STUDIES TOWARD A TOTAL SYNTHESIS OF DIGITOXIGENIN FROM THUJONE

by

JOHN WALLACE SOMERVILLE

B.Sc., The University of New Brunswick, 1984

A THESIS SUBMITTED IN PARTIAL FULFILMENT OF
THE REQUIREMENTS FOR THE DEGREE OF
MASTER OF SCIENCE

in

THE FACULTY OF GRADUATE STUDIES
(Department of Chemistry)

We accept this thesis as conforming
to the required standard

THE UNIVERSITY OF BRITISH COLUMBIA
August 1987

© John Wallace Somerville, 1987
In presenting this thesis in partial fulfilment of the requirements for an advanced degree at The University of British Columbia, I agree that the Library shall make it freely available for reference and study. I further agree that permission for extensive copying of this thesis for scholarly purposes may be granted by the Head of my Department or by his or her representatives. It is understood that copying or publication of this thesis for financial gain shall not be allowed without my written permission.

(Department of Chemistry)

The University of British Columbia
2075 Wesbrook Place
Vancouver, Canada
V6T 1W5

Date: August 1987
ABSTRACT

This thesis concerns studies directed towards a total synthesis of digitoxigenin 3c from thujone 1. The steroidal analog 85 had previously been synthesized from thujone and the strategy envisaged for the completion of the synthesis involved alkylation of 85 to produce 86a which, after deoxygenation of the C16-ketone followed by reduction of the nitrile, would produce the aldehyde 86b. Aldol condensation of the aldehyde 86b and glyoxylic acid (or an ester thereof) would result in the butenolide 87 which could be converted to the known digitoxigenin synthon 43 by hydrolysis of the C3-ethylene ketal.

The steroidal analog 85 was synthesized from the commercially available androst-4-ene-3,17-dione 6 by catalytic hydrogenation of the C4,5-double bond, to produce a mixture of the C5α-H and C5β-H diastereomers 88a and 88b, respectively. Separation by preparative liquid chromatography and protection of the C3-ketone of 88b as the ethylene ketal derivative resulted in the C17-ketone 89. Wittig reaction of 89 with methyltriphenylphosphonium bromide produced the C17-methylene derivative 91 which was oxidized with selenium dioxide to produce the allylic alcohol 92 and small amounts of the α,β-unsaturated ketone 93. Oxidation of 92 to the required α,β-unsaturated ketone 93 was achieved via the Swern methodology or, alternatively, by treatment with pyridinium dichlorochromate. Oxidation of the trimethylsilylenolether of 93 with DDQ resulted in the cross-conjugated dienone 85.

A model study was conducted at this time utilizing the α,β-unsaturated ketone 93 in order to develop the necessary chemistry for elaboration of the butenolide ring. Thus alkylation of 93 produced the nitrile 94, the C16-ketone of which was subsequently protected as the corresponding ethylene ketal derivative to
provide the required C17β-nitrile 95a. Nuclear Overhauser difference experiments on both 94 and 95a confirmed that both compounds had the required 17β-configuration. Reduction of the nitrile with diisobutylaluminum hydride resulted in the aldehyde 95b which was alkylated with mentholglyoxylate, followed by reduction to produce the butenolide 98 and the trans ester 99.

The approach to the synthesis of digitoxigenin was pursued by alkylation of the cross-conjugated diene 85 with diethylaluminum cyanide to produce 86a as a mixture of C17-epimers (ratio 2:1, on the basis of NMR). Separation of these isomers proved to be impractical and NOE-difference experiments on this mixture are consistent with the major isomer having the C17β-configuration.
# TABLE OF CONTENTS

Title page ........................................................................................................... i
Abstract .............................................................................................................. ii
Table of Contents ............................................................................................... iv
List of Figures ...................................................................................................... vi
List of Schemes ................................................................................................... vii
Acknowledgements ............................................................................................ viii
Abbreviations .................................................................................................... ix

1. Introduction .................................................................................................... 1
   1.1. Thujone .................................................................................................. 1
   1.2. Cardiac active glycosides ..................................................................... 3
   1.3. Problems associated with the synthesis of digitoxigenin ................. 6
   1.4. Review of the synthesis of digitoxigenin .......................................... 10

2. Scope of the present work ............................................................................ 28

3. Results and discussion ................................................................................ 33
   3.1. The Common Intermediate ................................................................ 33
      3.1.1. 5\beta-Androstane-3,17-dione 88b ........................................... 33
      3.1.2. Formation of the C3-ketal 89 ................................................ 35
      3.1.3. \alpha,\beta-Unsaturated ketone 93 ............................................. 37
      3.1.4. Cross-conjugated dienone 85 ................................................... 39
      3.1.5. Model studies of the aldol condensation of the C21-aldehyde 95b 40
   3.2. Digitoxigenin formal synthesis ......................................................... 50
      3.2.1. Hydrocyanation reaction ............................................................. 50

4. Experimental .................................................................................................. 55
   4.1. General .................................................................................................. 55
   4.2. 5\beta-Androstane-3,17-dione 88b ....................................................... 56
   4.3. 5\beta-Androstane-3,17-dione-3,3-ethyleneketal 89 ......................... 57
      4.3.1. Method A ..................................................................................... 57
      4.3.2. Method B ..................................................................................... 58
      4.3.3. Method C ..................................................................................... 58
   4.4. 17-Methylene-5\beta-androstane-3-one-3,3-ethyleneketal 91 ............. 60
   4.5. 16\alpha-Hydroxy-17-methylene-5\beta-androstane-3-one-3,3-ethyleneketal 92 60
   4.6. 17-Methylene-5\beta-androstane-3,16-dione-3,3-ethyleneketal 93 ....... 62
      4.6.1. Method A ..................................................................................... 62
      4.6.2. Method B ..................................................................................... 62
   4.7. 17\beta-Cyanomethyl-5\beta-androstane-3,16-dione-3,3-ethyleneketal 94 63
   4.8. 17\beta-Cyanomethyl-5\beta-androstane-3,16-dione-3,3,16-bis(ethyleneketal) 95a 64
4.9. 20-Oxo-5β-pregnane-3,16-dione-3,3,16,16-bis(ethyleneketal) 95b ........... 65
4.10. 5β-Card-20(22)-enolide-3,16-dione-3,3,16,16-bis(ethyleneketal) 98 ........ 66
4.11. 17-Methylene-5β-androst-14-ene-3,16-dione-3,3-ethyleneketal 85 .......... 67
4.12. 17-Cyanomethyl-5β-androst-14-ene-3,16-dione-3,3-ethyleneketal 86a ........ 68

5. References ........................................................................................................... 70
List of Figures

Figure 1: The chemistry of thujone ................................................................. 2
Figure 2: Some examples of cardenolides ......................................................... 4
Figure 3: Some common steroidal precursors ..................................................... 7
Figure 4: Intermediates 85 to 87 ................................................................. 32
Figure 5: The proton NMR spectrum of 88b ................................................. 35
Figure 6: 2D-COSY-HETCOR (1H, 13C) of 88b ............................................. 36
Figure 7: NOE-difference and decoupling experiments on 94 ......................... 42
Figure 8: 13C-NMR spectrum of 94 .......................................................... 43
Figure 9: NOE-difference experiments on 95a ............................................. 45
Figure 10: The synthesis of the cardenolide 98 ............................................ 47
Figure 11: The aldol condensation reaction of 95b ....................................... 49
Figure 12: 1H-NMR spectrum of 86a .......................................................... 51
Figure 13: NOE-difference experiments on 86a ............................................ 53
List of Schemes

Scheme 1: The Ruzicka approach to digitoxigenin 3c ........................................8
Scheme 2: The Engal and Bach approach to digitoxigenin 3c .................................9
Scheme 3: The first synthesis of digitoxigenin 3c ..................................................11
Scheme 4: The Pettit approach to digitoxigenin 3c ..............................................12
Scheme 5: Synthesis of the cardenolide 29 .............................................................14
Scheme 6: Digitoxigenin from 15α-hydroxycortexone 30a .....................................15
Scheme 7: The Kreiser synthesis of 39 .................................................................17
Scheme 8: The synthesis of 44 .............................................................................18
Scheme 9: The Wiesner synthesis of digitoxigenin 3c ...........................................19
Scheme 10: The Wicha approach to digitoxigenin 3c .............................................22
Scheme 11: The Wicha synthesis of 43 .................................................................25
Scheme 12: The Harnisch synthesis of 43 .............................................................26
Scheme 13: Intermediates 75 and 76 from thujone ...............................................29
Scheme 14: The synthesis of 80 ...........................................................................30
Scheme 15: The synthesis on of 2 ........................................................................31
Scheme 16: The synthesis of 93 ...........................................................................38
ACKNOWLEDGEMENTS

I wish to express my appreciation to Professor James P. Kutney for the opportunity to pursue this project and for his valuable advice, both during the progress of this research and in the preparation of this thesis.

I would also like to express my gratitude to Dr. Krystyna Piotrowska for her invaluable help and encouragement throughout this research project.

The technical expertise of the staff in the NMR, mass spectroscopy, and microanalytical service laboratories as well as the glass blowing, mechanical and electronics shops is very much appreciated. I would also particularly like to thank the staff of the NMR laboratory for their kind advice and patience.

Thanks must also go to Professor Ed Piers and his research group for the use of their infra-red spectrometers and preparative liquid chromatograph.

Last, but definitely not least, I wish to express my appreciation to the members (both past and present) of Dr. Kutney's research group for their advice, enlightening discussions and friendship. Finally I wish to thank Dr. Cam Boulet, Dr. Michael McHugh and Mr Malcolm Roberts for their meticulous proof reading and criticism of this thesis.
### ABBREVIATIONS

The following is a list of abbreviations that have been used throughout this thesis:

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ac</td>
<td>acetate</td>
</tr>
<tr>
<td>atm</td>
<td>atmosphere</td>
</tr>
<tr>
<td>bs</td>
<td>broad singlet</td>
</tr>
<tr>
<td>bz</td>
<td>benzyl</td>
</tr>
<tr>
<td>d</td>
<td>doublet</td>
</tr>
<tr>
<td>dd</td>
<td>doublet of doublets</td>
</tr>
<tr>
<td>dt</td>
<td>doublet of triplets</td>
</tr>
<tr>
<td>DDQ</td>
<td>2,3-dichloro-5,6-dicyanoquinone</td>
</tr>
<tr>
<td>GLC</td>
<td>gas liquid chromatography</td>
</tr>
<tr>
<td>h</td>
<td>hour</td>
</tr>
<tr>
<td>HPLC</td>
<td>high performance liquid chromatography</td>
</tr>
<tr>
<td>IR</td>
<td>infra red</td>
</tr>
<tr>
<td>max.</td>
<td>maxima</td>
</tr>
<tr>
<td>min.</td>
<td>minute</td>
</tr>
<tr>
<td>m</td>
<td>multiplet</td>
</tr>
<tr>
<td>NMR</td>
<td>nuclear magnetic resonance</td>
</tr>
<tr>
<td>NOE</td>
<td>nuclear Overhauser effect</td>
</tr>
<tr>
<td>Ph</td>
<td>phenyl</td>
</tr>
<tr>
<td>q</td>
<td>quartet</td>
</tr>
<tr>
<td>sat. aq.</td>
<td>saturated aqueous</td>
</tr>
<tr>
<td>s</td>
<td>singlet</td>
</tr>
<tr>
<td>THF</td>
<td>tetrahydrofuran</td>
</tr>
<tr>
<td>Term</td>
<td>Definition</td>
</tr>
<tr>
<td>----------</td>
<td>---------------------------------</td>
</tr>
<tr>
<td>t</td>
<td>triplet</td>
</tr>
<tr>
<td>p-TSA</td>
<td><em>para</em>-toluene sulphonic acid</td>
</tr>
<tr>
<td>UV</td>
<td>ultraviolet</td>
</tr>
<tr>
<td>v/v</td>
<td>ratio by volume</td>
</tr>
</tbody>
</table>
1. INTRODUCTION

1.1. THUJONE

The decline of the forest industry in Canada, and in particular British Columbia, has stimulated the search for more efficient uses of the available resources. The largest waste of any logging operation is the leftover bark, branches and leaves, generally termed "slash", which often must be removed before any re-forestation can begin and dry slash can also pose a considerable fire hazard. If the slash from Western red cedar is steam distilled, a leaf oil that contains as much as 88% thujone 1, depending on where the trees are grown and on the amount of rain, is obtained. This steam distillation can be done "on site" immediately after a logging operation, thus serving the dual purpose of removing the left-over "slash", and providing an inexpensive source of thujone. An important use for a chiral monoterpenoid like thujone would be the chemical synthesis of biologically important optically active molecules.

A great deal of synthetic work utilizing thujone as a chiral synthon has been done in our laboratory over the past few years. This work has shown that thujone can be a viable chiral synthon for synthesizing a variety of natural products and biologically important molecules such as: juvenile hormone analogs 2, pyrethroid insecticides 3, sesquiterpenes 4, and steroidal compounds 5 (see Figure 1).

From inspection of Figure 1, one can notice that the steroid 2, synthesized from thujone, has the rather unusual 5β-configuration. In the synthesis of this molecule, the reaction where this chiral center was created was completely stereospecific, thus producing only one diastereomer in very good yield. An important class of natural products, most of which have the 5β-configuration, are
Figure 1
the cardiac active glycosides\textsuperscript{6}. Typical examples\textsuperscript{7} of cardiac active glycosides used in modern medicine are digitoxin 3\textsuperscript{a} isolated from the Purple Foxglove \textit{Digitalis purpurea} and digoxin 3\textsuperscript{b} from \textit{D. lanata} (see Figure 2).

\textbf{1.2. CARDIAC ACTIVE GLYCOSIDES}

Various plant extracts containing cardiac glycosides have been used for centuries. Squilla, an extract of \textit{Urginea martima}, which contains the glycoside Proscillaridin A 4\textsuperscript{a}, was used as a medicine by the ancient Egyptians, and the Romans used it for various purposes such as: a diuretic, heart tonic, emetic and even as a rat poison\textsuperscript{7}.

A classic book published in 1785 by William Withering\textsuperscript{7} entitled "An Account of the Foxglove and some of its Medical Uses: With Practical Remarks on Dropsy and other Diseases" is the first description of the therapeutic uses of cardiac glycosides (in this case, extracts of the Purple Foxglove). Although Withering noted that foxglove was effective only for certain types of dropsy (the abnormal retention of body fluids in the body tissues, usually, but not always, because of heart failure) and also was aware of the effects on the heart ("It has power over the motion of the heart to a degree yet unobserved in any other medicine") he did not make any connection between these effects. It was not until a few years later (1799) that another physician (John Ferriar) realized that the main effect of foxglove was on the heart and that diuresis (and thus relief of dropsy) was a secondary effect due to the return of efficient pumping by the heart.

It was only in the last 60 years that it became generally accepted that the beneficial effects of these drugs were to reduce the symptoms of congestive heart failure. All of the beneficial effects of these drugs such as: increased cardiac output; decreased heart size, venous pressure, and blood volume; diuresis and relief of
edema can be explained on the basis of an increase in the force of contraction of
the heart muscle (positive inotropic effect).

The cardiac glycosides consist of a steroidal portion (aglycone) attached via
a glycoside linkage at C3 to a carbohydrate consisting of from one to four hexose
units. There are a very large number of naturally occurring cardiac active glycosides
that differ only in the carbohydrate portion. Thus hydrolysis of the glycoside
linkage of all the known cardiac glycosides produces relatively few aglycones.

The cardiac active steroids all have a common steroidal "backbone" (see
Figure 2), most having A/B and C/D cis ring junctions as well as 3β- and
14β-hydroxy groups and a lactone ring attached to the steroid at C17. There are
two major classes of cardiac active steroids, depending on the nature of the
17β-lactone moiety: the cardenolides have a butenolide ring and the bufadienolides

- **3a** digitoxin \( [R_1 = \text{tridigitoxose}, R_2 = H] \)
- **3b** digoxin \( [R_1 = \text{tridigitoxose}, R_2 = \text{OH}] \)
- **3c** digitoxigenin \( [R_1 = \text{OH}, R_2 = \text{OH}] \)
- **4a** proscillaridin A \( [R = \text{rhamnose}, \Delta^{4,5}] \)
- **4b** scillaren \( [R_1 = \text{OH}, \Delta^{4,5}] \)
- **4c** bufalin \( [R_1 = \text{OH}, C5\beta-H] \)
(such as scillaren 4b, and bufalin 4c) have an α-pyrone ring. The cardenolides and bufadienolides have similar effects on the heart but, in general, the bufadienolides are much more toxic.

Most of the biological activity of cardiac glycosides is associated with the aglycone, although the carbohydrate moiety is necessary. Comparison of two cardiac glycosides having the same carbohydrate moiety, such as the aforementioned digoxin and digitoxin is interesting. The plasma concentrations required for the desired therapeutic effect for digitoxin is in the range of 14-26 ng/ml whereas that for digoxin is only 0.8-1.6 ng/ml.

The most commonly used cardioactive glycoside in medicine is digoxin. It has been said that this drug is responsible for one of the highest rates of drug induced hospital admission and death. The therapeutic ratio (therapeutic/toxic dose) is very low for all cardiac active glycosides (in this case only 0.6). Also the plasma level is very dependent on the physical condition of the patient (especially with respect to kidney function) and so the dose must be carefully administered on an individual basis. It is this low margin of safety that has stimulated medicinal chemists and pharmacologists to attempt to develop safer analogs.

Structure activity studies have shown that the 17β-butenolide ring, 3β- and 14β-hydroxy groups are absolutely necessary for biological activity. Therefore, synthetic studies on a compound such as digitoxigenin, which incorporates the above features would be a valuable contribution to the development of a cardenolide type drug with an improved therapeutic ratio.
1.3. PROBLEMS ASSOCIATED WITH THE SYNTHESIS OF DIGITOXIGENIN

All of the approaches to cardenolides so far have been from steroidal precursors such as: progesterone 5, androstenedione 6, and testosterone 7 (see Figure 3), because these are readily available (although expensive) and one can envisage possible synthetic routes to incorporate the necessary functionality. The problems which must be overcome in such a partial synthesis are as follows:

1. The introduction of the 3β-hydroxy group from a 3-keto steroid.
2. Introducing the thermodynamically unfavourable A/B cis ring junction.
3. Introducing the 14β-hydroxy group and the thermodynamically unfavourable C/D cis ring junction.
4. In C/D cis steroids, it is generally true that a side chain at position C17 is thermodynamically favoured in the α-configuration.

Most of the steroidal precursors (such as the examples given above) have the Δ^4-3-keto functionality and most syntheses proceed via a catalytic hydrogenation of the C4,5 double bond producing a mixture of 5α- and 5β-isomers. The experimental conditions can often be modified to favour the desired 5β-isomer but complete stereoselectivity is very rare, thus, it will usually be necessary to separate these isomers. Similarly, with reduction of the 3-ketone to the desired 3β-configuration, a mixture of 3α- and 3β-hydroxy compounds often resulted. These problems generally caused low yields from the starting Δ^4-3-keto steroid, but this was not really a serious problem if these reactions were done at an early stage of the synthesis.

The problems of introducing the 14β-hydroxy group, and the 17β-butenolide ring foiled many of the earlier attempts to synthesize digitoxigenin 3c. The most common strategy is to introduce a 17β side chain (for elaboration of
the butenolide ring) on a 14α-steroid precursor because the 17β-configuration is thermodynamically favoured in these compounds, and introduce the 14β-hydroxy group in the later stages. One of the first attempts to synthesize a cardenolide was reported by Ruzicka et al. and this serves to illustrate the above points (see Scheme 1). Oxidation of the 14,16-diene with peracid gave the 14,15β-epoxide, which was then treated with hydrogen over platinum in ethanol or acetic acid to yield about 20% of the desired 14β-hydroxy, 17β-methylester and about 70% of the undesired 14β-hydroxy, 17α-methylester. In this case, once the C/D ring junction becomes cis, attack of hydrogen from the less hindered β-face of the steroid is kinetically favoured, thus producing the 17α-stereochemistry.

A few years later, a different approach was reported by Engel and Bach (see Scheme 2). Starting from 14-dehydrodigitoxigenin, attack of the
C14,15 double bond with hypobromous acid, generated in situ, gave the desired intermediate 14β-hydroxy-15α-bromide 13, which spontaneously (under these reaction conditions) cyclized to the 14,15β-epoxide 14. This epoxide was then opened by treatment with dry hydrochloric acid to give the 14β-hydroxy-15α-chloride 15. The chloride was then reduced with tri-(n-butyl)tin hydride to yield 3β-acetoxydigitoxigenin. Although the yields were not high, either this methodology or variations of it have become the standard way to introduce the 14β-hydroxy group into cardenolides.
Scheme 2
1.4. REVIEW OF THE SYNTHESIS OF DIGITOXIGENIN

The first synthesis of digitoxigenin was reported by Sondheimer et al.\textsuperscript{1,2} starting from methyl 3\textbeta\textbeta-acetoxy-14\textbeta-hydroxy-5\textbeta-eti anate 16 (Scheme 3) which was synthesized in 12 steps from 5\textbeta-androstane-3\textbeta-ol-17-one acetate 17\textsuperscript{1,3}. This compound was converted to 14\textbeta-hydroxy-5\textbeta-pregnan-20-one 18 by saponification to the corresponding hydroxy acid, which was acetylated at C3, in a total yield from 16 of 45%. As one can see, this starting material has most of the above mentioned problems already solved, only the lactone must be built up without the loss of the 14\textbeta-hydroxy group.

This method involved reacting 18 with lithium ethoxyacetylene to produce the \textalpha-acetylenic carbinol 19 followed by treatment with dilute sulphuric acid to effect a Meyer-Shuster\textsuperscript{1,4} type rearrangement producing the \textalpha,\textbeta-unsaturated ester 20a in a total yield of 45% from compound 18. Allylic oxidation of 20a with selenium dioxide produced the intermediate allylic alcohol 20b which was cyclized \textit{in situ} to the required butenolide ring. Digitoxigenin was then obtained in 24% overall yield from 16 by saponification of the 3\textbeta-acetate of compound 21.

An approach to a cardenolide compound starting from a 3\textbeta-acetoxy-20-oxo-pregnan-5-ene 22a based on a Wittig-Horner reaction was reported in 1970 by Pettit\textsuperscript{1,5}, (see Scheme 4). Treatment of the pregnane 22a with lead tetraacetate in the presence of boron trifluoride etherate resulted in the 3,21-diacetate 22b.

Treatment of this compound with the anion derived from diethylcyanomethylphosphonate followed, after removal of the solvent, by treatment with 2N hydrochloric acid/diethyl ether, resulted in 47% of the imino lactone 24 and 18% of the \textit{E}-unsaturated nitrile 23a. The imino lactone 24 could then be hydrolyzed, using 0.6N hydrochloric acid/methanol, to the lactone 25a in 90% yield
Scheme 3
Scheme 4
accompanied by 10% of the diene 26.

This same sequence was improved dramatically a few years later (1978) by Lenz. The diacetate 22b, was treated with the same Wittig reagent used above but instead of tetrahydrofuran, 1,2-dimethoxyethane was used as a solvent. After workup, the Z-unsaturated nitrile 23b resulted in 75% yield and no E-nitrile 23a was observed. Treatment of 23b with p-toluene sulfonic acid resulted in 95% yield of the 3β-hydroxy-cardenolide 25b.

This same reaction sequence was used on the enol ether of deoxycortexone 27, (Scheme 5) by the same authors. Their initial plan was to produce the unsaturated nitrile 28, saponify and cyclize to an imino lactone which could then be hydrolyzed to the lactone as above, but saponification of 28 was not successful. However treatment of 28 with p-toluene sulfonic acid in methanol resulted in conversion to the cardenolide 29 in 69% yield.

A digitoxigenin synthesis from 15α-hydroxycortexone 30a, (Scheme 6) was reported by Ruschig in 1969. The dimesylate derivative 30b was prepared and transesterified by the potassium salt of the malonic acid benzyl half ester to produce the intermediate 30c which spontaneously cyclized to produce the lactone 31. Pyrolysis of this compound in collidine in the presence of p-toluene sulfonic acid resulted in the elimination of the mesyl group to produce the C14,15 double bond, as well as the hydrolysis and decarboxylation of the benzyl ester to produce the required butenolide ring 32 in a yield of 25% from 30a. The C4,5 double bond was stereospecifically hydrogenated with hydrogen over palladium on charcoal to produce the Δ14-3-ketone 33a in 79% yield. The ketone was then reduced with lithium tri(tert-butoxy) aluminum hydride to produce the 3α-hydroxy compound 33b in 81% yield. This hydroxy compound was epimerized at C3 by
solvolysis (in sodium acetate/acetic acid) of the corresponding tosylate to give the required $3\beta$-acetoxy derivative $33c$ in 26% yield accompanied by the elimination product $34$. The $14\beta$-hydroxy group was introduced using a modification of the method developed by Engel and Bach\textsuperscript{11}. Thus, treatment of $33c$ with N,N-dibromobenzosulfonamide gave the bromohydrin $35$, then catalytic reduction over Raney nickel$\textsuperscript{®}$/palladium chloride mixture gave $3\beta$-acetoxy-digitoxigenin $21$, in 19% yield.

Scheme 5

27

28

29
\(30a \quad R_1, R_2 = H\)

\(30b \quad R_1, R_2 = \text{SO}_2\text{CH}_3\)

\(30c \quad R_1 = \text{OSO}_2\text{CH}_3, R_2 = \text{C(O)CH}_2\text{CO}_2\text{Bz}\)

\(31\)

\(32\)

\(33a \quad R_1, R_2 = \text{O}\)

\(33b \quad R_1 = \text{OH}, R_2 = H\)

\(33c \quad R_1 = H, R_2 = \text{OAc}\)

\(34\)

Scheme 6
A very convenient method for the construction of a butenolide ring system (from a C21 aldehyde) was developed by Kreiser and Nazir (see Scheme 7). This C21 aldehyde was synthesized via known methods from dehydroepiandrosterone. Aldol condensation of this aldehyde with glyoxylic acid monohydrate was studied. Under basic conditions, sodium acetate in glacial acetic acid (heating 68 hours at 67°C), the lactol was obtained in 27% yield and under acidic conditions (concentrated hydrochloric acid in glacial acetic acid, 36 hours at 60°C) the yield was 58%. The lactol was subsequently reduced with sodium borohydride and deacetylated to produce the cardenolide. Although the yields of the aldol condensation step are not high, the cardenolide ring is synthesized in only two steps from the aldehyde, and therefore this procedure is much more convenient than a method involving a lengthier series of reactions.

An interesting strategy for a digitoxigenin synthesis was employed by McMurry ef al. in their formal synthesis of digitoxigenin from 21-hydroxy-5β-pregnane-3,20-dione (Scheme 8). Treatment of with the mixed anhydride of trifluoroacetic acid and diethylphosphonoacetic acid gave the intermediate which, without isolation, was cyclized by an intramolecular Wittig or Horner-Emmons reaction to give the cardenolide in 70% yield from . Reduction of the 3-ketone to the required 3β-hydroxy group with L-Selectride® and, the latter after acetylation, afforded the 3β-acetoxy cardenolide in 89% yield. At first glance, this compound would seem to be a "dead end" because there seems to be no facile method to introduce the required 14β-hydroxy group. Their method of solving this problem was to utilize remote functionalization of steroids as developed by Breslow et al. Via this methodology was photolyzed in the presence of iodobenzene dichloride and this resulted in the production of
Scheme 7

$3\beta$-acetoxy-$5\beta$-carda-14,20(22)-dienolide 44 directly in 55% yield without the necessity of dehydrohalogenating the intermediate $14\alpha$-chloro compound. Surprisingly, only small amounts (about 10%) of the product 45 derived from oxidation of the tertiary allylic C17 position was observed. Since the compound 44 had previously$^{17}$ been transformed into digitoxigenin, this constitutes a formal synthesis from 40.

A synthesis employing a rather novel strategy starting from testosterone 7 was reported by Wiesner et al.$^{22}$ (see Scheme 9). The C4,5 double bond of testosterone was hydrogenated to produce mainly the required $5\beta$-isomer 46, and after protection of the C17 hydroxy group (as a tetrahydropyranyl ether), the C3
Scheme 8
Scheme 9
ketone was then reduced using a Meerwein-Ponndorf reaction to the alcohols 47a and 47b. The 3\(\beta\)-hydroxy compound 47b was transformed in 6 steps to the \(\alpha,\beta\)-unsaturated ketone 48. Attack of this ketone in the 1,2-sense from the \(\alpha\)-face of the molecule resulted in the tertiary allylic alcohol 49 with a yield of 93%. Treatment of this alcohol with acetic anhydride in pyridine to give the acetate, followed by refluxing in acetone containing calcium carbonate, resulted in allylic
rearrangement to the secondary allylic alcohol 50 in 87% yield. Hydrogenation over palladium on calcium carbonate in ethanol was stereospecific, thus producing the saturated alcohol 51 with the desired C17β-configuration in a yield of 92%. Oxidation of this compound with m-chloroperbenzoic acid in a mixture of chloroform, acetic acid, and sodium acetate produced the hydroxy lactone 52, which, without isolation, was reduced with sodium borohydride to give the 15β-hydroxy cardenolide 53 in 87% yield. Dehydration of this hydroxy group was effected by treatment with mesyl chloride in pyridine to produce the Δ14-compound 54 in 85% yield. The 14β-hydroxy group was then introduced via a modification of the aforementioned Engel and Bach method. Thus the olefin was treated with N-bromoacetamide in a mixture of acetic acid, water and acetone to produce the intermediate 14β-hydroxy, 15α-bromide, which, without isolation, was reduced with Raney nickel in a mixture of methylene chloride, methanol and potassium acetate to provide in 78% yield the 3β-benzyldigitoxigenin 55. The benzyl group was removed by hydrogenolysis to give digitoxigenin 3c in a 93% yield.

An interesting strategy for the construction of the butenolide ring based on a palladium-induced rearrangement of an allylic epoxide was employed by Wicha and Kabat in their synthesis of digitoxigenin from 3β-acetoxy-androst-5-ene-17-one 56 (see Scheme 10). The α,β-unsaturated ketone 57, prepared from 56 in overall yield of 40%, was oxidized with alkaline hydrogen peroxide to produce the 14,15β-epoxide 58 in 91% yield. After protection of the 3β-hydroxy group as the tetrahydropyranyl ether, the resulting compound was treated with methyl trimethylsilylacetate and n-butyllithium in ether to effect a Peterson olefination, giving the unsaturated ester 59a (as a mixture of geometric isomers at C20) in 75% yield. The mixture was reduced with lithium aluminum hydride in ether at -40°C to give
Scheme 10
mainly (80%) the allylic alcohol 59b, accompanied by some (10%) of the diol 60 resulting from ring opening of the epoxide. Esterification with (phenylthio)acetic acid in the presence of dicyclohexylcarbodiimide gave the isomeric esters 61 in 92% yield. Treatment of the ester with 10 mol% of tetrakis(triphenylphosphine) palladium(0) at 40°C in tetrahydrofuran solution resulted in 60% of the lactone 62. The C16,17 double bond was stereospecifically reduced using diimide generated in
situ from dipotassium azodicarboxylate and acetic acid in pyridine solution to produce the C17β-lactone 63a in 70% yield. Elimination of the thio ether was effected by oxidizing to the sulfoxide 63b and pyrolysis in refluxing toluene to give the cardenolide 64 in quantitative yield. The 15β-hydroxy group was eliminated in the usual way (mesyl chloride in pyridine) to give the unsaturated derivative 65 in 75% yield and mild acid hydrolysis removed the tetrahydropyranyl ether to give 14-anhydrodigitoxigenin in 80% yield. Since this compound has been\textsuperscript{1,7} transformed into digitoxigenin this constitutes a formal synthesis from 56.

Another approach to a cardenolide from an androstane was reported a few years later by the above research group\textsuperscript{2,4} (see Scheme 11). Treatment of the 3β-acetoxy-5β-androstane-17-one 17 with cyanoacetate in refluxing toluene in the presence of ammonium acetate resulted in the unsaturated nitrile 66 in 89% yield. Reduction of the double bond from the α-face of the molecule with sodium borohydride with simultaneous reduction of the ester followed by protection of the hydroxy group as a tetrahydropyranyl ether gave the saturated nitrile 67a in 96% yield. Diisobutyl aluminum hydride reduction of the nitrile gave the aldehyde 67b, which when treated with potassium cyanide and hydrochloric acid in methanol at room temperature followed by refluxing, resulted in the hydroxy lactone in 79% yield. Acetylation of both hydroxy groups followed by selective hydrolysis of the C22 acetate gave the mono-acetate 68 in 92% yield. Elimination of this hydroxy group by treatment with thionyl chloride in dimethylformamide at room temperature to produce the corresponding chloride, followed by addition of lithium carbonate and lithium chloride to the reaction mixture and refluxing, resulted in the cardenolide 43, which has already been transformed to a digitoxigenin synthon by McMurry et al.
Another synthesis of the digitoxigenin synthon 43 utilizing a novel strategy was reported by Harnisch et al. (see Scheme 12). The enol trifluoromethanesulfonate 69 was formed in 46% yield from 17 and alkylated with E-3-(methoxycarbonyl)-2-propenyl tetrahydropyranyl ether in the presence of palladium acetate triphenylphosphine to produce the intermediate \( \alpha, \beta \)-unsaturated ester 70. Treatment of this with an ion exchange resin (acid form) produced the butenolide 71 in 37% yield. Stereospecific hydrogenation of the C16,17 double bond
produced the desired C17β-cardenolide 43 in quantitative yield.

The above literature review has shown several examples of how many of the problems associated with the synthesis of cardenolides from common steroids can be overcome. For example, as was shown by McMurry et al., the reduction of the C3-ketone of 5β-steroids can be achieved stereospecifically utilizing complex reducing agents (e.g., L-Selectride) to produce mainly the 3β-hydroxy-5β-steroidal
precursor. The introduction of the 17β-configuration from androstane or testosterone type precursors (as opposed to the progesterone type that already have the 17β-configuration) is most often accomplished by taking advantage of the kinetically favoured attack of hydrogen (or some other reducing species) on ∆14-steroids from the less hindered α-face of the molecule. The introduction of the 14β-hydroxy group however, is done essentially by the method of Engel and Bach\textsuperscript{11} from the corresponding ∆14-steroid and often proceeds with a rather modest yield.
2. SCOPE OF THE PRESENT WORK

The chemistry of thujone 1, has been a major focus of this research group for the past 10 years and, as mentioned in the first part of this introduction, thujone has been shown to be a viable chiral synthon for a variety of biologically active molecules. As a result of the above research, a total synthesis of steroids such as the analog 2 (Figure 1) was achieved as follows\(^5\). Alkylation of thujone with methyl vinyl ketone 72 (Scheme 13) followed by cyclization of the resulting dione to yield the tricyclic $\delta,\gamma$-unsaturated ketone 74 in 48% yield from thujone. Alkylation of 74 with methyl acrylate resulted in a mixture of the carboxylic acid 75 and the isomeric keto-esters 76. Treatment of this mixture as shown in scheme 14 resulted in the enol lactones 77 and 78, both in 45% yield from 74. Alkylation of 77 and 78 with the Grignard reagent (as shown in Scheme 14) resulted in the compounds 79 and 80, respectively in 65% yield each. Compound 79 was readily converted to 80 by catalytic (H\(_2\)/Pd) reduction of the C8,14 double bond. Base catalyzed aldol condensation of 80 resulted in the tetracyclic enone 81 in 70% overall yield from 79. Birch reduction of 81 (Scheme 15), followed by cyclization resulted in 60% yield of the steroid analog 82 and hydrogenation of the C4,5-double bond resulted in a quantitative yield of the 5$\beta$-steroid analog 83. Acid catalyzed ring opening of the cyclopropane ring resulted in compound 84 (as a mixture of C17-epimers) and, after protecting the C3-ketone as an ethylene ketal, ozonolysis of the exocyclic double bond at C16 resulted in the steroid analog 2 in 46% overall yield from the $\alpha,\beta$-unsaturated ketone 82. As a continuation of this research, we wish to extend the chemistry of thujone by utilizing the steroidal analog 2 as a substrate for the synthesis of cardiac active steroids.

The 14$\beta$-steroidal analog 2 was converted to the cross-conjugated
Scheme 13

dienone 85 (Figure 4) via elimination with benzeneselenic anhydride in dichlorobenzene. If the dienone 85, can be selectively alkylated at the exocyclic methylene group with some type of aldehyde synthone (such as a nitrile), to produce an intermediate like 86a, then a direct cardenolide synthesis can be
Scope of the present work / 30

Scheme 14

75 + 76

1. OH\(^-\), 25°C
2. Ac\(_2\)O, NaOAc, reflux

77

1. OH\(^-\), 25°C
2. Pd/C, H\(_2\)
3. Ac\(_2\)O, NaOAc, reflux

78

79

80

ethyl ether, 0°C
Scheme 15
envisaged possibly via the aldehyde $86\text{b}$. This compound would be particularly well suited as a cardenolide or bufadienolide synthon because it already has the $\Delta^{14}$-functionality necessary for the introduction of the $14\beta$-hydroxy group and the C21 aldehyde can be used in an aldol condensation type reaction, similar to the method used by Kreiser$^{18}$ to construct the lactone ring. Therefore it should be possible to produce the cardenolide $87$ which, after removal of the ring-A ketal group, would yield the ketone $33\text{a}$ the latter having been already transformed$^{17}$ into digitoxigenin. In this manner, a formal synthesis from thujone would be achieved.
3. RESULTS AND DISCUSSION

3.1. THE COMMON INTERMEDIATE

As discussed earlier, the objective of this research project was to synthesize digitoxigenin from thujone possibly via the cross-conjugated dienone 85 and the C21-aldehyde 86b. The synthesis of the steroidal analog 85 from thujone is a rather lengthy process so we decided to produce this compound from a commercially available steroid such as androst-4-ene-3,17-dione 6. This synthetic route would also confirm the previously assigned absolute stereochemistry of the chiral centers as shown in 85.

3.1.1. 5\(\beta\)-Androstane-3,17-dione 88b

A synthesis of the cross-conjugated dienone from the androst-4-ene-3,17-dione 6 must, at some point, involve hydrogenation of the C4,5 double bond to obtain the desired 5\(\beta\)-isomer. Since this reaction often results in a mixture of the two possible isomers at C5 (5\(\beta\)- and 5\(\alpha\)-) the best strategy would be to perform this reaction first. Thus catalytic hydrogenation of 6, over palladium on calcium carbonate resulted in 5\(\alpha\)-androst-3,17-dione 88a and the desired 5\(\beta\)-androstane-3,17-dione 88b 28% and 60% yields respectively. These isomers have similar physical properties (such as melting point and specific rotation) so they were distinguished on the basis of circular dichromism\(^2\)\(^6\) and carbon-13 NMR.

Although 5\(\beta\)-androstane-3,17-dione is a known compound\(^2\)\(^7\), the proton NMR spectrum has not been discussed in the literature. Since the analysis of this spectrum would make it easier to assign the spectra of the subsequent compounds in this series, this spectrum was studied in detail (Figure 5). The two methyl group
signals (at 0.90 and 1.06 ppm) were assigned by comparison of this spectrum to that of the substrate, androst-4-ene-3,17-dione. The latter spectrum shows two methyl group singlets at 0.92 and 1.21 ppm due to the C18 and C19 methyl groups, respectively. Hydrogenation of the C4,5 double bond, causes one of the singlets (1.21 ppm) to shift to a higher field (1.06 ppm), while the other remains unchanged, therefore the singlet at 1.06 ppm must be due to the C19-methyl group. There are three well resolved multiplets, each integrating for one proton, at 2.32, 2.47 and 2.68 ppm in the spectrum. Decoupling experiments showed that these protons are not coupled to each other (see Figures 5b, 5c, and 5d) and on the basis of their chemical shift, they must be attached to carbons 2, 4 or 16. However it is not possible to say which proton is attached to which carbon. To solve this problem, a 2D-COSY-HETCOR experiment was performed. Since the carbon-13 NMR spectrum has been discussed in the literature this experiment would help to assign these signals. Examination of the 2D-HETCOR spectrum (Figure 6) and consideration of the coupling constants allows assignment of these multiplets as: 2.32 (C2β-hydrogen), 2.47 (C16α-hydrogen), and 2.68 ppm (C4α-hydrogen).
Results and discussion / 35

H-NMR (400 MHz, CDCl$_3$) of 5$_\beta$-androstan-3,17-dione 88b
a) normal 400 MHz spectrum.
b) homonuclear spin decoupling at 2.32 ppm.
c) homonuclear spin decoupling at 2.47 ppm.
d) homonuclear spin decoupling at 2.68 ppm.

Figure 5

3.1.2. Formation of the C3-ketal 89

The C3-ketone of 5$_\beta$-androstan-3,17-dione 88b was protected as an ethylene ketal, as in the thujone-derived steroid 2. Treatment of 88b with ethylene glycol in the presence of a catalytic amount of $p$-toluenesulfonic acid in refluxing benzene with azeotropic removal of water, resulted in 76% yield of the desired monoketal 89, 10% of the diketal 90 and 5% recovered substrate. Therefore, as one might expect (on the basis of steric arguments), the C3-ketone is much more reactive than the
Heteronuclear ($^{13}$C, $^1$H) 2D COSY spectrum (300 MHz, CDCl$_3$) of 5β-androstane-3,17-dione 88b.

Figure 6

C17-ketone. A more regioselective method for the formation of ethylene ketals of diones resulting from the use of 1,2-bis[(trimethylsilyl)oxy]ethane and catalytic amounts of trimethylsilyl trifluoromethane sulfonate was recently reported in the literature$^2$. In our hands, this method was not a significant improvement of the above method.
Several years ago, attempts were made to accomplish the selective ketalization of the C3-ketone in steroidal diones, for example, androst-4-ene-3,17-dione. This method worked very well on some 5α-steroids, so we thought it would be useful to try this method on our system. Heating a solution of 2-butanone-2,2-ethyleneketal and the dione in the presence of p-toluenesulfonic acid at reflux, resulted in complete conversion to the desired monoketal and the diketal in 70% and 30% yields, respectively. Although this method did not increase the yield of the desired product, it proved to be the most convenient for large scale preparations because the crude product could be used in the next reaction, and the diketal was easily recovered unchanged after the subsequent Wittig reaction with 89.

3.1.3. α,β-unsaturated ketone 93

Following similar procedures to those used by Trost et al., the C17-ketone was treated with methyltriphenylphosphonium bromide and potassium t-butoxide in dry tetrahydrofuran to yield 94% of the desired olefin (Scheme 16). Allylic oxidation of this compound with selenium dioxide, in the presence of t-butyl hydroperoxide, resulted in the allylic alcohol and the α,β-unsaturated ketone 93.
in 75% and 18% yields, respectively. The allylic alcohol 92, was oxidized by treatment with a solution of oxalylchloride in dimethylsulfoxide (Swern\textsuperscript{32} method) to the $\alpha,\beta$-unsaturated ketone 93 in 86% yield. Because of the technical difficulties involved in the above reaction, the Corey\textsuperscript{33} method was tried. Thus treatment of 92 with pyridinium chlorochromate resulted in only 74% yield of the $\alpha,\beta$-unsaturated ketone 93. Therefore, although the Swern reaction is more difficult, it is obviously the method of choice.
3.1.4. Cross-conjugated dienone 85

The last step in the synthesis of this dienone from thujone was the dehydration using benzeneselenic anhydride to produce both double bonds in a good yield\textsuperscript{26}. Therefore we decided to try this method on the \(\alpha,\beta\)-unsaturated ketone 93. In this case however, the yield was very low (only about 20%) with no recovered substrate. Because of this very disappointing result, we sought an alternative.

In a synthesis of the tricyclopentanoid (\(\pm\))-coriolin\textsuperscript{34}, one of the intermediates possesses a ring system very similar to the D-ring of the steroidal analog 2 and it was observed that two double bonds were introduced to produce a cross-conjugated dienone. Therefore this synthetic work could serve as a model for our system. Employing this methodology, the enolate of the \(\alpha,\beta\)-unsaturated ketone 93 was generated by treatment with lithium hexamethyl disilylamide in tetrahydrofuran at -40°C, and quenching of the latter with trimethylsilyl chloride gave the corresponding silylenol ether. Oxidation of this enol ether with palladium (II) acetate resulted in the desired cross-conjugated dienone 85 in only about 5% yield, with recovery of almost all of the remaining substrate. Several attempts to improve this yield were not successful, so we decided to search for an alternative method. Enol silyl ethers have also been oxidized\textsuperscript{34} with 2,3-dichloro-5,6-dicyanoquinone, and in this case the desired cross-conjugated dienone 85 resulted in 32% yield and 57% recovered substrate. While this reaction is far from ideal, the yields are similar to many examples in the literature for this reaction, so we decided to accept this result and proceed with the next step.
3.1.3. Model studies of the aldol condensation of the C21-aldehyde 95b

In order to evaluate the application of the Kreiser method mentioned earlier for synthesis of the required butenolide it was necessary to study the aldol condensation of a C21-aldehyde. For this purpose we decided to proceed with the 14α-steroid 93. To elaborate this α,β-unsaturated ketone to the required C21-aldehyde, we needed a one carbon aldehyde synthon, and the nitrile group appeared to be the most desirable. Hydrocyanation of the α,β-unsaturated ketone was done using the method developed by Nagata et al. for α,β-unsaturated ketones. Thus treatment of a dimethylformamide solution of the α,β-unsaturated ketone 93 with a water solution of potassium cyanide and ammonium chloride resulted in 80% yield of the nitrile 94. The proton (Figure 7a) and carbon-13 NMR (Figure 8) spectra show that this compound is a single isomer, not a mixture of epimers (at C17) as one might expect.

The nuclear Overhauser effect (NOE) can often be used to determine the configuration at specific centers of organic molecules. The NOE-difference experiment shows only the enhanced signals thereby simplifying the interpretation, and allowing the measurement of very small enhancements. This method can also aid in assigning the chemical shifts in regions of the spectrum where several multiplets overlap. The amount of enhancement decreases rapidly as the distance through space between the two protons is increased (\%\text{NOE} = 1/R^6, \text{where } R=\text{distance}). In the case of CH₃:H interactions this distance must be less than 3.7 Å in order to observe a measurable enhancement. Examination of a molecular model of the C17β-isomer of the nitrile 95 shows that the distance between the C18-methyl group and the C20-protons is much less than 3.7 Å and similarly, if the C17α-isomer is examined.
the distance between the C18-methyl and the C17β-hydrogen is also very small. Therefore irradiation of the C18-methyl signal should enable the determination of the configuration at C17.

Irradiation at 0.75 ppm (C18-methyl group) resulted in the enhancement of a quartet at 2.17 ppm (see Figure 7b) but not the AB quartet at 2.72 ppm (due to one of the C20-protons). Decoupling (see Figure 7c) of the signal at 2.17 ppm resulted in the collapse of the AB quartet at 2.72 ppm to a doublet ($J=4$ Hz) which is consistent with the 2.17 ppm signal being due to the other C20-proton. The observation that one of the C20-proton signals (at 2.17 ppm) is enhanced by irradiation at 0.75 ppm (C18-methyl group) is consistent with the compound having the required C17β-configuration.

The ketone at C16 was protected as the ethylene ketal derivative by heating a toluene/benzene solution of the nitrile 94 with an excess of ethylene glycol and a catalytic amount of p-toluenesulfonic acid for 48 h, with azeotropic removal of water, to yield 95% of the protected nitrile 95a. As above, the stereochemistry at C17 was investigated by NOE-difference spectroscopy (see Figure 9). Irradiation at 0.75 ppm (C18-methyl) resulted in enhancement of both the 2.25 and 2.35 ppm quartets (C20-hydrogens) but not the C17-proton resonance. This result is consistent with the nitrile 95a having the desired C17β-configuration.
Nuclear Overhauser difference and homonuclear spin decoupling experiments on 17β-cyanomethyl-5β-androstane-3,3-ethyleneketal 94 (400 MHz, CDCl₃).

a) off resonance spectrum
b) irradiation at 0.75 ppm
c) homonuclear spin decoupling (irradiation at 2.17 ppm)

Figure 7
$^{13}$C-NMR (75 MHz, CDCl$_3$) of 17β-cyanomethyl-5β-androstane-3,16-dione-3,3-ethyleneketal 94.

Figure 8
The nitrile 95a was reduced to the C21 aldehyde 95b in 66% yield by treatment with diisobutylaluminum hydride at room temperature. This reduction was done at room temperature because at lower temperatures, the reaction would not proceed to completion. Since the nitrile and the aldehyde could not be separated by chromatography on silica-gel, it was important to ensure a complete reaction even at the expense of a lower yield.

With the aldehyde 95b on hand we were ready to study methods for elaboration of the butenolide ring. As mentioned in the introduction, Kreiser et al.\(^1\) have developed a short synthesis of the butenolide ring from a C21-aldehyde. We could, however, not employ this method because of the drastic conditions involved. However, Pettit et al.\(^3\) have shown, that glyoxylate esters can be used in an aldol condensation reaction on pregnane type methyl ketones so we decided to try a variation of this method. In general, glyoxylate acids and esters are quite unstable, and form hydrates very easily (often the free aldehyde can not be isolated). Fortunately, the menthol ester of glyoxylic acid is stable and can be easily prepared in an anhydrous form by "Kugelrohr" distillation although, it too, forms the hydrate rapidly if exposed to moisture. The literature procedure\(^3\) for the preparation of this ester proceeds via an indirect route. Thus, treatment of menthol
Nuclear Overhauser difference experiments on 17β-cyanomethyl-5β-androstan-3,3,16,16-bis(ethyleneketal) 95a (400 MHz, CDCl₃).

a) off resonance spectrum
b) irradiation at 0.75 ppm

Figure 9
bromoacetate with silver nitrate to produce the corresponding nitrate followed by treatment with sodium acetate in dimethylsulfoxide, produces the crystalline menthol glyoxylate monohydrate.

It is known\textsuperscript{a,0}, that the lithium enolates of aldehydes are rather unreactive, but often the potassium enolates are much more reactive, so we decided to make the enolate using potassium hydride. The aldehyde 95b was added to a slurry of potassium hydride in tetrahydrofuran at room temperature, and after 30 min, a tetrahydrofuran solution of freshly distilled menthol glyoxylate was added. After about 2 h, the reaction was quenched by the addition of a solution of ammonium chloride (saturated aqueous). Work-up of this mixture, followed by column chromatography yielded a UV-active compound having the correct molecular weight (from mass spectrometry) of the desired ester 96 (Figure 10), in 32% yield. Repeated attempts to obtain an analytically pure sample were not successful, and the product consistently exhibited a menthol-like odor indicating that the compound was hydrolyzing to produce the hydroxy lactone 97 and menthol (possibly due to acid catalysis by silica-gel). Treatment of this compound with sodium borohydride in methanol resulted in the cardenolide 98 and the trans-ester 99 in yields of 17% and 70%, respectively. Because of the difficulties in working with the hydroxy-lactone we decided to reduce the crude aldol condensation reaction product with sodium borohydride in methanol to isolate the cardenolide 98 and the reduced trans ester 99 directly. Thus treatment of the crude reaction mixture (without work-up) with sodium borohydride in methanol resulted in the cardenolide 98, and the trans ester 99, in 17% and 24% yields, respectively. The trans menthol ester 99 could be hydrogenated over palladium on calcium carbonate and cyclized to produce the saturated lactone 100, thus providing indirect evidence that it was indeed the trans
Results and discussion / 47

Figure 10

96 \( R = \text{Menthol} \)

97

98

99 \( R = \text{Menthol} \)

100

Figure 10
Consideration of this reaction in more detail is interesting. Examination of molecular models leads to the conclusion that the aldehyde enolate would have the most favourable geometry as shown in Figure 11. Also, it is reasonable to assume that attack from the β-face of the enolate is not possible because the bulk of the C18-methyl group blocks this face of the enolate. The menthol glyoxylate would have a preferred conformation with the two carbonyl groups oriented in opposite directions, so that the aldehyde and the ester carbonyls define a plane. If the enolate attacks from below this plane (see Figure 11), and after the reaction the C20,21 bond is rotated so that $E_2$ elimination can occur, (the hydrogen and hydroxy groups are anti-periplanar) then a cis double bond will result. Similarly, if the enolate attacks from above the plane a trans double bond will result. In the transition state leading to the cis double bond, there would be repulsions between the C16-ketal oxygens and the ester carbonyl oxygen. The transition state leading to the trans double bond has steric crowding between the menthol ester and the rings B and C of the steroid. Therefore, relieving the repulsions due to the C16-ketal, and the bulk of the ester should both favour the transition state leading to the cis product.

The above synthesis has provided some experience with the aldol condensation of a C21-aldehyde, and we decided to proceed with the total synthesis of digitoxigenin from thujone.
bottom attack of enolate  

top attack of enolate

cis double bond  

trans double bond

Figure 11
3.2. DIGITOXIGENIN FORMAL SYNTHESIS

3.2.1. Hydrocyanation reaction

The work of Nagata et al.\textsuperscript{1} on the hydrocyanation reaction of some dienones to produce regioselective addition, and in some cases, stereoselective addition, led us to try their procedure using diethylaluminum cyanide. Thus treatment of the dienone 85 in an anhydrous benzene solution, with a solution of the diethylaluminum cyanide in toluene at 5°C, resulted in 50% yield of the desired product. Very slow addition of this reagent (diethylaluminum cyanide) to a dilute benzene solution of the dienone 85, resulted in lower yields of the desired product 86. The best results (75%) were obtained by fast addition of the reagent at room temperature.

The proton NMR spectrum, as well as gas chromatographic analysis, showed that this product 86, consists of a mixture of epimers at C17. The ratio of these isomers was about 2:1, although it was impossible to provide an accurate analysis because the GLC peaks were not well resolved. In similar fashion, the proton signals in the NMR spectrum were also poorly resolved. The NMR spectrum (see Figure 12) of this mixture, shows one of the C20 protons, and a poorly resolved signal is presumed to be due to the same proton of the minor isomer. These data make it difficult to accurately determine the relative amounts of the two isomers.

Extensive attempts to separate these isomers on silica-gel columns and even on reverse phase HPLC columns (CN, C18, and C8) were only partially successful. The proportion of the minor isomer was reduced enough so that it was not detected by NMR (see Figure 13a) but was still detected by gas chromatography, so the physical and spectral data are reported for this mixture.
$^1$H-NMR spectrum (400 MHz, CDCl$_3$) of the mixture of C17-epimers of 17-cyanomethyl-5β,14-ene-3,16-dione-3,3',16,16'-bis(ethyleneketal) 86.

Figure 12
Since the minor isomer was not detected in the NMR spectrum we decided to attempt the determination of the configuration at C17 of the major isomer, using NOE-difference experiments similar to those done on the above compounds 95 and 96a. Irradiation at 1.24 ppm (C18-methyl) (see Figure 13b), resulted in enhancement of a quartet at 2.26 ppm (8%, $J=18.12$ Hz), a doublet of doublets at 2.45 ppm (4%, $J=12.4$ Hz) and a multiplet at 2.49 ppm (6%). Another irradiation at 1.05 ppm (C19-methyl) (Figure 13c) resulted in enhancement (9%) of the 2.49 ppm multiplet, as above, but not the other signals. From these two experiments, and examination of a molecular model one can conclude that the multiplet at 2.49 ppm must be the allylic C8β-hydrogen because this proton is equidistant to the C18-methyl and the C19-methyl. Comparison to the proton NMR spectrum of the cross-conjugated dienone 85, which shows a signal at 2.46 ppm that is assigned to the C8β-hydrogen, confirms this assignment. Examination of molecular models of either 95, 96a, or 86 makes it reasonable, on the basis of steric grounds, to assume restricted rotation about the C17,20 bond, and therefore, unequal coupling constants between the C20-hydrogens and the C17-hydrogen. One of the coupling constants of the 2.26 ppm multiplet is equal to the large coupling of the doublet of doublets at 2.45 ppm. Since the two signals are coupled, and on the basis of their chemical shift, the multiplet at 2.26 ppm is assigned to one of the C20-protons of doublets at 2.45 ppm is due to the other C20-proton. The amount of enhancement of the C20-H signal (8%) is much larger than that of the C17-H resonance (4%), therefore the distance between the C18-methyl group and one of the C20-hydrogens (at 2.26 ppm) is much smaller than the distance to the C17-proton. These results are consistent with the side chain having the β-configuration.
Nuclear Overhauser difference experiments on 17β-cyanomethyl-5β-androst-4-ene-3,3-ethyleneketal 86 (400 MHz, CDCl₃).

- a) off resonance spectrum
- b) irradiation at 1.24 ppm
- c) irradiation at 1.05 ppm

Figure 13
In conclusion, the above studies have provided considerable chemistry relating to the synthesis of the target compound, digitoxigenin, from thujone-derived intermediates. It is clear that the information derived can now be applied to the further elaboration of the thujone-derived steroid analog 85 for completing the total synthesis of the cardenolide. Indeed, other researchers in our laboratory have already shown that 85, via the methodology described in this thesis, is a suitable intermediate for this purpose and the completion of the total synthesis is near at hand.
4. EXPERIMENTAL

4.1. GENERAL

The compounds were characterized by their melting points (with the recrystallization solvents given in brackets) on a Nalge melting point apparatus and are uncorrected, unless otherwise noted. The infrared spectra were recorded on a Perkin Elmer 710, 710B and 1710 (Fourier Transform I.R.) spectrometers in either chloroform solution (using NaCl cells of 0.1 mm path length) or as KBr pellets. The ultraviolet spectra were recorded on a Cary 15 spectrometer in 1 cm quartz cells the extinction coefficients are given in parenthesis and, the wavelength(s) of the maxima in nanometers. The mass spectra were recorded on AEI-MS-9 (low resolution) or KRATOS-MS-50 (high resolution) spectrometers, employing the electron impact ionization method. The $^1$H-NMR spectra were recorded on the following spectrometers: Bruker WH-400, Varian XL-300 or Bruker XHS-270 and the chemical shifts are reported in ppm relative to tetramethylsilane (internal standard). The optical rotations were recorded on a Perkin-Elmer 141 automatic polarimeter in a 10 cm cell at ambient temperature using the solvents and concentrations (in g/100 mL) indicated in parenthesis following the recorded rotation values. The molecular formulae of all the compounds were determined by combustion analysis by Mr P. Borda, Microanalytical Laboratory, University of British Columbia. Small scale column chromatography was performed using columns of silica gel (Merck 60G) with N$_2$ gas pressure to obtain a suitable flow rate. Large scale and more difficult separations were done using medium pressure liquid chromatography on a Waters LC-500 instrument (equipped with "Prep PAK-500/silica" columns). Petroleum ether
refers to the fraction boiling in the range 30-60 °C. Anhydrous tetrahydrofuran was prepared by distillation from a mixture containing sodium and benzophenone. Anhydrous benzene was prepared by distillation from a mixture of benzene and CaH₂. Androst-4-ene-3,17-dione was obtained from the Ujohn chemical company.

4.2. 5β-ANDROSTANE-3,17-DIONE 88b

A solution of androst-4-ene-3,17-dione 6 (5.72 g, 20 mmol) in 2-propanol (500 mL) containing 10% Pd/CaCO₃ (500 mg) was stirred under 1 atm. of H₂ for 5 h. (at 20°C). The catalyst was removed by filtration through Celite which was subsequently washed with methanol. The combined filtrate and washings were concentrated in vacuo to yield a viscous oil. Preparative liquid chromatography using CH₂Cl₂/ethyl acetate [95:5, v/v until 88a eluted, then 80:20 to elute 88b] to yield 1.60g (28%) pure 5α-androstane-3,17-dione 88a and 3.84g (67%) 5β-androstane-3,17-dione 88b.

The physical characteristics of 88a are as follows:
m.p. = 133.5-134.0°C (ligroin) (corrected value) [literature² value 132-134°C (ether/pentane)]. [α]D²⁴ = +116±2.5° (1.0266, CHCl₃) [literature value +105° (0.4-0.8,CHCl₃)]. IR ν max.(CHCl₃): 2930, 2815(C-H,stretch), 1735(C = O,stretch,C17-ketone), 1705(C = O,stretch,C3-ketone) cm⁻¹. ¹H-NMR (400 MHz, CDCl₃)δ: 0.81(1H,m), 0.89(3H,s,C18-H), 1.00(1H,m), 1.05(3H,s,C19-H), 1.21-2.56(20H,m). ms m/z: 288(M⁺), 273, 244, 229, 217. High resolution mass measurement: calculated for C₁₉H₂₈O₂: 288.2089 and found: 288.2093. Elemental analysis calculated for C₁₉H₂₈O₂: C 79.12, H 9.79; found: C 78.96, H 9.69.
The physical characteristics of 88b are as follows:

m.p. = 131.0-132.5°C (ligroin) (corrected value [literature value27 129-131°C ]).

$[\alpha]^D_{25} = +106.2 \pm 2^\circ$C (1.0165, CHCl$_3$) [literature value $[\alpha]^D_{25} = +87.4$ (1.0, CHCl$_3$).

IR $\nu$ max.(CHCl$_3$): 2950, 2875(C-H,stretch), 1725(C17 ketone), 1705(C3 ketone) cm$^{-1}$. $^1$H-NMR (400 MHz, CDCl$_3$)$\delta$: 0.90(3H,s,C18-H), 1.06(3H,s,C19-H), 1.15-2.23(16H,m), 2.32(1H,dt,$\gamma = 4,14$,C2a-H), 2.47(1H,dd,$\gamma = 8,20$,C16a-H), 2.68(1H,dt,$\gamma = 16$,C4a-H). $^13$C-NMR (75 MHz, CDCl$_3$)$\delta$:

13.910(C18), 20.564(C11), 21.865(C15), 22.692(C19), 24.802(C6), 26.408(C7), 31.710(C12), 35.124(C10), 35.173(C8), 35.942(C16), 36.996(C2), 37.202(C1), 41.039(C9), 42.296(C4), 44.233(C5), 47.872(C13), 51.417(C14), 212.632(C3), 220.200(C17). ms m/z: 288(M$^+$), 273, 255. High resolution mass measurement: calculated for C$_{19}$H$_{28}$O$_2$: 288.2089 and found: 288.2086. Elemental analysis: calculated for C$_{19}$H$_{28}$O$_2$: C 79.12 ,H 9.79; found: C 78.86, H 9.80.

4.3. 5$\beta$-ANDROSTANE-3,17-DIONE-3,3-ETHYLENEKETAL 89

4.3.1. Method A

To a solution of 88b (4.3g, 15 mmol) in benzene (200 mL) was added p-TSA (50 mg) and ethylene glycol (0.923 g, 14.9 mmol). The resulting mixture was heated at reflux, with azeotropic removal of water (using a "Dean-Stark" apparatus) for 3 h. The mixture was cooled, diluted with ethyl acetate (300 mL), washed with NaHCO$_3$ (200 mL, saturated aqueous solution), water (200 mL) then dried over MgSO$_4$, filtered, and the solvent removed in vacuo to yield a yellow oil. This residue was purified by preparative liquid chromatography using ethyl acetate/hexanes (1:4, v/v) to give the desired product 89 (3.77 g, 76%) and diketal 90.
(0.58 g, 10%) in addition to the unreacted starting ketone 88b (0.22 g, 5%).

4.3.2. Method B

To a solution of 5β-androstane-3,17-dione 88b (606.71 mg, 2.107 mmol) and 1,2-bis[(trimethylsilyl)oxy]ethane (0.62 mL, 2.5 mmol) in CH₂Cl₂ (3 mL) at -78°C was added trimethylsilyl trifluoromethanesulfonate (8.14 µL, 0.0421 mmol). After stirring the solution for 6 h. at -78°C under Ar atmosphere, the reaction was quenched by the addition of an equal volume of NaHCO₃ (sat. aq.) and allowed to warm up to room temperature. The mixture was diluted with CH₂Cl₂ (30 mL) and washed with NaHCO₃ (3X, 15 mL, sat. aq.), water (15 mL) and dried over MgSO₄. After filtration, the solvent was removed in vacuo to yield a white foam. This crude product was separated on a short silica-gel column using 10% ethyl acetate/petroleum ether to yield the desired monoketal 89 (544 mg, 78.0%), the diketal 90 (47.9 mg, 6.05%) and recovered substrate 88b (20.6 mg, 3.4%).

4.3.3. Method C

To a solution of 2-butanone-2,2-ethyleneketal was added 5β-androstane-3,17-dione 88b (7.08 g, 0.025 mmoles) and p-TSA (107 mg, 5.63x10⁻⁴ moles) and the resulting solution was refluxed for 1.25 h. Sodium bicarbonate (about 500 mg) was added and the excess 2-butanone-2,2-ethyleneketal was distilled off under reduced pressure (water aspirator). The resulting oil was re-dissolved in ethyl acetate (500 mL) and washed with water (3X, 100 mL). After drying over MgSO₄, filtering and concentrating in vacuo a brown oil resulted. Thin layer chromatography of this crude product showed that it contained mainly the monoketal 89 plus some of the corresponding diketal 90, so the crude product
was used in the next reaction, as is. The unreacted diketal (about 2.6 g) was
recovered from the next reaction unchanged, therefore the yield of the ketalization
step was about 70% of the desired 5β-androstane-3,17-dione-3,3-ethyleneketal 89 and
30% of the 5β-androstane-3,17-dione-3,3,17,17-bis(ethyleneketal) 90.

The physical properties of 89 are as follows:
m.p. = 130.5-131.0°C (hexanes). $[\alpha]_{D}^{25} = +96 \pm 4^\circ$ (0.2035, ethanol). IR $\nu$ max. (KBr):
2945, 2980(C-H, stretch), 1725(C17 ketone) cm$^{-1}$. $^1$H-NMR (400 MHz, CDCl$_3$)δ:
0.87(3H, s, C18-H), 0.98(3H, s, C19-H), 1.00-2.04(20H, m), 2.04-2.13(1H, m, C16β-H),
2.43(1H, dd, $\delta = 8.20$, C16α-H), 3.93(4H, s, C3-ketal). $^{13}$C-NMR (75 MHz, CDCl$_3$)δ:
13.855(C18), 20.384(C11), 21.823(C15), 23.076(C19), 25.187(C6), 26.480(C7),
30.082(C1), 31.758(C12), 32.195(C2), 34.761(C10), 35.233(C8), 35.693(C16), 35.904(C4),
40.123(C9), 40.852(C5), 47.820(C13), 51.484(C14), 64.035 and 64.184(C3-ethyleneketal),
109.686(C3), 220.768(C17). ms m/z: 332(M$^+$), 125(C$_7$H$_9$O$_2$), 99(C$_5$H$_7$O$_2$).
High resolution mass measurement: calculated for C$_{21}$H$_{32}$O$_3$: 332.2350 and found:
332.2350. Elemental analysis: calculated for C$_{21}$H$_{32}$O$_3$: C 75.92, H 9.71;
found: C 76.00, H 9.76.

The physical properties of 90 are as follows:
m.p. = 58.0-59.0°C (methanol). $[\alpha]_{D}^{25} = -6.19 \pm 0.7^\circ$ (1.115, CH$_2$Cl$_2$).
IR $\nu$ max. (CHCl$_3$): 2965(C-H, stretch) cm$^{-1}$. $^1$H-NMR (400 MHz, CDCl$_3$)δ:
0.85(3H, s, C18-H), 0.96(3H, s, C19-H), 1.00-2.10(22H, m), 3.80-4.00(8H, m, C3 and C17
ketals). ms m/z: 376(M$^+$), 125(C$_7$H$_9$O$_2$), 99(C$_5$H$_7$O$_2$). High resolution mass
measurement: calculated for C$_{23}$H$_{36}$O$_3$: 376.2614 and found: 376.2614. Elemental
analysis: calculated for C$_{23}$H$_{36}$O$_3$: C 73.47, H 9.65; found: C 73.75, H 9.16.
4.4. 17-METHYLENE-5β-ANDROSTANE-3-ONE-3,3-ETHYLENEKETAL 91

To a bright yellow mixture of methyltriphenylphosphonium bromide (15.70 g, 43.98 mmol) and potassium t-butoxide (5.42 g, 48.4 mmol) in dry THF (60 mL) was added ketone 89 (7.30 g, 22.0 mmol) in dry THF (100 mL). After refluxing for 3 h, the mixture was partitioned between hexanes (400 mL) and water (300 mL). The organic layer was washed with methanol (300 mL, 50% aq.) and brine (300 mL), dried over MgSO₄, filtered and concentrated in vacuo to yield a viscous brown oil. This oil was purified by preparative liquid chromatography using ethyl acetate/petroleum ether (5:95, v/v) to yield 6.86 g (94%) pure 91 as a colorless oil.

The physical properties of 91 are as follows:

b.p. = 220°C (at 0.22 mm Hg). [α]D²⁴ = +38±2° (0.2120, ethanol). IR ν max. (KBr): 2942(C-H, stretch), 1655(C= C, stretch), cm⁻¹. ¹H-NMR (400 MHz, CDCl₃)δ:
0.77(3H,s,C18-H), 0.98(3H,s,C19-H), 1.0-1.98(19H,m), 2.00(1H, t, J = 12, C15α-H), 2.24(1H,m,C16β-H), 2.48(1H,m,C16α-H), 3.94(4H,s,C3-ketal), 4.60(1H,bs,C20-H), 4.63(1H,bs,C20-H'). ms m/z: 330(M⁺), 315, 268, 125(C₇H₉O₂), 99(C₅H₇O₂). High resolution mass measurement: calculated for C₂₂H₃₁O₂: 330.2559 and found: 330.2559. Elemental analysis: calculated for C₂₂H₃₁O₂: C 80.01, H 10.38; found: C 80.20, H 10.50.

4.5. 16α-HYDROXY-17-METHYLENE-5β-ANDROSTANE-3-ONE-3,3-ETHYLENEKETAL 92

To a suspension of selenium dioxide (558 mg, 5 mmol) in CH₂Cl₂ (25 mL) at room temperature was added a solution of t-butylhydroperoxide (1.93 mL, 20 mmol, 70% aq.). The mixture was stirred at room temperature for 1 h. The steroidal olefin 91 (3.30 g, 10 mmol) in CH₂Cl₂ (55 mL) was then added and stirring was
continued for 5 h. The reaction mixture was diluted with diethyl ether (500 mL) and washed with sodium hydroxide (2X, 250 mL, 10% aq.) and water (250 mL). dried over MgSO$_4$, filtered and concentrated in vacuo to yield a white foam. Separation by preparative liquid chromatography using diethyl ether/petroleum ether (3:7, v/v) gave the allylic alcohol 92 (2.59 g, 75%) and the $\alpha,\beta$-unsaturated ketone 94 (0.61 g, 18%).

The physical properties of 92 are as follows:

m.p. = 167.0-168.0°C (ethanol). $\lbrack \alpha \rbrack _{D}^{23} = +2.4 \pm 0.3^\circ$ (2.852, CH$_2$Cl$_2$). IR $\nu$ max. (KBr): 3580(O-H, stretch), 2960, 2980(C-H, stretch) cm$^{-1}$. $^1$H-NMR (400 MHz, CDCl$_3$)$\delta$: 0.77(3H, s, C18-H), 0.97(3H, s, C19-H), 1.06-1.91(20H, m), 1.92(1H, t, $J = 12$, C15$\beta$-H), 3.92(4H, s, C3-ketal), 4.63(1H, bs, C16-H), 4.83(1H, d, $J = 2$, C20-H), 5.02(1H, d, $J = 2$, C20-H').

ms m/z: 326(M$^+$), 328(M-H$_2$O), 313, 125(C$_7$H$_9$O$_2$), 99(C$_5$H$_7$O$_2$). High resolution mass measurement: calculated for C$_{22}$H$_{34}$O$_3$: 330.2499 and found: 330.2503.

Elemental analysis: calculated for C$_{22}$H$_{34}$O$_3$: C 76.37, H 9.91; found: C 76.13, H 9.88.

The physical properties of 93 are as follows:

m.p. = 99.5-100.0°C (isopropanol). $\lbrack \alpha \rbrack _{D}^{25} = -119 \pm 0.3^\circ$ (0.2771, ethanol).

UV $\lambda$ max: 226 nm (4567, ethanol). IR $\nu$ max. (CHCl$_3$): 2950(C-H, stretch), 1720(C=O, stretch, C16) cm$^{-1}$. $^1$H-NMR (400 MHz, CDCl$_3$)$\delta$: 0.94(3H, s, C19-H), 1.01-2.07(19H, m), 2.24(1H, dd, $J = 8$, C15$\beta$-H), 3.94(4H, s, C3-ketal), 5.00(1H, s, C20-H), 5.79(1H, s, C20-H').

ms m/z: 324(M$^+$), 329, 287, 195, 125(C$_7$H$_9$O$_2$), 99(C$_5$H$_7$O$_2$). High resolution mass measurement: calculated for C$_{22}$H$_{33}$O$_3$: 324.2351 and found: 324.2351. Elemental analysis calculated for C$_{22}$H$_{32}$O$_3$: C 76.71, H 9.36; found: C 76.49, H 9.35.
4.6. **17-METHYLENE-5\(^\beta\)-ANDROSTANE-3,16-DIONE-3,3-ETHYLENEKETAL 93**

4.6.1. **Method A**
To a solution of oxalyl chloride (670\(\mu\)l, 7.68 mmol) in CH\(_2\)Cl\(_2\) (15 ml) at -78°C was added a solution of dimethylsulfoxide (1.7 mL, 15.36 mmol) in CH\(_2\)Cl\(_2\) (5 mL) and the mixture was stirred at -78°C for 5 min. A solution of the allylic alcohol 92 (2.39 g, 6.90 mmol) in CH\(_2\)Cl\(_2\) (5 mL) was added at -78°C. After stirring for 15 min, triethylamine (2.85 mL, 2.07 mmol) was added. The solution was then allowed to warm to -20°C and held at this temperature for 3 h. The reaction mixture was diluted with CH\(_2\)Cl\(_2\) (75 mL), washed with HCl (50 mL, 10% aq.), water (50 mL), saturated NaHCO\(_3\) (aq) (50 mL) and once again with water (50 mL). The organic layer was then dried over MgSO\(_4\), filtered and concentrated in vacuo, to yield a yellow-brown foam. This crude product was purified on a short silica-gel column using ethyl acetate/hexanes (1:4, v/v) to yield pure 93 (2.04 g, 86%).

4.6.2. **Method B**
To a solution of pyridinium chlorochromate (1.465 g, 6.796 mmol) and sodium acetate (0.011 g, 0.132 mmol) in CHCl\(_3\) (5 ml) at room temperature was added the allylic alcohol 92 (1.176 g, 3.399 mmol) in 5 ml CHCl\(_3\). The orange reaction mixture immediately turned black due to the precipitation of the reduced reagent. After stirring for 1 h at room temperature, the reaction mixture was added to 50 ml diethyl ether. The black precipitation was removed by filtration through florisil which was subsequently washed with diethyl ether (5X, 5 ml) and the solvents...
removed in vacuo to yield a white foam. This crude product was purified on a short silica-gel column using ethyl acetate/hexanes (1:4, v/v) to yield pure 93 (859.4 mg, 74%).

4.7. 17β-CYANOMETHYL-5β-ANDROSTANE-3,16-DIONE-3,3-ETHYLENEKETAL 94

To a solution of the α,β-unsaturated ketone 93 (2.75 g, 7.99 mmol) in dimethylformamide (83 mL), was added a solution of potassium cyanide (1.56 g, 24 mmol) and ammonium chloride (0.86 g, 15 mmol) in water (17 mL). The resulting mixture was refluxed for 3 h, cooled and diluted with an equal volume of water. The solution was then extracted with ethyl acetate (5X, 100 ml), dried over MgSO₄, filtered, and concentrated in vacuo to yield a brown foam. This crude product was purified on a short silica-gel column using ethyl acetate/hexanes (3:7, v/v) to yield pure 94 (2.72 g, 90%).

The physical properties of 94 are as follows:

m.p. = 162.0-162.5°C (ethanol). [α]₂⁵⁰ D = -126.3 ± 0.1° (1.3796, ethanol).

IR ν max. (CHCl₃): 2950(C-H, stretch), 2275(CN, stretch), 1740(C = O, stretch, C₁₆) cm⁻¹. ¹H-NMR (400 MHz, CDCl₃) δ: 0.77(3H, s, C₁₈-H), 0.99(3H, s, C₁₉-H), 1.12-1.95(17H, m), 2.01(1H, t, δ = 12, C₁₅α-H), 2.12-2.35(4H, m, C₃-ketal), 2.72(1H, dd, δ = 18, 4, C₂₀-H), 3.94(4H, s, C₃-ketal). ¹³C-NMR (75 MHz, CDCl₃) δ:

12.113(C₁₁), 13.158(C₁₈), 20.217(C₁₂), 22.879(C₁₉), 26.242(C₆), 29.886(C₁), 32.493(C₈), 33.678(C₂), 34.550(C₁₀), 35.477(C₄), 37.147(C₇), 37.404(C₂₀), 39.723(C₉), 40.422(C₅), 41.776(C₁₃), 50.066(C₁₄), 58.773(C₁₇), 63.826(C₁₅), 63.885 and
64.016(C3-ethyleneketal), 109.380(C3), 118.434(C21), 214.071(C16). ms m/z: 371(M+), 356, 314, 125(C7H9O2), 99(C5H7O2). High resolution mass measurement: calculated for C23H33O3N: 371.2460 and found: 371.2460. Elemental analysis: calculated for C23H33O3N: C 74.36, H 8.95, N 3.77; found: C 74.24, H 9.02, N 3.99.

4.8. 17β-CYANOMETHYL-5β-ANDROSTANE-3,16-DIONE

-3,3,16,16-BIS(ETHYLENEKETAL) 95a

To a solution of the ketone 95a (1.67 g, 4.5 mmol) in toluene/benzene (1:1, v/v) (200 mL) was added ethyleneglycol (3.1 g, 50 mmol) and p-TSA (50 mg). The mixture was heated at reflux with azeotropic removal of water for 48 h, then most of the solvent (all except about 50 mL) was distilled off. After cooling, the solution was diluted with ethyl acetate (300 mL) and washed with NaHCO3 (100 mL, 10% aq.) followed by water (100 mL). The organic solution was dried over MgSO4, filtered and concentrated in vacuo. The crude product was purified on a short silica-gel column with ethyl acetate/hexanes (3:7, v/v) to yield pure 95a (1.77 g, 95%).

The physical characteristics of 95a are as follows:
m.p. = 149.0-149.5°C (ethanol). [α]25D = +21.4±0.8° (2.9192, CH2Cl2). IR ν max. (CHCl3): 2937(C-H, stretch), 2252(CN, stretch) cm⁻¹. ¹H-NMR (400 MHz, CDCl3)δ:
0.76(3H, s, C18-H), 0.95(3H, s, C19-H), 1.00-2.20(21H, m), 2.25(1H, dd, J= 20, 4, C20-H), 2.35(1H, dd, J= 20, 4, C20-H'), 3.75(1H, q, J= 16, 8, O6-ketal), 3.88-3.97(2H, m, C16-ketal), 4.00-4.06(1H, m, C16-ketal), 3.94(4H, s, C3-ketal). ¹³C-NMR (75 MHz, CDCl3)δ:
11.804(C11), 12.753(C18), 20.376(C12), 23.062(C19), 26.187(C6), 26.518(C7), 30.029(C1), 33.991(C2), 34.675(C10), 34.798(C8), 35.628(C4), 37.775(C15), 39.385(C20), 39.893(C9), 40.729(C5), 41.984(C13), 51.953(C14), 55.422(C17),
63.456(C16-ethyleneketal), 64.028 and 64.156(C3-ethyleneketal), 65.266(C16-ethyleneketal), 115.543(C16), 109.716(C3), 119.635(C21). ms m/z: 415(M^+), 375, 125(C7H5O2), 99(C5H7O2). High resolution mass measurement: calculated for C_{25}H_{37}O_4N: 415.2722 and found: 415.2722. Elemental analysis: calculated for C_{25}H_{37}O_4N: C 72.26, H 8.97, N 3.37; found: C 72.36, H 9.10, N 3.40.

4.9. 20-OXO-5β-PREGNANE-3,16-DIONE-3,3,16,16-BIS(ETHYLENEKETAL) 95b

To a solution of the nitrile 95a (2.61 g, 6.3 mmol) in benzene (50 mL) was added a 1M solution of diisobutylaluminum hydride (in benzene solution) (18.89 ml, 18.9 mmol) at room temperature. The solution was stirred for 0.5 h, then quenched with NaHCO₃ (25 ml, sat. aq.). After diluting with ethyl acetate (50 mL) the layers were separated and the aqueous layer was extracted with ethyl acetate (5X, 25 mL). The combined organic layers were dried over MgSO₄, filtered and concentrated in vacuo to yield a brown oil. The crude product was purified by preparative liquid chromatography on silica-gel to yield pure aldehyde 95b (1.73 g, 66%).

The physical properties of 95b are as follows:
m.p. = 143.0-144.0°C (Hexanes). \([\alpha]_{D}^{25} = -10.7±0.9°\) (1.3567, CH₂Cl₂).
IR \(\nu\) max. (CHCl₃): 2937(C-H, stretch), 1695(C = O, stretch), cm⁻¹.
\(^1\)H-NMR (400 MHz, CDCl₃)δ: 0.74(3H, s, C18-H), 0.96(3H, s, C19-H), 1.03-1.92(9H, m), 2.00(1H, t, J=12, C17-H), 3.67-3.90(4H, m, C16-ketal), 3.93(4H, s, C3-ketal), 9.71(1H, m, C21-H). ms m/z: 418(+), 403, 390, 128, 125(C7H9O2), 99(C5H7O2). High resolution mass measurement: calculated for C_{25}H_{38}O₅: 418.2719 and found: 418.2719. Elemental analysis: calculated for C_{25}H_{38}O₅: C 71.74, H 9.15; found: C 71.92, H 9.27.
4.10. 5β-CARD-20(22)-ENOLIDE-3,16-DIONE-3,3,16,16-BIS(ETHYLENEKETAL) 98

To a suspension of potassium hydride (224 mg, 1.22 mmol, of 22% suspension in oil) in dry THF (6 ml) at room temperature was slowly added a solution of the aldehyde 95b (418 mg, 1mmol) in dry THF (6 ml). After hydrogen evolution had ceased, the turbid yellow solution was stirred for another 15 min, then freshly distilled anhydrous R(-)-menthylglyoxylate (318 mg, 1.5 mmol) in THF (2 ml) was added, and the mixture stirred for 2 h at room temperature. The reaction was then quenched with water (0.5 ml), diluted with methanol (5 ml) and sodium borohydride (190 mg, 5 mmol) was added to the mixture. After 30 min. at room temperature, the excess NaBH₄ was decomposed with acetone (5 ml) and the mixture was concentrated in vacuo. The residue was re-dissolved in ethyl acetate (200 ml) and washed with HCl (200 ml, 1M), NaHCO₃ (100 ml, sat. aq) and water (100 ml). The organic layers were then dried over MgSO₄, filtered and concentrated in vacuo to yield a brown oil (970 mg). The crude product was separated on a short silica-gel column using diethyl ether/petroleum ether (2:3, v/v) to yield the cardenolide 98 (78 mg, 17.0%) and the hydroxy ester 99 (147 mg, 24%).

The physical properties of the cardenolide 98 are as follows:
m.p. = 94.0-95.0°C (Hexanes). [α]²⁵°C = -17.5° (1.2505, ethanol). IR ν max. (CHCl₃): 2932(C-H,stretch), 1747(C=O,stretch) cm⁻¹. ¹H-NMR (400 MHz, CDCl₃) δ:
0.87(3H,c,C18-H), 0.97(3H,s,C19-H), 1.05-2.03(20H,m), 2.64(1H,s,C17-H), 3.72-3.95(4H,m,C6-ketal), 3.93(4H,s,C3-ketal), 4.77(2H,d, / = 2,C21-H), 6.04(1H,s,C22-H).
ms m/z: 458(M⁺), 125(C₇H₉O₂), 99(C₅H₇O₂). High resolution mass measurement:
calculated for C₂₇H₃₆O₆: 458.2970 and found: 458.2969. Elemental analysis:
calculated for C₂₇H₃₆O₆: C 70.74, H 8.30; found: C 70.98 H 8.49.
The physical properties of the ester 99 are as follows:
m.p. = 197.0-198.0°C (Heptane). \( [\alpha]_{D}^{25} = -11.3 \pm 0.7 \degree \) (2.26293, \( \text{CH}_2\text{Cl}_2 \)).

IR \( \nu \) max. (CHCl\(_3\)): 3610, 3415(O-H,stretch), 2950(C-H,stretch), 1700(C=O,stretch) cm\(^{-1}\).

\(^1\)H-NMR (400 MHz, CDCl\(_3\)) \( \delta \): 0.75(3H,d,\( J = 8 \),C\(_7\)'-H), 0.89(6H,t,\( J = 6 \),C\(_9\)'-H + Cl\(_{10}\)'-H), 0.94(3H,s,C\(_{18}\)-H), 0.95(3H,s,C\(_{19}\)-H), 0.80-2.10(29H,m), 2.28(1H,t,\( J = 8 \),C\(_{15}\a\)-H), 3.72-3.97(8H,m,C\(_3\)+C\(_{16}\)-ketal), 4.20(1H,dd,\( J = 16,8 \),C\(_{21}\)-H), 4.47(1H,s,C\(_{17}\)-H), 4.48(1H,dd,\( J = 16,8 \),C\(_{21}\)-H'), 4.78(1H,dt,\( J = 4,10 \),C\(_{3}'\)-H), 6.19(1H,s,C\(_{22}\)-H).

m/s m/z: 614(M\(^+\)), 552, 476, 125(C\(_7\)H\(_9\)O\(_2\)), 99(C\(_5\)H\(_7\)O\(_2\)). High resolution mass measurement: C\(_{37}\)H\(_{58}\)O\(_7\): 614.4196 and found: 614.4196. Elemental analysis:
calculated for C\(_{37}\)H\(_{58}\)O\(_7\): C 72.32, H 9.51; found: C 72.12, H 9.38.

4.11. 17-METHYLENE-5\(\beta\)-ANDROST-14-ENE-3,16-DIONE-3,3-ETHYLENEKETAL 85

A solution of lithium hexamethyldisilylamide was prepared by adding 5.67 ml (9.07 mmol) of a solution of \( n \)-butyllithium (1.6M, hexanes) to a solution of hexamethyldisilazane (3.34 ml, 15.1 mmol) in dry THF (15 mL) at -40°C (CH\(_3\)CN/CO\(_2\) bath), under Ar atmosphere. This solution was then added to a solution of the \( \alpha,\beta \)-unsaturated ketone 93 (2.60 g, 7.56 mmol) in dry THF (15 mL) under Ar atmosphere. After 15 min at -40°C the reaction was quenched with an excess of TMS-Cl (4.8 ml, 38 mmol) and allowed to warm up to room temperature. This solution was then added to a solution of 2,3-dicyano-4,5-dichloro benzoquinone (2.06 g, 9.07 mmol) in CH\(_3\)CN (150 mL) at room temperature. After stirring for 1 h the resulting black solution was evaporated to dryness, re-dissolved in 200 ml ethyl acetate and washed with NaOH (3X, 100 ml, 10% aq) and brine (2X, 100 mL). The solution was then dried over MgSO\(_4\), filtered and concentrated in vacuo to yield a brown foam. This crude product was then purified on a short
silica-gel column using ethyl acetate/toluene (5:95, v/v) to yield 87.6 mg (34%) of the cross-conjugated diene 85 and 1.48 g (57%) of recovered substrate.

The physical properties of 85 are as follows:

m.p. = 145.5-146.0°C (Hexanes). \([\alpha]^{25}_D = +221\pm6^\circ\) (0.2320, \(\text{CH}_2\text{Cl}_2\)).

UV \(\lambda_{max.}: 249\) nm (2.32x10\(^{-4}\), ethanol). IR \(\nu_{max.}\) (KBr): 2921(C-H,stretch), 1692(C\(_\text{C}\),C\(_\text{O}\),stretch), 1647, 1599(C=C,stretch) cm\(^{-1}\).

\(^1\text{H-NMR}\) (400 MHz, CDCl\(_3\))\(\delta: 1.07(3\text{H,c,C18-H}), 1.26(3\text{H,s,C19-H}), 1.32-2.06(16\text{H,m}), 2.46(1\text{H,dt,}J=4,10\text{,C18-H}), 3.93(4\text{H,s,C3-ketal}), 5.21(1\text{H,s,C15-H}), 5.95(2\text{H,s,C20-H}).\)

\(\text{ms m/z: 342(M}\,\,^+\text{), 125(C}\,\,7\text{H}_9\text{O}_2\text{), 99(C}\,\,5\text{H}_7\text{O}_2\text{). High resolution mass measurement: calculated for C}_{22}\text{H}_{30}\text{O}_3: 342.2194 and found: 342.2197. Elemental analysis: calculated for C}_{22}\text{H}_{30}\text{O}_3: C 77.21, H 8.83; found: C 76.92, H 8.72.\)

4.12. 17-CYANOMETHYL-5\(\beta\)-ANDROST-14-ENE-3,16-DIONE-3,3-ETHYLENEKETAL 86a

A solution of the diene 85 (443 mg, 130 mmol) in dry benzene (12 mL) was prepared at room temperature and to this was quickly added 1.30 ml (1.30 mmol) of a solution of diethylaluminum cyanide (1M, toluene). Upon addition of the diethylaluminum cyanide the solution turned deep orange and quickly faded (in about 30 sec) to a light orange color. After stirring for 30 min at room temperature, the reaction was quenched with NaOH (2 ml, 10% aq.) which caused the orange color to disappear. This mixture was then diluted with ethyl acetate (100 mL) and washed with NaOH (2X, 50 ml, 10% aq.), brine (2X, 50 mL), dried over MgSO\(_4\), filtered and concentrated \textit{in vacuo} to yield a white foam. This crude product was purified on a short silica-gel column using ethyl acetate/hexanes (1:4, v/v) to yield 360 mg (75%) of the unsaturated nitrile 86a as a mixture of C\(_{17}\) epimers. GLC analysis of this mixture showed that the ratio of isomers was
about 2:1. Repeated attempts to separate these isomers by column chromatography was only partially successful. It was possible to enrich the mixture in the major isomer enough that the minor isomer could not be detected by $^1$H-NMR (but could be detected by capillary gas chromatography). The data given here are for this mixture.

The physical properties of the nitrile 86 are as follows:

m.p. = 54.0-55.0°C (Hexanes). IR $\nu$ max. (KBr): 2944, 2874(C-H, stretch), 2360, 2342(CN, stretch), 1703(C=O, stretch), 1605(C=C, stretch) cm$^{-1}$. UV $\nu$ max.: 233nm (9.64x10$^3$, ethanol). $^1$H-NMR (400 MHz, CDCl$_3$) $\delta$: 1.05(3H, C18-H), 1.24(3H, s, C19-H), 0.83-2.35(17H, m), 2.26(1H, q, $J$ = 18, 16, C20-H).

2.45(1H, dd, $J$ = 16, 4, C17-H), 2.49(1H, m, C8β-H), 2.92(1H, dd, $J$ = 18, 4, C20-H'), 3.93(4H, s, C3-ketal), 5.80(1H, s, C15-H). ms m/z: 369($M^+$), 125(C$_7$H$_9$O$_2$), 99(C$_5$H$_7$O$_2$).

High resolution mass measurement: calculated for C$_{23}$H$_{31}$O$_3$N: 369.2292 and found: 369.2298.
5. REFERENCES


6. F. Sondheimer, *Chemistry in Britain*, 1, 454 (1965), and references therein.


