for optimum performance. The use of immobilized whole organisms of this type, or of crude extracts from them, would be the most efficient method for preparation of the required enzymatic activities.

Registry No. NADH, 58-68-4; NAD^+, 53-84-9; NADPH, 53-57-6; $NADP^+$, 53-59-8; ADH, 9031-72-5; AldDH, 9028-88-0; FDH, 9028-85-7; ethanol, 64-17-5; methanol, 67-56-1.

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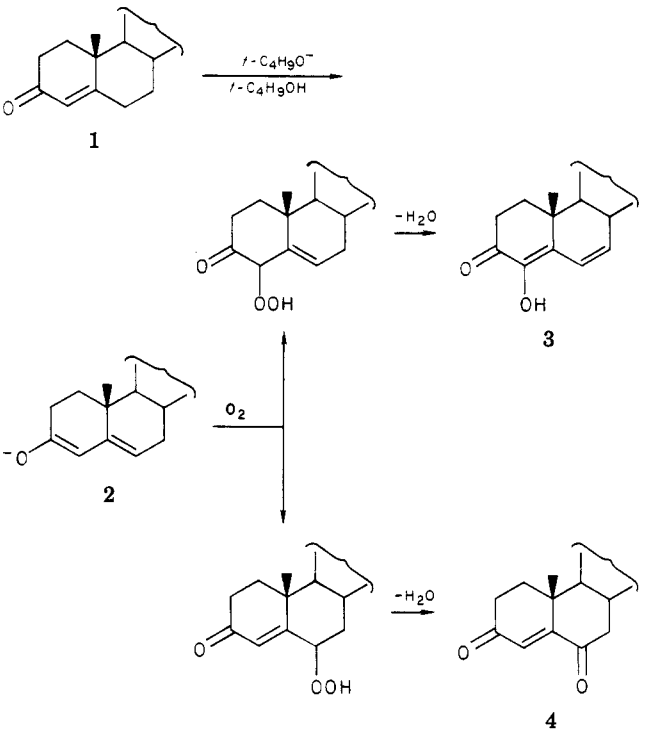
Superoxide-, *tert*-Butoxide-, and Hydroxide-Mediated Autoxidation of 3-Oxo-Δ^4 Steroids in Aprotic Media

Summary: The course of the base-catalyzed autoxidation of 3-oxo-Δ^4 steroids in aprotic media differs sharply both in yield and product distribution from that reported for protic solvents and involves the oxygenation of the kinetic dienolate.

Sir: The base catalyzed autoxidation[1] of various 3-oxo-Δ^4 steroids (1) in protic media was studied by Camerino and co-workers[2] in the early sixties. They reported that enones 1 react with oxygen in the presence of potassium *tert*-butoxide in *tert*-butyl alcohol, generating in low yield the corresponding 4-hydroxy-Δ^6-dehydro and 6-keto derivatives (ketones **3** and **4**, respectively). For example, when 4-cholesten-3-one is the substrate (1), enones **3** and **4** are isolated in 15% and 5% yield, respectively; no information is supplied by the authors as to the identity or fate of the remaining 80% of the product. In any case, it would seem clear that **3** and **4** result from oxygenation of the α(C-4) and γ(C-6) carbons of the thermodynamic[3] dienolate **2**.

Our interest in the base-catalyzed autoxidation of enones and enols in aprotic solvents,[4] particularly those mediated by the biologically important superoxide anion radical,[5] led us to examine the reaction of the steroidal analogues. As the results outlined in Scheme I indicate, the course of these oxidations differs substantially both in product distribution and yields from those reported by Camerino et al.[2] In a typical reaction, 4-cholesten-3-one,18-crown-6, and potassium superoxide (KO_2) in a 1:2:4 molar ratio were dissolved in dry benzene (100 mL/mmol of steroid) and



1 2 3 4

stirred under dry air at ambient temperature until all the steroid had been essentially consumed (~20 h). The reaction mixture was then acidified with 10% HCl and extracted 3 times with $NaHCO_3$ solution during which time an orange precipitate formed which floated in between the aqueous and organic layers. The organic phase was dried, concentrated, and chromatographed (preparative TLC on silica gel, using 25% acetone in hexane as eluent), yielding lactol **9** (30% yield) and a mixture of lactols **10** (4%) and **11** (6%). The latter were readily separable once they were converted by Ag_2O/CH_3I[6] to the corresponding keto esters **16** and **17**. The aforementioned precipitate was dissolved in 10% HCl (overnight) and the resulting solution was extracted with chloroform. Evaporation of the extracts gave a white precipitate (60% yield), which was treated with diazomethane. Separation of the resulting mixture of esters by preparative TLC gave **18**, **19**, and **20** in a molar ratio of 4:1:1. Esters **15–20** could be obtained directly from the reaction mixture by treating the latter with excess methyl iodide prior to aqueous workup. Similar results were obtained with testosterone.

Several important observations should be made regarding this reaction. Firstly, the course of this reaction remains essentially unchanged when KO_2 is replaced by KOH or potassium *tert*-butoxide except that the rate of reaction is fastest with the latter and slowest with KOH. Hence it is probable that the mechanism of the superoxide anion radical mediated process (like *tert*-butoxide and hydroxide) entails initial proton (not hydrogen-atom) abstraction,[4,7] which is followed by the various steps typical of base-catalyzed autoxidation.[1]

Secondly, when the reaction is quenched at shorter reaction times, in addition to starting material substantial amounts of enol **6** are obtained. The conversion of the latter to lactol **9** under the reaction conditions is quantitative and is likely to proceed by the mechanism suggested for 2-hydroxy-2,5-cyclohexadien-1-ones.[4b] Similarly lactol

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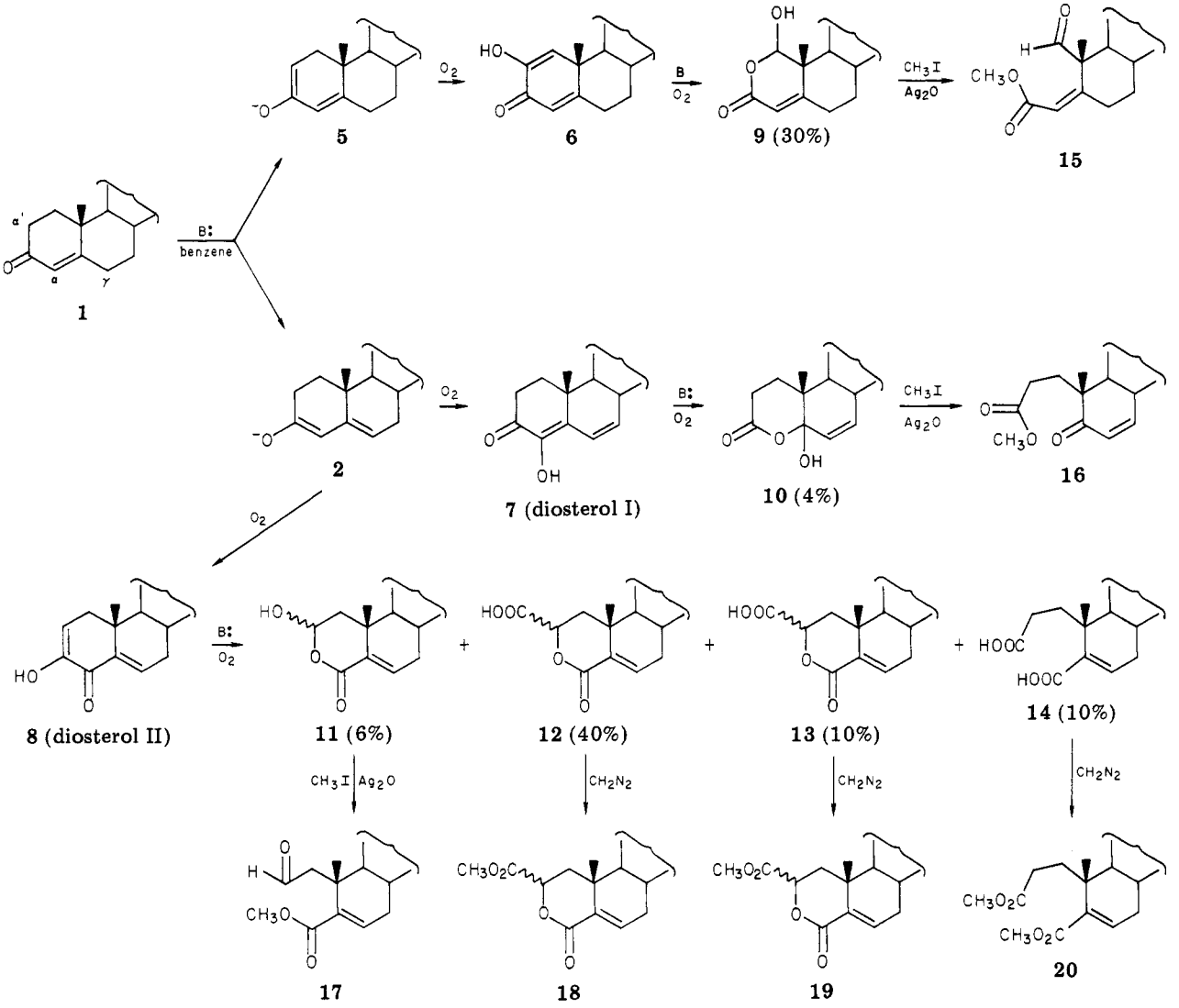
Scheme I. Base-Catalyzed Autoxidation of Δ^4-Cholestenone in Benzene



10 undoubtedly stems from diosterol I[8] (7), while lactol 11 and acids 12–14 are derived from diosterol II[8] (8). It is noteworthy, however, that while diosterols I and II result from the oxygenation of dienolate 2, enol 6 requires the intermediacy of the thermodynamically less stable but kinetically favored[3] dienolates 5. Indeed, when the reaction temperature is lowered, the rate of isomerization of 5 to 2 is slowed such that 5 can be trapped quantitatively. Thus when Δ^4-cholestenone is autoxidized with *tert*-butoxide in toluene at −17 °C for 2 h, enol 6 can be isolated in yields >90%. If instead of quenching the reaction to isolate enol 6, the reaction is allowed to continue at room temperature overnight, lactol 9 is obtained in yields >85%. Although the trapping of the kinetic dienolate in aprotic media is by no means new,[3] its application to autoxidative processes in general and to the synthesis[4c] of enol 6 and lactol 9 has until now, to the best of our knowledge, been unreported.

Finally, the total absence of diketone 4 either in the product mixture or during the course of the reaction (TLC) suggests that the oxygenation of enolate 2 in dry aprotic media occurs selectively at the α-position (C-4) in a kinetically controlled process. It is not surprising then that when the crown ether used is "old", i.e., wet[9], a complex product mixture results which contains no 6 or 9 but a 20% yield of diketone 4. Clearly the presence of a proton source allows for the rapid generation of the thermodynamic enolate (2) and in turn the thermodynamic product 4.

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Registry No. 1, 601-57-0; 2, 81971-28-0; 4, 984-84-9; 5, 81971-29-1; 6, 21936-15-2; 7, 3686-98-4; 8, 68138-42-1; 9, 81089-20-5; 10, 81971-30-4; 11, 81971-31-5; 12, 81971-32-6; 13, 81971-33-7; 14, 434-11-7; 15, 81971-34-8; 16, 30961-08-1; 17, 81971-35-9; 18, 81971-36-0; 19, 81971-37-1; 20, 55651-92-8; KO_2, 12030-88-5; KOH, 1310-58-3; potassium *tert*-butoxide, 865-47-4.

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(9) 18-Crown-6 in quite hygroscopic.

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