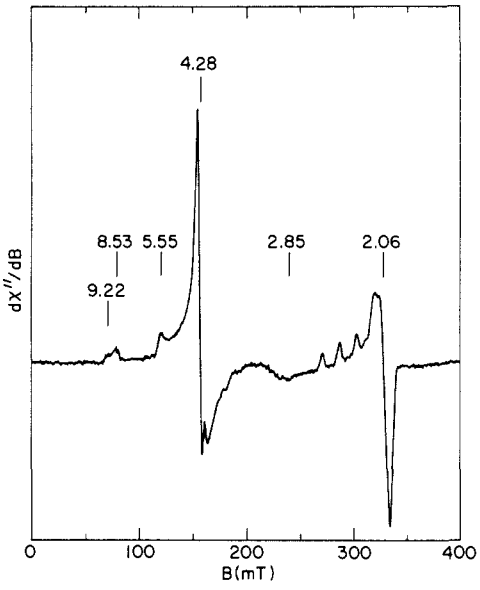


**Figure 2.** EPR spectrum of the native (Zn-Fe) form of red kidney bean purple phosphatase (**1**) at 4 K. Spectral conditions: microwaves 9.447 GHz at 20 $\mu$W; 100 kHz modulation at 2 $mT_{pp}$; $dB/dt$ = 2 mT/s; 10 scans in 2000 s.

enzyme has no EPR spectrum in the $g' = 1.74$ region but shows the $g = 4.3$ signal characteristic of isolated high spin Fe(III) with large rhombicity. From measurements at different temperatures and attempts to simulate the Fe(III) signals of Figure 2 it again appears that at least two different species are present: one with rhombicity $E/D \sim 0.25$ and $D/k \sim 1.5$ K giving rise to the $g \sim 9.22$ and $g \sim 4.28$ features and another one with $E/D \sim 0.14$, $D/k \sim 0.4$ K giving rise to the features at $g \sim 8.53$, 5.55, and 2.85. Since the sample contained 1.14 Fe atom and 0.76 Zn atom per subunit, not all the Fe(III) can be paired with a diamagnetic Zn(II). One therefore expects to observe at least two types of EPR signals, but it is not clear which one to assign to the native Fe(III)-Zn(II) pair. Owing to difficulties with the integration of the Cu(II) signal overlapping the highly rhombic iron signals, attempts to quantify the number of spins proved to be unreliable. Small $g = 4.3$ signals are present in the spectra of **2** and **3** (not shown) but account for only a very small percentage of the iron in the enzyme samples. Signals at $g = 4.3$ corresponding to trace amounts of high spin Fe(III) have been observed in other samples of pig allantoic fluid and beef spleen enzymes.[4,5,7] The spectrum of Figure 2 shows a sizeable and well-resolved Cu(II) signal; weaker signals have been noted in the spectra of Figure 1. Small amounts of copper have been found in some red kidney bean preparations, frequently $\leq$0.1 Cu per subunit, but in one preparation (of 16), as high as 0.56 Cu/subunit. The sample used in the current preparation was not analyzed for copper. Moreover, the absolute purity of the starting enzyme in no way alters the significance of the observations.

The EPR data on the Fe(II)-Fe(III) derivative of red kidney bean phosphatase therefore show that it contains an antiferromagnetically coupled binuclear iron complex. There is good evidence that the Zn(II) atom in the native enzyme occupies the same site as the Fe(II) atom in the Fe(II)-Fe(III) derivative: (i) binding studies have shown that there is only one strong binding site for divalent metal ions per subunit,[8] and (ii) the catalytic activity of the Fe(II)-Fe(III) derivative is similar to that of the Zn(II)-Fe(III) enzyme.[8,9] Therefore, the present evidence supports the existence of a binuclear zinc-iron complex in red kidney bean purple phosphatase, the first such complex to be reported.

The catalytic subunit of calcineurin, a phosphoprotein phosphatase from bovine brain, contains stoichiometric amounts of zinc and iron,[13] and it is possible that it may have an active-site structure similar to that of the red kidney bean enzyme. Several other purple phosphatases, including enzymes from sweet potato[14] and soybean,[15] have been purified from plants, and it will be of interest to determine their relationship to the red kidney bean enzyme. The only other enzyme known to contain zinc in close proximity to a transition metal ion is the Cu-Zn superoxide dismutase in which both metal ions are linked to the same imidazole group.[16]

The significance of a binuclear center in catalysis by phosphatases remains to be established, although roles for both metal ions of a model binuclear complex in hydrolysis of a phosphate ester have been established.[17] The reason why the animal purple phosphatases have an Fe-Fe center, whereas the plant enzyme has a Zn-Fe center, is also unknown. The catalytic efficiencies of the two systems are comparable, but they differ in their susceptibility to oxidation. One could speculate that the animal enzymes in contrast to the plant enzymes may be subject to reversible redox control of their phosphatase activity.

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**Registry No.** Phosphatase, 9013-05-2; acid phosphatase, 9001-77-8.

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## Silylation-Mediated Oxidation of 4-Aza-3-ketosteroids with DDQ Proceeds via DDQ-Substrate Adducts

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In connection with continuing interest from these laboratories in azasteroids[1] we wish to report an efficient, silylation-mediated DDQ (2,3-dichloro-5,6-dicyano-1,4-benzoquinone) oxidation of 4-aza-3-ketosteroids to the corresponding $\Delta^1$-lactams which proceeds via unprecedented DDQ-substrate adducts. The existing technology[2] for effecting such transformations involves several cumbersome steps or is often complicated by poor yields, unwanted byproducts, and use of selenium reagents. The new oxidation (**1** $\rightarrow$ **5**) provides a unique entry into a diverse spectrum of 17$\beta$-substituted $\Delta^1$-4-aza-5$\alpha$-androsten-3-ones (**5**) which are currently undergoing clinical evaluation for benign prostatic hypertrophy.[3] For example, treatment of **1a** with 1 mol of DDQ and 4 mol of BSTFA [bis(trimethylsilyl)trifluoroacetamide][4] in dioxane under

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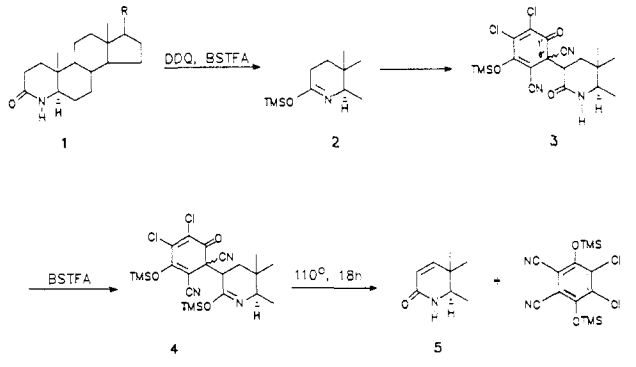
**Scheme I**[a]

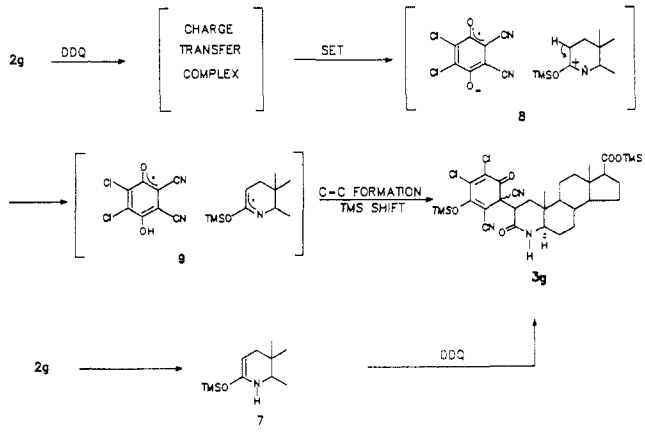



[a] **1a**; R = COOH, **1b**; R = $CONEt_2$, **1c**; R = CONH-*t*-butyl, **1d**; R = $COCH(CH_3)Et$, **1e**; R = $COOCH_3$, **1f**; R = CN, **1g**; R = COOTMS.

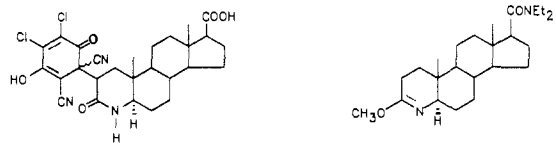
$N_2$ at 20 °C for 4 h followed by 110 °C for 18 h cleanly produced the dehydrogenated lactam **5a** in 85% yield.[5] These conditions were also applied successfully for conversion of the azasteroids **1b–g** to the corresponding $\Delta^1$-azasteroids **5b–g** with consistently high yields (85–90%).[5] Treatment of **1a** with BSTFA and DDQ at 20 °C showed rapid consumption of starting materials, but no product (**5a**) was detected. Gradual appearance of product was observed only when the reaction mixture was heated at reflux. Thus, action of DDQ and BSTFA on **1a** initially produced an intermediate which was undergoing thermolysis to **5a**. We present here the results of in situ NMR investigations where sequential intermediates in this reaction have been characterized.

The reaction of **1g** was examined with in situ NMR spectroscopy ($^{13}C$, $^{29}Si$, and $^1H$).[6] The earliest observation at 20 °C indicated that **1g** rapidly converts to the *O*-silyl imidate **2g** which then slowly reacts with DDQ to form a pair of diastereomeric (at $C_6'$) adducts **3g**. Then BSTFA resilylates **3g** to **4g** (Scheme I). Kinetic and $^{13}C/^{29}Si$ NMR evidence requires that **3g** be written as the free lactam shown. Key $^{13}C$ chemical shifts and long-range spin splittings, due to the 2-H, observed in situ in **3g** were $\delta_C$ = 180.1 ($^3J_{CH}$ = 3.5) and 181.9 ($^3J_{CH}$ = 5.8) for $C_1'$ and $\delta_C$ = 94.2 ($^3J_{CH}$ = 6.1) and 99.7 ($^3J_{CH}$ = 5.0) for $C_5'$. Pairing of $C_1'$ and $C_5'$ signals was ascribed to diastereoisomerism at $C_6'$, as were paired methine carbons at $\delta_C$ = 49.1 and 53.5 assigned to the newly substituted $C_2$. Comparable long-range proton splittings were found in **4g** for $C_1'$ ($\delta_C$ = 180.7, 180.8) and $C_5'$ ($\delta_C$ = 94.3, 98.5), while paired $C_3$ signals were relocated from 167.9 and 168.6 ppm in **3g** to 155.1 and 155.9 ppm in **4g**, reflecting the transformation from lactam to O-silylated imidate groups. Complete $^{13}C$ and $^{29}Si$ NMR data are given in the Supplementary Material. Terminating the reaction after 4 h at 20 °C enabled the isolation and independent characterization of unsilylated analogues **6**. The equatorial disposition of the DDQ-derived cyclohexadienone moiety at $C_2$ was established in **6** by $^1H$ NMR in DMSO-$d_6$ solvent [$\delta$ = 3.3, dd ($J$ = 12 Hz, 3.2 Hz) 1 H]. Other $^1H$ NMR features supportive of structures **6** included diasteriomerically split 19-$CH_3$ signals at 0.97 and 0.99 ppm and barely resolved 4-NH

**Scheme II**




singlets at 7.27 and 7.28 ppm. Mass spectroscopy (xenon FAB ionization, VG 20–250 quadrupole spectrometer) gave $(M + H)^+$ = 546, 548 (chlorine isotopic distribution) for **6**. Complete $^{13}C$




assignments for **6** appear in the Supplementary Material. Upon thermolysis **3g**, **4g**, and **6** produced the $\Delta^1$-analogues **5**, possibly via a concerted six-electron pathway.[7] The rates of thermolysis for the two diastereomers were significantly different.[8,9]

Formation of the diastereomeric adducts **3g** and **4g** could be explained either via single-electron-transfer mechanism[10] between DDQ and the O-silylated imidate **2g** or a nucleophilic attack of its O-silylated tautomer **7** on DDQ (Scheme II). Formation of the *O*-trimethylsilyl imidate is a prerequisite for the C–C bond formation. The corresponding *O*-methyl imidate **10** failed to react under similar conditions.

We have demonstrated a simple procedure for dehydrogenation of various 17-substituted 4-aza-3-ketosteroids in high yield. Furthermore, formation of the intermediates sheds important light on the mechanism of silylation-mediated quinone oxidations[10,11] which have been traditionally believed to proceed via hydride transfer. Formation of similar intermediates was also observed with quinones and TMS–enol ether derivatives of ketones as well as lactams. Details of this work along with further mechanistic studies will be the subject of future publications.

**Acknowledgment.** We thank J. L. Smith of these laboratories for mass spectroscopic investigations. We also thank R. A. Reamer, also of these laboratories, for complete $^{13}C$ assignments for **5c**.

**Supplementary Material Available:** Spectral data for compounds **1g–4g** and **6** (2 pages). Ordering information is given on any current masthead page.

(4) BSTFA is the preferred silylating agent in this oxidation in terms of yield and compatibility with DDQ.

(5) A typical procedure for the synthesis of **5a** follows. BSTFA (59.6 g, 231.4 mmol) was added to a stirred suspension of **1a** (18 g, 56.4 mmol) and DDQ (12.81 g, 56.4 mmol) in dioxane (180 mL) under $N_2$ at 20 °C. The light red reaction solution was stirred at 20 °C for 4 h and then at 110 °C for 18 h. The resulting dark red solution was poured into a stirred mixture of $CH_2Cl_2$ (300 mL) and 1% aqueous sodium bisulfite solution (80 mL). The heterogeneous mixture was filtered to remove the precipitated hydroquinone. The dark red organic layer was separated and washed with 100 mL of 2 N HCl. The product **5a** was crystallized from acetonitrile following removal of $CH_2Cl_2$ by distillation, producing 15.3 g of **5a** (85%).

(6) NMR spectra were obtained by using Varian XL-100 and Bruker WM250 spectrometers with broadband or composite pulse decoupling employed, respectively, in temperature-controlled $^{13}C$ studies. Silicon-29 observations were made with the INEPT technique (Burum, D. P.; Ernst, R. R. *J. Magn. Reson.* **1980**, *39*, 163–168) for optimum sensitivity. In the NMR studies a slight excess of DDQ was used, and BSTFA was added last.

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