water, 3 ml. of triethylamine, and 50 ml. of acetonitrile was stirred for 3 days at room temperature. After 2 days, the mixture became homogeneous. Water, 100 ml., was added, and the organic layer was separated, washed with 10% hydrochloric acid and water again, and dried over silica gel. Distillation gave 20.6 g. (58%) of a 1:1 complex of the alcohol with acetonitrile, b.p. 107°, $n^{25}D$ 1.3060.

Anal. Calcd. for $C_8H_5F_{12}NO$: C, 26.75; H, 1.40; F, 63.49. Found: C, 26.42; H, 1.52; F, 62.91.

Pure perfluoro(3H-2,3-dimethyl-2-butanol), b.p. 95–96°, $n^{25}D$ <1.3, was obtained from this complex by washing it with 20% oleum and redistilling. The proton n.m.r. showed a septet at 3.83 p.p.m. (J_{HF} = 8 c.p.s.) with an overlapping singlet (OH) at 3.7 p.p.m. The F^{19} n.m.r. showed a septet split to a doublet at −6.56 p.p.m. (J_{HF} = 8 c.p.s., J_{FF} = 8 c.p.s.) and a septet at +8.52 (J_{FF} = 8 c.p.s.) of equal area.

Anal. Calcd. for $C_6H_2F_{12}O$: C, 22.65; H, 0.64; F, 71.68. Found: C, 23.22; H, 0.87; F, 72.17.

This reaction was repeated using pyridine in place of the acetonitrile and triethylamine. In this case, a 1:1 complex between the alcohols and pyridine was formed, b.p. 90–91° (53 mm.), $n^{25}D$ 1.3632.

Anal. Calcd. for $C_{11}H_7F_{12}NO$: C, 33.26; H, 1.78; F, 57.41. Found: C, 33.78; H, 1.81; F, 57.48.

Complexes of Bis(perfluoroisopropyl)carbinol.—This alcohol forms a distillable 1:1 complex with pyridine, b.p. 44° (1.3 mm.), $n^{25}D$ 1.3526, infrared 3.8 μ (OH).

Anal. Calcd. for $C_7H_2F_{14}O \cdot C_5H_5N$: C, 32.23; H, 1.58; F, 59.48; N, 3.13. Found: C, 32.56; H, 1.72; F, 60.07; N, 3.25.

Bis(perfluoroisopropyl)carbinol also forms a distillable 1:1 complex with tetrahydrothiophene, b.p. 36° (12 mm.), $n^{25}D$ 1.3409, infrared 3.1 μ (OH).

Perfluoropinacol Dioxane Complex.—Perfluoropinacol forms a 1:1 complex with dioxane. This complex is obtained as long, colorless needles, m.p. 79–81°, when recrystallized from pentane.

Anal. Calcd. for $C_{10}H_{10}F_{12}O_2$: C, 28.45; H, 2.39; F, 54.05. Found: C, 28.48; H, 2.60; F, 54.37.

1-Chloro-1,1,2,2-tetrafluoroacetone.—Triethyl phosphite (168 g., 1.0 mole) was added dropwise to 200 g. (1.0 mole) of *sym*-dichlorotetrafluoroacetone, stirred and cooled in an ice bath. The addition required about 1 hr. One hundred ml. of 10% sulfuric acid was then added, and the reaction mixture was heated to reflux for 4 hr. The entire reaction mixture was then added dropwise to a stirred solution of 260 g. of phosphorus pentoxide in 600 ml. of concentrated sulfuric acid heated to 100–120°. The effluent gases were condensed in a trap cooled by ice–water. The condensate was redistilled to give 143 g. (87%) of 1-chloro-1,1,3,3-tetrafluoroacetone as a colorless liquid, b.p. 57°, $n^{25}D$ 1.3138. The F^{19} n.m.r. spectrum showed two general areas: a triplet (J_{FF} = 7 c.p.s.) centered at 0.98 p.p.m. (CF_2Cl) and a triplet (J_{FF} = 7 c.p.s.) split to a doublet (J_{FH} = 51 c.p.s.) centered at 61.6 p.p.m. (CF_2H).

Anal. Calcd. for C_3HClF_4O: C, 21.90; H, 0.61; Cl, 21.56; F, 46.20. Found: C, 22.08; H, 0.89; Cl, 21.96; F, 46.24.

***sym*-Tetrafluoroacetone.**—1-Chloro-1,1,2,2-tetrafluoroacetone was reduced in a similar manner with triethyl phosphite, except that 3 days was required for the hydrolysis step. *sym*-Tetrafluoroacetone was obtained in 87% yield as a colorless liquid, b.p. 58–59°, $n^{25}D$ 1.3005. The proton n.m.r. spectrum contained a triplet, and the F^{19} n.m.r. showed a doublet.

Anal. Calcd. for $C_3H_2F_4O$: C, 27.72; H, 1.56; F, 58.45. Found: C, 27.92; H, 1.71; F, 58.71.

Fluoroketone Hydrates.—The *gem*-diols listed in Table I were prepared by adding stoichiometric amounts of water to the corresponding anhydrous ketone.

[CONTRIBUTION FROM THE WORCESTER FOUNDATION FOR EXPERIMENTAL BIOLOGY, SHREWSBURY, MASSACHUSETTS]

Kinetics of the Oxidation of Allyl Alcohols with Dichlorodicyanoquinone. Conformational and Isotope Effects[1]

BY SUMNER H. BURSTEIN AND HOWARD J. RINGOLD

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The kinetic and thermodynamic parameters of the oxidation of a number of steroidal Δ^4-3-hydroxy steroids with dichlorodicyanoquinone (DDQ) have been studied. The reaction, which exhibits a first-order dependence on steroid and on quinone, is formulated as proceeding *via* a slow hydride transfer followed by a rapid proton loss. The primary deuterium isotope effects are large (*ca.* fivefold) as are the negative entropies of activation. The slower reaction rates in dioxane, as compared to those in *t*-butyl alcohol, appear to be caused by a relatively greater orientation of solvent in a charged transition state. Equatorial alcohols were found to be oxidized faster than the axial isomers, a fact which is attributed to favorable $\sigma-\pi$ overlap of the departing axial hydride in the case of the former. This effect, however, appears in the ΔS^* term for which an explanation is advanced.

2,3-Dichloro-5,6-dicyanoquinone (DDQ) is a high potential quinone which effects the oxidation of a number of organic substrates by what may be formulated as a straightforward hydride abstraction mechanism. Of considerable synthetic utility is the facile conversion[2] of a secondary allyl alcohol I, but not a saturated alcohol, to the corresponding ketone V, the probable mechanism[2a] being depicted in Fig. 1, path A. Abstraction by quinone (II) of the C_3-hydride, in the rate-determining step, yields the protonated ketone III and the hydroquinone anion IV. A rapid proton transfer then leads to the unsaturated ketone V and the hydroquinone VI. Although the unsaturated ketone may undergo further[3–5] oxidation by DDQ, the rate, except in the presence of strong acid,[4] is extremely slow and, proceeding from stoichiometric quantities of both reagents, V may be isolated in very high yield. A kinetic analysis of the allyl alcohol oxidation appeared to be of particular interest from the point of view of the effect of alcohol conformation on reactivity and for the determination of deuterium isotope effects.

Initial studies carried out with the readily available 3β,17β-dihydroxyandrost-4-ene[6] demonstrated that the reaction could be followed spectrophotometrically in the visible region by disappearance of the DDQ maximum. Although the oxidation was studied in a number of different solvents, aliquots of the reaction mixture were invariably diluted with benzene for the optical density determinations. In benzene the quinone absorbs at 410 mμ and the hydroquinone, which is formed during the course of the oxidation, absorbs at 362 mμ and does not interfere with determination of

(1) Supported in part by Grant T-185, American Cancer Society.

(2) (a) E. A. Braude, R. P. Linstead, and K. R. Woolridge, *J. Chem. Soc.*, 3070 (1956); (b) D. Burn, V. Petrow, and G. O. Weston, *Tetrahedron Letters*, **9**, 14 (1960).

(3) D. Burn, D. N. Kirk, and V. Petrow, *Proc. Chem. Soc.*, 14 (1960).

(4) H. J. Ringold and A. Turner, *Chem. Ind.* (London), 211 (1962).

(5) H. J. Ringold, M. Gut, M. Hayano, and A. Turner, *Tetrahedron Letters*, 835 (1962).

(6) F. Ungar, M. Gut, and R. I. Dorfman, *J. Biol. Chem.*; **224**, 191 (1957).

the quinone concentration. In a search for adequate solvents for the kinetic studies, methanol and ethanol were discarded owing to reaction with DDQ, and, also, it was found that the presence of water in organic solvents led to a rapid decomposition of quinone. Reasonable second-order kinetic curves (first-order in each component) were obtained with the seven solvents listed in Table I. It may be noted that of the solvents

TABLE I

APPROXIMATE RATE OF OXIDATION OF 3β,17β-DIHYDROXYANDROST-4-ENE WITH DICHLORODICYANOQUINONE (DDQ) IN VARIOUS SOLVENTS AT 27°

Solvent	Dielectric constant (20°)	Second-order rate constant, l. mole^{-1} sec.$^{-1}$ × 10^2
t-Butyl alcohol	12.3	1.9
2-Methyl-1-propanol	18.7	0.9
Nitrobenzene	36.1	.7
Acetone	21.4	.6
Benzene	2.3	.6
Dioxane	2.2	.5
Acetic acid	7.1 (17°)	.2

listed, the fastest rate was obtained with *t*-butyl alcohol while glacial acetic acid was the slowest. Nitrobenzene, acetone, and 2-methyl-1-propanol, with high dielectric constants, gave rates that were not markedly greater than benzene and dioxane. Dioxane and *t*-butyl alcohol were selected as solvents for the study of different substrates as being representative of a solvent of low and of moderately high dielectric constant.

Although satisfactory kinetics were obtained in dioxane, it was noted that a break and displacement of the curve appeared with all substrates at about 40% reaction. This break was found to be caused by precipitation of the hydroquinone, and therefore curves in this solvent were plotted only to the precipitation point. In *t*-butyl alcohol the hydroquinone remained soluble, and reactions were carried, in general, to about two half-lives. The addition of dichlorodicyanohydroquinone or of benzoyl peroxide, prior to reaction, was found to be without effect on the initial rate of reaction in either dioxane or *t*-butyl alcohol. *t*-Butyl hydroperoxide, in blank solution, promoted such a rapid decomposition of quinone that its influence on the rate of oxidation of the allyl alcohols could not be studied.

Four 3β-hydroxy-Δ^4 steroids, all bearing different substituents at C-17 (Table II), exhibited second-order rate constants of about 2×10^{-2} in *t*-butyl alcohol and of 0.5×10^{-2} in dioxane, demonstrating that the remote side chains had little, if any, influence on the rate of oxidation. The corresponding 3α-deuterio compounds underwent oxidation in *t*-butyl alcohol at only about one-fifth of the rate of the 3α-hydrogen compounds which established that this bond is broken in the rate-determining step. Two 3α-hydroxy-Δ^4-steroids, 3α-hydroxycholest-4-ene and 3α-hydroxyandrost-4-ene, exhibited rates in *t*-butyl alcohol of only 0.3×10^{-2}, demonstrating that in contrast to the chromic acid oxidation of saturated alcohols[7] the equatorial isomer is oxidized more readily than the axial isomer.

(7) J. Schreiber and A. Eschenmoser, *He'v. Chim. Acta*, **38**, 1529 (1955).

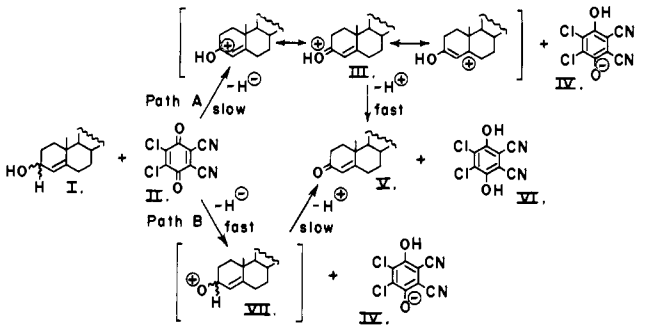


Figure 1.

In order to determine the thermodynamic parameters of this oxidation, the C-17 unsubstituted isomeric 3-hydroxyandrost-4-enes and their 3-deuterio analogs were carefully studied at 27 and at 47° in dioxane and

TABLE II

APPROXIMATE[a] RATES OF OXIDATION OF VARIOUS Δ^4-3-HYDROXY STEROIDS WITH DDQ AT 27°

Compound	Second-order rate constant, l. mole sec.$^{-1}$ *t*-Butyl alcohol	Dioxane, × 10^2
(1) (a) 3β-Hydroxycholest-4-ene	2.2×10^{-2}	0.5
(b) 3α-Deuterio-3β-hydroxycholest-4-ene	0.4×10^{-2}	...
(2) (a) 3β,17β-Dihydroxyandrost-4-ene	1.9×10^{-2}	0.5
(b) 3α-Deuterio-3β,17β-dihydroxyandrost-4-ene	0.5×10^{-2}	...
(3) (a) 3β,17β-Dihydroxy-17α-methylandrost-4-ene	2.2×10^{-2}	0.5
(b) 3α-Deuterio-3β,17β-dihydroxy-17α-methylandrost-4-ene	0.5×10^{-2}	...
(4) (a) 3β-Hydroxyandrost-4-ene[b]	2.0×10^{-2}	0.5
(b) 3α-Deuterio-3β-hydroxyandrost-4-ene	0.4×10^{-2}	...
(5) 3α-Hydroxycholest-4-ene	0.3×10^{-2}	...
(6) 3α-Hydroxyandrost-4-ene	0.3×10^{-2}	...
(7) 2α-Fluoro-3β,17β-dihydroxyandrost-4-ene	4×10^{-4} (47°)	...

[a] With the exception of 4a, 4b, and 6 for which precise values are given in Table III, duplicate or triplicate runs were made for each compound and the curves fitted by eye. [b] Rates in *t*-butyl alcohol and in dioxane were unchanged by the addition of 1 equiv. (based on quinone) of benzoic acid, of monochloroacetic acid, or of benzoyl peroxide, respectively.

in *t*-butyl alcohol. These rate data, which are in accord with the preliminary runs, are recorded in Table III with the blank spaces indicating rates that were too slow for accurate measurement. Considering first the solvent effect, it is apparent that the oxidation of either the 3α- or 3β-hydroxy isomer in *t*-butyl alcohol was 4.4–4.5 times faster than in dioxane. This solvent effect was shown, in the case of the 3β-hydroxy isomer, to be attributed to a more favorable entropy of activation of 3.5 cal./deg. (1.05 kcal. at 27°) in *t*-butyl alcohol, whereas the enthalpy of activation terms were essentially the same in both solvents.

With respect to conformation of the hydroxyl group, the 3β-hydroxy (equatorial) isomer was oxidized 6.4 times faster than the 3α- (axial) isomer in *t*-butyl alcohol at 27° and 6.8 times faster in either *t*-butyl alcohol or in dioxane at 47°. Determination of the thermodynamic parameters in *t*-butyl alcohol solution demonstrated that the reduced oxidation rate of the

TABLE III

RATES[a] OF OXIDATION AND THERMODYNAMIC CONSTANTS OF THE ISOMERIC ANDROST-4-EN-3-OLS AND 3-DEUTERIO ISOMERS WITH DICHLORODICYANOQUINONE

	3β-OH	3β-OH-3α-D	3α-OH	3α-OH-3β-D
		t-BuOH		
$k(27°)$	19.9 ± 0.2	3.93 ± 0.03	3.09 ± 0.02	...
k_H/k_D	5.06 ± 0.07		...	
$k(47°)$	81.3 ± 1.1	17.9 ± 0.2	11.8 ± 0.1	2.59 ± 0.03
k_H/k_D	4.54 ± 0.07		4.55 ± 0.07	
		Dioxane		
$k(27°)$	4.49 ± 0.05	0.788 ± 0.008	...	...
k_H/k_D	5.69 ± 0.09		...	
$k(47°)$	18.2 ± 0.2	3.61 ± 0.04	2.68 ± 0.02	0.494 ± 0.016
k_H/k_D	5.04 ± 0.06		5.42 ± 0.51	
		t-BuOH		
$\Delta F^*_{27°}$, kcal.	19.9	20.9	21.0	...
ΔH^*, kcal.	12.8 ± 0.2	13.9 ± 0.1	12.2 ± 0.1	...
ΔS^*, cal./deg.	−23.6 ± 0.5	−23.4 ± 0.4	−29.3 ± 0.3	...
$T\Delta S^*_{27°}$, kcal.	−7.08	−7.01	−8.78	...
		Dioxane		
ΔF^*, kcal.	20.8	21.8	...	...
ΔH^*, kcal.	12.7 ± 0.1	13.9 ± 0.1	...	...
ΔS^*, cal./deg.	−27.1 ± 0.5	−26.4 ± 0.5	...	...
$T\Delta S^*_{27°}$, kcal.	−8.13	−7.91	...	...

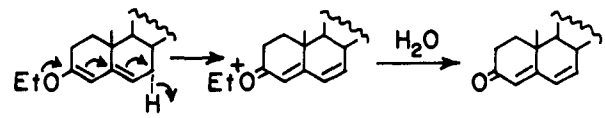
[a] Rates expressed as $k \times 10^3$, l. mole^{-1} sec.$^{-1}$ ± standard deviation.

axial compound could be attributed solely to a difference of −5.6 cal./deg. (1.7 kcal. at 27°) in the entropy of activation and that, in fact, the enthalpy factor was more favorable for the axial isomer by 0.6 kcal.

The kinetic isotope effect at 27° for the 3β-hydroxy isomer was 5.06 in *t*-butyl alcohol and 5.69 in dioxane. This same solvent difference of *ca.* 10% held at 47°, although the isotope effects were lower as expected (4.54 and 5.04). The isotope effects for the 3α- and 3β-hydroxy isomers were essentially identical at 47° in *t*-butyl alcohol. In dioxane, at this same temperature, the 3α-hydroxy compound showed a higher isotope effect than did the 3β-hydroxy steroid, but it is doubtful that this difference is significant owing to the high standard deviation in the rate of the 3α-hydroxy isomer.

Returning to the mechanism of the reaction, the high magnitude of the isotope effect leaves little doubt that there is a rate-determining rupture of the C_3-carbon–hydrogen bond. Assuming an ionic rather than a radical mechanism, oxidation may be pictured in the two extreme cases (Fig. 1) as proceeding by the removal of hydride from carbon followed by a fast proton loss from oxygen (path A) or by a rapid hydride transfer from oxygen followed by a slow proton loss from carbon (path B). The extremely facile oxidation of allyl alcohols in contrast to the stability of saturated alcohols can be readily rationalized *via* path A, which we strongly favor, on the basis of the resonance-stabilized intermediate III which is, in fact, simply the O-protonated unsaturated ketone. In contrast, the hypothetical oxonium species VII cannot undergo charge delocalization either through incipient carbonyl formation or through double bond participation. Furthermore, it is difficult to visualize the formation of VII in a fast step followed by a slow proton removal.[8]

Path A (Fig. 1) may also be compared with the DDQ oxidation of enol ethers[9] which is best explained on the



basis of hydride abstraction at an allylic position. The oxonium intermediate in the enol ether oxidation is formally analogous to the O-protonated ketone III.

Although there appears to be little doubt that the oxidation of allylic alcohols proceeds *via* electron-deficient carbon rather than electron-deficient oxygen, the question of hydride abstraction *vs.* a nonchain radical mechanism cannot be disposed of with certainty. As noted above, the hydride abstraction mechanism is particularly attractive from the point of view of double bond participation and of hydroxyl participation in the form of incipient carbonyl formation. A radical at C-3 can be delocalized by double bond and by hydroxyl participation but not by carbonyl formation unless accompanied by a simultaneous loss of H· from the hydroxyl group. The stereochemistry of the 1,4-quinone precludes a simultaneous loss of both hydrogens *via* a cyclic process while the first-order dependency on quinone precludes participation of a second molecule of DDQ in a rate-determining one-step loss of both hydrogens. Therefore, the ionic mechanism should be favored as a lower energy process than the hypothetical radical process.

Attempts to detect, by e.s.r., a free-radical steroid intermediate during the course of oxidation in *t*-butyl alcohol were unsuccessful since simple mixing of the quinone and hydroquinone led to the immediate appearance of a broad radical signal attributed to

(8) It should be noted that in principle the DDQ oxidation is quite different from chromic acid oxidations which probably proceed *via* a chromate ester followed by a concerted cyclic oxidation process. Although it is thought that the hydrogen atom on carbon is lost as a proton in chromic acid oxidation (F. H. Westheimer and Y. W. Chang, *J. Phys. Chem.*, **63**, 438 (1957)), no compelling evidence has yet been presented to establish that this is in fact the case.

(9) S. K. Pradhan and H. J. Ringold, *J. Org. Chem.*, **29**, 601 (1964).

semiquinone formation.[10] It need not be emphasized that the presence of semiquinone in no way dictates the participation of such a species in the dehydrogenation reaction.

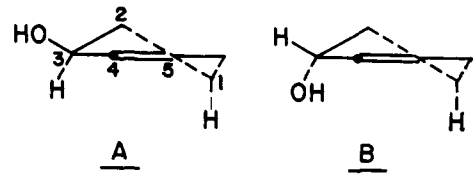
The mechanisms of the DDQ mediated dehydrogenation of unsaturated ketones[3-5] and of enol ethers[9] appear to implicate ionic intermediates, as have the extensive studies of Braude, Jackman, and co-workers[11] on the quinone dehydrogenation of hydroaromatic compounds. The solvent effects in the present study also point to ionic rather than to radical intermediates. The very slow rate in glacial acetic acid may be explained on the basis of hydrogen bonding of the C_3-hydroxyl group by acetic acid since placement of positive charge on the hydroxylic oxygen should retard abstraction of hydride. It should be noted in this respect, however, that the addition of an equivalent amount of benzoic acid or of monochloroacetic acid had no detectable influence on the rate of oxidation in *t*-butyl alcohol or in dioxane.[12] The failure to increase the rate of oxidation by the addition of benzoyl peroxide may also be cited against a radical mechanism.

Further support for a hydride-abstraction ionic mechanism comes from the high negative entropy of activation of -23 to -29 e.u. and from the fact that the decreased rate of oxidation in dioxane, as compared to *t*-butyl alcohol, reflects totally a less favorable entropy factor. This type of behavior is readily explicable if dioxane, with its low dielectric constant (Table I), undergoes a greater increase of orientation in a charge-transition state.

The extremely slow oxidation rate of a halogenated allyl alcohol, 2α-fluoro-3β,17β-dihydroxyandrost-4-ene, whose rate constant at 47° in *t*-butyl alcohol was only about $^1/_{200}$ of the nonfluorinated compound, is also consistent with a hydride abstraction mechanism. The inductive effect of fluorine should clearly retard initial abstraction of the C_3-hydride ion as well as destabilize the postulated protonated ketone intermediate III (Fig. 1). While fluorine would also be expected to retard formation of a radical at the α-carbon, it is intuitively felt that the effect on a radical process should not be as great as that actually observed (*ca.* 3.2 kcal.).

The high carbon–deuterium isotope effect of about fivefold appears to be greater than that normally anticipated for a hydride transfer reaction.[13] However, Dauben[14] has recently reported k_H/k_D values of 4 to 5 in the abstraction of hydride ion by carbonium ions, and it is apparent that our values are not inconsistent with hydride loss. In summary, all of our data support the ionic mechanism Ia, although a nonchain radical mechanism cannot be ruled out with certainty.

Of particular interest in this allylic alcohol oxidation study was the conformational effect whereby the pseudoequatorial 3β-hydroxy compounds (A) underwent reaction more than six times as rapidly as the corresponding pseudoaxial 3α-hydroxy isomers (B). *A priori*,



it might have been anticipated that the axial 3α-hydroxyl group, which is subject to a single, albeit minor, 1,3-diaxial interaction (1α-H–3α-OH), would undergo oxidation more readily than the 3β-hydroxyl which is free of nonbonded diaxial interactions. Such results would have been in accord with the concept of acceleration of oxidation rates by the relief of steric strain as advanced by Schreiber and Eschenmoser[7] to explain the rates of chromic acid oxidations. When it became apparent that there was, in fact, a reversal of the anticipated oxidation order, the unlikely possibility was entertained that ring A of the Δ^4-3-hydroxy steroids was not in the expected and highly favored (molecular models) half-chair conformation. The coupling pattern in the n.m.r.[15] of the C_4-proton with the C_3-proton was confirmatory for conformations A and B, however, since each of the 3α-hydroxy isomers exhibited the C_4-proton as a doublet (J = 5 c.p.s.) centered at 4.53–4.54 τ while the C_4-proton of the 3β-hydroxy compounds appeared as a broad but unsplit peak at 4.72–4.74 τ. The molecular models of A and B indicated that the pertinent dihedral angles for the C_4-proton with the 3β-proton and the 3α-proton are about 40 and 80°, respectively, for which coupling constants of about 5 and 0 c.p.s. would be predicted.[16]

Several alternate explanations for the seemingly anomalous oxidation rates were considered, one possibility being that strain relief is unimportant in DDQ oxidations perhaps attributed to a transition state resembling the starting alcohol. This was shown not to be the case, however, in a study[17] of the DDQ oxidation of the isomeric 3β-acetoxy-6-hydroxy-5α-cholest-7-enes; the axial 6β-hydroxy isomer, with a severe 1,3-

methyl–hydroxyl interaction, was oxidized about ten times as fast as the equatorial hydroxy compound. Therefore, sufficient strain relief does lead to an increased rate of reaction in common with chromic acid oxidations. It is also pertinent to mention at this point an independent study of chromic acid oxidations that is in progress in this laboratory.[17] With chromic acid in sodium acetate–buffered 90% acetic acid, 3β-hydroxyandrost-4-ene (equatorial hydroxyl) under-

(10) We wish to thank Dr. J. Gendell, Columbia University, for e.s.r. determinations.

(11) *Cf.* J. R. Barnard and L. M. Jackman, *J. Chem. Soc.*, 3110 (1960), E. A. Braude, L. M. Jackman, R. P. Linstead, and J. S. Shannon, *ibid.*, 3116 (1960); E. A. Braude, L. M. Jackman, R. P. Linstead, and G. Lowe, *ibid.*, 3123, 3133 (1960); A. M. Creighton and L. M. Jackman, *ibid.*, 3138 (1960); R. F. Brown and L. M. Jackman, *ibid.*, 3144 (1960); and earlier references therein.

(12) Under these conditions, the solvent itself should effectively compete with the steroid for the acid.

(13) K. Wiberg, *Chem. Rev.*, **55**, 713 (1955); C. G. Swain, R. A. Wiles, and R. F. W. Bader, *J. Am. Chem. Soc.*, **83**, 1945 (1961).

(14) H. J. Dauben, Jr., Abstracts, 142nd National Meeting of the American Chemical Society, Atlantic City, N. Y., Sept., 1962, Organic Section, p. 55Q.

(15) We are grateful to Mr. T. Wittstruck and to Mr. J. Cronin for n.m.r. determinations which were obtained with a Varian 4300 spectrometer at 60 Mc./sec. The samples were run in deuteriochloroform with tetramethylsilane as internal standard.

(16) M. Karplus, *J. Chem. Phys.*, **30**, 11 (1959). It is also pertinent that this same pattern of coupling constants was exhibited by the isomeric Δ^5-7-ols and Δ^7-6-ols where ring B is rigid and must remain in a half-chair conformation.

(17) S. Burstein and H. J. Ringold, unpublished data.

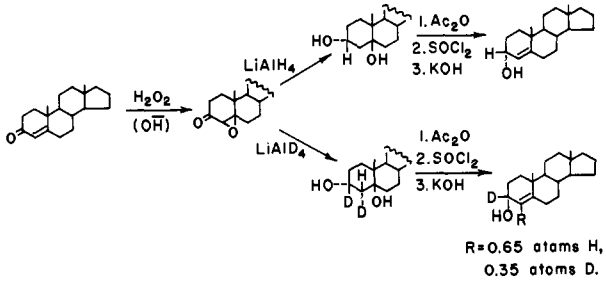


Figure 2.

went oxidation at about five times the rate of the 3α-hydroxy isomer. This indicated that with chromic acid or with DDQ there is an electronic or stereoelectronic[18] factor favoring the oxidation of equatorial allylic alcohols over the axial isomers and that when hydroxyl strain is minimal, this electronic effect is the dominant one. It is felt, however, that the primary effect leading to the more rapid oxidation of equatorial allyl alcohols is the fact that, in the rate-determining step, the hydrogen being removed from carbon is axial. By virtue of being essentially perpendicular to the double bond, the axial hydrogen, whether it is removed as proton or as hydride, will remain in continual overlap with the π-electrons of the double bond, thus minimizing the energy of the transition states.[19] Such overlap is obviously not possible with equatorial hydrogen loss.[20]

It may further be suggested that the same argument can be used, in reverse, for the metal hydride reduction of α,β-unsaturated ketones, in particular Δ^4-3-keto steroids. Formation of the equatorial isomer is quite favored despite the fact that the axial isomer is not significantly strained nor is steric approach of reagent markedly hindered from either side. Again, axial hydride attack and continuous $\sigma-\pi$ overlap would appear to offer a reasonable explanation for the observed stereospecificity.

The thermodynamic parameters (Table III) for the DDQ oxidation, however, are not what might have been anticipated for increased $\sigma-\pi$ overlap which, as a resonance effect, would have been expected to appear in the form of a more favorable ΔH^*. In fact, the increased oxidation rate of the 3β-hydroxy isomer is attributed solely to a more favorable (1.7 kcal.) entropy factor and is actually less favored by 0.6 kcal. with respect to the enthalpy of activation. Since orientation of solvent by a charged transition state would appear to account for the high negative ΔS^* of both isomers, we believe that the faster oxidation rate of the equatorial hydroxy isomer, although attributed primarily to the aforementioned resonance effect, is reflected in a lesser orientation of solvent. This is readily understandable if with the 3α-hydroxy (axial) isomer there is a greater development or a greater localization of charge in the transition state which could occur in two possible manners. First, it is quite probable that abstraction of the equatorial hydride requires a greater degree of bond stretching before the adjacent 4,5-double bond can undergo significant participation and consequently the transition state will be reached later and bear more charge than in the case of axial hydride loss. But, even assuming that the degree of hydride stretching is precisely the same with both isomers, there would be, in the case of the 3α-hydroxy isomer, a greater charge concentration on the C_3-carbon atom or on the incipient carbonyl oxygen since efficient charge delocalization by the carbon–carbon double bond can occur only with axial hydride removal. On the other hand, greater solvation should lower the heat content of the transition state and accounts in part for the somewhat lower ΔH^* of the 3α-hydroxy compound. It was hoped that differences in the degree of hydride stretching of the two isomers might be detected in the isotope effects, but no statistically significant difference was noted in either dioxane or *t*-butyl alcohol solution.

The preparation of the Δ^4-3β-ol and 3α-deuterio-Δ^4-3β-ol starting materials was straightforward and involved in each case the lithium aluminum hydride or deuteride reduction of the Δ^4-3-ketone which yielded predominantly the 3β-isomer. Obtention of the 3α-hydroxy isomers presented more of a problem since these substances could be isolated in only *ca.* 5–10% yield as the minor product of the hydride reduction and in general required repeated chromatography and/or digitonin separation of contaminating 3β-hydroxy isomer. Therefore an alternate synthesis was devised (Fig. 2) for preparation of the 3α-hydroxy-Δ^4-3-ols and the corresponding 3β-deuterio substances. Plattner, Heusser, and Kulkarni[21] had shown that lithium aluminum hydride reduction of 4β,5β-oxidocoprostan-3-one, which is readily obtained by the treatment of cholest-4-en-3-one with alkaline hydrogen peroxide, leads to the 3α,5β-diol. Dehydration of the 3-monoacetate of this diol with thionyl chloride–pyridine, followed by saponification, gave, in our hands, a good yield of 3α-hydroxycholest-4-ene. The same reaction sequence applied to androst-4-en-3-one led in reasonable yield to the desired 3α-hydroxyandrost-4-ene. When the 4β,5β-oxide was reduced with lithium aluminum deuteride, the same sequence led to the 3β-deuterio-3α-hydroxy substance, and a stereospecific *trans* elimination would have given the Δ^4-3α-ol free of deuterium at C-4. Mass spectroscopy indicated, however, that the product which was actually formed contained 35% of a dideuterated species which could only be the 3,4-dideuterio compound. This material did not show any noticeable difference from pure monodeuterio species in the rate of oxidation and was used for most of the kinetic determinations. It is possible that the accuracy of the analytical method was insufficient to detect a small secondary isotope effect.

(18) E. J. Corey and R. A. Sneen, *J. Am. Chem. Soc.*, **78**, 6269 (1956).

(19) For discussions of the importance of $\sigma-\pi$ overlap in the protonation of enols and of carbanions *cf.* Corey and Sneen, ref. 18; G. Stork and S. D. Darling, *J. Am. Chem. Soc.*, **86**, 1761 (1964); S. K. Malhotra and H. J. Ringold, *ibid.*, **86**, 1997 (1964).

(20) An apparent anomaly is the oxidation of 3β,6β-dihydroxycholest-4-ene with dichromate (L. F. Fieser and M. Fieser, "Steroids," Reinhold Publishing Corp., New York, N. Y., 1959, pp. 203, 225) or with DDQ (ref. 2) since the equatorial 3β-hydroxy group may be selectively oxidized despite the fact that the 6β-hydroxyl group is in a strong 1,3-diaxial interaction with the angular methyl group. Although it has been suggested (Fieser and Fieser, p. 225) to explain this finding that chromic acid oxidation of an allyl alcohol proceeds *via* a rate-determining formation of a carbonium ion, this has been shown (ref. 17) not to be the case by the observation of a substantial primary deuterium isotope effect in the chromic acid oxidation of simple 3α-deuterio-Δ^4-3β-ols. It appears likely that the slow rate of oxidation of a Δ^4-6β-ol is attributed to the fact that the product is an S-*cis* rather than the more stable S-*trans* form of α,β-unsaturated ketone.

(21) P. A. Plattner, H. Heusser, and A. B. Kulkarni, *Helv. Chim. Acta*, **31**, 1823 (1948).

Experimental[22]

Materials.—The preparation of the steroidal alcohols is described below. The 2,3-dichloro-5,6-dicyanoquinone was a gift from Eli Lilly and Co. and was recrystallized from benzene and dried *in vacuo* at 55° before use (m.p. 214–215°). The *t*-butyl alcohol was Baker analyzed reagent grade and did not require further purification. The dioxane was purified according to Fieser[23] and, to remove final traces of moisture, stored in the dark over Linde Molecular Sieves for at least 1 week prior to use. Without this final drying procedure, rates were not reproducible.

3β,17β-Dihydroxyandrost-4-ene was a gift from Dr. Marcel Gut[6] and was freed from traces of less soluble 3α-hydroxy isomer by fractional crystallization from acetone–hexane. The pure material exhibited m.p. 157–159° and appeared as a single spot on silica gel thin-layer chromatography in a benzene–ethyl acetate 7:3 system. The 3α-isomer was slightly more polar in this system.

3β-Hydroxycholest-4-ene was prepared by lithium aluminum hydride reduction of cholest-4-en-3-one and digitonin separation according to the directions of McKennis and Gaffnev.[24] The material, after recrystallization from aqueous methanol, exhibited m.p. 130–132° and appeared as a single spot on a silica gel plate (benzene–ethyl acetate, 19:1). In contrast to the Δ^4-3,17-diols, the 3β-hydroxy isomer was more polar than the 3α-axial isomer. In the n.m.r.[15] the C-4 proton appeared as a broad singlet at 4.74 τ.

3α-Hydroxycholest-4-ene was isolated according to McKennis and Gaffney[24] from the mother liquors of the cholest-4-en-3-one reduction after removal of the 3β-hydroxy isomer by digitonin separation. The 3α-hydroxy compound was formed in approximately 15% yield. The purified material, obtained in 7% yield after aqueous acetone crystallization, melted at 78–80° and appeared as a single spot on thin-layer chromatography (benzene–ethyl acetate, 19:1). The C_4-proton appeared as a doublet centered at 4.53 τ in the n.m.r., J = 5.0 c.p.s.

17α-Methyl-3β,17β-dihydroxyandrost-4-ene, prepared according to Bernstein, *et al.*,[25] by sodium borohydride reduction of 17α-methyltestosterone, exhibited m.p. 169–171° and appeared as a single spot on thin-layer chromatography (benzene–ethyl acetate, 4:1). In the n.m.r., the C_4-proton appeared as a singlet at 4.74 τ.

3β-Hydroxyandrost-4-ene.—Androst-4-en-3-one[26] (5.0 g.) was dissolved in methanol (50 ml.) and treated over a 10-min. period with solid sodium borohydride (2.5 g.). After standing for 4 hr. at room temperature, the solution was poured into 1 l. of water, and the products were extracted with ether. The crude mixture of alcohols was chromatographed on a column of neutral alumina (305 g.). Elution with pentane–benzene, 4:1, gave a mixture of alcohols in the early fractions and pure 3β-hydroxyandrost-4-ene in the latter fractions, which were pooled and crystallized from acetone–hexane, yielding 3.95 g. of pure material, m.p. 104–106°. On thin-layer chromatography (benzene–ethyl acetate, 9:1) the substance appeared as a single spot slightly more polar than the α-isomer. In the infrared, the substance showed hydroxyl absorption at 3350 and double-bond absorption at 860 cm.$^{-1}$; in the n.m.r., the C_4-proton was a singlet at 4.72 τ.

Anal. Calcd. for $C_{19}H_{30}O$: C, 83.15; H, 11.02. Found: C, 83.09; H, 11.12.

3α-Hydroxyandrost-4-ene.—The early fractions from the chromatogram described above, which consisted of a mixture of the two isomers, were chromatographed on preparative silica gel thin-layer plates (benzene–ethyl acetate, 4:1) and the less polar zones eluted with acetone. Repeated chromatography gave the pure 3α-hydroxy isomer in 5% yield. The analytical specimen from aqueous acetone melted at 37–39°. In the infrared, hydroxyl absorption was seen at 3400 and double bond absorption at 863 cm.$^{-1}$. The C_4-proton, in the n.m.r., appeared as a doublet (J = 5.0 c.p.s.) centered at 4.54 τ.

Anal. Calcd. for $C_{19}H_{30}O$: C, 83.15; H, 11.02. Found: C, 82.86; H, 10.79.

3α-Deuterio-3β-hydroxyandrost-4-ene.—A solution of androst-4-en-3-one (1.0 g.) in ether (50 ml.) was added to a slurry of lithium aluminum deuteride (0.6 g.) in ether (40 ml.) and boiled for 48 hr. The excess reagent was decomposed by the addition of saturated sodium sulfate solution, and the mixture of alcohols isolated by repeated ether extraction. Chromatography and crystallization as described above for the nondeuterated substance gave 0.7 g. of 3-deuterio compound, m.p. 104–106°. The material exhibited the same polarity as the nondeuterated substance on thin-layer chromatography and gave no melting point depression on admixture. In the infrared, a carbon–deuterium stretching band appeared at about 2120 cm.$^{-1}$.

Anal. Calcd. for $C_{19}H_{29}DO$: 3.33 atom % excess deuterium. Found[27]: 3.16%.

3β-Deuterio-3α-hydroxyandrost-4-ene.—Chromatographic separation on silica gel thin-layer plates of the early fractions from the alumina chromatograph of the 3α-deuterio-3β-hydroxy compound above gave 75 mg. of 3β-deuterio-3α-hydroxyandrost-4-ene, m.p. 35–39°. The infrared[28] showed a C–D stretching band at 2140 cm.$^{-1}$ ($CHCl_3$) and analysis by mass spectroscopy[29] indicated 97% of monodeuterated species.

3α-Deuterio-3β-hydroxycholest-4-ene was prepared by lithium aluminum deuteride reduction of cholest-4-en-3-one in the same manner as described for the nondeuterated substance except that the reduction period was extended to 48 hr. The analytical specimen exhibited m.p. 130–132°, undepressed on admixture with the nondeuterated 3β-ol. Mass spectroscopy[29] demonstrated the presence of 98% monodeuterio species.

3α-Deuterio-3β,17β-dihydroxyandrost-4-ene.—Reduction of testosterone for 48 hr. with lithium aluminum deuteride in ether solution, followed by sodium sulfate work-up and removal of the more insoluble 3α-hydroxy isomer by fractional crystallization from acetone–hexane, gave a 70% yield of the title compound, m.p. 158–160°. In the infrared, the C–D stretching band appeared at about 2120 cm.$^{-1}$.

Anal. Calcd. for $C_{19}H_{29}DO_2$: 3.33 atom % excess deuterium. Found[27]: 3.28%.

Alternate Procedures for 3α-Hydroxy-4-enes and 3β-Deuterio-3α-hydroxy-4-enes. 3α-Hydroxycholest-4-ene.—3α-Acetoxy-5β-hydroxycoprostane (300 mg.), prepared *via* lithium aluminum hydride reduction of 4β,5β-oxidocoprostan-3-one followed by acetylation according to the procedure of Plattner, *et al.*,[21] was dissolved in pyridine (15 ml.) and the solution cooled in an ice bath. Freshly distilled thionyl chloride (0.2 ml.) was added, and the mixture kept at 0–5° for 25 min. The yellow slurry was then poured onto crushed ice and diluted with water after the excess reagent had decomposed. The gummy product was filtered, then triturated with aqueous methanol, and finally saponified by heating under reflux for 1 hr. with 1% potassium hydroxide in methanol. Dilution with water and ether extraction followed by crystallization from aqueous acetone gave 175 mg. of 3α-hydroxycholest-4-ene, m.p. 78–80°, completely identical in all respects with the minor component obtained by lithium aluminum hydride reduction of cholest-4-en-3-one. No Δ^5-isomer could be detected by thin-layer chromatography or by n.m.r.

3α-Hydroxyandrost-4-ene.—Androst-4-en-3-one (3.0 g.) was dissolved in methanol (50 ml.) and cooled to 0°. Hydrogen peroxide (12 ml., 30%) and potassium hydroxide (12 ml., 4 N) were added simultaneously and the mixture was kept at 0° in the dark for 18 hr. The resulting suspension was diluted with water, filtered, and the product crystallized from aqueous methanol, yielding 2.0 g. of **4β,5β-oxidoandrostan-3-one**, m.p. 112–114°, ν_{max}^{KBr} 1720 cm.$^{-1}$.

Anal. Calcd. for $C_{19}H_{28}O_2$: C, 79.12; H, 9.79. Found: C, 78.86; H, 9.90.

The oxide (1.9 g.) in ether (60 ml.) was added to a slurry of lithium aluminum hydride (1 g.) in ether (100 ml.) and the mixture boiled for 4 hr. Saturated sodium sulfate solution was then added; the solids were filtered and washed thoroughly with acetone. The combined filtrates were taken to dryness, and the

(22) All melting points were determined on a Fisher–Johns melting point apparatus and are uncorrected. Infrared spectra were carried out in potassium bromide pellets on an Infracord except where noted otherwise. The kinetic runs were followed with a Perkin–Elmer 202 spectrophotometer using 3-ml. quartz cuvettes with a 1.00-cm. light path. The kinetic runs were carried out with the able technical assistance of Mrs. N. Ragaini.

(23) L. F. Fieser, "Experiments in Organic Chemistry," 3rd Ed., D. C. Heath and Co., Boston, Mass., 1955, p. 284.

(24) H. McKennis, Jr., and G. W. Gaffney, *J. Biol. Chem.*, **175**, 217 (1948); see also W. G. Dauben, R. A. Micheli, and J. F. Eastham. *J. Am. Chem. Soc.*, **74**, 3852 (1952).

(25) S. Bernstein, S. M. Stolar, and M. Heller, *ibid.*, **22**, 472 (1957).

(26) A. Butenandt. L. Karlson-Poschmann, G. Failer, U. Schmidt, and E. Biekert, *Ann.*, **575**, 123 (1952).

(27) Deuterium analysis by Mr. J. Nemeth, Urbana, Ill.

(28) Carried out on a Beckman IR-7 with Bausch and Lomb replica grating by Mr. G. Scrimshaw.

(29) Mass spectra by Dr. R. Ryhage, Karolinska Institutet, Stockholm, Sweden.

residue crystallized from chloroform–hexane, yielding 1.0 g. of 3α,5β-dihydroxyandrostane, m.p. 195–198°, no carbonyl absorption in the infrared. Thin-layer chromatography showed only a single spot.

Anal. Calcd. for $C_{19}H_{32}O_2$: C, 78.02; H, 11.03. Found: C, 77.76; H, 11.13.

Acetylation of this diol (1 g.) with acetic anhydride–pyridine gave 900 mg. of 3-monoacetate, m.p. 138–140°, after crystallization from aqueous methanol; ν_{max}^{KBr} 3560, 1720, and 1250 cm.$^{-1}$.

Dehydration of 200 mg. of 3α-acetoxy-5β-hydroxyandrostane with thionyl chloride–pyridine followed by saponification, as described for the alternate preparation of 3α-hydroxycholest-4-ene, gave, after crystallization from acetone–water, 120 mg. of the desired 3α-hydroxyandrost-4-ene, m.p. 38–40°, identical in all respects with the minor product obtained by hydride reduction of androst-4-en-3-one.

3β-Deuterio-3α-hydroxyandrost-4-ene.—4β,5β-Oxidoandrostan-3-one (1 g.) was treated exactly as described above except that lithium aluminum deuteride was substituted for the hydride and the reduction period was extended to 24 hr. Proceeding through the sequence of acetylation, thionyl chloride dehydration, saponification, and crystallization, 360 mg. of 3β-deuterio-3α-hydroxyandrost-4-ene, m.p. 38–40°, was obtained. The material was homogeneous on thin-layer chromatography and did not separate from the nondeuterated substance. In the carbon–deuterium stretching region in the infrared,[28] the major band, attributed to C_3-deuterium, appeared at 2140 cm.$^{-1}$ ($CHCl_3$) and a smaller band, attributed to C_4-deuterium, appeared at 2235 cm.$^{-1}$. Analysis by mass spectroscopy[29] indicated that 0.35 atom of deuterium remained on the double bond at C-4. Found: D_0, 2.0%; D_1, 63.3%; D_2, 34.7%.

2α-Fluoro-3β,17β-dihydroxyandrost-4-ene was prepared by lithium aluminum hydride reduction of 2α-fluorotestosterone; m.p. 180–181° and identical with material previously obtained by enzymatic reduction.[30]

Kinetic Determinations.—Oxidations were carried out at 27 or 47° and were followed spectrophotometrically by measuring the decrease in optical density of the quinone absorption in the visible region. The reactions were carried out in glass-stoppered volumetric flasks which were immersed in a thermostated constant-temperature water bath. Aliquots were withdrawn at suitable intervals, diluted into benzene, and the optical density read at 410 mμ, which is the maximum of the quinone in benzene solution. The hydroquinone which is formed during the reaction absorbs at 362 mμ in the visible and does not interfere with the quinone determination in benzene solution. In dioxane solution, the quinone absorbs at 390 mμ while the hydroquinone maximum is unchanged, so that there is some interference from the hydroquinone. A Perkin–Elmer 202 spectrophotometer and standard 1.00-cm. quartz cells were used in all runs.

Preliminary runs at a variety of concentrations established a first-order dependence on each reagent. The following initial molar concentrations of steroid and quinone were used in the kinetic runs for determination of the thermodynamic parameters reported in Table III. At least three separate runs were made at each temperature and with each solvent (Table IV).

A typical run 2.203×10^{-2} *M* in steroid and quinone is illustrated below. Finely powdered 3β-hydroxyandrost-4-ene (12.15 mg.) was weighed directly into a 2-ml. volumetric flask which was then placed in the constant-temperature bath. Dichlorodicyanoquinone (25.00 mg.) was weighed into a separate 5-ml. volumetric flask and dissolved in about 2 ml. of warm solvent.[31] The volumetric was then diluted to the mark with solvent at the desired temperature, thoroughly agitated, and the reaction initiated by addition of the quinone solution to the solid steroid.[30] The reaction volumetric was brought to volume, briskly shaken for a few seconds, and returned to the bath. At the desired time intervals, aliquots of 0.2 ml. were pipetted into a 10-ml. volumetric flask which was almost filled with benzene at 27°. The volumetric was diluted to the mark with benzene, and the optical density at 410 mμ read immediately.[32] Seven or eight points were usually obtained in this manner apart from the zero time reading which came from the original quinone solution. Reactions in *t*-butyl alcohol in which the hydroquinone was soluble gave satisfactory curves through about two half-lives of quinone, after which time occasional drifting occurred. In dioxane the reaction could only be followed to about 40% owing to precipitation of the hydroquinone at which point a displacement of the curve occurred.

TABLE IV

		t-Butyl alcohol		Dioxane	
		Steroid $\times 10^2$ *M*	Quinone $\times 10^2$ *M*	Steroid $\times 10^2$ *M*	Quinone $\times 10^2$ *M*
Δ⁴-3β-ol	27°	2.203	2.203	4.406	2.203
	47°	1.101	1.101	2.203	2.203
Δ⁴-3α-ol	27°	6.609	2.203	...	...
	47°	2.203	2.203	6.609	2.203
3D-Δ⁴-3β-ol	27°	6.609	2.203	6.609–13.218	2.203–4.406
	47°	2.203	2.203	6.609	2.203
3D-Δ⁴-3α-ol	27°	8.912	2.203	...	...
	47°	6.609	2.203	6.609	2.203

A typical set of points for 3β-hydroxyandrost-4-ene (2.203×10^{-2} *M*) in *t*-butyl alcohol at 27° is shown in Table V.

TABLE V

Time, min.	Opt. density	Time, min.	Opt. density
0	1.140	30	0.646
2	1.125	45	0.537
10	0.939	60	0.450
20	0.765	80	0.377

When steroid and quinone were run in equivalent concentrations, the reciprocal of optical density was plotted *vs.* time. The slope of this curve multiplied by (init. optical density/init. concentration) gave the second-order rate constant. For unequal concentrations of steroid (a) and quinone (b), $\log\left[\frac{a-x}{b-x}\left(\frac{b}{a}\right)\right]$, where x = moles of substance reacted at time t, was plotted *vs.* time and the slope multiplied by $(2.303/(a-b))$. In the runs reported in Table III, rate constants and standard deviations were determined by pooling all points from three or more individual runs and solving by least squares.[33]

Effects of Added Acid Hydroquinone or Benzoyl Peroxide.—To test for a possible effect of acid on the rate of reaction of 3β-hydroxyandrost-4-ene, monochloroacetic acid or benzoic acid was added to the DDQ solution in *t*-butyl alcohol and in dioxane to give a concentration of 2.203×10^{-2} *M*. The influence of dichlorodicyanohydroquinone was also tested at the same concentration in *t*-butyl alcohol, while in dioxane a saturated solution was utilized owing to the extreme insolubility of the hydroquinone. Benzoyl peroxide was tested as a possible accelerator at 2.203×10^{-2} *M* concentration. No significant influence on rate was noted with either acid, with the hydroquinone, or with the peroxide.

(30) H. J. Ringold, J. Graves, M. Hayano, and H. Lawrence, Jr., *Biochem. Biophys. Res. Commun.*, **13**, 162 (1963).

(31) This procedure was satisfactory for the 17-desoxy steroids, while with the less soluble 3,17-diols it was necessary to dissolve first the steroid in a known volume of the solvent of choice and to adjust the concentration of quinone accordingly.

(32) Prolonged standing of the quinone in very dilute medium introduced a substantial error attributed to autodecomposition. The concentrated solutions, however, which were used for zero-point determinations were found to undergo negligible decomposition over a period of several hours.

(33) We are grateful to Professor E. C. Trachtenberg, Department of Chemistry, Clark University, for setting up the data in satisfactory least-squares form and for aid in the calculations. We wish to thank Dr. J. Budde, Clark University, Computer Center, for computer time.