structure of **5**, were unsuccessful. Examination of molecular models reveals that the bridgehead carbon–hydrogen bonds are nearly parallel to the carbonyl group, with dihedral angles, calculated by MM2,[17,18] of 27° and 150°, so that the kinetic acidity of each is quite low.

Ketone **5** was also different from the previously reported *cis*-bicyclo[4.3.2]undecanone **14**[19] (by NMR and IR), which would have arisen from the "crossed" photoadduct **13** (Scheme III). To rule out the possibility that the product was the unknown *trans*-bicyclo[4.3.2]undecanone, an X-ray analysis was performed on the intermediate keto acid **11** (recrystallized as a monohydrate from methyl alcohol, mp 104–109 °C for the liberation of H_2O, then 137–139 °C), which confirmed the structural assignment shown for **5** (Chart I).[20] The formation of the trans isomer can be explained by a chairlike six-membered-ring transition state in the photocycloaddition. As shown in Chart I, the two bridgehead hydrogens are then necessarily trans.

In conclusion, we note that the intramolecular photocycloaddition of dioxolenones has important advantages over the more classical de Mayo diketone sequence.[21] Aside from the benefits of regiochemical control afforded by the use of the β-keto esters, this new methodology makes accessible the *trans*-bicyclo[5.3.1]undecane ring system **5**, which cannot be prepared by the standard de Mayo reaction.[22] The extension of this bicycloundecane ring construction to the synthesis of the taxanes, and the application of this methodology to the construction of other inside–outside bicycloalkanes, notably the *trans*-bicyclo[4.4.1]undecane ring system of the ingenane diterpenes,[23] is currently under way in our laboratories.

Acknowledgment. We thank Professor Edgar Warnhoff for a generous sample of *cis*-bicyclo[5.3.1]undecan-11-one (**12**). We also thank Professors Josef Fried and Raymond Funk for valuable discussions. Support from the donors of the Petroleum Research Fund, administered by the American Chemical Society, the National Institutes of Health (CA40250 to J.D.W., GM35982 to P.G.W., and GM07148 in the form of a training grant fellowship to J.P.H.), an American Cancer Society Institutional Grant, and Merck, Sharp and Dohme is gratefully acknowledged. The NMR instruments used were funded in part by the NSF Chemical Instrumentation Program and by the NCI via the University of Chicago Cancer Research Center (CA 14599).

Supplementary Material Available: Tables of atomic coordinates, bond lengths, bond angles, anisotropic thermal parameters, and hydrogen atom positions for the crystal structure of **11** (5 pages). Ordering information is given on any current masthead page.

(17) Calculated by using the Gajewski/Gilbert modification of the Allinger MM2 program (no. 395, Quantum Chemistry Program Exchange, Indiana University), which is commercially available through Serena Software, Bloomington, IN.

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Photosensitization of Cobalt Bleomycin

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Bleomycin (Blm), a glycopeptide-derived anticancer drug,[1] causes strand scission of DNA both in vivo and in vitro.[2] Cleavage of DNA by Blm has been extensively studied, with the finding that transition-metal ions such as Fe(II) must be present.[3] Blm has also been employed in combination chemotherapy with other drugs.[4] In vitro studies have shown that the DNA-cleaving activity of Blm is substantially modified by *cis*-diamminedichloroplatinum(II); a number of new cleavage sites are produced.[5] Hence the synergism between these drugs may be related to interactions at the level of DNA drug binding.

The chemically inert complex of Blm with cobalt(III), CoBlm, is known to accumulate in certain human cancers.[6] CoBlm also binds strongly to DNA in vitro ($K_d \approx 10^{-6}$ M) and can cause DNA strand breaks when irradiated with UV light.[7] The cleavage is sequence-dependent: pyrimidines attached to the 3′-side of guanine are attacked.[7b] Presumably, cleavage of DNA is a consequence of photoreduction of CoBlm (irradiation of Co(III) complexes in the ligand-to-metal charge-transfer region often leads to production of Co(II)[8]). CoBlm does not degrade DNA when irradiated with visible light. However, the extensive studies of electron transfer from ruthenium(II) polypyridyl complexes to various cobalt(III) ammine complexes[9] suggested to us that it might be possible to use ruthenium(II) tris(bipyridyl) as a sensitizer, to activate CoBlm in the presence of visible light.

Tris(2,2′-bipyridine)ruthenium(II), $Ru(bpy)_3^{2+}$, is a small cation which is electrostatically attracted to DNA[10] and may be expected

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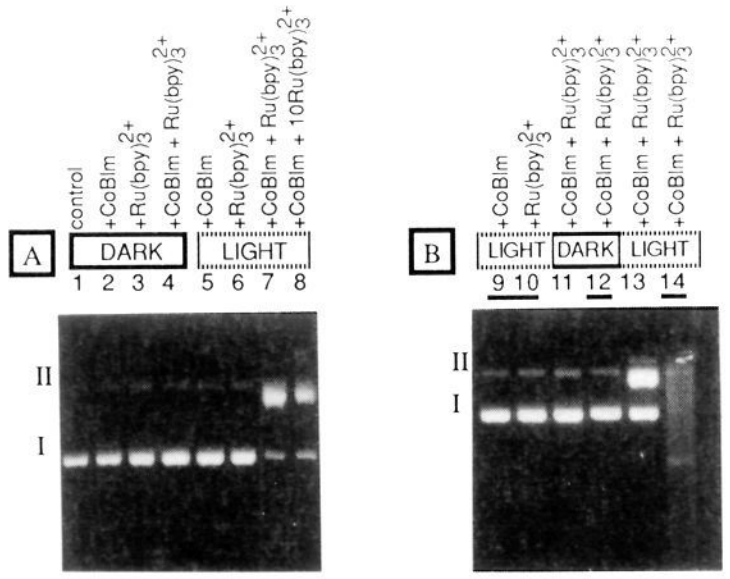

Figure 1. $Ru(bpy)_3^{2+}$-sensitized cleavage of ϕX174 RF I DNA by CoBLM, and production of alkali-labile sites. Reaction mixtures containing (in a total volume of 25 μL) 25 mM Tris borate, pH 8.1, 190 μM EDTA and 1 μg of ϕX174 RF I DNA were incubated (either in the dark or under illumination) with the indicated additions of CoBlm (final 11 μM) and/or $Ru(bpy)_3^{2+}$ (final 3 μM). Finally, the samples in lanes 9, 10, 12, and 14 (underlined) were maintained at pH 11.5 for 2 h in the dark following the addition of 10 μL of a sucrose/EDTA/dye mixture and 1 μL of 1.67 M NaOH/25 mM EDTA. Only the sample in lane 14 was hydrolyzed by this alkaline treatment. Electrophoresis was carried out in 1% (w/v) agarose, according to Chang and Meares.[7] DNA bands were visualized by ethidium fluorescence.

to diffuse one dimensionally along the chain.[11] Because of the enormous negative charge density of DNA, encounters between divalent cations and DNA-bound CoBlm are expected to be orders of magnitude more frequent than they would be in the absence of DNA.[12]

Under an ordinary 60-W incandescent light bulb for 1 h, supercoiled ϕX174 DNA (form I)[13] is almost quantitatively cleaved to nicked circular DNA (form II) in the presence of $Ru(bpy)_3^{2+}$ and CoBlm[14,15] (Figure 1A, lane 7). No DNA cleavage occurs in the absence of light (Figure 1A, lanes 2–4) or in the absence of $Ru(bpy)_3^{2+}$ (lane 5) or CoBlm (lane 6).[16] Further, using a 5′-^{32}P end-labeled 121-base-pair DNA restriction fragment as the substrate, polyacrylamide gel electrophoresis indicates that the cleavage fragments formed are essentially identical with those formed by UV irradiation of CoBlm (compare Figure 2, lanes 5 and 6).[17] Strand scission occurs mainly at the 3′-side of guanosine residues,[18] producing DNA cleavage fragments that migrate as

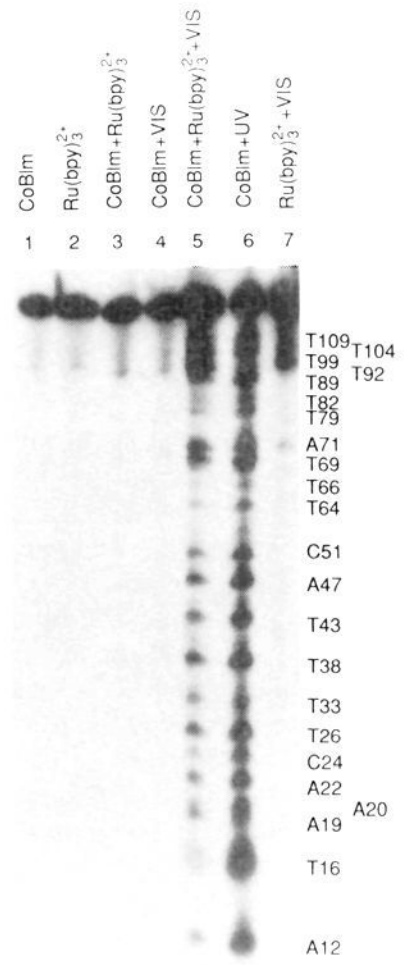

Figure 2. Sequence-dependent cleavage of a 121-base-pair T7-DNA restriction fragment[17] by Co^{III}Blm in the presence of $Ru(bpy)_3^{2+}$ and visible light. Each reaction mixture contained (in a total volume of 10 μL) 25 mM Tris borate, pH 8.1, 190 μM EDTA, and 5′-^{32}P-labeled double-stranded DNA. The reaction mixtures also contained the indicated additions of CoBlm (final 11 μM) and/or $Ru(bpy)_3^{2+}$ (final 3 μM), except that lane 6 was 1 μM CoBlm. Samples for lanes 4, 5, and 7 were irradiated with visible light; the sample in lane 6 was irradiated with approximately 100× more photons of 366-nm ultraviolet light.[23e] The other samples were incubated in the dark. Note that no nicking is observed in the absence of light, even in the presence of both $Ru(bpy)_3^{2+}$ and CoBlm (lane 3). At the end of the reaction the degraded DNA was ethanol precipitated twice and subjected to polyacrylamide gel electrophoresis and autoradiography.[7b] To identify the sites of DNA damage which are listed along the side, four sets of base-specific cleavage products were prepared[17] (not shown).

sharp bands on high-resolution gel electrophoresis.[19] The 121-base-pair DNA restriction fragment has the following sequence. Only the strand labeled with ^{32}P is shown: 5′-^{32}P-CTATTTAC-CA_{10} $GATTGTTAAA_{20}$ $GAGCGTCTTA_{30}$ $GGTCAGGTTT_{40}$ $CGTTAGACCG_{50}$ $CATCACCTTT_{60}$ $CAGTGTGGTG_{70}$ $ACTCACTGTC_{80}$ $GTTTCATGTC_{90}$ $GTACATCGTA_{100}$ $CCGTGTTTAC_{110}$ $TTCATGTTGT_{120}C_{121}$. The CoBlm–$Ru(bpy)_3^{2+}$ cleavage sites are in italics.

This treatment also produces sites cleavable by alkali. Alkali-labile sites, presumably formed by removal of nitrogeneous bases from DNA, are converted to single-strand breaks by exposure to high pH.[20] When ϕX174 DNA samples were treated with CoBlm, $Ru(bpy)_3^{2+}$, and light and then incubated for 2 h at pH 11.5 in the dark, the DNA was found to be degraded into smaller fragments, which appear as a diffuse band (Figure 1B; lane 14).

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(13) ϕX174 DNA, purchased from Bethesda Research Labs, was found to contain more than 95% form I DNA as shown by 1% agarose gel electrophoresis.

(14) CoBlm was prepared from $CoSO_4$ and Bleonoxane (gift sample from Bristol Labs, Syracuse, NY). The A_2 green fraction of CoBlm was separated by HPLC as described earlier[7] and was used for all these experiments. $Ru(bpy)_3^{2+}$ obtained from Johnson Matthey Inc. was purified by repeated precipitation with methanol from an aqueous solution.

(15) The concentrations of $Ru(bpy)_3^{2+}$ and CoBlm were determined by spectrophotometric absorption measurements. For $Ru(bpy)_3^{2+}$, $\epsilon_{452} = 14\,600$ M^{-1} cm^{-1};[9] for CoBlm, $\epsilon_{292} = 18\,000$ M^{-1} cm^{-1}.[7b] In cleavage reactions, $Ru(bpy)_3^{2+}$ was the last component added prior to irradiation.

(16) Reactions were carried out in siliconized and sterilized eppendorf vials by using a General Electric 60-W incandescent lamp (intensity ~ 7 $\mu W/cm^2$, at 450 nm $\leq \lambda \leq$ 460 nm) at a distance of about 10 cm. Care was taken not to expose the reaction mixtures to room light. All the apparatus used in this investigation was acid-washed and thoroughly rinsed with deionized, distilled water to avoid heavy metal contamination. The reaction vials were kept in crushed ice.

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(18) Similar sequence specificity of nicking was also observed when a 5′-^{32}P end-labeled 68-base pair DNA restriction fragment[7b] was used as the reaction substrate. Also, the thiobarbituric acid test[7b] indicates that there is no base–propenal formation. In addition, interstrand cross-linking of DNA was observed after visible irradiation of DNA in the presence of CoBlm and $Ru(bpy)_3^{2+}$.

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These results support the hypothesis that CoBlm cleavage of DNA is a consequence of the photoreduction of Co(III). It is interesting to note that forming CoBlm by air oxidation after mixing Co^{2+} and Blm in the presence of DNA is not accompanied by significant strand scission. Nor does irradiation of $Ru(bpy)_3^{2+}$ and $[Co(NH_3)_6]^{3+}$ in the presence of DNA cause cleavage.[21] Evidently, strand scission requires prior formation of the $[Co^{III}Blm]$–DNA complex. This complex is known to involve very close association of the cobalt center to the DNA[12] and is thought to involve intercalation of the Blm bithiazole moiety between DNA base pairs.[22]

These results show that $Ru(bpy)_3^{2+}$ sensitizes the cobalt–bleomycin-mediated cleavage of DNA in the presence of visible light, and the cleavage has the sequence specificity characteristic of bleomycin. Since CoBlm is known to accumulate preferentially in certain types of cancer cells in vivo,[6] such sensitized cleavage of DNA might be used in the light-mediated treatment of cancer.[23] We are investigating covalent conjugation of CoBlm with a suitable photosensitizer.

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Biosynthesis of Acivicin and 4-Hydroxyacivicin from Ornithine[1]

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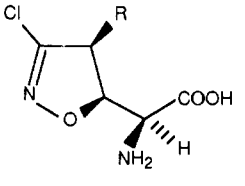
Acivicin (AT-125) (**1**), isolated[3] and characterized[4] from *Streptomyces sviceus* by researchers at the Upjohn Co., has potent anticancer activity[5] and has also found use as an important tool for studying xenobiotic metabolism involving glutathione.[6] 4-Hydroxyacivicin (**2**) is a cometabolite with roughly one-fifth the cytotoxic activity of **1**.[7] As an extension of our studies of secondary metabolism at the β-position of α-amino acids,[8–10] we have investigated the biosynthesis of **1** and **2** and report here aspects of their derivation from ornithine (**3**).



1, R=H

2, R=OH

A seed culture was prepared by inoculating 50 mL of broth[11] with a loopful of soil culture[12] and incubating the broths at 28 °C and 270 rpm in a gyrotory incubator shaker for 69 h. The seed culture was used to inoculate five 200-mL production broths[13] (2.5% v/v) in 1-L baffled[14] wide-mouth Erlenmeyer flasks, which were then incubated at 32 °C and 250 rpm. Acivicin first appeared in the fermentation broth[15] at 48 h and the concentration peaked at 120 h, whereupon the fermentation broth was worked up. After centrifugation (8800g, 15 min) the supernatant was decanted, and the pellet was resuspended in water and recentrifuged. The combined supernatants were adjusted to pH 7.8 and subjected to two ion-exchange chromatographies;[16] **1** and **2** were then separated from each other by flash chromatography.[17] The relevant fractions were lyophilized, resulting in solids that were each recrystallized (methanol–water) to purity, yielding 6 mg of **1** and 11.7 mg of **2**.

By recognition of the fact that a five-carbon amino acid should be the logical precursor, and considering the oxidation state of C-5 of the metabolites, DL-[1-^{14}C]glutamic acid (**4**) and L-[U-^{14}C]glutamine (**5**) were each fed to separate production broths (one flask each) at 48, 72, and 96 h after inoculation, and each fermentation was continued for a total of 120 h. All six experiments were worked up in standard fashion after 25.3 mg of **1** and 25.1 mg of **2** were added to each as carrier. In none of these experiments was either metabolite radioactive. However, when DL-[2-^{14}C]ornithine was fed at 48 h, workup yielded radioactive metabolites: the percent incorporation for **1** was 0.2 and for **2** it was 2.0, after recrystallization to constant specific activity.

In order to determine whether ornithine incorporation was specific and to simultaneously determine whether the α-amino groups were retained, 38.8 mg of DL-[5-^{13}C,5-^{15}N]ornithine (**3a**),[18,19] mixed with 13.2 μCi of DL-[5-^{14}C]-**3**, was fed in equal portions to five 200-mL production broths at 48 h, and these were worked up as usual after 120 h. The 100.6-MHz ^{13}C NMR spectrum[20] of each metabolite in D_2O, **1a**[21] and **2a**,[22] exhibited

(1) Studies of Nitrogen Metabolism Using ^{13}C NMR Spectroscopy. Part 6. For Part 5, see ref 10.

(2) Career Development Awardee of the National Cancer Institute (CA00880), 1979–1984.

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(11) The seed medium consisted of glucose (0.5 g), yeast extract (0.5 g), Difco peptone (0.12 g), and double-distilled water (50 mL) and was adjusted to pH 7.2.

(12) Antibiotic production in liquid culture was found to degenerate when *S. sviceus* was stored on agar [dextrin (10.0 g), beef extract (1.0 g), yeast extract (1.0 g), NZ Amine A (2.0 g), cobalt chloride (0.02 g), and agar (17.5 g) in 1 L of distilled H_2O at pH 7.5.], but stabilization was effected when the organism was maintained as spores on sterile soil: spores from a fresh slant were suspended in 0.9% NaCl + 0.01% Tween 80 and transferred to sterile soil in a culture tube; the tube was incubated at 26 °C for 2 weeks and then stored in a cold room (4 °C).

(13) The production medium consisted of cerelose (0.4 g), washed dry yeast (0.5 g), Kaysoy 200c (4 g), corn starch (2 g), NH_4Cl (1 g), and tap water (200 mL) and was adjusted to pH 7.2.

(14) One-liter wide-mouth Erlenmeyer flask with two sets of three fingerlike indentations (ca. 1 in.) in a vertical row.

(15) Production was checked by bioassay using *B. subtilis* UC-902 (obtained from The Upjohn Co.) as the test organism.

(16) Chromatographed first on Dowex 50WX4 (H^+, 100–200 mesh, 5 × 23 cm): washed with distilled H_2O and then eluted with 2.5 N NH_4OH. Relevant fractions were combined, adjusted to pH 7.0, and applied to Biorad AG3-X4A (100–200 mesh, 3 × 20 cm), washed with distilled H_2O, 50% MeOH, 90% MeOH, and then eluted with MeOH:H_2O:AcOH = 90:10:3.

(17) The sample in distilled H_2O was evaporated onto 0.4 g of silica gel 60, applied to the top of a silica gel 60 column (21 g), and eluted with methyl ethyl ketone:acetone:water = 65:20:15.

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