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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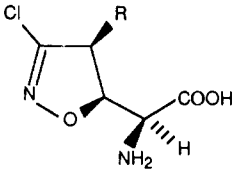
Acivicin (AT-125) (**1**), isolated[3] and characterized[4] from *Streptomyces sviceus* by researchers at the Upjohn Co., has potent anticancer activity[5] and has also found use as an important tool for studying xenobiotic metabolism involving glutathione.[6] 4-Hydroxyacivicin (**2**) is a cometabolite 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

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(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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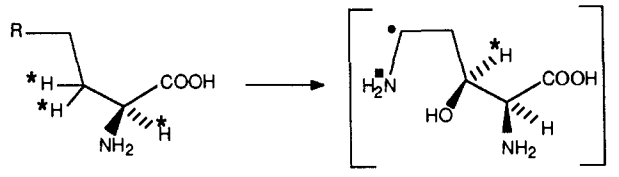
a spin-coupled doublet (J_{CN} = 2.7 Hz) for C-5 (152.6 and 154.7 ppm, respectively) with the lower field satellite superimposed on the natural-abundance singlet (1.4-Hz upfield isotope shift). Thus, both isotopes were retained and ornithine was demonstrated to be the primary precursor to both metabolites. This represents surprising apparent lack of metabolic economy since C-5 must subsequently be reoxidized to the amide oxidation state.

The subsequent metabolism of **3** has been probed with DL-[2,3,3-$^2H_3$]ornithine (**3b**),[23] this precursor having been prepared by exchange of **3** with D_2O in the presence of pyridoxal.[24] This feeding experiment afforded 3.6 mg of **1b** and 27 mg of **2b**. The

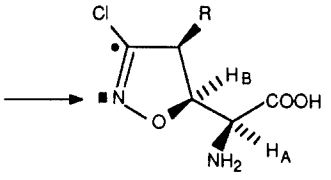


3, R=CH_2NH_2 **3a**, R=$^{13}CH_2^{15}NH_2$

4, R=COOH **3b**, R=CH_2NH_2, $^*H=^2H$

5, R=$CONH_2$

6



1a, R=H, •=^{13}C, ■=^{15}N

2a, R=OH, " "

1b, R=H, H_A=H, H_B=^2H

2b, R=OH, " "

61.4-MHz ^2H NMR spectrum of **2b**[25] (27693 scans)[26] showed resonances for residual HOD (δ 4.93), for *tert*-butyl alcohol added as a chemical shift and deuterium quantitation reference (δ 1.27), and for deuterium at C-3 (δ 5.19).[27] Absolutely no deuterium was detectable at C-2, even though the signal to noise ratio would have allowed detection of 2% retention relative to C-3.

It is noteworthy that a complete loss of deuterium had also been obtained during incorporation of a variety of arginines labeled with deuterium at C-2 in the biosynthesis of streptothricin F,[8,28] for which we have postulated the involvement of β-hydroxyarginine, and in both cases we believe that the loss of this hydrogen may be mechanism-based and related to the hydroxylation. These results are in contrast to our finding of only a 70-80% loss of deuterium from C-2 of labeled arginines that were fed to *S. griseochromogenes* and ultimately incorporated into blasticidin S[10] via a 2,3-aminomutase reaction; in this case the partial loss was probably coincidentally due to an arginine racemase activity[29] unrelated to the blasticidin biosynthesis. The further metabolism of **3**, including the potential involvement of β-hydroxyornithine **6**, is currently under study. Townsend and Ho[30] recently reported strong evidence for ornithine as the direct primary precursor to the C_5 unit of clavulanic acid; since C-3 of ornithine is eventually oxygenated in this pathway, **6** may also be involved in this somewhat more cryptic metabolism.

Acknowledgment. Dr. David Martin and Shirley Gerpheide of the Upjohn Co. are thanked for providing samples of acivicin and 4-hydroxyacivicin, slants of *S. sviceus*, and the design of the baffled flask. The work was supported by grants from the Public Health Service (GM 32110) and the Oregon State University Environmental Health Sciences Center (NIESH Grant ES 00210) to S.J.G. NMR spectra were obtained on a Bruker AM 400 spectrometer purchased in part by grants from the National Science Foundation (CHE-8216190) and from the M. J. Murdock Charitable Trust to Oregon State University.

(20) Sweep width, 25 000 Hz; acquisition time, 1.3 s; 64 K data points; chemical shifts referenced to CH_3CN (1.3 ppm).

(21) ^{13}C NMR of **1**: 170.2 (C-1), 152.6 (C-5), 80.7 (C-3), 56.4 (C-2), 40.2 ppm (C-4).

(22) ^{13}C NMR of **2**: 170.4 (C-1), 154.7 (C-5), 81.7 (C-3), 77.2 (C-4), 53.1 ppm (C-2).

(23) DL-[2,3,3-$^2H_3$]Ornithine (**3**), 88.9 mg, mixed with 12.0 μCi of DL-[5-^{14}C]ornithine was fed in equal portions to ten 200-mL production broths at 48 h.

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(25) ^1H NMR of **1**: δ 3.4 (m, 2 H), 4.0 (d, 1 H, *J* = 3.2 Hz), 5.2 (1 H, m). ^1H NMR of **2**: δ 5.2 (dd, 1 H, *J* = 3.7, 4.4 Hz), 5.3 (d, 1 H, *J* = 8.5 Hz), 4.4 (d, 1 H, *J* = 4.1 Hz).

(26) Sweep width, 586.2 Hz; acquisition time 1.7 s; no. of scans 27 693; 2K data points zero filled to 8K; 2.0-Hz line broadening.

(27) The ^2H NMR spectrum of **1b** similarly showed retention of deuterium only at C-3 although, due to the small amount of sample, the signal to noise was much poorer.

(28) We have recently synthesized [2,3,3,5,5-$^2H_5$]arginine. The ^2H NMR spectrum of streptothricin F biosynthetically derived from this showed resonances due only to the labels at C-5: Wityak, J.; Gould, S. J., unpublished results.

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Aryl-Substituted Molecular Metal Oxide Clusters

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Incorporation of transition metals into and onto metal oxide aggregates has received increasing attention owing to the analogies being developed between such molecular clusters and extended phase, bulk oxide surfaces.[1-4] Organic molecules are known to undergo chemical reactions on oxide surfaces and so metal oxide clusters bearing purely organic substituents offer the prospect of obtaining further insight into this chemistry. Few examples exist of hydrocarbyl-substituted oxymetalates. These include $CH_2Mo_4O_{15}H^{3-}$, obtained from formaldehyde and $Mo_2O_7^{2-}$, in which a CH_2 unit bridges two Mo-O-Mo units,[5] heteroatom-substituted clusters of the types $R_2P_2M_5O_{21}^{4-}$ (M = Mo, W),[6,7] $R_2As_2Mo_6O_{24}^{4-}$,[8] and $(CH_3O)_4Mo_8O_{24}^{4-}$, which contains two bridging and two terminal methoxy groups.[9-11] Pyrolysis of $(R_3O)_3PW_{12}O_{40}$ produces the trialkyl derivatives $R_3PW_{12}O_{40}$ (R = CH_3, C_2H_5) which, at higher temperatures, form $H_3PW_{12}O_{40}$.[12] As a further elaboration of our solid-state syntheses of derivatives of molecular metal oxide clusters,[13] we report here a *general* route to neutral, aryl-substituted heteropolyanions of the type $(aryl)_{8-n}XM_{12}O_{40}$ (X = P, Si; M = Mo, W; *n* is the formal oxidation state of heteroatom X).

Metathetical reaction of aryl diazonium salts with Keggin ions provides $(arylN_2)_{8-n}XM_{12}O_{40}$ which, when pyrolyzed, forms

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