# Eunicellin Diterpenes from Two Kenyan Soft Corals

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Six new eunicellin diterpenes designated klyxumines A and B (**1**, **2**) and epoxycladines A−D (**3**−**6**) were isolated from *Klyxum flaccidum* and *Cladiella kashmani*, collected in Kenya. The structures of the compounds were elucidated by interpretation of MS, COSY, HMQC, HMBC, and NOESY data.

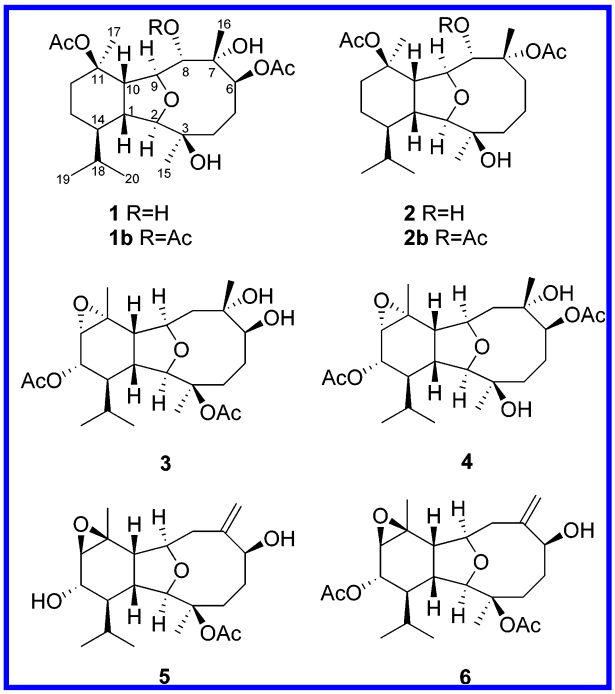
As part of our ongoing efforts to isolate biologically active compounds from marine invertebrates,[1] the constituents of the Kenyan soft corals *Klyxum flaccidum* and *Cladiella kashmani* were examined and six new eunicellin diterpenes were isolated, i.e., klyxumines A and B (**1**, **2**) from *Klyxum flaccidum* and epoxycladines A−D (**3**−**6**) from *Cladiella kashmani*. *Klyxum* is a new genus most recently formed from the soft coral *Alcyonium* by Alderslade, who gave arguments for this delineation.[2] The species classified to the new genus are tropical Indo-Pacific and contain symbiotic algae (zooxanthellae) in their tissue. The colonies are mostly very soft and highly contractile and may appear slightly translucent. All species that are now included in the genus *Klyxum* are listed by Alderslade.[2]

Eunicellin-based diterpenes are found both in soft corals and in gorgonian octocorals[3] and exhibit a wide range of biological activities that are of ecological, agrochemical, and pharmacological importance.[4] Examples of the ecological- and agrochemical-related activities exhibited by eunicellin-based diterpenes include molluscicidal activity and repellent activity against the muricid gastropods of the genus *Drupella*,[5] hemolytic activity,[6] inhibition of cell division in fertilized starfish eggs,[7] and insect growth inhibitory activity.[8] Several compounds have been reported to be antiinflammatory and antitumor agents,[9,10] exemplifying their pharmacological potential.

The petroleum ether extract of *K. flaccidum* was subjected to vacuum-liquid chromatography (VLC) over deactivated silica gel, using heptane with increasing proportions of ethyl acetate as eluent. Repeated chromatographies over silica gel, of fractions obtained from the first performed VLC, afforded compounds **1** and **2**.

The ethyl acetate extract of *C. kashmani* was subjected to VLC over deactivated silica gel, using heptane with increasing proportions of ethyl acetate as eluent. Repeated chromatographies over silica gel and Sephadex LH-20 columns of fractions obtained from the first performed VLC afforded compounds **3**−**6**.

The electrospray mass spectrum of klyxumine A (**1**) exhibited a molecular ion $[M + H]^+$ at *m/z* 457, and the base peak observed was a $[M + H - H_2O]^+$ peak at *m/z* 439. Comprehensive NMR analysis, summerized in Table 1 and Figures 1 and 2 as well as HRESMS experiments determined the molecular formula to be $C_{24}H_{40}O_8$. The compound was found to have a eunicellin skeleton with oxygen functionalities on C-3, C-6, C-7, C-8, and C-11, of which three are hydroxyls and two are acetate groups.




To establish the location of the acetates, compound **1** was acetylated to yield the 6,8,11-triacetate **1b**. In the $^1$H NMR spectrum of **1b**, a downfield shift was clearly observed for H-8 (3.93 ppm in **1** vs 5.22 ppm in **1b**), thus indicating that in **1** the oxygen functionality on C-8 is a secondary hydroxyl group. After acetylation no shift was observed for H-6; moreover, H-6 resonates at a relatively low field compared to a proton geminal to a hydroxyl group. Thus, it was established that one of the original acetate groups is connected to C-6. The location of the second acetate group of **1** was established by comparing the proton chemical shifts of the methyl groups attached to C-3, C-7, and C-11. Whereas the methyl groups on C-3 and C-7 appeared at $\delta_H$ 1.37 and 1.36 ppm, respectively, the methyl group on C-11 appeared at $\delta_H$ 1.54 ppm; therefore it was determined that the second acetate group of **1** is attached to C-11.[1] The relative configuration of the 10 asymmetric centers was determined on the basis of the coupling constants and correlations observed in the NOESY spectrum. H-14 is a double double triplet presenting 2.3, 4.7, and 12.1 Hz coupling constants, indicating that H-14 and H-1 must both be in the axial position. H-1 is a double doublet presenting 12.1 and 6.5 Hz coupling constants due to coupling to H-14 and H-10, respectively. The 6.5 Hz coupling constant (which is in close agreement with the respective coupling constant in previously reported eunicellin compounds)[11] suggested that H-10 is *cis* to H-1. The equatorial position

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**Table 1.** NMR Data for Klyxumines A (**1**) and B (**2**) and Klyxumine B Acetate (**2b**)

| | klyxumine A (**1**) | | klyxumine B (**2**) | | klyxumine B acetate (**2b**) | |
|---|---|---|---|---|---|---|
| no. | $\delta_C$, ppm[a,b] | $\delta_H$, ppm (mult)[c,d,e] | $\delta_C$, ppm [f,b] | $\delta_H$, ppm (mult)[g,d,e] | $\delta_C$ [f,b] | $\delta_H$, ppm (mult)[g,d,e] |
| 1 | 42.1 CH | 2.31 (dd, 12.1, 6.5) | 43.5 CH | 2.26 (m) | 42.4 CH | 2.58 (dt, 3.5, 7.7) |
| 2 | 89.2 CH | 3.59 (s) | 92.5 CH | 3.30 (s) | 91.5 CH | 3.45 (d, 3.2) |
| 3 | 85.7 C | | 86.4 C | | 85.0 C | |
| 4 | 34.1 $CH_2$ | 2.26 (dd, 14.3, 5.5) | 37.8 $CH_2$ | 2.56 (ddd, 12.5, 8.0, 1.9) | 36.2 $CH_2$ | 2.62 (dd, 14.5, 10.4) |
| | | 1.94 (m) | | 1.40 (m) | | 1.57 (m) |
| 5 | 27.8 $CH_2$ | 1.90 (m) | 19.6 $CH_2$ | 1.76 (m) | 19.6 $CH_2$ | 1.92 (m) |
| | | 1.66 (m) | | 1.69 (m) | | 1.78 (m) |
| 6 | 79.6 CH | 4.50 (d, 9.2) | 45.4 $CH_2$ | 2.41 (ddd, 15.6, 7.2, 2.1) | 44.4 $CH_2$ | 2.48 (ddd, 15.6, 6.8, 1.6) |
| | | | | 2.30 (m) | | 2.16 (m) |
| 7 | 85.6 C | | 86.2 C | | 79.5 C | |
| 8 | 76.5 CH | 3.93 (s) | 78.1 CH | 4.08 (d, 7.0) | 76.3 CH | 5.82 (d, 9.8) |
| 9 | 80.8 CH | 4.12 (dd, 12.4, 1.3) | 80.4 CH | 3.66 (s) | 78.5 CH | 3.85 (dd, 9.8, 4.9) |
| 10 | 45.3 CH | 3.47 (ddd, 12.0, 6.2, 1.6) | 52.5 CH | 3.67 (s) | 51.0 CH | 3.28 (dd, 5.3, 6.9) |
| 11 | 81.7 C | | 84.0 C | | 83.0 C | |
| 12 | 34.0 $CH_2$ | 1.92 (m) | 33.3 $CH_2$ | 2.20 (m) | 31.0 $CH_2$ | 2.39 (m) |
| | | 1.62 (m) | | 1.28 (m) | | 1.31 (m) |
| 13 | 18.7 $CH_2$ | 1.46 (m) | 18.7 $CH_2$ | 1.45 (m) | 18.9 $CH_2$ | 1.36 (m) |
| 14 | 40.9 CH | 1.31 (ddt 12.1, 4.7, 2.3) | 43.4 CH | 1.10 (dt, 3.1, 11.1) | 42.4 CH | 1.18 (dt, 3.0, 7.7) |
| 15 | 23.4 $CH_3$ | 1.37 (s) | 23.9 $CH_3$ | 1.33 (s) | 23.3 $CH_3$ | 1.44 (s) |
| 16 | 23.3 $CH_3$ | 1.36 (s) | 25.5 $CH_3$ | 1.60 (s) | 26.3 $CH_3$ | 1.64 (s) |
| 17 | 26.2 $CH_3$ | 1.54 (s) | 26.8 $CH_3$ | 1.81 (s) | 24.5 $CH_3$ | 1.51 (s) |
| 18 | 28.0 CH | 1.86 (m) | 29.9 CH | 1.61 (m) | 29.5 CH | 1.66 (m) |
| 19 | 21.8 $CH_3$ | 0.98 (d, 6.9) | 22.4 $CH_3$ | 0.87 (d) | 22.3 $CH_3$ | 0.92 (d) |
| 20 | 14.7 $CH_3$ | 0.82 (d, 6.9) | 16.3 $CH_3$ | 0.76 (d) | 16.6 $CH_3$ | 0.77 (d) |
| 21 | 170.4 C | | 170.0 C | | 169.5 C | |
| 22 | 22.1 $CH_3$ | 2.01 (s) | 22.5 $CH_3$ | 1.92 (s) | 20.9 $CH_3$ | 1.80 (s) |
| 23 | 170.3 C | | 170.1 C | | 170.0 C | |
| 24 | 22.1 $CH_3$ | 2.01 (s) | 22.8 $CH_3$ | 1.74 (s) | 21.9 $CH_3$ | 2.14 (s) |
| 25 | | | | | 169.0 C | |
| 26 | | | | | 22.1 $CH_3$ | 1.66 (s) |

[a] $CD_3COCD_3$, Bruker Avance-400 instrument, chemical shifts refer to $CD_3COCD_3$ ($\delta_C$ = 29.8, 205.7 ppm). [b] Multiplicities were determined by DEPT and HMQC experiments. [c] $CD_3COCD_3$, Bruker ARX-500 instrument, chemical shifts refer to TMS ($\delta_H$ = 0). [d] The CH correlations were assigned by a HMQC experiment. [e] Multiplicity and coupling constants are indicated in parentheses. [f] $C_6D_6$, Bruker Avance-400 instrument, chemical shifts refer to $C_6D_6$ ($\delta_C$ = 128.0). [g] $C_6D_6$, Bruker ARX-500 instrument, chemical shifts refer to TMS ($\delta_H$ = 0).

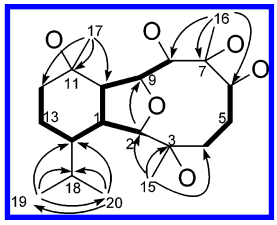

**Figure 1.** Selected COSY (—) and HMBC (↷) correlations in **1**.

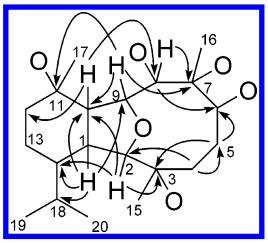

**Figure 2.** Additional HMBC correlations in **1**.

of Me-17 was determined on the basis of NOESY correlations (Figure 3) between the latter and H-8, H-9, and H-10. The above conclusions regarding the cyclohexane ring were further corroborated by NOESY correlations between H-1 and both H-10 and Me-20 as well as between H-2 and both H-14 and H-18. The α position of Me-15 was determined on the basis of a NOESY correlation between Me-15 and H-2. A NOESY correlation between H-8 and H-10 determined the $\beta$ position of H-8. Another correlation between Me-16 and both H-8 and H-10 established the $\beta$ position of Me-16. H-6 is a doublet presenting a coupling constant of 9.2 Hz; namely, H-6 is coupled to only one of the protons at position 5. To satisfy this condition, it is suggested that H-6 is in the α position. Thus the relative configuration of

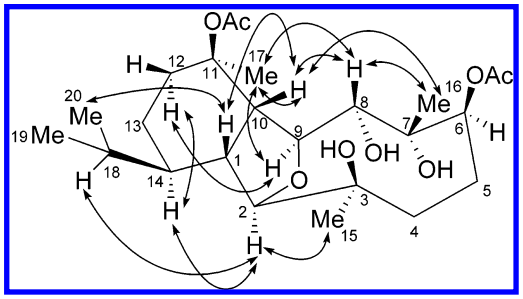

**Figure 3.** Selected NOESY correlations in **1**.

all chiral centers was determined to be 1*R**, 2*R**, 3*R**, 6*S**, 7*R**, 8*S**, 9*S**, 10*R**, 11*R**, 14*R**. It is of note that H-4A (in the following discussions A and B denote upfield and downfield resonances, respectively, of a geminal pair) resonates 0.32 ppm lower than H-4B and H-5A resonates 0.24 ppm lower than H-5B as a result of the spatial proximity of H-4A and H-5A to the oxygen atom of the ether bridge.

The electrospray mass spectrum of klyxumine B (**2**) exhibited a molecular ion $[M + Na]^+$ at *m/z* 463, and the molecular formula was determined to be $C_{24}H_{40}O_7$ on the basis of HRESMS. Comprehensive NMR analysis, summarized in Table 1 and Figures 4 and 5, determined the eunicellin skeleton with oxygen functionalities on C-3, C-7, C-8, and C-11, of which two are hydroxyls and two are acetates.

To establish the location of the acetate groups, compound **2** was acetylated to yield the triacetate **2b**. In the $^1H$ NMR spectrum of **2b** (Table 1), a downfield shift was clearly observed for H-8 (5.82 ppm in **2b** vs 4.08 ppm in **2**); moreover, upfield shifts were observed for C-7 and C-9 in

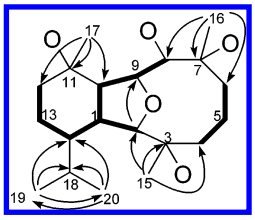

**Figure 4.** Selected COSY (—) and HMBC (↷) correlations in **2**.

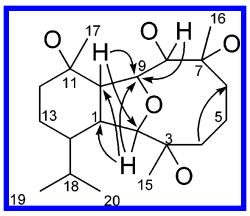

**Figure 5.** Additional HMBC correlations in **2**.

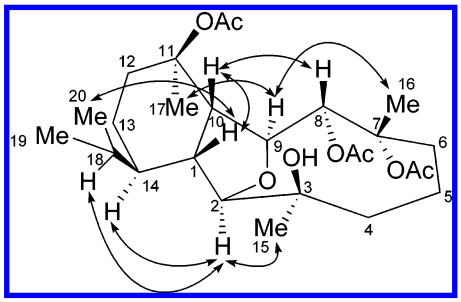

**Figure 6.** Selected NOESY correlations in **2b**.

**2b** in comparison with their respective chemical shifts in compound **2**, i.e., for C-7, 79.5 ppm in **2b** vs 86.2 ppm in **2**, and for C-9, 78.5 ppm in **2b** vs 80.4 ppm in **2**. Thus, it was concluded that in **2** the oxygen functionality on C-8 is a hydroxyl group. The three methyl groups 15, 16, and 17, which are geminal to an oxygen functionality, resonate at $\delta_H$ 1.33, 1.60, and 1.81, respectively (in compound **2**), indicating that the two acetate groups are attached at C-7 and C-11 and that the hydroxyl moiety is attached at C-3.[11] Compound **2** was thus concluded to be the 6-deacetoxy-7-acetyl analogue of compound **1**.

The relative configuration of the asymmetric centers was determined on the basis of the coupling constants and correlations observed in the NOESY spectrum of the acetate derivative **2b** (Figure 6) since in the case of **2b** the hydrogen resonances were better resolved. Analysis of the coupling constants and correlations observed in the NOESY spectrum of **2b** led to the conclusion that the relative configuration of C-3, C-7, and C-8 in compound **2b** is identical to that of the respective centers in compound **1**. A clearly observed correlation in the NOESY spectrum between H-1 and H-10 established the ring juncture as *cis*, as in **1**. When the coupling constants of H-1 and H-14 in **2b** were compared to the coupling constants of these same protons in **1**, major differences were observed. Whereas in **1** H-1 is a double doublet presenting 12.1 and 6.5 Hz coupling constants, in **2b** H-1 is a clear double triplet presenting coupling constants of 3.5 and 7.7 Hz, respectively. In **1**, H-14 is a double double triplet presenting 2.3, 4.7, and 12.1 Hz coupling constants, and in **2b** H-14 is a double triplet presenting 3.0 and 7.7 Hz coupling constants. It appears that no axial−axial coupling constants are exhibited by either H-1 or H-14 in **2b**; thus it was concluded that in **2b** both H-1 and H-14 are in the equatorial position toward the cyclohexane ring, while in **1** these same protons are axial. The *cis* ring juncture allows conformational freedom, and thus the whole system in **2b** adopts a different conformation than in **1**. Therefore, the relative configuration was established as 1*R**, 2*R**, 3*R**, 7*R**, 8*S**, 9*S**, 10*R**, 11*R**, 14*R**.

**Table 2.** NMR Data for Epoxycladines A (**3**) and B (**4**)

| | epoxycladine A (**3**) | | epoxycladine B (**4**) | |
|---|---|---|---|---|
| no. | $\delta_C$[a,b] | $\delta_H$ (mult)[c,d,e] | $\delta_C$[f,b] | $\delta_H$ (mult)[g,d,e] |
| 1 | 42.0 CH | 1.97 (m) | 43.2 CH | 2.15 (m) |
| 2 | 89.7 CH | 3.78 (br s) | 90.6 CH | 3.76 (br s) |
| 3 | 86.0 C | | 86.8 C | |
| 4 | 33.2 $CH_2$ | 2.31 (dd, 11.2, 13.2) | 34.7 $CH_2$ | 2.50 (dd, 9.8, 14.7) |
| | | 1.87 (m) | | 2.09 (m) |
| 5 | 30.6 $CH_2$ | 1.77 (m) | 29.0 $CH_2$ | 1.58 (m) |
| | | 1.44 (m) | | 1.43 (m) |
| 6 | 77.3 CH | 4.59 (br s) | 83.0 CH | 5.59 (d, 6.1) |
| 7 | 75.5 C | | 75.6 C | |
| 8 | 47.0 $CH_2$ | 1.86 (m) | 47.4 $CH_2$ | 2.02 (br d, 14.8) |
| | | 1.59 (m) | | 1.77 (dd, 14.5, 3.4) |
| 9 | 77.3 CH | 4.88 (dt, 3.6, 11.9) | 77.8 CH | 4.68 (m) |
| 10 | 47.7 CH | 1.94 (m) | 48.0 CH | 2.34 (m) |
| 11 | 58.9 C | | 59.6 C | |
| 12 | 60.9 CH | 3.13 (d, 1.7) | 62.2 CH | 3.22 (br s) |
| 13 | 70.8 CH | 5.21 (dd, 1.5, 8.4) | 71.3 CH | 5.15 (d, 10.0) |
| 14 | 39.8 CH | 2.03 (dt, 1.7, 8.5) | 38.9 CH | 1.88 (t, 10.6) |
| 15 | 22.9 $CH_3$ | 1.40 (s) | 23.3 $CH_3$ | 1.40 (s) |
| 16 | 22.9 $CH_3$ | 1.17 (s) | 22.7 $CH_3$ | 1.21 (s) |
| 17 | 21.9 $CH_3$ | 0.98 (s) | 23.3 $CH_3$ | 1.32 (s) |
| 18 | 29.5 CH | 1.58 (m) | 29.0 CH | 1.72 (m) |
| 19 | 22.9 $CH_3$ | 0.97 (d, 7.1) | 23.8 $CH_3$ | 1.13 (d, 6.6) |
| 20 | 16.2 $CH_3$ | 0.75 (d, 6.9) | 15.9 $CH_3$ | 0.84 (d, 6.8) |
| 21 | 169.6 C | | 174.5 C | |
| 22 | 20.4 $CH_3$ | 1.72 (s) | 22.3 $CH_3$ | 2.14 (s) |
| 23 | 168.1 C | | 170.9 C | |
| 24 | 21.3 $CH_3$ | 1.66 (s) | 21.4 $CH_3$ | 2.07 (s) |

[a] $C_6D_6$, Bruker Avance-400 instrument, chemical shifts refer to $C_6D_6$ ($\delta_C$ = 128.0 ppm). [b] Multiplicities were determined by DEPT and HMQC experiments. [c] $C_6D_6$, Bruker ARX-500 instrument, chemical shifts refer to TMS ($\delta_H$ = 0). [d] The CH correlations were assigned by a HMQC experiment. [e] Multiplicity and coupling constants are indicated in parentheses. [f] $CDCl_3$, Bruker Avance-400 instrument, chemical shifts refer to $CDCl_3$ ($\delta_C$ = 77.0). [g] $CDCl_3$, Bruker ARX-500 instrument, chemical shifts refer to TMS ($\delta_H$ = 0).

Oxygenation at C-8 is quite rare, and of all eunicellins, only four are oxygenated at C-8 and those are sclerophytins C and D,[12] litophynol B,[6] and sclerophytin C-6-ethyl ether,[13] which is considered an artifact. The relative stereochemistry of C-3, C-6, C-7, and C-8 in **1** is identical to that of the respective asymmetric centers in sclerophytins C and D[12] and litophynol B.[6] The absolute configuration of sclerophytin C was established by X-ray analysis, and the NMR data of the compound was reported in $CDCl_3$. The NMR data of klyxumine A, which was recorded in $CD_3COCD_3$, displayed a different set of multiplicities and coupling constants for the respective hydrogens. This fact, however, can be explained by suggesting that klyxumine A and sclerophytin C adopt different conformations in each solvent system. Compound **2** is the first compound reported in which C-8 is oxygenated while C-6 is not. It is of note that the relative stereochemistry of the asymmetric centers C-3, C-7, and C-8 in compound **2** is identical to that of the respective centers in scelerophytins C and D and in litophynol B.

From the second investigated soft coral, *C. kashmani*, we have isolated four new eunicellins, namely, epoxycladines A−D (**3**−**6**).

The CI mass spectrum of epoxycladine A (**3**) exhibited a molecular ion $[M + H]^+$ at *m/z* 455. Comprehensive NMR analysis, summerized in Table 2 and Figures 7 and 8, determined that the molecular formula was $C_{34}H_{38}O_8$ and

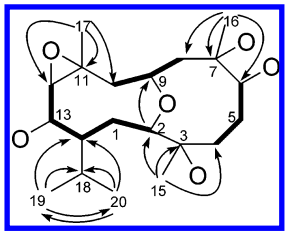

**Figure 7.** Selected COSY (—) and HMBC (↷) correlations in **3**.

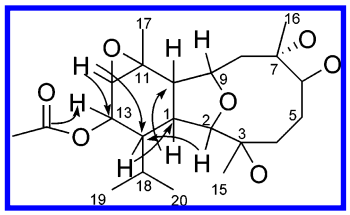

**Figure 8.** Additional HMBC correlations in **3**.

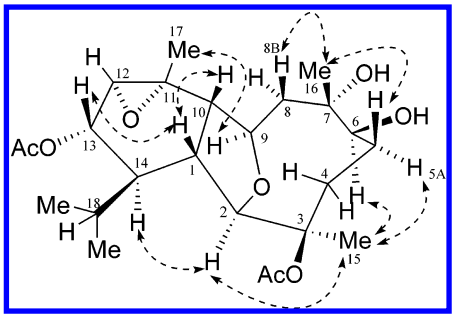

**Figure 9.** Selected NOESY correlations in **3**.

the compound had a eunicellin skeleton with oxygen functionalities on C-3, C-7, C-8, C-11, C-12, and C-13. C-11 and C-12, which resonate at 58.9 and 60.9 ppm, respectively, were suggested to comprise an epoxide moiety. Of the other oxygen functionalities, two were determined to be hydroxyls and two acetates. A correlation in the HMBC spectrum from H-13 to the carbonyl of an acetate group at $\delta_C$ 169.6 ppm implied that one acetate group is connected to C-13 (Figure 8).

In the $^1$H NMR spectrum of compound **3** in DMSO-$d_6$, the two hydroxyl protons were observed at 4.08 (s) and 4.02 (d) ppm. The former showed correlations in the HMBC spectrum to C-8, C-6, and C-7, and the latter showed a correlation in the COSY spectrum to H-6. Thus, it was concluded that the oxygen functionalities on C-6 and C-7 are in fact a secondary hydroxyl and a tertiary hydroxyl, respectively, and that the second acetate group was connected to C-3.

The relative configuration of the 11 asymmetric centers was determined on the basis of the coupling constants, correlations observed in the NOESY spectrum (Figure 9), and comparison to literature data of other eunicellin-based 11,12-epoxides. Clearly observed correlations in the NOESY spectrum between H-1 and H-10 established the ring juncture as *cis*. The $\beta$ position of H-13 was implied on the basis of a NOESY correlation between H-13 and H-1. H-14 is a double triplet presenting coupling constants of 1.7 Hz due to coupling to H-18 and 8.5 Hz due to couplings to both H-1 and H-13; thus it was concluded that H-14 is in the pseudoaxial α position. Correlations observed in the NOESY spectrum between H-14 and H-2, between H-2 and Me-15, and between Me-15 and both H-5A and H-6 implied that H-14, H-2, Me-15, H-5A, and H-6 are all in α positions. Correlations observed between H-5B and Me-16 as well as between Me-16 and H-8B implied that these protons are

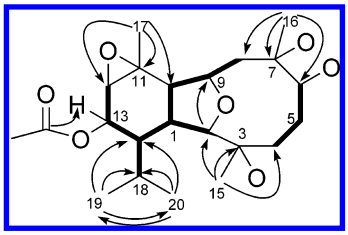

**Figure 10.** Selected COSY (—) and HMBC (↷) correlations in **4**.

all in the $\beta$ position. In this context it should be mentioned that a weak correlation in the NOESY spectrum was observed also between Me-16 and H-6, which is allowed if the C-6−C-7 bond rotates and the 10-membered ring adopts another conformation. At this stage the configuration of the epoxide ring was left to be determined, and this was done by comparing the chemical shift of H-9 in compound **3** to related compounds with the 11,12-epoxide in the α position vs compounds in which the epoxide is in the $\beta$ position relative to the plane defined by C-10, C-11, C-12, C-13, H-12, and C-17. In the $^1$H NMR spectrum of compound **3** in $CDCl_3$, H-9 resonates at $\delta_H$ 4.60 ppm. There is only one previously isolated compound in which the epoxide is in the α position. In the latter compound,[14] which is acetylated on both C-3 and C-6 but is not oxygenated on C-13, H-9 resonates at $\delta_H$ 4.63 ppm (relatively low field due to the effect of the epoxide oxygen atom). In contrast, in a synthetic analogue[12] of the latter in which the epoxide is in the $\beta$ position (and the oxygen is remote) H-9 resonates at $\delta_H$ 4.35 ppm. In calicophirin A,[15] in which the epoxide is also in the $\beta$ position, H-9 resonates at $\delta_H$ 4.45 ppm. Thus, the epoxide in compound **3** was determined to be in the α position and the relative configuration was determined to be 1*R**, 2*R**, 3*R**, 6*S**, 7*S**, 9*R**, 10*S**, 11*R**, 12*S**, 13*S**, 14*R**.

In the CI mass spectrum of epoxycladine B (**4**) a molecular ion was not observed; however, a base peak [M + H − $H_2O$]$^+$ was observed at *m/z* 436. Comprehensive NMR analysis, summerized in Table 2 and Figure 10, determined the compound to have a eunicellin skeleton with oxygen functionalities on C-3, C-7, C-8, C-11, C-12, and C-13. As in the case of compound **3**, C-11 and C-12, which resonate at 59.6 and 62.2 ppm, respectively, were suggested to comprise an epoxide moiety. Of the other oxygen functionalities, two were determined to be hydroxyls and two acetates. The $^{13}$C NMR spectrum of compound **4** highly resembles that of **3**, and the $R_f$ values of both compounds are also alike (ca. 0.4−0.5, Merck DC-Plastic folien Kieselgel 60 F254, 50% ethyl acetate in heptane); thus it was postulated that although the molecular ion peak was not observed, the actual molecular weight of compound **4** was 454 and the molecular formula of compound **4** was identical to that of **3**, namely, $C_{24}H_{38}O_8$. In addition, the $^1$H NMR spectrum of compound **4** in $CDCl_3$ was almost identical to the spectrum of **3**; the only major difference was observed in the oxygenated methines region. A clear downfield shift was observed for H-6, which resonated at $\delta_{H(CDCl3)}$ 4.40 ppm in compound **3** vs $\delta_{H(CDCl3)}$ 5.59 ppm in compound **4**. This implied that C-6 in compound **4** was acetylated. A correlation in the HMBC spectrum from H-13 to a carbonyl of an acetate implied that C-13 was acetylated as well, and the remaining oxygen functionalities on C-3 and C-7 were hydroxyls. Analysis of the coupling constants as well as interpretation of NOESY correlations (Figure 11) in the manner explained above in detail established that the relative configuration of **4** is identical to that of **3**, namely, 1*R**, 2*R**, 3*R**, 6*S**, 7*S**, 9*R**, 10*S**, 11*R**, 12*S**, 13*S**, 14*R**.

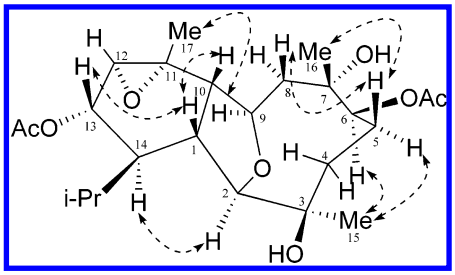

**Figure 11.** Selected NOESY correlations in **4**.

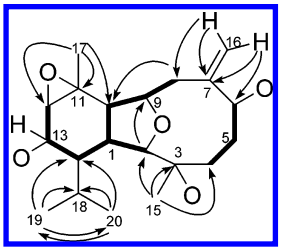

**Figure 12.** Selected COSY (—) and HMBC (↷) correlations in **5**.

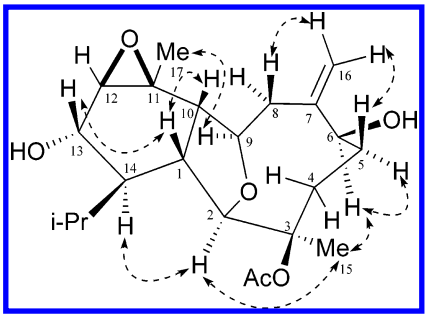

**Figure 13.** Selected NOESY correlations in **5**.

Comprehensive NMR analysis of compound **5** summerized in Table 2 and Figure 12 determined that the molecular formula was $C_{22}H_{34}O_6$ and the compound had a eunicellin skeleton with oxygen functionalities on C-3, C-7, C-11, C-12, and C-13. As in the case of compounds **3** and **4**, C-11 and C-12, which resonate at 58.9 and 65.8 ppm, respectively, were suggested to comprise an epoxide moiety. Of the other oxygen functionalities, two were determined to be hydroxyls and one was determined to be an acetate.

In compound **5**, H-13 resonates at $\delta_{H(CDCl3)}$ 3.97 ppm vs $\delta_{H(CDCl3)}$ 5.16 ppm in compound **4**, implying that the oxygen functionality on C-13 is a hydroxyl and that the acetate group could be connected either to C-3 or to C-6. The location of the acetate group was determined by comparing the chemical shifts of H-6 and Me-15 to these same protons in labiatin C[16] and palmonine B.[11] Both compounds contain an exocyclic double bond C-7−C-16, but whereas in labiatin C, C-6 is substituted by a hydroxyl group and C-3 is substituted by an acetate, in palmonine B, both positions are acetylated. In labiatin C, H-6 resonates at $\delta_{H(CDCl3)}$ 4.61 and Me-15 resonates at $\delta_{H(CDCl3)}$ 1.60, and in palmonine B, H-6 resonates at $\delta_{H(CDCl3)}$ 5.15 and Me-15 resonates at $\delta_{H(CDCl3)}$ 1.54. On the basis of these chemical shifts it can readily be deduced that in compound **5**, in which H-6 resonates at $\delta_{H(CDCl3)}$ 4.68 and Me-15 resonates at $\delta_{H(CDCl3)}$ 1.63, C-6 is hydroxylated and C-3 acetylated.

Analysis of coupling constants of the protons of the asymmetric centers as well as interpretation of the NOESY spectrum (Figure 13) led to the conclusion that the relative configuration of C-1, C-2, C-3, C-6, C-9, C-10, and C-13 in compound **5** is identical to that of the respective carbons in compounds **3** and **4**. The configuration of the epoxide was established on the basis of the chemical shift of H-9 as explained in detail above.[14] In contrast to compounds **3** and **4**, in compound **5**, the epoxide was determined to be in the $\beta$ position since H-9 resonates at 4.43 ppm (vs 4.60 and 4.68 ppm in **3** and **4**, respectively). Moreover, in compound **5** H-10 resonates at 2.52 ppm (vs 2.34 ppm in compound **4**). This downfield shift is caused by the spatial proximity between H-10 and the epoxide oxygen and thus further corroborates the suggested $\beta$ position for the epoxide. Thus, the relative configuration was determined to be 1*R**, 2*R**, 3*R**, 6*S**, 9*R**, 10*S**, 11*S**, 12*R**, 13*S**, 14*R**.

**Table 3.** NMR Data for Epoxycladines C (**5**) and D (**6**)

| | epoxycladine C (**5**) | | epoxycladine D (**6**) | |
|---|---|---|---|---|
| no. | $\delta_C$[a,b] | $\delta_H$ (mult)[c,d,e] | $\delta_C$[a,b] | $\delta_H$, ppm (mult)[c,d,e] |
| 1 | 42.3 CH | 2.12 (m) | 42.0 CH | 2.15 (m) |
| 2 | 88.8 CH | 3.71 (br s) | 88.8 CH | 3.73 (br s) |
| 3 | 84.4 C | | 84.5 C | |
| 4 | 27.4 $CH_2$ | 2.26 (m) | 27.5 $CH_2$ | 2.25 (m) |
| | | 1.61 (m) | | 1.59 (m) |
| 5 | 29.5 $CH_2$ | 2.22 (m) | 29.3 $CH_2$ | 2.20 (m) |
| | | 1.54 (m) | | 1.50 (m) |
| 6 | 85.9 CH | 4.68 (dd, 3.9, 11.4) | 86.0 CH | 4.68 (dd, 4.1, 11.6) |
| 7 | 146.2 C | | 146.2 C | |
| 8 | 41.9 $CH_2$ | 2.95 (dd, 4.5, 14.1) | 42.0 $CH_2$ | 2.95 (dd, 4.6, 13.8) |
| | | 2.52 (m) | | 2.50 (m) |
| 9 | 79.9 CH | 4.43 (dd, 4.2, 9.6) | 80.2 CH | 4.46 (dd, 4.8, 8.8) |
| 10 | 41.8 CH | 2.52 (m) | 42.0 CH | 2.50 (m) |
| 11 | 58.9 C | | 58.1 C | |
| 12 | 65.8 CH | 3.15 (br s) | 62.3 CH | 3.21 (br s) |
| 13 | 69.7 CH | 3.97 (d, 9.4) | 72.1 CH | 5.16 (d, 10.6) |
| 14 | 43.1 CH | 1.42 (m) | 39.4 CH | 1.77 (dt, 5.2, 13.0) |
| 15 | 21.6 $CH_3$ | 1.63 (s) | 21.6 $CH_3$ | 1.64 (s) |
| 16 | 117.2 $CH_2$ | 5.41 (s) | 117.3 $CH_2$ | 5.42 (s) |
| | | 5.22 (s) | | 5.22 (s) |
| 17 | 24.0 $CH_3$ | 1.41 (s) | 23.8 $CH_3$ | 1.39 (s) |
| 18 | 27.0 CH | 1.96 (m) | 26.7 CH | 1.97 (m) |
| 19 | 25.0 $CH_3$ | 1.15 (d, 7.2) | 24.3 $CH_3$ | 1.06 (d, 7.1) |
| 20 | 16.2 $CH_3$ | 0.96 (d, 6.7) | 16.3 $CH_3$ | 0.80 (d, 6.6) |
| 21 | 170.0 C | | 169.6 C | |
| 22 | 22.5 $CH_3$ | 1.92 (s) | 22.5 $CH_3$ | 1.91 (s) |
| 23 | | | 170.8 C | |
| 24 | | | 21.5 $CH_3$ | 2.13 (s) |

[a] $CDCl_3$, Bruker Avance-400 instrument, chemical shifts refer to $CDCl_3$ ($\delta_C$ = 77.0 ppm). [b] Multiplicities were determined by DEPT and HMQC experiments. [c] $CDCl_3$, Bruker ARX-500 instrument, chemical shifts refer to TMS ($\delta_H$ = 0). [d] The CH correlations were assigned by a HMQC experiment. [e] Multiplicity and coupling constants are indicated in parentheses.

The $^1$H NMR and $^{13}$C NMR spectra of compound **6** (Table 3) greatly resembled those of compound **5**. A major difference in the $^1$H NMR spectrum was observed in the oxygenated methines region. A clear downfield shift was observed for H-13, which resonated at $\delta_{H(CDCl3)}$ 5.16 in compound **6** vs $\delta_{H(CDCl3)}$ 3.97 in compound **5**. Furthermore, the $^{13}$C NMR spectrum of **6** revealed the presence of another acetate group, indicating that in compound **6** C-13 is acetylated. Another downfield shift was observed for H-14, which resonated at $\delta_{H(CDCl3)}$ 1.77 in compound **6** vs $\delta_{H(CDCl3)}$ 1.42 ppm in compound **5**, due to a $\gamma$ effect by the methyl of the C-13 acetate group.

Since compound **6** deteriorated rapidly, the NOESY experiment could not be performed. Still, the relative configuration could be determined for several of the asymmetric centers assuming the α position for H-2 and H-9 and the $\beta$ position for H-1 and H-10, which is the case in all eunicellin diterpenes. The epoxide in compound **6** was established to be in the $\beta$ position as in **5**, on the basis of the chemical shift of H-9 (4.46 ppm) and H-10 (2.50 ppm)

as outlined above for compound **5**. H-14 is a double triplet presenting coupling constants of 5.2 Hz due to coupling to H-18 and 10.6 Hz due to coupling to both H-1 and H-13; thus it was determined that H-13, H-14, and H-1 are all axial. The relative configuration of C-3 and C-6 in compound **6** was tentatively determined to be identical to their relative configuration in compound **5** on the basis of the close agreement of the chemical shifts of H-2, H-4A, H-4B, H-5A, H-5B, H-6, H-8A, and H-8B in compounds **5** and **6**.

Epoxycladines A−D (**3**−**6**) are all characterized by an 11,12-epoxide moiety and oxygenation on C-13. Each of these moieties is rare among eunicellins, and the only compound that features both is calicophirin A.[15] Due to the reactivity of the epoxide functionality, the epoxycladines decomposed rapidly. The relative configuration of the asymmetric centers C-3, C-6, and C-7 in compounds **3** and **4** as well as C-3 and C-6 in compounds **5** and **6** is identical to that of the respective centers in klyxumines A and B (**1** and **2**).

## Experimental Section

**General Experimental Procedures.** Optical rotations were obtained with a Jasco P-1010 polarimeter. IR spectra were obtained with a Bruker FTIR Vector 22 spectrometer. $^1$H and $^{13}$C NMR spectra were recorded on Bruker ARX-500 and Avance-400 spectrometers. $^1$H, $^{13}$C, COSY, HMQC, and HMBC spectra were recorded using standard Bruker pulse sequences. EIMS and CIMS measurements were recorded on a Fisons, Autospec Q instrument. Electrospray MS measurements were performed on an AppliedBiosystems Q-STAR Pulsar instrument.

**Biological Material.** The soft coral *Klyxum flaccidum* (Tixier-Durivault, 196**6**) was collected off Likoni, Shelly Reef, Kenya, 12−14 m in depth, February 8, 2003. A voucher specimen is deposited at the Zoological Museum, Tel Aviv University, Israel (ZMTAU CO 32221). The site has diverse assemblages of benthic organisms, mainly soft corals. This species has been originally described from Madagascar, and now its distribution encompasses numerous reefs throughout the Indian Ocean including the northern Red Sea. The aggregated colonies of this species are characterized by their dark brown polyps and the flabby lobes.

The soft coral *Cladiella kashmani* Benayahu & Schleyer, 1996 was collected in Kitagamwa, southern Kenya (February 5, 2003). A voucher specimen is deposited at the Zoological Museum, Tel Aviv University, Israel (ZMTAU Con 32222). This soft coral was collected on a patchy reef, at a depth of 8−12 m, characterized by rich fauna comprised of many other soft corals, sponges, and tunicates. This soft coral has been first described from KwaZulu-Natal, South Africa, and Bazaruto Island, Mozambique, and later was recorded in several reefs along the coast of Kenya.

**Extraction and Isolation.** Freeze-dried *K. flaccidum* (10 g) was homogenized and extracted with PE to give a brown gum (680 mg). The extract was subjected to VLC over deactivated silica gel, using heptane with increasing proportions of ethyl acetate as eluent, to afford 20 fractions. The fraction eluted with 20% ethyl acetate in heptane (30 mg) was subjected to another VLC separation over silica gel, and compound **2** (3.1 mg, 0.03%) was eluted with 10% ethyl acetate in heptane. The fraction eluted with 26% ethyl acetate in heptane in the first column (62 mg) was subjected to another VLC separation over deactivated silica gel. The fraction eluted with ethyl acetate in the latter separation (12 mg) was further chromatographed on a silica gel column, and compound **1** (6 mg, 0.06%) was afforded eluting with 80% ethyl acetate in heptane.

Freeze-dried *C. kashmani* (40 g) was extracted with ethyl acetate, and after filtration the crude extract was evaporated under vacuum to yield a green oily residue (1.00 g). The residue was repeatedly chromatographed on a Sephadex LH-20 column, eluting with petroleum ether−$CHCl_3$−MeOH (2:1:1), and was subjected to VLC over deactivated silica gel, using solvent mixtures of increasing polarity from petroleum ether to ethyl acetate, to yield compounds **3**−**6**. Compound **3** (9 mg, 0.022% dry wt) was eluted with 30% acetone in petroleum ether, compound **4** (6 mg, 0.015% dry wt) was eluted with 16% ethyl acetate in petroleum ether, compound **5** (3.1 mg, 0.021% dry wt) was eluted with 20% ethyl acetate in petroleum ether, and compound **6** (7 mg, 0.018% dry wt) was eluted with 30% acetone in petroleum ether.

**Compound 1:** colorless oil; $[\alpha]^{25}_D$ 10.5° (*c* 0.27, acetone); for $^1$H and $^{13}$C NMR data, see Table 1; ESMS *m/z* (%) 457 $[M + H]^+$ (4), 439 $[M + H - H_2O]^+$ (100), 379 $[M + H - H_2O - CH_3COOH]^+$ (35), 319 $[M + H - H_2O - 2CH_3COOH]^+$ (50); HRESMS *m/z* 457.2751 (calcd for $C_{24}H_{41}O_8$, 457.2795), *m/z* 439.2677 (calcd for $C_{24}H_{39}O_7$, 439.2690).

**Acetylation of 1 to 1b.** Compound **1** (3 mg) was dissolved in a mixture of pyridine−acetic anhydride (1:1) and was left to react overnight. Then the reaction mixture was evaporated to dryness to afford **1b**.

**Compound 1b:** colorless oil; $[\alpha]^{25}_D$ 6.8° (*c* 0.23, acetone); ESMS *m/z* (%) 499 $[M + H]^+$, 481 $[M + H - H_2O]^+$ (100), 439 $[M + H - CH_3COOH]^+$ (30), 361 (35).

**Compound 2:** colorless oil; for $^1$H and $^{13}$C NMR data, see Table 1; ESMS *m/z* (%) 463 $[M + Na]^+$ (50); HRESMS *m/z* 463.2677 (calcd for $C_{24}H_{40}O_7Na$, 453.2666).

**Acetylation of 2 to 2b.** Compound **2** (3 mg) was dissolved in a mixture of pyridine−acetic anhydride (1:1) and was left to react overnight. The reaction mixture was evaporated to dryness to afford **2b**.

**Compound 2b:** colorless oil; $[\alpha]^{25}_D$ 12° (*c* 0.067, acetone); for $^1$H and $^{13}$C NMR data, see Table 1; ESMS *m/z* (%) 505 $[M + Na]^+$ (50).

**Compound 3:** colorless oil; $[\alpha]^{25}_D$ −12° (*c* 0.34, $CHCl_3$); for $^1$H and $^{13}$C NMR data, see Table 2; CIMS *m/z* (%) 455 $[M + H]^+$ (10), 437 $[M + H - H_2O]^+$ (100), 419 $[M + H - 2H_2O]^+$ (15), 395 $[M + H - Ac]^+$ (70), 377 $[M + H - Ac - H_2O]^+$ (90), 359 $[M + H - Ac\text{-}2H_2O]^+$ (15), 335 $[M + H - 2Ac]^+$ (40), 317 $[M + H - 2Ac - H_2O]^+$ (50), 299 $[M + H - 2Ac - 2H_2O]^+$ (15).

**Compound 4:** colorless oil; $[\alpha]^{25}_D$ −11° (*c* 0.44, $CHCl_3$); for $^1$H and $^{13}$C NMR data, see Table 2; CIMS *m/z* (%) 437 $[M + H - H_2O]^+$ (40), 419 $[M + H - 2H_2O]^+$ (25), 377 $[M + H - Ac - H_2O]^+$ (100), 359 $[M + H - Ac - 2H_2O]^+$ (20), 335 $[M + H - 2Ac]^+$ (10), 317 $[M + H - 2Ac - H_2O]^+$ (55), 299 $[M + H - 2Ac - 2H_2O]^+$ (15).

**Compound 5:** colorless oil; $[\alpha]^{25}_D$ −9° (*c* 0.40, $CHCl_3$); for $^1$H and $^{13}$C NMR data, see Table 3; CIMS *m/z* (%) 393 $[M - H]^+$ (15), 377 $[M + H - H_2O]^+$ (25), 333 $[M - H - 60]$ (85), 317 $[M + H - Ac - H_2O]^+$ (50), 299 $[M + H - Ac - 2H_2O]^+$ (15).

**Compound 6:** colorless oil; $[\alpha]^{25}_D$ −21° (*c* 0.27, $CHCl_3$); for $^1$H and $^{13}$C NMR data, see Table 3; CIMS *m/z* (%) 419 $[M + H - H_2O]^+$ (40).

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**Supporting Information Available:** Additional details regarding structure elucidation and 2D NMR data for compounds **1**−**6**. This material is available free of charge via the Internet at http://pubs.acs.org.

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