This hypothesis was consistent with the fact that ^1H NMR did not reveal any peak for acid protons. On the other hand, until this work, due to the difficulty of purifying SbF_5, no experimental proof was available to exclude the participation of residual protons when the neat Lewis acid was used. In this case, the protolytic cleavage of the C-H bond could occur at a much faster rate in an autocatalytic way via reaction 4.

In our experiments, the simultaneous production of the protons and the carbocation in the presence of SbF_5 throws considerable doubt on both proposed reduction mechanisms.

In itself, the postulated direct hydride abstraction is not very plausible as already indicated by Olah, because a very strong C-H bond would be heterolytically cleaved to form the weak Sb-H bond. On top of this, the existence of a metal hydride, even as a short-lived intermediate, in the presence of strong electrophiles (such as the proton that is formed, the proton on the carbonyl, and the activated carbonyl group) without chemical reaction seems an unacceptable hypothesis. Unfortunately, this concept of hydride abstraction from saturated alkanes by Lewis acids has already found its way into the literature with extension to aluminum halides.[13]

Our experiments with acetone exclude also the hypothesis of the reduction of SbF_5 by the protonated alkane. In the presence of acetone, the protons are quantitatively trapped by the carbonyl group, no hydrogen is formed, and no protons are available for alkane protonation. Nevertheless, the formation of the ion and reduction of SbF_5 are verified. When deuterated isobutane (2-deuterio-2-methylpropane) was used as starting material in the presence of acetone, the quantitative formation of $(CH_3)_2COD^+$ could be followed by ^2H NMR. This experiment again proves that the proton is formed during the oxidation step of the hydrocarbon.

In 1976, Larsen[8] applied classical thermodynamics to the various superacid-catalyzed hydrocarbon oxidation processes proposed in the literature and found that the direct oxidation of isobutane by SbF_5 (reaction 1) should not occur because the reaction was strongly endothermic. In light of our results, this prediction seems puzzling, as in the same paper it was shown that SbF_5 was able to oxidize hydrogen and further that protons were

$$SbF_5 + H_2 \rightarrow SbF_3 + 2HF \qquad -33 \text{ kcal}$$

able to oxidize isobutane. This contradiction is probably due to the lack of data concerning the solvation enthalpy of the various species in superacid solution.

That SbF_5 itself was the oxidant has also been suggested earlier by Herlem[13] by comparison with HSO_3F-containing systems where the formation of SO_2 during the ionization process could be measured.

Formally the oxidoreduction process is best represented as

$$SbF_5 + 2e^- \rightarrow SbF_3 + 2F^-$$

$$RH - 2e^- \rightarrow R^+ + H^+$$

The excess SbF_5 accepts the fluoride ions to form polymeric anions, which are the well-known low-nucleophilic counterions in superacid media, whereas the proton will attack rapidly the strongest base present in the system. In the presence of acetone, it will be trapped by the carbonyl group and no hydrogen is formed. In the absence of acetone, the strongest base is the alkane and the protolytic ionization process will occur via protonation of the tertiary C-H bond in accord with the σ-bond reactivity sequence proposed by Olah,[11] tertiary C-H $\gg$ C-C $>$ secondary C-H $\gg$ primary C-H, and the stoichiometric amount of hydrogen (based on isobutane) will be recovered; the differentiation between Lewis and Brønsted acid cannot be established.

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Registry No. *i*-C_4H_{10}, 75-28-5; SBF_5, 7783-70-2.

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Iron(II)-Bleomycin-Mediated Reduction of O_2 to Water: An ^{17}O Nuclear Magnetic Resonance Study

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The bleomycins (BLMs) are antitumor agents believed to exert their therapeutic effects via DNA degradation.[1] Bleomycin-mediated DNA degradation requires O_2 and a redox-active metal ion such as Fe, Cu, or Mn.[2,3] Extensive mechanistic studies of DNA degradation have established two sets of products and suggested that both form by initial abstraction of the C-4′ H from deoxyribose.[3,4]

Although the formation of activated Fe·BLM involves the reduction of oxygenated Fe(II)·BLM,[5] much less is known about the stoichiometry of O_2 consumption or the fate of the O atoms when BLM is activated in the presence or absence of DNA. To characterize O_2 participation in greater detail, we have employed ^{17}O NMR spectroscopy[6] to monitor product formation concomitant with $^{17}O_2$ consumption by Fe(II)·BLM in the presence and absence of DNA.

Admixture of equimolar quantities of Fe(II) and BLM in oxygenated solution has been shown to afford equal amounts of Fe(III)·BLM and an activated Fe·BLM[3b,4f,5] believed to contain a reactive, coordinated oxygen.[3,5] In the absence of DNA, the activated species decays within minutes to Fe(III)·BLM,[5a-c] presumably with concomitant formation of H_2O. Formulation of the catalytic cycle as a $4e^-$ reduction of O_2 to H_2O, consistent with other metal-catalyzed O_2 reductions,[7] is supported by O_2 consumption data[5b] and the $2e^-$ titration of activated Fe·BLM to Fe(III)·BLM with I^- or thio-NADH.[8] The low natural abundance

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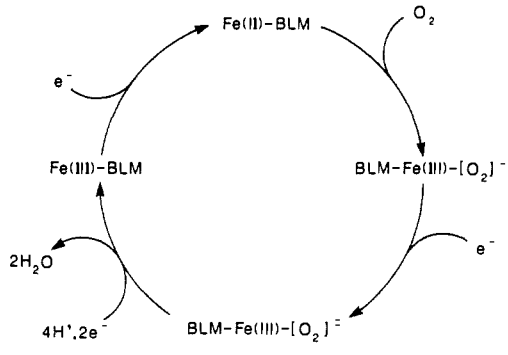
Table I. Production of $H_2{}^{17}O$ from Fe(II)·BLM + $^{17}O_2$[a]

Fe(II)·BLM, mM	reactant, mM	$H_2{}^{17}O$, mM calcd	$H_2{}^{17}O$, mM found	DNA products, mM base	DNA products, mM base propenal
20		10	9.4		
20	I^- (200)[b]	20	18.7		
5	calf thymus DNA (30)	6.1[c]	5.3	0.60	1.01
10	d(CGCTAGCG) (50)	7.3[c]	7.4	1.78	0.18

[a] BLM was dissolved in 400 μL of $H_2{}^{16}O$ (^{17}O-depleted) and placed in a 10-mm NMR tube. Sodium cacodylate was added (final concentration 50 mM), followed by 500 μL of dry THF. Argon was bubbled through the solution, and an ^{17}O NMR spectrum was recorded. The solution was then degassed, and $^{17}O_2$ (59% ^{17}O) was added (gas-tight syringe) followed by 100 μL of 0.2 M $Fe^{II}(NH_4)_2(SO_4)_2$ (deoxygenated $H_2{}^{16}O$). The reaction was maintained at 0 °C for 30 min, and a second ^{17}O NMR spectrum was recorded. Where a DNA substrate was employed, the reaction was carried out in 250 μL of 50 mM sodium cacodylate, in a glass tube inserted within the NMR tube. The DNA addition was made prior to recording of the first spectrum; an aliquot of the reaction mixture was removed for HPLC product analysis,[4f] and the remainder was used for ^{17}O NMR measurements over 5 days to measure exchange of ^{17}O-oxygenated intermediates with bulk solvent. [b] I^- was added, resulting in the production of 11.7 mM I_2 (vs 10 mM expected), as determined by starch–iodide titration. [c] The calculated yield of $H_2{}^{17}O$ is based on the observed quantities of base and base propenal formed and the assumption that all oxygenated DNA intermediates eventually exchange with solvent to produce $H_2{}^{17}O$. For complete utilization of 5 mM Fe(II)·BLM to form free bases, the theoretical yield of $H_2{}^{17}O$ would be 5 mM; the corresponding value for base propenal production would be 10 mM.

Scheme I. Production of H_2O from Fe(II)·BLM + O_2



of ^{17}O suggested that ^{17}O NMR might permit direct observation and quantification of the $H_2{}^{17}O$ putatively formed upon admixture of Fe(II)·BLM + $^{17}O_2$.[9] The increase in intensity of the $H_2{}^{17}O$ resonance[10] (Figure 1, supplementary material) could be investigated under a variety of conditions (Table I).

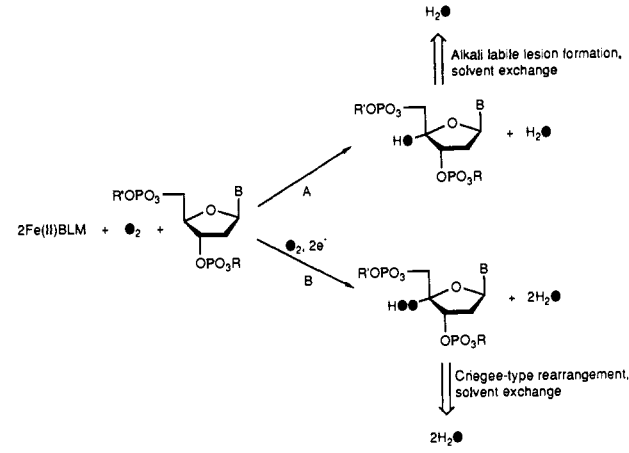
Absent DNA or added reductants, the reduction of O_2 by Fe(II)·BLM is envisioned to proceed as outlined in Scheme I. Thus the stoichiometry of H_2O production would be as indicated in eq 1, assuming that the requisite reducing equivalents all derive

$$4\text{Fe(II)·BLM} + O_2 + 4H^+ \rightarrow 4\text{Fe(III)·BLM} + 2H_2O \quad (1)$$

$$H_2O/\text{Fe·BLM} = 0.5$$

from Fe(II)·BLM. Accordingly, 20 mM Fe(II)·BLM would be expected to yield 10 mM $H_2{}^{17}O$; 9.4 mM $H_2{}^{17}O$ (94%) was actually observed (Table I).[11] As this analysis assumes that two Fe(II)·BLM's are required to reduce activated Fe·BLM, the finding that I^- can effect the same reduction[8] suggested that inclusion of I^- should alter the stoichiometry of $H_2{}^{17}O$ formation.[13] Experimentally, inclusion of excess I^- in the Fe(II)·BLM–$^{17}O_2$ reaction doubled the amount of $H_2{}^{17}O$ produced (Table I). Starch–iodide titration verified the production of I_2, essentially in the amount predicted. These data are consistent with the catalytic cycle outlined in Scheme I.

Scheme II. Disposition of ^{17}O Resulting from Oxidative DNA Degradation by Fe·BLM[a]



[a] ^{17}O-Labeled oxygen is represented by the darkened circles; double arrows represent exchange of oxygenated DNA intermediates with bulk solvent.

In the presence of DNA, net O_2 consumption is increased.[5b] The accepted mechanism of DNA degradation by Fe·BLM[3,4] (Scheme II) posits the partitioning of an initially generated C-4′ deoxyribose radical along two pathways, the proportion of which can be controlled by adjusting O_2 tension.[4g,14] Hydroxylation at C-4′ (pathway A) presumably results from the transfer of one O atom from activated Fe·BLM to DNA.[15] Collapse of this intermediate should result in the eventual release of two $H_2{}^{17}O$ molecules for every two Fe(II)·BLM's utilized (cf. Scheme II).

Pathway B, which leads to strand scission via the intermediacy of a C-4′ hydroperoxide, requires an additional equivalent of O_2 and can potentially produce four molecules of $H_2{}^{17}O$ (Scheme II).[16]

Experimentally, Fe(II)·BLM was incubated with the self-complementary octanucleotide d(CGCTAGCG) or calf thymus DNA, under limiting or saturating $^{17}O_2$, respectively. Reactions were analyzed for free base and base propenal formation; the extent of conversion of $^{17}O_2$ to $H_2{}^{17}O$ was determined by ^{17}O NMR spectroscopy. Degradation of 30 mM calf thymus DNA produced 0.60 mM total free bases and 1.01 mM base propenals. On the basis of the foregoing analysis, the amount of $H_2{}^{17}O$ produced was predicted to be 6.1 mM; 5.3 mM (87%) was actually detected.[17] Likewise, incubation of 50 mM d(CGCTAGCG) with Fe(II)·BLM produced 1.78 mM total free bases and 0.18 mM base propenals. The amount of $H_2{}^{17}O$ produced was predicted to be 7.3 mM; 7.4 mM $H_2{}^{17}O$ was observed.

In addition to demonstrating unequivocally that H_2O is a by-product of aerobic Fe·BLM activation and BLM-mediated DNA degradation, and providing support for the mechanisms outlined in Schemes I and II, ^{17}O NMR may permit the direct observation and quantification of putative oxygenated DNA reaction intermediates and thereby resolve certain mechanistic ambiguities.[15]

(9) Our experiments were carried out in ^{17}O-depleted (2.6 mM) $H_2{}^{16}O$.

(10) The increase in signal intensity was quantitated by comparison with added THF (resonance at ~20 ppm). One such spectrum is provided as supplementary material.

(11) A small percentage of activated Fe·BLM undergoes self-inactivation in the absence of substrate,[5d,12] presumably by oxygenation of the BLM molecule.

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(15) Alternatively, C-4′ hydroxylation could result from 1e$^-$ oxidation of the deoxyribose radical to a cation, followed by reaction with solvent H_2O. Direct exchange of an O atom from activated Fe·BLM with solvent would seem to be excluded by the finding that ^{18}O is transferred from activated BLM to olefinic substrates. See: Heimbrook, D. C.; Carr, S. A.; Mentzer, M. A.; Long, E. C.; Hecht, S. M. *Inorg. Chem.* **1987**, *26*, 3835.

(16) Presumably some $H_2{}^{17}O$ molecules would be formed by solvent exchange with DNA degradation intermediates. See, e.g.: McGall, G. H.; Rabow, L. E.; Stubbe, J.; Kozarich, J. W. *J. Am. Chem. Soc.* **1987**, *109*, 2836.

(17) If one of the four O atoms utilized in pathway B were not released as $H_2{}^{17}O$ during the course of this experiment, the predicted value would be 5.1 mM.

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Supplementary Material Available: ^{17}O NMR spectra for the Fe(II)·BLM-mediated formation of $H_2{}^{17}O$ from $^{17}O_2$ (2 pages). Ordering information is given on any current masthead page.

Stereostructure of Pimaricin

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Pimaricin, isolated in 1957 from *Streptomyces natalensis*,[1] is the first polyene macrolide whose correct covalent structure **1** was established[2] after a 10-year period of numerous revisions.[3,4] Its comparison with the very similar tetraenic antibiotic tetrin A[5] in which the *S* configuration at C_{25} was established[6] led to a confusing stereochemical situation for pimaricin for which the same *S* configuration at C_{25} was given.[7] Even more disconcerting was the description of the absolute configurations of other asymmetric centers which were by no means proven.[8] Pimaricin represents a prototype molecule of the glycosylated polyene macrolides,[9] important for antifungal therapy and promising for other properties including antiviral activity, stimulation of the immune response, and action in synergy with other antifungal drugs or antitumor compounds.[10] A long-standing lack of stereostructural information has been the major obstacle for interpreting structure–activity relationships. We now report the complete stereostructure of pimaricin,[11] whose convenient solution arose from our recent study on nystatin A_1.[12]

The basis of our approach includes (a) a combined use of phase-sensitive DQF-COSY,[13] NOESY,[14] and/or ROESY[15] 2D proton NMR experiments[16] for assessing relative configurational features, (b) a search for unambiguous proton–proton through-space contacts with the sugar D-mycosamine taken as an internal chiral probe to attain the absolute configuration, and (c) execution of minimal chemical modifications of the natural substance to identify configurations left unknown after the above procedures.

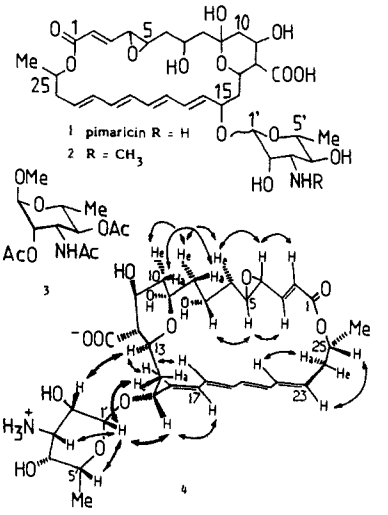

Figure 1. The double-headed arrows in structure **4** connect the pairs of hydrogens that are correlated by NOE (ROE) interactions. Only selected NOE (ROE) connectivities are shown; contacts between scalar coupled protons are not quoted. Ha(e) at C_6, C_8, C_{14}, and C_{24} refer to the pseudoaxial (a) or pseudoequatorial (e) orientation of these protons relative to the average plane of the macrocycle.

The D series of the mycosamine sugar[3a] was first confirmed by standard deglycosidation of pimaricin (HCl, MeOH, reflux, 2 h) and acetylation to di-*O*-acetate **3**, mp 139 °C, $[\alpha]_D$ +30°, identical with the compound obtained from nystatin A_1.[17] Analysis of phase-sensitive DQF-COSY experiments (10 mM MeOH-d_4 or DMSO-d_6 solutions) furnished the complete ^1H–^1H coupling pattern of pimaricin **1** and its *N*-acetyl derivative **2** (see supplementary material). This information combined with pertinent NOE contacts (see structure **4**, Figure 1) made the following structural assignments possible: (a) the chair conformation of the C_9–C_{13} segment with substituents at C_{11}, C_{12}, and C_{13} equatorials ($J_{10a,11}$ = 11.0, $J_{10e,11}$ = 4.8, $J_{11,12}$ = 10.5, and $J_{12,13}$ = 10.5 Hz) confirming previous observations;[18,19] (b) the axial orientation of OH_9, the first direct observation for structurally related polyene macrolides in solution ($J_{OH9-H10a}$ = 0.5–1 Hz in DMSO-d_6); (c) the diastereotopicity of the H_8 protons (see structure **4** for NOE contacts), hence the configuration at C_7 relative to the C_9–C_{13} tetrahydropyran; and (d) an accurate local geometry of the C_{13}–C_{16} segment by the *J* connectivities ($J_{13,14a}$ = 8.4, $J_{13,14e}$ = 1.0, $J_{14a,15}$ = 2.0, $J_{14e,15}$ = 3.5, and $J_{15,16}$ = 8.3 Hz) and observations of the NOE contacts, especially for the proton pairs H_{13}–H_{16} and H_{15}–H_{17}, defined the diastereotopicity of the H_{14} protons and thus the configuration at C_{15} relative to the one at C_{13}.

To attain the absolute configuration of this structural segment, the NOE map furnished three crucial data: the previously suggested[18a] β-configuration of the anomeric linkage at O_{15} of the aglycon ($H_{1'}$–$H_{3'}$ and $H_{1'}$–$H_{5'}$ NOE contacts), the proximity of the anomeric proton $H_{1'}$ to *both* H_{14e} and H_{15} of the aglycon ($H_{1'}$–H_{14e} and $H_{1'}$–H_{15} NOE contacts), and the sufficiently close location of $H_{2'}$ to H_{13} of the aglycon to observe a NOE connectivity. Only an *R* configuration at C_{15} can conform to the last

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