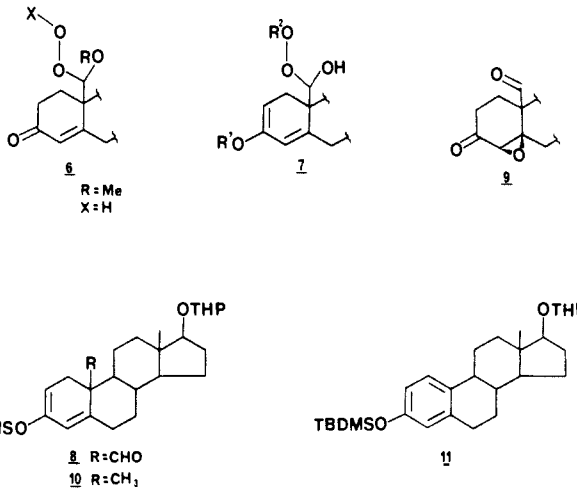

yield the 4β,5β-epoxide **9**. Treatment with *tert*-butyl hydroperoxide (70%) produced no reaction (TLC, ^{1}H NMR) under similar circumstances. Moreover, compounds **1a** and **2a** failed to react with hydrogen peroxide under these conditions. Taking into account the demonstrated facility[10] of the intramolecular epoxidation of compound **6**, the above results are plausibly explained by the intermediacy of a 19-hydroxy-19-hydroperoxide in the conversion of enone **3a** to epoxide **9**. The corresponding *tert*-butyl peroxide intermediate would be unable to react via Michael addition to the 4-en-3-one grouping. Such a 19-hydroperoxy intermediate in the case of compound **3a** is presumably reversibly formed since ^{1}H NMR data for 3β,17β-dihydroxy-19-oxoandrost-5-ene did not show diminishing of the aldehyde proton signal when the compound was treated with hydrogen peroxide in deuteriated solvent.

These results encouraged us to explore the reaction of compound **8** with hydrogen peroxide. Before embarking on a synthesis of compound **8**, it was shown that the known 10β-methyl dienol ether[12] **10** was unreactive to 30% hydrogen peroxide in $MeOH/CH_2Cl_2$ with $NaHCO_3$ present at 4 °C for several days (except for slight reketonization).[13] This demonstrated that hydrogen peroxide attack on the 19-oxo group should occur in preference to oxidation of the dienol system. We thus directed our attention to the construction of the 19-oxo derivative **8**.[14]

Treatment of the dienol ether **8** with 30% hydrogen peroxide (CH_2Cl_2/MeOH, $NaHCO_3$) resulted in rather slow but smooth aromatization affording the doubly protected estrogen derivative[16] **11** (62% yield after 3 days at 4 °C). Furthermore, production of approximately 1 equiv of formic acid[18] occurred per mol of estrogen derivative formed. Under similar conditions, *tert*-butyl hydroperoxide (70%) also reacted with compound **8** to afford protected estrogen **11** although at a somewhat slower rate (30% conversion of starting material to product after 3 days based on the ^{1}H NMR spectrum of the crude material). In the absence of peroxide agents less than 1% conversion occurred. In sum, it appears likely that the peroxide **7** is forming and subsequently decomposing to the aromatic derivative in a manner related to Scheme II. A potential aromatase model reaction has thus been created. The precise details of the mechanism of this model and its relationship to the enzymatic reaction are undergoing further study.

Acknowledgment. We thank Dr. J. Kachinski, Jr. for help with mass spectrometry and Syntex Co. for a generous gift of 19-hydroxydehydroepiandrosterone. We also appreciate the use of the 400 MHz NMR spectrometer at the Department of Chemistry, The Johns Hopkins University. This work was supported by the National Institutes of Health (HD-11840). We thank the Medical Scientist Training Program for a Grant to P.A.C. (GM-07309).

(12) Tanabe, M.; Crowe, D. F. *J. Chem. Soc., Chem. Commun.* **1973**, 564-565.

(13) The ^{1}H NMR spectrum revealed that about 10-20% reversion to the enone had occurred.

(14) (a) All new compounds gave satisfactory spectroscopic and analytical data. (b) Bisacetylation of 3β,19β-dihydroxyandrost-5-en-17-one with acetic anhydride and pyridine was followed by reduction of the 17-ketone with sodium borohydride. The resulting 17β-ol was protected as the THP ether and the acetate groups were removed with KOH/MeOH (overall yield 84%). Oxidation with Collins reagent followed by treatment with DBN in MeOH gave the desired 17β-(tetrahydropyranyl)oxyandrost-4-ene-3,19-dione in 45% yield. Treatment of this enone with TBDMS triflate and collidine[15] generated nearly exclusively the cisoid dienol ether **8** (95% yield after flash chromatography).

(15) Korenchuk, E. N.; Golubovskaya, L. E.; Pivnitskii, K. K. *Zh. Obsch. Khim.* **1985**, *55*, 2150-2151.

(16) Besides demonstrating the appropriate spectroscopic and analytical data, compound **11** matched identically with the tetrahydropyranylation product of known 3-((*tert*-butyldimethylsilyl)oxy)estradiol.[17]

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(18) Quantitation of formate was performed by using ^{1}H NMR integration with *n*-propanol employed as an internal reference. The formate was also derivatized as *p*-bromophenacyl formate which showed complete spectroscopic and chromatographic agreement with known material.[19] It was also shown that methyl formate hydrolysis (or other irrelevant pathways) was probably not an important source of the formic acid, as formic acid was not produced in significant quantity when methyl formate was submitted to the peroxide reaction conditions.

(19) Risley, J. M.; Van Etten, R. L. *J. Am. Chem. Soc.* **1980**, *102*, 4609-4614.

Rates of Specific Peptide Binding to the Glycopeptide Antibiotics Vancomycin, Ristocetin, and Avoparcin

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The glycopeptide antibiotics are of interest not only because of their clinical importance, as seen to date principally in vancomycin,[1] but also because they represent one of the smallest peptide-peptide binding systems where specific and tight (μM dissociation constants) interaction is achieved.[2] The structures in solution of several of these antibiotics and of their complexes formed with specific *N*-acyl-D-alanyl-D-alanine ligands have been elegantly investigated by NMR methods.[3]

The kinetics of the binding of these specific peptides to vancomycin and ristocetin have also been investigated by an NMR method[4] and appeared to reveal a striking difference between vancomycin and ristocetin, where the binding of *N,N'*-diacetyl-L-lysyl-D-alanyl-D-alanine to the former seemed much more rapid (10^{10} s^{-1} M^{-1}) than to the latter (3.8×10^6 s^{-1} M^{-1}). This result was interpreted[4a] in terms of the structural data. Thus, vancomycin was proposed to require a significant conformational change at the N-terminus on peptide binding[5] which is not necessary or possible in the more rigid ristocetin. Avoparcin represents an intermediate structure where the kinetics of binding have not yet been reported.

The experiments described below were initiated to explore in more detail the kinetics and mechanism of the binding process. We have recently described a new fluorescent ligand, ε-*N*-acetyl-α-*N*-dansyl-L-lysyl-D-alanyl-D-alanine (ADLAA),[6] which

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(2) Nieto, M.; Perkins, H. R. *Biochem. J.* **1971**, *123*, 789-803.

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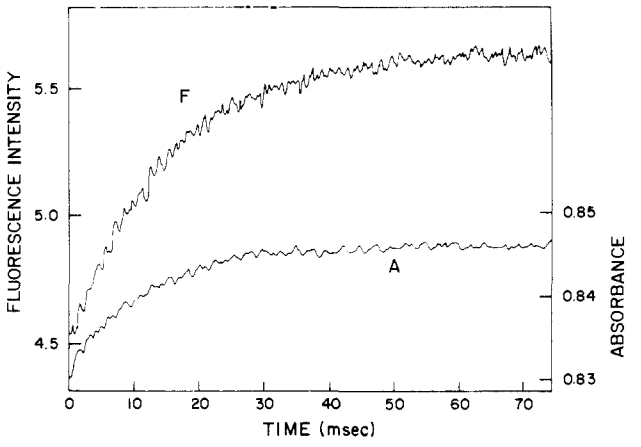

Figure 1. Kinetics of ADLAA binding to vancomycin. Recording of total fluorescence intensity beyond 400 nm (F) on mixing ADLAA and vancomycin (final concentrations 1.0 μM and 10.0 μM, respectively) in 0.1 M phosphate buffer, pH 7.0. Also shown is a recording of the absorbance at 550 nm (A) on mixing ADLAA and vancomycin (final concentrations 10.0 μM each) in a solution containing 33 μM phenol red at pH 7.6.

undergoes fluorescence enhancement on binding to these antibiotics. ADLAA was mixed in a stopped-flow spectrophotometer with a series of vancomycin solutions and the change in fluorescence[7] monitored. In all cases, monotonic increases were observed (Figure 1) which could be fitted[9] to a single-step binding process (eq 1, where D is ADLAA and V is vancomycin). This

$$D + V \underset{}{\overset{K_1(=k_1/k_{-1})}{\rightleftharpoons}} DV \qquad (1)$$

procedure yielded $k_1 = (9.3 \pm 1.6) \times 10^6$ s^{-1} M^{-1} and $k_{-1}(=k_1/K_1) = 31 \pm 5$ s^{-1}. Since the value of k_1 did not vary systematically over the concentration range employed,[7] the binding reaction is, kinetically, a bimolecular process under these conditions.

Similar results were obtained for ristocetin and α-avoparcin, leading to rate constants for binding (k_1) of $(7.2 \pm 2.1) \times 10^6$ and $(4.1 \pm 1.3) \times 10^6$ s^{-1} M^{-1}, respectively, and dissociation (k_{-1}) of (24 ± 7) s^{-1} and (28 ± 9) s^{-1}, respectively.

These results suggest that the same or a similar process is rate-determining in all three cases. That this slow step is not rearrangement of the dansyl group in a rapidly formed initial complex was demonstrated by the employment of a complementary measurement of the rates. At a pH above the pK_a of the N-terminal ammonium group, peptide binding is accompanied by proton uptake,[12] which can be followed by changes in the absorbance of phenol red at 550 nm,[13] as also shown in Figure 1. These data yielded the same rate constants within the experimental uncertainty limits, as did the fluorescence data, for the binding of ADLAA to both vancomycin and ristocetin, and similar rate constants were obtained for *N,N'*-diacetyl-L-lysyl-D-alanyl-D-alanine. Thus the rate-determining step in the binding of ADLAA does not involve the dansyl group but is apparently characteristic of specific binding of a L-lysyl-D-alanyl-D-alanine peptide. Since the step is common to the three antibiotics it cannot involve in any way the conformational change of the N-terminus specific to vancomycin.

The magnitude of the rate constant k_1 is remarkably, and probably impossibly, small for a diffusion-controlled combination of molecules of the size of ADLAA and the antibiotics.[14] Experiments in glycerol solutions bear this out—the rate constant k_1 of vancomycin is not affected by viscosity.[15] Hence a two-(at least) step binding process must obtain, where the first step involves diffusion-controlled formation of a weakly bound initial complex, which then rearranges in a slower step. The slow step might entail a common conformational change or perhaps a desolvation process.[16] We are looking into these possibilities.[17]

Acknowledgment. We are grateful to Wesleyan University for financial assistance.

(6) Popieniek, P. H.; Pratt, R. F. *Anal. Biochem.* **1987**, *165*, 108-113.
(7) Experiments with vancomycin were carried out in 0.1 M phosphate buffer at pH 7.0 and 25 °C. The stopped-flow system has been described previously.[8] The excitation wavelength was 330 nm, and total emission beyond 400 nm was monitored. The concentration of ADLAA was 1.0 μM, and the concentration of vancomycin was varied from 1.0 to 30.0 μM. Experiments with ristocetin and avoparcin A were performed similarly but at pH 6.5 and 6.0, respectively. Vancomycin B, ristocetin A, and α-avoparcin were generous gifts from Eli Lilly and Co., H. Lundbeck A/S, and American Cyanamid Co., Lederle Laboratories, respectively.
(8) Anderson, E. G.; Pratt, R. F. *J. Biol. Chem.* **1981**, *256*, 11401-11404.
(9) Measurements of fluorescence as a function of time were fitted by means of a nonlinear least-squares procedure[10] and an integrated rate equation.[11] Values for the equilibrium constants, K_1, were taken from fluorimetric titrations.[6]
(10) Johnson, M. L.; Halvorson, H. R.; Ackers, G. K. *Biochemistry* **1976**, *15*, 5363-5371.
(11) Rorabacher, D. B.; Turan, T. S.; Defever, J. A.; Nickels, W. G. *Inorg. Chem.* **1969**, *8*, 1498-1506.
(12) Brown, J. P.; Feeney, J.; Burgen, A. S. V. *Mol. Pharmacol.* **1975**, 11, 119-125.
(13) Reacting solutions at 25 °C contained 10.0 μM each of ADLAA and vancomycin or ristocetin, 33.0 μM phenol red, and 0.2 M potassium chloride and were adjusted to pH 7.6 after purging with nitrogen.
(14) Berg, O. G.; von Hippel, P. H. *Ann. Rev. Biophys. Biophys. Chem.* **1985**, 14, 131-160.
(15) Measurements of the equilibrium and rate constants of binding of ADLAA to vancomycin were made fluorimetrically as described above. The buffers included 30% and 60% (w/v) glycerol on one hand and, to give solutions of essentially unchanged (with respect to the aqueous conditions) viscosity but equivalent (to the glycerol solutions) dielectric constants, 15% and 30% (w/v) ethanol on the other. Both binding strength and rates were decreased but to the same extents, within experimental uncertainty, in the glycerol solutions as in the ethanol.
(16) Cram, D. J. *Angew. Chem., Int. Ed. Engl.* **1986**, *25*, 1039-1057.
(17) We do not understand the disparity between our results and those of Williamson et al.[4a] with respect to vancomycin. The aggregation of vancomycin and its peptide complex at the concentrations used (6 mM)[18] may have produced complications; however, spurred by a reviewer and an editor we undertook some 400 MHz ^{1}H NMR measurements (^2H_2O, 20 mM phosphate buffer, pD 6.5, 6 mM vancomycin or ristocetin, 12 mM *N,N'*-diacetyl-L-lysyl-D-alanyl-D-alanine) which showed the methyl group of the C-terminal D-alanine of the peptide to be in slow exchange at 60° in both the vancomycin and ristocetin complexes; the vancomycin observation is contrary to that of Williamson et al.[4a] but consistent with our fluorescence data.
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Nickel(0)-Promoted Cyclization of Enynes with Isocyanides: A New Route to Polycyclic Cyclopentenone Skeletons

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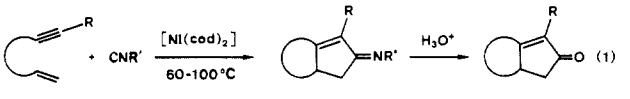
We report herein the first successful case of nickel(0)-promoted cyclization of enynes and isocyanides to form 1-imino-2-cyclopentenes which may be hydrolyzed to the corresponding cyclopentenones, as shown in eq 1.



Much interest has recently been directed toward transition-metal-promoted synthesis of cyclopentanoids from alkenes, alkynes, or enynes, and carbon monoxide.[1,2] Of these reactions, however,

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