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Bielschowskysin, a Gorgonian-Derived Biologically Active Diterpene with an Unprecedented Carbon Skeleton

Jeffrey Marrero,[†] Abimael D. Rodríguez,*[,†] Peter Baran,[†] Raphael G. Raptis,[†] Juan A. Sánchez,[‡] Eduardo Ortega-Barria,[§] and Todd L. Capson[⊥]

Department of Chemistry, University of Puerto Rico, P.O. Box 23346, U.P.R. Station, San Juan, Puerto Rico 00931-3346, Department of Systematic Biology & Laboratories of Analytical Biology, Smithsonian Institution, P.O. Box 37012, Washington, D.C. 20013-7012, Florida State University−Panamá, Cell and Molecular Biology of Pathogens Unit, Institute for Tropical Medicine and Health Sciences, La Boca 1034, Balboa, Ancón, Panamá, and Smithsonian Tropical Research Institute, P.O. Box 2072, Balboa, Ancón, Panamá

arodrig@cnnet.upr.edu

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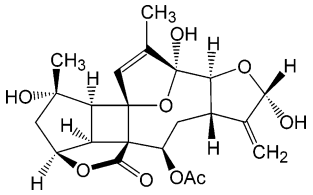
ABSTRACT



Bielschowskysin is a naturally occurring diterpene isolated from the Caribbean gorgonian octocoral *Pseudopterogorgia kallos*. Its highly oxygenated hexacyclic structure is based on a previously undescribed tricyclo[9.3.0.0^{2,10}]tetradecane ring system that was established through spectroscopic analysis and X-ray crystallographic analysis. Bielschowskysin was shown to exhibit antimalarial activity against *Plasmodium falciparum* as well as strong anticancer activity against two human cancer cell lines.

West Indian gorgonian octocorals (sea whips, sea feathers, sea plumes, and sea fans) are a prolific source of new terpenoids with diverse structures that often have interesting biological activities.[1] For instance, the gorgonian coral *Erythropodium caribaeorum* is a rich source of eleutherobin and a number of analogues that are microtubule-stabilizing antimitotic agents.[2] Another group of diterpene glycosides from *Pseudopterogorgia elisabethae*, the pseudopterosins and *seco*-pseudopterosins, possesses antiinflammatory and analgesic properties that exceed the potencies of existing drugs such as indomethacin.[3] Because of their unique structural features, biosynthesis, and biological activities, these families of potential drug leads have attracted much attention, thus spawning a large amount of effort directed toward their total synthesis. Despite the great interest in gorgonian corals belonging to the genus *Pseudopterogorgia*, these animals have not been thoroughly investigated for natural products.[1] In the course of our continuing investigations into the natural products chemistry of marine invertebrates from the West Indian region, we have studied extracts of *Pseudopterogorgia kallos* (Bielschowsky, 1918) collected near Old Providence Island located in the Southwestern Caribbean Sea.[4] Previously, we have reported on two diterpenoid lactones from

[†] University of Puerto Rico.
[‡] Smithsonian Institution.
[§] Florida State University−Panamá.
[⊥] Smithsonian Tropical Research Institute.

(1) Rodríguez, A. D. *Tetrahedron* **1995**, *51*, 4571−4618.
(2) Cinel, B.; Roberge, M.; Behrisch, H.; Van Ofwegen, L.; Castro, C. B.; Andersen, R. J. *Org Lett.* **2000**, *2*, 257−260.
(3) (a) Look, S. A.; Fenical, W.; Matsumoto, G. K.; Clardy, J. *J. Org. Chem.* **1986**, *51*, 5140−5145. (b) Look, S. A.; Fenical, W. *Tetrahedron* **1987**, *43*, 3363−3370. (c) Roussis, V.; Wu, Z.; Fenical, W. *J. Org. Chem.* **1990**, *55*, 4916−4922. (d) Ettouati, W. S.; Jacobs, R. S. *Mol. Pharmacol.* **1987**, *31*, 500−505.

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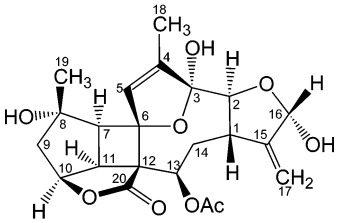
Table 1. ^{1}H NMR (500 MHz), ^{13}C NMR (125 Mz), ^{1}H−^{1}H COSY, NOESY, and HMBC Spectral Data of Bielschowskysin (**1**)[a]

position	δ_H, mult, intrgt (*J* in Hz)	δ_C (mult)[b]	^{1}H−^{1}H COSY	NOESY	HMBC[c]
1	2.69, m, 1H	40.8 (CH)	H2, H14*β*, H17α*β*	H14*β*	H16, H14α*β*, H17α*β*
2	4.07, d, 1H (9.8)	86.2 (CH)	H1	H14α	H16, H17α*β*
3		110.7 (C)			H2, H5, H_3-18
4		144.3 (C)			H2, H5, H_3-18
5	5.99, brd, 1H (1.5)	126.3 (CH)	H_3-18	H_3-18, H_3-19	H7, H_3-18
6		89.1 (C)			H5, H7, H11
7	2.81, dd, 1H (7.6, 1.7)	61.6 (CH)	H9α, H11	H11, H_3-19	H9α, H11, H_3-19
8		81.7 (C)			H7, H9α, H10, H11, H_3-19
9α	2.49, ddd, 1H (15.7, 8.1, 1.7)	49.7 (CH_2)	H7, H10	H9*β*, H10, H_3-19	H7, H11, H_3-19
9*β*	2.06, ddd, 1H (15.7, 8.1, 4.6)		H10	H9α, H11, H_3-19	
10	5.22, ddd, 1H (8.4, 8.1, 4.6)	84.8 (CH)	H9α*β*, H11	H7, H9α	H7, H9α*β*
11	3.18, dd, 1H (8.4, 7.6)	43.0 (CH)	H7, H10	H10	H7, H9α, H10, H13
12		59.2 (C)			H11, H13, H14α
13	5.69, dd, 1H (9.7, 7.4)	72.1 (CH)	H14α*β*	H14α	H11, H14α*β*
14α	2.02, ddd, 1H (13.5, 8.2, 7.4)	34.0 (CH_2)	H13	H2, H14*β*, H13	H2, H13
14*β*	2.27, ddd, 1H (13.5, 9.7, 3.8)		H13	H1, H14α, H17*β*	
15		155.1 (C)			H1, H17α*β*
16	5.54, brs, 1H	98.6 (CH)	H17α*β*		H17α*β*
17α	5.22, dd, 1H (2.9, 1.4)	109.3 (CH_2)	H1, H16, H17*β*	H17*β*	H16
17*β*	5.06, dd, 1H (2.6, 1.5)		H1, H16, H17α	H5, H14*β*, H17α	
18	1.82, d, 3H (1.5)	13.3 (CH_3)	H5	H5	H5
19	1.34, s, 3H	22.9 (CH_3)		H7, H9α*β*	H9*β*
20		177.2 (C)			H10, H11, H13
21		172.1 (C)			H13, H_3-22
22	2.05, s, 3H	20.9 (CH_3)			

[a] Spectra were recorded in CD_3OD at 25 °C. Chemical shift values are in parts per million relative to TMS. [b] ^{13}C NMR multiplicities were obtained from a DEPT-135 experiment. [c] Protons correlated to carbon resonances in the ^{13}C column.

this gorgonian specimen that have novel rearranged carbon skeletons.[5] From the same specimen, we now report the isolation and structure characterization of the highly oxygenated hexacyclic diterpene bielschowskysin (**1**), a novel compound with significant antiplasmodial activity. The structure of **1**, containing a tricyclo[9.3.0.0^{2,10}]tetradecane ring system, was elucidated on the basis of spectroscopic data and single-crystal X-ray diffraction analysis. The skeletal carbon framework of **1** is unprecedented in the field of natural products.



1

The partially air-dried animal specimens (1.07 kg) were frozen, lyophilized, cut into small pieces, and homogenized exhaustively using a mixture of CH_2Cl_2−MeOH (1:1). After concentration in vacuo, the dried extract (166 g) was subjected to our standard partitioning procedure, resulting in hexane, $CHCl_3$, and EtOAc fractions.[5] The EtOAc-soluble material (1.5 g) was purified by silica gel flash chromatography followed by normal-phase HPLC to yield pure **1** (39.6 mg; 0.024% based on the crude extract dry wt) as a colorless crystalline solid.[6]

The HREIMS of bielschowskysin (**1**) showed no molecular ion peak. Instead, a fragment ion peak at *m*/*z* 374.1368 [M − AcOH] suggested the molecular formula for **1** of $C_{22}H_{26}O_9$ (calcd for [M − AcOH], 374.1366) requiring 10 sites of unsaturation. The ^{13}C NMR spectrum (500 MHz, CD_3OD) of **1** showed 22 resolved resonances (Table 1). Four olefinic [δ 155.1 (C), 144.3 (C), 126.3 (CH), 109.3 (CH_2)] and two carbonyl [δ 177.2 (C), 172.1 (C)] resonances in the ^{13}C NMR spectrum accounted for four sites of unsaturation. Therefore, the remaining six sites of unsaturation required by the molecular formula had to be accounted for by rings. HMQC and DEPT-135 data showed that 23 of the 26 hydrogen atoms were attached to carbons (3 x CH_3, 3 x CH_2, 8 x CH); therefore, compound **1** had to have 3 OH groups.

The ^{1}H NMR spectrum of **1** acquired in CD_3OD at 500 MHz was well dispersed, which facilitated the straightfor-

(4) For the first in-depth study concerning the natural products chemistry of *P. kallos*, see: Look, S. A.; Burch, M. T.; Fenical, W.; Qi-tai, Z.; Clardy, J. *J. Org. Chem.* **1985**, *50*, 5741−5746.

(5) (a) Marrero, J.; Rodríguez, A. D.; Baran, P.; Raptis, R. G. *J. Org. Chem.* **2003**, *68*, 4977−4979. (b) Marrero, J.; Rodríguez, A. D.; Baran, P.; Raptis, R. G. *Org. Lett.* **2003**, *5*, 2551−2554.

(6) Bielschowskysin (**1**): colorless crystalline solid; mp 139−141 °C dec; $[\alpha]^{20}_D$ −17.3° (*c* 1.1, MeOH); IR (thin film) ν_{max} 3400, 1739, 1657, 1250, 1022 cm^{-1}; EIMS *m*/*z* 374 [M − AcOH]$^+$ (29), 356 (43), 346 (47), 328 (29), 287 (59), 269 (49), 153 (100). For experimental details pertaining to the isolation of **1**, see Supporting Information.

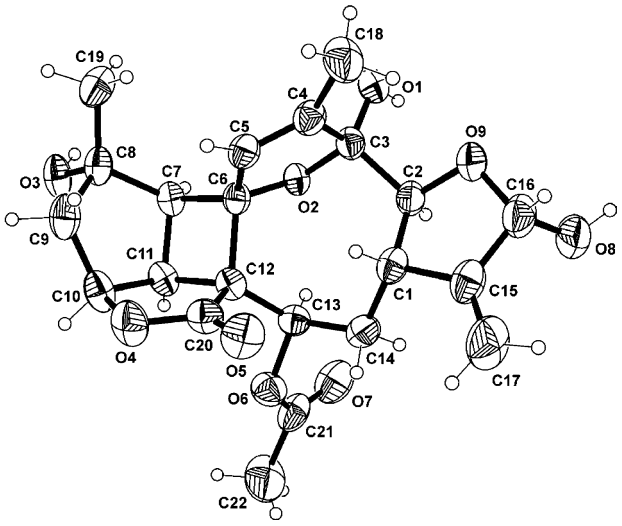


Figure 1. Computer-generated ORTEP drawing with atom labeling scheme and chemical drawing of one of the two crystallographically independent molecules of bielschowskysin (**1**). The absolute configuration shown is arbitrary. The carbon and oxygen atoms are drawn as 50% thermal ellipsoids.

ward identification of several features of the structure (Table 1). However, only a limited number of functional groups, one lactone, one acetate, two isolated carbon−carbon double bonds, and two cyclic hemiacetals, with limited connectivity to each other were apparent in two-dimensional NMR COSY experiments. Therefore, we elected to continue the structure determination using single-crystal X-ray diffraction techniques. Bielschowskysin (**1**) was recrystallized by slow evaporation of a mixture of EtOAc/MeOH to yield cubic colorless crystals of excellent quality.

The X-ray crystal structure, which defines only the relative configuration, is shown in Figure 1.[7] To verify the crystal structure and assign the NMR signals, HMBC experiments were conducted in CD_3OD. Important HMBC correlations were detected from H-2 to C-3 (hemiacetal quaternary carbon); from H-5 and H-7 to C-6 (spirocyclic carbon); from H-11 to C-7, 8, 9, 12, and 20 (lactone carbonyl carbon); and from H-13 to C-11, 12, 14, 20, and 21 (acetoxyl carbonyl carbon). These observations provided key connectivities among the partial structures within the complex tricyclo-[9.3.0.0^{2,10}]tetradecane nucleus of **1**. The relative configurations of the stereocenters of the highly strained ring system

(7) Crystal data for bielschowskysin **1**·0.6 H_2O at 298(2) K: $C_{22}H_{27.2}O_{9.6}$, M_r = 445.24, monoclinic, space group $P2_1$ (No. 4), a = 11.243(2), b = 15.640(3), c = 12.376(2) Å, β = 94.421(2)°, V = 2169.6(6) Å^3, Z = 4, ρ_{calc} = 1.363 Mg m^{-3}, F_{000} = 944, λ(Mo Kα) = 0.71073 Å, μ = 0.107 mm^{-1}. Data collection and reduction: crystal size, 0.50 × 0.39 × 0.34 mm^3, θ range, 1.82−27.99°, 19 350 reflections collected, 9362 independent reflections (R_{int} = 0.0291), final R indices ($I > 2\sigma(I)$): R_1 = 0.0372, wR_2 = 0.0951 for 589 variable parameters, GOF = 1.043. CCDC 230559 (**1**) contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB21EZ, UK; fax: (+44)1223-336-033; e-mail: deposit@ccdc.cam.ac.uk).

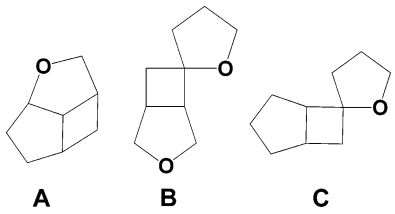
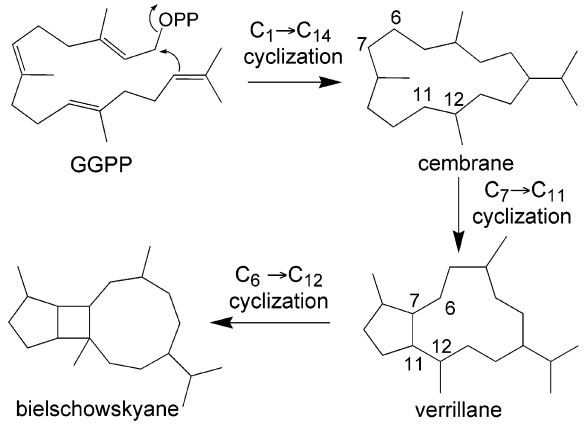


Figure 2. Tricyclic fused motifs **A**−**C**.

in bielschowskysin were confirmed on the basis of correlations observed in the NOESY NMR spectrum as well as through interpretation of NMR coupling constant data (Table 1). Thus, the overall relative stereochemistry for **1** with 10 chiral centers was assigned as 1*S**,2*S**,3*S**,6*S**,7*S**,8*S**,-10*S**,11*S**,12*R**,13*R**.

A careful substructure search of the Cambridge Structural Data Centre revealed no natural products composed of this specific tricyclic [5−4−9] ring architecture. The strained 4 + 9 fusion of **1**, long C6−C12 and C56−C62 distances of 1.602(2) and 1.604(2) Å, respectively, is also noteworthy (see ORTEP drawings of the two symmetry-independent molecules of **1** in Supporting Information). The tricyclic fused motif **A** (Figure 2) has been found in two synthetic compounds,[8] while motifs **B** and **C** have been identified only in natural products isolated from *Cystoseira balearica* and *Delisea elegans*, respectively.[9,10] Thus, bielschowskysin represents a new class of regular diterpene. We propose that the name bielschowskyane be used for this family of diterpenes. The co-occurrence of **1** with various furanocembranoid lactones within the same organism raises the possibility that bielschowskysin represents a further modification of an existing metabolite, thus suggesting the biogenetic pathway outlined in Scheme 1.[11] Although still unproven, the bielschowskyane ring system might be synthesized in vivo by subsequent cyclization of a suitable cembranoid precursor via successive [C7→C11] and [C6→C12] bond formation.[12]

Scheme 1. Proposed Biogenesis for the Bielschowskyane Skeleton



Bielschowskysin (**1**) exhibited antiplasmodial activity (IC_{50} = 10 μg/mL) when tested against *Plasmodium falciparum*.[13] Considering the ability of **1** to inhibit *P. falciparum*, bielschowskysin was also evaluated for cytotoxicity using the NCI's in vitro antitumor screen. Thus, compound **1** was found to display strong and specific in vitro cytotoxicity against the EKVX nonsmall cell lung cancer (GI_{50} < 0.01 μM) and CAKI-1 renal cancer (GI_{50} = 0.51 μM).

Acknowledgment. The staff of the Ministerio del Medio Ambiente (Bogotá, Colombia) assisted with the collection of *P. kallos*. The NCI provided in vitro cytotoxicity data for **1**. We gratefully acknowledge the NIH-MBRS SCORE /RISE Programs of the University of Puerto Rico and the ICBG #1 UO1TW-01021-01 from the Fogarty Center of the National Institutes of Health for partial financial support of this work.

Supporting Information Available: Detailed description of the experimental procedures, ORTEP drawings of the two symmetry-independent molecules of bielschowskysin (**1**), NMR spectra (^1H and ^{13}C), and tables of crystallographic data for **1** (crystal data and structure refinement, atomic coordinates, bond lengths and angles, anisotropic displacement parameters, and hydrogen coordinates). This material is available free of charge via the Internet at http://pubs.acs.org.

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(8) (a) Destro, R.; Merati, F.; Sisti, M. *Acta Crystallogr., Sect. C: Cryst. Struct. Commun*. **1987**, *C43*, 76−80. (b) De Gregori, A.; Jommi, G.; Sisti, M.; Gariboldi, P.; Merati, F. *Tetrahedron* **1988**, *44*, 2549−2568.

(9) Amico, V.; Cunsolo, F.; Piattelli, M.; Ruberto, G.; Fronczek, F. R. *Tetrahedron* **1984**, *40*, 1721−1725.

(10) McCombs, J. D.; Blunt, J. W.; Chambers, M. V.; Munro, M. H. G.; Robinson, W. T. *Tetrahedron* **1988**, *44*, 1489−1502.

(11) Complete details of the structures and biological activities of such furanocembranolides will be reported elsewhere.

(12) Rodríguez, A. D.; Shi, Y.-P. *J. Org. Chem*. **2000**, *65*, 5839−5842.

(13) Between 300 and 500 million clinical cases of malaria occur every year with over 1.2−2.7 million deaths. Prevention and treatment of *P. falciparum* malaria, its most severe form, are becoming more difficult because *P. falciparum* is increasingly resistant to various antimalarial drugs. Two chloroquine-sensitive strains (Sierra Leone clone D6 and Tanzania F32) and one chloroquine-resistant strain (Indochina clone W2) of *P. falciparum* were used for this study; see: Corbett, Y.; Herrera, L.; González, J.; Cubilla, L.; Capson, T. L.; Coley, P. D.; Kursar, T. A.; Romero, L. I.; Ortega-Barria, E. *Am. J. Trop. Med. Hyg.* **2004**, *70*, 119−124.