Autoxidation of Cholest-5-en-3-one, and its accompanying Isomerization, in Acetic Acid

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The rearrangement of cholest-5-en-3-one to cholest-4-en-3-one in acetic acid in the dark is of the first kinetic order and shows the characteristics expected for catalysis by acetic acid molecules. If no special precautions are taken to avoid the presence of oxygen and traces of metal ions, a smooth autoxidation, also of the first kinetic order, accompanies this rearrangement and gives 6α- and 6β-hydroperoxycholest-4-en-3-one together with cholest-4-ene-3,6-dione and other products of oxidation. The rate of autoxidation is not increased by five-fold increase in the concentration of oxygen nor by a 1 000-fold increase in the concentration of copper(II) ions; it can be reduced by the inclusion of free-radical chain inhibitors and stopped by the inclusion of ethylenediaminetetra-acetic acid, when the rate of isomerization is the same as that in the absence of oxygen. When autoxidation is proceeding, this isomerization is reduced in rate but is not completely eliminated. Similar reactions in ethanol or in carbon tetrachloride are much slower. These results show that isomerization proceeds by at least two heterolytic pathways, and that the rate of formation of an intermediate concerned in one of these can control the rate of an autoxidation by molecular oxygen catalysed by traces of metal ions. Study of primary (4-D) and solvent isotope effects shows that both mechanisms for isomerization are intermolecular, and involve stereoselective removal of 4β-H and introduction of 6β-D. The first may be formulated as a synchronous *syn*-S_E2' process. The second involves an intermediate which can be intercepted by oxygen in the presence of trace amounts of metal ions. The kinetic measurements show that in the absence of oxygen this intermediate gives more rapidly the unexchanged starting material than the rearranged enone; so it cannot be the free enol (which is known to be protonated much more rapidly at the 6- than at the 4-position), nor the enolate ion (which would give exchange at the 4-position). Isotope effects suggest that this intermediate should be formulated as an ion-pair, and establish also that exchange of both 4β- and 4α-hydrogen atoms with the solvent occurs at a rate which is significant but slower than those of the other reactions occurring in this solvent.

AERATION of cholest-5-en-3-one (1) in aprotic solvents with or without added dibenzoyl peroxide is known [1,2] to give the rearranged hydroperoxides (2; R = OH) and (3; R = OH); the corresponding reaction in pyridine in the presence of a photosensitizer gives cholesta-4,6-dien-3-one (4).[3] In methanol with triethylamine and a pyridinecopper(II) complex at 0°, cholest-4-ene-3,6-dione (5) is formed.[4]

We have now shown that when (1) in acetic acid is treated with air or oxygen, its rearrangement to cholest-4-en-3-one (6) is accompanied by the formation of (2; R = H or OH) and (3; R = H or OH), together with (5) and some other material containing no olefinic hydrogen atoms, probably an organic peroxide. The kinetics of the oxidation, however, are not those expected [5-8] for a free-radical oxidation involving the organic substrate directly.

EXPERIMENTAL AND RESULTS

Some of the materials and general methods have been described elsewhere; [9] additional information is provided in Supplementary Publication No. SUP 21895 (13 pp.).* Acetic [^{2}H]acid was prepared by reaction of deuterium oxide with acetyl chloride, b.p. 116—117 °C.

4β-Deuteriocholest-5-en-3-one was prepared as follows. A mixture of 5α,6β-dibromocholestan-3-one [10] (11 g), acetic [^{2}H]acid (1.20 g), deuterium oxide (15 ml), zinc dust (2.0 g), and dry ether (200 ml) was refluxed for 13 h and then shaken with anhydrous sodium carbonate (2 g). The ethereal solution was then decanted off and washed with water, dried ($MgSO_4$), and evaporated to dryness under reduced pressure. The solid residue (2.9 g) after three recrystallizations from acetone, had m.p. 127—129 °C, isotopic purity (by ^{1}H n.m.r. spectroscopy) 4α, 15 ± 5% D; 4β, 83 ± 3% D. 4,4-Dideuteriocholest-5-en-3-one was prepared similarly, refluxing being continued for 40 h, m.p. 127—129 °C, 4α, >90% D; 4β, >95% D.

6β-Hydroperoxycholest-4-en-3-one was prepared by Cox's method,[2] m.p. 178—180° (lit.,[2] 180—181°). In our hands, attempts to isolate 6α-hydroperoxycholest-4-en-3-one (2; R = OH) failed because the product decomposed on recrystallization or during t.l.c. giving (5). However, its presence (*ca.* 40%) in the reaction mixture after treating cholest-5-en-3-one with dibenzoyl peroxide and oxygen in cyclohexane for 24 h at 50 °C for preparation of the β-isomer [2] was evident from the ^{1}H n.m.r. spectrum. This mixture was converted into the corresponding mixture of alcohols (2; R = H) and (3; R = H) by treatment of it in ether (15 ml) with a solution of KI (2 g) in MeOH (30 ml) and a few drops of acetic acid for 1 h, and these were separated by t.l.c. and characterised for recognition in mixtures. Cholest-4-ene-3,6-dione was prepared by Volger and Brackman's method.[4] The properties of these various products obtainable by oxidation of cholest-5-en-3-one are given in SUP 21895.

Products formed during the Reaction of Cholest-5-en-3-one

* For details of Supplementary Publications see Notice to Authors No. 7 in *J.C.S. Perkin II*, 1975, Index issue.

[1] L. F. Fieser, T. W. Green, F. Bischoff, G. Lopez, and J. J. Rupp, *J. Amer. Chem. Soc.*, 1955, **77**, 3928.

[2] A. J. Cox, *J. Org. Chem.*, 1965, **30**, 2052.

[3] A. Nickon and W. L. Mendelson, *J. Org. Chem.*, 1965, **30**, 2087.

[4] H. C. Volger and W. Brackman, *Rec. Trav. chim.*, 1965, **84**, 579; H. C. Volger, W. Brackman, and J. W. F. M. Lemmers, *ibid.*, p. 1203.

[5] K. U. Ingold, *Chem. Rev.*, 1961, **61**, 563.

[6] O. L. Mageli and C. S. Sheppard, in ' Organic Peroxides,' ed. D. Swern, Interscience, New York, 1970, vol. 1, pp. 15*ff*.

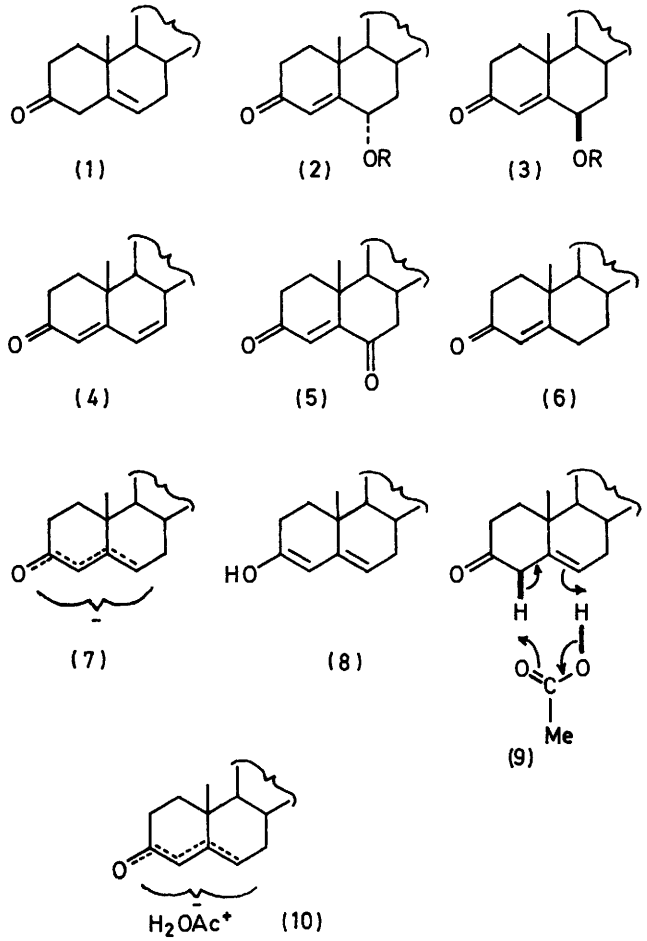
[7] G. A. Russell, in ' Peroxide Reaction Mechanisms,' ed. J. O. Edwards, Interscience, New York, 1962, pp. 107—128.

[8] J. Betts, *Quart. Rev.*, 1971, **25**, 265.

[9] P. B. D. de la Mare and B. N. B. Hannan, *J.C.S. Perkin II*, 1973, 1586.

[10] L. F. Fieser, *Org. Synth.*, 1963, Coll. Vol. IV, 195.

in Acetic Acid.—A solution of (1) (1.54 g) in acetic acid (200 ml) was placed in an air-filled flask (2 l) and set aside at 20° in the dark for 39 h. Acetic acid was removed from one portion (20 ml), which was worked up by addition of an equal volume of benzene, careful removal of the solvent under reduced pressure, and addition of a further volume of benzene, which was removed similarly. 2,4-Dibromoanisole (0.034 0 g) was added to the residue (the ^{1}H n.m.r. signal of the methoxy-protons was found to be a convenient stable standard for quantitative analysis using peak integrals). The ^{1}H n.m.r. spectrum of the product



mixture had a series of signals in the range τ 4.4—3.7 which could be recognised as those of the vinylic 4-protons in (6) (4.26, m), (3; R = H) (4.17, s), (3; R = OH) (4.09, s), (2; R = OH) (3.88, d, *J ca.* 2 Hz), (5) (3.83, s), and (2; R = H) (3.80, d, *J ca.* 2 Hz) in relative proportions 0.30 : 0.06 : 0.14 : 0.03 : 0.15 : 0.04 (total mole fraction 0.72). The peak integral of the broad multiplet (τ 5.8—5.2) exceeded that expected from the combined effect of the C-6 hydrogens of (2; R = H and OH) and (3; R = H and OH) by the relative mole fraction 0.28. This we attribute to the presence of other products of oxidation, the nature of which is indicated by the following experiment. An aliquot part (10 cm^3) of the product mixture was worked up as usual, and to the residue was added ether (3 cm^3) and solid CO_2 (*ca.* 1 g). The mixture was gently warmed to remove air, and a solution of KI (0.5 g) in MeOH (10 cm^3; similarly deaerated) and acetic acid (*ca.* 0.5 cm^3) was added. The flask was stoppered, allowed to stand (45 min), and titrated with 0.010M-$Na_2S_2O_3$, sodium starch glycolate being used as indicator. A control determination showed that no correction was needed. The I_2 liberated by the product mixture (4.0×10^{-4} mol) amounted to 1.05×10^{-4} mol; this exceeds significantly the amount expected (0.72×10^{-4} mol) from the quantities of the hydroperoxides present. The unidentified material therefore, probably includes dialkyl peroxides.

The proportions of the (2; R = H) and (3; R = H) present in the reaction mixture from several such estimations were determined by ^{1}H n.m.r. spectroscopy, and accorded with the amounts expected. Samples of these, of (5), and of (6) were isolated from the reaction mixture by t.l.c., and were identical with authentic samples.

The original product mixture was examined by t.l.c., a number of different solvent mixtures being used for development. In each case the mixture separated into six components: a poorly resolved pair having the same R_F values as (5) and (6); another pair having the same R_F values as (2; R = OH) and (3; R = OH); and a third pair with the same R_F values as (2; R = H) and (3; R = H).

The effects of various conditions of reaction and of added reagents on the products of reaction of cholest-5-en-3-one (0.005—0.04M) in the dark, and of a number of subsidiary and control experiments, are given in Tables S1 and S2 of SUP 21895.

Kinetics of Oxidation and Isomerization of Cholest-5-en-3-one (1) *in Acetic Acid at* 20.0 °C.—Acetic acid (200 cm^3), finely ground cholest-5-en-3-one (1.54 g), and the appropriate amounts of other reagents were stirred mechanically in a round-bottomed air-filled flask (2 l), held at 20.0° in a thermostat. The mixture needed rapid stirring at first to dissolve the reactant; thereafter slow stirring was sufficient. At appropriate intervals, aliquot parts (20 cm^3) were removed for work-up and analysis by ^{1}H n.m.r. spectroscopy. Peak integrals were used for analysis; added 2,4-dibromoanisole was used as standard. The results of a typical kinetic run are given in Table S3 of SUP 21895, together with product proportions after *ca.* 50% for the reaction in the presence of 4-hydroxy-3,5-di-t-butylanisole.

The rate of disappearance of starting material followed a first-order kinetic law to a good approximation; mean k_1 4.5×10^{-5} and 4.4×10^{-5} s^{-1}. When 4-hydroxy-3,5-di-t-butylanisole (0.000 5M) was added, a significantly reduced value (3.2×10^{-5} s^{-1}) was obtained, and the ratio [Ox] : [(6)] of total oxidation products to cholest-4-en-3-one (last column in Table S3) had a mean value reduced from 4.3 to 1.8.

The rate of disappearance of starting material, and the ratio [Ox] : [(6)], was unchanged within experimental error by the presence of sodium acetate (0.012M), or by the supply of oxygen instead of air to the reaction mixture. In the presence of HBr, however, isomerization was very fast and no products of oxidation could be detected.

A typical reaction mixture, when analysed for metal ions by atomic absorption spectrometry, contained copper (10^{-6}M), iron (10^{-6}M), zinc (10^{-7}M), calcium (10^{-6}M), and magnesium (10^{-6}M), probably leached from the glass apparatus. Oxidation was reduced to zero when the tetrasodium salt of ethylenediaminetetra-acetic acid [added as the dihydrate of its disodium salt (0.01M) and sodium acetate (0.02M)], was included in the reaction mixture; the first-order rate coefficient for the isomerization then had the value $k_1 = 1.65 \times 10^{-5}$ s^{-1}. Inclusion also of copper(II)

acetate (0.002 5M in excess of the amount of the sodium salt of ethylenediaminetetra-acetic acid) restored the rate of oxidation, and the proportion [Ox] : [(6)], to their original values; under these conditions, the oxidation product consisted almost exclusively of the enedione (5).

The kinetics of isomerization were determined similarly in acetic acid and in acetic acid with added base in an atmosphere of oxygen-free nitrogen at 20.0 °C. No products of oxidation were detected; the reactions were within experimental error first order in starting material over at least three half-lives, and proceeded virtually to completion [(6) : (1) > 10^3]. The rate coefficients for the isomerizations were as follows: $10^5k_1/s^{-1}$ = 1.58, 1.65 (HOAc); 1.76 (0.01M-NaOAc); 1.89 (0.079M-NaOAc); 2.34 (0.15M-NaOAc); 2.87 (0.20M-NaOAc). In the presence of added HBr (0.01M), the rearrangement was very rapid.

The corresponding reactions of the 5-enone in acetic [^{2}H]acid and of its 4-deuteriated derivatives in acetic acid were examined also. For the 5-enone after partial reaction the proportion of 4-H and its distribution between the α- and β-positions could be determined from the integrals of the relevant signals relative to that for 6-H. For the 4-enone, the proportion of 4-H could be determined similarly; when 6β-D was present, the signal for 4-H, instead of being a multiplet ($W_{\frac{1}{2}}$ 3.6 Hz), became a relatively sharp singlet ($W_{\frac{1}{2}}$ 1.6 Hz), as is expected because the allylic coupling between 4- and 6-H should be much greater for the axial 6β- than for the equatorial 6α-H.

The results are summarised in Tables 1 and 2. The

TABLE 1

Product proportions, isomeric compositions, and derived rates of isomerization of cholest-5-en-3-one and deuteriated derivatives in acetic acid at 20 °C under nitrogen in the dark

Cholest-5-en-3-one (isomeric composition) of starting material	4,4-H_2		4β-H [a]	4,4′-D_2 [b]
Solvent	HOAc	DOAc	HOAc	HOAc
t/h	20	5.0	20	20
Recovered starting material				
Proportion of total product	0.31	0.85	0.63	0.67
4α-H (%)	100	100	~70	~10
4β-H (%)	100	~95	~20	~15
Rearranged 4-enone (6)				
Proportion of total product	0.69	0.15	0.37	0.33
$W_{\frac{1}{2}}$(4-H)/Hz	3.6	1.6	3.5	Signal absent
4-H (%)	100	100	50	
6β-H (%)	100	~0	~100	~100
$10^5k_1/s^{-1}$	1.61	0.90 [c]	0.64	0.56

[a] 85% 4α-H, 17% 4β-H. [b] <10% 4α-H, <5% 4β-H. [c] After 73 h (79% reaction), the unchanged 5-enone contained much 4-deuterium; the rearranged product contained substantially only deuterium at the 6β-position.

separate rate coefficients for oxidation and rearrangement follow directly from the observed rates and the proportion [Ox] : [(6)] of oxidation to isomerization. The rates of reaction of the 4β-deuterio- and 4,4-dideuterio-enones are very similar, and accord even better if correction is made for incomplete deuteriation of the former compound, a result which indicates that the 4α-hydrogen atom is concerned only to a minor degree in the main reactions of oxidation and isomerization. A slower exchange reaction at the 4-position, however, affects the results when reaction is allowed to proceed beyond the first half-life. This reaction, which partly scrambles any isotopic labelling between the 4α- and 4β-positions, is not interfered with by concurrent oxidation.

Although the isotopic compositions as determined by ^{1}H n.m.r. spectroscopy must be regarded as approximate only, they give clearcut indications of the magnitude of the primary and solvent isotope effects and of the considerable

TABLE 2

Product proportions, isotopic compositions, and derived rates of isomerization of cholest-5-en-3-one in acetic [^{2}H]acid and of cholest-5-en-3-one and its 4-deuteriated derivatives in acetic acid at 20 °C in the presence of air and traces of metal ions in the dark

Cholest-5-en-3-one (isomeric composition of starting material)	4,4-H_2		4β-D [a]	4,4-D_2 [b]
Solvent	HOAc	DOAc	HOAc	HOAc
t/h	6.9	6.9	26.8	26.8
Recovered starting material (1)				
Proportion of total product	0.33	0.40	0.37	0.48
4α-H (%)	100	~100	~35	~8
4β-H (%)	100	~85	~20	~12
Rearranged 4-enone (6)				
Proportion of total product	0.13	0.035	Some	Some
$W_{\frac{1}{2}}$(4-H)/Hz	3.6	1.6	3.5	3.5
4-H (%)	100	100	Large	Small
6β-H (%)	100	~0	~100	~100
$10^5k_1/s^{-1}$	4.5	3.7	1.0	0.8
[Ox] : [(6)]	4.1	16	*c*	*c*
$10^5k_1/s^{-1}$ (oxidation)	3.6	3.5	*c*	*c*
$10^5k_1/s^{-1}$ (direct isomerization)	0.9	0.2	*c*	*c*

[a] 85% 4α-H, 17% 4β-H. [b] <10% 4α-H, <5% 4β-H. [c] Values of [Ox] : [(6)], and hence the derived values for the rates of oxidation and direct isomerization, were for these deuteriated compounds not determinable experimentally by our method of analysis.

stereoselectivity of the proton-transfer processes, and in our opinion sustain the conclusions drawn in the Discussion section.

DISCUSSION

(a) *Acid- and Base-catalysed Isomerization of Cholest-5-en-3-one in Acetic Acid.*—In an aprotic solvent, without an added catalyst, cholest-5-en-3-one does not rearrange. In acetic acid, however, it undergoes isomerization smoothly by a reaction which is first order in starting material over at least 90% reaction, and in the absence of oxygen and unbound metal ions gives complete rearrangement within experimental error.

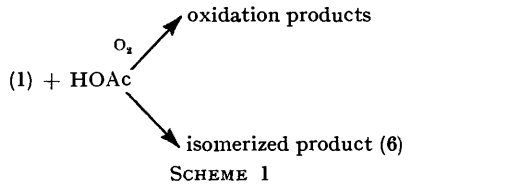
The rate of rearrangement is very much increased by strong acid. The addition of sodium acetate increases the rate by an amount which at high concentrations of sodium acetate rises more rapidly than the stoicheiometric concentration of sodium acetate, and is reasonably well represented by a dependence on $[NaOAc]^{3/2}$. Such behaviour has been noted in other base-catalysed prototropic rearrangements,[11] and can be interpreted directly if the basic species involved in attack on the substrate has the composition $[Na(OAc)_2]^-$, *i.e.* that of an acetate ion solvated by a sodium acetate molecule, or of an ion

[11] P. B. D. de la Mare and A. Singh, *J.C.S. Perkin II*, 1973, 59.

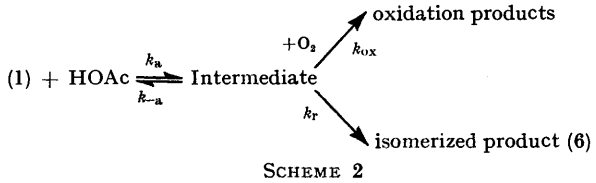
triplet. The mechanism of these specific acid- and base-catalysed isomerizations are probably those normally concerned in such reactions, and involve respectively the conjugate acid of (1) and the mesomeric enolate ion (7).

(b) *Isomerization of Cholest-5-en-3-one catalysed by Acetic Acid.*—When a small amount of sodium acetate is added to acetic acid, the concentration of H_2OAc^+ must be reduced from *ca.* 5×10^{-7}M as estimated from the autoprotolysis constant (2.5×10^{-13} mol^2 l^{-2} at 25 °C) [12] to a very much smaller value. The rate measured under nitrogen in the absence of sodium acetate (k_1 1.6×10^{-5} s^{-1}) contains not only a negligible contribution from attack by the acetate ion or a solvated form of this, but also only a very minor contribution from specific acid catalysis. It involves, therefore, catalysis by one or more acetic acid molecules.

When oxygen and free metal ions are present, the rate of isomerization is still of the first kinetic order, but the first-order rate coefficient is reduced to $10^5k_1/s^{-1} = 0.9$, and a faster first-order oxidation accompanies the rearrangement. We ask first, are the two processes independent first-order reactions, taking the form of Scheme 1 or 2?



SCHEME 1



SCHEME 2

Both of these formulations require that the total rate of reaction (with oxygen, $10^5k_1/s^{-1} = 4.5$) be a composite of two rates, one of which is that of the independently measured rearrangement. The product ratio, however, shows that the first-order rate coefficient for the rearrangement in the presence of oxygen contributes only $10^5k_1/s^{-1} = 0.9$ to the total rate. The value in the absence of oxygen is 1.6; the discrepancy exceeds considerably the experimental error. Neither Scheme 1 nor 2 can, therefore, alone account for the observed rate and product measurements; the isomerization must be able to proceed by two independent pathways, one being that of Scheme 2 ('indirect' isomerization) and the second being a 'direct' isomerization (1) ⟶ (6).

(c) *The 'Direct' Isomerization catalysed by Acetic Acid.*—The 'direct' isomerization remains as a first-order process whether or not oxygen is allowed to interfere with the 'indirect' isomerization. The rates given in the last line of Table 2 show that this 'direct' isomerization has a moderately large normal solvent deuterium isotope effect (k^{HOAc}/k^{DOAc} *ca.* 4.5). Its 4β-deuterium isotope effect is in the 'normal' direction, since the rate for this compound of the isomerization exceeds those of the total isomerization of the deuterio-compounds (last line of Table 1). We have not been able to establish its value experimentally, since our ^{1}H n.m.r. analyses of the products of oxidation of the deuterio-compounds could not be made quantitative, but a value $k^{4\beta\text{-H}}/k^{4\beta\text{-D}} = 2$ would be consistent with the values of the rate coefficients for the other processes.

In the transition state for isomerization by this pathway, therefore, the 6β-bond is being formed and the 4β-bond is being broken. The cyclic formulation (9) represents a single molecule of acetic acid acting as a catalyst for a concerted intermolecular proton transfer, and is analogous with that proposed [13] for the corresponding reaction in benzene catalysed by amines and by phenols. The results do not establish whether one or more than one molecule of catalyst is involved in the concerted proton-transfer, which is an example of the S_E2' rearrangement, and appears to be strongly stereoselective in the sense that the entering proton is *syn* to the leaving proton. Despite some theoretical speculations to the contrary,[14] it appears that this stereochemistry is favoured for the S_E2' reactions of relatively rigid systems of this kind involving axial entering and leaving groups.[15]

Although stereospecific proton transfers from the 4β- to the 6β-position have been recognised for the corresponding enzyme-catalysed rearrangements,[16] the latter reactions differ from that involved in the present isomerization in that they are intramolecular, a circumstance which requires or at least is very favourable for a *syn*-transfer of a proton. In our isomerizations, however, the proton entering at the 6β-position is clearly derived from the solvent.

(d) *Isomerization of Cholest-5-en-3-one catalysed by Acetic Acid; the Indirect Pathway involving an Intermediate trappable by Oxygen.*—The total rate of first-order rearrangement of cholest-5-en-3-one under nitrogen has a rate-coefficient $10^5k_1/s^{-1} = 1.6$, of which only 0.9 can be attributed to the 'direct' isomerization. The remainder, $10^5k_1/s^{-1} = 0.7$, is eliminated when oxygen and metal ions are present; it has the further characteristic that its rate-coefficient in acetic [^{2}H]acid is $10^5k_1/s^{-1}$ $= (0.9 - 0.2) = 0.7$, so it differs from the first mode of isomerization in being subject only to a small solvent deuterium isotope effect ($k^{HOAc}/k^{DOAc} = ca.$ 1). Its primary (4β-D) deuterium isotope effect could not be determined exactly; the mechanism which we are proposing requires that it should be the same as that for

[12] I. M. Kolthoff and A. Willman, *J. Amer. Chem. Soc.*, 1934, **56**, 1007, 1014.
[13] A. Fauve, A. Kergomard, and M. F. Renard, *Tetrahedron Letters*, 1973, 607.
[14] N. T. Anh, *Chem. Comm.*, 1968, 1089.
[15] I. M. Cunningham and K. H. Overton, *J.C.S. Perkin I*, 1975, 2140.
[16] S. K. Malhotra and H. J. Ringold, *J. Amer. Chem. Soc.*, 1965, **87**, 3228.

the related oxidation. The group entering at the 6-position is derived from the solvent, and enters stereoselectively on the β-face of the molecule.

Oxidation supervenes when oxygen is supplied and traces of metal ions are present. It proceeds at a rate substantially faster ($10^5k_1/s^{-1} = 3.6$ in acetic acid) than that of the isomerization ($10^5k_1/s^{-1} = 0.9$) The rate of this oxidation is not increased by increasing the concentration of oxygen five-fold, nor by increasing the concentration of Cu^{2+} by a factor of 2.5×10^3. Its solvent deuterium isotope effect ($k^{HOAc}/k^{DOAc} = 3.6/3.5 = ca.\ 1$) is small, as for the associated indirect isomerization. Its primary (4β-) deuterium isotope effect is large: certainly not less than $k^{4\beta\text{-}H}/k^{4\beta\text{-}D} = 3.6/1.0 = 3.6$; and, for the reasons already given (since the value 1.0 is composite, including a component from the residual 4β-H and from rearrangement), more probably *ca.* $3.6/0.5 \sim 7$. The observed rate of reaction of the 4,4-dideuterioenone ($10^5k_1/s^{-1} = 0.8$, including a component of isomerization) is reasonably consistent with this estimate.

Scheme 1 is not an adequate representation of this pathway, since it does not provide an explanation why increase in the concentration of oxygen does not increase the rate of oxidation. Scheme 2, on the other hand, is consistent with the kinetic results provided that allowance is made for the additional direct isomerization. Under nitrogen, or when free metal ions are absent, the intermediate is formed reversibly, and the measured rate coefficient $k_1 = k_a/(1 + k_{-a}/k_r)$. When oxidation proceeds in the presence of metal ions, the intermediate is carried immediately on to products of oxidation (k_{obs} being very much greater than both k_r and k_{-a}), and the measured rate coefficient is that for formation of the intermediate, k_a. The rates give the partition ratio for the intermediate, k_{-a}/k_r, as $(3.6/0.7) - 1 = 4.1$ in acetic acid and $(3.5/0.7) - 1 = 4.0$ in acetic [^{2}H]acid. It is reasonable that these values should be approximately the same, since the competing processes available to this very reactive intermediate must be approaching the limit of diffusion control (see below).

The nature of the intermediate, and of its formation and decomposition, can now be considered. It is formed by a reaction subject to a considerable primary 4β-deuterium isotope effect, but to no substantial solvent deuterium isotope effect. In the rate-determining stage, therefore, the proton is being removed from the 4β-position by acetic acid acting as a base. It would be natural, therefore, to presume that the intermediate is the enol (8), analogous with that concerned in many acid- and base-catalysed reactions of ketones. Such a compound might be expected to undergo attack by triplet oxygen under catalysis by some intermediate carbocationic species, by analogy with reactions described recently by Barton *et al.*[17] The kinetic results, however, do not accord with this proposal. The free enol is known [16] to undergo protonation predominantly at the 6- rather than at the 4-position. The value estimated for k_{-a}/k_r (4) indicates that the intermediate when formed reversibly is reprotonated preferentially in the reverse sense.

Another attractive formulation of the intermediate is that it is the enolate ion (7). This also can be excluded for its reprotonation at the 4-position, relatively fast when it is formed in stationary concentration (*e.g.* under nitrogen), would allow exchange of the 4-proton with the solvent, and this reaction, though a contributor to the complex of processes observed in this solvent, is slow relative to the other reactions under observation.

We propose, therefore, that the intermediate is the ion-pair (10). This can be captured by oxygen and metal ions more rapidly than it is protonated at either carbon atom or at oxygen. We presume, since the latter reaction must be very fast, that the reactions of this intermediate represented by k_{-a}, k_r, and k_{ox} in Scheme 2, must all be close to the limit of diffusion control, and that the hydrogen bonding between the solvent acetic acid and cholest-5-en-3-one must provide a configuration somewhat unfavourable for formation of the enol.

In the above discussion we have treated the accompanying slow exchange and scrambling between the 4α- and 4β-positions as a separate reaction, since this exchange, like one component of the isomerization, is not interfered with by oxygen. It could of course be allowed for in Scheme 2 by including a second intermediate formed at an early stage in the reaction. We should make it clear also that we accept the known importance of hydrogen-bonded complexes in both biochemical [16] and chemical [13,16] transformations of ketones, and think it probable that the transition states of all the reactions considered here are derived from such complexes formed in rapid pre-equilibrium.

For reactions (including isomerizations, proton exchange reactions, and halogenations) of ketones in aqueous solvents, the most commonly encountered intermediates are the isomeric enol [*e.g.* (8)] and the derived enolate ion [*e.g.* (7)]. Enolisation is often held [16] to be concerned in the corresponding enzyme-catalysed transformations, in which the catalysis is thought [16] to involve complexing of the substrate at more than one site with acidic and basic sites on the surface of the enzyme. Related reactions carried out in solvents of lower dielectric constant, however, are becoming recognised as able to involve intramolecular proton transfers involving ion-pairs, as for example in the general-base-catalysed rearrangement of t-butyl thiobut-3-enoate to t-butyl thiobut-2-enoate discussed by Fedor and Gray.[18] These authors point out that the possibility that enzyme-catalysed isomerizations may proceed through paths other than rate-determining enolisation deserves serious consideration. It is likely that some reactions in solvents of low dielectric constant may be better models for enzyme-catalysed processes than are the corresponding reactions in water, because specific partitioning of intermediates between several pathways

[17] D. H. R. Barton, R. K. Haynes, G. Leclerc, P. D. Magnus, and I. D. Menzies, *J.C.S. Perkin I*, 1975, 2055.

[18] L. Fedor and P. H. Gray, *J. Amer. Chem. Soc.*, 1976, **98**, 783.

can occur both in reactions of ion-pairs and on surfaces. The major difference between the enzyme-catalysed isomerization and the acetic acid-catalysed processes described in this paper is that the former gives intramolecular proton transfer, whereas the latter allow the solvent to provide the new proton.

(e) *Pathways leading to Products of Oxidation.*—It is clear from the above discussion that the oxidation of cholest-5-en-3-one by oxygen in acetic acid has its rate controlled by heterolysis of the 4β-proton, being of the first kinetic order, subject to a substantial primary (4β-) kinetic isotope effect, and not subject to a large solvent deuterium isotope effect. The intermediate, probably the ion-pair (10), can be rapidly and completely trapped by oxygen with metal ions in trace amounts. We have outlined only the more obvious features of the subsequent stages involving oxidation. The hydroperoxides (2; R = OH) and (3; R = OH) are first formed rather unselectively by a homolytic process catalysed by traces of metal ions. The corresponding alcohols and the enedione (5) are secondary products derived primarily from reaction of (2; R = OH) with reactant and product, probably also by homolytic processes. When copper(II) acetate is present in more than trace concentration (*e.g.* 0.001 25M), the enedione is the sole product of oxidation, but the rate of disappearance of starting material is unchanged. This autoxidation, therefore, is still rate-controlled by heterolytic fission to give a reactive intermediate; it is likely that heterolytic control of homolytic processes may be important in some biological oxidations involving molecular oxygen and unbound trace metals.

Some further comments are given in SUP 21895.

More conventional autoxidations occur if benzoyl peroxide is used as a catalyst, and then the reaction becomes autocatalytic.

Oxidation of cholest-5-en-3-one with air or oxygen in carbon tetrachloride at 20° was found to be slow [$10^5 k_1/s^{-1}$ (initial) *ca.* 0.007], autocatalytic, catalysed by dibenzoyl peroxide, and inhibited by 3,5-di-t-butylanisole; the products are (2; R = OH) and (3; R = OH), as in the corresponding reaction in cyclohexane [2] at 50 °C. The similar reaction in ethanol was also slow [$10^5 k_1/s^{-1}$ (initial) *ca.* 0.05]. The products were entirely those of oxidation, (2; R = H and OH), (3; R = H and OH), and (5); no rearrangement accompanied oxidation under these conditions.

We thank Dr. F. J. B. Aggett for analyses by atomic absorption spectroscopy, Dr. G. A. Bowmaker for e.s.r. measurements, Professor T. F. Slater, Brunel University, for valuable comments and criticism, and the University of Otago for e.s.r. facilities.

[6/120 *Received*, 19*th January*, 1976]