Sarcodictyin A and Two Novel Diterpenoid Glycosides, Eleuthosides A and B, from the Soft Coral *Eleutherobia aurea*

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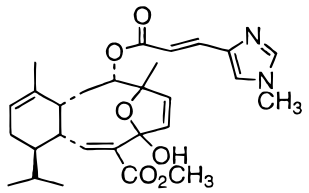
Sarcodictyin A and two novel diterpenoid glycosides, eleuthosides A and B, have been isolated from the soft coral *Eleutherobia aurea* collected from the Kwazulu−Natal Coast, South Africa. The structure determination of all three compounds was based mainly on 1D and 2D NMR spectroscopy and MS.

Alcyonarians of the order Alcyonacea, Gorgonacea, and Pennatulacea are known to contain a variety of terpenoids.[1] The order Alcyonacea, family Alcyoniidae, contains two closely related genera, *Alcyonium* Linnaens and *Eleutherobia* Pütter. Both genera are unbranched digitiform-to-cylindrical soft corals with monomorphic polyps whose status has not yet been completely resolved.

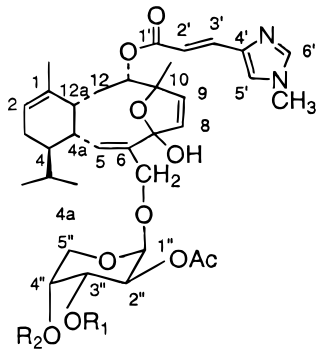
Recently we reported the isolation of two new xenicane diterpenoids, zahavins A and B, from a newly described cylindrical, gold Alcyoniidae Indo-Pacific soft coral.[2] A literature survey led to the conclusion that the latter newly described species from the Kwazulu−Natal Coast, South Africa, *Eleutherobia aurea* sp. nov., should belong to the *Eleutherobia* genus rather than *Alcyonium*.[3] Clearly, thorough research is still required to clarify the identification of these two closely related genera. Whereas chemical studies of the genus *Alcyonium* are well documented,[1,4,5] investigation of the less common genus *Eleutherobia* has not been reported.

During the past 2 years we have undertaken a periodical chemical analysis of several soft corals including *E. aurea*. More than 100 gram-scale samples from one colony of *E. aurea* have been examined, and remarkable chemical changes have been observed. In several of the examined samples we observed lowfield resonances in the δ_H 6.0−7.5 ppm region of the proton NMR spectra, leading to the previously reported discovery of sarcodictyin A (**1**) from the Stolonifer *Sarcodictyon roseum*.[4] In addition, several other new terpenoid glycosides were isolated in minute amounts. The structures of two of these glycosides, eleuthoside A and eleuthoside B, are the subject of this report.

A specimen of *E. aurea* (10 g) collected from the Kwazulu−Natal Coast during June 1995, (TASA 428) was extracted with EtOAc to afford a crude extract. Successive solvent partitioning between aqueous MeOH and hexane, CCl_4, and $CHCl_3$ gave a $CHCl_3$ fraction containing a mixture of compounds **1**−**3**. Silica gel chromatography of this mixture yielded compounds **1**−**3** (ca. 2 mg each) (0.02% dry wt). The ^{1}H-NMR spectra of these three compounds had numerous common features.



1 Sarcodictyin A



2 Eleuthoside A R_1=Ac, R_2=H

3 Eleuthoside B R_1=H , R_2=Ac

Compound **1** had the highest mass peak at *m*/*z* 496. HR MS revealed the composition $C_{28}H_{36}N_2O_6$, which is in full agreement with the 28 resonances in the ^{13}C-NMR spectrum. The latter signals, together with the ^{1}H-NMR data (Table 1), unequivocally identified compound **1** as sarcodictyin A, a hydroxy diterpenoid methyl ester esterified with (*E*)-*N*(1)-methylurocanic acid.[5]

The second compound, eleuthoside A (**2**), had FABMS and NMR data consistent with a composition $C_{36}H_{48}N_2O_{11}$. Comparison of the NMR data of **2** with the data of **1** (Table 1) implied the presence of the same oxygen-bridged bicyclo[8,4,0]tetradecatriene skeleton as in **1**, as well as the presence of the hemiacetal and the *N*-methylurocanate ester, and the absence of the C(6) carbomethoxy group. The *N*-methylurocanate moiety ($C_7H_7N_2O$) was unequivocally confirmed by fragments of *m*/*z* 135 (100%) and *m*/*z* 153 ($C_7H_9N_2O_2$) as well as M − 135 and M − 152 in the MS of **2**, as previously reported,[4] and also measured by us for **1**. The ^{1}H- and ^{13}C-NMR spectra of **2** contained two additional oxymethylene groups, three oxymethine groups, one characteristic acetal group (δ_C 112.3 s) and two acetates. There was a change in the chemical shift of H-5 from 6.97 to 5.83 ppm, together with an upfield shift of C(5)

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Table 1. ^{13}C- and ^{1}H-NMR Data (125 and 500 MHz) Including HH- and CH-Correlations of **1** and **2**[a,b]

	1			**2**			
position	δ_C[c]	δ_H[c]	δ_H[c]	δ_C[d]	δ_H[d]	COSY (H to H)	HMBC (H to C)
1	134.33 s			134.1 s			
2	121.78 d	5.26 br s	5.32br s	121.3 d	5.28 m	12a	
3	24.58 t	a 2.38 br d	a 2.42 br d	24.4 t	a 2.33br d	3b, 4, Me_2CH	
		b 1.94 br d	b 1.90 br s		b 2.02 m	3a, 4, Me_2CH	
4	42.09 d	1.17 m	1.10 m	42.1 d	1.25 m	3, 4a, Me_2CH	
4a	34.92 d	4.58 ddd	4.43 m	34.3 d	4.05 m	4, 5, 12a	
5	143.91 d	6.97 d	5.83 (d,9.7)	137.9 d	5.52 (d,9.2)	4a	4,7,$CH_2C(6)$
6	135.54 s			133.3 s			
7	112.28 s			112.3 s			
8	134.66 d	7.12 d	6.23 (d,5.7)	133.2 d	6.08 (d,5.6)	9	7, 9,10
9	132.97 d	6.28 d	6.76 (d,5.7)	132.1 d	6.13 (d,5.6)	8	7, 8, 10
10	89.64 s			90.3 s			
11	81.77 d	5.18 br d	5.22 (d,7.3)	81.1 d	4.82 (d,7.3)	12	9,10,12a,1′, MeC(10)
12	32.24 t	a 1.98 br d	a 1.55 m	31.6 t	a 1.77 m	11, 12a	1, 4a, 12a
		b 1.76 ddd	b 1.30 m		b 1.32 m		
12a	39.22 d	2.95 br d	2.93 br s	38.7 d	2.64 br d	2,5,11	
Me_2CH	29.04 d	1.43 m	1.45 m	29.1 d	1.60 m	3,4,Me(ProR), Me(ProS)	
Me(ProS)	20.38 q	0.91 d	0.96 (d,6.5)	20.5 q	1.01 (d,6.5)	Me_2CH	4,Me (ProR) Me_2CH
Me(ProR)	22.23 q	0.81 d	0.84 (d,6.5)	22.2 q	0.95 (d,6.5)	Me_2CH	4,Me (ProS) Me_2CH
Me C(1)	22.14 q	1.58 br s	1.70 s	22.0 q	1.60 s		1, 2
Me C(10)	25.88 q	1.53 s	1.55 s	25.6 q	1.51 s		9, 10, 11
C−C(6)	167.95 s						
MeO	51.75 q	3.65 s					
1′	167.18 s			166.8 s			
2′	115.31 d	7.14 d	7.33 (d,15.5)	115.9 d	6.56 (d,15.5)	3'	4′
3′	138.04 d	8.05 d	8.02 (d,15.5)	136.4 d	7.53 (d,15.5)	2'	1′, 5′
4′	138.32 s			138.0 s			
5′	124.50 d	7.35 br s	7.72 s	122.7 d	7.09 s	6'	6′
6′	140.36 d	7.68 br s	7.67 s	139.2 d	7.46 s	5', NMe	
MeN	33.26 q	3.40 s	3.4 s	33.6 q	3.72 s		5′, 6′
$CH_2C(6)$			a 4.8 m	71.9 t	a 4.26 (d,11.2)	$CH_2bC(6)$	5, 6, 7, 1″
			b 4.38 (d,12)		b 3.94 (d,11.2)	$CH_2aC(6)$	
1″			5.53 br s	95.5 d	5.04 (d,1.5)	2″	3″
2″			6.03 (dd,10.5, 3.5)	67.9 d	5.30 m	1″, 3″ Ac (2″)	170.5
3″			5.83 (d,9.7)	69.9 d	5.26 m	2″, 4″, Ac (3″)	1″, 170.5
4″			4.57 br s	67.9 d	4.13 br s	3″, 5″	
5″			a 4.13(d,11)	62.2 t	a 3.90 br s	4″, 5″b	1″, 4″
			b 3.96(d,11)		b 3.75 br s	4″, 5″a	
Ac (2″)				170.5 s			Ac (2″)
			1.95 s	20.8 q	2.06 s	2″	
Ac (3″)				170.5 s			Ac (3″)
			2.13 s	20.8 q	2.10 s	2″	

[a] Bruker ARX 500 instrument, chemical shifts refer to TMS ($\delta = 0$) and $CDCl_3$ ($\delta_C = 77.0$). [b] Assignments aided by HMQC, HMBC, and homo-COSY experiments. [c] Pyridine C_5D_5N. [d] $CDCl_3$.

and C(6) from 143.9 and 135.5 ppm to 137.9 and 133.3 ppm, respectively. Most important, a CH-correlation was observed between H-5 to one of the two new CH_2O− groups. These data suggested the replacement of the carbomethoxyl group of **1** by an oxymethylene group in **2**. Unequivocal proof for this suggestion came from the mass spectral fragment at *m/z* 217 (55%) due to the cleavage of the C(6)CH_2O−C(1″) bond. This intense $C_9H_{12}O_6$ fragment ion (*m/z* 217) together with COSY and HMBC data (Table 1) implied the existence of a diacetylated −OCH_2CH(O−)CH(O−)CH(O−)O− moiety, namely a pentose diacetate located on the C(6)CH_2O− functionality. The various coupling constants and carbon chemical shifts[6−8] of the pentose indicated arabinose. Furthermore, the proton and carbon chemical shifts assigned the 2″,3″-diacetyl arabinose structure.

Eleuthoside B (**3**) possessed very similar spectroscopic data to those of **1**. From the NMR data (Experimental Section) it was evident that **3** and **2** were isomers of very similar structure. Changes in the ^{1}H and ^{13}C chemical shifts of the saccharide atoms (H-3″ shifted from δ_H 5.83 to 4.17, H-4″ from 4.57 to 5.15, C-3″ from 69.9 to 66.4, and C-4″ from 67.9 to 72.0), together with the COSY and HMBC correlations of this ring allowed determination of the structure of **3** as the 2″,4″-diacetoxy arabinose isomer of **2**. The small amounts of **2** and **3** available precluded hydrolysis to obtain free arabinose for determination of absolute configuration.

In Pietra's work on sarcodictyin A,[4] the possibility that histidine may be the source of urocanic acid was pointed out, although this is rare in secondary metabolites. Production of urocanic acid by a histidase is well known for many bacteria.[9] For example, the presence of urocanic acid in preserved fish is believed to be caused by bacteria and has been proposed as a spoilage index.[9] A study of the chemical composition of the periodically collected samples of *E. aurea* (see above) as well as the structure of the other glycosides is ongoing. So far, despite the fact that all samples were handled identically (i.e., frozen immediately upon collection), it is clear that not all contain the eleuthosides reported here.

Experimental Section

General Experimental Procedures. IR spectra were recorded on a Nicolet 205 FT-IR spectrophotometer. MS (low resolution and high resolution) were recorded on a Fisons Autospec Q instrument. ^{1}H- and ^{13}C-NMR spectra were recorded on Bruker AMX-360 and ARX-500 spectrometers, respectively. All chemical shifts are reported with respect to TMS ($\delta_H = 0$) or

$CDCl_3$ ($\delta_C = 77.0$). Optical rotations were measured on a Perkin-Elmer Model 141 polarimeter using a 1-cm microcell.

Biological Material. *E. aurea* Benayahu and Schleyer (1995)[3] (class Octocorallia, order Alcyonacea, family Alcyoniidae) (No. TASA 428) was collected from the Kwazulu−Natal Coast, South Africa, by scuba at a depth of 27-30 m during June 1995. The colonies are upright and digitiform, and the polyparium bears numerous polyps. The species has spheroid, radiate, and double deltoid sclerites, this last being the most conspicuous and abundant. The living colonies are bright yellow and golden in color with long white polyps. *E. aurea* has no symbiotic zooxanthellae. A voucher sample is deposited in the Zoological Department at Tel Aviv University.

Isolation Procedures. After collection, the soft coral was immediately frozen at −25 °C. A sample of freeze-dried soft coral (10 g) was then extracted with EtOAc to give a brown gum (290 mg). This crude gum was partitioned between 10% aqueous MeOH and hexane, 20% aqueous MeOH and CCl_4, and finally 30% aqueous MeOH and $CHCl_3$ to give a brown residue (50 mg), in the $CHCl_3$ phase, containing compounds **1**−3. The latter residue was chromatographed on Si gel H (VLC), eluted with EtOAc and EtOAc−MeOH 95:5 to 9:1 to afford compounds **1**−**3**, (ca. 2 mg of each).

Compound 1. Compound **1** was identical in all respects, including its optical activity,[4] to sarcodictyin A; DCIMS *m/z* [MH^+] 497 (35), 479 (MH − H_2O, 22), 345 (M − 152, 46), 327 (345 − H_2O, 58), 153 (33), 135 ($C_7H_7N_2O$, 100).

Eleuthoside A (2): oil; $[\alpha]_D$ −9° (*c* 0.2, $CHCl_3$) ν_{max} (neat) 3413, 2925, 1736, 1712, 1631, 1370, 1245, 1147, 1065, 1036, 998 cm^{-1}; HRFABMS *m/z* 685.3362 (calcd for $C_{36}H_{49}N_2O_{11}$ 685.3338); FABMS (m−NBA) *m/z* [MH^+] 685 (100), 668 (25), 289 (30), 217 (55), 153 (50); FABMS(+Na^+), *m/z* [MNa^+] 707(45), 176 (153 + Na, 100); EIMS *m/z* [M] 514 (8), 422 (7), 298 (12), 217 (15), 153 (35), 135 (100); ^1H and ^{13}C NMR, see Table 1.

Eleuthoside B (3): oil; $[\alpha]_D$ −9° (*c* 0.2, $CHCl_3$); ν_{max} (neat) 3380, 2931, 1737, 1710, 1633, 1375, 1244, 1156, 1037 cm^{-1}; HRFABMS *m/z* 685.3356 (calcd for $C_{36}H_{49}N_2O_{11}$ 685.3338). FABMS (m−NBA) *m/z* 685 (60), 667 (35), 427 (80), 391 (50), 273 (50), 217 (100), 153 (50). FABMS(+Na^+) [MNa^+] 707 (75), 176 (153 + Na, 100); EIMS *m/z* [M] 422 (5), 298 (5), 217 (15), 153 (23), 135 (100); ^1H-NMR ($CDCl_3$, 500 MHz) δ 7.57 (1H, d, J = 15.6 Hz, H-3′), 7.47 (1H, s, H-5′), 7.10 (1H, s, H-6′), 6.56 (1H, d, J = 15.6 Hz, H-2′), 6.14 (1H, d, J = 5.7 Hz, H-9), 6.10 (1H, d, J = 5.7 Hz, H-8), 5.52 (1H, d, J = 9.2 Hz, H-5), 5.28 (1H, br s, H-2), 5.15 (1H, br d, J = 1.5 Hz, H-4″), 5.07 (1H, m, H-2″), 5.01 (1H, d, J = 1.5 Hz, H-1″), 4.82 (1H, d, J = 7.2 Hz, H-11), 4.25 [1H, d, J = 11.3 Hz, H−CH_2(a)C(6)], 4.17 (1H, dd, J = 10.0, 3.5 Hz, H-3″), 4.04 (1H, m, H-4a), 3.94 [1H, d, J = 11.3 Hz, H-CH2(b)C(6)], 3.86 (1H, d, J = 15.0 Hz, H-5″a), 3.77 (1H, d, J = 15.0 Hz, H-5″b), 3.72 (3H, s, NMe), 2.66 (1H, m, H-12a), 2.31 (1H, m, H-3a), 2.13 [3H, s, Ac (3″)], 2.13 [3H, s, Ac(4″)], 2.06 (1H, m, H-3b), 1.60 (1H, m, H-Me_2CH), 1.60 (1H, m, H-12a), 1.53 [3H, s, MeC(1)], 1.49 (3H, s, MeC10), 1.30 (1H, m, H-12b), 1.30 (1H, m, H-4), 1.00 [3H, d, J = 6.4 Hz, Me(Pro *S*)], 0.95 [3H, d, J = 6.4 Hz Me(Pro *R*)]; ^{13}C NMR ($CDCl_3$), 125 (MHz) δ 170.0 [s, Ac(2″)], 170.0 [s, Ac (4″)], 166.0 (s, C-1′), 139.2 (d, C-5′), 138.0 (s, C-4′), 138.0 (d, C-5), 136.0 (d, C-3′), 134.0 (s, C-1), 133.3 (d, C-6), 133.2 (d, C-8), 132.0 (d, C-11), 122.0 (d, C-6′), 121.0 (d, C-2), 116.0 (d, C-2′), 112.0 (s, C-7), 95.4 (d, C-1″), 90.3 (s, C-10), 81.1 (d, C-11), 72.0 (d, C-4″), 71.9 (t, CH_2C(6)), 71.3 (d, C-2″), 66.4 (d, C-3″), 60.5 (t, C-5″), 42.2 (d, C-4), 38.6 (d, C-12a), 34.3 (d, C-4a), 33.6 (q, NMe), 31.6 (t, C-12), 29.1 (d, Me_2CH), 24.8 (q, MeC(10)), 24.4 (t, C-3), 22.2 [q, Me(*pro R*)], 21.9 (q, MeC(1)), 21.1 [q, Ac(3″)], 21.0 [q, Ac(4″)], 20.5 [q, Me(*pro S*)].

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