Incubation of 1b with the Enzyme Inactivated by the "k_{cat}" Inhibitor. A 1000-unit sample of pure bacterial enzyme was inactivated[16a] by a solution of **3** in dioxanne.[17] After incubation with this inactivated enzyme for 5 min, **1b** was reduced and treated as previously described.[14] The amount of epimerized deuterium is 7%.

Isomerization of 1b with a Limiting Amount of Enzyme. A 30-mg sample of **1b** (0.95 D) was dissolved in 30 mL of EtOH and then added to 1050 mL of 0.03 M phosphate buffer, pH 7. A 12-unit sample of pure enzyme was then added in order to isomerize 40% of the mixture of **1** and **2**. It is not possible to purify this mixture by chromatography since the label may be epimerized during this step.[14] The Δ^5-3-keto group in **1** was selectively reduced with a limited amount of $LiAlH(O(t\text{-}Bu))_3$, yielding 3β-hydroxyandrost-5-ene-17-one which could be easily separated from **2**. After reduction of the 17-keto group, the 3β,17β-dihydroxyandrost-5-ene was treated as previously described—12% of the initial deuterium has been exchanged and 18% of the remaining deuterium has been epimerized.

Incubation of the Other Substrates in Phosphate Buffer without Enzyme. 3b (25 mg) dissolved in 100 mL of EtOH and 1500 mL of 0.03 M phosphate buffer was left for 5 h at 25 °C. After extraction with benzene, the ^1H NMR spectrum showed a slight exchange (6%).

4b (8 mg) in 8 mL of EtOH and 280 mL of 0.03 M phosphate buffer, *pH* 7, was left for 30 min at 25 °C. After extraction with benzene and chromatography on silica gel the ^1H NMR spectrum showed a slight epimerization (9%).

10β-Chloro-17β-hydroxyestr-5-ene-3-one (9). **9** was prepared according to ref 27 (47%): mp 144 °C; NMR ($CDCl_3$) δ 5.7 (m, 1 H, 4-H), 3.55 (t, 1 H, 17 α-H), 0.82 (s, 3 H, 18-Me).

3α,4β-Dideuterio-3β,17β-dihydroxyestr-5(10)-ene (10b). A 470 mg sample of **9** dissolved in 10 mL of a 1:1 mixture of ether–benzene was added to 470 mg of $LiAlD_4$ in 2 mL of anhydrous ether and heated to reflux for 2 h. After one further hour at room temperature the mixture was hydrolyzed with EtOAc, diluted with HCl and extracted with EtOAc, yielding 380 mg of crude product, which was purified by chromatography on silica gel (benzene–EtOAc, 8:2). A 270-mg sample of pure **10b** was obtained (64%): mp 150 °C. Mass Spectrometry: D_2, 0.95, D_1, 0.05. NMR ($CDCl_3$): δ 3.55 (t, 1 H, 17α-H), 0.88 (s, 3 H, 18-Me), no olefinic proton.

4β-Deuterioestr-5(10)-ene-3,17-dione (4b). A slow stream of N_2 was bubbled in 0.79 mL of Jones reagent for a few minutes, and this reagent was added to 130 mg of **10b** in 30 mL of acetone previously distilled on $KMnO_4$. After 15 min at 0 °C, 0.66 mL of 2-propanol was added. The mixture was then poured into iced water and extracted with benzene, yielding 84.7 mg of crude product. Purification on silica gel (benzene–EtOAc, 95:5) yielded 12 mg of **4b** (10%).

NMR (270 MHz):[52] amount of incorporated deuterium ≥ 0.95 (no A.B. signal corresponding to the 4α- and 4β-protons), large singlet at 2.70 ppm, little shoulder at 2.64 ppm (relative areas 93/7); 93% of the deuterium has the 4β stereochemistry.

5,6-Oxido-3β,20β-dihydroxypregnane Dibenzoate (12). A solution of 7.1 g of 3β,20β-dihydroxypregn-5-ene dibenzoate (mp 226 °C) and 5.8 g of *m*-chloroperbenzoic acid in 80 mL of methylene chloride was left overnight at room temperature. The mixture was washed with aqueous $NaHCO_3$ and water, dried, and concentrated in vacuo to yield 6.3 g of a mixture of the 5α,6α- and 5β,6β-epoxides. This mixture could not be purified by conventional chromatographic treatments.

6β-Chloro-3β,5α,20β-trihydroxypregnane 3,20-Dibenzoate (13a). A 10.3-mL sample of concentrated HCl (37%) was added to a solution of 6.3 g of **12** in 250 mL of acetone. The solution was allowed to stand for 30 min at room temperature and slowly diluted with water to yield 6.6 g of a mixture of chlorhydrins which were purified by chromatography on silica gel (hexane–EtOAc, 2:1) to yield 4.4 g of **13a** (65%), mp 140 °C.

NMR ($CDCl_3$): δ 0.75 (s, 3 H, 18-Me), 1.3 (s + d, 6 H, 21-Me + 19-Me), 3.9 (m, 1 H, 6α-H), 5.3 (m, 2 H, 3α- + 20α-H). This isomer was identified by comparison of the chemical shifts of the 3α- and 6α-protons with those obtained in the chlorhydrin described by Malhotra and Ringold[7] in the synthesis of **1b**.

6β-Chloro-3β,20β-dihydroxypregn-4-ene Dibenzoate (14). A 8-mL sample of $SOCl_2$ was added dropwise to an ice-cooled solution of 4 g of chlorhydrin **13a** in 80 mL of dry pyridine. After being left to stand for 30 min at 0 °C, the solution was poured into crushed ice and the product was filtered off, washed, and dried. Recrystallization (acetone) afforded pure material, mp 216 °C (80%).

NMR ($CDCl_3$): δ 0.75 (s, 3 H, 18-Me), 1.25 (d, 3 H, 21-Me, J = 7 Hz), 1.35 (s, 3 H, 19-Me).

4β-Deuterio-3β,20β-dihydroxypregn-5-ene (15). A mixture of 1 g of **14** and 380 mg of $LiAlD_4$ in 20 mL of anhydrous benzene and ether was boiled under reflux with N_2 for 2 h and then stirred overnight at room temperature. The excess of hydride was decomposed by addition of a saturated solution of Na_2SO_4 and the product isolated by extraction with EtOAc. One crystallization (acetone) yielded 402 mg of **15** (71%), mp 202 °C (identical with the nondeuterated compound). M^+ = 319 (1 D).

4β-Deuteriopregn-5-ene-3,20-dione (3b). A 0.8-mL sample of 8 N Jones reagent was added with stirring to an ice-cooled solution of 120 mg of **15** in 40 mL of acetone. After the solution was stirred for another 4 min, 0.4 mL of 2-propanol was added, the resulting solution was filtered on a cotton plug, ice water was added, and nitrogen was allowed to bubble into the solution. The product was then filtered, washed, and dried in vacuo, yielding 60 mg of **3b**.

NMR ($CDCl_3$): δ 0.6 (s, 3 H, 18-Me), 1.13 (s, 3 H, 19-Me), 2.08 (s, 3 H, 21-Me), 2.76 (s, 1 H, 4α-H), 5.33 (m, 1 H, 5-H).

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(52) The 4α- and 4β-protons are not differentiated at 100 MHz, but they appear as an AB system at 2.64 and 2.71 ppm (J = 20 Hz) at 270 MHz.

(53) **Note Added in Proof:** From this complex mixture, a small amount of estr-5-ene-3,17-dione containing 1 D per molecule could be isolated. However, the NMR spectrum showed that the deuterium was distributed on several carbon atoms. The kinetic experiments reported by H. Weintraub et al. (*Biochem. J.* **1980**, *185*, 723–732), without our agreement, were carried out on this preliminary sample and should be run again.

Communications to the Editor

Transition-Metal Binding Site of Bleomycin. Cobalt(III) Bleomycin

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The study of the metal binding properties of the glycol peptide antibiotic bleomycin (**1**) is an active area of research.[1] Although certain radioactive metal complexes of the drug have been synthesized and used as diagnostics in nuclear medicine for some time,[2] the recent realization that an iron ion cofactor is very likely required for the in vivo anticancer activity of bleomycin[3] has stimulated renewed and intense interest in the metal binding properties of the drug. In vitro as well as in vivo experiments with

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(1) J. C. Dabrowiak in "Metal Ions in Biological Systems", H. Sigel, Ed., Vol. 11, Marcel Dekker, Inc., New York, p 305; *J. Inorg. Biochem.*, **13**, 317 (1980).

(2) D. M. Taylor and M. F. Cottral, *Radiopharm.*, [*Int. Symp.*], 458 (1975).

(3) E. A. Sausville, J. Peisach, and S. B. Horwitz, *Biochem. Biophys. Res. Commun.*, **73**, 814 (1976).

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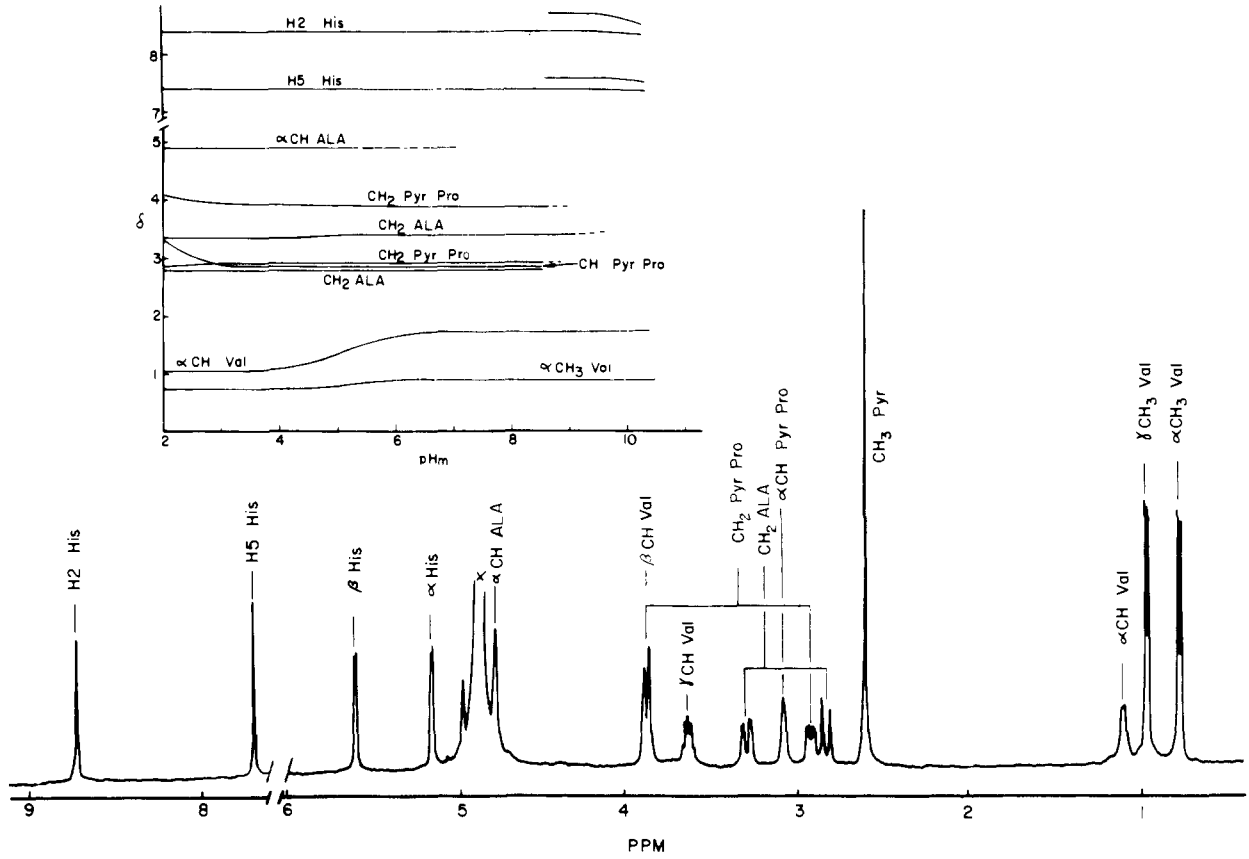

Figure 1. The 400-MHz ^1H NMR spectrum of Co(III)-**2** at pH_m 2.7 in D_2O is shown. pH_m is the pH meter reading without correction for the dueterium isotope effect. The pH_m dependency of the chemical shifts of selected resonances is shown in the inset.

bleomycin have shown that the drug is capable of degrading DNA.[4] The recent observations that the process is base sequence specific[5] strongly suggest that stereochemical factors associated with a metal ion–drug–DNA complex play an important role in the degrading process.

Theoretical and spectroscopic investigations have generated a number of possible transition-metal binding sites for the antibiotic.[6,7] However, the most definitive evidence forwarded to date on the manner in which bleomycin binds transition-metal ions has been provided by the X-ray structural analysis of Cu^{II}(P-3A).[8] But, since this complex was isolated from a culture broth and it contains a biologically inactive fragment of bleomycin, its relevance to the actual metal binding site of the intact, active antibiotic is subject to question. In an approach analogous to that used to study the metal binding sites of certain metalloenzymes,[9] we have used the labile and exchange-inert cation pair, Co(II,III), to elucidate the structure of the metal binding site of bleomycin. The similarity in the coordination chemistries of both Fe(II) and Co(III) makes Co(III)-**1** a useful vehicle for probing the structure of the biologically important iron complex and for studying the nature of the interaction between a metallated bleomycin and DNA. In this report we present the results of a spectroscopic investigation of the major acid hydrolysis product of Co(III)-**1**. The structure of the hydrolysis product, which has preserved the ligating properties of the intact drug, was found to be analogous to the structure of Cu^{II}(P-3A).[8]

Reaction of 1.0 mmol of **1**[10] with 1 equiv of $Co^{II}(ClO_4)_2 \cdot 6H_2O$ in water at pH 7.0 under nitrogen yielded the brown air-sensitive compex Co(II)-**1**. ESR, electrochemical, and potentiometric titration studies have shown that the cobalt ion in Co(II)-**1** occupies the same binding site as does Fe(II) in Fe(II)-**1**.[1,11] Aerobic oxidation of Co(II)-**1** at 25 °C yielded the substitution-inert green complex, Co(III)-**1**.[12] Mild hydrolysis of this compound (6 N HCl, 43 °C, 5 days) and workup involving $CHCl_3$ and 1-butanol washes, CM-Sephadex chromatography [0.01 M $(NH_4)_2CO_3$, pH, 6.5], and finally QAE-Sephadex chromatography (0.01 N HCl) yielded 300 mg (yield 40%, based on Co) of a red hydrolysis product and four other cobalt-containing minor components. The major product was subsequently identified as Co(III)-**2**. Thin-layer chromatography unequivocally identified one of the minor components (12% Co) as unhydrolyzed Co(III)-**1**.[13]

Elemental analysis of Co(III)-**2** was consistent with the stoichiometry $[C_{24}H_{33}N_9O_{10}ClCo] \cdot 3H_2O$, M_r 756.[14] ^{13}C NMR of Co(III)-**2** (25.2 MHz, D_2O) showed that the complex is missing the gulose, mannose, and bithiazole moieties of bleomycin.[15] Furthermore, the 24 carbon resonances of Co(III)-**2** were equally

(4) H. Umezawa, *Antibiotics (NY)*, **3**, 21 (1975).
(5) M. Takeshita, A. P. Grollman, E. Ohtsubo, and H. Ohtsubo, *Proc. Natl. Acad. Sci. U.S.A.*, **75**, 5983 (1978); A. D. D'Andrea and W. A. Haseltine, *ibid.*, **75**, 3608 (1978).
(6) J. C. Dabrowiak, F. T. Greenaway, W. F. Longo, M. Van Husen, and S. T. Crooke, *Biochim. Biophys. Acta*, **517**, 517 (1978); H. Murakami, H. Mori, and S. Taira, *J. Theor. Biol.*, **59**, 1 (1976); M. Kunishima, T. Fujii, Y. Nakayama, T. Takita, and H. Umezawa, *J. Antibiot.*, **29**, 853 (1976); C. M. Vos, G. Westera, and D. Schipper, *J. Inorg. Biochem.*, **13**, 165 (1980).
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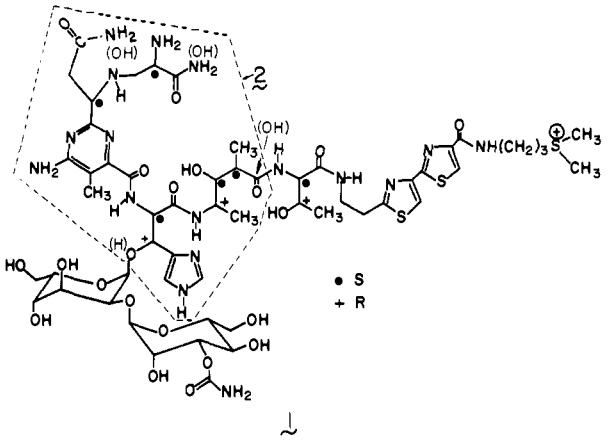
(9) J. I. Legg, *Coord. Chem. Rev.*, **25**, 103 (1978); E. P. Kang, C. B. Storm, and F. W. Carson, *J. Am. Chem. Soc.*, **97**, 6723 (1975).
(10) The commercial mixture of bleomycin, Blenoxane, was used in the synthesis.
(11) Y. Sugiura, *J. Antibiot.*, **31**, 1206 (1978); Y. Sugiura, K. Ishizu, and M. Miyoshi, *ibid.*, **32**, 453 (1979); J. C. Dabrowiak and F. S. Santillo, *J. Electrochem. Soc.*, **126**, 2091 (1979); Y. Sugiura, *J. Am. Chem. Soc.*, **102**, 5216 (1980).
(12) L. H. DeRiemer, C. F. Meares, D. A. Goodwin, and C. I. Diamanti, *J. Med. Chem.*, **22**, 1019 (1979).
(13) Silica gel, ethanol–5% ammonium acetate (2:1). R_f Co(III)-**2**, 0.56; Co(III)-**1**, 0.31.
(14) Anal. Calcd: 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.
(15) H. Naganawa, Y. Muraoka, T. Takita, and H. Umezawa, *J. Antibiot.*, **30**, 388 (1977).



divided between aromatic/carbonyl and aliphatic type resonances. A high-resolution (400 MHz) ^1H NMR spectral analysis of Co(III)-**2** (Figure 1) revealed that the complex contained 22 nonexchangeable protons, 6 of which could be attributed to methyl groups attached to asymmetric carbon atoms (D_2O, TSP, δ 0.954, d, 6.3 Hz; δ 0.765, d, 6.8 Hz, pH_m 2.7) and a single resonance accounted for the 3 protons of a methyl group attached to an aromatic ring (δ 2.601, s). The above spectroscopic data are consistent with the hydrolysis product Co(III)-**2** containing pseudotetrapeptide A of bleomycin, **2**.[15] Spin-decoupling experiments and a study of the ^1H and ^{13}C NMR spectra of Co(III)**2** as a function of pH_m (pH_m 2.0–11.5) confirmed the signal assignments shown in Figure 1.

Once the size and structure of the peptide fragment bound to the cobalt ion was established, the identity of the metal donor atoms was sought. The relatively simple visible absorption spectrum [λ_{max} (ϵ, M), 440 (356), $^1A_1 \rightarrow {}^1A_2$; 550 nm (136) $^1A_1 \rightarrow {}^1E$) and the circular dichroism spectrum [λ_{max} ($\Delta\epsilon$), 442 (3.90) and 530 nm (0.69)] of Co(III)-**2** at pH 6.2 indicated that the Co(III) ion resides in a crystal field of tetragonal symmetry. A calculation of the in-plane field strength yielded a value of 2650 cm^{-1} for Dq_{xy}.[16,17] Since this value is comparable to that found for tetraaza macrocyclic complexes[18] as well as for other in-plane tetraaza acyclic systems,[17] an in-plane array of four nitrogen atoms was assigned to Co(III)-**2**.

The calculated out-of-plane crystal field Dq_z (D_{4h}), 1744 cm^{-1}, indicated that weaker ligands occupied the axial sites of Co(III)-**2**. The relatively low crystal field strength of Cl^-[17] and the fact that AgCl readily precipitated from aqueous solutions of Co(III)-**2** upon addition of soluble Ag(I) salts to the solution ruled out axial ligand combinations involving Cl^-. Oxygen donor atoms were assigned to the axial sites. Since absorption studies showed that CN^- would displace only one of the axial ligands of Co(III)-**2** to form a monocyano derivative, a water molecule was assigned to one of the axial sites.[19]

^1H and ^{13}C NMR studies of Co(III)-**2** as a function of pH as well as potentiometric titrations[20] helped to further define the structure of Co(III)-**2**. The compound exhibited two acidic pK_a's at 2.73 (4) and 4.77 (4) which through NMR were assigned to proton loss from the pyrimidinyl propionamide and methyl valerate carboxyl functions, respectively (Figure 1, inset). None of the NMR resonances of Co(III)-**2** were affected by pH in the pH_m range 6.4–8.0. However, above this range, the molecule appeared to undergo a significant structural alteration.

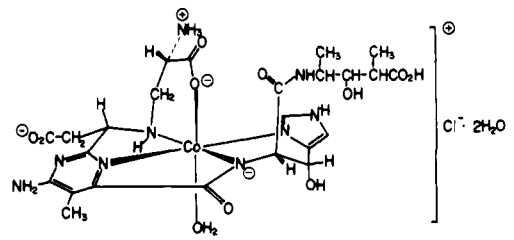


Figure 2. The structure of Co(III)-**2** at pH 3.8 is shown.

In the pH_m range 8.0–10.0, ^1H and ^{13}C NMR showed that Co(III)-**2** partially converts to a second complex which is in slow exchange with Co(III)-**2** on the NMR time scale. The ^1H and ^{13}C NMR spectra of the mixture are complex, the latter exhibiting 43 distinct resonance lines. Optically the partial conversion of Co(III)-**2** to a new compound is accompanied by a slight shift of the $^1A_1 \rightarrow {}^1E$ transition to lower energy, indicative of a decrease in the axial crystal field of the complex.[17] Efforts to characterize the new complex by using NMR were complicated by a second chemical event involving the imidazole. At pH_m values above ~10 the imidazole signals of both Co(III)-**2** (δ 8.591, H-2; δ 7.551, H-5) (Figure 1) and those of the new complex (δ 8.731, H-2; δ 7.611, H-5) shifted to high field. By analogy with Zn(II)-**1**[21] the imidazoles of both Co(III) complexes are deprotonating at high pH_m to form the imidazolium anion. Finally, conductance measurements on Co(III)-**2** showed that at pH 6.5 the compound is a nonelectrolyte in aqueous media.[22]

The above-presented evidence and the structures of **1** and **2** lead us to conclude that the structure of Co(III)-**2** is that shown in Figure 2. The binding of the pyrimidine and the imidazole (from NMR) and the requirement of an in-plane all-nitrogen environment limit the secondary amine and the β-hydroxyhistidine amide nitrogens to the positions indicated in Figure 1. Model studies show that the pyrimidine must bind by N(1) in order for other nitrogen atoms of **2** to also bind to the cation. This configuration of groups generates adjacent chelate rings having five, five, and six members, respectively, and it appears more favorable than a previously proposed alternate structure having a nine-membered chelate ring.[7] The relatively high in-plane nitrogen crystal field[18] also argues in favor of the chelate ring configuration shown in Figure 2. Charge balance and the structure of numerous metal–peptide–amide complexes[23] require that the β-hydroxyhistidine amide be deprotonated in Co(III)-**2**. In addition to the water molecule, the remaining axial site of Co(III)-**2** is occupied by the carboxylate of the β-aminoalanine moiety. This assignment was based on the axial crystal-field requirement and the observation that the deprotonation of this carboxylate does not appear in the titration curve of Co(III)-**2**. Precedence for this carboxyl group of bleomycin binding to a metal ion has appeared earlier.[24] Deamidobleomycin, a bleomycin having a β-aminoalanine moiety identical with that of **2**, utilizes this carboxyl group for binding Co(II) and Cu(II) at neutral pH. By analogy with deamidobleomycin, the high pH behavior of Co(III)-**2** is very likely due to the displacement of the carboxyl from the metal by the adjacent primary amine group. Charge balance requires that this amine function be protonated in Co(III)-**2**. Although we are unable to unequivocally choose between the structure shown in Figure 2 and a second possible isomer wherein the β-aminoalanine carboxyl and the water molecule have interchanged positions, the structure shown in Figure 2 appears to be the most favorable. Model studies show that the alternate structure would not have allowed the carbamoyl of mannose in **1** to bind to the cation. Although this group was not incorporated into Co(III)-**2**, its

(16) The parameters for the crystal-field calculation as defined in ref 17 were C, 3800 cm^{-1}; Dt, 519 cm^{-1}.

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

(18) A. M. Tait and D. H. Busch, *Inorg. Chem.*, **15**, 197 (1976).

(19) Displacement of a carboxyl function by cyanide is also possible. However, both the chelate effect and the fact that the carboxylate is negatively charged argue in favor of displacement of the water molecule.

(20) Co(III)-**2**, 3.1 mM; titrant, 0.998 (1) KOH; supporting electrolyte, 0.15 KCl; 25 ± 0.3 °C. Data analysis was accomplished by using the Bjerrum half-$\bar{n}$ method with the aid of a Fortran computer program called STBLTY (A. Avdeef, personal communication). The data were refined by using a nonlinear least-squares program.

(21) N. J. Oppenheimer, L. O. Rodriguez, and S. M. Hecht, *Biochemistry*, **18**, 3439 (1979).

(22) 7.01×10^{-4} M; $\Lambda \sim 0$ Ω^{-1} cm^2 mol^{-1}.

(23) H. Freeman in "Inorganic Biochemistry", G. L. Eichhorn, Ed., Elsevier, New York, 1973, p 121; E. J. Evans, J. E. Grice, C. J. Hawkins, and M. R. Heard, *Inorg. Chem.*, **19**, 3496 (1980).

(24) Y. Sugiura, Y. Muraoka, A. Fujii, T. Takita, and H. Umezawa, *J. Antibiot.*, **32**, 756 (1979).

importance in bleomycin coordination chemistry has been previously documented.[1]

The manner in which Co(III)-**2** was synthesized and isolated establishes the compound as an important structural model for the biologically significant iron-bleomycins.

Acknowledgment. We thank S. T. Crooke and Bristol Laboratories for supplying **1**. We also thank R. Grulich (Bristol Laboratories) and Y. Kuroda (University of Rochester) for obtaining ^{13}C and ^{1}H NMR data. We acknowledge the National Institutes of Health for support of this work (Grant CA 25112-01).

Total Synthesis of (±)-Dihydroperiphylline

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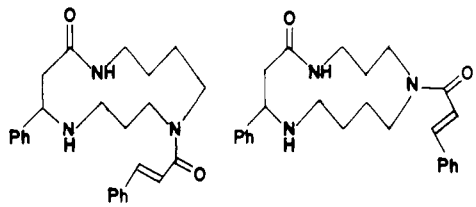
Among the polyamine alkaloids recently isolated from plant sources are dihydroperiphylline (**1**)[1] and the isomeric lactam celacinnine (**2**),[2] both of which incorporate spermidine and cinnamic acid residues in 13-membered rings. These and related



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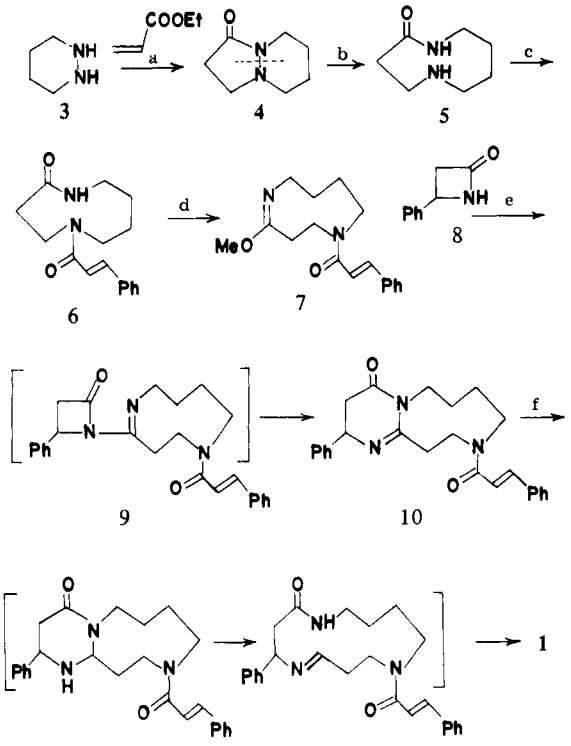
macrocyclic lactams are of special interest because of the notable biological activity shown by members of the polyamine family.[3] In two recent syntheses of celacinnine, the parent ring system was formed by direct amino acid cyclization[4] or by a transamidation process involving an amino lactam.[5] Preferred acylation of the less hindered secondary amino group then yielded the natural product. Other synthetic work leading to the formation of 13-membered lactams in this series has been reported by Husson.[6]

We now describe a total synthesis of dihydroperiphylline by an efficient six-step sequence in which the macrocyclic ring is formed by successive ring expansions of smaller heterocyclic units. Our procedure (Scheme I) permits clear-cut differentiation of the two secondary amino groups in the 13-membered lactam system by selective acylation in an early step.

Piperidazine (**3**) was condensed with ethyl acrylate to form 7-oxo-1,6-diazabicyclo[4.3.0]nonane (70%) (**4**)[7] which could be

Scheme I[a]



[a] (a) 25 °C, 1 h; 180 °C, 12 h (70%); (b) Na/NH_3 (3 equiv), 1.75 h (87%); (c) *trans*-PhCH=CHCOCl, 4-dimethylaminopyridine, CH_2Cl_2, 25 °C, 10 h (95%); (d) $Me_3O^+BF_4^-$, CH_2Cl_2, 25 °C, 23 h; 50% aqueous K_2CO_3 (83%); (e) PhCl, reflux, 21 h (67%); (f) $NaBH_3CN$ (3 equiv), 25 °C, 3 h; 50 °C, 2 h; then 25 °C, 12 h (93%).

readily cleaved with sodium in liquid ammonia under the conditions reported by Kemp[8] to form the amino lactam (**5**)[7] (87%, mp 82-84 °C). Treatment of **5** with *trans*-cinnamoyl chloride in methylene chloride in the presence of 4-(dimethylamino)pyridine yielded **6**[7] (95%, mp 147-148 °C). Conversion of **6** to the imino ether (**7**)[7] (83%) was achieved by using Meerwein's reagent (Me_3O^+, BF_4^-) followed by workup in 50% aqueous potassium carbonate.[9]

(1) Dihydroperiphylline is one of six alkaloids isolated from the leaves of *Peripterygia marginata*. All of these alkaloids contain a 13-membered ring system derived from dicinnamoylspermidine. R. Hocquemiller, A. Cavé, and H.-P. Husson, *Tetrahedron*, **33**, 645 (1977).

(2) (a) S. M. Kupchan, H. P. J. Hintz, R. M. Smith, A. Karim, M. W. Cass, W. A. Court, and M. Yatagai, *J. Chem. Soc., Chem. Commun.*, 329 (1974); (b) *J. Org. Chem.*, **42**, 3660 (1977).

(3) (a) "Advances in Polyamine Research", Vol. 1 and 2, R. A. Campbell, D. R. Morris, D. Bartos, G. D. Daves, and F. Bartos, Eds., Raven Press, New York, 1978. (b) M. M. Badawi, K. Bernauer, P. Van den Broek, G. Gröger, A. Guggisberg, S. Johne, L. Kompis, F. Schneider, H.-J. Veith, M. Hesse, and H. Schmid, *Pure Appl. Chem.*, **33**, 81 (1973).

(4) J. S. McManis and B. Ganem, *J. Org. Chem.*, **45**, 2041 (1980).

(5) H. H. Wasserman, R. P. Robinson, and H. Matsuyama, *Tetrahedron Lett.*, 3493 (1980).

(6) R. Hocquemiller, A. Cavé, and H.-P. Husson, *Tetrahedron*, **33**, 653 (1977).

(7) Spectroscopic and analytical data for new compounds are provided as follows: **4**: IR (neat) 1680 cm^{-1}; NMR (90 MHz, $CDCl_3$) δ 4.35-2.25 (8 H, m including d at 2.55), 1.95-1.35 (4 H, m, N-C-CH_2). **5**: IR (neat) 3340, 1650, 1550 cm^{-1}; NMR (90 MHz, $CDCl_3$) δ 6.80 (1 H, br s, CONH), 3.70 (1 H, **m**, CONH-C*H*), 3.50-2.35 (5 H, m), 2.15 (2 H, t, J = 6 Hz, NH-CH_2), 1.90-1.25 (5 H, m, N-C-CH_2, NH). Anal. Calcd for $C_7H_{14}N_2O$: C, 59.13; H, 9.92; N, 19.70. Found: C, 59.38; H, 10.11; N, 19.94. **6**: IR ($CDCl_3$) 3320, 1650, 1600 cm^{-1}; NMR (90 MHz, $CDCl_3$) δ 7.90-6.70 (7 H, m, phenyl, vinyl), 5.80-5.40 (1 H, br s, CONH), 3.90-3.00 (5 H, m, CON-CH_2, CONH-C*H*), 2.90-2.30 (3 H, m, $COCH_2$, CONH-C*H*), 2.00-1.40 (4 H, m, N-C-CH_2). Anal. Calcd for $C_{16}H_{20}N_2O_2$: C, 70.56; H, 7.40; N, 10.29. Found: C, 70.40; H, 7.40; N, 10.17. **7**: IR ($CDCl_3$) 1670, 1650, 1600 cm^{-1}; NMR (90 MHz, $CDCl_3$) δ 7.69 (1 H, d, J = 16 Hz, =C*H*Ph), 7.50-7.30 (5 H, m, phenyl), 6.99 (1 H, d, J = 16 Hz, =C*H*CO), 3.70-3.30 (9 H, OCH_3, CONC*H*$_2$, C=NC*H*$_2$), 2.80-2.60 (2 H, m, =CCH_2), 2.00-1.40 (4 H, m, $NCCH_2$). **10**: IR ($CDCl_3$) 1690, 1640, 1600 cm^{-1}; NMR (270 MHz, $CDCl_3$, 50 °C) δ 7.72 (1 H, d, J = 15.4 Hz, =C*H*Ph), 7.54-7.25 (10 H, m, phenyl), 6.97 (1 H, d, J = 15.4 Hz, =CHCO), 4.65 (1 H, dd, J = 4.8, 13.4 Hz, PhC*H*N), 4.34-2.57 (10 H, m), 2.11-1.39 (4 H, $NCCH_2$). Anal. Calcd for $C_{25}H_{27}N_3O_2$: C, 74.79; H, 6.78; N, 10.47. Found: C, 74.56; H, 6.62; N, 10.43. **1**: IR ($CDCl_3$) 3300, 1660 (sh), 1648, 1600, 1546, 1523 cm^{-1}; NMR (270 MHz, $CDCl_3$, 50 °C) δ 7.67 (1 H, d, J = 15.4 Hz, =C*H*Ph), 7.47-7.16 (11 H, m, phenyl, NHCO), 6.79 (1 H, d, J = 15.4 Hz, =C*H*CO), 3.91 (1 H, m, C*H*Ph), 3.89-3.06 (6 H, m, CH_2NCO), 2.68-2.55 (1 H, **m**, C*H*NH), 2.50-2.32 (3 H, m, C*H*NH, CH_2CO), 2.01-1.49 (7 H, m, $NCCH_2$, N*H*). **14**: IR ($CDCl_3$) 3430, 3300, 1660 (sh), 1640 (sh), 1625, 1550 cm^{-1}; NMR (90 MHz, $CDCl_3$) δ 7.70 (1 H, br s, NHCO), 7.40-7.10 (10 H, **m**, phenyl), 4.00-2.00 (15 H, m), 1.90-1.30 (7 H, $NCCH_2$, NH).

(8) Cleavage of 1,2-diacylhydrazides with Na/NH_3 has been reported by D. S. Kemp, M. D. Sidell, and T. J. Shortridge, *J. Org. Chem.*, **44**, 4473 (1979).

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