Kallolide A, a New Antiinflammatory Diterpenoid, and Related Lactones from the Caribbean Octocoral *Pseudopterogorgia kallos* (Bielschowsky)

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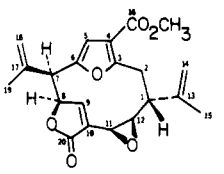
Received April 1, 1985

Four new representatives of the rare pseudopterane skeleton are reported from the Caribbean gorgonian *Pseudopterogorgia kallos* (Bielschowsky). The structure of the sole crystalline metabolite, kallolide A acetate (3), was provided by single-crystal X-ray diffraction analysis. Structures for kallolides A–C (2, 4, 5) were subsequently established on the basis of spectral analyses and chemical interconversions. Kallolide A (2), the major metabolite, shows potent antiinflammatory activity.

The genus *Pseudopterogorgia* is widely distributed throughout the tropical Atlantic Ocean and the Caribbean Sea, and these organisms are known to produce a diversity of novel secondary metabolites.[2-8] As part of a research program emphasizing the characterization of biologically active and structurally unique natural products from marine octocorals, we have focused our attention on this conspicuous and abundant genus of sea whips. Our earlier work on *Pseudopterogorgia acerosa* resulted in the discovery of pseudopterolide (1), a potent cytotoxic di-



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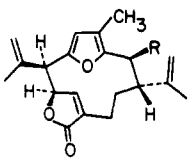
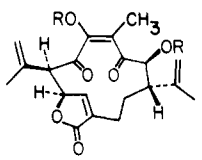


2 R = OH
3 R = OAc
4 R = H



5 R = H
9 R = Ac

terpenoid possessing the irregular pseudopterane carbon skeleton.[7] We now report the structures of four closely related lactones, kallolides A, A acetate, B, and C (2–5) isolated from the Caribbean gorgonian *Pseudopterogorgia kallos*. The major metabolite, kallolide A (2), shows marked antiinflammatory activity,[9] thereby extending the range of biological activity recognized for these 12-membered ring furanolactones.

Table I. ^{13}C NMR Assignments for Kallolides A, A Acetate, B, and C (2–5)[a]

carbon	kallolide A (2)	kallolide A acetate (3)	kallolide B (4)	kallolide C (5)
1	48.6	46.0	42.1	47.6
2	65.1	66.5	30.1[b]	67.0
3	149.4[b]	147.1[b]	149.6[c]	202.3
4	119.8	121.6	116.4	100.4[b]
5	112.2	112.2	112.4	100.0[b]
6	151.1[b]	151.7[b]	150.0[c]	183.8
7	49.6	48.6	48.8	51.0
8	80.9	80.7	81.2	79.9
9	146.5	146.2	147.1	145.3
10	136.9	137.2	136.8	135.3
11	21.9	21.9	23.1	21.6
12	33.0	33.2	34.5[b]	28.7
13	141.7[c]	141.6[c]	148.2[d]	139.8[c]
14	114.7[d]	114.7[d]	110.7[e]	115.9[d]
15	17.3[e]	18.0[e]	19.5[f]	17.4[e]
16	9.6	9.5	9.7	6.5
17	144.2[c]	143.7[c]	142.7[d]	143.5[c]
18	116.3[d]	114.8[d]	114.5[e]	119.2[d]
19	21.6[e]	21.6[e]	21.8[f]	23.5[e]
20	175.6	175.4	175.6	174.6
		170.5[g]		
		20.9[g]		

[a] The ^{13}C NMR spectra were recorded in $CDCl_3$ at 50 MHz. Multiplicities were obtained by single-frequency off-resonance decoupling, and assignments were made based on J_R values and/or comparison to models. [b–f] Signals within a column may be reversed. [g] Signals associated with an acetate ester.

An abundant reef-dwelling species found in the Caribbean Sea, *P. kallos* grows as small, bushy plumose colonies that may reach a height of only 15–20 cm or less. At Channel Cay, Little Harbor Cay, and Chub Cay in the Bahama Islands, *P. kallos* was one of the most common gorgonians growing at depths of 5–15 m. Freshly collected animals were stored frozen and subsequently extracted with chloroform and then ethyl acetate. Standard silica gel chromatography of the combined organic extracts followed by separation using high-performance liquid chromatography (HPLC) led to the purification of pseudopteranes 2–5. Kallolide A (2) was the major metabolite isolated from the gorgonian, comprising 2.0% of the organic extract. Small quantities of metabolites 3–5 were isolated, which together represented less than 2.0% of the crude lipid extract.

Kallolide A (2) was isolated as an amorphous solid after purification by HPLC. A molecular formula of $C_{20}H_{24}O_4$

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(9) Research in progress in the laboratory of Dr. R. S. Jacobs, University of California, Santa Barbara. Complete details of the pharmacology of kallolide A will be published at a later date.

0022-3263/85/1950-5741$01.50/0

Table II. ^{1}H NMR Assignments for Kallolides A, A Acetate, B, and C (2-5)[a]

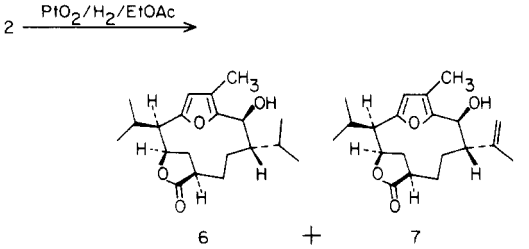
carbon	kallolide A (2)	kallolide A acetate (3)	kallolide B (4)	kallolide C (5)
1	2.86 (1 H, ddd, J = 1.0, 3.8, 11.2)	3.08 (1 H, ddd, J = 1.0, 7.5, 11.8)	2.69 (1 H, m)	2.42 (1 H, ddd, J = 1.0, 4.3, 11.1)
2	4.36 (1 H, d, J = 11.2)	5.57 (1 H, d, J = 11.8)	2.43 (1 H, d, J = 12.3) 2.46 (1 H, dd, J = 4.4, 12.0)	4.48 (1 H, d, J = 11.1)
3				
4				
5	5.90 (1 H, s)	5.89 (1 H, s)	5.85 (1 H, s)	
6				
7	3.80 (1 H, d, J = 4.6)	3.79 (1 H, d, J = 4.5)	3.74 (1 H, d, J = 4.0)	3.44 (1 H, d, J = 5.4)
8	5.40 (1 H, d, J = 4.6)	5.41 (1 H, d, J = 4.5)	5.37 (1 H, d, J = 4.0)	5.10 (1 H, d, J = 5.4)
9	6.65 (1 H, br s)	6.65 (1 H, br s)	6.68 (1 H, s)	6.67 (1 H, s)
10				
11	2.11 (1 H, ddd, J = 1.0, 2.0, 15.8)	2.13 (1 H, ddd, J = 1.0, 1.0, 13.0)	2.16 (1 H, ddd, J = 1.0, 1.0, 13.0)	2.23 (1 H, ddd, J = 1.0, 2.0, 12.5)
	2.40 (1 H, ddd, J = 1.0, 10.5, 15.8)	2.44 (1 H, ddd, J = 2.4, 12.0, 13.0)	2.38 (1 H, ddd, J = 2.4, 10.6, 13.4)	2.49 (1 H, ddd, J = 3.4, 12.0, 12.5)
12	0.43 (1 H, dddd, J = 1.0, 1.0, 10.5, 12.0)	0.45 (1 H, dddd, J = 1.0, 2.4, 10.0, 12.0)	0.85 (1 H, dddd, J = 1.0, 2.4, 10.0, 13.4)	1.13 (1 H, dddd, J = 1.0, 1.0, 12.0, 14.1)
	1.63 (1 H, dddd, J = 1.0, 2.0, 3.8, 12.0)	1.66 (1 H, m)	1.69 (1 H, m)	1.72 (1 H, dddd, J = 1.0, 3.4, 4.3, 14.1)
13				
14	5.00 (1 H, s)[b]	5.15 (1 H, s)[b]	5.00 (1 H, d, J = 1.0)[b]	5.11 (1 H, s)[b]
	5.27 (1 H, s)[b]	5.00 (1 H, s)[b]	4.97 (1 H, d, J = 1.0)[b]	4.99 (1 H, s)[b]
15	1.79 (3 H, s)[c]	1.68 (3 H, s)[c]	1.77 (3 H, s)[c]	1.79 (3 H, s)[c]
16	1.99 (3 H, s)	1.96 (3 H, s)	1.96 (3 H, s)	2.08 (3 H, s)
17				
18	4.80 (1 H, s)[b]	4.86 (1 H, s)[b]	4.87 (1 H, s)[b]	5.41 (1 H, s)[b]
	4.99 (1 H, s)[b]	4.76 (1 H, s)[b]	4.75 (1 H, s)[b]	5.32 (1 H, s)[b]
19	1.96 (3 H, s)[c]	1.96 (3 H, s)[c]	1.87 (3 H, s)[c]	1.79 (3 H, s)[c]
20				
		2.05 (3 H, s)[d]		

[a] The ^{1}H NMR spectra were recorded at 360 MHz in $CDCl_3$. Assignments were aided by spin-decoupling and NOEDS experiments. J values are given in Hertz, and chemical shifts are reported in δ units (ppm downfield from Me_4Si). [b] Signals within a column may be reversed. [c] Associated with the acetate ester functional group.

was established for this metabolite by mass spectral analysis and by interpretation of ^{13}C NMR (Table I). This molecular composition suggested the presence of 9° of unsaturation. An infrared absorption at 3550 cm^{-1}, coupled with a resonance in the ^{13}C NMR spectrum at δ 65.1 (d) and a proton NMR signal (Table II) at δ 4.36 (1 H, d, J = 11.2 Hz), suggested the presence of a secondary alcohol. Acetylation (acetic anhydride in pyridine) confirmed this assignment, producing monoacetate **3** in quantitative yield. Ester **3**, also isolated as a natural product, showed carbonyl infrared absorption at 1720 cm^{-1}. A resonance in the ^{1}H NMR spectrum (Table II) of kallolide A acetate (**3**) at δ 2.05 (3 H, s) was assigned to the acetate methyl. The α-hydroxyl methine observed in the spectrum of **2** at δ 4.36 was shifted downfield to δ 5.57 (1 H, d, J = 11.8 Hz) in the proton NMR spectrum of **3**.

Consideration of other spectral features defined the nature of the remaining oxygen atoms in kallolide A (**2**). A carbonyl absorption in the IR spectrum of **2** at 1755 cm^{-1}, together with a one-proton resonance at δ 6.65 in the ^{1}H NMR spectrum and ^{13}C NMR signals at δ 175.6 (s), 146.5 (d), 136.9 (s), and 80.9 (d), suggested the presence of an α-substituted α,β-unsaturated γ-lactone functionality. This structural feature was assigned confidently by comparison of spectral data with a number of natural products,[7,10-14] including pseudopterolide (**1**),[7] which also contains this functional group. The remaining oxygen atom in **2** was assigned to an α,α'-dialkylated β-methyl furan constellation on the basis of familiar ^{13}C NMR resonances at δ 151.1 (s), 149.4 (s), 119.8 (s), 112.2 (d), and 9.6 (q) and two singlets in the proton NMR spectrum at δ 5.90 (1 H, s) and 1.99 (3 H, s).

Further examination of the NMR data showed that two isopropenyl groups were present in the molecule. Resonances in the ^{13}C NMR spectrum at δ 144.2 (s), 141.7 (s), 114.7 (t), and 116.3 (t), along with four ^{1}H NMR signals at δ 5.27 (1 H, s), 5.00 (1 H, s), 4.99 (1 H, s), and 4.80 (1 H, s), confirmed the presence of two terminal olefins in **2**. Spin-decoupling experiments more precisely defined each terminal olefin as methyl substituted. Irradiation of the signals at δ 5.27 and 5.00 sharpened a resonance at δ 1.79 (3 H, s) assigned to an olefinic methyl. Likewise, irradiation of the bands at δ 4.99 and 4.80 removed a small allylic coupling from another olefinic methyl resonance at δ 1.96 (3 H, s). Catalytic hydrogenation of kallolide A (**2**) over PtO_2 yielded two products, one of which confirmed the presence of two isopropenyl groups in **2**. A hexahydro derivative (**6**) and a tetrahydro derivative (**7**) were obtained



6 + 7

from this reaction. Inspection of spectral data showed that the α,β-unsaturated γ-lactone present in **2** had been saturated in both products; the carbonyl absorption in the IR spectrum of each product was shifted to 1780 cm^{-1},

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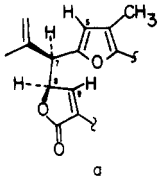
indicative of fully saturated γ-lactones. Furthermore, the β proton of the α,β-unsaturated γ-lactone, observed in the ^{1}H NMR spectrum of **2** as a low-field singlet, was absent in the spectra of **6** and **7**. In the case of **6**, four newly formed doublet methyls [δ 1.13 (3 H, d, J = 6.5 Hz), 1.06 (3 H, d, J = 7.0 Hz), 0.99 (3 H, d, J = 6.5 Hz), 0.95 (3 H, d, J = 7.0 Hz)] were observed. Irradiation of a one-proton muliplet caused two of the doublet methyls to collapse to singlet resonances. The other two doublet methyls were also decoupled following irradiation of a signal at high field due to another methine proton. These results established the assignment of two isopropyl groups in **6** and, therefore, of two isopropenyl functionalities in kallolide A (**2**).

From these data, eight of the 9° of unsaturation could be accounted for, demonstrating that **2** was monocarbocyclic. Furthermore, the ring was concluded to be 12-membered from consideration of the observed functional groups. The presence of two isopropenyl functionalities in the molecule excluded regularly isoprenoid ring systems for the carbon skeleton of **2** as this can arise only from an irregular joining of isoprene units. While both the cubitane[15] and pseudopterane carbon skeletons might have been considered for the 12-membered monocarbocyclic ring system of **2**, the pseudopterane skeleton with the isopropyl groups oriented 1,7 was obviously favored on biogenetic grounds. Metabolite **2** compared favorably to pseudopterolide (**1**), possessing several similar functional groups and NMR features.

Results from ^{1}H NMR nuclear Overhauser enhancement difference spectroscopy (NOEDS) studies defined the relative spatial orientation and stereochemistry of the γ-lactone, one isopropenyl group, and the furan in kallolide A. Enhancement of the C-9 proton, as well as enhancement of the C-7 isopropenyl methine, was observed following irradiation of the C-8 lactone proton at δ 5.40. These results demonstrated that the lactone proton (H-8) and the isopropenyl methine (H-7) were wthin nOe proximity on the same face of the molecule. Similarly, irradiation of the isopropenyl methine at C-7 resulted in enhancement of the β furan proton (H-5) [δ 5.90 (1 H, s)], the β proton on the α,β-unsaturated γ-lactone (H-9), and the lactone methine proton (H-8). The spatial arrangement of the furan proton, the lactone methine, and the isopropenyl proton were thereby established. On the basis of these results, a partial structure as in **a** could be constructed accounting for 13 carbons in the skeleton of **2**.



a

On the other side of the ring in **2**, results from spin-decoupling experiments established coupling constants and connectivities between protons associated with the remaining functional groups in the molecule. Since the multiplicity of the α-hydroxyl methine did not change in the ^{1}H NMR spectrum of the hexahydro derivative (**6**) [δ 4.38 (1 H, d, J = 10.6 Hz)], the alcohol in **6** and, therefore in **2**, was perceived to be positioned at C-2, adjacent to the quaternary center produced by the furan, not the lactone. Irradiation of a methine proton at δ 2.86, assigned to the remaining α-isopropenyl methine, decoupled the α-hydroxy proton and simplified signals due to a methylene at δ 1.63 and 0.43. This methylene was further coupled to two methylene protons at δ 2.11 and 2.40 that were adjacent to a quaternary center, proposed to be that due to the lactone. Hence, the isopropenyl group was oriented at C-1, and carbons 11 and 12 were assigned as methylenes. The relative stereochemistries, however, of the α-hydroxyl methine (C-2) and the isopropenyl methine (C-1) could not be determined unambiguously. Also, relative stereochemical configurations of the spatially distant asymmetric centers C-1,2 and C-7,8 could obviously not be assigned from these NMR data. On the basis of this major limitation, the structure of kallolide A acetate (**3**) was securely determined by X-ray crystallographic methods. Figure 1 is a computer-generated perspective drawing of the final X-ray model of kallolide A acetate less hydrogens. The X-ray experiment did not define the absolute configuration, so the enantiomer shown is in arbitrary choice. There is some evidence for distortion in the α,β-unsaturated γ-lactone ring since the C8–C9–C10–C11 torsional angle is 167° rather than the ideal 180°. Since kallolide A acetate was also produced from kallolide A itself, the structure of **2** was also fully confirmed by the X-ray experiment.

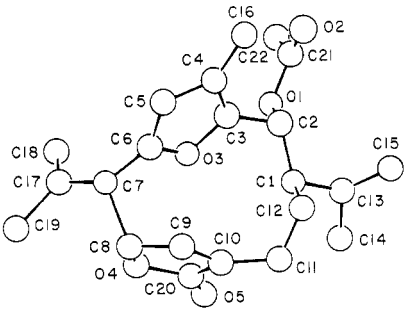
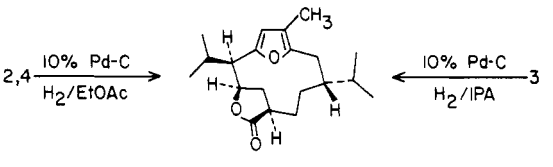


Figure 1. Computer-generated perspective drawing of the final X-ray model of kallolide A acetate (**3**). Hydrogens are omitted for clarity, and no absolute configuration is implied. The structure shown is the enantiomer of the structure drawn as **3**.

Once the structures of pseudopteranes **2** and **3** had been determined, a structure for the less polar metabolite, kallolide B (**4**), could be proposed based mainly upon spectral comparisons. Kallolide B analyzed for $C_{20}H_{24}O_3$ by high-resolution mass spectrometry and showed infrared absorption that suggested the presence of the analogous α,β-unsaturated lactone functionality found in kallolide A. The NMR features (Tables I and II) of **4** also compared favorably to those of **2** and **3**, except that the NMR resonance due to functionalization at C-2 was replaced in kallolide B by the bands due to a methylene. On the basis of this very favorable comparison, **4** was perceived to be the C-2 deoxo derivative of kallolide A. A hexahydro derivative, **8** was obtained from **4** by catalytic hydrogen-



8

ation. Similarly, hydrogenation of **2** and **3**, using 10% Pd–C as a catalyst, resulted in hydrogenolysis at C-2 and yielded a product in each case that was identical in all respects with **8**. This result conclusively interrelated kallolide B with kallolides A and A acetate at all comparable chiral centers.

Kallolide C (**5**), a minor and relatively unstable compound, was isolated by HPLC from the same fractions of the initial chromatography as kallolide A. A molecular formula of $C_{20}H_{24}O_6$ was proposed for this metabolite the

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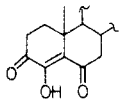
basis of data from low-resolution mass and ^{13}C NMR spectrometry (Table I). The molecule also showed infrared absorptions that suggested the presence of hydroxyl and α,β-unsaturated γ-lactone functionalities. A comparison of the NMR features of **5** and **2** revealed additional similarities between the two molecules. Two trisubstituted terminal olefins, identified by ^{13}C and ^1H NMR resonances (Table II) were defined in kallolide C (**5**) as part of two isopropenyl groups, as in kallolide A. Results from spin-decoupling experiments and ^1H NOEDS studies suggested that metabolite **5** possessed the same substituents and relative stereochemistries at C-11, C-12, C-1, and C-2 as in **2**. Resonances in the ^{13}C NMR spectrum of **5** also compared favorably to **2** at carbons 1, 2, 7–15, and 17–19. Kallolide C, therefore, was proposed to be identical to **2** at all carbons except for those of the furan constellation that was obviously not present.

The major difference between the two molecules was initially most apparent from consideration of other NMR data. The familiar furan proton was not present in the spectrum of **5**. Analysis of the ^{13}C NMR data (Table I) showed that, in place of the resonances for a furan, four new signals were present in the spectrum of kallolide C (**5**) at δ 202.3 (s), 183.8 (s), 100.4 (s), and 110.0 (s). Also, a ^{13}C NMR resonance at δ 6.5 (q) was assigned to an unusually shielded methyl group in the molecule (C-16). These data suggested that the furan had been replaced by an oxidation product possessing two ketones, one conjugated [δ 202.3 (s)] and the other nonconjugated [δ 183.8 (s)]. UV absorption at 286 nm (ϵ 4030) compared favorably to UV data [λ_{max} 275 nm (ϵ 5000)] for a steroidal compound that possessed the chromophore shown in **b**.[16] On the



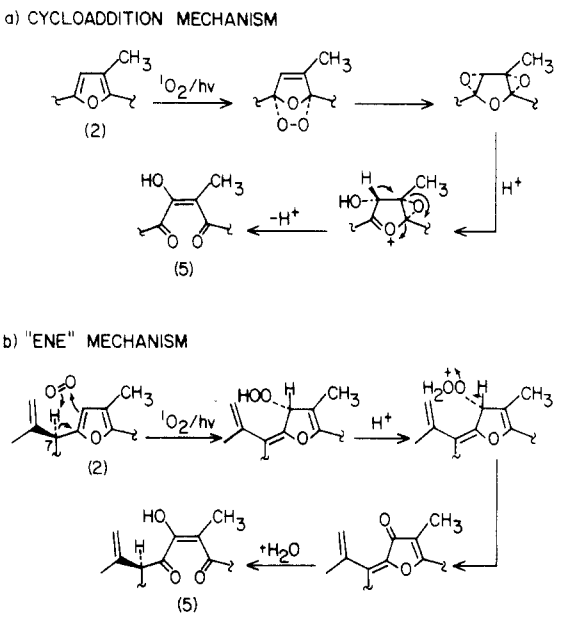
b

basis of this comparison and considering the functional groups that remained to be assigned, a structure for **5** was formulated as shown. Proof for the presence of the C-5 enol hdyroxyl in **5** came from acetylation to yield **9**. In the ^1H NMR spectrum of this derivative, the C-2 acetoxyl methine was shifted downfield to δ 5.55 (1 H, d, J = 12.1 Hz). In addition, two new singlets due to acetate methyls were observed at δ 1.97 (3 H, s) and 1.95 (3 H, s).

Additional support for the structural formulation of kallolide C as **5** was provided by the preparation of kallolide C from kallolide A (**2**). Singlet oxygen photooxidation of kallolide A (polymer-bound Rose Bengal/CH_2Cl_2/incandescent irradiation/12 h) gave a product identical in all respects with kallolide C as the major component of the reaction mixture (10% overall yield). Since kallolide C could be produced from kallolide A (**2**) in this fashion, it may not be a true secondary metabolite.

The mechanism of this conversion is intriguing, and several possibilities may be considered. Normally singlet oxygen reacts rapidly with furans in a $\pi^4s^+\pi^2s$ fashion to give transannular peroxides.[17] Such transannular peroxides have been isolated, but they normally participate in a variety of chemical reactions. Addition of a nucleophilic solvent, rearrangement to dicarbonyl compounds, Baeyer–Villiger type rearrangements, and rearrangement to bisepoxides are all known [i.e., Scheme I a].[17] However,

Scheme I



in all of this chemistry there is no precedent for formation of products like **5**. A novel mechanism that accounts nicely for the formation of **5** is presented in Scheme Ib. It begins with the reaction of **2** and singlet oxygen in an ene fashion either in a direct manner or through a perepoxide intermediate. The ene reaction of furans with singlet oxygen is unprecedented to our knowledge, but there are structural features of **2** that make it plausible. In **2** the participating C7–H bond is both tertiary and allylic, and it can be easily oriented coplanar with the furan π system, the geometry needed for the ene reaction. The 4+2 mode of addition is sterically more difficult. One face of the furan is shielded by the lactone-containing ring, while the other face is partially shielded by the isopropenyl at C7 and the hydroxyl at C2. Similar ene reactions with singlet oxygen have been proposed for pyrroles and indoles.[17] Once the hydroperoxide is formed, fragmentation to **5** is straightforward [Scheme Ib]. Further experiments to establish the mechanism of this reaction are in progress.

Kallolides A–C possess the pseudopterane skeleton first identified in pseudopterolide, a unique inhibitor of cell division.[7] Pharmacological testing of these new pseudopterane derivatives has yielded interesting results. Kallolide A, for example, inhibits phorbol ester induced inflammation (PMA, 4-β-phorbol-12-myristate-13-acetate) in the mouse ear assay[18] at concentrations and with efficacies equivalent to indomethacin, a potent nonsteroidal antiinflammatory drug.

Experimental Section

Infrared spectra were recorded on a Perkin-Elmer Model 137 spectrophotometer. Ultraviolet spectra were obtained in MeOH on a Beckman Acta XIV spectrophotometer. Proton NMR spectra were recorded in $CDCl_3$ solution at 360 MHz on a spectrometer constructed from an Oxford narrow-bore magnet and a Nicolet-1180E Fourier transform data system by Dr. John Wright for the UCSD NMR facility; all chemical shifts are reported with respect to Me_4Si (δ 0). All samples prepared for NOEDS were degassed by bubbling Ar through the solution for 45–60 min and then sealed around the cap with parafilm. Solutions were made up in $CDCl_3$ such that after degassing and a loss of a significant volume of $CDCl_3$ the final concentration was 0.03–0.05 M. Carbon-13 NMR spectra were recorded in $CDCl_3$ solution on a Nicolet-Oxford Magnetics 50-MHz wide-bore spectrometer; all

(16) Dorfman, L. *Chem. Rev.* **1953**, *53*, 47 (see p 83 specifically for the example shown in the text).

(17) Wasserman, H. H.; Lipshutz, B. H. In "Singlet Oxygen"; Wasserman, H. H., Murray, R. W., Eds.; Academic Press: New York, 1979; pp 431–443, 463–467.

(18) Van Arman, C. G. *J. Clin. Pharm. Ther.* **1974**, *16*, 900.

chemical shifts are reported with respect to Me_4Si (δ 0). Low-resolution mass spectra were recorded at 70 eV on a Hewlett-Packard Model 5930A mass spectrometer. High-resolution mass measurements were supplied by Dr. A. Burlingame, University of California—Berkeley, and by the mass spectrometry service laboratory, University of Minnesota. Optical rotations were measured on a Perkin-Elmer Model 141 polarimeter with a 10-cm microcell. Melting points were determined on a Fisher-Johns apparatus and are reported uncorrected. All solvents used were distilled from glass prior to use. Final purifications of all metabolites and reaction products were achieved by high-performance liquid chromatography on silica gel using mixtures of ethyl acetate and isooctane.

Collection and Extraction. *P. kallos* (Bielschowsky) (BA-73, BA-80, BA-93) was collected by hand using scuba in the Bahamas at Channel Cay, Little Harbor Cay, and Chub Cay in Sept 1981. The gorgonian was found primarily in shallow water (5–15-m depth); however, at Chub Cay some specimens were also collected at 30–35-m depth. Animals were stored frozen until workup, when they were homogenized and repeatedly extracted with $CHCl_3$ and then EtOAc. The $CHCl_3$ and EtOAc extracts were combined, filtered, and evaporated in vacuo to yield a residue that was partitioned between saturated brine and $CHCl_3$. The resulting $CHCl_3$ extract was dried ($MgSO_4$) and filtered, and the solvent was evaporated to yield 111 g of crude organic extract (from 820 g, dry weight, of the gorgonian). The extract (44 g) was applied to a silica gel column, and fractions werre eluted with a solvent gradient that began with petroleum ether and continued through mixtures of EtOAc and MeOH. Pseudopteranes **2–5** were isolated from less polar fractions of the initial chromatography; kallolide B (**4**) was the least polar of the four pseudopteranes, eluting from the column slightly after the hydrocarbons with 15–20% EtOAc in petroleum ether. Pseudopterane **3** was eluted next from the column with 20–25% EtOAc in petroleum ether. Fractions eluted from the column with 25–35% EtOAc in petroleum ether contained metabolites **2** and **5**.

Kallolide A (2). Separation by HPLC (μ-Porasil, using 45% and 40% EtOAc in isooctane) yielded 850 mg (1.9% of the crude extract) of alcohol **2** as an amorphous solid. Kallolide A showed $[\alpha]^{20}_D$ +145° (*c* 0.67, $CHCl_3$) and exhibited the following spectral characteristics: UV (MeOH) λ_{max} 218 nm (ϵ 13 000); IR ($CHCl_3$) 3550, 3020, 2920, 1750, 1440, 1210 cm^{-1}; HRMS, M^+, *m/z* (relative intensity) obsd 328.1682 (8.0), $C_{20}H_{24}O_4$ calcd 328.1675, 310.1584 ($M^+ - H_2O$, 3.1), 163.0757 ($C_{10}H_{11}O_2$, 11).

Kallolide A Acetate (3). Kallolide A acetate (**3**) crystallized from diethyl ether after purification by HPLC (μ-Porasil, using 30% and 25% EtOAc in isooctane). Repeated recrystallization gave 300 mg (0.7% of the crude extract) of kallolide A acetate (**3**). Metabolite **3**, mp 198–199.5 °C (discolors >185 °C), showed $[\alpha]^{20}_D$ +55° (*c* 0.65, $CHCl_3$) and exhibited the following spectral characteristics: UV (MeOH) λ_{max} 221 nm (ϵ 9300); IR ($CHCl_3$) 3020, 2920, 1755, 1720, 1440, 1370, 1210 cm^{-1}; HRMS, M^+, *m/z* (relative intensity) obsd 370.1774 (58.7), $C_{22}H_{26}O_5$ calcd 370.1780, 310.1572 (M^+ – HOAc, 100).

Kallolide B (4). Purification by HPLC (μ-Porasil, using 25% EtOAc in isooctane) yielded 100 mg (0.2% of the crude extract) of **4**. Kallolide B, an oil, showed $[\alpha]^{20}_D$ +123° (*c* 0.81, $CHCl_3$) and exhibited the following spectral characteristics: UV (MeOH) λ_{max} 216 nm (ϵ 13 000); IR ($CHCl_3$) 3020, 2920, 1750, 1440, 1220 cm^{-1}; HRMS, M^+, *m/z* (relative intensity) obsd 312.1728 (44), $C_{20}H_{24}O_3$ calcd 312.1725, 216.1515 ($C_{15}H_{20}O$, 15), 201.1261 ($C_{14}H_{17}O$, 17), 147.0811 ($C_{10}H_{11}O$, 100).

Kallolide C (5). Purification by HPLC (μ-Porasil, using 45 and 40% EtOAc in isooctane) gave 250 mg (0.6% of the crude extract) of diol **5**. Kallolide C (**5**), an amorphous solid, showed $[\alpha]^{20}_D$ –14° (*c* 0.91, $CHCl_3$) and showed the following spectral characteristics: UV (MeOH) λ_{max} 286 nm (ϵ 4030); IR ($CHCl_3$) 3300–3550, 1760, 1715, 1670, 1440, 1220, 1100 cm^{-1}; HRMS, M^+, *m/z* (relative intensity) obsd 360.1579 (4), $C_{20}H_{24}O_6$ calcd 360.1573, 342.1471 ($C_{20}H_{22}O_5$, 15), 287.1612 ($C_{18}H_{23}O_3$, 8), 233.0886 ($C_{17}H_{12}O$, 11), 194.0906 ($C_{11}H_{14}O_3$, 28), 139.0738 ($C_8H_{11}O_2$, 100).

Acetylation of 2 To Yield Ester 3. To 20 mg of alcohol **2**, dissolved in 2 mL of dry pyridine, was added excess acetic anhydride with stirring. Stirring was continued overnight at room temperature, and then the reaction was quenched with ice and extracted with $CHCl_3$ (3 × 20 mL). The combined $CHCl_3$ layers were washed with 5% HCl (3 × 20 mL), water, and 5% $NaHCO_3$ (20 mL), then dried ($MgSO_4$), filtered, and evaporated to yield, quantitatively, ester **3**. Purification by HPLC (with μ-Porasil, using 25% EtOAc in isooctane), followed by repeated recrystallization of the ester from diethyl ether, gave a product, identical in all respects, including its melting point (197–198.5 °C, with yellowing above 185 °C), with the natural product kallolide A acetate (**3**).

Catalytic Hydrogenation of 2 To Yield the Hexahydro (6) and Tetrahydro (7) Derivatives. Compound **2** (44.8 mg, 0.14 mmol) was dissolved in EtOAc (20 mL) and added to a 25-mL Erlenmeyer suction flask containing a stirring bar and a catalytic amount of PtO_2. A septum and balloon were wired to the reaction vessel. The flask was then purged with hydrogen, and the balloon was filled. After the mixture was stirred at room temperature for 2 h, the hydrogen was removed, the solution was filtered through Celite, and the solvent was evaporated. Separation by HPLC (μ-Porasil, using 40% EtOAc in isooctane) yielded two products, 17.4 mg (38% from **2**) of the hexahydro derivative, **6**, and 17.6 mg (39% from **2**) of the tetrahydro derivative, **7**. Compound **6** showed $[\alpha]^{20}_D$ +5° (*c* 0.5, $CHCl_3$) and exhibited the following spectral features: UV (MeOH) λ_{max} 226 nm (ϵ 5400); IR ($CHCl_3$) 3350, 2960, 2920, 1780, 1460 cm^{-1}; LRMS, M^+, *m/z* (relative intensity) 334 (5) for $C_{20}H_{30}O_4$; ^1H NMR (360 MHz, $CDCl_3$) δ 5.94 (1 H, s, C-5), 4.98 (1 H, m, C-8), 4.38 (1 H, d, *J* = 10.6 Hz, C-2), 2.74 (1 H, m), 2.38 (1 H, dd, *J* = 3.1, 10.1 Hz, C-7), 2.03 (3 H, s), 1.13 (3 H, d, *J* = 6.5 Hz), 1.06 (3 H, d, *J* = 7.0 Hz), 0.99 (3 H, d, *J* = 6.5 Hz), 0.95 (3 H, d, *J* = 7.0 Hz). Derivative **7** showed $[\alpha]^{20}_D$ –23° (*c*, 1.0, $CHCl_3$) and had the following spectral characteristics: UV λ_{max} (MeOH) 226 nm (ϵ 1000); IR ($CHCl_3$) 3500, 2960, 1725–1780, 1640, 1450, 1370 cm^{-1}; LRMS, M^+, *m/z* (relative intensity) 332 (21) for $C_{20}H_{26}O_4$; ^1H NMR (360 MHz, $CDCl_3$) 5.96 (1 H, s, C-5), 5.22 (1 H, brs, (C-14), 5.05 (1 H, brs, C-14), 5.00 (1 H, m, C-8), 4.39 (1 H, d, *J* = 10.5 Hz, C-2), 2.87 (1 H, m), 2.39 (1 H, dd, *J* = 3.4, 9.9 Hz, C-7), 2.04 (3 H, s, C-16), 1.77 (3 H, s, C-15), 1.13 (3 H, d, *J* = 6.5 Hz, C-18), 0.97 (3 H, d, *J* = 6.5 Hz, C-19).

Single-Crystal X-ray Diffraction Analysis of Kallolide A Acetate (3). Preliminary X-ray photographs of crystals of kallolide A acetate showed orthorhombic symmetry and accurate lattice constants of a = 6.603 (2) Å, b = 15.517 (3) Å, and c = 20.355 (4) Å were determined by a least-squares fit of 15 diffractometer measured 2θ values. The systematic extinctions, chirality, and density were uniquely accommodated by space group $P2_12_12_1$ with one molecule of $C_{22}H_{26}O_5$ forming the asymmetric unit. All unique reflections with $2\theta \leq 114°$ were collected on a computer-controlled four-circle diffractometer using a variable speed, 1° ω scan, and graphite-monochromated Cu Kα (1.541 78 Å) radiation. After correction for Lorentz, polarization, and background effects, 1498 (91%) of the 1647 reflections were judged observed ($|F_o| \geq 3\sigma(F_o)$). The structure was solved routinely by direct methods.[19] Block-diagonal least-squares refinements with anisotropic non-hydrogen atoms and isotropic hydrogens have converged to a standard crystallographic residual of 0.052 for the observed data. Consult the paragraph at the end of this paper for additional crystallographic data.

Catalytic Hydrogenation of Kallolide B (4) To Yield the Hexahydro Derivative 8. Kallolide B (**4**; 15.0 mg, 0.048 mmol), dissolved in EtOAc (10 mL), was added to a 25-mL Erlenmeyer suction flask containing a catalytic amount of 10% Pd–C and a stirring bar. To the reaction flask were secured a balloon and

(19) All crystallographic calculations were done on a Prime 850 computer, operated by the Cornell Chemistry Computing Facility. Principal programs employed were REDUCE and UNIQUE, data reduction programs: Lewonowicz, M. E. Cornell University, 1978. Main, P.; Hull, S. E.; Lessinger, L.; Germain, G.; Declercq, J.-P.; Woolfson, M. M. "MULTAN 78, A System of Computer Programs for the Automatic Solution of Crystal Structures from X-ray Diffraction Data" [direct methods programs and fast Fourier transformation routine (locally modified to perform all Fourier calculations including Patterson syntheses)]; University of York: York, England, 1978. Weeks, C. M. "NQEST" [CYBER 173 version negative quartets figure of merit calculations]; Medical Foundation of Buffalo, Inc.: Buffalo, Aug 1976. Hirotsu, K.; Arnold, E. "BLS78A" [Anisotropic block-diagonal least-squares refinement]; Cornell University: 1980. Johnson, C. K. Ithaca, "ORTEP" [crystallographic illustration program]; Oak Ridge National Laboratory: Oak Ridge, TN, June 1965; Report ORNL 3794.

septum. The flask was then purged with hydrogen, and the balloon was filled. After the mixture was stirred for 8 h at room temperature, the hydrogen was removed, the solution was filtered through Celite, and the solvent was evaporated to yield the hexahydro derivative, **8**, quantitatively (14.8 mg, 97% from **4**) as an oil. Compound **8** was purified by HPLC (μ-Porasil, using 15% EtOAc in isooctane). Derivative **8** showed $[\alpha]^{20}_D$ +24° (*c* 0.5, $CHCl_3$) and exhibited the following spectral features: UV (MeOH) λ_{max} 226 nm (ϵ 8400); IR ($CHCl_3$) 2960, 1780, 1460, 1380, 1360, 1215 cm^{-1}; HRMS, M^+, *m/z* (relative intensity) obsd 318.2148 (40), $C_{20}H_{30}O_3$ requires 318.2195, 275.1646 ($C_{17}H_{23}O_3$, 45), 247.1684 ($C_{16}H_{23}O_2$, 100); ^1H NMR (360 MHz, $CDCl_3$) δ 5.93 (1 H, s), 4.98 (1 H, m), 1.89 (3 H, s), 1.13 (3 H, d, *J* = 6.6 Hz), 1.03 (3 H, d, *J* = 6.6 Hz), 0.94 (3 H, d, *J* = 6.8 Hz), 0.90 (3 H, d, *J* = 6.8 Hz).

Catalytic Hydrogenation and Hydrogenolysis of Kallolide A (2) To Yield 8. Kallolide A (**2**; 23.1 mg, 0.07 mmol) was dissolved in EtOAc (15 mL) and transferred to a 25-mL Erlenmeyer suction flask that contained a catalytic amount of 10% Pd–C and a stirring bar. The reaction vessel was equipped for hydrogenation as outlined above, and after stirring for 3 h at room temperature under a hydrogen atmosphere, the reaction was terminated by removal of hydrogen. The solution was then filtered through Celite and evaporated to yield an oil. Purification of the product by HPLC (μ-Porasil, using 15% EtOAc in isooctane) yielded **8** (17.1 mg, 76% from **2**) as an oil, identical in all respects, including its optical properties ($[\alpha]^{20}_D$ +25° (*c* 0.9, $CHCl_3$)) with the product obtained from the hydrogenation of **4**.

Catalytic Hydrogenation and Hydrogenolysis of Kallolide A Acetate (3) To Yield 8. Kallolide A acetate (**3**; 35.8 mg, 0.097 mmol), dissolved in 15 mL of isopropyl alcohol (IPA), was equipped for hydrogenation as outlined above with 10% Pd–C as the catalyst. After 6 h, the reaction was terminated, the solution was filtered through Celite, and the solvent was evaporated to yield an oil that was purified by HPLC (μ-Porasil, using 15% EtOAc in isooctane). Compound **8** the major product of the reaction (25.2 mg, 82% from **3**), was identical in all respects, including its optical properties ($[\alpha]^{20}_D$ +26° (*c* 0.7, $CHCl_3$)), with the product obtained from hydrogenation of kallolide B (**4**).

Acetylation of Kallolide C (5) To Yield Diacetate 9. Excess acetic anhydride was added to a stirred solution of **5** (15.2 mg, 0.042 mmol) dissolved in 2 mL of dry pyridine. The reaction mixture was stirred at room temperature overnight, next quenched with ice, and extracted with $CHCl_3$. The combined $CHCl_3$ layers were washed with 5% $NaHCO_3$ (20 mL), dried over $MgSO_4$, filtered, and evaporated to yield, quantitaively, diacetate **9**. Diester **9** exhibited the following spectral features: UV (MeOH) λ_{max} 275 nm (ϵ 3600); IR ($CHCl_3$) 2960, 1760, 1730, 1650, 1480, 1450, 1360 cm^{-1}; LRMS, M^+, *m/z* (relative intensity) 384 (1.7) for $C_{22}H_{24}O_6$ (M^+ – OAc), 342 (1.7), 217 (3.0); ^1H NMR (360 MHz, $CDCl_3$) δ 6.75 (1 H, s), 5.55 (1 H, d, *J* = 12.1 Hz), 5.09 (1 H, s), 5.10 (1 H, brs), 5.08 (1 H, s), 5.02 (1 H, s), 4.86 (1 H, s), 3.40 (1 H, d, *J* = 4.4 Hz), 2.10 (3 H, s), 1.97 (3 H, s), 1.95 (3 H, s), 1.87 (3 H, s), 1.69 (3 H, s).

Photooxidation of Kallolide A (2) To Yield Kallolide C (5). Kallolide A (319 mg, 0.97 mmol), dissolved in CH_2Cl_2 (1.4 mL), was added to a flask containing 9.4 mg of polymer-bound Rose Bengal[20] and a stirring bar. A balloon was attached to the reaction vessel. The flask was then purged with pure O_2, the balloon was filled, and the stirred reaction was irradiated with a 100-W light bulb. After 12 h, when the Rose Bengal had bleached, the O_2 was removed, the solution was filtered through washed cotton, and the solvent was removed in vacuo. Chromatography of the reaction mixture (HPLC, Lichroprep Si 60 using 50% EtOAc in isooctane) gave 35 mg (.097 mmol, 10% yield from **2**) of a major product that was identical in all respects, including its optical properties ($[\alpha]^{27}_D$ –14° (*c* 1.08, $CHCl_3$)), with the natural product kallolide C (**5**).

Acknowledgment. Research at the Scripps Institution was supported by the NSF, Chemistry Division, under Grants CHE81-11907 and CHE83-15546. We also thank the NSF for financial support to operate the ORV Calanus (University of Miami) in the Bahamas. Portions of this work (Traineeships to S.A.L. and M.T.B.) were supported by the U.S. Department of Commerce, Sea Grant Program. Research at Cornell University was provided under NIH Grant CA24487 and the NSF under Grant INT-8117327. Mass spectra were provided by the Mass Spectrometry Resource Center, School of Pharmacy, University of California, San Francisco (A. L. Burlingame, Director) supported by NIH Grant RR00719. We thank Dr. Frederick M. Bayer, Smithsonian Institution, for identification of *P. kallos*.

Supplementary Material Available: Tables of fractional coordinates, thermal parameters, bond distances, and bond angles for kallolide A acetate (4 pages). Ordering information is given on any current masthead page.

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