and *fac*-IrH_3P_3, it is the heat of protonation of NEt_3 that drives the endergonic *fac* → *mer* transformation. Nevertheless, the fact that substoichiometric $IrH_4P_3^+$ can convert not only *fac* to *mer* but also the reverse indicates that this system lacks the stereospecificity that characterizes the transition state $(P_3IrH_4\cdots NEt_3^+)^\ddagger$. One possibility is that the proton transfer occurs not from $IrH_4P_3^+$ but instead from the unsaturated $IrH_2P_3^+$ whose existence we have demonstrated (eq 3). It is well established that unsaturated complexes condense with hydride complexes to form hydride bridged dimers.[13,14] Such reactions are fast, and fragmentation of $(P_3IrH_2\cdots H_3IrP_3)^+$ (eq 5) need not occur with the

$$IrH_2P_3^+ + \textit{mer}\text{-}Ir^*H_3P_3 \rightarrow P_3IrH_2\cdots H_3Ir^*P_3^+ \rightarrow Ir^*H_2P_3^+ + \textit{fac}\text{- and }\textit{mer}\text{-}IrH_3P_3 \quad (5)$$

same stereoselectivity as shown by $(P_3IrH_4\cdots NEt_3)^+$. This mechanism has the added advantage that it is less susceptible to the steric rate reduction reported previously for proton transfer between a saturated transition-metal hydride and its conjugate base $(HMo(CO)_2(dppe)_2^+$ with $Mo(CO)_2(dppe)_2$).[15] Discrimination between mechanistic alternatives for this unusual reaction is the focus of current work.

Acknowledgment. This work was supported by NSF Grant CHE-8305281 and by a grant from the Gulf Oil Foundation. We thank Johnson Matthey Co. for a loan of chemicals, Eric Westerberg for a generous gift of *fac*-$IrH_3(PMe_2Ph)_3$, and Robert Crabtree for an enlightening observation.

Supplementary Material Available: A listing of spectroscopic data for the cations $IrH_2L(PMe_2Ph)_3^+$, L = N_2, CO, MeCN, and THF (2 pages). Ordering information is given on any current masthead page.

(13) Venanzi, L. M. *Coord. Chem. Rev.* **1982**, *43*, 251.
(14) Compare $2CpRhH_2(PR_3) + 1H^+ \rightarrow [Cp(PR_3)Rh]_2(\mu\text{-}H)_3^+ + H_2$: Werner, H.; Wolf, J. *Angew. Chem., Int. Ed. Engl.* **1982**, *21*, 296.
(15) Hanckel, J. M.; Darensbourg, M. Y. *J. Am. Chem. Soc.* **1983**, *105*, 6979.

Oxygen Transfer by Bleomycin Analogues Dysfunctional in DNA Cleavage

Robert E. Kilkuskie, Hosbett Suguna, Benjamin Yellin, Natesan Murugesan, and Sidney M. Hecht*

Departments of Chemistry and Biology
University of Virginia
Charlottesville, Virginia 22901
Received July 17, 1984

The bleomycins are a family of glycopeptide-derived antitumor antibiotics used clinically for the treatment of squamous cell carcinomas and malignant lymphomas.[1] At least three metallobleomycins mediate oxidative DNA strand scission,[2] and it is this property of the bleomycins that is believed to be responsible for their therapeutic effects. Bleomycin-mediated DNA cleavage is sequence selective[3] and is generally thought to result from DNA recognition and binding by the bithiazole moiety and C-terminal substituent of BLM,[4] and metal chelation and oxygen activation by the N-terminus,[1c,5] although there is only limited direct supporting evidence. The appearance of several recent reports containing data whose interpretation appears inconsistent with this view[6] prompts us to describe experiments that employ bleomycin analogues lacking the putative DNA binding domain. Presently, we demonstrate that the C-terminus of bleomycin is required for DNA strand scission, and that oxygen activation can be effected by the N-terminus alone. Also illustrated for the first time is the transfer of oxygen from an activated Fe complex to a cis olefin with preferential formation of the *trans*-epoxide.

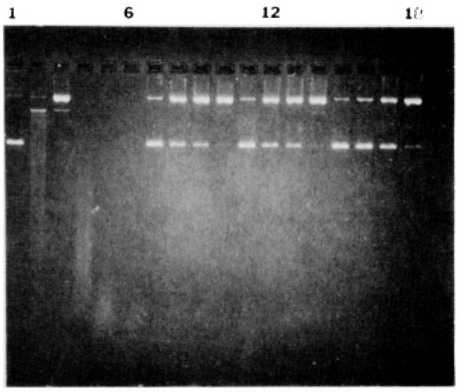
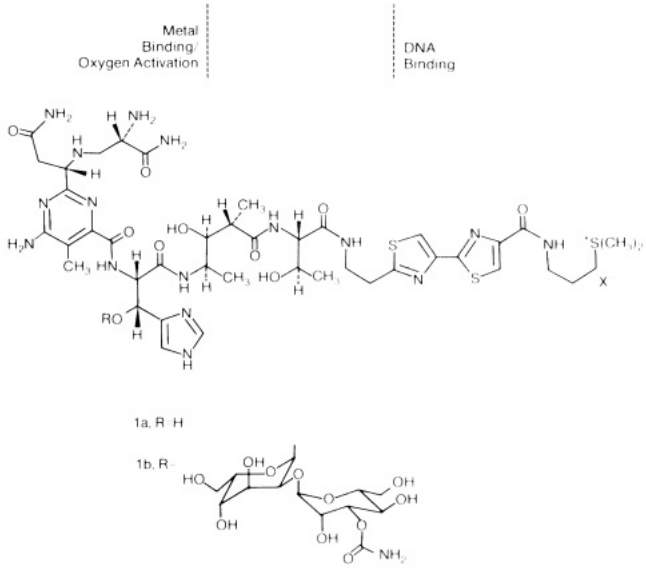


Figure 1. DNA cleavage by bleomycin analogues. Reaction mixtures contained 15 μM SV40 DNA in 20 mM sodium cacodylate, pH 7.0 (lane 1), plus 0.5 μM Fe^{II}·BLM A_2 (lane 2), 1, 5, 10, and 50 μM Fe^{II}·deglyco-BLM A_2 (lanes 3–6, respectively), 1, 5, 10, and 50 μM $Fe(NH_4)_2(SO_4)_2$ (lanes 7–10), 1, 5, 10, and 50 μM Fe^{II}·**2** (lanes 11–14), or 1, 5, 10, and 50 μM Fe^{II}·**3** (lanes 15–18). Lanes 4–6 reflect extensive DNA degradation by deglyco-BLM A_2.

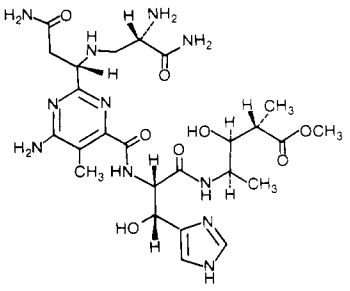
Bleomycin derivatives lacking the carbohydrate moiety (e.g., deglycobleomycin A_2 (**1a**)) bind metal ions and activate oxygen



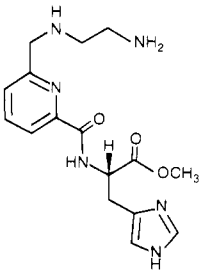
(4) (a) Chien, M.; Grollman, A. P.; Horwitz, S. B. *Biochemistry* **1977**, *16*, 3641. (b) Povirk, L. F.; Hogan, M.; Dattagupta, N. *Ibid.* **1979**, *18*, 96. (c) Kross, J.; Henner, W. D.; Haseltine, W. A.; Rodriguez, L.; Levin, M. D.; Hecht, S. M. *Ibid.* **1982**, *21*, 3711.
(5) (a) Oppenheimer, N. J.; Rodriguez, L. O.; Hecht, S. M. *Proc. Natl. Acad. Sci. U.S.A.* **1979**, *76*, 5616. (b) Takahashi, K.; Ekimoto, H.; Aoyagi, S.; Koyu, A.; Kuramochi, H.; Yoshioka, O.; Matsuda, A.; Fujii, A.; Umezawa, H. *J. Antibiot.* **1979**, *32*, 36. (c) Murugesan, N.; Ehrenfeld, G. M.; Hecht, S. M. *J. Biol. Chem.* **1982**, *257*, 8600. (d) Sugiura, Y.; Suzuki, T.; Otsuka, M.; Kobayashi, S.; Ohno, M.; Takita, T.; Umezawa, H. *Ibid.* **1983**, *258*, 1328.
(6) These include suggestions that the bithiazole moiety may be a metal ligand (Sheridan, R. P.; Gupta, R. K. *J. Biol. Chem.* **1981**, *256*, 1242), that binding of BLM to DNA results in helix shortening rather than elongation[4b] (Langley, K. H.; Patel, M. R.; Fournier, M. J. In "Biomedical Applications of Laser Light Scattering"; Satelle, D. B., Lee, W. I., Ware, B. R., Eds.; Elsevier Biomedical: Amsterdam, 1982; pp 37–49), and that BLM analogues lacking the bithiazole moiety retain some DNA cleavage activity[5d] (Umezawa, H.; Takita, T.; Sugiura, Y.; Otsuka, M.; Kobayashi, S.; Ohno, M. *Tetrahedron* **1984**, *40*, 501).

(1) (a) Umezawa, H. *Biomedicine* **1973**, *89*, 459. (b) Umezawa, H. In "Bleomycin: Current Status and New Developments"; Carter, S. K., Crooke, S. T., Umezawa, H., Eds.; Academic Press: New York, 1978; pp 15 ff. (c) Hecht, S. M. In "Bleomycin: Chemical, Biochemical and Biological Aspects"; Hecht, S. M., Ed.; Springer-Verlag: New York, 1979; pp 1 ff.
(2) (a) Ishida, R.; Takahashi, T. *Biochem. Biophys. Res. Commun.* **1975**, *66*, 1432. (b) Sausville, E. A.; Peisach, J.; Horwitz, S. B. *Biochemistry* **1978**, *17*, 2740. (c) Oppenheimer, N. J.; Chang, C.; Rodriguez, L. O.; Hecht, S. M. *J. Biol. Chem.* **1981**, *256*, 1514. (d) Ehrenfeld, G. M.; Rodriguez, L. O.; Hecht, S. M.; Chang, C.; Basus, V. J.; Oppenheimer, N. J. *Biochemistry*, in press. (e) Ehrenfeld, G. M.; Murugesan, N.; Hecht, S. M. *Inorg. Chem.* **1984**, *23*, 1496.
(3) (a) D'Andrea, A. D.; Haseltine, W. A. *Proc. Natl. Acad. Sci. U.S.A.* **1978**, *75*, 3608. (b) Takeshita, M.; Grollman, A.; Ohtsubo, E.; Ohtsubo, H. *Ibid.* **1978**, *75*, 5983.

nearly as well as the respective bleomycins (bleomycin A_2 (**1b**)).[7] They have been shown to mediate DNA strand scission with the same sequence specificity as the respective bleomycins;[5d] following anaerobic activation with C_6H_5IO both bleomycin and deglycobleomycin converted *cis*-stilbene to *cis*-stilbene oxide.[5c,7] For the present study we employed an analogue of deglycobleomycin (compound **2**[8]) lacking the putative DNA binding domain, as well



2



3

as a structurally simpler analogue (**3**) reported by Hénichart et al.[10]

Shown in Figure 1 is the attempted cleavage of SV40 form I DNA using **2** and **3** in the presence of Fe(II) and O_2.[11] At concentrations of Fe^{II}·**2** (lanes 11–14) and Fe^{II}·**3** (lanes 15–18) up to 50 μM, no conversion to form II (nicked circular) DNA or form III (linear duplex) DNA was noted beyond that produced by Fe(II) alone (lanes 7–10). In contrast, Fe(II)–deglycobleomycin produced extensive DNA degradation when tested over the same concentration range (lanes 3–6).

Although the lack of activity of Fe(II) + **2** or **3** in DNA strand scission seemed likely to be due to the absence of the putative DNA binding domain, it was also possibly due to lack of Fe(II) binding by **2** or **3** or to an inability to activate or transfer oxygen. Accordingly, the formation of Fe^{II}·**2** and Fe^{II}·**3** was established by spectral determination,[12] and each was utilized for the attempted epoxidation of *cis*-stilbene following activation with C_6H_5IO, a transformation already established for bleomycin[5c] and deglycobleomycin.[7] When employed at 0.57 mM concentration, Fe^{III}·**2** and Fe^{III}·**3** both effected epoxidation of *cis*-stilbene; the yields were ~150% in each case, based on added ligand.[13] Similar yields of *trans*-epoxide were obtained when Fe^{II}·**2** or Fe^{II}·**3** were incubated in the presence of *cis*-stilbene + O_2 + ascorbate. This confirmed the activation and transfer of oxygen by **2** and **3** in more traditional bimolecular reactions and served to define those structural components of BLM required for oxygen activation.

One remarkable feature of *cis*-stilbene oxidation by **2** and **3** was the finding that *trans*-stilbene oxide was the predominant product. Previous studies using cytochrome P-450 and related model compounds containing ligated Fe have shown the cis isomer of stilbene to be the preferred substrate for epoxidation and *cis*-stilbene oxide to be the predominant product.[14] Analogous findings for three metallobleomycins[2e,5c] and two metallodeglycobleomycins[7] have reinforced these observations, as well as the mechanistic similarities between bleomycin and cytochrome P-450 as regards oxygen activation and transfer. The present finding parallels the observation by Valentine and co-workers that *trans*-stilbene oxide was produced from *cis*-stilbene via the agency of $Cu(NO_3)_2 + C_6H_5IO$.[15] It seems reasonable to suggest that the stereoselectivity noted previously for *cis*-stilbene finds its basis in the greater steric accessibility of this isomer to the bulky epoxidizing agents.[16]

Acknowledgment. We thank Dr. Peter Dervan for a helpful discussion at the outset of this work. This work was supported by Research Grants CA-27603 and CA-29235, awarded by the National Cancer Institute, Department of Health and Human Services.

(7) Aoyagi, Y.; Suguna, H.; Murugesan, N.; Ehrenfeld, G. M.; Chang, L.-H.; Ohgi, T.; Shekhani, M. S.; Kirkup, M. P.; Hecht, S. M. *J. Am. Chem. Soc.* **1982**, *104*, 5237.

(8) Prepared by DCC-mediated coupling of BOC-pyrimidoblamic acid[9] with methyl (2*S*,3*S*,4*R*)-4-(L-*erythro*-β-hydroxyhistidylamino)-3-hydroxy-2-methylvalerate,[7] followed by deblocking (CF_3COOH, CH_3SCH_3, 25 °C, 1 h).[7]

(9) Umezawa, Y.; Morishima, H.; Saito, S.; Takita, T.; Umezawa, H.; Kobayashi, S.; Otsuka, M.; Narita, M.; Ohno, M. *J. Am. Chem. Soc.* **1980**, *102*, 6631. (b) Arai, H.; Hagmann, W. K.; Suguna, H.; Hecht, S. M. *Ibid.* **1980**, *102*, 6633.

(10) Hénichart, J.-P.; Houssin, R.; Bernier, J.-L.; Catteau, J.-P. *J. Chem. Soc., Chem. Commun.* **1982**, 1295.

(11) Reaction mixtures (40 μL) containing 15 μM SV40 DNA, 1–50 μM $Fe(NH_4)_2(SO_4)_2$, and 1–50 μM **1a**, **2**, or **3** in 20 μM sodium cacodylate, pH 7.0, were incubated at 25 °C for 1 h. The reaction was terminated (1 mM EDTA) and samples were loaded onto 1.2% agarose gels containing 1 μg/mL ethidium bromide for electrophoretic analysis (16 h at 40 V in 40 mM Tris-OAc, 5 mM NaOAc, 1 mM EDTA, pH 7.8).

(12) For both **2** and **3**, the addition of Fe(II) in increasing concentrations up to 1 equiv caused increased absorption at the observed λ_{max} (282 and 268 nm, respectively), analogous to changes noted for BLM.

(13) An anaerobic solution (O_2-free argon) containing 0.12 μmol of **2** or **3** and 5 μg of $Fe(ClO_4)_3$ (0.12 μmol) in 25 μL of H_2O was incubated (10 min, 25 °C) and then treated with *cis*-stilbene (2 mg, 11.1 μmol) in 135 μL of CH_3OH. Iodosobenzene (0.8 mg, 3.6 μmol) was added dropwise (50 μL CH_3OH) over a period of 10 min. After an additional 1 h at 25 °C, the reaction was subjected to extractive workup and analyzed by HPLC.[2e]

(14) (a) Groves, J. T.; Nemo, T. E.; Myers, R. S. *J. Am. Chem. Soc.* **1979**, *101*, 1032. (b) Groves, J. T.; Nemo, T. E. *Ibid.* **1983**, *105*, 5786.

(15) Franklin, C. C.; Van Atta, R. B.; Fan Tai, A.; Valentine, J. S. *J. Am. Chem. Soc.* **1984**, *106*, 814.

(16) Consistent with this suggestion was the observation that variation in porphyrin or olefin substitution significantly altered chiral induction during the epoxidation of prochiral olefins: Groves, J. T.; Myers, R. S. *J. Am. Chem. Soc.* **1983**, *105*, 5791.

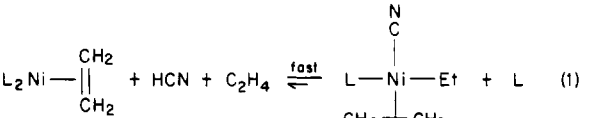
(Ethylene)ethylnickel Cyanide Complex Intermediate in Catalytic Hydrocyanation of Ethylene. Reductive Elimination by an Associative Process

Ronald J. McKinney* and D. Christopher Roe

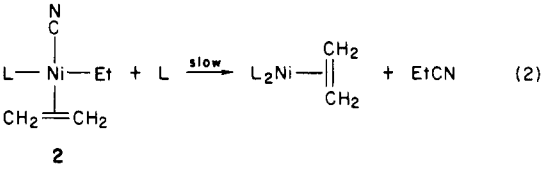
Contribution No. 3609
Central Research & Development Department
E. I. du Pont de Nemours and Company
Experimental Station, Wilmington, Delaware 19898

Received October 1, 1984

The reaction of (ethylene)bis(tri-*o*-tolyl phosphite)nickel, $(C_2H_4)L_2Ni(0)$ [L = P(O-*o*-tolyl)$_3$] (**1**), with ethylene and hydrogen cyanide at −40 °C produces $(C_2H_4)L(CN)(C_2H_5)Ni(II)$ (**2**) quantitatively (eq 1). Reaction of **2** with tri-*o*-tolyl phosphite



(1)



(2)

(L) causes reductive elimination of propionitrile and regenerates **1** (eq 2).

As part of our continuing studies of olefin hydrocyanation, we carried out kinetic measurements of the previously reported nickel-catalyzed hydrocyanation of ethylene,[1] eq 3, at low tem-

$$HCN + C_2H_4 \xrightarrow{Ni(0)} C_2H_5CN \qquad (3)$$

perature utilizing proton NMR spectroscopy. Starting with the ethylene complex **1** rather than the $[(o\text{-tolyl-O})_3P]_3Ni$ previously

(1) Tolman, C. A.; Seidel, W. C.; Druliner, J. D.; Domaille, P. J. *Organometallics* **1984**, *3*, 33–38.

0002-7863/85/1507-0261$01.50/0