N-HETEROCYCLIC ANALOGUES OF STEROIDS.

by


A Thesis presented for the Degree of Doctor of Philosophy

University of Edinburgh

October 1976.
TO MY PARENTS
Declaration

I declare that this thesis is my own composition, that the work of which it is a record has been carried out by myself and that it has not been submitted in any previous application for a Higher Degree.

This thesis describes results of research carried out in the Department of Chemistry, University of Edinburgh under the supervision of Dr. P.J. Sykes between October 1973 and September 1976.
Acknowledgements.

I should like to express my thanks to Dr. P.J. Sykes for his guidance and help during this work, to Professor J.I.G. Cadogan for the provision of technical and library facilities and to my mother for typing the manuscript. The award of maintenance grants from the Science Research Council and the University of Edinburgh is gratefully acknowledged.
The following Postgraduate Lecture Courses were attended:

1. Thermal Analysis (5 lectures) Drs. B. Lowe and H.P. Leach (University of Edinburgh).
2. Industrial Research and Development (5 lectures) Dr. B. Gravenor (University College, Swansea).
3. Carbonium Ions (5 lectures) Dr. K. Capon (University of Glasgow).
5. Molecular Rearrangements (5 lectures) Dr. G. Tennant (University of Edinburgh).
6. High Speed Liquid Chromatography (5 lectures) Prof. J. Knox and Dr. J. Done (University of Edinburgh).
7. Biomimetic Chemistry (5 lectures) Dr. M. Paton (University of Edinburgh).
8. N.M.R. Spectroscopy (5 lectures) Dr. R.K. Harris (University of East Anglia).
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Abbreviations

\< \quad \text{extinction coefficient}

h \quad \text{hours}

i.r. \quad \text{infra-red}

J \quad \text{coupling constant}

\lambda_{\text{max}} \quad \text{wavelength of maximum absorption}

lit \quad \text{literature}

m/e \quad \text{mass to charge ratio}

m.p. \quad \text{melting point}

M^+ \quad \text{molecular (parent) ion}

n.m.r. \quad \text{nuclear magnetic resonance}

ppm \quad \text{parts per million}

R(\ ) \quad \text{the ratio of the distance travelled by a component on a t.l.c. plate to the distance travelled by a standard component on the same plate, the identity of the standard being given in parenthesis}

\delta \quad \text{chemical shift}

sh \quad \text{shoulder}

t.l.c. \quad \text{thin layer chromatography}

u.v. \quad \text{ultra violet}

\nu_{\text{max}} \quad \text{frequency of absorption maximum}
Nomenclature.

The naming of steroids with heterocyclic rings fused to the nucleus of the steroid, the syntheses of some of which are described in this present work, is somewhat complicated. The steroid fragments of these compounds have been named in accordance with IUPAC rules while the heterocyclic ring system has been named after the analogous heterocyclic system prepared by the condensation of simple aliphatic $\beta$-dicarboxylic acids and 2-aminopyridinium iodide. This procedure can be justified by the similarity in the spectral properties of the steroid heterocycles and the simple model compounds.

Thus since structure (13) is known as 2,4-dimethyl-pyrido-[1,2-a]pyrimidin-5-ium iodide, the steroid (59) has been assigned the name 17$\beta$-hydroxy-5$\alpha$-androst-2-eno-[2,3-e]pyrido-[1,2-a]pyrimidin-5$'$.ium iodide. [2,3-e] describes the fusion of the steroid fragment to the pyrimidine ring of the pyrido-[1,2-a]pyrimidinium fragment, the numerals referring to the A-ring of the steroid and the letter $e$ to the side of the pyrimidine ring fused to the steroid. [1,2-a] describes the fusion of the pyridine ring to the pyrimidine ring. The location of the positive charge on the bridgehead quaternized nitrogen is described with respect to the whole pyrido-[1,2-a]pyrimidinium system (i.e. position 5').

Similarly structure (146) is known as 2-methyl-4H-pyrido-[1,2-a]pyrimidin-4-one and the analogous steroid heterocycle (108) has been given the name, 17$\beta$-hydroxy-17$\alpha$-methyl-5$\alpha$-androst-2-eno-[3,2-d]-4$'$.H-pyrido-[1,2-a]pyrimidin-4-one. [3,2-d] describes the fusion of the steroid fragment to the pyrimidine ring of the pyrido-[1,2-a]pyrimidin-4-one fragment. The position of the carbonyl group is described in relation to the whole pyrido-[1,2-a]pyrimidine system (i.e. position 4').
Summary.

The reaction of 2-aminopyridinium iodide with several steroidal β-dicarbonyls was studied.

17β-hydroxy-2-hydroxymethylene-5α-androstan-3-one condensed with 2-aminopyridine to give 17β-hydroxy-2(2'-pyridyl-aminomethylene)-5α-androstan-3-one. The analogous 2-anilino-methylene derivative was similarly prepared. These aromatic amines condense with the formyl carbonyl of the steroid in contrast to hydrazine which condenses with the ketone carbonyl. 17β-acetoxy-2(2'-pyridyl-aminomethylene)-5α-androstan-3-one cyclized in an acid catalyzed reaction to give 17β-acetoxy-5α-androst-2-eno-[2,3-e] pyrido-[1,2-a] pyrimidin-5'-ium iodide. 17β-hydroxy-5α-androst-2-eno-[2,3-e] pyrido-[1,2-a] pyrimidin-5'-ium iodide was obtained in a one step reaction when 17β-hydroxy-2-hydroxymethylene-5α-androstan-3-one was condensed with 2-aminopyridinium iodide.

3β-hydroxy-16-hydroxymethylene-androst-5-en-17-one condensed with 2-aminopyridine to give 3β-hydroxy-16(2'-pyridyl-aminomethylene)-androstan-5-en-17-one. This enaminone showed less hydrogen bonding than was found in 17β-hydroxy-2(2'-pyridyl-aminomethylene)-5α-androstan-3-one. It was not possible to obtain 3β-hydroxy-androst-5,16-dieno-[2,3-e] pyrido-[1,2-a] pyrimidin-5'-ium iodide by cyclization of the enaminone or by the condensation of 3β-hydroxy-16-hydroxymethylene-androst-5-en-17-one and 2-aminopyridinium iodide, probably for mechanistic reasons.

17β-acetoxy-2α-acetyl-5α-androstan-3-one condensed with 2-aminopyridinium iodide to give 17β-acetoxy-5α-androst-2-eno-[2,3-e] -2'-methyl-pyrido-[1,2-a] pyrimidin-5'-ium iodide in very low yield. The condensation product of 17β-acetoxy-2α-acetyl-5α-
androstan-3-one and 2-aminopyridine was not isolated and it was concluded that this reaction was inhibited by steric hindrance caused by the methyl group of the 2-acetyl fragment of the steroid.

17β-hydroxy-2(2',pyridyl-aminomethylene)-androst-4-en-3-one was obtained by condensation of 17β-hydroxy-2-hydroxymethylene-androst-4-en-3-one and 2-aminopyridine. The 17β-acetate of this enaminone was cyclized to give 17β-acetoxy-androst-2,4-dieno-[2,3-e] pyrido-[1,2-a] pyrimidin-5′-ium iodide. When 17β-hydroxy-2-hydroxymethylene-androst-4-en-3-one and 2-aminopyridinium iodide were condensed two products were obtained, 17β-hydroxy-androst-2,4-dieno-[2,3-e] pyrido-[1,2-a] pyrimidin-5′-ium iodide and another which was not definitely identified but which may be 4',17β-dihydroxy-androst-2,5-dieno-[2,3-e] pyrido-[1,2-a] pyrimidin-5′-ium iodide.

2α-carbomethoxy-17β-hydroxy-17α-methyl-5α-androstan-3-one and 2-aminopyridinium iodide condensed to give 17β-hydroxy-17α-methyl-5α-androst-4-en-3-one. The formation of this steroid was shown to occur by condensation of 2-aminopyridine with 2α-carbomethoxy-17β-hydroxy-17α-methyl-5α-androstan-3-one to give 17β-hydroxy-17α-methyl-2α(2'-pyridyl-amido)-5α-androstan-3-one which then condensed at the 3-ketone position with another molecule of 2-aminopyridine and cyclized by loss of the original 2-aminopyridine molecule to give the steroidal [3,2-d]-4'H-pyrido-[1,2-a] pyrimidin-4′-one. 17β-hydroxy-17α-methyl-2α(2'-pyridyl-amido)-5α-androstan-3-one was cyclized in the presence of acid to give 17β-hydroxy-17α-methyl-5α-androst-2-eno-[2,3-e]-2H-pyrido-[1,2-a] pyrimidin-2′-one.
1. Introduction

Kockakian,¹ in 1935, first observed that the androgens, the male sex hormones, possessed the ability to stimulate the synthesis of cellular protein, associated with the secondary male characteristic of muscle development. It was quickly appreciated that compounds with this property would provide valuable medications in the treatment of conditions involving abnormally low tissue protein content. However, the natural steroids which possess this anabolic effect were unacceptable for clinical use, particularly with women and children, because of the virilising androgen effect.²

An assay of the relative anabolic and androgenic ratio was developed by Eisenberg and Gordan⁴ and later modified by Herschberger et al.⁵ Examination of the natural androgens showed that almost all had an anabolic/androgenic ratio of ca. 1,0, so that if strongly androgenic they were also strongly anabolic but if only weak androgens the anabolic effect was weak. The opinion of Kruskemper⁵ that "the androgenic effect differs from the anabolic effect only in its location and not in its essence, thus anabolic steroids which possess no androgenic character cannot exist" is not universally shared and anabolic steroids with no androgenic action are still sought.⁶

The need for an anabolic agent with few or no androgenic or progestational side effects led to attempts to vary endocrine activity patterns by means of alteration within, or substitutions on the steroid nucleus. It was assumed by most workers in this field that the target receptors (enzyme systems, for example) at the sites of anabolic and androgenic activities differed sufficiently to make at least theoretically feasible the synthesis of a compound which would "fit" only one of these cellular receptor systems. In 1961,
(1) R = H
(2) R = CH₃
(3) R = H
(4) R = CH₃
(5) R = H
(6) R = CH₃
Clinton et al.\textsuperscript{7} in an attempt to alter the type or stability of receptor site bonding by substituting an "aromatic" nitrogen for oxygen at the C-3 position and thus change the nucleophilic environment of the area, prepared a series of $\Delta^4$-androstano-$[3,2-c]$ pyrazoles (1) and their $\Delta^4$ analogues (2) by the reaction of hydrazine or a substituted hydrazine on the 2-hydroxy-methylene derivatives of 17$\beta$-hydroxy-5$\Delta$-androst-3-one and androst-4-en-3-one respectively. The introduction of a $[3,2-c]$ pyrazole ring on to a steroid was found to produce quite pronounced changes in endocrinological activity especially in the altered anabolic/androgenic ratios and in the development of new types of activity. For example 17$\beta$-hydroxy-5$\Delta$-androst-3-one (3) and its 17$\alpha$-methyl derivative (4) have little clinical application as they are both anabolic and androgenic. The corresponding $[3,2-c]$ pyrazoles on the other hand exhibit a much greater separation of activities.\textsuperscript{8}

Both anabolic and androgenic activities fell off abruptly in the 17$\alpha$-alkyl-17$\beta$-hydroxy-5$\Delta$-androstan-3-one-$[3,2-c]$ pyrazoles when the 17$\alpha$-alkyl group was larger than ethyl indicating rather severe restrictions on "fit" to the cellular receptor site in the presence of the $[3,2-c]$ pyrazole ring. N-methylation also decreased both androgenic and anabolic activities in comparison with the parent steroidal $[3,2-c]$ pyrazole while acylation of the pyrazole ring imparted a low degree of estrogenicity to the androstano-$[3,2-c]$-pyrazoles without affecting the anabolic/androgenic ratios.

The series of 17$\beta$-hydroxy-androst-4-eno-$[3,2-c]$ pyrazoles (2) had anabolic/androgenic ratios comparable to their saturated analogues in the 5$\Delta$-androstano-$[3,2-c]$ pyrazole series.

Some 17$\alpha$-alkyl-17$\beta$-hydroxy-2-hydroxymethylene-5$\Delta$-androstan-3-ones (5) and their $\Delta^4$ analogues (6) show a good degree
of oral anabolic activity and have good anabolic/androgenic ratios but in all cases the anabolic activities of the 2-hydroxymethylene-3-keto precursors have been found to be less favourable than their [3,2-c] pyrazole derivatives. 17β-hydroxy-2-hydroxymethylene-17α-methyl-5α-androstan-3-one (oxymetholone)⁹ (5′,R=CH₃) is one such potent anabolic agent with minimal androgenic effect. A model of this steroid shows that the hydrogen bonding between the hydroxymethylene hydroxyl group and the C-3 carbonyl group leads to the formation of a flat six-membered "ring" fused to the A-ring and that this additional "ring" is bent towards the α-side of the molecule. The 2-hydroxymethylene-3-keto steroids are similar, with respect to the additional flat "ring", to their [3,2-c] pyrazole derivatives and to other anabolic steroids in which a heterocyclic ring is fused to the C-2 and C-3 of the ring. It is possible that the ability of oxymetholone (5′,R=CH₃) and its heterocyclic derivatives to produce anabolic activity may be related to their geometry at the A-ring end of each molecule. This hypothesis is in accordance with the concept that different structural features of the molecule may be responsible for different requirements for hormonal activity.¹⁰

3β-hydroxy-5α-androstano- and androst-5-eno-[17,16-c] pyrazoles (7) have been synthesized by the action of hydrazine hydrate on the corresponding 16-hydroxymethylene-17-ketones¹¹ but have been found not to exhibit any androgenic or anabolic activity. However a number of 5α-androstano- and androst-5-eno-[17,16-c]-5′ methyl pyrazoles (8) synthesized from pregnan- and pregn-ene-16, 20-diones (9) and hydrazine have been shown to be useful as diuretic and anabolic steroids.¹²

Steroids exhibiting high anabolic but low androgenic activity have also been produced when an isoxazole ring is fused at the 4 and 5 positions to the C-2 and C-3 of a steroid nucleus.¹³
These 17β-hydroxy-androst-2-eno-[2,3-d] isoxazoles (10) were prepared by the reaction of the corresponding 2-hydroxymethylene-3-keto steroid and hydroxylamine hydrochloride in ethanol. The 17β-(3-cyclohexyl-propionyloxy) derivative of 17β-hydroxy-5α-androst-2-eno-[2,3-d] isoxazole (10) was found to be a very potent anabolic agent with long duration of action and minimal androgenicity. The isomeric [3,2-c] isoxazole (11) has been synthesized either by the condensation of the 2-hydroxymethylene-3-keto steroid and hydroxylamine hydrochloride in pyridine or by the reaction of the 2-cyano-3-oxo-steroid and hydroxylamine hydrochloride in ethanol.

There is no report in the literature of the synthesis of androst-16-eno-[16,17-d] isoxazoles (12) but the preparation of the isomeric androst-16-eno[17,16-d]-3'-methyl-isoxazole (13) has been described.

Steroidal [3,2-d] pyrimidines have been prepared by the reaction of a 2-hydroxymethylene-3-keto steroid with a lower alkylamidine hydrochloride in the presence of strong base. 17β-hydroxy-5α-androstano-[3,2-d]-2'-methyl-pyrimidine (14), for example, was synthesized from 17β-hydroxy-2-hydroxymethylene-5α-androstan-3-one (5, R=H) and acetamidine hydrochloride. 17β-hydroxy-5α-androstano-[3,2-d]-2',6'-dimethyl-pyrimidine (15) and 17β-hydroxy-androst-4-eno-[3,2-d]-2'-methyl-pyrimidine (16) have also been synthesized from 2α-acetyl-17β-hydroxy-5α-androstan-3-one (17) and 17β-hydroxy-2-hydroxymethylene-androst-4-en-3-one (6, R=H) respectively. The use of a guanidine salt in place of acetamidine hydrochloride gave the analogous [3,2-d]-2'-amino-pyrimidines (18). One of these steroids, 17β-hydroxy-17α-methyl-5α-androstano-[3,2-d]-2'-amino-pyrimidine, has been found to decrease nitrogen excretion in male and female humans but to increase 17-keto steroid excretion in the female and
decrease it in the male. 17β-hydroxy-5α-androstano-[3,2-d]-2',6'β-diamino-pyrimidines (19) and their Δ[4] analogues have been prepared by the reactions of cyanoguanidine and 3-ketosteroids at 230-250°. These steroids have been reported to be androgenic and active against gram negative and gram positive bacteria.

3α-hydroxy-5α-androstano-[17,16-d]-2'-methyl-pyrimidine (20) which possesses hormonal activity has been synthesised from the 16β-hydroxy-methylene-17-keto steroid and acetamidine hydrochloride in the presence of sodium methoxide.

Greater variation in the substituents at the 2 and 6 positions in the pyrimidine ring has been obtained by the modification of 2-cyano-3-keto steroids prior to either reaction with ammonia or hydroxylamine, or cyclization at high temperature. De Ruggieri et al. prepared a series of androstano-[3,2-d]-6'-amino-2'-hydroxy-pyrimidines (21) from 2-cyano-3-keto steroids by conversion of the latter to the 2-cyano-3-aminourethano-androst-2-ene (22) in two stages followed by cyclization at 130° in an autoclave. This mode of synthesis was further developed by the same workers who having prepared an androstano-[3,2-c]-5'-aminoisoazole (23) by condensing hydroxylamines and a 2-cyano-androst-3-one, opened the isoxazole ring by hydrogenation to give the enamine (24), which was then treated with ethyl orthoformate to give the androstano-[3,2-d]-6'-hydroxy-pyrimidine (25). The 17β-O-carbomethoxy derivative of this steroid has been prepared more recently by treatment of 2x,17β-O-dicarbomethoxy-3-ethoxyformimido-5α-androstane-2-ene (26), derived from 2x,17β-O-dicarbomethoxy-5α-androst-3-one, with a saturated ethanolic ammonia solution. Formation of the 5'-N-ethylurethane derivative (27) of the steroidal [3,2-c]-5'-aminoisoazole (23) followed by reduction to give the enamine (28) and then treatment with base yielded the androstano-[3,2-c]-2',6'-dihydroxy-
More steroidal pyrimidines, many of which are useful as antibacterial and antiviral agents and also in the treatment of cardiovascular diseases and hyperlipidemia, were obtained by the treatment of 2-carbamoyl-3(\textit{N}-ethoxy-methylene)-amino-5\&-androst-2-ones (30) with ammonia or hydroxylamine to give the \([3,2-\text{d}]-6'\text{-hydroxy-pyrimidine}\) (25) and the \([3,2-\text{d}]-2',6'\text{-dihydroxypyrimidine}\) (29) derivatives respectively and by refluxing 2-formyl- or 2-acetyl-carbamoyl-3-amino-17\&-acetoxy-5\&-androst-2-ones (31) and ethylene glycol to give the \([3,2-\text{d}]-6'\text{-hydroxy-pyrimidine}\) (25) and \([3,2-\text{d}]-6'\text{-hydroxy-2'-methyl-pyrimidine}\) (32) respectively.

N-heterocyclic analogues of anabolic steroids with two ring systems are also known. Bardos et al.\(^2\) prepared 17\&-acetoxy-5\&-androstano-[4,3-\text{g}]-2',4'\text{-diamino-pteridine}\) (33), which was found to be a folic acid antagonist with lipid solubility, by heating 4-chloro-17\&-hydroxy-androst-4-en-3-one in a mixture of absolute ethanol and acetic acid with the bisulphite of 2,4,5,6-tetraaminopyrimidine. The analogous \([2,3-\text{g}]-2',4'\text{-diamino-pteridine}\) (34), synthesised from 2\&-bromo-17\&-hydroxy-5\&-androstano-3-one, has oral anti-androgenic activity in rat and chick assays, Yoneda et al.\(^2\) developed an alternative synthesis of 17\&-hydroxy-5\&-androstano-[2,3-\text{g}]-2',4'\text{-diamino-pteridine}\) by fusing 5-\(1,2\text{-diethoxycarbonylhydrazino}\)-2,4,6-triamino-pyrimidine\(^3\) with 17\&-hydroxy-3-morpholino-5\&-androst-2-ene. The \([2,3-\text{g}]-2'\text{-amino-4'\text{-hydroxy-pteridine}}\) derivative was similarly prepared. In a later paper\(^3\), the same authors describe improved methods of synthesis and pteridino steroids with a variety of substituents on the pteridine ring system.

More recently the synthesis of steroidal pyrido-[2,3-\text{d}]
pyrimidines has been described\(^4,5\). 17\&-hydroxy-2-hydroxymethylene-5\&-androst-3-one (5;\(R=\text{H}\)) and 17\&-hydroxy-2-hydroxymethylene-androst-
(35) \( X = 0, S, NH \).

(36) \( X = 0, S, NH \).

(37)

(38)

(39)
-4-en-3-one (6, R=H) on condensation with 4-aminouracils in aqueous acetic acid gave 17β-hydroxy-5α-androstan-2,3-g-1',2',3',4'-tetrahydro-pyrido-[2,3-d] pyrimidine (35) \(^{32}\) and the \(^1\)D analogue \(^{33}\) respectively. The analogous 3β-hydroxy-5α-androst-16-en-17-one-1',2',3',4'-tetrahydro-pyrido-[2,3-d] pyrimidine (36) \(^{34}\) was synthesised from the 16-aminomethylene-17-keto derivative of 3β-hydroxy-16-hydroxymethylene-5α-androstan-17-one.

**Steroids containing a bridgehead nitrogen atom.**

In 1965 Doorenbos and Wu \(^{35}\) reported the synthesis of cholestane-4-aza-5-eno-[4,3-a]-1',4',5',6'-tetrahydropyrimidine (37, R\(^1=\)C\(_8\)H\(_{17}\), R\(^2=\)H) and 17β-hydroxy-17α-methyl-androst-4-eno-[4,3-a]-1',4',5',6'-tetrahydropyrimidine (37, R\(^1=\)OH, R\(^2=\)CH\(_3\)) by treating 3,5-seco-14-norcholestan-5-on-3-oic acid (38, R\(^1=\)C\(_8\)H\(_{17}\), R\(^2=\)H) and 17β-hydroxy-17α-methyl-3,5-seco-17β-methylandrostane-5-on-3-oic acid (38, R\(^1=\)OH, R\(^2=\)CH\(_3\)) with 1,3-diaminopropane at 210°. The cholestane derivative (37, R\(^1=\)C\(_8\)H\(_{17}\), R\(^2=\)H) is a potent inhibitor of cholesterol biosynthesis, an anti-inflammatory agent and a diuretic when administered subcutaneously but inactive when administered orally. The androstane derivative has little activity.

Catsoulacos and Souli \(^{36}\) prepared 3β-acetoxy-5α-androst-16-en-17-one-7'-methyl-imidazo-[1,2-a] pyridine (39) from 3β-acetoxy-16β-bromo-5α-androstan-17-one and 2-amino-4-methyl-pyridine while more recently Yamazaki et al. \(^{37}\) synthesised a 9,14-diazasteroid system (41) by reduction of the pyrrolidylquinoline (40) to the alcohol followed by quaternization.

This present work describing the synthesis and attempted synthesis of androstane derivatives with a pyrido-[1,2-a] pyrimidine ring system which contains a bridgehead nitrogen atom fused to the steroid nucleus is based on a study made by Potts et al. \(^{38}\) on the condensation reaction of 2-aminopyridinium iodide (42) \(^{39}\) with aliphatic
dicarbonyls.

Potts et al. 38 condensed acetyl acetone and 2-aminopyridinium iodide by refluxing them in pyridine to yield 2,4-dimethylpyrido-[1,2-a] pyrimidin-5-ium iodide (43). 5-methyl-1,2,3,4-tetrahydro-pyrido-[1,2-a] quinazolin-11-ium iodide (44) was similarly prepared from 2-acetyl cyclohexanone. The condensation of 2-aminopyridinium iodide and ethyl acetoacetate gave 1,4-dihydro-2-methyl-4-oxopyrido-[1,2-a] pyrimidin-1-ium iodide which on treatment with sodium hydroxide readily gave 2-methyl-1H-pyrido-[1,2-a] pyrimidin-4-one (46).

Many pyrido-[1,2-a] pyrimidin-2- and 4-ones have been prepared by the reaction of 2-aminopyridines with a variety of acetylenic esters and 2-ketoesters, but relatively few pyrido-[1,2-a] pyrimidinium salts not bearing an oxo- or an imino- substituent have been described. Nesmeianov and his co-workers 41 isolated a series of compounds which they suggested were acylethylidenaminopyridines (47) by the reaction of 2-aminopyridine with acylacetalddehyde acetics at 140° in sealed tubes. Treatment of these derivatives with acid yielded the corresponding pyrido-[1,2-a] pyrimidinium salts which could be hydrolysed back to the supposed acylethylidenamines by base. Sawyer and Wibberley 44 prepared several compounds of this type under milder conditions by refluxing the reactants in xylene and suggested that the products were better represented as the tautomeric hydrogen-bonded 2-(2-acetylvinylamino) pyridines (48). These products showed no normal ketone carbonyl absorption in their i.r. spectra but exhibited three strong lower frequency absorptions which correspond to the enamino system. 1H n.m.r. spectra showed chemical shifts and coupling constants which fell within the range reported for this type of compound.

The product of the reaction between ethyl acetoacetate and 2-aminopyridine at 160° was originally regarded as 2H-1-methyl-
(49)  

(50)  

(51)  

(52)
-pyrido-[1,2-a]-pyrimidin-2-one (49), but Antaki and Petrow\textsuperscript{50} showed that the product was in fact the 2-methyl-4-keto isomer (46) by virtue of its alternative synthesis from 2-bromopyridine and ethyl $\beta$-aminocrotonate. The 4-keto structure (46) was confirmed by Adams and Pachter\textsuperscript{51} who compared the u.v. spectrum of the product obtained from the reaction of ethyl acetoacetate and 2-aminopyridine with the u.v. spectra of 2H-pyrido-[1,2-a] pyrimidin-2-one and 1H-pyrido-[1,2-a] pyrimidin-4-one, both of whose structures had been established independently.

Uncertainty existed in the literature for a long period as to the possible intermediates formed in the reaction of ethyl acetoacetate and 2-aminopyridine. Khitrik\textsuperscript{52} and Antaki and Petrow\textsuperscript{50} found that 2-acetoacetamidopyridine ($50; R^1=H, R^2=CH_3$), formed by heating the reactants at 100°, could only be cyclized to the pyrimidin-4-one (46) by treatment with hot concentrated sulphuric acid and then only in low yields while Kucherov\textsuperscript{53,54} showed that, when N-(5-chloro-2-pyridyl)-$\beta$-(5'-chloro-2'-pyridylamino)-crotonamide ($51, R^1=R^2=5$-Cl) was treated with sulphuric acid a 7-chloro-pyrido-[1,2-a] pyrimidinone was formed, which he incorrectly regarded as the 2-keto isomer. Shur and Ismailatam\textsuperscript{55} prepared a number of symmetrical and unsymmetrical crotonamides ($51$).

Symmetrical crotonamides were obtained by the interaction of 2-aminopyridines and the 2-acetoacetamidopyridine obtained from it and unsymmetrical crotonamides from 2-aminopyridines and a 2-acetoacetamidopyridine derived from a different 2-aminopyridine according to Khitrik\textsuperscript{52} and Kucherov\textsuperscript{54}. The conversion of the crotonamides into the pyrido-[1,2-a]pyrimidin-4-ones was effected by heating them with polyphosphoric acid (PPA). The 1H-pyrido-[1,2-a] pyrimidin-4-ones obtained from the crotonamides were also
$R^1 \quad \text{CH}_3\text{COCH}_2\text{COOR}^2 \quad \text{H}^+$

$R^1 \quad \text{CH}_3\text{C}═\text{CHCOOR}^2$

$(52)$

$R^1 \quad \text{CH}_3$

$(46)$

$R^1 = 3-\text{CH}_3, 5-\text{Cl}, 5-\text{Br}, 3,5-\text{di-Br}$.

$R^2 = \text{CH}_3, \text{C}_2\text{H}_5$.

Figure 1.
\[ R^1 \text{Py} + \text{CH}_3\text{COCH}_2\text{COOR}^2 \xrightarrow{-R^2\text{OH}} \text{PyNHCOCH}_2\text{COCH}_3 \]

\((50)\)

\[ \text{PyNHCOCH}_2\text{COCH}_3 \xrightarrow{\text{PPA}} \text{PyNHCOCH} = \text{C} = \text{C} = \text{NH} \text{Py} \]

\((51)\)

\[ R^1 \text{PyNH}_{2} + \left[ R^1 \text{PyNH} = \text{C} = \text{CHCOOH} \right] \]

\((53)\)

\((54)\)

\[ R^1 = \text{H, 4-CH}_3, 5-\text{CH}_3, 6-\text{CH}_3 \]

\(\text{Figure 2.}\)
obtained by the cyclization of alkyl \( \beta \)-pyridylaminocrotonates (52) and 2-acylacetamidopyridines (50) in the presence of PPA.

Since the crotonates (52) had been shown to undergo cyclization to pyrimidin-4-ones when heated in PPA it was assumed by Shur and Israelstam\(^{55}\) that the mechanism of the direct synthesis in cases where the 2-aminopyridine is known to give a crotonate is straightforward (Fig. 1). Crotonates are generally formed by the reaction of the 2-aminopyridine and acetoacetic ester in the presence of an acid catalyst\(^{54}\). On the other hand, the mechanism for the formation of those pyrimidin-4-ones, derived from 2-aminopyridines, which form 2-acylacetamidopyridines (50) appeared to be more complicated. It was suggested that in such cases a crotonamide (51) is formed although no crotonamide was isolated in these reactions. The mechanism shown in Fig. 2 was proposed for the direct synthesis of 1H-pyrido-\([1,2-a]\) pyrimidin-4-ones from such 2-aminopyridines. The conversion of crotonamides (51) to the pyrido-\([1,2-a]\) pyrimidin-4-one probably occurs in two stages. The first has been shown by investigation of the products obtained by cyclization of unsymmetrical crotonamides to be hydrolytic fission of the bond at \( a \) (Fig. 2) to give the aminopyridine (53) and the \( \beta \)-pyridylaminocrotonic acid (54) which cyclizes in the second stage to give the pyrido-\([1,2-a]\) pyrimidin-4-one (46).

In a later work\(^{56}\), Yale disputes the possibility of the participation of 2-acetoacetamido derivatives (50) and (51) as intermediates on the basis of the failure of 2-acetoacetamido(pyridine to cyclize under any conditions other than in the presence of concentrated sulphuric acid while 2-aminopyridine condenses with either ethyl acetoacetate or ethyl \( \beta \)-aminocrotonate at 160-220\( ^\circ \) to give 1H-2-methyl-pyrido-\([1,2-a]\) pyrimidin-4-one (46). The findings reported by Shur and Israelstam\(^{55}\) are ignored. Yale found that 2-\( \alpha \)-amino-3-\((\alpha\)-bromobenzyl)oxy) pyridine reacted with ethyl acetoacetate
(55) \[ \text{Structure} \]

(56) \[ \text{Structure} \]

(5) \[ \text{Structure} \]

(6) \[ \text{Structure} \]

(17) \[ \text{Structure} \]
to give two products in low combined overall yield. These products were identified as the enamine (55) and the pyrido-[1,2-a] pyrimidin-4-one (56). The cyclization of the pure enamine (55) to give the pyrido-[1,2-a] pyrimidin-4-one (56) is given as evidence of the participation of an enamine in the formation of the pyrido-[1,2-a] pyrimidin-4-one by the reaction of 2-aminopyridines with acetoacetic ester.

The present work describes the reactions of 2-aminopyridine and its iodide with the following steroidal β-dicarbonyls: 17β-hydroxy-2-hydroxymethylene-5α-androstan-3-one (5,R=H) and its 17α-methyl derivative (5,R=CH₃), 17β-hydroxy-2-hydroxymethylene-androst-4-en-3-one (6), 2-acetyl-17β-acetoxy-5α-androstan-3-one (17) and 3β-hydroxy-16-hydroxymethylene-androst-5-en-3-one (57). The reaction of 2-aminopyridinium iodide with the steroidal β-ketoester, 2-carboxymethoxy-17β-hydroxy-17α-methyl-5α-androstan-3-one (58) is also described.

In the unsymmetrical steroidal β-ketoaldehydes and β-diketone ambiguity arises in respect of the identity of the reaction product because of the two possible sites on the steroid precursor for anil formation to occur. Thus, theoretically, 17β-hydroxy-2-hydroxymethylene-5α-androstan-3-one (5,R=H), for example, can condense with 2-aminopyridinium iodide to give either 17β-hydroxy-5α-androst-2-eno-[2,3-c] pyrido-[1,2-a] pyrimidin-5'-ium iodide (59) or 17β-hydroxy-5α-androstano-[3,2-d] pyrido-[1,2-a] pyrimidin-5'-ium iodide (60). (see note on nomenclature at the beginning of this work). The need to be able to differentiate between the two possible reaction products, (59) and (60), necessitates the synthesis and determination of the structure of the anil formed by the condensation of 2-aminopyridine and the steroidal β-dicarbonyl.

The product of the condensation of 2-aminopyridinium iodide
\[ (61) \]

\[ (62) \]
and the steroidal $\beta$-ketoester, 2&carbomethoxy-17$\beta$-hydroxy-17$\alpha$-
-methyl-5&androstan-3-one (58) would be expected to be 17$\beta$-hydroxy-
-17$\alpha$-methyl-5&androst-2-ene-[3,2-d]-1',4'-dihydro-4'-oxo-pyrido-
-[1,2-a]pyrimidin-1'-ium iodide (61) if the reaction proceeds by the
same pathway as the condensation of 2-aminopyridinium iodide and
ethyl acetoacetate$^3$. Condensation of the steroid in the opposite
sense would be expected to give the [2,3-e]-1',2'-dihydro-2'-oxo-
-pyrido-[1,2-a]pyrimidin -1'-ium iodide (62). The structure of the
intermediate formed by the reaction of 2-aminopyridine and the
steroidal $\beta$-ketoester (58) will also be of interest in attempting to
determine the reaction pathway and in indicating whether rearrangement
takes place during reaction.
2. General Experimental Procedures.

Melting points were determined on a Kofler hot stage apparatus and are uncorrected.

Infra-red spectra of bromoform solutions were recorded on a Perkin Elmer 157G spectrometer.

Ultra-violet spectra were recorded on a Unicam SP800 spectrometer or Perkin Elmer 402 spectrometer.

Nuclear Magnetic Resonance spectra were recorded in deuterochloroform solution, using tetramethylsilane as internal standard on a Nuclear Magnetic Resonance Ltd. EM-360 (60MHz) spectrometer or a Varian HA 100 (100MHz) spectrometer (operated by Mr. J. Millar).

Mass spectrometry was carried out on an AEI MS 902 instrument (operated by Mr. D. Thomas).

Thin Layer Chromatography was performed on Merck silica gel GF 254.

The eluting system was benzene-ethanol (9:1). The plates were developed with sulphuric acid in ethanol (1:19) spray and then heated. Where appropriate inspection of the plate was made with an ultra-violet lamp prior to developing.

2-aminopyridinium iodide (42)

2-aminopyridine (10g, 1.06 x 10^-1 mole) dissolved in absolute ethanol (30ml) was treated with 55% w/w hydroiodic acid (2ml) at -20° to -30° for 2h. The product was precipitated by addition of diethyl ether. The precipitate was filtered and dried before being taken up in ethanol and precipitated again with ether. Recrystallization from absolute ethanol gave 2-aminopyridinium iodide (14.7g, 6.6 x 10^-2 mole, 62%), m.p. 145-147°.
3. The reaction of 17β-hydroxy-2-hydroxymethylene-5α-androstan-3-one with 2-aminopyridine and with 2-aminopyridinium iodide.

3.1 Synthesis of 17β-hydroxy-2-hydroxymethylene-5α-androstan-3-one (5).

17β-hydroxy-2-hydroxymethylene-5α-androstan-3-one was synthesised to provide a suitable steroidal β-ketoaldehyde for condensation with 2-aminopyridinium iodide. The preparation of 17β-hydroxy-2-hydroxymethylene-5α-androstan-3-one was first described by Ringold et al. 9 and was achieved by condensation of ethyl formate with the Δ²-enolate anion of 17β-hydroxy-5α-androstan-3-one. The product was precipitated as the enolate salt from the non-polar solvent and then acidified 58 to give the free hydroxymethylene compound.

I.r. studies 59 on keto-enol systems indicate that the grouping R.CO.CH=CH.OH is capable of forming a resonance stabilised intramolecular hydrogen bond of considerable strength. This results in a very large shift of the carbonyl absorption, whereas normal hydrogen bonding rarely produces shifts of more than 10cm⁻¹. The i.r. spectrum of hydroxymethylene steroid exhibited the characteristic broad band of the enolicβ-ketoaldehyde system at 1660-1540cm⁻¹. The spectrum also shows a band of medium intensity at 1700cm⁻¹ which can be attributed to the dicarbonyl form of the steroid (63) and indicating that the keto-enol form of the steroid exists in equilibrium with small amounts of the dicarbonyl form.

The n.m.r. spectrum of the hydroxymethylene steroid showed a singlet at 68.56 and a very broad signal between 613.00 and 614.80. The resonance at 68.56 has been assigned 60 to the average of the signals from the "aldehydic" proton of the formyl-enol form of the steroid (64) and the "vinyl" proton of the hydroxymethylene-ketone form (5), these two forms existing in equilibrium in deuterochloroform solution. The broad signal at 613.00-14.80 was assigned to an enolic
proton. There are no previous reports in the literature concerning the chemical shift and characteristics of this signal but it appears to be considerably broader than the signal for the enolic proton in 2-hydroxymethylene-5α-cholestan-3-one.

3.2 The reaction of 17β-hydroxy-2-hydroxymethylene-5α-androstan-3-one with 2-aminopyridine.

17β-hydroxy-2-hydroxymethylene-5α-androstan-3-one (5) and 2-aminopyridine, on standing in pyridine overnight gave a yellow precipitate. Recrystallization of this precipitate from ethanol gave a pale yellow solid (65, R1 = R2 = H), m.p. 242–247°C, in 51% yield. The same product was obtained using 2-aminopyridinium iodide instead of 2-aminopyridine, and in 65% yield by refluxing the 2-hydroxymethylene steroid and 2-aminopyridine in methanol for five hours, the latter conditions being those used in the condensation of hydrazine hydrate and 17β-hydroxy-2-hydroxymethylene-5α-androstan-3-one.

Mass spectrometry indicated that the product had a molecular formula of C25H34N2O2. The u.v. spectrum showed λmax at 234nm (logε 4.10), 290 (3.99) and 353 (4.49) and the i.r. spectrum absorptions at 3600, 16140, 1590, 1545, 1475, 1414 and 1415 cm⁻¹. The product was not sufficiently soluble in deuteriochloroform to permit a n.m.r. spectrum to be obtained.

Acetylation of the product gave a monoacetate (65, R1 = CH₃CO, R2 = H) with identical u.v. spectrum to its precursor and identified by its i.r. spectrum as the 17β-acetate. The n.m.r. spectrum of this compound showed signals at δ 6.67–6.92, 6.75, 6.72 and 6.22 associated with the four hydrogens of a monosubstituted pyridine ring and doublets at δ 7.82 and δ 11.88 each with spin coupling constant, J = 11.5 Hz.

The analogous 17β-hydroxy-17α-methyl derivative (65, R1 = H, R2 = CH₃)
was synthesised from 17β-hydroxy-2-hydroxymethylene-17α-methyl-5α-
-androstan-3-one (5, R=CH₃). A sample of the product was recrystall-
ized from diethyl ether to give crystals of approximately 1 mm in
length suitable for analysis by x-ray crystallography.

3.3 The reaction of 17β-hydroxy-2-hydroxymethylene-17α-methyl-5α-
-androstan-3-one and aniline.

A solution of 17β-hydroxy-2-hydroxymethylene-17α-methyl-
-5α-androstan-3-one (5, R=CH₃) and aniline in methanol was refluxed
for five hours. The product (67) obtained after extraction of the
reaction mixture with diethyl ether, and recrystallization from
diethyl ether had \( \lambda_{\text{max}} \) at 235 nm (log \( \varepsilon \) 4.00), 290 (3.53) and 355
(4.28) in its u.v. spectrum. The i.r. spectrum showed absorptions
at 3570, 1630, 1590 and 1545 cm⁻¹ while the n.m.r. spectrum showed
resonances at 6.96–7.53 associated with five aromatic protons
and doublets at 6.7.10 and 6.11.92 each with coupling constant \( J=12 \text{Hz} \).

3.4 The reaction of the condensation product of 17β-hydroxy-2-
-hydroxymethylene-5α-androstan-3-one and 2-aminopyridine
with hydrazine hydrate.

A solution of the product (65, \( R^1=R^2=R \)), obtained by the
condensation reaction of the 2-hydroxymethylene steroid and
2-aminopyridine, and hydrazine hydrate in methanol was allowed to
stand at room temperature overnight. The resulting product,
m.p. 123–125°, had \( \lambda_{\text{max}} \) in its u.v. spectrum at 221 nm (log \( \varepsilon \) 3.67)
and a molecular formula determined by mass spectrometry of \( C_{20}H_{30}N_{2}O \)
Comparison with literature data \(^7\) indicated that the product was
17β-hydroxy-5α-androstano-[3,2-c] pyrazole (1).

3.5 Discussion.

The product obtained by the condensation of 17β-hydroxy-
-2-hydroxymethylene-5α-androstan-3-one (5) with 2-aminopyridine
shows no normal ketone carbonyl absorption in its i.r. spectrum but exhibits three strong lower frequency absorptions at 1614, 1590 and 1545 cm⁻¹. In a study of the i.r. spectra of 2,3-unsaturated β-ketoamines, Holtzclaw et al. found three similar strong frequency absorptions which it was shown corresponded to an enaminone system. The first absorption in the 1630–1610 cm⁻¹ region was assigned to vibrations characteristic of a hydrogen bonded conjugated carbonyl in a quasi six-membered ring. The other two absorptions in the 1590–1570 cm⁻¹ region and in the 1500 cm⁻¹ region were not definitely assigned but were taken as being characteristic of the enaminone system. No N-H stretching frequency was observed in the usual region around 3300 cm⁻¹ for this type of stretch. The N-H band in hydrogen bonded compounds was assumed to have been broadened and lowered to around 3000 cm⁻¹ where it would be hidden by the C-H bands. The condensation product obtained from the steroid appears from its i.r. spectrum to be an enaminone. In the condensation of 2-aminopyridine with β-dicarbonyl compounds ambiguity in the identity of the products results from the two possible reaction sites. Therefore on the basis of the i.r. spectrum two possible structures, (65) and (66), were postulated for the steroidal enaminone.

Sawyer and Wibberley found that the n.m.r. spectrum of 2(2-acetylvinylamino)pyridine (48a) where the enaminone system corresponds to that in the steroid structure (65), showed two doublets, at 67.93 and 611.69, both with spin coupling constant, J=12.0 Hz. The signal at 67.93 was assigned to the vinyl proton and the signal at 611.69 to the proton of the amine. The n.m.r. of 2(2-formyl-1-methylvinylamino)-6-methylpyridine (48b), where the enaminone system is similar to that in the steroid structure (66), showed a signal at 69.09 due to the aldehydic proton and a broad singlet at 612.76 due to the amino proton. The n.m.r. spectrum of
steroidal enaminoone with doublets at $\delta 7.82$ and $\delta 11.88$ and coupling constants, $J=1.5\text{Hz}$ gives good correlation with that of 2(2-acetyl-vinylamino)pyridine (1b8a). The signal at $\delta 7.82$ was assigned to a vinyl proton coupled to a proton on an adjacent nitrogen, the latter proton giving rise to the signal at $\delta 11.88$. The n.m.r. spectrum of the steroid shows chemical shifts and coupling constants which fall within the range reported for this type of compound17,18. On the evidence of the i.r. and n.m.r. spectra the product of the condensation of 17$\beta$-hydroxy-2-hydroxymethylene-5$\alpha$-androstan-3-one (5) and 2-aminopyridine is 17$\beta$-hydroxy-2(2'-pyridylaminomethylene)-5$\alpha$-androstan-3-one (65, $R^1=R^2=H$).

The magnitude of the coupling constant (11.5Hz) between the amino proton and the vinyl proton on the adjacent carbon is consistent with these two protons being trans to one another18, as shown in structure (65). The coupling constant, if the two protons were cis, would be expected to be much smaller (0-2Hz)18. This is consistent with a structure which takes into account intramolecular hydrogen bonding between the amino hydrogen and the carbonyl oxygen. Furthermore a Dreiding model indicates that there would be steric hindrance between the pyridine ring and the carbonyl group if the amino proton and vinyl proton were in a cis conformation. A Dreiding model also shows that in structure (65) the flat hydrogen bonded six-membered "ring" fused to the A-ring is bent towards the $\alpha$-side of the molecule. The pyridine ring conjugated to the rest of the enaminoone system also lies in the same plane.

The system of conjugated double bonds consisting of the pyridine ring and the enaminoone system constitutes the longest chromophore in the molecule and can be assumed to give rise to the
highest $\lambda_{\text{max}}$ in the u.v. spectrum at 353 nm. In the flat hydrogen-bonded six-membered ring of the enaminoone, a quasi-"aromatic" system which will contribute towards decreasing the energy of the $\Pi \rightarrow \Pi^*$ transition of the chromophore is created by delocalization of the $\Pi$ electrons around the ring.

The spectra obtained from the product of the reaction between 17\(^\beta\)-hydroxy-2-hydroxymethylene-17\(\alpha\)-methyl-5\(\alpha\)-androstan-3-one (5, R=CH\(_3\)) and aniline gives good correlation with the spectra of 17\(^\beta\)-hydroxy-2(2'-pyridyl-aminomethylene)-5\(\alpha\)-androstan-3-one (65) and its 17\(^\beta\)-acetate, and can be formulated as 2-aminomethylene-17\(^\beta\)-hydroxy-17\(\alpha\)-methyl-5\(\alpha\)-androstan-3-one (67). The reactions of aniline and 2-aminopyridine with 2-hydroxymethylene-3-keto steroids suggest that anil formation with primary amines, in general, may proceed by condensation of the formyl carbonyl in contrast to hydrazone formation where it has been shown that attack is at the 3-ketone.

It has been shown that the direction of enolization of cyclic \(\beta\)-ketoaldehydes is strongly dependent on structure and that the trends may be rationalized in terms of the strain in double bonds (\(\Pi\) strain). The application by Bhacca and Williams of the method devised by Garbisch to the n.m.r. spectrum of 17\(^\beta\)-hydroxy-2-hydroxymethylene-17\(\alpha\)-methyl-5\(\alpha\)-androstan-3-one (5, R=CH\(_3\)), determined in deuterochloroform, in order to estimate the equilibrium between the hydroxymethylene-ketone and formyl-enol tautomers showed that the steroid existed as a mixture of 22% hydroxymethylene-ketone (5) and 78% formyl-enol (64). This is consistent with the observation that tautomers with double bonds in six-membered rings are, in general, favoured in comparison with those with semicyclic double bonds.

In contrast, 17\(^\beta\)-hydroxy-2(2'-pyridyl-aminomethylene)-5\(\alpha\)-androstan-
-3-one (65) from the evidence of its i.r. and n.m.r. spectra exists predominantly in the enaminone form (65) rather than in the imino-enol form (68). However, integration of the signal at δ11.88 in the n.m.r. spectrum of the steroidal enaminone (65) indicated that only 0.8 of a proton was giving rise to the signal. This suggests that the enaminone form (65) exists in equilibrium with small quantities of the imino-enol (68) in deuterochloroform solution.

It has been observed that the anilinomethylene derivatives of both cyclic and acyclic β-diketones prefer to exist in the enaminone form rather than the imino-enol form. This could be expected as imines, in general, form enamines more readily than ketones enolise. It appears that the conformational influence met in the formyl-enol (64) is outweighed by other factors in the enaminone.

It was found that 17β-acetoxy-2-acetoxyomethylene-5α-androstan-3-one (69) prepared by the acetylation of 17β-hydroxy-2-hydroxymethylene-5α-androstan-3-one (5) would not react with 2-aminopyridine under the same conditions that were used to condense 17β-hydroxy-2-hydroxymethylene-5α-androstan-3-one, that is on standing in pyridine solution overnight at room temperature, indicating that the 2-acetoxyomethylene moiety may be blocking anil formation.

The reaction of 17β-hydroxy-2(2'-pyridyl-aminomethylene)-5α-androstan-3-one (65, R¹=R²=H) with hydrazine hydrate at room temperature to give the [3,2-c] pyrazole (1) provides some more chemical evidence that 2-aminopyridine condenses with the formyl carbonyl of the hydroxymethylene steroid thus leaving the keto carbonyl intact and able to react with hydrazine. Cyclization will occur by expulsion of the 2-aminopyridine. This suggests that the C-N bond in 17β-hydroxy-2(2'-pyridyl-aminomethylene)-5α-androstan-3-one is labile making the aminopyridine fragment a good leaving
Kuzmenko et al. \textsuperscript{52} condensed 17\(\beta\)-hydroxy-2-hydroxymethylene-17\(\alpha\)-methyl-5\(\alpha\)-androstan-3-one (5, \(R=CH_3\)) in refluxing methanol to obtain 3-hydrazono-17\(\beta\)-hydroxy-2-hydroxymethylene-17\(\alpha\)-methyl-5\(\alpha\)-androstan which was then cyclised by dehydration under reduced pressure at 140-150\(^\circ\) to yield 17\(\beta\)-hydroxy-17\(\alpha\)-methyl-5\(\alpha\)-androstan-[3,2-c]-pyrazole (1). The first part of this work was repeated so that the n.m.r. spectrum of the 3-hydrazono-2-hydroxymethylene steroid could be obtained and the chemical shifts and coupling constants of the hydroxymethylene fragment could be compared with those obtained from the condensation product of 17\(\beta\)-hydroxy-2-hydroxymethylene-5\(\alpha\)-androstan-3-one and 2-aminopyridine with a view to eliminating 17\(\beta\)-hydroxy-2-hydroxymethylene-3(2'pyridyl-amin)-5\(\alpha\)-androstan-2-one (66) as a possible product. However it was found that after refluxing the hydroxymethylene steroid (5) and hydrazine hydrate in ethanol for 5 hours, the product was not the 3-hydrazono-2-hydroxymethylene derivative but the [3,2-c]pyrazole (1).

3.6 The synthesis of 5\(\alpha\)-androst-2-eno-[2,3-e] pyrido-[1,2-a] pyrimidin-5\(^{\prime}\)-ium iodides.

Since the anil formed from the reaction of 17\(\beta\)-hydroxy-2-hydroxymethylene-5\(\alpha\)-androstan-3-one (5) and 2-aminopyridine had been identified as 17\(\beta\)-hydroxy-2(2'-pyridyl-aminomethylene)-5\(\alpha\)-androstan-3-one (65, \(R^1=R^2=H\)) it was predicted that the product obtained by acid catalyzed cyclization of this enaminoe would be 17\(\beta\)-hydroxy-5\(\alpha\)-androst-2-eno-[2,3-e] pyrido-[1,2-a] pyrimidin-5\(^{\prime}\)-ium iodide (59) and that the same compound would be obtained by the reaction of the hydroxymethylene steroid (5) and 2-aminopyridinium iodide (42) as it was highly likely that the first step in this reaction would be anil formation.
3.7 The synthesis of 17β-acetoxy-5α-androst-2-eno-[2,3-e]pyrido-[1,2-a] pyrimidin-5′-ium iodide (70).

(a) by cyclization of 17β-acetoxy-2(2′-pyridyl-aminomethylene)-5α-androstan-3-one.

17β-acetoxy-2(2′-pyridyl-aminomethylene)-5α-androstan-3-one (65, R1 = CH₃O, R2 = H) dissolved in the minimum volume of ethanol containing two drops of 55% w/w hydroiodic acid gave yellow crystals on standing overnight at room temperature. A sample of the product dissolved in ethanol gave a fluffy precipitate on the addition of a few drops of ethanolic silver nitrate indicating that the product was an iodide. Mass spectrometry indicated that the product had a molecular formula of C₂₇H₁₅N₂O₂I while the u.v. spectrum showed λmax at 217nm (log ε 4.30), 234 (4.50), 240 (4.46), 314 (3.77) and 326 (3.85). The u.v. spectrum of 2,4-dimethylpyrido-[1,2-a]-pyrimidin-5-ium iodide (43) shows λmax at 212nm (log ε 4.68), 227 (4.81), 305 (3.95), 312 (3.89) and 317 (4.01). Despite the presence of a shoulder at 240nm in the steroid spectrum not found in the spectrum of 2,4-dimethylpyrido-[1,2-a]pyrimidin-5-ium iodide (43) and the absence in the steroid spectrum of the maxima at 312nm found in the latter compound, the u.v. spectrum of the steroid molecule correlates sufficiently well with that of the latter compound to indicate the presence of a pyrido-[1,2-a]pyrimidinium iodide in the steroid molecule and to conclude that the steroid is 17β-acetoxy-5α-androst-2-eno-[2,3-e]pyrido-[1,2-a]pyrimidin-5′-ium iodide (70).

(b) by the reaction of 17β-acetoxy-2-acetoxymethylene-5α-androstan-3-one and 2-aminopyridinium iodide.

17β-hydroxy-2-hydroxymethylene-5α-androstan-3-one (5, R=H) was acetylated with acetic anhydride in pyridine to give
17β-acetoxy-2-acetoxymethylene-5α-androstan-3-one (69) 70. The diacetate was refluxed gently for 10 minutes in pyridine with 2-aminopyridinium iodide to give a yellow precipitate identified by its mass spectrum and u.v. spectrum as 17β-acetoxy-5α-androst-2-eno-[2,3-e] pyrido-[1,2-a] pyrimidin-5'-ium iodide (70).

3.8 The synthesis of 17β-hydroxy- and 17β-hydroxy-17α-methyl-5α-androst-2-eno-[2,3-e] pyrido-[1,2-a] pyrimidin-5'-ium iodide (59).

17β-hydroxy-2-hydroxymethylene-5α-androstan-3-one (5, R=H) and 2-aminopyridinium iodide in pyridine solution were refluxed gently for 10 minutes to give a yellow precipitate in 51% yield, which in ethanolic solution gave a fluffy precipitate on the addition of ethanolic silver nitrate solution. The product was found by mass spectrometry to have a molecular formula of C_{25}H_{33}N_{2}O_{1}, while the u.v. spectrum confirmed the presence of the pyrido-[1,2-a] pyrimidinium chromophore. The reaction product was therefore taken to be 17β-hydroxy-5α-androst-2-eno-[2,3-e] pyrido-[1,2-a] pyrimidin-5'-ium iodide (59, R=H).

The synthesis of 17β-hydroxy-17α-methyl-5α-androst-2-eno-[2,3-e] pyrido-[1,2-a] pyrimidin-5'-ium iodide (59, R=CH₃) was achieved by the reaction of 17β-hydroxy-2-hydroxymethylene-17α-methyl-5α-androstan-3-one (5, R=CH₃) and 2-aminopyridinium iodide. This reaction was effected at room temperature whereas under such conditions 17β-hydroxy-2-hydroxymethylene-5α-androstan-3-one gave the intermediate anil (65, R¹=R²=H) and not the cyclised product (59, R=H).

A Dreiding model of 17β-hydroxy-5α-androst-2-eno-[2,3-e] pyrido-[1,2-a] pyrimidin-5'-ium iodide (59) showed that, as in the case of 17β-hydroxy-2-(2'-pyridyl-aminomethylene)-5α-androstan-3-one (65), the heterocyclic ring system is bent towards the α-side of the ring.
3.9 Experimental

3.9.1 17β-hydroxy-2-hydroxymethylene-5α-androstan-3-one (5, R=H).

Sodium hydride (3g, 80% dispersion in oil, washed with dry benzene) was added to a solution of 17β-hydroxy-5α-androstan-3-one (3.0g, 10⁻³ mole) in dry benzene (150ml) and ethyl formate (6ml). The mixture was stirred for 5h, then methanol (2ml) was added to destroy excess sodium hydride. The sodium salt of the hydroxymethylene derivative was filtered, washed with hexane and dried in vacuo. The free enol was liberated by adding the salt in small quantities to ice cold 30% hydrochloric acid (100ml). The lumps formed were crushed, filtered, washed with water and dried. Recrystallization from petroleum ether (60:80) gave white crystals of 17β-hydroxy-2-hydroxymethylene-5α-androstan-3-one (3.0g, 9.9 x 10⁻³ mole, 94%); m.p. 140-142 °C (lit 9 140-146 °C); n.m.r. (100 MHz), δ 0.76 (C-18 methyl), δ 0.80 (C-19 methyl), δ 3.63 (multiplet, C-17 hydrogen), δ 8.56 (singlet, average of H=CH=OH and H=CH=O), δ 13.70-14.80 (broad signal, enolH); i.r. (CHBr₃), ν_max 3660 (enolic OH), 3595 (OH), 1700 (weak, C=O), 1635 (C=O), 1580cm⁻¹ (CO,CH=OH, ν_C=O).

3.9.2 17β-acetoxy-2-acetoxyxethylene-5α-androstan-3-one (69).70

A solution of 17β-hydroxy-2-hydroxymethylene-5α-androstan-3-one (1.0g, 3.1 x 10⁻³ mole) and acetic anhydride (1ml) in pyridine (5ml) was allowed to stand at room temperature overnight. The reaction mixture was then taken up in diethyl ether, washed with 5% hydrochloric acid, saturated sodium bicarbonate solution and water. The ether solution was dried over anhydrous magnesium sulphate and the solvent evaporated to dryness. Analysis of the residue by t.l.c. indicated that the major product was 17β-acetoxy-2-acetoxyxethylene-5α-androstan-3-one while 17β-acetoxy-2-hydroxymethylene-5α-androstan-3-one...
was present as a minor product. Separation of the two components was achieved by column chromatography using 10% deactivated alumina as the stationary phase and benzene as eluent. The recovered diacetate was recrystallized from petroleum ether (60:80) to give $17\beta$-acetoxy-2-acetoxyxymethylene-5x-androstan-3-one (0.7g, 1.7 x 10^{-3} mole, 55%) \\
\text{m.p. 146-50° (lit 70 156-163°); n.m.r. (60MHz), δ 0.83 (C-18 methyl),} \\
\δ 0.86 (C-19 methyl), δ2.04 (17β-acetate), δ 2.24 (2'-acetate), \\
δ4.60 (triplet, C=17x hydrogen), δ 8.13 (CH_{3}CO_{2}C=O); i.r. (ClBr), \\
\nu_{max} 1770 (ester O=O), 1715 (ester C=O), 1690 (C=O), 1613cm^{-1} (C=O). \\
3.9.iii $17\beta$-hydroxy-2(2'-pyridyl-aminomethylene)-5x-androstan-3-one \\
(65, R^1=H^2=H).

(a) A solution of $17\beta$-hydroxy-2-hydroxymethylene-5x-androstan-3-one (490mg, 1.54 x 10^{-3} mole) and 2-aminopyridine (290mg, 3.08 x 10^{-3} mole) in pyridine (1ml) was allowed to stand at room temperature overnight. The resulting yellow precipitate was filtered, dried and recrystallized from ethanol to give $17\beta$-hydroxy-2(2'-pyridyl-aminomethylene)-5x-androstan-3-one (310mg, 7.86 x 10^{-3} mole, 51%); m.p. 242-247°; u.v. (CH_{3}OH), \lambda_{max} 234nm (log ε 4.10), \\
290 (3.99), 353 (4.49); i.r. (ClBr), \nu_{max} 3600 (OH), 1640, 1590, 1545, 1475, 1140, 1415cm^{-1}; mass spectrum, M^+, m/e 394, calculated for C_{25}H_{34}N_{2}O_{2} 394.26201, found 394.26238, error less than 1 ppm.

(b) The same product was obtained when (a) was repeated using 2-aminopyridinium iodide instead of 2-aminopyridine.

(c) A solution of $17\beta$-hydroxy-2-hydroxymethylene-5x-androstan-3-one (220mg 7.0 x 10^{-4} mole) and 2-aminopyridine (320mg, 3.40 x 10^{-3} mole) in methanol (15ml) was refluxed for 5h. On cooling a precipitate formed, which was filtered, dried and recrystallized from ethanol to give $17\beta$-hydroxy-2(2'-pyridyl-aminomethylene)-5x-androstan-3-one (180mg, 4.57 x 10^{-4} mole, 65%).
A solution of 17β-hydroxy-2-hydroxymethylene-17α-methyl-5α-androstan-3-one (103mg, 3.10 x 10⁻⁴ mole) and 2-aminopyridine (31mg, 3.30 x 10⁻⁴ mole) in methanol (10ml) was refluxed for 5h. On cooling, water was poured into the reaction mixture which was then extracted with diethyl ether. The ether extract was washed with water, dried over anhydrous magnesium sulphate and evaporated to dryness. Recrystallization from diethyl ether gave 17β-hydroxy-17α-methyl-2(2'-pyridyl-aminomethylene)-5α-androstan-3-one (60mg, 1.47 x 10⁻⁴ mole, 47%); m.p. 102-104°C; u.v. (CH₃OH), λ<sub>max</sub> 235nm (log ε 3.69), 292 (3.57), 353 (4.13); mass spectrum, M<sup>+</sup>, m/e 1408.

A sample of the product was recrystallized from a volume of diethyl ether greater than the minimum volume usually employed in recrystallization to give crystals of approximately 1mm in length suitable for x-ray crystallography.

A solution of 17α-acetoxy-2(2'-pyridyl-aminomethylene)-5α-androstan-3-one (300mg, 7.61 x 10⁻⁴ mole) and a few drops of acetic anhydride in pyridine (10ml) was refluxed for 2h. The solvent was evaporated in vacuo and the residue taken up in diethyl ether. The ether solution was washed with water, 5% hydrochloric acid, saturated sodium bicarbonate solution and water again before being dried over anhydrous magnesium sulphate and evaporated to dryness. The residue was recrystallized from ethanol to give 17α-acetoxy-2(2'-pyridyl-aminomethylene)-5α-androstan-3-one (180mg, 4.12 x 10⁻⁴ mole, 54%); m.p. 181-183°C; u.v. (CH₃OH), λ<sub>max</sub> 235nm (log ε 3.72), 291 (3.84), 352 (4.44); n.m.r. (100 MHz), δ 0.80 (c-18 methyl, C-19 methyl), 82.01 (17α-acetate), 8 4.60 (triplet, 17α-hydrogen), 86.67-86.92 (2H, multiplet, pyridine ring, 3-H, 5-H).
doublet, pyridine ring, $^4$H), 57.82 (1H, doublet, $J=11.5$Hz, 
$\text{-NH-CH}=C$, 58.22 (1H, doublet, pyridine ring, $^6$H), 511.38 broad 
($^8$H, doublet, $J=11.5$ Hz, $^N$H), i.r. (CHBr$_3$), $\nu^\text{max}$ 1715 (ester $C=O$), 
1640 (C=O), 1590, 1545, 1147, 1141cm$^{-1}$; mass spectrum, $M^+$, m/e 436.

3.9.i 

$2\text{-anilinomethylene-17\text{\$\beta$}-hydroxy-17\text{\$\alpha$}-methyl-5\text{\$\alpha$}-androstan-3\text{-one}}$ (67).

A solution of 17\text{\$\beta$}-hydroxy-2-hydroxymethylene-17\text{\$\alpha$}-methyl-
-5\text{\$\alpha$}-androstan-3\text{-one} (270mg, 8.13 x $10^{-4}$ mole) and aniline (0.1ml) in 
methanol (20ml) was refluxed for 5h. The solvent was removed in vacuo 
and the residue taken up in diethyl ether. The ether solution was 
was washed with 20% hydrochloric acid to remove excess aniline and then 
with water. The solution was dried over anhydrous magnesium sulphate 
and evaporated to dryness. The residue was recrystallized from 
diethyl ether to give 2-anilinomethylene-17\text{\$\beta$}-hydroxy-17\text{\$\alpha$}-methyl-5\text{\$\alpha$}-
-androstan-3\text{-one} (173mg, 4.25 x $10^{-4}$ mole, 52%); m.p. 99-101$^\circ$; u.v. 
(CH$_3$OH), $\lambda^\text{max}$ 235nm (log $\epsilon$ 4.00), 290 (3.53), 355 (4.28); n.m.r. 
(60 MHz), $\delta$ 0.86 (c-18 methyl), $\delta$ 0.93 (c-19 methyl), $\delta$ 1.26 (c-17 methyl), 
86.96-87.53 (6H, aromatic hydrogen), $\delta$ 7.10 (1H, doublet, $J=12$Hz, $^\text{-NH-CH}=C$), 
becomes a singlet at $\delta$ 7.06 on addition of D$_2$O), $\delta$ 11.92 broad (1H, doublet, 
$J=12$Hz, $^N$H, disappears on addition of D$_2$O); i.r. (CHBr$_3$), $\nu^\text{max}$ 3570 (OH), 
1630 (C=O), 1590, 1545cm$^{-1}$; mass spectrum, $M^+$, m/e 407, calculated for 
$C_{27}H_{37}NO_2$ 407.2824, found 407.2775, error less than 12 ppm.

3.9.ii 

The reaction between 17\text{\$\beta$}-hydroxy-2(1\text{\$\beta$}-pyridyl-aminomethylene)-
-5\text{\$\alpha$}-androstan-3\text{-one} (65) and hydrazine hydrate.

(Formation of 1\text{\$\beta$}-hydroxy-5\text{\$\alpha$}-androstan-[3,2-c] pyrazole (1)).

To a solution of 17\text{\$\beta$}-hydroxy-2(1\text{\$\beta$}-pyridyl-aminomethylene)-
-5\text{\$\alpha$}-androstan-3\text{-one} (102mg, 2.59 x $10^{-4}$ mole) in methanol (15ml) a 
few drops of hydrazine hydrate were added and the mixture allowed to 
stand at room temperature overnight. The reaction mixture was diluted
with water and extracted with chloroform. The organic extract was washed with water, dried over anhydrous magnesium sulphate and evaporated to dryness. Purification by preparative t.l.c. gave 17β-hydroxy-5α-androstan-[3,2-c] pyrazole (20mg, 6.37 x 10⁻⁵ mole, 25%); m.p. 123-125° (lit 7 127-128°); u.v. (CH₃OH), λ max 224nm (log ε 3.67) (lit 7 224nm (log ε 3.70)); mass spectrum, M⁺, m/e 314, calculated for C₂₀H₃₀N₂O₃ 312, found 314.235666, error less than 1 ppm.

3.9.viii 17β-hydroxy-17α-methyl-5α-androstano-[3,2-c] pyrazole(1). A mixture of 17β-hydroxy-2-hydroxymethylene-17α-methyl-5α-androstan-3-one (147mg, 1.442 x 10⁻⁴ mole) and hydrazine hydrate (0.5ml) in methanol (5ml) was refluxed for 5h. On cooling the solution was diluted with water (20ml) and left standing overnight. The precipitate was filtered off, washed with water and dried at room temperature. It was then heated with diethyl ether (3ml), filtered off, and dried in a dessicator to give 17β-hydroxy-17α-methyl-5α-androstan-[3,2-c] pyrazole (140mg, 1.426 x 10⁻⁴ mole, 91%); m.p. 114.7-114.9° (lit 7 114.8°); mass spectrum, M⁺, m/e 328.

3.9.ix 17β-acetoxy-5α-androst-2-eno-[2,3-e] pyrido-[1,2-a]-pyrimidin-5'-ium iodide (70).

(a) 17β-acetoxy-2(2'-pyridyl-aminomethylene)-5α-androstan-3-one (178mg, 1.43 x 10⁻⁴ mole) was dissolved in the minimum volume of absolute ethanol. Two drops of 55% v/w hydroiodic acid were added. On standing overnight a precipitate had formed. The precipitate was filtered and dried to give 17β-acetoxy-5α-androst-2-eno-[2,3-e] pyrido-[1,2-a] pyrimidin-5'-ium iodide (118mg, 2.16 x 10⁻⁴ mole, 49%); u.v. (CH₃OH), λ max 217nm (sh) (log ε 4.30), 234 (4.50), 240 (sh) (4.46), 314 (3.77), 326 (3.85); i.r. (CHBr₃), V max 1715 (ester C=O), 1631, 1620, 1580 cm⁻¹; mass spectrum, M⁺ (-HI),
m/e 418, calculated for $C_{27}H_{34}N_2O_2$, found 418.262014, error less than 2 ppm.

(b) 17β-acetoxy-2-acetoxymethylene-5α-androstan-3-one (205mg, 5.12 x 10^{-4} mole) and 2-aminopyridinium iodide (115mg, $5.18 \times 10^{-4}$ mole) in pyridine (1ml) were refluxed gently for 10 min. On cooling, sufficient diethyl ether was added to the solution to give complete precipitation. The precipitate was filtered, washed with ether and dried. Recrystallization from acetone gave 17β-acetoxy-5α-androst-2-eno-[2,3-e] pyrido-[1,2-a] pyrimidin-5'-ium iodide (93mg, $2.22 \times 10^{-4}$ mole, 43%); u.v. (CH$_3$OH), $\lambda_{max}$ 217nm (sh) ($\log \varepsilon = 4.28$), 234 (4.49), 240 (sh) (4.56), 313 (3.79), 326 (3.84); mass spectrum, $M^+$(-HI), m/e 418.

3.9 x 17β-hydroxy-5α-androst-2-eno-[2,3-e] pyrido-[1,2-a] pyrimidin-5'-ium iodide (59 R=CH$_3$).

A solution of 17β-hydroxy-2-hydroxymethylene-5α-androstan-3-one (305mg, 9.60 x 10^{-4} mole) and 2-aminopyridinium iodide (214mg, $9.61 \times 10^{-4}$ mole) in pyridine (1ml) was refluxed gently for 10 min. The yellow precipitate which had formed was filtered, washed with ether and dried. Recrystallization from ethanol gave 17β-hydroxy-5α-androst-2-eno-[2,3-e] pyrido-[1,2-a] pyrimidin-5'-ium iodide (206mg, $5.48 \times 10^{-4}$ mole, 57%); u.v. (CH$_3$OH), $\lambda_{max}$ 216nm (log $\varepsilon = 4.48$), 234 (4.49), 241 (sh) (4.56), 318 (3.89), 326 (3.93); mass spectrum; $M^+$(-HI), m/e 376, calculated for $C_{25}H_{32}N_2O_3$ 376.251451, found 376.251217, error less than 1 ppm.

3.9 x 17β-hydroxy-17α-methyl-5α-androst-2-eno-[2,3-e] pyrido-[1,2-a] pyrimidin-5'-ium iodide (59 R=CH$_3$).

17β-hydroxy-2-hydromethylene-17α-methyl-5α-androstan-3-one (300mg, 9.15 x 10^{-4} mole) and 2-aminopyridinium iodide (210mg, $9.45 \times 10^{-4}$ mole) were dissolved in pyridine (1ml) and allowed to stand overnight.
at room temperature. Sufficient diethyl ether was added to cause complete precipitation. The precipitate was filtered, washed with ether and dried. Recrystallization from ethanol gave 17β-hydroxy-17α-methyl-5α-androst-2-eno-[2,3-e]-pyrido-[1,2-a] pyrimidin-
5'-ium iodide (73mg, 1.41 x 10⁻⁴ mole, 14.9%); u.v. (CH₃OH), λ max 215nm (log ε 4.44), 233 (4.54), 240 (sh)(4.48), 313 (3.77), 326 (3.86); mass spectrum, M⁺(-H), m/e 390, calculated for C₂₆H₃₄N₂O
390.267100, found 390.267959, error less than 6 ppm.
4. The reaction of 3β-hydroxy-16-hydroxymethylene-androst-5-en-17-one with 2-aminopyridine and 2-aminopyridinium iodide.

4.1 Synthesis of 3β-hydroxy-16-hydroxymethylene-androst-5-en-17-one (57).

3β-hydroxy-16-hydroxymethylene-androst-5-en-17-one was prepared by the condensation of the Δ16-enolate anion of 3β-hydroxy-androst-5-en-17-one. The product is obtained as the enolate salt and then acidified to give the free hydroxymethylene compound. The i.r. spectrum exhibited absorptions at 1710 cm⁻¹ and 1625 cm⁻¹.

4.2 The reaction of 3β-hydroxy-16-hydroxymethylene-androst-5-en-17-one with 2-aminopyridine.

A solution of 3β-hydroxy-16-hydroxymethylene-androst-5-en-17-one (57) and 2-aminopyridine in pyridine was allowed to stand at room temperature for two days. The product was recovered by evaporation of the solvent and extraction of the residue by diethyl ether. Recrystallization of the residue, obtained on evaporation of the ether, from ethanol gave a white solid, m.p. 144-146⁰, in 51% yield.

Mass spectrometry indicated that the product had a molecular formula of C₂₅H₃₂N₂O₂. The u.v. spectrum showed λmax at 232 nm (log ε 3.75), 291 (4.04), 340 (4.51) and the i.r. spectrum had absorptions at 3590, 3395, 1685, 1610, 1580, 1565, 1500, 1465 and 1435 cm⁻¹.

Acetylation of the product gave a monoacetate identified by its i.r. spectrum as the 3β-acetate. The n.m.r. spectrum of the acetate showed signals at δ6.60-6.96, δ7.52 and δ8.20 associated with the four hydrogens of a monosubstituted pyridine ring and doublets at δ7.88 and δ10.70 each with spin coupling constants, J = 11 Hz.
4.3 Discussion

By analogy with the formation of 17β-hydroxy-2(2'-pyridyl-aminomethylene)-5α-androstan-3-one (65, R^1 = R^2 = H) from the condensation of 17β-hydroxy-2-hydroxymethylene-5α-androstan-3-one (5, R = H) and 2-aminopyridine, the anil formed by the reaction of 3β-hydroxy-16-hydroxymethylene-androst-5-en-17-one (57) and 2-aminopyridine was expected to be 3β-hydroxy-16(2'-pyridyl-aminomethylene)-androstan-5-en-17-one (71, R = H). The doublets at δ 7.88 and δ 10.70 in the n.m.r. spectrum of the 3β-acetates of the condensation product (71) were thus assigned to a vinyl proton and an adjacent amino proton respectively although an upfield shift of approximately 1 ppm was noted for the latter resonance relative to the chemical shift of the corresponding resonance in 17β-acetoxyl-2(2'-pyridyl-aminomethylene)-5α-androstan-3-one (65) and 2-(2-acetylvinylnamino)-pyridine (48a). The coupling constant (J=11Hz) between the two doublets was consistent with those previously reported for these two resonances and indicated that the two protons were trans to one another. The n.m.r. spectrum confirmed the prediction that the product of the reaction between 3β-hydroxy-16-hydroxymethylene-androst-5-en-17-one (57) and 2-aminopyridine was the enamionone, 3β-hydroxy-16(2'-pyridyl-aminomethylene)-androstan-5-en-17-one (71, R = H).

Apart from the difference in chemical shift for the amino proton in 3β-acetoxyl-16(2'-pyridyl-aminomethylene)-androstan-5-en-17-one (71, R=CH_3CO) compared to chemical shift of the amino proton in 17β-acetoxyl-2(2'-pyridyl-aminomethylene)-5α-androstan-3-one (65) both the i.r. and u.v. spectra of the former steroid (71) differed significantly from that of the latter (65).

The i.r. spectrum of 3β-hydroxy-16(2'-pyridyl-aminomethylene)-
-androst-5-en-17-one (71, R=H) showed an absorption at 3370 cm\(^{-1}\) not present in the i.r. spectrum of 17\(\beta\)-hydroxy-2(2'-pyridyl-aminomethylene)-5\(\alpha\)-androstan-3-one (65), while the absorption in the latter steroid at 1640 cm\(^{-1}\) which was assigned to vibrations characteristic of a hydrogen bonded conjugated carbonyl appeared to have moved up to 1685 cm\(^{-1}\) in the former steroid. This indicated that since hydrogen bonding causes a lowering of the "normal" ketone absorption at about 1700 cm\(^{-1}\) to the 1630-1610 cm\(^{-1}\) region\(^{46}\), the 3-ketone in 3\(\beta\)-hydroxy-16(2'-pyridyl-aminomethylene)--androst-5-en-17-one (71) was only weakly hydrogen bonded. This was borne out by the appearance of a non-hydrogen bonded N-H stretch at 3370 cm\(^{-1}\) in the i.r. spectrum.

As suggested in section 3.5 (page 19), the absorption maximum at 353 nm (\(\log \varepsilon = 4.49\)) in the u.v. spectrum of 17\(\beta\)-hydroxy-2(2'-pyridyl-aminomethylene)-5\(\alpha\)-androstan-3-one (65) could be assigned to a chromophore incorporating the pyridine ring and the quasi "aromatic" hydrogen bonded six-membered ring of the enaminone. The occurrence of the corresponding absorption maximum at 340 nm (\(\log \varepsilon = 4.51\)) in the u.v. spectrum of 3\(\beta\)-hydroxy-16(2'-pyridyl-aminomethylene)-androst-5-en-3-one (71) indicated that the pyridine-enaminone chromophore in this steroid was less conjugated than that in 17\(\beta\)-hydroxy-2(2'-pyridyl-aminomethylene)-5\(\alpha\)-androstan-3-one (65).

The conclusion drawn from the i.r. and u.v. spectra was that the amino proton was too far from the carbonyl oxygen for effective hydrogen bonding to take place. As a result the i.r. absorptions for the carbonyl and N-H stretches occurred at frequencies near those normally associated with their non-hydrogen bonded stretches\(^{47}\). In the u.v. spectrum, the absence of a significant amount of hydrogen bonding prevented the creation of a quasi
"aromatic" enaminone system with subsequent loss of electron delocalisation and resonance energy in the pyridine-enaminone system. As a result the energy of the $\pi \rightarrow \pi^*$ transition was increased relative to the energy of the corresponding transition in the hydrogen bonded chromophore of the 17$\beta$-hydroxy steroid (65) and the wavelength at which the transition occurred was therefore lowered$^{65}$. The upfield chemical shift of the amino hydrogen in the n.m.r. spectrum of 3$\beta$-acetoxy-16(2'-pyridyl-aminomethylene)-androst-5-en-17-one (71, R = CH$_3$CO) relative to the corresponding chemical shift in 17$\beta$-acetoxy-2(2'-pyridyl-aminomethylene)-5$\alpha$-androstan-3-one (65) was attributed to the amino proton in the former steroid having a lower electron density in its vicinity because of the presence of only weak hydrogen bonding to the 3-ketone in the enaminone.

The most likely reason for the absence of significant hydrogen bonding in 3$\beta$-hydroxy-16(2'-pyridyl-aminomethylene)-androst-5-en-17-one (71) appeared to be that the adjacent five-membered D-ring of the steroid was imposing a conformation on the enaminone fragment such that the carbonyl oxygen and amino proton were not close enough to permit hydrogen bonding between them. Dreiding models showed that while the D-ring allowed the enaminone to adopt a more planar conformation without imposing further strain on the D-ring than was the case in 17$\beta$-hydroxy-2(2'-pyridyl-aminomethylene)-5$\alpha$-androstan-3-one (65), because the angle between the C=C double bond on the C-16 and the C-16 - C-17 bond was greater in 3$\beta$-hydroxy-16(2'-pyridyl-aminomethylene)-androst-5-en-17-one (71) than the angle between the C=C double bond on the C-2 and the C-2 - C-3 bond in its 17$\beta$-hydroxy analogue (65), the distance between the amino proton and the carbonyl was greater in the 3$\beta$-hydroxy steroid (71) than in the 17$\beta$-hydroxy steroid (65).
The attempted synthesis of 3β-hydroxy- or 3β-acetoxy-androst-5,16-dieno-[16,17-e] pyrido-[1,2-a] pyrimidin-5'-ium iodide (72).

It was predicted that since the anil formed from 3β-hydroxy-16-hydroxymethylene-androst-5-en-17-one (57) and 2-aminopyridine was 3β-hydroxy-16-(2′-pyridyl-aminomethylene)-androst-5-en-17-one (71, R=H), the product obtained from the cyclization of the enamino would, if the reaction were possible, be 3β-hydroxy-androst-5,16-dieno-[16,17-e] pyrido-[1,2-a] pyrimidin-5'-ium iodide (72, R=H).

(a) The reaction of 3β-hydroxy-16-hydroxymethylene-androst-5-en-17-one (57) and 2-aminopyridinium iodide.

A solution of 3β-hydroxy-16-hydroxymethylene-androst-5-en-17-one (57) and 2-aminopyridinium iodide in pyridine was refluxed gently for 10 minutes. The major product, isolated by extraction with diethyl ether was identified by its u.v., i.r., and mass spectrum as 3β-hydroxy-16(2′-pyridyl-aminomethylene)-androst-5-en-17-one (71, R=H). A small amount of material precipitated on the addition of diethyl ether to the cooled reaction mixture was not fully characterized but sufficient evidence was obtained to show that it was probably either 2-aminopyridinium iodide or products caused by its decomposition and not steroid.

(b) The attempted cyclization of 3β-acetoxy-16(2′-pyridyl-aminomethylene)-androst-5-en-17-one (71, R=CH₃CO).

A saturated solution of 3β-acetoxy-16(2′-pyridyl-aminomethylene)-androst-5-en-17-one (71, R=CH₃CO) in absolute ethanol containing a drop of 55% w/w hydroiodic acid was stirred at room temperature for three days by which time a small quantity of solid had precipitated. The u.v. spectrum (see Experimental)
of the solid showed $\lambda_{\text{max}}$ which were attributed to starting material (or its deacetylation product) and to 2-aminopyridine (or its iodide). The spectrum also showed $\lambda_{\text{max}}$ at 218,316 and 330 nm which it was thought could be attributed to the pyrido-[1,2-α] pyrimidinium chromophore in 3β-acetoxy-androst-5,16-dieno-[16,17-e] pyrido-[1,2-α]-pyrimidin-5'-ium iodide (72, R=CH₃CO), indicating that some cyclization of the starting material might have occurred.

On returning the precipitate to the filtrate, the mixture was refluxed for one hour. The volume of solvent was reduced and on addition of a little diethyl ether a precipitate formed. The u.v. spectrum of this precipitate indicated that it was similar in composition to the precipitate obtained when the mixture had been allowed to stand at room temperature. The mass spectrum showed peaks at m/e 254, 128 and 94 corresponding to iodine, hydrogen iodide and 2-aminopyridine respectively. A number of peaks at m/e 418, 417, 403, 358, 357 and 343 with low ion pressures were also present. The calculated m/e ratio for 3β-acetoxy-androst-5,16-dieno-[16,17-e] pyrido-[1,2-α]-pyrimidin-5'-ium iodide (72, R=CH₃CO) is 416 and it was thought that the peaks at m/e 418 and 417 might be due to the cyclized derivative. The peak at m/e 358 might be due to 3β-acetoxy-16-hydroxymethylene-androst-5-en-3-one formed by the hydrolysis of the starting material (71).

A further sample of 3β-acetoxy-16(2'-pyridyl-aminomethylene)-androstan-5-en-17-one (71, R=CH₃CO) dissolved in absolute ethanol containing concentrated hydrochloric acid was refluxed for one hour. After evaporation of the solvent the residue was partitioned between diethyl ether and water. The fractions were separated and evaporated to dryness. The u.v. spectrum of the ether fraction indicated that it consisted predominantly of
starting material while the spectrum of the aqueous fraction showed it consisted mainly of 2-aminopyridine or its chloride salt.

4.5 Discussion.

It appears from the results of the above experiments that the 16(2'-pyridyl-aminomethylene)-androst-5-en-17-one system (71) cannot be readily cyclized by an acid catalyzed reaction to form an androst-5,16-dieno-[16,17-e] pyrido-[1,2-a] pyrimidin-5'-ium iodide (72). The evidence from the experiments indicates that cyclization may occur to a small degree but also that hydrolysis of the enaminone to give 2-aminopyridine and 3β-acetoxy-16-hydroxymethylene-androst-5-en-17-one may occur as well, as shown by the presence of \( \lambda_{\text{max}} \) corresponding to 2-aminopyridine in the u.v. spectrum of the reaction mixture and the peak at \( m/e \) 358, possibly corresponding to 3β-acetoxy-16-hydroxymethylene-androst-5-en-17-one, in the mass spectrum of the material precipitated by diethyl ether dilution of the reaction mixture. As the C-N bond in 17β-hydroxy-2(2'-pyridyl-aminomethylene)-5α-androstane-3-one (65) is considered to be fairly labile (section 3.5, page 20), it is not surprising that in 3β-acetoxy-16(2'-pyridyl-aminomethylene)-androst-5-en-17-one (71), if cyclization does not occur or only proceeds very slowly, hydrolysis also occurs in a competing reaction. The explanation as to why 3β-acetoxy-16(2'-pyridyl-aminomethylene)-androst-5-en-17-one (71) is not readily cyclized to 3β-acetoxy-androst-5,16-dieno-[16,17-e] pyrido-[1,2-a] pyrimidin-5'-ium iodide (72) in an acid catalyzed reaction appears to lie with the mechanism of cyclization rather than with any instability of an aromatic heterocyclic ring system fused to the D-ring of a steroid. Bouchon et al. have reported the synthesis of a 3β-hydroxy-5α-androst-16-eno-[7,16-f]-1',2',3',4'-tetrahydro-pyrido-[2,3-d] pyrimidine (36) by the reaction of 16-aminomethylene-
(36) $X = O, S, NH$. 

(20)
-3β-hydroxy-5α-androstan-17-one and a l-aminouracil, while
Ringold and Mancera\textsuperscript{23} have synthesised 3α-hydroxy-5α-androstane-
-\([17,16-d]\)-2'-methyl-pyrimidine (20) by the condensation of
3α-hydroxy-16-hydroxymethylene-5α-androstan-17-one and acetamidine
hydrochloride.

Possible explanations as to why 3β-acetoxy-16(2'-pyridyl-
aminomethylene)-androst-5-en-one (71) does not cyclize as readily
as 17β-acetoxy-2(2'-pyridyl-aminomethylene)-5α-androstan-3-one (65)
will be discussed in greater detail later (section 7).
4.6 Experimental.

4.6.1 3α-hydroxy-16-hydroxymethylene-androst-5-en-17-one (57).

Sodium methoxide was prepared from sodium (1.5g) and absolute methanol (30ml). The excess methanol was removed in vacuo. A solution of 3α-acetox-androst-5-en-17-one (1.8g, 5.0 x 10⁻³ mole) and ethyl formate (1.5ml) in dry benzene (25ml) was added and the mixture stirred for 2h followed by a further 2h under reflux. The mixture was then poured into water (50ml). The benzene layer was discarded and the aqueous phase washed with diethyl ether and free of ether by bubbling air through it. Acidification of the aqueous solution with concentrated hydrochloric acid gave a precipitate which was filtered and dried. Recrystallization from ethanol gave 3α-hydroxy-16-hydroxymethylene-androst-5-en-17-one (1.0g, 3.4 x 10⁻³ mole, 68%); m.p. 238-239 ° (lit 71 238-239 °); i.r. (CHBr₃), Vmax 1710 (C=O), 1635 cm⁻¹.

4.6.ii 3α-hydroxy-16(2'-pyridyl-aminomethylene)-androst-5-en-17-one (71, R=H).

A solution of 3α-hydroxy-16-hydroxymethylene-androst-5-en-17-one (200mg, 6.34 x 10⁻⁴ mole) and 2-aminopyridine (60mg, 6.39 x 10⁻⁴ mole) in pyridine (5ml) was allowed to stand at room temperature for two days. The solvent was removed in vacuo and the residue taken up in diethyl ether. The ether solution was washed with 5% hydrochloric acid and water. It was then dried over anhydrous magnesium sulphate and the solvent evaporated to dryness. Recrystallization of the residue from ethanol gave 3α-hydroxy-16(2'-pyridyl-aminomethylene)-androst-5-en-17-one (127mg, 3.24 x 10⁻³ mole, 51%); m.p. 144-146 °; u.v. (CH₃OH), λmax 232nm (log ε 3.75), 291 (4.04), 340 (4.51); i.r. (CHBr₃), Vmax 3550 (OH), 3395 (NH), 1685 (C=O), 1610, 1580, 1665, 1500, 1465 and 1435 cm⁻¹; mass spectrum, M⁺, m/e 392, calculated for
C$_{25}$H$_{32}$N$_2$O$_2$ 392.246365, found 392.246377, error less than 1 ppm.

4.6.iii 3β-acetoxy-16(2' -pyridyl-aminomethylene)-androst-5-en-17-one (71, $R=CH_3 CO$).

A solution of 3β-hydroxy-16(2' -pyridyl-aminomethylene)-androst-5-en-17-one (100mg, 2.55 x 10$^{-4}$ mole) in pyridine (10ml) containing a few drops of acetic anhydride was refluxed for 2h. The solvent was evaporated in vacuo and the residue taken up in diethyl ether. The ether solution was washed with water, 5% hydrochloric acid, saturated sodium bicarbonate solution and water again before being dried over anhydrous magnesium sulphate and evaporated to dryness. Recrystallization of the residue from ethanol gave 3β-acetoxy-16(2' -pyridyl-aminomethylene)-androst-5-en-17-one (51mg, 1.17 x 10$^{-4}$, 46%); m.p. 223-226°; u.v. (CH$_3$OH), $\lambda_{max}$ 230nm (log ε 3.67), 290 (3.98), 344 (4.48); n.m.r. (100 MHz), $\delta$ 0.914 (c-18 methyl), 1.06 (c-19 methyl), 2.02 (3β-acetate), 5.40 (1H, doublet, C-6 vinyl-H), 5.66-5.96 (2H, multiplet, pyridine ring, 3-H, 5-H), 7.53 (1H, doubly degenerate doublet, pyridine ring, 4-H), 7.88 (1H, doublet, J=11.5Hz, NH-C=CH=), 8.20 (1H, doublet, pyridine ring, 6-H), 10.70 broad (0.7H, doublet, J=11Hz, N-H), i.r. (CHBr$_3$), $\nu_{max}$ 1705, 1685, 1610, 1580 and 1555cm$^{-1}$.


(a) The reaction of 3β-hydroxy-16-hydroxymethylene-androst-5-en-17-one and 2-aminopyridinium iodide.

A solution of 3β-hydroxy-16-hydroxymethylene-androst-5-en-17-one (115mg, 3.66 x 10$^{-4}$ mole) and 2-aminopyridinium iodide (81mg, 3.78 x 10$^{-4}$ mole) in pyridine (15ml) was refluxed gently for 10 minutes. The addition of diethyl ether caused the precipitation of a small amount of material which was filtered and dried.
The u.v. spectrum showed an intense $\lambda_{\text{max}}$ at 220nm and smaller $\lambda_{\text{max}}$ at 251, 256 and 263nm while the mass spectrum showed peaks corresponding to m/e 128, 127, 94 and 75. It was concluded that the precipitate probably consisted of 2-aminopyridinium iodide and artefacts resulting from its decomposition.

The pyridine-diethyl ether solution was evaporated to dryness and the residue taken up in diethyl ether. The ether solution was washed with 5% hydrochloric acid and water before being dried over anhydrous magnesium sulphate and evaporated to dryness. The residue was recrystallized from ethanol to give 3β-hydroxy-16-(2'-pyridyl-aminomethylene)-androst-5-en-17-one (100mg, 2.81 x $10^{-4}$ mole, 77%); m.p. 145-147°C.

(b) The attempted cyclization of 3β-acetoxy-16(2'-pyridyl-aminomethylene)-androst-5-en-17-one (71).

A solution of 3β-acetoxy-16(2'-pyridyl-aminomethylene)-androst-5-en-17-one (27mg, 6.89 x $10^{-5}$ mole) in absolute ethanol (10ml) containing a drop of 55% w/w hydroiodic acid was stirred for three days at room temperature by which time a small quantity of solid had precipitated. The u.v. spectrum of the solid showed a broad $\lambda_{\text{max}}$ at 228nm with a shoulder at 235nm and other $\lambda_{\text{max}}$ of lower intensity at 290, 316, 330 and 346nm. It was thought that the $\lambda_{\text{max}}$ at 228, 316 and 330nm might be due to the formation of 3β-acetoxy-androst-5,16-dieno-[16,17-e] pyrido-[1,2-a] pyrimidin-5'-ium iodide (the u.v. spectrum of 2,4-dimethylpyrido-[1,2-a] pyrimidin-5-ium (43) shows $\lambda_{\text{max}}$ at 212, 227, 305, 312 and 317nm). The $\lambda_{\text{max}}$ at 235nm and 290nm were attributed to 2-aminopyridine (lit73 u.v. $\lambda_{\text{max}}$ 230, 296nm) or its iodide while the low intensity $\lambda_{\text{max}}$ at 346 was attributed to starting material (or the deacetylated derivative).
The precipitate was dissolved in a little absolute ethanol and returned to the original solution. Another drop of 55% w/w hydroiodic acid was added and the solution refluxed for 1h. On cooling no precipitate formed so the solvent was reduced in volume on the rotary evaporator. On addition of a little diethyl ether a precipitate formed which was filtered. The u.v. spectrum showed $\lambda_{\text{max}}$ at 218, 235, 280, 317, 326 and 351 nm and it was concluded that it consisted of a mixture of starting material, 2-aminopyridine (or its iodide) and possibly the required steroidal pyrido-[1,2-a] pyrimidin-5'-ium iodide. The mass spectrum showed peaks at m/e 418, 417, 403, 358, 357 and 343 as well as peaks at m/e 254, 128 and 94 corresponding to iodine, hydrogen iodide and 2-aminopyridine. The u.v. spectrum of the filtrate indicated that it consisted predominantly of starting material or its deacetylation product.

A further sample (12 mg) of 3β-acetoxy-16(2′-pyridylaminomethylene)-androst-5-en-17-one was dissolved in absolute ethanol (10 ml) containing concentrated hydrochloric acid (0.5 ml) and the mixture refluxed for 1 h. On cooling, most of the solvent was removed on the rotary evaporator. Diethyl ether and water were added to the residue. The aqueous layer was removed and the ether layer washed with a small portion of water. The aqueous extracts were combined and evaporated to dryness. The ether extract was dried over anhydrous magnesium sulphate and evaporated to dryness.

The u.v. spectrum of the ether extract showed $\lambda_{\text{max}}$ at 234, 290 and 345 nm indicating that the extract consisted of starting material (or the deacetylation product). The u.v. spectrum of the aqueous extract showed $\lambda_{\text{max}}$ at 230 nm and 298 nm probably corresponding to 2-aminopyridine or its chloride salt.
5. The reaction of 17β-acetoxy-2α-acetyl-5α-androstan-3-one with 2-aminopyridinium iodide.

5.1 The synthesis of 17β-acetoxy-2α-acetyl-5α-androstan-3-one (17, R=CH₃CO).

17β-acetoxy-2α-acetyl-5α-androstan-3-one (17, R=CH₃CO) was synthesised by the method described by Fujimoto and Ledeen. 17β-acetoxy-5α-androstan-3-one (73) was acetylated at the C-2 position using Hauser's inverse-addition method with acetic anhydride and boron trifluoride. The product was recovered as the borofluoride complex (74). The complex was decomposed by refluxing a methanolic solution with sodium acetate and acetic acid for 2 hours. Upon cooling, the β-diketone crystallized.

From spectral data 17β-acetoxy-2α-acetyl-5α-androstan-3-one appears to exist entirely in the cyclic hydrogen bonded enolic form which is formulated as structure (17a). It has a broad band at 1640-1570 cm⁻¹ in the i.r. spectrum characteristic of enolic β-diketones. The n.m.r. spectrum shows a singlet at δ15.76 which was assigned to an enolic proton.

A sample of 17β-acetoxy-2α-acetyl-5α-androstan-3-one was readily deacetylated on standing at room temperature in a methanol-acetone solution containing hydrochloric acid to give 17β-hydroxy-2α-acetyl-5α-androstan-3-one (17, R=H).

5.2 The reaction of 17β-acetoxy-2α-acetyl-5α-androstan-3-one (17, R=CH₃CO) and 2-aminopyridinium iodide.

By analogy with the reaction of 17β-hydroxy-2-hydroxymethylene-5α-androstan-3-one (5) and 2-aminopyridinium iodide (section 3.8, page 23) it was predicted that the product of the reaction between 17β-acetoxy-2α-acetyl-5α-androstan-3-one (17, R=CH₃CO) and 2-aminopyridinium iodide would be 17β-acetoxy-5α-androst-2-eno-[2,3-α]-2'-methyl-pyrido-[1',2-α]-pyrimidin-5'-ium iodide (75).
A solution of 17β-acetoxy-2α-acetyl-5α-androstan-3-one (17, R=CH₃CO) and 2-aminopyridinium iodide in pyridine was refluxed for 65 hours. After removal of the solvent the residual tar was taken up in water and washed with diethyl ether. Evaporation of the aqueous phase and recrystallization of the residue from ethanol; water (1:1) gave 11mg of a yellow solid (75). A sample of the product dissolved in ethanol gave a fluffy precipitate on the addition of a few drops of ethanolic silver nitrate indicating that the product was an iodide. Mass spectrometry indicated that the product had a molecular formula of C₂₈H₃₇N₂O₂I while the u.v. spectrum had λ_max at 216nm (log ε 4.39), 233 (4.55), 239 (sh)(4.52), 309 (3.75) and 321 (3.81). The u.v. spectrum gave good agreement with the spectrum of 17β-hydroxy-5α-androst-2-eno-[2,3-e] pyrido-[1,2-a] pyrimidin-5′-ium iodide (59) (section 3.9.x, page 29). The yellow product was therefore formulated as 17β-acetoxy-5α-androstan-2-eno[2,3-e]-2'-methyl-pyrido-[1,2-a] pyrimidin-5′-ium iodide (75).

The ether fraction was worked up and the residue recovered identified by analytical t.l.c. and i.r. spectroscopy as starting material, 17β-acetoxy-2α-acetyl-5α-androstan-3-one (17, R=CH₃CO).

5.3 The attempted reaction between 17β-acetoxy-2α-acetyl-5α-androstan-3-one (17) and 2-aminopyridine.

A solution of 17β-acetoxy-2α-acetyl-5α-androstan-3-one (17, R=CH₃CO) and 2-aminopyridine in pyridine was allowed to stand at room temperature overnight. After removal of the solvent, the residue was taken up in diethyl ether and worked up. However, only the starting material 17β-acetoxy-2α-acetyl-5α-androstan-3-one, was recovered.

The experiment was repeated using first pyridine and then ethanol as solvent and refluxing the reaction mixture for 2 days. In both instances only starting material was recovered.
5.4 The attempted reaction of 17β-hydroxy-2α-acetyl-5α-androstane-3-one (17, R=H) and 2-aminopyridine in a solution of N hydrochloric acid in ethanol.

A solution of 17β-hydroxy-2α-acetyl-5α-androstane-3-one (17, R=H) and 2-aminopyridine in a solution of N hydrochloric acid in absolute ethanol was refluxed for 5 hours. On evaporation of the solvent the residue was taken up in diethyl ether which was washed with water. Both the ether and aqueous phases were evaporated and the residues analysed by u.v. spectroscopy. The ether residue had λ\text{max} at 291 nm in its u.v. spectrum. Analysis by t.l.c. confirmed that the residue was 17β-hydroxy-2α-acetyl-5α-androstane-3-one (17, R=H) and this was confirmed by a n.m.r. spectrum. The u.v. spectrum of the aqueous residue had λ\text{max} at 233 nm and 305 nm corresponding to 2-aminopyridine or its chloride salt.

5.5 Discussion.

In contrast to 17β-hydroxy-2-hydroxymethylene-5α-androstane-3-one (5), the reaction of 17β-acetoxy-2α-acetyl-5α-androstane-3-one (17) and 2-aminopyridinium iodide in pyridine gives only very low yields (ca. 5%) of its [2,3-e] pyrido-[1,2-a] pyrimidin-5′-ium iodide derivative (75). All attempts to isolate the condensation product (76) of the 2-acetyl-3-keto steroid and 2-aminopyridine in pyridine or ethanol failed but it must be assumed that, since the [2,3-e] pyrido-[1,2-a] pyrimidin-5′-ium iodide derivative (75) can be synthesised, anil formation occurs and that the problem is to isolate very small quantities of the anil from the reaction mixture which contains a preponderance of starting material. It is also obvious that it is the inability of the 2-acetyl-3-keto steroid (17) to condense readily with 2-aminopyridine which is causing low yields of the cyclic derivative (75).
The i.r. spectrum of 17β-hydroxy-2-acetyl-5α-androstan-3-one (17, R=H) while having the characteristic broad β-diketone band at 1640-1540 cm\(^{-1}\) does not absorb in the "normal" carbonyl region from 1750 to 1700 cm\(^{-1}\). In contrast to this, both 2-acetyl-cyclohexanone and acetylacetone have absorptions in the 1700 cm\(^{-1}\) region of medium intensity, which from their positions are attributable to the keto forms. Inspection of the i.r. spectrum of 17β-hydroxy-2-hydroxymethylene-5α-androstan-3-one (5) also reveals a medium intensity absorption at 1700 cm\(^{-1}\) (section 3, page 114). Furthermore the u.v. peak (290 nm) arising from the cyclic hydrogen bonded enolic diketone in the 2-acetyl steroid occurs at a longer wavelength than that reported for 2-hydroxymethylene-3-keto steroids (ca. 282 nm) or for simple aliphatic β-diketones (ca. 270 nm). It would therefore appear that the cyclic hydrogen bonded enolic form (17β) is more stable in the 2-acetyl steroid than in either 17β-hydroxy-2-hydroxymethylene-5α-androstan-3-one (5) or 2-acetyl-cyclohexanone. This may be due to greater conformational lability in the latter two compounds, resulting in a greater tendency to open to the diketo form. Since acetylacetone, 2-acetyl-cyclohexanone and 17β-hydroxy-2-hydroxymethylene-5α-androstan-3-one (5) (section 3, page 23) have all been shown to condense readily with 2-aminopyridinium iodide in pyridine to form pyrido-pyrimidinium iodides, the inability of 17β-acetoxy-2-acetyl-5α-androstan-3-one (17) to condense readily with 2-aminopyridinium iodide may be due in part to the fact that the cyclic hydrogen bonded enolic β-diketone is more stable in the 2-acetyl steroid than in the 2-hydroxymethylene steroid or the aliphatic β-diketones.

Another related reason for the slowness of the reaction is the probable steric hindrance caused by the methyl group of the
2-acetyl fragment. In anil formation the carbon of the acetyl
carbon will become tetrahedral in the transition state (77). In this
transition state steric hindrance arises because of the close
proximity of the methyl protons and the pyridine ring.

The u.v. spectrum of 1\(^{7}\alpha\)-acetoxy-2\(\alpha\)-acetyl-5\(\alpha\)-androstan-3-one (17)
in a N solution of hydrochloric acid in ethanol shows in addition to
the \(\lambda_{\text{max}}\) at 290nm another \(\lambda_{\text{max}}\) at 232nm (\(\varepsilon=2370\)). The intensity of the
peak at 290nm decreases on going from neutral solution (\(\varepsilon=9600\)) to acid
solution (\(\varepsilon=3970\)). The form of the 2-acetyl steroid attributable to the
absorption at the lower wavelength has not been determined. The attempt
described in section 5.4 (page 45) to condense 1\(\beta\)-hydroxy-2\(\alpha\)-acetyl-
-5\(\alpha\)-androstan-3-one (17) and 2-aminopyridine in a solution of N
hydrochloric acid in ethanol was based on the assumption that the \(\lambda_{\text{max}}\)
at 232nm represented a form of the 2-acetyl steroid in which the
\(\beta\)-dicarbonyl is less strongly hydrogen bonded than in the cyclic
hydrogen bonded enolic form represented by the \(\lambda_{\text{max}}\) at 290nm and
that in this less stable form the acetyl ketone might have been more
accessible for reaction with the amine. However, this experiment
also failed to bring about any detectable amount of condensation
with 2-aminopyridine.
5.6 Experimental.

5.6.1 17β-hydroxy-5α-androstan-3-one (73).

A solution of 17β-hydroxy-5α-androstan-3-one (3.4g, 1.2 x 10^-2 mole) and acetic anhydride (1ml) in pyridine (15ml) was refluxed for 1h. On cooling, the mixture was poured into diethyl ether, washed successively with water, 5% hydrochloric acid, 5% sodium hydroxide and finally with water again. The ether solution was dried over anhydrous magnesium sulphate and evaporated to dryness to give 17β-acetoxy-5α-androstan-3-one (3.89 g, 1.1 x 10^-2 mole, 92%); m.p. 154-156° (lit 158-160°).

5.6ii 17β-acetoxy-2α-acetyl-5α-androstan-3-one (17, R=CH3, CO)74.

(a) Borofluoride complex:

A solution of glacial acetic acid (5.3ml) in ethylene chloride (10ml) was cooled on an ice bath. Boron trifluoride gas, washed through concentrated sulphuric acid, was passed into the stirred solution until saturation was reached. To the resulting white paste kept under nitrogen with continued cooling and stirring, a solution of 17β-acetoxy-5α-androstan-3-one (3.39 g, 1.1 x 10^-2 mole) and acetic anhydride (1ml) in ethylene chloride (8ml) was added. The addition required 15 minutes during which time the mixture became homogenous. After a further 30 minutes in the ice bath the solution was allowed to stand at room temperature for 3h. The solution was then washed with water, saturated sodium bicarbonate solution and water again. The organic phase was dried over anhydrous magnesium sulphate and evaporated to dryness to give the borofluoride complex (4.1g, 1.0 x 10^-2 mole, 91%); m.p. 254-264° (lit 265-277°).

(b) 17β-acetoxy-2α-acetyl-5α-androstan-3-one.

A mixture of borofluoride complex (4.1g, 1.0 x 10^-2 mole) in methanol (170ml) containing sodium acetate (1.58g), glacial
acetic acid (1.09ml) was refluxed for 2.5h. On cooling and refrigeration crystals formed. These were filtered to give 17β-acetoxy-2α-acetyl-5α-androstan-3-one (3.3g, 8.8 x 10⁻³ mole, 88%); m.p. 181-182° (lit 71 180-181°); n.m.r (60MHz), δ 0.80 (C-18 methyl), δ 0.83 (C-19 methyl), δ 2.06 (17α-acetate), δ 2.13 (2-acetyl), δ 4.60 (multiplet, C-17 hydrogen), δ 15.76 (singlet, enol hydrogen); i.r. (CHBr₃), \( \nu_{\max} \) 1705 (ester C=O), 1640-1570cm⁻¹ (broad, –COCH₂CO–, enol form, chelated).

5.6.iii 2α-acetyl-17β-hydroxy-5α-androstan-3-one (17, R=H)₇⁹.

A solution of 17β-acetoxy-2α-acetyl-5α-androstan-3-one (211mg, 5.65 x 10⁻⁴ mole) in methanol (30ml) and acetone (15ml) containing concentrated hydrochloric acid (2ml) was allowed to stand at room temperature for 3 days. After the addition of water, the mixture was extracted with methylene chloride and the latter phase washed with dilute sodium bicarbonate solution and water. The methylene chloride solution was dried over anhydrous magnesium sulphate and evaporated to dryness. Recrystallization of the residue from petroleum ether (60:80) yielded 2α-acetyl-17β-hydroxy-5α-androstan-3-one (143mg, 4.31 x 10⁻⁴ mole, 76%); m.p. 148-149° (lit 71 150-152°).

5.6.iv 17β-acetoxy-5α-androst-2-eno-[2,3-e]-2'-methyl-pyrido-[1,2-a]-pyrimidin-5'-ium iodide (75).

A solution of 17β-acetoxy-2α-acetyl-5α-androstan-3-one (130mg, 4.01 x 10⁻⁴ mole) and 2-aminopyridinium iodide (200mg, 9.0 x 10⁻⁴ mole) in pyridine (25ml) was refluxed for 65h. Some of the pyridine was removed on the rotary evaporator. The resulting tar was taken up in water and washed with diethyl ether. The aqueous phase was evaporated to dryness and recrystallization of the
residue from ethanol; water (1:1) gave 17β-acetoxy-5α-androst-2-eno-
-[2,3-e]-2'-methyl-pyrido-[1,2-a] pyrimidin-5'-ium iodide
(11mg, 1.95 x 10^{-5} mole, 4.9%); u.v. (CH$_3$OH), $\lambda_{\text{max}}$ 216nm (log $\varepsilon$ 4.39),
233 (4.55), 239 (4.52), 309 (3.75), 321 (3.81); mass spectrum,
$M^+$ (-HI), m/e 432, calculated for C$_{26}$H$_{36}$N$_2$O$_2$ 432.277633, found
432.277291, error less than 1 ppm.

The ether fraction was washed with water, dried over
anhydrous magnesium sulphate and evaporated to dryness. The residue
was identified by analytical t.l.c. and i.r. spectroscopy as
17β-acetoxy-2αacetoyl-5α-androstan-3-one.

5.6.v The attempted reaction of 17β-acetoxy-2αacetoyl-5α-androstan-
-3-one (17, R=CH$_3$CO) and 2-aminoypyridine.

(a) A solution of 17β-acetoxy-2αacetoyl-5α-androstan-3-one
(220mg, 5.65 x 10^{-4} mole) and 2-aminoypyridine (130mg, 1.38 x 10^{-4} mole)
in pyridine (5ml) was allowed to stand at room temperature overnight.
The solvent was removed on the rotary evaporator and the residue taken
up in diethyl ether. The ether solution was washed with 5% hydrochloric
acid and water. It was then dried over anhydrous magnesium sulphate
and the solvent evaporated to dryness. Recrystallization of the
residue from ethanol gave 90mg of a white solid, m.p. 178-179°C
(lit.$^7_{14}$ m.p. of starting material 180-181°C). The i.r. and n.m.r.
spectra confirmed the identity of the solid as the starting material,
17β-acetoxy-2αacetoyl-5α-androstan-3-one.

(b) A solution of 17β-acetoxy-2αacetoyl-5α-androstan-3-one
(140mg, 3.74 x 10^{-4} mole) and 2-aminoypyridine (105mg, 1.16 x 10^{-3} mole)
in pyridine (30ml) was refluxed for 2 days. On being worked up as in
(a) above, only starting material was recovered.

(c) The experiment was repeated using ethanol as solvent.
The reaction mixture was again refluxed for 2 days. Only starting
material was recovered.
The attempted reaction of 17\(\beta\)-hydroxy-2\(\alpha\)-acetyl-5\(\alpha\)-androstan-3-one (17, \(R=H\)) and 2-aminopyridine in a solution of N hydrochloric acid in ethanol.

A solution of 17\(\beta\)-hydroxy-2\(\alpha\)-acetyl-5\(\alpha\)-androstan-3-one (1.43 mg, 4.31 x 10^{-4} mole) and 2-aminopyridine (60 mg, 6.38 x 10^{-4} mole) in a solution of N hydrochloric acid in absolute ethanol (20 ml) was refluxed for 5 h. The solvent was removed on the rotary evaporator and the residue taken up in diethyl ether. The ether solution was washed with water, dried over anhydrous magnesium sulphate and evaporated to dryness. The aqueous washings were also evaporated to dryness. The u.v. spectrum of the ether residue had \(\lambda_{\text{max}}\) at 291 nm and was found to co-chromatograph with 17\(\beta\)-hydroxy-2\(\alpha\)-acetyl-5\(\alpha\)-androstan-3-one on a t.l.c. plate. The u.v. spectrum of the aqueous residue had \(\lambda_{\text{max}}\) at 233 nm and 305 nm corresponding to 2-aminopyridine or its chloride salt.
6. The reaction of 17β-hydroxy-2-hydroxymethylene-androst-4-en-3-one with 2-aminopyridine and with 2-aminopyridinium iodide.

6.1 Synthesis of 17β-hydroxy-2-hydroxymethylene-androst-4-en-3-one (6).  
17β-hydroxy-2-hydroxymethylene-androst-4-en-3-one (6) was prepared from 17β-hydroxy-androst-4-en-3-one by the method described by Weisenborn, Remy and Jacobs. The experimental procedure is similar to that for 17α-hydroxy-2-hydroxymethylene-5α-androstan-3-one (5) (section 3.1).

6.2 The reaction of 17β-hydroxy-2-hydroxymethylene-androst-4-en-3-one (6) with 2-aminopyridine.  
A solution of 17β-hydroxy-2-hydroxymethylene-androst-4-en-3-one (6) and 2-aminopyridine in pyridine was allowed to stand at room temperature for 2 days. The product was recovered by evaporation of the solvent and extraction of the residue by diethyl ether. Recrystallization of the residue, obtained on evaporation of the ether, from acetone: petroleum ether (60:80) (1:4) gave a yellow solid (78, R=H), m.p. 235-238°C in 76% yield. Mass spectrometry indicated that the product had a molecular formula of C_{25}H_{32}N_{2}O_{2}. The u.v. spectrum showed λ_{max} at 257nm (log ε 4.34) and 377nm (4.37) and the i.r. spectrum had absorptions at 3600, 1645, 1594, 1550, 1473, 1435 and 1417 cm^{-1}. The n.m.r. spectrum showed signals at δ 6.62-6.90, 8.752 and 8.82 associated with the four protons of a monosubstituted pyridine ring and doublets at δ 7.82 and 8.13 each with spin coupling constant, J=12Hz.

The 17β-acetoxy derivative (78, R=CH_{3}CO) was readily obtained by acetylation of the steroid using acetic anhydride in pyridine.
6.3 Discussion.

The i.r. and n.m.r. spectra obtained for the condensation product of the reaction between 17β-hydroxy-2-hydroxymethylene-androst-4-en-3-one (6) and 2-aminopyridine resemble closely the i.r. and n.m.r. spectra obtained for 17β-hydroxy- and 17β-acetoxy-2(2′-pyridyl-aminomethylene)-5α-androstan-3-one (65) so that the product can be formulated as 17β-hydroxy-2(2′-pyridyl-aminomethylene)-androst-4-en-3-one (78, R=H). In the n.m.r. spectrum the magnitude of the coupling constant (J=12Hz) between the amino proton and the vinyl proton on the adjacent carbon atom indicates that the two protons lie trans to one another while the similarity with respect to the i.r. absorption frequencies and n.m.r. shifts between the spectra of 17β-hydroxy-2(2′-pyridyl-aminomethylene)-androst-4-en-3-one (78) and 17β-acetoxy-2(2′-pyridyl-aminomethylene)-5α-androstan-3-one (65) suggests that both enaminones contain approximately the same degree of internal hydrogen bonding between the amino proton and the C-3 carbonyl.

The application by Huynh and Julia of the method devised by Garbsch to the n.m.r. spectrum of 2-hydroxymethylene-cholest-4-en-3-one (79), determined in deuterochloroform solution, in order to estimate the equilibrium between the hydroxymethylene-ketone and the formyl-enol tautomers showed that the steroid existed in solution as a mixture of 75% hydroxymethylene-ketone (79) and 25% formyl-enol (80). The chemical shifts for the "aldehydic" protons in 2-hydroxy-cholest-4-en-3-one (79) and 17β-hydroxy-2-hydroxy-cholest-4-en-3-one (6) are identical and it is reasonable to assume that the equilibrium between the hydroxymethylene-ketone and formyl-enol tautomers is the same in both steroids. The equilibrium in 17β-hydroxy-2-hydroxymethylene-androst-4-en-3-one (6) is thus the
converse of that in 17β-hydroxy-2-hydroxymethylene-17α-methyl-5,α-androstan-3-one (5), the difference being due probably to the stability conferred on the hydroxymethylene-ketone tautomer by the Δ4 double bond. The conformation of 17β-hydroxy-2(2'-pyridyl-aminomethylene)-androst-4-en-3-one (78) and its hydroxymethylene precursor (6) are therefore the same.

6.4 The cyclization of 17β-acetoxyl-2(2'-pyridyl-aminomethylene)-androst-4-en-3-one (78). Formation of 17β-acetoxyl-androst-2,14-dieno-[2,3-d]pyrido-[1,2-a]pyrimidine-5'-ium iodide (81, R=CH3CO).

17β-acetoxy-2(2'-pyridyl-aminomethylene)-androst-4-en-3-one (78, R=CH3CO) dissolved in the minimum volume of absolute ethanol containing five drops of 55% w/w hydroiodic acid gave an orange precipitate on being stirred for 7 days at room temperature. A sample of the product dissolved in ethanol gave a fluffy precipitate on the addition of a few drops of ethanolic silver nitrate indicating that the product was an iodide. Mass spectrometry indicated that after loss of a hydrogen iodide moiety the product had a molecular formula of C_{27}H_{32}N_2O_2. The u.v. spectrum had λ_{max} at 218nm (log ε = 4.27), 250sh (3.88), 272 (3.77), 294(sh) (3.65), 360 (3.93) and 374 (3.92). The cyclic product was formulated as 17β-acetoxyl-androst-2,4-dieno-[2,3-e]pyrido-[1,2-a]pyrimidine-5'-ium iodide (81, R=CH3CO).

6.5 The reaction of 17β-hydroxy-2-hydroxymethylene-androst-4-en-3-one (6) and 2-aminopyridinium iodide.

(a) A solution of 17β-hydroxy-2-hydroxymethylene-androst-4-en-3-one (6) and 2-aminopyridinium iodide in pyridine was refluxed gently for 15 minutes. After evaporating the solvent, water was added
to the residue and the resultant mixture extracted with diethyl ether. The ether extract was worked up and the residue on evaporation of the solvent, recrystallized from acetone: petroleum ether (60:80) to give 17β-hydroxy-2(2′-pyridyl-aminomethylene)-androst-4-en-3-one (78, R=H) in 70% yield. On evaporation, the residue from the aqueous phase was recrystallized from ethanol to give 16mg of orange material, a small sample of which when dissolved in ethanol gave a precipitate on the addition of a few drops of ethanolic silver nitrate. The molecular formula of the solid was found by mass spectrometry to be C_{25}H_{31}N_{2}O_{2}I while the u.v. spectrum showed λ_{max} at 213nm (log ε 4.37), 231 (ε 4.43), 238 (ε 4.35), 313 (ε 3.64) and 327 (ε 3.70).

(b) The reaction of 17β-hydroxy-2-hydroxymethylene-androst-4-en-3-one (6) with 2-aminopyridinium iodide was repeated. This time the reaction mixture was refluxed for 2 hours, in anticipation that the product ratio would be more favourable towards the cyclized product with a longer reaction time than in (a). The reaction was also carried out under nitrogen in an attempt to exclude moisture. On cooling, ether was poured in and the resulting precipitate filtered and dried in vacuo. The yellow-orange solid was then washed with ether to remove any neutral material and dried. A well defined u.v. spectrum was not obtained but it was concluded that the solid was a mixture of 17β-hydroxy-androst-2,4-dieno-[2,3-e] pyrido-[1,2-a] pyrimidin-5′-ium iodide (81, R=H), 2-aminopyridinium iodide and the steroid, C_{25}H_{31}N_{2}O_{2}I, also found in (a) above.

An attempt was made to recrystallize the solid from absolute ethanol. The u.v. spectrum of the recrystallized material had λ_{max} at 225, 241, 275, 314, 327 and 370nm suggesting that the
(78)

(81)

(6)

(59)
material was a mixture of 17β-hydroxy-androst-2,4-dieno-[2,3-e]-pyrido-[1,2-a] pyrimidin-5'-ium iodide (81) and the steroid, C_{25}H_{31}N_{2}O_{2}I.

The reaction of 17β-acetoxy-androst-2,4-dieno-[2,3-e]-pyrido-[1,2-a] pyrimidin-5'-ium iodide (81) with water.

A solution of 17β-acetoxy-androst-2,4-dieno-[2,3-e]-pyrido-[1,2-a] pyrimidin-5'-ium iodide (81, R=CH_3CO) in aqueous ethanol containing a drop of 55% w/w hydroiodic acid was refluxed for 1 hour. On reducing the volume of the solvent a precipitate formed. The precipitate had an u.v. spectrum which showed \( \lambda_{\text{max}} \) at 215 nm (log \( \varepsilon \) 4.25), 231 (4.32), 238 (4.26), 313 (3.60) and 325 (3.67). Mass spectrometry gave the molecular formula as C_{27}H_{33}N_{2}O_{3}I.

Discussion.

17β-acetoxy-2(2'-pyridyl-aminomethylene)-androst-4-en-3-one (78) cyclizes on protonation of the pyridine ring nitrogen to give a product formulated as 17β-acetoxy-androst-2,4-dieno-[2,3-e] pyrido-[1,2-a] pyrimidin-5'-ium iodide (81, R=CH_3CO). However when 17β-hydroxy-2-hydroxymethylene-androst-4-en-3-one (6) and 2-aminopyridinium iodide were refluxed in pyridine for 15 minutes, similar conditions to those employed in the synthesis of 17β-hydroxy-5α-androst-2-eno-[2,3-e] pyrido-[1,2-a] pyrimidin-5'-ium iodide (59) from 17β-hydroxy-2-hydroxymethylene-5α-androstan-3-one (5), the major product was the 2(2'-pyridyl-aminomethylene) derivative (78, R=H). Furthermore the small quantity of cyclized product which was recovered from the aqueous phase had an u.v. spectrum characteristic of the 10 electron chromophore found in the 5α-androst-2-eno-[2,3-e] pyrido-[1,2-a] pyrimidin-5'-ium iodide (59).
and not as had been predicted the same u.v. spectrum as obtained for 17β-acetoxy-androst-2,4-dieno-[2,3-e] pyrido-[1,2-a] pyrimidin-5'-ium iodide (81, R=CH₃CO). The mass spectrum of the cyclized product indicated that it had one oxygen atom more than the androst-2,4-dieno-[2,3-e] pyrido-[1,2-a] pyrimidin-5'-ium iodide (81).

A sample of 17β-acetoxy-androst-2,4-dieno-[2,3-e] pyrido-[1,2-a] pyrimidin-5'-ium iodide (81) in aqueous ethanol containing a trace of hydroiodic acid on refluxing for an hour gave a small quantity of solid, whose u.v. spectrum was characteristic of the androst-2-enol-[2,3-c] pyrido-[1,2-a] pyrimidin-5'-ium iodide system (59) and whose mass spectrum showed that the molecular formula was C₂₇H₃₃N₂C₃I. It was concluded that this steroid was the 17β-acetate of the unidentified steroid, C₂₅H₃₁N₂O₂I found as one of the products of the reaction of 17β-hydroxy-2-hydroxymethylene-androst-4-en-3-one (6) and 2-aminopyridinium iodide.

When the reaction of 17β-hydroxy-2-hydroxymethylene-androst-4-en-3-one (6) and 2-aminopyridinium iodide was repeated with the reaction time being prolonged to two hours and precautions being taken to exclude water both during the reaction and the work up, the u.v. spectrum of the product obtained indicated that it was a mixture of 17β-hydroxy-androst-2,4-dieno-[2,3-e] pyrido-[1,2-a] pyrimidin-5'-ium iodide (81, R=H) and the steroid, C₂₅H₃₁N₂O₂I, whose u.v. spectrum showed the presence of the 10 electron chromophore.

It appears that 17β-hydroxy-androst-2,4-dieno-[2,3-e] pyrido-[1,2-a] pyrimidin 5'-ium iodide (81, R=H) cannot be synthesised from 17β-hydroxy-2-hydroxymethylene-androst-4-en-3-one (6) and 2-aminopyridinium iodide as the only pyrido-pyrimidinium product unless water can be completely excluded from the reaction mixture and work up. This situation appears to be very difficult to obtain
and it seems that the most convenient route to the synthesis of
the androst-2,4-dieno-[2,3-e] pyrido-[1,2-a] pyrimidin-5'-ium
iodide system (81) is by acid catalysed cyclization of the
2(2'-pyridyl-aminomethylene)-androsten-3-one (78) in absolute
ethanol at room temperature.

The molecular formula of the steroid, C_{27}H_{33}N_{2}O_{3}I,
indicates that it differs from that of 17β-acetoxy-androst-2,4-
dieno-[2,3-e] pyrido-[1,2-a] pyrimidin-5'-ium (81, R=CH_{3}CO) in
having one more oxygen atom. The u.v. spectrum of the steroid,
C_{27}H_{33}N_{2}O_{3}I, shows that it is an androst-2-eno-[2,3-e]
pyrido-[1,2-a] pyrimidin-5'-ium iodide (82). It appears that this steroid
has been formed by the reaction of water on the Δ^4 double bond of
its precursor (81). However the structure of the steroid,
C_{27}H_{33}N_{2}O_{3}I has not been definitely established. Insufficient
material was recovered to allow an i.r. spectrum to be obtained.

Epoxide formation can be eliminated on the grounds that the
reaction conditions were not those normally associated with epoxide
formation. The formation of a carbonyl group at C-4 on the
A-ring which would be conjugated to the aromatic heterocyclic ring
system can be dismissed on the evidence of the u.v. spectrum.
The only other structures that can be postulated are those in which
there is a hydroxyl group on either the C-4 or C-5 of the steroid
and the double bond has moved out of conjugation into the B-ring.
One such possibility is 17β-acetoxy-45-hydroxy-androst-2,5-dieno-
-[2,3-e] pyrido-[1,2-a] pyrimidin-5'-ium iodide (83). The formation
of this steroid can be postulated on consideration of the possible
mechanism of cyclization of the steroidenal enamine (78) and it will
be discussed in greater detail along with possible mechanisms of
cyclization in section 7.2.
6.8 Experimental.

6.8.1 17α-hydroxy-2-hydroxymethylene-androst-4-en-3-one (6)\(^80\).

Sodium hydride (1.9g, 80% dispersion in oil, washed with dry benzene) was added to 17β-hydroxy-androst-4-en-3-one (5.0g, 1.7 x 10^{-2} mole) dissolved in dry benzene (100ml) and ethyl formate (5ml) and the mixture stirred at room temperature for 3 days. Methanol (5ml) was then added to destroy excess sodium hydride. Water (150ml) was added and the layers separated. The aqueous solution was extracted with diethyl ether to remove any neutral material and then acidified with 30% hydrochloric acid (40ml). The liberated enol was extracted with diethyl ether and the organic layer washed with water, dried over anhydrous magnesium sulphate and the solvent evaporated to dryness. Trituration of the residue with chloroform : diethyl ether (1:1) gave 17β-hydroxy-2-hydroxymethylene-androst-4-en-3-one (3.2g, 10^{-2} mole, 60%); m.p. 167-9° (lit\(^80\) 165-6°); u.v. (C\(_2\)H\(_5\)OH), \(\lambda\)\(_{\text{max}}\) 252nm (log \(\varepsilon\) 4.06); 306 (3.76); n.m.r. (60MHz), \(\delta\) 0.73 (C-18 methyl), 1.03 (C-19 Methyl), 3.60 (multiplet, C-17 hydrogen), 5.70 (C-4 vinyl hydrogen), 7.30 (singlet, average of -CO.C=CH.OH and -CO.H=CH.C=O)\(^81\); i.r. (CHBr\(_3\)), \(\nu\)\(_{\text{max}}\) 3600 (OH), 16\(_{\text{HC}}\)cm\(^{-1}\) (CO.CH\(_2\).CO\(_{-}\), enol form, chelated).

6.8ii 17β-hydroxy-2'(2'-pyridyl-aminomethylene)-androst-4-en-3-one (78, R=H).

A solution of 17β-hydroxy-androst-4-en-3-one (250mg, 7.91 x 10^{-4} mole) and 2-aminopyridine (77mg, 8.20 x 10^{-4} mole) in pyridine (1ml) was allowed to stand at room temperature for 2 days. The solvent was removed on the rotary evaporator and the residue taken up in diethyl ether, washed with 5% hydrochloric acid and water, dried over anhydrous magnesium sulphate and evaporated to dryness.
Recrystallization of the residue from acetone and petroleum ether 
(60:80) (1:1) gave 17β-hydroxy-2(2'-pyridyl-aminomethylene)-
androst-4-en-3-one (237mg, 6.05 x 10^{-4} mole, 76%); m.p. 235-238°C; u.v. (CH₃OH), \( \lambda_{\text{max}} \)
257nm (log ε 4.34), 377 (4.37); n.m.r. (100MHz), δ 0.80 (C-18 methyl),
δ 1.07 (C-19 methyl), δ 3.58 (multiplet, C-17 hydrogen), δ 5.78 
(C-4 vinyl hydrogen), δ 6.62-δ.90 (2H, multiplet, pyridine ring,
3-H, 5-H), δ 7.52 (1H, doubly degenerate doublet, pyridine ring, 4-H),
δ 7.80 (1H, doublet, \( J=12Hz,\text{-NH-C}_{\text{H}}-\text{C}- \)), δ 8.22 (1H, doublet, pyridine 
ring, 6-H), δ 11.36 broad (1H, doublet, \( J=12Hz,\text{-N-H} \)); i.r. (CHBr₃),
\( \nu_{\text{max}} \) 3600 (OH), 1645 (C=O), 1592,1549,1470,1435,1415cm⁻¹; mass 
spectrum, \( M^+ \), m/e 392, calculated for \( C_{25}H_{32}N_2O_2 \) 392.214365, found 
392.214753, error less than 1 ppm.

6.8.iii 17β-acetoxy-2(2'-pyridyl-aminomethylene)-androst-4-en-3-one 
(78,\text{R}=\text{CH}_{\text{2}}\text{CO}).

17β-hydroxy-2(2'-pyridyl-aminomethylene)-androst-4-en-3-one 
(120mg, 4.11 x 10^{-4} mole) was acetylated using the method described 
for 17β-acetoxy-2(2'-pyridyl-aminomethylene)-5α-androstan-3-one 
(section 3.9.v) to give its 17β-acetate. Recrystallization of the 
crude product from methanol gave 17β-acetoxy-2(2'-pyridyl-aminomethylene)-
androst-4-en-3-one (95mg, 2.19 x 10^{-4} mole, 53%); 
m.p. 245-250°C; u.v. (CH₃OH), 257nm (log ε 2.30), 378 (4.37); i.r. 
(CHBr₃), \( \nu_{\text{max}} \) 1720 (ester C=O), 1645 (C=O), 1594,1550,1473,1435, 
1417cm⁻¹; mass spectrum, \( M^+ \), m/e 434.

6.8.iv 17β-acetoxy-androst-2,4-dieno-[2,3-e]pyrido-[1,2-a]
pyrimidin-5'-ium iodide (81,\text{R}=\text{CH}_{\text{2}}\text{CO}).

17β-acetoxy-2(2'-pyridyl-aminomethylene)-androst-4-en-3-one 
(80mg, 1.85 x 10^{-4} mole) was dissolved in absolute ethanol (50ml). 
Five drops of 55% w/w hydroiodic acid were added and the solution
was stirred for 7 days. The precipitate which had formed was collected and dried to give \( \text{17\textbeta-acetoxy-androst-2,14-dieno-[2,3-e]pyrido-[1,2-a]pyrimidin-5'-ium iodide} \) (80mg, \( 1.47 \times 10^{-4} \) mole, 79%);

\[ \text{UV (CH}_3\text{OH), } \lambda_{\text{max}} \text{ 218nm (log e 4.27), 250 (sh) (3.88), 272 (3.77), 294 (sh) (3.65), 360 (3.93), 374 (3.92); mass spectrum, } M^+(-HI), m/e 416, \text{ calculated for } C_{27}H_{32}N_2O_2 416.2146365, \text{ found 416.2145610, error less than 1 ppm.} \]

The reaction of \( \text{17\textbeta-hydroxy-2-hydroxymethylene-androst-4-en-3-one and 2-aminopyridinium iodide} \).

(a) A solution of \( \text{17\textbeta-hydroxy-2-hydroxymethylene-androst-4-en-3-one} \) (110mg, \( 3.48 \times 10^{-4} \) mole) and 2-aminopyridinium iodide (100mg, \( 4.50 \times 10^{-4} \) mole) in pyridine (2ml) was refluxed gently for 15 minutes. Some of the pyridine was removed on the rotary evaporator. Water was added to the residue and the mixture was extracted with diethyl ether. The aqueous phase was evaporated to dryness and recrystallized from ethanol to give a brownish-red solid,

\[ \text{molecular formula } C_{25}H_{31}N_2O_2 \text{I}, (16mg, } 3.08 \times 10^{-5} \text{ mole, 8.9%}; \] \[ \text{UV (CH}_3\text{OH), } \lambda_{\text{max}} \text{ 213nm (log e 4.37), 231 (4.43), 238 (4.35), 313 (3.64), 327 (3.70); mass spectrum, } M^+(-HI), m/e 390, \text{ calculated for } C_{25}H_{30}N_2O_2 390.2307159, \text{ found 390.229451, error less than 4 ppm.} \]

The ether extract was washed with water, dried over anhydrous magnesium sulphate and evaporated to dryness. The residue was recrystallized from acetone : petroleum ether (60:80)(1:4) to give \( \text{17\beta-hydroxy-2-(2'-pyridyl-aminomethylene)-androst-4-en-3-one} \)

(b) A solution of \( \text{17\beta-hydroxy-2-hydroxymethylene-androst-4-en-3-one} \) (105mg, \( 3.32 \times 10^{-4} \) mole) and 2-aminopyridinium iodide (75mg, \( 3.38 \times 10^{-4} \) mole) in pyridine (3ml) was refluxed gently for 2h under nitrogen. On cooling, diethyl ether was poured in and the
resulting precipitate filtered and dried in vacuo. The solid was then added to diethyl ether and the mixture stirred for 2 days. The solid was then filtered off and dried. The u.v. spectrum of the solid was not well defined but showed \( \lambda_{\text{max}} \) at 223, 252, 280, 326 and 371 nm. It was concluded that the solid was a mixture of 17β-hydroxy-androst-2,4-dieno-[2,3-e] pyrido-[1,2-a] pyrimidin-5'-ium iodide, the unidentified steroid \( \text{C}_{25} \text{H}_{31} \text{N}_{2} \text{O}_{2} \text{I} \) found in (a) above and 2-aminopyridinium iodide. An attempt was made to recrystallize the solid from absolute ethanol. The u.v. spectrum of the recrystallized material had \( \lambda_{\text{max}} \) at 225, 241, 275, 314, 327 and 370 nm. It was concluded that the recovered material was a mixture of 17β-hydroxy-androst-2,4-dieno-[2,3-e] pyrido-[1,2-a] pyrimidin-5'-ium iodide and the unidentified steroid \( \text{C}_{25} \text{H}_{31} \text{N}_{2} \text{O}_{2} \text{I} \).

6.8.vi The reaction of 17β-acetoxy-androst-2,4-dieno-[2,3-e] pyrido-[1,2-a] pyrimidin-5'-ium iodide (81) with hot aqueous hydroiodic acid.

A solution of 17β-acetoxy-androst-2,4-dieno-[2,3-e] pyrido-[1,2-a] pyrimidin-5'-ium iodide (20 mg, \( 3.67 \times 10^{-5} \) mole) in aqueous ethanol (15 ml) containing 55% w/w hydroiodic acid (1 drop) was refluxed for 1 h. The volume of the solvent was reduced on the rotary evaporator. The precipitate which had formed was filtered and washed with a little cold diethyl ether to give a red solid, (moleculer formula \( \text{C}_{27} \text{H}_{33} \text{N}_{2} \text{O}_{3} \text{I} \)), (2 mg, \( 4.17 \times 10^{-6} \) mole, 12.5%); u.v. (CH\(_3\)OH), \( \lambda_{\text{max}} \) 215 nm (log e 4.25), 231 (4.32), 238 (sh)(4.26), 313 (3.60), 325 (3.67); mass spectrum, \( \text{M}^+ \) (-HI), m/e 432, calculated for \( \text{C}_{27} \text{H}_{32} \text{N}_{2} \text{O}_{3} \) 432.241279, found 432.242787, error less than 4 ppm.
Scheme 1.

(5) → (61)

(63) → (84)

(85) → (65)
Possible mechanisms for the condensation of $\beta$-dicarbonyl steroids with 2-aminopyridine.

The reaction of steroidal $\beta$-dicarbonyls with 2-aminopyridine as described in the preceding sections almost certainly proceeds by the accepted mechanism for anil formation (scheme 1)\(^3\). This requires that the hydroxymethylene (or acetyl) moiety exists in the carbonyl form and is not hydrogen bonded. The i.r. spectrum of 17$\beta$-hydroxy-2-hydroxymethylene-5$\alpha$-androstan-3-one (5) in addition to the broad absorption at 1640-1540 cm\(^{-1}\) also shows an absorption of medium intensity at 1700 cm\(^{-1}\) which can be attributed to the formyl-keto form (63) of the $\beta$-keto-aldehyde. This suggests that small amounts of formyl-keto form (63) exist in equilibrium with the keto-enol forms (5, 64). It is reasonable to assume that this is the form of the hydroxymethylene steroid undergoing condensation with 2-aminopyridine. The i.r. spectrum of 3$\beta$-hydroxy-16-hydroxymethylene-androstan-5-en-17-one (57) shows a similar absorption at 1710 cm\(^{-1}\) which again can be attributed to the formyl-keto form (66).

As noted earlier (section 5.5, page 46), the absence of any absorption in the 1750-1700 cm\(^{-1}\) region of the i.r. spectrum of 2$\alpha$-acetyl-17$\beta$-hydroxy-5$\alpha$-androst-3-one (17, R=H) indicated that as the cyclic hydrogen bonded enolic form (17a) of the $\beta$-dicarbonyl appears to be more stable in the 2-acetyl steroid than in the 2-hydroxymethylene steroid, there would be less tendency for the former steroid to open to the diketo form (17). The fact that the 2-acetyl steroid reacts considerably more slowly with 2-aminopyridine than the 2-hydroxymethylene steroid does and that 2-acetyl cyclohexanone which exhibits an i.r. absorption at 1700 cm\(^{-1}\) also reacts readily with 2-aminopyridine\(^3\) points to the non-hydrogen bonded diketo form being the form of the $\beta$-dicarbonyl steroid that condenses with 2-aminopyridine.
The i.r. spectrum of 17β-hydroxy-2-hydroxymethylene-androst-
-4-en-3-one (6) is difficult to interpret in a similar way to that of
17β-hydroxy-2-hydroxymethylene-5α-androstan-3-one (5). It exhibits
only a very weak absorption at 1725cm⁻¹ and does not show an intense
broad absorption in the 1640-1570cm⁻¹ region characteristic of the
cyclic hydrogen bonded enolic form of β-ketoaldehyde but rather it
shows a relatively narrow basorption at 1640cm⁻¹. However, if it is
the non-hydrogen bonded formyl-keto form of the hydroxymethylene steroid
that condenses with 2-aminopyridine it must be assumed that the cyclic
hydrogen bonded form of 17β-hydroxy-2-hydroxymethylene-androst-
-4-en-3-one can open out to the diketo form (87).

A possible reason for condensations involving 2-aminopyridine
or aniline occurring with the formyl carbonyl rather than with the
keto carbonyl may be that if anil formation occurred at the C-3 ketone,
there would be steric hindrance in both the intermediate (88) and the
product (66) because of the proximity of the pyridine ring to the
A-ring of the steroid. Steric hindrance in intermediate (84) formed
in the course of condensation with the less crowded formyl group
would be expected to be less. However, when the formyl group is
replaced by an acetyl group as in 17β-acetoxy-2α-acetyl-5α-androstan-
-3-one (17) steric hindrance may arise in the intermediate (77) of
anil formation and as remarked earlier (section 5.5, page 146), this
could be one reason why anil formation in the 2-acetyl steroid (17)
proceeds less readily than in the hydroxymethylene steroids.

Shaw⁸⁴ reported the formation of ethyl 2-cyano-3-anilino-
-acyrloyl-carbamate (91) from ethyl 2-cyano-3-ethoxy-acyrloyl-
-carbamate (89) and aniline in cold ethanol. In this reaction, the
anilino group has displaced an ethoxy group by the mechanism shown
in scheme 2. The formation of ethyl N(2-pyridinyl)-aminoethylene-
Scheme 4.

(65) $\xrightarrow{H^+} (95)$

(96) $\xrightarrow{\text{H}_2\text{O}} (98) \xrightarrow{-\text{H}_2\text{O}} (59)$
-cyanoacetate (93) by the reaction of ethyl ethoxymethylene-
cyanoacetate (92) and 2-aminopyridine has also been reported\textsuperscript{48}. The participation of the hydroxymethylene-ketone form (5) of 17β-hydroxy-2-hydroxymethylene-5α-androstan-3-one in anil formation by analogy to the earlier work cited above can be postulated (scheme 3). This mechanism would be more likely to operate in polar solvents such as methanol where the intramolecular hydrogen bonding in the hydroxymethylene steroid is replaced by hydrogen bonding between the steroid and the solvent. Moreover the polar solvent will be able to accommodate the charge separation of the intermediate (94) more readily than less polar solvents such as pyridine.

7.2 Possible mechanisms of cyclization of the 2(2'-pyridyl-
-aminomethylene)-3-keto system.

Schemes 4 and 5 show two possible mechanisms by which the 2(2'-pyridyl-aminomethylene)-3-ketone system may cyclize. In both mechanisms rotation about the C-N bond must occur while the molecule is in enaminone form (95) so as to bring the pyridine ring nitrogen into the proximity of the carbonyl group. This involves the destruction of the hydrogen bonded six-membered ring with the associated loss of free energy.

In the first mechanism (scheme 4), the enaminone (96) then equilibrates to the iminoenol (97) and cyclization occurs through formation of a bond between the pyridine nitrogen and the carbonyl carbon (98) followed by loss of water to give the aromatic system (59). A feature of this mechanism is that the formation of the C-N bond (97 and 98) involves the disruption of the aromatic system of the pyridine ring. This would involve energy being put into the system and may not be consistent with the observation that cyclization
Scheme 5.

(65) $\xrightarrow{\text{H}^3} \quad \text{(95)} \quad \text{(95)}$

(99) $\xrightarrow{\text{?}} \quad \text{(100)}$

(101) $\xrightarrow{\text{?}} \quad \text{(59)}$
occurs readily at room temperature. On the other hand, it can be argued that since disruption of the aromatic system occurs in nucleophilic substitution, the possibility of break up of the aromatic system of the pyridine ring during cyclization cannot be entirely dismissed.

Scheme 5 envisages an equilibrium existing between structure (96) where the proton is on the pyridine ring and structure (99) where it is on the carbonyl oxygen. On the formation of a bond between the nitrogen and C-3 carbon by nucleophilic attack by the former, the enamine (100) equilibrates to the imine (101) to permit the formation of a double bond across the C-2 and C-3 of the steroid by loss of water to give the aromatic system (59). The formation of the imine (101) is necessary as loss of water in the enamine (100) is improbable. Its formation can be further justified by the fact that it leads to a conjugated system of eight \( \pi \) electrons, instead of six as in the enamine (100) and will contribute towards stabilising the positive charge on the bridgehead nitrogen.

It was suggested in Section 4.5 (page 37) that it appeared likely that 3\( \beta \)-acetoxy-16(2'-pyridyl-aminomethylene)-androst-5-en-17-one (71) did not cyclize to 3\( \beta \)-acetoxy-androst-5,16-dien-[16,17-\( \alpha \)]-pyrido-[1,2-a] pyrimidin-5'-ium iodide (72) for mechanistic reasons even though aromatic systems fused to the D-ring of the steroid are known\(^{23,34} \). It can be assumed that cyclization would in theory proceed by analogous mechanisms to those postulated in scheme 4 and scheme 5. Examination of schemes 4 and 5 reveal only two possible explanations. The first, in the mechanism analogous to that postulated in scheme 4, is that since \( \beta \)-unsaturated cyclopentanones where the C-C double bond is exocyclic are known to be less highly enolized than the corresponding cyclohexanone derivatives\(^{85} \), the equilibrium
Scheme 6.

Scheme 7.
(36) $x = O, S, NH$.

(35) $x = O, S, NH$.

(5)
between the tautomeric intermediates, (102) and (103) (scheme 6),
may lie sufficiently to the right for the rate of cyclization to be
seriously retarded by the slow rate of enolization of the enaminone (102)
to the iminoenol (103).

The second possible explanation relates to the analogous
mechanism to that shown in scheme 5. In the intermediate (104)
(c.f. intermediate (101) in scheme 5) the hydroxyl group and the
hydrogen on the adjacent carbon will not be able to take up a confor-
mation antiperiplanar to one another which may be necessary to permit
expulsion of a water molecule to give the cyclic aromatic system (72).
In the analogous case with the six-membered ring (101) the hydroxyl
group and the hydrogen on the adjacent carbon will most likely be
axial and antiperiplanar to the A-ring of the steroid with the
heterocyclic rings equatorial. However, in the five-membered D-ring,
the heterocyclic rings will be fused in such a way that they are either
above or below the plane of the D-ring while the hydroxyl group and
the hydrogen will be on the opposite side of the plane of the D-ring
and approximately synperiplanar.

It is interesting to note that a mechanism can be postulated
for the formation of 3β-hydroxy-5α-androst-16-en-17-one (35) by the reaction of
16-aminomethylene-3β-hydroxy-5α-androstan-17-one and a 4-aminouracil
which does not involve either a tautomeric equilibrium with a double
bond in the D-ring or loss of water across adjacent carbon atoms in
the D-ring. Furthermore the synthesis of the analogous 17β-hydroxy-
5α-androstan-3-one (5) with a 4-aminouracil has also been reported.
Scheme 8.

(78) $H^+$

(105)

(81)

(83)

(83) $\sim H_2O$
In section 6.7 (page 58), it was suggested that 17β-acetoxy-4'-hydroxy-androst-2,5-diene-[2,3-e]-pyrido-[1,2-a] pyrimidin-5'ium iodide (83) might be the product formed when water reacted with the Δ4 double bond of 17β-acetoxy-androst-2,4-diene-[2,3-e] pyrido-[1,2-a] pyrimidin-5'ium iodide (81). The formation of structure (83) is postulated on the assumption of two competitive reactions occurring during cyclization of the 2(2'-pyridyl-aminomethylene)-androst-4-en-3-one (78). If the enamino (78) cyclizes by the mechanism postulated in scheme 4, on protonation the Δ2,4'-enol (105) (scheme 8) will be formed. However by analogy with the enolization of cholest-4-en-3-one 86 in similar conditions it is reasonable to assume that the Δ2,4'-enol (105) will be quickly converted to the more stable Δ3,5'-enol (106). Both the Δ2,4' and Δ3,5' enols can cyclize and lose a molecule of water to give the androst-2,4-diene-[2,3-e]-pyrido-[1,2-a] pyrimidin-5'ium iodide (81). The formation of another steroidal product (83) with one more oxygen atom than the steroid (81) can thus be explained by the attack of water on the Δ3,5'-enol and simultaneous cyclization to give the 4'-hydroxy-2,5-diene-[2,3-e] pyrido-[1,2-a] pyrimidin-5'ium iodide (83).
8. The reaction of 2α-carbomethoxy-17β-hydroxy-17α-methyl-5α-
androstan-3-one with 2-aminopyridinium iodide, and with
2-aminopyrididine.

8.1 The synthesis of 2α-carbomethoxy-17β-hydroxy-17α-methyl-
-5α-androstan-3-one (58).

2α-carbomethoxy-17β-hydroxy-17α-methyl-5α-androstan-
-3-one (58) was prepared by the method described by de Ruggiere
et al. 25. The synthesis was effected by the condensation of dimethyl
carbonate with the Δ2-enolate ion of 17β-hydroxy-17α-methyl-5α-
-androstan-3-one in dimethyl sulfoxide. The 2α-carbomethoxy-3-
keto steroid was precipitated out of the reaction mixture by
water and recrystallized from methanol. The i.r. spectrum of the
product exhibited strong absorptions at 1655 and 1615 cm⁻¹. The
first absorption has been assigned to the ester carbonyl stretching
vibration in an β-unsaturated ester and the second to a C-C double
bond stretch. The n.m.r. spectrum gave a singlet at δ 12.00 which
was assigned to an enolic proton. The β-keto ester exists
predominantly in the cyclic hydrogen bonded enolic form (107).

8.2 The reaction of 2α-carbomethoxy-17β-hydroxy-17α-methyl-5α-
-androstan-3-one (58) with 2-aminopyridinium iodide.

A solution of 2α-carbomethoxy-17β-hydroxy-17α-methyl-5α-
-androstan-3-one (58) and 2-aminopyridinium iodide in pyridine was
refluxed for 4 hours. After removing the solvent on the rotary
evaporator the residue was taken up in chloroform and washed with
water. Analysis of the residue obtained on evaporation of the
chloroform by t.l.c. indicated the presence of three components.
Two were identified by co-chromatography as the starting material,
2α-carbomethoxy-17β-hydroxy-17α-methyl-5α-androstan-3-one (58) and
17β-hydroxy-17α-methyl-5α-androstan-3-one. The third component had a \( R(\text{starting material}) \) value of 0.58. The component \( R(\text{starting material}) = 0.58 \) was readily isolated from the other two components by preparative t.l.c. and subsequently recrystallized from ethanol to give a white solid (108) in 22% yield, m.p. 245-250°C.

Mass spectrometry indicated that the product had a molecular formula of \( C_{26}H_{34}N_2O_2 \) and the u.v. spectrum showed \( \lambda_{\text{max}} \) at 207 nm (log ε 3.95), 216 (4.13), 238 (4.10), 244 (4.03), 253 (3.94) and 335 (3.95). The i.r. spectrum exhibited absorptions at 3570, 1650, 1625, 1570, and 1525 cm\(^{-1}\) while in the n.m.r. spectrum apart from singlets corresponding to the C-17, C-18 and C-19 methyl protons, there was a multiplet at \( \delta 7.00 \) (1 proton), another multiplet at \( \delta 7.58 \) (2 protons) and a doublet at \( \delta 8.90 \) (1 proton).

The experiment was repeated using 2-amino-4-methyl-pyridinium picrate instead of 2-aminopyridinium iodide. Analysis by t.l.c. of the organic extract revealed the presence of three components. Again the starting material, 2α-carboxymethoxy-17β-hydroxy-17α-methyl-5α-androstan-3-one (58) and 17β-hydroxy-17α-methyl-5α-androstan-3-one were found. The third component had a \( R(\text{starting material}) \) value of 0.79. It was not found possible to separate the component \( R(\text{starting material}) = 0.79 \) completely from the other two components on the first attempt at preparative t.l.c. However a pure sample (110) in 5% yield was obtained at a second attempt. Mass spectrometry indicated that the product had molecular formula \( C_{27}H_{36}N_2O_2 \) and the u.v. spectrum showed \( \lambda_{\text{max}} \) at 211 nm (log ε 4.30), 218 (sh) (4.20), 237 (4.06), 242 (4.08), 251 (3.98), 328 (4.03) and 341 (4.02). The n.m.r. spectrum in addition to the signals for the C-17, C-18 and C-19 methyl protons showed singlets at \( \delta 2.13 \) (3 protons) and \( \delta 7.26 \) (1 proton), and doublets at \( \delta 6.86 \) and \( \delta 8.83 \) (1 proton each).
\( (61) \)

\[ \text{Diagram of compound 61} \]

\( (62) \)

\[ \text{Diagram of compound 62} \]

\( (108) \)

\[ \text{Diagram of compound 108} \]

\( (109) \)

\[ \text{Diagram of compound 109} \]

\( (46) \)

\[ \text{Diagram of compound 46} \]

\( (49) \)

\[ \text{Diagram of compound 49} \]
Steroidal

Pyrido-pyrimidone

(108)

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*Table 1.*
It was remarked in the Introduction (section 1, page 12) that there are two possible products (61, 62) of the condensation between a 24-carbomethoxy-3-keto steroid and 2-aminopyridinium iodide. In contrast to the reaction of ethyl acetoacetate and 2-aminopyridinium iodide in pyridine, where the product is obtained as the iodide salt, the steroid is in fact isolated as the free base. The molecular formula obtained from mass spectrometry of the reaction product confirms that the product is the free base (108 or 109) of one of the two structures (61, 62) postulated earlier. The i.r., n.m.r. and u.v. spectra of the steroidal product were compared with those reported for 2-methyl-1H-pyrido-[1,2-a] pyrimidin-4-one (46) and 4-methyl-2H-pyrido-[1,2-a] pyrimidin-2-one (49). The i.r. spectrum of the steroid does not correlate sufficiently well with the i.r. spectrum of either of the model compounds (46, 49) to be of any use in determining the structure of the steroid. The signals at 87.00, 87.68 and 88.90 in the n.m.r. spectrum of the steroid correlate better with the signals for the C-9 (87.16), C-7 and C-8 (87.66), and C-6 (89.05) protons in the n.m.r. spectrum of the pyrido-[1,2-a] pyrimidin-4-one (46) than for the corresponding resonances in the pyrido-[1,2-a] pyrimidin-2-one (49), (87.23 (9-H), 87.75 (7-H, 8-H) and 88.25 (6-H)). The most convincing evidence as to the structure of the steroidal pyrido-pyrimidinone is obtained by comparison of the u.v. spectra of the steroid and the model compounds (46, 49). The relevant data is given in Table 1. With the exception of the $\lambda_{\text{max}}$ at 216 nm in the steroid, the u.v. spectrum of the steroid gives good correlation with the u.v. spectrum of 2-methyl-1H-pyrido-[1,2-a] pyrimidin-4-one (46). The u.v. spectrum of the steroid bears little resemblance to that of 4-methyl-2H-pyrido-[1,2-a] pyrimidin-2-one (49), so that structure (109) can
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Table 2.
be eliminated as a possible product. On this basis, the steroid was formulated as 17β-hydroxy-17α-methyl-5α-androst-2-eno-[3,2-d]-4′H-pyrido-[1,2-a] pyrimidin-4′-one (108).

The u.v. spectrum of the condensation product of the reaction between 2α-carbomethoxy-17β-hydroxy-17α-methyl-5α-androst-3-one (58) and 2-amino-4-methyl-pyridinium picrate correlated well with the u.v. spectrum reported for 2,8-dimethyl-4H-pyrido-[1,2-a]-pyrimidin-4-one (111) (Table 2), although the λ_max found in the u.v. spectrum of the steroid at 211 and 218 nm have not been reported in the model compound (111). The steroid can therefore be formulated as 17β-hydroxy-17α-methyl-5α-androst-2-eno-[3,2-d]-8′-methyl-4′H-pyrido-[1,2-a] pyrimidin-4′-one (110). The considerably lower yield obtained in the condensation of 2-amino-4-methyl-pyridinium picrate with the steroidal β-ketoester (58) than in the analogous reaction of 2-aminopyridinium iodide is attributed mainly to the difficulties encountered in isolation of the product by preparative t.l.c. due to the similar R values of all three components of the reaction mixture.

The u.v. spectra of the two steroidal [3,2-d]-4′H-pyrido-[1,2-a] pyrimidin-4′-ones (108, 110) are broadly similar. They both exhibit a broad band at about 335 nm which has been ascribed to the pyridone-2-imine chromophore and another broad band at about 245 nm which has been attributed to the -C=O chromophore of the pyrimidine moiety.

The formation of 17β-hydroxy-17α-methyl-5α-androst-3-one (4) as a minor product in the reaction of 2-aminopyridinium iodide with the 2α-carbomethoxy-3-keto steroid (58) will be discussed later, (section 8.6).
The reaction of $\alpha$-carbomethoxy-17\(\beta\)-hydroxy-17\(\alpha\)-methyl-5\(\alpha\)-androstan-3-one (58) with 2-aminopyridine.

A solution of $\alpha$-carbomethoxy-17\(\beta\)-hydroxy-17\(\alpha\)-methyl-5\(\alpha\)-androstan-3-one (58) and 2-aminopyridine in pyridine was refluxed for 4 hours. On removal of the solvent, the residue was extracted with chloroform and washed with water. Analysis by t.l.c. of the residue, after evaporation of the chloroform, indicated the presence of the starting material, $\alpha$-carbomethoxy-17\(\beta\)-hydroxy-17\(\alpha\)-methyl-5\(\alpha\)-androstan-3-one (58), 17\(\beta\)-hydroxy-17\(\alpha\)-methyl-5\(\alpha\)-androstan-3-one (41), and a third component, $R$(starting material) = 0.82. This third component was isolated by preparative t.l.c. and further purified by recrystallization to give in 9.7% yield a white solid (112), m.p. 230-231°. Mass spectrometry indicated that the product had a molecular weight of 424 (C\(_{26}\)H\(_{36}\)N\(_2\)O\(_3\)) and the u.v. spectrum exhibited \(\lambda_{max}\) at 240 nm (log \(\varepsilon\) 3.88), 277 (3.95) and 290 (4.11). The low yield of the product obtained was ascribed partly to the difficulties of separating three components of similar \(R\) values by preparative t.l.c.

A much improved yield (43%) was obtained when toluene was used as a solvent and the mixture was refluxed for 2 days. On cooling, the solvent was removed on the rotary evaporator and recrystallization of the residue from ethanol gave, as sole product, a white solid (112), m.p. 238-240° with molecular formula, C\(_{26}\)H\(_{36}\)N\(_2\)O\(_3\). The u.v. spectrum of the product showed \(\lambda_{max}\) at 240 nm (log \(\varepsilon\) 4.09), 275 (4.15) and 288 (4.23) while the i.r. spectrum exhibited absorptions at 3610, 3570, 3400, 1680, 1630, 1585, 1565, 1510 and 1425 cm\(^{-1}\). The product was not sufficiently soluble in deuteriochloroform to permit a n.m.r. spectrum to be obtained.

The analogous 17\(\beta\)-0 carbomethoxy-17\(\alpha\)-hydrogen derivative (115) was prepared by refluxing \(2\alpha,17\beta\)-0 dicarbomethoxy-5\(\alpha\)-androstan-3-one\(^\text{25}\).
and 2-aminopyridine in toluene for 2 days. The recovered product had a molecular formula of $C_{27}H_{36}N_2O_5$. The u.v. spectrum showed $\lambda_{max}$ at 241 nm ($\log \varepsilon = 3.91$), 275 (4.02) and 288 (4.09). The n.m.r. spectrum, in addition to the signals associated with the C-18 and C-19 methyl protons and the C-17α proton, showed a multiplet at $\delta 7.01$ (1 proton), a multiplet at $\delta 7.67$ (1 proton), and a multiplet at $\delta 8.04-8.50$ (2 protons) arising from the aromatic protons of the pyridine ring. The n.m.r. spectrum also showed a singlet at $\delta 7.78$ (1 proton), a singlet at $\delta 13.80$, integrating for 0.86 of a proton and a signal at $\delta 9.74$, integrating for 0.14 of a proton, all of which disappeared when the n.m.r. spectrum was re-run after the deuteriochloroform solution had been shaken with a little deuterium oxide.

8.5 **Synthesis of 2-acetoacetamido-pyridine (50)**

2-acetoacetamidopyridine (50) was prepared from ethyl acetoacetate and 2-aminopyridine by the method described by Antaki and Petrov in order that the u.v., i.r. and n.m.r. spectra might be obtained for comparison with the spectra obtained from the product of the reaction between 2α-carbomethoxy-17β-hydroxy-17α-methyl-5α-androstan-3-one (58) and 2-aminopyridine.

8.6 **Discussion.**

The molecular formula of the product obtained by the reaction of 2α-carbomethoxy-17β-hydroxy-17α-methyl-5α-androstan-3-one (58) and 2-aminopyridine indicated that amide formation had occurred between the steroidal ester group and the amine. This is consistent with the formation of 2-acetoacetamidopyridine (50) by the reaction of ethyl acetoacetate and 2-aminopyridine at similar temperatures. The conventionally most plausible structure would be expected to be $17\beta$-hydroxy-17α-methyl-2α(2'-pyridyl-amido)-5α-androstan-3-one (112).
The u.v., i.r. and n.m.r. spectra of the steroidal amide differ sufficiently from those of 2-acetoacetamidopyridine (50) for it to be readily concluded that the two amides exist in different forms. The i.r. and n.m.r. spectra of 2-acetoacetamidopyridine (50), by comparison with those of acetoacanilide \(^{93,94}\), indicate that it exists predominantly as a conventional amide, i.e. (50). While the u.v. spectrum of 2-acetoacetamidopyridine (50) and that of the steroidal amide both exhibit \(\lambda_{\text{max}}\) of nearly equal intensity at \(\text{ca. 245nm and ca. 275nm}\), the latter compound shows a \(\lambda_{\text{max}}\) at 288nm \((\log \varepsilon = 4.23)\) while 2-acetoacetamidopyridine (50) shows a much less intense \(\lambda_{\text{max}}\) at 298nm \((\log \varepsilon = 3.45)\). The conventional amide structure (112) can therefore be eliminated as a possible major form of the steroidal amide.

The i.r. spectrum of the steroidal amide shows a weak absorption at 3640cm\(^{-1}\) which can be ascribed to an enolic hydroxyl group and an absorption at 3400cm\(^{-1}\) attributable to a N-H stretch \(^{95}\). The frequency at which this N-H stretch occurs indicates that it is not hydrogen bonded to a carbonyl oxygen \(^{145}\). Therefore structures in which there is hydrogen bonding involving the amino proton can be eliminated, for example structure (112). The broad absorption of medium intensity at 1685cm\(^{-1}\) from its position can be assigned to a carbonyl vibration. Difficulty arises in assigning this absorption to one or other of the two carbonyls present in the molecule. While the absorption occurs at a frequency within the range reported for an amido carbonyl \(^{95}\) it could also be assigned to a hydrogen bonded C-3 carbonyl on the A-ring \(^{59}\). This absorption is much less intense than the vibration found at the same frequency in the i.r. spectrum of 2-acetoacetamidopyridine (50) and also less intense than vibrations normally associated with
amido carbonyl vibrations. The intensity of the vibration suggests that the carbonyl giving rise to it is perhaps in equilibrium with its enol form.

Similar difficulties arise from the strong absorption at 1630 cm\(^{-1}\) which could be assigned to a strongly hydrogen bonded carbonyl vibration\(^ {96}\), a C-N double bond\(^ {97}\) or a C-C double bond. The strong i.r. absorption at 1638 cm\(^{-1}\) in ethyl N-anilino-crotonate (113)\(^ {93}\) and 1,4-dihydro-pyrid-4-one (114)\(^ {98}\) were considered to be due to the -N-C=CO- fragment and it is possible that this is also the case with the steroidal amide. This absorption does not appear in the i.r. spectrum of either 2-acetoacetamidopyridine (50) or acetoacatanilide\(^ {93}\).

Overall, the i.r. spectrum suggests that in solution the steroidal amide exists as an equilibrium mixture of more than one form.

When the n.m.r. spectrum of the analogous 17β-0 carboxethoxy-17α-hydrogen derivative (115) was run in deuterochloroform solution after shaking with a little deuterium oxide the signals at δ7.78, 6.9.74 and 6.13.80 disappeared, indicating the presence of two enolizable protons. Since the i.r. spectrum has shown that the amino proton in the steroidal amide is not hydrogen bonded to a carbonyl, its chemical shift would be expected to be further upfield than the chemical shift at δ11.88 assigned to the deshielded hydrogen bonded amino proton in 17β-acetoxy-2(2'-pyridyl-aminomethylene)-5α-androstan-3-one (65, R\(^{1}\)=CH\(_3\)CO, R\(^{2}\)=H) (section 3.5, page 18). Therefore it appears to be reasonable to assign the signal at δ7.78 to the amino proton of the amide. This signal is also within the range of chemical shifts reported for amino protons in simple non-hydrogen bonded amides\(^ {99}\). The resonance at δ13.80 falls within the range reported for the chemical shifts of hydrogen bonded enolic protons.
in such steroidal &-dicarbonyls as 2\&-carbomethoxy-17\&-hydroxy-
-17\&-methyl-5\&-androstan-3-one (58) (δ12.00) (section 8.1, page 69)
and 2\&-acetyl-17\&-hydroxy-5\&-androstan-3-one (17) (δ15.76)
(section 5.1, page 43) and it would seem feasible that this resonance
arises from an enolic proton hydrogen bonded to a carbonyl in a
\&-ketoenol system.

The presence of a signal in the n.m.r. spectrum at δ9.74
integrating for about 0.14 of a proton is difficult to interpret.
However, the n.m.r. spectra of 2-acetoacetamidopyridine (50) and
acetoacetanilide\textsuperscript{94} show signals due to amino protons at δ9.85 and
δ9.34 respectively and it may be that the resonance at δ9.74 in
the n.m.r. spectra of the steroidal amide can be assigned to an
amino proton hydrogen bonded to a carbonyl in the conventional form
of the amide (112), which may exist in small amounts, in equilibrium
with the more predominant forms (118,119).

The n.m.r. spectrum of the monosubstituted pyridine ring
in the steroidal amide is similar to that found in 2-acetoacetamido-
pyridine (50) but is different from that of 17\&-acetoxy-2(2\&-pyridyl-
-aminomethylene)-5\&-androstan-3-one (65) in that in the amide the
resonance (δ8.04-δ8.50) for the proton at either C-3 or C-5 in the
pyridine ring is further downfield than the corresponding resonance
(δ6.67-δ6.92) in the anaminone (65). Since the proton at C-3 on the
pyridine ring is closest to the amide linkage it is probably this
proton that is being deshielded. This infers that the pyridine ring
is being held in a fixed conformation. This is not surprising as
the C-N bond of the amide can be expected to exhibit some double
bond character.

Two possible equilibra (116,117 and 118,119) can be
postulated as the predominant forms of the steroidal amide on the
Scheme 9.

(107) \rightarrow \text{RNH}_2 \rightarrow \text{CH}_3O\rightarrow \text{(58)}

(112) \rightarrow \rightarrow \rightarrow \rightarrow \rightarrow \rightarrow

(113) \leftrightarrow \leftrightarrow \leftrightarrow \leftrightarrow \leftrightarrow

(119)
basis of the spectroscopic evidence reported above. It is probably not possible to differentiate between the two possibilities using spectroscopic evidence. However, a Dreiding model of both structure (116) and structure (117) shows that steric hindrance could be expected in both between the C-3 proton of the pyridine ring and the oxygen at the C-3 of the steroid. Furthermore, the fact that it has been shown that the C-3 carbonyl on the A-ring of the steroidal amide can condense with another molecule of 2-aminopyridine and subsequently cyclize by loss of the 2'-pyridyl-amino group to give the \([3,2-\text{d}]-4'-\text{pyrido}-[1,2-a] \text{pyrimidin-4'-one} \) (108) (section 8.8) would suggest that the predominant forms of the amide are most likely to be represented as an equilibrium mixture of structures (118) and (119) where the pyridine ring is far enough removed from the C-3 carbonyl not to hinder the approach of another 2-aminopyridine molecule to the C-3 carbonyl. The fact that structures (118) and (119) are the predominant forms of the amide rather than the conventional form (112) can be explained by the increased stability in these forms due to the creation of a 12-electron conjugated system from the pyridine ring to the C-3 oxygen atom which is not possible in the conventional amide (112).

In conclusion, the condensation of 2-aminopyridine with 2α-carbomethoxy-17β-hydroxy-17α-methyl-5α-androstan-3-one (58) gives the amide, 17β-hydroxy-17α-methyl-2α(2'-pyridyl-amido)-5α-androstan-3-one (112) which exists in solution mainly as a mixture of the two enolic forms, (118,119), and of possibly, small amounts of the conventional amide (112). Qualitatively the formation of the amide appears to proceed by the accepted mechanism for amide formation involving nucleophilic substitution at the carbonyl carbon of the 2α-carbomethoxy group of the steroid (58) (scheme 9).
The formation of 17β-hydroxy-17α-methyl-5α-androstan-3-one (I) as a product of the reaction of the 2α-carbomethoxy-3-keto steroid (58) with either 2-aminopyridine or its iodide in pyridine suggests that a decomposition reaction is also taking place in the reaction mixture as well as the condensation reaction. The most likely explanation is that the amide linkage in the steroid (112) is hydrolysed by traces of water present in the solvent and simultaneously decarboxylates forming 17β-hydroxy-17α-methyl-5α-androstan-3-one (I). The mechanism is probably similar to that of the decarboxylation of β-ketoacids and is shown in scheme 10. This reaction suggests that the amide linkage is fairly labile.

8.7 The reaction of 17β-hydroxy-17α-methyl-2α(2'-pyridyl-amido)-5α-androstan-3-one (112) with 2-amino-4-methyl-pyridinium picrate.

A solution of 17β-hydroxy-17α-methyl-2α(2'-pyridyl-amido)-5α-androstan-3-one (112) and 2-amino-4-methyl-pyridinium picrate in pyridine was refluxed for 5 hours. On removal of the solvent the residue was taken up in chloroform and washed with water. Analysis by t.l.c. of the residue obtained on evaporation of the chloroform indicated the presence of two components, 17β-hydroxy-17α-methyl-5α-androstan-3-one (I) and a component R(starting material) = 0.59. A small quantity (10mg) of the component R(starting material) = 0.59 was isolated by preparative t.l.c. The u.v. spectrum of this material was identical to that obtained previously for 17β-hydroxy-17α-methyl-5α-androst-2-eno-[3,2-d]-8'-methyl-4'-H-pyrido-[1,2-a]-pyrimidin-4'-one (110). However, further analysis by t.l.c. indicated the presence, as well, of 17β-hydroxy-17α-methyl-5α-androst-2-eno-[3,2-d] pyrido-[1,2-a] pyrimidin-4'-one (108). This was confirmed by mass spectrometry.
The formation of \(17\beta\)-hydroxy-17\(\alpha\)-methyl-2\(\alpha\)(2'\'-pyridyl-amido)-5\(\alpha\)-androst-3-one (112) as the product of the reaction of 2\(\alpha\)-carbomethoxy-17\(\beta\)-hydroxy-17\(\alpha\)-methyl-5\(\alpha\)-androst-3-one (58) and 2-aminopyridine in pyridine indicates that it is an intermediate in the synthesis of \(17\beta\)-hydroxy-17\(\alpha\)-methyl-5\(\alpha\)-androstan-2-ene-[3,2-\(d\)]-4\(H\)-pyrido-[1,2-\(a\)]pyrimidin-4\(H\)-one (108). This indicates that a rearrangement must occur on going from the amide (112) to the cyclized product (108) with the amide linkage being broken. The reaction of \(17\beta\)-hydroxy-17\(\alpha\)-methyl-2\(\alpha\)(2'\'-pyridyl-amido)-5\(\alpha\)-androst-3-one (112) with 2-amino-4-methyl-pyridinium picrate to give a mixture of \(17\beta\)-hydroxy-17\(\alpha\)-methyl-5\(\alpha\)-androstan-2-ene-[3,2-\(d\)]-6\(\alpha\)-methyl-4\(H\)-pyrido-[1,2-\(a\)]pyrimidin-4\(H\)-one (110) and the [3,2-\(d\)]-4\(H\)-pyrido-[1,2-\(a\)]pyrimidin-4\(H\)-one (108) indicates that the [3,2-\(d\)]-4\(H\)-pyrido-[1,2-\(a\)]pyrimidin-4\(H\)-one system (108) is formed by the condensation of a molecule of 2-aminopyridine with the C-3 carbonyl of the amide (112) to give the 2(2'\'-pyridyl-amido)=-3(2'\'-pyridyl-amino)-5\(\alpha\)-androstan-2-ene (120, R=H or CH\(_3\)) and then cyclization involving loss of the original aminopyridine molecule. The formation of \(17\beta\)-hydroxy-17\(\alpha\)-methyl-5\(\alpha\)-androstan-2-ene-[3,2-\(d\)]-4\(H\)-pyrido-[1,2-\(a\)]pyrimidin-4\(H\)-one (108) in this reaction is explained by 2-aminopyridine, formed by the fission of \(17\beta\)-hydroxy-17\(\alpha\)-methyl-2(2'\'-pyridyl-amido)-3(2'\'-(4\(H\)-methyl-pyridyl)-amino)-5\(\alpha\)-androstan-2-ene (120, R=CH\(_3\)), condensing with the C-3 carbonyl of the amide (112) and then cyclizing to the pyrido-[1,2-\(a\)]pyrimidin-4\(H\)-one (108).

No trace has been found of \(17\beta\)-hydroxy-17\(\alpha\)-methyl-2(2'\'-pyridyl-amido)-3(2'\'-pyridyl-amino)-5\(\alpha\)-androstan-2-ene (120, R=H) or the 3(2'\'-(4\(H\)-methyl-pyridyl)-amino)-analogue (120, R=CH\(_3\)) in any
Scheme 11.

R

(58) \[ \rightarrow \]

R

(112)

R = \text{I, CH}_3

(120)

R = \text{I, CH}_3

H

(106) R = H

(110) R = \text{CH}_3
of the experiments performed although its formation is inferred from
the reaction of 17β-hydroxy-17α-methyl-2α(2'-pyridyl-amido)-5α-
androstan-3-one (112) and 2-amino-4-methyl-pyridinium picrate.

On the evidence of the experiments described the formation
of 17β-hydroxy-17α-methyl-5α-androst-2-ene-[3,2-d]-4'H-pyrido-[1,2-a]-
pyrimidin-4'-one (108) from 2α-carbomethoxy-17β-hydroxy-17α-methyl-
-5α-androstan-3-one (58) and 2-aminopyridinium iodide seems to
proceed by the same mechanism as has been proposed by Shur and
Israelstam55 for the formation of 2-methyl-4H-pyrido-[1,2-a]-
pyrimidin-4-one (46) from acetoacetic ester and 2-aminopyridine
(section 1, page 10). This mechanism is shown for the steroidal
β-ketoester (58) in scheme 11.

8.9 The attempted cyclization of 17β-hydroxy-17α-methyl-2α-
-(2'-pyridyl-amido)-5α-androstan-3-one (112) by acid catalysis.
(a) The reaction of 17β-hydroxy-17α-methyl-2α(2'-pyridyl-
amido)-5α-androstan-3-one (112) with 5% w/w aqueous hydroiodic
acid.

A solution of 17β-hydroxy-17α-methyl-2α(2'-pyridyl-amido)-
-5α-androstan-3-one (112) in ethanol containing a drop of 5% w/w
hydroiodic acid was refluxed for 2 hours. After removal of the
solvent the residue was taken up in methylene chloride, washed with
dilute sodium hydroxide and water. Analysis of the residue obtained
on evaporation of the solvent by t.l.c. showed the presence of two
components, R(starting material) = 0.73 and 0.95, the first component
being the major one.

The experiment was repeated on a further sample of 17β-hydroxy-
-17α-methyl-2α(2'-pyridyl-amido)-5α-androstan-3-one (112). The
reaction mixture was refluxed overnight. Analysis of the residue
by t.l.c. after it had been freed of acid showed that it consisted of the two components, R(starting material) = 0.73 and 0.95, the latter component being the major one. 33mg of a mixture of the components, R(starting material) = 0.73 and 0.95 and 31mg of the component, R(starting material) = 0.73 were obtained by preparative t.l.c. Mass spectrometry indicated that the molecular formula of the component, R(starting material) = 0.73 was C_{26}H_{36}N_{2}O_{4} and that of the component, R(starting material) = 0.95 was C_{26}H_{34}N_{2}O_{3}.

The u.v. spectrum of the steroid, C_{26}H_{36}N_{2}O_{4}, showed $\lambda_{\text{max}}$ at 238nm (log ε 4.05) and 275nm (3.77) while its n.m.r. spectrum, apart from the signals associated with the C-18, C-19 and C-17 methyl groups and the aromatic protons of the pyridine ring, showed resonances at δ 9.10 and δ 9.35, both integrating for 0.5 of a proton and both disappearing when the n.m.r. spectrum was re-run after the deuterochloroform solution had been shaken with a little deuterium oxide.

The i.r. spectrum showed absorptions at 3560, 3340, 1670 and 1560cm$^{-1}$.

It was not possible to obtain a pure sample of the steroid, C_{26}H_{34}N_{2}O_{3}, and as a result, no i.r., u.v. or n.m.r. spectra were recorded for this steroid.

(b) The cyclization of 17β-hydroxy-17α-methyl-2α(2'-pyridyl-amido)-5α-androstan-3-one (112) by hydrochloric acid in absolute ethanol.

A solution of 17β-hydroxy-17α-methyl-2α(2'-pyridyl-amido)-5α-androstan-3-one (112) in absolute ethanol containing concentrated hydrochloric acid was refluxed for 4 hours. On evaporation of the solvent, the residue was taken up in methylene chloride and filtered to remove extraneous material. The organic solution was washed with sodium bicarbonate solution to remove any traces of acid. Evaporation of the organic solvent gave 22mg of material, m.p. > 250°.
Mass spectrometry indicated that the product (109) had a molecular formula of $\text{C}_{26}\text{H}_{34}\text{N}_2\text{O}_2$. The u.v. spectrum showed $\lambda_{\text{max}}$ at 205 nm (log $\varepsilon$ 4.05), 240 (4.23), 248 (4.18), 274 (3.95), 286 (3.92) and 330 (3.31). It was not possible to obtain a satisfactory n.m.r. spectrum because of insufficient material. However, the material recovered from deuterochloroform solution was found to be different from the material recovered from the reaction mixture. It was found by mass spectrometry to now have a molecular formula of $\text{C}_{26}\text{H}_{34}\text{N}_2\text{O}_3$ while its u.v. spectrum showed $\lambda_{\text{max}}$ at 206 nm (log $\varepsilon$ 3.86), 235 (3.96), 247 (3.86), 274 (3.61) and 284 (3.59). The i.r. spectrum showed absorptions at 3570, 3400, 3340, 1670, 1620, 1575, 1555, 1505, 1475 and 1415 cm$^{-1}$.

8.10 Discussion.

The effect of acid on 17$\beta$-hydroxy-17$\alpha$-methyl-2$\alpha$-(2$'$-pyridyl-\-amido)-5$\alpha$-androstan-3-one (112) was investigated. The purpose of this was to see whether the $[3,2-\alpha]-4'\text{H}-\text{pyrido-[1,2-\alpha]}$ pyrimidin-$4'$-one (108) might be obtained in an acid catalyzed reaction as had been suggested by Antaki and Petrow for the cyclization of 2-acetoacetamidopyridine (50) or whether the steroidal amide (112) would cyclize to give 17$\beta$-hydroxy-17$\alpha$-methyl-5$\alpha$-androst-2-eno-[2,3-$\varepsilon$]-2$'\text{H}$-pyrido-$[1,2-\alpha]$ pyrimidin-$2'$-one (109) in a reaction analogous to the cyclization of 17$\beta$-hydroxy-2(2$'$-pyridyl-aminomethylene)-5$\alpha$-androstan-3-one (65) to give 17$\beta$-hydroxy-5$\alpha$-androst-2-eno-[2,3-$\varepsilon$]-pyrido-$[1,2-\alpha]$ pyrimidin-$5'$-ium iodide (59).

The reaction was attempted using aqueous hydroiodic acid in ethanol and hydrochloric acid in absolute ethanol. The reaction of aqueous hydroiodic acid with the steroidal amide (112) gave two major products, one with molecular formula, $\text{C}_{26}\text{H}_{36}\text{N}_2\text{O}_4$, and the other with molecular formula, $\text{C}_{26}\text{H}_{34}\text{N}_2\text{O}_3$. When the reaction was carried
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<th><img src="108" alt="Steroid Structure" /></th>
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**Table 3**
out using hydrochloric acid in absolute ethanol, the mass spectrum of the product obtained indicated that the amide (112) had lost a molecule of water in the reaction. It was concluded that cyclization of the amide had occurred. The most likely cyclized product, provided no rearrangement had occurred, would be expected to be 17β-hydroxy-17α-methyl-5α-androst-2-eno-[2,3-e]-2'H-pyrido-[1,2-a]-pyrimidin-2'-one (109). The u.v. spectrum of the cyclized product was compared with that of 17β-hydroxy-17α-methyl-5α-androst-2-eno-[3,2-d]-4'H-pyrido-[1,2-a]-pyrimidin-4'-one (108), 2-methyl-4'H-pyrido-[1,2-a]-pyrimidin-4-one (46) and 4-methyl-2H-pyrido-[1,2-a]-pyrimidin-2-one (49). The relevant data are shown in Table 3. No definite conclusions can be drawn from the comparison of the spectra. However, the u.v. spectrum of the cyclized product bears a greater resemblance, especially at the higher λ_max (298, 274, 286 and 330 nm) to the u.v. spectrum of 4-methyl-2H-pyrido-[1,2-a]-pyrimidin-2-one (49). There is, unfortunately, less correlation between the two spectra at lower wavelengths. Nevertheless it is reasonable to conclude from the u.v. spectrum and the mass spectrum of the cyclized product that it is 17β-hydroxy-17α-methyl-5α-androst-2-eno-[2,3-e]-2'H-pyrido-[1,2-a]-pyrimidin-2'-one (109). The i.r. spectrum of the product (109) in nujol shows absorptions at 1640 and 1595 cm⁻¹. The absorption at 1640 cm⁻¹ can be attributed to a carbonyl stretch and the absorption at 1595 cm⁻¹ to a C-C double bond stretch. The i.r. spectrum correlates fairly well with that of 4-methyl-2H-pyrido-[1,2-a]-pyrimidin-2-one (49) which shows absorptions at 1670 cm⁻¹ (C=O) and 1600 cm⁻¹ (C=C)³.⁸

The mechanism of the cyclization can be postulated to occur by either of the two pathways shown in scheme 12. The mechanisms are analogous to those postulated for the cyclization of 17β-acetoxyc-
-2(2'-pyridyl-aminomethylene)-5α-androstan-3-one (65) earlier (schemes 4 and 5, page 65), except that in the case of the cyclization of the amide (112), there is no need for a quaternary bridgehead nitrogen (121) as the more stable pyrimidin-2-one system (109) can be formed.

After an unsuccessful attempt to obtain a satisfactory n.m.r. spectrum of the cyclized product (109), probably due to insufficient material, the material recovered from the deuteriochloroform solution was found not to be the cyclized product (109). The molecular formula was found by mass spectrometry to be C_{26}H_{34}N_2O_3 while the u.v. spectrum has $\lambda_{\text{max}}$ at the same wavelengths as 17β-hydroxy-17α-methyl-5α-androst-2-eno-[2,3-e]-2'H-pyrido-[1,2-a] pyrimidin-2'-one (109) except that the $\lambda_{\text{max}}$ at 330nm found in the latter steroid (109) is absent. The i.r. spectrum shows absorptions at 3140cm^{-1} and 3340cm^{-1} which can be assigned to N-H stretches and an absorption of medium intensity at 1670cm^{-1} which can be assigned to a carbonyl stretch. The steroid appears to contain two amino protons.

A steroid of the same molecular formula (C_{26}H_{34}N_2O_3) is also found along with a steroid, C_{26}H_{36}N_2O_4, when 17β-hydroxy-1α-methyl-2(2'-pyridyl-amido)-5α-androstan-3-one (112) is treated with aqueous hydroiodic acid. It seems reasonable to conclude that the steroid, C_{26}H_{34}N_2O_3 is formed by the reaction of water on 17β-hydroxy-17α-methyl-5α-androst-2-eno-[2,3-e]-2'H-pyrido-[1,2-a] pyrimidin-2'-one (109). (The deuteriochloroform solution for the n.m.r. spectrum had been shaken with a little deuterium oxide and had therefore been washed with water prior to evaporation of the deuteriochloroform and recovery of the steroid.) It would seem therefore that on treatment with acid, the amide (112) cyclizes to 17β-hydroxy-17α-methyl-5α-androst-2-eno-[2,3-e]-2'H-pyrido-[1,2-a] pyrimidin-2'-one (109)
Scheme 13.

\[ \text{m/e 424} \quad (112) \quad \xrightarrow{\text{H}_2\text{O}} \quad \text{m/e 406} \quad (109) \]

\[ \xrightarrow{\text{C}_{26}H_{34}N_2O_3 \quad +\text{H}_2\text{O}} \quad \text{m/e 422} \quad \xrightarrow{\text{C}_{26}H_{36}N_2O_4} \quad \text{m/e 440} \]

(122)
which reacts with water to give the steroid, C$_{26}$H$_{34}$N$_2$O$_3$, which in turn reacts with water to give the steroid, C$_{26}$H$_{36}$N$_2$O$_4$ (scheme 13). The disappearance of the $\lambda_{\text{max}}$ at 330nm on going from 17$\beta$-hydroxy-17$\alpha$-methyl-5$\alpha$-androst-2-eno-[2,3-e]-2$'\text{H}$-pyrido-[1,2-a] pyrimidin-2$'$-one (109) to the steroid, C$_{26}$H$_{34}$N$_2$O$_3$, suggests that the longest chromophore in the former steroid (109) is being disrupted in the reaction. Similarly the steroid, C$_{26}$H$_{36}$N$_2$O$_4$ is less conjugated than the steroid, C$_{26}$H$_{34}$N$_2$O$_3$, indicating that a molecule of water probably reacts with part of the longest chromophore of the latter steroid.

The n.m.r. spectrum of the steroid, C$_{26}$H$_{36}$N$_2$O$_4$, shows signals at $\delta$ 9.10 and $\delta$ 9.35 which between them integrate for one proton and both of which disappeared when the spectrum was run after the deuterochloroform solution had been shaken with a little deuterium oxide. It seems unlikely that these signals constitute a doublet, coupling constant, $J=12$Hz, as a corresponding doublet cannot be found in the spectrum. The i.r. spectrum shows an absorption at 3340cm$^{-1}$, due to an N-H stretch, although the absorption at 3400cm$^{-1}$ found in the i.r. spectrum of the steroid, C$_{26}$H$_{34}$N$_2$O$_3$, is absent in this steroid. The i.r. spectrum also shows a strong absorption at 1670cm$^{-1}$ which can be assigned to a carbonyl stretch.

It has not been possible either to deduce the structures of the steroids, C$_{26}$H$_{34}$N$_2$O$_3$ and C$_{26}$H$_{36}$N$_2$O$_4$, from the available data or to postulate any theoretically possible structures with these molecular formulae. In aqueous acid solution it could be expected that 17$\beta$-hydroxy-17$\alpha$-methyl-5$\alpha$-androst-2-eno-[2,3-e]-2$'\text{H}$-pyrido-[1,2-a] pyrimidin-2$'$-one (109) might be hydrolysed with cleavage of the carbonyl-N bond. If this were the case, the first product would be structure (122). While this molecule has a $m/e$ ratio of
424, two more than the m/e ratio determined experimentally for one of the products, its structure is not inconsistent with either the i.r. or u.v. spectra.
8.11 Experimental.

8.11.1 2α-carbomethoxy-17β-hydroxy-17α-methyl-5α-androstan-3-one (58) 25.

A solution of 17β-hydroxy-17α-methyl-5α-androstan-3-one (4.5 g, 1.5 x 10⁻² mole) in dry dimethyl sulfoxide (90 ml) was stirred with freshly prepared sodium methoxide (5 g) for 30 minutes. A solution of dimethyl carbonate (10 ml) in dimethyl sulfoxide (10 ml) was added and the mixture stirred for 2 h. On dilution with water the steroidal product was precipitated. The precipitate was filtered, dried and recrystallized from methanol to give 2α-carbomethoxy-17β-hydroxy-17α-methyl-5α-androstan-3-one (1.96 g, 5.62 x 10⁻³ mole, 36%); m.p. 161-163 °C (lit 25 m.p. 162-164 °C; n.m.r. (60 MHz), δ 0.76 (2-carbomethoxy methyl), δ 0.86 (3-carbomethoxy methyl), δ 1.20 (C-18 methyl), δ 3.70 (enol H); i.r. (CHBr₃), ν max 3595 (CH₃O·00·C·0H·V), 1655 (C=C·00·C·0H·C=C·0H), 1615 cm⁻¹ (CH₃O·C=C·OH·V·C=0).

8.11.11 17β-hydroxy-17α-methyl-5α-androstan-2-one (108).

A solution of 2α-carbomethoxy-17β-hydroxy-17α-methyl-5α-androstan-3-one (225 mg, 6.21 x 10⁻⁴ mole) and 2-aminopyridinium iodide (213 mg, 9.60 x 10⁻⁴ mole) in pyridine (5 ml) was refluxed for 7 hours. The solvent was removed in vacuo and the residue taken up in chloroform. The organic solution was washed with water, dried over anhydrous magnesium sulphate and evaporated to dryness. Analysis of the residue by t.l.c. indicated the presence of the following components:-

(a) 2α-carbomethoxy-17β-hydroxy-17α-methyl-5α-androstan-3-one,
(b) 17β-hydroxy-17α-methyl-5α-androstan-3-one,
(c) R(2α-carbomethoxy-17β-hydroxy-17α-methyl-5α-androstan-3-one)=0.58.

The component R(2α-carbomethoxy-17β-hydroxy-17α-methyl-5α-androstan-3-one)=0.58 was separated from the other components by
preparative t.l.c. and recrystallized from ethanol to give
17α-hydroxy-17β-methyl-5α-androst-2-eno-[3,2-d]-4′-H-pyrido-[1,2-a]-
pyrimidin-4′-one (55mg, 1.34 x 10⁻⁴ mole, 22%); m.p. 245-250°;

u.v. (CH₃OH), λ_max 207nm (log ε 3.95), 216 (4.13), 238 (4.10),
248 (4.03), 253 (3.94), 335 (3.95); n.m.r. (100 MHz), δ 0.80
(6-18 methyl), δ 0.90 (6-19 methyl), δ 1.22 (6-17x methyl), δ 7.00
(1H, multiplet, pyrido-[1,2-a] pyrimidin-4′-one, 9-H), δ 7.58
(2H, multiplet, pyrido-[1,2-a] pyrimidin-4′-one, 7-H, 8-H), δ 8.90
(1H, doublet, pyrido-[1,2-a] pyrimidin-4′-one, 6-H); i.r. (CHBr₃),
λ_max 3570 (OH), 1650 (C=O), 1625 (C=N), 1570, 1525 cm⁻¹; m.e.s
spectrum, M⁺, m/e 406, calculated for C₂₆H₃₄N₂O₂ 406.2620114, found
406.259713, error less than 6ppm.

8.11.iii 17α-hydroxy-17β-methyl-5α-androst-2-eno-[3,2-d]-8′-methyl-
-4′H-pyrido-[1,2-a] pyrimidin-4′-one (110).

A solution of 2α-carbomethoxy-17β-hydroxy-17α-methyl-5α-
androstan-3-one (528mg, 1.46 x 10⁻³ mole) and 2-amino-4-methyl-
pyridinium picrate (424mg, 1.85 x 10⁻³ mole) in pyridine (10ml)
was refluxed for 4h. The solvent was removed on the rotary
evaporator and the residue was taken up in chloroform, washed with
water, dried over anhydrous magnesium sulphate and evaporated to
dryness. Analysis by t.l.c. of the residue indicated the presence
of the following components:

(a) 2α-carbomethoxy-17β-hydroxy-17α-methyl-5α-androstan-3-one,
(b) 17β-hydroxy-17α-methyl-5α-androstan-3-one,
(c) R(2α-carbomethoxy-17β-hydroxy-17α-methyl-5α-androstan-3-one)=0.79.

An attempt was made to isolate the component R(2α-carbomethoxy-
-17β-hydroxy-17α-methyl-5α-androstan-3-one)=0.79 by preparative t.l.c.
A fraction containing the required component and minor amounts of the
other two components was recovered. This fraction was further purified by preparative t.l.c. to give $\beta\beta$-hydroxy-$\alpha\alpha$-methyl-$5\alpha$-androst-2-eno-[3,2-$\alpha$]-8'-methyl-$4\beta$-pyrido-[1,2-$\alpha$] pyrimidin-$4\beta$-one (31mg, $7.38 \times 10^{-5}$ mole, 5%); m.p. 135-136°; u.v. (CH$_2$OH), $\lambda$ max 211nm (log $E$ 4.30), 218 (sh) (4.20), 237 (4.06), 242 (4.08), 251 (3.98), 310 (3.86), 328 (4.03), 341 (4.02); n.m.r. (60 MHz), $60.83$ (C-18 methyl), $60.93$ (C-19 methyl), $61.26$ ($\gamma$-17 ethyl), $62.143$ (3H, singlet, pyrido-[1,2-$\alpha$] pyrimidin-$4\beta$-one, 8'-methyl), $66.86$ (1H, doublet, $J=5$Hz, pyrido-[1,2-$\alpha$] pyrimidin-$4\gamma$-one, 7-H), $67.26$ (1H, singlet, pyrido-[1,2-$\alpha$] pyrimidin-$4\beta$-one, 9-H), $8.83$ (1H, doublet, $J=4$Hz, pyrido-[1,2-$\alpha$] pyrimidin-$4\beta$-one, 5-H); mass spectrum, M$^+$, m/e 420, calculated for C$_{27}$H$_{36}$N$_2$O$_2$ 420.277663, found 420.2799014, error less than 6ppm.

8.11.iv 17$\beta$-hydroxy-$\alpha\alpha$-methyl-$2\alpha$(2'-pyridyl-amido)-$5\alpha$-androst-3-one (112):

(a) A solution of $2\alpha$-carbomethoxy-$\beta\beta$-hydroxy-$\alpha\alpha$-methyl-$5\alpha$-androst-3-one (185mg, $5.1 \times 10^{-4}$ mole) and 2-amino-pyridine (101ng, $1.11 \times 10^{-3}$ mole) in pyridine (3ml) was refluxed for 1h. On cooling the solvent was removed in vacuo, the residue taken up in chloroform, washed with water, dried over anhydrous magnesium sulphate and evaporated to dryness. Analysis of the residue by t.l.c. showed the following components:

(a) $2\alpha$-carbomethoxy-$\beta\beta$-hydroxy-$\alpha\alpha$-methyl-$5\alpha$-androst-3-one,
(b) $\alpha\beta$-hydroxy-$\alpha\alpha$-methyl-$5\alpha$-androst-3-one,
(c) R($2\alpha$-carbomethoxy-$\beta\beta$-hydroxy-$\alpha\alpha$-methyl-$5\alpha$-androst-3-one) = 0.82.

The component R($2\alpha$-carbomethoxy-$\beta\beta$-hydroxy-$\alpha\alpha$-methyl-$5\alpha$-androst-3-one) = 0.82 was isolated from the other components by preparative t.l.c. and recrystallized from ethanol to give $\beta\beta$-hydroxy-$\alpha\alpha$-methyl-$2\alpha$(2'-pyridyl-amido)-$5\alpha$-androst-3-one (21mg, $4.95 \times 10^{-5}$ mole, 9.7%); m.p. 230-231°; u.v. (CH$_3$OH), 240nm (log $E$ 3.88),
(b) A solution of 2α-carbomethoxy-17β-hydroxy-17α-methyl-5α-
androst-3-one (338mg, 9.34 x 10⁻⁴ mole) and 2-aminopyridine
(182mg, 1.94 x 10⁻³ mole) in toluene (15ml) was refluxed for 2 days.
The solvent was then removed on the rotary evaporator and the
residue recrystallized from ethanol to give 17β-hydroxy-17α-methyl-
-2α(2'-pyridyl-amido)-5α-androst-3-one (170mg, 4.01 x 10⁻⁴ mole,
lit5); m.p. 238-240⁰; u.v. (CH₃OH), λ max 240nm (log ε 4.09),
275 (sh)(4.15), 288 (4.23); i.r. (CHBr₃), ν max 3640
(weak, enol OH),
3570 (OH), 3400 (NH), 1685,1630,1585,1565,1510,1425cm⁻¹; mass
spectrum, M⁺, m/e 424, calculated for C₂₆H₃₆N₂O₃ 424.270081, found
424.270081, error less than 6ppm.

8.11.v 2α, 17β-O dicarbomethoxy-5α-androstan-3-one²⁵.
To a solution of 17β-hydroxy-5α-androstan-3-one
(2.0g, 6.9 x 10⁻³ mole) in boiling dimethyl carbonate (20ml), freshly
prepared sodium methoxide (2.59) was added and the mixture refluxed
with stirring for 3h. Hexane was added to the resultant paste and
the sodium salt filtered and dried. It was then added in small
portions to cold 4N hydrochloric acid (50ml). The steroid was
extracted with diethyl ether, washed with water until acid free, dried
over anhydrous magnesium sulphate and evaporated to dryness.
Recrystallization of the residue from methanol gave 2α,17β-O dicarbo-
methoxy-5α-androstan-3-one (1.4g, 3.88 x 10⁻³ mole, 56%); m.p. 145-147⁰
(lit²⁵ m.p. 145-149⁰); n.m.r. (60 MHz), δ 0.76 (C-18 methyl), δ 0.83
(C-19 methyl), δ 3.73 (2α-carbomethoxy, methyl), δ 3.76 (17α-carbomethoxy,
methyl), δ 4.50 (multiplet, C-17 H), δ 12.06 (enolic H); i.r. (CHBr₃),
ν max 1730 (ester C=O), 1655 (CH₂C=O=CH₂, ν C=O), 1615cm⁻¹
(CH₂CO=CH₂, ν C=O).
8.11.vi 17β-0-carbomethoxy-2(1'-pyridyl-amido)-5α-androstan-3-one (115).

A solution of 2α,17β-carbomethoxy-5α-androstan-3-one (1.43 mg, 3.50 x 10^{-4} mole) and 2-aminopyridine (87 mg, 9.25 x 10^{-4} mole) in toluene (15 ml) was refluxed for two days. On cooling the solvent was evaporated on the rotary evaporator and the residue taken up in diethyl ether, washed with water, dried over anhydrous magnesium sulphate and evaporated to dryness. Recrystallization of the residue gave 17β-0-carbomethoxy-2(1'-pyridyl-amido)-5α-androstan-3-one (7.7 mg, 1.64 x 10^{-4} mole, 47%); m.p. 165-167°; u.v. (CH₃OH), \( \lambda_{max} \) 241 nm (log ε 3.91), 275 (4.02), 288 (4.09); n.m.r. (100 MHz), \( \delta \) 0.80 (6H, C-18 methyl, C-19 methyl), 3.74 (3H, 17β-O carbomethoxy, methyl), 4.43 (1H, triplet, 17β-H), 7.01 (1H, multiplet, pyridine ring, 5-H), 7.67 (1H, multiplet, pyridine ring, 4-H), 7.78 (1H, singlet, disappears on addition of D₂O, N-H), 8.04-8.50 (2H, multiplet, pyridine ring, 3-H, 6-H), 9.74 broad (0.14H, disappears on addition of D₂O, enol H), 13.80 broad (0.86 H, singlet, disappears on addition of D₂O); i.r. (CHCl₃), \( \gamma_{max} \) 3390 (NH), 1720 (ester C=O), 1685, 1630, 1585, 1565, 1500, 1425 cm⁻¹; mass spectrum, \( M^+ \), m/e 468, calculated for C₂₇H₃₆N₂O₅ 468.2621406, found 468.262824, error less than 1 ppm.

8.11.vii 2-acetoacetamidopyridine (50).50

A mixture of ethyl acetoacetate (5 g, 3.64 x 10^{-2} mole) and 2-aminopyridine (1.8 g, 1.91 x 10^{-2} mole) was refluxed for 4 h. On cooling excess reactants were removed by vacuum distillation. Recrystallization of the residue from ethanol gave 2-acetoacetamidopyridine (65 mg, 3.45 x 10^{-4} mole, 1%); m.p. 109-111° (lit 113°); u.v. (CHCl₃), \( \lambda_{max} \) 246 nm (log ε 3.53), 277 (3.97), 298 (3.45); n.m.r. (60 MHz), \( \delta \) 2.33 (3H, singlet, methyl), 3.66 (2H, singlet, -CH₂-), 7.10 (1H, multiplet, pyridine ring, 5-H), 7.70 (1H,
multiplet, pyridine ring, l H ), 8.26 ( 2H, multiplet, pyridine ring, 
3- 3, 6-3), 59.90 ( 1H, broad, disappears on addition of D 2 0, N-H );
i.r. (CHBr 3 ), v max 3400, 2970, 1710, 1685, 157505 2 0 and 1435 cm -1.

8.11.viii The reaction of 17β-hydroxy-17β-methyl-2α(2'-pyridyl-
amido)-5α-androst-3-one (112) with 2-amino-4-

A solution of 17β-hydroxy-17β-methyl-2α(2'-pyridyl-amido) -
5α-androst-3-one (136mg, 3.21 x 10 -4 mol) and 2-amino-4-methyl-
pyridinium picrate (77mg, 3.36 x 10 -4 mol) in pyridine (10ml) was 
refluxed for 5h. The solvent was removed on the rotary evaporator 
and the residue taken up in chloroform, washed with water, dried 
over anhydrous magnesium sulphate and evaporated to dryness. 
Analysis of the residue by t.l.c. indicated that the following 
components were present:

(a) 17β-hydroxy-17β-methyl-5α-androst-3-one,

(b) R(17β-hydroxy-17β-methyl-2α(2'-pyridyl-amido)-5α-androst-
3-one)=0.59.

The component R(17β-hydroxy-17β-methyl-2α(2'-pyridyl-
amido)-5α-androst-3-one)=0.59 was isolated by preparative t.l.c. 
to give 10mg of material. T.l.c. analysis of this material indicated 
that it consisted of two components of very similar R(17β-hydroxy-
17β-methyl-2α(2'-pyridyl-amido)-5α-androst-3-one) values. The 
two components were identified by co-chromatography as 17β-hydroxy-
17β-methyl-5α-androst-2-eno-[3,2-d]-8'-methyl-5H-pyrido-[1',2'-a]- 
pyrimidin-4'-one and 17β-hydroxy-17β-methyl-5α-androst-2-eno-
- [3,2-d]-5H-pyrido-[1,2-a] pyrimidin-4'-one; u.v. (CH 3 OH) λ max 
210nm (log ε 4.31), 218 (4.21), 238 (4.07), 243 (4.07), 252 (3.96), 
312 (3.86), 329 (4.02), 340 (4.01); mass spectrum, M + , m/e 420,406, 
calculated for C 27 H 36 N 2 O 2 420.277663, found 420.276592, error less than 3ppm, 
calculated for C 26 H 34 N 2 O 2 406.262104, found 406.260108, error less than 5ppm.
The attempted cyclization of 17β-hydroxy-17α-methyl-2α(2'-pyridyl-amido)-5α-androstan-3-one (112) using aqueous hydroiodic acid.

(a) 17β-hydroxy-17α-methyl-2α(2'-pyridyl-amido)-5α-androstan-3-one (280mg) was dissolved in ethanol (80ml). Two drops of 55% w/w hydroiodic acid were added and the mixture refluxed for 2h. The solvent was removed on the rotary evaporator. The residue was taken up in methylene chloride, washed with 10% sodium hydroxide solution, then with water, dried over anhydrous magnesium sulphate and evaporated to dryness. Analysis by t.l.c. showed the presence of the following components:

- (a) \( R(\text{starting material}) = 0.73 \),
- (b) \( R(\text{starting material}) = 0.95 \).

The component \( R(\text{starting material}) = 0.73 \) was the major component.

(b) The reaction was repeated on a further 190mg of steroid and the reaction mixture refluxed overnight. Analysis of the product by t.l.c. showed the presence of the following components:

- (a) \( R(\text{starting material}) = 0.73 \),
- (b) \( R(\text{starting material}) = 0.95 \).

The component \( R(\text{starting material}) = 0.95 \) was the major component.

Purification of the two reaction mixtures by preparative t.l.c. gave 33mg of a mixture of the components, \( R(\text{starting material}) = 0.73 \) and 0.95, and 31mg of the component, \( R(\text{starting material}) = 0.73 \).

Spectral data:

- (a) \( R(\text{starting material}) = 0.73 \); u.v. (CH\(_3\)OH); \( \lambda_{\text{max}} \) 208nm (log ε 3.73), 238 (4.05), 275 (3.77); n.m.r. (100 MHz), \( \delta \) 0.80 (C-18 methyl), 0.82 (C-19 methyl), 1.06 (C-17 α-methyl), 7.04 (1H, multiplet, pyridine ring, 5-H), 7.56 (1H, multiplet, pyridine ring, 4-H), 8.10 (1H, multiplet, pyridine ring, 3-H), 8.29
(1H, multiplet, pyridine ring, 6-\(H\)), \(\delta 9.10\) (0.5H, broad signal, disappears on addition of \(D_2O\)), \(\delta 9.35\) (0.5H, broad signal, disappears on addition of \(D_2O\)); i.r. (CHCl\(_3\)), \(\nu_{\text{max}}^{\text{IR}}\) 3560 (OH), 33140 (NH), 1670 (C=O), 1560cm\(^{-1}\); mass spectrum, \(M^+\), m/e 440, calculated for \(C_{26}H_{34}N_2O_4\) 440.2673; found 440.267352, error less than 1ppm.

(b) R(starting material) = 0.95; mass spectrum, \(M^+\), m/e 422, calculated for \(C_{26}H_{34}N_2O_3\) 422.2547; found 422.25476, error less than 6ppm.

8.11.x The reaction of 17\(\beta\)-hydroxy-17\(\alpha\)-methyl-2\(\alpha\)-(2'-pyridyl-amido)-5\(\alpha\)-androstan-3-one (112) and hydrochloric acid in absolute ethanol.

A solution of 17\(\beta\)-hydroxy-17\(\alpha\)-methyl-2\(\alpha\)(2'-pyridyl-amido)-5\(\alpha\)-androstan-3-one (88mg, 2.07 x 10\(^{-4}\) mole) dissolved in absolute ethanol (40ml) containing concentrated hydrochloric acid (1ml) was refluxed for 1h. On cooling, the solvent was removed on the rotary evaporator. The residue was taken up in methylene chloride and the insoluble material which remained was filtered off. The organic solution was washed with saturated sodium bicarbonate solution and water, before being dried over anhydrous magnesium sulphate. The solvent was evaporated to give 17\(\beta\)-hydroxy-17\(\alpha\)-methyl-5\(\alpha\)-androstan-2\(\beta\)-eno[2,3-\(e\)]-2'H-pyrido-[1,2-a]pyrimidin-2'-one (22mg, 5.41 x 10\(^{-5}\) mole), (26%); m.p. > 250\(^{\circ}\); u.v. (CH\(_3\)OH), \(\lambda_{\text{MAX}}\) 205nm (log \(\epsilon\) 4.05), 240 (4.23), 248 (4.18), 274 (3.95), 286 (3.92), 330 (3.31); i.r. (nujol), \(\nu_{\text{MAX}}^{\text{IR}}\) 16140, 1595, 1580, 1520cm\(^{-1}\); mass spectrum, \(M^+\), m/e 406, calculated for \(C_{26}H_{34}N_2O_2\) 406.2601; found 406.2608; error less than 3ppm. It was not possible to obtain a satisfactory n.m.r. spectrum due to insufficient material. An attempt to obtain an n.m.r. spectrum after the addition of a drop of \(D_2O\) to the deuterochloroform solution was also unsuccessful.
solution was washed with water, dried over anhydrous magnesium sulphate and the solvent evaporated. The recovered material was found not to be 17β-hydroxy-17α-methyl-5α-androst-2-eno-[2,3-e]-2'H-pyrido-[1,2-a] pyrimidin-2'-one. The following spectral data was recorded for the recovered material:

u.v. (CH$_3$OH); $\lambda_{max}$ 206 nm (log $\varepsilon$ 3.86), 235 (3.96), 247 (3.86), 274 (3.64), 284 (3.59); i.r. (CHBr$_3$), $\nu_{max}$ 3570 (OH), 3400 (NH), 3340 (NH), 1670 (C=O), 1620, 1575, 1535, 1500, 1475 and 1415 cm$^{-1}$; mass spectrum, M$^+$, m/e 422, calculated for C$_{20}$H$_{20}$N$_2$O$_3$ 422.256926, found 422.256232, error less than 2 ppm.
References


35. N.J. Doorenbos and M.S. Wu, Chem. and Ind., 1965, 12, 650.


