fumaronitrile, 764-42-1; squaric acid, 2892-51-5; allyl alcohol, 107-18-6; $K_4[Pt_2(pop)_4]$, 79716-40-8.

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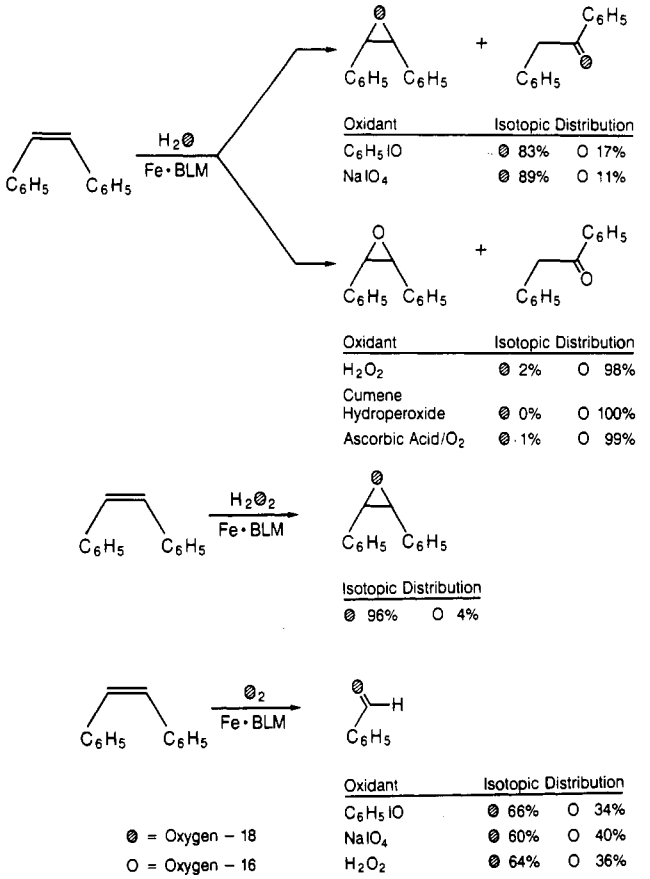
Mechanism of Oxygenation of *cis*-Stilbene by Iron Bleomycin

Sir:

The bleomycins (BLM's) are a family of antitumor antibiotics isolated from *Streptomyces verticillus*.[1] They mediate DNA strand scission in the presence of O_2 and certain metal ions;[2] this transformation is believed to constitute the basis for their antitumor activity.[3] Metallobleomycins may also be activated for DNA strand scission by the use of oxidants such as iodosobenzene.[4] In addition to mediating destruction of DNA, activated metallobleomycins have been shown to effect the oxidation of several low molecular weight olefinic substrates.[4a,4c,5]

Although the products[1a,6] and chemical mechanism[1a,6,7] of DNA cleavage by bleomycin have been studied in some detail, as have the products resulting from BLM-mediated oxidation of small molecules,[4a,4c,5] the only published oxygen-labeling experiments identified the aqueous medium as the source of oxygen in *cis*-stilbene oxide formed in the C_6H_5IO-supported oxidation of *cis*-stilbene by FeBLM.[5b] Presently, we report the results of a more thorough study in which we employed oxygen labeling to help define the mechanism of small-molecule oxidation by BLM. The results of this study suggest that the source of oxygen transferred in the formation of *cis*-stilbene oxide is a metal–oxo species, the oxygen in which is derived from the oxidant employed for metallobleomycin activation.

Scheme I. Isotopic Distribution of ^{18}O and ^{16}O in Products Resulting from FeBLM-Dependent Oxidation of *cis*-Stilbene in the Presence of Various Oxidants[10]



When carried out under aerobic conditions, the FeBLM-mediated oxidation of *cis*-stilbene afforded a number of products, including *cis*-stilbene oxide and benzaldehyde.[4,5,8] Previous work suggested that these two products resulted from two different oxidation pathways: one of these was suggested to be an oxygen-transfer reaction leading to *cis*-stilbene oxide; the other, an electron-abstraction pathway yielding the stilbene cation radical, which reacted with O_2 to form benzaldehyde.[8] The oxygen-18-labeling data presented in Scheme I are consistent with this hypothesis.[9] For each of the oxidants tested, the benzaldehyde oxygen was derived primarily from O_2.[10] In contrast, the source of oxygen in *cis*-stilbene oxide depended on the oxidant used to support the reaction. In all cases, the labeling of deoxybenzoin

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(9) In a typical $H_2{}^{18}O$-labeling experiment, 22 μL of an aqueous solution containing preformed 9.1 mM Fe^{III}BLM was added to an (aerobic) solution that had been formed by admixture of 150 μL of methanol containing 100 mM *cis*-stilbene and 285 μL of 4:1 CH_3OH–$H_2{}^{18}O$ (50% ^{18}O content in H_2O). The reaction was initiated by the addition of 50 μL of 4:1 CH_3OH–$H_2{}^{16}O$ containing 40 mM oxidant. The reaction mixture was maintained at 25 °C for 1 h and then treated with 2 mL of 0.5% aqueous NaCl. The reaction mixture was extracted with 1 mL of CH_2Cl_2, and the organic extract was concentrated and analyzed on a 15 m DB-17 column in a Finnigan-MAT 4610 gas chromatograph–mass spectrometer equipped with an INCOS data system. Reactions conducted under $^{18}O_2$ (^{18}O content 98%) were performed similarly, with the exception that the reaction mixtures were degassed by alternate cycles of vacuum/argon purging in capped 1-mL reacti-vials (Wheaton Scientific) prior to addition of the oxidant. Following addition of $^{18}O_2$ via gastight syringe to a final pressure of approximately 1 atm, a degassed solution of oxidant was added to initiate the reaction.
(10) In addition to the exchange of the C_6H_5CHO oxygen with H_2O under the reaction conditions (23%), some C_6H_5CHO formation was also observed to occur in a BLM- and Fe-independent fashion. Therefore, the ^{18}O-labeling efficiency of C_6H_5CHO by the oxidative pathway envisioned[8] is significantly understated in Scheme I.

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Table I. ^{18}O Content of $(C_6H_5)_3P{=}O$ Formed by $(C_6H_5)_3P$ Oxidation following C_6H_5IO/H_2O Exchange[a]

incubation time, min	solvent system: 5% $H_2{}^{18}O$[b]–95% CH_3OH	solvent system: 40% $H_2{}^{18}O$[c]–60% CH_3OH
0	0	0
0.5[d]	90	
15	91	82
45	92	82
75	89	82

[a] Also included in the incubation was $(CH_3C_6H_4)_3P{=}{}^{16}O$ to assess possible exchange of triarylphosphine oxide with water; no exchange was observed. All experiments were carried out at 25 °C, except as noted. [b] 97 atom % ^{18}O. [c] 35 atom % ^{18}O. [d] Carried out at −10 °C.

was qualitatively and quantitatively the same as for *cis*-stilbene oxide (cf. ref 8). In the H_2O_2 and cumene hydroperoxide supported reactions, for example, no ^{18}O incorporation was observed from either water or O_2, suggesting strongly that the source of epoxide oxygen was the oxidant itself. In fact, when the epoxidation was repeated with $H_2{}^{18}O_2$,[11] the derived *cis*-stilbene oxide was found to contain predominantly ^{18}O (Scheme I). The source of the epoxide oxygen was also established directly where $Fe^{III}BLM$ was activated in the presence of ascorbate + $^{18}O_2$; the formed *cis*-stilbene oxide contained ≃90% ^{18}O in the epoxide oxygen atom. In the C_6H_5IO- and $NaIO_4$-supported reactions, however, the epoxide oxygen was derived primarily from water.

One possible reason for the presence of H_2O-derived oxygen in *cis*-stilbene oxide in the C_6H_5IO and $NaIO_4$-supported reactions could be preequilibration of the oxidant oxygens with water prior to FeBLM activation. Although C_6H_5IO has been reported not to exchange with water,[5b,12] few of the reports contain any experimental detail; one of those that does has subsequently been questioned,[12e] and other studies have reported oxygen exchange.[13] Accordingly, we sought to measure oxygen exchange directly, using $CH_3{}^{16}OH{-}H_2{}^{18}O + C_6H_5I^{16}O$ under conditions comparable to those employed in the BLM-mediated oxygen-transfer experiments reported here.[8] The exchange reactions were quenched by the addition of triphenylphosphine; mass spectrometric analysis of triphenylphosphine oxide provided a measure of oxygen exchange. As shown in Table I, under the conditions of our experiment, exchange with H_2O was complete within 30 s.[14] Also studied by using the same technique was the exchange of periodate with $H_2{}^{18}O$; consistent with an earlier report,[15] exchange was found to occur rapidly. Clearly, the incorporation of ^{18}O into *cis*-stilbene oxide from $H_2{}^{18}O$ will depend on the relative rates of oxygen exchange into the oxidant vs formation of the metal–oxo species. The accumulated data suggest that the observed incorporation of ^{18}O into *cis*-stilbene oxide from $H_2{}^{18}O$ during the C_6H_5IO- and $NaIO_4$-supported FeBLM reactions is consistent with the direct transfer of oxygen to *cis*-stilbene from a high-valent metal–oxo intermediate. Accordingly, the present ^{18}O-labeling study supports the mechanistic scheme proposed earlier,[8] in which FeBLM was suggested to mediate the transformation of *cis*-stilbene both by electron-abstraction and oxygen-transfer pathways.

It has been noted previously that the products formed from small substrate molecules with FeBLM are strikingly similar to those obtained with cytochrome P-450 and related model systems.[5b] The ^{18}O-labeling patterns observed here further extend this analogy. For example, the 5-exo-hydroxylation of camphor by *Pseudomonas putida* cytochrome P-450 was accomplished by ^{18}O incorporation from $H_2{}^{18}O$ when C_6H_5IO was the exogenous oxidant, but not when activation was carried out with $NADH/O_2$, *m*-chloroperbenzoic acid, or cumene hydroperoxide.[12c] Essentially the same results were obtained for the hydroxylation of cyclohexane by liver microsomal cytochrome P-450.[12d] In total, these results reinforce earlier suggestions that FeBLM-mediated oxidation of small molecules shares many common characteristics with cytochrome P-450.[16]

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