NEW REACTIONS IN STEROID CHEMISTRY

A thesis presented by

JOHN FINTAN McGARRITY

in partial fulfilment of the

requirements for the

DIPLOMA OF IMPERIAL COLLEGE

Hofmann Laboratory
Chemistry Department
Imperial College
London SW7

October 1971

ABSTRACT

The research described in this thesis is divided into four parts:-

Part 1 is concerned with the synthesis of several Δ^7 ergostanol derivatives for use in biosynthetic experiments.

Part 2 outlines the introduction and removal of the methoxymethyl ether protecting group for the steroidal 3β hydroxyl function.

Part 3 describes the isolation and structural investigation of a new triterpenoid isolated from yeast.

Part 4 relates the development of new reactions for the introduction of double bonds into ring A of steroidal 3-aryl hydrazones. Several novel methods for the regeneration of the starting ketone from the aryl hydrazone via 3-gem disubstituted derivatives. A kinetically controlled isomerisation of a p-nitrophenylhydrazone to the less stable aryl azo isomer is introduced.

The synthetic significance of this section is placed in perspective by a review of the known methods of introduction of unsaturation into ring A of 3 keto steroids.

Acknowledgements

I thank Professor D.H.R. Barton F.R.S. for his direction and encouragement of this research. I also wish to thank Dr. D.A. Widdowson for his invaluable advice and guidance.

Mr. K.I. Jones and his staff are thanked for their exoellent analytical service. Mrs. A.I. Boston and Mr. P.N. Jenkins are also thanked for their supply of n.m.r. spectra, and also Mrs. J. Lee and Mr. J. Bilton for providing mass spectra. The technical assistance of Mr. A.P. Coleman and his staff is gratefully acknowledged.

I wish to acknowledge the receipt of a Northern Ireland Ministry of Education Postgraduate Studentship for 1968-1971, and also a Queens University of Belfast Postgraduate Foundation Studentship for the same period.

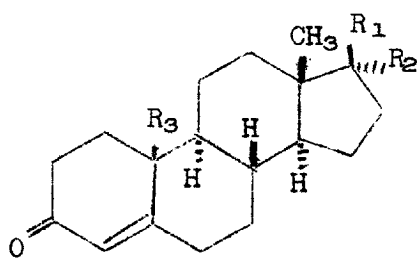
Contents

REVIEW

Methods for the introduction of double bonds into ring A of steroidal 3-ketones

INTRODUCTION

Most of the physiologically potent steroids in the adrenocortical series, possess a $\triangle$4-3-keto grouping, as in cortisol (1), the major hormone secreted by the human adrenal cortex. The major male sex hormone, testosterone (2) and the most important of the gestogens (one class of female sex hormones) progesterone (3), also exhibit this structural feature in ring A. The estrogens, the other female sex hormone type typified by estradiol , (4) have an aromatic ring A which is synthetically accessible from a 1, 4 -dien-3-one, ring A,[1] by a dienone-phenol rearrangement.

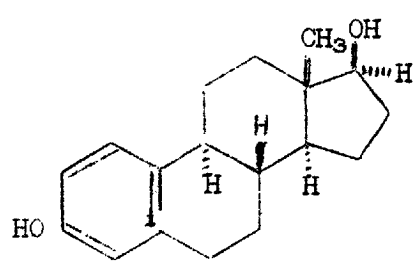


(1) R_1=$COCH_2OH$, R_2=OH, R_3=CH_3

(2) R_1=OH, R_2=H, R_3=CH_3

(8) R_1=OH, R_2=H, R_3=H

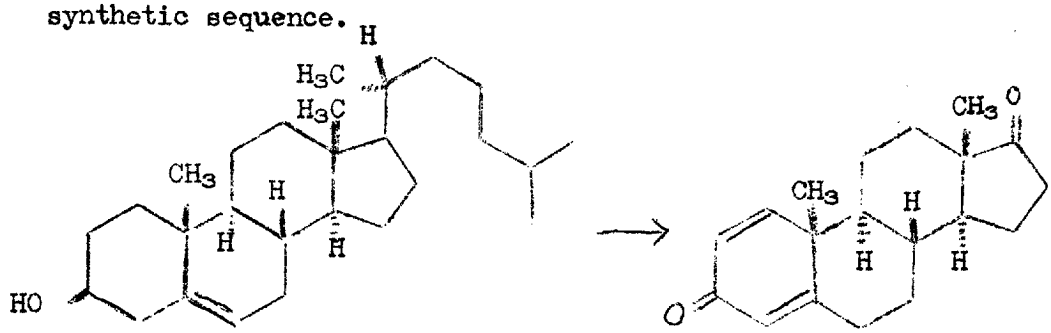
(3) R_1=$COCH_3$, R_2=H, R_3=CH_3



(4)

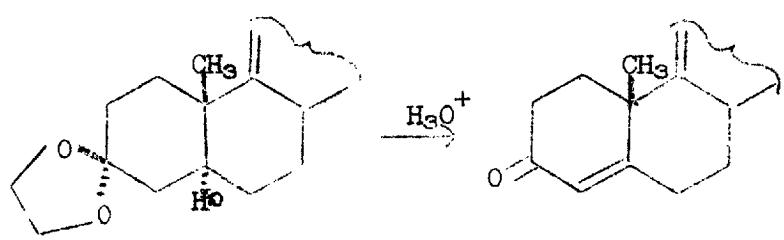
Consequently, the intr duction of unsa uration into ring A, in conjugation with a 3-keto function, has been of major importance in the synthetic chemistry of these steroids. Whereas in future, it may be possible to combine this, and other synthetic stages, without isolation of intermediates - micro-organisms have been used[2] to degrade cholesterol (5), with simultaneous oxidation, double bond isomerization, and dehydrogenation, to yield andostra - 1, 4-dien-3, 17-dione (6) - generally, the introduction of the double bond occurs at the end of the

synthetic sequence.

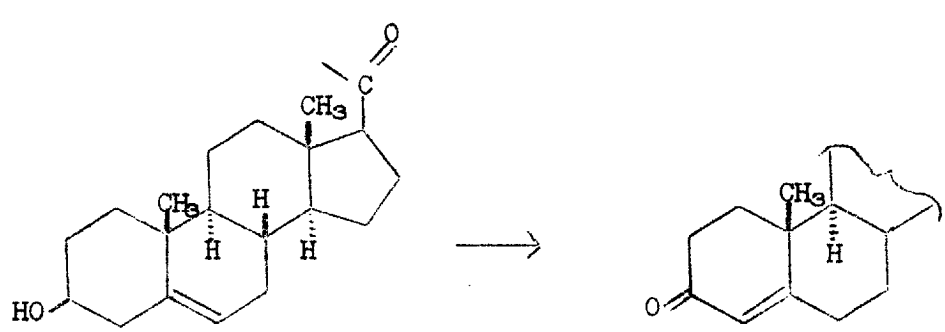


(5) (6)

Syntheses can be designed to have substituents present, which can give an easy introduction of the double bond by a particular method at the last step. In a recent example of cortico steroid total synthesis[3], a 3-ketal group was hydrolysed with simultaneous dehydration involving a 5α -hydroxy group to give the 4-en-3-one.

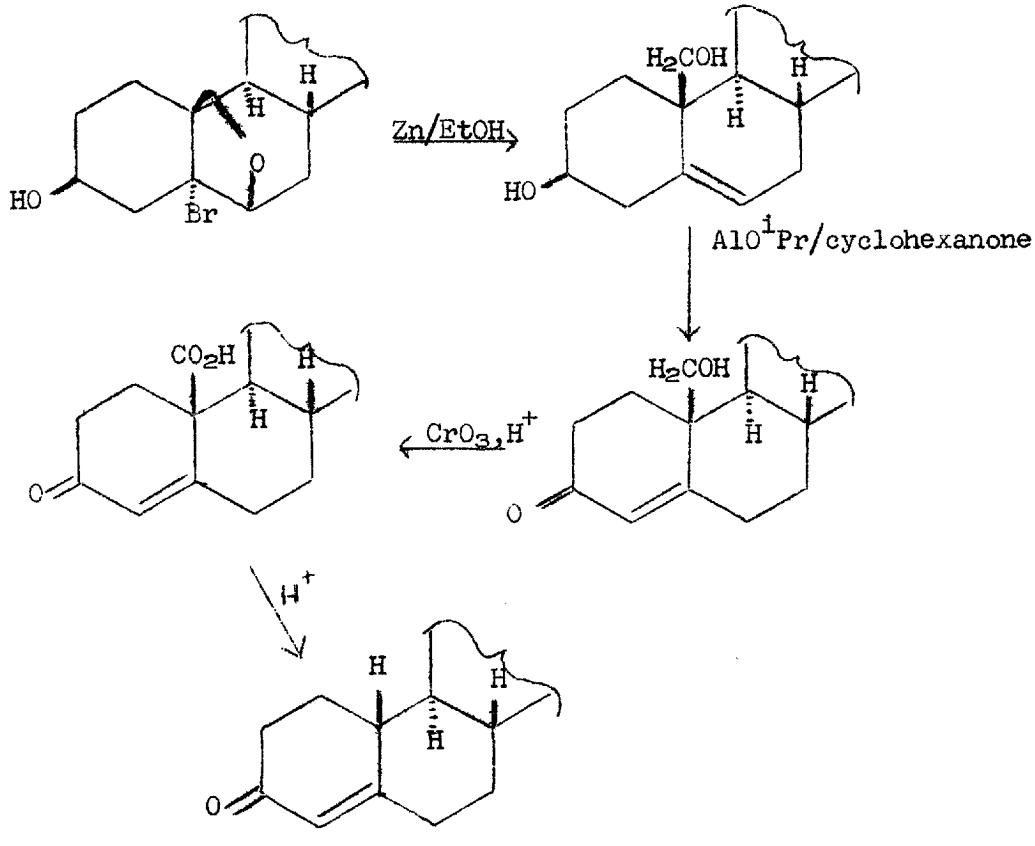


Many of the earlier steroid hormone syntheses[4] involved Oppenauer oxidation[5], of a 3-hydroxy-5-olefin to the 4-en-3 one system. One of the original conversions,[6] was that of pregnenolone (7) to progesterone (3) in 70% yield.

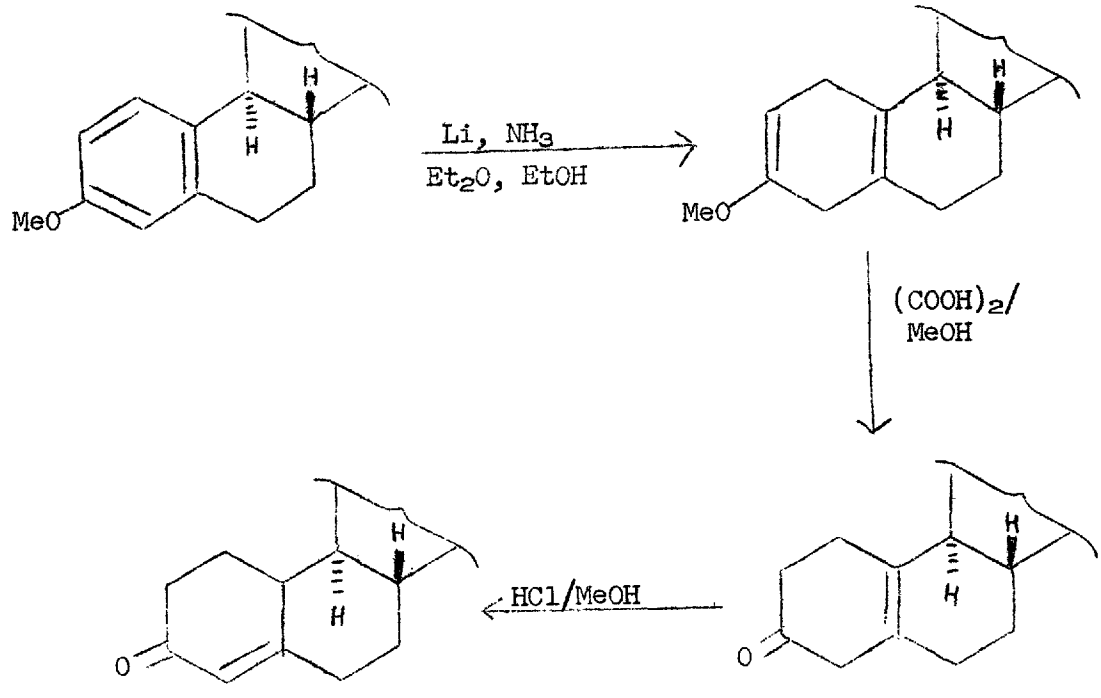


(7)

The Oppenauer oxidation is well suited for use at the end of a synthetic sequence, as it frequently gives high yields (80 - 95%), and can be quite specific in its action. Due to steric inhibition, an 11β hydroxyl group will not react during oxidation of a 3 β [7] or 20[8] hydroxyl group. Acid labile groups e.g. acetals,[9] are unaffected by the Oppenauer conditions. A more recent example of its use is in the synthesis of 19-nor-steroids.[10]



The most common and simple route to 19-nor-steroids is by use of the Birch reduction[11] of steroids with an aromatic ring A. Most of the applications of this reaction to steroids, have been in the hormone series, this was occasioned by the usually enhanced biological activity of the 19-nor-analogues of the steroid hormones. In the synthesis of 19-nor-testosterone[12] (8), the reduction was achieved in an overall yield of 66%.


MeO
H
H
Li, NH3
Et2O, EtOH
MeO
H
H
(COOH)2/
MeOH
O
H
H
HCl/MeOH
O
H
H

Relative Stability of Enols from 3-Ketones[13]

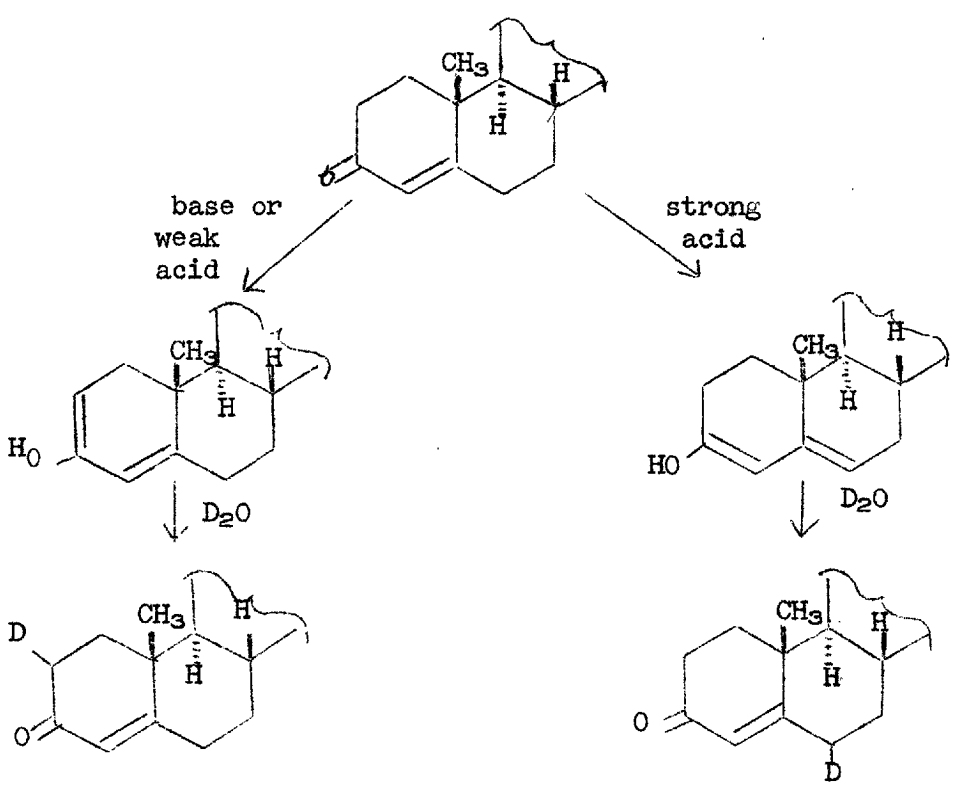
The reactions under review later, all involve the intermediacy of the enol form of the 3-keto group. The direction of enolisation is subject to both kinetic and thermodynamic control, depending on reaction conditions, which thus determine the position of α functionalisation. Under conditions of thermodynamic equilibrium, 5α -3-ketones give predominantly 2-enols,[14] whereas 5β -3-ketones prefer the 3-enols.

In the latter case, this preference may be largely due to relief of non-bonded interactions between the 4α -hydrogen, and the 7α -and 9α -hydrogens, when a trigonal centre is formed at C-4.[15] Calculations of H-H and CH_3-H non-bonded interactions for this system indicated an enthalpy difference of 1.9 kcal/mole, which corresponds to an equilibrium mixture of 96% 3-enol and 4% 2-enol. The experimentally determined equilibrium ratio from enol acetylation studies was 93.5% 3-enol and 6.5% 2 enol.[16]

In the 5α series the preferential formation of the 2-enol parallels the greater stability of the 2-ene relative to the 3-ene. Differences in the heats of hydrogenation of the Δ^2 and Δ^3 5α -cholestenes, show the former to be more stable by 2.1 kcal/mole.[17] This stability difference has been rationalised [18] as resulting from the greater steric compression of the 6β-hydrogen and the 19-methyl group in the Δ^3 isomer. Enol acetylation studies on 19-nor-5α -3-oxo-steroids[19] have reinforced this interpretation, by demonstrating that removal of the 19-methyl group, leads to increased formation of the 3-enol acetate. Longer range substituent effects may also disturb the equilibrium ratio. For instance, in the 5 β series, the introduction of an 11β acetoxyl group, shifts the equilibrium percentage of the 3-enol acetate from 94% to 75%.[20]

Unsubstituted Δ^4-3-oxo-steroids react under acid-catalysed equilibrium enol acetylation conditions, to give exclusively the 3, 5-dienol 3-acetate.[21] Thus the free energy difference between the two dien-3-ols cannot be estimated in this way. Alkyl[22] or aryl[21] substitution at C-2 leads, as expected, to increased formation of the 2, 4-dienol acetate. Once again the enol equilibrium is sensitive to long range effects. Introduction of a 9, (11) double bond changes the equilibrium ratio of 3, 5-dien-3-acetate to 2, 4-dien-3-acetate for a 2α-phenyl substituted 4-en-3-one, from 60 : 40 to 43 : 57.[23]

The thermodynamically preferred formation of the 3, 5 dienol is not always realised, however. It has been shown, through kinetic and deuterium-exchange experiments,[24] that enolisation of an unsubstituted Δ^4-3-ketone in the presence of strong acid, leads to moderate favouring of the more stable 3, 5-dienol as the kinetically determined product. With weak acid, or with sodium deuteroxide, formation of the less stable 2, 4-dienol and dienolate anion are heavily preferred.



With base catalysis the most acidic proton is first removed. In the above system this will be the C-2 proton, which, being directly adjacent to the carbonyl group, will be more subject to its inductive effect than will be the competing C-6 proton. It has been concluded[25] that in either acid or base catalysed enolisation, the methylenic C-H bond length in the transition state decreases with increase in base strength - i.e. the stronger the base, the closer the transition state resembles the original ketone. Thus only with a very weak base, will the transition state for enolisation bear resemblance to the enolate anion with consequent reflection of thermodynamic stability relationships.

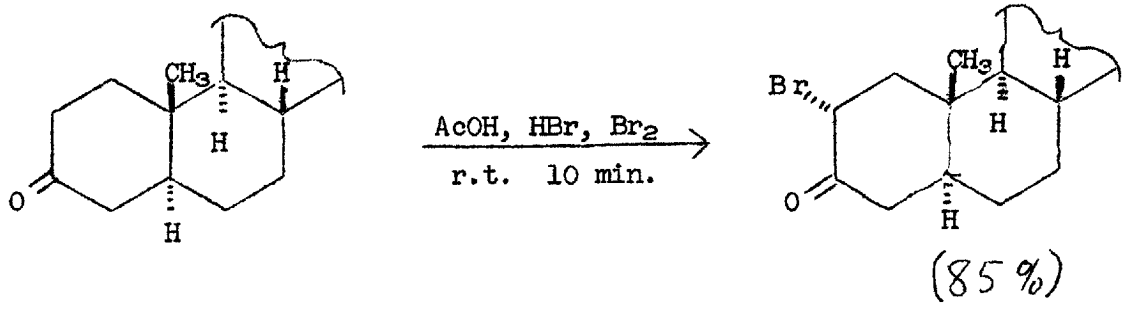
With acid catalysis, protonation of the carbonyl leads to a lengthening of the C-O bond - and a greater resemblance to enol. In a comparison of reactions in an aqueous medium of a weak acid, e.g. acetic acid, and a strong acid, e.g. hydrochloric acid, and assuming carbonyl protonation by hydronium ion in each case, the transition state determining factor will be the strength of the base removing the methylenic proton. In the case of acetic acid the base will be the relatively strong one, acetate ion-which will require relatively little C-H stretching, and the transition state will therefore resemble protonated ketone more than enol. In dilute hydrochloric acid, the strongest base present will be water which will require considerable C-H bond stretching and will lead to a transition state with a greater resemblance to enol. In this latter case, the acidity of the methylenic protons will assume little importance relative to the stability of the respective enols.

The above study[24] also revealed preferential loss of the 2β and 6β axial protons, rather than their α counterparts. This confirms the importance of overlap of the axial substituents' σ bonding

orbital and the carbonyl π orbital in the enolisation transition state.[18] A more dramatic confirmation is derived from the observation that the 19-nor-analogue showed a 300 fold preference for axial proton removal in the absence of steric hindrance by the 19-methyl group.[26]

Bromination of 3-Keto Steroids

The earliest and most common method of introducing unsaturation in conjugation with a steroidal 3-keto group, is by bromination followed by dehydrobromination. 5α -Cholestan-3-one was first monobrominated with acid catalysis to yield the 2α-bromoketone.[27]



(85 %)

The introduction of the bromine at C-2 is expected from comparison of the stabilities of the Δ^2 and Δ^3 enols. The situation of the bromine substituent in the 2α - i.e. equatorial conformation, is also expected under reaction conditions with strong acid catalysis, which would allow ready epimerisation of a less stable axial substituent. It was noticed[28] in those cases where both epimers were isolated, for example, in the bromination at C-4 of a 5α , 6β - dibromoketone, that the unstable one - with axial bromine, seemed to be formed initially. This was in accordance with Corey's concept[18] of stereoelectronic control of axial approach of the substituent to the intermediate enol. However, when a 2-en-3-ol acetate was brominated under conditions of kinetic control, i.e. in the presence of a buffer to consume the hydrobromic acid formed, the 2α -epimer was again produced,[29] in apparent contradiction of the above rule. It appears[30] that during bromination, axial attack on the enol which constrains ring A in the 'half chair' conformation, (fig.I) cannot occur on the β face, because of steric compression by the 19-methyl group. Attack on the α face gives bromine axially substituted in a ring in the 'boat'

conformation. Re-attainment of the favoured chair conformation renders the 2 α substituent equatorial.

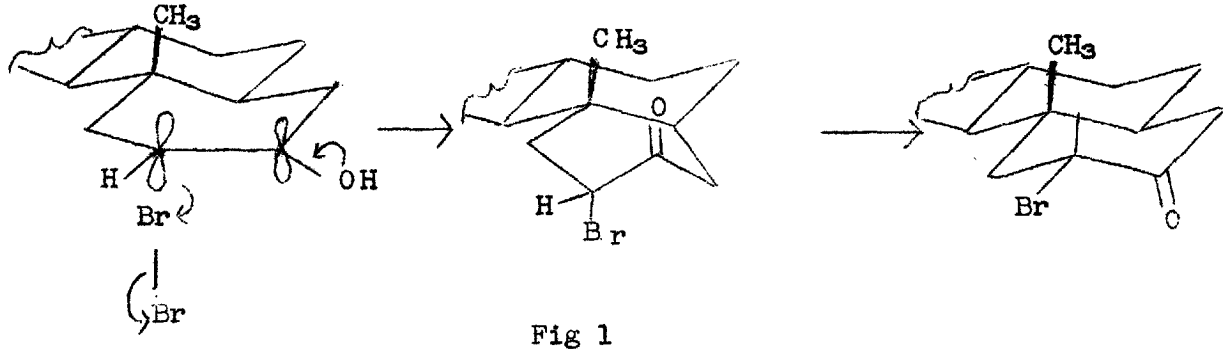


Fig 1

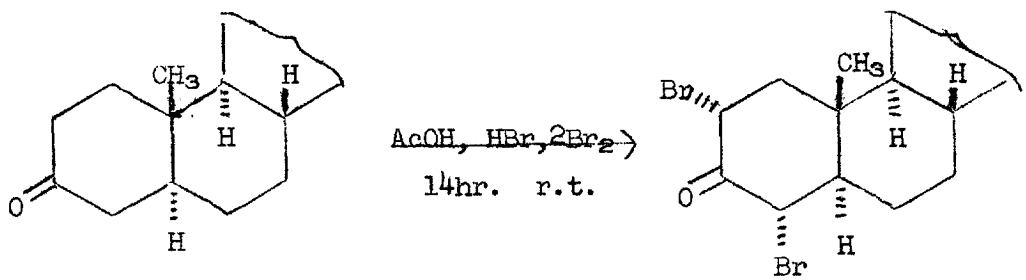
If the steric crowding on the β face is removed as in a 19-nor-steroid, the predicted initial 2 α-bromination is observed. Similarly, introduction of a 5 α -methyl group, to compress the α face leads to the formation of the 2 β-bromide. An indication that the 2 α -bromo-5 α -ketone is indeed the kinetically, as well as thermodynamically controlled product, comes from the results of bromination of a 2,2,4,4 tetra-deuterated-3-ketone in buffered acetic acid.[31] The resulting 2 α bromoketone retained 43% of its deuterium in the 2 β position. If epimerization had occurred at C-2, the expected deuterium loss would have been 100%.

3-keto-steroids have been brominated under a large variety of reaction conditions other than the usual one of acetic acid/bromine.[32] Of note among these is the use of pyridine hydrobromide perbromide[33] - which provides easily measurable quantities of bromine for small scale reactions, and also the use of N-bromo-succinimide in carbon tetrachloride,[34] which has the advantage of not producing any acid in the course of the reaction. Both these alternative methods provide the same monobromination products and yields as the usual method.

In the 5β series, the 4-bromo-3-ketones can be obtained in 60-70% yield, from bromination in buffered acetic acid.[35] This

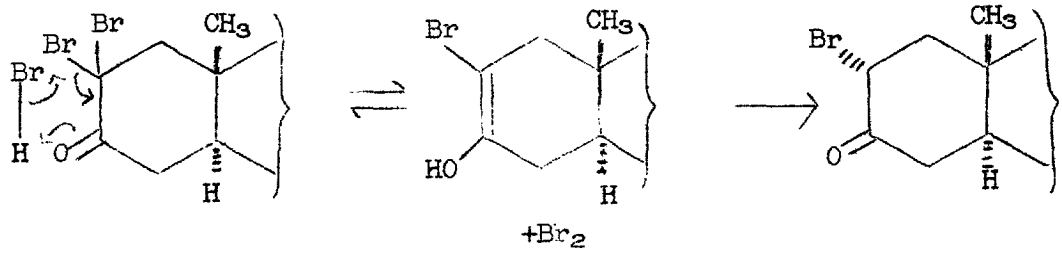
yield has been increased to 80% through the use of di-methyl formamide as bromination solvent.[36]

When 5 α -cholestan-3-one is treated with two moles of bromine in the presence of acetic acid and hydrobromic acid, the 2 α , 4 α - dibromoketone is formed[37] via the 2,2-dibromo isomer.[38]



The initial formation of gem-dihalides with acid catalysis, appears to be general in the absence of steric constraints, as evidenced by the formation of 2 α -bromo-2 β -chloro-3-ketones[39] and 2,2-dichloro-3-ketones,[40] which are stable under the reaction conditions.

The rearrangement of the 2,2-dibromo ketone to its 2α, 4α - isomer has been explained as an allylic rearrangement in the enolic form.[41] Djerassi[38(a)] implicated hydrobromic acid as a catalyst for the rearrangement, by the observations that: (a) in the presence of the acid, water inhibited the isomerisation, presumably by decreasing the acid strength of the catalyst in acetic acid; (b) in the absence of hydrogen bromide, sodium acetate and hydrogen peroxide inhibited the rearrangement, by removing traces of the acid formed by dehydrobromination of the 2,2 dibromide. The involvement of hydrobromic acid was confirmed by kinetic studies,[42] which revealed that the reaction was first order in hydrobromic acid. No rearrangement was observed in acetic acid, with or without the addition of other strong acids - hydrochloric or perchloric. On this basis, a mechanism involving the intermediacy of molecular bromine was proposed.

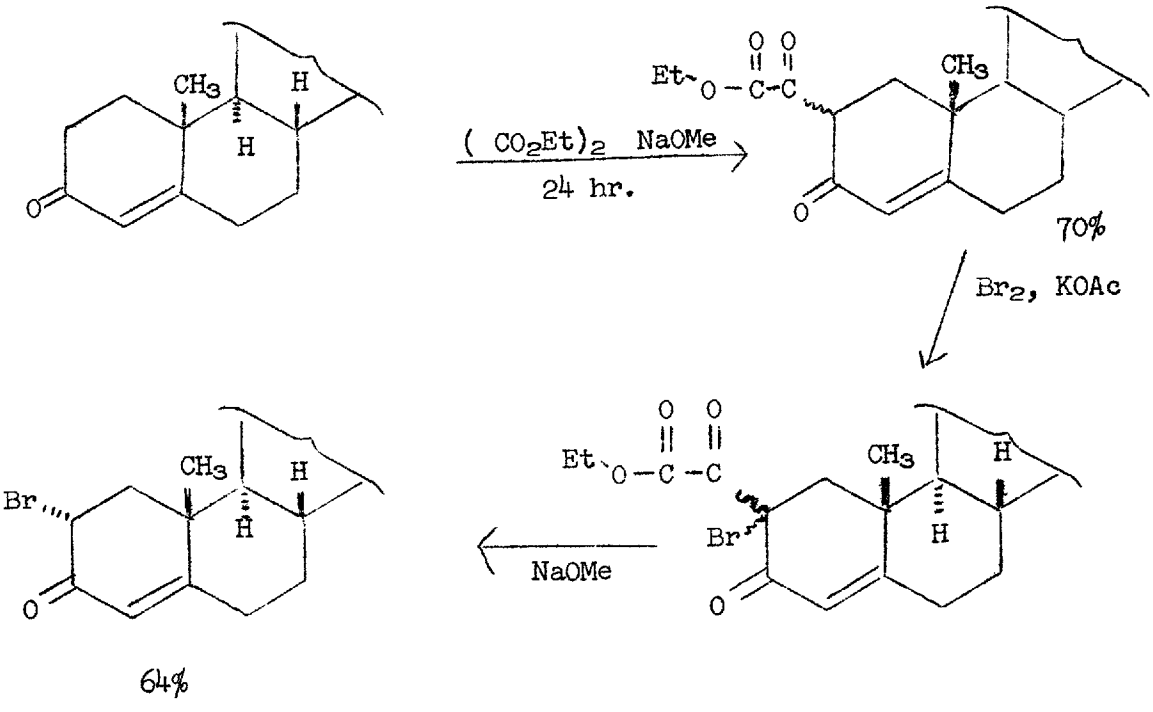


This mechanism received support from the trapping of the intermediate bromine by β -napthol during the rearrangement to give 1-bromo-2-napthol and 2 α -bromoketone.[42]

5 β -cholestan-3-one reacts in a similar fashion to yield initially the 4β -monobromoketone, and eventually in 90% yield, the the 2 β , 4β -dibromo-5β -cholestan-3-one.[43]

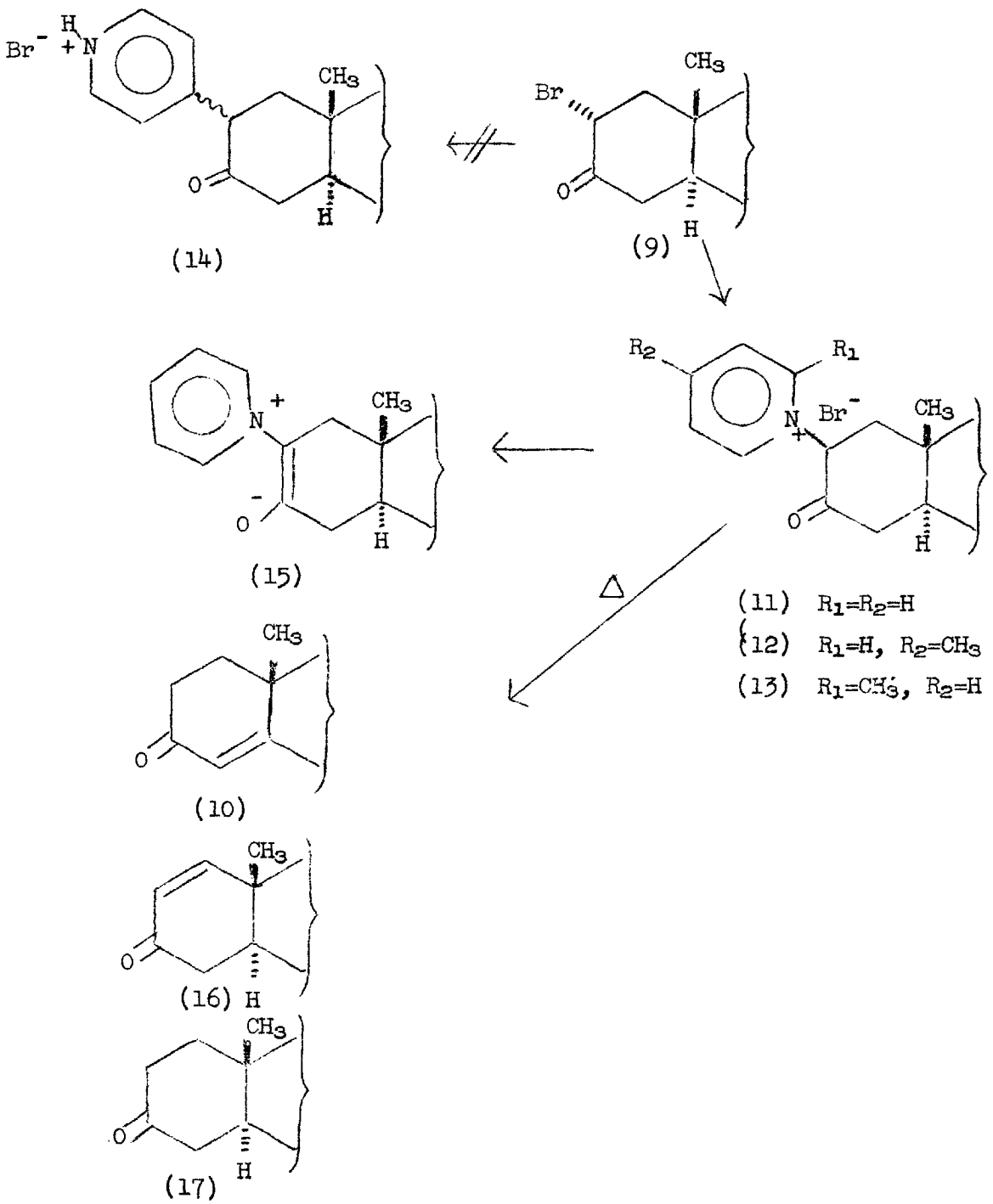
The 2,2-dibromo ketones can be obtained in the 5α series by use of N-bromo-succinimide[34] in 51% yield or by base-catalysed bromination,[42] which though ineffective for brominating unsubstituted 3-ketones, proceeds efficiently (57%) with a 2α -bromo-3-ketone, to give no trace of the 2α , 4α -dibromo product.

Bromination of steroidal Δ^4 -3-ketores in acetic acid leads to the 6-bromo, . . , -- . , and then (in 80% yield) to the 2,6-dibromo - Δ^4-3-ketones.[44] In the presence of a proton acceptor, allylic bromination is suppressed, and 4-bromo- Δ^4 - 3-ketones are produced.[45] The preparation of 2-bromo-4-en-3-ones is best achieved through the 2-ethoxaloyl derivative.[46] α -Functionalisation by this means can be quite selective, as it is dependent on the steric congestion of the reaction site.


CH3
H
H
O
(CO2Et)2 NaOMe
24 hr.
Et
O
C
C
O
O
CH3
O
70%
Br2, KOAc
Br
CH3
H
H
O
64%
NaOMe
Et
O
C
C
O
O
Br
CH3
H
H
O

Dehydrobromination of α -bromoketosteroids

When 2α -bromo-cholestan-3-one (9) is heated in refluxing pyridine, a pyridinium salt is formed.[47] This, on pyrolysis was reported to yield cholest-4-en-3-one(10). The structure of the salt was variously considered to be a 1-pyridinium(11)[47] and a 4-pyridinium salt[48] (14). The former structure was shown to be correct by the elimination of hydrogen bromide to form a betaine[49] (15).

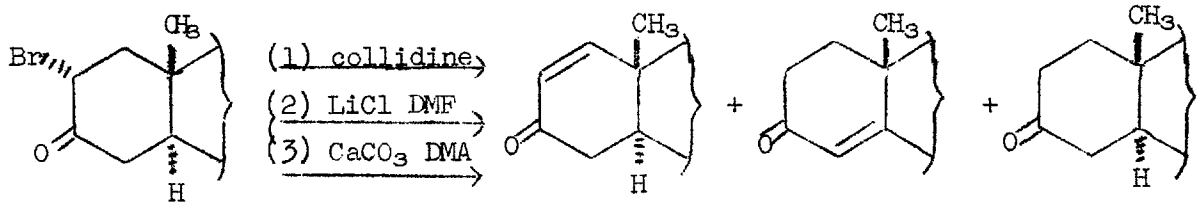


Thermal decomposition of the analogous γ picolinium salt (12) gave the 4-en-3-one (10), and also the 1-en-3-one (16) and saturated ketone (17) in a ration of 3:1:2.[50] Treatment of (9) with refluxing collidine gave a mixture of the same three ketones. The ratio of (10):(16):(17) formed in this reaction has been found to be 40%: 20%:0 [38a] and 30%:40%:10%.[51] The non-intermediacy of the salts of the type (11), (12), (13), in the dehydrobromination with substituted pyridines, has been demonstrated: (a) rather doubtfully, by the failure to detect (11) during the formation of (10) and (16) with 2-methyl pyridine;[49] and (b) by the observation that the quaternary salt (12), is recovered unchanged after subjection to the reflux conditions under which dehydrobromination proceeds.[50]

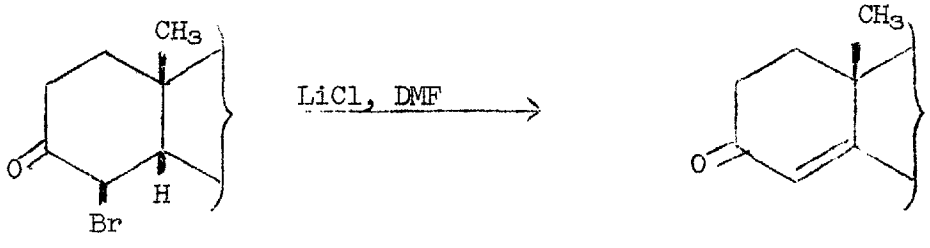
A comparison of the reactions of (9) with different pyridine bases showed that, in the absence of an α substituent, quaternary salt formation is the predominant course, whereas the presence of an α methyl group suppresses salt formation completely in favour of dehydrobromination.[50] The presence of two α methyl groups leads to increased reductive debromination at the expense of dehydrohalogenation. This was attributed to the increased hindrance of approach of the nitrogen to the C-1 hydrogens. With other haloketones the reduction reaction is more significant. For example, treatment of 3α-bromocholestan-2-one with 2,6-lutidine gave 28% of the saturated ketone.[51]

Competitive reductive debromination has not been noted in dehydrobrominations with lithium halides in aprotic solvents. Thus 4β-bromo-17α-hydroxy-21-acetoxy-5β-pregnan-3,11,30-trione yielded 73% of cortisone acetate when treated with lithium chloride in dimethyl formamide.[36] With 2-halo-3-ketones though no saturated ketone is produced, once again a mixture of product enones is observed. Thus in a recent dehydrobromination in a linear steroid analogue[52] a

2α -bromo-3-ketone gave, with lithium chloride and di-methyl formamide, 52% of 1-en-3-one and 22% of 4-en-3-one. This contrasts markedly with the reaction of 2α -bromo-5α -pregnan-3,11,30-trione under the same conditions to give 11-keto progesterone in 40% yield with less than 5% of the Δ^1 isomer being detected.[53] However, the first product ratio corresponds with the 48% yield of 1-en-3-one and 31% 4-en-3-one obtained on treating 2α -chloro-cholestan-3-one with the same reagent.[54] An isomerically pure product can be obtained by the use of calcium carbonate in di-methyl acetamide. With this system, 2α -bromo cholestan-3-one was converted to the 1-en-3-one in 82% yield.[55]



(1)	40%	) 30%	10%
(2)	52%	22%	—
(3)	80%	—	—



Scheme 2

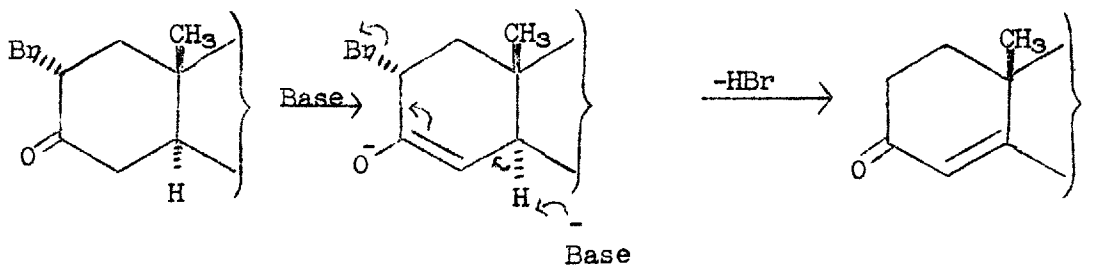
In the dehydrobrominations of 2,4-dibromoketones, the formation of the desired 1,4-dien-3-one was attended by the simultaneous formation of 4,6-dien-3-ones. Thus the yield of cholesta-1,4-dien-3-one formed by collidine treatment of 2α,4α -dibromo-cholestan-3-one was found to be 49-53%.[56] A more close examination[57] of the dehydrobromination products from this compound in collidine revealed

that the $\Delta^{4,6}$ isomer was formed in 15% yield compared with 60% yield of the $\Delta^{1,4}$ isomer. This same study revealed that the use of lithium bromide in di-methyl formamide, in the presence of lithium carbonate, on the same substrate, formed the 4,6 dienone in 39% yield, and the 1,4-diene isomer in 31%. This result is an apparent contradition of the claim[58] that the use of lithium carbonate prevents the formation of the 4,6-dienone-presumably by removing any acid formed. This claim was based on several reactions including the formation of the 1,4-dien-3-one in 90% yield from a 2 α, 4β-dibromoketone in the 5 α series, and also the dehydrobromination of a 2 β , 4 β -dibromoketone in the 5 α series yielding 80% of the cross-conjugated product. Yields comparable to these - (81%) - have been obtained for a 2 α, 4 α-dibromo-5 α -3-ketosteroid analogue, with lithium chloride, lithium carbonate and di-methyl acetamide.[52]

The usually accepted mechanism for elimination of hydrogen bromide from an α -bromo ketone is via an E2 <u>trans</u> elimination. Thus dehydrobromination of 16 α -deuterio-17 α -bromo-pregnan-11,20-dione with pyridine, and with lithium chloride in di-methyl formamide, gave complete retention of deuterium.[59] However, a study of the reaction of 1 α -deuterio-2 α -bromo-cholestan-3-one, with calcium carbonate in di-methyl acetamide, showed loss of deuterium, i.e. the axial proton.[60] This suggests that the mechanism is a <u>cis</u> 1 α , 2α elimination, or else a <u>trans</u> diaxial loss, after isomerisation of the 2 α -bromo substituent to the 2 β conformation.

This reaction may not proceed through the same mechanism as the lithium halide/di-methyl formamide, or collidine promoted eliminations, as unlike the above reactions, they lead to a mixture of the product enones. That the 4-en-3-one was not derived from

the Δ^1 isomer during the reaction has been shown by the stability of the 1-enone under the reaction conditions.[50] The 4-en-3-one must arise directly from the bromo-ketone, this could occur by a 1,4 - elimination from the 3-enol.[61]

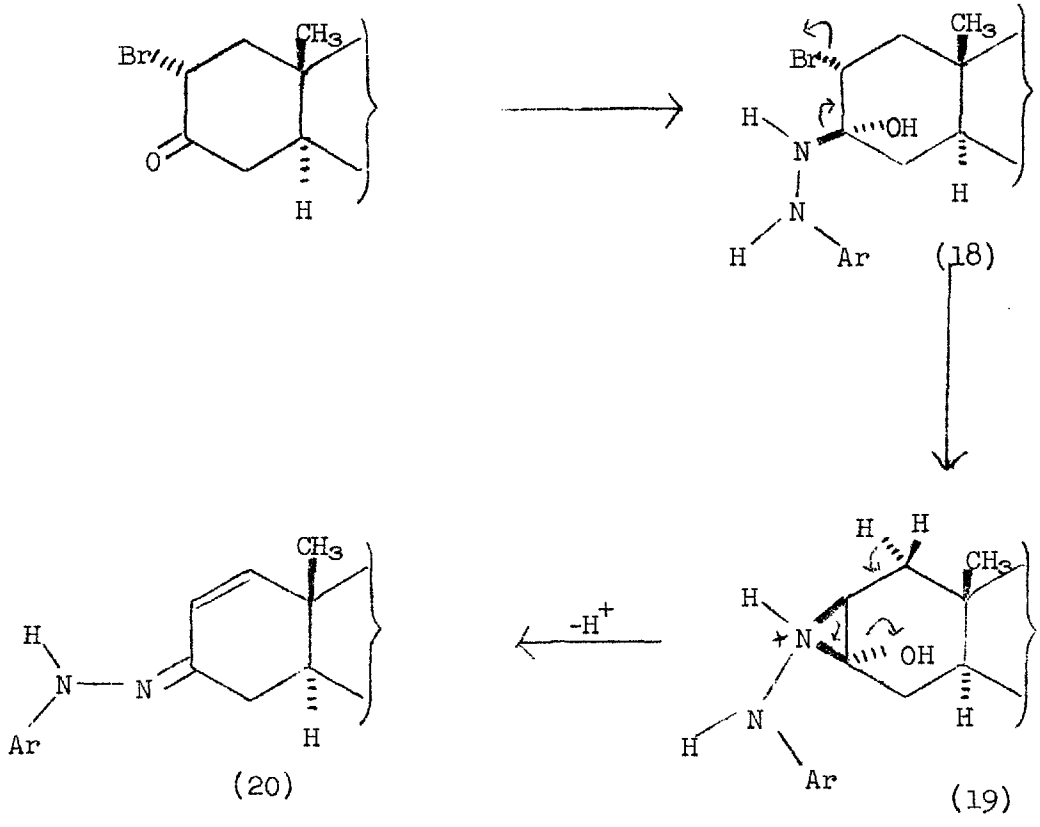


Investigations of the mechanisms of the reactions in aprotic solvents is complicated by the uncertainty in the exact mode of action of the lithium halides. It has been found[36] that magnesium, beryllium and aluminium chlorides, are almost as efficient as lithium chloride in effecting dehydrohalogenation in di-methyl formamide, but that sodium, ammonium or calcium chlorides gave no significant reaction. The same investigations showed that the rate was dependent on the lithium chloride concentration, and that the reaction rate with a lithium chloride catalyst was greater than that with lithium iodide. The importance of the halide anion is evidenced by the fact that, whereas the dehydrobromination of a 2 α, 4 α-dibromo-5 β-cholan-3-one, proceeds with just lithium carbonate in di-methyl formamide, the addition of lithium bromide increases the yield from 42% to 90%.[58] Investigation of the kinetics of the lithium halide catalysed reaction, revealed that the rate depended on the concentration of the bromide ions.[62] This suggests that in the transition state, there is considerable C-H bond cleavage. In contrast, the elimination of hydrogen halides, with lithium halides, indicated transition states

of low C-H fission.[63] It is probable that the removal of the bromine substituent is concerted with proton loss when a *trans* relationship exists, or else is subsequent to it when a *cis* elimination is operative.[61(b)]

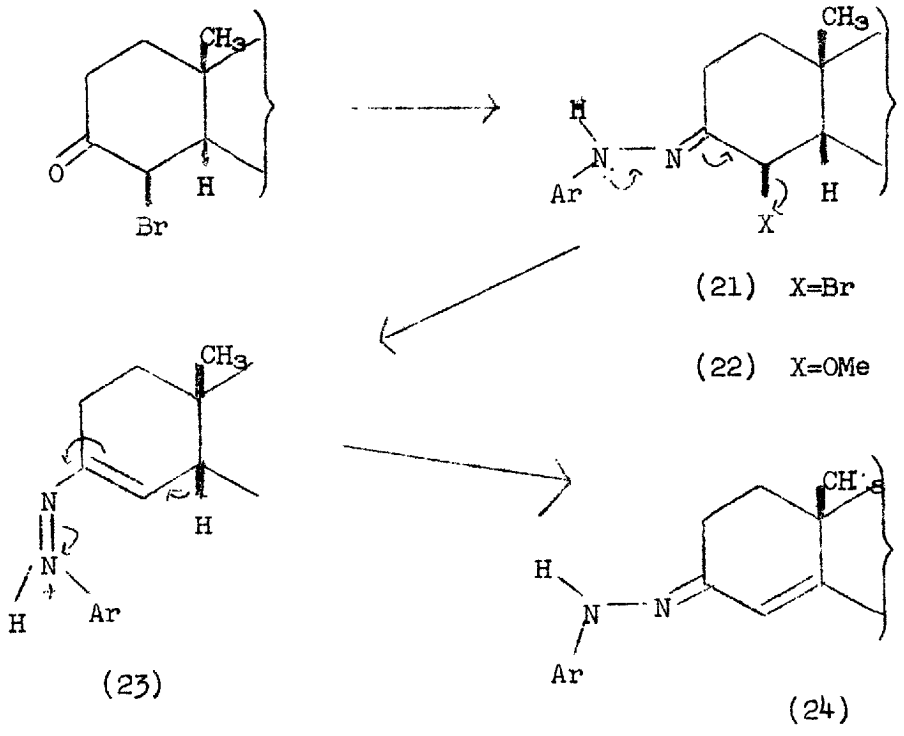
Dehydrobromination effected through Hydrazone Formation

The formation of the di-nitrophenylhydrazone of an α-bromo-ketone in acetic acid, is attended by rapid elimination of hydrogen bromide. By this reaction 4,12-dibromo-5β-cholan-3,11-dione was converted to the Δ^4-3-di-nitrophenylhydrazone in 90% yield.[64] This is paralleled in the 5 α series by the formation, from a 2 α-bromo-3-ketone, in 80% to 90% yield of the 1-en-3-di-nitrophenyl hydrazone, uncontaminated by the Δ^4 isomer.[65] The mechanism originally proposed for this transformation[65] (Scheme 3), involved initial formation of the intermediate (18), followed by neighbouring group displacement of the bromine by nitrogen, β to the phenyl group, to form a cyclic immonium intermediate(19). Elimination of water and a proton should then yield the product (20).



Scheme 3

A subsequent proposal[66] (Scheme 4) suggested that the α -bromo aryl hydrazone (21) was initially formed, and that the nitrogen α to the phenyl group was the principal electron source in the subsequent displacement, thus assuming a positive charge in the unsaturated azo-intermediate(22). This intermediate could be attacked by a nucleophilic species, e.g. methanol to yield (23), or could suffer proton loss to give the observed product (24).



Scheme 4

This mechanism was supported: (a) by the isolation of a 4-methoxy--di-nitrophenylhydrazone (22), when the reaction was carried out in the presence of methanol; and (b) by the observation that the 2-bromo-arylhydrazone (21), which compound, was prepared by the bromination of the 3-aryl hydrazone in chloroform solution, was stable until introduced into the presence of acetic acid, when immediate elimination of hydrogen bromide occurred.[66] The intermediacy of (21) was proved by its later isolation from the reaction of a 4-bromo-3-ketone with dinitrophenylhydrazine in acetic acid/chloroform at 0°C.[67] The proposed unsaturated azo-intermediate (23) could not be isolated from the above

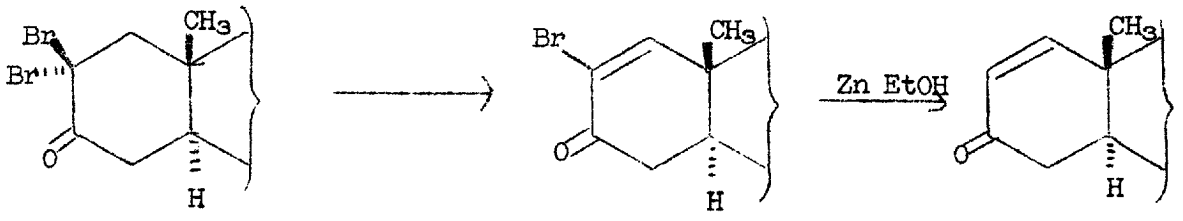
reaction, but has been obtained in the case of the analogous reaction of phenylhydrazine,[68] p-nitrophenylhydrazine, and semicarbazide.[69] The existence of a π bonded intermediate such as (23) explains the observation[60] that for a 2α-bromoketone, the 1 α , i.e. axial, proton is preferentially lost in the reaction with semicarbazide.

The use of semicarbazide, instead of di-nitrophenylhydrazine, to effect dehydrobromination, is advantageous in the ease with which the product ketone can be generated.[69] With two equivalents of pyruvic acid in 70% acetic acid, the yield of enone is quantitative. The original disadvantage inthe use of semicarbazide - the tendency to substitution at C-4 - has been avoided by using solvents which do not have an easily substitutable anion, e.g. in tertiary butanol, the yield of cortisone acetate - 3 - semicarbazone from the 4-bromo-4,5-dihydrocortisone acetate is reported to be 93%.[69]

The above reaction has been found to be of little use in the preparation of dienones.[65] This is because of incomplete dehydrobromination of the 2,4-dibromides, and also because of the difficulty in generating the product dienone from its derivative, in,for example, the conversion of a 2-bromo-4-en-3-ketone to the 4,6-dien-3-di-nitrophenylhydrazone in 46% yield.[65]

Conversion of Dibromides into 1-and 4-en-3-ones

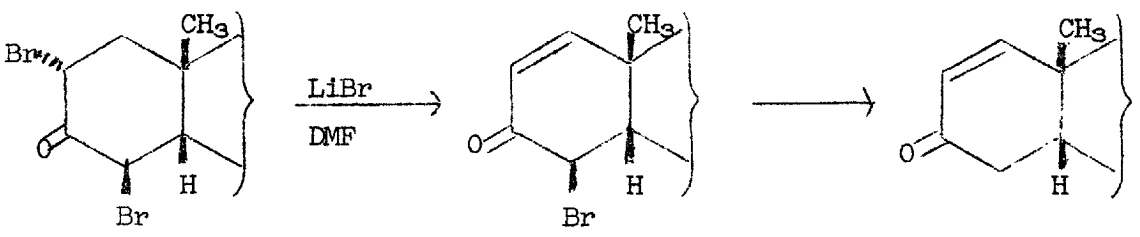
The Mattox Kendall reaction (discussed above) has been used to debrominate 2,2 dibromo-3-ketones[65] (25) to 2-bromo-1-en-3-ones (26) in 60% yield. A similar yield was obtained when the reaction was carried out with collidine.[70]



(25) (26)

Debromination of (26) with zinc and ethanol produced the 1-en-3-one in 89% yield.[38a]

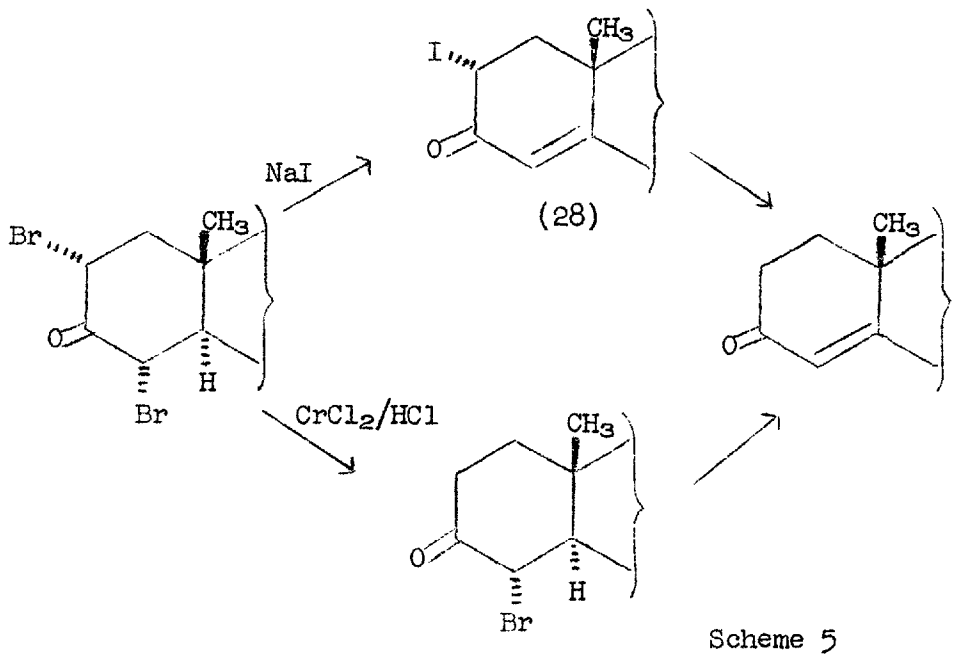
Analogues of this compound in the 5 β series have been obtained by careful dehydrohalogenation of the 2 α , 4 β dibromo-3-ketone, with lithium bromide in di-methyl formamide, under thermally less vigorous conditions than are generally used, to give in 70% yield the 4 β -bromo-1-en-3-one (27) from which the 1-en-3-one obtained on treatment with zinc and acetic acid.[71]



(27)

The commercially more attractive 4-en-3-ones were first produced via the 2 α-bromo-4-en-3-ones, obtained by partial dehydrobromination with collidine, of the 2 α,4 α dibromo ketones in the 5 α series[72] in 39% yield,[38(a)] with subsequent debromination with zinc-ethanol (90% yield).

A superior method of formation was based on the findings[73] that: (a) 2 α-iodo-3-ketones are dehydroiodinated in 70% yield in collidine; and (b) treatment of a 2 α,4 α-dibromo ketone with sodium iodide in acetone, leads to the 2 α-iodo-4-en-3-one (28). This can be converted to the desired product in a yield of 60% based on the starting dibromide. This reaction is used widely on substrates with stable side chains and which lack a C-11 substituent. It is less efficient however, in the preparation of adrenal hormone analogues.[74] Higher yields are obtained by carefully controlled reduction of a 2 α,4 α dibromo-3-ketone with chromous or titanous salts in hydrochloric acid, to the 4 α bromo-3-ketone[75] (29) (92% yield). This can be converted efficiently into the 4-en-3-one by the Mattox Kendall procedure.

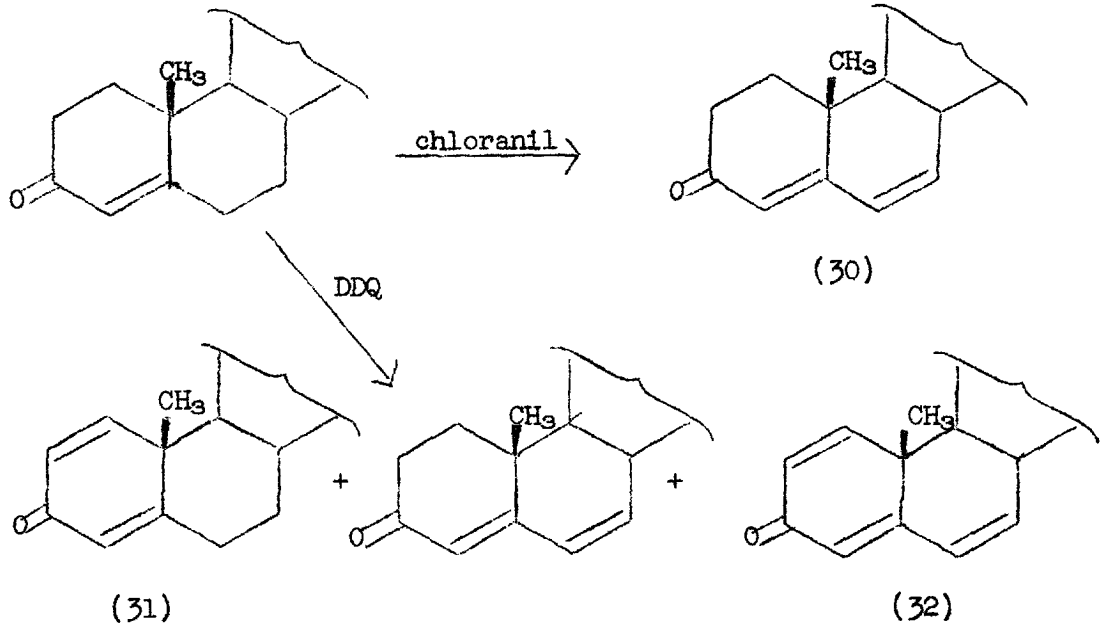


Scheme 5

Recently a promising method of dehydrobromination was introduced.[76] The heating of an α bromo-ketone in hexamethyl phosphotriamide leads to an α, β unsaturated ketone without concurrent formation of the α', β' unsaturated isomer. Thus, 2 α-bromo-cholestan-3-one yielded 75% of 1-en-3-one. In the lanostane series yields of 90% were obtained.

Dehydrogenations of Steroid Ketones with Dichlorodicyanobenzoquinone (D.D.Q.)

The dehydrogenation of 3-keto-steroids with hydride abstractors was first demonstrated by the conversion of hydrocortisone acetate, and other steroidal 4-en-3-ones, to a 4,6-dien-3-one (30) in 66% yield with chloranil.[77] Later it was reported[78] that the reaction of 4-en-3-ones with 2-3-dichloro-5,6-dicyano-benzoquinone (D.D.Q.) gives the isomeric 1,4-dien-3-ones (31). Yields for this latter reaction are high, e.g. testosterone was dehydrogenated to the 1,2 dehydro analogue with 70% conversion.[79]



Scheme 6

The byproducts formed consist of the 4,6 dien-3-one (30) and the 1,4,6-trien-3-one (32). The trienone has been shown to be derived from the 4,6-dien-3-one, and not from the 1,4-isomer, which is inert to the quinone in refluxing benzene.[78]

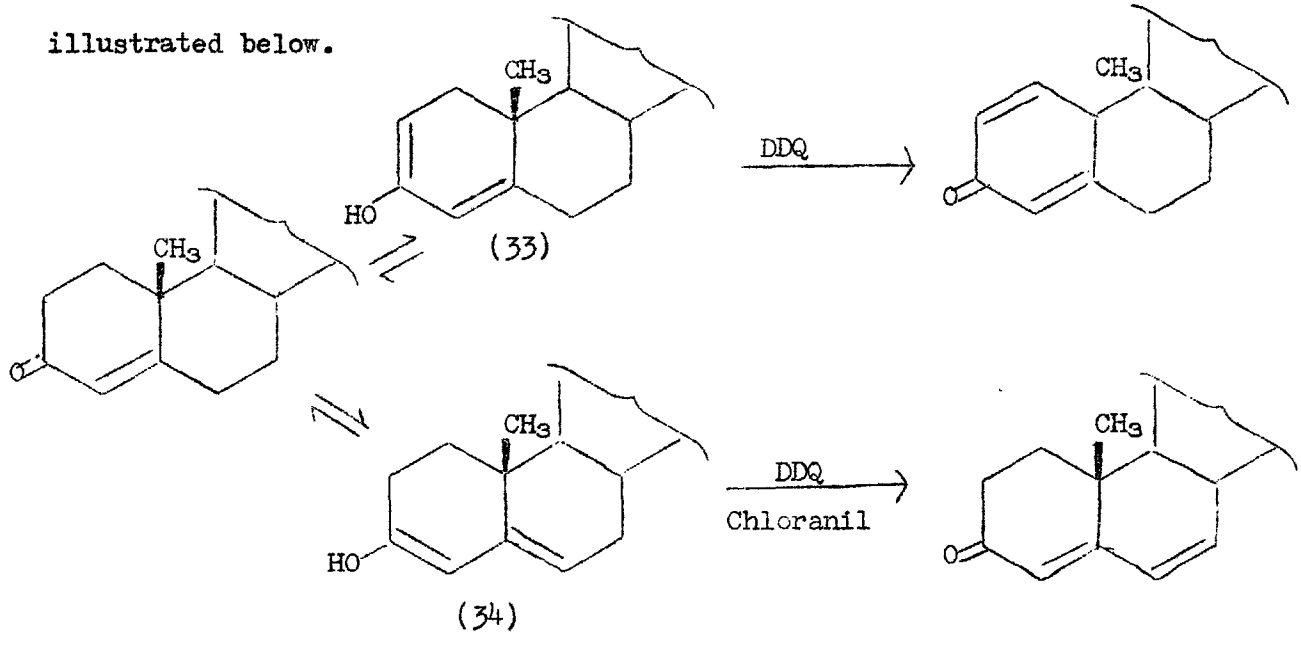
The D.D.Q. dehydrogenation was more specific in benzene than in dioxan.[80] * The optimum ratio of (31) to (30) formed is 11:1 in

*Footnote:- The results quoted here, unless referenced otherwise, are all taken from reference 80. In many cases they derive from more careful examination of work originally published by the same group. References to the original publications are cited in reference 81.

the former and 5:1 in the latter solvent.[26]

The presence of hydrated toluene-p-sulphonic acid accelerated the reaction in dioxan, but markedly increased the proportion of 4,6-dien-3-oneproduced. Under these conditions, both the 1,4- and 4,6-dien-3-ones were further dehydrogenated to the 1,4,6-trien-3-one. With anhydrous toluene-p-sulphonic acid or anhydrous hydrogen chloride as catalyst, very rapid, exclusive.(98%) formation of the 4,6-dienone was observed. In benzene,weak acids accelerated the D.D.Q. reaction without drastically altering the product ratios, with the exception of benzoic acid, which rendered the 1,2-dehydrogenation more pronounced,- the ratio of 1,4- to 4,6-isomers was 12:1. The effect of the weak acids was almost negligiblein dioxan.

The rate enhancement observed with acid implies that the rate determining step in the reaction is slow enolisation, followed by rapid abstraction of hydride from the intermediate enol. This is supported by the observation that preformed enol ethers react rapidly with D.D.Q. at room temperature.[82] It was noticed that the reaction rate was dependent on the quinone concentration in the uncatalysed reaction,but independent of it in the presence of benzoic acid, implying that the quinone itself can catalyse the enolisation. The most commonly accepted mechanism is illustrated below.



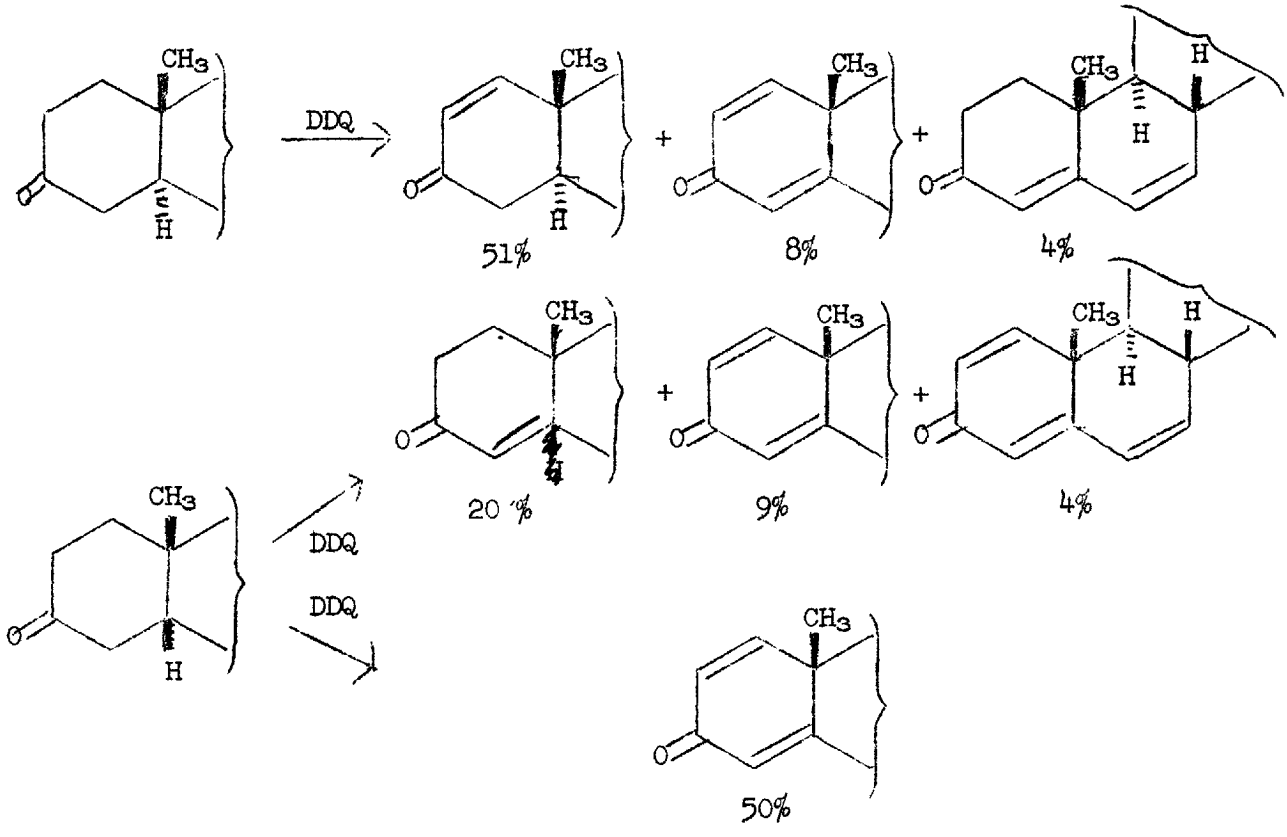
The 4-en-3-one gives rise to two possible enols (33), (34). In the absence of acid, the kinetically determined enol (33) undergoes a C-1, hydride abstraction to give (31). Chloranil lacks sufficient redox potential to abstract a hydride from this enol, and so the 2,4-dienol isomerises to the more stable 3,5-dienol which undergoes hydride abstraction at C-7 to furnish (30). In the presence of weak acids, enolisation though accelerated, is still the slow step, and hydride abstraction is still faster than enol equilibration. Under the influence of hydrogen chloride and other strong acids, enolisation is dramatically accelerated, and in the absence of water, the 3,5-dienol becomes kinetically as well as thermodynamically, favoured.

It is worthy of note that when the proportion of 2,4-dienol in the equilibrium mixture is increased by a neighbouring, or distant, substituent, e.g. a 9(11) double bond[23], the yield of 1,4-dien-3-one will be increased. Thus 17 α -methyl- $\Delta^{9(11)}$ -testosterone on reaction with D.D.Q. in dioxan yielded 84% of 1,4-dehydro-testosterone.[83]

Δ^{1} -Steroidal-3-ketones were more resistant to attack by the quinone and required strong acid catalysis to effect the reaction at a reasonable rate.

Reactions of 5 α -and 5 β -3-oxo-steroids with D.D.Q. follow both possible directions of enolisation. The most specific conditions for 1,2-dehydrogenation of 5 α -androstane-3,17-dione were in refluxing anhydrous dioxan in which the yield was 51%. The reaction of 5 α -3-ketones was accelerated by acids but the product ratios were not significantly altered. It appears that the yields are reduced by the Michael addition of the product hydroquinone to the product α , β unsaturated ketone. In the 5 β series, use of benzene as solvent leads to preferential 4,5-dehydrogenation, but this is not sufficiently pronounced to be of synthetic use. The conversion of 5 β -3-ones to

1,4 dienones is of more utility however, and has been accomplished in 50% yield.[84]



The lack of reactivity of 1 α -methyl-5 α -androstan-3-one, in contrast to the lability of the 1 β -methyl isomer suggests that 1,2 dehydrogenation involves trans diaxial elimination with enolisation occurring by loss of the 2 β proton, and hydride abstraction following from the 1 α position. This was confirmed by deuteration studies which showed that a 2 β -deuterio-4-en-3-one lost 56% of its deuterium despite a substantial kinetic isotope effect. The deuterated material reacted more slowly than the unsubstituted ketone and isotope effects of 2.3. and 2.6 were found. A similar isotope effect was observed in benzoic acid catalysed reactions.

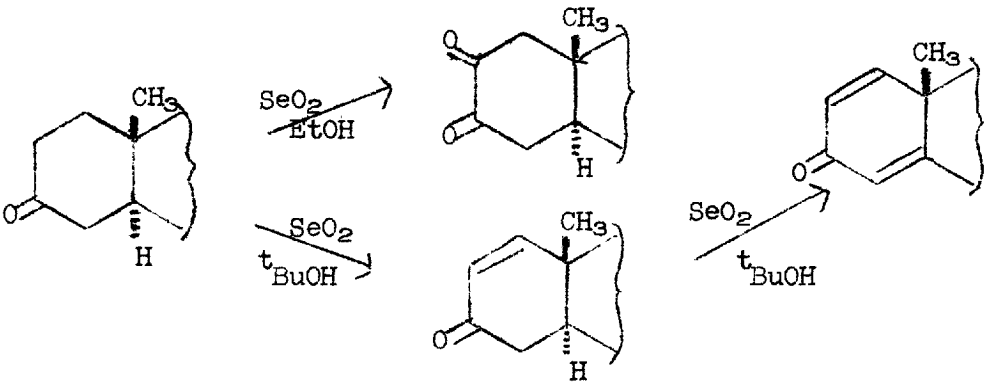
When the same deuterated ketone was dehydrogenated by D.D.Q. in the presence of toluene-p-sulphonic acid, the resulting 6,7-dehydro-compound showed only a small loss of isotope, thereby demonstrating

that the 3,5-dienol is formed directly, and not by equilibration of the 2,4-dienol.

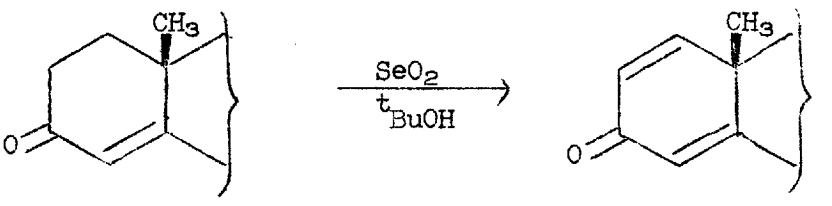
In experiments with 1 α -deuterated substrates, dehydrogenation involved 68% of deuterium loss. This result reinforces the previous indication of 1α proton loss from tritium labelled ketones.[85]

Selenium Dioxide Dehydrogenations

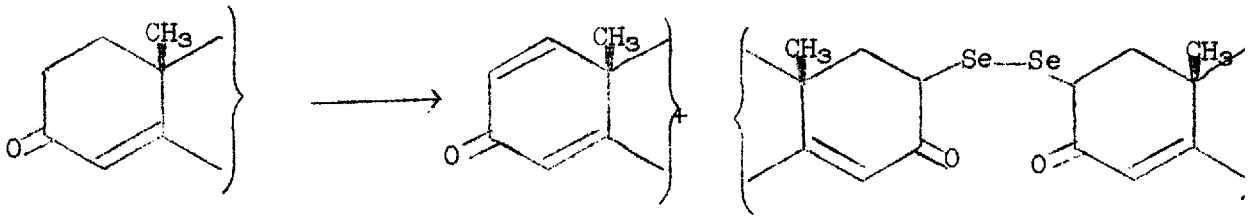
The extensive use of D.D.Q. as a dehydrogenating agent has largely diminished interest in the similar action of selenium dioxide.[86] Whereas in a primary alcoholic solvent, selenium dioxide oxidises cholestan-3-one to cholesta-2,3-dione,[87] in a tertiary alcohol, dehydrogenation ensues. Thus with this system, dihydro-cortisone acetate yields the 1-en-3-one, which can further react to provide the 1,4-dien-3-one(in unspecified yield).[88]



The direction of attack on a saturated ketone is determined by the direction of enolisation, as in the 5 β series, the first double bond is introduced in the 4,5 position.[88] The product 4-en-3-one also reacts readily with the oxide. Thus 1,2-dehydro-oortisone acetate is obtained in 50-60% yield. Yields of up to 80% oan be obtained in this type of conversion under optimum conditions,[89] but appear to be very dependent on substituents, e.g. 1,2-dehydrocortisol-21-acetate is obtained in 78% yield, whereas 1,2-dehydro-cortisone is only produced in 36% yield.



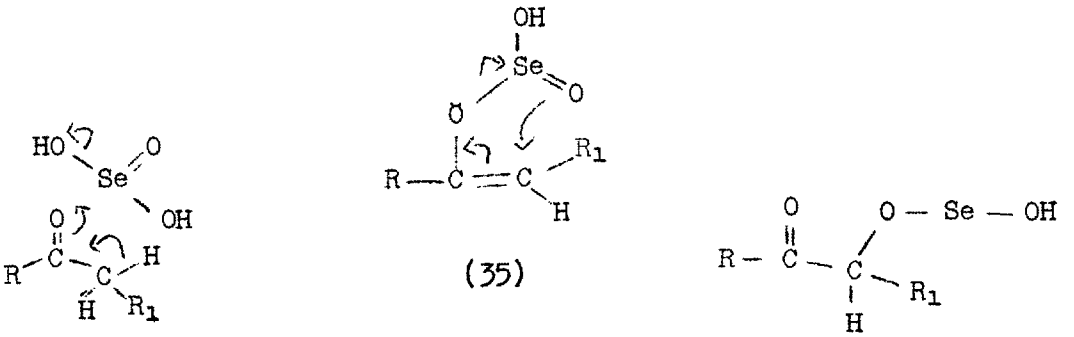
The generally inferior yields of selenium dioxide reactions, relative to D.D.Q. ones derive from the greater tendency of the former to indulge in side reactions. A substantial proportion of product loss is caused by entry of the selenium into the steroid nucleus. In the dehydrogenation of testosterone, as well as 53% of the desired product, there was 24% formation of a disteroidal diselenide.[90]



As in the D.D.Q. reaction, saturated 3-ketones yield a mixture of isomeric enones with selenium dioxide. In the treatment of 5α -androstan-3,17-dione, yields of the 1-en-3-one, 4-en-3-one and 1,4-dien-3-one were 13.3%, 8.1% and 5.4%.[91] The demonstration that the Δ^4 compound arose from a side reaction and not by isomerisation of the expected Δ^1 product was achieved by carrying out the reaction with a 1α -deuterio-ketone. In the Δ^1 product, there was 93% removal of the deuterium, and in the Δ^4 isomer there was 84% retention.

A kinetic study of the dehydrogenation of cortisone acetate showed that the reaction was first order in both the ketone and the dioxide.[92] This parallels the earlier observation that in the oxidation of desoxybenzoin to the α -diketone, the rate depended on the concentration of ketone, oxide and acid.[93] A primary isotope effect of 6 was noticed, and Hammett constants of ρ (ArCO) = - 0.56 and ρ ($ArCH_2$) = + 0.25 were recorded. This data led to the postulate of electrophilic attack of protonated selenic acid on the carbonyl oxygen as the first step, in a

process akin to enolisation, to furnish the enol selenite (35) which could then rearrange to give (36).

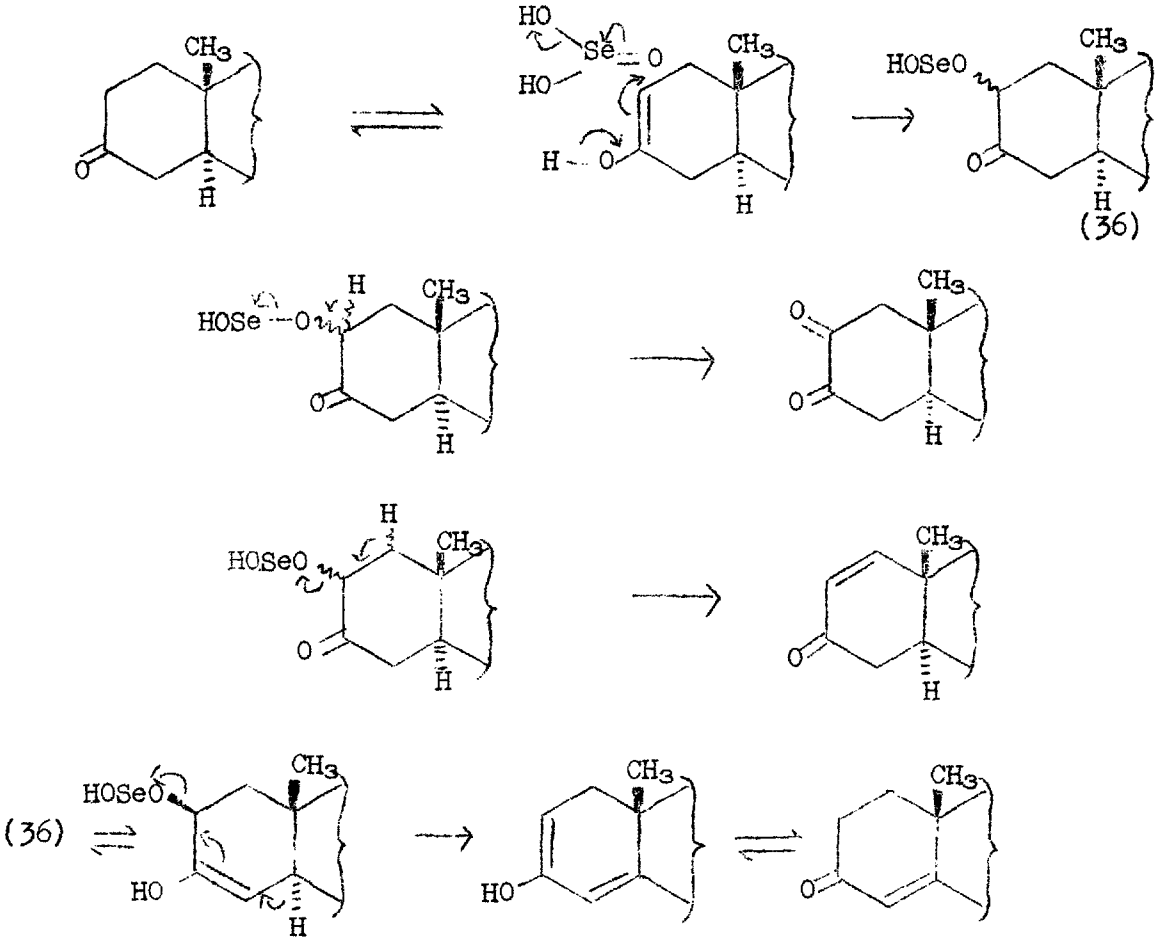


(35)

(36)

Scheme 8

An alternative proposal[94] is that (36) is formed directly by attack of selenous acid on the enol (Scheme 9). Decomposition can proceed by loss of the α proton leading to the α-diketone, or by loss of the β-proton to give the α,β-unsaturated ketone.

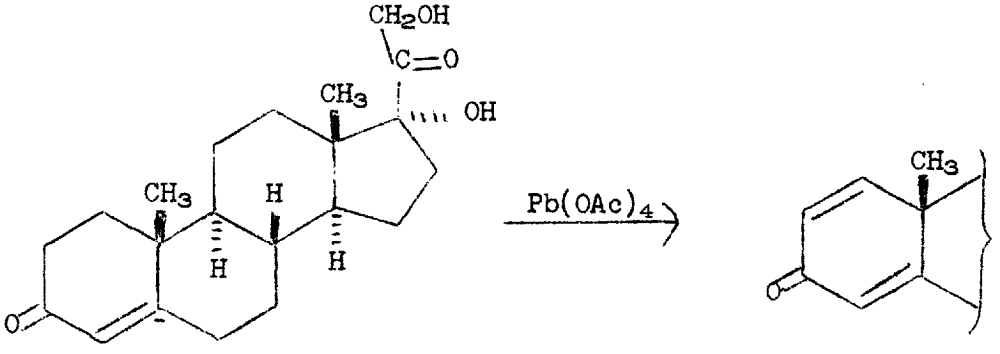


Scheme 9

The role of the solvent in determining the reaction course has been explained as arising from the increased bulk of the selenous acid due to solvation in the primary alcohol, favouring α attack.[95] This leads to equatorial substitution with its consequent resistance to α, β elimination. If the enolisation is not the rate determining step, then the 4-en-3-one may arise by a 1,4 elimination from the 3-en-3-ol analogous to that proposed for bromine elimination.[61(a)]

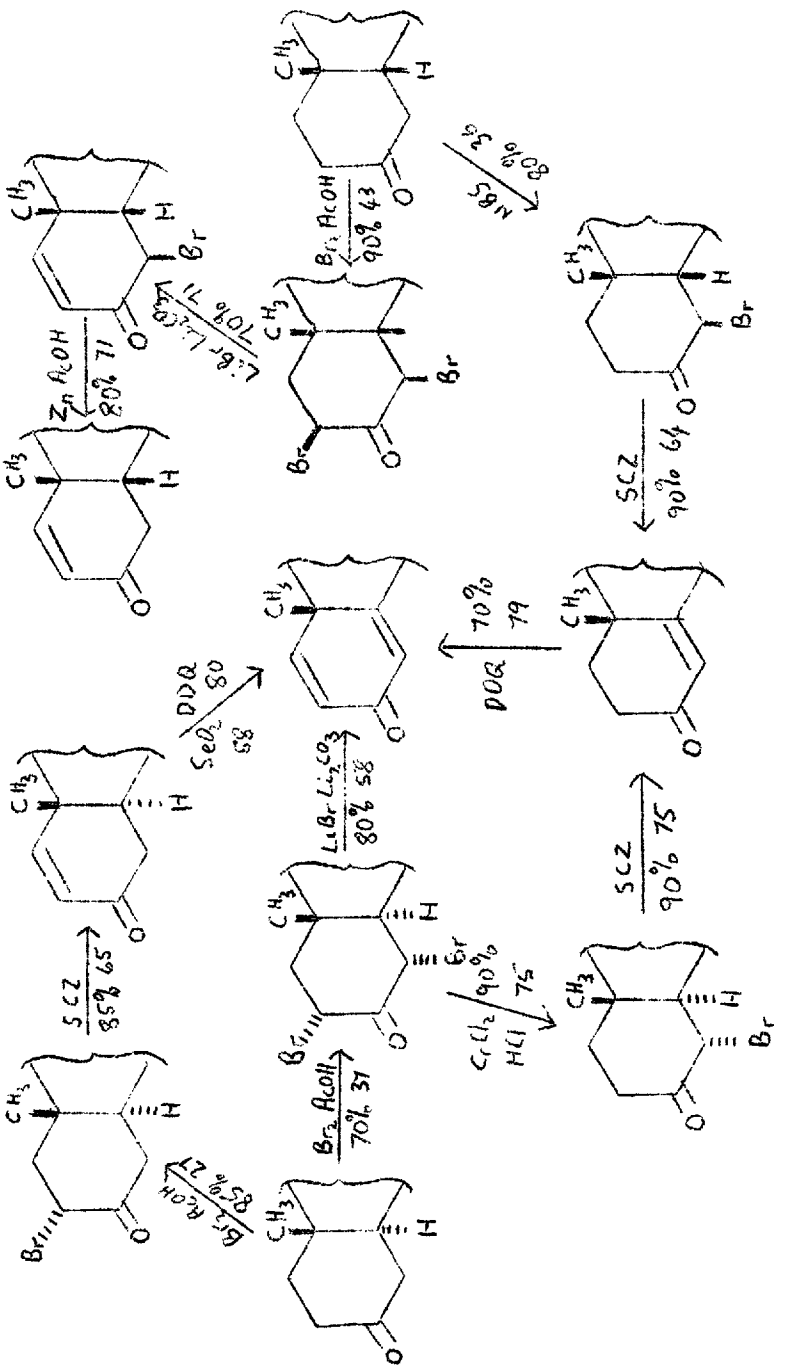
Lead Tetra-acetoate Dehydrogenations

In an investigation of the reaction of lead tetra-acetate with progesterone,[96] the formation of 21-acetoxy-1,4-pregnadien-3,20-dione in 8% yield was noted. A similar yield was obtained with desoxy-corticosterone acetate. With cortisol-21-acetate, the yield of 1,4-dien-3-one was 10%. With an extended reaction time this product was consumed. No subsequent attempts to have been made to improve the yield of this reaction.



Conclusion

Scheme 10 summarises the most efficient methods of transformation reviewed here. In general terms, when the starting material is a saturated 3-ketone, the bromination-dehydrobromination sequence is best for introducing one or two double bonds. The Mattox-Kendall procedure is most efficient for mono-dehydrobromination, and the lithium bromide, di-methyl formamide system is superior for the elimination of two molecules of hydrogen bromide. The formation of 4-en-3-one and 1,4-dien-3-one systems is most efficient when the starting material is in the 5 β series. The introduction of a 1,2-double bond into a 4-en-3-one is best accomplished with D.D.Q. or exceptionally selenium dioxide.



Scheme 10

The numbers beside the percentage yield figures denote the references detailing the reaction conditions.

References

1(a) E.B. Hershberg, M. Rubin and E. Schwenk, J.Org.Chem., 1950, 15, 292

1(b) H.L. Dryden, jun., G.M. Webber, and J.J. Wieczorek, J.Amer.Chem.Soc., 1964, 86, 742

2 W.F. Van der Waard, J. Doodeward, J. De Flines and S. Van der Weele, Chemical Abstracts, 1969, 71, 57875j

3. J.C. Gasc and L. Nedelec, Tetrahedron Letters, 1971, 2005

4(a) A. Wettstein, Experimentia, 1947, 3, 185

4(b) J. Von Euw and T. Reichstein, Helv.Chim.Acta, 1946, 29, 1919

5 C. Djerassi, Organic Reactions, 1957, 6, 207

6 L.V. Oppenauer, Rec.Trav.Chim., 1937, 56, 137

7 G. Rosenkranz, J. Patakai and C. Djerassi, J.Org.Chem., 1954, 17, 290

8 J. Von Euw, A. Lardon and T. Reichstein, Helv.Chim.Acta., 1944, 27, 821

9 V. Schindler, H. Frey and T. Reichstein, Helv.Chim.Acta., 1941, 22, 360.

10 O. Halpern, I. Delfin, L. Magana and A. Bowers, J.Org.Chem., 1966, 31, 693

11 A.J. Birch, J.Chem.Soc., 1944, 430

12 A.L. Wilds and N.A. Nelson, J.Amer.Chem.Soc., 1953, 75, 5366

13 D.N. Kirk and M.P. Hartshorn, Steroid Reaction Mechanisms, Elsevier, Amsterdam, 1968, pp 154-163

14 W.G.Dauben, R.A. Mitchell and J.F. Eastham, J.Amer.Chem.Soc., 1952, 74, 3852

15 A.S. Dreiding, Chem.Ind., 1954, 1419

16 A.J. Liston, J.Org.Chem., 1966, 31, 2105

17 R.B. Turner, W.R. Meador and A.E. Winkler, J.Amer.Chem.Soc., 1957, 79, 4122

18 E.J. Corey and R.A. Sneen, J.Amer.Chem.Soc., 1955, 77, 2505

19 B. Kerkoz, E.P. Chavez and C. Djerassi, J.Chem.Soc., 1962, 1323

20 A.J. Liston and M. Howarth, J.Org.Chem., 1967, 32, 1034

21 A.J. Liston and P. Toft, Chem.Comm., 1970, 111

22 idem. J.Org.Chem., 1969, 34, 2288

23 idem. Tetrahedron, 1971, 27, 969

24 S.K. Malhotra and H.J. Ringold, J.Amer.Chem.Soc., 1964, 86, 1997

25 G.C. Swain and A.S. Rosenberg, J.Amer.Chem.Soc., 1961, 83, 2154

26 G. Subrahmangan, S.K. Malhotra and H.S. Ringold, ibid 1966, 88

27 A. Butenandt and A. Wolff, Chem.Ber., 1935, 68, 2091

28 E.J. Corey, J.Amer.Chem.Soc., 1954, 76, 175

29 R. Villotti, H.J. Ringold and C. Djerassi, ibid, 1960, 82, 5693

30(a) J. Valls and E. Toromonoff, Bull.Soc.Chim.France., 1961, 758

30(b) Reference 13, pp 163-168.

31 R.A. Jerassi, J.Org.Chem., 1965, 30, 1650

32 C. Djerassi, Steroid Reactions, Holden Day, San Francisco, 1963, pp. 181-191

33 C. Djerassi and C.R. Scholz, J.Amer.Chem.Soc., 1948, 70, 417

34 idem, Experimentia, 1947, 3, 107

35 V.R. Mattox and E.C. Kendall, J.Biol.Chem., 1950, 185, 593

36 R.P. Holysz, J.Amer.Chem.Soc., 1953, 75, 4432

37 L. Ruzicka, W. Bosshard, W.H. Fischer and W. Wirz, Helv.Chim. Acta., 1936, 19, 1147

38(a) C. Djerassi and C.R. Scholz, J.Amer.Chem.Soc., 1947, 69, 2404

38(b) C. Doree, J.Chem.Soc., 1909, 648

39 E.W. Warnhoff, J.Org.Chem., 1963, 28, 887

40 A. Ellis and V. Petrow, J.Chem.Soc., 1953, 3869

41 H.H. Inhoffen and G. Zaelsdorff, Chem.Ber., 1943, 76, 233

42 C.W.P. Crowe, R.M. Evans, G.F.H. Green and A.G. Long., J.Chem.Soc., 1956, 4351

43 C. Djerassi and G. Rosenkranz, Experimentia, 1951, 7, 93

44 C. Djerassi, G. Rosenkranz, J. Romo, St. Kaufmann and J. Pataki, J.Amer.Chem.Soc., 1950, 72, 4534

45 D.N. Kirk, D.K. Patel and V. Petrow, J.Chem.Soc., 1956, 627

46(a) K. asuda, Chem.and Pharm.Bull.(Japan) 1964, 12, 1217

46(b) G.R.Allen Jnr. and M.J. Weiss, J.Amer.Chem.Soc., 1960, 82, 2840

47 L. Ruzicka, P.A. Plattner and R. Aeschbocher, Helv.Chim.Acta, 1938, 21, 866

48 H.H.Inhoffen, G. Zuhlsdorff and Huang-Minlon, Chem.Ber., 1940, 73, 451

49 G. Frangatos and A. Taurins, Canad.J.Chem., 1961, 39, 410

50 E.V.Warnhoff, J.Org.Chem., 1962, 27, 4587

51 E.W.Warnhoff and D.R. Marshall, ibid, 1967, 32, 2000

52 S. Aoyama and K. Sasaki, Chem. and Pharm. Bull.(Japan), 1970, 18, 1310

53 B.J.Magerlein, J.Org.Chem., 1959, 24, 1564

54 J.J. Beereboom and C. Djerassi, ibid, 1954, 19, 1196

55 G.F.H. Green and A.G. Long, J.Chem.Soc., 1961, 2532

56 A.L. Wilds and C. Djerassi, J.Amer.Chem.Soc., 1946, 68, 1712

57 B. Pelc, S. Hermanek and J. Holubek, Coll. Czech. Chem. Comm., 1961, 26, 1852

58 R. Joly, J. Warnant, G. Romine and D. Bertin, Bull.Soc.Chim. France, 1958, 366.

59 N.L. Wendler, D. Taub and H. Kuo, J.Amer.Chem.Soc., 1960, 82, 5701

60 F.J. Schmitz and W.S. Johnson, Tetrahedron Letters, 1962, 15, 647

61(a) M.E. Kuehne, J.Amer.Chem.Soc., 1961, 83, 1492

61(b) Reference 13, p 109-110

62 V.Delaroff, R. Smolik, M. Bolla and M. Legrand, Bull.Soc.Chim. France, 1963, 966

63 R.A. Bartsch, J.Org.Chem., 1970, 35, 1023

64 V.R. Mattox and E.C. Kendall, J.Amer.Chem.Soc., 1948, 70, 882

65 C. Djerassi, ibid, 1949, 71, 1003

66 V.R. Mattox and E.C. Kendall, ibid, 1950, 72, 2290

67 W.F. McGuckin and E.F. Kendall, ibid, 1952, 74, 3951

68 J. Buckingham and R.D. Guthrie, J.Chem.Soc., 1968, 3079

69 W.F. McGuckin and E.C. Kendall, J.Amer.Chem.Soc., 1952, 74, 5811

70 C. Djerassi and C.R. Scholz, J.Org.Chem., 1948, 13, 697

71 R. Joly and J. Warnant, Bull.Soc.Chim.France, 1958, 367

72 H.H. Inhoffen and G. Zuehlsdorff, Chem.Ber., 1943, 76, 233

73 G. Rosenkranz, O. Mancera, J. Gratica and C. Djerassi, J.Amer.Chem.Soc., 1950, 72, 4077

74 F.Sondheimer, G. Rosenkranz, O. Mancera and C. Djerassi, ibid, 1953, 75, 2601

75 R.M. Evans, J.C. Hamlet, J.S. Hunt, P.G. Jones, A.G. Long, J.F. Oughton, L. Stephenson, T. Walker and B.M. Wilson, J.Chem.Soc., 1956, 4356.

76 R.Hanna, Tetrahedron Letters, 1968, 2105

77 E.J. Agnello and G.D. Laudbach, J.Amer.Chem.Soc., 1960, 82, 4293

78 D. Burn, D.N. Kirk and V. Petrow, Proc.Chem.Soc., 1960, 14

79 J. Vida and M. Gut, J.Med.Chem., 1963, 6, 792.

80 A.B. Turner and H.J. Ringold, J.Chem.Soc.(C)., 1967, 1720

81 D.Walker and J.D. Hilbert, Chem.Rev., 1967, 67, 153

82 S.K.Pradham and H.J. Ringold, J.Org.Chem., 1964, 29, 601

83 W. Brown and A.B. Turner, Chem.Comm., 1968, 561

84 G. Muller, J. Martel and C. Huynh, Bull.Soc.Chim.France, 1961, 2000

85 H.J. Brodie, M. Hayano and M. Gut, J.Amer.Chem.Soc., 1962, 84, 3766

86 Reference 32, pp 233-235

87 E.T. Stiller and C. Rosenheim, J.Chem.Soc., 1938, 353

88 S.A. Szpilfogel, T.A.P. Posthumus, M.S. DeWinter and D.A. Van Dorp, Rec.Trav.Chim., 1956, 75, 475

89 C.H. Meystre, H.Frey, W. Voser and A. Wettstein, Helv. Chim. Acta., 1956, 39, 734

90 J.S. Baran, J.Amer.Chem.Soc., 1958, 80, 1687

91 R.A. Jerassi and D.A. Speyer, J.Org.Chem., 1966, 31, 244

92 G. Langbein, J. Prakt. Chem., 1962, 18, 244

93 E.J. Corey and J.P. Schaefer, J.Amer.Chem.Soc., 1960, 82, 918.

94 W.A. Waters, Mechanism of Oxidation of Organic Compounds, Wiley New York, 1964, p 94.

95 Reference 13, pp 190-192.

96 R.L. Clarke, K. Dobriner, A. Mooradian and C.M. Martini, J.Amer.Chem.Soc., 1955, 77, 661

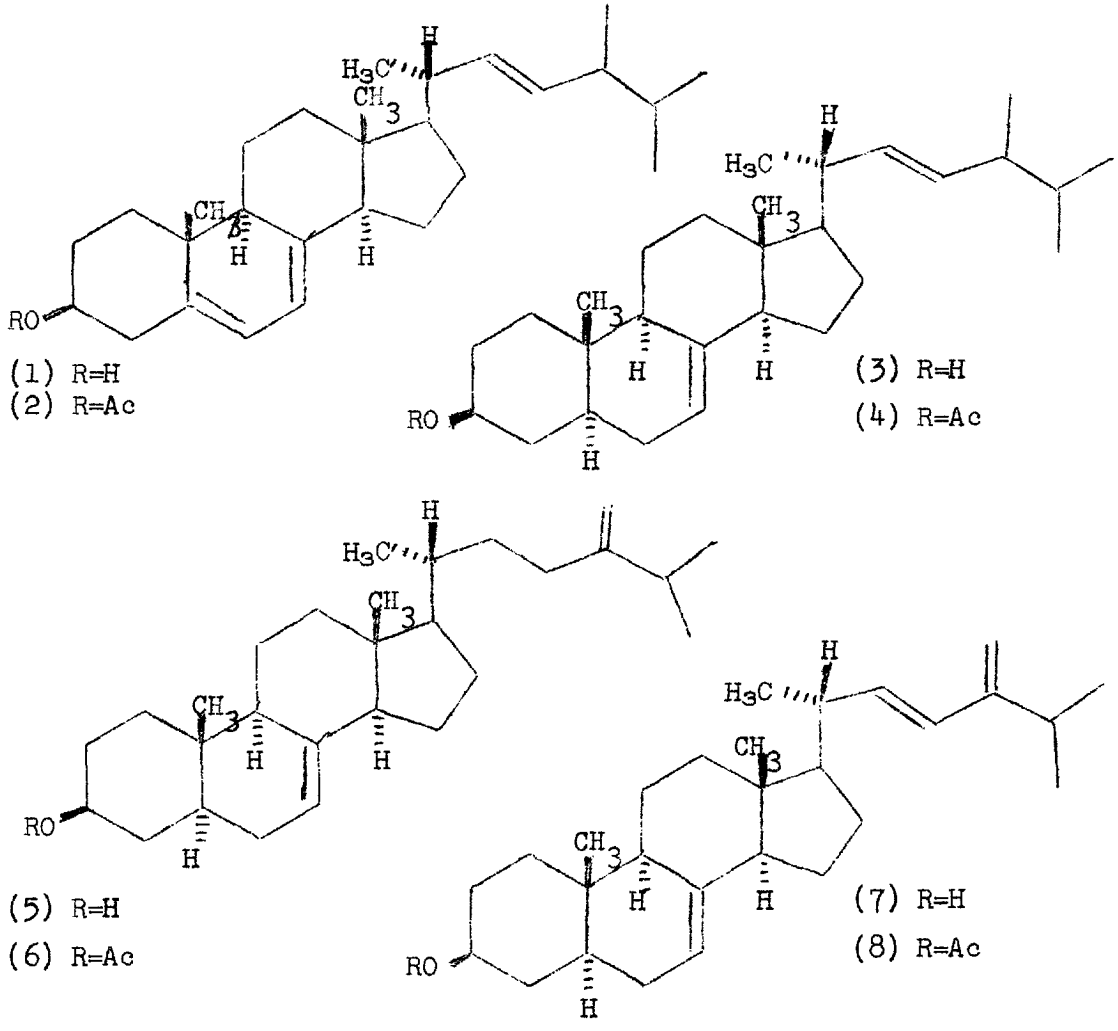
97 S. Burnstein and R.I. Dorfman, ibid, 1955, 77, 4668

RESULTS AND DISCUSSION

PART I

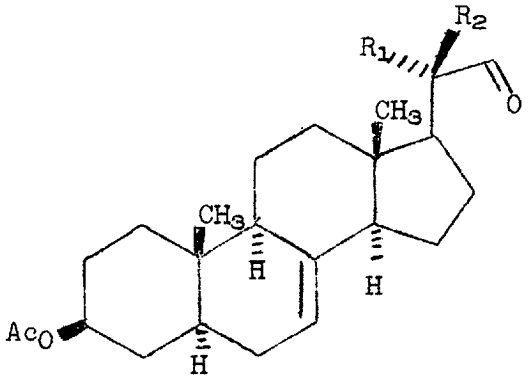
Partial Synthesis of some Δ^7 Steroids

In connection with investigations in this laboratory[1] into the biosynthesis of ergosterol (1) in yeast, it was of interest to determine the role of the Δ^7 sterols: 5α -ergosta-7,22-dien-3β -ol (3), 5α -ergosta-7,24(28)-dien-3β -ol (5) and 5α -ergosta-7,22, 24(28)-trien-3β -ol (7). All three of these sterols have recently been isolated during the course of these studies, as minor companions of ergosterol in the sterol mixture obtained from the cell debris of Saccharomyces cerevisiae[2].



1. Synthesis of 3β-Acetoxy-5α-ergosta-7,22,24(28)-triene(8)

The proposed synthetic route involved hydrogenation of the 5,6 double bond in (2) to give (4), followed by selective ozonolysis of the 22, 23 double bond to provide 3β-acetoxy-23,24-dinor-5α-chol-7-en-22-al (9). Addition of the appropriate side chain by a Wittig reaction could lead to (8).



(9) R_1=H R_2=CH_3

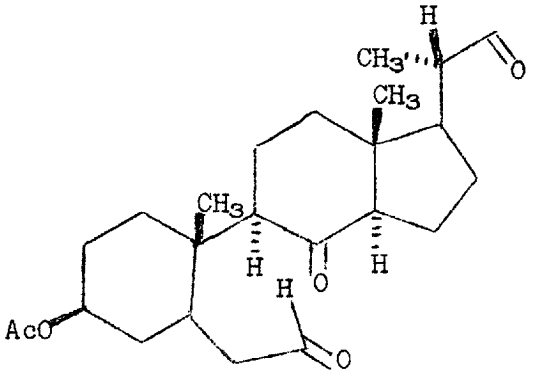
(10) R_1=CH_3 R_2=H

Laubach[3] used W-2 Raney nickel catalyst in dioxan (to hydrogenate the 5,6 double bond in ergosterol), whereas Anderson[4] had success with W-6 catalyst in ethyl acetate.

In this study, the more simply prepared W-2 catalyst was used. The choice of ethyl acetate as solvent rather than dioxan further simplified the preparation of the catalyst system. It was found that a very satisfactory, active catalyst was formed by following the approved procedure,[5] except for the reduction of the number of washings of the catalyst with distilled water from forty to two, before washing with ethanol. The catalyst prepared in this way still probably contained base, but it effected the 5,6 hydrogenation very rapidly, quantitatively and specifically for both the acetate (2) and free sterol (1).

The ozonolysis of the 22,23 double bond of 5-dihydroergosterol was first carried out[6] at low temperature with subsequent decomposition of the product in zinc dust and acetic acid reportedly in 80-85% yield. However, later investigation revealed that a yield of only 40% was obtained in 1% pyridine in dichloromethane - reputed to be the best solvent system for this reaction,[8] at -70°C. Accordingly, this solvent system was employed for the ozonolysis of (4) under different reaction conditions. The reactions were followed by thin layer chromatography (t.l.c.).

When initially investigated at -70°C with a gaseous ozone flow through the solution in 1% pyridine in dichloromethane, there was formation of a product which was more polar than the starting material, and which was developed yellow with dinitrophenylhydrazine spray. After fifteen minutes, a second, much more polar product, appeared. This with the visualising spray showed a deep orange colour. With continuing ozone flow the originally formed product, - which on later isolation proved to be (9), - was consumed and the reaction mixture eventually contained only the later product, presumed to be (11), and material at the origin of the chromatogram.



(11)

Further ozonolyses were conducted at -90°C with varying pyridine concentrations and with varying ozone flow rates. The lowering of the reaction temperature from -70°C to-90°C did not appear to enhance the selectivity of the reaction. Pyridine concentrations of less than 1% by volume led to more rapid formation of (11), relative to (9), than was evident with the original solvent ratio. With no pyridine the reaction became dirty much more quickly and (9) appeared to be consumed as rapidly as it was formed. A slow gas flow rate gave a much more controlled reaction than did a fast flow - indicating the importance of avoiding a high local concentration of ozone.

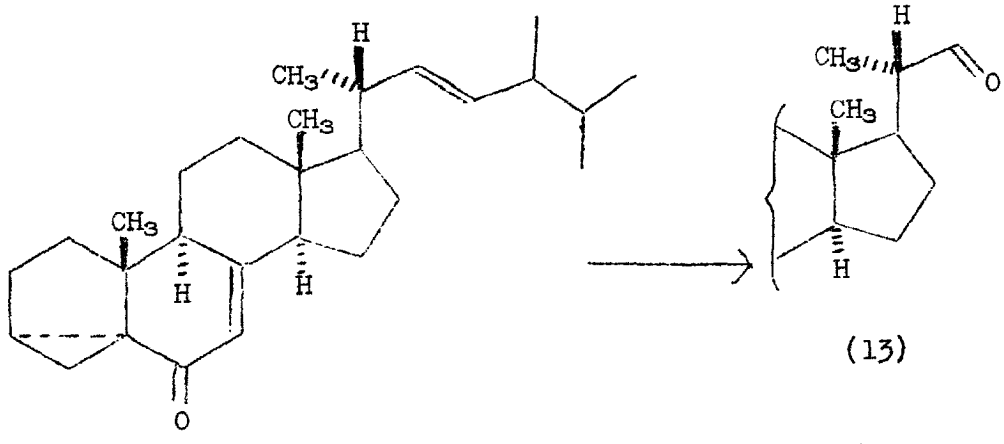
Thus it appears that the presence of pyridine in solution increases the selectivity of attack at the Δ^{22} bond. This may be due to decrease in electrophilicity of the ozone - it has been shown that the reaction of ozone with aldehydes, in which it behaves as an electrophile,[9] is considerably retarded in pyridine.[10] If this is the case, it is difficult to see why the attack on the more electron dense Δ^{7} bond is not favoured. If, however, coordination with pyridine increased the effective bulk of the ozone, then the origin of the selectivity may be the greater steric crowding of the nuclear double bond.

In the preparative experiment, over-reaction due to high local concentration of ozone was avoided by adding the electrophile as a saturated solution in the reaction solvent, at-70°C with rapid stirring. This method was first employed by Jones,[11] but only for small scale experiments. The addition was conveniently monitored by t.l.c. rather than by the usual potassium iodide titration. By use of this method, there was no formation of (11) until after 40 - 50% formation of (9). It was advantageous to stop the reaction at this

stage and work up, because with only starting material (4) and (9) present in the crude reaction product the diene acetate (4) could readily be crystallised out, by the addition of methanol, in 50% recovery. Dilution of the methanol solution with water, yielded the product (9) (40% after recrystallisation).

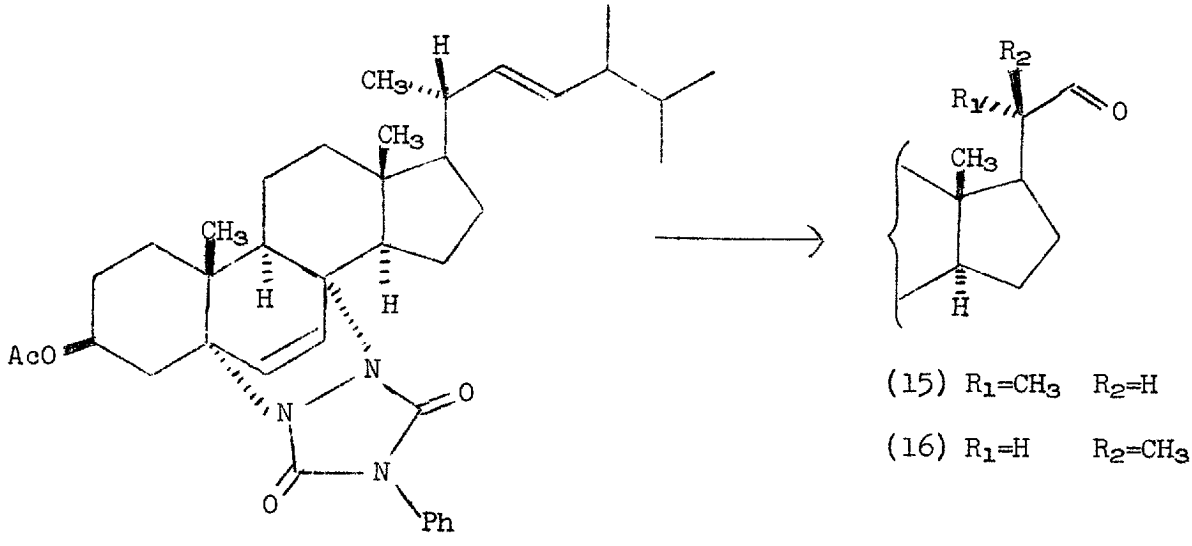
Thus the addition of ozone in solution, although superficially more laborious than the gaseous addition, is the more efficient procedure, as it gives almost complete recovery of unused starting material, as well as a cleaner product, enabling simplification of the work up procedure.

The p.m.r. spectrum of the product aldehyde obtained without a reductive work up, showed a shoulder at 9·45 τ on the C-18 methyl resonance at 9·41 τ. Also the low field aldehyde proton appeared as two doublets in the ratio 1:4. This indicated that 20% epimerisation of the aldehyde at C-20 had occurred.[25] This complication was obviated by the addition of zinc and acetic acid, before the reaction mixture was allowed to warm to room temperature. This treatment was unnecessary in the ozonolysis of (12) which gave (13) without epimerisation under the same reaction conditions.[12]



(12) (13)

In contrast, in the ozonolysis of (14), there was epimerisation of (15) to (16) even with a zinc-acetic acid work up.[1a]



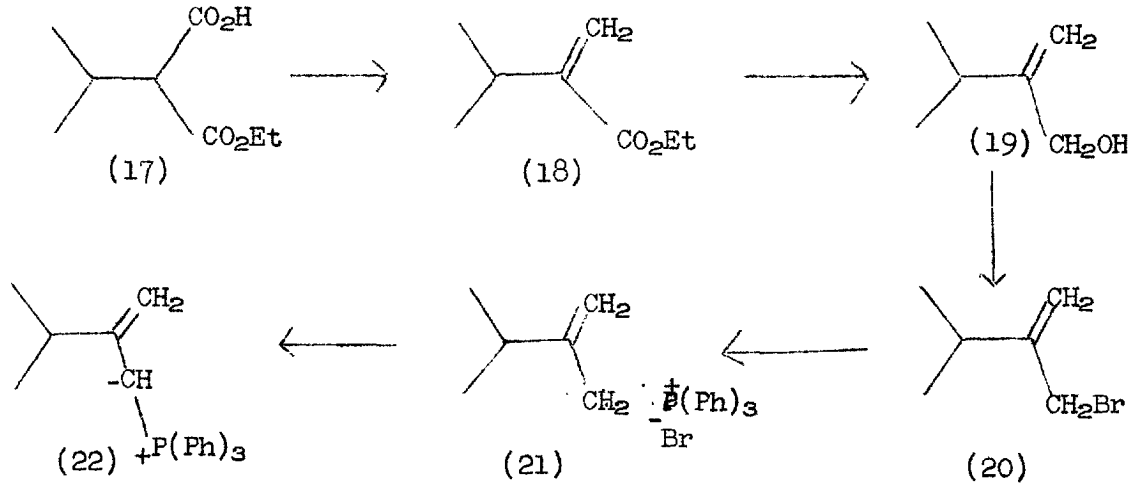
(14)

(15) $R_1=CH_3$ $R_2=H$

(16) $R_1=H$ $R_2=CH_3$

Epimerisation at C-20 in 22-aldehydes reportedly requires potassium t-butoxide.[13] Pyridine has not been reported as catalysing this transformation in any examples other than those above. The different rates of epimerisation with pyridine in compounds (9), (13), (15) may be ascribed to long range effects as yet not understood.

The final step in the synthesis of (8) - the addition of the side chain, was achieved by the Wittig reaction of (9) with the phosphorane (22) derived from 3-methyl-2-methylene-butyl-triphenyl-phosphonium bromide (21). The phosphonium salt was prepared by the following route developed by Dr. T. Shioiri.[14]



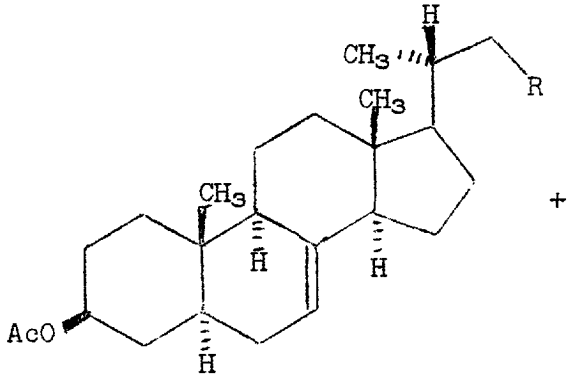
Ethyl 2-isopropyl acrylate[15] (18) was prepared by a Mannich reaction of methyl isopropyl malonate (17) with formalin in diethylamine. Reduction with lithium aluminium hydride and aluminium chloride in diethyl ether,[16] gave 3-methyl-2-methylene butan-1-ol (19) in 64% yield. Treatment of (19) with phosphorus tribromide in diethyl ether, yielded the corresponding bromide (2) (60%) which readily reacted with triphenylphosphine in refluxing benzene to form (21) (95%).

The Wittig reaction[17] of (9) with the phosphorane (22) produced by treating (21) with methyl lithium in tetrahydrofuran proceeded smoothly, to furnish after reacetylation (8) the required sterol acetate in 85% yield. It was necessary to ensure than an excess of methyl lithium was not present, as this caused epimerisation at C-20.

The product (8) was identical in spectral data and melting point to that derived from the natural sterol (7) isolated from yeast.[2]

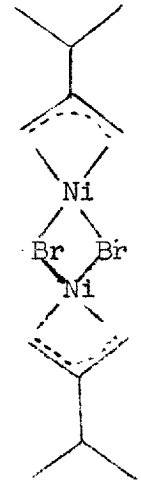
2. Attempted Synthesis of Episterol

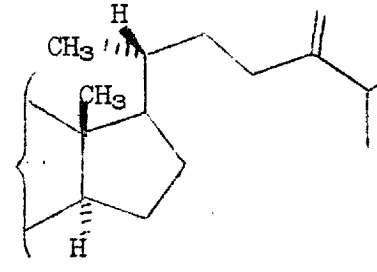
It was decided to introduce the side chain of episterol (7) onto the 7-cholene skeleton by using Corey's coupling reaction[18] of an allyl halide with an alkyl halide. In this proposed reaction, the 22-iodo-23,24-dinor-5 α -chol-7-en-3 β -yl acetate (23) is replaced by the π ligand of the allyl complex, formed between nickel carbonyl and (20).



(23) R=I

(24) R=OH





(6)

23,24-Dinor-5 α -chol-7-en-3,22-diol-3β - acetate (24) was prepared from (9) by reduction with sodium borohydride in ethanol in good yield (87%). The iodination of (24) to give (23) was carried out by using triphenylphosphite and methyl iodide as recommended by Landauer.[19] This gave a rather messy reaction mixture, which was worked up by preparative thick layer chromatography (p.l.c.) to yield 73% of (23). Apparently higher yields in this type of iodination can be obtained by use of the pre-formed triphenoxymethyl - phosphonium iodide in anhydrous tetrahydrofuran.[20]

Model experimental attempts to couple allyl bromide and n-butyl iodide without isolation of the intermediate π allyl complex were unsuccessful. This synthetic route was abandoned as episterol was prepared by another reaction sequence in this laboratory.[14]

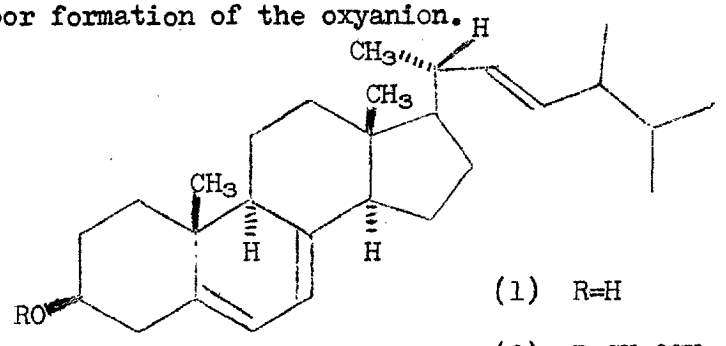
PART II

Protection of the Steroidal 3 β -Hydroxyl Function

Steroidal hydroxyl functions are normally protected as acetates, benzoates, sulphonates or tetrahydro-pyranyl ethers.[21] The ester protecting groups are not stable to alkaline conditions that are often found in synthetic steroid chemistry. Although tetrahydropyranyl ethers are stable to base, their formation from secondary alcohols by the addition of dihydropyran, generally gives rise to a mixture of stereoisomers and also requires acid conditions which can give rise to complications in the sterol nucleus of the ethers.

The methoxymethyl ether protecting group is generally used for the protection of phenolic hydroxy groups,[21] and in fact, its only use in steroid chemistry is on the aromatic ring A of estrane derivatives.[22] It was decided to extend its use to the protection of the 3 steroidal hydroxyl function as a new method of removal under non-acidic conditions was foreseen (vide-infra).

Initial preparative experiments[14] into the reaction of ergosterol (1) with methoxymethyl chloride under a variety of conditions including sodium hydride-tetrahydrofuran, sodium shot-dimethoxyethane, sodium hydride-dimethyl sulphide, gave a complex reaction mixture. The optimum yield of the methoxymethyl ether (2) obtained with methyl lithium in tetrahydrofuran was 36%. The difficulties encountered were ascribed to poor formation of the oxyanion.

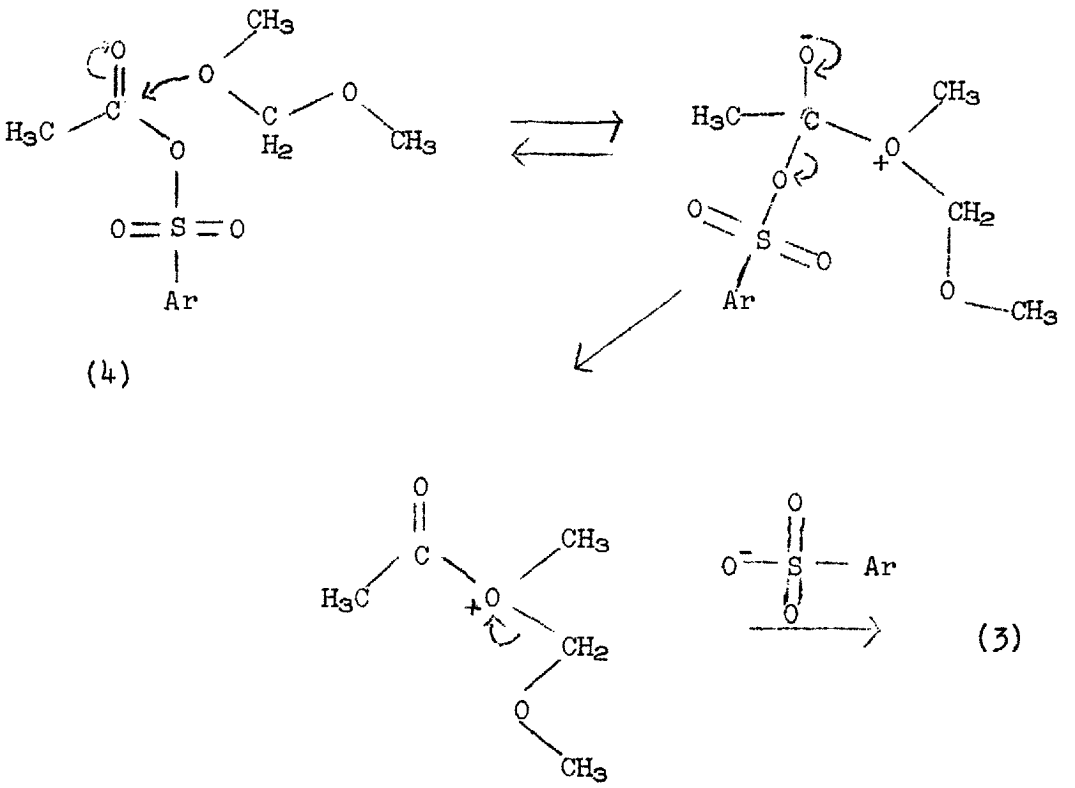


(1) R=H

(2) R=CH_2OCH_3

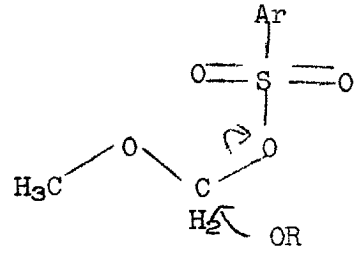
It was hoped that this limitation could be avoided by using the much more reactive methoxymethyl toluene-p-sulphonate (3) to methoxymethylate the sterol, by analogy with the facile reactions of methoxymethyl methane-sulphonate with primary and secondary alcohols.[23]

The very simple preparation of (3) (Scheme I) involved reaction of anhydrous toluene-p-sulphonic acid with acetyl chloride to form acetyl toluene-p-sulphonate (4) (95%). This mixed anhydride reacted very readily[24] with dimethoxymethane to yield (3) in quantitative yield. The toluene-p-sulphonate was used in the expectation that the crystalline intermediates in the preparation would be easily purified. It was found that both (3) and (4) were so sensitive to water vapour that it was best not to isolate them, but to use them immediately after formation without purification. This was found not to affect the yields adversely.

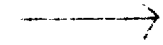


(4) (3)

Scheme 1



(3)



(2)

The reaction of (3) with the sterol (1) was attempted under different conditions:

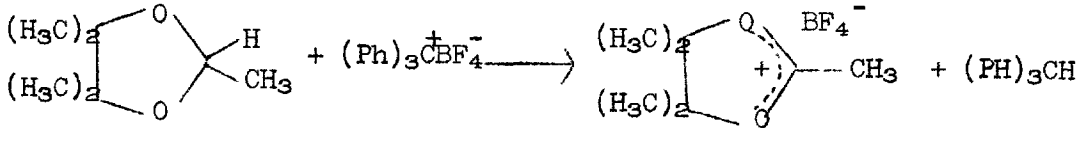
1) With the sodium salt of ergosterol, produced with an excess of sodium hydride in anhydrous ether, under nitrogen at 0°C, the reaction proceeded very rapidly to give the required product (2) in 60% yield. On later repetition this was increased to 80%.
2) With no base at 15°C the reaction was clean at the start but later, after 20% reaction, became messy. With no base at 0°C the slower reaction was about 50% complete when decomposition products appeared.

It seemed evident from the above experiments that the reaction without base was slower than with base, and that the toluene-p-sulphonic acid produced in the reaction was causing the decomposition. An attempt to eliminate this by the addition of sodium carbonate instead of sodium hydride, to remove the acid without affecting the susceptible tosylate, did not improve the reaction significantly.

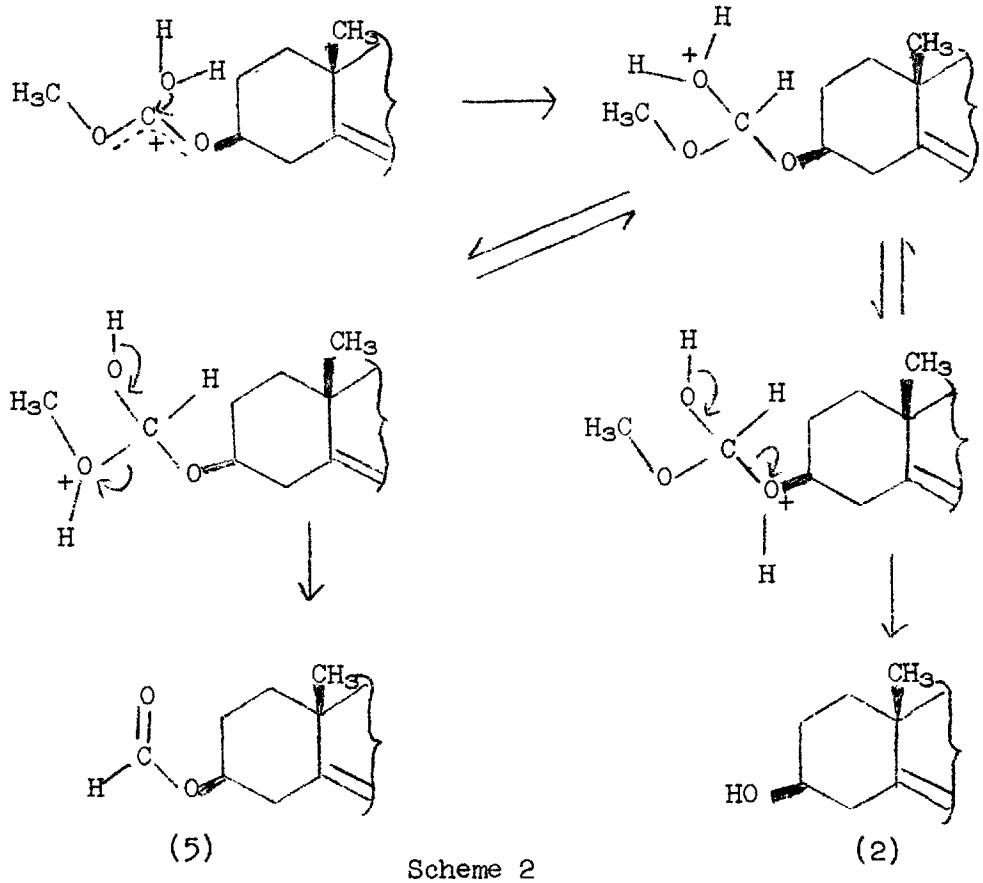
The protecting group was stable to base - the ether (2) was recovered unchanged after 24 hours refluxing in 2% ethanolic

potassium hydroxide. It was also stable to mild acid, being unaffected by acetic acid at room temperature and at 110°C. The addition of toluene-p-sulphonic acid to the acetic acid solution did not cause reaction.

A possible mode of cleavage under neutral conditions was based on the observation that triphenylmethyl cation abstracts a hydride ion readily from a carbon substituted with an ethereal oxygen,[25] and extremely rapidly from a gem-disubstituted carbon, e.g. with 1,3-dioxalans, stable dioxolonium salts have been isolated.[26]



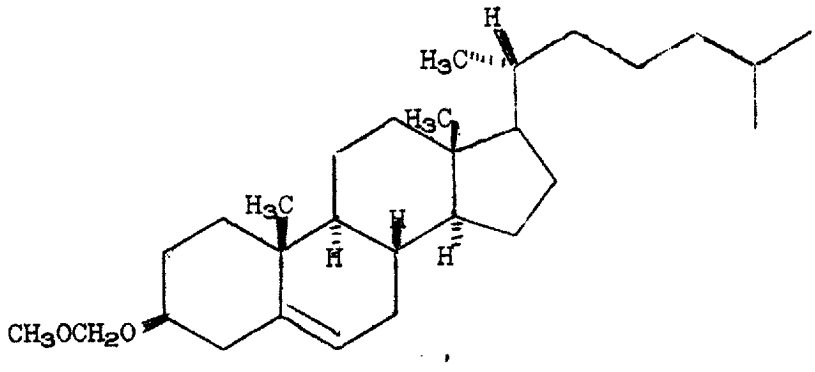
Hydrolysis of an analogous stabilised carbonium ion species derived from (2) (Scheme 2) could proceed in two ways to yield: (a) ergosterol and methyl formate; or (b) ergosteryl formate (5) and methanol. If (5) should be formed, it could readily provide the alcohol (1) on mild base treatment.



(5) (2)

Scheme 2

Treatment of ergosteryl methoxymethyl ether with triphenylmethyl fluoroborate in anhydrous dichloromethane at room temperature, under nitrogen gave a 45% yield of ergosterol. At 20°C, 0°C and -30°C, decomposition became apparent after the reaction was 40% complete. The decomposition appeared to be of the ergosterol, probably due to traces of hydrofluoroboric acid, contaminating the triphenyl-methyl salt, or generated from it by traces of water in the solvent. The starting material (2) was stable to hydrofluoroboric acid at -30°C in contrast to the free sterol which was rapidly attacked. When this reaction was applied to the analogous cholesteryl methoxymethyl ether (6) - prepared by the same method, there was no decomposition, and an 80% yield of product sterol was obtained.[27]



(6)

The utility of the methoxymethyl protecting group for other 3 hydroxysteroids is under further investigation in these laboratories, as is the method of removal described above. If triphenylmethyl fluoroborate can be prepared free from acid, then this method could be of great synthetic use.

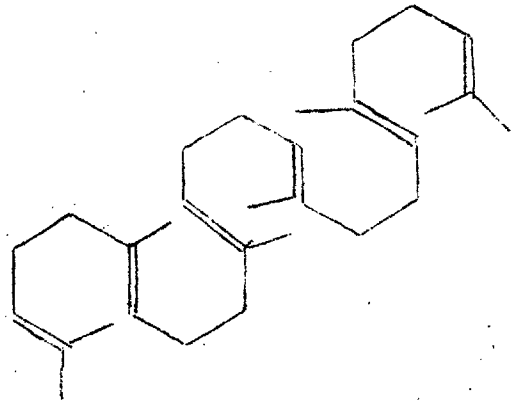
PART III

Attempted Identification of a Triterpenoid Isolated from Yeast

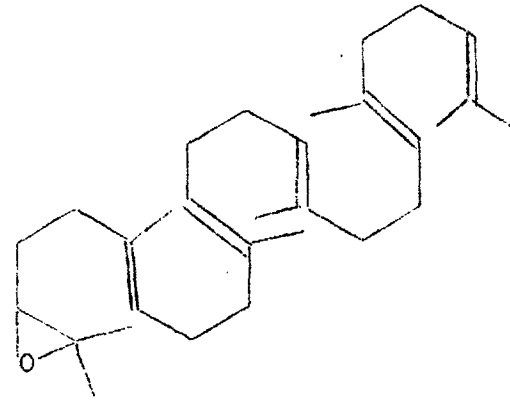
Column chromatography of crude residues from Saccharomyces cerevisiae on deactivated aluminium oxide (Grade 3) gave a separation into two oily fractions and three sterol fractions.[1] The first, least polar, oil was shown to be squalene (1). The composition of the sterol fractions was exhaustively studied by Dr. U.M. Kempe.[2]

The second oil analysed correctly for a molecular formula of $C_{30}H_{50}O$.

It was shown not to be squalene-2,3-oxide(2) which has this molecular composition, by t.l.c. comparison. In 2% ethyl acetate in light petroleum on silica GF, squalene, squalene oxide and the unknown had R_F 0.7, 0.3 and 0.06 respectively.



(1)



(2)

The infra-red spectrum in nujol showed C-H absorptions, C=C absorption and a very weak, broad absorption at 3350 cm^{-1}. In dilute carbon tetrachloride solution this was replaced by a weak, sharp absorption at 3640 cm^{-1},

The ultra-violet spectrum showed only end absorption.

The p.m.r. spectrum was similar to that of squalene. It showed four vinylic protons at 4.92 τ , an envelope of allylic CH_2 protons

at ca 7.6 τ , and two groups of protons from methyl groups substituted on double bonds at 8.33 τ and 8.39 τ . However, in the squalene spectrum, the higher field resonance (8.39 τ) is more intense than the lower one at 8.32 τ by a factor of 3, whereas for fraction 2 the lower field resonance is the more intense, by a factor of 4:1. Also, in the latter spectrum, the integral of both methyl resonances together was approximately equal to that of the methylene envelope. For squalene, the ratio of methyl to methylene integral was 6:5.

Another difference was the existence in the spectrum of fraction 2 of a well defined triplet at 6.38 τ integrating for 1H. This was assigned to the proton α to a secondary hydroxyl group, which must be flanked by a CH_2 group and a quaternary centre.

The mass spectrum showed a molecular ion of 426, confirming the molecular composition of $C_{30}H_{50}O$. Other important peaks were: M-18 (loss of water), M-44 (loss of C_2H_4O or C_3H_8) M-59 (loss of C_2H_4O or C_3H_8, then CH_3), M-155 (loss of $C_{11}H_{17}O$ or $C_{12}H_{21}$), M-157 (loss of $C_{11}H_{19}O$ or $C_{12}H_{23}$), and the base peak at M-309.

The compound had zero optical rotation, this was confirmed by o.r.d. measurement.

The alcohol was labile to acid. It was decomposed by hydrochloric acid, and treatment with acetic acid in benzene gave conversion to a less polar product and decomposition material. Stability to base was demonstrated by its recovery, unchanged, after treatment with 2% ethanolic sodium hydroxide at room temperature. It was, however, readily attacked by oxygen. On storage under nitrogen, it decomposed to give very polar material and one product corresponding, on t.l.c., to the compound produced on acetic acid treatment.

The hydroxyl group could be acetylated with acetic anhydride in pyridine. In the p.m.r. spectrum the α proton triplet now appeared

at 5.95 τ , in confirmation of this assignment. An attempted oxidation with manganese dioxide and chloroform was not successful, indicating the absence of an allylic hydroxyl group.

Oxidation did occur, however, with chromic anhydride in pyridine[28] in poor yield. The infrared spectrum of the product showed a strong carbonyl absorption at 1723 cm^{-1}, and a doubtful aldehyde absorption at 2714 cm^{-1}. There was no hydroxyl absorption.

The p.m.r. spectrum showed no aldehyde proton, and no low field triplet. Apart from this, the spectra of the alcohol and ketone were very similar. The ketone did not have a significant ultra-violet absorption, and treatment with base did not convert it to an α,β unsaturated ketone.

On hydrogenation of the alcohol over platinum in ethyl acetate, 4.8 equivalents of hydrogen were consumed. The infra-red spectrum of the product showed no C=C absorption. The p.m.r. spectrum showed no vinylic protons, and the mass spectrum molecular ion of m/e = 436 confirmed the uptake of 5 moles of hydrogen.

A comparison of the t.l.c. behaviour of squalene and the alcohol before and after hydrogenation is given in the table.

T.L.C. of Squalene and the Unknown Compound

	10% $AgNO_3$/Silica GF	Silica GF
Unknown	R_F = 0.03	R_F = 0.69
Hydrogenated unknown	R_F = 0.68	R_F = 0.68
Squalene	R_F = 0.29	R_F = 0.83

Solvent:- ethyl acetate: benzene 1:4

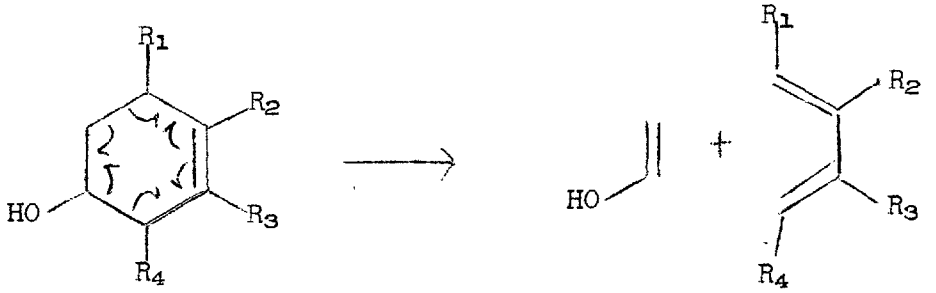
It can be seen that the alcohol complexed with the silver ions more strongly than did squalene. This strong binding was not due to the interaction of the hydroxyl group with silica, because the R_Fs of

the hydrogenated unknown, on silica and silver nitrate/silica, and the unknown alcohol on silica were the same.

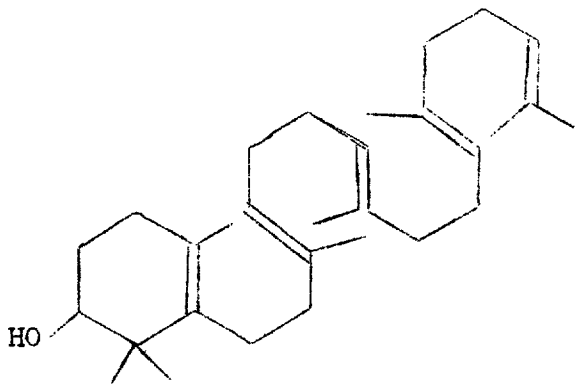
The mass spectrum showed, apart from the molecular ion at 436, strong peaks at M-15 (loss of CH_3), M-18 (loss of water) and base peak M-16 which could be due to hydrogenolysed material. There was no M-44 peak.

The molecular formula of $C_{30}H_{50}O$ demands, in addition to the 5 double bonds revealed by hydrogenation, and the hydroxyl group, the presence of one ring. The introduction of a ring into a squalenoid structure, would provide the quaternary carbon required by the p.m.r. results.

A significant difference in the mass spectra of the original alcohol and hydrogenated alcohol was the absence of M-44 and M-59 peaks in the latter. Both these fragmentations, arising from the loss of C_2H_4O or C_3H_8, are dependent on the presence of at least one double bond in the molecule near to the hydroxyl group. The loss of a C_3 fragment from a squalenoid structure is unlikely. The loss of C_2H_4O, however can be accomodated by a retro-Diels Alder fragmentation from a six membered ring containing a double bond, as in fig.1.



Structure (3) accomodates the conclusions from the mass spectral and p.m.r. results.



(3)

This structure is also consistent with several other observations:-

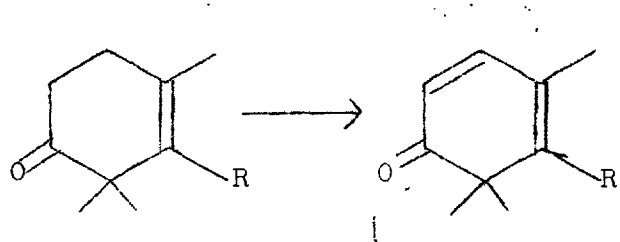
(a) The number of vinylic protons in the p.m.r. spectrum was 4, whereas the number of double bonds from the hydrogenation was 5.

(b) The different environment of the 2 methyl groups relative to the other 6 may explain the ca 4:1 ratio of the vinylic methyl signals, and also the observed ratio of vinylic methyls: allylic protons of ca 1:1.

The zero rotation of the alcohol indicates that if structure (3) is correct, then the compound could well be an artefact, as enzymic cyclisations of natural and presumably optically pure squalene oxide give steroeospecifically the 3β conformation in sterols.[29]

Structure (3) has been reported as a product of the Lewis acid catalysed cyclisation of squalene oxide.[30] This work was repeated, but no compound corresponding to the alcohol from yeast could be detected; furthermore although the other compounds reported in the product mixture were isolated, the compound to which structure (3) was assigned could not be found.

This evidence was not considered adequate to eliminate structure (3) as possible for the alcohol, so attempts were made to c nfirm it by the introduction of a double bond, under non-acidic conditions, into the 1,2 position of the oxidation product (presumably (4)), to form a readily identifiable conjugated system.

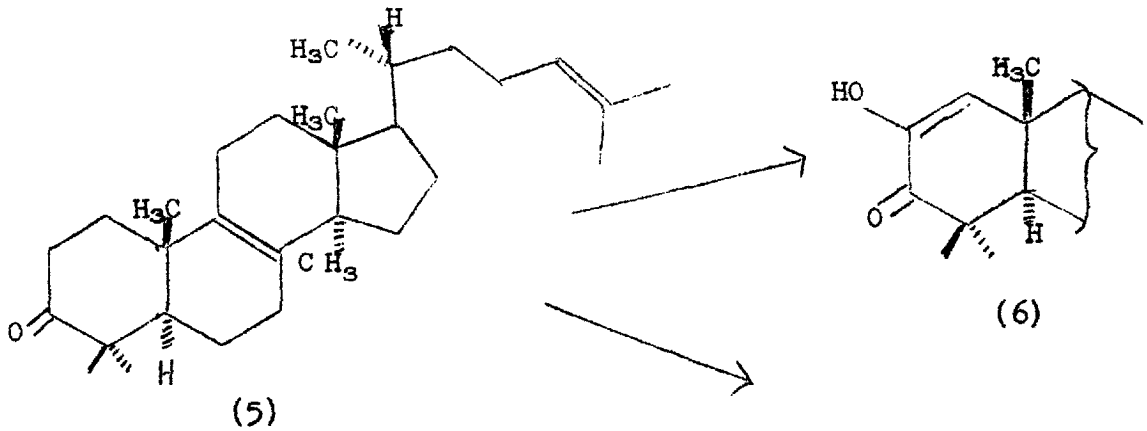


(4)

A positive Zimmerman test[31] on (4) indicated the presence of acidic hydrogens α to the carbonyl group. Autoxidation with potassium tert.-butoxide and oxygen,[32] which with lanost-8,24-dien-3-one (5) gave the dios-phenol (6), led when applied to (4) to a continuous uptake of over two moles of oxygen, and a variety of products which were revealed on t.l.c.

Similarly, an attempt to employ the formylation and dehydrogenation described by Edwards[33] for 3-keto-steroids was abortive. The formylation[34] which was successful with (5) to give (7) yielded, with (4), one squalenoid product. This was isolated by p.l.c. but did not have the expected infra-red or ultra-violet spectra.

These failures promoted investigation of a new type of reaction to introduce a 1,2 double bond into 3-keto-steroids under mild conditions. The development of these reactions is described in Part IV.



(5)

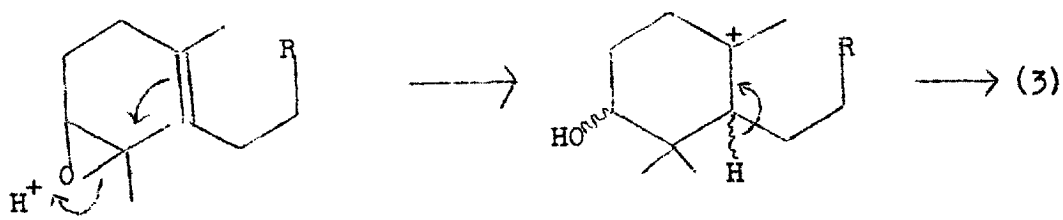
(6)

(7)

Structure (3) has not been definitely assigned to the alcohol isolated from yeast residues. This alcohol was isolated from fresh yeast cells in the course of a separate experiment in this laboratory.[35]

The work-up involved no acid treatment. The sample thus obtained had no optical rotation. If the alcohol is an artefact, then structure (3) is readily derived by acid catalysed cyclisation of squalene oxide

It is difficult to visualise a method of formation from the base treatment which is involved in the work-up of yeast.

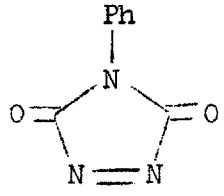


(3)

Thus the status of this compound is still in doubt.

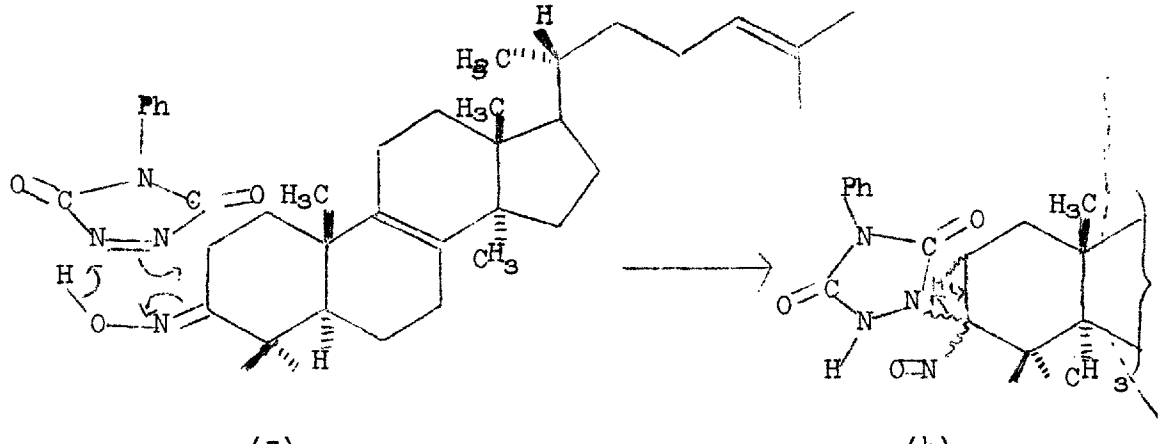
PART IV

Dehydrogenation of Steroidal 3-Ketone Derivatives
Reactions with 4-Phenyl-1,2,4-triazoline-3,5-dione(1)

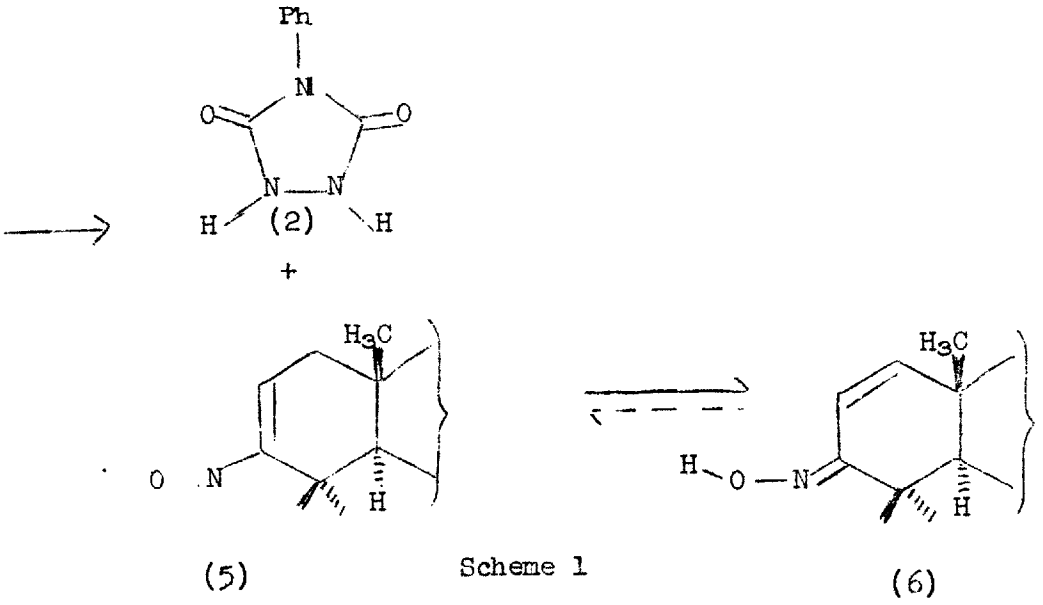


(1)

The triazoline(1)[36] which is used in this laboratory as a dienophile to protect the ergosterol ring B diene system,[1a] also undergoes the ene-reaction with mono-olefins with allylic hydrogens.[37] It was envisaged that a similar adduct formed by addition of (1) to a lanosta-8,24-dien-3-one carbonyl derivative such as the oxime(3) (Scheme (1)) would undergo facile thermal elimination of N-phenyl urazole (2) to introduce a new double bond. With such a derivative, tautomerisation to the dehydro analogue of the starting material would complete the sequence.



(3) (4)

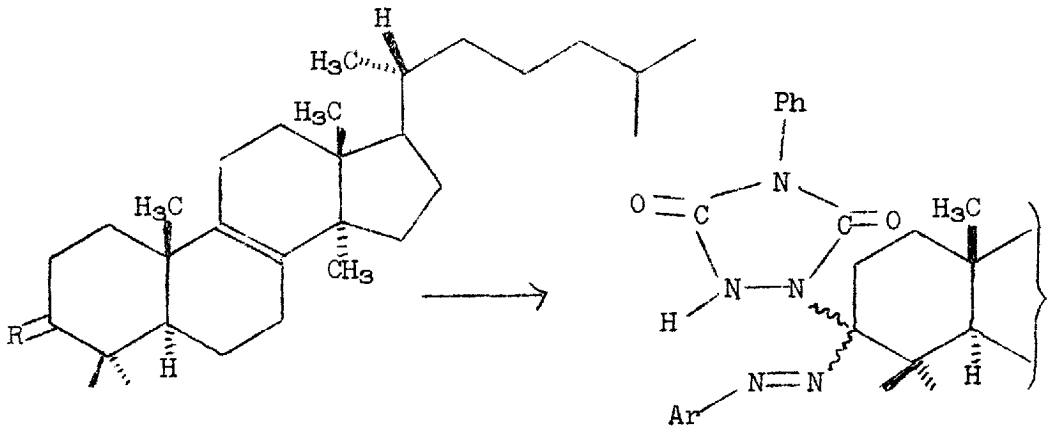


(5) Scheme 1 (6)

The reaction was attempted with (3) and an excess of triazoline (1). At 35°C in dichloromethane the adduct (4) was formed readily but proved to be very labile and decomposed to the product (6) before all of the starting oxime had reacted. (6) reacted further with the excess triazoline at the side chain double bond and at the C=N bond giving rise to many products.

With one equivalent of triazoline, the thermal decomposition of (1)[38] appeared to proceed at a rate comparable to that of the addition to form (4), as the red azo colour was exhausted before the reaction was 50% complete. Reaction at 0°C was slow and incomplete, probably due to the competing ene-reaction of the side chain. This was eliminated by reacting lanost-8-en-3-oxime (7) with (1). The reaction was stopped before all the starting oxime was consumed. The product, which was slightly less polar (on silica gel t.l.c.) than the starting material, had the correct ultra-violet absorption for an α,β unsaturated oxime, but was formed in very poor yield (ca 10%). The intermediate adduct corresponding to (4), was resolved in t.l.c. - into several products, which indicated the further reaction of (1) with the oxime function of the product. In order to avoid this consecutive reaction,

it was decided to react the triazoline with the phenyl hydrazone (8), which was expected to react initially more easily as it had a higher electron density in the double bond.



(7) R=N-OH

(8) R=N-N(H)Ph

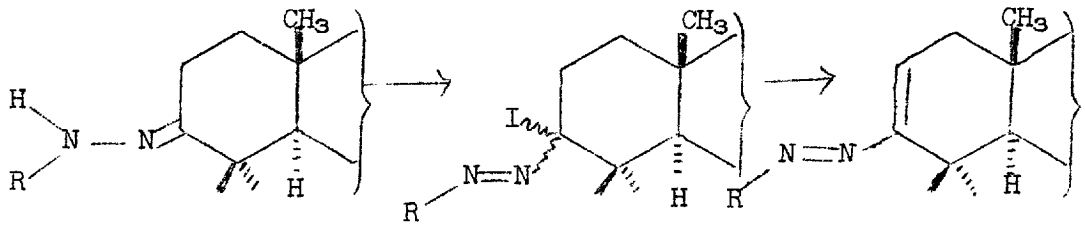
(10) R=O

(9)

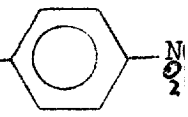
The reaction with one mole of triazoline was rapid and yielded 40% of a product the spectral data of which supported the structure (9). Attempts to eliminate the urazole (2) from this thermally, by refluxing in chloroform, led to a multiplicity of products. Attempted elimination with acetic acid gave three products one of which was the 3-ketone (10) again, however, in low yield. Thus neither the oxime nor the phenylhydrazone proved suitable for oxidation in this fashion.

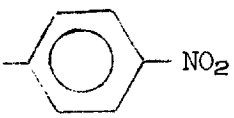
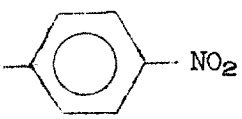
Reactions with Iodine

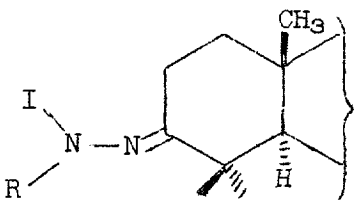
A sequence of reactions similar to the above could occur with iodine as oxidant, (Scheme 2). When iodine and triethylamine were reacted with hydrazone (11)[39] the elimination of hydrogen iodide from the iodo-intermediate - originally considered to be (14)[39] but probably better formulated as (13) (see below) yielded a diazoalkane which reacted further. It was hoped that with the phenylhydrazone (8), where this is impossible, α,β elimination would occur under the influence of mild base to yield (15) and hence (16).



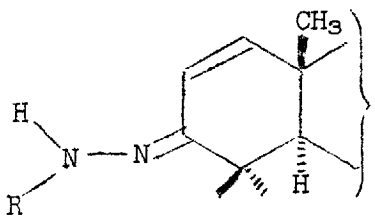
(8) R = C_6H_5 (12) R = C_6H_5 (15) R = C_6H_5

(11) R = H (13) R = H (19) R = 

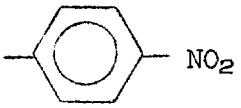
(17) R=  (18) R = 



(14) R = H



(16) R= C_6H_5

(20) R= 

Scheme 2

The reaction of (8) with one mole of iodine and two moles of triethylamine yielded two products, less polar on t.l.c. than the starting material. The least polar product proved to be 3-phenylazo-5 α -lanosta-2,8-diene (15). The other product on p.l.c. work-up decomposed to lanostenone (1). It is presumed to be (12), (see below).

On stirring (15) with triethylamine there was no apparent conversion to 5 α -lanosta-1,-8-diene-3-phenylhydrazone (16). This was in accord with the findings of Hassner,[40] and Guthrie,[41] that the corresponding cholestene phenylazo system would not isomerise with base treatment - sodium methoxide in methanol, or acetic acid treatment, in contrast to the readily isomerised cholestane phenylazo analogue.[42] The desired isomerisation in the cholestene system is reported to proceed, however, on recrystallisation from acetone, or in the presence of phenylhydrazine hydrobromide, but not with phenylhydrazine alone.[40] This indicated to the authors the necessity of combined acid-base catalysis.

This was tested by investigating the effect of different base strength on the oxidation reaction:-

With no base in ether the dehydrogenated phenylazo compound was formed after the appearance of the unstable intermediate.

With pyridine, the reaction was more rapid to give the same results.

With potassium tert.-butoxide in glyme, the reaction was very rapid to give a third product which ran just in front of the starting material on a t.l.c. plate, and appeared after the other two. This on isolation had infra-red bands at 3,500 cm^{-1} and 1,600 cm^{-1} and an ultra-violet absorption at 385 nm. Repeated reactions gave the

same three products, the final isomerisation could not be forced at higher temperatures or with excess base.

It was anticipated that with the p-nitrophenyl hydrazone (17), the final isomerisation should proceed more readily, as the p-nitro group should increase the acidity of the γ -hydrogen atom of the azo-intermediate and stabilise the nitrogen anion of the phenylhydrazone product.

Reaction of (17) with iodine in the presence of potassium tert.-butoxide was very rapid and gave an 85% yield of the desired 5α -lanosta-,8-diene-3-p-nitrophenylhydrazone (3), identical to that prepared from authentic 1,8-dien-3-one. The arylazo intermediates (18) and (19) were visible on t.l.c. but were consumed much more rapidly than in the phenylhydrazone case.

The initial rapidity of the reactions in glyme could arise from a solvent effect, or from the faster reaction of iodine with the anions of the phenylhydrazones. To clarifiy this, the reaction was repeated with both arylhydrazones in glyme in the absence of base. In both cases there was no reaction until butoxide was added. For the p-nitro derivative there was no reaction in the presence of triethylamine, which did not form the red colour characteristic of the anion. So it was evident that in glyme the initial iodine substitution only occurs with the arylazo-anion. (Ar-N-N-C$\langle$) The reaction in ether, without base, must therefore have proceeded through a different mechanism of iodine attack. As no reaction occurred in glyme freshly distilled from lithium aluminium hydride under nitrogen, it must involve radical initiation - perhaps by a peroxide in the ether, rather than a cyclic transition state analogous to that operative in the N-phenyl triazoline-3,5-dione -ene reaction. This was investigated by stirring iodine and the

phenylhydroazone in peroxide free ether under nitrogen in the dark - there was no reaction after 12 hours. On the introduction of light, reaction proceeded.

The most probable mechanism involves abstraction of a hydrogen radical, by either peroxide or iodine radical to form a pseudo-allylic species of the type (21) which can couple with an iodine radical at C.-3.

($C{=}N{-}\dot{N}{-}Ar \longleftrightarrow \dot{C}{-}N{=}N{-}Ar$)

This species is reported as an intermediate in the autoxidation of arylhydrazones,[43] in which reaction radical coupling occurred at carbon.

During the reactions with the phenylhydrazone (8), it was critical to exclude oxygen from the reaction system, as otherwise a quantitative yield of lanostenone (10) was obtained. This arose through attack on the α -iodo azo compound (12) by oxygen. The instability of this type of compound, to nucleophiles at least, is evidenced by the formation of 2-phenylazo-2-alkoxy propanes, when iodine is reacted with acetone phenylhydrazone in alcoholic solution.[44]

The alternative oxidation pathway via the mesomeric cation analogous to the radical and anion above was briefly investigated by treating the arylhydrazones with hydride abstracters. The triphenyl-methyl cation did not react, D.D.Q. did react slowly, but formation of the phenylazo compound (15) was accompanied by appreciable decomposition, so the reaction was investigated no further.

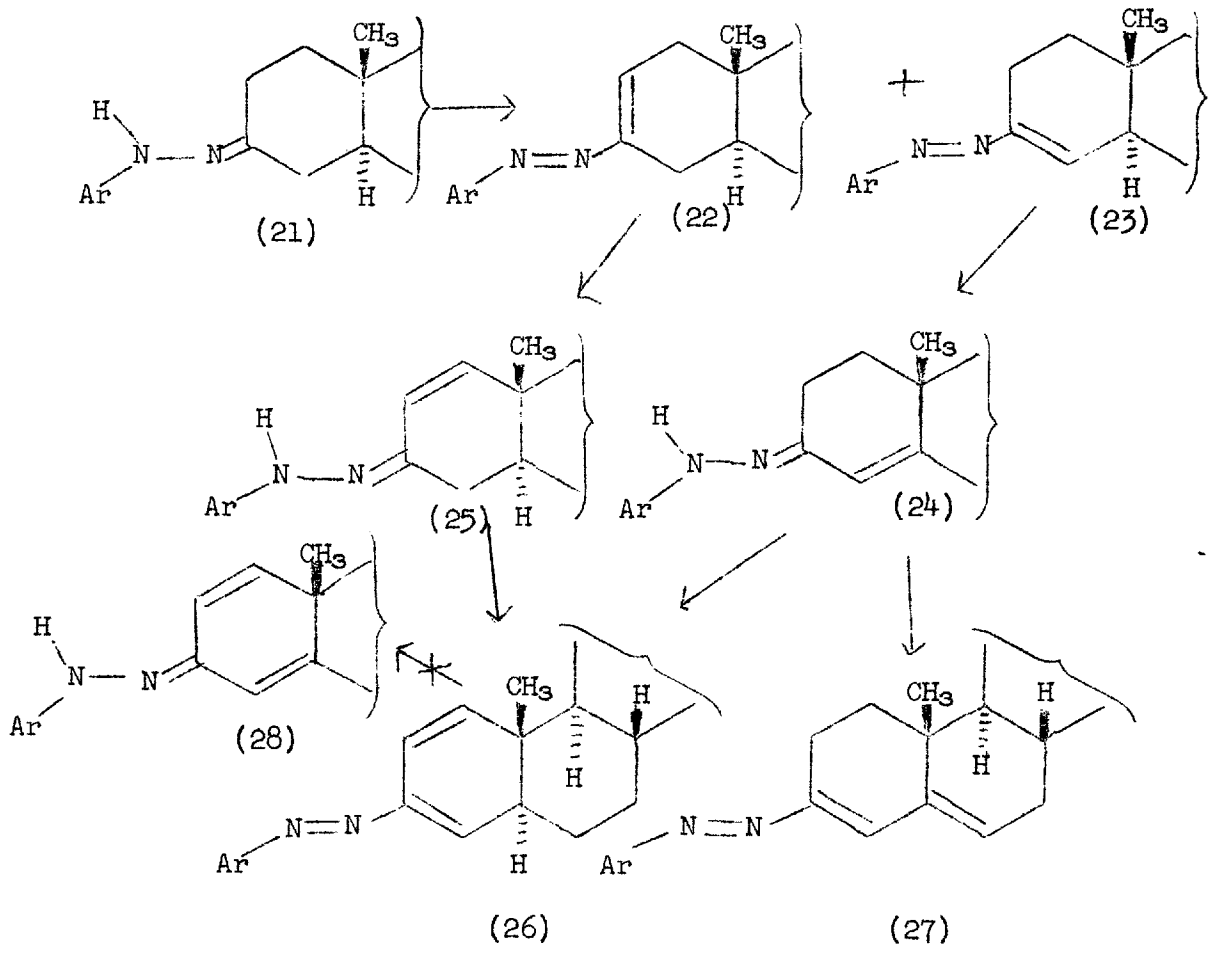
The iodine dehydrogenation reaction was extended into the cholestane series in the hope that sequential introduction of double bonds could be achieved.

5α -cholestane-3-p-nitrophenylhydrazone (21) was reacted with one equivalent of iodine and two equivalents of base (Scheme 2). After two

hours at room temperature, t.l.c. showed two non-polar products - shown to be the phenylazo-isomers (22) and (23) by their spectral data, and one phenylhydrazone product. This appeared to be (24). Its p.m.r. spectrum showed one vinylic proton at 6.24 τ . The other isomer (25) was isolated from a subsequent reaction and had a well-defined AB quartet for two vinylic protons at 3.64 and 3.95 τ . Thus apparently isomerisation is more rapid for (23) than for (22). The isomerisation rate of both (22) and (23) is much less than that of the analogue (19) in the lanostene series.

The reaction was repeated with initial addition of 1 mole of iodine, followed by one mole of base, which rapidly gave (22) and (23). Addition of another equivalent of base gave after 30 minutes isomerisation, one spot on t.l.c. corresponding to (24) and (25). Another mole iodine was added, followed by another of base. This gave complete conversion to a less polar product with similar R_F to (22) and (23). Addition of six moles of base produced after two hours of this treatment, a compound with R_F similar to that of the starting material in high yield (82%). This proved to be the phenylazo compound (27). It was difficult to crystallise and so may be contaminated with (28) though this was not evident in the p.m.r. spectrum.

A large excess of base was avoided as it was found to remove the iodine to form a dark red oil, which was held at the origin of a t.l.c. plate. The final addition of excess butoxide was necessary to accelerate the final elimination of hydrogen iodide, as otherwise, with prolonged reaction, there was appreciable decomposition.



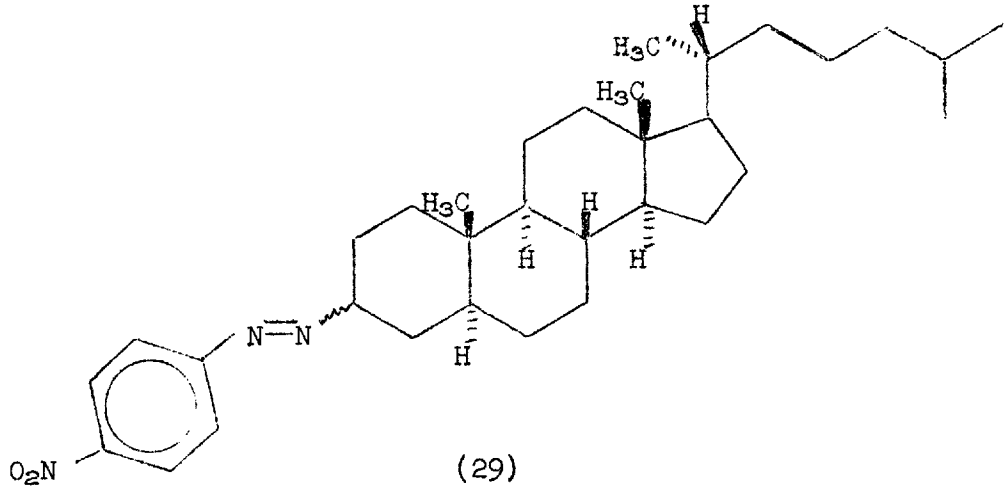
Scheme 2

Isomerisation of (26) to (28) was attempted with potassium tert-butoxide in glyme, and with sodium methoxide in refluxing methanol, but was unsuccessful, although the anion was formed in both these cases as evidenced by the deep purple or red colour. With potassium tert-butoxide, decomposition to very polar material was quite rapid - possibly due to oxygen intrusion. Treatment of (26) with acetic acid gave several products, none of which corresponded to the authentic cholesta-1,4-dien-3-p-nitrophenyl-hydrazone.

Dehydrogenation of p-nitrophenylhydrazones with iodine and potassium tert-butoxide is synthetically useful in the lanostane series. The limitation to the extension of the reaction in the cholestane series exists in the final isomerisation. This limitation is undergoing further investigation in this laboratory.

Isomerisation of 5α -cholestane-3-p-nitrophenylhydrazone

It was noticed that treatment of the arylhydrazone (21) with potassium tert-butoxide in glyme gave rise to a less polar product. This proved to be 3-p-nitrophenylazo-5α -cholestane (29), formed in 30% yield, with 50% recovery of starting material.

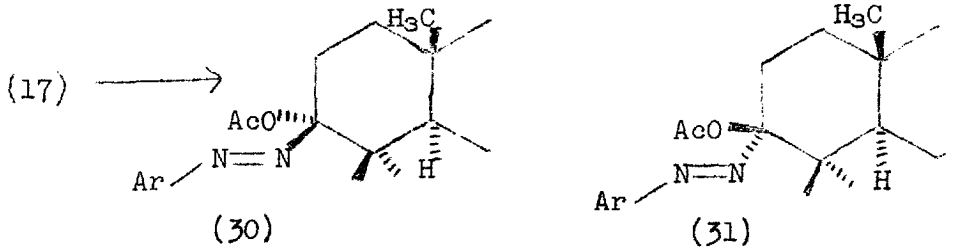


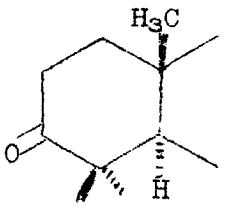
(29)

As it has been shown that cholestane phenylhydrazone is the much preferred isomer at equilibrium,[42] and also that for the unsaturated systems in the lanostane series above, the p-nitro group increases this preference, this result must arise from kinetically controlled protonation. When excess sodium hydride was used in glyme there was significant formation of the anion as evidenced by the ultra-violet absorption at 490 nm, but there was no formation of (29) on the t.l.c. plate or on work-up. This may be another example of the cation affecting the kinetically controlled electron density distribution in ambident anions.[45]

Removal of the Arylhydrazone Group

As an alternative to the usual methods of converting arylhydrazones to the parent carbonyl compound,[46] it was decided to attempt the generation of lanostenone (10) by treatment of the azoacetates (30) and (31) with base.[47] Reaction of lanost-8-ene-3-p-nitrophenylhydrazone (17) with lead tetra-acetate[48] gave an immediate conversion to 3α -acetoxy-3β -p-nitrophenylazo-5α -lanost-8-ene (30) and the 3β -acetoxy-3α -arylazo isomer (31) in a ratio of 3:1.





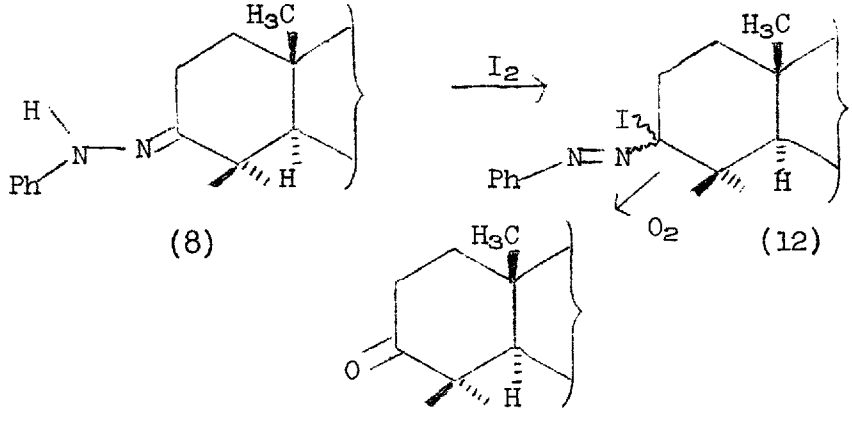
(10)

The two isomers could be distinguished by their t.l.c. polarity and p.m.r. spectra. The less polar had one methyl group at 8.89 τ , downfield from the main methyl group envelope, whereas the more polar had two methyl groups downfield at 8.78 τ and 8.85 τ . Inspection of Dreiding models reveals that when the arylazo group is α , the 4 α group is much nearer to it than is the 4 β methyl group; when the arylazo group is β , then both methyl groups at C-4 are approximately equidistant from it. Thus the more polar isomer was assigned the structure (30).

Both azo-acetates could be hydrolysed to the ketone with base. Surprisingly, drastic conditions, refluxing ethanol/potassium hydroxide, were necessary.

An attempt was made to utilise the susceptibility of the iodo-azo-intermediate (12) to oxygen attack which led to degradation to the ketone (10), by use of iodine as a catalyst for the removal of the arylhydrazone group with oxygen.

When the phenylhydrazone (8) was shaken in benzene with half a mole of iodine in an atmosphere of oxygen, both the iodo - and hydroperoxy - azo compounds (12) and (32) respectively were formed. However, iodination of (8) in the absence of oxygen, and subsequent exposure to an oxygen atmosphere produced a smooth conversion to the lanostenone.

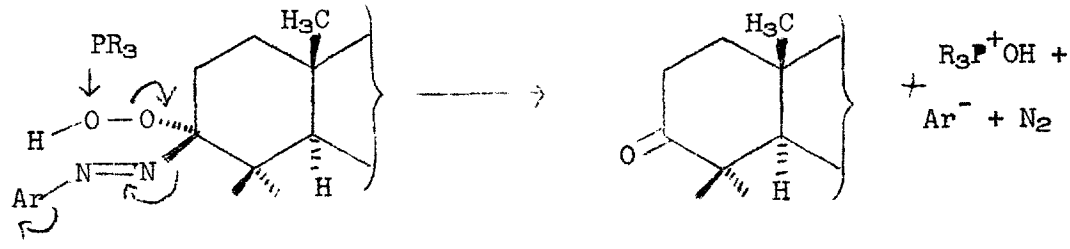


(8) (12)

(10)

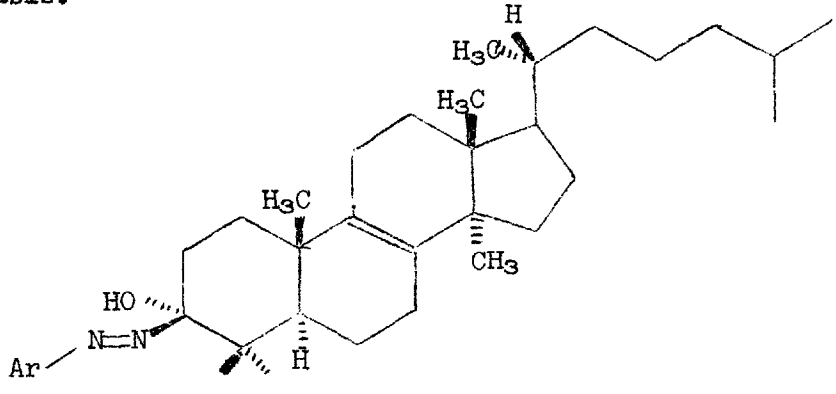
The facile autoxidation of arylhydrazones[49] was also utilised to effect this conversion.

It was anticipated that abstraction of oxygen from the hydroperoxide (32) by a phosphine would leave a system which would collapse to the ketone with expulsion of nitrogen and an aryl fragment.



(32)

In the event of treatment of (32) with tris-diethyl-triamino-phosphine, there was immediate and quantitative formation of a compound to which the structure (33) was assigned on the following basis.

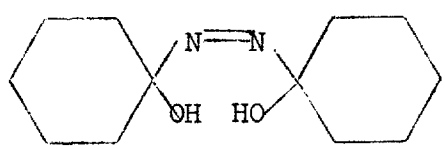


(33)

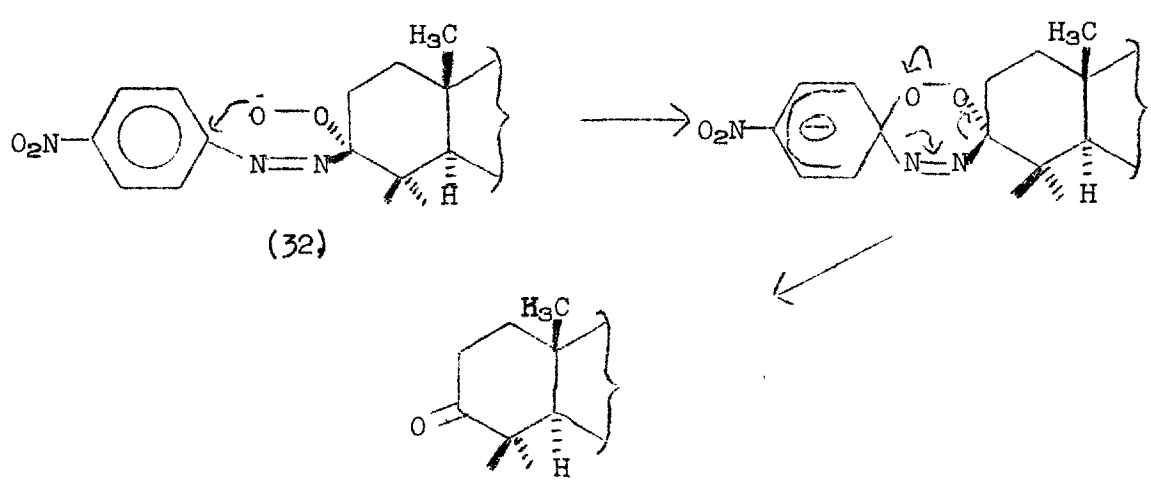
The analysis supported the molecular formula $C_{36}H_{55}O_3N_3$. The infra-red spectrum was virtually identical to that of the hydro-peroxide with an O-H absorption at 3441 cm^{-1} compared to 3436 cm^{-1}. (The O-O stretch, reported[49] at 830 cm^{-1} was not visible in either spectrum.) The ultra-violet spectrum had absorptions at 284.5 and 396 nm, whereas that of (32) showed peaks at 281 nm and 425 nm. The only significant difference in the p.m.r. spectra was the position of the O-H proton at 0.95 τ in (32) and at 5.36 τ in

(33). A significant similarity was the siting of the aromatic AB quartet at 1.54 τ and 2.15 τ in the former and 1.61 τ and 2.16 τ in the latter. For 3-p-nitrophenylazo-cholestane, these resonances are at 1.74 τ and 2.22 τ .

The p.m.r. spectra of (33), as with (32) showed low field 4,4 dimethyl signals, and was assigned the β configuration for the phenylazo group. The gem-hydroxyazo structure has been shown to be stable in e.g.[50]



It seemed conceivable that with a favourable geometry, the 3-p-nitro group in the hydroperoxide (32) could facilitate nucleophilic attack on the 1 aromatic position by the hydroperoxide anion, with subsequent collapse to a phenol, nitrogen and ketone.

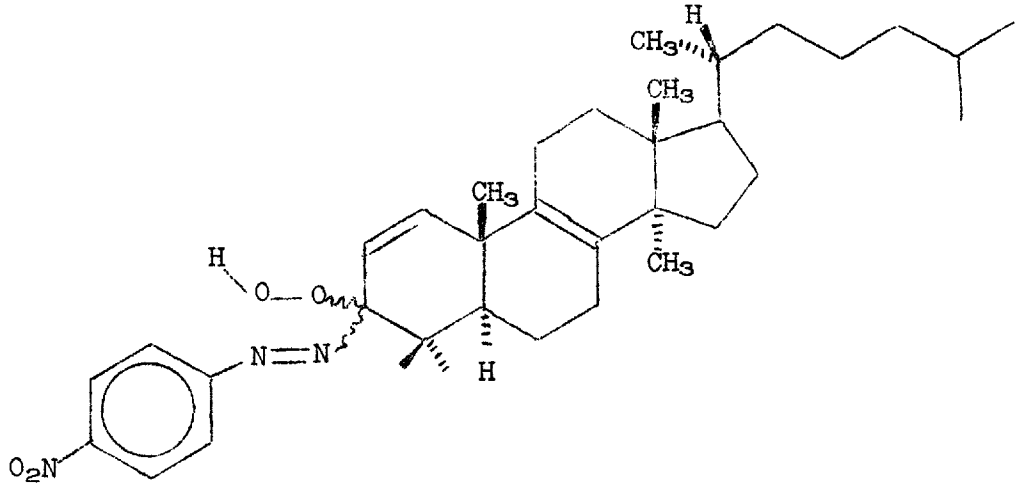


(32)

Evidence for a similar type of collapse came from the mass spectrum of (32) which showed a molecular ion of 426, corresponding to lanostenone. Also, evolution of a gas was noted during melting point determinations, the melt on cooling ran identically to lanostenone on t.l.c.

The trans → cis isomerisation and the fragmentation were attempted both photochemically and thermally. After photolysis for one hour in benzene in a Pyrex vessel with a high pressure mercury lamp, (32) was converted to lanostenone together with further decomposition products. After the same period of time of reflux in benzene the hydroperoxide was unchanged. 48 Hours reflux of (32) in benzene, however, gave the ketone (70%) and 4-nitro diphenyl. In refluxing ethanol, conversion was complete in 12 hourse, to give the ketone and nitrobenzene. In 2% potassium hydroxide in refluxing ethanol all the hydroperoxide was removed in one minute, with formation of the ketone. There was no p-nitro-phenol or nitrobenzene revealed in the ultra-violet spectrum of the product mixture.

The gem -hydroxy-phenylazo steroid (33) was also converted into lanostenone on refluxing for 48 hours in benzene. The Δ^1 analogue of (32) i.e. (34) was thermally stable. It was unaffected by 7 days reflux in benzene; it also gave the appropriate molecular ion in the mass spectrum and not that of the thermal elimination product. Treatment of (34) with 2% potassium hydroxide in refluxing ethanol, did cause rapid reaction, however, to give two products, neither of which was the desired unsaturated ketone.



(34)

The aromatic fragments isolated in these reactions without base indicate an aryl radical is formed. If the initial step in the hydroperoxide fragmentation were O-O bond cleavage, it is hard to explain why the hydroxy compound (33) also undergoes the same reaction. If the N-aryl bond should first cleave homolytically, which may explain the acceleration on changing from benzene to ethanol as solvent,[51] then the reason for the resistance of (34) to this reaction is not clear. Further work on this process is required to delineate the mechanism and is being undertaken in this department.

REFERENCES

1(a) D.H.R. Barton, T.Shiori and D.A. Widdowson, J.Chem.Soc.(C) 1971, 1968

1(b) Part 6, D.H.R. Barton, U.M. Kempe and D.A. Widdowson, in press

2 U.M. Kempe, Ph.D. thesis, University of London, 1971.

3 G.D. Laubach and K.J. Brunings, J.Amer.Chem.Soc., 1952, 74, 705

4 R.C. Anderson, R. Stevenson and F.S. Spring, J.Chem.Soc., 1952 2901

5 R. Mozingo, Organic Syntheses, Coll.Vol.3, p 181

6 D. MacLean, W.S. Strachan and F.S. Spring, Chem.Ind. 1953, 1259

7 K. Sakai and K. Tsuda, Chem. and Pharm. Bull. (Japan), 1963, 11, 529

8 G. Slomp Jnr. and J.L. Johnson, J.Amer.Chem.Soc., 1958, 80, 915

9 H.M. White and P.S. Bailey, J.Org.Chem., 1965, 30, 3037

10 R.E. Erickson, D. Bakalik, C. Richards, M. Scanlon and G. Huddleston, ibid, 1966, 31, 461

11 P. Bladon, H.B. Henbest and E.R.H. Jones, J.Chem.Soc., 1952, 4890

12 D.H.R. Barton, P.G. Feakins, J.P. Poyser and P.G. Sammes, J.Chem.Soc.(C), 1970, 1584

13 W. Sucrow, Chem.Ber., 1967, 100, 259

14 T. Shioiri, D.I.C. thesis, Imperial College of Science and Technology, 1970

15(a) I.N. Nazarov, Yu.A. Titov and A.I. Kuznetsova, Bull.Acad of Sciences of the U.S.S.R., 1959, 1362

15(b) R.E. Stube, Organic Syntheses, Coll.Vol.4., p 417

16 cf: M.J. Jorgenson, Tetrahedron Letters, 1962, 559

17 A. Maercker, Organic Reactions, 1965, 14, 270

18 E.J. Corey and M.F. Semmelhack, J.Amer.Chem.Soc., 1967, 89, 2755

19 S.R. Landauer and H.N. Rydon, J.Chem.Soc., 1953, 2224

20 U. Wrzeciono, C.F. Murphy, G. Ourisson, S. Corsano, J.D. Ehrhardt, M.F. Lhomme and G. Teller, Bull.Soc.Chim.France, 1970, 966

21 J.F.W. McOmie, Advances in Organic Chemistry, 1963, Vol.3, p 191

22 H.C. Shah, R.P. Stein and H. Smith, Chemical Abstracts, 1971, 74, 142181 m

23 M.H. Karger and Y. Mazuk, J.Amer.Chem.Soc., 1969, 91, 5663

24 M.H. Karger and Y. Mazuk, J.Amer.Chem.Soc., 1968, 90, 3878

25 M. Gomberg, J.Amer.Chem.Soc., 1913, 35, 200

26 H. Meerwein, V. Hederich, H. Marshel and K. Wunderlich, Ann., 1960, 635, 1

27 G. Leclaerc, private communication

28 I. Poos, G.E. Arth, R.E. Beyler and L.H. Sarett, J.Amer.Chem. Soc., 1953, 75, 422

29 J.D. Willett, K.P. Sharpless, K.E. Lord, E.E. van Tamelen and R.B. Clayton, J. Biol. Chem., 1967, 242, 4182

30 Y. Kitahara, T. Kato and M. Kishi, Chem. and Pharm. Bull. (Japan)., 1968, 16, 2216

31 E. Zimmerman, Biochem.J., 1938, 32, 1312

32 E.J. Bailey, D.H.R. Barton, J. Elks and J.F. Templeton, J.Chem. Soc., 1962, 1578

33 J.A. Edwards, M.C. Calzada, L.C. Ibanez, M.E. Cabazas Rivera, R. Urquiza, L. Cardona, S.C. Orr and A. Bowers, J.Org.Chem., 1964, 29, 3481

34 R.O. Clinton, A.J. Manson, H.C. Noumann, R.L. Clarke, D.F. Page, W.B. Dickenson and Clarissa Carabateas, J.Amer. Chem.Soc., 1961, 83, 1478

35 J.J. Wright, private communication

36 R.C. Cookson, S.S.H. Gilani and I.D.R. Stevens, Tetrahedron Letters, 1962, 615

37 W.H. Pirkle and J.C. Stickler, Chem.Comm., 1967, 760.

38 J.C. Stickler and W.H. Pirkle, J.Org.Chem., 1966, 31, 3444

39(a) D.H.R. Barton, R.E. O'Brien and S. Sternhell, J.Chem.Soc., 1962, 470

39(b) A. Pross and S. Sternhell, Aust.J.Chem., 1970, 23, 989

40 A. Hassner and P. Catsoulalos, Chem. Comm., 1967, 121

41 J. Buckingham and R.D. Guthrie, J.Chem.Soc., 1968, 3079

42 A.J. Bellamy and R.D. Guthrie, J.Chem.Soc., 1965, 3528

43 R. Criegee and G. Lohaus, Chem.Ber., 1951, 81, 213

44 J. Schantl, Tetrahedron Letters, 1970, 3785

45 cf. H.O. House and V. Kramer, J.Org.Chem., 1963, 28, 3362

46 J. Buckingham, Quart.Rev., 1969, 37

47 cf. J.T. Edward and S.A. Samad, Can.J.Chem., 1963, 4. 1638

48(a) D.C. Iffland, L. Salisbury and W.R. Schafer, J.Amer.Chem. Soc., 1961, 83, 747

48(b) M.J. Harrison, R.O.C. Norman and W.A.F. Gladstone, J.Chem. Soc.(C), 1967, 735

49 A.J. Bellamy and R.D. Guthrie, J.Chem.Soc., 1965, 2788

50 E. Schmitz, R. Ohme and S. Schramm, Angew. Chemie., 1963, 75, 208

51 E.S. Huyser, Adv.Free Radical Chem., 1965, 1, 77

EXPERIMENTAL

Unless otherwise stated, the following procedures were adopted in the experiments described in this section.

Melting points were determined on a Kofler hot stage apparatus, and are uncorrected. Rotations were measured in chloroform, in a 10cm. cell (0.5ml.) at the sodium D line wavelength on a Perkin Elmer 141 polarimeter.

Infra-red (i.r.) spectra were recorded on a Unicam SP200 or Perkin Elmer 257 spectrometer, of solutions in chloroform or carbon tetrachloride, or of mulls with nujol. Ultra-violet (u.v.) spectra were measured of ethanol solutions, using a Unicam SP800 spectrometer. N.m.r. spectra were measured of solutions in deuteriochloroform, with tetramethylsilane as an internal standard, on Varian A60, T60, or H.A.100 spectrometers. The following abbreviations apply to the n.m.r. data.

Hz. = Hertz

s = singlet

d = doublet

t = triplet

m = multiplet

b = broad

AB = AB quartet

Reactions were followed by thin layer chromatography (t.l.c.) on Merck GF_{254} silica plates. Preparative t.l.c. was carried out on 0.01x20x20 (small), or 0.1x20x60 cm^3 (large) plates of the same material.

Organic solvent extracts of aqueous solutions were dried with anhydrous sodium sulphate.

Petroleum refers to that fraction boiling in the range 60°C-80°C, unless otherwise specified.

Part 1

3β-Acetoxy-23,24-dinor-5α-chol-7-en-22-al. (9)

3β-Acetoxy-5α-ergosta-7,22-diene (4)[1] (1.4g. 3.2 m. mole.) was dissolved in 99% dichloromethane and 1% pyridine (20 ml.). To this solution, stirred rapidly at -70°C, was added a saturated solution of ozone in the same solvent mixture (7x100 ml.), at the same temperature, at a rate of 20 ml. per second. Zinc powder (3g.) and acetic acid (25 ml.) were added, and the mixture was allowed to warm to room temperature, with continuous stirring. After filtration the solution was washed with water (200 ml.), dried, and evaporated to yield a light brown oil. Addition of methanol (50 ml.) to this oil, caused the separation of the starting material,(0.7g 50%) as fine plates. Addition of water (50 ml.) to the methanolic solution, precipitated the aldehyde (9), (0.47g. 40%) as needles m.p. (from acetone) 144-147°C, $(\alpha)_D^{20}$ -14.5 (c, 0.71), (lit.[2] m.p. 139-141°C, (α_D) -18.7)

3β-Acetoxy-5α-ergosta-7,22,24(28)-triene (8)

Triphenyl-(2-methylene-3-methyl)-butyl-phosphonium bromide (21), (1.31g 2.7 m.mole.), was stirred under dry nitrogen in tetrahydrofuran (50 ml.). Addition of a 1.10 N solution of methyl lithium in ether (2.2 ml.), to give an immediate dark red colouration, was followed by addition of the aldehyde (9), (1.00g. 2.7 m.mole.), in tetrahydrofuran (5 ml.). T.l.c. of the solution, after overnight stirring, showed the desired product, and approximately 10% of hydrolysed product. Water (50 ml.) was added to the brown red solution, before extraction with ether (3x150 ml.). The ether extract was washed with water (100 ml.), dried and evaporated to leave a brown residue. This was dissolved in pyridine (5 ml.) and acetic-anhydride (2.5 ml.). After overnight standing, the yellow solution was poured into ice-water (20 ml.), and extracted with ether (3x20 ml.). The

ether extract was washed with water (20 ml.), dried, and evaporated to give the acetate (8) as a brown foam. (1.03g. 85%) m.p. (from chloroform-methanol) 132-134°C $(\alpha)_D^{25}$ +7.3 (c, 0.104), ν_{max} 2920, 2865, 1720, 890 $cm.^{-1}$; λ_{max} 227 (21,500), 232 (23,500), 241 nm. (16250) τ, 9.5 (3H, s, H-18), 9.21 (3H, s, H-19), 8.96 (9H, d J 6.5 Hz., H-21,26,27), 8.08 (3H, s, O-Ac),5.22 (2H, d. J 2Hz, H-28), 4.90 (1H, b.s. H-7), 4.48 (1H, d.d. J 16,8.5 Hz. H-22), 4.10 (1H, d. J 16Hz. H-23)

23,24-Dinor-5α-chol-7-en-3,22-diol 3β-acetate (24)

A solution of sodium borohydride (0.10g. 2.8 m.mole), in ethanol (10 ml.),was added to a solution of the aldehyde (9), (1.03g. 2.7 m.mole), in ethanol (10 ml.), and was allowed to stir at room temperature for 1.5 minutes. The solution was then poured into aqueous ammonium chloride solution (80 ml.), and was extracted with ether (3x100ml.). The ether extract was washed with water (100ml.), dried, and evaporated to give the diol mono-acetate (24), (0.88g. 87%). m.p. (from ethanol) 164-166°C $(\alpha)_D^{26}$ -8.95 (c, 1.23), υ_{max} 3500,2920,2800,1720 $cm.^{-1}$ τ, 9.44 (3H, s. H-18), 9.19 (3H, s. H-19), 8.95 (3H, d. J 6Hz. H-21), 7.97 (3H, s. O-Ac.), 5.5 (2H, m. H-22), 5.32 (1H, b.s. H-3), 4.85 (1H, s. H-7)

[Found C, 76.75, H, 10.38, Calculated for $C_{24}H_{38}O_3$, C, 76.96, H, 10.23]

22-Iodo-23,24-dinor-5α-chol-7-en-3β-yl acetate (23)

The diol mono-acetate (24), (0.53g. 1.09m.mole), and triphenyl-phosphite (0.44g. 1.6 m.mole), were refluxed in methyl iodide (30ml.), for 16 hours. The solution volume was reduced to 2 ml., and was chromatographed on two large plates with benzene as eluent. The second band (Rf. 0.53) was stripped with chloroform which yielded the iodide (23) as plates (0.41g. 73%). m.p. (from benzene-methanol) 158-160°C $(\alpha)_D^{26}$ +22.1 (c, 0.83), υ_{max} 2940,2860,1725,1030 $cm.^{-1}$ τ, 9.43 (3H, s. H-18), 9.19 (3H, s. H-19), 7.98 (3H, s. O-Ac.), 6.75 (2H, m. H-7)

[Found, C, 59.64% H, 7.83% I, 26.31%. Calculated for $C_{24}H_{37}O_2I$ C, 59.50% H, 7.70% I, 26.19%.]

Part 2

Acetyl-p-toluenesulphonate (4)

Anhydrous p-toluene sulphonic acid was prepared by recrystallisation of the mono-hydrate from acetone-benzene, followed by heating at 100°C overnight,under vacuum, to give a light brown liquid which solidified on cooling.

The anhydrous p-toluene sulphonic acid (1.27g. 0.07 mole) was dissolved in acetyl chloride (50 ml.). The solution was refluxed for two hours, when hydrochloric acid gas ceased to be evolved. The solvent was removed under reduced pressure to yield the solid mixed anhydride (4), (15g. 95%) τ, 6.81 (3H, s. Ph-Me), 2.02,2.57 (4H, AB J 8Hz. aromatic-H), 6.50 (3H, s. O-Ac)

Methoxymethyl-p-toluenesulphonate (3)

Dimethoxymethane (15 ml. 0.17 mole) was added to acetyl-p-toluene-sulphonate (15g. 0.070 mole), under nitrogen. The solution was stirred for 30 minutes, then the solvent was removed under reduced pressure to yield a light brown oil. (14.8g 98%) τ ,7.55 (3H, s. O-Me), 6.65 (3H, s. Ph-Me), 4.66 (2H, s. O-CH_2-O), 2.08,2.59 (4H, AB J 8Hz. aromatic-H)

5α-Ergosta-5,7,22-trien-3β-yl-methoxymethyl ether (2)

Ergosterol (1), (100mg. 0.25 m.mole), was stirred with sodium hydride (35mg, 14.0 m.mole), in anhydrous ether (2 ml.), under nitrogen, for 60 minutes, until the evolution of hydrogen ceased. To this solution was added a solution of methoxymethyl-p-toluenesulphonate (3), (155mg. 0.8 m.mole)in ether (2 ml.) under nitrogen. The reaction mixture was stirred

for 10 minutes, then ethanol (1 ml.) was added and the solvent was removed in vacuuo. The residue was shaken with benzene (5 ml.). The benzene extract was washed with water (5 ml.), dried, and chromatographed on 5 small silica plates with benzene, to yield the ether (2) (Rf = 0.6) (89mg. 80%), colourless scales from dichloromethane-methanol. m.p. 108-109°C, $(\alpha)_D^{23}$ -66 (c, 0.56), ν_{max} 1255,1115,1055,980 $cm.^{-1}$ λ_{max} 261.5 (7,500), 271.5 (9400), 282 (11700), 293.5 (6,550) nm. τ, 9.36 (3H, s. 18-H), 9.22 (3H, s. 19-H), 6.58 (3H, s. O-Me), 5.26 (2H, s. O-CH_2-O), 4.75 (2H, m. 22,23-H), 4.48 (2H, d. J 7.0Hz. 6,7-H)

[Found C, 82.0% H, 11.0% Calculated for $C_{30}H_{48}O_2$ C, 81.8% H, 11.0%]

Removal of the methoxymethly protecting group.

To a solution of ergosteryl-3β-methoxymethyl ether (2), (30mg. 0.07 m.m mole), in anhydrous dichloromethane (2 ml.), was added triphenylmethyl fluoroborate (45mg. 0.13 m.mole). The mixture was stirred for one hour under nitrogen, at room temperature, then quenched with saturated sodium carbonate solution (1 ml.). The organic layer was washed with water (2 ml.) dried, and chromatographed on two small silica plates with acetone-petrol, 1:9, to yield ergosterol (1), (Rf 0.4), (12mg. 45%).

Part 3

Column Chromatography of Sterol Residues.

Sterol residues of Saccharomyces cerivisiae, (supplied by Koninkelijke Nederlandsce Gist en Spiritus Fabriek N.V.Holland), (45g.), were stirred in a 20% mixture of petrol in benzene (300 ml.), for 30 minutes, to give a suspension which was centrifuged. The supernatent solution was evaporated. The resultant residue was taken up in 50% petrol-benzene, (300 ml.). This solution was centrifuged, and added to a column of 6cm. diameter, packed with aluminium oxide, (Grade 3), (1500g), suspended in 50% petrol-benzene. The column was eluted with 50% petrol-benzene, (3 litres), until the first four fractions had been removed, then with benzene (10 litres). and finally, dichloromethane (4 litres). Fractions of 20 ml. each were collected with a fraction collector. The fractions were examined by t.l.c. on silica plates with a solvent system of 20% ethyl acetate in benzene.

TABLE 1

Fraction	Solvent			Yield
	type	ratio V/V	volume in ltr.	
	b-p	50-50	3	
1	"	"	1.5	0.6 g.
2	"	"	6.5	0.45 g.
3	"	"	12	5.9 g.
3+4	"	"	3	1.2 g.
4	"	"	5	1.2 g.
4+5	b_1	100	10	2.5 g.
5	d	100	4	15.0 g.

Abbreviations used; b. benzene, p. petrol, d. dichloromethane.

Fractions 1,2,4,5 were shown to be squalene, 4,4-dimethylsterols, 4α-mono-methylsterols, and 4-desmethylsterols, by comparison of t.l.c. and spectral properties, with those of samples previously isolated and

identified.[4]

Investigation of Fraction 2

Fraction 2 was a light yellow oil. υ_{max} (CCl_4) 3650,2950,2880, 1660,1460,1382 $cm.^{-1}$ τ, ($CDCl_3$) 4.92 (4H,(?) s.), 6.38 (1H, t. J 3.5Hz) 7.72-7.80 (4H,(?) m.), 7.94,7.98 (16H,(?) 2s.), 8.33,8.39 (16H,(?) 2s.). τ, (C_6D_6) 4.82 (4H,(?) s.), 6.62 (1H, t. J 3.5 Hz.), 7.90 (18H,(?) s.), 8.31,8.45 (16H,(?) 2s.) m/e 426, (M+) (7.2%), 408 (7.2%), 382 (12.0%), 367 (7.2%), 271 (8.4%), 269 (14.5%), 117 (100%)

[Found C, 84.47% H, 11.71% $C_{30}H_{50}O$ requires C, 84.44% H, 11.81%.]

Hydrogenation of Fraction 2

Platinum oxide (17mg.), was shaken in ethyl acetate (8ml.) in an atmosphere of hydrogen, until reduction was complete. The unknown oil (21mg. 0.05 m.mole) was added. The suspension was shaken until uptake of hydrogen ceased, after the consumption of 4.8 equivalents of hydrogen. The suspension was filtered, and the solvent was evaporated to leave an oil, (20.8mg. 97%). υ_{max} (film), 3630,2490,2860,1360,1465,1160,1050 $cm.^{-1}$ τ, 8.74 (31-35H, m.), 9.14 (15H, m.), 8.03 (16H, m.) 8.37 (6H, m.) m/e 436 (M^+) (13.1%), 421 (41%), 420 (100%), 418 (59%).

Acetylation of Fraction 2

The alcohol (50mg. 0.12 m.mole), was stirred with acetic anhydride (1ml) and pyridine (1 ml.), at room temperature for two minutes. Water (5 ml.) was added. The suspension was extracted with benzeze (3x5ml.). The extract was dried, concectrated to 1 ml. and chromatographed on two small silica plates in petrol:benzene, 1:1. The band at Rf. 0.9 was extracted with dichloromethane, to yield the acetate (14mg. 24%). υ_{max} (film) 3020,2980,2890,1724,1660,1460,1365 $cm.^{-1}$ τ, 4.93 (4-5H, s.), 5.95 (1H, t. J 6Hz.), 7.96 (3H, s. O-Ac), 8.00 (16H,(?) s.), 8.34 (12H,(?) s.)

8.42 (3H, s.).

Oxidation of Fraction 2

Chromic anhydride (0.12g. 12,5 m.mole) was added, slowly, with stirring and cooling at 0°C, to pyridine (1 ml.). To the suspension so formed was added fraction 2 (0.1 g. 0.24 m.mole), in benzene (1ml.). The mixture was stirred at room temperature for two hours, before addition to water (10 ml.), and extraction with benzene (3x10 ml.). The organic extract was washed with water (10 ml.), dried, concentrated to 1 ml., and chromato - graphed on one large silica plate, with a solvent system of benzene:petrol 1:1. The band at Rf 0.6 was eluted withacid-free dichloromethane, to yield an oil, (15mg. 15%). υ_{max} (film) 3032,2960,2910,2850,2714,1719,1660, 1430,1382 $cm.^{-1}$ τ, 4.97 (4-5H, s.), 8.06 (20H,(?) s.), 8.39 (15H,(?) s.), 8.46 (3H, s.).

Autoxidation of ketone from Fraction 2 [5]

The ketone (15mg. 0.03 m.mole), was shaken with a 1 N solution of potassium tert. butoxide in tert. butanol (2.5 ml.), for 30 minutes, in O_2 ir which time there was continuous absorption of two moles of oxygen. The solution was poured into pH 6 buffer (10 ml.), and extracted with ether (2x10 ml.). T.l.c. showed a continuous series of products. The crude reaction mixture showed only end absorption in the u.v. spectrum.

Formylation of the Ketone from Fraction 2 [6]

The ketone (13mg. 0.03 m.mole), in dry pyridine (1 ml.), was treated under nitrogen, with ethyl formate (0.08 ml. 1 m.mole), followed by a solution of sodium (6.6 mg. 0.3 m.mole), in methanol (0.06 ml.). The solution was stirred overnight before being poured into acetic acid (o.75ml) in water (7 ml.). The suspension was extracted with benzene (2x5 ml.). After the usual treatment,this was chromatographed on one small silica plate. The only band visualised with H_2SO_4 showed no absorption in the u.v.

Part 4

Reaction of 5α-Lanost-8-ene-3-oxime with 4-Phenyl-1,2,4-triazoline-3,5,-dione

To the oxime (7)[7], (35mg. 0.08 m.mole), in anhydrous dichloromethane (3ml.) under nitrogen, was added a solution of the triazoline (1)[8] (14mg. 0.08 m.mole), in dichloromethane (1 ml.), at room temperature. The red solution was stirred at 40°C for two hours, until the colour was exhausted. T.l.c. showed (a) starting material, (b) one less polar product, (c) one product more polar than (7), and material at the origin. The cloudy solution was filtered, concentrated to one ml., and chromatographed on three small silica plates, in 20% ethyl acetate in benzene . Band (c), Rf 0.2, was removed with chloroform, and yielded a

(4mg. 11%) υ_{max} 3380,1760,1720,1705,1600,1510 cm.$^{-1}$ λ_{max} 236 (16,000)

5α-Lanost-8-ene-3-phenylhydrazone (8)

To 5α-lanost-8-en-3-one (10)[7], (1.02g. 2.4 m.mole), in refluxing ethanol (10 ml.), acetic acid (1 ml.), and water (0.2 ml.), was added phenylhydrazine (0.8g. 7.5 m.mole). The solution was refluxed under nitrogen for 20 minutes and cooled to yield pale yellow plates (1.10 g. 92%). M.p. (from ethanol) 126-128°C, $(\alpha)_D^{25}$ +18.7 (c, 0.15), ν_{max} 3400,2950,1605, 1514,1252,760,700 cm.$^{-1}$ λ_{max} 274.7 (24,500),nm. τ, 2.65 (2H, m. aromatic H) 2.80 (1H, s. N-H), 2.94 (3H, m. aromatic H), 8.70 (3H, s. 19-H) 8.88,8.91 (6H, 2s. 4,4-H), 9.11 (3H, s. 14-H), 9.12 (9H, d. J 6 Hz. 21,26,27-H), 9.28 (3H, s. 18-H). m/e 516 M^+ (100%), 411 (36%), 215 (40%), 171 (46%).

Found C, 83.78% H, 10.80%, N, 5.37%. Calculated for $C_{36}H_{56}N_2$, C, 83.66, H, 10.92%, N, 5.42%.

5α-Lanost-8-ene-3-p-nitrophenylhydrazone (17)

p-Nitrophenylhydrazine (0.55g. 3.7 m.mole), was added to lanostenone (10)

(1.55 g.3.6 m.mole), in refluxing ethanol (15 ml.), acetic acid (1 ml.) and water (0.3 ml.). The solution was refluxed under nitrogen for 20 minutes, and cooled to yield a yellow solid (1.98g. 97%). m.p. (from ethanol), 144-146°C $(\alpha)_D^{25}$ +13.4 (c, 0.167), υ_{max} 3300, 2950,1600, 1338,1272,1120,856 $cm.^{-1}$ λ_{max} 250.5 (10,500), 395 (23,800) nm. τ, 1.89,2.97 (4H AB J 9.0 Hz. aromatic-H), 2.24 (1H, s. N-H), 8.71 8.88,9.06,9.11,9.16,9.28 (24H, 4,4,19,14,21,26,27,18-H). m/e 561 (M^+) (16%), 546 (8%), 531 (6%), 410 (5%), 260 (100%).

[Found C,76.96% H, 9.87% N, 7.48%. Calculated for $C_{36}H_{55}N_3O_2$ C, 76.67% H, 9.75% N, 7.40%.]

Reaction of 5α-Lanost-8-ene 3-phenylhydrazone with 4-Phenyl-1,2,4-triazoline-3,5-dione

The phenylhydrazone (8), (133mg. 0.26 m.mole), was stirred in acid-free dichloromethane (5 ml.), with the triazoline (1), (45mg. 0.26 m.mole) for five minutes, at room temperature. The solution was concentrated to 2 ml. and chromatographed on one large silica plate in acetone-petrol, 1:9. The band Rf 0.2 was extracted with dichloromethane, to give a glass (67mg.) which resisted crystallisation λ_{max} 271.5 (9,660) 402 (120) nm. τ, 2.4-2.7 (9H, m. aromatic H), 8.80 (6H, s. 4,4-H), 8.88 (3H, s. 19-H) 9.11 (3H, s. 14-H), 9.15 (9H, d. J 5 Hz. 21,26,27-H), 9.38 (3H, s. 18-H)

Reaction of 5α-Lanost-8-ene 3-phenylhydrazone withIodine and Triethylamine

The phenylhydrazone (8), (195mg. 0.38 m.mole), in tetrahydrofuran (9 ml) and triethylamine (0.72 ml.), was treated with a solution of iodine (700mg 2.7 m.mole), in the same solvent (3.6 ml.). The solution was stirred for 15 minutes before filtration of the white precipitate,formed in the reaction The brown filtrate was evaporated, and the residue was taken up in benzene

(10 ml.), leaving a thick red oily residue. The benzene solution was concentrated to 2 ml. and chromatographed on one large silica plate in 5% acetone in petrol. The least polar, red, band Rf 0.9, was removed with dichloromethane. Evaporation yielded 3-phenylazo-5α-lanosta-2,8-diene (15) (68mg. 35%). M.p. 129-131°C, $(\alpha)_D^{26}$ -11.1 (c, 0.15), υ_{max} 2880,1636, 1475,1460,1390,1380,700, $cm.^{-1}$ λ_{max} 313 (9,800), 437 (440) nm.

[M^+ at 514,4268 Calculated for $C_{36}H_{54}N_2$ 514.4287]

The second product showed a u.v. absorption at λ_{max} 273 nm., but decomposed before any further measurment could be made.

Reaction of 5α-Lanost-8-ene-3-phenylhydrazone with Iodine and Potassium Tert. Butoxide

To a suspension of potassium tert. butoxide (35mg.), in glyme (4ml.) - distilled from lithium aluminium hydride under nitrogen,-was added the phenylhydrazone(61mg. 0.13 m.mole), then iodine (29mg. 0.12 m.mole). The colour of the solution initially dark red, faded to brown then progressed to reddish brown in one minute. T.l.c. showed the two products obtained in the triethylamine reaction, and another, slightly less polar than the starting material. The solution was poured into water (10 ml.), and extracted with benzene (3x10 ml.). This extract was washed with water (20 ml.), dried, concentrated to 2 ml. and chromatographed on four small silica plates, with 5% ethyl acetate in petrol. The third band Rf 0.4 was extracted to yield a brown glass (6mg. 10%)

υ_{max} 3450,2950,1600,1503,1476,1390 $cm.^{-1}$ λ_{max} 385 (9,000 (?)) nm.

m/e 514 (M^+)

Reaction of 5α-Lanost-8-ene-3-p-nitrophenylhydrazone with Iodine and Potassium Tert. Butoxide

The arylhydrazone (17), (50mg. 0.09 m.mole) was added to potassium tert. butoxide (70mg. 0.6 m.mole), in glyme (5 ml.), to give an

immediate red colouration. Iodine (22.5mg. 0.09 m.mole), was added after two minutes, to produce a purple colour. After five minutes stirring at room temperature, the solution was poured into 5% aqueous acetic acid (10ml) The suspension was extracted with benzene, (3x15 ml.). The extract was washed with water (20 ml.), dried, and evaporated to yield 5α-lanosta-1,8-diene-3-p-nitrophenylhydrazone (59mg. 85%), m.p. (from ethanol) 178-181°C, $(\alpha)_D^{26}$ +14.1 (c, 0.09), ν_{max} 3320,1600,1475,1310,1275,1115, 876 $cm.^{-1}$, λ 235 (9,700), 297(5,010), 327(4,750), 405(29,950)nm., τ, 2.07 (1H, s. N-H), 1.87,2.96 (4H, AB J 9Hz. aromatic H), 3.39 (1H, s. H-2), 3.60 (1H, s.(?) H-1), 8.70 (3H, s. H-4), 8.83 (6H, s. H-4,19) 9.08 (9H, d. J 6Hz. H-21,26,27), 9.09 (3H, s. H-14), 9.27 (3H, s. H-18) m/e 559, (M^+), (14%), 504 (2%), 529 (14%), 408 (20%), 189 (100%)

[Found C, 77.06% H, 9.39% N, 7.39% Calculated for $C_{38}H_{53}N_3O_2$
C, 77.24% H, 9.54% N, 7.51%]

The melting point was identical to, and undepressed on admixture with, the p-nitrophenylhydrazone of authentic 5α-lanosta-1,8-dien-3-one.[9]

Reaction of 5α-Cholestane-3-p-nitrophenylhydrazone with Iodine (1mole) and Potassium Tert. Butoxide.

To the arylhydrazone (21), (115mg. 0.22 m.mole), in dry,oxygen-free glyme (5 ml.), was added potassium tert. butoxide (120mg. 1.1 m.mole), and iodine (65mg. 0.25 m.mole). T.l.c. after 30minutes showed two products less polar than the starting material, and one slightly more polar. The solution was poured into 5% aqueous acetic acid, and extracted with benzene (3x15 ml.). The extract,after washing as above, was chromatographed on six small silica plates with 25% ethylacetate in petrol. Three bands were extracted.

Band 1 Rf 0.9 λ_{max} 399 nm.

Band 2 Rf 0.85 λ_{max} 404 nm.

Band 3 Rf 0.4 λ_{max} 250,399 nm.

Band 3 υ_{max} 3410,3000,1600,1520,1120,860 $cm.^{-1}$ τ, 2.40 (1H, s. N-H), 1.90,3.02 (4H, AB J 9Hz. aromatic H), 6.24 (1H,(?) s.(?) H-4(?)), 8.90 (3H, s. H-19), 9.15 (9H, d. J 6Hz. H-21,26,27), 9.30 (3H, s. H-18). m/e 519 (M^+)

Reaction of 5α-Cholestane-3-p-nitrophenylhydrazone with Iodine (2 moles) and Potassium Tert. Butoxide.

To the arylhydrazone (21), (101mg. 0.19 m.mole), in dry, oxygen-free glyme (6 ml.), was added iodine (49mg. 0.19 m.mole), to give a brown solution. Potassium tert. butoxide (44mg. 0.38 m.mole), on addition, coloured the solution initially dark red, and after two minutes, reddish-brown. T.l.c. -with 25% ethyl acetate in petrol,- showed two products Rf 0.8,0.9 (Rf of starting material 0.6). After 20 minutes, potassium tert. butoxide (22mg. 0,19 m.mole), was added, to give an immediate purple colour. T.l.c. after three minutes showed partial formation of a product corresponding to the unsaturated arylhydrazones (24), (25). After 20 minutes, this was the only product visible. Iodine (59mg. 0.19 m.mole) was added to colour the solution dark brown. T.l.c. of the reaction mixture 10 minutes after the iodine addition, showed one less polar product, and some brown material at the origin. Potassium tert. butoxide (150mg. 0.94 m.mole), was added to regenerate the purple colour. T.l.c. after 30 minutes, showed one product Rf 0.6 , and material at the origin. The reaction was worked up by pouring into 5% aqueous acetic acid, (10 ml.), extracting with benzene (3x15 ml.), and filtering through a 5 cm. column of aluminium oxide (Grade 3), with benzene. Evaporation of the solvent left a red gum, which was solidified from ethanol-water. (86mg. 85%) m.p. (from nitromethane) 164-169°C, $(\alpha)_D^{25}$ +10.8 (c, 0.104). υ_{max} 2960, 1600,1478,1336,1110,840 $cm.^{-1}$ λ_{max} 226.6(16,350), 248(11,900), 420.5 (39,800) nm. τ, 1.86,2.96 (4H, AB J 9Hz. aromatic-H), 3.50 (0-1H, s.(?)), 3.76 (1-2H, m.), 9.07 (3H, s. H-19), 9.14 (9H, d. J 6Hz.

H-21,26,27), 9.30 (3H, s. H-18). m/e 517 (M^+).

3-p-Nitrophenylazo-5α-cholestane (29)

5α-Cholestane-3-p-nitrophenylhydrazone (21),[10] (69mg. 0.13 m.mole) in glyme (5 ml.), was stirred at room temperature for 10 minutes with potassium tert. butoxide (200mg. 1.7 m.mole). The reaction was quenched with water (25 ml.), and extracted with ether (40 ml.). The extract was washed with water (2x20 ml.), dried, and chromatographed on four small silica plates, with 15% ethyl acetate in petrol. The more polar band, yellow in colour, yielded the starting material (29mg. 43%). The less polar, red, band after extraction with dichloromethane, yielded the phenylazo isomer (19mg.29%) as red plates. m.p. (from ethanol) 203-204°C, $(\alpha)_D^{26}$ +58 (c, 0.44) υ_{max} 2950,1640,1538,1470,1350,870 $cm.^{-1}$, λ_{max} 328(25,750), 436(372) nm. τ, 1.74 2.22 (4H, AB J 9Hz. aromatic H), 3.0 (1H,(?) s.(?) H-3), 9.15 (9H, d. J 7 Hz. H-21,26,27), 9.21 (3H, s. H-19), 9.33 (3H, s. H-18). m/e 519 (M^+), (100%), 489,(22%), 382,(11%), 383, (11%).

[Found C, 75.73% H,9.69% N, 7.99%. Calculated for $C_{33}H_{51}N_3O_2$
C, 75.96% H, 9.85% N, 8.05%.]

Reaction of 5α-Lanost-8-ene-3-p-nitrophenylhydrazone with Lead Tetraacetate.

The arylhydrazone (17), (90mg. 0.16 m.mole), in benzene (5 ml.),and pyridine(0.5 ml.),was stirred for two minutes with lead tetraacetate (90 mg. 0.20 m.mole). After filtration, the pyridine was azeotroped off with toluene. The residue in toluene(1 ml.) was chromatographed on five small silica plates with 10% ethyl acetate in petrol. The two yellow bands, Rf 0.6, 0.5 were extracted with dichloromethane. The less polar band yielded 3β-acetoxy-3α-p-nitrophenylaxo-lanost-8-ene (31), (11.3mg. 11%), m.p. (from ethanol), 173-176°C, $(\alpha)_D^{25}$ +12.5 (c, 0.08). λ_{max} 282(17;650), 420)364) nm. τ, 1.71,2.28 (4H, AB J 9 Hz. aromatic H),

7.86 (3H, s. O-Ac), 8.89 (3H, s. H-4α), 9.01 (3H, s. H-19),9.08 (3H, s. H-14), 9.12 (9H, d. J 5 Hz H21,26,27), 9.16(?) (3H, s. H-4β) 9.29 (3H, s. H-18), m/e 561 , (35%), 559 (25%), 544(25%), 260 (100%)

[Found C, 73.69% H, 9.28% N, 6.78%. Calculated for $C_{38}H_{57}N_3O_4$
C, 73.63% H, 9.27% N, 6.78%.]

The more polar band yielded 3α-acetoxy-3β-p-nitrophenylazo-lanost-8-ene (30) (40mg. 44%) m.p. (from ethanol) 217-219°C, $(\alpha)_D^{26}$ -14.5 (c, 0.09) λ_{max} 282.5(17500), 420(294) nm. τ, 1.66,2.30 (4H, AB J 9 Hz.), 7.83 (3H, s. O-Ac), 8.78 (3H, s. H-4β), 8.85 (3H, s. H-4α), 9.10 (3H, s. H-14), 9.13 (9H, d. J 6 Hz. H-21,26,27,), 9.25 (3H, s. H-19), 9.36 (3H, s. H-18). m/e 559 (6%), 543 (6%), 469 (12%), 427 (60/), 409 (100%).

[Found C, 73.62% H, 9.23% N, 6.89%. Calculated for $C_{38}H_{57}N_3O_4$
C, 73.63% H, 9.27% N, 6.78%.]

Regeneration of 5α-Lanost-8-en-3-one from the p-Nitrophenylhydrazone via the Azoacetates.

The crude mixture of azoacetates, prepared by treatment of 5α-lanost-8-ene-3-p-nitrophenylhydrazone (17), (60mg. 0.10 m.mole), with lead tetraacetate (45mg. 0.10 m.mole), as described above, was refluxed in 2% potassium hydroxide in ethanol (10 ml.), for two hours. Addition of water (1 ml.), precipitated a white solid, after cooling. Recrystallisation from ethanol yielded the ketone (10), (32mg. 80%).

Regeneration of 5α-Lanost-8-en-3-one from the p-Nitrophenylhydrazone with Iodine and Oxygen.

The arylhydrazone (17), (13mg. 0.025 m.mole), in ether (2 ml.), was stirred with iodine (6.3mg. 0,025 m.mole), for five minutes. Benzene (3 ml) was added, and the solution was shaken in an oxygen atmosphere for 10 minutes. Filtration of the solution through aluminium oxide (15g), (Grade 3)

with benzene (40 ml.),yielded lanostenone (8.8mg. 85%).

Autoxidation of 5α-Lanost-8-ene-3-p-nitrophenylhydrazone.

The arylhydrazone (17), (57mg. 0.1 m.mole), in benzene (5 ml.), was shaken in an atmosphere of oxygen, until absorption ceased in two hours. The solution was evaporated to yield the 3α-hydroperoxy-3β-p-nitrophenylazo-lanost-8-ene (32), (60mg 99%), m.p. (From ethanol) 151-153°C (dec) $(\alpha)_D^{26}$ + 26.6 (c, 0.236), ν_{max} 3420,2950,1610,1600,1540,1475,1382,1356, 875 $cm.^{-1}$ λ_{max} 281(20,580), 425(348) nm. τ,0.95 (1H, s. O-H), 1.54,2.15 (4H, AB J 9Hz. aromatic-H (, 8.74 (3H, s. H-4β), 8.80 (3H, s. H-4α), 9.11 (3H, s. H-14), 9.12 (3H, d. J 6 Hz. H-21,26,27) 9.16 (3H s. H-19) 9.26 (3H, s. H-18). m/e 426

[Found C, 73.01% H, 9.40% N, 6.82%. Calculated for $C_{36}H_{55}N_3O_4$
C, 72.81% H, 9.34% N, 7.08%.]

3α-Hydroxy-3β-p-nitrophenylazo-5α-lanost-8-ene ( )

The hydroperoxide (32), (28mg. 0.046 m.mole),in benzene (1 ml.), was stirred with tris-diethyl-triaminophosphine (0.1 ml.), for 30 seconds. The solution was filtered through aluminium oxide (10 g.), (Grade 3), with benzene (50 ml.). Evaporation yielded the yellow azo alcohol (26mg. 96%) m.p. (from ethanol), 149-151°C (dec.), $(\alpha)_D^{26}$ + 23.2 (c, 0.47), ν_{max} 3460,2950,1618,1550,1480,1385,1360,875 $cm.^{-1}$, λ_{max} 284.5 (18,800), 396 (376) nm. τ, 1.61,2.16 (4H, AB J 9 Hz. aromatic H), 5.36 (1H, s. O-H), 8.75 (3H, s. H-4β), 8.81 (3H, s. H-4α), 9.13 (3H, s. H-14), 9.14 (9H, d. J 6 Hz. H-21,26,27), 9.27 (3H, s. H-19), 9.39 (3H, s. H-18). m/e 426

[Found C, 74.80% H, 9.56% N,7.27%. Calculated for $C_{36}H_{55}N_3O_3$
C,74.83% H, 9.59% N,7.27%.]

Autoxidation of 5α-Lanosta-1,8-diene-3-p-nitrophenylhydrazone

The arylhydrazone (20), (23mg. 0.05 m.mole), was autoxidised as described above, for 12 hours, to give the hydroperoxide, (24 mg. 96%), m.p. (from ethanol), 190-193°C, $(\alpha)_D^{25}$ - 19.4 (c, 0.04), ν_{max} 3600, 3000, 1600,1350,870 $cm.^{-1}$ λ_{max} 281 (19,800), 327 (33,400), 346 (26,000) nm. m/e 591

[Found C, 72.90% H, 8.87% N, 6.99%. Calculated for $C_{36}H_{53}N_3O_4$
C, 73.06% H, 9.03% N, 7.10.]

Pyrolysis of 3α-Hydroperoxy-3α-p-nitrophenylazo-lanost-8-ene

(a) in benzene

The hydroperoxide (32), (60mg. 0.10 m.mole), was refluxed in benzene (5 ml.) for 48 hours. The solution was chromatographed on one large silica plate, with four developments in 4% ethyl acetate-petrol. The intense u.v. opaque band at Rf 0.8, and the less intense band following, were both extracted with dichloromethane. Band 1 yielded 4-nitrodiphenyl (15mg. 70%) m.p. (from ethanol) 112-115°C (lit.[11] m.p. 113.7), υ_{max} 3080,1600,1360,868 868 $cm.^{-1}$ λ_{max} 223 (9,000), 305 (15,000) nm. , 1.73,2.30 (4H, AB J 9 Hz.) 2.45-2.50 (5H, m.). m/e 199

(b) in ethanol

The same decomposition was carried out in refluxing ethanol for 12 hours. The same chromatographic work-up yielded nitrobenzene (6mg. 50%) , identical to an authentic sample, in t.l.c. behaviour, and spectral data. In both the above pyrolyses, lanostenone was obtained in 70% yield. m.p. was undepressed on admixture with an authentic sample.

References.

1 R.C.Anderson, R.Stevenson and F.S.Spring, J.Chem.Soc., 1952, 2901.

2 K.Sakai and K.Tsuda, Chem.and Pharm. Bull. (Japan), 1963, 11, 529.

3 M.H.Karger and Y.Mazur, J. Amer. Chem. Soc., 1968, 90, 3878.

4 U.M.Kempe, Ph.D thesis, University of London, 1971.

5 E.J.Bailey, D.H.R.Barton, J.Elks and J.F.Templeton, J. Chem. Soc., 1962, 1578.

6 R.O.Clinton, A.J.Manson, H.C.Neumann, R.L.Clarke, D.F.Page, W.B.Dickinson and Clarissa Carabateas, J. Amer. Chem. Soc., 1961, 83, 1478.

8 J.C.Stickler and W.H.Pirkle, J.Org. Chem., 1966, 31, 3444.

9 D.H.R.Barton, D.A.Lewis, and J.F.McGhie, J. Chem. Soc., 1957, 2907.

7 L.Ruzicka, R.Denss, and O.Jeger, Helv. Chim. Acta., 1945, 28, 759

10 J.Buckingham and R.D.Guthrie, J. Chem. Soc., 1968, 1445.

11 M.Gomberg and W.E.Bachmann, J. Amer. Chem. Soc., 1929, 49, 250.