Cembrane Diterpenes from the Southern African Soft Coral *Cladiella kashmani*

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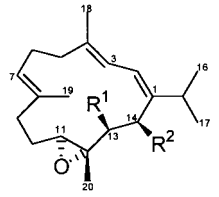
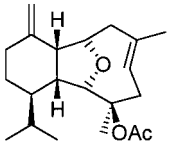
Two known cembrane diterpenes, flaccidoxide (**1**) and (1*Z*,3*E*,7*E*,11*S*,12*S*,14*S*)-11,12-epoxy cembra-1,3,7-trien-14-ol (**2**), and the new cembrane diterpene flaccidoxide-13-acetate (**3**) were isolated from specimens of *Cladiella kashmani* collected off Ponto do Oura, Mozambique. The modified Mosher's method established the previously unassigned absolute configuration of **1** as (1*Z*,3*E*,7*E*,11*S*,12*S*,13*S*,14*R*)-14-acetoxy-11,12-epoxy cembra-1,3,7-trien-13-ol. Acetylation of **1** yielded **3** and thus confirmed the structure of **3** as (1*Z*,3*E*,7*E*,11*S*,12*R*,13*S*,14*R*)-13,14-diacetoxy-11,12-epoxy cembra-1,3,7-triene. All three diterpenes were toxic to the brine shrimp *Artemia salina*.

In continuation of our search for biologically active metabolites from southern African marine soft corals,[1] we have examined, from Mozambique, a specimen of the recently described octocoral species *Cladiella kashmani* Benayahu and Schleyer (1996).[2] An EtOAc extract of this organism yielded the known cembranoids flaccidoxide (**1**)[3] and (1*Z*,3*E*,7*E*,11*S*,12*S*,14*S*)-11,12-epoxy cembra-1,3,7-trien-14-ol (**2**).[4] In addition to these two compounds, a new acetylated derivative of flaccidoxide, flaccidoxide-13-acetate (**3**), was isolated from the *C. kashmani* extract. Application of the modified Mosher's method to **1** followed by acetylation of **1** to give **3** established the absolute configuration of these two compounds. This is the first reported isolation of cembranoids from *Cladiella*, a genus that, until now, has yielded predominantly tricyclic eunicellane (or cladiellane) diterpenes[5,6] (e.g., cladiellin, **4**). Although the occurrence of cembranoids in *Cladiella* is unusual, it is possible that the biosynthesis of eunicellane diterpenes may involve an internal cyclization of a cembrane precursor.[6,7]



1 R^1 = OH, R^2 = OAc
2 R^1 = H, R^2 = OH
3 R^1 = R^2 = OAc



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Specimens of *C. kashmani* were collected using scuba from the Malangan Reef, Ponto do Oura, Mozambique, in Spring 1995. The frozen soft coral was freeze-dried and steeped in EtOAc. *Artemia salina* larvicidal bioassay-guided[8] fractionation of a portion of the EtOAc extract yielded flaccidoxide (**1**, 0.014% dry wt), (1*Z*,3*E*,7*E*,11*S*,12*S*,-14*S*)-11,12-epoxy cembra-1,3,7-trien-14-ol (**2**, 0.006% dry wt), and flaccidoxide-13-acetate (**3**, 0.013% dry wt) as colorless oils.

The molecular formula of the most polar metabolite **1**, $C_{22}H_{34}O_4$, was determined from HREIMS data. The ^{13}C NMR data indicated that this compound possessed an 11,12-epoxy cembranoid skeleton incorporating an acetoxy and a hydroxy moiety. The placement of these functionalities followed from HMQC and HMBC data, and the structure of **1** was determined to be (1*Z*,3*E*,7*E*)-14-acetoxy-11,12-epoxy cembra-1,3,7-trien-13-ol, the spectral data of which were consistent with the spectral data ($[\alpha]_D$, UV, IR, NMR, MS) reported by Kashman et al.[3] for flaccidoxide.

Table 1. ^{13}C and ^1H NMR Data for Compound **3**[a]

carbon	δ_C[b]	δ_H[c]
1	139.8 s	
2	125.0 d	6.26 d 1H (11.8)
3	121.3 d	6.05 d 1H (11.8)
4	139.5 s	
5	40.1 t	2.11 m 1H, 2.25 m 1H
6	25.9 t	2.09 m 1H, 2.29 m 1H
7	126.3 d	5.17 m 1H
8	134.0 s	
9	36.5 t	2.19 m 2H
10	24.6 t	1.66 m 1H, 1.33 m 1H
11	58.4 d	3.08 dd 1H (8.8, 2.6)
12	59.9 s	
13	73.2 d	5.50 d 1H (9.7)
14	68.6 d	5.75 d 1H (9.7)
15	28.3 d	2.59 septet 1H (6.8)
16	24.7 q	1.03 d 3H (6.8)
17	24.8 q	1.03 d 3H (6.8)
18	16.2 q	1.75 s 3H
19	15.0 q	1.44 s 3H
20	16.8 q	1.27 s 3H
13-OAc	170.6 s	
	20.7 q	2.12 s 3H
14-OAc	169.1 s	
	20.8 q	1.94 s 3H

[a] Values in ppm, spectra acquired in $CDCl_3$. [b] 100 MHz, multiplicity by DEPT. [c] 400 MHz, coupling constants (Hz) in parentheses.

HREIMS data also provided the molecular formula of **2** ($C_{20}H_{32}O_2$). Comparison of the spectral data of **2** (UV, IR, NMR, MS) with those reported previously[4,9] for (1*Z*,3*E*,7*E*,-11*S*,12*S*,14*S*)-11,12-epoxy cembra-1,3,7-trien-14-ol confirmed the structure of this compound. The large, positive optical rotation obtained for **2** (+203°) is consistent with that reported by Bowden et al.[4] ($[\alpha]_D$ +229°), confirming the 11*S*, 12*S*, and 14*S* absolute stereochemistry of the compound isolated from *C. kashmani*.

Although the ^1H and ^{13}C NMR spectra of **3** (Table 1) were very similar to those of **1**, the spectra of the former compound contained extra signals in accordance with the

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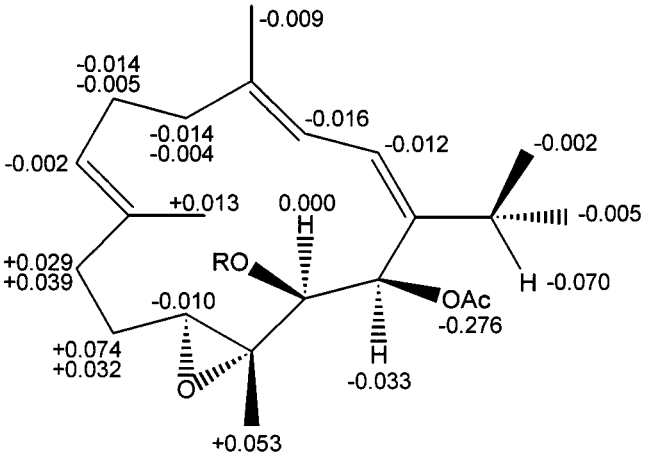

Figure 1. $\Delta\delta$ values ($\delta_S - \delta_R$) for (*R*)- and (*S*)-MTPA esters of **1**. Spectra recorded at 400 MHz, values reported in ppm (R = MTPA ester moiety).

presence of an additional acetate moiety [δ_H 2.12, 3H (s) and δ_C 170.6 (s) and 20.7 (q)]. The molecular formula of **3**, $C_{24}H_{36}O_5$, determined by HREIMS, confirmed the inference made from the NMR data and suggested that **3** was the 13-acetate ester of flaccidoxide (**1**). Accordingly, acetylation of **1** yielded an oil spectroscopically indistinguishable from **3**, unequivocally establishing the structure of **3** as (1*Z*,3*E*,7*E*)-13,14-diacetoxy-11,12-epoxy cembra-1,3,7-triene.

The absolute configuration of flaccidoxide (**1**) was unknown, and we consequently tackled the stereochemistry of **1** using the modified Mosher's method of Ohtani et al.[10] Cognisant of possible anomalies in the application of Mosher's method to hindered alcohol functionalities on the cembrane skeleton,[11] we approached the interpretation of the Mosher's data with caution. The ^{1}H NMR and COSY spectra of the (*R*)- and (*S*)-2-methoxy-2-phenyl-2-(trifluoromethyl)acetic acid (MTPA) esters of **1** were assigned, and the calculated $\Delta\delta$ values [δ of protons in the (*S*)-MTPA ester $-$ δ of the corresponding protons in the (*R*)-MTPA ester] are shown in Figure 1. None of the $\Delta\delta$ incongruities observed by Kusumi *et al.*[11] for hindered cembranoid alcohols was apparent, and the arrangement of positive and negative $\Delta\delta$ values around the cembranoid ring were consistent, with only a single anomalous negative $\Delta\delta$ value obtained for H-11 proving to be an exception. This anomaly was investigated as follows. Molecular modeling studies[12] of the (*R*)-MTPA ester of **1** revealed that, in the 'ideal' Mosher's conformation,[10] H-11 lies both close to, and in, the plane of the aromatic ring and is therefore slightly deshielded and not shielded as expected. The 0.02-ppm downfield chemical shift of H-11 in the (*R*)-MTPA ester of **1** (cf **3**) possibly lends support to this argument. A similar molecular modeling study of the (*S*)-MTPA ester showed that the H-11 proton is the closest proton to the OMe moiety of the MTPA ester and is accordingly also weakly deshielded. The net result of these findings is that H-11 is more deshielded in the (*R*)-MTPA ester of **1** than in the (*S*)-MTPA ester, which results in a small negative $\Delta\delta$ value and explains the observed anomoly. The 13*S* stereochemistry, thus established for **1**, was related to the other stereogenic centers in this compound from a combination of 1D NOEDS experiments (Table 2) and molecular modeling studies (Figure 2)[12] to assign an 11*S*,12*S*,13*S*,14*R* stereochemistry for **1**. The absolute configuration of flaccidoxide (**1**) and flaccidoxide-13-acetate (**3**) were shown to be the same from optical rotation measurements, with authentic **3** giving a rotation of +158° and **3** obtained from

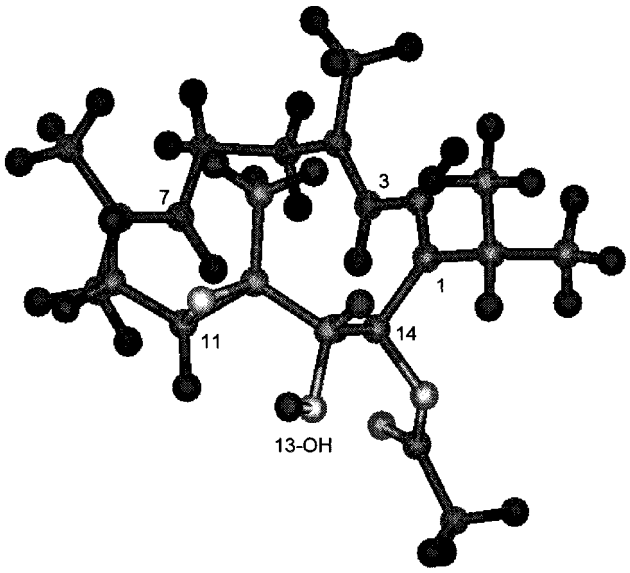

Figure 2. Energy minimized conformation of flaccidoxide (**1**).

Table 2. Observed NOE Enhancements for Compound **1**

irradiated ^{1}H	NOE correlated protons
H-11	H-3, H-7, H-9, H-13, H-14
H-13	H-15, H-20
H-14	H-3, H-11, 14OAc
H-15	H-13
H-20	H-7, H-10, H-13, H-14
14-OAc	H-11, H-13, H-14

acetylation of **1**, a rotation of +162°. Taking into account the Cahn−Ingold−Prelog priority reversal at C-12, arising from acetylation at C-13, the absolute configuration of **3** is assigned as 11*S*,12*R*,13*S*,14*R*. These stereochemical assignments are consistent with those reported for 13-functionalized[13] and 14-functionalized[3,9] 11,12-epoxy cembranoids.

All three diterpenes were toxic to *A. salina* and displayed an interesting range in activity. The LC_{50} values estimated by probit analysis[14] were flaccidoxide-13-acetate (**1**), 180 ppm; flaccidoxide (**2**), 50 ppm; and compound **3**, 110 ppm.

Experimental Section

General Experimental Procedures. IR and UV spectra were recorded on a Perkin-Elmer Spectrum 2000 FT-IR spectrometer and a GBC UV/vis 916 spectrometer, respectively. The ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AMX400 spectrometer. Optical rotations were measured on a Perkin-Elmer 141 polarimeter, and low resolution mass spectra were recorded on a Finnigan GCQ mass spectrometer. High-resolution mass spectra were obtained by Dr. P. Boshoff of the Mass Spectrometry Unit, Cape Technikon, Cape Town. Semi-preparative HPLC separations were performed on a Whatman Magnum 9 Partisil 10 column.

Animal Material. *C. kashmani* Benayahu and Schleyer (1996) (class Octocorallia, order Alcyonacea, family Alcyoniidae)[2] was collected at a depth of 13 m from the Malangan Reef, off Ponto do Oura, Mozambique (26° 46.8′ S, 32° 53.9′ E) in October 1995. A voucher specimen of *C. kashmani* is located in the marine invertebrate collection housed at Rhodes University (MOZ 95−021).

Isolation Procedures. The soft coral was immediately frozen after collection and later freeze-dried (440 g). All of the freeze-dried soft coral was extracted with EtOAc to give a brown gum (11.7 g), a portion of which (5.0 g) was initially flash chromatographed on Si gel (gradient elution; 1:1, 3:2, and 4:1 EtOAc/hexane and 100% EtOAc). Excessive amounts of cholesterol were removed from several of the flash chroma-

tography fractions by crystallization from MeOH. Subsequent fractionation was bioassay-guided, and further Si gel column chromatography (gradient elution; 100% hexane, 9:1, 4:1, and 7:3 hexane/EtOAc, and 100% EtOAc) was necessary before selected fractions could be subjected to normal-phase HPLC (8:2 and 7:3 hexane/EtOAc) to yield compounds **1** (27 mg), **2** (11 mg), and **3** (25 mg).

Assessment of Biological Activity. *A. salina* larvicidal bioassays were performed as described by Solis et al.[8] Estimates of median lethal concentration for each of the compounds were obtained by probit analysis[14] of *A. salina* mortality data from 12 solutions across a concentration range of 400−12.5 μg/mL.

(1*Z*,3*E*,7*E*,11*S*,12*R*,13*S*,14*R*)-14-Acetoxy-11,12-epoxy cembra-1,3,7-trien-13-ol (1): colorless oil; $[\alpha]^{21}_D$ +104.2° (*c* 0.17, $CHCl_3$); UV, IR (film), MS, and ^{1}H, ^{13}C NMR data consistent with literature values;[3] HREIMS *m*/*z* 362.2459 (calcd for $C_{22}H_{34}O_4$, 362.2457); toxic to *A. salina*, LC_{50} = 42 μg/mL (95% confidence levels 38, 47), equivalent to 0.12 mM (95% confidence levels 0.10, 0.13).

Acetylation of 1. Compound **1** (10.8 mg) was dissolved in pyridine (0.5 mL) and Ac_2O (0.5 mL) and stirred at room temperature for 48 h. Excess pyridine and Ac_2O were removed under reduced pressure to give a brown oil (12.0 mg). Normal-phase HPLC (7:3 hexane/EtOAc) of the crude product yielded a yellow oil (2.6 mg, $[\alpha]^{21}_D$ +162°), which was identical to the diacetate **3** in all respects.

Preparation of the (*R*)- and (*S*)-MTPA Esters of 1. (*R*)-MPTA (23.6 mg), dicyclohexylcarbodiimide (35.5 mg), and 4-dimethyl aminopyridine (7.1 mg) were added to a solution of **1** (6.2 mg) in dry CH_2Cl_2 (1.0 mL). The solution was stirred overnight at room temperature, diluted with EtOAc (5.0 mL) and H_2O (0.5 mL), and filtered. The resulting solution was washed with 0.2 M HCl (5.0 mL), H_2O (5.0 mL), saturated $NaHCO_3$ (5.0 mL), and H_2O (5.0 mL). The EtOAc solution was dried over anhydrous Na_2SO_4, the solvent removed under reduced pressure, and the resultant oil further purified by normal-phase HPLC (4:1 hexane/EtOAc) to yield the (*R*)-MTPA ester of **1** as a colorless oil (1.2 mg): ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.30 (1H, d, J = 11.5 Hz, H-2), 6.09 (1H, d, J = 11.5 Hz, H-3), 5.82 (1H, d, J = 9.2 Hz, H-14), 5.70 (1H, d, J = 9.1 Hz, H-13), 5.18 (1H, m, H-7), 3.10 (1H, br d, J = 8.8 Hz, H-11), 2.66 (1H, m, H-15), 2.30 (1H, m, H-6a), 2.23 (1H, m, H-9a), 2.20 (1H, m, H-5a), 2.15 (1H, m, H-9b), 2.13 (1H, m, H-6b), 2.09 (1H, m, H-5b), 1.80 (3H, s, 14-OAc), 1.76 (3H, s, 3H-18), 1.62 (1H, m, H-10a), 1.42 (3H, s, 3H-19), 1.25 (1H, m, H-10b), 1.20 (3H, s, 3H-20), 1.07 (3H, d, J = 6.8 Hz, 3H-16), 1.05 (3H, d, J = 6.8 Hz, 3H-17).

The (*S*)-MTPA ester of **1** (0.6 mg) was prepared in the same manner as above: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.29 (1H, d, J = 11.6 Hz, H-2), 6.07 (1H, d, J = 11.7 Hz, H-3), 5.79 (1H, d, J = 9.2 Hz, H-14), 5.70 (1H, d, J = 9.2 Hz, H-13), 5.18 (1H, m, H-7), 3.09 (1H, br d, J = 8.6 Hz, H-11), 2.59 (1H, m, H-15), 2.29 (1H, m, H-6a), 2.26 (1H, m, H-9a), 2.19 (1H, m, H-5a), 2.19 (1H, m, H-9b), 2.11 (1H, m, H-6b), 2.09 (1H, m, H-5b), 1.76 (3H, s, 3H-18), 1.65 (1H, m, H-10a), 1.52 (3H, s, 14-OAc), 1.44 (3H, s, 3H-19), 1.32 (1H, m, H-10b), 1.25 (3H, s, 3H-20), 1.06 (3H, d, J = 6.8 Hz, 3H-16), 1.05 (3H, d, J = 6.8 Hz, 3H-17).

(1*Z*,3*E*,7*E*,11S,12S,14S)-11,12-Epoxy cembra-1,3,7-trien-14-ol (2): colorless oil; $[\alpha]^{21}_D$ +203.3° (*c* 0.33, $CHCl_3$); UV, IR (film), MS and ^{1}H, ^{13}C NMR data are consistent with literature values;[4,9] HREIMS *m*/*z* 304.2413 (calcd for $C_{20}H_{32}O_2$, 304.2402); toxic to *A. salina* LC_{50} = 107 μg/mL (95% confidence levels 91, 127) equivalent to 0.35 mM (95% confidence levels 0.30, 0.42).

(1*Z*,3*E*,7*E*,11S,12R,13S,14R)-13,14-Diacetoxy-11,12-epoxy cembra-1,3,7-triene (3): colorless oil; $[\alpha]^{21}_D$ +157.8° (*c* 0.78, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 252 (4.30) nm; IR (film) ν_{max} 2962, 2931, 1745, 1437, 1372, 1243, 1224, 1028, 965 cm^{-1}; ^{1}H NMR and ^{13}C NMR data, see Table 1; EIMS (70 eV) *m*/*z* 404 [M$^+$] (3), 233 (18), 213 (25), 191 (24), 152 (33), 137 (29), 121 (31), 119 (25), 109 (76), 95 (38), 93 (23), 81 (30), 43 (100); HREIMS *m*/*z* 404.2551 (calcd for $C_{24}H_{36}O_5$, 404.2562); toxic to *A. salina*, LC_{50} = 180 μg/mL (95% confidence levels 142, 243) equivalent to 0.45 mM (95% confidence levels 0.35, 0.60).

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(12) Molecular modeling was performed on a Silicon Graphics O2 computer employing the MSI Cerius^2 4.0 platform. A molecular dynamics routine was used to explore conformational space and establish global energy minima for various possible isomeric structures and conformers of **1** and its (*R*)- and (*S*)-MTPA esters.

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