New Oxygenated Africanenes from the Soft Coral *Sinularia dissecta*†

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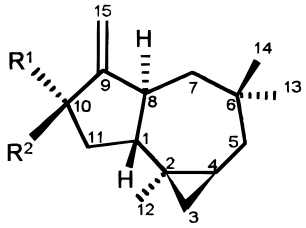
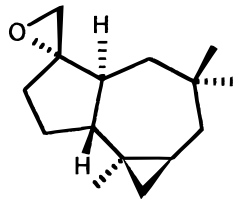
Three new oxygenated africanenes (**1**−**3**) have been isolated from the soft coral *Sinularia dissecta* and were characterized by spectral and chemical studies.

Soft corals are a rich source of terpenoids having a variety of biological activities.[1,2] In continuation of a search for biologically active compounds from marine organisms, we examined the soft coral *Sinularia dissecta* Tixier Durivault (Alcyoniidae) collected from the Mandapam coast of India during June 1996. In previous studies, we reported polyoxygenated furanocembrane-derived diterpenes from *S. dissecta*.[3,4] In the early 1980s, Kashman et al.[5] and Braekman et al.[6] independently reported a novel tricyclic sesquiterpene, $\Delta^{9(15)}$-africanene (**4**), from the soft corals *Sinularia erecta* and *Sinularia polydactila*, respectively. In this paper we report three novel oxygenated $\Delta^{9(15)}$-africanene natural products (**1**−**3**).



1 R^1 = OH R^2 = H
1a R^1 = OAc R^2 = H
1b R^1 = R^2 = O



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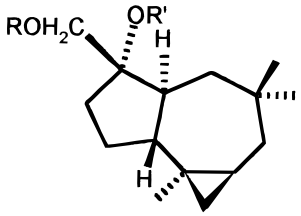
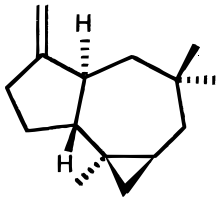


3 R = R' = H
3a R = Ac R' = H
3b R = Ac R' = Ac



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The 1:1 dichloromethane-methanol extract of the soft coral *S. dissecta* was partitioned between water and ethyl acetate. The concentrated ethyl acetate extract was subjected to gel filtration chromatography followed by silica gel chromatography to afford the known compounds $\Delta^{9(15)}$-africanene (**4**),[5,6] β-elemene,[7] batyl alcohol,[8] an isomandapamate,[9] and three oxygenated africanenes (**1**−**3**).

10α-Hydroxy-$\Delta^{9(15)}$-africanene (**1**) was obtained as a solid, mp 98 °C, $[\alpha]^{25}_D$ +19.1° (*c* 1.2, $CHCl_3$), which was transparent in its UV spectrum and analyzed for $C_{15}H_{24}O$ by HREIMS. The IR spectrum showed bands for the presence of alcohol (3500 cm^{-1}), terminal methylene (1620, 890 cm^{-1}), and geminal dimethyl groups (1375 cm^{-1}). Compound **1** formed a monoacetate (**1a**) upon acetylation with Ac_2O/pyridine.

The ^{1}H NMR spectrum of compound **1** showed signals for the presence of an exocyclic methylene at δ 5.15 (1H, d, J = 1.5 Hz) and 4.90 (1H, d, J = 1.5 Hz), an allylic methine-bearing hydroxyl at δ 4.45 (1H, br t, $W_{1/2}$ = 6 Hz), three tertiary methyls at δ 1.05 (3H, s), 0.95 (3H, s), and 0.90 (3H, s), and a trisubstituted cyclopropane ring at δ 0.54 (2H, m) and 0.25 (1H, m). The spectral data were similar to those of $\Delta^{9(15)}$-africanene (**4**)[5,6] except for the signal at δ 4.45, which was shifted downfield to δ 5.50 (1H, br t, $W_{1/2}$ = 6 Hz) in the corresponding acetate **1a**. Pyridinium chlorochromate (PCC) oxidation of compound **1** yielded an α,β-unsaturated ketone (**1b**) [UV λ_{max} (MeOH) 232 (9,800) nm], thus establishing the allylic nature of the alcohol, and this was also supported by ^{13}C NMR spectral signals[10] in the spectrum of **1** at δ 161.0 (s), 108.6 (t), and 75.5 (d) (Table 1). The stereochemistry of the OH group was established from ^{1}H−^{1}H COSY and NOESY correlations. The ^{1}H−^{1}H COSY spectrum revealed that the C-15 methylene protons at δ 5.15 and 4.90 showed correlations with the C-8 allylic methine proton at δ 2.46 (1H, m, H-8$_\alpha$), which in turn showed correlations with the C-1 methine proton at δ 1.88 (1H, m, H-1$_\beta$) and the C-7 methylene protons at δ 1.18 and 1.80 (each 1H, m, H-7), respectively. Further, the C-4 cyclopropane methine proton at δ 0.54 (1H, m) showed correlations with the C-5 methylene protons at δ 1.22 and 1.80 (each 1H, m, H-5) and one of the C-3 methylene protons at δ 0.25 (1H, m). In the NOESY spectrum, the C-10 allylic carbinol proton at δ 4.45 (1H, br t, $W_{1/2}$ = 6 Hz) showed strong correlations with the C-1 methine proton at δ 1.88 (1H, m, H-1$_\beta$) and did not show any correlation with the C-8 methine proton at δ 2.46 (1H, m), indicating that the hydroxyl group at C-10 is α oriented. Thus, the structure of compound **1** was established as 10α-hydroxy-$\Delta^{9(15)}$-africanene.

9α,15-Epoxyafricanane (**2**) was obtained as a solid, mp 125 °C, $[\alpha]^{25}_D$ +16.2° (*c* 1.1, $CHCl_3$), which showed no absorptions in the UV spectrum and analyzed for $C_{15}H_{24}O$ by elemental analysis. It was also found to be a sesquiterpene and its ^{1}H NMR spectrum was reminiscent of that of $\Delta^{9(15)}$-africanene (**4**). The ^{1}H NMR spectrum of compound **2** showed signals for the presence of cyclopropyl protons at δ 0.21 (1H, m, H-3$_\beta$) and 0.55 (2H, m, H-3$_\alpha$, H-4$_\alpha$), three tertiary methyls at δ 0.92 (3H, s, H-13), 0.95 (3H, s, H-12), and 1.0 (3H, s, H-14), and two methylene group signals at δ 3.57 (1H, d, J = 13.5 Hz, H-15) and 3.46 (1H, d, J = 13.5 Hz, H-15), respectively, and was devoid of exocyclic methylene protons at δ 4.65 (1H, brs) and 4.82 (1H, brs) as

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Table 1. ^{13}C NMR Data of Compounds **1**−**3** in $CDCl_3$

carbon	1	2	3
1	48.9 d	49.8 d	50.1 d
2	18.9 s	19.7 s	19.7 s
3	23.5 t	23.6 t	23.8 t
4	22.1 d	21.8 d	23.3 d
5	43.3 t	43.2 t	43.5 t
6	33.9 s	33.3 s	33.5 s
7	51.6 t	44.4 t	44.4 t
8	41.3 d	44.4 d	48.2 d
9	161.0 s	82.9 s	82.6 s
10	75.5 d	29.6 t	29.6 t
11	36.3 t	37.3 t	36.8 t
12	24.2 q	24.2 q	23.6 q
13	20.4 q	19.7 q	21.7 q
14	33.9 q	33.9 q	34.0 q
15	108.6 t	68.9 t	65.3 t

observed for $\Delta^{9(15)}$-africanene (**4**).[5,6] The methylene signals at δ 3.57 and 3.46 were attributed to the presence of an exocyclic epoxide bridging the C-9 and C-15 carbons of $\Delta^{9(15)}$-africanene. Further, the presence of an epoxide was also inferred from the ^{13}C NMR signals[10] at δ 82.9 (s) and 68.9 (t). The stereochemistry of the epoxide was established from $^1H-^1H$ COSY and NOESY correlations. The $^1H-^1H$ COSY spectrum revealed that one of the C-7 methylene protons at δ 1.44−1.38 (1H, m, H-7$_\beta$) showed correlations with the C-8 methine proton at δ 1.66−1.58 (1H, m, H-8$_\alpha$), which in turn showed a correlation with the C-1 methine proton at δ 1.76−1.66 (1H, m, H-1$_\beta$). Further, the C-4 cyclopropane methine proton at δ 0.55 (1H, m, H-4$_\alpha$) showed correlations with the C-5 methylene protons at δ 1.21−1.02 and 1.86−1.76 (each 1H, m, H-5) and one of the C-3 methylene protons at δ 0.21 (1H, m, H-3$_\beta$). In the NOESY spectrum, one of the C-15 methylene protons at δ 3.57 showed a strong correlation with one of the C-7 methylene protons at δ 1.44−1.38 (1H, m, H-7$_\beta$) but did not show any correlation with the C-8 methine proton at δ 1.66−1.58 (1H, m). The other C-15 methylene proton at δ 3.46 showed a strong correlation with the C-10 methylene proton at δ 1.76−1.66 (1H, m, H-10$_\beta$), indicating that the epoxide group is α-oriented. Thus, the structure of compound **2** was established as 9α,15-epoxyafricanane. The structure of compound **2** was further confirmed by reacting *m*-chloroperbenzoic acid with $\Delta^{9(15)}$-africanene (**4**) to yield a chromatographically inseparable C-9 epimeric mixture of compound **2**. The synthetically prepared epoxy compounds showed identical ^1H NMR and mass spectral data with those of natural product.

9α,15-Dihydroxyafricanane (**3**) was obtained as a crystalline solid, mp 113 °C, $[\alpha]^{25}_D$ +13.8° (*c* 1.25, $CHCl_3$) and analyzed for $C_{15}H_{26}O_2$ by elemental analysis. The IR spectrum (ν_{max} 3500 and 1375 cm^{-1}) indicated the presence of a hydroxyl group, and showed no absorptions in the UV spectrum. The ^1H NMR spectrum of compound **3** showed the presence of a trisubstituted cyclopropyl ring at δ 0.21 (1H, m) and 0.55 (2H, m), three tertiary methyls at δ 0.91 (3H, s), 0.96 (3H, s), and 1.05 (3H, s), and two protons at δ 3.56 (1H, d, J = 13 Hz, H-15) and 3.43 (1H, d, J = 13 Hz, H-15), which could be attributed to a methylene group bearing an oxygen atom. Further, compound **3** formed a monoacetate (**3a**) [^1H NMR δ 4.02 (2H, ABq, J = 13 Hz), and 2.01 (3H, s, −OCOCH$_3$)] and diacetate **3b** [^1H NMR δ 4.62 (1H, d, J = 13 Hz), 4.12 (1H, d, J = 13 Hz), 2.02 (3H, s, −OCOCH$_3$), and 2.04 (3H, s, −OCOCH$_3$)] upon acetylation with Ac_2O/pyridine, suggesting the presence of two hydroxyl groups. The ^{13}C NMR spectrum of **3** (Table 1) supported the presence of two oxygenated carbon signals at δ 82.6 (s) and 65.3 (t). The foregoing spectral data and a literature survey established the structure of compound **3** as 9,15-dihydroxyafricanane. Further, to establish the structure of compound **3**, $\Delta^{9(15)}$-africanane (**4**) was subjected to OsO_4 oxidation, to yield 9α,15-dihydroxyafricanane, whose structure was previously established by X-ray crystallography.[6] The physical (TLC and mp) and spectral data (^1H NMR and MS) of the synthetically prepared compound were found to be identical with those of the isolated natural diol **3**. Thus, the structure of compound **3** was established as 9α, 15-dihydroxyafricanane. The previous authors did not report $[\alpha]_D$ and ^{13}C NMR spectral data[6] for compound **3**. This is the first report of compound **3** as a natural product.

Experimental Section

General Experimental Procedures. Optical rotations were measured with a JASCO DIP-370 polarimeter. UV and IR spectra were recorded on Shimadzu and Perkin-Elmer 240C instruments. ^1H and ^{13}C NMR spectra were recorded on Varian Unity 400 MHz and Varian Gemini 200 MHz spectrometers using TMS as internal standard. Chemical shifts are reported in parts per million, and coupling constants (J) are expressed in Hz. Mass spectra were recorded on VG Auto Spec-M instrument. Elemental analyses were carried out on a Perkin-Elmer 240C instrument.

Animal Material. The soft coral *S. dissecta* (Tixier Durivault) (IIC-233) was collected on the Mandapam coast in the Gulf of Mannar, India during June 1996, and a voucher specimen (IIC-233) is on deposit at the National Institute of Oceanography, Goa, India.

Extraction and Isolation. The freshly collected specimen (1.5 kg dry weight after extraction) was extracted with CH_2Cl_2−MeOH (1:1, 3 × 1.5 L) at room temperature. The combined extracts were filtered and the solvent was removed under reduced pressure to yield a greenish gum (90 g). The crude extract was partitioned between water and ethyl acetate. The organic layer was concentrated under vacuum and subjected to gel filtration chromatography (Sephadex LH-20; 1:1 dichloromethane−methanol) followed by silica gel chromatography eluting with hexane, hexane/ethyl acetate mixtures, and finally with ethyl acetate to afford the known compounds $\Delta^{9,15}$-africanene (**4**, 500 mg), *β*-elemene (20 mg), batyl alcohol (200 mg), and isomandapamate (100 mg), and the new compounds **1** (40 mg), **2** (30 mg), and **3** (25 mg).

10α-Hydroxy-$\Delta^{9(15)}$-africanene (1): obtained as colorless needles (hexane); mp 98 °C; $[\alpha]^{25}_D$ +19.1° (*c* 1.2, $CHCl_3$); IR (KBr) ν_{max} 3500, 1620, 1375, 890 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 5.15 (1H, d, J = 1.5 Hz, H-15$_a$), 4.90 (1H, d, J = 1.5 Hz, H-15$_b$), 4.45 (1H, br t, $W_{1/2}$ = 6 Hz, H-10$_\beta$), 2.46 (1H, m, H-8$_\alpha$), 1.88 (1H, m, H-1$_\beta$), 1.80 (4H, m, H-5$_\beta$, H-7$_\beta$, H-11$_\alpha$, H-11$_\beta$), 1.22 (1H, m, H-5$_\alpha$), 1.18 (1H, m, H-7$_\alpha$), 1.05 (3H, s), 0.95 (3H, s), 0.90 (3H, s), 0.54 (2H, m, H-3$_\alpha$, H-4), 0.25 (1H, m, H-3$_\beta$); ^{13}C NMR ($CDCl_3$, 50 MHz), see Table 1; EIMS *m*/*z* 220 [M]$^+$ (8), 205 (16), 187 (12), 123 (30), 105 (43); HREIMS *m*/*z* 220.3673 (calcd for $C_{15}H_{24}O$, 220.3550).

Acetylation of Compound 1. A solution of **1** (5 mg) in Ac_2O/pyridine (0.25 mL) was allowed to stand at room-temperature overnight. After the usual workup, the crude product was chromatographed on silica gel to give the monoacetate **1a**: IR (neat) ν_{max} 1740, 1620, 1375, 780 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 5.50 (1H, br t, $W_{1/2}$ = 5.3 Hz), 5.25 (1H, d, J = 2 Hz), 5.0 (1H, br s), 2.1−2.2 (2H, m), 2.01 (3H, s), 1.5−1.9 (6H, m), 1.05 (3H, s), 0.95 (3H, s), 0.92 (3H, s), 0.56 (2H, m), 0.25 (1H, m); EIMS *m*/*z* 202 [M$^+$ − AcOH] (10), 187 (10), 167 (10), 149 (50), 123 (10), 105 (20), 43 (100).

Oxidation of Compound 1. To an ice cold solution of compound **1** (3 mg) in dry dichloromethane was added pyridinium chlorochromate (5 mg). This mixture was stirred for 6 h. After the usual workup, the ketone was isolated by silica gel column chromatography to yield compound **1b**: UV (MeOH) λ_{max} 232 (ϵ 9800) nm; IR ν_{max} (neat) 1680, 1620, 1040, 980 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 6.05 (1H, br s), 5.25

(1H, br s), 2.75 (2H, m), 1.45−1.85 (6H, m), 1.02 (3H, s), 0.96 (3H, s), 0.91 (3H, s), 0.55 (2H, m), 0.24 (1H, m); EIMS *m*/*z* 218 $[M]^+$ (10), 163 (5), 149 (15), 125 (70), 109 (20), 95 (30), 81 (40), 77 (80), 55 (60), 41 (100).

9α,15-Epoxyafricanane (2): obtained as an amorphous solid; mp 125 °C; $[\alpha]^{25}_D$ +16.2° (*c* 1.1, $CHCl_3$); IR (KBr) ν_{max} 2950, 1375, 1080 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 3.57 (1H, d, J = 13.5 Hz, H-15), 3.46 (1H, d, J = 13.5 Hz, H-15), 1.86−1.76 (2H, m, H-10$_\alpha$, H-5$_\alpha$), 1.76−1.66 (2H, m, H-10$_\beta$, H-1$_\beta$), 1.66−1.58 (2H, m, H-8$_\alpha$, H-11$_\beta$), 1.44−1.38 (1H, m, H-7$_\beta$), 1.25 (1H, m, H-11$_\alpha$), 1.21−1.02 (2H, m, H-7$_\alpha$, H-5$_\beta$), 1.00 (3H, s, H-14), 0.95 (3H, s, H-12), 0.92 (3H, s, H-13), 0.55 (2H, m, H-3$_\alpha$, H-4$_\alpha$), 0.21 (1H, m, H-3$_\beta$); ^{13}C NMR ($CDCl_3$, 50 MHz), see Table 1; EIMS *m*/*z* 189 $[M^+ - 31]$ (20), 123 (25), 95 (45); *anal.* C 81.74%, H 11.15%, calcd for $C_{15}H_{24}O$, C 81.76%, H 10.97%.

Epoxidation of $\Delta^{9(15)}$-Africanene (4). To a solution of compound **4** (10 mg) in dichloromethane (10 mL) was added *m*-chloroperbenzoic acid (15 mg) was added and the solution was stirred at 0 °C for 6 h. The reaction mixture was washed with saturated sodium bicarbonate solution and the organic layer was evaporated to give a C-9 epimeric mixture of compound **2**. The ^1H NMR and mass spectral data of the synthetically prepared epoxy compound was found to be identical with those of the isolated molecule (**2**).

9α,15-Dihydroxyafricanane (3): obtained as an crystalline solid; mp 113 °C; $[\alpha]^{25}_D$ +13.8° (*c* 1.25, $CHCl_3$); IR (neat) ν_{max} 3500, 2900, 1375 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 3.56 (1H, d, J = 13 Hz, H-15), 3.43 (1H, d, J = 13 Hz, H-15), 2.00 (2H, m), 1.86−1.76 (4H, m), 1.64−1.54 (2H, m), 1.38−1.22 (2H, m), 1.05 (3H, s), 0.96 (3H, s), 0.91 (3H, s), 0.55 (2H, m), 0.21 (1H, m); ^{13}C NMR ($CDCl_3$, 50 MHz), see Table 1; EIMS *m*/*z* 207 $[M^+ - 31]$ (2), 107 (35), 95 (40), 81(54); *anal.* C 75.58%, H 11.21%, calcd for $C_{15}H_{26}O_2$, C 75.52%, H 10.99%.

OsO_4 Oxidation of $\Delta^{9(15)}$-Africanene (4). To a solution of compound **4** (20 mg) in acetone/water (2 mL, 9:1) was added a catalytic amount of OsO_4 and the mixture was allowed to stir at room temperature. After 1 h, *N*-methylmorpholine *N*-oxide (20 mg) was added to the reaction mixture which was stirred at room-temperature overnight. The reaction mixture was quenched with saturated sodium bisulfite solution and acetone was evaporated under vacuum. The residue was extracted with ethyl acetate and the organic layer was washed with brine, and dried over sodium sulfate. Concentration of the organic layer, followed by silica gel column chromatography afforded the diol **3** (15 mg) as a crystalline solid. The physical (TLC and mp) and spectral data (^1H NMR and mass) of the synthetically prepared compound were found to be identical with those of the naturally isolated diol **3**.

Acetylation of Compound 3. A solution of **3** (10 mg) in Ac_2O/pyridine (0.2 mL) was allowed to stand at room temperature for 12 h. After the usual workup, the crude product was chromatographed on silica gel to give the monoacetate **3a** [7 mg; IR (neat) ν_{max} 3500, 1735, 1095 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 4.02 (2H, ABq, J = 13 Hz), 2.01 (3H, s), 1.6−1.9 (7H, m), 1.2−1.4 (3H, m), 0.95 (3H, s), 0.94 (3H, s), 0.89 (3H, s), 0.52 (2H, m), 0.21 (1H, m); EIMS *m*/*z* 280 $[M]^+$ (2), 220 (3), 207 (20), 133 (10), 107 (20), 95 (40), 55 (100)] and diacetate **3b** [4 mg; IR (neat) ν_{max} 1735, 1095 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 4.62 (1H, ABq, J = 13 Hz), 4.12 (1H, ABq, J = 13 Hz), 2.04 (3H, s), 2.02 (3H, s), 1.5−1.9 (7H, m), 1.1−1.4 (3H, m), 0.95 (3H, s), 0.90 (3H, s), 0.88 (3H, s), 0.52 (2H, m), 0.18 (1H, m); EIMS *m*/*z* 262 $[M^+ - AcOH]$ (2), 220 (5), 207 (10), 202 (30), 187 (20), 159 (30), 133 (10), 107 (20), 95 (40), 55 (100)].

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