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On the reactivity of bromoperoxidase I (*Ascophyllum nodosum*) in buffered organic media: Formation of carbon bromine bonds*,**

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Abstract: Peroxidase (PO) activity of vanadate(V)-dependent bromoperoxidase (BPO) I (*Ascophyllum nodosum*) [V_{Br}PO(*An*I)] was retained with a half-life time of ~60 days, if stored in H_2O_2-incubated, morpholin-4-ethane sulfonic acid (MES)-buffered aqueous alcoholic solutions. These conditions were applied for converting bromide and, e.g., methyl pyrrole-2-carboxylate into bromopyrroles with an almost quantitative peroxide yield. δ,ε-unsaturated alcohols furnished β-bromohydrins and products of bromocyclization, i.e., tetrahydrofurans and tetrahydropyrans (70–84 % mass balance), if treated with H_2O_2, KBr, and V_{Br}PO(*An*I) in phosphate-buffered, CH_3CN-diluted media.

Keywords: haloperoxidase; vanadium; oxidation catalysis; arene bromination; peroxide chemistry; bromocyclization; bromohydrin; functional haloperoxidase model.

INTRODUCTION

Oxidation of bromide, either from mineralized deposits or from ocean water (c_{Br^-} ~ 0.9 mM) poses an important step in the natural bromine cycle. While formation of molecular bromine dominates in the marine boundary layer, hypobromous acid (HOBr) is preferentially formed in aqueous environments [1–3]. Both reagents are able to bromofunctionalize π-nucleophiles (e.g., olefins or arenes) [4–6] and thus provide adequate reactivity for transforming a large variety of metabolites into naturally occurring organobromine compounds (Fig. 1) [7–9].

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**Dedicated to Prof. Dr. Klaus Hafner in recognition of his contribution to the chemistry of nonbenzenoid aromatic and antiaromatic compounds.

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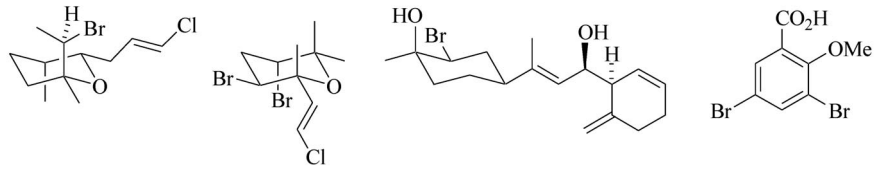


kumepaloxane — *Haminoea cymbalum*; aplysiapyranoid A — *Aplysia kurodai*; rogioldiol B — *Laurencia microcladia*; *Didiscus sp.*

Fig. 1 Examples of acetogenine-, terpene-, and shikimate-derived naturally occuring organobromine compounds [10–13].

Oxidation of bromide in solution generally is accomplished with peroxides. This type of reaction consumes aliquots of, e.g., H_2O_2 and H^+ per equivalent of oxidized Br^- (Scheme 1). It is an exothermic process that provides HOBr or Br_2/H_2O with notable rates under strongly acidic conditions [14]. The significance of acid-catalysis for peroxide activation gradually fades as the proton concentration decreases [15]. In pH-neutral media, the rate of bromide oxidation is negligibly small. Recent advances in peroxidase (PO) chemistry have provided evidence that notable time-yield factors for bromoelectrophile formation from Br^- at pH 6–7 should in principle be attainable using haloperoxidases, e.g. vanadium-dependent bromoperoxidase (BPO) I of the brown alga *Ascophyllum nodosum* [$V_{Br}PO(AnI)$, EC 1.11.1.10] (Fig. 2), as catalyst for H_2O_2 activation [16–21]. This enzyme has been reported to retain its catalytic activity in alcoholic media (EtOH, *i*PrOH, *i*BuOH), even at elevated temperatures [22]. It requires no additional cofactor, in order to catalyze the oxidation of Br^- with H_2O_2 at a rate that exceeds existing best nonenzymatic alternatives by 3–4 orders of magnitude [23–25].

• *aqueous media*

$$Br^- + H_2O_2 + H^+ \xrightarrow[-H_2O]{V_{Br}PO(AnI)\ pH\ 6.3} \underset{favored}{HOBr} \underset{-H^+/Br^-}{\overset{H^+/Br^-}{\rightleftharpoons}} Br_2 + H_2O$$

• *anhydrous non alcoholic media*

$$2\,Br^- + ROOH + H^+ \xrightarrow[-H_2O\ -ROH]{VOL(OEt)\ e.g.\ \mathbf{1}} \underset{favored}{Br_2} \underset{-Br^-}{\overset{Br^-}{\rightleftharpoons}} Br_3^-$$

reagent	HO**Br**	Br_2	Br_3^-
reactivity	hydroxybromination (Ad_E)	dibromination (Ad_E)	dibromination (Ad_E)
	hydrogen-bromine exchange (S_EAr)	hydrogen-bromine exchange (S_EAr)	leaving group displacement (S_N)

Scheme 1 Synopsis of reactivity associated with peroxide-mediated bromide oxidation in aqueous and nonaqueous environment (for depiction of vanadium compounds, refer to Fig. 2; e.g., R = *t*Bu).

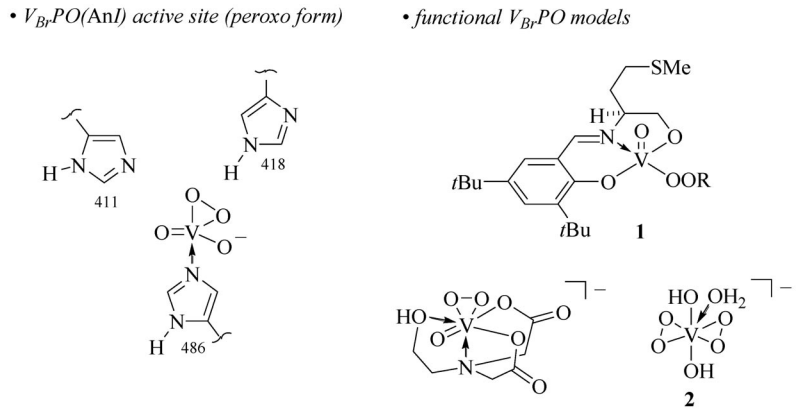


Fig. 2 Schematic drawing of vanadium active center in vanadium-dependent BPO I (*A. nodosum*) [$V_{Br}PO(AnI)$] (left; numbers refer to position of amino acids related to depicted side-chain residues) and structure formulas of functional $V_{Br}PO$ models (right) [23,28,30,31].

The active site in $V_{Br}PO(AnI)$ is composed of a histidine-bound vanadate(V) located in proximity to a number of peptide side chains, such as imidazole subunits from histidines 411 and 418, which are essential for acid/base catalysis in peroxide loading and bromide oxidation (Fig. 2). Additional binding of the vanadate cofactor to the apoenzyme occurs via hydrogen bridges (K_{Diss} = 35–55 nM at pH 8.5) [23,26]. The active site of $V_{Br}PO(AnI)$ is located at the bottom of a substrate funnel that is ~15 Å deep, ~12 Å wide at its entrance. It narrows to ~8 Å at the apical O^- substituent at vanadium, which is considered to be the location of one of the peroxide O-atoms in the H_2O_2-loaded enzyme (Fig. 2, left).

Kinetic and selectivity data of $V_{Br}PO(AnI)$-catalyzed bromide oxidation were interpreted in terms of HOBr formation in the initial step [23,24]. Bromide, which was identified in close proximity to the active site via K-edge extended X-ray absorption fine structure (EXAFS) [27], is expected to participate in equilibria between HOBr/Br^-, Br_2, and Br_3^- (Scheme 1). Acid equivalents required for converting HOBr and Br^- into Br_2 are available from protonated peptide side chains. Efforts directed toward understanding elementary steps in $V_{Br}PO(AnI)$-catalyzed bromide oxidation with H_2O_2 led to the development of several functional BPO models (e.g., Fig. 2, right) [21,28–32]. Most synthetic reagents, however, require low pH for effectively catalyzing bromide oxidation [28], which causes acid activation of the peroxide to significantly interfere with vanadium-based catalysis. The use of oxovanadium(V) inner complexes, such as **1**, as catalyst for, e.g., constructing the heterocyclic core of aplysiapyranoid A (for structure, see Fig. 1) in BPO model reactions, required alkyl hydroperoxides as terminal oxidants and anhydrous organic solvents (e.g., CH_2Cl_2, CH_3CN), in order to effectively occur [31,33].

Based on its potential in sustainable chemistry, we became interested in exploring the chemistry of $V_{Br}PO(AnI)$ in order to prepare building blocks relevant to naturally occurring organobromine compounds [34]. The present report summarizes major results of a study directed toward determining preferred season for collecting *A. nodosum* for most effectively isolating $V_{Br}PO(AnI)$ and the use of the enzyme in the synthesis of bromoarenes, bromohydrins, and bromocyclization products. The results were supplemented by reactivity and selectivity data from NH_4VO_3/H_2O_2-mediated bromide oxidation, i.e., transformations in aqueous solutions containing bis(peroxo)vanadate **2**, with the aim to gain deeper insight into the nature of bromoelectrophile(s) formed in $V_{Br}PO(AnI)$-catalyzed reactions.

BROMOPEROXIDASE ACTIVITY IN *A. NODOSUM*

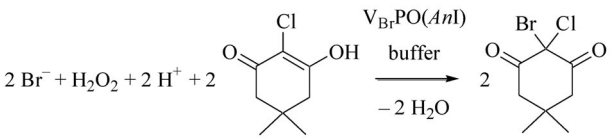
For covering the demand of BPOs, the question about a preferred season of *A. nodosum* harvest from a site selected for algal supply [Station Biologique Roscoff/Brittany (France)] and thus $V_{Br}PO(AnI)$ isolation was addressed in a survey lasting from April 2005 to March 2006. *A. nodosum* was collected, lyophylized, and homogenized to 1-μm particle size in a milling process performed below 30 °C in order to minimize PO-activity loss. The material was subjected to a liquid–liquid partitioning process [35], which was followed by dialysis vs. Tris-(hydroxymethyl)-aminomethane-buffered aqueous $NaVO_3$ solution for restoring catalytic activity of the mixture that consisted of isoenzymes $V_{Br}PO(AnI)$ and $V_{Br}PO(AnII)$. Combined PO activity showed seasonal variation by a factor of ~4. A maximum value of 190 ± 13 $U\ g^{-1}$ dry mass (dm) (triiodide assay, Scheme 2) was found for specimen collected in April 2005. In the following month, PO activity slightly decreased. This trend continued in June (52 ± 5 $U\ g^{-1}$ dm) and reached a level that prevailed until August 2005. In the succeeding months, a moderate increase in PO activity was noted, which was followed by a steep ascent in January 2006 (160 ± 11 $U\ g^{-1}$ dm) and a high activity phase that lasted until the end of the study in March 2006 (140 ± 12 $U\ g^{-1}$ dm). These data suggest preferentially collecting *A. nodosum* from midwinter to early spring for obtaining the most significant yields of vanadium-dependent BPOs from specimen growing in the intertidal region close to Roscoff, France.

• *Triiodide assay*

$$2\,I^- + H_2O_2 + 2\,H^+ \xrightarrow[-2\,H_2O]{V_{Br}PO(AnI),\ buffer} I_2 \underset{-I^-}{\overset{I^-}{\rightleftharpoons}} I_3^-$$

$\lambda_{max} = 350$ nm

• *Monochlorodimedone assay*



$\lambda_{max} = 290$ nm

Scheme 2 Stoichiometry associated with spectrophotometric assays for quantifying PO and BPO activity of $V_{Br}PO(AnI)$ (MCD = monochlorodimedone).

For continuing the project, BPO isoenzymes were separated by hydrophobic interaction chromatography on Phenyl Sepharose™ 6 FF and subsequent size exclusion chromatography on Sephacryl™ S-300 HR, furnish pure $V_{Br}PO(AnI)$. Its identity was verified via matrix-assisted laser desorption/ionization with time-of-flight (MALDI-TOF) mass analysis of trypsine-digested samples, and database comparison of peptide fragments with the known primary structure of the enzyme [23]. The $V_{Br}PO(AnI)$ preparation obtained from the isolation procedure outlined above showed an activity of 693 $U\ mg^{-1}$ (triiodide assay pH 6.2), which corresponded to 172 $U\ mg^{-1}$ in the monochlorodimedone (MCD) assay (pH 6.5) (Scheme 2).

BROMOPEROXIDASE STABILITY IN BUFFERED ORGANIC MEDIA

For economically applying $V_{Br}PO(AnI)$ in synthesis, buffers and additives were varied with the aim to identify conditions that were adequate for retaining PO activity in aqueous organic solutions. Time de-

pendency of PO activity [U in μmol min^{-1}, triiodide assay] was monitored in Tris-(hydroxymethyl)-aminomethane (Tris)-HCl- (pH 9.0), imidazole- (pH 7.0), phosphate- (pH 6.3), 4-(2-hydroxyethyl)-piperazin-1-ethane sulfonic acid (HEPES)- (pH 6.3), and morpholin-4-ethane sulfonic acid (MES)-buffered solutions (pH 6.2). Control experiments indicated that changes in titer of stock solutions used for the assay (200 mM of KI, 27 mM of H_2O_2) remained insignificant, if reagents were kept no longer than three days (Δc_{I^-} = –0.02 %, potentiometry; $\Delta c_{H_2O_2}$ = –0.02 %, manganometry). PO activity decreased upon enzyme storage in all selected buffers. A more precise data analysis was attainable by fitting time dependency of PO activity decay with the first-order relationship $\ln(U/U_0) = kt$, where U denotes PO activity at the time t, U_0 the initial PO activity, and k a reaction constant, thus leading to correlation coefficients between 0.99 and 0.85. Likewise approximated PO activity half-life times increased along the series of buffers phosphate < MES < Tris-HCl. Addition of H_2O_2 (~1 μM) to $V_{Br}PO(AnI)$ stock solution led to a marked extension of PO activity for MES-buffered solutions, showing an extrapolated half-life time of ~60 days. The addition of NaCl (50 μM) similarly extended PO activity beyond values obtained for the enzyme in the absence of the salt. The latter effect, however, was less pronounced than the one exerted by H_2O_2. PO activity half-life time in imidazole- and HEPES-buffered solutions fell short of values obtained for MES-buffered, H_2O_2-preincubated $V_{Br}PO(AnI)$ solutions. The data were therefore not included in Table 1.

Table 1 Monitoring PO activity of $V_{Br}PO(AnI)$ (triiodide assay) in buffered solutions.[a]

		$t_{1/2}$/days[c]		
Entry	Buffer (pH)[b]	Standard[d]	H_2O_2-incubated[e]	NaCl-incubated[f]
1	Tris-HCl (9.00)	120	2.0	10
2	Phosphate (6.27)	0.2	13	0.4
3	MES (6.22)	8.8	60	24

[a]~9 μM stock solution of $V_{Br}PO(AnI)$ (M = 120 514 Da) in Tris buffer (50 mM); triiodide assay performed at 20.0 ± 0.5 °C.
[b]1 ml of buffer volume; Tris = tris-(hydroxymethyl)-aminomethane; phosphate = K_2HPO_4/citric acid monohydrate/HCl; MES = morpholin-4-ethane sulfonic acid.
[c]Calculated from a linear correlation of $\ln(U/U_o)$ vs. storage time at 4 °C; half-life times above 15 days were extrapolated and are considered reliable within a relative precision of ±10 %.
[d]Addition of H_2O_2 and I^- at the onset of spectrophotometric PO activity measurement.
[e]Storage of $V_{Br}PO(AnI)$ in the given buffer and 1 μM H_2O_2 for at least 5 min prior to I^- addition and UV-spectrophotometric monitoring of PO activity.
[f]Storage of $V_{Br}PO(AnI)$ in the given buffer and 50 μM of NaCl for 6 days prior to I^- addition and UV-spectrophotometric monitoring of PO activity.

The addition of *i*PrOH, *t*BuOH, DMSO, CH_3CN, acetone, or dimethyl carbonate led to a gradual $V_{Br}PO(AnI)$ activity loss as the volume percentage of the organic cosolvent increased (not shown). The least significant effects were noted for H_2O_2-preincubated, MES-buffered solutions. The addition of *i*PrOH, CH_3CN, or 1,4-dioxane to the latter mixture furnished solutions with extrapolated PO activity half-life times of more than 30 days, even as the degree of cosolvent reached a level of 50 % (*v*/*v*) (Table 2). These parameters finally were considered satisfactory for exploring $V_{Br}PO(AnI)$ reactivity under turnover conditions in the second part of the project.

Table 2 Monitoring $V_{Br}PO(AnI)$ activity (triiodide assay) in buffered solutions.[a]

Entry	Cosolvent	Buffer[b]	$t_{1/2}$/days[c]	
			25 % (*v*/*v*) of cosolvent	50 % (*v*/*v*) of cosolvent
1	*i*PrOH	Tris-HCl	13	9.7
2	*i*PrOH	Phosphate	–[d]	0.1
3	*i*PrOH	MES	84	46
4	CH_3CN	MES	80	31
5	1,4-dioxane	MES	42	31

[a]$V_{Br}PO(AnI)$ (M = 120 514 Da) stock solution (~9 μM) in Tris buffer incubated with H_2O_2 (~1 μM).
[b]1 ml of buffer volume; for pH, see Table 1.
[c]Calculated from the slope of a linear correlation of $\ln(U/U_o)$ vs. time; half-life times above 15 days were extrapolated and are considered reliable within a relative precision of ±10 %.
[d]Not determined.

USE OF BROMOPEROXIDASE I AND FUNCTIONAL MODELS IN ARENE BROMINATION

The quest for efficiency of $V_{Br}PO(AnI)$-catalyzed bromide oxidation in combination with organic substrate bromination was pursued by exploring effects of temperature, nature, and amount of organic cosolvent, reaction time, H_2O_2 concentration, substrate/enzyme ratio, and substrate/peroxide ratio. Bromination of substituted (hetero)arenes was selected as reporter reaction for referencing selectivity data obtained from $V_{Br}PO(AnI)$-catalyzed reactions to those of known processes (Scheme 3).

$$\mathrm{Ar{-}H} \xrightarrow[\textit{V-catalyst}]{\mathrm{H^+/\,Br^-/\,H_2O_2}} \mathrm{Ar{-}Br} + 2\,\mathrm{H_2O}$$

Scheme 3 Stoichiometry for arene bromination in vanadium-catalyzed oxidations [V-catalyst = $V_{Br}PO(AnI)$ and functional enzyme models].

In the absence of $V_{Br}PO(AnI)$, methyl pyrrole-2-carboxylate (**3**) was recovered in 95 % yield, if stirred for 5.5 h at 22 °C in MES-buffered solutions containing 25 % (*v*/*v*) of *t*BuOH, 1 equiv of H_2O_2, and 3 equiv of NaBr (not shown). *t*BuOH was selected as a low-cost nontoxic solvent that is comparably inert toward peroxides. It is miscible with H_2O at any ratio. A high concentration of, e.g., alkali bromides, however, induces phase separation. The degree of phase separation of a 2/1-mixture of *t*BuOH/H_2O, for example, gradually increased as the KBr concentration of the aqueous phase was raised from ~1.0 M (homogeneous solution) to 4.5 M (biphasic system; Fig. 3). In between (1.4 M < c^{aq}_{KBr} < 2.5 M) partial phase separation was noted.

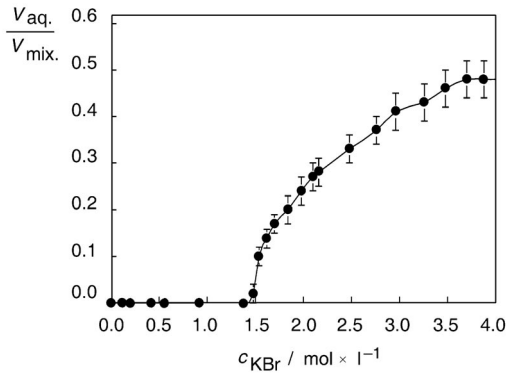
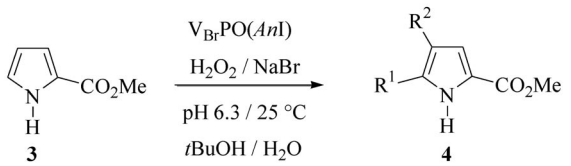


Fig. 3 Quantifying phase separation of *t*BuOH/H_2O [67/33 (*v*/*v*)] mixtures upon addition of KBr (20 °C). c_{KBr} refers to applied aqueous solution prior to *t*BuOH addition.

In the presence of $V_{Br}PO(AnI)$, a significant reaction between methyl pyrrole-2-carboxylate (**3**), H_2O_2, and NaBr occurred (MES-buffered solution). The degree of conversion in the temperature range of 25–60 °C using 50 % (*v*/*v*) of cosolvents *i*PrOH, CH_3CN, or 1,4-dioxane, however, remained far from quantitative (gas chromatography, GC), if 2 equiv of H_2O_2 were added right from the start of the reaction. Similar observations were made if 2-isopropyl-5-methylphenol (thymol) served as substrate. With substrate **3**, slow addition of 1 equiv of H_2O_2 over a period of ~3 h, an increase of $V_{Br}PO(AnI)$ concentration to 5×10^{-4} mol % (2.5 *U*), and a change of organic cosolvent to *t*BuOH finally led to a quantitative substrate conversion within 5.5 h. The reaction afforded a 13/87-mixture of brominated pyrroles **4a**–**b** (68 %) in addition to 9 % of dibromide **4c** (Scheme 4). The peroxide yield of the reaction was 86 %. A final check of the reaction mixture indicated that PO activity of $V_{Br}PO(AnI)$ had retained and pH had increased to 8.

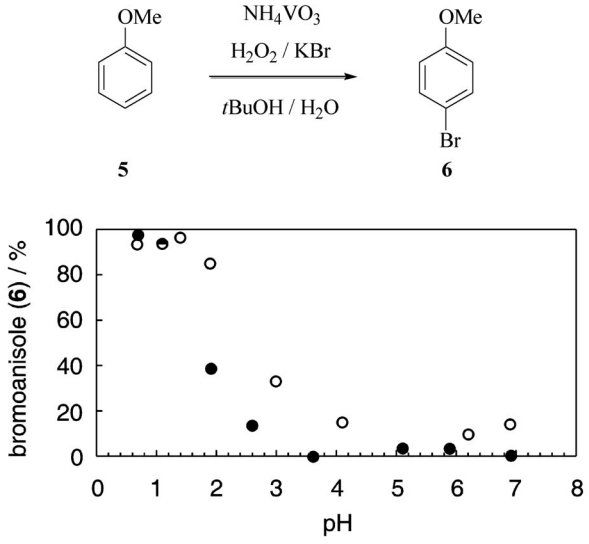


3 **4**

4	R^1	R^2	yield / %
a	Br	H	9
b	H	Br	59
c	Br	Br	9

Scheme 4 Bromination of methyl pyrrole-2-carboxylate (**3**) in MES-buffered solutions [2.5 *U* of $V_{Br}PO(AnI)$ (triiodide assay); yields determined via GC].

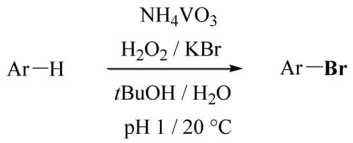
For classifying the nature of in situ-generated bromination reagent(s) in $V_{Br}PO(AnI)$-catalyzed oxidations under the selected conditions, aromatic substrates were treated with the reagent combination of NH_4VO_3, H_2O_2, and KBr in *t*BuOH/H_2O. pH and vanadium concentration were varied while adhering to the homogeneous *t*BuOH/H_2O system (Scheme 5) [32]. Based on results from supplementary studies (not shown), 2 equiv of H_2O_2 were added as terminal oxidant and 8.7 equiv of KBr as bromide source (c_0^{KBr} = 0.9 M) [33]. Bromination of anisole **5** under such conditions occurred *para*-selectively to provide exclusively (GC) the product of monobromination (Scheme 5). This information agreed with selectivity of the familiar Br_2/HOAc-mediated bromination of substrate **5**, which proceeds under kinetic

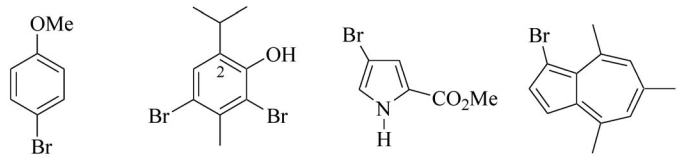


Scheme 5 pH dependency of bromoanisole formation from NH_4VO_3/H_2O_2/KBr-mixtures (c_0^{KBr} = 0.9 M) in *t*BuOH/H_2O (2/1) (○, 0.54 equiv of NH_4VO_3; ●, 0.25 equiv of NH_4VO_3). Reaction parameter: 2.0 equiv of H_2O_2, 8.7 equiv of KBr, 25 °C, 1 h reaction time. pH refers to the aqueous phase prior to *t*BuOH addition.

control [34,35]. Yields of bromoanisole **6** in vanadium-catalyzed reactions were strongly dependent on acid concentration (Scheme 5). Maximum reactivity was found, if pH of the aqueous phase was adjusted with $HClO_4$ to 1 prior to *t*BuOH addition. Minimum yield of bromoanisole **6** was detected as pH of the aqueous phase set to 5 (Scheme 5). Data from supplementary ^{51}V NMR investigations [36,37] indicated that NH_4VO_3 was almost instantaneously converted into bisperoxocomplex **2** [–688 ppm in H_2O; –679 ppm in *t*BuOH/H_2O = 2/1 (*v*/*v*); referenced vs. $VOCl_3$ as external standard] at pH 1 (adjusted with $HClO_4$). The conversion of NH_4VO_3 into peroxoide **2** at pH 5.3 was slow. It remained far from quantitative, even after 15 min of reaction time [37]. In the absence of vanadate, 9 % of bromoanisole **6** was obtained from substrate **5**, H_2O_2, and KBr in *t*BuOH/H_2O (pH 1) after a reaction time of 3 h. This information pointed to low but notable background reactivity from acid-catalyzed peroxide activation in this type of V_{Br}PO-model reaction.

The parameters deduced for anisole bromination were adapted for bromofunctionalization of selected aromatic compounds (Scheme 6). Thymol afforded only products of electrophilic aromatic substitution (^1H NMR). The fact that 4,6-dibromothymol was formed as a major product pointed to consumption of more than 1 equiv of H_2O_2 in this instance, leading to a peroxide yield of 78 %. Methyl pyrrole-2-carboxylate provided 73 % of monobrominated derivative **4b**, i.e., the major product obtained from the V_{Br}PO(*An*I)-catalyzed reaction (see Scheme 4). Bromination of 4,6,8-trimethylazulene (purple) was evident from a change in color toward blue [38]. Thin-layer chromatography (TLC) analysis and NMR spectra pointed to formation of 1- and 1,3-dibromo-4,6,8-trimethylazulenes. Attempts to separate bromination products via chromatography or sublimation failed due to an inherent lability of these compounds. Treatment of 4,6,8-trimethylazulene with *N*-bromosuccinimide (NBS) in a separate experiment furnished the same labile mixture of brominated 4,6,8-trimethylazulenes in addition to unreacted substrate [42].



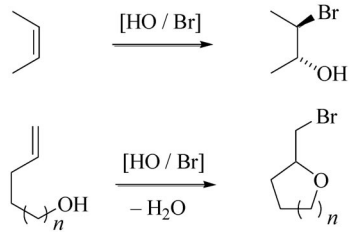


6a (98 %) [a] 74 % (+ 8 %) [b] **4b** (73 %) n.d. [c]

Scheme 6 Yields of bromoarenes formed from NH_4VO_3/H_2O_2/KBr-mixtures. Reagents and conditions: NH_4VO_3 (0.25 equiv), H_2O_2 (2.0 equiv), KBr (8.7 equiv, c_0 = 0.9 M), pH 1, *t*BuOH/H_2O [67/33 (*v*/*v*)], 3–18 h. [a]*ortho*:*para* < 1:99. [b]Figure in parentheses refers to yield of 4-bromothymol. [c]Not determined (see text).

VICINAL BROMOHYDROXYLATION AND BROMOCYCLIZATION OF ALKENOLS

The pursuit of bromohydrin synthesis and formation of bromocyclization products (Scheme 7) in V_{Br}PO(*An*I)-catalyzed reactions [39,40] required the availability of guidelines for reliably distinguishing positions of C,O and C,Br bond formation. This issue was addressed in a NMR-spectroscopic investigation prior to conducting enzyme-catalyzed reactions.



Scheme 7 Basic structural units associated with hydroxybromination of olefins and intramolecular bromoetherification of alkenols.

Synthesis of vicinal bromohydrins was accomplished by treatment of olefins or alkenols with a ~0.2–0.3 M bromide-free HOBr solution [1] in *t*BuOH/H_2O (2/1). Yields of likewise prepared compounds ranged between 16 and 41 % [2]. Slightly higher yields (22–47 %) were obtained, using suspensions of NBS in H_2O. Control experiments indicated that the selected work-up procedure accounted to some extent for the low yields of bromohydrins. In a recovery experiment using an equimolar mixture of bromohydrins **7a** and **7b** (Fig. 4), 57 % of a 33/67-mixture of **7a/7b** was obtained after chromatography (SiO_2, petroleum ether/Et_2O = 1/3 (*v*/*v*)]. Subsequent elution of the SiO_2 column with CH_3OH afforded an additional 17 % of isomer **7a**. The latter step had for obvious reasons not been performed in case of compound purification from reaction mixtures of bromohydrin syntheses.

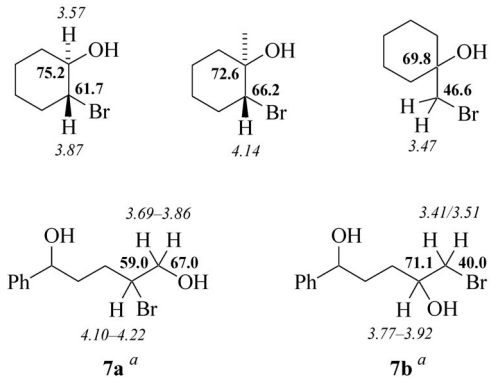


Fig. 4 NMR shift values ($CDCl_3$, 20 °C) for assignment of bromine and hydroxyl group positioning in β-bromohydrins [7–10,41,46]. Figures in italics refer to ^{1}H NMR chemical shifts ($CDCl_3$, 20 °C), bold numbers (except compound numbering) to ^{13}C NMR shift values ($CDCl_3$, 20 °C). [a]50/50-Mixture of diastereomers.

According to ^{13}C NMR investigations (20 °C, $CDCl_3$), resonances of hydroxyl-substituted carbons were consistently lowfield-shifted, if compared to bromine carbon bonds in similar chemical environment. In ^{1}H NMR spectra (20 °C, $CDCl_3$), protons attached to primary hydroxyl-substituted C-atoms were stronger deshielded than those attached to CH_2Br groups. For CHX entities (X = OH, Br), the situation was reversed (Figs. 4 and 5).

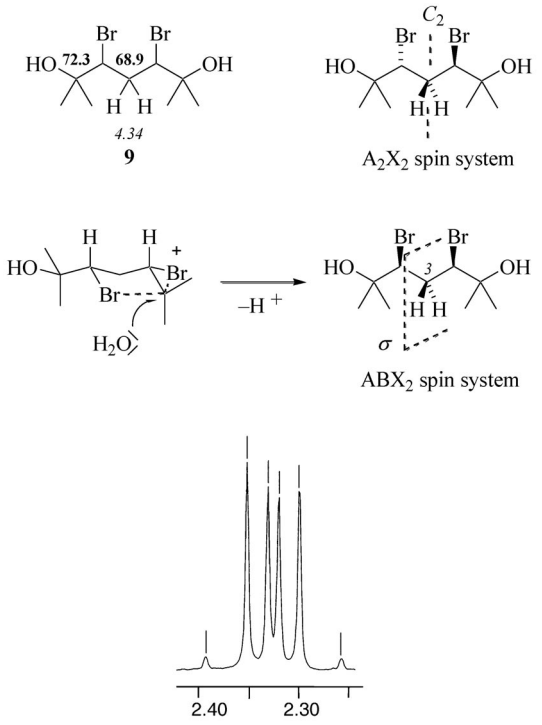
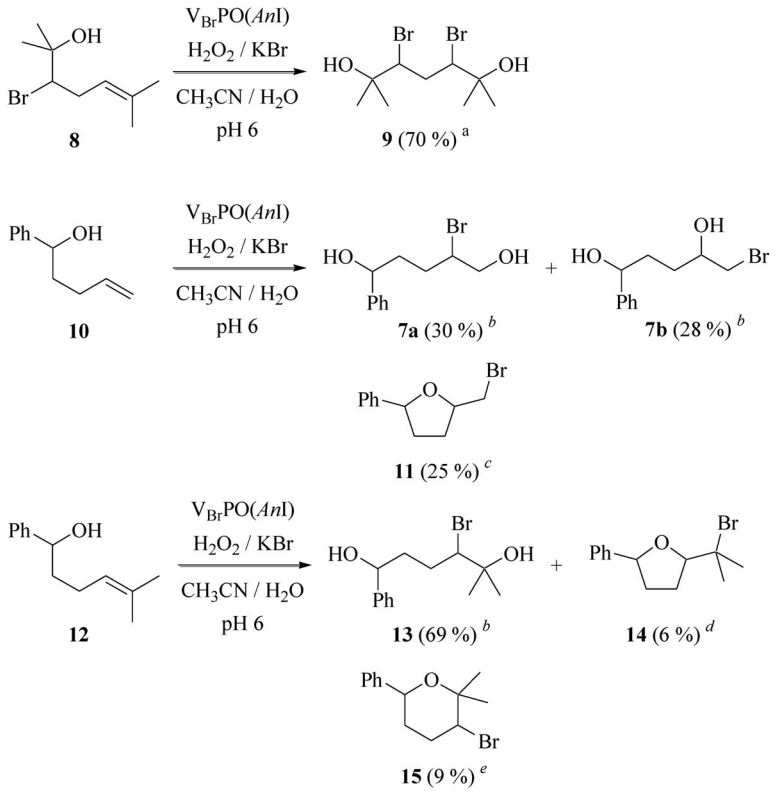


Fig. 5 Symmetry and transition structure-related considerations for rationalizing selectivity in bromohydrin formation (top and center) and part of the ^{1}H NMR spectrum (600 MHz, $CDCl_3$, 20 °C) showing resonances of protons attached to C3 in product **9** [Figures in italics refer to ^{1}H NMR chemical shifts ($CDCl_3$, 20 °C), bold numbers (except compound numbering) to ^{13}C NMR shift values ($CDCl_3$, 20 °C)].

$V_{Br}PO(AnI)$-catalyzed oxidation of Br^- for transforming δ,ε-unsaturated alcohols in H_2O_2/KBr-containing media was performed in aqueous phosphate buffer (pH 6), which had been diluted with 25 % (*v*/*v*) of CH_3CN for facilitating organic substrate solubilization. Separate experiments had shown that quantitative alkenol conversion (GC) under such conditions was attainable, if the reaction was consecutively treated five times in intervals of 1 h with KBr (0.6 mmol), H_2O_2 [30 % (*w*/*w*), 0.4 mmol] and $V_{Br}PO(AnI)$ (8 μl of a 0.16 mM, Tris-HCl-buffered solution). The reaction mixture was afterwards allowed to stir for 18 h at 20 °C. In view of difficulties in mass balancing of reaction mixtures associated with bromohydrin formation outlined above, an alternative analytical procedure for product identification and quantitation was developed. Organic products were extracted with CH_2Cl_2. Product analysis was performed via NMR spectroscopy using pentachlorobenzene as internal standard. According to results from an independent recovery experiment, likewise determined yields are considered to be reliable within a relative precision of ±10 %.

Conversion of alkenols **8**, **10**, and **12** using established conditions furnished bromohydrins **9**, **7a/7b**, and **13** as major products (58–70 %, Scheme 8). Constitutionally symmetric bromohydrin **9** was formed as diastereomerically pure product (NMR), whereas compounds **7a/7b** and **9** were obtained as 50/50-mixtures of diastereomers. Assignment of relative configuration of bromohydrin **9** was feasible via analysis of $^3J_{H,H}$-coupling constants associated with H-atoms attached to C3 (Fig. 5). C_2-symmetry in **9** would have given rise to homotopic environments at either side of C3 and thus an A_2X_2 spin system for proton resonances attached to this site. The experimental spectrum, however, showed an



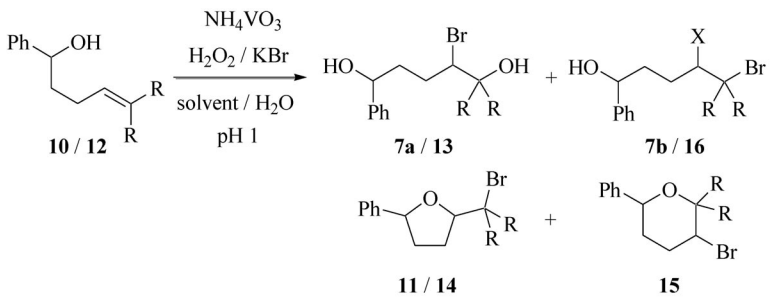
Scheme 8 Selectivity in intramolecular bromoetherification and/or bromohydroxylation of δ,ε-unsaturated alcohols in $V_{Br}PO(AnI)$-catalyzed oxidations of bromide in phosphate-buffered (pH 6, KH_2PO_4/K_2HPO_4) solutions [CH_3CN/H_2O (1/3), 25 °C; >95 % conversion]. [a]Diastereomerically pure (NMR). [b]50/50 ratio of diastereomers (^1H NMR). [c]*cis*:*trans* = 40:60 (^1H NMR). [d]*cis*:*trans* = 47:53 (^1H NMR). [e]*cis*:*trans* < 5:95 (^1H NMR).

ABX_2 spin system for these protons, thus pointing to heterotopic subunits and therefore to C_s symmetry of bromohydrin **9** (Fig. 5). The origin of selectivity may be explained on the basis of a stereochemical model that takes a C,Br-neighboring group effect in an energetically favored conformer of a postulated bromonium ion intermediate into account (Fig. 5, center).

Brominated cyclic ethers **11**, **14**–**15** were identified as additional products in $V_{Br}PO(AnI)$-catalyzed bromide oxidations conducted in the presence of alkenols **10** and **12** (Scheme 8). Yields of bromocyclization products differed (15–25 %) and seemed to be dependent on substitution at reacting entities. An inherent low stereoselectivity for tetrahydrofuran synthesis (**11** and **14**) on one side, and formation of diastereomerically pure 3,6-*trans*-3-bromo-2,2-dimethyl-6-phenyltetrahydrofuran *trans*-**15** (^{1}H NMR) on the other, pointed to diagnostic selectivities of Br_2-mediated ring closures of respective alkenols [32,43,44]. Selectivity of bromohydrin formation, on the other hand, corresponded to trends observed in reactions between the substrates and HOBr.

Finally, selectivity in $V_{Br}PO(AnI)$-catalyzed transformations was compared to data obtained from conversions of alkenols **10** and **12** with H_2O_2, KBr, and NH_4VO_3 in homogeneous [*t*BuOH/H_2O = 2/1 (*v*/*v*)]) and in heterogeneous mixtures [$CHCl_3$/H_2O = 2/1 (*v*/*v*)]. 1-Phenyl-pent-4-en-1-ol (**10**) furnished 2-bromomethyl-5-phenyltetrahydrofuran **11** in yields that ranged between 53–30 % (Table 3, entries 1–2) and mixtures of regioisomeric bromohydrins **7a** and **7b** (15–34 %), both as 50/50-mixtures of diastereomers. Bromination of 5-methyl-1-phenylhex-4-en-1-ol (**12**) under those conditions afforded 2-(2-bromo-2-propyl)-5-phenyltetrahydrofuran **14** (5–12 %), 3-bromo-2,2-dimethyl-6-phenyltetrahydropyran **15** (12–35 %), bromohydrin **13** (27–29 %), and dibromide **16** (20–22 %). Yields of bromocyclization products **11**, **14**–**15** obtained in the biphasic system ($CHCl_3$/H_2O) [28] were slightly higher than those obtained in homogenous solutions (*t*BuOH/H_2O, Table 3, entries 1–5) [45]. Stereo and product selectivity were similar but not identical to results obtained from $V_{Br}PO(AnI)$-catalyzed reactions (Scheme 8). In a similar manner, yields from both sets of experiments diverged to some extent.

Table 3 Oxidative transformation of alkenols in the presence of NH_4VO_3/H_2O/KBr.



Entry	R	Solvent	**7a**, **13**[a]/%	**16**[a]/% X = Br	**7b**[a]/% X = OH	**11**, **14**/% (*cis*:*trans*)	**15**/% (*cis*:*trans*)
1	H	$CHCl_3$[b]	**7a**: 7	–[c]	**7b**: 9	**11**: 53 (36:64)	–[c]
2	H	*t*BuOH[d]	**7a**: 14	–[c]	**7b**: 20	**11**: 30 (40:60)	–[c]
3	CH_3	$CHCl_3$[e]	**13**: 29	**16**: 20	–[c]	**14**: 12 (46:54)	**15**: 35 (14:86)
4	CH_3	*t*BuOH[f]	**13**: 27	**16**: 22	–[c]	**14**: 5 (48:52)	**15**: 12 (10:90)

[a]50/50 ratio of diastereomers.
[b]80 % substrate conversion.
[c]Not detected (^{1}H NMR and GC).
[d]82 % substrate conversion.
[e]>95 % substrate conversion.
[f]77 % substrate conversion.

CONCLUDING REMARKS

BPO I isolated from the brown alga *A. nodosum* [V_{Br}PO(*An*I)] certainly belongs to the group of enzymes that are expected to play an important role for future development of sustainable bromination chemistry. The enzyme is readily available. Its PO activity in MES-buffered solutions shows half-life times of ~60–80 days and is largely retained in the presence of ~25 % (*v*/*v*) of organic cosolvent (e.g., *t*BuOH, *i*PrOH, CH_3CN, 1,4-dioxane) for organic substrate solubilization. The enzyme unfolds a reactivity in these media that is applicable for preparing brominated (hetero)aromatic compounds, vicinal bromohydrins, and products of alkenol bromocyclization. The next challenge to take for uncovering the full potential of BPOs in synthesis will probably be associated with an increase of turnover numbers and time-yield factors, using reaction media of similar salinity as ocean water.

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