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Isolation and Characterization of Kallosin A, a Novel Rearranged Pseudopterane Diterpenoid from the Caribbean Sea Plume *Pseudopterogorgia kallos* (Bielschowsky)

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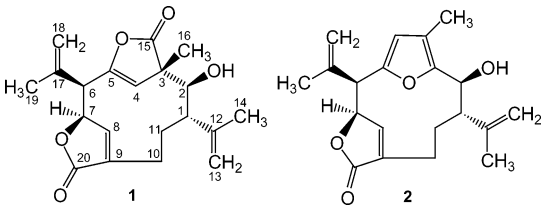
Abstract: The Caribbean alcyonacean *Pseudopterogorgia kallos* is shown to contain a novel rearranged pseudopterane diterpene, kallosin A (**1**), possessing several unusual structural features. In addition to having two distinct 2(3*H*)- and 2(5*H*)-furanone moieties, kallosin A is based on a new carbon skeleton. The structural assignment of **1** was based mainly on 1D and 2D NMR spectral data and was further supported by accurate mass measurement and single-crystal X-ray diffraction analysis.

Caribbean gorgonian octocorals belonging to the genus *Pseudopterogorgia* have attracted considerable attention as a source of novel natural products with unique structures and biological properties.[1,2] In particular, terpenes are thought by many to be the largest pool of chemical diversity and physiologically interesting metabolites.[3] Surprisingly, despite the great interest in *Pseudopterogorgia* octocorals, there appears to be only one in-depth study concerning the natural products chemistry of *Pseudopterogorgia kallos* (Bielschowsky, 1918).[4] In this paper, we report the isolation and characterization by ^{1}H and ^{13}C NMR, IR, UV, FABMS, and X-ray crystallography of kallosin A (**1**), a new diterpene isolated from a Colombian specimen of *P. kallos* possessing a previously undescribed carbon skeleton.



A chloroform extract of sun-dried *P. kallos* collected at depths of 83−93 ft near Old Providence Island, Colombia, was chromatographed on silica gel and divided into 32 fractions of differing polarity. Repeated silica gel column chromatography of the residual oil (1.34 g) left over from fraction 12 after concentration furnished kallosin A (**1**, 6.1 mg) and the known pseudopterane diterpene kallolide A (**2**, 359 mg).[4]

Kallosin A (**1**), $[\alpha]^{20}_D$ −48.1 (*c* 0.33, $CHCl_3$), was obtained as a colorless crystalline solid. The IR spectrum (thin film deposited on a diamond cell in an IR microscope) showed strong absorptions at ν_{max} 3521, 1771, 1752, and 1647 cm^{-1} suggesting the presence of hydroxyl, lactone, and olefin functionalities. The UV spectrum of **1** in MeOH showed an absorption maximum centered at λ_{max} 207 nm (ϵ 7500). An intense pseudomolecular ion at *m/z* 345.1702 $[M + H]^+$ corresponded to a molecular formula of $C_{20}H_{24}O_5$ and indicated a hydrogen deficiency of 9. The ^{1}H NMR and ^{13}C NMR (Table 1) indicated two terminal double bonds [δ_H 5.09 (br d, 1H), 5.29 (br d, 1H); δ_C 143.0 (C), 119.3 (CH_2) and δ_H 5.31 (br s, 1H), 5.11 (br s, 1H); δ_C 138.6 (C), 115.6 (CH_2)], two polarized trisubstituted double bonds [δ_H 5.43 (s, 1H); δ_C 149.3 (C), 111.8 (CH) and δ_H 7.08 (br s, 1H); δ_C 147.8 (CH), 137.3 (C)], and two lactones [δ_C 180.4 (C) and 174.9 (C)], which together accounted for six degrees of unsaturation. Spectral evidence thus demanded that compound **1** was tricyclic with four olefins and two lactone carbonyl groups. The ^{1}H NMR spectrum of **1** showed two broad methyl singlets at δ_H 1.92 and 1.71, which together with the four broad singlets at δ_H 5.31, 5.29, 5.11 and 5.09 (each 1H), were indicative of two $-C(CH_3)=CH_2$ groups, a sharp three-protons singlet at δ_H 1.44 indicating a tertiary methyl group, a one-proton broad multiplet at δ_H 5.44 suggesting a hydrogen atom on the carbon bearing a lactone oxygen, and a one-proton doublet at δ_H 3.64 (J = 10.8 Hz) indicative of a hydrogen on a carbon bearing a secondary hydroxyl group. Other salient features of the spectrum included a one-proton broad singlet at δ_H 3.42 and a one-proton doublet of doublet at δ_H 2.12 (J = 10.4, 10.8 Hz) ascribable to bis allylic and allylic methines, respectively.

The ^{13}C NMR and DEPT spectra of **1** exhibited 20 carbon signals (3CH_3, 4CH_2, 6CH, and 7C) whose chemical shift values and multiplicity confirmed the presence of a 2(3*H*)- and 2(5*H*)-furanones [δ_C 180.4 (C), 149.3 (C), 111.8 (CH), 53.2 (C) and δ_C 174.9 (C), 147.8 (CH), 137.3 (C), 79.3 (CH), respectively],[5] two isopropenyl side chains [δ_C 143.0 (C), 119.3 (CH_2), 17.0 (CH_3) and δ_C 138.6 (C), 115.6 (CH_2), 22.7 (CH_3)], and one hydroxyl-bearing secondary carbon [δ_C 73.3 (CH)] as the partial structures. The ^{1}H and ^{13}C NMR signals of **1**, which were assigned by DEPT, ^{1}H−^{1}H COSY, HMQC, and HMBC, are listed in Table 1. The relative positions of all the partial structures and functional groups present in **1** were clearly supported by these experiments.

The planar structure of kallosin A (**1**) was confirmed and the relative stereochemistry assigned from a single-crystal X-ray diffraction analysis. Subsequent ^{1}H−^{1}H NOE experiments supported by coupling constant analysis and molecular modeling studies fully agreed with the proposed relative stereochemistry.[6]

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TABLE 1. ^{1}H NMR (500 MHz), ^{13}C NMR (125 MHz), ^{1}H−^{1}H COSY, and HMBC Spectral Data of Kallosin A (1) in $CDCl_3$[a]

position	δ_H, mult, intgrt (*J* in Hz)	δ_C (mult)[b]	^{1}H−^{1}H COSY	HMBC[c]
1	2.12, dd, 1H (10.4, 10.8)	50.7 (CH)	H-2, H-11α	H-2, H-10α*β*, H-11*β*, H-13α*β*, H_3-14
2	3.64, d, 1H (10.8)	73.3 (CH)	H-1	H-1, H-11*β*, H_3-16
3		53.2 (C)		H-1, H-4, H_3-16
4	5.43, s, 1H	111.8 (CH)		H-2, H-6, H_3-16
5		149.3 (C)		H-4, H-6, H-7
6	3.42, br s, 1H	49.2 (CH)	H-7, H-8, H-18α*β*	H-18α*β*, H_3-19
7	5.44, br m, 1H	79.3 (CH)	H-6, H-8	H-8
8	7.08, br s, 1H	147.8 (CH)	H-6, H-7	H-7, H-10α*β*
9		137.3 (C)		H-7, H-8, H-10α*β*, H-11α*β*
10α	2.26, ddd, 1H (3.8, 4.1, 13.2)	21.3 (CH_2)	H-10*β*, H-11α*β*	H-1, H-8, H-11*β*
10*β*	2.32, ddd, 1H (2.0, 2.4, 13.2)		H-10α, H-11α*β*	
11α	1.75, m, 1H	31.6 (CH_2)	H-1, H-10α*β*, H-11*β*	H-1, H-10α*β*
11*β*	0.84, ddd, 1H (3.0, 11.8, 12.3)		H-10α*β*, H-11α	
12		143.0 (C)		H-1, H-11*β*, H-13α*β*, H_3-14
13α	5.09, br d, 1H (1.5)	119.3 (CH_2)	H-13*β*, H_3-14	H-1, H_3-14
13*β*	5.29, br d, 1H (1.6)		H-13α, H_3-14	
14	1.71, br s, 3H	17.0 (CH_3)	H-13α*β*	H-1, H-13α*β*
15		180.4 (C)		H-4, H_3-16
16	1.44, s, 3H	23.6 (CH_3)		
17		138.6 (C)		H-6, H-18α*β*, H_3-19
18α	5.31, br s, 1H	115.6 (CH_2)	H-6, H-18*β*, H_3-19	H-6, H_3-19
18*β*	5.11, br s, 1H		H-6, H-18α, H_3-19	
19	1.92, br s, 3H	22.7 (CH_3)	H-18α*β*	H-6, H-18α*β*
20		174.9 (C)		H-7, H-8, H-10α*β*
2-OH	2.17, s, 1H (exchangeable)			

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

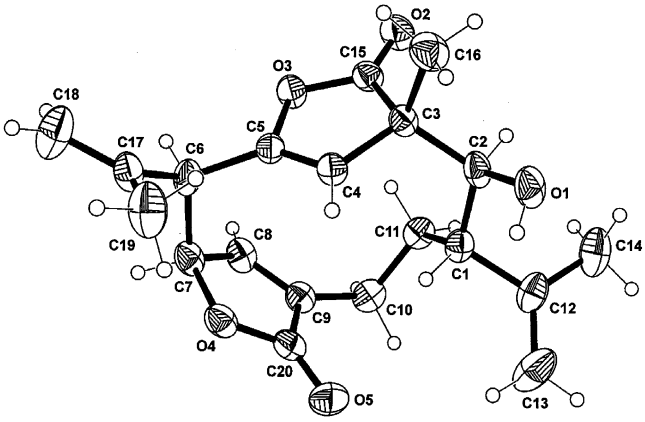

FIGURE 1. Molecular structure of kallosin A (**1**), which defines only the relative configuration, with atom labeling scheme. The carbon and oxygen atoms are drawn as 30% thermal ellipsoids.

The X-ray crystal structure (Figure 1) reveals that the kallane carbon skeleton, which has not been previously reported, appears to be structurally related to the *Pseudopterogorgia*-derived pseudopterane family of diterpenes[7] by a ring contraction process requiring the migration of the C2−C3 *σ* bond of a pseudopterane precursor to the C4 position (Figure 2). Thus, kallosin A (**1**) can be envisioned as a rearranged pseudopterane diterpenoid based on the novel kallane skeleton. Specifically, the present compound is considered to arise from kallolide A (**2**) through oxidation of the double bonds in the furan ring followed by homolytic cleavage of the peroxide linkage and concomitant migration of the C2−C3 bond

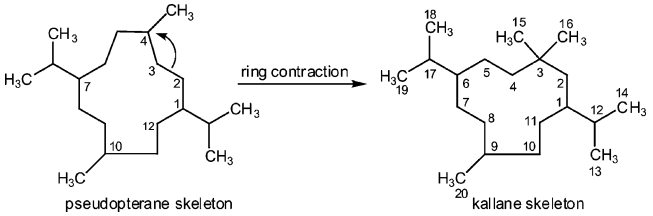

FIGURE 2. Novel kallane skeleton with numbering system and its proposed biogenetic interrelation with the pseudopterane skeleton upon migration of the C2−C3 *σ* bond to the C4 position.

to the C4 position, epoxide ring opening, and dehydration (Scheme 1). Although kallosin A is the first member of this novel class of marine-derived diterpenes to be discovered, there exist a few reports describing the isolation of 2(3*H*)-furanones (or their corresponding congeners bearing an epoxide group) presumably arising from a furanoterpenoid precursor following a similar biogenetic route.[8,9]

At 500 *μ*g/mL, kallosin A (**1**) displayed no in vivo cytotoxicity in the brine shrimp lethality bioassay (BSLT), whereas kallolide A (**2**) showed a 64% death response at the same concentration after a 24 h count period.[10] Further investigations regarding the possible antiinflammatory properties of kallosin A and the biogenetic relationship between diterpenes **1** and **2** are in progress.[11]

Experimental Section

Collection and Extraction Procedures. Kallosin A (**1**) was isolated from the gorgonian octocoral *P. kallos* (Bielschowsky,

(6) Selected NOEs for kallosin A (**1**): H-1/H-4, H-1/H-13*β*, H-2/H-11α*β*, H-2/H_3-16, H-4/H_3-16, H-6/H-7, H-6/H-8, H-6/H-18α, H-6/H_3-19, H-7/H-8, H-8/H-10α, H-10*β*/H-13*β*, H13α/H_3-14, H-18*β*/H_3-19.

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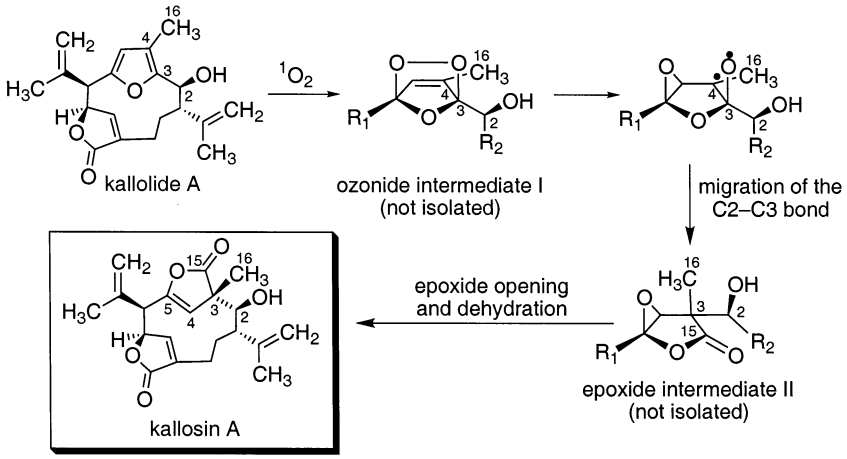
SCHEME 1



1918) collected in March 2002 at depths of 83−93 ft near Old Providence Island, Colombia. A voucher specimen is stored at the Chemistry Department of the University of Puerto Rico. The sun-dried animal (1.07 kg) was blended with MeOH−CH_2Cl_2 (1:1) (20 × 1 L), and after filtration, the crude extract was evaporated under vacuum to yield a green gummy residue (166 g). After the crude extract was partitioned between hexane and H_2O, the aqueous suspension was extracted with $CHCl_3$ (3 × 2 L). The resulting extract was concentrated in vacuo to yield 39 g of a brown solid that was chromatographed over silica gel (673 g) using a step gradient of EtOAc−hexane (0−100%) and thus separated into 32 fractions (I−XXXII) on the basis of TLC and NMR analyses. Purification of fraction XII (1.34 g) by silica gel (70 g) column chromatography with 1% acetone in $CHCl_3$ yielded 13 subfractions, denoted A−M. Purification of subfraction G (113 mg) by silica gel (15 g) chromatography with 1% acetone in $CHCl_3$ led to pure kallosin A (**1**) (6.1 mg, yield = 3.7×10^{-3}%). Furthermore, purification of subfraction H (770 mg) on a silica gel (40 g) column using a mixture of 17% EtOAc in hexane as eluant afforded known kallolide A (**2**) (359 mg, 0.22%).[4]

Kallosin A (1): colorless crystalline solid; $[\alpha]^{20}_D$ −48.1 (*c* 0.33, $CHCl_3$); IR (thin film) 3521, 3112, 3095, 2977, 2954, 2918, 2853, 1771, 1752, 1647, 1410, 1197, 1090, 1037, 1016, 962 cm^{-1}; UV λ_{max} (MeOH) 207 nm (ϵ 7500); ^1H NMR (500 MHz, $CDCl_3$) see Table 1; ^{13}C NMR (125 MHz, $CDCl_3$) see Table 1; HRFAB-MS (glycerol) *m*/*z* $[M + H]^+$ 345.1702 (calcd for $C_{20}H_{25}O_5$, 345.1702).

(11) By the end of this work ,we did not have enough natural product left to test for antiinflammatory activity. It is quite conceivable, however, that kallosin A (**1**) is a strong antiinflammatory agent insofar as kallolide A (**2**), its most likely biogenetic precursor, has been described as a potent inhibitor of phorbol ester induced inflammation in the mouse ear assay (see ref 4). We are currently attempting to procure additional quantities of **1** to explore this contention further.

X-ray Single-Crystal Structure Determination of Kallosin A (1) at 298(2) K. Crystal Data: $C_{20}H_{24}O_5$, M_r = 344.39, monoclinic, space group *C2* (No. 5), *a* = 16.749(4) Å, *b* = 6.248(2) Å, *c* = 17.816(4) Å, β = 106.363(5)°, *V* = 1788.8(7) $Å^3$, *Z* = 4, ρ_{calc} 1.279 Mg m^{-3}, F_{000} = 736, λ(Mo Kα) = 0.71073 Å, μ= 0.091 mm^{-1}. **Data collection and reduction:** crystal size, 0.14 × 0.07 × 0.05 mm^3, θ range, 1.19−28.04°, 6370 reflections collected, 4154 independent reflections (R_{in}= 0.0311), final *R* indices ($I > 2\sigma(I)$): R_1= 0.0534, wR_2 = 0.1091 for 230 variable parameters, GOF = 1.038.

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Supporting Information Available: General experimental paragraph, ^1H and ^{13}C NMR spectra for kallosin A, and tables of crystal 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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