

Bebryazulene, a New Guaiane Metabolite from the Indian Ocean Gorgonian Coral, *Bebryce grandicalyx*

Maurice Aknin,[†] Amira Rudi,[‡] Yoel Kashman,[‡] and Emile Marcel Gaydou^{*,†}

Laboratoire de Phytochimie de Marseille, Faculté des Sciences et Techniques de Saint-Jérôme, Avenue Escadrille Normandie Niemen, F-13397 Marseille Cedex 20, France, and School of Chemistry, Tel Aviv University, Tel Aviv 69978, Israel

Received February 26, 1998

Bebryazulene (**1**), a new sesquiterpene having the guaiane skeleton, was isolated from the methanol–chloroform 1:2 extract of the Indian Ocean gorgonian coral *Bebryce grandicalyx* (Kuekenenthal) collected in the lagoon of Mayotte. Structure assignment was based on interpretation of spectroscopic data. This new metabolite was very labile and reacted with 4-phenyl-3*H*-1,2,4-triazoline-3,5-dione to yield an unexpected adduct.

Guaiazulene and related sesquiterpenes are recognized metabolites of some species of gorgonian.^{1–11} Most of these compounds are part of the origin of the brilliant colors of tropical marine gorgonians, including many shades of blue. Some of them are extremely sensitive when exposed to light and air during normal laboratory operations. Two of the more resistant compounds were first identified as guaiazulene¹ and linderazulene.² The former compound has been isolated from a gorgonian for the first time from a Japanese specimen of the gorgonian *Euplexora erecta*, while the latter was isolated from the gorgonian *Paramuricea chamaelon*, collected in the sea of Marmara near Istanbul, both from shallow waters. They were also isolated at the same time from a deep sea gorgonian, family Paramuriceidae, collected in Hawaiian waters.³ Three other azulenes, a halogenated azulene,⁴ a new ketolactone linderazulene, ehuazulene,⁵ the origin of a yellow pigment, and a nitrogenous azulene derivative,⁶ were also isolated. 2,3-Dihydro-linderazulene⁷ was obtained from *Acalygcorgia* sp. and a tetrahydro-linderazulene, echinofuran,⁸ from *Echinogorgia praelonga* (Ridley), both Japanese specimens. The two new condensed guaiazulenoid pigments gorgiabisazulene and gorgiaallylazulene were also isolated from the same genus.⁹ Recently, 11 new sesquiterpenes having the guaiane skeleton were isolated from the Caribbean gorgonian coral *Pseudopterogorgia americana* (Gmelin) collected in Puerto Rico.^{10,11}

In the course of our continuing study of biologically active metabolites from marine invertebrates in the lagoon of Mayotte,¹² Comoros islands, northwest of Madagascar in the Indian Ocean, we investigated the lipophilic extract of the gorgonian *Bebryce grandicalyx* (Kuekenenthal). In this paper, we describe the isolation and structure elucidation of a new guaiane metabolite from this gorgonian *B. grandicalyx*.

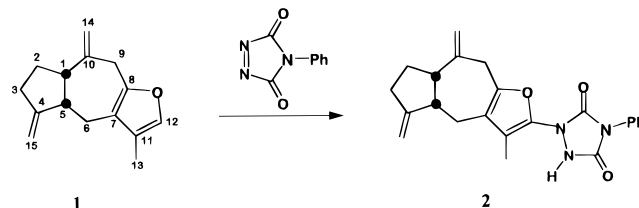
A specimen of *B. grandicalyx* was collected at a depth of 50 m at Prevoyante Reef in the lagoon of Mayotte. The CHCl₃–MeOH (2:1) extract of the gorgonian was found to contain a new sesquiterpene (5% yield), named bebryazulene.

The new sesquiterpene **1** showed a pseudomolecular ion at *m/z* 215 (M + H)⁺(100) in the CI mass spectra, which indicated a formula of C₁₅H₁₈O with seven degrees of unsaturation. The ¹³C NMR spectrum (Table 1) showed

signals for 15 atoms, including eight sp² carbons, suggesting **1** to have four double bonds in a tricyclic sesquiterpene system. Two double bonds were exomethylene (δ_C 112.8, 149.4, and 105.9 t, 156.7 s), and the two others (δ_C 119.0, 145.2, and 120.6 s, 136.0 d) were indicated to be part of a furan ring. The presence of a furan ring was also supported by IR absorptions at 1100, 1137, and 3070 cm⁻¹, the UV spectrum in MeOH at λ_{max} 230, and an ¹H NMR signal at δ_H 7.10 s (H-12).

The structures of the two other rings, one five membered and one seven membered, were determined by HMBC and H/H COSY correlations as indicated in Table 1. The five-membered ring unit was deduced to be a 4-methylenecyclopentane residue. Two methylene groups of the cycloheptane unit were directly attached to the furan ring; one to the oxygen bearing quaternary and the other to the olefin carbon signal at δ_C 119.0. Finally, position of the second exomethylene group was deduced to be in **10**, which was confirmed by HMBC correlations. This sequence established the cycloheptane unit and confirmed that furanoguaia-4(15),10(14)-diene was a tricyclic sesquiterpene. The stereochemistry of **1** was found to be *cis*, as indicated by an NOE between H-1 and H-5 (4.7%). This structure was unequivocally confirmed by additional NOE's observed between H-6 and H-15b (2.8%), H-6 and H-13 (2.8%), H-5 and H-6 (1.6%), H-1 and H-9b (2.5%), H-9a and H-14a (1.5%), and H-9a and H-9b (5.5%).

The spontaneous reaction of **1** with a reactive dienophile such as 4-phenyl-3*H*-1,2,4-triazoline-3,5-dione yielded an unexpected adduct **2** in which H-12 disappeared. The



molecular ion *m/z* 389 [MH⁺] in the FABMS, which indicated a formula of C₂₃H₂₃N₃O₃, suggested the formation of an adduct, but the presence of two fragments at *m/z* 213 (85%) and *m/z* 176 (52%) indicated a proton transfer from the sesquiterpene (H-12) to the dienophile moiety. Long-range CH correlations, observed in an HMBC experiment, confirmed the structure of **2**, showing among others the

* To whom correspondence should be addressed. Tel.: + 33 4 91 28 93 35. Fax: + 33 4 91 28 86 47. E-mail: emile.gaydou@iut-chimie.u-3mrs.fr.

[†] Faculté des Sciences et Techniques de Saint-Jérôme.

[‡] Tel Aviv University.

Table 1. ^1H NMR (500 MHz) and ^{13}C NMR (125 MHz) Data Including H–H and C–H Correlations of Bebryazulene^a

position	δ_{H} , ppm, mult	J , Hz	δ_{C} , ppm	HMBC (C to H)	COSY (^1H – ^1H)
1	2.90, tq	1.8, 8.5	49.5, d	2a, 6a, 6b, 9a, 9b, 14a, 14b	2a, 2b, 5
2a	1.96, m		28.1, t	1, 3a, 3b, 5	3a, 3b
b	1.80, m				
3a	2.45, m		32.7, t	2a, 2b, 5, 9a, 9b, 15a, 15b	2a, 2b
b	2.35, m				15a, 15b
4			156.7, s	2a, 3a, 5, 6a, 6b, 15a, 15b	
5	3.05, dt	6.2, 8.5	47.6, d	1, 2a, 6a, 6b, 15a, 15b	6a, 6b
6a	2.50, m		25.4, t	1, 5	5
b	2.50, m				
7			119.0, s	5, 6a, 6b, 9a, 9b, 12, 13 ^b	
8			145.2, s	6a, 6b, 9a, 9b, 12	
9a	3.75, d	15.2	36.4, t	14a, 14b	
b	3.45, d	15.2			
10			149.4, s	1, 2b, 9a, 9b, 14b	
11			120.6, s	6a, 6b, 12, 13 ^b	
12	7.10, s	136.0 d	13		
13	1.98, s		8.1, q	12	
14a	5.10, s		112.8, t	1, 9a, 9b	
b	5.00, s				
15a	5.20, s		105.9, t	4, 5	
b	5.15, s				

^a Bruker ARX 500 instrument, chemical shifts refer to TMS ($\delta_{\text{H}} = 0$) for ^1H NMR and to C_6D_6 ($\delta_{\text{C}} = 128$) for ^{13}C NMR. ^b These correlations were confirmed by an INAPT experiment.

correlation between the amido proton and the corresponding amido carbon.

Experimental Section

General Experimental Procedure. IR spectra were recorded on a Nicolet 205 FT-IR spectrophotometer. UV spectrum was taken on Unikom 931 spectrophotometer. CI mass spectrum was recorded on a Fisons Autospec Q instrument. ^1H and ^{13}C NMR spectra were recorded on Bruker AMX-360 and ARX-500 spectrometers. All chemical shifts are reported with respect to TMS ($\delta_{\text{H}} = 0$) and C_6D_6 ($\delta_{\text{C}} = 128$).

Biological Material. *B. grandicalyx* (Kuekenthal) (family Plexauridae) was collected at Prevoyante Reef, Lagoon of Mayotte, Comoros islands, by scuba at a depth of 50 m during April 1995. A voucher sample is deposited at the Forschungsinstitut und Naturmuseum Senckenberg, Frankfurt am Main. The freeze-dried gorgonian was homogenized and extracted with $\text{MeOH}-\text{CHCl}_3$ 1:2 (200 mL \times 5) to give a brown gum (6.5 g) after evaporation.

This crude extract (120 mg) was subjected to partition by the method of Kupchan et al.¹³ The petroleum ether fraction (50 mg) was chromatographed several times on Si gel columns, eluting with hexane–EtOAc mixtures to afford the labile bebryazulene **1** (R_f value 0.8, EtOAc–hexane 1:3).

Bebryazulene 1: unstable oil; IR ν_{max} (neat) 3080, 2925, 2850, 1640, 1440, 1420, 1400, 1137, 1100, 890 cm^{-1} ; UV (MeOH) λ_{max} 230 (ϵ 5890), 279 nm (ϵ 1113); ^1H and ^{13}C NMR, see Table 1; CIMS m/z ($\text{M} + \text{H}$)⁺ 215 (100), 183 (10), 169 (24), 155 (32), 103 (46). Unfortunately, compound **1** decomposed before optical rotation and high-resolution MS could be obtained.

Triazolinedione Adduct of Bebryazulene (1). Compound **1** (2.0 mg) was dissolved in CCl_4 (1.0 mL), and 4-phenyl-3*H*-1,2,4-triazoline-3,5-dione (5.0 mg) was added to give a spontaneous reaction yielding compound **2**: ^1H NMR δ 7.38 d (2H), 7.25 t (2H), 7.14 t (1H), 6.15 brs (*NH*), 4.79 s (1H), 4.74 s (1H), 4.71 s (1H), 4.67 s (1H), 3.24 brs (2H), 2.80 m (1H), 2.76 m (1H), 1.71 s (3H); ^{13}C NMR δ 177 (carbonyl); HMBC HN/CO; FABMS m/z 389 (M^+ , 100), 286 (75), 228 (60), 213 ($\text{C}_{15}\text{H}_{17}\text{O}$, 85), 176 ($\text{C}_8\text{H}_6\text{N}_3\text{O}_2$, 52).

Acknowledgment. We gratefully acknowledge Dr. M. Grasshoff, Senckenberg Naturmuseum, for the gorgonian identification.

References and Notes

- Fusetani, N.; Matsunaga, S.; Konosu, S. *Experientia* **1981**, *37*, 680–681.
- Imre, S.; Thomson, R. J.; Yalhi, B. *Experientia* **1981**, *37*, 442–443.
- Okuda, R. K.; Klein, D.; Kinnel, R. B.; Li, M.; Scheuer, P. J. *Pure Appl. Chem.* **1982**, *54*, 1907–1914.
- Li, M. K. W.; Scheuer, P. J. *Tetrahedron. Lett.* **1984**, *25*, 587–590.
- Li, M. K. W.; Scheuer, P. J. *Tetrahedron. Lett.* **1984**, *25*, 2109–2110.
- Li, M. K. W.; Scheuer, P. J. *Tetrahedron. Lett.* **1984**, *25*, 4707–4708.
- Sakemi, S.; Higa, T. *Experientia* **1987**, *43*, 624–625.
- Tanaka, J.; Miki, H.; Higa, T. *J. Nat. Prod.* **1992**, *55*, 1522–1524.
- Ochi, M.; Kataoka, K.; Tatsukawa, A.; Kotsuki, H.; Shibata, K. *Chem. Lett.* **1993**, 2003–2006.
- Rodriguez, A. D.; Boulanger, A. *J. Nat. Prod.* **1996**, *59*, 653–657.
- Rodriguez, A. D.; Boulanger, A. *J. Nat. Prod.* **1997**, *60*, 207–211.
- Rudi, A.; Akinin, M.; Gaydou, E. M.; Kashman, Y. *J. Nat. Prod.* **1997**, *60*, 700–703.
- Kupchan, S. M.; Komoda, Y.; Branfman, A. R.; Sneden, A. T.; Court, W. A.; Thomas, G. J.; Hintz, H. P. J.; Smith, R. M.; Karim, A.; Howie, G. A.; Verma, A. K.; Nagao, Y.; Dailey, R. G., Jr.; Zimmerly, V. A.; Summer, W. C., Jr. *J. Org. Chem.* **1977**, *42*, 2349–2357.

NP980063J