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# A mechanistic investigation of bromoperoxidases mimicking systems. Evidence of a hypobromite-like vanadium intermediate from experimental data and ab initio calculations

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**Abstract**

A two-phase ($H_2O$-$CHCl_3$ or $H_2O$-$CH_2Cl_2$) system in which $NH_4VO_3$, KBr and $H_2O_2$ are dissolved in the aqueous phase and the substrate, e.g., an aromatic compound or an olefin, in the organic one, has been shown to mimic the chemistry of bromoperoxidases. These enzymes catalyze the bromination of organic compounds. When the substrate is styrene or substituted stilbenes, both dibromoderivatives and halohydrins are formed. This is taken as evidence for the formation of a hypobromite-like vanadium complex. Ab initio calculations confirm the plausibility of the involvement of such a complex.

*Keywords:* Bromoperoxidase; Peroxovanadium complex; Aromatics and olefins bromination; Hypobromite-like vanadium complex

## 1. Introduction

Vanadium is present in plants and living organisms, including humans and its involvement in biological processes is documented [1–4]. However, the role played by the metal is still poorly understood [1–5]. Only fragmentary information is available even for systems under active investigation, i.e., haloperoxidases which are vanadium-dependent enzymes first isolated from brown algae [6–9]. Based on a series of data, including EXAFS experiments [10], a general picture of the active site of haloperoxidases (Fig. 1) and of the mode of action of the enzymes (Scheme 1), is available.

The active site of haloperoxidases contains vanadium, in its +5 oxidation state, arranged in a distorted octahedral geometry. Due to the intrinsic uncertainty of the EXAFS technique [10] the nature of the aminoacids of the proteic chain of the enzyme, acting as ligands, is not well established. By contrast, the presence of the oxo-oxygen and of aquo ligands appears to be assessed [8,10]. As shown in Scheme 1, the vanadium complex adds hydrogen peroxide forming a monoperoxovanadium complex [6–8]. Both mono and diperoxovanadium complexes could be formed in acidic aqueous solutions [5,11–14]. However, the former are considered

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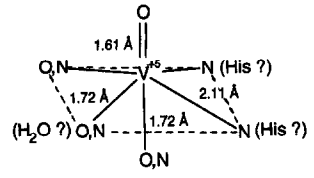
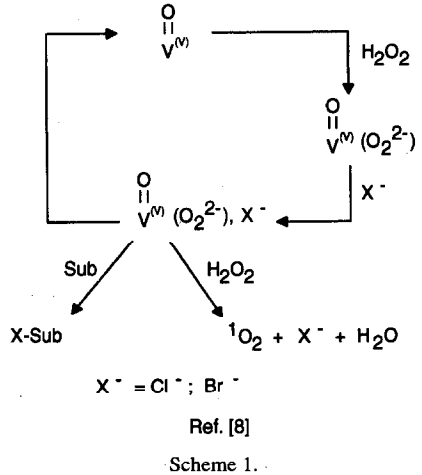



Fig. 1. active site of V-BrPO from EXAFS analysis [10].

more likely than the latter. In fact, a large excess of hydrogen peroxide over vanadium is not expected in biological systems. Moreover, monoperoxovanadium complexes are usually more reactive than the diperoxo derivatives toward organic and inorganic substrates [11–19]. According to Scheme 1, the monoperoxovanadium complexes oxidize halides, either bromide or chloride ion [15–17]. The mechanism of the oxidation and the nature of the product, which is commonly indicated as a bromine or chlorine equivalent, are essentially unknown, even though different hypotheses have been suggested [6–8]. The intermediate takes part in two different processes. In the presence of a suitable substrate it acts as a halogenating agent. Alternatively, it may react with a second molecule of hydrogen peroxide. The latter process is, in fact, a decomposition reaction which leads to the original vanadium precursor and to singlet oxygen which is, likely, rapidly quenched in the system [8].




Scheme 1.

Our attention toward the chemistry of haloperoxidases was attracted by the lack of a detailed mechanistic information [6–9]. In addition, we have a long-standing interest [11,12,18,19] in the oxidative behavior of peroxovanadium complexes which may be considered ideal models in the study of haloperoxidases [13,14]. In this paper we present results concerning the oxidation of bromide ion by simple peroxovanadium complexes and the subsequent bromination of a series of organic substrates. These results, which have been collected both in acidic aqueous solution and in a two-phase, water-$CHCl_3$ or $CH_2Cl_2$ system, allow us to propose a more detailed description of the chemistry of haloperoxidases. In addition, the high yields obtained in the bromination of organic substrates suggest a synthetic relevance of the system examined. Part of the results reported here has been the subject of previous communications [20,21].

## 2. Experimental section

### 2.1. Reagents and solvents

Anhydrous $NH_4VO_3$ (99.9% Fluka puriss. p.a.) and $H_2O_2$ (30%, w/v, Carlo Erba) were used without further purification. All ligands and substrates, commercially available products, were used without further purification. Deionized water was passed trough a Milli-Q/Organex-Q system (Millipore). Hydrogen peroxide solutions were prepared by dissolving the appropriate amount of 30% (w/v) $H_2O_2$ in Milli-Q water. The organic solvents $CHCl_3$ or $CH_2Cl_2$ were purified by using standard procedures.

### 2.2. Methodologies

The peroxide content was determined by standard iodimetric titration. pH measurements (0.02) have been obtained with a pH-meter Metrohm 632 standardized at pH 4.0 and 7.0,

respectively, before each measurements. The disappearance of the peroxide content for the various vanadium peroxocomplexes has been monitored by UV-VIS spectrophotometry following the decrease in absorbance at the $\lambda_{max}$ values of each species: **1** = 459.0 nm, **2** = 443.4 nm, **3** = 452.2 nm, **4** = 438.9 nm. The amount of dioxygen evolved in the decomposition reactions was determined by means of a thermostated, 10 ml maximum capacity, gas-burette. TLC analyses were carried out on Kieselgel $F_{254}$ thin-layer plates (Merck). The eluent was a mixture of ethyl acetate/2-propanol/water 75:16:9, and the developing agent was a $NaOH$-$AgNO_3$-$NH_4OH$ solution in water [22]. The HPLC analyses were carried out on a Waters Millipore instrument by using a Merck LiChrospher 3 m (4.6 mm × 15 cm) column. The eluent was a 95:5 mixture of water and methanol. For all the products a response factor (thymine internal standard) was previously determined. The quantitative GC analyses (benzophenone internal standard) have been performed on a 3% FFAP on Chromosorb WAW DMCS (0.5 m × 2.5mm) column. The GC-MS analyses have been carried out on a 15 m SE 30 capillary column, 0.25 mm i.d. In the reaction of peroxocomplex **1** (0.003 M) in aqueous acid solution in the presence of KBr (0.05 M, pH 1.1, $t$ = 30°C, see Fig. 3) the formation of bromine has been quantitatively determined by using an amperometric technique performed with a rotating disk electrode and an applied potential of 1.00 V vs. SCE (eluent: Milli-Q water KBr 0.1 M, $HClO_4$ 0.1 M).

### 2.3. Bromination reaction in water solution

In a typical reaction, weighted amounts of $NH_4VO_3$, KBr and uracil are dissolved in ca. 8 ml of water in the presence of acid (pH 1, $HClO_4$). To this solution, thermostated at 37°C, the appropriate volume of a standardized (iodometry) $H_2O_2$ solution (11.04 M in water) is added. The reaction mixture is then made up to 10 ml. Under these conditions, all vanadium is present as the oxo-monoperoxo aquo complex $[VO(O_2)(H_2O)_n]^+$, as revealed by $^{51}$V-NMR spectroscopic analysis [13]. The formation of 5-bromouracil has been established by TLC comparison with an authentic sample and also by HPLC analysis. The yield of 5-bromouracil has been determined by quantitative HPLC analysis (thymine internal standard).

### 2.4. Bromination reaction in a two-phase system

In a typical reaction carried out in a two-phase system ($H_2O/CHCl_3$) or ($H_2O/CH_2Cl_2$) the acid (pH 0.90, $HClO_4$) aqueous phase (20 ml) contains KBr (1 mmol), $H_2O_2$ (0.4 mmol) and $NH_4VO_3$ (0.2 mmol). All vanadium is present as the oxo-monoperoxo aquo complex $[VO(O_2)(H_2O)_3]^+$, as revealed by $^{51}$V-NMR spectroscopic analysis [13]. In the organic phase 0.2 mmol of the substrate are dissolved. The two phases are added together, and the reaction is carried out at 25°C under stirring. The formation of products is monitored by GC-MS analysis of the organic phase. The yields are calculated by GC-MS analysis after complete disappearance of the substrate in the reactions in the presence of an excess of hydrogen peroxide. In all the other reactions the yields are determined by quantitative GC analyses (benzophenone internal standard). The identity of the reaction products has been confirmed by comparison of their MS and $^1$H-NMR spectra with authentic samples.

### 2.5. Instrumentation

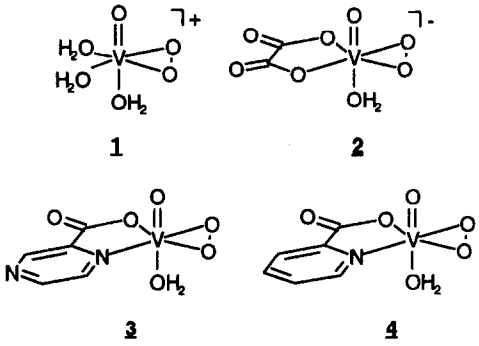
The $^{51}$V-NMR spectra were recorded on a 4.69 Tesla Bruker AC, 200 MHz spectrometer for $^1$H [13,14]. The electronic spectra were obtained with a Lambda 5 Perkin Elmer instrument with a temperature control better than 0.05°C. The quantitative determination of bromine has been done by using a Waters ACTION, Millipore chromatographic system equipped with a Water 966 diode-array coupled

with a amperometric detector EG and G PAR 400. The GC and GC-MS analyses were carried out on a Varian 3700 instrument equipped with a Hewlett-Packard 3365 integration system and on a Hewlett-Packard 5890 gas chromatograph connected with a 5970 mass selective detector, respectively.

## 3. Results and discussion

### 3.1. Reactions in acidic aqueous solutions

Based on our previous $^{51}$V-NMR studies, the conditions for obtaining the monoperoxocomplexes **1**–**4** in aqueous solution as the only vanadium species present have been used [13,14].




Details are given in the Section 2: Experimental section. These complexes undergo a slow self-decomposition which proceeds through a radical chain mechanism [23,24]. The products of the reaction are dioxygen and V(v) derivatives. Typical sigmoid-shaped reaction profiles are shown in Fig. 2.

The decomposition of the aqua complex **1** is the fastest one in agreement with its superior ability to act as a one-electron acceptor [14]. In fact, the presence of organic ligands increases the electron density on vanadium in compared with $H_2O$, thus reducing the attitude of the complex to accept the electron. This has been directly proved by recently reported $^{51}$V-NMR

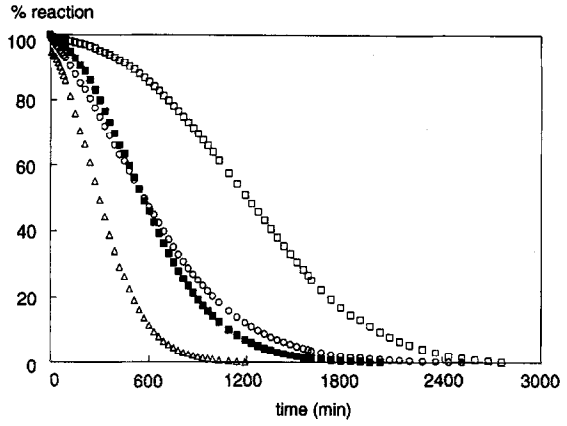



Fig. 2. Disappearance of the oxidant as a function of time, measured by the decrease in absorbance at the $\lambda_{max}$ values, for the decomposition of $5\times10^{-3}$M of **1** (Δ), **2** (■), **3** (○) and **4** (□), in water ($HClO_4$, pH 1) at 37°C.

studies [14]. The oxidizing ability of **1**–**4** toward organic substrates has been examined by using uracil as model substrate. All complexes are able to carry out the oxidation of uracil as demonstrated by the detection of the corresponding *cis*-diol together with minor amounts of overoxidized products, e.g., 1-formyl-5-hydroxyhydantoin [22]. Attempts to quantitatively estimate the amount of *cis*-diol produced have not been made at this stage of the investigation. Also in this oxidation, **1** appears to be more reactive than the other complexes containing organic ligands, as indicated by the fact that the active oxygen disappearance is faster than in the oxidations carried out by **2**–**4**. When 0.05 M KBr is added to a 0.003 M solution of **1** at pH 1.1, the oxidant is rapidly consumed and bromine is formed. However, as shown in Fig. 3, the amount of bromine produced, quantitatively estimated by an electrochemical procedure, see Section 2: Experimental section for details, is only ca. 20% of the active oxygen consumed.

This might simply indicate that, under the conditions adopted, the reaction between the 'bromine equivalent' intermediate and hydrogen peroxide, see Scheme 1, predominates but it is likely that other processes are taking place in the system. In fact, it is observed that if uracil is

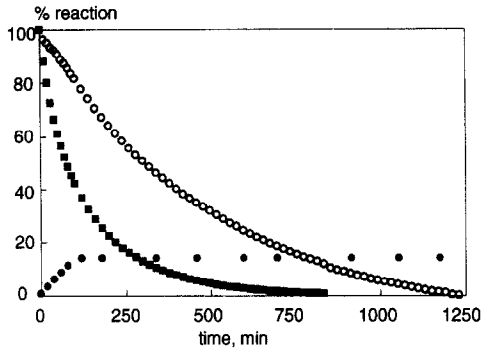



Fig. 3. Reaction profile for the decomposition of **1** ($3\times10^{-3}$ M) (○) in water ($HClO_4$, pH 1) at 30°C. Disappearance of the oxidant (■) and formation of $Br_2$ (●) in the reaction of **1** ($3\times10^{-3}$ M) with KBr ($5\times10^{-2}$ M), in water ($HClO_4$, pH 1) at 30°C.

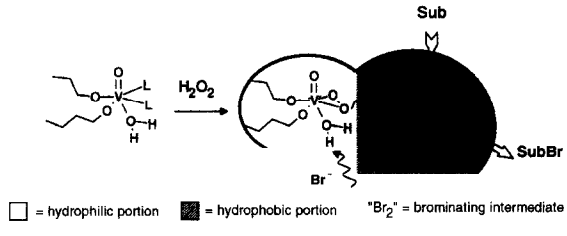
added to the solution containing **1** and KBr, 5-bromo-uracil is produced in about 25% yield, but the hydroxylation reaction leading to the *cis*-diol of uracil, is almost suppressed. In fact, HPLC analysis of the reaction mixture shows only traces of oxidized products deriving from uracil. Again, this might simply indicate that both the decomposition reaction and the bromination of uracil are faster than the hydroxylation of the substrate. However, this rationale does not agree with the data obtained by substituting **1** with the other monoperoxo vanadium complexes **2**–**4** which, under conditions identical to those of Fig. 3, do not form bromine even though their decomposition is accelerated. In addition the hydroxylation of uracil does not take place. The two observations taken together suggest that $Br^-$ is involved in the decomposition chain of all four complexes. In particular, it may be envisaged, also on the basis of literature data [15–17], that more steps involving the oxidation of $Br^-$ to $Br\cdot$ and the reduction of $Br\cdot$ to $Br^-$ should be added to the decomposition reaction scheme established [23,24] in the absence of $Br^-$. Further oxidation of $Br\cdot$ to the 'bromine equivalent' intermediate is observed only for **1** in agreement with the fact that the oxidizing ability of **1** is larger than that of **2**–**4** [14]. The intervention of $Br^-$ in the decomposition chain of **1**–**4** results in the suppression of the reaction leading to uracil *cis*-diol. The complexity of the system deserves a more accurate investigation which is, however, outside the aim of the present work. The experimental fact which is here of interest is that the oxidation of $Br^-$ by vanadium monoperoxocomplexes in water appears to be a rather inefficient process. Bromine is formed only when **1** is the oxidant and the yield is poor. This may indicate that our model system, i.e., the oxidation in water, is a too simplified one compared with the actual reactions of bromoperoxidases.

### 3.2. Reactions in a two-phase system

In the light of the results presented in the previous paragraph, and, in particular, of the accelerating effect of $Br^-$ on the decomposition reaction of peroxovanadium complexes even when $Br_2$ is apparently not formed, it may be envisaged that, in biological systems, the oxidation of $Br^-$ and the bromination of organic substrates occur in different portions of the enzymes [20]. In particular, it is likely that the former process takes place in a hydrophilic environment and the latter in a lipophilic one. This hypothesis is pictorially shown in Scheme 2.

If the real situation is that depicted in Scheme 2, a chemical model mimicking the mode of action of bromoperoxidases might be the two-phase system shown in Scheme 3 [20,21].

In such a system the formation of the monoperoxovanadium complex and the oxidation of $Br^-$ take place in water. The resulting 'bromine equivalent' intermediate is transferred




Scheme 2.

under stirring into $CHCl_3$ or $CH_2Cl_2$ in which the reaction of the organic substrate occurs.

We have used this procedure for the bromination of a series of organic substrates either aromatic compounds or olefins. The results are reported in Tables 1 and 2.

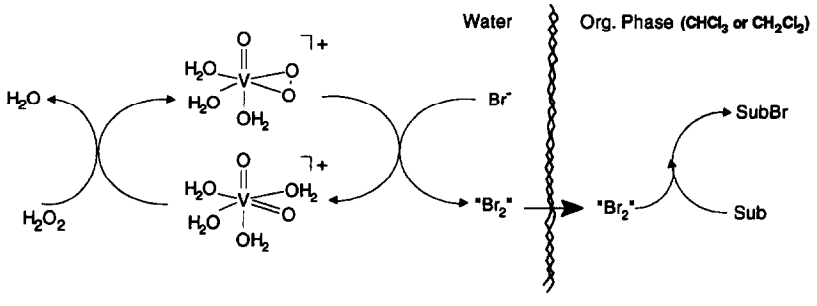
It may be seen that, for the majority of the substrates examined, the reaction is rather selective. Moreover the yields, calculated on hydrogen peroxide, are in many cases almost quantitative. This indicates that in such a system, $Br^-$ is quantitatively oxidized at variance with the experiments carried out in water. Lower yields are found with particularly resistant substrates, such as benzene, phenanthrene, 4-chlorostyrene, stilbene and 4,4′-dimethoxystilbene. On the other hand, no other products are observed, thus suggesting that in particularly slow reactions, decomposition of hydrogen peroxide becomes a competitive process. The results obtained with styrene and stilbene derivatives deserve particular attention. Also for these substrates, the yields of bromine containing material are rather high but, together with the expected dibromoderivatives, also bromohydrins are formed. In the case of styrene (run 10–12, Table 2), bromohydrin is the major product. In view of the mechanistic information which could be provided by a better understanding of the behavior of styrene and stilbene derivatives, we have studied the bromination of styrene in some more detail. Thus, we have ruled out, by direct experiments, that under our experimental conditions the halohydrin can be formed by hydrolysis of the dibromostyrene. Then, we have tested the behavior of classical brominating systems under the two-phase conditions. The results are collected in Table 3.

In all cases dibromostyrene is, by far, the major product, even in the case in which the system $BrO_3^-/Br^-$ is used. These data suggest that the 'bromine equivalent' intermediate formed in our system behaves differently from bromine. We have also examined the effect of the rate of stirring on products distribution. The results for styrene are reported in Table 4.

Table 1
Bromination of aromatic substrates with $H_2O_2$ and KBr, catalyzed by $NH_4VO_3$ in a two phase system, 20 ml $H_2O$/20 ml $CHCl_3$, at 25°C [a]

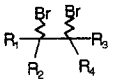
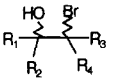
| No. | Substrate (mmol) | Yield (%) | Products% |
|---|---|---|---|
| 1 | benzene | 40 [b] | bromobenzene |
| 2 | methoxybenzene | > 98 [c] | 1-bromo-4-methoxybenzene |
| 3 | phenanthrene | 50 [b] | 9-bromophenanthrene |

[a] $H_2O_2$ (0.4 mmol) and KBr (1 mmol) and $NH_4VO_3$ (0.2 mmol) in water, pH 0.9; substrate (0.2 mmol) in $CHCl_3$, stirring rate 500 rpm.
[b] Conversion of the substrate after complete disappearance of hydrogen peroxide. No other products are detected, reaction time 24 h.
[c] Yield calculated by GC-MS after complete disappearance of the substrate. The identity of the products has been confirmed by comparison of their MS and $^1$H-NMR spectra with authentic samples.




Scheme 3.

Table 2
Reaction of alkenes (0.2 mmol) with $H_2O_2$ and KBr (1 mmol) catalyzed by $NH_4VO_3$ (0.2 mmol) in a two-phase system, 20 ml $H_2O$/20 ml organic solvent, at 25°C

| # |  | Solvent | r.p.m. | $H_2O_2$ mmol | yield (%) |  |  |
|---|---|---|---|---|---|---|---|
| | $R_1$, $R_2$, $R_3$, $R_4$, | | | | | % | % |
| 1 | $C_4H_9$, H, H, H | $CHCl_3$ | 500 | 0.4 | >98[a] | 100 | 0 |
| 2 | $C_6H_{13}$, H, H, H | $CHCl_3$ | 500 | 0.4 | 95[a] | 100 | 0 |
| 3 | $C_3H_7$, H, H, $C_3H_7$ | $CHCl_3$ | 500 | 0.4 | >98[a] | 100 | 0 |
| 4 | $C_5H_{11}CHOH$, H, H, H | $CHCl_3$ | 500 | 0.4 | >98[a] | 100 | 0 |
| 5 | $C_8H_{17}$, H, $C_7H_{14}$,COOH,H | $CHCl_3$ | 500 | 0.4 | >98[a] | 100 | 0 |
| 6 | $C_6H_5$, H, H, $C_6H_5$ | $CHCl_3$ | 500 | 0.4 | 95[a] | 100, anti:syn = 85:15 | 0 |
| 7 | $C_6H_5$, H, H, $C_6H_5$ | $CH_2Cl_2$ | 700 | 0.2 | 58[b] | 100, anti:syn = 80:20 | 0 |
| 9 | (4-$OCH_3$)-$C_6H_5$, H, H, (4-$OCH_3$)-$C_6H_5$ | $CH_2Cl_2$ | 700 | 0.2 | 48[b] | 38, anti:syn = n.d. | 62, anti:syn = 77:23 |
| 10 | $C_6H_5$, H, H, H | $CHCl_3$ | 500 | 0.4 | 96[b] | 73 | 27 |
| 11 | $C_6H_5$, H, H, H | $CH_2Cl_2$ | 500 | 0.4 | 96[b] | 42 | 58 |
| 12 | $C_6H_5$, H, H, H | $CH_2Cl_2$ | 700 | 0.2 | 79[b] | 37 | 63 |
| 13 | (4-Cl)-$C_6H_5$, H, H, H | $CH_2Cl_2$ | 700 | 0.2 | 52[b] | 100 | 0 |
| 14 | $C_6H_5$, H, H, $CH_3$ | $CH_2Cl_2$ | 700 | 0.2 | 83[b] | 42, anti:syn 87:13 | 58, anti:syn = n.d. |
| 15 | $C_6H_5$, $CH_3$, H, H, | $CH_2Cl_2$ | 700 | 0.2 | 85[b] | 34 | 66 |

[a] Yield calculated by GC-MS after complete disappearance of the substrate, reaction time 24 h. The identity of the products has been confirmed by comparison of their MS and $^1$H-NMR spectra with authentic samples.
[b] Yields calculated by quantitative GC analysis of the organic phase (benzophenone internal standard), reaction time 24 h.

At low stirring rate, the dibromoderivative is the major product. The concentration of bromohydrin increases with increasing the rate of stirring so that, when such a rate is rather large, the bromohydrin predominates. We have also observed that an increase of the volume of the organic phase, or a decrease of the volume of the water solution, leads to an increase of bromohydrin while the total yield of the two products is almost the same.

A plausible rationale of the observations reported so far is that there are two different

Table 3
Reaction of styrene with various brominating reagents, in a two phase system 20 ml $H_2O$/20 ml $CH_2Cl_2$, 700 rpm, at 25°C

| No. | Reagent | Yield(%) [b] | 2-Bromo,1-hydroxy,1-phenylethane(%) [c] | 1,2-Dibromo,1-phenylethane(%) [c] |
|---|---|---|---|---|
| 1 | $BrO_3^-/Br^-/H^+$ | 99 | 25 | 75 |
| 2 | HOBr($BrO^-/H^+$) | 72 | 20 | 80 |
| 3 | HOBr($Br^-$/HOCl) | 99 | 20 | 80 |
| 4 | *N*-Br-succinimide | 72 | 10 | 90 |
| 5 | $Br_2$ | 99 | 8 | 92 |
| 6 | $Br_2$ [d] | 95 | 7 | 93 |

[a] In all runs the reagents are added in order to produce 0.2 mmol of $Br_2$.
[b] Determined at the disappearance of the red color of bromine, reaction time 6 h.
[c] Determined by GC (benzophenone internal standard).
[d] Aqueous $Br_3^-$solution added dropwise in 4 h.

Table 4
Reaction of styrene with $H_2O_2$ and KBr, catalyzed by $NH_4VO_3$ in a two phase system 20 ml $H_2O$/20 ml $CH_2Cl_2$, at increasing stirring rates, at 25°C. [a]

| No. | rpm | Yield % [b] | 2-Bromo, 1-hydroxy, 1-phenylethane (%) [c] | 1,2-Dibromo, 1-phenyl-ethane (%) [c] |
|---|---|---|---|---|
| 1 | 30 | 33 | 18 | 82 |
| 2 | 300 | 52 | 46 | 54 |
| 3 | 700 | 79 | 63 | 37 |
| 4 | 900 | 72 | 68 | 32 |
| 5 | 1300 | 66 | 79 | 21 |

[a] $H_2O_2$ (0.2 mmol) and KBr (1 mmol) and $NH_4VO_3$ (0.2 mmol) in water, pH 1; substrate (0.2 mmol) in $CH_2Cl_2$.
[b] Determined at complete consumption of the peroxide, reaction time 24 h.
[c] Determined by GC (benzophenone internal standard).

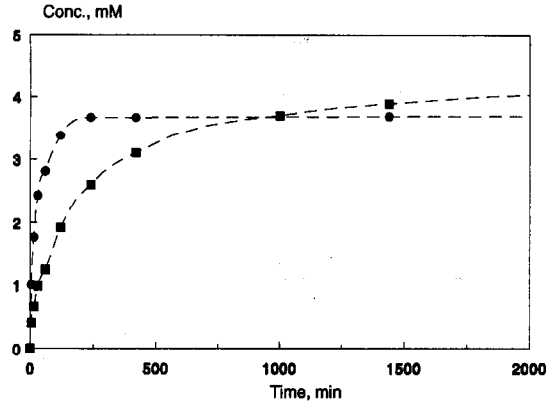



Fig. 4. Formation of 2-bromo,1-phenyl,1-hydroxyethane (■) and 1,2-dibromo,1-phenylethane (●) from styrene as a function of the time. The reaction is carried out in the typical two-phase ($H_2O$-$CHCl_3$) system (see text).

species reacting with olefins producing either dibromoderivatives or bromohydrins. The former species is easily transferred into the organic phase while the latter requires stirring. The substituents on the double bond play a major role in driving the reaction toward one or the other of the two products. In particular, electrondonating substituents favor bromohydrin over dibromoderivative formation (runs 9 and 13, Table 2).

The hypothesis of two parallel reactions is supported by the data presented in Fig. 4 which show that the two products are formed from the very beginning at different rates.

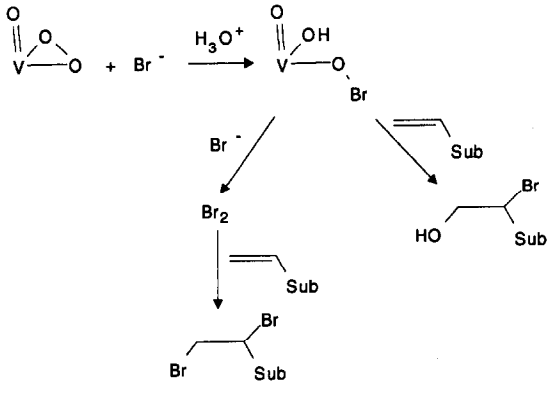
We have also observed that under conditions identical to those of Fig. 4 an increase in KBr from 0.4 mmol to 1.6 mmol causes the total yields of products to increase from 47% to 86% and the ratio dibromoderivative/bromohydrin to increase from 0.55 to 0.94.

A mechanistic scheme which accounts for all the experimental facts so far available is shown in Scheme 4.

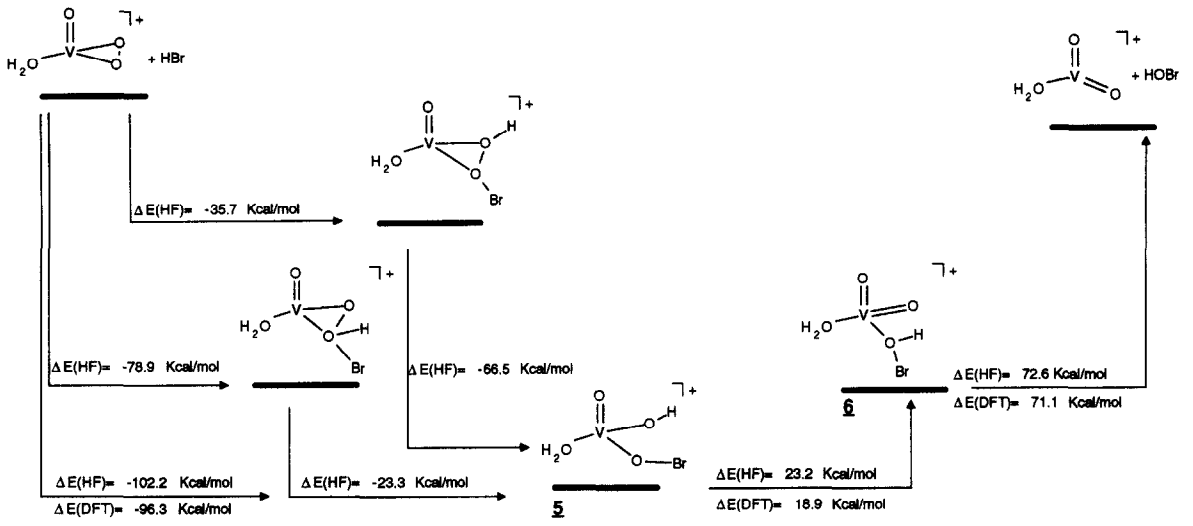
It may be mentioned that the occurrence of a hypobromite-like intermediate in the catalytic cycle of bromoperoxidases has been previously suggested though in the absence of experimental evidence [21].

Since Scheme 4 may be considered, at this stage, rather speculative, we looked for further evidence supporting the mechanistic proposal.

A detailed kinetic investigation, which would provide useful information, is planned but it has not yet been carried out also in view of the complexity of the two-phase system. Alternatively, we have carried out ab initio calculations aimed at establishing the plausibility of the hypobromite-like intermediate. The calculations have been performed by optimizing all geometries without geometry constraints by using RHF/3–21G(*) basis set [25]. Vibrational frequencies calculations at the RHF 3–21G(*)




Scheme 4.




Scheme 5.

level were used to characterize all minima stationary points (zero imaging frequencies).

The results of such ab initio calculations are graphically shown in Scheme 5 [26].

It may be seen that the suggested hypobromite-like intermediate lies in a deep hole of potential energy in comparison with the reactants, $[VO(O_2)(H_2O)^+]$/HBr and the couple $[VO_2(H_2O)^+]$/HBrO. Therefore, even by taking into account all the limitations of theoretical calculations when they are used for interpreting reacting systems in solution, it may be concluded that a species such as **5** can, indeed, exist.

We are currently looking for further support to these results by using the Moeller-Plesset perturbation theory (MP2), the Density Functional Theory (DTF) and a larger basis set employing effective core potentials (ECP) [27,28]. Preliminary data are encouraging as they confirm the general picture established here. As an example, by using B3LYP/LANL2DZ density functional calculations [27,28], which take into account correlation effects, the difference of energy between reactants and **5** is $-96.3$ kcal $mol^{-1}$ and between **5** and **6** 18.9 kcal $mol^{-1}$. The difference in energy between **5** and the oxocomplex-HOBr is 90.0 kcal $mol^{-1}$ (see Scheme 5).

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