Ciereszkolide: Isolation and Structure Characterization of a Novel Rearranged Cembrane from the Caribbean Sea Plume *Pseudopterogorgia kallos*

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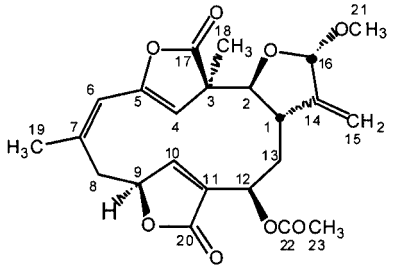
Dedicated to the memory of Professor Leon S. Ciereszko[‡]

Keywords: Terpenoids / Natural products / X-ray diffraction / Ring contraction / Rearrangement

An investigation of the chloroform extract of a Colombian specimen of *Pseudopterogorgia kallos* has led to the isolation of a novel rearranged cembrane, ciereszkolide (**1**). The structure of **1** is based on a new carbon skeleton that possesses several unusual structural features, namely, two butyrolactones [one 2(3*H*)- and one 2(5*H*)-furanone] across a 13-membered macrocycle, a (*Z*)-trisubstituted olefin, and a five-membered *trans*-fused cyclic ketal. The structure of **1** was resolved by interpretation of 1D and 2D NMR spectroscopic data supported by HRFAB-MS, IR, UV, and a single-crystal X-ray diffraction analysis.

Introduction

Gorgonian octocorals represent a seemingly inexhaustible source of structurally complex biologically active metabolites.[1,2] Particularly, Caribbean gorgonian octocorals of the genus *Pseudopterogorgia* have been recognized as one of the most inventive producers of biologically active compounds, namely, anti-inflammatory,[3] anti-mycobacterial,[4] anti-malarial,[5] and anti-tumor.[6] Their exquisite structural architecture and biomedical potential have rendered these natural products irresistible to natural product and synthetic chemists alike.[7] Surprisingly, the natural products chemistry of some *Pseudopterogorgia* species, in particular that of the least conspicuous species, remains largely unexplored. In 1985, Fenical and co-workers reported the isolation and structural characterization of four pseudopterane diterpenes with significant anti-inflammatory properties from the Caribbean sea plume *Pseudopterogorgia kallos* (Bielschowsky, 1918).[3a] For almost two decades this report remained as the only in-depth account of the natural products chemistry of this animal. In 2003, we began a chemical reinvestigation of *P. kallos*, which, thus far, has led to the isolation and structure elucidation of various diterpenes of novel structure and diverse bioactivity.[8] As part of our continuing effort to find biologically active compounds with novel carbon architecture we now wish to report the discovery of a rearranged cembrane diterpene based on a new carbon skeleton, ciereszkolide (**1**). Indeed, a careful literature search has revealed that the skeletal carbon framework of **1** is unprecedented in the field of natural products.



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Results and Discussion

After collection by scuba diving near Old Providence Island, Colombia, *P. kallos* colonies were partially air-dried on-site, frozen, and later lyophilized prior to their extraction. The dry animal material (1.07 kg) was cut in small pieces and homogenized exhaustively using a 1:1 blend of CH_2Cl_2/MeOH. After filtration and concentration in vacuo, the crude extract (166 g) was subjected to our standard partitioning procedure thereby affording crude hexane, $CHCl_3$, and EtOAc extracts.[8] The $CHCl_3$ extract (39.3 g) was purified by repeated silica gel column chromatography and HPLC to afford 4.5 mg (0.0027% based on the weight of the animal crude extract) of ciereszkolide (**1**). The molec-

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Table 1. ^{1}H NMR (500 MHz), ^{13}C NMR (125 MHz), ^{1}H-^{1}H COSY, HMBC and NOESY spectroscopic data for ciereszkolide (**1**); NMR spectra were recorded in $CDCl_3$ at 25 °C; ^{1}H and ^{13}C NMR chemical shift values are in ppm and are referenced to the residual $CHCl_3$ (δ = 7.26 ppm) or $CDCl_3$ (δ = 77.0 ppm) signals

	δH, mult., integ. (*J* in Hz)	δC (mult.)[a]	^{1}H-^{1}H COSY	HMBC[b]	NOESY[c]
1	1.83, br. m, 1H	41.7 (CH)	H2,H13αβ,H15	H2, H13α, H_2-15, H16	H13β, H_2-15
2	4.01, d, 1 H (2.8)	85.5 (CH)	H1, H4	H_2-13, H16, H_3-18	H4,H12,H13α,H_3-18
3		53.2 (C)		H4, H_3-18	
4	5.16, d, 1 H (1.5)	110.6 (CH)	H2	H2, H_3-18	H2, H10, H_3-18
5		150.2 (C)		H4, H6	
6	5.99, q, 1 H (1.5)	117.2 (CH)	H_3-19	H4, H_2-8, H_3-19	H_3-19
7		143.4 (C)		H_2-8, H_3-19	
8α	2.85, dd, 1 H (12.3, 6.0)	40.6 (CH_2)	H8β, H9	H6, H9, H_3-19	H8β, H9, H_3-19
8β	2.50, dd, 1 H (12.3, 11.3)		H8α, H9		H8α
9	5.09, ddd, 1 H (11.3, 6.0, 1.4)	77.6 (CH)	H8αβ, H10	H10, H_2-8	H8α, H10, H_3-19
10	7.46, d, 1 H (1.4)	157.9 (CH)	H9	H9, H12, H13αβ	H4, H9, H12, H_3-19
11		128.7 (C)		H9, H10, H12, H_2-13	
12	5.45, dd, 1 H (10.7, 4.6)	67.5 (CH)	H13αβ	H10, H_2-13	H2, H10, H_2-13
13α	2.12, ddd, 1 H (14.3, 10.4, 4.6)	38.3 (CH_2)	H1, H12, H13β	H2, H12	H2, H12, H13β
13β	2.57, ddd, 1 H (14.3, 10.7, 3.4)		H1, H12, H13α		H1, H12, H13α
14		148.4 (C)		H2, H_2-15, H_2-13, H16	
15α	5.26, br. s, 1 H	112.4 (CH_2)	H1	H16	H1, H_3-21
15β	5.25, br. s, 1 H				H1, H_3-21
16	5.39, br. s, 1 H	105.5 (CH)		H2, H_2-15, H_3-21	H_3-21
17		177.5 (C)		H2, H4, H_3-18	
18	1.45, br. s, 3 H	20.5 (CH_3)		H2	H2, H4
19	1.98, d, 3 H (1.5)	24.1 (CH_3)	H6	H6, H_2-8	H6, H8α, H9, H10
20		169.5 (C)		H9, H10, H12	
21	3.39, s, 3 H	54.4 (CH_3)		H16	H_2-15, H16
22		170.7 (C)		H12, H_3-23	
23	2.05, s, 3 H	21.0 (CH_3)			

[a] ^{13}C NMR multiplicities were deduced from a DEPT NMR experiment. [b] Proton resonances correlated to carbon resonances in the ^{13}C column. [c] Selected NOESY correlations.

ular structure of the crystalline solid was established by ^{1}H NMR, ^{13}C NMR, DEPT, ^{1}H-^{1}H COSY, TOCSY, HMQC, HMBC, and 2D NOESY spectroscopic experiments. Applying these combined NMR spectroscopic methods resulted in the unambiguous assignment of all the hydrogen and carbon atoms as listed in Table 1. A single-crystal X-ray structure analysis subsequently allowed us to confirm the proposed structure for **1**.

Ciereszkolide (**1**) was isolated as a colorless crystalline solid, $[\alpha]_D^{20} = +13.7$ (c = 0.7, $CHCl_3$). The UV spectrum of **1** in MeOH shows two absorption maxima centered at λ_{max} = 203 nm (ε = 21900) and λ_{max} = 238 nm (ε = 8900). Eleven degrees of unsaturation were deduced from its empirical formula $C_{23}H_{26}O_8$, established from HRFAB-MS analysis of the pseudomolecular ion $[M + H]^+$ at m/z = 431.1701 (calcd. for $C_{23}H_{27}O_8$: M = 431.1706). The IR spectrum (thin film deposited on a diamond cell in an IR microscope) shows strong absorptions at $\tilde{\nu}$ = 1796, 1745, and 1639 cm^{-1}, suggesting the presence of two distinct lactone and olefin functionalities, respectively, in **1**. The ^{13}C NMR spectrum recorded in $CDCl_3$ exhibits all 23 signals (Table 1), and a DEPT NMR experiment indicates the presence of four methyl, three methylene and eight methine groups, and eight quaternary carbon atoms. The presence of eleven sp^2-hybridized carbon atoms in the molecule, as deduced from the ^{13}C and DEPT NMR spectra, corresponding to four carbon−carbon and three carbon−oxygen double bonds indicates that compound **1** is tetracyclic.

The ^{1}H and ^{13}C NMR (Table 1) spectroscopic data identify four oxygen-bearing methine groups [δ_C = 105.5 (d), 85.5 (d), 77.6 (d), 67.5 (d); δ_H = 5.45 (dd, J = 10.7, 4.6 Hz), 5.39 (br. s), 5.09 (ddd, J = 11.3, 6.0, 1.4 Hz), 4.01 (d, J = 2.8 Hz)]; three ester carbonyl groups [δ_C = 177.5 (s), 170.7 (s), 169.5 (s)]; eight vinyl carbon atoms, of which four are quaternary [δ_C = 150.2 (s), 148.4 (s), 143.4 (s), 128.7 (s)], three are tertiary [δ_C = 157.9 (d), 117.2 (d), 110.6 (d); δ_H = 7.46 (d, J = 1.4 Hz, 1 H), 5.99 (q, J = 1.5 Hz, 1 H), 5.16 (d, J = 1.5 Hz, 1 H)], and one is terminal [δ_C = 112.4 (t); δ_H = 5.26 (br. s, 1 H), 5.25 (br. s, 1 H)]; two sp^3-methylene groups [δ_C = 40.6 (t); δ_H = 2.85 (dd, J = 12.3, 6.0 Hz, 1 H), 2.50 (dd, J = 12.3, 11.3 Hz, 1 H) and δ_C = 38.3 (t); δ_H = 2.12 (ddd, J = 14.3, 10.4, 4.6 Hz, 1 H), 2.57 (ddd, J = 14.3, 10.7, 3.4 Hz, 1 H)]; four methyl groups, three of which are attached to quaternary carbon atoms [δ_C = 24.1 (q), 21.0 (q), 20.5 (q); δ_H = 1.45 (br. s, 3 H), 1.98 (d, J = 1.5 Hz, 3 H), 2.05 (s, 3 H)] and one bears an oxygen atom [δ_C = 54.4 (q); δ_H = 3.39 (s, 3 H)]; and a conspicuous quaternary sp^3 carbon atom [δ_C = 53.2 (s)]. Since these spectroscopic data are not reminiscent of any known class of alcyonarian metabolites, it appeared that the structure of compound **1** is based on a novel carbon skeleton.[1,2]

Three partial structures (**A**−**C**) were deduced from extensive analyses of the 2D NMR spectroscopic data of **1**

including COSY, TOCSY, NOESY, HMQC, and HMBC spectra in $CDCl_3$ (Figure 1). The ^1H-^1H COSY, HMBC, and NOESY correlations for ciereszkolide (**1**) are summarized in Table 1. Units **A**−**C** were subsequently connected using a number of key HMBC NMR correlations (see Table 1).

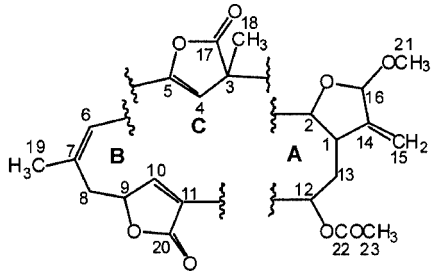

Figure 1. Partial structures of ciereszkolide (**1**) deduced from the ^1H-^1H COSY, HMQC, and HMBC NMR experiments

Fortunately, the recrystallization of compound **1** generated high-quality crystals, and an X-ray analysis of a single crystal provided the structure with relative stereochemistry (Figure 2). The relative configuration of the six stereocenters about the tetracyclic nuclei of ciereszkolide (**1**) was confirmed on the basis of ^1H NMR coupling constant and NOESY data (Table 1) supported by molecular modeling studies. Thus, the overall relative stereochemistry for ciereszkolide (**1**) was assigned as (1*S**,2*S**,3*R**,9*S**,12*R**, 16*S**). The absolute configuration of **1** could not be inferred from the X-ray crystallographic analysis.

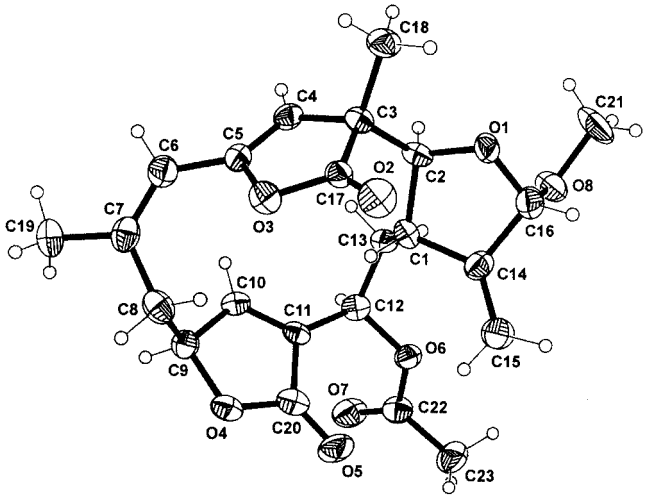

Figure 2. ORTEP diagram of ciereszkolide (**1**); the thermal ellipsoids are drawn at the 30% probability level; the crystallographic model of **1** shows the crystallographic atom-numbering scheme and the relative stereochemistry

Conclusion

The novel carbon skeleton of **1**, which we have named ciereszkane, represents a new class of marine-derived diterpenoid. From the structural viewpoint, ciereszkolide appears to be related to the *Pseudopterogorgia*-derived cembrane family of diterpenes by a ring contraction process requiring the overall migration of the C2−C3 σ-bond of a suitable cembrane precursor to the C4 position (Figure 3). Earlier, we have suggested a similar ring contraction for the conversion of a pseudopterane diterpene, kallolide A,[3a] leading to kallosin A.[8a] The mechanisms for the cembrane−ciereszkane and the pseudopterane−kallane cycloisomerizations are intriguing, and several possibilities may be considered. For instance, the proposed C2−C3 σ-bond migration taking place in each process could well be a concerted transformation, but a diradical mechanism cannot be ruled out as definite stereochemical information is lacking. On the other hand, as ciereszkolide and kallosin A appear to be the sole isomer resulting from these skeletal interconversions, these rearrangements must be intrinsically stereospecific.[9]

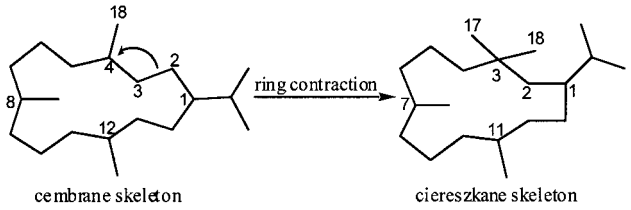

Figure 3. Novel ciereszkane skeleton and its proposed biogenetic interrelation with the cembrane skeleton upon migration of C2−C3 σ-bond to the C4 position

Ciereszkolide (**1**) did not display in vivo cytotoxicity in the brine shrimp lethality bioassay (BSLT), even at the highest concentration (500 μg/mL) after a 24 h count period. Moreover, in an in vitro anti-tuberculosis screen against *Mycobacterium tuberculosis* H37Rv at 6.25 μg/mL, compound **1** caused 0% inhibition.

Experimental Section

General Remarks: 1D and 2D NMR spectroscopic data were recorded with Bruker DPX-300 and DRX-500 FT NMR spectrometers. Infrared and UV spectra were obtained with a Nicolet Magna FT-IR 750 and a Shimadzu UV-2401PC UV/Vis spectrometer, respectively. The optical rotation was recorded with an Autopol IV automatic polarimeter. Column chromatography was performed on silica gel (35−75 mesh), and TLC analyses were carried out using glass precoated silica gel plates. HPLC separations were performed using a Polar-Bonded Cyano Ultrasphere semipreparative column (5.0 mm × 25 cm). All solvents used were either spectral grade or were distilled from glass prior to use. The percentage yield of ciereszkolide (**1**) is based on the weight of the crude CH_2Cl_2/MeOH extract.

Collection of *Pseudopterogorgia kallos*: Healthy colonies of *P. kallos* (Bielschowsky) were collected by SCUBA at depths of 25−28 m in Old Providence Island, Colombia, on March 15−16, 2002. A voucher specimen is stored in the Chemistry Department of the University of Puerto Rico, Río Piedras Campus.

Extraction and Isolation Procedures: The organism was partially air-dried, frozen, and lyophilized prior to its extraction. The dry

specimens (1.07 kg) were blended using a mixture of CH_2Cl_2/MeOH (1:1) (20 × 1 L). After filtration, the crude extract was concentrated and stored under vacuum to yield a greenish gum (166 g). The crude extract was partitioned between hexane and H_2O, and the aqueous suspension was extracted with $CHCl_3$ (3 × 2 L). The resulting $CHCl_3$ extract was concentrated in vacuo to yield 39.3 g of a brown amorphous solid, which was separated into 32 fractions (A−FF) by column chromatography on silica gel (673 g). Fraction U (849 mg) was separated into 12 fractions by column chromatography on silica gel (60 g) with a mixture of 4% EtOAc in $CHCl_3$ as eluent. The fifth fraction (90.6 mg) was purified by normal-phase HPLC (Ultrasphere Polar-Bonded Cyano column with 15% 2-propanol in hexane) to afford ciereszkolide (**1**) (4.5 mg, yield 0.0027%) as a colorless crystalline solid.

Ciereszkolide (1): $[\alpha]_D^{20} = +13.7$ ($c = 0.7$, $CHCl_3$). IR (thin film): $\tilde{\nu}$ = 3093, 2989, 2956, 2906, 2879, 2825, 1796, 1745, 1675, 1666, 1639, 1437, 1361, 1250, 1186, 1088, 1031, 1020 cm^{-1}. UV (MeOH): λ_{max} = 203 nm (ε = 21900), λ_{max} = 238 nm (ε = 8900). ^1H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz) spectroscopic data see Table 1. HRFAB-MS (glycerol): *m/z* $[M + H]^+$ = 431.1701 (calcd. for $C_{23}H_{27}O_8$: 431.1706).

X-ray Crystallographic Study: Suitable crystals for the X-ray crystallographic studies of ciereszkolide (**1**) were obtained as colorless needles by slow concentration of a $CHCl_3$/MeOH/EtOAc solution (3:3:1). The crystals were mounted on the tip of a glass fiber with epoxy glue. The X-ray data were collected at 298 K with a Bruker SMART 1 K CCD diffractometer equipped with a graphite monochromator and Mo-K_α radiation (λ = 0.71073 Å) using the SMART[10] software. Final values of the cell parameters were obtained from least-squares refinement of the positions of 686 reflections. A total of 1271 80-s frames were collected in three sets with a 0.3° ω-scan. The frames were then processed using the SAINT software[11] to give the *hkl* file corrected for Lorentz and polarization effects. No absorption correction was applied. The structure was solved by direct methods with the SHELX-90[12] program and refined by least-squares methods on F^2, SHELXTL-93,[13] incorporated in SHELXTL, Version 5.1.[14] The initial E-maps yielded all non-hydrogen atom positions. All non-hydrogen atoms were refined anisotropically, and the H atoms were positioned geometrically and treated as riding, with C−H distances in the range 0.93−0.98 Å and with $U_{iso}(H) = 1.2$ or $1.5\ U_{eq}(C)$. CCDC-232104 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge at www.ccdc.cam.ac.uk/conts/retrieving.html [or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; Fax: (internat.) + 44-1223-336-033; E-mail: deposit@ccdc.cam.ac.uk].

Crystal Data for Ciereszkolide (1): $C_{23}H_{26}O_8$, M_r = 430.44, space group monoclinic, $P2_1$ (No. 4). Unit cell parameters: a = 12.545(3) Å, b = 6.174(2) Å, c = 14.186(3) Å, β = 90.863(4)°, V = 1098.6(4) $Å^3$, Z = 2, $\rho_{calcd.}$ = 1.301 $Mg·m^{-3}$, F_{000} = 456, μ(Mo-K_α) = 0.098 mm^{-1}. Crystal dimensions 0.16 × 0.03 × 0.01 mm. A total of 5630 reflections collected, 3811 independent reflections (R_{int} = 0.0910), final R_{int} [$I > 2\sigma(I)$]: R_1 = 0.0726, wR_2 = 0.1670 for 284 variable parameters $\{w = 1/[\sigma^2F_o^2 + (0.0855P)^2 + 0.0000P]$, where $P = (F_o^2 + 2F_c^2)/3\}$. $(\Delta/\sigma)_{max} < 0.001$, $\Delta\rho_{max}$ = 0.20 $e·Å^{-3}$, $\Delta\rho_{min}$ = 0.22 $e·Å^{-3}$, GOF = 0.997.

Supporting Information: ^1H and ^{13}C NMR spectra for ciereszkolide (**1**) are available, see the footnote on the first page of this article.

Acknowledgments

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