J|A|C|S
ARTICLES
Published on Web 10/27/2004

Vanadium Bromoperoxidase-Catalyzed Biosynthesis of Halogenated Marine Natural Products

Jayme N. Carter-Franklin and Alison Butler*

Contribution from the Department of Chemistry and Biochemistry, University of California, Santa Barbara, California 93106-9510

Received April 10, 2004; E-mail: butler@chem.ucsb.edu

Abstract: Marine red algae (Rhodophyta) are a rich source of bioactive halogenated natural products. The biogenesis of the cyclic halogenated terpene marine natural products, in particular, has attracted sustained interest in part because terpenes are the biogenic precursors of many bioactive metabolites. The *first* enzymatic asymmetric bromination and cyclization of a terpene, producing marine natural products isolated from red algae, is reported. Vanadium bromoperoxidase (V-BrPO) isolated from marine red algae (species of *Laurencia, Plocamium, Corallina*) catalyzes the bromination of the sesquiterpene (*E*)-(+)-nerolidol producing α-, β-, and γ-snyderol and (+)-3β-bromo-8-epicaparrapi oxide. α-Snyderol, β-snyderol, and (+)-3β-bromo-8-epicaparrapi oxide have been isolated from *Laurencia obtusa*, and each have also been isolated from other species of marine red algae. γ-Snyderol is a proposed intermediate in other bicyclo natural products. Single diastereomers of β-snyderol, γ-snyderol, and mixed diastereomers of (+)-3β-bromo-8-epicaparrapi oxide (de = 20−25%) are produced in the enzyme reaction, whereas two diastereomers of these compounds are formed in the synthesis with 2,4,4,6-tetrabromocyclohexa-2,5-dienone (TBCO). V-BrPO likely functions by catalyzing the two-electron oxidation of bromide ion by hydrogen peroxide producing a bromonium ion or equivalent in the active site that brominates one face of the terminal olefin of nerolidol. These results establish V-BrPO's role in the biosynthesis of brominated cyclic sesquiterpene structures from marine red algae for the first time.

Introduction

The sheer abundance of halogenated compounds isolated from the marine environment distinguishes marine natural products from terrestrial natural products. In particular, halogenated compounds from marine red algae (Rhodophyceae) comprise some of the most frequently reported metabolites.[1−3] Brominated compounds such as thyrsiferyl acetate (**I**, see Figure 1),[4−6] halomon (**II**),[7,8] ma'ilione (**III**),[9,10] furanones (e.g., **IV**),[11−13] cyclized sesquiterpenes (e.g., **V**, **VI**),[14] and acetogenins (e.g., **VII**),[14] all from marine red algae, exhibit wide-ranging biological activities including protein phosphatase inhibition (**I**), antitumor and cytotoxic function (**II**), anthelminthic properties

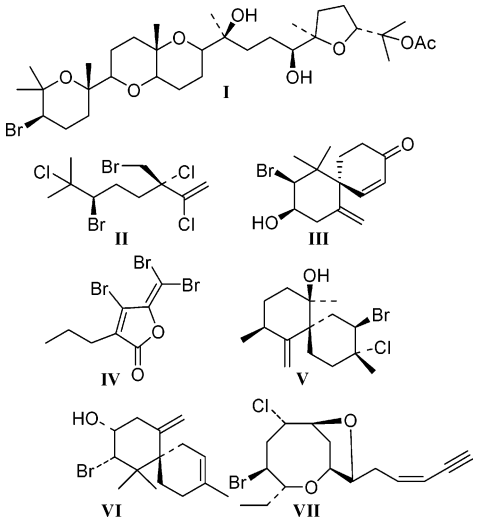


Figure 1. Halogenated natural products from marine red algae. Thyrsiferyl acetate (**I**),[4−6] halomon (**II**),[7,8,11] ma'ilione (**III**),[9,10] brominated furanone (**IV**),[11−13] brominated cyclized sesquiterpenes (**V**, **VI**)[14] and brominated cyclized acetogenin (**VII**).[14]

(1) Fenical, W. *J. Phycol.* **1975**, *11*, 245−259.
(2) Gribble, G. W. *Acc. Chem. Res.* **1998**, *31*, 141−152.
(3) Faulkner, D. J. *Nat. Prod. Rep.* **2002**, *19*, 1−48 and references therein.
(4) Suzuki, T.; Suzuki, M.; Furusaki, A.; Matsumoto, T.; Kato, A.; Imanaka, Y.; Kurosawa, E. *Tetrahedron Lett.* **1985**, *26*, 1329−1332.
(5) Matsuzawa, S. I.; Suzuki, T.; Suzuki, M.; Matsuda, A.; Kawamura, T.; Mizuno, Y.; Kikuchi, K. *FEBS Lett.* **1994**, *356*, 272−274.
(6) Schonthal, A. H. *Front. Biosci.* **1998**, *3*, 1262−1273.
(7) Fuller, R. W.; Cardellina, J. H., II; Kato, Y.; Brinen, L. S.; Clardy, J.; Snader, K. M.; Boyd, M. R. *J. Med. Chem.* **1992**, *35*, 3007−3011.
(8) Fuller, R. W.; Cardellina , J. H., II; Jurek, J.; Scheurer, P. J.; Alvarado-Lindner, B.; McGuire, M.; Gray, G. N.; Steiner, J. R.; Clardy, J.; Menez, E.; Shoemaker, R. H.; Newman, D. J.; Snader, K. M.; Boyd, M. R. *J. Med. Chem.* **1994**, *37*, 4407−4411.
(9) Juagdan, E. G.; Kalidindi, R.; Scheuer, P. J. *Tetrahedron* **1997**, *53*, 521−528.
(10) Davyt, D.; Fernandez, R.; Suescun, L.; Mombru', A. W.; Saldana, J.; Dominguez, L.; Coll, J.; Fujii, M. T.; Manta, E. *J. Nat. Prod.* **2001**, *64*, 1552.
(11) Kazlauskas, R.; Murphy, P. T.; Quinn, R. J.; Wells, R. J. *Tetrahedron Lett.* **1977**, *1*, 37−40.
(12) De Nys, R.; Wright, A. D.; Konig, G. M.; Sticher, O. *Tetrahedron* **1993**, *49*, 11213−11220.
(13) Maximllien, R.; De Nys, R.; Holmstrom, C.; Gram, L.; Givskov, M.; Crass, K.; Kjelleberg, S.; Steinberg, P. D. *Aquat. Microb. Ecol.* **1998**, *15*, 233−246.
(14) Suzuki, M.; Daitoh, M.; Vairappan, C. S.; Abe, T.; Masuda, M. *J. Nat. Prod.* **2001**, *64*, 597−602.

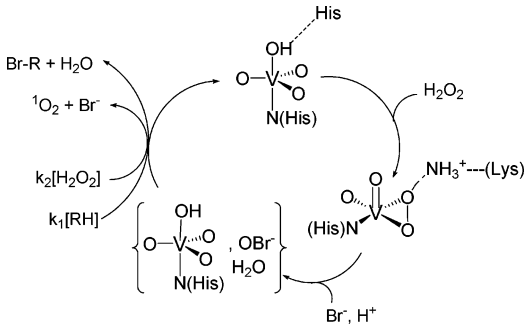
10.1021/ja047925p CCC: $27.50

(**III**), anti-fouling properties (**IV**), as well as broad spectrum antimicrobial (**V**−**VII**) and antiviral (**I**) activities (Figure 1).

Within the vast array of halogenated compounds, it is the biosynthesis of halogenated cyclic terpenes that has attracted sustained interest, in part because terpenes are the biogenic precursors of many bioactive natural products. Early investigations on the synthesis of halogenated terpenes were carried out at a time when the existence of halogenating enzymes had only been hypothesized. Van Tamelen and Hessler demonstrated that *N*-bromosuccinamide reacts with methyl farnesate in an aqueous tetrahydrofuran mixture to produce a bromo-bicyclic ester of methyl farnesate. This reaction was the first demonstration of a bromonium-ion-induced cyclization of a terpene forming a bromocyclic product.[15,16] Subsequently, reagents have been developed to produce brominated cyclized terpenes including bromine in the presence of Lewis acids such as $AlBr_3$, $SnBr_4$, or $AgBF_4$,[17−19] 2,4,4,6-tetrabromocyclohexa-2,5-dienone (TBCO),[20−22] the acid-catalyzed cyclization of terpene-containing terminal bromohydrins,[23,24] as well as mercuric trifluoroacetate in the presence of molecular bromine.[25−27] Through these investigations a consensus developed that brominated cyclized terpene natural products likely resulted from a bromonium-ion-induced cyclization of an acyclic terpene precursor.[15−17,19,28−31]

Since the time of the initial investigations described above, vanadium haloperoxidase[32−34] and FeHeme haloperoxidase[35−37] enzymes have been isolated from marine organisms. Vanadium bromoperoxidase (V-BrPO), which appears to be the more prevalent of the two classes of enzymes, is an abundant and robust enzyme found in all classes of marine algae, including species that produce chiral halogenated natural products. In the active site of V-BrPO, the vanadate ion (V^V) is coordinated to the protein scaffold by a single histidine residue positioned at the bottom of a deep active-site channel.[38,39] Multiple amino acid side chains hydrogen bond to the vanadate oxygen atoms. The ligand histidine and the amino acids that participate in hydrogen bonding are conserved in V-BrPO isolated from both red (Rhodophyta) and brown (Phaeophyta) marine algae, although little sequence homology exist in the rest of the active-site channel.

Scheme 1



V-BrPO catalyzes the oxidation of halides (I^-, Br^-, Cl^-) by the peroxo complex of V-BrPO (Scheme 1). The oxidized halogen intermediate can halogenate an appropriate organic substrate or oxidize a second equivalent of hydrogen peroxide, producing dioxygen in the singlet excited state to complete the catalytic cycle (Scheme 1).[34,38,40] Bromination reactions catalyzed by V-BrPO proceed via an electrophilic mechanism (i.e., Br^+), as opposed to a radical ($Br^\bullet$) process.[41]

While the numbers of halogenated metabolites isolated from marine organisms continue to increase, the biochemical pathways by which these products are synthesized within the algae have not been fully elucidated.[42−44] We recently reported that V-BrPO isolated from marine red algae (e.g., *Corallina officinalis*, *Laurencia pacifica*, and *Plocamium cartilagineum*) catalyzes the bromination and cyclization of monoterpenes geraniol and nerol to cyclic structures that are found in many bromocyclic terpene marine metabolites,[45] although these bromocyclic monoterpene compounds are not known, marine natural products. Several bromosesquiterpene metabolites isolated from the red alga *L. obtusa* provide an attractive target for biosynthetic studies because all of these compounds could originate from a single substrate. Herein we report the first enzymatic asymmetric bromination and cyclization of a sesquiterpene, (*E*)-(+)-nerolidol (**1**), by V-BrPO producing marine natural products α-snyderol (**2**), *β*-snyderol (**3**), and (+)-3*β*-bromo-8-epicaparrapi oxide (**5**), and we establish V-BrPO's likely role in the biosynthesis of brominated cyclic sesquiterpene structures from marine red algae.

Methods and Materials

General Methods. Vanadium bromoperoxidase from the marine red algae *C. officinalis*, *P. cartilagineum*, and *L. pacifica* were purified as previously described for *C. officinalis* with minor modifications.[46,47] *C. officinalis* and *P. cartilagineum* were collected off the coast of Santa

(15) vanTamelen, E.; Hessler, E. J. *Chem. Commun.* **1966**, 411−413.
(16) vanTamelen, E. *Acc. Chem. Res.* **1968**, *1*, 111−120.
(17) Wolinksky, L. E.; Faulkner, D. J. *J. Org. Chem.* **1976**, *41*, 597−600.
(18) Hoye, T. R.; Kurth, M. J. *J. Org. Chem.* **1978**, *43*, 3693−3697.
(19) Gonzalez, A. G.; Darias, J.; Diaz, A.; Fourneron, J. D.; Martin, J. D.; Perez, C. *Tetrahedron Lett.* **1976**, *35*, 3051−3054.
(20) Kato, T.; Ishii, K.; Ichinose, I.; Nakai, Y.; Kumagai, T. *J. Chem. Soc., Chem. Commun.* **1980**, 1106−1108.
(21) Shieh, H.; Prestwich, G. D. *Tetrahedron Lett.* **1982**, *23*, 4643−4646.
(22) Gonzalez, I. C.; Forsyth, C. J. *J. Am. Chem. Soc.* **2000**, *122*, 9099−9108.
(23) Murai, A.; Abiko, A.; Kato, K.; Tadashi, M. *Chem. Lett.* **1981**, 1125−1128.
(24) Murai, A.; Abiko, A.; Masamune, T. *Tetrahedron Lett.* **1984**, *25*, 4955−4958.
(25) Hoye, T. R.; Kurth, M. J. *J. Org. Chem.* **1979**, *44*, 3461−3467.
(26) Gopalan, A.; Prieto, R.; Mueller, B.; Peters, D. *Tetrahedron Lett.* **1992**, *33*, 1679−1682.
(27) Hoye, T. R.; Caruso, A. J.; Kurth, M. J. *J. Org. Chem.* **1981**, *46*, 6, 3550−3552.
(28) Faulkner, D. J. *Pure Appl. Chem.* **1976**, *48*, 25−28.
(29) Gonzalez, A. G.; Martin, J. D.; Perez, C.; Ramirez, M. A. *Tetrahedron Lett.* **1976**, *2*, 137−138.
(30) Kato, T.; Kanno, S.; Kitahara, Y. *Tetrahedron* **1970**, *26*, 4287−4292.
(31) Kato, T.; Ichinose, I.; Kamoshida, A.; Kitahara, Y. *J. Chem. Soc., Chem. Commun.* **1976**, 518−519.
(32) Vilter, H. *Bot. Mar.* **1983**, *26*, 429−435.
(33) Wever, R.; de Boer, E. *Biochem. Biophys. Acta* **1985**, *830*, 181−186.
(34) Butler, A.; Walker, J. V. *Chem. Rev.* **1993**, *93*, 1937−1944.
(35) Manthey, J. A.; Hager, L. P. *J. Biol. Chem.* **1985**, *260*, 9654−9659.
(36) Manthey, J. A.; Hager, L. P. *Biochemistry* **1989**, *28*, 3052−3057.
(37) Roach, M. P.; Chen, Y. P.; Woodin, S. A.; Lincoln, D. E.; Lovell, C. R.; Dawson, J. H. *Biochemistry* **1997**, *36*, 2197−2202.
(38) Weyand, M.; Hecht, H. J.; Kiess, M.; Liaud, M. F.; Vilter, H.; Schomburg, D. *J. Mol. Biol.* **1999**, *293*, 595−611.
(39) Isupov, M. N.; Dalby, A. R.; Brindley, A. A.; Izumi, Y.; Tanabe, T.; Murshudov, G. N.; Littlechild, J. A. *J. Mol. Biol.* **2000**, *299*, 1035−1049.
(40) Everett, R. R.; Soedjak, H. S.; Butler, A. *J. Biol. Chem.* **1990**, *265*, 15671−15679.
(41) Soedjak, H. S.; Walker, J. V.; Butler, A. *Biochemistry* **1995**, *34*, 12689−12696.
(42) Fukuzawa, A.; Aye, M.; Nakamura, M.; Tamura, M.; Murai, A. *Chem. Lett.* **1990**, 1287−1290.
(43) Fukuzawa, A.; Takasugi, Y.; Murai, A.; Nakamura, M.; Tamura, M. *Tetrahedron Lett.* **1992**, *33*, 2017−2018.
(44) Fukuzawa, A.; Aye, M.; Takasugi, Y.; Nakamura, M.; Tamura, M.; Murai, A. *Chem. Lett.* **1994**.
(45) Carter-Franklin, J.; Parrish, J. D.; Tschirret-Guth, R. A.; Little, R. D.; Butler, A. *J. Am. Chem. Soc.* **2003**, *125*, 3688−3689.
(46) Carter, J. N.; Beatty, K. E.; Simpson, M. T.; Butler, A. *J. Inorg. Biochem.* **2002**, *91*, 59−69.
(47) Brindley, A. A.; Dalby, A. R.; Isupov, M. N.; Littlechild, J. A. *Acta Crystallogr.* **1998**, *D54*, 454−457.

Barbara, CA. *L. pacifica* was collected from an intertidal region off the coast of La Jolla, CA. Bromoperoxidase activity was determined by monitoring the bromination of 50 μM monochlorodimedone (MCD) spectrophotometrically at 290 nm under conditions of 0.1 M KBr, and 1 mM H_2O_2 in 0.1 M sodium phosphate buffer pH 6.00. The extinction coefficient difference at 290 nm between brominated MCD and MCD is 19 900 cm^{-1} M^{-1}.[48]

^1H NMR and ^{13}C NMR spectra were recorded on a Varian Inova instrument (500 and 125 MHz, respectively) using deuteriochloroform as the solvent (internal reference $\delta = 7.27$ ppm, $CHCl_3$). Consumption and formation of reaction substrates and products were monitored by gas chromatography (GC). Chiral GC analysis was performed using a Cyclosil B capillary column (J & W Scientific).

Aqueous bromine was prepared by dilution of bromine vapors in 0.1 M NaOH and standardized by tri-iodide formation (I_3^-; λ_{max} 353 nm, $\Delta\epsilon = 26\,600$ cm^{-1} M^{-1}) by reaction with 0.05 M KI in 0.1 M citrate phosphate buffer pH 4.5. The predominant species in solution at pH 6−7 is HOBr; however, multiple oxidized bromine species are present in solution which are best represented as $OBr^- = HOBr = Br_2 = Br_3^-$,[49] hereafter referred to as "aqueous bromine".

Normal-phase HPLC was used to purify products **2**, **3**, and **4** which often eluted as overlapping peaks in reversed-phase mode. Using a silica HPLC column (semipreparative 250 mm × 10 mm, YMC-Sil, YMC Inc.), products were eluted isocratically with 0.3% 2-propanol/hexanes, and detected by UV absorbance (214 nm). Reversed-phase HPLC was used to purify product **5**. Samples were applied to a C_{18} column (semipreparative 250 mm × 10 mm or analytical 250 mm × 4.5 mm, ODS-AQ, YMC Inc.), and products were eluted using gradient mixtures of acetonitrile and water. The UV absorbance of the eluent was monitored at 214 nm. Flash column chromatography was performed using 230−400 mesh silica gel (EM Science) and mixtures of ether and pentane as eluent. (*E*)-(+)-Nerolidol (**1**) was purchased from Indofine Chemical Company and used as received. TBCO was used as obtained from Acros.

V-BrPO-Catalyzed Reaction with (*E*)-(+)-Nerolidol (1). (*E*)-(+)-Nerolidol (**1**) (0.5 mM) was dissolved in ethanol and added to 0.15 M phosphate buffer (pH 5.7) containing 40% v/v ethanol and 40 mM KBr. Enzymatic reactions containing 23 nM V-BrPO, were initiated by addition of 1 or 2 mol equiv of H_2O_2 via syringe pump with respect to **1**. After 2.5 h, the reactions were extracted with 3 volumes of hexanes. Alcohol and ether products were separated by elution on a silica Sep-pak (Waters Corp.) with 10% ether/pentane. Bromo alcohol and bromoether products were resuspended in 100% ethanol and injected directly on a C_{18} analytical column for product profile and retention time analysis by reversed-phase HPLC.

To obtain products **2**, **3**, **4**, and **5** for spectroscopic characterization, reactions were run on a scale of 7 mM **1** with 55 nM V-BrPO. Upon reaction completion (ca. 2.5 h), products were extracted with 3 volumes of hexanes, washed with brine, dried with $MgSO_4$, and reduced to a small volume in vacuo. Crude extracts were first fractioned by flash chromatography followed by HPLC purification as described above. Spectroscopic data for **2**, **3**, **4**, **5**, and **5′** are identical to reported literature values.[17,50,51]

The relative stereochemistries of **2**, **3**, **4**, and **5** were determined from coupling constants and by one-dimensional nOe analysis. Diastereomeric excesses were established by chiral GC analysis using an isotherm of 155 °C for **2**, **3**, **4**, and 165 °C for **5**. Chiral GC retention times for **3** and **4** were 75.7 and 91.0 min, respectively. Retention times for diastereomers **5** and **5′** were 27.6 and 26.8 min, respectively.

Nonenzymatic Reactions with (*E*)-(+)-Nerolidol (1). Reaction of **1** (0.5 mM) with 1 or 2 mol equiv of aqueous bromine was carried out under the same reaction conditions as described above for V-BrPO-catalyzed reactions (i.e., 0.04 M KBr in 0.15 M phosphate buffer (pH 5.7) containing 40% ethanol), although without addition of V-BrPO or H_2O_2. Aqueous bromine was added in a controlled manner via syringe pump at a rate approximating the bromination rate of MCD by V-BrPO (all reactions were performed in the dark with stirring). Bromination of **1** with TBCO was performed in nitromethane with stirring in the dark.[20−22]

Competitive Substrate Kinetics. Competitive kinetics for the bromination of phenol red (50 μM) catalyzed by V-BrPO (5 nM) or carried out by the stoichiometric addition of aqueous bromine as a function of the concentration of **1** were performed at 25 °C in the presence of 40 mM KBr in 0.1 M phosphate buffer (pH 5.7) containing 40% ethanol. V-BrPO-catalyzed experiments were initiated by the addition of 0.5 mM H_2O_2. Nonenzymatic competition experiments were initiated by the addition of aqueous bromine (5 mM stock solution) in 10-μL aliquots at 30 s intervals. UV absorbance was measured 20 s after each addition of aqueous bromine. Production of bromophenol blue was monitored at 596 nm.

Results and Discussion

V-BrPO-Catalyzed Reaction with (*E*)-(+)-Nerolidol (1) versus Reaction with Aqueous Bromine or TBCO. To explore whether V-BrPO could be involved in the biosynthesis of halogenated marine natural products, the reactivity of V-BrPO toward (*E*)-(+)-nerolidol (**1**) was investigated. When (*E*)-(+)-nerolidol (7 mM) (**1**) reacts with V-BrPO (55 nM) in phosphate buffer (pH 6.5) containing 40% organic cosolvent (e.g., EtOH, ^{i}PrOH), bromide (40 mM), and 1 or 2 equiv of hydrogen peroxide, a mixture of bromoether, bromo alcohol, bromohydrin, and epoxide species is produced.

V-BrPO-Catalyzed Biosynthesis of α-, β-, and γ-Snyderols. The V-BrPO-catalyzed reaction with **1** produces the known bromo alcohols α-, β-, and γ-snyderols (**2**, **3**, and **4**), where **2** and **3** are natural products isolated from the marine red alga *L. obtusa*.[50] Snyderol products **2**−**4** are not produced in the absence of V-BrPO or of one or more reaction substrates (e.g., Br^- or H_2O_2), indicating that oxidation of bromide by a peroxo−V-BrPO complex is necessary for the bromination and cyclization of **1** to produce **2**−**4**. In addition, the presence of organic cosolvent (≥40%) is necessary for the formation of snyderols, where the use of 2-propanol is preferred to minimize competing nucleophilic reactions by the cosolvent.

The formation of **2**−**4** in the V-BrPO-catalyzed reaction results from selective bromination of the C10−C11 olefinic bond of **1**, producing a bromonium−nerolidol adduct (Scheme 2). The proposed bromocarbenium ion intermediate is subsequently attacked by the electron-rich internal olefin, followed by one of three different elimination reactions leading to **2**, **3**, and **4**. Brominated snyderols **2**−**4** comprise 10% of the total isolated products of the reaction.[52] The proposed mechanism of formation of **2**, **3**, and **4** is analogous to the V-BrPO-catalyzed reactions with geraniol and geranyl acetate, producing bromocyclized monoterpenes.[17,45]

In contrast to the V-BrPO reaction, only trace quantities of **2**, **3**, and **4** are formed in the reaction of **1** with aqueous bromine (1−2 equiv) (Figure 2). Similar results were observed previously with the monoterpenes, nerol and geraniol, where brominated cyclized products were only produced in the enzyme-catalyzed reactions and absent from reactions with aqueous bromine.[45]

(48) Hager, L.; Morris, D.; Brown, F.; Eberwein, H. *J. Biol. Chem.* **1966**, *241*, 1769−1777.
(49) Ziderman, I. *Isr. J. Chem.* **1972**, *11*, 7−20.
(50) Howard, B. M.; Fenical, W. *Tetrahedron Lett.* **1976**, *1*, 41−44.
(51) Faulkner, D. J. *Phytochemistry* **1976**, *15*, 1993−1994.

(52) The remaining 90% of isolated product included brominated cyclic ethers (30%) and bromohydrin and epoxide species (60%).

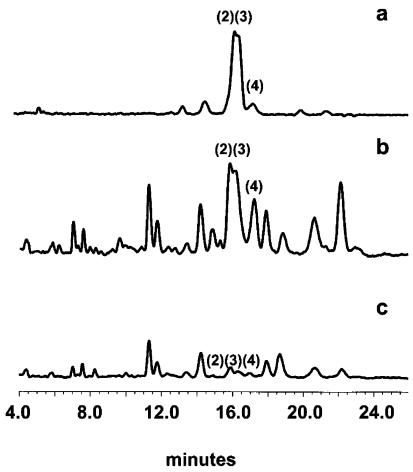
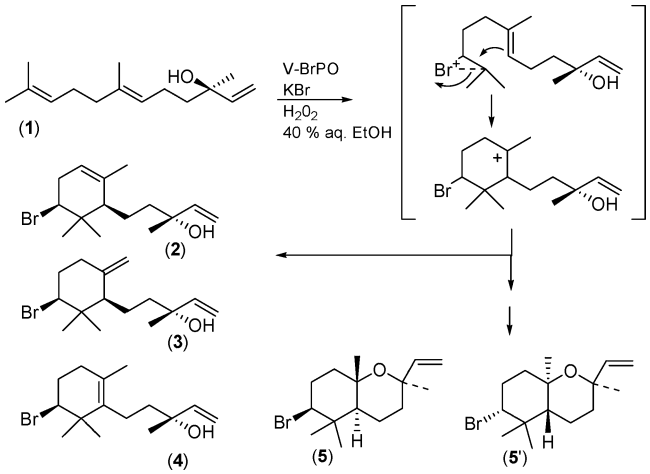


Figure 2. Reversed-phase HPLC separation of bromo alcohol containing fractions formed in the reactions of V-BrPO and aqueous bromine with **1**. (a) Mixture of authentic sample of α-, *β*-, *γ*-snyderol (**2**, **3**, and **4**) (i.e., synthesized using TBCO in nitromethane[20,31]). (b) Bromo alcohol containing fraction of the V-BrPO-catalyzed reaction. (**c**) Bromo alcohol containing fraction of the aqueous bromine reaction. Peaks are denoted with numbers corresponding to their structures. The reaction mixtures contained 0.04 M KBr and 0.5 mM **1** in 0.1 M sodium phosphate, pH 5.7 with 40% v/v ethanol. Enzymatic reactions were initiated by the addition of H_2O_2 (1 mM) and allowed to react at 24 °C for 60 min. Aqueous bromine reactions were initiated by the addition of NaOBr to a final concentration of 1mM and reacted at 24 °C for 60 min.

Scheme 2. Proposed Mechanism for V-BrPO-Catalyzed Biosynthesis of the Marine Natural Products α-, *β*-, and *γ*-Snyderol (**2**, **3**, and **4**), and (+)-3*β*-Bromo-8-epicaparrapi Oxide (**5** and **5′**)



On the other hand, reaction of **1** with TBCO carried out in non-nucleophilic solvents (e.g., methylene chloride or nitromethane) produces **2**−**4** as previously reported.[20,31]

The relative stereochemistry of the proton adjacent to the bromine in **2**−**4** is indicated by a doublet of doublets signal at 4.17 ppm for α-snyderol ($J = 9$, 7 Hz), 4.10 ppm for *β*-snyderol ($J = 11$, 5 Hz), and 4.22 ppm for *γ*-snyderol ($J = 10$, 4 Hz) in the ^{1}H NMR spectrum, which is indicative of an axial proton and thus an equatorial bromine.[17] The stereochemistry of **2**−**4** is consistent with our previous results of a V-BrPO-catalyzed reaction with geraniol to produce bromocyclogeraniol, in which the bromine is also found in the equatorial position.[45] In addition, the stereochemistry of **2**−**4** is in agreement with the stereochemistry determined for the isolated marine natural products.[50] The equatorial position of bromine in cyclized terpenes has been observed in many cationic cyclization reactions, where the arrangement of the bromonium−terpene adduct appears to control the conformation of the transition state in the cyclization reaction.[21,23]

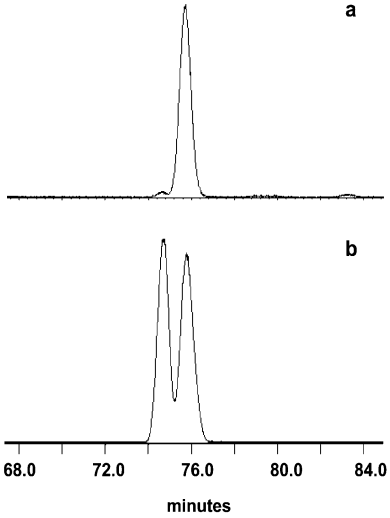


Figure 3. Chiral GC−MS chromatogram for **3** isolated from the (a) V-BrPO-catalyzed reaction and the (b) TBCO reaction with **1**. The retention time for the single diastereomer of **3** isolated from V-BrPO reactions was 75.7 min. Diastereomers isolated from TBCO reactions eluted with retention times of 74.1 and 75.7 min, respectively. Gas chromatography was performed using 90 min, 155 °C isotherm runs.

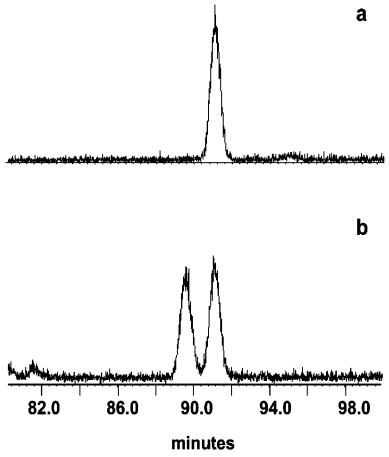
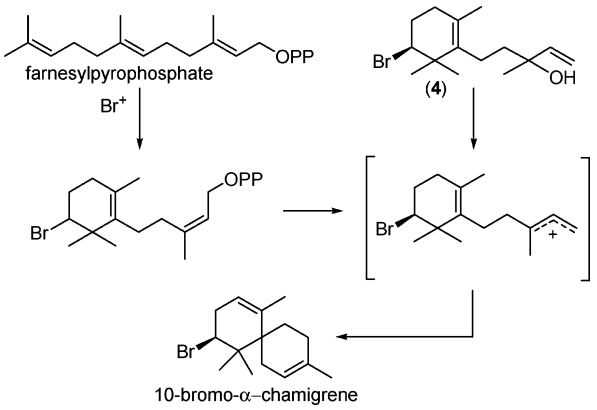


Figure 4. Chiral GC−MS chromatogram for **4** isolated from the (a) V-BrPO-catalyzed reaction and the (b) TBCO reaction with **1**. The retention time for the single diastereomer of **4** isolated from V-BrPO reactions was 91.0 min. Diastereomers isolated from TBCO reactions eluted with retention times of 89.6 and 91.0 min, respectively. Gas chromatography was performed using 90 min, 155 °C isotherm runs.

Chiral GC−MS analysis of snyderols **2**−**4** demonstrates that V-BrPO catalyzes the asymmetric bromination of the C10−C11 double bond of **1** (Figures 3 and 4). A single peak is observed in the chiral GC chromatogram of *β*-snyderol (**3**) and *γ*-snyderol (**4**) formed in the enzyme reaction, with a retention time equal to that of one of the two diastereomers formed in the TBCO reaction (Figures 3 and 4). Snyderols **3** and **4** obtained from TBCO reactions, as expected, showed the formation of equal amounts of the two diastereomers.[20,31] The diastereomers of α-snyderol (**2**) could not be separated by chiral GC using the conditions employed for **3** and **4** nor under any of the other conditions investigated. The fact that single diastereomers of **3** and **4** are obtained in the V-BrPO reactions implies that

Scheme 3



bromination of the terminal olefin of **1** occurs within the active-site cavity, and not outside the enzyme active site via a freely diffusible brominating species (e.g., HOBr, Br_2, Br_3^-, etc). Thus, we propose that **1** orients in a favorable conformation within the active site suitable for asymmetric bromination at the C10−C11 double bond, possibly assisted by some protein interaction with the allylic alcohol moiety of **1**. The ability of V-BrPO to specifically bind and orient substrates has been reported and has resulted in the regioselective brominative oxidation of substituted indoles,[53] as well as the non-halide-dependent enantiospecific oxidation of sulfides.[54−56]

The diastereoselective formation of γ-snyderol (**4**) is striking when one considers that this is the first report of an enzymatic bromonium-ion-induced cyclization forming a tetra-substituted olefin. V-BrPO-catalyzed formation of **4** supports previous proposals of an enzyme-catalyzed cyclization of farnesyl pyrophosphate to 10-bromo-α-chamigrene via a tetra-substituted monobromocyclofarnesyl pyrophosphate intermediate[1,28,57] (Scheme 3). Cyclization of the brominated monocyclofarnesyl pyrophosphate is assumed to proceed through an allylic cation. Previously, Wolinksky and Faulkner demonstrated in a biomimetic synthesis of 10-bromo-α-chamigrene the conversion of racemic **4** to the chamigrene skeleton.[17] 10-Bromo-α-chamigrene was not detected in our biosynthetic studies with V-BrPO; however, reactions with (*E*)-farnesyl pyrophosphate are under investigation.

V-BrPO-Catalyzed Biosynthesis of (+)-3β-Bromo-8-epicaparrapi Oxide. The marine natural product (+)-3β-bromo-8-epicaparrapi oxide (**5**), and its corresponding diastereomer (**5′**), was obtained from the bromoether-containing fractions of the V-BrPO-catalyzed reaction with **1**. As was observed with the snyderol products, **5** and **5′** are produced only when all substrate components of the enzyme reaction are present (i.e., V-BrPO, KBr, H_2O_2, 40% cosolvent). The presence of the C-8 epimer of **5** and **5′** is not observed, indicating that epimerization of the allylic alcohol during the reaction does not occur. The optical rotation measurements of **5**, (i.e., $[\alpha]_D$ + 31.8; c = 1.52 in EtOH) isolated from the V-BrPO-catalyzed reaction are similar

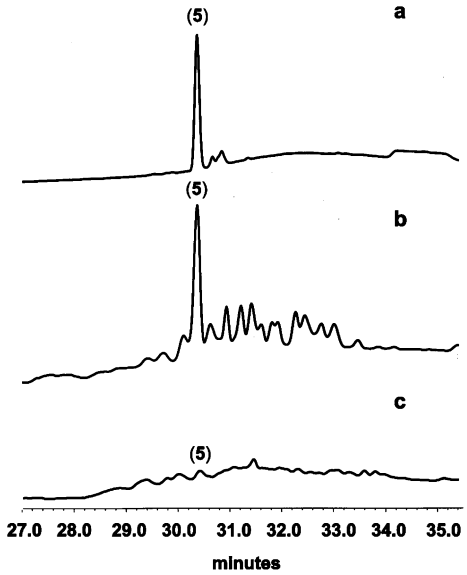


Figure 5. Reversed-phase HPLC separation of bromoether-containing fractions from the reactions of V-BrPO and aqueous bromine with **1**. (a) Authentic sample of (+)-3β-bromo-8-epicaparrapi oxide (**5**) and **5′** (i.e., synthesized using TBCO[20,31]). (b) V-BrPO-catalyzed reaction. (c) Aqueous bromine reaction. Peaks are denoted with numbers corresponding to their structures. The reaction mixture contained 0.04 M KBr and 0.5 mM **1** in 0.1 M sodium phosphate (pH 5.7) with 40% v/v ethanol. Enzymatic reactions were initiated by the addition of H_2O_2 (1 mM) and allowed to react at 24 °C for 60 min. Aqueous bromine reactions were initiated by addition of NaOBr (1mM) and reacted at 24 °C for 60 min.

to those of the natural product isolated from *L. obtusa*, (i.e., $[\alpha]_D$ + 30.4; c = 1.46 in EtOH).[51]

The V-BrPO-catalyzed formation of **5** and **5′** is proposed to occur via bromination of the terminal olefin of **1** followed by stereospecific cyclization to ring closure (Scheme 2). Nucleophilic trapping of the proposed bromocarbenium ion by the allylic alcohol leading to **5** and **5′** is supported by cationic cyclization reactions of terpenes previously reported and used to study the concertedness of the overall ring-formation process.[26,27,58,59]

Reaction of **1** with aqueous bromine (1 or 2 equiv) produces only trace quantities of **5** and **5′** (Figure 5). TBCO reactions with **1** produces **5** and **5′** as previously described.[20] In addition, five- and six-membered cyclic bromoether species (i.e., 25%) were also present within the bromoether-containing fractions. These brominated cyclic ethers have been previously identified;[20] the specificity of bromination of these products is under investigation. (+)-3β-Bromo-8-epicaparrapi oxide (**5**) was isolated in ∼5% yield, and the remaining cyclic bromoether compounds were produced in 25% isolated yield.

The stereochemistry of **5** at the bromine bearing carbon was determined by the presence of H_{ax} signal as a doublet of doublets at δ 3.99 (J = 12.5 Hz). The stereochemistry of the bridgehead methyl was determined by the presence of a nOe signal to the vinylic proton at 6.0 ppm, thus indicating that the cyclization reaction produces a *trans*-fused bicyclo[4.4.0] system. The stereochemistry of **5** is consistent with that previously reported in cyclization reactions where the four chiral centers of **5** are introduced stereospecifically following bromination of **1**, in

(53) Martinez, J. S.; Carroll, G. L.; Tschirret-Guth, R. A.; Altenhoff, G.; Little, R. D.; Butler, A. *J. Am. Chem. Soc.* **2001**, *123*, 3289−3294.
(54) Andersson, M.; Willetts, A.; Allenmark, S. *J. Org. Chem.* **1997**, *62*, 2, 8455−8458.
(55) ten Brink, H. B.; Tuynman, A.; Dekker, H.; Hemrika, W.; Izumi, Y.; Oshiro, T.; Schoemaker, H. E.; Wever, R. *Inorg. Chem.* **1998**, *37*, 6780−6784.
(56) Andersson, M.; Allenmark, S. *Tetrahedron* **1998**, *54*, 15293−15304.
(57) Martin, J. D.; Palazon, J. M.; Perez, C.; Ravelo, J. L. *Pure Appl. Chem.* **1986**, *58*, 395−406.
(58) Garst, M.; Cheung, Y.; Johnson, W. *J. Am. Chem. Soc.* **1979**, *101*, 4404−4406.
(59) Snowden, R.; Eichenberger, J.; Linder, S.; Sonnay, P.; Vial, C.; Schulte-Elte, K. *J. Org. Chem.* **1992**, *57*, 955−960.

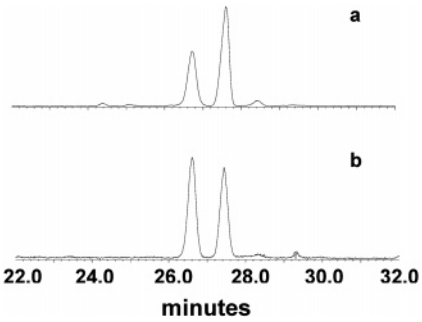
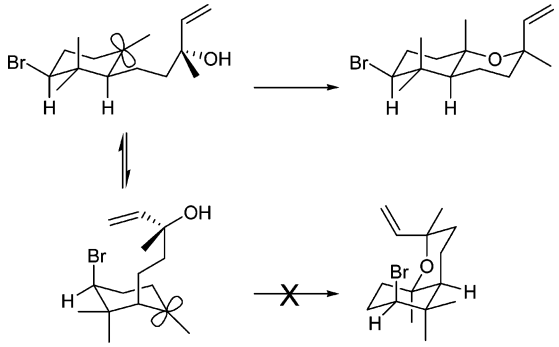


Figure 6. Chiral GC−MS chromatogram for **5** and **5′** isolated from the (a) V-BrPO-catalyzed reaction and the (b) TBCO reactions with **1**. The retention time of **5′** and **5** isolated from V-BrPO reactions was 26.8 and 27.6 min, respectively. Diastereomers isolated from TBCO reactions eluted with retention times of 26.8 and 27.6 min, respectively. Gas chromatography was performed using 90 min, 165 °C isotherm runs.

Scheme 4



which the set configuration of the carbon alcohol (C-3) bond uniquely determines the remaining three stereocenters.[20,21,25] The biosynthesis of the *trans*-fused ring system with exclusion of the *cis*-fused ring system can be rationalized by assuming the planar bromocarbenium ion is quenched by internal nucleophilic trapping on the empty *p*-orbital by the allylic alcohol in an equatorial position (Scheme 4). Formation of the *cis*-fused ring system is hindered by unfavorable isomerization of the bromine from the equatorial to the axial position leading to 1,3-diaxial interactions.[18] V-BrPO-catalyzed bromination and cyclization of **1** to **5** is considered consistent with the Stork−Eschenmoser hypothesis for a synchronous cyclization mechanism initiated by bromination at the C10−C11 olefin of **1**.[60,61]

V-BrPO catalyzed the asymmetric bromination of the terminal olefin of **1** followed by successive cyclization to produce (+)-3β-bromo-8-eqicaparrapi oxide (**5**) as a mixture of diastereomers (de = 20−25%), whereas equal amounts of the two diastereomers are observed for the corresponding TBCO reaction[20] (Figure 6). In an analogous reaction, enzymes such as terpene cyclases control the stereospecific cyclization of isoprenoids by the asymmetric protonation of the terminal olefin, followed by successive cyclizations and stabilization of intermediates and conformations within enzyme cavities lined with hydrophobic residues.[62−65] These results suggest a "terpene cyclase"-like cyclization along the hydrophobic substrate channel in V-BrPO. Cyclization is initiated by the asymmetric bromination of the terminal olefin to generate a bromonium-ion adduct of nerolidol on the terminal olefin which controls the stereoselectivity of the cyclization reaction.

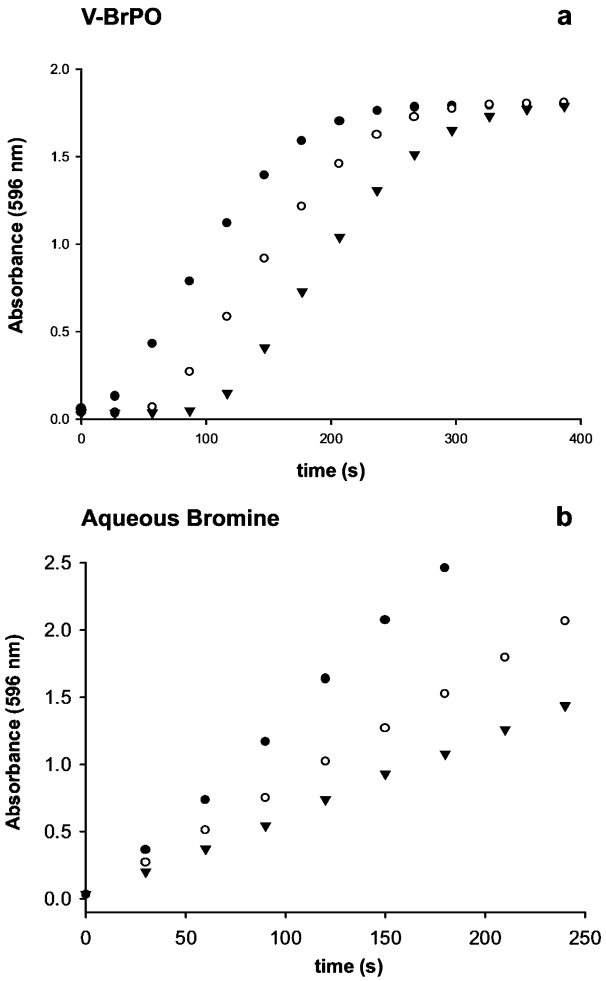


Figure 7. Time course for the bromination of phenol red as a function of the (*E*)-(+)-nerolidol (**1**) concentration (a) catalyzed by V-BrPO or (b) by reaction with aqueous bromine. The reactions were performed at 24 °C in the presence of 50 μM phenol red and 40 mM KBr in 0.1 M sodium phosphate buffer (pH 5.7) with 40% v/v ethanol. V-BrPO reactions were initiated by addition of 0.5 mM H_2O_2 and 5 nM V-BrPO. Aqueous bromine reactions were initiated by addition of NaOBr (stock solution 5 mM in 0.02 M NaOH) in 10 μL aliquots at 30 s intervals. Production of bromophenol blue was monitored at 596 nm. Concentration of **1**: ●, 0 μM; ○, 50 μM; ▼100 μM.

Competition Kinetics for the Bromination of (*E*)-(+)-Nerolidol (1) versus Phenol Red. Vanadium bromoperoxidase preferentially catalyzes the bromination of **1** when presented with a mixture of **1** and phenol red as organic substrates (Figure 7). The preferential bromination of **1** is concentration dependent as indicated by the increase in the lag phase for the bromination of phenol red as the concentration of **1** is increased (Figure 7a). Following the consumption of **1**, the rate of bromination of phenol red occurs at the same rate as in the absence of added **1**. Furthermore, a concentration-dependent lag phase is absent in reactions with aqueous bromine under the same conditions as V-BrPO, indicating that **1** and phenol red are brominated simultaneously (Figure 7b). The difference in reactivity of **1** in the V-BrPO/H_2O_2/Br^- system versus reaction with aqueous

(60) Stork, G.; Burgstahler, A. W. *J. Am. Chem. Soc.* **1955**, *77*, 5068−5077.
(61) Eschenmoser, A.; Ruzicka, L.; Jeger, O.; Arigoni, D. *Helv. Chim. Acta* **1955**, *38*, 1890−1904.
(62) Croteau, R.; Cane, D. E. *Methods Enzymol.* **1985**, *110*, 383−405.
(63) Cane, D. E. *Chem. Rev.* **1990**, *90*, 1089−1103.
(64) Dougherty, D. A. *Science* **1996**, *271*, 163−168.
(65) Davis, E. M.; Croteau, R. *Top. Curr. Chem.* **2000**, *209*, 53−95.

bromine, is consistent with an enzyme-trapped or enzyme-bound brominating species, and not a freely diffusible brominating species.[53,66]

Conclusions

Vanadium bromoperoxidase catalyzes the asymmetric bromination and cyclization of (*E*)-(+)-nerolidol (**1**) to produce single diastereomers of the marine natural products β- and γ-snyderol (**3** and **4**) and a mixture of diastereomers of (+)-3β-bromo-8-epicaparrapi oxide (**5**). In contrast, reaction of **1** with aqueous bromine produced only minimal quantities of these brominated cyclized products, and reaction of **1** with TBCO in nitromethane produced an equal mixture of each diastereomeric product of **3**, **4**, and **5**. Diastereomers of **2** could not be resolved by chiral GC. The observed diastereoselectivity is the first report of V-BrPO-catalyzed enantiospecific bromination and cyclization of sesquiterpenes, forming chiral brominated marine natural products, and establishes a role for V-BrPO in the biogenesis of halogenated metabolites in marine algae. The high specificity of these V-BrPO-catalyzed reactions suggests that **1** docks within the active-site channel of V-BrPO in a specific orientation and is not randomly binding within the active-site cavity. In the case of random substrate binding, one would expect symmetric bromination of the C10−C11 olefinic bond of **1**, leading to equal distribution of both diastereomers of the different natural products. V-BrPO functions by coordination of H_2O_2 followed by oxidation of Br^- to produce the equivalent of a bromonium ion (Br^+) that attacks one face of the terminal olefin of **1**. The nature of the brominating species as enzyme bound (e.g., V−OBr) or enzyme trapped (e.g., Br^+, HOBr, OBr^-, etc.) is not known and cannot be addressed by these experiments, although the competitive kinetic data shows that the brominating species is not consistent with release from the active site.

The X-ray structure of V-BrPO from *C. officinalis* shows that the vanadium site resides at the bottom of a 20-Å deep substrate channel.[39] Hydrophobic patches of residues dominate the substrate channel leading to the vanadium site. With the exception of the amino acids involved in hydrogen bonding to the vanadate ion, only three other hydrophilic residues (Glu124, Arg395, Asp292) are positioned within 7.5 Å of the vanadate oxygen atoms. Thus, the hydrophobic surface of the substrate cavity, as well as certain charged residues, likely provides the environment necessary for docking and bromination of **1**.

In early studies of V-BrPO, the apparent lack of selectivity led to the suggestion that V-BrPO produced a diffusible oxidized halogen intermediate such as the equilibrium mixture of HOBr = OBr^- = Br_2 = Br_3^- that would carry out a molecular bromination reaction outside the enzyme active-site.[67−69] Subsequent kinetic studies showing sequential bromination or brominative oxidation of different substrates,[66] as well as different selectivity,[53,70] suggested that some substrates bind to the V-BrPO active site and block the release of the oxidized halogen intermediate. The discovery of direct asymmetric oxidation of bicyclic sulfides catalyzed by V-BrPO, producing the corresponding sulfoxides with high enantioselectivity,[54,56] bolstered the hypothesis that V-BrPO can participate in asymmetric halogenation reactions. Finally, the bromination and cyclization of model terpene precursors[45] demonstrated that V-BrPO can catalyze the bromination and cyclization of terpenes, producing cyclic structures that make up parts of halogenated marine natural products. Now, as we demonstrated above, V-BrPO has been shown to catalyze the asymmetric bromination and cyclization of (*E*)-(+)-nerolidol (**1**), forming known marine natural products. While compounds **2**, **3**, and **5** are all natural products that have been isolated from *Laurencia obtusa*,[50,51,71] these compounds have each also been isolated singly from other species of *Laurencia* (i.e., *L. intricata*, *L. synderiae*), and the product distribution of these compounds within different algae is not yet understood. Additional experiments are under way to further understand the interactions between (*E*)-(+)-nerolidol (**1**) and V-BrPO that promote the cyclization process, both for sesquiterpenes as reported here and also for longer-chain terpenes. Other cyclic brominated terpenes are also metabolites produced by marine red algae that likely contain haloperoxidases with different substrate reactivity or substrate selectivity or that work in concert with other enzymes such as terpene cyclases, etc. Thus, with the discovery of the asymmetric halogenation catalyzed by V-BrPO, many interesting questions can now be investigated.

Acknowledgment. We are particularly thankful to Prof. R. Daniel Little (UCSB) for thoughtful discussions on the project.We are grateful for support from NSF CHE 0213523 (A.B.), California Sea Grant NA06RG0142, Project R/MP-94 (A.B.), and a Sea Grant Traineeship for J.C.F. funded by a grant from the National Sea Grant College Program, National Oceanic and Atmospheric Administration, and the U.S. Department of Commerce.

JA047925P

(66) Tschirret-Guth, R. A.; Butler, A. *J. Am. Chem. Soc.* **1994**, *116*, 411−412.
(67) Itoh, N.; Izumi, Y.; Yamada, H. *J. Biol. Chem.* **1987**, *262*, 11982−11987.
(68) de Boer, E.; Wever, R. *J. Biol. Chem.* **1988**, *263*, 12326−12332.
(69) Itoh, N.; Hasan, A.; Izumi, Y.; Yamada, H. *Eur. J. Biochem.* **1988**, *172*, 477−484.
(70) Butler, A.; Tschirret-Guth, R. A. In *Mechanisms of Biohalogenation and Dehalogenation*; Janssen, D. B., Soda, K., Wever, R., Eds.; Royal Netherlands Academy of Arts and Sciences: Amsterdam, The Netherlands, 1996; pp 55−68.
(71) Topcu, G.; Zeynep, A.; Imre, S.; Goren, A. C.; Pezzuto, J. M.; Clement, J. A.; Kingston, D. G. *J. Nat. Prod.* **2003**, *66*, 1505−1508.