Isolation, Structural Characterization, and Synthesis of a Naturally Occurring Bisfuranopseudopterane Ether: Biskallolide A. Evidence for a Carbocation Intermediate during the Facile Conversion of Kallolide A and Isokallolide A into Various Solvolysis Products

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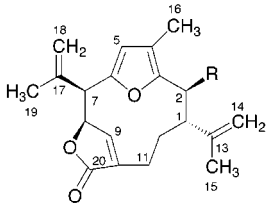
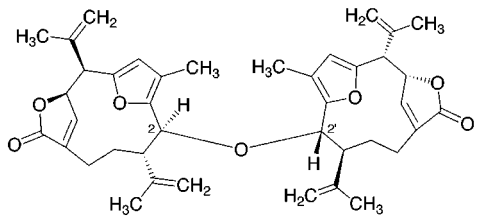
The West Indian alcyonacean *Pseudopterogorgia bipinnata* (Verrill, 1864) is shown to contain a novel bisditerpenoid ether: biskallolide A (**2**). The structural assignment of **2** was mainly based on 1D and 2D NMR and MS spectral data and was further confirmed by synthesis. The 2-*C*-alkoxylation of furanopseudopteranes kallolide A (**1**) and isokallolide A (**8**) occurs spontaneously in some solvents and involves replacement of the C2 hydroxyl with an alkoxyl group to yield solvolysis products that display net retention of configuration. The facile solvolytic 2-*C*-acyloxylation of kallolide A was achieved readily under similar circumstances to afford kallolide A acetate (**4**) as the sole product. Mechanistic details in conversion of alcohols **1** and **8** into various solvolysis products, including dimeric ethers **2** and **9**, were investigated in this study. Solvolysis of kallolide A and isokallolide A in [^{18}O]-labeled solvent demonstrated that the C2 alkoxyl of the solvolysis products originated from the solvent, suggesting that these conversions may proceed through an S_N1 mechanism with generation of a carbocation intermediate. The chemical structures of kallolide A derivatives **3**−**7** and those of isokallolide A congeners **9**−**11** were established by detailed analysis of the spectral data.

Introduction

The ability of tropical and subtropical species of alcyonaceans in making terpenoids is quite remarkable indeed. Since the early 1960s, both the abundance and ease of collection of these marine animals have fostered an extensive investigation of their secondary metabolism.[1] Octocorals of the genus *Pseudopterogorgia*, for instance, represent a rich source of novel terpenoids, which have stimulated considerable interest because of their chemical complexity and biological activity.[2] The common West Indian gorgonian octocoral *Pseudopterogorgia bipinnata* (Verrill, 1864) produces diterpenoids of the cembrane, pseudopterane, and gersolane families, and some of these metabolites possess interesting pharmacological properties.[3−5] Thus kallolide A (**1**), a major metabolite of *P. bipinnata* and *P. kallos* based on the 12-membered carbocyclic skeleton of pseudopterane, possesses antiinflammatory properties that exceed the potency of existing drugs such as indomethacin.[5,6] We have recently reported on a series of highly oxygenated cembranolides, bipinnatins G−I and bipinnatolides F−J, as well as a rare pseudopterane, bipinnapterolide A, from a Caribbean specimen of *P. bipinnata* collected near San Andrés Island, Colombia.[7] Continuing our study of this gorgonian species, we have now isolated a novel bisditerpenoid ether, namely, biskallolide A (**2**). The structure



kallolide A (**1**) R = -OH
2-*O*-methylkallolide A (**3**) R = -OCH_3
kallolide A acetate (**4**) R = -$OCOCH_3$
2-[4-iodobenzoyl]kallolide A (**5**) R = -$OCOC_6H_4I$
2-*O*-ethylkallolide A (**6**) R = -OCH_2CH_3
2-[^{18}O]-methylkallolide A (**12**) R = -$^{18}OCH_3$



biskallolide A (**2**)

assignment of furanopseudopterane ether **2** was based

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(1) Faulkner, D. J. *Nat. Prod. Rep.* **1999**, *16*, 155−198 and previous reports in this series.
(2) Fenical, W. *J. Nat. Prod.* **1987**, *50*, 1001−1008.
(3) Rodríguez, A. D. *Tetrahedron* **1995**, *51*, 4571−4618 and references therein.
(4) Wright, A. E.; Burres, N. S.; Schulte, G. K. *Tetrahedron Lett.* **1989**, *30*, 3491−3494.
(5) (a) Rodríguez, A. D.; Shi, J.-G. *J. Org. Chem.* **1998**, *63*, 420−421. (b) Rodríguez, A. D.; Shi, J.-G.; Huang, S. D. *J. Org. Chem.* **1998**, *63*, 4425−4432.
(6) (a) Bandurraga, M. M.; Fenical, W.; Donovan, S. F.; Clardy, J. *J. Am. Chem. Soc.* **1982**, *104*, 6463−6465. (b) Look, S. A.; Burch, M. T.; Fenical, W.; Qi-tai, Z.; Clardy, J. *J. Org. Chem.* **1985**, *50*, 5741−5746.

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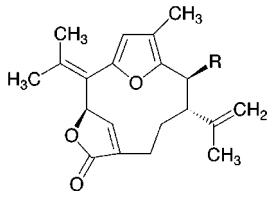
on extensive analyses of its ^1H and ^{13}C NMR data, combined with 1D and 2D NMR experiments, IR, UV, and HRFAB−MS spectral data, and comparisons with kallolide A (**1**). Biskallolide A (**2**) appears to be produced in nature by dehydration of two units of alcohol **1**. Unambiguous proof of structure was ultimately achieved when kallolide A (**1**) was directly transformed into biskallolide A (**2**), thus definitively establishing their biogenetic relationship. As part of our investigations aimed at the synthesis of natural product **2** it was observed that the 2-*C*-alkoxylation and 2-*C*-acyloxylation of kallolide A (**1**) occurred spontaneously in some solvents to yield solvolysis products that display net retention of configuration. Several mechanistic aspects in these and other related conversions were investigated in this study. From the chemical data gathered, it appears that these facile conversions proceed through an S_N1 mechanism via a relatively stable carbocation intermediate. The biological implications of these findings are discussed in this report.

Results and Discussion

Conventional chromatographic methods yielded pure biskallolide A (**2**) from the $CHCl_3$ extract of *P. bipinnata*. Biskallolide A, mp 248−266 °C dec, $[\alpha]^{24}_D$ +88° (*c* 0.50, $CHCl_3$), presented at first a number of puzzling features. The ^1H and ^{13}C NMR spectra, which appear to indicate a C_{20} compound, suggested a close structural similarity with kallolide A (**1**), but the signals in the ^1H NMR spectrum were, however, unusually broad. Except for the conspicuous absence of an intense absorption above 3100 cm^{-1} (otherwise ascribable to a hydroxyl functionality), the IR spectrum of **2** indicated that this compound contained many of the same functional groups as **1**, i.e., olefin, γ-lactone, and furan moieties.[8] Moreover, because there were no significantly large differences in both ^1H and ^{13}C chemical shift values at most positions in **2** when compared to **1**, it was quickly thought that **2** could be a symmetrical ether. An explanation for these unusual features was provided when it was shown by HRFAB mass spectroscopy that biskallolide A (**2**) analyzed for $C_{40}H_{46}O_7$, corresponding to two diterpenoid units minus one unit of H_2O. Thus, the NMR and UV spectra and optical activity established that the two C_{20} units were structurally and configurationally identical. The broadening of the ^1H signals can be attributed to the reduced rate of tumbling of this rather large molecule in solution. However, no attempt was made to run the NMR spectra at higher temperatures. The assignment of relative stereochemistry at C2 was based mainly on a strong NOE response between H2 and the Me-15 group located on the bottom face of the molecule. Moreover, the large ^1H NMR coupling constant of the oxymethine proton H2 resonating at δ 3.94 (d, 1H, J = 11.1 Hz) indicated a trans relationship to H1. We concluded that kallolide A (**1**) and biskallolide A (**2**) have identical relative stereochemistry at C2. Despite the paucity of material and the difficulties associated with the size of **2**, its molecular structure was established by extensive 1D and 2D NMR experiments ($^1H-^1H$ COSY, long-range COSY, DEPT, NOESY, HMQC, and HMBC). Detailed interpretation of these data permitted the assignment of all carbon and hydrogen atoms in biskallolide A unambiguously.

Additional support for the structural formulation of biskallolide A as **2** was provided by the preparation of biskallolide A from kallolide A (**1**). At first sight, kallolide A would appear to possess appropriate functionality and stereochemistry for straightforward conversion to biskallolide A by routine acid-mediated dehydration of alcohols, which may proceed by an S_N1 and/or S_N2 pathway. However, in the case of alcohol **1**, if one assumes that the species from which the leaving group departs is ROH_2^+, an S_N1 pathway with little or no tendency toward epimerization would have to prevail in order to obtain biskallolide A. The S_N1 pathway was also predicted as molecular calculations showed that the isopropenyl substituent adjacent to the hydroxyl functionality of kallolide A (**1**) adopts an axial-like orientation, effectively blocking the α-face approach to ROH_2^+. Notwithstanding, when alcohol **1** was treated with catalytic amounts of *p*-toluenesulfonic acid hydrate (PTSA·H_2O) in $CDCl_3$, the conjugated isopropylidene isomer, isokallolide A (**8**), was



isokallolide A (**8**) R = -OH
2-*O*-methyl-isokallolide A (**10**) R = $-OCH_3$
2-*O*-ethyl-isokallolide A (**11**) R = $-OCH_2CH_3$
2-[^{18}O]-methyl-isokallolide A (**13**) R = $-^{18}OCH_3$

formed instead as the sole product in 78% isolated yield within 30 min at room temperature. It was now clear that our methodology based on the dehydration of alcohols was not sufficiently energetic to overcome the steric factors associated with the formation of a strained bisfuranopseudopterane ether such as **2**.[9] These results and the fact that a solution of **1** in 1:1 MeOH−$CHCl_3$ left stirring for 2 weeks at room temperature led only to recovered starting material demonstrated that biskallolide A was not an artifact of kallolide A.

As we were unable to produce bisfuranether **2** by the conventional procedure, we decided to proceed along previous lines, but this time we conducted the reaction at elevated temperatures in the absence of solvent and without an acid catalyst. To that end, crystalline kallolide A was heated in a sandy bath at 115 °C for 45 min and afforded exclusively biskallolide A (**2**) in 56% yield after chromatographic purification. We suspect that the lability

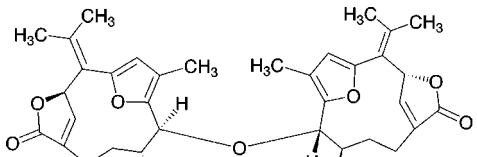
(7) Rodríguez, A. D.; Shi, J.-G.; Huang, S. D. *J. Nat. Prod.* **1999**, *62*, 1228−1237.

(8) (a) Tinto, W. F.; Chan, W. R.; Reynolds, W. F.; McLean, S. *Tetrahedron Lett.* **1990**, *31*, 465−468. (b) Chan, W. R.; Tinto, W. F.; Laydoo, R. S.; Machand, P. S.; Reynolds, W. F.; McLean, S. *J. Org. Chem.* **1991**, *56*, 1773−1776. (c) Tinto, W. F.; Lough, A. J.; Reynolds, W. F.; McLean, S. *Tetrahedron Lett.* **1991**, *32*, 4661−4664. (d) Tinto, W. F.; John, L.; Reynolds, W. F.; McLean, S. *Tetrahedron* **1991**, *47*, 8679−8686. (e) Tinto, W. F.; Laydoo, R. S.; Miller, S. L.; Reynolds, W. F.; McLean, S. *J. Nat. Prod.* **1995**, *58*, 1975−1977. (f) Rodríguez, A. D.; Soto, J. J. *Chem. Pharm. Bull.* **1996**, *44*, 91−94. (g) Rodríguez, A. D.; Soto, J. J. *Tetrahedron Lett.* **1996**, *37*, 2687−2690. (h) Rodríguez, A. D.; Soto, J. J. *J. Org. Chem.* **1996**, *61*, 4487−4490. (i) Rodríguez, A. D.; Soto, J. J. *J. Nat. Prod.* **1998**, *61*, 401−404.

(9) We noticed from the HREI−MS spectrum that alcohol **1** could not readily undergo dehydration to afford the strained conjugated olefin, as the [$M^{+\bullet}$ − 18] ion peak, representing loss of a water molecule, was among the least abundant (relative intensity = 3.1%) ion fragments.

of furans to air oxidation may account for the lower than expected yield.[10] Interestingly, the reaction proceeded with retention of stereochemistry at C2. This result, which was suggestive of an S_N1 process, was reminiscent of the work of Abramson and co-workers, who had shown that certain furanocembranes undergo S_N1-type solvolysis affording products that display net retention of configuration at C2.[11] Moreover, previous work by Marshall aimed at the total synthesis of **1** had shown that kallolide A is substantially lower in energy than its C2 epimer.[12,13] Accordingly, we expected the C2 epimer of biskallolide A to be significantly higher in energy than **2**, a contention supported by our molecular mechanics calculations (vide infra). The success of this synthesis prompted our investigation of a parallel route to kallolide A analogues. Initially, it was predicted that the 2-*C*-alkoxylation of kallolide A (**1**) could occur spontaneously in some solvents, involving replacement of the C2 hydroxyl with an alkoxyl group to yield solvolysis products that display net retention of configuration. In accord with this prediction, the expected products were formed in reasonably high yields.

We first attempted the conversion of kallolide A (**1**) to ether **3** with methanol at 23 °C for 3 days without success. However, upon addition of a few drops of $CDCl_3$ and stirring at room temperature overnight, the expected methyl ether was formed in ca. 74% isolated yield as a single C2 epimer.[14] A NOESY NMR experiment and ^{1}H NMR coupling constant analysis ($^3J_{H1,H2}$ = 11.1 Hz) supported our assignment of stereochemistry at C2 as indicated. In agreement with Marshall's results, the stereospecificity of this reaction reflects the ~2.2 kcal/mol energy difference between the α- and *β*-isomers, predicted by molecular mechanics calculations.[12] To pursue the notion that a carbocation might be involved in the preceding conversion, it was found that isokallolide A (**8**), which displays extended conjugation, can be readily converted in methanol, without addition of $CDCl_3$, to ether **10** as the sole product in 58% yield upon stirring at room temperature overnight. Moreover, in sharp contrast to our previous findings with kallolide A (**1**), prolonged exposure of isokallolide A (**8**) in $CDCl_3$ led to bisisokallolide A (**9**) without the need for elevated temperatures. Although bisfuranether **9** was obtained in only 11% yield, no other byproduct could be detected by TLC analysis.[15] Thus, it soon became apparent that kallolide A (**1**) was more resistant to ether formation, requiring traces of acid or elevated temperatures, conditions not required to convert alcohol **8** into the corresponding ethers. We surmised that this difference in reactivity was suggestive of an S_N1 process clearly reflecting the 9.8 kcal/mol energy difference between carbocations **I** and **II** predicted by molecular mechanics calculations (Figure 1).[16] While the carbocations involved in these reactions are relatively long-lived when compared to aliphatic carbocations, presumably because they can be stabilized by resonance with the furan ring, they are not equally stable and must therefore exhibit different rates of formation.[17] Carbocation **I**, for instance, is relatively less stable in solution than **II** and thus will be expected to display a slower rate of formation.[18] It was now clear that despite the introduced strain into the ring system, carbocation **II** is relatively more stable than **I** since **II** is more highly conjugated. For calibration purposes, carbocation **III**, which has the same degree of conjugation as **II**, is nonetheless more stable by over 8 kcal/mol since it is based on the less strained furanocembrane ring system.



bisisokallolide A (**9**)

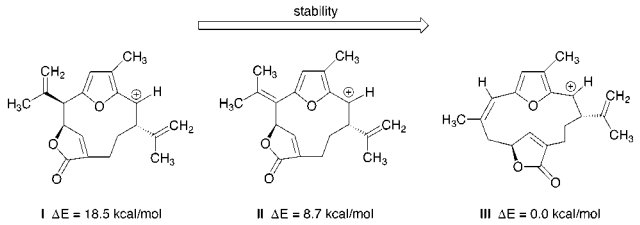


Figure 1. Calculated minimum energies for furanopseudopteryl carbocations **I** and **II**. The calculated minimum energy for furanocembryl carbocation **III** was included because of its close analogy to the carbocations **I** and **II**.

Because the conversions just described are presumably dependent on the presence of a relatively stable carbocation intermediate for their success, we next subjected kallolide A (**1**) to solvolytic 2-*C*-acyloxylation with AcOH as "trapping agent" to demonstrate the formation of a stable carbocation with a mean lifetime greater than 10^{-9} s.[17] As predicted, solvolysis of **1** upon stirring at room temperature for 30 h led to acetate **4** as the sole product in 48% yield. The IR, UV, HREI−MS, and ^{1}H and ^{13}C NMR spectra of **4** were superimposable with those of natural product kallolide A acetate whose structure was defined by X-ray methods.[6b] Despite the rather low yield

(10) Marshall, J. A.; Bartley, G. S.; Wallace, E. M. *J. Org. Chem.* **1996**, *61*, 5729−5735.

(11) (a) Hyde, E. G.; Boyer, A.; Tang, P.; Xu, Y.; Abramson, S. N. *J. Med. Chem.* **1995**, *38*, 2231−2238. (b) Hyde, E. G.; Thornhill, S. M.; Boyer, A. J.; Abramson, S. N. *J. Med. Chem.* **1995**, *38*, 4704−4709.

(12) Marshall, J. A.; Liao, J. *J. Org. Chem.* **1998**, *63*, 5962−5970.

(13) This realization allowed Marshall to develop a strategy whereby the epimerization at the C2 position of a kallolide A precursor produced kallolide A (**1**) in high yield under S_N1 solvolysis conditions with none of the C2 epimer.

(14) A solution of kallolide A (**1**) in 100% $CDCl_3$ set aside for several months at room temperature did not show any signs of decomposition. On the other hand, a solution of **1** in methanol containing a few drops of $CDCl_3$ slowly decomposes to yield ether **3** as traces of acid present in $CDCl_3$ catalyze the reaction. Naturally, we found that this methodology can be improved dramatically upon adding to the reaction mixture 1 or 2 drops of $CDCl_3$ previously saturated with dry HCl.

(15) We did not examine the dehydration of alcohol **8** at elevated temperatures because we felt that at high temperatures the inherent sensitivity of **8** (and **9**) to air oxidation would be higher, leading to partial or complete decomposition; see refs 6b and 10.

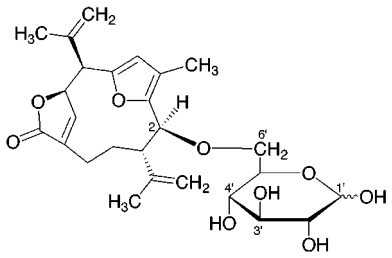
(16) The program Insight II (version 98.0) was employed for these calculations. Global minimum multiple conformer searching was achieved with the Steepest Descents minimizer through multiple step interations (500) until the minimum energy conformer was found multiple times.

(17) (a) Richard, J. P.; Rothenberg, M. E.; Jencks, W. P. *J. Am. Chem. Soc.* **1984**, *106*, 1361−1372. (b) Islam, N. B.; Gupta, S. C.; Yagi, H.; Jerina, D. M.; Whalen, D. L. *J. Am. Chem. Soc.* **1990**, *112*, 6363−6369. (c) Richard, J. P.; Yeary, P. E. *J. Am. Chem. Soc.* **1993**, *115*, 1739−1744.

(18) (a) Ta-Shma, R.; Rappoport, Z. *J. Am. Chem. Soc.* **1983**, *105*, 6082−6095. (b) Richard, J. P. *J. Org. Chem.* **1994**, *59*, 25−29.

the preceding reaction showed no byproducts according to TLC analysis. We suspect that the lability of kallolide A acetate (**4**) to silica gel chromatography may account for the lower than expected yield. As a matter of interest, we thought that alcohol **1** could otherwise be protected, albeit more efficiently, under typical S_N2 acyl substitution conditions. However, treatment of kallolide A (**1**) with 4-iodobenzoyl chloride and pyridine at room temperature led to *p*-iodobenzoate **5** in only 52% isolated yield. Of course, here base-promoted protection of the hydroxyl moiety is likely to proceed less efficiently as a result of steric factors associated with the large size of the reagent used.

Remarkably, treatment of kallolide A (**1**) with catalytic amounts of PTSA·H_2O in ethyl acetate gave, in 78% yield, a 1.3:1 mixture of ethers **6** and **11**, favoring the former, upon stirring at room temperature for 30 min. The relative stereochemistry at C2 of ethers **6** and **11**, which was confirmed by NOESY NMR experiments and ^{1}H NMR coupling constant analysis, remained unchanged when compared to that of kallolide A. We reasoned that in this case acid-mediated transesterification of ethyl acetate leads initially to kallolide A acetate (**4**), which then undergoes ethanolysis following an S_N1 pathway. To test this idea, we treated a solution of **4** in methanol using dry HCl as a catalyst and obtained ether **3** smoothly in 85% yield. As no intermediates could be isolated during the synthesis of **6** and **11**, we could not ascertain whether acid-promoted hydrolysis or transesterification of ethyl acetate occurred prior to solvolysis. On the other hand, we were pleased to find that **1**, when treated with α-*D*-glucose in DMSO-d_6 containing traces of $CDCl_3$, smoothly underwent 2-*C*-alkoxylation after 4 days at 23 °C to afford glycoside **7** as a 2:1 mixture of β/α anomers. None of the C2 epimer was formed in this



2-*O*-*D*-[glucopyranose-6'-yl]kallolide A (**7a,b**)

reaction. The isolation of nearly equal amounts of anomers **7a** and **7b** suggests that equilibration at C1′ most likely occurs after ether formation. In this case, we believe that the conversion of kallolide A (**1**) to its 2-*C*-glucopyranose-6′-yl derivative (rather than its 2-*O*-glycosylation congener) can be envisioned as suggestive of an S_N1 process mediated, not by a glycosyl carbocation but a carbocation intermediate such as **I** (Figure 1).[19] Multiple attempts to effect separation of the mixture of hemiacetals by normal-phase silica gel chromatography led to either partial or complete decomposition with recovery of kallolide A. A parallel investigation employing a penta-*O*-protected-β-D-glucose derivative as a scavenging nucleophile led to recovered starting material or, under more forcing conditions, to decomposition.

The presence of relatively stable furanopseudopteryl carbocations during the foregoing synthetic transformations (Figure 1) can be supported further by incorporation of ^{18}O from the solvent into the compound. Indeed, solvolysis of kallolide A (**1**) in ^{18}O-enriched methanol containing traces of $CDCl_3$ at room temperature for 5 h gave ether **12** in 70% isolated yield. The ^{1}H and ^{13}C NMR spectra and TLC retention time of the latter compound were identical to those recorded earlier for ether **3**, generated in unenriched methanol. In addition, the HREI−MS of ether **12** was similar to that of **3**, but the former produced ion peaks that were 2 mass units heavier than those observed in the HREI−MS of **3** (m/z = 344 vs 342, respectively). Similarly, the solvolysis of isokallolide A (**8**) conducted in ^{18}O-enriched methanol having traces of $CDCl_3$ was completed at room temperature in 10 min, affording **13** as the only product in 66% isolated yield. These results are consistent with an S_N1 reaction mechanism involving a carbocation intermediate, since the C2 oxygen of **12** and **13** clearly originated from the solvent rather than from kallolide A or isokallolide A.

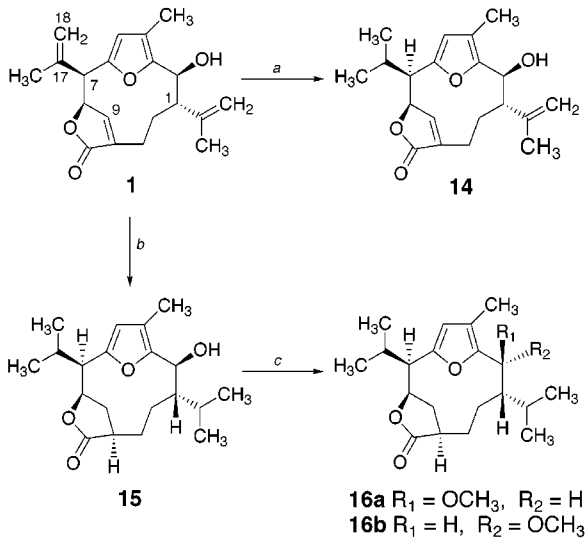
At first sight, it might appear that the imperviousness of optically pure **1** and **8** to epimerization is inconsistent with an S_N1 reaction mechanism. While epimerization is usual for simple acyclic carbocations, it is not unexpected for macrocyclic systems such as kallolide A or isokallolide A, where the two sides of the ring are not symmetrical. In fact, molecular mechanics calculations suggest that the butenolide ring in **1** and **8** would likely enforce a preferred conformation on the macrocyclic pseudopterane ring, thereby significantly shielding the bottom face of carbocations **I** and **II** (Figure 1). Moreover, we speculated that the stereochemistry of the products could be interpreted, at least in part, as indicating that the nearby π-system of the C1 isopropylene group assists in the departure of the leaving group from the bottom face of the molecule. Such anchimeric assistance from the nearby π bond provides an alternate explanation as to why the original stereochemistry of the C2 carbon remains unchanged during solvolysis, as scavenging nucleophiles will not be in a favorable position for backside attack. To test our prediction, we embarked upon a synthesis of hexahydro derivative **15** (Scheme 1).[6b] Hydrogenation of kallolide A using 10% Pd−C as a catalyst produced dihydro adduct **14** in ca. 77% yield; no other byproduct could be detected.[20] Clearly, of the two isopropylene groups in **1** the one at C1 is the most sterically hindered. Unexpectedly, hydrogenation of **1** in EtOAc during 1 h at room temperature, using PtO_2 as a catalyst, afforded exclusively the hexahydro derivative **15** in 88% yield.[6b] Our suspicions were confirmed upon solvolytic methoxylation of alcohol **15** with MeOH using dry HCl as a catalyst, whereupon ether **16** was produced as a 4:1 mixture of C2 epimers in 85% yield favoring the β isomer (Scheme 1).[21] Interestingly, this reaction proceeds with approximately 20% inversion of stereochemistry at C2. The contrasting behavior of macrocyclic alcohol **15** when compared to **1** and **8** suggests that, while steric encumbrance is still the main factor controlling the stereochemistry at C2, to a significant extent, there exist such remote stereocontrol during the solvolysis of kallolide A and isokallolide A.

(19) Hanessian, S. In *Preparative Carbohydrate Chemistry*; Hanessian, S., Ed.; Marcel Dekker: New York, NY, 1997; pp 381−388.

(20) Prolonged exposure with Pd−C as a catalyst also resulted in concomitant hydrogenolysis of **1** at C2; see also ref 6b.

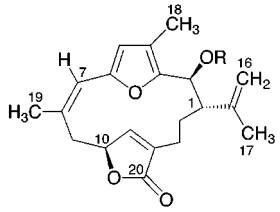
(21) The 4:1 mixture of **16a**/**16b** was determined on the basis of the ^{1}H NMR data for H-2.

Scheme 1[a]



[a] Reaction conditions: (a) 10% Pd−C, H_2, EtOAc, 23 °C, 30 min, 77%; (b) PtO_2, H_2, EtOAc, 23 °C, 1 h, 88%; (c) MeOH, $CDCl_3$ containing traces of dry HCl, 23 °C, 2 h, 85%.

As a matter of interest, we also isolated the furanocembrane 2-*O*-methylbipinnatin J (**18**) from the same



bipinnatin J (**17**) R = H
2-*O*-methylbipinnatin J (**18**) R = CH_3

extracts of *P. bipinnata* as a minor component (yield = 1.25×10^{-2}%). However, since ether **18** could be produced from bipinnatin J (**17**)[5a] under mild conditions (1:1 MeOH/$CDCl_3$, rt, 24 h), the former may not be a true secondary metabolite. We suspect that the foregoing 2-*C*-methoxylation sequence, whereupon methyl ether **18** was produced as the sole product, proceeds with the fastest rate of solvolysis, but we could not be certain. Support for this contention stems from molecular mechanics calculations, which revealed the furanocembryl carbocation **III** to be the most stable by over 18 kcal/mol (Figure 1).[16]

Biological Significance. Although detailed physicochemical examinations, such as, for example, rate studies, isotope effects, effects of substituents, and solvent effects, have not been carried out, and the only available information is product analysis, molecular mechanics calculations, and the incorporation of ^{18}O from the solvent, a plausible mechanistic interpretation could be to invoke the intermediacy of a stable carbocation species. This has biological significance as the pharmacological activities that a number of known metabolites exhibit can be linked to the formation of a reactive carbocation intermediate.[11] Abramson, for instance, has shown that carbocation intermediates may be involved in the biological activation of a well-known family of nicotinic acetylcholine receptor antagonists known as lophotoxins.[22] The lophotoxins, formally a group of furanocembranes possessing many of the same structural features as pseudopteranes kallolide A (**1**) and isokallolide A (**8**), are to date the only natural toxins known to irreversibly inhibit a neurotransmitter receptor by utilizing a covalent mechanism of action.[23] Our studies have helped to establish the subtle reactivity engendered by the C2 oxygen substituent of furanopseudopterane alcohols **1** and **8** and may explain the ability of these compounds to spontaneously generate stable carbocations. In addition, they lead us to predict that these gorgonian metabolites and possibly some of their solvolysis products may represent a novel family of highly potent antagonists of the nicotinic acetylcholine receptor. Experiments to assess the potential ability of compounds **1** and **8** to interact with the nicotinic acetylcholine receptor are currently underway. On the other hand, neither kallolide A (**1**), biskallolide A (**2**), bipinnatin J (**17**), nor 2-*O*-methylbipinnatin J (**18**) showed relevant inhibitorial activity against *Mycobacterium tuberculosis* $H_{37}Rv$, the aetiological agent that causes tuberculosis, at a drug concentration of 12.5 μg/mL. Follow-up biological screening of kallolide A (**1**), bipinnatin J (**17**), and 2-*O*-methylbipinnatin J (**18**) in the National Cancer Institute's (NCI) 60-cell-line tumor panel indicated no significant in vitro cancer cell cytotoxicity.

Experimental Section

General Experimental Procedures. ^1H and ^{13}C NMR were measured either at 300 and 75 MHz or at 500 and 125 MHz, respectively. IR spectra were determined as thin films, and UV spectra were recorded in MeOH solution. Melting points were determined on a capillary apparatus and are uncorrected. Column chromatography was performed on silica gel (35−75 mesh), and TLC analyses were carried out using glass precoated silica gel plates. HPLC was performed using a 10 mm silica gel Partisil 10 semipreparative column (9.4 mm × 50 cm). Molecular mechanics calculations were performed on INSIGHT-II 3.0/Discover Packages (Biosym Technologies, 9685 Scranton Rd., San Diego, CA 92121-2777). All solvents used were either spectral grade or were distilled from glass prior to use. The percentage yield of natural products **1**, **2**, **17**, and **18** is based on the weight of the crude gorgonian extract. Reactions were monitored by thin-layer chromatography (TLC) on silica gel plates (0.25 mm) and visualized using UV light and I_2 vapors. Reaction temperatures were measured externally. Unless otherwise noted, reagents were obtained from commercial suppliers and were used as provided. *p*-Toluenosulfonic acid hydrate, 4-iodobenzoyl chloride, glacial acetic acid, methanol, ethyl acetate, α-D-glucose, β-D-glucose pentaacetate, DMSO-d_6, deuteriochloroform, 10% palladium on activated carbon, and platinum(IV) oxide were purchased from Aldrich Chemical Co. Ltd. ^{18}O-Enriched methanol [normalized, 95 atom % ^{18}O] was purchased from Medical Isotopes, Inc. Yields refer to chromatographically and spectroscopically (IR, UV, MS, ^1H and ^{13}C NMR) pure materials.

Collection and Extraction Procedures. Kallolide A (**1**), biskallolide A (**2**), bipinnatin J (**17**), and 2-*O*-methyl bipinnatin J (**18**) were isolated from the gorgonian octocoral *P. bipinnata*

(22) (a) Culver, P.; Burch, M.; Potenza, C.; Wasserman, L.; Fenical, W.; Taylor, P. *Mol. Pharmacol.* **1985**, *28*, 436−444. (b) Abramson, S. N.; Li, Y.; Culver, P.; Taylor, P. *J. Biol. Chem.* **1989**, *264*, 12666−12672. (c) Abramson, S. N.; Trischman, J. A.; Tapiolas, D. M.; Harold, E. E.; Fenical, W.; Taylor, P. *J. Med. Chem.* **1991**, *34*, 1798−1804. (d) Groebe, D. R.; Dumm, J. M.; Abramson, S. N. *J. Biol. Chem.* **1994**, *269*, 8885−8891.

(23) Besides lophotoxin itself, isolated from various species of *Lophogorgia*, the "lophotoxins" include all of the furanocembranolides known collectively as bipinnatins that have been isolated from Caribbean specimens of *P. bipinnata*. Thus, strictly speaking, bipinnatin J (**17**) is also a member of the lophotoxin family of neurotoxins.

(Verrill, 1864) collected in May, 1996 near San Andrés Island, Colombia.[5] A voucher specimen is stored at the Chemistry Department of the University of Puerto Rico. The dry animal (2.1 kg) was blended with MeOH−$CHCl_3$ (1:1) (5 × 1 L), and after filtration, the crude extract was evaporated under vacuum to yield a green residue (167.5 g). After partitioning the crude extract between hexane and H_2O, the aqueous suspension was extracted with $CHCl_3$ (4 × 1 L). The resulting extract was concentrated in vacuo to yield 43.3 g of an oil, which was chromatographed over silica gel (400 g), and separated into 30 fractions (I−XXX) on the basis of TLC analyses. Subsequent purification of fraction III (42.1 mg) by column chromatography over silica gel (10 g) eluting with 15% EtOAc in hexane yielded 2-*O*-methylbipinnatin J (**18**) (21.0 mg; yield = 1.25×10^{-2}%). Purification of fraction VIII (8.2 g) by column chromatography over silica gel (400 g) using a step gradient of EtOAc−hexane as eluant afforded 3.76 g (yield = 2.24%) of kallolide A (**1**) and 67.0 mg (yield = 0.04%) of bipinnatin J (**17**). Purification of fraction IX (0.89 g) by size exclusion chromatography on a Bio-Beads SX-3 column using toluene as eluant led to three subfractions. The last subfraction (0.26 g) was purified by normal-phase HPLC [Partisil 10 M9/50 silica gel with 15% 2-propanol in hexane] to afford biskallolide A (**2**) (2.6 mg, yield = 1.55×10^{-3}%).

Kallolide A (1) and Bipinnatin J (17). The physical and spectroscopic properties of metabolites **1**[6b] and **17**[5a] have been described previously in the literature.

Biskallolide A (2): colorless crystals, mp 248−266 °C dec; $[\alpha]^{24}_D$ +88.0° (*c* 0.50, $CHCl_3$); IR (film) 3099, 3077, 1747, 1646, 1447, 1216, 1189, 1091 cm^{-1}; UV λ_{max} (MeOH) 220 nm (ϵ 6400); ^1H NMR (300 MHz, $CDCl_3$) δ 2.71 (dd, 1H, J = 7.2, 11.1 Hz, H-1), 3.94 (d, 1H, J = 11.1 Hz, H-2), 5.86 (s, 1H, H-5), 3.76 (br d, 1H, J = 4.5 Hz, H-7), 5.31 (br d, 1H, J = 4.5 Hz, H-8), 6.53 (br s, 1H, H-9), 1.99 (ddd, 1H, J = 1.8, 3.0, 13.2 Hz, H-11α), 2.32 (ddd, 1H, J = 2.4, 13.2, 13.5 Hz, H-11*β*), 0.30 (ddd, 1H, J = 1.8, 13.5, 13.5 Hz, H-12α), 1.43 (dddd, 1H, J = 2.4, 3.0, 7.2, 13.5 Hz, H-12*β*), 4.70 (br s, 1H, H-14α), 4.94 (br d, 1H, J = 0.9 Hz, H-14*β*), 1.50 (br s, 3H, Me-15), 1.86 (s, 3H, Me-16), 4.89 (br s, 1H, H-18α), 5.01 (br d, 1H, J = 1.0, H-18*β*), 1.99 (br s, 3H, Me-19); ^{13}C NMR (75 MHz, $CDCl_3$) δ 48.0 (d, C-1), 71.1 (d, C-2), 149.4 (s, C-3), 120.5 (s, C-4), 112.0 (d, C-5), 151.9 (s, C-6), 49.2 (d, C-7), 80.8 (d, C-8), 145.5 (d, C-9), 137.1 (s, C-10), 22.2 (t, C-11), 33.6 (t, C-12), 145.5 (s, C-13), 112.8 (t, C-14), 18.7 (q, C-15), 10.0 (q, C-16), 141.7 (s, C-17), 114.6 (t, C-18), 21.8 (q, C-19), 174.8 (s, C-20); HRFAB−MS (3-NBA) *m*/*z* $[M + 1]^+$ 639.3322 (calcd for $C_{40}H_{47}O_7$, 639.3322).

Synthesis of Biskallolide A (2). A small amount of crystalline kallolide A (**1**) (32.0 mg, 0.10 mmol) was placed inside an NMR tube and heated in a sandy bath at 115 °C for 45 min. The tube was cooled, 0.7 mL of $CDCl_3$ was added, and the ^1H NMR spectrum of the oily residue was recorded, showing no starting material left unreacted. A concentrated solution of this oil was chromatographed over silica gel (10 g) eluting with 4% EtOAc in $CHCl_3$ to afford colorless crystals of pure biskallolide A (17.3 mg, 56% yield), which was identified by comparison of its melting point, MS, and ^1H and ^{13}C NMR data to those of the authentic natural product.

Synthesis of 2-*O*-Methylkallolide A (3). After a solution of kallolide A (8.6 mg, 0.026 mmol) in dry methanol (2.0 mL) was magnetically stirred for 3 days at 23 °C, TLC analysis revealed that no reaction had occurred. The mixture was then treated with deuteriochloroform (0.5 mL), allowed to stir at room temperature overnight, and concentrated in vacuo. The residue obtained was chromatographed on silica gel (1.5 g, 11% acetone in hexane) to give 6.6 mg (74%) of 2-*O*-methylkallolide A. Data for **3**: white solid; mp 150−151 °C; $[\alpha]^{24}_D$ +97.9° (*c* 1.1, $CHCl_3$); IR (film) 3073, 1750, 1644, 1450, 1089, 901 cm^{-1}; UV λ_{max} (MeOH) 224 nm (ϵ 11400); ^1H NMR (300 MHz, $CDCl_3$) δ 2.97 (dd, 1H, J = 7.5, 11.1 Hz, H-1), 3.99 (d, 1H, J = 11.1 Hz, H-2), 5.90 (br s, 1H, H-5), 3.77 (d, 1H, J = 4.5 Hz, H-7), 5.37 (br d, 1H, J = 4.8 Hz, H-8), 6.60 (br s, 1H, H-9), 2.06 (ddd, 1H, J = 2.7, 3.3, 13.2 Hz, H-11α), 2.42 (ddd, 1H, J = 2.7, 13.2, 13.5 Hz, H-11*β*), 0.38 (ddd, 1H, J = 3.3, 13.5, 13.5 Hz, H-12α), 1.52 (m, 1H, H-12*β*), 4.88 (br dd, 1H, J = 1.2, 2.1 Hz, H-14α), 5.20 (br d, 1H, J = 2.1 Hz, H-14*β*), 1.74 (br s, 3H, Me-15), 2.00 (s, 3H, Me-16), 4.75 (br d, 1H, J = 0.6 Hz, H-18α), 4.98 (br dd, 1H, J = 1.2, 2.1 Hz, H-18*β*), 1.95 (br s, 3H, Me-19), 3.17 (s, 3H, O*CH*$_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 46.5 (d, C-1), 74.0 (d, C-2), 147.7 (s, C-3), 121.6 (s, C-4), 111.9 (d, C-5), 151.6 (s, C-6), 48.8 (d, C-7), 80.8 (d, C-8), 145.9 (d, C-9), 137.4 (s, C-10), 21.8 (t, C-11), 33.6 (t, C-12), 145.0 (s, C-13), 114.0 (t, C-14), 17.5 (q, C-15), 9.7 (q, C-16), 141.9 (s, C-17), 114.7 (t, C-18), 21.6 (q, C-19), 175.4 (s, C-20), 55.5 (q, O*CH*$_3$); EI−MS *m*/*z* $[M]^+$ 342 (9), 310 (17), 214 (14), 178 (100), 163 (9), 135 (14); HREI−MS *m*/*z* $[M]^+$ 342.1818 (calcd for $C_{21}H_{26}O_4$, 342.1831).

Synthesis of Kallolide A Acetate (4). A solution of kallolide A (5.2 mg, 0.016 mmol) in glacial acetic acid (2.0 mL) was stirred vigorously at 23 °C for 30 h and concentrated to leave a residue that was chromatographed on silica gel (1.5 g). Elution with 20% acetone in hexane yielded 2.8 mg (48%) of **4** as a white crystalline solid, mp 198−199 °C. Compound **4** turned out to be identical in all respects (mp, IR, UV, $[\alpha]^{24}_D$, HREI−MS, ^1H and ^{13}C NMR) to natural product kallolide A acetate.[6b]

Conversion of Kallolide A Acetate (4) to Ether 3. After a solution of kallolide A acetate (13.0 mg, 0.039 mmol) in dry methanol (0.5 mL) was stirred for 3 days at 23 °C, TLC analysis revealed no reaction. The mixture was then treated with deuteriochloroform (2 drops), which had been previously saturated with dry HCl, allowed to stir at room temperature for 3 h, and concentrated in vacuo. In this way, we obtained 11.6 mg (85%) of pure 2-*O*-methylkallolide A (**3**).

Synthesis of 2-[4-Iodobenzoyl]kallolide A (5). To a solution of kallolide A (10.0 mg, 0.030 mmol) in pyridine (2.0 mL) was added 4-iodobenzoyl chloride (30 mg, 0.11 mmol), and the resulting solution was stirred overnight at 25 °C. The solvent was rotoevaporated under vacuum to leave an oil, which was chromatographed over silica gel (10 g) eluting with 2% EtOAc in $CHCl_3$. There was isolated 8.9 mg (52%) of pure benzoate **5**.

Synthesis of 2-*O*-Ethylkallolide A (6) and 2-*O*-Ethyl-isokallolide A (11). To a solution of kallolide A (20.7 mg, 0.063 mmol) in EtOAc (2.0 mL) was added a small crystal of *p*-toluenesulfonic acid hydrate (0.2 mg). After the solution was stirred at 25 °C for 30 min, powdered $NaHCO_3$ (2.0 mg) was added, and the mixture was stirred for another 5 min. After rotoevaporation the oil obtained was chromatographed over silica gel (5 g) using 2% acetone in $CHCl_3$ as eluant to yield 10.0 mg (45%) of 2-*O*-ethylkallolide A and 7.5 mg (33%) of 2-*O*-ethyl-isokallolide A.

Synthesis of 2-*O*-D-[Glucopyranose-6′-yl]kallolide A (7a,b). A solution of kallolide A (10.5 mg, 0.032 mmol) and α-D-glucose (8 mg, 0.044 mmol) in DMSO-d_6 (0.5 mL) was treated with deuteriochloroform (2 drops), stirred vigorously at room temperature for 4 days, and freeze-dried to leave a residue that was chromatographed on reversed-phase ODS silica gel (1.5 g). Elution with 33% methanol in H_2O yielded 5.3 mg (34%) of 2-*O*-D-[glucopyranose-6′-yl]kallolide A as a 2:1 mixture of *β*/α anomers, respectively. Data for **7a,b**: colorless gum; $[\alpha]^{24}_D$ +72.3° (*c* 1.3, MeOH); IR (film) 3358, 3076, 1738, 1654, 1451, 1046 cm^{-1}; UV λ_{max} (MeOH) 220 nm (ϵ 17000), 258 nm (ϵ 24500); ^1H NMR (500 MHz, CD_3OD) (*β*-anomer) δ 2.88 (dd, 1H, J = 7.5, 11.1 Hz, H-1), 4.33 (d, 1H, J = 11.1 Hz, H-2), 6.00 (br s, 1H, H-5), 3.98 (d, 1H, J = 4.2 Hz, H-7), 5.50 (br d, 1H, J = 4.8 Hz, H-8), 6.84 (br d, 1H, J = 0.6 Hz, H-9), 2.06 (br d, 1H, J = 13.7 Hz, H-11α), 2.33 (br dd, 1H, J = 13.3, 13.3 Hz, H-11*β*), 0.44 (ddd, 1H, J = 2.4, 13.2, 13.2 Hz, H-12α), 1.52 (m, 1H, H-12*β*), 4.78 (br s, 1H, H-14α), 5.09 (br s, 1H, H-14*β*), 1.77 (br s, 3H, Me-15), 2.02 (s, 3H, Me-16), 4.71 (br s, 1H, H-18α), 4.93 (br s, 1H, H-18*β*), 1.95 (br s, 3H, Me-19), 4.37 (d, 1H, J = 7.8 Hz, H-1′), 3.15 (m, 1H, H-2′), 3.23 (m, 1H, H-3′), 3.06 (m, 1H, H-4′), 3.78 (m, 1H, H-5′), 3.40 (dd, 1H, J = 6.8, 10.7 Hz, H6′), 3.70 (br d, 1H, J = 10.8 Hz, H-6″); ^{13}C NMR (125 MHz, CD_3OD) (*β*-anomer) δ 48.7 (d, C-1), 74.8 (d, C-2), 148.9 (s, C-3), 123.6 (s, C-4), 113.4 (d, C-5), 153.5 (s, C-6), 49.3 (d, C-7), 83.0 (d, C-8), 149.5 (d, C-9), 137.6 (s, C-10), 22.5 (t, C-11), 34.7 (t, C-12), 147.2 (s, C-13), 113.7 (t, C-14), 18.0 (q, C-15), 9.7 (q, C-16), 144.0 (s, C-17), 114.6 (t, C-18), 21.8 (q, C-19), 178.0 (s, C-20), 98.0 (d, C-1′), 78.1 (d, C-2′), 77.2 (d, C-3′), 76.2 (d, C-4′),

72.1 (d, C-5′), 69.3 (t, C-6′); ^{1}H NMR (500 MHz, CD_3OD) (α-anomer) δ 5.01 (d, 1H, J = 3.6 Hz, H-1′), 3.58 (m, 1H, H-2′), 3.23 (m, 1H, H-3′), 3.15 (m, 1H, H-4′), 3.66 (m, 1H, H-5′), 3.45 (dd, 1H, J = 5.7, 10.6 Hz, H-6′), 3.62 (br d, 1H, J = 10.7 Hz, H-6″); ^{13}C NMR (125 MHz, CD_3OD) (α-anomer) δ 93.8 (d, C-1′), 74.8 (d, C-2′), 73.7 (d, C-3′), 72.5 (d, C-4′), 72.4 (d, C-5′), 69.2 (t, C-6′); HRFAB−MS (glycerol) m/z $[M + Na]^+$ 513.2119 (calcd for $C_{26}H_{34}O_9Na$, 513.2100).

Attempted Glucosylation of Kallolide A (1). A solution of kallolide A (8.9 mg, 0.027 mmol) and β-D-glucose pentaacetate (14.0 mg, 0.036 mmol) in $CDCl_3$ was treated with 1 drop of a freshly prepared solution of dry HCl in $CDCl_3$ and stirred vigorously for 1 week at 23 °C. Periodic TLC and ^{1}H NMR analyses only revealed unreacted starting material.

Synthesis of Isokallolide A (8). A mixture of kallolide A (15 mg, 0.046 mmol) and *p*-toluenesulfonic acid hydrate (0.2 mg) in $CDCl_3$ (0.7 mL) was shaken inside an NMR tube. The progress of the reaction was monitored upon measuring routine ^{1}H NMR spectra every 5 min. After 30 min all of the signals ascribed to kallolide A had disappeared, powdered $NaHCO_3$ (2 mg) was added, and the tube was shaken for 2 min. The reaction solution was chromatographed over silica gel (5 g) eluting with 3% acetone in $CHCl_3$ to afford pure isokallolide A. There was isolated 11.7 mg (78%) as a colorless gum: $[\alpha]^{24}_D$ +26.5° (*c* 0.34, $CHCl_3$); IR (film) 3475, 3074, 1750, 1655, 1644, 1455, 1197, 1053, 940 cm^{-1}; UV λ_{max} (MeOH) 210 nm (ϵ 18300), 264 nm (ϵ 10600); ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.84 (dd, 1H, J = 7.2, 11.1 Hz, H-1), 4.38 (d, 1H, J = 11.1 Hz, H-2), 5.90 (s, 1H, H-5), 5.95 (br s, 1H, H-8), 6.55 (br s, 1H, H-9), 2.14 (ddd, 1H, J = 2.7, 3.0, 13.2 Hz, H-11α), 2.43 (ddd, 1H, J = 2.7, 13.2, 13.2 Hz, H-11β), 0.59 (ddd, 1H, J = 2.7, 13.2, 13.2 Hz, H-12α), 1.67 (dddd, 1H, J = 2.7, 3.0, 7.2, 13.2 Hz, H-12β), 5.01 (br dd, 1H, J = 1.5, 1.8 Hz, H-14α), 5.26 (br dd, 1H, J = 1.5, 1.8 Hz, H-14β), 1.80 (br s, 3H, Me-15), 2.02 (br s, 3H, Me-16), 1.99 (s, 3H, Me-18), 2.07 (br s, 3H, Me-19); ^{13}C NMR (75 MHz, $CDCl_3$) δ 49.6 (d, C-1), 65.2 (d, C-2), 148.0 (s, C-3), 119.9 (s, C-4), 113.4 (d, C-5), 149.8 (s, C-6), 142.6 (s, C-7), 78.9 (d, C-8), 147.5 (d, C-9), 137.4 (s, C-10), 21.8 (t, C-11), 33.4 (t, C-12), 144.0 (s, C-13), 116.7 (t, C-14), 17.3 (q, C-15), 9.5 (q, C-16), 120.4 (s, C-17), 23.0 (q, C-18), 20.6 (q, C-19), 175.6 (s, C-20); EI−MS m/z $[M]^+$ 328 (54), 310 (100), 295 (62), 285 (22), 260 (39), 231 (22), 215 (27), 201 (30), 164 (52), 163 (55), 149 (42), 135 (21); HREI−MS m/z $[M]^+$ 328.1674 (calcd for $C_{20}H_{24}O_4$, 328.1675).

Synthesis of Bisisokallolide A (9). A solution of isokallolide A (**8**) (11.7 mg, 0.036 mmol) in $CDCl_3$ was kept inside an NMR tube at 25 °C for 2 weeks. The ^{1}H NMR spectrum and TLC analysis of the solution using 6% EtOAc in $CHCl_3$ indicated that a minor product was formed. Preparative TLC separation of the minor product afforded 1.3 mg (11%) of bisisokallolide A as a white crystalline solid: mp 237−247 °C dec; $[\alpha]^{24}_D$ +17.5° (*c* 0.50, $CHCl_3$); IR (film) 3106, 3075, 1745, 1652, 1558, 1544, 1049, 1012, 944 cm^{-1}; UV λ_{max} (MeOH) 208 nm (ϵ 40800), 264 nm (ϵ 20600); ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.72 (dd, 1H, J = 7.8, 11.1 Hz, H-1), 4.08 (d, 1H, J = 11.1 Hz, H-2), 5.70 (s, 1H, H-5), 5.86 (br d, 1H, J = 1.2 Hz, H-8), 6.43 (br s, 1H, H-9), 2.02 (m, 1H, H-11α), 2.37 (ddd, 1H, J = 2.7, 13.5, 13.5 Hz, H-11β), 0.41 (ddd, 1H, J = 2.7, 13.5, 13.5 Hz, H-12α), 1.46 (m, 1H, H-12β), 4.72 (br dd, 1H, J = 1.0, 1.2 Hz, H-14α), 4.98 (br d, 1H, J = 1.2 Hz, H-14β), 1.52 (br s, 3H, Me-15), 1.95 (s, 3H, Me-16), 1.89 (s, 3H, Me-18), 2.06 (br s, 3H, Me-19); ^{13}C NMR (75 MHz, $CDCl_3$) δ 47.4 (d, C-1), 70.0 (d, C-2), 148.1 (s, C-3), 119.6 (s, C-4), 113.1 (d, C-5), 149.5 (s, C-6), 141.7 (s, C-7), 78.5 (d, C-8), 147.1 (d, C-9), 137.5 (s, C-10), 21.8 (t, C-11), 34.0 (t, C-12), 145.1 (s, C-13), 113.7 (t, C-14), 18.0 (q, C-15), 10.0 (q, C-16), 121.1 (s, C-17), 22.8 (q, C-18), 20.5 (q, C-19), 175.3 (s, C-20); HRFAB−MS m/z $[M + Li]^+$ 645.3390 (calcd for $C_{40}H_{46}O_7$-Li, 645.3403).

Synthesis of 2-*O*-Methyl-isokallolide A (10). After a solution of isokallolide A (3.0 mg, 0.01 mmol) was stirred overnight in methanol (2.0 mL) at 25 °C, the solvent was removed under N_2. The residue obtained was chromatographed over silica gel (5 g) eluting with 3% acetone in $CHCl_3$ to yield 1.8 mg (58%) of 2-*O*-methyl-isokallolide A as a colorless gum: $[\alpha]^{24}_D$ +30.0° (*c* 1.8, $CHCl_3$); IR (film) 3071, 1750, 1646, 1447, 1088, 1101, 1050 cm^{-1}; UV λ_{max} (MeOH) 210 nm (ϵ 46700), 262 nm (ϵ 33200); ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.94 (dd, 1H, J = 7.5, 11.4 Hz, H-1), 4.00 (d, 1H, J = 11.4 Hz, H-2), 5.92 (s, 1H, H-5), 5.93 (br s, 1H, H-8), 6.52 (br s, 1H, H-9), 2.11 (ddd, 1H, J = 2.4, 3.0, 13.5 Hz, H-11α), 2.47 (ddd, 1H, J = 2.4, 13.5, 13.5 Hz, H-11β), 0.54 (ddd, 1H, J = 2.4, 13.5, 13.5 Hz, H-12α), 1.57 (dddd, 1H, J = 2.4, 3.0, 7.5, 13.5 Hz, H-12β), 4.89 (br dd, 1H, J = 1.5, 1.8 Hz, H-14α), 5.19 (br d, 1H, J = 1.8 Hz, H-14β), 1.75 (br s, 3H, Me-15), 2.04 (s, 3H, Me-16), 2.01 (s, 3H, Me-18), 2.07 (br s, 3H, Me-19), 3.18 (s, 3H, OCH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ 46.6 (d, C-1), 74.2 (d, C-2), 146.2 (s, C-3), 120.7 (s, C-4), 113.1 (d, C-5), 150.3 (s, C-6), 142.4 (s, C-7), 78.7 (d, C-8), 147.2 (d, C-9), 137.7 (s, C-10), 21.7 (t, C-11), 33.9 (t, C-12), 145.0 (s, C-13), 114.0 (t, C-14), 17.6 (q, C-15), 9.7 (q, C-16), 121.4 (s, C-17), 23.1 (q, C-18), 20.6 (q, C-19), 175.3 (s, C-20), 55.6 (q, OCH_3); EI−MS m/z $[M]^+$ 342 (50), 310 (11), 274 (37), 230 (15), 178 (100), 163 (28), 147 (9); HREI−MS m/z $[M]^+$ 342.1828 (calcd for $C_{21}H_{26}O_4$, 342.1831).

Synthesis of 2-[^{18}*O*]-Methylkallolide A (12). A solution of kallolide A (13.7 mg, 0.042 mmol) in ^{18}O-enriched methanol (0.25 g) was treated with deuteriochloroform (0.5 mL), stirred vigorously at 23 °C for 5 h, and concentrated to leave a residue that was chromatographed on silica gel (1.0 g). Elution with 15% acetone in hexane yielded 9.7 mg (70%) of 2-[^{18}*O*]-methylkallolide A as a white solid. Data for **12**: mp 150−151 °C; EI−MS m/z $[M]^+$ 344 (8), 311 (4), 310 (6), 275 (3), 214 (8), 180 (100), 178 (10), 165 (6), 149 (2), 137 (5), 129 (5); HREI−MS m/z $[M]^+$ 344.1870 (calcd for $C_{21}H_{26}{}^{16}O_3{}^{18}O$, 344.1873), 311.1649 ($C_{20}H_{23}{}^{16}O_3$, $M^+ - {}^{18}OCH_3$), 310.1576 ($C_{20}H_{22}{}^{16}O_3$, $M^+ - H^{18}OCH_3$), 180.1026 (100%, $C_{11}H_{14}{}^{16}O^{18}O$).

Synthesis of 2-[^{18}*O*]-Methyl-isokallolide A (13). A solution of isokallolide A (3.3 mg, 0.010 mmol) in ^{18}O-enriched methanol (0.25 g) was treated with deuteriochloroform (0.2 mL), stirred vigorously at 23 °C for 10 min, and concentrated to leave a residue that was chromatographed on silica gel (1.0 g). Elution with 15% acetone in hexane yielded 2.3 mg (66%) of 2-[^{18}*O*]-methyl-isokallolide A as a white solid. Data for **13**: mp 134−135 °C; EI−MS m/z $[M]^+$ 344 (53), 311 (8), 310 (10), 276 (38), 232 (18), 180 (100), 165 (24), 149 (13), 147 (8), 91 (6); HREI−MS m/z $[M]^+$ 344.1928 (calcd for $C_{21}H_{26}{}^{16}O_3{}^{18}O$, 344.1873), 311.1630 ($C_{20}H_{23}{}^{16}O_3$, $M^+ - {}^{18}OCH_3$), 310.1576 ($C_{20}H_{22}{}^{16}O_3$, $M^+ - H^{18}OCH_3$), 180.1052 (100%, $C_{11}H_{14}{}^{16}O^{18}O$), 178.0999 ($C_{11}H_{14}{}^{16}O_2$).

Catalytic Hydrogenation of Kallolide A (1).[6b] Path A. A solution of compound **1** (20 mg, 0.061 mmol) in EtOAc (7 mL) was stirred at room temperature for 30 min under hydrogen at atmospheric pressure in the presence of catalytic amounts of 10% Pd on activated carbon. After removal of the catalyst by filtration the excess solvent was evaporated under reduced pressure. The residue obtained was chromatographed over silica gel (1.2 g) eluting with 15% acetone in hexane to yield 15.6 mg (77%) of dihydro derivative **14**.

Path B. A solution of kallolide A (**1**) (45.0 mg, 0.14 mmol) in EtOAc (10 mL) was stirred at room temperature for 1 h under hydrogen at atmospheric pressure in the presence of catalytic amounts of PtO_2. After removal of the catalyst by filtration the excess solvent was evaporated under reduced pressure. The homogeneous oil obtained (40.2 mg, 88% crude yield) turned out to be identical in all respects (IR, UV, $[\alpha]^{25}_D$, LREI−MS, and ^{1}H NMR) to the hexahydro derivative **15** reported by Fenical and co-workers.[6b] Partial NMR data for **15**: ^{13}C NMR (75 MHz, $CDCl_3$) δ 46.5 (d, C-1), 68.1 (d, C-2), 149.9 (s, C-3), 118.3 (s, C-4), 113.5 (d, C-5), 151.8 (s, C-6), 50.8 (d, C-7), 77.8 (d, C-8), 25.9 (t, C-9), 38.6 (d, C-10), 31.6 (t, C-11), 20.9 (t, C-12), 27.6 (d, C-13), 20.1 (q, C-14), 17.3 (q, C-15), 9.5 (q, C-16), 28.6 (d, C-17), 22.4 (q, C-18), 20.7 (q, C-19), 180.6 (s, C-20).

Methanolysis of Hexahydro Derivative 15 To Afford Ether 16a,b. A solution of hexahydro adduct **15** (35.0 mg, 0.105 mmol) in dry methanol (1 mL) was stirred overnight at room temperature, after which TLC analysis revealed no reaction. The mixture was then treated with deuteriochloroform (2 drops) containing dry HCl, allowed to stir at room temperature for 2 h, and concentrated in vacuo to leave a white solid residue that was chromatographed on silica gel (2.5 g).

Elution with 10% acetone in hexane afforded 31.0 mg (85%) of a 4:1 mixture of epimers **16a,b**.

2-*O*-Methylbipinnatin J (18): white crystalline solid; mp 180−181 °C; $[\alpha]^{24}_D$ −13.2° (*c* 0.53, $CHCl_3$); IR (film) 3079, 1754, 1647, 1450, 1098, 1063, 1017, 973 cm^{-1}; UV λ_{max} (MeOH) 206 nm (ϵ 8800), 282 nm (ϵ 7600); ^1H NMR (500 MHz, $CDCl_3$) δ 2.41 (dd, 1H, J = 11.1, 11.1 Hz, H-1), 4.10 (d, 1H, J = 11.1 Hz, H-2), 6.04 (s, 1H, H-5), 6.11 (br s, 1H, H-7), 2.70 (dd, 1H, J = 4.5, 11.7 Hz, H-9α), 3.22 (dd, 1H, J = 11.7, 11.7 Hz, H-9*β*), 4.95 (m, 1H, H-10), 6.78 (br s, 1H, H-11), 2.05 (m, 1H, H-13α), 2.47 (ddd, 1H, J = 3.0, 14.4, 14.4 Hz, H-13*β*), 0.85 (ddd, 1H, J = 3.6, 13.5, 13.5 Hz, H-14α), 1.53 (dddd, 1H, J = 3.0, 11.1, 13.5, 14.4 Hz, H-14*β*), 4.94 (br s, 1H, H-16α), 5.01 (br s, 1H, H-16*β*), 1.76 (br s, 3H, Me-17), 2.07 (s, 3H, Me-18), 2.00 (br s, 3H, Me-19), 3.13 (s, 3H, OCH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ 48.5 (d, C-1), 74.5 (d, C-2), 147.3 (s, C-3), 122.4 (s, C-4), 113.5 (d, C-5), 152.0 (s, C-6), 117.3 (d, C-7), 129.4 (s, C-8), 39.7 (t, C-9), 78.6 (d, C-10), 151.4 (d, C-11), 132.8 (s, C-12), 19.5 (t, C-13), 30.3 (t, C-14), 143.0 (s, C-15), 115.4 (t, C-16), 18.1 (q, C-17), 9.8 (q, C-18), 25.9 (q, C-19), 174.2 (s, C-20), 55.8 (q, OCH_3); EI−MS *m*/*z* $[M]^+$ 342 (13), 311 (2), 273 (2), 178 (100), 163 (5), 135 (8); HREI−MS *m*/*z* $[M]^+$ 342.1841 (calcd for $C_{21}H_{26}O_4$, 342.1831).

Synthesis of 2-*O*-Methylbipinnatin J (18). Bipinnatin J (**17**) (7.0 mg, 0.021 mmol) was dissolved in a 1:1 mixture of methanol and $CDCl_3$ (1.0 mL), and the solution was stirred at 25 °C for 24 h. The solvent was removed under a stream of N_2 to afford a white solid residue, which was chromatographed over silica (10 mg) using 15% EtOAc in $CHCl_3$ as eluant to give 5.3 mg (73%) of 2-*O*-methylbipinnatin J. The spectral data obtained for the product were identical to those of natural **18**.

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Supporting Information Available: Spectral data for compounds **5**, **6**, **11**, **14**, and **16a,b**. Copies of the ^1H and ^{13}C NMR spectra for biskallolide A (**2**) and compounds **3**, **4**, **7**, **9**, **11**, **14**, and **18**, as well as the HREI−MS spectral data for ethers **3** and **12**. This material is available free of charge via the Internet at http://pubs.acs.org.

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