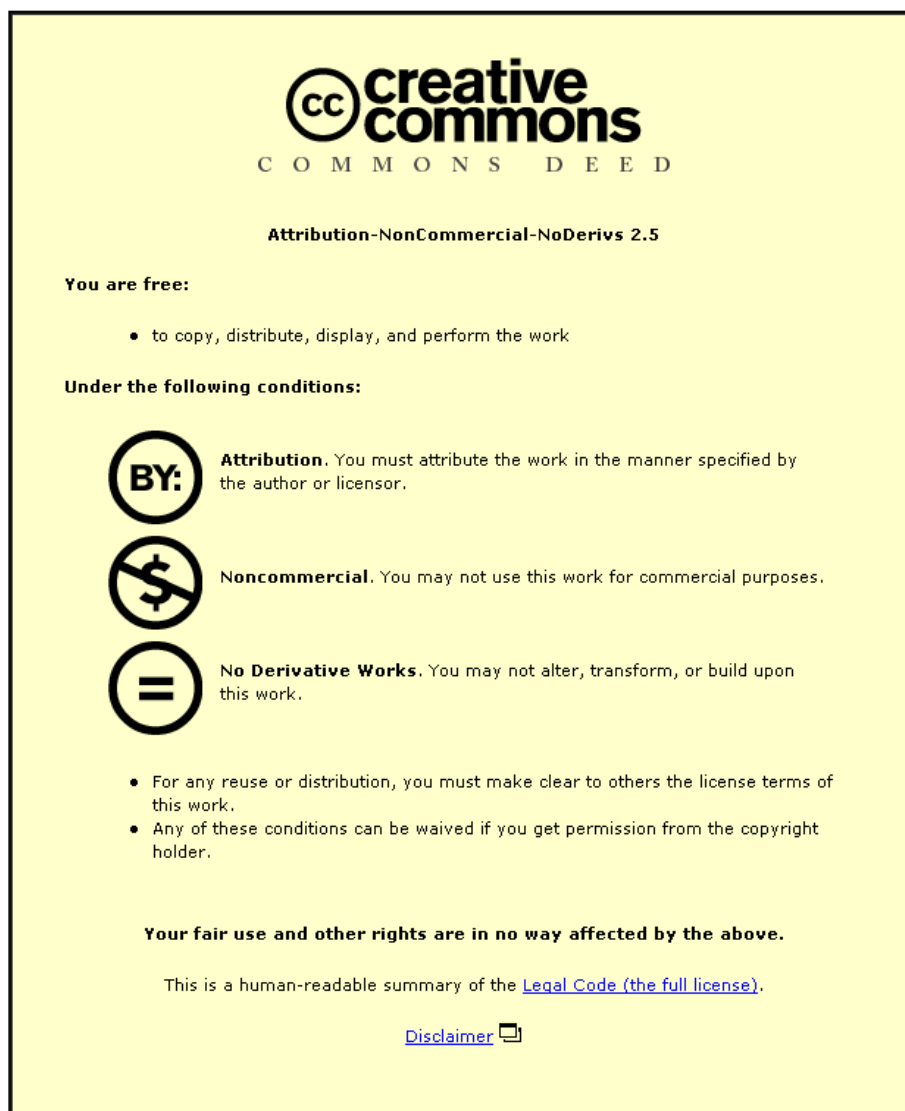


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**SYNTHESIS OF SOME
ANTHRASTEROIDS
AS POTENTIAL
ANTI-TUMOUR AGENTS**

by

Susie Seok Si Toh-Lewis

A thesis submitted in partial fulfillment of the requirements for the award
of

Doctor of Philosophy
of Loughborough University of Technology

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To God Be The Glory!

*If a man
does not keep pace with his companions,
perhaps
it is because
he hears a different drummer.
Let him step
to the music which he hears,
however measured or far away.*

Henry D. Thoreau

***"I devoted myself to study and to explore by wisdom all that is done under
heaven. What a heavy burden God has laid on men!"***

***"What is crooked cannot be straightened;
what is lacking cannot be counted."***

Ecclesiastes

Summary

It has recently been discovered that 1, 25-dihydroxyvitamin D₃, the active form of vitamin D, in addition to its important role in calcium transport activity in bone, intestine and kidney, it is also found to be capable of suppressing cell proliferation and inducing cell differentiation of certain tumour cells such as malignant melanoma, breast cancer, and myeloid leukemia. The utility of 1,25-dihydroxyvitamin D₃ and other vitamin D analogues as drugs in the treatment of these cancers has been restricted in part due to their potent calcemic effects. As a consequence, there has been enhanced interest in the development of structurally modified analogues of vitamin D with high cell differentiating ability and low calcemic effects. Selected hydroxylated anthrasteroids were designed to bind to the vitamin D receptors present in certain cancers, thereby inducing cell differentiation and inhibiting cell proliferation with reduction and/or even elimination of the potent calcemic effects of vitamin D.

Following a proposed scheme, the 1 (10→6) abeo-ergosta-5, 7, 9, 22-tetraen-3-one (**6**), a key intermediate in the synthesis of the target molecule, was synthesized starting with ergosterol. Acetylation of ergosterol gave the acetate, which was protected with 4-phenyl-1,2,4-triazoline-3,5-dione (PTAD). Rearrangement of the adduct with BF₃-ether gave the anthrasteroid-3-acetate. Hydrolysis of the acetate gave the alcohol. Oxidation of the alcohol gave 1 (10→6) abeo-ergosta-5,7,9,22-tetraen-3-one (**6**). The overall yield obtained over the 5-steps was 50 %.

After a number of unsuccessful attempts to functionalise at C-2, we were able to prepare 1(10→6) abeo-2-carbomethoxyergosta-5,7,9,22-tetraen-3-one (**39**), that had allowed access to our target molecules (**59**). Aromatisation of (**39**) with pyrrolidone hydrotribromide (PHT) gave the phenolic ester, followed by methylation with methyl iodide gave the methoxy ester in good yield. Reduction with lithium aluminium hydride gave the methoxy alcohol. Oxidation of the alcohol with tetra-n-propylammonium perruthenate (TPAP) gave the aldehyde in good yield. A 2-C extension *via* a Horner-Wadsworth-Emmons reaction with triethylphosphonoacetate gave the side chain unsaturated ethoxy ester in moderate yield. Catalytic hydrogenation of the ester with Pd/C gave the saturated ester in quantitative yield. Lastly, reduction of the saturated ester with lithium aluminium hydride gave 3-[1 (10→6) abeo-3-methoxyergosta-1,3,5,7,9-pentaen-2-yl]-1-propanol (**59**), our target molecule in good yield.

The target molecule (**59**) was synthesized over 14- steps with an overall yield of 10 %.

List of Abbreviations

| | |
|-------|--|
| BCM | bone calcium mobilisation |
| dba | dibenzylideneacetone |
| DCC | dicyclohexylcarbodiimide |
| DCM | dichloromethane |
| DHT | dihydrotachysterol |
| DIBAL | diisobutylaluminium hydride |
| DHCC | dihydroxycholecalciferol |
| DMF | dimethylformamide |
| DMSO | dimethylsulphoxide |
| DNA | deoxyribose nucleic acid |
| EI | electron impact |
| HCC | hydroxycholecalciferol |
| HMPA | hexamethylphosphoramide |
| HWE | Horner-Wadsworth-Emmons |
| ICA | intestinal calcium absorption |
| IR | infrared |
| MeOH | methanol |
| MMC | magnesium methoxycarbonate |
| m.p | melting point |
| NOE | Nuclear Overhauser Effect |
| NMO | 4-Methylmorpholine N-oxide |
| n.m.r | nuclear magnetic resonance |
| PCC | pyridinium chlorochromate |
| PDC | pyridinium dichromate |
| PHT | pyrrolidone hydrotribromide |
| PTAD | 4-Phenyl-1, 2, 4-triazoline-3, 5-dione |
| PTH | parathyroid hormone |
| R.T | room temperature |
| tBDMS | tert-butyldimethylsilyl |
| THF | tetrahydrofuran |
| TMS | tetramethylsilane |
| TLC | thin layer chromatography |
| TPAP | tetra-propyl ammonium perruthenate |
| UV | ultraviolet |

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Chapter One

1.1 Background

1.1.1 Historical Aspects

It is recognised that vitamin D is not a single compound, but a family of compounds that exhibit antirachitic activity. The most important vitamin D compounds are ergocalciferol (vitamin D₂) and cholecalciferol (vitamin D₃) (Fig. 1).

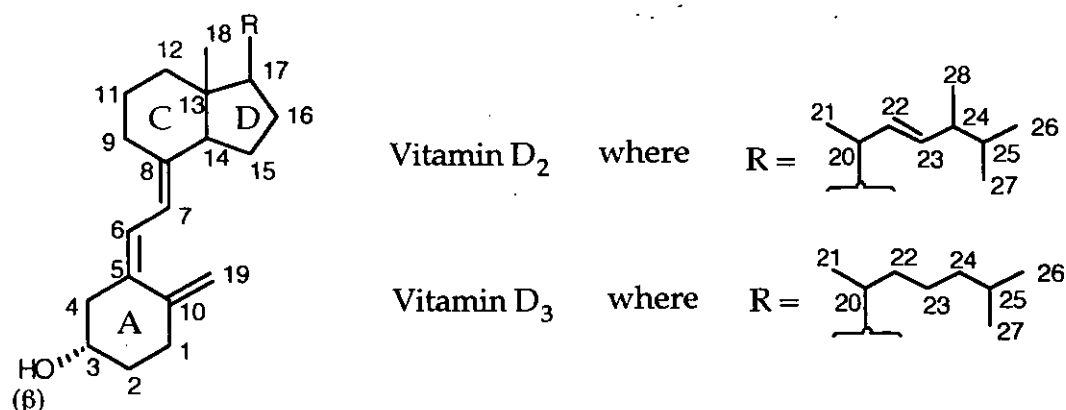


Fig. 1

Between 1931 and 1932, Askew¹ *et al* and Windaus² *et al* both identified the first antirachitic substance, as ergocalciferol. This was derived by irradiation of a plant sterol, ergosterol. In 1937, Windaus³ *et al* synthesised 7-dehydrocholesterol and upon irradiation produced the other major form of vitamin D, namely cholecalciferol.

Physiologically, vitamin D₃ is produced in the epidermis of the skin by ultraviolet irradiation. UV irradiation brings about isomerisation of 7-dehydrocholesterol to previtamin D₃ and other photoisomers, lumisterol and tachysterol. The previtamin D₃ undergoes thermal isomerisation to vitamin D₃ to yield an equilibrium favouring vitamin D₃ (Fig. 2).

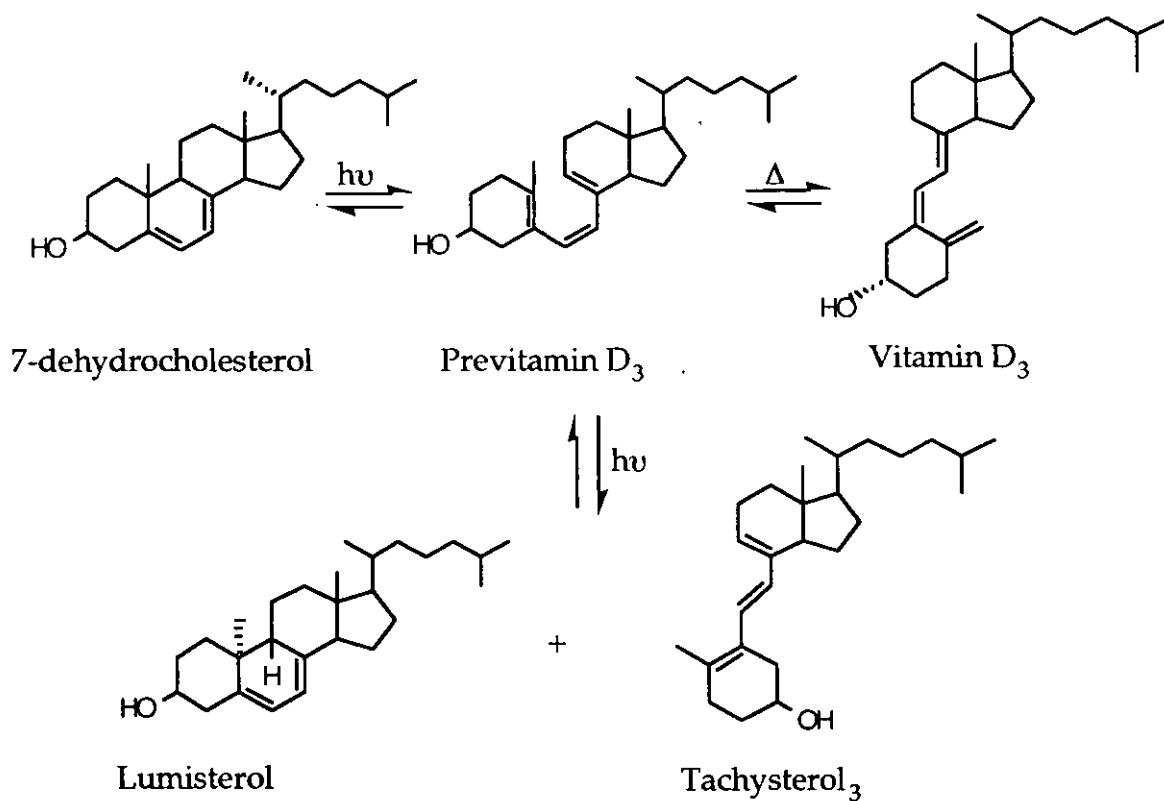


Fig. 2

1.1.2 Vitamin D Pathways

As vitamin D₃ is produced, it is bound by α -globulin, the plasma transport protein, and transported in the blood to the liver. In the liver it undergoes hydroxylation at carbon 25 and is converted to 25-hydroxyvitamin D₃ (25 HCC). 25 HCC is not accumulated in the liver and is transported by the plasma protein to the kidney. In the kidney, 25 HCC is hydroxylated at carbon 1 to yield 1 α ,25-dihydroxylcholecalciferol (1,25 DHCC) which is the active hormonal form^{4,5} of vitamin D (Fig. 3).

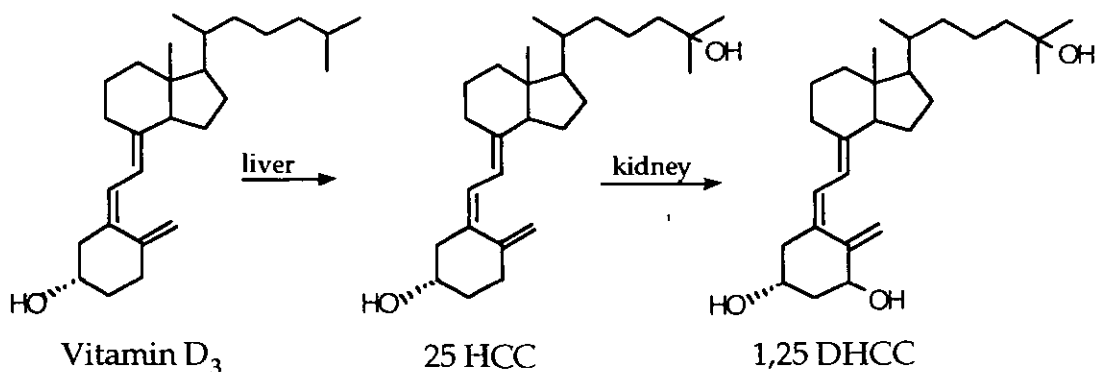


Fig. 3

1,25 DHCC acts as a hormone in the following way, it interacts with hormone receptors at target tissues, the hormone-receptor complex causes the formation of intracellular messenger molecules which stimulate or depress some characteristic biochemical activity of the target cell and, it is regulated by a feedback mechanism.

After formation in the kidney, 1,25 DHCC is then transported to the intestine, to bone and elsewhere in the kidney where it initiates the target organ responses.⁶⁻⁹ In the intestine it stimulates intestinal calcium and phosphate transport. In the bone it stimulates the mobilisation of calcium and phosphorus from the bone compartment to the plasma. In the kidney it stimulates renal reabsorption of calcium. These target organ responses result in elevation of plasma calcium and phosphorus to normal and supersaturated levels that are required for normal mineralisation of bone and for neuromuscular function.

1.1.3 Molecular Mechanism of Action of 1,25 DHCC

Although the precise molecular mechanisms of the steroid hormone still remains unknown, it has been suggested that 1,25 DHCC binds to a protein, i.e. the vitamin D receptor, in the nuclear compartment of target cells, and as a receptor-complex, it initiates target organ responses *via* a nucleus mediated mechanism involving transcription and translation.¹⁰⁻¹⁶ Protein synthesis is involved and the receptor-complex causes transcription of specific genomes that code for calcium and phosphorus transport proteins.¹⁷⁻¹⁹

The vitamin D receptor belongs to a superfamily²⁹⁻³¹ of steroid hormone receptors including those for glucocorticosteroids, progesterone, testosterone, oestrogen, retinoic acid and thyroxine. A schematic diagram (Fig. 4) of a generalised structure of the receptor¹⁷ shows that it is organised into functionally distinct regions, comprising of a variable N terminal region, a short cysteine-rich DNA binding region, and a C terminal which is the ligand binding domain (Fig. 4).

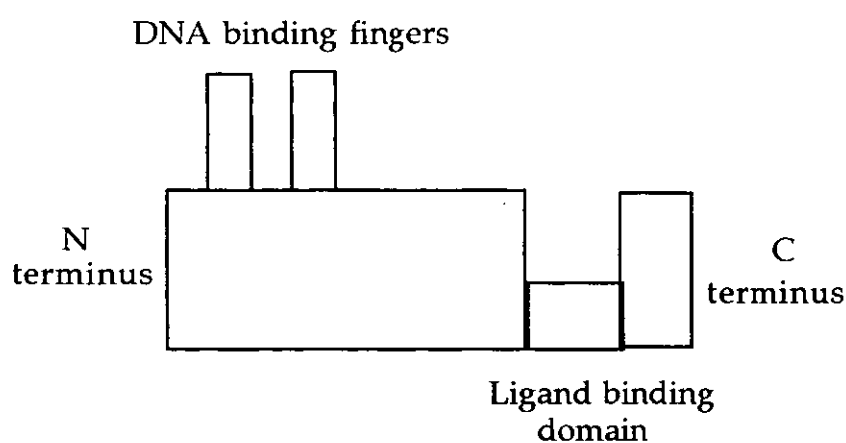


Fig. 4: Vitamin D Receptor

It has also been suggested that 1,25 DHCC may also act on other target organs^{20-28,33} such as malpighian cells of the skin,²⁰ islet cells of the pancreas,^{29,32} endocrine cells of the pituitary, thyroid, thymus, parathyroid cells,³² and some cancer cells.³⁴

1.1.4 Regulation of Vitamin D Metabolism

The production of the hormone 1,25 DHCC is regulated by the need for calcium or the need for phosphorus (Fig. 5). It is markedly dependent upon plasma calcium concentration. This regulation by plasma calcium concentration is mediated by the parathyroid glands. Under conditions of hypocalcemia, parathyroid hormone (PTH) is secreted.

Parathyroid hormone (PTH) has among its other functions the stimulation of renal 25 HCC-1 α -hydroxylase, which causes 1 α -hydroxylation of 25 HCC. The 1,25 DHCC formed then proceeds to the intestine whereby intestinal calcium absorption is stimulated.³⁵ In the bone it acts to allow the bones to respond to PTH to cause mobilisation of calcium from bone. In the kidney, 1,25 DHCC probably acts in a similar manner and stimulates renal reabsorption of calcium.³⁶ The plasma calcium concentration is thereby raised and the feedback mechanism suppresses PTH secretion and thereby shuts down the calcium mobilising system.

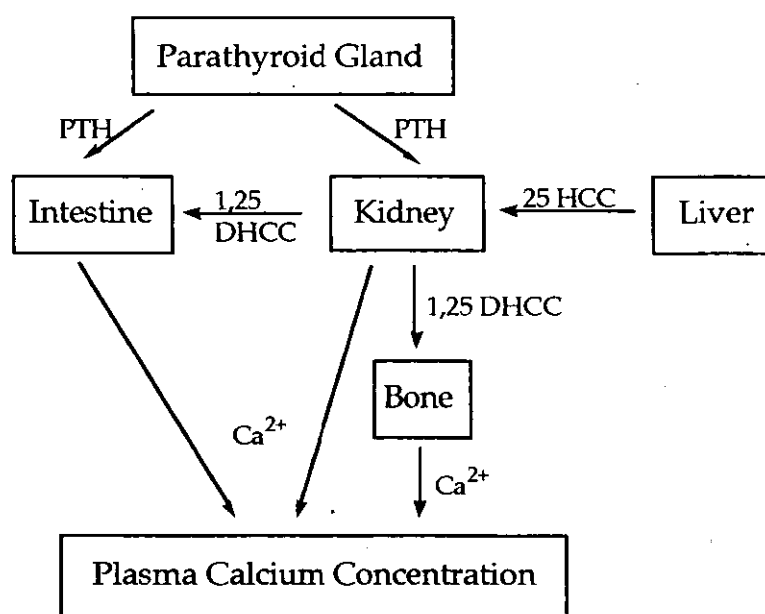


Fig. 5

Besides regulation by calcium and the parathyroid hormone, blood phosphorus has a profound effect on vitamin D metabolism.^{37,38} Low blood phosphorus stimulates accumulation of 1,25 DHCC in the plasma. Whether this is the result of stimulation of the 25 HCC-1 α -hydroxylase or blocking the removal of 1,25 DHCC remains to be determined. The increased levels of 1,25 DHCC results in an increased calcium and phosphorus absorption thereby causing a rise in blood phosphorus.

1.1.5 Disorders in Vitamin D metabolism: Hypercalcemia

Hypercalcemia is seen in a variety of circumstances including hyperthyroidism, vitamin D intoxication, malignant tumours of the breasts and other tissues. Hypercalcemia occurs when the plasma serum calcium level is above 3.0 mmol/litre. This could result from a rise in plasma 1,25 DHCC or chronic ingestion of large doses of vitamin D (150,000 units/day). 1,25 DHCC, in hypercalcemic patients, causes mobilisation of calcium from bone resulting in demineralisation of bone, calcium deposition in the kidney, and inducing metastatic calcification of soft tissues. The symptoms of hypercalcemia include muscular weakness, polyuria, thirst, anorexia, nausea, drowsiness, and confusion.

1.2 Rationale for the use of Vitamin D metabolites and analogues as Anti-tumour agents.

In the past, chemotherapy has improved the survival of many cancer patients, but alternative approaches are still needed for many malignancies.

In most cancer cells, there is a block in their ability to undergo terminal differentiation. The cells remain in the proliferative pool providing a growth advantage over the normal cells. Induction of terminal differentiation of these cancer cells may be an alternative therapeutic approach.

Differentiation therapy is based on the observation that many cancer cells are arrested at an early, immature stage of development and that a number of chemical entities are able to stimulate these cells to differentiate into their mature forms, where upon they stop proliferating. The best known example of this kind of compound is the vitamin A derivatives, the retinoids. Recently, however, vitamin D metabolites and analogues have shown promise in this area of cell differentiation (Table 1).

| Tumour cell | Origin | Proposed biological action of vitamin D metabolites |
|---------------------------------------|---------------|--|
| breast cancer ¹⁵ | human | inhibition of cell growth |
| malignant melanoma ¹⁶ | human | suppression of cell growth |
| myeloid leukemia ^{28,42} | human | suppression of cell growth and induction of cell differentiation |
| non-Hodgkin's lymphomas ⁴³ | human | suppress tumour growth |

Table 1

1,25 DHCC exerts its effects by binding to specific receptors found in classical target organs such as bone, kidney, and intestine. Recently, it has been shown that these specific receptors²⁰⁻²⁸ are distributed in an ubiquitous manner in a variety of tissues in both classical and non-classical target sites namely, skin, muscle, pancreas, pituitary, and cancer cells.^{20-31,39} There has been an increasing body of evidence to support the role of 1,25 DHCC in cellular growth and differentiation processes.⁴⁰

Recent observations⁴¹ showed that 1,25 DHCC was the most potent of the vitamin D analogues to cause cell differentiation of myeloid leukemia cells in mice, followed successively by 1 α HCC, 25 HCC, and 24R,25 DHCC. Subsequently, it was also reported that 1,25 DHCC was also capable of inducing differentiation and inhibit proliferation of a variety of human acute myeloid leukemia cell lines.^{28,42}

It was also shown that addition of 1,25 DHCC in nM concentrations to cell cultures of breast cancer cells, colon cancer cells, prostate carcinoma cells, melanoma cells, leukemic cells and many other cancer cells lines, caused the growth of the cells to be inhibited. In some cell types terminal differentiation was achieved.

1,25 DHCC and some of its synthetic analogues have therefore been shown to suppress tumour growth, inhibit metastasis and prolong survival of experimental animals. Preliminary clinical studies suggest that oral administration of 1,25 DHCC or 1α HCC may be beneficial in treating myelofibrosis, myelodysplastic syndromes and non-Hodgkin's lymphomas.⁴³

Unfortunately, the use of highly active vitamin D₃ derivatives to control cell proliferation and promote cell differentiation is limited by their potent calcemic effects. Hypercalcemia is induced by systemic doses of higher than a few μg per day. Hypercalcemia can also be found in a proportion of patients with malignancies such as carcinomas of the bronchus and breast, and reticuloendothelial disorders. In patients with bone metastases, hypercalcemia is attributed to local release of skeletal calcium by invading tumour at a rate exceeding the renal capacity for its excretion.⁴⁴ Alternatively, hypercalcemia may be caused by humoral factors secreted by the malignant cells which in turn causes mobilization of calcium from bone and prevents renal clearance of excess calcium. For example, myeloid cells secrete a lymphokine which is capable of local bone reabsorption or in reticuloendothelial disorders secrete prostaglandins and interleukin I which causes bone reabsorption.⁴⁵⁻⁴⁷ Some solid tumours such as breast carcinomas also produce prostaglandins which are capable of bone reabsorption. More recently,⁴⁸ several growth factors derived from tumours have been identified and shown to be capable of bone reabsorption.

In view of the widespread distribution of vitamin D receptors in a variety of cancer cell lines, and the ability of some vitamin D metabolites and analogues to regulate growth and differentiation of these cells, the rationale for their use in cancer therapy is therefore well established.

1.3 Structure Activity Relationships

The biological activity of vitamin D₃ and its metabolites, namely 25 HCC, 1,25 DHCC, and others can be attributed to the following component parts; (a) hydroxyl groups on ring A which are associated with intestinal calcium absorption (ICA), (b) orientation of ring A in relation to rings C and D, (c) the side chain, which exerts effect on bone calcium mobilization (BCM).

1.3.1 Minimum Structure for Vitamin D Activity

It has been suggested on the basis of analogue activity studies,⁴⁹ that vitamin D₃ has the minimal structure producing detectable activity without prior metabolism to more active forms.⁵⁰ Vitamin D₃ is approximately 10⁶ times less active than 1,25 DHCC. Other studies have shown that 24-nor-25 HCC is weakly active *in vitro*, but inactive *in vivo* and 26,27-bisnor-25 HCC is weakly active *in vivo*.⁵¹ (Fig. 6). These studies suggest that for minimal detectable activity, 25-OH group is not essential, although the presence of it enhances activity. In all three analogues, the 3-hydroxyl group is present, which suggests that in the absence of any other hydroxyl groups, the 3-OH is essential for detectable vitamin D activity.

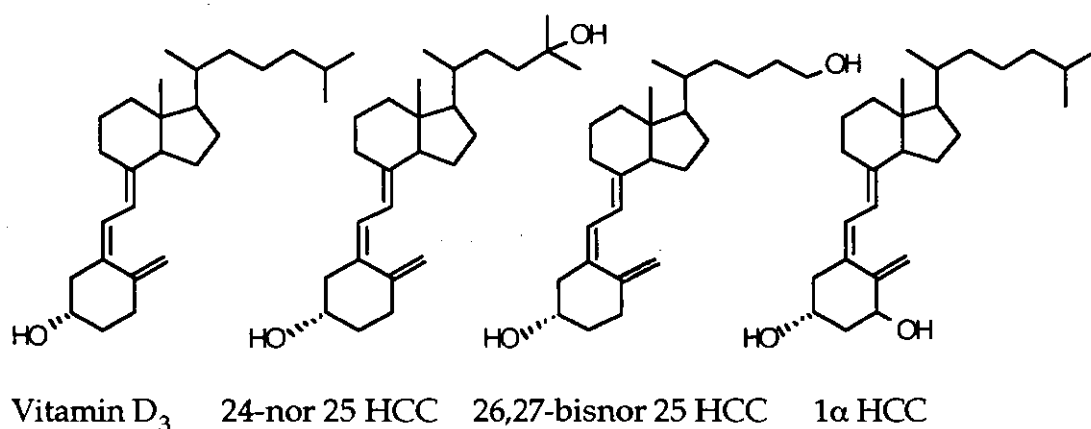


Fig. 6

1α HCC seems to possess the minimal structural requirements for maximal vitamin D activity without prior metabolism to active forms. It is approximately 10² times less effective than 1,25 DHCC in binding to the receptor site and inducing calcium reabsorption.⁵² It appears that the 1α-hydroxyl group provides better binding to the receptor sites and is more effective than vitamin D₃ in competing for receptor binding sites.

1.3.2 Structural Requirements for Receptor Binding

Haussler,^{53, 54} Norman,^{55, 56} and DeLuca⁵⁷ have made comparative studies investigating the binding affinities between various vitamin D analogues (ligands) and the intestinal vitamin D receptors. Binding to receptors is thought to be an essential preliminary step for expression of biological activity.

(a) Hydroxyl Groups

Affinity for the receptor is determined primarily by the spatial arrangement of hydroxyl functions on the ligand. Amongst the three hydroxyl groups of 1,25 DHCC, those at C-1 and C-25 exert the most profound effect. By comparing the binding affinities⁵² of 1,25 DHCC with vitamin D₃, it was shown that 1,25 DHCC was 10⁶ times more active than vitamin D₃. The addition of a 25-hydroxyl group to vitamin D₃ improved the binding affinity by the order of 10² times. The addition of 1-hydroxyl group to vitamin D₃ improved the binding affinity by 10³ times.

The binding affinity is only moderately sensitive to the exact position of the side chain hydroxyl group.^{52,56} 24(R) HCC and 25 HCC both exhibit similar binding affinities, as do 1,24(R) DHCC and 1,25 DHCC (Fig. 7). 24-nor-1,25 DHCC was shown to compete equally with 1,25 DHCC for receptor sites. These studies show that the binding site could accommodate a 24(R) or 25-hydroxyl group with approximately equal facility.

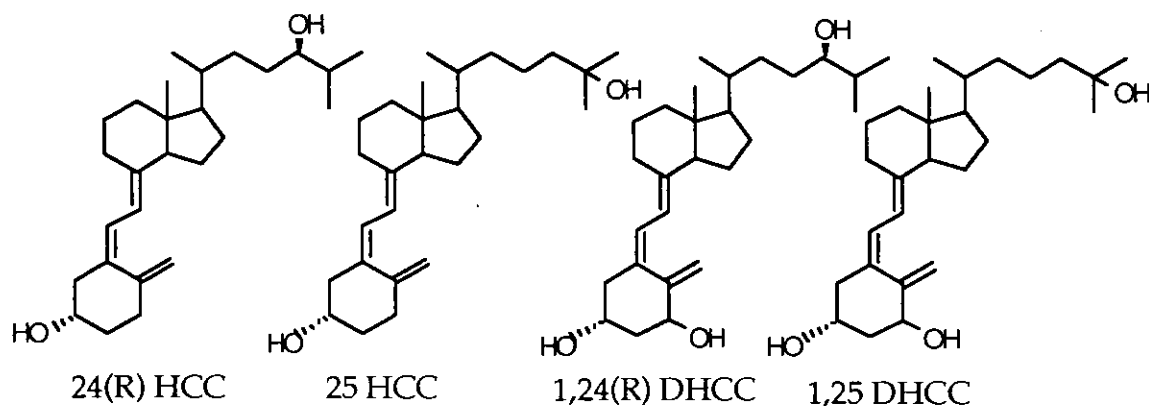


Fig. 7

The stereochemistry of the 1-hydroxyl group is also important in binding affinity. It has been shown that 1β HCC is 10^3 times less effective than 1α HCC in competing for receptor binding sites⁵⁸ *in vitro*, and is inactive *in vivo*. 1-Deoxy compounds such as 25 HCC, and 24,25 DHCC were found to be 10^3 times less active than 1,25 DHCC.

Structurally, dihydrotachysterols (DHT) and 5,6-*trans* vitamin D₃ has its A ring rotated 180° and this places the 3-hydroxyl group in a pseudo 1α -hydroxyl position (Fig. 8). Both these compounds are 10-100 times less active than 1,25 DHCC but are still capable of stimulating intestinal calcium absorption (ICA) and bone calcium mobilization (BCM) in anephric rats.⁵⁹

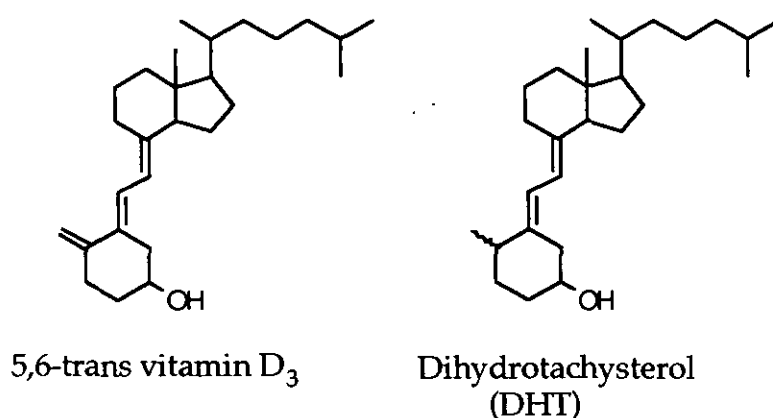


Fig. 8

It has been shown that the 3β -hydroxyl is not an essential functional group as long as there is a hydroxyl group at C-1 position. This is confirmed by studies involving DHT and 5,6-*trans* vitamin D where by a pseudo C-1 hydroxyl is present and also by 3-deoxy-1 HCC (Fig. 9) which were shown to be biologically active. 3-Deoxy-1HCC was found to be capable of stimulating ICA and BCM as well as stimulating growth and calcification of bone, *in vivo*, and is estimated to approximate the potency of 1,25 DHCC *in vivo*, which implies 3-deoxy-1 HCC probably undergoes metabolic conversion to 3-deoxy-1,25-DHCC *in vivo*. Studies involving 3-deoxy-1,25 DHCC also showed that binding affinity when compared with 1,25 DHCC was only reduced by a factor of 10.^{55, 56}

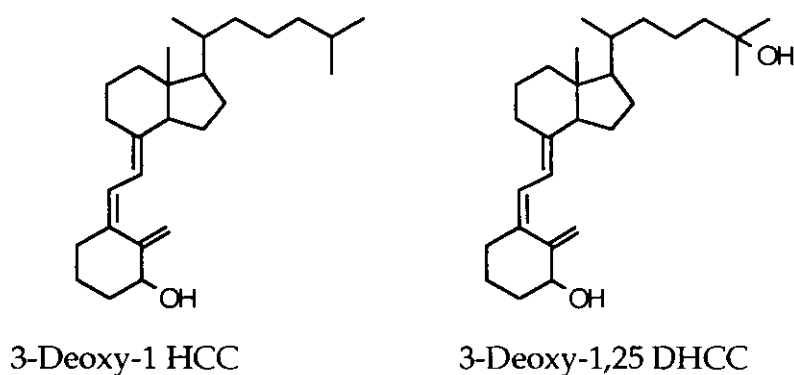


Fig. 9

(b) Orientation of Ring A in relation to Ring C and D

The orientation of the triene system has significant but not dramatic effect on vitamin D activity. 5,6-trans-25 HCC is shown to be 50 times less effective than 3-deoxy-1,25 DHCC in competing for receptor sites⁵⁶ (Fig. 10).

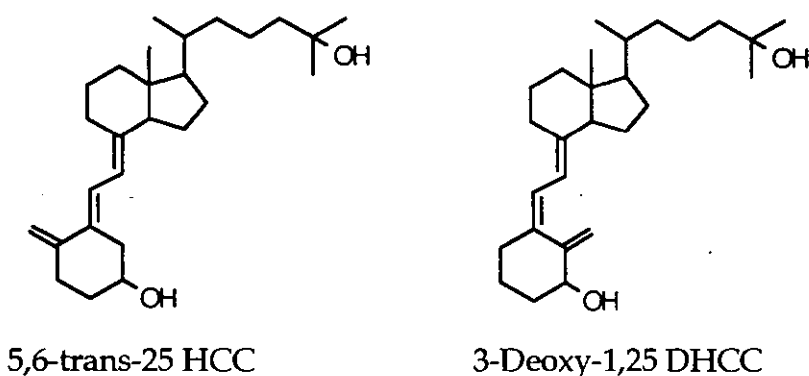


Fig. 10

The 5,6-cis triene and C-19 methylene are not an absolute requirement for recognition by the receptors in the intestine and bone since both 5,6-trans vitamin D and DHT are biologically active too.

(c) The Side Chain

Replacing the vitamin D₃ side chain with the vitamin D₂ side chain, Fig. 1, has no measurable difference in vitamin D activity. This suggests that the exact side chain structure may be of secondary consequence provided the

appropriate chain length is preserved to allow for introduction of a 24(R) or 25-hydroxyl group.

Removal of 1 or 2 carbons from the length of the side chain drastically reduces the biological potency. Studies by DeLuca⁶¹ and Norman⁶² showed that analogues with a side chain of 5, 6, 7, or 9 carbons and a terminal tertiary hydroxyl group were found to have no significant vitamin D activity (Fig. 11).

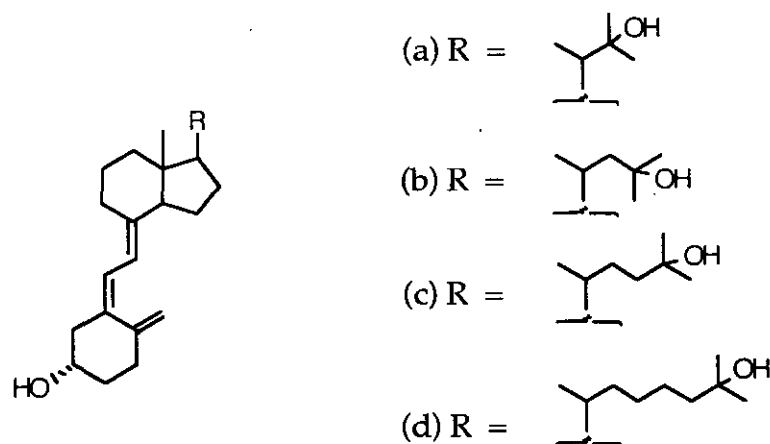


Fig. 11

Replacement of the entire side chain^{61,62} with a hydroxyl group eliminates all activity as seen in pregnacalciferol and, 1-hydroxypregnacalciferol, and pentanor-25-HCC (Fig. 12).

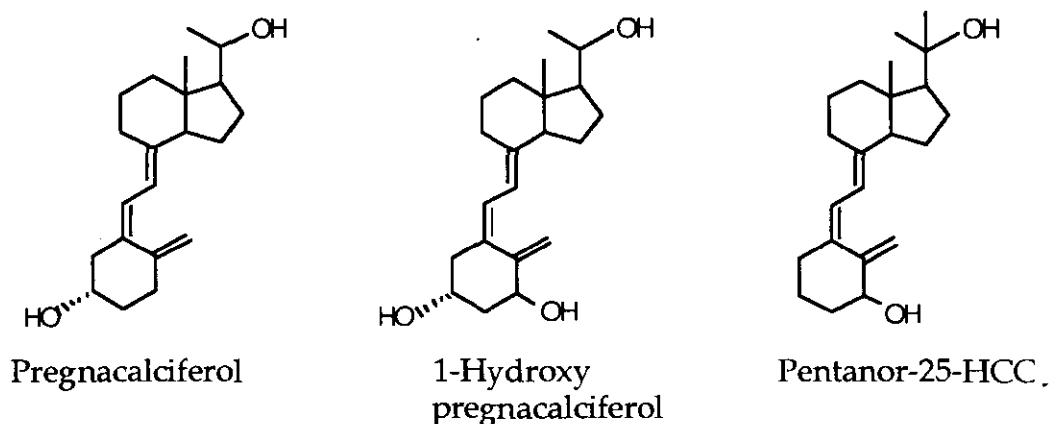


Fig. 12

1.3.3 Summary of Structure Activity Relationships

- (a) 1,25 DHCC is the most active naturally occurring vitamin D₃ metabolite.
- (b) Elimination of C-1 or C-25 hydroxyl groups drastically reduces the binding affinity for the receptor and reduces the calcium absorption ability by 2 to 3 orders of magnitude.
- (c) Elimination of 3 β -hydroxyl group only reduces activity by a factor of 10.
- (d) The stereochemistry of the C-1 hydroxyl group is important as 1 α HCC is 10³ times more active than 1 β HCC.
- (e) Orientation of the triene system has a significant but not drastic effect on vitamin D activity. This is evident in that 5,6-trans vitamin D is about 10 times less active than the 3-deoxy-25 HCC in competing for receptor sites.
- (f) Elimination of the entire side chain and replacement with a hydroxyl group eliminates all activity.
- (g) A shift of the side chain hydroxyl group from C-25 to C-24 has hardly any effect on vitamin D activity, though stereochemistry at C-24 is important.
- (h) Shortening the side chain by 1,2,3 or 6 carbons with retention of terminal tertiary hydroxyl group also eliminates all activity.

1.4 Development of New Vitamin D Analogues

The aim of synthesizing new compounds is to develop compounds that will (a) retain or increase the effects of cell regulatory processes such as cell differentiation and cell proliferation, (b) decrease or eliminate the potent effects of calcium metabolism to avoid toxic calcemic side effects.

In vitro and *in vivo* studies^{41, 43} on 1,25 DHCC and 1 HCC have shown that both compounds are capable of inducing cell differentiation and inhibiting cell proliferation as well as prolonging survival time of mice with tumours. The use of these compounds was limited by their potent calcemic effects. Hence using

these compounds as models, various groups have synthesized a number of promising new analogues, including some potential vitamin D antagonists.

1.4.1 Anti-vitamin D Analogues

A true vitamin D antagonist or anti-vitamin D may be defined as a vitamin D analogue capable of binding to vitamin D receptor without inducing calcium metabolism and other vitamin D responses. It also prevents 1,25 DHCC from binding to the receptor site by occupying or partially occupying the receptor site itself. A number of researchers⁶³⁻⁷² have also used the term vitamin D antagonists to mean antimetabolites, which are not true antagonists in that they do not inhibit vitamin D activity by binding to the receptor site. Instead they act as competitive inhibitors of the vitamin D-25-hydroxylase enzyme. These antimetabolites have a blocked C-25 position, and it was reasoned that the failure of these compounds to be 25-hydroxylated should prevent their subsequent 1-hydroxylation and thereby render them devoid of vitamin D activity. It was further suggested that analogues that are effective 25-hydroxylase inhibitors and are biologically inactive themselves should be antagonists of vitamin D action.

In the following discussion, both views of vitamin D antagonist and anti-vitamin D activity are presented.

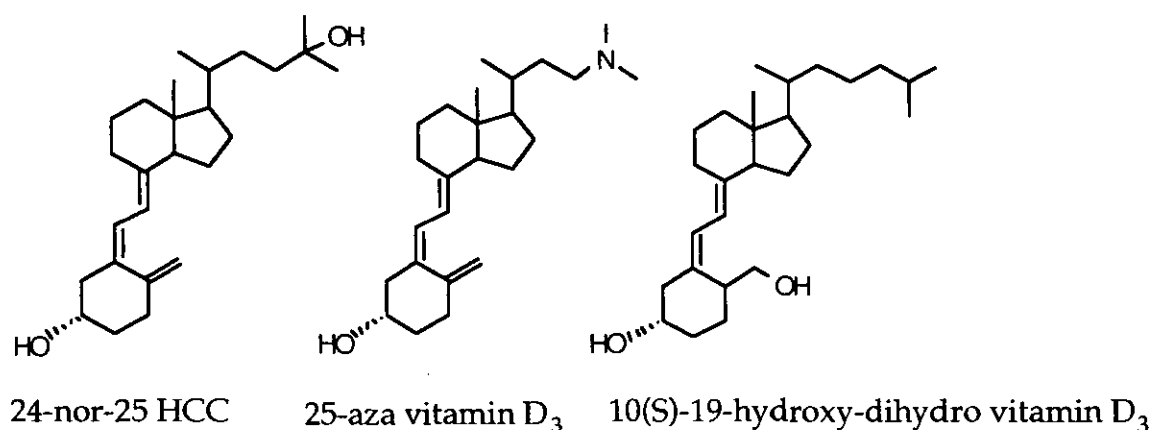


Fig. 13

The first 'anti-vitamin D' compound (Fig. 13) made was 25-aza vitamin D₃.^{56, 63, 64, 71} It acts as a 25-hydroxylase inhibitor, thereby blocking 25-hydroxylation of vitamin D₃. An approximately 1600 fold excess of 25-aza vitamin D₃ is required to block target organ responses to vitamin D, hence making it a poor 'antagonist'.

10(S)-19-hydroxy-dihydrovitamin D₃^{69,70} (Fig. 13) was also found to be an inhibitor of 25-hydroxylase, it is more effective than 25-aza vitamin D₃, but even then it is still a poor 'antagonist'.

24-nor-25 HCC (Fig. 13) was synthesized by Norman⁷¹ *et al* and was found to have no agonist activity *in vivo*, but is weakly active *in vitro*. It was observed to inhibit vitamin D₃ activity, but it does not inhibit vitamin D analogues which were already 25-hydroxylated, such as 25 HCC, and 1,25 DHCC. This suggests that the antagonism of vitamin D activity is mediated by the inhibition of conversion of vitamin D₃ to its metabolically active form in the liver, i.e. 25-hydroxylation is prevented.

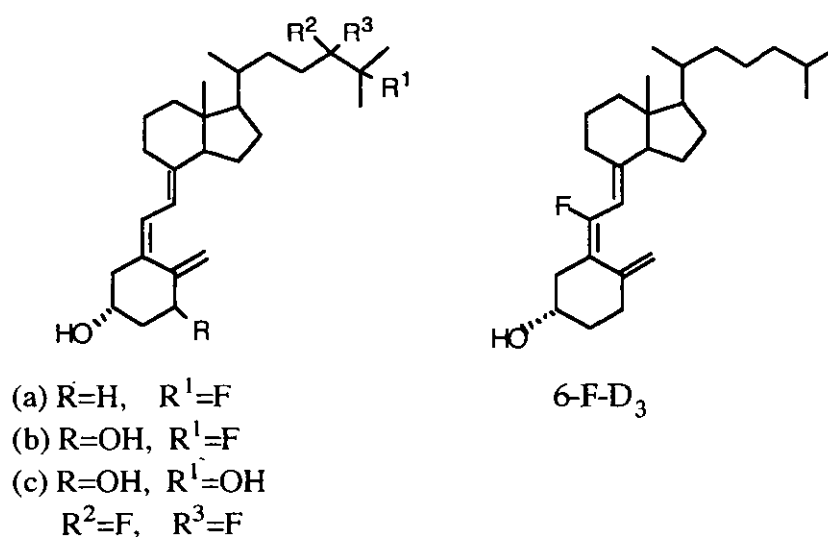


Fig. 14

A number of fluorinated vitamin D₃ analogues were also synthesized⁶⁵⁻⁶⁸ as potential 'antagonists'. 25-F vitamin D₃ (Fig. 14a) was found to be a more effective 25-hydroxylase inhibitor, but metabolism *in vivo* to the 1, 24-hydroxylated derivative renders the analogue biologically active. Other

fluorinated compounds such as 1-hydroxy-25-fluoro vitamin D₃ (Fig. 14b) and 24,24-difluoro-1,25 DHCC (Fig. 14c) were also synthesized but were found to be 5-10 times more potent than 1,25 DHCC *in vivo*. A possible explanation^{66,69} could be that the change in polarity of the molecule brought about significant binding affinity to the receptor.

More recently, a true antagonist, 6-fluoro vitamin D₃ (6-F-D₃)⁷² (Fig. 14) was synthesized and found to show significant direct interaction with the vitamin D receptor site, thereby inhibiting intestinal calcium absorption and bone calcium mobilization. It was suggested that the change in the polarity of the triene moiety by the electronegative fluorine atom at C-6 was responsible for the direct ligand-receptor interaction.

Of all the anti-vitamin D compounds synthesized so far, it seems that 6-F-D₃ may satisfy the requirement of an anti-vitamin but further work is necessary to validate its use in cancer therapy.

1.4.2 New Vitamin D Analogues

Recently Leo Pharmaceuticals⁷³ tested a vitamin D analogue MC 903 (Fig. 15a) for its effects on cell differentiation and cell proliferation *in vitro* using human histiocytic lymphoma cell line U937 and *in vivo* on calcium metabolism in rats. They found MC 903 to be a potent inducer of cell differentiation and an inhibitor of cell proliferation and DNA synthesis. It was also found to be 100 times less potent than 1,25 DHCC in causing hypercalcemia, hypercalciuria and inducing bone calcium mobilization. MC 903 was considered a promising agent for systemic treatment of cancer. From *in vivo* studies however, it was found that the low calcemic effect of MC 903 was mainly related to the rapid rate of metabolic clearance. Therefore MC 903 is now considered more suitable for topical use^{74,75} and today it is developed as an effective therapy for topical treatment of psoriasis.

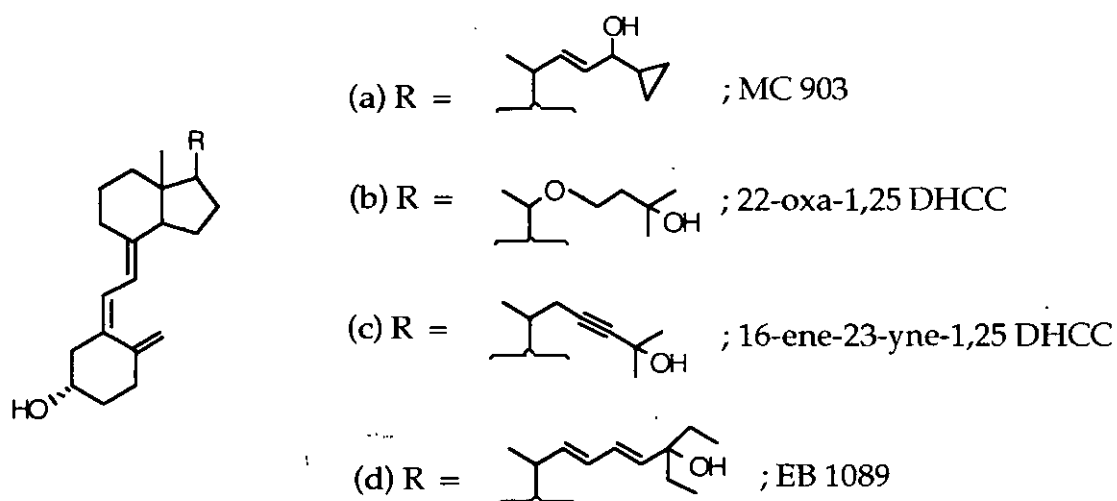


Fig. 15

Development of vitamin D analogues suitable for systemic use in patients with hyperproliferative disease have so far led to three compounds, namely 22-oxa-1,25 DHCC (**Fig. 15b**), developed by Chugai Pharmaceuticals, which is able to delay the growth of implanted breast tumour cells in thymic mice.⁷⁶ The second compound is 16-ene-23-yne-1,25 DHCC⁷⁷ (**Fig. 15c**), developed by Hoffman-La Roche which was shown to prolong survival of mice injected with leukemia cells. The third compound is EB 1089 (**Fig. 15d**), developed by Leo Pharmaceuticals, is able to inhibit the growth of rat mammary tumours induced by the carcinogen nitrosomethylurea.⁷⁸

1.5 Conclusion

From the discussion, it is apparent that the majority of the analogues synthesized so far, have modifications carried out on the side chain of the 9, 10-secosteroid parent molecule. In our research we are concerned with modifications to the ring structure of the molecule, using the anthrasteroid skeleton and modifying the substituents on ring A. In this way, we hope to be able to design potential vitamin D antagonists with anti-tumour activity.

Chapter Two

2.1 Aim of Project

It is our aim to develop new vitamin D₃ analogues with high cell differentiating ability and potent effects on suppressing cell proliferation, but with low risk of inducing vitamin D associated side effects of hypercalcemia, hypercalciuria, and induction of bone reabsorption. In the long-term management of malignant hypercalcemia, the development of vitamin D antagonists may well provide a new dimension of counteracting the oestoclastic activity of bone reabsorption as well as reducing intestinal absorption of calcium by antagonising the intestinal actions of 1,25 DHCC.

2.2 Proposed Synthesis

It has been proposed to synthesize analogues of vitamin D as potential vitamin D antagonists as well as anti-tumour agents. If the analogy of the use of anti-oestrogens in the treatment of breast cancer is considered, then perhaps analogues of vitamin D may act in a similar manner.

Vitamin D antagonists could act in the tumour cells by occupying vitamin D receptor sites, thereby prevent the binding of 1,25 DHCC to the receptors and blocking end organ responses. We propose to design antagonists that would mimic the actions of 1,25 DHCC of inducing cell differentiation and suppressing cell proliferation, without exhibiting potent calcemic effects. Alternatively, they may act as anti-tumour agents via some direct lethal effect of the receptor-complex itself.

Following from the previous discussion on structure activity relationships, we proposed to synthesize some selected hydroxyanthrasteroids (11), (28), and (33) shown in Fig. 16, as suitable substrates designed to bind to vitamin D receptors found in a number of cancer cell lines.

Using the Sybyl 6 computer programme, with initial optimisation using the Simplex method, followed by energy minimisation using the Powell method to carry out three dimensional modelling (Fig. 17), it is possible to view the similarity, especially of the ring structure, of 1,25 DHCC with that of the proposed anthrasteroid target molecules, (11), (28), and (33). The near planarity of the ring structure is maintained by the aromatic ring B of the anthrasteroids.

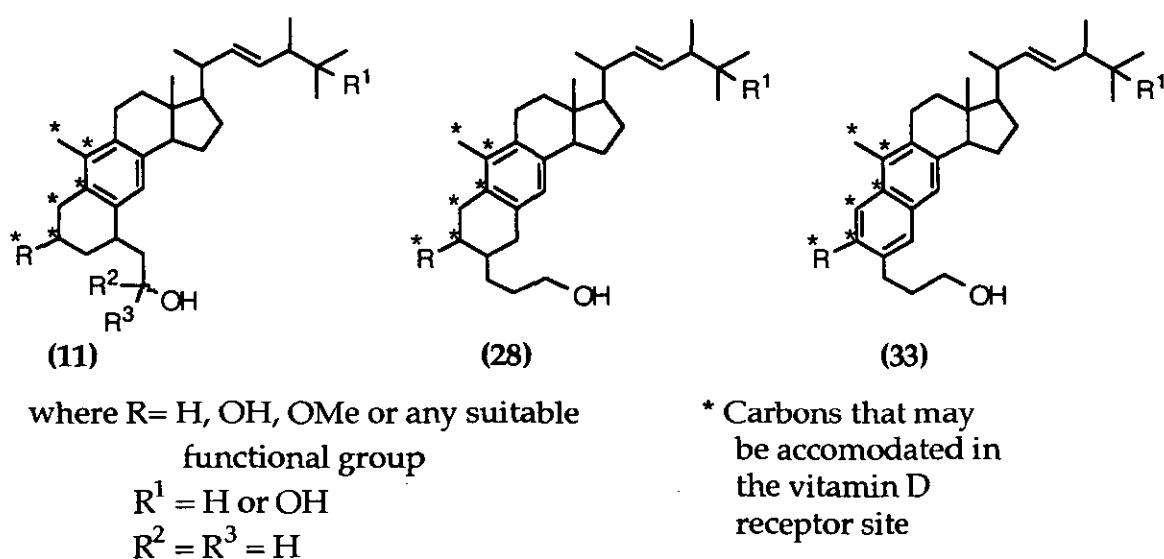


Fig. 16

The important structural features that the hydroxyanthrasteroids have in common with 1,25 DHCC are (a) the hydroxyl groups are in the key positions, and (b) the near planarity of the molecule (Fig. 17). As seen from structure-activity relationship studies on 5,6-trans vitamin D and dihydrotachysterol (DHT), Section 1.3.2(b), it appears that the transposition of C-10 and C-19 methylene group is not an absolute requirement for recognition by the receptor. This suggests that the carbon atoms C* may be accommodated in the receptor sites.

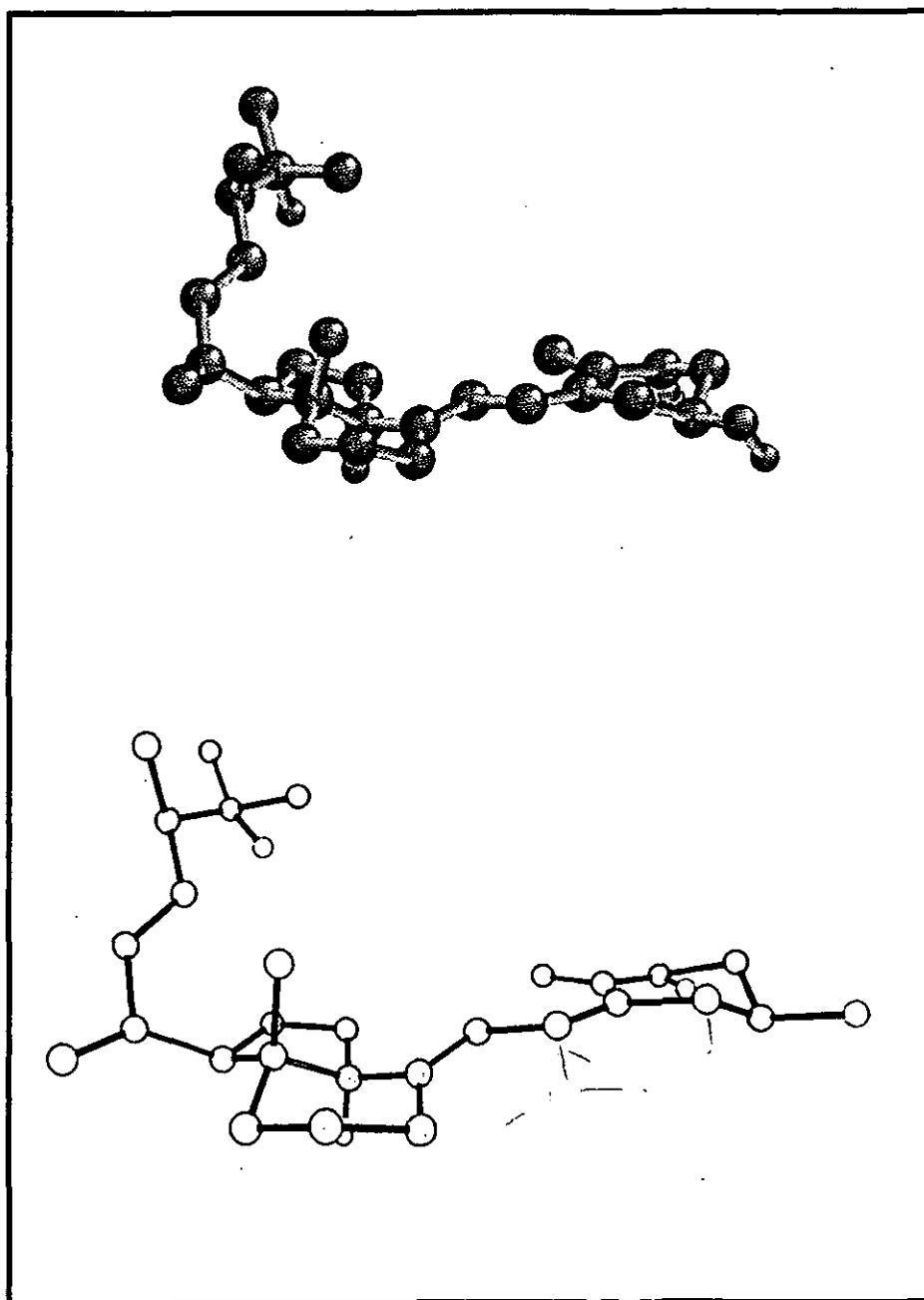


Fig. 17a: Three dimensional model of 1,25 DHCC

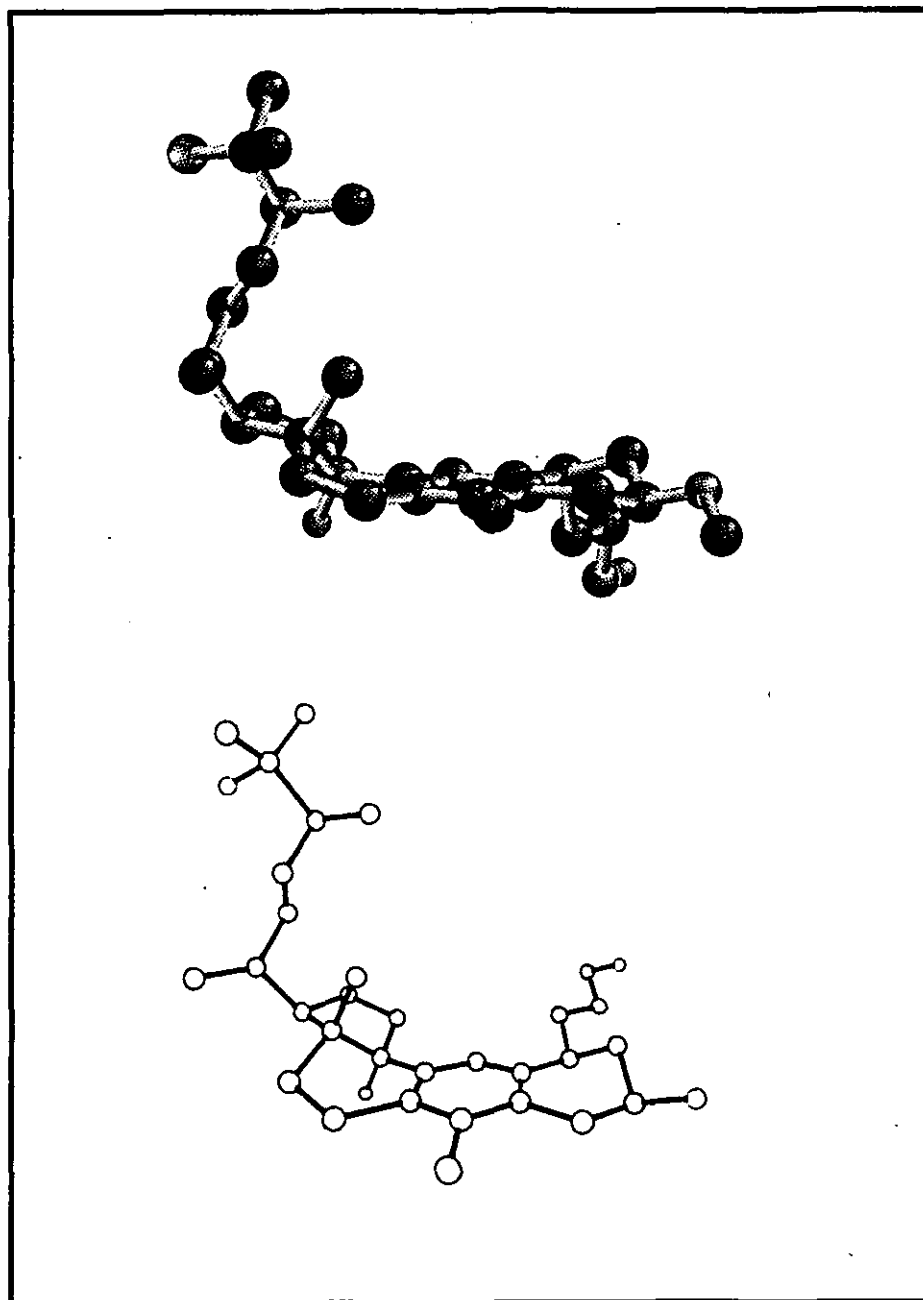


Fig. 17b: Three dimensional model of proposed anthrasteroid (11)

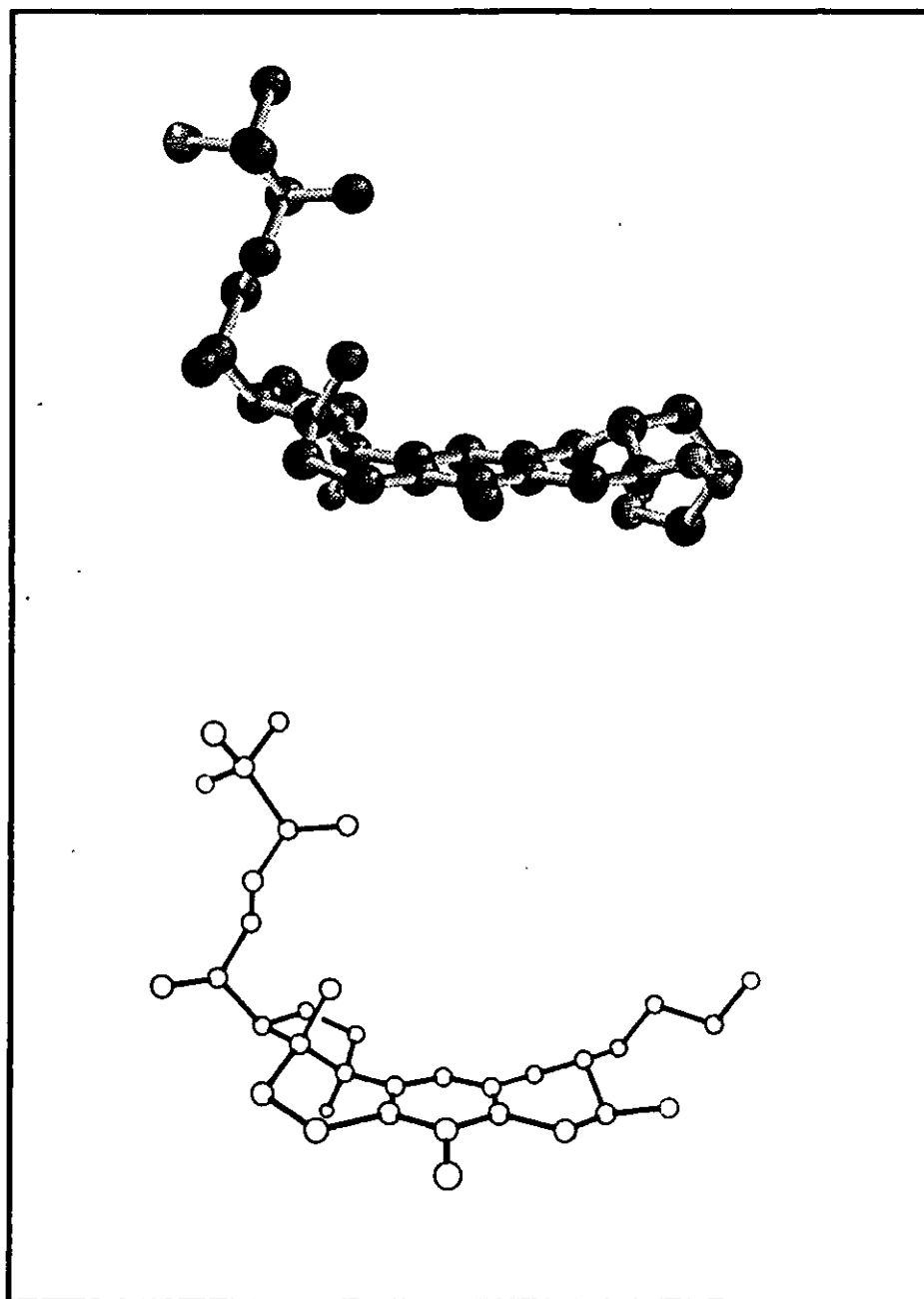


Fig. 17c: Three dimensional model of proposed anthrasteroid (28)

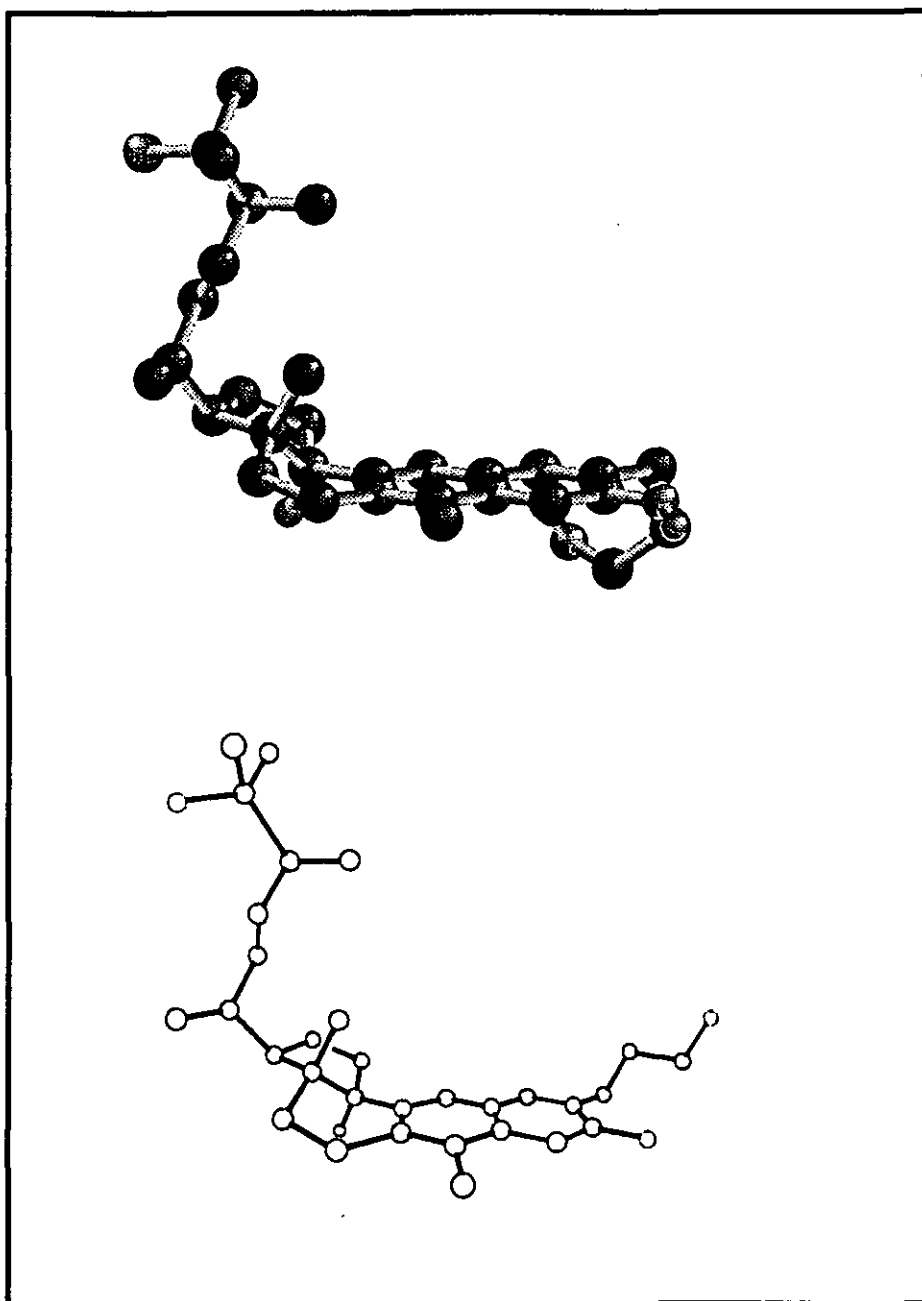
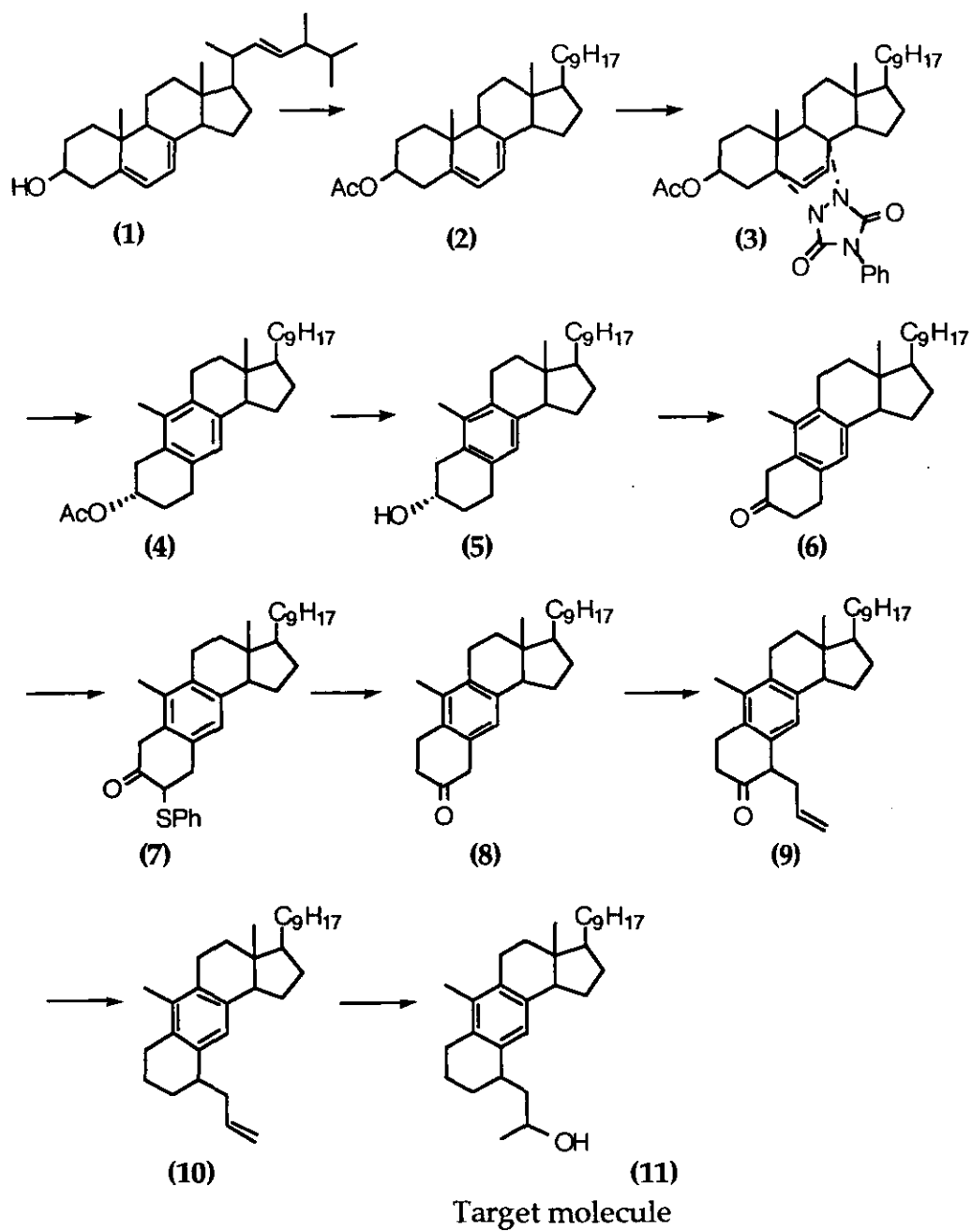


Fig. 17d: Three dimensional model of proposed anthrasteroid (33)

Our initial approach to target **(11)** is shown in **Scheme 2.1**.

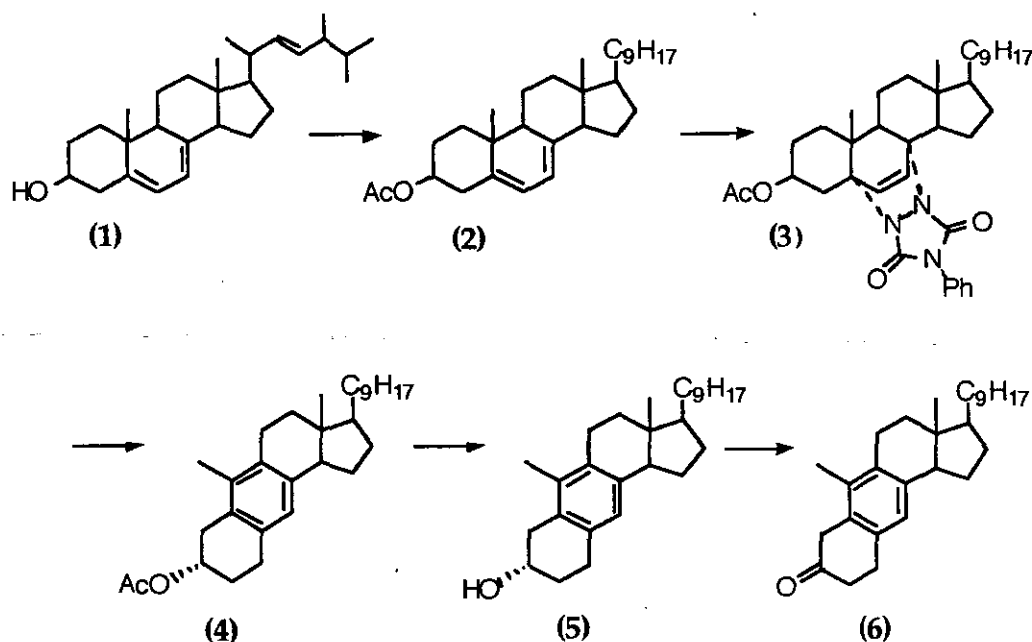


Scheme 2.1

Chapter Three

3.1 Introduction

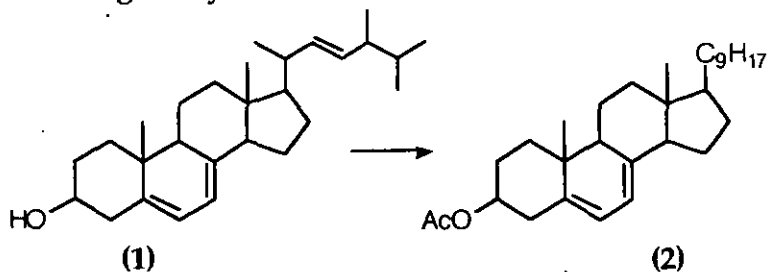
The anthrasteroid ketone (**6**) is a key compound in the proposed route, and its synthesis is shown in **Scheme 3.1**. This route essentially follows that proposed by Whalley⁸² for the cholesterol series, with a few modifications to improve the yields obtained at each intermediate step. The ketone (**6**) serves as an important intermediate from which further modifications on ring A can be carried out. It was important to be able to synthesize the ketone in very good overall yield and the use of 4-phenyl-1,2,4-triazoline-3,5-dione (PTAD), introduced by Whalley, gave access to the anthrasteroid molecule [i.e. (**3**) → (**4**)] in good yield.



Scheme 3.1

Ergosterol was chosen as the starting material because it is relatively cheap and the side chain C-22 alkene would allow for suitable modification at a later stage.

3.2 Synthesis of Ergosteryl Acetate (2)

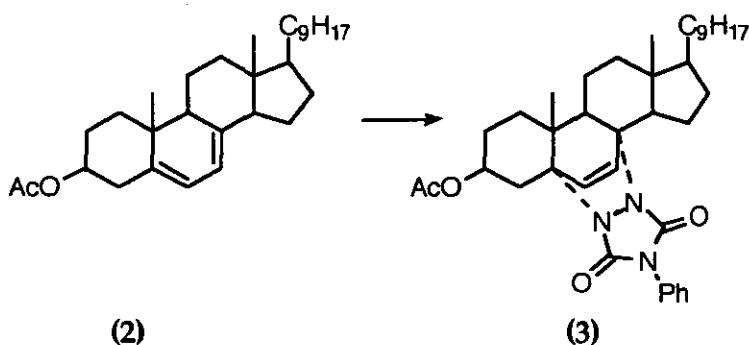


Scheme 3.2

Ergosteryl acetate (2) was prepared in quantitative yield by acetylating ergosterol with acetic anhydride and pyridine, Scheme 3.2.

The crude acetate (2) was recrystallised from boiling methanol. The melting point was 170 - 173°C (lit.,⁷⁹ 173-175°C). The yield was quantitative. From the IR spectrum, the carbonyl stretching frequency for the acetate (2) appeared as a sharp absorption peak at 1734 cm⁻¹. The ¹H n.m.r spectrum of (2) showed a three-proton singlet at δ 2.02, which corresponded to the methyl protons in the acetate group.

3.3 Synthesis of 3', 5'-Dioxo-4'-phenyl-5 α , 8 α -[1', 2'] 1', 2' 4'-triazolidinoergosta-1, 6, 22-trien- 3 β -yl Acetate (3)



Scheme 3.3

The protective group, PTAD⁸⁰ was prepared by dissolving 4-Phenylurazole in dioxan and treating it with *t*-butylhypochlorite. The crude product obtained was further purified by sublimation on a cold finger, to give red carmine needles. Reacting the acetate (2) with PTAD,⁸¹⁻⁸³ gave the Diels-Alder adduct (3) in quantitative yield, Scheme 3.3. The melting point of the compound recrystallised from methanol was 172-175°C (lit.,⁸¹ 173 - 175°C).

The spectroscopic data for (3) was consistent with literature values⁸¹ for analogous adducts. The IR spectrum of the adduct (3) showed two sharp absorption peaks at 1736 cm⁻¹ and 1757 cm⁻¹, which corresponded to the stretching frequencies of the two carbonyl groups in the triazoline ring. The other stretching frequency at 1707 cm⁻¹ was assigned to the carbonyl group of the acetate. An AB quartet was observed at δ 6.21 and δ 6.40, with a coupling constant J 8 Hz, this signal was assigned to the olefinic protons at C-6 and C-7. The chemical shifts for these two olefinic protons have been shifted downfield. This could easily be explained by the anisotropic effect of the neighbouring amide carbonyl group of the triazoline ring which has a deshielding effect on the C-6 and C-7 protons. The multiplet centred at δ 7.37 was assigned to the five aromatic protons in PTAD.

Anthrasteroid Rearrangement

3.4 Synthesis of 1 (10→6) abeo-3 α -Acetoxyergosta-5, 7, 9, 22-tetraene (4)

3.4.1 Introduction

Initial studies on the transformation of certain steroids to anthrasteroids were carried out by Nes and Mosettig⁸⁴ in 1953. In the past⁸⁵⁻⁸⁸ anthrasteroids were synthesized from unsaturated steroids, steroidal alcohols and ketones of the cholesterol and ergosterol series by vigorous treatment with acidic reagents. The yields obtained from these acid-catalysed reactions were between 10-40%, which was deemed unsatisfactory. Anthrasteroids were also made by total synthesis⁸⁹ starting with 6-methoxytetralone, the overall yields obtained from this method were just as poor, between 10-20 %.

More recently, a much superior route of synthesizing anthrasteroids from steroidal 5,7-dienes was reported by Whalley^{82,83} *et al.* Our proposed scheme of synthesis of the key intermediate, the anthrasteroid-3-acetate (4), is largely based on this route. Although we have, in the course of synthesis, made a few modifications to the route to further improve the yields obtained.

3.4.3 Mechanism of transformation

It has been proposed^{82,90} that the transformation involves an oxidative rearrangement, Fig. 18. The rearrangement proceeds *via* the intermediate formation of a spiran compound (3b). The inductive effect of the acetoxy group causes the 1(5)- bond to be more prone to shift towards the electron deficient C-6 centre, thereby forming the 3 α -substituted product (4).

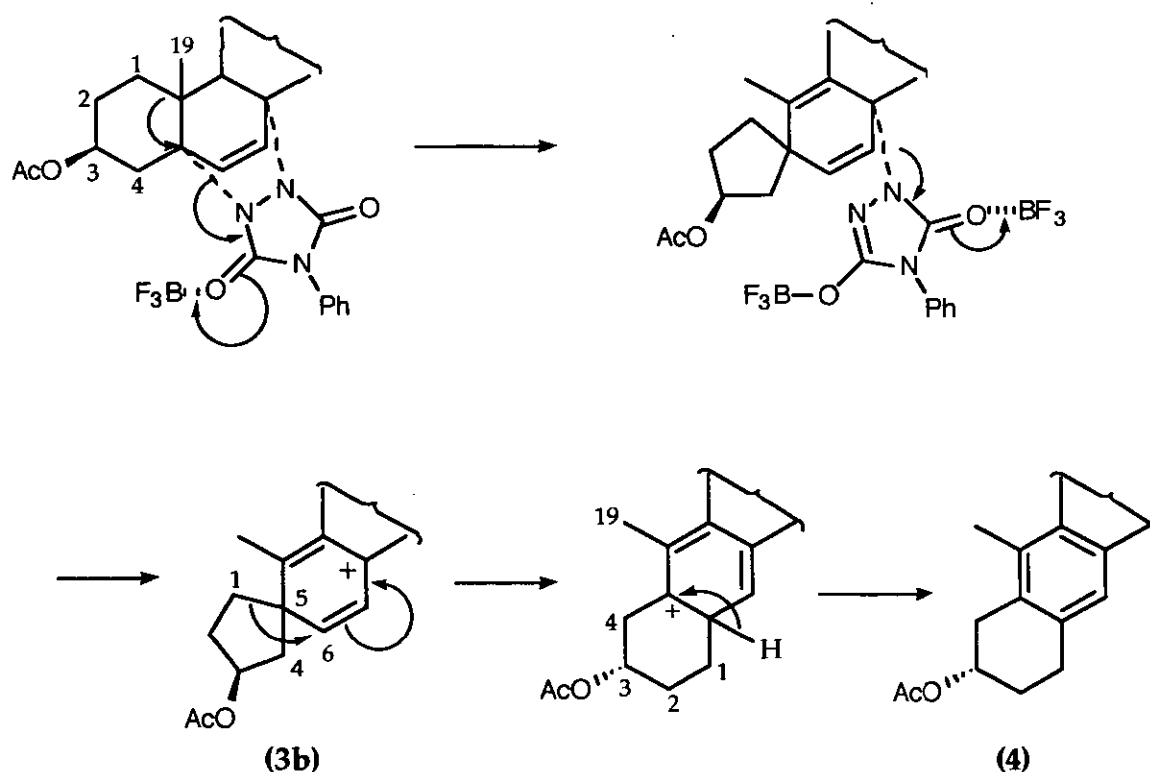
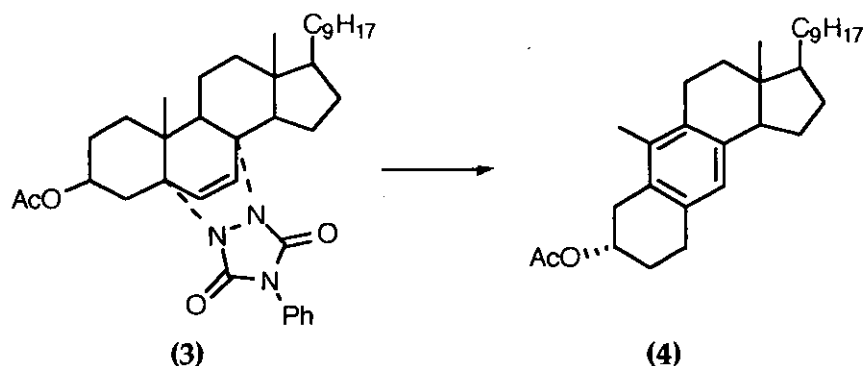


Fig. 18

Alternatively, the rearrangement could have proceeded with the 4(5)-bond of the spiran (3b) shifting to C-6, resulting in a 2 α -substituted product instead. Whalley⁸² *et al* deduced that the mechanism of the rearrangement had proceeded by shifting of the 1(5)-bond of the spiran compound to give the 3 α -substituted product (4) by identifying the end product and proving the structure by X-ray crystallography, as well as studies involving n.m.r spectroscopy and circular dichroism data.⁹¹ Our own spectroscopic evidence supports this mechanism.

3.4.2 Synthesis

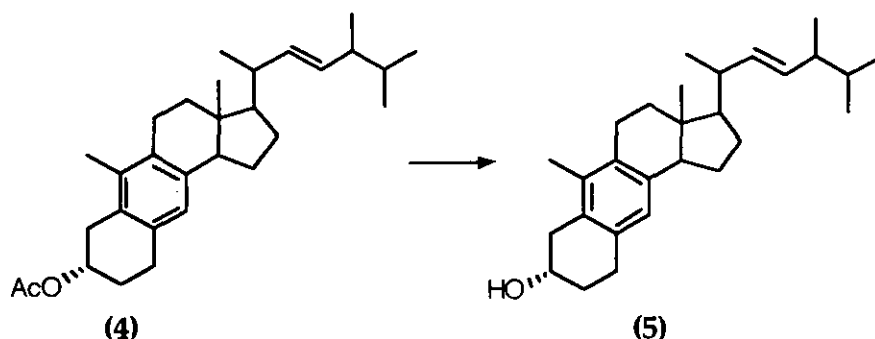


Scheme 3.4

Treatment of the PTAD adduct (3) with a boron trifluoride-ether complex⁸² gave the anthrasteroid acetate (4) in 70 % yield. The acetate was recrystallised from methanol, afforded white crystals with melting point of 80-82°C. The elemental analysis obtained for (4) was consistent with the molecular formula C₃₀H₄₄O₂.

The spectroscopic data was consistent with the structure of the anthrasteroid-3-acetate (4). The IR spectrum showed a sharp absorption peak at 1740 cm⁻¹ which corresponded to the acetate group. The ¹H n.m.r spectrum showed two singlets at δ 2.04 and δ 2.07, each integrating for three protons, corresponding to the methyl protons of the acetate group at C-3 and at C-10, both signals were shifted downfield compared with the adduct (3), where the chemical shifts for the acetate group and 10-Me were at δ 2.00 and δ 0.97, respectively. The aromatic proton at C-7 appeared as a singlet at δ 6.65. From the 400 MHz proton spectrum, the multiplet at δ 5.22 was assigned to the C-3 proton. The NOE experiments showed that the 3-H was probably in the equatorial position (i.e. β face), coupling with both C-2 and C-4 protons, where J=5 Hz, . Thereby suggesting that the acetate group at C-3 is in the α position. The mass spectrum by electron impact (EI) gave *m/z* of the major fragment as 376 which corresponded to the molecular ion having lost the acetate group. The accurate mass for C₃₀H₄₄O₂ was found to be 436.3342.

3.5 Synthesis of 1 (10→6) abeo-3 α -Hydroxyergosta-5, 7, 9, 22-tetraene (5)



Scheme 3.5

Alkaline hydrolysis of the anthrasteroid-3-acetate (4) gave the anthrasteroid-3-ol (5) in good yield, 90 %. Previous workers⁸² were unable to obtain the 3 α -ol (5) in a crystalline form, but recrystallisation of the product from methanol-acetone mixture, gave white crystals with a melting point of 122-124°C. The elemental analysis was consistent with the molecular formula C₂₈H₄₂O.

The spectroscopic data was consistent with the structure (5). The IR spectrum showed a broad absorption peak at 3355 cm⁻¹, corresponding to the hydroxyl group. The ¹H n.m.r spectrum showed a multiplet centred at δ 4.09, integrating for one proton, corresponding to the C-3 proton. The olefinic protons at C-22 and C-23 appeared as a multiplet at δ 5.23.

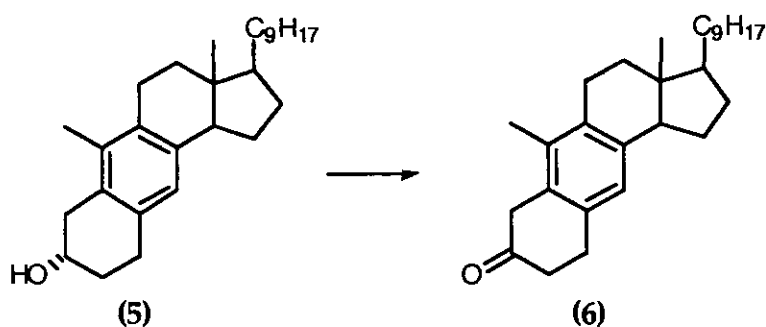
3.6 Synthesis of 1 (10→6) abeo -Ergosta-5, 7, 9, 22-tetraen-3-one (6)

3.6.1 Introduction

A large number of oxidants have been used to oxidise alcohols to ketones. The most widely used of the transition metal oxidants are Cr(VI)-based reagents. For simple unfunctionalised alcohols, oxidations can be carried out using Jones' reagent, which is an acidic aqueous solution of chromic acid. The alcohol is converted to the ketone very rapidly and over-oxidation is minimal. The chromium trioxide-pyridine complex (Collin's reagent) is useful in

situations where other functional groups may be susceptible to oxidation or when the molecule is sensitive to strong acid. Other useful reagents namely pyridinium chlorochromate (PCC) and pyridinium dichromate (PDC) have also been used to oxidise secondary alcohols to ketones. Another useful group of procedures has been developed which involves dimethylsulphoxide (DMSO) in the presence of suitable activators such as dicyclohexylcarbodiimide (DCC), oxalyl chloride, acetic anhydride or sulphur trioxide. These methods are suitable for use in oxidation of molecules that are sensitive to the more powerful transition metal oxidants. Manganese dioxide is another useful oxidant. This reagent preferentially attacks allylic and benzylic hydroxyl groups and therefore is more selective. Ruthenium tetroxide is another reagent that has been used to oxidise certain alcohols to ketones when a number of other methods have failed, however it is a potent oxidant and readily attacks carbon-carbon double bonds. The development of the CrO_3 -pyridine and DMSO-based methods has decreased the instances when older oxidation techniques such as the Oppenauer oxidation which requires aluminium isopropoxide and cyclohexanone, are used. However the reaction conditions are non-acidic, and may be useful in very acid-sensitive molecules.

3.6.2 Synthesis



Scheme 3.6

Oxidation of the alcohol (5) to the ketone (6) using the Moffat reagent did not give the high yields quoted by Whalley⁸² *et al.* Use of dicyclohexylcarbodiimide (DCC) in a 50 % mixture of benzene and dimethylsulphoxide (DMSO) to effect oxidation gave less than 20 % of the desired ketone (6). One of the problems encountered included contamination of the product with dicyclohexylurea. In the work up process, dicyclohexylurea was precipitated. It was presumed that filtration through a

short column of celite would remove the contaminant. However, even though the product was recrystallised twice, dicyclohexylurea still remained a contaminant.

Various oxidation methods were carried out in an attempt to find the procedure that would produce high yields of the desired ketone (6). Swern oxidation, using oxalyl chloride in DMSO was attempted, but failed to give good yields of the desired ketone (6). Instead an intractable oil was obtained. Upon purification on silica, only a very small amount of the ketone (6) was isolated. Next, we attempted the Jones oxidation, the yields obtained were again poor. Oxidation with pyridinium dichromate (PDC) did not give satisfactory yields of the ketone (6). One of the problems encountered was the difficulty in eliminating a chromium complex from the final compound. Purification by column chromatography did not completely remove the contaminant from the ketone (6). Oxidation with pyridinium chlorochromate (PCC) gave quite satisfactory yields, approximately 50 % yield of the ketone (6). The last oxidation method we did attempt was the Oppenauer oxidation. This procedure gave even better yields of the ketone (6) than that obtained from the PCC oxidation, we obtained yields of between 70 and 90 %.

The spectroscopic data for the ketone (6) were consistent with literature values.⁸² The IR spectrum showed a strong carbonyl stretching at 1722 cm⁻¹. The mass spectrum showed a 100 % molecular ion peak at 392. The accurate mass was found to be 392.3077 which was consistent with the molecular formula C₂₈H₄₀O.

The only disadvantage of the Oppenauer oxidation compared with the PCC oxidation is that the work up was more tedious. The difficulty in removing cyclohexanol at the end of the reaction was more troublesome than expected. Most of the cyclohexanol was removed by evaporation under high vacuum but traces still remained, which was removed by column chromatography.

3.7 Conclusion

The first step in the synthesis involved quantitative acetylation of ergosterol, followed by 1,4-Diels Alder addition of 4-phenyl-1,2,4-triazoline-3,5-dione (PTAD) to adduct (3) in quantitative yield. Oxidative rearrangement of the adduct led to the anthrasteroid acetate (4) in 70 % yield. Alkaline hydrolysis of the anthrasteroid acetate gave the alcohol (5) in quantitative yield. Oxidation of the alcohol yielded the ketone (6), the key intermediate in 75 % yield.

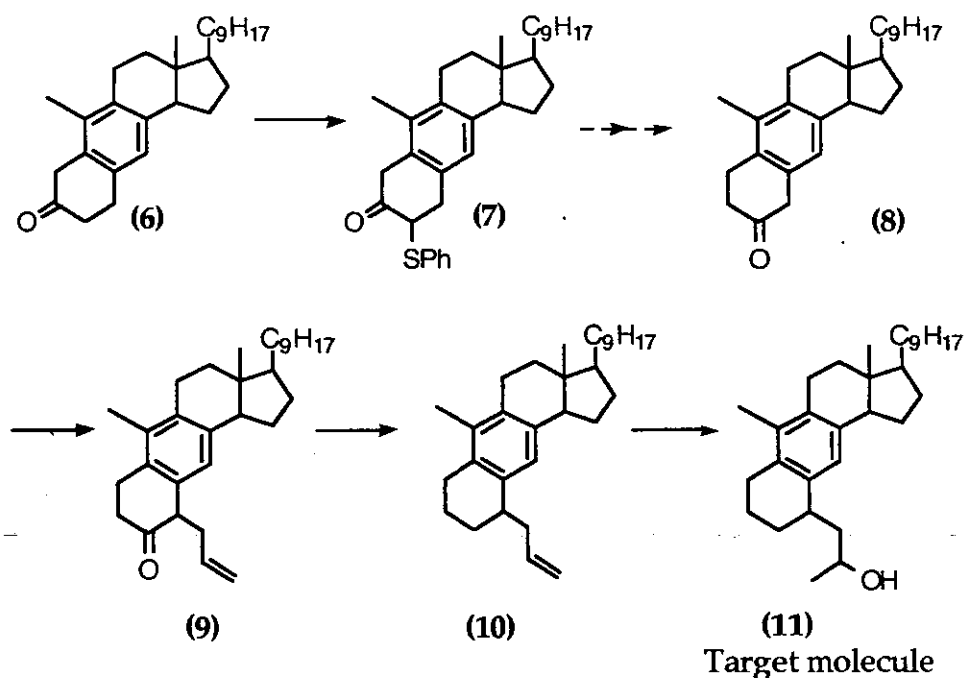
The synthesis according to **Scheme 3.1** has been achieved. The objectives were to synthesize the key intermediate, 1 (10→6) abeo-ergosta-5, 7, 9, 22-tetraen-3-one (6), and to optimise the yields obtained for the intermediary compounds at each stage of the synthesis. Both these objectives were realised. The overall yield for the 5-step synthesis was 45 -50 %.

Chapter Four

4.1 Attempted Sulphenylation

4.1.1 Introduction

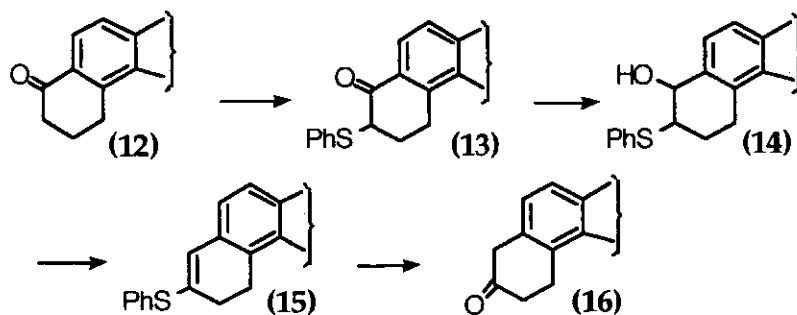
The proposed synthesis, **Scheme 4.1**, involves a 1,2-carbonyl transposition of (6) to (8), followed by alkylation at C-1, then reduction of the ketone (9) and lastly hydration of double bond to give the hydroxylated anthrasteroid (11), which is one of the target molecules.



Scheme 4.1

4.1.2 The 1,2-Carbonyl Transposition

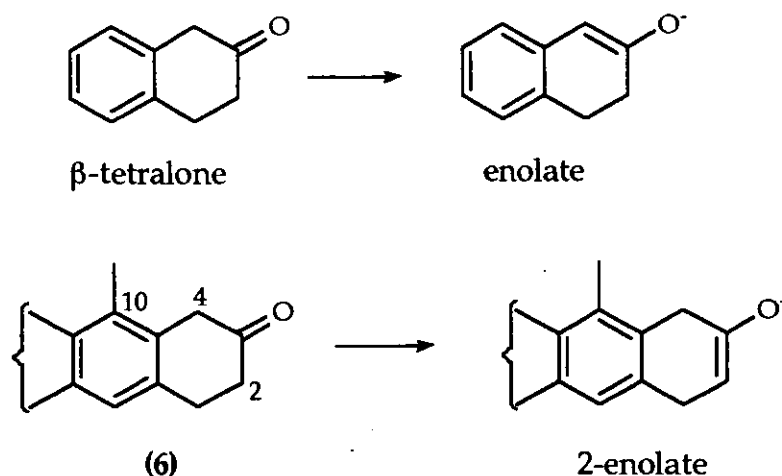
The proposed transposition sequence follows the procedure by Trost,⁹² **Scheme 4.2**.



Scheme 4.2

The first step according to Trost, involved sulphenylation of the ketone (12) with phenyl disulphide, then reduction with sodium borohydride in methanol to the sulphenyl alcohol (14). Dehydration of the alcohol and hydrolysis of the resultant enol thioether (15) gave the 1,2-transposed ketone (16).

It is hoped that by applying the Trost procedure to the proposed synthesis, 1,2-carbonyl transposition of the anthrasteroid ketone (6) could be effected. The key reaction involved direct sulphenylation of the ketone at C-2. Studies⁹³ have shown that in the case of β -tetralone, enolisation tends to proceed towards the α position, Scheme 4.3.



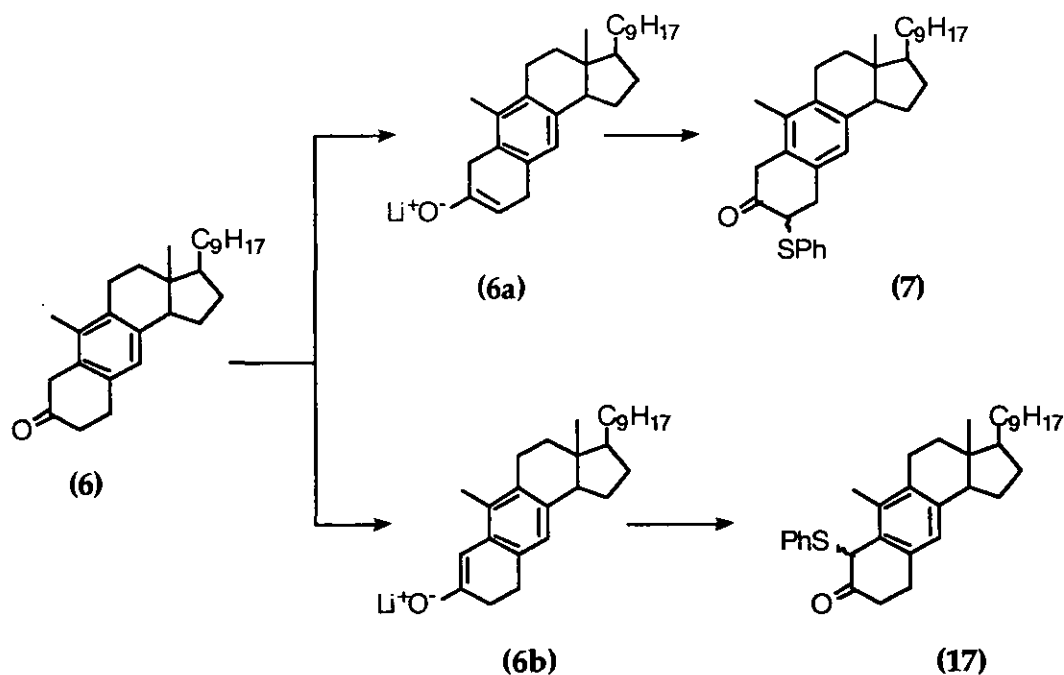
Scheme 4.3

However, it was thought that the methyl group at C-10 of the anthrasteroid (6) might hinder abstraction of the proton at C-4, by a sterically hindered base and that the more accessible proton at C-2 would be deprotonated instead, *via* a kinetically controlled reaction, to give the desired 2-enolate, Scheme 4.3.

4.1.3 Synthesis

The synthesis according to Scheme 4.4, was carried out by treating the ketone (6) with a highly hindered base such as lithium hexamethyldisilazide, $[\text{LiN}(\text{SiMe}_3)_2]$, followed by phenyl disulphide. We had hoped to obtain the

2-sulphenylated product (7) *via* the kinetic enolate (6a), as shown in Scheme 4.4. From spectroscopic data it was apparent that a mixture of compounds was obtained, with the thermodynamically controlled product i.e. the 4-sulphenylated product (17), being the major product.



Scheme 4.4

The IR spectrum of (17), showed a carbonyl absorption at 1705 cm^{-1} . From the ^1H n.m.r spectrum of the crude product, a mixture of products was obtained. Column chromatography was carried out on the crude compound on grade 3 alumina, eluting with 5 % diethyl ether in n-hexane. A number of fractions were recovered, the predominant product recovered being the 4-sulphenylated compound (17). Evidence was obtained from the proton n.m.r spectrum of (17). The signal at δ 4.8 appeared as a singlet, integrating for one proton, corresponded to the methine proton at C-4. In the original ketone (6), the methylene at C-4 appeared as a singlet at δ 3.52, indicating the identical chemical shifts of the C-4 protons. The 4-sulphenylated compound (17) is a 50:50 mixture of both α and β isomers, as indicated by the two singlets at δ 0.51 and δ 0.60, corresponding to the C-13 methyl protons and at δ 2.21 and δ 2.25, corresponding to the C-10 methyl protons. The isomers were not separable. The accurate mass was found to be 500.3125 which was consistent with the

molecular formula $C_{34}H_{44}OS$. The other fractions recovered contained a mixture of the starting ketone (6) and the 2-sulphenylated compound (7), with poor yield. The proton n.m.r of the 2-sulphenylated compound (7) showed a triplet, integrating for one proton at δ 3.75, corresponding to the methine proton at C-2. The methylene protons at C-4 appeared as a singlet at δ 3.55, integrating for two protons.

The reaction was repeated a number of times, each time varying the conditions of the reaction. The ratio of base used varied between 1 to 2 equivalents. This did not affect the outcome of the reaction. A mixture of products was recovered each time. Next, the duration of the reaction was varied between 1 to 5 hours stirring at $-78^{\circ}C$, this was monitored by tlc. It was found that on one occasion when the reaction mixture was allowed to react for only 1 hour, the 2-sulphenylated product (7) was isolated in very low yield from the reaction mixture which consisted mainly of unreacted ketone. When the reaction was allowed to carry on for another 30 minutes, the predominant product was the 4-sulphenylated compound (17).

By using a different hindered base such as lithium isopropyl cyclohexylamide, we had hoped to be able to obtain the kinetically controlled product i.e. the 2-sulphenylated product (7) in better yield. The result of the reaction was similar to that obtained for the previous reaction, the major product isolated was the thermodynamically controlled product, i.e. the 4-sulphenylated product (17).

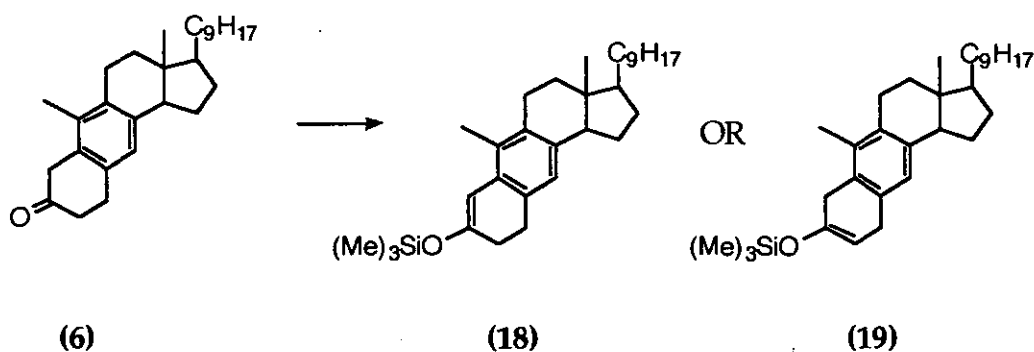
It was conceivable that the thermodynamically controlled lithium enolate (6b), Scheme 4.4 was formed in preference to the kinetic enolate (6a), even though ideal conditions for generating the kinetic enolate was used. Although the expected enolate (6a) may have formed first, the predominant product was the thermodynamically more stable product, i.e. the 4-sulphenylated compound (17). The driving force for this is undoubtedly that the 4-enolate (6b) formed was in conjugation with the aromatic ring B, and possibly there is insufficient steric hindrance from the C-10 methyl group to hinder approach of even a very bulky base to the C-4 protons.

4.1.4 Conclusion

As we were unable to prepare the desired 2-sulphenylated compound (7) in good yield, we could not carry out the proposed synthesis, **Scheme 4.1**, according to Trost. Therefore an alternative route had to be investigated.

4.2 Direction of Enolisation

In an attempt to confirm that enolisation of the ketone (6) had proceeded towards C-4 instead of C-2, the ketone (6) was reacted with trimethylchlorosilane, in the presence of lithium hexamethyldisilazide, trapping it as a silyl enolate.



Scheme 4.5

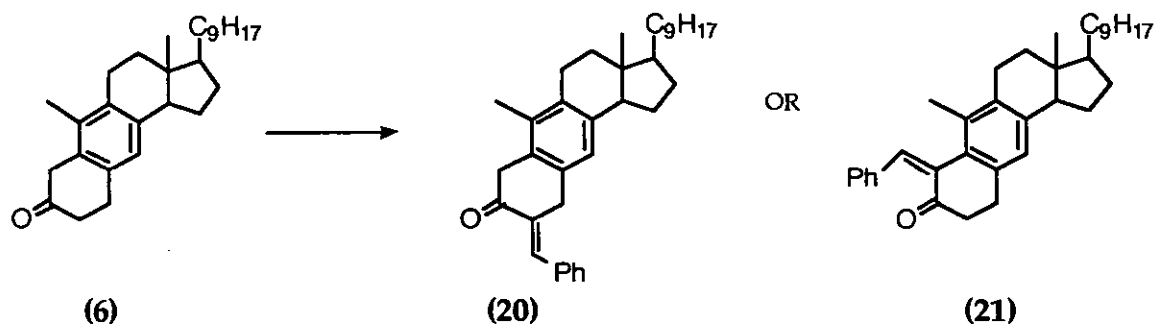
The silylation reaction was done in THF at -78°C , with lithium hexamethyldisilazide as base. A mixture of the starting ketone (6) and the silylated product was observed by TLC of the crude product. Spectroscopic data of the crude product, showed evidence of the presence of the thermodynamic product, i.e. the 4-silyl enol ether (18). The ^1H n.m.r showed a singlet at δ 5.9, integrating for one proton, which corresponds to the olefinic proton at C-4. The presence of the silyl group was shown by the large singlet at δ 0.15. If the kinetic 2-silyl enol ether (19) was formed then we would expect to see a triplet at approximately δ 5.5, integrating for one olefinic proton, corresponding to C-2 and the methylene protons at C-4 would appear at approximately δ 3.5 as a singlet. These signals were not present in the proton n.m.r of the crude product. From the IR spectrum, the Si-O stretching

frequency was detected in the finger print region of 910 cm^{-1} , and the Si-Me₃ stretching frequency was detected at 736 cm^{-1} . On work-up and further purification, the product was too unstable, and was hydrolysed and we recovered the ketone (6) instead.

4.3 Blocking Position 4 of 1 (10→6) abeo-Ergosta-5,7,9,22-tetraen-3-one (6)

4.3.1 Blocking with Benzaldehyde

As the enolisation seemed to proceed towards C-4 instead of C-2, we proposed to block the C-4 position with a bulky group first, then proceed with the 1,2-carbonyl transposition *via* the method suggested by Trost. The ketone (6) was treated with benzaldehyde and base. The spectroscopic data suggested that the 2-substituted product (20) had been obtained instead of the expected 4-substituted product (21), Scheme 4.6.

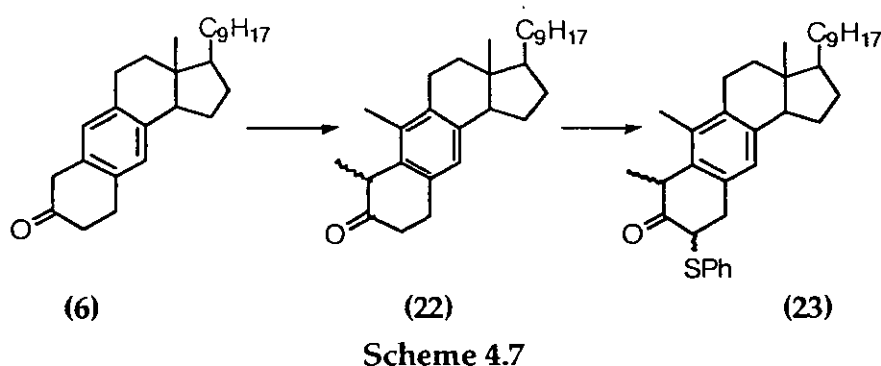


Scheme 4.6

From the ¹H n.m.r spectrum of the product, the presence of the C-4 methylene protons at $\delta\ 4.05$ indicated that condensation with benzaldehyde gave the 2-substituted product (20). The benzylic proton gave a singlet at $\delta\ 7.35$ and the multiplet, integrating for five protons at $\delta\ 7.15$, corresponded to the phenyl group. This result was very interesting, it could be that the benzaldehyde molecule was too large and steric hindrance from the C-10 methyl group prevented interaction with C-4 to form the condensation product. Whereas, in the case of proton abstraction at C-4 by a highly hindered base such as lithium hexamethyldisilazide, co-ordination of the ketone with the lithium cation enabled the removal of the C-4 proton, even though there is still steric hindrance from C-10 methyl group.

Therefore an alternative substituent is needed to block position 4 of the anthrasteroid molecule.

4.3.2 Blocking with Methyl Groups



The ketone was treated with sodium hydride and methyl iodide to effect methylation. The reaction was repeated numerous times, under different reaction conditions (Table 2). The factors that were investigated included the addition sequence of the reagents, reaction time, temperature, the ratio of reagents used and the type of base used. Of the sixteen reactions carried out, only two were successful in methylating the ketone, the yield obtained for these reactions were less than 10 %. However this was not reproducible. The product isolated from the crude mixture was found to be the monoalkylated product (22), Scheme 4.7. Even when a large excess of base was used, as well as an excess of methyl iodide, dimethylation of the ketone did not occur. When we attempted to alkylate the monoalkylated product (22) again, the reaction failed to yield the dimethylated product.

| Base used | Methyl iodide | Reaction conditions |
|---|--------------------|---|
| NaH, 1-5 equivalents | 2.5-10 equivalents | 2 h at R.T, then reflux 1 h Method (a)* |
| NaH, 1-5 equivalents | 1-3 equivalents | 1.5 h at R.T, then reflux 3 h, Method (b)* |
| K metal/ <i>t</i> -BuOH, 4 equivalents | 8 equivalents | 4 h at R.T |

*Method (a): Methyl iodide was added, then followed by NaH.

*Method (b): NaH was added, then followed by methyl iodide.

Table 2

The spectroscopic data showed that we had isolated the monoalkylated ketone (22). The IR spectrum showed the carbonyl stretching frequency at 1704 cm^{-1} . From the ^1H n.m.r spectrum, it was evident that we had obtained the 4-methylated ketone (22). The 4-methyl group appeared as a singlet at δ 1.33, integrating for three protons. The methine proton at C-4 appeared as a quartet at δ 3.70, integrating for one proton. Further evidence for (22), was shown by decoupling experiments. Irradiation of 4-H at δ 3.7, showed the collapse of the signal at δ 1.33, which corresponded to 4-Me. The presence of α and β isomers was also evident as shown by the appearance of two singlets very close to each other at δ 0.58 and δ 0.60, corresponding to the C-13 methyl, at δ 2.13 and δ 2.14, corresponding to the C-10 methyl, and at δ 1.32 and δ 1.33, corresponding to the methyl group at C-4. From the mass spectrum, the accurate mass was found to be 406.3233, which is consistent with the formula $\text{C}_{29}\text{H}_{42}\text{O}$.

Although the yield for the monoalkylated product (22) could not be further improved, the next step of the synthesis was carried out, that is to effect sulphenylation at C-2 with C-4 position blocked.

The suphenylation step was carried with phenyl disulphide, but the reaction failed to give the desired C-2 sulphenylated product (23), Scheme 4.7. Instead an intractable polymeric tar was obtained. Examination by TLC showed that numerous products were present without any one spot being the predominant one. At this stage it was felt that we had exhausted our effects in attempting to make this reaction viable for our purpose.

4.4 Conclusion

In theory, the strategy according to Scheme 4.1, to effect 1,2-carbonyl transposition, followed by alkylation, then reduction to remove the carbonyl group and then hydration of double bond to yield the hydroxylated anthrasteroid (11), seemed straightforward especially as there was literature precedence. However, from actual synthesis work, the route was difficult to accomplish. The problems encountered included low yields of the intermediates, especially of the 4-methylated compound (22), Scheme 4.7, and the failure to obtain the desired 2-sulphenylated product (7) and (23).

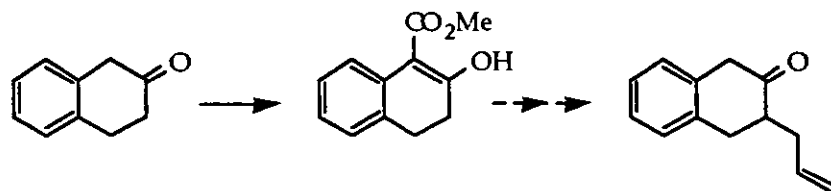
Therefore, an alternative route to synthesize the target molecule had to be investigated.

4.5 Dianion Alkylation Approach

4.5.1 Introduction

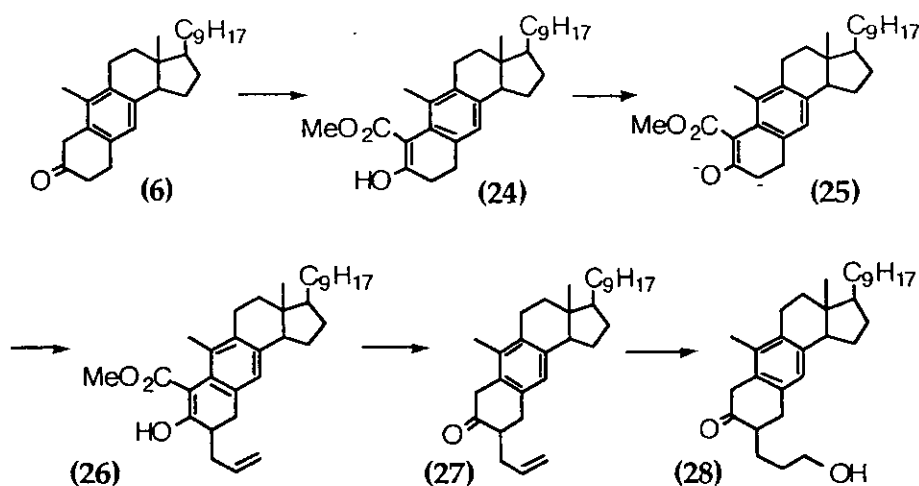
After searching through the literature, it was found that previous researchers⁹⁴ had worked on similar systems such as β -tetralone, where C-3 alkylation was desired. Aristoff⁹⁴ *et al* had investigated a dianion alkylation approach. It was proposed that we should apply this approach to our synthesis.

Aristoff *et al* found that direct dianion formation and alkylation of β -tetralone was unsuccessful but if converted to the β -keto ester then the dianion generated could be alkylated by a variety of reagents, **Scheme 4.8**, to give the desired C-3 alkylated product.



Scheme 4.8

The proposed route of synthesis, **Scheme 4.9**, to the target molecule would therefore involve the preparation of the β -keto ester (**24**), followed by generation of the dianion (**25**), alkylation with allyl bromide, then Krapcho's decarboxylation procedure⁹⁵ would yield the desired C-2 alkylated product (**27**). Then hydroboration followed by oxidation would give the hydroxylated target molecule (**28**).



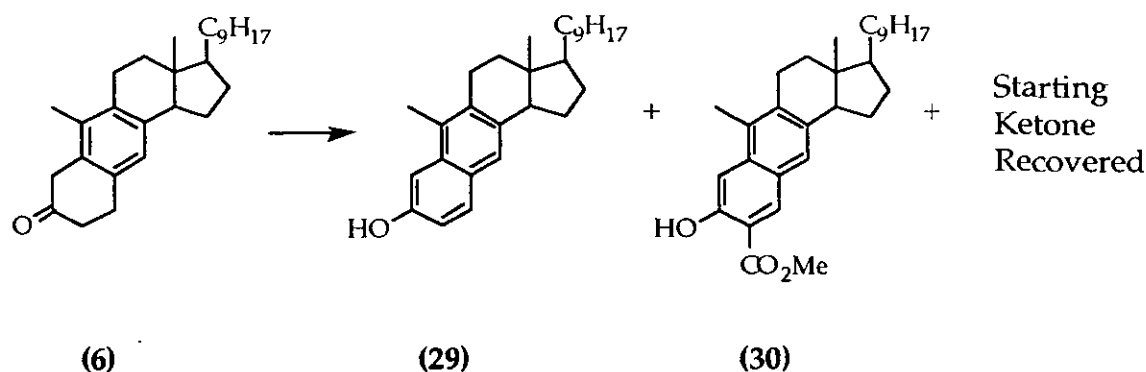
Scheme 4.9

4.5.2 Synthesis

The preparation of the β -keto ester (24), Scheme 4.9, was attempted by reacting the ketone with sodium methoxide in dimethyl carbonate. From preliminary observations on TLC it seemed that a mixture of products was obtained. The reaction was repeated several times, varying the reaction conditions each time, without success. In another attempt, using sodium hydride (60 % in oil) as base, we failed to form the β -keto ester. Changing the solvent to diethyl carbonate did not affect the reaction. Heating the reaction for longer times did not yield the desired product (24). Instead, it was apparent that we had obtained some aromatised products.

The crude black tar material obtained was purified by column chromatography on silica. Three fractions were isolated, the rest of the material was retained on the column. The spectroscopic data showed that one of the fractions isolated was the starting ketone (6), 8 % yield, the other was a phenolic compound (29), Scheme 4.10, 3 % yield, and the third fraction (which is not always isolated), was an aromatised keto ester (30), with less than 5 % yield. The ¹H n.m.r spectrum of the phenolic compound (29), showed signals centred at δ 7.64, δ 7.26, and δ 7.01, each integrating for one proton, indicating the presence of an aromatic ring. A broad singlet at δ 4.97, integrating for one proton corresponded to the hydroxyl group. The IR spectrum also showed a broad stretching frequency at 3416 cm⁻¹, attributed to the presence of a hydroxyl group. Evidence for the aromatised keto ester (30), was from the

^1H n.m.r spectrum, which gave a sharp singlet at δ 4.00, corresponding to the methoxy group of the ester function and singlets at δ 7.26, δ 7.45, and δ 8.37, indicating the presence of an aromatic ring with three protons. A singlet at δ 10.32 corresponded to the hydroxyl group.



Scheme 4.10

It could be that the aromatised products may have resulted from slow base-catalysed auto-oxidation reaction in the presence of air.

4.6 Conclusion

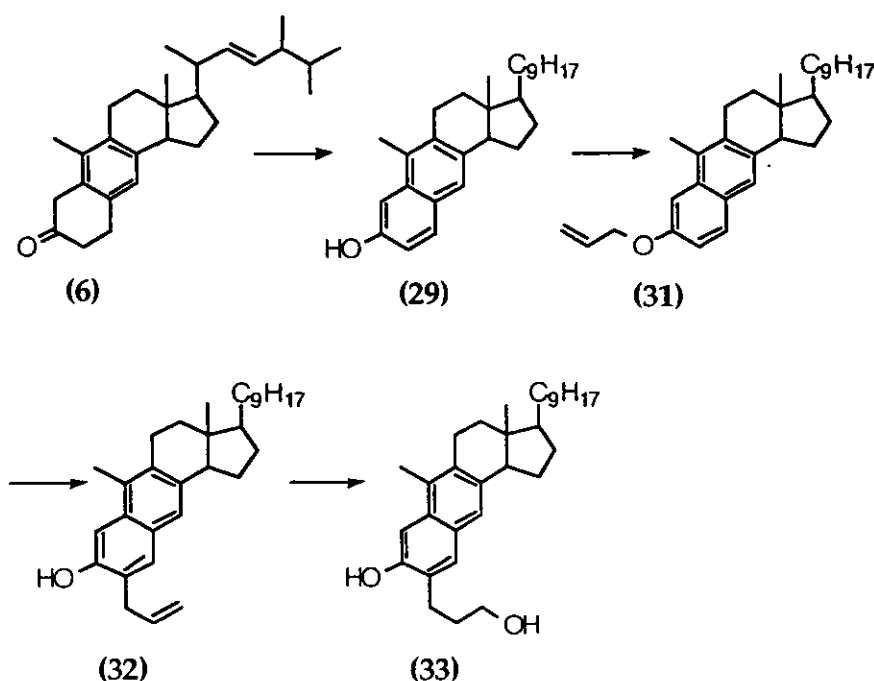
As we failed to prepare the β -keto ester (24), we were unable to generate the dianion (25), Scheme 4.9. It is apparent that this proposed route was not a viable option for us to proceed. We need to further investigate other procedures for synthesizing β -keto esters, which will be discussed in chapter 6.

Chapter Five

5.1 Introduction

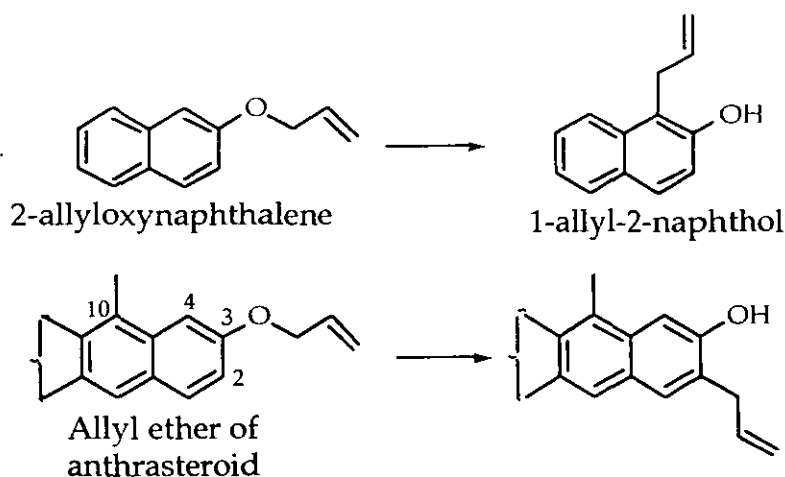
We had observed that there was a tendency for ring A to aromatised as discussed in the previous chapter. It was proposed that we should take advantage of this tendency and prepare the phenolic compound (29) in good yield. As indicated in Chapter 2, Section 2.2, one of the proposed target molecules for synthesis was (33) where both ring A and ring B are aromatic. Such compound should still have a reasonable overall conformation to fit the vitamin D receptor sites.

The proposed synthesis, Scheme 5.1 involved aromatisation of ring A, alkylation of the phenol (29) to the allyl ether (31), followed by Claisen rearrangement of the allyl ether. Selective hydroboration followed by oxidation would afford the hydroxylated target molecule (33).



Scheme 5.1

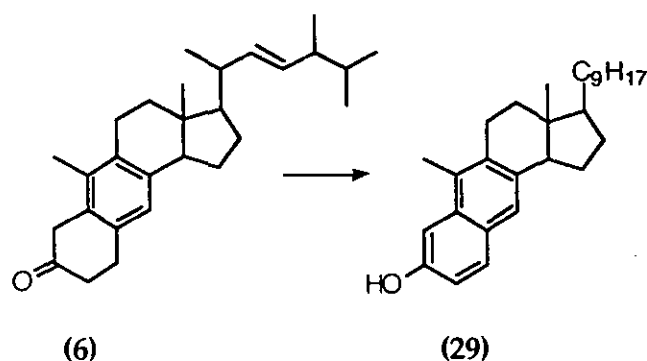
The Claisen rearrangement was first discovered in 1912,⁹⁶ it showed that 2-allyloxynaphthalene rearranged on heating to give 1-allyl-2-naphthol, Scheme 5.2.



Scheme 5.2

As direct alkylation at C-2 position of the anthrasteroid molecule was not possible, as shown in the previous chapter, we thought that the C-10 methyl of the anthrasteroid molecule might influence the Claisen rearrangement by hindering migration of the allyl side chain towards C-4 and re-direct rearrangement to C-2 instead, Scheme 5.2.

5.2 Synthesis of 1 (10 \rightarrow 6) abeo-3-Hydroxyergosta-1, 3, 5, 7, 9, 22-hexaene (29)

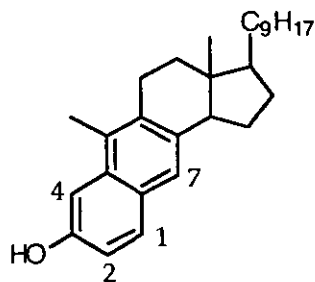


Scheme 5.3

The phenolic compound (29) was formed in good yield, 77 %. The aromatisation of ring A had proceeded satisfactorily. The ketone (6) was dissolved in THF and treated with pyrrolidone tribromide (PHT) at 0°C for 1 hour. The crude product was purified by column chromatography on silica.

The isolated compound was further purified by recrystallisation from methanol, to give white crystals, m. p. 140-141°C which is consistent with literature values.⁸²

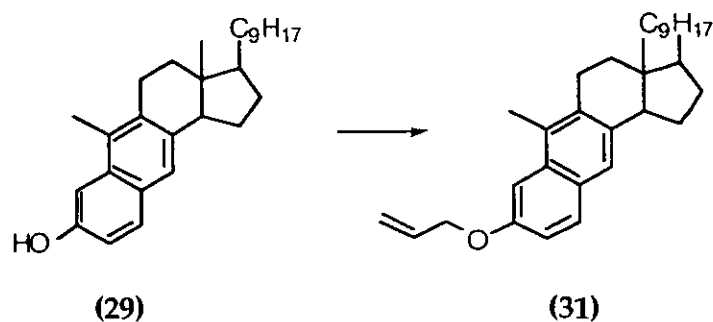
The identity of (29) was confirmed by spectroscopic data. The aromatic nature of the compound was evident from the ^1H n.m.r spectra, a singlet at δ 7.24, corresponded to the aromatic proton at C-7. The aromatic protons at C-1 and C-4 appeared as doublets at δ 7.64, where $J=9$ Hz and δ 7.31, where $J=3$ Hz, respectively. The aromatic proton at C-2 was coupled to 1-H and 4-H, appearing as a double doublet at δ 6.99, with coupling constants 3 Hz, and 9 Hz. Decoupling experiments were also carried out to confirm the signals that were coupling with each other. It was found that by irradiating at δ 7.0, which corresponded to 2-H, it was observed that the doublets at δ 7.64, corresponding to 1-H, and δ 7.31, corresponding to 4-H were decoupled to give singlets. The aromatic nature of (29) was further confirmed by evidence from the carbon n.m.r spectrum, which showed ten signals between δ 106-152, corresponding to the aromatic region of the spectrum. The phenol (29) gave a characteristic absorption peak for the hydroxyl function at 3424 cm^{-1} in the IR spectrum. Further evidence came from carrying out D_2O exchange on the n.m.r sample, showed a disappearance of the broad singlet at δ 4.88 which corresponded to the hydroxyl group.



(29)

We also attempted to aromatise ring A with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ). The yield obtained was only 39 %.

5.3 Synthesis of 3-(prop-2-enyloxy)-ergosta-1, 3, 5, 7, 9, 22-hexaene (31)



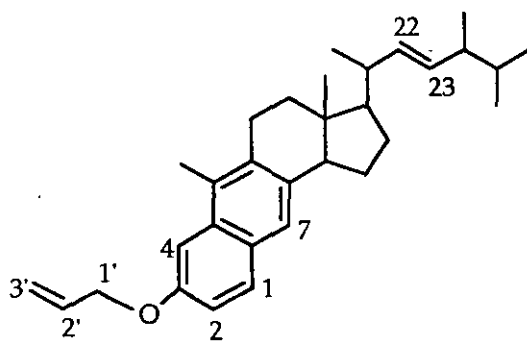
Scheme 5.4

The next step involved alkylation of (29) with allyl bromide to yield the allyl ether (31). We attempted numerous experiments (Table 3). Addition of 1.5 equivalent sodium metal to the phenol in ethanol to generate the base *in situ* gave the best yields of (31), 74 %.

| Base used | Solvent used | Reaction conditions | Results: | |
|--------------------------------|--------------|---------------------|---------------|------|
| | | | Compound (29) | (31) |
| 1.5 equiv. NaOEt | Ethanol | 1.5 h at R.T | 0 % | 74 % |
| | | 2.5 h at reflux | | |
| K ₂ CO ₃ | Acetone | 0.5 h at 0°C | 0 % | 51 % |
| | | 1 h at R.T | | |
| | | 2.5 h at reflux | | |
| 2 % solution NaOEt | Ethanol | 1.5 h at R.T | 70 % | 6 % |
| | | 1.5 h at reflux | | |
| 1.5 equiv. NaH | THF | 1.5 h at R.T | 80 % | 2 % |
| | | 1.5 h at reflux | | |
| 1.5 equiv. NaH | DMF | 1.5 h at R.T | 50 % | 0 % |
| | | Reflux overnight | | |
| 4 equiv. KOH | DMSO | 24 h at R.T | 80 % | 0 % |

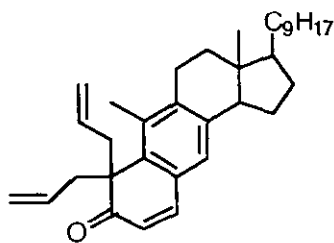
Table 3

The alkylation reaction was not a clean reaction. The crude oily product was purified by column chromatography on silica and a number of side products were also isolated. The spectroscopic data confirmed the structure of (31). The absorption peaks at 1626 cm^{-1} and 1602 cm^{-1} in the IR spectrum, corresponded to the C=C stretching frequencies of the molecule. The ^1H n.m.r spectrum showed a double doublet at δ 7.08, corresponding to the aromatic proton at C-2 coupling with 1-H and 4-H. A singlet at δ 7.26, corresponding to C-7 and two doublets at δ 7.30, and δ 7.65, corresponding to 4-H and 1-H, respectively. The side chain olefinic protons at C-22 and C-23 appeared as a multiplet centred at δ 5.24 and the geminal olefinic protons at C-3' appeared as double doublets centred at δ 5.32 and δ 5.47, where one of the geminal proton is *trans* coupled to 2'-H, where $J=17.0\text{ Hz}$, and the other is *cis* coupled to 2'-H, where $J=10\text{ Hz}$. The 2'-H appeared as a multiplet centred at δ 6.12, and the methylene protons at C-1' appeared as a multiplet centred at δ 4.65.



(31)

The other minor components isolated were the starting material (29) and a diallyl compound (34). The diallyl product (34) was not always isolated, but on the two occasions when it was isolated, which constituted for less than 5 % of the yield recovered. The spectroscopic data (which will be discussed in greater detail later) confirmed the identity of the diallyl compound (34).



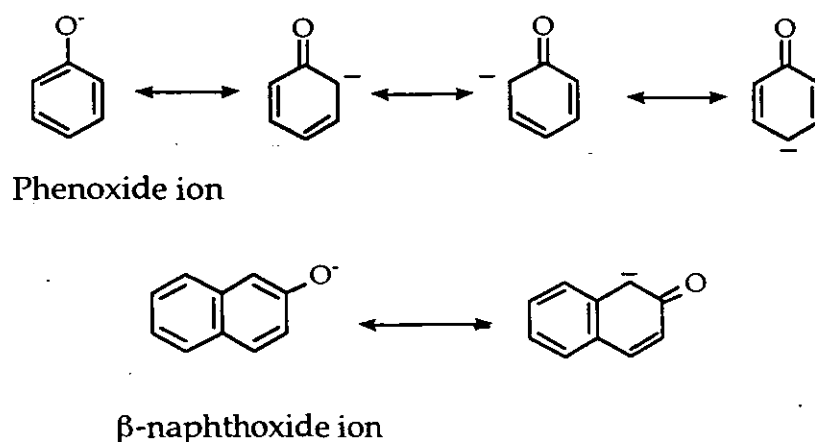
(34)

5.3.1 Mechanism of Alkylation

The reaction proceeds *via* the deprotonation of the phenol by a suitable base, then the phenoxide ion reacts with an electrophile, in an S_N2 manner. The alkylating agent must be reactive toward nucleophilic displacement.

Ordinarily, the alkylation of phenolic salts in solution produces the ether (*i.e.* O-alkylation), in quantitative yields.⁹⁷ However, the alkylation of ketone enolates has led to formation of polyalkylated products, which is a major source of difficulty in these alkylations.⁹⁸

Kornblum^{99,100} *et al* carried out studies on solvation as a factor in the alkylation of ambident anions, *i.e.*, anions possessing capability for covalent bond formation at either of two alternative positions. For example, the phenoxide ion is capable of bond formation at oxygen or at the *ortho* and *para* ring carbon atoms, or the example of the β -naphthoxide ion, which is capable of covalent bond formation at oxygen, or at the α -carbon, **Scheme 5.5**.



Scheme 5.5

The conclusions drawn from their studies were that the course of alkylation is highly dependent on the type of solvent used in the reaction. This can be attributed to two factors namely, the ability of the solvent to solvate the ions and the dielectric constant of the solvent. In experiments employing sodium β -naphthoxide, in aprotic solvents with high dielectric constant such as DMF and DMSO, they were able to obtain exclusively the O-alkylated product. While using aprotic solvents with relatively low dielectric constant, such as

THF, gave them a mixture of O-alkylated product, 60 % and C-alkylated product, 36 %. Both DMF and DMSO are good cation solvators and poor anion solvators, so co-ordination to the enolate anion is much less effective, than to the metal cations, Fig. 19, thereby providing a medium in which the enolate-metal ion pair are dissociated to give a less hindered, and more reactive enolate, which favours O-alkylation.

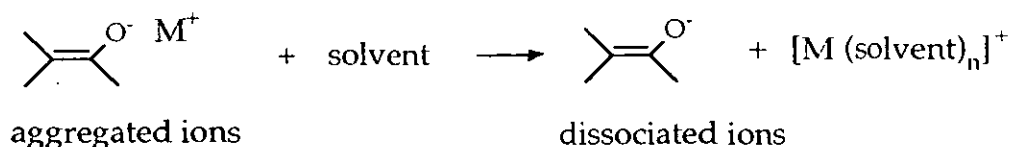


Fig 19

In contrast, the reactions carried out in aqueous solutions of trifluoroethanol and water gave exclusively the C-alkylated product. These protic solvents form particularly strong hydrogen bonds with the oxygen atom of the enolate anion Fig. 20. This strong solvation decreases the reactivity at oxygen and therefore favours C-alkylation.

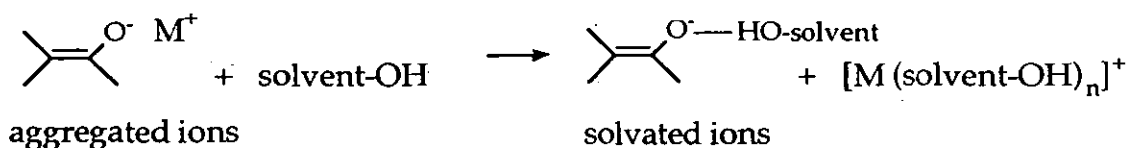


Fig. 20

In our synthesis, the allyl ether (31) was the major product obtained, O-alkylation was favoured, but the reaction conditions favouring the O-alkylated product were sodium metal in ethanol and potassium carbonate in acetone. In this case it seemed that the protic solvents used were yielding the O-alkylated product contrary to what is expected, *i.e.* the use of protic solvents usually favours C-alkylation. Phenoxide ions are a special case, whereby O-alkylation is preferred. This is because aromaticity of the phenolic ring is maintained in the transition state when it undergoes O-alkylation, whereas C-alkylation disrupts the aromatic conjugation of the ring in the intermediate state, Fig. 21.

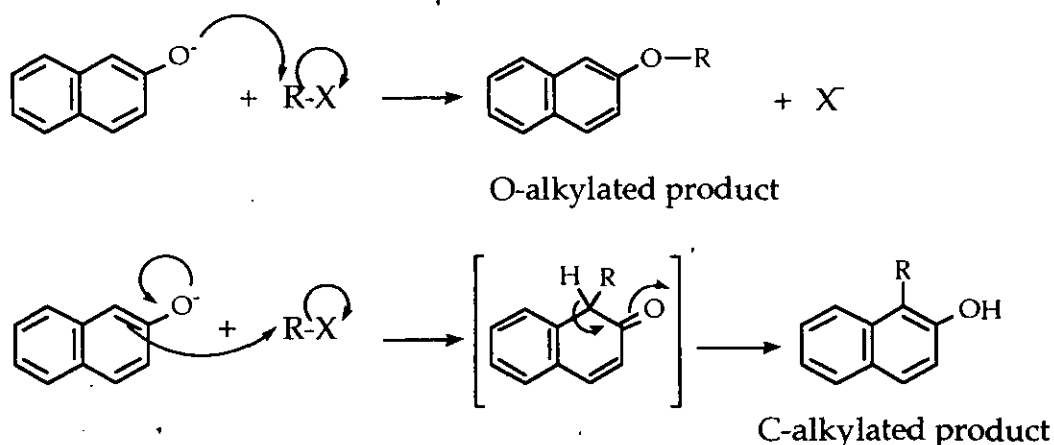


Fig. 21

5.4 Claisen Rearrangement

5.4.1 Introduction

The Claisen rearrangement is a [3,3]-sigmatropic process involving a concerted reorganisation of electrons during which a group attached by a σ bond migrates to a more distant terminus of an adjacent π -electron system. There is a simultaneous shift of electrons. Further evidence were provided by studies^{101,102} on allyl ethers of phenols and the following mechanism was proposed Fig. 22.

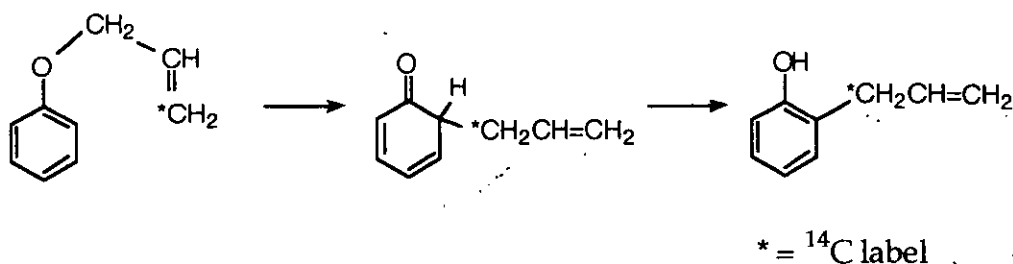
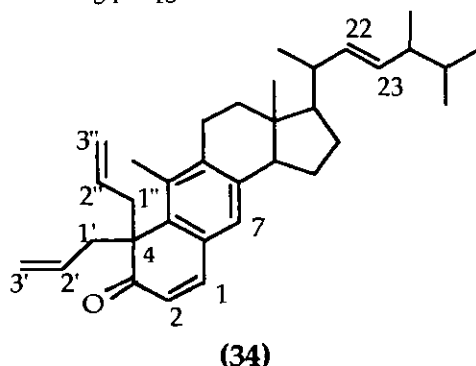
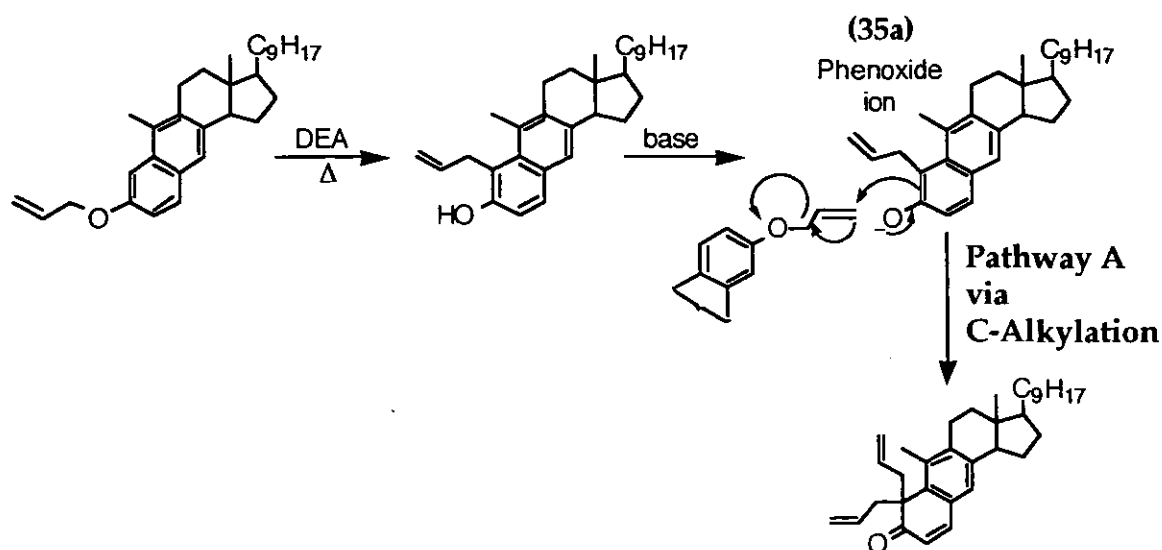


Fig. 22

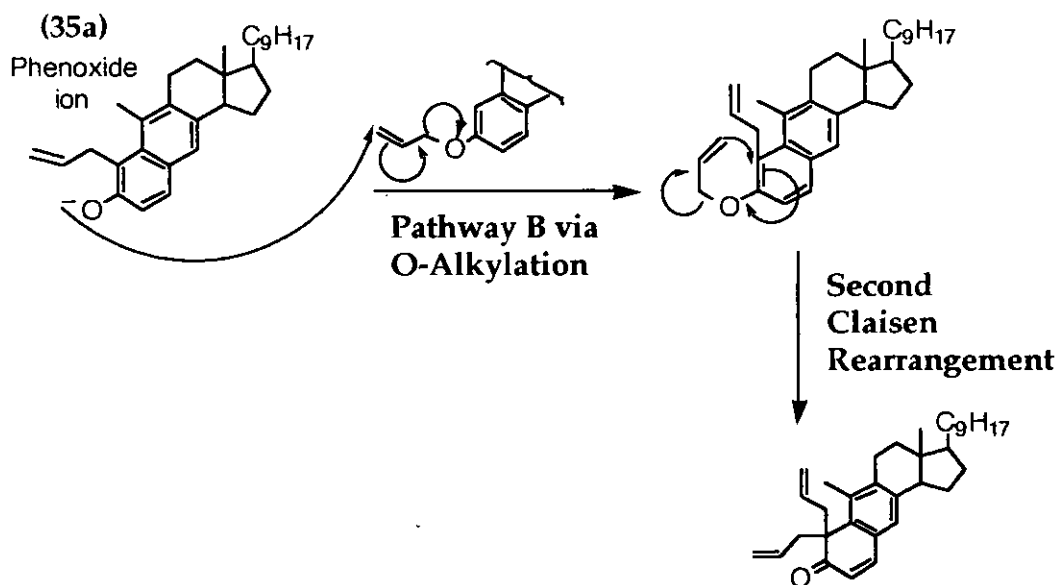
integrating for four protons corresponded to the methylene protons at C-1' and C-1'' of the allyl side chains. Two doublets at δ 6.11 and δ 7.33, each integrating for one proton, corresponded to the olefinic protons at C-2 and C-1, respectively, where the coupling constant is J 10 Hz. The aromatic proton at C-7 appeared as a singlet at δ 6.81. The accurate mass was found to be consistent with the formula $C_{34}H_{46}O$.



A possible explanation for the observed products was proposed. It could be that Claisen rearrangement had taken place, but as soon as the Claisen product was formed, the basic conditions of the reaction caused deprotonation and the phenoxide anion (35a) was generated. The allyl ether acts as an alkylating agent at C or O *via* either pathway A, Scheme 5.8 or pathway B shown in Scheme 5.9 to give the diallyl compound (34). The driving force for the formation of a diallyl compound may be attributed to the need to relieve steric hindrance between the allyl group at C-4 and the methyl group at C-10.

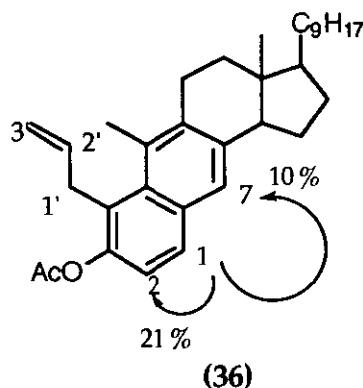


Scheme 5.8



Scheme 5.9

To prove that the Claisen rearrangement did take place, we proposed to trap the Claisen product as the acetate (36). Addition of a few drops of acetic anhydride to the refluxing solution of allyl ether in diethylaniline gave the acetate as white crystals with m.p. 129.7-130.8°C, in 85 % yield. The identity of the acetate (36) was confirmed by spectroscopic evidence.



An absorption peak at 1750 cm⁻¹ was detected on the IR spectrum of (36), indicating the presence of a carbonyl function. From the ¹H n.m.r spectrum, a singlet at δ 2.30, integrating for three protons was assigned to the methyl protons of the acetate group at C-3 and the other singlet at δ 2.64, integrating for three protons was assigned to C-10 methyl. The methylene protons at C-1' on the allyl side chain were assigned to the multiplet at δ 3.80. The geminal protons at C-3' appeared as double doublets at δ 5.00 and δ 5.15, which is *trans*

coupled, $J=17$ Hz, and *cis* coupled, $J=11$ Hz, to 2'-H, respectively. The olefinic proton at C-2' appeared as a multiplet centred at δ 6.12, integrating for one proton. The aromatic protons at C-1, and C-2 were assigned to the doublets at δ 7.63 and δ 7.07, respectively, with each doublet integrating for one proton, where coupling constant $J=9$ Hz. The 7-H aromatic proton appeared as a singlet at δ 7.29. Further evidence confirming the structure of the acetate (**36**) was from NOE experiments and COSY plots. Irradiation at δ 7.6, corresponding to 1-H, showed enhancement of signals at δ 7.3 by 10 %, corresponding to 7-H, and at δ 7.0 by 21 %, corresponding to 2-H. When 7-H was irradiated at δ 7.3, signal enhancement was only observed at δ 7.6, corresponding to 1-H and irradiation of 2-H at δ 7.0, signal enhancement was observed only at δ 7.6, corresponding to 1-H. The C-10 methyl group and C-3 acetate were also irradiated, but there was no observed enhancement of any other signals. The accurate mass was found to be consistent with the formula $C_{33}H_{44}O_2$.

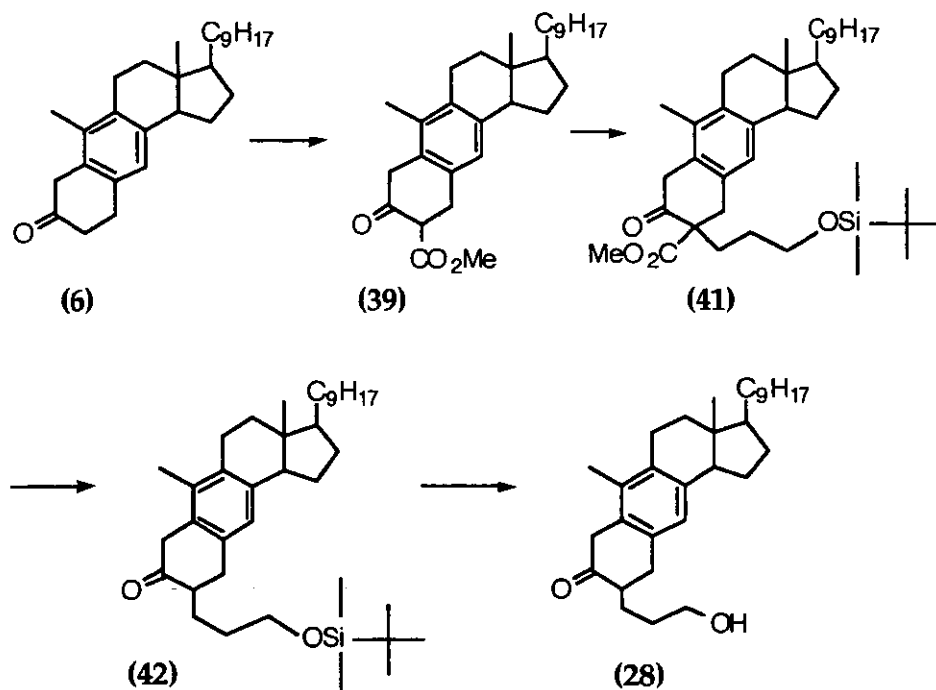
5.5 Conclusion

The strategy of employing the Claisen rearrangement to effect C-2 alkylation did not proceed as expected. The rearrangement proceeded towards C-4 instead of C-2. The 2-allyl Claisen product (**32**) was not isolated, instead we obtained a diallyl compound (**34**). As the Claisen rearrangement failed to yield the desired intermediate (**32**), we were unable to synthesize the target molecule *via* this proposed route, **Scheme 5.1**. An alternative route had to be investigated.

Chapter Six

6.1 Introduction

An alternative route to synthesize the target molecule (28) is proposed, **Scheme 6.1**. Carbomethoxylation of the ketone (6) would yield the β -keto ester (39). Then alkylation would give the product (41). Decarboxylation of (41) would afford the 2-alkylated product (42), and then hydrolysis of (42) would yield the hydroxylated target molecule (28).



Scheme 6.1

6.1.2 Regiospecific Carbomethoxylation of the Anthrasteroid Ketone (6)

In the 2-tetralone system, Fig. 23, carbomethoxylation can occur at either C-1 or C-3 position depending on the nature of the acyl group and of the solvent system employed.^{109,110}

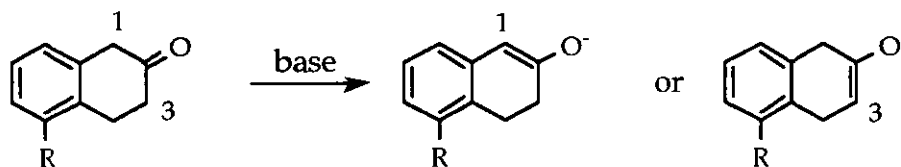


Fig. 23

Colvin¹⁰⁴ *et al* showed that treatment of 2-tetralone with sodium hydride and dimethyl carbonate gave exclusively the 1-carbomethoxy product *i.e.* the thermodynamically stable product. Pelletier¹⁰³ *et al* showed that the 3-carbomethoxy product *i.e.* the kinetically controlled product can be obtained by treating the tetralone with magnesium methoxy carbonate, although very low yields were reported.

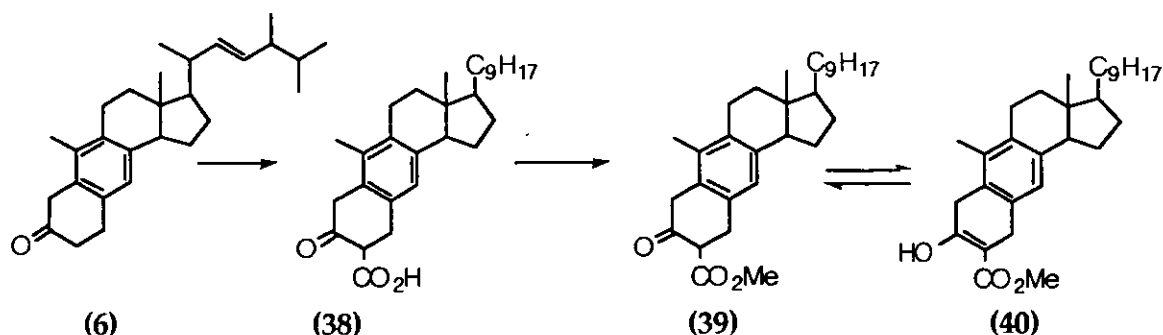
In Chapter 4, Section 4.5, attempts to prepare the β -keto ester (24), **Scheme 4.9**, by treatment of the anthrasteroid ketone (6) with sodium methoxide or sodium hydride and dimethyl carbonate failed to give the expected thermodynamically controlled product, contrary to what the literature suggests^{94,104} for certain 2-tetralones. Other workers¹⁰³ had also tried conventional methods for example using ethyl chloroformate or diethyl oxalate to carboethoxylate certain 2-tetralones and failed to obtain any carboethoxylated products.

A literature search revealed an elegant method by Stiles,^{105,106} using magnesium methoxy carbonate (MMC) to effect carbomethoxylation at C-3 position of certain 2-tetralones. Other researchers^{103,107} had also utilized MMC and had some success with carbomethoxylation of certain 2-tetralones.

The MMC method of carbomethoxylation was applied to the anthrasteroid system. This provided the means to prepare the desired kinetically controlled β -keto ester (39), in the proposed synthesis, **Scheme 6.1**, in good moderate yield.

6.2 Synthesis of 1 (10 \rightarrow 6) abeo-2-Carbomethoxyergosta-5, 7, 9, 22-tetraen-3-one (39)

The carbomethoxylation was carried out by treating the ketone (6) with MMC in DMF and refluxing the mixture for 4 hours. The crude carboxylic acid (38) was precipitated in ice-cold dilute hydrochloric acid and dried in a vacuum oven and used immediately in the next step. Methylation with diazomethane afforded the β -keto ester (40), between 60-80 % yield.

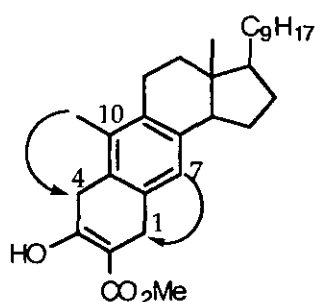


Scheme 6.2

This was not an easy reaction to carry out. The high yields were not always reproducible. The initial attempts to carry out this reaction failed to yield any desirable products, but modifications to the reaction conditions gave better results. These modifications included preparing fresh reagents each time the reaction was done. The solvents DMF and THF used in the reaction had to be dried and freshly distilled. The reflux temperature must be maintained throughout the reaction time. The slow rate of addition of the ketone (6), to the reaction mixture must be at a constant rate, over 30 minutes. The precipitation of the crude carboxylic acid must be done slowly and in an ice-cold solution of 10 % dilute hydrochloric acid and the resulting mixture had to be stirred in an ice bath for at least one hour before collecting the precipitated carboxylic acid (38). Methylation of the acid (38) with diazomethane was carried out immediately, no further purification was attempted as the acid decomposed rapidly. The yields obtained for the β -keto ester, 60-80 %, was a great improvement over the literature yields of 10-30 %, for similar tetralone systems.

The identity of the β -keto ester (39) was confirmed by spectroscopic data. From the IR spectrum, the typical carbonyl stretching frequency of an ester at 1730 cm^{-1} was shifted to a lower frequency at 1669 cm^{-1} . This lowering of frequency is also typical of enolised β keto-carbonyl systems.¹⁰⁷ The ^1H n.m.r spectrum indicated the presence of the enolic form (40) of the ester, by a sharp singlet at δ 12.17, integrating for one proton, corresponded to the hydroxyl group at C-3. This was confirmed by carrying out a D_2O exchange on the sample and a disappearance of the singlet at δ 12.17 was observed. A singlet at δ 3.81, integrating for three protons was assigned to the methyl group on the ester function at C-2. The methylene protons at C-1 and C-4 appeared as multiplets at δ 3.61, and δ 3.52 respectively, each multiplet integrating for two

protons, respectively. The aromatic proton at C-7 appeared as a singlet at δ 6.74. The carbon n.m.r spectrum gave further evidence of the presence of the enolised ester (40), the C-3 carbonyl carbon was assigned δ 172.3 and the carbonyl carbon of the ester group at C-2 was assigned δ 169.3. NOE experiments were also carried out to confirm the identity of (40). The molecule was irradiated at δ 6.74, corresponding to 7-H and enhancement was observed δ 3.61, corresponding to 1-H. A second irradiation was carried out at δ 2.12, corresponding to C-10 methyl, and enhancement was observed at δ 3.52, corresponding to 4-H.

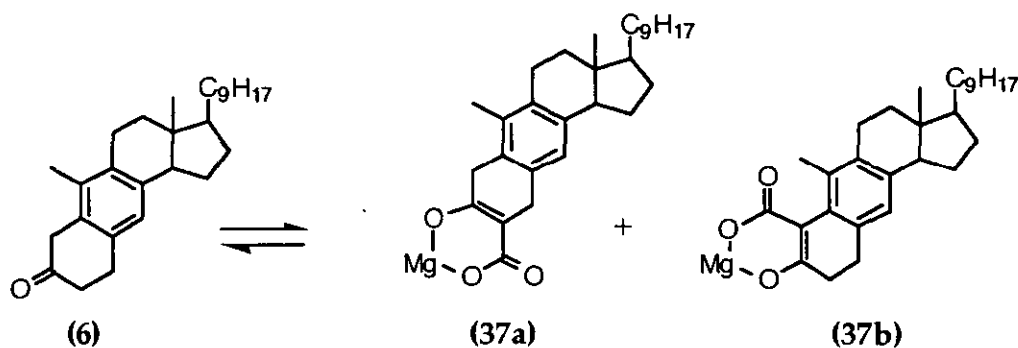


(40)

The accurate mass of the molecule was consistent with the formula $C_{30}H_{42}O_3$. The mass spectrum gave a 100 % molecular ion peak at 450.

6.2.1 Mechanism of Carbomethoxylation

It has been suggested^{103,107} that the MMC forms a magnesium chelate with the ketone (Scheme 6.3). The magnesium chelate-assisted carboxylation occurs under equilibrium conditions and that the 2-carboxy magnesium chelate (37a) is favoured over the 4-carboxy magnesium chelate (37b), due to steric hindrance from C-10 methyl group.



Scheme 6.3

6.2.2 Conclusion

The use of magnesium methoxy carbonate (MMC) in the carboxylation reaction with the ketone (6), gave moderate yields of the β -keto ester (40). This had been a major breakthrough in the synthesis of the target molecule, the hydroxylated anthrasteroid (28). The functionalisation at C-2 allowed for synthetically useful functional groups to be positioned at C-2 thereby giving access to the synthesis of the target molecule (28).

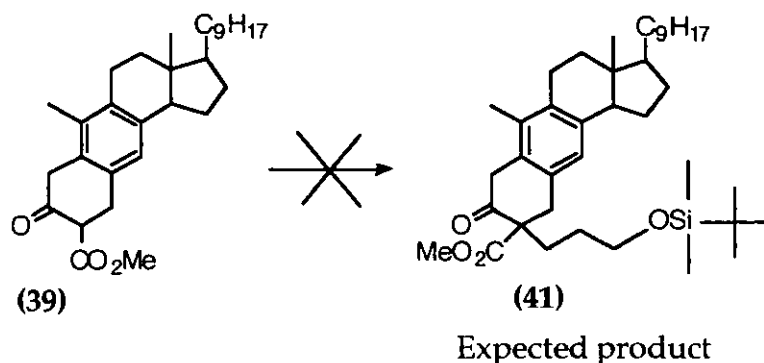
6.3 Attempted Alkylations

6.3.1 Introduction

The next step in the synthesis, **Scheme 6.1**, involved alkylation at C-2. Various alkylation procedures were attempted, **Table 4**, **Table 5**, and **Table 6**, summarises the results obtained in the alkylations.

In the β -keto ester (39), the presence of two electron withdrawing groups, namely the carbonyl group and the ester group, favours formation of the enolate resulting from deprotonation by base of the acidic proton from the carbon situated between them, *i.e.* at C-2. The enolate formed would react with the alkylating agent to give the C-2 alkylated product.

6.3.2 Alkylation with 3-*tert*-butyldimethylsilyloxy iodopropane



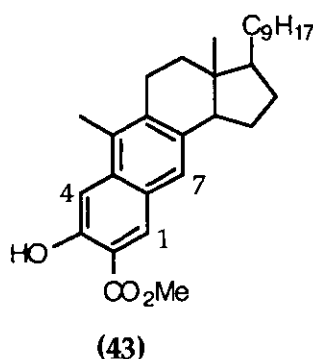
Scheme 6.4

A general alkylation procedure¹⁰⁸ was followed, **Scheme 6.4**. Alkylation was carried out on the β -keto ester (**39**), using various bases and different alkylating agent, **Table 4**.

| Base | Solvent | Alkylating agent | Results: Yields | | |
|-----------------------|---------|--------------------------|-----------------|-----|------|
| | | | 39 | 41 | 43 |
| NaH, 1.2 equiv., | THF | 3-(tBDMS)-1-bromopropane | 7 % | 0 % | 39 % |
| NaH, 1.2 equiv., | THF | 3-(tBDMS)-1-iodopropane | 0 % | 0 % | 32 % |
| Na metal, 1.5 equiv., | MeOH | 3-(tBDMS)-1-iodopropane | 0 % | 0 % | 41 % |

Table 4

As shown in the results, we were unable to obtain the desired alkylated product (**41**). The only product isolated from these attempted alkylations with 3-*tert*-butyldimethylsiloxy iodopropane [3-(tBDMS)-1-iodopropane], or the bromopropane was the naphthalene (**43**), between 30-40 % yield. Aromatisation of ring A may have resulted from base-catalysed auto-oxidation in presence of air trapped in the reaction mixture.

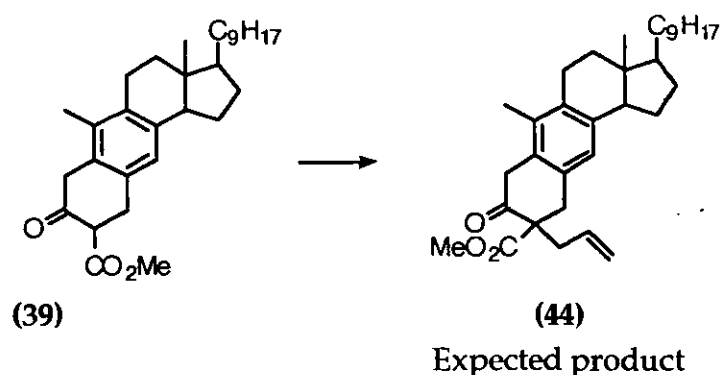


The identity of (**43**) was confirmed by spectroscopic data. The IR spectrum of (**43**) gave a broad absorption peak at 3264 cm^{-1} , indicating the presence of a hydroxyl group. The carbonyl stretching frequency appeared as a broad absorption peak at 1676 cm^{-1} . The ^1H n.m.r spectrum showed three aromatic protons appearing as singlets at δ 7.29, δ 7.45 and δ 8.38,

corresponding to 4-H, 7-H and 1-H respectively. The hydroxyl group appeared as a sharp singlet at δ 10.32. The methyl protons of the ester group appeared as a singlet at δ 4.00. The accurate mass was found to be consistent with the molecular formula $C_{30}H_{40}O_3$. The mass spectrum gave a 100 % molecular ion peak at 448.

6.3.3 Alkylation with Allyl Bromide

The next set of alkylation reactions we attempted, utilized allyl bromide as alkylating agent. It was intended to alkylate at C-2 and obtain the 2-allyl product (44), Scheme 6.5.

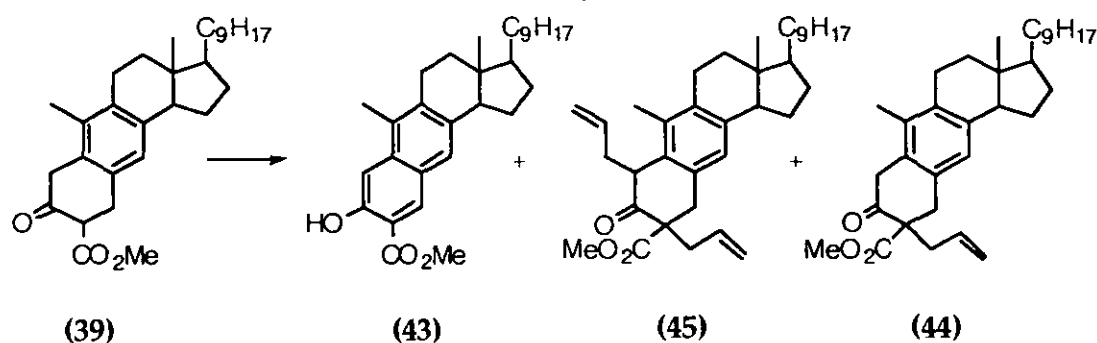


Scheme 6.5

Various bases and different reaction conditions were used to effect alkylation of the keto ester (39), the results were summarized in Table 5.

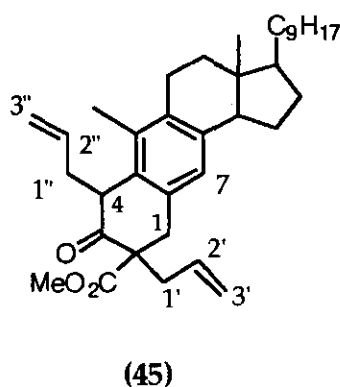
| Base/ Solvent | Alkylating agent: Allyl Bromide | Reaction conditions | Results: Yields | | |
|-------------------------|------------------------------------|------------------------|-----------------|------|------|
| | | | (43) | (45) | (44) |
| NaH, 1.5 equiv., DMF | 5 equivalents | 2 h, R.T | 3 % | 15 % | 0 % |
| NaH, 1 equiv., THF | 1 equivalent | 2 h, R.T | 16 % | 20 % | 3 % |
| Na, 1.5 equiv., MeOH | 1.5 equivalents | 1h, R.T 2 h, Reflux | 10 % | 20 % | 0 % |
| Na, 1.5 equiv., MeOH | 1.5 equivalents | 6 h, R.T | 14 % | 38 % | 0 % |

Table 5



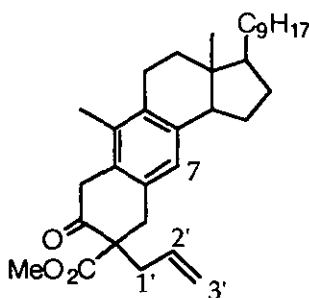
Scheme 6.6

The alkylation reaction did not proceed as expected. It was not a clean reaction. The crude mixture was purified by column chromatography on flash silica. A mixture of products were isolated, **Scheme 6.6**, the naphthalene (43), between 3-16 % yield, a bisallyl product (45), between 15-38 % yield, and on one occasion the 2-allyl product (44) was isolated, but in very poor yield, 3%. The remaining material was a polymeric tar that was retained on the chromatographic column,



The identity of the fractions was confirmed by spectroscopic evidence. The predominant compound being the bisallyl product (45). From the IR spectrum, the carbonyl stretching frequency of the ester function and ketone function appeared at 1728 cm^{-1} , and 1708 cm^{-1} respectively. The ^1H n.m.r spectrum showed two multiplets centred at δ 5.59 and δ 5.79, each integrating for one proton were assigned to the olefinic protons on the allyl side chains at C-2'' and C-2' respectively. The methylene protons at C-1' appeared as a multiplet corresponding to δ 3.02, and the methylene protons at C-1'' were probably hidden under a broad multiplet at δ 2.68. The olefinic geminal

protons at C-3' and C-3'' appeared as a multiplet, integrating for four protons centred at δ 5.13. The methine proton at C-4 appeared as a triplet at δ 3.88, with coupling constant of 8 Hz. The aromatic proton at C-7 appeared as a singlet at δ 6.67. The methyl protons of the ester group appeared as a singlet at δ 3.33.



(44)

The other minor component isolated was the 2-allyl product (44). The IR spectrum showed two carbonyl absorption peaks at 1730 cm^{-1} and 1710 cm^{-1} , corresponding to the ester and ketone functions respectively. From the ^1H n.m.r spectrum, the C-3' geminal olefinic protons appeared as a multiplet centred at δ 5.10, integrating for two protons. The olefinic proton at C-2', appeared as a multiplet centred at δ 5.78, integrating for one proton and the methylene protons at C-1' appeared as a doublet at δ 3.80 where $J=15\text{ Hz}$, integrating for two protons. The aromatic proton at C-7 appeared as a singlet at δ 6.70 and the methyl protons on the ester group appeared as a singlet at δ 3.60. The methylene protons at C-4 appeared as a singlet at δ 3.53, integrating for two protons.

A possible explanation for the observed products *i.e.* the bisallyl compound (45) and the monoallyl compound (44) could be that alkylation can occur at two positions, that is at C-2 and/or C-4 of the β -keto ester (39), Fig. 24. It was expected that the C-2 proton of (39) would be quite acidic being α to the carbonyl group as well as the carbomethoxy group and would be deprotonated in preference to the methylene protons at C-4 by a suitable base. The carbanion formed then reacts with the alkylating agent to give the 2-monoallyl product (44) which was isolated. Alternatively, the C-4 position is also susceptible to C-alkylation, being benzylic the C-4 protons are also quite acidic and the enolate formed is in conjugation with ring B of the anthrasteroid molecule.

Perhaps it is not surprising that both the bisallyl and monoallyl products were isolated in this series of alkylation reactions.

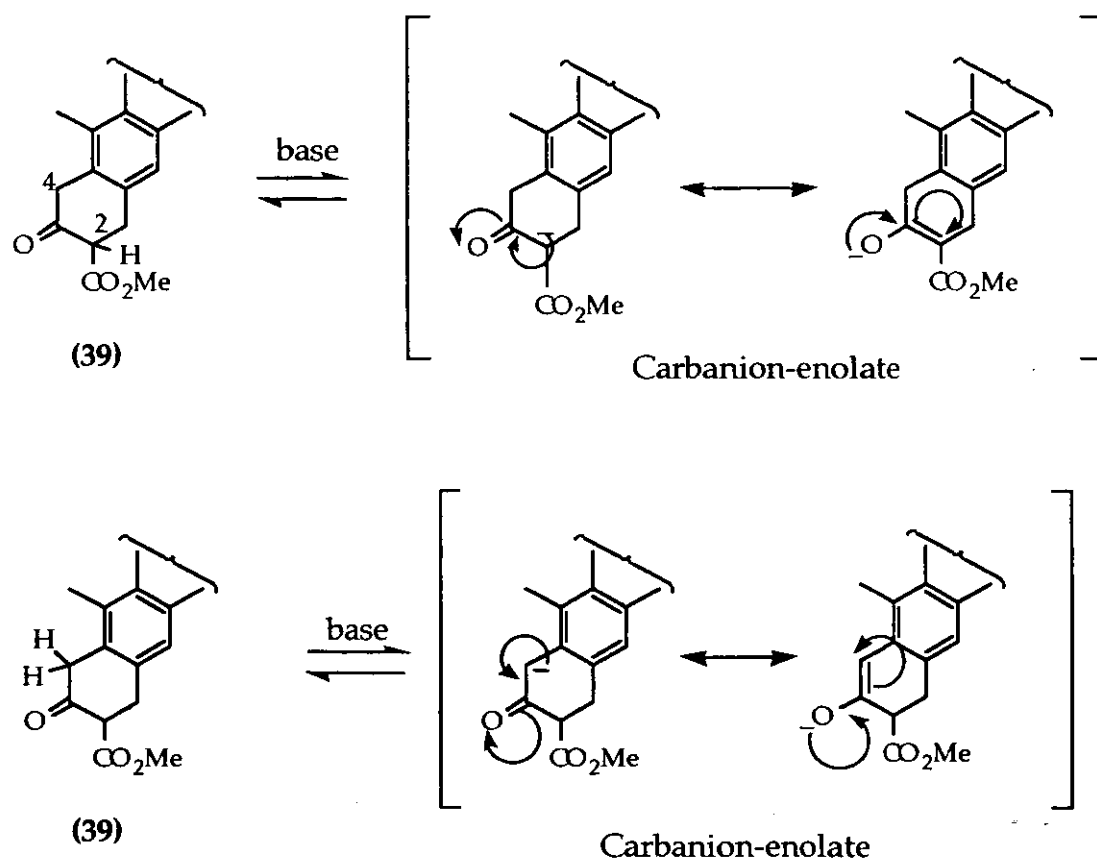


Fig. 24

6.3.4 Alkylation with Palladium Catalyst and Allyl Acetate

As we failed to obtain the desired 2-allyl product (**44**) in good yield via alkylation with allyl bromide, we decided to use tri-(dibenzylideneacetone)-dipalladium-chloroform-triphenyl phosphine catalyst [$\text{Pd}_2(\text{dba})_3 \cdot \text{CHCl}_3 \cdot \text{PPh}_3$], to effect C-2 alkylation. There has been much literature precedent for the use of palladium catalysts¹¹²⁻¹¹⁴ in Pd(0)-directed C-alkylation, Fig. 25.

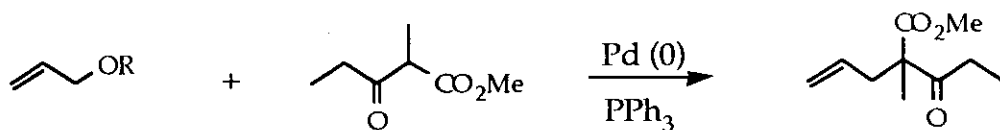


Fig. 25

Allyl acetate react with Pd(0) complexes to form π -allylpalladium complexes *in situ* as intermediates, which, without being isolated, reacts with C-nucleophiles.^{112,115}

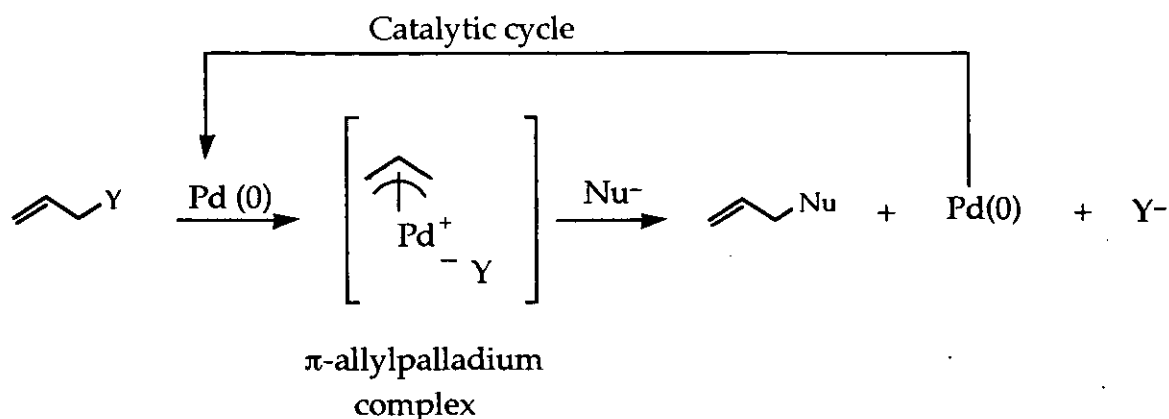


Fig. 26

The palladium-phosphine catalyst was prepared by reacting dibenzylideneacetone (dba), with palladium chloride in chloroform and the precipitate was collected and dried in a vacuum oven. It was then mixed with triphenyl phosphine in THF just before use. The alkylation reaction was repeated three times under different reaction conditions, Table 6.

| NaH/ THF | Allyl acetate | Palladium catalyst | Reaction conditions | Results: Yields | | |
|-------------|---------------|--------------------|-----------------------------|-----------------|------|------|
| | | | | (43) | (45) | (44) |
| 1.5 equiv., | 2 equiv., | 2.5 mol % | 1 h, R.T 30°C, 40 mins., | 11 % | 32 % | 4 % |
| 0.5 equiv., | 2 equiv., | 2.5 mol % | 30 mins., R.T | 11 % | 12 % | 20 % |
| 0.5 equiv., | 2 equiv., | 2.5 mol % | 3 h, R.T | 17 % | 0 % | 16 % |

Table 6

From the results, it was apparent that the reaction using palladium-phosphine catalyst was not selective enough, we had again obtained a mixture of products.

6.4 Conclusion

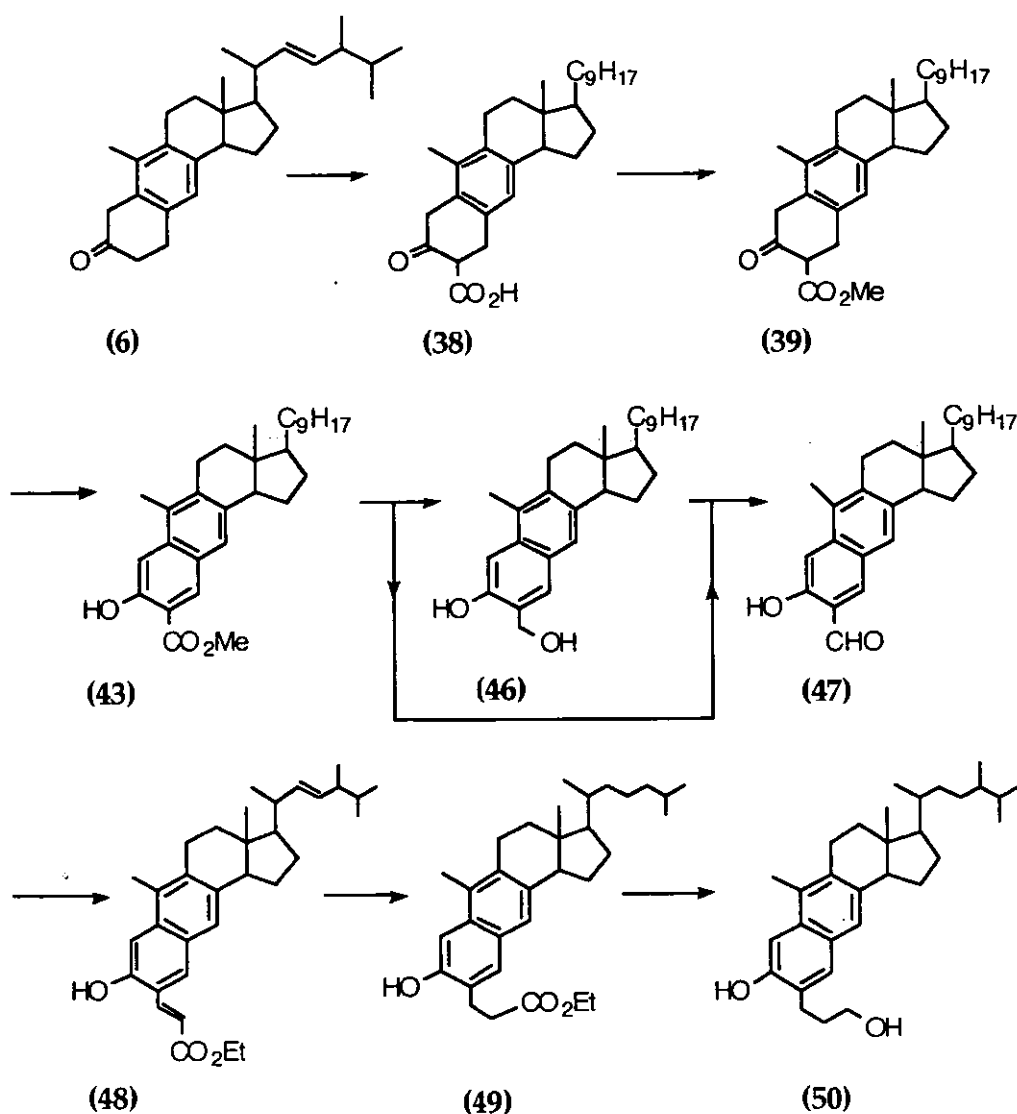
The procedure using magnesium methoxy carbonate (MMC) to prepare the β -keto ester (40) in good yields was a major breakthrough that allowed access to C-2 functionalisation of the anthrasteroid molecule. Although alkylation with various alkylating agents such as 3-(tBDMS)-1-iodopropane, allyl bromide and allyl acetate and a palladium catalyst, all had failed to give the desired 2-allyl product (44) in good yield. It was interesting that the alkylation reactions carried out with the iodopropane alkylating agent gave the naphthalene compound (43) as the major product and the alkylations with allyl bromide and allyl acetate with palladium catalyst yielded the bisallyl compound (45) as the major product. It has been known that alkylation of ketone enolates has led to formation of polyalkylated products, which is a major problem in these alkylations.

An alternative route to synthesize the target molecule had to be investigated.

Chapter Seven

7.1 Introduction

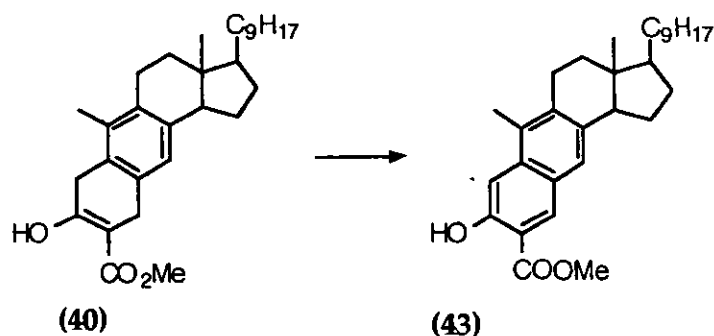
In view of the difficulties in developing a target molecule with a non-aromatic ring A, it was proposed that we should concentrate on developing a target molecule with both rings A and B being aromatic *i.e.* with a naphthalene ring system. As indicated in Chapter 2, Section 2.2, where one of the proposed target molecules for synthesis had ring A and ring B aromatic, this region of the molecule is quite planar, **Fig 17**, and could still be accommodated by the vitamin D receptor site. A new route to synthesize the target molecule (**50**) was proposed, **Scheme 7.1**.



Scheme 7.1

According to **Scheme 7.1**, carbomethoxylation of the ketone (**6**) would afford the β -keto ester (**39**), which followed by aromatisation of ring A would give the naphthalene ester (**43**). Partial reduction¹¹⁵ of the ester (**43**) was expected to give the aldehyde (**47**), directly or alternatively, reduction of (**43**) would yield the diol (**46**), then followed by oxidation would afford the aldehyde (**47**). A two carbon extension *via* a Horner-Wadsworth-Emmons (HWE) reaction¹¹⁶⁻¹²¹ would yield the ester (**48**), which followed by catalytic hydrogenation and reduction would yield the target molecule (**50**).

7.2 Synthesis of **1** (10 \rightarrow 6) abeo -2-Carbomethoxy-3-hydroxyergosta-1, 3, 5, 7, 9, 22-hexaene (**43**)



Scheme 7.2

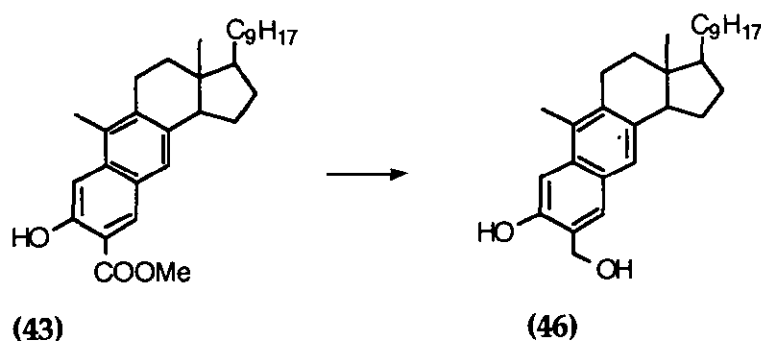
The β -keto ester (**40**) was treated with pyrrolidone hydrotribromide (PHT) in THF at 0°C for 1 hour, afforded the naphthalene ester (**43**) in quantitative yield. The identity of (**43**) was confirmed by spectroscopic evidence. From the ^1H n.m.r spectrum, the three aromatic protons at C-1, C-4 and C-7 appeared as singlets at δ 8.37, δ 7.29, and δ 7.45. The hydroxyl group appeared as a broad singlet at δ 10.31, which was confirmed by carrying out a D_2O exchange on the n.m.r sample. The singlet at δ 4.00 corresponded to the methoxy group of the ester. The accurate mass was found to be 448.2967, which was consistent with the molecular formula $\text{C}_{30}\text{H}_{40}\text{O}_3$.

7.3 Synthesis of 1 (10→6) abeo-2-Hydroxymethyl-3-hydroxyergosta-1, 3, 5, 7, 9, 22-hexaene (46)

7.3.1 Introduction

It was thought that partial reduction of the ester (43) to the aldehyde (47) without over-reduction to the alcohol (46), could be effected, **Scheme 7.1**. The ester was treated with di-isobutylaluminium hydride (DIBAL)¹¹⁵ at low temperatures. The desired aldehyde was not obtained, instead the starting ester (43) was recovered. It could be that DIBAL was not reactive enough to reduce (43) to the aldehyde. There was no trace of over-reduction to the alcohol. Therefore it was decided to reduce the ester (43) completely to the alcohol (46) and then oxidise (46) to the aldehyde (47), **Scheme 7.1**.

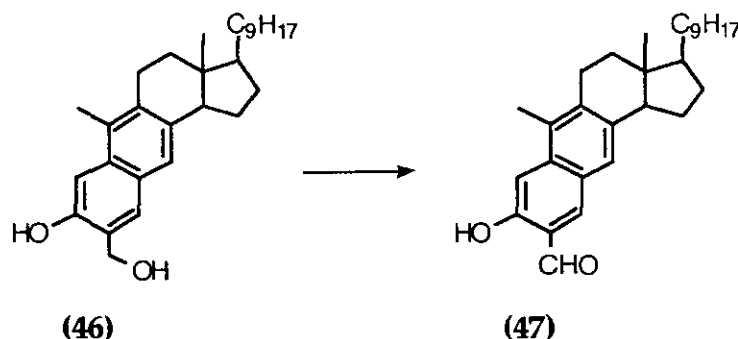
7.3.2 Synthesis



Scheme 7.3

Treating the ester (43) with lithium aluminium hydride (LiAlH_4), afforded the diol (46) in 85 % yield. The reduction was also carried out using lithium borohydride, the yields obtained were not as good as with LiAlH_4 . From the IR spectrum, a broad absorption peak at 3415 cm^{-1} corresponded to the hydroxyl groups present. The ^1H n.m.r spectrum gave a broad singlet at $\delta 7.16$, corresponding to the benzylic hydroxyl group, carrying out a D_2O exchange showed the disappearance of the singlet at $\delta 7.16$, confirming the presence of the hydroxyl function. The benzylic protons appeared as a singlet, integrating for two protons at $\delta 4.94$. The accurate mass was found to be 420.3027 which was consistent with the molecular formula $\text{C}_{29}\text{H}_{40}\text{O}_2$.

7.4 Synthesis of 1 (10→6) abeo-2-Formyl-3-hydroxyergosta-1, 3, 5, 7, 9, 22-hexaene (47)

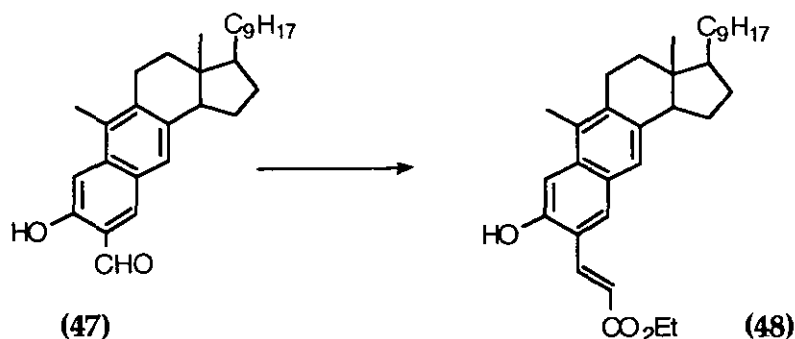


Scheme 7.4

The diol (46) was oxidised to the aldehyde (47) using a variety of reagents. Oxidation with tetrapropylammonium perruthenate (TPAP), a catalytic oxidant, gave the best yield, which was 30 %. Manganese dioxide (MnO₂) oxidation gave a yield of 23 %. The advantage of using TPAP over MnO₂ is that the reaction is quick, and catalytic amount of reagent is used. The reaction required only one hour of stirring at room temperature, whereas with MnO₂, the reaction required 48 hours and a large excess of the reagent was used.

The identity of (47) was confirmed by spectroscopic evidence. The IR spectrum showed a sharp absorption peak at 1720 cm⁻¹ corresponding to the carbonyl group and a broad absorption peak at 3276 cm⁻¹, corresponding to the hydroxyl group. The ¹H n.m.r showed that the aromatic protons at C-1, C-4, C-7 have been shifted downfield to δ 8.03, δ 7.35 and δ 7.42 respectively. The singlets at δ 10.03 and δ 10.28, integrating for one proton each, corresponded to the carbonyl group and the hydroxyl group, respectively. D₂O exchange showed that the signal at δ 10.28, corresponding to the hydroxyl group had disappeared, indicating that the assignment was correct.

7.5 Synthesis of Ethyl 3-[1 (10→6) abeo-3-Hydroxyergosta-1, 3, 5, 7, 9, 22-hexaen-2-yl] propenoate (48)



Scheme 7.5

The HWE reaction was carried out by treating the aldehyde (**47**) with triethylphosphonoacetate.^{117,121} The phosphonate carbanion was generated by deprotonation with sodium hydride in benzene, followed by slow addition of a solution of (**47**) in benzene. The crude product was purified by column chromatography on silica and the HWE product (**48**) was isolated as an oil, 16 % yield. The reaction was repeated several times, varying the reaction conditions each time, but the yield could not be improved.

From spectroscopic data, the IR spectrum showed a sharp absorption peak at 1707 cm^{-1} , corresponding to the carbonyl group, and a broad hydroxyl absorption peak at 3309 cm^{-1} . The ^1H n.m.r spectrum showed a triplet at δ 1.35, integrating for three protons, and a quartet at δ 4.28, integrating for two protons, corresponding to the methyl group and the methylene group, respectively, of the ester function. A broad singlet at δ 5.61, corresponded to the hydroxyl group and the olefinic protons at C-1' and C-2' appeared as doublets at δ 8.04 and δ 6.75, respectively, with a *trans* coupling constant of 16 Hz. The accurate mass was found to be 488.3295 which is consistent with the formula $\text{C}_{33}\text{H}_{44}\text{O}_3$.

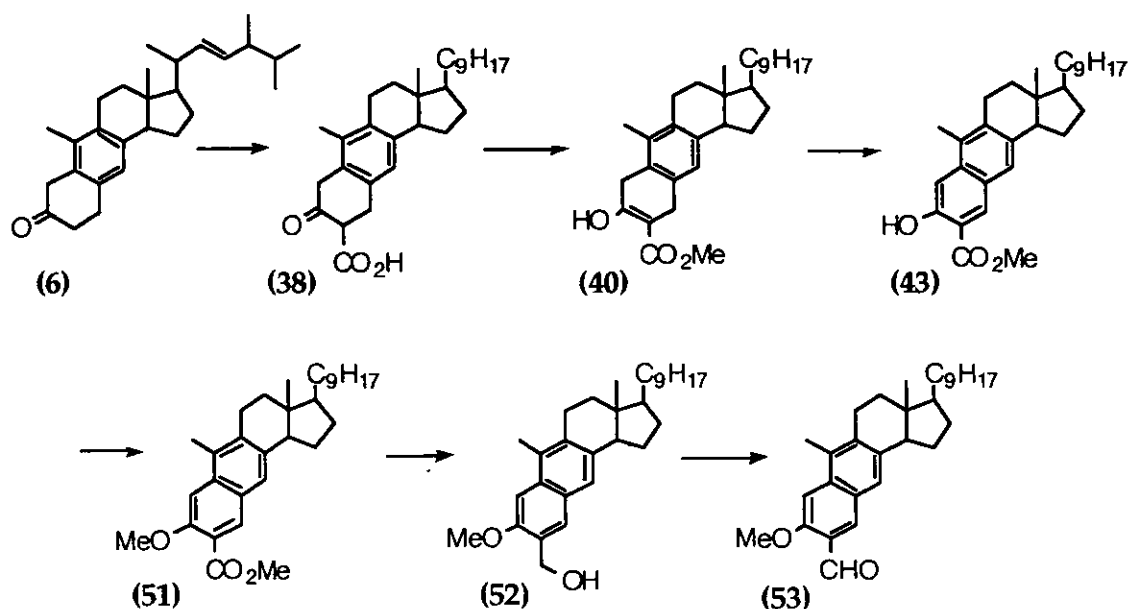
7.6 Conclusion

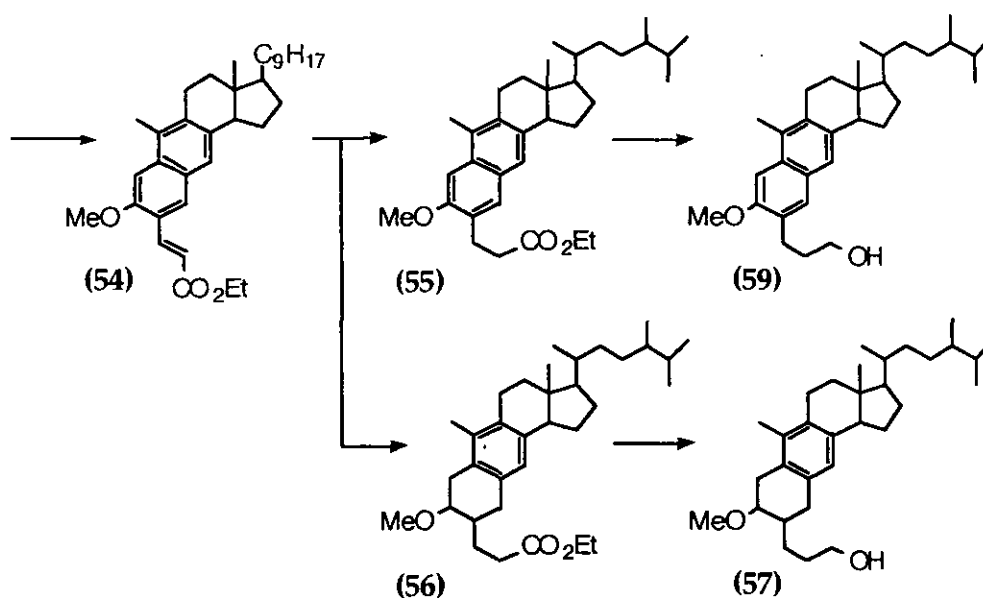
The synthesis according to **Scheme 7.1**, has been successful up to this stage, but the later stages afforded poor yields. The naphthalene ester (**43**) was obtained in quantitative yield. Reduction of (**43**) to the diol (**46**) proceeded quantitatively. Oxidation of the diol to the aldehyde (**47**) using either manganese dioxide or TPAP gave low yields of (**47**), 20-30%. Subsequent coupling of the aldehyde (**47**) with triethylphosphonoacetate gave the HWE product (**48**) in 16 % yield. The last two steps, *i.e.* oxidation and two carbon chain extension, were extremely low yielding steps. The overall yield for the 6-step sequence starting from (**39**) to (**48**), **Scheme 7.1**, was 4 %. The objective was to design a synthetic route that would give maximum yields for each intermediary compound. In this 6-step synthesis, the last two steps gave unsatisfactory yields. Therefore an alternative route was investigated to improve the overall yield.

7.7 Synthesis of 1 (10→6) abeo-2-Carbomethoxy-3-methoxyergosta-1,3,5,7,9,22-hexaene (**51**)

7.7.1 Introduction

An improved route to synthesizing the target molecules (**57**) and (**59**) was proposed, **Scheme 7.6**.

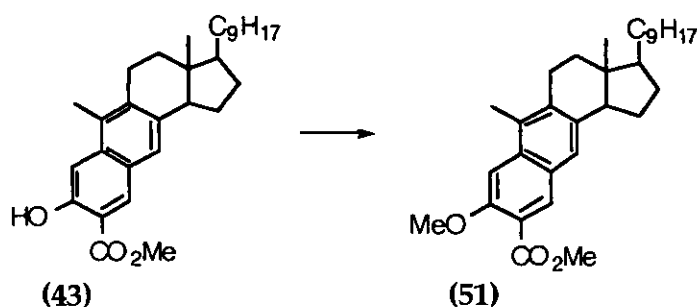




Scheme 7.6

The improved route, **Scheme 7.6**, is essentially the same as **Scheme 7.1**, the modification involved synthesizing the methoxy derivative (51) of the ester (43), **Scheme 7.6**. Reduction of (51) would afford the alcohol (52), then followed by oxidation and Horner-Wadsworth-Emmons (HWE) reaction would yield the methoxy ester (54). Catalytic hydrogenation of (54) would afford (55) and (56), then followed by reduction would yield the target molecules (57) and (59).

7.7.2 Synthesis

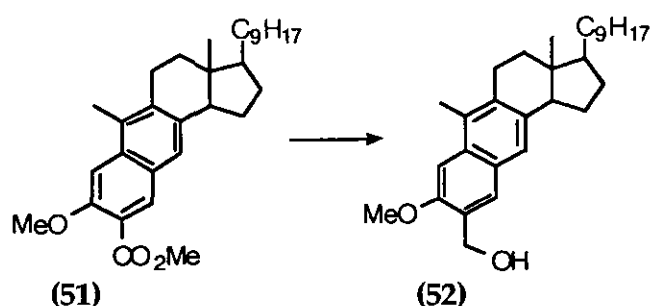


Scheme 7.7

Alkylation of the ester (43) with methyl iodide gave the methoxy ester (51) in good yield. The identity of (51) was confirmed by spectroscopic data. The ¹H n.m.r spectrum showed a singlet at δ 3.99, integrating for three protons,

corresponded to the methoxy group at C-3. The O-methyl group of the ester function has been shifted slightly upfield relative to its position in the original hydroxyester (43) and appeared as a singlet at δ 3.94, integrating for three protons. The aromatic protons at C-1, C-7 have also been shifted upfield and appeared as singlets at δ 8.25 and δ 7.30. The accurate mass was consistent with the molecular formula $C_{31}H_{42}O_3$ and the mass spectrum by electron impact gave a 100 % molecular ion peak at 462.

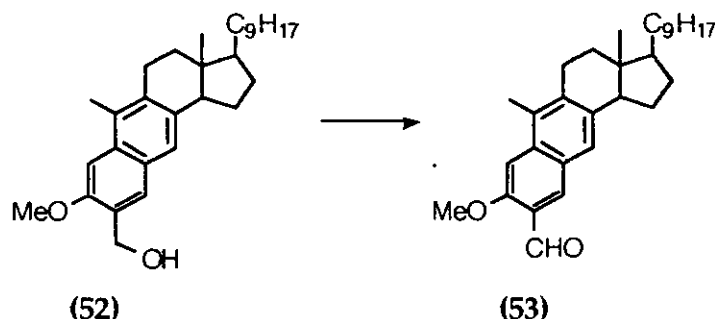
7.8 Synthesis of 1 (10→6) abeo-2-Hydroxymethyl-3-methoxyergosta-1, 3, 5, 7, 9, 22-hexaene (52)



Scheme 7.8

Reduction with lithium aluminium hydride gave the alcohol (52) in 90 % yield. From the IR spectrum, a broad absorption peak at 3435 cm^{-1} corresponding to the hydroxyl function was observed. The ^1H n.m.r spectrum showed a singlet at δ 3.91, integrating for three protons, corresponding to the OMe group at C-3. The benzylic protons appeared as a singlet at δ 4.76, integrating for two protons. The three aromatic protons at C-1, C-4, and C-7 appeared as singlets at δ 7.58, δ 7.16 and δ 7.22, respectively. The accurate mass was found to be 434.3184 which was consistent with the molecular formula $C_{30}H_{42}O_2$.

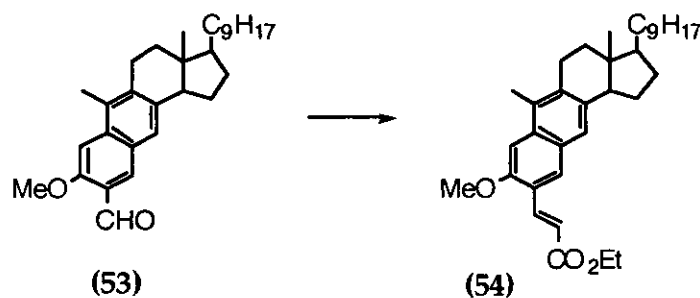
7.9 Synthesis of 1 (10→6) abeo-2-Formyl-3-methoxyergosta-1, 3, 5, 7, 9, 22-hexaene (53)



Scheme 7.9

Oxidation of (52) with TPAP gave the aldehyde (53) in 80 % yield. This was an improvement over the oxidation of the diol (46) to (47) in **Scheme 7.4**, where the best yield was 30 %. The IR spectrum showed a broad carbonyl stretching frequency at 1685 cm^{-1} . The ^1H n.m.r spectrum showed a singlet at δ 10.53, integrating for one proton, which corresponded to the aldehyde group. The aromatic protons at C-1, C-4, and C-7 were shifted downfield to δ 8.24, δ 7.24, and δ 7.28 respectively. The accurate mass was consistent with the molecular formula, $\text{C}_{30}\text{H}_{40}\text{O}_2$.

7.10 Synthesis of Ethyl 3-[1 (10→6) abeo-3-Methoxyergosta-1, 3, 5, 7, 9, 22-hexaen-2-yl] propenoate (54)

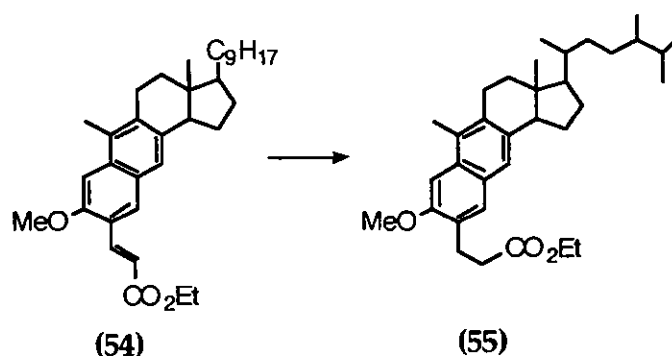


Scheme 7.10

A two carbon chain extension was carried out on (53) using Horner-Wadsworth-Emmons (HWE) reaction, previously discussed in Section 7.5.

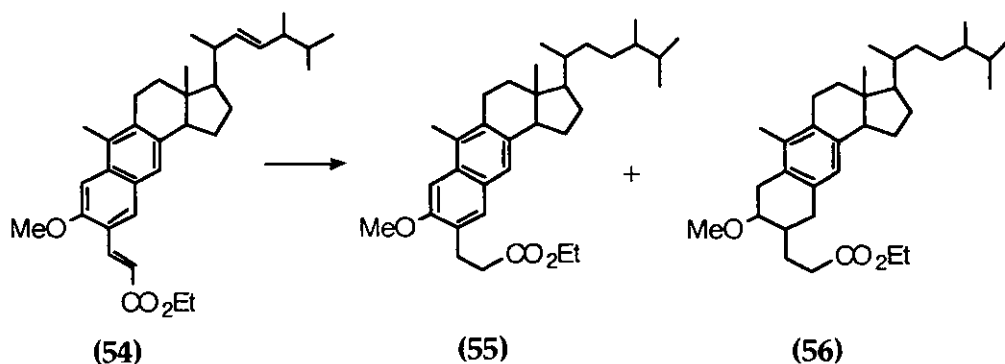
Triethylphosphonoacetate was treated with sodium hydride to generate the phosphonate carbanion which when reacted with the aldehyde (53) afforded the HWE product (54) in 78 % yield. This was a vast improvement over the previous reaction, with the hydroxy derivative (48), **Scheme 7.5**, where the yield obtained was 16 %. The IR spectrum showed a sharp carbonyl absorption at 1702 cm^{-1} , corresponding to the ester group. The ^1H n.m.r spectrum gave a triplet at δ 1.35, integrating for three protons, corresponding to the methyl group on the ester function and a quartet at δ 4.27, integrating for two protons, corresponding to the methylene protons on the ester function. The olefinic protons at C-1' and C-2' appeared as doublets, with a *trans* coupling constant, $J=16\text{ Hz}$, at δ 8.05 and δ 6.68, respectively.

7.11 Synthesis of Ethyl 3-[1 (10→6) abeo-3-Methoxyergosta-1, 3, 5, 7, 9-pentaen-2-yl] propanoate (55) and Ethyl 3-[1 (10→6) abeo-3-Methoxyergosta-5, 7, 9-trien-2-yl] propanoate (56)



Scheme 7.11

Catalytic hydrogenation of (54) in freshly distilled ethyl acetate with 10 % palladium on carbon gave the unsaturated ester (55) in 84 % yield. When the reaction was carried out in ethyl acetate that has been allowed to stand for some time, a mixture of products was obtained. Purification by column chromatography on silica gave two products. From spectroscopic evidence, two esters were obtained, one was (55), in which the ring A was aromatic, and the other was (56), where ring A was saturated, **Scheme 7.12**.



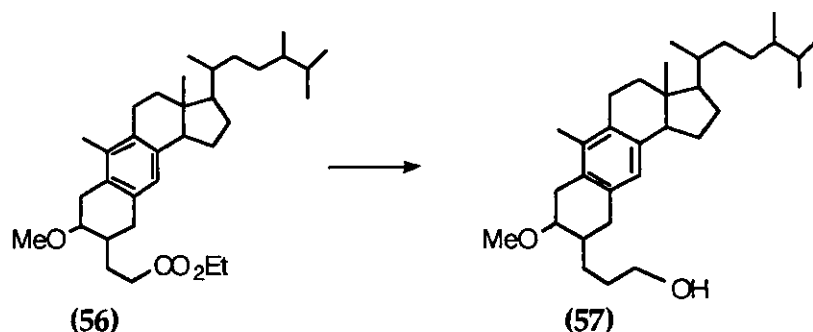
Scheme 7.12

It could be that in the older ethyl acetate there was traces of acetic acid due to oxidation in air when it was allowed to stand for some time on the bench. The acid present in the solvent aided hydrogenation of the aromatic ring affording the saturated compound (56). The experiment was repeated again using freshly distilled and dried solvent, the only product recovered was the aromatic compound (55).

From the ^1H n.m.r of (55), the absence of a multiplet at δ 5.25, indicated that the side chain olefin at C-22 had been reduced. The aromatic protons at C-1, C-4, and C-7 appeared at δ 7.49, δ 7.18 and δ 7.23, respectively. The methylene protons at C-1' and C-2' of the C-2 substituted side chain, appeared as triplets at δ 2.66 and δ 3.03, respectively, with coupling constant of 8 Hz.

From the ^1H n.m.r spectrum of (56), the aromatic signals for C-1 and C-4 were absent, as was the multiplet at δ 5.25, indicating that they had been reduced. The methine proton at C-3 appeared as a multiplet centred at δ 3.63. The signal for the aromatic proton at C-7 was shifted upfield to δ 6.61. The accurate mass for both compounds, (55) and (56), were consistent with their molecular formula.

7.12 Synthesis of 3-[1 (10→6) abeo-3-Methoxyergosta-5, 7, 9-trien-2-yl]-1-propanol (57) and 3-[1 (10→6) abeo-3-Methoxyergosta-1, 3, 5, 7, 9-pentaen-2-yl]-1-propanol (59)



Scheme 7.13

Reduction of the ester (56) with lithium aluminium hydride yielded the alcohol (57) as an oil in 40 % yield. The IR spectrum of (57) showed a broad absorption peak at 3435 cm^{-1} , indicating the presence of a hydroxyl function. The methylene protons 1'-H and 2'-H on the propanol side chain at C-2, were probably hidden under a broad multiplet between $\delta\ 2.71\text{--}2.79$ and the methylene protons at C-1 and C-4 were also probably hidden under a broad multiplet between $\delta\ 1.00$ and $\delta\ 2.00$. The multiplet centred at $\delta\ 3.64$, integrating for three protons corresponded to C-3' methylene protons and the methine proton at C-3.

The alcohol (57) was derivatised as the acetate (58), Fig. 28, by reacting (57) with pyridine and acetic anhydride. The crude product was recrystallised from methanol to yield white crystals, m.p. $48\text{--}50^\circ\text{C}$. The IR spectrum showed a sharp carbonyl stretching frequency at 1727 cm^{-1} . The ^1H n.m.r spectrum showed a singlet at $\delta\ 2.07$, integrating for three protons, corresponding to the acetate group. The methylene protons at C-3' appeared as a triplet at $\delta\ 4.07$, integrating for two protons.

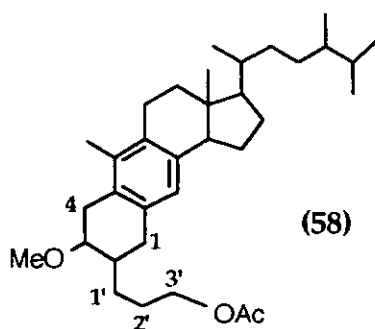
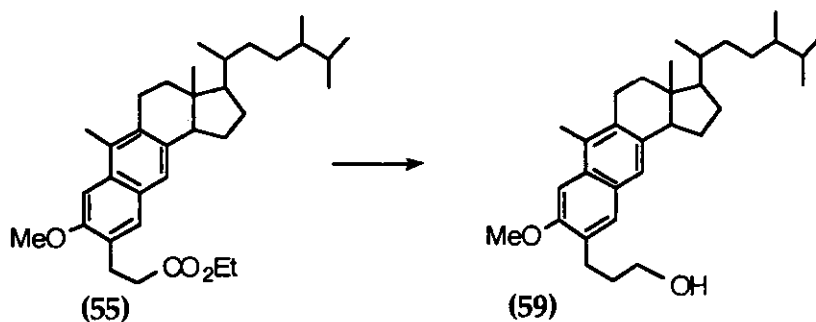


Fig. 28

Reduction of (55) was carried out in a similar manner to yield the aromatic alcohol (59) in 82 % yield, **Scheme 7.14**. The IR spectrum of (59) showed a broad absorption at 3488 cm^{-1} , corresponding to the hydroxyl group. From the ^1H n.m.r spectrum, the methylene protons on the C-2 substituted side chain appeared as multiplets centred at δ 1.91, δ 2.83, and δ 3.61, each multiplet integrating for two protons. The elemental analysis was found to be C 82.58, H 10.66 which calculated for $\text{C}_{32}\text{H}_{48}\text{O}_2$, required C 82.70 and H 10.41.



Scheme 7.14

7.13 Conclusion

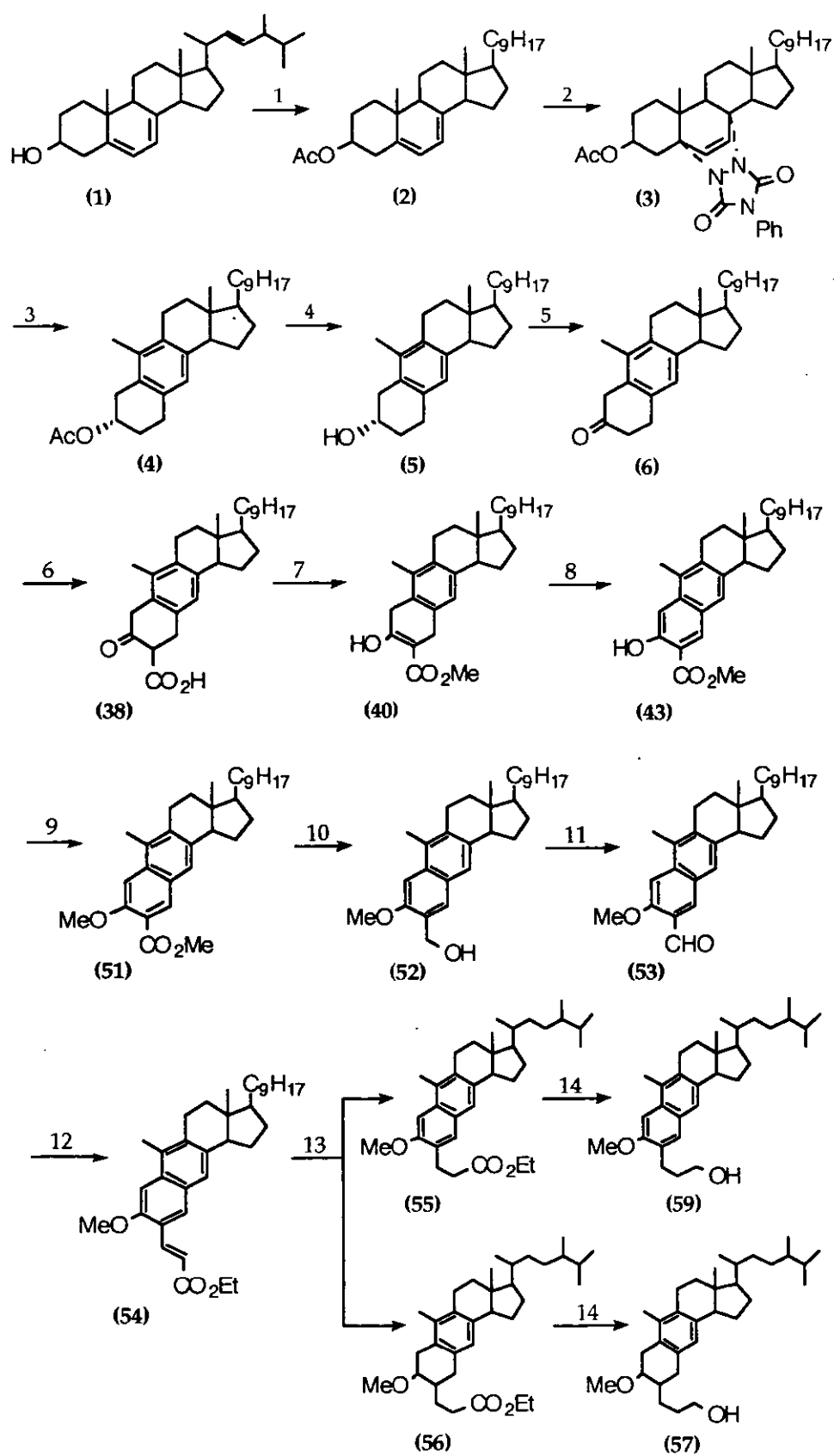
We have achieved the synthesis of two target molecules, (57), and (59). The overall yield for the 9-step sequence, **Scheme 7.6**, from (6) to (59), was 24 %.

Chapter Eight

8.1 Final Conclusion

The objective of this project was to synthesize hydroxylated anthrasteroids as potential anti-tumour agents. It was our aim to develop new vitamin D antagonists that would mimic the action of 1,25 DHCC of inducing cell differentiation and suppressing cell proliferation without exhibiting potent calcemic effects, which has limited the use of 1,25 DHCC in cancer therapy.

The synthesis of two target hydroxyanthrasteroids, namely 3-[1 (10→6) abeo-3-methoxyergosta-5, 7, 9-trien-2-yl]-1-propanol (**57**) and 3-[1 (10→6) abeo-3-methoxyergosta-1, 3, 5, 7, 9-pentaen-2-yl]-1-propanol (**59**) have been achieved. The best route of synthesis of (**59**), **Scheme 8.1**, involved a 14-step sequence starting with ergosterol. Acetylation of ergosterol (**1**) gave (**2**) in quantitative yield, followed by Diels Alder reaction afforded the adduct (**3**) in quantitative yield. Oxidative rearrangement of (**3**) gave 1 (10→6) abeo-3 α -acetoxyergosta-5, 7, 9, 22--tetraene (**4**) in 75 % yield. Alkaline hydrolysis gave the alcohol (**5**) in quantitative yield, followed by oxidation to the ketone (**6**) in 80 % yield. Reacting (**6**) with MMC and diazomethane afforded a β -keto ester (**40**) in 80 % yield. Aromatisation of (**40**) gave (**43**) in quantitative yield, followed by alkylation which yielded the methoxy derivative (**51**) in 90 % yield. Reduction of (**51**), followed by oxidation of (**52**) with TPAP gave the aldehyde (**53**) in moderate yield. Carrying out a Horner-Wadsworth-Emmons reaction afforded (**54**) in 78 % yield. Catalytic hydrogenation of (**54**), gave (**55**) in 85 % yield, followed by reduction gave the target molecule (**59**) in 80 % yield. Alternatively, when catalytic hydrogenation of (**54**), was carried out with ethyl acetate that had been allowed to stand for some time, (**56**) was obtained in 64 % yield, followed by reduction to give the saturated target molecule (**57**) in 40 % yield. The overall yield of 12 % for the 14-step sequence was satisfactory.

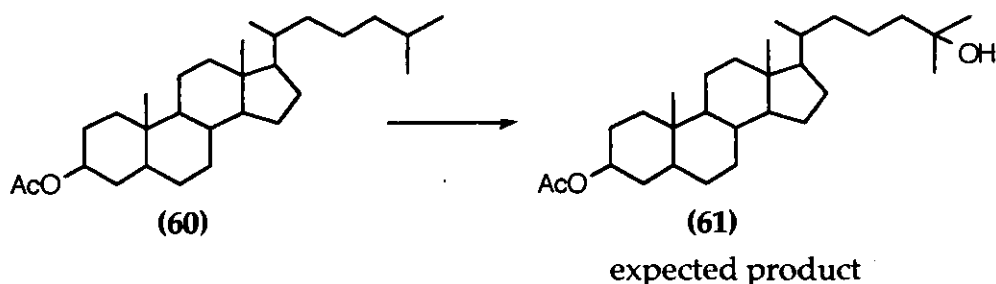


Scheme 8.1

8.2 Future Work

It was proposed that 25-hydroxylation of the target compounds (59) and (57) could be effected with dioxirane reagents. There has been literature precedence for the use of such reagents.^{122,123}

Some preliminary work had been carried out to investigate direct insertion of a hydroxyl group at C-25. Model studies were carried out on dihydrocholesterol-3-acetate (60), Scheme 8.2.



Scheme 8.2

Acetylation of dihydrocholesterol afforded dihydrocholesterol-3-acetate in quantitative yield. Dihydrocholesterol-3-acetate (60) was treated with dimethyl dioxirane¹²⁴ and stirred overnight at 0 °C. We failed to obtain the desired 25-hydroxyl compound (61). We attempted the reaction again using the dioxirane from cyclohexanone, i.e. 1,2-dioxaspiro[2.5] octane,¹²⁵ to effect 25-hydroxyl insertion. The desired product (61) was not obtained. From spectroscopic data, starting material (60) was recovered in each case. Initial attempts to effect direct hydroxylation at C-25 seemed to have failed, further investigation is required.

Chapter Nine

EXPERIMENTAL

General Procedures

All solvents were distilled before use. Tetrahydrofuran (THF) was distilled from sodium in the presence of benzophenone. Dichloromethane (DCM) was distilled from phosphorus pentoxide. Diethyl ether (Et_2O), ethyl acetate (EtOAc) and petroleum ether (40-60°C) were distilled from calcium chloride. Dimethylformamide (DMF) and dimethylsulphoxide (DMSO) were dried over calcium hydride, distilled under reduced pressure and stored over molecular sieves under nitrogen. Methanol (MeOH) and ethanol (EtOH) were dried over dry magnesium turnings with a trace of iodine, distilled and stored over molecular sieves under nitrogen. Chloroform (CHCl_3) was distilled from phosphorus pentoxide. Pyridine was dried over potassium hydroxide and distilled. Toluene and benzene (C_6H_6) were distilled from sodium hydride and stored over sodium wire. All solutions of compounds were dried over anhydrous magnesium sulphate unless otherwise stated. Glassware used in the reactions was usually flamed dried under nitrogen and reactions were carried out under inert atmosphere of nitrogen or argon unless otherwise stated.

Analytical TLC was carried out on aluminium backed plates coated with Merck Kieselgel 60 F₂₅₄ and compounds were visualised by acidic ammonium molybdate (IV) or iodine as appropriate. Preparative TLC was performed on 1 m by 20 cm plates or 20 cm by 20 cm plates coated with Merck Kieselgel 60 PF₂₅₄ + 366 (0.75 mm thickness). Flash chromatography was performed on Matrex® Silica 60 (35-70 micron).

Melting points (uncorrected) were determined on a Kofler hot-stage and digital melting point apparatus.

Ultraviolet spectra were recorded on a Shimadzu UV-160 spectrophotometer, using 1 cm silica cells and ethanol as solvent.

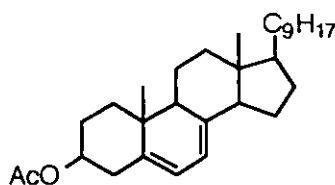
Infra-red spectra were recorded on a Nicolet-205 FTIR, Philips PU-9510 or Perkin-Elmer 1600 series FTIR spectrophotometers. The technique used was either nujol mull or a solution in chloroform or neat film.

Proton NMR spectra (^1H n.m.r) were recorded at 60, 90, 250 or 400 MHz on either a Varian EM-390, Perkin-Elmer R32, Bruker 250AC or Bruker 400AC spectrometers. NOE experiments and COSY plots were carried out at the Centre for Nuclear Magnetic Resonance, University of Warwick. Carbon NMR were recorded at 63 MHz or 100 MHz on either a Bruker 250AC or Bruker 400AC spectrometers. The samples were dissolved in deuterated chloroform and tetramethylsilane (TMS) was used as internal reference. Coupling constants (J) were measured in Hz.

Mass spectra were recorded on a Kratos MS-80 spectrometer using electron impact (EI).

Elemental analyses were sent to Medac Ltd., Department of Chemistry Brunel University Uxbridge Middlesex, UB8 3PH.

Experimental for Chapter 3

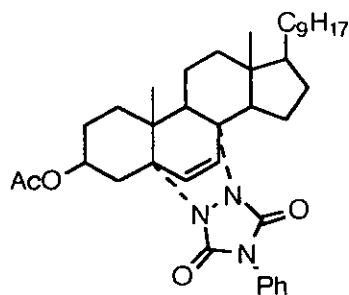


Ergosta-5, 7, 22-trien-3 β -acetate (2)

Ergosterol (1.0 g, 2.52 mmol) was dissolved in dry pyridine (10 ml, 62 mmol) and acetic anhydride (10 ml, 52 mmol) was added. The mixture was allowed to stand at room temperature for 18 hours, and then added to ice and allowed to stand for a further 1 hour. The precipitate was then filtered and washed with 2M hydrochloric acid, saturated sodium hydrogen carbonate, water and dried in a vacuum oven. The product was recrystallised from methanol affording the acetate (2) as white crystals. Yield (1.0 g, 90 %), m.p. 170-173°C (lit.,⁷⁹ 173-175°C). ν_{\max} (nujol) 2927, 2954, 2869, 2854, 1734, 1460, 1376 and 1367 cm^{-1} ; δ_{H} (250 MHz; CDCl_3) 0.62 (3 H, s, 13-Me), 0.81-0.85 (6 H, m, 25-Me), 0.90 (3 H, s 10-Me), 0.94 (3 H, d, J 6, 24-Me), 1.03 (3 H, d, J 6, 20-Me), 2.02 (3 H, s, OAc), 2.37 (1 H, m, 4 β -H), 2.50 (1 H, m, 4 α -H), 4.63-4.75 (1 H, m, 3 α -H), 5.17-5.25 (2 H, m, 22-H and 23-H), 5.36-5.37 (1 H, m, 7-H) and 5.56-5.57 (1 H, m, 6-H); δ_{C} (63 MHz; CDCl_3) 170.3 (C-29), 141.3 (C-8), 138.4 (C-5), 135.5 (C-22), 131.9 (C-23), 120.2 (C-6), 116.3 (C-7), 72.7 (C-3), 55.7 (C-17), 54.4 (C-14), 46.0 (C-9), 42.8 (C-13), 42.7 (C-24), 40.4 (C-20), 39.0 (C-12), 37.9 (C-1), 36.6 (C-4), 33.0 (C-25), 28.2 (C-16), 28.1 (C-2), 22.9 (C-15), 21.1 (C-30), 21.1 (C-21), 20.5 (C-11), 19.9 (C-27), 19.6 (C-26), 17.6 (C-28), 16.1 (C-19), and 12.0 (C-18); (Found: M^+ , 438.3500. $\text{C}_{30}\text{H}_{46}\text{O}_2$ requires 438.3497); m/z (EI) 438 (M^+ , 2 %), and 378 ($M^+ - \text{OAc}$, 100).

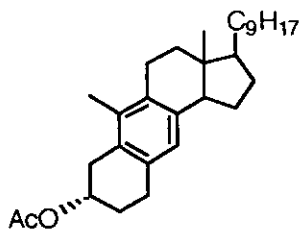
Preparation of 4- Phenyl-1, 2, 4-triazoline-3, 5-dione (PTAD)

4-phenylurazole (9.92g, 0.014 mol) was dissolved in 1,4-dioxan (280 ml) and *t*-butylhypochlorite (7.4 ml, 0.014 mol) was added. The reaction mixture was stirred at room temperature for 1 hour. The solvent was removed *in vacuo* in a water bath at temperatures not exceeding 40°C. The crude product obtained was further purified by sublimation on a cold finger. The red carmine needles were collected and stored in a dark container and kept in the refrigerator.



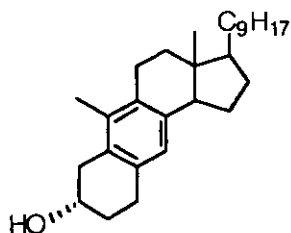
3',5'-Dioxo-4'-phenyl-5 α ,8 α -[1',2']1',2',4'-triazolindinoergosta-6,22-dien-3 β -acetate (3)

Ergosteryl acetate (2) (1.0 g, 2.27 mmol) was dissolved in dichloromethane (10 ml, 0.156 mol). The solution was titrated at room temperature with a solution of 4-phenyl-1,2,4-triazoline-3,5-dione (PTAD) in acetone until a pink coloration persisted. The solvent was removed *in vacuo* and recrystallised from methanol gave white crystals. Yield (1.26 g, 92%). m.p. 170-175°C (lit.,⁸¹ 173-175°C). ν_{\max} . (nujol) 2955, 2926, 2868, 1757, 1736, 1707, 1503, 1458, 1397, 1378, and 1241 cm^{-1} ; δ_{H} (250 MHz; CDCl_3) 0.79 (3 H, s, 13-Me), 0.81-0.84 (6 H, m, 25-Me), 0.89 (3 H, d, J 6, 24-Me), 0.97 (3 H, s, 10-Me), 1.02 (3 H, d, J 6, 20-Me), 2.00 (3 H, s, OAc), 3.23 (1 H, dd, J 5, 14, 4-H), 5.18-5.22 (2 H, m, 22-H and 23-H), 5.39-5.50 (1 H, m, 3 α -H), 6.21 (1-H, d, J 8, 6-H), 6.40 (1 H, d, J 8, 7-H), and 7.27-7.46 (5 H, m, Ph); δ_{C} (63 MHz; CDCl_3) 169.9 (C-29), 149.0 (C-5'), 146.4 (C-3'), 135.1 (C-6), 135.0 (C-22), 132.3 (C-23), 131.7, 129.1 (C-7), 128.7, 127.6, 126.1, 70.4 (C-3), 65.2 (C-5), 64.8 (C-8), 55.0 (C-17), 52.7, 49.2, 43.8, 42.7 (C-24), 41.0 (C-13), 39.5 (C-12), 38.0 (C-4), 33.6 (C-1), 33.0 (C-25), 30.9, 27.5, 25.9, 23.3, 22.3, 21.3 (C-30), 21.2 (C-21), 19.9 (C-27), 19.6 (C-26), 17.5 (C-28), 17.4 (C-19), and 13.2 (C-18). [Found; (M-OAc-PTAD), 378.3264. $\text{C}_{27}\text{H}_{43}\text{O}_2$ requires 378.3264]; m/z (EI) 613 (M^+ , <0.1 %), 378 [$(\text{M}^+ - \text{OAc-PTAD})$, 30], 177 (PTAD, 100).



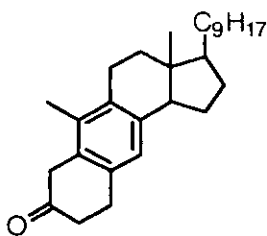
1 (10→6) abeo-3 α -Acetoxyergosta-5, 7, 9, 22-tetraene (4)

3',5'-Dioxo-4'-phenyl-5 α ,8 α -[1',2']1',2',4'-triazolindinoergosta-6,22-dien-3-acetate (3) (1 g, 1.62 mmol) was dissolved in dried benzene (25 ml) and boron-trifluoride-ether (5 ml) was added. The mixture was stirred for 3 hours under nitrogen at room temperature. The reaction mixture was diluted with water (100 ml) and the product was extracted with diethyl ether. The ether layer was washed with saturated sodium hydrogen carbonate and water and dried over anhydrous magnesium sulphate. The solvent was removed *in vacuo*. An oily residue was obtained and was recrystallised from methanol to give (4). Yield (0.51 g, 70 %). m.p. 80-82°C; (Found: C, 82.9; H, 10.3. Calculated for C₃₀H₄₄O₂: C, 82.51; H, 10.16 %); ν_{\max} . (nujol) 2955, 2926, 2870, 2854, 1740, 1462, 1377, 1366, and 1244 cm⁻¹; δ_H (400 MHz; CDCl₃) 0.57 (3 H, s, 13-Me), 0.81-0.88 (6 H, m, 25-Me), 0.93 (3 H, d, J 7, 24-Me), 1.08 (3 H, d, J 7, 20-Me), 2.04 (3 H, s, OAc), 2.07 (3 H, s, 10-Me), 2.63-2.75 (3 H, m, 4-H and 11-H), 2.82-2.85 (2 H, m, 1-H), 3.04 (1 H, dd, J 6, 15, 4-H), 5.18-5.28 (3 H, m, 3-H, 22-H and 23-H), and 6.65 (1 H, s, 7-H); δ_C (63 MHz; CDCl₃) 170.8 (C-29), 137.9, 135.6 (C-22), 134.1, 132.4, 132.1, 132.0 (C-23), 129.1, 123.8 (C-7), 70.6 (C-3), 55.1 (C-17), 51.8, 42.8 (C-24), 41.7 (C-13), 40.5 (C-20), 37.1 (C-10), 33.1 (C-25), 32.7, 29.2, 27.5 (C-16), 26.8, 25.8, 24.2, 21.4 (C-30), 21.0 (C-21), 19.9 (C-27), 19.6 (C-26), 17.6 (C-28), 14.5 (C-19), and 11.3 (C-18); (Found; M⁺, 436.3342. C₃₀H₄₄O₂ requires 436.3341). *m/z* 436 (M⁺, <0.3%), 376 [(M-OAc), 100].



1 (10→6) abeo-3 α -Hydroxyergosta-5, 7, 9, 22-tetraene (5)

1 (10→6) abeo-3 α -acetoxyergosta-5, 7, 9, 22-tetraene (**4**) (1.0 g, 2.29 mmol) was dissolved in 2% w/v potassium hydroxide in ethanol (65 ml). The reaction was stirred for 1 hour. After which the product was taken up in diethyl ether and washed with 2M hydrochloric acid, water, saturated sodium hydrogen carbonate and water again until neutral to litmus paper and dried over anhydrous magnesium sulphate. The solvent was removed *in vacuo*. The crude product was recrystallised from methanol, affording white crystals of (**5**). Yield (0.82 g, 90 %). m.p. 122-124°C. (Found: C, 85.5; H, 10.9. C₂₈H₄₂O requires C, 85.22; H, 10.73 %); ν_{\max} (nujol) 3355, 2964, 2930, 1463, 1376, 1260 and 1056 cm⁻¹; δ_{H} (250 MHz; CDCl₃) 0.58 (3 H, s, 13-Me), 0.81-0.86 (6 H, m, 25-Me), 0.94 (3 H, d, J 7, 24-Me), 1.08 (3 H, d, J 7, 20-Me), 2.01 (3 H, s, 10-Me), 2.82 (2 H, m, 1-H), 3.03 (2 H, dd, J 5, 15, 4-H), 4.06-4.13 (1 H, m, 3 β -H), 5.21-5.24 (2 H, m, 22-H and 23-H) and 6.63 (1 H, s, 7-H); δ_{C} (63 MHz; CDCl₃) 137.7, 135.6 (C-22), 134.1, 132.5, 132.1, 132.0 (C-23), 129.7, 123.8 (C-7), 68.1 (C-3), 55.1 (C-17), 51.8, 42.8 (C-24), 41.7 (C-13), 40.5 (C-20), 37.1 (C-10), 36.5 (C-4), 33.1 (C-25), 31.3, 29.2, 27.6, 25.7, 24.2, 21.0 (C-21), 19.9 (C-27), 19.6 (C-26), 17.6 (C-28), 14.6 (C-19), and 11.3 (C-18). (Found; M^+ , 394.3245. C₂₈H₄₂O requires 394.3235). m/z 394 (M^+ , 100%).



1 (10→6) abeo-Ergosta-5, 7, 9, 22-tetraen-3-one (6)

Method (a) Oppenauer oxidation

A solution of cyclohexanone (3 ml, 28.5 mmol) in toluene (17 ml, 0.16 mmol) was refluxed for 1 hour in a Dean Stark apparatus. When no more water collected, 1 (10→6) abeo-3 α -hydroxyergosta-5, 7, 9, 22-tetraene (5) (1.0 g, 2.5 mmol) was added to the reaction mixture and refluxed for 1 hour. Aluminium isopropoxide (0.275 g, 13.3 mmol) was added rapidly and refluxing was continued for 20 minutes. The reaction mixture was cooled to 15-20°C and then poured into a separating funnel. The reaction mixture was washed with 2M hydrochloric acid (100 ml), with vigorous shaking. The aqueous layer was re-extracted with diethyl ether (2 x 25 ml). The combined organic layers were washed with water, saturated sodium hydrogen carbonate, water again and dried over anhydrous magnesium sulphate. The solvents were removed *in vacuo* to give an oily residue. The crude product (6) was recrystallised from methanol. Column chromatography on silica, eluting with 20 % ethyl acetate in petroleum ether (40-60°C) may be carried out to further purify the product. Yield (0.746 g, 75 %). m.p. 95-97°C (lit.,⁸² 97-99°C). ν_{max} . (nujol) 2954, 2927, 2855, 1722, 1460, and 1376 cm^{-1} ; δ_{H} (250 MHz; CDCl_3) 0.59 (3 H, s, 13-Me), 0.82-0.85 (6 H, m, 25-Me), 0.93 (3 H, d, J 8, 24-Me), 1.09 (3 H, d, J 8, 20-Me), 2.06 (3 H, s, 10-Me), 3.00 (2 H, t, J 8, 1-H), 3.52 (2 H, s, 4-H), 5.21-5.25 (2 H, m, 22-H and 23-H), and 6.74 (1 H, s, 7-H); δ_{C} (63 MHz; CDCl_3) 211.4 (C-3), 138.4, 135.4 (C-22), 133.3, 133.0, 132.9, 132.0 (C-23), 128.6, 123.0 (C-7), 54.9, 51.7, 42.7, 41.9, 41.5 (C-13), 40.4, 38.6, 36.8, 32.9, 29.0, 28.9, 25.8, 24.0, 20.8 (C-21), 19.8 (C-27), 19.5 (C-26), 17.5 (C-28), 14.5 (C-19), and 11.1 (C-18). (Found; M^+ , 392.3077. $\text{C}_{28}\text{H}_{40}\text{O}$ requires 392.3079); m/z 392 (M^+ , 100%).

Method (b)

To solution of 1 (10→6) abeo-3 α -hydroxyergosta-5, 7, 9, 22-tetraene (5) (1.0 g, 2.5 mmol) in dichloromethane (20 ml) was added celite (1.0g) and in one portion pyridinium chlorochromate (PCC) (2.0 g, 1.5 equivalent). The resulting mixture was stirred at room temperature for 1 hour. After which, the solvent was removed *in vacuo* and the crude product (6) was purified by column chromatography on silica. Yield obtained was 0.463 g, 46.6%.

Method (c)

To a solution of pyridinium dichromate (PDC) (1.43 g, 1.5 equivalent) in dichloromethane (3.6 ml) and pyridinium trifluoroacetate (0.4 equivalent) was added 1 (10→ 6) abeo-3 α -hydroxyergosta-5, 7, 9, 22-tetraene (5) (1.0 g, 2.5 mmol). The reaction mixture was stirred at 25°C for 3 hours. After which, the reaction mixture was diluted with diethyl ether and the solution was filtered through a small amount of magnesium sulphate. The solvents were removed *in vacuo*. The crude product was purified by column chromatography. The yield obtained was very poor.

Method (d) Moffat Oxidation

A solution of 1 (10→ 6) abeo-3 α -hydroxyergosta-5, 7, 9, 22-tetraene (5) (0.79 g, 2 mmol) in 50 % mixture of benzene (3 ml) and DMSO (3 ml), pyridine (0.16 ml) and trifluoroacetic acid (0.08 ml). The reaction mixture was then treated with dicyclohexylcarbodiimide (DCC) (1.24 g, 6 mmol) and the reaction flask was sealed and stirred overnight. The product was taken up in diethyl ether and treated with a solution of oxalic acid (0.54 g, 6 mmol) in methanol (5 ml). Gas was evolved, and after 30 minutes, the mixture was washed with water (50 ml). An insoluble precipitate of dicyclohexylurea was formed, which was removed by filtration. The organic layer was washed with saturated sodium hydrogen carbonate, water, and dried. The solvents were removed *in vacuo*. The product obtained was crystallised from a mixture of diethyl ether and methanol (1:4). The yield obtained was very poor.

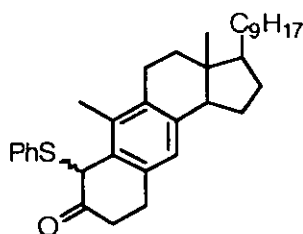
Method (e): Swern Oxidation

To a solution of dichloromethane (25 ml) and oxalyl chloride (2 ml, 22 mmol) was added DMSO (3.4 ml) in dichloromethane (5 ml) at -10°C and the solution was stirred for 2 minutes. After which, a solution of 1 (10→6) abeo-3α-hydroxyergosta-5, 7, 9, 22-tetraene (5) (3.95 g, 10 mmol) in dichloromethane (10 ml) was added slowly over 5 minutes to the reaction mixture, stirring was continued for another 15 minutes at -10°C. Then triethylamine (7 ml) was added and the reaction mixture was stirred for 5 minutes and allowed to warm to room temperature. Then water (50 ml) was added and the aqueous layer was re-extracted with dichloromethane (2 x 25 ml). The combined organic phase was then washed with water, 1 % hydrochloric acid, 5 % sodium sulphite, water, and dried over magnesium sulphate. The solvent was removed *in vacuo* to give an intractable oil. Attempts to purify the crude oil failed.

Method (f) Jones Oxidation

A solution of 1 (10→6) abeo-3α-hydroxyergosta-5, 7, 9, 22-tetraene (5) (1.0 g, 2.5 mmol) in acetone (120 ml) was stirred at 0°C and treated with 8N Jones reagent (1 ml), dropwise. The reaction mixture was stirred for 5-10 minutes. After which the mixture was treated with methanol to remove excess Jones reagent and diluted with water. Solvents were removed *in vacuo*. The residue was taken up in diethyl ether and washed with water, saturated sodium hydrogen carbonate, water and dried. The product was crystallised from methanol and further purified by column chromatography on alumina. The yield obtained was very poor.

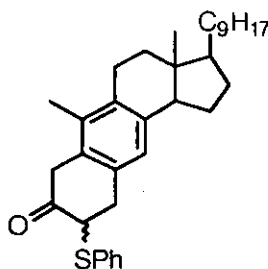
Experimental for Chapter 4



1 (10→6) abeo-4-Sulphenylergosta-5, 7, 9, 22-tetraen-3-one (17)

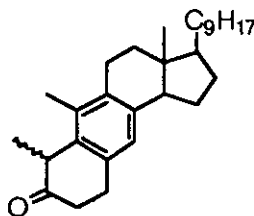
1 (10→6) abeo-ergosta-5, 7, 9, 22-tetraen-3-one (**6**) (0.4 g, 1.0 mmol), was dissolved in THF (5 ml), and cooled to -78°C . Lithium hexamethyldisilazide (1.0 M solution in hexane) (0.46 ml, 2.0 mmol) was then added dropwise to the reaction mixture and the mixture was stirred for 30 minutes. Alternatively, lithium isopropylcyclohexylamide (2 equivalents) was used as a base. Then hexamethylphosphoramide (HMPA) (0.17 ml, 1.0 mmol) was added and stirred at -78°C for another 1 hour. Phenyl disulphide (0.22 g, 1.0 mmol) was then added and stirring was continued for 1 hour at -78°C and then at room temperature for 1 hour. The reaction was monitored by TLC (5 % diethyl ether in hexane). After 5 hours, the crude product obtained was taken up in diethyl ether and washed with 2M hydrochloric acid, water, saturated sodium hydrogen carbonate, water and dried over anhydrous magnesium sulphate. The solvent was removed *in vacuo* and a thick viscous gum was obtained. Preparative TLC on alumina grade 3, eluting with 5 % diethyl ether in n-hexane. The major fraction was obtained as a 50:50 mixture of α and β isomers of 1 (10→6) abeo-4-sulphenylergosta-5, 7, 9, 22-tetraen-3-one (**17**), a yellow gum (0.153 g, 30 %). No further attempt was made to separate the isomers which had very similar R_f values. ν_{max} (nujol) 3060, 2960, 2870, 1705, 1460, and 1380 cm^{-1} ; δ_{H} (60 MHz; CDCl_3) 0.51, 0.60 (3 H, s, 13-Me), 0.75-0.85 (6 H, m, 25-Me), 1.00 (3 H, d, J 5, 24-Me), 2.21, 2.25 (3 H, s, 10-Me), 4.80 (1 H, s, 4-H), 5.21-5.31 (2 H, m, 22-H and 23-H), 6.75 (1 H, s, 7-H), and 7.21-7.45 (5 H, m, Ph); (Found: M^+ 500.3125. $\text{C}_{34}\text{H}_{44}\text{OS}$ requires 500.3112); m/z (EI) 500 (M^+ , <1 %), 391 [(M-SPh), 20], 109 (SPh, 100).

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1 (10→6) abeo-2-Sulphenylergosta-5, 7, 9, 22-tetraen-3-one (7)

The other minor components isolated were the starting ketone (6), and the 2-sulphenylated product (7), as a brown oil, in very poor yield, < 5%. ν_{max} (nujol) 3060, 2960, 2870, 1705, 1580, 1460, 1380 cm^{-1} ; δ_{H} (60 MHz; CDCl_3) 0.55 (3 H, s, 13-Me), 0.80-0.90 (6 H, m, 25-Me), 1.00-1.05 (3 H, m, 24-Me), 1.15 (3 H, m, 20-Me), 2.10 (3 H, s, 10-Me), 3.55 (2 H, s, 4-H), 3.75 (1 H, t, J 6, 2-H), 5.20-5.30 (2 H, m, 22-H, 23-H), 6.75 (1 H, s, 7-H), 7.20-7.40 (5 H, m, Ph).



1 (10→6) abeo-4-Methylergosta-5, 7, 9, 22-tetraen-3-one (22)

Method (a)

1 (10→6) abeo-ergosta-5, 7, 9, 22-tetraen-3-one (6) (1.0 g, 2.55 mmol) was dissolved in dried benzene (4 ml) and methyl iodide (3 ml, 2.5 equivalents) was added dropwise to the solution. The reaction mixture was stirred at 0°C for 15 minutes. Sodium hydride (80 % in oil) (0.196 g, 3.2 equivalents) was slowly added to the reaction mixture and the reaction temperature was kept below 25°C. The mixture was stirred for another 2 hours at room temperature and then refluxed for 1 hour, cooled and washed with 2 M hydrochloric acid then extracted with diethyl ether. The organic layer was washed with water, saturated sodium hydrogen carbonate, water and dried over anhydrous magnesium sulphate. The solvent was removed *in vacuo* to give an intractable brown oil. Column chromatography on silica was carried out in an attempt to purify the crude product, eluting with 20 % ethyl acetate in petroleum ether (40-60°C). The product (22) was obtained as a 50:50 mixture of α and β isomers

in very low yield (0.96 g, 9.3 %). ν_{\max} . (CHCl_3) 3036, 2960, 2872, 1704, 1598, 1456 and 1380 cm^{-1} ; δ_{H} (250 MHz; CDCl_3) 0.58, 0.60 (3 H, s, 13-Me), 0.82-0.88 (6 H, m, 25-Me), 1.05 (3 H, d, J 7, 24-Me), 1.07 (3 H, d, J 7, 20-Me), 1.32, 1.33 (3 H, s, 4-Me), 2.13, 2.14 (3 H, s, 10-Me), 3.70 (1 H, q, J 8, 4-H), 5.21-5.25 (2 H, m, 22-H and 23-H), and 6.75 (1 H, s, 7-H); (Found: M^+ 406.3233. $\text{C}_{29}\text{H}_{42}\text{O}$ requires 406.3235); m/z (EI) 406 (M^+ , 40%).

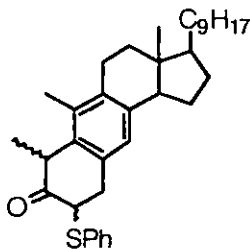
Method (b)

To a solution of 1 (10→6) abeo-ergosta-5, 7, 9, 22-tetraen-3-one (**6**) (0.5 g, 0.127 mmol), in benzene (5 ml) was added sodium hydride (80 % in oil) (0.1 g, 3.3 equivalents). The reaction mixture was stirred at room temperature for 30 minutes, after which methyl iodide (0.2 ml, 2.5 equivalents) was added dropwise over 30 minutes. The mixture was stirred at room temperature for 1.5 hours, then refluxed for 3 hours and monitored by TLC. The reaction mixture was cooled and washed with 2 M hydrochloric acid then extracted with diethyl ether. The organic layer was washed with water, saturated sodium hydrogen carbonate, water and dried over anhydrous magnesium sulphate. The solvent was removed *in vacuo* to give an intractable brown oil. Purification by column chromatography on silica failed to yield any of the desired product.

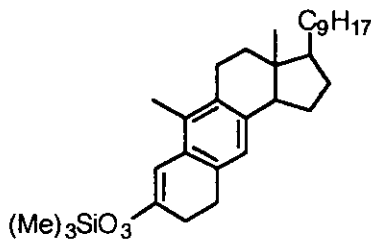
Method (c)

To a solution of potassium (0.4 g, 4 equivalents) in *t*-butylalcohol (20 ml) was added 1 (10→6) abeo-ergosta-5, 7, 9, 22-tetraen-3-one (**6**) (1.0 g, 2.5 mmol). The reaction mixture was stirred until all the ketone had dissolved. Methyl iodide (1.3 ml, 8 equivalents) was added and stirring was continued for 4 hours. The reaction was monitored by TLC until alkylation was completed. After 4 hours the reaction mixture was diluted with water. The product was taken up in diethyl ether and washed with 2M hydrochloric acid, water, saturated sodium hydrogen carbonate, water again, and then dried. The solvent was removed *in vacuo*. An oily product was obtained, further purification by preparative TLC was attempted, but this failed to yield any of the desired product.

Attempted Synthesis of 1 (10→6) abeo-4-Methyl-2-sulphenylergosta-5, 7, 9, 22-tetraen-3-one (23)



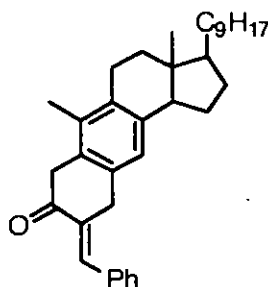
1 (10→6) abeo-4-methylergosta-5, 7, 9, 22-tetraen-3-one (**22**) (0.100 g, 0.246 mmol) was dissolved in THF, dried and freshly distilled, (5 ml). Lithium hexamethyldisilazide (0.055 ml, 1.2 equivalents) was added dropwise to the reaction solution at -78°C and the reaction mixture was stirred for 1 hour. After which, phenyl disulphide (0.053 g, 0.246 mmol) in THF (10 ml) was added, and the reaction mixture was stirred for 2.5 hours at -78°C . The product was extracted with diethyl ether, the organic layer was washed with water, saturated sodium hydrogen carbonate, water and dried over anhydrous magnesium sulphate. The solvent was removed *in vacuo* to give an oily residue. From TLC of the crude product, there were numerous spots and more than one predominant product. Preparative TLC was carried out, eluting with 5 % ethyl acetate in petroleum ether ($40-60^{\circ}\text{C}$), in an attempt to purify the crude oily residue, but this failed to give good separation. Different solvent systems were also tried but they failed to give good separation. No further attempt was made to separate the numerous components obtained.



1 (10→6) abeo-3-(Trimethylsilyloxy) ergosta-3, 5, 7, 9, 22-pentaene (18)

1 (10→6) abeo-ergosta-5, 7, 9, 22-tetraen-3-one (**6**) (0.100 g, 0.254 mmol) was added slowly to a solution of lithium hexamethyldisilazide (0.057 g, 0.243 mmol) in THF (10 ml), dried and freshly distilled at -78°C . The reaction

mixture was stirred for 30 minutes. Trimethylchlorosilane (0.064 ml, 0.500 mmol) was then added rapidly and stirring was continued for 2 hours. The solvent was removed *in vacuo*. The crude product was extracted with pentane. The organic layer was washed with water and dried over anhydrous magnesium sulphate. The solvent was removed *in vacuo*. A brown oil was obtained. From TLC, a mixture of products was observed. Preparative TLC on silica was carried out. The product was unstable and upon purification on silica decomposed. The following spectroscopic data is of the crude product. ν_{\max} (CHCl_3) 2956, 2868, 1634, 1458, 910, 736 cm^{-1} ; δ_{H} (60 MHz, CDCl_3) 0.15 (9 H, br s, SiMe_3), 0.55 (3 H, s, 13-Me), 0.75-0.80 (6 H, m, 25-Me), 0.90 (3 H, d, J 3, 24-Me), 1.02 (3 H, d, J 3, 20-Me), 2.05 (3 H, s, 10-Me), 5.15-5.25 (2 H, m, 22-H, and 23-H), 5.90 (1 H, s, 4-H), 6.70 (1 H, s, 7-H).



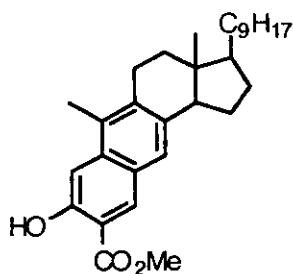
1 (10→6) abeo-2-(Phenylethenoyl) ergosta-5, 7, 9, 22-tetraen-3-one (20)

1 (10→6) abeo-ergosta-5, 7, 9, 22-tetraen-3-one (**6**) (0.100 g, 0.254 mmol) was dissolved in ethanol (10 ml) and potassium hydroxide pellets (0.0224 g, 0.4 mmol) was added to the solution. The mixture was stirred until the potassium hydroxide had dissolved. Benzaldehyde (0.04 ml, 0.4 mmol) was then added. The reaction mixture was refluxed for 2 hours. The crude product was extracted with diethyl ether and washed with water, 2M hydrochloric acid, water, and dried over anhydrous magnesium sulphate. The solvent was removed *in vacuo*. A brown oil was obtained, 0.3 g, 60 %. ν_{\max} (CHCl_3) 2956, 2928, 2872, 1726, 1602, 1490, 1452, and 1382 cm^{-1} ; δ_{H} (60 MHz; CDCl_3) 0.55 (3 H, s, 13-Me), 0.75-0.85 (6 H, m, 25-Me), 0.95-1.00 (3 H, m, 24-Me), 2.35 (3 H, s, 10-Me), 1.1 (3 H, br s, 20-Me), 4.05 (2 H, br s, 4-H), 5.15-5.25 (2 H, m, 22-H, and 23-H), 7.15 (5 H, m, Ph), and 7.35 (1 H, s, Ph-H). (Found: M^+ , 480.3393. $\text{C}_{35}\text{H}_{44}\text{O}$ requires 480.3392; m/z (EI) 480 (M^+ , 20%).

Attempted Carboalkylation of 1 (10→6) abeo-Ergosta-5, 7, 9, 22-tetraen-3-one (6)

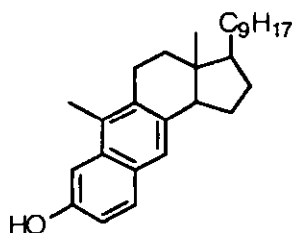
Method (a)

A solution of 1 (10→6) abeo-ergosta-5, 7, 9, 22-tetraen-3-one (**6**) (0.4 g, 1.0 mmol) in dimethyl carbonate (2 ml) at 0°C was treated with 25 % w/v methanolic sodium methoxide. The reaction mixture was refluxed at 70°C for 18 hours, then cooled to 0°C and 2M hydrochloric acid was added. The crude product was extracted with diethyl ether and washed with water, saturated sodium hydrogen carbonate, water and dried over anhydrous magnesium sulphate. The solvent was removed *in vacuo* to give a brown oil. Preparative TLC on silica, eluting with 20% ethyl acetate in petroleum ether (40-60°C) showed that a mixture of products had been obtained. Three major fractions were recovered. Two of the major fractions were identified and the third contained the starting ketone (**6**).



1 (10→6) abeo-2-Carbomethoxy-3-hydroxyergosta-1, 3, 5, 7, 9, 22-hexaene (**30**)

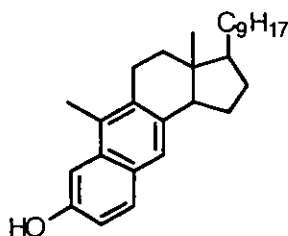
Fraction (1): ν_{max} (CHCl₃) 3268, 2956, 2868, 1706, 1676, 1628, 1456, 1440 and 1380 cm⁻¹; δ_{H} (250 MHz; CDCl₃) 0.56 (3 H, s, 13-Me), 0.83-0.87 (6 H, m, 25-Me), 0.92-0.96 (3 H, m, 24-Me), 1.08-1.11 (3 H, m, 20-Me), 2.47 (3 H, s, 10-Me), 4.00 (3 H, s, CO₂Me), 5.23-5.26 (2 H, m, 22-H, and 23-H), 7.26 (1 H, s, 4-H), 7.45 (1 H, s, 7-H), 8.37 (1 H, s, 1-H), 10.32 (1 H, s, OH).



1 (10→6) abeo-3-Hydroxyergosta-1, 3, 5, 7, 9, 22-hexaene (**29**)

Fraction (2): ν_{max} (CHCl₃) 3416, 3028, 2956, 2868, 1732, 1628, 1602, 1458, and 1380 cm⁻¹; δ_{H} (250 MHz, CDCl₃) 0.55 (3 H, s, 13-Me), 0.83-0.88 (6 H, m, 25-Me),

0.95 (3 H, d, J 7, 24-Me), 1.11 (3 H, d, J 7, 20-Me), 2.45 (3 H, s, 10-Me), 4.97 (1 H, br s, OH), 5.21-5.28 (2 H, m, 22-H, and 23-H), 6.98 (1 H, dd, J 3, 10, 2-H), 7.26 (1 H, s, 7-H), 7.31 (1 H, d, J 3, 4-H), 7.64 (1 H, d, J 10, 1-H).

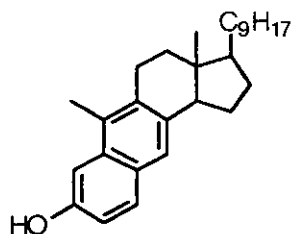


1 (10→6) abeo-3-Hydroxyergosta-1,3,5,7,9,22-hexaene (29)

Method (b)

A mixture of 1 (10→6) abeo-ergosta-5,7,9,22-tetraen-3-one (6) (0.25 g, 0.64 mmol) and sodium hydride (60 % in oil) (0.073 g, 1.5 equivalent), in dimethyl carbonate (4 ml) with a catalytic amount of methanol, was refluxed under nitrogen for 30 hours, the reaction mixture was cooled and 2M hydrochloric acid was added. The crude product was extracted with diethyl ether and washed with water, saturated sodium hydrogen carbonate, water and dried over anhydrous magnesium sulphate. The solvent was removed *in vacuo* to give a black oil. Column chromatography on silica, eluting with 20 % ethyl acetate in petroleum ether (40-60°C), afforded a brown oil as the major product. ν_{\max} (CHCl₃) 3588, 3416, 2956, 2868, 1728, 1602, 1458, and 1380 cm⁻¹; δ_{H} (250 MHz; CDCl₃) 0.55 (3 H, s, 13-Me), 0.83-0.87 (6 H, m, 25-Me), 0.94 (3 H, d, J 7, 24-Me), 1.10 (3 H, d, J 7, 20-Me), 2.42 (3 H, s, 10-Me), 5.07 (1 H, br s, OH), 5.22-5.28 (2 H, m, 22-H and 23-H), , 6.98 (1 H, dd, J 3, 9, 2-H), 7.24 (1 H, s, 7-H), 7.28 (1 H, d, J 2, 4-H), and 7.60 (1 H, d, J 9, 1-H);

Experimental for Chapter 5



1 (10→6) abeo-3-Hydroxyergosta-1, 3, 5, 7, 9, 22-hexaene (29)

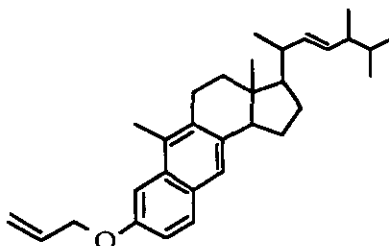
Method (a)

A solution of 1 (10→6) abeo-ergosta-5, 7, 9, 22-tetraen-3-one (**6**) (0.5 g, 1.27 mmol), pyrrolidine hydrotribromide (PHT) (0.32 g, 1.2 mmol) in THF (20 ml), dried and freshly distilled, was stirred at 0°C for 1 hour. After which, the crude product was extracted with diethyl ether. The organic layer was washed with water and dried over anhydrous magnesium sulphate. The solvent was removed *in vacuo*. Column chromatography on silica, eluting with 20 % ethyl acetate in petroleum ether (40-60°C) was carried out to give a light brown solid. Yield (0.383 g, 77%). m.p. 141.2-142.5°C (lit.,⁸² 145°C). ν_{max} . (nujol) 3424, 2956, 2852, 1626, 1600, 1458, 1376, and 1236 cm^{-1} ; δ_{H} (250 MHz; CDCl_3) 0.57 (3 H, s, 13-Me), 0.82-0.86 (6 H, m, 25-Me), 0.95 (3 H, d, J 7, 24-Me), 1.11 (3 H, d, J 7, 20-Me), 2.47 (3 H, s, 10-Me), 4.88 (1 H, s, OH), 5.23-5.26 (2 H, m, 22-H and 23-H), 6.99 (1 H, dd, J 3, 9, 2-H), 7.24 (1 H, s, 7-H), 7.31 (1 H, d, J 3, 4-H), and 7.64 (1 H, d, J 9, 1-H). δ_{C} (63 MHz; CDCl_3) 152.7 (C-3), 136.9, 135.5 (C-22), 133.4, 132.3, 132.1 (C-23), 129.6 (C-1), 128.8, 127.4, 122.1 (C-7), 116.0 (C-2), 106.1 (C-4), 55.4, 52.3, 42.8, 41.9 (C-13), 40.5, 37.2, 33.1, 29.2, 26.2, 24.3, 21.0 (C-21), 19.9 (C-27), 19.6 (C-26), 17.6 (C-28), 14.2 (C-19), and 11.7 (C-18). (Found; M^+ , 390.2931. $\text{C}_{28}\text{H}_{38}\text{O}$ requires 390.2922); m/z (EI) 390 (M^+ , 100%).

Method (b)

A mixture of 1 (10→6) abeo-ergosta-5, 7, 9, 22-tetraen-3-one (**6**) (0.257 g, 0.64 mmol), 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) (0.19 g, 0.84 mmol) and *p*-toluenesulphonic acid (0.16 g, 0.84 mmol) in 1,4-dioxane (25 ml) was refluxed for 8 hours. The reaction mixture was cooled and the residual DDQ was removed by filtration and the solvent was removed *in vacuo*. The crude product obtained was dissolved in diethyl ether and was washed

with sodium sulphite, 5 % sodium hydroxide and water and dried over anhydrous magnesium sulphate. The solvent was removed *in vacuo*. Column chromatography on silica, eluting with 20 % ethyl acetate in petroleum ether (40-60°C), was carried out to give a brown oil. Yield (0.101 g, 39 %). ν_{\max} . (CHCl₃) 3468, 3016, 2960 and 1216 cm⁻¹; δ_{H} (250 MHz; CDCl₃) 2.46 (3 H, s, 10-Me), 5.24-5.32 (2 H, m, 22-H and 23-H), 6.98 (1 H, dd, J 3, 9, 2-H), 7.24 (1 H, s, 7-H), 7.31 (1 H, s, 4-H), and 7.64 (1 H, d, J 9, 1-H).



1 (10→6) abeo-3-(Prop-2-enoyloxy) ergosta-1, 3, 5, 7, 9, 22-hexaene (31)

Method (a)

1 (10→6) abeo-3-hydroxyergosta-1, 3, 5, 7, 9, 22-hexaene (**29**) (0.4 g, 1.0 mmol) was dissolved in ethanol (2 ml) and sodium metal (0.035 g, 1.5 mmol) was added to the reaction mixture. When the sodium metal had dissolved, allyl bromide (0.13 mol, 1.5 mmol) was added to the reaction mixture. The mixture was stirred at room temperature for 1 hour. After which, a further 0.5 equivalent of allyl bromide was added and the reaction mixture was refluxed for 2.5 hours. The product was extracted with diethyl ether and washed with 2M hydrochloric acid, water and dried over anhydrous magnesium sulphate. The solvent was removed *in vacuo* to give a brown oil. Preparative TLC on silica, eluting with 2.5 % ethyl acetate in petroleum ether (40-60°C) was carried out. The major product was the desired allyl ether (**31**) which was recrystallised from methanol, affording a white solid. Yield (0.318 g, 74 %). m.p. 71.5-72.7°C. ν_{\max} . (CHCl₃) 3072, 2956, 2868, 1626, 1602, 1496, 1458, and 1024 cm⁻¹; δ_{H} (400 MHz; CDCl₃) 0.57 (3 H, s, 13-Me), 0.82-0.86 (6 H, m, 25-Me), 0.95 (3 H, d, J 7, 24-Me), 1.11 (3 H, d, J 7, 20-Me), 2.49 (3 H, s, 10-Me), 2.83 (1 H, m, 14-H), 2.99 (2 H, m, 11-H), 4.65-4.66 (2 H, m, 1'-H), 5.23-5.26 (2 H, m, 22-H and 23-H), 5.32 (1 H, dd, J 2, J_{cis} 10, 3'-H), 5.47 (1 H, dd, J 2, J_{trans} 17, 3'-H), 6.07-6.18 (1 H, m, 2'-H), 7.08 (1 H, dd, J 3, 10, 2-H), 7.26 (1 H, s, 7-H), 7.30 (1 H, d, J 3, 4-H), and 7.65 (1 H, d, J 9, 1-H); δ_{C} (63 MHz; CDCl₃) 155.9 (C-3), 137.2, 135.6 (C-22), 133.4, 133.2, 132.1 (C-22), 129.4, 129.0, 127.6,

122.0, 117.7, 116.9, 104.1, 77.3, 69.0 55.6, 52.3, 42.8, , 41.9 (C-13), 40.6, 37.4, 33.1, 29.1, 26.3, 24.3, 21.0 (C-21), 19.9 (C-27), 19.6 (C-26), 17.6 (C-28), 14.4 (C-19), and 11.6 (C-18); (Found: M^+ , 430.3222. $C_{31}H_{42}O$ requires 430.3235); m/z (EI) 430 (M^+ , 100 %).

Method (b)

A mixture of the 1 (10→6) abeo-3-hydroxyergosta-1, 3, 5, 7, 9, 22-hexaene (29) (0.500 g, 1.28 mmol) potassium carbonate (1 equivalent) and acetone (1 ml) was cooled to 0°C, and 1 equivalent of allyl bromide was added to the mixture and stirred for 30 minutes. The reaction mixture was allowed to warm up to room temperature and stirred for 1 hour and another 0.5 equivalent of allyl bromide was added. The mixture was then refluxed for 2.5 hours. The reaction mixture was cooled and the crude product was extracted with diethyl ether and washed with dilute hydrochloric acid, and water until neutral to litmus and dried over anhydrous magnesium sulphate. The crude product was further purified by column chromatography on silica, eluting with 10 % ethyl acetate in petroleum ether (40-60°C). The allyl ether (31) was obtained in moderate yield (0.288 g, 50.8%), m.p. 70-71°C. ν_{\max} ($CHCl_3$) 2956, 2868, 1626, 1602, 1496, 1458 and 1394 cm^{-1} ; δ_H (250 MHz; $CDCl_3$) 0.57 (3H, s, 13-Me), 0.83-0.87 (6 H, m, 25-Me), 0.94 (3 H, d, J 8, 24-Me), 1.09 (3 H, d, J 8, 20-Me), 2.47 (3 H, s, 10-Me), 2.82 (1 H, m, 14-H), 2.98 (2 H, m, 11-H), 4.63-4.66 (2 H, m, 1'-H), 5.23-5.25 (2 H, m, 22-H and 23-H), 5.31 (2 H, dd J 3, J_{cis} 10, 3'-H), 5.46 (1 H, dd, J 3, J_{trans} 17, 3'-H), 6.06-6.17 (1 H, m, 2'-H), 7.08 (1 H, dd, J 3, 9, 2-H), 7.27 (1 H, s, 7-H), 7.30 (1 H, d, J 3, 4-H), and 7.63 (1 H, d, J 9, 1-H); (Found: M^+ , 430.3222. $C_{21}H_{42}O$ requires 430.3236); m/z 430 (M^+ , 100).

Method (c)

1 (10→6) abeo-3-hydroxyergosta-1, 3, 5, 7, 9, 22-hexaene (29) (0.500 g, 1.28 mmol) was dissolved in 2% solution of sodium in ethanol (10 ml). The reaction mixture was stirred at room temperature for 30 minutes. Allyl bromide (0.11 ml, 1 equivalent) was added dropwise, and was stirred at room temperature for 1 hour. A further 0.5 equivalent of allyl bromide was added after 1 hour and the reaction mixture was refluxed at 80-85°C for 1.5 hours. The solvents were removed *in vacuo*. The crude product was extracted with diethyl ether and washed with 2M hydrochloric acid, water, saturated sodium hydrogen carbonate, water again and dried over anhydrous magnesium sulphate. The solvent was removed *in vacuo*. Preparative TLC on silica, eluting

with 5 % ethyl acetate in petroleum ether (40-60°C) was carried out to give two products. The major component recovered was starting material (**29**) (0.35 g, 70 %) and the other, was the desired allyl ether (**31**) (0.035 g, 6.4 %). ν_{max} . (CHCl₃) 3072, 2956, 2868, 1628, 1602, 1458, and 1382 cm⁻¹; δ_{H} (250 MHz; CDCl₃) 0.56 (3 H, s, 13-Me), 0.83-0.87 (6 H, m, 25-Me), 0.94 (3 H, d, J 8, 24-Me), 1.09 (3 H, d, J 8, 20-Me), 2.47 (3 H, s, 10-Me), 4.63-4.66 (2 H, m, 1'-H), 5.23-5.25 (2 H, m, 22-H and 23-H), 5.30 (1 H, dd, J 2, 10, 3'-H), 5.46 (1 H, dd, J 3, 17, 3'-H), 6.06-6.17 (1 H, m, 2'-H), 7.08 (1 H, dd, J 3, 9, 2-H), 7.27 (1 H, s, 7-H), 7.30 (1 H, d, J 3, 4-H), 7.64 (1 H, d, J 9, 1-H).

Method (d)

1 (10→6) abeo-3-hydroxyergosta-1, 3, 5, 7, 9, 22-hexaene (**29**) (0.382 g, 0.98 mmol) was dissolved in THF (5 ml), dried and freshly distilled, and sodium hydride (1.5 equivalent) was added. The mixture was stirred at room temperature for 30 minutes and allyl bromide (0.13 ml, 1.5 equivalent) was added. The reaction mixture was stirred at room temperature for 1 hour and then refluxed for 1.5 hours. The reaction was cooled and excess sodium hydride was removed with 2M hydrochloric acid. The product was extracted with diethyl ether and washed with water, saturated sodium hydrogen carbonate, water and dried over anhydrous magnesium sulphate. The solvent was removed *in vacuo*. Preparative TLC on silica, eluting with 5 % ethyl acetate in petroleum ether (40-60°C) was carried out. A mixture of products was obtained. The major component recovered was starting material (**29**) (0.305g, 80 %), and the other, was the allyl ether (**31**) in very poor yield (0.009 g, 2 %).

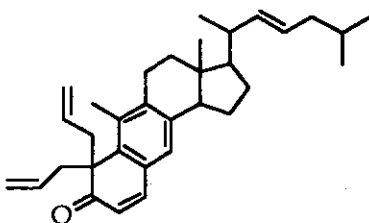
Method (e)

1 (10→6) abeo-3-hydroxyergosta-1, 3, 5, 7, 9, 22-hexaene (**29**) (0.100 g, 0.256 mmol) was dissolved in DMF (2 ml) and sodium hydride (1.5 equivalents) was added. The mixture was stirred at room temperature for 30 minutes. Allyl bromide (0.034 ml, 1.5 equivalents) was then added and the reaction mixture was refluxed overnight. The product was extracted with diethyl ether, and washed with 2M hydrochloric acid, water, saturated sodium hydrogen carbonate, water and dried over anhydrous magnesium sulphate. The solvent was removed *in vacuo* to give a brown oil. Preparative TLC on silica, eluting with 2.5 % ethyl acetate in petroleum ether (40-60°C) was carried out. A mixture of products was obtained. The desired allyl ether was obtained in very

low yield, (0.012 g, 11 %). The major component recovered was that of the starting material **(29)** (0.05 g, 50 %).

Method (f)

1 (10→6) abeo-3-hydroxyergosta-1, 3, 5, 7, 9, 22-hexaene **(29)** (0.200 g, 0.51 mmol) was dissolved in DMSO (10 ml) and potassium hydroxide (4 equivalents) was added. The mixture was stirred for 30 minutes, then allyl bromide (4 equivalents) was added and the reaction mixture stirred at room temperature for 24 hours. The reaction mixture was neutralised with 2M hydrochloric acid and the product was extracted with diethyl ether. The organic layer was washed with water (5 x 20 ml), saturated sodium hydrogen carbonate, water until all of the DMSO was removed, and dried over anhydrous magnesium. The solvent was removed *in vacuo* to give a brown oil. From TLC, a mixture of products was observed. Preparative TLC on silica afforded primarily, the starting material **(29)** (0.160 g, 80 %), with very little of the allyl ether **(31)**.

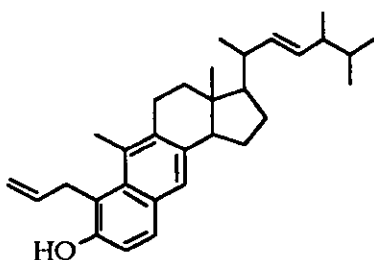


1 (10→6) abeo-4, 4-(Prop-2-enoyl) ergosta-1, 5, 7, 9, 22-pentaen-3-one **(34)**

1 (10→6) abeo-3-(prop-2-enoyloxy) ergosta-1, 3, 5, 7, 9, 22-hexaene **(31)** (0.1 g, 0.23 mmol) was dissolved in diethylaniline (1 ml). The solution was refluxed under nitrogen for 26 hours. The product was extracted with diethyl ether and washed with water, 2M hydrochloric acid, water, saturated sodium hydrogen carbonate, water and dried over anhydrous magnesium sulphate. The solvent was removed *in vacuo* to give a brown oil was obtained. Preparative TLC was carried out on silica, eluting with 10 % ethyl acetate in petroleum ether (40-60°C). Four fractions were isolated. The predominant product (fraction 2) isolated was a diallyl compound **(34)** (0.0273 g, 25 %). ν_{max} . (CHCl_3) 2948, 2868, 1718, 1640, 1456, and 1372 cm^{-1} ; δ_{H} (250 MHz; CDCl_3) 0.56 (3 H, s, 13-Me), 0.83 0.87 (6 H, m, 25-Me), 0.94 (3 H, d, J 7, 24-Me), 1.10 (3 H, d, J 7, 20-Me), 2.42 (3 H, s, 10-Me), 2.70-2.77 (3 H, m, 11-H and 14-H),

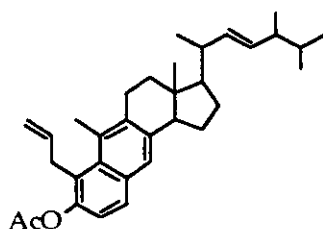
2.88-3.07 (4 H, m, 1'-H and 1''-H), 4.70 (2 H, d, J 10, 3'-H and 3''-H), 4.83 (2 H, dd, J 7, 20, 3'-H and 3''-H), 5.16-5.26 (4 H, m, 22-H, 23-H, and 2'-H and 2''-H), 6.11 (1H, d, J 10, 2-H), 6.81 (1 H, s, 7-H) and 7.33 (1 H, d, J 10, 1-H); δ_C (63 MHz; CDCl₃) 204.5 (C-3), 147.9 (C-2), 139.1, 138.9, 137.8, 135.3 (C-22), 135.0, 133.4 (C-2'), 133.3 (C-2''), 132.2 (C-23), 129.1, 126.5 (C-1), 124.1 (C-7), 116.9 (C-3' and C-3''), 104.1 (C-4), 57.2, 55.1, 51.6, 43.7, 42.7, 41.3 (C-13), 40.5, 37.0, 33.0, 29.2, 28.7, 23.9, 20.9 (C-21), 19.9 (C-27), 19.6 (C-26), 18.0 (C-19), 17.5 (C-28), and 11.3 (C-18); (Found: M^+ 470.3588. C₃₄H₄₆O requires 470.3549); m/z (EI) 470 (M^+ , 12 %).

The other minor components recovered were the starting allyl ether (fraction 1) (**31**) (0.0169 g, 17 %) and the phenolic compound (fraction 3) (**29**) (0.0135 g, 15 %).



1 (10→6) abeo-3-Hydroxy-4-(prop-2-enoyl) ergosta-1, 5, 7, 9, 22-pentaene (35)

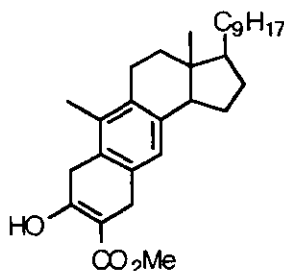
Fraction 4 contained a trace amount of the Claisen product (**35**) (0.008 g, 8 %). ν_{\max} . (CHCl₃) 3484, 3028, 2940, 2864, 1734, 1666, and 1456 cm⁻¹; δ_H (250 MHz; CDCl₃) 0.59 (3H, s, 13-Me), 0.83-0.87 (6 H, m, 25-Me), 0.94 (3 H, d, J 7, 24-Me), 1.10 (3 H, d, J 7, 20-Me), 2.55 (3 H, s, 10-Me), 4.86-4.95 (2 H, m, 3'-H), 5.22-5.24 (2 H, m, 22-H and 23-H), 5.24-5.45 (1 H, m, 2'-H), 6.11 (1 H, dd, J 2, 9, 2-H), 6.63 (1 H, s, 7-H), 7.34 (1 H, dd, J 2, 10, 1-H), and 8.92 (1 H, br s, OH); (Found: M^+ , 430.3213. C₃₁H₄₂O requires 430.3235); m/z (EI) 430 (M^+ , 430 %).



1 (10→6) abeo-3-Acetoxy-4-(prop-2-enoyl) ergosta-1, 3, 5, 7, 9, 22-hexaene (36)

1 (10→6) abeo-3-(prop-2-enoyloxy) ergosta-1, 3, 5, 7, 9, 22-hexaene (31) (0.100g, 0.23 mmol) was dissolved in diethylaniline (1 ml) and a few drops of acetic anhydride was added. The solution was refluxed under nitrogen for 26 hours. The product was extracted with diethyl ether and washed with water, 2M hydrochloric acid, water, saturated sodium hydrogen carbonate, water and dried over anhydrous magnesium sulphate. The solvent was removed *in vacuo* to give a brown oil was obtained. Preparative TLC on silica, eluting with 10 % ethyl acetate in petroleum ether (40-60°C), afforded the major product as the acetate (36) (0.085 g, 85 %). The acetate (36) was recrystallised from methanol gave white crystals. m.p. 129.7-130.8°C. ν_{\max} (CHCl₃) 2956, 2868, 1750, 1636, 1600, 1484, 1458 and 1370 cm⁻¹; δ_{H} (400 MHz; CDCl₃) 0.58 (3 H, s, 13-Me), 0.83-0.87 (6 H, m, 25-Me), 0.95 (3 H, d, J 7, 24-Me), 1.11 (3 H, d, J 7, 20-Me), 2.30 (3 H, s, OAc), 2.64 (3 H, s, 10-Me), 3.79-3.82 (2 H, m, 1'-H), 5.00 (1 H, dd, J 2, J_{trans} 17, 3'-H), 5.15 (1 H, dd, J 2, J_{cis} 11, 3'-H), 5.23-5.26 (2 H, m, 22-H and 23-H), 6.10-6.14 (1 H, m, 2'-H), 7.07 (1 H, d, J 9, 2-H), 7.29 (1 H, s, 7-H), 7.63 (1 H, d, J 9, 1-H); (Found; M⁺, 472.3339. C₃₃H₄₄O₂ requires 472.3341); *m/z* (EI) 472 (M⁺, 38 %), 430 [(M-C₃H₆), 81].

Experimental for Chapter 6



1 (10→6) abeo-2-Carbomethoxy-3-hydroxyergosta-2, 5, 7, 9, 22-pentaene (40)

1 (10→6) abeo-ergosta-5, 7, 9, 22-tetraen-3-one (**6**) (0.500 g, 1.27 mmol) was dissolved in THF (0.5 ml) and was added dropwise to a stirred solution of magnesium methoxy carbonate (MMC) (5 ml). The resulting mixture was heated under nitrogen at 130°C for 4 hours. The reaction mixture was then cooled to 10°C and was slowly poured with vigorous stirring into 10 % aqueous hydrochloric acid (50 ml) kept at -10°C throughout the addition. Evolution of carbon dioxide was vigorous and a tan solid separated. The mixture was allowed to stand in ice for 45 minutes, after which the solid was collected on a filter and washed with large amounts of ice-cold water. The tan solid (**38**) was dried quickly in a vacuum dessicator and used immediately in the next step. No attempt was made to characterise this solid (storage of the crude acid overnight in a vacuum dessicator resulted in extensive decomposition of product). An ice-cold solution of diazomethane in diethyl ether (6 ml) was added dropwise at a rapid rate to the stirred suspension of the crude carboxylic acid (**38**) in a 3:1 mixture of diethyl ether in methanol (4 ml). After which the clear reddish brown solution was stirred in an ice bath for 1 hour. The solvent was then removed *in vacuo*, yielded the crude ester as a reddish brown solid. The crude product was further purified by column chromatography on silica eluting with 10 % ethyl acetate in petroleum ether (40-60°C). The product (**40**) was recrystallised from methanol. Yield (0.329 g, 56 %), m.p. 136-137°C. ν_{max} . (CHCl₃) 3375, 2957, 2877, 1669, 1622, 1576, 1450, and 1376 cm⁻¹; δ_{H} (250 MHz; CDCl₃) 0.58 (3 H, s, 13-Me), 0.82-0.88 (6 H, m, 25-Me), 0.94 (3 H, d, J 7, 24-Me), 1.09 (3 H, d, J 7, 20-Me), 2.12 (3 H, s, 10-Me), 3.52 (2 H, m, 4-H), 3.61 (2 H, m, 1-H), 3.81 (3 H, s, CO₂Me), 5.21-5.25 (2 H, m, 22-H and 23-H), 6.74 (1 H, s, 7-H), and 12.17 (1 H, s, OH); δ_{C} (63 MHz; CDCl₃) 172.3 (C-3),

169.3 (C-29), 138.5, 135.5 (C-22), 133.0, 132.4, 132.1 (C-23), 130.0, 128.9, 123.3 (C-7), 95.4, 55.1, 51.8, 51.6, 42.8, 41.7 (C-13), 40.5, 37.0, 33.0, 32.0, 29.2, 28.4, 25.7, 24.1, 21.0 (C-21), 19.9 (C-27), 19.6 (C-26), 17.6 (C-28), 14.7 (C-19), and 11.2 (C-18); (Found: M^+ , 450.3138. $C_{30}H_{42}O_3$ requires 450.3133); m/z (EI) 450 (M^+ , 100 %).

Preparation of Magnesium Methoxy Carbonate (MMC)

MMC was generated under nitrogen by refluxing magnesium turnings (10.9 g, 0.46 g. atom) with anhydrous methanol (250 ml) until hydrogen gas was evolved. The mixture was stirred until all the magnesium had reacted. Methanol was then removed by distillation under reduced pressure. The gray solid residue was slurried in DMF (225 ml) and dry carbon dioxide was bubbled into the mixture at room temperature for 2 hours. The resulting cloudy, faintly yellow solution was cooled to room temperature and stored under carbon dioxide.

Preparation of Diazomethane

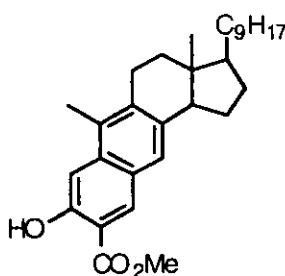
Ethanol (95 %, 40 ml) was added to a solution of potassium hydroxide (20 g) in water (32 ml). The reaction vessel was warmed to 65°C in a water bath. A solution of Diazald® (20 g) in diethyl ether (180 ml) was added slowly to the reaction vessel, from a dropping funnel. The rate of addition should approximate the rate of distillation of diazomethane into the collecting flask, which was cooled in an ice bath. When the Diazald® was added, another 40 ml of diethyl ether was added slowly and distillation continued until the distillate was colourless. The diazomethane collected was stored in the freezer.

Procedure for the Preparation of *t*-butyldimethylsiloxy iodopropane

A stirred solution of 3-chloropropyl iodide in DMF (2 ml/g alcohol) was treated with *t*-butyldimethylsilylchloride (1.2 equivalents) and imidazole (2.5 equivalents) under nitrogen at room temperature. The reaction mixture was stirred for 2 days. The reaction mixture was poured into water, and extracted with petroleum ether (40-60°C). The organic phase was washed with 10 % hydrochloric acid, and with water to neutrality, dried, and the solvent was evaporated. The above chloride was then dissolved in acetone (1 ml, 0.55 mmol chloride) and treated with sodium iodide (2.2 equivalent). The solution was heated under reflux under nitrogen for 3 days. The

precipitate was filtered off and washed with dichloromethane. The combined filtrate and washings were diluted with dichloromethane, washed with water, 10 % aqueous sulphite, and brine, dried, and evaporated to give the *t*-butyldimethylsiloxy iodopropane as a colourless oil. ν_{max} . (film) 2952, 2928, 2896, 2856, 1470, 1438, 1424, 1386, 1360, 1256, 1182, 1136 and 1098 cm^{-1} ; δ_{H} (250 MHz; CDCl_3) 0.1 [6 H, s, Si (CH_3)₂], 0.95 [9 H, s, Si (CH_3)₃], 2.0 (2 H, q, J 6, $\text{OCH}_2\text{CH}_2\text{CH}_2\text{I}$), 3.30 (2 H, t, J 7, CH_2I), 3.65 (2 H, t, J 6, $\text{OCH}_2\text{CH}_2\text{CH}_2\text{I}$);

Attempted Alkylations on 1 (10→6) abeo-2-Carbomethoxy-3-hydroxyergosta-2, 5, 7, 9, 22-pentaene (40)



1 (10→6) abeo-2-Carbomethoxy-3-hydroxyergosta-1, 3, 5, 7, 9, 22-hexaene (43)

Method (a)

A solution of 1 (10→6) abeo-2-carbomethoxy-3-hydroxyergosta-2, 5, 7, 9, 22-pentaene (40) (0.100 g, 0.222 mmol) in THF (2 ml) was added slowly to a suspension of sodium hydride (0.011 g, 60 % in mineral oil, 1.2 equivalents) in THF (0.6 ml) containing hexamethylphosphoramide (HMPA) (0.05 ml, 1.2 equivalents) at room temperature under nitrogen. The reaction mixture was stirred at room temperature for 1 hour, then 3-(*t*-butyldimethylsiloxy)-1-bromopropane (0.286 g, 5 equivalents) was added dropwise to the mixture. The reaction mixture was refluxed at 80°C for 14 hours. The crude product was extracted with diethyl ether. The organic phase was washed with water, and dried over potassium carbonate. The solvent was removed *in vacuo*. The crude product was an oil. Column chromatography on silica, eluting with 10 % ethyl acetate in petroleum ether (40-60°C) gave a mixture of products, none of which was the desired alkylated product. The major component recovered was the naphthalene ester (43) in 39 % yield (0.039 g). ν_{max} . (CHCl_3) 3220, 2952, 2868, 1676, 1626, 1440 cm^{-1} ; δ_{H} (250 MHz; CDCl_3)

0.56 (3 H, s, 13-Me), 0.83-0.89 (6 H, m, 25-Me), 0.94 (3 H, d, J 7, 24-Me), 1.11 (3 H, d, J 8, 20-Me), 2.47 (3 H, s, 10-Me), 4.00 (3 H, s, OMe), 5.23-5.26 (2 H, m, 22-H and 23-H), 7.29 (1 H, s, 4-H), 7.45 (1 H, s, 7-H), 8.38 (1 H, s, 1-H), and 10.32 (1 H, br s, OH); (Found; M^+ , 448.2967. $C_{30}H_{40}O_3$ requires 448.2977); m/z (EI) 448 (M^+ , 100 %).

The minor component recovered was the starting β -keto ester (**40**) (0.007 g, 7 %).

Method (b)

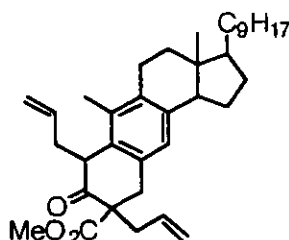
A solution of **1** (10 \rightarrow 6) abeo-2-carbomethoxy-3-hydroxyergosta-2, 5, 7, 9, 22-pentaene (**40**) (0.100 g, 0.22 mmol) in THF (0.15 ml) was added slowly to a suspension of sodium hydride (60 % in mineral oil) (0.11 g, 1.2 equivalents) in THF (0.4 ml) containing HMPA (0.05 ml, 1.2 equivalents) at room temperature under nitrogen. The reaction mixture was stirred at room temperature for 1 hour, then 3-(*t*-butyldimethylsiloxy)-1-iodopropane (0.286 g, 5 equivalents) was added dropwise to the mixture. The reaction mixture was refluxed at 80°C for 14 hours. The product was extracted with diethyl ether. The organic layer was washed with water, and dried over potassium carbonate. The solvent was removed *in vacuo*. The crude product was an oil. Preparative TLC was carried out on silica to purify the crude product. The major product recovered was the naphthalene ester (**43**) (0.032 g, 32 %). ν_{\max} . ($CHCl_3$) 3249, 2964, 2864, 1682, 1626, and 1450 cm^{-1} ; δ_H (250 MHz; $CDCl_3$) 0.56 (3 H, s, 13-Me), 0.82-0.86 (6 H, m, 25-Me), 0.94 (3 H, d, J 7, 24-Me), 1.11 (3 H, d, J 7, 20-Me), 2.42 (3 H, s, 10-Me), 3.90 (3 H, s, OMe), 5.23-5.27 (2 H, m, 22-H and 23-H), 7.31 (1 H, s, 4-H), 7.45 (1 H, s, 7-H), and 8.37 (1 H, s, 1-H);

Method (c)

To a solution of methanol (1 ml) was dissolved sodium metal (0.008 g, 1.5 equivalents) was added dropwise a solution of **1** (10 \rightarrow 6) abeo-2-carbomethoxy-3-hydroxyergosta-2, 5, 7, 9, 22-pentaene (**40**) (0.102 g, 0.225 mmol). The suspension was stirred at room temperature for 1 hour. Then 3-(*t*-butyldimethylsiloxy)-1-iodopropane (0.286 g, 5 equivalents) was added to the mixture and the reaction mixture was refluxed for 20 hours. The crude product was extracted with diethyl ether and washed with water, 2M hydrochloric acid, water, saturated sodium hydrogen carbonate, water and dried. The solvent was removed *in vacuo*. The crude product was

purified by column chromatography on silica, eluting with 10 % ethyl acetate in petroleum ether (40-60°C). The major product recovered was the naphthalene ester (**43**) (0.042 g, 41 %). ν_{\max} . (CHCl₃) 3264, 2944, 2864, 1676, 1600, 1496, 1440, 1380, and 1364 cm⁻¹; δ_{H} (250 MHz; CDCl₃) 0.56 (3 H, s, 13-Me), 0.83-0.89 (6 H, m, 25-Me), 0.94 (3 H, d, J 7, 24-Me), 1.11 (3 H, d, J 7, 20-Me), 2.47 (3 H, s, 10-Me), 4.00 (3 H, s, OMe), 5.23-5.26 (2 H, m, 22-H and 23-H), 7.29 (1 H, s, 4-H), 7.45 (1 H, s, 7-H), 8.38 (1 H, s, 1-H), and 10.32 (1 H, br s, OH); (Found; M^+ , 448.2990. C₃₀H₄₀O₃ requires 448.2977); m/z (EI) 448 (M^+ , 100 %).

Attempted alkylation with allyl bromide



1 (10→6) abeo-2-Carbomethoxy-2, 4-(prop-2-enoyl) ergosta-5, 7, 9, 22-tetraen-3-one (45**)**

Method (a)

To a suspension of sodium hydride (0.025 g, 1.5 equivalents) in DMF (5 ml) was added a suspension of 1 (10→6) abeo-2-carbomethoxy-3-hydroxy ergosta-2, 5, 7, 9, 22-pentaene (**40**) (0.246 g, 0.546 mmol) in DMF (2 ml). The reaction mixture was stirred at room temperature for 1 hour. Then allyl bromide (0.235 ml, 5 equivalents) was added dropwise. The reaction mixture was stirred at room temperature for 2 hours. The crude product was purified by column chromatography on silica eluting with 10 % ethyl acetate in petroleum ether (40-60°C). A mixture of products were recovered. The predominant product was a brown oil, a bisallyl compound (**45**) (0.044 g, 15 %). ν_{\max} . (CHCl₃) 3060, 2944, 2864, 1728, 1708, 1636, and 1456 cm⁻¹; δ_{H} (250 MHz; CDCl₃) 0.51 (3 H, s, 13-Me), 0.82-0.86 (6 H, m, 25-Me), 0.94 (3 H, d, J 7, 24-Me), 1.08 (3 H, d, J 7, 20-Me), 2.10 (3 H, s, 10-Me), 3.02 (2 H, m, 1'-H), 3.33 (3 H, s, OMe), 3.88 (1 H, t, J 8, 4 H), 4.90-5.16 (4 H, m, 3'-H, and 3''-H), 5.21-5.25 (2 H, m, 22-H, and 23-H), 5.59 (1 H, m, 2''-H), 5.79 (1 H, m, 2'-H), and 6.67 (1 H, s, 7-H); (Found: M^+ 530.3767. C₃₆H₅₀O₃ requires 530.3759); m/z (EI) 530 (M^+ , <3 %).

The other component was the naphthalene ester (**43**) (0.007 g, 3%).

Method (b)

To a suspension of sodium hydride (0.013 g, 1 equivalent) in THF (5 ml), was added dropwise a solution of 1 (10→6) abeo-2-carbomethoxy-3-hydroxyergosta-2, 5, 7, 9, 22-pentaene (**40**) (0.150 g, 0.333 mmol) in THF. The reaction mixture was stirred at room temperature for 1 hour and allyl bromide (0.029 ml, 1 equivalent) was added dropwise over 30 minutes. The resulting reaction mixture was stirred for another 2 hours at room temperature, monitoring the reaction by TLC. The crude product was purified by preparative TLC on silica, eluting twice with 10 % ethyl acetate in petroleum ether (40-60°C). The predominant product recovered was a bisallyl product (**45**) (0.035 g, 20 %), the other components were the naphthalene ester (**43**) (0.023 g, 16 %) and the monoallyl product (**44**) (0.005 g, 3 %).

Method (c)

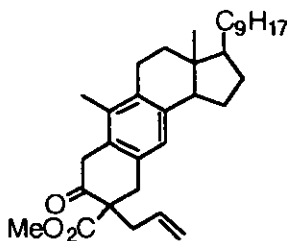
To a mixture of sodium metal (0.016 g, 1.5 equivalents) dissolved in methanol (2 ml), was added dropwise a suspension of the 1 (10→6) abeo-2-carbomethoxy-3-hydroxyergosta-2, 5, 7, 9, 22-pentaene (**40**) (0.205 g, 0.455 mmol) in methanol. The reaction mixture was stirred at room temperature for 1 hour, and allyl bromide (0.06 ml, 1.5 equivalents) was added dropwise over 10 minutes to the reaction mixture. The reaction mixture was refluxed for 2 hours, monitoring by TLC. The crude product was purified by column chromatography on silica, eluting with 5 % ethyl acetate in petroleum ether (40-60°C). A mixture of products was obtained. The predominant product was a bisallyl compound (**45**) (0.048 g, 20 %), the other component was the naphthalene ester (**43**) (0.020 g, 10 %).

Method (d)

The above procedure was repeated, but instead of refluxing the reaction mixture, it was stirred at room temperature for 6 hours, monitoring the reaction by TLC. The crude material was purified by preparative TLC on silica, eluting with 10 % ethyl acetate in petroleum ether (40-60°C). The predominant product recovered was a bisallyl compound (**45**) (0.090 g, 38 %), and the other component was the naphthalene ester (**43**) (0.028 g, 14 %).

Attempted Alkylation with Palladium-phosphine catalyst and Allyl acetate Method (a)

The 1 (10→6) abeo-2-carbomethoxy-3-hydroxyergosta-2, 5, 7, 9, 22-pentaene (**40**) (0.225 g, 0.5 mmol) was added to a suspension of sodium hydride (60 % in mineral oil, prewashed with n-hexane) (0.030 g, 1.5 equivalents) in THF (1 ml). The reaction mixture was stirred for 1 hour and added to a solution of palladium catalyst (0.013 g, 2.5 mol %) and triphenyl phosphine (0.026 g, 0.1 mmol) in THF. Then allyl acetate (0.100 g, 2 equivalents) was added to the reaction mixture. The reaction mixture was stirred for 40 minutes at 30°C under nitrogen. The reaction was monitored by TLC. The crude product was purified by column chromatography on silica, eluting with 10 % ethyl acetate in petroleum ether (40-60°C). The predominant product recovered was a bisallyl compound (**45**) (0.084 g, 32 %), and the other component was the naphthalene ester (**43**) (0.024 g, 11 %).



1 (10→6) abeo-2-Carbomethoxy-2-(prop-2-enoyl) ergosta-5, 7, 9, 22-tetraen-3-one (**44**)

The third component was a monoallyl compound (**44**) (0.009 g, 4 %). ν_{\max} (CHCl_3) 2948, 2868, 1730, 1710, and 1454 cm^{-1} ; δ_{H} (250 MHz; CDCl_3) 0.56 (3 H, s, 13-Me), 0.82-0.86 (6 H, m, 25-Me), 0.94 (3 H, d, J 7, 24-Me), 1.08 (3 H, d, J 7, 20-Me), 2.24 (3 H, s, 10-Me), 3.53 (2 H, s, 4-H), 3.60 (3-H, s, OMe), 3.80 (2 H, d, J 15, 1'-H), 5.06-5.15 (2 H, m, 3'-H), 5.23-5.25 (2 H, m, 22-H, and 23-H), 5.78 (1 H, m, 2'-H), 6.70 (1 H, s, 7-H); (Found; M^+ , 490.3425. $\text{C}_{33}\text{H}_{46}\text{O}_3$ requires 490.3446); m/z (EI) 490 (M^+ , 1.7 %), 44 (CO_2 , 100).

Method (b)

The above procedure, method (a), was repeated using 0.5 equivalent of sodium hydride and stirring the reaction mixture for 30 minutes. A mixture of products was recovered. The predominant product was the

monoallyl compound (**44**) (20 %), the other products were the naphthalene ester (**43**) (11 %), and the bisallyl compound (**45**) (11 %).

Method (c)

Method (a) was repeated using 0.5 equivalent of sodium hydride, and the reaction mixture was stirred 3 hours. The products recovered were the monoallyl product (**44**) (16 %), and the aromatised ester (**43**) (17 %).

Preparation of Palladium Catalyst: Tris (dibenzlideneacetone) dipalladium (Chloroform), Pd₂(dba)₃. CHCl₃

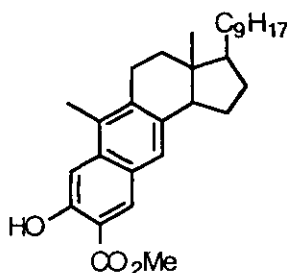
(a) Preparation of dibenzlideneacetone (dba)

A solution of sodium hydroxide (100 g) in water (1000 ml) and ethanol (800 ml) was cooled and stirred vigorously at 20°C. Then half of the mixture of benzaldehyde (106 g, 1 mol) and acetone (29 g, 0.5 mol) was added to the reaction mixture and stirred for 3 minutes. A yellow flocculent precipitate was formed. After 15 minutes, the rest of the mixture of benzaldehyde and acetone was added to the reaction mixture. Vigorous stirring was continued for 30 minutes. The precipitate was collected by filtration through a large Buchner funnel and the precipitate was washed with distilled water and dried at room temperature to constant weight. m.p. 104-107°C. The crude material was recrystallised from boiling ethyl acetate, m.p. 110-111°C.

(b) Preparation of Pd₂(dba)₃. CHCl₃

To a hot solution (50°C) of methanol (150 ml), dba (4.6 g, 19.6 mmol), sodium acetate (3.9 g, 47.5 mmol) was added palladium chloride (1.05 g, 5.92 mmol). The mixture was stirred for 4 hours at 40 °C. A reddish-purple precipitate was formed and the reaction mixture was cooled to room temperature to allow for complete precipitation. The precipitate was collected by filtration and washed successively with water and acetone and dried *in vacuo*. The precipitate (3.39 g) was dissolved in hot chloroform (120 ml), and filtered to give a deep violet solution. To the solution was added diethyl ether (170 ml) slowly, deep purple needles were precipitated. The precipitate was collected by filtration and washed with diethyl ether and dried *in vacuo*. m.p. 122-124°C (decomposed).

Experimental for Chapter 7



1 (10→6) abeo-2-Carbomethoxy-3-hydroxyergosta-1, 3, 5, 7, 9, 22-hexaene (43)

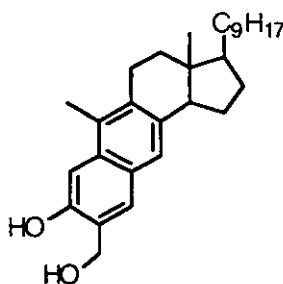
To a solution of the 1 (10→6) abeo-2-carbomethoxy-3-hydroxyergosta-2, 5, 7, 9, 22-pentaene (**40**) (1.0 g, 2.22 mmol) in THF (20 ml) was added in one portion PHT (1.14 g, 1 equiv.). The reaction mixture was stirred at 0°C for 1 hour. The product was extracted with diethyl ether and washed with water until neutral. The solvent was removed *in vacuo*. The crude product was recrystallised from methanol and gave yellow crystals. Yield (0.835 g, 84 %). m.p. 137.6-138.6°C; ν_{max} (CHCl₃) 3249, 2964, 2864, 1682, and 1450 cm⁻¹; δ_{H} (250 MHz; CDCl₃) 0.56 (3 H, s, 13-Me), 0.83-0.86 (6 H, m, 25-Me), 0.95 (3 H, d, J 7, 24-Me), 1.11 (3 H, d, J 7, 20-Me), 2.46 (3 H, s, 10-Me), 2.73 (1 H, m, 14-H), 2.95 (2 H, m, 11-H), 4.00 (3 H, s, CO₂Me), 5.23-5.26 (2 H, m, 22-H and 23-H), 7.29 (1 H, s, 4-H), 7.45 (1 H, s, 7-H), 8.37 (1 H, s, 1-H), and 10.31 (1 H, s, OH); δ_{C} (63 MHz; CDCl₃) 170.4 (C-29), 155.5 (C-3), 137.6, 136.8, 136.0, 135.5 (C-22), 132.1 (C-1), 132.0 (C-23), 128.7, 125.8, 123.3 (C-7), 112.5, 108.0 (C-4), 55.4, 52.3, 52.2 (C-30), 42.8, 41.8 (C-13), 40.5, 37.0, 33.0, 29.2, 26.4, 24.2, 21.0 (C-21), 20.0 (C-27), 19.6 (C-26), 17.6 (C-28), 14.3 (C-19), and 11.7 (C-18); (Found; M^+ , 448.2967. C₃₀H₄₀O₃ requires 448.2977); m/z (EI), 448 (M^+ , 100 %).

Attempted reduction of 1 (10→6) abeo-2-Carbomethoxy-3-hydroxyergosta-1, 3, 5, 7, 9, 22-hexaene (43) to the aldehyde (47)

A solution of the 1 (10→6) abeo-2-carbomethoxy-3-hydroxyergosta-1, 3, 5, 7, 9, 22-hexaene (**43**) (0.200 g, 0.446 mmol) in toluene was stirred at -20°C and diisobutylaluminium hydride (DIBAL) (0.33 ml, 1 equivalent) was added, dropwise over 10 minutes. The reaction mixture was stirred for 2 hours at -20°C and monitored by TLC. The reaction was quenched by addition of methanol and the mixture was allowed to warm to room

temperature. Then water was added and the reaction mixture was transferred to a round bottom flask and the solvents removed by evaporation under reduced pressure. The solid residue was triturated with warm diethyl ether and the ether extracts was dried over anhydrous magnesium sulphate. The solvent was removed *in vacuo*. The component recovered was the starting material. The aldehyde was not formed.

The above procedure was repeated several times by varying the temperature between -20°C and -5°C, using between 1 equivalent to 3 equivalents of DIBAL. We failed to obtain the desired aldehyde.



1 (10→6) abeo 2-Hydroxymethyl-3-hydroxyergosta-1, 3, 5, 7, 9, 22-hexaene (46)

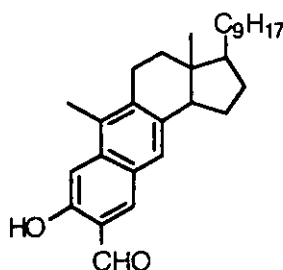
Method (a)

To a suspension of lithium aluminium hydride (0.085 g, 2 equivalents) in diethyl ether was added dropwise, a solution of 1 (10→6) abeo-2-carbomethoxy-3-hydroxyergosta-1, 3, 5, 7, 9, 22-hexaene (43) (0.500 g, 1.11 mmol) in diethyl ether. The reaction mixture was refluxed for 2 hours. The reaction was cooled to room temperature and the excess lithium aluminium hydride was destroyed with wet ethyl acetate (2 ml) added to the cooled reaction mixture and stirred for 30 minutes. The crude product was extracted with diethyl ether, washed with water, 2M hydrochloric acid, and water again and dried. The solvents were removed *in vacuo*. The crude product was further purified by column chromatography on silica eluting with 50 % ethyl acetate in petroleum ether (40-60°C). The product was recrystallised from ethanol, yielded brown crystals (0.398 g, 85 %). m.p. 136-138°C. ν_{max} . (CHCl₃) 3415, 2954, 2930, 2872, 1642, 1609, 1502, 1492, 1459, and 1382 cm⁻¹; δ_{H} (250 MHz; CDCl₃) 0.55 (3 H, s, 13-Me), 0.83-0.89 (6 H, m, 25-Me), 0.95 (3 H, d, J 7, 24-Me), 1.11 (3 H, d, J 7, 20-Me), 2.45 (3 H, s, 10-Me), 4.94 (2 H, s, PhCH₂OH), 5.23-5.25 (2 H, m, 22-H and 23-H), 7.16 (1 H, br s, OH), 7.21 (1 H, s, 4-H), 7.38 (1 H, s, 7-H), and 7.43 (1 H, s, 1-H); δ_{C} (63 MHz; CDCl₃)

152.7 (C-3), 137.0, 135.4 (C-22), 133.4, 132.2, 132.0 (C-23), 128.6, 127.1 (C-1), 126.9, 125.5, 121.8 (C-7), 107.3 (C-4), 64.0 (C-29), 55.3, 52.1, 42.7, 41.8 (C-13), 40.4, 36.5, 33.0, 29.1, 26.0, 24.2, 20.9 (C-21), 19.9 (C-27), 19.6 (C-26), 17.4 (C-28), 14.0 (C-19), and 11.6 (C-18); (Found; M^+ , 420.3027. $C_{29}H_{40}O_2$ requires 420.3028); m/z (EI) 420 (M^+ , <1.0 %), 404 (42), 41 (100).

Method (b)

To a solution of lithium borohydride (0.024 g, 2.5 equivalents) in THF/MeOH (5 ml: 0.04 ml) was added dropwise over 5 minutes, a solution of 1 (10→6) abeo-2-carbomethoxy-3-hydroxyergosta-1, 3, 5, 7, 9, 22-hexaene (43) (0.200 g, 0.446 mmol) in THF (1 ml). The reaction mixture was refluxed for 1 hour. The reaction was quenched with 1M hydrochloric acid and cooled in an ice-bath. The reaction mixture was extracted with dichloromethane and washed with water and dried and the solvent was removed *in vacuo*. The crude product was further purified by preparative TLC on silica, eluting with 50 % diethyl ether in petroleum ether (40-60°C). The product was recrystallised from methanol which yielded brown crystals. Yield (0.117 g, 62.5 %).



1 (10→6) abeo-2-Formyl-3-hydroxyergosta-1, 3, 5, 7, 9, 22-hexaene (47)

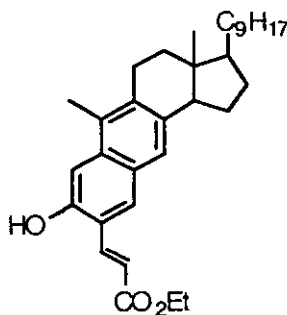
Method (a)

A solution of 1 (10→6) abeo-2-hydroxymethyl-3-hydroxyergosta-1, 3, 5, 7, 9, 22-hexaene (46) (0.155 g, 0.37 mmol) in diethyl ether was added dropwise to a thoroughly wet suspension of manganese dioxide (1.0 g, 30 equivalents). The reaction mixture was stirred for 2 days at room temperature. The reaction mixture was filtered through a short column of celite to remove the manganese dioxide and the residue was washed with diethyl ether. The combined filtrate was dried over anhydrous magnesium sulphate and the solvent was removed *in vacuo*. The crude product was purified by column chromatography on silica, eluting with 50 % ethyl acetate in petroleum

ether (40-60°C) which gave a yellow oil. Yield (0.036 g, 23.3 %). ν_{max} . (CHCl_3) 3276, 2960, 2872, 1720, 1684, 1661, 1638, 1600, 1459, 1424, and 1372 cm^{-1} ; δ_{H} (250 MHz; CDCl_3) 0.57 (3 H, s, 13-Me), 0.83-0.89 (6 H, m, 25-Me), 0.94 (3 H, d, J 7, 24-Me), 1.10 (3 H, d, J 7, 20-Me), 2.48 (3 H, s, 10-Me), 5.23-5.27 (2 H, m, 22-H and 23-H), 7.35 (1 H, s, 4-H), 7.42 (1 H, s, 7-H), 8.03 (1 H, s, 1-H), 10.03 (1 H, s, CHO), and 10.28 (1 H, s, OH); (Found; M^+ , 418.2835. $\text{C}_{29}\text{H}_{38}\text{O}_2$ requires 418.2871): m/z (EI) 418 (M^+ , 64 %), 29 (CHO , 100).

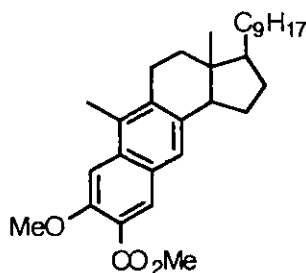
Method (b)

A mixture of 1 (10→6) abeo 2-hydroxymethyl-3-hydroxyergosta-1, 3, 5, 7, 9, 22-hexaene (**46**) (0.100 g, 0.237 mmol), 4-methylmorpholine N-oxide (NMO) (0.042 g, 1.5 equivalents), and powdered 4Å molecular sieves (500 mg/mmol) in dichloromethane was stirred at room temperature under nitrogen for 10 minutes. Then tetra-propyl ammonium perruthenate (TPAP) (0.008 g, 10 mole %) was added in one portion to the reaction mixture. The reaction mixture was stirred at room temperature for 1 hour. The solvent was removed *in vacuo*. The crude product was chromatographed through a short column of flash silica eluting with 50 % diethyl ether in petroleum ether (40-60°C). The starting diol (**46**) (0.050 g, 50 %) was also recovered from the crude mixture. The aldehyde was obtained as a yellow oil. Yield (0.030 g, 30 %).



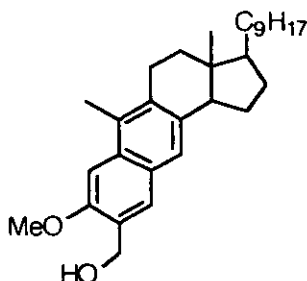
**Ethyl 3-[1 (10→6) abeo-3-Hydroxyergosta-1, 3, 5, 7, 9, 22-hexaen-2-yl]
propenoate (48)**

To a suspension of sodium hydride (60 % in mineral oil) (0.023 g, 2.5 equivalents) in benzene (5 ml) at 0°C, was added, dropwise, triethyl phosphonoacetate (0.053 ml, 1.2 equivalents). The mixture was stirred at 5°C for 30 minutes, then a solution of 1 (10→6) abeo-2-formyl-3-hydroxyergosta-1,3,5,7,9,22-hexaene (47) (0.100 g, 0.239 mmol) in benzene (3 ml) was added slowly to the reaction mixture. The reaction mixture was then left stirring at room temperature overnight. The crude product was extracted with diethyl ether and washed with saturated sodium hydrogen carbonate and water and dried. The solvent was removed *in vacuo*. The crude product was further purified by column chromatography on silica eluting with 50 % diethyl ether in petroleum ether (40-60°C). The product was obtained as an oil. Yield (0.018 g, 15.5 %). ν_{max} (CHCl₃) 3309, 2961, 2931, 2873, 1707, 1624, 1463, and 1370 cm⁻¹; δ_{H} (250 MHz; CDCl₃) 0.56 (3 H, s, 13-Me), 0.83-0.87 (6 H, m, 25-Me), 0.95 (3 H, d, J 7, 24-Me), 1.11 (3 H, d, J 7, 20-Me), 1.35 (3 H, t, J 8, CO₂CH₂CH₃) 2.44 (3 H, s, 10-Me), 4.28 (2 H, q, J 8, CO₂CH₂CH₃), 5.23-5.26 (2 H, m, 22-H and 23-H), 5.61 (1 H, br s, OH), 6.75 (1 H, d, J 16, 2'-H), 7.86 (1 H, s, 1-H), and 8.04 (1 H, d, J 16, 1'-H); (Found; M^+ , 488.3295. C₃₃ H₄₄ O₃ requires 488.3290); m/z (EI) 488 (M^+ , 20 %), 44 (CO₂, 100).



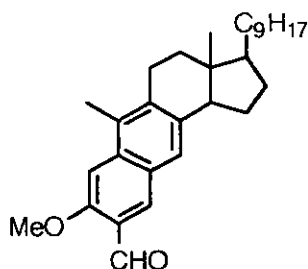
1 (10→6) abeo-2-Carbomethoxy-3-methoxyergosta-1, 3, 5, 7, 9, 22-hexaene (51)

To a suspension of potassium hydroxide (0.311 g, 5.55 mmol) in DMSO (10 ml) was added 1 (10→6) abeo-2-carbomethoxy-3-hydroxyergosta-1, 3, 5, 7, 9, 22-hexaene (**43**) (0.500g, 1.11 mmol). The suspension was stirred at room temperature for 30 minutes. Then methyl iodide (0.274 ml, 4 equivalents) was added dropwise, to the suspension. The reaction mixture was stirred for 2 hours. The crude product was extracted with diethyl ether and washed with water (5 x 20 ml), and dried. The solvent was removed *in vacuo*. The crude product was recrystallised from acetone to give white crystals. Yield (0.469 g, 91 %). m.p. 144-144.5°C. ν_{max} (CHCl₃) 3010, 2959, 2873, 1720, 1628, 1601, 1499, 1464, and 1437 cm⁻¹; δ_{H} (250 MHz; CDCl₃) 0.55 (3 H, s, 13-Me), 0.82-0.87 (6 H, m, 25-Me), 0.95 (3 H, d, J 7, 24-Me), 1.11 (3 H, d, J 7, 20-Me), 2.48 (3 H, s, 10-Me), 2.74 (1 H, m, 14-H), 2.96 (2 H, m, 11-H), 3.94 (3 H, s, CO₂Me), 3.99 (3 H, s, OMe), 5.22-5.25 (2 H, m, 22-H and 23-H), 7.25 (1 H, s, 4-H), 7.30 (1 H, s, 7-H), and 8.25 (1 H, s, 1-H); δ_{C} (63 MHz; CDCl₃) 166.7 (C-29), 155.2 (C-3), 137.9, 136.0, 135.4 (C-22), 133.9, 133.1 (C-1), 132.2 (C-23), 128.8, 126.1, 123.1, 119.7, 103.2, 55.9, 55.4, 52.2, 52.1, 42.8, 41.9 (C-13), 40.5, 37.1, 33.1, 29.1, 26.4, 24.2, 21.0 (C-21), 19.9 (C-27), 19.6 (C-26), 17.6 (C-28), 14.4 (C-19), and 11.7 (C-18); (Found; M^+ , 462.3144. C₃₁H₄₂O₃ requires 462.3134); m/z (EI) 462 (M^+ , 100 %).



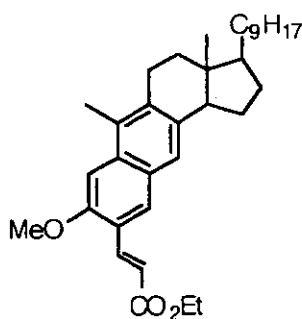
**1 (10→6) abeo-2-Hydroxymethyl-3-methoxyergosta-1, 3, 5, 7, 9, 22-hexaene
(52)**

To a suspension of lithium aluminium hydride (0.062 g, 1.5 equivalents) in diethyl ether (10 ml) was added dropwise, a solution of the 1 (10→6) abeo-2-carbomethoxy-3-methoxyergosta-1, 3, 5, 7, 9, 22-hexaene (51) (0.500 g, 1.08 mmol) in diethyl ether (5 ml). The reaction mixture was refluxed for 2 hours. The reaction was quenched by adding wet ethyl acetate (2 ml) to the cooled reaction mixture and stirred for 30 minutes. The crude product was extracted with diethyl ether and washed with 2M hydrochloric acid, water, saturated sodium hydrogen carbonate, water again, and dried. The solvent was removed *in vacuo*. The crude product was recrystallised from acetone to give white crystals. Yield (0.418 g, 89 %). m.p. 109-110.5°C; ν_{max} . (CHCl_3) 3435, 3009, 2960, 2872, 1636, 1602, 1498, 1465, 1382, and 1238 cm^{-1} ; δ_{H} (250 MHz; CDCl_3) 0.54 (3 H, s, 13-Me), 0.83-0.86 (6 H, m, 25-Me), 0.95 (3 H, d, J 7, 24-Me), 1.09 (3 H, d, J 7, 20-Me), 2.47 (3 H, s, 10-Me), 2.69-2.78 (1 H, m, 14-H), 2.90-2.95 (2 H, m, 11-H), 3.91 (3 H, s, OMe), 4.76 (2 H, s, CH_2OH), 5.22-5.25 (2 H, m, 22-H and 23-H), 7.16 (1 H, s, 4-H), 7.22 (1 H, s, 7-H), and 7.58 (1 H, s, 1-H); δ_{C} (63 MHz; CDCl_3) 155. (C-3), 137.2, 135.5 (C-22), 133.2, 132.1 (C-23), 131.6, 128.8, 128.7, 127.6 (C-1), 127.2, 122.1, 101.4 (C-4), 62.3 C-29), 55.3, 55.1, 52.2, 42.8, 41.8 (C-13), 40.5, 37.3, 33.1, 29.2, 26.2, 24.3, 21.0 (C-21), 19.9 (C-27), 19.6 (C-26), 17.6 (C-28), 14.4 (C-19), and 11.6 (C-18); (Found; M^+ , 434.3184. $\text{C}_{30}\text{H}_{42}\text{O}_2$ requires 434.3184); m/z (EI) 434 (M^+ , 100 %).



1 (10→6) abeo-2-Formyl-3-methoxyergosta-1, 3, 5, 7, 9, 22-hexaene (53)

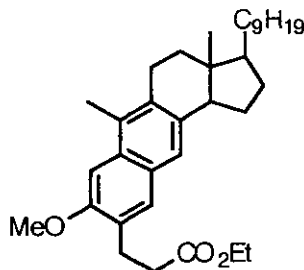
A mixture of 1 (10→6) abeo-2-hydroxymethyl-3-methoxyergosta-1, 3, 5, 7, 9, 22-hexaene (52) (0.500 g, 1.15 mmol), NMO (0.199 g, 1.7 mmol), and 4Å molecular sieves (0.577g, 500 mg/mmol) in dichloromethane (6 ml) was stirred at room temperature for 30 minutes. Then TPAP (0.040 g, 10 mole %) was added in one portion to the mixture. The reaction mixture was stirred at room temperature for 1 hour. The solvent was removed *in vacuo* and the crude product was filtered through a short column of flash silica eluting with 30 % ethyl acetate in petroleum ether (40-60°C). The product was recrystallised from methanol to give yellow crystals. Yield (0.398, 80 %). m.p. 166-167°C; ν_{max} (CHCl₃) 2960, 2872, 1685, 1622, 1600, 1496, 1464, and 1220 cm⁻¹; δ_{H} (250 MHz; CDCl₃) 0.56 (3 H, s, 13-Me), 0.83-0.88 (6 H, m, 25-Me), 0.95 (3 H, d, J 7, 24-Me), 1.11 (3 H, d, J 7, 20-Me), 2.50 (3 H, s, 10-Me), 4.00 (3 H, s, OMe), 5.22-5.25 (2 H, m, 22-H and 23-H), 7.24 (1 H, s, 4-H), 7.28 (1 H, s, 7-H), 8.24 (1 H, s, 1-H), and 10.53 (1 H, s, CHO); δ_{C} (63 MHz; CDCl₃) 190.0 (CHO), 157.0 (C-3), 138.2, 137.1, 135.3 (C-22), 135.2, 132.2 (C-23), 131.1 (C-1), 129.0, 126.4, 124.2 (C-7), 102.4 (C-4), 55.4, 52.1, 52.0, 42.8, 41.9 (C-13), 40.5, 37.0, 33.1, 29.1, 26.6, 24.2, 21.0 (C-21), 19.9 (C-27), 19.6 (C-26), 17.6 (C-28), 14.4 (C-19), and 11.7 (C-18); (Found; M^+ , 432.3019. C₃₀H₄₀O₂ requires 432.3028); m/z (EI) 432 (M^+ , 100 %).



**Ethyl 3-[1 (10→6) abeo-3-Methoxyergosta-1, 3, 5, 7, 9, 22-hexaen-2-yl]
propenoate (54)**

A suspension of sodium hydride (60 % in mineral oil) (0.110 g, 2.5 equivalents) in benzene (2 ml) was stirred at 0°C for 10 minutes. After which triethylphosphonoacetate (0.357 ml, 1.5 equivalents) was added dropwise to the suspension, keeping the temperature at 0°C. The reaction mixture was stirred for 30 minutes. Then a solution of the 1 (10→6) abeo-2-formyl-3-methoxyergosta-1, 3, 5, 7, 9, 22-hexaene (53) (0.500 g, 1.16 mmol) was added dropwise to the reaction mixture and the resulting mixture was stirred at room temperature overnight. The crude product was extracted with diethyl ether, washed with 2M hydrochloric acid, water, saturated sodium hydrogen carbonate, water, and dried. The solvent was removed *in vacuo*. The product was further purified by column chromatography on silica eluting with 50 % diethyl ether in petroleum ether (40-60°C) and recrystallised from ethanol to give yellow crystals. Yield (0.453 g, 78 %). m.p. 84-85°C; ν_{max} (CHCl₃) 3010, 2960, 2933, 2872, 1702, 1622, 1602, 1496, 1464, and 1280 cm⁻¹; δ_{H} (250 MHz; CDCl₃) 0.57 (3 H, s, 13-Me), 0.83-0.87 (6 H, m, 25-Me), 0.95 (3 H, d, J 7, 24-Me), 1.10 (3 H, d, J 7, 20-Me), 1.35 (3 H, t, J 7, CO₂CH₂CH₃), 2.47 (3 H, s, 10-Me), 3.96 (3 H, s, OMe), 4.27 (2 H, q, J 7, CO₂CH₂CH₃), 5.22-5.26 (2 H, m, 22-H and 23-H), 6.68 (1 H, d, J 16, 2'-H), 7.18 (1 H, s, 4-H), 7.24 (1 H, s, 7-H), 7.86 (1 H, s, 1-H), and 8.05 (1 H, d, J 16, 1'-H); δ_{C} (63 MHz; CDCl₃) 167.6 (C-3'), 155.4 (C-3), 140.7 (C-1'), 137.8, 135.5 (C-22), 134.9, 132.9, 132.1 (C-23), 129.8 (C-2'), 128.8, 126.9, 123.5, 122.6 (C-7), 119.1 (C-1), 102.0 (C-4), 60.3 (C-4'), 55.4, 55.3, 52.2, 52.1, 42.8, 41.9 (C-13), 40.5, 37.1, 33.6, 29.2, 26.4, 24.3, 21.0 (C-21), 19.9 (C-27), 19.6 (C-26), 17.6 (C-28),

14.4 (C-19), and 11.7 (C-18); (Found; M^+ , 502.3452. $C_{34}H_{46}O_3$ requires 502.3447); m/z (EI) 502 (M^+ , 100 %).



Ethyl 3-[1 (10→6) abeo-3-Methoxyergosta-1, 3, 5, 7, 9-pentaen-2-yl] propanoate (55)

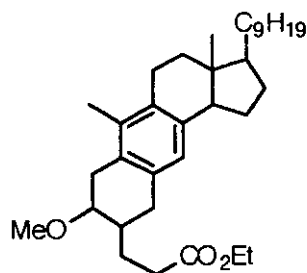
Method (a)

Ethyl 3-[1 (10→6) abeo-3-methoxyergosta-1, 3, 5, 7, 9, 22-hexaen-2-yl] propanoate (54) (0.100 g, 0.199 mmol) was dissolved in freshly distilled ethyl acetate (20 ml) and added to thoroughly wet palladium on 10 % carbon. The reaction vessel was evacuated several times and filled with hydrogen gas. The reaction was stirred at room temperature for 24 hours. The reaction mixture was then filtered through a short column of silica and the crude product was further purified by preparative TLC on silica. An oil was obtained. Yield (0.0836 g, 83 %). ν_{\max} (film) 3003, 2959, 2872, 1725, 1635, 1602, 1497, 1415, and 1376 cm^{-1} ; δ_{H} (250 MHz; CDCl_3) 0.55 (3 H, s, 13-Me), 0.79-0.85 (6 H, m, 25-Me), 0.88 (3 H, d, J 7, 24-Me), 1.02 (3 H, d, J 7, 20-Me), 1.24 (3 H, t, J 7, $\text{CO}_2\text{CH}_2\text{CH}_3$), 2.50 (3 H, s, 10-Me), 2.66 (2 H, t, J 7, 1'-H), 2.74-2.84 (1 H, m, 14-H), 2.91-3.00 (2 H, m, 11-H), 3.03 (2 H, t, J 7, 2'-H), 3.94 (3 H, s, OMe), 4.13 (2 H, q, J 7, $\text{CO}_2\text{CH}_2\text{CH}_3$), 7.18 (1 H, s, 4-H), 7.23 (1 H, s, 7-H), and 7.49 (1 H, s, 1-H); δ_{C} (63 MHz; CDCl_3) 173.4 (C-3'), 155.6 (C-3), 137.0, 132.7, 131.1, 129.1, 128.8, 128.5 (C-4), 127.2, 122.0 (C-7), 101.2 (C-1), 60.2 (C-1'), 55.5, 55.1, 52.2, 42.0 (C-13), 39.1, 38.8, 37.3, 34.3, 33.6, 31.5, 30.6, 28.9, 26.3, 26.2, 24.3, 20.5 (C-21), 18.9, 17.6 (C-28), 15.4, 14.3 (C-19), 14.2, and 11.5 (C-18); (Found; M^+ , 506.3765. $C_{34}H_{50}O_3$ requires 506.3760); m/z (EI) 506 (M^+ , 100 %).

Method (b)

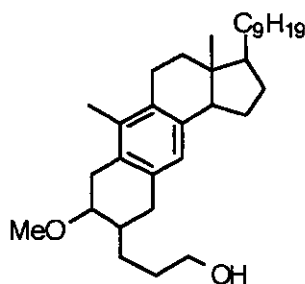
The above procedure was repeated using ethyl acetate, that had been left standing for some time, as solvent in the catalytic hydrogenation reaction instead of freshly distilled solvent. The crude product was purified by column chromatography on silica. Two fractions were recovered.

Fraction (1): 55 was obtained as an oil. Yield (0.0131 g, 13 %).



Ethyl 3-[1 (10→6) abeo-3-Methoxyergosta-5, 7, 9-trien-2-yl] propanoate (56)

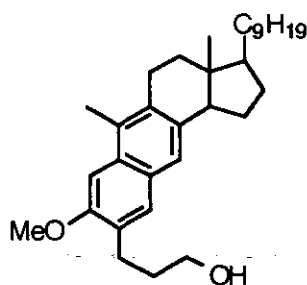
Fraction (2): 56 was obtained as an oil. Yield (0.065 g, 64 %). ν_{\max} (CHCl₃) 3004, 2958, 2932, 2873, 1725, 1465, 1376, and 1095 cm⁻¹; δ_{H} (250 MHz; CDCl₃) 0.56 (3 H, s, 13-Me), 0.78-0.83 (6 H, m, 25-Me), 0.87 (3 H, d, J 7, 24-Me), 0.99 (3 H, d, J 6, 20-Me), 1.24 (3 H, t, J 7, CO₂CH₂CH₃), 2.07 (3 H, s, 10-Me), 3.40 (3 H, s, OMe), 3.62-3.64 (1 H, m, 3-H), 4.11 (2 H, q, J 7, CO₂CH₂CH₃), and 6.61 (1 H, s, 7-H); δ_{C} (63 MHz; CDCl₃) 173.7 (C-3'), 137.6, 133.8, 131.8, 131.7, 129.0, 124.2 (C-7), 78.3 (C-3), 60.1, 56.5, 55.1, 51.7, 41.8 (C-13), 39.0, 37.2, 36.5, 35.7, 33.6, 32.3, 31.8, 31.4, 30.5, 30.2, 28.8, 25.7, 24.5, 24.1, 20.5 (C-21), 18.9, 17.5 (C-28), 15.4, 14.5 (C-19), 14.2, and 11.0 (C-18); (Found; M^+ , 510.4095. C₃₄H₅₄O₃ requires 510.4073); m/z (EI) 510 (M^+ , <0.1 %), 478 [(M-OMe-H), 100].



3-[1 (10→6) abeo-3-Methoxyergosta-5, 7, 9-trien-2-yl]-1-propanol (57)

To a suspension of lithium aluminium hydride (0.012 g, 1.5 equivalents) in diethyl ether (10 ml) was added dropwise a solution of ethyl 3-[1 (10→6) abeo-3-methoxyergosta-5, 7, 9-trien-2-yl] propanoate (56) (0.100 g, 0.196 mmol) in diethyl ether (5 ml). The reaction mixture was refluxed for 2 hours. The reaction was quenched with wet ethyl acetate

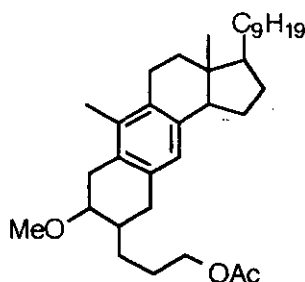
(5 ml) and the crude product was extracted with diethyl ether. The organic phase was washed with 2M hydrochloric acid, water, saturated sodium hydrogen carbonate, water, and dried. The solvent was removed *in vacuo*. The crude product was purified by preparative TLC on silica, eluting with 50 % diethyl ether in petroleum ether (40-60°C) gave an oil. Yield (0.0367 g, 40 %). ν_{\max} . (CHCl₃) 3435, 3005, 2957, 2934, 2874, 1463, 1383, 1220, and 1090 cm⁻¹; δ_{H} (250 MHz; CDCl₃) 0.56 (3 H, s, 13-Me), 0.78-0.83 (6 H, m, 25-Me), 0.87 (3 H, d, J 7, 24-Me), 0.99 (3 H, d, J 7, 20-Me), 2.07 (3 H, s, 10-Me), 3.40 (3 H, s, OMe), 3.61-3.66 (3 H, m, CH₂OH and 3 α -H), and 6.61 (1 H, s, 7-H); δ_{C} (63 MHz; CDCl₃) 137.6, 133.8, 132.0, 131.8, 129.2, 124.3 (C-7), 78.8 (C-3), 63.2, 56.6, 55.1, 51.8, 41.8 (C-13), 39.0, 37.2, 36.5, 36.3, 33.6, 32.2, 31.4, 30.7, 30.6, 30.3, 28.8, 25.8, 25.0, 24.1, 20.5 (C-21), 18.9, 18.2, 15.4, 14.5 (C-19), and 11.1 (C-18); (Found; M⁺, 468.3969. C₃₂H₅₂O₂ requires 468.3967); *m/z* (EI) 468 (M⁺, <1 %), 436 [(M - OMe-H), 100].



3-[1 (10→6) abeo-3-Methoxyergosta-1, 3, 5, 7, 9-pentaen-2-yl]-1-propanol (59)

Reduction with lithium aluminium hydride was carried out in a similar manner as the previous procedure. Ethyl 3-[1 (10→6) abeo-3-methoxy ergosta-1, 3, 5, 7, 9-pentaen-2-yl] propanoate (55) (0.100 g, 0.197 mmol) was used in the reduction. The crude product obtained was an oil and recrystallisation from methanol gave white crystals. Yield (0.075 g, 82 %). m.p. 130-131°C; (Found: C, 82.58; H, 10.66. Calculated for C₃₂H₄₈O₂: C, 82.70; H, 10.41 %); ν_{\max} . (CHCl₃) 3488, 3007, 2958, 2938, 2873, 1632, 1603, 1496, and 1465 cm⁻¹; δ_{H} (250 MHz; CDCl₃) 0.54 (3 H, s, 13-Me), 0.79-0.84 (6 H, m, 25-Me), 0.88 (3 H, d, J 7, 24-Me), 1.00 (3 H, d, J 7, 20-Me), 1.87-1.95 (2 H, m, CH₂CH₂CH₂OH), 2.48 (3 H, s, 10-Me), 2.80-2.86 (2 H, m, CH₂CH₂CH₂OH), 2.92-2.96 (2 H, m, 11-H), 3.56-3.63 (2 H, m, CH₂CH₂CH₂OH), 3.93 (3 H, s, OMe), 7.18 (1 H, s, 4-H), 7.21 (1 H, s, 7-H), and 7.48 (1 H, s, 1-H); δ_{C} (63 MHz; CDCl₃)

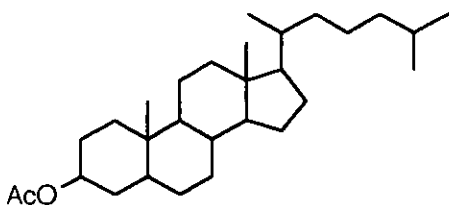
155.7 (C-3), 137.1, 132.6, 130.9, 129.8, 128.7, 128.6, (C-1), 127.4, 121.5, (C-7), 101.3 (C-4), 62.0 ($\underline{\text{CH}_2\text{OH}}$), 55.3, 55.2, 52.2, 42.0 (C-13), 39.0, 37.3, 37.2, 36.6, 33.6, 33.0, 31.5, 30.6, 28.8, 26.2, 24.3, 20.5 (C-21), 18.9, 17.6 (C-28), 15.4, 14.4 (C-19), and 11.5 (C-18); (Found; M^+ , 464.3653. $\text{C}_{32}\text{H}_{48}\text{O}_2$ requires 464.3654); m/z (EI) 464 (M^+ , 12%), 200 [(M -264), 100].



3-Acetoxy-1-[1 (10→6) abeo-3-Methoxyergosta-5, 7, 9-trien-2-yl] propane (58)

The alcohol 3-[1 (10→6) abeo-3-methoxyergosta-5, 7, 9-trien-2-yl]-1-propanol (57) (0.020 g, 0.0426 mmol) was dissolved in pyridine (1 ml) and acetic anhydride (1 ml) was added. The reaction mixture was stirred at room temperature overnight. The crude mixture was extracted with diethyl ether and washed with saturated copper sulphate solution, water, and dried. The solvent was removed *in vacuo*. The crude product was recrystallised from methanol to give white crystals. Yield (0.019 g, 90 %). m.p. 48-50°C; ν_{max} . (CHCl_3) 3005, 2958, 2932, 2873, 1727, 1466, 1367, and 1255 cm^{-1} ; δ_{H} (250 MHz; CDCl_3) 0.57 (3 H, s, 13-Me), 0.78-0.83 (6 H, m, 25-Me), 0.87 (3 H, d, J 7, 24-Me), 0.99 (3 H, d, J 7, 20-Me), 2.03 (3 H, s, 10-Me), 2.07 (3 H, s, OAc), 3.41 (3 H, s, OMe), 3.64-3.65 (1 H, m, 3-H), 4.07 (2 H, t, J 13, $\underline{\text{CH}_2\text{OAc}}$), and 6.62 (1 H, s, 7-H); δ_{C} (63 MHz; CDCl_3) 170.7 (C-4'), 137.6, 133.8, 131.9, 131.8, 129.0, 124.2 (C-7), 78.5 ($\underline{\text{CH}_3\text{CO}_2\text{R}}$), 64.7, 56.6, 55.0, 51.7, 41.7 (C-13), 39.0, 37.2, 36.5, 36.1, 33.6, 31.9, 31.4, 30.5, 30.2, 28.8, 26.5, 25.7, 25.2, 24.1, 20.9 (C-21), 20.4, 18.9, 17.5 (C-28), 15.3, 14.5 (C-19), and 11.0 (C-18); (Found; M^+ , 510.4079. $\text{C}_{34}\text{H}_{54}\text{O}_3$ requires 510.4073); m/z (EI) 510 (M^+ , 17 %), 478 [(M -32), 100].

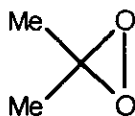
Experimental for Chapter 8



(60)

Dihydrocholesterol (3.0 g, 7.72 mmol) was dissolved in pyridine (30 ml) and acetic anhydride (30 ml) was added. The mixture was allowed to react overnight at room temperature, then ice was added to the reaction mixture and the acetate was collected by filtration. The precipitate was washed with dilute hydrochloric acid, water and dried in a vacuum oven. The acetate (**60**) was recrystallised from methanol to give colourless crystals, m.p. 110.0-110.8°C. Yield (3.1 g, 95 %). ν_{\max} . (CHCl₃) 2952, 2925, 2854, 1737, 1465, 1376, 1239, 1026 cm⁻¹; δ_{H} (250 MHz; CDCl₃) 0.64 (3 H, s, 13-Me), 2.01 (3 H, s, OAc), 4.61-4.74 (1 H, m, 3 α -H); δ_{C} (63 MHz; CDCl₃) 170.65 (C-28), 73.75 (C-3), 56.40, 56.25, 54.21, 44.6, 42.57 (C-13), 39.97, 39.51, 36.75, 36.16, 35.81, 35.46, 34.02, 31.97, 28.60, 28.25, 28.01, 27.46, 24.20, 23.84, 22.83, 22.57, 21.46, 21.19, 18.66, 12.21, 12.06; (Found: M^+ 430.3812. C₂₉H₅₀O₂ requires 430.3810) m/z (EI) 430 (M^+ , 45%)

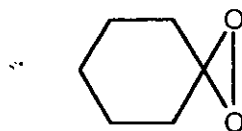
Preparation of Dimethyl dioxirane



A 1000 ml, three-necked, round bottomed reaction flask was equipped with an efficient mechanical stirrer and an additional funnel for solids, connected by means of a U tube to a two-necked receiving flask, the latter cooled at -78°C by means of a dry ice/ethanol bath. The reaction flask was charged with a mixture of water (127 ml), acetone (96 ml), and sodium hydrogen carbonate (29 g), and cooled at 5-10°C, with help of ice/water bath. Solid Oxone® (60 g, 0.0975 mol), was added in small portions over 15 minutes, to a vigorously stirred reaction mixture, which was kept cooled. After 3 minutes of the last addition, a moderate vacuum (80-100 Torr) was applied, the cooling ice/water bath was removed. The dimethyl dioxirane /acetone

solution was distilled and collected in the cooled receiving flask. The dioxirane was stored in the freezer. The molarity of the dioxirane was determined by titration against sodium thiosulphate solution (0.01M).

Preparation of 1,2-Dioxaspiro[2.5] octane



A mixture of cyclohexanone (60 ml), phosphate buffer solution (50 ml), and ice (21 g) was stirred at 0°C (ice-salt bath). Cooled Oxone® (135 g) was added as a slurry in water (200 ml) over 35 minutes. 5M potassium hydroxide solution was added simultaneously to maintain the pH at 7-8.5. A yellow colour was formed immediately upon combining the reagents. The mixture was stirred vigorously for 2-3 minutes and then poured into a beaker containing a cooled mixture (4: 2: 1) of anhydrous NaSO₄, NaH₂PO₄·H₂O, and Na₂HPO₄·7H₂O. The combined mixture was stirred vigorously in an ice-salt bath. The liquid phase was transferred rapidly to a cooled separating funnel, and the aqueous phase separated out. The dark yellow organic phase was dried over anhydrous sodium sulphate and stored in the freezer. The molarity was determined by titration against sodium thiosulphate solution 0.01M.

Attempted 25-hydroxylation on Dihydrocholesterol-3-acetate (60)

Dihydrocholesterol-3-acetate (**60**) (0.100g, 0.232 mmol) was dissolved in acetone and the solution was stirred in an ice bath, maintaining the temperature between 0-5°C. The dioxirane reagent (2-4 equivalents) was added dropwise to the reaction mixture and was stirred for 2 hours, monitoring the reaction by TLC. The reaction mixture was allowed to overnight between 0-5°C. After 24 hours, the solvent was removed by evaporation *in vacuo*. The crude product was recrystallised from methanol to yield white crystals (0.095 g), m.p. 109-110°C. The spectroscopic data (IR, proton and carbon n.m.r, and *m/z*) was identical to the starting material (**60**).

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