CHEMICAL AND MICROBIOLOGICAL STUDIES OF ALICYCLIC SYSTEMS

A Thesis submitted to the

Board of the Faculty of Physical Sciences

in partial fulfilment

of the requirements for the degree of

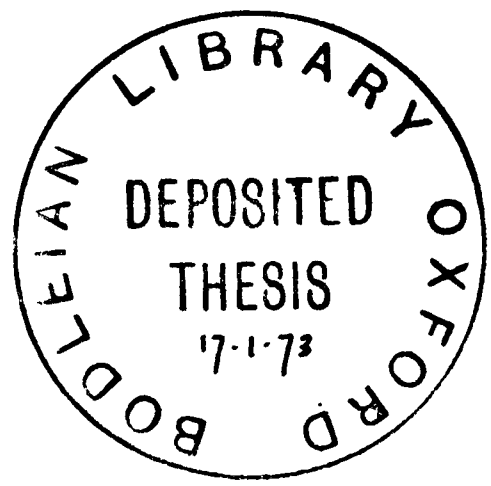
Doctor of Philosophy

in the

University of Oxford

by

Ian Maxwell Clark



Balliol College,

Oxford.

The Dyson Perrins Laboratory,

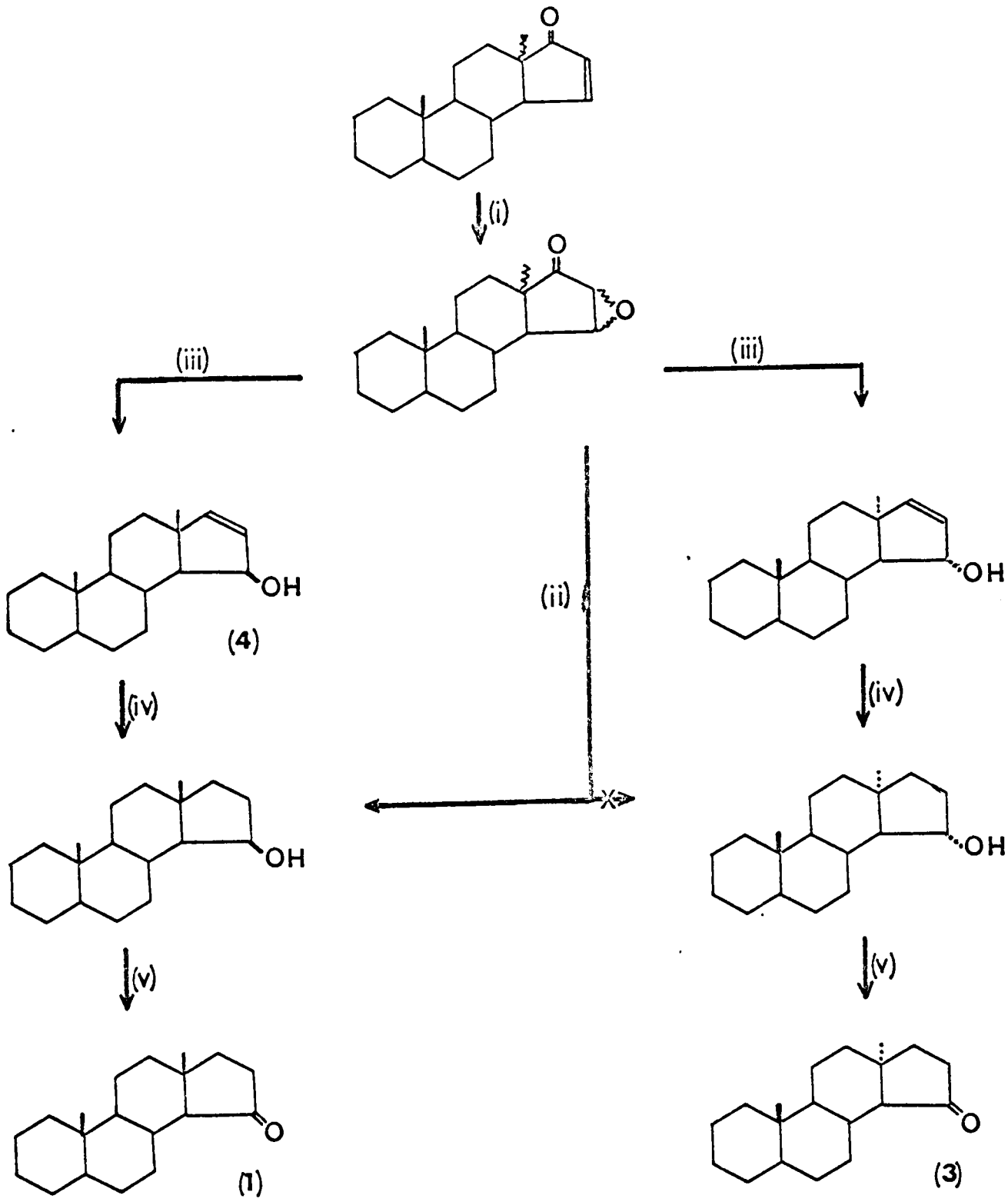
Oxford.

August 1972

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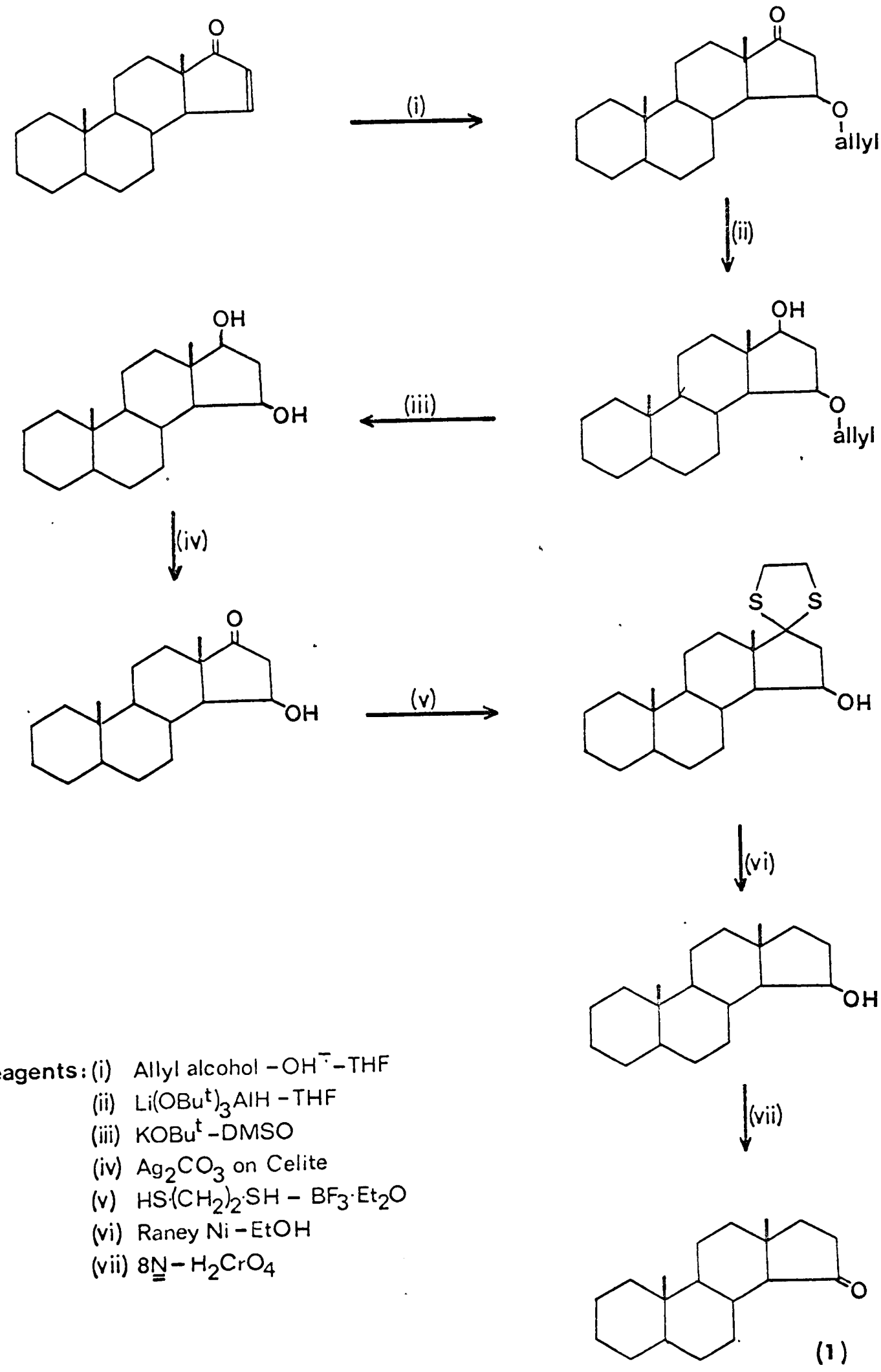
ABSTRACT

SCHEME 1.



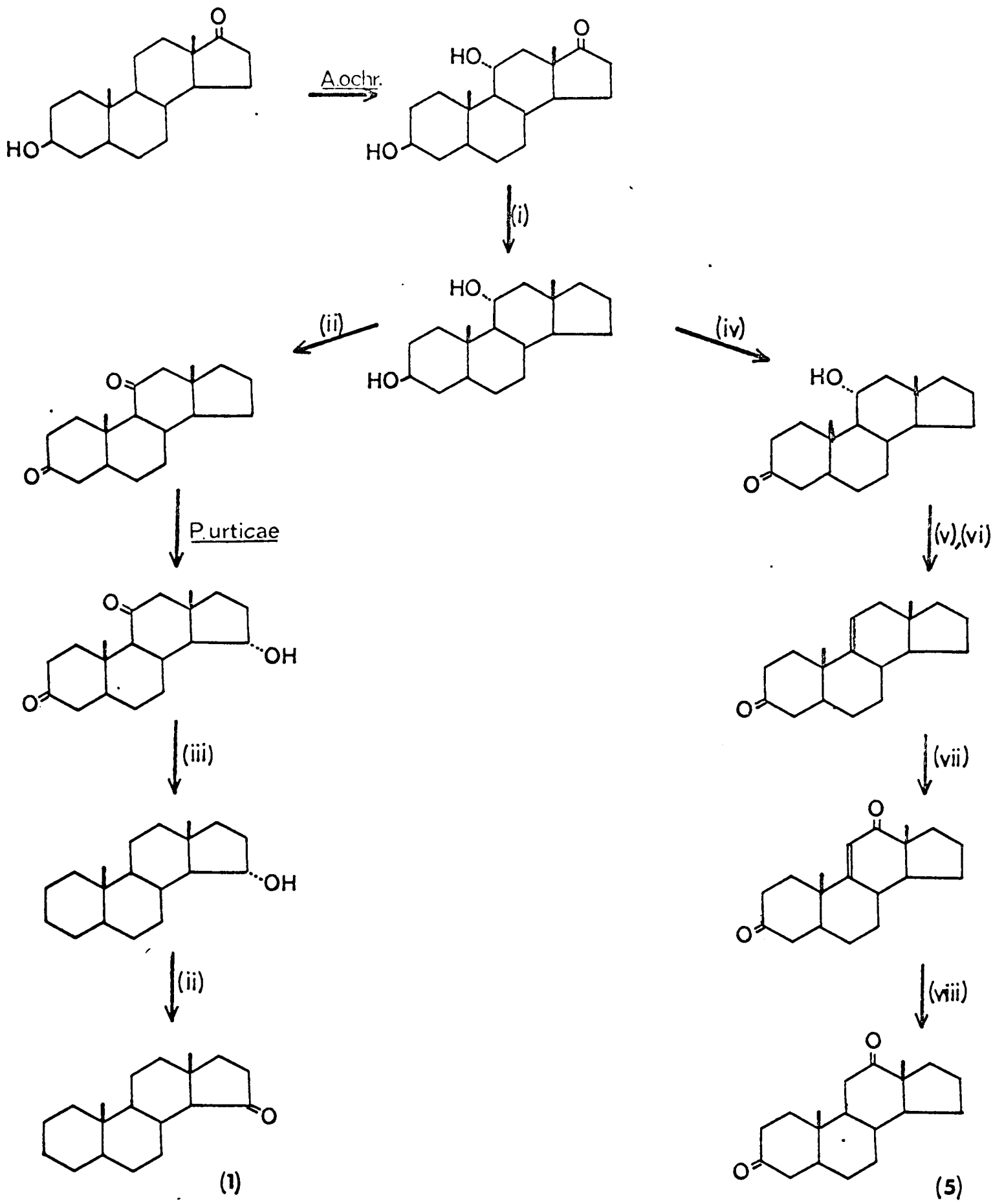
Reagents: (i) $H_2O_2 - OH^-$
(ii) $N_2H_4 - H^+$; air
(iii) $N_2H_4 - H^+$; N_2
(iv) Pd on C – H_2
(v) 8N – H_2CrO_4

SCHEME 2.



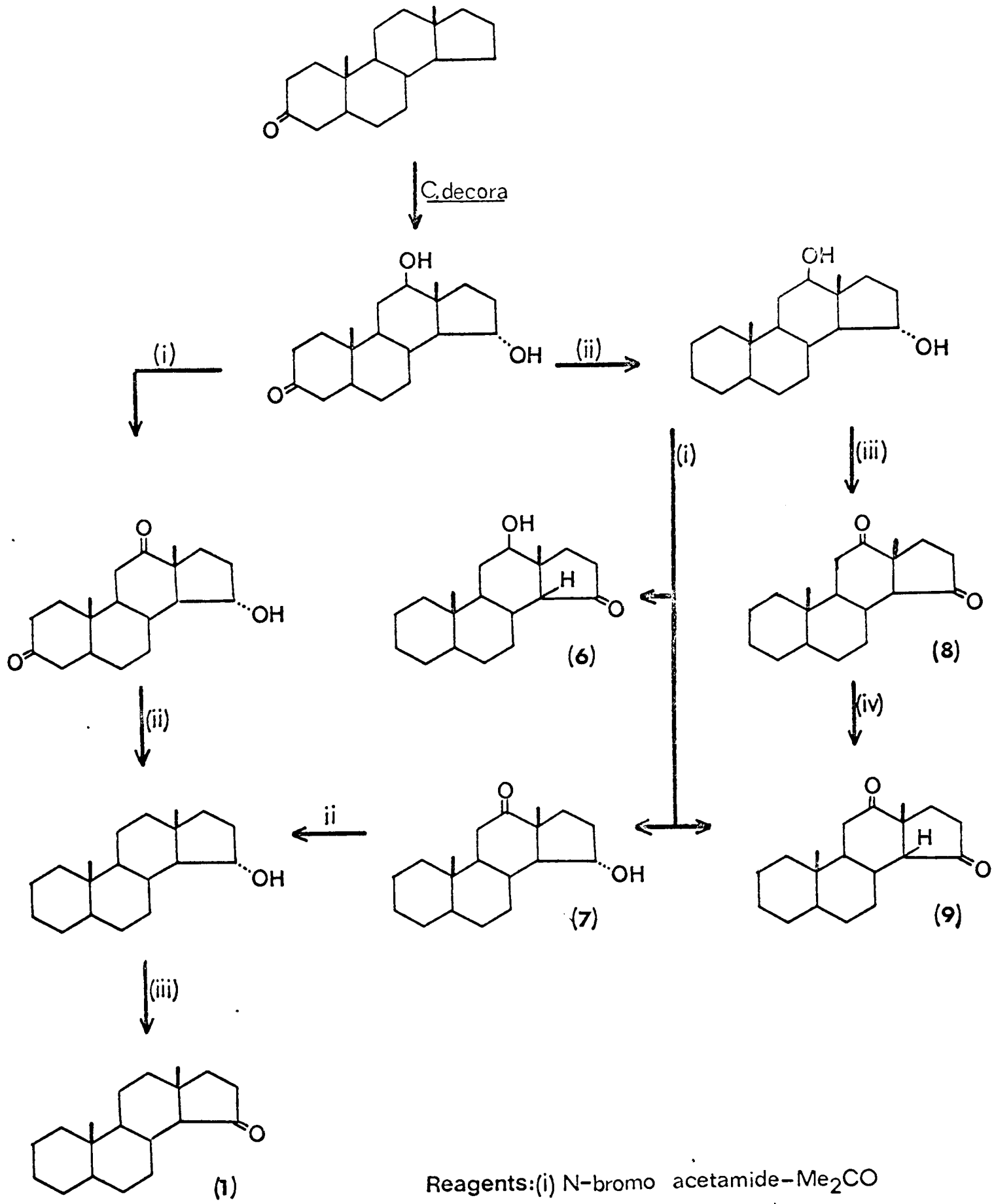
Reagents: (i) Allyl alcohol $-OH^-$ - THF
(ii) $Li(OBu^t)_3AlH$ - THF
(iii) $KOBu^t$ - DMSO
(iv) Ag_2CO_3 on Celite
(v) $HS{\cdot}(CH_2)_2{\cdot}SH - BF_3{\cdot}Et_2O$
(vi) Raney Ni - EtOH
(vii) $8\underline{N} - H_2CrO_4$

SCHEME 3.



Reagents: (i) Huang–Minlon reduction
(ii) 8N – H_2CrO_4
(iii) Huang–Minlon reduction using forcing conditions
(iv) N-bromo acetamide – Me_2CO
(v) TsCl – C_5H_5N
(vi) LiCl – Li_2CO_3 – DMF
(vii) CrO_3 – AcOH – Ac_2O
(viii) Pd on C – H_2

SCHEME 4.



Reagents: (i) N-bromo acetamide–Me_2CO
(ii) Huang-Minlon reduction
(iii) $8\underline{N}-H_2CrO_4$
(iv) KOH-MeOH

In the microbiological studies initiated in this department nine years ago, an attempt has been made to gain more understanding of the processes relating structural variations in a steroidal substrate with the positions of microbiological hydroxylation. To this end, oxygenated steroids (mainly androstanes) have been prepared and incubated with selected micro-organisms.

The primary purpose of the present work was to synthesise 5α-androstan-15-one [1], 5α,14β-androstan-15-one [2], 5α,13α-androstan-15-one [3], and a series of simple oxygenated androstanes of unnatural configuration, and to incubate these compounds with the fungus Calonectria decora.

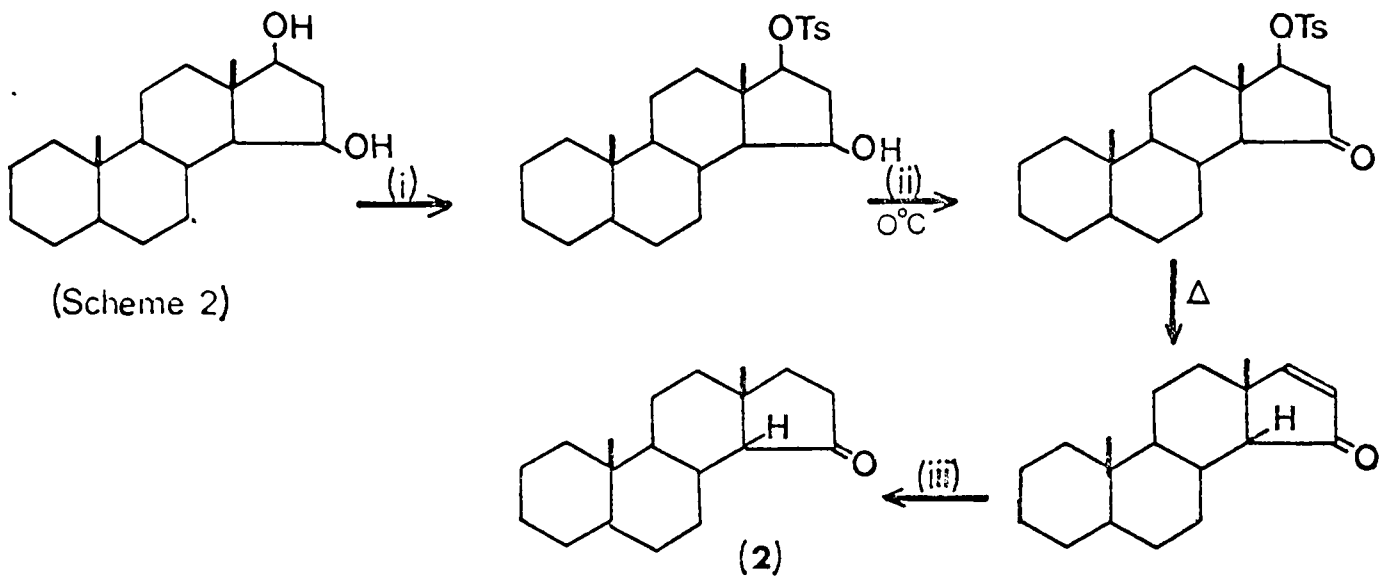
A related aim was to augment the utility of n.m.r. spectroscopy in elucidating the structures of steroid metabolites by a revision and an extension of the incremental C-methyl shift data recently published from this department.[1]

Synthesis of substrates

By modifying the procedures of Djerassi et al.[2] and Nambara et al.,[3] the isomeric 15-ketones [1] and [3] were prepared in satisfactory yields (Scheme 1). A key factor in the improved yields of 5α-androstan-15-one [1] was the development of conditions for an unusual in situ diimide reduction of 5α-androst-16-en-15β-ol [4].

Three new syntheses of 5α-androstan-15-one [1] were devised (Schemes 2, 3, and 4), all giving yields comparable with the previously reported method.[2] The alternative new chemical synthesis of 5α-androstan-15-one [1] is outlined in Scheme 2. The other two utilised the hydroxylating capacity of the fungi Aspergillus ochraceus and Penicillium urticae (Scheme 3) or Calonectria decora (Scheme 4); this work demonstrated that with a judicious choice of substrate and

SCHEME 5.

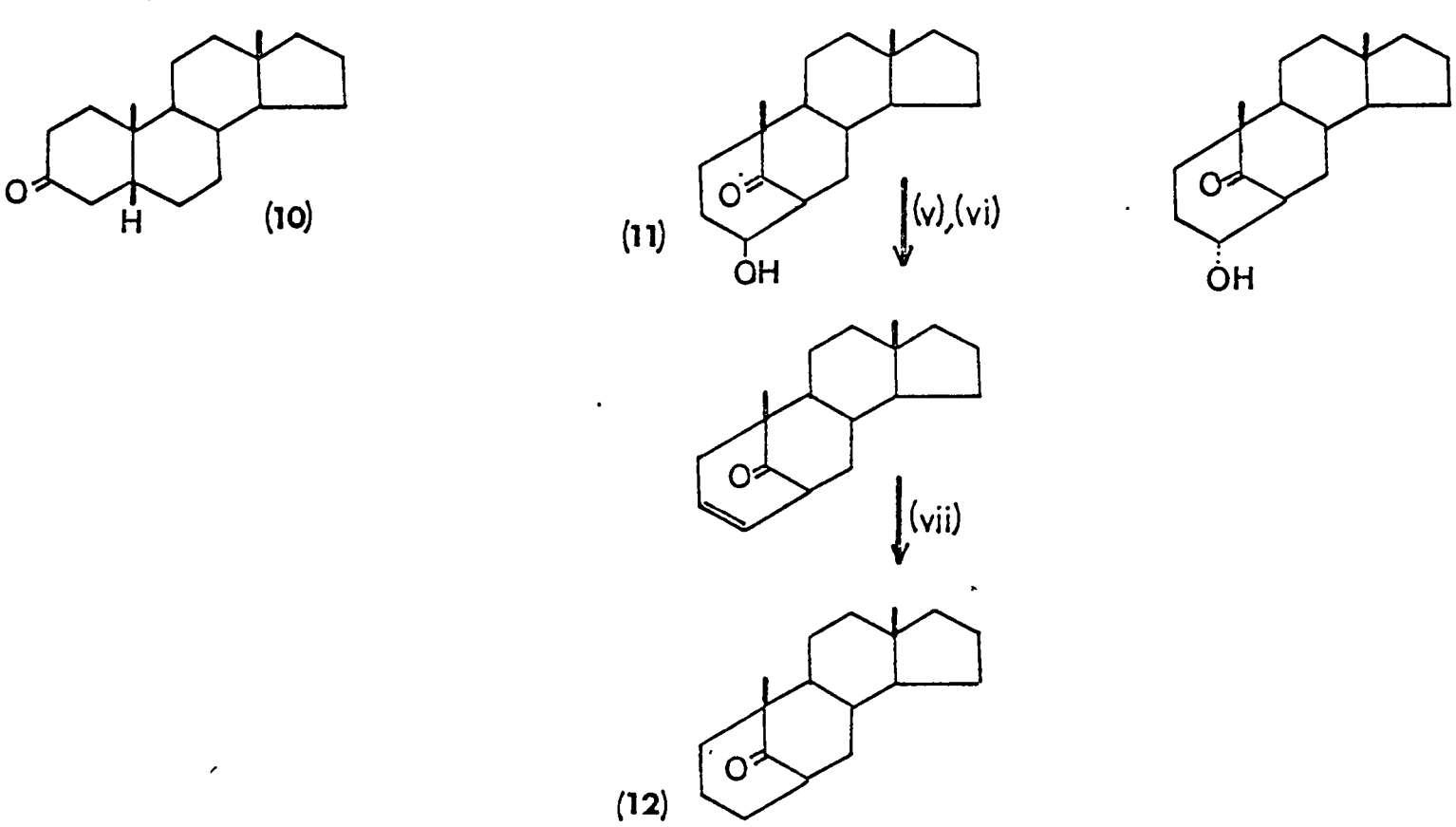


(Scheme 2)

(2)

Reagents: (i) TsCl – C_5H_5N; (ii) 8N – H_2CrO_4; (iii) Pd on C – H_2

SCHEME 6.



Reagents: (i) Pd on $CaCO_3$ – H_2 (ii) O_3–EtOAc; $NaHCO_3$ – H_2O_2
(iii) AcCl – Ac_2O (iv) $Li(OBu^t)_3AlH$ – THF
(v) TsCl – C_5H_5N (vi) LiCl – Li_2CO_3 – DMF
(vii) Pd on C – H_2

conditions, microbiological hydroxylation can often be effectively employed in synthetic work. Associated with the work in Scheme 3, optimum conditions for large scale fermentations with the fungus A.ochraceus were developed, and a synthesis of 5α-androstane-3,12-dione [5] much superior to that previously reported[4] was devised. Associated with the work in Scheme 4 were syntheses of the substrates 12β-hydroxy-5α,14β-androstan-15-one [6], 15α-hydroxy-5α-androstan-12-one [7], 5α-androstane-12,15-dione [8], and 5α,14β-androstane-12,15-dione [9].

A high-yield synthesis of 5α,14β-androstan-15-one [2] is shown in Scheme 5.

Syntheses of 5β-androstan-3-one [10], A-nor-(5⟶6β)abeo-androstan-5-one [12] and its 3β-hydroxy derivative [11] followed from the simple modification of established procedures,[5,6] and are outlined in Scheme 6.

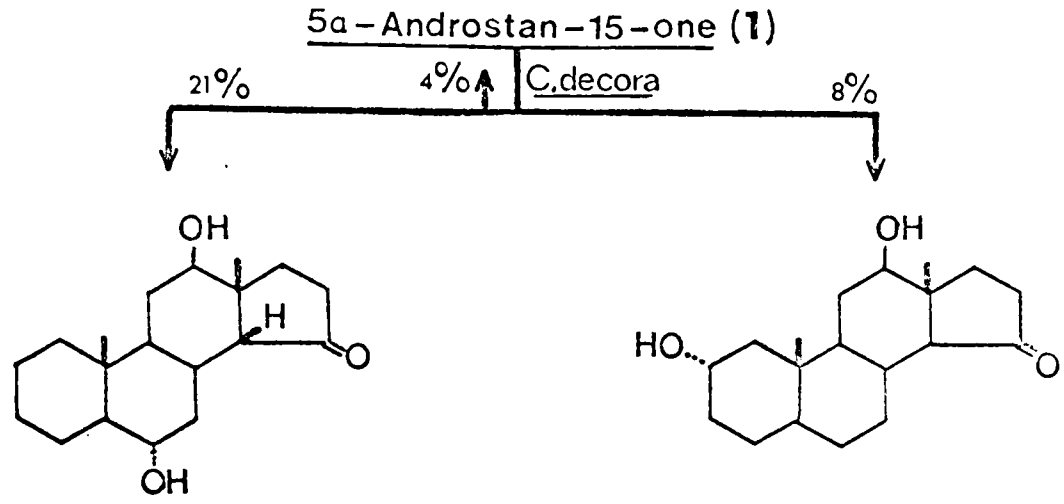
Hydroxylations with C.decora

The structures of the products of incubations with this fungus were assigned after an examination of the spectra of the compounds and their derivatives, and where possible, conversion of the products to known compounds.

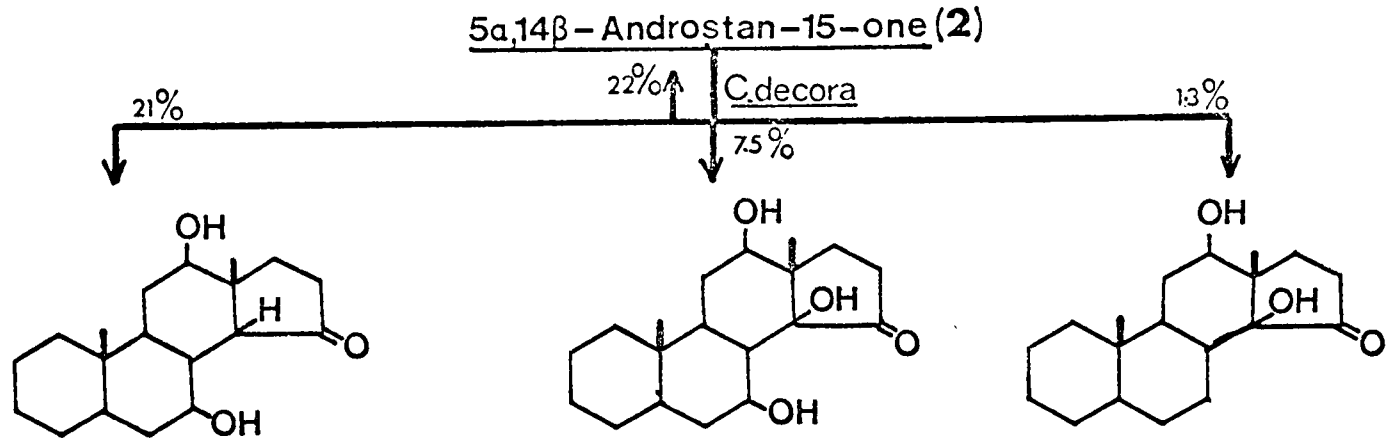
The incubation of 5α-androstan-15-one [1] with C.decora (Scheme 7) constituted the final experiment in a series related to the hydroxylation of 5α-androstane mono-ketones, where the position of the carbonyl group had been varied systematically around the androstane skeleton. It was surprising that in the hydroxylation of 5α-androstan-15-one [1], the major product was isolated as the 14β-isomer. The results for the whole series have been discussed in a recent paper,[7] and are not repeated here.

The result of incubating 5α,14β-androstan-15-one [2] (Scheme 8) prompted a more systematic study into the hydroxylation of substrates of unnatural

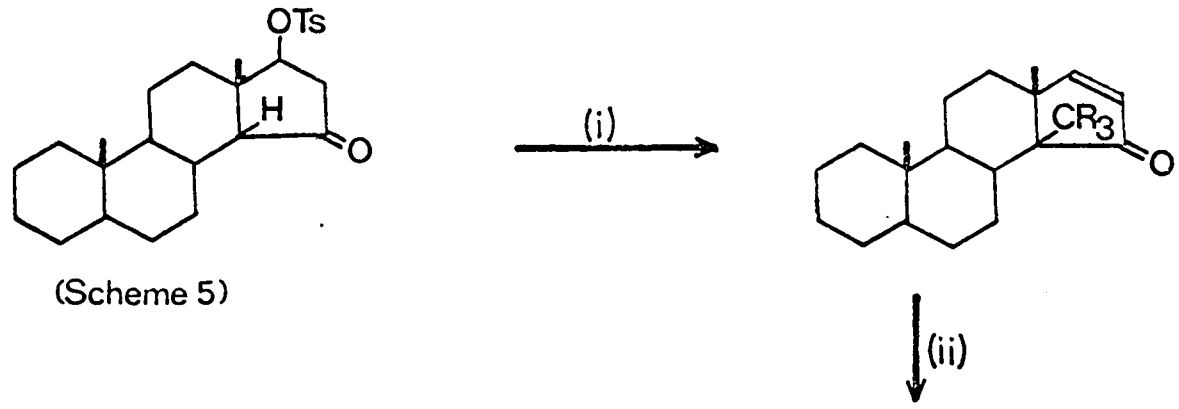
SCHEME **7.**



SCHEME **8.**



SCHEME **9.**



(Scheme 5)

Reagents: (i) $KOBu^t - Bu^tOH - CR_3I$
(ii) Pd on C - H_2
(iii) Huang-Minlon reduction using forcing conditions

(13) R=H
(14) R=D

configuration. Significant results in this work are summarised in Table 1. Only those groups introduced or modified by the fungus are listed in the Product(s) column.

Table 1

Substrate	Product(s)	
5α,13α-Androstan-15-one [3]	6α,12β-$(OH)_2$	12.5%
12β-Hydroxy-5α,14β-androstan-15-one [6]	7β-OH	20%
	7β,14-$(OH)_2$	7%
5β-Androstan-3-one [10]	3β-OH	8%
	3α-OH	1.5%

No clear-cut conclusions could be made from a consideration of these results and a few isolated examples from previous work;[8] with the binding site out of the plane of the steroid skeleton, unpredictable variations (usually a spatial restriction) in the pattern of hydroxylation were found to occur.

Related topics

In connection with the microbiological and mass spectral studies in this laboratory, it was necessary to characterise the complete series of mono-oxygenated 5α-androstanes. A number of the alcohols (including a few of unnatural configuration) were synthesised and a series of acetates were prepared; the principal constants for all 5α-androstane mono-alcohols and their acetate derivatives were tabulated, for later reference in the microbiological work. Some aspects of the mass spectra of the acetates were examined, and an interesting feature was the expulsion of a fragment of 29 mass units, especially dominant in

5α-androstane 2-acetates.

It has been difficult to assess the contribution of the C_{14}-methyl group in the initial mass spectral fragmentation of triterpene compounds.[9] With the availability of suitable precursors, it was hoped to examine this effect in the [M-15] peak of 14-methyl-5α,14β-androstane [13] and its 14-methyl-d_3 analogue [14], prepared as in Scheme 9. It was found that in these compounds, the initial fragmentation leading to the [M-15] peak was dominated (>90%) by a loss of the 14-methyl group.

The development of unusual in situ conditions for the reduction with diimide of 5α-androst-16-en-15β-ol [4] is shown in Scheme 1. In an extension of this work, a number of readily available A- and D-ring olefins and allylic alcohols were reacted under the neutral conditions ("P") described by Corey et al.[10] Within the limited range studied, it was found that facile reduction is unique to the 16-olefin of the 5α,14α-androstane series.

N.m.r. spectroscopy of steroids

The structural determination of steroid metabolites has been very dependent on n.m.r. spectroscopy; a consideration of the angular methyl signals in $CDCl_3$, of the position and multiplicity of >CH-OR proton signals, and of the difference of angular methyl signals of derived polyketones in CCl_4 and benzene has usually provided sufficient data to define the structure of a new metabolite.[1,8]

The most recent refinement of Zürcher's original treatment[11] comes from a study of 411 oxygenated steroids prepared in this department,[1,12] and other notable contributions have been made by French[13] and Japanese[14] groups. An additional 300 steroids (mostly new, and many of unnatural configuration) have been characterised in this department, and some anomalies, usually arising from

structural interactions, have become apparent in the published data. In the present work, use has been made of the now complete series of mono-oxygenated 5α-androstanes to devise a new set of C-methyl incremental values. Account has been taken of interacting functional groups, and many anomalies have been accommodated. A number of previously unrecorded shift values have also been deduced.

To facilitate use, the new set of incremental values has been printed in tabular form, and will appear in Part VIII of the Oxford series, "Microbiological Hydroxylation of Steroids".[15]

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W. A. Denny, Sir Ewart R.H. Jones, V. Kumar, G. D. Meakins, and V.E.M. Thomas, J.Chem.Soc. (Perkin I), 1972, 492; (c) I. M. Clark, A. S. Clegg, W. A. Denny, Sir Ewart R.H. Jones, G. D. Meakins, and A. Pendlebury, J.Chem.Soc. (Perkin I), 1972, 499.

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I am grateful to the technical staff of the Laboratory who determined the greater part of the physical data. In particular, I am indebted to Mrs. E. E. Richards for recording n.m.r. spectra, Mrs. R. Foster for i.r. spectra and optical rotation data, and Mr. J. W. Keeping, who carried out the work associated with growing the micro-organisms.

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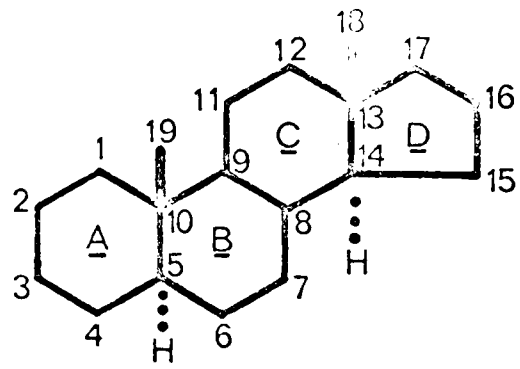
I am indebted to Miss P. A. Wilmot for her cheerfulness and restraint during the typing of this Thesis.

Finally, I should like to thank my friends, especially those of the Perkin Coffee Club, for having made my stay in Oxford so interesting and enjoyable.

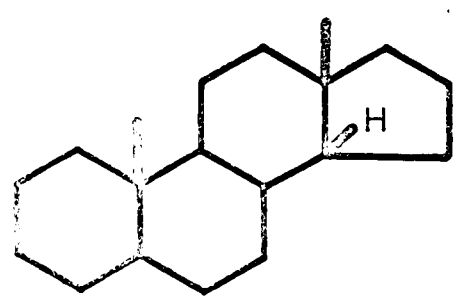


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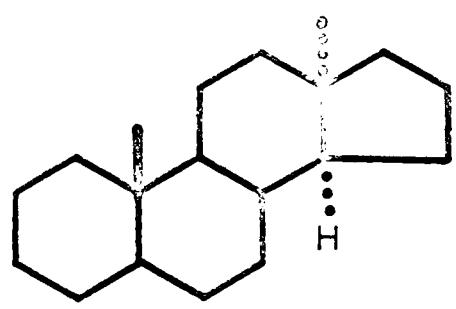
5α-androstane



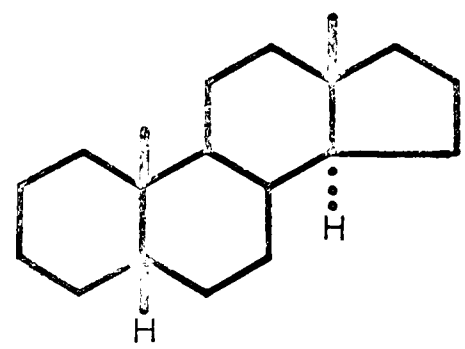
5α,14β-androstane



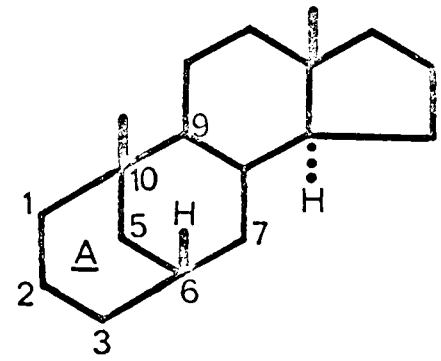
5α, 13α-androstane



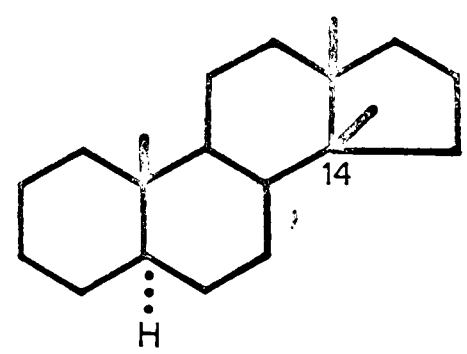
5β-androstane



A-nor-(5⟶6β) abeo-androstane



14-methyl-5α, 14β-androstane



INTRODUCTION

The microbiological hydroxylation of steroids was first demonstrated by Horvath and Kramli,[1] who in 1948 reported the 7-hydroxylation of cholesterol. The discovery[2] in 1952 that a hydroxyl group could be introduced in high yield at the 11α-position of progesterone using a culture of the fungus Rhizopus arrhizus led to viable syntheses of cortico-steroids, and established the value of this technique in the pharmaceutical industry.

Interest in the hydroxylation process has subsequently been commercially oriented, and little effort has been made to correlate structural variations in the substrate with positions of hydroxylation; nearly all of the vast literature on the process, comprehensively reviewed until 1965,[3,4] refers to substrates oxygenated at position 3 which are often complicated by the presence of other substituents.

The aim of work carried out in this laboratory has been to obtain information about the spatial relationships between binding and hydroxylation sites, and to determine structural requirements for efficient hydroxylation; this work has involved a study of the action of a variety of micro-organisms on simple mono- and di-oxygenated androstanes, where the positions of the substituents have been varied systematically around the steroid nucleus. By the application of a knowledge of some of the patterns of hydroxylation, it has been possible to devise syntheses of general value.

Accounts of some of the work have been published,[5-8] and a detailed description of the remainder is reported in seven theses.[9] Other groups have concurrently sought similar objectives, by the study of hydroxylation in cell-

free systems[10,11] or by the use of non-steroidal substrates.[12,13]

The starting point of the work described in this thesis was to devise a practicable synthesis of the relatively unavailable 5α-androstan-15-one [1], which was required as a final substrate in the systematic investigation of the hydroxylation of 5α-androstane mono-ketones by the fungus Calonectria decora.*

A surprising variation in the pattern of hydroxylation of the 15-ketones caused by a change of configuration of the D-ring prompted the initiation of a more systematic study of the effect of Calonectria decora on other substrates having a binding site out of the plane of the steroid skeleton.

The work is presented in six sections.

Section I describes the synthesis of 15-oxygenated substrates, and includes the development of large scale fermentation techniques. The preparation of other steroidal substrates of unnatural configuration is also presented.

Section II outlines the fermentation with Calonectria decora of the substrates prepared, and attempts to relate the results to those obtained in previous work* for mono-oxygenated androstanes of natural configuration.

Section III records a series of experiments arising from, but not directly relevant to, the previous sections.

Section IV presents in tabular form the n.m.r., mass spectral, and circular dichroism Cotton effect data for compounds described in this Thesis.

* An account of this work has been accepted for publication.[14]

Section V makes use of the data of some 300 additional compounds, many containing unique combinations of functional groups, to revise and extend the n.m.r. studies previously reported by this group.[9d, 15]

Section VI. Experimental

SECTION I

THE SYNTHESIS OF STEROIDAL SUBSTRATES

A. THE CHEMICAL SYNTHESIS OF 15-OXYGENATED ANDROSTANES,* AND RELATED REACTIONS

5α-Androstan-15-one [1] was not readily available at the time when it was required in the hydroxylation studies described. Concurrently with the development of a microbiological route to 5α-androstan-15α-ol [53],** possible variations of existing syntheses and new variations were sought with the aim of developing a method suited to large scale operations.

A number of previous workers have considered the problem of functionalizing the 15-position, but the most direct access to [1], *via* conversion of the oxido ketone [16] to the allylic alcohol [18] (Scheme 3) with hydrazine and acid,[18] was reported[19] to give low and variable yields. In more recent studies in the 13α-series,[32b] similar low yields were obtained. A longer sequence depending on a reported facile Michael addition of benzyl alcohol to 5α-androst-15-en-17-one [10][21,22,23,28] was of potential applicability.

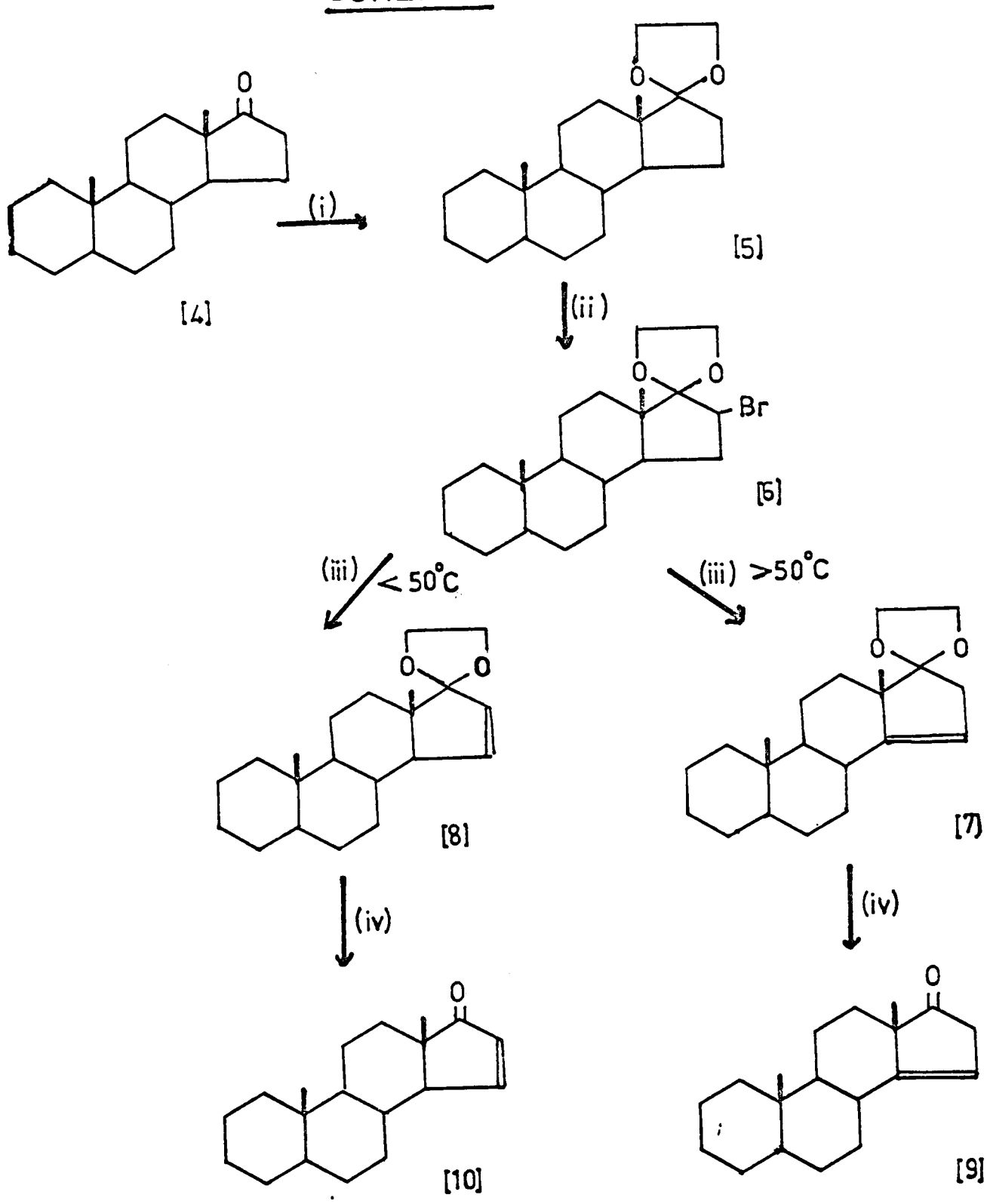
The allylic functionalisation of 16-olefins and the allylic rearrangement of 15-en-17β-alcohols had not been reported but preliminary experiments proved discouraging and are not described here.

The preparation of 15-ketones *via* acid-catalysed rearrangement of 14,15-epoxides was known.[24,25] Previous studies of the hydroboration of 14-olefins suggested that the stereochemistry of attack was dependent on 17β-substitution; all studies were confined to 17-substituted systems[22,26,27] and

* The bulk of the work described has been accepted for publication.[16]

** See Section IB; reference 17.

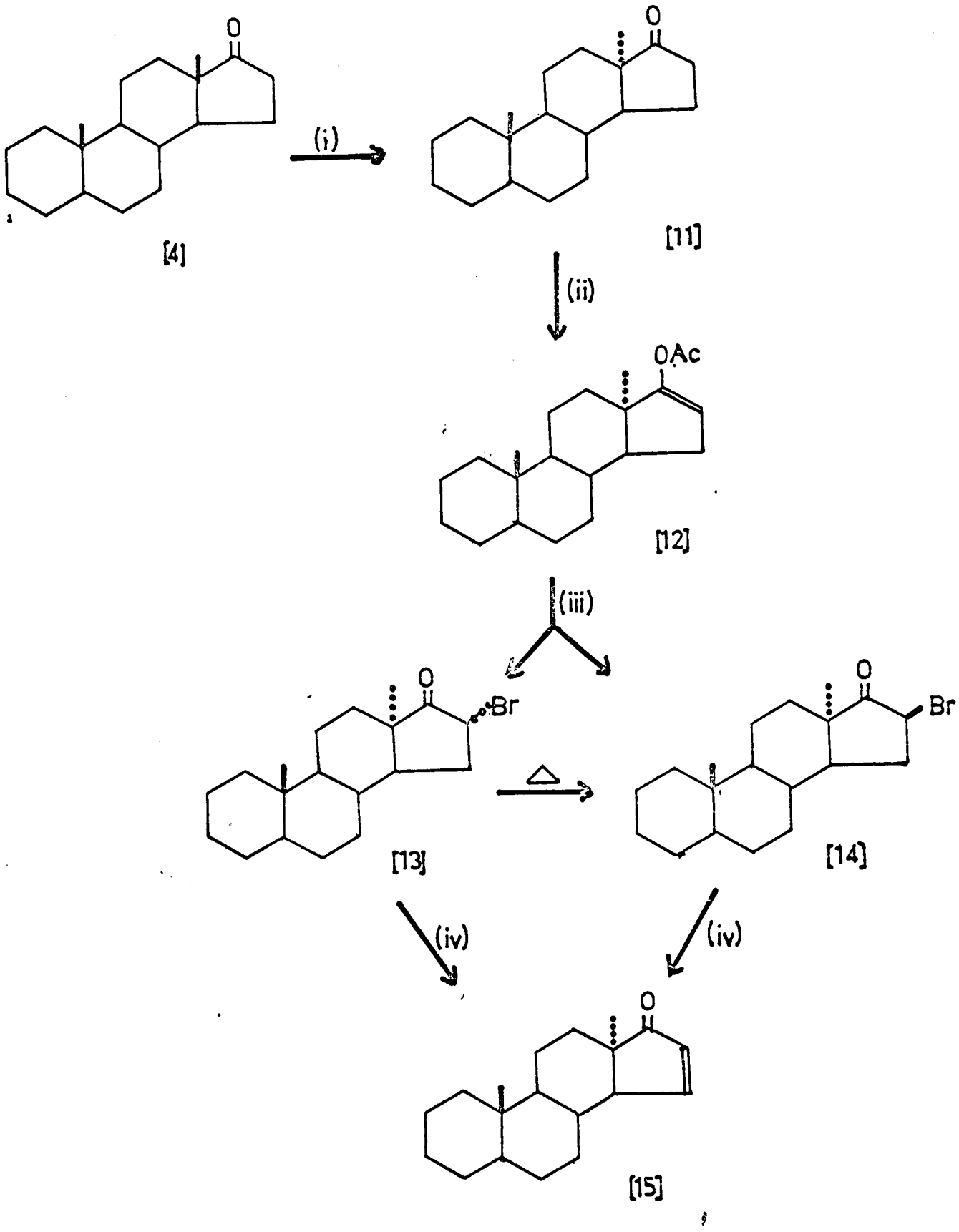
SCHEME 1.



Reagents: (i), $HO(CH_2)_2OH$ – TsOH – PhMe ; (ii) $PhNMe_3.Br_3$ – THF

(iii) $KOBu^t$ – DMSO ; (iv) Me_2CO – TsOH

SCHEME 2.



Reagents : (i) $h\nu$ – Et_2O ; (ii) i-PrOAc – H^+ ; (iii) Br_2 – CCl_4

(iv) LiBr – Li_2CO_3 – DMF

this has been shown to affect significantly the stereochemistry of ring D reactions.[28,29] The allylic oxidation of 8(14)-olefins gave in the cholestane series only low yields of 15-oxygenated product.[30] Furthermore, the preparation of 8(14)- and 14-olefins is tedious.[30,31]

From these considerations, it appeared most profitable to confine studies to the 15-functionalisation of 5α-androst-15-en-17-one [10]. Emphasis was to be on the development of high yield reactions and it was not intended to pursue low yield reactions.

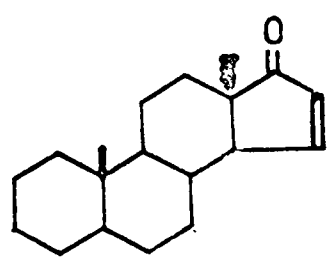
The results are presented in Schemes 1-6. The structures (in particular the stereochemical features) of new steroids are described in detail only when they do not follow from their relationships to known compounds or from the interpretation of n.m.r. (Section IV; Tables 19-21), i.r., or circular dichroism (c.d.) (Section IV; Table 22) data.

Synthesis of 5α-androst-15-en-17-one [10] and 5α,13α-androst-15-en-17-one [15] (Schemes 1 and 2)

5α-Androst-15-en-17-one [10] was prepared in the established manner.[19] In the present work, dehydrobromination of the bromoacetal [6] to the Δ^{15}-acetal [8] required careful temperature control to avoid rearrangement to the Δ^{14}-acetal [7].

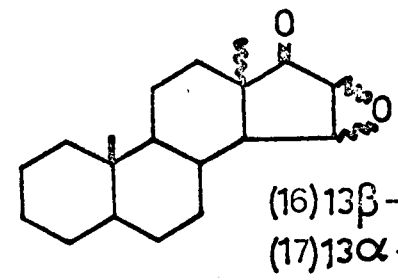
Nambara et al.[32] recently found that whereas in the C/D trans steroids dehydrobromination of a 16-bromo-17-ketone is inhibited,[33] it proceeds with ease in the 13α-series. The relative ease may be ascribable to a favourable geometrical alignment of the reaction centres in the cis-C/D modification. By using the method described,[32] 5α,13α-androst-15-en-17-one [15] was obtained in high yield.

SCHEME 3.



(10) 13β-Me
(15) 13α-Me

(i)

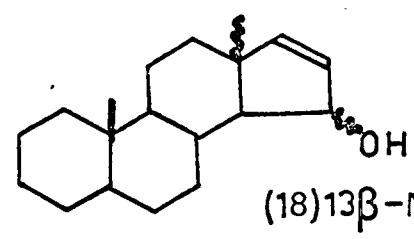


(16) 13β-Me ; β-oxido
(17) 13α-Me ; α-oxido

(ii) ; N_2

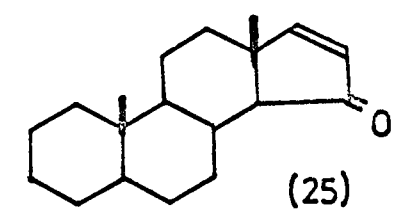
(16)

(ii) ; O_2



(18) 13β-Me ; 15β-OH
(19) 13α-Me ; 15α-OH

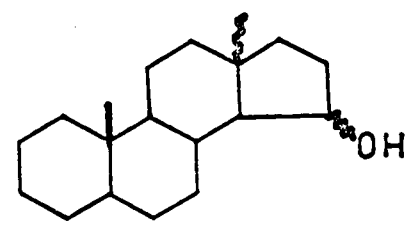
(iv) 0°C



(25)

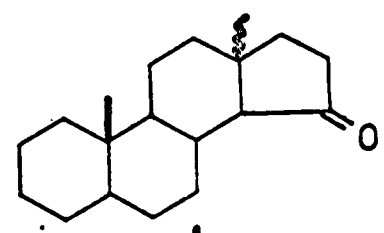
(iii)

(20)



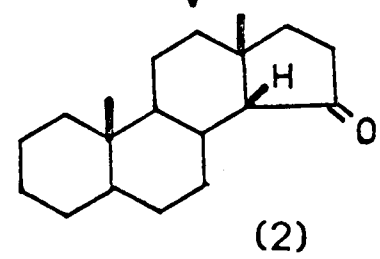
(20) 13β-Me ; 15β-OH
(21) 13α-Me ; 15α-OH

(iv)



(1) 13β-Me
(3) 13α-Me

(v)



(2)

(vi)

14β-H-15α-OH [23]
14β-H-15β-OH [24]

Reagents: (i) H_2O_2-OH^- ; (ii) N_2H_4-H^+-MeOH ; (iii) Pd/C-H_2 ;
(iv) 8N-H_2CrO_4 ; (v) MeOH-NaOMe ; (vi) $Li(OBu^t)_3AlH$-THF

Preparation of 5α-androstan-15-one [1], 5α,14β-androstan-15-one [2] and 5α,13α-androstan-15-one [3]: The Hydrazine Rearrangement (Scheme 3)

The transformation [16] ⟶ [18], reported by Djerassi et al.[19] to give variable, and at best 30%, yields gave consistently 40-43% of product under anhydrous conditions (Table 1). Catalytic hydrogenation of [18] gave 5α-androstan-15β-ol [20] subsequently oxidised with 8N-chromic acid at 0°C to 5α-androstan-15-one [1]. The ketone [1] was readily isomerised in base to 5α,14β-androstan-15-one [2].

In an attempt to inhibit the large number of competing reactions, the rearrangement [16] ⟶ [18] was repeated at 0°C, with no significant improvements. Further variations (Table 1) showed that both the allylic and saturated alcohols [18] and [20] were obtained depending on the atmosphere (N_2 or air) in which the reaction was conducted. The use of toluene-p-sulphonic acid monohydrate as a proton source caused more efficient reduction and suggested that a diimide mechanism was involved. The unique character of this reaction is discussed in section III.

Because of its low solubility in methanol, rearrangement of the 13α-oxido-ketone [17]* was carried out at 20°C. In an atmosphere of air, complete decomposition of the product resulted, and unlike the 13β,14α-series, reaction of the allylic alcohol [19] with hydrazine and toluene-p-sulphonic acid mono-

* The 15α,16α-configuration of the epoxide ring was deduced from its positive c.d. Cotton effect.[32b] The sign of the Cotton effect of an oxido-ketone follows the "reversed octant rule", and depends on the sign of the octant in which the oxido-oxygen is located.[34]

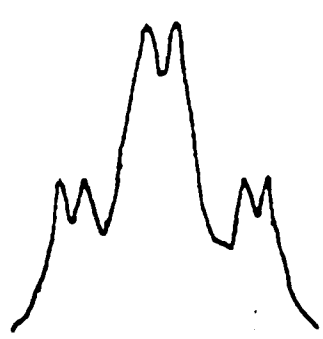
TABLE 1

The reaction of oxido-ketones and allylic alcohols in the presence of hydrazine and acid

Compound (500 mg)	Solvent	Acid	Temp. (°C)	Atmos.	Weight (mg) of Product [18]	[20]
[16]	MeOH	AcOH (200 mg)	20	N_2	205	
	Bu^tOH	"	20	N_2	163	
	"	"	0	N_2	211	
	"	"	0	air	40	165
	MeOH	$TsOH.H_2O$ (500 mg)	0	N_2	204	
	"	"	0	air		243
[18]	MeOH	"	0	air		482
					[19]	[21]
[17]	MeOH	$TsOH.H_2O$ (500 mg)	20	air	-	-
	"	"	20	N_2	192	
[19]	"	"	0	air		-

FIGURE 1. Signal shapes of >CH-OH protons in the n.m.r. spectra of 15-hydroxy androstanes.

5α-androstan-15α-ol (**53**)



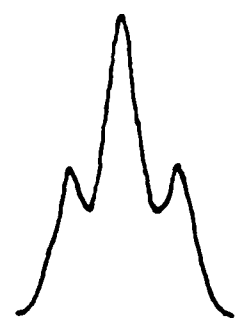
τ 5.92 (J 3,9,9 Hz ; $W_{1/2}$ 19 Hz)

5α-androstan-15β-ol (**20**)



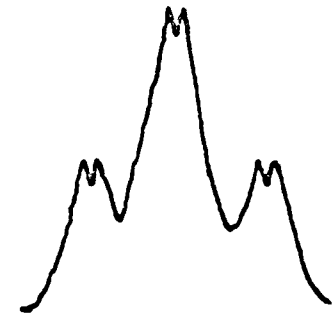
τ 5.69 (J 1.5,7,7 Hz ; $W_{1/2}$ 14 Hz)

5α,14β-androstan-15α-ol. (**23**)



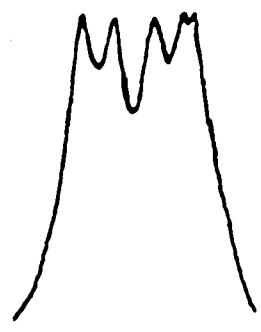
τ 5.61 (J 5,5 Hz ; $W_{1/2}$ 12 Hz)

5α,14β-androstan-15β-ol (**24**)



τ 5.65 (J 1.5,8.5,8.5 Hz ; $W_{1/2}$ 19 Hz)

5α,13α-androstan-15α-ol (**21**)



τ 5.90 (J 3,7.5 Hz ; $W_{1/2}$ 13 Hz)

---

hydrate, in air at 0°C, gave a polar product;* no "diimide reduction" to the less polar 5α,13α-androstan-15α-ol [21] was detected. Under N_2, yields of [19] were much improved on those reported by Nambara et al.[32b] Catalytic hydrogenation of [19], and oxidation of 5α,13α-androstan-15α-ol [21] with 8N-chromic acid afforded the ketone [3] in good yield.

Reduction of 5α,14β-androstan-15-one [2] with lithium tri-t-butoxy-aluminium hydride in THF gave a mixture (4:1) of 5α,14β-androstan-15α-ol [23] and 5α,14β-androstan-15β-ol [24]; the configurations of which were assigned on the expectation that the CH-OH proton would be more strongly coupled in the 15β-alcohol, as is found in the 14α-H-15α-OH system[15] (Figure 1).

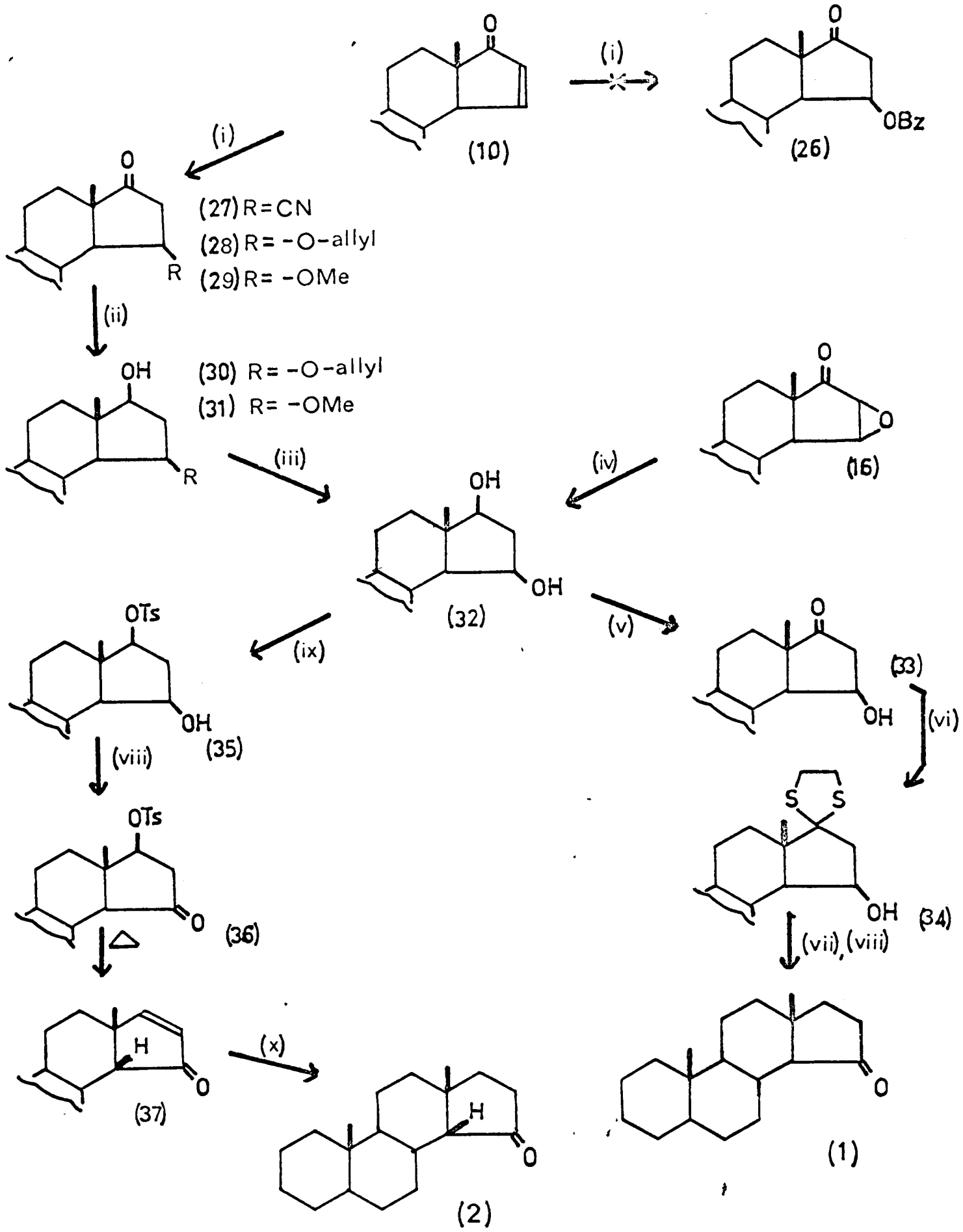
Alternative syntheses of 5α-androstan-15-one [1] and 5α,14β-androstan-15-one [2] (Scheme 4)

The addition of benzyl alcohol to 5α-androst-15-en-17-one [10], a key step in the synthesis of 5α,14β-androstan-15-one [2] and C-17-substituted 15-ketones by Djerassi et al.[21] could not be repeated.** However, addition of the nucleophiles CN^-, OMe^-, and O-allyl$^-$ proceeded in almost quantitative yields. Reduction of the 15β-substituted products [28] and [29] with lithium tri-t-butoxyaluminium hydride gave [30] and [31]. 5α-Androstane-15β,17β-diol [32] was obtained from base-catalysed cleavage[37] of the allylether [31],

* It is noted that the reaction of oxido-ketones with toluene-p-sulphonyl hydrazine at elevated temperatures results in ring cleavage.[35] Lack of material precluded further investigation of this result.

** Thanks are due to Dr. J. M. Evans for his confirmation of this negative result.

SCHEME 4.



Reagents: (i) NaCN-THF, or ROH - OH^- - THF; (ii) $Li(OBu^t)AlH$ - THF; (iii) $KOBu^t$ - DMSO[37]; (iv) $LiAlH_4$ - Et_2O; (v) Ag_2CO_3 - Celite[36]; (vi) $HS(CH_2)_2SH$ - $BF_3.Et_2O$; (vii) Raney Ni[38] - EtOH; (viii) 8N - H_2CrO_4; (ix) TsCl - C_5H_5N; (x) Pd/C - H_2

and was identical with the product of metal hydride reduction of the oxido-ketone [16]. Oxidation of the diol [32] with silver carbonate on Celite[36] gave 15β-hydroxy-5α-androstan-17-one [33]. Although the Huang-Minlon reduction of 15α-hydroxy-17-ketones proceeds in high yield,[9c] that of the thermodynamically less stable 15β-hydroxy-17-ketone [33] gave a polar product. Controlled thio-ketalisation of [33], then desulphurisation, gave 5α-androstan-15β-ol [20].

Mono-tosylation of the diol [32] was more selective than a similar acetylation procedure of Djerassi et al.,[21] but elimination of the 15-oxo-17β-yl tosylate [36] even under mild conditions gave 5α,14β-androst-16-en-15-one [37]. Subsequent hydrogenation afforded 5α,14β-androstan-15-one [2] in high yield.

Figure 2: Ring cleavage of a β-hydroxy toluene-p-sulphonate

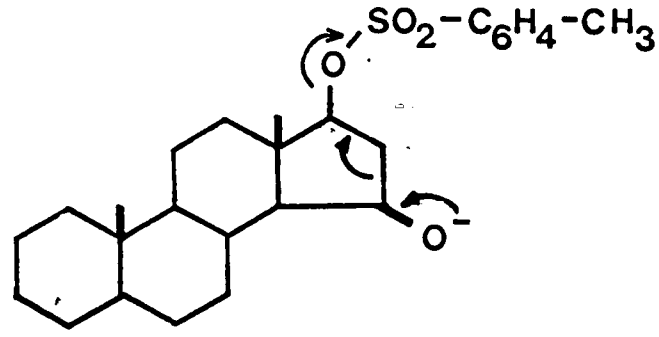
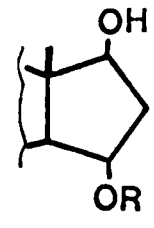
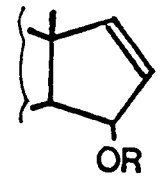
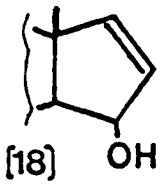
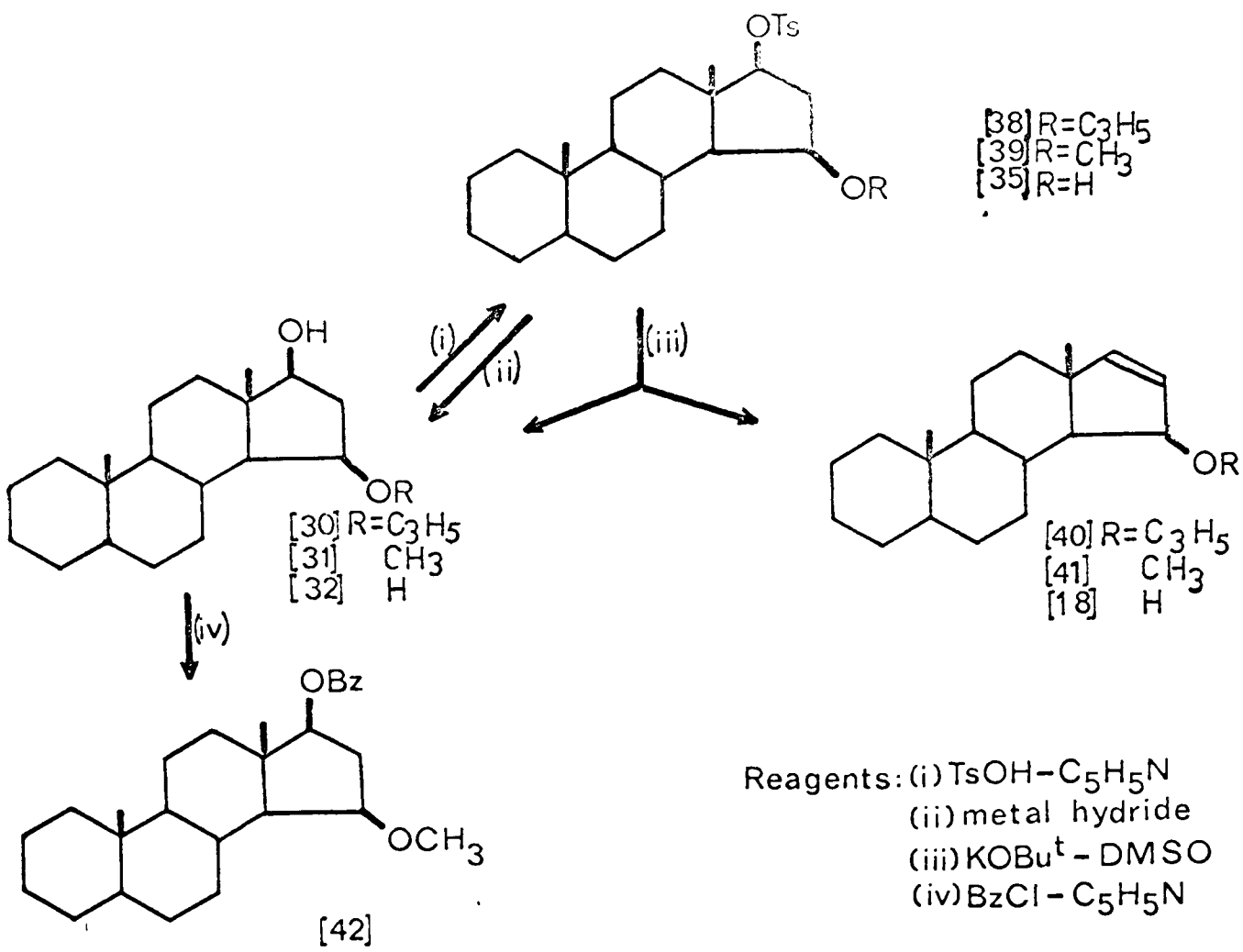


TABLE 2

Elimination of toluene-p-sulphonates [38] and [39] with Bu^tOH in DMSO

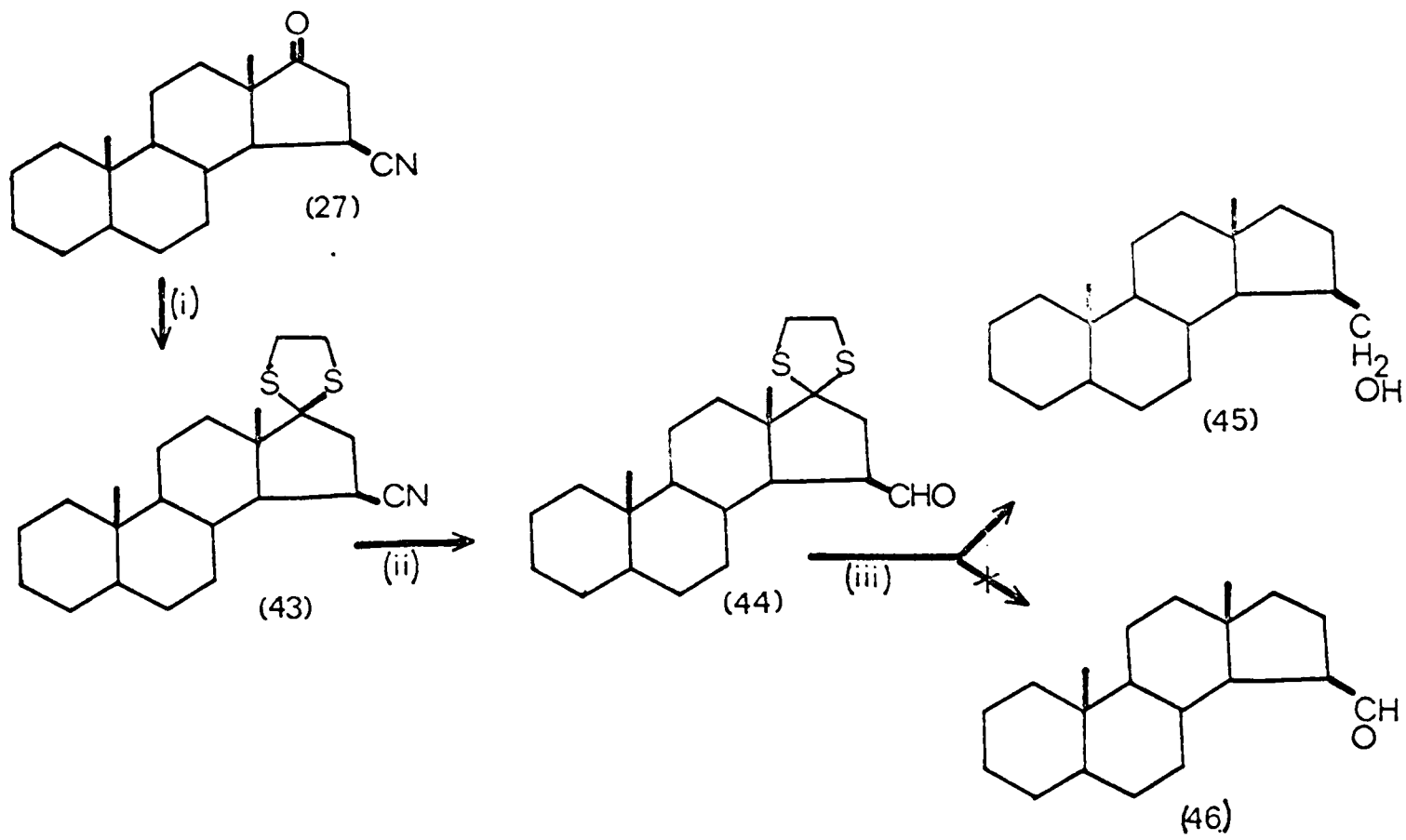
Starting material	Recovered s.m. (%)	Products (%)			Other
				 [18]	
[38] R = -allyl	0	8	6	5	polar
[39] R = $-CH_3$	20	40	28	-	-

SCHEME 5.



Reagents: (i) $TsOH-C_5H_5N$
(ii) metal hydride
(iii) $KOBu^t$ – DMSO
(iv) $BzCl-C_5H_5N$

SCHEME 6.



Reagents: (i) $HS(CH_2)_2SH - BF_3.Et_2O$
(ii) $LiAlH_4$ – THF
(iii) Raney Ni[38] – EtOH

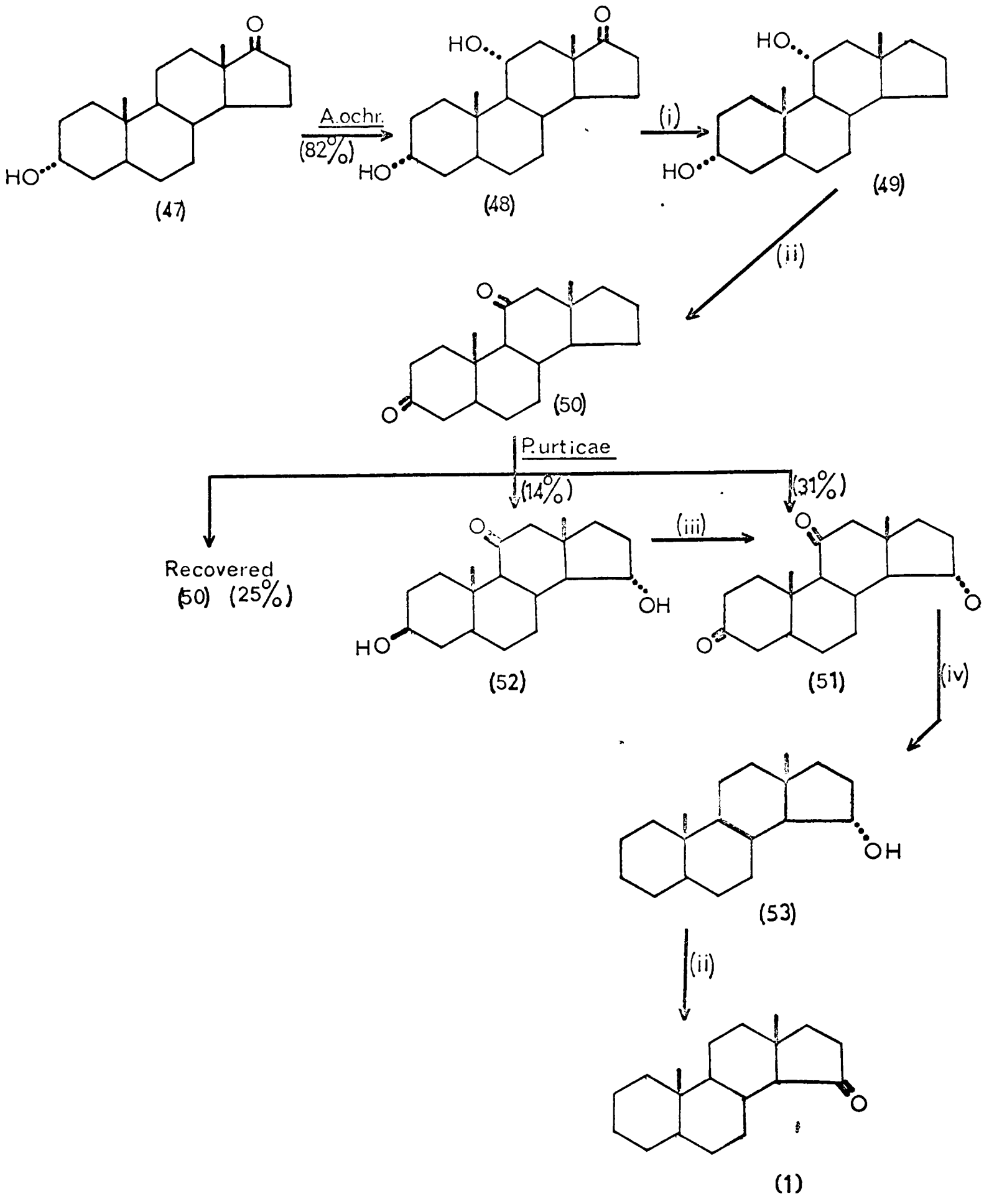
Related synthetic work (Schemes 5 and 6)

The reductive C-O cleavage of a steroidal ring A-tosylate by metal hydride reagents is well known.[39] Similarly, cleavage of 14ξ-methyl-15α-yl and 14ξ-methyl-15β-yl tosylates has been reported.[40] Nambara et al.[41] were unable to cleave a 17β-yl mesylate, and to our knowledge the reaction with a 17β-yl tosylate is not known. Cleavage was attempted on the tosylates [35], [38] and [39] using various conditions (see experimental section); almost invariably S-O cleavage resulted.

The reaction of the hydroxy-ester [35] with sodium methoxide in DMSO gave a polar product probably resulting from ring cleavage (Figure 2), and with potassium t-butoxide in DMSO, reaction of the esters [38] and [39] gave only poor yields of the desired elimination products (Table 2). 15β-Methoxy-5α-androst-16-ene [41] was surprisingly stable to rearrangement and cleavage;[37] even with harsh conditions there was no reaction. Pyrolytic elimination of the benzoate [42] could not be induced even at 320°C; because of the stability of the allylic ether [41], no attempt was made to improve this elimination by using alternative reagents or derivatives.

The hindered nature of the nitrile in [43] was demonstrated by the strong shielding of one -S-CH_2-group in the n.m.r. spectrum, and its inertness to reaction with bulky metal hydride reagents.[42] Reaction with an excess of lithium aluminium hydride at 25°C was slow, and with forcing conditions the 15β-methal derivative [44] was obtained in low yield. Desulphurisation of [44] with deactivated Raney nickel[38] gave a product (50%) spectroscopically consistent with 15β-hydroxymethyl-5α-androstane [45]; the expected 15β-methal-5α-androstane [46] was not detected.

SCHEME 7.



Reagents: (i) Huang–Minlon reduction[45]; (ii) 8N – H_2CrO_4;
(iii) Ag_2CO_3 – Celite[36];
(iv) Huang–Minlon reduction under forcing conditions.[46]

B. THE MICROBIOLOGICAL SYNTHESIS OF 12- AND 15-OXYGENATED ANDROSTANES

An important aim of the work in this department has been to develop microbiological hydroxylation as a useful synthetic technique, initially with respect to steroid transformations. By learning something of the hydroxylating character of selected fungi and by careful choice of substrate, organism, and incubation conditions, it was hoped to design microbiological syntheses to give optimum yields of desired compounds. This concept is illustrated by the development of gram-scale syntheses of 12- and 15-oxygenated androstanes.

The Incubation of 5α-androstane-3,11-dione [50] with Penicillium urticae*

Utilisation of the specific 15α-hydroxylating capacity of the fungus *Penicillium urticae* has led to a convenient route to 5α-androstan-15-one [1] (scheme 7).

Although several instances of 15-monohydroxylation had been recorded,[3] the nature of the substrate or the paucity of the yield excluded these as practical approaches to 15-oxygenated androstanes. Related work in this laboratory[48] suggested that 5α-androstane-diones might be suitable substrates, and during screening experiments an efficient conversion of 5α-androstane-3,11-dione [50] into its 15α-hydroxy derivatives [51] and [52] was found to occur with *P.urticae*. Extensive screenings of this and related fungi under a variety of conditions including artificial media[49] were judged by t.l.c. not to be so effective.

Selective oxidation of the dihydroxy ketone [52] to the hydroxy-diketone

* This work has been published.[17]

[51] occurred with silver carbonate on Celite,[36] and Huang-Minlon reduction of [51] under forcing conditions[46] afforded 5α-androstan-15α-ol [53], readily oxidised to 5α-androstan-15-one [1].

5α-Androstane-3,11-dione [50] was not easily accessible by chemical means, but microbiological hydroxylation of 3α-hydroxy-5α-androstan-17-one [47] with A.ochraceus gave the 11α-hydroxy derivative [48].* Huang-Minlon reduction of [48], followed by oxidation with 8N-chromic acid afforded 5α-androstane-3,11-dione [50] in high yield.

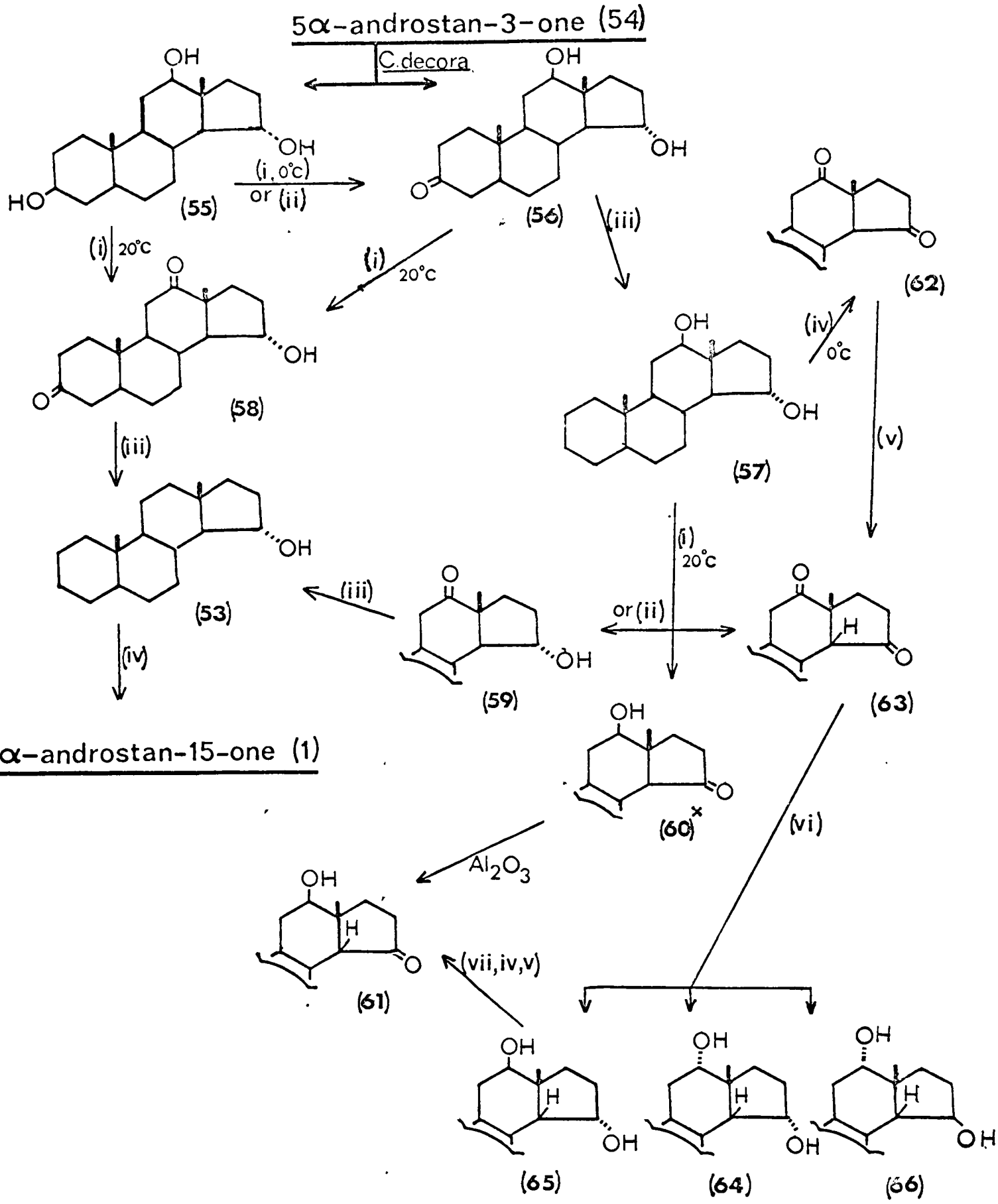
This work illustrates an important general feature of microbiological hydroxylation of steroids, viz. that, in certain cases, the site and efficiency of hydroxylation by a particular micro-organism is strongly influenced by the structure of the substrate, and by appropriate choice of organism and substrate it is possible to produce a variety of desired hydroxylation patterns. While direct hydroxylation of readily available materials is impractical as a route to some steroidal types (e.g. 15-oxygenated androstanes), others (e.g. 11-hydroxy compounds) are readily obtained and may then be used to generate substrates (such as the 3,11-diketone employed here) which are satisfactorily hydroxylated at the required site.

Transformation of 12β,15α-dihydroxy-5α-androstan-3-one [56] (Scheme 8)

A combination of microbiological and chemical results utilising the well established 12β,15α-dihydroxylating capacity of C.decora with 5α-androstan-

* Later large scale fermentations employed 3β-hydroxy-5α-androstan-17-one [67] (Section IC).

SCHEME 8.



Reagents:-(i) N-bromo acetamide - Me_2CO; (ii) Ag_2CO_3 - Celite[36] ;(iii)Huang-Minlon; (iv) 8N-H_2CrO_4 ;(v)KOH-MeOH ;(vi)$Li(OBu^t)_3AlH$ -THF (vii) Ac_2O - C_5H_5N.

x Spectroscopic evidence only.

3-one [54][14] gave an efficient four step synthesis for the transposition of a keto group from the A to the D-ring. Such a transposition can be compared to that of "remote oxidation" accomplished by photochemical means[51] and an efficient alternative using standard chemical reactions is hard to envisage.

The conversion of 5α-androstane-3β,12β,15α-triol [55] to 12β,15α-dihydroxy-5α-androstan-3-one [56] and to 15α-hydroxy-5α-androstane-3,12-dione [58] depended on the different rates at which the hydroxyl-groups (3 > 12 > 15) were attacked. Oxidation of the trisubstituted compounds [55] and [56] with N-bromo acetamide was more selective than that of the diol [57], where a 2:1 mixture of 15α-hydroxy-5α-androstan-12-one [59] and 12β-hydroxy-5α,14β-androstan-15-one [61] resulted. However, with silver carbonate on Celite,[36] oxidation of the diol [57] was totally selective. Huang-Minlon reduction of the hydroxy-dione [58] or the hydroxy-ketone [59] gave 5α-androstan-15α-ol [53], oxidised with 8N-chromic acid to 5α-androstan-15-one [1].

Oxidation of the diol [57] with 8N-chromic acid gave 5α-androstane-12,15-dione [62] which was readily isomerised to the 14β-modification [63]. Reduction of 5α,14β-androstane-12,15-dione [63] with lithium tri-t-butoxy-aluminium hydride gave a mixture of the stereoisomeric diols [64], [65] and [66]. The configurational assignment of the products was based on strong H-bonding stretching frequencies ($\nu_{max.}$ 3618, 3440, and 3250 cm^{-1}) observed in the i.r. spectrum of [64], as expected for a 14βH-12α,15α-diol system, and on the expectation that in the n.m.r. spectra of the 14β-compounds, the CH-OH proton would be more strongly coupled in the 15β-alcohols (Figure 1). Partial acetylation of [65], oxidation with 8N-chromic acid, and saponification of the product gave 12β-hydroxy-5α,14β-androstan-15-one [61].

C. A LARGE SCALE FERMENTATION TECHNIQUE

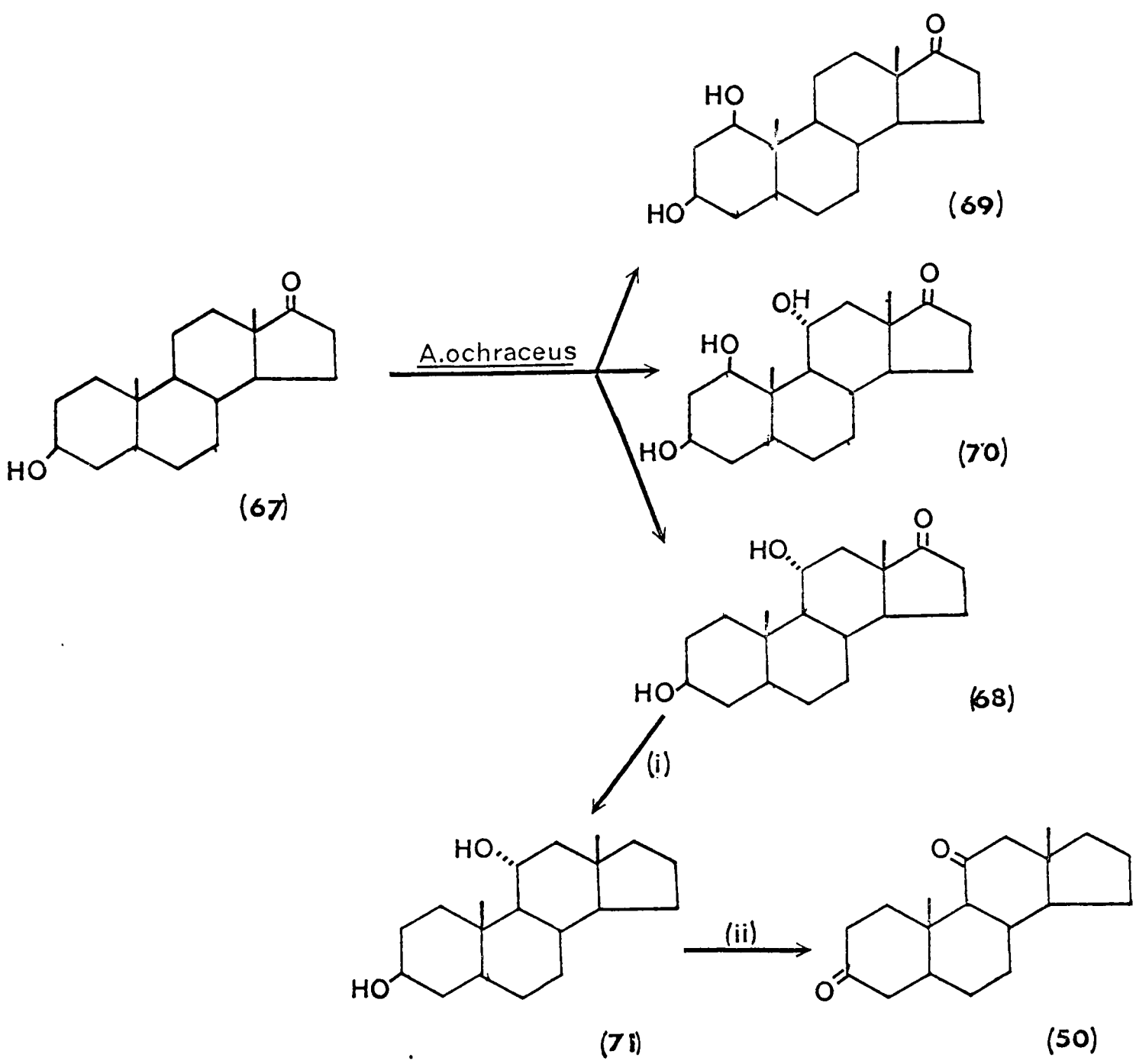
Syntheses described in the previous chapter required the preparation of gram amounts of 3,11- and 12,15-oxygenated steroids arising from the hydroxylation of 3β-hydroxy-5α-androstan-17-one [67] with A.ochraceus and 5α-androstan-3-one [54] with C.decora respectively. The preceding shake-flask incubations (Schemes 7 and 8) were scaled up in a "Biotech" Model FL110 fermentor system containing 5l of broth.

The major considerations in the choice of suitable fermentation conditions were the requirement of a high yield and a high concentration of product. That is, it was necessary to overcome the limitation of steroidal solubility, to induce sufficient enzyme to convert large amounts of substrate and to reduce the adverse effects of toxicity of the solvent supporting the steroid and the possible repression of hydroxylase formation by excessive amounts of nutrient.

With A.ochraceus the stepwise addition of substrate allowed final concentrations of up to (and possibly beyond) 4 g/l, but with C.decora a maximum of only 2 g/l was possible. The substrate was invariably introduced in ethanolic solution so that with the continuously aerated system solvent concentrations could be kept below a lethal level.* In order to maintain high steroid recovery (up to 95%) it was necessary to add glucose to the medium at regular intervals; no attempt was made to assess the effect of the nutrient addition on enzyme induction and cell growth.

* The presence of small amounts of ethanol has been shown to favour the induction of Δ^1-dehydrogenase but inhibit the growth of A.simplex.[53]

SCHEME 9.



Reagents: (i) Huang-Minlon reduction ; (ii) 8$\underline{N}$-H_2CrO_4

Patterns of hydroxylation proved similar to those found for shake-flask incubations, but in general hydroxylation was more rapid. After several trials, near optimum conditions were established for fermentations with A.ochraceus, but using C.decora results were less successful and only some guide-lines were established.

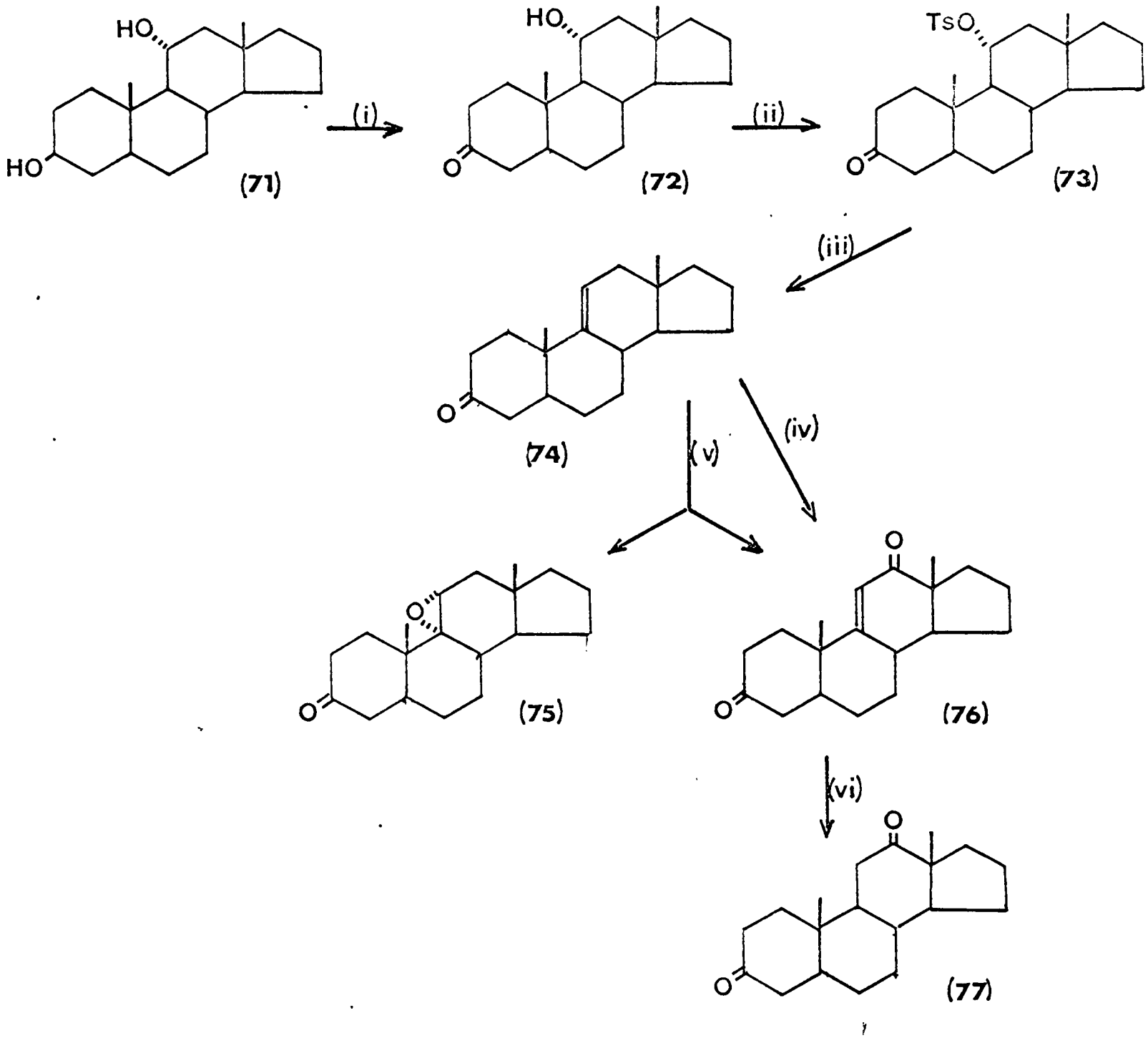
Fermentation of 3β-hydroxy-5α-androstan-17-one [67] with A.ochraceus (Scheme 9)

Optimum conditions (see experimental) using the less concentrated medium A afforded starting material [67] (22%), 3β,11α-dihydroxy-5α-androstan-17-one [68] (70%) and a mixture of polar products (2.5%). Later variations utilizing the concentrated medium B* gave superior results and allowed the determination of structures of the minor, more polar, products. Fermentation afforded starting material [67] (2%), 3β,11α-dihydroxy-5α-androstan-17-one [68] (89%), 1β,3β-dihydroxy-5α-androstan-17-one [69] (1%), and 1β,3β,11α-trihydroxy-5α-androstan-17-one [70] (1%).

An interesting feature of the fermentations was the isolation of [69]: 1β-monohydroxylation by A.ochraceus has not previously been observed, and the result suggests that 1β,11α-dihydroxylation by A.ochraceus is not a strictly sequential process but may instead result from independent attack at the two positions. Product [68] was transformed to 5α-androstane-3,11-dione [50] as previously described (Scheme 7).

* In collaboration with Dr. W. Müller.[54]

SCHEME 10.



Reagents: (i) NBA–Me_2CO ; (ii) TsCl–C_5H_5N ; (iii) LiCl–Li_2CO_3–DMF ;
(iv) Na_2CrO_4–$HOAc$–Ac_2O ; (v) CrO_3–AcOH–Ac_2O ; (vi) Pd/C–H_2

The Synthesis of 5α-androstane-3,12-dione [77] (Scheme 10)

In connection with other work in this group,[55] 5α-androstane-3,12-dione [77] was required as a microbiological substrate. The compound had been prepared[9e] from hecogenin acetate in low (2.5%) yield, by a modification of the tedious method originally described by Djerassi and Tökes.[56] With the ready availability of large amounts of 5α-androstane-3β,11α-diol [71] a synthesis of the dione was devised (Scheme 10).

Selective oxidation of the diol [71] with N-bromo-acetamide afforded 11α-hydroxy-5α-androstan-3-one [72] which, via the 11α-yl tosylate [73] was transformed to 5α-androst-9(11)-en-3-one [74]. Allylic oxidation of the olefin* with chromium trioxide or t-butyl-chromate resulted in low yields of the ene-dione [76], and significant epoxidation, giving the 9α,11α-oxido-3-ketone [75]. Oxidation with sodium chromate in acetic acid-acetic anhydride gave the ene-dione [76] in 23% yield, which compared favourably with the nitrosyl fluoride method previously reported.[57] Hydrogenation of [76] gave 5α-androstane-3,12-dione [77] in 12% overall yield from 3β-hydroxy-5α-androstan-17-one [67].

Modifications of this route can be envisaged, whereby the synthesis of a variety of 12-oxygenated substrates should be possible.

Fermentation of 5α-androstan-3-one [54] with C.decora (Scheme 11)

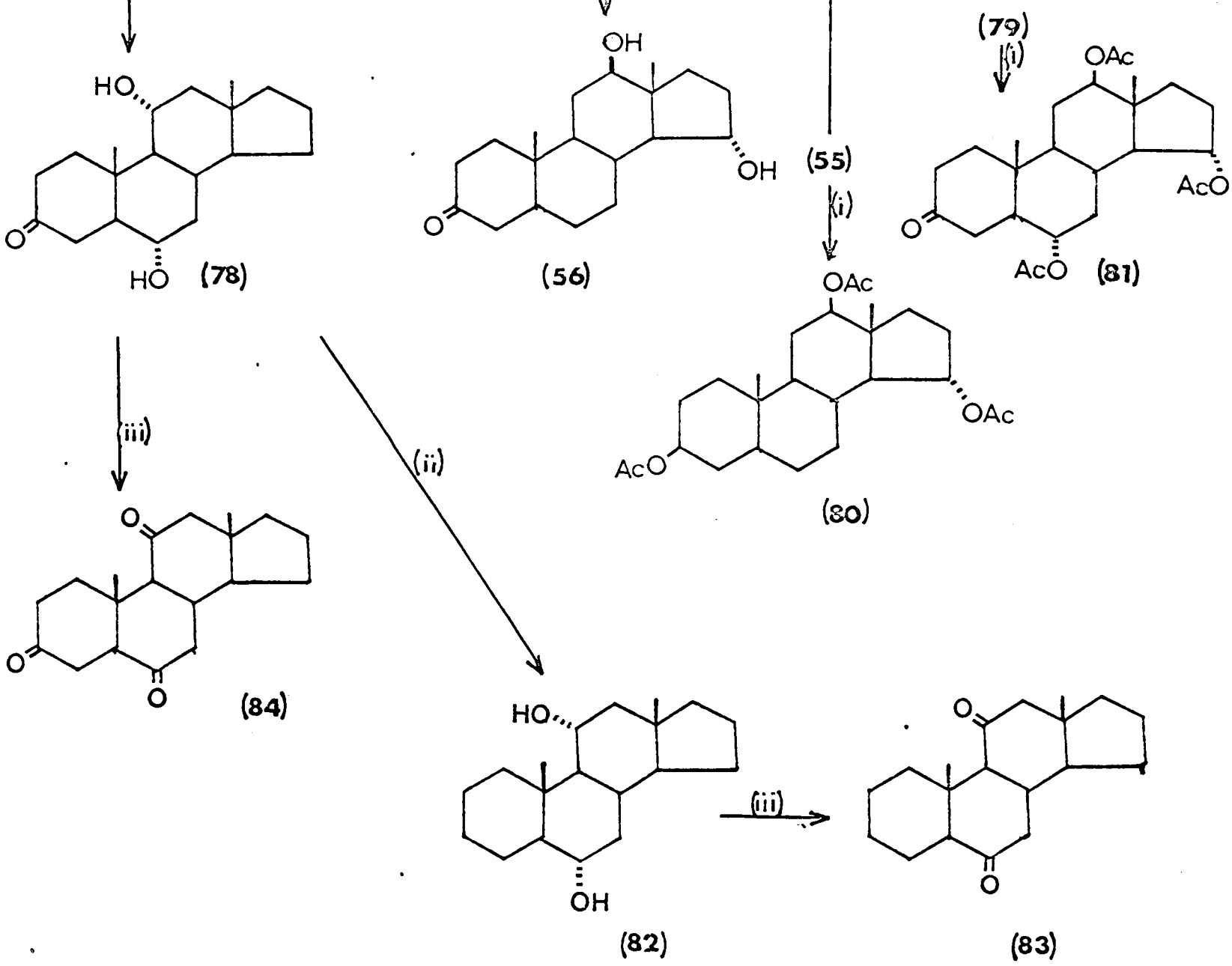
A problem in fermentor experiments with C.decora was an inability to obtain a consistently healthy growth of the culture; it was necessary that the culture be pre-grown and that the fermentor medium be very heavily inoculated.

* In collaboration with Dr. A. Bell.

SCHEME 11.

5α-androstan-3-one (54)

C.decora

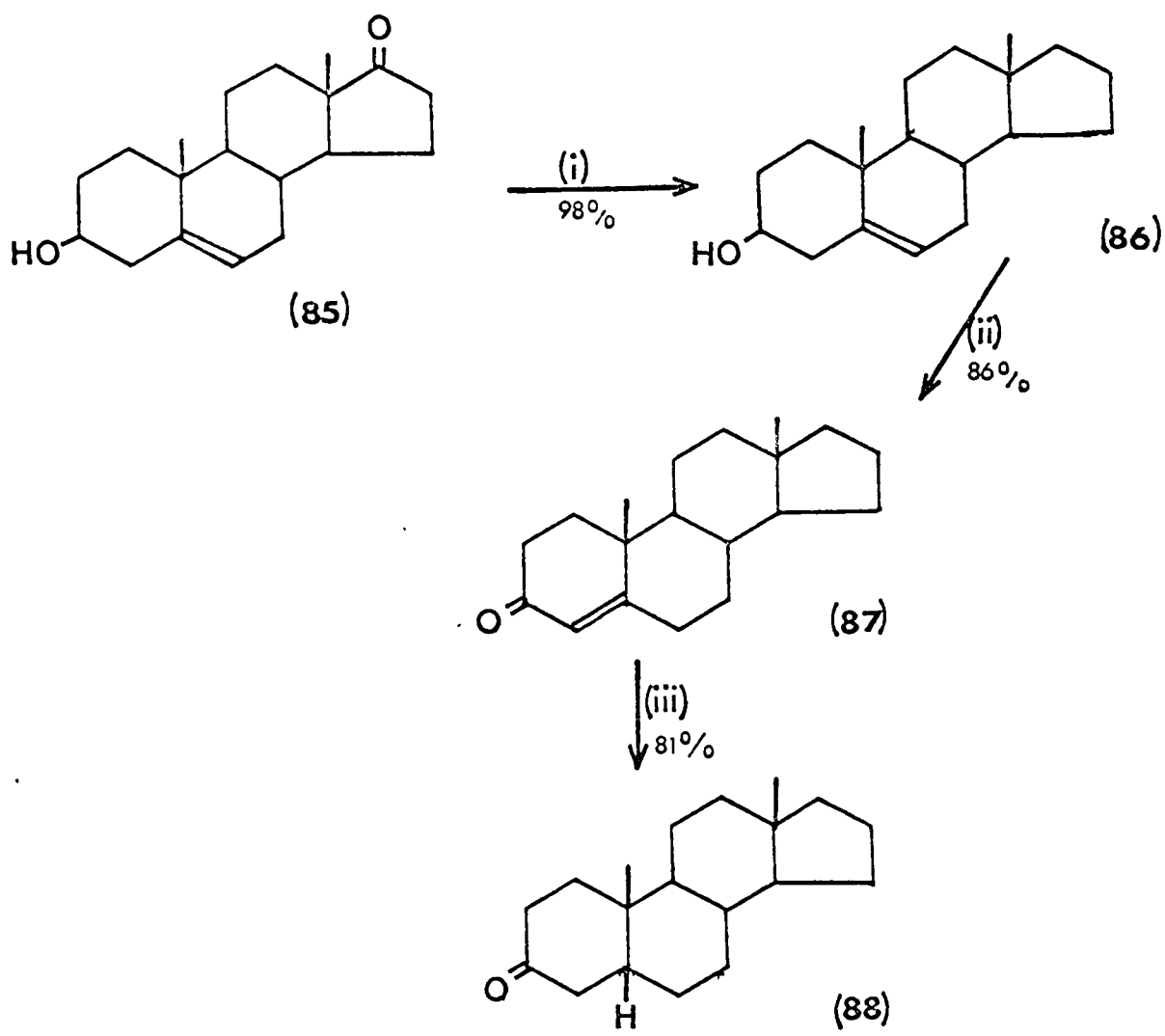


Reagents: (i) $Ac_2O - C_5H_5N$; (ii) Huang-Minlon reduction ;
(iii) $8\underline{N} - H_2CrO_4$.

Healthy cultures, invariably grown in the less concentrated medium A, did not support more than 2 g/l substrate concentration.

Utilising a healthy growth of C.decora, fermentation of [54] afforded starting material (6.5%), 12β,15α-dihydroxy-5α-androstan-3-one [56] (32%) and 5α-androstane-3β,12β,15α-triol [55] (7.6%). Minor products included 6α,11α-dihydroxy-5α-androstan-3-one [78] (4.4%) and 6α,12β,15α-trihydroxy-5α-androstan-3-one [79] (8%). The restricted hydroxylation pattern in [78] suggests that with this fungus a competition between two enzyme systems may be involved; this feature will be discussed in Section II.

SCHEME 12.

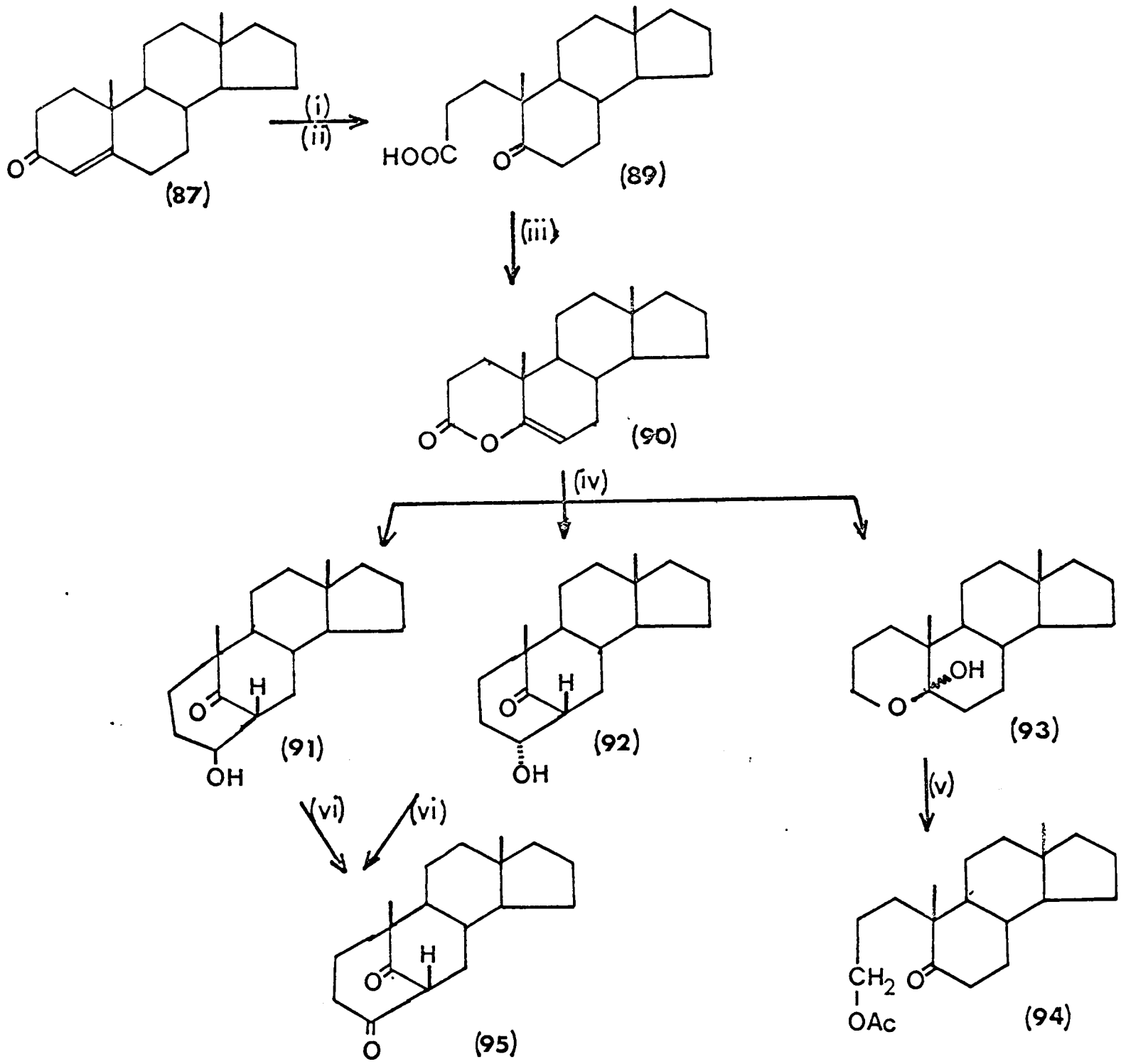


Reagents: (i) Huang-Minlon reduction

(ii) Oppenauer oxidation[61]

(iii) 5% Pd on $CaCO_3$ - Acetonitrile - H_2.[62]

SCHEME 13.



Reagents: (i) O_3 – EtOAc ; (ii) $NaHCO_3$ – H_2O_2 ; (iii) AcCl – Ac_2O
(iv) $Li(OBu^t)_3AlH$ – THF ; (v) Ac_2O – C_5H_5N ; (vi) 8N – H_2CrO_4

D. THE SYNTHESIS OF SUBSTRATES OF UNNATURAL CONFIGURATION

Synthesis of 5β-androstan-3-one [88]

This ketone was prepared by the conventional method, outlined in Scheme 12.

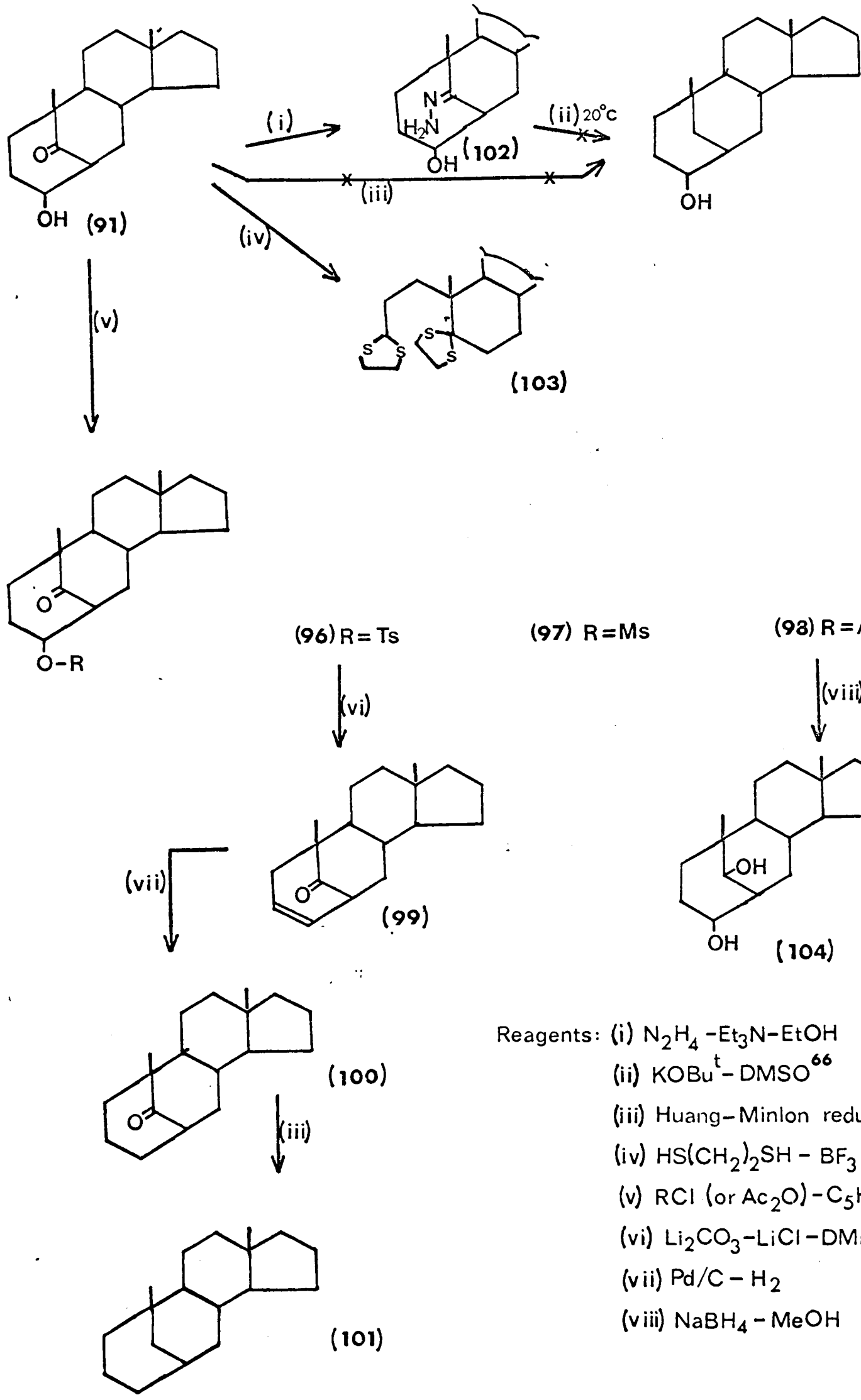
Synthesis of A-nor-(5 → 6β)abeo-androstane derivatives (Schemes 13 and 14)

The isomeric bridged ketols [91] and [92] were prepared by simple modification of the existing method.[64] Ozonolysis of 5α-androst-4-en-3-one [87] gave an acid spectrally consistent with A-nor-3,5-seco-5-oxo-androstan-3-oic acid [89]. Dehydration of the crude acid with acetyl chloride and acetic anhydride[65] gave the enol lactone [90] in good yield.

Reduction of [90] with lithium tri-t-butoxyaluminium hydride gave a 1:4 ratio of 3α-hydroxy- [91] and 3β-hydroxy-A-nor-(5 → 6β)abeo-androstan-5-one [92], and was considerably less stereo-selective than a similar reduction of 17β-hydroxy-4-oxa-androst-5-en-3-one where Martin et al.[64] report a 1:24 isomeric mixture. A non polar product of the reaction was consistent by n.m.r. and i.r. spectra with a tertiary-hydroxy-acetal structure, and exhibited a mass spectral molecular ion at m/e 278. Acetylation gave A-nor-3-acetoxy-3,5-seco-androstan-5-one [94], and the structure of the hydroxy-acetal can be rationalised as the isomeric mixture [93] attributable to an excess of reducing agent. Oxidation of the hydroxy-ketone [91] with 8N-chromic acid at 0°C gave the 3,5-dione [95].

Stable tosylate, mesylate and acetate derivatives [96], [97] and [98] of the 3β-hydroxy-5-ketone [91] were readily formed, and subsequent transformation of the tosylate [96] afforded the bridged mono-ketone [100] in high

SCHEME 14.


(i)
(ii) 20°C
(iii)
(iv)
(v)
(vi)
(vii)
(viii)
OH
(91)
(102)
(103)
(96) R = Ts
(97) R = Ms
(98) R = Ac
O-R
(99)
(100)
(101)
(104)
Reagents: (i) N_2H_4 -Et_3N-EtOH
(ii) $KOBu^t$- DMSO[66]
(iii) Huang-Minlon reduction
(iv) $HS(CH_2)_2SH$ - $BF_3.Et_2O$
(v) RCl (or Ac_2O) - C_5H_5N
(vi) Li_2CO_3-LiCl-DMF
(vii) Pd/C - H_2
(viii) $NaBH_4$ - MeOH

yield. Huang-Minlon reduction of [100] gave A-nor-(5 → 6β)abeo-androstane [101], useful as a basis for n.m.r. spectral correlations (Section IV).

In an attempt to prepare the bridge-head ketone, Huang-Minlon reduction of [91] caused ring opening, even under mild conditions[66] and thioketalisation of [91] in $BF_3.Et_2O$ gave a single product spectrally consistent with the structure [103]. Reduction of the acetoxy-ketone [98] with sodium borohydride proceeded slowly, and caused cleavage of the ester to give the diol [104]; it had been hoped to remove the 5-functionalisation by preparation of, followed by the reductive cleavage[39] of, a 3β-acetoxy-5ξ-toluene-p-sulphonate derivative.

SECTION II

INCUBATIONS WITH

CALONECTRIA DECORA

Table 3: Hydroxylation of 5α-androstane mono-ketones with C.decora*

Substrate	Substrate recovered (%)	Hydroxylation Products Major (%)		Minor (%)	
1-CO [105]	75	complex mixture			
2-CO [106]	13	**6**α,**12**β-$(OH)_2$	(26)	**6**α,**11**α-$(OH)_2$	(11)
3-CO [54]	10	**12**β,**15**α-$(OH)_2$	(52)	3β,**12**β,**15**α-$(OH)_3$	(9)
4-CO [107]	ca. 10	**12**β,**15**α-$(OH)_2$	(36)	**11**α,**15**α-$(OH)_2$	(36)
6-CO [108]	80	<10% hydroxylation			
7-CO [109]	72			**12**β-(OH)	(1)
11-CO [110]	40	**1**β,**6**α-$(OH)_2$	(11)	**15**α-OH-**6**-CO	(0.3)
12-CO [111]	9	**6**α,**15**α-$(OH)_2$	(15)	**1**β,**6**α,**15**α-$(OH)_3$	(12)
15-CO [1]		see Table 4			
16-CO [112]	31	**6**α,**11**α-$(OH)_2$	(33)	**1**β,**6**α-$(OH)_2$	(9)
17-CO [4]	40	**1**β,**6**α-$(OH)_2$	(47)		

* Adapted from reference 14

Previous investigation of the hydroxylating capacity of C.decora, one of the first micro-organisms to be investigated by this group[9b, 14] made use of 3-oxygenated steroidal substrates, often containing an oxygenated 17β-side chain. Almost invariably 12β,15α-dihydroxylated products were obtained[3] and prior to the current work[14] only one example of 11α-hydroxylation had been quoted.

Initial work in this laboratory demonstrated that the presence of an oxygenated 17β-side chain had little influence on the pattern of steroidal hydroxylation,[9b] and subsequent experiments have shown that in fact with an appropriate choice of the position of a polar group (usually a carbonyl) on the substrate, equatorial dihydroxylation can be introduced on most positions of the androstane skeleton (Table 3).

From the table, and additional data,[67] some general features of the hydroxylation of steroidal substrates with C.decora can be summarised:

(1) Androstanes having carbonyl groups on the terminal rings (positions 3, 4, 16 and 17) are transformed to one or two dihydroxylated products. The lack of effective hydroxylation with 5α-androstan-1-one [105] can be attributed to excessive shielding effects of the 10-methyl group and the C-ring. Similarly, in the case of dioxygenated substrates, hydroxylation is inhibited by a group in a position adjacent to that at which hydroxylation would be expected to occur.

(2) Androstanes with carbonyl groups in rings B and C (with the exception of the 12-oxo group) are less efficiently hydroxylated than if these binding sites are situated in rings A and D.

(3) Hydroxyl groups are invariably introduced equatorially.

Table 4: Incubation of 15-oxygenated 5α-androstanes with C.decora

Substrate	Substrate recovered (%)	Products	
		Major (%)	Minor (%)
15-CO [1]	4	14β-H-**6**α,**12**β-$(OH)_2$ (21)	**2**α,**12**β-$(OH)_2$ (8)
15α-OH-12-CO [59]	25	**6**α-OH (10.5)	-
12,15-$(CO)_2$ [62]	34	-	-

Table 5: Incubation of some steroidal ketones of unnatural configuration, and some steroidal ketals

Substrate	Substrate recovered (%)	Products (%)		Ref.
13α-Me-17-CO [11]	10	**1**β,**6**α-$(OH)_2$ (10)	(10)	9e
5β-H-17-CO [113]	56	**12**β,**15**α-$(OH)_2$	(6)	9b
17,17-O.$[CH_2]_2$O [5]	53	**7**β,**12**β,**15**α-$(OH)_3$	(22)	48
		7β,**12**β-$(OH)_2$	(3)	
		1β,**7**β,**12**β-$(OH)_3$	(10)	
16,16-O.$[CH_2]_2$O [114]	29	**6**α,**12**β-$(OH)_2$	(40);	9g
		12β-OH-**6**-CO	(2.8)	
3,3-O.$[CH_2]_2$O [115]	37	**6**α,**12**β-$(OH)_2$	(31)	48
		12β,**15**α-$(OH)_2$	(16.5)	

(4) A three-site enzyme-substrate complex[14] can be envisaged where each substrate is hydroxylated at the same enzyme site, and where the position of the polar group of the substrate controls its orientation with respect to the enzyme surface. In the case of 3, 4, 16 and 17-ketones the correspondence is so close to be of predictive value: hydroxyl groups are introduced to carbon atoms approximately 4Å apart and there is only a slight variation (4.5Å to 7.5Å) in the distances from the carbonyl group. The appearance of a second product in some cases clearly shows that the complex should not be regarded as a strictly rigid system; in terms of a flexible model, even the 19-hydroxylation of 5α-androstan-17-one [4] can be rationalised.[9f]

Despite a preponderance of C.decora toward 15α-hydroxylation, and although the 15-oxo group lay at a position where a transition in the pattern from 1β- to 12β-hydroxylation was evident, difficulties in the preparation of substantial quantities of 5α-androstan-15-one [1] had precluded its earlier use as a substrate. The initial object of the present work was to study the incubation of this substrate.

Kumar[9e] had found that the hydroxylation of 5α-androstan-12-one [111] with C.decora gave 6α,15α-dihydroxy-5α-androstan-12-one [130] and 1β,6α,15α-trihydroxy-5α-androstan-12-one but had detected no 1β,15α- or 2α,15α-dihydroxylated products. In the hope of inter-relating this result with that of 5α-androstan-15-one [1], 15α-hydroxy-5α-androstan-12-one [59] and 5α-androstane-12,15-dione [62] were used as substrates. (The results, Table 4, are discussed later.)

The incubation of 5α,14β-androstan-15-one [2] with C.decora gave products, also discussed later, which demonstrated a spatially restricted

Table 6: Incubation of oxygenated androstanes of unnatural configuration with *C.decora*

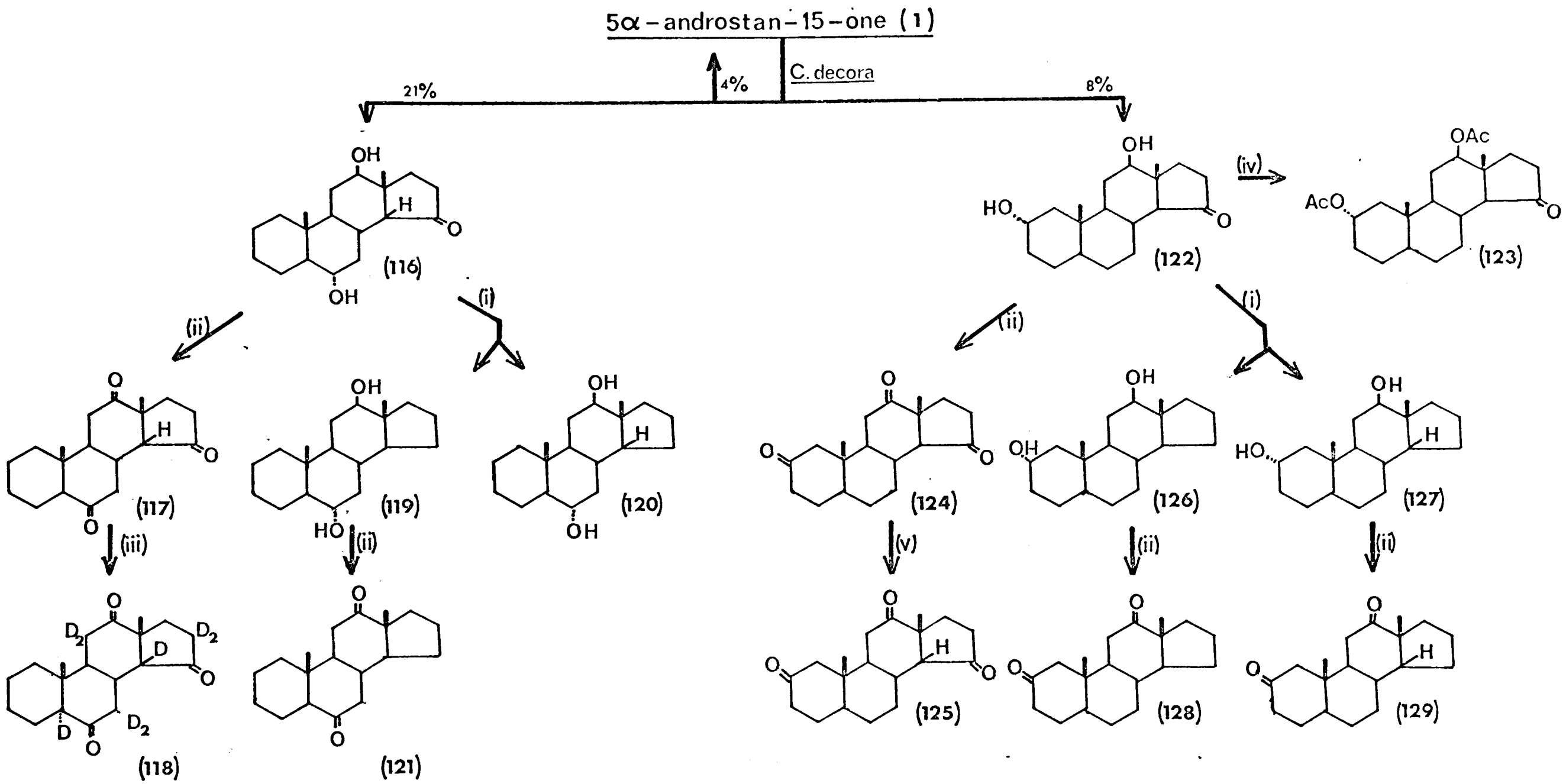
Substrate		Recovered substrate (%)	Products(%)
14β-H-15-CO	[2]	22.5	**7**β,**12**β-$(OH)_2$ (21); **7**β,**12**β,**14**β-$(OH)_3$ (7.5)
12β-OH-14β-H-15-CO	[61]*	-	as above
14β-H-12,15-$(CO)_2$	[63]	4	complex mixture
13α-Me-15-CO	[3]*+	-	**6**α,**12**β-$(OH)_2$ (15)
5β-H-3-CO	[88]	71	3α-OH (8.1); 3β-OH (1.5)
A-nor-(5→6β)abeo-5-CO	[100]+	41.5	**11**α,**15**α-$(OH)_2$ (12.5); **6**β,**11**α-$(OH)_2$ (6.5)
3β-OH-5-CO	[91]+	11.0	**12**β-OH (23.5); **7**β-OH (13); **11**α-OH (6.4); **15**α-OH-3-CO (1.2)

* Small scale incubations

\+ Structures not unambiguously characterised

hydroxylation pattern (Table 6). A similarly restricted pattern observed in the products of the hydroxylation of 5β-androstan-17-one [113],[9b] 17,17-ethylenedioxy-5α-androstane [5][48] and 16,16-ethylenedioxy-5α-androstane [114][9g] (Table 5) suggested a breakdown of the enzyme-substrate model when the planarity of the androstane skeleton relative to the binding site was lost. Conversely, Kumar[9e] found that the incubation of 5α,13α-androstan-17-one [11] resulted in 1β,6α-dihydroxylation, as with the 13β-isomer (though in reduced yield). In the present work, a more systematic investigation was initiated of the hydroxylation of substrates where, relative to the steroidal binding site, other rings had been projected out of plane. Substrates incubated and the results are listed (Table 6). Some incubations, which are clearly indicated, were conducted only on a trial scale, and low yields (or, in the case of A-nor-(5 → 6β)abeo-androstanes, an instability to standard transformations) precluded an unambiguous structural determination of the products. In the following work, the structural determination of products of the incubations (Tables 4 and 6) is initially described; then follows a brief interpretation of these results.

SCHEME 15.



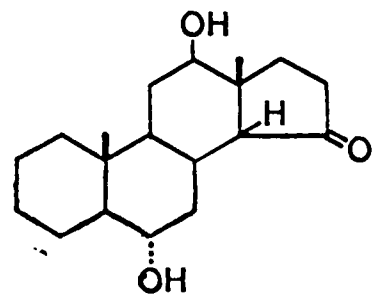
Reagents: (i) Huang-Minlon reduction; (ii) 8N-H_2CrO_4; (iii) Na-MeOD-D_2O; (iv) Ac_2O-C_5H_5N; (v) Na-MeOH

Incubation of 5α-androstan-15-one [1] with C.decora (Scheme 15)

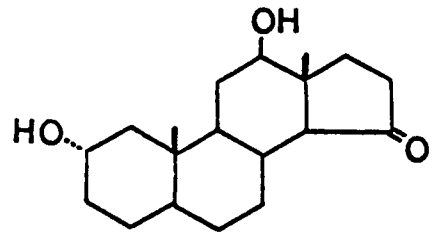
A 6-day incubation of the ketone in DMSO (7.5%) gave:-

starting material [1] 4%

5α,14β-androstan-15-one [2] 1.3%



6α,12β-dihydroxy-5α,14β-androstan-15-one [116] 21%



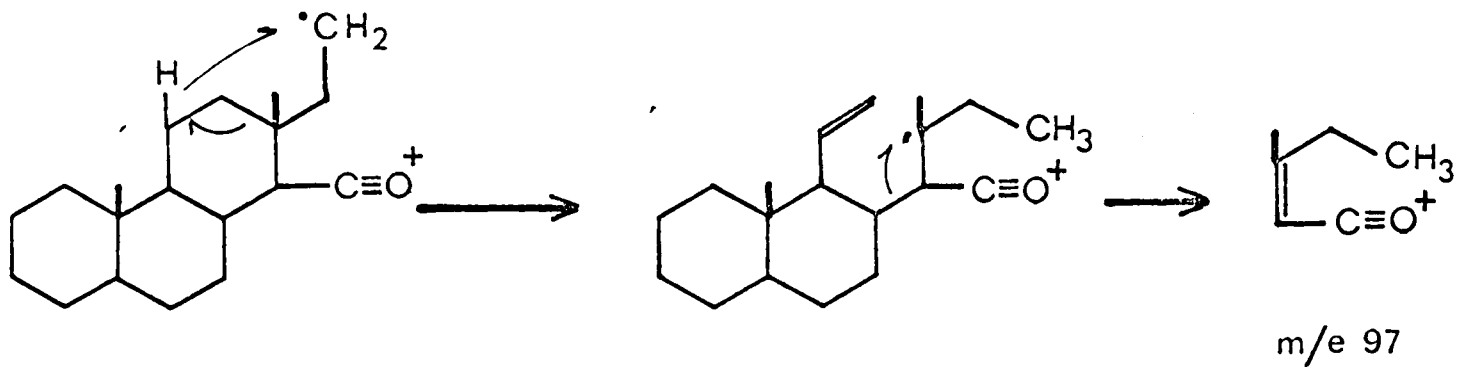
2α,12β-dihydroxy-5α-androstan-15-one [122] 8%

and 7 minor products which were not investigated.

A carbonyl stretching frequency at $\nu_{max.}$ 1740 cm^{-1}, a mass spectral molecular ion at m/e 306, and a characteristic base peak at m/e 97, probably arising as shown in Scheme 16,[19] confirmed that both major products were dihydroxy-ketones and retained the 15-oxo group. A strong negative c.d. Cotton effect in [116] (Figure 3) was characteristic of a 14β-H-15-oxo structure[19] and conversely the 14α-configuration was assigned to the diacetate [123] of [122], on the basis of its strong positive Cotton effect.

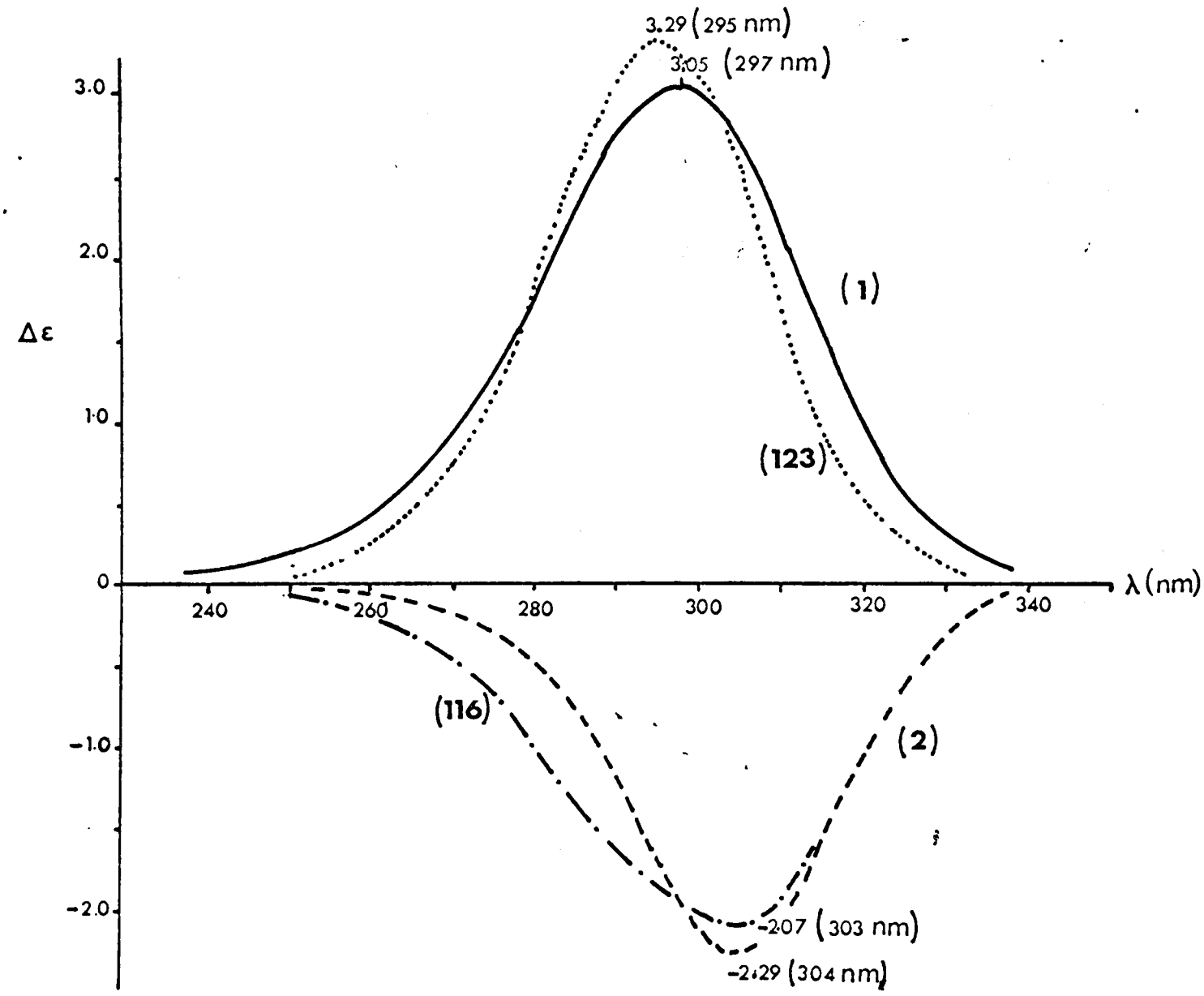
Oxidation of the major product [116] with 8N-chromic acid gave 5α,14β-androstane-6,12,15-trione [118] which exhibited in its n.m.r. spectrum a low-field quartet at τ 6.22 (J 12, 12.5 Hz), integrating to one proton, and absent

SCHEME 16. Possible fragmentation of androstane 15-ketones.[19]



m/e 97

FIGURE 3.



C.d. curves (in MeOH) of the 15-ketones **(1)**, **(2)**, **(116)** and **(123)**

FIGURE 4.

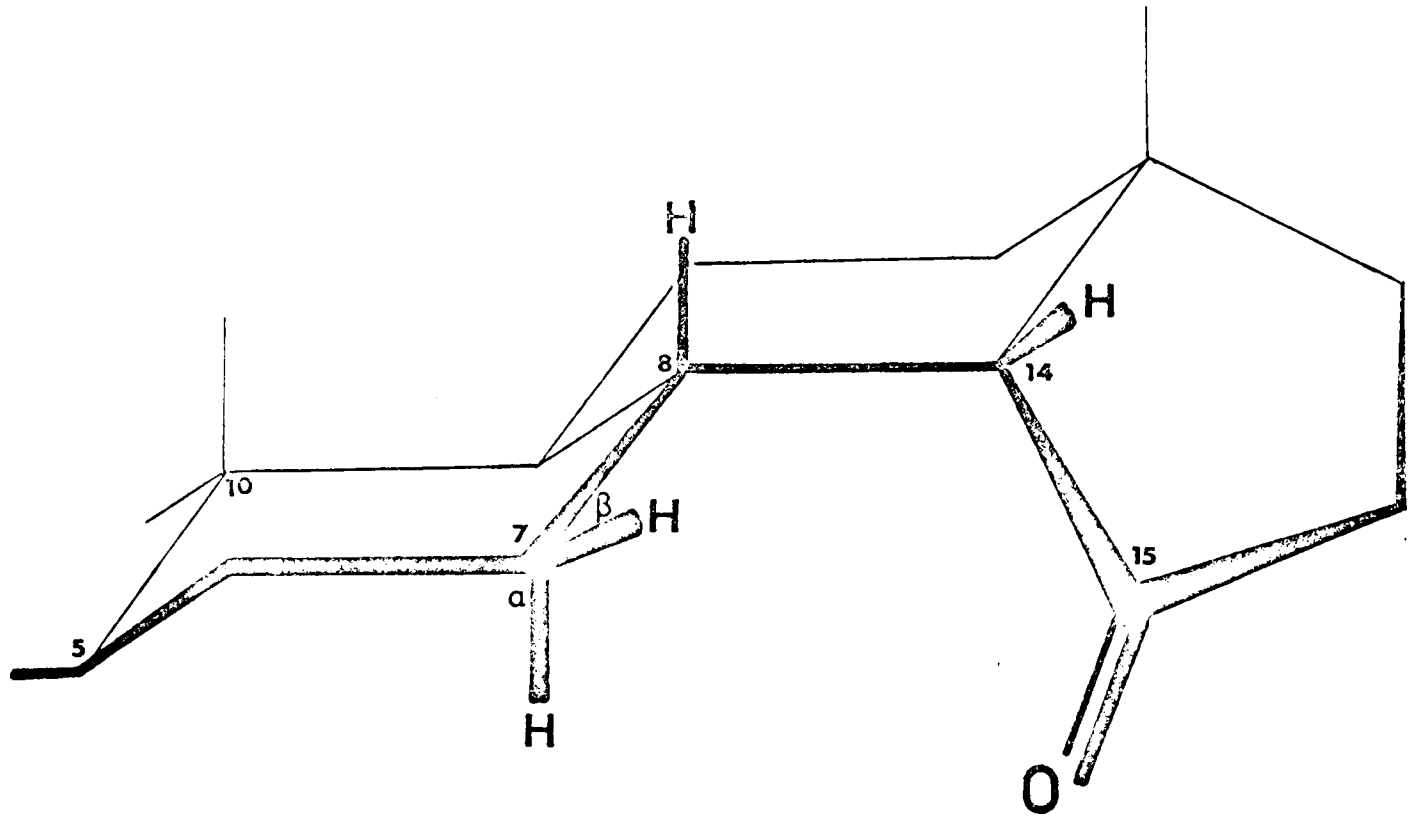
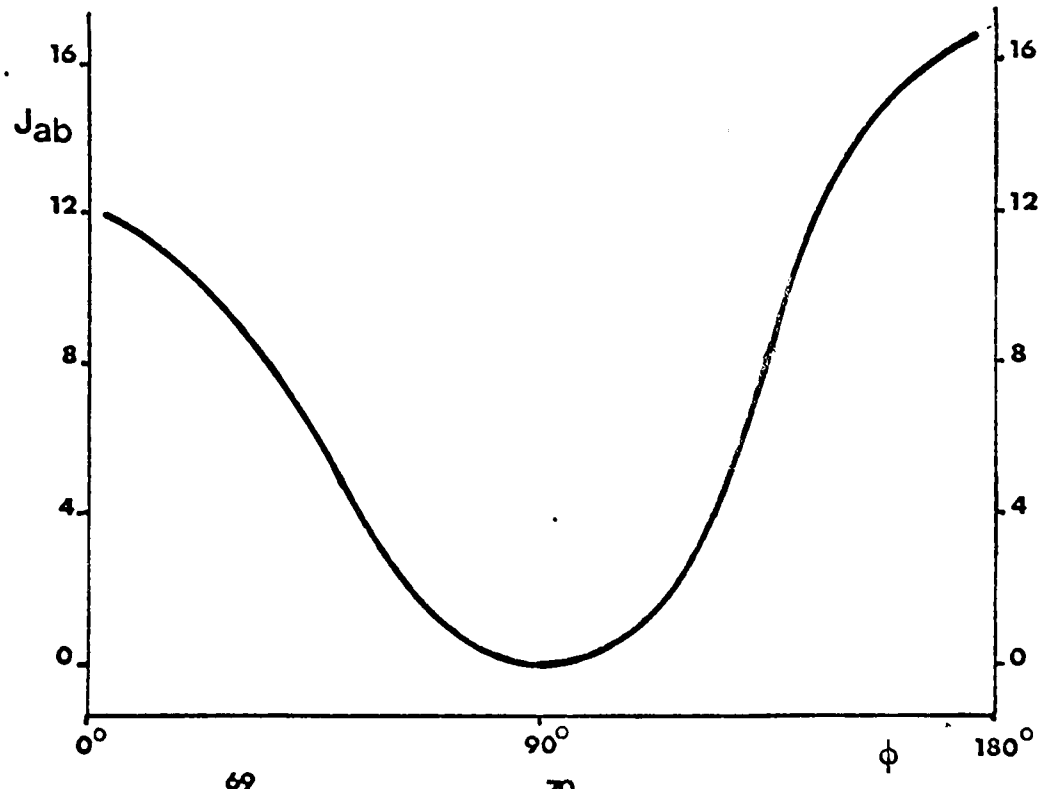
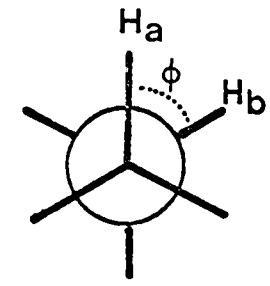
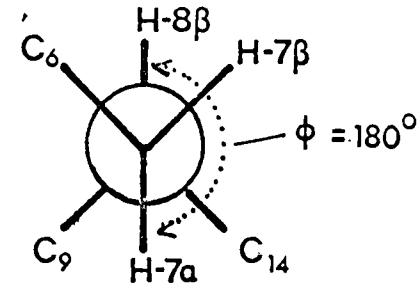


FIGURE 5.





The modified[69] Karplus relationship.[70] Coupling constant, J(Hz) vs. dihedral angle (ϕ) between protons a and b on adjacent carbon atoms.



The dihedral relationship between 7α- and 8β-protons in 5α-androstane (See also Figure 4)

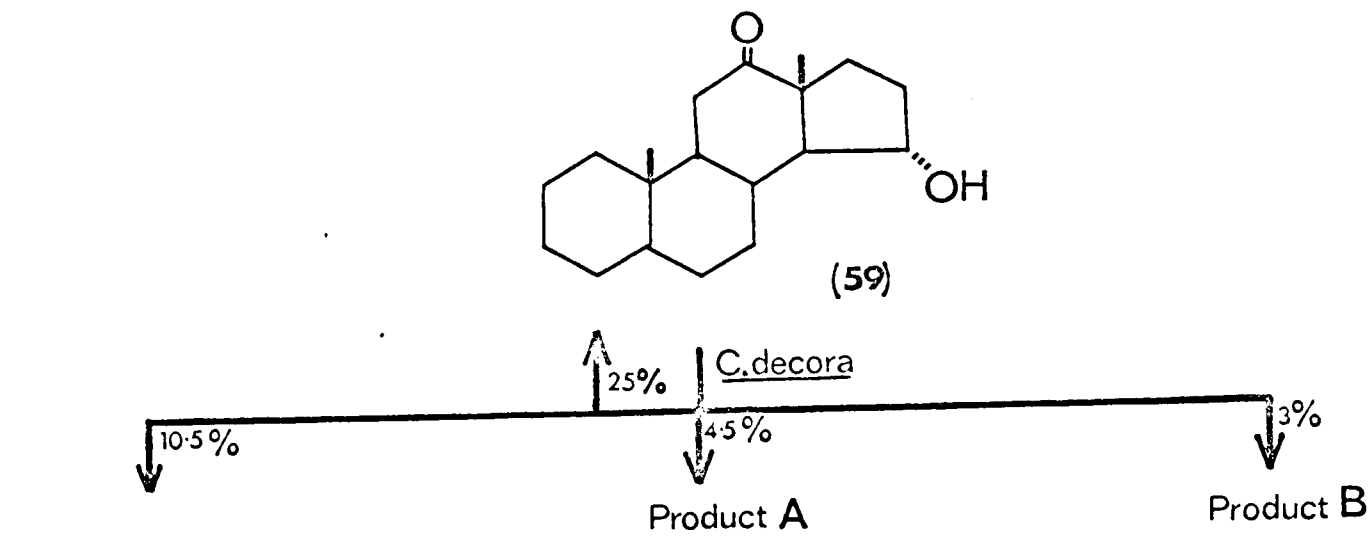
after equilibration of the trione with sodium in O-deuteriomethanol and deuterium oxide.[68] [This equilibration resulted in an uptake of eight deuterium atoms ($\underline{M}^+$ 310)]. Shielding of the 7α-proton by a 14β-H-15-ketone has been well established[19] (Figure 4) and an additional downfield shift is consistent with the presence of an adjacent ketone. The large splitting is as expected for a dihedral angle of 180° between the 7α- and 8β-protons (Figure 5) and a large geminal coupling.[71]

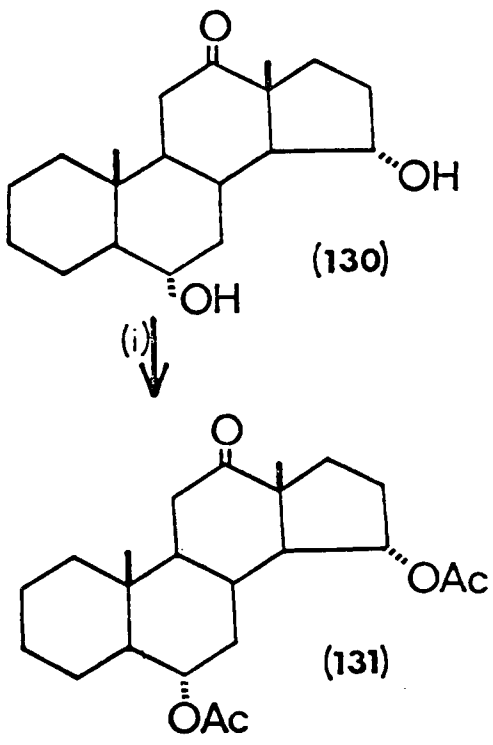
Huang-Minlon reduction of [116] under forcing conditions[46] gave a mixture (2:1) of 14α- and 14β-diols [Part III D]. 5α-Androstane-6β,12β-diol [119], separated by selective crystallisation, and the dione [121] were identified by direct comparison.[47]

The positions and profiles of the two lowfield signals in the n.m.r. spectrum of the minor product [122] were consistent with 2α,12β- or 4α,12β-dihydroxylation.[15] Oxidation of [122] with 8$\underline{\underline{N}}$-chromic acid at 0°C afforded a trione which was isomerised in methanolic potassium hydroxide to its 14β-isomer. Both triones showed split C_{19}-$\underline{H}$ n.m.r. resonance, characteristic of 2-keto androstanes,[9d] and C-methyl solvent shift values in agreement with those calculated for 5α-androstane-2,12,15-trione [124] and 5α,14β-androstane-2,12,15-trione [125].

Huang-Minlon reduction of [122] gave two products [Part III E] spectroscopically consistent with the structures 5α-androstane-2α,12β-diol [126] and 5α,14β-androstane-2α,12β-diol [127]. Oxidation with 8$\underline{\underline{N}}$-chromic acid gave the corresponding 2,12-diones [128] and [129] whose n.m.r. split C_{19}-$\underline{H}$ resonance and solvent shift values were consistent with the structures [128] and [129] proposed. Insufficient material prevented full characterisation of these products.

SCHEME 17.





Reagent: i $Ac_2O-C_5H_5N$

Incubation of 15α-hydroxy-5α-androstan-12-one [59] with C.decora (Scheme 17)

Incubation of the ketone [59] in DMSO (7.5%) for 4 days gave:

starting material [59] 25%,

6α,15α-dihydroxy-5α-androstan-12-one [130], isolated as the diacetate [131], and identified by direct comparison with an authentic sample[9e] 10.5%,

product A (unidentified) 4.5%,

product B (unidentified) 3%,

and two other products isolated as their diacetate derivatives <2%.

Products A and B showed mass spectral molecular ions at m/e 306, and were therefore dihydroxy-ketones.

Product A, in addition to a low-field signal consistent with 15α-hydroxylation,[15] showed in its n.m.r. spectrum a broad unresolved low-field signal. Strong hydrogen bonding evident from its i.r. spectrum,the ready formation of an unidentified acetonide derivative, and its decomposition during oxidation with 8N-chromic acid precludes A-ring hydroxylation and suggests a 7,15-dihydroxylated structure.

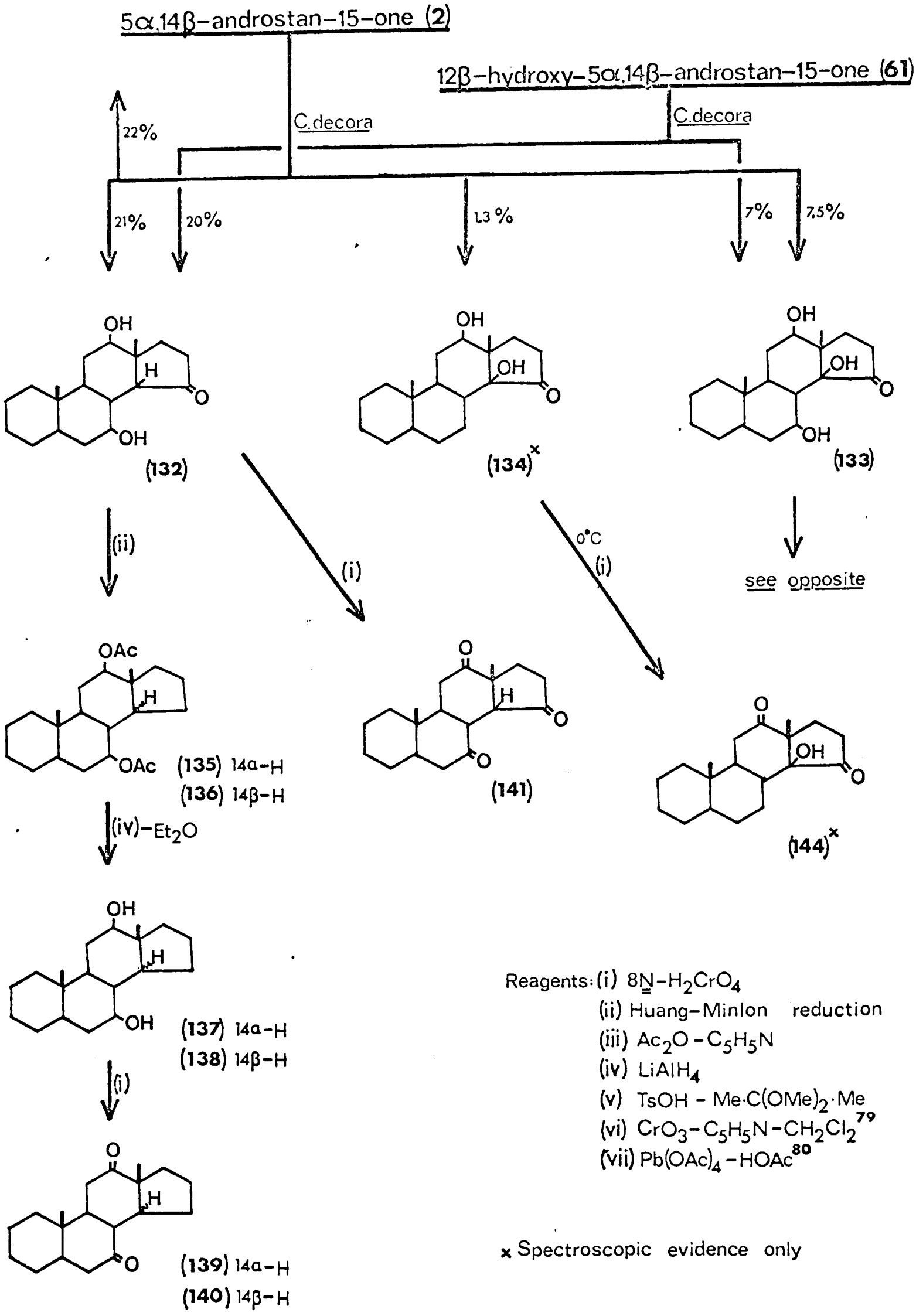
Product B showed in its n.m.r. spectrum a low-field sextet consistent with 15α-hydroxylation, but a second low-field geminal proton could not be resolved. Reaction of the dihydroxy-ketone with 8N-chromic acid at 0°C resulted in extensive decomposition which would be unlikely if A-ring hydroxylation were present.

Incubation of 5α-androstane-12,15-dione [62] with C.decora

Incubation of the dione [62] for 2 days in DMSO (7.5%) gave:-

starting material [62] 34%,

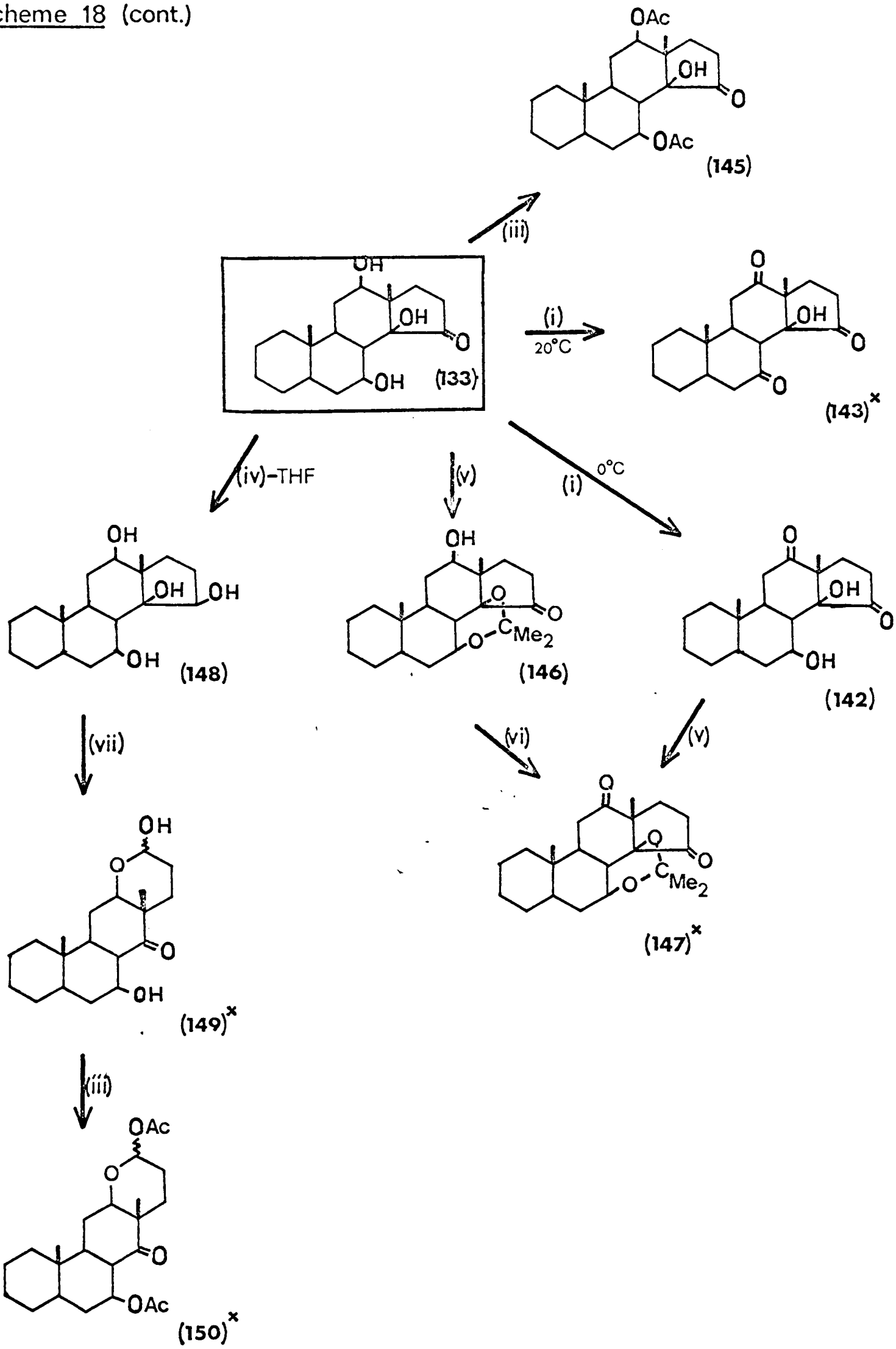
SCHEME 18.



Reagents: (i) 8N-H_2CrO_4
(ii) Huang-Minlon reduction
(iii) $Ac_2O - C_5H_5N$
(iv) $LiAlH_4$
(v) TsOH - $Me{\cdot}C(OMe)_2{\cdot}Me$
(vi) $CrO_3 - C_5H_5N - CH_2Cl_2$ [79]
(vii) $Pb(OAc)_4$ - HOAc [80]

x Spectroscopic evidence only

Scheme 18 (cont.)



and a complex mixture of polar products.

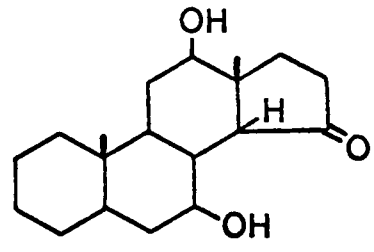
The major product (ca. 10%), isolated as its tetra-acetate, and seen from its n.m.r. spectrum to have substantial olefinic content, was not characterised.

Incubation of 5α,14β-androstan-15-one [2] and 12β-hydroxy-5α,14β-androstan-15-one [61] with C.decora (Scheme 18)

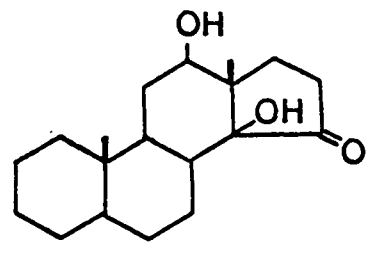
Incubation of [2] for 6 days in DMSO (7.5%) gave:-

starting material [2] 22.5%

5α-androstan-15-one [1] 0.6%

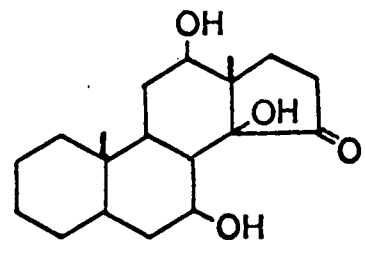


7β,12β-dihydroxy-5α,14β-androstan-15-one [132] 21%



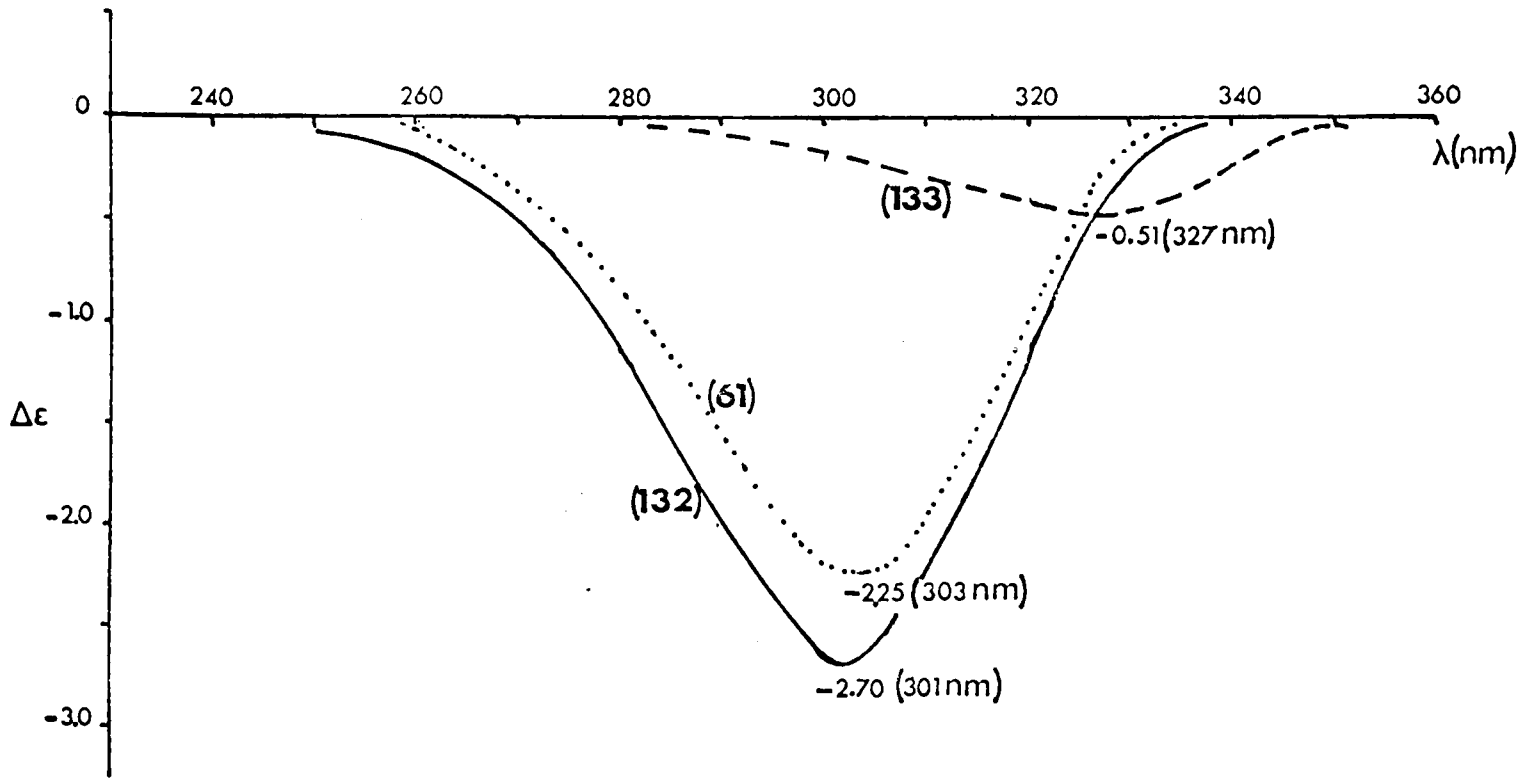
12β,14-dihydroxy-5α,14β-androstan-15-one [133] 1.3%

and



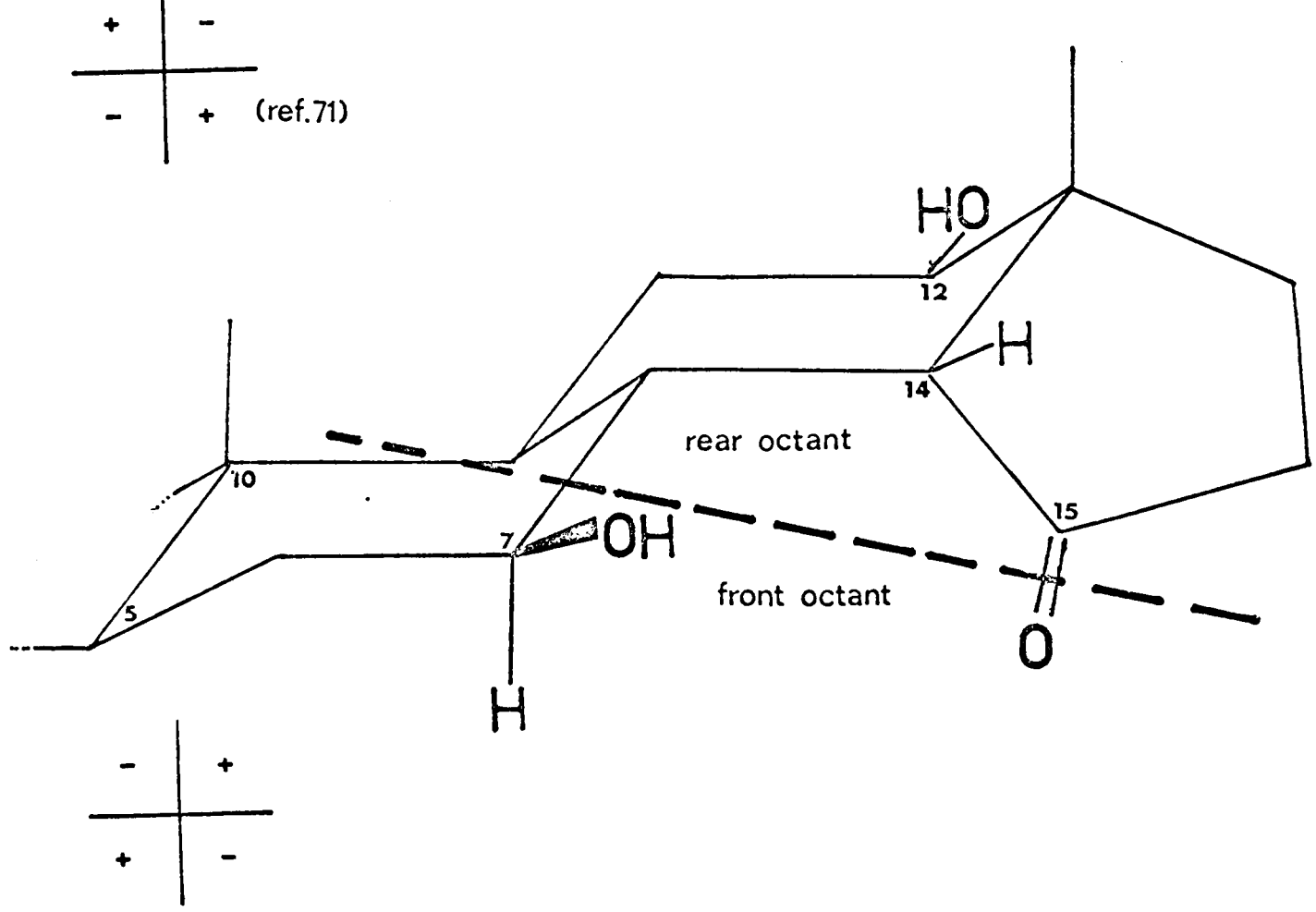
15-oxo-5α,14β-androstane-7β,12β,14-triol [134] 7.5%

FIGURE 6.



C.d. curves (in MeOH) of the 12β-hydroxy-15-ketones **(61)**, **(132)** and **(133)**.

FIGURE 7.



Application of the Octant Rule to 14β-H-15-oxo androstanes, demonstrated with product **(132)**.

A small scale incubation of 12β-hydroxy-5α,14β-androstan-15-one [61] for 4 days in DMSO (7.5%) afforded the products [132] (20%) and [133] (7%), and confirmed the presence of a 12β-hydroxy group in these products.

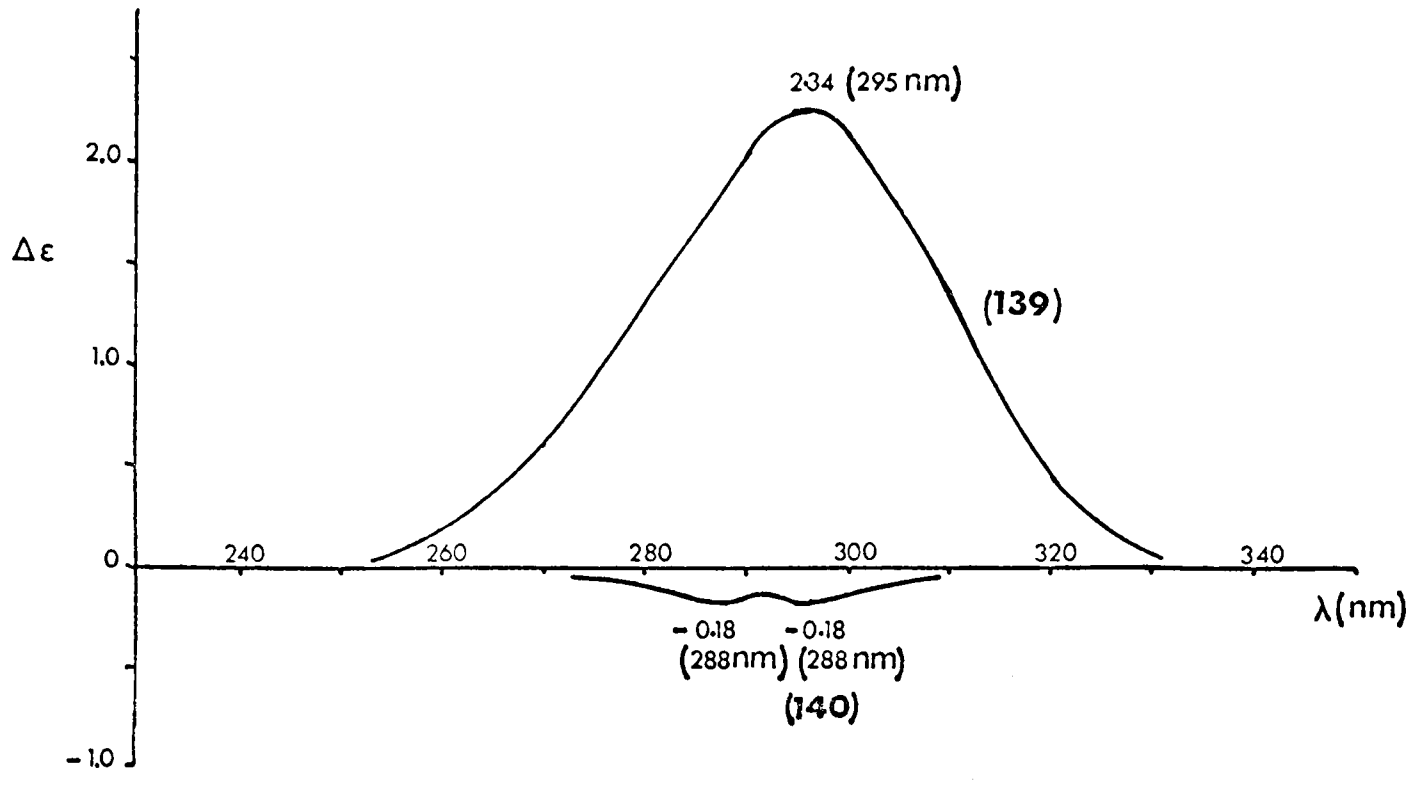
The major product, 7β,12β-dihydroxy-5α,14β-androstan-15-one [132], with a mass spectral molecular ion at m/e 306, a carbonyl stretching frequency at $\nu_{max.}$ 1733 cm^{-1}, and a non-bonded hydroxyl stretching frequency at $\nu_{max.}$ 3626 cm^{-1} was clearly a dihydroxylated structure retaining the carboxyl group at the 15-position. The profile of the n.m.r. sextet at τ 5.38 (J 10, 10, 5 Hz) was as expected for a proton geminal to an equatorial 7β-hydroxy group,[15] and the unusually low field of resonance was consistent with an additional shielding effect by a 14β-H-15-oxo group (Figure 4). A quartet at τ 6.20 (J 4, 12 Hz) was consistent with 12β-hydroxylation.

An increased intensity of the negative Cotton effect of [132] (Figure 6), characteristic of a 14β-15-oxo system, when compared with that of 12β-hydroxy-5α,14β-androstan-15-one [59], was attributed to a further negative contribution by the 7β-hydroxy group, situated in the front octant (Figure 7).

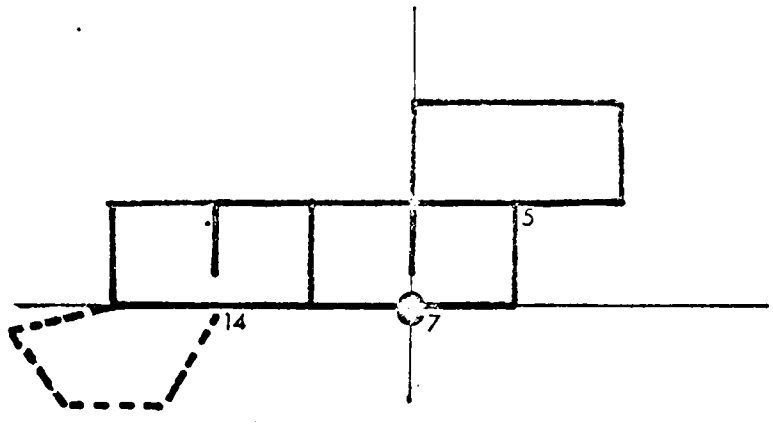
A significant mass spectral fragment of m/e 207 [M-99; 40%], absent in other 12β-hydroxylated 14β-15-ketones, indicated interaction of the hydroxyl group with the basic 15-oxo fragmentation (Scheme 16), and was further evidence for the presence of a 7-hydroxyl group in [132].

Huang-Minlon reduction of the dihydroxy-ketone [132] gave a mixture (1:3; Section IIID) of 5α-androstane-7β,12β-diol [137] and 5α,14β-androstane-7β,12β-diol [138], separable as their acetates [135] and [136], and oxidised to the diones [139] and [140]. The structures of the natural isomers, 5α-androstane-7β,12β-diol [137] and 5α-androstane-7,12-dione [139], were confirmed by comparison with authentic samples.[48] A feature of the i.r. spectra of the diols

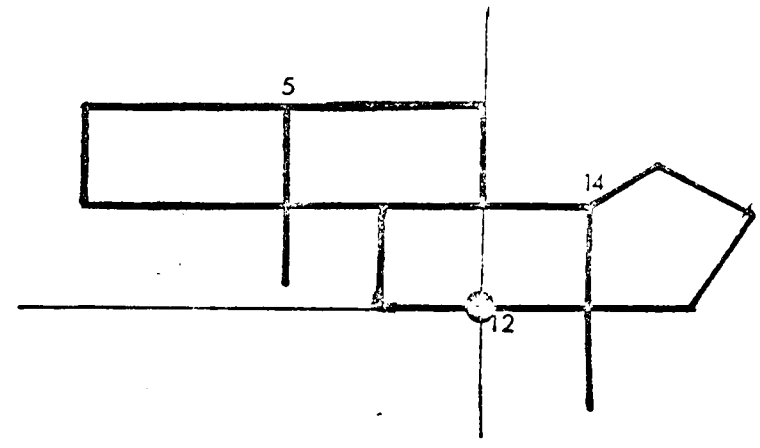
FIGURE 8.



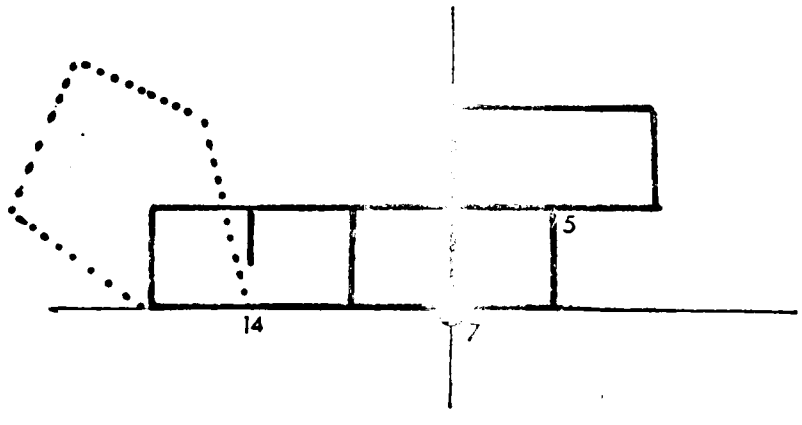
C.d. curves (in MeOH) of 5α-androstane-7 12-dione **(139)** and 5α,14β-androstane-7,12-dione **(140)**.



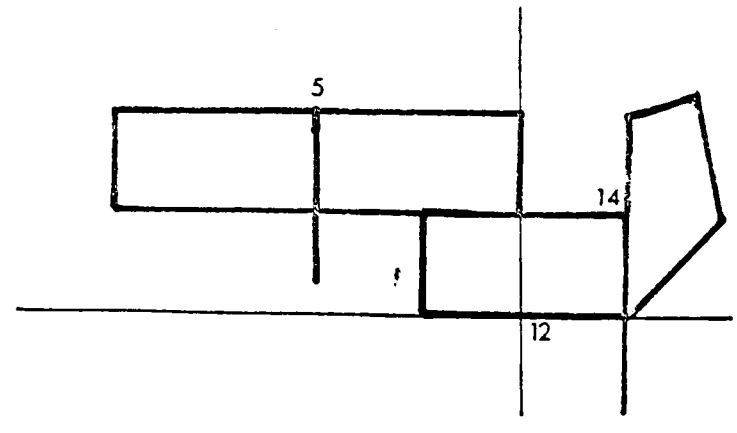
14α-H-7-CO



14α-H-12-CO



14β-H-7-CO

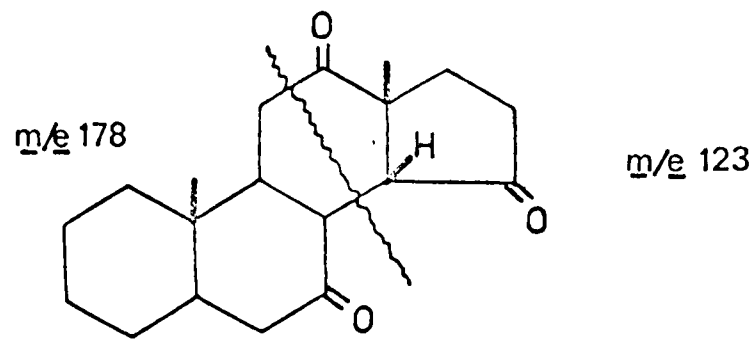


14β-H-12-CO

Octant projections of the respective ketones

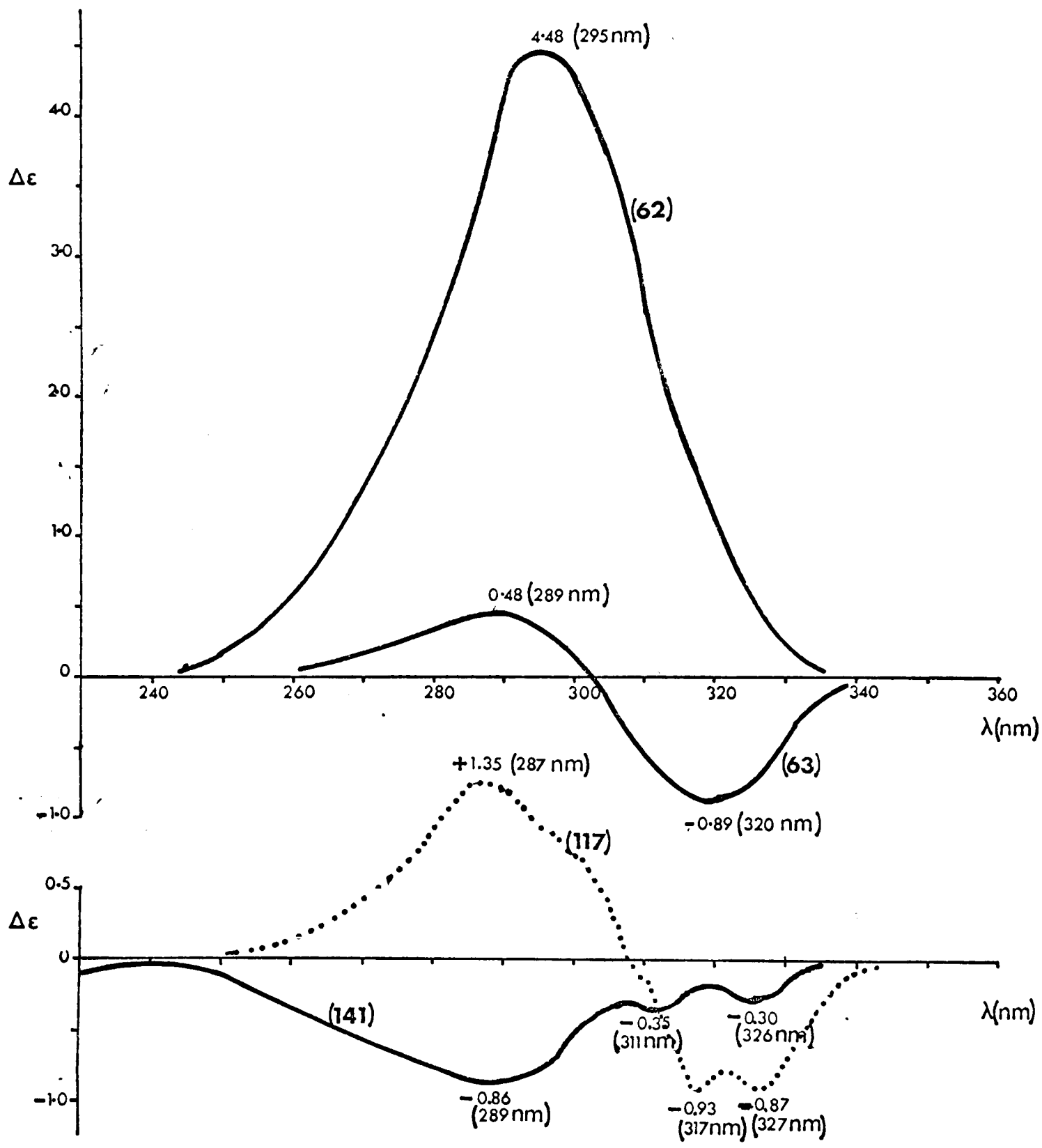
---------- Front octant

FIGURE 9.



Possible mass spectral fragmentation of 5α,14β-androstane-7,12,15-trione **(141)**

FIGURE 10.



C.d. curves (in MeOH) of: **(62)** 5α-androstane-12,15-dione
(63) 5α,14β- " - "
(117) " " -6,12,15-trione
(141) " " -7,12,15-trione

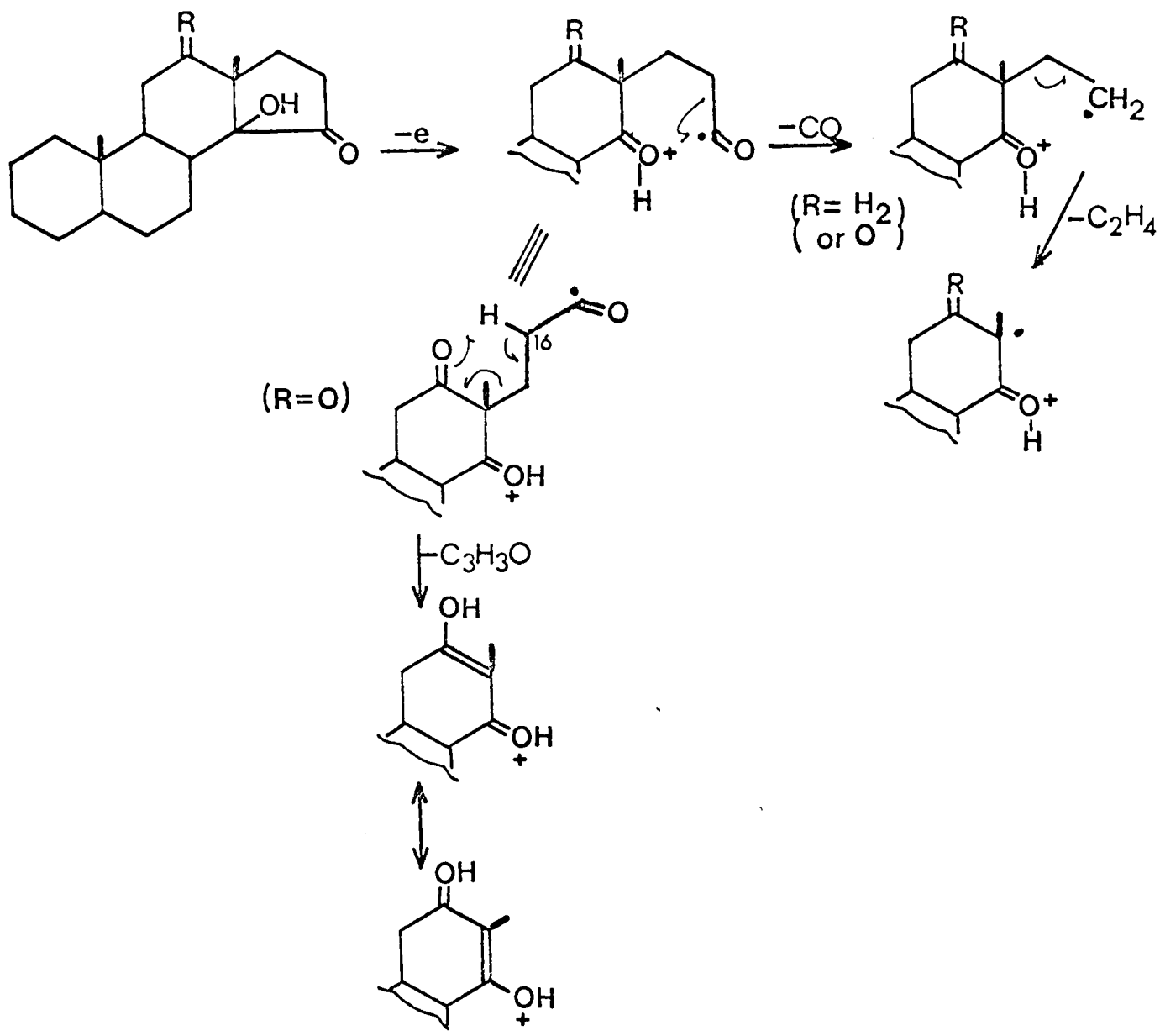
and their acetates was that a singlet C-H stretch at $\nu_{max.}$ 2860 cm^{-1} for the 14α-isomers was replaced by a well resolved doublet at $\nu_{max.}$ 2860 and 2880 cm^{-1} in the cases of the 14β-isomers. A comparison of the c.d. curves of the diones [139] and [140] (Figure 8) was in support of the configurations assigned.

Oxidation of the dihydroxy-ketone [132] gave 5α,14β-androstane-7,12,15-trione [141]. A low field doublet at τ 6.69 (J 4 Hz) in its n.m.r. spectrum was assigned to the 14β-proton, shielded by the 7-oxo group. In addition to the intense loss of a carbonyl fragment [M-28; 80%] in its mass spectrum, the trione [141] gave dominant fragments at m/e 178 [M-124; 100%] and m/e 123 [M-179; 92%] probably arising from cleavage of the C_{11-12} and C_{8-14} linkages (Figure 9). The complex c.d. curve, which bore no likeness to those of other 14β-12,15-diones (Figure 10) was evidence of strong electronic interaction between the 7- and 15-oxo groups.

The second major product [133] showed in its n.m.r. spectrum only two protons geminal to a hydroxyl group although the mass spectrum [M^+ 322] indicated trihydroxylation. Similarly the minor product [134] displayed in its n.m.r. spectrum only one geminal proton, characteristic of an equatorial 12β-hydroxyl group,[15] but the molecular ion [M^+ 306] corresponded to a dihydroxy ketone; in both cases tertiary hydroxylation was evident.

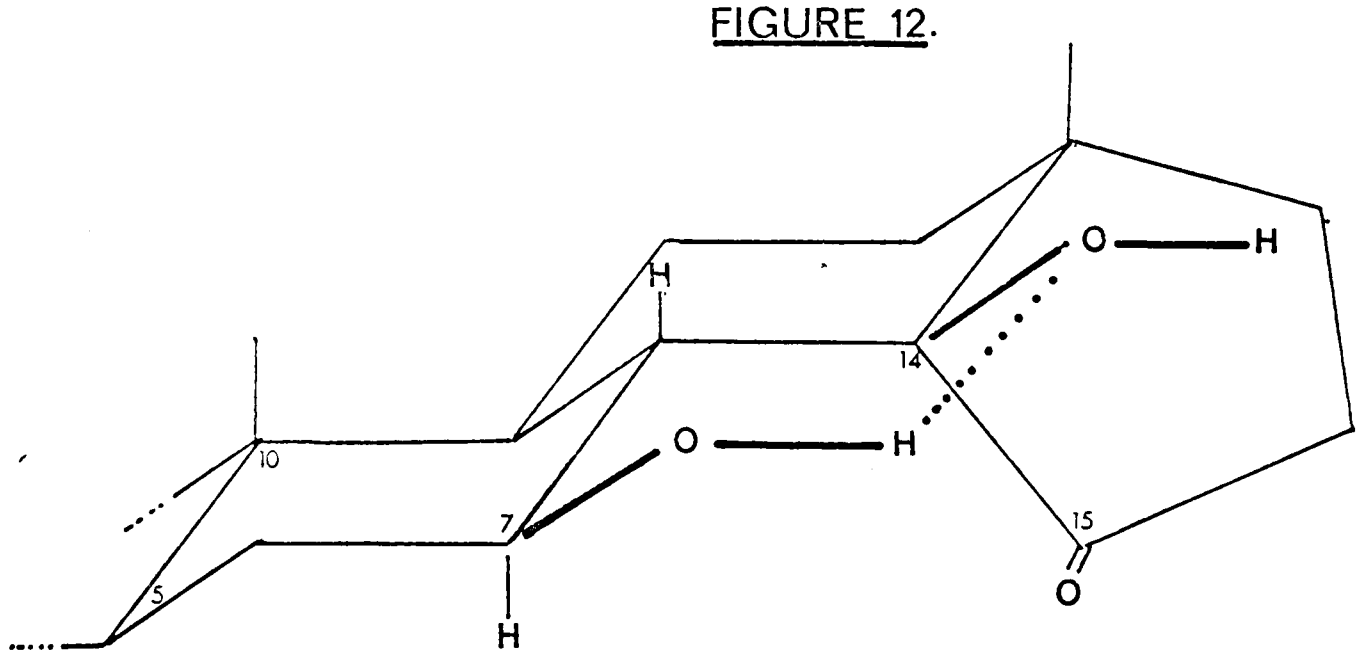
The negative c.d. Cotton effect of [133] (Figure 6), characteristic of a 14β-15-oxo group but of higher wavelength and reduced intensity when compared with [132] could be rationalised only in terms of an axial substituent adjacent to the keto group;[74,75] that is, 14β-hydroxylation. The mass spectra of [133], [134], and all transformation products were dominated by a loss of 56 mass units,

FIGURE 11.



Possible fragmentation paths in the mass spectra of 14β-hydroxy-15-oxo androstanes. (Adapted from ref. 73)

FIGURE 12.



attributable to C_{14-15} and C_{13-17} cleavage (Figure 11). Such D-ring cleavage has been conclusively established in the 14β-hydroxy-15-oxo digacetigenin series.[73]

The trihydroxy-ketone [133] showed in its n.m.r. spectrum a sextet at τ 5.0 ($W_{\frac{1}{2}}$ 27 Hz) which, after comparison with the spectrum of [132], was similarly associated with the hydrogen geminal to a 7β-hydroxyl group and shielded by a 14β-15-oxo function.

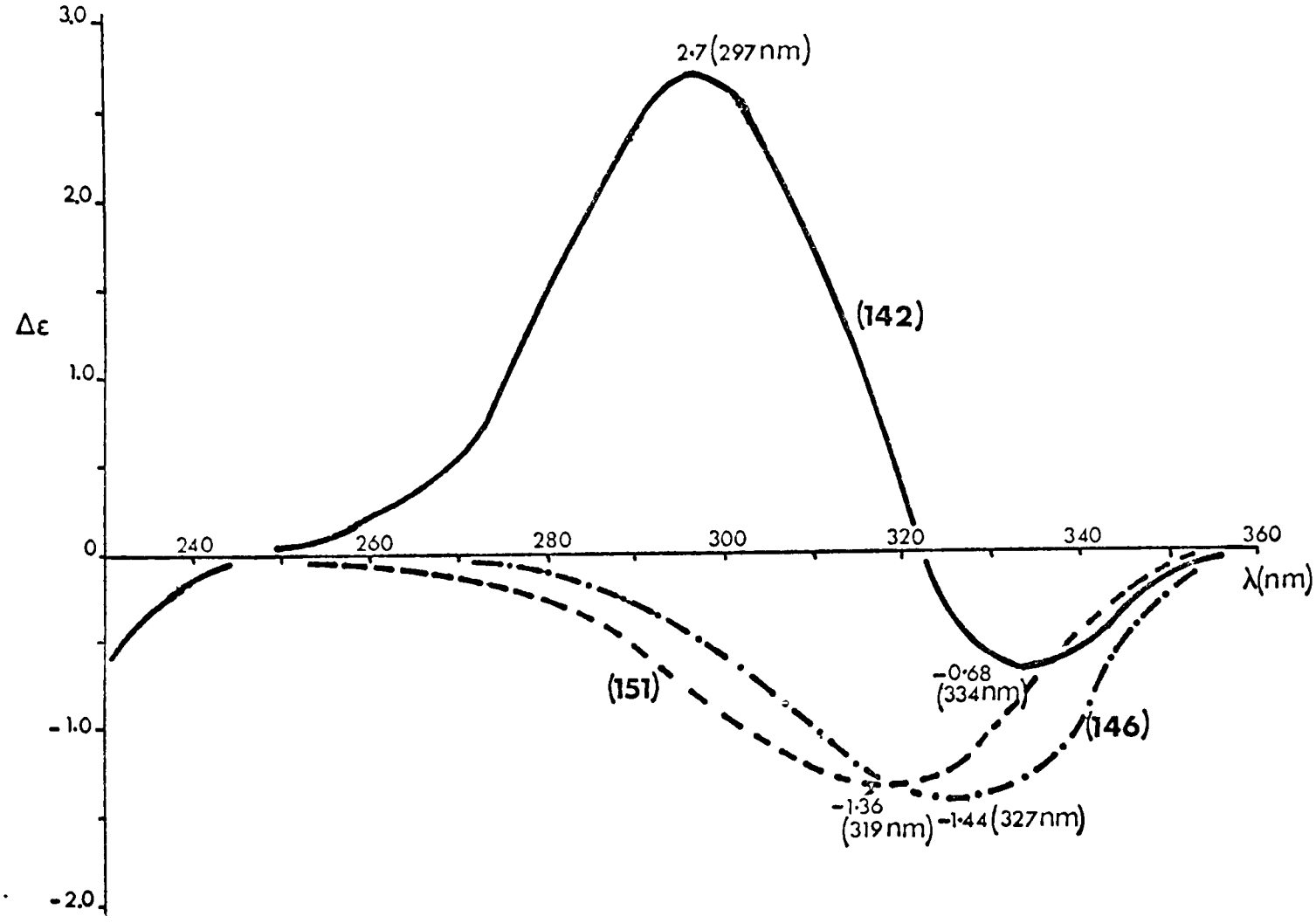
Oxidation of [133] with 8N-chromic acid at 0°C afforded 7β,14-dihydroxy-5α,14β-androstane-12,15-dione [142], and at 20°C gave a mixture from which a hydroxy-trione, spectroscopically consistent with 14-hydroxy-5α,14β-androstane-7,12,15-trione [143] was isolated. Attempted elimination of the 14-hydroxyl group in [143] resulted in a complex mixture; the 8β,14β-geometry is unfavourable for elimination.[76]

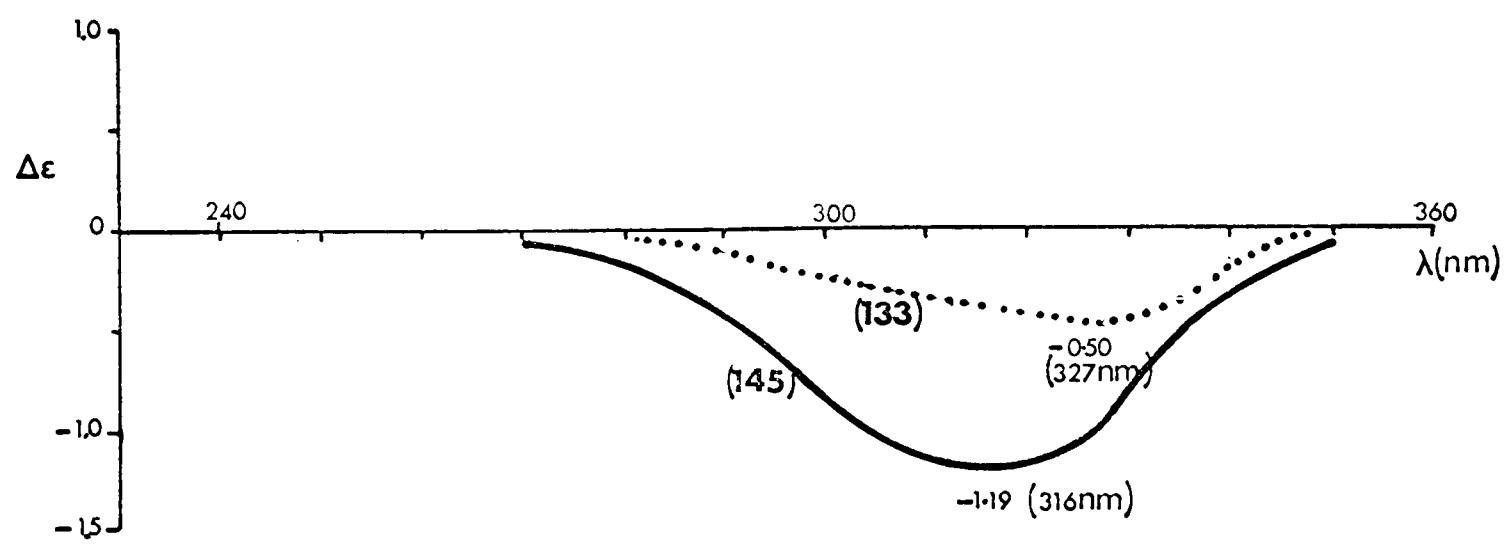
The i.r. spectra of [133] and [142] showed strongly bonded hydroxyl stretching at $\nu_{max.}$ 3405 cm^{-1}. The axes of the equatorial 7β-C-O and 14β-C-O bonds are parallel and should be ideally situated to afford by acetonide formation or by H-bonding a perfect chair conformation (Figure 12). Such H-bonding would account for the incomplete oxidation observed.

Acetylation of the trihydroxy-ketone [133] gave 15-oxo-5α,14β-androstane-7β,12β,14-triol 7,12-diacetate [145] in whose i.r. spectrum the hydroxyl stretching frequency had shifted to 3524 cm^{-1}. The shift of 120 cm^{-1} was in close agreement with that observed in the hydroxylation products of digitoxigenin[77] and similar to the 3β,5β-dihydroxy- and 3α,5α-dihydroxy-systems reported by Meakins et al.[78]

The trihydroxy-ketone [133] formed an isopropylidene derivative [146] in quantitative yield. Oxidation of [139] by the Sarett procedure[79] gave a product

FIGURE 13.





C.d. curves (in MeOH) of the 14β-hydroxy-15-oxo derivatives **(133)** **(142)** **(145)** **(146)** and **(151)**

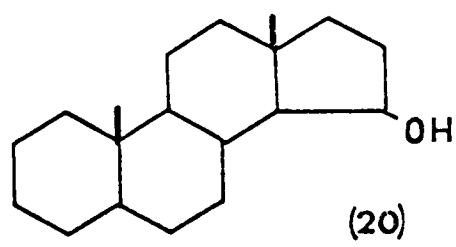
of R_F identical with that of the isopropylidene derivative [147] of 7β,14-dihydroxy-5α,14β-androstane-12,15-dione [142].

Reduction of [133] with lithium aluminium hydride gave the 7β,12β,14β,15β-tetraol [148] which after oxidation with lead tetra-acetate in acetic acid[80] gave a product consistent (spectroscopically) with the (1:1) lactone mixture [149], presumedly formed by condensation of the aldehyde. Acetylation of the lactone mixture gave a product spectroscopically consistent with the acetate mixture [150].

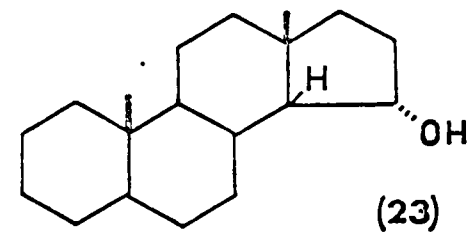
The unambiguous determination of the structures of 5α,14β-androstane derivatives already described in this Thesis and elsewhere[81] allowed the tentative assignment of Zürcher additive values[15,82] for a number of substituents associated with the 5α,14β-androstane system [Table 46; Section V]. The synthesis of 14-hydroxy-5α,14β-androstan-15-one [151] (Scheme 19) allowed the determination of a Zürcher value for this function. Final support for the 14β-hydroxylated structures proposed came from a detailed examination of the position of resonance of the C-methyl protons in their n.m.r. spectra; the observed values were with the exception of the tetra-ol [148] in surprising agreement with the calculated values (Table 20; Section IV).

The high wavelength Cotton effect of [133] and its isopropylidene derivative [146] when compared with 14-hydroxy-5α,14β-androstan-15-one [151] (Figure 13) can be attributed to strong 7β-O-14β-O bonding, while the lower wavelength Cotton effect of the diacetate [145] can be ascribed to an expected steric interaction within the system.

SCHEME 19.



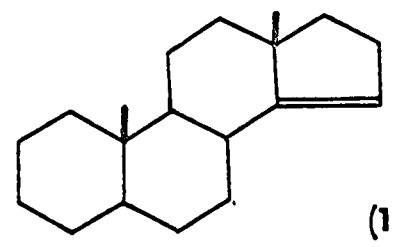
(20)



(23)

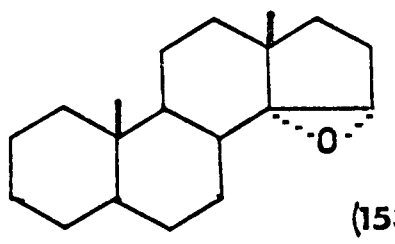
(i)

(i)

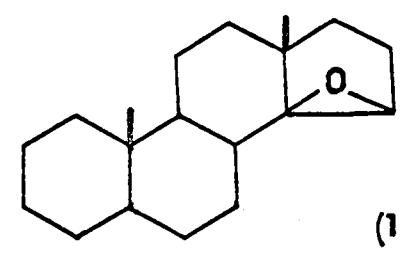


(152)

(ii)



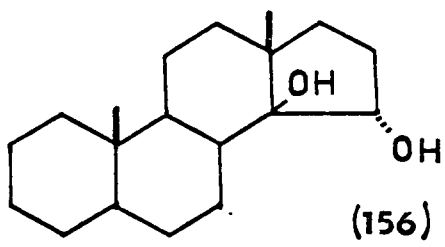
(153)



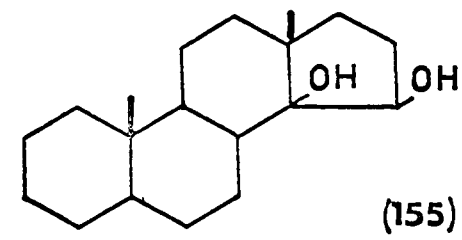
(154)

(2) ← (iii)

(iii) → (1)+(2)



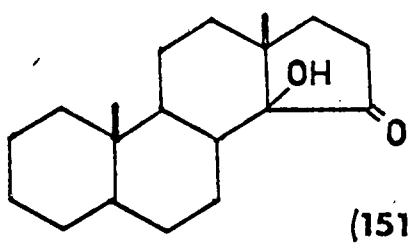
(156)



(155)

(iv) 0°C

(iv) 20°C



(151)

?

Reagents: (i) $MsCl - C_5H_5N$

(ii) Perpthalic acid – Et_2O [85]

(iii) H_3O^+

(iv) 8N – H_2CrO_4

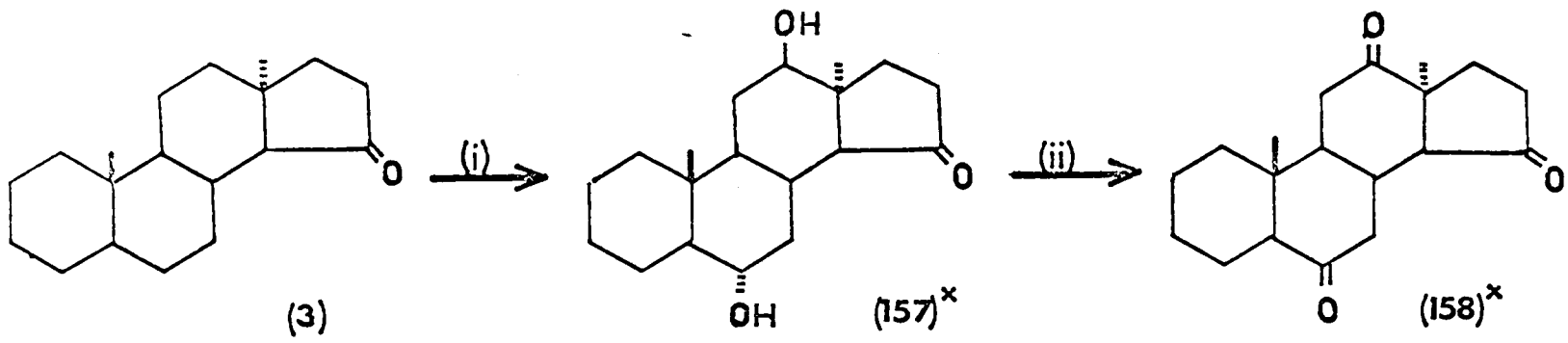
Synthesis of 14-hydroxy-5α,14β-androstan-15-one [151] (Scheme 19)

The synthesis of the steroidal 14β-hydroxy-15-oxo function via the hydrolytic cleavage of a 14α,15α-epoxide is well documented[24b, 83, 84] but has been confined mainly to cardenolide compounds.

5α-Androst-14-ene [152] was prepared by reaction of 5α-androstan-15β-ol [20] or 5α,14β-androstan-15α-ol [23] with mesyl chloride in pyridine. Oxidation of the olefin [152] with excess monoperphthalic acid[85] gave a separable mixture (11:9) of the 14α,15α-oxido- and 14β,15β-oxido-5α-androstanes [153] and [154]. This epoxidation was surprisingly non-selective; the selectivity of epoxidation is known to be very dependent on D-ring conformation[22, 29, 86] and the higher yields of 14α,15α-epoxides previously reported[24b, 83, 84] may well be a consequence of substitution at the 17β-position.

The hydrolytic cleavage of a 14β,15β-epoxide has not been reported, but on mild reaction of 14β,15β-oxido-17β-isobufadienolide with perchloric acid in acetone, Nambara and Shimada[24b] obtained the thermodynamically less stable 14α-H-15-oxo product; the reaction presumably operated via a hydride shift mechanism. In the present work, the application of these conditions to 14,15β-oxido-5α,14β-androstane [154] resulted in a mixture of the 14α- and 14β-isomers [1] and [2]. With milder conditions, cleavage afforded 5α,14β-androstane-14,15β-diol [155]; the cis-nature of the diol was assigned on the basis of a bonded i.r. hydroxyl stretching frequency at $\nu_{max.}$ 3520 cm^{-1}. Rearrangement of the more strained 14α,15α-epoxide [146], under the mild conditions used above, gave 5α,14β-androstan-15-one [2]. Very mild hydrolysis gave 5α,14β-androstane-14,15α-diol [156]; the configurational assignment of this product was based on the precedent of a β-attack at the tertiary centre.[42b, 83, 84]

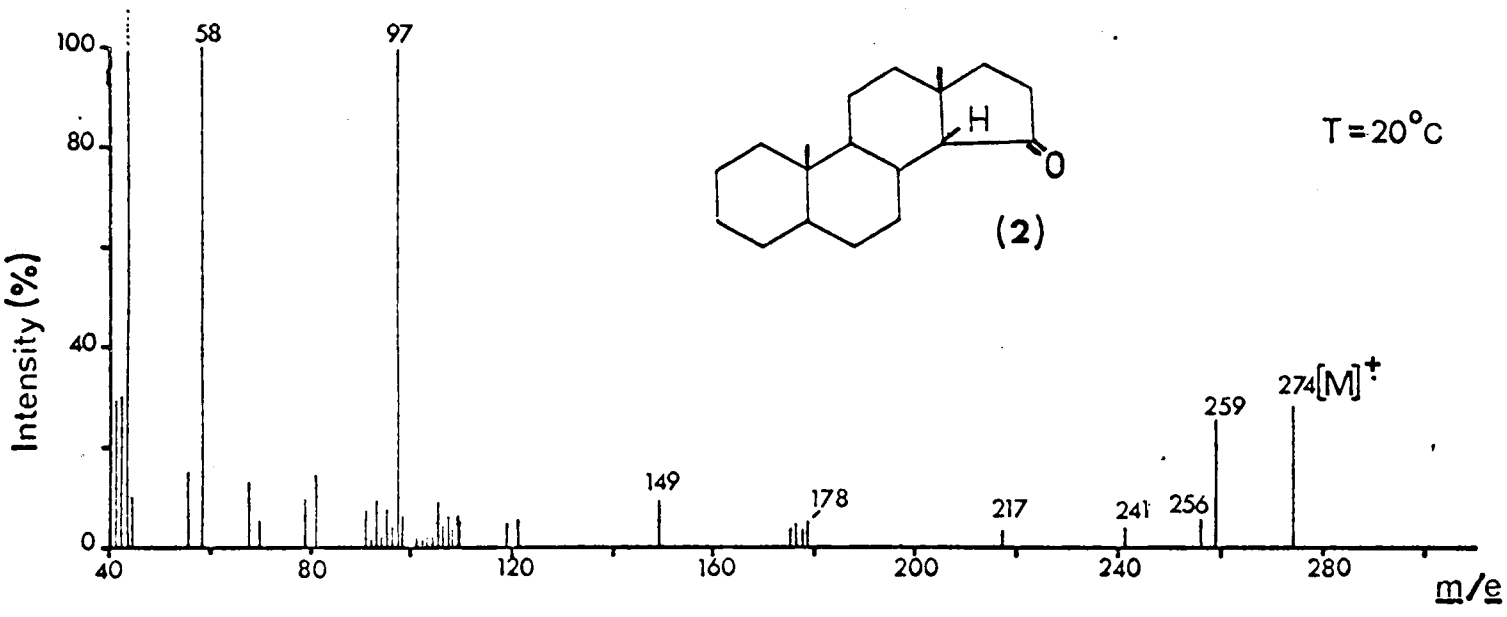
SCHEME 20.

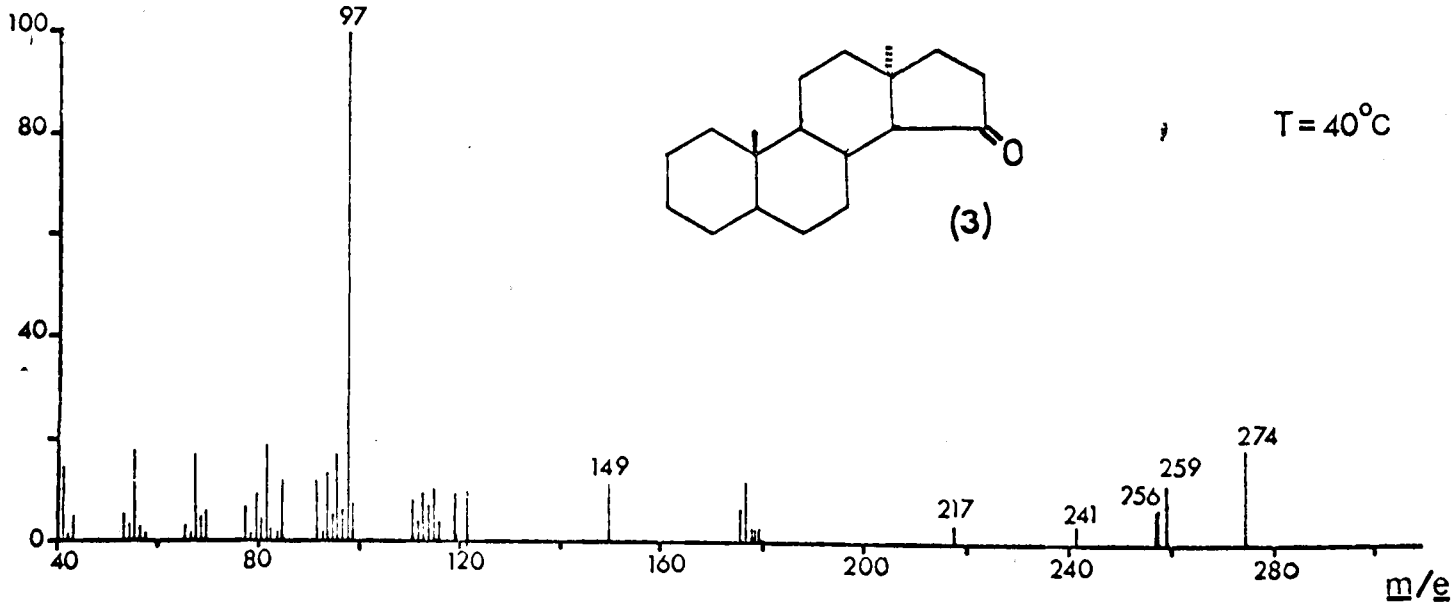


Reagents: (i) C.decora; (ii) 8N – H_2CrO_4

× Spectroscopic evidence only.

FIGURE 14. Mass spectral comparison of the isomeric 15–ketones **(2)** and **(3)**.





Treatment of the cis-diol [155] with 8N-chromic acid at 0°C caused no reaction, but at 20°C a mixture of polar compounds resulted, probably after ring opening via a cyclic intermediate. Ring cleavage of 4,4'-dimethyl-5α,14β-androstane-14,15β-diol, prepared by osmium tetraoxide oxidation of the 14-olefin, has been reported[87] in its oxidation with chromium trioxide in acetic acid.

Oxidation of the trans-diol [156] with 8N-chromic acid at 0°C gave 14-hydroxy-5α,14β-androstan-15-one [151].

Incubation of 5α,13α-androstan-15-one [3] with C.decora (Scheme 20)

A small-scale (80 mg) 6-day incubation of the ketone [3] in DMSO (7.5%) gave as the major component, a product formulated as 6α,12β-dihydroxy-5α,13α-androstan-15-one [157] (ca. 15%), and at least seven other unidentified steroidal products.

A mass spectral molecular ion at m/e 306, and i.r. stretching frequencies at 1740 and 3630 cm^{-1}, suggested that the product [157] was dihydroxylated, and retained the 15-oxo group. The positions and profiles of the two low field signals in its n.m.r. spectrum were consistent with either 1β,6α-, or 12β,6α-dihydroxylated structures. Oxidation of [157] with 8N-chromic acid afforded the trione [158]. If a 1-oxo group were present, a downfield shift of the order of 0.40 τ would be expected in the C-19 proton resonance of [158]; this was not observed. Although no values are available for 6- or 12-oxygenated 5α,13α-androstanes, the C-methyl chemical shifts and solvent shift values were of the order expected for 5α,13α-androstane-6,12,15-trione [158].

In the mass spectra of 5α,14β-androstane-12,15-dione [63], and 5α,14β-

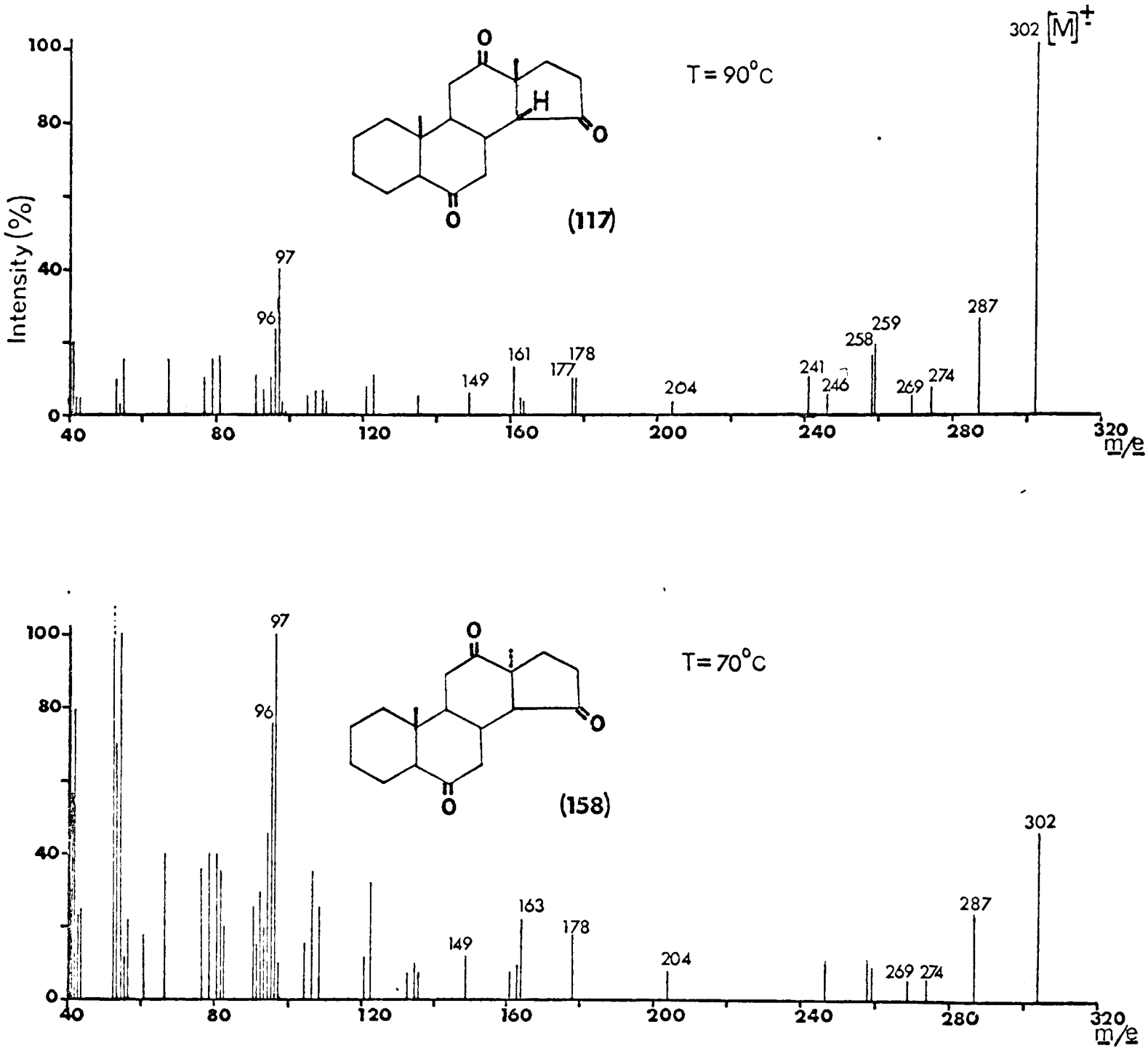


FIGURE 15. Mass spectral comparison of the isomeric 6,12,15-triketo-androstanes **(117)** and **(158)**

androstane-6,12,15-trione [117] fragmentation is clearly dominated by the characteristic D-ring cleavage arising from the 15-oxo group. From strain considerations, it would be expected that fragmentation of the C/D-cis-15-ketones [2] and [3] would be similar; figure 14 confirms this feature. A finger-print comparison of the spectrum of 5α,14β-androstane-6,12,15-trione [117] with the 13α-isomer [158] (Figure 15) constitutes strong evidence in support of the proposed 6,12,15-trioxygenated structures.

Incubation of 5α,14β-androstane-12,15-dione [63] with C.decora

A 4-day incubation of the dione [63] in ethanol (2.5%) gave starting material [63] (4%), and six unidentified products (<3%).

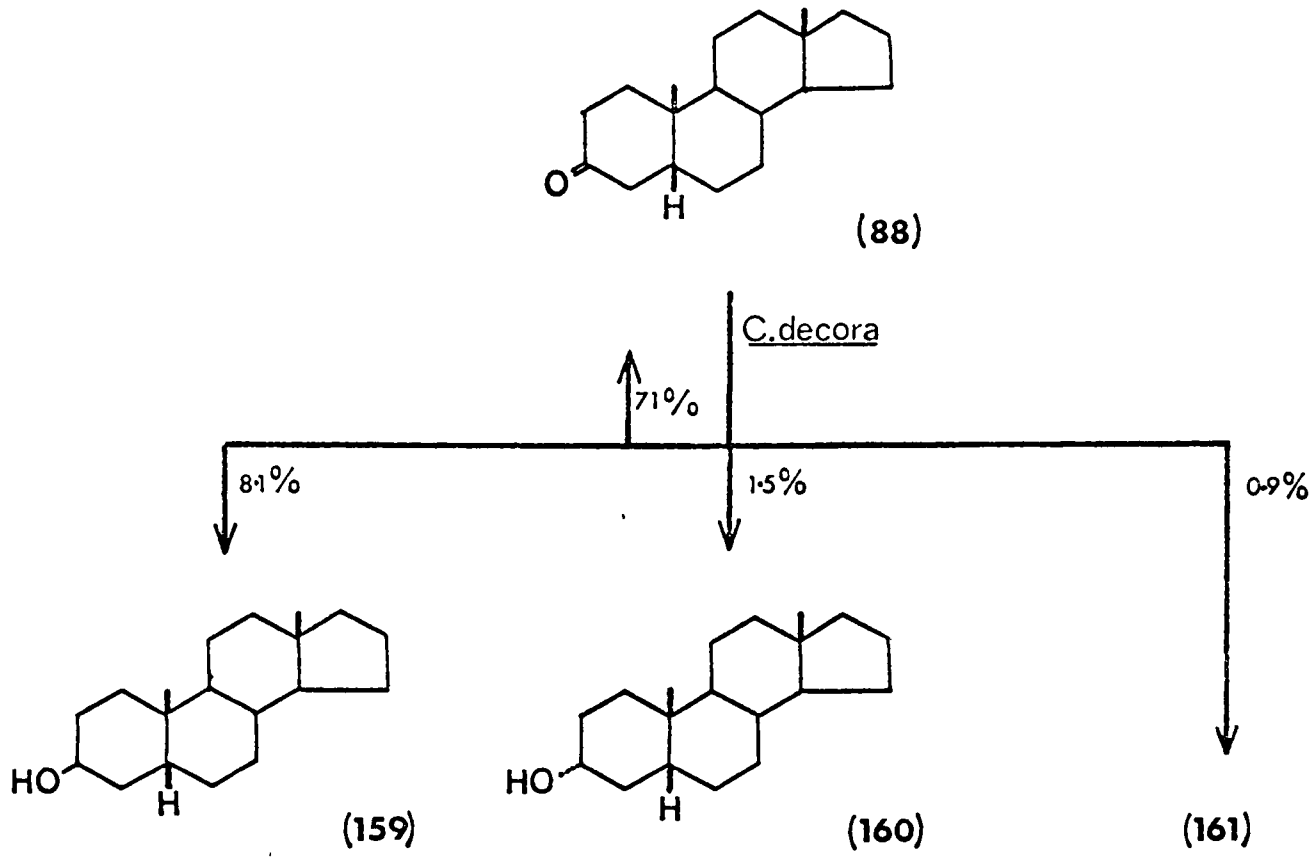
Incubation of 5β-androstan-3-one [88] with C.decora (Scheme 21)

A 3-day incubation of [88] in ethanol (2.5%) gave:-

starting material [88]	71%,
5β-androstan-3β-ol [159]	8.1%,
5β-androstan-3α-ol [160]	1.5%,
and a minor product [161]	0.9%.

The minor product [161], which was shown from mass spectral and i.r. data to be an androstane-diol, could not be characterised.

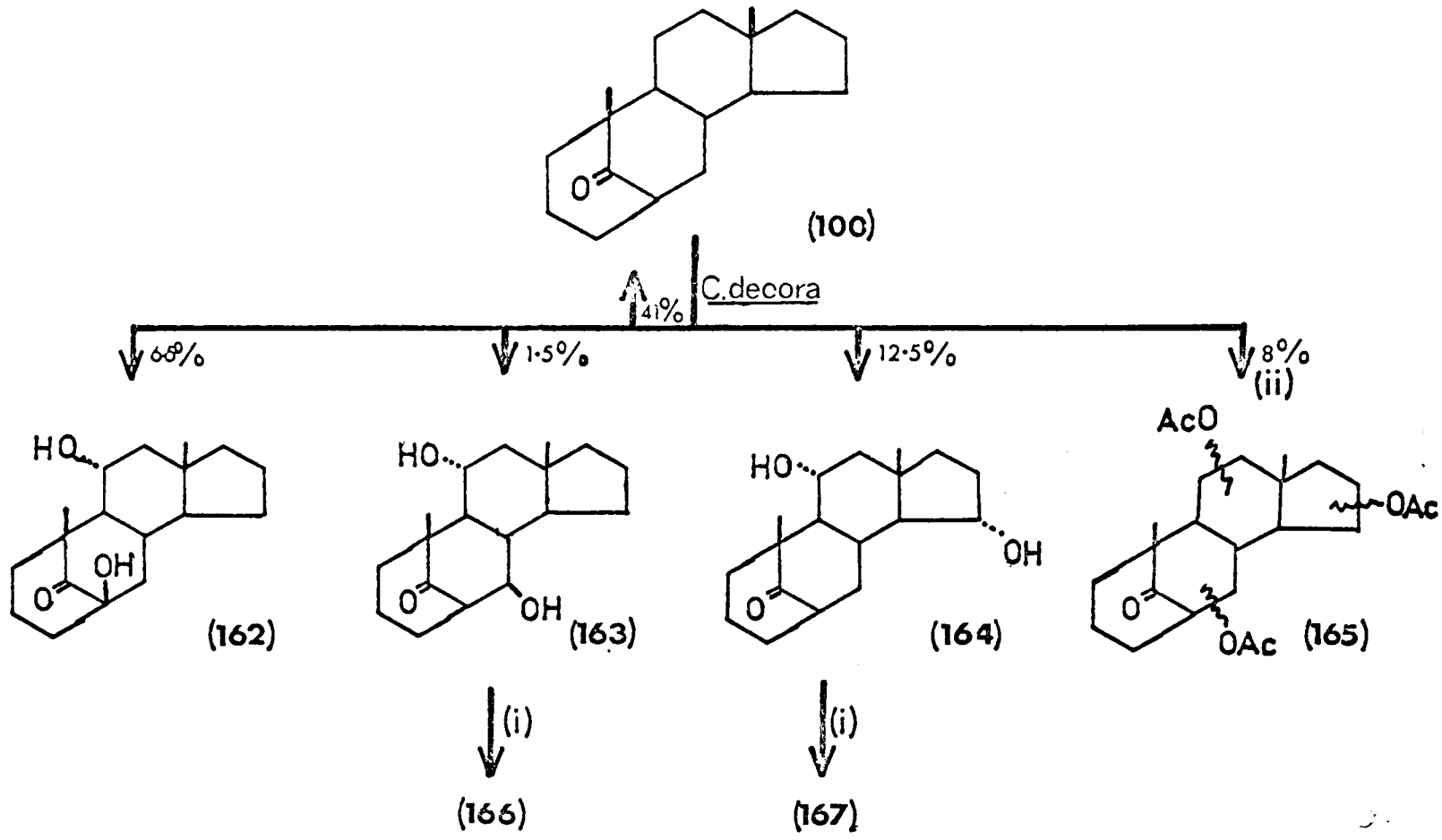
SCHEME 21.



Incubations of A-nor-(5 → 6β)abeo-androstan-5-one [100] and 3β-hydroxy-A-nor-(5 → 6β)abeo-androstan-5-one [91] with C.decora (Schemes 22 and 23)

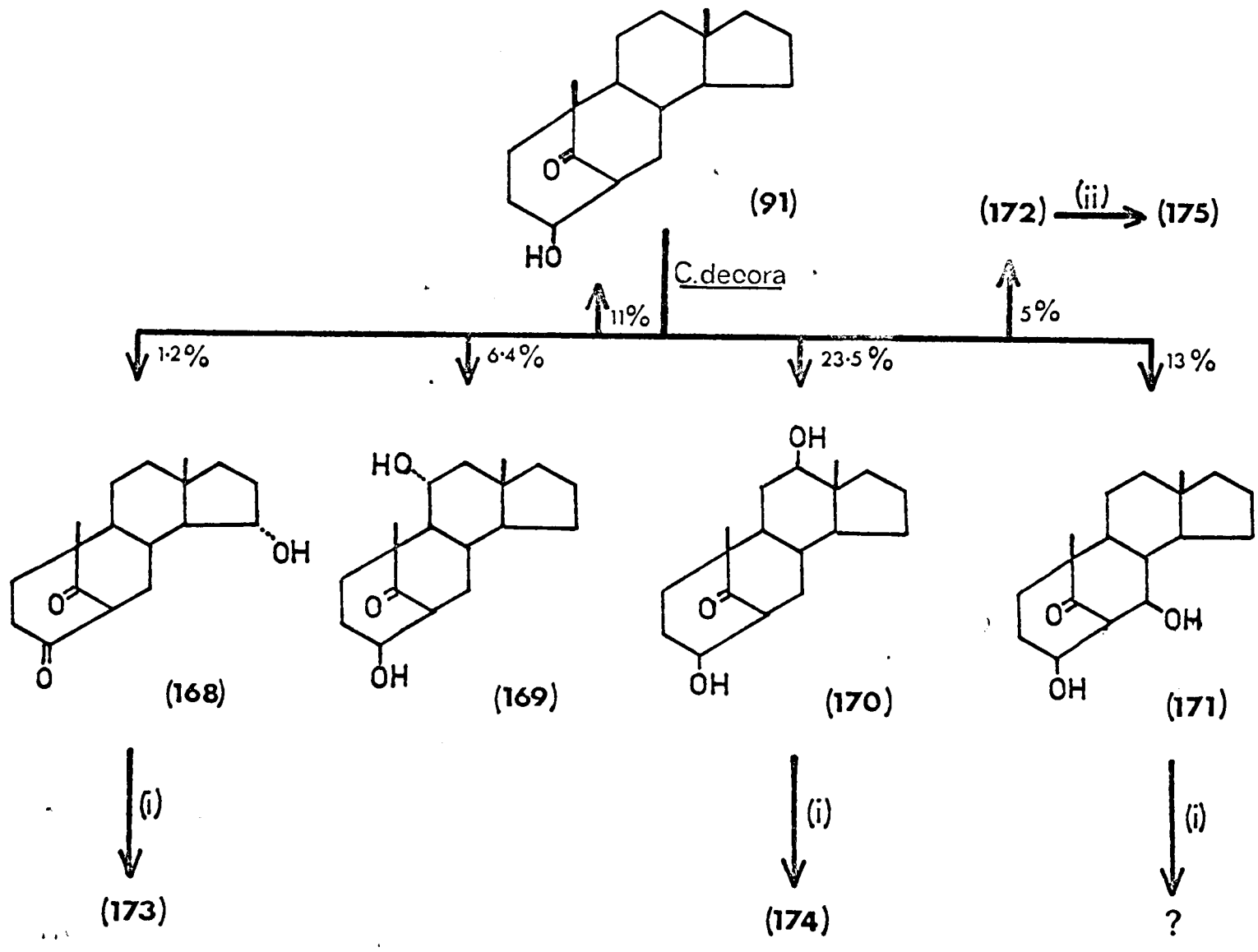
In these feedings, separation of the metabolites proved difficult, and satisfactory analytical results could not be obtained for all the products. Structural determination was complicated because of the instability of most of the products to oxidation with 8N-chromic acid or to Huang-Minlon reduction; because of the small quantities of available material, structures were assigned after consideration of the i.r., n.m.r., and mass spectra of the compounds, and (where possible) the products of oxidation. The only spectral results available of comparative use in this bicyclononane type of androstane series were deduced from examination of the spectra of the synthetic compounds described in Section I; from these data, a

SCHEME 22.



Reagents: (i) 8N-H_2CrO_4 ;(ii) $Ac_2O - C_5H_5N$

SCHEME 23.



Reagents: as above

reasonable prediction of the effects of a functional group in rings A or B could be made.

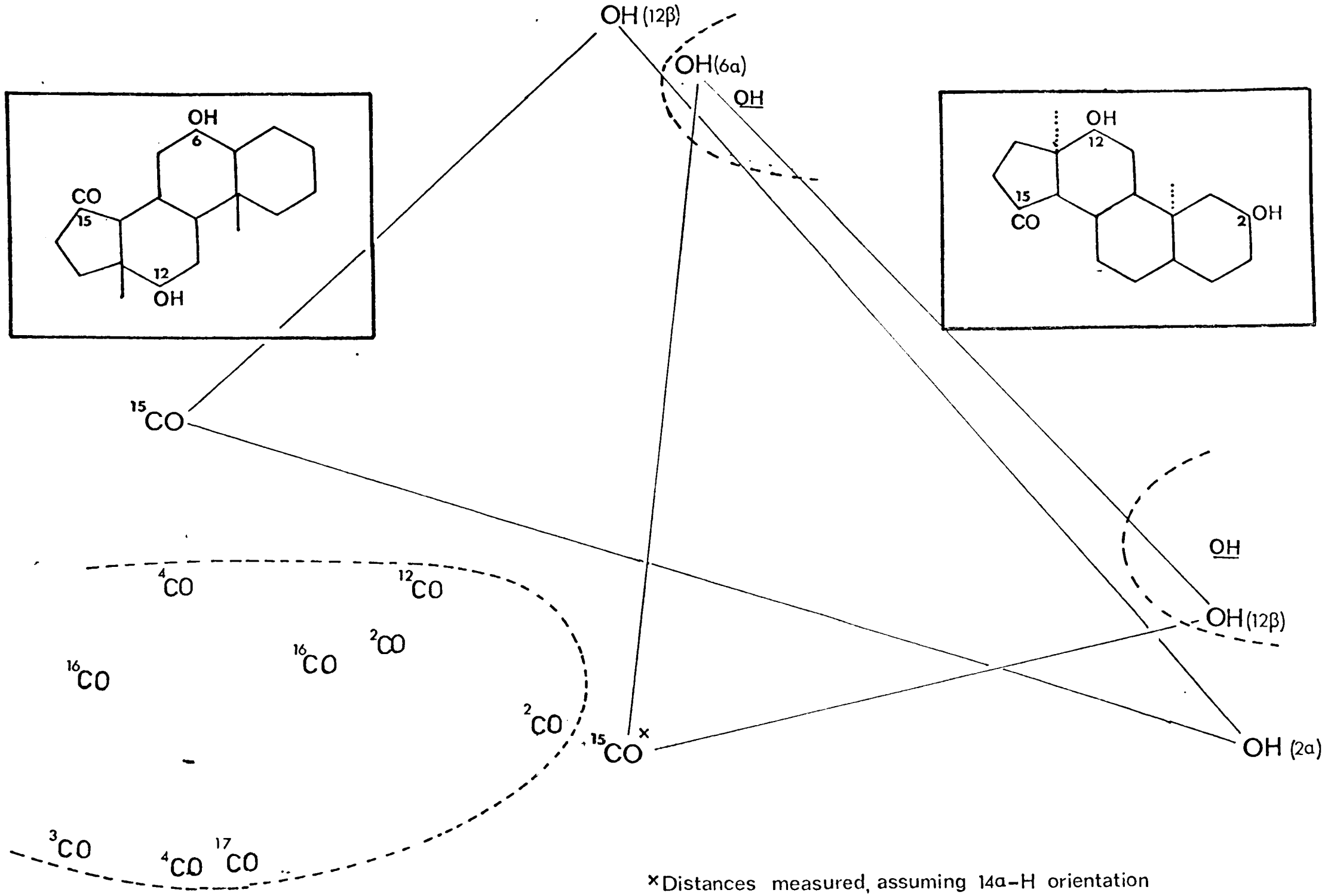
Tables 7 and 8 list the products obtained, in order of increasing polarity, and the tentative structures assigned. (Those compounds which were not obtained analytically pure are indicated by an asterisk).

Table 7: Incubation (5 day; 7.5% DMSO) of [100] with C.decora (Scheme 22)

Product	Structure	Yield (%)
(100)	starting material	41.5
Product 1 (162)*	**6**β,**11**α-$(OH)_2$-5-CO [(a)]	6.5
Product 2 (163)	**7**β,**11**α-$(OH)_2$-5-CO	1.5
Product 3 (164)*	**11**α,**15**α(?)-$(OH)_2$-5-CO	12.5
Product 4 (165)*	Isolated as keto-triacetate [(b)]	8

(a) Spectral data does not distinguish between 6β or 8β hydroxylation; on the precedent that C.decora hydroxylates in an equatorial mode, the 6β-hydroxylation is proposed.

(b) N.m.r. spectrum in C_6H_6 shows an inseparable 1:1 mixture of triacetoxy ketones. Examination of the complex low field protons in n.m.r. spectra ($CDCl_3$ and C_6H_6) suggest the presence of 12β-, and 11α-hydroxylation products.



×Distances measured, assuming 14α-H orientation

FIGURE 16. Distances between carbon atoms hydroxylated by C.decora and directing carbonyl group. (Adapted from ref. 14)

Table 8: Incubation (4 day; 2.5% EtOH) of [91] with C.decora (Scheme 23)

Product	Structure	Yield (%)
(91)	starting material	11.0
Product 1 (168)	**15**α-OH-**3**,5-$(CO)_2$	1.2
Product 2 (169)	3β,**11**α-$(OH)_2$-5-CO	6.4
Product 3 (170)	3β,**12**β-$(OH)_2$-5-CO	23.5
Product 4 (171)	3β,**7**β-$(OH)_2$-5-CO	13.0
Product 5 (172)*	Isolated as keto-triacetate[(a)]	5.1

(a) N.m.r. spectrum in C_6H_6 indicated a 1:1 mixture. From the complex low field signals, peaks consistent with 12β- and 15α-hydroxylation could be resolved.

Discussion of Results

The incubation of 5α-androstan-15-one [1] with C.decora constituted the final experiment in a systematic study of the action of this organism on 5α-androstane mono-ketones. The results of the complete study have been outlined earlier in this section, and are discussed in full elsewhere.[14]

Figure 16 illustrates the 3-site enzyme-substrate model deduced from the results of incubations of other A- and D-ring ketones.[14] Superimposed is the spatial relationship found in the products [116] and [122] of the incubation described. 2α,12β-Dihydroxylation falls well outside the limits imposed by this model; the distance of 6.5Å between the hydroxylated carbon atoms, otherwise noted to be constant at ca. 4Å, is especially large. The 6α,12β-dihydroxylation of the major product [116] cannot be properly compared, since it is isolated in

the more stable 14β-configuration; the sites attacked are much closer to the binding site than with other D-ring ketones, although diequatorial substitution is observed at carbon atoms separated by about the usual distance.

This example of a specific configurational inversion in the presence of C.decora is unique, but it is not clear whether the transformation is due to enzymic or structural factors. In a "blank run" no isomerisation of the substrate resulted, but hydroxylation of the 14β-isomer [2] does not cause 6α,12β-dihydroxylation; inversion must occur either during or after hydroxylation.

A comparison of the results with those of the incubations of 15α-hydroxy-5α-androstan-12-one [59] and 5α-androstan-12-one [111][9e] suggests some specificity of C.decora toward a 3-point 6,12,15-attack.

In the hydroxylation of steroids of unnatural configuration, and those of ethylene ketals (Tables 5 and 6), C.decora continues to give di-equatorial hydroxylated products; even with the unique example of tertiary hydroxylation adjacent to the binding site, the 14β-hydroxyl group lies in an equatorial position relative to rings A, B, and C.

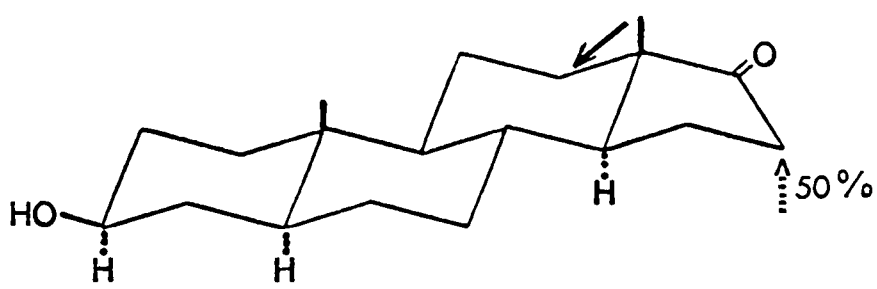
An examination of the results available suggests that when the binding site is moved from the plane of the androstane skeleton, steric factors may inhibit the "usual" hydroxylation pattern. Models show that with the 13α- and 14β-D-ring ketones, a hydroxylase attack from the β-face of the steroid would be hindered only in the 14β-series; in fact the 13α-compounds are hydroxylated analogously to their natural counterparts. With 5β-androstan-17-one [113], A-ring attack would be hindered, and with 5β-androstan-3-one [88] B, C, and D-ring attack would be restricted (in this example, it is noted that reductase activity remains

constant, though less specificity is observed). It is interesting to note that in the incubation of 3β-hydroxy-5α-androstan-17-one [67][9c] hydroxylation is directed by the 3β-hydroxyl group, but with 3α-hydroxy-5α-androstan-17-one [47][9g] the major directive influence is exerted by the 17-keto group (Figure 17).

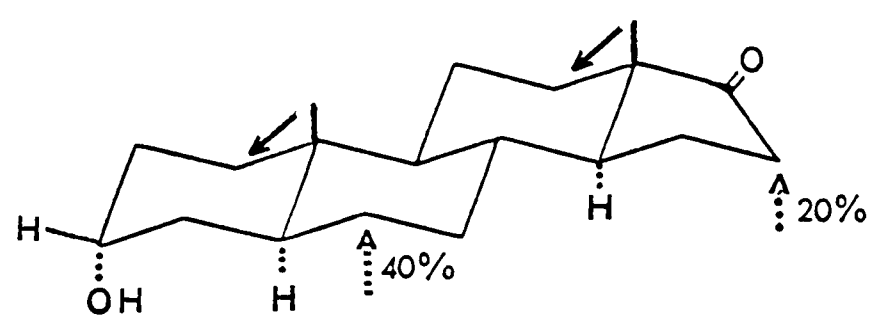
It is not within the scope of this work to argue in favour of the induction of alternative hydroxylating enzymes. (This must await the study of isolated hydroxylase systems.) However, it is notable that with the incubation of 3,3-ethylenedioxy-5α-androstane [115], 6α,12β-dihydroxylation was dominant, but with the large scale incubation of 5α-androstan-3-one [54] (Scheme 11) only a trace of the 6α,11α-dihydroxylated product [78] was isolated.

The somewhat inconclusive results of incubating the A-nor-(5 ⟶ 6β)abeo-androstanes [100] and [91] are best compared with those of substrates having a binding site in rings B or C (Table 3), in that directional specificity is clearly reduced. The relatively high conversions obtained could be due to the exposed nature of the 5-oxo group in the chair-conformation of ring B.

FIGURE 17: The action of C.decora on 3β-hydroxy-5α-androstan-17-one [67] and 3α-hydroxy-5α-androstan-17-one [47]



(67)

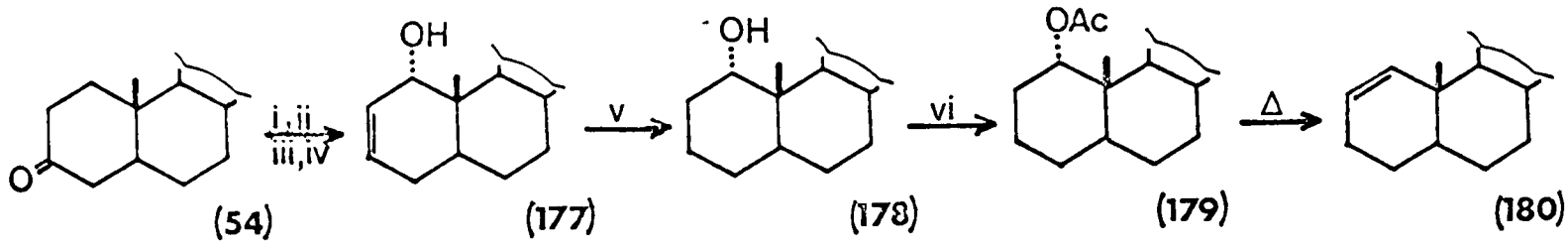


(47)

SECTION III

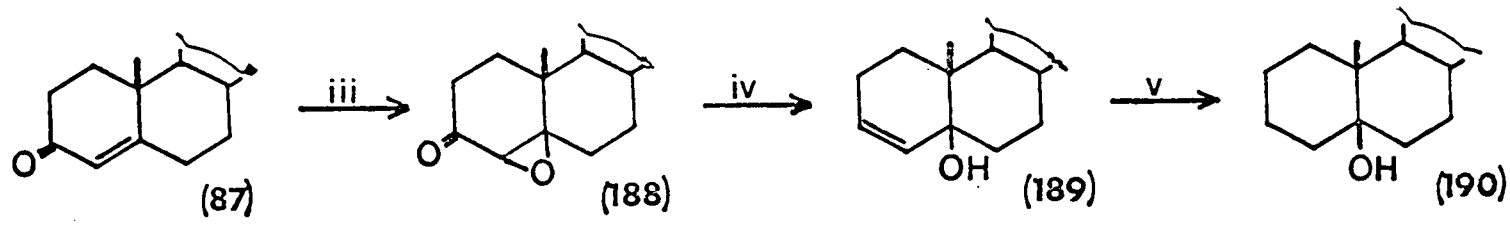
SCHEME 24.

(a).



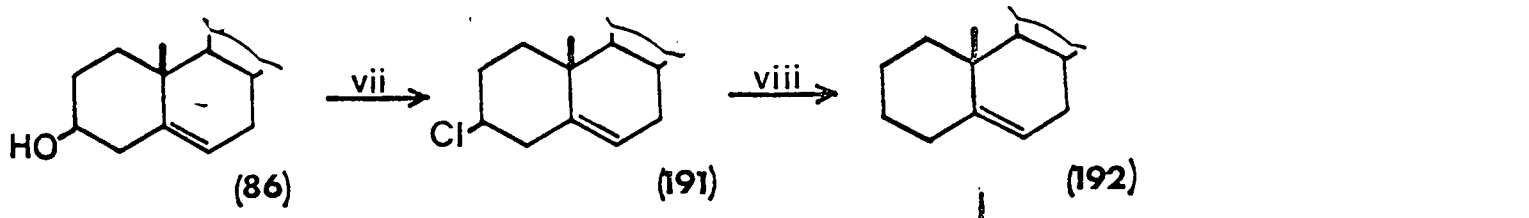
(54) (177) (178) (179) (180)

(b).



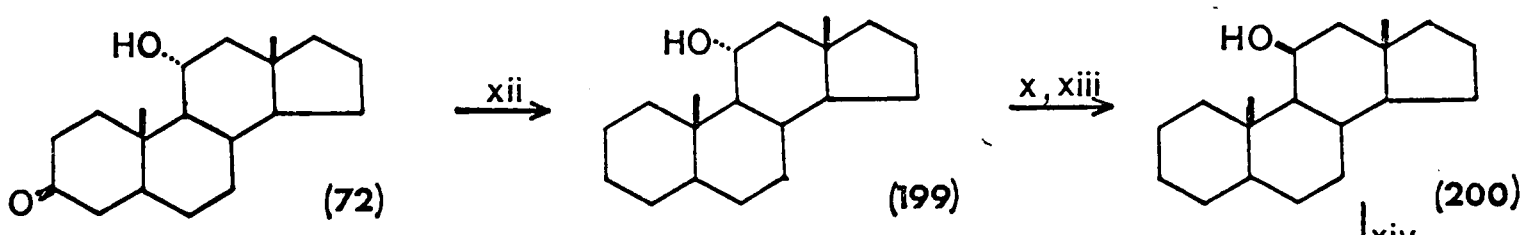
(87) (188) (189) (190)

(c).



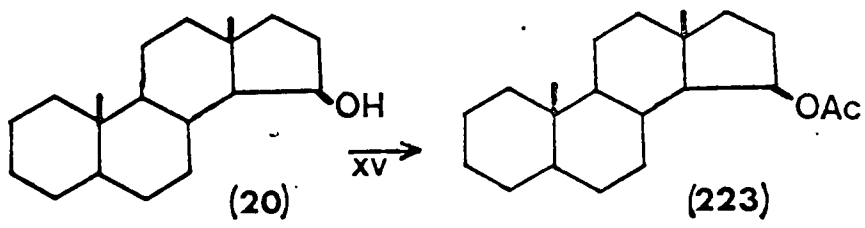
(86) (191) (192) (196) (195) (193) (194)

(d).



(72) (199) (200) (201)

(e).



(20) (223)

Reagents:

(i) Br_2–HOAc
(ii) LiCl–Li_2CO_3–DMF
(iii) H_2O_2–NaOH–EtOH
(iv) N_2H_4–TsOH.H_2O–MeOH
(v) Pd on C–H_2
(vi) Ac_2O–C_5H_5N
(vii) $SOCl_2$–$PhNMe_2$–$CHCl_3$–Et_2O
(viii) Na–NH_3–Et_2O
(ix) $NaBH_4$–diglyme–BF_3·Et_2O
(x) $8\underline{N}$–H_2CrO_4
(xi) $Li(OBu^t)_3AlH$–THF
(xii) Huang–Minlon reduction
(xiii) $LiAlH_4$–THF ; reflux
(xiv) Ac_2O–HOAc–TsOH·H_2O–Δ
(xv) Ac_2O–C_5H_5N–Δ

SECTION III

A number of topics arising out of the principal work of this Thesis are discussed in the following section.

[A] The Synthesis and Physical Properties of Monohydroxy-androstanes and their Acetates

In connection with the microbiological studies in this laboratory, it was necessary to characterise the complete series of mono-oxygenated 5α-androstanes. These substances were required to serve as substrates (Table 3; Section II) or as reference compounds in the structural elucidation of microbiological products. It is surprising that prior to the publication of these results[88] so few of the acetates had been described.

The synthesis of a number of alcohols is described here (Scheme 24) or in other parts of this Thesis (the source is given by the scheme no. in which the preparation appears). Where the preparation of the alcohol is not described, the source of data within this group is acknowledged. With the exceptions of 5α-androstan-11β-yl acetate [201] and 5α-androstan-15β-yl acetate [223] (Scheme 24) the usual acetylation procedure was followed.

The physical constants of the complete series of secondary monohydroxy-5α-androstanes and their acetates are tabulated in Tables 9 and 10. Details of n.m.r. spectra appear in Table 20 (Section IV) and mass spectra in Table 11; some aspects of the mass spectra are discussed. Data for some alcohols of unnatural configuration are included.

TABLE 9. MONO-HYDROXYLATED ANDROSTANES

Compound		Source	m.p. (°C)	$[\alpha]_D$	(c)	$\nu_{max.}^{+}$ (cm^{-1})	lit.
5α-androstan-1α-ol	[178]	Sch.24	107-109	+17	(0.8)	3628	89
1β	[181]	9e	120-122	-19	(0.7)	3655,3619	89
2α	[182]	47	128-129	+5	(1.0)	3621.	
2β	[183]	47	133-134	+10	(1.0)	3626	90
3α	[184]	9b	146-148	+4	(0.6)	3626	92
3β	[185]	9c	150-151	-2	(0.8)	3620	93
4α	[186]	9e	166-169	-29	(0.6)	3630,3606	
4β	[187]	48	121	+7	(1.1)	3630	
6α	[195]	Sch.24	97-99	+23	(0.8)	3631,3607	9c
5α-androstan-6β-ol	[196]	Sch.24	78-79	-13	(1.0)	3630	
7α	[197]	9e	109-111	-13	(1.0)	3629	94
7β	[198]	9b	125-127	+34	(1.0)	3648,3618	94
11α	[199]	Sch.24	108-109	-26	(0.9)	3658,3610	95
11β	[200]	Sch.24	89-90	+17	(1.0)	3645,3621	95
12α	[203]	9e	123-126	+41		3634	
12β	[203]	9e	126-128	+1	(0.6)	3622	56
15α	[53]	Sch.7	160-161	+41	(1.0)	3630,3612	19
15β	[20]	Sch.3	79-81	-36	(0.9)	3627	19
16α	[204]	47	161-162	-6	(1.5)	3623	96,97
16β	[205]	47	110-112	-3	(1.0)	3626	96,97
17α	[206]	58	153-154	-10	(1.1)	3625	98
17β	[207]	58	164-166	+14	(1.0)	3627	93
5β-androstan-3α-ol	[160]	Sch.21	146-147	-0.5	(0.7)	(3620)	63
-3β-ol	[159]	Sch.21	143-144	-0.3	(1.1)	(3625)	63
5β-androstan-5-ol	[190]	Sch.24	78	+10	(0.2)	(3625)	
-6β-ol	[193]	Sch.24	oil	-		(3620)	9c
5α,13α-androstan-15α-ol	[21]	Sch.3	108-110	-40	(0.4)	(3635)	
5α,14β-androstan-15α-ol	[23]	Sch.3	81-82	+67	(1.0)	(3640)	
5α,14β-androstan-15β-ol	[24]	Sch.3	134-138	-8.5	(0.1)	(3640)	

+ Recorded under conditions of ref.52. Bracketed results recorded under usual conditions.

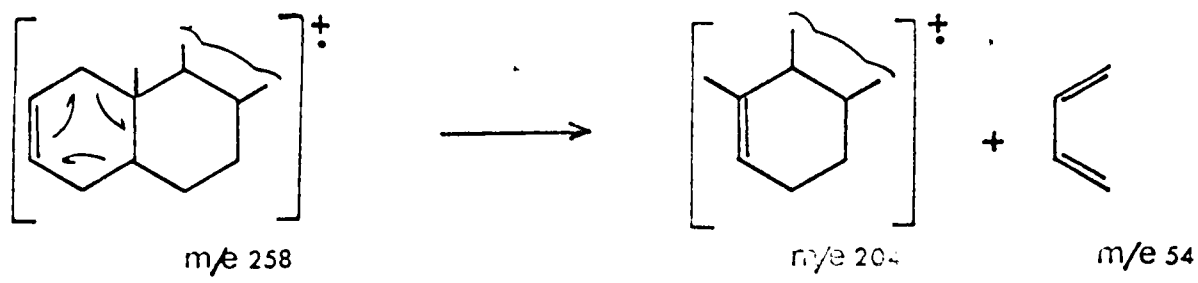
TABLE 10. ANDROSTANE MONO-ACETATES

Compound		Source	m.p. (°C)	$[\alpha]_D$ (c)	Analysis** C	Analysis** H	lit.
5α-androstan-1α-yl acetate	[179]	Sch.24	oil	+34 (1.8)	79.5	10.2	
1β	[208]	*	oil	-11 (0.8)	79.4	10.6	
2α	[209]	47	153-155	-23 (0.9)			
2β	[210]	47	83-84	+11 (0.9)			
3α	[211]	9b	130-133	-			103
3β	[212]	*	88-89	-9 (1.0)			102,103
4α	[213]	48	116-118	-5 (0.2)			
4β	[214]	48	107-109	+12 (0.9)			
6α	[215]	*	112-113	+66 (0.9)	79.2	10.8	
6β	[216]	*	64-66	-36 (0.8)	79.2	10.7	
7α	[217]	*	120-121	-49 (0.9)	79.4	10.9	94
7β	[218]	*	88-89	+50 (0.8)	79.3	10.9	94
11α	[219]	*	oil	-50 (1.5)	79.0	10.55	26
11β	[201]	Sch.24	70-72	+33 (0.9)			101
12α	[220]	9e	90-93	+59 (0.3)			
12β	[221]	9e	61-64	-15 (0.6)			
15α	[222]	*	96-97	+46 (0.5)	79.2	10.8	
15β	[223]	Sch.24	oil	-57 (3.3)	78.9	10.55	19
16α	[224]	47	120-122	-13 (0.8)			96
16β	[225]	47	111-112	+5 (0.6)			96
17α	[226]	58	94-97	-9 (0.3)			97
17β	[227]	*	79-80	+7 (1.0)			97
5β-androstan-6β-yl acetate	[194]	Sch.24	80-81	-7 (0.9)			

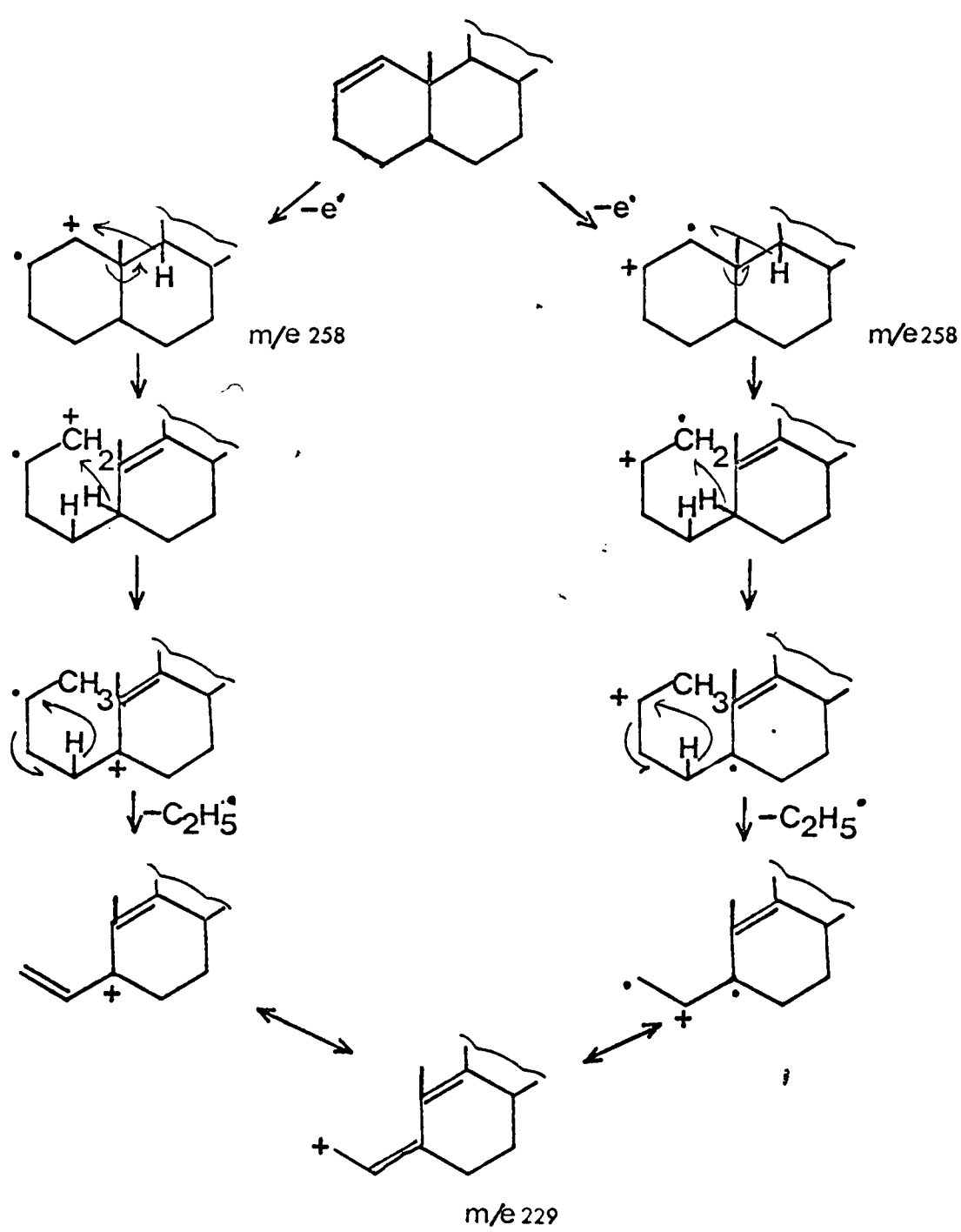
* Prepared by the usual acetylation procedure

** $C_{21}H_{34}O_2$ requires C, 79.2; H, 10.8%

SCHEME 25. The retro-Diels-Alder mass-spectral fragmentation of 5α-androst-2-ene (**228**)[104]



SCHEME 26. Possible fragmentation of 5α-androst-1-ene (**180**)



The mass spectral fragmentation of androstane mono-acetates

The present study of the complete series of mono-acetates was prompted by the observation by Woodgate,[47] that the fragmentation of 5α-androstane 2-acetates gave a strong peak at m/e 229, corresponding to loss of 29 mass units from the [M-60] fragment. Woodgate supplemented this result with the study of a series of deuterated derivatives (Table 13), and further work has shown that this peak is also present in the mass spectrum of 5α-androst-1-ene [180] and its 1-d_1-analogue. Table 11 shows that the peak in question is present, but to a lesser extent, in the mass spectra of most other acetates.

The spectrum of 5α-androst-2-ene [288] (Table 12) does not show a peak at m/e 229; instead, the spectrum is dominated by a peak at m/e 204, resulting from retro-Diels-Alder decomposition (Scheme 25).[104] Apparently there is no significant double bond isomerisation preceding this fragmentation,[105] and the A-ring cleavage of 5α-androst-1-ene [180] gives a more dominant peak at m/e 202. A thermally-induced 1,2-elimination of acetic acid is known to occur in the mass spectra of most steroidal acetates,[104,106] and an examination of the peak intensities at m/e 202 and m/e 204 in the spectra of the 2-acetates [182] and [183] suggests that the elimination proceeds mainly toward the Δ^1-olefin. In the spectra of 5α-androst-1-ene [180] and 1-d_1-5α-androst-1-ene the expulsion of fragments of 29 and 30 mass units respectively confirms that the strong peak does arise from the olefin, and probably results from the loss of an ethyl-radical after C_{1-10} cleavage (Scheme 26). It is thus surprising that the spectra of the 1-acetates [179] and [208] show only weak peaks at m/e 229. The appearance of a peak of increased intensity at m/e 203 supports the suggestion that under the conditions used only a fraction of the acetate is eliminated in the accepted

TABLE 11. Initial fragments in the mass spectra of androstane acetates

[* denotes spectra recorded by Woodgate[47]]

	Compound	M^+ (m/e 318) %	M-59 %	M-60 %	M-60-15 %	M-60-28 %	M-60-29 %	m/e 217 %	m/e 204 %	m/e 203 %	m/e 202 %	Temp °C
	5α-androstan-1α-yl acetate	3.5	24	100	41	2.5	5	1.5	3	**15**	14	50
	1β	2.5	22	100	47	3	7	2	4	**18**	20	45
*	2α	0.5	21	100	59	8	**32**	12	31	19	55	
*	2β	-	24	100	35	6	**24**	2	21	13	31	
*	3α	-	22	100	48	2	2.5	7	25	8	5	
*	3β	-	25	100	42	-	3.5	6	34	8	6	
*	4α	-	22	100	75	2	3	2	-	1	2	
*	4β	-	29	100	72	1	2	4	1	4	5	
	6α	-	20.5	80	100	2	3.5	1	0.5	1.5	4	45
	6β	-	19	80	100	2	5	1	-	1.5	5	35
	5β-androstan-6β-yl acetate	-	20	100	92	1	3	1.5	-	0.5	3	55
	5α-androstan-7α-yl acetate	-	21	87	100	-	4	-	-	-	4	55
	7β	-	18	80	100	2	3	1	-	1	3.5	55
	11α	0.5	20	96	100	1	4.5	1	-	1.5	5.5	55
	11β	-	23	89	100	1.5	3.5	1.5	-	-	4	65

continued over....

5α-androstan-12α-yl acetate		21	100	83	0.5	1.5	0.5	0.5	1.5	3.5	55
12β		22	92	100	1	2	1.0	0.5	2.5	8.0	35
15α	1.0	**67**	95	100	5	10	**12**	2	6	13	55
15β	3.0	**62**	85	100	4	9	**11**	2	6	14	30
16α	-	13	57	100	-	-	**85**	1	2	7	65
16β	0.15	**31**	100	100	0.3	0.4	**52**	2	10	13	100
17α	14	**30**	100	91	9	8	**45**	4	9	10	60
17β	35	23	100	65	6	2.5	**30**	2	7	5	55

Table 12: Fragmentation of some steroidal olefins

Compound	$M^{+\cdot}$	M-28	M-29	M-30	m/e			
	258	230	229	228	204	203	202	201
5α-androst-1-ene [180]	55	5	**21.5**	-	8	8	**16**	4
* 1-d_1-5α-androst-1-ene	(259) 100	(231) 1	(230) 9	(229) **34**	4	12	**31**	2
5α-androst-2-ene [228]	95	2	2.5	-	**100**	10	7	9
5α-androst-14-ene [152]	58	-	-	-	-	-	2	6
	370	342	341		316	315	314	313
* 5α-cholest-1-ene	100	5	**13**		7	6	16	-
* 5α-cholest-2-ene	100	-	1		32	3	1	-
* 5α-cholest-3-ene	100	-	1		2	1	2	1
* 5α-cholest-4-ene	100	1	1		6.5	1	2.5	1

Table 13: Fragmentation of deuterated 1- and 2-acetoxy 5α-androstanes[47]

Compound	M^+		M-60		M-61		M-60-29		M-60-30		M-61-30	
	m/e	%	m/e	%	m/e	%	m/e	%	m/e	%	m/e	%
1-d_1-1β-OAc	319	6	259	100	258	6	230	2	229	2.5		
2-d_1-2α-OAc	319	-	259	100	258	23	230	9.5	229	5.5		
2-d_1-2β-OAc	319	-	259	100	258	5.5	230	11.5	229	7.5		
3α-d_1-2β-OAc	319	-	259	95	258	8	230	29	229	3		
1,1,3,3-d_4-2α-OAc	322	3.5	262	27	261	100	233	4	232	6	231	15
1,1,3,3-d_4-2β-OAc	322	2.5	262	24	261	95	233	5	232	7	231	19
1,1,2,3,3-d_5-2α-OAc	323	1	263	29	262	100	234	4	233	11	232	8
1,1,2,3,3-d_5-2β-OAc	323	0.5	263	23.5	262	100	234	2	233	4	232	10.5

1,2-mode. (Steroidal alcohols are known to eliminate to a greater extent via a 1,3- or a 1,4-mode,[106] and the spectra of many androstane mono-alcohols show only very weak peaks at m/e 229;[107] this is therefore in accord with the above suggestion.)

In the decomposition of D-ring acetates, a significant fragment at m/e 217 corresponds to a loss of 41 mass units [(M-60)-C_3H_5], and in the case of the 17-acetates a loss of 28 mass units (m/e 230) is more dominant than that of 29 units (m/e 229).

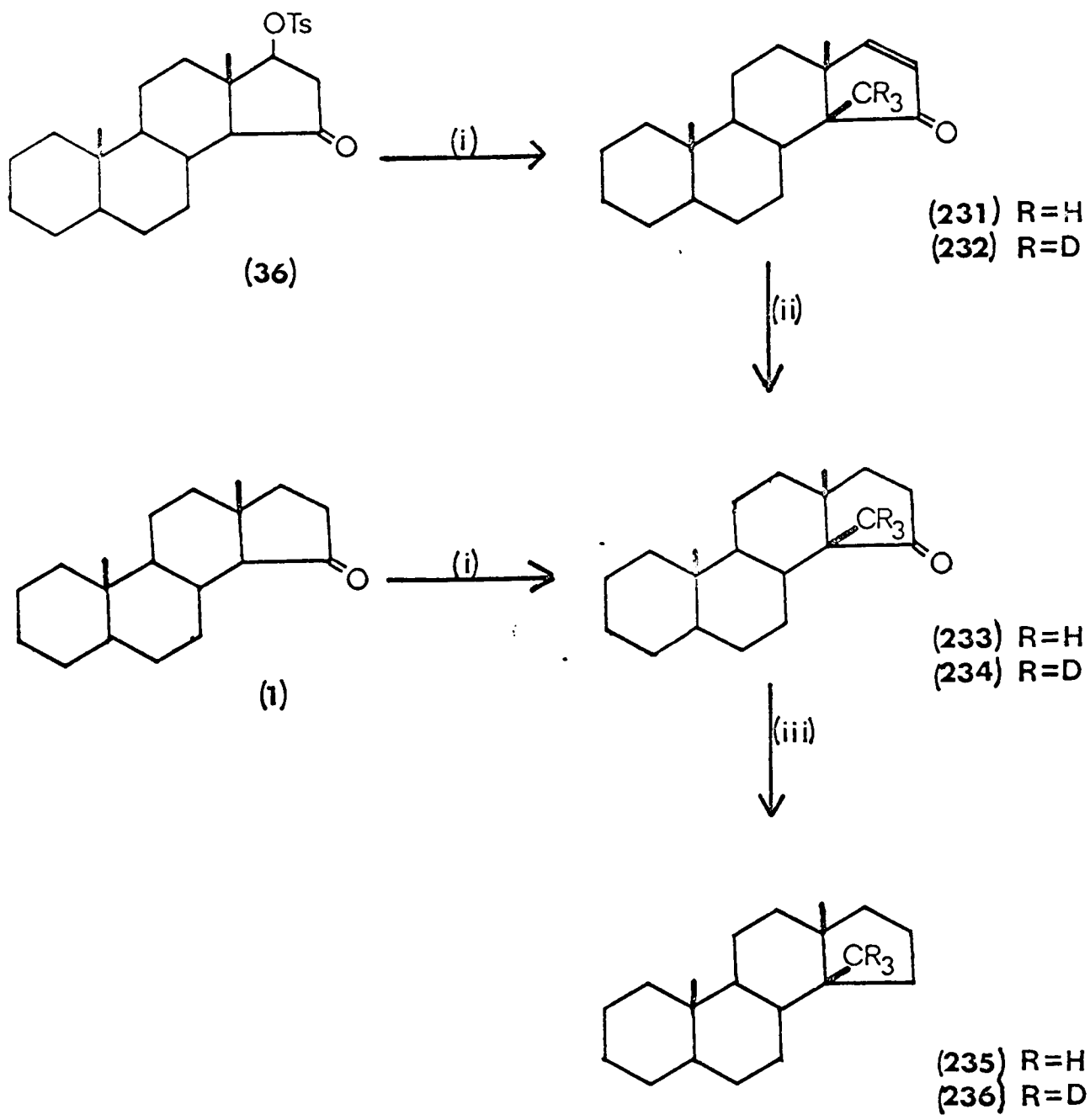
The thermal elimination of 15-acetates is known to be facile, and the appearance of a more intense peak at m/e 229 in their mass spectra is surprising especially because subsequent fragmentation is similar to that of 5α-androst-14-ene [152] (Table 12). However, strong peaks at m/e 259 and 244 correspond to the loss of the acetate function as the radical $CH_3COO^{\cdot}$, and it is possible that the peaks at m/e 229 arise via an alternative mechanism.

[B] Preparation of 14-Methylated Androstanes (Scheme 27)

A problem in the study of the mass spectra of triterpenes has been to define the position of the tertiary-methyl substituent contributing most to the (M-15) fragment.[106] This peak has been thought to arise from a cleavage of the 14-methyl group, but the relative inaccessibility of suitable precursors has precluded the synthesis of deuterated model compounds. With the availability of 5α-androstan-15-one [1] (Section I) it was clear that the introduction of a labelled 14α-methyl group could provide useful mass spectral data.

The 14α-orientation of the methyl group in Δ^8-lanostenyl has been confirmed from X-ray studies,[108,109] and in its synthetic correlation with

SCHEME 27.

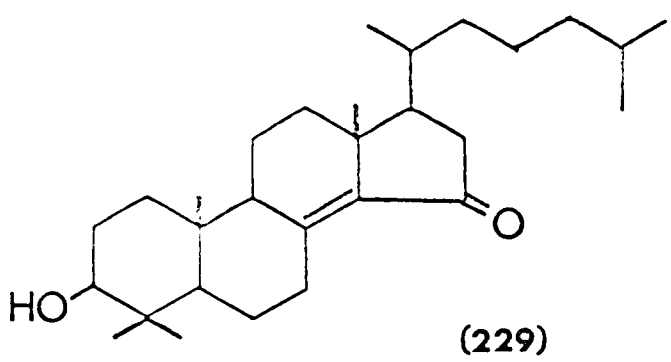


Reagents: (i) $KOBu^t - Bu^tOH - CR_3I$

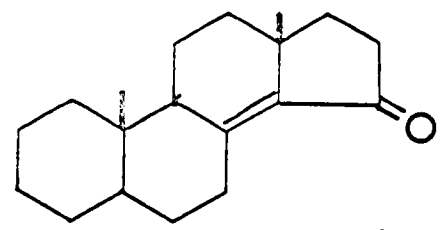
(ii) $Pd/C - H_2$

(iii) Huang-Minlon reduction under forcing conditions[46].

cholesterol, Woodward et al.[31a] introduced the group by C-methylation of the $\Delta^{8(14)}$-15-ketone [229]. Bromination of 5α-androstan-15-one gives a 1:1 mixture



(229)



(230)

of the 14α- and 14β-bromo derivatives, but only the 14α-isomer is geometrically favourable for elimination to 5α-androst-8(14)-en-15-one [230];[76] therefore this method was not used.

Beal[110] and Bannister,[40] in patented work, report that the reaction of a 15-ketone with methyl iodide and potassium t-butoxide affords a 14α-methylated product, but no evidence for the configurational assignments has been given. Similarly, Petit and Brown[23] claim that this reaction with a 16-en-15-ketone gives the 14α-methyl derivative; in this case the structure is assigned on the basis of limited n.m.r. and rotatory dispersion evidence.

In a modification (Scheme 27) of the procedure of Petit and Brown,[23] methyl and deuteromethyl groups were introduced at the 14-position. However a comparison of the c.d. curve of the saturated ketone [233] with those of 5α-androstan-15-one [1] and 5α,14β-androstan-15-one [2] (Figure 19) was suggestive of a 14β-configuration in the methylated compound [233]. Difficulty in applying the octant rule to a flexible ring-D ketone is well known,[111-114] but if the diagrams corresponding to extreme envelopes in 5α-androstan-15-one [1],

FIGURE 18. Octant diagrams of extreme conformations of (1), (2), and their 14-methylated derivatives.

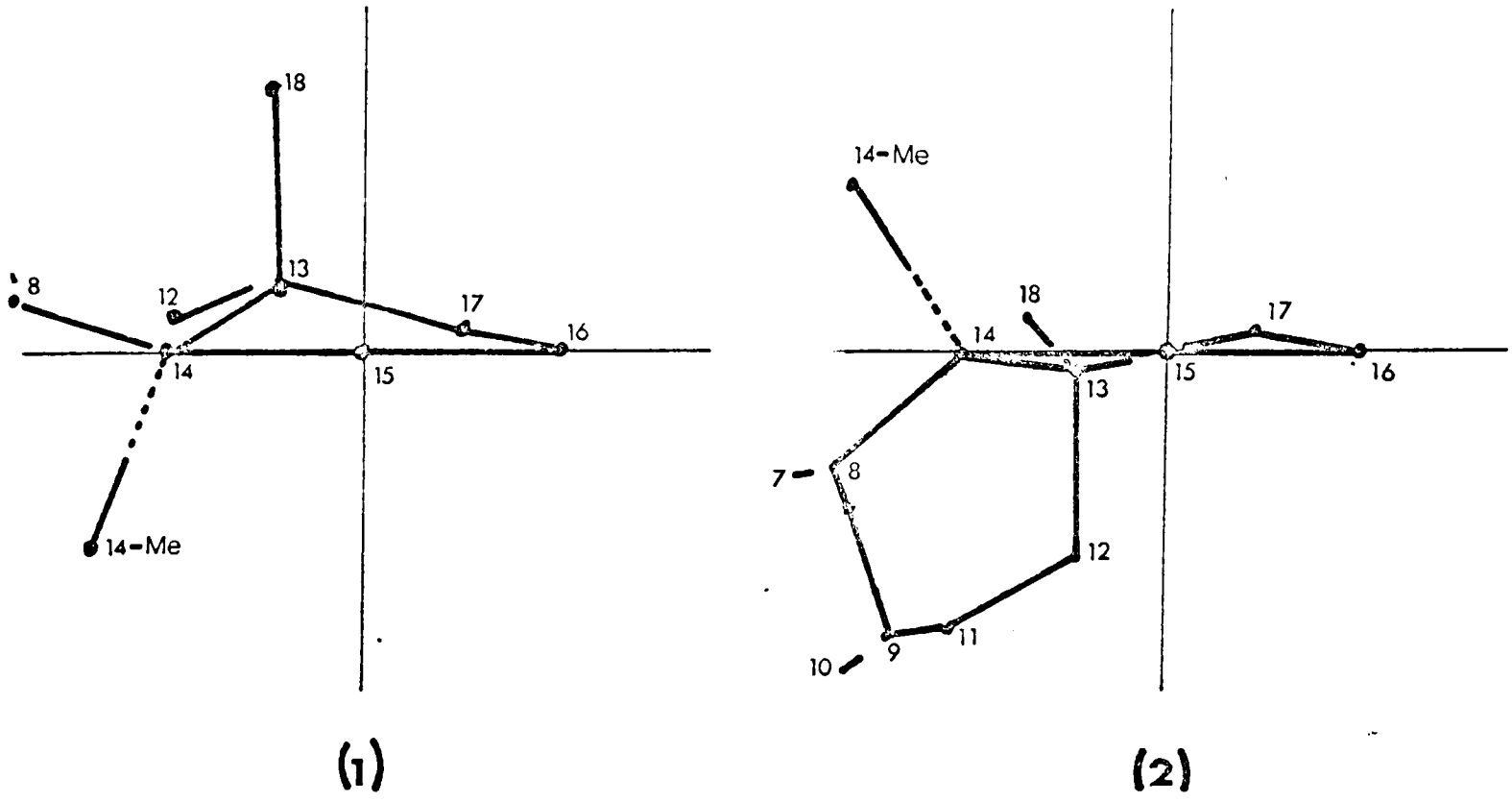
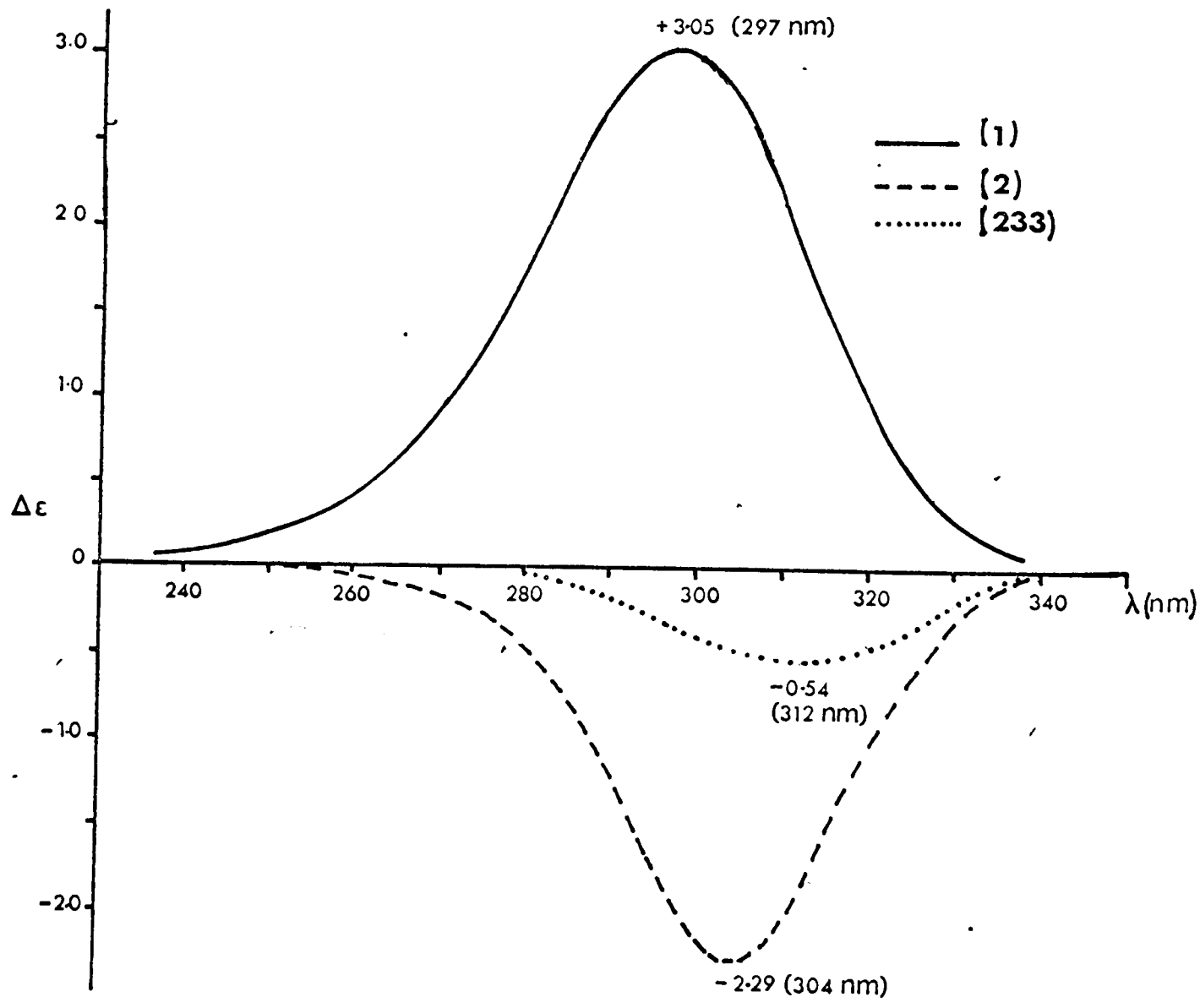


FIGURE 19. C.d.-curves (in MeOH) of the 15-ketones (1), (2) and (211)



5α,14β-androstan-15-one [2], and their 14-methylated derivatives are considered [Figure 18], no inversion of the sign of the respective Cotton effects, but a reduction of amplitude would be expected.

Table 14 : Chemical shifts (τ_2) of 19-, 18- and 14-methyl protons of 14-methylated and related androstanes and lanostanes

Functional group	τ_2 (14β-Me) (14β-CD_3)			τ_2 (14α-H)		τ_2 (14β-H)	
	19-H	18-H	14-Me	19-H	18-H	19-H	18-H
Δ^{16}-15-CO	9.27	8.93	8.93	9.15	9.02	9.24	8.79
	9.27	8.93					
15-CO	9.28	9.00	9.02	9.21	9.21	9.27	8.85
	9.28	9.00					
hydrocarbon	9.25	9.12	9.20	9.22	9.31	9.23	9.01
	9.25	9.12					
4,4-Me_2-cholestane[115]	9.16	9.36					
lanostane[115]	9.08	9.24	9.20				

Table 14 compares the 19-, 18-, and 14-Me proton resonances of the 14-methylated compounds prepared, with the corresponding 14α-H and 14β-H derivatives. Included is a comparison of these resonances in 4,4-dimethyl cholestane and lanostane.[115] It would be expected that a 14-methyl substituent would cause a consistent shift in C-19 and C-18 proton resonance. This is clearly not so if a 14α-methylated structure is considered, but an average upfield shift of +0.02 τ and +0.13 τ is compatible with a 14β-methyl structure.[114] On the basis of this evidence, in the present work a 14β-configuration was assigned to all methylated compounds.

A β-attack on the enolate anion is not surprising; models show that especially in $\Delta^{14,16}$ systems, the β-face at position 14 is no longer shielded by the 13β-methyl group, and therefore attack from this direction may be kinetically more favourable. Furthermore, there is ample precedent for β-attack on these systems.[28,76,116,117] An examination of models for $\Delta^{8(14)}$-15-CO, Δ^{7}-14-Me-15-CO, and $\Delta^{7,14}$-15-enolate systems shows that the α-face is more exposed, and also that the relief of D-ring strain caused by a 14β-configuration is less evident.

In an attempt to obtain the 14α-methylated derivatives, the procedure of Beal[110] was repeated, but only the 14β-methylated product [233] was obtained. Photo-irradiation of [233] according to the method of Botts[118] did not result in inversion of the 14-methyl substituent.

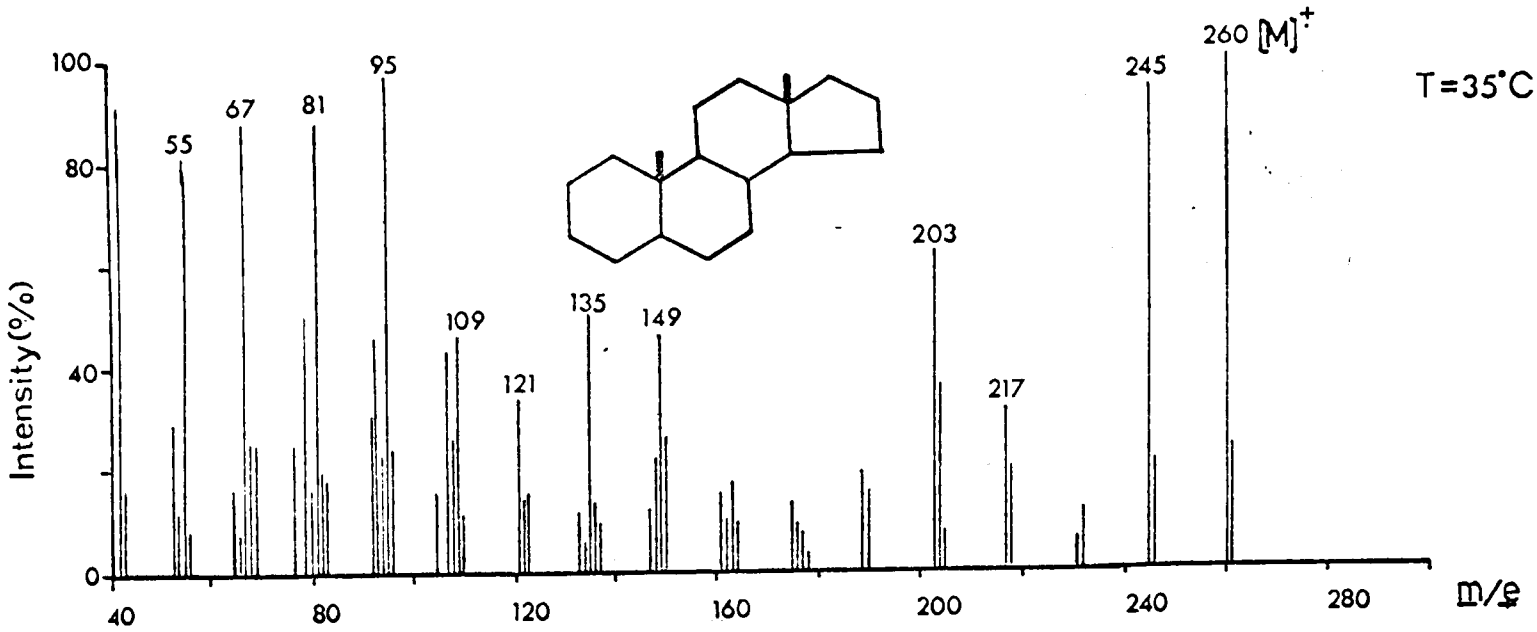
Mass Spectra of 14-Methylated Androstanes

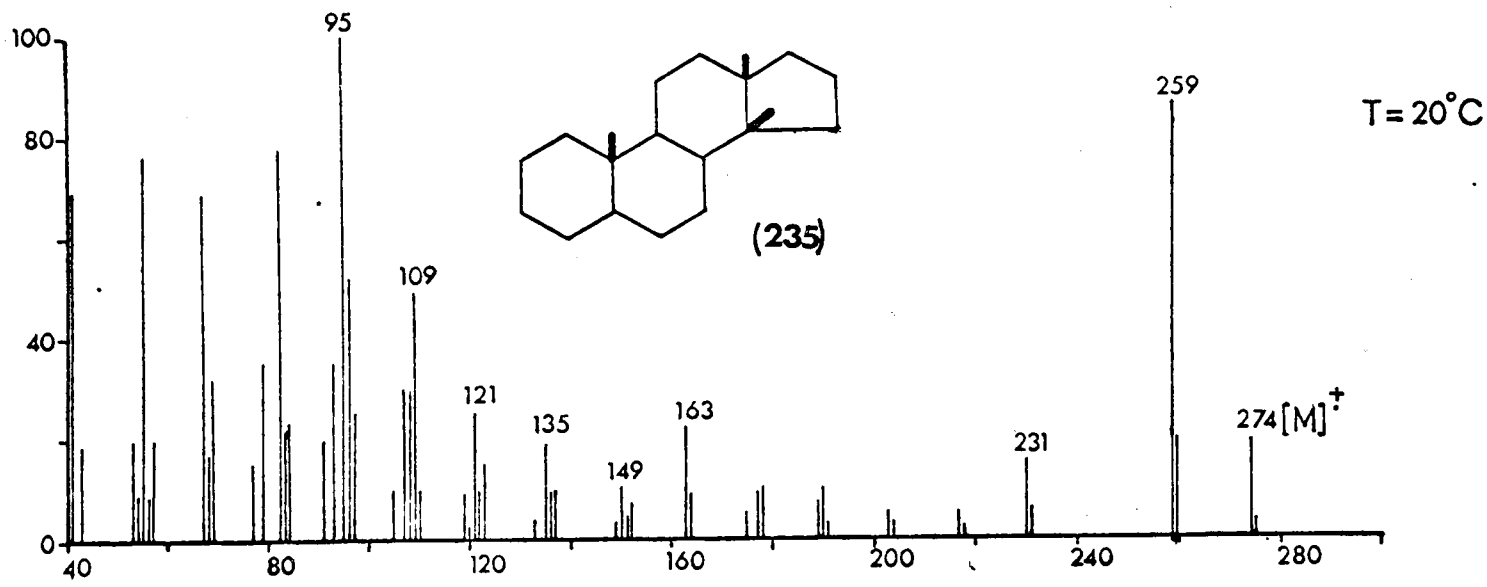
Table 15 lists the major fragments in the mass spectra of 14-methyl-5α,14β-androst-16-en-15-one [231], 14-methyl-5α,14β-androstan-15-one [233] and their deuteriated analogues [232] and [234]. The fragmentation of [233] is very similar to that found in the 15-ketones [1], [2], and [3], and the same D-ring cleavage process (Scheme 16) appears to operate.

Table 15

Compound no.	m/e (%)	Temp (°C)
[231]	286 [M^+; 21]; 271 (13); 176 (29); 111 (100)	55
[232]	289 [M^+; 6]; 274 (4); 176 (28); 114 (100)	70
[233]	288 [M^+; 5]; 273 (1); 178 (1); 111 (100)	70
[234]	291 [M^+; 5]; 274 (3.5); 259 (4); 178 (2.5); 114 (100)	60

FIGURE 20 Mass spectra of some androstanes





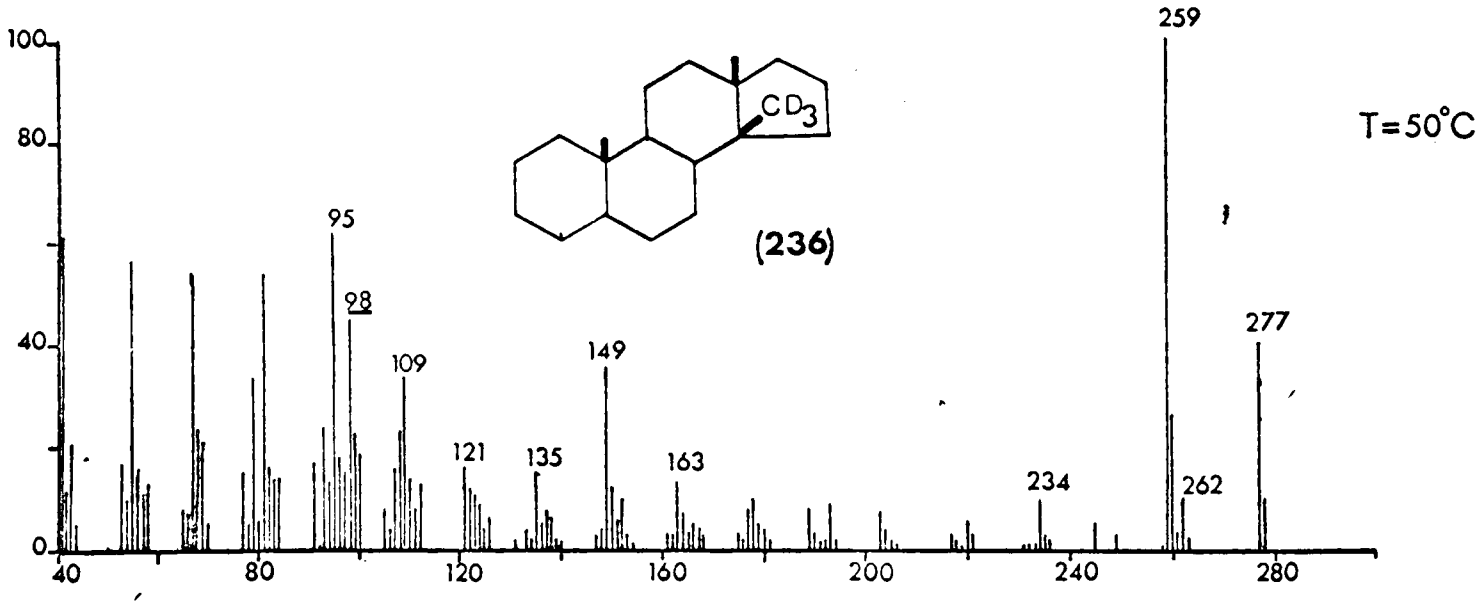


Figure 20 shows the spectra of 5α-androstane, 14-methyl-5α,14β-androstane [235] and 14-methyl-d_3-5α,14β-androstane [236]. It is clear that in the fragmentation of [235], the 14-methyl group contributes at least 90% toward the initial C-methyl cleavage. The spectra of lanostane and its 14β-methyl isomer are not appreciably different[119] and it is reasonable to expect no significant difference in the spectrum of 14-methyl-5α,14α-androstane.

[C] Reduction with Diimide

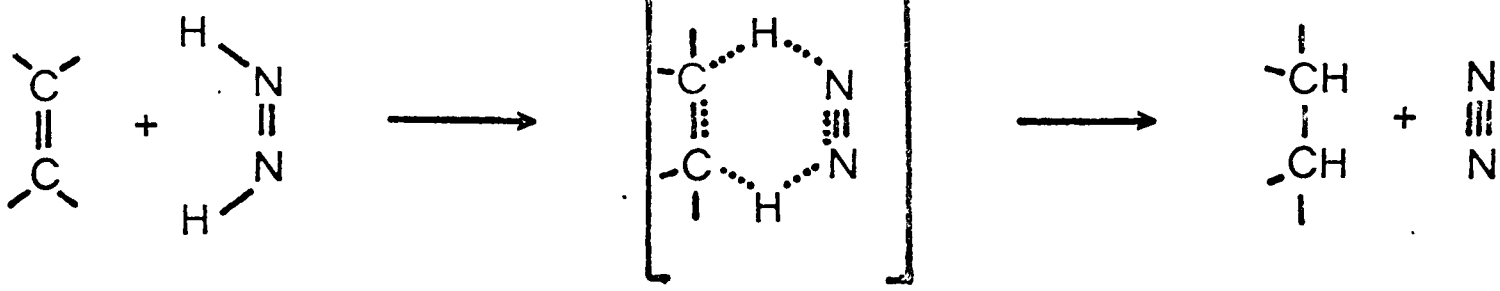
Hydrazine has long been regarded as an effective reducing agent. As early as 1905 the reduction of oleic acid to stearic acid with hydrazine was reported.[120] More recently the reaction of gibberellic acid with hydrazine gave the tetrahydro-derivative,[121] and it was noted that the Wolff-Kischner reduction of some unsaturated keto-acids resulted in reduction of the olefin.[122,124]

With the recognition that the presence of an oxidising agent was necessary for reduction with hydrazine, it was apparent that the function of the oxidant was to convert the hydrazine into something capable of reducing double bonds. A number of workers[123-126] simultaneously concluded that an intermediate reagent, diimide (N_2H_2), was responsible for reductions formerly attributed to hydrazine. Conclusive evidence for the existence of diimide in solution soon followed,[123c,127-129] but attempts to isolate the reagent as a Diels-Alder complex were unsuccessful.[123a,130]

The mechanism for the stereoselective[123b,125c] and exothermic[123a] reduction is believed to involve a 'synchronous transport' from the syn-form of diimide via a cyclic transition state[123b,124,125b] (Scheme 28).

The development of methods for the in situ generation of diimide now permits the choice of a wide range of reaction conditions to suit the stability

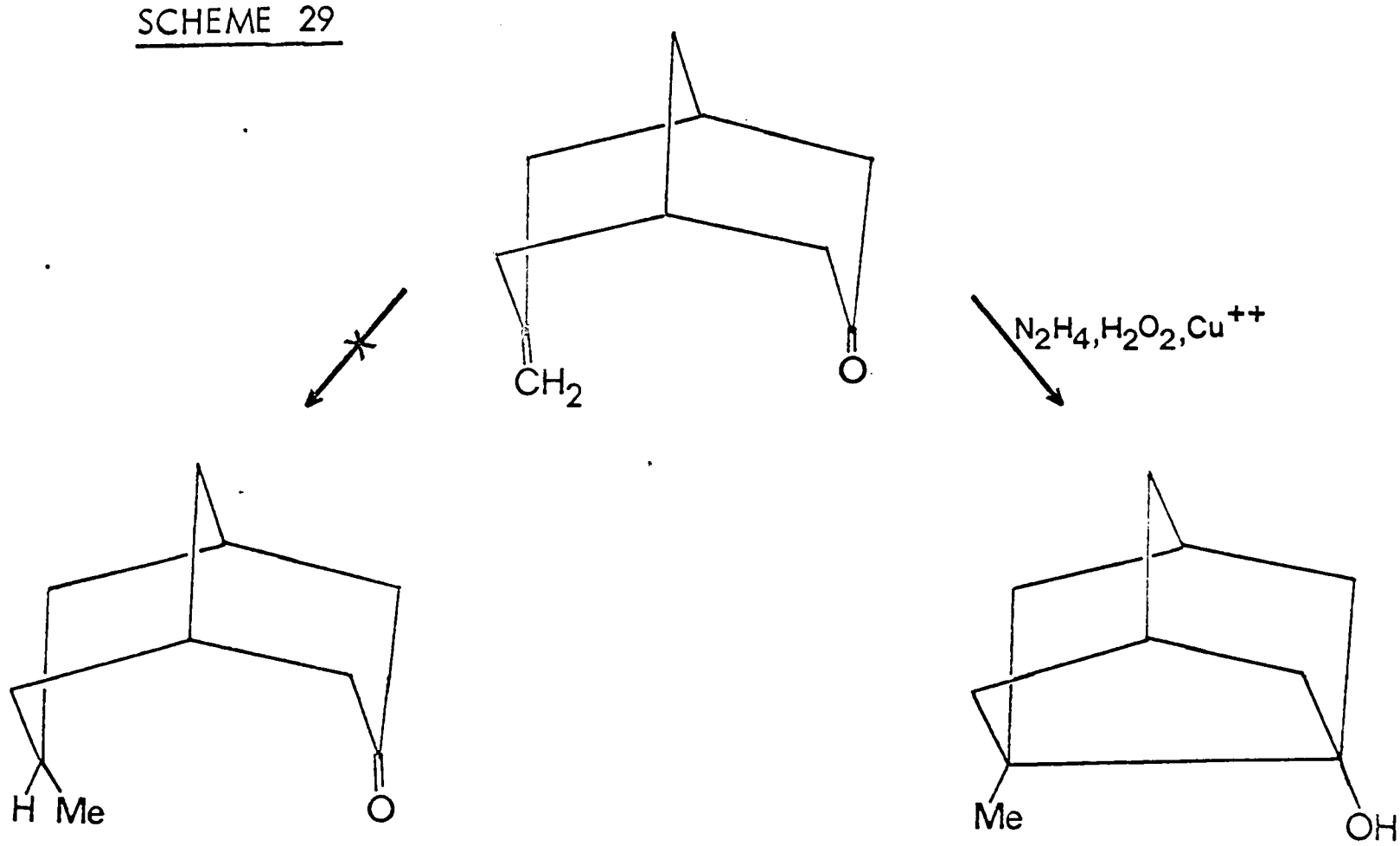
SCHEME 28: The Mechanism of Reduction with Diimide



requirements of the reacting species,[131,132] and the versatility of the reaction is much increased.

The chemistry of diimide has been comprehensively reviewed,[126,131-133] and more recently the stereospecific reduction of allene systems has been studied.[134] An unprecedented transannular reductive condensation between C-C and C-O double bonds has also been reported,[135] although the mechanism for the reaction (Scheme 29) is open to discussion.

SCHEME 29



In the present work [Scheme 3] it was found that using modified conditions for the hydrazine rearrangement of an oxido-ketone,[18a] and in the presence of air, the product [18] of rearrangement of 15β,16β-oxido-5α-androstan-17-one [16] was reduced in situ to 5α-androstan-15β-ol [20]. Under the same conditions, 5α-androst-16-en-15β-ol [18] was reduced almost quantitatively to [20]. Surprisingly these conditions when applied to 15α,16α-oxido-5α,13α-androstan-17-one [17] or to 5α,13α-androst-16-en-15α-ol [19] gave only an unidentified polar product. With the same conditions, there was no reduction of 5α-androst-2-en-1α-ol [177] (Scheme 24).

A comparative study with a limited number of readily available olefins and using the neutral "P" conditions of Corey et al.[123a] was initiated, with two aims:

(a) to determine whether the difference of reactivity of A- and D-ring olefins was general, and

(b) to establish the effect of the presence of an allylic oxygen function.

Results are listed in Table 16.

Table 16: Reduction of Steroidal Olefins with Diimide

Compound	Olefinic Position	Percentage Reduction
5α-androst-16-en-15β-ol [18]	16	90
-2-en-1α-ol [177]	2	0
-1-en-3β-ol [237]	1	6.8
-3-en-5β-ol [189]	3	0
5α-androst-16-ene [240]	16	51
-2-ene [228]	2	0
-5-ene [192]	5	0
Cholest-5-ene [241]*	5	0

* Corey et al.[123a] report that the "O" conditions result in 20% reduction of cholesterol.

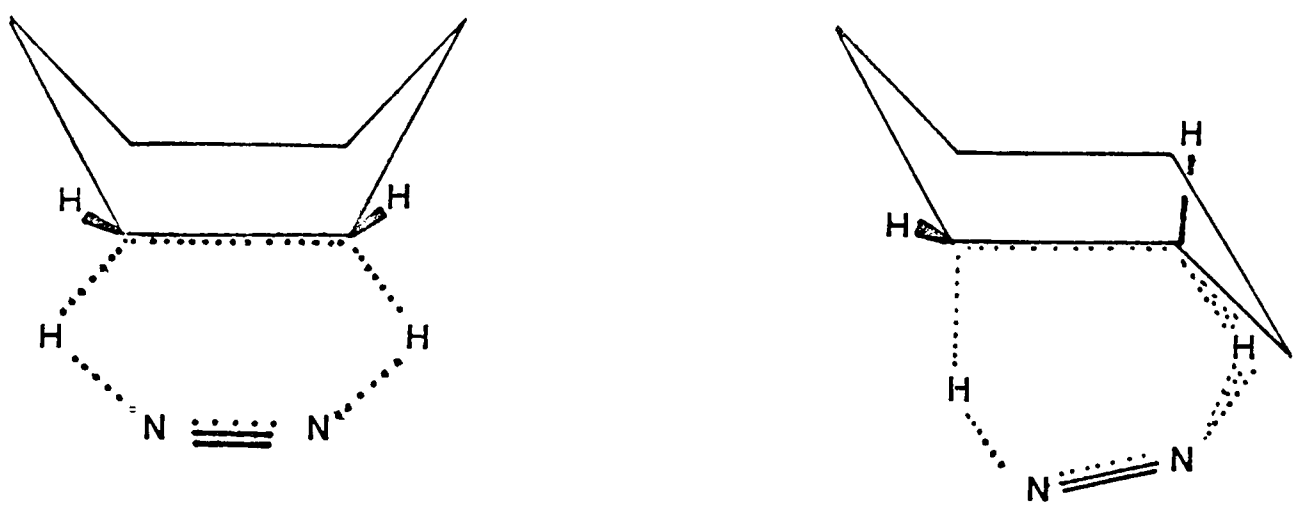
From the two positive results, it is suggested that allylic oxygenation may encourage hydrogenation. This function is also present in gibberellic acid.[121]

A surprising difference observed in the reactivity of the A- and D-ring olefins may be attributed to strain within the 5-membered ring system. Closer inspection of previously reported diimide hydrogenations of cis-substituted olefins* shows that high yield reductions occur almost exclusively in straight chain or 5-membered ring systems.[126,131,132] Because of the observed reactivity of unstrained non-cyclic systems, it is tempting to relate the lack of reactivity of a non-planar 6-membered ring olefin to a possible distortion of an sp_3 reaction intermediate (Figure 21). However, in view of Hammond's postulate for fast exothermic reactions,** such a model must be considered with caution. It would be interesting to compare the reductions of 6-membered ring olefins restricted to a chair conformation.

* In the transition state, bond angles of the olefin are compressed from ca. 120° to ca. 109°, and the substituents on the double bond increase the activation energy. cis-Substituents are more strongly affected by this change in bond angle than trans-substituents, and cis-olefins react 3 to 10 times more slowly than their trans-isomers.

** If a one-step reaction is fast and highly exothermic, the structure of the transition state should resemble that of the reactants more closely than it does of the products. Moreover, if the rate of the overall reaction is comparable to that of the formation of the intermediate complex, then the complex and the transition state should closely resemble each other.[136,137]

FIGURE 21



[D] Removal of a 15-Oxo Group

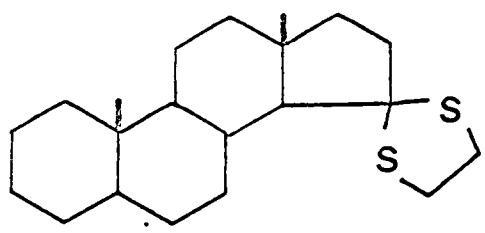
The principal methods for the removal of a ketone from the steroidal skeleton have been the Huang-Minlon reduction[45] and the Raney nickel desulphurisation of an ethylene thio-ketal derivative.[38b] Fetizon and Golfier[87] found that the Huang-Minlon reduction of 4,4'-dimethyl-5α,14β-androstan -15-one gave 4,4'-dimethyl-5α,14β-androstane in low yield. This is the only precedent for the removal of a 15-oxo group, necessary for the structural determination of microbiological products described in this thesis.

In a series of experiments described in section II, only forcing Huang-Minlon conditions[46] ensured complete reduction, and it was interesting to find that competition between steric and kinetic controls determined the isomeric composition of the product. The results (Table 17) do not require comment.

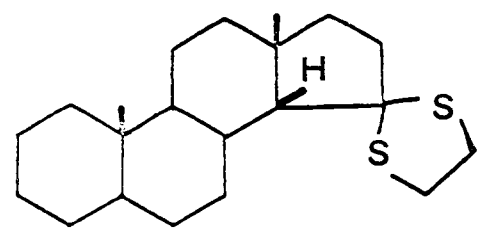
Table 17: Huang-Minlon reduction of 15-keto steroids

Starting Material	Reaction Product (%)		Comment
	14α-isomer	14β-isomer	
5α,14β-androstan-15-one [2]	45	55	Analysed by g.l.c.
2α,12β-dihydroxy-5α-androstan-15-one [122]	50	50	
6α,12β-dihydroxy-5α,14β-androstan-15-one [116]	65	35	
7β,12β-dihydroxy-5α,14β-androstan-15-one [132]	15	85	Separable as diacetates

Thioketalisation of 5α-androstan-15-one [1] gave a quantitative yield of 5α-androstan-15-one ethylene thioketal [242], but with 5α,14β-androstan-15-one [2] an inseparable mixture (1:1) of the 14α- and 14β-isomers [242] and [243] resulted.



(242)



(243)

It is likely that, using more carefully controlled reaction conditions, equilibrium mixtures could be forced toward the 14α-modification, but an investigation of the possibilities of this method was not pursued.

[E] Incubation of 5α-androstan-15-one [1] and 5α,14β-androstan-15-one [2] with Aspergillus ochraceus and Rhizopus nigricans

Incubation of the ketones [1] and [2] with A.ochraceus afforded no steroidal products. The incubations with R.nigricans resulted in small amounts of steroidal products, but these were not characterised. Results are summarised in Table 18.

Table 18

Substrate	Culture	Conditions	% s.m.	Products
[1]	A.ochraceus	A, 7.5 DMSO, 4 d	53	nil
[2]	"	"	37	nil
[1]	R.nigricans	"	59	3 prod<1.5%
[2]	"	"	53	4 prod<3%

SECTION IV

SPECTRAL DATA OF

COMPOUNDS STUDIED

The n.m.r., c.d., and mass spectra of compounds described in this Thesis are recorded in the following Tables.

(1) N.m.r. Spectra

It is clear from the preceding sections that the structural elucidation of the products obtained from microbiological hydroxylation is based primarily on n.m.r. spectroscopy. A consideration of the angular methyl signals in $CDCl_3$, of the position and multiplicity of >CH-OR proton signals (a reproduction[9d] of these characteristic signal shapes is shown in Figure 22; see also Figure 1), and of the difference in angular methyl signals of derived polyketones in CCl_4 and benzene, has usually allowed the unambiguous determination of steroidal products. The application of this technique will be further discussed in Section V.

Tables 19-21 record the n.m.r. spectra of all compounds described in this Thesis. The format of the tables, and the abbreviations used, have the usual meaning, and are defined elsewhere.[15] Calculated values for the chemical shifts of the methyl groups are obtained from the modified version of Zürcher's additive values, derived in Section V, and summarised in Tables 46 and 47.

(2) C.d. Spectra

The main use of the c.d. spectra of compounds studied in the preceding sections was to define or to confirm the orientation of the D-ring at position 14 of 15-oxo androstanes. Table 22 summarises the Cotton effect data of some 30 compounds.

(3) Mass Spectra

Although the mass spectral fragmentation processes of most mono-

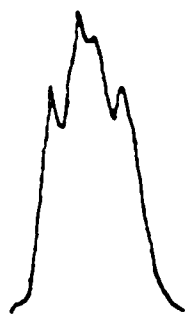
oxygenated androstanes have been elucidated, the unambiguous location of the position of keto, hydroxyl, or olefinic groups from the mass spectrum of a polyfunctional steroid is difficult or impossible, and the recent work of Vetter et al.[139] serves to redefine some of the complex problems involved in the mass spectral analysis of a polyfunctional steroid. An electron deficiency at a potentially reactive site is a prerequisite for fragmentation, but the amount of charge density required at a particular site depends on the fragmentation processes involved; these may vary substantially in the presence of other functional groups.

Nevertheless, from an exhaustive literature search, Spiteller et al. have been able to tabulate the major fragmentation patterns corresponding to a particular, or to a combination of functional groups in the steroid series.[140,141]

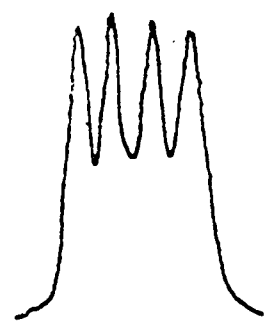
Table 23 summarises the major fragments in the mass spectra of 132 compounds described in the preceding sections. No attempt has been made to assign structures to the fragments eliminated, but generally these are in accord with those predicted from the correlations of Spiteller et al.

FIGURE 22. >CH.OR Signals (100 MHz) [9d]

Coupling constants and half-band widths given in Hz



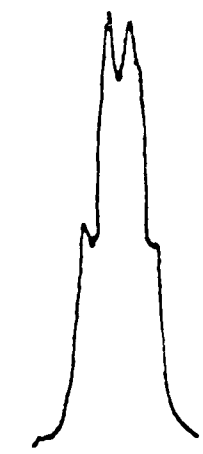
1α-OR
J = 3,3
$W_{\frac{1}{2}}$ ~ 10



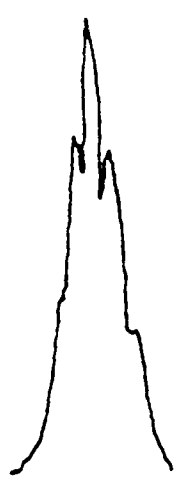
1β-OR
J = 10,5-7
$W_{\frac{1}{2}}$ ~ 18



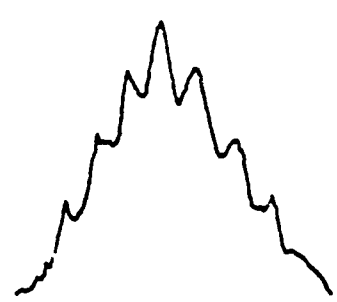
2α-OR
J = 11,11,5,5
$W_{\frac{1}{2}}$ ~ 22



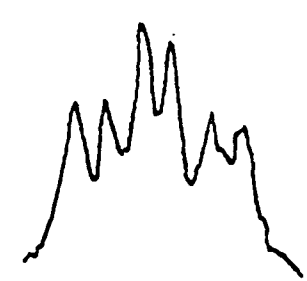
2β-OR
$W_{\frac{1}{2}}$ ~ 9



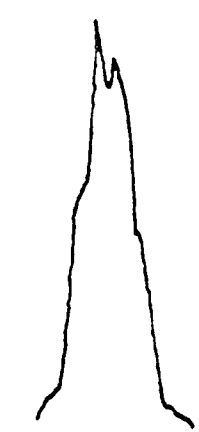
3α-OR
J = 2.5,2.5,2.5,2.5
$W_{\frac{1}{2}}$ ~ 8



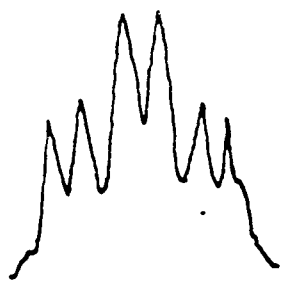
3β-OR
J = 10,10,5,5
$W_{\frac{1}{2}}$ ~ 22



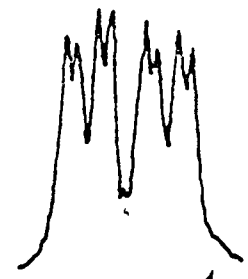
4α-OR
J = 10,10,5
$W_{\frac{1}{2}}$ ~ 20



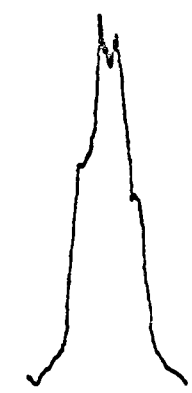
4β-OR
$W_{\frac{1}{2}}$ ~ 7



6α-OR
J = 10,10,5
$W_{\frac{1}{2}}$ ~ 20



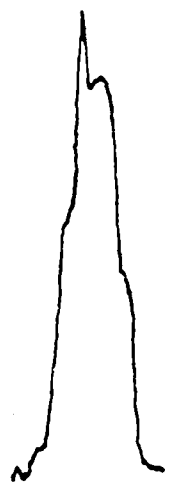
6α-OR (Δ^4)
J = 10,5,2
$W_{\frac{1}{2}}$ ~ 18



6β-OR
J = 3,3,3
$W_{\frac{1}{2}}$ ~ 7



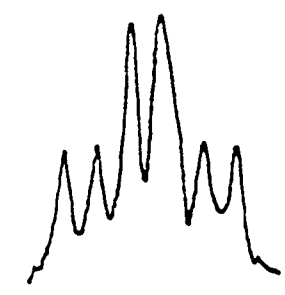
6β-OR (Δ^4)
J = 2.5,2.5
$W_{\frac{1}{2}}$ ~ 7



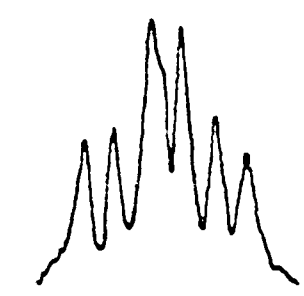
7α-OR
$W_{\frac{1}{2}}$ ~ 7



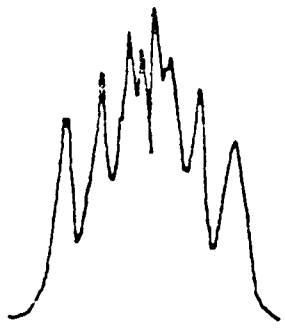
7β-OH
$W_{\frac{1}{2}}$ ~ 20



7β-OAc
J = 10,10,5
$W_{\frac{1}{2}}$ ~ 20



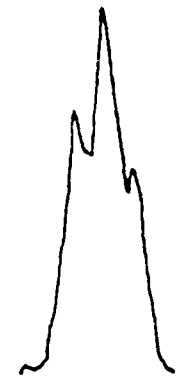
11α-OR
J = 10,10,5
$W_{\frac{1}{2}}$ ~ 20



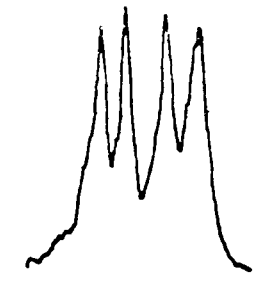
11α-OR (10β-H)
J = 10, 9, 5
$W_{\frac{1}{2}}$ ~ 20



11β-OR
J = 3, 3, 3
$W_{\frac{1}{2}}$ ~ 12



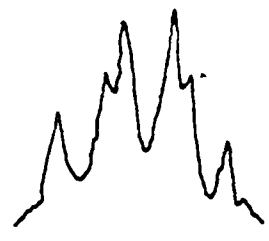
12α-OH
J = 3, 3
$W_{\frac{1}{2}}$ ~ 12



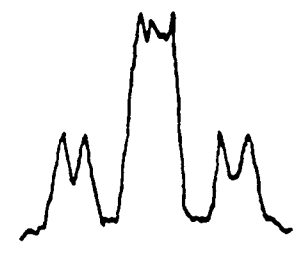
12β-OR
J = 10-12, 4-5
$W_{\frac{1}{2}}$ ~ 18



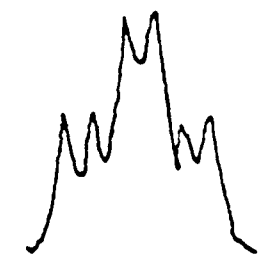
15α-OH
J = 8-10, 8-10, 3-4
$W_{\frac{1}{2}}$ ~ 17



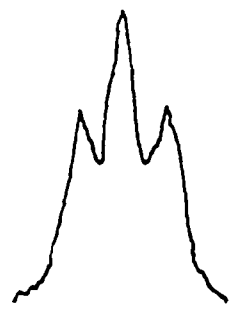
15α-OH (17C=O)
J = 8, 8, 7-8
$W_{\frac{1}{2}}$ ~ 15



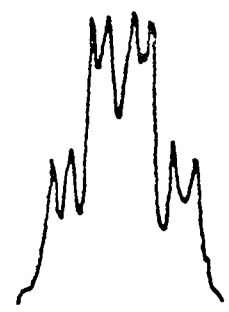
15α-OAc
J = 10, 9, 3
$W_{\frac{1}{2}}$ ~ 20



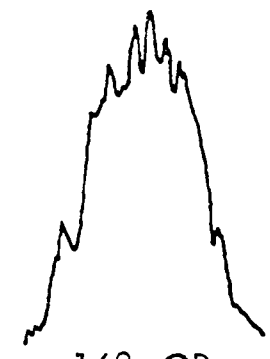
15α-OAc (17C=O)
J = 8, 8, 4-3
$W_{\frac{1}{2}}$ ~ 15



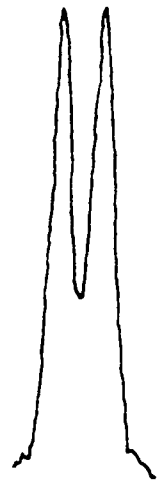
15β-OH
J = 6, 6
$W_{\frac{1}{2}}$ ~ 12



16α-OR
J = 6, 6, 6, 3
(acetate, J = 7, 7, 7, 4-3)
$W_{\frac{1}{2}}$ ~ 18



16β-OR
J = 8-6, 6-5, 6-5, 5-4
$W_{\frac{1}{2}}$ ~ 18



17α-OH
J = 7-6
$W_{\frac{1}{2}}$ ~ 10



17β-OH
17β-OAc (15α-OR)
J = 8, 8
$W_{\frac{1}{2}}$ ~ 15



17β-OAc
J = 10-9, 8-7
$W_{\frac{1}{2}}$ ~ 15

TABLE 19: KETONES and ETHYLENE ACETALS. Chemical shifts (τ-values) and solvent shifts (Δ-values) of 19- and 18-protons. Subscript numerals of τ refer to the solvent, 1, CCl_4; 2, $CDCl_3$; C_6H_6. $\Delta_1^3 = \tau_3 - \tau_1$.

No.	Compound	τ_1		τ_2		τ_2 (calc.)		τ_3		Δ_1^3	
		19	18	19	18	19	18	19	18	19	18
105	5α-Androstan-1-one	8.88	9.31	8.84	9.30	8.84	9.30	9.13	9.31	+0.25	0.00
106	5α-Androstan-2-one	9.26	9.30	9.24	9.31	9.24	9.31	9.42	9.39	+0.16	+0.09
154	5α-Androstan-3-one	8.99	9.28	8.98	9.27	8.98	9.27	9.37	9.34	+0.38	+0.06
88	5β-Androstan-3-one	8.97	9.28	8.96	9.27	8.96	9.27	9.27	9.36	+0.30	+0.08
115	3,3-Ethylenedioxy-5α-androstane	9.19	9.30	9.19	9.31	9.19	9.31	9.28	9.34	+0.09	+0.04
176b	5α-Androst-1-en-3-one	8.99	9.32	8.98	9.26	8.98	9.26	9.34	9.37	+0.35	+0.05
87	Androst-4-en-3-one	8.81	9.24	8.80	9.24	8.80	9.24	9.24	9.37	+0.43	+0.13
74	5α-Androst-9(11)-en-3-one	8.87	9.33	8.84	9.31	8.84	9.31	9.22	9.33	+0.35	+0.00
107	5α-Androstan-4-one	9.27	9.30	9.25	9.30	9.25	9.30	9.40	9.38	+0.13	+0.08
100	A-Nor-(5→6β)abeo-androstan-5-one	9.12	9.30	9.04	9.33			8.93	9.48	−0.19	+0.18
99	A-Nor-(5→6β)abeo-androst-2-en-5-one			8.98	9.33						
108	5α-Androstan-6-one	9.25	9.27	9.26	9.29	9.26	9.29	9.37	9.44	+0.12	+0.17
109	5α-Androstan-7-one	8.96	9.33	8.95	9.31	8.95	9.31	9.26	9.38	+0.30	+0.05
110	5α-Androstan-11-one	9.01	9.33	8.98	9.33	9.00	9.34	8.84	9.44	−0.17	+0.11
111	5α-Androstan-12-one	9.14	9.03	9.13	8.99	9.13	8.99	9.36	9.24	+0.22	+0.21
1	5α-Androstan-15-one	9.20	9.22	9.21	9.21	9.21	9.21	9.31	9.42	+0.11	+0.20
3	5α,13α-Androstan-15-one	9.26	9.08	9.26	9.08	9.26	9.08	9.29	9.38	+0.03	+0.30
2	5α,14β-Androstan-15-one	9.14	8.64	9.27	8.85	9.27	8.85	9.17	8.93	+0.03	+0.29
233	14-Methyl-5α,14β-androstan-15-one			9.26	9.01						
25	5α-Androst-16-en-15-one			9.15	9.02	9.15	9.02				
37	5α,14β-Androst-16-en-15-one			9.24	8.79	9.24	8.79				
231	14-Methyl-5α,14β-androst-16-en-15-one			9.27	8.93						
112	5α-Androstan-16-one	9.19	9.13	9.18	9.13	9.18	9.13	9.32	9.44	+0.13	+0.31
114	16,16-Ethylenedioxy-5α-androstane			9.22	9.10	9.22	9.10				
4	5α-Androstan-17-one	9.19	9.19	9.19	9.14	9.19	9.14	9.32	9.37	+0.31	+0.18
11	5α,13α-Androstan-17-one	9.34	9.07	9.37	9.03	9.37	9.03	9.44	9.20	+0.10	+0.17
113	5β-Androstan-17-one	9.06	9.20	9.05	9.15	9.05	9.15	9.16	9.38	+0.10	+0.18
9	5α-Androst-14-en-17-one			9.16	8.89	9.16	8.98				
10	5α-Androst-15-en-17-one	9.15	9.01	9.14	8.93	9.14	8.93	9.10	9.34	−0.05	+0.33
15	5α,13α-Androst-15-en-17-one			9.33	8.92	9.33	8.92				
—	5α,14β-Androst-15-en-17-one			9.24	8.91	9.24	8.91				
13	16α-Bromo-5α,13α-androstan-17-one			9.39	8.84						
14	16β-Bromo-5α,13α-androstan-17-one			9.32	8.97						
6	16α-Bromo-5α-androstan-17-one-ethylene ketal			9.22	9.12						
5	17,17-Ethylenedioxy-5α-androstane	9.22	9.23	9.22	9.17	9.22	9.17	9.23	9.03	+0.01	−0.20
7	17,17-Ethylenedioxy-5α-androst-14-ene			9.19	8.92	9.19	8.92				
8	17,17-Ethylenedioxy-5α-androst-15-ene			9.18	9.08	9.19	9.08				
Two	>C = O										
128	5α-Androstane-2,12-dione	9.18 (d)	9.02	9.16 (d)	8.98	9.15	8.99	9.57 (d)	9.34	+0.39	+0.32
95	A-Nor-(3→5β)abeo-androstane-3,5-dione	8.96	9.30	8.89	9.31			9.01	9.58	+0.05	+0.28
50	5α-Androstane-3,11-dione	8.81	9.30	8.78	9.30	8.76	9.30	8.97	9.49	+0.16	+0.19
77	5α-Androstane-3,12-dione	8.90	8.98	8.89	8.95	8.89	8.95	9.49	9.27	+0.59	+0.29
76	5α-Androst-9(11)-ene-3,12-dione			8.71	9.04	8.71	9.04				
83	5α-Androstane-6,11-dione	9.08	9.31	9.03	9.31	9.04	9.32	8.96	9.59	−0.12	+0.28
121	5α-Androstane-6,12-dione	9.19	8.99	9.17	8.97	9.17	8.97	9.50	9.39	+0.31	+0.40
139	5α-Androstane-7,12-dione	8.86	9.03	8.87	8.99	8.86	8.99	9.39	9.31	+0.53	+0.28
140	5α,14β-Androstane-7,12-dione	8.96	8.83	8.95	8.79	8.95	8.79	9.48	9.04	+0.53	+0.21
62	5α-Androstane-12,15-dione	9.12	8.93	9.12	8.88	9.12	8.89	9.45	9.36	+0.33	+0.43
63	5α,14β-Androstane-12,15-dione	9.20	8.67	9.18	8.63	9.18	8.63	9.45	9.10	+0.25	+0.43

Table 19 (cont.)

No.	Compound	τ_1 19	τ_1 18	τ_2 19	τ_2 18	τ_2(calc.) 19	τ_2(calc.) 18	τ_3 19	τ_3 18	Δ_1^3 19	Δ_1^3 18
Three	C = O										
124	5α-Androstane-2,12,15-trione	9.16 (d)	8.93	9.16 (d)	8.90	9.14	8.89	9.65 (d)	9.45	+0.49	+0.52
125	5α,14β-Androstane-2,12,15-trione	9.24	8.65	9.22	8.62	9.20	8.63	9.64	9.15	+0.40	+0.53
84	5α-Androstane-3,6,11-trione	8.87	9.27	8.83	9.28	8.80	9.28	9.07	9.63	+0.20	+0.36
158	5α,13α-Androstane-6,12,15-trione	9.26	8.85	9.24	8.80			9.63	9.02	+0.37	+0.17
117	5α,14β-Androstane-6,12,15-trione	9.26	8.63	9.22	8.60	9.22	8.60	9.59	9.23	+0.33	+0.60
141	5α,14β-Androstane-7,12,15-trione			9.02	8.62	8.99	8.63				
173	A-Nor-(5→6β)abeo-androstane-3,5,15-trione	8.94	9.22	8.88	9.23			9.08	9.73	+0.14	+0.51
166	A-Nor-(5→6β)abeo-androstane-5,7,11-trione	8.69	9.32	8.61	9.35			8.51	9.71	−0.18	+0.39
167	A-Nor-(5→6β)abeo-androstane-5,11,15-trione	8.86	9.16	8.74	9.19			8.43	9.78	−0.43	+0.62

TABLE 20: ALCOHOLS, ACETATES, and some ETHERS. Chemical Shifts (τ_2-values) of 19-, 18-, and CH-OR protons. For CH-OR signals the multiplicity [s(singlet), d, t, q, qn, sx, sp, o(octet)] is followed by the coupling constant(s) (J, Hz); for an unresolved multiplet (m), the halfwidth ($W_{1/2}$, Hz) is given.

No.	Compound	τ_2 19	τ_2 18	τ_2(calc) 19	τ_2(calc) 18	Assign-ment	>CH-OR τ	>CH-OR Form	Assign-ment	τ	Form
178	5α-Androstan-1α-ol	9.20	9.30	9.20	9.30	H–1	6.40	m(6)			
179	5α-Androstan-1α-yl acetate	9.16	9.32	9.16	9.32	H–1	5.17	m(5)			
177	5α-Androst-2-en-1α-ol	9.28	9.28	9.28	9.28	H–1	5.26	m(9)			
181	5α-Androstan-1β-ol	9.17	9.31	9.17	9.31	H–1	6.61	q(10,5)			
208	5α-Androstan-1β-yl acetate	9.05	9.32	9.05	9.32	H–1	5.4	m(18)			
182	5α-Androstan-2α-ol	9.20	9.30	9.20	9.30	H–2	6.21	sp(11,11,5,5)			
209	5α-Androstan-2α-yl acetate	9.15	9.31	9.15	9.31	H–2	5.09	sp(11,11,5,5)			
183	5α-Androstan-2β-ol	8.96	9.30	8.96	9.30	H–2	5.83	m(9)			
210	5α-Androstan-2β-yl acetate	9.06	9.31	9.06	9.31	H–2	4.90	m(9)			
184	5α-Androstan-3α-ol	9.21	9.30	9.21	9.30	H–3	5.95	m(7)			
160	5β-Androstan-3α-ol	9.07	9.31	9.07	9.30	H–3	6.30	m(23)			
211	5α-Androstan-3α-yl acetate	9.20	9.30	9.20	9.30	H–3	4.98	m(7)			
185	5α-Androstan-3β-ol	9.19	9.31	9.19	9.31	H–3	6.45	sp(10,10,5,5)			
159	5β-Androstan-3β-ol	9.02	9.30	9.02	9.30	H–3	5.85	t(3,3)			
212	5α-Androstan-3β-yl acetate	9.17	9.31	9.17	9.31	H–3	5.30	sp(10,10,5,5)			
237	5α-Androst-1-en-3β-ol	9.09	9.29	9.09	9.29	H–3	5.67	m(17)			
86	Androst-5-en-3β-ol	8.97	9.28	8.97	9.28	H–3	6.47	sp(10,10,6,5)			
186	5α-Androstan-4α-ol	9.20	9.31	9.20	9.31	H–4	6.57	sx(10,10,5)			
213	5α-Androstan-4α-yl acetate	9.15	9.31	9.15	9.31	H–4	5.32	sx(10,10,5)			
187	5α-Androstan-4β-ol	8.96	9.31	8.96	9.31	H–4	6.18	m(7)			
214	5α-Androstan-4β-yl acetate	8.99	9.31	8.99	9.31	H–4	5.05	m(7)			
190	5β-Androstan-5-ol	9.10	9.31	9.10	9.31						
189	5β-Androst-3-en-5-ol	9.02	9.30	9.02	9.30						
195	5α-Androstan-6α-ol	9.20	9.30	9.20	9.30	H–6	6.60	sx(10,10,4.5)			
215	5α-Androstan-6α-yl acetate	9.15	9.31	9.15	9.31	H–6	5.3	sx(10,10,4.5)			
196	5α-Androstan-6β-ol	8.99	9.27	8.99	9.27	H–6	6.19	m(6)			
193	5β-Androstan-6β-ol	8.84	9.28	8.88	9.28	H–6	6.26	t(3,3)			
216	5α-Androstan-6β-yl acetate	9.02	9.26	9.02	9.26	H–6	5.05	m(6)			
94	5β-Androstan-6β-yl acetate	8.99	9.26	8.99	9.26	H–6	5.26	m(7)			
197	5α-Androstan-7α-ol	9.21	9.30	9.21	9.30	H–7	6.14	m(7)			
217	5α-Androstan-7α-yl acetate	9.20	9.32	9.20	9.32	H–7	5.05	m(5)			
198	5α-Androstan-7β-ol	9.19	9.28	9.19	9.28	H–7	6.65	m(21)			
218	5α-Androstan-7β-yl acetate	9.17	9.28	9.17	9.28	H–7	5.4	m(15)			
199	5α-Androstan-11α-ol	9.06	9.29	9.08	9.30	H–11	6.04	sx(10,10,5)			
219	5α-Androstan-11α-yl acetate	9.10	9.24	9.12	9.25	H–11	5.32	sx(10,10,5)			
200	5α-Androstan-11β-ol	8.96	9.07	8.96	9.07	H–11	5.65	q(7,4)			
201	5α-Androstan-11β-yl acetate	9.12	9.18	9.12	9.18	H–11	4.62	q(6,3)			
202	5α-Androstan-12α-ol	9.21	9.26	9.21	9.26	H–12	6.20	t(3,3)			
220	5α-Androstan-12α-yl acetate	9.22	9.22	9.22	9.22	H–12	5.01	t(3,3)			
203	5α-Androstan-12β-ol	9.20	9.29	9.20	9.29	H–12	6.60	q(10,5)			
221	5α-Androstan-12β-yl acetate	9.20	9.23	9.20	9.23	H–12	5.34	q(11,5)			
53	5α-Androstan-15α-ol	9.20	9.25	9.21	9.27	H–15	5.92	o(10,9,3)	See Figure 1		
21	5α,13α-Androstan-15α-ol	9.30	8.89	9.30	8.89	H–15	5.90	q(7.5,3)	See Figure 1		
23	5α,14β-Androstan-15α-ol	9.26	8.98	9.26	8.98	H–15	5.60	t(5,5)	See Figure 1		
222	5α-Androstan-15α-yl acetate	9.21	9.23	9.21	9.23	H–15	5.06	o(10,9,3)			
19	5α,13α-Androst-16-en-15α-ol	9.33	8.85	9.33	8.85	H–15	5.60	m(4)			
20	5α-Androstan-15β-ol	9.16	9.00	9.16	9.00	H–15	5.69	sx(7,7,1.5)	See Figure 1		
24	5α,14β-Androstan-15β-ol	9.23	8.97	9.23	8.97	H–15	5.65	sx(8,8,1.5)	See Figure 1		
223	5α-Androstan-15β-yl acetate	9.16	9.05	9.16	9.05	H–15	4.82	sp(7.5,5.5,2)			
18	5α-Androst-16-en-15β-ol	9.13	8.91	9.13	8.91	H–15	5.45	q(4,2.5)			
40	15β-Allyloxy-5α-androst-16-ene	9.15	8.93			H–15	5.85	q(4,2.5)			
41	15β-Methoxy-5α-androst-16-ene	9.15	8.95	9.15	8.95	H–15	5.99	q(5,2)			
204	5α-Androstan-16α-ol	9.22	9.30	9.22	9.30	H–16	5.53	o(6,6,6,3)			
224	5α-Androstan-16α-yl acetate	9.22	9.27	9.22	9.27	H–16	4.78	o(7,7,7,4)			
205	5α-Androstan-16β-ol	9.20	9.06	9.20	9.06	H–16	5.63	m(15)			
225	5α-Androstan-16β-yl acetate	9.21	9.12	9.21	9.12	H–16	4.85	m(16)			
206	5α-Androstan-17α-ol	9.20	9.34	9.20	9.34	H–17	6.28	m(10)			
226	5α-Androstan-17α-yl acetate	9.21	9.27	9.21	9.27	H–17	5.16	d(6)			
207	5α-Androstan-17β-ol	9.21	9.27	9.21	9.27	H–17	6.37	t(8,8)			
227	5α-Androstan-17β-yl acetate	9.21	9.22	9.21	9.22	H–17	5.41	q(9,7.5)			
—	5α-Androst-15-en-17β-ol	9.19	9.19	9.19	9.19	H–17	5.67	s($W_{1/2}$ 7)			
12	5α,13α-Androst-16-en-17-yl acetate	9.33	9.00	9.33	9.00	H–16	4.55	t(2,2)			

Table 20 (cont.)

No.	Compound	τ_2 19	τ_2 18	τ_2(calc) 19	τ_2(calc) 18	Assignment	τ	>CH-OR Form	Assignment	τ	Form
One-OR and one >C = 0 (or -S-[CH_2]$_2$-S-) group											
92	3α-Hydroxy-A-nor-(5→6β) abeo-androstan-5-one	9.04	9.32			H–3	6.12	sx(10,10,5)			
47	3α-Hydroxy-5α-androstan-17-one	9.19	9.14	9.18	9.13						
91	3β-Hydroxy-A-nor-(5→6β)abeo-androstan-5-one	9.01	9.34			H–3	5.77	m(7)			
98	5-Oxo-A-nor-(5→6β)abeo-androstan-3β-yl acetate	8.99	9.34			H–3	4.82	m(6)			
97	5-Oxo-A-nor-(5→6β)abeo-androstan-3β-yl mesylate	8.98	9.34			H–3	not recorded.				
96	5-Oxo-A-nor-(5→6β)abeo-androstan-3β-yl toluene p-sulphonate	9.02	9.37			H–3	5.05	m(8)			
67	3β-Hydroxy-5α-androstan-17-one	9.16	9.13	9.16	9.14	H–3	6.41	sp(10,10,5,5)			
85	3β-Hydroxy-androst-5-en-17-one	8.97	9.12	8.94	9.11	H–3	6.50	m(25)			
72	11α-Hydroxy-5α-androstan-3-one	8.86	9.25	8.84	9.26	H–11	6.02	sx(10,10,5)			
73	3-Oxo-5α-androstan-11α-yl toluene p-sulphonate	8.88	9.22			H–11	4.85	sx(10,10,5)			
60	12β-Hydroxy-5α-androstan-15-one	9.18	9.20	9.19	9.19	H–12	6.70	q(11,5)			
61	12β-Hydroxy-5α,14β-androstan-15-one	9.24	8.87	9.24	8.87	H–12	6.64	q(11,4)			
151	14-Hydroxy-5α,14β-androstan-15-one	9.25	8.95	9.25	8.95	–					
59	15α-Hydroxy-5α-androstan-12-one	9.10	8.94	9.12	8.95	H–15	5.73	sx(8,8,4)			
33	15β-Hydroxy-5α-androstan-17-one	9.14	8.82	9.13	8.83	H–15	5.4	m(7)			
34	17,17-Ethylenedithio-5α-androstan-15β-ol	9.16	8.83			H–15	5.47	o(7.5,7,3)			
28	15β-Allyloxy-5α-androstan-17-one	9.13	8.84			overlap with ether protons					
29	15β-Methoxy-5α-androstan-17-one	9.15	8.90			H–15	6.06	t(5,5)			
36	15-Oxo-5α-androstan-17β-yl toluene-p-sulphonate	9.22	9.17			H–17	5.38	t(8,8)			
One-OR and two >C = 0 groups											
174	3β-Hydroxy-A-nor-(5→6β)abeo-androstane-5,12-dione	9.00	9.00			H–3	5.75	m(7)			
144	14-Hydroxy-5α,14β-androstane-12,15-dione	9.17	8.74	9.16	8.73						
168	15α-Hydroxy-A-nor-(5→6β)abeo-androstane-3,5-dione	8.88	9.28			H–15	5.96	sx(9,9,4)			
51	15α-Hydroxy-5α-androstane-3,11-dione	8.76	9.26	8.75	9.26	H–15	5.82	sx(9.5,9.5,3)			
58	15α-Hydroxy-5α-androstane-3,12-dione	8.89	8.91	8.88	8.91	H–15	5.70	sx(9,9,4)			
One-OR and three C = 0 groups											
143	14-Hydroxy-5α,14β-androstane-7,12,15-trione	9.03	8.70	8.97	8.73						
Two-OR groups											
126	5α-Androstane-2α,12β-diol	9.19	9.29	9.18	9.29	H–2	6.24	sp(11,11,5,5)	H–12	6.59	q(12,5)
127	5α,14β-Androstane-2α,12β-diol	9.19	9.03	9.18	9.02	H–2, 12		not clear			
49	5α-Androstane-3α,11α-diol	9.07	9.28	9.07	9.29	H–3	5.95	m(overlap)	H–11	6.03	sx(10,10,5)
104	A-Nor-(5→6β)abeo-androstane-3β,5β-diol	8.96	9.27			H–3	6.11	m(6)	H–5	6.23	d(3)
71	5α-Androstane-3β,11α-diol	9.05	9.28	9.05	9.30	H–3	6.45	sp(10,10,5,5)	H–11	6.35	t(8,8)
82	5α-Androstane-6α,11α-diol	9.04	9.28	9.06	9.29	H–6	6.62	m(18)	H–11	6.09	m(27)
119	5α-Androstane-6α,12β-diol	9.18	9.28	9.18	9.28	H–6	6.60	m(overlap)	H–12	6.58	q(11,5)
120	5α,14β-Androstane-6α,12β-diol	9.18	9.02	9.18	9.02			weak spectrum			
137	5α-Androstane-7β,12β-diol	9.19	9.27	9.17	9.26	H–7		obscured	H–12	6.58	q(12,4)
138	5α,14β-Androstane-7β,12β-diol	9.19	9.03	9.17	9.01	H–7, 12	6.6	m(20)			
135	7β,12β-Diacetoxy-5α-androstane	9.16	9.21	9.15	9.20	H–7		obscured	H–12	5.40	q(11,4.5)
136	7β,12β-Diacetoxy-5α,14β-androstane	9.17	8.96			H–7, 12	5.35	m(28); overlap			
64	5α,14β-Androstane-12α,15α-diol	9.27	8.91	9.25	8.96	H–12	6.46	m(7)	H–15	5.75	t(5,5)
66	5α,14β-Androstane-12α,15β-diol	9.22	8.95	9.22	8.95	H–12	6.48	m(9)	H–15	5.55	o(8.5,8,4)
57	5α-Androstane-12β,15α-diol	9.19	9.25	9.19	9.25	H–12	6.54	q(10,5)	H–15	5.82	sx(9,9,3)

Table 20 (cont.)

No.	Compound	τ_2 19	τ_2 18	τ_2(calc) 19	τ_2(calc) 18	Assignment	τ	$\geq$CH-OR Form	Assignment	τ	Form
65	5α,14β-Androstane-12β,15α-diol	9.24	8.98	9.23	9.20	H—12	6.06	q(12,4)	H—15	5.61	sx(4,4,1)
156	5α,14β-Androstane-14,15α-diol	9.25	9.00	9.25	9.00	H—15	5.83	d(8)			
155	5α,14β-Androstane-14,15β-diol	9.18	8.78	9.18	8.78	H—15	5.89	d(5)			
153	14,15α-Oxido-5α-androstane	9.19	9.08	9.19	9.08	H—15	6.56	m(3)			
154	14,15β-Oxido-5α,14β-androstane	9.19	8.96	9.19	8.96	H—15	6.62	m(3)			
32	5α-Androstane-15β,17β-diol	9.17	8.99	9.15	8.96	H—15	5.78	sp(7,5,2.5)	H—17	6.43	t(8,8)
30	15β-Allyloxy-5α-androstan-17β-ol	9.18	9.03			H—15	obscured		H—17	obscured	
38	15β-Allyloxy-5α-androstan-17β-yl toluene-p-sulphonate	9.21	9.00			H—15	obscured		H—17	5.75	t(8,8)
35	15β-Hydroxy-5α-androstan-17β-yl toluene-p-sulphonate	9.20	8.95			H—15	obscured		H—17	5.80	t(8,8)
31	15β-Methoxy-5α-androstan-17β-ol	9.18	9.07			H—15, 17	6.42	overlap			
42	15β-Methoxy-5α-androstan-17β-yl benzoate	9.18	8.88			H—15	6.3	m(15)	H—17	5.2	t(9,9)
39	15β-Methoxy-5α-androstan-17β-yl toluene p-sulphonate	9.21	9.02			H—15	6.43	m(14)	H—17	5.79	t(8,8)
Two-OR and one $\geq$C = 0 group											
69	1β,3β-Dihydroxy-5α-androstan-17-one	9.14	9.15	9.11	9.14	H—1	6.57	q(11,5)	H—3	6.37	sp(10,10,5,5)
123	2α,12β-Diacetoxy-5α-androstan-15-one	9.14	9.14	9.12	9.13	H—2	5.1	obscured	H—12	5.14	q(11,5)
122	2α,12β-Dihydroxy-5α-androstan-15-one	9.18	9.18	9.17	9.18	H—2	6.3	obscured	H—12	6.59	q(11,5)
48	3α,11α-Dihydroxy-5α-androstan-17-one	9.05	9.13	9.04	9.12	H—3	5.99	m(8)	H—11	6.03	sx(10,10,5)
171	3β,7β-Dihydroxy-A-nor-(5→6β) abeo-androstan-5-one	9.00	9.30			H—3	5.76	m(8)	H—7	6.01	m(20)
169	3β,11α-Dihydroxy-A-nor-(5→6β) abeo-androstan-5-one	8.76	9.35			H—3	5.76	m(8)	H—11	6.06	sx(10,10,5)
170	3β,12β-Dihydroxy-A-nor-(5→6β) abeo-androstan-5-one	9.01	9.34			H—3	5.79	m(7)	H—12	6.62	q(11,4)
68	3β,11α-Dihydroxy-5α-androstan-17-one	9.04	9.12	9.02	9.13	H—3	6.38	m(22)	H—11	5.99	sx(10,10,5)
52	3β,15α-Dihydroxy-5α-androstan-11-one	8.96	9.29	8.96	9.30	H—3	6.43	m(21)	H—15	5.85	sx(9,9,4)
185	4β,5-Oxido-5β-androstan-3-one	8.86	9.27	8.86	9.27	H—4	7.04	s(2)			
162	6,11α-Dihydroxy-A-nor-(5→6β) abeo-androstan-5-one	8.66	9.34			H—11	6.06	sx(10,10,5)			
78	6α,11α-Dihydroxy-5α-androstan-3-one	8.85	9.25	8.82	9.25	H—6	6.55	sx(10,10,5)	H—11	6.02	sx(10,10,5)
157	6α,12β-Dihydroxy-5α,13α-androstan-15-one	9.23	8.92			H—6	6.68	sx(10,10,4)	H—11	6.35	q(11,5)
116	6α,12β-Dihydroxy-5α,14β-androstan-15-one	9.21	8.84	9.22	8.86	H—6, 12	6.7	m(20 Hz)			
131	6α,15α-Diacetoxy-5α-androstan-12-one	9.05	8.91	9.06	8.91	H—6	5.27	sx(9,9,5)	H—15	4.87	sx(9,9,4)
16	7β,11α-Dihydroxy-A-nor-(5→6β) abeo-androstan-5-one	8.76	9.32			H—7	6.34	q(9,3)	H—11	6.01	sx(10,10,5)
132	7β,12β-Dihydroxy-5α,14β-androstan-15-one	9.21	8.85	9.21	8.85	H—7	5.40	m(16)	H—12	6.64	q(12,4)
75	9α,11α-Oxido-5α-androstan-3-one	8.73	9.25	8.73	9.25	H—11	6.86	d(5)			
164	11α,15α-Dihydroxy-A-nor-(5→6β) abeo-androstan-5-one	8.77	9.30			H—11) H—15)	6.05	m(28); overlapping signals			
134	12β,14-Dihydroxy-5α,14β-androstan-15-one	9.23	8.98	9.22	8.97	H—12	6.78	q(12,4)			
56	12β,15α-Dihydroxy-5α-androstan-3-one	8.95	9.22	8.95	9.21	H—12	6.48	q(11,5)	H—15	5.76	m(20)
17	15α,16α-Oxido-5α,13α-androstan-17-one	9.36	8.86	9.36	8.86	H—15	6.68	d(25)	H—16	6.22	d(2.5)
16	15β,16β-Oxido-5α-androstan-17-one	9.16	8.85	9.16	8.85	H—15	6.73	d(3)	H—16	6.17	d(3)
Two-OR and two $\geq$C = 0 groups											
142	7β,14-Dihydroxy-5α,14β-androstan-12,15-dione	9.13	8.71	9.13	8.71			weak spectrum			

Table 20 (cont.)

No.	Compound	τ_2 19	τ_2 18	τ_2(calc) 19	τ_2(calc) 18	Assignment	τ	$\geq$C<u>H</u>-OR Form	Assignment	τ	Form
Three-OR groups											
55	5α-Androstane-3β,12β,15α triol	9.16	9.22	9.16	9.25			weak spectrum			
80	3β,12β,15α-Triacetoxy-5α-androstane	9.16	9.16	9.14	9.15	H—3, 12	5.29	q(11,5) + overlap	H—15	4.79	m(18)
Three-OR and one $\geq$C = 0 (or acetal) group											
79	3-Oxo-5α-androstane-6α,12β,15α-triol	8.93	9.20	8.93	9.20			weak spectrum			
81	6α,12β,15α-Triacetoxy-5α-androstan-3-one	8.92	9.09	8.88	9.11	H—15 H—6	4.92 5.38	sx(9,9,4) sx(10,10,5)	H—12	5.29	q(10,4)
133	15-Oxo-5α,14β-androstane-7β,12β,14-triol	9.19	8.96	9.18	8.95	H—7	5.05	m(28)	H—12	6.82	q(12,4)
146	12β-Hydroxy-7β,14-isopropylidene-dioxy-5α,14β-androstan-15-one	9.18	9.01	9.18	9.01	H—7	5.45	m(20)	H—12	6.85	q(12,4)
145	7β,12β-Diacetoxy-14-hydroxy-5α,14β-androstan-15-one	9.16	8.87	9.19	8.90	H—7	4.1	m(27)	H—12	5.47	q(12,4)
165	? (mixture)	9.05	9.19				5.1	m(40); 3 protons			
172	? (mixture)	8.99	9.29					weak spectrum			
175	? (mixture)	9.00	9.20			H—3	4.74	m(8)		5.15	m(50); 2 protons
Four-OR groups											
148	5α,14β-Androstane-7β,12β,14,15β-tetraol	9.16	9.01	9.12	8.73			weak spectrum and complex			

TABLE 21: HYDROCARBONS and MISCELLANEOUS ANDROSTANES. Chemical shifts (τ-values) of 19- and 18-protons, and low-field proton signals.

No.	Compound	τ_2 19	τ_2 18	Other signals
201	A-nor-(5→6β)abeo-androstane	9.19	9.29	
235	14-Methyl-5α,14β-androstane	9.25	9.12	9.20 (s, CH$_3$-14)
180	5α-Androst-1-ene	9.14	9.28	4.09 (d; J9Hz; W½15Hz; H-1); 4.46 (m, W½18Hz; H-2)
228	5α-Androst-2-ene	9.23	9.29	4.19 (m; W½5Hz; H-2 and H-3)
192	Androst-5-ene	9.00	9.28	4.75 (m; W½8Hz; H-6)
191	3β-Chloro-androst-5-ene	8.97	9.27	6.25 (m; W½20Hz; H-3); 4.65 (d; J5Hz; H-6)
152	5α-Androst-14-ene	9.19	8.99	4.84 (q; J2,4Hz; H-15)
240	5α-Androst-16-ene	9.17	9.25	4.36 (sx; J 6.3, 1.8, 1.8; H-16); 4.22 (q; J 6.3, 1.2; H-17)
239	17-Iodo-5α-androst-16-ene	9.19	9.29	
27	15β-Cyano-5α-androstan-17-one	9.13	8.82	6.75 (sp; J9,8,2Hz; H-15)
43	15β-Cyano-17,17-ethylenedithio-5α-androstane	9.15	8.84	7.11 (2H, s,-S·CH$_2$·); 6.26 (3H, m, W½7Hz,-S·CH$_2$· and H-15)
44	17,17-Ethylenedithio-5α-androstane-15β-carbaldehyde	9.20	9.00	6.78 (4H, s,-S·[CH$_2$]$_2$·S-); 6.55 (1H, d, J17Hz, H-15); 0.39 (1H, d, J2Hz, -CHO)
45	15β-Hydroxymethyl-5α-androstane	9.22	9.20	7.93 (2H, d, J6Hz, -CH$_2$-OH)
90	4-Oxa-androst-5-en-3-one	8.88	9.23	4.72 (q; J6,2Hz; H-6)
93	4-Oxa-5ξ-androstan-5-ol	9.06	9.30	6.02 (1H, m, W½28Hz); 6.37 (2H, m, W½20Hz)
94	A-Nor-3-acetoxy-3,5-seco-androstan-5-one	8.90	9.22	5.95 (3H; t; J6.5,6.5Hz; H-3)
149	7β,12ξ-Dihydroxy-D-homo-15a-oxa-(14→12β)abeo-androstan-14-one	9.10) 9.10)	(8.81 (8.77	complex
150	7β,12ξ-Diacetoxy-D-homo-15a-oxa-(14→12β)abeo-androstan-14-one	9.08) 9.08)	(8.74 (8.71	complex

TABLE 22: Circular Dichroism Cotton Effects of the Compounds Studied

Spectra were recorded in methanolic solutions at ambient temperatures in the range 22-24°C.

Compound No.	Compound	Cotton Effects λ (nm) [Δε]
185 [a]	4β,5-Oxido-5β-androstan-3-one	219 [+0.50]; 310 [+3.3]
100 [b]	A-Nor-(5→6β)abeo-androstan-5-one	295 [+0.17]
92 [b]	3α-Hydroxy-A-nor-(5→6β)abeo-androstan-5-one	294 [+0.84]; 301 [+0.83]; 311(sh) [+0.47]
91 [b]	3β-Hydroxy-A-nor-(5→6β)abeo-androstan-5-one	294 [+0.19]
59	15α-Hydroxy-5α-androstan-12-one	290 [+1.43]
1	5α-Androstan-15-one	297 [+3.05]
3	5α,13α-Androstan-15-one	298 [+2.18]
2	5α,14β-Androstan-15-one	304 [-2.29]
233	14-Methyl-5α,14β-androstan-15-one	312 [-0.54]
61	12β-Hydroxy-5α,14β-androstan-15-one	303 [-2.25]
151	14-Hydroxy-5α,14β-androstan-15-one	319 [-1.36]
123	2α,12β-Diacetoxy-5α-androstan-15-one	295 [+3.29]
116	6α,12β-Dihydroxy-5α,14β-androstan-15-one	303 [-2.07]
132	7β,12β-Dihydroxy-5α,14β-androstan-15-one	301 [-2.70]
133	15-Oxo-5α,14β-androstane-7β,12β,14-triol	327 [-0.50]
145	7β,12β-Diacetoxy-14-hydroxy-5α,14β-androstan-15-one	316 [-1.19]
146	7β,14-Isopropylidenedioxy-12β-hydroxy-5α,14β-androstan-15-one	327 [-1.44]
231	14-Methyl-5α,14β-androst-16-en-15-one	233 [+0.49]; 339 [+1.24]

11	5α,13α-Androstan-17-one	270 [+0.09]; 299(sh) [-0.53]; 308 [-0.59]
13 [c]	16α-Bromo-5α,13α-androstan-17-one	211 [+1.45]; 320 [-1.74]
14 [c]	16β-Bromo-5α,13α-androstan-17-one	218 [-3.56]; 314 [+0.44]
17 [a]	15α,16α-Oxido-5α,13α-androstan-17-one	317 [+3.35]

Two >C=O Groups

95 [b]	A-Nor-(5→6β)abeo-androstane-3,5-dione	296.5(sh) [+1.13]; 304 [+1.27]; 312(sh) [+1.02]
139	5α-Androstane-7,12-dione	295 [+2.34]
140	5α,14β-Androstane-7,12-dione	288 [-0.18]; 295 [-0.18]
62	5α-Androstane-12,15-dione	295 [+4.48]
63	5α,14β-Androstane-12,15-dione	289 [+0.48]; 320 [-0.89]
142	7β,14-Dihydroxy-5α-androstane-12,15-dione	297 [+2.5]; 334 [-0.68]

Three >C=O Groups

117	5α,14β-Androstane-6,12,15-trione	287 [+1.35]; 317 [-0.93]; 327 [-0.87]
141	5α,14β-Androstane-7,12,15-trione	289 [-0.86]; 311 [-0.35]; 326 [-0.30]

[a] See footnote, page 6

[b] A c.d. study of the equivalent A-nor-(5→6β)abeo-cholestanes is the subject of a recent paper of Sntazke and Kinsky.[138]

[c] For the "α-haloketone rule", see reference 72, page 79.

TABLE 23: Major Peaks in the Mass Spectra of the Compounds Studied

The compound number corresponds to that in the text of this Thesis, and the peaks are given in mass units (m/e) followed by the abundance (percentage of the base peak) in parentheses.

Compound	M^+ (%)	m/e (%) Other peaks
1	274 (100)	97 (80), 109 (46), 259 (38), 217 (33), 190 (32), 245 (28)
2	see Figure 14	
3	see Figure 14	
11	274 (100)	124 (60), 110 (60), 259 (28), 230 (18), 149 (16), 217 (14)
12	316 (8)	274 (100), 260 (94), 241 (25), 109 (19), 95 (16), 149 (10.5)
13	354 (19)	109 (100), 139 (94), 273 (87), 121 (84), 95 (81),
14	352 (19)	218 (78)
15	272 (100)	97 (91), 257 (38), 149 (34), 135 (32), 176 (23)
17	288 (32)	111 (100), 95 (31), 97 (28), 135 (16.5), 149 (15), 150 (12)
19	274 (100)	95 (95), 110 (84), 109 (68), 241 (42), 149 (40), 147 (40)
21	276 (40)	243 (100), 217 (100), 95 (81), 97 (81), 258 (51), 149 (43), 148 (39)
23	276 (2)	243 (100), 258 (50), 148 (41), 149 (32), 95 (35), 109 (30)
24	276 (15)	243 (100), 95 (64), 109 (64), 258 (55), 218 (53), 149 (30), 148 (29)
27	299 (100)	95 (90), 110 (71), 109 (68), 242 (50), 284 (33)
28	330 (100)	109 (77), 218 (56), 95 (56), 108 (48), 203 (32)
29	304 (100)	109 (50), 289 (30), 108 (27), 218 (18), 128 (18)
30	332 (4)	274 (100), 103 (90), 99 (50), 149 (39), 259 (39), 230 (37)
31	306 (5)	274 (100), 109 (18), 149 (12), 230 (11), 256 (10), 259 (9)
33	290 (46)	109 (100), 149 (53), 175 (46), 274 (41), 135 (40)
34	366 (26)	147 (100), 148 (80), 119 (21), 120 (20), 338 (15)
42	410 (0.25)	105 (100), 97 (34), 288 (28), 256 (21), 274 (19)
43	375 (13)	157 (100), 118 (12), 109 (11), 160 (9), 219 (3)
44	378 (12)	57 (100), 160 (30), 86 (26), 260 (16), 118 (11), 131 (11)
50	288 (100)	124 (82), 151 (42), 109 (38), 164 (37), 177 (34)
51	304 (100)	95 (24), 109 (17), 107 (16), 149 (13), 276 (13), 165 (12)
52	306 (100)	193 (48), 147 (23), 118 (21), 180 (18)
59	290 (60)	96 (100), 97 (60), 272 (42), 247 (36), 257 (23)
61	290 (4)	97 (100), 98 (7), 109 (5), 176 (4), 267 (3)
62	288 (92)	97 (100), 96 (99), 110 (66), 121 (25), 149 (24)
63	288 (31)	97 (100), 96 (86), 110 (29), 190 (23), 121 (21)
64	292 (14)	256 (100), 241 (66), 106 (52), 93 (45), 95 (43)
65	292 (-)	259 (100), 274 (90), 109 (35), 256 (30), 241 (28)

71	292 (14)	274 (100), 94 (69), 147 (64), 260 (60), 241 (50)
74	272 (52)	247 (100), 95 (60), 105 (40), 149 (39)
77	288 (100)	93 (50), 270 (46), 147 (44), 111 (39), 121 (36)
82	292 (7)	96 (100), 188 (90), 241 (65), 256 (60), 135 (50)
83	288 (67)	151 (100), 191 (58), 164 (49), 138 (48), 273 (38)
84	302 (74)	273 (100), 205 (28), 141 (26), 109 (25), 93 (25)
90	274 (76)	135 (100), 163 (50), 219 (44), 218 (34), 200 (22)
91	276 (55)	135 (100), 220 (70), 149 (70), 219 (40), 232 (18)
92	276 (100)	149 (98), 135 (76), 220 (36), 219 (21), 232 (18)
93	278 (17)	95 (100), 260 (87), 245 (71), 135 (50), 219 (35), 203 (30)
94	320 (-)	245 (100), 95 (70), 110 (67), 220 (40), 260 (37)
95	274 (91)	135 (100), 163 (46), 219 (40), 218 (35), 149 (23)
98	318 (10)	230 (100), 135 (53), 258 (35), 127 (22), 215 (21)
99	258 (100)	135 (71), 243 (52), 149 (52), 163 (30), 215 (19), 189 (19)
100	260 (100)	149 (70), 135 (19), 245 (7), 189 (6), 227 (5), 232 (2)
101	246 (50)	95 (100), 136 (59), 231 (52), 203 (44), 121 (25)
102	290 (66)	272 (100), 257 (53)
103	428 (95)	400 (100), 105 (97), 131 (74), 186 (44)
104	278 (-)	260 (100), 227 (100), 245 (95), 149 (70), 135 (50)
108	274 (100)	259 (86), 123 (20), 256 (19), 135 (19), 219 (18)
116	306 (1.3)	97 (100), 270 (32), 288 (20)
117	Figure 15	
118	310 (69)	99 (100), 100 (97), 182 (50), 149 (43), 247 (30), 262 (30), 295 (25)
121	288 (67)	151 (100), 191 (58), 164 (49), 138 (48), 273 (38)
122	306 (54)	97 (100), 288 (71), 273 (37), 180 (28), 270 (12)
123	390 (3)	270 (100), 97 (98), 255 (42), 330 (35), 214 (28)
124	302 (22)	96 (100), 97 (54), 110 (15), 123 (13)
131	390 (1)	330 (100), 122 (26), 315 (23), 107 (21), 147 (16)
132	306 (6)	97 (100), 207 (45)
133	322 (2.5)	266 (100), 304 (20), 109 (17), 149 (14)
134	306 (50)	250 (100), 100 (42), 232 (37), 231 (36), 149 (24)
138	292 (0.2)	81 (100), 95 (85), 111 (47), 259 (46), 274 (34)
140	288 (47)	81 (100), 122 (87), 247 (65), 192 (40)
141	302 (40)	178 (100), 123 (92), 274 (85), 97 (68), 260 (48)
142	320 (6)	264 (45), 274 (30), 302 (30)
143	318 (30)	262 (100), 140 (22), 125 (19)
145	406 (2)	230 (100), 290 (71), 350 (48), 346 (24), 258 (22)
148	324 (2)	262 (100), 95 (75), 149 (56), 245 (53), 249 (53)
149	322 (28)	95 (100), 97 (95), 149 (34), 220 (28), 191 (20)
150	406 (-)	95 (100), 97 (34), 286 (27), 204 (21), 149 (20)
151	290 (8)	234 (100), 262 (15)
152	see Table 12	
153	274 (100)	95 (44), 109 (44), 121 (40), 217 (39), 189 (34)
154	274 (100)	109 (90), 95 (59), 121 (42), 217 (38), 189 (35)
155	292 (-)	189 (100), 109 (8), 216 (66), 256 (50)
156	292 (1.5)	274 (100), 163 (57), 123 (55), 234 (40)

157	306 (0.4)	97 (100), 117 (44), 119 (44), 270 (36), 125 (18)
158	see Figure 15	
159	276 (-)	243 (100), 258 (83), 108 (81), 95 (80), 93 (65)
160	276 (-)	95 (100), 97 (95), 243 (64), 258 (57)
161	292 (0.1)	274 (100), 219 (52), 163 (52), 218 (40), 161 (32)
162	292 (19)	220 (100)
163	292 (100)	All other peaks < 5%
164	292 (86)	111 (100), 110 (61), 264 (13), 256 (10), 241 (10), 203 (9)
167	288 (46)	110 (100), 178 (43), 149 (32), 270 (25), 164 (18)
168	290 (12)	120 (100), 272 (28), 217 (25)
170	292 (100)	236 (63), 147 (42), 109 (39), 133 (35)
171	292 (100)	121 (85), 236 (62), 207 (40), 217 (37)
172	308 (100)	215 (93), 252 (81), 145 (70), 149 (59), 147 (56)
173	288 (48)	149 (100), 95 (65), 215 (37), 109 (35), 137 (30)
174	290 (100)	149 (80), 120 (46), 163 (34), 234 (32)
175	434 (3)	286 (100), 226 (100), 254 (34), 346 (29), 314 (27), 339 (17); a mixture
179	see Table 11	
180	see Table 12	
189	274 (100)	124 (100), 95 (93), 109 (71), 256 (65), 241 (49)
190	276 (12)	243 (100), 258 (81), 220 (86), 135 (48), 95 (47)
193	see Table 11	
201	see Table 11	
208	see Table 11	
↕		
227		
228	see Table 12	
231-4	see Table 14	
235	see Figure 20	
236	see Figure 20	

SECTION V

The Nuclear Magnetic Resonance Spectra of Steroids

The importance of nuclear magnetic resonance in the structural elucidation of oxygenated steroids has been demonstrated in this Thesis and other recent publications.[9,14,15,17,59,88]

Zürcher's survey[82] of steroidal n.m.r. spectra, in which he confirmed the additivity of substituent effects on C-methyl proton resonances, has been augmented in various ways,[142-147,172] and similar considerations have been extended to steroids of unnatural configuration.[113,145a,c,e,148-150] The recently published data[15,17,52,59,88] of 411 oxygenated steroids prepared in this laboratory provides, in addition to the most recent and comprehensive review of C-methyl contributory shifts, valuable generalisations of the positions and profiles of protons geminal to hydroxyl groups.

The use of shift reagents[151-153] in the analysis of low field proton signals has been highlighted by the recent widespread utilisation of the paramagnetic shifts induced by europium complexes,[154-157] and ^{13}C-n.m.r. spectroscopy[158] has provided another valuable source of information of molecular environments. However, neither technique has been used in the present work.

In addition to those published some 300 new steroids, many of unnatural configuration, have been characterised in this laboratory and where additive contributions have been defined, their n.m.r. spectra have usually confirmed the consistency of the published data.[15] However, some notable exceptions, to a lesser extent apparent at the time of publication, appear.

This section attempts to accommodate these anomalies, to establish (in some cases tentative) C-methyl incremental values for previously unrecorded functional groups or "interacting units", and to consider the spectra of some steroids of unnatural configuration. To facilitate the practical value of this

work, results are summarised at the end of the chapter [Tables 46 and 47]. The tables include some data from literature sources.

[A] 5α,14α-Androstanes

Despite early evidence of long-range conformational interactions,[159] the rigidity of the steroidal skeleton has been accepted; its use as a rigid model in mechanistic studies has not been disputed, and for this reason substituent effects on C-methyl proton resonance have been found to be additive. However it is known[15,82,142a] that contributory values are no longer valid when the field exerted by a substituent on a C-methyl proton is altered by the interaction of some other functional groups, or a change in skeletal conformation consequent on the introduction of that group.

The contribution of a particular functional group to C-methyl proton resonance has almost invariably been derived from a consideration of data for polyfunctional compounds, using as a basis the "naked" value for the 3-oxo group (that is, the shift of the C-methyl proton resonance caused by a 3-oxo group when it alone appears on the androstane skeleton). The effects of small structural variations have been "averaged out" to give a "best fit" presentation of data. These are referred to as "average" values. In the past, the aim has been to predict the C-methyl chemical shifts for a new structure; many compounds have come within the acceptable limits of ±0.03 τ,* but some major discrepancies

* Instrumental errors lie within ±0.015 τ, but variations must be expected as a result of coordination of the solvent ($CDCl_3$) with polar functional groups. Solubility factors preclude the general use of CCl_4 as a solvent.

TABLE 24. Chemical shifts (τ_2; $CDCl_3$) of 19- and 18-protons of some 5α, 14α-androstanes where no structural interactions would be expected to be reflected in the spectra

Compound	τ_2 (obs.)		τ_2 (calc.) Average[15]		τ_2 (calc.) Naked (Table 26)	
	19-H	18-H	19-H	18-H	19-H	18-H
5α-androstane-3,12-dione	8.89	8.95	8.88	8.89	8.89	8.95
5α-androstane-7,12-dione	8.87	8.99	8.84	8.92	8.86	8.99
5α-androstane-12,15-dione	9.12	8.88	9.11	8.85	9.12	8.89
5α-androstane-3β,12β-diol	9.17	9.28	9.18	9.25	9.17	9.29
5α-androstane-7β,12β-diol	9.18	9.27	9.18	9.23	9.17	9.26
12β-hydroxy-5α-androstan-7-one	8.94	9.30	8.93	9.25	8.93	9.29
6β-hydroxy-5α-androstan-3-one	8.77	9.23	8.74	9.22	8.76	9.23
3β,6α-diacetoxy-5α-androstan-17-one	9.08	9.14	9.10	9.12	9.08	9.14
3β,16α-diacetoxy-5α-androstan-7-one	9.22	9.25	9.21	9.20	9.21	9.25
3β,6α,15α-triacetoxy-5α-androstane	9.11	9.27	9.13	9.22	9.11	9.27
3β,6α,17α-triacetoxy-5α-androstane	9.10	9.27	9.15	9.26	9.10	9.27

TABLE 25. Distortions arising from presence of a 14-olefin grouping*

Compound	τ_2 (obs.)		τ_2 (calc.; naked from Table 26)	
	19-H	18-H	19-H	18-H
5α-androst-14-ene	9.19	8.99		
44'-Me_2 "	9.11	9.01	9.11	9.01
5α-androst-14-en-12β-ol	9.18	9.00	9.17	8.97
5α-androst-14-en-17β-ol	9.17	8.91	9.18	8.95
5α-androst-14-en-17-one	9.16	8.89	9.16	8.82
17,17-ethylenedioxy-5α-androst-14-ene	9.19	8.93	9.19	8.85

* Fetizon et al.[30] have observed an 8(14)-olefinic interaction with a 12-oxo contribution.

have appeared in systems where no structural interactions would be expected. Table 24 illustrates a few such examples.

The field exerted by certain functional groups at some positions on the steroid skeleton, on one or both of the tertiary methyl groups can be expected to be especially susceptible to slight steric variations. It is the premise of the present work that sufficient data now exists for it to be permissible to take note of these skeletal interactions.

The presence of an sp^2-system, especially at a tertiary centre, would serve to magnify any variation in the position of a functional group relative to the C-methyl protons. In spite of the large number of compounds now available, lack of data still precludes the systematic search for long range substituent effects, but the well known Δ^4-,* Δ^5- and Δ^{14}-systems cause considerable distortion** [Table 25]. The flexibility of a five-membered ring[161] means that values for functional groups within the D-ring could be subject to larger variations than in other rings. (The theoretical treatment[161] has been augmented by the analysis of

* Clegg[9d] notes the effect of a Δ^4-bond on the coupling associated with an 11α-hydroxy substituent.

** Direct deshielding effects of a double bond are observed only in the core of the axis of the bond, and it is reasonable to assume that conformational changes play the more important part. Clegg[9d] has dealt comprehensively with the effect of a double bond on CH-OR proton resonances in its vicinity, and Tori et al.[160] have investigated the effect of bulky 6β-substituents in Δ^4-3-oxo systems.

coupling constants in a variety of 17-substituted androstanes[149,162]). Dipole-dipole interactions between functional groups (particularly where one of the groups lies in a position 1,3-diaxial to the methyl protons[19,142a]) could also result in conformational distortions.*

The position of the 19-protons relative to a substituent at the 11-position could be varied even by long range conformational changes.** Nevertheless, the readily available 11-oxygenated androstanes have often been used in the derivation of contributory constants for other functional groups.

With work described elsewhere in this Thesis [Tables 19-21], or by other members of this group,[9,15,47,48] it has been possible to establish for the first time "naked" C-methyl contributory values for all oxygenated mono-functional 5α-androstanes [Table 26] and for a variety of simple interacting units [Table 27]. A comparison of these values with previous average values is generally within ±0.03 τ, but some examples (notably 12-oxygenated steroids) show surprising variation.

An attempt has been made to derive new constants based on naked contributions, averaging as few values as possible to allow for interactions. In

* Snatzke et al.[163] report that c.d. studies reveal vicinal interactions in 5β-4,7-diketones, but not in the 5α-series.

** See footnote *, previous page. Zürcher[82] has previously noted the effect of A-ring substituents on additivity values of substituents at the 11-position.

polyfunctional compounds which do not contain obviously interacting groups* it has been surprising to find that only values for 11- and 15-oxygenated positions require modification from the naked results [Table 28]. Table 29, listing C-methyl τ_2 values for a selection of non-interacting 12-oxygenated steroids, compares calculated τ_2 values based on the naked contributions (modified for positions 11 and 15) with those based on the previous average values, and demonstrates the validity of the new assignments. Similar calculations for other oxygenated androstanes have supported these conclusions.

* It is clear that constants for some simple (e.g. 7-ketal) and many interacting units must still be derived from polyfunctional compounds. A discussion of these values follows Table 29, and the shift values are included in the final summary [Table 46].

TABLE 26: The effect of substituents ($\Delta\tau_2$, $CDCl_3$) on 19- and 18-protons of 5α,14α-androstane mono-ketones, mono-alcohols, and mono-acetates. Chemical shifts (τ_2 values) are recorded in Tables 19 and 20. [Chemical shifts of 19- and 18-protons of 5α-androstane are 9.22 and 9.31 respectively].

Ketones

Posn.	$\Delta\tau_2$ (naked)		$\Delta\tau_2$ (average)[15]		Posn.	$\Delta\tau_2$ (naked)		$\Delta\tau_2$ (average)[15]	
	19-H	18-H	19-H	18-H		19-H	18-H	19-H	18-H
1	-0.38	-0.01	-0.38	-0.02	11	-0.24	+0.02	-0.22	+0.03
2	+0.02	0.00	+0.03	-0.01	12	-0.09	-0.32	-0.10	-0.38
3	-0.24	-0.04	-0.24	-0.04	15	-0.01	-0.10	-0.01	-0.08
4	+0.03	-0.01	+0.04	+0.01	16	-0.04	-0.18	-0.04	-0.17
6	+0.04	-0.02	+0.05	-0.02	17	-0.03	-0.17	-0.02	-0.17
7	-0.27	0.00	-0.28	-0.01					

Alcohols

Posn.	$\Delta\tau_2$ (naked)		$\Delta\tau_2$ (average)[15]	
	19-H	18-H	19-H	18-H
1α	-0.02	-0.01	-0.01	0.00
1β	-0.05	0.00	-0.05	0.00
2α	-0.02	-0.01	-0.02	0.00
2β	-0.26	-0.01	-0.26	-0.01
3α	-0.01	-0.01	-0.00	-0.01
3β	-0.03	0.00	-0.03	-0.01
4α	-0.02	0.00	-0.02	0.00
4β	-0.26	0.00	-0.24	0.00

Acetates

$\Delta\tau_2$ (naked)		$\Delta\tau_2$ (average)[15]	
19-H	18-H	19-H	18-H
-0.06	0.00	-0.06	+0.01
-0.17	+0.01	-0.14	-0.01
-0.07	0.00	-0.08	-0.02
-0.16	0.00	-0.16	0.00
-0.02	-0.01	-0.02	-0.01
-0.05	0.00	-0.05	-0.02
-0.07	0.00	-0.06	-0.02
-0.23	0.00	-0.23	0.00

6α	-0.02	-0.01	-0.03	0.00	-0.06	0.00	-0.03	0.00
6β	-0.23	-0.04	-0.23	-0.04	-0.20	-0.05	-0.16	-0.06
7α	-0.01	-0.01	0.00	-0.01	-0.02	+0.01	-0.02	-0.02
7β	-0.03	-0.03	-0.03	-0.03	-0.05	-0.03	-0.05	+0.02
11α	-0.16	-0.02	-0.12	-0.03	-0.12	-0.07	-0.08	-0.07
11β	-0.26	-0.24	-0.26	-0.25	-0.10	-0.13		
12α	-0.01	-0.05	-0.01	-0.05	-0.00	-0.09		
12β	-0.02	-0.02	-0.02	-0.05	-0.02	-0.08	-0.02	-0.08
15α	-0.02	-0.06	-0.01	-0.03	-0.01	-0.08	0.00	-0.07
15β	-0.06	-0.31	-0.02	-0.28	-0.06	-0.26		
16α	0.00	-0.01	+0.01	-0.01	0.00	-0.04	-0.01	-0.07
16β	-0.02	-0.25	-0.02	-0.25	-0.01	-0.19	-0.02	-0.16
17α	-0.02	+0.03	+0.02	+0.06	-0.01	-0.04		
17β	-0.01	-0.04	0.00	-0.03	-0.01	-0.09	-0.02	-0.08

TABLE 27: Chemical shifts (τ_2; $CDCl_3$) and the effect of substituents ($\Delta\tau_2$) of simple oxygenated 5α-androstanes

Substituents	τ_2		$\Delta\tau_2$		Substituents	τ_2		$\Delta\tau_2$	
	19-H	18-H	19-H	18-H		19-H	18-H	19-H	18-H
-	9.22	9.31			Δ^5	9.80	9.28	-0.02	-0.03
Δ^1	9.14	9.28	-0.08	-0.03	Δ^{14}	9.19	8.99	-0.03	-0.32
Δ^2	9.23	9.29	+0.01	-0.02	Δ^{16}	9.22	9.18	0.00	-0.13
Δ^2-1-CO	8.94	9.28	-0.28	-0.03	Δ^{16}-15-CO	9.15	9.02	-0.07	-0.29
Δ^1-3-CO	8.98	9.26	-0.24	-0.05	15,15-S.$(CH_2)_2$.S	9.17	9.11	-0.05	-0.30
Δ^4-3-CO	8.80	9.24	-0.42	-0.07	16,16-$(OMe)_2$	9.21	9.13	-0.01	-0.09
$\Delta^{4,6}$-3-CO	8.87	9.19	-0.35	-0.12	16,16-O.$(CH_2)_2$.O	9.22	9.10	0.00	-0.21
3,3-$(OMe)_2$	9.21	9.32	-0.01	+0.01	Δ^{14}-17-CO	9.16	8.89	-0.06	-0.42
3,3-$(OEt)_2$	9.22	9.32	0.00	+0.01	Δ^{15}-17-CO	9.14	8.94	-0.08	-0.38
3,3-O.$(CH_2)_2$.O	9.19	9.31	-0.03	0.00	17,17-O.$(CH_2)_2$.O	9.22	9.17	0.00	-0.14
3,3-S.$(CH_2)_2$.S	9.20	9.32	-0.02	+0.01	Δ^{14}-17,17-O.$(CH_2)_2$.O	9.19	8.93	-0.03	-0.29
3,3-O.$(CH_2)_2$.O-Δ^5	8.96	9.27	-0.26	-0.05	Δ^{15}-17,17-O.$(CH_2)_2$.O	9.18	9.08	-0.04	-0.23
Δ^5-7-CO	8.81	9.28	-0.41	-0.03	$\Delta^{3,5}$-2,7-$(CO)_2$	8.72	9.25	-0.50	-0.06
$\Delta^{3,5}$-7-CO	8.87	9.25	-0.35	-0.06	Δ^4-3,6-$(CO)_2$	8.83	9.22	-0.39	-0.09
					12,17-$(CO)_2$	9.11	8.79	-0.11	-0.52

Δ^{2}-1α-OH	9.28	9.28	+0.06	-0.03	Δ^{16}-15β-OH	9.13	8.91	-0.09	-0.40
Δ^{2}-1β-OH	9.21	9.29	-0.01	-0.02	Δ^{16}-15β-OMe	9.15	8.95	-0.07	-0.36
3α-OMe	9.22	9.32	0.00	+0.01	16β-OMe	9.22	9.13	0.00	-0.18
3β-OMe	9.20	9.31	-0.02	0.00	16α-OEt	9.22	9.31	0.00	-0.01
Δ^{1}-3α-OH	9.18	9.28	-0.04	-0.03	16β-OEt	9.22	9.10	0.00	-0.21
Δ^{1}-3β-OH	9.09	9.29	-0.13	-0.02	17β-OEt	9.21	9.25	-0.01	-0.06
Δ^{2}-3-OMe	9.26	9.30	+0.04	-0.01	Δ^{15}-17β-OH	9.19	9.19	-0.03	-0.12
Δ^{2}-3-OEt	9.25	9.30	+0.03	-0.01	Δ^{16}-17-OAc	9.19	9.11	-0.03	-0.20
$\Delta^{3,5}$-3-OMe	9.06	9.26	-0.16	-0.05					
Δ^{4}-3α-OH	9.01	9.27	-0.21	-0.04	2α-OAc-3-CO	8.87	9.28	-0.35	-0.03
Δ^{4}-3β-OH	8.93	9.26	-0.29	-0.05	5α-OH-3-CO	8.82	9.27	-0.40	-0.05
Δ^{5}-3β-OH	8.97	9.28	-0.25	-0.03	6β-OH-Δ^{4}-3-CO	8.62	9.20	-0.60	-0.11
Δ^{5}-3β-OAc	8.95	9.29	-0.27	-0.02	17β-OH-16-CO	9.28	9.17	+0.06	-0.14
Δ^{5}-3β-OTs	9.02	9.30	-0.20	-0.01	17β-OAc-16-CO	9.18	9.18	-0.04	-0.13
Δ^{5}-3β-OMe	8.98	9.28	-0.24	-0.03	16α-OH-17-CO	9.19	9.05	-0.03	-0.26
					16α-OAc-17-CO	9.19	9.04	-0.03	-0.27
					16β-OAc-17-CO	9.18	9.04	-0.04	-0.27
$3\beta,5\text{-}(OH)_2$	8.99	9.29	-0.23	-0.02	16α-OAc-17β-OH	9.21	9.19	-0.01	-0.12
$12\beta,17\beta\text{-}(OH)_2$	9.18	9.18	-0.04	-0.13	$16\alpha,17\beta\text{-}(OAc)_2$	9.20	9.22	-0.02	-0.09
$15\beta,17\beta\text{-}(OH)_2$	9.17	8.99	-0.04	-0.32	16β-OAc-17β-OH	9.19	9.19	-0.03	-0.12
$16\alpha,17\alpha\text{-}(OH)_2$	9.22	9.31	0.00	0.00	$16\beta,17\beta\text{-}(OH)_2$	9.21	9.21	-0.01	-0.10

TABLE 28: A modification of the "naked" contributions ($\Delta\tau_2$) for 11- and 15-oxygenated substituents

Substituent	$\Delta\tau_2$ (naked)		$\Delta\tau_2$ (modified)		Applicable in the presence of:-
	19-H	18-H	19-H	18-H	
11-CO*	-0.24	+0.02	-0.22	+0.02	A or B ketones A, B, or D alcohols
			-0.22	+0.05	D-ketone
			-0.22	**+0.03**	overall average (Table 46)
11α-OH*	-0.16	-0.02	-0.16	-0.02	B or D alcohols
			-0.13	-0.02	A or B ketone A alcohol
			-0.13	0.00	D alcohol
			-0.14	**-0.01**	overall average (Table 46)
11α-OAc	-0.12	-0.07	-0.08	-0.04	17β-acetate
			-0.10	**-0.03**	overall average (Table 46)
11β-OH	-0.26	-0.24	**-0.25**	**-0.23**	overall average (Table 46)
11β-OAc	-0.10	-0.13	insufficient examples studied		
15-CO	-0.01	-0.10	insufficient examples studied		
15α-OH	-0.02	-0.06	**-0.01**	**-0.03**	overall average (Table 46)

* Small variations can be expected in the presence of 4- or 5-olefins.

TABLE 29: 12-Oxygenated Androstanes. A comparison of observed, calculated (naked), and calculated (average[15]) chemical shifts (τ_2; $CDCl_3$) of 19- and 18-protons

Substituents	τ_2 ($CDCl_3$) observed		calc. (naked)		calc. (average[15])	
	19-H	18-H	19-H	18-H	19-H	18-H
2,12-$(CO)_2$	9.16	8.96	9.15	8.99	9.15	8.93
3,12-$(CO)_2$	8.89	8.95	8.89	8.95	8.88	8.89
6,12-$(CO)_2$	9.17	8.97	9.17	8.97	9.17	8.91
7,12-$(CO)_2$	8.87	8.99	8.86	8.99	8.84	8.82
Δ^5-7,12-$(CO)_2$	8.72	8.94	8.72	8.96	8.71	8.91
12,15-$(CO)_2$	9.12	8.88	9.12	8.89	9.11	8.85
2,12,15-$(CO)_3$	9.16	8.89	9.14	8.89	9.14	8.84
3,6,12-$(CO)_3$	8.94	8.95	8.93	8.93	8.93	8.87
3,7,12-$(CO)_3$	8.62	8.92	8.62	8.95	8.60	8.88
3,12,15-$(CO)_3$	8.89	8.86	8.88	8.85	8.87	8.81
Δ^4-3,12,15-$(CO)_3$	8.72	8.84	8.70	8.82	8.69	8.77
4,12,15-$(CO)_3$	9.15	8.88	9.15	8.87	9.15	8.86
6,12,16-$(CO)_3$	9.12	8.78	9.13	8.79	9.13	8.74
12-CO-15α-OH	9.10	8.94	9.11	8.93	9.11	8.90
12-CO-3β,15α-$(OH)_2$	9.08	8.93	9.09	8.95	9.08	8.89
12-CO-6α,15α-$(OH)_2$	9.11	8.95	9.09	8.93	9.08	8.89
12-CO-6α,15α-$(OAc)_2$	9.06	8.92	9.06	8.91	9.09	8.86
12-CO-1β,6α,15α-$(OH)_3$	9.05	8.93	9.04	8.93	9.03	8.89
12-CO-1β,6α,15α-$(OAc)_3$	8.90	8.93	8.89	8.94	8.95	8.85
3β,12α-$(OAc)_2$	9.18	9.22	9.17	9.22	9.17	9.20
3β,12α-$(OH)_2$-7-CO	8.93	9.26	8.91	9.26	8.92	9.24
3β,12α-$(OAc)_2$-7-CO	8.88	9.21	8.90	9.22	8.92	9.20
3β,12α-$(OH)_2$-Δ^5-7-CO	8.81	9.24	8.77	9.22	8.77	9.26

12β-OH-3-O.$(CH_2)_2$.O	9.15	9.27	9.17	9.29	9.18	9.26
12β-OH-7-CO	8.94	9.30	8.93	9.29	8.93	9.25
12β-OH-Δ^5-7-CO	8.79	9.26	8.79	9.26	8.81	9.27
12β-OH-$\Delta^{3,5}$-7-CO	8.84	9.22	8.84	9.23	8.84	9.22
12β-OH-3,15-$(CO)_2$	8.96	9.16	8.95	9.15	8.96	9.14
12β-OH-2,6,16-$(CO)_3$	9.22	9.08	9.22	9.09	9.25	9.06
2α,12β-$(OH)_2$	9.19	9.29	9.18	9.28	9.19	9.26
3β,12β-$(OH)_2$	9.17	9.28	9.17	9.29	9.18	9.25
6α,12β-$(OH)_2$	9.18	9.28	9.18	9.28	9.18	9.26
7β,12β-$(OH)_2$	9.18	9.27	9.17	9.26	9.18	9.23
7β,12β-$(OAc)_2$	9.16	9.21	9.15	9.20	9.16	9.28
12β,15α-$(OH)_2$	9.19	9.25	9.18	9.23	9.20	9.23
2α,12β-$(OH)_2$-15-CO	9.18	9.18	9.17	9.18	9.18	9.18
2α,12β-$(OAc)_2$-15-CO	9.14	9.14	9.12	9.13	9.11	9.13
3β,12β-$(OH)_2$-7-CO	8.90	9.29	8.90	9.29	8.90	9.24
3β,12β-$(OH)_2$-Δ^5-7-CO	8.78	9.26	8.76	9.26	8.78	9.26
3β,12β-$(OAc)_2$-Δ^5-7-CO	8.74	9.19	8.75	9.18	8.80	9.27
6α,12β-$(OH)_2$-2-CO	9.21	9.28	9.20	9.28	9.21	9.25
6α,12β-$(OH)_2$-3,3-O.$(CH)_2$.O	9.16	9.29	9.15	9.28	9.15	9.26
6α,12β-$(OH)_2$-16-CO	9.15	9.11	9.14	9.10	9.14	9.09
12β,15α-$(OH)_2$-3-CO	8.96	9.23	8.95	9.21	8.96	9.19
12β,15α-$(OH)_2$-3-O.$(CH_2)_2$.O	9.16	9.25	9.16	9.25	9.16	9.23
12β,15α-$(OH)_2$-4-CO	9.24	9.26	9.22	9.24	9.21	9.21
12β,15α-$(OH)_2$-$\Delta^{3,5}$-7-CO	8.84	9.17	8.83	9.19	8.83	9.19
6α,12β-$(OH)_2$-2,16-$(CO)_2$	9.18	9.11	9.16	9.10	9.17	9.08
3β,12β,15α-$(OH)_3$	9.16	9.22	9.16	9.25	9.17	9.25
3β,12β,15α-$(OAc)_3$	9.16	9.16	9.14	9.15	9.15	9.14
3β,12β,15α-$(OAc)_3$-Δ^5	8.92	9.14	8.92	9.11	8.94	9.19
6α,12β,15α-$(OH)_3$	9.17	9.23	9.16	9.22	9.17	9.23

[B] The evaluation of shift values arising from group interactions or conformational distortions in 5α,14α-androstanes

The evaluation of the C-methyl proton resonance shifts caused by interacting functional units or by conformational distortions has been complicated by a limited number of suitable examples, many of which contain other functional groups which could conceivably take part in the interactions observed.

Using naked contributions where possible, an attempt has been made to take account of specific interactions which have been shown to exist. In some systems values calculated from naked additive contributions were in surprisingly close agreement with observed C-methyl proton shifts, and these are also discussed.

Contributory shifts evaluated must be considered tentative, but are included in the final table of substituent effects (Table 46) if the interactions involved result in variations of greater than ±0.04 τ from the naked results.

18- and 19-Oxygenated Compounds

The limited data available precludes the evaluation of any consistent trends. 18-Hydroxylated compounds show variations which could be attributed to a rotational freedom of the hydroxyl group in question; the effect of an 18-acetyl group appears to be more consistent. The two 19-hydroxylated compounds are complicated by the presence of a 1-oxo group. Table 30 lists data, and the apparent contribution of the tertiary hydroxyl group, for the compounds studied.

TABLE 30: 18- and 19-proton resonance values of 5α,14α-androstanes hydroxylated at angular C-methyl positions

Compound	τ_2 19-H	τ_2 18-H	$\Delta\tau_2$ contribution of 18-OR group*
18-OH-2,7-$(CO)_2$	8.92	6.44 $\underline{4}$ (12,2) 6.72 $\overline{a}$ (12)	-0.05
18-OH-3,6-$(CO)_2$	9.03	6.38 $\underline{4}$ (12,2) 6.72 $\overline{d}$ (12)	+0.01
18-OH-3,7-$(CO)_2$	8.73	6.36 $\underline{4}$ (12,3) 6.70 $\overline{\underline{4}}$ (12,3)	+0.02
3β,18-$(OH)_2$-7-CO	8.92	6.42 d (10) 6.77 d (10)	0.00
16β,18-$(OH)_2$-2,7-$(CO)_2$	8.96	insoluble	+0.01
3β,16β,18-$(OH)_3$-7-CO	8.90	6.35 m (25) (3α-H overlap)	0.00
3β,16β,18-$(OH)_3$-11-CO	8.98	6.37 d (12) 6.58 d (12)	+0.01
18-OAc-3,7-$(CO)_2$	8.69	5.87 $\underline{4}$ (12,2) 6.27 $\overline{d}$ (12)	-0.02
** 3β,18-$(OAc)_2$-7-CO	8.78	?	-0.12
16β,18-$(OAc)_2$-2,7-$(CO)_2$	8.95	5.84 $\underline{4}$ (12,2) 6.02 $\overline{d}$ (12)	-0.01
16β,18-$(OAc)_2$-3,7-$(CO)_2$	8.67	5.60 $\underline{4}$ (12,2) 5.96 $\overline{d}$ (12)	-0.03
3β,16β,18-$(OAc)_3$-7-CO	8.87	5.67 $\underline{4}$ (12,2) 6.01 $\overline{d}$ (12)	-0.02
6α,19-$(OH)_2$-1,17-$(CO)_2$		9.18	
6α,19-$(OAc)_2$-1,17-$(CO)_2$		9.12	

* Based on naked contributions for other groups

** The large variation observed here must throw doubt on the structural assignment of the compound.

Interactions between 7- and D-ring-substituents

It has been noted that the flexibility of the D-ring could be expected to cause a variation of contributory $\Delta\tau_2$ values for groups within the ring. Table 31 compares the C-methyl proton resonances of a number of 7,17- and 7,16-oxygenated androstanes with the values calculated from naked values. The number of examples are limited, but interactions are evident in 7α-acetoxy-16-oxo-, 7α-acetoxy-17-oxo-, and 7β-acetoxy-17-oxo-5α-androstanes.

Revised values are presented in Table 32 (7,15-dioxygenated androstanes will be considered separately).

TABLE 31: Chemical shifts (τ_2; $CDCl_3$) of 19- and 18-protons of 5α,14α-androstanes oxygenated at 7- and D-ring positions

Compound	τ_2 (observed)		τ_2 (calc.; naked)	
	19-H	18-H	19-H	18-H
7α-OAc-17-CO	9.17	9.10	9.17	9.13
7β-OH-17-CO	9.16	9.10	9.16	9.11
7β-OAc-17-CO	9.14	9.16	9.14	9.11
17β-OH-7-CO	8.94	9.26	8.94	9.27
17β-OAc-7-CO	8.93	9.21	8.94	9.22
7α-OH-2,16-$(CO)_2$	9.21	9.12	9.19	9.12
17β-OAc-$\Delta^{3,5}$-7-CO	8.85	9.14	8.83	9.17
2α,7α-$(OH)_2$-16-CO	9.17	9.13	9.15	9.11
3α,7β-$(OH)_2$-17-CO	9.17	9.12	9.15	9.10
3α,17β-$(OH)_2$-7-CO	8.94	9.26	8.93	9.26
3β,7α-$(OH)_2$-16-CO	9.14	9.11	9.14	9.12
3β,7α-$(OAc)_2$-16-CO	9.11	9.08	9.11	9.14
3β,7β-$(OH)_2$-17-CO	9.14	9.11	9.13	9.11
3β,7β-$(OAc)_2$-17-CO	9.09	9.17	9.09	9.11
3β,17β-$(OH)_2$-7-CO	8.89	9.25	8.91	9.27

TABLE 32: Revised 7-acetoxy C-methyl contributions allowing for presence of 16- and 17-oxo groups

Substituent	$\Delta\tau_2$ ($CDCl_3$) 19-H	18-H	
7α-OAc	-0.02	+0.01	(naked values)
" [16-CO] or [17-CO]	-0.02	-0.04	
7β-OAc	-0.05	-0.03	(naked values')
" [17-CO]	-0.05	+0.02	

Tertiary-hydroxyl substituents

Table 33 summarises the 19- and 18-proton resonances for the tertiary-hydroxylated 5α-androstanes available. Table 34 lists the contributory effects derived from the above data, and includes some results from literature sources.

TABLE 33: Chemical shifts ($CDCl_3$) of 19- and 18-protons of 5α-androstanes containing tertiary-hydroxyl substituents

Compound	τ_2 (observed) 19-H	18-H	Compound	τ_2 (observed) 19-H	18-H
5-OH-3-CO	8.82	9.26	5,17β-$(OH)_2$-11-CO	8.32	9.31
5-OH-3,11,16-$(CO)_3$	8.61	9.14	3β,5,6β-$(OH)_3$	9.26	9.27
3β,5-$(OH)_2$	8.99	9.29	3β,6β-$(OAc)_2$-5-OH	8.84	9.28
3β,5-$(OH)_2$-17-CO	8.98	9.13	3β,5,16β-$(OH)_3$-11-CO	8.81	9.09
5,17β-$(OH)_2$-3-CO	8.81	9.22	3β,16β-$(OAc)_2$-5-OH-11-CO	8.80	9.15
9α-OH-3-CO	8.86	9.25	9α,17β-$(OH)_2$-3-CO	8.86	9.22
9α-OH-3,7-$(CO)_2$	8.66	9.26	9α-OH-17β-OAc-3-CO	8.86	9.18
9α-OH-3,15-$(CO)_2$	8.86	9.15	3β,9α-$(OH)_2$-Δ^5-7,17-$(CO)_2$	8.62	9.11
9α-OH-3,17-$(CO)_2$	8.82	9.12	6α,9α-$(OH)_2$-11,17-$(CO)_2$	8.91	9.17
9α-OH-$\Delta^{3,5}$-7,17-$(CO)_2$	8.72	9.03	7α,9α-$(OH)_2$-3,17-$(CO)_2$	8.86	9.11
9α-OH-3,11,16-$(CO)_3$	8.69	9.12	9α,16α-$(OH)_2$-3,11-$(CO)_2$	8.75	9.28
3β,9α-$(OH)_2$-7-CO	8.80	9.30	9α,16β-$(OH)_2$-3,11-$(CO)_2$	8.72	9.04
3β,9α-$(OH)_2$-Δ^5-7-CO	8.62	9.11	3β,9α,17β-$(OH)_3$	9.05	9.24
3β-OAc-9α-OH-7-CO	8.78	9.30	9α-OH-3β,17β-$(OH)_2$	9.04	9.21
3β,9α-$(OH)_2$-17-CO	9.04	9.13	3β-OAc-7α,9α-$(OH)_2$-17-CO	9.05	9.15
3β-OAc-9α-OH-17-CO	9.02	9.14	9α-OH-1α,3α,17β-$(OAc)_3$	9.00	9.17
9α,15β-$(OH)_2$-3-CO	8.83	8.95			
*14-OH-3,7-$(CO)_2$	8.72	9.12	3β,14-$(OH)_2$-Δ^5-7,17-$(CO)_2$	8.76	9.01
14-OH-3,17-$(CO)_2$	8.95	8.99	3β,7α-$(OAc)_2$-14-OH-17-CO	9.16	8.97
14-OH-Δ^4-3,17-$(CO)_2$	8.78	8.96	3β-OAc-9α,14-$(OH)_2$-17-CO	9.03	9.01
*3β,14-$(OH)_2$-7-CO	8.92	9.20	3β,12β,14-$(OH)_3$-17-CO	9.13	8.98
3β,14-$(OH)_2$-Δ^5-7-CO	8.78	9.16	3β,12β-$(OAc)_2$-14-OH-17-CO	9.07	8.97
3β,14-$(OH)_2$-17-CO	9.16	9.01	17β-OAc-9α,14-$(OH)_2$-3-CO	8.87	9.07

* These values appear inconsistent; it is surprising that an A-ring conformational change should so markedly alter 18-proton resonance.

TABLE 34: Tentative effects ($\Delta\tau_2$) of tertiary-hydroxyl substituents on 19- and 18-proton signals of 5α-androstanes

	$\Delta\tau_2$		$\Delta\tau_2$ (lit.)		
	19-H	18-H	19-H	18-H	(ref.)
5α-OH			-0.18	-0.01	(82)
[3β-OH]	-0.19	-0.01			
[3-CO]	-0.16	-0.01	-0.17	0.00	(142d)
[6-CO]			-0.06	-0.01	(")
[11-CO]	-0.17	+0.02			
[3β-OR-11-CO]	-0.14	+0.01			
[3,11-$(CO)_2$]	-0.11	+0.03			
3β,6β,5α-$(OH)_3$	+0.04	-0.04			
3β,6β-$(OAc)_2$-5α-OH	-0.38	-0.03			
5α-OAc			-0.20	0.00	(142d)
9α-OH*	-0.12	-0.02	-0.14	0.00	(82)
[Δ^4]			-0.15	-0.01	(160)
[7α-OH]	-0.08	0.00			
[7-CO]	no interaction				
[14α-OH]	no interaction				
9α-OH-11-CO	-0.24	+0.02			
14α-OH			-0.00	-0.12	(142d)
			-0.02	-0.17	(113)
[7-CO]	values inconsistent (3 examples)				
[17-CO]	0.00	-0.12	+0.01	-0.11	(113)
12β,14α-$(OH)_2$-17-CO	-0.06	-0.33			
12β-OAc-14α-OH-17-CO	-0.10	-0.34			

* Interaction between 6- and 9-oxygenated groups is probable, but cannot be confirmed from the data available.

α-Disubstituted units

In the work of this group, few α-disubstituted 5α-androstanes have been characterised (Tables 35 and 36). The results of Nambara et al.,[149] derived from extensive D-ring conformational studies, are a valuable supplement to these data, and are included in the final table of substituent effects. Insufficient data are available for a generalisation of the coupling associated with protons geminal to the hydroxyl groups; this however is usually self evident and has been documented in recent publications.[149,164]

The n.m.r. spectra of epoxides and epithio groups - their C-methyl contributions and low field proton characteristics - have been published[142a] and require no special comment. Several α,β-oxido-ketones are described in other parts of this Thesis (Table 20) and their naked substituent effects are included in Table 46.

TABLE 35: Chemical shifts (τ_2; $CDCl_3$) of 19- and 18-protons of 2,3-, 11,12- and 16,17-dioxygenated 5α-androstanes

Substituents	τ_2		Substituents	τ_2	
	19-H	18-H		19-H	18-H
2α-OAc-3-CO	8.87	9.28	16α-OAc-17-CO	9.19	9.04
17β-OAc-3β-OH-2-CO	9.22	9.26	16β-OAc-17-CO	9.18	9.04
3β,17β-$(OAc)_2$-2-CO	9.22	9.22	17β-OAc-16-CO	9.18	9.18
			16α,17α-$(OH)_2$	9.22	9.31
6α,11α,12β-$(OAc)_3$-3,3-O.$(CH_2)_2$.O*	9.06	9.17	16α,17β-$(OH)_2$	9.21	9.25
11α,12β,15α-$(OH)_3$-3-CO	8.86	9.20	16α-OAc-17β-OH	9.19	9.21
11α,12β,15α-$(OH)_3$-3,3-O.$(CH_2)_2$.O	9.05	9.23	16α,17β-$(OAc)_2$	9.20	9.22
			16β-OAc-17α-OH	9.18	9.22
16α-OH-17-CO	9.19	9.05	16β-OAc-17β-OH	9.19	9.19

* This value is not used to derive the 11α,12β-$(OAc)_2$-contribution because a 3,3-ethylenedioxy-6α-acetate would be expected to interact (see Table 40).

TABLE 36: The effect ($\Delta\tau_2$; $CDCl_3$) of α-disubstituted units on 19- and 18-proton resonance of 5α,14α-androstanes

Substituent	$\Delta\tau_2$ 19-H	18-H
2-CO-3β-OH	+0.01	+0.04
2-CO-3β-OAc	+0.01	0.00 *
11α,12β-$(OH)_2$ [3-CO]	-0.11	-0.03
[3-O.$(CH_2)_2$.O]	-0.13	-0.04
16-CO-17β-OAc	-0.04	-0.13 *
17-CO-16α-OH	-0.03	-0.26 *
17-CO-16α-OAc	-0.03	-0.27 *
17-CO-16β-OAc	-0.04	-0.27 *
16α,17α-$(OH)_2$	0.00	0.00 *
16α,17β-$(OH)_2$	-0.01	-0.06
16α-OAc-17β-OH	-0.03	-0.10
16α,17β-$(OAc)_2$	-0.02	-0.09
16β-OAc-17α-OH	-0.04	-0.09
16β-OAc-17β-OH	-0.03	-0.12

* Naked values; duplicated from Table 27

β-Disubstituted units

With the data available, 1,3-, 12,17-, and 15,17-dioxygenated systems can be considered. The three groups differ in terms of ring size, and relative patterns of substituents; in some cases significant interactions are noted and in others none are apparent. Table 37 lists the compounds from which the relevant

substituent effects [Table 38] are derived. Most of the compounds are heavily substituted, and Table 39 illustrates two anomalies from the data derived.

TABLE 37: Chemical shifts (τ_2; $CDCl_3$) of 19- and 18-protons of 1,3-, 12,17- and 15,17-dioxygenated 5α,14α-androstanes

Substituents	τ_2 19-H	τ_2 18-H	Substituents	τ_2 19-H	τ_2 18-H
1,3,17-$(CO)_3$	8.73	9.10	7β,12β-$(OH)_2$-17-CO	9.17	9.04
3α-OMe-1,6-$(CO)_2$	8.88	9.29	12α,17β-$(OH)_2$-6-CO	9.28	9.27
3α-OMe-1,6,17-$(CO)_3$	8.86	9.13	7β,12β-$(OH)_2$-3,17-$(CO)_2$	8.99	9.02
1α,3α-$(OAc)_2$	9.16	9.31[89]	1β,7β,12β-$(OH)_3$-17-CO	9.04	9.11
1α,3β-$(OAc)_2$	9.10	9.31[89]	3β,12β,14-$(OH)_3$-17-CO	9.13	8.98
1α,3β-$(OH)_2$-17-CO	9.18	9.15	3β,12β-$(OAc)_2$-14-OH-17-CO	9.07	8.97
1α-OH-3β-OAc-17-CO	9.16	9.14			
1α,3β-$(OAc)_2$-17-CO	9.08	9.15	3β,12β,15α-$(OH)_3$-17-CO	9.14	9.03
1α,17β-$(OH)_2$-3-CO	8.99	9.23	7β,12β,15α-$(OH)_3$-17-CO	9.15	9.02
1α,17β-$(OAc)_2$-3-CO	8.93	9.20	3β-OMe-12β,15α-$(OH)_2$-17-CO	9.16	9.04
1β,3β-$(OH)_2$-7-CO	8.87	9.31			
1β,3β-$(OH)_2$-17-CO	9.14	9.15	Δ^4-3,15,17-$(CO)_3$	8.77	8.92
1β,6α-$(OH)_2$-3α-OMe	9.16	9.30	15α-OH-17-CO	9.17	9.11
1β,6α-$(OH)_2$-3α-OMe-17-CO	9.14	9.14	15β-OH-17-CO	9.14	8.82
			15β-OMe-17-CO	9.15	8.90
1α,3β,6α-$(OAc)_3$-17-CO	8.97	9.14	15α-OH-Δ^4-3,17-$(CO)_2$	8.77	9.05
1α,3α,7α-$(OAc)_3$-17-CO	9.08	9.19	3β-OMe-15α-OH-17-CO	9.16	9.10
			15α,17β-$(OH)_2$-Δ^4-3-CO	8.78	9.13
12,17-$(CO)_3$	9.11	8.79	15α,17β-$(OH)_2$-estra-Δ^4-3-CO		9.08
12β-OH-17-CO	9.19	9.06			
12β-OAc-17-CO	9.17	9.01	15β,17β-$(OH)_2$	9.17	8.99
12β,17β-$(OH)_2$	9.18	9.18	15β-OMe-17β-OH	9.18	9.07
3β,12β-$(OH)_2$-17-CO	9.14	9.02	1β,3α,15α-$(OH)_3$-17-CO	9.18	9.05

TABLE 38: The effect of 1,3-, 12,17-, and 15,17-dioxygenated units on 19- and 18-proton resonance of 5α,14α-androstanes

[* naked value]

Substituent	$\Delta\tau_2$ (or comments) 19-H	18-H	
1,3-$(CO)_2$	-0.46	-0.04	
1α,3α-$(OR)_2$	slight interaction		
1α,3α-$(OAc)_2$ [9α-OH]	no interaction; see Table 34		
1α,3β-$(OR)_2$	If 1α-OH, slight interaction		
1β,3α-$(OH)_2$	not defined; see Table 39		
1β,3α-$(OAc)_2$	slight interactions		
1α-OH-3-CO	-0.22	-0.04	
1α-OAc-3-CO	slight interactions		
1β-OH-3-CO	-0.10	+0.01	; 1 example
1β-OAc-3-CO	-0.33	-0.01	; 1 "
12,17-$(CO)_2$	-0.11	-0.52*	; 1 example
12α,17β-$(OH)_2$	+0.03	+0.01	; 1 "
12β,17β-$(OH)_2$	-0.04	-0.13*	; 1 "
12β-OH-17-CO	-0.03	-0.25*	
12β-OAc-17-CO			; no examples
12β,14-$(OH)_2$-17-CO	-0.06	-0.33	; 1 example
12β-OAc-14-OH-17-CO	-0.10	-0.34	; 1 "
12β,15α-$(OH)_2$-17-CO	-0.04	-0.26	
15,17-$(CO)_2$	no interaction		
15α-OH-17-CO	"		
15α-OH-17β-OH	"		
15β-OH-17-CO	"		
15β,17β-$(OH)_2$	-0.05	-0.32*	; 1 example

TABLE 39: Two examples illustrate a breakdown of correlations from Table 38, and the unexpected distortions occurring in some poly-substituted systems

Compound	τ_2 (found)		τ_2 (calc.)	
	19-H	18-H	19-H	18-H
17-oxo-5α-androstane-1β,3α,15α-triol	9.18	9.05	9.12	9.09
1α,3β,7α-triacetoxy-5α-androstan-17-one	9.08	9.19	9.06	9.15

γ-Disubstituted units

From the limited data available (Table 40) it appears that only those groups involving direct dipole-dipole interactions (i.e. 1,11- and 7,15-dioxygenated compounds) cause conformational changes which are significantly reflected in their n.m.r. C-methyl proton resonance.

A number of the 1,11-dioxygenated compounds were polysubstituted pregnane derivatives.[9d] A comparison of the observed C-methyl resonance values with those calculated (using increments derived from 1,11-dioxygenated androstanes; Table 41) showed irregularities which were particularly evident with Δ^5-11-oxygenated pregnanes (Table 42). On the other hand, values calculated for 1β,11α-isopropylidene-pregnane derivatives were in close agreement.

TABLE 40: Chemical shifts (τ_2; $CDCl_3$) of 19- and 18-protons of 1,11-, 3,6-, and 7,15-dioxygenated androstanes

Substituents	τ_2 19-H	τ_2 18-H
1,6,11-$(CO)_3$	8.68	9.33
1,6,11,17-$(CO)_4$	8.66	9.10
6α-OH-1,11-$(CO)_2$	8.64	9.35
1α,6α-$(OH)_2$-11-CO	8.98	9.33
1α,6α-$(OH)_2$-11,17-$(CO)_2$	8.91	9.17
3β-OH-1α,11α-O.CMe_2.O-17-CO	9.10	9.15
1β,11α-.O.-3,3,17,17-[O.$(CH_2)_2$.O]$_2$	9.13	9.24
3,6-$(CO)_2$	9.03	9.26
Δ^4-3,6-$(CO)_2$	8.83	9.22
3,6,11-$(CO)_3$	8.83	9.28
Δ^4-3,6,11-$(CO)_3$	8.61	9.23
3,6,15-$(CO)_3$.	9.03	9.16
6β-OH-Δ^4-3-CO	8.62	9.20
15α-OH-3,6-$(CO)_2$	9.02	9.22
16β-OH-3,6-$(CO)_2$	9.01	9.03
6β,11α-$(OH)_2$-Δ^4-3-CO	8.48	9.17
6β,16β-$(OH)_2$-Δ^4-3-CO	8.59	8.95
6β,17β-$(OH)_2$-Δ^4-3-CO	8.62	9.19
6β-OH-17β-OAc-Δ^4-3-CO	8.60	9.13
3β,16α-$(OH)_2$-Δ^4-6-CO	8.99	9.25
3β,16β-$(OH)_2$-Δ^4-6-CO	8.97	9.00
3β,6β,15α-$(OAc)_3$	8.98	9.18
7α,15α-$(OAc)_2$-3-CO	8.97	9.23
*12β,15α-$(OH)_2$-7-CO	8.81	9.29
*12β,15α-$(OAc)_2$-7-CO	8.94	9.13
*12β,15α-$(OH)_2$-$\Delta^{3,5}$-7-CO	8.84	9.17
7β,12β,15α-$(OH)_3$	9.18	9.23
12β-OH-7β,15α-O.CMe_2.O	9.18	9.22
3β,12β,15α-$(OAc)_3$-7-CO	8.80	9.12
3β-O-allyl-7β,12β,15α-$(OAc)_3$	9.13	9.13
3β-O-propyl-7β,12β,15α-$(OH)_3$	9.10	9.22

* structures questionable[165]

TABLE 41: The effect of 1,11-, 3,6-, and 15,17-dioxygenated units on 19- and 18-proton signals of 5α-androstanes [* naked values]

Substituent	$\Delta\tau_2$ 19-H	18-H	
1β-OH-11-CO	no interaction		
1α-OH-11-CO	"	"	
1,11-$(CO)_2$	-0.56	+0.04	
1,11-$(CO)_2$ [D-ring CO]	-0.56	-0.02	one example
1β,11α-O-CMe_2-O-	-0.07	+0.01	
1β,11α-oxido	-0.06	-0.03	
3,6-$(CO)_2$	no interaction		
Δ^4-3,6-$(CO)_2$	-0.39	-0.09*	
Δ^4-3-CO-6β-OH	-0.60	-0.11*	
Δ^4-6-CO-3β-OH	-0.23	-0.06	
6-CO-3β-OH			
3-CO-6α-OH	no interactions		
3-CO-6β-OH			
3-ketal-6β-OH	-0.26	-0.04	
3,6-$(OH)_2$	no examples		
3β,6α-$(OAc)_2$	no interaction		
7β,15α-O-CMe_2-O-	-0.02	-0.07	
7,15-$(CO)_2$	-0.27	-0.06	
7α,15α-$(OH)_2$			
7β,12β,15α-$(OH)_3$			
7β,12β,15α-$(OAc)_3$			
7-CO-15α-OH	no interactions		
7-CO-15α-OAc			
Δ^5-7-CO-15α-OAc			
$\Delta^{3,5}$-7-CO-15α-OH			

TABLE 42: Chemical shifts (τ_2) at 19- and 18-protons of 1,11-dioxygenated pregnanes. Calculated values are derived from Tables 41 and 46

Compound	τ_2 (found)		τ_2 (calc.)	
	19-H	18-H	19-H	18-H
1β,3β-$(OH)_2$-Δ^5-11,20-$(CO)_2$	8.79	9.36	8.71	9.39
1β,3β,11α-$(OH)_3$-20-CO	9.09	9.39	9.02	9.39
1β-OAc-3β,11α-$(OH)_2$-20-CO	9.07	9.39	8.90	9.39
1β-OH-3β,11α-$(OAc)_2$-20-CO	9.09	9.38	9.12	9.34
1β,3β-$(OAc)_2$,11α-(OH)-20-CO	8.86	9.41	8.88	9.39
1β,3β,11α-$(OAc)_3$-Δ^5-20-CO	8.81	9.33	8.69	9.33
1β,11α-O-CMe_2-O-3β-OH-20-CO	9.11	9.38	9.13	9.41
" -3β-OH-16-Me-20-CO	9.14	9.04	9.13	9.07
" -3β-OH-Δ^5-20-CO	8.89	9.37	8.91	9.38
" -3β-OH-Δ^{16}-20-CO	9.08	9.11	9.09	9.13
" -3,20-$(CO)_2$	8.95	9.37	8.92	9.37

[C] 5β,14α-Androstanes

Models indicate that the out-of-plane A-ring of the 5β-androstane skeleton allows considerably more dipole-dipole interaction than its 5α-counterpart. Recent c.d. measurements[163] have indicated vicinal interactions in 5β-4,7-dioxo structures, and ring strain considerations have been used to explain differing mass spectral fragmentation patterns of 5α- and 5β-1,6-dioxo compounds.[166]

Tables 43 and 44 list data for the few 5β-androstanes prepared by this group;

most of the compounds are mono-oxygenated and provide naked $\Delta\tau_2$ values. However, it is not surprising that these are unsatisfactory for use as additive increments. Tentative average increments which adequately fit the observed data at hand have been derived, and are supplemented in the final summary (Table 46) by data from literature sources. The examples of 5β-androstan-17-one and 12β,15α-dihydroxy-5β-androstan-17-one support the validity of the assumption that for non-interacting substituents far removed from the centre of conformational variation, additive increments derived from the natural isomer lie within the limits of error.

TABLE 43: Chemical shifts (τ_2; $CDCl_3$) and the effect of substituents [$\Delta\tau_2$ (naked) and $\Delta\tau_2$ (average)] of mono-oxygenated 5β,14α-androstanes

Compound	τ_2		$\Delta\tau_2$ (naked)		$\Delta\tau_2$ (average)	
	19-H	18-H	19-H	18-H	19-H	18-H
5β-androstane	9.08	9.31				
5β-androstan-1-one	8.86	9.32	-0.22	+0.01	-0.20	+0.02
5β-androstan-3-one	8.96	9.27	-0.12	-0.04	—	
5β-androstan-4-one	8.89	9.32	-0.19	+0.01	—	
5β-androstan-6-one	9.16	9.29	+0.08	-0.02	-0.10	-0.02
5β-androstan-11-one	8.83	9.33	-0.25	-0.02	-0.22	+0.02
5β-androstan-17-one	9.05	9.15	-0.03	-0.16	—	
5β-pregnan-20-one	9.08	9.40				
5β-androstan-1β-ol	8.94	9.31	-0.14	0.00	—	
5β-androstan-3α-ol	9.07	9.31	-0.01	0.00	—	
5β-androstan-3β-ol	9.02	9.30	-0.06	-0.01	—	
5β-androstan-5-ol	9.10	9.31	+0.02	0.00	—	
5β-androst-3-en-5-ol	9.02	9.30	-0.06	-0.01	—	
5β-androstan-6α-ol	9.08	9.31	0.00	0.00	—	
5β-androstan-6β-ol	8.84	9.28	-0.24	-0.03	-0.20	-0.03
5β-androstan-6β-yl acetate	8.99	9.26	-0.09	-0.05	—	
5β-androstan-11α-ol	8.94	9.29	-0.14	-0.02	—	
5β-estran-11β-ol		9.08		-0.23	—	

TABLE 44: Chemical shifts (τ_2; $CDCl_3$) and calculated values (from Table 43) of the 19- and 18-protons of di- and tri-oxygenated 5β,14α-androstanes

Compound	τ_2		τ_2 (calc.)	
	19-H	18-H	19-H	18-H
5β-androstane-3,11-dione	8.75	9.30	8.74	9.29
5β-androstane-1,6,16-trione	8.94	9.11	8.94	9.14
6β-hydroxy-5β-androstan-3-one	8.77	9.24	8.76	9.24
1β-hydroxy-5β-androstane-6,17-dione	9.01	9.12	9.01	9.12
6-oxo-5β-androstane-5,11α,17β-triol	9.07	9.26		
5β-androstane-3α,6β-diol	8.88	9.27	8.87	9.28
5β-androstane-3β,6β-diol	8.84	9.27	8.82	9.27
*12β,15α-dihydroxy-5β-androstan-17-one	9.04	9.05	9.04	9.05

* derived from incremental values for 5α,14α-androstanes (Table 46). See text.

[D] 5α,13α-Androstanes

The only 5α,13α-androstanes prepared in this department are contained in this Thesis, and n.m.r. data appear in Section IV. These data have allowed the determination of naked $\Delta\tau_2$ increments for a number of D-ring substituents which agree with the results of Nambara et al.[32,149b] based on 3-oxygenated analogues. These are included in Table 46.

[E] 5α,14β-Androstanes

The n.m.r. data listed in Table 45 supplement those of the 5α,14β-androstanes contained in this Thesis (Tables 19 and 20). Using the naked C-methyl contributions of 15-oxygenated and 14β,15-dioxygenated substituents it has been possible to derived averaged $\Delta\tau_2$ values for a number of functional groups. These incremental values appear in Table 46; included in the table are additional $\Delta\tau_2$ values derived by Fetizon et al.,[145e] Nambara et al.[149a] and Hol.[113]

The observed and the calculated τ_2 values (Tables 19 and 20) for the 5α,14β-androstanes described in this Thesis are in close agreement, and a comparison of these results supports the validity of the assumptions governing positions far removed from the centre of conformational change.

TABLE 45: Chemical shifts (τ values: 1, CCl_4; 2, $CDCl_3$; 3, C_6H_6) of the 19- and 18-protons of some 5α,14β-androstane ketones. Calculated τ_2 values are derived from averaged substituent effects listed in Table 46.

Substituents	τ_1		τ_2		τ_2 (calc.)		τ_3	
	19-H	18-H	19-H	18-H	19-H	18-H	19-H	18-H
17-CO			9.22	8.93	9.22	8.93		
3β-OMe-12,15-$(CO)_2$	9.18	8.66	9.17	8.63	9.16	8.63	9.50	9.09
3,11,15-$(CO)_3$	8.81	8.81	8.84	8.84	8.81	8.84	9.04	9.30
3,12,15-$(CO)_3$	8.99	8.65	8.96	8.59	8.94	8.59	9.61	9.12
Δ^4-3,12,15-$(CO)_3$	8.80	8.62	8.77	8.55	8.75	8.56	9.47	9.13
5α,14β-estrane-3,12,15-$(CO)_3$	-	8.63	-	8.58	-	8.59	-	9.02

[F] Other Steroidal Types

Clegg[9d] has prepared most of the estranes available for use in the present study. He demonstrated that the rigidity of the skeleton was preserved and that the absence of a 10-methyl group had little influence on proton resonance at the 13-methyl position; the use of incremental values derived for the relevant androstane series was therefore justified.

In the present work, insufficient examples have been studied for a proper assessment of the effect of a pregnane side chain and pregnane derivatives have not been used in the derivation of additive increments. Table 42 lists data of a few 1,11-dioxygenated pregnanes where the observed C-methyl proton resonance has significantly deviated from values calculated (using increments derived in the androstane series). In a computerised regression analysis, however, Cohen and Rock[172] demonstrated non-divergent results for the 17-substituents β-C_8H_{17}; β-$CH(OH)CH_3$; β-$CH(OAc)CH_3$; β-$COCH_2OAc$; β-$COCH_2OH$; β-$COCH_3$ and α-$COCH_3$.

TABLES 46 and 47: The Effect of Substituents ($\Delta\tau_2$; $CDCl_3$) on 19- and 18-Proton Signals, and the observed solvent shift values (Δ_1^3) of Androstane Mono-ketones

The preceding evaluation of the effect of substituents on the 19- and 18-proton signals of 5α-androstanes has taken account of the naked (Table 26) and modified (Table 28) values of oxygenated substituents, and of a number of interacting substituent units (Tables 30-42). The effect of some substituents in androstanes of unnatural configuration has been evaluated (Tables 42-45).

Table 46 summarises for practical application the results of the present work

and, where applicable, those of other workers. When considering the interactions arising from poly-substituted units, only those effects deviating by greater than ±0.04 τ from the combined individual contributions have been included.

Use of the table: (a) The entry for a particular substituent is determined by its position on the steroid skeleton. Thereafter, precedence is defined in the order α-OR > β-OR > CO > ethylene ketal > acetal > acetonide > ethylene thioketal > Δ (endo) > Δ (exo) (i.e. methylene) > epoxide.

(b) Interacting units are considered in the more convenient of the two forms illustrated by example.

(i) As a combined unit, the entry corresponds to the lower numbered position.

	19-H	18-H
1-CO	-0.38	-0.01
-3-CO	-0.46	-0.04

(ii) As a separate unit, the entry appears in its usual position. In the following example, the effect of a 6β-hydroxyl substituent in the presence of a 4-olefin is (-0.19, -0.04), and this must be added to the existing Δ^4-value.

6β-OH	-0.23	-0.04
[-Δ^4]	-0.19	-0.04

(c) When calculating the expected chemical shifts of an unknown androstane of unnatural configuration, it is reasonable to assume that for non-interacting substituents far removed from the centre of conformational variation, additive increments derived from the natural isomer are within the limits of error (±0.30 τ).

(d) A positive $\Delta\tau_2$ value indicates a shift to higher field.

Table 47 summarises the observed solvent shifts ($\Delta_1^3 = \tau_{C_6H_6} - \tau_{CCl_4}$) of a variety of androstane mono-ketones.

TABLE 46: The Effect of Substituents ($\Delta\tau_2$, $CDCl_3$) on 19-H and 18-H Signals

		5α-androstane		5β-androstane		5α,13α-androstane		5α,14β-androstane	
		19-H	18-H	19-H	18-H	19-H	18-H	19-H	18-H
Hydrocarbon (basic value)		9.22	9.31	9.08	9.31	9.31	9.14	9.23	9.01
Substituent									
1α-OH		−0.02	−0.01	−0.21	−0.01[a]				
	-Δ²	+0.06	−0.03						
1α-OAc		−0.06	+0.01	−0.05	0.00[a]				
1β-OH		−0.05	0.00	−0.14	0.00				
	-3-CO*	−0.10	+0.01						
1β-OAc		−0.17	+0.01	0.00	−0.01[a]				
	-3-CO*	−0.33	−0.01						
1-CO		−0.38	−0.01	−0.20	+0.02				
	-3-CO	−0.46	−0.04						
	-Δ²	−0.28	−0.03						
	-Δ²-3-Me	−0.27	−0.01						
1β,11α-O·CMe_2·O		−0.07	+0.01						
Δ¹		−0.08	−0.04	−0.18	+0.02[a]				
1 = CH_2		−0.17	−0.04[c]						
1α,11α-O		−0.10[b]							
1β,11α-O		−0.08	−0.03						
2α-OH		−0.02	−0.01	0.00	0.00[a]				
	-3-CO-Δ⁴	−0.53	−0.08[a]						
2α-OAc		−0.07	0.00	−0.01	0.00[a]				
	-3-CO	−0.35	−0.03						
	-3-CO-Δ⁴	−0.53	−0.06[a]						
2β-OH		−0.26	−0.01[a]	−0.03	+0.01[a]				
	-3-CO-Δ⁴	−0.43	−0.06[a]						
2β-OAc		−0.16	0.00	−0.07	0.00				
	-3-CO-Δ⁴	−0.43	−0.07[a]						
2-CO		+0.02	0.00	−0.15	0.00[a]				
	-3β-OH	+0.01	+0.04						
	-3β-OAc	+0.01	0.00						
	-Δ³·⁵-7-CO	−0.50	−0.06						
2,2-O·$(CH_2)_2$·O		−0.12	−0.01						
Δ²		+0.01	−0.02	−0.05	−0.02[a]				
	-3-OMe	+0.04	−0.01						
	-3-OEt	+0.03	−0.01						
	-3-OAc	−0.03	0.00						
2 = CH_2		+0.10	+0.01[c]						
2α,3α-O		+0.01	0.00[d]						
2β,3β-O		−0.07	+0.01						
3α-OH		−0.01	−0.01	−0.01	0.00				
	-Δ¹	−0.04	−0.03						
	-Δ⁴	−0.21	−0.04						
3α-OAc		−0.02	−0.01	+0.01	+0.02[a]				
	-Δ⁴	−0.23	−0.03[b]						
3α-OMe***		−0.01	+0.01						
3β-OH		−0.03	0.00	−0.06	−0.01				
	-6-CO-Δ⁴	−0.23	−0.06						
	-Δ¹	−0.13	−0.02						
	-Δ²	−0.29	−0.05						
	-Δ⁵	−0.25	−0.03						
3β-OAc		−0.05	0.00	−0.06	−0.01[b]				
	-Δ⁴	−0.29	−0.04[b]						
	-Δ⁵	−0.27	−0.02						
3β-OMe***		−0.02	0.00						
	-Δ⁵	−0.24	−0.03						
3-CO		−0.24	−0.04	−0.12	−0.04				
	-6-CO-Δ⁴	−0.39	−0.09						
	-Δ¹	−0.24	−0.05						
	-Δ¹·⁴	−0.46	−0.10[b]						
	-Δ¹·⁴·⁶	−0.43	−0.15[b]						
	-Δ⁴	−0.42	−0.07						
	-Δ⁴·⁶	−0.35	−0.12						
	-1α,2α-O	−0.06	0.00						
	-4α,5α-O	−0.27	−0.04						
	-4β,5β-O			−0.22	−0.04				

TABLE 46 (cont.)

Substituent		5α-androstane 19-H	5α-androstane 18-H	5β-androstane 19-H	5β-androstane 18-H	5α,13α-androstane 19-H	5α,13α-androstane 18-H	5α,14β-androstane 19-H	5α,14β-androstane 18-H
3,3-O·$(CH_2)_2$·O		−0.03	0.00						
3,3-(OR_2);R=Me,Et		−0.01	+0.01	−0.02	0.00				
3,3-S·$(CH_2)_2$·S		−0.02	+0.01						
Δ^3		+0.04	−0.01						
$\Delta^{3,5}$		−0.20	−0.06[b]						
	-3-OMe	−0.16	−0.03						
3 = CH_2		−0.09	−0.02	−0.01	0.00[c]				
3α,4α-O		+0.04	+0.01						
3β,4β-O				+0.04	−0.01				
4α-OH		−0.02	0.00	−0.01	−0.01[b]				
4α-OAc		−0.07	0.00						
4β-OH		−0.26	0.00						
4β-OAc		−0.23	0.00	−0.08	0.00[a]				
4-CO		+0.03	−0.01	−0.19	+0.01				
Δ^4		−0.21	−0.02						
4 = CH_2		+0.08	0.00[c]						
5-OH		−0.18	−0.01[a]	+0.02	0.00*				
	-3β,6β-$(OH)_2$	+0.04	−0.04						
	-3β,6β-$(OAc)_2$	−0.38	−0.03						
	[-3β-OR-11-CO]	−0.14	0.00						
	[-3-CO]	−0.16	−0.01						
	[-6-CO]	−0.06	−0.01[b]	+0.12	0.00*				
	[-3,11-$(CO)_2$]	−0.11	+0.02						
Δ^5				−0.06	−0.01				
5-OAc		−0.20	0.00[a]						
Δ^5		−0.22	−0.03						
$\Delta^{5,7}$		−0.14	+0.03[b]						
$\Delta^{5(10)}$			−0.03[a]						
5α,6α-O		−0.12	+0.04						
5β,6β-O				−0.03	+0.02				
6α-OH		−0.02	−0.01	0.00	0.00				
	[-Δ^4]	+0.01	−0.01[a]						
	[-Δ^4-3-CO]	+0.02	−0.02						
6α-OAc		−0.06	0.00	−0.04	−0.01[a]				
	[-Δ^4]	−0.04	−0.01[a]						
6β-OH		−0.23	−0.04	−0.20	−0.03				
	[-Δ^4]	−0.19	−0.04[a]						
	[-Δ^4-3-CO]	−0.18	−0.04						
6β-OAc		−0.20	−0.05	−0.09	−0.05				
	[-Δ^4]	−0.09	−0.04						
6-CO		+0.04	−0.02	+0.10	−0.02			+0.04	−0.03
	[-Δ^2-1-CO]	−0.01	−0.03						
	-$\Delta^{3,4}$	−0.19	−0.02[j]						
	-Δ^4	−0.13	+0.01[j]						
	-Δ^7	0.00	+0.08[j]						
Δ^6		+0.04	−0.03[f]						
6 = CH_2		+0.11	+0.01[c]						
6β,19-O			−0.03[b]						
7α-OH		−0.01	−0.01	+0.01	+0.01[b]				
	-Δ^5	−0.23	−0.02[a]						
	-Δ^5-[-3βOH]	−0.19	−0.03						
7α-OAc		−0.02	+0.01	−0.01	0.00[a]				
	[-16-CO or -17-CO]	−0.02	−0.04						
7β-OH		−0.03	−0.03	−0.03	−0.04[a]			−0.03	−0.02
	-Δ^5	−0.26	−0.07						
7β-OAc		−0.05	−0.03						
	[-17-CO]	−0.05	+0.02						
	-Δ^5	−0.28	−0.08						
7-CO		−0.27	0.00					−0.19	0.00
	[-11-CO-8α,9α-O]	−0.41	−0.06[g]						
	-15-CO	−0.27	−0.06						
	-$\Delta^{3,5}$	−0.35	−0.06						
	-$\Delta^{3,5}$[-17β-OAc]	−0.35	−0.09						
	-$\Delta^{3,5,8(9)}$	−0.55	0.00*						
	-Δ^5	−0.41	−0.03						
7,7-O·$(CH_2)_2$·O		−0.03	−0.01						
7β,14β-O·CMe_2·O, [-12β-OH-15-CO]								−0.06	+0.14*
7β,15α-O·CMe_2·O		−0.02	−0.07						

TABLE 46 (cont.)

Substituent		5α-androstane 19-H	5α-androstane 18-H	5β-androstane 19-H	5β-androstane 18-H	5α,13α-androstane 19-H	5α,13α-androstane 18-H	5α,14β-androstane 19-H	5α,14β-androstane 18-H
Δ^7		0.00	+0.11[f]						
$\Delta^{7,9}$		−0.09	+0.15[b]						
7 = CH_2		−0.13	−0.03[c]						
8β-OH		−0.16	−0.28[a]					−0.18	−0.18[b]
$\Delta^{8(9)}$		−0.13	+0.05[f]						
$\Delta^{8(14)}$		+0.12	−0.17[f]						
8α,9α-O		+0.10[b]							
8α,14α-O		−0.07	−0.30[g]						
8β,14β-O								−0.10	−0.03[g]
9α-OH		−0.12	−0.02						
	[-7α-OH]	−0.08	0.00						
	-11-CO	−0.24	+0.02						
$\Delta^{9(11)}$		−0.14	+0.04	−0.12	+0.07[a]				
9α,11α-O		−0.20	−0.09[d]						
	*[-3-CO]	−0.25	−0.02						
9β,11β-O		−0.12	−0.18[b]						
10β-OH	[-Δ^4]		−0.04[b]						
11α-OH		−0.14	−0.01	−0.14	−0.02				
	-12β-OH	−0.11	−0.03						
	[-$\Delta^{1,4}$-3-CO]	−0.18	−0.02[a]						
11α-OAc		−0.10	−0.06	−0.11	−0.04[a]				
11β-OH		−0.24	−0.24	−0.26	−0.24[b]				
	[-$\Delta^{1,4}$-3-CO]	−0.23	−0.21[a]						
11β-OAc		−0.10	−0.13	−0.07	−0.12[b]				
	[-$\Delta^{1,4}$-3-CO]	−0.05[a]							
11-CO		−0.22	+0.03	−0.22	+0.02			−0.20	+0.03
	-1-CO	−0.56	+0.04						
	-1-CO[-17-CO]	−0.56	−0.02						
	-$\Delta^{8(9)}$	−0.28	−0.03[b]						
Δ^{11}		+0.03	−0.08[b]	+0.03	−0.06[f]				
11α,12α-O		−0.07	−0.12						
11β,12β-O		−0.17	−0.09[d]						
12α-OH		−0.01	−0.05					−0.01	−0.02
	*-17β-OH	+0.03	+0.01						
12α-OAc		0.00	−0.09						
12β-OH		−0.02	−0.02					−0.03	+0.02
	-17β-OH	−0.04	−0.13						
	-17-CO	−0.03	−0.25						
12β-OAc		−0.02	−0.08						
12-CO		−0.09	−0.32					−0.09	−0.22
	-17-CO	−0.11	−0.52						
	-$\Delta^{9(11)}$	−0.27	−0.23						
14α-OH		−0.02	−0.17[e]			−0.02	−0.10[e]		
	-12β-OH-17-CO	−0.06	−0.33						
	-12β-OAc-17-CO	−0.10	−0.34						
	[-17-CO]	0.00	−0.12						
14β-OH								−0.02	+0.03[b]
	-15α-OH							+0.02	−0.01
	-15β-OH							−0.03	−0.23
	-15-CO							+0.02	−0.06
Δ^{14}		−0.03	−0.32						
$\Delta^{14,16}$*	[-17-CO]	−0.03	−0.25						
		−0.11	−0.32						
14,15-O*		−0.03	−0.23					−0.03	−0.05
15α-OH		−0.01	−0.04			−0.01	−0.23	+0.03	−0.03
	-Δ^{16}					+0.02	−0.29		
15α-OAc		−0.01	−0.08						
15β-OH		−0.06	−0.31					0.00	−0.04
	-Δ^{16}	−0.09	−0.40						
15β-OAc		−0.06	−0.23						
15β-OMe	-Δ^{16}	−0.07	−0.36*						
15-CO		−0.01	−0.10			−0.05	−0.06	+0.04	−0.16
	-Δ^{16}	−0.07	−0.29					+0.01	−0.22*
	-Δ^7	+0.01	−0.21					+0.01	−0.14[b]*

TABLE 46 (cont.)

Substituent	5α-androstane 19-H	5α-androstane 18-H	5β-androstane 19-H	5β-androstane 18-H	5α,13α-androstane 19-H	5α,13α-androstane 18-H	5α,14β-androstane 19-H	5α,14β-androstane 18-H
15,15-S·$(CH_2)_2$·S *	−0.05	−0.20						
Δ^{15}							+0.04	−0.13
					+0.04	−0.05[l]		
16α-OH	0.00	−0.01						
-17α-OH	0.00	0.00			+0.01	−0.24[l]	+0.01	+0.01[k]
-17β-OH	−0.01	−0.03						
-17-CO	−0.03	−0.26						
16α-OAc	0.00	−0.04			+0.07	−0.13[l]		
-17β-OH	−0.03	−0.10			+0.01	−0.20[l]	0.00	0.00[k]
-17β-OAc	−0.02	−0.09			0.00	−0.14[l]		
-17-CO	−0.03	−0.26						
16α-OEt	0.00	−0.01			+0.08	−0.18[l]	−0.02	−0.15[k]
16β-OH	−0.02	−0.25						
-17-CO					−0.02	+0.03[l]	−0.02	−0.01[k]
16β-OAc	−0.01	−0.19			+0.07	−0.18[l]		
-17α-OH	−0.04	−0.09			−0.04	−0.02[l]	−0.03	−0.03[k]
-17β-OH	−0.03	−0.12			−0.02	−0.05[l]		
-17-CO	−0.04	−0.27						
16β-OMe	0.00	−0.18			+0.06	−0.18[l]	−0.02	−0.15[k]
16β-OEt	0.00	−0.21						
16-CO	−0.04	−0.18						
-17α-OH					−0.01	−0.16[l]	−0.03	−0.19[k]
-17α-OAc					−0.03	+0.07[l]		
-17β-OAc	−0.04	−0.13			+0.05	−0.04[l]	−0.04	−0.19[k]
* -Δ^{14}	−0.13	−0.55			−0.01	−0.26[l]		
16,16-O·$(CH_2)_2$·O	0.00	−0.21						
16,16-$(OMe)_2$	−0.01	−0.09						
Δ^{16}	−0.04	−0.09			+0.05	−0.04[l]	−0.02	−0.10[k]
-17-OAc	−0.03	−0.20			+0.02	−0.14	−0.01	−0.07
-17-COMe	−0.02	−0.18						
-17-COMe-16-Me	−0.03	−0.27						
16 = CH_2	−0.03	−0.05[c]						
16α,17α-O	0.00	−0.03			0.00	−0.26	+0.05	−0.15
16β,17β-O	0.00	−0.12			0.00	+0.02	+0.01	−0.23
17α-OH	−0.02	+0.03			−0.03	0.00[l]	−0.01	−0.02[k]
17α-OAc	−0.01	−0.04			−0.04	−0.07[l]	−0.01	0.00[k]
17β-OH	−0.01	−0.04			−0.03	+0.02[l]	−0.01	−0.04[k]
* -Δ^{14}	−0.05	−0.40						
* -Δ^{15}	−0.03	−0.12						
17β-OAc	−0.01	−0.09			−0.03	−0.02[l]	−0.02	+0.04[k]
17β-OEt	−0.01	−0.06						
17-CO	−0.03	−0.17	−0.03	−0.16	+0.06	−0.11	−0.01	−0.08
* -Δ^{14}	−0.06	−0.42						
* -Δ^{15}	−0.08	−0.38			+0.02	−0.22	+0.01	−0.10
* -15α,16α-O					+0.05	−0.23		
* -15β,16β-O	−0.06	−0.46						
17,17-O·$(CH_2)_2$·O	0.00	−0.14						
* -Δ^{14}	−0.03	−0.20						
* -Δ^{15}	−0.03	−0.23			−0.01	−0.08[l,*]		
17,17-S·$(CH_2)_2$·S							0.00	−0.13[k]
17 = CH_2	−0.02	−0.08[c]	−0.02	−0.08[c]				
17α-$COCH_3$	−0.01	−0.20					+0.04	−0.25[a]
17β-$COCH_3$	+0.01	+0.08	0.00	+0.09			−0.03	+0.03[a]
18-OH**	+0.01							
18-OAc	−0.02							
19-OH		−0.01[a]						

* One example only

** Subject to variation

*** Data for less common ethers appear in ref. 88

(a) Ref. 142d;(b) Ref. 82;(c) Ref. 145b;(d) Ref. 142a;(e) Ref. 113;(f) Ref. 167;(g) Ref. 168;(h) Ref. 169

(i) Ref. 170;(j) Ref. 171;(k) Ref. 149a;(l) Ref. 149b.

TABLE 47: Observed solvent shift values (Δ_1^3) for androstane mono-ketones.

Substituent		5α,14α		5β,14α		5α,13α		5α,14β	
		19-H	18-H	19-H	18-H	19-H	18-H	19-H	18-H
1-CO		+0.20	+0.02	−0.14	+0.10				
1-CO	$-\Delta^2$	+0.11	+0.03						
2-CO		+0.17	+0.09						
3-CO		+0.34	+0.05	+0.20	+0.08				
	$-\Delta^1$	+0.37	+0.11						
	$-\Delta^{3,4}$	+0.34	+0.22						
		+0.41	+0.11						
4-CO		+0.11	+0.09	+0.49	+0.06				
6-CO		+0.10	+0.17	+0.08	+0.16			+0.08	+0.17
7-CO		+0.03	+0.08					+0.30	+0.07*
11-CO		−0.20	+0.10	−0.20	+0.11				
12-CO		+0.22	+0.24					+0.22	+0.14*
15-CO		+0.11	+0.19			+0.12	+0.21	+0.03	+0.20
16-CO		+0.13	+0.30						
17-CO		+0.12	+0.18	+0.10	+0.18	+0.10	+0.13		
	Δ^{15}	+0.05	+0.33						

* Derived from poly-oxygenated compounds.

SECTION VI

EXPERIMENTAL

Melting points were determined on a Kofler hot stage, and are uncorrected. Optical rotations were measured at the sodium D-line on a Perkin-Elmer photoelectric polarimeter at 20°C, using $CHCl_3$ solutions in 10 cm cells unless otherwise stated. Optical rotatory dispersion (o.r.d.) and circular dichroism (c.d.) spectra were recorded in MeOH solutions, by Dr. P. M. Scopes, Westfield College, London. Infrared (i.r.) spectra were recorded on a Perkin-Elmer 257 grating spectrometer in 1 mm cells in CCl_4 solutions, unless otherwise indicated. When greater resolution was required, a Perkin-Elmer 521 instrument was employed, using dilute solutions in CCl_4 in 1 mm cells. Ultra-violet (u.v.) spectra were recorded on a Cary 14M spectrophotometer using EtOH as solvent. Nuclear magnetic resonance (n.m.r.) spectra were recorded as solutions in $CHCl_3$ or $CDCl_3$ (unless otherwise stated) using 0.2% $SiMe_4$ as internal reference on Perkin-Elmer R10 (60 MHz) or Perkin-Elmer R14 (100 MHz) instruments. Mass spectra were recorded by an A.E.I. MS-9 or Varian MAT CH-7 mass spectrometer using a direct insertion probe. Micro-analyses were carried out by Drs. Weiler and Strauss of these Laboratories.

Alumina used in column chromatography was 'Camag MFC' neutral alumina, Brockman activity 1, deactivated as required by shaking with the appropriate weight of distilled water. Silica gel used in chromatography was of grade M60, similarly deactivated as appropriate. Kieselgel $HF_{254+366}$ layers of 0.1 mm were used for analytical thin layer chromatography (t.l.c.). In preparative layer chromatography (p.l.c.), large (100 cm x 20 cm) or small (20 cm x 20 cm) plates were coated with a 1 mm layer of Kieselgel $PF_{254+366}$.

The description of the technique is abbreviated for a form such as "p.l.c. [6 large plates, 6 x Me_2CO-petrol (1:9)]".

Solvents and Reagents

Et_2O and C_6H_6, when required dry, were stored over sodium for at least 48 h prior to use. 'Petrol' refers to the light petroleum fraction boiling in the range 40°-60°, and was purified by fractionation over P_2O_5. Pyridine was purified by fractionation and stored over Type 4A molecular sieve in well stoppered bottles. Tetrahydrofuran (THF) and dioxan were filtered through active alumina (1 g/ml solvent) and fractionated from $LiAlH_4$ immediately prior to use. Dimethylsulfoxide (DMSO), when required dry, was stored over CaH_2 for 24 h, and distilled from the suspension.

Jones' reagent[44] (8N-chromic acid) was prepared by adding a solution of chromium trioxide (107 g) in concentrated sulphuric acid (92 ml) to water (308 ml).

Fetizon's reagent[36] was prepared by stirring a suspension of $AgNO_3$ (20 g), and Celite (14.8 g) in H_2O (290 ml) with Na_2CO_3 (6.5 g) for 5 min and then azeotropically distilling to dryness.

Lithium tri-t-butoxyaluminium hydride [$Li(OBu^t)_3AlH$]

t-BuOH (12.6 g) was added to a suspension of $LiAlH_4$ (2.05 g) in Et_2O (70 ml) at $0^\circ C$. The mixture was stirred under N_2 for 2 h and then evaporated to dryness to give $Li(OBu^t)_3AlH$ (12.8 g).

GENERAL PROCEDURES

Standard work-up

The reaction mixture was diluted with H_2O or poured onto ice and extracted twice with a suitable solvent. The organic extracts were washed with brine and dried over $MgSO_4$. Filtration, and evaporation of solvent gave the crude product which was then purified.

Jones' oxidation[44] (Oxidation with 8N-chromic acid)

The steroid in the minimum amount of Analar Me_2CO was treated at 20°C with Jones' reagent until a permanent brown colouration persisted. The mixture was poured into H_2O, extracted twice with Et_2O, washed with aqueous $NaHCO_3$ solution and dried over $MgSO_4$. Evaporation of the solvent gave the crude product which was recrystallised from a suitable solvent.

Huang-Minlon reduction[45]

The steroid (4.04 g, 15 mmol) in diethylene glycol (freshly distilled; 140 ml) containing $N_2H_4.H_2O$ (30 ml) was heated at 140°C for 1.5 h. The condensor was then removed and the excess of H_2O and N_2H_4 were distilled off to a pot temperature of 200°C. The mixture was cooled, KOH (10 g) was added and the solution was heated at 200°C for 3 h. After this time the mixture was poured into iced H_2O and acidified with 2N-HCl. Filtration gave the crude product (3.7 g) which was recrystallised from a suitable solvent.

Huang-Minlon reduction under forcing conditions[46]

$N_2H_4.H_2O$ (95%; 200 ml) was heated under reflux for 3 h with NaOH (10 g) and distilled. The procedure was repeated for 6 h, and fractionation gave

anhydrous N_2H_4.[43]

The ketone (500 mg) was dissolved in a solution prepared by warming Na (1.2 g) with diethylene glycol (freshly distilled; 60 ml). Anhydrous N_2H_4 (12 ml) was added and the solution was heated at 170°C for 4 h. The solution was distilled until it reached 210°C, and kept at 210° for 24 h. The mixture was poured into iced H_2O, acidified with 2N-HCl, and treated as appropriate.

Acetylation

The hydroxysteroid was kept at 20°C for 15 h in the minimum volume of C_5H_5N-Ac_2O (1:1). The mixture was poured into iced H_2O, extracted with Et_2O, washed with aqueous $NaHCO_3$, dried and evaporated as usual.

INCUBATIONS OF OXYGENATED ANDROSTANES

Growing the micro-organisms

Cultures of the micro-organisms were obtained from the Centraalbureau Voor Schimmelcultures, Baarn, Netherlands. Slopes were prepared from 3% malt extract containing 2% agar in ion-free distilled water. These were inoculated with the organism from the master slope and developed for 10-15 days at 25°. (A continuous supply of slopes was maintained by preparing new ones from the existing slopes 2 weeks after the appearance of a healthy development.) The slopes were used as a source of spore suspension for inoculating the flasks of medium described later.

Two nutrient media were used. Medium A was prepared from malt extract (1 g), beef extract (1 g), yeast (Difco) (1 g), cornsteep liquor (1 ml), glucose

(5 g), and ion-free distilled water (1 l): for medium B the amounts of the first four ingredients were doubled. The pH was brought to 5.5 with 2N-HCl and sucrose (2 g) was added. The medium was introduced into 500 ml conical flasks (200 ml per flask for A, 80 ml for B) which were then sterilised in steam.

The flasks were inoculated with spore suspensions (3 or 4 flasks per slope), under sterile conditions, plugged with cotton wool, and swirled (200 rev min^{-1}) on a New Brunswick gyrorotatory shaker at 25°C for 2 days.

The larger scale feedings (Section IC) carried out in a Biotech (Model FL 110-01) fermentor, used 5 l of the appropriate medium which was similarly inoculated. Details are described in the appropriate section.

Addition of the steroid

The substrate was dissolved in dimethyl sulphoxide (DMSO) or ethanol and this solution [6 ml of DMSO/flask or 2 ml of EtOH/flask] was introduced, under sterile conditions, into the flasks containing the micro-organisms. The flasks were then swirled at 200 r.p.m. at 25°C for the specified time.

Extraction of products

Different extraction procedures (I, II, and III) were used according to the type of substrate and micro-organism involved.

Extraction I. The contents of the flasks were combined and filtered with use of appropriate filter-aids. (A thick layer of coarse salt was effective in many cases.) The mycelium was extracted by repeated soaking in Me_2CO and decantation. After evaporation of the Me_2CO the residue was partitioned between $CHCl_3$ (or EtOAc) and H_2O. The organic layer afforded the 'mycelial extract'. The culture fluid (i.e. the filtrate) was saturated with NaCl, and extracted by

shaking with CH_2Cl_2. The CH_2Cl_2 layer was dried and evaporated, to give the 'broth extract'. (The CH_2Cl_2 layer was not washed with H_2O, since this led to a considerable loss of polar products.)

Extraction II. This differed from extraction I in one respect. The culture fluid, after saturation with NaCl, was extracted with EtOAc, either by shaking or continuously.

Extraction III. This procedure avoids the filtration (which is difficult with some micor-organisms). The total material from the incubation was extracted by rolling in drums with CH_2Cl_2. Treatment of the CH_2Cl_2 layer as in extraction I gave the 'total extract'.

Separation of products

The extracts, obtained as described above, were separated either by column or preparative layer chromatography (p.l.c.). In some cases a combination of both column and preparative layer chromatography was necessary.

The following abbreviated form will be used to describe incubations:- 'Incubation with micro-organism X: 400 mg in EtOH (20 ml), 10 flasks, medium B, 4 d, extraction II gave 200 mg mycelial extract and 550 mg broth extract. Chromatography of mycelial extract on neutral Al_2O_3 (15% deactivated, 10 g). This indicates:- 'A solution of the steroid (400 mg) in EtOH (20 ml) was incubated for 4 days with the micro-organism grown in 10 flasks of medium B. Extraction procedure I gave the specified extracts, and the mycelial extract was chromatographed on a neutral Al_2O_3 column.'

SECTION IA

5α-Androst-15-en-17-one [10] (Scheme 1)

A solution of 5α-androstan-17-one [4] (25 g), $TsOH.H_2O$ (1.8 g) and ethylene glycol (25 ml) in toluene (320 ml) was heated under reflux, under a Dean-Stark separator, for 48 h. Work-up gave 17,17-ethylenedioxy-5α-androstane [5], m.p. 144-147° (from EtOAc) (25.6 g), $[\alpha]_D$ -20° (dioxan, c 1.1) (lit.,[19] m.p. 140-145°, $[\alpha]_D$ -20°), $\nu_{max.}$ 1165 and 1172 cm^{-1}.

Bromination of the ketal [5] (22 g) in THF (220 ml) with phenyltrimethylammonium bromide perbromide (27 g) as described by Djerassi et al.[19] gave 16α-bromo-17,17-ethylenedioxy-5α-androstane [6], m.p. 149-151° (from EtOH) (23.5 g), $[\alpha]_D$ -44° (c 1.0) (lit.,[19] m.p. 145-149°), $\nu_{max.}$ 632 cm^{-1}.

A solution of the acetal [6] (23 g) and $KOBu^t$ [freshly sublimed; from K (5.6 g)] in Me_2SO (700 ml) was stirred under N_2 at 45°C for 30 h. Standard manipulation gave the Δ^{15}-acetal [8] (15.1 g), m.p. 120-121.5° (from MeOH), $[\alpha]_D$ -80° (c 0.7) (lit.,[19] m.p. 120-121°, $[\alpha]_D$ -120°), $\nu_{max.}$ 3050 cm^{-1}. A similar experiment carried out at 55°C afforded material, shown by t.l.c. to contain approximately equal amounts of the Δ^{15}-acetal [8] and another compound. Chromatography on Al_2O_3 and elution with petrol-Et_2O (10:1) gave 17,17-ethylenedioxy-5α-androst-14-ene [7], m.p. 82-83° (from MeOH), $[\alpha]_D$+2° (c 1.0) (Found: C, 79.6; H, 10.4. $C_{21}H_{32}O_2$ requires C, 79.8; H, 10.2%), $\nu_{max.}$ 3060 and 1647 cm^{-1}.

A solution of the Δ^{15}-acetal [8] (22 g) and $TsOH.H_2O$ (1.5 g) in Me_2CO (2 l)-H_2O (100 ml) was stirred at 20°C for 6 h. Work-up gave a solid (21 g) which was chromatographed on silica gel (5% deact.; 500 g). Elution with

petrol-Et_2O (9:1) gave 5α-androst-15-en-17-one [10] (15.1 g), m.p. 99-101° (from C_6H_{14}), $[\alpha]_D$ -79° (c 1.0) (lit.,[19] m.p. 98-101°, $[\alpha]_D$ -80°), $\lambda_{max.}$ 232.5 nm (ε 7,820), $\nu_{max.}$ 1710 cm^{-1}.

A solution of the Δ^{14}-acetal [7] (1.67 g) and $TsOH.H_2O$ (150 mg) in Me_2CO (150 ml)-H_2O (10 ml) was kept at 20°C for 48 h. Work-up gave 5α-androst-14-en-17-one [9] (1.42 g), m.p. 56-57° (from MeOH-H_2O), $[\alpha]_D$ +142° (c 1.0) (lit.,[20] m.p. 56-57°), $\nu_{max.}$ 3062, 1752, 1640 cm^{-1}.

5α,13α-Androst-15-en-17-one [15] (Scheme 2)

5α-Androstan-17-one [4] (3 g) in dry Et_2O (200 ml) was irradiated at 25°C for 50 min using a Hanovia 450 w medium pressure mercury arc with a pyrex filter. The products of 7 separate irradiations were combined to give an oil (21.4 g) which was oxidised with 8N-chromic acid in Me_2CO. Short path distillation (184°/0.5 mm) of the product gave a distillate (15.1 g) which was chromatographed on Al_2O_3 (300 g). Elution with petrol-Et_2O (20:1) gave 5α,13α-androstan-17-one [11], m.p. 123-124° (from MeOH) (7.35 g), $[\alpha]_D$ -102° (c 1.0) (lit.,[9e] m.p. 121.5-122.5°, $[\alpha]_D$ -103°), $\nu_{max.}$ 1744 cm^{-1}.

A solution of [11] (7 g) in isopropenyl acetate (250 ml) was reacted as described by Nambara et al.[32a] Chromatography of the residue on SiO_2 (150 g) and elution with petrol-Et_2O (100:1) gave 5α,13α-androst-16-en-17-yl acetate [12] (7.2 g), m.p. 111-113° (from MeOH), $[\alpha]_D$ -88° (c 1.0) (Found: C, 79.8; H, 10.1. $C_{21}H_{32}O_2$ requires C, 79.7; H, 10.2%), $\nu_{max.}$ 1761 and 1647 cm^{-1}.

Br_2 (3.8 g) dissolved in CCl_4 was added dropwise with stirring at 0°C to a solution of [12] (7.5 g) in CCl_4 (250 ml) containing anhydrous K_2CO_3 (7 g). The mixture was washed with $NaHSO_3$ solution, and the usual work-up gave a residue which was purified by chromatography on SiO_2 (150 g). Elution with

petrol gave 16α-bromo-5α,13α-androstan-17-one [13] (1.7 g), m.p. 134-136° (from MeOH), $[\alpha]_D$ -152° (c 0.7) (Found: C, 65.0; H, 8.2. $C_{19}H_{29}OBr$ requires C, 64.8; H, 8.3%), $\nu_{max.}$ 1765 cm^{-1}, followed by 16β-bromo-5α,13α-androstan-17-one [14] (4.9 g), m.p. 105-128° (from petrol) (isomerisation), $[\alpha]_D$ -16.4° (c 1.0) (Found: C, 64.6; H, 8.1%), $\nu_{max.}$ 1765 cm^{-1}. Attempted crystallisation of [14] in MeOH caused isomerisation to a mixture of the 16α- and 16β-bromo isomers in a ratio of ca. 3:1. A solution of this mixture (6.2 g), Li_2CO_3 (27 g) and LiBr (6 g) was heated under reflux in an atmosphere of N_2 for 2.5 h. Work-up gave 5α,13α-androst-15-en-17-one [15] (5.01 g), m.p. 61-62° (from MeOH), $[\alpha]_D$ -174° (c 0.7) (Found: C, 82.9; H, 10.6. $C_{19}H_{28}O$ requires C, 83.0; H, 10.8%), $\lambda_{max.}$ 228 nm (ε 2,260), $\nu_{max.}$ 1712 cm^{-1}.

5α-Androstan-15-one [1] and 5α,14β-androstan-15-one [2] (Scheme 3)

H_2O_2 (30%; 5.6 ml) was added dropwise to a stirred solution of 5α-androst-15-en-17-one [10] (1.5 g) and 4N-NaOH (2.7 ml) in Bu^tOH (200 ml) such that the temperature was kept at less than 15°C. The mixture was stirred for 18 h, brine and Et_2O were added, and the Et_2O extract was washed with dil. $FeSO_4$, H_2O, dil. AcOH, H_2O, satd. $KHCO_3$, and brine. The usual work-up gave a residue (1.3 g) which was filtered through SiO_2 (5% deactivated; 25 g) with petrol-Et_2O (97:3) to give 15β,16β-epoxy-5α-androstan-17-one [16] (940 mg), m.p. 137-138° (from MeOH), $[\alpha]_D$ -48.5° (c 1.0) (lit.,[19] m.p. 137-138°, $[\alpha]_D$ -48°), $\nu_{max.}$ 1750 cm^{-1}.

A solution of N_2H_4 (anhydrous; 280 mg) in MeOH (3 ml) was added dropwise to a solution of the epoxy-ketone [16] (500 mg) in dry MeOH (20 ml) which was stirred under N_2 at 20°C. After 5 min, AcOH (200 mg) in MeOH (3 ml) was added, and the yellow solution was stirred for 18 h. Evaporation at

2 cm/20°C to half volume and work-up gave material (493 mg) which was chromatographed on Al_2O_3 (5% deact., 20 g). Petrol-Et_2O (99:1) eluted 5α-androst-16-en-15β-ol [18] (205 mg), m.p. 75-77° (from C_6H_{14}), $[\alpha]_D$ -70° (c 0.4) (lit.,[19] m.p. 76-77°, $[\alpha]_D$ -71°). Hydrogenation of this alcohol (200 mg) in EtOAc (20 ml) over 5% Pd on C (pre-reduced; 130 mg) gave 5α-androstan-15β-ol [20] (188 mg), m.p. 75.5-77° (from MeOH), $[\alpha]_D$ -33° (c 0.6) (lit.,[19] m.p. 75.5-77°, $[\alpha]_D$ -34°).

The results of similar experiments carried out with the epoxy-ketone [16] (500 mg) or the allylic alcohol [18] (500 mg) and N_2H_4 (280 mg) are shown below, the result of the experiment already described being included as the first entry. In the fourth experiment the mixture of alcohols produced was separated by chromatography on Al_2O_3 (7% deact.; 20 g). Elution with petrol-Et_2O (99:1) gave the saturated alcohol [20] and then the allylic alcohol [18].

Compound	Solvent	Acid	Temp/°C	Atmosphere	Wts. (mg) of 18	20
[16]	MeOH	AcOH (200 mg)	20	N_2	205	
[16]	Bu^tOH	"	20	N_2	168	
[16]	"	"	0	N_2	211	
[16]	"	"	0	air	40	165
[16]	"	$TsOH.H_2O$ (500 mg)	0	N_2	204	
[16]	"	"	0	air		243
[18]	MeOH	"	0	air		482

Oxidation of 5α-androst-16-en-15β-ol [18] (100 mg) with 8N-H_2CrO_4 at 0°C and p.l.c. [1 small plate; 1 x petrol-Et_2O (9:1)] of the residue (96 mg) gave 5α-androst-16-en-15-one [25] (56 mg), m.p. 74-76° (from MeOH), $[\alpha]_D$ +5° (c 0.5) (lit.,[19] m.p. 75-77°, $[\alpha]_D$ +4°), $\lambda_{max.}$ 222.5 nm (ε 6,800), $\nu_{max.}$ 1696, 1650 cm^{-1}.

Oxidation of 5α-androstan-15β-ol [20] with 8N-H_2CrO_4 at 0°C gave 5α-androstan-15-one [1], m.p. 89-92° (from MeOH-H_2O), $[\alpha]_D$ +30° (c 0.9) (lit.,[19] m.p. 92-93°, $[\alpha]_D$ +30°), $\nu_{max.}$ 1740 cm^{-1}.

A mixture of 5α-androstan-15-one [1] (300 mg), Na (190 mg), MeOH (9 ml) and H_2O (1 ml) was heated under reflux for 3 h. P.l.c. [3 small plates; 1 x petrol-Et_2O (19:1)] of the residue (290 mg) gave s.m. [1] (36 mg) and 5α,14β-androstan-15-one [2] (234 mg), m.p. 75-76° (from MeOH), $[\alpha]_D$ -38° (c 1.0) (lit.,[19] m.p. 72-73°, $[\alpha]_D$ -37°), $\nu_{max.}$ 1739 cm^{-1}.

A solution of 5α,14β-androstan-15-one [2] (1.25 g) in THF (30 ml) was stirred with $Li(OBu^t)_3AlH$ [from $LiAlH_4$ (300 mg)] in THF (10 ml) at 0°C for 3 h, and then at 20°C for 4 h. Work-up gave an oil (1.3 g) which was chromatographed on Al_2O_3 (5% deact.; 50 g). Elution with petrol-Et_2O (19:1) gave 5α,14β-androstan-15α-ol [23] (940 mg), m.p. 81-82° (from MeOH), $[\alpha]_D$ +67° (c 1.0) (Found: C, 82.8; H, 11.6. $C_{19}H_{32}O$ requires C, 82.54; H, 11.67%), $\nu_{max.}$ 3640 cm^{-1}, then 5α,14β-androstan-15β-ol [24] (230 mg), m.p. 134-138° (from C_6H_4), $[\alpha]_D$ -8.5° (c 0.1) (Found: C, 82.5; H, 11.7%), $\nu_{max.}$ 3640 cm^{-1}.

5α,13α-Androstan-15-one [3] (Scheme 3)

H_2O_2 (30%; 20 ml) was added dropwise to a stirred solution of 5α,13α-androst-15-en-17-one [15] (4.7 g) and 4N-NaOH (9.5 ml) in MeOH (500 ml) at 0°C. The mixture was stirred at 5°C for 4 h and allowed to stand at 5°C

overnight. Work-up gave a residue (4.8 g). Crystallisation from MeOH afforded 15α,16α-oxido-5α,13α-androstan-17-one [17] (4.4 g), m.p. 118-119°, $[\alpha]_D$ -13.3° (c 0.81) (Found: C, 79.3; H, 9.7. $C_{19}H_{28}O_2$ requires C, 79.1; H, 9.8%), $\nu_{max.}$ 1745 cm^{-1}.

A solution of the oxido-ketone [17] (3 g) and N_2H_4 (anhydrous; 1.7 g) in MeOH (150 ml) was stirred under N_2 at 20°C for 30 min. $TsOH.H_2O$ (3 g) was added, and the yellow solution was stirred for 12 h. Evaporation at 2 cm/20°C to half volume and work-up gave material (3.09 g) which was chromatographed on SiO_2 (120 g). Petrol-Et_2O (9:1) eluted 5α,13α-androst-16-en-15α-ol [19] (1.06 g), m.p. 124-126° (from petrol), $[\alpha]_D$ -1.4° (c 0.9) (Found: C, 82.9; H, 11.1. $C_{19}H_{30}O$ requires C, 83.2; H, 11.0%), $\nu_{max.}$ 3635, 3050 and 1630 cm^{-1}.

When the experiment was carried out with the oxido-ketone [17] (500 mg) at 20°C or the allylic alcohol [19] (50 mg) at 0°C, in air, a polar product resulted.

Hydrogenation of the allylic alcohol [19] (1.3 g) in EtOAc (150 ml) over 10% Pd on C (pre-reduced; 1.0 g) gave 5α,13α-androstan-15α-ol [21] (1.08 g), m.p. 108-110° (from MeOH-H_2O), $[\alpha]_D$ -40° (c 0.4) (Found: C, 82.25; H, 11.3. $C_{19}H_{32}O$ requires C, 82.5; H, 11.7%), $\nu_{max.}$ 3635 cm^{-1}.

Oxidation of the alcohol [21] (1.0 g) with 8N-H_2CrO_4 at 0°C gave 5α,13α-androstan-15-one [3] (980 mg), m.p. 114-115° (from MeOH-H_2O), $[\alpha]_D$ +50° (c 0.4) (Found: C, 83.1; H, 10.9. $C_{19}H_{30}O$ requires C, 83.2; H, 11.0%), $\nu_{max.}$ 1740 cm^{-1}.

An alternative synthesis of 5α-androstan-15-one [1] (Scheme 4)

KOH (80 mg) was added to a solution of 5α-androst-15-en-17-one [10] (100 mg) in BzOH (4 ml) and the mixture was stirred under N_2 at 20°C for 6 h. EtOAc addition (50 ml) precipitated a salt, which was filtered; the filtrate was

concentrated and excess BzOH removed by steam distillation. Work-up gave a residue (150 mg) and p.l.c. [1 large plate; 8 x petrol-Et_2O (99:1)] afforded major products at R_F 0.3 (30 mg) and 0.5 (21 mg) as oils. Repeated p.l.c. caused equilibration between the two products.

A solution of 5α-androst-15-en-17-one [10] (1 g) and NaCN (2.5 g) in THF (100 ml)-H_2O (2 ml) was refluxed for 2.5 h. Work-up, and crystallisation (MeOH) of the product (1.02 g) gave 15β-cyano-5α-androstan-17-one [27] (840 mg), m.p. 188-190°, $[\alpha]_D$ +0.5° (c 1.1) (Found: C, 80.0; H, 9.5; N, 4.4. $C_{20}H_{29}NO$ requires C, 80.3; H, 9.8; N, 4.5%), $\nu_{max.}$ 2235 and 1750 cm^{-1}.

Solutions of 5α-androst-15-en-17-one [10] (1 g) in THF (11 ml) and of NaOH (110 mg) in H_2O (2 ml)-MeOH (100 ml) were mixed and kept at 20°C for 2 h. Work-up gave 15β-methoxy-5α-androstan-17-one [29] (880 mg), m.p. 109-110° (from MeOH), $[\alpha]_D$ -12° (c 1.0) (Found: C, 79.4; H, 10.5. $C_{20}H_{32}O_2$ requires C, 79.0; H, 10.6%), $\nu_{max.}$ 1742 cm^{-1}.

A similar experiment using allyl alcohol afforded 15β-allyloxy-5α-androstan-17-one [28] (950 mg), m.p. 109-110° (from MeOH-H_2O), $[\alpha]_D$ -25° (c 0.9) (Found: C, 80.0; H, 10.45. $C_{22}H_{34}O_2$ requires C, 80.1; H, 10.4%), $\nu_{max.}$ 1745 cm^{-1}.

A solution of the 15β-methoxy-17-ketone [29] (1 g) in THF (10 ml) was stirred with $Li(OBu^t)_3AlH$ [from $LiAlH_4$ (200 mg)] in THF (10 ml) at 0°C for 3 h, and at 20°C for 4 h. Work-up gave 15β-methoxy-5α-androstan-17β-ol [31] (950 mg), m.p. 124-125° (from Me_2CO-C_6H_{14}), $[\alpha]_D$ -6° (c 1.0) (Found: C, 76.8; H, 10.1. $C_{20}H_{34}O_2$ requires C, 76.6; H, 10.1%), $\nu_{max.}$ 3620 cm^{-1}.

Similarly to the preceding experiment, reduction of [28] gave 15β-allyloxy-

5α-androstan-17β-ol [30] (960 mg), m.p. 119-119.5° (from MeOH-H_2O), $[\alpha]_D$ -52° (c 1.0) (Found: C, 79.6; H, 11.1. $C_{22}H_{36}O_2$ requires C, 79.6; H, 10.9%), $\nu_{max.}$ 3620 cm^{-1}.

A solution of 15β-allyloxy-5α-androstan-17β-ol [30] (1 g) and $KOBu^t$ (560 mg) in dry DMSO (20 ml) was kept at 100°C under N_2 for 1 h. 5N-HCl (5 ml) was added, and the heating continued for 10 min. Work-up, then chromatography on Al_2O_3 (10% deact.; 20 g) and elution with petrol-Et_2O (9:1) gave 5α-androstane-15β,17β-diol [32] (740 mg), double m.p. 146-149° and 155-156° (from Me_2CO-C_6H_4), $[\alpha]_D$ -21° (c 0.8) (lit.,[21] m.p. 148-149°, $[\alpha]_D$ -21.5°), $\nu_{max.}$ 3625 cm^{-1}.

A solution of 15β,16β-oxido-5α-androstan-17-one [16] (400 mg) in THF (20 ml) was refluxed with $LiAlH_4$ (240 mg) for 3 h. Work-up gave the 15β,17β-diol [32] (347 mg) identical with the preceding product.

A solution of the diol [32] (1 g) in dry C_6H_6 (250 ml) was refluxed with Ag_2CO_3 on Celite[36] (7 g) for 7 h. More reagent (7 g) was added, and the refluxing was continued for a further 14 h. The mixture was filtered through $MgSC_4$, the insoluble material was washed with $CHCl_3$, and the combined filtrates were evaporated. Chromatography on Al_2O_3 (10% deact.; 30 g) and elution with petrol-Et_2O (5:1) gave 15β-hydroxy-5α-androstan-17-one [33] (847 mg), m.p. 173-174° (from Me_2CO-C_6H_{14}), $[\alpha]_D$ +52° (c 1.0) (lit.,[21] m.p. 172-173.5°, $[\alpha]_D$ +60°), $\nu_{max.}$ 3625 and 1745 cm^{-1}.

Huang-Minlon reduction of the hydroxy-ketone [33] (15 mg) in diethylene glycol (4 ml) and $N_2H_4.H_2O$ (0.8 ml) gave a product containing no trace (by t.l.c. comparison with an authentic sample) of 5α-androstan-15β-ol [20] and which was not further investigated.

A solution of the hydroxy-ketone [33] (100 mg) and $BF_3.Et_2O$ (0.12 ml) in $HS[CH_2]_2SH$ (0.12 ml) was shaken for 5 min at 20°C, diluted with $CHCl_3$ (3 ml), kept for 4 h at 20°C, diluted with more $CHCl_3$ (3 ml) and washed with saturated $NaHCO_3$ solution. Work-up and p.l.c. [1 small plate; 2 x petrol-Et_2O (9:1)] gave 17,17-ethylenedithio-5α-androstan-15β-ol [34] (98 mg), m.p. 184-185° (from MeOH-H_2O), $[\alpha]_D$ -47° (c 0.9) (Found: C, 68.6; H, 9.3. $C_{21}H_{34}OS_2$ requires C, 68.8; H, 9.3%), $\nu_{max.}$ 3635 cm^{-1}. A solution of this thioacetal [34] (120 mg) in dry EtOH (20 ml) was refluxed with Raney Ni (freshly prepared;[38] 700 mg of sludge in EtOH) for 20 min. P.l.c. [2 small plates; 1 x petrol-Et_2O (9:1)] gave starting material (26 mg) and 5α-androstan-15β-ol [20] (59 mg), m.p. and mixed m.p. 79-81°.

An alternative synthesis of 5α,14β-androstan-15-one [2] (Scheme 4)

A solution of the diol [32] (550 mg) and TsCl (325 mg) in C_5H_5N was kept at 0°C for12 h. More TsCl (75 mg) was added, and the solution was kept at 0°C for 12 h, then at 20°C for 12 h. Chromatography on SiO_2 (1% deact.; 20 g) and elution with petrol-Et_2O (19:1) gave 15β-hydroxy-5α-androstan-17β-yl toluene-p-sulphonate [35] (559 mg), m.p. 179.5-181° (from Et_2O), $[\alpha]_D$ -1° (c 0.5) (Found: C, 69.7; H, 8.8; S, 7.2. $C_{26}H_{36}O_4S$ requires C, 70.0; H, 8.6; S, 7.2%), $\nu_{max.}$ 3632 cm^{-1}.

Oxidation of the hydroxy-ester [35] (200 mg) in Me_2CO with 8N-H_2CrO_4 at 0°C gave 15-oxo-5α-androstan-17β-yl toluene-p-sulphonate [36] (185 mg), m.p. 111.5-112.5° (from MeOH), $[\alpha]_D$ +31° (c 1.0) (Found: C, 69.6; H, 7.9; S, 7.7. $C_{26}H_{36}O_2S$ requires C, 70.2; H, 8.2; S, 7.2%), $\nu_{max.}$ 1752 cm^{-1}.

Heating of the keto-ester [36] (250 mg) for 1 h at 90°C under N_2 afforded 5α,14β-androst-16-en-15-one [37] (144 mg), m.p. 80-81.5° (from C_6H_{14}),

$[\alpha]_D$ +120° (c 1.0) (lit.,[21] m.p. 79-80.5°, $[\alpha]_D$ +120°), $\nu_{max.}$ 1706 cm^{-1}. The conjugated ketone [37] (61 mg) was also obtained by refluxing a solution of the keto-ester [36] (150 mg) in MeOH (50 ml) with $NaHCO_3$ (30 mg) for 4 h and purifying the product by p.l.c. [1 small plate; 5 x petrol-Et_2O (19:1)].

Hydrogenation of the conjugated ketone [37] (150 mg) in EtOAc (40 ml) over 10% Pd on C (100 mg) for 4 h gave 5α,14β-androstan-15-one [2] (125 mg), m.p. 72-73° (from MeOH-H_2O), $\nu_{max.}$ 1739 cm^{-1}.

Related synthetic work. Attempted ester elimination from the 17β-position (Scheme 5)

Treatment of the hydroxy-ethers [31] and [30] with TsCl-C_5H_5N at 0°C for 1 d, and then at 20°C for 1 d gave 15β-methoxy-5α-androstan-17β-yl toluene-p-sulphonate [39] (85% yield), m.p. 155-156° (from C_6H_{14}), $[\alpha]_D$ -30° (c 0.9) (Found: C, 70.5; H, 8.9; S, 7.1. $C_{27}H_{40}O_4S$ requires C, 70.5; H, 8.8; S, 7.1%), and 15β-allyloxy-5α-androstan-17β-yl toluene-p-sulphonate [38] (81% yield), m.p. 98-99° (from C_6H_{14}), $[\alpha]_D$ -30° (c 0.9) (Found: C, 71.3; H, 8.7. $C_{29}H_{42}O_4S$ requires C, 71.6; H, 8.9%).

When solutions of the esters [39] and [38], and the hydroxy-ester [35] were reacted with metal hydride reducing agents under a variety of conditions, the products were the respective hydroxy-ethers [31] and [30] or the diol [32]. Results and conditions are summarised below; yields were estimated from the weight of product after purification by p.l.c. and the products were in every case identified by spectral comparison with an authentic sample.

(a) A solution of the ester (100 mg) in THF, Et_2O or dioxan (12 ml) was added dropwise to a stirred suspension of $LiAlH_4$ (100 mg) in the solvent (10 ml) at 0°C. After reaction as appropriate, usual work-up gave a residue which was

purified by p.l.c.

(b) A solution of the ester (100 mg) in $Na(OC_2H_4OCH_3)_2AlH_2$ (120 mg) in dry C_6H_6 (3 ml) was heated under reflux for 2 h. The usual work-up gave a residue which was purified by p.l.c.

(c)[39b] — A solution of the ester (100 mg) and $NaBH_4$ (25 mg) in DMSO (2 ml) was heated at 80-85° under N_2 for 4 h. Work-up and purification was as above.

Starting material	Reagent	Conditions		Product	(%)
(38)	$LiAlH_4$ in Et_2O	reflux	1 h	[30]	(95)
		20°	3 h		(80)
	$LiAlH_4$ in THF	reflux	3 h	[30] + mixture	(51)
	$LiAlH_4$ in dioxan	reflux	8 h	[30] + complex mixture	(34)
	$NaBH_4$ in DMSO	-		[30]	(100)
	$Na(OC_2H_4OMe)_2AlH_2$ in C_6H_6	-		[30]	(95)
(39)	$LiAlH_4$ in Et_2O	reflux	1 h	[31]	(100)
		20°	3 h	[31]	(85)
(35)	$LiAlH_4$ in Et_2O	reflux	1 h	[32]	(92)
		20°	3 h	[32]	(83)
	$LiAlH_4$ in THF	20°	2 h	[32]	(95)

A solution of the hydroxy-ester [35] (50 mg) and NaOMe (13 mg) in DMSO (3 ml) was heated at 100°C under N_2 for 40 min, 2N-HCl (0.5 ml) was added, and heating was continued for a further 5 min. The usual work-up gave a polar oil (35 mg) which was not further investigated.

A solution of the allyloxy-ester [38] (215 mg) and $KOBu^t$ (100 mg) in DMSO (5 ml) was heated under N_2 at 100°C for 7 h, 5N-HCl (1 ml) was added, and heating was continued for a further 10 min. Work-up and p.l.c. [2 small plates; 1 x petrol-Et_2O (4:1)] gave, in order of decreasing R_F values, an oil (17 mg), $\nu_{max.}$ 3090, 3039 and 1648 cm^{-1}, which could not be crystallised but was spectrally consistent with 15β-allyloxy-5α-androst-16-ene [40], 5α-androst-16-en-15β-ol [18] (9.5 mg), m.p. 64-71°, mixed m.p. 66-73°, $\nu_{max.}$ 3620 cm^{-1}, 15β-allyloxy-5α-androstan-17β-ol [30] (15 mg), m.p. and mixed m.p. 117-119°, and polar material (120 mg) which was not investigated.

Reaction of the methoxy-ester [39] (260 mg) and $KOBu^t$ (68 mg) as described in the preceding experiment gave, after p.l.c. [2 small plates; 1 x petrol-Et_2O (4:1)], 15β-methoxy-5α-androst-16-ene [41] (46 mg) as an oil (Found: C, 83.1; H, 11.0. $C_{20}H_{32}O$ requires C, 83.4; H, 11.2%), $\nu_{max.}$ 3050 and 1650 cm^{-1}, starting material [39] (52 mg), and 15β-methoxy-5α-androstan-17β-ol [31] (67 mg), m.p. and mixed m.p. 121-124°, identified by i.r. examination.

A solution of 15β-methoxy-5α-androst-16-ene [41] (40 mg) and $KOBu^t$ (30 mg) in DMSO was reacted as described above. Work-up gave an oil (51 mg) and p.l.c. [1 small plate; 1 x petrol-Et_2O (97:3)] afforded starting material [41] (34 mg), identified by i.r. comparison.

A solution of 15β-methoxy-5α-androstan-17β-ol [31] (300 mg) in C_5H_5N

(8 ml) and benzoyl chloride (800 mg) was allowed to stand at 0°C for 1 d, then at 20°C for 1 d. Work-up gave 17β-benzoyloxy-5α-androstane-15β-yl methyl ether [42] (362 mg), m.p. 104-106° (from C_6H_{14}), $[\alpha]_D$ -2° (c 0.6) (Found: C, 78.9; H, 9.3. $C_{27}H_{38}O_3$ requires C, 79.1; H, 9.3%), $\nu_{max.}$ 1722 cm^{-1}. The benzoate [42] (100 mg) was heated under reflux (10 cm Hg/320°C) in a N_2 stream for 10 min. The flask was cooled, walls were washed with Et_2O, and the procedure was repeated. The crystalline residue, m.p. 103-106°, was identical to the starting material.

Related synthetic work. Reactions of 15β-cyano-5α-androstan-17-one [27] (Scheme 6)

A solution of the 15β-cyano-5α-androstan-17-one [27] (500 mg) and $BF_3.Et_2O$ (0.7 ml) in $HS.[CH_2]_2.SH$ (0.7 ml) was shaken for 5 min at 20°C, diluted with $CHCl_3$ (12 ml), kept for 12 h at 20°C, diluted with more $CHCl_3$ (10 ml), and washed with saturated $NaHCO_3$ aq. Work-up gave 15β-cyano-17,17-ethylenedithio-5α-androstane [43] (605 mg), m.p. 191-191.5° (from Me_2CO-C_6H_{14}), $[\alpha]_D$ -82° (c 0.9) (Found: C, 70.8; H, 8.8; N, 3.9. $C_{22}H_{33}NS_2$ requires C, 70.5; H, 8.9; N, 3.7%), $\nu_{max.}$ 2240 cm^{-1}, m/e 375 (M^+, 15%).

A solution of the cyano-thioacetal [43] (370 mg) in THF (10 ml) was added during 15 min to a stirred suspension of $LiAlH_4$ (190 mg) in THF (15 ml) at 20°C, and the mixture was stirred for 2 h. Work-up and p.l.c. [1 large plate, 3 x petrol-Et_2O (9:1)] afforded 17,17-ethylenedithio-5α-androstane-15β-carbaldehyde [44] (167 mg), m.p. 125-127° (from C_6H_{14}), $[\alpha]_D$ +12° (c 1.2) (Found: C, 69.9; H, 8.9. $C_{22}H_{34}OS_2$ requires C, 69.9; H, 9.0%), $\nu_{max.}$ 2705 and 1728 cm^{-1}.

A solution of the ethylenedithio-carbaldehyde [44] (65 mg) in dry EtOH (10 ml) was refluxed with deactivated Raney Ni (freshly prepared;[38] 350 mg sludge in EtOH) for 15 min. P.l.c. [1 small plate; 1 x petrol.-Et_2O (9:1)] gave the dominant product as an oil (18 mg), $\nu_{max.}$ 3618 cm^{-1}, spectrally consistent with 15β-hydroxymethyl-5α-androstane [45].

SECTION 18

Synthesis of 5α-androstan-15-one [1] via 5α-androstane-3,11-dione [50] (Scheme 7)

Incubation of 3α-hydroxy-5α-androstan-17-one [47] with Aspergillus ochraceus. The hydroxy-ketone [47] (1 g) in Me_2CO (50 ml), 25 flasks, medium A, 2 d, extraction II → 1.5 g broth extract. Crystallisation from Me_2CO gave 3α,11α-dihydroxy-5α-androstan-17-one [48] (864 mg), m.p. 193-194°, $[\alpha]_D$ -47° (c 0.4) (Found: C, 74.5; H, 9.9. $C_{19}H_{30}O_3$ requires C, 74.5; H, 9.9%), $\nu_{max.}$ 3625 and 1740 cm^{-1}.

Huang-Minlon reduction of the dihydroxy-ketone [48] (1.1 g) gave 5α-androstane-3α,11α-diol [49] (0.96 g), m.p. 163-164° (from Me_2CO-C_6H_{14}), $[\alpha]_D$ -25° (c 0.4) (Found: C, 77.6; H, 10.8. $C_{19}H_{32}O_2$ requires C, 78.0; H, 11.0%), $\nu_{max.}$ 3640 cm^{-1}.

Oxidation of the diol [49] (0.9 g) with 8N-H_2CrO_4 gave 5α-androstane-3,11-dione [50] (0.81 g), m.p. 120-122° (from C_6H_{14}), $[\alpha]_D$ +84° (c 1.0) (lit.,[50] m.p. 123-124°, $[\alpha]_D$ +83°), $\nu_{max.}$ 1715 cm^{-1}.

Incubation of 5α-androstane-3,11-dione [50] with Penicillium urticae. The dione [50] (400 mg) in EtOH (50 ml), 10 flasks, medium A, 5 d, extraction II → 210 mg mycelial extract + 520 mg broth extract. Chromatography of mycelial extract on Al_2O_3 (5% deact.; 10 g). Petrol-$CHCl_3$ (4:1) gave s.m. [50] (99 mg). Chromatography of broth extract on Al_2O_3 (5% deact.; 20 g). Petrol-$CHCl_3$ (3:1) gave 15α-hydroxy-5α-androstane-3,11-dione [51], m.p. 168-170° (from Me_2CO-C_6H_{14}) (132 mg), $[\alpha]_D$ +102° (c 0.7) (Found: C, 74.9; H, 9.2. $C_{19}H_{28}O_3$ requires C, 74.9; H, 9.3%), $\nu_{max.}$ 3625 and

1715 cm^{-1}. Further elution gave 3β,15α-dihydroxy-5α-androstan-11-one [52] (60 mg), m.p. 228-230° (from Me_2CO-C_6H_{14}), $[\alpha]_D$ +67° (c 0.7) (Found: C, 74.3; H, 9.6. $C_{19}H_{30}O_3$ requires C, 74.5; H, 9.9%), $\nu_{max.}$ 3620 and 1712 cm^{-1}.

A solution of the dihydroxy-ketone [52] (108 mg) in dry C_6H_6 (50 ml) was heated under reflux with Ag_2CO_3 on Celite[36] (1 g). After 5 h more reagent (500 mg) was added and the refluxing was continued for a further 5 h. Work-up and p.l.c. [2 small plates; 3 x petrol-Me_2CO (3:1)] gave the hydroxy-diketone [51] (64 mg), m.p. and mixed m.p. 164-166°.

Huang-Minlon reduction of the hydroxy-diketone [51] (234 mg) with anhydrous hydrazine[46] gave a residue (215 mg). P.l.c. [1 large plate; 2 x petrol-Et_2O (9:1)] gave 5α-androstan-15α-ol [53] (129 mg), m.p. 160-161° (from MeOH), $[\alpha]_D$ +41° (c 1.0) (lit.,[19] m.p. 161-163°, $[\alpha]_D$ +42°), $\nu_{max.}$ 3630 and 3612 cm^{-1}.

Oxidation of the 15α-ol [53] (120 mg) with 8$\underline{\underline{N}}$-$H_2CrO_4$ at 0°C gave 5α-androstan-15-one [1] (110 mg), m.p. (from MeOH-H_2O) and mixed m.p. 90-92°, $\nu_{max.}$ 1740 cm^{-1}.

Transformations of 12β,15α-dihydroxy-5α-androstan-3-one [56]* (Scheme 8)

A stirred suspension of 5α-androstane-3β,12β,15α-triol [55] (440 mg) and Ag_2CO_3 on Celite reagent (2.4 g)[36] in Ph.Me (120 ml) was refluxed for 1 h under a Dean-Stark separator. More reagent (2.4 g) was added and the heating was continued for 2 h. Work-up and p.l.c. [1 large plate; 1 x petrol-Me_2CO (1:1)] gave the dihydroxy-ketone [56] (360 mg), m.p. and mixed m.p.

* For the preparation of [55] and [56], see Section IC (Scheme 10).

172-173° (from Me_2CO-C_6H_{14}) and starting material (30 mg).

A solution of AcNHBr (1.6 g) in H_2O (20 ml) was added to a stirred solution of the triol [55] (800 mg) in Me_2CO (50 ml) at 20°C. More Me_2CO (50 ml) was added (to dissolve the precipitate), and the solution was stirred at 20°C for 4 h. After work-up, the product was chromatographed on Al_2O_3 (10% deact.; 35 g). Elution with $CHCl_3$-Et_2O (1:1) gave 15α-hydroxy-5α-androstane-3,12-dione [58], m.p. 220-222° (from Me_2CO-C_6H_{14}) (498 mg), mixed m.p.[9e] 220-222°, $\nu_{max.}$ 3630, 3613, 1713 and 1707 cm^{-1} (conditions of ref. 52).

The hydroxy-diketone [58] (170 mg) was also obtained by oxidising the dihydroxy-ketone [56] (200 mg) with AcNHBr (250 mg).

Huang-Minlon reduction of the hydroxy-diketone [58] (100 mg) afforded 5α-androstan-15α-ol [53] (79 mg), m.p. and mixed m.p. 160-161°, identified by i.r. examination. Oxidation of the alcohol [53] (100 mg) with 8N-H_2CrO_4 at 0°C gave 5α-androstan-15-one [1] (90 mg), m.p. and mixed m.p. 91-92°.

Huang-Minlon reduction of 12β,15α-dihydroxy-5α-androstan-3-one [56] (4.3 g) gave 5α-androstane-12β,15α-diol [57] (4.1 g), m.p. 139-140°, then 170-171° (from Me_2CO-C_6H_{14}), $[\alpha]_D$ +35° (c 0.6) (lit.,[9b] m.p. 139-140°, then 170-172°, $[\alpha]_D$ +40°), $\nu_{max.}$ 3625 cm^{-1}.

The diol [57] (3 g) in Me_2CO (total volume 180 ml) was oxidised with AcNHBr (4 g) in H_2O (50 ml) at 20°C for 4 h. Work-up, chromatography on Al_2O_3 (5% deact.; 150 g) and elution with petrol-Et_2O (4:1) gave in order of increasing polarity, 5α,14β-androstane-12,15-dione [62] (741 mg), m.p. 124-126° (from petrol), $[\alpha]_D$ +14° (c 0.9) (lit.,[9b] m.p. 126-128°, $[\alpha]_D$ +12°), $\nu_{max.}$ 1740 and 1720 cm^{-1}, 15α-hydroxy-5α-androstan-12-one [59] (1.7 g), m.p. 181-182°

(from Me_2CO-C_6H_{14}), $[\alpha]_D$ +95° (c 1.0) (Found: C, 78.5; H, 10.2. $C_{19}H_{30}O_2$ requires C, 78.6; H, 10.3%), $\nu_{max.}$ (conditions of ref.52) 3628, 3612 and 1709 cm^{-1}, and 12β-hydroxy-5α,14β-androstan-15-one [61] (170 mg), m.p. 151-153° (from MeOH-H_2O), $[\alpha]_D$ -32° (c 1.0) (Found: C, 78.3; H, 10.4. $C_{19}H_{30}O_2$ requires C, 78.6; H, 10.3%), $\nu_{max.}$ 3630 and 1740 cm^{-1}.

A solution of 5α-androstane-12β,15α-diol [57] (300 mg) in C_6H_6 (300 ml) was refluxed for 36 h with Ag_2CO_3 on Celite[36] (3 g) under a Dean-Stark separator. The mixture was filtered through $MgSO_4$, the insoluble material was washed with C_6H_6 (150 ml), and the combined filtrates were refluxed for12 h with more reagent (3 g). Work-up and p.l.c. [3 small plates; 1 x petrol-Et_2O (1:1)] gave 15α-hydroxy-5α-androstan-12-one [59] (194 mg), m.p. and mixed m.p. 181-182°.

Huang-Minlon reduction of 15α-hydroxy-5α-androstan-12-one [59] (100 mg) gave 5α-androstan-15α-ol [53] (84 mg), m.p. and mixed m.p. 160-161°.

Oxidation of 5α-androstane-12β,15α-diol [57] (1.3 g) with 8N-H_2CrO_4 at 0°C gave 5α-androstane-12,15-dione [62] (1.2 g), m.p. 195-197° (from MeOH-H_2O), $[\alpha]_D$ +111° (c 1.0) (lit.,[9b] m.p. 192-193°, $[\alpha]_D$ +113°), $\nu_{max.}$ 1740 and 1720 cm^{-1}.

A solution of the dione [62] (900 mg) in MeOH-5% NaOH (30 ml) was heated under reflux for 1 h. Work-up gave a residue (840 mg) which was chromatographed on Al_2O_3 (5% deact.; 40 g). Elution with petrol-Et_2O (19:1) gave 5α,14β-androstane-12,15-dione [63] (760 mg), m.p. and mixed m.p. 125-127°.

Reduction of 5α,14β-androstane-12,15-dione [63] (400 mg) in THF (10 ml) with $Li(OBu^t)_3AlH$ (from 75 mg $LiAlH_4$) gave a mixture (410 mg). P.l.c. [1 large plate; 5 x petrol-Et_2O (3:1)] gave in order of decreasing R_F values, 5α,14β-

androstane-12α,15α-diol [64] (72 mg), m.p. 220-222° (from Me_2CO-C_6H_{14}), $[\alpha]_D$ +102° (c 0.4) (Found: C, 78.0; H, 11.0. $C_{19}H_{32}O_2$ requires C, 78.0; H, 11.0%), $\nu_{max.}$ (conditions of ref. 32) 3618, 3440 and 3250 cm^{-1}, 5α,14β-androstane-12β,15α-diol [65] (148 mg), m.p. 149-150° (from Me_2CO-C_6H_{14}), $[\alpha]_D$ +55° (c 1.0) (Found: C, 78.0; H, 11.0%), $\nu_{max.}$ 3635 cm^{-1}, and 5α,14β-androstane-12α,15β-diol [66] (122 mg), m.p. 81-89° unchanged after repeated crystallisation from MeOH-H_2O, $[\alpha]_D$ +42° (c 0.8), $\nu_{max.}$ 3633 cm^{-1}.

Treatment of the 12β,15α-diol [65] (100 mg) with Ac_2O (5 ml)-C_5H_5N (5 ml) at 20°C for 12 h gave an oil (106 mg), $\nu_{max.}$ 3635 and 1745 cm^{-1}, which was dissolved in Me_2CO and oxidised with 8N-H_2CrO_4. A solution of the product in MeOH (20 ml)-KOH (1 g) was kept at 20°C for 24 h. Work-up and p.l.c. [2 small plates; 1 x petrol-Et_2O (1:1)] gave 12β-hydroxy-5α,14β-androstan-15-one [61] (76 mg), m.p. and mixed m.p. 150-152°.

SECTION 1C

Fermentation of 3β-hydroxy-5α-androstan-17-one [67] with Aspergillus ochraceus (Scheme 9)

Method (i): The hydroxy-ketone [67] (20.0 g) in EtOH (400 ml) added 1 g/1.5 h, glucose (3 g) in H_2O (50 ml) added after 10, 13 and 16 h; 5 l medium A, 60 h, extraction II ⟶ 15.9 g mycelial extract and 5.2 g broth extract. Chromatography of the total extract on Al_2O_3 (5% deact.; 250 g) and elution with $CHCl_3$ gave s.m. [67] (4.4 g). Further elution gave 3β,11α-dihydroxy-5α-androstan-17-one [68] (14.1 g), m.p. 103-106° (from Me_2CO), $[\alpha]_D$ +46° (c 1.0) (lit.,[60] m.p. 104-106°, $[\alpha]_D$ +47°), $\nu_{max.}$ 3620, 1750 cm^{-1}. Elution with $CHCl_3$-MeOH (9:1) gave a mixture of two polar products.*

Method (ii):** The hydroxy-ketone [67] (20.0 g) in EtOH (400 ml) added 1 g/1.5 h, glucose (3 g) in H_2O (50 ml) added after 10, 13 and 16 h; 5 l medium B, 66 h, extraction II ⟶ 22 g mycelial extract and 4 g broth extract. Purification[54] gave s.m. [67] (430 mg), 11α-hydroxy-5α-androstan-3,17-dione (65 mg), 3β,11α-dihydroxy-5α-androstan-17-one [68] (18.75 g), 1β,3β-dihydroxy-5α-androstan-17-one [69] (213 mg) and 17-oxo-5α-androstane-1β,3β,11α-triol [70] (197 mg).

Huang-Minlon reduction of 3β,11α-dihydroxy-5α-androstan-17-one [68] (13 g) gave 5α-androstane-3β,11α-diol [71] (12.4 g), m.p. 187.5-189° (from Me_2CO-C_6H_{14}), $[\alpha]_D$ -28.7° (c 1.0) (Found: C, 78.2; H, 11.1. $C_{19}H_{32}O_2$

* Passed to Dr. W. Müller

** The experiment described was carried out by Dr. W. Müller.[54]

requires C, 78.15; H, 11.05%), $\nu_{max.}$ 3640 cm^{-1}.

Oxidation of the diol [71] (12 g) with 8N-H_2CrO_4 gave 5α-androstane-3,11-dione [50] (11.6 g), m.p. 121-123° (from petrol), $[\alpha]_D$ +83° (c 1.0) (lit.,[50] m.p. 123-124°, $[\alpha]_D$ +83°), $\nu_{max.}$ 1715 cm^{-1}.

5α-Androstane-3,12-dione [77] (Scheme 10)

A solution of AcNHBr (4.0 g) in H_2O (100 ml), added to a stirred solution of 5α-androstane-3β,11α-diol [71] (4.3 g) in Me_2CO (300 ml) was stirred at 0°C for 3 h. Work-up, chromatography of the product on Al_2O_3 (5% deact.; 100 g) and elution with petrol-Et_2O (1:1) gave 11α-hydroxy-5α-androstan-3-one [72] (3.4 g), m.p. 124-125° (from MeOH-H_2O) $[\alpha]_D$ -12° (c 0.9) (Found: C, 78.4; H, 10.25. $C_{19}H_{30}O_2$ requires C, 78.6; H, 10.35%), $\nu_{max.}$ 3640, 1720 cm^{-1}.

A solution of the hydroxy-ketone [72] (3.15 g) and TsCl (2.25 g) in C_5H_5N (30 ml) was kept at 0°C for 12 h and then at 20°C for 24 h. Work-up gave 3-oxo-5α-androstan-11α-yl tosylate [73] (4.3 g), m.p. 148-149° (from MeOH), $[\alpha]_D$ -5.4° (c 1.5) (Found: C, 69.8; H, 8.15. $C_{26}H_{34}SO_4$ requires C, 70.25; H, 8.15%), $\nu_{max.}$ 1720 cm^{-1}.

A solution of the tosylate [73] (4.2 g), Li_2CO_3 (2.1 g) and LiCl (2 g) in DMF (120 ml) was heated under reflux in an atmosphere of N_2 for 30 min. Work-up gave 5α-androst-9(11)-en-3-one [74] (2.45 g), m.p. 98-101° (from MeOH), $[\alpha]_D$ +32.5° (c 1.0) (Found: C, 84.0; H, 10.2. $C_{19}H_{28}O$ requires C, 83.8; H, 10.35%), $\nu_{max.}$ 3050, 1720 cm^{-1}.

5α-Androst-9(11)-ene-3,12-dione [76]*

(a) The non-conjugated enone [74] (200 mg) was stirred with anhydrous Na_2CrO_4 (225 mg) in glacial HOAc (2 ml)-Ac_2O (1 ml) at 35°C for 72 h. Work-up gave a gum (107 mg). P.l.c. [2 small plates; 1 x petrol-Et_2O (2:3)] gave 5α-androst-9(11)-ene-3,12-dione [76] (50 mg), m.p. 195-196° (from Me_2CO-C_6H_{14}), $[\alpha]_D$ +54.3° (c 1.0) (Found: C, 79.8; H, 9.2. $C_{19}H_{26}O_2$ requires C, 79.7; H, 9.1%), $\nu_{max.}$ 1720, 1680 cm^{-1}.

(b) CrO_3 (16.5 g) was added in portions to Bu^tOH (39 ml) such that the temperature was maintained below 30°C. The mixture was extracted with CCl_4 (100 ml) and the extract (dried over Na_2SO_4) reduced in vacuo (so that the temperature was maintained below 40°C) to a volume of 65 ml. A solution of $Bu^t_2Cr_2O_7$ (1 ml) in glacial HOAc (0.5 ml)-Ac_2O (0.25 ml) was added dropwise to a stirred solution of the enone [74] (200 mg) in CCl_4 (5 ml) at 75°C. After 5 h, oxalic acid (250 mg) in H_2O (3 ml), then solid oxalic acid (200 mg) was added to the solution. Work-up with CCl_4 (an emulsion was cleared by filtration through Celite) gave a gum (118 mg). P.l.c. [2 small plates; 1 x petrol-Et_2O (1:1)] gave starting material [74] (73 mg) and 5α-androst-9(11)-ene-3,12-dione [76] (26 mg).

(c) Oxidation of the enone [74] (400 mg) with CrO_3 (700 mg) in HOAc (4 ml)-Ac_2O (2 ml) for 72 h at 20°C gave a gum (126 mg). P.l.c. [2 small plates; 1 x petrol-Et_2O (7:3)] gave starting material [74] (50 mg), 5α-androst-9(11)-ene-3,12-dione [76] (40 mg) and 9α,11α-epoxy-5α-androstan-3-one [75] (18 mg), m.p. 130-132° (from Me_2CO-petrol), $[\alpha]_D$ +8° (c 1.0) (Found: C, 78.85; H, 9.8. $C_{19}H_{28}O_2$ requires C, 79.1; H, 9.8%), $\nu_{max.}$ 1720 cm^{-1}. Per-oxidation of 5α-androst-9(11)-en-3-one [74] with m-chloro-perbenzoic acid in CH_2Cl_2 for 30 min at 20°C gave the epoxy ketone [75], m.p. and mixed m.p. 130-132°.

Hydrogenation of the ene-dione [76] (100 mg) in EtOAc (12 ml) over 5% Pd on C (90 mg) for 1 h gave 5α-androstane-3,12-dione [77] (93 mg), m.p. 214-216° (from Me_2CO-C_6H_{14}), $[\alpha]_D$ +73° (c 0.5) (lit.,[9e,59] m.p. 212-215°, $[\alpha]_D$ +71°), $\nu_{max.}$ 1715 cm^{-1}.

Fermentation of 5α-androstan-3-one [54] with Calonectria decora (Scheme 11)

The ketone [54] (10.0 g) in EtOH (200 ml), added 1 g/2.5 h; glucose (3 g) in H_2O (50 ml) added after 5, 10 and 15 h; 5 l medium A, 50 h, extraction II ⟶ 1.4 g mycelial extract and 9.8 g broth extract. Chromatography of the mycelial extract on Al_2O_3 (5% deact.; 50 g) and elution with petrol-Et_2O (19:1) gave s.m. [54] (650 mg). Chromatography of the broth extract on Al_2O_3 (5% deact.; 250 g) and elution with $CHCl_3$-MeOH (19:1) gave 12β,15α-dihydroxy-5α-androstan-3-one [56] (3.6 g), m.p. (from EtOAc) and mixed m.p.[9b] 172-174°, $[\alpha]_D$ +62° (c 0.9), $\nu_{max.}$ 3630, 1720 cm^{-1}, and 6α,11α-dihydroxy-5α-androstan-3-one [78] (510 mg), m.p. 137-138°, then 180-182° (from Me_2CO-C_6H_{14}), $[\alpha]_D$ +1.9° (c 1.0) (Found: C, 74.3; H, 9.6. $C_{19}H_{30}O_3$ requires C, 74.45; H, 9.85%), $\nu_{max.}$ 3625 and 1720 cm^{-1}. Further elution gave a mixture of polar products (3.5 g) which were immediately acetylated. P.l.c. of the product (3.2 g) [6 large plates; 2 x petrol-Me_2CO (4:1)] gave 3β,12β,15α-triacetoxy-5α-androstane [80] (2.15 g), m.p. (from C_6H_{14}) and mixed[9b] m.p. 141-142°, $[\alpha]_D$ +15° (c 0.9), and 6α,12β,15α-triacetoxy-5α-androstan-3-one [81] (950 mg), m.p. and mixed m.p.[58] 182-184° (from Et_2O), $[\alpha]_D$ +96° (c 0.8), $\nu_{max.}$ 1735, 1720 cm^{-1}.

Huang-Minlon reduction of 6α,11α-dihydroxy-5α-androstan-3-one [78] (100 mg) gave 5α-androstane-6α,11α-diol [82] (94 mg), m.p. 160-161° (from Me_2CO-C_6H_{14}), $[\alpha]_D$ -5.3° (c 1.0) (Found: C, 77.95; H, 11.0. $C_{19}H_{32}O_2$

requires C, 78.05; H, 11.05%), $\nu_{max.}$ 3620 cm^{-1}. Oxidation of the diol [82] (50 mg) with 8$\underline{N}$-H_2CrO_4 gave 5α-androstane-6,11-dione [83] (44 mg), m.p. (from C_6H_{14}) and mixed[9b] m.p. 173-174°, $[\alpha]_D$ +52° (c 1.0), $\nu_{max.}$ 1720 cm^{-1}.

Oxidation of the dihydroxy-ketone [78] (50 mg) with 8$\underline{N}$-H_2CrO_4 gave 5α-androstane-3,6,11-trione [84] (41 mg), m.p. and mixed m.p.[9c] 188-189° (from C_6H_{14}), $[\alpha]_D$ +48° (c 0.1) (lit.,[9c] m.p. 188-190°, $[\alpha]_D$ +57°), $\nu_{max.}$ 1720 cm^{-1}.

SECTION ID

Synthesis of 5β-androstan-3-one [88] (Scheme 12)

Huang-Minlon reduction of 3β-hydroxy-androst-5-en-17-one [85] (30 g) gave androst-5-en-3β-ol [86] (28 g), m.p. (from MeOH) and mixed m.p. 135-137° (lit.,[9f] m.p. 132-134°), $\nu_{max.}$ 3620 cm^{-1}.

Oppenauer oxidation[61] of androst-5-en-3β-ol [86] (21.6 g) gave androst-4-en-3-one [87], m.p. 104-106° (from MeOH) (19.5 g) (lit.,[9f] m.p. 103-106°), $[\alpha]_D$ +106° (c 1.0), $\nu_{max.}$ 1695 cm^{-1}.

Hydrogenation of the unsaturated ketone [87] (3 g) in acetonitrile (600 ml) over Pd on $CaCO_3$ (freshly prepared;[62] 3 g) gave an oil (2.95 g). P.l.c. [6 large plates; 8 x petrol-CH_2Cl_2 (19:1)] gave 5β-androstan-3-one [88] (2.42 g), m.p. 59-60° (from MeOH-H_2O), $[\alpha]_D$ +14.6° (c 0.7) (lit.,[63] m.p. 59-60°, $[\alpha]_D$ +17°), $\nu_{max.}$ 1715 cm^{-1}, and 5α-androstan-3-one [54] (420 mg), m.p. (from MeOH) and mixed m.p. 103-104°.

Synthesis of A-nor-(5 ⟶ 6β)abeo-androstane derivatives (Schemes 13 and 14)

5α-Androst-4-en-3-one [87] (25.7 g) in EtOAc (900 ml) was ozonised (12 l/h; 2%) at -60°C until a permanent blue colouration resulted. The suspensior was purged with N_2 and stirred vigorously with $NaHCO_3$ (5% aqueous; 450 ml) and H_2O_2 (30%; 225 ml) at 0°C for 24 h and at 20°C for 24 h. The aqueous phase was separated and the organic phase extracted with 2N-NaOH (4 x 100 ml). The combined extracts were acidified and extracted with $CHCl_3$. Work-up gave an oil (30.1 g). P.l.c. [1 large plate; 1 x $CHCl_3$-MeOH (19:1)] of a portion (500 mg) gave a product (290 mg), which could not be crystallised. The crude material (23.7 g) in Ac_2O (300 ml) and AcCl (160 ml) was heated under reflux

for 30 h. Solvents were removed in vacuo at 40°C and the residue was taken up in Et_2O (1 l) and washed with $NaHCO_3$ (5% aqueous; 2 x 200 ml). Chromatography of the product (18.8 g) on SiO_2 (250 g) and elution with petrol-Et_2O (9:1) gave 4-oxa-androst-5-en-3-one [90] (11.9 g), m.p. 90-91° (from C_6H_{14}), $[\alpha]_D$ -98° (c 1.0) (Found: C, 78.8; H, 9.4. $C_{18}H_{28}O_2$ requires C, 78.8; H, 9.5%), $\nu_{max.}$ 3060, 1760 and 1690 cm^{-1}.

A solution of the enol-lactone [90] (11.9 g) and $Li(OBu^t)_3AlH$ (12 g) in THF was stirred at 0°C for 2 h and then at 20°C for 2 h. Work-up gave an oil which was purified by chromatography on SiO_2 (300 g). Elution with petrol-Et_2O (4:1) and p.l.c. of the product (2.33 g) [5 large plates; 1 x petrol-Et_2O (1:1)] gave an isomeric mixture of 4-oxa-5ξ-androstan-5-ol [93] as an oil, $\nu_{max.}$ 3610 cm^{-1}, m/e 278 (M^+, 28%) and 260 (100%). Acetylation of the acetal [93] (120 mg) and p.l.c. of the product [1 small plate; 2 x petrol-Et_2O (4:1)] gave A-nor-3,5-seco-5-oxo-androstane-3-yl acetate [94] (77 mg) as an oil, $[\alpha]_D$ +20° (c 1.0) (Found: C, 75.15; H, 9.85. $C_{20}H_{32}O_3$ requires C, 74.95; H, 10.05%), $\nu_{max.}$ 1740 and 1708 cm^{-1}, m/e 260 [$(M-60)^+$, 30%] and 245 (100%). Elution with petrol-Et_2O (1:1) and crystallisation of the product (6.6 g) from Me_2CO-C_6H_{14} gave 3β-hydroxy-A-nor-(5 → 6β) abeo-androstan-5-one [91] (3.9 g), m.p. 182-183° (from Me_2CO-C_6H_{14}), $[\alpha]_D$ -27° (c 1.0) (Found: C, 78.4; H, 10.1. $C_{18}H_{28}O_2$ requires C, 78.2; H, 10.2%), $\nu_{max.}$ 3620, 3426 and 1718 cm^{-1}. P.l.c. [6 large plates; 3 x petrol-Et_2O (1:1)] of the mother liquors gave [91] (1.46 g) and the less polar 3α-hydroxy-A-nor-(5 → 6β) abeo-androstan-5-one [92] (1.25 g), m.p. 155-156° (from Me_2CO-C_6H_{14}), $[\alpha]_D$ -7.6° (c 0.5) (Found: C, 78.1; H, 10.1%), $\nu_{max.}$ 3625 and 1718 cm^{-1}.

Oxidation of the hydroxy-ketone [91] (1 g) in Me_2CO with 8N-H_2CrO_4 at 0°C gave A-nor-(5 → 6β) abeo-androstane-3,5-dione [95] (720 mg), m.p. 115-118° (from petrol at -40°C), $[\alpha]_D$ -26° (c 0.5) (Found: C, 78.7; H, 9.4. $C_{18}H_{26}O_2$ requires C, 78.8; H, 9.5%), $\nu_{max.}$ 1740, 1710 cm^{-1}.

Acetylation of the hydroxy-ketone [91] (170 mg) at 20°C for 3 d gave 3β-acetoxy-A-nor-(5 → 6β) abeo-androstan-5-one [98], m.p. 165-166° (from C_6H_{14}) (163 mg), $[\alpha]_D$ -28° (c 1.0) (Found: C, 75.7; H, 9.7. $C_{20}H_{30}O_3$ requires C, 75.4; H, 9.5%), $\nu_{max.}$ 1745, 1725 cm^{-1}.

Treatment of the hydroxy-ketone [91] (2 g) with TsCl (4.5 g) and C_5H_5N (40 ml) at 20°C for 3 d gave 5-oxo-A-nor-(5 → 6β) abeo-androstan-3β-yl toluene p-sulphonate [96] (3.05 g), m.p. 182-184° (from C_6H_{14}), $[\alpha]_D$ -15° (c 1.0) (Found: C, 70.0; H, 8.1; S, 7.2. $C_{25}H_{34}SO_4$ requires C, 69.75; H, 8.0; S, 7.4%), $\nu_{max.}$ 1723, 1180 and 1170 cm^{-1}.

A solution of the hydroxy-ketone [91] (100 mg) and MsCl (1 ml) in C_5H_5N (1 ml) and Me_2CO (3 ml), kept at 0°C for 12 h and 20°C for 1 d, gave an oil (300 mg). Chromatography on SiO_2 (20 g) and elution with petrol-Et_2O (1:1) gave the mesylate [97], m.p. 164-166° (from petrol) (98 mg), $[\alpha]_D$ -23° (c 0.9) (Found: C, 64.7; H, 8.7; S, 9.2. $C_{19}H_{30}SO_4$ requires C, 64.4; H, 8.5; S, 9.0%), $\nu_{max.}$ 1722 and 1170 cm^{-1}.

A suspension of the tosylate [96] (2.4 g), Li_2CO_3 (1.2 g) and LiCl (1.7 g) in DMF (65 ml) was heated under reflux, in an atmosphere of N_2, for 2 h. Work-up gave A-nor-(5 → 6β) abeo-androst-2-en-5-one [99] (1.56 g), m.p. 133-134° (from petrol), $[\alpha]_D$ -185° (c 1.0) (Found: C, 83.9; H, 10.3. $C_{18}H_{26}O$ requires C, 83.7; H, 10.1%), $\nu_{max.}$ 3040, 1725 and 1645 cm^{-1}.

Hydrogenation of [99] (1.5 g) in EtOAc (150 ml) over 5% Pd on C (1.0 g)

gave A-nor-(5 → 6β)abeo-androstan-5-one [100] (1.42 g), m.p. 139-140° (from MeOH), $[\alpha]_D$ -26° (c 1.0) (Found: C, 83.1; H, 10.9. $C_{18}H_{28}O$ requires C, 83.0; H, 10.8%), $\nu_{max.}$ 1715 cm^{-1}.

Huang-Minlon reduction of the ketone [100] (55 mg) and p.l.c. [1 small plate; 1 x petrol] of the product gave A-nor-(5 → 6β)abeo-androstane [101] (26 mg), m.p. 92-94° (from MeOH), $[\alpha]_D$ -24° (c 0.3) (Found: C, 87.9; H, 12.1. $C_{18}H_{30}$ requires C, 87.7; H, 12.3%).

Huang-Minlon reduction of the hydroxy-ketone [91] (240 mg) gave a non-polar product which rapidly oxidised in air. P.l.c. [3 small plates; 1 x petrol-Et_2O (4:1)] gave an oil (167 mg), $\tau_{max.}$ 9.30 (3H, s), 9.15 (3H, s) and 4.89 (1H, s, $W_{\frac{1}{2}}$ 5 Hz).

The hydroxy-ketone [91] (150 mg), Et_3N (0.2 ml) and $N_2H_4.H_2O$ (0.7 ml) in EtOH (1 ml) were heated under reflux for 4 h and allowed to cool slowly, giving the hydrazone [102] (137 mg), m.p. 99-102° (from EtOH), $\nu_{max.}$ 3350-3620 cm^{-1}, m/e 290 (M^+, 62%) and 272 (100%). The hydrazone [102] (100 mg) was added to a stirred solution of $KOBu^t$ (65 mg) in DMSO (0.5 ml) at 20°C, at the rate of 20 mg/h.[66] Work-up after 8 h gave unidentified polar material.

A solution of the hydroxy-ketone [91] (100 mg) in $BF_3.Et_2O$ (0.2 ml) and $HS.[CH_2]_2.SH$ (0.3 ml) was shaken for 5 min at 20°C, diluted with $CHCl_3$ (3 ml) and kept for 3 h at 20°C. Work-up and p.l.c. of the product [2 small plates; 1 x petrol-Et_2O (17:3)] gave an oil (103 mg), $\nu_{max.}$ 1380, 1280 cm^{-1}, $\tau_{max.}$ 9.31 (3H, s), 8.94 (3H,s), 6.79 (2H, s), 6.77 (6H, s) and 5.08 (1H; t; J 6,6 Hz), m/e 428 (M^+, 96%) and 400 (100%).

A solution of the acetoxy-ketone [98] (160 mg) and $NaBH_4$ (6 mg) in MeOH (20 ml) was stirred at 20°C for 12 h. Work-up gave a residue (151 mg)

which from t.l.c. examination showed only a trace of polar product. Reaction of the residue with $NaBH_4$ (40 mg), as above, gave an oil (153 mg). P.l.c. [2 small plates; 2 x Et_2O] gave in order of decreasing R_F, starting material [90] (51 mg), an unidentified product as an oil (20 mg), $\tau_{max.}$ 9.30 (3H, s), 9.03 (3H, s), 6.48 (1H, q, J 6,6,6 Hz), 6.22 (1H, m, $W_{\frac{1}{2}}$ 15 Hz), 6.05 (1H, m, $W_{\frac{1}{2}}$ 10 Hz), m/e 304 (M^+, 14%), 204 (100%), and A-nor-(5 → 6β)abeo-androstane-3β,5β-diol [104] (71 mg), m.p. 116-119° (from C_6H_{14}), $[\alpha]_D$ -22° (c 0.9) (Found: C, 77.5; H, 11.0. $C_{18}H_{30}O_2$ requires C, 77.65; H, 10.85%), $\nu_{max.}$ ($CHCl_3$) 3300-3450 cm^{-1}.

SECTION II

Incubation of 5α-androstan-15-one [1] with C.decora (Scheme 15)

(a) The ketone [1] (1 g) in Me_2SO (150 ml), 25 flasks, medium B, 6 d, extraction II ⟶ 2.76 g combined extract. Chromatography on Al_2O_3 (5% deact.; 60 g). $CHCl_3$ gave fractions A (320 mg), B (610 mg) and C (350 mg) which were purified by p.l.c. Fraction A [1 large plate; 2 x petrol-Et_2O (9:1)] gave s.m. [1] (39 mg) and 5α,14β-androstan-15-one [2] (13 mg). Fraction B [2 large plates; 3 x petrol-Me_2CO (4:1)] gave 6α,12β-dihydroxy-5α,14β-androstan-15-one [116] (228 mg), m.p. 149-151° (from Me_2CO-C_6H_{14}), $[\alpha]_D$ +0.9° (c 1.0) (Found: C, 74.5; H, 9.8. $C_{19}H_{30}O_3$ requires C, 74.5; H, 9.9%) and 3 steroidal products (61 mg, 62 mg, 49 mg) which were not characterised. Fraction C [1 large plate; 3 x petrol-Me_2CO (2:1)] gave 2α,12β-dihydroxy-5α-androstan-15-one [122] (90 mg), m.p. 189-191° (from Me_2CO-C_6H_{14}), $[\alpha]_D$ +43° (c 0.2) (Found: C, 74.2; H, 10.1. $C_{19}H_{30}O_3$ requires C, 74.5; H, 9.9%), and 4 steroidal products not characterised.

The ketone [1] (80 mg) in Me_2SO (6 ml), 1 flask, medium B not inoculated, 6 d, extraction II ⟶ unchanged substrate [1] (74 mg). No trace of the 14β-isomer [2] was detected by t.l.c. examination.

(b) Huang-Minlon reduction of 6α,12β-dihydroxy-5α,14β-androstan-15-one [116] (60 mg) using forcing conditions[46] gave a residue (46 mg). Fractional crystallisation gave 5α-androstane-6α,12β-diol [119] (5 mg), m.p. 196-198° (from Me_2CO-C_6H_{14}) and mixed m.p. 195-198°.[14,47] Oxidation of the mother liquors (30 mg) with 8N-H_2CrO_4 gave a residue (27 mg) and p.l.c. [1 small plate; 1 x petrol-Et_2O (1:1)] gave 5α-androstane-6,12-dione [121] (14 mg), m.p. 180-182°

(from C_6H_{14}) and mixed m.p. 180-183°.[14,47]

Oxidation of the dihydroxy-ketone [116] (100 mg) with 8N-H_2CrO_4 gave 5α,14β-androstane-6,12,15-trione [117] (96 mg), m.p. 205-206° (from C_6H_{14}) (Found: C, 75.3; H, 8.7. $C_{19}H_{26}O_3$ requires C, 75.5; H, 8.7%), $\nu_{max.}$ 1720 and 1740 cm^{-1}.

A solution of the trione [117] (50 mg) and Na (25 mg) in CH_3OD (10 ml) and D_2O (5 ml) was heated under reflux for 3 h. Work-up and p.l.c. [1 small plate; 1 x petrol-Et_2O (1:1)] gave 5α-androstane-d_8-6,12,15-trione [118] (40 mg), m.p. 191-194° (from Me_2CO-C_6H_{14}) (14 mg), m/e 310 (M^+, 69%).

Acetylation of 2α,12β-dihydroxy-5α-androstan-15-one [122] (40 mg) gave 2α,12β-diacetoxy-5α-androstan-15-one [123] (43 mg), m.p. 172-174° (from C_6H_{14}), $[\alpha]_D$ -11° (c 1.0) (Found: C, 70.6; H, 8.9. $C_{23}H_{34}O_5$ requires C, 70.4; H, 8.8%).

Oxidation of the dihydroxyketone [122] (50 mg) with 8N-H_2CrO_4 at 0°C gave a product (36 mg), m.p. 191-193° (from C_6H_{14}), $\nu_{max.}$ 1720, 1740 cm^{-1}, consistent with 5α-androstane-2,12,15-trione [124].

The trione [124] (30 mg) and Na (20 mg) in MeOH (10 ml) were heated under reflux for.3 h. Work-up as usual gave a product (20 mg) consistent by n.m.r. with 5α,14β-androstane-2,12,15-trione [125].

Huang-Minlon reduction of 2α,12β-dihydroxy-5α-androstan-15-one [122] (30 mg) with anhydrous hydrazine (1 ml) gave a residue (24 mg). P.l.c. [1 small plate; 3 x petrol-Et_2O (1:1)] gave products spectroscopically consistent with 5α-androstane-2α,12β-diol [126] (12 mg) and 5α,14β-androstane-2α,12β-diol [127] (8 mg).

Oxidation of [126] (12 mg) with 8N-H_2CrO_4 gave a residue which with

p.l.c. [½ small plate; 1 x petrol-Et_2O (1:1)] afforded a product (8 mg) formulated as 5α-androstane-2,12-dione [128].

Incubation of 15α-hydroxy-5α-androstan-12-one [59] with C.decora (Scheme 17)

The ketone [59] (800 mg) in Me_2SO (120 ml), 20 flasks, medium B, 4 d, extraction II ⟶ 1.23 g total extract. P.l.c. [3 large plates; 3 x petrol-Me_2CO (3:1)] gave two products (25 mg, 41 mg) which were not characterised, and polar material (275 mg) which was immediately acetylated. P.l.c. of the product [3 small plates; 3 x petrol-Et_2O (2:1)] gave 6α,15α-diacetoxy-5α-androstan-12-one [131] (111 mg), m.p. 221-223° (from Me_2CO-C_6H_{14}), $[\alpha]_D$ +69° (c 1.0) (lit.,[9e] m.p. 222-225°, $[\alpha]_D$ +60°), $\nu_{max.}$ 1720, 1735 cm^{-1}.

Incubation of 5α-androstane-12,15-dione [62] with C.decora

The dione [62] (520 mg) in Me_2SO (78 ml), 13 flasks, medium B, 2 d, extraction II ⟶ 330 mg broth extract + 560 mg mycelial extract. P.l.c. [1 large plate; 1 x petrol-Et_2O (1:1)] of the mycelial extract gave s.m. [62] (177 mg). Acetylation of the broth extract gave a glass (200 mg) from which no steroidal products were separated by p.l.c. [2 small plates; 3 x petrol-Et_2O (1:1)].

Incubation of 5α,14β-androstan-15-one [2] and 12β-hydroxy-5α,14β-androstan-15-one [61] with C.decora (Scheme 18)

(a) The ketone [1] (2 g) in Me_2SO (300 ml), 50 flasks, medium B, 6 d, extraction II ⟶ 2.3 g broth extract + 850 mg mycelial extract. Chromatography of mycelial extract on neutral Al_2O_3 (5% deact.; 20 g). Petrol-Et_2O (9:1) gave a residue (550 mg) which with p.l.c. [1 large plate; 3 x petrol-Et_2O (9:1)] gave s.m. (450 mg) and 5α,14α-androstan-15-one [1] (12 mg). Filtration of broth extract through neutral Al_2O_3 (5% deact.; 20 g) with $CHCl_3$ gave a residue

(980 mg) which with p.l.c. [2 large plates; 3 x Me_2CO-petrol (1:4)] gave 7β,12β-dihydroxy-5α,14β-androstan-15-one [132] (585 mg), m.p. 169-170° (from Me_2CO-C_6H_{14}), $[\alpha]_D$ -34° (c 1.0) (Found: C, 74.3; H, 9.7. $C_{19}H_{30}O_3$ requires C, 74.5; H, 9.9%), 15-oxo-5α,14β-androstane-7β,12β,14-triol [133] (179 mg), m.p. 146-148° (from Me_2CO-C_6H_{14}), $[\alpha]_D$ -16.4° (c 0.5) (Found: C, 70.9; H, 9.3. $C_{19}H_{30}O_4$ requires C, 70.8; H, 9.4%) and a product formulated from spectral evidence as 12β,14-dihydroxy-5α,14β-androstan-15-one [134] (29 mg). 7 minor products were not isolated.

(b) The hydroxy-ketone [61] (80 mg) in Me_2SO (12 ml), 2 flasks, medium B, 4 d, extraction II ⟶ 181 mg total extract. P.l.c. [2 small plates; 1 x petrol-Me_2CO (2:1)] gave the dihydroxy-ketone [132] (18 mg), m.p. and mixed m.p. 167-169° (from Me_2CO-C_6H_{14}) and [133] (7 mg), $\nu_{max.}^{52}$ 1739, 3406, 3592, 3616, 3628 cm^{-1}, m/e 322 (M^+, 3%), 266 (100%).

(c) Transformations: (Scheme 18).

Huang-Minlon reduction of the dihydroxy-ketone [132] (250 mg) using forcing conditions,[46] and p.l.c. of the acetylated product [1 large plate; 4 x petrol-Et_2O (9:1)] gave 7β,12β-diacetoxy-5α-androstane [135] (73 mg), $\nu_{max.}$ 1735 cm^{-1}, but which could not be crystallised, and 7β,12β-diacetoxy-5α,14β-androstane [136] (157 mg), m.p. 104-106° (from MeOH-H_2O), $[\alpha]_D$ +33.4° (c 1.0) (Found: C, 73.5; H, 9.5. $C_{19}H_{36}O_4$ requires C, 73.4; H, 9.6%), $\nu_{max.}$ 1735 cm^{-1}.

Saponification of [135] (46 mg) with $LiAlH_4$ (10 mg) in Et_2O (10 ml) gave 5α-androstane-7β,12β-diol [137] (36 mg), m.p. 144-145° (from C_6H_{14}), $[\alpha]_D$ +36° (c 0.4) (lit.,[48] m.p. 144-145°, $[\alpha]_D$ +14°). Similar saponification of [136] (140 mg) gave 5α,14β-androstane-7β,12β-diol [138] (116 mg),

m.p. 169-170° (from C_6H_{14}), $[\alpha]_D$ +48.5° (c 0.8) (Found: C, 78.0; H, 11.0. $C_{19}H_{32}O_2$ requires C, 78.0; H, 11.0%).

Oxidation of the respective diols [137] and [138] with 8N-H_2CrO_4 gave 5α-androstane-7,12-dione [139] (26 mg), m.p. 170-172° (from C_6H_{14}), $[\alpha]_D$ -34° (c 0.5) (lit.,[48] m.p. 168-170°, $[\alpha]_D$ -31°), and 5α,14β-androstane-7,12-dione [140] (80 mg), m.p. 142-144° (from C_6H_{14}), $[\alpha]_D$ +125° (c 0.6) (Found: C, 78.9; H, 9.6. $C_{19}H_{28}O_2$ requires C, 79.1; H, 9.6%), $\nu_{max.}$ 1720 cm^{-1}.

Oxidation of the dihydroxy-ketone [132] (30 mg) with 8N-H_2CrO_4 gave 5α,14β-androstane-7,12,15-trione [141] (28 mg), m.p. 175-177° (from C_6H_{14}), $[\alpha]_D$ -28.5° (c 0.2) (Found: C, 75.6; H, 8.7. $C_{19}H_{26}O_3$ requires C, 75.5; H, 8.7%), $\nu_{max.}$ 1720, 1740 cm^{-1}.

Oxidation of 15-oxo-5α,14β-androstane-7β,12β,14-triol [133] (20 mg) with 8N-H_2CrO_4 at 0°C gave 7β,14-dihydroxy-5α,14β-androstane-12,15-dione [142] (16 mg), m.p. 194-196° (Found: C, 71.0; H, 8.7. $C_{19}H_{28}O_4$ requires C, 71.2; H, 8.8%), $\nu_{max.}$ 1712, 1744, 3405, 3620 cm^{-1} (conditions of ref.52). Oxidation of [133] (30 mg) at 20°C gave a complex mixture; p.l.c. (1 small plate; 1 x Et_2O) gave a product (10 mg), $\nu_{max.}$ 1715, 1750, 3620, 3380 cm^{-1}, consistent with 14-hydroxy-5α,14β-androstane-7,12,15-trione [143]. This product [143] (6 mg) in methanolic KOH (2 ml; 2%) was stirred at 20°C for 5 min. Work-up afforded a complex mixture which was not investigated.

Acetylation of [133] (50 mg) and p.l.c. of the residue [1 small plate; 1 x petrol-Et_2O (1:1)] gave 15-oxo-5α,14β-androstane-7β,12β,14-triol 7,12-diacetate [145] (43 mg), m.p. 97-99° (from MeOH-H_2O), $[\alpha]_D$ +91° (c 0.9) (Found: C, 67.8; H, 8.5. $C_{23}H_{34}O_6$ requires C, 67.9; H, 8.4%),

$\nu_{max.}$ 1740, 1744, 1757, 3524, 3709 cm^{-1} (conditions of ref.52).

A solution of the trihydroxy-ketone [133] (60 mg) and $TsOH.H_2O$ (8 mg) in 2,2-dimethoxypropane (freshly distilled, 6 ml) was stirred at 20°C for 30 min. Work-up gave a residue (84 mg) and p.l.c. [1 small plate; 2 x petrol-Et_2O (1:1)] gave 12β-hydroxy-7β,14-isopropylidenedioxy-5α,14β-androstan-15-one [146] (62 mg) as a glass, $[\alpha]_D$ -55° (c 0.6) (Found: C, 73.2; H, 9.3. $C_{22}H_{34}O_4$ requires C, 72.9; H, 9.4%), $\nu_{max.}$ 1742, 3630 cm^{-1}.

The dihydroxy-dione [142] (10 mg) and $TsOH.H_2O$ (1.5 mg) in 2,2-dimethoxypropane (2 ml), reacted as above, gave a residue (10 mg), $\nu_{max.}$ 1710, 1740 cm^{-1}.

A solution of the isopropylidenedioxy-derivative [146] (10 mg) in dry CH_2Cl_2 (2 ml) was added at 0°C to a solution of C_6H_5N (0.4 ml) and CrO_3 (30 mg) in CH_2Cl_2 (2 ml), and the mixture was stirred at 0°C for 24 h. Work-up, and t.l.c. of the residue (12 mg) [petrol-Et_2O (1:1)] showed a dominant spot of r.f. identical with the product of the preceding experiment.

The trihydroxy-ketone [133] (60 mg) in THF (20 ml) was reduced with $LiAlH_4$ (30 mg). Work-up gave 5α,14β-androstane-7β,12β,14,15β-tetraol [148] (54 mg), m.p. 269-272° (from Me_2CO-C_6H_{14}), $[\alpha]_D$ (EtOH) +7.4° (c 0.2) (Found: C, 70.5; H, 9.9. $C_{19}H_{32}O_4$ requires C, 70.3; H, 9.9%), $\nu_{max.}$ 3370, 3450 cm^{-1} ($CHCl_3$).

A mixture of $Pb(OAc)_4$ (90 mg) and the tetraol [148] (41 mg) in HOAc (3.6 ml) and H_2O (0.4 ml) was stirred at 20°C for 4 h. Work-up and p.l.c. [1 small plate; 2 x Et_2O] afforded a glass (29 mg), $\nu_{max.}$ 1698, 3544, 3575, 3615 cm^{-1}, consistent with the (1:1) lactone mixture [149]. Acetylation of [149] (20 mg) and p.l.c. [1 small plate; 1 x petrol-Et_2O (1:1)] of the residue gave a

product (16 mg), $\nu_{max.}$ 1712, 1739, 1755 cm^{-1}, consistent with the (1:1) mixture [150].

Synthesis of 14-hydroxy-5α,14β-androstan-15-one [151] (Scheme 19)

A solution of 5α-androstan-15β-ol [20] (1.2 g), C_5H_5N (12 ml) and MsCl (12 ml) in Me_2CO (25 ml) was kept at 20°C for 18 h. Work-up, chromatography of the residue (1.35 g) on SiO_2 (40 g), and elution with petrol gave an oil (960 mg). P.l.c. [1 large plate; 5% $AgNO_3$ impregnated in $HF_{254+366}$; 2 x petrol-Et_2O (200:1)] gave 5α-androst-14-ene [152] (520 mg), m.p. 38-39° (from MeOH), $[\alpha]_D$ +32° (c 1.0) (Found: C, 88.5; H, 11.5. $C_{19}H_{30}$ requires C, 88.3; H, 11.3%), $\nu_{max.}$ 3060, 1647 cm^{-1}.

A similar procedure with 5α,14β-androstan-15α-ol [23] (800 mg) afforded the olefin [152] (640 mg). Purification with $AgNO_3$-impregnated SiO_2 was not necessary.

Perphthalic acid (6.4 g) in Et_2O (80 ml)[85] was added at 0°C to a stirred solution of [152] (760 mg) in Et_2O (25 ml). After 1 h, work-up and p.l.c. [2 large plates; 3 x petrol-Et_2O (49:1)] of the residue gave 14,15α-oxido-5α-androstane [153] (252 mg), m.p. 75-76° (from MeOH), $[\alpha]_D$ +21.5° (c 1.0) (Found: C, 83.2; H, 10.9. $C_{19}H_{30}O$ requires C, 83.2; H, 11.0%), $\nu_{max.}$ 3022 cm^{-1}, 14,15β-oxido-5α,14β-androstane [154] (187 mg), m.p. 57-59° (from MeOH), $[\alpha]_D$ -4° (c 1.1) (Found: C, 83.2; H, 10.9%), $\nu_{max.}$ 3035 cm^{-1}, and an intermediate mixture (1:1) of [153] and [154] (251 mg).

Epoxide opening:

(a) The β-epoxide [154] (20 mg) in MeCOEt (1 ml) was stirred for 5 min at 20°C with a solution (0.1 ml) prepared from $HClO_4$ (0.1 ml; 60% w/v) and MeCOEt (2.5 ml). Work-up and p.l.c. [½ small plate; 1 x petrol-Et_2O (9:1)]

gave 5α-androstan-15-one [1] (6 mg) and 5α,14β-androstan-15-one [2] (9 mg).

(b) The β-epoxide [154] (80 mg) and 2N-HCl (2 ml) in THF (7.5 ml) and H_2O (7.5 ml) was stirred at 20°C for 2 h. Work-up and p.l.c. [1 small plate; 3 x petrol-Et_2O (1:1)] afforded s.m. [154] (52 mg) and 5α,14β-androstane-14,15β-diol [155] (24 mg), m.p. 119-122° (from MeOH-H_2O), $[\alpha]_D$ -15° (c 0.5) (Found: C, 77.7; H, 10.9. $C_{19}H_{32}O_2$ requires C, 78.0; H, 11.0%), $\nu_{max.}$ 3520, 3634 cm^{-1}.

(c) The β-epoxide [154] (50 mg) reacted as above, but with $HClO_4$ (0.2 ml; 60% w/v) gave s.m. [154] (5 mg) and the cis-diol [155] (40 mg).

(d) The α-epoxide [153] (20 mg), treated as in (a) above, gave 5α,14β-androstan-15-one [2] (17 mg).

(e) Treated as in (b) above, the α-epoxide [153] (100 mg) gave 5α,14β-androstan-15-one [2] (36 mg) and 5α,14β-androstane-14,15α-diol [156] (53 mg), m.p. 168-169° (from MeOH-H_2O), $[\alpha]_D$ +107° (c 0.5) (Found: C, 78.0; H, 10.9. $C_{19}H_{32}O_2$ requires C, 78.0; H, 11.0%), $\nu_{max.}$ 3630 cm^{-1}.

(f) The α-epoxide [153] (60 mg) and 2N-HCl (5 drops) in THF-H_2O (5 drops) was stirred at 20°C for 2 h. Work-up and purification gave [2] (10 mg) and the trans-diol [156] (48 mg).

Treatment of 5α,14β-androstan-14β,15β-diol [155] (10 mg) with 8N-H_2CrO_4 at 0°C caused no reaction; at 20° oxidation gave a mixture of polar products which were discarded.

Oxidation of 5α,14β-androstane-14,15α-diol [156] (70 mg) with 8N-H_2CrO_4 at 0°C gave 14-hydroxy-5α,14β-androstan-15-one [151] (60 mg), m.p. 128-130° (from MeOH-H_2O), $[\alpha]_D$ +50° (c 0.1) (Found: C, 78.7; H, 10.5.

$C_{19}H_{30}O_2$ requires C, 78.6; H, 10.3%), $\nu_{max.}$ 3610, 1740 cm^{-1}.

Incubation of 5α,13α-androstan-15-one [3] with C.decora (Scheme 20)

(a) The ketone [3] (80 mg) in Me_2SO (12 ml), 2 flasks, medium B, 6 d, extraction II ⟶ 127 mg combined extract. P.l.c. [2 small plates; 2 x petrol-Me_2CO (3:1)] and repeated p.l.c. [1 small plate] afforded the major product (16 mg) as a mixture. Repeated p.l.c. as above gave a non-crystalline product consistent with 6α,12β-dihydroxy-5α,13α-androstan-15-one [157] (10 mg), $\nu_{max.}$ 1740 and 3629 cm^{-1}.

(b) Oxidation of [157] with 8N-H_2CrO_4 gave a product formulated as 5α,13α-androstane-6,12,15-trione [158], m.p. 218-221° (from Me_2CO-C_6H_{14}) (4 mg), $\nu_{max.}$ 1720, 1740 cm^{-1}.

Incubation of 5α,14β-androstane-12,15-dione [63] with C.decora

The dione [63] (1 g) in EtOH (50 ml), 25 flasks, medium B, 4 d, extraction II ⟶ 1.04 g broth extract + 600 mg mycelial extract. P.l.c. [2 large plates; 1 x petrol-Et_2O (1:1)] of the mycelial extract gave s.m. (40 mg). Acetylation of the broth extract gave a residue (930 mg). P.l.c. [2 large plates; 2 x Me_2CO-petrol (1:4)] gave six products (31, 19, 26, 29, 14, and 21 mg in order of decreasing polarity) which were not characterised.

Incubation of 5β-androstan-3-one [88] with C.decora (Scheme 21)

The ketone [88] (1.04 g) in EtOH (52 ml), 26 flasks, medium A, 3 d, extraction II ⟶ 1.27 g broth extract + 600 mg mycelial extract. Chromatography of the combined extract on Al_2O_3 (5% deact.; 75 g) gave fractions A (940 mg), B (260 mg) and C (40 mg) which were purified by p.l.c. Fraction A [2 large plates; 2 x petrol-Et_2O (1:1)] gave s.m. [88] (707 mg). Fraction B [3 small plates; 5 x petrol-Et_2O (9:1)] gave 5β-androstan-3β-ol [159] (81 mg),

m.p. 143-144° (from MeOH-H_2O), $[\alpha]_D$ -0.3° (c 1.1) (lit.,[63] m.p. 143-143.5°, $[\alpha]_D$ +2°), $\nu_{max.}$ 3625 cm^{-1}, and 5β-androstan-3α-ol [160] (15 mg), m.p. 146-147° (from MeOH-H_2O), $[\alpha]_D$ -0.5° (c 0.7) (lit.,[63] m.p. 146-146.5°, $[\alpha]_D$ +7°), $\nu_{max.}$ 3620 cm^{-1}. Fraction C [1 small plate; 1 x petrol-Me_2CO (3:1)] gave a product [161], m.p. 210-215° (from $CHCl_3$-C_6H_{14}) (9 mg), $\nu_{max.}$ ($CHCl_3$) 3410 and 3620 cm^{-1}, m/e 274 (M^+, 100%).

Incubation of A-nor-(5 → 6β)abeo-androstan-5-one [100] with C.decora (Scheme 22)

(a) The ketone [100] (1 g) in Me_2SO (150 ml), 25 flasks, medium B, 5 d, extraction II → 960 mg broth extract + 560 mg mycelial extract. Chromatography of the mycelial extract on SiO_2 (20 g) and elution with petrol-Et_2O (19:1) gave s.m. [100] (415 mg). Chromatography of the broth extract on SiO_2 (50 g) and elution with petrol-$CHCl_3$ (3:1) gave fractions A (137 mg), B (110 mg) and C (408 mg) which were purified by p.l.c. Fraction A [2 small plates; 1 x petrol-Me_2CO (3:1)] gave product 1 [162] (79 mg) as a non-crystalline solid after repeated p.l.c. [1 small plate; 1 x Et_2O] (69 mg), m.p. 68-90°, $\nu_{max.}$ 1715, 3500, 3625 cm^{-1}. Fraction B [2 small plates; 2 x petrol-Me_2CO (2:1)] gave product 2 [163] (18 mg), m.p. 211-215° (from Me_2CO-C_6H_{14}) (6 mg) (Found: C, 73.7; H, 9.4. $C_{18}H_{28}O_3$ requires C, 73.9; H, 9.65%), $\nu_{max.}$ 1720, 3625 cm^{-1}, and product 3 [164] (51 mg) which was combined with a later fraction. Fraction C [1 large plate; 2 x petrol-Me_2CO (3:1)] gave product 3 [164] (85 mg) which was combined with the previous product, m.p. 183-184° (from Me_2CO-C_6H_{14}), $[\alpha]_D$ -62° (c 0.3) (Found: C, 72.6; H, 9.7. $C_{18}H_{28}O_3$ requires C, 73.9; H, 9.65%), $\nu_{max.}$ ($CHCl_3$) 1710, 3400, 3620 cm^{-1}, and product 4 [165] (98 mg) which was immediately acetylated. P.l.c. of the product [2 small

plates; 3 x petrol-Et_2O (3:1)] gave the keto-triacetate mixture [165] (81 mg), m.p. 164-169° (from C_6H_{14}), $\nu_{max.}$ 1710, 1735 cm^{-1}, $\tau_{C\text{-}methyl}$(C_6H_6) 8.82, 9.02, 9.45, 9.56.

(b) Oxidation of product 2 [162] (8 mg) with 8N-H_2CrO_4 afforded a hydroxy-dione [166] (2.5 mg), $\nu_{max.}$ 1710, 1740, 3630 cm^{-1}. Oxidation of product 3 [164] (50 mg) with 8N-H_2CrO_4 and p.l.c. of the mixture [1 small plate; 2 x petrol-Me_2CO] gave a trione [167] (16 mg), m.p. 167-169° (from C_6H_{14}), $\nu_{max.}$ 1710, 1720 and 1750 cm^{-1}.

Incubation of 3β-hydroxy-A-nor(5 ⟶ 6β)abeo-androstan-15-one [91] with C.decora (Scheme 23)

(a) The ketone [91] (1 g) in EtOH (50 ml), 25 flasks, medium B, 4 d, extraction II ⟶ 850 mg broth extract + 390 mg mycelial extract. P.l.c. of the mycelial extract [1 large plate; 1 x Et_2O] gave s.m. [91] (104 mg). Chromatography of the broth extract on SiO_2 (35 g) and elution with petrol-$CHCl_3$ (1:1) gave fraction A (37 mg). $CHCl_3$ gave fractions B (394 mg) and C (132 mg). $CHCl_3$-MeOH (9:1) gave fraction D (136 mg). These fractions were purified by p.l.c. Fraction A [1 small plate; 1 x Et_2O] gave 15α-hydroxy-A-nor-(5 ⟶ 6β)abeo-androstane-3,5-dione [168] (12 mg), m.p. 185-187° (from C_6H_{14}), $[\alpha]_D$ +30° (c 0.1) (Found: C, 74.3; H, 9.2. $C_{18}H_{26}O_3$ requires C, 74.4; H, 9.0%), $\nu_{max.}$ 1710, 1740, 3630 cm^{-1}. Fraction B [1 large plate; 2 x petrol-Me_2CO (3:1)] gave product 2 [169] (64 mg) as an oil, $[\alpha]_D$ -84° (c 0.3) (Found: C, 73.8; H, 9.8. $C_{18}H_{28}O_3$ requires C, 73.9; H, 9.65%), $\nu_{max.}$ 1720, 3630 cm^{-1}, and product 3 [170] (234 mg), m.p. 185-187° (from Me_2CO-C_6H_{14}), $[\alpha]_D$ -36.5° (c 0.4) (Found: C, 74.2; H, 9.9. $C_{18}H_{28}O_3$ requires C, 73.9; H, 9.65%), $\nu_{max.}$ 1720, 3630 cm^{-1}. Fraction C [2 large

plates; 5 x petrol-Me_2CO (3:1)] gave product 4 [171] (130 mg), m.p. 191-192° (from Me_2CO-C_6H_{14}), $[\alpha]_D$ +7.6° (c 0.5) (Found: 74.5; H, 9.8. $C_{18}H_{28}O_3$ requires C, 73.9; H, 9.65%), $\nu_{max.}$ 1720, 3630 cm^{-1}. Fraction D [1 large plate; 2 x petrol-Me_2CO (1:1)] gave product 5 [172] (68 mg), m.p. 135-175°, then 213-216° (from Me_2CO-C_6H_{14}) (Found: C, 66.0; H, 9.3. $C_{18}H_{28}O_4.H_2O$ requires C, 66.2; H, 9.3%), $\nu_{max.}$ 1720, 3630 cm^{-1}.

(b) Oxidation of [169] (6 mg) with 8$\underline{\underline{N}}$-$H_2CrO_4$ at 0°C afforded a product formulated as $\underline{\underline{A}}$-nor-(5 → 6β)abeo-androstane-3,5,15-trione [173] (3 mg), $\nu_{max.}$ 1710, 1740, 1750 cm^{-1}. Oxidation of product 3 [170] (60 mg) with 8$\underline{\underline{N}}$-$H_2CrO_4$ at 0°C, and p.l.c. of the residue [1 small plate; 2 x Et_2O] gave a hydroxy-dione [174] (23 mg), $\nu_{max.}$ 1718, 3625 cm^{-1}. Similar oxidation of product 4 [171] (50 mg) afforded a mixture, and with p.l.c. [1 small plate; 2 x Et_2O] the dominant product was isolated as an oil, $\nu_{max.}$ 1710, 1740, 3480 (v. str.), 3620 cm^{-1}.

Acetylation of product 5 [172] (100 mg) and p.l.c. of the product [1 small plate; 3 x petrol-Et_2O (3:1)] gave a keto-triacetate mixture [175] (92 mg) as an oil, $\nu_{max.}$ 1720, 1735 cm^{-1}, $\tau_{C\text{-methyl}}$ (C_6H_6) 8.82, 9.02, 9.47, 9.58.

EXPERIMENTAL. SECTION III.

[A]. The Synthesis of Mono-hydroxy Androstanes and their Acetate Derivatives

(Scheme 24)

5α-Androstan-1α-ol [178]. Br_2 (6.1 g) was added dropwise to a stirred solution of 5α-androstan-3-one [54] (10 g) and HBr (1 drop) in HOAc (1 l). Work-up gave 2α-bromo-5α-androstan-3-one [176a], m.p. (from $CHCl_3$) and mixed m.p.[99] 208-213° (8.92 g). A suspension of the bromo-ketone [176a] (8.92 g), LiCl (9.8 g) and Li_2CO_3 (8.4 g) in DMF (250 ml) was refluxed for 16 h under N_2. Work-up, chromatography of the product (6.8 g) on SiO_2 (3% deact.; 220 g) and elution with petrol-Et_2O (19:1) gave 5α-androst-1-en-3-one [176b] (4.91 g), m.p. 104-106° (from Me_2CO), $[\alpha]_D$ +39° (c 1.0) (lit.,[89] m.p. 104-106°, $[\alpha]_D$ +44°), $\nu_{max.}$ 3040 and 1678 cm^{-1}, and 5α-androst-4-en-3-one [87] (0.94 g), m.p. and mixed m.p. 103-104°.

A solution of the enone [176b] (2.6 g), H_2O_2 (30%; 20 ml) and NaOH (40%; 8 drops) in EtOH (50 ml) was refluxed for 10 min. Work-up gave 1α,2α-oxido-5α-androstan-3-one [176c], m.p. 103-105° (from EtOH-H_2O), $[\alpha]_D$ +102° (c 1.3) (lit.,[89] m.p. 105-106°, $[\alpha]_D$ +113°), $\nu_{max.}$ 1715 and 875 cm^{-1}.

A solution of N_2H_4 (anhydrous; 280 mg) in MeOH (3 ml) was added dropwise to a stirred solution of the epoxy-ketone [176c] (500 mg) in MeOH (20 ml) at 0°C under N_2. After 5 min, TsOH.H_2O (500 mg) was added and the yellow solution was stirred for 18 h at 20°C. Work-up gave a product (470 mg) which was chromatographed on Al_2O_3 (5% deact.; 15 g). Elution with petrol-Et_2O (19:1) gave 5α-androst-2-en-1α-ol [177] (372 mg), m.p. 105-106° (from EtOH-H_2O), $[\alpha]_D$ +136° (c 1.0) (lit.,[89] m.p. 103°, $[\alpha]_D$ +133°), $\nu_{max.}$ 3618, 3025,

1650 cm^{-1}. A similar reaction in air gave the unsaturated alcohol [177] (271 mg).

Hydrogenation of the unsaturated alcohol [177] (200 mg) in EtOAc (50 ml) over 10% Pd on C catalyst (100 mg) for 3 h gave 5α-androstan-1α-ol [178] (190 mg), m.p. 107-109° (from MeOH-H_2O), $[\alpha]_D$ +17° (c 0.8) (lit.,[89] m.p. 108-109°, $[\alpha]_D$ +18°), $\nu_{max.}$ 3628 cm^{-1}.

Acetylation of the alcohol [178] (150 mg) gave 5α-androstan-1α-yl acetate [179] (143 mg) as an oil, $[\alpha]_D$ +34° (c 1.8) (Found: C, 79.5; H, 10.4. $C_{21}H_{34}O_2$ requires C, 79.2; H, 10.75%), $\nu_{max.}$ 1735 cm^{-1}.

5α-Androstan-1α-yl acetate [179] (500 mg) was heated under N_2, in a Wood's alloy bath preheated to 380°C, for 10 min. P.l.c. of the residue [1 large plate; 1 x petrol-Et_2O (99:1)] and further purification by p.l.c. [2 small plates; 5% $AgNO_3$ impregnated in $HF_{254+366}$; 1 x petrol-Et_2O (99:1)] gave 5α-androst-1-ene [180] (220 mg), m.p. 40-41° (from MeOH-H_2O), $[\alpha]_D$ -26° (c 1.1) (Found: C, 88.2; H, 11.5. $C_{19}H_{30}$ requires C, 88.3; H, 11.7%), $\nu_{max.}$ 3025 and 1644 cm^{-1}.

5β-Androstan-5-ol [190]. A solution of 5α-androst-4-en-3-one [87] (1.3 g), H_2O_2 (30%; 10 ml) and NaOH (40%; 4 drops) in EtOH (25 ml) was heated under reflux for 10 min. Work-up followed by p.l.c. [3 large plates; 4 x petrol-Et_2O (19:1)] gave starting material [87] (120 mg) and 4β,5-oxido-5β-androstan-3-one [188] (585 mg), m.p. 115-116° (from EtOH-H_2O), $[\alpha]_D$ +129° (c 1.1) (Found: C, 79.2; H, 9.7. $C_{19}H_{28}O_2$ requires C, 79.1; H, 9.8%), $\nu_{max.}$ 1715 cm^{-1}.

A solution of N_2H_4 (anhydrous; 280 mg) in MeOH (3 ml) was added dropwise to a stirred solution of the oxido-ketone [188] (500 mg) in MeOH (20 ml) at 0°C in air. After 5 min, TsOH.H_2O (500 mg) was added and the yellow solution was stirred for 18 h at 20°C. Work-up and p.l.c. of the residue (503 mg) [1 large

plate; 3 x petrol-Et_2O (9:1)] gave 5α-androst-3-en-5β-ol [189] (274 mg), m.p. 120-121° (from EtOH), $[\alpha]_D$ +108° (c 0.8) (Found: C, 83.1; H, 11.15. $C_{19}H_{30}O$ requires C, 83.15; H, 11.0%), $\nu_{max.}$ 3620, 3020, 1650 cm^{-1}.

Hydrogenation of [189] (70 mg) in EtOAc (50 ml) over 10% Pd on C catalyst (50 mg) gave an oil. P.l.c. [1 small plate; 1 x petrol-Et_2O (19:1)] gave a non polar oil (34 mg), shown by n.m.r. to be a mixture (2:1) of 5α- and 5β-androstane, and 5β-androstan-5-ol [190] (26 mg), m.p. 78° (from MeOH-H_2O), $[\alpha]_D$ +10° (c 0.16) (Found: C, 82.3; H, 11.6. $C_{19}H_{32}O$ requires C, 82.5; H, 11.7%), $\nu_{max.}$ 3625 cm^{-1}.

Preparation of [193], [195] and [196]. Thionyl chloride (6 ml) was added to a mixture of androst-5-en-3β-ol [86] (13.0 g) and $PhNMe_2$ (10 ml) in $CHCl_3$ (100 ml) and Et_2O (100 ml) at 0°C. After 1 h, work-up and filtration of the Et_2O extract through Al_2O_3 gave 3β-chloro-androst-5-ene [191] (12.0 g), m.p. 114-115.5° (from MeOH) (lit., [9c] m.p. 114.5-115.5°). The chloro-olefin [191] (12.0 g) in Et_2O (140 ml) was added to a stirred solution of Na (4.2 g) in liquid NH_3 (130 ml). After 1 h excess NH_3 was allowed to evaporate, residual Na was destroyed with EtOH (50 ml), and the solution was concentrated to half volume. Work-up and filtration of the product through Al_2O_3 with petrol gave androst-5-ene [192] (9.5 g), m.p. 77-78° (from MeOH), $[\alpha]_D$ -128° (c 1.0) (lit., [9c] m.p. 77-78°, $[\alpha]_D$ -128°).

A solution of the olefin [192] (800 mg) and $BF_3.Et_2O$ (1.5 ml) in Et_2O (5 ml) was added dropwise to a solution of $NaBH_4$ (200 mg) in diglyme (20 ml) at 0°C. After 1 h, H_2O was added and work-up gave material (920 mg) which was dissolved in THF (10 ml) and treated with 4N-NaOH (20 ml) and H_2O_2 (30%; 10 ml) for 12 h. Work-up and p.l.c. of the product [2 large plates;

2 x petrol-Et_2O (19:1)] gave, (i) starting material (154 mg), (ii) 5β-androstan-6β-ol [193] (120 mg) as an oil, $\nu_{max.}$ 3620 cm^{-1}, and confirmed as the acetate [194], m.p. 80-81° (from MeOH), $[\alpha]_D$ -7° (c 0.9) (lit.,[9c] m.p. 80-81°), $\nu_{max.}$ 1735 cm^{-1}, and (iii) 5α-androstan-6α-ol [195] (380 mg), m.p. 97-99° (from MeOH), $[\alpha]_D$ +22° (c 0.8) (lit.,[9c] m.p. 99-100°, $[\alpha]_D$ +23°), $\nu_{max.}$ 1735 cm^{-1}.

Oxidation of [195] (380 mg) with 8N-H_2CrO_4 gave 5α-androstan-6-one [108] (340 mg), m.p. 95-96° (from MeOH), $[\alpha]_D$ -26° (c 0.9) (lit.,[91] m.p. 94-95°, $[\alpha]_D$ -26°).

The ketone [108] (300 mg) in THF (5 ml), added to a solution of $Li(OBu^t)_3AlH$ [from $LiAlH_4$ (75 mg)] in THF (5 ml), was stirred at 0°C for 3 h and at 20°C for 3 h. Work-up, and p.l.c. [1 large plate; 2 x petrol-Et_2O (9:1)] gave 5α-androstan-6β-ol [196] (270 mg), m.p. 77.5-78.5° (from MeOH), $[\alpha]_D$ -13.5° (c 1.0) (Found: C, 82.6; H, 11.5. $C_{19}H_{32}O$ requires C, 82.6; H, 11.7%), $\nu_{max.}$ 3620 cm^{-1}.

Preparation of [199], [200] and [201]. Huang-Minlon reduction of 11α-hydroxy-5α-androstan-3-one [72] (1 g) gave 5α-androstan-11α-ol [199] (920 mg), m.p. 107-109° (from MeOH), $[\alpha]_D$ -26° (c 0.8) (lit.,[95] m.p. 108-109°), $\nu_{max.}$ 3620 cm^{-1}.

Oxidation of [199] (500 mg) gave 5α-androstan-11-one [110] (467 mg), m.p. 48-50°, $[\alpha]_D$ +60° (c 0.9) (lit.,[100] m.p. 49-50°, $[\alpha]_D$ +65°), $\nu_{max.}$ 1710 cm^{-1}.

A mixture of the ketone [110] (300 mg) and $LiAlH_4$ (400 mg) in THF (5 ml) was heated under reflux for 2.5 h. Work-up gave 5α-androstan-11β-ol [200] (286 mg), m.p. 89-90° (from MeOH), $[\alpha]_D$ +17° (c 1.0) (lit.,[26] m.p. 91-92°), $\nu_{max.}$ 3645 and 3621 cm^{-1}.

A solution of the alcohol [200] (100 mg) and $TsOH.H_2O$ (10 mg) in HOAc (1 ml) and Ac_2O (0.2 ml) was kept at 100°C for 10 h and 20°C for 5 d. Work-up gave 5α-androstan-11β-yl acetate [201] (83 mg), m.p. 70-72° (from MeOH), $[\alpha]_D$ +33° (c 0.9) (lit.,[101] m.p. 70-72°, $[\alpha]_D$ +35°), $\nu_{max.}$ 1735 cm^{-1}.

Preparation of 5α-androstan-15β-yl acetate [223]. A solution of 5α-androstan-15β-ol [20] (100 mg) in C_5H_5N (3 ml) and Ac_2O (3 ml) was heated at 70°C for 5 h. Work-up gave 5α-androstan-15β-yl acetate [223] (92 mg), as an oil, $[\alpha]_D$ -57° (c 3.3) (lit.,[19] $[\alpha]_D$ -45°) (Found: C, 78.95; H, 10.6. $C_{21}H_{34}O_2$ requires C, 79.2; H, 10.8%), $\nu_{max.}$ 1735 cm^{-1}.

[B] The Synthesis of 14-Methylated Androstanes (Scheme 27)

A solution of 15-oxo-5α-androstan-17β-yl toluene-p-sulphonate [36] (500 mg) and $KOBu^t$ [from K (2 g)] in $HOBu^t$ (60 ml) was stirred under N_2 at 20°C for 1.5 h. MeI (5.4 ml) was added at 10°C, and the suspension was stirred at 20°C for 5 h. Work-up and p.l.c. [1 large plate; 4 x petrol-Et_2O (19:1)] gave 14β-methyl-5α-androst-16-en-15-one [231] (260 mg), m.p. 103-104° (from MeOH-H_2O), $[\alpha]_D$ +90° (c 1.0) (Found: C, 83.8; H, 10.7. $C_{20}H_{30}O$ requires C, 83.9; H, 10.6%), $\lambda_{max.}$ 230.6 nm (ε 7060), $\nu_{max.}$ 3040, 1705 cm^{-1}.

Similar reaction of [36] (220 mg) with CD_3I (2 g) afforded 14β-methyl-d_3-5α-androst-16-en-15-one [232] (118 mg), m.p. 101-103° (from MeOH-H_2O), $[\alpha]_D$ +88° (c 0.9), $\nu_{max.}$ 3040, 2240, 1705 cm^{-1}.

Hydrogenation of the enone [231] (150 mg) in EtOAc (25 ml) over 10% Pd on C catalyst (100 mg) for 4 h gave 14β-methyl-5α-androstan-15-one [233] (138 mg), m.p. 114-116° (from MeOH-H_2O), $[\alpha]_D$ -18° (c 1.1) (Found: C, 83.2; H, 11.3. $C_{20}H_{32}O$ requires C, 83.4; H, 11.2%), $\nu_{max.}$ 1740 cm^{-1}.

Similar hydrogenation of the deuteriated enone [232] afforded 14β-methyl-d_3-5α-androstan-15-one [234], m.p. 113-114° (from MeOH-H_2O), $\nu_{max.}$ 2230, 1740 cm^{-1}.

A solution of 5α-androstan-15-one [1] (500 mg) and MeI (12 ml) in C_6H_6 (150 ml) at 5°C, was added dropwise to a solution of $KOBu^t$ (10 g) in C_6H_6 (150 ml) and the mixture was stirred under N_2 at 20°C for 8 h. Work-up gave an oil (700 mg) and p.l.c. [2 large plates; 1 x petrol-Et_2O (9:1)] afforded 14β-methyl-5α-androstan-15-one [233] (190 mg), m.p. and mixed m.p. 113-115°.

Huang-Minlon reduction of the ketone [233] (75 mg) under forced conditions[46] afforded 14β-methyl-5α-androstane [235] (41 mg), m.p. 57-59° (from MeOH), $[\alpha]_D$ -31.4° (c 0.24) (Found: C, 87.5; H, 12.3. $C_{20}H_{34}$ requires C, 87.5; H, 12.5%). Similar reduction of the deuteriated ketone [234] (80 mg) gave 14β-methyl-d_3-5α-androstane [236] (20 mg), m.p. 56-58° (from MeOH), $\nu_{max.}$ 2225 cm^{-1}.

14β-Methyl-5α-androstan-15-one [233] (190 mg) in Et_2O (20 ml) was irradiated in a 'Rayonet' reactor at 3,500A for 4.5 h. P.l.c. of the residue [2 small plates; 4 x petrol-Et_2O (19:1)] gave starting material [233] (128 mg), m.p. and mixed m.p. 112-114°, and a complex mixture of polar products. Irradiation of the ketone [233] (200 mg) for 16 h gave starting material (92 mg).

[C] Reduction with Diimide

General Procedure: $CuSO_4$ (1% aqueous; 0.1 ml) was added to a solution of the steroidal olefin (1 mmole), H_2O_2 (30%; 0.7 ml) and N_2H_4 (160 mg) in MeOH (2.5 ml) at 0°C, and the mixture was stirred for 30 min. Work-up gave a residue which was purified by p.l.c. (1 large plate). Yields quoted were

calculated from the weight of product obtained. Products were identified by m.p. and spectral comparison with an authentic sample.

5α-Androst-1-en-3β-ol [237]. A solution of 5α-androst-1-en-3-one [176b] (500 mg) in Et_2O (15 ml) was added dropwise to a stirred suspension of $LiAlH_4$ (150 mg) in Et_2O (15 ml) at 0°C and the mixture was stirred at 0°C for 2 h, then at 20°C for 2 h. Work-up followed by p.l.c. [1 large plate; 3 x petrol-Et_2O (4:1)] gave 5α-androst-1-en-3β-ol [237] (405 mg), m.p. (from C_6H_{14}) and mixed m.p.[88] 120-122°, $\nu_{max.}$ 3610 cm^{-1}.

5α-Androst-16-ene [240]. A solution of 5α-androstan-17-one [4] (4 g), $N_2H_4.H_2O$ (20 ml) and Et_3N (6 ml) in EtOH (30 ml) was refluxed for 2 h. The mixture was poured into H_2O (800 ml), and filtration gave 5α-androstan-17-one hydrazone [238] (3.64 g), m.p. 151-155° (from EtOH) (lit.,[9e] m.p. 154-156°).

A solution of I_2 in THF (25%; 22 ml) was added dropwise to a stirred solution of the hydrazone [238] (3.6 g) and Et_3N (26 ml) in THF (85 ml) until the iodine colour persisted. Saturated $Na_2S_2O_3$ (25 ml) was added, and THF removed in vacuo at less than 40°C. Work-up gave 17-iodo-5α-androst-16-ene [239], m.p. 124-126° (from Me_2CO) (2.55 g) (lit.,[9e] m.p. 124-126°), $\nu_{max.}$ 3065 cm^{-1} (CS_2).

Na (5.1 g) was added portionwise to a refluxing solution of the iodo-olefin [239] (2.55 g) in dry i-PrOH (130 ml). After 3.5 h, work-up gave a residue (1.73 g), which was filtered through Al_2O_3 (50 g) with petrol to give 5α-androst-16-ene [240], m.p. 77-78.5° (from MeOH) (1.35 g), $[\alpha]_D$ +13° (c 1.0) (lit.,[167] m.p. 78-79°, $[\alpha]_D$ +15°), $\nu_{max.}$ 3050 cm^{-1}.

[D] The Problem of Removal of a 15-Oxo Group

A solution of 5α-androstan-15-one [1] (100 mg) and $BF_3.Et_2O$ (0.3 ml) in $HS[CH_2]_2SH$ (0.5 ml) and $CHCl_3$ (5 ml) was kept at 20°C for 12 h, diluted with $CHCl_3$ (10 ml) and washed with saturated $NaHCO_3$ solution. Work-up gave 15,15-ethylenedithio-5α-androstane [242] (120 mg), m.p. 149-150° (from MeOH), $[\alpha]_D$ -15° (c 0.9) (Found: C, 72.2; H, 9.8; S, 18.2. $C_{21}H_{34}S_2$ requires C, 71.95; H, 9.8; S, 18.25%), $\tau_{max.}$ 9.17 (3H, 19-H), 9.11 (3H, 18-H) and 6.5-6.9 (4H, m).

5α,14β-Androstane-15-one [2] (100 mg), reacted as above, gave a product (127 mg), m.p. 148-152° (from MeOH), $\tau_{max.}$ 9.26 (1 unit), 9.17 (1 unit), 9.11 (1 unit) and 8.95 (1 unit).

[E] Feedings of 15-ketones [1] and [2] with A.ochraceus and R.nigricans

Incubation of 5α-androstan-15-one [1] with A.ochraceus: The ketone [1] (920 mg) in Me_2SO (138 ml), 23 flasks, medium B, 4 d, extraction II ⟶ 1.15 g broth extract + 920 mg mycelial extract. P.l.c. of the mycelial extract [2 large plates; 1 x petrol-Et_2O (9:1)] gave s.m. (491 mg). P.l.c. of the broth extract [3 large plates; 1 x petrol-Me_2CO (2:1)] afforded no steroidal products.

Incubation of 5α,14β-androstan-15-one [2] with A.ochraceus: The ketone [2] (1 g) in Me_2SO (150 ml), 25 flasks, medium B, 4 d, extraction II ⟶ 720 mg total extract. Chromatography on Al_2O_3 (5% deact.; 20 g) and elution with petrol-Et_2O (20:1) gave s.m. (372 mg). Elution with $CHCl_3$ afforded no steroidal material.

Incubation of 5α-androstan-15-one [1] with R.nigricans: The ketone [1] (1 g) in Me_2SO (150 ml), 25 flasks, medium B, 4 d, extraction II ⟶ 1.6 g broth

extract + 1.24 g mycelial extract. P.l.c. of the mycelial extract [3 large plates; 1 x petrol-Et_2O (9:1)] gave s.m. [1] (593 mg). P.l.c. of the broth extract [3 large plates; 1 x petrol-Me_2CO (2:1) gave 3 steroidal products (11 mg, 16 mg, 9 mg) which were not characterised.

Incubation of 5α,14β-androstan-15-one [2] with R.nigricans: The ketone [2] (1 g) in Me_2SO (150 ml), 25 flasks, medium B, 4 d, extraction II ⟶ 400 mg broth extract + 1.04 g mycelial extract. Chromatography of the mycelial extract on Al_2O_3 (5% deact.; 30 g) and elution with petrol-Et_2O (20:1) gave s.m. (530 mg). P.l.c. of the broth extract [1 large plate; 2 x petrol-Me_2CO (3:1)] gave 4 steroidal products (19, 33, 11 and 11 mg) which were not characterised.

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