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Lehualides E−K, Cytotoxic Metabolites from the Tongan Marine Sponge *Plakortis* sp.

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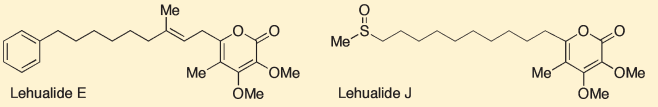
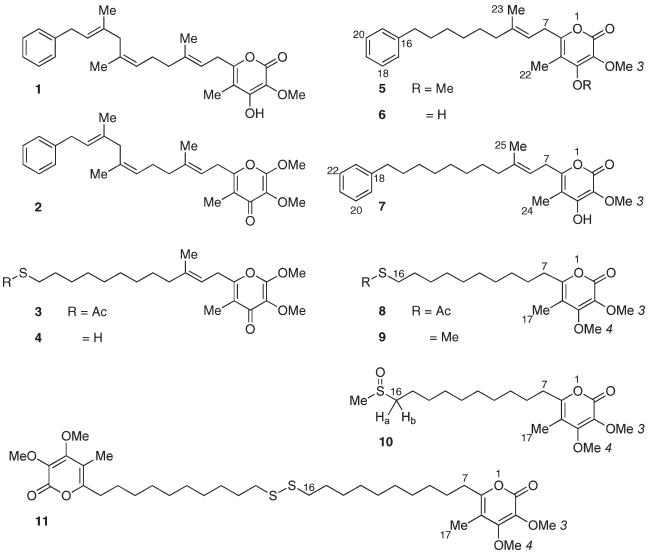
Supporting Information

ABSTRACT: Spectroscopy-guided chemical analysis of a marine sponge from the genus *Plakortis*, collected in Tonga, yielded seven new metabolites of polyketide origin, lehualides E−K (**5**−**11**), four of which incorporate various sulfur functionalities. The structures of compounds **5**−**11** were elucidated by interpretation of spectroscopic data and spectral comparison with model compounds. The biological activities of compounds **6**−**9** were investigated against human promyeloid leukemic HL-60 cells and two yeast strains, wild-type and a drug-sensitive mutant.



Marine sponges of the genus *Plakortis* (family Plakinidae) are known to be rich sources of structurally unique and biologically active secondary metabolites. Frequently isolated from *Plakortis* extracts, the antibacterial plakortin[1] and other polyketides containing cycloperoxide rings[2] exhibit wide-ranging biological effects including antiparasitic activity[3] and promotion of Ca^{2+} uptake in the cardiac sarcoplasmic reticulum.[4]

The common plakortin carbon skeleton is frequently augmented by further functionalities, including γ-lactone moieties,[2b,4,5] tetrahydrofuran rings,[6] and furanoester groups.[7] Another common motif of *Plakortis* isolates is a long alkyl chain with varying degrees of saturation. This feature is exemplified by the epiplakinic acids, isolated from Palauan collections of *Plakortis nigra*,[2b] and is characteristic of lehualides A−D (**1**−**4**).[8] Reported in 2005 from a Hawai'ian *Plakortis* specimen, these cytotoxic polyketides incorporate α- or γ-pyrone moieties, coupled with thioacetate or thiol functionalities.[8] Seven additional members of this class, lehualides E−K (**5**−**11**), are reported herein.



RESULTS AND DISCUSSION

A *Plakortis* sponge specimen, collected from a cave off the coast of 'Eua Island, Tonga, was extracted in MeOH and partitioned over reversed-phase polystyrene divinyl benzene resin with mixtures of acetone and water. Subsequent iterative, NMR-guided chromatography on dihydroxypropoxypropyl-derivatized silica gel (DIOL, CH_2Cl_2/EtOAc) and reversed-phase HPLC (C-18, MeCN/H_2O) of the 100% and 75% acetone fractions provided lehualides E−K (**5**−**11**) as colorless oils or glassy solids. The structure of lehualide K (**11**) was elucidated from fractions containing persistent impurities (ca. 15%) of compounds **7** and **8**.

The positive-ion mode HRESIMS data of lehualide E (**5**) showed a $[M + Na]^+$ pseudomolecular ion peak at m/z 385.2399, consistent with the molecular formula $C_{24}H_{32}O_4$, requiring seven degrees of unsaturation. Of a possible 24, the ^{13}C NMR spectrum contained 22 distinct resonances, including 11 sp^2 centers, indicating some molecular symmetry, while the HSQC spectrum accounted for all of the 32 protons.

Immediately apparent in the $CDCl_3$ ^1H NMR spectrum were the resonances of a mono-alkyl-substituted benzene ring (δ_H 7.27−7.16), an olefinic methine (δ_H 5.18), two methoxy singlets (δ_H 4.19, 3.81), a deshielded methylene doublet and triplet (δ_H 3.17, 2.57), two olefinic methyl singlets (δ_H 1.85, 1.66), and an aliphatic methylene pocket integrating for 12 protons. Three spin systems were identified from the COSY spectrum: a mono-alkyl-substituted phenyl ring, an isolated methyl-substituted alkene, and an extended aliphatic chain.

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COSY and HMBC correlations allowed the facile assembly of the mono-alkyl-substituted benzene ring C-16−C-21 [C-16 (δ_C 143.1); CH-17, CH-21 (δ_C 128.3, δ_H 7.27 2H); CH-18, CH-20 (δ_C 128.5, δ_H 7.17 2H); CH-19 (δ_C 125.7, δ_H 7.16 1H)]. The ring accounted for four of the 11 observed sp^2 resonances, four of the degrees of unsaturation required by the molecular formula, and the element of molecular symmetry indicated by the ^{13}C spectrum. HMBC correlations were observed between the protons of the methylene triplet CH_2-15 (δ_C 36.1, δ_H 2.57) and C-16, while reciprocal correlations between the methines CH-17/-21 and CH_2-15 established the attachment point of the aromatic ring.

The protons of the olefinic methyl singlet CH_3-23 (δ_C 16.4, δ_H 1.66) showed HMBC correlations to two other sp^2 carbons, CH-8 (δ_C 118.2, δ_H 5.18) and C-9 (δ_C 138.7). A further HMBC correlation from H_3-23 to the methylene carbon CH_2-10 (δ_C 39.7, δ_H 1.96) and a COSY correlation from H-8 to the protons of another methylene, CH_2-7 (δ_C 30.2, δ_H 3.17), established this olefinic system as an isolated double bond. The *E*-geometry of the double bond was established from NOE enhancements of H-8/H_2-7, H_3-23/H_2-11, H_3-23/H_2-7, and H-8/H_2-10 following selective irradiation of the resonances.

The remaining five nonprotonated sp^2 carbons, C-2 (δ_C 162.7), C-3 (δ_C 128.3), C-4 (δ_C 159.0), and C-5 (δ_C 108.3), could be accounted for only by two fully substituted carbon-carbon double bonds and a carbonyl. HMBC correlations were observed from H_2-7 to C-5 and C-6, indicating the double-allylic character of H_2-7, consistent with its chemical shift. An HMBC correlation from H-8 to C-6 established the C-7−C-6 bond. Further HMBC correlations were observed from the protons of another olefinic methyl, CH_3-22 (δ_C 10.1, δ_H 1.85), to C-4, C-5, and C-6, which together with a homoallylic COSY correlation between H_2-7 and H_3-22 established the methyl attachment at C-5 and the C-5−C-4 and C-5−C-6 connections. The substructure was extended by an HMBC correlation from the protons of the methoxy OCH_3-4 (δ_C 60.5, δ_H 4.19) to C-4, while another methoxy, OCH_3-3 (δ_C 60.4, δ_H 3.81), correlated with C-3, revealing a final carbon-carbon double bond. Reciprocal NOE correlations between OCH_3-3 and OCH_3-4 established the C-3−C-4 connectivity, while further NOE correlations between OCH_3-4 and H_3-22 and between H_2-7 and H_3-22 confirmed the tetrasubstituted diene C-3−C-6, with CH_2-7 and CH_3-22 on the same side of the C-5−C-6 double bond. The remaining sp^2 center, C-2 (δ_C 162.7), which must be a carbonyl, was therefore assigned as an α,β-unsaturated ester on the basis of its shielded chemical shift. The shielded chemical shift of the oxygenated C-3 (δ_C 126.2) is consistent with the attachment of the carbonyl.[8,9] All but one degree of unsaturation associated with the molecular formula were accounted for by the phenyl ring, the isolated double bond C-8−C-9, and the C-2−C-6 dienenoate. Furthermore the four oxygen atoms required by the molecular formula are accounted for by the two oxymethyls and the ester functionality. The deshielded chemical shift of C-6 (δ_C 154.8) is consistent with oxygen substitution, and therefore an α-pyrone moiety, C-2−C-6, similar to that of lehualide A (**1**), is established. Absorption maxima observed in the UV spectrum (λ_{max} 291 nm) and stretching frequencies in the IR spectrum (ν_{max} 1686, 1649 cm^{-1}) support this assignment,[10] while the chemical shifts of the sp^2 carbon resonances are consistent with those reported for similar systems.[8]

A series of sequential COSY and HMBC correlations beginning at the H_2-15 methylene triplet and terminating with H_2-10 established a C_6 methylene chain linking the mono-alkyl-substituted benzene ring and the C-2−CH_2-10 segment. Selective irradiation of both methylene centers in a series of 1D TOCSY experiments confirmed the connection, completing the structure of **5**.

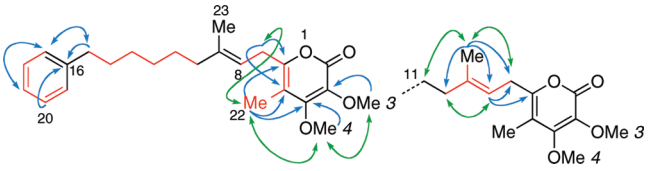

Figure 1. Structural elucidation of lehualide E (**5**). COSY couplings are shown in red, and key NOE and HMBC correlations are shown in green and blue, respectively. Double-headed arrows depict reciprocal correlations.

Lehualide F (**6**) was isolated as a white solid. Positive-ion mode HRESIMS analysis of **6** (*m/z* 393.2042 $[M + Na]^+$) indicated the molecular formula $C_{23}H_{30}O_4$, differing from that of **5** by 14 mass units. The multiplicity-edited HSQC spectrum accounted for 32 of the 33 protons, indicating the presence of one exchangeable proton, while the ^1H spectrum of **6** appeared essentially identical to that of **5** except for the presence of only one methoxy.

An α-pyrone (λ_{max} 298 nm) similar to that of **5** was quickly identified. The oxy-substituted double bond between C-5 and C-6 was established on the basis of the strong HMBC correlations from the protons of the olefinic methyl H_3-21 and methylene H_2-7 to both C-5 and C-6, and weak homoallylic COSY coupling between H_3-21 and H_2-7. A further HMBC correlation from H_3-22 to C-4 (δ_C 162.1) was observed; however the methoxy observed in **5** was absent, suggesting hydroxy substitution of C-4. This accounted for the exchangeable proton (IR: ν_{max} 3250 cm^{-1}, δ_H 6.50 ppm). HMBC correlations from protons of the lone methoxy OCH_3-3 (δ_C 60.0, δ_H 3.86) to C-3 (δ_C 125.0) were observed as in **5**. The α-pyrone system was completed by the α,β-unsaturated ester carbonyl C-2 (δ_C 160.4). All other chemical shifts and correlations observed in the NMR spectra were essentially identical to those of **5**, thereby establishing **6** as the C-4 *des*-methoxy congener of **5**.

A pseudomolecular ion peak observed in the positive-ion mode HRESIMS spectrum for lehualide G (**7**) (*m/z* 421.2355 $[M + Na]^+$) indicated a molecular formula of $C_{25}H_{34}O_4$, differing from **6** by two methylene units. Detailed analysis of the 1D and 2D NMR spectra of **6** and **7** determined that the two were chain-length congeners. The only perceivable differences between the spectra of the two compounds were the methylene regions of the ^1H and ^{13}C spectra. The 1D and 2D NMR data of **7** were consistent with the α-pyrone moiety present in **6**, and UV and IR spectroscopic data supported the assignment. The extended methylene chain of **7**, C-10−C-17, was established by selective 1D TOCSY irradiation of H_2-17 (C-17: δ_C 36.1, δ_H 2.61). As the mixing time was increased from 0 to 120 ms, the methylene resonances H_2-16−H_2-10 (C-10: δ_C 40.5, δ_H 2.01) were sequentially revealed. Analogous 1D TOCSY irradiation of H_2-10 coupled with COSY and HMBC correlations within the chain provided confirmation of this assignment and the connection between the methylenes. HSQC-TOCSY analysis of **7** in CD_3OD over a selected bandwidth (δ_C: 10−50 ppm) allowed elucidation of the aliphatic chain: [C-12−C-15 (δ_C 32.8, 30.6, 30.5, 30.3, δ_H 1.25−1.29) and C-16 (δ_C 30.1, δ_H 1.22)]. Using this method the methylene resonances C-12−C-16 were clearly

resolved, and the TOCSY correlations aided concise ordering of the resonances.

Positive-ion mode HRESIMS analysis of lehualide H (**8**) generated a pseudomolecular ion peak at m/z 407.1864 $[M + Na]^+$ suitable for the formula $C_{20}H_{32}O_5S$, requiring five degrees of unsaturation, and incorporation of a sulfur atom. Analysis of the ^1H NMR spectrum of compound **8** revealed, in contrast with the lehualides E−G, the absence of the phenyl, olefinic proton, and doubly allylic methylene signals. Similar 2D NMR, IR, and UV spectroscopic data to those of **5** and the presence of two methoxy signals indicated retention of the dimethoxy α-pyrone, therefore accounting for four of the five oxygen atoms, and four degrees of unsaturation. As with **5**, the nonprotonated α-pyrone ring was assigned on the basis of HMBC and NOE correlations of H_2-7, H_3-21, and the methoxy groups OCH_3-4 and OCH_3-3. The doubly allylic methylene doublet of **5**, CH_2-7 (δ_C 30.2, δ_H 3.17), is replaced in **8** by a singly allylic methylene triplet, CH_2-7 (δ_C 30.9, δ_H 2.44), as evidenced by homoallylic COSY correlation with H_3-21 and HMBC correlations with C-5 and C-6. This confirmed the absences of the isolated double bond and associated olefinic methyl of the previously described structures. The long alkyl chain characteristic of the lehualides extended between CH_2-7 and CH_2-16 (δ_C 29.2, δ_H 2.86) and selective 1D TOCSY irradiations of both methylenes H_2-7 and H_2-16, with increasing mixing times (0−200 ms), established the connection between the two terminating methylenes. The final substructure began at the deshielded methyl singlet terminus (δ_C 30.8, δ_H 2.35). Strong HMBC correlations from the protons of both the methyl and H_2-16 to an ester carbonyl (δ_C 196.3) were observed. The large $^1J_{CH}$ coupling constant and low ^1H and ^{13}C chemical shifts of the C-16 methylene (δ_C 29.2, δ_H 2.86, $^1J_{CH}$ 141) indicated sulfur attachment.[11] The chemical shift of the acetate carbonyl (δ_C 196.3) is consistent with a thioacetate moiety, as previously observed in lehualide C (**3**) and *Dysidea* sp.[12] Reciprocal weak HMBC correlations observed from the protons of methylene H_2-16 and the methyl terminus to the respective carbons confirmed the thioester linkage. The elucidation of this substructure accounted for the sulfur atom, the fifth oxygen atom, and the final degree of unsaturation required by the molecular formula.

Positive-ion mode HRESIMS analysis of lehualide I (**9**) (m/z 379.1920 $[M + Na]^+$) established the molecular formula as $C_{19}H_{32}O_4S$. Analysis of the 1D and 2D NMR spectroscopic data, in conjunction with IR and UV spectra, confirmed the same C-6 alkyl-substituted α-pyrone system present in **8**, accounting for the four degrees of unsaturation and the four oxygen atoms required by the molecular formula. The alkyl chain was again elucidated by irradiation of methylenes H_2-16 and H_2-7 using a 1D TOCSY pulse with various mixing times. Similar to **8** a methyl singlet was observed (δ_C 15.6, δ_H 2.09), and inspection of 2D NMR spectroscopic data revealed weak HMBC and COSY cross-peaks with the CH_2-16 methylene (δ_C 34.3, δ_H 2.49), indicating the presence of a heteroatom between them. The high $^1J_{CH}$ values and shielded chemical shifts of the aliphatic centers CH_2-16 ($^1J_{CH}$ 137) and the methyl terminus ($^1J_{CH}$ 137) established the linkage as a sulfur atom.[11] This was corroborated by a weak C−S stretch present in the IR spectrum at 698 cm^{-1}. Methyloctyl sulfide was prepared from octyl bromide[10a] and was found to have identical spectroscopic characteristics to the aliphatic portion of the natural product, confirming the structure of **9** as a methyl sulfide.

The molecular formula of lehualide J (**10**) was established by positive-ion mode HRESIMS analysis as $C_{19}H_{32}O_5S$ (m/z 395.1868 $[M + Na]^+$). As with compounds **8** and **9** the C-6 alkyl-substituted α-pyrone system was identified through detailed analysis of both 1D and 2D NMR data and corroborated by UV and IR spectroscopic data. The extended aliphatic chain, typical of the latter lehualides, terminates in a deshielded diastereotopic methylene, CH_2-16 (δ_C 54.9, δ_H a 2.73, b 2.66). A deshielded methyl singlet (δ_C 38.7, δ_H 2.56) was observed at significantly higher chemical shift than that of the methyl sulfide **8**, indicating a change of functionality. Reciprocal HMBC correlations between these two centers established their linkage through a heteroatom bridge. Again the large $^1J_{CH}$ values of CH_2-16 ($^1J_{CH}$ Ha 137, Hb 135) and the methyl terminus ($^1J_{CH}$ 137) indicated a sulfur bridge.[11a] An oxygen-bearing stereogenic sulfoxide center is consistent with the diastereotopic nature of CH_2-16 and the remaining oxygen suggested by the pseudomolecular ion. The IR stretch of the functionality was present at 1027 cm^{-1}.[13] It is unclear, however, whether the apparent lack of optical activity ($[\alpha]^{19.1}_D$ 0.00 (c 3.69 g mL^{-1} E^{-5}, $CHCl_3$)) is due to the natural occurrence of **10** as a racemic mixture or small sample size. Stoichiometric oxidation of methyloctyl sulfide with both H_2O_2 and KIO_4 provided the sulfoxide product,[14] which proved to be a spectroscopic match for the aliphatic portion of the natural product in both chemical shift and $^1J_{CH}$ coupling. For further spectroscopic comparison with both **9** and **10**, methyloctyl sulfone was prepared via oxidation of the sulfide with activated MnO_2 and $KMnO_4$[15] and displayed significant spectroscopic differences.

The ^1H and ^{13}C NMR spectra of lehualide K (**11**) are remarkably similar to those of **8**, except for the absence of the acetyl group. Detailed analysis of both the 1D and 2D NMR spectra suggested that **11** is the thiol analogue of **8**, although the signal attributed to the S−H proton diminished during the purification process. The positive-ion mode HRESIMS spectrum showed a $[M + Na]^+$ pseudomolecular ion peak at m/z 705.3477. This was consistent with the molecular formula $C_{36}H_{58}O_8S_2$ and indicated that the species had formed a disulfide dimer. In light of the isolation of the thiol lehualide D (**4**), dimerization of **11** during the isolation process is suspected. As is the case with compounds **8**−**10**, the C-16 methylene has a large $^1J_{CH}$ value (δ_C 39.3, δ_H 2.67, $^1J_{CH}$ 139) and shielded chemical shift. Compound **11** was not isolated in purity exceeding ~85%, as persistent impurities (compounds **6** and **8**) remained despite multiple normal-phase column chromatographic purifications

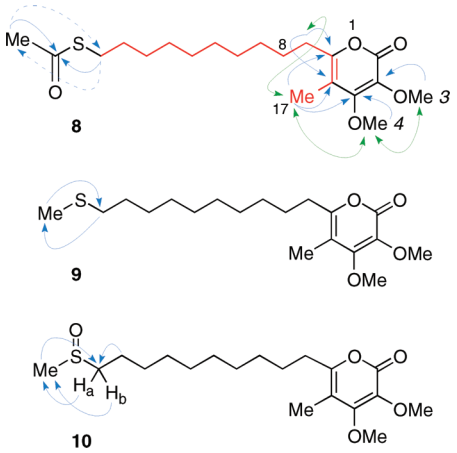

Figure 2. Lehualides H−J (**8**−**10**). COSY couplings are shown in red, and key NOE and HMBC correlations are shown in green and blue, respectively. Double-headed arrows depict reciprocal correlations.

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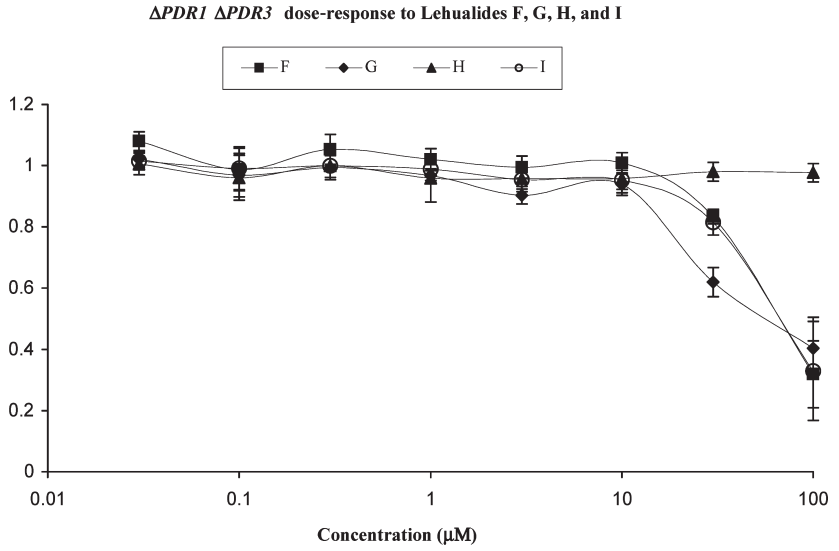

Figure 3. Dose-dependent growth inhibition of drug-sensitive yeast to lehualides F, G, H, and I.

and attempted isolation by C-18 and DIOL HPLC under various solvent conditions.

A cell-based dose-response assay was used to assess the susceptibility of yeast to lehualides F (**6**), G (**7**), H (**8**), and I (**9**). The compounds were tested against wild-type yeast or a drug-sensitive (DS) mutant strain that is deleted for the genes encoding *PDR1* and *PDR3*, transcription factors that act as master regulators of the pleiotropic drug resistance (PDR) network in yeast.[16] The DS strain is crippled in its ability to upregulate the PDR response and is up to 200 times more sensitive to xenobiotics[17] than wild-type yeast. Compounds **6**, **7**, and **9** inhibited growth of the DS strain by 60%, 68%, and 67%, respectively, at the highest concentration tested (Figure 3). Interestingly, lehualide H (**8**) did not inhibit yeast growth at any concentration, suggesting that this compound either is inactive in yeast, has a potency profile that is not captured in the tested concentration range, or is highly unstable. In contrast, none of the compounds inhibited the growth of wild-type yeast at any concentration tested (data not shown). The increased sensitivity of the DS mutant suggests the lehualides are substrates for one of the PDR efflux pumps. To more accurately gauge the potency of the lehualides, the dose-response experiment was repeated with **6** using a higher concentration range. Lehualide F (**6**) exhibited an IC_{50} of 44 μM. Furthermore, a 48% growth inhibition of the wild-type strain at the highest concentration was observed, indicating a half log decrease in sensitivity compared to the DS strain and supporting the hypothesis that the lehualides are substrates for the PDR efflux pumps.

Lehualides F−I (**6**−**9**) also displayed cytotoxicity against the human promyelocytic leukemia (HL-60) cell line. Using a standard 2-day cell proliferation assay, compounds **6** and **7** exhibited IC_{50} values for growth inhibition of 6.2 and 5.4 μM, respectively, while interestingly the thioacetate and sulfide compounds **8** and **9** showed weaker inhibition of the same cell line with respective IC_{50} values of 14.6 and 10.8 μM.

While the sulfur functionalities of lehualides H (**8**) and K (**11**) have been previously encountered in compounds **3** and **4**, the methyl sulfide and sulfoxide functionalities present in **9** and **10** are thought to be unprecedented in metabolites from sponges of this genus. Sulfoxides and sulfides have rarely been reported from marine organisms, although there have been numerous examples reported from terrestrial plants including methionine and cysteine sulfoxides from brassica and alliaceous vegetables[13,18] and trisulfides from the roots of some angiosperms.[19] Examples from sponge literature include sulfoxides psammaplin N,[20] eudistomin K,[21] and didemnolines C and D[11b] and the sulfide benzylthiocrellidone from *Crella spinulata*.[22] The moderate biological activity displayed by compounds of the lehualide class has prompted synthetic investigations, and the succinct synthesis of compound **2** was recently reported.[23] The isolation of lehualides E−K (**5**−**11**), particularly in light of the S-functionalities present in compounds **8**−**11**, adds to the diversity of chemical functionality obtained from *Plakortis* sponges.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were obtained on a Autopol II automatic polarimeter, UV spectra were acquired on an Agilent 8453 UV/visible spectrometer, while IR spectra were recorded using a Perkin-Elmer Spectrum One FT-IR spectrometer. NMR spectra were obtained using a 600 MHz Varian DirectDrive spectrometer equipped with a triple resonance HCN cryogenic probe, operating at 600, 150, and 60 MHz for ^{1}H, ^{13}C, and ^{15}N nuclei, respectively. Spectra were recorded in CD_3OD (Cambridge Isotopes) and $CDCl_3$ (Merck), with chemical shifts δ (ppm) referenced to the residual solvent peak [(CD_3OD: δ_C 49.00, δ_H 3.31) and ($CDCl_3$: δ_C 77.16, δ_H 7.26)].[24] HREIMS measurements were obtained using a Waters Q-TOF Premier Tandem mass spectrometer. Silica gel plates were used for analytical thin-layer chromatography. 2,3-Dihydroxypropoxypropyl-derivatized silica gel was used for all normal-phase column chromatography, while Supelco HP20 and HP20SS polystyrene(divinylbenzene) resins were used for reversed-phase column chromatography. HPLC-grade CH_2Cl_2 (Merck) and MeCN (Fluka) were used generally and for HPLC purification; all other solvents were distilled from glass immediately prior to use. All chemical reagents were purchased from Sigma-Aldrich and Penta International Corporation and used without further purification.

Animal Material. The sponge was collected from the horizontal ceiling of a large cave at a depth of 12−15 m, on ʻEua Island, Kingdom of Tonga. It was identified as a species of *Plakortis* (order Homosclerophorida, family Plakinidae), similar to *P. ceylonica* (Dendy, 1905). The sponge formed small oval pendant encrustations about 3 cm thick; the

Table 1. NMR Spectroscopic Data of Lehualide E (5) ($CDCl_3$, ^1H 600 MHz; ^{13}C 150 MHz)

position	δ_C, mult.	δ_H (J, Hz)	COSY	HMBC (H→C)	NOE[a]
2	162.7, C				
3	128.3, C				
OCH_3 3	60.5, CH_3	3.81, s		3	OCH_3 4
4	159.0, C				
OCH_3 4	60.6, CH_3	4.19, s		4	OCH_3 3; 22
5	108.3, C				
6	154.8, C				
7	30.3, CH_2	3.17, d (6.9)	7; 22[b]	8; 6; 23; 5[b]	
8	117.5, CH	5.17, t (7.2)	6; 23	7; 23; 6[b]	10
9	138.7, C				
10	39.7, CH_2	1.96, t (7.5)	10; 23	11; 23; 9; 8	8
11	27.8, CH_2			11; 9	10; 12; 9
12	29.9, CH_2				
13	29.3, CH_2	1.33, quin (7.3)	13; 11		
14	31.6, CH_2	1.59, quin (8.1)	14; 12	15; 16; 13	
15	36.1, CH_2	2.57, t (7.7)	13	14; 16; 17; 21	
16	143.0, C				
17	128.5, CH	7.19, d (7.4)	18	19; 21	
18	128.4, CH	7.29, t (7.0)	17; 19	16; 20	
19	125.7, CH	7.19, t (7.8)	18; 20	17; 21	
20	128.4, CH	7.29, t (7.0)	19; 21	16; 18	
21	128.5, CH	7.19, d (7.4)	20	17; 19	
22	10.3, CH_3	1.85, s	7[b]	5; 4; 6; 7[b]	OCH_3 3; 7
23	16.4, CH_3	1.66, s		9; 8; 10	11; 7

[a] Selected correlations. [b] Weak correlation.

Table 2. NMR Spectroscopic Data of Lehualides F and G (6, 7) ($CDCl_3$, ^1H 600 MHz; ^{13}C 150 MHz)

	6		7	
position	δ_C, mult.	δ_H (J, Hz)	δ_C	δ_H (J, Hz)
2	160.3, C			160.4, C
3	125.0, C			125.0, C
OCH_3 3	59.8, CH_3	3.90, s	60.0, CH_3	3.86, s
4	157.9, C			158.0, C
5	105.6, C			105.7, C
6	157.3, C			157.3, C
7	30.2, CH_2	3.20, d (7.2)	30.3, CH_2	3.21, d (7.0)
8	117.2, CH	5.17, t (7.0)	117.1, CH	5.16, t (7.0)
9	139.0, C			139.1, C
10	39.7, CH_2	1.97, t (8.4)	39.7, CH_2	1.97, t (7.4)
11	27.8, CH_2	1.38, quin (7.6)	27.8, CH_2	1.36, quin (7.1)
12	29.29, CH_2	1.32, quin (8.0)	29.4, CH_2	1.20 − 1.33, m
13	29.26, CH_2	1.24 − 1.29, m	29.55, CH_2	1.20 − 1.33, m
14	31.5, CH_2	1.59, q (8.0)	29.58, CH_2	1.20 − 1.33, m
15	36.1, CH_2	2.57, t (7.2)	29.45, CH_2	1.20 − 1.33, m
16	143.0, C		31.7, CH_2	1.59, quin (6.9)
17	128.5, CH	7.17, d (8.2)	36.1, CH_2	2.61, t (7.8)
18	128.4, CH	7.22, t (7.7)	143.1, C	
19	125.6, CH	7.16, t (6.0)	128.5, CH	7.17, d (7.4)
20	128.4, CH	7.22, t (7.7)	128.3, CH	7.27, t (7.3)
21	128.5, CH	7.17, d (8.2)	125.7, CH	7.16, t (6.3)
22	9.4, CH_3	1.96, s	128.3, CH	7.27, t (7.3)
23	16.3, CH_3	1.68, s	128.5, CH	7.17, d (7.4)
24			9.5, CH_3	1.96, s
25			16.5, CH_3	1.68, s
OH		6.50, bs		6.50, bs

texture is cork-like and rubbery and covered in wide shallow nodules about 3 mm high. The sponge was dark chocolate-purplish brown, with a dense interior. Diod spicules of approximately 180−250 μm long were found. A voucher specimen (PTN3_20A) has been deposited in the collection of Dr. James Bell at the School of Biological Sciences, VUW, Wellington.

Extraction of *Plakortis* and Isolation of Lehualides E−K (5−11). The frozen sample (36.0 g) was extracted twice for 14 h in 100 mL of MeOH at room temperature. The second extract, followed by the first, were passed through a column of 40 mL of HP20 resin, and the eluents were combined, diluted with 200 mL of H_2O, passed through the column again, further diluted to 25% MeOH, and passed through the column once more. The column was washed with H_2O, then eluted, generating three 100 mL fractions of 30%, 75%, and 100% Me_2CO/H_2O. The 75% and 100% Me_2CO/H_2O fractions were analyzed by 1D and 2D NMR, then subjected to further reversed-phase purification using HP20SS resin, generating fractions of 30%, 40%, 50%, 60%, 70%, 80%, 90%, and 100% Me_2CO/H_2O. Further purification of the 70−100% Me_2CO/H_2O fractions by DIOL oil chromatography (hexanes/CH_2Cl_2/EtOAc) and reversed-phase HPLC (C-18, 85% MeCN/H_2O) yielded lehualides E−I (**5**−**9**) in varying ratios (see compound data) and lehualide K (**11**) with persistent impurities of compounds **7** and **8**. DIOL oil chromatography (hexanes/CH_2Cl_2/EtOAc) and HPLC (C-18, 95% MeCN/H_2O) purification of the 60% Me_2CO/H_2O HP20SS fraction afforded lehualide J (**10**).

Biological Testing. A dose-response assay was performed using two *Saccharomyces cerevisiae* strains: wild-type YCG117 (*MAT*α *ura3*Δ*0::NAT* derived from strain Y7092[25]) and YCG198 (*MAT*α *PDR1* Δ*::NAT PDR3* Δ*::URA3*, derived from strain Y8205[25]), a drug-sensitive mutant strain that lacks the two main transcription factor genes that regulate the expression of pleiotropic drug resistance efflux pumps.[16] For the assay, yeast cultures were grown to stationary phase in synthetic complete (SC) media[26] and diluted to 5×10^5 cells/mL in fresh SC, and 100 μL aliquots were added to 96-well tissue culture plates. Seven point, half-log serial dilutions of compounds **6**−**9** (10 to 0.1 mM) were prepared from 10 mM working stocks dissolved in dimethyl sulfoxide (DMSO). A 1 μL volume of diluent was added per well, giving a final concentration range of 100 to 0.01 μM. DMSO (1% final concentration) was added to one well as a negative control. To achieve the highest concentration for compound **6** in the dose-response experiment, 3 μL of working stock was added to give a final concentration of 300 μM L^{-1} along with a control well containing 3% DMSO. Plates were mixed by vortexing and incubated at 30 °C for 18 h. Cell growth was quantified by measuring optical density (OD) at 590 nm using a Wallac EnVision 2102 multilabel plate reader (Perkin-Elmer). Residual growth (%) was calculated for each concentration using the formula (OD_{exp}/OD_{DMSO}) × 100. Results are reported as the mean ± standard error for two independent experiments performed in triplicate. The IC_{50} values were determined using Prism (Graphpad Software).

Tests for inhibition of growth of human HL-60 promyeloid leukemic cells were carried out using the MTT cell proliferation assay as previously described.[27]

Lehualide E (5): clear oil, 540 μg; UV (MeOH) λ_{max} (log ε), 235 (3.80), 295 (3.73); IR (film) ν_{max} 3250, 2928, 2853, 1687, 1647, 1561, 1440, 1221, 1094 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; HRESIMS m/z 385.2399 $[M + Na]^+$ (calcd for $C_{24}H_{32}O_4Na$, 385.2398; Δ 0.2 ppm).

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Lehualide F (6): white solid, 2.79 mg; UV (MeOH) λ_{max} (log ε), 235 (3.81), 282 (3.70) nm; IR (film) ν_{max} 3250, 2929, 2854, 1686, 1648, 1560, 1439, 1220, 1094 cm^{-1}; ^1H and ^{13}C NMR data, see Table 2; HRESIMS *m/z* 393.2042 $[M + Na]^+$ (calcd for $C_{23}H_{30}O_4Na$, 393.2042; Δ 0.0 ppm).

Table 3. ^1H and ^{13}C NMR Spectroscopic Data of Lehualides H and K (8 and 11) ($CDCl_3$, ^1H 600 MHz; ^{13}C 150 MHz)

	8		11	
position	δ_C, mult.	δ_H (*J*, Hz)	δ_C	δ_H (*J*, Hz)
2	162.7, C			162.7, C
3	127.9, C			128.4, C
OCH_3 3	60.4, CH_3	3.81, s	60.5, CH_3	3.81, s
4	158.9, C			158.9, C
OCH_3 4	60.6, CH_3	4.16, s	60.6, CH_3	4.17, s
5	108.5, C			108.5, C
6	155.9, C			155.9, C
7	30.9, CH_2	2.44, t (7.7)	30.9, CH_2	2.44, t (7.7)
8	27.5, CH_2	1.58, quin (7.5)	27.5, CH_2	1.59, quin (7.5)
9	29.3, CH_2	1.28, m	29.33, CH_2	1.27, m
10	29.4, CH_2	1.27, m	29.62, CH_2	1.28, m
11	29.54, CH_2	1.25, m	29.59, CH_2	1.28, m
12	29.55, CH_2	1.25, m	29.55, CH_2	1.28, m
13	29.48, CH_2	1.23, m	29.44, CH_2	1.28, m
14	28.9, CH_2	1.35, m	28.6, CH_2	1.37, quin (7.6)
15	29.48, CH_2	1.55, t (7.2)	29.3, CH_2	1.66, quin (7.4)
16	29.2, CH_2	2.86, t (7.3)	39.2, CH_2	2.67, t (7.4)
SC═*O*CH_3	196.3, C			
SC═O*C*H_3	30.8, CH_3	2.35, s		
17	10.5, CH_3	1.84, s	10.4, CH_3	1.84, s

Lehualide G (7): white solid, 18.32 mg; UV (MeOH) λ_{max} (log ε), 235 (3.44), 291 (3.55) nm; IR (film) ν_{max} 3250, 2926, 2854, 1686, 1649, 1563, 1439, 1276, 1094 cm^{-1}; ^1H and ^{13}C NMR data, see Table 2; HRESIMS *m/z* 421.2355 $[M + Na]^+$ (calcd for $C_{25}H_{34}O_4Na$, 421.2355; Δ 0.0 ppm).

Lehualide H (8): white solid, 1.40 mg; UV (MeOH) λ_{max} (log ε), 255 (3.68), 297 (4.29) nm; IR (film) ν_{max} 2925, 2853, 1709, 1690, 1651, 1569, 1458, 1227, 670 cm^{-1}; ^1H and ^{13}C NMR data, see Table 3; HRESIMS *m/z* 407.1864 $[M + Na]^+$ (calcd for $C_{20}H_{32}O_5SNa$, 407.1868; Δ −1.0 ppm).

Lehualide I (9): clear oil, 646 μg; UV (MeOH) λ_{max} (log ε), 249 (3.12), 297 (3.96) nm; IR (film) ν_{max} 2926, 2854, 1712, 1652, 1570, 1460, 1370, 1229, 699 cm^{-1}; ^1H and ^{13}C NMR data, see Table 4; HRESIMS *m/z* 379.1920 $[M + Na]^+$ (calcd for $C_{19}H_{32}O_4SNa$, 379.1919; Δ 0.3 ppm).

Lehualide J (10): clear oil, 1.95 mg; $[\alpha]^{19.1}_D$ 0.00 (*c* 3.69 g mL^{-1} E^{-5}, $CHCl_3$); UV (MeOH) λ_{max} (log ε), 249 (2.88), 288 (3.52) nm; IR (film) ν_{max} 2925, 2855, 1713, 1652, 1569, 1458, 1371, 1228, 1098, 699 cm^{-1}; ^1H and ^{13}C NMR data, see Table 4; HRESIMS *m/z* 395.1868 $[M + Na]^+$ (calcd for $C_{19}H_{32}O_5SNa$, 395.1868; Δ 0.0 ppm).

Lehualide K (11): clear oil, 2.12 mg (impure); UV (MeOH) λ_{max} (log ε), 240 (3.41), 296 (3.60) nm; IR (film) ν_{max} 2926, 2852, 1690, 1651, 1569, 1458, 1227, 672 cm^{-1}; ^1H and ^{13}C NMR data, see Table 3; HRESIMS $[M + Na]^+$ *m/z* 705.3477 (calcd for $C_{36}H_{58}O_8S_2Na$, 705.3471; Δ 0.9 ppm).

Preparation of Methyloctyl Sulfide. Methyloctylthiol (0.5 mL, mmol) was stirred under ambient conditions in 20 mL of MeOH. One equivalent of MeI was added, and the reaction was heated at reflux for 20 min, affording the monomethylation product in a 40% yield.

Methyloctyl sulfide: colorless liquid; IR (film) ν_{max} 698 cm^{-1}; ^1H NMR ($CDCl_3$, 600 MHz) δ 2.48 (2H, t, *J* = 7.2, H-2), 2.09 (3H, s, H-1), 1.58 (2H, quin, *J* = 7.2, H-3), 1.37 (2H, quin, *J* = 7.2, H-4), 1.23−1.30 (8H, m, H-5−H-8), 0.88 (3H, t, *J* = 7.2, H-9); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 34.2 (C-2), 31.8 (C-8), 29.15 (C-5), 29.13 (C-7), 29.1 (C-3), 28.8 (C-4), 22.6 (C-6), 15.5 (C-1), 14.0 (C-9).

Table 4. ^1H and ^{13}C NMR Spectroscopic Data of Lehualides I and J (9, 10) ($CDCl_3$, ^1H 600 MHz; ^{13}C 150 MHz)

	9		10	
position	δ_C, mult.	δ_H (*J*, Hz)	δ_C	δ_H (*J*, Hz)
2	162.6, C			162.7, C
3	127.7, C			127.8, C
OCH_3 3	60.4, CH_3	3.85, s	60.5, CH_3	3.81, s
4	158.8, C			158.9, C
OCH_3 4	60.5, CH_3	4.21, s	60.6, CH_3	4.17, s
5	108.3, C			108.5, C
6	155.8, C			155.9, C
7	30.8, CH_2	2.45, t (7.7)	30.9, CH_2	2.44, t (7.7)
8	27.4, CH_2	1.59, quin (7.2)	27.5, CH_2	1.59, quin (7.7)
9	29.5, CH_2	1.28, m	29.36, CH_2	1.28, m
10	29.4, CH_2	1.28, m	29.28, CH_2	1.30, m
11	29.3, CH_2	1.28, m	28.9, CH_2	1.44, m
12	29.2, CH_2	1.28, m	29.44, CH_2	1.24, m
13	29.2, CH_2	1.28, m	29.39, CH_2	1.27, m
14	28.8, CH_2	1.37, quin (8.0)	29.24, CH_2	1.32, m
15	29.2, CH_2	1.58, quin (7.3)	22.7, CH_2	1.76, quin (7.0)
16	34.3, CH_2	2.49, bt (7.7)	54.9, CH_2	a 2.73, ddd (13.0, 9.0, 6.1)
				b 2.66, ddd (12.7, 9.2, 6.6)
*S*Me; O═*S*Me	15.6, CH_3	2.09, bs	38.7, CH_3	2.56, s
17	10.3, CH_3	1.85, s	10.4, CH_3	1.85, s

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Preparation of Methyloctyl Sulfoxide A. Methyloctyl sulfide (0.5 mL, 2.6 mmol) was stirred in 30 mL of MeOH, after which 1.5 equiv (4.0 mmol) of KIO_4 in 3 mL of H_2O was added, and the cloudy solution stirred at room temperature for 12 h. Methyloctyl sulfoxide was produced in 100% yield.

Preparation of Methyloctyl Sulfoxide B. Methyloctyl sulfide (0.5 mL, 2.6 mmol) was stirred in 30 mL of MeOH and treated with 1.5 equiv (4.0 mmol) of 30% H_2O_2 aqueous solution. The reaction was stirred for 12 h under ambient conditions, affording the sulfoxide product in 100% yield.

Methyloctyl sulfoxide: white, crystalline solid; IR (film) ν_{max} 1027, 748 cm^{-1}; ^1H NMR ($CDCl_3$, 600 MHz) δ 2.73 (1H, ddd, J = 14.9, 9.8, 5.8, H-2a), 2.64 (1H, ddd, J = 16.2, 9.3, 5.8, H-2b), 2.56 (3H, s, H-1), 1.75 (2H, quin, J = 7.0, H-3), 1.44 (2H, sep, J = 8.4, H-4), 1.22−1.34 (8H, m, H-5−H-8), 0.88 (3H, t, J = 7.1 Hz, H-9); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 54.9 (C-2), 38.7 (C-1), 31.9 (C-8), 29.3 (C-5), 29.2 (C-7), 28.9 (C-4), 22.8 (C-6), 22.7 (C-3), 14.2 (C-9); HRESIMS $[M + Na]^+$ m/z 199.1133 (calcd for $C_9H_{20}OS$, Δ −2.0 ppm); HRESIMS $[M + H]^+$ m/z 159.1207 (calcd for $C_9H_{20}S$, Δ 1.3 ppm).

Preparation of Methyloctyl Sulfone. Methyloctyl sulfide (0.5 mL, 2.6 mmol) was stirred in 25 mL of CH_2Cl_2 with excess 3:1 activated $MnO_2/KMnO_4$. The suspension was stirred for 96 h, then filtered, and the residue was washed with a 1:1 mixture of MeOH and CH_2Cl_2. The filtrate was evaporated, affording a white crystalline solid in 100% yield.

Methyloctyl sulfone: white, crystalline solid; IR (film) ν_{max} 1273, 1141, 1128, 1117, 765, 749 cm^{-1}; ^1H NMR ($CDCl_3$, 600 MHz) δ 3.00 (2H, t, J = 8.4 Hz, H-2), 2.89 (3H, s, H-1), 1.85 (2H, quin, J = 7.8 Hz, H-3), 1.44 (2H, quin, J = 7.2 Hz, H-4), 1.24−1.34 (8H, m, H-5 − H-8), 0.88 (3H, t, J = 7.2 Hz, H-9); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 55.0 (C-2), 40.5 (C-1), 31.8 (C-8), 29.14 (C-5), 29.05 (C-6), 28.5 (C-4), 22.7 (C-7), 22.6 (C-3), 14.2 (C-9).

■ ASSOCIATED CONTENT

Supporting Information. ^1H, ^{13}C, gCOSY, HSQCad, and gHMBC NMR spectra for compounds **5**−**11** and the bsHSQC-TOCSYad spectrum for compound **7**. Above-water photograph of *Plakortis* specimen. This information is available free of charge via the Internet at http://pubs.acs.org.

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