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FURTHER EVIDENCE FOR A METAL-OXO INTERMEDIATE IN THE OLEFIN EPOXIDATION BY IRON- OR MANGANESE-BLEOMYCIN COMPLEXES ASSOCIATED WITH $KHSO_5$, AN OXYGEN ATOM DONOR

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The use of iodosylbenzene with the iron-bleomycin complex in the olefin epoxidation leads to the questionable role of iron-oxo in this oxygenation reaction (*Tetrahedron Letters*, 1985, **26**, 4699-4702). Oxygenation of olefins is also observed when potassium hydrogen persulfate, $KHSO_5$, is used as oxygen donor and the reported data support a possible role of metal-oxo species in these oxygen transfers mediated by manganese- or iron-bleomycin complexes.

Bleomycin is one of the rare antitumor agents for which all authors agree that the mechanism of action is related to the chelation of metal salts (*e.g.* iron) by this glycopeptide molecule, followed by a reductive activation of molecular oxygen by the bleomycin-metal complex.[1] The activated form of bleomycin[2] is able to release free nucleic bases and base propenals from DNA, the key-step being the abstraction of the hydrogen atom at the C'4 position of deoxyribose.[3] A concomitant DNA cleavage is observed during these reactions. "Activated BLM" has been assigned to a high-valent bleomycin-iron complex, two oxidizing equivalents above the stable iron(III) complex.[2] Its chemical reactivity is closer to that of the oxidized form of chloroperoxidase than of cytochrome P-450 when the chlorination of monochlorodimedone or the demethylation of N,N-dimethylaniline are used as reaction tests.[4] However, Hecht *et al.* have recently evidenced the epoxidation of olefins with iodosylbenzene, PhIO, in the presence of the bleomycin-iron complex.[5,6] Among the multiple pathways postulated for these olefin oxidations by BLM-Fe(III)-PhIO, one can be related to a "P-450-like" route.[6,7]

$$BLM.Fe^{III} \xrightarrow{\text{oxygen donor}} \text{"activated BLM"}\ (BLM.Fe^{V}{=}O \text{ or } BLM.Fe^{IV}\text{-}O^{\bullet})$$

(oxygen donor = PhIO or $KHSO_5$).

Such a hypothesis is further supported by the DNA cleavage observed when bleomycin-Fe(III) is associated with a water-soluble oxygen donor, $KHSO_5$ or potassium hydrogen persulfate.[8a] Because of the dual epoxide formation - *i.e.* P-450-like route[5,6] or Lewis acid catalysis[7] - when PhIO is associated with BLM.Fe(III) and olefins in aqueous methanol, we report here the olefin epoxidation by the bleomycin-iron complex and $KHSO_5$ as oxygen-donor. This peroxidic compound behaves as a very efficient oxygen donor in hydroxylation reactions catalyzed by manganese-porphyrins.[8b]

Typical experiments were run as follows. To 400 nmol of bleomycin (Roger Bellon, Paris) containing 70% of BLM A_2 and 30% of BLM B_2 and 5 molar excess of a metal salt (2 µmol of $Fe(ClO_4)_3$, $Mn(OAc)_3,2H_2O$ or $Zn(OAc)_2,2H_2O$) were added 200 µL of bi-distilled water and subsequently 700µL of acetonitrile. After stirring for 5 min in order to ensure complete formation of the BLM-metal complex, *cis*-stilbene (5 µL, 28 µmol) was injected into this homogeneous phase prior to the addition of 100 µL of an aqueous solution of $KHSO_5$ (5,6 µmol obtained from a mother solution prepared by dissolving 20 mg of Oxone® in 1 mL of NaOH 30 nM ; pH 7.5). The final reaction volume was 1 mL. After stirring for 1 h at room temperature, aliquots were injected into a capillary GLC column (Chrompack CP Wax 51; 25 m x 0.22 mm) with an appropriate standard (indole). The molar ratio for the different reactants is BLM/$KHSO_5$/olefin =1/15/70. In the particular case of $Mn(OAc)_3$, the solubilization of this salt in the mixture acetonitrile/water is favoured by a few seconds of sonication. All reactions were performed in air.

Table1. Product distribution[a] in the oxidation of *cis*-stilbene[b] by BLM / Mn^{III}, Fe^{III} or Zn^{II} / $KHSO_5$ in acetonitrile-water (v/v 70/30)

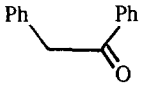
	Mn^{III}/BLM (%)	Fe^{III}/BLM (%)	Zn^{II}/BLM (%)
	100	50	< 5
	65	65	-
PhCH(OH)-CH(OH)Ph	165	60	-
	75	145	-
PhCHO	20	*ca.* 200	-
Total yield	425	520	< 5

Table 2. Case of *trans*-stilbene

Mn^{III}/BLM(%)	Fe^{III}/BLM(%)
475[c,d]	265
100	25
<10	ca.150
585	430

[a] Yields are expressed with respect to the initial amount of BLM (400 nmol). In a blank experiment without BLM, all the yields were 10 to 20 times lower than those indicated in the present Table. [b] The *cis*-stilbene used in the present study contained 1.8% of the *trans* isomer. No significant modification of concentration of *trans* isomer was observed during the reactions. For a possible isomerization process of *cis*-stilbene by Fe/BLM/PhIO, see reference 6). [c] In a blank experiment without metal salt, the *trans*-stilbene oxide yield is less than 2% with respect to BLM. [d] The traces of *cis*-stilbene oxide detected in these reactions correspond to the traces of *cis*-stilbene (0.1%) initially present in the used *trans*-stilbene.

The product distribution after 1 h of reaction in the oxidation of *cis*-stilbene by BLM/Mn^{III}, Fe^{III} or Zn^{II}/$KHSO_5$ is reported in Table I. First, it has to be noted that zinc salts chelated by BLM do not show a catalytic activity when associated to a good oxygen-donor such as $KHSO_5$. In that case, an oxidation process by $KHSO_5$ through the Lewis acid assistance of Zn^{II}/BLM can be precluded, whereas such a reaction pathway is possible when PhIO is used as oxidant.[7] On the opposite, the Mn- and Fe-BLM complexes are able to catalyze the oxidation of *cis*-stilbene. The total yield of detected products is 520% and 425% for Fe^{III} and Mn^{III}/BLM/$KHSO_5$, respectively, *i.e.* more than 5 and 4 catalytic cycles are performed by the iron- and manganese-bleomycin complexes. Same turnover numbers values have been observed when PhIO is the oxygen donor [1b].

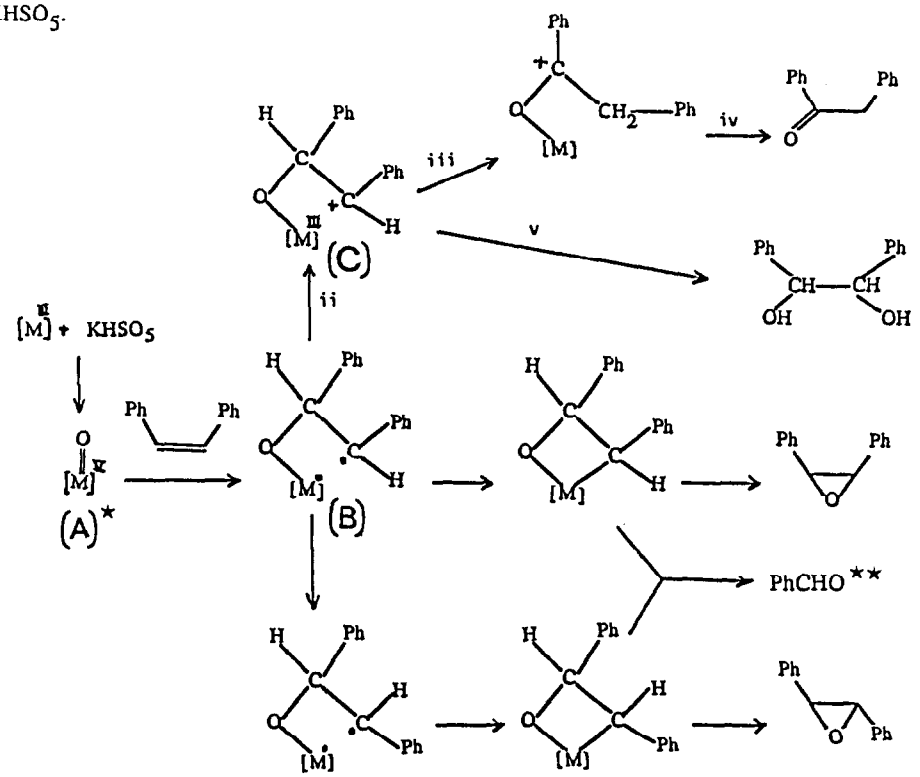
Compared to metalloporphyrin catalysts for which hundreds of catalytic cycles are possible when associated with $KHSO_5$,[8] the present low catalytic activity of metal-bleomycin complexes suggests that bleomycin is partially self-destroyed by the active metal-oxo species (In the absence of a good oxygen acceptor such as olefin, iron-bleomycin is quickly bleached by $KHSO_5$). However, the facts are not against the possible role of a "bleomycin-metal-oxo" as active species in the oxidative cleavage of DNA since, in this case, DNA breaks can be associated with a suicide activity of bleomycin. Furthermore, the self-destruction of bleomycin by the metal-oxo route might also be considered as a way to destroy *in vivo* the excess of the drug which is not necessary to the pharmacological activity.

For both metals, the two isomers of stilbene oxide are formed during the catalytic reaction. We checked that these two epoxides are stable in the reaction conditions. The loss of stereoselectivity during the epoxidation of *cis*-stilbene may also be observed during the catalytic epoxidation of *cis*- stilbene by metalloporphyrins.[9] The cis/trans epoxide ratio is 1.53 for Mn/BLM and 0.76 for Fe/BLM, lower than that one observed with PhIO (2.5 for Fe/BLM[5]). This poor stereoselective epoxidation of *cis*-stilbene is an evidence for the formation of an intermediate such as (B) subsequent to the addition of *cis*-stilbene to the metal-oxo species (A) (Scheme 1). After rotation around the C-C bond, the ring closure leads to two isomeric oxo-metallacyclobutanes. A reductive elimination process then gives the corresponding epoxide, while a C-C bond cleavage of these same four-membered rings might explain the formation of benzaldehyde without the necessity for an external electron as evoked by Hecht *et al.*[6], since a strong oxidant such as $KHSO_5$ may clean the reaction mixture from all traces of reducing equivalents.From (B), an intramolecular electron transfer would give a carbocation intermediate (C) for which two possible decomposition pathways are possible: a hydride transfer and the consequent formation of deoxybenzoïn (pathway iii), or the reaction with water and subsequent diol formation (pathway v). The important yield observed for the three compounds, *i.e.* hydrobenzoin, deoxybenzoin and benzaldehyde, indicates that the routes leading to their formation are not minor, contrarily to what has been observed in the metalloporphyrin-catalyzed epoxidation of *cis*-stilbene.[10]

Table 2 summarizes the data obtained for the epoxidation of *trans*-stilbene by Mn^{III} or Fe^{III} / BLM / $KHSO_5$ under the same reaction conditions as for the *cis*-olefin. Two main features are observed for both metals: (i) the total yield is as high as for *cis*-stilbene, 585 and 430% for Mn and Fe, respectively, and (ii) the epoxide yield is better than in the case of *cis*-olefin. Such a high epoxide yield is not observed with PhIO as oxygen donor[5]. Finally, it should be noticed that for both stilbene isomers, the highest yield of benzaldehyde is obtained for the iron catalysis. This large amount could be attributed to the presence of molecular oxygen[6].

Before concluding, it must be added that in pure water and with a water-soluble olefin such as 2,5-dihydrofuran, no epoxide is detected with Fe^{III} or Mn^{III} / BLM / $KHSO_5$, whereas a small amount of epoxide is formed in water by the reaction with $KHSO_5$ alone. These results indicate that the catalytic decomposition of $KHSO_5$ by metal-bleomycin, probably *via* a metal-oxo complex, and its reaction with water is faster than the oxygen transfer from the chelated metal to the substrate. Therefore, in pure water, metal-oxo entities may avoid their quenching by water molecules provided a substrate with a strong affinity for the metal complex is present when the highly-reactive metal-oxo complex is generated. Such hypothesis has recently been supported by the oxidative cleavage of DNA in water by metalloporphyrins and $KHSO_5$.[11] Hence, metal-oxo entities may behave as oxygenating species in biological medium, not only inside the hydrophobic pocket of cytochrome P-450.

Scheme 1. Different possible intermediates and observed products during the oxidation of *cis*-stilbene by Fe^{III} or Mn^{III} / BLM / $KHSO_5$.



[ ] refers to the bleomycin ligand. (i) C-C bond rotation, (ii) intramolecular electron transfer, (iii) hydride transfer, (iv) oxidative cleavage (?), (v) hydrolysis step.

* or [M^{IV}- O•]. For a recent review on the possible structure of metal-oxo entities, see reference 10.

** a cleavage of the C-C bond in these oxometallacyclobutane intermediates may give straightforward a molecule of PhCHO and an unstable metal-carbene complex.

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References

1. a) L. F. Porvik, in "Molecular Aspects of Anti-cancer Drug Action", S. Neidle and M. J. Waring, Eds., Verlag,Weinheim, 1983, p. 157-181.
   b) S. M. Hecht, *Acc. Chem. Res.*, 1986, **19**, 383-391.
2. R. M. Burger, J. S. Blanchard, S. B. Horwitz and J. Peisach, *J. Biol. Chem.*, 1985, **260**, 15406-15409, and references therein.
3. J. C. Wu, J. Stubbe and J. W. Kozarich, *Biochemistry*, 1985, **24**, 7569-7573, and references therein.
4. G. Padbury and S. S. Sligar, *J. Biol. Chem.*, 1985, **260**, 7820-7823.
5. N. Murugesan and S. M. Hecht, *J. Amer. Chem. Soc.*, 1985, **107**, 493-500.
6. D. C. Heimbrook, R. L. Mulholland, Jr. and S. M. Hecht, *J. Amer. Chem. Soc.*, 1986, **108**, 7839-7840.
7. R. M. Moriarty, R. Penmasta and I. Prakash, *Tetrahedron Lett.*, 1985, **26**, 4699-4702.
8. a) G. Pratviel, J. Bernadou and B. Meunier, *Biochem. Biophys. Res. Comm.*, 1986, **136**, 1013-1020,
   b) B. De Poorter, M. Ricci and B. Meunier, *Tetrahedron Lett.*, 1985, **37**, 4459-4462.
9. B. Meunier, E. Guilmet, M. E. de Carvalho et R. Poilblanc, *J. Amer. Chem. Soc.*, 1984, **106**, 6668-6676.
10. B. Meunier, *Bull. Soc. Chim. Fr.*, 1986, 578-594.
11. E. Fouquet, J. Bernadou and B. Meunier, manuscript in preparation.

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