Pergamon

Tetrahedron Letters, Vol. 36, No. 15, pp. 2675-2678, 1995
Elsevier Science Ltd
Printed in Great Britain
0040-4039/95 $9.50+0.00

0040-4039(95)00331-2

Vanadium Bromoperoxidases Mimicking Systems: Bromohydrins Formation as Evidence of the Occurrence of a Hypobromite-Like Vanadium Complex.

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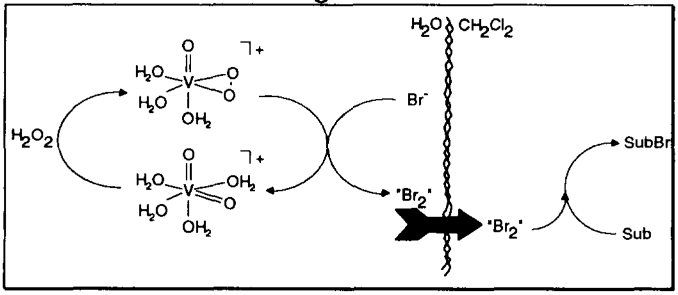
Abstract: In the aqueous phase of a two-phase (H_2O-CH_2Cl_2) system mimicking the hydrophilic and the hydrophobic portions of vanadium dependent bromoperoxidases, (V-BrPO), a mono peroxovanadium complex, formed in situ by addition of H_2O_2 to NH_4VO_3, oxidizes Br^- to a species whose nature is still largely unknown. Such species displays a bromine-like reactivity toward organic substrates dissolved in CH_2Cl_2. The observation that styrene, 1-methyl- and 2-methyl styrenes afford, together with dibromo-compounds, appreciable amounts of bromohydrins, suggests that a hypobromite-like species is also formed. Evidence is presented that such a species is a vanadium complex.

Vanadium dependent bromoperoxidases (V-BrPO) are enzymes that catalyze the oxidation of bromide ion by hydrogen peroxide.[1-3] The catalytic effect is due to the formation of a peroxovanadium complex which is a stronger oxidant than hydrogen peroxide.[4-7] The nature of the species resulting from the oxidation is still largely unknown.[8-10] The term of "bromine-equivalent" intermediate has been coined to account for its ability to brominate organic compounds.[8-10] Bromination competes with the decomposition of hydrogen peroxide.[11,12] In a recent paper[13] we proposed that the formation of the "bromine-equivalent" species occurs in a hydrophilic portion of the enzyme whereas substrate bromination takes place in a hydrophobic one. As a result, hydrogen peroxide decomposition is minimized. This may be mimicked in the two-phase system shown in Figure 1.

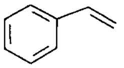
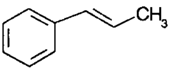
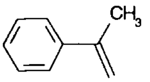
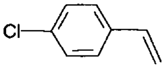
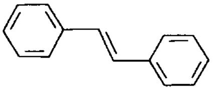
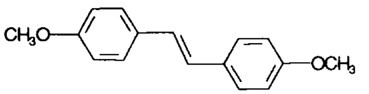
Figure 1



High yields of dibromoderivatives have been obtained with a number of olefins.[10] In the attempt to enlarge the scope of the procedure other olefins were examined.

The data obtained under the experimental conditions provided in note 14 are collected in Table 1.

Table 1: Bromination of olefins 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), 700 rpm, at 25°C.[a]

#	substrate	yield % [b]	bromohydrin % [c]	dibromo derivative %[c]
1		79	63	37
2		83	58 *anti*:*syn*= n.d.[c]	42 *anti*:*syn*=87:13
3		85	66	34
4		52	-	100
5		58	-	100 *anti*:*syn*=80:20
6		48	38 *anti*:*syn*= n.d.[d]	62 *anti*:*syn*=77:23

[a] See note 14. [b] determined at complete consumption of the peroxide. c determined by GC (internal standard benzophenone). [d] not determined.

The selectivity observed is low. In fact, together with dibromoderivatives, bromohydrins are also formed. The latter are, in some cases, the major products. As an example, in the reaction with styrene, the ratio bromohydrin-dibromo styrene is ≈2. The same products distribution is observed by changing the Br^- initial concentration from 1 to 20 mmol/20 ml water. The presence of substituents in the aromatic ring (entries # 4 and 6) greatly affects the selectivity of the reaction. In the light of the facts listed below these results provide useful hints on the nature of the species occurring in the system. It appears rather unlikely that, under the acidic conditions employed,[14] bromohydrins can be formed by reaction of bromonium ion and HO^-. Furthermore, direct experiments rule out that bromohydrins are formed by hydrolysis of the dibromo derivatives. Finally, bromohydrins cannot derive from cleavage of epoxides which under our experimental conditions are not formed. Preliminary data[15] indicate that bromohydrins and dibromo derivatives are formed via two parallel processes. In the reaction of styrene the concentration of the two products regularly and continuously increases as a function of time.[15]

The behavior of classical brominating systems[16-20] toward styrene under experimental conditions identical with those employed in the two-phase vanadium catalyzed procedure is illustrated by the data of Table 2.

It appears that when the species, either present or formed in aqueous solutions, is bromine the reaction with styrene predominantly affords dibromostyrene. Entry 3 (Table 2) shows that a slow addition of bromine to the reaction mixture does not affect the products distribution. On this basis, we believe that our data provide the first direct evidence[8-10,21] that the species resulting from the vanadium-catalyzed oxidation of KBr by H_2O_2 behaves differently from bromine.

Table 2: Bromination of styrene with known brominating reagents, in a two phase system 20 ml H_2O/20 ml CH_2Cl_2, 700 rpm, at 25°C

#	System[a]	yield % [b]	bromohydrin %[c]	dibromo derivative %[c]
1	BrO_3^- / Br^- / H^+	99	25	75
2	Br_2	99	8	92
3	Br_2[d]	95	7	93
4	N-Br-succinimide	72	10	90
5	HOBr (BrO^- / H^+)	72	20	80
6	HOBr (Br^- / HOCl)	99	20	80

[a] in all the systems the reagents are equivalent 0.2 mmoles of Br_2 as in the vanadium catalyzed reactions. [b] determined at the disappearance of the red colour of bromine. [c] determined by GC (internal standard benzophenone). [d] Br_2, as aqueous Br_3^- solution, was added dropwise over a period of 4 hrs.

The results of Table 3 provide more information by showing that in the reaction with styrene the products distribution is a function of the rate of stirring.

Table 3: Bromination 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 various stirring rates, at 25°C.[a]

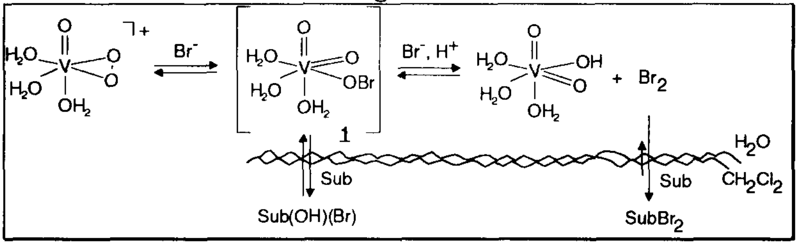
#	rpm	yield %[b]	bromohydrin %[c]	dibromo derivative %[c]
1	30	33	18	82
2	300	52	46	54
3	700	79	63	37
4	900	72	68	32
5	1300	66	79	21

[a] See note 14. [b]determined at complete consumption of the peroxide. [c] determined by GC (internal standard benzophenone).

In particular, the dibromoderivative, which is presumably formed by reaction with bromine, is the major product at low stirring rate whereas, at high stirring rate bromohydrin is predominantly formed. Taking into account that bromine is readily transferred into the organic phase even in the absence of stirring, the results of Table 3 suggest that, in the aqueous phase, together with bromine, another species is formed which reacts with olefins yielding the bromohydrins. The tendency of such species to migrate into the organic phase is lower than that of bromine. A plausible scheme which provides a rationale to the observations presented here is shown in Figure 2.

It is envisaged that the first interaction of the peroxovanadium complex with the bromide ion produces intermediate **1** whose resemblance with a hypobromite derivative is evident. This is the species responsible of bromohydrin formation. Further reaction of **1** with Br^- leads to bromine which brominates the substrate. The competition between bromohydrin formation and dibromination implies that the product distribution should depend not only on the rate of stirring but also on the rates of the various reactions and hence on the nature of the substrates. This is indeed the case as revealed by an inspection of the date of Table 1. It may be seen that the presence in the aromatic ring of styrene of the electronwithdrawing Cl group, which decreases the nucleophilicity of the substrate, drives the reaction toward the quantitative formation of the dibromoderivative (entries # 1 and 4). Thus it appears that bromohydrin formation is more sensitive to electronic effect than the formation of the dibromoderivative. In turn, two electrondonating groups, OCH_3, in the aromatic rings of stilbene, allow the onset of bromohydrin formation (entries # 5 and 6). In the light of our results, the occurrence of species such as **1** in the chemistry of bromoperoxidases is, at least, worthy of further investigation.

Figure 2



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\# Erasmus Student from University of Gøteborg.

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14. In the acid (pH=1, $HClO_4$) aqueous phase (20 ml) KBr (1 mmole), H_2O_2 (0.2 mmoles) and NH_4VO_3 (0.2 mmoles) are dissolved. Under these conditions vanadium is present as the oxo-monoperoxo aquo complex **1** as revealed by ^{51}V-NMR analysis (see ref. 7). Table 1 collects the results obtained with some selected substrates (0.2 mmoles in 20 ml of CH_2Cl_2), at 25°C under stirring (700 rpm). In the absence of vanadium only traces of brominated products are detected. All reactions have been done in the presence of excesses of Br^- in order to avoid kinetic effects on the formation of bromine.
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(*Received in UK* 10 *January* 1995; *revised* 10 *February* 1995; *accepted* 17 *February* 1995)