Oxidations of Enone Systems in Steroids by Oxidizers with Reversible Redox Potential

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The oxidation of steroid enones by oxidizers with reversible redox potentials (mostly tetrazolium salts) is described. Products containing an oxo or hydroxy group at the position γ with respect to the oxo of the enone group were isolated in relatively high yields. The results of kinetic studies on the redox processes are reported and a reaction mechanism is proposed.

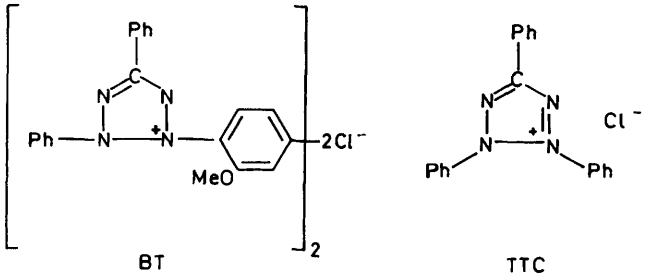
There are few reports of oxidation reactions of steroid enones in alkaline media.[1-3] Camerino *et al.*,[2] reported that cholest-4-en-3-one reacts with oxygen in the presence of potassium t-butoxide in protic media, generating in low yield the corresponding 4-hydroxy and 6-oxo derivatives. Langenbach and Knoche[3] found a small amount of 6-oxoprogesterone as a product of the reaction of progesterone with oxygen in alkaline methanol.

The 6-oxo derivative was not detected when the alkaline treatment was carried out under anaerobic conditions.

We report here results bearing on the oxidizability of enone groups in steroids by compounds with reversible redox potentials (mostly tetrazolium salts) in the presence of potassium hydroxide and under an inert atmosphere (nitrogen). We studied the reaction pathway as well as its products, and carried out kinetic studies of the redox reaction; moreover, we investigated the influence of the medium on the course of the redox processes and undertook studies aimed at elucidating the mechanism of oxidation by these oxidizers. In earlier papers,[4] we have described the oxidation of an α-ketol group in corticosteroids by oxidizers with thermodynamically reversible redox potentials in mild alkaline media. In a one-step reaction we obtained high yields of the respective mono-, di-, and trihydroxycorticosteroid acids and, in lower yields, the respective α-oxo acids or ring *D* oxo derivatives.

Results and Discussion

As oxidizers we used tetrazolium salts and indophenol salts, as well as diphenylamines and alloxans. The course of the oxidation of steroid enones was found to be invariant, but the yields of oxidation products differed. The best oxidizers with respect to yield and easy separation of the coloured products of reduction were Blue Tetrazolium (BT) and 2,3,5-triphenyltetrazolium chloride (TTC). In this paper, we discuss the yields



BT TTC

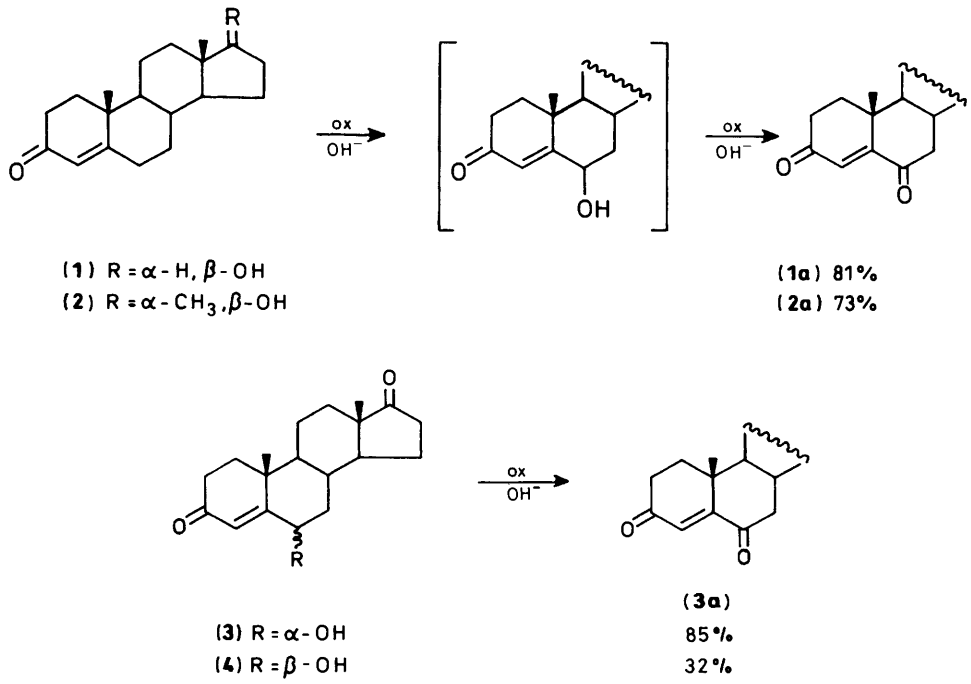
obtained with BT as oxidizer (denoted as 'ox' in the Schemes). In the Experimental section we also give the yields obtained with TTC. The tetrazolium salts used are analytical reagents well known for their use in biological redox reactions; during the reaction the colourless form is reduced to a deeply coloured formazan.[5]

Steroids with a sterically unhindered 4-en-3-one group served as model compounds for which we determined the optimal conditions for oxidation. These compounds, 17β-hydroxyandrost-4-en-3-one (**1**) and 17β-hydroxy-17α-methylandrost-4-en-3-one (**2**), underwent oxidation in relatively high yield to the respective 6-oxo derivatives (**1a**) (81%) and (**2a**) (73%). T.l.c. studies with appropriate standards showed a 6-hydroxy steroid to arise in the first step of the reaction (Scheme 1). Confirmation of the hydroxy intermediate was obtained in the oxidation of 6α-(**3**) and 6β-hydroxyandrost-4-ene-3,17-dione (**4**). In the case of the 6α-hydroxy isomer (**3**), the 6-oxo enone (**3a**) was isolated in 85% yield. In that of the 6β-hydroxy isomer (**4**), the same reaction yielded 32% of the same 6-oxo enone (**3a**) (Scheme 1). Longer reaction times enhanced the yield to 46%; however the product required recrystallisation to obtain pure crystals. The known alkaline rearrangement of 6β-hydroxy-Δ^4-3-ones to 5α-3,6-diones[6] probably accounts for the low yield. A small amount of 5α-androstane-3,6,17-trione was found in the remaining reaction mixture (by t.l.c. analysis, with a standard for comparison).

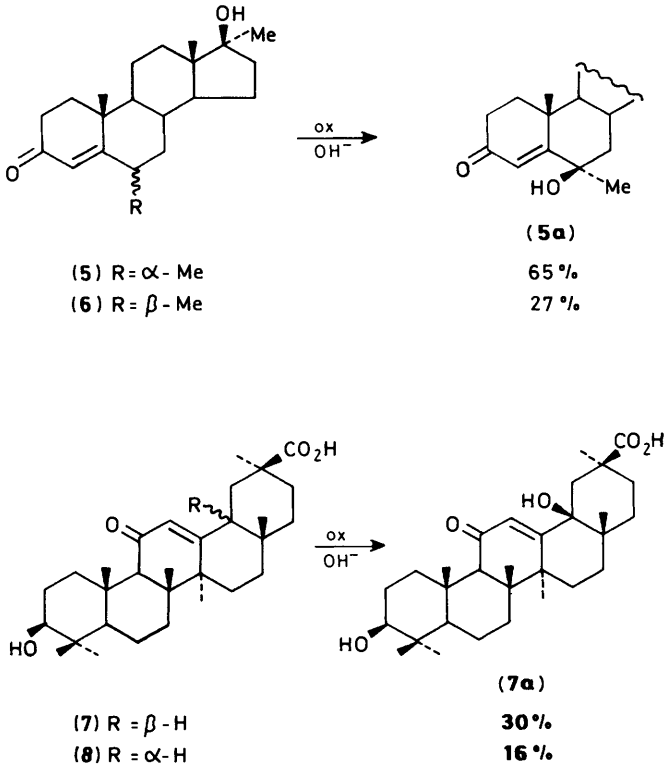
A divergence from the previous results occurred when 11-hydroxy-4-en-3-ones were oxidised. An 11α-hydroxy substituent had little influence on the course and yield of the reaction was observed. 11α17β-Dihydroxy-17α-methylandrost-4-en-3-one and 11α-hydroxyandrost-4-ene-3,17-dione gave the respective 6-oxo derivatives in 68 and 75% yield, respectively. However, the 11β-hydroxy substituent in 11β-hydroxyandrost-4-ene-3,17-dione unexpectedly deactivated the enone group; the reaction gave very low yields of oxidation product and starting material predominated in the reaction mixture. A similar suppression of the redox reaction was found with 17β-hydroxy-4-methylandrost-4-en-3-one. The 2α-methyl group in 2α-methylpregn-4-ene-3,11,20-trione did not hinder the reaction; the respective 6-oxo compound was isolated in ~54% yield.

The 19-nor steroid 17α-ethynyl-17β-hydroxy-19-norandrost-4-en-3-one showed enhanced reactivity. The reaction was complete in half the time required for the model enone (**1**). The expected 6-oxo derivative, 17α-ethynyl-17β-hydroxy-19-norandrost-4-ene-3,6-dione, was obtained in 36% yield. In addition, a 6-oxo compound with an aromatic ring *A*, 17α-ethynyl-3,17β-dihydroxyestra-1,3,5-(10)-trien-6-one, was isolated, in 42% yield. T.l.c. analysis showed traces of three other products; however, we refrained from determining their structure as unimportant for the clarification of the redox processes under investigation. Their formation and the aromatisation of ring *A* probably reflected the lability of 19-nor-3-oxo steroids in alkaline media.[7]

In cases when the carbon atom γ with respect to the enone group (C-6) possessed a tertiary structure, as in 6α, 17α- (**5**) and



Scheme 1.



Scheme 2.

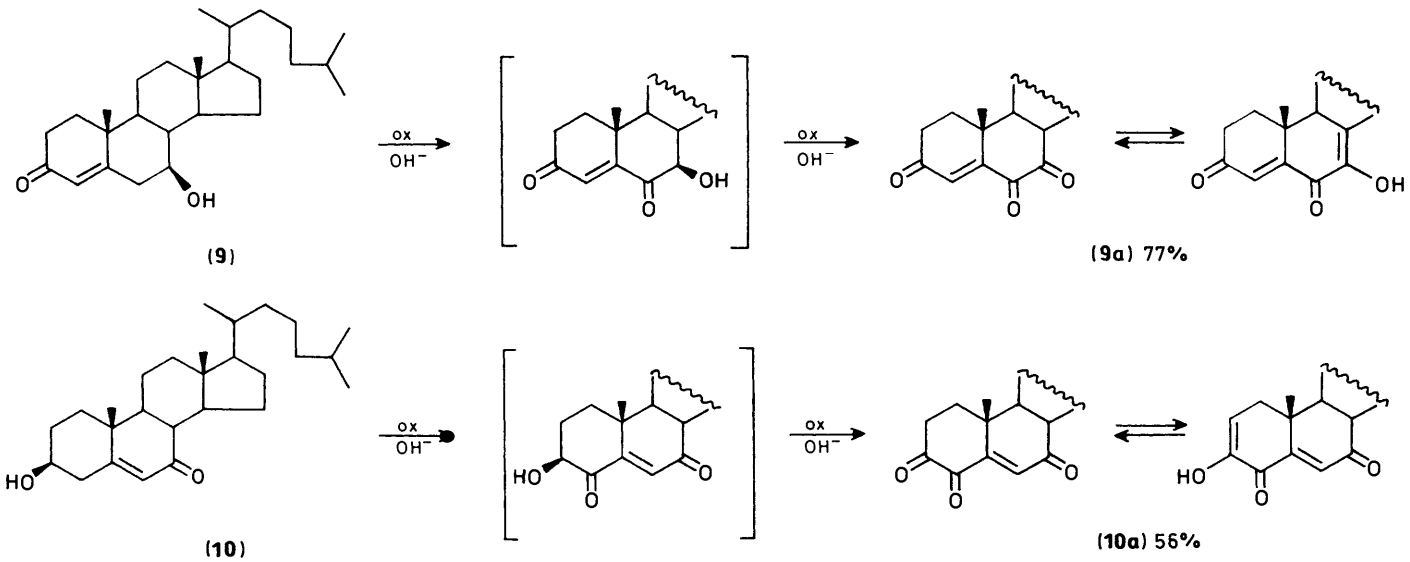
6β,17α-dimethyl-17β-hydroxyandrost-4-en-3-one (**6**), the reaction permitted the isolation of the intermediate 6-hydroxy derivatives (Scheme 2). From the 6α-methyl isomer (**5**) the 6β-hydroxy enone (**5a**) was obtained in 65% yield; under identical conditions the 6β-methyl isomer (**6**) yielded the same product but in only 27% yield. An extension of the reaction time raised the yield to 39%; however the compound thus obtained required additional purification. The isolated product of oxidation (**5a**) is further proof of the role of intermediate hydroxy forms in the production of the 6-oxo derivatives.

The glycyrrhetinic acid isomers (**7**) and (**8**) with tertiary C-18 (γ to the enone group) gave the hydroxy derivatives in low yield. In the case of the 18β-isomer (**7**), the 18β-hydroxy compound (**7a**) was isolated in 30% yield, whereas the same product arose from the 18α-isomer (**8**) in only 16% yield, (Scheme 2). The configuration of the 18-hydroxy substituent was assumed as β on the basis of spectral data and by analogy with the substance (**5a**).

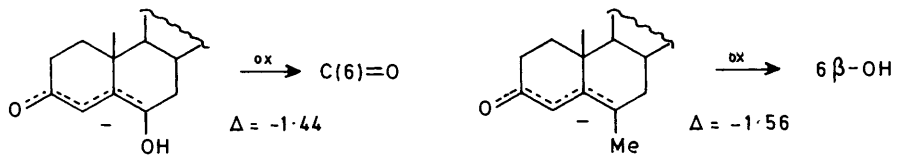
The *cis*-8-en-15-one 15-oxochol-8(14)-en-24-oic acid exhibited weak reactivity. The 7-oxo derivative was obtained in 33% yield. A rise in alkalinity of the reaction medium or an increase in temperature had no marked influence on yield.

Studies on the oxidation of the hydroxy-enones 7β-hydroxycholest-4-en-3-one (**9**) and 3β-hydroxycholest-5-en-7-one (**10**) showed that in order to achieve complete reaction the concentration of the oxidizer had to be higher than for the 'pure' enone groups as in the steroid (**1**). Presumably, the oxidation reactions of these compounds proceed in two steps (Scheme 3). First the ketone function is introduced on the carbon atom γ with respect to the enone group, thus leading to an α-ketol highly susceptible to further oxidation; we did not succeed in isolating these compounds. In a second step, the α-ketol group immediately undergoes reoxidation, giving α-dioxo products, which we succeeded in isolating as enol derivatives in high yields [77% for cholest-4-ene-3,6,7-trione (**9a**) and 56% for cholest-5-ene-3,4,7-trione (**10a**)]. Confirmation of the high activity of α-ketol groups in cyclic compounds with respect to oxidizers with reversible redox potentials was also obtained with the model compound cyclohex-2-en-1-one. In the redox process, it was converted into the cyclohexane-1,2-dione, which exists in the enol form only.[8]

On the other hand, steroids with extended dienone-type conjugation (androsta-1,4-diene-3,17-dione and androsta-4,6-diene-3,17-dione) proved totally 'inactive' with regard to our oxidizers. Enhanced alkalinity of the medium or a protracted



Scheme 3.



Scheme 4.

Table. Rate constants for enolisation and oxidation of steroid enones

Substance	$\log k_{enol}/s^{-1}$	$\log k^{ox}_{obs.}/s^{-1}$ [a]	$\Delta = \log k_{enol} - \log k^{ox}_{obs.}$
(3) 6α-OH	−4.88	−3.45	−1.43
(4) 6β-OH	−5.09	−3.65	−1.44
(5) 6α-CH_3	−5.11	−3.55	−1.56
(6) 6β-CH_3	−5.25	−3.70	−1.55

[a] Total for the enolisation and oxidation processes.

reaction failed to make oxidation effective. Drastic conditions led only to decomposition of the oxidizers.

Mechanistic Considerations.—Our earlier studies on the redox reaction between oxidizers with reversible redox potentials and corticosteroids showed that the alkaline medium transformed the α-ketol group of the corticosteroids into the enediol form, defined as the 'active form'. The presence of their form determined the further course of the oxidation.[4] The enone groups of the present steroids, under identical conditions (0.056M KOH, room temperature)[4] did not undergo oxidation. However, by increasing the pH (0.5—1.5M KOH) and raising the temperature (70 °C), we achieved satisfactory oxidation of the enone groups of the compounds studied. On the basis of numerous literature data[9] and our kinetic studies on the influence of the medium on these redox reactions,[10] the enone group of the steroids can be assumed to undergo enolisation, thus producing enolate ion (the requisite 'active form') which is necessary for oxidation. In addition we performed kinetic spectrophotometric studies of the redox reactions between BT and selected steroid enones: (**1**), (**3**)—(**6**), 11α-hydroxyandrost-4-ene-3,17-dione (**11**), 11β-hydroxyandrost-4-ene-3,17-dione (**12**), 2α-methylpregn-4-ene-3,11,20-trione (**13**), and 17α-ethynyl-17β-hydroxy-19-norandrost-4-en-3-one (**14**). Pseudo-first-order rate constants were obtained for all these enones (see Experimental section). A plot of the kinetic data (Figure) shows that the rate of oxidation depends strongly on the steric structure of the molecule. β-Substituents close to the enone

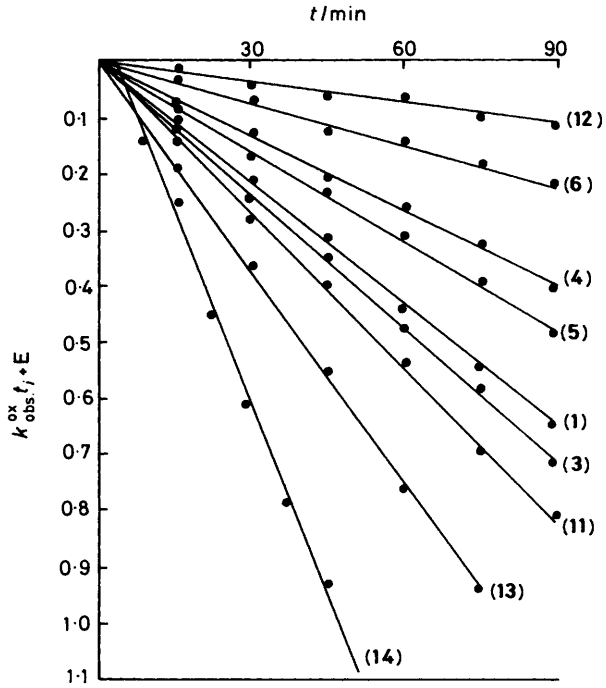
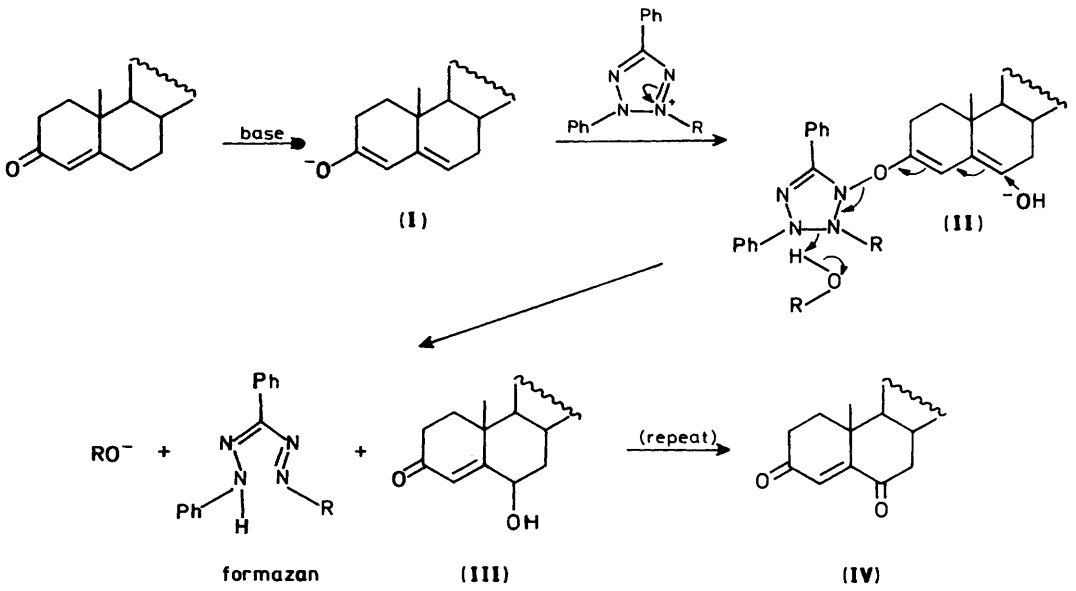


Figure. Reaction rates of typical enone steroids with BT; see equation (i)



Scheme 5. Proposed mechanism of oxidation of steroid enones

chromophore [as in (**4**), (**6**), and (**12**)] lower the rate. In no cases did α-substituents [as in (**3**), (**5**), and (**11**)] cause a decrease in reaction rate as compared with the enone (**1**), in which the enone chromophore is sterically unhindered. In certain cases [enones (**13**) and (**14**)] the rate was even higher than for compound (**1**). We therefore assume that the rate-controlling step is the formation of the 3,5-dienolate, a process involving attack by base from the β-side of the molecule.

We also carried out comparative kinetic studies on the processes of enolisation (25 °C; M KOH–EtOH) and oxidation (25 °C; M KOH–EtOH; BT) for the isomers (**3**)/(**4**) and (**5**)/(**6**) (see Table).

The differences in the enolisation rates (log k_{enol}) for 6α- and 6β-isomers can be interpreted by analogy with literature data [11] as due to stereoelectronic effects causing the proton with β-configuration at C-6 to be more easily removed in the enolisation process than the α-proton. Rate differences (Δ = log k_{enol} − log $k^{ox}_{obs.}$) are the same for isomers (**3**)/(**4**) and (**5**)/(**6**). This is evidence that the following reaction (oxidation of the dienol) is common to both isomers (Scheme 4). Also, this explains why the isomers (**5**) and (**6**) give exclusively 6β-hydroxy derivatives.

Proof of the ionic nature of the reaction between enone steroids and the tetrazolium salts was obtained by monitoring the reactions of the enones (**1**) and (**2**) with BT and TTC by e.s.r., and from kinetic studies of the redox processes in the presence of iodine (a radical scavenger which inhibits the radical reaction; for results see Experimental section). These studies suggested that the oxidation did not proceed by a free radical mechanism. Hence it can be assumed that the reactions are bimolecular, in which electrons are transferred from the steroid to the oxidizer.

When conducting the redox reaction in different solvents, both hydrated and anhydrous {EtOH, Me_2SO, $(MeO[CH_2]_2)_2O$}, we found that anhydrous solvents suppressed the oxidation completely in the case of enones (**1**) and (**2**). We also measured the rate of BT reduction by the enones (**1**) and (**2**) in $(MeO[CH_2]_2)_2O$ and in CH_3OD with a 5% added D_2O. The rate showed a moderate decrease, suggesting that at a post-enolisation stage a certain role with regard to the rate of the process as a whole may be played by general acid catalysis caused by D_2O (dissociation of the O–D bond) and, by analogy, by H_2O (dissociation of the O–H bond) under standard conditions.[10]

From considerations related to our experimental results as well as to the literature data for enolisation reactions [12] it can be assumed that the mechanism of oxidation of steroid enones follows the path shown in Scheme 5. Formation of the dienolate ion (I) is followed by its addition to the tetrazolium salt, with formation of an intermediate adduct (II). The weak N–O bond in (II) is disrupted by β-attack of the base (OH^-), with possible general acid catalysis (ROH). The 6β-hydroxy enone (III) is further oxidized to the 6-oxo derivative (**IV**) by repetition of the previous sequence of reactions.

Experimental

All reactions were run under nitrogen. M.p.s were determined with a PHMK apparatus. ^1H N.m.r. spectra were recorded with a Varian EM-360 60 MHz spectrometer, with $(CD_3)SO$ as solvent. Chemical shifts are reported (δ_H) relative to Me_4Si as internal standard. I.r. spectra were determined for KBr tablets with a Specord M-80 spectrophotometer. U.v. spectra were determined for solutions in ethanol with a Specord M-40 spectrometer equipped with a constant-temperature cell compartment and cell holder to control the temperature at 25, 35, 45, or 50 ± 0.5 °C. Electron spin resonance studies were carried out with a Varian 4502-13 instrument. Microanalyses were carried out with a Perkin-Elmer 240 elemental analyser.

Materials.—Enones were prepared by published procedures: (**3**), (**4**), (**5**), (**9**), (**10**), and 15-oxo-5β-chol-8(14)-en-24-oic acid. Blue Tetrazolium (BT), 2,3,5-triphenyltetrazolium chloride (TTC), and other enone steroids used were commercially available.

General Procedure for Oxidation of Enones by Tetrazolium Salts.—A solution of the enone (0.3 mmol) and BT (0.8 mmol, 0.582 g) or TTC (1.2 mmol, 0.402 g) in 95% ethanol (15 ml) was placed in a three-neck flask with a stirrer and dropping funnel. The flask was flushed with oxygen-free nitrogen, the solution was heated to boiling, and 6M potassium hydroxide in ethanol (5 ml) was added (reaction solution 1.5M in KOH). From the moment the base was added, the reaction ran for 30 min at the boiling temperature of the solution. Water (7 ml) was then added, the mixture was cooled to *ca.* 5 °C, and the precipitated coloured formazans (reduction product of BT or TTC) were

removed by filtration. The filtrate was neutralized with 5M hydrochloric acid (*ca.* 6 ml) and then water (30 ml) was added. The mixture was extracted with chloroform (3 × 60 ml) and benzene (1 × 50 ml). The combined organic extract was evaporated under reduced pressure to 1/3 of its original volume ('organic phase'). Next, the 'organic phase' was shaken with water (2 × 20 ml) and gently shaken with 0.5M KOH (1 × 10 ml) in order to remove unchanged tetrazolium salt. The water and 0.5M alkaline extracts were then combined ('aqueous phase').

Organic phase. The phase was exhaustively extracted with Claisen's alkali[13] (3 × 30 ml); the extracts were combined and acidified with conc. hydrochloric acid, and extracted with chloroform (3 × 100 ml). The combined chloroform extracts were dried ($MgSO_4$), filtered, and evaporated to dryness under reduced pressure. The crude product was purified on a chromatographic column (30 × 1.5 cm) containing silica gel (20—70 mesh; Merck). Eluants are specified later. The appropriate combined eluate fractions were evaporated to leave the residual crude products. In general, the oxidation product was obtained in almost pure form, and was purified by recrystallisation. In certain cases the product had to be purified by further column chromatography or recrystallization.

Aqueous phase. The combined extracts were acidified with 5M hydrochloric acid and extracted with chloroform (2 × 50 ml). The extracts were dried and evaporated to dryness. By t.l.c. the composition of the extract was checked for undesirable steroids [silica gel; methyl ethyl ketone–chloroform (1:9)]. The remaining aqueous phase was rejected.

The following products were obtained.

17β-Hydroxyandrost-4-ene-3,6-dione (**1a**), from the enone (**1**) (0.087 g). Chromatography eluant chloroform–benzene (2:1). Recrystallization from acetone–hexane gave yellow needles of the 6-oxo enone (**1a**) (0.073 g, 81%), from oxidation with BT. In the TTC oxidation, additional recrystallization from hexane gave (**1g**) (0.059 g, 65%); m.p. 206—207 °C (lit.,[14] 203—205 °C) (Found: C, 75.8; H, 8.6. Calc. for $C_{19}H_{26}O_3$: C, 75.5; H, 8.7%); $\lambda_{max.}$ 252 nm (log ε 4.03); $\nu_{max.}$ 3 580, 1 691, 1 675, and 1 610 cm^{-1}; δ_H 0.67 (s, 18-H_3), 1.19 (s, 19-H_3), 3.65 (m, 17-H), and 5.83 (s, 4-H).

17β-*Hydroxy*-17α-*methylandrost*-4-*ene*-3,6-*dione* (**2a**), from the enone (**2**) (0.091 g). Chromatography eluant chloroform–ethyl acetate (2:1). Recrystallization from acetone–hexane gave pale yellow crystals of the 6-oxo enone (**2a**) (0.069 g, 73%), from oxidation with BT. In the TTC oxidation, additional recrystallization from acetone–ether gave (**2a**) (0.048 g, 51%); m.p. 209—210 °C (Found: C, 75.7; H, 9.1. $C_{20}H_{28}O_3$ requires C, 75.9; H, 8.9%); $\lambda_{max.}$ 253 nm (log ε 4.10); $\nu_{max.}$ 3 595, 1 690, 1 672, and 1 613 cm^{-1}; δ_H 0.72 (s, 18-H_3), 1.22 (s, 19-H_3), 1.48 (s, 17α-Me), and 5.72 (s, 4-H).

Androst-4-ene-3,6,17-trione (**3a**), from the enone (**3**) (0.091 g). Eluants in chromatography: chloroform–ethyl acetate (1:1). Recrystallization from hexane gave pale yellow leaflets of the 6-oxo enone (**3a**) (0.077 g, 85%), from oxidation with BT. TTC oxidation gave (**3a**) (0.048 g, 54%); m.p. 218—219 °C (lit.,[14] 216—217 °C) (Found: C, 75.6; H, 7.9. Calc. for $C_{19}H_{24}O_3$: C, 76.0; H, 8.05%); $\lambda_{max.}$ 252 nm (log ε 4.08); $\nu_{max.}$ 1 730, 1 692, and 1 612 cm^{-1}; δ_H 0.91 (s, 18-H_3), 1.22 (s, 19-H_3), and 5.95 (s, 4-H). The same product was obtained from the enone (**4**) (0.091 g). Oxidation with BT gave 0.029 g (32%); oxidation with TTC yielded 0.023 g (26%); m.p. 217—218 °C (Found: C, 76.2; H, 8.1%).

11α,17β-*Dihydroxy*-17α-*methylandrost*-4-*ene*-3,6-*dione* was obtained from 11α, 17β-dihydroxy-17α-methylandrost-4-en-3-one (0.095 g). Eluant in chromatography: ethyl acetate. Recrystallization from acetone–ether gave light yellow needles (0.068 g, 68%) from BT oxidation and [0.053 g (53%) from oxidation with TTC]; m.p. 187—189 °C (Found: C, 72.6; H, 8.6. $C_{20}H_{28}O_4$ requires C, 72.3; H, 8.5%); $\lambda_{max.}$ 253 nm (log ε 4.12); $\nu_{max.}$ 3 520, 1 691, 1 677, and 1 615 cm^{-1}; δ_H 1.05 (s, 18-H_3), 1.41 (s, 19-H_3), 1.55 (s, 17α-Me), 5.95 (s, 4-H), and 4.32 (m, 1 H, 11β-H).

11α-*Hydroxyandrost*-4-*ene*-3,6,17-*trione* was obtained from 11α-hydroxyandrost-4-ene-3,17-dione (0.091 g). Eluant in chromatography: chloroform–ethyl acetate (1:1). Recrystallization from acetone–hexane or hexane gave yellow needles (0.071 g, 75%) from BT oxidation [0.040 g (42%) from TTC oxidation]; m.p. 210—212 °C (Found: C, 71.9; H, 7.4. $C_{19}H_{24}O_4$ requires C, 72.1; H, 7.6%); $\lambda_{max.}$ 251 nm (log ε 4.01); $\nu_{max.}$ 3 510, 1 735, 1 692, 1 673, and 1 612 cm^{-1}; δ_H 0.89 (s, 18-H_3), 1.29 (s, 19-H_3), and 5.95 (s, 4-H).

2α-*Methylpregn*-4-*ene*-3,6,11,20-*tetraone* was obtained from 2α-methylpregn-4-ene-3,11,20-trione (0.103 g). Eluant in column chromatography: ethyl acetate. Crystallization from acetone–light petroleum gave a light yellow glass (0.068 g, 64%) from BT oxidation [0.052 g (49%) from TTC oxidation]. Additional recrystallization gave a sample of m.p. 239—240 °C (Found: C, 74.4; H, 7.7. $C_{22}H_{28}O_4$ requires C, 74.1; H, 7.9%); $\lambda_{max.}$ 250 nm (log ε 3.98); $\nu_{max.}$ 1 710—1 699s, 1 680s, and 1 619 cm^{-1}; δ_H 0.71 (s, 18-H_3); 1.25, 1.51, and 2.11 (s, d, 19-H_3, 20-Me and 2α-Me), and 5.75 (s, 4-H).

17α-*Ethynyl*-17β-*hydroxy*-19-*norandrost*-4-*ene*-3,6-*dione* and 17α-*ethynyl*-3,17β-*dihydroxyestra*-1,3,5(10)-*trien*-6-*one* were obtained from 17α-ethynyl-17β-hydroxy-19-norandrost-4-en-3-one (0.089 g). The combined Claisen's alkaline extracts from the 'organic phase' gave crude material; column chromatography (chloroform) and recrystallization from acetone–ether gave 17α-ethynyl-17β-hydroxy-19-norandrost-4-ene-3,6-dione (0.034 g, 36%). The 'organic phase' left after extraction with Claisen's alkali was washed with 3M hydrochloric acid (15 ml), dried, and evaporated under reduced pressure. Digestion of the residue with ether yielded 17α-*Ethynyl*-3,17β-*dihydroxyestra*-1,3,5(10)-*trien*-6-*one* (0.039 g, 42%). Recrystallization from methanol gave the analytical sample.

The 19-*nor*-4-*ene*-3,6-*dione* had m.p. 201—202.5 °C (Found: C, 76.5; H, 7.9. $C_{20}H_{24}O_3$ requires C, 76.9; H, 7.7%); $\lambda_{max.}$ 251 nm (log ε 4.05); $\nu_{max.}$ 3 501, 3 275, 1 685, and 1 675sh cm^{-1}; δ_H 0.79 (s, 18-H_3), 2.63 (s, HC≡C), and 5.78 (s, 4-H).

The *estratrien*-6-*one* had m.p. 262—263 °C (Found: C, 77.9; H, 6.6. $C_{20}H_{22}O_3$ requires C, 77.4; H, 7.14%); $\lambda_{max.}$ 255 (log ε 3.50) and 328 nm (3.95); $\nu_{max.}$ 3 498, 3 280—3 250, 1 668, and 1 605 cm^{-1}; δ_H 0.82 (s, 18-H_3), 2.71 (s, HC≡C), and 6.8—7.2 (m, Ar).

6β,17β-*Dihydroxy*-6α,17α-*dimethylandrost*-4-*en*-3-*one* (**5a**) was obtained from the enone (**5**) (0.095 g). Eluant in chromatography: chloroform–ethyl acetate (2:1). Recrystallization from acetone–hexane gave light yellow fine-grained crystals of the 6-hydroxy enone (**5a**) (0.065 g, 65%) from oxidation with BT [TTC oxidation gave 0.052 g (52%) after additional recrystallization]. The configuration of the 6-hydroxy group is inferred on the basis of the u.v. maximum (*cf.* refs. 15 and 16). The product had m.p. 247—248.5 °C (Found: C, 75.6; H, 9.9. $C_{21}H_{32}O_3$ requires C, 75.9; H, 9.7%); $\lambda_{max.}$ 239—240 nm (log ε 4.09); $\nu_{max.}$ 3 530s, 1 682, and 1 614 cm^{-1}; δ_H 0.78 (s, 18-H_3), 1.19 (s, 19-H_3), 1.25, 1.40 (s, 6α- and 17α-Me), and 5.49 (s, 4-H).

The same product was obtained from 0.095 g of the enone (**6**). Oxidation with BT gave 0.027 g (27%); oxidation with TTC yielded 0.017 g (17%); m.p. 246—247 °C (Found: C, 76.0; H, 9.5%).

3β,18β-*Dihydroxy*-11-*oxo*-20β-*olean*-12-*en*-29-*oic acid* (18β-hydroxyglycyrrhetinic acid) (**7a**), was obtained from the isoprenoid enone (**7**) (0.144 g). Eluant in chromatography: chloroform. Recrystallization from chloroform–ethanol gave, from oxidation with BT, light yellow crystals of the 18β-hydroxy enone (**7a**) (0.044 g, 30%), m.p. 227—280 °C (Found: C, 74.6; H, 9.05. $C_{30}H_{46}O_5$ requires C, 74.0; H, 9.5); $\lambda_{max.}$ 247 nm

(log ε 3.95); $\nu_{max.}$3 450, 2 640, 1 690, and 1 620 cm^{-1}; δ_H 0.78, 0.94, 1.05, 1.22, 1.45 (singlets, Me groups), and 5.55 (s, 12-H). Oxidation with TTC was not investigated. The same product (0.023 g, 16%) was obtained from 0.144 g of the isoprenoid enone (**8**); m.p. 276—279.5 °C (Found: C, 74.3; H, 9.9%).

7,15-*Dioxo*-5β-*chol*-8(14)-en-24-*oic acid* was obtained from 15-oxo-5β-chol-8(14)-en-24-oic acid (0.108 g). Eluant in chromatography: ethyl acetate–chloroform (1:1). Recrystallization from AcOH gave the 7-ketone (0.037 g, 33%) m.p. 230—232 °C (Found: C, 73.3; H, 8.9. $C_{23}H_{34}O_4$ requires C, 73.8; H, 9.15%); $\lambda_{max.}$257 nm (log ε 4.05); $\nu_{max.}$2 633, 1 710, 1 685, 1 671, and 1 622 cm^{-1}; δ_H 0.72 (s, 18-H_3), 1.17 (s, 19-H_3), and 4.25 (s, OH of acid). Oxidation with TTC was not investigated.

Cholest-4-*ene*-3,6,7-*trione* (**9a**) was obtained from the hydroxy enone (**9**) (0.120 g) and BT (1.09 g, 1.5 mmol). Eluant in chromatography: chloroform. Recrystallization from acetone–methanol gave yellow fine-grained crystals of (**9a**) (0.095 g, 77%). Oxidation with 2 mmol of TTC gave 0.063 g (51%) of pure (**9a**); m.p. 172—174 °C (Found: C, 78.8; H, 9.5. $C_{27}H_{40}O_3$ requires C, 78.6; H, 9.8%); $\lambda_{max.}$289 nm (log ε 3.90); $\nu_{max.}$3 460, 1 685, 1 674sh, and 1 611 cm^{-1}; δ_H 0.76 (s, 18-H_3), 0.89, 1.08, and 1.23 (s, d, m, 19-H_3 and other Me groups), 6.15 (s, 4-H), and 6.31 (s, enol).

Cholest-5-*ene*-3,4,7-*trione* (**10a**) was obtained from the hydroxy enone (**10**) (0.120 g) and BT (1.09 g, 1.5 mmol). Eluant in chromatography: chloroform. Recrystallization from acetone–ethanol gave yellow crystals of (**10a**) (0.069 g, 56%). Oxidation with 2 mmol of TTC gave 0.057 g (47%) of (**10a**); m.p. 148—150.5 °C (Found: C, 78.4; H, 9.6. $C_{27}H_{40}O_3$ requires C, 78.6; H, 9.8%); $\nu_{max.}$3 490, 1 680, 1 672sh, 1 622, and 1 616 cm^{-1}; δ_H 0.82 (s, 18-H_3), 0.97, 1.10, 1.16, and 1.26 (s, d, m, 19-H_3 and other Me groups), 6.18 (s, 6-H), and 6.34 (m, 2-H + OH); $\lambda_{max.}$281 (log ε 3.65) and 335 nm (3.60) (the band at 335 nm may indicate the presence in solution of a 2,4,6-triene-3,4,6-triol structure).

Rate Studies on the Redox Reactions of the Enones (**1**), (**3**)—(**6**), *and* (**11**)—(**14**) *with Blue Tetrazolium* (*BT*).—The absorbance of a solution of the enone and BT (concn. 3×10^{-5}M) in 1.5M potassium hydroxide (in ethanol) at 45 on 50 °C was recorded automatically at appropriate time intervals. In kinetic calculations we determined the increases in absorbance maximum at 525 nm (corresponding to the reduced form of BT). The rate constants were obtained by a least-squares regression of equation (i), where $k^{ox}_{obs.}$ is the observed reaction rate, A_i is the

$$\ln[1 - A_i/A'] = k^{ox}_{obs.}t_i + E \qquad (i)$$

absorbance at any time (t_i), $A'$is the optimum absorbance and E is the intercept of the regression line (greater than 0.99 in most cases).

Comparative Rate Studies on Enolisation and Oxidation Reactions.—(*a*) *Rate of enolisation of enones* (**3**)—(**6**). The absorbance of a solution of the enone (concn. 3×10^{-5}M) in M potassium hydroxide (in ethanol) at 25 °C was recorded at appropriate time intervals. In our kinetic calculations we determined the increases in absorbance maximum at 375 nm. The rate constants for first-order enolisation reactions were calculated from equation (ii), where: k is the rate, a is the stoicheiometric coefficient, A_1 and A_2 are absorbances and t_1 and t_2 are times.

$$k = \frac{2.303}{a(t_2 - t_1)} \log \frac{A_1}{A_2} \qquad (ii)$$

(b) *Rate of oxidation of the enones* (**3**)—(**6**) *by BT*. The method was the same as that described for rate studies on the redox reaction of enones with BT. The measurements were performed at 25 °C in M potassium hydroxide (in ethanol). The best rectilinear time-dependance of the product concentration was obtained for a first-order reaction. The rate constants of the redox process were calculated in the same way as for the enolisation (a).

Electron Spin Resonance Study.—The enone (**1**) or (**2**) (0.1 mg ml^{-1}) in ethanol was treated by the procedure described for rate studies on the redox reaction of enones with BT. Immediately after addition of KOH, the sample was scanned. After the scan, the solution was swept with N_2 and rescanned for 20 min. Scans for comparison were made with alcohol, and alcohol with the appropriate enone. The e.s.r. spectrum showed the presence of an unpaired electron; its intensity was a function of the concentration of the enone used and did not change during the 40 min required for completion of the redox process.

Effect of Free Radical Inhibitor.—To the enone (**1**) or (**2**) (0.55 μmol) and BT (0.95 μmol) was added iodine (0.41 mol) in ethanol (4 ml). 1.5M Potassium hydroxide (5 ml) was then added; this solution showed an absorbance of 0.511 at 525 nm after 34 min; the absorbance of a duplicate without iodine was 0.506 after the same time.

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References

1 A. Frimer, P. Gilinsky-Sharon, J. Hameiri, and G. Aljadeff, *J. Org. Chem.*, 1982, **47**, 2818.
2 B. Camerino, B. Patelli, and R. Sciaky, *Tetrahedron Lett.*, 1961, 544; *Gazz. Chim. Ital.*, 1962, **92**, 693.
3 R. J. Langenbach and H. W. Knoche, *Steroids*, 1968, **11**, 123.
4 M. A. Smoczkiewicz and J. Jasiczak, *Synthesis*, 1980, 739; J. Jasiczak and M. A. Smoczkiewicz, *ibid.*, 1981, 804; *Tetrahedron Lett.*, 1985, 5221.
5 S. Görög, 'Quantitative Analysis of Steroids,' Akad. Kiado, Budapest, 1983, pp. 187—189; M. A. Smoczkiewicz and J. Jasiczak, *Chem. Anal. (Warsaw)*, 1971, **19**, 1091.
6 L. F. Fieser and M. Fieser, 'Steroids,' Reinhold, New York, 1959, p. 204.
7 J. Kalvoda, K. Hensler, and A. Wettstein, *Helv. Chim. Acta*, 1963, **46**, 1017; H. Hagiwara, S. Naguchi, and M. Nishikawa, *Chem. Pharm. Bull.*, 1960, **8**, 84.
8 J. Jasiczak, *Tetrahedron Lett.*, 1987, 4323.
9 H. J. Ringold and S. K. Malhotra, *J. Am. Chem. Soc.*, 1962, **84**, 3402; S. K. Malhotra and H. J. Ringold, *ibid.*, 1963, **85**, 1538; 1964, **86**, 1997.
10 J. Jasiczak, in preparation.
11 G. Subrahmangam, S. K. Malhotra, and H. J. Ringold, *J. Am. Chem. Soc.*, 1966, **88**, 1332; E. J. Corey and R. A. Sneen, *ibid.*, 1956, **78**, 6269.
12 D. N. Kirk and M. P. Hartshorn, 'Steroid Reaction Mechanisms,' Elsevier, Amsterdam, 1968, pp. 168—188 and refs. therein.
13 L. F. Fieser and M. Fieser, 'Reagents for Organic Synthesis,' Wiley, New York, 1967, p. 153.
14 A. Butenand and H. Riegel, *Ber.*, 1936, **69**, 1163.
15 R. Sciaky and A. Consonni, *Gazz. Chim. Ital.*, 1962, **92**, 547.
16 R. Gardi and A. Lusignani, *J. Org. Chem.*, 1967, **32**, 2647.

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