SYNTHETIC APPROACHES TO ANTI-HORMONAL STEROIDS

BY

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I would like to dedicate this

work to my parents in gratitude

for their support and encouragement.

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ABSTRACT

The first section of this thesis presents a review of the literature on hormones and anti-hormones, with particular emphasis on the structures and functions of the naturally occurring male and female sex steroids and on the preparation and properties of their synthetic analogues which have been found to function as anti-hormones.

The second section of the thesis presents the results of a small project carried out to synthesise two 7-methylated derivatives of 19-nortestosterone for biological evaluation as potential anti-androgens. One of these, 17β-hydroxy-7-methylestra-4,6-dien-3-one, was prepared by the oxidation of 7α-methyl-19-nortestosterone, whereas hitherto oxidation had been successfully achieved only with the 7β-methyl isomer. 7β-methyl-19-nortestosterone, the second of the two methyl-steroids required, was synthesised in poor yield, along with the 7α-isomer, by conjugate methylcuprate addition to 6-dehydro-19-nortestosterone. The separation of the 7-methyl epimers proved to be difficult and was achieved by development of a preparative HPLC system using a large laboratory-made column.

The third section of the thesis describes the major part of the research work and concerns the development of a route for the synthesis of 11-aryl-A-nor steroids. These steroids have been designed to explore their potential as anti-progestins. It is demonstrated that a thallium-mediated ring contraction of steroidal 4-en-3-ones carrying an 11-keto group provides entry to a functionalised A-nor steroid skeleton which can then be arylated using organolithium reagents. Manipulation of the functionalities led to the successful synthesis of 11-phenyl-A-nor-5β-androst-11-ene-2,17-dione, which should be a valuable intermediate for the generation of a set of 11-arylated A-nor steroids for biological evaluation.

CONTENTS

CHAPTER 1

Introduction - Hormones and Anti-hormones

1.1 The Endrocrine Hormones

Hormones are chemical messengers produced within an organism. They have target sites which are distant from the site of their synthesis and in animals they usually reach their targets via circulation in the blood stream. Their general functions are to co-ordinate the activities of the many different cells that comprise multi-cellular organisms. About 50 hormones have now been identified and new ones are being discovered every year.[1-3]

Chemically, the hormones in the human body can be classified into three groups[4]: peptides, with chains varying in length from 3 to ca. 200 amino acids and with some of the larger peptides also being associated with carbohydrate strands - e.g. the glycopeptides Follicle Stimulating Hormone (FSH) and Leutinizing Hormone (LH); tyrosine-derived phenylethylamine derivatives - e.g. the catecholamine hormone adrenalin and the thyroid hormone thyroxine; and the steroid hormones synthesised in the adrenal cortex and the gonads.

The majority of hormones are synthesised in special glands called endocrine (ductless) glands, which secrete their

products directly into the circulation via the blood supply with which they are in contact. Important endocrine glands are found in the neck - e.g. thyroid and parathyroid - and in the abdomen and groin - e.g. adrenals, pancreas and gonads (testes in the male and ovaries in the female).

There are two fundamentally different biochemical mechanisms by which hormones express their activities[5,6] when they interact at their target tissues. The polar and very water-soluble peptide hormones cannot easily enter the cell and instead they interact with hormone receptors which are specialised proteins embedded in the cell surface membrane. Binding occurs with a very high association constant and generally results in a conformational change in the receptor, which in turn leads to activation of an effector molecule with which it is associated in the cell membrane. Typically, the effector is the enzyme adenylate cyclase, which then amplifies the signal of the single hormone molecule binding to its receptor by converting a number of molecules of ATP to cAMP on the inside of the cell. The cAMP produced then acts as a co-factor for some enzyme (protein kinase) within an adjacent compartment of the cell, so that the net effect of binding of the hormone at the cell surface receptor is the activation of the enzyme reaction which leads to an increased metabolic turnover, the signal being amplified and carried by the second messenger, cAMP. This general mechanism (Figure 1.1) is intrinsically a fast response

process and is used by the body in many situations where rapid adjustment in metabolism is needed to meet short-term demands. For example, as well as the pancreatic peptides insulin and glucagon (which constantly regulate the critical concentration of glucose in the bloodstream by balancing its interconversion with glycogen), the "fight-or-flight" hormone adrenalin works by this type of mechanism.

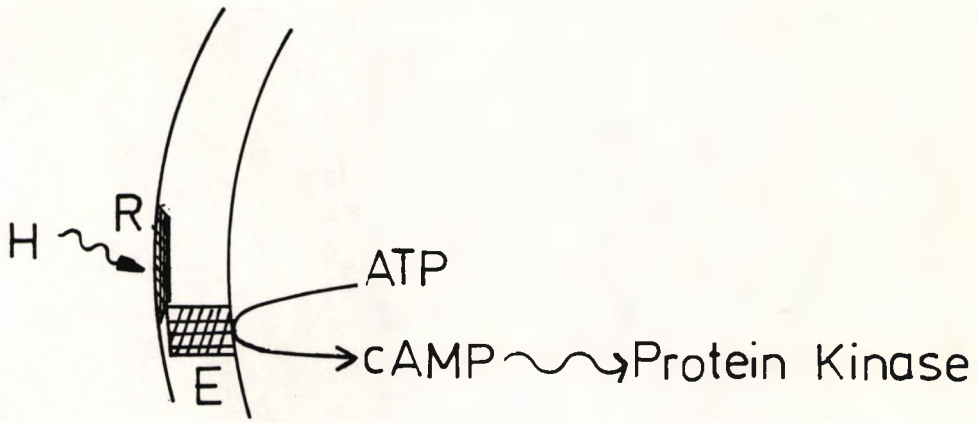


Fig. 1.1 Mechanism of hormone action by interaction at cell surface

The hormone (H) binds to a cell membrane receptor (R), leading to activation of an effector (E) on the internal surface. In this case the effector is adenylate cyclase, which converts ATP to the second messenger cAMP, providing an essential co-factor for a protein kinase.

The second general mechanism applies to the less polar hormones such as thyroxine and all the steroids, which are able to passively diffuse into the body's cells. Within the target cells there are special hormone receptor proteins to which they bind tightly. Evidence from the addition of

radio-labelled steroids to cultured cells, followed by cell lysis and sedimentation, suggested originally that the free receptors are generally located in the cell cytosol and that the bound hormone-receptor complex is then translocated into the cell nucleus. This view has been widely accepted, but some recent work now suggests that the free receptors may actually reside in the nucleus all the time and that the detection of unbound receptors in the cytosol was an experimental artefact due to leakage there from the nucleus during work-up. In any event, the tight hormone-receptor complex interacts with the chromosomal proteins (chromatin) associated with the nuclear DNA and the result is the activation of transcription of a segment of genetic code, copied as mRNA. The translation of this genetic code by the cell's ribosomes leads to synthesis of new proteins, which are generally enzymes and which in many cases utilise cAMP as a co-factor. Thus, the net result of binding of hormones such as the steroids to their intracellular (possibly intranuclear) receptors is again the induction of additional enzyme activity and is often again associated with elevated intracellular levels of cAMP (Figure 1.2). Crucial differences to the first mechanism described above are: firstly, that the induced enzyme(s) need not necessarily have been present at all, prior to arrival of the hormone, and secondly, that this is intrinsically a much slower response mechanism and is never used when the body needs a fast reaction such as adrenalin-stimulated activity.

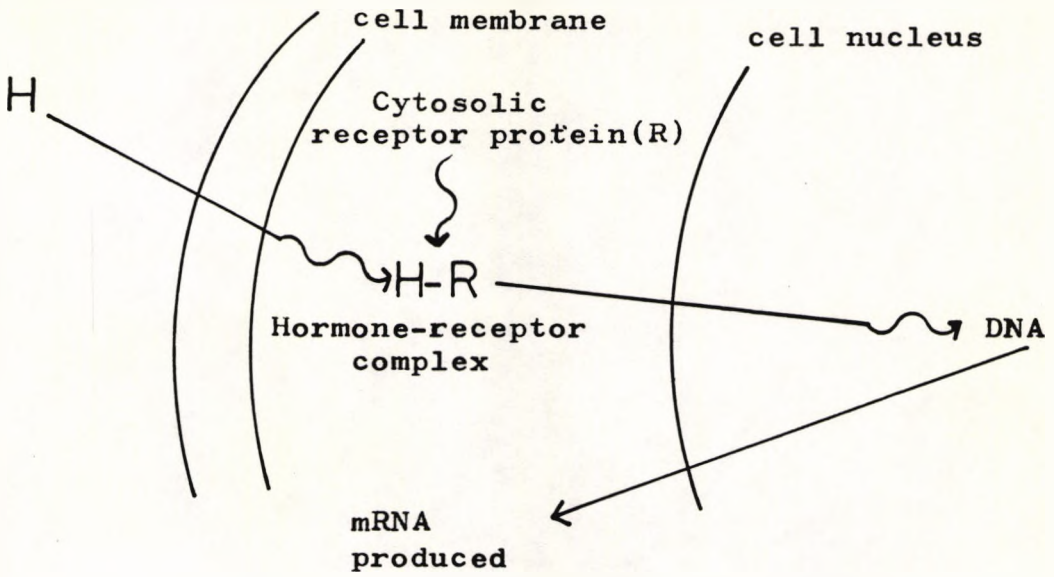


Fig. 1.2 Mechanism of hormone action for hormones entering the cell

One of the principal types of steroid hormones is the class of "corticosteroids" synthesised in the adrenal cortex. These are subdivided into the glucocorticoid class (e.g. cortisone, cortisol) which regulate aspects of amino acid, carbohydrate and lipid metabolism and also inflammatory responses, and the mineralcorticoid class (e.g. aldosterone) which regulate salt-water balance in the kidneys and also have a pressure effect which contributes to maintenance of blood pressure.

A further type of steroid hormones is synthesised in the ovaries and testes and these gonadal steroids are responsible for regulation of sexual function in women and men, respectively.

1.2 Pituitary and Hypothalamic Regulation

For the majority of the endocrine hormones, including all of the sex steroids, there is a 3-tiered mechanism which controls their circulating concentrations (Figure 1.3). At the lowest level, the plasma concentration of each steroid depends on its rate of synthesis in and release from the gonadal tissues where it originates. These gonadal processes are regulated by gonad-stimulating hormones (gonadotrophins) produced by the pituitary gland at the base of the skull. For example, the pituitary hormones FSH and LH are respectively responsible for controlling the release of estrogen and progesterone in the ovaries. In turn, the release of such stimulating hormones from the pituitary gland is controlled by further releasing hormones produced in the hypothalamus. This gland at the base of the brain, is situated immediately above the pituitary and is connected to it by a short stalk through which chemical products can be carried in a direct flow of blood. Thus, for example, rapid pulses of Gonadotrophin Releasing Hormone, known as Gn-RH or

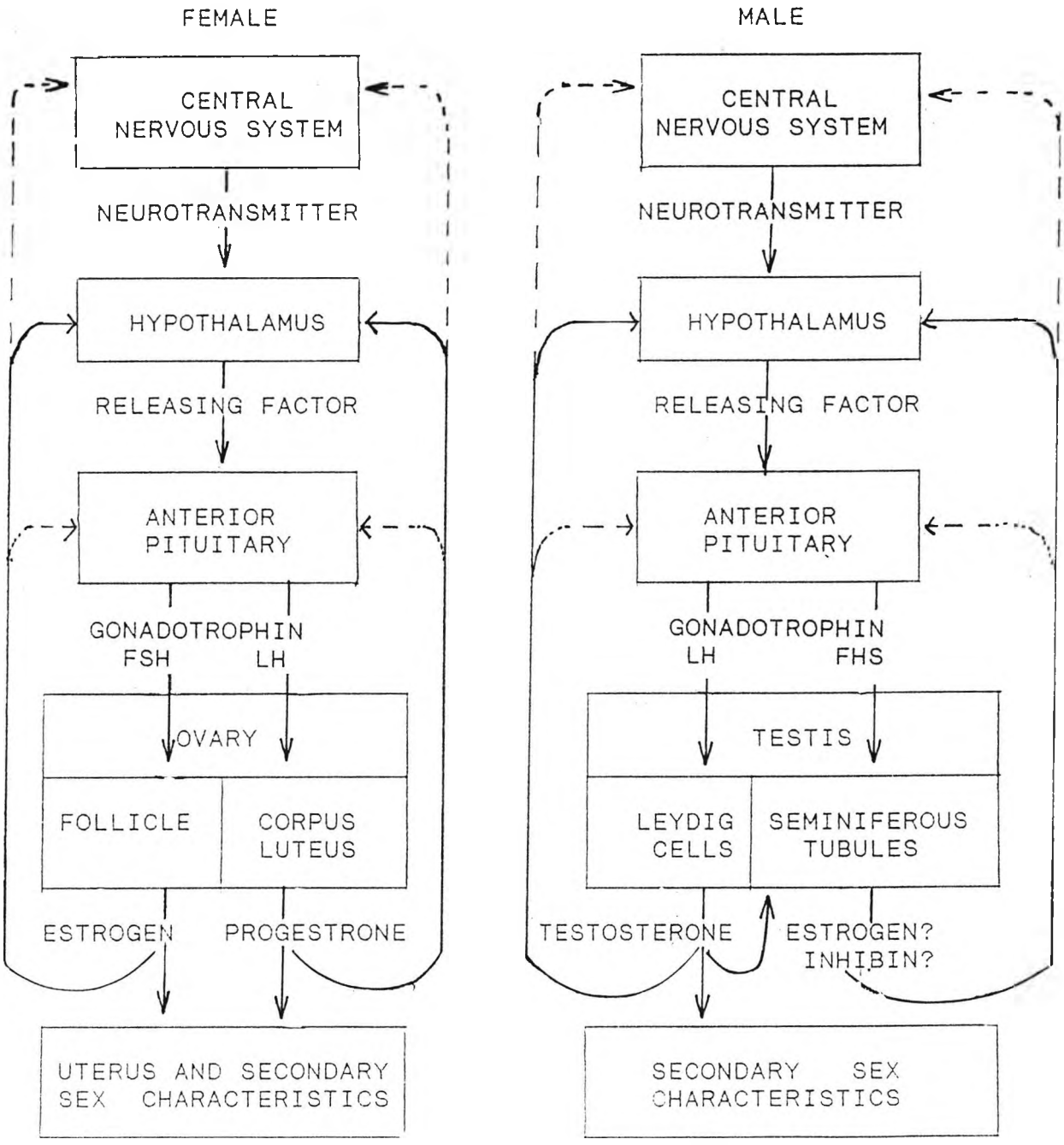

FEMALE
CENTRAL NERVOUS SYSTEM
NEUROTRANSMITTER
HYPOTHALAMUS
RELEASING FACTOR
ANTERIOR PITUITARY
GONADOTROPHIN
FSH
LH
OVARY
FOLLICLE
CORPUS LUTEUS
ESTROGEN
PROGESTRONE
UTERUS AND SECONDARY SEX CHARACTERISTICS
MALE
CENTRAL NERVOUS SYSTEM
NEUROTRANSMITTER
HYPOTHALAMUS
RELEASING FACTOR
ANTERIOR PITUITARY
GONADOTROPHIN
LH
FHS
TESTIS
LEYDIG CELLS
SEMINIFEROUS TUBULES
TESTOSTERONE
ESTROGEN?
INHIBIN?
SECONDARY SEX CHARACTERISTICS

Fig. 1.3

LH-RH, from the hypothalamus regulate the release of pituitary FSH and LH which are targeted on the gonads. There are several opportunities for feed-back regulation in this 3-tiered system, so that circulating levels of the pituitary or endocrine hormones or metabolic products connected with their activities can interact with receptors at higher tiers, thus allowing the whole system to maintain a finely balanced responsiveness to physiological and metabolic requirements. Moreover, the close proximity of the highest tier to the brain undoubtedly results in some direct neural inputs as well.

1.3 Hormonal Aspects of Sexual Function in the Female

In the female, estrogens (e.g. estradiol: 1.1a) produced in the granulosa cells in the ovaries under the controlling influence of pituitary FSH during the first half of the monthly cycle stimulate the thickening and vascularization of the lining of the uterus (endometrium), preparing it for implantation. Following ovulation at the mid-point of the monthly cycle, the ovum (oocyte) released from its burst follicle on the ovarian surface travels down the fallopian tube towards the uterus. In the course of its fallopian transport, the ovum may meet spermatozoa and fuse with one of them in the process of fertilization. A fertilized ovum will begin to grow and divide and the resulting group of cells (blastocyst) may then become attached to the surface of

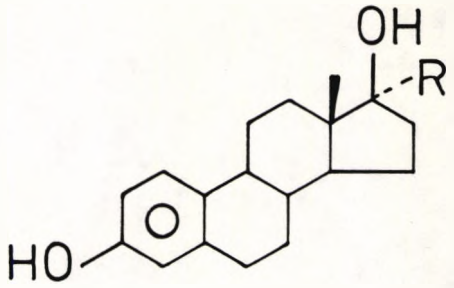
the uterus, implanting in the endometrium (conception can be defined as occurring at this point) and developing into an embryo and later into a fetus, which is interfaced with the maternal blood system through the placenta that develops during the early stages of pregnancy.[5]

Following the release of the ripened ovum at the mid-point in the monthly cycle, the burst follicular sack is transformed into a solid secretory body called the corpus luteum. Under the controlling influence of pituitary LH, the corpus luteum secretes progesterone (1.2) which maintains the receptivity of the uterine endometrium to implantation and also is essential to maintain the implantation as pregnancy develops. During pregnancy, progesterone also performs several other vital hormonal functions, including stimulation of the development of mammary tissue for later lactation and inhibition of the release of any further ovulation (which could obviously have serious consequences, if a second conception occurred some months after the first). So important is a high level of progesterone during pregnancy that, in addition to its continued secretion by the corpus luteum, it also begins to be made in large quantities by the placenta as the pregnancy progresses.

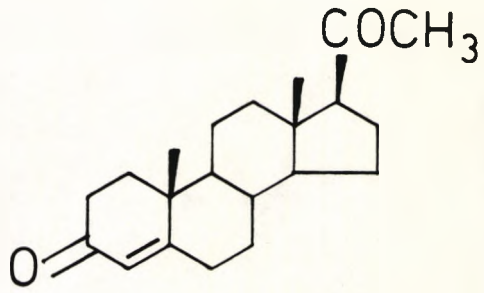
As the blastocyst implants and begins its development, it emits a signalling substance, human chorionic gonadotrophin

or hcg (the chorion is an outer membrane that encapsulates the developing fetus before birth) whose presence ensures the maintenance of the corpus luteum. (In fact, the newest and most sensitive pregnancy-testing kits rely on the detection of traces of hcg in the urine within a few days of pregnancy becoming established). If this signal fails to appear, the levels of LH decline steeply at the end of the monthly cycle, the corpus luteum breaks down (luteolysis), progesterone levels drop and the endometrium is shed in a flow of blood (menstruation). This leaves a fresh, thin endometrial surface and the whole cycle of events leading to ovulation can begin again.

It was the recognition of the key rôle of progesterone in inhibiting ovulation which led to the development of oral contraceptives (the "Pill") in the mid-1950s. Progesterone itself is unsuitable as an oral drug, since it is rapidly metabolised by the "first pass" effect, in which substances are absorbed across the intestinal wall into the blood supply in the portal vein, from where they are carried directly to the liver and are met by an array of metabolising enzymes. A search for analogues of the natural compound with progesterone-like activity (progestins) and with the ability to survive first-pass hepatic metabolism resulted in the observations that removal of the 19-methyl group and introduction of 17β-hydroxyl-17α-acetylenic functionality in place of the natural 17β-acetyl group separately led to



(1.1) (a) R=H
(b) R=C≡CH



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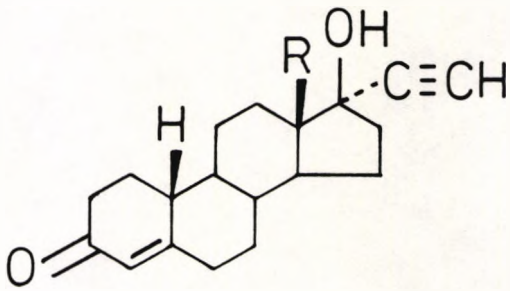
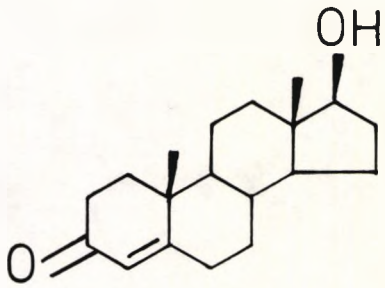


(1.3) (a) R=Me
(b) R=Et



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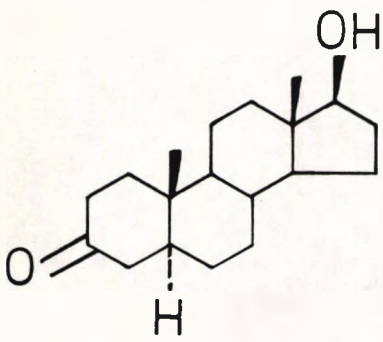


(1.5)

increased progestin potency and oral activity. These properties proved to be additive, so that their combination into the semi-synthetic steroid norethisterone (1.3a) led to the first generation of oral contraceptives. It was soon found in clinical studies that better control of cyclicity and bleeding and reduction in side effects could be achieved by combining the progestin with an orally active estrogen. A typical example is 17α-ethynylestradiol (1.1b) with the acetylenic unit again providing protection against first-pass metabolism. During the 1960s a further synthetic progestin, levonorgestrel (1.3b) was discovered to be 80 times more potent than norethisterone. Combinations of levonorgestrel and ethynylestradiol have been the most popular formulations in oral contraceptives used in the West for more than a decade.

As well as having a direct effect on the endometrium, estrogens are also important in stimulating the development of secondary sexual characteristics (breasts, deposition of fat on hips and thighs, hair, etc.) in the female at puberty.

1.4 Hormonal Aspects of Sexual Function in the Male

The testes in the male contain very long, highly convoluted seminiferous tubules, within which spermatogenesis occurs.[5,6] The primary germ cells (spermatogonia, containing 46 chromosomes) undergo several successive

divisions, eventually producing spermatocytes, each of which contains 23 chromosomes that will pair with 23 chromosomes in the ovum on fertilization. The spermatocytes gradually transform into round spermatids which eventually develop a tail, midpiece and compact head in the final spermatozoa. During these development stages, the sperm cells remain in close contact with Sertoli cells which line the inner walls of the seminiferous tubules and maintain and nourish the growing sperm. After about 10 weeks of development within the seminiferous tubules, the spermatozoa are released and are carried out of the testes in a flow of fluid, passing slowly (1-2 weeks transit time) through a very long, coiled duct called the epididymis. During the epididymal transport stage the spermatozoa undergo a number of biochemical changes both in internal metabolism and, especially, in the nature of the external surface coating, which leads to them acquiring motility and prepares them for the eventual process of fusion with the ovum. On ejaculation, the contents of the epididymis pass through a connecting tube (vas deferens) and out through the urethra, acquiring additional fluids on the way which are contributed to the seminal fluid by ducts from the prostate and Cowper's glands.

The Sertoli cells require two control agents to be present in order to function correctly, one being FSH from the pituitary gland and the other the androgenic steroid hormone, testosterone (1.4). The latter is synthesised in Leydig

(interstitial) cells which are found in the spaces between the seminiferous tubules. Production of testosterone in the Leydig cells is under the control of the pituitary hormone LH and, as in the female, the secretion of both the gonadotrophins FSH and LH in the male pituitary is regulated by pulsatile signals of Gn-RH (LH-RH) from the hypothalamus.

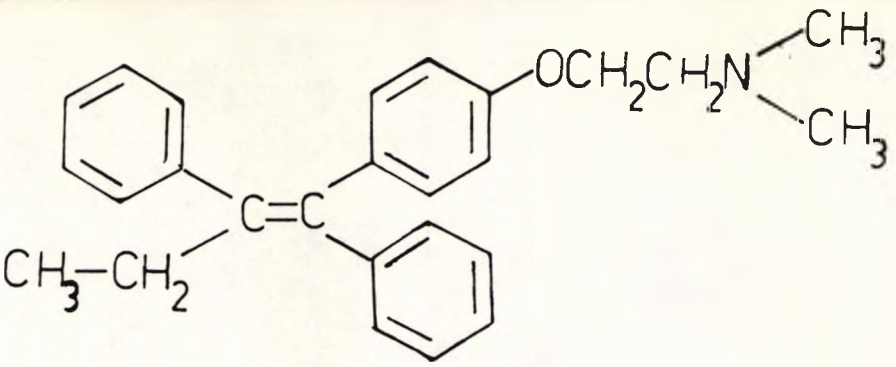
Some testosterone is enzymatically reduced to 5α-dihydrotestosterone (DHT; 1.5) in circulation, in peripheral tissues (e.g. in skin) and in target tissues (including cells in the testes and in accessory glands such as the prostate) and it is the reduced form of the androgen which binds to the androgen receptor in many tissues. In addition to the primary function of maintaining spermatogenesis, androgens serve several other hormonal functions in the male. In particular, their continuous presence in a sufficiently high plasma concentration is essential for the maintenance of secondary sexual characteristics (body musculature, hair, and voice) and libido and potency.

1.5 Anti-hormones

Substances which mimic the action of a natural substance, being able to bind to its natural receptor and elicit, at least qualitatively, the same type of response, are termed agonists.[2] Many agonists of the natural, human hormones have been synthesised and have proved invaluable in a variety

of therapies: e.g. powerful glucocorticoid agonists such as the semi-synthetic steroids dexamethasone, betamethasone and prednisolone are used for treating chronic inflammatory conditions and allergies; agonists of the natural progestin, such as levonorgestrel, are used for the suppression of ovulation; estrogen agonists are used for hormone replacement therapy in post-menopausal women to prevent bone loss leading to osteoporosis (thin, fragile bones) in later life.

Substances which bind strongly to a biological receptor but fail to elicit the natural agonistic response are termed antagonists. Antagonism of hormone receptors can also be used as a basis for a variety of therapies. Amongst the important applications of this type of approach are the uses of anti-hormones in cancer chemotherapy. Many tumours which originate in tissues of the reproductive system have a strong requirement for supplies of the hormone naturally associated with that tissue and can be prevented from growing by starving them of that hormone. For example, breast tumours often have a requirement for estrogens and their growth can be inhibited by anti-estrogens such as ICI's non-steroidal compound tamoxifen (1.6).[7] Recently, some steroidal compounds such as (1.7) have been shown to be pure anti-estrogens[8-10]



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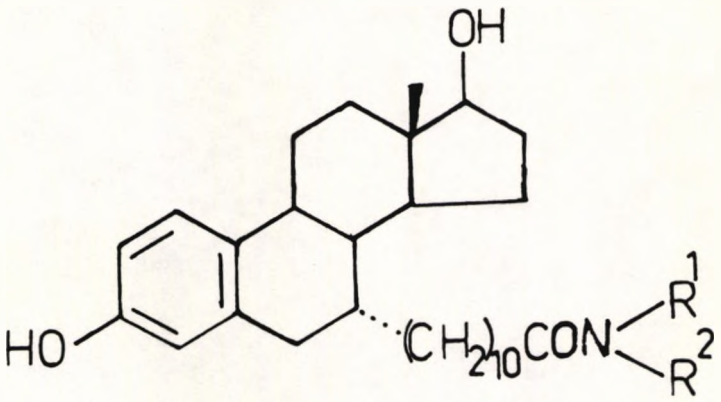


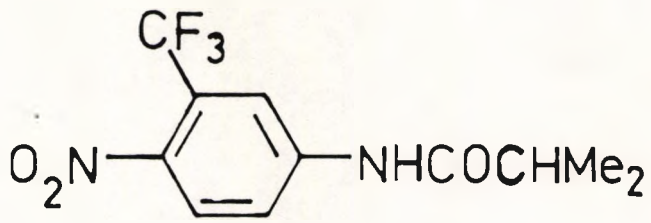
$R^1 = CH_3$

$R^2 = {}^nC_4H_9$

(1.7)

Another example is the prostate gland, which may become larger due to benign growth or due to cancer. Carcinoma of the prostate is particularly difficult to cure and there are often metasteses (secondary tumours formed by cells of the original growth having split off and lodged elsewhere in the body) in inaccessible locations such as the spine. Both the primary and secondary tumours are generally androgen-dependent and can be inhibited by completely shutting off the

supply of androgens. Originally this was achieved by castration, a traumatic event for men already faced with a terminal illness. Nowadays, there are a number of options referred to as "chemical castration" which achieve the same objective non-surgically. For example, the use of antagonists of the natural LH-RH will switch off secretion of FSH and LH from the pituitary and hence lead to collapse of both testosterone production by the Leydig cells and sperm biosynthesis by the Sertoli cells. Alternatively, an anti-androgen can be used which blocks the steroidal hormone receptors in the target tissues. Examples include the non-steroidal drug flutamide (1.8) and the steroid cyproterone acetate (1.9).



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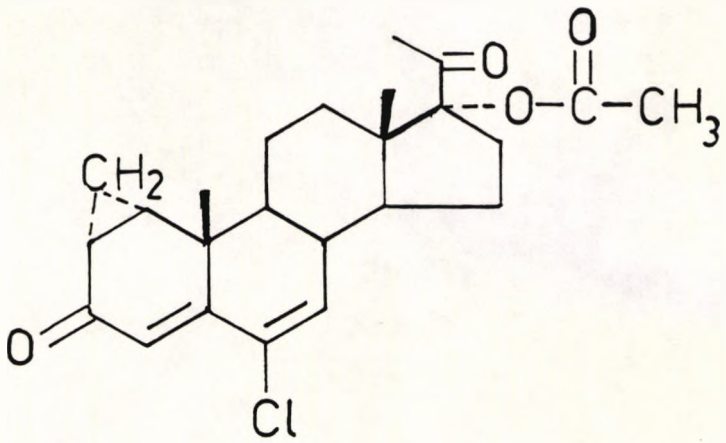

(1.9)

The applications of anti-androgens and anti-progestins are discussed in detail in the following chapters.

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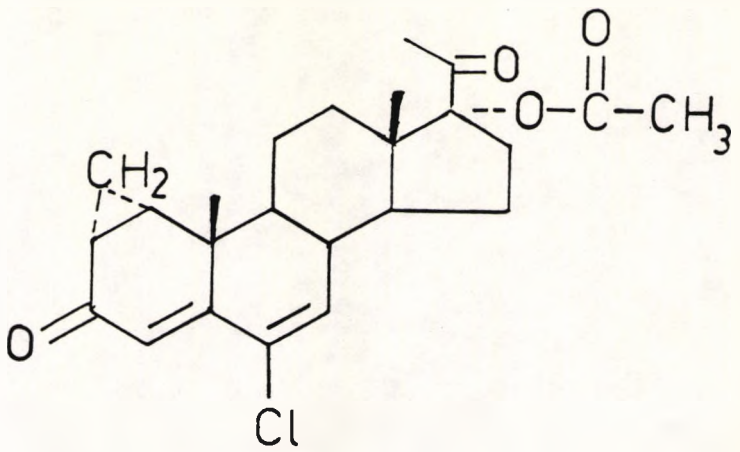
CHAPTER 2

Steroidal Anti-androgens

2.1 Biological Studies of Anti-androgens

Two compounds which have long been known to possess weak anti-androgenic activity are dodecahydrophenanthrene[1] and A-norprogesterone.[2] However, the first major clinical advance in this field resulted from studies of the 1,2-methylene-substituted steroid cyproterone acetate (2.1). This had been synthesised as a potential progestin and was indeed found to have powerful progestational effects, but in the course of the evaluation it was also discovered to have strong anti-androgenic character. In the mature rat, the compound causes atrophy of the seminal vesicles, prostate gland and other androgen-responsive organs, as well as cellular changes in the pituitary gland that are typical of castration with increased gonadotropin secretion (this latter effect is not seen in men).[3] The testes are unaffected by small doses, but large doses can cause decreases in Leydig cell function and spermatogenesis. As a result of these characteristic anti-androgenic effects, cyproterone acetate has been extensively investigated and used clinically for the management of prostate carcinoma; for reduction of acne in both sexes and hirsutism in women; in the therapy of precocious puberty, to delay premature sexual and physical maturation; and for reduction of libido in men with severe (and criminal) deviations in sexual behaviour.[4]



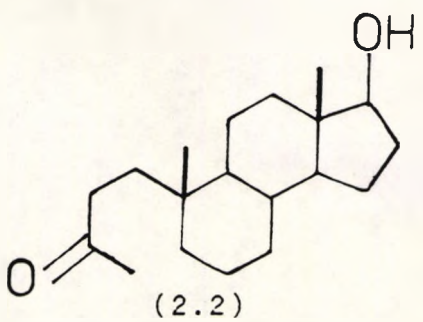
(2.1)

Cyproterone acetate has also been investigated as a male contraceptive agent. The combination of direct anti-androgenic effects (antagonist binding to dihydrotestosterone receptors) and feed-back inhibition of the pituitary gland by this steroid results in down-regulation of FSH and LH secretion and shut-down of spermatogenesis. However, disadvantages of cyproterone acetate include consequent loss of testosterone production from the Leydig cells, leading to loss of potency and libido, and the fact that this steroid is also a powerful progestin causing breast changes in the male.[4,5]

Apart from cyproterone acetate and A-norprogesterone (for treatment of acne), many other steroids have been investigated and/or used for their anti-androgenic character. However, whilst more than 200 anti-androgens have been reported, only a few have progressed further than the initial identification of their activity.[6-9] The ring-A oxa-steroid, 6α-bromo-17β-hydroxy-17α-methyl-4-oxa-5α-androstan-

3-one (BOMT) exhibits anti-androgenic effects in all androgen-dependent tissues outside the central nervous system,[10,11] competing for DHT binding sites slightly more effectively than does cyproterone acetate. Other steroids with high effectiveness in competing for DHT receptors include 17β-hydroxy-2-oxa-4,9-estradien-3-one and 17β-hydroxy -17α-methyl-2-oxa-4,9-estradien-3-one,[12] although they showed some androgenic as well as anti-androgenic character. The highest degree of inhibition seemed to be associated with compounds showing a general flattening of ring geometry.

A variety of B-norandrostanes have been shown to possess little or no androgenic character and amongst these, B-nor-17α-methyltestosterone was active as an anti-androgen and B-nor-deoxycorticosterone and its acetate were marginally anti-androgenic.[13] The anti-androgenic activity of 17β-acetoxy-4α,5-cyclo-A-homo-B-nor-5α-androst-1-en-3-one was found to be comparable with that of cyproterone acetate,[14] as was the activity of the 4,5-secosteroid (2.2).[15]



(2.2)

Alkylations of the A-ring of testosterone appear to mainly give rise to compounds with varying degrees of androgenicity.[16] However, 2α-methyltestosterone with a 17α-ethynyl or 17α-vinyl group are claimed to show progestational and anti-androgenic qualities.[17]

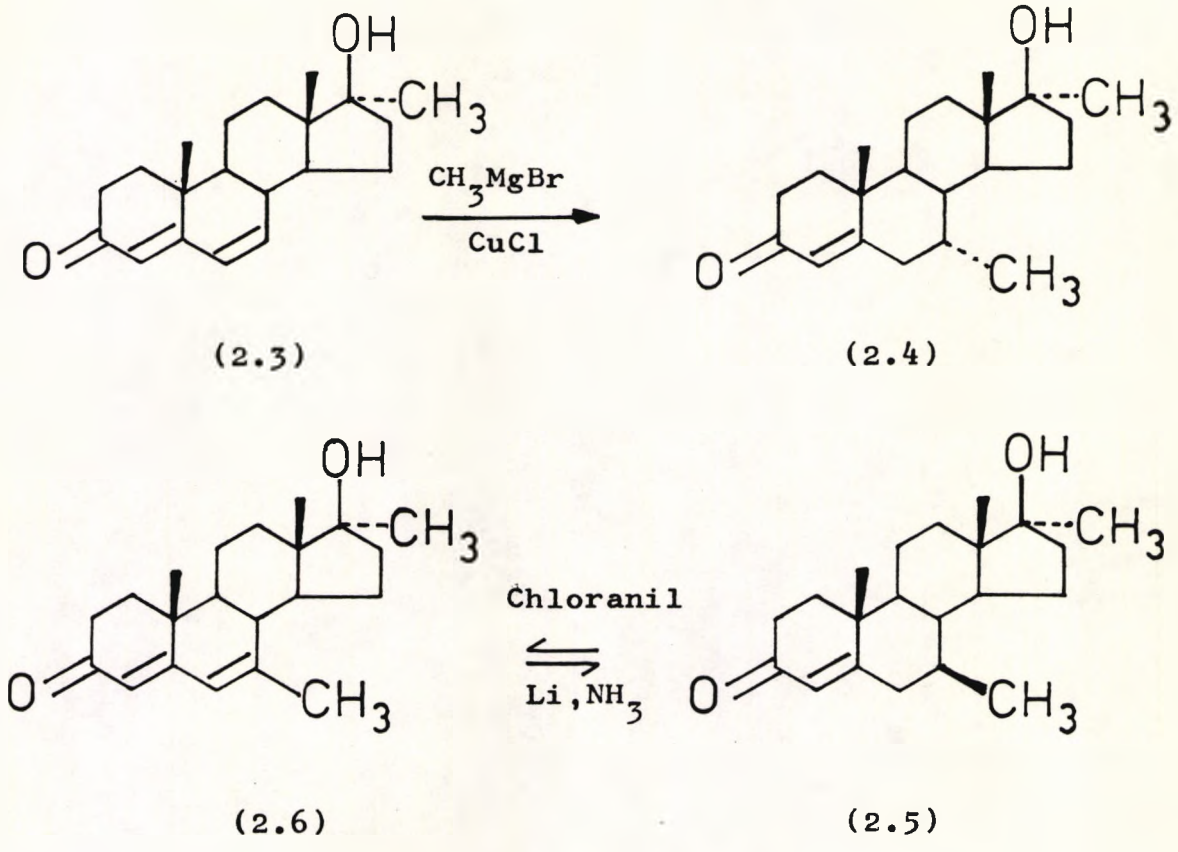
Based on evidence from earlier, limited structure-activity correlations, a Japanese group synthesised 16β-ethyl-19-nortestosterone (known as TSAA-291), which was shown to be an anti-androgen (but less powerful than cyproterone acetate) with reduced or negligible hormonal activities of other types and has been studied in clinical trials for the treatment of prostate cancer.[18]

A group at Upjohn observed that introduction of a 7α-methyl group onto 19-nortestosterone resulted in increases in both anabolic and androgenic activity.[19] The subtle effects of stereochemistry are very important for determination of agonist or antagonist actions following receptor binding. Thus, 7α,17α-dimethyl-5β,17β-dihydroxy-19-norandrostan-3-one is anabolic but anti-androgenic,[20] whereas 7α-methylestr-4-ene-3α,17β-diol is anti-progestational.[21] Recently, attention has focussed on the possible anti-androgenic, anti-fertility effects of 7β-methyl-19-nortesterone.[22]

2.2 Synthetic Routes to 7-alkylated Steroids as Potential Anti-androgens

As discussed in the previous Section, recent interest has focussed on the production of 7-methylandrogens in the androstane and pregnane series.

Various methods for preparing 7-alkylated steroids have been reported in the literature.[23-26] The method described by Campbell and Babcock[3] involving Michael addition of methylmagnesium bromide to 6-dehydro-17α-methyl-testosterone in the presence of cuprous chloride gave 7α,17α-dimethyl-testosterone (2.4) as the main product and a small amount of the 7β-epimer (2.5) (Scheme 2.1). On dehydrogenation of the crude product with chloranil, the minor component (2.5) was converted into 6-dehydro-7,17α-dimethyltestosterone (2.6), which was separated from 7α-methylenone (2.4) and then reduced back to 7β-methyl-enone (2.5) with Li-NH_3. The 7α-epimer does not undergo dehydrogenation with chloranil.

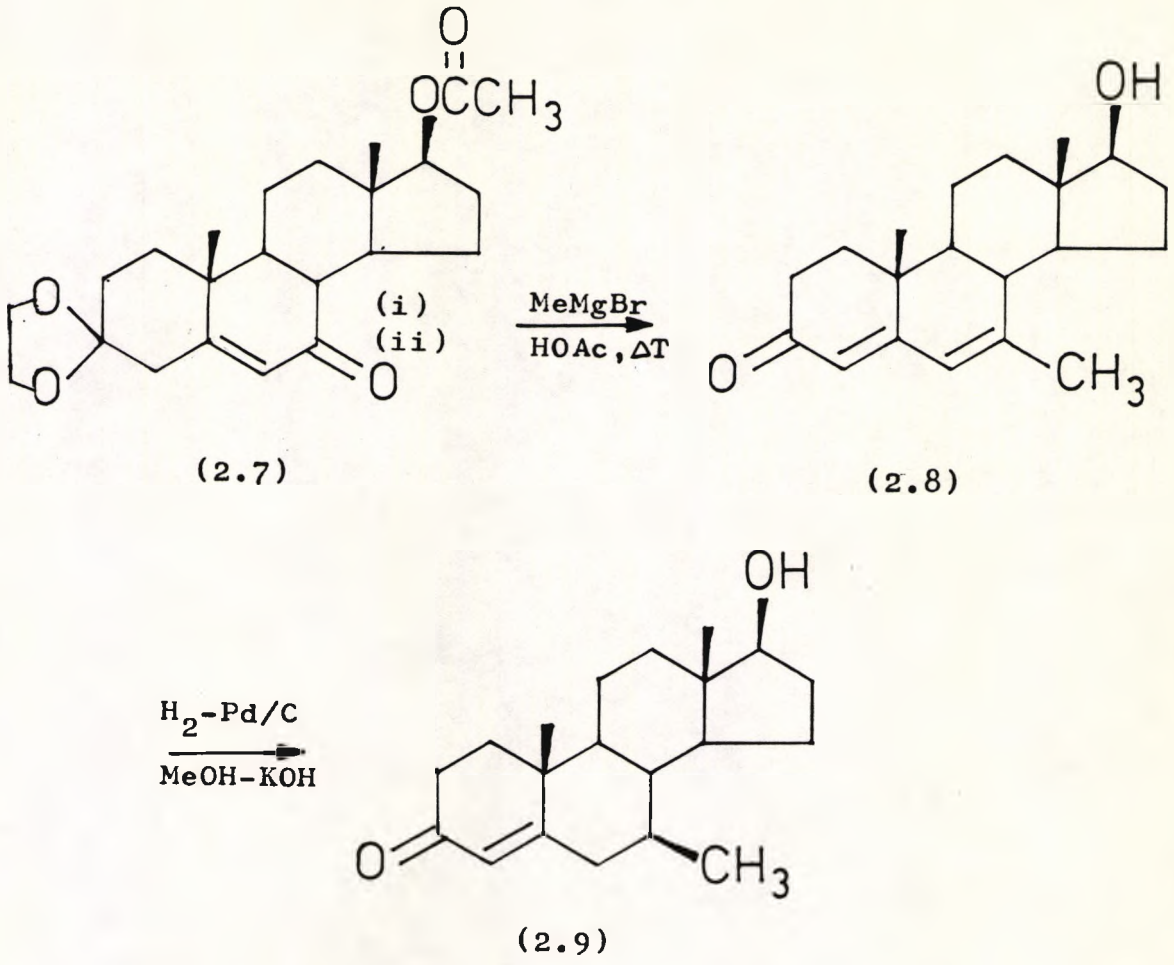


(2.3) (2.4)

(2.6) (2.5)

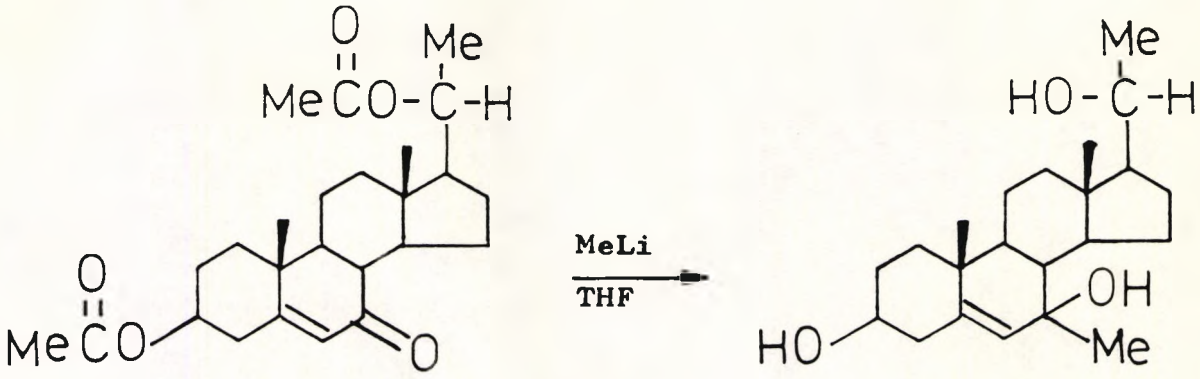
Scheme 2.1

7β-Methyltestosterone has been prepared by Zderic and Ringold[24], by addition of methyl Grignard to 7-ketotestosterone ethylene ketal acetate (2.7) (Scheme 2.2.). When the total crude product of the Grignard reaction was treated with hot aqueous acetic acid, 7-methyl—6-dehydro-testosterone (2.8) was obtained in excellent yield. This was hydrogenated with 5% palladium carbon catalyst in methanol-KOH mixture to give 7β-methyltestosterone (2.9).

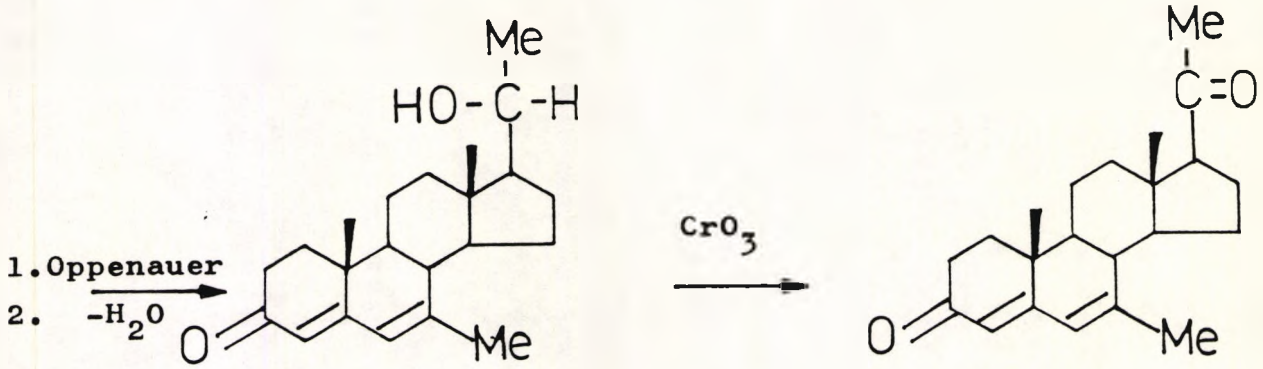


Scheme 2.2

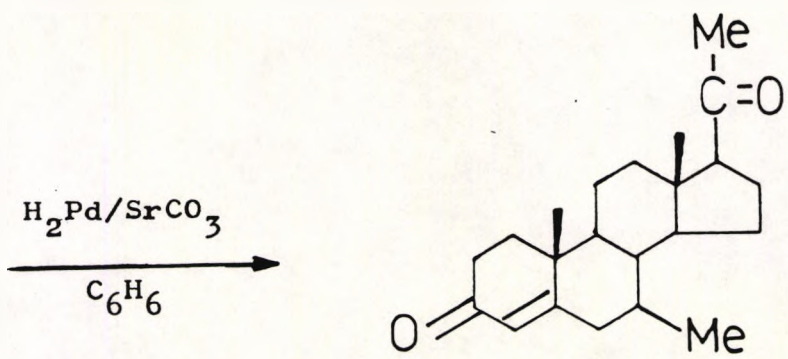
Another method for preparing 7β-alkylated steroids and 6-dehydro-7-methyl steroids has been described by Robinson and co-workers[25] (Scheme 2.3). When the 7-ketone (2.10) was treated with methylmagnesium iodide or, preferably, lithium methyl in ether-tetrahydrofuran mixtures, compound (2.11) was formed. This triol underwent Oppenauer oxidation and dehydration to give dienone (2.12) which was oxidised with chromic acid to compound (2.13), 7-methylpregna-4,6-dien - 3,20-dione, in good yield. Finally hydrogenation of the diene (2.13) in benzene with palladium-strontium carbonate catalyst[27] gave 7β-methylprogesterone (2.14) in poor yield.



(2.10) (2.11)



(2.12) (2.13)

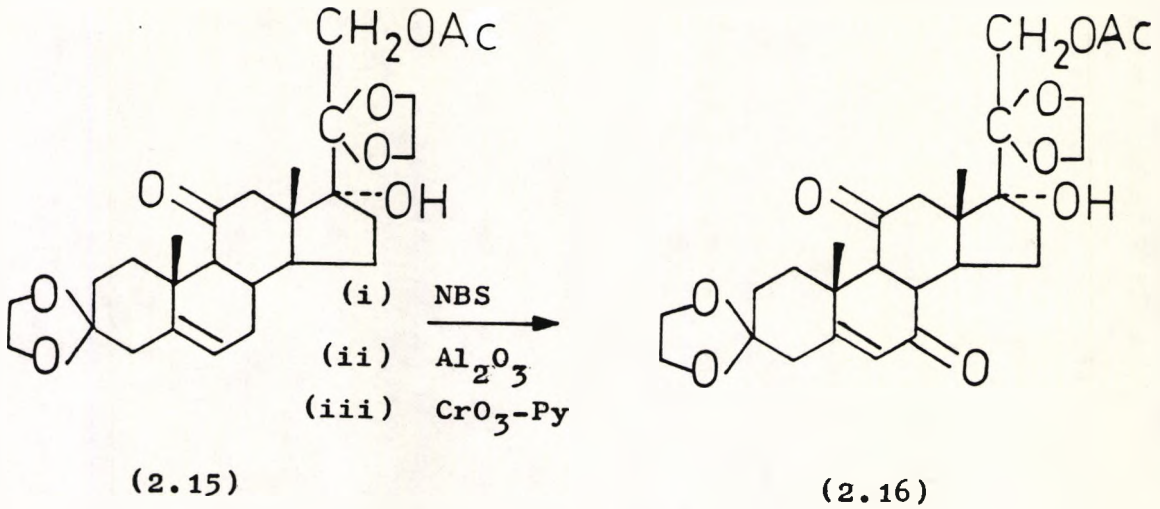


(2.14)

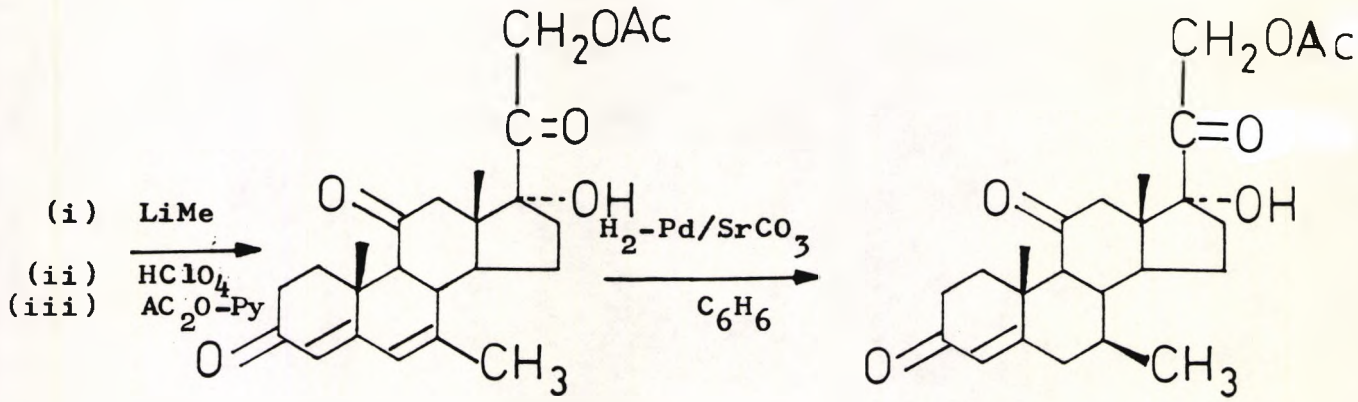
Scheme 2.3

7β-Alkylated steroids can also be prepared using another method described by Robinson and his colleagues[26] (Scheme 2.4).

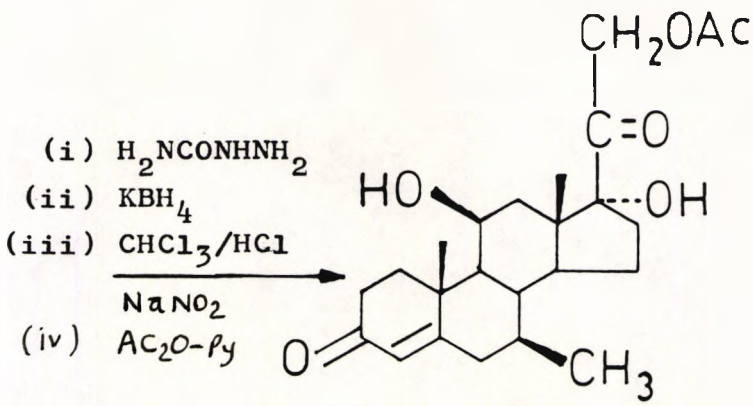
The 7-ketobisketal (2.16) was prepared from (2.15) by successive Zeigler bromination, treatment with alumina, and chromic acid-pyridine oxidation.[28,29] When compound (2.16) was treated with lithium methyl in tetrahydrofuran followed by treatment with perchloric acid in methanol at room temperature and then acid anhydride-pyridine acetylation, compound (2.17) was obtained. Hydrogenation of the dienone (2.17) in benzene solution with palladium strontium catalyst[7] gave 7β-methylcortisone acetate (2.18). Conversion of this to the hydrocortisone derivative (2.19) was achieved by potassium borohydride reduction[30] of the 3,20-bis-semicarbazone followed by regeneration of the carbonyl groups at C-3 and C-20, using a two phase chlorform aqueous hydrochloric acid procedure,[31] and finally reacetylation at C-21.



(2.15) (2.16)



(2.17) (2.18)



(2.19)

Scheme 2.4

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CHAPTER 3

Anti-progestins

3.1 Introduction

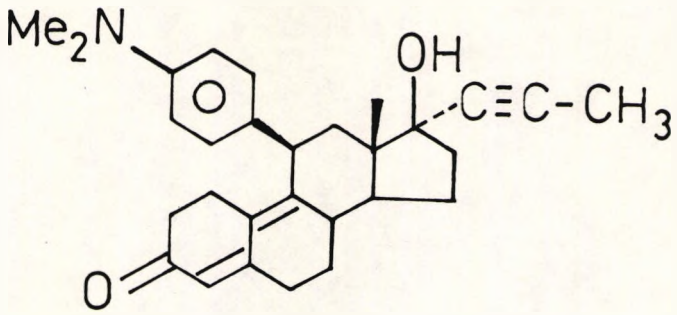
During the 1970's, several groups of workers made efforts to develop anti-progestational compounds and a number of steroids were subjected to screening procedures to determine what structural features would lead to inhibition of binding of radio-labelled progesterone or other progestins to the progesterone receptor.[1-3] However, the breakthrough eventually came as the result of an accident, when a group working at Roussel-Uclaf in France synthesised some 11β-aryl steroids in the search for anti-glucocorticoid activity. Some of these new compounds, which included RU 38486, indeed proved to be somewhat active as anti-glucocorticoids, but more remarkably they also proved to be very potent anti-progestins.

The potential use of anti-progestins as a means of terminating pregnancy, based on the blockage of the essential function of progesterone in maintaining gestation (see Chapter 1) was quickly appreciated by Roussel-Uclaf. Following toxicological studies which established the safety of the new steroid, it was extensively investigated in clinical trials. Most of these studies have focused on the

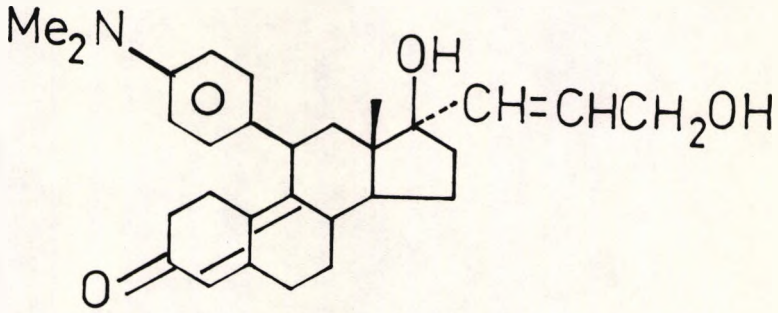
use of the compound for medical termination of early pregnancy. Somewhat unexpectedly, the compound's efficacy to induce complete abortion was lower than might have been anticipated. However, success rates that compare favourably with the currently available alternatives, vacuum aspiration and prostaglandin administration, can be achieved if the anti-progestin treatment is complemented with a low dose of a synthetic prostaglandin analogue. Such combination regimens are relatively free of side-effects.

Preliminary results suggest that RU 38486 has potential also as an emergency post-coital contraceptive with a wider range of action than the currently used hormonal preparations which need to be administered within 48-72h after unprotected intercourse. Nevertheless, treatment as early as possible seems advisable because it would appear that efficacy decreases after implantation has commenced.

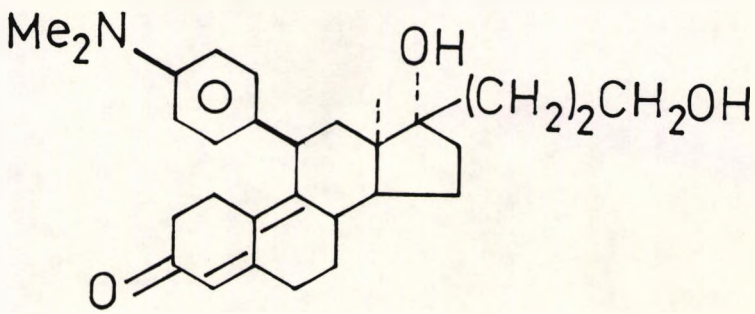
The discovery of the first competitive progesterone antagonist RU 38486 (3.1) has initiated an intense search for more potent and more selective anti-progestins. Among several hundreds of compounds under preliminary investigation by Schering[4] are ZK 98734 (3.2) and ZK 98299 (3.3). Similarly related compounds to RU 38486 have been synthesised by Organon.[5] Among the Organon compounds, compounds (3.4), (3.5) and (3.6) are the most interesting ones. All these compounds do not only differ in relative potency, but are clearly distinguished by their different behaviour in various animal models.



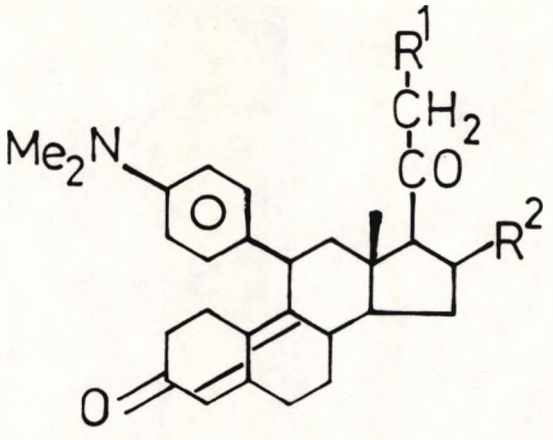
(3.1) = RU 38486



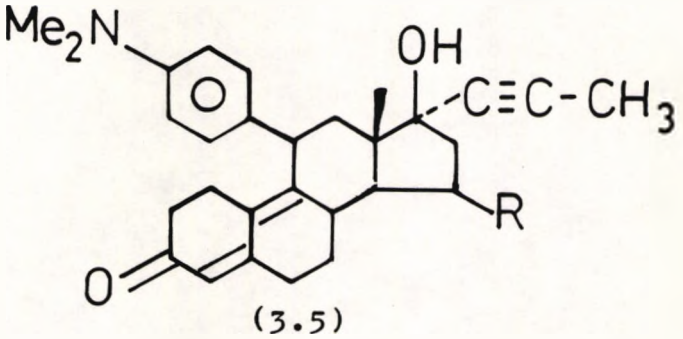
(3.2) = ZK 98734



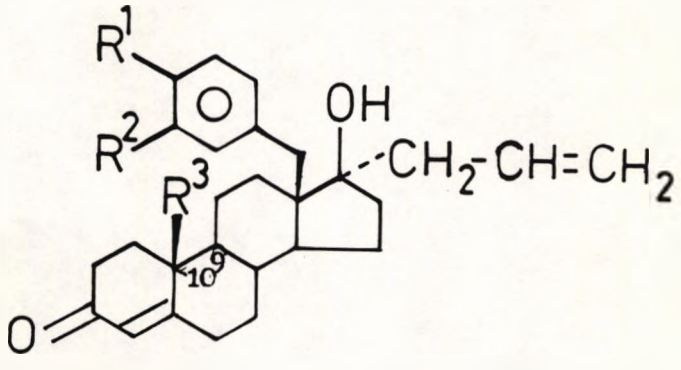
(3.3) = ZK 98299



(3.4)



(3.5)



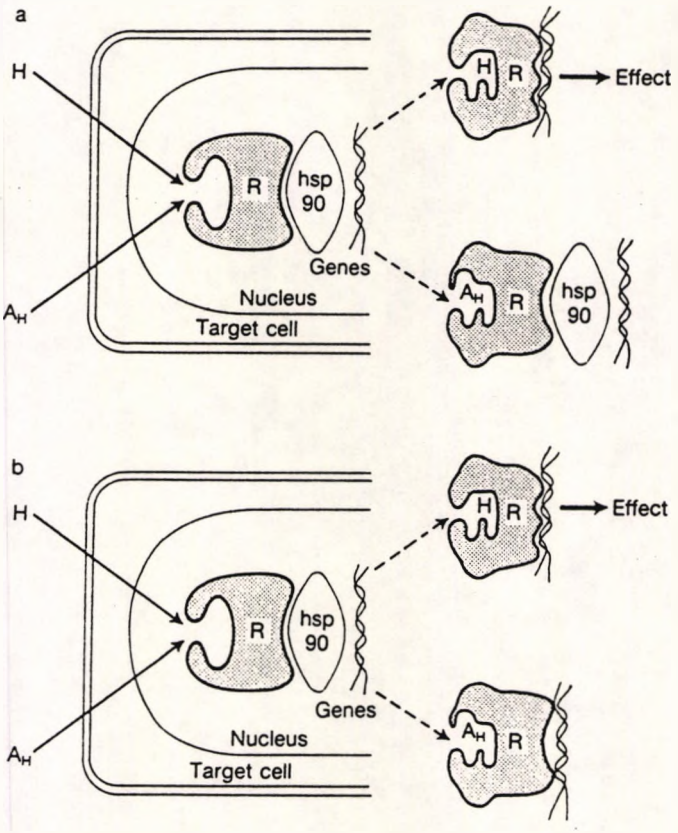
	R^1	R^2	R^3	9, 10
3.6a	NMe_2	H	CH_3	Saturated
b	NMe_2	H	-	Unsaturated
c	H	NMe_2	CH_3	Saturated
d	H	NMe_2	-	Unsaturated

3.2 Mechanism of Action of Anti-progestins

3.2.1 Cellular Mechanism of Action

The mechanism of action of antiprogestins at the cellular level has not been fully elucidated. In order to explain the absence of significant agonist activity despite the high receptor binding affinity of these compounds, a number of studies have been conducted on the physiochemical characteristics and DNA-binding ability of glucocorticoid and progesterone receptors bound to antiprogestin.[6-8] These studies have yielded conflicting results which may be due in part to the fact that the ligand-receptor interactions are usually studied under non-physiological, cell-free conditions rather than in intact cells.[9]

However, Grojer and co-workers,[10] from their studies with the chick glucocorticoid receptor, have recently proposed a very elegant model of the molecular basis of hormone antagonism which reconciles some of the divergent findings reported previously. In this model (Fig. 3.1a) the unliganded, non DNA-binding 8S form of the receptor is a hetero-oligomer consisting of the 4S steroid-binding unit 'R' and the non steroid-binding, non DNA-binding heat-shock protein 'hsp 90' which is common to all classes of 8S receptors.[11] Binding of the native hormone or homone agonist 'H' to the receptor triggers a conformational change



Models of the molecular basis of hormone antagonism at receptor level. For explanation see text (adapted from Baulieu *et al.* 1987).

Fig. 3.1

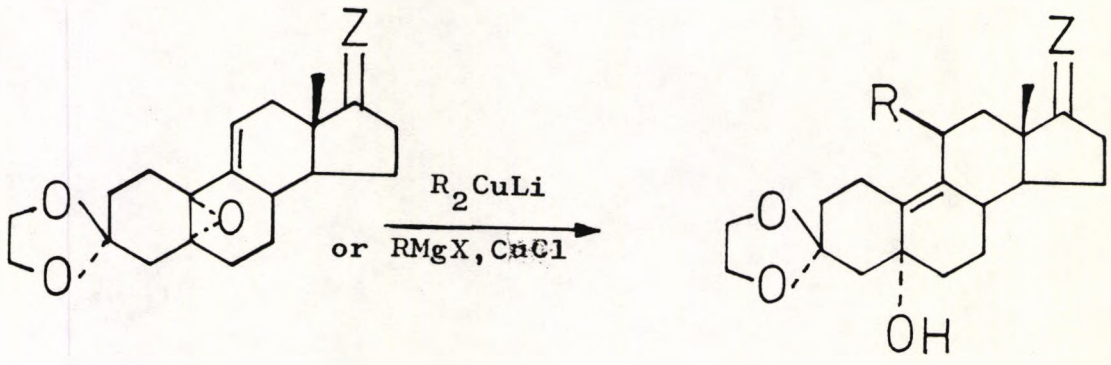
with release of the hsp 90 sub-unit and unmasking of the DNA-binding site on the 'activated' 4S receptor. In contrast, when the antihormone 'A_H' binds to the receptor, the R-hsp 90 complex is stabilized, hsp 90 is not released and DNA-binding does not occur. The alternative model, depicted in Fig. 3.1b, proposes that binding of the antihormone to the receptor is capable of provoking a transformation with dissociation of hsp 90 from the 4S subunit. However, the latter does not undergo the conformational change required for optimal DNA-binding and induction of gene transcription.

It should be noted that both models are not necessarily mutually exclusive and that both mechanisms may well be operative in vivo, either concomitantly or at different times depending on the physiological status of the cell.

Interestingly, two Organon compounds (3.6a) and (3.6c) which are equipotent with RU 38486 as regards pregnancy termination in rats after oral administration, are probably metabolised and activated in the gastrointestinal tract before they become biologically active. Unlike RU 38486 they display no antiglucocorticoid activity.

3.3 Synthesis of Anti-progestins

A general method for the synthesis of 11β-substituted 19-norsteroids, using the conjugate opening of allylic epoxides by organo-copper reagents (Scheme 3.1), has been reported by Teutsch and his co-workers.[12]

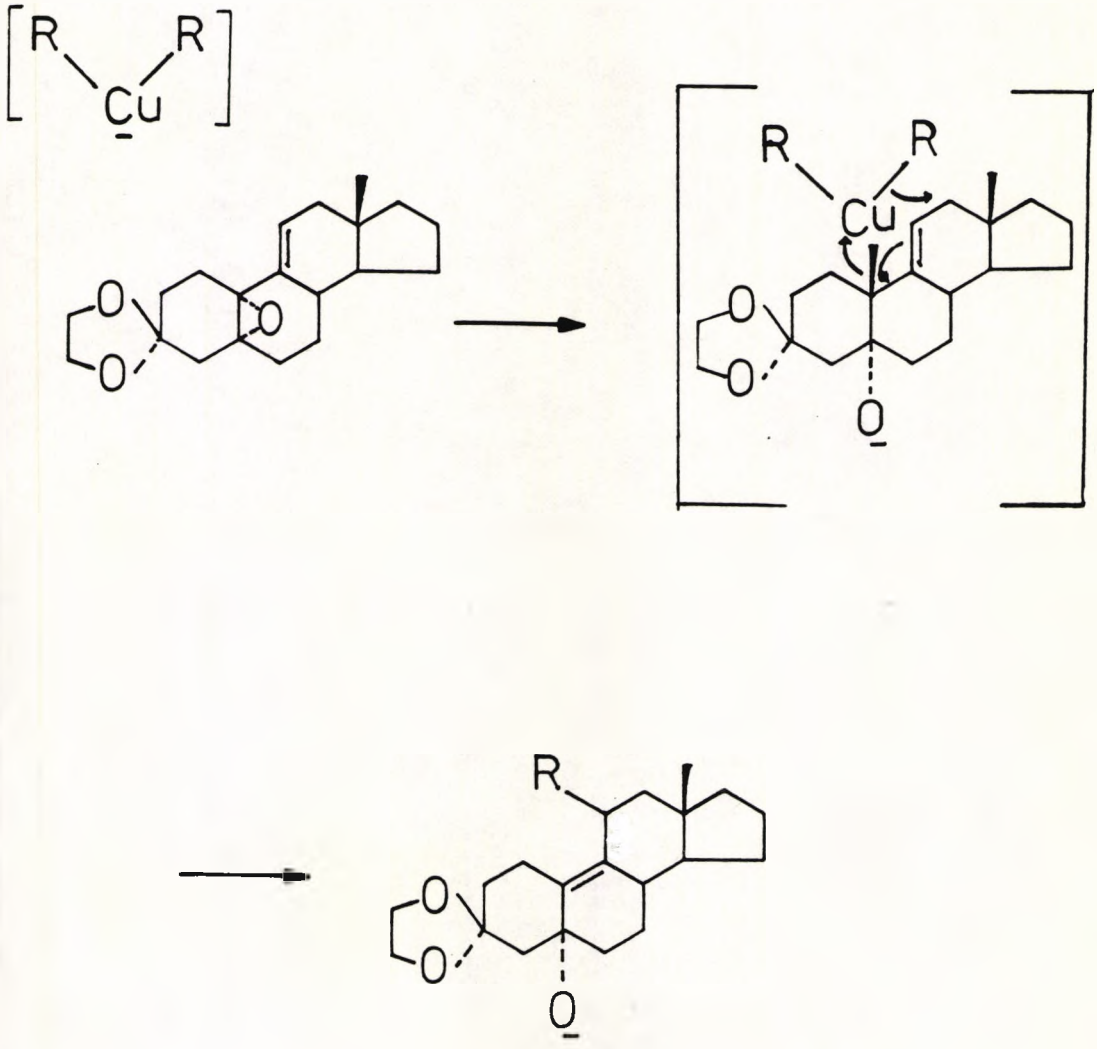


Scheme 3.1

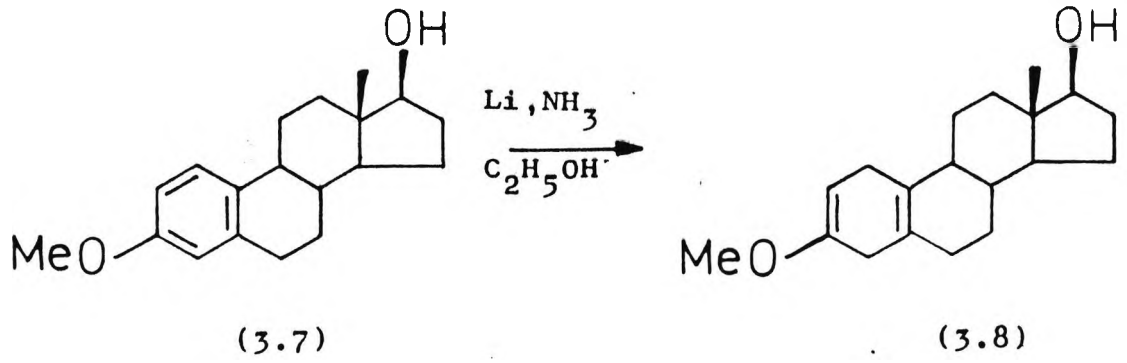
The suggested mechanism for the organocuprate reagent is shown in Scheme 3.2 and involves initial nucleophilic displacement of the epoxide at C_{10} followed by an allylic re-arrangement.

The reported method for the preparation of RU 38486 involves the initial preparation of the intermediate compound (3.13) from estradiol methyl ether (3.7) (Scheme 3.3). This is followed by the introduction of the 11β-substituent

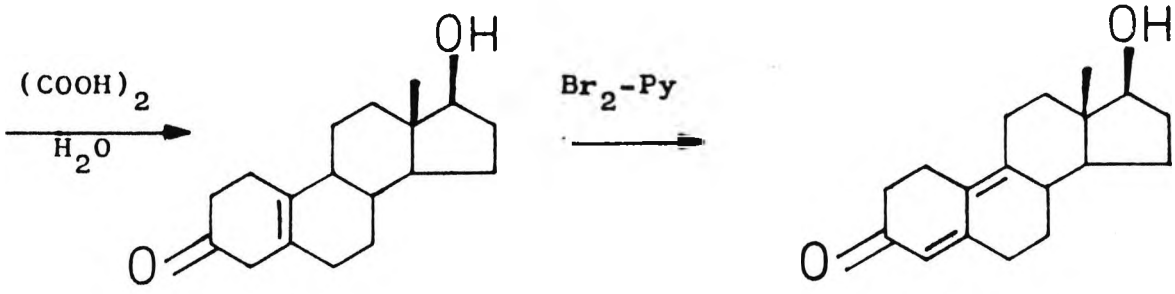
following Teutsch's method[12] to afford compound (3.14). Treatment of compound (3.14) with suitable lithium-acetylide gives compound (3.15). Finally, appropriate hydrolysis of compound (3.15) affords RU 38486 (3.1).



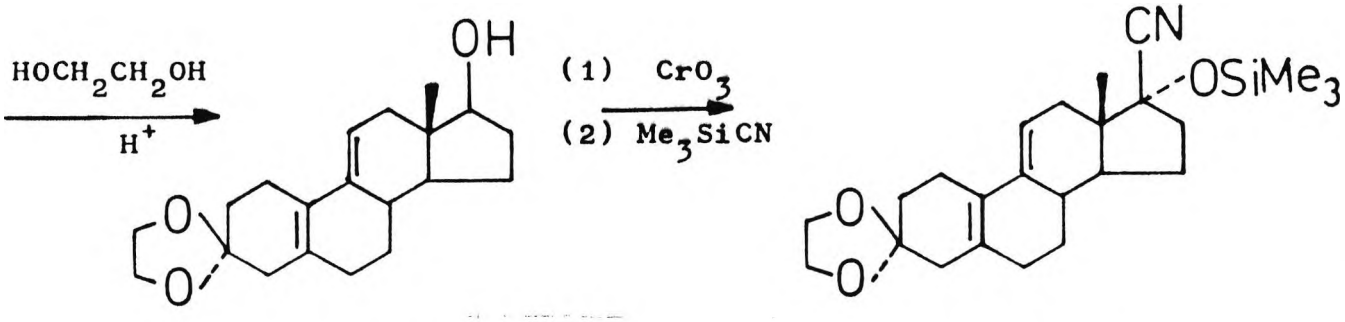
Scheme 3.2



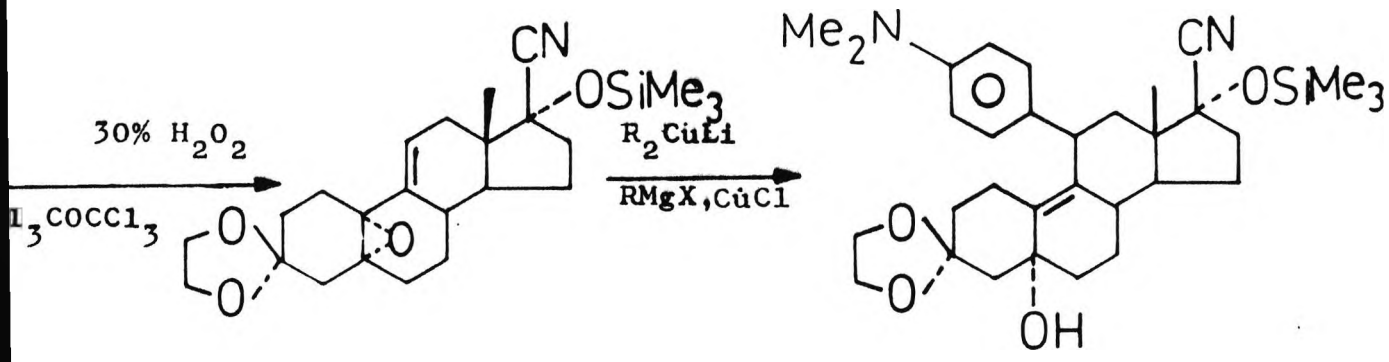
(3.7) (3.8)



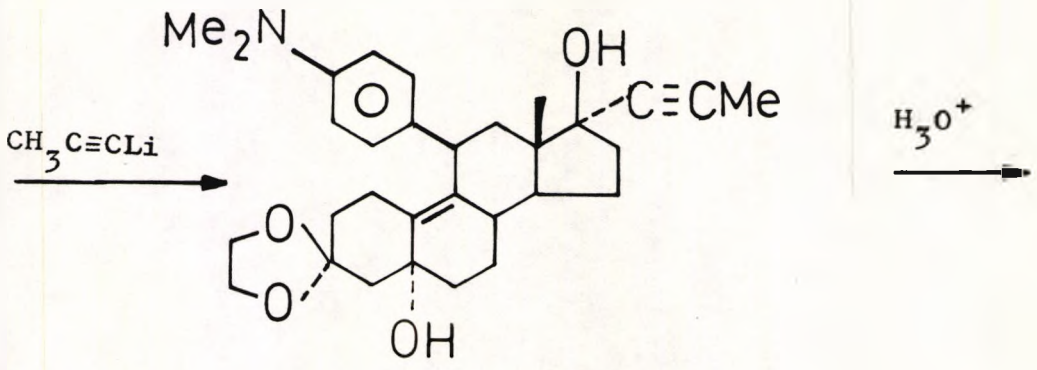
(3.9) (3.10)



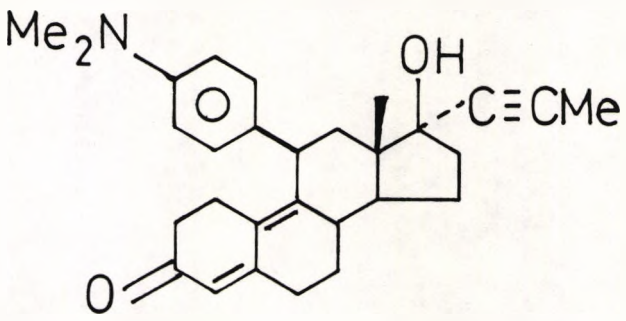
(3.11) (3.12)



(3.13) (3.14)



(3.15)



(3.1)

RU 38486

Scheme 3.3

Synthesis of related compounds (3.2) (ZK 98734) and (3.3) (ZK 98299) by Schering has been described [4] and the routes are outlined in Scheme 3.4

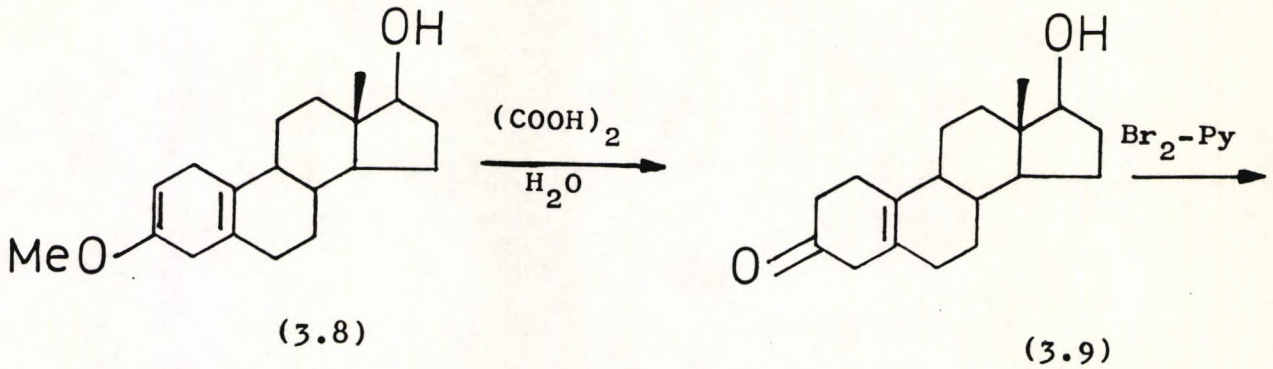
For the preparation of ZK 98734, the method involves the general and efficient strategy developed by Teutsh et al.[12] for the synthesis of 11β-substituted estra-4,9-dienes. However, part of this strategy is modified for the preparation of ZK 98299 (Scheme 3.4).

For both compounds, the starting material is the Birch reduction product of estradiol methyl ether,[13] for which appropriate hydrolysis leads to the deconjugate enone (3.9), which by a bromination-dehydrobromation sequence is transformed to dienone (3.10). Ketalization of (3.10) proceeds with a double bond shift to form 5(10),9(11)-diene (3.16) which is submitted to expoxidation to form 5α,10α-epoxide (3.17). The next step is the introduction of the 11β-substituent which is conveniently achieved by a copper (1)-catalysed Grignard reaction. Oppenauer oxidation of compound (3.18) gives (3.19). Treatment of (3.19) with the lithio derivative of propargyl terahydropyranyl ether gives compound (3.20). Subsequent hydrogenation followed by acid-catalysed deprotection-dehydration leads to compound ZK 98734 (3.2).

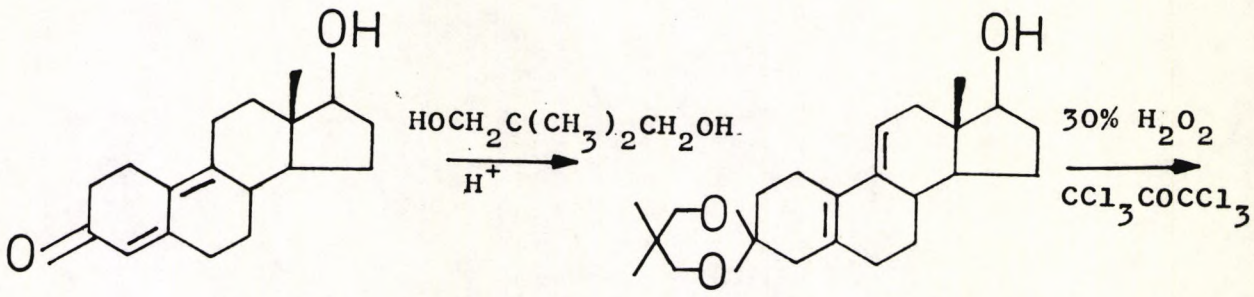
Alternatively, after Oppenauer oxidation, the next step in the preparation of ZK 98299 is the photolysis of the

intermediate (3.19) with the full spectrum of a mercury high pressure lamp in dioxane-acid. By Norrish-type 1 cleavage and recombination, 13α-methyl-gonane (3.22) is obtained in a good yield. The side chain at C-17 is constructed by adding the lithio derivative of propargyl tetrahydropyranyl ether. In contrast to the behaviour observed in the natural estrane series, nucleophilic attack at C-17 in the ketone (3.22) is not very highly stereoselective, giving both isomers with predominant formation of 17β-adduct (3.23). Hydrogenation of the 17β-adduct (3.23) followed by acid-catalysed deprotection-dehydration gives compound ZK 98299 (3.3).

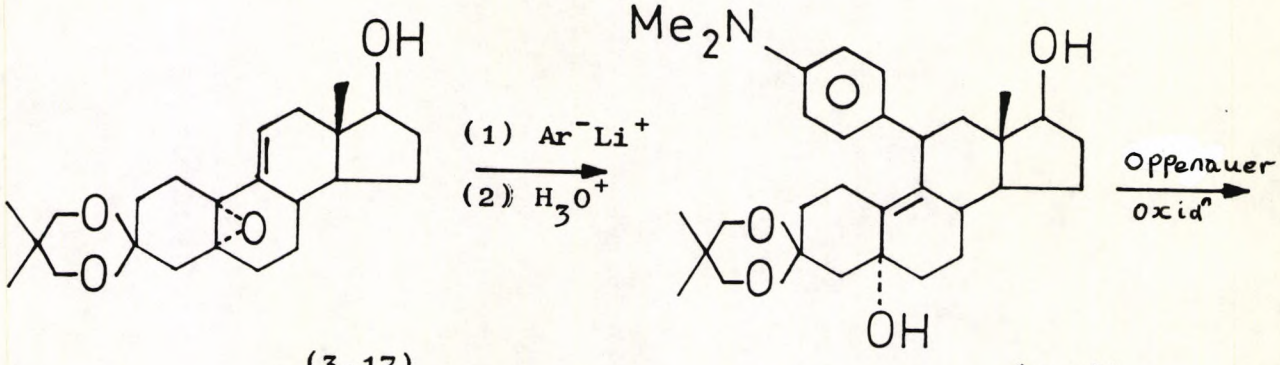
In these synthetic routes for preparation of both ZK 98734 and ZK 98299, a different procedure is used for epoxidation of diene (3.16) which is better than the original one described by Teutsch et al.[12] (see Scheme 3.5) The solution presented by Teutsch et al.[14] (H_2O_2, hexachloroacetone) proved to be highly regioselective, however stereoselectivity was not as good since a mixture of 5α,10α-epoxide (3.17a) (65%) and the corresponding 5β,10β-isomer (3.17b) (35%) was formed (Scheme 3.5). Wiechert and Neef established that taking Fe(11)-phthalocyanine, a cheap and readily accessible pigment, as catalyst in combination with iodosylbenzene as oxygen source, considerably promoted formation of 5α,10α-epoxide (3.26a).[15] A free hydroxy group at C-17 must be protected by ether formation or should be oxidized.



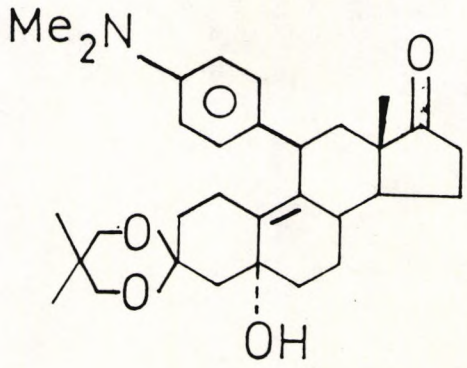
(3.8) (3.9)



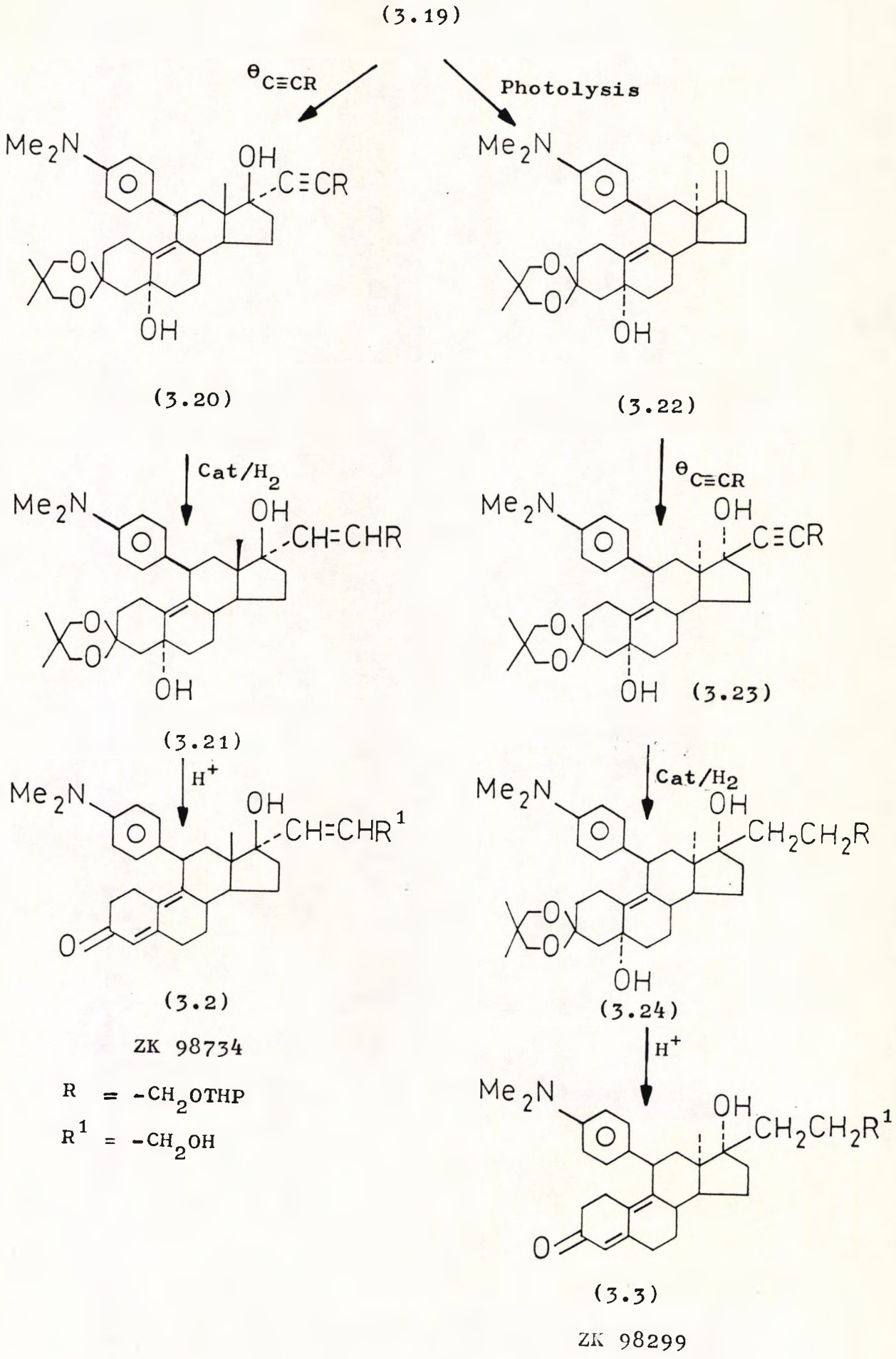
(3.10) (3.16)



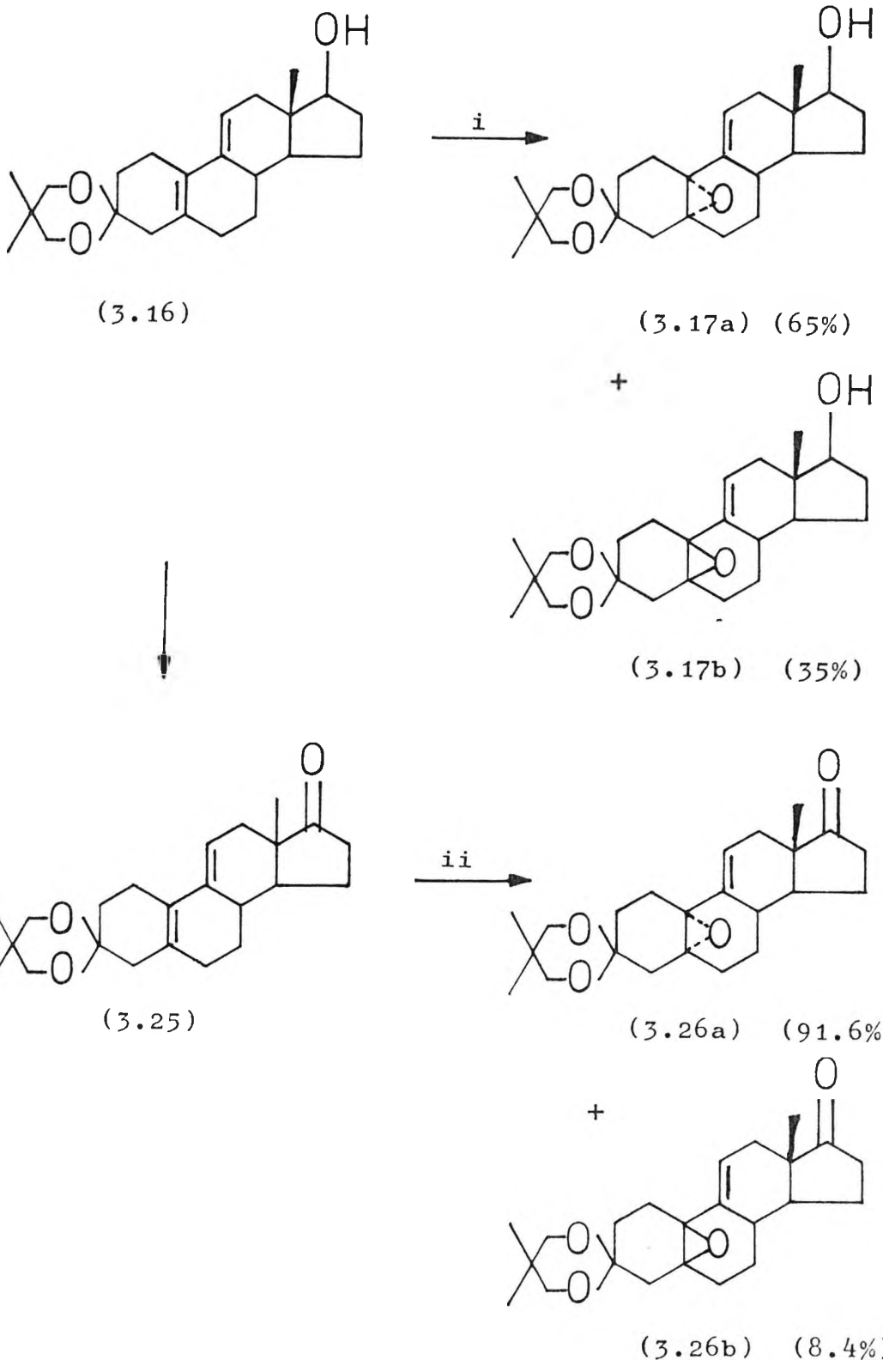
(3.17) (3.18)



(3.19)


(3.19)
θC≡CR
Photolysis
Me2N
OH
C≡CR
O
OH
(3.20)
(3.22)
Cat/H2
CH=CHR
(3.21)
(3.23)
H+
CH=CHR1
(3.2)
ZK 98734
R = -CH2OTHP
R1 = -CH2OH
CH2CH2R
(3.24)
CH2CH2R1
(3.3)
ZK 98299

Scheme 3.4



i:30% H_2O_2, CCl_3-$\overset{O}{\overset{\|}{C}}$-$CCl_3$; ii: Fe(11)-phthalocyanine iodosylbenzene

Scheme 3.5

3.4 References

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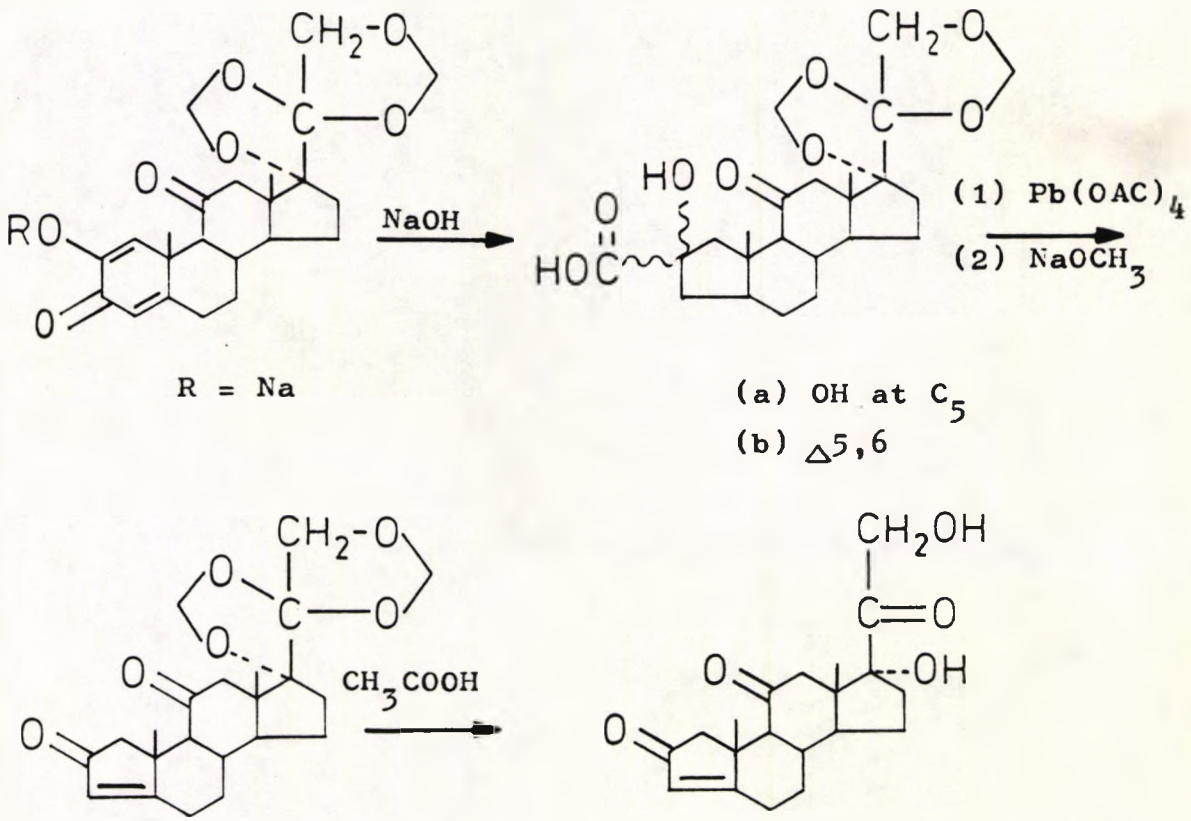
CHAPTER 4

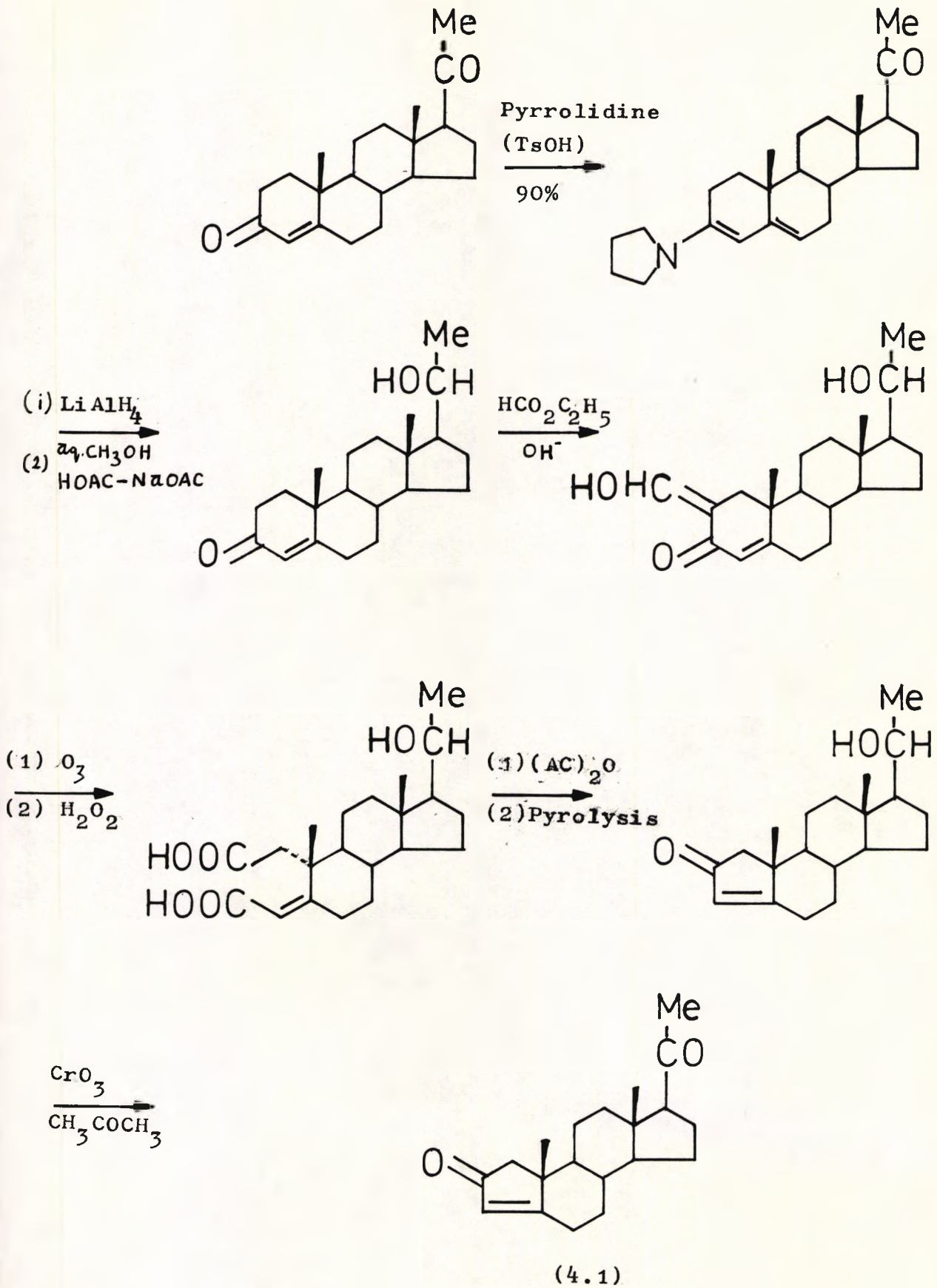
A-nor-steroids

4.1 Introduction

A number of A-nor-steroids (i.e. steroids with one carbon atom missing from the A-ring) have been prepared by ring contraction procedures[1-7] (e.g. Schemes 4.1-4.3) carried out on derivatives of testosterone, 19-nortestosterone, progesterone, cortisone and other hormones and some have been reported to show anti-hormonal properties. For example, A-nor-progesterone (4.1) possesses anti-androgenic activity.[1,3]

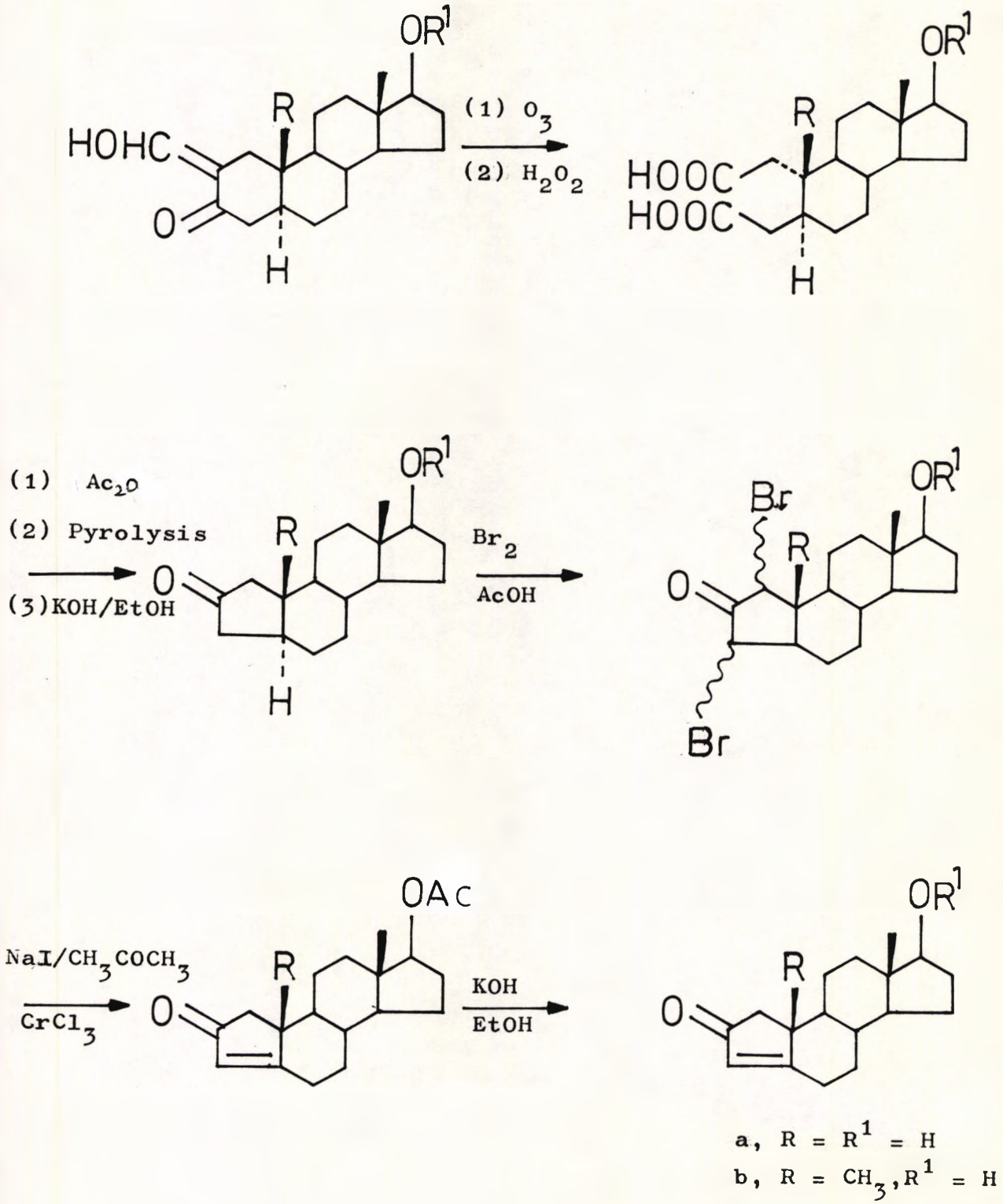


Scheme 4.1


Me
CO
O
Pyrrolidine
(TsOH)
90%
N
(i) LiAlH4
(2) aq.CH3OH
HOAC-NaOAC
HOCH
HCO2C2H5
OH⁻
HOHC
(1) O3
(2) H2O2
HOOC
HOOC
(1)(AC)2O
(2)Pyrolysis
CrO3
CH3COCH3

(4.1)

Scheme 4.2



a, $R = R^1 = H$

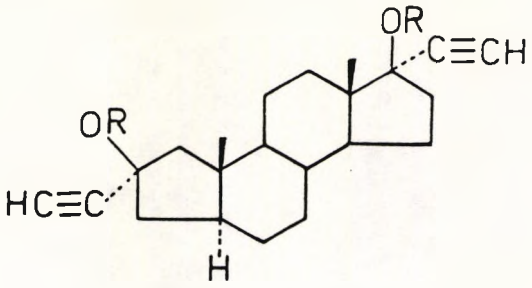
b, $R = CH_3, R^1 = H$

Scheme 4.3

4.2 Anordrin

Early work by Pincus (who carried out the first clinical studies with oral contraceptives in the 1950's) and his co-workers[8-11] established that some A-nor-steroids exhibit both anti-progestational and estrogenic activity when administered to rats and mice, opening up the possibility of the use of such compounds as anti-implantation agents. Chang and Yanagimachi[12] examined the effects of 2 ,17α-diethynyl-2β,17β-dihydroxy-A-nor-androstane (4.2) which has also been referred to in the literature as H241, AF-45 and anordiol, finding it to be as effective as estrogens in preventing pregnancy in rabbits and hamsters when administered in the period 1-3 days after mating (but before implantation has occurred). Subsequently, the dipropionate ester of this A-nor-steroid, named anordrin (4.3) and also known as AF-53, was extensively investigated in China during the 1960's and 1970's, both in animals and in women and was found to be a clinicially effective anti-implantation agent when taken post-coitally (so-called "visiting pill", popular with Chinese couples working apart and meeting only infrequently).[13]

Anordrin has a relatively small estrogenic activity (about 5% that of estradiol) whilst showing anti-estrogen, anti-progestin and anti-androgen character as a result of competitive binding to the respective hormone receptors.[14,15]



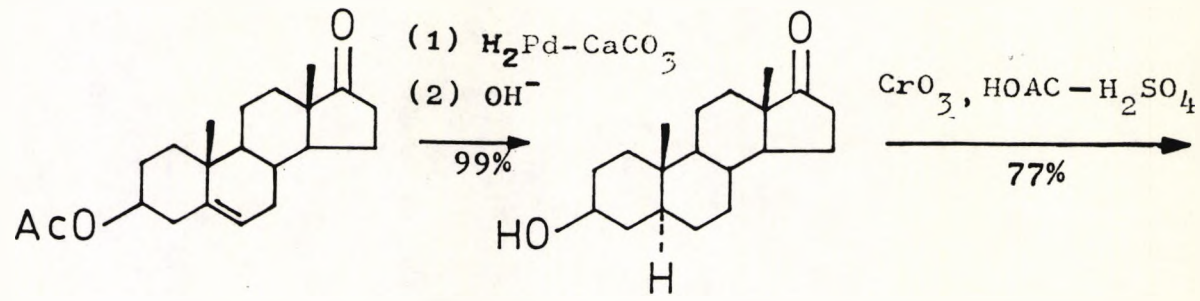
4.2, R = H (Anordiol)

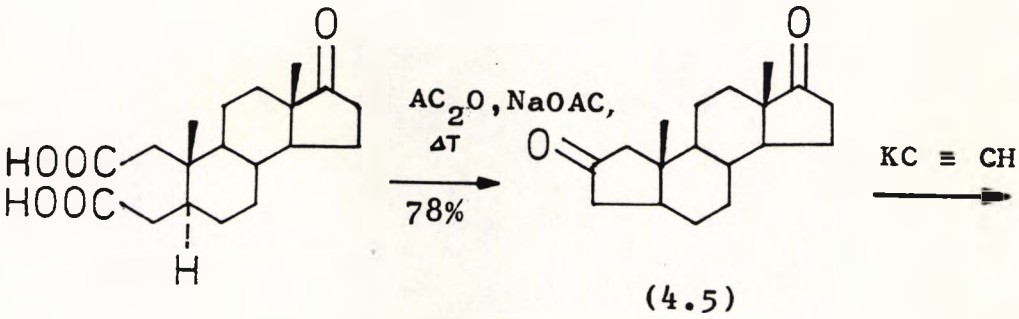
4.3, R = COEt (Anordrin)

4.4, R = $COCH_2CH_2CO_2H$

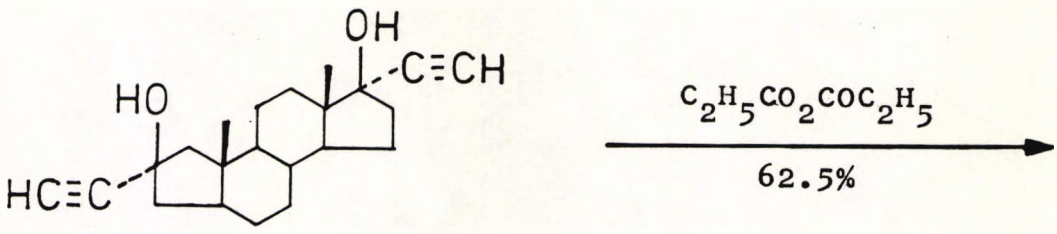
It seems clear that anordrin and also its bis-hemisuccinate analogue (known as SIPPR-113 or AF-57) (4.4) are hydrolysed in vivo to anordiol (4.2), but in view of the complex hormonal/anti-hormonal profile of these steroids their precise mechanism of action as postcoital anti-fertility agents has remained controversial and the wider adoption of anordrin as a clinical contraceptive drug has been discouraged by concerns about the possible health risks of estrogenic steroids.[16,17]

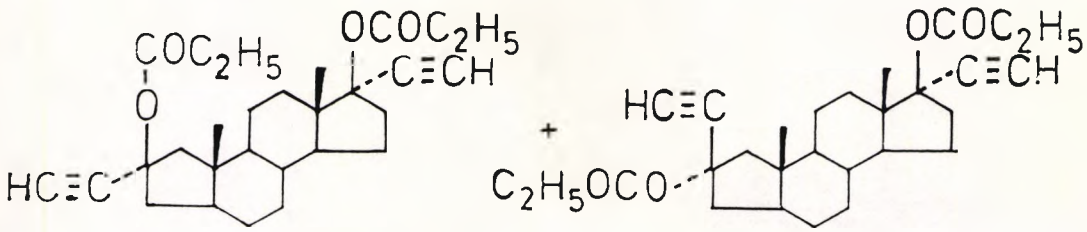
The synthesis of anodrin in China has been carried out by the route shown in Scheme 4.4. When potassium acetylide reacts with A-nor-androstane-2,17-dione (4.5), attack occurs exclusively from the alpha face of C-17 due to the steric effect of the 18-methyl group, but rather unselectively at the less hindered 2-position, producing a mixture of 2α-ethynyl (4.3) and 2β-ethynyl (4.6) isomers with the former predominating. The 2α-isomer is considered to be the more important anti-fertility agent.





(4.5)





2α-Anordrin (4.3)

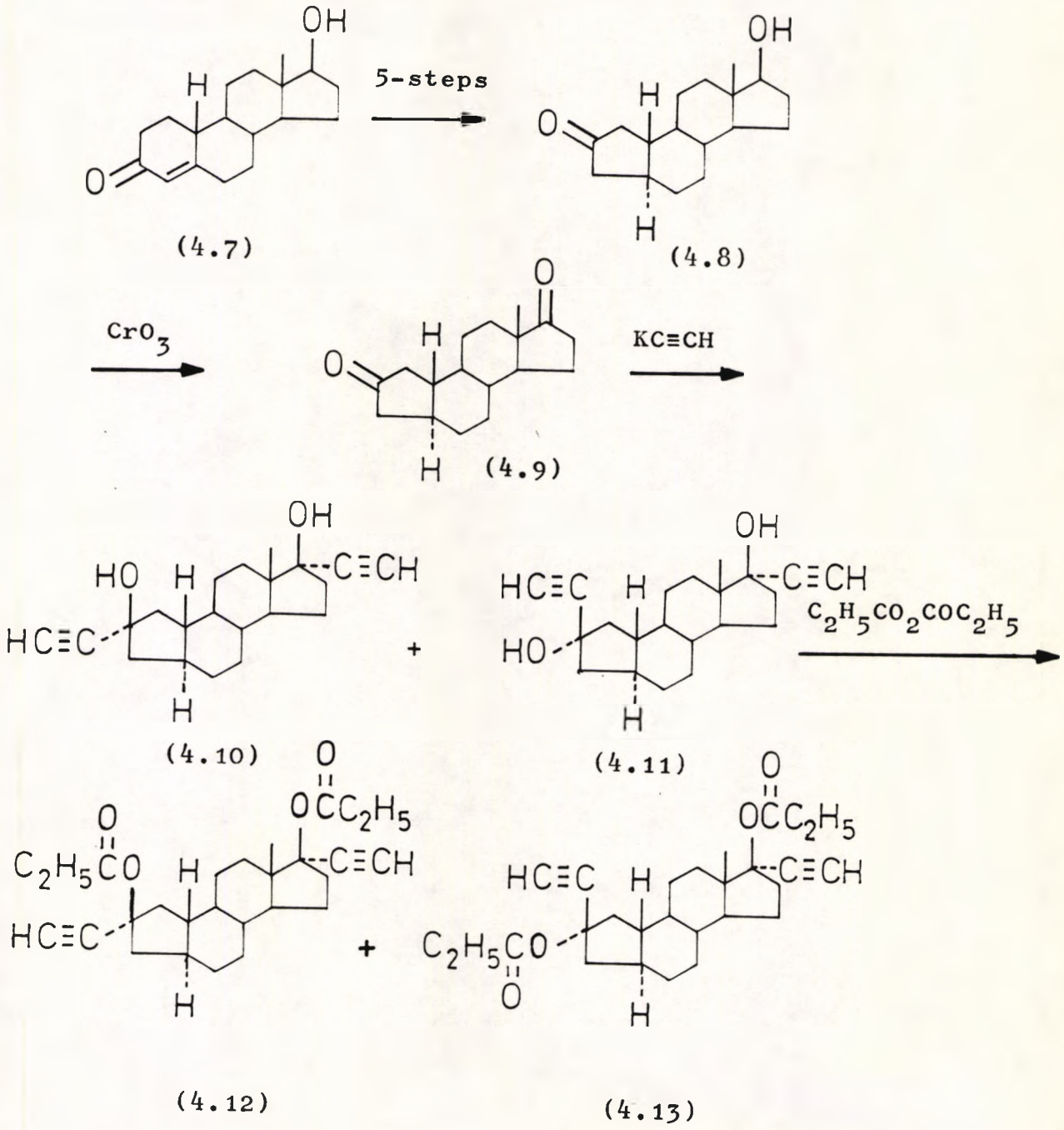
2β-Anordrin (4.6)

Scheme 4.4

Crabbe et al.[18] have synthesised the two isomers of anordrin and also synthesised the analagous compounds lacking the 19-methyl group, which they named dinordrin I and II (Scheme 4.5). The starting material was 19-nor-testosterone (4.7), which was converted to 2-keto-dinor-steroid (4.8) by conventional techniques.[19] Oxidation of the 17-hydroxyl group in the intermediate (4.8) provided the corresponding 2,17-diketo-dinor-steroid (4.9) which was treated with an excess of lithium acetylide-ethylenediamine complex to afford 3:2 mixture of the 2α-ethynyl compound (4.10) and its 2β-epimer (4.11) separated by preparative thin layer chromatography (TLC).

Esterification of the tertiary hydroxyl groups of diols (4.10) and (4.11) with propionic anhydride provided the dipropionates (4.12) and (4.13).

The anti-implantation activity of dinordrin I in rats was 10 times higher than that of anordrin, while in turn, anordrin was more active than dinordrin II. The estrogenic activity of the different configurational isomers of both anordrin and dinordrin was very low.

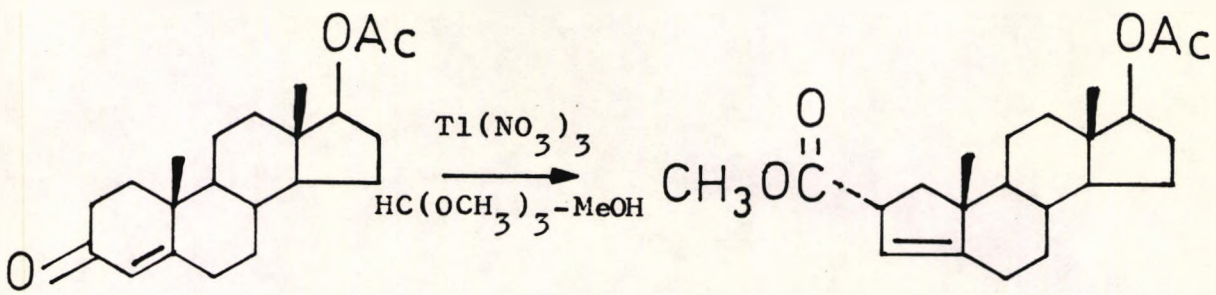

OH
H
O
5-steps
OH
H
O
H
(4.7)
(4.8)
CrO_3
O
H
O
H
(4.9)
KC≡CH
OH
HO
H
C≡CH
HC≡C
H
(4.10)
+
OH
C≡CH
HC≡C
H
HO
H
(4.11)
$C_2H_5CO_2COC_2H_5$
O
OCC_2H_5
C_2H_5CO
H
C≡CH
HC≡C
H
(4.12)
+
O
OCC_2H_5
HC≡C
H
C≡CH
C_2H_5CO
O
H
(4.13)

Scheme 4.5

Recently, a new method has been developed by an Italian group[20,21] which involves a "one-step" inversion of cyclic enones into β,γ-unsaturated nor-esters. This method eliminates protecting and deprotecting steps of the functional groups that are involved in the Chinese method of synthesising A-nor-steroids and should therefore improve the overall yields of A-nor-steroids.

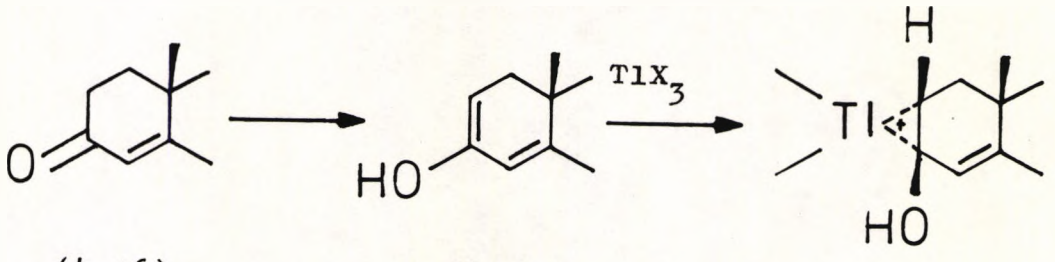
For example (Scheme 4.6), testosterone acetate (4.14) was reacted with thallium (111) nitrate at 0°C in an alcoholic medium to give A-nor-2α-methoxycarbonyl-androst-3-en-17β-ylacetate (4.15) in 75% yield.

The proposed mechanism for this reaction is shown in Scheme 4.7. It is assumed that, as for the mechanism of contraction of saturated ketones[22], the electrophilic thallium nitrate adds to the Δ^2,4-dienol (4.17) from the less-hindered α face of the steroid. The subsequent elimination of thallium from intermediate (4.18) gives the contraction product (4.19)

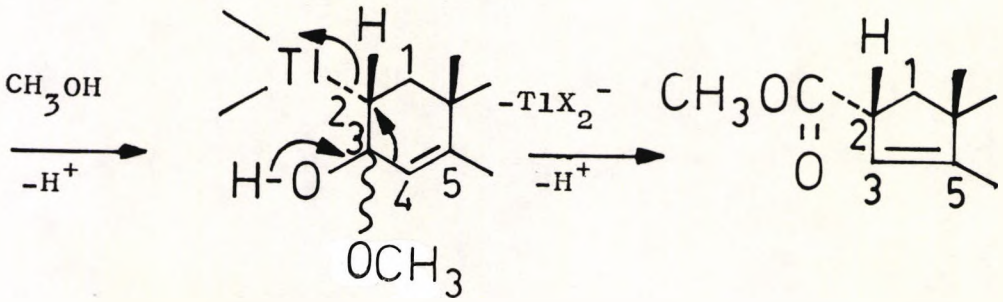


(4.14) (4.15)

Scheme 4.6



(4.16) (4.17)



(4.18) (4.19)

Scheme 4.7

4.3 References

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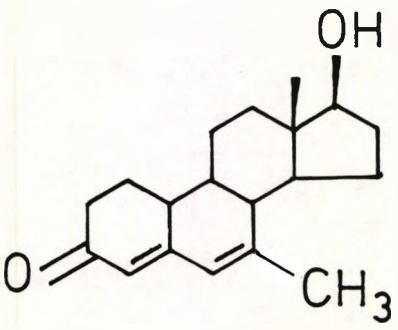
CHAPTER 5

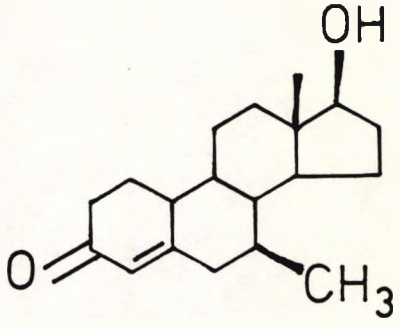
Synthesis of 7-methyl-steroids

5.1 Introduction

As discussed in Chapter 2.1, there has been considerable interest in recent years in the possible anti-androgenic anti-fertility effects of certain alkylated steroids and general methods[1-3] for their synthesis were described. As part of a collaboration with the Population Council of the Rockefeller Foundation, we were requested to synthesise 7-methyl-4,6-diene (5.1) and the 7β-methyl-19-nortestosterone (5.2) for detailed biological investigation.



(5.1)

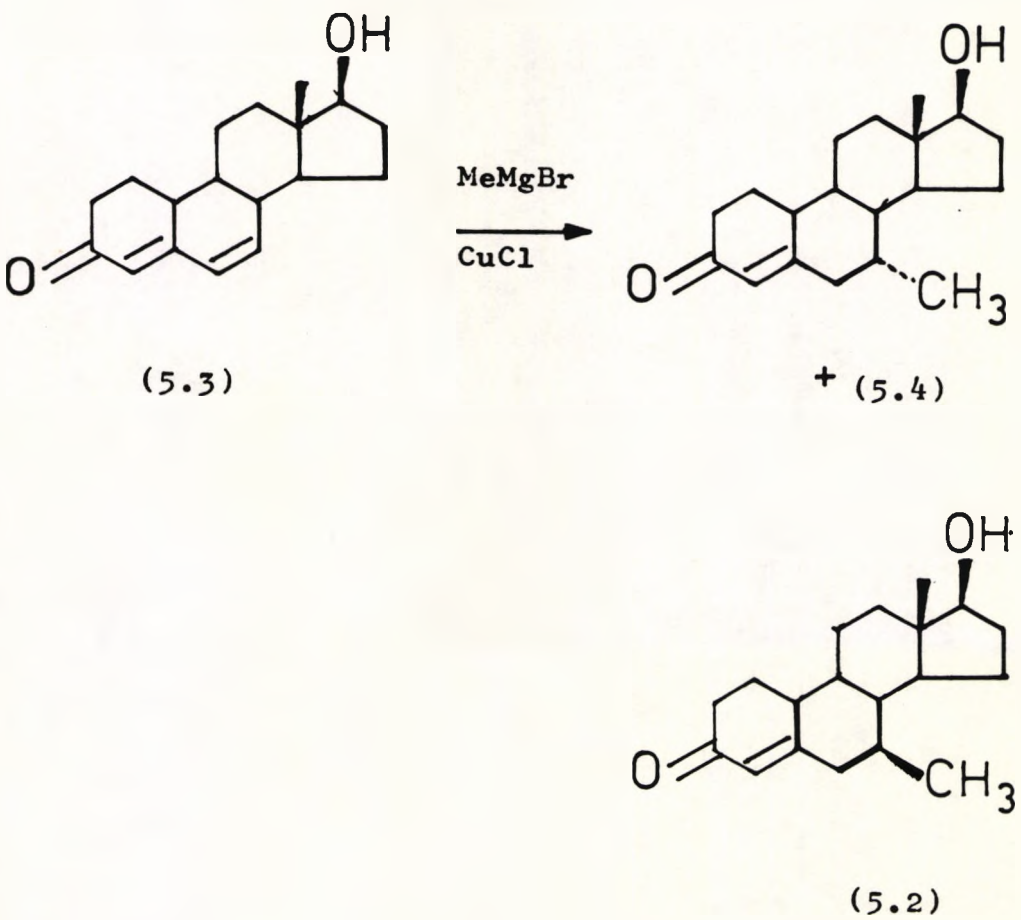


(5.2)

In this chapter, specific methods for the preparation of 7-methylated androstanes will be reviewed and their application to the synthesis of the desired compounds will be described.

Campbell and Babcock[1] have reported a procedure for 7-alkylation of 17α-methyltestosterone, shown in Scheme 2.1, p.25.

In a patent[4] in 1967, Campbell and Babcock also reported the application of this methodology in the 19-nortestosterone series. Thus copper-catalysed conjugate addition[6,7] of methyl Grignard to the 19-nor dienone (5.3) afforded the 7α- and 7β-methyl steroids (5.4) and (5.2) in yields of 50% and 12%, respectively (Scheme 5.1). In this series, it was claimed that the epimers could be separated by column chromatography using gradient elution.

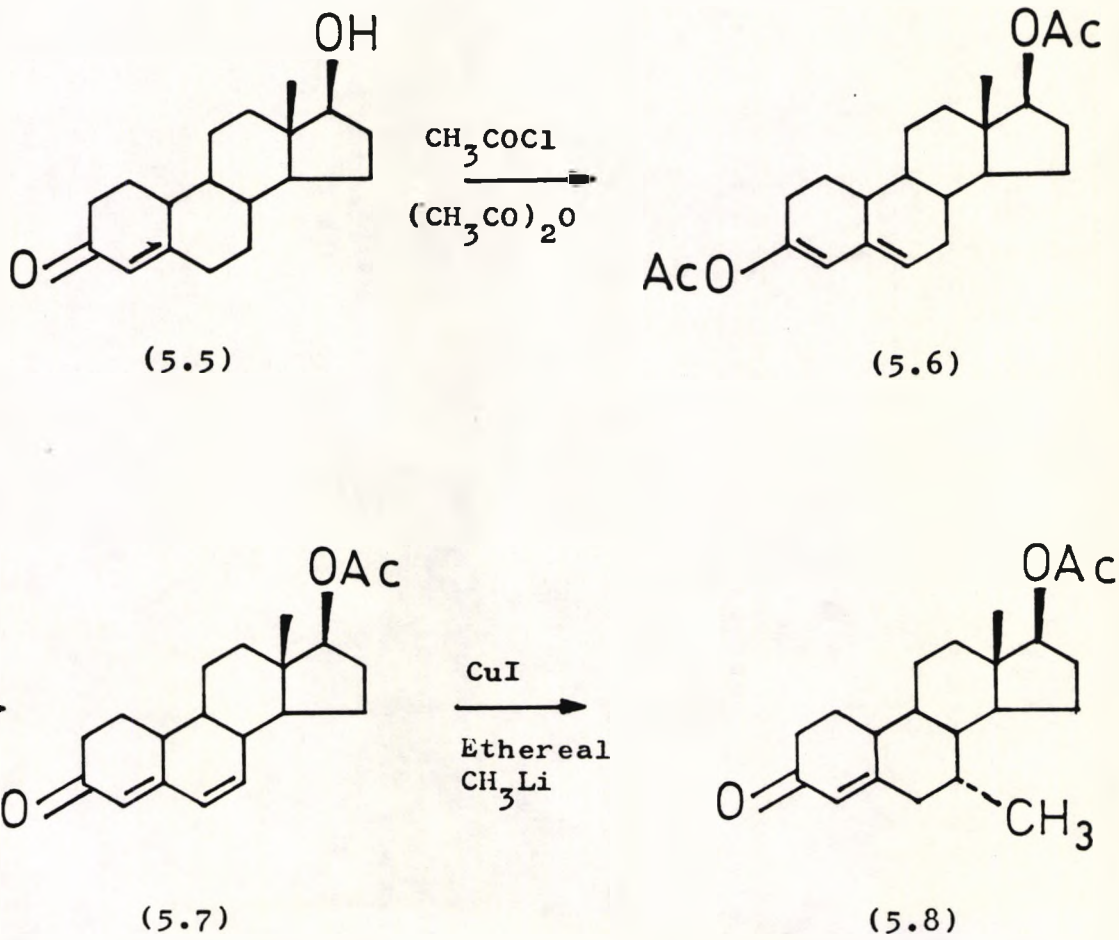


(5.3) + (5.4)

(5.2)

Scheme 5.1

Alternative methodology has been reported by Grunwell and Benson[5]. 19-Nortestosterone (5.5) was acetylated to give the dienyl acetate (5.6), which was oxidised (bromination/elimination) to the dienone (5.7) and conjugate addition was effected using MeLi-CuI. Under the latter condition, only the 7α-methyl product (5.8) was observed (Scheme 5.2).

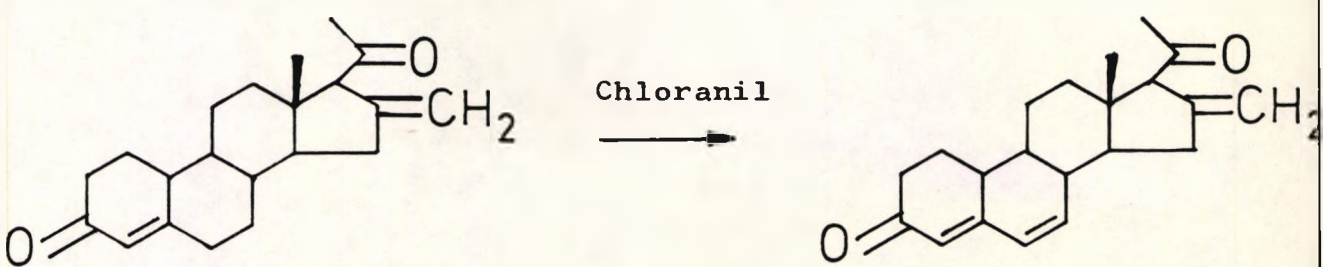


Scheme 5.2

5.2 Results and Discussion

For the proposed synthesis of 7-methyl-17β-hydroxy-estra-4,6-diene-3-one (5.1) and 7β-methyl-19-nortestosterone (5.2), the simplest route based on the above literature reports appeared to be the direct 1-step oxidation of 19-nortestosterone using chloranil, followed by conjugate addition of Grignard reagent and further oxidation.

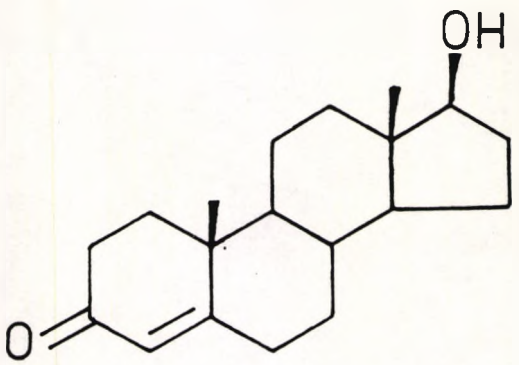
In addition to the oxidation of 17α-methyl testosterone by Campbell and Babcock[1] discussed in Section 2.2, other groups have also reported the successful oxidation of steroidal 4-en-3-ones to 4,6-dien-3-ones using chloranil. For example, good yields were obtained in the corticosteroid field using this method.[8,9] However, the only reference to the application of this reaction in the 19-norsteroid field which could be found was in a patent[10] claiming the oxidation of the 16α-methylene derivative (5.9) to dienone (5.10), for which no yield was reported.



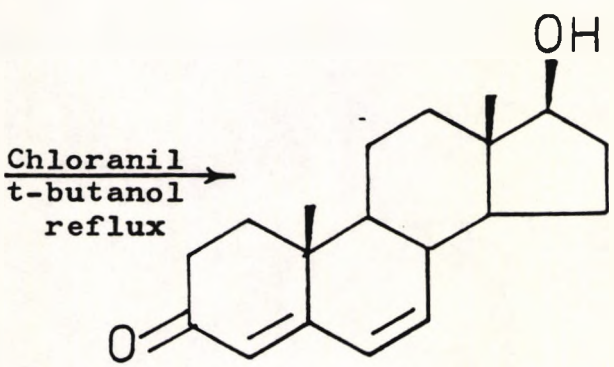
(5.9) (5.10)

The lack of such examples may be of significance, since in the 19-nor series an alternative site for allylic oxidation exists and may lead to competing formation of an A-ring phenol. Consequently, it was decided to first carry out the oxidiation reaction in the testosterone series in order to establish working conditions, before attempting direct oxidation of 19-nortestosterone.

When testosterone was refluxed with chloranil in t-butanol, the dienone (5.12) was obtained in 67% yield.

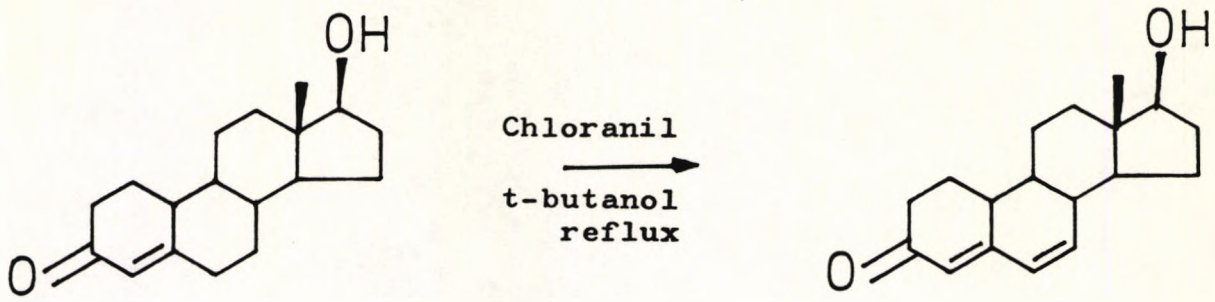


(5.11)



(5.12)

Following the success of this model reaction, the method was then investigated with 19-nortestosterone (5.5). However, the expected product (5.13) was not formed cleanly. ^{1}H NMR showed that the crude product contained a mixture of starting material (5.5) and product (5.13). These proved difficult to separate from one another and from other, unidentified by-products. Attempts to improve the conversion by the use of more chloranil for a longer time led



(5.5) (5.13)

to a considerable increase in the formation of by-products. Consequently, it was decided to abandon this approach.

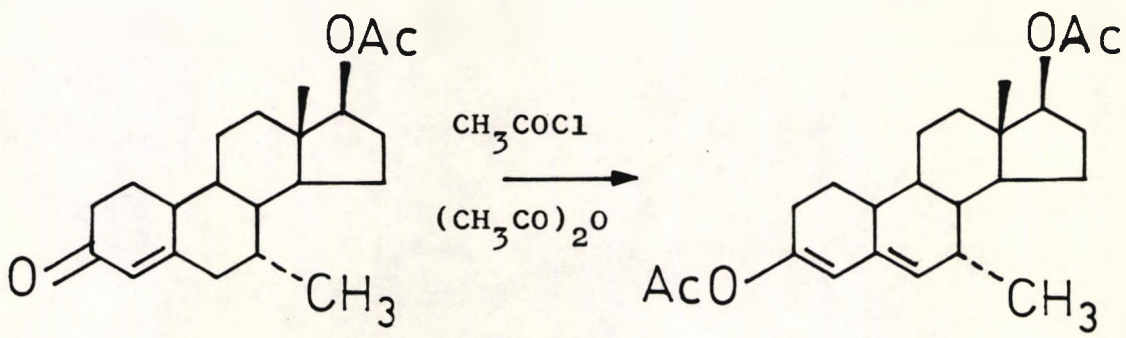
Attention was then turned to the method of Grunwell and Benson[5] (Scheme 5.2). 19-Nortestosterone was acetylated, giving the dienyl acetate (5.6) in 65% yield (Lit: 71% yield). Following bromination and elimination, the desired 17-acetoxy dienone (5.7) was obtained in 57.9% yield (Lit: 65.7%).

When the 17-acetoxydienone (5.7) was treated with MeLi-CuI, 7α-methyl-19-nortestosterone acetate (5.8) was obtained in 28% yield (Lit: not given).

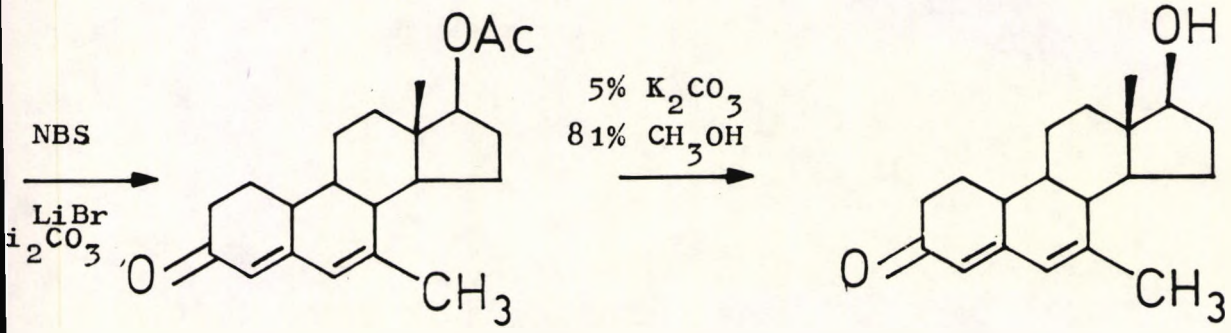
It was noted that Campbell and Babcock[1] had observed that only the 7β-methyl testosterone derivative (2.5) and not the 7α-methyl isomer (2.4) was oxidised to a 4,6-dien-3-one with chloranil (Scheme 2.1) (Page 25). However, we reasoned that the NBS oxidation of dienylacetate would not be so sensitive

to stereochemistry at the C-7 position and that it should therefore be possible to utilise this process for the synthesis of the required 7-methylestradienone (5.1). Accordingly (Scheme 5.3), 7α-methyl-19-nortestosterone acetate (5.8) was acetylated and the dienyl acetate (5.14) then treated with NBS followed by LiBr-Li_2CO_3, which afforded the 17-acetoxy dienone (5.15). This was hydrolysed to give the required compound (5.1). The structure of the compound was fully supported by spectroscopic evidence and the purity was demonstrated by HPLC.

Thus, in contrast to the previously published methodology, in which only the 7β-methyl-4-en-3-ones have been oxidised to 7-methyl-4,6-dien-3-ones, the procedure which we adopted, via oxidation of the dienyl acetate, allows the 7α-methyl-4-en-3-one to be converted into the 7-methyl-4,6-dien-3-one.



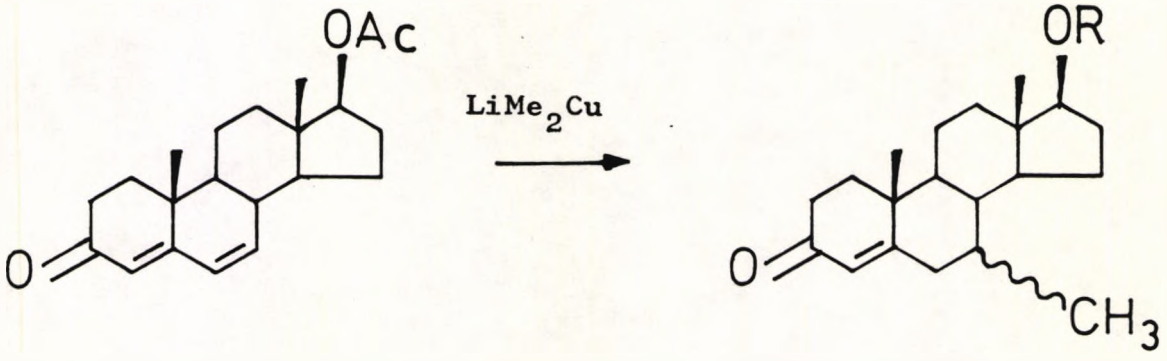
(5.8) (5.14)



(5.15) (5.1)

Scheme 5.3

The synthesis of 7β-methyl-19-nortestosterone (5.2) should, in principle, be readily achievable by the copper-catalysed, conjugate addition of methyl Grignard reagent to the dienone (5.7) which is readily available as an intermediate in the synthesis of 7-methyldienone (5.1) It was noted earlier (Section 5.1) that conjugate addition to steroidal 4,6-dien-3-ones using MeMgBr-CuCl favoured α rather than β attack (ratio 50:12) and that MeLi-CuI favoured exclusively α attack. Furthermore, it has been reported by Gasparrini et al.[3] that attack of $LiMe_2Cu$ on 17-acetoxy dienone (5.16) gave 7-methylated esters (5.17; 7α 41%, 7β 36%) and 7-methylated alcohols (5.18 ; 7α 7%, 7β 8%)



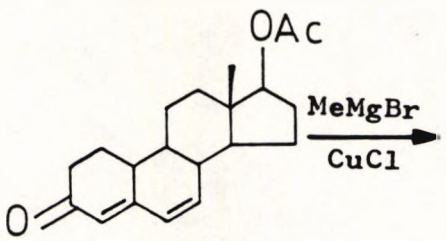
(5.16)

(5.17) ; R = Ac

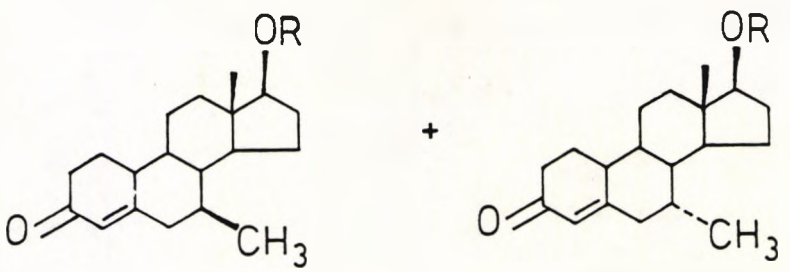
(5.18) ; R = H

Consequently, it appeared that it would be fruitful to explore the reactions of the 19-nordienone (5.7) with methylating reagents under a variety of conditions in order to optimise the yield of the desired 7β-epimer.

When 19-nordienone (5.7) was treated with methylmagnesium bromide in the presence of copper (1) chloride at 0-17°C following Babcock and Campbell's method[5] a crude product was obtained which on HPLC analysis was shown to be a mixture of four components. The alcohols (5.4 and 5.2) could be resolved from the acetates (5.8 and 5.19) by conventional column chromatography (CC). However, in contrast to the claim by Babcock and Campbell,[4] the mixture could not be separated into individual epimers by classical liquid chromatography (LC). The isomeric mixtures were separated by preparative HPLC (see Chapter 6) and identified by ^{1}H NMR as the expected epimeric pairs of acetates and alcohols (Scheme 5.4). From HPLC analysis of the crude reaction in Scheme 5.4 it was determined that the yields were: 7α-methyl-17-acetate, 13.8% ; 7β-methy-17-acetate, 2.1% ; 7α-methyl-17-alcohol, 10.2% ; 7β-methyl-17-alcohol, 3.2%. Thus in this reaction the overall β : α selectivity was 5.3 : 24.



(5.7)



(5.2) R = H (5.4) R = H

(5.19) R = Ac (5.8) R = Ac

Scheme 5.4

Conditions were then investigated for optimising the β-selectivity of Campbell and Babcock's method.[4] Changes in reaction temperature and rates of addition of reagents were made and the results monitored by HPLC, but no significant improvement in β-selectivity was observed.

The reaction was then carried out on a larger scale following Campbell and Babcock's method[4], to give a mixture of 7-methyl-19-nortestosterone and 7-methyl-19-nortestosterone acetate. These products were resolved into their α- and β-isomers by preparative HPLC to isolate the desired 7β-methyl-19-nortestosterone (5.2), along with the three other by-products, 7α-methyl-19-nortestosterone (5.4), 7α-methyl-19-nortestosterone acetate (5.8) and 7β-methyl-19-nortestosterone acetate (5.19).

The 7β-methyl-17-acetate (5.19) was hydrolysed using methanolic potassium carbonate solution under reflux to furnish further quantities of the required 7β-methyl-17-alcohol (5.2) in an overall yield of 4.8% from the dienone (5.7). Pure samples of the two required compounds (5.1) and (5.2) were submitted to the Population Council for detailed biological investigation of their hormonal properties.

5.3 Experimental

3,17β-Diacetoxyestra-3,5-diene (5.6)

19-Nortestosterone (2g, 0.0073 mole) was refluxed under nitrogen in a mixture of acetic anhydride (7.5 ml) and acetyl chloride (7.5 ml) for a period of 3 hr. The solvent was removed under reduced pressure. The solid residue which remained was triturated with ice water, filtered, washed with cold aqueous sodium bicarbonate solution, rinsed with water and dried. Recrystallization of this residue from acetone gave 3,17β-diacetoxyestra-3,5-diene (5.6) (1.85g, 65%; Lit:[5] 71%) which melted at 163-165°C (Lit:[5] 165-71°C). ^{1}H NMR ($CDCl_3$)δ: 0.80 (3H, S), 1.2 (14H, m, methylene envelope), 2.0 (3H,S), 2.08 (3H, S), 4.68 (1H,t) 5.52 (1H, d), 5.84 (1H, d); ν_{max}(nujol) cm^{-1} 1756, 1729 : EI-MS : m/z 358.2307 (M^+, 1.3%; $C_{22}H_{30}O_4$ requires 358.2187), 315 (0.8%).

17β-Hydroxyestra-4,6-dien-3-one acetate (5.7)

A solution of 3,17β-diacetoxyestra-3,5-diene (5.6) (4g, 0.00105 mole) in acetone (212 mo), water (54.4 ml), acetic acid (5.44 ml), pyridine (1.2 ml) and sodium acetate (5.44g, 0.065 mole) was cooled to 0-5°C using a salt-methanol-ice bath. N-bromosuccinimide (2.14g, 0.012 mole) was then added to this cooled solution. The reaction mixture was shielded from light by means of aluminium foil and stirring continued for a period of 3 hr. at 0-5°C. The solution was poured

onto cold brine (800 ml) and the product extracted with ether (200 ml). The ether extract was washed with water, dried over anhydrous magnesium sulfate and concentrated under vacuum below 20°C. The amber oil residue was dissolved in dimethylformamide (5 ml) and rapidly added to a vigorously refluxing suspension of dimethylformamide (50 ml), lithium bromide (4g, 0.046 mole) and lithium carbonate (4g, 0.03 mole) under nitrogen. Residual ether was permitted to evaporate and the reaction mixture was refluxed for a period of 1 hr. After cooling, the suspension was filtered and the filtrate was poured into an ice water mixture. The product was extracted into ether and the combined ether extracts were washed with 5% sodium hydroxide (150 ml) solution and washed again with water. The solution was dried over anhydrous magnesium sulfate, filtered and concentrated under vacuum to yield a yellow solid which was layered with hexane and filtered to give pure 17β-hydroxy-estra-4,6-dien-3-one acetate (5.7) (2.30g, 57.9%). M.p. 100-102°C ; ^{1}H NMR ($CDCl_3$)δ: 0.88 (3H, S), 1.16-1.88 (10H, m, methylene envelope), 4.57 (3H, t), 6.08 (2H, S) ; ν_{max}(nujol)cm^{-1} 1732, 1664 ; EI-MS : m/z 314.1714 (M^+, 25.26%; $C_{20}H_{26}O_3$ requires 314.1920), 255 (6.2%).

<u>17β-Hydroxy-7α-methylestr -4-en-3-one acetate (5.8)</u>

A solution of lithium dimethylcuprate was prepared under nitrogen by the addition of 1.6M ethereal methyllithium (9 ml, 0.014 mole) to a slurry of cuprous iodide (1.4g, 0.0073

mole) in anhydrous ether (14.28 ml) at 0°C. The solution was stirred at this temperature for 5 min and a solution of 17β-hydroxyestra-4,6-dien-3-one acetate (5.7) (0.5g, 0.0015 mole) in anhydrous tetrahydrofuran (4.2 ml) was added over a 10 min. period. The reaction mixture was stirred for an additional 15 min. at 0°C and poured into a saturated aqueous ammonium chloride solution. Benzene was added and the resulting mixture was rapidly filtered through filter aid. The organic layer was washed with a saturated aqueous ammonium chloride solution, then with water, dried over anhydrous magnesium sulfate and evaported to dryness.

The oily yellow crude product was dissolved in methanol (9.7 ml) to which water (0.4 ml) and concentrated hydrochloric acid (0.6 ml) had been added. The solution was stirred for a period of 2 hr and poured onto a mixture of ice-water. The ether extract was washed with water, dried over magnesium sulfate and evaporated to dryness. The crude product was purified by preparative t.l.c. on silica gel and developed with CH_2Cl_2-CH_3CN (80:20) to give 0.1476g (28%) of the desired 17β-hydroxy-7α-methylestr-4-en-3-one acetate (5.8). M.p. 108-110°C. ^1H NMR ($CDCl_3$)δ: 0.76 (3H, d), 0.84 (3H, S), 1-1.84 (3H, S), 1-1.84 (14H, m, methylene envelope), 2.00 (3H, S), 4.6 (1H, t), 5.76 (1H, S) ; ν_{max}($CHCl_3$) cm^{-1} : 1725, 1660, 1615 ; EI-MS : m/z 330.2316 (M^+, 51.54% ; $C_{21}H_{30}O_3$ requires 330.2238).

3,17β-Diacetoxy-7α-methylestra-3,5-diene (5.14)

17β-Hydroxy-7α-methylestr-4-en-3-one acetate (5.8) (0.5g, 0.0034 mole) was refluxed under nitrogen in a mixture of acetic anhydride (1.9 ml) and acetyl chloride (1.9 ml) for a period of 3 hr. The solvent was removed under reduced pressure. The solid which remained was triturated with ice water, filtered and washed with cold aqueous sodium bicarbonate solution. The residue was recrystallized from acetone to yield pure 3,17β-diacetoxy-7α-methylestra-3,5-diene (5.14) (0.1775g, 35.5%). M.p. 90-92°C; ^{1}H NMR ($CDCl_3$)δ; 0.85 (3H, d), 0.90 (3H, S), 1.00-2.0 (12H, m, methylene envelope), 2.10 (3H, S), 2.2 (3H, S), 4.8 (1H, t), 5.68 (1H, d), 5.92 (1H, d) ; ν_{max}($CHCl_3$) cm^{-1} 1730, 1680, 1618 ; EI-MS: m/z 372.2309 (M^+, 8.83% ; $C_{23}H_{32}O_4$ requires 372.2347), 357 (1.25%), 342 (27.21%).

17β-Hydroxy-7-methylestra-4,6-dien-3-one acetate (5.15)

3,17β-Diacetoxy-7α-methylestra-3,5-diene (5.14) (1.6003g, 0.0043 mole) was placed in solution containing acetone (84.82 ml), water (21.7 ml), acetic acid (2.2 ml), pyridine (0.48 ml) and sodium acetate (2.18g, 0.026 mole). The solution was cooled to 0°-5°C using a salt-methanol-ice bath and N-bromosuccinimide (0.86g, 0.0087 mole) was added at one time. The reaction mixture was shielded from light by means of aluminium foil and stirring continued for a period of 3 hr.

at 0^{o}-5^{o}C. The solution was poured onto cold brine (320 ml) and the product extracted with ether (200 ml). The ether extract was washed with water, dried over anhydrous magnesium sulfate and concentrated under vacuum below 20^{o}C. The amber oily residue was dissolved in dimethylformamide (2 ml) and rapidly added to a vigorously refluxing suspension of dimethylformamide (20 ml), lithium bromide (1.6003g, 0.0184 mole) and lithium carbonate (1.6003g, 0.0217 mole) under nitrogen. Residual ether was permitted to evaporate and the reaction mixture was refluxed for a period of 1 hr. After cooling, the suspension was filtered and the filtrate was poured into an ice-water mixture. The product was extracted into ether and the combined ether extracts were washed with 5% sodium hydroxide solution (53 ml) and washed again with water. The solution was dried over anhydrous magnesium sulfate and concentrated under vacuum to yield a yellow solid which was layered with hexane and filtered to give 1.2g of the crude product. This was purified by preparative t.l.c. on silica and developed with CH_2Cl_2-CH_3CN (90:10) to yield 0.156g (11%) of compound (5.15). M.p. 140-145^{o}C ; ^{1}H NMR($CDCl_3$)δ: 0.84 (3H, S), 1.2-1.9 (12H, m, methylene envelope), 1.89 (3H, S), 2.00 (3H, S), 4.48 (1H, t), 5.48 (1H, S), 5.84 (1H, S) ;ν_{max}($CHCl_3$) cm^{-1} : 1725, 1655, 1615, 1585 ; EI-MS : m/z 328.1995 (M^{+}, 16.50% ; $C_{21}H_{28}O_3$ requires 328.2079).

17β-Hyroxy-7-methyl-estra-4,6-dien-3-one (5.1)

(6-dehydro-7-methyl-19-nortestosterone (5.1))

A solution of 17β-hydroxy-7-methylestra-4,6-dien-3-one acetate (0.0984g, 0.0003 mole) in a mixture of 5% aqueous potassium carbonate (1.312 ml) and 80% aqueous methanol (12 ml) was heated under reflux in an atmosphere of nitrogen for 2 hr. The reaction mixture was extracted with ether and the ethereal extract was washed with water and dried over anhydrous magnesium sulfate. The dried ether solution was filtered and evaporated to dryness. The residue was purified by preparative t.l.c. on silica gel and developed with CH_2Cl_2-CH_3CN (90:10) to yield 0.0454g (52%) of pure 6-dehydro-7-methyl-19-nortestosterone (5.1). M.p. 178-180°C ^1H NMR ($CDCl_3$)δ: 0.8 (S, 3H),1.88 (S, 3H), 3.54 (t, 1H), 5.10 (S, 1H), 5.84 (S, 1H) ;ν_{max}(nujol) cm^{-1} 3500 (S), 1650, 1610 ; EI-MS: m/z 286.1992 (M^+, 22.86%; $C_{19}H_{26}O_2$ requires 286.1970).

7β-Methyl-19-nortestosterone (5.2); 7α-methyl-19-nortestosterone (5.4); 7α-methyl-19-nortestosterone acetate (5.8); 7β-methyl-19-nortestosterone acetate (5.19)

To a suspension of copper (1) chloride (0.2678g, 0.0014 mole) in tetrahydrofuran (50 ml) under nitrogen was added 3M-ethereal MeMgBr (14 ml). The mixture was cooled to 0°-17° and 6-dehydro-19-nortestosterone acetate (2.3481g, 0.0074

mole), dissolved in tetrahydrofuran (50 ml) was added. After 30 mins, 10% hydrochloric acid solution (17 ml) was added. The reaction product was extracted with a mixture of toluene and dichloromethane and the organic phase washed successively with dil. sodium hydroxide, dil. hydrochloric acid and water to neutral, dried and evaporated under reduced pressure. The residue was first purified by preparative t.l.c. on silica gel and developed with CH_2Cl_2-CH_3CN (80:20) to give 7-methyl-19-nortestosterone (0.2758g, 12.81%) and 7-methyl-19-nortestosterone acetate (0.7880g, 31.93%) respectively. The fractions were then further separated by preparative HPLC, using a reversed phase column 5μODS-hypersil and MeOH-H_2O (75:25) as the mobile phase to yield pure 7β-methyl-19-nortestosterone (5.2) (0.0681g, 3.16%), 7α-methyl-19-nortestosterone (5.4) (0.2200g, 10.21%) and 7α-methyl-19-nortestosterone acetate (5.8) (0.34034g, 13.8%) and 7β-methyl-19-nortestosterone acetate (5.19) (0.0524g, 2.12%) respectively.

7β-Methyl-19-nortestosterone (5.2) : M.p. 130-132^{o}C (Lit:[3] 134-134.2^{o}C) ; ^{1}H NMR ($CDCl_3$)δ: 0.84 (3H,S), 1.04 (3H, d), 1.12-2.4 (14H, m, methylene envelope), 3.56 (1H, b), 5.76 (1H, S); ν_{max}(nujol) cm^{-1} 3380, 1655, 1610 ; EI-MS : m/z 288.2142 (M^+, 89% ; $C_{19}H_{28}O_2$ requires 288.2129), 273 (5.4%), 255 (5.8%).

7α-Methyl-19-nortestosterone (5.4) M.p. 95-97°C ; ^{1}H NMR ($CDCl_3$)δ; 0.76 (3H, d), 0.82 (3H, S), 1.04-2.6 (14H, m, methylene envelope), 3.72 (1H, t), 5.80 (1H, S) ; ν_{max}(nujol) cm^{-1} 3380, 1655, 1610 ; EI-MS m/z 288.2142 (M^+, 89%; $C_{19}H_{28}O_2$ requires 288.2129), 273 (5.4%), 270 (12.1%).

7α-Methyl-19-nortestosterone acetate (5.8) : M.p. 107-110° (Lit.[1] 108-110) ; ^{1}H NMR ($CDCl_3$)δ: 0.76 (3H,d) 1.06 (3H,S), 1-1.96 (14H, m, methylene envelope), 4.56 (1H,t), 5.76 (1H,S) ;ν_{max}($CHCl_3$) cm^{-1} 1725, 1665, 1620 ; EI-MS : 330.2316 (M^+, 51.54% ; $C_{21}H_{30}O_3$ requires 330.2238).

7β-Methyl-19-nortestosterone acetate (5.19) : oil ; ^{1}H NMR ($CDCl_3$)δ: 0.84 (3H, S), 1.04 (3H, d), 1.12-1.96 (14H,m, methylene envelope), 4.56 (1H,t), 5.76 (1H,S); ν_{max}($CHCl_3$) cm^{-1} 1725, 1665, 1620 ; EI-MS : m/z 330.2316 (M^+, 7.64%, $C_{21}H_{30}O_3$ requires 330.2238).

HPLC analysis of the four compounds above using a reversed phase system : mobile phase, MeOH-H_2O (70:30) ; λ, 244nm; ab, 1Aufsd ; flow-rate, 2 ml/min ; column, SM6 5μ ODS Hypersil, 12.5 x 0.45 cm, showed that all samples were at least 98-99% pure.

7β-Methyl-19-nortestosterone (5.2)

A solution of 7β-methyl-19-nortestosterone acetate (5.19) (0.0524 g, 0.00016 mole) in a mixture of 5% aqueous K_2CO_3 (0.7 ml) and 80% aqueous MeoH (6 ml) was heated under reflux in an atmosphere of nitrogen for 2 hr. The reaction mixture was extracted with ether, dried over anhydrous magnesium sulfate, filtered and evaporated to dryness. The residue was purified by preparative t.l.c. on silica gel and developed with CH_2Cl_2-CH_3CN (90:10) to yield pure 7β-methyl-19-nortestosterone (5.2) (0.0349g, 76%). M.p. 128-130°C. ^1H NMR ($CDCl_3$)δ: 0.84 (S, 3H), 1.04 (d, 3H), 1.12-2.4 (m, methylene envelope, 14 H), 3.56 (t, 1H), 5.76 (S, 1H). ν_{max}(nujol)cm^{-1} 3380, 1655, 1610. EI-MS m/z 288.2196 (M^+, 100% ; $C_{19}H_{28}O_2$ requires 288.2129 (4.90%, 255 (5.00%).

5.4 References

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CHAPTER 6

HPLC Studies of 7-methyl-steroids

6.1 Introduction

The history of chromatography is one of periodic advances that have followed major innovations: partition and paper chromatography in the 1940's, gas and thin layer chromatography in the 1950's, and the various gel or size exclusion methods in the early 1960's. A few years later the practice of chromatography was revolutionised by a technique called "modern liquid chromatography", generally accepted as high performance liquid chromatography (HPLC)[1], which offered improvements over traditional column chromatography in equipment, materials, techniques and in terms of results, advantages in convenience, accuracy and speed. Undoubtedly the technique had a large impact in chemical analysis and is a major method for separation, quantitation and identification of chemical substances.

To-day, the development of new types of stationary phases for HPLC is continuously and rapidly growing to improve separation and selectivity.

6.2 The development of HPLC

HPLC was introduced as a practical analytical technique in the mid-1960's and to-day is used both in an analytical and a preparative scale. Its development had been anticipated by Martin and Synge[2] in 1941. Their paper described the theory of chromatography, introducing the concept of 'height equivalent to a theoretical plate' (HETP) to express column efficiency, introduced liquid-liquid (or partition) chromatography and predicted the advantage of a gaseous mobile phase, as in GC, and of a high pressure liquid phase with a small particle size, as in HPLC.

The differences between modern HPLC and column chromatography involve the use of narrower columns, smaller particle sizes, and higher flow rates produced by higher column inlet pressure. In comparison to conventional liquid chromatography, HPLC offers several advantages, such as higher resolution, greater reproducibility, and shorter retention times, in addition the column can be used many times without regeneration. Compared to GC, HPLC can be used for the non-volatile, thermally labile and polar compounds which are unsuited to GC analysis.

The four basic liquid chromatographic methods which feature in HPLC are briefly summarised:-

(1) Liquid-Liquid (or partition) Chromatography (LLC) where the stationary phase is a liquid coating upon a solid support, the liquid being immiscible with the mobile phase.

(2) Liquid-Solid (or adsorption) Chromatography (LSC) which has a high surface area solid as the stationary phase onto which the solute is adsorbed.

(3) Ion Exchange Chromatography (IEC) where the stationary phase is an ion exchanger with fixed ionic groups that have counter ions in the mobile phase. Retention of sample ions is by ion exchange of the sample and the counter ions.

(4) Size Exclusion Chromatography (SEC) in which retention is due to the size and shape of the molecule and longest retention occurs for the smallest molecules.

Bonded phase chromatography (BPC) is where the stationary phase is chemically bonded to a solid support in place of the mechanically-held liquid coating of LLC. It has increasingly supplanted both LLC and LSC, in the case of LSC because firstly the polarity of the surface can be controlled and secondly it responds to changes in solvent composition more rapidly, while the advantages of BPC over LLC are summarised below:-

(1) In BPC, mobile and stationary phases are completely immiscible, whereas in LLC this rarely occurs and pre-saturation of the mobile phase by the stationary phase is required. The stationary phase is chemically bonded to the support and is not, as in LLC, a mechanically held film which is susceptible to being stripped off at high flow rates.

(2) Gradient elution is possible in BPC but not in LLC.

(3) Contamination of the eluent cannot occur in BPC as it usually does in LLC. As a consequence, scale up and isolation of components in preparative scale work is more convenient with BPC than with LCC.

6.3 Theoretical aspects of HPLC

Chromatography involves the separation of a mixture, achieved through the differential distribution of sample compounds between the mobile and stationary phases. This separation is dependent upon the composition of the two phases, the efficiency of the column, the column temperature, and mass transfer effects regarding the two phases. Therefore, control of the separation is primarily through alteration of the composition of the mobile phase, which can be varied by gradient elution, and by the choice of column. There are two types of mobile and stationary phase combinations, either

normal-phase or reverse-phase. In normal-phase chromatography (NPLC), the stationary phase is polar and the mobile phase is non-polar, therefore polar compounds are retained and non-polar compounds elute first. With reverse-phase chromatography (RPLC) polar compounds are retained less, since the mobile phase is more polar than the stationary phase. To decrease retention times in RPLC a less polar mobile phase is required, for NPLC it is the opposite i.e. an increase in the polarity of the eluting mixture is required.

The following concepts have been developed to quantitatively describe a chromatogram such as that shown in Fig. 6.1 and 6.2.

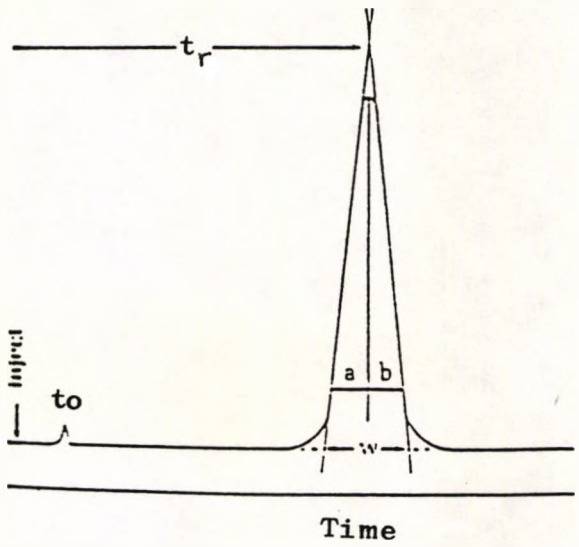


Fig. 6.1 Peak parameters

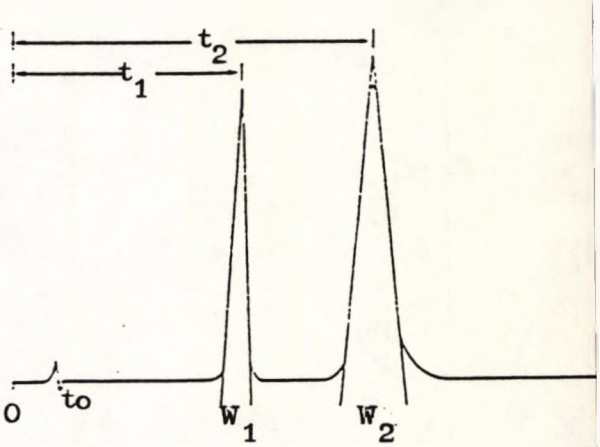


Fig. 6.2 Two peaks

(1) Capacity factor, k'

This is the most suitable way of defining solute retention

$$k' = \frac{n_s}{n_m}$$

Where n_s = number of mols solute in the stationary phase

n_m = number of mols solute in the mobile phase

The k' value is also given by the equations,

$$k' = \frac{V_R - V_o}{V_o}$$

$$k' = \frac{t_r - t_o}{t_o}$$ when the flow rate is constant

V_o , t_o = volume and retention time of unretained solute

V_R , t_r = volume and retention time of solute

The capacity factor is affected both by the temperature and the composition of the mobile phase.

(2) Resolution, R_s

This measures the degree of separation of peaks. See Fig. 6.2.

$$R_s = \frac{\text{Difference in retention time (or volume)}}{\text{average peak width (in time or volume units)}}$$

$$= \frac{2(t_2 - t_1)}{(W_1 + W_2)}$$

Where t_1, t_2, W_1 and W_2 are, respectively, the retention times and base widths of peaks 1 and 2

Selectivity, α

for two solutes a and b,

$$\alpha = \frac{k'_2}{k'_1}$$

Separation only occurs when $\alpha > 1$, with higher values indicating easier separation. α is dependent upon the nature of the two phases and upon the temperature.

(4) The Asymmetry Factor, A_s

This is a measure of the asymmetry of a peak (see Fig. 6.1)

$$A_s = a/b$$

N.B. a and b are measured on the baseline.

(5) Efficiency, N

Column efficiency is given by the number of theoretical plates, N, that the column possesses.

$$N = 16\left(\frac{t_r}{W}\right)^2$$

Where W is the base width of the peak.

OR:

$$N = 5.54\left(\frac{t_r}{W_n}\right)^2$$

Where W_n is the peak width at half height.

The column efficiency can only be modified by repacking the column.

(6) Height equivalent to a theoretical plate, HETP.

The HETP (or plate height, H) is given by

$$HETP = \frac{L}{N}$$

Where L is the length of the column.

HETP is small for efficient columns.

(7) The General Resolution Equation

This describes the resolution of two solutes in terms of the column efficiency N, selectivity α, and capacity factors.

$$R_S = \frac{1}{4}\left[\frac{\alpha - 1}{\alpha}\right]\left[\frac{k'}{1 + k'}\right]N^{0.5}$$

, k' = average of k'_1 and k'_2

The optimisation of a chromatographic separation involves optimisation of the above values by changing either the column or the mobile phase parameters, although only certain improvements are possible i.e. changing the mobile phase of an inefficient column is usually ineffective in improving resolution.

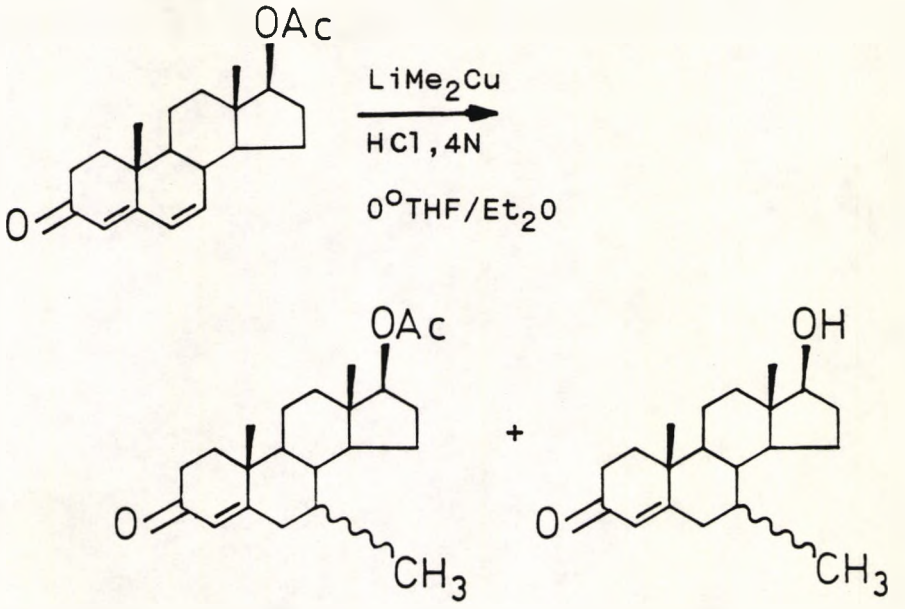
6.4 Reversed-phase preparative high-performance liquid chromatography separation of 7-methyl-steroids

6.4.1 Literature methods for separation of 7-methyl steroid epimers

In the last 20 years, high-performance liquid chromatography (HPLC) has become an increasingly important technique for the analytical and preparative separation of structurally similar organic compounds.[3-5]

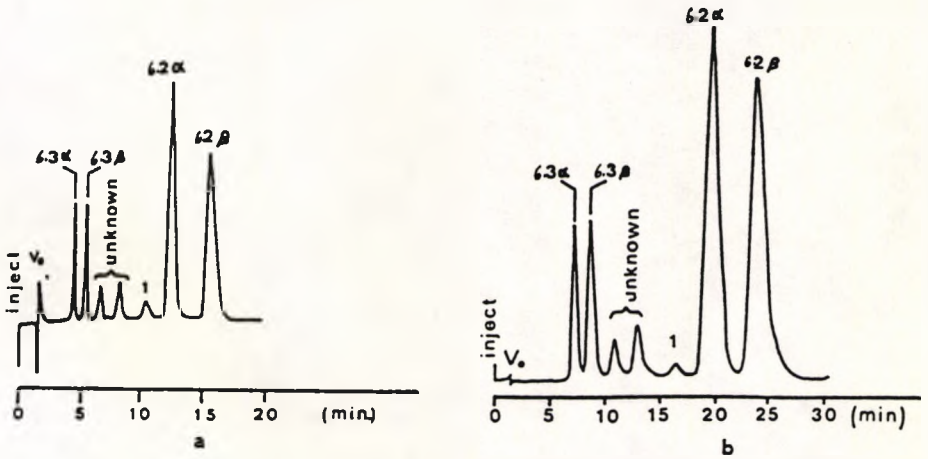
It had been reported[7] that a mixture of 7α-and 7β-methyl-steroids were difficult to separate by traditional column chromatography or by paper-chromotographic techniques. In most cases the 7β-steroids in the 7α/7β mixture were first selectively dehydrogenated with chloranil and then chromatographic separation afforded unchanged 7α-methyl-steroids and a small amount of 6-dehydro-7-methylsteroids. This difficulty has been overcome by high performance chromatography.

Gasparrini and co-workers[6] have reported that the resolution of 7-methylsteroid epimers could be achieved by HPLC and they applied this technique to the separation of the products of methylation of 6-dehydrotestosterone acetate (Scheme 6.1). Analytical separation of the components of the reaction mixture was obtained using reversed phase conditions on C_{18} or C_8 phases (Fig. 6.1).



6.2 :α(41%)+β(36%) : 6.3 :α(7%)+β(8%)

Scheme 6.1

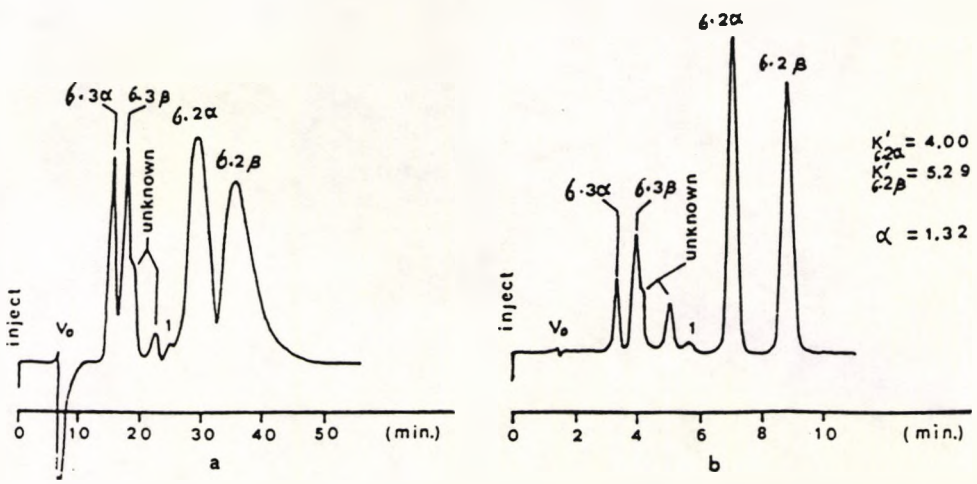


Results for reaction mixture using reversed-phase analytical LC conditions. (a) Packing: LiChrosorb RP-18 (7 μm). Column: 25 cm × 4.6 mm I.D. Solvent: methanol–water (75:25). Flow-rate: 2.0 ml/min. Detector: RI (×8). Temperature: ambient. (b) Packing: LiChrosorb RP-8 (10 μm). Column: 25 cm × 4.6 mm I.D. Solvent: methanol–water (70:30). Flow-rate: 2.0 ml/min. Detector: RI (×8). Temperature: ambient.

Fig.6.1

It was emphasised that there were two advantages of the RP-18 column over the RP-8 column: (1) RP-18 allowed a shorter analysis time and a higher selectivity. (2) The separation on the RP-18 column utilized a mobile phase enriched in methanol; from a preparative standpoint this factor increases the loading capacity of the column.

In their preparative work, separation was effected by reversed-phase HPLC using axially compressed, high-efficiency 2- or 4-cm I.D columns, packed with LiChrosorb RP-18 (10μm) or LiChroprep RP-18 (25-40μm), respectively. The separation attained with the 2-cm I.D column is illustrated in Fig. 6.2a, while the corresponding analytical separation, using the same eluent is shown in Fig. 6.2b. In a typical preparative run, carried out by Gasparrini and co-workers, about 0.7g of mixture was purified in less than 50 min on the 4 cm I.D column with a 95% recovery of products with purity greater than 99% being attained.

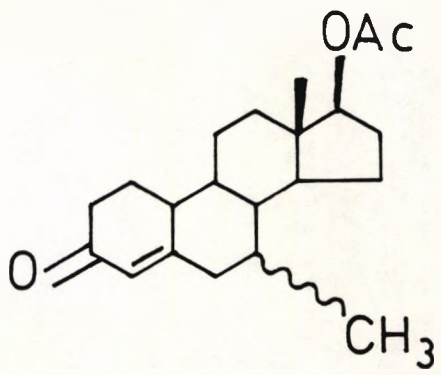


(a) Result for reaction mixture using preparative LC conditions. Packing: LiChrosorb RP-18 (10 μm), 30 g. Column: 20 cm × 2.0 cm I.D. (P_i = 13 bar). Solvent: methanol-water (80:20). Detector: RI (×20). Flow-rate: 5.5 ml/min (P_e = 7.2 bar). Amount: 0.120 g (1.2 ml of methanol). Temperature: ambient. (b) Results for reaction mixture using analytical LC conditions. Packing: LiChrosorb RP-18 (10 μm). Column: 25 cm × 4.6 mm I.D. Solvent: methanol-water (80:20). Detector: RI (×8). Flow-rate: 2.0 ml/min. Temperature: ambient.

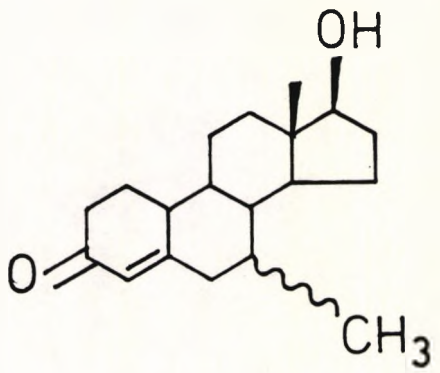
Fig. 6.2

6.4.2 Results and Discussions

As described in Chapter 5, reaction of 17β-hydroxyestra-4,6-dien-3-one acetate with methylmagnesium bromide[8,9] in the presence of copper (I) chloride gave a mixture of epimeric 7-methylated steroidal acetates (6.4) and alcohols (6.5) which required separation for identification and determination of product yields.



(6.4)



(6.5)

Initially, we attempted to separate the components of the reaction mixture by means of conventional chromatography as reported by Babcock and Campbell.[8] This approach, using preparative t.l.c. with CH_2Cl_2-CH_3CN (80:20, v/v) as mobile phase, only led to partial purification in which a pair of epimeric acetates (6.4) were separated from a pair of epimeric alcohols (6.5). The resolution of the epimeric pairs of 7-methylsteroids was then investigated by reversed-phase HPLC. We preferred to use 5-μm Hypersil-ODS, a small diameter, spherical silica derivative which gives better performance than the 10μm, irregular microparticulate phases described by Gasparrini et al.[6] with MeOH-H_2O as mobile

phase. Each pair of epimers was found to contain two main peaks with minor impurities (Fig. 6.3). When the crude mixture was analysed by normal and reversed-phase HPLC under isocratic conditions (Figs. 6.4 and 6.5), the best separation of the components was obtained on ODS-silica. The large α values (1.20 & 1.26) obtained in the epimer separations on ODS-Hypersil indicated that this stationary phase would be well suited to scale-up for preparative HPLC.

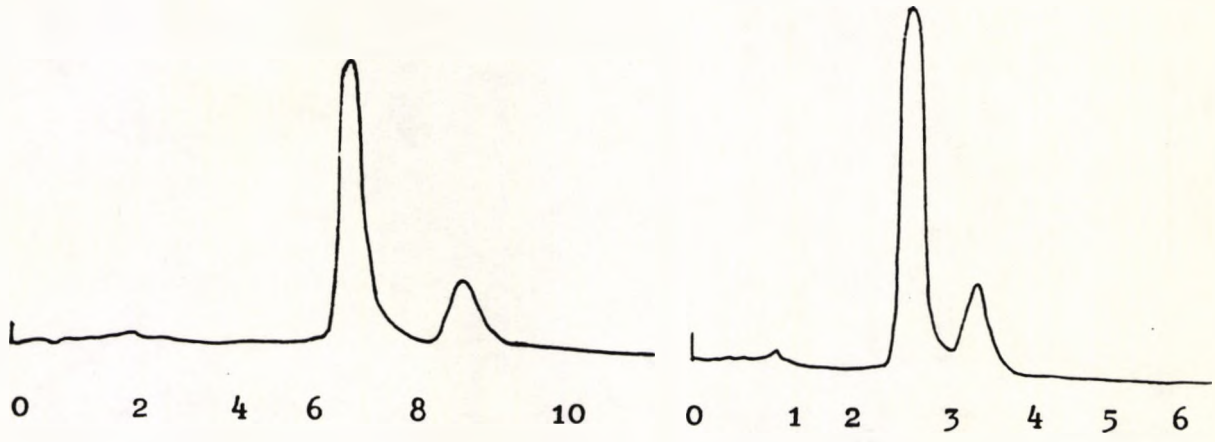


Fig. 6.3a Fig. 6.3b

Fig.6.3 Analysis of the fractions: (a) containing epimers (6.4),(b) containing epimers (6.5), on 12.5 x 4.5 cm i.d. 5μm ODS-Hypersil, eluted with MeOH-H_2O, (70:30) at 2.0 ml/min. Detector, 244 nm x 0.5 AUfsd. Injections in 20μl CH_2Cl_2.

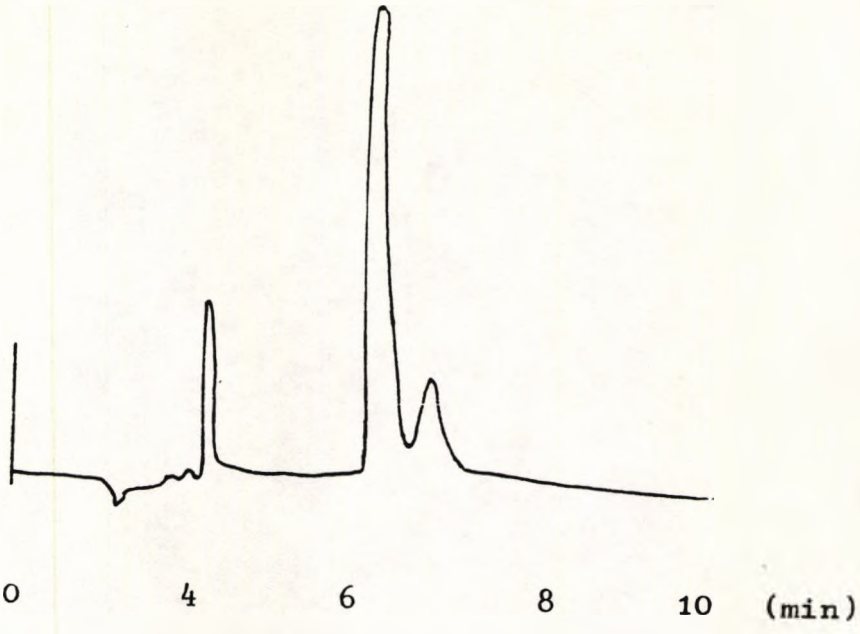


Fig.6.4 Analysis of reaction mixture (6.4 and 6.5).
Column: 25 x 0.45 cm i.d. 5μm Hypersil, eluted with CH_2Cl_2-MeCN (90:10 v/v) at 2.0 ml/min. Detection at 244 nm x 2 AUfsd.
Injections in 20μl CH_2Cl_2.

For convenience the crude product was initially subjected to preparative t.l.c using CH_2Cl_2-CH_3CN (80:20, v/v) as mobile phase which provided each pair of epimers (6.4) and (6.5) as a mixture.

A large preparative column (33 cm x 22 mm I.D.) was constructed, for economy the packing material being

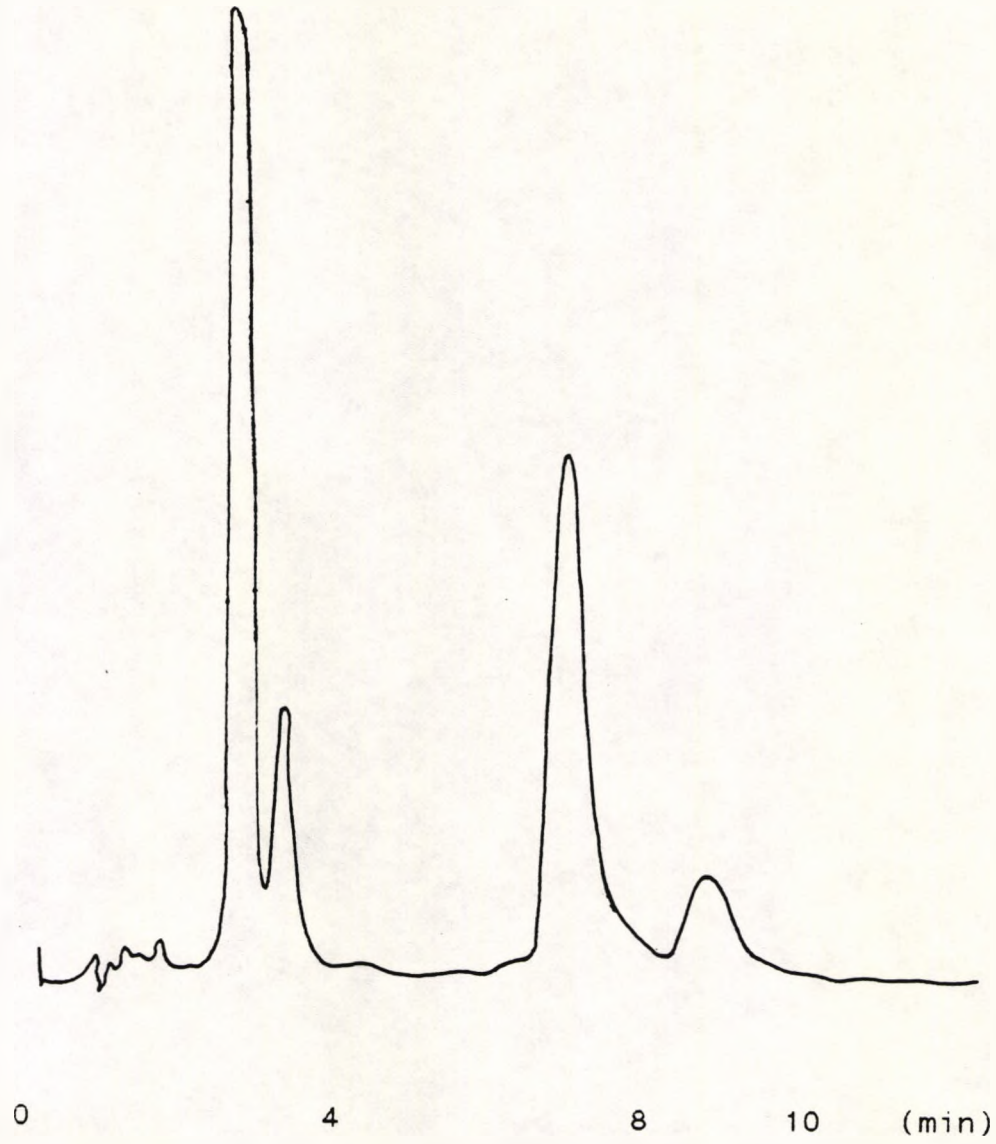


Fig.6.5 Analysis of reaction mixture (6.4 and 6.5).

Column: 25 x 0.45 cm i.d. 5μm ODS-Hypersil, eluted with MeOH-H_2O (70:30 v/v) at 2.0 ml/min. Detection at 244 nm x 2 AUfsd.

Injections in 20μl MeOH.

synthesized in our laboratory by reaction of 5μm Hypersil with octadecyltrichlorosilane.[9] On this column, 250 mg of each pair of mixed epimers could be separated in one run taking just over 37 min. (Figs. 6.6a and 6.7a). The fractions collected were re-analysed and showed satisfactory results, with a 95% recovery of products and with purity greater than 99% (Figs. 6.6b and 6.7b).

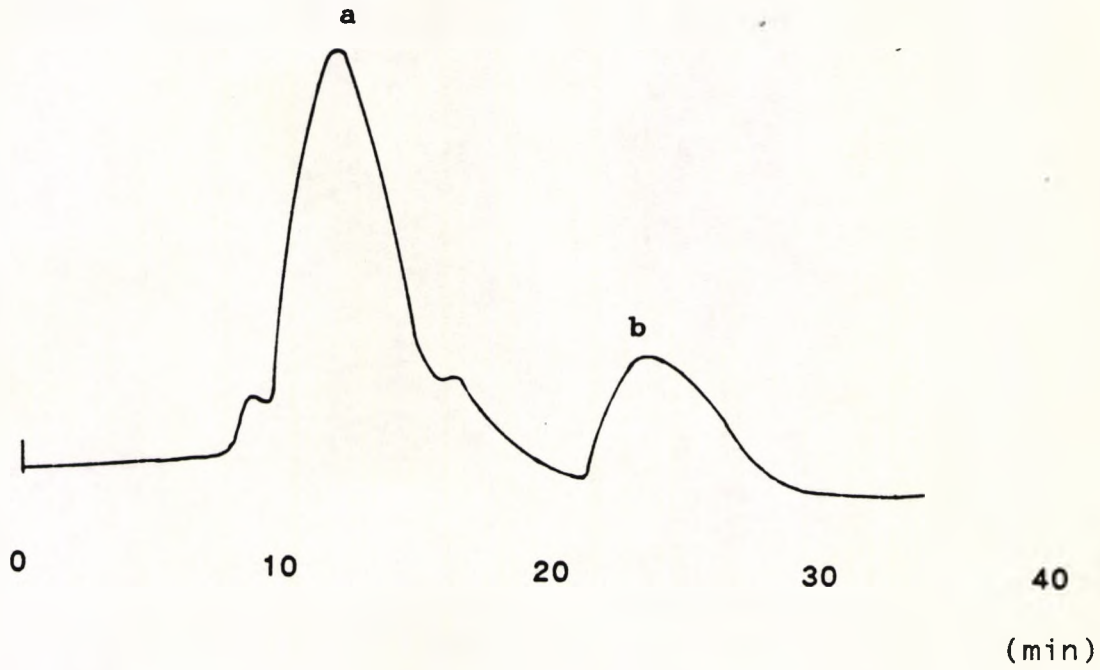


Fig.6.6a Preparative HPLC separation of compound (6.4).
Column: 33 x 2.2 cm i.d. 5μm ODS-Hypersil, eluted with MeOH-H_2O (75:25 v/v) at 15 ml/min. Detection at 267 nm x 2 AUfsd.
Injections in 100μl MeOH.

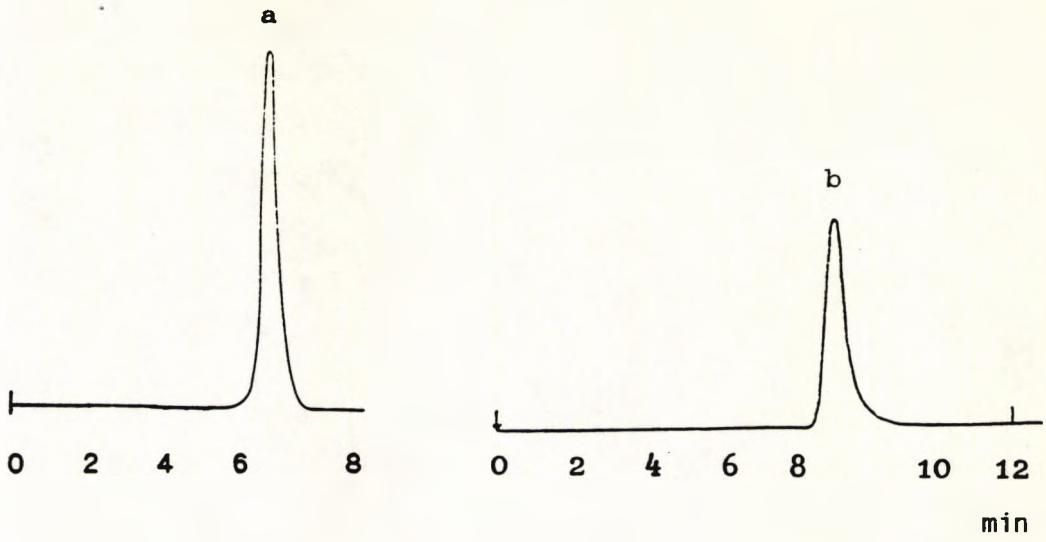


Fig.6.6b Analysis of fractions a and b from preparative HPLC of compound (6.4).

Column: 25 x 0.45 cm i.d. 5μm ODS-Hypersil, eluted with MeOH-H_2O (70:30 v/v) at 2.0 ml/min. Detection at 244 nm x 2 AUfsd.

Injections in 20μl MeOH-H_2O (70:30).

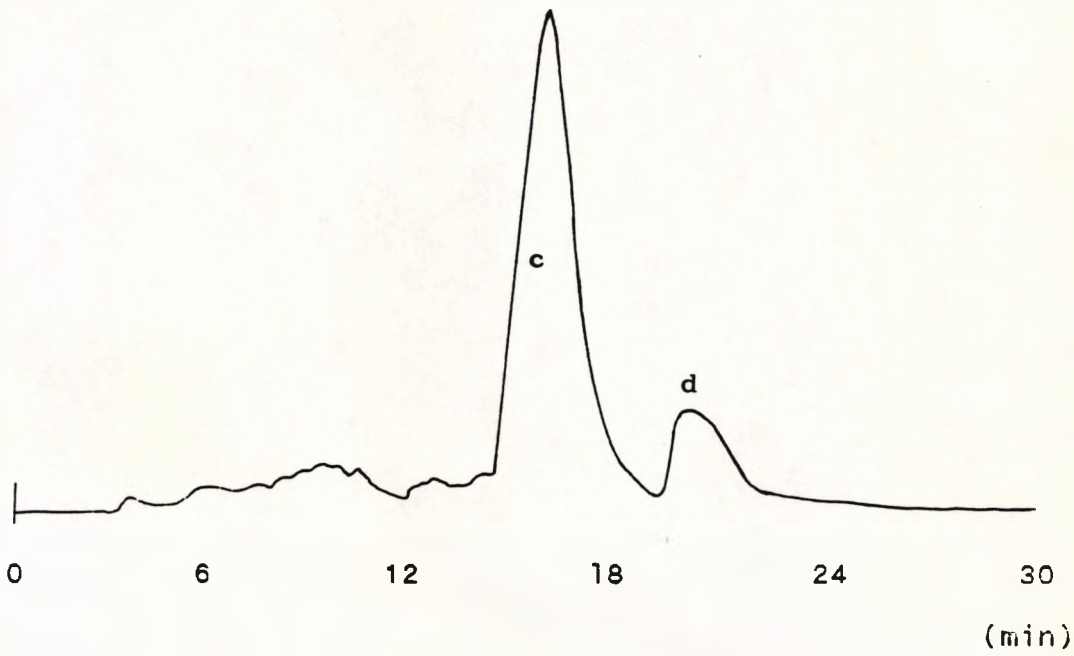


Fig.6.7a Preparative HPLC separation of compound (6.5).

Column: 33 x 2.2 cm i.d. 5μm ODS-Hypersil, eluted with MeOH-H_2O (70:30 v/v) at 20 ml/min. Detection at 272 nm x 2 AUfsd.

Injections in 100μl MeOH.

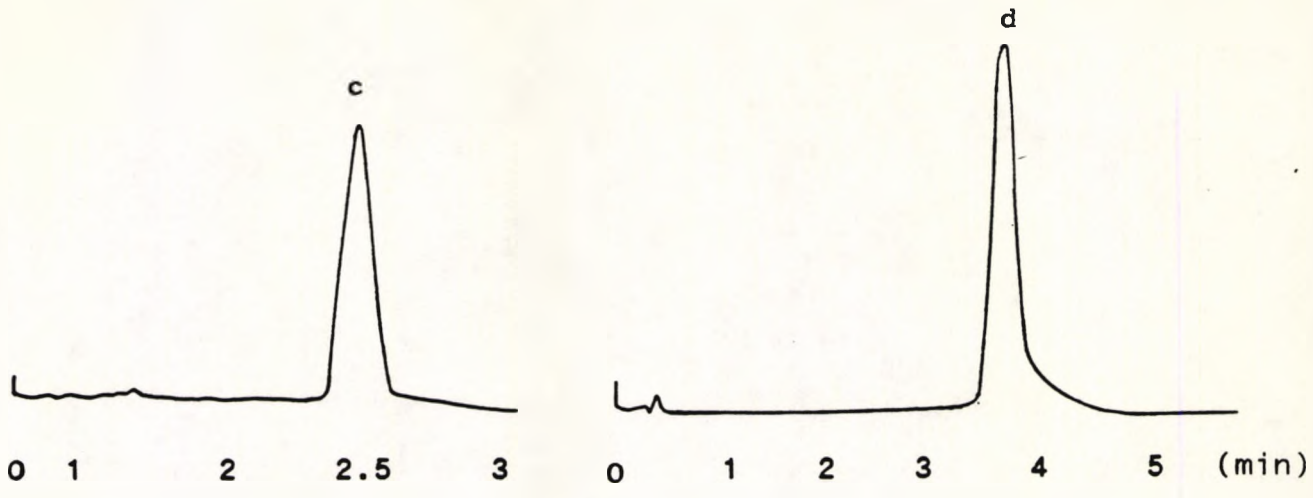


Fig.6.7b Analysis of fractions c and d from preparative HPLC of compound (6.5).

Column : 25 x 0.45 cm i.d. 5μm ODS-Hypersil, eluted with MeOH-H_2O (70:30 v/v) at 2 ml/min. Detection at 243 nm x 2 AUfsd.

Injections in 20μl MeOH-H_2O (70:30).

6.5 Experimental

The HPLC equipment consisted of a Cecil CE1000 pump, Rheodyne 7125 injector fitted with a loop (25μl for analytical separations and 200μl for preparative separations) and a Cecil 1200 variable wavelength detector (used at 244 nm x 2 AUfsd for analytical separations and in the range 244-272 nm x 2 AUFsd for preparative separations).

Analytical separations were performed on columns (25 x 0.45 cm i.d.) packed with 5μm ODS-Hypersil and eluted at 2 ml/min with MeoH: H_2O (70:30 v/v).

Preparative separations were performed on columns (30 x 2 cm i.d.) packed with 5μm ODS-Hypersil and eluted at 16 ml/min with MeOH: H_2O (70:30 v/v).

Water was purified in a Milli-Q apparatus (15-18 Mohm, 0.22μm pore size, Millipore Corp. Bedford, MA). All solvents were purchased as HPLC grade. Mobile phases were degassed ultrasonically before use.

All samples were dissolved in methanol.

6.6 References

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CHAPTER 7

Strategy for design and synthesis of 11β-aryl-A-nor-steroids

7.1 Introduction

The search for new drugs which function by interaction with specific biological receptors may, in principle, be carried out in a number of different ways. The least scientific method is to establish a random screening programme. This method is very expensive and has a relatively poor success rate (typically one in several thousand compounds randomly screened will prove useful), but is sometimes used by pharmaceutical companies in the absence of other good leads and has the one distinct merit that it may turn up activity in entirely novel structural types since it makes no presumptions about structure-activity relationships.

A second approach is to take an already-identified lead compound and make as many as possible of its close structural analogues. This approach is also very expensive but is frequently used by pharmaceutical companies, both in the course of lead optimization and for the purposes of comprehensive patent coverage. In the case of anti-progestins, Roussel-Uclaf's patents cover several hundred close relatives of RU 38486 and a further several hundred compounds are claimed by the rival Schering patents. Consequently, there is little prospect of other, outside

groups finding much scope for original compounds by this approach. Moreover, it is unlikely that any work closely related to the existing compounds already covered will lead to the discovery of a new drug with dramatically increased potency.

A rational approach which can now be applied to drug design and discovery, since the development of modern computer hardware and the related software for molecular modelling, is to explore the shape of the active site within a receptor and design a molecule with appropriate steric and electronic parameters to fit this site. In cases where the receptor is a protein which can be crystallized, X-ray crystallography can be used to provide a very detailed and realistic picture of the active site as a basis for modelling and in recent times a number of drugs (e.g. angiotensin converting enzyme inhibitors) have been designed in this way. When the protein cannot be crystallized but its primary sequence is known, attempts can be made to "guess" its 3D structure by using sequence analogues with folding patterns in previously X-rayed proteins. However, this type of approach is still very speculative and is still in its infancy. Moreover, conformational predictions have so far been applied only to relatively low molecular weight (<20,000 Daltons) proteins.

The primary sequence of the progesterone receptor has been established as a result of cloning and decoding of the cDNA

which is responsible for its biosynthesis. The progesterone receptor is a protein of 933 amino acid residues and a molecular weight of about 100,000 Daltons, which is far beyond the scope of current methodologies for calculating 3D structures.

When the 3D structure of the biological receptor is unknown, it is still possible to develop useful maps of important stereochemical and polar features of the active site region. This can be done by studying receptor interactions with a number of substrates and using the results to gauge the shape, size and character of the spaces into which it binds. This form of blind mapping has been very successful in building up pictures of receptor regions for many kinds of drugs: the morphine receptors in the nervous system are a good example.

In the present work, an attempt has been made to use the information available from published studies of anti-progestins in order to design a novel structure which it is hoped will show improved receptor binding. An important element of the reasoning behind the approach adopted is that individual structural features built into a steroid framework will have additive and/or synergistic effects when combined together.

There are good historical precedents to support the argument that the effects of individual structural elements

on the biological activity of steroids can sometimes be additive. One of the earliest examples of this phenomenon was seen in the development of the first oral progestin, when Djerassi's group at Syntex combined the beneficial effects of removal of the 19-methyl group and attachment of the 17-ethynyl function. The incorporation of these functionalities resulted in the desired combination of high progestational potency and good oral activity in norethisterone, used in the contraceptive pill. Similarly, combinations of structural features (1,2-double bond, 9-fluro-11-hydroxy, 16-alkyl, etc.) have been very successful in the development of highly potent and selective glucocorticoids.

In the light of these precedents, it seems to be a rational approach to search for new anti-progestins having increased potency, compared with RU 38486, by seeking structural features which may contribute to strong, antagonistic binding to the progesterone receptor.

7.2 Target design

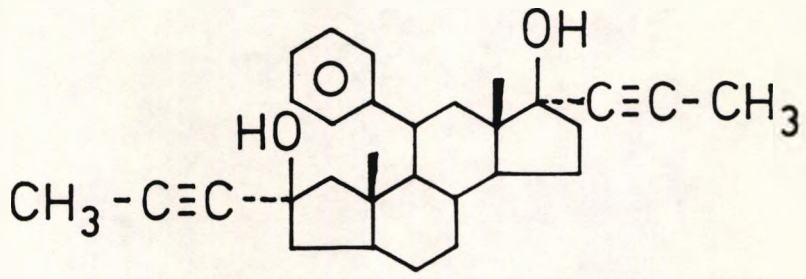
In the present work, the following structural elements have been selected as being important to the goal of developing a new, potent, orally-active anti-progestin:

1. In common with the vast body of earlier work on both progestins and anti-progestins, it is assumed that 17α-alkynyl-17β-hydroxy functionality is desirable for good oral potency.

2. All the powerful anti-progestins so far identified have had an aryl substituent located either at 11β or on the 18-methyl group. It is evident that there must be a pocket of substantial size located in the receptor above the C ring of the steroid, into which the aryl group can fit. It is therefore desirable that any new anti-progestin should retain the presence of an aryl (or similar) function projecting into this sector of space.

3. In searching for additional structural features to supplement those already incorporated in the Ru and ZK compounds, attention was drawn to the A-nor steroids reviewed in Chapter 4. Amongst these compounds, anordrin in particular is striking as the only steroid other than RU 38486 which has been extensively studied and clinically used on a large scale as an anti-implantation agent. Although the clinical mechanism of action of anordrin remains in some doubt, there is clear evidence for its anti-hormonal properties. It was therefore decided that the ring A pattern of anordrin should be incorporated into the new design: namely, A-nor ring with a 2α-alkynyl-2β-hydroxy substitution pattern.

Combining these three types of structural elements, it was decided to synthesise a series of A-nor-androstanes having 11β-aryl,2α,17α-dialkynyl-2β,17β-diol substituents (7.1) for investigation of their anti-progestin properties.



(7.1)

7.3 Synthetic strategy

The application of the process of retro-synthetic analysis to the target structure (7.1) conditioned by the requirement that the synthesis must be based on a relatively cheap and readily available steroid if it is going to be practical, leads to the following requirements for the starting material:

- the steroid which is selected for modification will have a normal, 6-membered A-ring (economy) with functionality that permits ring contraction and subsequent loss of the extruded C atom;

- it will carry an oxygen substituent at C-11, in the form of a hydroxyl or ketone group, so as to make possible the introduction of an aryl substituent by nucleophilic attack;

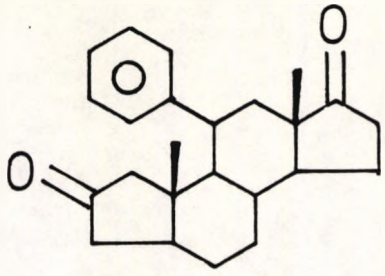
- it will have C-17 functionality in the form of a masked ketone, e.g. hydroxyl derivative or carbon chain which can be oxidatively removed.

- the A-ring and D-ring functionalities must be capable of manipulation so that, at the appropriate time, they can be converted into ketone functions which will be the subject of attack by suitable acetylenic reagents.

Further consideration led to the following additional requirements:

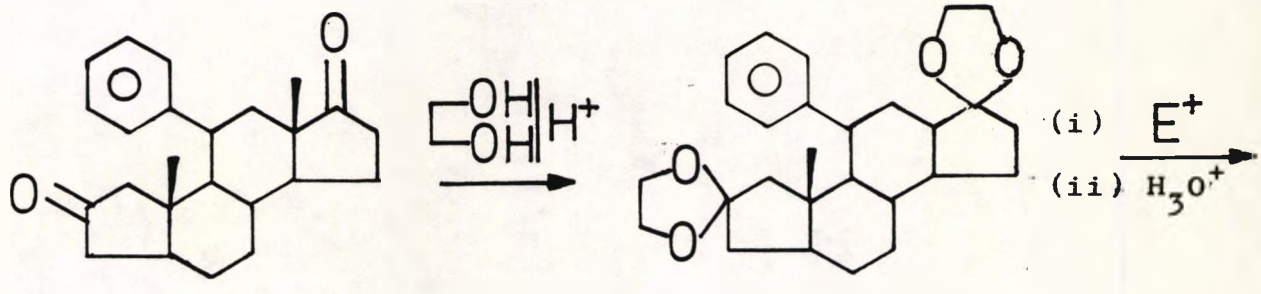
- In order that a significant number of new compounds can be prepared for testing with the minimum amount of effort, the synthesis should be a divergent one: there should be a single route to a common intermediate, as late in the stepwise scheme as possible, from which all the differently substituted analogues can be prepared in relatively few additional stages.

These considerations led us to identify the 11β-phenyl-A-nor-androstanedione (7.2) as the intermediate goal for this work. It was reasoned that, if the synthesis of this compound could

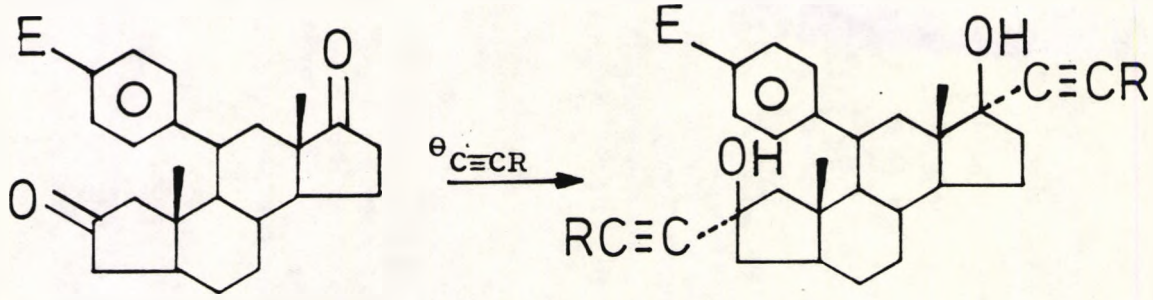


(7.2)

be achieved with adequate efficiency on a large (multi-gram) scale, it would serve as a suitable precursor to all our required products. After suitable protection of the carbonyls, electrophilic aromatic substitution and further manipulation of the substituents on the benzene ring could provide a series of aryl steroids, with the last steps being the unmasking of the carbonyl groups and their combination with alkyne-derived carbanions (Scheme 7.1).



(7.2)



Scheme 7.1

7.5 References

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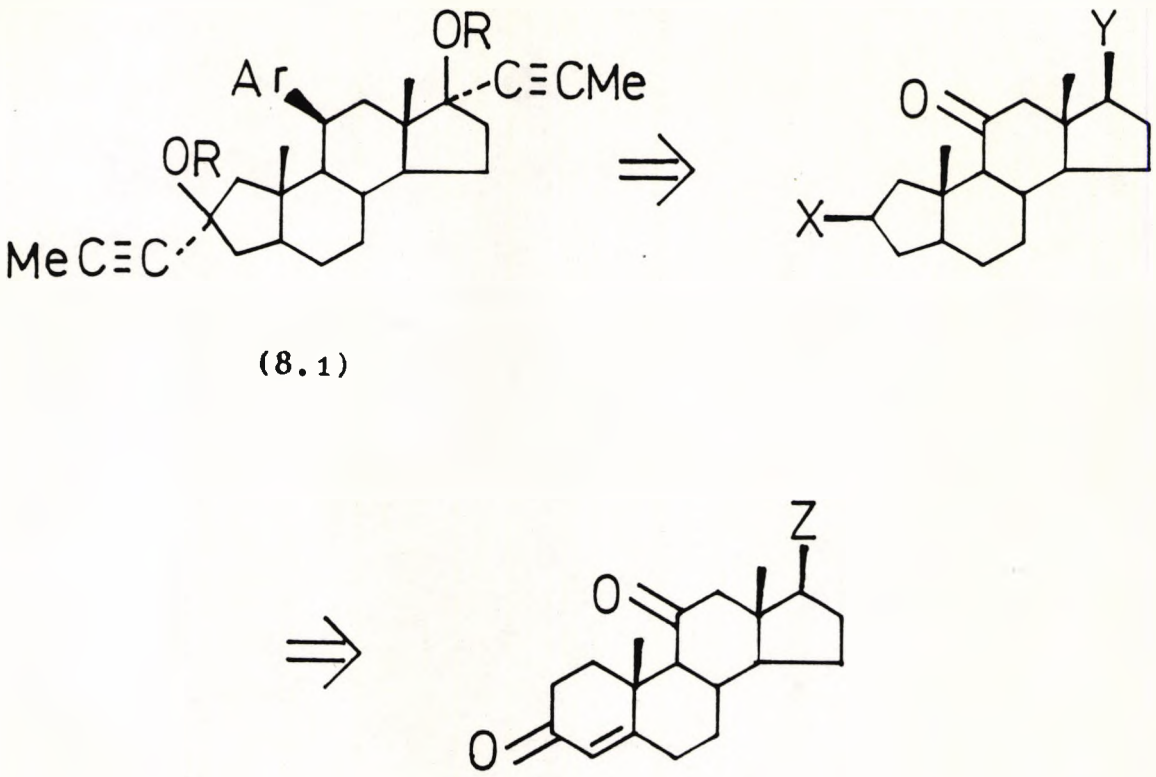
CHAPTER 8

Studies of the ring contraction of steroidal 4-en-3-ones

8.1 Introduction

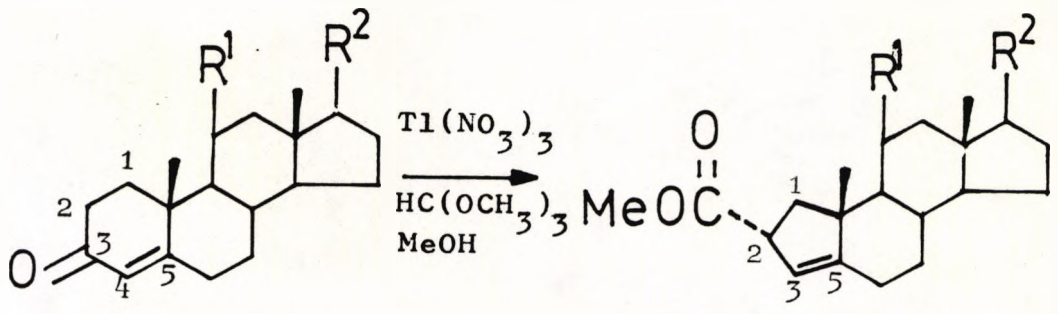
Changes in the basic carbon skeleton of a steroid may cause striking changes in its physiological activity. During the past three decades, various laboratories have investigated the preparation of A-nor analogues of biologically active steroidal materials,[1-5] and it has been found, for example, that the A-norderivative of hydrocortisone is inactive[4] but that A-norprogesterone is an anti-androgen[7] (see Chapter 4).

As discussed in Chapter 7, consideration of the biological activities associated with A-norsteroids such as anordrin[6] and with 11β-aryl steroids such as RU 38486[7] led us to propose that an 11β-aryl-A-norsteroid (8.1) would be an interesting target as a potential anti-hormonal drug. Retrosynthetic analysis (Scheme 8.1) led us to the requirement for ring contraction of an 11-keto steroid.



(8.1)

Scheme 8.1

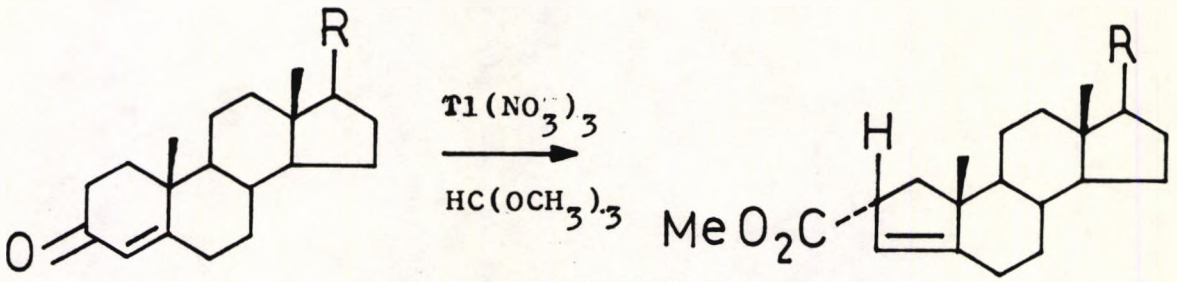


(8.2) (8.3)

Scheme 8.2

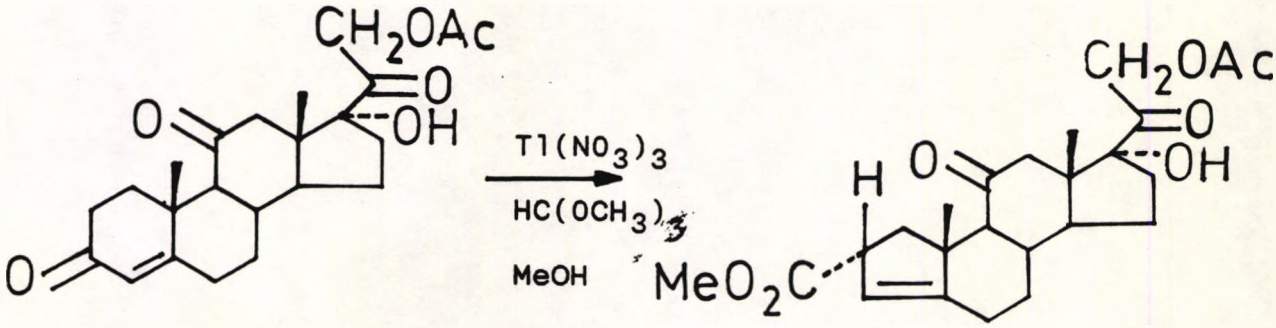
An Italian group[8,4] has reported that steroidal 4-en-3-ones can be converted in a single step into A-norsteroids using thallium nitrate (Scheme 8.2). The reported mechanism for this reaction has already been discussed in Chapter 4.

The results of the work by the Italian group [8,9] are summarised in Scheme 8.3.



(8.4) a, R = OAc
b, R = COMe

(8.5) a,R = OAc 75%
b,R = $COCH_3$ 65%



(8.6)

(8.7) 66%

Scheme 8.3

8.2 Results and Discussion

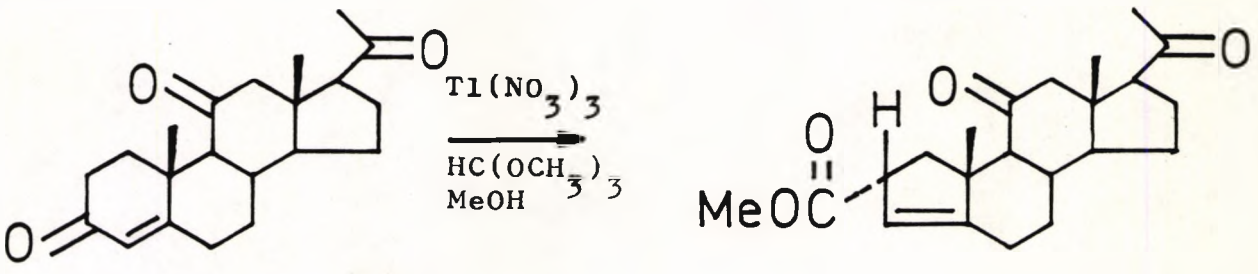
The ring contraction procedure was first examined using progesterone (8.4b) as a cheap and convenient model compound. The Italian group[8,9] reported that this steroidal enone underwent ring contraction to give the A-norester (8.5b) in 65% yield (Scheme 8.3).

In our hands, a product was obtained in 25% yield and its purity and structure (8.5b) were confirmed by its analytical and spectroscopic data. However, we were unable to reproduce the yield reported in the literature.[8,9]

Another model compound we examined was cortisone acetate (8.6). A yield of 66% of A-norester (8.7) was reported[9] when compound (8.6) underwent ring contraction (Scheme 8.3). When we repeated this procedure, compound (8.7) was obtained in 45% yield and its structure was confirmed by IR, ^1H NMR and MS.

The same method was then used for the ring contraction of 11-ketoprogesterone (8.8) (Scheme 8.4). A product was obtained in 48% yield, which was an improvement over the yields obtained in the model reactions. The structure of the new compound (8.9) is in accordance with the following spectroscopic data : the MS spectrum shows a molecular ion at m/z = 358 ($C_{21}H_{26}O_5$) ; the 100 MHz ^1H NMR spectrum exhibits a singlet at 83.6 ppm (3H) and a broad

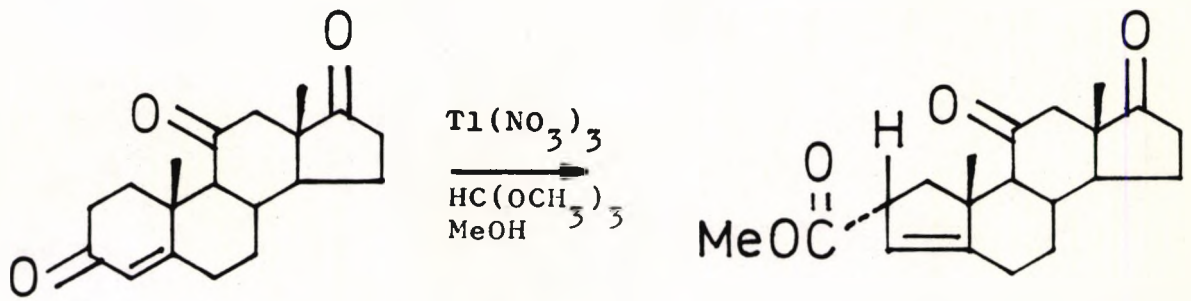
singlet at δ5.2 ppm (1H, $W^1/2$ = 5Hz) which can be attributed to the methoxycarbonyl group C(2) and the olefinic proton C(3), respectively; the IR spectrum shows the absorbance of the saturated ester at 1730 cm^{-1}.



(8.8) (8.9)

Scheme 8.4

Adrenosterone (8.10) was treated with thallium nitrate in a similar manner. This reaction gave 16% yield of A-norester (8.11) (Scheme 8.5).

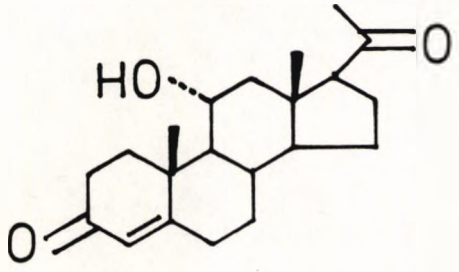


(8.10) (8.11)

Scheme 8.5

In the ^1H NMR spectrum of the product (8.11) the same typical signals as for compound (8.9) were observed: singlet of the methoxycarbonyl group at δ3.68 and a broad singlet at δ5.18 ($W^1/2$ = 5Hz). In the IR spectrum, ν_{max} : 1730 cm^{-1} (ester group) and 1705, and 1725 (keto groups); (MS parent peak at m/z = 328 ($C_{20}H_{24}O_4$)).

Three out of four reactions discussed above employed an 11-keto-steroid with oxygen at C-11 position as starting material. We also examined the use of a steroid with a hydroxyl group at the C-11 position, namely 11α-hydroxy-progesterone (8.12). An attempt to react this with thallium nitrate in the same manner gave a complex mixture from which no identifiable products were obtained. This indicated that replacement of a keto group at C-11 by a hydroxyl group was unsatisfactory and this approach was not pursued further.



(8.12)

Both 11-ketoprogesterone and cortisone acetate gave similar yields (48 and 45%, respectively) of A-noresters and either of these would be suitable as starting material for the purpose or our work. 11-Ketoprogesterone was selected because it is significantly cheaper.

8.3 Experimental

(General Procedure)

Thallium (III) nitrate (230.9 mg, 0.58 mmol) was dissolved in a mixture of trimethylorthoformate (TMOF) (1.9 ml) and MeOH (1.4 ml) and the resulting solution left stirring at 0°C for 30 min. A cold solution of the steroid (0.5 mmol) in a mixture of TMOF (1.4 ml) and MeOH (1.2 ml) was quickly added. After 10 min a white precipitate of thallium (I) nitrate was formed. After 40 min the reaction mixture was neutralized with saturated aqueous sodium carbonate solution then filtered and extracted with diethyl ether.

The crude reaction mixture was chromatographed on a silica gel column. On elution with a mixture of hexane-ether (20:80, v/v), the desired A-norsteroid was obtained as follows:

(a) From progesterone (200 mg) was obtained 2α-methoxy-carbonyl-A-norpregn-3-en-20-one (8.5b) (56 mg, 25%) ; M.p. 106-108°C; ^{1}H NMR ($CDCl_3$)δ: 0.65 (3H, S), 0.98 (3H, S), 2.1 (3H, S), 3.68 (3H, S), 5.18 (1H, S) ; ν_{max}($CHCl_3$)cm^{-1} : 1730, 1710 ; EI-MS : m/z 344.2547 (M^+, 40% ; $C_{22}H_{32}O_3$ requires 344.2398).

(b) From cortisone-21-acetate (200 mg) was obtained 2α-methoxycarbonyl-A-norpregn-3-ene-17α, 21-diol-11, 20-dione 21-acetate (8.7) (96.9 mg, 45%) ; M.p. 170-172°C ; ^{1}H NMR ($CDCl_3$)δ:0.62 (3H, S), 1.08 (3H, S), 2.08 (3H, S), 3.56 (3H, S), 5.02 (1H, S) ;ν_{max}($CHCl_3$) cm^{-1} 1730-1701 ; EI-MS; m/z 432.2166 (M^+ 13% ; $C_{24}H_{32}O_7$, requires 432.2194), 417 (14%).

(c) From 11-ketoprogesterone (200 mg) was obtained 2α-methoxycarbonyl-A-norpregn-3-ene-11, 20-dione (8.9) (96.3 mg, 48%) ; M.p. 100-102°C ; ^{1}H NMR ($CDCl_3$)δ: 0.56 (3H, S), 1.06 (3H, S), 3.52 (3H, S), 5.08 (1H, S) ;ν_{max}($CHCl_3$)cm^{-1} 1700-1725 ; EI-MS : m/z 358.2136 (M^+, 11.95% ; $C_{21}H_{26}O_5$ requires 358.1818), 343 (100%).

(d) From adrenosterone (200 mg) was obtained 2α-methoxycarbonyl-A-norandrost-3-ene-11,17-dione (8.13) (37.5 mg,16.02%) ; M.p. oil ; ^{1}H NMR ($CDCl_3$)δ: 0.84 (3H, S), 1.12 (3H, S), 3.48 (3H, S), 5.04 (1H, S) :ν_{max}($CHCl_3$)cm^{-1} : 1740-1725 : EI-MS ; m/z 328.1524 (M^+, 8.24% ; $C_{20}H_{24}O_4$ requires 328.1709).

8.4 References

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CHAPTER 9

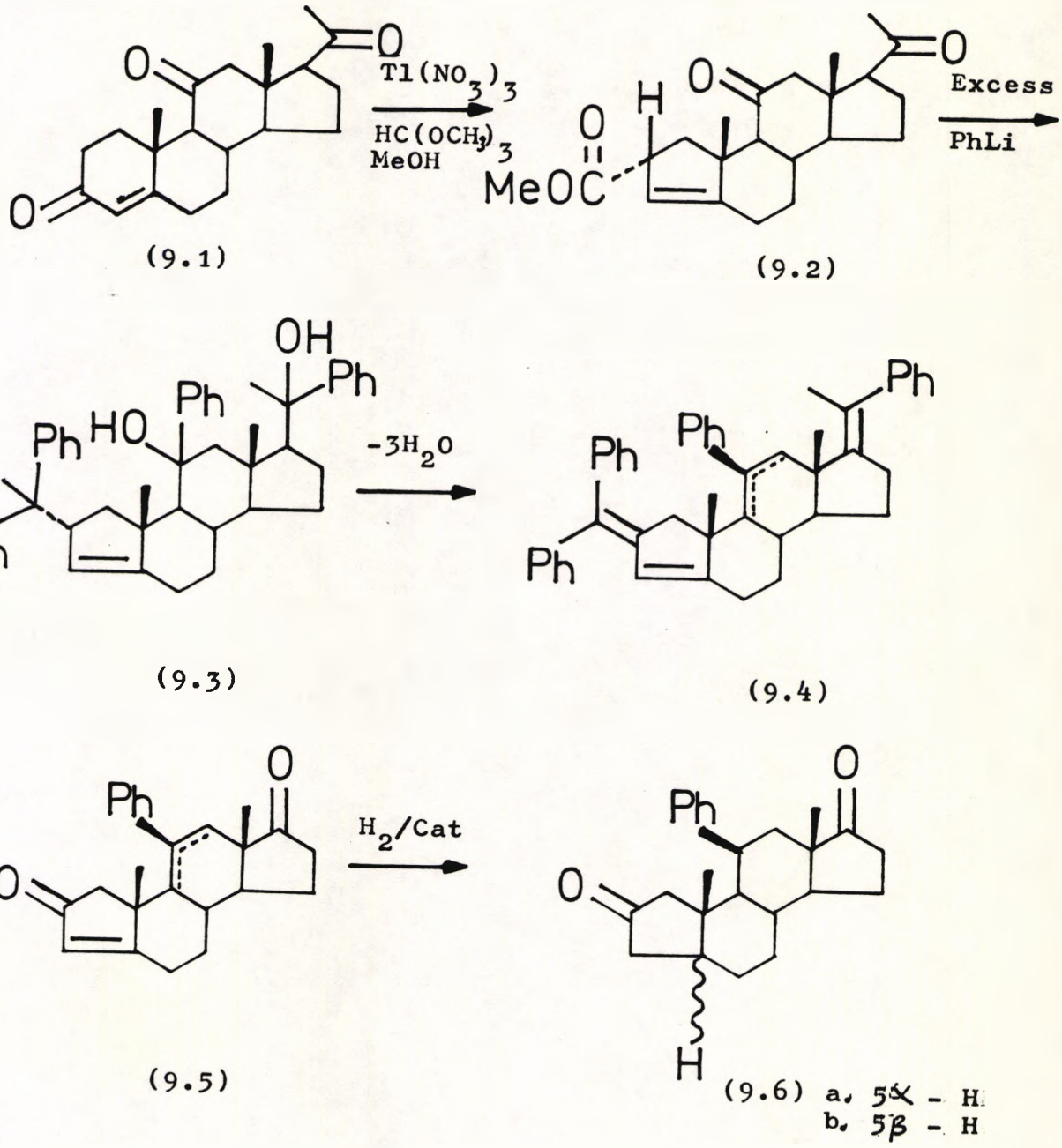
Synthetic approaches to 11β-aryl-A-nor-androstanes

9.1 Introduction

It was demonstrated in Chapter 8 that 11-ketoprogesterone (9.1) underwent ring contraction induced by thallium(III) nitrate in trimethyl orthoformate-methanol mixture to give the A-norester (9.2). From 200 mg of 11-ketoprogesterone, 96.3 mg (48%) of A-norester (9.2) was obtained. This appeared to be a suitable procedure for application to the synthesis of the 11β-phenyldiketone intermediate (9.6). For this purpose it was necessary to scale up this reaction to make a large amount of the ring contracted steroid (9.2) and then to examine the use of this steroid in the strategy given in Scheme 9.1.

9.2 Results and Discussion

The ring contraction reaction on 11-ketoprogesterone (9.1) was successfully carried out on a large scale, 25 g of this steroid furnishing 13.1 g (48%) of the pure 11-keto A-norester (9.2) following chromatography on a silica column. The structure of the bulk product (9.2) was confirmed by ^{1}H and ^{13}C NMR spectroscopy. In particular, the observation of


Tl(NO3)3
HC(OCH3)3
MeOH
Excess
PhLi
-3H2O
O3
H2/Cat
(9.1)
(9.2)
(9.3)
(9.4)
(9.5)
(9.6) a. 5α - H
b. 5β - H

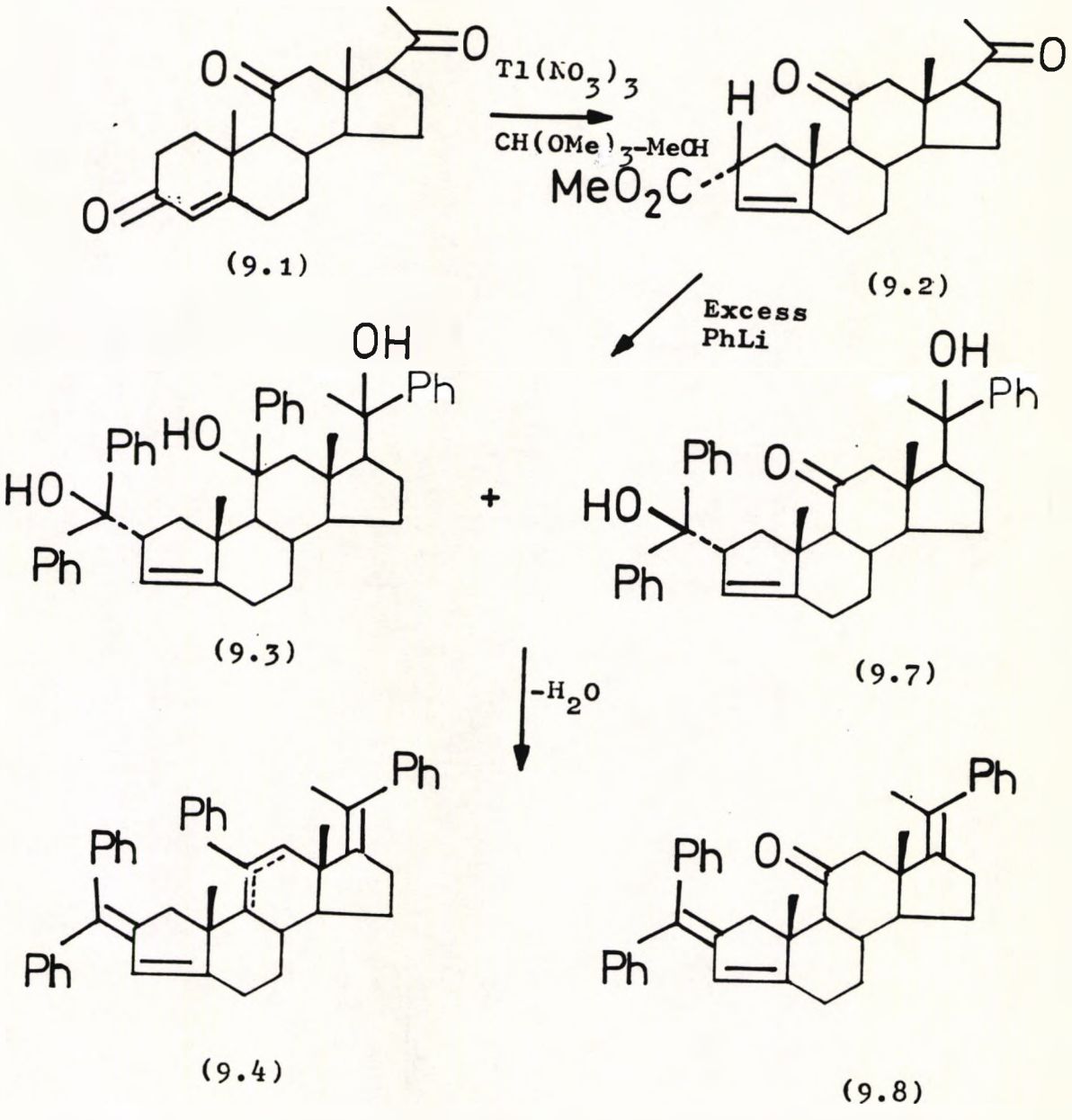
Scheme 9.1

an n.O.e. between the CH_3 group attached to C-10 and the H atom at C-2 confirmed their cis-1,3-diaxial relationship and thus proved that the MeO_2C substituent has a 2α orientation.

An attempt to prepare compound (9.3) using PhMgBr failed, a mixture of starting material and unidentified complex compounds being obtained. However, treatment of compound (9.2) with excess 2M PhLi at -78°C gave a mixture of compounds (9.3) and (9.7) (ratio : 1:1) (Scheme 9.2), which were separated by preparative HPLC.

The structure of compound (9.3) was supported by its IR, ^1H NMR and mass spectra. The IR spectrum showed absence of an ester group but there was a new peak at 1600 cm^{-1} corresponding to the phenyl group. In the ^1H NMR there was loss of the methoxycarbonyl group at δ3.52 and presence of a new multiplet signal at δ7.16 due to the four phenyl groups. The mass spectrum did not exhibit a molecular ion, expected at m/z 638, but showed M^+-18 (m/z 620) due to the loss of one molecule of H_2O.

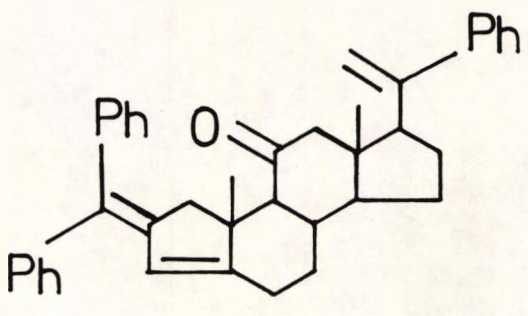
Similarly, the structure of compound (9.7) was supported by its IR, ^1H NMR and mass spectra. Additional confirmation of this structure was given by its ^{13}C NMR, where the presence of the carbonyl group was shown by a signal at δ197. Furthermore, when pure compound (9.7) was treated with more PhLi at -78°C a mixture of compounds (9.3) and (9.7) was


O
O
O
Tl(NO3)3
CH(OMe)3-MeOH
MeO2C
H
O
O
(9.1)
(9.2)
Excess
PhLi
OH
Ph
Ph
HO
Ph
HO
Ph
+
OH
Ph
Ph
O
HO
Ph
(9.3)
(9.7)
-H2O
Ph
Ph
Ph
Ph
Ph
Ph
O
Ph
(9.4)
(9.8)

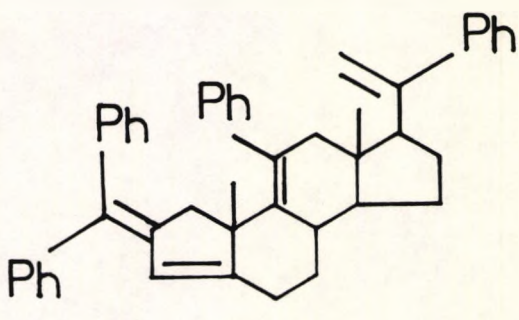
Scheme 9.2

again obtained. These results were in agreement with previous reports on incomplete phenylation of polycarbonyl compounds using PhLi[7].

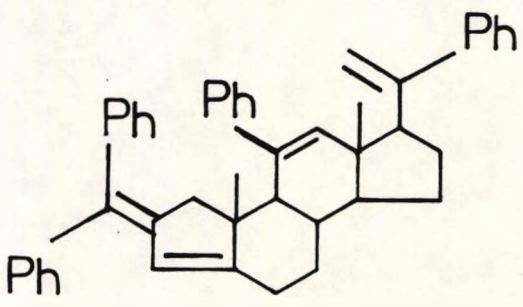
The next step of the proposed route Scheme 9.1 required the dehydration of the enetriol (9.3) to the tetraolefin (9.4), with the expectation that the two phenylmethylene side chains could then be oxidatively removed (e.g. by ozonolysis) to generate the diketosteroid (9.5). For convenience, it was decided to explore the dehydration reaction using an unseparated mixture of triol (9.3) and dihydroxyketone (9.7). Several dehydrating agents, including MsCl-Py, phosphorus oxychloride-pyridine, and $FeCl_3$-SiO_2 gave complex mixtures which were difficult to separate, but in which the presence of the α-styryl compounds (9.8b, 9.9, 9.10) was suggested by the appearance of olefinic signals at δ5.14.



(9.8b)



(9.9)



(9.10)

The cleanest dehydration conditions proved to be the use of freshly distilled thionyl chloride in pyridine at -5°C. However, this yielded the α-styryl compounds (9.8b, 9.9 and 9.10) and not the desired phenethylidene isomers (9.4, 9.8) (Scheme 9.2).

The structure of compound (9.10) was shown by its ^{1}H NMR spectrum. The protons of the vinyl group at C-20 gave rise to a multiplet of eight lines centred at δ 5.14, representing a pair of double doublets due to the coupling between the two vinyl protons and also allylic coupling between each vinyl proton and the proton on C-17. There also appeared in the spectrum two olefinic doublets at δ5.65 and δ 5.70 respectively, for the protons C-3 and C-12, with each again showing allylic coupling.

Similarly, the structure of compound (9.8b) was supported by its ^{1}H NMR. However, there was no allylic coupling between the two vinyl protons and C-17 proton, although each of the two vinyl protons resonated at δ5.13 and δ 5.16 respectively. The doublet that appeared at δ5.86 in the spectrum was due to the olefinic proton on C-3 coupling, allylically, with one proton on C-6.

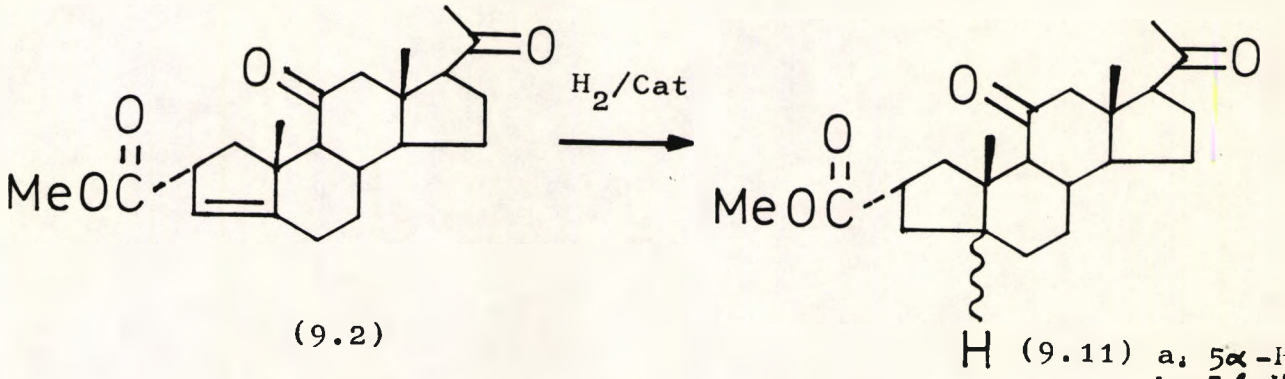
The failure of compound (9.3) to generate an exomethylene group at C-17 on dehydration was a disappointment. Presumably the conformation of the 1-phenyl-1-hydroxyethyl

substituent is such that dehydration towards the C-21 methyl is kinetically preferred over loss of the 17-H. Thermodynamically, the more substituted exomethylene product (9.4) should have been favoured by Saytzeff's Rule.

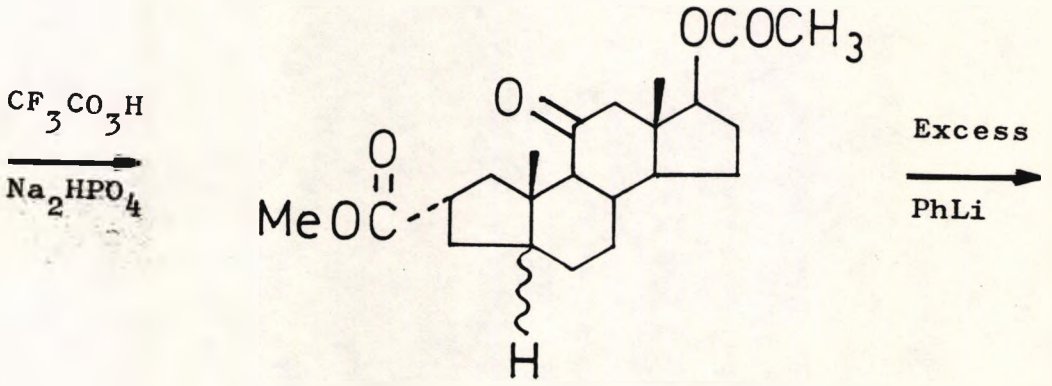
In view of this result, it was decided to modify the approach, as shown in Scheme 9.3, so that the 17-substituent would be transformed by a Baeyer-Villiger oxidation prior to reaction of the ester and 11-keto functions with PhLi, thus avoiding altogether the need for dehydration of the D-ring substituent. However, it was now necessary to reduce the A-ring double bond at an earlier stage, to avoid its oxidation by the Baeyer-Villiger reagent.

Accordingly (Scheme 9.3), compound (9.2) was hydrogenated with Pd-C in EtOH-HOAC (2:1) for two days, affording compound (9.11) in good yield (89.5%).

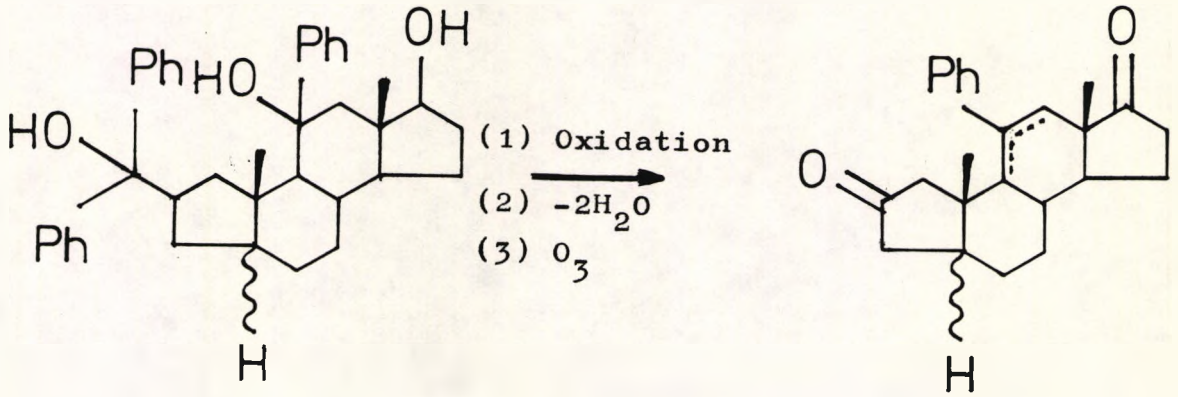
Catalytic hydrogenation of a steroidal 3-ene normally proceeds from the α-face, due to the steric effect of the β-methyl groups. Thus, the A-nor-3-ene (9.2) might be expected to give the trans ring junction, structure (9.11a). However, the NMR spectrum of the hydrogenation product was difficult to analyse in the region of the H-5 proton due to the close proximity of various signals. Stereochemistry of this compound is proved by the more detailed analysis on a later compound in the reaction sequence (see compound (9.21) on page 135).



(9.2) (9.11) a. 5α-H
b. 5β-H



(9.12) a. 5α-H
b. 5β-H



(9.13) a. 5α-H
b. 5β-H

(9.14) a. 5α-H
b. 5β-H

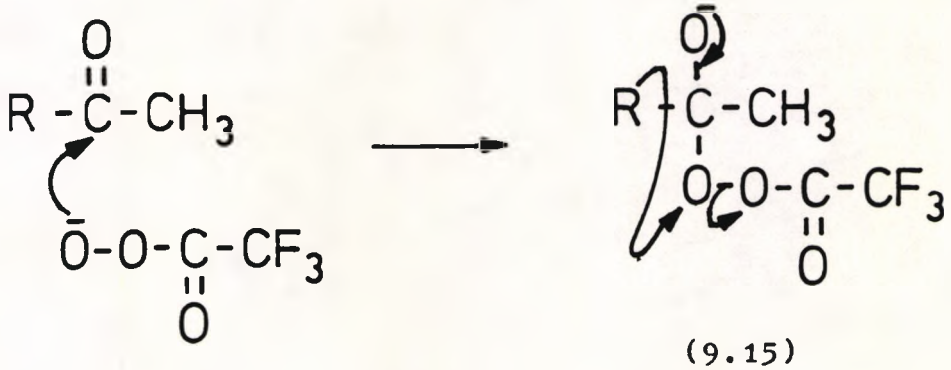
Scheme 9.3

Considerable effort was required to establish conditions suitable for the Baeyer-Villiger oxidation of compound (9.11) to the acetate (9.12) (Scheme 9.3). A variety of procedures employing perbenzoic acid, m-chloroperbenzoic acid and per acetic acid, with and without acid catalysts, were examined without success. In general, either unchanged starting material or complex mixtures of products were isolated. Eventually, it was found that treatment of compound (9.11) with freshly prepared peroxytrifluoroacetic acid[3,4] in methylene chloride containing a slurry of sodium phosphate as a buffering agent, gave the acetate (9.12) in 62% yield.

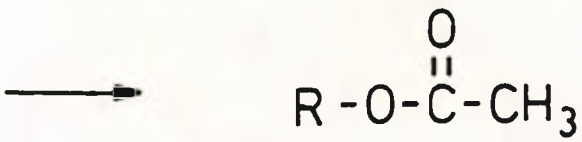
The identity of compound (9.12) was confirmed by its NMR spectrum, which showed a new signal at δ4.64 due to the 17-H of the 17-acetate. The triplet nature of the signal arises due to coupling of the 17-H with the two geminal protons on C-16.

The mechanism of the Baeyer-Villiger reaction has been extensively discussed in the literature[5,6] and there is little doubt that the reaction proceeds through decomposition of the peroxytrifluoroacetic acid-ketone adduct (9.15) (Scheme 9.4), which by loss of trifluoroacetate anion and the concerted migration of an alkyl group yields the ester (9.16). The effectiveness of peroxy-trifluoroacetic acid in this reaction is due to the facile heterolysis of the oxygen-oxygen bond induced by the highly electronegative trifluoroacetic substitutent.

It has also been established that structural features in the migrating group which are best able to accommodate a positive charge facilitate the re-arrangement. This is presumably due to the build-up of positive charge on the peroxy oxygen in the transition state[5,6], as the leaving group departs. On this basis, then, the migrating group will normally be that which is most stabilized by hyperconjugative electron release, favouring alkyl migration in the order 3>2>1.



(9.15)



(9.16)

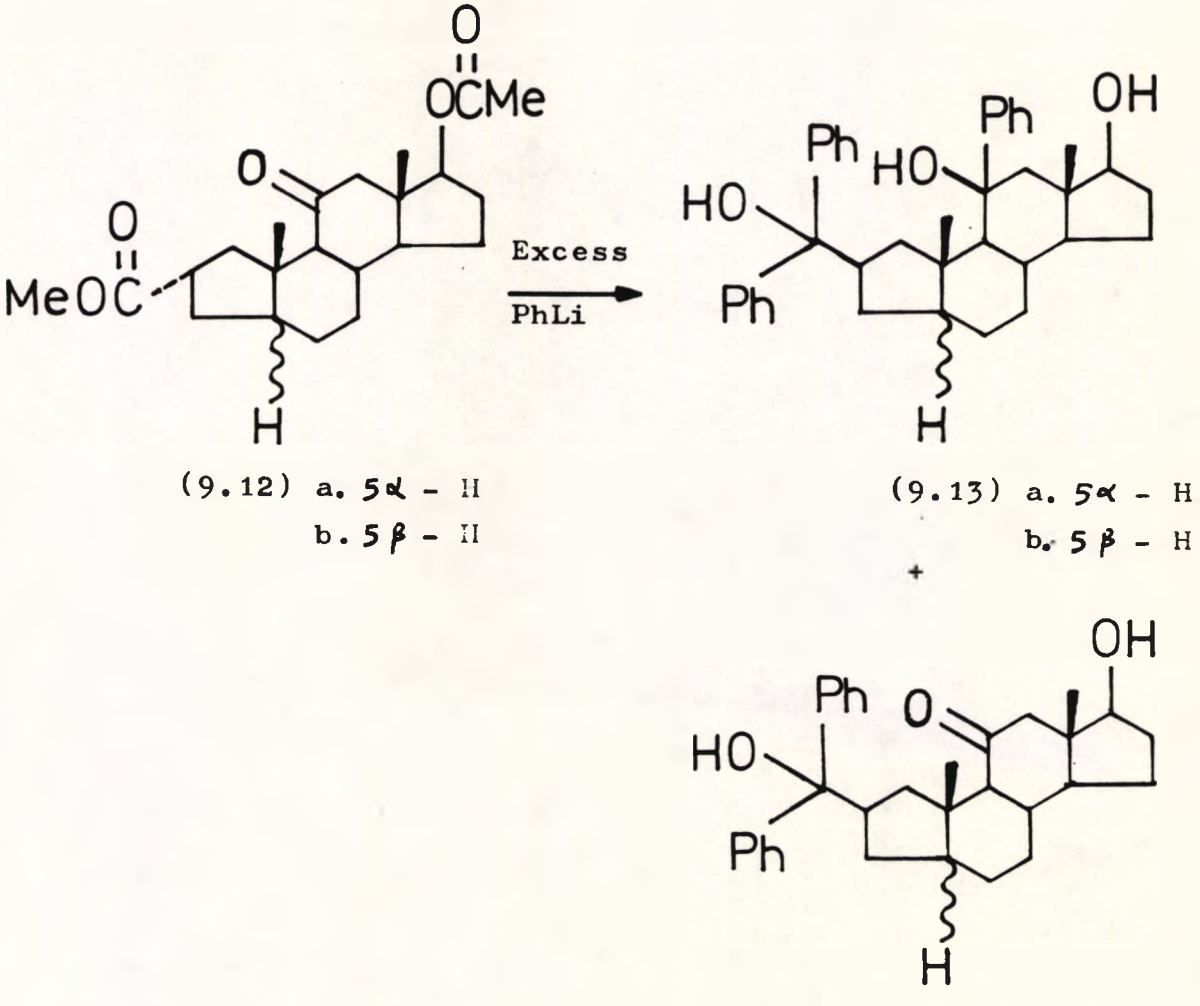
R = Steroid

Scheme 9.4

Thus, in the oxidation of compound (9.11), the steroidal (secondary) group is the one that migrates, giving an acetate ester rather than a methyl ester.

Treatment of the 11-keto-diester (9.12) with excess PhLi gave a mixture of compounds (9.13) and (9.17) (Scheme 9.5), which were separated by column chromatography.

The structural assignment of compounds (9.13) and (9.17) was made on the basis of their analytical and spectral data (IR, ^{1}H NMR, MS). Although in the mass spectra of both compounds their molecular ions were not present, in both cases, $M^{+}-H_2O$ ions were observed. Structure (9.17) was further confirmed by its ^{13}C NMR spectrum, showing signal at δ210 due to the carbon of the C=0 group.



(9.12) a. 5α - H
b. 5β - H

(9.13) a. 5α - H
b. 5β - H

+

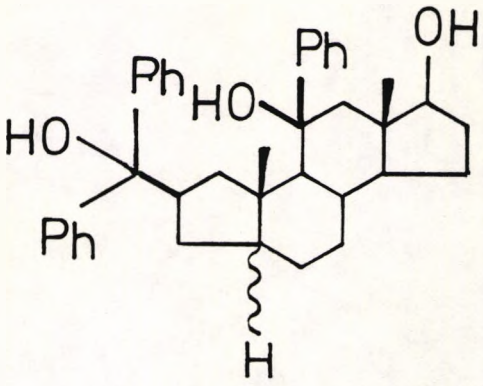
(9.17) a. 5α - H
b. 5β - H

Scheme 9.5

It appeared that compound (9.17) was obtained as a result of incomplete reaction. However, when the reaction was repeated and temperature was increased from -78 to 50°C after the addition of compound (9.12) and also using 100% excess PhLi, there was no change in yields of compounds (9.13) and (9.17). However, when pure compound (9.17) was further treated with more PhLi at -78°C, a mixture of compounds (9.13) and (9.17) was again obtained. These results were in agreement with previous reports on incomplete phenylation of polycarbonyl compounds using PhLi[7]. It has been shown that when organolithium reagents[7] react with a carbonyl compound there exists a competition between addition, reduction and enolization, and that at -78°C reduction and enolization are minimized. However, in the present case the only possible competition would be between addition and enolization simply because of the nature of the organolithium used, i.e. PhLi; a β-hydrogen is required in the organolithium for reduction to take place.

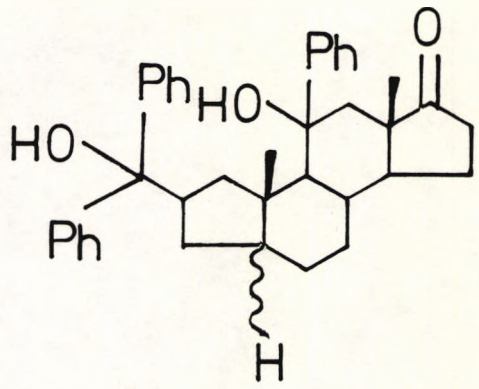
Oxidation of compound (9.13) using dipyridinium dichromate gave compound (9.18) in good yield[8,9] (91.3%) (Scheme 9.6). The infrared spectrum of compound (9.18) possessed the expected band at 1730 cm^{-1} due to the carbonyl group at C-17.

Amongst several procedures investigated, the method described by Nishiguchi et al.[10] for the dehydration of secondary and tertiary alcohols was found to be most suitable



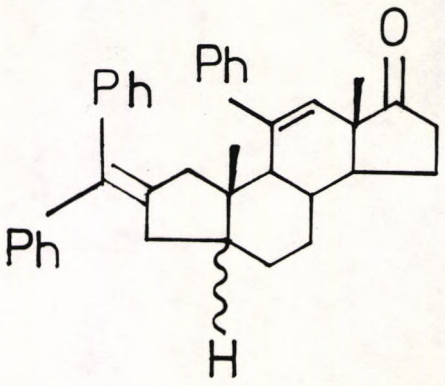
(9.13) a. 5α- H
b. 5β- H

$R_2Cr_2O_7^{2-}$



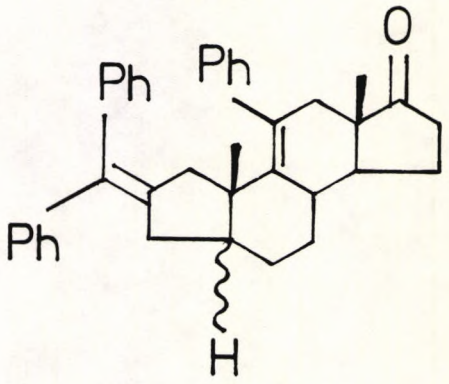
(9.18) a. 5α- H
b. 5β- H

$CuSO_4/SiO_2$



(9.19) a. 5α- H
b. 5β- H

+



(9.20) a. 5α- H
b. 5β- H

R = $(C_5H_6N^+)_2$

Scheme 9.6

for dehydrating compound (9.18). Thus, treatment of compound (9.18) with freshly prepared $CuSO_4/SiO_2$ in carbon tetrachloride under reflux gave two isomeric compounds (9.19) and (9.20) which were separated by preparative t.l.c. The ^1H NMR spectra of the two isomers confirmed their structures. A singlet olefinic signal appeared at δ6.03 in the ^1H NMR spectrum of compound (9.19) but was not present in the ^1H NMR spectrum of compound (9.20).

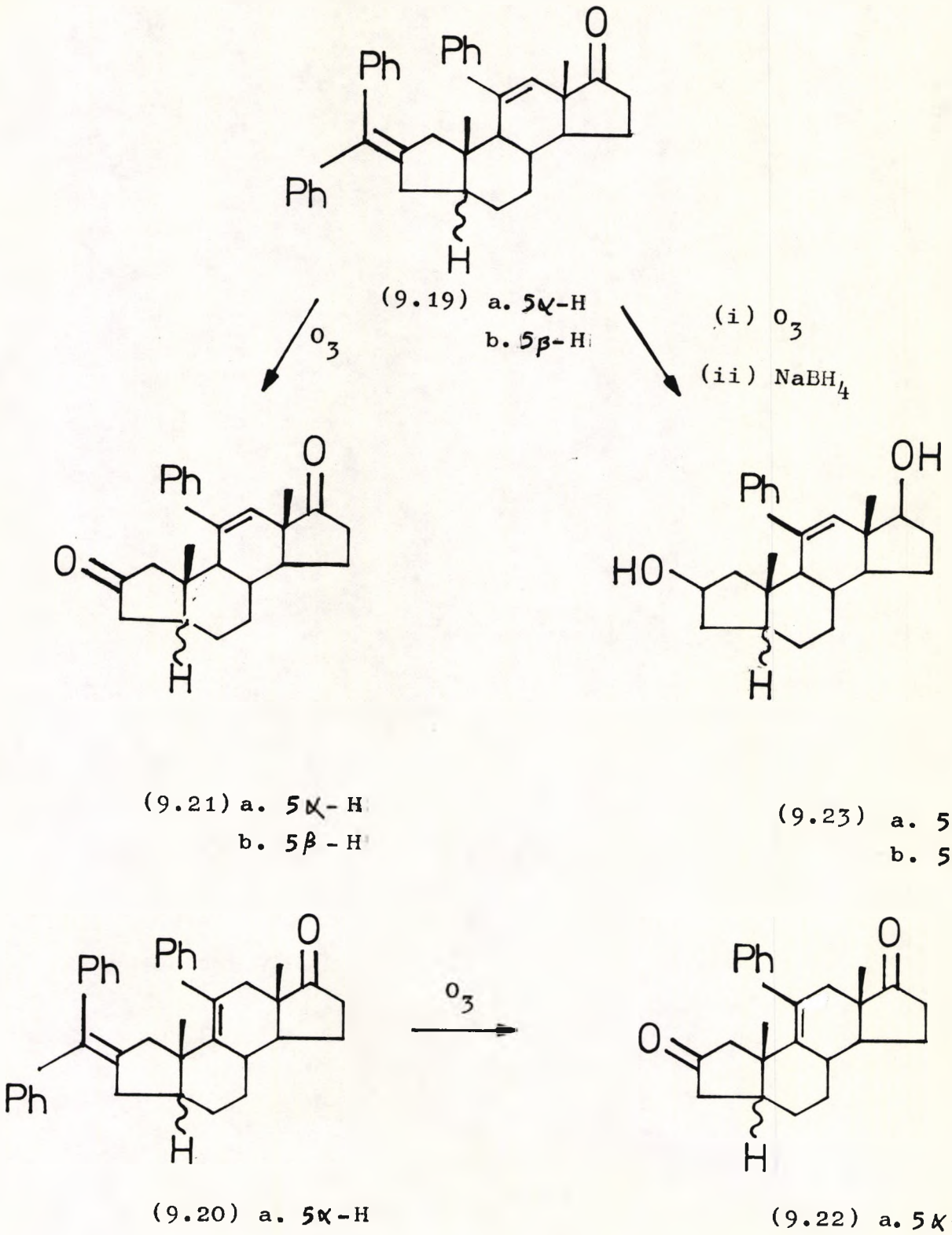
It was observed that when the dehydration reaction mixture was refluxed gently, compound (9.19) and (9.20) were obtained in ratio 6:1 yield. However, when the reaction mixture was refluxed vigorously, the ratio obtained was 1:6. These results can be explained by the kinetic preference for abstraction of H-12 rather than H-9. The thermodynamic isomer preferred is compound (9.20).

Several methods were examined for the oxidative cleavage of the diphenylmethylene side chain in compounds (9.19) and (9.20). Procedures employing chromium trioxide[11] and potassium permanganate with 18-crown ether[12] were investigated, but gave low yields and furnished compounds (9.21) and (9.22) mixed with other by-products which were very difficult to separate. Eventually, the ozonolysis method described by Shepherd et al.[13] for the synthesis of ketones was found to be suitable. Thus, when ozone was

passed into separate solutions of compounds (9.19) and (9.20) in methylene chloride and pyridine (225:1 ratio), good yields of compounds (9.21) and (9.22) respectively were obtained (Scheme 9.7).

The structural assignments of the isolated compounds (9.21) and (9.22) were made on the basis of their analytical and spectral data (IR, ^{1}H NMR, ^{13}C NMR, MS) : the IR spectra of compounds (9.21) and (9.22) showed a broad band at 1735 cm^{-1} due to the two carbonyl groups at positions C-2 and C-17; their mass spectra both showed a molecular ion peak at 348; the ^{13}C NMR spectra of both compounds showed two C=O signals at δ206 and δ209; the ^{1}H NMR spectra of both compounds were almost the same but the ^{1}H NMR spectrum of compound (9.21) showed an additional doublet signal at δ6.06 due to the olefinic proton at C-12; also in the ^{1}H NMR spectra of both compounds there appeared a multiplet signal at δ7.2 which integrated for 5H's. This evidence indicates the loss of two phenyl groups which is in agreement with the expected results.

Up to this point, the stereochemistry of the 5-H in the series of compounds (9.11-9.22) had been tentatively assumed to be 5α on the basis that steroidal hydrogenation usually occurs from the underside. The complexity of the 400 MHz ^{1}H NMR spectra in early members of this series had made detailed analysis of the 5-H signal difficult. However, the



(9.19) a. 5α-H
b. 5β-H

(9.21) a. 5α-H
b. 5β-H

(9.23) a. 5α-H
b. 5β-H

(9.20) a. 5α-H
b. 5β-H

(9.22) a. 5α-H
b. 5β-H

Scheme 9.7

introduction of the carbonyl group in the A-nor ring and the flattening effect of a double bond in the C ring appeared to make the product (9.21) a suitable compound in which to explore this point further and detailed ^1H NMR investigations were carried out.

Two distinct approaches can be considered in the examination of the C-5 stereochemistry in a steroid such as (9.21). The first is the use of the Karplus equation to correlate the observed coupling constants with the dihedral angles between 5-H and the nearby protons which are 3β, 3α, 6β and 6α. However, because of the complexity of the 400 MHz ^1H NMR and 2D COSY spectra, the only interaction that could be clearly seen was that between the 3-H and 5-H protons. A nice doublet of doublets of doublets (ddd) was observed for 3β and 3α protons respectively due to interaction with 5-H and the two protons on C-1 as a result of coupling through the C-2 carbonyl from both ^1H NMR and the 2D COSY spectra. The observed $^J3\beta\text{-}5 = {}^J3\alpha\text{-}5 = 9$. These values indicated dihedral angles for $^J3\text{-}5 = 30\text{-}45$ and $^J3\text{-}5 = 160\text{-}170$. However, examination of models showed that both 5α and 5β isomers (9.21a) and (9.21b) could adopt conformations consistent with these angles and consequently no conclusive decision could be reached from this approach.

The second method examined was the case of the technique of nuclear Overhauser enhancement difference spectroscopy (nOeds). When two protons are in close proximity to one

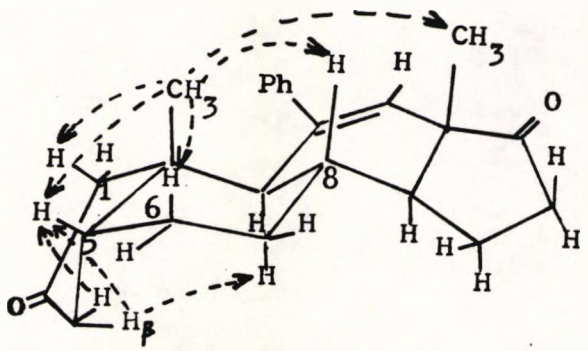
another in a molecule and their spins are contributing to one another's NMR relaxation, double irradiation of one proton causes an increase in signal intensity for the other. This nuclear Overhauser enhancement can reach as much as 50% of the original signal size, but is often much smaller (due to factors of distance and the relaxation contributions of all other adjacent protons). The power of the nOeds technique, in which the normal spectrum is substracted from the enhanced one on double irradiation, lies in the ability to sensitively detect small enhancements.

For the two possible structures (9.21a) and (9.21b), the most obvious feature to search for is the presence of nOe's to and from the 19-Me group. In particular, nOe's between this group and C_5-H would be indicative of cis stereochemistry (9.21b). When a series of nOe experiments were run on compound (9.21) in $CDCl_3$ (Table 9.1), a clear nOe was observed at H-5 on irradiation of 19-Me, consistent with cis stereochemistry (9.21b).

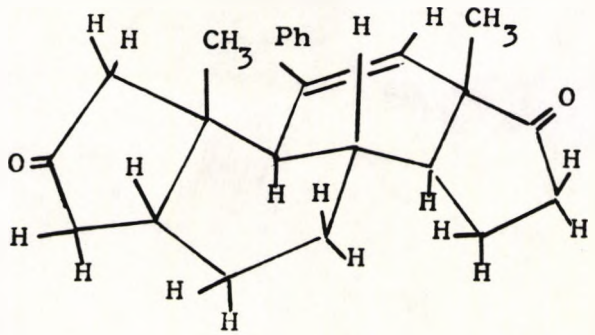
There were also nOe's from 19-Me to H-1β, H-6β, H-8β and 18-Me which were consistent with the conformation (X). Interestingly, when the nOe difference spectra were collected on (9.21) in deuterated toluene no nOe could be observed for 19-Me to H-6β. Models suggest that the alternative, β-ring boat conformation (Y) is accessible for the cis-fused isomer and this could be more strongly favoured in some solvents.

Table 9.1 ^{1}H NMR and nOeds results for (9.21) in $CDCl_3$

Assignment	δ(PPM)	No. of hydrogen	Multiplicity	NOeS TO
1β	1.28	1	dd	1α, 19β, 3β
3β	2.4	1	ddd	5, 3α, 7β
3α	1.96	1	ddd	6α, 5
7α	1.85	1	multiplet	7β, 14α, 9α, 8β, 6α
14α	1.75	1	multiplet	9α, 16α, 8, 12, 7α
18β	1.09	3	S	12, 15β, 16β?, 19β?
19β	0.8	3	S	5, 6β, 1β, 8? Ph(2',6'), 18β
9α	2.6	1	multiplet	1α, 14α, 7α Ph(2',6')



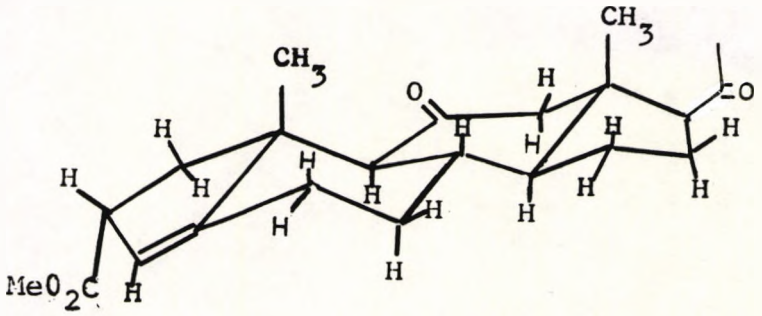
(X)



(Y)

In summary, the nOeds evidence clearly indicates that compound (9.21) has 5-H stereochemistry (9.21b) with a cis fusion of the A and B rings. Since none of the processes involved in the conversion of the diketo ester (9.11) into compound (9.21b) could possibly have resulted in inversion of the stereochemistry at C-5, it follows that all the series (9.11) to (9.21) has this same stereochemistry i.e. (9.11b) to (9.21b) throughout Schemes 9.3 to 9.7.

The formation of the cis-fused diketo ester (9.11b) from the 3-ene (9.2) may have two possible explanations. Firstly, it is known that when hydrogenation is carried out in an acidic medium, as in the present case, it is possible for trans-hydrogenation to occur by the addition of one hydrogen atom from the catalyst surface and capture of a proton from the opposite face of the olefin. However, in the present case an alternative explanation seems reasonable: due to the ring contraction to an A-nor steroid and the presence of the methoxycarbonyl group at C-2, the steric effects of the 19-Me are counterbalanced by those of the ester group. Models suggest that the conformation (Z) of the substrate (9.2) may make approach to the catalyst surface more favourable from the β-face.

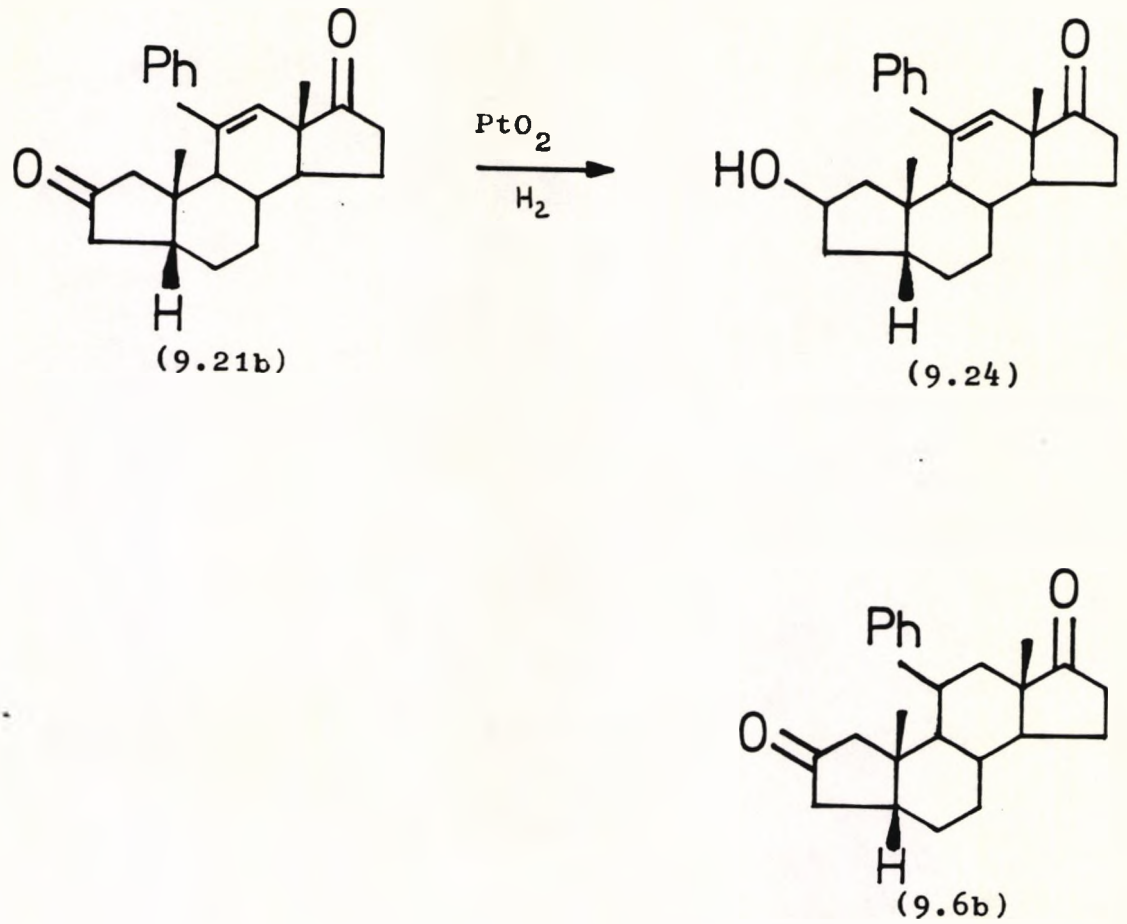


(Z)

Since the original intention of this project had been to synthesise a 5 -A-nor steroid combining structural elements from anordrin and RU-38.486, the unexpected acquisition of the 5 series required re-evaluation. Inspection of models suggested that this inversion of configuration at C-5 would not dramatically alter the overall shape of the final steroid or its ability to bind to the progesterone receptor. It was encouraging to recall that the inversion of stereochemistry at the C/D ring junction in ZK-98.299 did not lead to loss of antiprogestational activity. It was therefore decided to continue the previously planned synthesis, but with the 5 series.

Further quantities of the diphenylmethylene steroid (9.19b) were prepared and the ozonolysis reaction repeated to provide larger amounts of 2-keto steroid (9.21b). During one run, olefin (9.19b) was ozonised and the reaction worked up using $NaBH_4$ instead of zinc dust. As a consequence[14], further reduction at the C-2 and C-17 sites also took place and the 2,17-dihydroxy-A-nor steroid (9.23b) was obtained.

Hydrogenation of compound (9.21a) using PtO_2[15] as a catalyst in ethyl acetate gave compound (9.24) instead of the expected compound (9.6b) (Scheme 9.8).



Scheme 9.8

The structural assignment of compound (9.24) was made on the basis of its ^1H NMR and mass spectra; the mass spectrum showed a molecular ion at 350 as expected; the ^1H NMR spectrum showed in addition to the phenyl group multiplet signal at δ 7.2, two other low field signals at δ 3.82 and δ 5.97 respectively. The signal at δ 3.82 was a multiplet

(dddd) due to the proton of the hydroxyl group at C-2. The signal would have been a triplet, had the hydroxyl group been in position C-17, as in the case of compound (9.13b). The signal at δ5.97 was a doublet for the olefinic proton at C-12, and was slightly upfield from the corresponding signal at δ6.06 in the precursor (9.21a).

The formation of alcohol (9.24) evidently reflects the greater ease of reduction of the unhindered A-ring carbonyl group compared to the C-ring olefin.

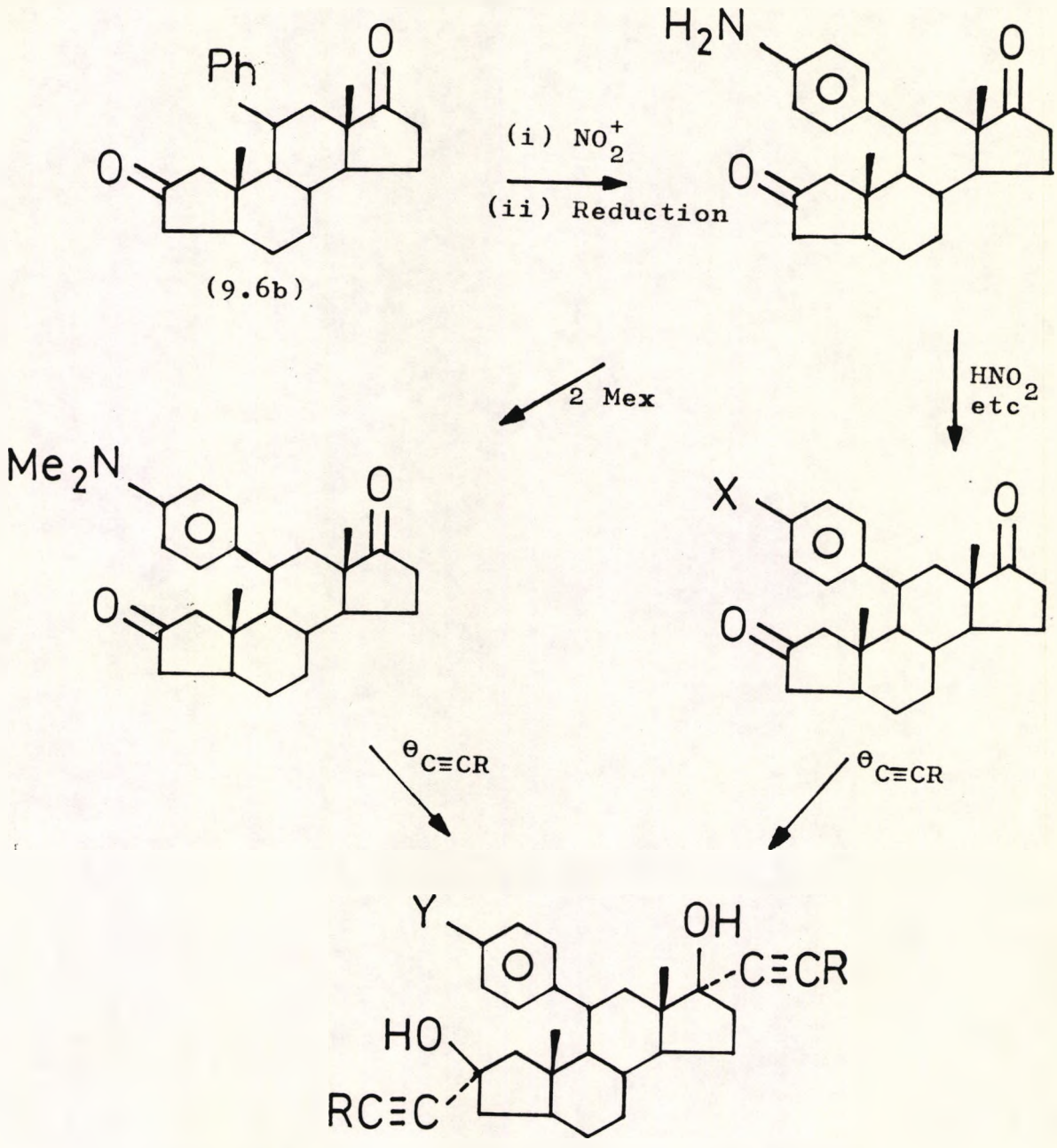
Work on this project was terminated at this point, owing to lack of further time.

Conclusion and suggestions for further work

The work described in this chapter has led to a successful route being established for the synthesis of 11-phenyl-A-nor-5β-androstene-2,17-diones having a double bond in the C-ring. The double bond needs to be reduced to furnish the 11β-phenyl-diketone, which is required for the synthesis of novel compounds for testing as anti-hormonal agents.

Reduction of the C-ring olefin may be achievable by using a more selective hydrogenation catalyst [e.g. the homogeneous Wilkinson's catalyst]. Alternatively, the two carbonyl groups could be protected as ketals, or reduced to the alcohols and then re-oxidised after the double bond has been removed by a reducing agent (e.g. H_2/cat at high pressure, or diborane or diimide).

Once the 11β-phenyl diketone (9.6b) is available, it is anticipated that it can be converted into the final products as shown in Scheme 9.9.


Ph
O
O
(9.6b)
(i) NO_2^+
(ii) Reduction
H_2N
O
O
2 Mex
HNO_2 etc
Me_2N
O
O
X
O
O
$\ominus_{C\equiv CR}$
$\ominus_{C\equiv CR}$
Y
OH
$C\equiv CR$
HO
$RC\equiv C$

Scheme 9.9

9.3 Experimental

2α-methoxycarbonyl-A-norpregn-3-ene-11, 20-dione (9.2) (Large Scale Preparation)

Thallium (III) nitrate (32g, 42.3 mmol) was dissolved in a mixture of TMOF (265 ml) and MeOH (204 ml) and the resulting solution left under stirring at 0°C for 30 min. A cold (0°C) solution of 11-ketoprogesterone (9.1) (20 g, 49 mmol) in a mixture of TMOF (401.6 ml) and MeOH (306.1 ml) was quickly added. After ten minutes a white precipitate of thallium (I) nitrate was formed. After 40 min. the reaction solution was neutralised with a saturated aqueous sodium carbonate, then filtered and extracted with diethyl ether. The crude reaction mixture was chromatographed on a silical gel column. On elution with a mixture of hexane-ether (20:80 v/v) pure 2α-methoxycarbonyl-A-norpregn-3-ene-11, 20-dione (9.2) was afforded (9.6 g, 48%); M.P. 100-102°C : ^{1}H NMR($CDCl_3$)δ: 0.56 (3H, S), 1.06 (3H, S), 3.52 (3H, S), 5.08 (1H, S) ; ν_{max}($CHCl_3$)cm^{-1} 1700-1725, 1520 : EI-MS ; m/z 358.2136 (M^+, 11.95% ; $C_{21}H_{26}O_5$ requires 358.1818), 343 (100%).

2-(1'-hydroxy-1',1'-diphenylmethyl)-17-(1"-hydroxy-1"-phenyl-ethyl)-11α-hydroxy-11β-phenyl-A-norandrost-3-ene) (9.3) ; 2-(1'-hydroxy-1', 1'-diphenyl-methyl)-17-(1"-hydroxy-1"-phenyl-ethyl)-A-norandrost-3-en-11-one, (9.7)

A solution of 2α-methoxycarbonyl-A-norpregn-3-ene-11, 20-dione (9.2) (500mg,1.40 mmol) in dry ether (5 ml) was added to 2M phenyllithium (50 ml, 100 mmol) in dry hexane/ether (90:10 V:V) at -78°C under N_2. The resulting mixture was allowed to warm up to room temperature and then stirred for one hour. The reaction mixture was cooled in an ice bath and a cold concentrated ammonium chloride solution was added dropwise with stirring. The ether layer was separated and the aqueous layer extracted with ether. The combined ether extracts were dried ($MgSO_4$) and evaporated to yield a brown oil. The crude brown oil was partially purified by preparative t.l.c. using hexane as solvent to give a mixture of compounds (9.3) and (9.7) (818.9 mg). The mixture was further purified by preparative HPLC (Column: 30 x 2 cm I.D. Packing : 5μm Apex silica Hypersil. Mobile phase: CH_2Cl_2-iPrOH (99.8 : 0.2 V:V) λ= 244 nm. Flow-rate: 15 ml/min. to give pure compounds (245.1 mg, 28.5%) and (252.6 mg, 32.3%). Compound (9.3) : M.P. >>230°C; ^{1}H NMR ($CDCl_3$)δ: 1.2 (3H,S), 1.27 (3H,S), 1.53 (3H,S), 3.6 (1H,m) 4.73 (1H,S), 7.16 (20H,m); ν_{max} ($CHCl_3$) cm^{-1}: 3520, 3450, 1600;

EI-MS : m/z 602.3809 (M^+-2H_2O, 15.86% ; $C_{45}H_{46}O$ requires 602.3615); CI-MS(NH_3) : m/z 621.3733(M+H-H_2O, 95%; $C_{45}H_{49}O_2$ requires 621.3804). Compound (9.7) : M.P. 135-140°C ; ^1H NMR ($CDCl_3$) : 0.83 (3H, S), 1.16 (3H, S), 1.6 (3H, S), 4.06 (1H, m), 4.93 (1H, S), 7.26 (15H, m) ; ν_{max} ($CHCl_3$) cm^{-1} : 1700, 1600 ; EI-MS : m/z 542.3184 (M^+-H_2O, 14.62% ; $C_{39}H_{42}O_2$ requires 542.3246) ; CI-MS(NH_3) : m/z 543.3263 (M+H-H_2O, 95% ; $C_{39}H_{43}O_2$ requires 543.3325).

2-(1',1'-diphenylmethylene)-17-(1"-phenylethenyl)-A-nor-androst-3-en-11-one, (9.8b); 2-(1',1'-diphenylmethylene)-17-(1"-phenylethenyl)-11-phenyl-A-norandrosta-3,11-diene, (9.10).

A partially purified product containing compounds (9.3) and (9.7) (1.56 g, 2.45 mmol) was dissolved in dry pyridine (78 ml), cooled to -5°C and thionyl chloride (15.6 ml) was added. The mixture was allowed to stand at -5°C for 20 hrs, and then poured into ice-water. The oily mixture was extracted with ether (500 ml), the extract washed with saturated saline solution, dried and evaporated under pressure. The residue was purified by preparative t.l.c. to give compounds (9.8b) (146 mg, 30%) and (9.10) (116 mg, 20%). Compound (9.8b): M.P. 225-230°C ; ^1H NMR ($CDCl_3$)δ: 0.58 (3H,S), 1.12 (3H, S), 5.08 (1H, S), 5.21 (1H, S), 5.83 (1H,d), 7.19 (15H, m) ; ν_{max} ($CHCl_3$) cm^{-1} : 1690 ; EI-MS : m/z

524.3194. (M^+, 68.51% ; $C_{39}H_{40}O$ requires 524.3137). Compound (9.10): M.P. 231-233°C ; ^1H NMR ($CDCl_3$)δ: 0.74 (3H,1H), 0.86 (3H, 1H), 5.22 (2H, m) 5.64 (1H, d), 5.70 (1H, d), 7.17 (20 H, m) : $\nu_{max}(CHCl_3)cm^{-1}$: 1600 ; EI-MS : m/z 584.3471 (M^+, 2.1% : $C_{45}H_{44}$ requires 584.3507).

2α-methoxycarbonyl-A-nor-5β-pregnan-11, 20-dione (9.11b).

A solution of compound (9.2) (500 mg, 0.0014 mmol) in ethanol (30 ml) and glacial acetic acid (15 ml) was hydrogenated at atmospheric pressure, using Pd/C (130 mg) as a catalyst, for 2 days with stirring. On completion of the reaction Pd/C was filtered off and the filtrate was evaporated to dryness to give compound (9.11b) (450 mg, 89.5%), M.P. 125-127°C ; ^1H NMR($CDCl_3$)δ; 0.52 (3H, S), 1.20 (3H, S), 2.08 (3H, S), 3.64 (3H, S) ;$\nu_{max}(CHCl_3)$ cm^{-1} : 1700-1725 ; EI-MS : m/z 360.2377 (M^+, 159.6% ; $C_{22}H_{32}O_4$ requires 360.2347), 345 (58.5%).

2α-methoxycarbonyl-A-nor-5β-androstan-11-one 17β-acetate (9.12b).

A solution of peroxytrifluoroacetic acid was prepared by adding 60% hydrogen peroxide (1.8 ml, 1.08 mmol) dropwise to a well stirred cold solution of trifluoroacetic anhydride (8 ml, 104 mmol) in dichloromethane (11.2 ml). After 20

minutes addition time, the mixture was then allowed to warm up to room temperature and then cooled in an ice-bath for 3 mins.

This solution was added over a 15-minute period to a well-stirred mixture of disodium hydrogen phosphate (13 g, 91.6 mmol) and compound (9.11b) (500 mg, 1.4 mmol) in methylene chloride (50 ml). After addition was completed the mixture was heated under reflux at 41°C for 4 hrs. The mixed salts were then collected on a sintered funnel and washed with methylene chloride. The combined filtrates were washed with 150 ml of 10% sodium carbonate and dried over magnesium sulfate. The solvent was removed under reduced pressure to afford compound (9.12b) (323.5 mg : 62%) : M.P. oil : ^{1}H NMR($CDCl_3$)δ: 0.68 (3H, S), 1.12 (3H, S), 1.92 (3H, S), 3.48 (3H, S), 4.64 (1H, t) ;ν_{max}($CHCl_3$) cm^{-1} : 1700-1730 ;EI-MS : m/z 376.2308 (M^+, 3.95% ; $C_{22}H_{32}O_5$ requires 376.2296).

<u>2-(1'-hydroxy-1',1'-diphenylmethyl)-11 κ-hydroxy-11β-phenyl-A-nor-5β-androstan-17β-ol (9.13b) ; 2-(1'-hydyroxy-1',1'-diphenylmethyl)-A-nor-5β-androstan-17β-ol-11-one, (9.17b)</u>

A cold solution of compound (9.12b) (1 g, 2.65 mmol) in dry ether (10 ml) was syringed into a stirred solution of

2M phenyllithium (100 ml, 200 mmol) in dry hexane-ether mixture (90:10) under N_2 at -78°C. After the addition was complete, the reaction mixture was allowed to warm up to room temperature and then stirred for 1 hr. The reaction mixture was cooled in an ice-bath and a cold saturated ammonium chloride solution was added dropwise with stirring. The ether layer was separated and the aqueous layer extracted with ether. The combined ether extracts were dried ($MgSO_4$) and evporated to yield a brown oil. The crude product was chromatographed on a silica gel column. Elution with 2:8 hexane-Et_2O gave compounds (9.13b) (380 mg, 26.66%) and (9.17b) (370 mg, 30.37%) respectively. These two compounds were further purified by preparative HPLC on a normal phase system using (0.2 : 99.8 v/v) iPrOH-CH_2Cl_2 as mobile phase. Compound (9.13b) : white solid, M.P. 243-245°C; ^1H NMR($CDCl_3$)δ: 1.0 (3H, S), 1.24 (3H, S), 2.72 (1H, m), 3.36 (1H, t), 6.8 (15H, m) ;ν_{max}($CHCl_3$) cm^{-1} : 3396 ; EI-MS : m/z 518.3241 (M^+-H_2O, 22.63% ; $C_{37}H_{42}O_2$ requires 518.3246); CI-MS (NH_3); m/z 519.3263 (M^++H-H_2O, 95% ; $C_{37}H_{43}O_2$ requires 519.3325). Compound (9.17b) : white solid, M.P. 122-128°C; ^1H NMR($CDCl_3$)δ: 0.34 (3H, S), 1.2 (3H, S), 3.2 (1H, m), 3.8 (1H, t), 7.24 (10H, m) ;ν_{max}($CHCl_3$)cm^{-1} : 1700, 1600 ; EI-MS : m/z 440.2670 (M^+-H_2O, 11.65% $C_{31}H_{36}O_3$ requires 440.2767); CI-MS : m/z 476.3165 (M^++NH_4, 10%; $C_{31}H_{42}NO_3$ requires 476.3225), 458 (M^+, 70%), 441 (M^++H-H_2O, 100%).

2-(1'-hydroxy-1',1'-diphenylmethyl)-11α-hydroxy-11β-phenyl-A-nor-5β-androstan-17-one (9.18b)

To a solution of compound (9.13b) (11 g, 21 mmol) in dichloromethane (1.4 l) at room temperature was added pyridinium dichromate (45.29 g, 130 mmol). The reaction mixture was stirred by means of a mechanical stirrer for 4 hrs. Dilution of the reaction mixture with ether, removal of the easily filterable precipitate, and concentration of the filtrate under reduced pressure gave the crude compound (9.18b) (10 g, 91.25%). Some of the crude product (1 g) was purified first by preparative t.l.c. using hexane-ether (20:80 v/v) as solvent and then by preparative HPLC on a normal phase column (30 x 2 cm I.D,) packed with 5μm Hypersil) using hexane-CH_2Cl_2 (10:90 v/v) as mobile phase: M.P. 245-250°C; ^{1}H NMR($CDCl_3$)δ: 1.20 (3H, S), 1.36 (3H, S), 2.88 (1H, m), 7.24 (15H, m); ν_{max}($CHCl_3$)cm^{-1} : 1730, 1600; EI-MS, m/z 534.3469 (M^+, 0.5%) $C_{37}H_{42}O_3$ requires 534:3195, 516 (2%).

Preparation of $CuSO_4/SiO_2$ solid phase dehydrating reagent

The reagent was prepared by mixing chromatographic silica gel (230-400 mesh) (5 g,0.083 mole) with a solution of copper (II) sulfate pentahydrate (3.32 g, 0.013 mole) in water (20

ml). The water was evaporated under reduced pressure (0.5mm/Hg) at 128°C for 4 hrs. At this stage there was no further water condensation in a trap cooled with liquid nitrogen.

2-(1',1'-diphenylmethylene)-11-phenyl-A-nor-5β-androst-11-en-17-one, (9.19b) 2-(1',1'-diphenylmethylene)-11-phenyl-A-nor-5β-androst-9-en-17-one(9.20b)

To a solution of compound (9.18b) (50 mg, 0.995 mmol) in carbon tetrachloride (5 ml) was added anhydrous copper (II) sulfate on silica gel (509 mg, 3.32 mmol). The resulting reaction mixture was refluxed gently (90-95°C) for 1.1/4 hr. The reaction was monitored by analytical t.l.c. After the completion of the reaction, the crude products were isolated from the solid reagent by filtration. The filtrate was evaporated to dryness under reduced pressure and the residue was purified by preparative t.l.c. using CH_2Cl_2 as solvent, to afford compounds (9.19b) (30 mg, 64%) and (9.20) (5 mg, 10.66% respectively.

Compound (9.19b): white solid, M.P. 114-119°C, ^1H NMR($CDCl_3$)δ: 0.93 (3H, S), 1.03 (3H, S), 6.03 (1H, S), 7.13 (15H, m) ; $\nu_{max}(CHCl_3)cm^{-1}$: 1740 ; EI-MS : m/z 498.2935 (M^+, 41.57% ; $C_{37}H_{38}O$ requires 498.2978), 483 (8.94%).

Compound (9.20b): white solid, M.P. 125-127°C; ^1H NMR ($CDCl_3$)δ: 1.03 (3H, S), 1.2 (3H, S), 7.10 (15H, m) ; $\nu_{max}(CHCl_3)cm^{-1}$: 1734. EI-MS : m/z 498.2926 (M^+, 25.04% ; $C_{37}H_{38}O$ requires 498.2978), 483 (11.73%).

<u>11-phenyl-A-nor-5β-androst-11-ene-2, 17-dione (9.21b);</u>

<u>11-phenyl-A-nor-5β-androst-9-ene-2, 17-dione (9.22b).</u>

A solution of compound (9.19b) (300 mg, 0.62 mmol) in dry dichloromethane (13.5 ml) and dry pyridine 0.06 ml) was ozonized for 45 mins by passing through a stream of ozone-oxygen with stirring, at -78°C. By this time the colour of the reaction mixture had changed from deep orange to yellow. To this pale yellow reaction solution was added zinc dust (350 mg, 5.35 mmol) and glacial acetic acid (1.52 ml) and stirring was continued for 15 mins at 0°C, and, finally for 5 mins at 35°C. The bright yellow solution was removed from the zinc by filtration and was washed with two 5 ml portions of water. It was then cooled by the addition of ice and washed with 20 ml and 10 ml of cold 10% sodium carbonate, 10 ml of cold 10% sodium hydroxide and four 50-ml portions of cold water, all aqueous washes being back-washed with 50 ml of methylene chloride. The fine white precipitates which formed at the interfaces during the extraction were separated with the aqueous phases and discarded. The combined

methylene chloride solutions were dried over sodium sulfate, filtered and concentrated to dryness at 40°C under a reduced pressure to produce a crude oily yellow compound. This was purified by preparative t.l.c. using ether-hexane (80:20 v/v) as solvent to give compound (9.21b) (41.5 mg, 20%) : M.p. 190-192; ^{1}H NMR($CDCl_3$)δ: 0.9 (3H, S), 1.00 (3H, S), 6.06 (1H, d), 7.13 (5H, M); ν_{max}($CHCl_3$)cm^{-1} : 1740 ; EI-MS : m/z 348.2058 (M^+, 56% ; $C_{24}H_{28}O_2$ requires 348.2129).

In the same experimental conditions, compound (9.20) gave compound (9.22b) (26%).

Compound (9.22b): M.P. 145-150°C ; ^{1}H NMR($CDCl_3$)δ: 1.00 (3H, S), 1.3 (3H, S), 7.18 (5H, m) ;ν_{max}($CHCl_3$)cm^{-1} : 1735; EI-MS: m/z 348.2047 (M^+, 100% ; $C_{24}H_{28}O2$ requires 348.2129), 333 (23%).

<u>11-phenyl-A-nor-5β-androst-11-ene-2,17-diol (9.23b)</u>

A solution of compound (9.19b) (200 mg, 0.42 mmol) in dry dichloromethane (9 ml) and anhydrous pyridine (0.04 ml) was ozonised for 45 mins by passing through a stream of ozone-oxygen, with stirring, at -78°C. At this reaction time the colour of the reaction mixture changed from deep orange to yellow. The ozonide formed was reduced by adding a solution

of sodium borohydride (267.6 mg, 7.43 mmol) in cold 50% aqueous ethanol (2 ml) slowly to the stirred ozonide mixture. The temperature of the reaction mixture was maintained at 25°C by occasional ice-bath cooling. The reaction mixture was then warmed in a water bath for 2.5 hrs with continued stirring. After standing overnight at room temperature, the mixture was acidified with 10% sulfuric acid, the dichloromethane layer separated, and the aqueous layer further extracted with dichloromethane. The combined extracts were dried over anhydrous magnesium sulfate, filtered, and the dichloromethane removed under reduced pressure. The oily residue was subjected to preparative t.l.c., using a mixture of ether/hexane (80:20 v/v) as mobile phase, to yield pure compound (9.23b) (37.5 mg, 26.6%) : M.P. 168-70°C ; ^{1}H NMR($CDCl_3$)δ: 0.86 (3H, S), 1.00 (3H, S) ; 3.62 (1H, t) ; 3.94 (1H, m), 6.0 (1H, S) 7.23 (5H, m) ; ν_{max}($CHCl_3$) cm^{-1} 3265, 3198 ; EI-MS: m/z 352.2371 (M^+, 61.4%; $C_{24}H_{32}O_2$ requires 352.2449) 334 (M^+-H_2O, 14%).

2α-hydroxy-11-phenyl-A-nor-5β-androst-11-en-17-one (9.24)

A solution of compound (9.21b) (21 mg, 0.603 mmol) in ethyl acetate (5 ml) was hydrogenated using platinium oxide catalyst (1.16 mg), at atmosphere pressure, for 31 hr. Filtration and evaporation gave a crude product which was

purified by preparative HPLC to afford compound (9.24) (18 mg, 85%) ; M.P. >>290^{o}C, ^{1}H NMR($CDCl_3$) δ: 1.00 (3H, S), 1.26 (3H, S), 3.82 (1H, m), 5.80 (1H, d) 7.2 (5H, m) : ν_{max}($CHCl_3$)cm^{-1} 3360, 1730, EI-MS : m/z 350.2231 (M^{+}, 29% ; $C_{24}H_{30}O_2$ 350.2289).

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