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# New Isocyano and Isothiocyanato Terpene Metabolites from the Tropical Marine Sponge *Acanthella cavernosa*

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**Abstract**—Spectroscopic data are reported for 10-isothiocyanato-4-cadinene (**4**), for 8-hydroxy-isokalihinol F (**8**) and for two isocyanobifloradiene epoxides, (**9**) and (**10**), isolated from Great Barrier Reef specimens of *Acanthella cavernosa*. 

## Introduction

Marine sponges (Porifera) have been a focal point for structural investigations by natural products chemists because of the range of unique, bioactive structures isolated from this phylum.[1] Examples of this distinctive sponge chemistry include terpenes functionalised by isocyanide, isothiocyanate, isocyanate, thiocyanate and dichloroimine substituents,[2,3] which possess potent antibacterial, cytotoxic, antimalarial or antifouling activity.[4] The observation, first made in our research group, that inorganic cyanide is incorporated into isocyanide and isothiocyanate metabolites in marine sponges[5] is extraordinary since cyanide is poisonous to many forms of life.[6] In our more recent biosynthetic work, we have confirmed that thiocyanate is also implicated in the biosynthesis of these two functional groups in marine sponges,[5c,d] and that thiocyanate and dichlorimine substituents also have this unusual biosynthetic origin.[7]

The sponge genus *Acanthella* (order Halichondrida, family Axinellidae) is characterised by the presence of terpene isocyanides, isothiocyanates and related compounds and has been the subject of numerous chemical and taxonomic studies,[8,9] in addition to biosynthetic investigations.[5c,d] We now report the structures of four new terpene metabolites from *Acanthella cavernosa*, which are pertinent to our ongoing biosynthetic studies.

## Results and Discussion

### Sponge chemistry

Sponge samples were collected at depths of −15 m at various dive sites at Heron Island on the Great Barrier Reef. The specimens were extracted into DCM/MeOH 1:1, then the combined organic extracts were concentrated in vacuo and subjected to silica flash chromatography using a step gradient of hexane/EtOAc (from 5–100% EtOAc). Individual fractions were purified by NPHPLC using ethyl acetate in hexane. Sponge sample 29-9-96-1-1 was extracted to give axisonitrile-3 (**1**) and axisothiocyanate-3 (**2**)[10] as the major metabolites together with the isothiocyanate (**3**)[8i] and a new sesquiterpene 10-isothiocyanato-4-cadinene (**4**). The sponge sample 29-9-96-1-5 was extracted to give the diterpene isokalihinol F (**5**)[9d,f] as the major metabolite together with 1-*epi*-kalihinene (**6**),[9g,h,l] kalihipyran (**7**),[9g] the new diterpenes 8-hydroxy-isokalihinol F (**8**) and the isocyanobifloradiene epoxides (**9**) and (**10**).

### Sesquiterpenoids

The known sesquiterpenes (**1**)–(**3**) were identified by comparison of their spectroscopic data with the literature.[5d,8i,10] The new sesquiterpene had a molecular formula of $C_{16}H_{25}NS$ by HREIMS. Interpretation of DQFCOSY, geHSQC and geHMBC data (Table 1) established the planar structure as that of (**4**) rather than the isomeric (**11**);[8g] in particular COSY connectivities from H-6 through to H-9 were inconsistent with (**11**). The isothiocyanato group was positioned at C-10 since this carbon had a chemical shift of 64.7 ppm; however, the small amount of material available precluded detection of the isothiocyanato carbon, even by $^{13}C$ using a 13 s pulse delay or by geHMBC. The relative stereochemistry of (**4**), suggested by the singlet nature of H-5,[9g,h] was confirmed by comparison with data for 10-isothiocyanato-4-amorphene (**12**)[8g,11] and 10-isocyano-4-cadinene (**13**).[12] The H-1–H-6 coupling is 4 Hz in amorphene (**12**) and 10.8 Hz in cadinene (**13**). Hence the *trans* ring junction in (**4**) was evident from the 10.6 Hz coupling between H-1 and H-6. The 13.0 Hz coupling

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**Table 1.** $^{13}C$ NMR data and long-range $^{13}C$–$^{1}H$ correlations for 10-isothiocyanato-4-cadinene (**4**)

| # | $\delta$ $^{13}C$[a] | $\delta$ $^{1}H$[b] | COSY | HMBC[c,d] |
|---|---|---|---|---|
| 1 | 48.7 (d) | 1.48 (ddd, 12.3, 10.6, 1.9) | 2ax, 2eq, 6 | 10 |
| 2 | 24.1 (t) | eq 1.94 (dddd, 12.3, 6.0, 1.9, 1.9) | 1, 2ax, 3 | 4 |
| | | ax 1.32 (dddd, 12.3, 12.3, 12.3, 5.8) | 1, 2eq, 3 | 3 |
| 3 | 30.6 (t) | 2.02, 1.97 (2H, m) | 2ax, 2eq | – |
| 4 | 135.2 (s) | – | – | – |
| 5 | 121.3 (d) | 5.44 (br s) | 3, 15 | 3, 15 |
| 6 | 38.4 (d) | 1.72 (m, 10.6, 10.6, 1.5) | 1, 7 | – |
| 7 | 46.2 (d) | 1.04 (dddd, 13.0, 10.6, 3.2, 3.0) | 6, 8ax, 8eq, 11 | – |
| 8 | 20.6 (t) | eq 1.58 (m, 13.0, 4.3, 3.0, 3.0) | 7, 8ax, 9ax, 9eq | – |
| | | ax 1.12 (dddd, 13.5, 13.0, 13.0, 3.6) | 7, 8eq, 9ax, 9eq | – |
| 9 | 40.7 (t) | eq 2.01 (ddd, 13.0, 3.6, 3.0) | 9ax, 8ax, 8eq | – |
| | | ax 1.76 (ddd, 13.5, 13.0, 4.3) | 8ax, 8eq, 9eq | 10 |
| 10 | 64.7 (s) | – | – | – |
| 11 | 25.9 (d) | 2.13 (m, 7.0, 7.0, 3.2) | 12, 13 | – |
| 12 | 15.0 (q) | 0.73 (3H, d, 7.0) | 11 | 7, 11, 13 |
| 13 | 21.4 (q) | 0.89 (3H, d, 7.0) | 11 | 7, 11, 12 |
| 14 | 20.0 (q) | 1.27 (3H, s) | – | 1, 9, 10 |
| 15 | 23.7 (q) | 1.65 (3H, s) | 3, 5 | 3, 4, 5 |
| 16 | [e] | – | – | – |

[a] Inverse detection at 500 MHz (geHSQC); solution in $CDCl_3$; $^{13}C$=77.0 ppm.
[b] 500 MHz; solution in $CDCl_3$ referenced at $^{1}H=\delta$ 7.25; 1H unless stated.
[c] Inverse detection at 500 MHz; correlations observed when $^{1}J$ $^{13}C$–$^{1}H$=135 Hz and long range $^{n}J$ $^{13}C$–$^{1}H$=8 Hz.
[d] From $^{1}H$ to $^{13}C$.
[e] Not observed.

between H-7 and H-8 established that the isopropyl group of (**4**) was equatorial, while the $^{13}C$ NMR shift for C-14 of 20.0 ppm indicated this methyl group was axially orientated.[8g] NOESY correlations from H-1 to H-7 and from H-6 to H-14 confirmed the proposed relative stereochemistry; thus (**4**) has ($1R^*$, $6R^*$, $7S^*$, $10R^*$) stereochemistry. Literature data suggest that the sign and magnitude of optical rotation in a terpene is insensitive to replacement of –NC by –NCS;[10,13] since isothiocyanate (**4**) has the same sign and magnitude of optical rotation as (**13**), we propose it has the same absolute stereochemistry (Fig. 1).

## Diterpenoid metabolites

Isokalihinol F (**5**), 1-*epi*-kalihinene (**6**) and kalihipyran (**7**) were identified by comparison of their spectroscopic data with the literature.[9d,g,h] The new diterpene (**8**) had a molecular formula of $C_{23}H_{33}N_3O_3$ by HREIMS and was fully investigated by 2D NMR including DQFCOSY, geHSQC and geHMBC (summarised in Table 2 and in Experimental). There were five deshielded methyl singlets, four of which appear as broadened singlets due to coupling with the isocyano nitrogen. Three singlets at 151.7, 153.3 and 154.9 ppm, together with triplets ($J$=5 Hz) at 61.9, 59.9 and 60.1 ppm, were consistent with the structure containing three isocyanide groups, as in isokalihinol F. The secondary hydroxyl function at C-5 (76.6 ppm, $\delta$ 3.61 (dd)), coupled to the adjacent OH at $\delta$ 6.34, was equatorially disposed given the 9.5 Hz coupling from H-5 to H-6. Extensive signal overlap prevented measurement of the H-1–H-6 coupling constant; however, the *trans* ring junction was suggested by the different sets of NOESY correlations shown by H-1 and H-6, respectively. Thus H-1 showed nOe's to H-7 and

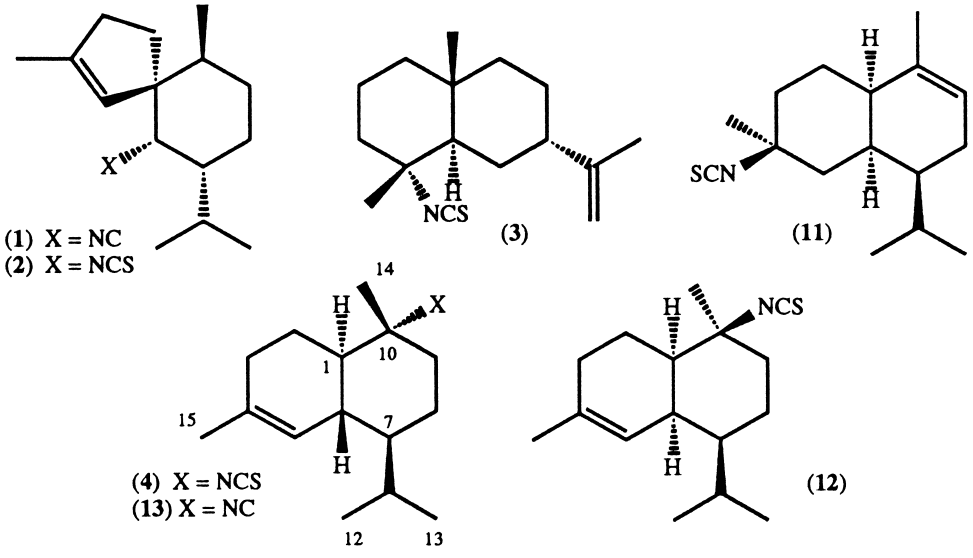



**Figure 1.** Selected sesquiterpenes from *Acanthella cavernosa*.

**Table 2.** $^{1}H$ and $^{13}C$ NMR data for the diterpenes (**8**)–(**10**)

| # | δ $^{13}C^{a}$ (**8**) | δ $^{1}H^{b}$ (**8**) | δ $^{13}C^{a}$ (**9**) | δ $^{1}H^{b}$ (**9**) | δ $^{13}C^{a}$ (**10**) | δ $^{1}H^{b}$ (**10**) |
|---|---|---|---|---|---|---|
| 1 | 48.5 (d) | 1.57 | 47.7 (d) | 1.53 | 47.7 (d) | 1.48 |
| 2 | 21.4 (t) | α 1.36 | 23.5 (t) | α 1.38 | 23.4 (t) | α 1.30 |
| | | β 1.99 | | β 2.08 | | β 2.05 |
| 3 | 37.1 (t) | α 2.12 β 1.90 | 30.6 (t) | 2.05 (2H) | 30.7 (d) | 2.03 |
| 4 | 61.9 (s) | – | 135.1 (s) | – | 135.8 (s) | – |
| 5 | 76.6 (d) | 3.61 (dd 9.5 3.5) | 121.5 (d) | 5.64 (bs) | 120.9 (d) | 5.34 |
| 6 | 37.7 (d) | 1.90 | 37.7 (d) | 2.00 | 38.0 (d) | 1.87 |
| 7 | 56.4 (d) | 1.61 | 45.0 (d) | 1.24 (ddd 13 13 3) | 49.5 (d) | 0.92 |
| 8 | 68.0 (d) | 4.22 (t 2.5) | 25.1 (t) | α 1.29 | 23.8 (t) | α 1.46 |
| | | | | β 1.57 | | β 1.81 |
| 9 | 48.0 (t) | α 2.20 | 40.4 (t) | α 2.07 | 40.2 (t) | α 2.09 |
| | | β 2.04 | | β 1.57 | | β 1.82 |
| 10 | 59.9 (s) | – | 60.2 (s) | – | 59.5 (s) | – |
| 11 | 88.5 (s) | – | 60.1 (s) | – | 59.5 (s) | – |
| 12 | 38.0 (t) | 1.88 (2H) | 62.2 (d) | 2.61 (t 6.5) | 29.3 (t) | 1.66 |
| 13 | 25.3 (t) | α 2.03 β 2.21 | 27.0 (t) | 2.22 (2H) | 23.6 (t) | 1.88 |
| 14 | 82.1 (d) | 3.85 | 119.2 (d) | 5.17 (dqq 7.5 1.5 1.5) | 123.7 (d) | 5.04 |
| 15 | 60.1 (s) | – | 134.3 (s) | – | 132.2 (s) | – |
| 16 | 25.9 (q) | 1.35 (3H bs) | 25.7 (q) | 1.71 (3H bs) | 25.7 (q) | 1.67 (3H bs) |
| 17 | 25.7 (q) | 1.46 (3H bs) | 18.0 (q) | 1.61 (3H bs) | 17.7 (q) | 1.58 (3H bs) |
| 18 | 21.2 (q) | 1.45 (3H s) | 18.4 (q) | 1.22 (3H s) | 53.3 (t) | 2.87 (d 4.5) 2.54 (d 4.5) |
| 19 | 19.5 (q) | 1.44 (3H bs) | 23.5 (q) | 1.68 (3H bs) | 23.6 (q) | 1.65 (3H bs) |
| 20 | 24.1 (q) | 1.52 (3H bs) | 20.0 (q) | 1.33 (3H bs) | 20.1 (q) | 1.32 (3H bs) |
| N*C* | 154.9 (s) | – | 152.6 (s) | – | 152.5 (q) | – |
| N*C* | 153.3 (s) | – | | | | |
| N*C* | 151.7 (s) | – | | | | |
| 5-O*H* | | 6.34 | | | | |
| 8-O*H* | | 1.70 | | | | |

[a] Inverse detection at 500 MHz (geHSQC); solution in $CDCl_3$; $^{13}C$=77.0 ppm.
[b] 500 MHz; solution in $CDCl_3$ referenced at $^{1}H$=δ 7.25.

H-9β, while H-6 showed an nOe to H-20, which was nOe correlated to H-9α rather than to H-9β. The $^{13}C$ value for the C-19 methyl group of 19.5 ppm suggested this group was axial rather than equatorial;[12] although the C-20 methyl was also axial, the $^{13}C$ shift value of 24.1 ppm for this carbon was to lower field than expected, suggesting nearby structural modification. Other small differences in the NMR data for C-6, C-7 and C-9 when compared with that of (**5**) suggested a second secondary hydroxyl function (68.0 ppm, δ 4.22 (t); OH at δ 1.70) was positioned at C-8. The stereochemistry shown at C-8 was deduced from the 2.5 Hz coupling of H-8 to the adjacent protons and confirmed by NOESY correlations from H-8 to H-7 and H-9β. The remaining α and β protons were assigned from

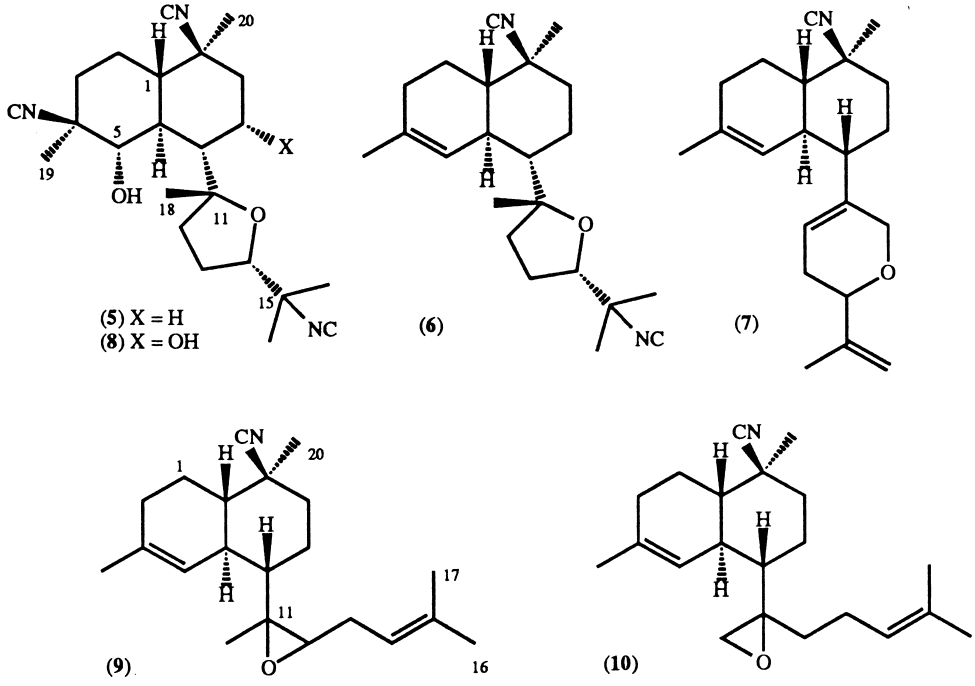
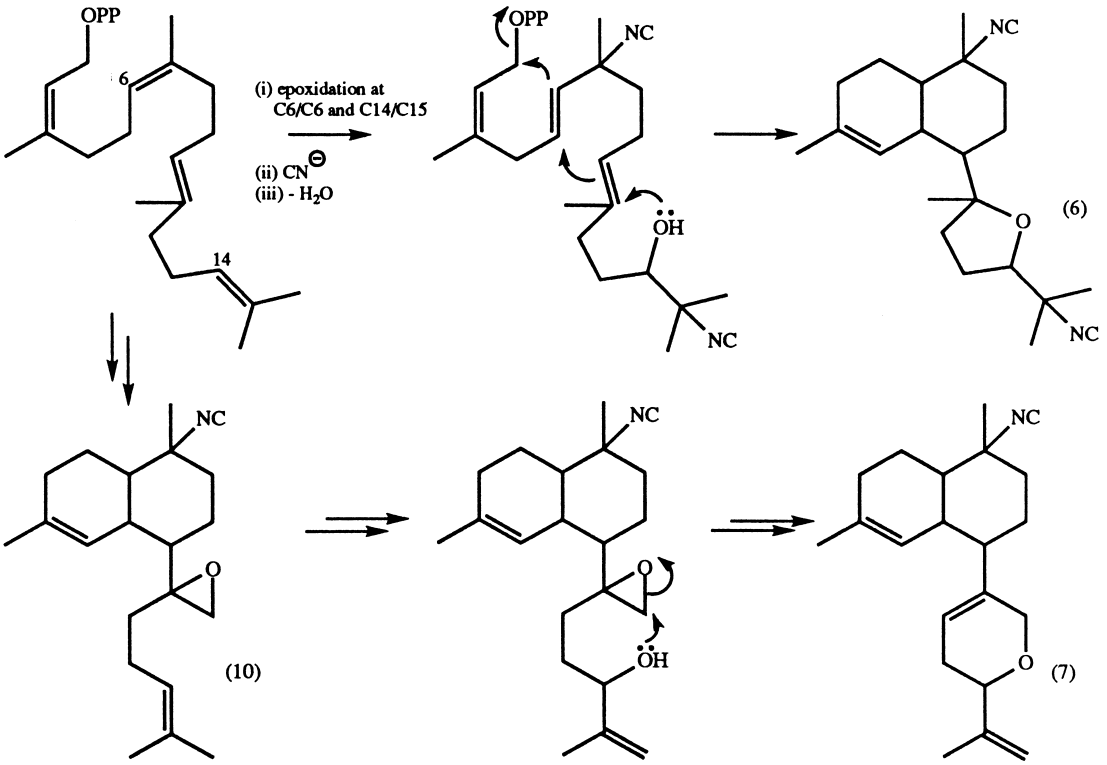



**Figure 2.** Selected kalihinol diterpenes from *Acanthella cavernosa*.




**Scheme 1.** Biosynthetic schemes to selected diterpene metabolites of *Acanthella cavernosa*.

coupling constant values or NOESY data. The stereochemistry at C-11, and of the tetrahydrofuranyl ring, was confirmed by NOESY correlations from the C-11 methyl group to both H-7 and H-14.[9g] From its $[\alpha]_D$ value, 8-hydroxy isokalihinol F has the same absolute stereochemistry as isokalihinol F.

Complete 2D NMR data sets were also obtained for the epoxides (**9**) and (**10**). The epoxide (**9**) had a molecular formula of $C_{21}H_{31}NO$ established by mass measurement of a 2M+H ion and possessed a trisubstituted double bond (135.1, 121.5 ppm; $\delta$ 5.64 bs) and an isopropylidene group (134.3, 119.2 ppm; $\delta$ 1.71 (3H, s), 1.61 (3H, s) and 5.17 (1H, t). There was a single isocyano substituent (broad triplet at 60.2 ppm) while the 11,12 epoxy group was suggested by a one proton triplet at $\delta$ 2.61 (H-12) linked to an oxygenated carbon at 62.2 ppm[3a] and by key HMBC correlations from H-18 to C-12 and from H-7 to C-18. The bicyclic ring system was apparent from the DQFCOSY and geHMBC data (summarised in Experimental) and from comparison of $^1$H and $^{13}$C NMR with that of 1-*epi*-kalihinene,[9g,h] notably the bs at $\delta$ 5.64, characteristic of *trans* fused $\Delta^4$-cadinenes[9h] and the triplet nature of H-6 ($J$=11 Hz).[9g,l] Thus (**9**) was an 11,12-epoxy-10-isocyano-4,14-bifloradiene.[14,15] The relative stereochemistry shown was confirmed by nOe's between H-6 and H-20 and between H-1 and H-7. Assignment of the *E* and *Z* methyls attached to C-15 at 25.7 and 18.0 ppm, respectively,[16] was confirmed by an nOe between H-14 and H-16 ($\delta$ 1.71; correlated to 25.7 ppm). The second epoxide (**10**), whose molecular weight was deduced as $C_{21}H_{31}NO$ by mass measurement of a 2M+Na ion, contained similar spectroscopic features to (**9**), including an isocyano group (broad triplet at 59.5 ppm), but showed only four methyl groups. Mutually-coupled protons at $\delta$ 2.54 and 2.87, linked to a carbon at 53.3 ppm and HMBC-correlated to C-7 (49.5 ppm) and C-11 (also at 59.5 ppm), were assigned to a terminal epoxide group. The analysis of DQFCOSY and geHMBC data (Table 2) were entirely in accordance with the proposed structure, while the stereochemistry shown was confirmed by NOESY data. It was not possible to deduce the relative stereochemistry at C-11. Thus (**10**) is an 11,18-epoxy-10-isocyano-4,14-bifloradiene (Fig. 2).[14,15]

The biosynthetic origin[9f,17] of the furanyl and pyranyl kalihinol diterpenes may involve epoxidation at the terminal double bond of a bifloradiene precursor, followed by nucleophilic attack at either end of the epoxide by cyanide ion to give a hydroxyisocyanide, which could then initiate cyclisation to form the bicyclic system (Scheme 1). Epoxides (**9**) and (**10**) represent alternative epoxidation products, but epoxide (**10**) can be envisaged as a precursor to the kalihipyran ring system.

## Experimental

### Isolation of metabolites

Sponge samples were collected using SCUBA at −15 m at Heron Island. Voucher samples of *Acanthella cavernosa* (G303455) were identified by Mr John Kennedy and are held at The Queensland Museum, Brisbane.

Frozen sponge (105 g wet wt.), collected at Heron Island (23°27′S, 151°55′E) on the Great Barrier Reef, was cut into pieces and left in DCM/MeOH 1:1(3×300 mL) at room temperature. The organic solution was filtered through a

plug of cotton wool and the solvent removed by rotary evaporation to give an orange semi-solid (1.42 g), which was further purified by flash chromatography on silica using a step gradient from hexane/EtOAc 95:5 to 100% EtOAc and finally MeOH. Early eluting fractions containing sesquiterpene metabolites were purified by silica HPLC using 0.25% EtOAc in hexane to give 21 mg of (**1**) $[\alpha]_D$=+43 ($c$=0.006, $CH_2Cl_2$), 13 mg of (**2**) $[\alpha]_D$=+152 ($c$=0.003, $CH_2Cl_2$), 3.2 mg of (**3**) $[\alpha]_D$=+42 ($c$=0.003, $CH_2Cl_2$), and 1.4 mg of (**4**). A second sample of sponge (60 g wet wt.), collected at Wistari 3 dive site, was worked up in identical fashion to give a crude extract (0.8 g), which was further purified by flash chromatography using a step gradient as above. The first column fraction containing diterpenes was purified by NPHPLC using 5% EtOAc in hexane to give 21.1 mg of (**7**), $[\alpha]_D$=+61.3 ($c$=0.0035, $CH_2Cl_2$), and the epoxides (**9**) (3.3 mg) and (**10**) (1.1 mg), while NPHPLC of a second diterpene fraction using 5% EtOAc in hexane gave 12.4 mg of (**6**), $[\alpha]_D$=+1 ($c$=0.009, $CH_2Cl_2$). NPHPLC of a more polar diterpene fraction using 35% EtOAc in hexane gave 63.5 mg of (**5**), $[\alpha]_D$=+8 ($c$=0.055, $CH_2Cl_2$), together with 25.2 mg of the new kalihinol metabolite (**8**).

**10-Isothiocyanato-4-cadinene (4).** $[\alpha]_D$=+3 ($c$=0.0015, $CH_2Cl_2$); HREIMS, found 263.1700, calcd for $C_{16}H_{25}NS$ 263.1702 ($M^+$) (−0.71 ppm); $^1H$ and $^{13}C$ NMR, see Table 1.

**8-Hydroxy-isokalinihol F (8).** $[\alpha]_D$=+14 ($c$=0.018, $CH_2Cl_2$); HRESMS, 422.2428 calcd for $C_{23}H_{33}N_3O_3Na$ 422.2414 ($M+Na^+$) (+3.37 ppm); $^1H$ and $^{13}C$ NMR, see Table 2; HMBC correlations: from H-1 to C-2; H-3α to C-2, C-4, C-5 and C-19; from H-3β to C-2 and C-19; from H-5 to C-3, C-4, C-6, C-7, C-8 and C-19; from H-6 to C-1, C-5 and C-7; from H-7 to C-4, C-5, C-8, C-11 and C-18; from H-8 to C-6, C-7 and C-10; from H-9α to C-10, C-11 and C-20; from H-9β to C-1, C-7, C-8, C-10, C-11 and C-20; from H-12 to C-13; from H-13α to C-3, C-12 and C-14; from H-13β to C-3, C-12 and C-14; from H-14 to C-12, C-13 and C-15; from H-16 to C-14, C-15 and C-17; from H-17 to C-14, C-15 and C-16; from H-18 to C-11 and C-12; from H-19 to C-3, C-4 and C-5; from H-20 to C-1, C-9 and C-10; from 5-O*H* to C-4.

**Epoxide (9).** $[\alpha]_D$=+17.3 ($c$=0.0033, $CH_2Cl_2$); HRESMS, 627.4914 calcd for $C_{42}H_{63}N_2O_2$ 627.4884 ($2M+H^+$) (+4.70 ppm); $^1H$ and $^{13}C$ NMR, see Table 2; HMBC correlations: from H-1 to C-6 and C-20; from H-2α to C-3 and C-6; from H-2β to C-1, C-3 and C-6; from H-3 to C-2 and C-4; from H-5 to C-1, C-3, C-6, C-7 and C-19; from H-6 to C-1, C-4 and C-7; from H-7 to C-8; from H-8α to C-9; from H-8β to C-7 and C-9; from H-9α to C-8; from H-9β to C-8 and C-20; from H-12 to C-13, C-14 and C-18; from H-13 to C-12 and C-14; from H-14 to C-12, C-13, C-16 and C-17; from H-16 to C-12, C-14 and C-17; from H-17 to C-14 and C-16; from H-18 to C-7 and C-12; from H-19 to C-4 and C-5; from H-20 to C-1 and C-9.

**Epoxide (10).** $[\alpha]_D$=+7.3 ($c$=0.0011, $CH_2Cl_2$); HRESMS, 649.4720 calcd for $C_{42}H_{62}N_2O_2Na$ 649.4703 ($2M+Na^+$) (+2.59 ppm); $^1H$ and $^{13}C$ NMR, see Table 2; HMBC correlations: from H-1 to C-2, C-6, C-10 and C-20; from H-2α to C-3 and C-6; from H-2β to C-3 and C-6; from H-3 to C-4; from H-5 to C-1 and C-3; from H-8α to C-9; from H-9α to C-1 and C-10; from H-9β to C-8 and C-20; from H-12 to C-11 and C-18; from H-13 to C-12, C-14 and C-16; from H-14 to C-17; from H-16 to C-13, C-14 and C-17; from H-17 to C-14 and C-16; from H-18 to C-7 and C-11; from H-19 to C-3, C-4 and C-5; from H-20 to C-1, C-9 and C-10.

## NMR experiments

All 1D and 2D NMR spectra were recorded on a Bruker DRX 500 MHz spectrometer, using $CDCl_3$ as solvent referenced at $\delta$ 7.25/77.0 ppm. All 2D spectra were acquired using 2K×512 complex data matrix which was zero filled once in each dimension and a π/2 shifted sine-squared bell window function was applied in both dimensions before Fourier transformation. The HMBC and the phase sensitive geHSQC spectra were acquired with 64 and 24 transients, respectively. The evolution delay was set for $^nJ_{CH}$ of 8 Hz (geHMBC) and $^1J_{CH}$ of 135 Hz (geHSQC). The DQFCOSY spectra were acquired with 16 or 24 transients per increment while the NOESY spectra were acquired with 32 transients per increment. A mixing time of 1.14 s was used in the NOESY experiment on (**4**) while that on (**8**)–(**10**) used 800 ms.

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