2OTf$^+$), 151 (doubly charged cation). For spectral data, see Table I.

Electrochemical Study. Cyclic voltammetry was performed with 10-mL portions of 2 mM solutions of sulfide in CH_3CN and 0.1 M tetrabutylammonium perchlorate. The CV cell was equipped with a Iwaki Glass SCE reference electrode in a reference well separated from the analyte by a cracked glass bead junction, a Pt wire counter electrode, and a Pt disk working electrode polished before use with alumina. All sulfides studied were purified by preparative liquid chromatography.

Registry No. **1**, 112399-00-5; **2**, 112421-52-0; **3**, 112399-01-6; **3a**, 112399-02-7; **4**, 112399-04-9; **6**, 112399-05-0; **7**, 108428-22-4; **8**, 108428-23-5; **9**, 108428-24-6; **9a**, 112399-06-1; **10**, 108428-25-7; **13**, 112399-07-2; **14**, 112399-08-3; **15**, 112335-85-0; **16**, 112399-09-4; **17**, 608-28-6; **18**, 118-72-9; **19**, 52805-90-0; **20**, 112399-10-7; **21**, 112399-11-8; **22**, 54088-93-6; **23**, 112399-12-9; **24**, 112399-13-0; PhSH, 108-98-5; MeSH·Na, 5188-07-8; *o*-$PhSC_6H_4CH_2Br$, 37660-43-8; *m*-$(CH_2Br)_2C_6H_4$, 626-15-3.

Communications to the Editor

A Peroxide Model Reaction for Placental Aromatase

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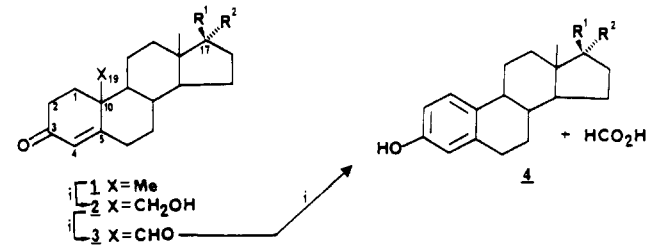
The conversion of androgens **1** to estrogens **4** is catalyzed by the cytochrome P-450 enzyme system estrogen synthetase (aromatase). The mechanism of this transformation has recently attracted attention both because of the chemical novelty of the reaction and the potential medical importance of aromatase inhibitors.[1] Three separate steps are apparently involved in the transformation (see Scheme I), and formic acid is ultimately produced as a byproduct. Three molar equivalents of NADPH and O_2 are required overall.[2] Two stereospecific hydroxylations occur at C-19 to afford the 19-OH **2** and 19-oxo **3** intermediates. The first equivalent of oxygen consumed is incorporated into compound **3** and eventually formic acid.[3] The third equivalent of oxygen consumed also is incorporated into formic acid[4] as is one of the original C-19 hydrogens. The 1β,2β-hydrogens of compound **3** are lost to the aqueous medium.[5]

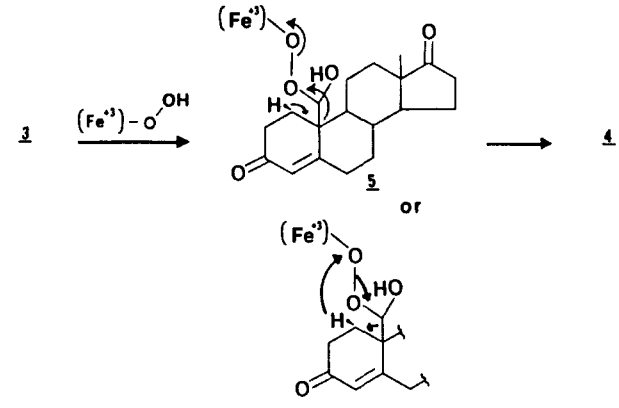
Despite intensive investigation, the nature of the third oxidative step catalyzed by aromatase remains unknown. Theories involving 2β-hydroxylation,[6] Baeyer-Villiger oxygen insertion,[4] and 4,5-epoxidation[7] have been shown to be unlikely. A proposal suggesting heme ferric peroxide attack of the 19-oxo group to yield the corresponding α-hydroxyferric peroxide **5** (see Scheme II) has remained viable but not well studied.[8] The peroxide **5** was envisioned to fragment either by a hydride transfer[8] or proton shift[9]

Scheme I[a]



[a] (i) NADPH, O_2; a: R^1 = O, R^2 = O; b: R^1 = OH, R^2 = H.

Scheme II



pathway to produce the aromatic ring. Recently, we sought to model this intermediate and synthesized the corresponding α-methoxyhydroperoxide **6** by ozonolysis of the appropriate vinyl ether.[10] This relatively unstable compound failed to afford estrone under a variety of conditions. One possible explanation for the observed lack of reactivity was the absence of a driving force for 1β-hydrogen removal. It was hypothesized that concomitant enolization of the 3-ketone could lower this energy barrier.[10] We desired to test this idea by exploring the reactivity of a chemical model such as compound **7**.

It was expected that ozonolysis of the appropriate 10β-vinyl analogue to diene **10** in a manner employed[10] for the synthesis of peroxide **6** would be nonselective. Instead we envisaged the reaction of hydrogen peroxide with the dienol ether **8** as a route to the hydroperoxide **7** (R^1 = TBDMS, R^2 = H).[11] Indeed treatment of the 19-aldehyde **3a** with excess 30% hydrogen peroxide in the absence of strong base (MeOH, $NaHCO_3$, 4 °C, 2 h) led to rapid and stereospecific epoxidation to afford in 60%

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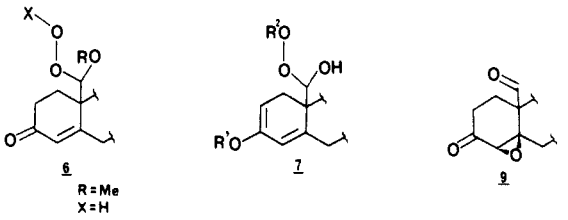
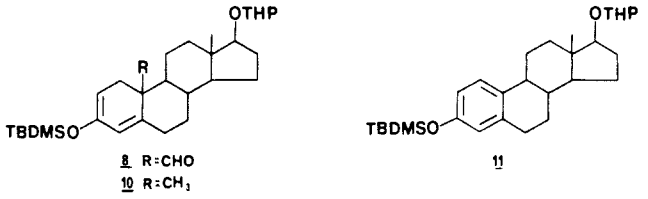


6
R=Me
X=H

7

9



8 R=CHO
10 R=CH$_3$

11

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).

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(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).

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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.

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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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