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Intricarene, an Unprecedented Trispiropentacyclic Diterpene from the Caribbean Sea Plume *Pseudopterogorgia kallos*

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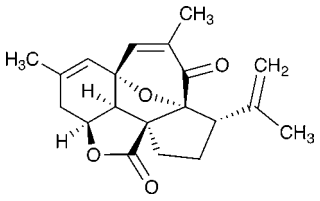
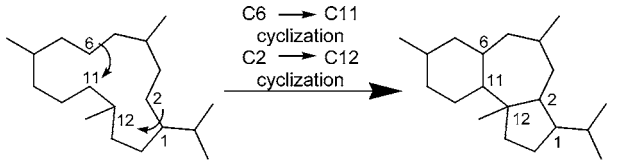
ABSTRACT



A novel trispiropentacyclic diterpene, intricarene (1), was isolated from the hexane extract of the Caribbean gorgonian octocoral *Pseudopterogorgia kallos*. Its highly entangled structure was established by interpretation of NMR, IR, UV, and HREIMS data and subsequently confirmed by X-ray diffraction analysis. The unprecedented carbon skeleton of 1 constitutes a new addition to the already impressive architectural diversity of the diterpene class of marine secondary metabolites.

Gorgonian corals of the genus *Pseudopterogorgia* have proven to be a rich source of terpenoid secondary metabolites that frequently exhibit interesting biological activities.[1] As part of an ongoing program to screen marine invertebrate extracts for antitubercular metabolites with low cytotoxicity, we have investigated the MeOH/$CHCl_3$ extract of a Colombian specimen of the gorgonian coral *Pseudopterogorgia kallos* (Bielschowsky, 1918).[2] Fractionation of the crude extract led subsequently to the isolation of the novel diterpenoid metabolite, intricarene (**1**), whose complex structure is based on the unprecedented intricarane carbon skeleton (Scheme 1).[3] In this paper, we present details of the isolation, structure determination, proposed biogenesis, and biological activity of **1**.

Scheme 1. Proposed Biogenesis for the Intricarane Skeleton



cembrane carbon skeleton — intricarane carbon skeleton

Specimens of *P. kallos* were collected by hand using scuba on reefs off the coast of Providencia (Old Providence) Island, Colombia, partially air-dried on site, frozen, lyophilized (1.07 kg), cut in small fragments, and homogenized exhaustively with a 1:1 mixture of CH_2Cl_2−MeOH. The green gum obtained (166 g) by evaporating the combined CH_2Cl_2−MeOH extracts was partitioned between hexane and H_2O. The hexane extract was concentrated under reduced pressure,

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(1) (a) Fenical, W. *J. Nat. Prod.* **1987**, *50*, 1001−1008. (b) Rodríguez, A. D. *Tetrahedron* **1995**, *51*, 4571−4618.

(2) For the first report on 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.

(3) From the Latin word *intricare*, to entangle, perplex.

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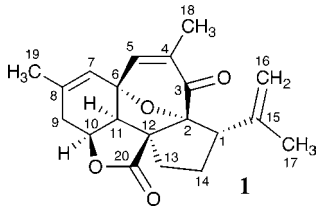
Table 1. ^{1}H NMR (500 MHz), ^{13}C NMR (125 Mz), ^{1}H−^{1}H COSY, NOESY, and HMBC Spectral Data for Intricarene (**1**)[a]

position	δ_H, mult, intrgt (*J* in Hz)	δ_C (mult)[b]	^{1}H−^{1}H COSY	NOESY	HMBC[c]
1	3.38, dd, 1H (11.9, 5.7)	46.4 (CH)	H14α*β*	H13*β*, H14*β*, H16*β*, H_3-17	H13α*β*, H16α*β*, H_3-17
2		102.8 (C)			H1, H13α, H14α*β*
3		193.0 (C)			H5, H_3-18
4		137.2 (C)			H_3-18
5	6.27, q, 1H (1.6)	147.3 (CH)	H_3-18	H9*β*, H_3-18	H11, H_3-18
6		84.6 (C)			H5, H10, H11
7	6.41, q, 1H (1.6)	128.1 (CH)	H_3-19	H_3-19	H9α, H11, H_3-19
8		135.2 (C)			H9α*β*, H_3-19
9α	2.82, dd, 1H (18.6, 8.6)	35.7 (CH_2)	H9*β*, H10	H9*β*, H10, H_3-19	H7, H11, H_3-19
9*β*	2.41, br d, 1H (18.6)		H9α, H10	H5, H9α	
10	4.77, ddd, 1H (8.6, 5.3, 2.6)	70.7 (CH)	H9α*β*, H11	H9α, H11	H9α*β*, H11
11	2.54, d, 1H (5.3)	58.0 (CH)	H10	H10, H13α	H9α*β*, H13α*β*
12		64.2 (C)			H11, H13*β*, H14α*β*
13α	2.01, m, 1H	29.3 (CH_2)	H13*β*, H14α*β*	H11, H13*β*	H11
13*β*	1.92, m, 1H		H13α, H14α*β*	H1, H13α	
14α	2.11, m, 1H	28.5 (CH_2)	H1, H13α*β*	H11	H1, H13α*β*
14*β*	1.91, m, 1H		H1, H13α*β*	H1	
15		141.8 (C)			H1, H16*β*, H_3-17
16α	4.92, q, 1H (1.3)	113.3 (CH_2)	H16*β*, H_3-17	H1, H_3-17	H_3-17
16*β*	4.86, br s, 1H		H16α		
17	1.75, br s, 3H	23.1 (CH_3)	H16α	H1, H16*β*	H16α*β*
18	1.76, d, 3H (1.6)	14.4 (CH_3)	H5	H5	H5
19	1.84, t, 3H (1.2)	23.0 (CH_3)	H7	H7, H9α	H7, H9α
20		177.9 (C)			H11, H13*β*

[a] Spectra were recorded in $CDCl_3$ 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 ^{13}C column.

and the oily residue (71.9 g) obtained was fractionated via size-exclusion chromatography over Bio-Beads SX-3 in toluene followed by silica gel chromatography using step gradient elution (hexanes to EtOAc) to afford pure intricarene (**1**) as a white crystalline solid (4.0 mg; 0.0024% based on the crude extract dry wt).[4]



1

Intricarene (**1**) was isolated as optically active crystals ($[\alpha]^{20}_D$ +50.0° (*c* 0.7, $CHCl_3$)) that gave an $[M]^+$ ion at *m*/*z* 326.1524 in the HREI-MS appropriate for a molecular formula of $C_{20}H_{22}O_4$ (calcd 326.1518), requiring 10 sites of unsaturation.[5] The infrared spectrum of **1** showed bands characteristic of γ-lactone and α,*β*-unsaturated ketone carbonyl groups (ν_{max} = 1767 and 1690 cm^{-1}). Twenty resonances (8 × C; 5 × CH; 4 × CH_2; 3 × CH_3) were observed in the ^{13}C NMR spectrum of **1** (Table 1). Four deshielded resonances in the ^{1}H NMR spectrum [δ 6.41, q, J = 1.6 Hz (H-7); 6.27, q, J = 1.6 Hz (H-5); 4.92, q, J = 1.3 Hz (H-16α); 4.86, br s (H-16*β*)], three sharp methyl signals [δ 1.84, t, J = 1.2 Hz (H_3-19); 1.76, d, J = 1.6 Hz (H_3-18); 1.75, br s (H_3-17)], and six deshielded resonances in the ^{13}C NMR spectrum [δ 147.3 (CH, C-5); 137.2 (C, C-4); 135.2 (C, C-8); 128.1 (CH, C-7); 141.8 (C, C-15); 113.3 (CH_2, C-16)] were readily assigned to three methyl-bearing olefins (two trisubstituted and one 1,1-disubstituted) by analysis of their ^{1}H scalar coupling patterns and the corresponding ^{1}H−^{1}H COSY, HMQC, and HMBC correlations (Table 1). Two additional carbonyl resonances [δ 193.0 (C-3); 177.9 (C-20)] in the ^{13}C NMR spectrum accounted for five sites of unsaturation, and the absence of ^{13}C NMR evidence for further unsaturated functionality indicated the presence of five additional rings in **1**.

Comprehensive analysis of two-dimensional NMR data, including the results of ^{1}H−^{1}H COSY, HMQC, and HMBC experiments, enabled the complete planar structure of intricarene to be constructed, as in **1**, on a [6−7−5] tricarbocyclic framework substituted with methyl groups and an isopropenyl side chain. To establish these structural features of **1**,

(4) For our prior work in this series, see: (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. (c) Marrero, J.; Rodríguez, A. D.; Baran, P.; Raptis, R. G.; Sánchez, J. A.; Ortega-Barria, E.; Capson, T. L. *Org. Lett*. **2004**, *6*, 1661−1664. (d) Marrero, J.; Rodríguez, A. D.; Baran, P.; Raptis, R. G. *Eur. J. Org. Chem*. **2004**, 3909−3912.

(5) Data for intricarene (**1**): white crystals; $[\alpha]_D$ +50.0° (*c* 0.7, $CHCl_3$); UV (MeOH) λ_{max} 245 nm (6200); IR (film, NaCl) ν_{max} 3078, 2947, 1767, 1690, 1641, 1627, 1440, 1380, 1282, 1182, 1166, 1002, 973 cm^{-1}; LREIMS *m*/*z* (%) 326 (M^+, 74), 311 (23), 298 (13), 281 (6), 230 (100), 216 (40), 215 (46), 91 (33); HREI-MS *m*/*z* 326.1524, calcd for $C_{20}H_{22}O_4$ 326.1518.

exhaustive NMR measurements were conducted in $CDCl_3$. In this solvent, the three methyl signals (a broad singlet for C-17, a doublet for C-18, and a triplet for C-19) were readily resolved. HMBC experiments conclusively placed the methyl groups at strategic locations on the tricarbocyclic ring structure of intricarene. Correlations from the H_3-17 protons to C-1, C-15, and C-16 placed the isopropenyl side chain at C-1. Correlations from the H_3-18 methyl protons to C-3 and C-5 allowed this methyl group to be placed at C-4, and likewise, correlations from the H_3-19 methyl protons to C-7, C-8, and C-9 allowed this methyl group to be placed at C-8. HMBC correlations observed between a carbon resonance at δ 102.8 (C-2) and both the methine resonance at δ 3.38 (H-1) and the methylene resonances at δ 2.01 (H-13α) and 2.11/1.91 (H-14$\alpha\beta$) showed that the tertiary carbon C-2, bearing an ether oxygen and a ketone carbonyl, had to be bridged by a saturated ethylene group through C-1 and C-12 to give a cyclopentane ring (C-1 to C-2 and C-12 to C-14). HMBC correlations between the pivotal methine resonance at δ 2.54 (H-11) and all of the ^{13}C resonances at δ 177.9 (C-20), 147.3 (C-5), 128.1 (C-7), 84.6 (C-6), 70.7 (C-10), 64.2 (C-12), 35.7 (C-9), and 29.3 (C-13) demonstrated that such a key bridgehead carbon (C-11) had to be flanked by the spirocyclic carbons C-6 and C-12 and by the oxymethine carbon C-10. A series of HMBC correlations between the olefinic resonance at δ 6.27 (H-5) and the ^{13}C resonances at δ 193.0 (C-3), 84.6 (C-6), and 14.4 (C-18) showed that the spirocyclic carbon atoms C-2 and C-6 had to be bridged by an ether oxygen and the α,β-unsaturated ketone bearing the methyl (C-18) group to give an 8-oxabicyclo[3.2.1]octane ring system (C-2 to C-6 and C-11 to C-12). Similarly, HMBC correlations between δ 6.41 (H-7) and both 35.7 (C-9) and 23.0 (C-19) and between 1.84 (H_3-19) and 135.2 (C-8), 128.1 (C-7), and 35.7 (C-9) accounted for the cyclohexene ring in **1** (C-6 to C-11). Last, HMBC correlations between δ 177.9 (C-20) and both δ 2.54 (H-11) and 1.92 (H-13β) indicated that C-20 had to be bonded to a quaternary carbon (C-12) and to C-11 through the oxymethine carbon at δ 70.7 (C-10) to complete the final ring required in **1**.

Portions of the relative stereochemistry of intricarene were readily assigned by NOE NMR spectral methods. However, while the relative configurations about the three spirocyclic carbons in **1** (C-2, C-6, and C-12) could be assigned indirectly on the basis of the overall correlations observed in the NOESY NMR spectrum, confirmation of the entire structure of intricarene by single-crystal X-ray diffraction analysis was highly desirable. Thus, recrystallization of **1** by slow evaporation from a mixture of MeOH/$CHCl_3$/acetone gave crystals of excellent quality that were amenable to X-ray crystallographic analysis. The X-ray crystal structure, which defines only the relative configuration, is shown in Figure 1.[6] Hence, the overall relative stereochemistry for the six stereocenters within the highly strained ring systems in intricarene was assigned as 1*S**, 2*R**, 6*R**, 10*S**, 11*S**, and 12*S**. The assigned configurations, which were in full agreement with the correlations observed in the NOESY spectrum of **1** (Figure 2), were confirmed through interpretation of NMR coupling constant data (Table 1).

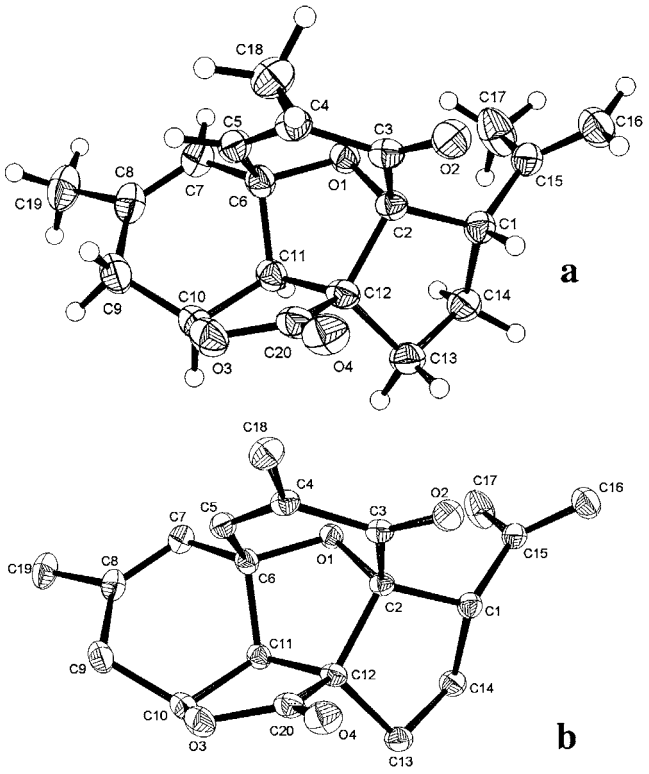


Figure 1. Computer-generated ORTEP drawings of intricarene (**1**) with atom labeling scheme. The absolute configuration shown is arbitrary. In (a) the carbon and oxygen atoms are drawn as 50% thermal ellipsoids; in (b) the hydrogen atoms are omitted for clarity, and the carbon and oxygen atoms are drawn as 30% thermal ellipsoids.

Intricarene (**1**) has an unprecedented regular diterpenoid carbon skeleton that can be formally derived from the "cembrane" skeleton found in many *Pseudopterogorgia* metabolites by sequential cyclization via [C6 → C11] and [C2 → C12] bond formation.[7] The co-occurrence of **1** with various furanocembranoid lactones within the same organism suggests the biogenetic pathway outlined in Scheme 1.[8] We suggest the semisystematic name "intricarane" to define this new carbon skeleton and a numbering scheme that preserves the C1−C20 numbering of the cembrane skeleton.

Intricarene is exceptional in several respects. While other diterpenes based on a [6−7−5] tricarbocyclic system have

(6) Crystal data for intricarene (**1**) at 298(2) K: $C_{20}H_{22}O_4$, M_r = 326.38, monoclinic, space group $P2_1$, a = 7.1134(4), b = 12.6053(6), c = 9.4510(5) Å, β = 105.5470(2)°, V = 816.43(6) Å^3, Z = 2, ρ_{calc} = 1.328 Mg m^{-3}, F_{000} = 348, λ(Mo Kα) = 0.71073 Å, μ = 0.092 mm^{-1}. Data collection and reduction: crystal size, 0.50 × 0.25 × 0.25 mm^3, θ range, 2.24−27.16°, 5909 reflections collected, 1882 independent reflections (R_{int} = 0.0291), final R indices ($I > 2\sigma(I)$); R_1 = 0.0322, wR_2 = 0 for 220 variable parameters, GOF = 1.030. CCDC 265687 (**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).

(7) (a) Wright, A. E.; Burres, N. S.; Schulte, G. K. *Tetrahedron Lett.* **1989**, *30*, 3491−3494. (b) Rodríguez, A. D.; Shi, J.-G.; Huang, S. D. *J. Nat. Prod.* **1999**, *62*, 1228−1237.

(8) Complete details of the isolation, purification, structure elucidation, and biological activities of all of the new furanocembranolides coisolated during the present study will soon be reported.

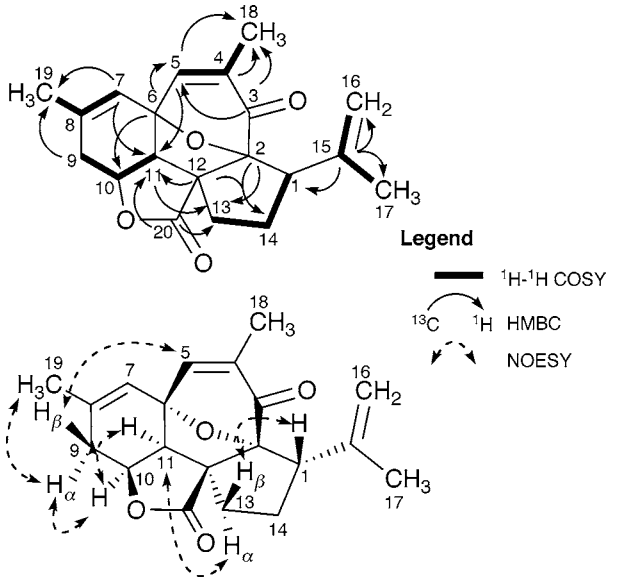


Figure 2. Key ^{1}H−^{1}H COSY, HMBC, and NOESY correlations for intricarene (**1**).

been reported, the structural core of **1** is unprecedented in a natural product. It is the combination of an oxabridge across the seven-membered ring in **1** (between C-2 and C-6) leading to a complex oxapolycyclic system, the three spirocyclic systems (two of them adjoined), and the methylation/isopropylation substitution pattern that makes the carbon skeleton of intricarene unique. Thus, intricarene (**1**) represents the first example of a new structural class of diterpenes. The closest structural relatives to **1** are the dolastane- and the guanacastepene-based diterpenes, isolated from marine and terrestrial sources, respectively. These families of compounds also possess a [6−7−5] tricarbocyclic core but have a distinct methylation/isopropylation substitution pattern.[9]

Compound **1** was tested for its inhibitory activity toward the growth of *Mycobacterium tuberculosis* H_{37}Rv (ATCC 27294) in the microplate Alamar Blue assay.[10] The highest percentage of inhibition attained (15%) was detected at a concentration of 128 μg/mL, thus suggesting a lack of appreciable toxicity against this bacterium strain. Unfortunately, the paucity of this material prevented us from conducting further biological screenings.

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Supporting Information Available: Detailed description of the experimental procedures, copies of the NMR (^{1}H and ^{13}C), and HREI-MS spectra of intricarene (**1**), a partial mechanistic interpretation of the mass-spectral fragmentation data, 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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