H.; Natori, T.; Binnen, L.; Clardy, J. *Tetrahedron Lett.* **1990**, *31*, 3599−3602.
(5) (a) Okino, T.; Yoshimura, E.; Hirota, H.; Fusetani, N. *Tetrahedron Lett.* **1995**, *36*, 8637−8640. (b) Hirota, H.; Tomono, Y.; Fusetani, N. *Tetrahedron* **1996**, *52*, 2359−2368. (c) Okino, T.; Yoshimura, E.; Hirota, H.; Fusetani, N. *J. Nat. Prod.* **1996**, *59*, 1081−1083.
(6) König, G. M.; Wright, A. D.; Angerhofer, C. K. *J. Org. Chem.* **1996**, *61*, 3259−3267.
(7) Sharma, H. A.; Tanaka, J.; Higa, T.; Lithgo, A.; Bernardinelli, G.; Jefford, C. W. *Tetrahedron Lett.* **1992**, *33*, 1593−1596.
(8) Rodriguez, J.; Nieto, R. M.; Hunter, L. M.; Diaz, M. C.; Crews, P. *Tetrahedron* **1994**, *50*, 11079−11090.
(9) Chang, C. W. J.; Scheuer, P. J. *Comp. Biochem. Physiol.* **1990**, *97B*, 227−233.
(10) Trimurtulu, G.; Faulkner, D. J. *J. Nat. Prod.* **1994**, *57*, 501−506.
(11) Pettit, G. R.; Cichacz, Z.; Barkoczy, J.; Dorsaz, A. C.; Herald, D. L.; Williams, M. D.; Doubek, D. L.; Schmidt, J. M.; Tackett, L. P.; Brune, D. C.; Cerny, R. L.; Hopper, J. N. A.; Bakus, G. J. *J. Nat. Prod.* **1993**, *56*, 260−267.
(12) Burke, S. D.; Junge, K. W.; Phillips, J. R.; Perri, R. E. *Tetrahedron Lett.* **1994**, 703−706.
(13) Malik, S.; Djerassi, C. *Steroids* **1989**, *53*, 271−284.
(14) Higa, T.; Tanaka, J.; Kitamura, A.; Koyama, T.; Takahashi, M.; Uchia, T. *Pure Appl. Chem.* **1994**, *66*, 2227−2230.
(15) Chang, C. W. J.; Scheuer, P. J. In *Topics in Current Chemistry*, Scheuer, P. J., Ed. Springer: Berlin, 1993; Vol. 167, pp 33−75.
(16) Demarco, P. V.; Farkas, E.; Doddrell, D.; Mylari, B. L.; Wenkert, E. *J. Am. Chem. Soc.* **1968**, *90*, 5480−5486.

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