CITY UNIVERSITY

DEPARTMENT OF CHEMISTRY

A SYNTHETIC APPROACH TO ANTIPROGESTATIONAL STEROIDS

BY FARAH AYERMAN

SEPTEMBER 1996

A Thesis submitted in partial fulfilment of the requirements for the

Degree of Doctor of Philosophy of City University

ACKNOWLEDGEMENTS

I would like to express my profound thanks to my supervisor, Professor S.A. Matlin for giving me the opportunity to carry out this research.

I would like to thank SERC Mass Spectrometry Service Centre, University of Swansea for running high resolution mass spectra, I.K. Katyal for running mass spectra, and Mr. J.J. Hastings for running NMR spectra.

I would like to thank Dr. Charles Thomas for his assistance at the outset of this project which is based upon his original work. I am grateful to Dr. Hanak Singh Rayat for his support.

Also, I would like to thank my parents and husband for their encouragement and support, without which I could not have completed my thesis.

DECLARATION

I grant powers of discretion to the University Librarian to allow this thesis to be copied in whole or in part without further reference to me. This permission covers only single copies made for study purposes, subject to normal conditions of acknowledgement.

ABSTRACT

The nature and uses of some steroidal hormones and anti-hormones are reviewed, with an emphasis on the anti-progestins which have anti-implantation activity. A new type of steroid structure has been designed which combines two types of structural elements, namely an A-nor skeleton and an 11-aryl substituent, which are separately known to display anti-implantation properties.

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-5ß-androst-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 **Page**

CHAPTER 1

Introduction - Hormones and Anti-hormones

1.1 The Endocrine Hormones

The endocrine system consists of a number of ductless glands that produce highly active chemical regulators called hormones. These chemical messengers are secreted in the bloodstream and carried to certain target organs where they act upon specific receptor cells[1].

Hormones regulate metabolism and assist in the perpetuation of the species by influencing a wide variety of biological systems to perform several functions;

a) To maintain a constant internal environment in the body fluids i.e. homeostasis

b) To regulate the general growth and development of the body

c) To aid sexual maturation, to maintain sexual rhythms, and to facilitate the reproductive process.

d) To regulate energy production and to stabilize the metabolic rate.

e) To help the body adjust to stressful or emergency situations.

There are three main functional types of hormones. The first of these are the releasing factors from the hypothalamus, which stimulate the secretion of some anterior pituitary hormones. Releasing factors act only upon the anterior pituitary. The second functional type of hormone is known as a tropic hormone. These are produced by the pituitary gland and stimulate the growth and activity of specific

endocrine glands[2]. They include thyrotropin TSH, a hormone that causes the thyroid gland to produce thyroxine, adrencorticotropin ACTH, which stimulates the adrenal cortex to produce cortisol, and the gonadotropins, FSH and LH, which stimulate the ovaries to produce estrogen and the testes to produce testosterone, respectively. The other main functional type are the effector or nontropic hormones which are mainly produced by the endocrine glands and exert their metabolic effects upon nonendocrine tissues. Gonadal hormones affect a wide variety of tissues during puberty to produce the typical secondary sex characteristics for each sex; thyroid hormones affect the metabolic rates of all tissues, growth hormone stimulates skeletal growth and affects many metabolic processes.

1.2 Chemical Types

There are five general chemical types of hormones; glycoproteins, proteins or long-chain polypeptides, short-chain polypeptides, amino acid derivatives and steroids. The anterior pitutary hormones TSH, FSH and LH are either glycoproteins or long-chain polypeptides, and the posterior pituitary hormones ADH and all of the hypothalamus releasing factors are short-chain peptides. Hormones from the adrenal cortex and the gonads are steroids, whilst epinephrine and norepinephrine from the adrenal medulla are amines derived from the amino acid tyrosine[3].

The production and secretion of hormones by an endocrine gland may be initiated by one or more of several different types of signals: for example, by the stimulation of the cerebral cortex by thoughts, emotions, stress and by chemical transmitters active at nerve

junctions. It may also be caused by a variation in blood osmolality or volume e.g. secretion of antidiuretic hormone, ADH, by the posterior pituitary when the plasma osmolality increases and secretion of renin by the kidney in response to low blood volume. It can also be initiated by the release of hormones in the gastrointestinal tract in the presence of various foods.

1.3 Hypothalamic Regulation

The control and regulation of the hormones that exert such profound effects on so many tissue cells are very complicated. Figure (1.1) is a schematic representation of reproducive hormonal control, where the primary control is believed to rest in the hypothalamus, which is a small gland and nerve centre situated above the pituitary.

The isolation, characterisation and synthesis of hormone-releasing factors have firmly established their role in the physiology of endocrine secretion. Some of these peptides (TRH, thyroid-releasing hormone and GnRH, gonadotropin releasing hormone) release more than one hormone and others inhibit release (PIF, peptide inhibit factor, somatostatin). In addition, these peptide hormones influence behaviour and affect neuronal excitability within the hypothalamus[4].

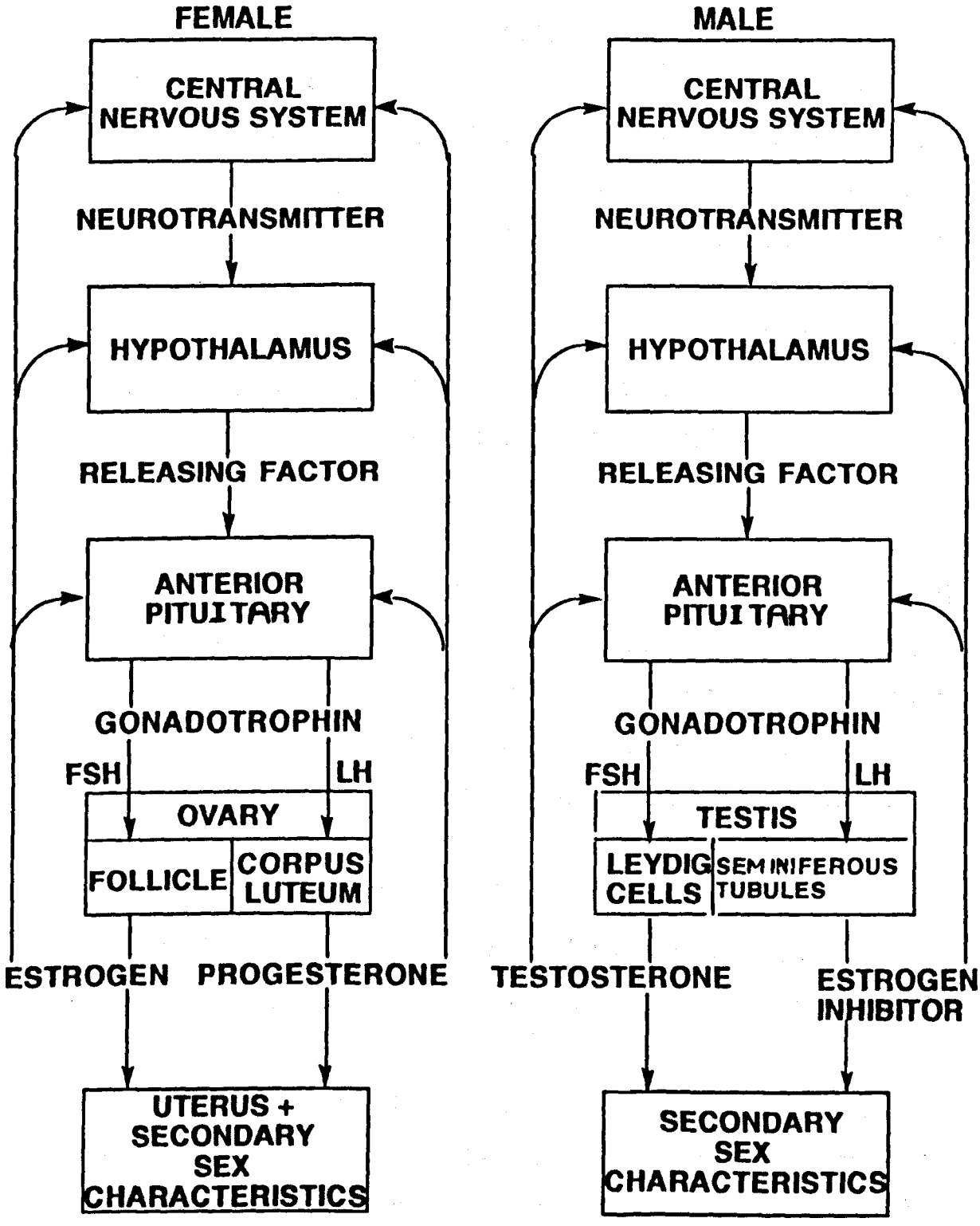

FEMALE
CENTRAL NERVOUS SYSTEM
NEUROTRANSMITTER
HYPOTHALAMUS
RELEASING FACTOR
ANTERIOR PITUITARY
GONADOTROPHIN
FSH
LH
OVARY
FOLLICLE
CORPUS LUTEUM
ESTROGEN
PROGESTERONE
UTERUS + SECONDARY SEX CHARACTERISTICS
MALE
CENTRAL NERVOUS SYSTEM
NEUROTRANSMITTER
HYPOTHALAMUS
RELEASING FACTOR
ANTERIOR PITUITARY
GONADOTROPHIN
FSH
LH
TESTIS
LEYDIG CELLS
SEMINIFEROUS TUBULES
TESTOSTERONE
ESTROGEN INHIBITOR
SECONDARY SEX CHARACTERISTICS

Fig.1.1

Thyroid-stimulating hormone (TSH) is the primary regulator of the thyroid gland, and it is secreted in response to TRH. TRH stimulates thyrotrophs and lactotrophs to release TSH and prolactin respectively, through an adenylate cyclase-dependent mechanism. Thyroid hormone inhibits this effect at the thyrotropin-producing cell, but only partially decreases the effect on prolactin secretion. Somatostatin also blocks TRH-induced secretion of TSH. In some types of pituitary abnormalities, such as in acromegaly or in certain tumours, TRH also stimulates release of growth hormone.

Corticotropin releasing factor (CRF) is a potent hypothalamic regulator of ACTH formation and release. CRF has been identified in multiple sites in the central nervous system and also in peripheral tissues and some tumours. It is used clinically to test for abnormalities of the hypothalamic-pituitary-adrenal axis. Patients with Cushings disease exhibit a normal or exaggerated release of ACTH in response to exogenous CRF, in contrast to those with ectopic ACTH secretion who fail to respond. CRF is also used to evaluate ACTH release in depressed people and in patients with anorexia nervosa[5].

GnRH is the gonadotropin releasing hormone, also known as luteinising hormone-releasing hormone LHRH. It is a decapeptide that is responsible for regulating the secretion of the pituitary gonadotropins FSH and LH.

GnRH is used to treat hypothalamic amenorrhea, isolated gonadotropin deficiency and carcinoma of the prostate. Intermittent

administration of the hormone causes sustained secretion of both FSH and LH and administration of GnRH to men with primary GnRH deficiency has been proved to result in growth of the testes and a gradual increase in sperm count.

GnRH antagonists act as competitive inhibitors at GnRH receptors. Through blockade of the actions of endogenous GnRH, they also suppress secretion of gonadal steroids. In contrast to the effect of GnRH agonists, the hypogonadal state is produced without initial pituitary stimulation.

The growth hormone-releasing hormone (GHRH) along with somatostatin, directly controls the synthesis and release of growth hormone from pituitary somatotrophs. Although it has only fairly recently been recognised, GHRH is a very useful agent in the diagnosis and treatment of growth hormone deficiency.

Because hypothalamic lesions decrease GH production and retard growth, research on control of GH secretion by GRF has led to isolation and purification of somatostatin, a tetradecapeptide that inhibits GH release. This peptide has an extensive distribution within the nervous system and in many peripheral tissues, including the gastrointestinal tract and endocrine and exocrine glands. Like many other regulatory factors, somatostatin is synthesised in a longer form that is also active.

Somatostatin is a potent suppresser of GH release and it blocks the GH response to exercise, insulin-induced hypoglycohemia and levodopa

injection. Neurotransmitters and neuropeptides that modify somatostatin release also affect GH concentration.

Somatostatin is also found in many cells that have nothing to do with GH, and it inhibits secretion of several other pituitary and peripheral hormones, such as TSH, prolactin, ACTH, glucagon, insulin, and gastrin. Inhibition of hormone secretion and also secretion of peptides from cancer cells may depend on a common mechanism such as interaction with a guanine nucleotide regulatory protein that inhibits adenylate cyclase. Also somatostatin is believed to be involved in virtually all sensory systems.

Growth hormone (GH), is the most abundant hormone of the anterior pituitary, and it has important effects on the metabolism. By decreasing glucose utilisation, antagonising insulin, increasing amino acid transport and protein synthesis, GH promotes protein synthesis at the expense of carbohydrate and fat.

A deficiency of GH in children causes dwarfism and an excess causes giantism. A hypersecretion of GH occurring in adulthood after closure of the long bones results in large, gross-featured persons, a condition known as acromegaly.

1.4 Female sex hormones

The reproductive system in females is more complex than that in males. This is due to the cyclical events that take place during the menstrual cycle and the even greater changes that occur during a pregnancy. Two different chemical types of steroid hormones are

produced and secreted by the ovary in non-pregnant females. During pregnancy, the same hormones are produced by the ovary, but in different proportions.

The placenta also makes the hormones that are necessary for the maintenance of pregnancy[6].

One group of female sex hormones are the estrogens which originate in the ovarian follicles. The estrogens participate in the menstrual cycle and are essential for the development and maintenance of the reproductive organs and secondary sex characteristics. The second group consists of progesterone and its metabolites, which are formed in the corpus luteum, the body that develops from the ruptured ovarian follicle.

The ovarian cycle in the mature female reflects changes in gonadotropins, in steroid synthesis, and in the histology of the ovaries and endometrium. The underlying mechanism that coordinates these events is the acquisition of specific hormone receptors that enable cells to respond to the circulating hormones. The hormonal control of menstruation is illustrated in Fig (1.2).

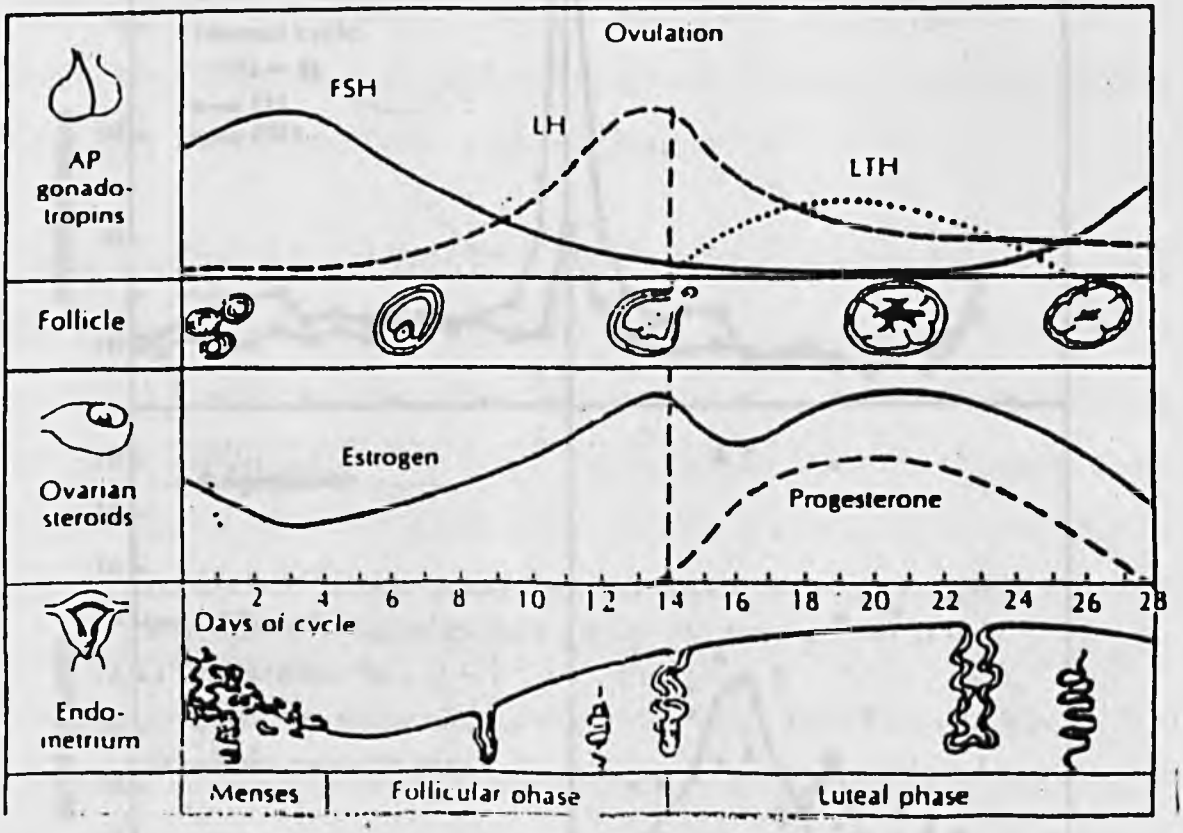


Fig. 1.2

The actual measurements of serum hormone concentrations are shown in Fig(1.3). These concentrations of progesterone are plotted against mean concentrations of FSH and LH determined in normal women and centred according to the day of LH peak i.e. day 0.

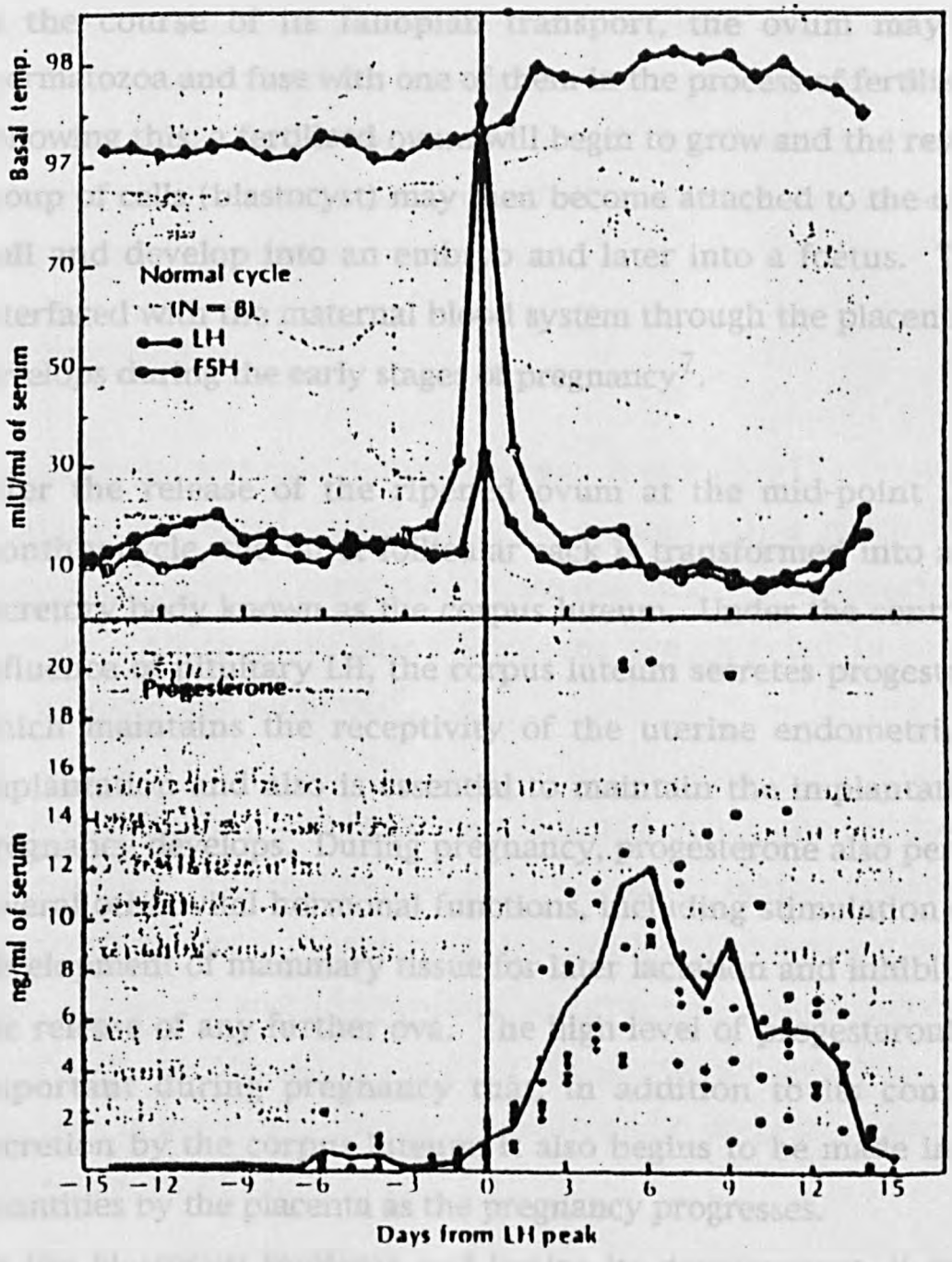


Fig. 1.3

In the female, estrogens such as estradiol stimulate the thickening and vascularization of the lining of the uterus (endometrium), preparing it for implantation. After ovulation at the mid-point of the monthly cycle, the ovum 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. Following this, a fertilized ovum will begin to grow and the resulting group of cells (blastocyst) may then become attached to the uterine wall and develop into an embryo and later into a foetus. This is interfaced with the maternal blood system through the placenta that develops during the early stages of pregnancy[7].

After 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 known as the corpus luteum. Under the controlling influence of pituitary LH, the corpus luteum secretes progesterone, 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 ova. The high level of progesterone is so important 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.

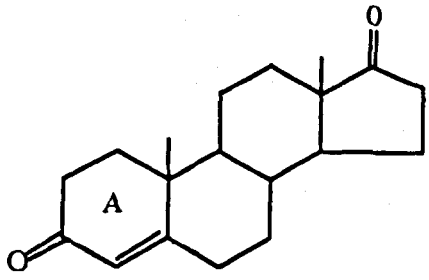
As the blastocyst implants and begins its development, it emits a signalling substance known as human chorionic gonadotropin (HCG).

This is a glycoprotein hormone that resembles pituitary LH in that they have identical∝chains but different ß chains; they have similar hormonal action in stimulating the corpus luteum to produce progesterone, which helps to maintain the pregnancy.
The formation of HCG by the chorion of the developing placenta begins shortly after implantation of the fertilized egg in the uterine wall. Its concentration in plasma and urine rises steadily from the first week after conception for the next 10 to 12 weeks. It was the recognition of the early presence of HCG in urine that became the basis for some pregnancy testing kits.

1.5 Estrogens

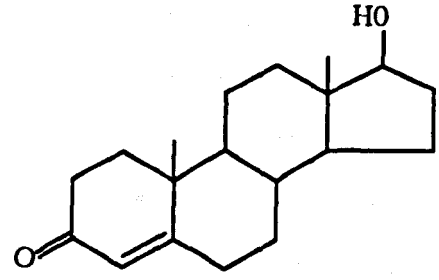
The principal and most potent estrogen is estradiol-17ß (4),which is partially converted in the body to estrone (3), a much weaker hormone. Estriol, a metabolite of estrone, also has some estrogenic activity. All three of these estrogens are excreted in urine as glucuronides and sulphates[8].

Biosynthetically, estrogens are formed from the androgenic precursors androstenedione (1) and testosterone (2). Androstenedione is converted by the ovary to testosterone, which is then aromatised and demethylated to estrogens. Aromatisation of the A ring of the steroid is required for estrogenic activity. If this reaction is defective, circulating androgenic precursors can cause virilization Fig (1.4).



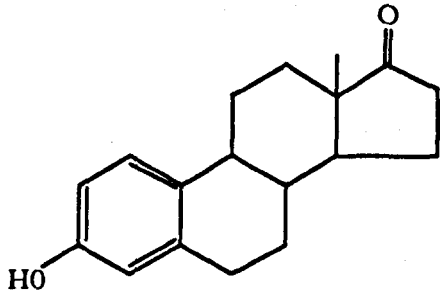
Androstenedione

(1)



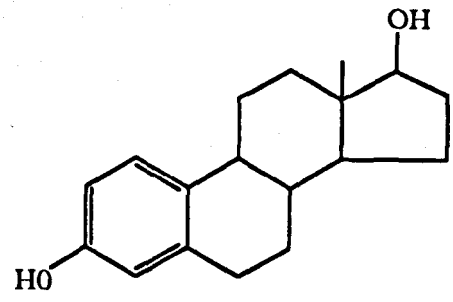
Testosterone

(2)



Estrone

(3)



Estradiol

(4)

Fig. 1.4

Estrogens are the female sex hormones and are basically responsible for the development and maintenance of accessory sex organs and secondary sexual characteristics in the female. They induce proliferation in the epithelium of the fallopian tubes, endometrium, cervix and vagina. Especially in the preovulatory phase, estrogens produce changes in the tubular mucosa and stimulate the contraction and motility of the fallopian tubes, which promote the transport of the ovum. The growth of the endometrial mucosa is accompanied by an increased blood supply to this tissue produced by increased coiling of the spiral arterioles.

Estrogens, along with pituitary factors and progesterone, stimulate mammary growth. While the proliferation of the lobular-alveolar system in primates is under progesterone control, estrogens are responsible for the growth of the stroma and duct system. The growth and pigmentation of the nipples as well as of the labia of the vagina are stimulated by estrogens. In women, the distribution of subcutaneous fat and of body hair and the smooth character of the skin are likewise controlled by estrogens. Estrogens induce the opening of the vaginal introitus in rodents, and estriol has been shown to increase the size of the vagina in rabbits. It is likely that this hormone increases the lumen of the birth canal in preparation for delivery[9].

Estrogen secretion is under the control of pituitary gonadotropins. Conversely, large doses of estrogens may act on the hypothalamus to inhibit the release of the follicle-stimulating and luteinising hormones and may therefore inhibit ovulation and eventually lead to involution of accessory sex organs. During pregnancy, the estrogens act with progesterone to aid nidation, maintain gestation, block the production of lactogenic hormone and finally facilitate parturition. Excessive doses of hormone may depress growth hormone production and lead to stunted growth and premature closure of the epiphyses. The rise in body temperature at ovulation may be due to an effect of estrogens on the basal metabolic rate. Estrogens also have some effect on salt and water retention and may raise the blood pressure. They decrease clotting time and are also clinically useful in the control of bleeding.

Folic acid is necessary for the response of the genital tract to estrogens. The androgenic adrenocortical and progestational hormones antagonise some of the effects of estrogens. Estrone and estradiol inhibit the effect of testosterone on comb growth in the capon. Estrogens have been linked in the past with tumours in certain animals, but in other cases they have had the opposite effect in that estrogen treatment has benifited some patients with cancer of the breast or prostate. Estrogen treatment is also used in female hypogenitalism, in certain cases of menstrual dysfunctions, for menopausal symptoms, for engorged breasts, in senile vaginitis and in pruritus vulvae. The toxic side effects, such as nausea and vomiting, are similar to those sometimes observed in pregnancy. In primates, withdrawal of estrogen treatment produces bleeding similar to menstruation.

1.6 Estrogen receptors

The actions of estrogens on target tissues are mediated through specific tissue receptors. High concentrations of estrogen-binding protein are found in the uterus, vagina and mammary glands, but estrogens are also found in many other tissues. These receptors, like those for androgens and glococorticoids, belong to a superfamily of proteins, all of which are regulators of gene transcription. Fig (1.5) shows how the steroids from the circulation diffuse into the cells, but only target cells have the appropriate receptors to retain them[10].

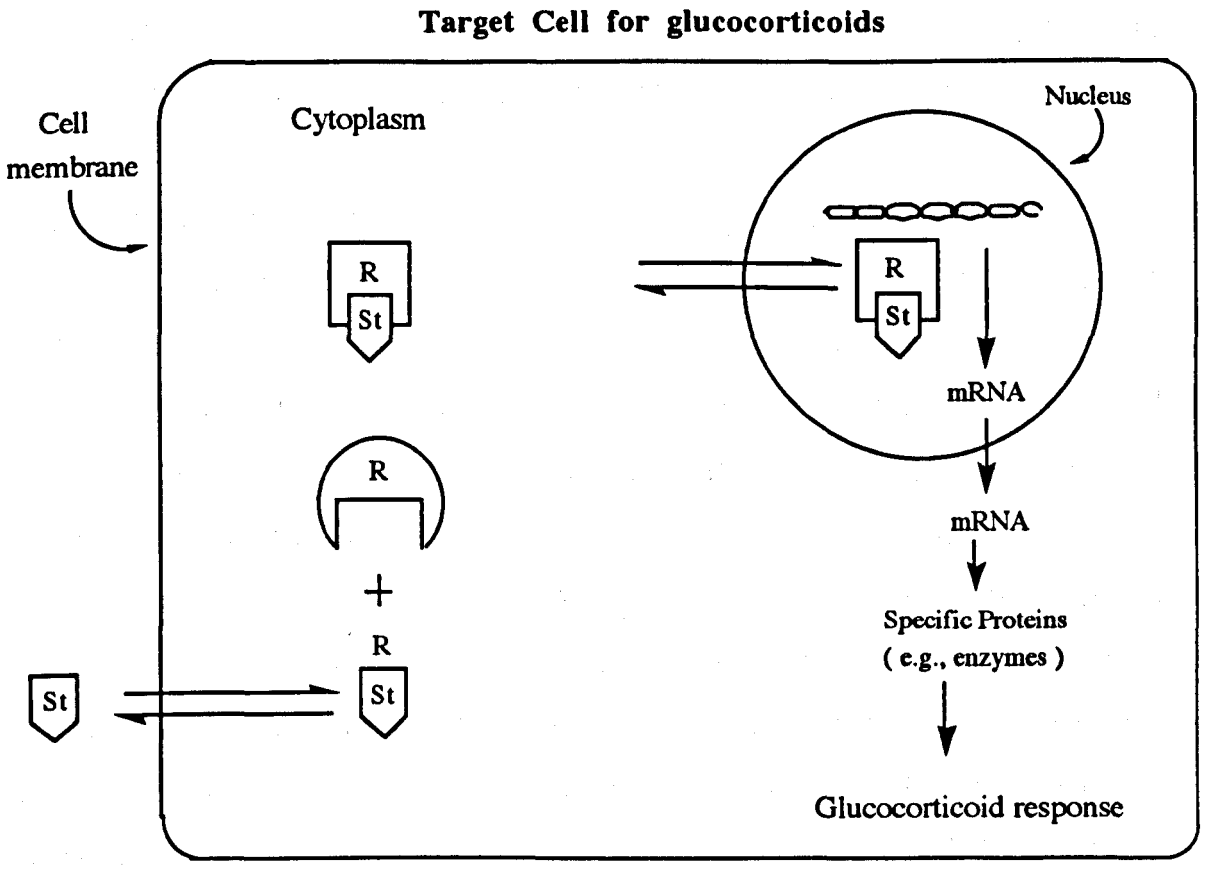


Fig. 1.5

The steroid hormone binds to the receptor and activates a change that allows the complex to bind to nuclear chromatin. Binding of the steroid receptor complex to a specific nuclear acceptor site alters gene transcription.

1.6.1 Therapeutic uses

The main use of estrogens is in combination with progestins in oral contraceptive preparations. The constant concentrations of circulating hormones suppress secretion of FSH and LH and also the

midcycle surge of LH just before ovulation. Therefore, follicle maturation is retarded and the stimulus for ovulation is suppressed.

Estrogens are used as replacement therapy in ovarian failure or at menopause to prevent osteoporosis and to relieve the symptoms of estrogen deficiency. When estrogen secretion decreases at menopause, estrogen-responsive target tissues can cause several symptoms such as vasomotor instability, drying of the vaginal mucosa, insomnia, irritability and other mood changes. As ovarian function declines with age, androstenedione from the adrenal cortex becomes the primary source of estrogen, in the form of estrone. Because the naturally occurring concentration of androstenedione and the efficiency of its conversion may vary considerably among individuals, estrogen replacement therapy is often used, in order to ease the transition into menopause.

Estrogens are also used to prevent or to treat osteoporosis, which is a reduction of bone mass causing fragility or risk of fracture, and they are equally effective in retarding bone loss in menopausal women, but up to now, studies have shown that they are only effective with continual administration.

Estrogens are used as androgen antagonists in certain androgen-sensitive cancers although the doses required are much higher than those needed for hormone replacement, and there is also a danger of adverse effects.

All compounds with estrogenic activity have virtually the same side effects and risks. Nausea is the most common side effect and others include fluid retention and breast tenderness. The severity of the symptoms, which usually subside with continued use, is related to the potency of the compound used. Recently there have been several studies which indicate an increase in the relative risk for endometrial or breast cancer in women who take estrogens as hormone replacement. Other serious risks of estrogen therapy include an increased tendency toward thromboembolic disease, and there is also a small risk of hypertension in a few people.

1.7 Progestins

The synthetic oral progestins are derivatives of testosterone. If the 19-methyl group of testosterone is removed, its androgenic properties are significantly reduced and the product reveals progestational activity. Addition of an acetylene group at the 17∝ position also conveys progestational activity and produces compounds that are less rapidly metabolised by the liver. Fig (1.6) shows the chemical structures of important progestins related to testosterone and 19-nortestosterone that are used as progestational agents[4]. Arrows point where the basic norethindrone structure has been modified to produce new compounds.

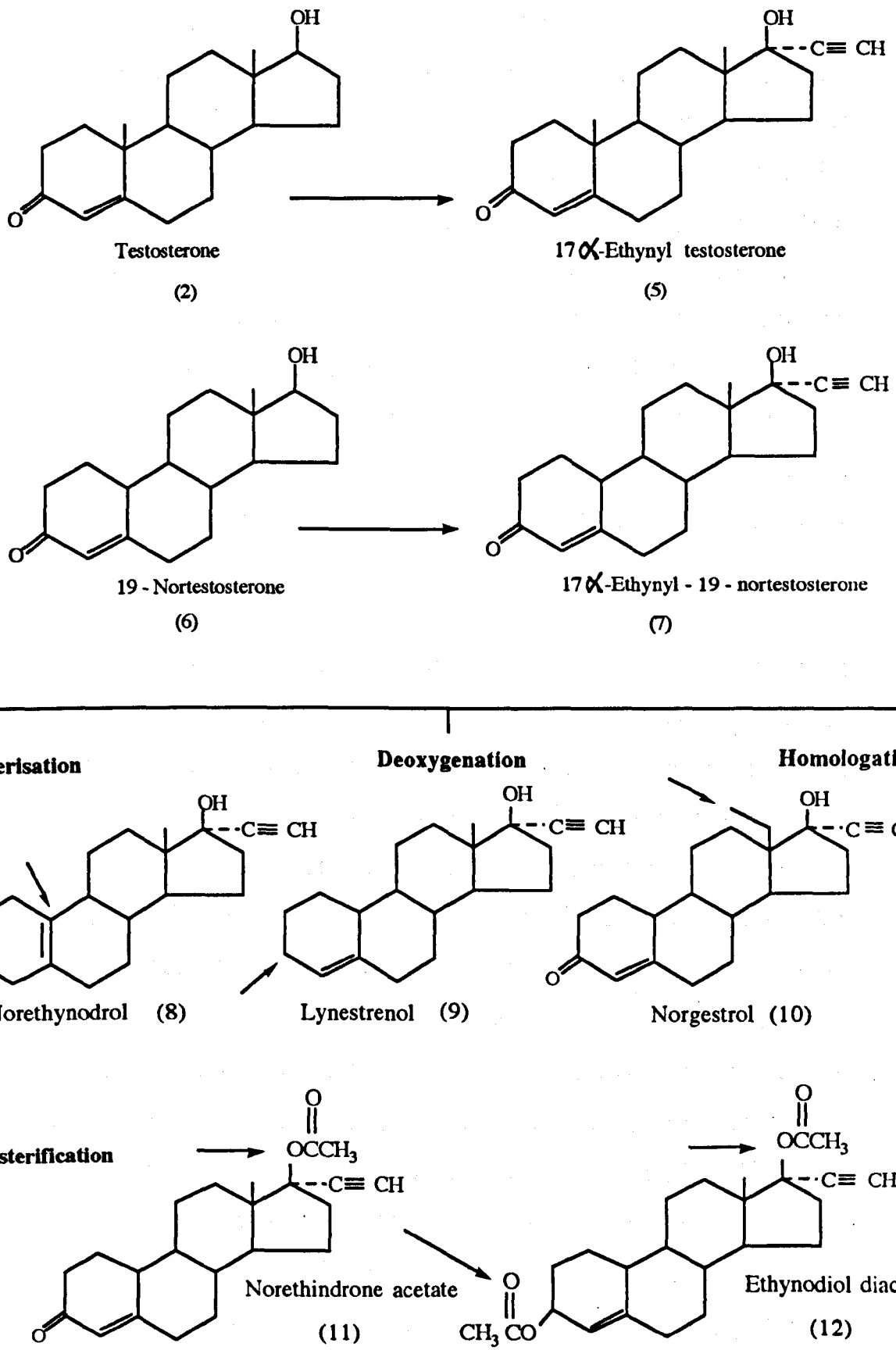

OH
C≡ CH
O
Testosterone
(2)
17∝-Ethynyl testosterone
(5)
19 - Nortestosterone
(6)
17∝-Ethynyl - 19 - nortestosterone
(7)
Isomerisation
Deoxygenation
Homologation
Norethynodrol (8)
Lynestrenol (9)
Norgestrol (10)
Esterification
OCCH3
Norethindrone acetate
(11)
CH3 CO
Ethynodiol diacetate
(12)

Fig. 1.6

`The effects of progesterone are generally opposite to those of estrogen. For example, it decreases myometrial contractions, increases glandular development of the breast and endometrium and promotes secretion of a viscous mucus from cervical glands. However, like estrogens, it inhibits LH secretion through a negative feedback on the hypothalamic-anterior pituitary axis. There is more than one way by which the synthetic progestational hormones prevent conception. Firstly, alteration of cervical mucus from a watery, non-viscous secretion to a viscous, cellular secretion physically blocks sperm penetration. As well as this, progestational stimulation early in the ovarian cycle causes premature development of endometrial glands and endometrial involution.

The main use of progestins is in oral contraceptive formulations, and they are administered along with estrogens for treatment of menopausal symptoms. In most oral contraceptive combinations, progestins are combined with a semisynthetic estrogen. Some compounds can be used alone, but oral contraceptives that contain only a progestin have a higher failure rate and may cause irregular bleeding. Progestins are also used to treat protracted uterine bleeding, dysmenorrhea, amonorrhea and endometriosis. The addition of a progestin can help repair the necrotic endometrium, which allows natural shedding once the hormone is withdrawn.
There are several side effects associated with progestational agents, as indeed there are with any oral contraceptives and these are similar to symptoms associated with pregnancy. These effects include a gain in weight, depression and fatigue. These are probably

due to its androgenic action, and in contrast to the nausea caused by estrogens, the symptoms do not usually subside with continued use.

Although it has often been thought in the past that there are certain links between long-term progestin use and cancer, there is no convincing evidence that this is the case and in fact, oral contraceptives actually appear to prevent some forms of cancer. In a review of this issue[11], it has been suggested that blockade of ovulation by pregnancy, lactation, or hormonal suppression may protect against endometrial and ovarian cancer. It also indicates however, that the case for cervical cancer is not so clear.

There are many compounds which have progestational activity. Semisynthetic derivatives of progesterone include medroxyprogesterone acetate and hydroxyprogesterone caproate. These have little estrogenic or androgenic activity and can be used by the injection method or in the case of medroxyprogesterone acetate, also in tablet form.

The 19-norsteroids (6) have some estrogenic potency and this is probably because of metabolism to estrogenic compounds. Some also have androgenic actions and the most commonly used agents are those included in oral contraceptives such as ethynodiol diacetate (12), norethindrone acetate(11), norethynodrol(8),and norgestrol(10).

1.8 Progesterone antagonists

It is necessary to keep up progesterone levels in order to maintain pregnancy and because of this, antagonism of progesterone receptors

early in pregnancy can cause abortion. Mifepristone, also known as RU 486, is an antagonist at progesterone and glucocorticoid receptors, and is used to terminate early pregnancy. Another drug, epostane, terminates pregnancy through inhibition of progesterone synthesis, blocking the conversion of pregnenolone to progesterone by the enzyme 3-hydroxysteroid dehydrogenase.

1.9 Biosynthesis of steroids

One of the main actions of gonadotropins is to promote synthesis of enzymes that catalyse various steps in the synthesis of steroid hormones. Androstenedione (1), which is the common precursor of androgens and estrogens, is formed by the same mechanism in testis, ovary and adrenal cortex. The synthetic scheme is shown in Fig (1.7).

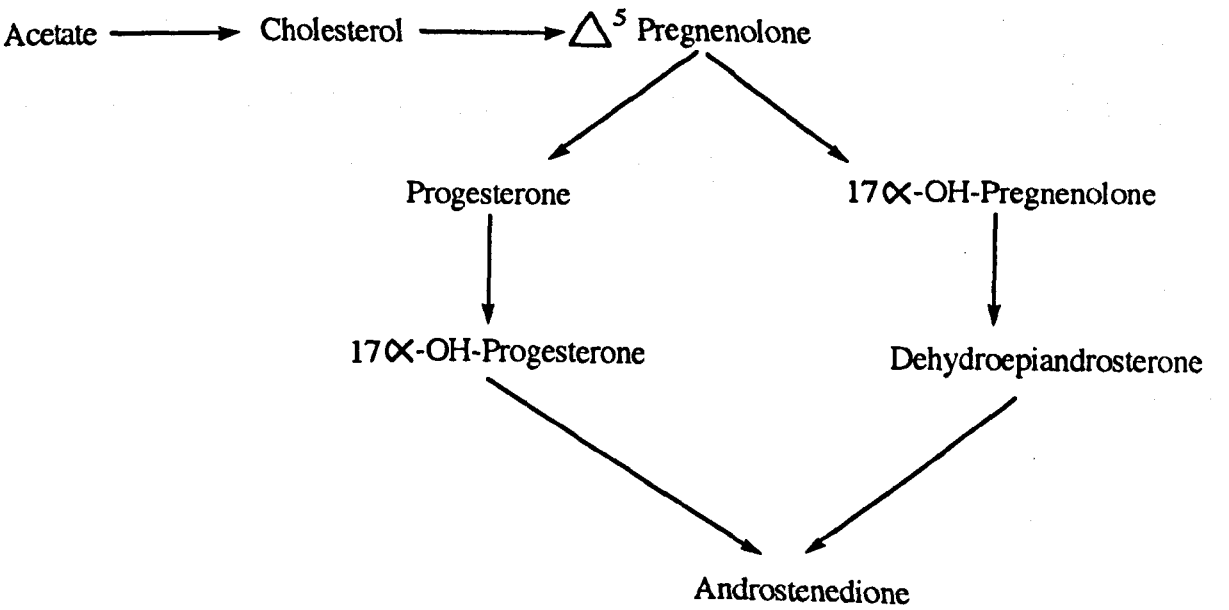
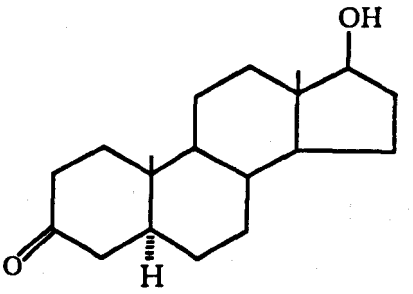


Fig. 1.7

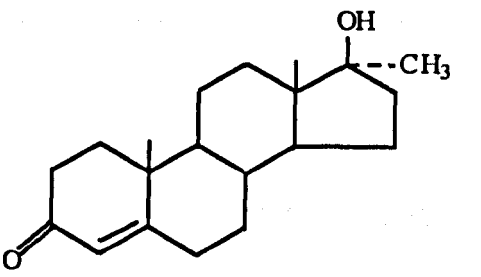
1.10 Androgens

Androgens are the male sex hormones and the principal naturally occurring one is testosterone (2). This is formed in the Leydig cells under the influence of LH. It is continuously secreted during adult life and is responsible for the development, function and maintenance of the secondary male characteristics, the male accessory sex organs and for spermatogenesis. The androgens are also secreted from the adrenal cortex and the ovaries and they cause retention of water, nitrogen, potassium, sodium, calcium, chloride, sulphate and phosphorus. They also increase protein anabolism[12].

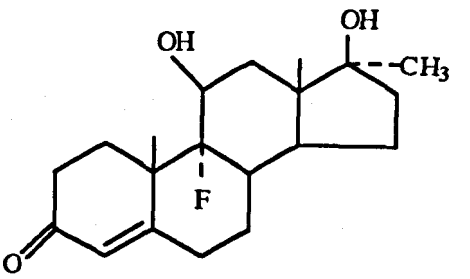
Testosterone is converted in most parts of the body to a more active metabolite, dihydrotestosterone (13). This acts at a cellular level like other steroid hormones by binding to a cytoplasmic receptor, which leads to the increased synthesis of RNA and protein. Dihydrotestosterone is more potent than testosterone and mediates most of the androgenic effects attributed to testosterone[13]. Orally effective derivatives of testosterone include methyltestosterone (14), fluoxymesterone (15) and methandrostenolone (16) Fig (1.8).



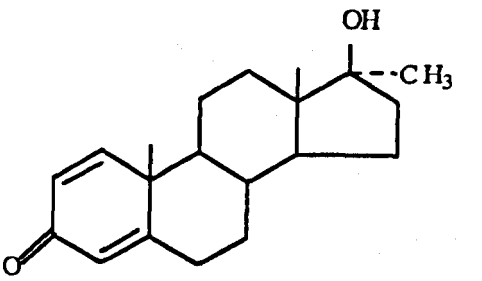
Dihydrotestosterone
(13)



Methyltestosterone
(14)



Fluoxymesterone
(15)



Methandrostenolone
(16)

Fig. 1.8

1.10.1 Actions and uses of androgens

Testosterone has both androgenic and anabolic actions. The androgenic actions, as stated previously, are responsible for changes associated with sexual maturation and for stimulation of spermatogenesis. In the early stages of embryonic life, androgens promote development of the male phenotype. They are also responsible for the sudden growth at puberty and for the muscle mass in the maturing male. In addition to this, androgens stimulate growth and secretion of sebaceous glands and growth of facial hair[14].

The most important use of androgens is replacement therapy when normal testosterone secretion is reduced or absent. They have been used in hypogonadism in the male and in some types of infertility. Anabolic steroids are used in conditions in which there is a negative nitrogen balance such as in wasting diseases, malnutrition, severe anaemia or severe trauma. they also have a certain use in the treatment of growth deficits or osteoporosis. Anabolic steroids are widely used by athletes in order to try to improve their strength and performance, or by bodybuilders who want to increase muscle weight. However, this can result in hepatic abnormalities, an elevated concentration of LDL cholesterol or even coronary artery disease.

1.11 Male sex hormones

The hormonal control of reproduction in the male is relatively simple compared to the female. The male gonads (testes) like the ovaries, have a double function; they produce and secrete the male hormone, testerone, and they also produce the spermatozoa which carry out the fertilization of the ovum in the reproductive process. 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 ten 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 through a very long, coiled duct called the epididymis. During their epididymal transport

stage the spermatomoza 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 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 (2).

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 from the hypothalamus. Some testosterone is enzymatically reduced to 5∝-dihydrotestosterone in circulation, in peripheral tissues 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 continous 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.

1.12 Oral contraceptives

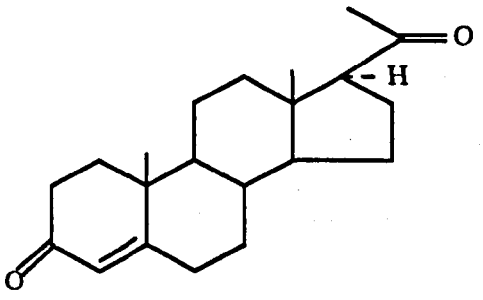
It was the recognition of the key role of progesterone (17) in inhibiting ovulation which led to the development of oral contraceptives in the mid-1950's. Progestational steroids act as antifertility compounds by suppression of oestrus or prevention of ovulation, via a feedback effect upon the secretion of gonadotrophins by the anterior pituitary. There are many different types of oral contraceptive and the most common of these is a combination preparation which contains both progestin and estrogen. Single entity preparations are also available. A progestin alone has come to be known as the "minipill", while an estrogen alone is a postcoital or "morning after" pill. The minipills were introduced to eliminate the estrogen, which is the agent in combined preparations thought to be responsible for most of the side effects of oral contraceptives. However, these preparations are not as popular as the combined preparations since their efficiency is not so high and also the menstrual cycles are not as regular[15].

The administration of an estrogen and a progestin in combination preparations could interfere with fertility in several ways. It is clear though, that the mixture inhibits ovulation. Ovulation could be prevented either by inhibiting the ovulatory stimulus or by preventing the growth of follicles, and this agrees with the experimental observation that follicular growth and ovulation can be prevented by either estrogen or progesterone given singly. The orally active progestins alone have been administered because of the fear that estrogen may have harmful effects. Continuous

administration of a progestin in sufficient dose abolishes the cycle for as long as it is given and leads to ovarian and endometrial atrophy. Very small doses may alter the structure of the endometrium and the consistency of the cervical mucus without disrupting the cycle or inhibiting ovulation.

Long acting progestins, given by intramuscular injection, are also effective. Additional forms of contraception also utilise the highly active, purely progestational compounds to provide vaginal absorption, in intrauterine devices, or in plastic capsules for subcutaneous application.

There are many side effects connected to the use of oral contraceptives, some of which are quite major and others which are less significant such as nausea, vomiting and headaches. The most important side effects to be aware of are the cardiovascular side effects and the induction or promotion of tumours. Although these seem alarming, they are not well substantiated and in fact the oral contraceptive can actually lower the incidence of certain disorders. It can be said that in most ways, the advantages of the oral contraceptives outweigh the disadvantages.

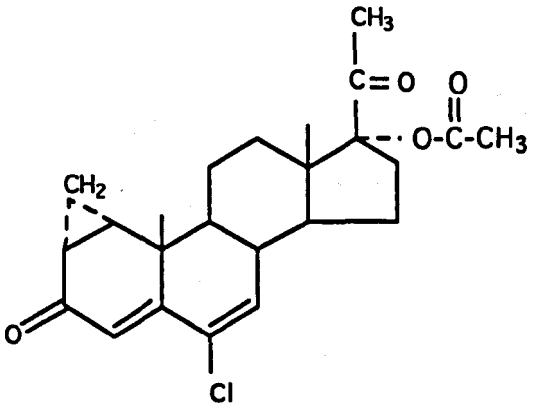


Progesterone
(17)

1.13 Anti-hormones

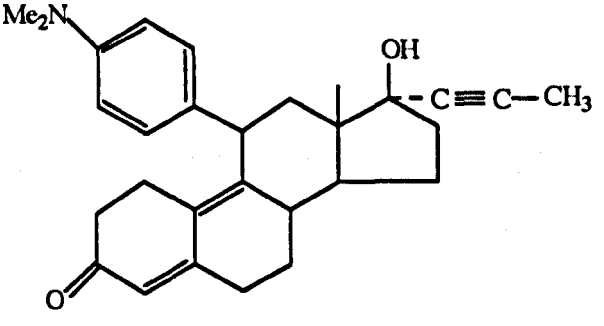
Steroid hormone receptor antagonists can be defined as compounds which bind with high affinity to the receptors so as to exclude the native steroid hormone from occupying its binding site and also which do not themselves lead to any hormonal agonist effect. Compounds of this kind which bind to the receptors for estrogens, androgens or mineralocorticoids have been in clinical use for some time, but until recently receptor antagonists for progesterone and glucocorticoids have been more elusive.

The first major clinical advance in the field of anti-androgens resulted from studies of the 1,2-methylene substituted steroid cyproterone acetate (18).

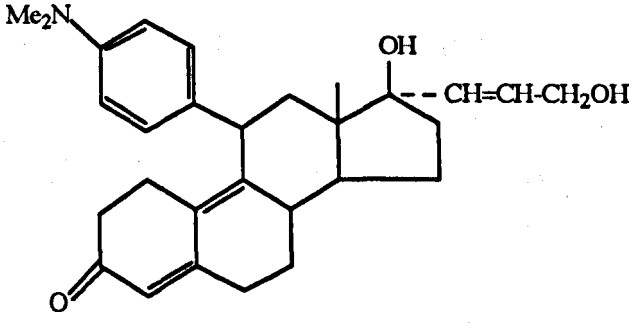


Cyproterone Acetate

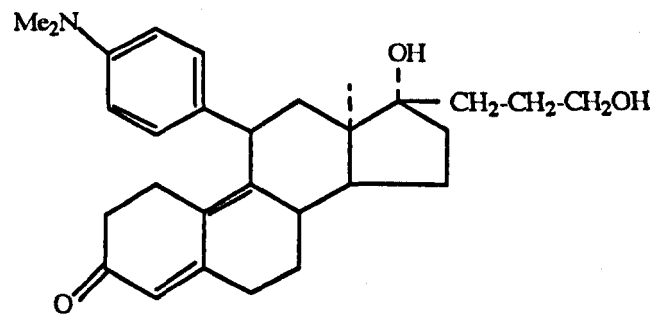
(18)



(19) = RU 38486



(20) = ZK 98734



(21) = ZK 98299

Many other steroids have been investigated for their anti-androgenic characteristics, but few have progressed further then the initial identification of their activity[16].

Early attempts[17,18] to synthesize progesterone antagonists by introducing bulky substituents at positions 1, 7 and 17 (4) of active progestins failed.

Researchers at Roussel-Uclaf made a major breakthrough in the search for effective progesterone antagonists. They found that certain 11-aryl steroids showed both antiglucocorticoid and antiprogestional activities. This indicated the existence of a much larger 'pocket' than expected in the receptor molecule above C-11 of the bound steroid[19]. Similar results were also reported by workers at Schering[20].

Several hundred compounds with 11-aryl substitution have been sythesized [21,22] and among these 3 derivatives, RU 38486,(19) ZK 98.734,(20) and ZK 98.299, (21) showed particularly strong properties as antiprogestins. Progesterone is crucial to the maintenance of pregnancy, and the analogues can serve as a means of interrupting pregnancy.

Under the name 'mifepristone'. RU 38486 is administered orally (600mg), followed after 2 days by a small dose of a prostaglandin (e.g. sulprostone or gemeprost), which increases the frequency and strength of the uterine contractions needed to expel the embryo. In many countries including the UK, the drug combination is now approved for ending pregnancies of up to 49 days duration (counting from the first day of the last menstrual period). This procedure is now a favourable approach to the termination of pregnancy.

Many women in developing nations and, to a lesser extent, in industrialized countries, rely on pregnancy interruption for birth control. Although legal surgical methods are safe and effective, they have well-known drawbacks. In the first three months of pregnancy, vacuum aspiration (sometimes preceded by dilation of the cervix) is the usual method of choice. In this approach, suction is applied to remove the embryo and the endometrial tissue in which it is embedded. After about three months of pregnancy, the required precedures generally become more complex. As pregnancy progresses, the risks of infection, haemorrhage, scarring and impaired fertility increase. In developing nations, where surgical facilities are often inadequate, the danger is greater. What is worse, where legally operated facilities are not readily accessible, many women die from having unsafe abortions, typically because of uncontrolled bleeding or infection.

Results also suggest that RU 38486 has potential as an emergency post-coital contraceptive with a wider range of action than the currently used hormonal preparations which need to be administered

within 48-72 hours after unprotected intercourse. Nevertheless, treatment as early as possible seems advisable because it would appear that efficiency decreases after implantation has commenced.

Binding to steroid hormone receptor and plasma proteins

1.14 Progesterone receptor

It has been demonstrated that RU 38486 (19), ZK 98.734 (20) and ZK 98.299 (21) show specific, high-affinity binding to progesterone receptor preparations from a variety of species including rat ovary,[23] rabbit uterus,[24] calf uterus and human endometrium and myometrium.

In general the relative binding affinity of these antiprogestins is lower than that of progesterone in assays involving a relatively short (1-3 hours) period of incubation. However, RU 38486 dissociates more slowly from the receptor than progesterone[25] and its relative binding affinity is substantially greater than that of progesterone when incubation period is prolonged. It is unclear whether or not the presence of biologically active metabolities influences the antiprogestational activity of RU 38486.

1.15 Glucocorticoid receptor

RU 38486 (19), ZK 98.734 (20) and ZK 98.299 (21) also bind very strongly to the glucocorticoid receptor of various tissues and species. The ability of antiprogestins[26] to interact with both the progesterone

and glucocorticoid receptor is not surprising in view of the more than 50 percent homology in amino acid composition that exists between these two receptors in their C-terminal end, the region which is thought to be involved in steroid binding.

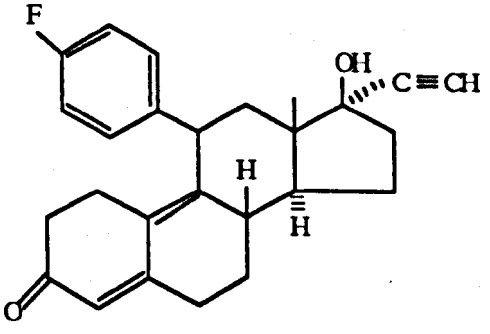
Because of its antiglucocorticoid propeties, RU 38486 has been employed orally in clinical treatment of Cushing's syndrome and adrenal cancer, while topical application of the compound may have potential in the therapy of certain forms of glaucoma and ocular hypertension.

1.16 Other steroid hormone receptors

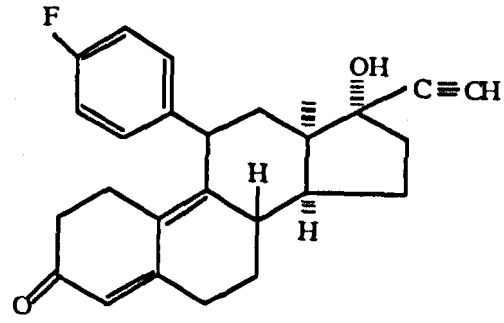
The synthetic steroid analogue RU 38486 (19) binds to the androgen receptor of the rat prostate and rat ovary but with lower affintiy than testosterone when given orally to castrated male rats. RU 38486 causes a dose-dependent inhibition of the increase in weight of seminal vesicles and prostate induced by testosterone propionate but this antiandrogenic activity is 20-30 times weaker than the compound's antiprogestational and antiglucocorticoid activities in the rat. Both of the synthetic steroids ZK 98.734 and ZK 98.299 also exhibit weak binding affinity for the androgen receptor of rat prostate, but are potent antiprogestins. The ability of antiprogestins to bind to the androgen receptor is probably due to the structural homology that exists in the putative steroid binding region of rat and human androgen receptor and, on the other hand, the human progesterone receptor.

1.17 Receptor binding by 13∝-methyl steroids

There is a high affinity of the 13∝ -methyl compounds for both progesterone and glucocorticoid receptors[27]. Results obtained with a large number of synthetic steroids with normally configured skeletons provide information concerning the atoms most critical for binding to the receptor protein. For example, Seeley et al[28] demonstrated that, in addition to hydrogen bonding with the 3 and 20 ketone groups, van der Waals' interactions at carbons 2,4,7,9,12,18 and 19 can normally be expected. The conformation of the A and B rings in particular can therefore be expected to influence receptor binding. In the case of the 11-aryl derivatives, it might also be anticipated that the allowed variation in orientation of the phenyl ring with respect to the rest of the molecule will be limited by the size and shape of the hydrophobic domain on the protein with which this group interacts. In order to gain a more detailed analysis of these structural differences, X-ray crystallographic analysis of a 13-epimeric pair has been examined. For this purpose, the 4-fluorophenyl derivatives (22) and (23) were chosen.



(22)



(23)

The basic results indicate a close similarity in molecular shape (Fig 1.9) and it can be seen that the relative positions of the 3-keto group and carbon atoms 3-6, 10 and 11 are virtually unchanged, which suggests that tha A-ring will be able to interact quite normally with the receptor. The relative orientations of the fluorophenyl group in the two compounds are also quite similar. The major changes involve the C and D-rings, as would be expected, and carbon atoms 7 and 8. From both X-ray crystallographic and protein-binding studies, it is known that these regions of the molecule are less intimately involved in receptor interactions (Seeley and Duax et al)[28,29], so that the main features of these two steroids are compatible with current notions of the receptor protein. It is worth noting however, that the 17-hydroxyl groups in these compounds, 17ß in the natural epimer and 17∝in the 13∝-methyl derivative, occupy quite different positions. The clear differences found in the biological activities of all these compounds indicate that these groups play an important role in their activity, most likely at the receptor level, and this implies that there are receptor sites for hydogen bonding with the hydroxyl groups of the 13∝-methyl isomers which do not interact with progesterone.

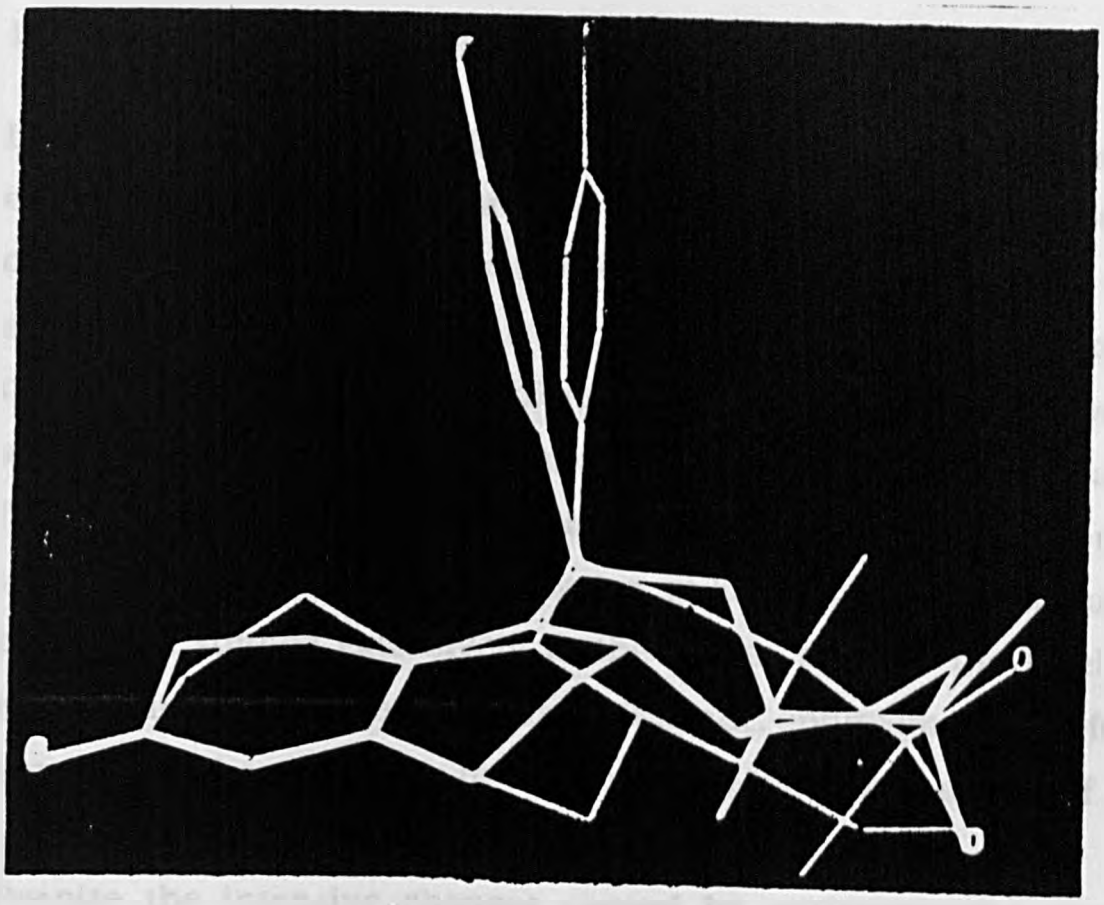


Fig. 1.9

1.18 Termination of early pregnancy

Oral administration of RU 38486 either in a single dose or for three consecutive days starting on the day of implantation (day 6 post-coitum) is capable of terminating pregnancy in the rat and mouse[30]. A reduction in the 100% effective dose can be achieved by using alternative routes of drug administration (subcutaneous, intramuscular and intravaginal). Comparative studies have shown that RU 38486, ZK 98.734 and ZK 98.299 have comparable potency at this early stage of pregnancy in rats and guinea pigs.

1.19 Termination of more advanced pregnancy

In the rat, oral doses of RU 38486 similar to those that interrupt early pregnancy also terminate more advanced pregnancies except on day 15, when there appears to exist a relative insensitivity to the abortifacient activity of the compounds[30]. The lack of dose-dependency and the higher efficiency of sequential treatment with antiprogestins and the prostaglandin analogue sulprostone are also found in human pregnancy termination. The mechanisms through which antiprogestins induce abortion at later stages of pregnancy have been studied extensively in the guinea pig. At the level of myometrium, antiprogestins cause a dramatic, approximately 30-fold increase in the sensitivity to sulprostone which, in the case of ZK 98.299 is maintained for 24 hours

Despite the intensive changes caused by antiprogestins alone, abortion only occurs several days later and it is often incomplete or does not take place at all. This situation which seems somewhat contradictory, is called the 'antigestagen syndrome' and is generally considered to be due to the lack of mymometrial contractions resulting from insufficient endogenous PG production which, in turn, is thought to be due to the inhibition of progesterone by the administered antiprogestin.

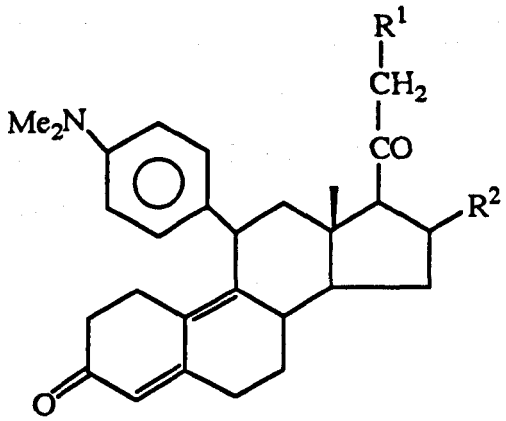
CHAPTER 2

Anti-progestins

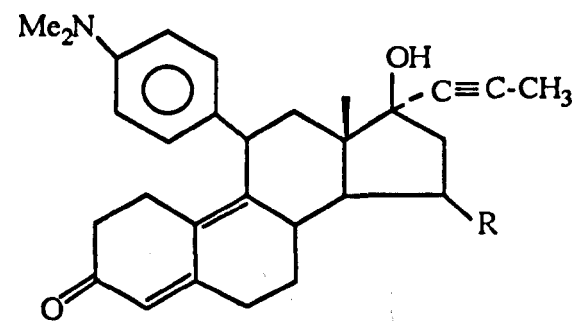
2.1 Introduction

Anti-progestional compounds were developed in the 1970's, and a number of steroids were subjected to screening procedures in order to determine what structural features would lead to inhibition of binding of radio-labelled progesterone or other progestins to the progesterone receptor. The Roussel-Uclaf group in France synthesised some11-aryl steroids in the search for anti-glucocorticoid activity. Some of these new compounds, which included RU 38486, proved to be active as anti-glucocorticoids but more notably, they also proved to be very potential anti-progestins.

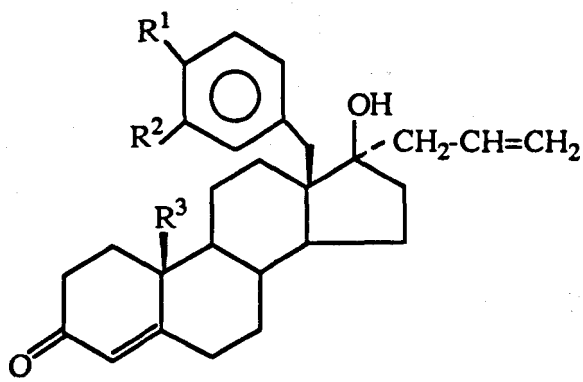
Following the discovery of the first competitive progesterone antagonist RU 38486, there has been an intense search for more potent and more selective anti-progestins. Among several hundreds of compounds under preliminary investigation by Schering are ZK 98734 and ZK 98299. Similarly related compounds to RU 38486 have been synthesised by Organon. Among the Organon compounds, compounds (2.1), (2.2) and (2.3) 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.



(2.1)



(2.2)

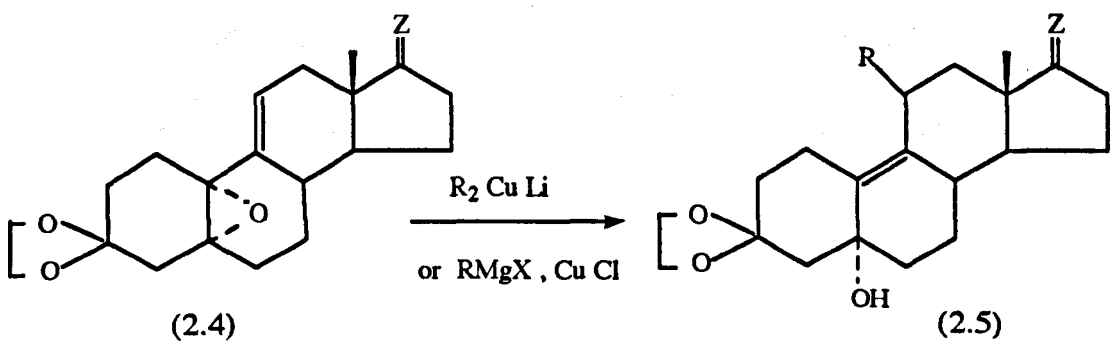


(2.3)

		R1	R2	R3	9,10
2.3	a	NMe_2	H	CH_3	Saturated
	b	NMe_2	H	___	Unsaturated
	c	H	NMe_2	CH_3	Saturated
	d	H	NMe_2	___	Unsaturated

2.2 Synthesis of anti-progestins

Teutsch and his co-workers devised a general method for the synthesis of 11-substituted 19-norsteroids, using the conjugate opening of allylic epoxides by organo copper reagents [31] Fig(2.1).



(2.4) (2.5)

Fig. 2.1

The suggested mechanism for the organocuprate reagent is shown in Fig (2.2) and involves initial nucleophilic displacement of the epoxide at C10 followed by an allylic rearrangement.

The preparation of RU 38486 involves the initial preparation of the intermediate compound (2.16) from estradiol methyl ether (2.10) Fig (2.3). This is followed by the introduction of the 11-substituent following Teutsch's method to afford compound (2.17). Treatment of compound (2.17) with a suitable lithium acetylide gives compound

(2.18). Finally, appropriate hydrolysis of compound (2.18) affords RU 38486 (2.19) (Fig 2.3).

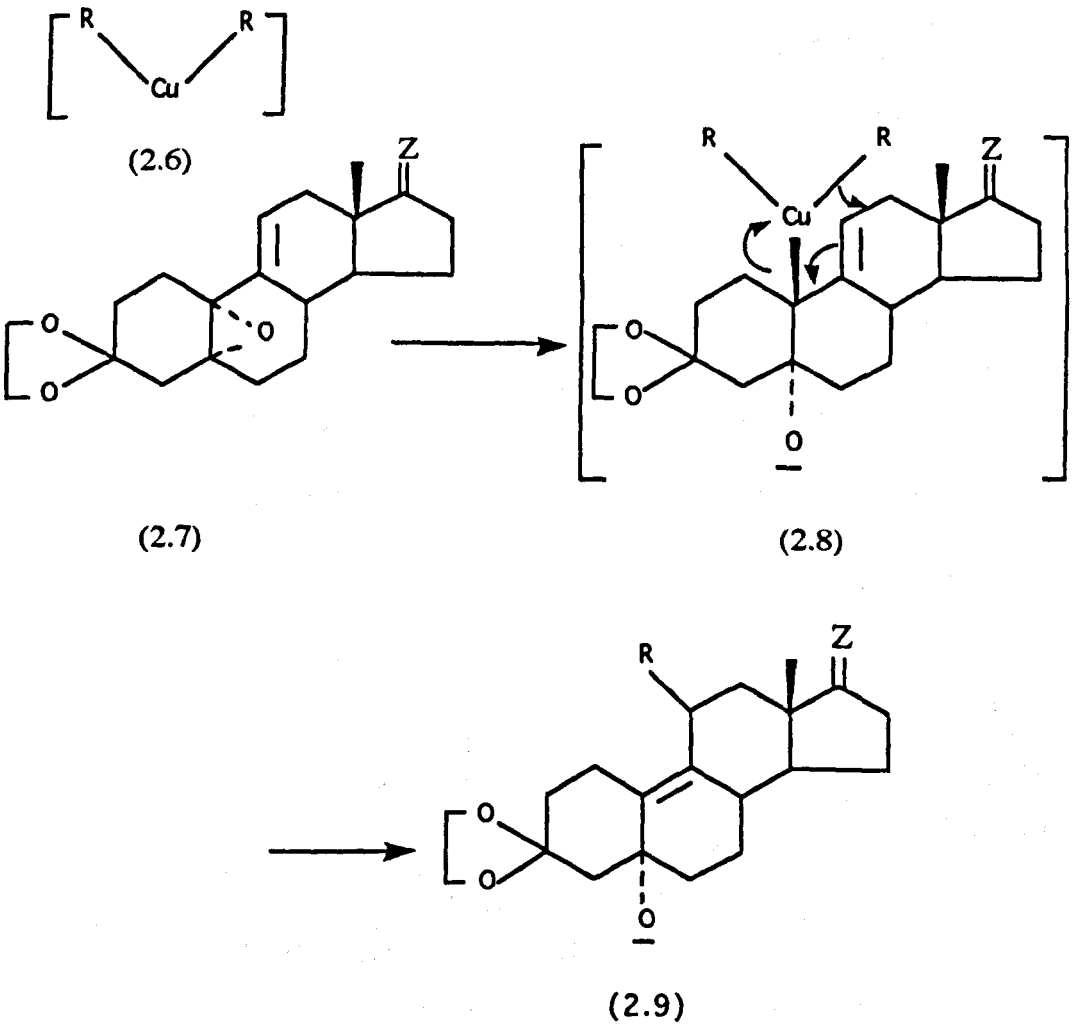
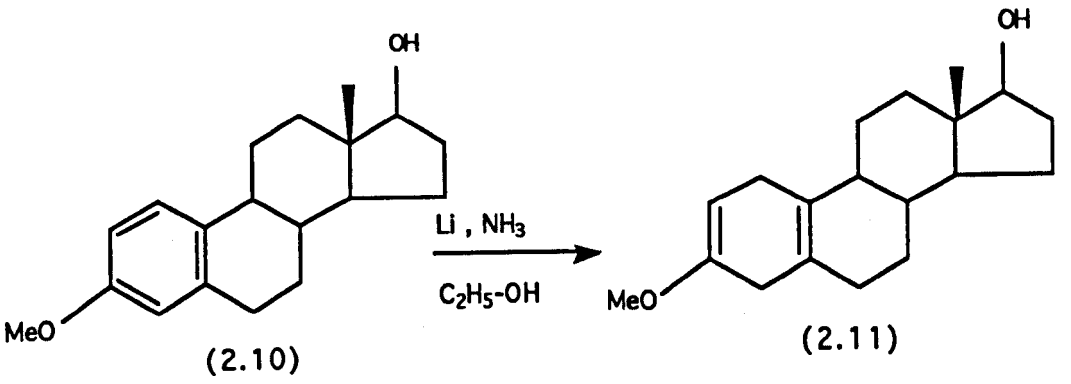
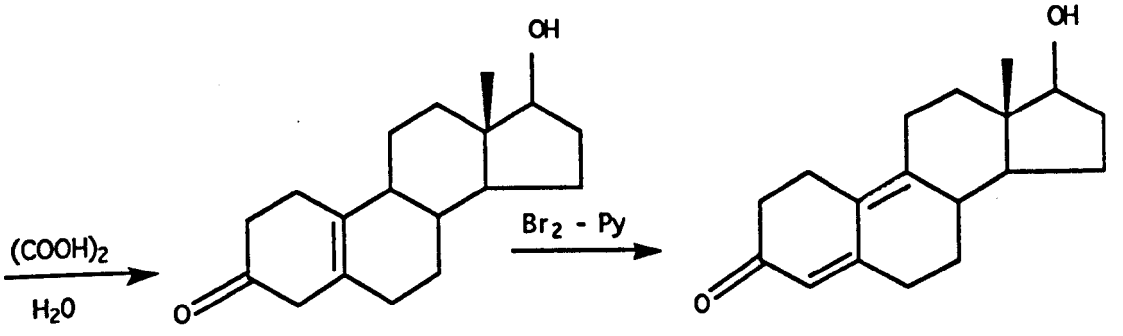


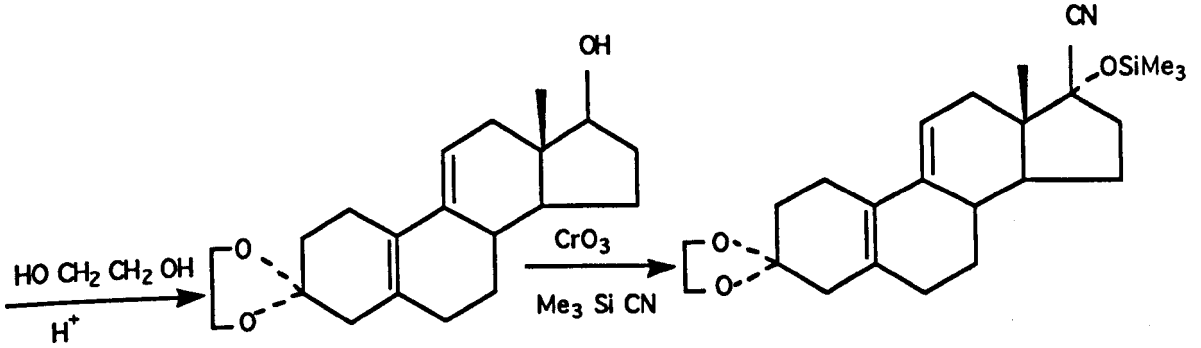
Fig. 2.2



(2.10) (2.11)



(2.12) (2.13)



(2.14) (2.15)

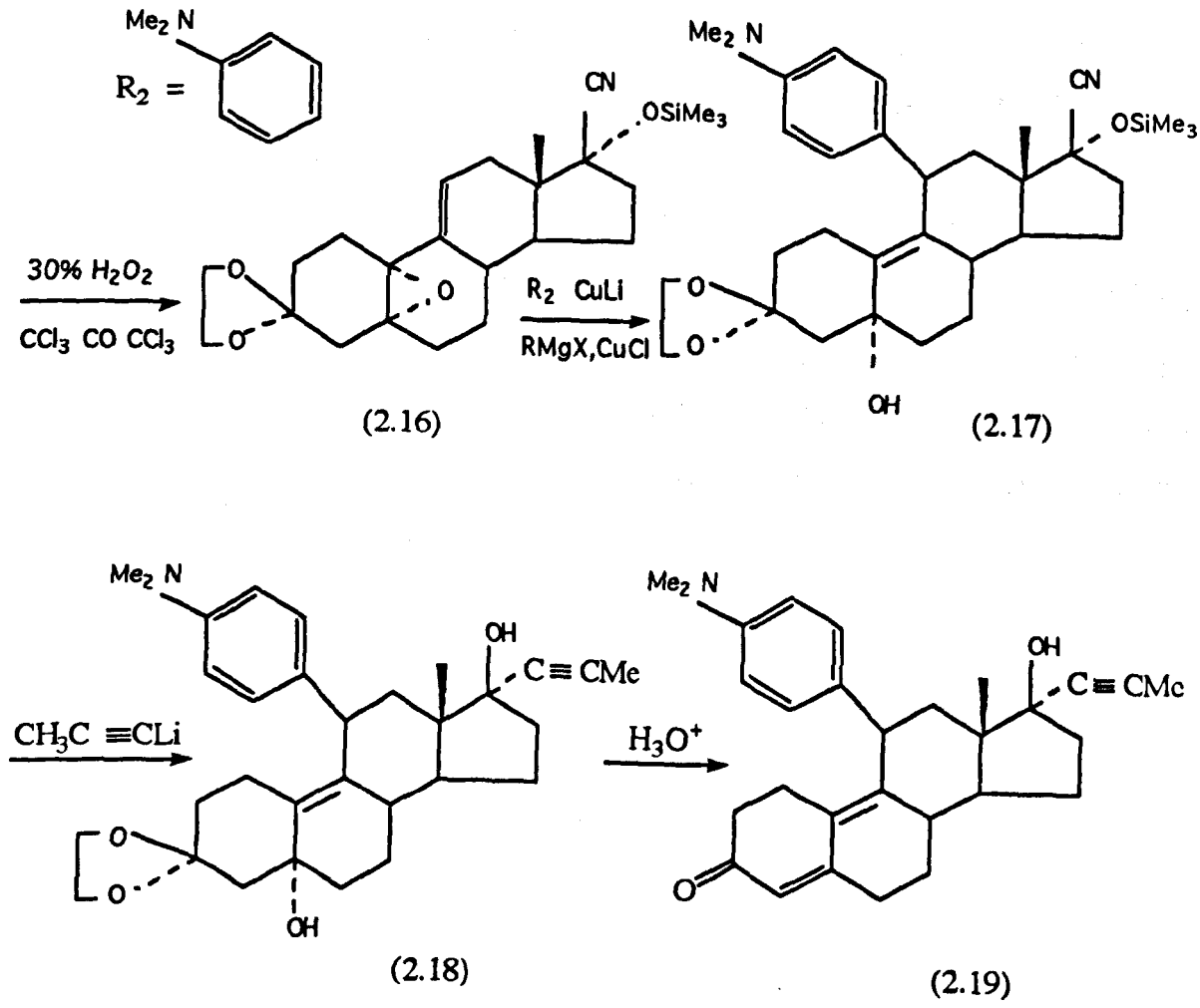
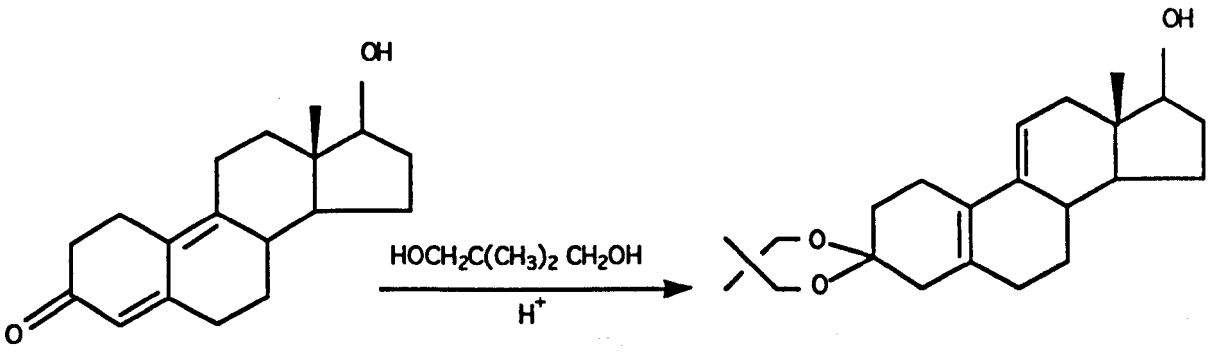


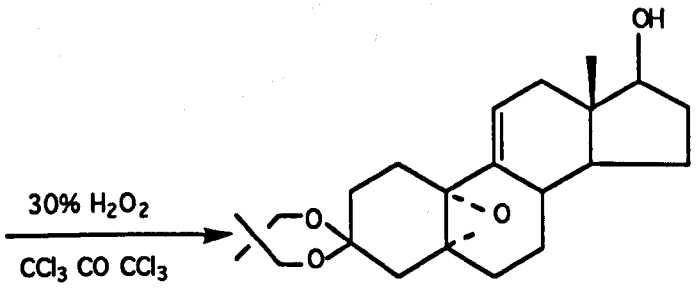
Fig. 2.3

For preparation of ZK 98734, the method involves a modification of the general and efficient strategy devised[21] for the synthesis of 11-substituted estra-4, 9-dienes. The starting material is the Birch reduction product of estradiol methyl ether, for which appropriate hydrolysis leads to the deconjugated enone (2.12), which by a bromination-dehydrobromation sequence is transformed to the dienone (2.13) (Fig 2.3). Ketalization of this compound (Fig 2.4)

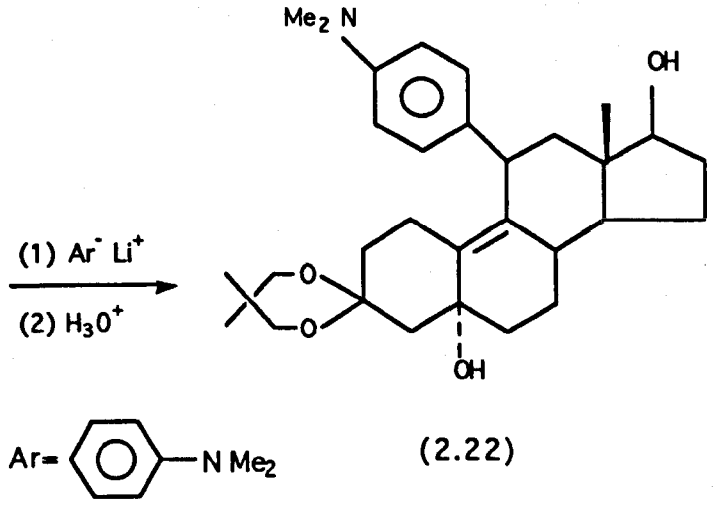
proceeds with a double bond shift to form the 5(10), 9(11)-diene (2.20), which is epoxidised to form 5∝,10∝- epoxide (2.21). The next step is the introduction of the11ß-substituent which is conveniently achieved by reaction with*p*-dimethyl amino phenyl lithium. Compound (2.22) undergoes Oppenauer oxidation to the ketone (2.23), which is treated with the lithio derivative of propargyl terahydropyranyl ether to give compound (2.24)(R= CH_2OTHP). Hydrogenation followed by acid-catalysed deprotection-dehydration[32] leads to compound ZK 98734 (20).



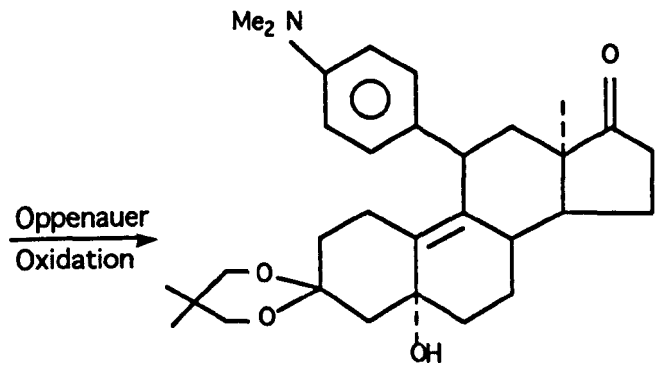
(2.13) (2.20)



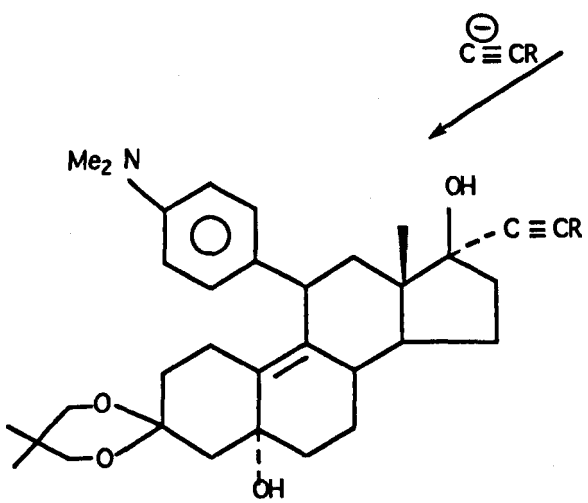
(2.21)



(2.22)

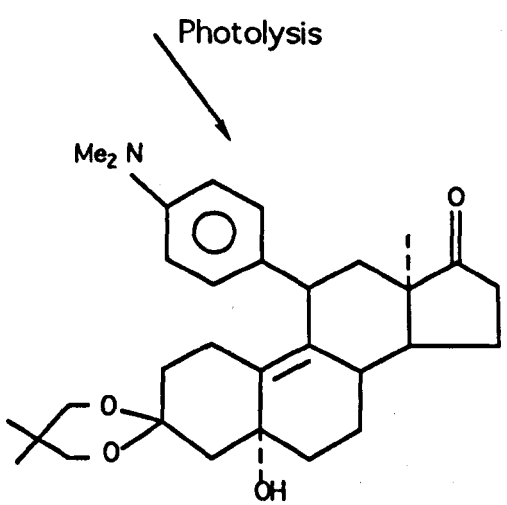


(2.23)



R= CH_2 OTHP

(2.24)



(2.26)

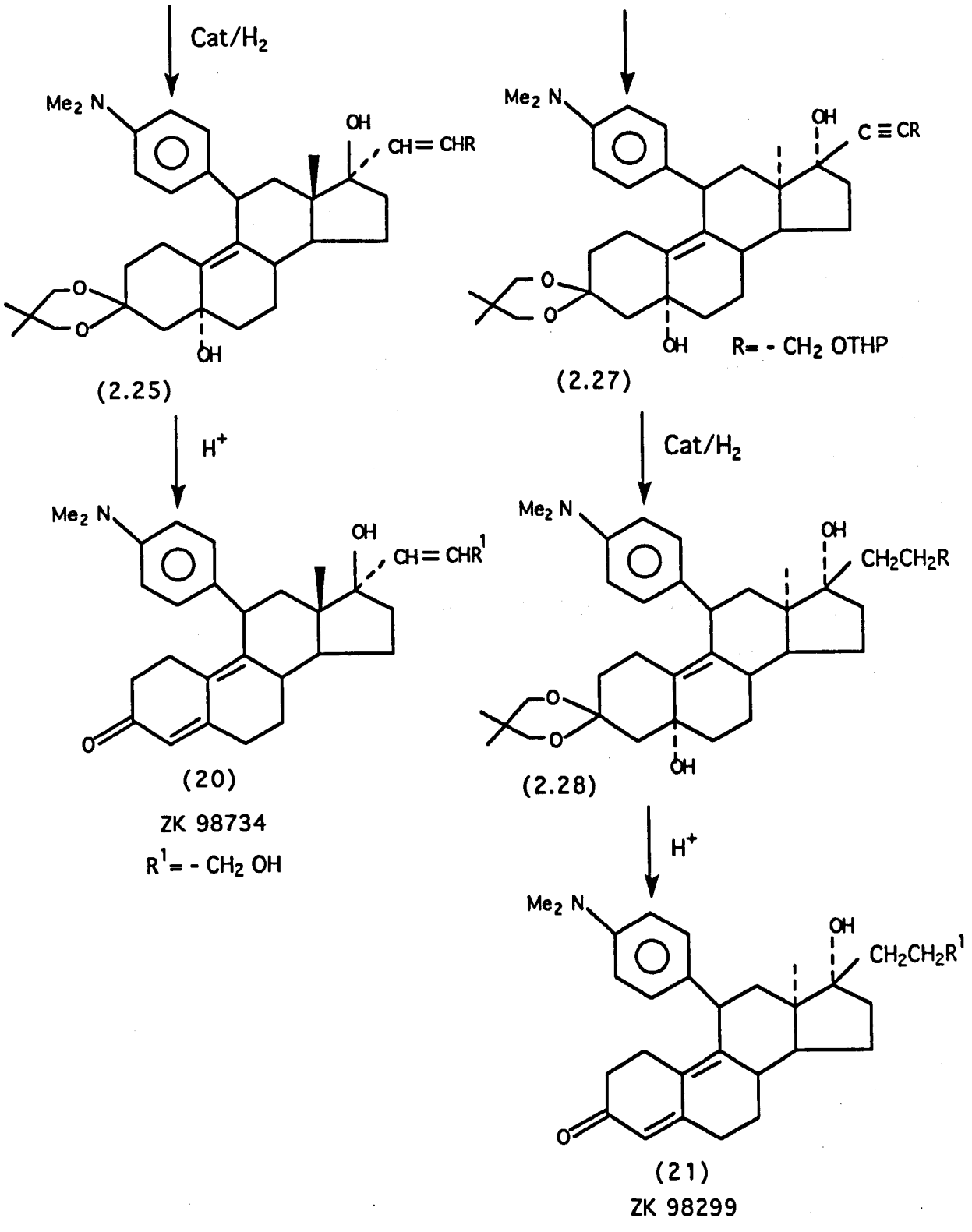

Cat/H2
Me2 N
OH
CH= CHR
O
O
OH
(2.25)
H+
Me2 N
OH
CH= CHR1
O
(20)
ZK 98734
R1= - CH2 OH
Me2 N
OH
C ≡ CR
O
O
OH
R= - CH2 OTHP
(2.27)
Cat/H2
Me2 N
OH
CH2CH2R
O
O
OH
(2.28)
H+
Me2 N
OH
CH2CH2R1
O
(21)
ZK 98299

Fig. 2.4

Alternatively, after Oppenauer oxidation,the next step in the preparation of the ZK 98299 is the photolysis of the intermediate (2.23) with the full spectrum of a mercury high pressure lamp in acidified dioxan. By Norrish-type 1 cleavage and recombination, 13α-methyl-gonane^{20}(2.26) is obtained in 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 (2.26) is not very highly stereoselective, giving isomers with predominant formation of the 17ß-adduct (2.27).

Hydrogenation of this compound followed by acid catalysed deprotection-dehydration gives compound ZK 98299 (21).

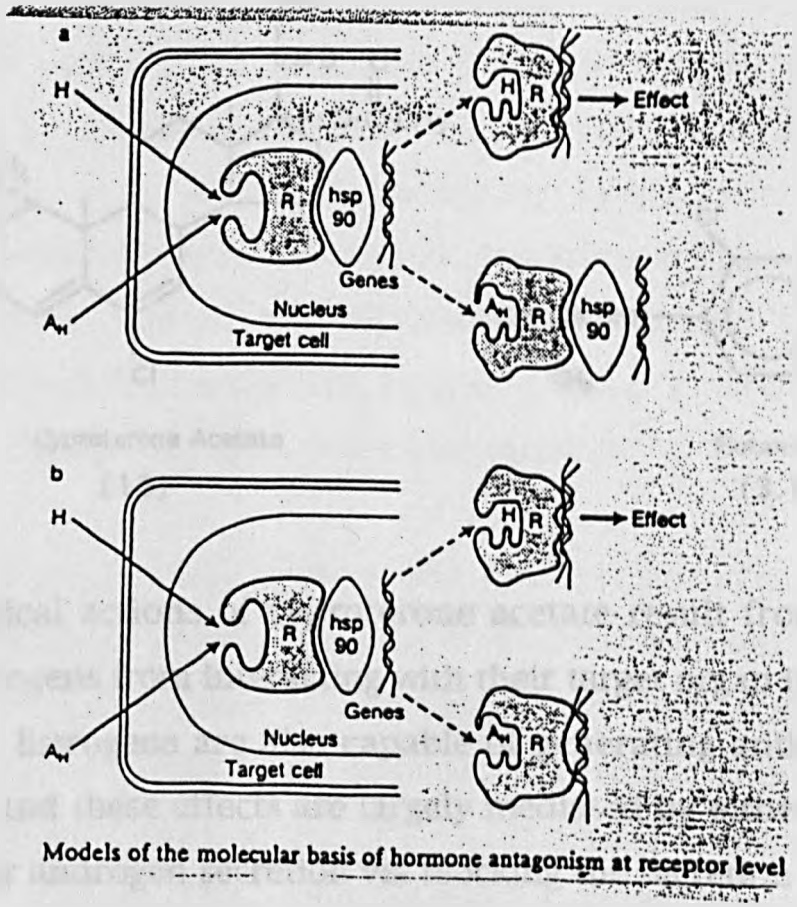
2.3 Mechanism of action of anti-progestins

The cellular mechanism of action of anti-progestins is not particularly clear. Several studies have been carried out in order to explain the absence of significant agonist activity despite high receptor binding affinity of these compounds, These studies have been conducted on the physiochemical characteristics and DNA-binding ability of glucocorticoid and progesterone receptors bound to antiprogestin. Because the ligand-receptor interactions are usually studied under non-physiological, cell-free conditions as opposed to in intact cells, the studies have yielded conflicting results[33].

Recently, a model of the molecular basis of hormone antagonism has been proposed by Groger et al,[34] which reconciles some of the divergent findings reported previously. In this model (Fig 2.5), 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. Binding of the native hormone or hormone agonist 'H' to the receptor triggers a conformational change with release of the hsp 90 sub-unit and unmasking of the DNA-binding site on the 'activated' 4S receptor. In contrast to this, the R-hsp 90 complex is stabilised when the antihormone 'A_H' binds to the receptor. Also the hsp is not released and DNA-binding does not occur. In (Fig 2.5), an alternative model suggets 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 is important to note that both models are not necessarily mutually exclusive and that both mechanims can be operative in vivo, either concomitantly or at different times, depending on the physiological status of the cell.



Models of the molecular basis of hormone antagonism at receptor level

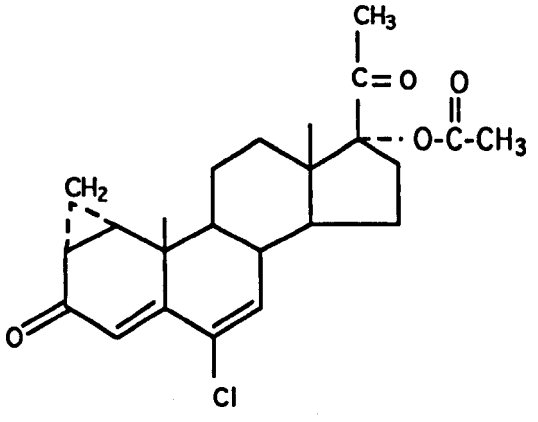
Fig. 2.5

CHAPTER 3

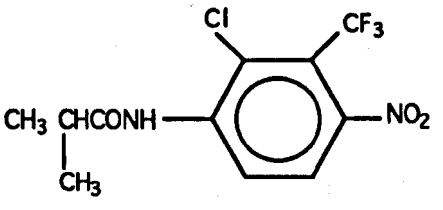
Steroidal Anti- androgens

3.1 Biological Studies of Steroidal Anti-androgens

The most common anti-androgens include cyproterone acetate (18) and flutamide (3.1). It can be seen from (18) that cyproterone acetate, like many anti-androgens, has a basic steroid skeleton whereas flutamide is not a steroid.



Cyproterone Acetate
(18)



Flutamide
(3.1)

The biological actions of cyproterone acetate result from blocking active androgens from interacting with their target organ intracellular receptors. Estrogens are also capable of generating antiandrogenic responses and these effects are largely mediated by either inhibition of testicular androgen secretion via blocking the secretion of LH or by a direct suppression of testosterone synthesis by Leydig cells. Cyproterone acetate has progestational activity, which enables it to have an inhibitory feedback effect on the hypothalamus, as well as

giving it the properties of an androgen antagonist. In contrast, cyproterone itself has no progestational activity and promotes hypothalamic stimulation by inhibiting the negative feedback inhibition of testosterone. Cyproterone is much less active as an anti-androgen than its acetate[35].

Cyproterone acetate has been extensively investigated and used clinically for the management of prostate carcinoma, for the 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 the reduction of libido in men with severe deviations in sexual behaviour. Cyproterone acetate has been investigated as a male contraceptive agent, shutting down spermatogenesis. However, all anti-androgens have many undesirable side effects and these include consequent loss of testosterone production from the Leydig cells, leading to loss of potency and libido, and breast changes in the male (gynaecomastia).

Apart from cyproterone acetate and A-norprogestron, 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[36-39]. The ring-A oxa-steroid,6α-bromo-17ß-hydroxy-17α-methyl-4-oxa-5α-androstanan-3-one exhibits anti-androgenic effects in all androgen dependent tissues outside the central nervous system[40,41], competing for DHT binding

sites slightly more selectively than does cyproterone acetate. Other steroids with high effectiveness in competing for DHT receptors include 17ß-hydroxy-2-oxa-4,9-estradien-3-one and 17ß-hydroxy-17∝-methyl-2-oxa-4,9-estradien-3-one, 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.

Alkylations of the A-ring of testosterone appear to mainly give rise to compounds with varing degrees of androgenicity. However, 2∝-methyltestosterones with a 17-∝-ethynyl or 17∝-vinyl group are claimed to show progestational and anti-androgenic qualities[14].

A group at Upjohn observed that introduction of a 7∝-methyl group onto 19-nortestosterone resulted in increases in both anabolic and androgenic activity[42]. Recently, attention has focussed on the possible anti-androgenic, anti-fertility effects of 7ß-methyl-19-nortestosterone[43].

Flutamide is a non-steroidal antiandrogen that has no other hormonal activity. The predominant effect of flutamide is the enhancement of the frequency of pulses of LH secretion. Therefore, while the drug is a pure antiandrogen, the rise in plasma testosterone serves to limit its antiandrogenic effects. Consequently, flutamide is most useful to inhibit the action of adrenal androgens in castrated men or in men receiving GnRH continuously or in situations in which LH production is

not under predominant control of androgen. The principal clinical application of flutamide at present is in the treatment of prostatic cancer, usually in conjunction with GnRH blockade or estrogen.

It has also been used experimentally in combination with an oral contraceptive for the treatment of hirsutism in women.

3.2 Synthesis of anti-androgens

Various methods for preparing 7-alkylated steroids have been reported in the literature[44-47]. The method described by Campell and Babcock[42] involving Michael addition of methylmagnesium bromide to 6-dehydro-17∝-methyl-testosterone (3.2) in the presence of cuprous chloride gave 7∝, 17∝-dimethyl-testosterone (3.3) as the main product and a small amount of the 7ß-epimer(3.4) (Fig3.1). On dehydrogenation of the crude product with chloranil, the minor component (3.4) was converted into 6-dehydro-7, 17∝-dimethyltestosterone (3.5) which was separated from 7∝-methyl enone (3.3) and then reduced back to 7ß-methyl enone(3.4) with Li-NH_3. The 7∝-epimer does not undergo dehydrogenation with chloranil.

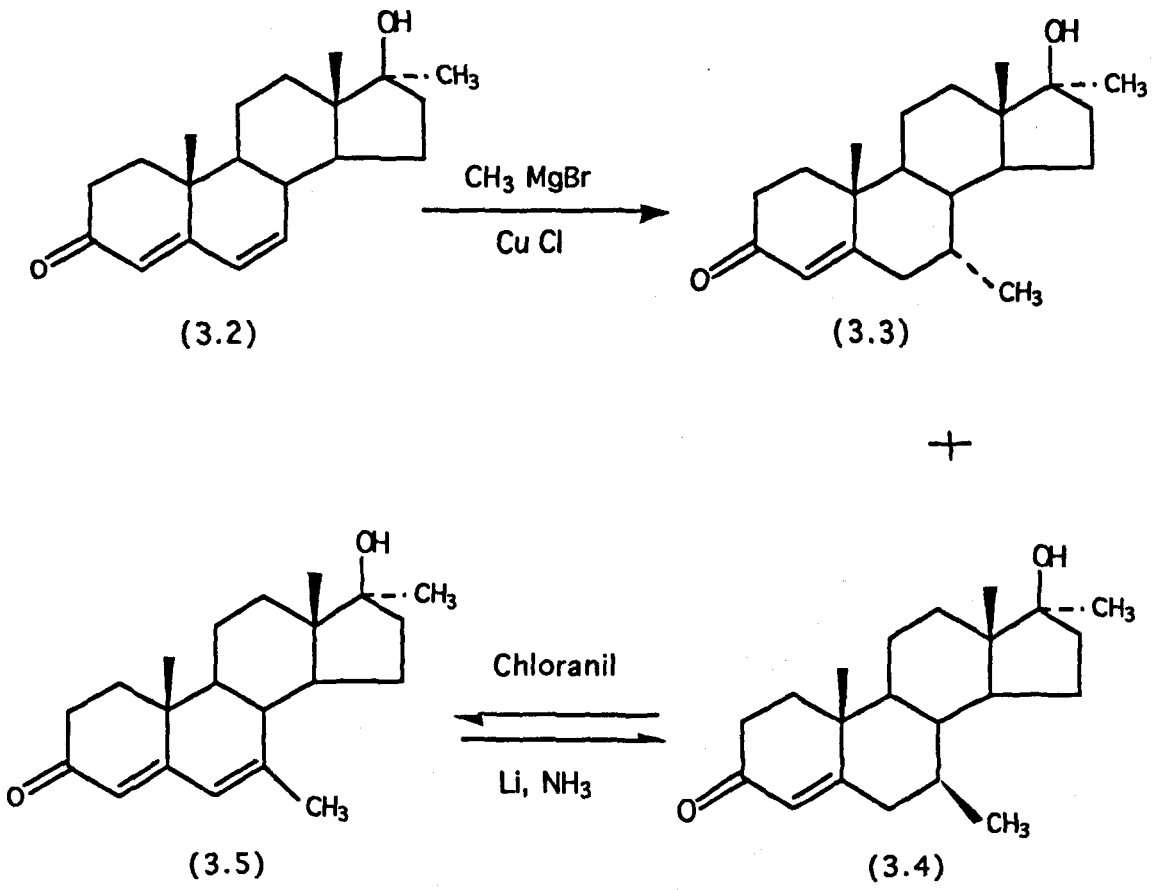


Fig. 3.1

7ß-Methyltestosterone has been prepared by Zderic and Ringold[45] by the addition of methyl Grignard reagent to 7-ketotestosterone ethylene ketal acetate (3.6) (Fig3.2).

When the total crude product of the Grignard reaction was treated with hot aqueous acetic acid , 7-methyl-6-dehydrotestosterone (3.7) was obtained in excellent yield. This was hydrogenated with 5% palladium carbon catalyst in methanol-KOH mixture to give 7ß-methyltestosterone (3.7).

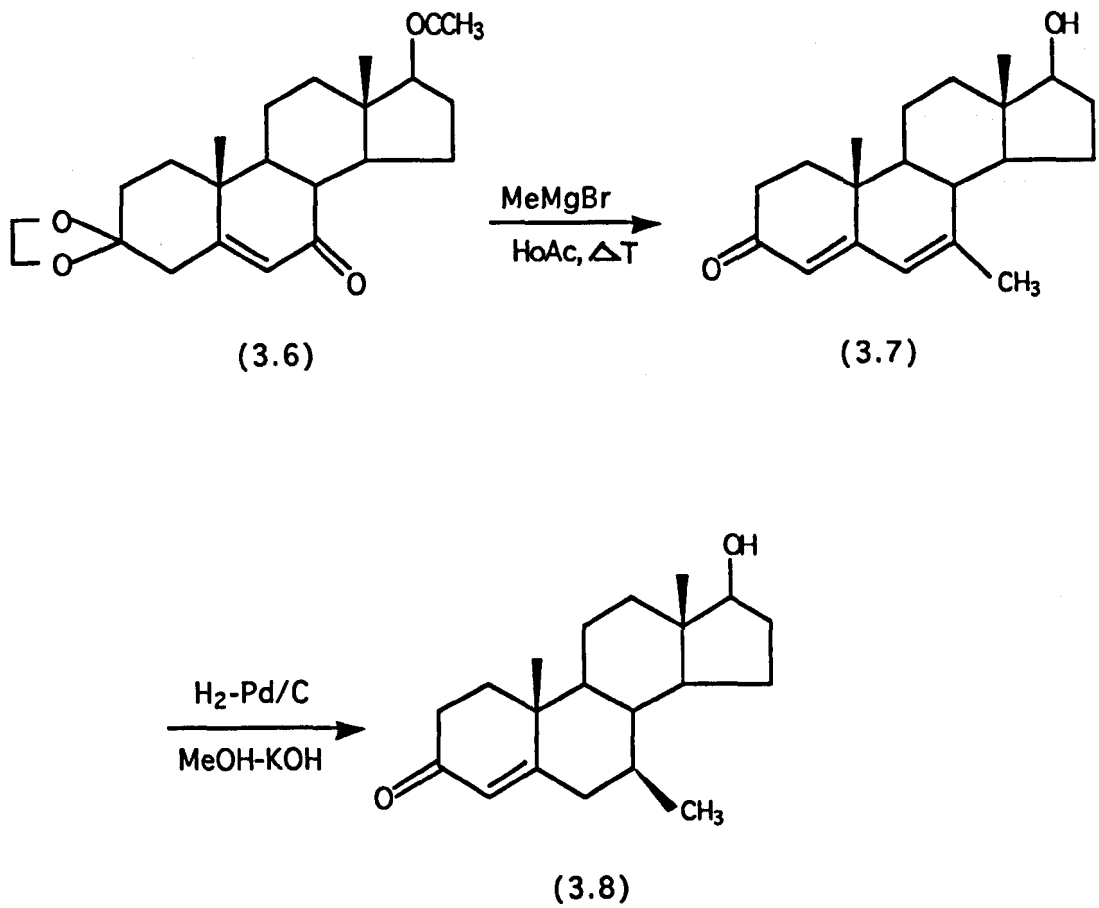
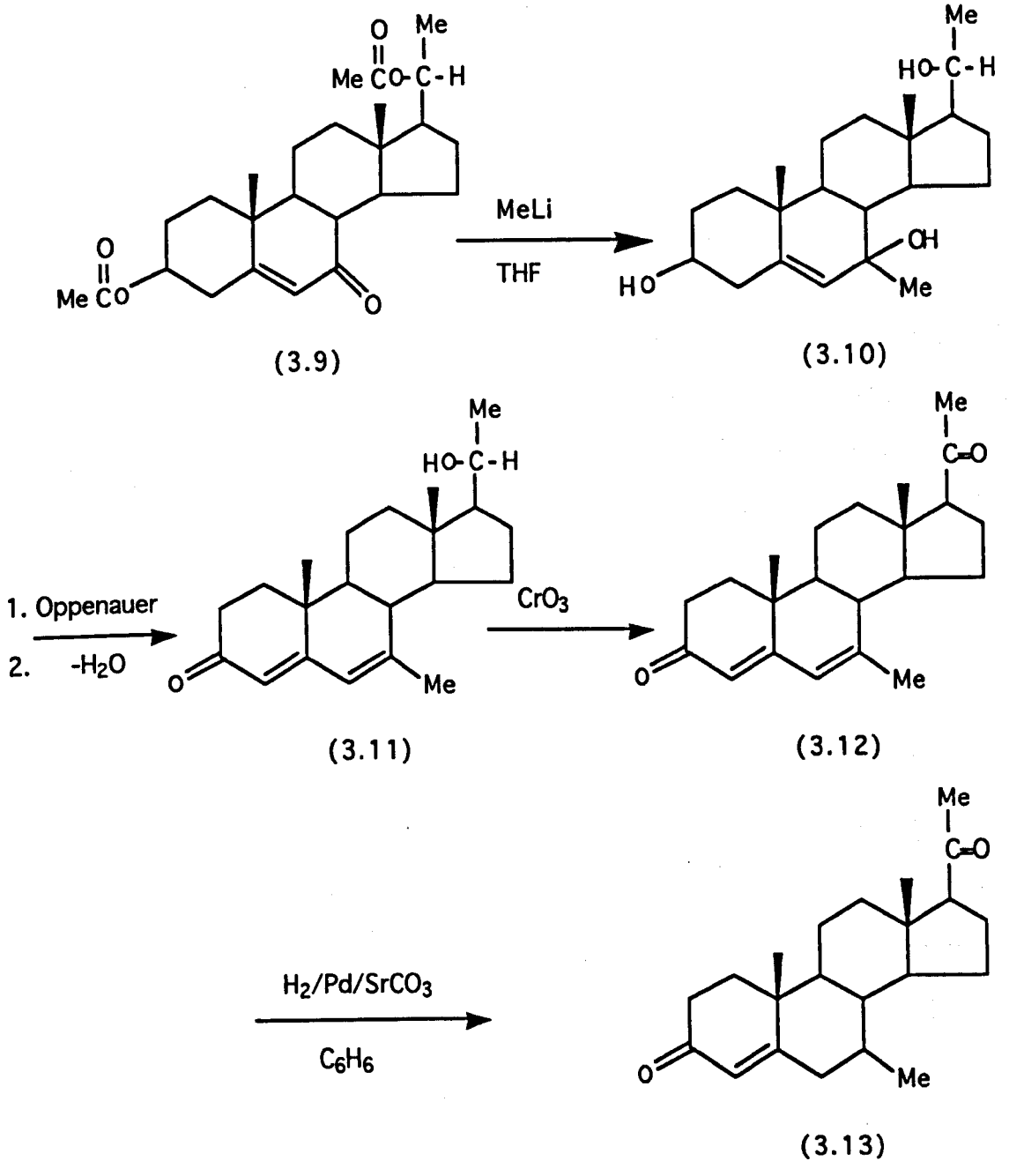


Fig. 3.2

Another method for preparing 7ß-alkylated steroids and 6- dehydro-7-methyl steroids has been described by Robinson and co-workers[46] Fig (3.3). When the 7-ketone (3.9) was treated with methyl-magnesium iodide or, preferably, methyl lithium in ether-terahydrofuran mixtures, compound (3.10) was formed.

This triol underwent Oppenauer oxidation and dehydration to give dienone (3.11) which was oxidised with chromic acid to compound (3.12) 7-methylpregna-4,6-dien-3,20-dione, in good yield. Finally hydrogenation of the diene (3.12) in benzene with palladium strontium carbonate catalyst gave 7ß-methylprogesterone (3.13) in poor yield.



(3.9) (3.10) (3.11) (3.12) (3.13)

Fig. 3.3

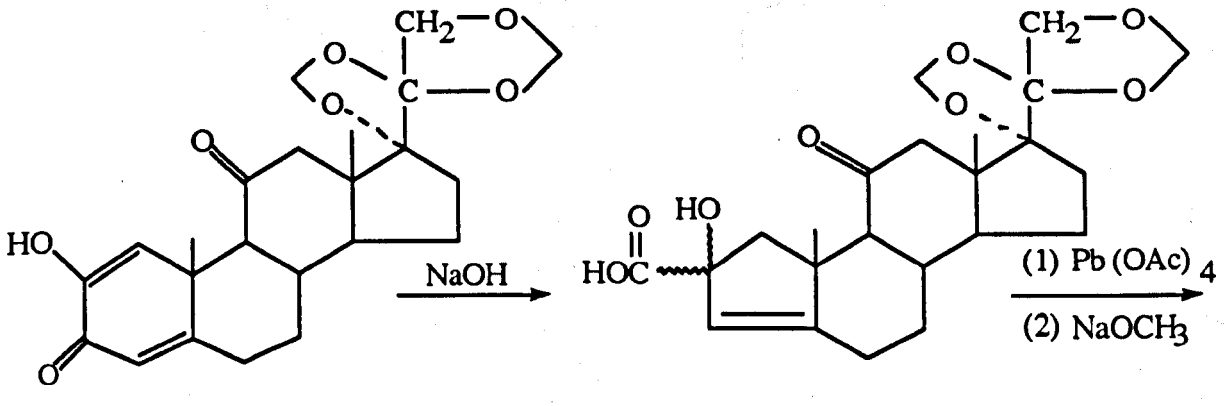
CHAPTER 4

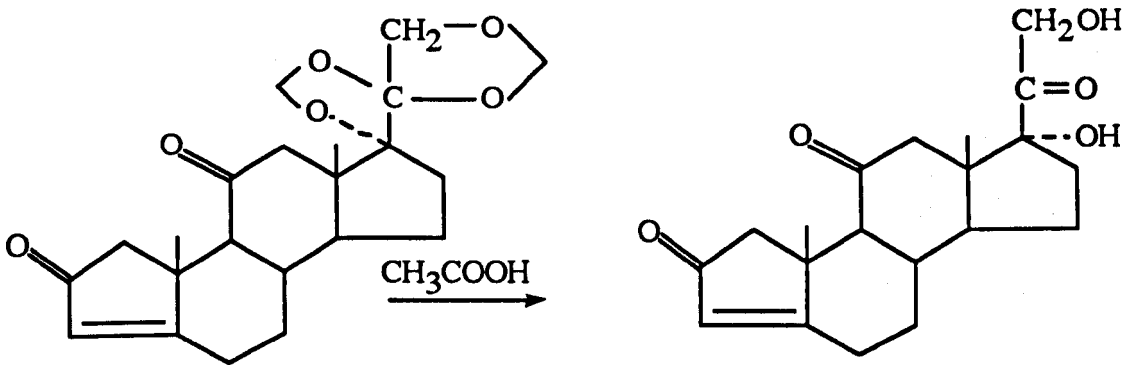
Nor-steroids

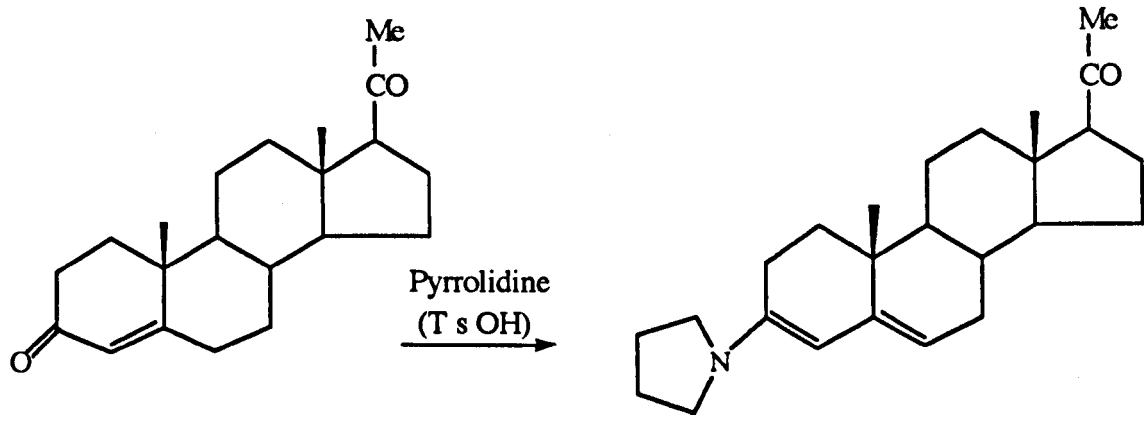
4.1 A- Nor- steroids

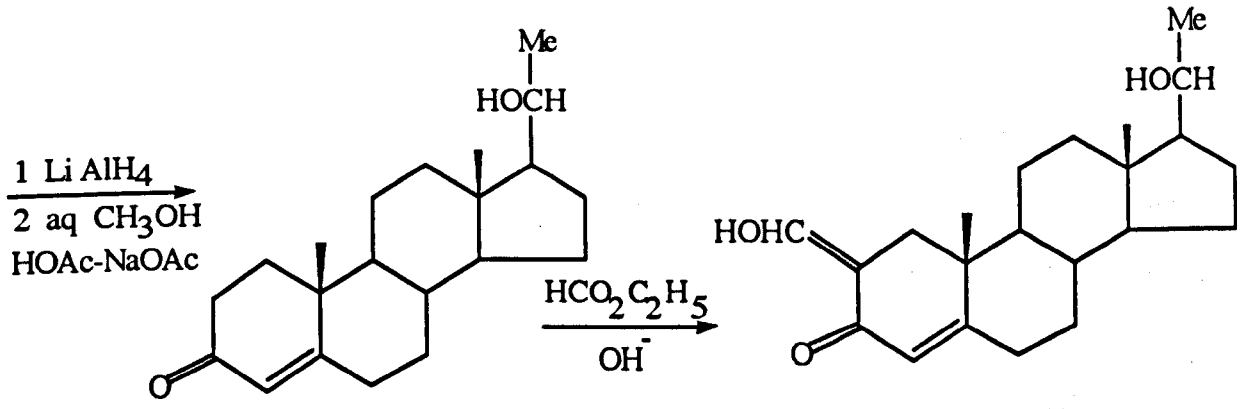
A-nor- steroids with one carbon atom missing from the A ring

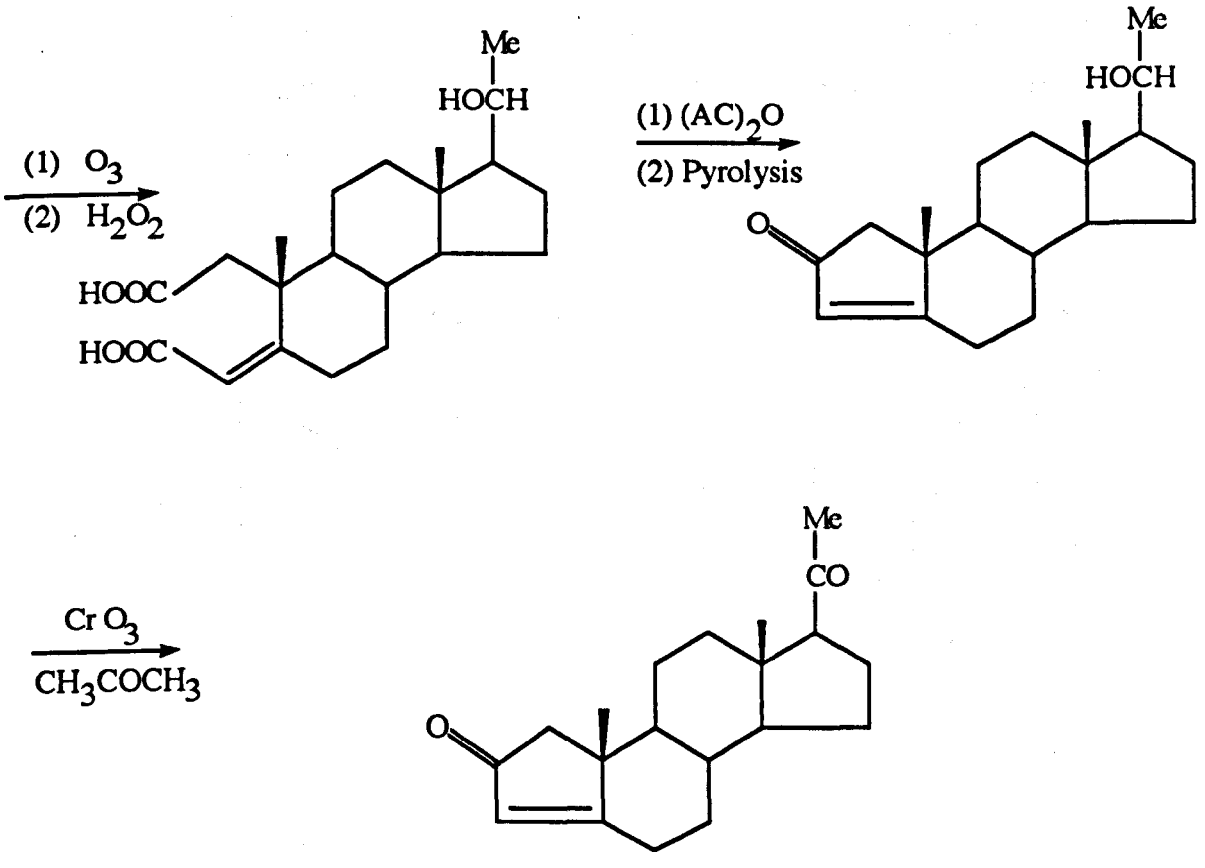
Several A-nor steroids have been prepared by ring contraction procedures carried out on derivatives of testosterone, 19-nortestosterone, progesterone, cortisone and others, and have shown anti-hormonal properties[49]. For example, A-nor-progesterone (4.1) possesses anti-androgenic activity.




CH2—O
O
C
O
O
CH3COOH
CH2OH
C=O
OH


Me
CO
Pyrrolidine
(T s OH)
N


1 Li AlH4
2 aq CH3OH
HOAc-NaOAc
Me
HOCH
HCO2C2H5
OH-
HOHC



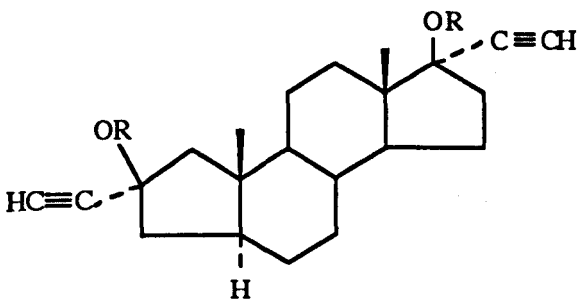
(4.1)

Fig. 4.1

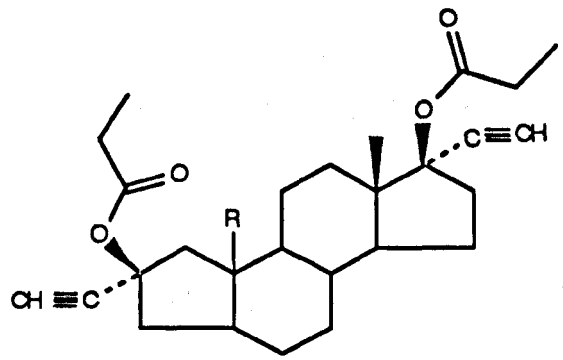
It has been observed by Raynaud et al[50] that a certain class of antiestrogens appear to exert their antagonistic activity from the fast dissociation of the complex they form with the estrogen receptor compared to that of the natural hormone. The relative binding activity of such compounds is high but it declines with prolonged incubation.

Antifertility effectiveness in adult cycling female baboons was investigated using 2∝, 17∝-diethynyl-A-nor-5∝-androstane-2ß, 17ß-

diol (anordrin 4.2) and 2∝, 17∝-diethynyl-A-nor-5∝-estrane-2ß, 17ß-diol (dinordrin 4.3), or its 2ß-ethynyl-isomer (dinordrin II).



(4.2) , R = COEt (Anordrin)



(4.3)

4.2 B-Norsteroids

B-Nor analogues of testosterone, dehydroepiandrosterone, 17∝-methyltestosterone, 4-androstene-3,17-dione, 5-androstene-3ß,17ß-diol, 3∝-hydroxy-5∝-androstan-17-one and 17ß-hydroxy-5∝-androstan-3-one were either extremely weak androgens in chick comb test or fully inactive in biological assay. These findings indicate a considerable decrease in androgenicity as a result of elimination of one carbon atom from ring B. However, the B-nor form of 17∝-methyltestosterone was active as an antiandrogen and marginally active antiandrogens were free B-nor-deoxycorticosterone and its acetate[51].

Potential hormonal activity was sought in various B-norsteroid, analogues of corticosteroids, estrogens and androgens, by examining

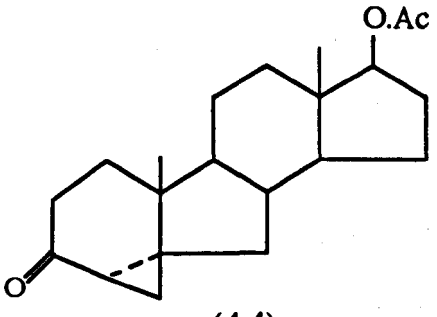
the displacement ability of an appropriate radioligand from receptor binding. The binding properties were compared with parent hormones with normal steroid nucleus. Glucocorticoids B-norcortisol and B-nor-11-deoxycortisol, if compared in terms of their binding to rat liver cytosol, show a decrease in binding ability of about three orders of magnitude relative to corticoids without contraction of ringB.

The binding of 11ß-hydroxy- and 11-oxo-B-norprogesterone was quite negligible in contrast with a relatively high binding of 11ß-hydroxyprogesterone[51].

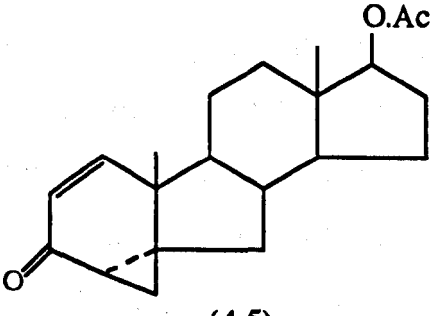
A similar situation was found for estrone and estradiol-17ß and their noranalogues. In B-norestrogens, a lower ability to displace ^{3}H-estradiol-17ß from uterine cytosol receptors was found than in C_{18}-estrogens[52].

4,5-Cyclo-A-homo-B-nor-androstane derivatives (Fig4.2, compounds 4.4-4.6) were assayed in vivo on mice for their androgenic and anti-androgenic activity, and the effect was compared with that of cyproterone acetate. The inhibition of dihydrotestosterone binding to rat prostate cytosol by the compound correlated with the in vivo effects. The antiandrogenic activity[53,54] of 17ß-acetoxy-4∝,5-cyclo-A-homo-B-nor-5∝-androst-1-en-3-one was found to be comparable with that of cyproterone acetate. It was also antirenotrophic but unlike cyproterone acetate it was lacking antianabolic activity and corticoid-like action on relative weight of spleen.

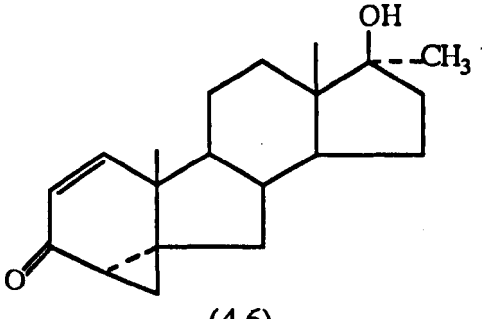
A lower antiandrogenic activity was found for 17ß-hydroxy-17∝-methyl-4∝,5-cyclo-A-homo-B-nor-5∝-androst-1-en-3-one, whilst the remaining three compounds (4.1,4.2,4.3) with saturated ring A were only very weak competitors for dihydrotestosterone binding in prostate and for testosterone action in vivo in accessory sex organs[55].



(4.4)



(4.5)



(4.6)

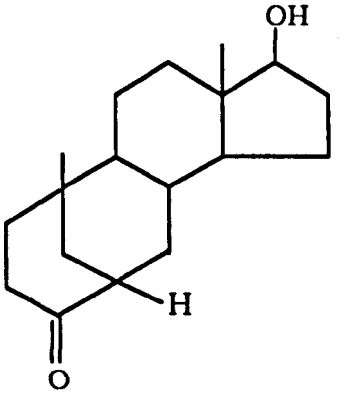
Fig. 4.2

4.3 A- or B- homosteroids

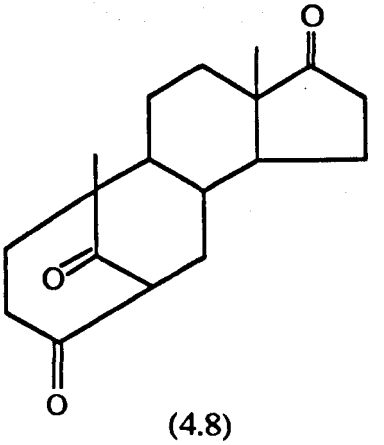
There have only been three reports[56,57,58] on a series of A-homo- and B-homo-androstane and pregnane derivatives (Fig 4.3,compounds 4.7-4.18).

The steroids with expanded rings A or B were tested for potential androgenic or antiandrogenic activity by binding assay on rat prostate cytosol receptors. The binding to sex hormone binding ß-globulin and the inhibition of 5α-reductase was measured as well.

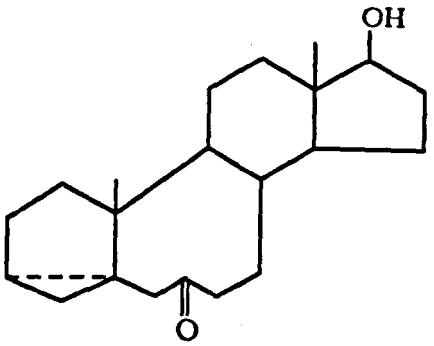
The results of the in vitro screening were compared with in vivo bioassay. Though a very weak binding activity and reductase inhibition was found in several steroids with expanded rings A or B, a weak androgenic rather than antiandrogenic activity was observed in them. It was found that, in general, compounds with deformed planar configuration due to A- or B-ring expansion or to a change in A/B-ring annellation (4.7 and 4.8), do not show much agreement of the bioassay[59] results with the data obtained with screening by in vitro tests for the biological activity[60].



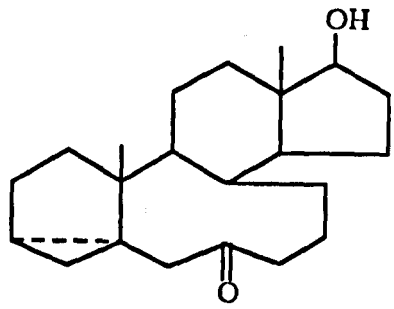
(4.7)



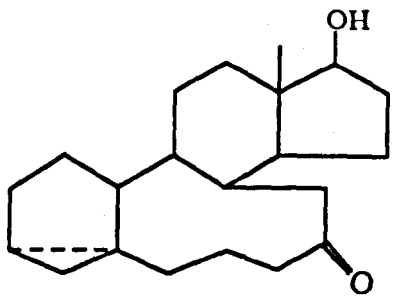
(4.8)



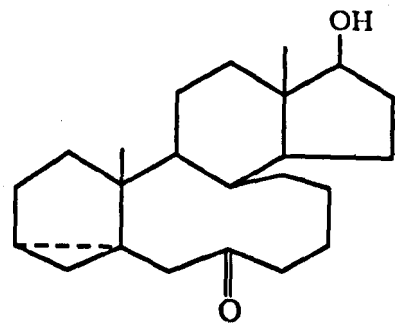
(4.9)



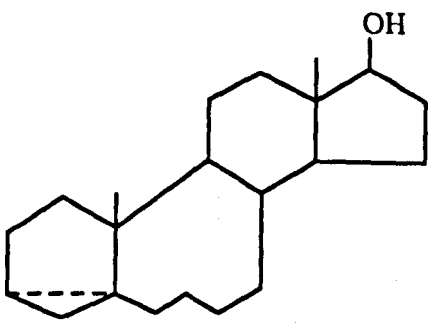
(4.10)



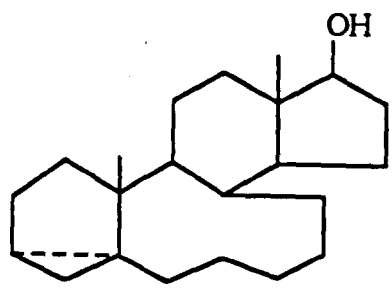
(4.11)



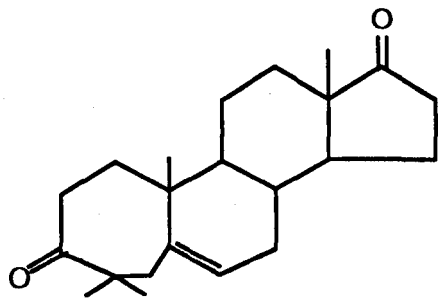
(4.12)



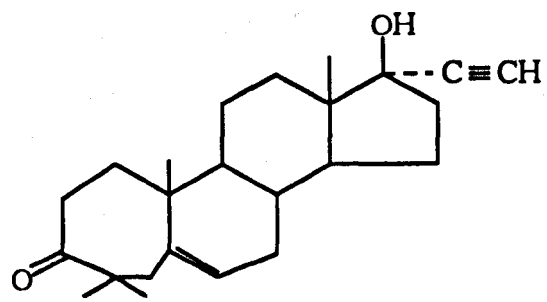
(4.13)



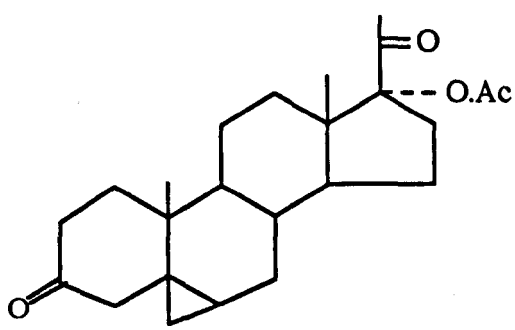
(4.14)



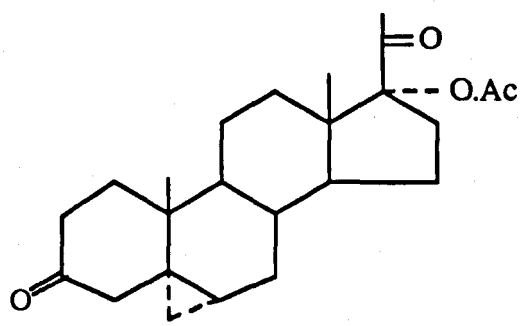
(4.15)



(4.16)



(4.17)



(4.18)

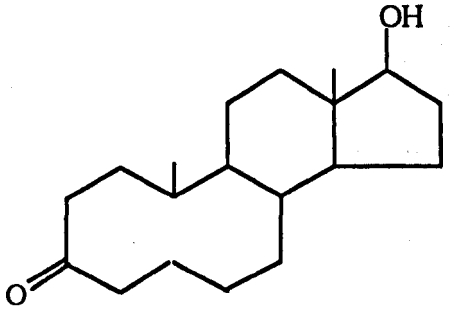
Fig. 4.3

4.4 Secosteroids

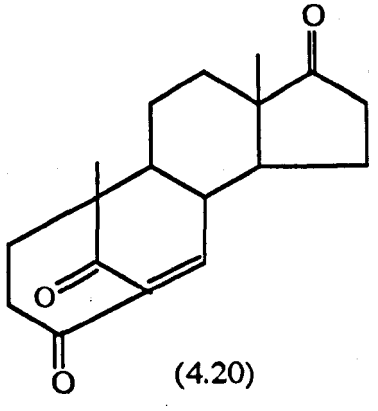
The importance of the features of planarity and molecular rigidity[61] both for receptor affinity and for biological activity was demonstrated in a series of 5,6-seco-and9,11-secoestradiols, representing flexible analogues of the parent prototype/17ß-estradiol. The relative binding affinities of these derivatives were compared with their uterotrophic and anti-implantation activity. A considerable decrease of relative binding affinity was found for d,1-5,6 seco-17ß-estradiol (95% compared with estradiol) and for d-9,11-seco-17ß-estradiol (25.2%) which have an increased degree of rotational and vibrational freedom

in the region of rings B and C compared to that of estradiol. Comparable relative binding affinities of isomers of d, 1-5,6-seco-estradiol (8ß-H 7.95% and 8∝-H 5.06%) are noteworthy, since the latter correspond to 8-iso-17ß-estradiol which has been found to have fairly high receptor affinity[50].

Antiandrogenic activity was tested by bioassay for 3-oxo-5,10-seco-steroids(4.19). Their antihormonal activity is the expression of their irreversible inhibition of 5∝-reductase[62]. They are powerful non-competitive inhibitors of rat epididymal and human sex skin fibroblast 5∝-reductase but very poor inhibitors of 3∝-hydroxysteroid dehydrogenase[63]. For 5∝-reductase the inhibition constant for 5 ,10 seco-estra-4 ,5-diene-3,10,17-trione(4.20) was 5.47mol/l in epididymus and 1.60 mol/l in fibroblasts, and for 5,10,20- trione 0.90 mol/l and 0.53 mol/l per litre, respectively. The former secosteroid has a closely similar conformation to that of 4-androstene-3, 17-dione[64] and for the latter conformational similarity analogous to progesterone was presumed[65].



(4.19)

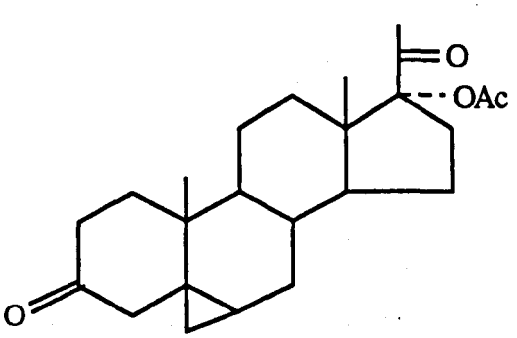


(4.20)

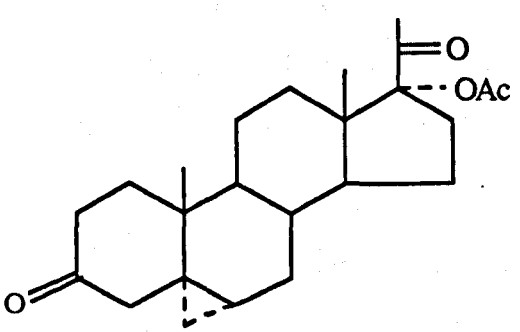
4.5 Other Modifications of Ring A or B.

A 6-membered ring A attached to ring B in position 6 in 17ß-hydroxy-3, 6-cyclo-A-nor-3,5- seco-6ß-androstan-3-one, compound (4.7) caused a high binding to rat prostate cytosol receptors for dihydrotesterone and relatively high antiandrogenic activity[66].

A lower antidrogenic activity was exhibited by its 5-oxo derivatives (4.8) and by two compounds related structurally to cyproterone acetate, namely 17∝-acetoxy-5,7ß cyclo-B-homo-5ß-pregnane-3,20-dione (4.21) and its 5,7∝- cycloisomer (4.22). Various 3,5 and 5,7∝-cyclosteroids were checked for antiandrogenic activity[67] and binding to rat prostate cytosol or sex hormone binding globulin and in some also for inhibition of 5∝-reductase with the aim to find structures similar to cyproterone acetate with comparable useful biological effects[68]. In spite of the demonstration of several relatively potent antiandrogens, cyproterone acetate remains the superior compound among the antiandrogens with a wide application for therapeutic use as testified by numerous publications[69].



(4.21)



(4.22)

4.6 C- Norsteroids

The quest for anti-inflammatory steroids possessing increased activity or lacking side-effects has included several modifications of the hydrocortisone molecule. Of these, the introduction of 6∝- or 16-methyl substituents has been particularly effective[70]. Also the demonstrated enhancement of activity due to the 12∝ -halogen substituent provided further impetus to the synthesis of a steroidal dervative methylated at position 12.

Although several procedures for the insertion of the 12∝-methyl functionality might be envisioned, the addition of a methyl organometallic to an 11ß-12ß-epoxide appeared to be the method of choice. The known diaxial opening of 5,6-epoxide with methyl Grignard reagents suggested that the desired 11ß-hydroxy-12∝ -methyl grouping would be the expected product of such an addition. Furthermore, the anticipated conversion via the 11-ketone to the equatorial 12ß-epimer, which was expected to be the thermodynamically more stable isomer, also would make the 12ß-methyl derivatives available. Accordingly, 11ß,12ß-epoxypregnane-3,20-dione 3,20-bis-(ethylene ketal) was prepared by the following route.12∝-Bromopregnane-3,11,20-trione[71] was converted to the corresponding 3,20-bis(ethylene ketal) with ethylene glycol and *p*-toluenesulphonic acid[72]. Because of the loss of bromine during reduction of steroidal bromo-ketones with certain other hydrides[73,74] lithium borohydride was used in the preparation of the bromohydrin. This compound was not isolated as a crystalline

intermediate, but was transformed immediately to the desired 11ß,12ß-epoxide (4.25).

The action of methyl Grignard reagents in opening 5,6-epoxides has been the subject of several investigations[75], but their action upon other steroidal epoxides has remained largely unexplored. When (4.25) was treated with methylmagnesium iodide in refluxing benzene, a crystalline product was obtained which gave the correct analysis for the desired bisketal (4.26).

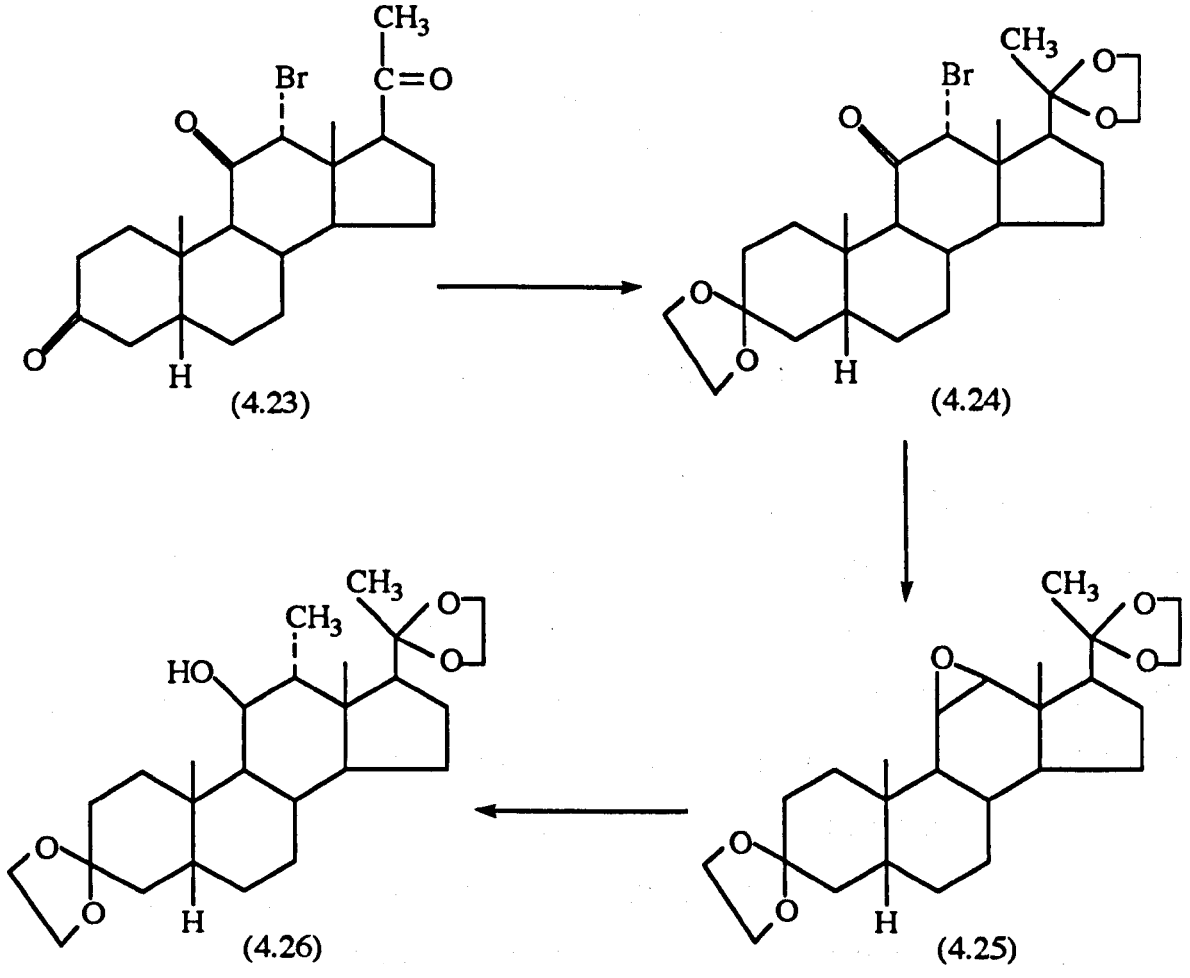
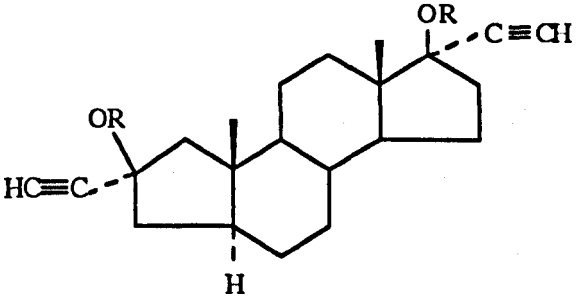


Fig. 4.4

4.7 Anordrin

As early as 1962, Pincus at al., reported that some A-nor-steroids exhibit both anti-progestational and estrogenic activity when administered to rats and mice, opening up possibilities of their use as anti-implantation agents[76-79].



4.27 , R = H (Anordiol)

4.2 , R = COEt (Anordrin)

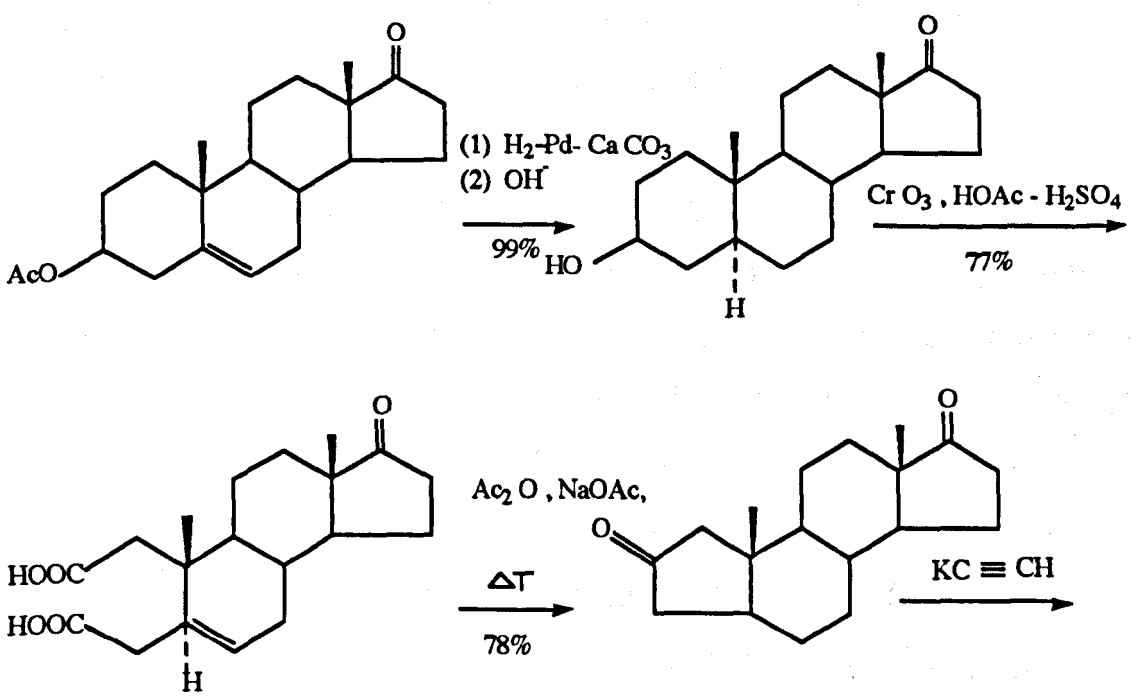
4.29 , R = $COCH_2CH_2CO_2H$

Since the late 1970's, there has been a revival of interest in the biological properties associated with these A-nor-steroids, following publication by Chinese investigators who reported that a significant anti-fertility activity[80] is associated with the dipropionate of 2,17∝-diethynyl-A-nor-5-androstane-2ß,17ß-diol(4.2), used as a mixture of isomers at position 2. It was reported 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[81].

Anordrin has a relatively small estrogenic activity whilst showing anti-estrogen, anti-progestin and anti-androgen character as a result

of competitive binding to the respective hormone receptors. Anordrin and also its bis-hemisuccinate analogue are hydrolysed in vivo to anordiol,(4.27) 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.

The synthesis of anordrin[82] is described in Fig(4.5) involving a reaction between potassium acetylide and A-nor-androstane-2,17-dione (4.30) in which an attack occurs selectively from the alpha face of C-17 due to the steric effect of the 18-methyl group at the less hindered C-2 position, producing a mixture of 2∝-ethynyl (4.2) and 2ß-ethynyl (4.31) isomers.



(4.30)

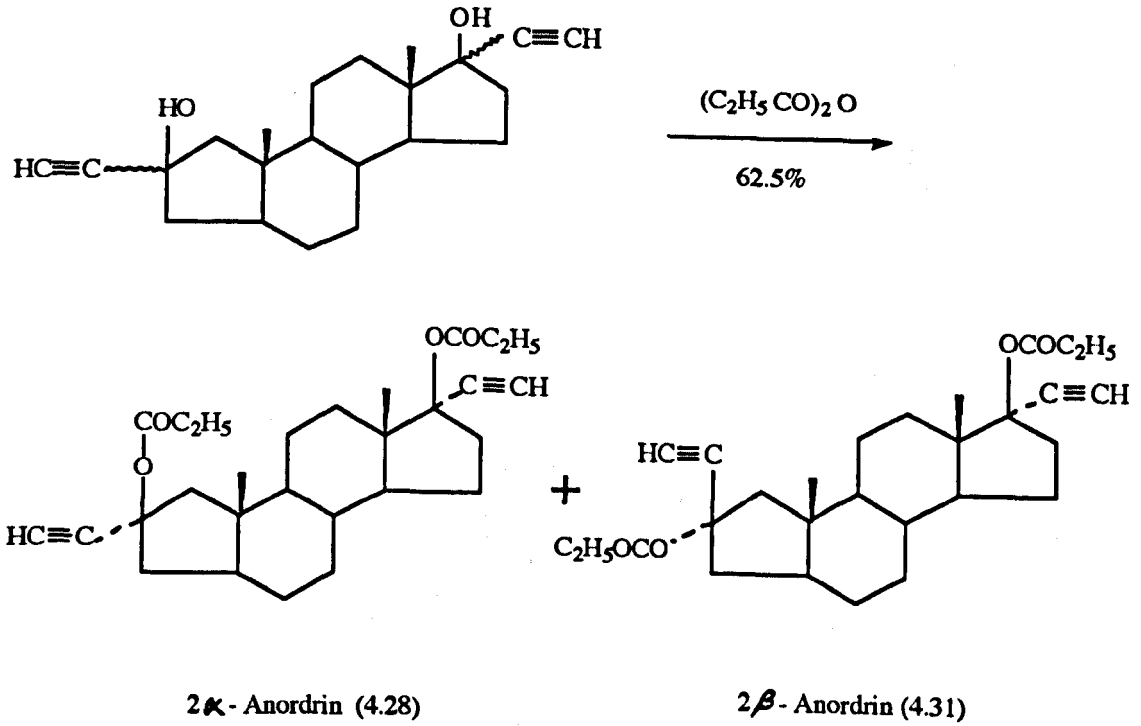


Fig. 4.5

Crabbe et al[83] have prepared the two separate isomers of anordrin and also synthesised the analogous compounds lacking the 19-methyl group, which they named dinordrin I and II.

The starting material for the dinordrins was 19-nor-testosterone (Fig 4.6, compound 4.32), which was converted to 2-keto-dinor-steroid (4.33) by conventional techniques. Oxidation of the 17-hydroxyl group in the intermediate (4.33) provided the corresponding 2,17-diketo-dinor-steroid (4.34) which was treated with an excess of lithium acetylide-ethylenediamine complex to afford a 3:2 mixture of th 2α-ethynyl compound (4.35) and its 2ß-epimer (4.36) separated by preparative TLC. Esterification of the tertiary hydroxyl groups of diols (4.35) and (4.36) with propionic anhydride provided the dipropionates (4.37) and (4.38).

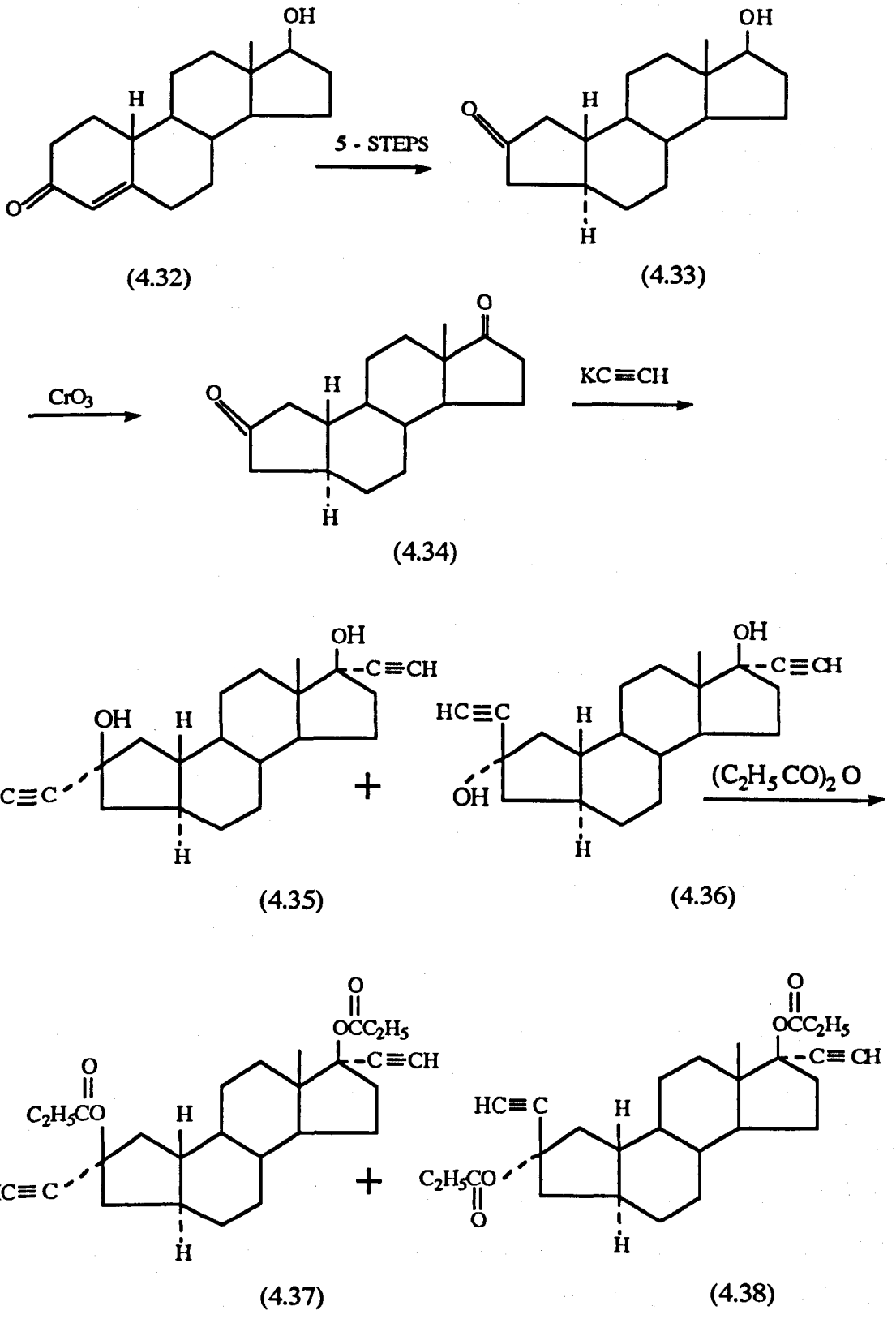

OH
H
O
5 - STEPS
(4.32)
(4.33)
CrO_3
$KC\equiv CH$
(4.34)
$HC\equiv C$
$-C\equiv CH$
(4.35)
(4.36)
$(C_2H_5CO)_2O$
OCC_2H_5
C_2H_5CO
(4.37)
(4.38)

Fig. 4.6

4.8 Postcoital use of anordrin and RU 38486 for prevention of implantation in the rat

A study by Chang, Wang and Bardin[84] was recently carried out to determine whether RU 38486, anordrin or anordiol, alone or in combination, could prevent pregnancy when administered prior to implantation on postcoital day 2.

RU 38486 synergises with anordrin and the diol which is its hydrolysed metabolite to terminate pregnancy . The interaction of these agents was studied at two days postcoitally in the rat. RU 38486 at a dose of 4 mg/kg did not prevent pregnancy, as determined by autopsy 12 days post insemination. A non-effective dose of RU 38486 (2-4 mg/kg) combined with a non-effective dose of anordrin (1.25-2.5 mg/kg) prevented pregnancy in all animals treated and there was no evidence of implantation sites or embryos when the animals were examined on day 12 post insemination. The same synergistic effect was observed when a small dose of RU 38486 (1 mg/kg) was combined with 0.6 mg/kg anordiol.

When investigating pregnancy prevention, animals were treated two days post coitally with 4 mg/kg of RU 38486 and 2.5 mg/kg of anordrin or 2 mg/kg of RU 38486 and 0.6 mg/kg of anordiol, and were killed at short intervals after treatment. These drugs had no effect after 6 hours, but the numbers of embryos in oviducts were significantly reduced 12 hours after treatment. By 24 hours following treatment, no embryos were recovered from either the oviduct or the

uterus. Progesterone and estradiol levels in serum collected 24 hours after treatment were not significantly different from those of controls.

These findings demonstrate that combinations of RU 38486 with either anordrin or anordiol synergise to prevent pregnancy in the rat when administered postcoitally.

CHAPTER 5

STRATEGY FOR THE SYNTHESIS OF POTENTIAL NEW ANTI-PROGESTINS

This research project involves the synthesis of a new steroid which it is hoped will function as an anti-implantation agent.

5.1 BACKGROUND

The first orally active, synthetic progestin was discovered 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.

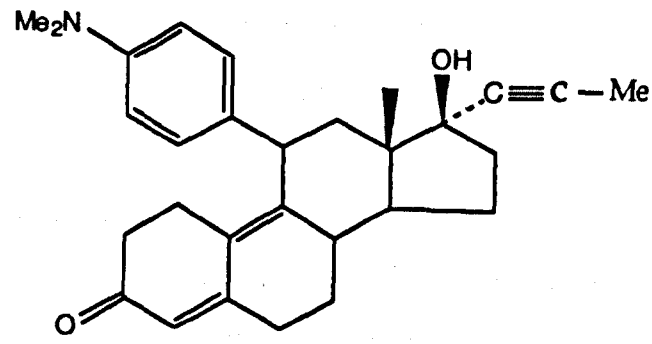
Similarly, combinations of structural features have been very successful in the development of highly potent and selective glucocorticoids. Therefore it seems to be a rational approach to search for new anti-progestins having increased potency, compared with RU 38486, by seeking to combine individual structural features which may contribute to strong, antagonistic binding to the progesterone receptor.

There are a number of different ways to try to identify new drugs which function by interaction with specific biological receptors. The least scientific method is to establish a random screening program. This method is very expensive and does not have a particularly good success rate. However, it is sometimes used by pharmaceutical companies in the absence of other good leads and has the one distinct merit that it may turn up entirely novel structural types since it makes no presumptions about structure- activity relationships.

Another approach is to take an already identified lead compound and make as many as possible of its close structural analogues. This method is also is very expensive but is frequently used by pharmaceutical companies.

Two important developments in the field of post-coital contraceptives have taken place in recent years.

1. The Roussel-Uclaf pharmaceutical company in France discovered that the introduction of a 4 - (N,N-dimethylamino) - phenyl group at the 11-position of certain steroids gives them anti-hormonal properties. In particular, RU 38486 (19) is a potent anti-glucocorticoid and is also an anti-progestin. It is now in widespread clinical use as an anti-implantation agent.



(19)

2. In the Peoples' Republic of China, a compound called anordrin (4.3,R=Me) has been developed and has been used clinically as a "Visiting Pill" or "Vacation Pill". It is popular with many Chinese couples who are assigned work in different towns and meet only *infrequently for short periods.* It is taken for a few days after intercourse and has a high effectiveness, apparently acting as an anti-implantation agent. However, anordrin possesses some estrogenic character which is undesirable, since estrogens are associated with nausea, breast swelling and a possibly increased risk of thrombosis and cancer. The 19-nor-analogue has also been made and is an even more potent anti-implantation agent, known as dinordrin(4.3,R=H).

The primary sequence of the progestrone receptor has been established as a result of cloning and the coding of the cDNA which is responsible for its biosynthesis. The progestrone 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 in to 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 antiprogestins 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 in to a steroid framework will have additive and / or synergistic effects when combined together.

5.2 TARGET DESIGN

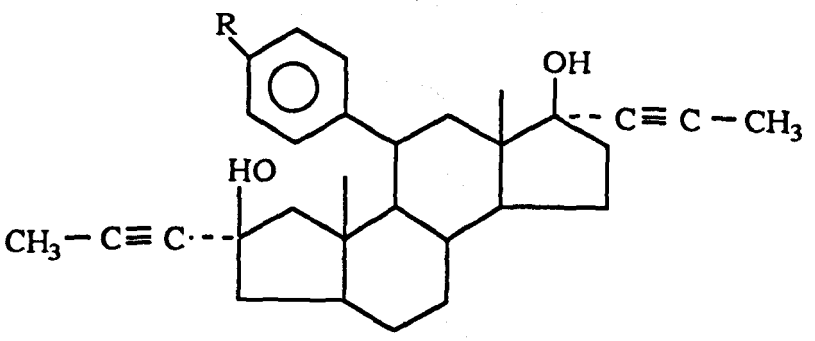
In the present work, the following structural elements have been selected as being important to the goal of developing a new, potent, orally-active anti-progestin:

1. From the very large body of work on both progestins and anti-progestins, it is assumed that 17 ∝ -alkynyl-17ß-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 11ß 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. When looking 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 the 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, an A-nor ring with a 2 ∝ -alkynyl-2ß-hydroxy substitution pattern. Combining these three types of structural elements, it was decided to synthesise a series of A-nor-androstanes having 11ß-aryl,2 ∝ ,17 ∝ -dialkynyl-2ß,17ß-diol substituents (5.1) for investigation of their anti-progestin properties.



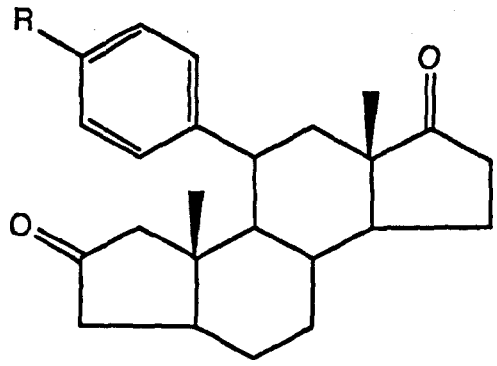
(5.1)

5.3 PROPOSED WORK

The A-nor structure and 11-[4-(dimethylamino)-phenyl] group both independently confer anti-implantation character on the steroid nucleus. There is ample precedent to show that the biological effects of separate functional groups in steroids are often additive (eg. the currently used oral contraceptives and anti-inflammatory steroids were developed by combining favourable substituents).

It is therefore hoped that by combining the A-nor and 11-aryl function a powerful new anti-implantation agent will be obtained.

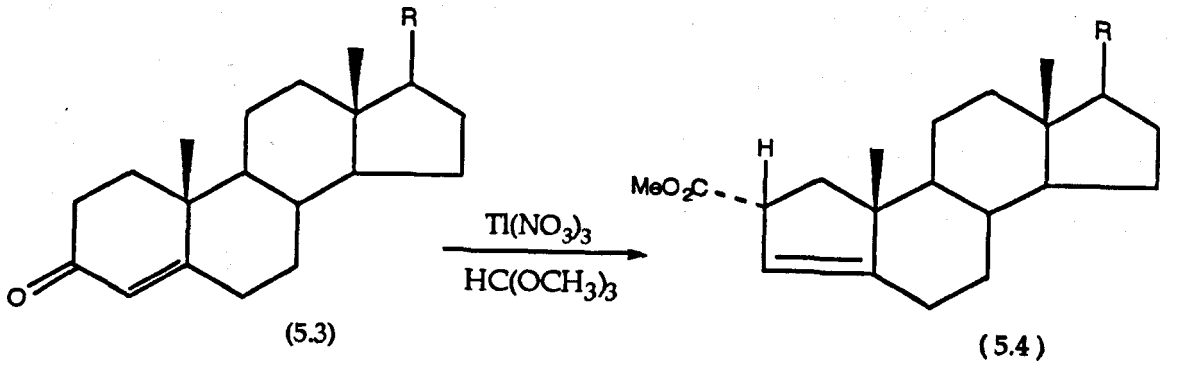
The strategy involves the synthesis of an 11-aryl A-nor diketone (5.2) to which various nucleophiles can be added at the carbonyl groups.



(5.2)

Initially, the steroid(5.2,R=H) will be prepared, because this should be easier to prepare in the absence of a substituent on the phenyl group. By aromatic substitution reactions, a wide variety of substituents, including NMe_2 , can be introduced to give a large series of compounds for testing.

In our own work, a ring contraction method was first examined using the cheap and convenient model compound 11-Ketoprogesterone . An Italian group has reported[85] that this steroidal enone undergoes ring contraction using thallium nitrate to give the A-norester (5.4) in 65% yield.

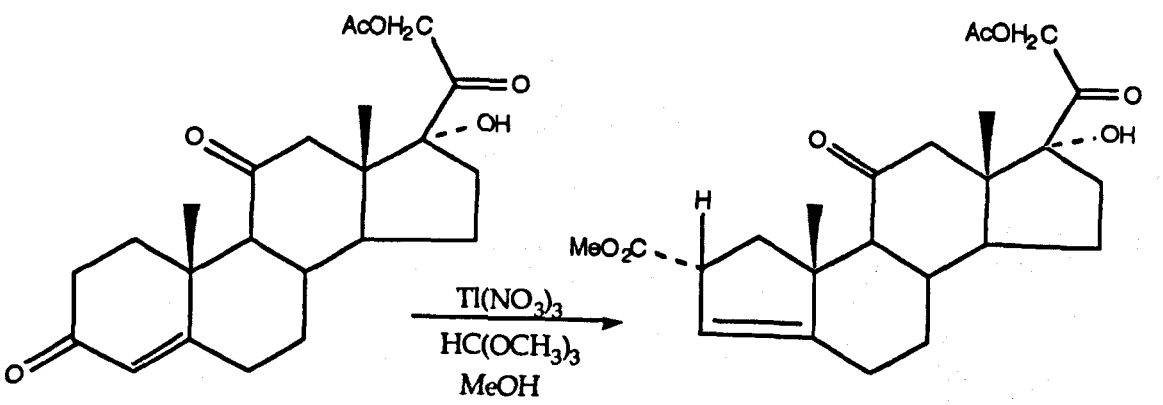


(5.3) (5.4)

R = $COCH_3$ R = $COCH_3$

Fig. 5.1

My colleague, Charles Thomas[86], was able to repeat the reaction, obtaining the A-nor ester (5.4) but only in 25% yield. The structure and purity of this compound (5.4) were confirmed by analytical and spectroscopic data. Unfortunately, the reported literature yield was not reproduced.

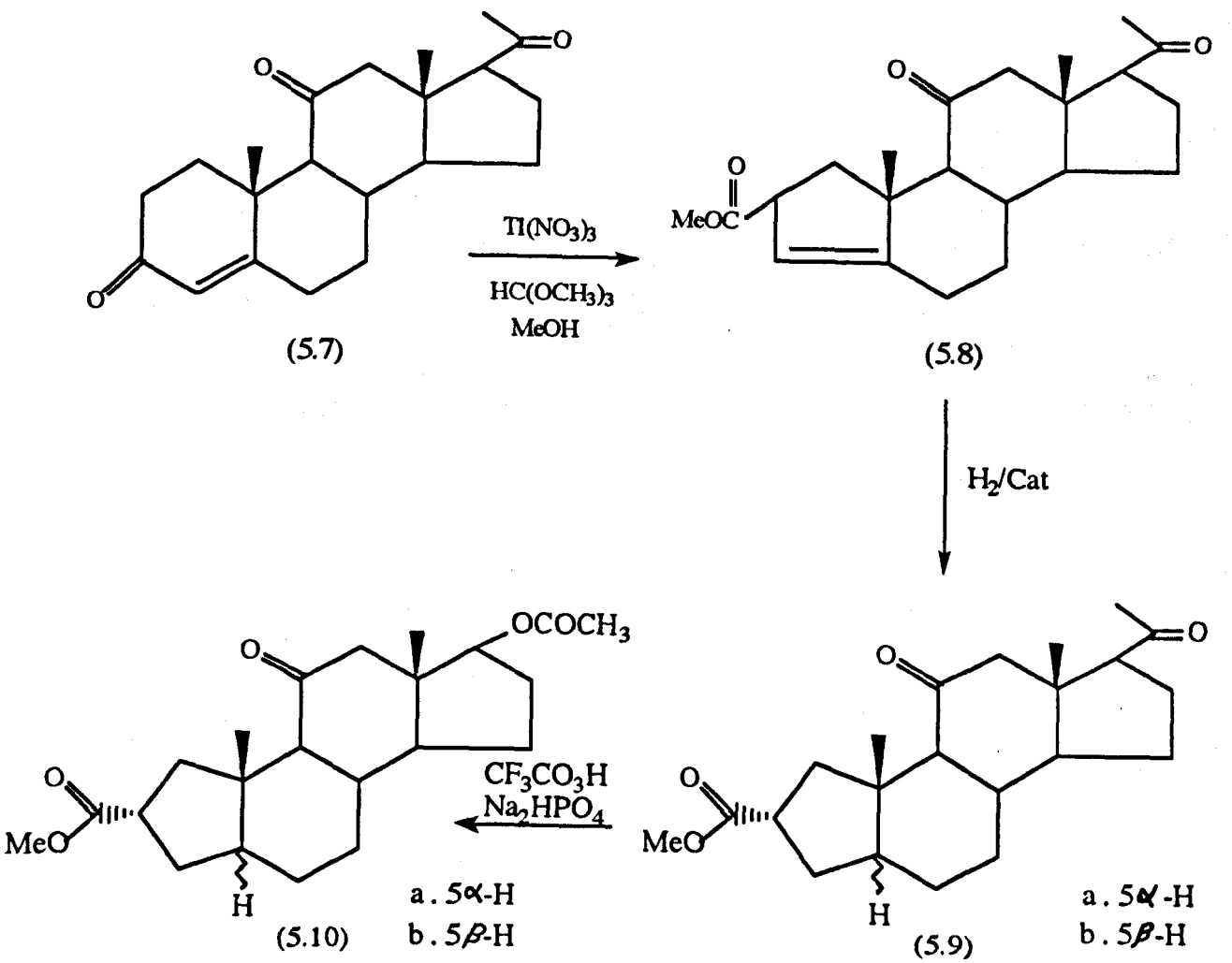


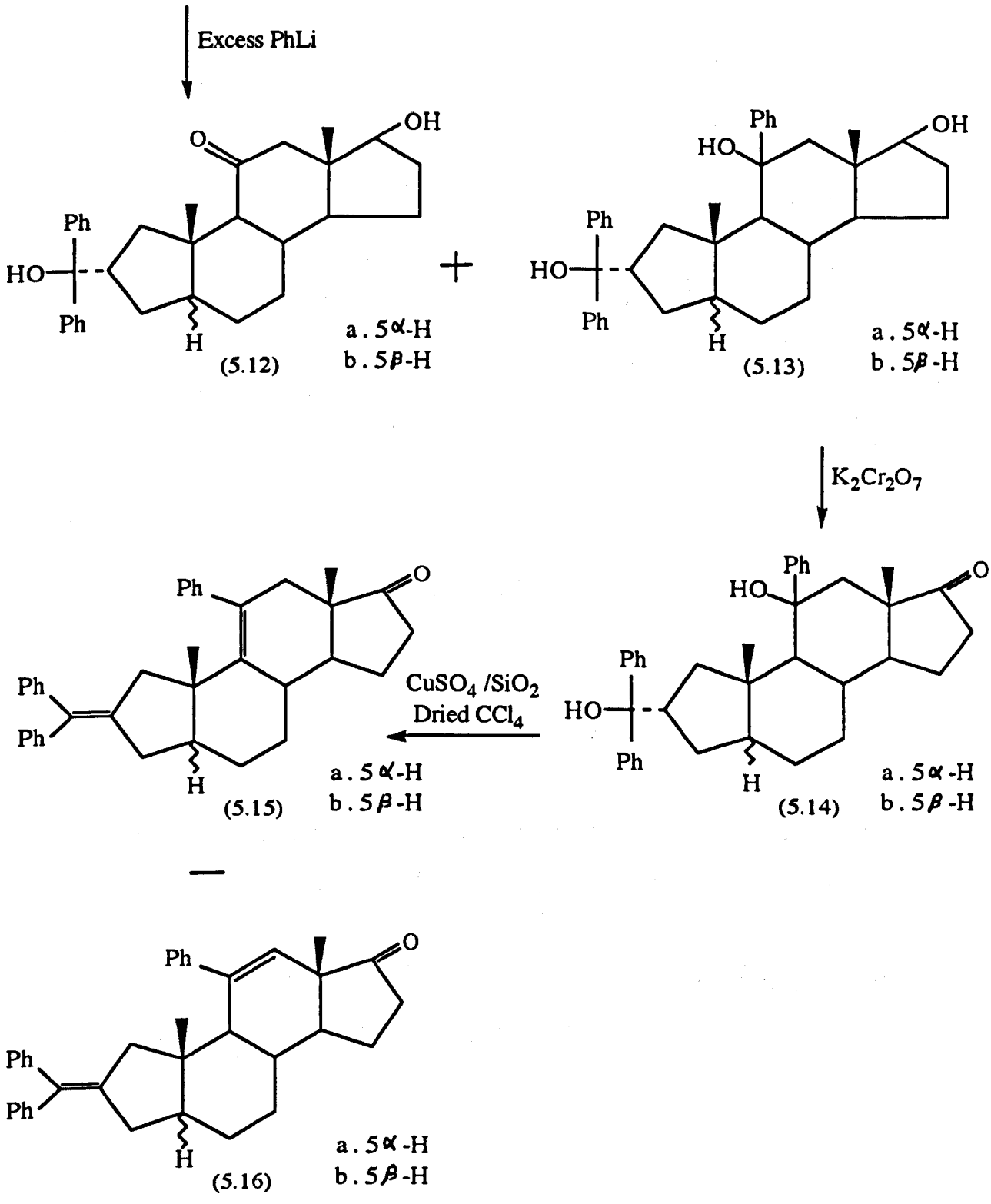
(5.5) (5.6)

Fig. 5.2

Thomas also examined cortisone acetate (5.5) as a model compound, to afford a 45% yield of the ring contraction product (5.6). The reported yield in the literature[87] was 66%. Thomas also carried out the ring contraction of 11-ketoprogesterone(5.7) on a 5g scale, obtaining a yield of 50% of the diketo ester(5.8).

Thomas carried forward the synthesis from the ring-contracted ester (5.8) (Fig 5.3). He was able to obtain a few milligrams of the 11-phenyl A-nordiketone (5.20) (Fig 5.4).




Excess PhLi
OH
O
Ph
HO
Ph
H
(5.12)
a. 5α-H
b. 5β-H
+
Ph
HO
OH
Ph
HO
Ph
H
(5.13)
a. 5α-H
b. 5β-H
K2Cr2O7
Ph
HO
O
Ph
HO
Ph
H
(5.14)
a. 5α-H
b. 5β-H
CuSO4 /SiO2
Dried CCl4
Ph
O
Ph
Ph
H
(5.15)
a. 5α-H
b. 5β-H
—
Ph
O
Ph
Ph
H
(5.16)
a. 5α-H
b. 5β-H

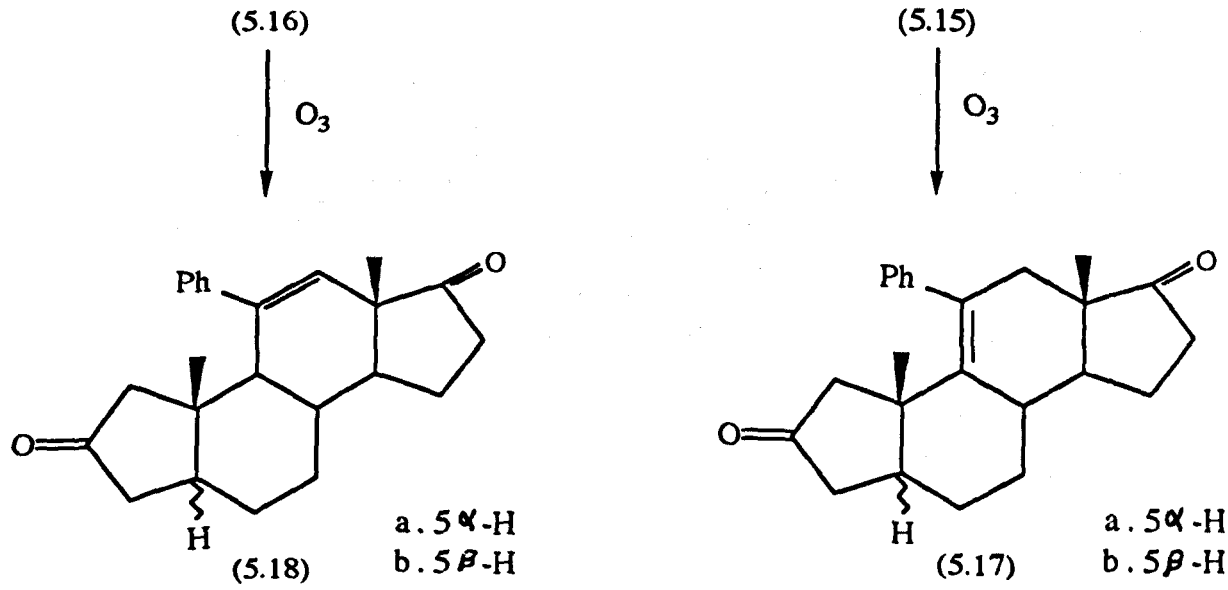


Fig. 5.3

The overall objectives of my work were therefore:

1) to carry out the reaction sequence on a much larger scale, in order to obtain large quantities of the 11-phenyl steroidal enediones (5.18).

2) to investigate methods for the selective reduction of the double bond in (5.18b) (Fig 5.4) in order to obtain the required11ß-phenyl substitution.

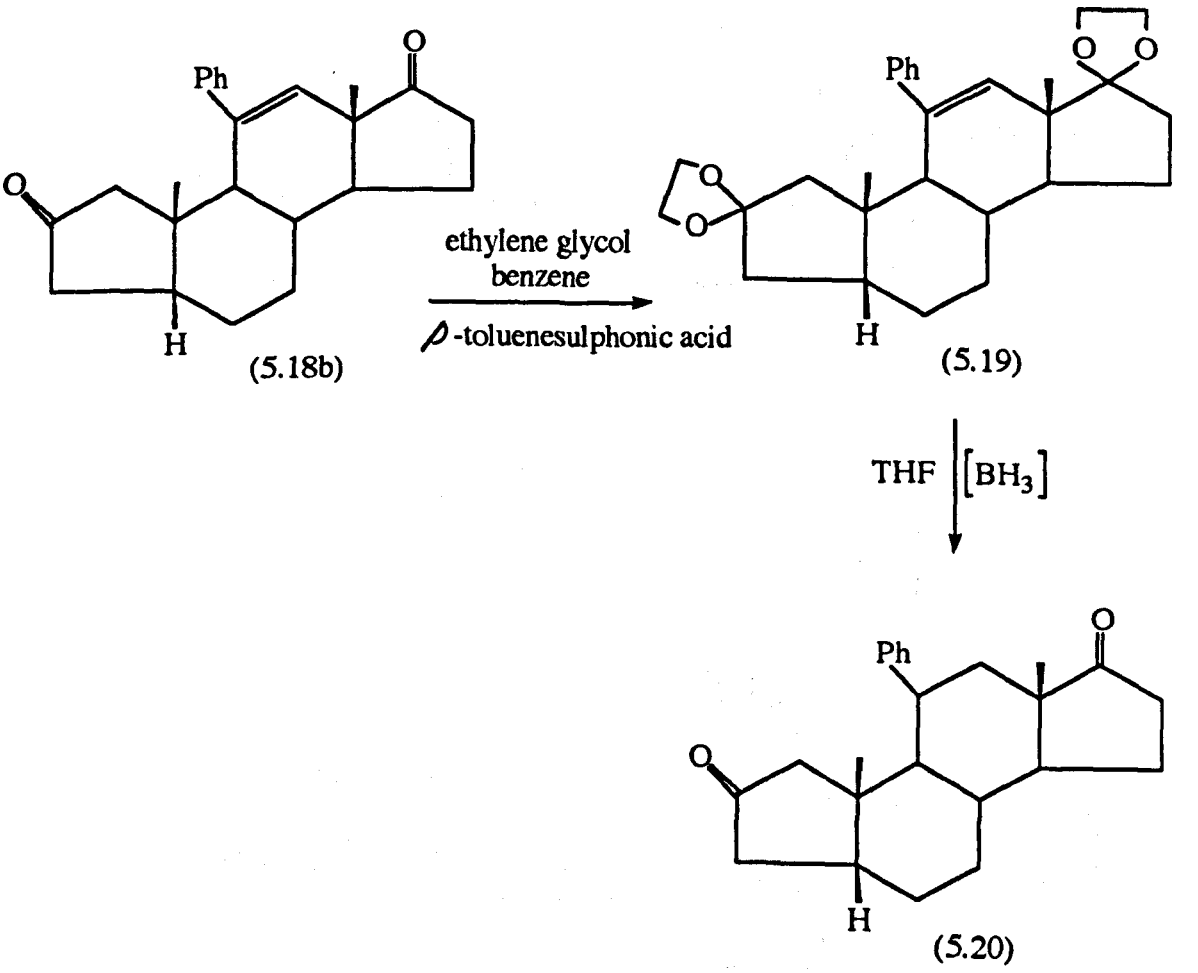


Fig. 5.4

3) to convert this intermediate11ß-phenyl diketone into a series of substituted analogues for biological testing (Fig 5.5).

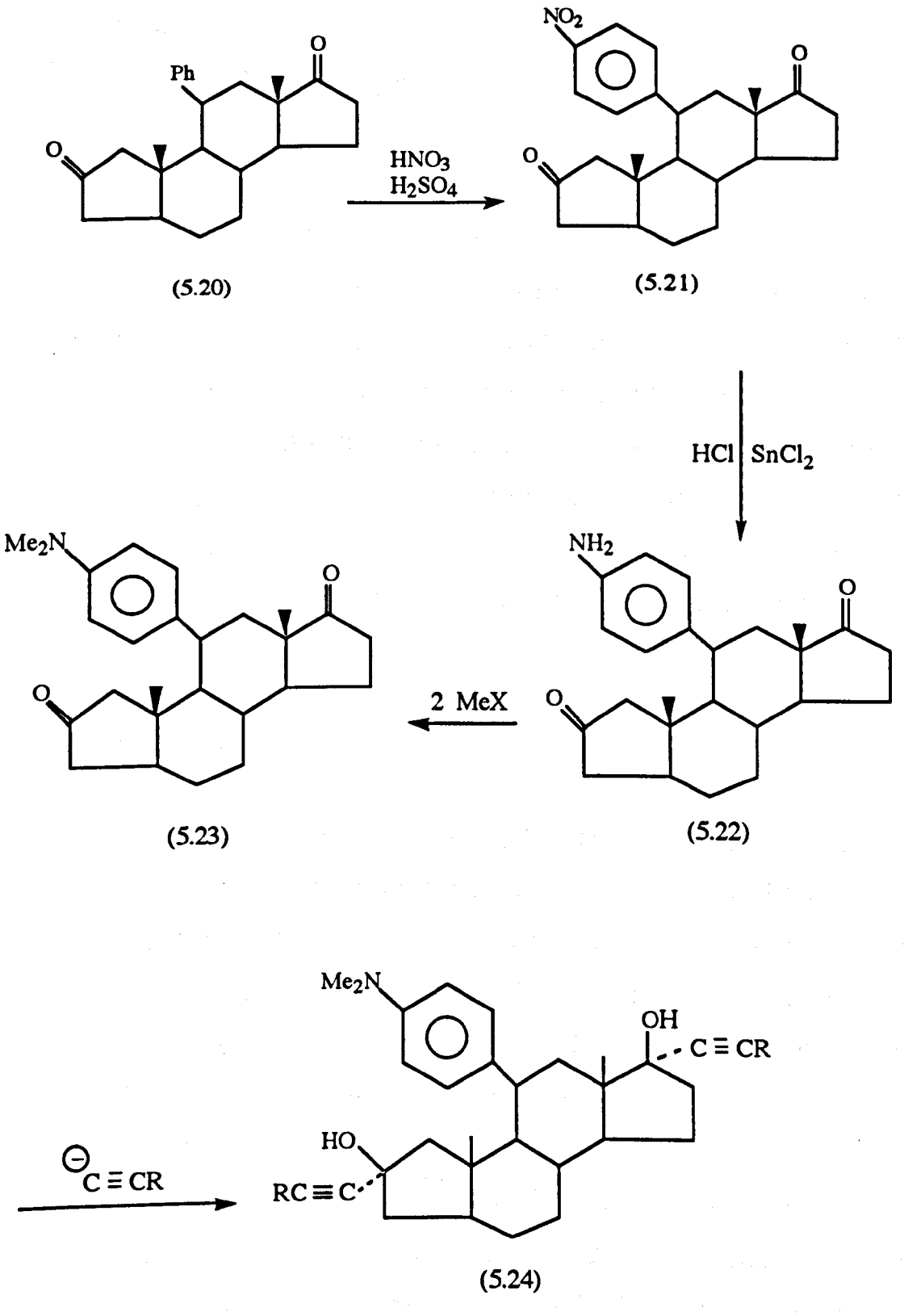

Ph
O
O
HNO3
H2SO4
NO2
O
O
(5.20)
(5.21)
HCl SnCl2
NH2
O
O
Me2N
O
O
2 MeX
(5.22)
(5.23)
Me2N
OH
C ≡ CR
HO
RC ≡ C
⊖C ≡ CR
(5.24)

Fig. 5.5

CHAPTER 6

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

6.1 Introduction

The method developed by C.Thomas[86] in this laboratory was repeated on a larger scale, carrying out the ring contraction of 11-ketoprogesterone, using thallium nitrate in a mixture of trimethyl-orthoformate and methanol to give the A-norester (5.8).
This appeared to be a satisfactory method for the introduction of the A-nor-structure. For this reason, it was necessary to scale up this reaction even further to make a large amount of the ring contraction steroid (5.20) as an intermediate for the synthesis of the target compound.

6.2 Results and Discussion

The ring contraction reaction on 11-ketoprogesterone (5.7) was successfully carried out on a large scale The purity and structure of the 11-keto A-norester (5.8) was confirmed by analytical (TLC) and spectroscopic (IR, NMR, MS) data. The ^{1}H NMR spectrum of the reaction product (5.8) showed a broad lH singlet at 5.10 ppm and a 3H singlet at 3.55 ppm (CO_2 Me). Also there were bands at 0.57 (CH_3) ppm and 1.10 (CH_3)ppm. A singlet at 2.11 ppm was attributed to the CH_3 next to the carbonyl group[85]. In addition, the infra-red spectrum showed a saturated ester at1724 cm^{-1} . The mass spectrum of the reaction product showed a peak at m/z 358 corresponding to the required molecular ion.

The mechanism of the ring contraction reaction is shown in Fig 6.1.

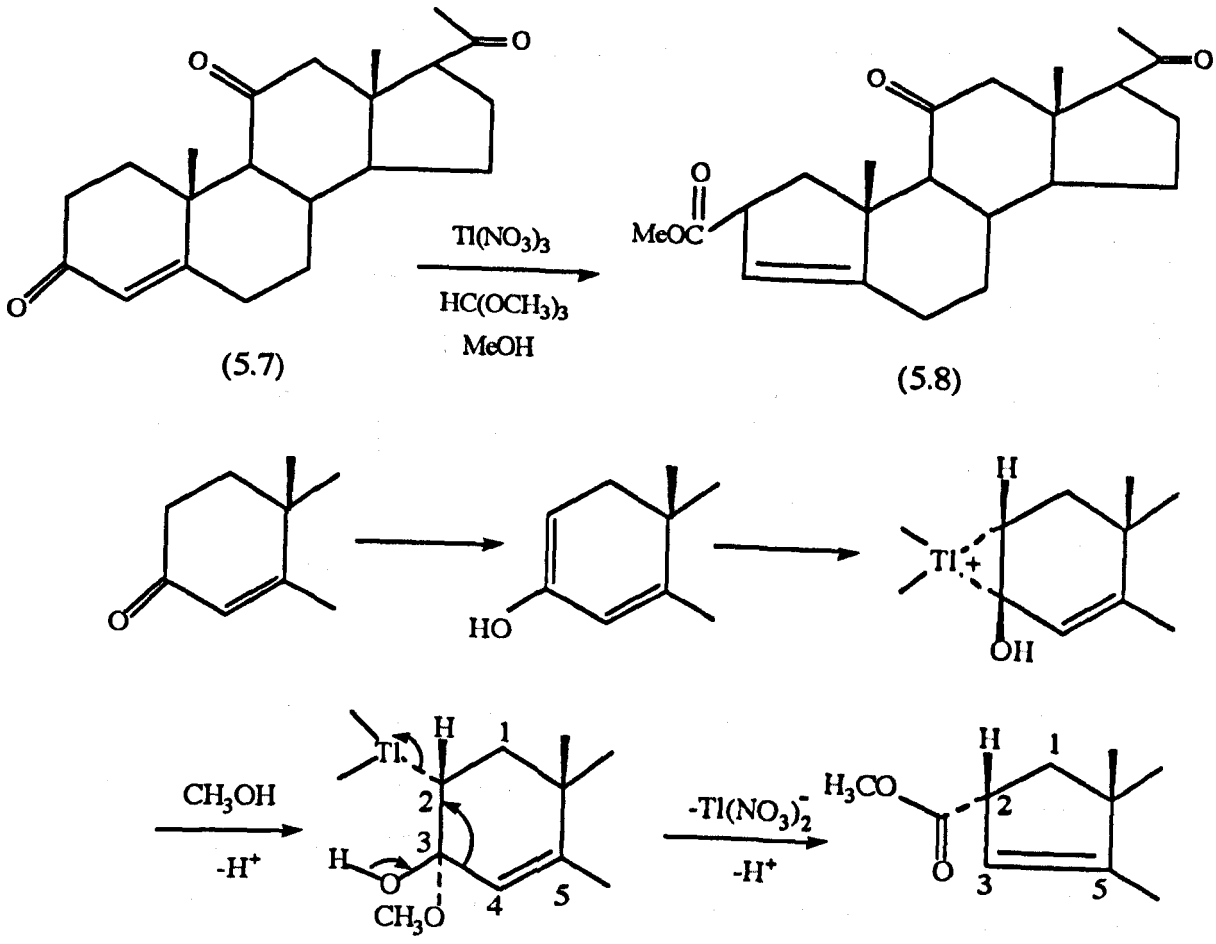
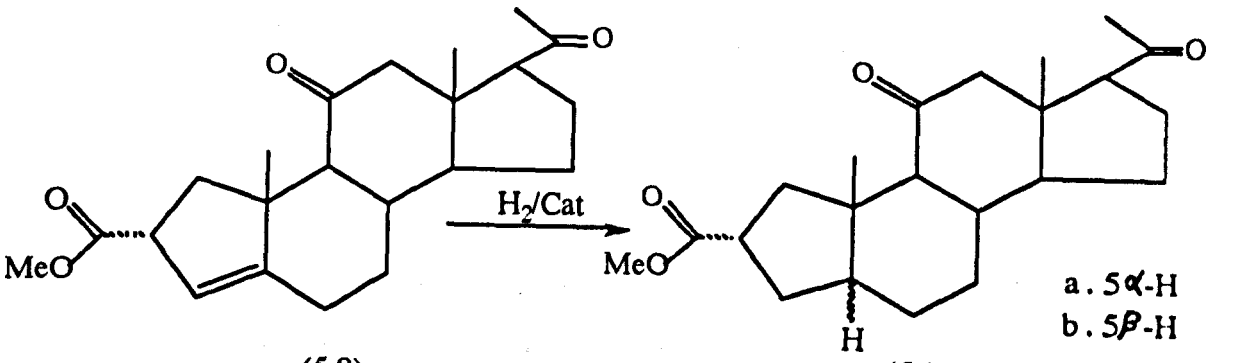


Fig. 6.1

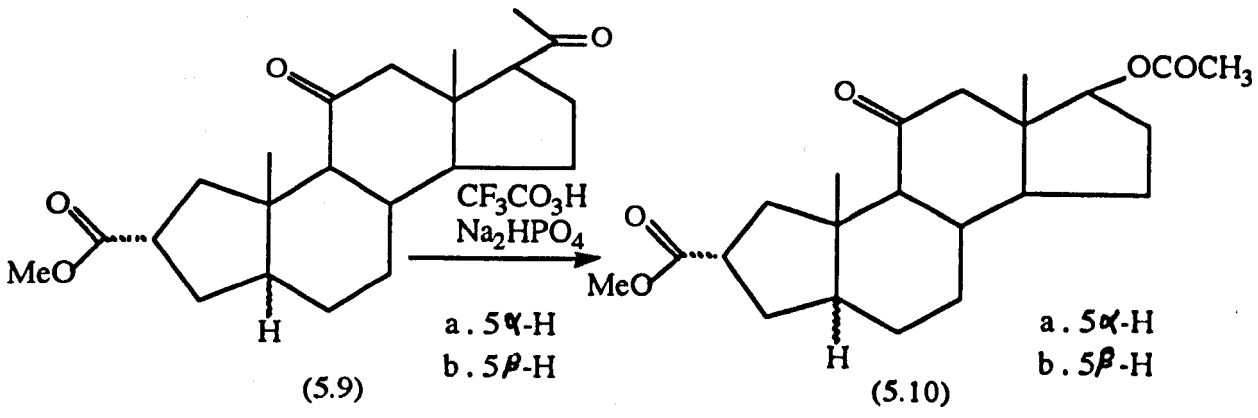
In the next step, compound (5.8) was hydrogenated with palladium on activated charcoal in absolute ethanol and acetic acid (2:1) for two days, affording compound (5.9) in good yield (85%). Catalytic hydrogenation of a steroidal 4-ene normally proceeds from the α -face, due to the steric effect of the ß-methyl groups. Therefore, the A-nor-3-ene (5.8) would be expected to give the trans ring junction. The structure of compound (5.9a) was supported by its ^1H NMR and mass spectra. However, the NMR spectrum of the hydrogenation

product was difficult to analyze in the region of the H-5 proton due to the close proximity of various signals. The mass spectrum of the reaction product showed a molecular ion peak at m/z 360.



(5.8) (5.9)

Considerable effort was required to establish conditions suitable for the Baeyer-Villiger oxidation of compound (5.9) to the acetate (5.10). A variety of procedures employing perbenzoic acid or m-chloroperbenzoic acid, with and without acid catalysts, were examined without success. In general, either unchanged starting material or complex mixtures of products were isolated. Finally, it was found that treatment of compound (5.9) with freshly prepared peroxytrifluoroacetic acid[88,89] in methylene chloride containing a slurry of sodium phosphate as a buffering agent, gave the acetate (5.10) in 80% yield.



The identity of compound (5.10) was confirmed by its ^1H NMR spectrum, which showed a new signal at 4.68 ppm due to the 17-H of the 17 acetate.

The mechanism of the Baeyer-Villiger reaction has been discussed in the literature[90,91] and the reaction proceeds through decomposition of the peroxytrifluoroacetic acid-ketone adduct (5.11) (Fig6.2), which by loss of the trifluoroacetate anion and the concerted migration of an alkyl group yields the ester (5.10). The effectiveness of peroxytrifluoroacetic acid in this reaction is due to the facile heterolysis of the oxygen-oxygen bond induced by the highly electronegative trifluoroacetate substitutent.

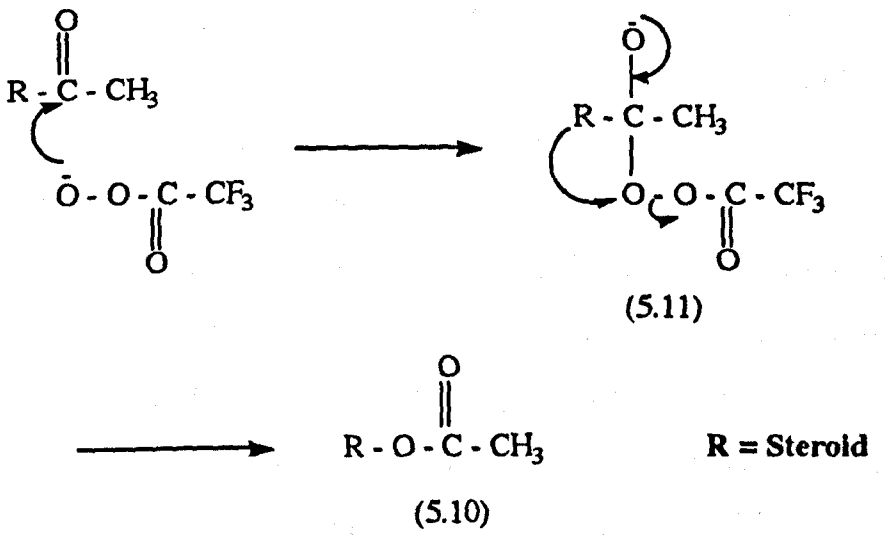
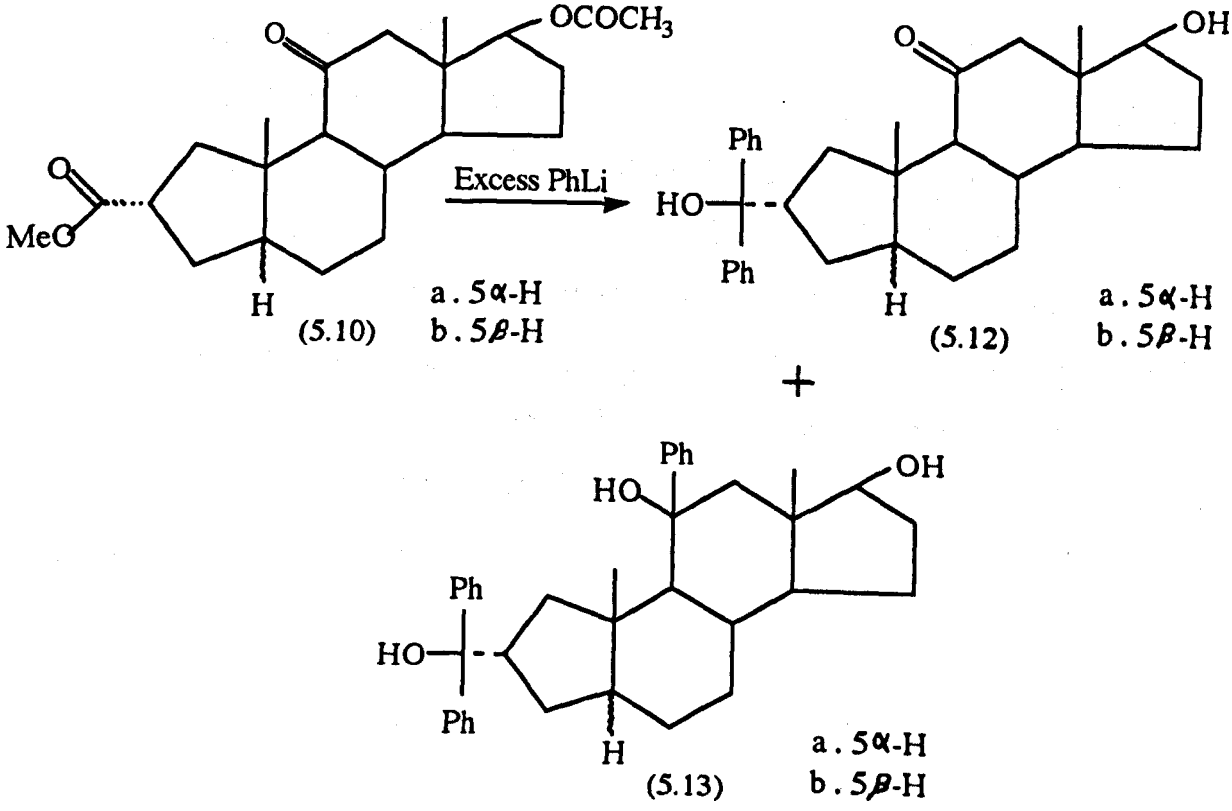


Fig.6.2

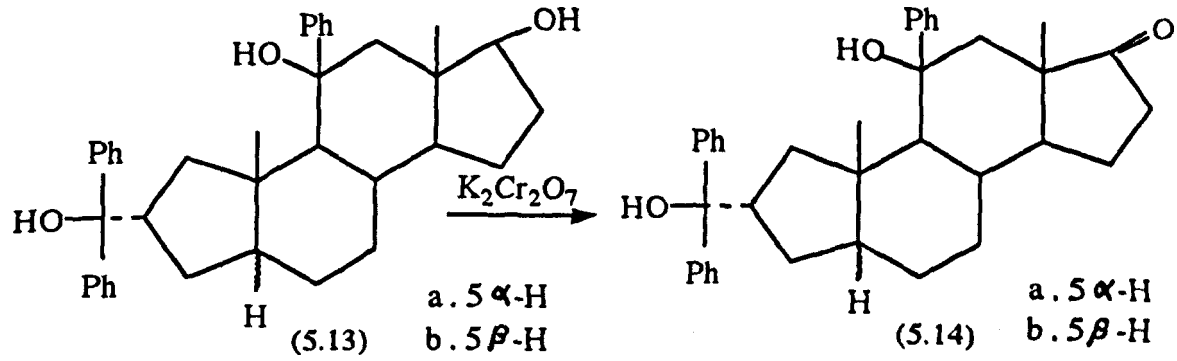
It has been established that structural features in the migrating group which are best able to accommodate a positive charge facilitate the rearrangement. This is due to the build up of positive charge on the peroxy oxygen in the transition state, as the leaving group departs, the relative ease of electron release favouring alkyl migration in the order 3°>2°>1°. Therefore on oxidation of compound (5.9), the steroidal (secondary) group is the one that migrates, giving an acetate ester rather than methyl ester.

The treatment of the 11-ketodiester (5.10) with excess phenyl lithium[92] gave a mixture of compounds (5.12) and (5.13) in a ratio of about 5:6, which were separated by preparative T.L.C. The structures were assigned on the basis of their spectroscopic data. Although in the mass spectra of both compounds their molecular ions were not present, in both cases, $M^+ - H_2O$ ions were observed. Structure (5.12) was further confirmed by its ^{13}C NMR spectrum, showing a signal at 210 ppm due to the carbon of the C=O group.

When the reaction was repeated and the temperature was increased from -78 to 50 °C after the addition of compound (5.10) and also using 100% excess PhLi, there was no change in yield of compounds (5.12) and (5.13). When pure compound (5.13) was further treated with more PhLi at -78 °C, a mixture of compounds (5.13) and (5.12) was obtained. When organolithium reagents react with a carbonyl compound, there exists a competition between addition, reduction and enolization, and at -78 °C reduction and enolization are minimized. But in this case the only possible competition would be between addition and enolization simply because of the nature of the organolithium used, i.e. PhLi, a ß -hydrogen is required in the organolithium for reduction to take place.

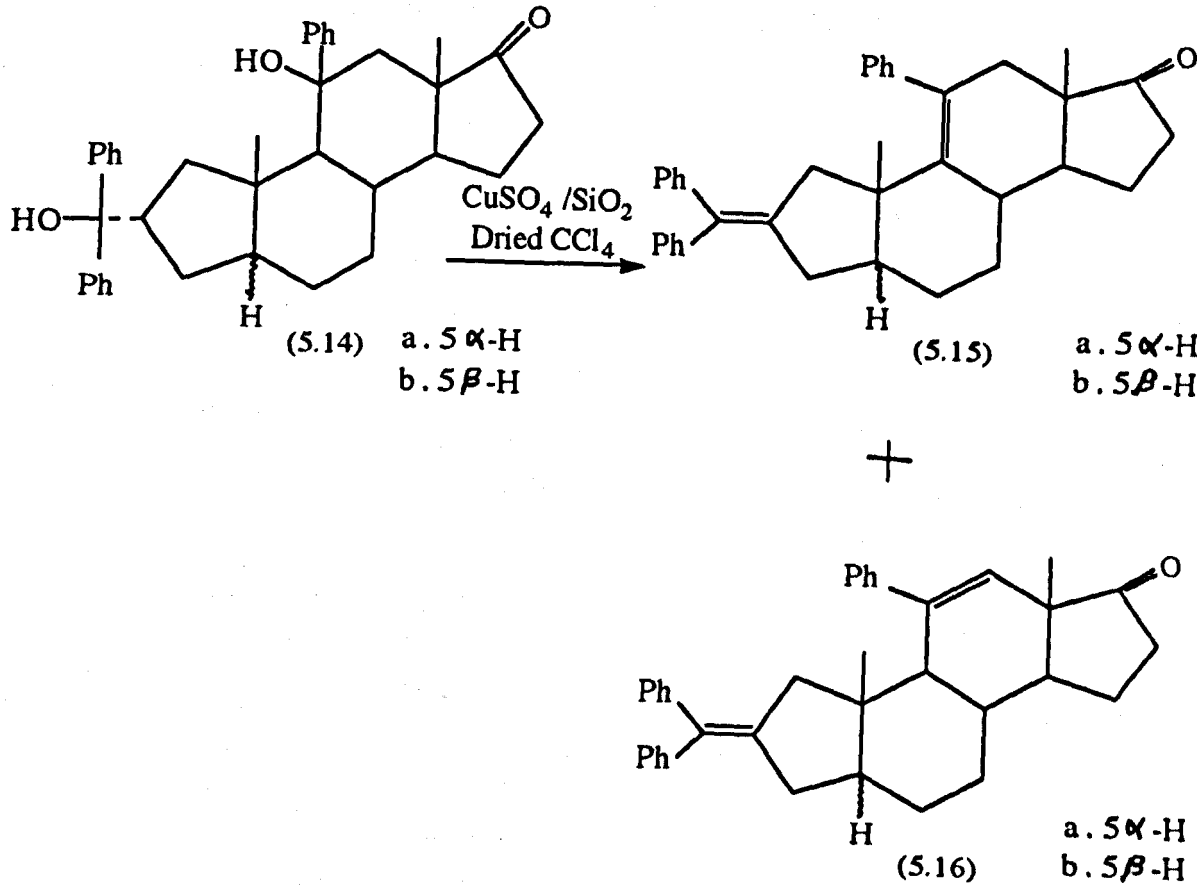


Oxidation of compound (5.13) using dipyridinium dichromate[93,94] gave compound (5.14) in good yield (4.2g, 84%). The infrared spectrum of compound (5.14) showed the expected band at1730 cm^{-1} due to the carbonyl group at C-17.



(5.13) (5.14)

The most suitable method for dehydration[95] of secondary and tertiary alcohols was found to be the treatment of compound (5.14) with freshly prepared CuSO4/SiO2 in dried cyclohexane under reflux which gave two isomeric compounds, (5.15) and (5.16), which were separated by preparative TLC. The ^1H NMR spectra of the two isomers confirmed their structures. A singlet signal appeared at 6.0 ppm in the ^1H NMR spectrum of compound (5.16) but was not present in the ^1H NMR spectrum of compound (5.15).



(5.14) a. 5α-H b. 5β-H

(5.15) a. 5α-H b. 5β-H

+

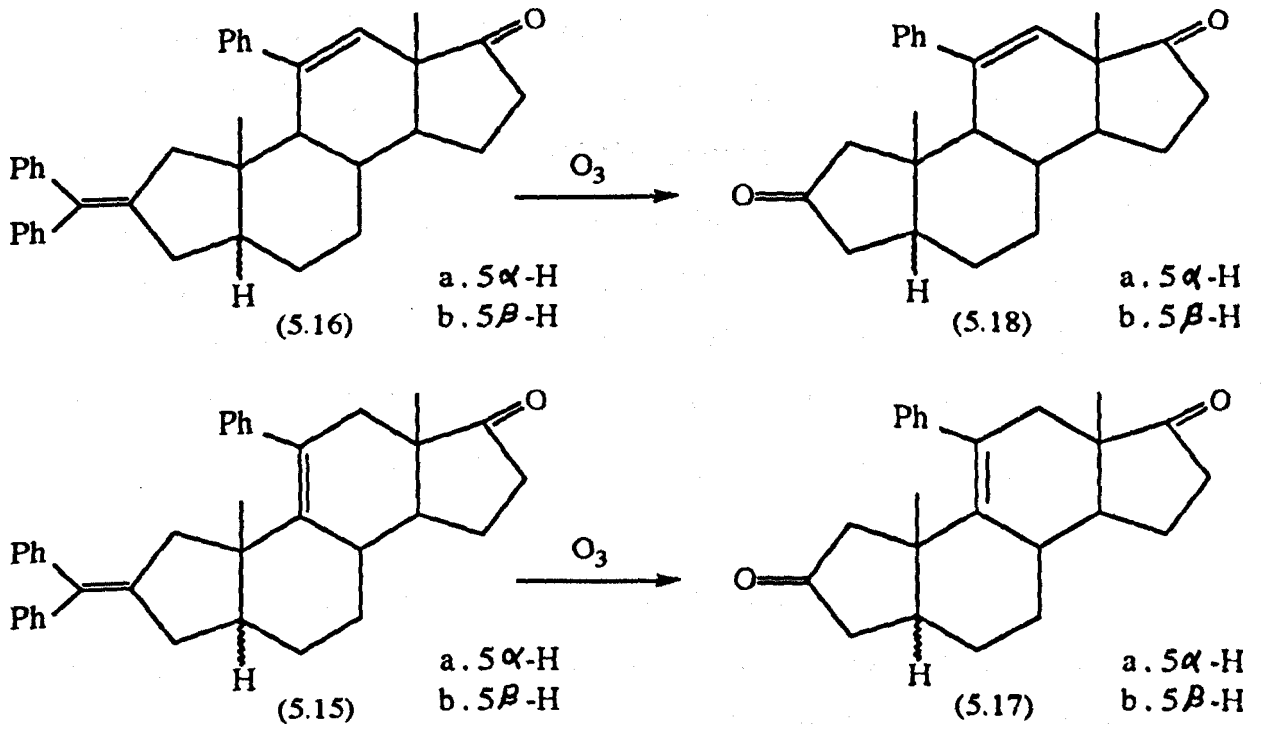
(5.16) a. 5α-H b. 5β-H

It was observed that when the dehydration reaction mixture was refluxed gently, compounds (5.16) and (5.15) were obtained in a ratio of 6:1. However, when the reaction mixture was refluxed vigorously, the ratio obtained was 1:6.

These results can be explained by kinetic preference for abstraction of the H-12 rather than H-9 . The thermodynamic isomer preferred is compound (5.15) as it is more stable.

Ozonolysis[96] was found to be suitable for the oxidative cleavage of the diphenylmethylene side chain in compounds (5.15) and (5.16). Ozone was passed into separate solutions of compounds (5.15) and (5.16) in methylene chloride and after work-up with zinc dust and

glacial acetic acid, the products (5.17) and (5.18) respectively,were isolated by preparative TLC.



The structural assignments of the isolated compounds (5.17) and (5.18) were made on the basis of their spectral data (IR, ^{1}H NMR, MS). The IR spectra of compounds (5.17) and (5.18) each showed a strong band at 1740 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 m/z 348. The ^{1}H NMR spectra of both compounds were almost the same but the ^{1}H NMR spectrum of compound (5.18) showed an additional doublet signal at 6.0 ppm due to the olefinic proton at C-12. Also, in the ^{1}H NMR spectra of both compounds there was a multiplet signal at 7.16 ppm 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 (5.9-5.17) 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 introduction of the carbonyl group in the A-nor ring and the effect of a double bond in the C- ring appeared to make the product (5.18) a suitable compound in which to explore this point further and detailed ^{1}H NMR investigations were carried out.

In the work by my colleague Charles Thomas, two distinct approaches were used in the examination of the C-5 stereochemistry in a steroid such as (5.18). The first was 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 3ß, 3α, 6ß and 6α.

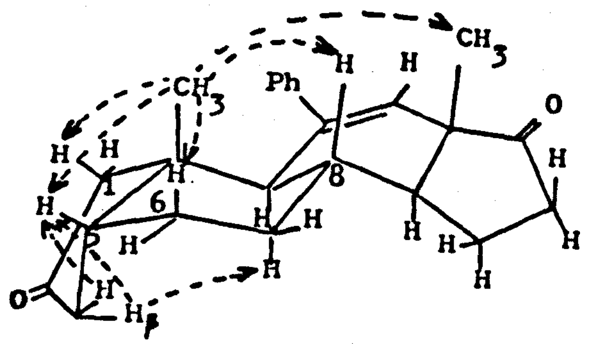
However, because of the complexity of the 400 MHZ ^{1}H NMR and 2D COSY spectra, the only interaction that could be clearly seen was that between the 3-H and 5-H protons. A nice doublet of doublets of doublets (ddd) was observed for 3ß and 3α protons respectively due to interaction with 5-H and the two protons on C-1 as a result of coupling through the C-2 carbonyl from both ^{1}H NMR and the 2D COSY spectra. Thomas observed $J_{3ß-5} = J_{3α-5} = 9$ Hz. These values indicated dihedral angles for $J_{3ß-5} = 30\text{-}45°$ and $J_{3α-5} = 160\text{-}170°$.

However, examination of models showed that both 5α and 5ß isomers (5.18a) and (5.18b) could adopt conformations consistent with these angles and consequently no conclusive decision could be reached from this approach.

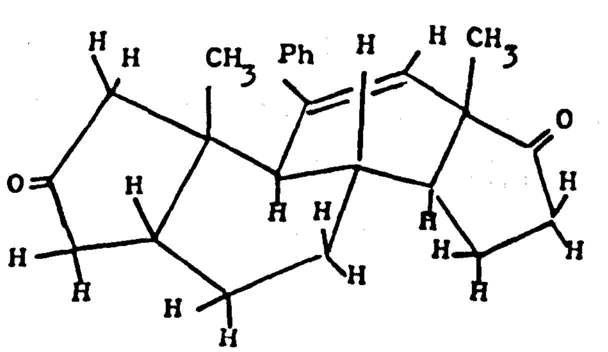
The second method examined was the use of the technique of nuclear Overhauser enhancement difference spectroscopy (nOeds)[86]. When two protons are in close proximity to one another in a molecule and their spins are contributing to one another's NMR relaxation, double irradiation of one proton causes 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 subtracted from the enhanced one on double irradiation, lies in the ability to sensitively detect small enhancements.

Table 6.1 ^{1}H NMR and nOeds results for (5.20b) in $CDCl_3$[86]

Assign-ment	=	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)

For the two possible structures (5.18a) and (5.18b), 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 (5.18b).

When a series of nOe experiments were run on compound (5.18b) in $CDCl_3$ (table 6.1), a clear nOe was observed[86] at H-5 on irradiation of19-Me, consistent with cis stereochemistry (5.18b).

There were also nOe's from 19-Me to H-1ß, H-6ß, H-8ß and 18-Me which were consistent with the conformation (X). Interestingly, when the nOe difference spectra were collected on (5.18) in deuterated toluene, no nOe could be observed for 19-Me to H-6ß. Models suggest that the alternative, ß-ring boat conformation (Y) is accessible for the cis-fused isomer and this could be more strongly favoured in some solvents.

In summary, the nOeds evidence clearly indicates that compound (5.18) has 5-ß stereochemistry (5.18b) with a cis fusion of the A and B rings.
Since none of the processes involved in the conversion of the diketo ester (5.8) into compound (5.18b) could possibly have resulted in inversion of the stereochemistry at C-5, it follows that all the series (5.8) to (5.18) have this same stereochemistry i.e. (5.8b) to (5.18b) .

The formation of the cis-fused diketo ester (5.9b) from the 3-ene (5.7) 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 methoxy carbonyl group at C-2, the steric effects of the 19-Me are counterbalanced by those of the ester group.

Models suggest that the conformation Fig (6.3) of the substrate (5.7) may make approach to the catalyst surface more favourable from the ß-face. On the basis of the evidence for the 5ß configuration in the diketo ester (5.9b), it was concluded that the structures of all compounds (5.9)-(5.18) must be assigned to the 5ß series.

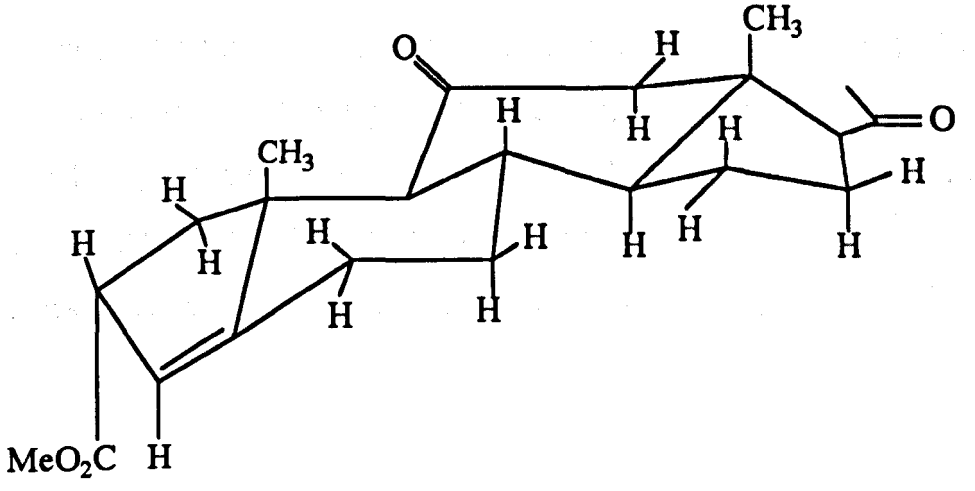


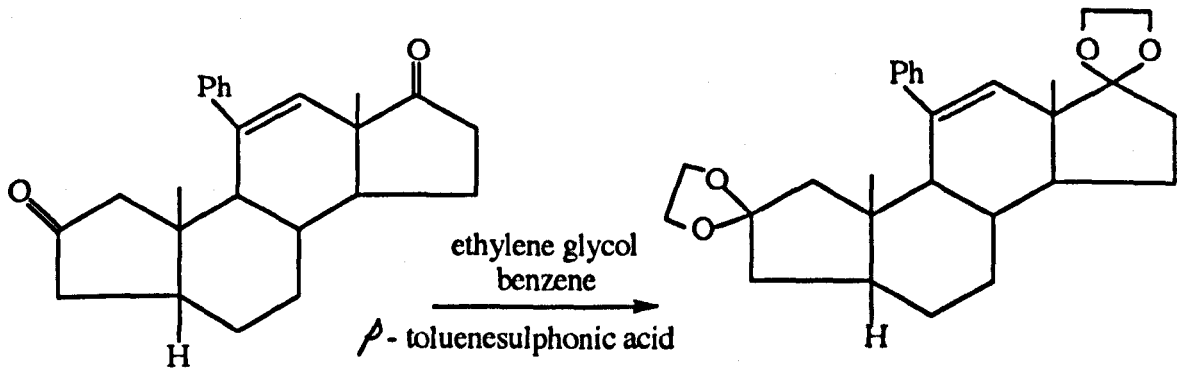
Fig. 6.3

Since the original intention of this project had been to synthesise a 5 -A-nor steroid combining structural elements from anordin and

RU 38486, the unexpected acquisition of the 5ß series required re-evaluation. The model 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 also encouraging that the inversion of stereochemistry at the C/D ring junction in ZK 98299 did not lead to loss of antiprogestational activity. It was therefore decided to continue the previously planned synthesis, but with the 5ß series.

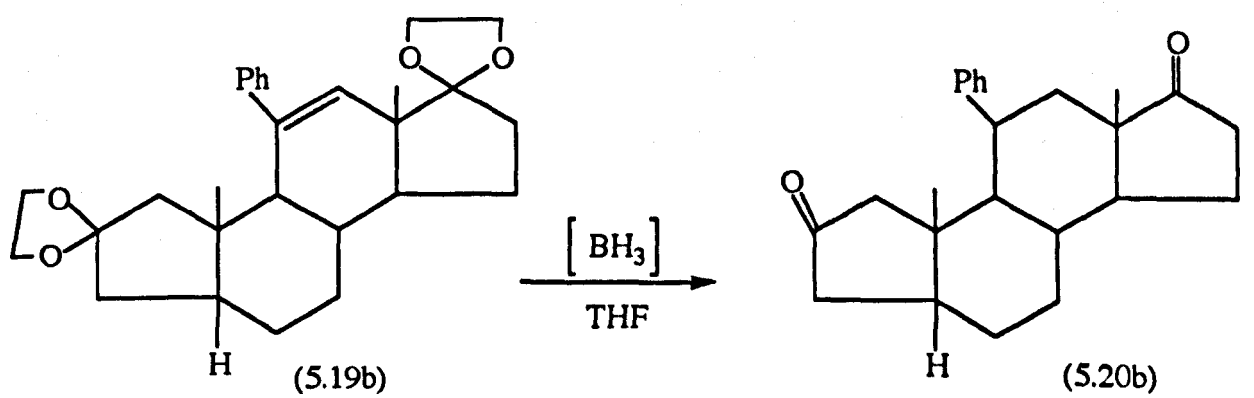
Following the literature[97] method for the ketalization of progesterone, 11-phenyl-A-nor-5β androst-11-en-2,17-dione (5.18b) was refluxed for 3 hours with ethylene glycol, p-toluene sulphonic acid as a catalyst and dry benzene in a Dean and Stark apparatus, to afford 2,2-ethylenedioxy-11-phenyl-17,17-ethylenedioxy-5-norandrostane (5.19), which was isolated and identified by its spectra. The ^{1}H NMR spectrum indicated the appearance of a multiplet between 3.9-3.65 ppm corresponding to 8 hydrogen atoms in two ketal functionalities. Also present was a 1H singlet at 6.06 ppm corresponding to the C-12 H. The IR spectrum of the compound did not show a band at 1740 cm^{-1}, confirming the loss of two carbonyl groups at positions C-2 and C-17. Its mass spectrum showed a molecular ion peak at m/z 436.



(5.18b) (5.19b)

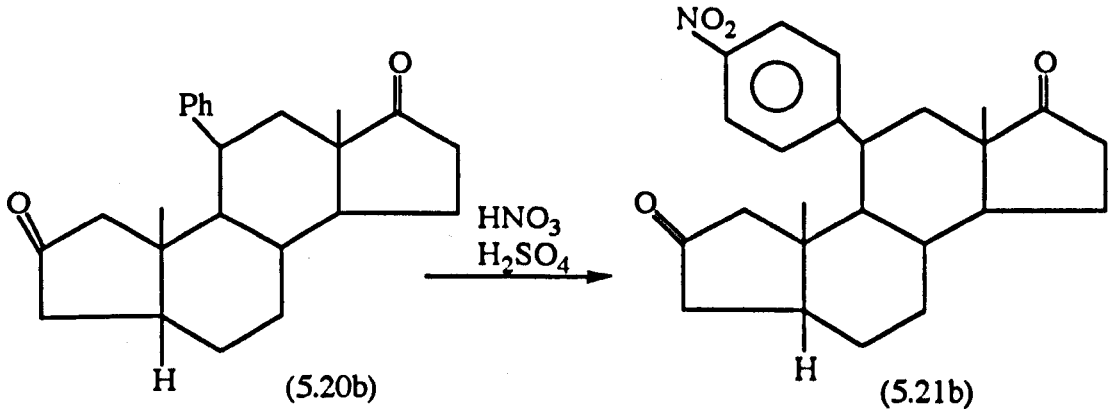
Hydroboration of compound (5.19b) using diborane[98] in tetrahydrofuran gave compound (5.20). The structure of the compound was confirmed by its NMR spectrum which showed an absence of the singlet olefin signal that was present in the ^1H NMR spectrum of compound (5.19b).

It was also observed that the ^1H NMR spectrum of compound (5.20) showed the absence of the ketal signals that were present in the ^1H spectrum of compound (5.19b) at 3.9-3.65 ppm.



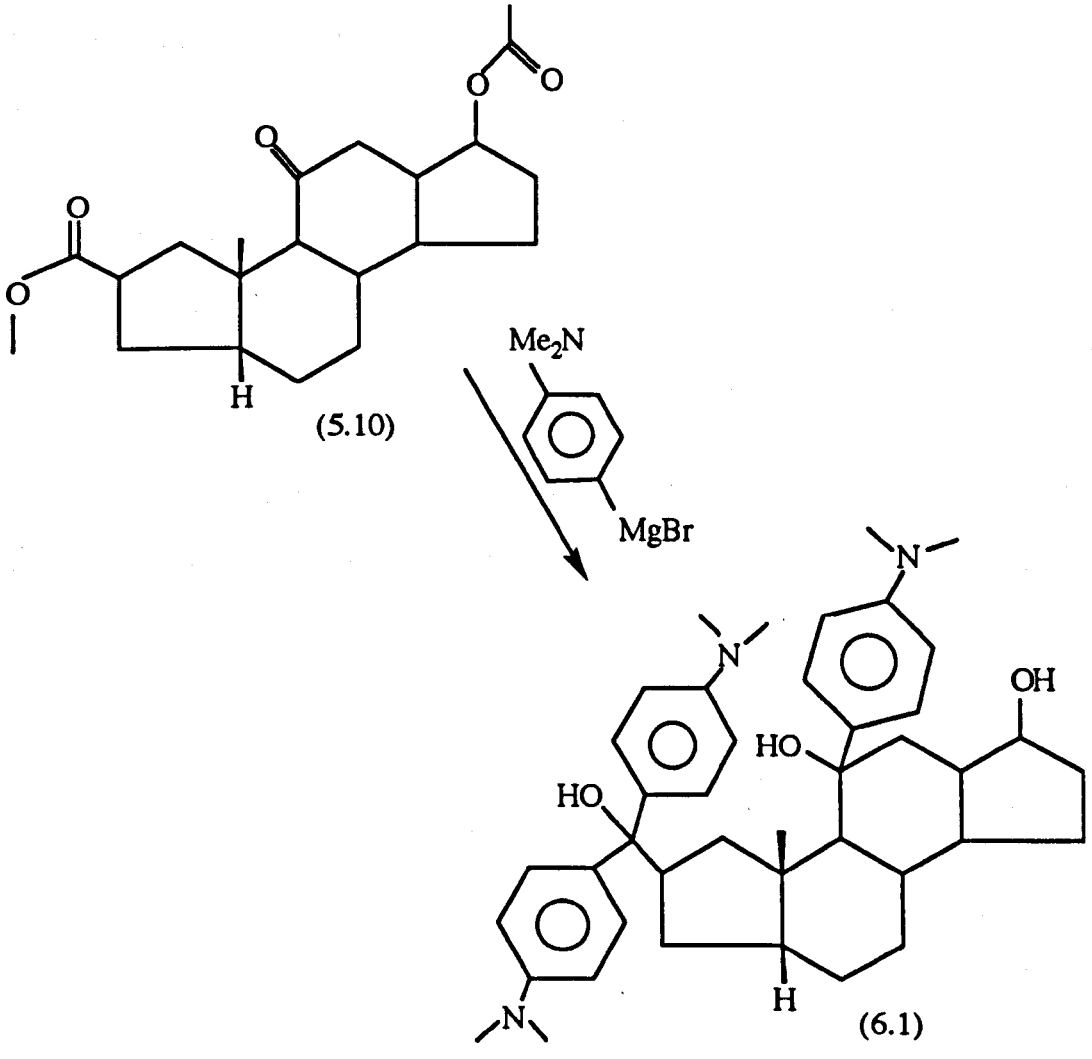
(5.19b) (5.20b)

A number of attempts were made to nitrate compound (5.20b) with concentrated nitric acid and sulphuric acid in acetic acid[99,100]. In none of these attempts were we able to isolate the desired product (5.21b) or any other side product from the reaction mixture.



(5.20b) (5.21b)

The strategy reported by Kirk and Petrow[101] involved the action of organometallic reagents on an 11-keto steroid followed by dehydration and a catalytic hydrogenation procedure. This methodology was further utilised in the synthesis of 11ß -ethyl estrone and 11ß -ethyl-n-propyl and-n-butyl estrones[101]. In the hope of achieving a similar result, the keto diester (5.10) was treated with an excess of 4-(N,N-dimethyl) phenyl magnesium bromide, to try to produce the triol (6.1). Unfortunately we were not able to isolate the desired product.


O
O
O
O
O
H
(5.10)
Me2N
MgBr
N
N
OH
HO
HO
N
H
(6.1)

6.3 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-5ß-androstene-2, 17-diones having a double bond in the C-ring. The double bond needs to be reduced to furnish the 11ß -phenyl-diketone, which is required for the synthesis of novel compounds for testing as anti-hormanal agents.

Reduction of the C-ring olefin was achieved by using diborane in tetrahydrofuran. Alternatively, the two carbonyl groups could be protected as ketals.

Once the 11ß -phenyl diketone (5.20) is available, it is anticipated that it can be converted into the final products as shown in Fig 5.5.

CHAPTER 7

EXPERIMENTAL

All chemicals and solvents were purchased from Aldrich, Fluka, Jansens and Sigma. The solvents were of SLR grade and dried where necessary according to the procedures cited in the literature[102]. Melting points were determined on a Gallenkamp Melting point Apparatus and are uncorrected. Infrared spectroscopy was performed on samples in the individual methods on a Perkin Elmer instrument (FT-IR 1720X spectrometer) and recorded as wavenumber (cm^{-1}). ^{1}H NMR and ^{13}C NMR were performed on a Bruker AC 250 MHz or a Bruker AC 400 MHz spectrometer in $CDCl_3$ solutions. Chemical shifts are quoted in ppm(δ) and TMS was used as an internal standard. Low resolution EI-MS,CIMS($NH4^+$) and FAB-MS were obtained on a Kratos MS-80 mass spectrometer. High resolution EI-MS and CI-MS($NH4^+$) were obtained from the SERC Mass Spectrometry Service Centre, University College of Swansea. Column chromatography was carried out using Kieselgel 60 (particle size 40-63μm). Organic solvents were removed under reduced pressure on a Büchi rotary evaporator.

2α-METHOXYCARBONYL-A-NORPREGN-3-ENE-11, 20-DIONE (large scale preparation) (5.8).

Thallium (III) nitrate (25g, 49 mmole) was dissolved in a mixture of trimethyl orthoformate (206.7ml) and methanol (159ml), and the resulting solution left stirring at 0 °C for 30 minutes. A cold (0 °C) solution of 11-ketoprogesterone (5.7) (15.6g, 38.2mmole) in a mixture of trimethyl orthoformate (312.7ml) and MeOH (238.2ml) was quickly added. After 30 mins, the reaction solution was neutralised with saturated aqueous sodium carbonate, then filtered and extracted with diethyl ether. The ether was then evaporated and a white oily solid was formed. The product was purified using silica column chromatography. On elution with a mixture of hexane-ether (20:80), pure 2α-methoxycarbonyl-A-norpregn-3-ene-11,20-dione (5.8) was afforded (10g, 64%); M.P. 99-102 °C. Analytical TLC showed Rf=0.673. ^{1}H NMR ($CDCl_3$)δ: 5.06 (1H,s),3.55 (3H,s), 2.11 (3H,s), 1.10 (3H,s) and 0.57 (3H,s). νmax ($CHCl_3$) cm^{-1} 1700-1727, 1521: EI-MS; m/z: 358.4709 (M^+,15.89%, $C_{21}H_{26}O_5$ requires 358.4778), 356, 345, 329, 328, 313, 301, 300, 285, 257, 244, 240, 219, 206, 193, 175, 165, 161, 159, 151, 135, 133, 131, 123, 121, 119, 109, 108, 107, 105, 95, 94, 93, 91, 85, 81, 80, 79, 77, 71, 69, 67, 59, 55 (100%), 53.

2α-METHOXYCARBONYL-A-NOR-5β-PREGNANE-11,20-DIONE (5.9b).

A solution of compound (5.8) (5.0g, 13.9 mmol) was added to palladium on activated charcoal (1.3g, 10%) suspended in glacial acetic acid (150 ml) and ethanol (300 ml) and the mixture was stirred magnetically under hydrogen. The hydrogenation took place over a period of two days, and after this the mixture was filtered and evaporated giving a yellow solid (5.9b), (4.3g 85% yield), MP 124-128 °C, ^{1}H NMR ($CDCl_3$)δ: 3.62 (3H,s), 2.10 (3H,s),1.22 (3H,s) and 0.55 (3H,s); ν max($CHCl_3$) cm^{-1} 1700-1728; EI-MS: m/z :360.4735 (M^+, 137.5%; $C_{22}H_{32}O_4$ requires 360.4715), 345, 285, 258, 219, 206, 133, 107, 95, 93, 91, 85, 81 (100%), 79, 77.

2α-METHOXYCARBONYL-A-NOR-5β-ANDROSTAN-17β-OL-11-ONE ACETATE (5.10b).

A solution of peroxytrifluoroacetic acid was prepared by adding 60% hydrogen peroxide (3.6 ml, 75.6mmol) dropwise to a well stirred, cold solution of trifluroacetic anhydride (16ml, 207.7mmol) in dichloromethane (22.4 ml). The mixture was then allowed to warm up to room temperature with stirring and then cooled in an ice bath for 3 mins. This solution was added over 15 mins to a well stirred mixture of disodium hydrogen phosphate (26g, 183mmol) and compound (5.9b) (1g, 2.8mmol) in methylene chloride (100 ml).

After addition was completed, the mixture was heated under reflux for 4 hours. The product was then filtered and washed with methylene chloride. The combined filtrates were washed with 300 ml of 10% sodium carbonate and dried over magnesium sulphate and then filtered. The filtrate was collected and evaporated. The crude product was then dried, and obtained as a pale yellow oil. Compound (5.10b), 0.8g (80%). ^{1}H NMR ($CDCl_3$) δ: 4.68 (1H,t), 3.65 (3H,s), 1.93 (3H,s), 1.14 (3H,s) and 0.66 (3H,s); νmax ($CHCl_3$) cm^{-1}: 1700-1730; EI: MS m/z: 376.2446 (M^+, 5.67%; $C_{22}H_{32}O_5$ requires 376.2441), 361, 360, 329, 302, 301, 284, 283, 235, 222, 209, 149, 135, 133, 121, 119, 109, 107, 105, 97, 95, 93, 91, 84, 82, 81, 80, 79, 77, 69, 67, 59, 57, 55 (100%), 55, 53.

2-(1'-HYDROXY-1',1'-DIPHENYLMETHYL)-11α-HYDROXY-11β PHENYL A-NOR-5β-ANDROSTAN-17β-OL(5.13b): 2-(1'-HYDROXY-1',1'- DIPHENYLMETHYL)-A-NOR-5β-ANDROSTAN-17β-OL-11-ONE,(5.12b).

A cold solution of compound (5.10b) (1.0g, 2.6 mmol) in dry ether (10 ml) was added by syringe into a stirred solution of 2M phenyl lithium (100 ml, 200mmol) in dry hexane-ether mixture (90:10) under nitrogen at -78 °C. After the addition was complete the reaction 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 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 over magnesium sulphate and evaporated to yield a brown oil. The crude product was purified by preparative TLC using ether-hexane (8:2) as a solvent to give compounds (5.12b) (320mg, 23.55%) and (5.13b), (350mg, 29.90%) respectively. Compound (5.13b): white solid, M.P.244-246 °C ^{1}H NMR ($CDCl_3$) δ: 1.02 (3H,s), 1.3 (3H,s), 2.74 (1H,m), 3.32 (1H,t), 6.78 (15H,m), ν max($CHCl_3$) cm^{-1} 3393: EI-MS : m/z 518.3161 (M^+-H_2O, 10.28% $C_{37}H_{42}O_2$ requires 518.3197). Compound (5.12b) white solid M.P.123-129 °C; ^{1}H NMR ($CDCl_3$) δ 700, 1597: EI-MS : m/z 440.2973 (M^+-H_2O, 8.92%, $C_{31}H_{38}O_3$ requires 440.2959). The reaction was then repeated twice on a large scale and a total of 8 g of compound (5.13b) accumulated.

2-(1'HYDROXY-1',1'-DIPHENYLMETHYL)-11α-HYDROXY-11β-PHENYL-A-NOR-5β- ANDROSTAN-17-ONE (5.14b).

Pyridinium dichromate (20.58g, 39.4mmol) was added to a solution of compound (5.13b), (5g, 10 mmol) in dichloromethane at room temperature. The reaction mixture was stirred for four hours. Dilution of the reaction mixture with ether, removal of the precipitate and concentration of the filtrate under reduced pressure gave the crude compound (5.14b),(4.2g, 84%). Some of the crude product (100mg) was purified by preparative TLC using (2:8v/v) hexane-ether as solvent, M.P.244-251 °C. ^{1}H NMR ($CDCl_3$) :1.21 (3H,s), 1.38 (3H,s),

as solvent, M.P.244-251 °C. ^{1}H NMR ($CDCl_3$)δ:1.21 (3H,s), 1.38 (3H,s), 2.87 (1H,m), 7.26(15H,m);νmax ($CHCl_3$) cm^{-1} 1730 (s), 1600; EI-MS, m/z 534.2563 (M^+, 1.2%, $C_{37}H_{42}O_3$ requires 534.2549).

PREPARATION OF $CUSO_4/SiO_2$ SOLID PHASE DEHYDRATING REAGENT.

The reagent was prepared by mixing chromatographic[95] silica gel (230-400 mesh) (25.0g, 420 mmole) with copper (II) sulphate pentahydrate (16.60g, 70 mmole) in water (20 ml). The water was evaporated under reduced pressure and the solid dried for two hours at over 200°C. At this stage there was no further water condensation in a trap cooled with liquid nitrogen. The product was then allowed to cool slowly over night in a vacuum desiccator over phosphorus penta oxide.

2-(1'-1'-DIPHENYLMETHYLENE)-11-PHENYL-A-NOR-5β-ANDROST-11-EN-17-ONE,(5.16b)-2-(1',1'-DIPHENYLMETHYLENE)-11-PHENYL-A-NOR-5β-ANDROST-9-EN-17-ONE (5.15b).

To a solution of compound (5.14b),(50mg, 0.095 mmol) in carbon tetrachloride (5ml) was added copper (II) sulphate on silica gel (509mg, 3.32 mmol). The resulting reaction mixture was refluxed at a temperature between 90-95 °C for 75 mins. The reaction was monitored by analytical TLC, and after the completion of the reaction, the crude products were isolated from the solid by filtration.

The filtrate was evaporated to dryness under reduced pressure and the residue was purified by preparative T.L.C. using dichloromethane as solvent, to afford two olefinic compounds (5.16b), (29mg, 58%) and (5.15b), (9 mg, 18%) respectively. Compound (5.16b) white solid, M.P.113-118 °C; ^{1}H NMR ($CDCl_3$) δ: 0.94 (3H,s), 1.02 (3H,s), 6.05 (1H,s), 7.16 (15H,m); νmax ($CHCl_3$) cm^{-1} :1739, EI-MS: m/z 498.2938 (M^+ ,11.35%; $C_{37}H_{38}O$ requires 498.2922), compound (5.15b): white solid, M.P. 124-128 °C; ^{1}H NMR ($CDCl_3$) δ: 1.01 (3H,s), 1.23 (3H,s), 7.12 (15H,m); νmax ($CHCl_3$) cm^{-1} : 1735 EI-MS: m/z 498.2926 (M^+,21.12%; $C_{37}H_{38}O$ requires 498.2939.

11-PHENYL-A-NOR-5β-ANDROST-11-ENE-2,17-DIONE (5.18b).

A solution of compound(5.16b), (300mg, 0.62mmol) in dry dichloromethane (13.5ml) and dry pyridine (0.06ml) was ozonized for 45 minutes using 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, zinc dust (350mg, 5.35mmol) and glacial acetic acid (1.52ml) were added 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 5ml portions of water. It was then cooled by the addition of ice and washed with 20ml and 10ml of cold 10% sodium carbonate, 10ml of cold 10% sodium hydroxide and four 50ml portions of cold water, all aqueous washes being back-washed with 50ml 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 sulphate, filtered and concentrated to dryness at 40°C under reduced pressure to produce a crude oily yellow compound. This was purified by preparative T.L.C. using ether-hexane (80:20 v/v) to give compound (5.18b), (44.5mg, 15%) M.P. 191-193°C: ^{1}H NMR ($CDCl_3$)δ :0.96 (3H,s),1.08 (3H,s), 6.03 (1H,d), 7.16 (5H,m); νmax ($CHCl_3$) cm^1:1740; EI-MS: m/z 348.2054 (M^+, 22%, $C_{24}H_{28}O_2$ requires 348.2125).

In the same experimental conditions, compound (5.15b) gave compound (5.17b) (50.2mg, 17%). Compound (5.17b): M.P.142-148°C; ^{1}H- NMR($CDCl_3$)δ:1.02(3H,s),1.28(3H,s), 7.15(5H,m):νmax($CHCl_3$) cm^{-1}: 1735; EI-MS: m/z 348.2042(M^+, 100%, $C_{24}H_{28}O_2$ requires 348.2129).

3,3 :17,17-BIS (ETHYLENE DIOXY).11-PHENYL-A-NOR-5 -ß ANDROST-11-ENE (5.19b).

A mixture of compound (5.18b), (1.0 g 2.9 mmol), ethylene glycol (5ml), p-toluenesulphonic acid (0.025 g), and benzene (20 ml) was heated under reflux for 48 hours in a 1-L round bottom flask equipped with a Dean -Stark trap. Saturated sodium bicarbonate solution (2.5 ml) was added to the cooled mixture and the benzene layer was separated, washed with water (2 x 5 ml) dried (Na_2SO_4), and concentrated to dryness under reduced pressure. Recrystallization from ethanol gave

pure compound (5.19b), (0.7 g, 70% yield): mp 185-187.5 °C. ^{1}H-NMR($CDCl_3$) δ:0.94 (3H, s), 1.15 (3H, s), 6.06 (1H, d), 7.18 (5H, m), 3.65-3.98 (8H, m - OCH_2CH_2O-) ; νmax ($CHCl_3$) cm^{-1} 1438, 1370, 1313, 1102; EI - MS: m/z 436.2185 (M^+ , 35%, $C_{28}H_{36}O_4$ requires 436.2176).

11-PHENYL-A-NOR-5 ß-ANDROST-2,17- DIONE (5.20b).

In a three-neck flask was placed the compound (5.19b), (0.1 g, 0.23 mmol) in 0.1 ml of tetrahydrofuran. The flask was immersed in an ice-bath and a standard solution of diborane in tetrahydrofuran, 0.1 ml, was added dropwise by means of a syringe to the compound (5.19b) in tetrahydrofuran. The reaction mixture was permitted to remain for 2 hours at 0-5°C.

The residual diborane was destroyed with ethylene glycol. After addition of 0.3 ml of glacial acetic acid, the flask was permitted to remain overnight at room temperature. The reaction mixture was poured in to ice-water. The upper layer was separated, washed with sodium hydroxide solution and then with a saturated sodium chloride solution, dried and concentrated to dryness under reduced pressure. The crude product (0.06g) was purified by preparative TLC using 100% dichloromethane to give compound (5.20b), (0.04 g, 40% yield). ^{1}H NMR ($CDCl_3$)δ: 1.02 (3H, s), 1.12(3H, s), 7.14 (5H, m);νmax ($CHCl_3$) cm^{-1}1742 EI-MS : m/z 350.2245 (M^+, 25% $C_{24}H_{30}O_2$ requires 350.223).

7.1 REFERENCES

1) L.J. Lerner, Recent Progress in Hormone Res, 1964,**20**,435.

2) L.O. Randall and J.J. Selitto, Endocrinology, 1958,**62**, 693.

3) F. Newmann , Acta Endocrinol. 1966,**53**,53.

4) F. Murad and R.C. Haynes Jun., in: The Pharmacological Basis of Therapeutics, A.G.G. Iman, L.S. Goodman, T.W. Rall and F.Murad eds. Macmillan Publishing Co, N.Y, 1985, 7th Edn ,1454.

5) L.J. Lerner, Pharmacol. Ther. B, 1975,**1**,217.

6) J. Tuomisto and P .Mannisto, Neurotransmitter Regulation of Anterior Pituitary Hormones, Pharmacol. Rev, 1985,**37**,249.

7) P. M. Conn, D. Staley, C. Harris, Mechanism of Action of Gonadotropin Releasing Hormone, Ann. Rev. Physiol, 1986,**48**,495.

8) J.L. Durand and R. Bressler, Clinical Pharmacology of the Steroidal Oral Contraceptives, Adv. Intern. Med, 1979,**24**,97.

9) K. J. Ryan, Ann . Rev. Med, 1982,**33**,171.

10) J . D. Baxter and P .H. Forsham, Am. J . Med, 1972,**53**,573.

11) P. G. Stubblefield, . J. Reprod. Med, 1984,**29**,524.

12) M.O .Thorner, Hosp. Pract, 1986, **21(12)**, 63.

13) E. Marshall, The drug of champions, Science, 1988,**242**,183.

14) H.J. Ringold and G. Rosenkranz, U.S. Patent, 2852, 537 (Chem Abstr. 1959,**53**,4361c).

15) M.K. Agarwal (ed), Hormone Antagonists, Walter de Gruyter, Berlin, 1982.

16) U. Verma and K. Laumas, J. Steroid Biochem , 1987,27,851.

17) B. Beyer, L. Terenius, R.W. Brueggemeier, V.V. Ranade, R.E. Counsell , Synthesis of potential antiprogestins. Steroids,1976,27,123.

18) B. Beyer, L. Terenius, R.E. Counsell, Synthesis of potential anti-progestins, Steroids, 1980,35,481.

19) D.L. Healy, Clinical Reproduction and Fertility, 1985,3,277.

20) R. Wiechert and G. Neef, J. Steroid Biochem, 1987,27,851.

21) G. Teutsch, in: Adrenal Steroid Antagonism, M.K. Agarwal (ed), Walter de Gruyter, Berlin, 1984, p.43.

22) H.J. Kloosterboer, G.H.J. Deckers, M.J. Van der Herrel and H.J.J. Loozen, J. Steroid Biochem, 1988,31,567.

23) J.R. Schreiber, A.J.W. Hsueh and E.E. Baulieu, Binding of the anti-progestin RU 38486 to rat ovary steroid receptors, Contraception, 1983,28,77.

24) D. Philibert, Hormone Antagonists, M. K. Agarwal (ed), Walter de Gruyter, Berlin, 1984, p.77.

25) D. Philibert, M. Moguilewsky, I. Mary, D. Lecaque, C. Tournemine, J. Secchi and R. Deraedt, Pharmacological profile of RU 38486 in animals,In:The Antiprogestin Steroid RU 38 486 and Human Fertility Control (ed. E.E. Baulieu and S.J. Segal), Plenum Press, NewYork, 1985, p.49.

26) A. Groger, Y. Le Bouc, I . Joab, C. Radanyi, J.M. Renoir , P. Robel and E.E. Baulieu . European Journal of Biochemistry, 1985,**149**,445.

27) G. Neef, G. Sauer, A.T. Seeger and R. Wiechert, Tet. Lett, 1984,**25**, 3425.

28) D.H. Seeley, W.Y. Wang, and H.A. Salhanik, Journal of Biological Chemistry, 1982,**257**,13359-66.

29) W.L. Duax, J.F. Griffin, D.C. Rohrer, and C.M. Weeks, Steroid agonists and antagonists, M.K. Agarwal(ed) , Walter de Gruyter, Berlin,New York, 1982, pp3-24

30) W. Elger, M. Fahnrich, S. Beier, S.S. Quig and K. Chwalisz, American Journal of Obstetrics and Gynaecology, 1987,**157**,1065.

C. Chang , J. Kokontis and S. Liao, Science,New York,1988,**240**,324.

31) A. Belanger, D. Philibert and G. Teutsch, Steroids, 1981,**37**,361.

32) H.L. Dryden, G.M. Weber, J. Org. Chem, 1961,**26**,3237.

33) E.J. Rajpert, F.P. Lemargre, P.H. Eliard, M. Place, D.A. Lafontaine, I.V. Economidis, A. Belayen, J.A. Material and G.G. Rousseau, J. Steroid Biochem, 1987,**26**,513.

34) A. Groger, G. Schwenzer - Groyer , F. Cadepond , M. Mariller and EE. Baulieu , Nature, 1987,**328**, 624.

35) R.I. Dorfman, R.I.Fajkos, J. Joska, Steroids, 1964,**3**, 675.

36) F. Neumann, R. Von Berswordt-Wallrabe, W. Elger, W. Steinbeck, J.D. Hahn and M. Kramer, Recent progr. in Hormone Res,1970, **26**, 337.

37) R.I. Dorfman, Proc. Sec. Exp. Biol. Med, 1964,**116**,1055.

38) F. Neumann, R. Von Berswordt-Wallrabe, W. Elger, and W. Steinbeck, Mosbacher Kolloq. Ges. Biol. Chem, 1967,**18**, 218.

39) L. J. Lerner, Pharmacol. Ther. B, 1975,**1**, 217.

40) H.L. Saunders, K. Holden and J.F. Kerwin, Steroids, 1964,**3**, 687.

41) A. Zarate, VB. Mahesh and R.B. Greenblatt, J. Clin. Endocrin. Metab,1966,**26**,1394.

42) J.A. Campbell, S.C. Lyster, G.W. Duncan and J.C. Babcock,Steroids, 1963,**1**,317.

43) D.E. Wishart, J. Reprod. Fert, 1972,**30**,333.

44) J.A. Campbell and J.C. Babcock, J. Am. Chem. Soc, 1959,**81**,4069.

45) J.A. Zderic, H. Carpio, and H.J. Ringold, J. Am. Chem. Soc, 1959,**81**, 432.

46) C.H. Robinson, O.Gnoj, W. Charney, M.L. Gilmore and E.P. Oliveto, J. Am. Chem. Soc, 1959,**81**,408.

47) C.H. Robinson, O. Gnoj and E.p. Oliveto, J. Org. Chem,1959,**24**,121.

48) G. Nomine, R. Bucourt and A. Pierdet, C.R. Acad. Sci, 1962,**254**, 1823.

49) B.Camerino and U. Valcavi, Brit. Patent 887, 007(Chem. Abst. 1962, **57**,918c).

50) J.P. Raynaud, M.M. Bouton, M. Moguilewsky, T. Ojasoo, P.A. Philibert, J . Steroid Biochem, 1980,**12**,143.

51) R.I. Dorfman, J. Fajkos, J. Joska, Steroids, 1964,**3**,675.

52) L. Stark, M. Alexandrova, R. Hampl, V. Sanda, Endocrin, 1980,**14**,45.

53) F. Neumann, R. Von Berswordt - Walrabe, W. Elger, H. Steinbeck, J.D. Hahn, M. Kramer, Rec. Progr. Hormone Res, 1970,**26**,337.

54) L. Starka, J. Sulcova, P.D. Broulik, J. Joska, J. Fajkos, M. Doskocil J. Steroid Biochem, 1977,**8**,939.

55) W.L. Duax, G.D. Smith, D.C. Swenson, P.D. Strong, C.M. Weeks, S.N. Ananchenko, V.V. Egorova, J. Steroid Biochem, 1981,**14**,1.

56) G.A. Chernayev, T.I. Barkova, V.V. Egorova, I.B. Sorakina, S.N. Ananchenko, G.D. Matatadze, N.A. Sokolova, V.B. Rozen, J. Steroid Biochem, 1975,**6**,1483.

57) L. Starka, R. Hampel, M. Bicikova, V. Gerny, J. Fajkos, A. Kasal, P. Kocovsky, L. Kohout, H. Velgova . J. Steroid Biochem,1980,**13**, 455.

58) S. Durani, A.K. Agarwal, R. Saxena, B.S. Setty, R.C.Gupt,P.L.Kolne, S. Ray, N. Anand. J. Steroid Biochem, 1979,**11**,667.

59) W. Voigt, A. Castro, D.F. Covery, C.H. Robinson . Acta Endocrin, 1978,**87**,668.

60) P. Crabbe, H. Fillion, Y. Letourneaux, E. Diczfalusy, A.R. Aestro, J.W. Goldzieher, A.A. Shaikh, V.D. Castracone, Steroids,1979,**33**,85.

61) R.F. Dorfman, J. Fajkos, J. Joska, Steroids, 1964,**3**,675.

62) F.H. Batzold, D.F. Covery, C.H.Robinson,Cancer Treat Rep,1977,**61**, 2.

63) B. Robaire, D.F. Covery, C.H. Robinson, L.L. Ewing. J.Steroid Biochem,1977,**8**,307.

64) N.A. Zerhaini, M. Maes, C.H. Sultan, S. Rothwell, C.J. Migeon, J. Steroids Biochem, 1977,**8**,307.

65) H.L. Carrel, J.P. Glusker, C.H. Robinson, D.F. Covery, F.H. Batzold, J. Am. Chem. Soc, 1978,**100**,4284.

66) R.W.S. Skinner, R.V. Pozaderac, R.E. Counsell, C.F. Hsu, P.A. Weinhold, Steroids, 1979,**30**,15.

67) R.W.S. Skinner, R.V. Pozaderac, R.E. Counsell, C.F. Hsu, P.A. Weinhold, Steroids, 1975,**25**,189.

68) H.L. Carrel, J.P. Glusker, C.H. Robinson, D.F. Covey, F.H. Batzold, J. Am. Chem. Soc, 1978,**100**,4282.

69) F. Neumann, K. Steinbeck, Androgen II and Antiandrogens. Springer Verlag, Berlin, Heidelberg, New York 1974, p. 235-484.

70) R.E. Beyler, F. Hoffman and L.H. Sarett, J. Org. Chem, 1960,**25**.352.

71) D. Taub, R.D. Hoffsommer and N.I. Wendler, Steroids, 1957,**79**,452.

72) L.F. Fieser and M. Fieser, Steroids, Reinhold Publishing Corp, New York, 1959,p. 682.

73) P. Hegner and T. Reichstein, Helv. Chim. Acta, 1943,**26**,726.

74) R. A. Antonueei, S. Bernstein, R. Lenhard,K. J. Sax and J.H.William, J. Org. Chem, 1952,**17**,1369.

75) J. Schmidlin and A. Wettstein, Helv. Chim. Acta,1953,**36**,1241. J. N. Cornforth, J.M. Osbond and G.H. Phillips, J.Chem. Soc,1954, 970.

76) G. Pincus, U.K. Banik, Excerpta Medica Int Congr, 1963,**111**,595.

77) G. Pincus, Steroids, 1964,**4**,657.

78) G. Pincus, Anat. Rec, 1967,**53**,157.

79) Z.P. Gu, M.C. Chang, Contraception, 1979,**20**,549.

80) R.R. Mehta, J.M. Jenco, J.R. Chalterton, Steroids, 1981,**38**,679.

81) R.R. Mehta , J.M. Jenco , J.R. Chalterton , D.Venton, Steroids,1982, **40**,65.

82) J.F. Biellmann and M. Rajic, Bull. Soc. Chem. Japan, 1962, p.441.

83) P. Crabbe , H. Fillion , Y. Le Tourneaux , E. Diczfalusy , A.R. Aedo, A.A. Goldzieher , V.D. Shikh ,Steroids, 1979,**33**,35.

84) C.M. Chang, C. Wang, C.W. Bardin, Degeneration of ova in the rat and rabbit, Endocrinology, 1959,**65**,339.

85) E. Mincione , P. Barraco , M.L. Forcellese, Gazz Chimica Italiana, 1980,**110**,515.

86) C. Thomas , PhD thesis, City University, 1993.

87) E. Mincione, P. Barraco, and M.L. Forcellese, Gazz Chimica Italiana, 1985,**37**,115.

88) W.D. Emmons , G.B. Lucas , J. Am. Chem. Soc, 1955,**77**,2287.

89) W.D. Emmons , A.S. Pagano , J. Am. Chem. Soc, 1955,**77**,89.

90) W. Von , E. Doering , L. Speers , J. Am. Chem. Soc, 1950,**72**,5515.

91) R.B. Turner , J. Am. Chem. Soc, 1950,**72**,878.

92) J.D. Buhler, J. Org. Chem., 1973,**38**,905.

93) W.W. Hess, F.J. Frank, Tet. Lett, 1968,**30**,3363.

94) E.J. Corey, G. Schmidt, Tet. Lett, 1979,p.399.

95) T. Nishiguchi, N. Machida, E. Yamamoto, Tet. Lett, 1987,28,4565.

96) D.A. Shepherd, R.A. Doria, J.A. Campbell, B.A. Johnson , R.P. Holsz, J.E. Stafford , R.L. Pederson , J. Am. Chem. Soc.,1954,**77**, 1212.

97) A.J. Pearson , Y.S.S. Chen , A.R. Han , S.Y. Hsu , R.Tapan ,J. Chem. Soc, 1985,287.

98) C.D. Harries and K. Oppenheim, Chem. Zentr, 1916,**87II**,993.

99) F. Anselm, F. Zuckmayer, Ber, 1899,**32**,3283.

100) T.Y Shen , G.L. Walford , B.E. Witzel , W.V. Ger. offen , Chem. Abstr, 1971,**74**,125270x.

101) D.N. Kirk and V. Petrow, J. Chem . Soc 1961, 2091.

102) D.D. Perrin, W.L.F. Armarego, D.R. Perrin, Purification of Laboratory Chemicals, Pergamon Press, Oxford,1982.