Cobalt(III) Complex of Pseudotetrapeptide A of Bleomycin

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Abstract: The major metal-containing hydrolysis product of both orange and green cobalt(III) bleomycin has been studied by spectroscopic as well as chemical means. With use of ^{1}H NMR (at 400 MHz), absorption, and circular dichroism spectroscopies and other physical techniques, the complex obtained from the hydrolysis of either isomeric cobalt(III) bleomycin was shown to contain pseudotetrapeptide A of bleomycin bound to the cation in a manner identical with that of the peptide in the bleomycin metabolite complex, Cu^{II}(P-3A). The hydrolysis product is a $Co^{III}(N_5O)$-type complex having the primary amine group of the peptide and a water molecule occupying the two axial positions of the compound with the secondary amine, N(1) of the pyrimidine, the deprotonated amide nitrogen atom of the β-hydroxyhistidine, and N(3) of the imidazole occupying the planar coordination sites. The observation that both isomers yield the same acid hydrolysis product indicates that the bleomycin functional group (unidentified) which differentiates the two isomeric Cobalt(III) bleomycins is not part of pseudotetrapeptide A of the antibiotic. The similarity in coordination chemistry between Co(III) and Fe(II) and the method used to synthesize cobalt(III) bleomycin make the hydrolysis product an important structural model for the biologically significant iron bleomycins.

The study of the metal-binding properties of the antitumor antibiotic bleomycin, BLM (**1**), has attracted considerable attention.[1-3] Although it has been known for some time that the antibiotic is capable of degrading DNA,[4] recent evidence shows that a metal complex of the drug, Fe^{II}BLM, is the important species in the degrading process.[5] It has been proposed that DNA degradation by bleomycin proceeds through a ternary complex, Fe^{II}–BLM–DNA. Since Fe^{II}BLM is capable of radical production via an oxidative mechanism, it is thought that the radicals which are produced in the oxidation of this complex while it is bound to DNA are the chemical species responsible for DNA damage. Moreover, studies have also shown that the degradation process is specific for GC and GT sequences of DNA,[6] suggesting that stereochemical factors associated with the ternary complex are important in the degradation process.

The structural role that metal ions play in the mechanism of action of bleomycin has been actively studied. Based in part on earlier chemical evidence presented by Umezawa[2] and with the help of new spectroscopic information, we described the bleomycin binding site for Cu(II) and Zn(II).[7,8] However, the subsequent revision in the structure of the antibiotic[9] forced abandonment of the originally proposed structures for these metal complexes.[1] Using single-crystal X-ray analysis, Iitaka et al.[10] determined the structure of a Cu(II) complex of the bleomycin metabolite, P-3A. Since the structure of the metabolite was nearly identical with that of the metal-binding region of bleomycin, Cu^{II}(P-3A) was used to assign the structures of a number of metallobleomycins, Fe(II), Co(II), Ni(III), and Cu(II).[11-14] However, the validity of at least two of the structures assigned in this manner has been seriously questioned and alternate structures for Cu^{II}- and Fe^{II}BLM have been proposed.[15,16] Extensive NMR studies on Co^{III}BLM[17] and Zn^{II}BLM[18] have also lead to binding sites which are inconsistent with the structural elements encompassed by Cu^{II}(P-3A).

For several reasons the cobalt(III) bleomycins appear uniquely suited for gaining detailed information on the manner in which the intact biologically active antibiotic binds transition metal ions. (i) Spectroscopic evidence suggests that Co^{II}BLM is isostructural with Fe^{II}BLM.[1,14] Since oxidation of Co^{II}BLM would probably not alter ligand geometry, Co^{III}BLM is very likely isostructural with the divalent complexes. (ii) Co(III) yields kinetically stable complexes. Thus, it is possible to subject Co^{III}-**1** to hydrolysis and remove a section of the bleomycin molecule containing the cobalt ion and the peptide fragment important in metal binding. Since the size of the resulting cobalt(III) peptide complex is

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(1) Dabrowiak, J. C. In "Metal Ions, in Biological Systems"; Sigel, H., Ed.; Marcel Dekker: New York, 1980; Vol. 11, p 305.
(2) Dabrowiak, J. C. *J. Inorg. Biochem.* **1980**, *13*, 317.
(3) Hecht, S., Ed. "Bleomycin: Chemical, Biochemical and Biological Aspects"; Springer-Verlag: New York, 1979.
(4) Umezawa, H. In "Antibiotics. Mechanism of Action of Antimicrobial and Antitumor Agents"; Corcoran, J. W., Hahn, F. E., Eds; Springer-Verlag: New York, 1975; Vol. III, p 21.
(5) Sausville, E. A.; Peisach, J.; Horwitz, S. B. *Biochem. Biophys. Res. Commun.* **1976**, 73, 814. Sausville, E. A.; Horwitz, S. B. In "Effects of Drugs on the cell Nucleus"; Busch, H., Crooke, S. T., Daskal, Y., Eds.; Academic Press; New York, 1979; p 181 Lown, J. W., Sim, S. *Biochem. Biophys. Res. Commun.* **1977**, 77, 1150.
(6) Takeshita, M.; Grollman, A. P.; Ohtsubo, E.; Ohtsubo, H. *Proc. Natl. Acad. Sci. U.S.A.* **1978**, *75*, 5983. D'Andrea, A. D.; Haseltine, W. A. *Ibid.* **1978**, *75*, 3608.
(7) Dabrowiak, J. C.; Greenaway, F. T.; Longo, W. E.; Van Husen, M.; Crooke, S. T. *Biochim. Biophys. Acta* **1978**, *517*, 517.
(8) Dabrowiak, J. C.; Greenaway, F. T.; Grulich, R. *Biochemistry* **1978**, *17*, 4090.
(9) Takita, T.; Muraoka, Y.; Nakatani, T.; Fjuii, A.; Umezawa, Y.; Naganawa, H.; Umezawa, H. *J. Antibiot.* **1978**, *31*, 801.
(10) Iitaka, Y.; Nakamura, H.; Nakatani, T.; Muraoka, Y.; Fujii, A.; Takita, T.; Umezawa, H. *J. Antibiot.* **1978**, *31*, 1070.
(11) Takita, T.; Muraoka, Y.; Natkatani, T.; Fujii, A.; Iitaka, Y.; Umezawa, H., *J. Antibiot.* **1978**, *31*, 1073.
(12) Umezawa, H.; Takita, T. *Struct. Bonding (Berlin)* **1980**, *40*, 73.
(13) Sugiura, Y.; Mino, Y. *Inorg. Chem.* **1979**, *18*, 1336.
(14) Sugiura, Y. *J. Am. Chem. Soc.* **1980**, *102*, 5216.
(15) Oppenheimer, N. J.; Rodriguez, L. O.; Hecht, S. M. *Proc. Natl. Acad. Sci. U.S.A.* **1979**, *76*, 5616.
(16) Bereman, R. D.; Winkler, M. E. *J. Inorg. Biochem.* **1980**, *13*, 95.
(17) Vos, C. M.; Westera, G.; Shipper, D. *J. Inorg. Biochem.* **1980**, *13*, 165.
(18) Lenkinski, R.; Dallas, J. *J. Am. Chem. Soc.* **1979**, *101*, 5902.

0002-7863/81/1503-7543$01.25/0

smaller than Co^{III}-**1**, it should be possible to use standard spectroscopic techniques to obtain relatively detailed structural information on the compound. This structural information will be useful in assigning the structure of the larger complex Co^{III}-**1**. (iii) Co(III) complexes are normally diamagnetic and they can be easily studied by using NMR. In addition, the relationship between electronic structure and stereochemistry for this cation is highly developed.[19] Thus, the additional spectroscopic techniques of absorption and circular dichroism spectroscopy can also be used to gain structural information on the complex.

The structural elucidation of Co^{III}BLM is also important from the standpoint of nuclear medicine.[20,21] Experiments with the radiolabeled complex, $^{57}Co^{III}$BLM, have shown that the two isomeric forms of the complex orange and green Co^{III}-**1** exhibit dramtically different in vivo distributions. The concentration of the green isomer which is found in blood and in tumor cells after injection of the radiolabled compound is more than twice that of the orange complex.[20] Since the structural difference between the two isomers almost certainly involves the cobalt polyhedron, the structures of the compounds warrant investigation.

In a previous brief report,[22] we established that hydrolysis of green Co^{III}BLM yields a Co(III) complex of pseudotetrapeptide A of bleomycin (**2**). Arguments based on a variety of spectroscopic measurements established that the structure of the hydrolysis product was analogous to that of the Cu^{II}(P-3A). In this report we show that both orange and green Co^{III}BLM produce the same major acid hydrolysis product. However, an analysis of the ^1H NMR data (400 MHz) for the complex in acidic D_2O and in Me_2SO solution revealed that the orginally proposed structure for the complex[22] should be revised. The revised structure is identical with that earlier proposed except the primary amine group, and not the carboxylate of the β-amino moiety of **2**, occupies one of the axial coordination sites of the complex. Thus, from the standpoint of the nature of the donor atoms utilized by the peptide and their arrangement of the metal polyhedron, the hydrolysis product and Cu^{II}(P-3A) are isostructural.

Experimental Section

Physical Measurements. High-resolution ^1H NMR spectra of the complexes in D_2O were determined by using a Brucker WH-400 FT NMR spectrometer. Data were collected in 5-mm tubes containing ~0.5 mL of a ~1 mM solution of the complex. Prior to data collection, the complex was twice lyophilized with 20 mL of D_2O in order to replace all exchangeable protons with deuterons. The standard electrode correction of pD, meter reading +0.4,[23] was employed. The pD of the NMR solutions was adjusted by addition of microliter quantities of 0.1 N NaOD or DCl to the NMR sample. The internal standard used in the study was TSP, sodium 3-(trimethylsilyl)propionate-*2,2,3,3-d*$_4$. The chemical shifts below pD ~5 have been corrected for the 0.017-ppm downfield shift of the $(CH_3)_3Si$ resonance of TSP as the carboxyl function protonates (pK_a = ~5). Unless otherwise noted, the error in chemical shift is ±0.002 ppm. For the purpose of plotting the chemical shifts as a function of pD, spectra were recorded at the following pD values: 0.9, 2.1, 2.6, 3.1, 3.4, 4.4, 5.0, 5.9, 6.4, 6.7, and 8.0.

In order to observe the NH resonances of the complex in D_2O, we dissolved the protonated form of the compound in D_2O and adjusted the pD of the solution immediately to 2.1 with 0.1 N DCl. The chemical shifts were referenced to internal TSP. NMR solutions of the protonated form of the complex (~1 mM) in Me_2SO-d_6 containing ~5μL of trifluoroacetic acid were prepared in an inert atmosphere box. Chemical shifts were intially referenced to the small amount of Me_2SO-d_5 present in the NMR solvent and finally to Me_4Si by using the expression $\delta(Me_2SO) - \delta(Me_4Si) = 2.550$ ppm.

The potentiometric titration data were collected on 3.0 mM solutions of the complex by using a digital Corning Model 130 pH meter at 25 ± 0.01 °C. The Beckman combination electrode (Beckman No. 39501) was calibrated at two pH values, pH 4.008 and pH 6.865, by using NBS buffer solutions. Titrant was delivered to the titration vessel by using a Gilmont digital ultraprecise micrometer buret. The ESD associated with titrant delivery was 0.1 μL. Potassium chloride (0.15 M) was used as a supporting electrolyte. The computer program STABLY, employing the Bjerrum half $\bar{n}$ method,[24] was used for computation and refinement of the pK_a values of the hydrolysis product. Poor electrode response below pH 2.5 and above pH 10.5 prevented analysis at pH extremes. On the basis of two runs, the determined pK_a values for the hydrolysis product were 2.73 (4) and 4.77 (4).

Absorption data on the compound, in water, were collected by using a Cary 219 spectrophotometer. Circular dichroism spectra were recorded by using a Durrum-Jasco Model J-20 spectropolarimeter. The absorption and CD spectra of the complex were not significantly affected by pH in the pH range 0.9–8.0.

The conductance of the hydrolysis product was measured by using a Yellow Springs Instrument Model 31 conductivity bridge. Dissolution of the hydrolysis product in water (7.01×10^{-4} M) followed by the addition of 1 equiv of NaOH to adjust the pH to 6.5 resulted in the deprotonation of the γ-amino-α-methylvaleric acid carboxyl moiety of the complex. A determination of the equivalent conductance of the resulting solution yielded a value of 128 Ω^{-1} cm^2 mol^{-1} which after correction for the presence of 1 equiv of NaCl, 126.5 Ω^{-1} cm^2 mol^{-1}, resulted in an equivalent conductance of the hydrolysis product of ~0 Ω^{-1} cm^2 mol^{-1}.

Synthesis of Co^{III}-2. The preparation of the divalent cobalt complex Co^{II}-**1** was carried out under nitrogen in an inert atmosphere box. Combining 1.0 mmol of Blenoxane sulfate with 1 equiv of $Co^{II}(ClO_4)_2$ in water followed by the adjustment of the pH of the solution of 7.0 (0.1 N NaOH) yielded Co^{II}BLM. After 15 min, the brown solution was removed from the box and it was stirred in the atmosphere for 10 h. The color of the solution changed from brown to green indicative of the oxidation of Co^{II}BLM to green Co^{III}BLM.[20] After removal of water at reduced pressure, the green residue was taken up in 180 mL of 6 N HCl and allowed to stand at 43 °C for 5 days. Following the same procedure, a second portion of Blenoxane sulfate (1.0 mmol) was combined with $Co^{II}(ClO_4)_2$. However, after oxidation to green Co^{III}BLM, and just prior to hydrolysis, the solution was heated at 100 C for 2 h (pH 7.0) to convert green Co^{III}BLM to orange Co^{III}BLM.[20] The hydrolysis products from both preparations were subjected to the following separation procedure.

After hydrolysis, the solvent and HCl were removed at reduced pressure and the residue which remained was taken up in 60 mL of water (pH 7.0). The organic components present in the hydrolysis mixture were removed by extraction with 150 mL of $CHCl_3$ and finally with 200 mL of *n*-butyl alcohol. After removal of water in vacuo, the residue was taken up in 50 mL of water and 200 mL of methanol was added to induce precipitation of a brown colored solid. The solid was removed by filtration and the filtrate was evaporated to dryness in vacuo. The above precipitation procedure was repeated to remove an additional amount of methanol-insoluble material. Thin-layer chromatography showed that the yellow methanol-insoluble material contained several components, and its separation was not pursued. The components soluble in methanol–water, red-brown in color, were separated by using column chromatography.

The red-brown residue was taken up in water and placed on a CM-Sephadex-C-25-120 (16 × 4 cm) column in the NH_4^+ form. Elution with 0.01 M $(NH_4)_2CO_3$ (pH 6.5) resulted in the appearance of three bands. The major band, red-brown in color, was eluted first from the column. Thin-layer chromatography (silica gel, ethanol–5% ammonium acetate (2:1)) showed that this band contained a mixture of three components, one major component with $R_f = 0.56$, and two minor components with $R_f = 0.68$ and ~0. The slowest moving band off the Sephadex column was shown by TLC to contain unreacted Co^{III}BLM ($R_f = 0.31$). Separation of the major component from the two minor components present in the fastest eluting band was accomplished by using anion-exchange chromatography.

After removal of $(NH_4)_2CO_3$ in vacuo the fastest eluting band off the CM Sephadex column was taken up in the water and placed on a column containing QAE-Sephadex (28 × 1.5 cm) in the Cl^- form. Elution with 3 mN HCl resulted in the separation of three components, with the central band being the major component (~90% of the material). After removal of the solvent in vacuo, the residue containing the major fraction, red-brown in color, was taken up in 5 mL of water and reprecipitated by the slow addition of acetone to yield the pure form of the hydrolysis product. A ^1H NMR spectral analysis of the major acid hydrolysis product from both the orange and green preparations showed that both isomers produce the same major hydrolysis product. NMR spectra were

(19) Hawkins, C. J. "Absolute Configuration of Metal Complexes"; Wiley-Interscience: New York, 1971.
(20) DeRiemer, L. H.; Meares, C. F.; Goodwin, D. A.; Diamanti, C. I. *J. Med. Chem.* **1979**, *22*, 1019.
(21) Kakinuma, J.; Kagiyama, R.; Hirotake, O. *Eur. J. Nucl. Med.* **1980**, *5*, 159.
(22) Tsukayama, M.; Randall, C.; Santillo, F. S.; Dabrowiak, J. C. *J. Am. Chem. Soc.* **1981**, *103*, 458.
(23) Glasoe, P. K.; Long, F. A. *J. Phys. Chem.* **1960**, *64*, 188.
(24) Avdeef, A., personal communication.

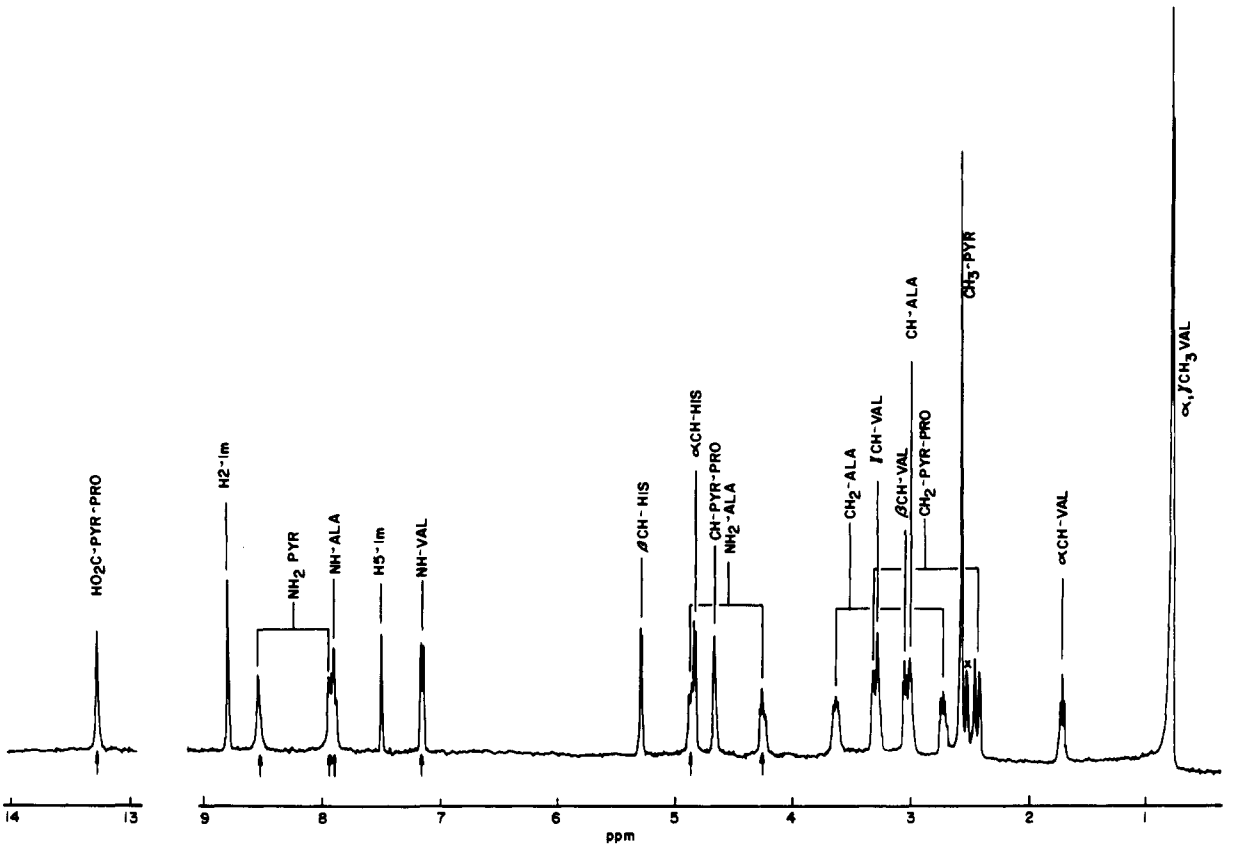


Figure 1. The 400-MHz ^1H NMR spectrum of the hydrolysis product and the signal assignments in Me_2SO are shown. The signals indicated by arrows are resonances associated with exchangeable protons of the complex.

determined on individual solutions of each of the major products and on a mixture of the major products isolated from both (the orange and green) hydrolysis reactions. The yield from either preparation was ~300 mg (40% Co).

Anal. Calcd for $C_{24}H_{33}N_9O_{10}ClCo\cdot 3H_2O$: C, 38.12; H, 5.21; N, 16.67, Cl, 4.68. Found: C, 38.25; H, 5.08; N, 16.33; Cl, 4.62.

A check of the stability of the hydrolysis product to the conditions used for the hydrolysis showed the complex partially converts to a second unidentified species in strongly acidic media. After 5 days in 6N HCl (43 °C) ~95% of the unaltered starting material was recovered from the reaction mixture.

Synthesis of Co^{III}-2-Trimethyl Ester. Dry HCl gas was passed through 30 mL of a heated methanol solution (65 °C) containing 40 mg of **2**. After 12 h, the green solution was cooled and the solvent removed in vacuo. The residue was taken up in 1 mL of water, and 100 mL of actone was slowly added to induce precipitation of the crude ester. The crude product pale green in color was loaded onto a QAE Sephadex anion-exchange resin in the Cl^- form, elution with water resulted in the separation of four bands with the second band off the column containing the bulk of the material. Thin-layer chromatography (silica gel, ethanol–5% ammonium acetate (4:7)) showed that the second band consisted of at least three components with the major component being (~80%) Co^{III}-**2**-trimethyl ester (yield 22 mg). Because of the low purity of the compound and the fact that it readily decomposed to a mono ester material in weakly basic media (pH ~8), the triester was only partailly characterized: ^1H NMR (D_2O, TSP, pD 3.3) δ 1.420 (6 H, Val CH_3's), 2.600 (3 H, pyr CH_3), 3.667 (3 H, OCH_3), 3.796 (3 H, OCH_3), 3.863 (3 H, OCH_3), 6.723 (1 H, β-CH His), 7.845 (1 H, H5 Im), 9.907 (1 H, H2 Im).

Results and Discussion

Assignment of the ^1H NMR Resonances. In a brief communication we earlier reported[22] that the carboxyl group of the β-aminoalanine moiety of **2** occupies an axial coordination site of the hydrolysis product. This assignment was to significant degree based on the assignment of the ABX NMR patterns associated with the pyrimidinylpropionic acid and β-aminoalanine moieties of the peptide. On the basis of more recent NMR studies of the hydrolysis product in Me_2SO solution, under conditions where the N–H resonances of the complex can be observed, it is now evident that the original NMR assignment should be reversed and that the axial ligating group is the primary amine moiety of the peptide.

Table I. ^1H NMR Chemical Shift Data

		hydrolysis product		
	2, pH 6.0	pD 2.1	pD 6.4	Me_2SO^a
	Nonexchangeable Protons			
CH_3-pyr	2.48	2.580	2.600	2.600
α-CH_3-Val	1.51	0.744	0.895	0.794
γ-CH_3-Val	1.57	0.928	0.939	0.794
CH_2-pyr-pro	3.91[b]	2.808	2.868	2.461
		3.281	3.354	3.35
α-CH-Val	2.86	1.083	1.699	1.729
γ-CH-Val	4.39	3.604	3.527	3.319
CH_2-Ala	3.29[c]	2.888	2.969	2.751
		3.935	3.708	3.668
CH-Ala	4.87[d]	3.202	2.904	3.039
α-CH-His	5.26	5.095	5.093	4.872
β-CH-His	5.73	5.540	5.563	5.339
CH-pyr-pro	4.55[e]	4.642	4.73	4.702
β-CH-Val	4.17	3.827	3.303	3.080
CH5-Im	7.90	7.609	7.567	7.538
CH2-Im	9.05	8.630	8.605	8.837
	Exchangeable Protons			
NH-Val		7.775		7.190
NH_2-Ala		4.403		4.296
		5.357		4.918
NH-Ala		7.827		7.944
NH_2-pyr				8.582
				7.987
HO_2C-pyr-pro				13.318

[a] In Me_2SO-d_6 containing ~5 μL of trifluoroacetic acid. [b] Doublet, J = 6.5 Hz. [c] Center of the AB part of an ABX pattern. [d] Triplet, J = 6 Hz. [e] Triplet, J = 6.5 Hz.

The high-resolution ^1H NMR spectrum (400 MHz) of the hydrolysis product in acidified Me_2SO is shown in Figure 1, and the chemical shift and coupling constant data for the complex are collected in Table I and II, respectively. With the help of homonuclear spin-decoupling, chemical shift data for pseudotetrapeptide A of bleomycin[25] and by observing

Table II. Coupling Constant Data (Hz)[a]

			hydrolysis product			
			pD 6.4	pD 2.1	Me_2SO	2, pH 6.0
$CH-NH_2$-Ala	^3J	upfield NH		~5	~5	
	^3J	downfield NH		~0	~0	
	^2J			−11.3	−10.7	
$CHCH_2$-Ala	^3J	upfield CH	5.8	5.6	6.0	
	^3J	downfield CH	5.1	1.6	2.0	
	^2J		−13.2	−13.1	−13.0	
$NHCH_2$-Ala	^3J	upfield CH		6.0	6.6	
	^3J	downfield CH		6	6	
NH-Ala-CH-pyr-pro	^3J			~0	~0	
$CHCH_2$-pyr-pro	^3J	upfield CH	2.0 } (>70% gg)	2.8 } (>70% gg)	~0 (>90% gg)	
	^3J	downfield CH	4.9	4.4	4.3	
	^2J		−18.3	−18.7	−16.5	
His	$^3J_{\alpha-\beta}$		3.2	3.2	3.0	7.8
Val α-CH_3	^3J		6.8	7.2	6.6	6.5
γ-CH_3	^3J		6.8	6.4	6.6	6.5
	$^3J_{\alpha-\beta}$		~6	3.2 (<10% trans)	6.7 (~40% trans)	6.0
	$^3J_{\beta-\gamma}$		~6	9.1 (85% trans)	~6	6.0
	$^3J_{\gamma-NH}$			9.2	6.6	

[a] The rotamer populations were calculated by using the approach described in ref 33. The constants used in the calculation were J_{gauche} = 2.5 Hz and J_{trans} = 11.0 Hz.

which of the resonances disappear as D_2O is added to the Me_2SO NMR sample, we have assigned all of the 22 proton resonances of the complex in Me_2SO. NMR studies of the protonated form of the compound in acidified D_2O and the fully exchanged form of the complex in D_2O as a function of pD confirmed the NMR assignments obtained in pure Me_2SO.

The resonances indicated by arrows in Figure 1 are associated with exchangeable protons of the complex. Of these seven resonances, the signal at lowest field, δ 13.318 (1 H), has been assigned to the proton of the protonated pyrimidinylpropionic acid group (HO_2C-pyr-pro) of the compound. The signal occurs in a region where carboxylic acid proton resonances are expected in Me_2SO,[26] and NMR studies of the complex in D_2O as a function of pD clearly show that this functional group does not undergo ionization in the pD range 0.9–8.0. The broad signals at 7.987 (1 H) and 8.582 (1 H) ppm are associated with the NH_2 group of the pyridmidine moeity, NH_2-pyr (Table I). Since setting the NMR decoupler frequency to the position of either resonance results in the loss of *both* NMR signals, the amino group is in slow rotation on the NMR time scale. The resonance on the high-field side of the high-field pyrimidine NH_2 signal has been assigned to the secondary amine proton of the complex (NH-Ala). Decoupling experiments clearly show that this signal is spin coupled to *only two* other resonances in the spectrum of the compound and that the coupled resonances are the AB part of an ABX pattern. This observation unequivocally assigns the signals at 2.751 (1 H) and 3.668 (1 H) ppm to the CH_2 group of the β-aminoalanine fragment of the peptide. The magnitude of the *geminal* coupling constant for the CH_2-Ala group of the hydrolysis product (Table II) is similar to that observed for the same group in Zn^{II}**1**.[27] The lack of observed coupling between the NH resonance and the methine proton of the pyrimidinylpropionic acid moiety, CH-pyr-pro, is important in establishing the absolute configuration of the coordinated secondary amine nitrogen atom (discussed below). The amide hydrogen atom of the γ-amino-α-methylvaleric acid moiety, NH-Val, occurs as a double at 7.190 (1 H) ppm. The chemical shift of this resonance is similar to those reported for bleomycin A_2 and an acylated analogue of the antibiotic in Me_2SO.[27]

Decoupling experiments show that the remaining two resonances associated with exchangeable protons at 4.296 (1 H) and 4.918 (1 H) ppm are coupled to each other and that the high-field resonance is also coupled to the methine proton of the β-aminoalanine moiety, CH-Ala (Table I and II). Thus, these resonances must be due to the primary amine group of the peptide, NH_2-Ala. The remaining resonances of the complex were assigned by using decoupling techniques and by reference to the structure and the chemical shift data for **2**.[25] It is noteworthy that the amide hydrogen of the β-hydroxyhistidine moiety of **2** was not observed in Me_2SO solution. Arguments presented below show that the amide nitrogen atom is deprotonated and that it is bound to the cation.

Addition of D_2O to the Me_2SO NMR solution caused HO_2C-pyr-pro, NH_2-pyr, and NH-Val to undergo facile deuterium exchange. However,

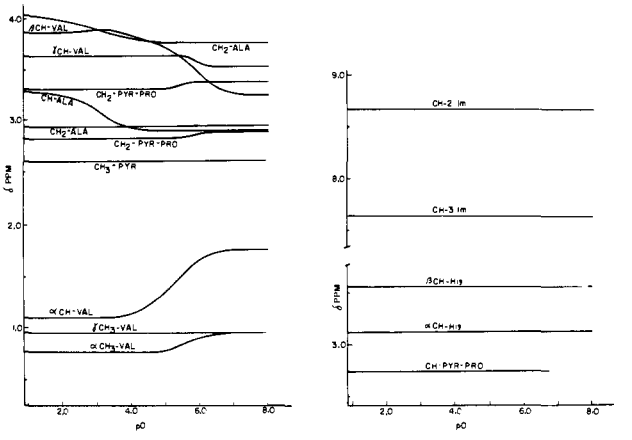


Figure 2. The chemical shift data for the complex in D_2O as a function of pD are shown.

the observation that NH-Ala and NH_2-Ala were relatively slow to exchange in acidified Me_2SO solution indicated that both the primary and secondary amine are bound to the metal ion. Addition of D_2O to the Me_2SO solutions also caused most of the signals to shift to new positions (Table I). The direction of the individual shifts and the ^1H NMR studies of the protonated form of the complex in acidified D_2O allowed the complete assignment of the resonances of the complex in pure D_2O.

^1H NMR Spectra of the Hydrolysis Product in D_2O. The ^1H NMR spectral data of the hydrolysis product as a function of pD in the pD range 0.9–8.0 in D_2O are shown in Figure 2, and the chemical shift data for the complex at pD 2.1 and 6.4 as well as those for **2** at pH 6.0 are collected in Table I. The ^1H NMR spectrum of the fully exchanged complex possessing 22 protons at pD 3.1, and indicating the revised assignments for the ABX patterns, is shown in Figure 3.

No attempt was made to explain the direction (primarily upfield) or magnitude of the chemical shifts which occur for **2** upon binding of the peptide to the cobalt ion. Such an analysis was complicated by two factors. First, titration data on **2** have not been reported and as a consequence little is known about the state of ionization of the peptide at pH 6.0, the pH value at which the NMR data for **2** were recorded.[25] If a specific metal donor site of **2** were actually protonated at pH 6.0, it would be difficult to predict the direction of the shift upon substitution of the proton for the cation at the donor atom of the peptide. Second, the anisotropy in the electron density about Co(III) has been shown to strongly affect the shielding constants of protons in the vicinity of the metal ion.[28] This effect is dependent on the energy separation between the ground state and low-lying excited states of the cobalt ion. It exhibits

(25) Naganawa, H. "Bleomycin: Chemical, Biochemical and Biological Aspects"; Hecht, S., Ed.; Springer-Verlag: New York, 1979; p 106.
(26) Mohacsi, E. *J. Chem. Educ.* **1964**, *41*, 38.
(27) Oppenheimer, N. J.; Rodriguez, L. O.; Hecht, S. *Biochemistry* **1979**, *18*, 3439.
(28) Gore, E. S.; Dabrowiak, J. C.; Busch, D. H. *J. Chem. Soc., Chem. Commun.* **1972**, 923. Trogler, W. C.; Stewart, R. C.; Epps, L. A.; Marzilli, L. J. *Inorg. Chem.* **1974**, *13*, 1564.

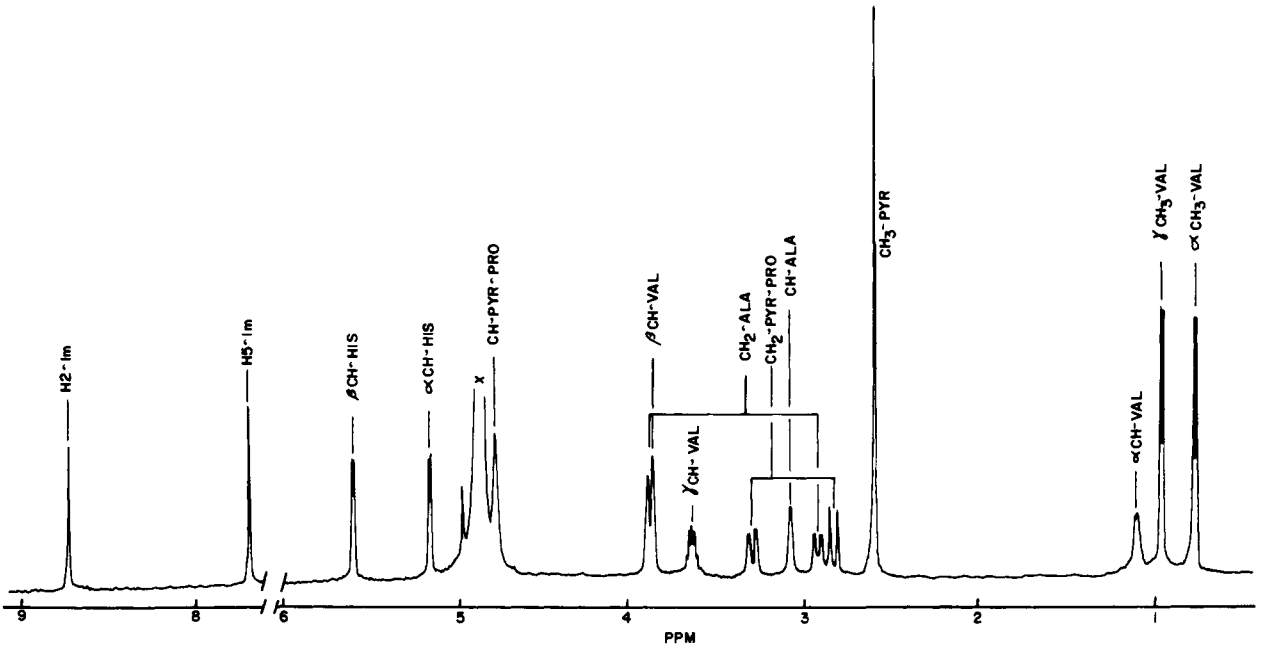
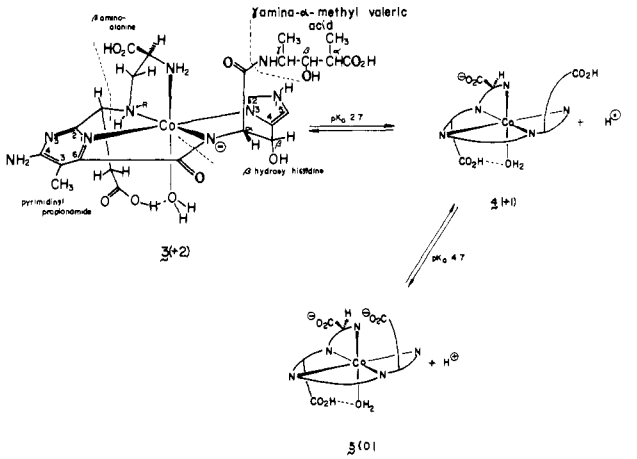


Figure 3. The 400-MHz ^1H NMR spectrum of the hydrolysis product with the signal assignments in D_2O at pD 3.1 is shown.

Scheme I. The Structure of the Hydrolysis Product in Various pH Ranges Is Shown[a]

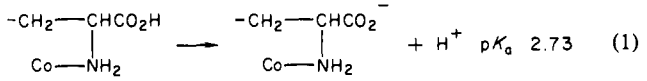


[a] The charge on the complex is given in parentheses below the structure.

a distance and angular dependence, the latter factor giving rise to both shielding and deshielding effects.

The structural changes which occur in the hydrolysis product as a function of pH are shown in Scheme I. At this point no attempt will be made to rigorously defend the structures shown in the scheme. However, as will be evident below, the structures depicted are the ones most consistent with *all* of the observed data for the hydrolysis product and reference to them at this point greatly simplifies the discussion of the ^1H NMR data of the complex.

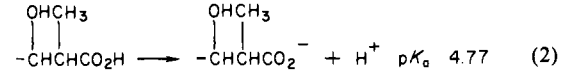
Potentiometric titration studies on the hydrolysis product showed that in the pH range 2.5 to ~3.5 the complex undergoes a single deprotonation having a pK_a value of 2.73 (4). ^1H NMR data for the complex in this pD range (Figure 2) indicate that the event is associated with the deprotonation of the β-aminoalanine acid function of the complex (eq 1).



The conversion of **3** to **4** (Scheme I) causes two of the proton resonances of the β-aminoalanine group, the low field β-methylene proton and α-methine proton, to shift *upfield* 0.2–0.3 ppm. *Upfield* shifts of this magnitude are expected as the adjacent carboxyl group deprotonates.[29]

In addition to the *upfield* shifts, the *vicinal* coupling constant between the low-field methylene proton and adjacent methine proton changes from 1.6 to 5.1 Hz (Table II) indicative of a conformational change in this part of the compound. Except for a relatively small shift of the high-field methylene proton of this ABX pattern (~0.05 ppm), none of the other protons of the complex experienced shifts or changes in coupling constants in the pD range 0.9–3.5 (Figure 2).

Above pH ~3.5 the complex undergoes a second ionization having a pK_a of 4.77 (4). ^1H NMR studies in the pD range ~3.5 to ~6.5 show that the second ionization is due to a loss of a proton from the γ-amino-α-methylvaleric acid carboxyl function (eq 2). This ionization affects



a number of the proton resonances of the complex. While the β and γ-methine protons of the γ-amino-α-methylvaleric acid moiety exhibit unexpectedly large *upfield* shifts for protons relatively remote from the site of ionization, the α-methine and the α-methyl groups shift in a direction opposite to that expected (downfield shifts are observed) for deprotonation of the terminal carboxyl group. In addition to the shifts, the coupling constants in this residue of the hydrolysis product change markedly as the ionization occurs (Table II). In the same pD range, the methylene protons of the pyrimidinylpropionic acid fragment of the compound also experience a small *downfield* shift (~0.05 ppm), an event which is accompanied by a slight change in conformation about the CH_2CH bond of this fragment (Table II). These observations show that ionization causes a change in the conformation of the γ-amino-α-methylvaleric acid moiety and that in its ionized form the carboxyl group is near the cobalt ion. It is reasonable to expect that the terminal carboxylate, which is separated from the cobalt ion by a relatively long alkyl chain, is involved in an ion-pair type of relationship with the positively charged remainder (1+) of the complex. The ionization of the third carboxyl group, HO_2C-pyr-pro, was not observed in the pD range 0.9–8.0, indicating that its pK_a for ionization is above ~8. Comparison of the ionization properties of the hydrolysis product with the higher order ionizations of the polycarboxylate EDTA[30] shows that $pK_a > 8$ for the ionization of the HO_2C-pyr-pro is not unreasonable. The deprotonation of constants water molecules bound to Co(III) vary widely, and values for the ionization of this group which have $pK_a > 8$ are known.[31]

Analysis of the Coupling Constant Data. Analysis of the coupling constant data for the complex in Me_2SO and D_2O afforded a detailed view of the stereochemistry of the hydrolysis product. Of major concern

(29) Evans, E. J.; Grice, J. E.; Hawkins, C. J.; Heard, M. R. *Inorg. Chem.* **1980**, *19*, 3496. Casey, A. F. "PMR Spectroscopy in Medicinal and Biological Chemistry; Academic Press: New York, 1971.

(30) Martell, A. E.; Sillén, L. G. "Stability Constants of Metal Complexes"; Chemical Society: London, 1964.

(31) Springborg, J. Schaffer, C. E. *Acta Chem. Scand.* **1973**, *27*, 3312; Burgess, J. and Horwood, E., Metal Ions in Solution, Halsted Press, New York, New York, 1978.

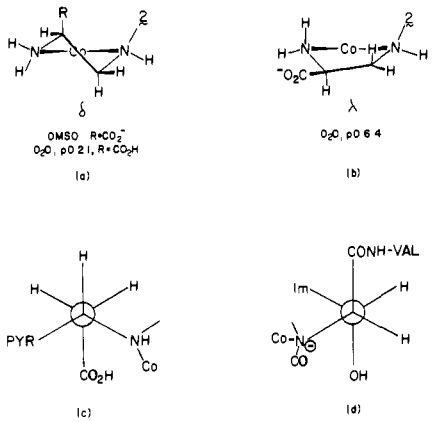


Figure 4. (a) and (b) The conformations of the coordinated β amino alanine fragment. (c) The most abundant rotomer for the CH_2CH fragment of the pyrimidinyl propionic acid moiety. (d) The conformation of the coordinate β-hydroxy histidine.

was the disposition of the chelate arm involving the β-aminoalanine fragment of the peptide. On the basis of ^{13}C and ^1H NMR data, structures proposed for CoIII-**1**[17] and Fe^{II}**1**(CO)[15] have positioned this arm of **1** in the plane occupied by the secondary amine and the pyrimidine, a geometry which is different than that observed for Cu^{II}(P-3A).[10]

With the binding of the primary and secondary amine groups and N(1) of the pyrimidine to the cation, two types of coordination, *facial* and *meridional*, for this tridentate fragment of the peptide are possible. Each type of coordination has in turn two permitted absolute configurations for the coordinated secondary amine nitrogen atom. Analysis of the coupling constant data shows the coupling between CH-pyr-pro and NH-Ala is ~0 Hz. Although the magnitude of the vicinal coupling constant as a function of dihedral angle for Co^{III}-NH-CH fragments has been little explored, the function is expected to obey the Karplus expression.[32] Thus, the dihedral angle in this part of the molecule is ~90°. Molecular models show that *only* the structure having a facial arrangement of groups and a coordinated secondary amine nitrogen atom with the *R* absolute configuration exhibits a dihedral angle of ~90 (Scheme I). The disposition of the chelate arms and the absolute configuration of the nitrogen atom is ultimately based on the fact that the configuration of the methine carbon atom of the pyrimidinylpropionic acid group of **2** is *S*.[3] Models further show that the meridional possibilities are strained and thus are thermodynamically less favored than the facial isomers, making them less likely possibilities[15] for metal complexes involving the entact antibiotic.

The conformation of the five-membered chelate ring involving the β-aminoalanine residue appears to be dependent on the state of ionization of the attached carboxylic acid moiety and on the nature of the solvent (Figure 4a,b). In Me_2SO when the carboxylic acid moiety is *deprotonated* and in D_2O at low pD when it is protonated the coupling constant data, for CH_2-CH-Ala, show that the five-membered ring has the δ conformation (Table II). However, as the pD is raised and as the carboxyl group undergoes ionization, the coupling constants in this region of the compound change. When the ionization is complete, the conformation of the ring has inverted to give what appears to be (from coupling constant data and the structure of the peptide) a distorted λ-type conformation (Figure 4b). Restricted movement about the coordinated secondary amine nitrogen prevents the chelate ring from adopting a regular λ conformation.

Calculation of the rotomer population[33] about the $CHCH_2$ bond of the pyridmidinylpropionic acid moiety shows that the fragment primarily exits in the gauche-gauche conformation in the pD range 0.9-8.0 (Figure 4c). Since this conformation places the protonated carboxyl group near the water molecule which occupies the axial site of the compound, the carboxylate is probably hydrogen bonded to the coordinated water molecule. As was mentioned earlier, the coupling constants and chemical shifts of the protons in pyr-pro change slightly as the γ-amino-α-methylvaleric acid carboxyl group ionizes. The reason for this is not clear, but it may reflect electrostatic changes which occur on the axis of the compound as the valeric acid carboxyl group enters into ion pair formation with the remainder of the complex.

Table III. Absorption and CD Data for the Hydrolysis Product[a]

absorption		CD	
nm (ϵ, M)	assignt	nm ($\Delta\epsilon$)	assignt
550 (136)	$^1A_1 \rightarrow {}^1E$	530 (0.69)	$^1A_1 \rightarrow {}^1E$
440 (~400, sh)	$^1A_1 \rightarrow {}^1A_2$	442 (3.90)	$^1A_1 \rightarrow {}^1A_2$
324 (6500)	CT (?)		
310 (5900)	$n \rightarrow \pi^*$, pyr (?)		
254 (13 000)	$\pi \rightarrow \pi^*$, pyr		

[a] At pH 6.1.

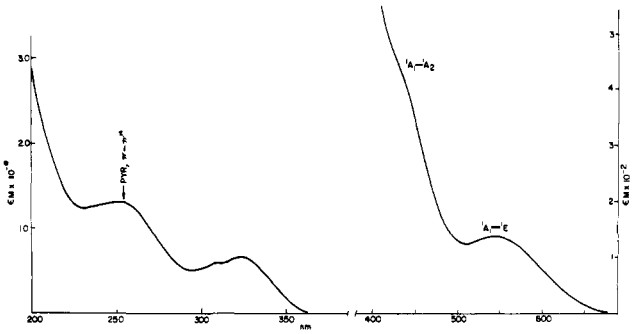


Figure 5. The absorption spectrum of the hydrolysis product at pH 6.1 is shown.

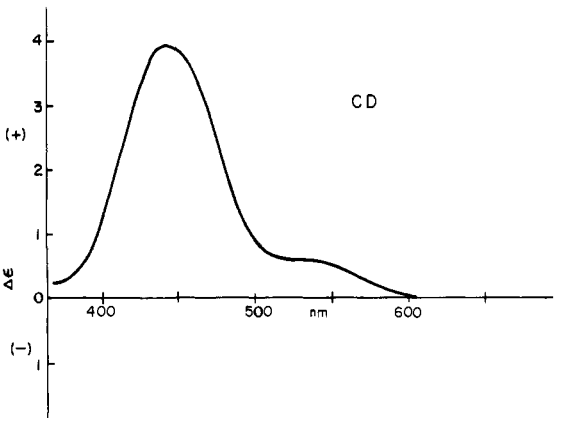


Figure 6. The circular dichroism spectrum of the hydrolysis product at pH 6.1 is shown.

The magnitude of the *vicinal* coupling across the CHCH bond of the β-hydroxyhistidine is indicative of the gauche conformation. Since the deprotonated amide and N(3) of the imidazole are coordinated to the cobalt ion, the gauche conformation shown in Figure 4d is present. This conformation has been previously found for Zn^{II}**1**,[27] Fe^{II}**1**(CO),[15] and Cu^{II}(P-3A),[10] and its presence is a requirement for the ion-pair interaction observed for the hydrolysis product. Due to the number of accessible degrees of freedom, a detailed conformational analysis of the valeric acid moiety with either the protonated or deprotonated form of the terminal acid group was not attempted.

The Donor Atoms of the Complex—Optical and Chemical Studies. The absorption and circular dichroism spectra of the hydrolysis product are shown in Figures 5 and 6, and the data are collected in Table III. In the visible region of the spectrum, the hydrolysis product exhibits two broad absorptions which have intensities consistent with Laporte-forbidden, spin-allowed, transitions.[34] The fact that only two absorption bands are observed and that only two Gaussian-shaped envelopes are present in the CD spectrum of the complex (Figure 6) strongly suggests that the site symmetry about the cobalt ion is high and that the system can be analyzed by using either C_{4v} or D_{4h} symmetry.[35] In either of these point groups, the first excited electronic state of the low-spin d^6 ion splits into an $^1A_2$ and an ^1E state. Consideration of the positions of the two absorption bands for the complex shows that the crystal field on the axis

(32) Bystrov, V. F.; Portnova, S. L.; Tsetlin, V. I.; Ivanov, V. T.; Ovchinnikov, V. A. *Tetrahedron* **1969**, *25*, 493. Legg, J. I.; Cooke, D. W. *Inorg. Chem.* **1966**, *4*, 594. Ellis, P., personal communication.

(33) Fenney, J. *J. Magn. Reson.* **1976**, *21*, 473.

(34) Lever, A. B. P. "Inorganic Electronic Spectroscopy", Elsevier: Amsterdam, 1968.

(35) Wentworth, R. A. D.; Piper, T. S. *Inorg. Chem.* **1965**, *4*, 709.

of the compound is less than that in the plane and thus the ^1E state is lowest in energy. Using the approach developed by Wentworth and Piper[35] for D_{4h} symmetry systems, we determined the in-plane crystal field strength of the hydrolysis product to be 2650 cm^{-1}.[22] The large magnitude of the crystal field, and the fact that the site symmetry about the cobalt ion is high, imply that four nitrogen atoms of **2** occupy the planar positions of the cobalt polyhedron. Substitution of one of the in-plane nitrogen atoms for an oxygen atom would have the effect of shifting the A_2 transition to lower energy and also very likely lift the degeneracy of the E state.

Pseudotetrapeptide A of bleomycin possesses nine nitrogen atoms five of which are located in the two heterocyclic residues of the molecule, three in the pyrimidine, and two in the imidazole. The shifted ^1H NMR resonances for both of these residues as well as the altered electronic absorption spectrum of the pyrimidine (Table III) indicate that both of these groups are bound to the cobalt ion. This observation and the structure of the peptide dictate that N-1 of the pyrimidine and not N-3 or the amine group on C-4 of the heterocycle is bound to the cobalt ion. The ligation of either of the latter two nitrogen atoms would not allow the imidazole to bind to the cation. Although models show that the pyridimidine and imidazole can be either cis or trans to one another on the octahedron, the stipulation that in all nitrogen in plane field is present ultimately requires that the two heterocycles occupy trans positions. This arrangement allows the amide nitrogen atom of the β-hydroxyhistidine moiety to occupy the site between the two heterocycles, thus producing three nitrogen atoms on an edge of the octahedron. The stereochemistry of the peptide further specifies that N-3 of the imidazole is ligated to the cation. Since no evidence for the protonation of the secondary amine of **2** was found, $pK_a = 2.7$ for **1**,[36] the secondary amine nitrogen atom must also be ligated to the cation. The structure of **2** constrains the secondary amine to be coplanar with the other three in-plane nitrogens of the pyrimidine, the amide, and the imidazole.

While the binding of the secondary amine, the pyrimidine, and the imidazole of bleomycin to transition-metal cations has not been generally disputed, the ligation of the β-hydroxyhistidine amide and its role in the coordination chemistry of the anitbiotic has been controversial. On the basis of spectroscopic evidence, binding sites for Fe^{II}-**1**(CO)[15] and Co^{III}-**1**[17] have been proposed which exclude amide binding and position the pyrimidine and imidazole on cis sites of the metal polyhedron. Cis coordination of these residues of bleomycin results in the formation of a thermodynamically unfavored nine-membered chealate ring.[37] The alternate possibility described above for the hydrolysis product and one which is consistent with the structure of Cu^{II}(P3-A)[10] is that the pyridmidine, the amide nitrogen, and the imidazole span an edge of the polyhedron, thus generating contiguous highly favored five- and six-membered chelate rings. Aside from the size of the chelate rings other factors strongly infer that the latter possibility is in face the case for the hydrolysis product. First, the observed high in plane crystal field for the hydrolysis product reflects the presence of the deprotonated amide group. Spectral studies on numerous copper(II) peptide complexes[38] have shown that the deprotonated amide nitrogen is a strong metal-binding group and that it lies above imidazole and primary amines in the spectrochemical series. Since this series is independent of the nature of the cation, the strongest ligating atom on Co(III) in the hydrolysis product is the amide and its presence is the reason for the high in-plane crystal field. Although the bleomycin donor set is acyclic, its crystal field is similar to those generated by unsaturated tetraaza macrocyclic ligands which are generally strong metal-ligating groups.[39]

Second, titration studies on the precursor complex Co^{II}**1** have shown that metal binding to the antibiotic causes four protons to be relased to solution.[36] Three protons orignate from the primary and secondary amines and the imidazole while the fourth originates from a functional group which does not appear in the titration curve of the metal-free antibiotic. The most logical source for the fourth proton is a metal-bound amide function. The binding of amides to cobalt(III) results in facile amide hydrogen loss, $pK_a \sim 0$.[40] Reprotonation does not remove the amide from the metal ion but instead generates a bound iminol function. Potentiometric titration and NMR studies show that the reprotonation of the amide for the hydrolysis product must be below pH ~2. The amide proton resonance, NH–His amide, does not appear in the spectrum of the hydrolysis product in Me_2SO solution.

Third, conductance measurements on the hydrolysis product have shown that at pH 6.5 the compound is a nonelectrolyte in solution and that its charge must be zero. Since **2** has only three carboxyl functions and one is protonated at pH 6.5 (HO_2C–pyr–pro), charge balance requires that the amide group is deprotonated. The rapid precipitation of AgCl from solutions of the complex upon addition of soluble silver salts to the medium indicates the chloride ion acts only as a counterion and that it is not bound to the cobalt ion.

Determining the nature of the peptide donor atom which occupies one of the axial sites of the complex proved to be difficult. The existence of the relatively low crystal field strength on the axis of the complex (1745 cm^{-1})[22] combined with the fact that one of the carboxyl groups of the complex does not undergo ionization in the pH range 0.9–8.0 lead us to conclude initially that one of these functions in its deprotonated form is bound to the cation. The disposition of the three carboxyl functions of the complex was ultimately elucidated by chemical means. Since the reaction of the hydrolysis product with methanol–HCl resulted in the formation of the triester of the compound, none of the acid functions are bound to the cation. The kinetic inertness of Co(III) would be expected to prevent a metal-bound carboxylate from entering into esterficiation.

The NMR data of the protonated from a compound in Me_2SO and in acidic D_2O clearly show that the primary amine function of the peptide is bound to the cation. Chemical conformation of this conclusion was obtained by the failure of the complex to undergo acylation (with benzoyl chloride) by using Schotten Bauman conditions. Since the unligated primary amine group of bleomycin is known to be susceptible to acylation,[27] its resistance to acyl formation is indicative of metal binding.

The low axial crystal field strength of the complex, 1745 cm^{-1}, suggests that groups weaker than the in-plane nitrogen atoms occupy the axial positions of the compound. Although primary amine functions bound to Co(III) are normally considered to be strong metal donor groups, $Dq = \sim 2400$ cm^{-1},[35] the primary amine group of **2** which occupies the axial site of the comlex has a relatively low crystal field strength, a propery which appears to be directly attributable to the structure of the peptide. The crystal field strength of L in the complex ion, $[Co^{III}(NH_3)_5L]^{3+}$ where L is in the N-bonded methyl ester of (*S*)-alanine, a model ligand for the primary amine function of the β-aminoalanine moiety of **2**, is only 1970 cm^{-1}.[41] Apparently the carbonyl group, which is α to the amine, is capable of significnatly reducing the donor strength on the amine nitrogen atom. Thus, the primary amine functions of **1** and **2** are expected to be weaker metal donors than simple aliphatic primary amines. The positions of the nitrogen donor atoms of the peptide probably further lower the effective donor strength of the primary amine. The X-ray crystallographic analysis of Cu^{II}(P-3A)[10] shows that chelation produces a strained structure. Specifically, the central ring angle, secondary amine–Cu(II)–primary amine is ~81° for Cu^{II}(P-3A).[10] This angle is somewhat lower than expected,[42] and if it persists in the Co(III) complex, its primary effect would be to further reduce the crystal field strength on the axis of the compound. These observations and the fact that the crystal field strength of H_2O on Co(III), the remaining axial group, is ~ 1880 cm^{-1}[35] are consistent with the observed magnitude of the *total* axial crystal field strength of the complex.

The hydrolysis product exhibits three strong absorptions at high energy which do not appear to be d–d electronic transitions (Figure 5). Of these, the band at 254 nm can be assigned to the $\pi \rightarrow \pi^*$ electronic transition of the bound pyrimidine function. This assignment is consistent with numerous other metallobleomycins.[2] The origin of the bands at 324 and 310 nm are uncertain, but the higher energy band is very likely that of the shifted $n \rightarrow \pi^*$ electronic transition of the bound pyrimidine function. The remaining band appears to be a charge-transfer transition association with one of the heterocycles or the amide group of the complex. Assignment of either of these bands to charge-transfer transitions associated with the aqua and the primary or secondary amine groups of the complex can be eliminated, since charge-transfer transitions for these groups are expected to occur below ~250 nm.[34]

In summary, we have presented evidence that the major product obtained from the hydrolysis of the either orange or green Co^{III}-**1** is a cobalt complex of pseudotetrapeptide A of bleomycin. Chemical and spectroscopic evidence indicate that from the standpoint of the nature of the donor atoms and their arrangement on the metal polyhedron, the Co(III)-containing hydrolysis product and Cu^{II}(P-3A)[10] are isostructural. Since both orange and green Co^{III}**1** give the same hydrolysis product, the functional group of **1** which differentiates between the two isomeric bleomycins is not part of pseudotetrapeptide A of bleomycin. In view of

(36) Sugiura, Y.; Ishizu, K.; Miyoshi, J. *J. Antibiot.* **1979**, *32*, 453. Santillo, F. S.; Dabrowiak, J. C., unpublished results.
(37) Purcell, K. F.; and Kotz, J. C. "Inorganic Chemistry"; W. B. Saunders Co.: Philadelphia, Pa., 1977.
(38) Billo, E. J. *Inorg. Nucl. Chem. Lett.* **1974**, *10*, 613.
(39) Tait, A. M.; Busch, D. H. *Inorg. Chem.* **1976**, *15*, 197.
(40) Buckingham, D. A.; Foster, D. M.; Sargeson, A. M. *J. Am. Chem. Soc.* **1969**, *91*, 3451.
(41) Dellenbaugh, G. G.; Douglas, B. E. *Inorg. Nucl. Chem. Lett.* **1973**, *9*, 1255. The value of *DqL* was calculated from the absorption data of the complex.
(42) Enemark, J. H.; Quinby, M. S.; Reed, L. L.; Steuk, M. J.; Walthers, K. K. *Inorg. Chem.* **1970**, *9*, 2397.

the different in vivo distributions exhibited by the two isomeric bleomycins, it is evident that metal coordination of this unidentified group has a dramatic affect on drug delivery. Work in progress on the behavior of the hydrolysis product at high pH and on the spectroscopic properties of the entact cobalt(III)bleomycins will more clearly define the coordination properties of the entact biologically active antibiotic.

Acknowledgment. We are especially indebted to C. J. Hawkins for helpful advice on several aspects of the work presented in the manuscript. We also wish to thank J. I. Legg, C. W. Storm, D. W. Margerum, and K. Hyde for helpful discussions. The help of Y. Kuroda of the Univeristy of Rochester NMR facility in obtaining the NMR data is also appreciated. Special thanks to F. S. Santillo and C. Randall for their help in obtaining the potentiometric titration and optical data for the complex. Finaly, we acknowledge the National Institutes of Health for support of this work (Grant CA 25112-01).

Palladium-Catalyzed 1,3-Oxygen-to-Carbon Alkyl Shifts. Basic Studies

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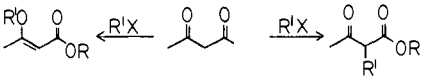
Contribution from the McElvain Laboratories of Organic Chemistry, Department of Chemistry, University of Wisconsin, Madison, Wisconsin 53706. *Received March 27, 1981*

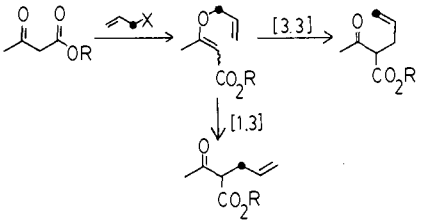
Abstract: [1-(Carbomethoxy)alkylidene]-5-vinyltetrahydrofurans which arise from preferential O-alkylation upon cyclization of β-keto esters smoothly rearrange to the desired C-alkylation products, 2-(carbomethoxy)-3-vinylcyclopentanones, with catalysis by Pd(0). With the methyl-substituted analogue, i.e., 2-(5-vinyltetrahydrofuran-2-ylidene)propionate, the major product is (*Z*)-2-(carbomethoxy)-2-methyl-3-vinylcyclopentanone. On the other hand, 1-(benzenesulfonyl)-1-(5-vinyltetrahydrofuran-2-ylidene)ethane rearranged to (*E*)-2-(benzenesulfonyl)-2-methyl-3-vinylcyclopentanone with high stereoselectivity. Conformational considerations account for these observations. This reaction constitutes the equivalent of a [1.3] rearrangement of an allyl vinyl ether and thus complements the normal [3.3] thermal rearrangement.

The chemistry of β-keto esters continues to play a major role in synthetic design. Unfortunately the major problem of O- vs. C-alkylation of such species plagues this important C–C bond-forming process (eq 1).[1] In the special case of allylating agents,



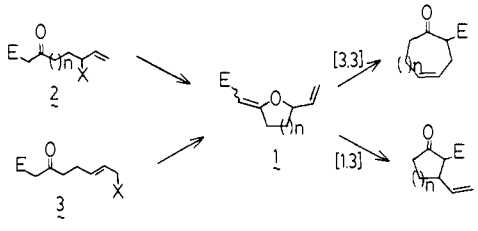
(1)

O-alkylation can be rectified by [3.3] sigmatropic rearrangement (eq 2).[2] Nevertheless, such a solution requires an allyl inversion.



(2)

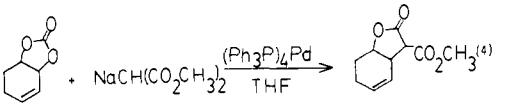
For substituted allyl systems, the substitution pattern of the product will be different, and most importantly, for intramolecular cases such as **1**, different ring sizes arise (eq 3).[3] This aspect



(3)

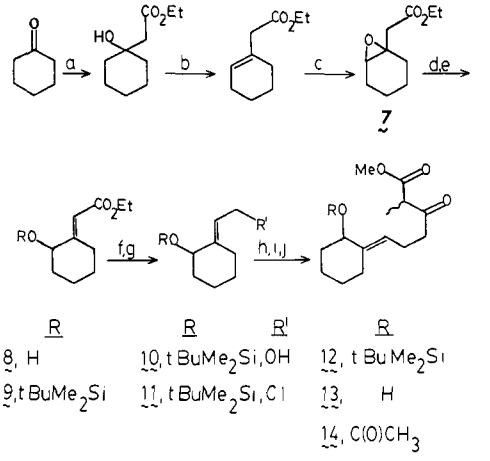
is particularly troublesome since the problem of O- vs. C-alkylation is especially pronounced for substrates like **2** or **3** ($n = 1$) which lead exclusively to products of O-alkylation.[1,4]

Work in our laboratories indicated the feasibility of vinyl carbonates such as **4** serving in palladium-catalyzed allylic al-



(4)

Scheme I. Synthesis of (*E*)-2-Acetoxy[[3-oxo-4-(carbomethoxy)-pentyl]methylidene]cyclohexane



7

R	R	R'	R
8, H	**10**, t BuMe$_2$Si, OH		**12**, t BuMe$_2$Si
9, t BuMe$_2$Si	**11**, t BuMe$_2$Si, Cl		**13**, H
			14, C(O)CH$_3$

[a] Zn, BrCH$_2$CO$_2$Et, Et$_2$O/PhH, Δ_X, 1 h. [b] *p*TSA, Dean-Stark, 48 h, 76%. [c] MCPBA, CH$_2$Cl$_2$/0.5M NaHCO$_3$, 25 °C, 3 h, 80%. [d] *t*-BuOK, THF, −105 °C, 2 h. [e] *t*-BuMe$_2$SiCl, imidazole, DMF, 25 °C, 2 h, 43% from **7**. [f] *i*-Bu$_2$AlH, PhCH$_3$, −78–25 °C, 1.5 h, 96%. [g] HMPA, CCl$_4$, Et$_2$O, 0 °C, 1 h, 84%. [h] CH$_3$COCH(CH$_3$)-CO$_2$CH$_3$, NaH, BuLi, 0.8M in THF, 0 °C, 1 h, 62%. [i] *n*-Bu$_4$NF, PhCO$_2$H, THF, 25 °C, 90 h. [j] AcCl, 4-DMAP, CH$_2$Cl$_2$, 25 °C, 1 h, 73% from **12**.

(1) E.g., Martel, J.; Blade-Font, A.; Marie, C.; Vivat, M.; Toromanoff, E.; Buendia, J. *Bull. Soc. Chim. Fr.* **1978**, *II*, 131. Bartlett, P. A.; Jernstedt, K. K. *Tetrahedron Lett.* **1980**, 1602.

(2) Rhoads, S. J.; Raulins, N. R. *Org. React. (N.Y.)* **1975**, *22*, 1.

(3) Rhoads, S. J.; Watson, J. M. *J. Am. Chem. Soc.* **1971**, *93*, 5813. Demole, E.; Enggish, P. *Chem. Commun.* **1969**, 264.

(4) Cf., Maxwell, E. N.; Titterington, D. *Tetrahedron Lett.* **1980**, 2123.

0002-7863/81/1503-7550$01.25/0