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New bioactive hydrogenated linderazulene-derivatives from the gorgonian *Echinogorgia complexa*

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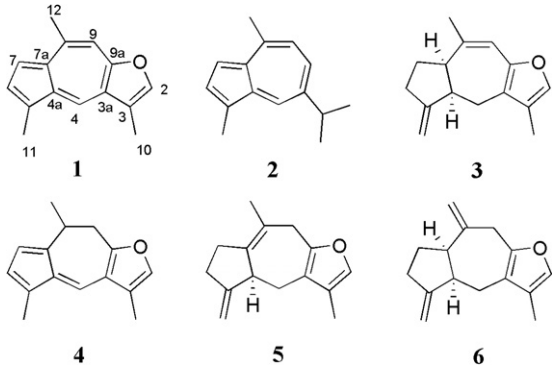
Abstract—Chemical analysis of the secondary metabolite pattern of the gorgonian *Echinogorgia complexa*, collected along South Indian coasts, showed the presence of two new tricyclic guaiane furanosesquiterpenes, *iso*-echinofuran (**3**) and 8,9-dihydro-linderazulene (**4**), co-occurring with the known echinofuran (**5**) and structurally related to the pigment linderazulene (**1**). The unprecedented molecules **3** and **4** were characterized by spectral methods, mainly by NMR techniques. Compounds **3**–**5** displayed moderate activity in the mitochondrial respiratory chain inhibition assay.

Azulene sesquiterpenes are typical secondary metabolites of marine octocorals of the order *Gorgonacea*.[1–11] Most of them are responsible of the brilliant colors of gorgonians and in addition various bioactivities including cytotoxic, antimicrobial, antifungal, immunostimulatory and cell division inhibitory properties have been reported for azulene sesquiterpenes.[7,8,10] Linderazulene (**1**)[1,3,5] and guaiazulene (**2**),[2,3,10,12] previously reported from terrestrial sources,[13,14] were the first azulene metabolites isolated from marine organisms. Subsequently, several related sesquiterpenes have been found in gorgonian species belonging to different genera (*Paramuricea*, *Acalycigorgia*, *Bebryce*, *Echinogorgia*, *Pseudopterogorgia*).[3–11,15,16] Guaiane azulenes have been found to be quite labile and easily decomposable on exposure to air and light during the work-up. A photo artifact from linderazulene (**1**) has been also reported.[17]

In this Letter we describe isolation, structure elucidation and biological activity of two new hydrogenated linderazulene derivatives designated as tricyclic furanosesquiterpenes **3** and **4**, co-occurring with the known echinofuran (**5**)[8] in the gorgonian *Echinogorgia complexa* Nutting collected from subtidal regions of West Indian coasts (Goa).

Although previous studies showed a strong antifouling activity of the alcoholic extract prepared from *E. complexa*,[18,19] active compounds were not characterized.



A sample of the gorgonian *E. complexa* (dry weight 54 g) was collected in December 2005, immediately frozen at −20 °C and subsequently transferred to ICB for the chemical studies. The frozen animal was extracted exhaustively with acetone (4 × 200 mL). The Et_2O soluble fraction of the acetone extract was concentrated under reduced pressure to furnish a residual orange oil

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(600 mg), which was analyzed by TLC chromatography (light petroleum ether/diethyl ether in different ratio) showing mainly the presence of sterols and lipids. The less polar part of the extract was found to contain three yellow spots between R_f 0.35–0.55 (*n*-hexane). The extract was then purified on a silica-gel column (petroleum light ether with increasing amount of diethyl ether) to obtain the mixture of yellow components (18 mg). A preliminary NMR analysis of this mixture suggested the presence of furano-sesquiterpenes. Subsequent comparison with available literature data indicated structures related to the linderazulene framework. The mixture was further purified on semi-preparative silica-gel plate, using *n*-hexane as eluent, to afford echinofuran (**5**, 1.0 mg), *iso*-echinofuran (**3**, 0.7 mg) and 8,9-dihydro-linderazulene (**4**, 0.7 mg) in order of increasing polarity.

(−)-Echinofuran (**5**), previously isolated from *Echinogorgia praelonga*,[8] was identified by comparison of ^{1}H NMR, mass spectral data and $[\alpha]_D^{25}$ with the literature values.[20] The ^{13}C NMR values of **5**, not previously assigned, were also assigned by performing 2D-NMR experiments.[21] Due to high unstability of these molecules in chloroform, NMR analysis of **5** was carried out in deuterated benzene. Compounds **3** and **4**, structurally related to **5**, were also analyzed in the same manner.

Compound **3**[22] had a molecular formula $C_{15}H_{18}O$, which was determined by HR-APCIMS giving a pseudo-molecular ion $[M+H]^+$ *m/z* 215.1431, and ^{13}C NMR spectrum (Table 1). The ^{1}H NMR spectrum of **3** showed strong analogies with that of echinofuran (**5**). An aliphatic methine proton (δ 2.35, m, H-7a) and an olefinic proton (δ 6.33, br s, H-9) replaced the methylene group H_2-9 resonating at δ 3.59 (1H, br d, $J = 17.8$ Hz, H-9) and 3.02 (1H, d, $J = 17.8$ Hz, H-9) in **5**. The ^{13}C NMR spectrum showed signals attributable to seven sp^3 carbons (two vinyl methyls at δ 7.8 and δ 22.2; three methylenes at δ 28.6, 29.8 and 32.0; two methine carbons at δ 50.9 and 45.9) and eight sp^2 carbons attributable to four double bonds: two tri-substituted (δ 136.0, 121.8, 137.3 and 116.8), a tetra-substituted (δ 149.0 and 120.0), and an exocyclic double bond (δ 105.6 and 156.1). These data indicated that compound **3** was an isomer of **5**, with a double bond at C-8/C-9 position instead of a double bond at C-7a/C-8. The cis A,B-ring junction was suggested by comparison with bebryazulene (**6**). In fact, the carbon values of the 5-membered ring of both compounds[11] were very similar. Unfortunately, **3** degraded before recording NOE difference experiments, which had been planned to confirm this suggestion. The proton and carbon values of **3** were assigned by 2D-NMR experiments (Table 1).

The molecular formula $C_{15}H_{16}O$ of compound **4**[23] was determined by HR-APCIMS which gave a pseudo-molecular ion $[M+H]^+$ *m/z* 213.1520 and ^{13}C NMR values (Table 1). The ^{1}H NMR spectrum displayed olefinic signals at δ 6.75 (1H, s, H-4) and 6.02 (2H, br s, H-6 and H-7), a singlet at δ 6.77 due an α-furan proton (H-2), two double doublets at δ 2.82 (1H, dd, $J = 16.4$, 2.9 Hz, H-9) and 2.66 (1H, dd, $J = 16.4$, 9.4 Hz, H-9) attributed to a methylene coupled to a methine at δ 2.83 (1H, m, H-8), which was further correlated to a secondary methyl signal resonating at δ 1.08 (3H, d, $J = 6.8$ Hz, H_3-12), and finally two methyl broad singlets occurring at δ 1.73 (H_3-10) and 2.06 (H_3-11). The ^{13}C NMR spectrum contained signals due to ten sp^2 carbons according to the presence of an additional unsaturation with respect to compound **3** (Table 1). Careful comparison of spectral data of **4** with those of linderazulene-related compounds[1,5,7,8,11] suggested that it was a dihydro-linderazulene. A series of 2D-NMR experiments (^{1}H–^{1}H COSY, HMQC, HMBC) led to structure

Table 1. NMR spectroscopic data[a] (400 MHz, C_6D_6) for compound (**3**) and compound (**4**)

Position	**3**		**4**	
	δ_C Mult.	δ_H (*J* in Hz)	δ_C Mult.	δ_H (*J* in Hz)
2	136.0 CH	6.94 br s	137.3 CH	6.77 br s
3	121.8 qC	—	120.6 qC	—
3a	120.0 qC	—	119.5 qC	—
4	29.8 CH_2	2.80 dd (16.4, 3.9) 2.39 dd (16.4, 3.1)	122.4 CH	6.75 s
4a	45.9 CH	2.56 m	133.2 qC	—
5	156.1 qC	—	144.1 qC	—
6	32.0 CH_2	2.28 m 2.11 m	125.6 CH	−6.02 br s
7	28.6 CH_2	1.67 m 1.32 m	125.6 CH	−6.02 br s
7a	50.9 CH	2.35 m	138.1 qC	—
8	137.3 qC	—	31.5 CH	2.83 m
9	116.8 CH	6.33 br s	34.1 CH_2	2.82 dd (16.4, 2.9) 2.66 dd (16.4, 9.4)
9a	149.0 qC	—	158.1 qC	—
10	7.8 CH_3	1.73 br s	7.4 CH_3	1.73 br s
11	105.6 CH_2	5.00 br s 4.91 br s	12.2 CH_3	2.06 br s
12	22.2 CH_3	1.67 br s	19.8 CH_3	1.08 d (6.8)

[a] Assignments aided by COSY, HSQC, HMBC.

4 in full agreement with the assigned values of proton and carbon signals (Table 1).

Interesting biological activities have been reported in the literature for several guaianoid azulene metabolites.[7,8,10] In particular, echinofuran (**5**) was found to inhibit cell division of fertilized sea urchin eggs.[8] Compounds **3**–**5**, isolated in the present study, have been tested in the mitochondrial respiratory chain assay. They displayed a moderate activity for the inhibition of the integrated electron transfer chain (NADH oxidase activity) in beef heart sub-mitochondrial particles (SMP).[24] IC_{50} values were 4.3 ± 0.15 μM, 2.5 ± 0.04 μM and 2.2 ± 0.31 μM for compounds **3**, **4** and **5** respectively, whereas full inhibition of rotenone sensitive NADH oxidase activity was achieved at approximately 2 μM.[25]

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References and notes

1. Imre, S.; Thomson, R. H.; Yalhi, B. *Experientia* **1981**, *37*, 442–443.
2. Fusetani, N.; Matsunaga, S.; Konosu, S. *Experientia* **1981**, *37*, 680–681.
3. Okuda, R. K.; Klein, D.; Kinnel, R. B.; Li, M.; Scheuer, P. J. *Pure Appl. Chem.* **1982**, *54*, 1907–1914.
4. Li, M. K. W.; Scheuer, P. J. *Tetrahedron Lett.* **1984**, *25*, 587–590.
5. Li, M. K. W.; Scheuer, P. J. *Tetrahedron Lett.* **1984**, *25*, 2109–2110.
6. Li, M. K. W.; Scheuer, P. J. *Tetrahedron Lett.* **1984**, *25*, 4707–4708.
7. Sakemi, S.; Higa, T. *Experientia* **1987**, *43*, 624–625.
8. Tanaka, J. I.; Miki, H.; Higa, T. *J. Nat. Prod.* **1992**, *55*, 1522–1524.
9. Ochi, M.; Kataoka, K.; Tatsukawa, A.; Kotsuhi, H.; Shibata, K. *Chem. Lett.* **1993**, 2003–2006.
10. Seo, Y.; Rho, J.-R.; Geum, N.; Yoon, J. B.; Shin, J. *J. Nat. Prod.* **1996**, *59*, 985–986.
11. Aknin, M.; Rudi, A.; Kashman, Y.; Gaydou, E. M. *J. Nat. Prod.* **1998**, *61*, 1286–1287.
12. Howard, B. M.; Fenical, W. *Tetrahedron Lett.* **1978**, 2453–2456.
13. Haagen-Smit, A. J. *Fortschr. Chem. Org. Naturst.* **1948**, *5*, 40–71.
14. Nozoe, T.; Ito, S. *Fortschr. Chem. Org. Naturst.* **1961**, *19*, 32–119.
15. Rodrìguez, A. D.; Boulanger, A. *J. Nat. Prod.* **1996**, *59*, 653–657.
16. Rodrìguez, A. D.; Boulanger, A. *J. Nat. Prod.* **1997**, *60*, 207–211.
17. Alpertunga, B.; Imre, S.; Cowe, H.; Cox, P. J.; Thomson, R. H. *Tetrahedron Lett.* **1983**, *24*, 4461–4462.
18. Wilsanand, V.; Wagh, A. B.; Bapuji, M. *Indian J. Mar. Sci.* **1999**, *28*, 280–284.
19. Wilsanand, V.; Wagh, A. B.; Bapuji, M. *Microbios* **2001**, *104*, 131–140.
20. Due to the easy degradation of compound **5** it is conceivable that the optical rotation value may be inaccurate. ^{1}H NMR spectrum was also recorded in $CDCl_3$ as reported in Ref. 8.
21. Compound **5**: $R_f = 0.50$ (*n*-hexane); $[\alpha]_D^{25}$ −14 (*c* 0.1, *n*-hexane); lit.[8] $[\alpha]_D^{20}$ −91 ($CHCl_3$); APCIMS: *m*/*z* 215.1440 (calculated for $C_{15}H_{18}OH^+$: 215.1436); ^{1}H NMR (400 MHz, C_6D_6): δ 6.93 (1H, br s, H-2), δ 4.98 (1H, br s, H-11a), δ 4.90 (1H, br s, H-11b), δ 3.59 (1H, br d, $J = 17.8$ Hz, H-9), δ 3.26 (1H, m, H-4), δ 3.02 (1H, d, $J = 17.8$ Hz, H-9), δ 2.52 (1H, dt, $J = 3.42$, 3.42, 15.7 Hz, H-7a), δ 2.38 (2H, m, H-6), δ 2.28 (2H, m, H-4), δ 2.24 (1H, m, H-7b), δ 1.72 (3H, br s, H_3-10), δ 1.52 (3H, br s, H_3-12). ^{13}C NMR (C_6D_6): δ 156.3 (C-5, C), δ 149.2 (C-9a, C), δ 140.5 (C-7a, C), δ 135.7 (C-2, CH), δ 124.5 (C-8, C), δ 121.1 (C-3, C), δ 119.3 (C-3a, C), δ 105.6 (C-11, CH_2), δ 46.0 (C-4a, CH), δ 33.7 (C-9, CH_2), δ 32.8 (C-4, CH_2), δ 30.2 (C-6, CH_2), δ 29.9 (C-7, CH_2), δ 21.3 (C-12, CH_3), δ 8.8 (C-10, CH_3).
22. Compound **3**: $R_f = 0.45$ (*n*-hexane); $[\alpha]_D^{25}$ −2.8 (*c* 0.07, *n*-hexane); APCIMS: *m*/*z* 215.1431 (calculated for $C_{15}H_{18}OH^+$: 215.1436); ^{1}H and ^{13}C NMR data are given in Table 1.
23. Compound **4**: $R_f = 0.40$ (*n*-hexane); $[\alpha]_D^{25}$ −38.2 (*c* 0.07, *n*-hexane); APCIMS: *m*/*z* 213.1520 (calculated for $C_{15}H_{16}OH^+$: 213.1517); ^{1}H and ^{13}C NMR data are given in Table 1.
24. Fato, R.; Estornell, D.; Bernardo, S.; Pallotti, F.; Parenti-Castelli, G.; Lenaz, G. *Biochemistry* **1996**, *35*, 2705–2716.
25. Fontana, A.; Cimino, G.; Gavagnin, M.; Gonzalez, M. C.; Estornell, E. *J. Med. Chem.* **2001**, *44*, 2362–2365.