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AN INVESTIGATION OF
POTENTIAL STEROIDAL
CARCINOGENS

by

Julie Carolyn Gill

A thesis submitted in partial fulfilment of the requirements for the award of Doctor of Philosophy, Department of Chemistry of the Loughborough University of Technology.


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I would like to thank my Supervisor, Dr. Brian Marples for the guidance and encouragement he has given me throughout my studies.

I would also like to express my gratitude to the academic and technical staff of the Organic Chemistry Section for all their help, in particular Mr. Mike Harris and Mrs. Hansa Madha. A special note of thanks goes out to all my research colleagues, for all their help and companionship.

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Many thanks to Miss Carol Kilcullen for her help with this thesis and finally I would like to thank Mr. Mike Burford for his help, both with the typing of this thesis and with the computer produced diagrams. I would also like to thank him for all his support during this period of my life.
ABSTRACT

An Investigation of Potential Steroidal Carcinogens.

Author: Julie Carolyn Gill.

Various potential metabolites of steroids with alkylating properties were synthesised, in order that their reaction with important cellular constituents and their mutagenicity could be investigated by Dr. Grover of the Chester Beatty Research Institute. Promising compounds were also investigated by Dr. Traynor, of Loughborough University, as part of an ongoing project to find selectively toxic compounds to estrogen dependant tumours.

The majority of the electrophilic species synthesised have been epoxides. A series of 3-oxo-4β,5β-epoxy-steroids have been synthesised from their 3-oxo-Δ4-counterparts. The 6α,7α-epoxide of estradiyl diacetate was prepared by epoxidation of 6,7-dehydroestriadiyl diacetate, which was in turn prepared via a novel route employing the Shapiro reaction.

20,21-Dehydronorethisterone and 17α-vinylestradiol have been prepared by reduction of norethisterone and 17α-ethynylestradiol respectively. Epoxidation gave the
20,21-epoxides. Payne rearrangement of the (20R)- and (20S)-3,17β-dihydroxy-20,21-epoxy-19-norpregna-1,3,5(10)-triene (prepared from 17α-ethynylestradiol) gave (20S)- and (20R)-3,21-dihydroxy-17β,20-epoxy-19-norpregna-1,3,5-(10)-triene respectively. The reactions of these 20,21- and 17β-20-epoxides with nucleophiles (namely acid hydrolysis and addition of thiophenol and various amines) were investigated for comparison with their reactions with biological nucleophiles.

A novel synthesis to 2-hydroxyestrogens was developed with a view to preparing 6,7-epoxy-2-hydroxyestrogens.
Some work contained in this thesis has been previously published, as detailed below:

J.C. Gill, P.M. Lockey, B.A. Marples and J.R. Traynor,

(2) Regioselective 2-hydroxylation of 3-methoxyestratrienes via chromium carbonyl complexes.
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References
INTRODUCTION

1. Steroids as carcinogens.

Many steroids, both endogenous and exogenous, are suspected of being carcinogens. For example, Reddy and Wynder found that patients with cancer of the colon exhibited higher levels of cholesterol (1), coprostanol (2), coprostanone (3), total bile acids, deoxycholic acid (4) and lithocholic acid (5) in their faecal excretory products than controls. They proposed that colonic epithelial cells may possibly interact with bile acids and cholesterol metabolites, and that this may be relevant in colonic carcinogenesis.

The aetiology of breast cancer has also been linked with steroids. Bulbrook et al. conducted a study on 4,850 normal women aged 25-55 years. During a five year period beginning 1961, 24-hour urine samples were taken. By 1970, 27 of these women had developed breast cancer. Each of these women exhibited low excretion of androgen metabolites, particularly etiocholanolone (6), when compared with the unaffected women, and these subnormal levels were detected five months to nine years before diagnosis. Bulbrook considered that the low androgen excretion had no aetiological significance in itself, but
was an index of other endocrine or constitutional characteristics of more direct relevance.

A reduction in the risk of breast cancer is associated with late menarche, early menopause and oophorectomy\textsuperscript{2,5}. As each of these conditions is associated with low estrogen levels, it would appear to suggest a possible aetiologic role for estrogens in human breast cancer. In apparent contrast to these observations, a lower risk of breast cancer is also associated with women who have had an early full-term pregnancy. Increased levels of estriol (7) are associated with pregnancy. Women produce three major estrogens - estrone (8), estradiol (9) and estriol (7). Whereas estrone (8) and estradiol (9) have repeatedly produced mammary tumours in rats and mice, estriol (7) has generally failed. Estriol (7) impedes some effects of the more active estrogens, such as the promotion of uterine growth, and, in common with other 'antiestrogens', estriol (7) also protects the rat against tumour induction by dimethylbenzanthrene. Estriol (7) also competes with estradiol (9) for cytoplasmic binding sites in cells from estrogen target organs and inhibits estradiol (9) incorporation by nuclei of a chemically induced rat breast tumour. It is therefore possible that estriol (7) reduces the risk of breast cancer by competing for estrogen binding sites. Lemon\textsuperscript{6,7} has suggested that women with a low ratio of estriol (7) to other estrogens have a high risk of breast cancer,
although results from several case-controlled studies of this hypothesis have been conflicting. Indeed, more recent work by Fishman et al. has indicated that women with breast cancer produce a greater quantity of 16α-hydroxylated estrogens when compared with normal subjects. All studies, however, suggest that estrogens and estrogen metabolism may play a role in the induction of breast cancer.

Estradiol (9) and other estrogens have been found to induce renal carcinoma in male Syrian hamsters, and the metabolite 4-hydroxyestradiol (10), was found to be as carcinogenic as estradiol (9). The other catechol metabolite, 2-hydroxyestradiol (11), did not induce renal carcinoma, however, but this is probably due to its rapid methylation to 2-methoxyestradiol (12) and metabolic clearance. The high carcinogenic activity of 4-hydroxyestradiol (10) is consistent with the idea that catechol estrogens play a part in estrogen induced carcinoma. This theory will be discussed in more detail below.

There is also concern that exogenous steroids may be carcinogenic. It is virtually certain that exposure in utero to diethylstilbestrol (DES, 13) [a nonsteroidal estrogen given to women to prevent threatened abortion] can cause clear cell adenocarcinomas of the vagina and cervix. The evidence that exogenous estrogens, given for
(7) R=H
(8) R=Me
(11), R=H
(12), R=Me
(13)
the treatment of menopausal symptoms, contribute to an increased risk of endometrial cancer is nearly as strong, and there is also evidence that sequential oral contraceptives may be associated with endometrial cancer.

Oral contraceptives have also been linked with cervical cancer, although the evidence for this was not conclusive. There is strong evidence, however, that oral contraceptives may reduce the risk of benign breast disease. They have also been linked with hepatic adenoma and it has been suggested that particular risk is associated with mestranol (14), which may be due to attempts by the liver to demethylate it to ethynylestradiol (15).

It has been suggested that testosterone (16) may play a role in the aetiology of prostate cancer. [Testosterone (16) and its metabolite, dihydrotestosterone (17), are the principal trophic hormones that regulate growth and function of epithelial prostate tissue.] It has been observed that prostate cancer is almost twice as likely to develop in blacks as compared to whites in the United States. As this 2:1 ratio is already apparent at the age
of 45 years (the age at which the earliest prostate cancer cases occur) it would appear to suggest that the factors responsible for the difference occur early in life. A study undertaken in Los Angeles on young male college students' showed the mean testosterone (16) levels in blacks to be 19% higher than in whites, and free testosterone (16) levels to be 21% higher in blacks. Even after adjustments for time of sampling, age, weight, alcohol use, cigarette smoking and use of prescription drugs, blacks had 15% higher testosterone (16) levels and 13% higher free testosterone (16) levels. A 15% difference in circulating testosterone (16) levels could readily explain a twofold difference in prostate cancer risk.

As a further example of steroids as carcinogens the formation of the cholesterol epoxide (18) has been suggested as the cause of ultra-violet carcinogenesis of the skin\textsuperscript{[17]}. 

\begin{center}
\includegraphics[width=0.8\textwidth]{images.png}
\end{center}
2. Reactions with cellular constituents.

Most chemical carcinogens appear to bind covalently with critical cellular constituents. The reaction of many steroids with proteins and DNA has therefore been studied.

Not surprisingly, DES (13) has been studied extensively. Blackburn et al. found that DES (13) covalently bound to calf thymus DNA \textit{in vitro} as a result of activation by longwave ultra-violet light or by oxidation with iodine. This led to binding levels directly comparable with those established for carcinogenic polycyclic aromatic hydrocarbons. Blackburn also found a 2:1 selectivity by DES (13) for binding to purine rather than pyrimidine bases. DES (13) was also found to bind covalently with DNA \textit{in vitro} as a result of incubation with a liver microsomal preparation and on incubation with primary mouse foetal cells in culture. The extent of the binding was, however, low compared with other known carcinogens. It therefore seems possible that DES (13) would exhibit little if any toxicity were it not that hormone-target tissues possess remarkable receptor properties and specific transport mechanisms for hormones into the nucleus.

Blackburn et al. also found estradiol (9) and estrone (8) to bind covalently with DNA \textit{in vitro} as a result of
activation either chemically with iodine or hydrogen peroxide, or by incubation with a rat liver microsomal preparation. Duncan and Brookes\textsuperscript{21}, however, challenge the fact that estradiol (9) will covalently bind to DNA when incubated with rat liver microsomal preparations and suggest an estrogen/DNA/RNA or protein complex may be formed.

2-Hydroxyestradiol (11), 2-hydroxyestrone (19) and 2-hydroxy-17\textalpha;-ethynylestradiol (20) are oxidative metabolites of both the naturally occurring and synthetic estrogens (9, 8 and 15). These 2-hydroxyestrogens were found to be converted by rat liver microsomes into reactive metabolites that became irreversibly bound to microsomal protein\textsuperscript{22}, as were their original estrogens. This irreversible binding required microsomes, oxygen and NADPH. Hepatic microsomal cytochrome P-450 has been shown to form superoxide anions, and it was thought that the estrogens may bind via the formation of a superoxide anion. A decrease in binding when superoxide dismutase was present indicated that binding may be mediated in part by superoxide anions. However, the decrease in binding was only 20-35\%, indicating that some other oxidative pathway was also involved. When NADPH is omitted from the microsomal preparations and the superoxide generated by the soluble enzyme xanthine oxidase, only the 2-hydroxyestrogens are irreversibly bound. This reflects the requirement for prior
hydroxylation of the estrogens by microsomal NADPH cytochrome P-450 oxygenase if binding is to take place via a superoxide anion.

Kappus, Bolt and Remmer also found that 17α-ethynylestradiol (15) (a constituent of the contraceptive pill) bound irreversibly to microsomal proteins when incubated in vitro with rat liver microsomes. In vivo experiments also showed irreversible binding to liver proteins, and a comparison between [6,7-3H]ethynylestradiol and [6,7-3H]estradiol in vivo showed twice as much ethynylestradiol (15) bound to liver microsomes than estradiol (9). Further in vivo studies showed that metabolites of ethynylestradiol (15) can also readily bind to organs such as lung, spleen and kidney.
Ethynylestradiol (15) and estradiol (9) have also been shown to bind covalently to rat liver DNA in vivo. The binding of ethynylestradiol (15) is slightly greater than estradiol (9), possibly because metabolic degradation at C-16 is retarded. The binding for both steroids, however, is in the same order as for benzene and about 10,000 times below the binding of a typical liver carcinogen such as aflatoxin B, or N,N-dimethylnitrosamine.

As mentioned above, estrogens will induce renal carcinomas in Syrian hamsters. Syrian hamsters were treated with estrogens as structurally diverse as estradiol (9), 11β-methyl-17α-ethynylestradiol (21), 11β-ethyl-17α-ethynylestradiol (22), 11β-methoxy-17α-ethynylestradiol (23), DES (13) and hexestrol (24), and their organs later examined for DNA adducts. These adducts were investigated by 32P-postlabelling followed by tlc. Each of these estrogens produced an identical set of covalently modified nucleotides. The authors propose that the exogenous estrogens or their metabolites do not themselves bind covalently to DNA, but rather induce the formation of an unknown DNA adduct. The possibility that the exogenous estrogens modified cellular metabolites so that endogenous estrogens were converted to DNA-reactive metabolites was not ruled out. These DNA alterations were only induced in hamsters treated with estrogens, and not with other steroids such as progesterone (25) or deoxycorticosterone acetate (26).
Also, the DNA alterations were only found in the hamster kidney, suggesting that these observed alterations might play a role in estrogen-induced carcinogenesis.

The epoxide (27) is a known metabolite of norethisterone (28), another constituent of many contraceptive pills. When the epoxide (27) was incubated with several proteins and nucleic acids it bound irreversibly to proteins containing free sulphydryl groups but not to constituents without SH-groups, such as concanavalin A, gamma-globulin, DNA and RNA. When the epoxide (27) was incubated with rat hepatic microsomes, addition of NADPH increased the amount of irreversible binding to microsomal proteins and on addition of glutathione and cytosol only the NADPH-dependent protein binding decreased. These results indicate that as well as binding directly to proteins, the epoxide (27) is converted by hepatic microsomal enzymes to another metabolite which can also react with proteins.

The cholesterol epoxide (18), a known carcinogenic metabolite of cholesterol (1), has also been shown to bind covalently with calf thymus DNA.
(21), R=Me
(22), R=Et
(23), R=MeO

(24)

(25)

(26)

(27)

(28)

It may be noted that, in order to react with particular cellular constituents, it is generally required that the steroids are metabolised into electrophilic species, in particular epoxides. In order to predict how a steroid may behave in the body, with respect to its carcinogenicity, it is useful to be able to ascertain the type of metabolite that may be formed.

Much work has been done concerning the metabolites of DES (13). Metzler\textsuperscript{25} has identified some oxidative metabolites which may react with cellular constituents, e.g. (29)-(32).

![Chemical structures](image_url)
Metzler has also proposed that 3,4-epoxyDES (33) may be an intermediate in the metabolic pathway leading to 4-hydroxypropiophenone (HPP, 34).

![Chemical Structure of 3,4-epoxyDES (33)](image1)

![Chemical Structure of 4-hydroxypropiophenone (HPP, 34)](image2)

DES-quinone (35) has also been postulated as a metabolic intermediate, leading to the formation of Z,Z-dienestrol (29). The existence of DES-quinone (35) as a metabolic intermediate of DES (13) has not yet been established, but its chemical instability and reactivity have led to suggestions that it might play a role in DES-induced carcinogenesis.

![Chemical Structure of DES-quinone (35)](image3)

DES-quinone (35) has been shown to bind to DNA, although no DES-quinone (35) adducts have been isolated and the binding was found to decrease with increasing reaction times. An unstable covalent interaction with DNA was suggested and Schiff base-type intermediates, which ultimately and irreversibly rearrange to Z,Z-dienestrol.

-15-
were postulated (fig.1). DES-quinone (35) has also been shown to form covalent adducts with sulphhydryl-rich proteins.

![Chemical structures](image)

Fig. 1:

As described above, several different estrogens produced the same DNA-adducts in Syrian hamster kidneys. It was postulated that these adducts arose from an estrogen induced covalent linkage of some unknown endogenous metabolite to DNA. It was further postulated that the unknown substance is activated by radical reactions. DES-quinone (35) possibly participates in the generation of radicals as shown in fig.2. Oxidation of DES (13) and reduction of DES-quinone (35) by single electron transfer would lead to the formation of the DES-semiquinone radical. This, in turn, could be responsible for damaging DNA directly, or it may react with molecular oxygen to produce superoxide anion radical ($O_2^-$) and thus
regenerate the quinone (35). This type of quinone redox cycling and concurrent generation of $\cdot O_2^-$ has been shown to be mutagenic in the Ames Test. The superoxide anion radicals and/or semiquinone radicals may thus cause the DNA modification observed by Liehr et al in the Syrian hamster kidney.

![Diagram](image)

Fig. 2. 1. peroxidase $H_2O_2$; 2. PASO reductase, NADPH.

Studies on the metabolism of DES (13) with prostaglandin synthase$^{30}$ have provided evidence for the formation of DES-quinone (35); the UV spectra recorded in incubations of DES (13) with ram seminal vesicle gland microsomes in the presence of arachidonic acid, closely resemble published spectra of DES-quinone (35). Arguments are also given for the formation of an intermediate radical.

ω-Hydroxy-dienestrol (31) is a major metabolite of DES$^{31}$. The alkylating potential of its acetate ester was assessed by incubating with 4- (p-nitrobenzyl)pyridine, as was the alkylating potential of $\omega,\omega'$-diacetoxy-β-dienestrolacetate (36). The di-$\omega$-substituted compound
(36) was found to alkylate at twice the rate of the mono-ω-substituted compound (31).

The compounds (31) and (36) have similarities with the carcinogenic metabolites of safrole and estragole, 1-hydroxysafrole (37) and 1-hydroxyestragole (38). From indirect experimental evidence Phillips and Miller\textsuperscript{32,33} have proposed the following metabolic pathway for (37) and (38) (fig. 3.), leading to three major metabolites.

Fig. 3.
Much work has also been performed to identify the metabolites of steroidal estrogens. Estrone (8) and estradiol (9) are interconvertible by a 17β-hydroxysteroid dehydrogenase. Their metabolism includes hydroxylation at C-6 and C-16 (to give 39, 40, 41 and 42) and further oxidation to 6-oxo- and 16-oxo-estrone and estradiol (43, 44, 45 and 46). Hydroxylation also takes place to give the catechols 2-hydroxy- and 4-hydroxy-estrone and estradiol (19, 11, 47 and 10). These catechols are methylated to give the 2-methoxy- and 4-methoxy-steroids (48, 12, 49 and 50). Methylation also takes place at C-3. Knuppen et al. found that 2-hydroxyestrone (19) was methylated in vitro to 2-methoxyestrone (48) and 2-hydroxyestrone 3-methyl ether (51) by human liver, and that the two isomers were formed in approximately equal amounts. In vivo studies, however, found the concentration of 2-hydroxyestrone 3-methyl ether (51) in pregnancy urine to be approximately one-twentieth of that of the isomeric 2-methoxyestrone (48). No explanation is given for the divergence between these in vitro and in vivo results.

Li and coworkers argue that the formation of catechols may be pertinent in the induction of carcinomas by estrogens, and in particular in the induction of renal carcinoma in Syrian hamsters. They found that different steroidal estrogens exhibited decreasing catechol formation with hamster kidney microsomal preparations in
(39), \( R = O \)
(40), \( R = \overset{\text{OH}}{\text{H}} \)

(41), \( R = O \)
(42), \( R = \overset{\text{OH}}{\text{H}} \)

(43), \( R = O \)
(44), \( R = \overset{\text{OH}}{\text{H}} \)

(45), \( R = O \)
(46), \( R = \overset{\text{OH}}{\text{H}} \)

(19), \( R^1 = R^2 = H; \ R^3 = O \)
(11), \( R^1 = R^2 = H; \ R^3 = \overset{\text{OH}}{\text{H}} \)
(48), \( R^1 = H; \ R^2 = \text{Me}; \ R^3 = O \)
(12), \( R^1 = H; \ R^2 = \text{Me}; \ R^3 = \overset{\text{OH}}{\text{H}} \)
(51), \( R^1 = \text{Me}; \ R^2 = H; \ R^3 = O \)

(47), \( R^1 = H; \ R^2 = O \)
(10), \( R^1 = H; \ R^2 = \overset{\text{OH}}{\text{H}} \)
(49), \( R^1 = \text{Me}; \ R^2 = O \)
(50), \( R^1 = \text{Me}; \ R^2 = \overset{\text{OH}}{\text{H}} \)
the following order: estrone (8) > equilenin (52) >
estriadiol (9) > equilin (53) > ethynylestradiol (15) >
estriol (7). Except for \(Z,\overline{Z}\)-dienestrol (29), the stilbene
estrogens revealed levels of catechol formation that were
similar to estradiol (9). These results may explain the
weak carcinogenic activity of ethynylestradiol (15),
estriol (7), and \(Z,\overline{Z}\)-dienestrol (29) in Syrian hamsters,
as they are poor substrates for hamster renal estrogen 2-
and 4-hydroxylase (ESH). Conversely, the potent
carcinogens DES (13), \(E,\overline{E}\)-dienestrol (54), and hexestrol
(24) exhibited substantial levels of \(\alpha\)-hydroxylation.

The greater susceptibility of hamsters to renal
tumorigenesis as compared to rats may be explained by the
fact that the ESH activity in hamster kidney microsomes
was found to be substantially greater than in rat kidney

-21-
microsomes, and consequently more catechol intermediates are formed in the hamster kidney. Further evidence that catechols may play a part in the induction of carcinomas was found in the fact that a 3.5- to nearly 6-fold decline in catechol formation was observed in hamster kidney, but not in hamster liver, when the animals had been exposed to α-naphthoflavone. α-Naphthoflavone has been reported to inhibit the induction of renal tumours by estrogens. The amount of catechol formation does not, however, explain the weak carcinogenicity of equilenin (52) in hamster kidney or the fact that estrone (8) is not as potent as either DES (13) or estradiol (9) in effecting transformation in the hamster kidney. These results would appear to indicate that other aspects of estrogen metabolism as well as estrogenic potency contribute significantly to the ultimate carcinogenicity of estrogens.

These arguments for the role of catechols in estrogen induced carcinoma conflict with those of Liehr and co-workers (reported above), who suggest that the formation of a DES-quinone (35) may be important in the induction of carcinoma by DES (13).²⁹

Le Quesne et al.²⁶ suggest that the estrogen catechols may be formed via an epoxide intermediate, and that this intermediate may be important in estrogen carcinogenicity by binding to cytoplasmic and nuclear receptors. As
reported above estradiol (9) metabolises to the catechols (10) and (14). Le Quesne and co-workers suggest that these may be formed either via oxirane hemiacetals such as (55) and (56), or via dienol epoxides and their keto tautomers e.g. (57)-(59), and (60)-(62). Whilst (55) and (56) would be expected to be of such a highly reactive and transient nature that proof of their existence may be difficult and equivocal, the intermediates (57) and (60) are capable of stabilization as the keto tautomers (58), (59), (61), and (62). The 17-O-acetyl derivatives of (59) and (62) were found to aromatize to the tri-O-acetyl derivatives of (10) and (11) respectively when treated with p-toluenesulphonic acid in acetic anhydride. Interestingly, the 17-O-acetyl derivative of compound (59a) was shown to be as active as the highly mutagenic 3-methylcholanthrene in inducing chemical transformations of mouse fibroblast cells, and at least two orders of magnitude more effective than estradiol (9). The 17-O-acetyl derivative of (59b) and (62), however, were inactive in the same concentrations as used for the 17-O-acetyl derivative of (59a).

Estrogen epoxides and estrogen-α-quinones/semiquinones have been proposed as being responsible for the genotoxicity of estrogens. Abul-Hajj\textsuperscript{37} carried out a study to determine whether the 1,2-epoxyestrogens (e.g. 60) [enol tautomers of 1,2-epoxy-4-estrene-3-one (e.g. 62)] would bind to macromolecules and form water-soluble
\( R^1 = R^2 = H \)  
\( R^1 = H, R^2 = OH \)  
\( R^1 = OH, R^2 = H \)

\( a = \alpha\text{-epoxide}; b = \beta\text{-epoxide.} \)
metabolites, and to determine whether conjugation with sulphydryl groups would lead to the same products as derived from microsomal metabolism of estradiol.

Radio labelled estradiol (9) and 1α,2α-epoxy-4-estrene-3-one-17β-ol (62a) were incubated with rat liver microsomes and rat liver cytosol. The radioactivity irreversibly bound to the protein, and the aqueous-soluble and organic-soluble fractions was determined. Addition of SKF 525A (which inhibits P-450 mediated hydroxylations) was found to reduced the radioactivity bound to the protein and the aqueous-soluble fraction with estradiol (9), but had no effect with 1,2-epoxyestrenolone (62a). Addition of ascorbic acid (which inhibits the oxidation of catechols to the o-semiquinones and quinones) decreased the binding to the protein and the aqueous-soluble fraction with both estrogens in the incubations with microsomes, but had no effect with the cytosol fraction. Incubations of both estrogens with microsomes containing cysteine resulted in an aqueous soluble product which had a HPLC retention time similar to that of the di-cysteine adduct (63). These results suggest that both estradiol (9) and 1,2-epoxyestrenolone (62a) are transformed to catechols, followed by oxidation to the o-semiquinone/quinone which reacts with cysteine to form the 2-hydroxyestradiol-1(4)-thioether (63) (fig.4).
Incubations with rat liver cytosol in the presence of thiol showed no aqueous soluble metabolites with estradiol (9). Using 1,2-epoxyestrenolone (62a) as a substrate, however, produced estradiol-2-thioether (64) and another compound, tentatively identified as 1α-hydroxy-4-estrene-3-one-17β-ol-2β-yl-cysteine (65). These results indicate that these metabolites are formed by enzymatic catalysis with glutathione-S-transferase, since incubations with cysteine or glutathione in the absence of cytosol showed no transformation.

The di-thioether adducts (63) appear to arise from non-enzymic additions to the quinone/semiquinone. On the other hand, the formation of estradiol-2-thioether (64) requires the presence of glutathione-S-transferase. In order to determine whether 1,2-epoxyestradiol (60) may be involved as an active intermediate, an incubation of estradiol (9) with microsomes in the presence of cysteine and rat liver cytosol was carried out. Only di-thioether adducts (63) were formed, suggesting that 1,2-epoxyestradiol (60) may not be formed as an active intermediate. Further support for these results is obtained from in vivo studies, in which no estradiol-2-thioether analogues (64) have been observed. Whilst these results do not rule out 1,2-epoxyestradiol (60) as an intermediate in estrogen metabolism, it does suggest that the major pathway for irreversible binding of estrogens to macromolecules involves the estrogen
Fig. 4.
α-semiquinone and quinone (66) and not the postulated 1,2-epoxyestrogen. (60)

Support for the proposal that epoxide intermediates lead to the catechol estrogens, is afforded from a study of the metabolism of polycyclic aromatic hydrocarbons. The carcinogen benzo(a)pyrene (67) has been shown to metabolise, via the 7,8-dihydrodiol (68), to 7,8-dihydroxy-9,10-epoxy-7,8,9,10-tetrahydro-benzo(a)pyrene (69)39,40.

![Chemical structures](image)

Benzo(a)pyrene (67) was found to form optically pure (-)7β,8α-dihydroxy-7,8-dihydrobenzo(a)pyrene (71) on incubation with rat liver microsomes39. This trans-diol (71), which was thought to be formed via the epoxide (70, fig.5), was further metabolised to, predominantly,
7β,8α-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydrobenzo(a)-pyrene (72). Other unidentified metabolites were also formed. Racemic trans-dihydroxy-7,8-dihydrobenzo(a)pyrene (71 and its enantiomer) metabolised to both the 9α,10α-epoxide and the 9β,10β-epoxide (72,73 and their enantiomers).

![Diagram](image_url)

Fig. 5.

Experiments involving the induction of skin tumours in mice with various optically pure enantiomers of *trans*-7,8-dihydroxy-9,10-epoxy-7,8,9,10-tetrahydrobenzo(a)pyrene⁴⁰ (69) concluded that (+)7β,8α-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydrobenzo(a)pyrene (72) is the ultimate carcinogenic form of benzo(a)pyrene (67). 7,8-Dihydroxy-9,10-epoxy-7,8,9,10-tetrahydrobenzo(a)pyr-
ene (69) has been found to react with DNA even in unfavourable conditions such as those found when adding Mg$^{2+}$ to DNA or when denaturing the DNA.$^{41}$

Metabolism of chrysene (74a) has been found to form the triol-epoxide, trans-1,2-dihydro-1,2-dihydroxy-3,4-epoxy-9-hydroxychrysene (74)$^{42}$. This triol-epoxide metabolite bears some structural resemblance to 4,5-epoxy-9-hydroxybenzo(a)pyrene (75) which, in some biological situations, is also involved in the metabolic activation of benzo(a)pyrene(67).

![Chemical Structures](image)

Both contain a distant phenolic OH-group which could enhance the chemical reactivity of the hydrocarbon epoxides. The phenolic OH-groups will stabilize the reaction transition states by donating electronic charge to the incipient carbonium ions, thereby enhancing chemical reaction rates. They may also stabilize the resulting carbonium ion to such an extent that a new and distinct chemical species, quinone-methides (e.g. 76, fig 6), may be postulated. It may be these quinone-methides
are the alkylating species which react with cellular macromolecules.

Fig. 6.

As indicated above, bay-region diol-epoxides derived from polycyclic aromatic hydrocarbons have been implicated as 'ultimate carcinogens' responsible for the carcinogenic behaviour of certain hydrocarbons. Their solution chemistry has been of considerable interest, as this
may give some indication of how they will react with cellular constituents.

The acid-catalysed hydration of diol-epoxides (77) and (78) and tetrahydro-epoxides (79) have been studied. Interestingly, the trans-diol-cis-epoxides (78a-c) underwent predominantly trans hydration, whereas the trans-diol-trans-epoxides (77a-c) and the tetrahydro-epoxides (79a-d) gave varied cis/trans hydration ratios. It was found that the amount of cis-hydration decreased from a to d e.g. (77a) gave 80% cis hydration and (77b) gave only 50% cis-hydration. Perhaps not surprisingly it appears that the amount of cis-hydration increases as the ability of the aryl group to stabilize positive charge at the benzylic position increases.

![Chemical structures]

\[ (77) \quad (78) \quad (79) \]

a, benzo(a)pyrene; c, phenanthrene;
b, chrysene; d, naphthalene.

In order to prove this hypothesis, the hydrolysis of 1.2-epoxytetralin (80a) was compared with that of 6-methoxy-1.2-epoxytetralin (80b). The 6-methoxy group would be expected to stabilize positive charge at the
benzylic position, as do additional aromatic rings. It was found that acid-catalysed hydrolysis of (80a) produced 94% of the trans-hydration product (82a), whereas (80b) produced 81% of the cis-diol (81b). Thus, substitution of methoxyl at the 6-position of (80a) has the same effect as the substitution of additional aromatic rings, indicating that stabilization of the benzylic cation is an important factor in determining the hydrolysis products.

\[ \begin{align*}
(80a) & \quad X=H; \quad b, \quad X=\text{O}Me. \\
(81a) & \quad X=\text{OH}; \\
(82a) & \quad X=\text{OH}.
\end{align*} \]

From 250-MHz nmr spectra, the structure of (80a) and (80b) was established to be conformation (83). Protonation of (83) would lead to the intermediate benzyl cations, (84). (Evidence for the production of an intermediate cation in the acid-catalysed hydration of (80a) has been provided by the fact that in solutions containing chloride ion, the product distribution is different than when chloride ion is absent despite the fact that there is no kinetic dependence on chloride ion at sufficiently low pH. Therefore, an intermediate must be trapped by chloride ion subsequent to the
rate-limiting step.) Unsubstituted cyclohexenyl cations are known to undergo preferential pseudoaxial attack by solvent at a rate faster than conformational isomerisation of the ion, and therefore, the unstabilised ion (84a) might be expected to undergo a similar attack by solvent to yield the trans-diol (82a). The more stable cation (84b), however, could undergo conformation isomerization to the more stable conformation (85b) which would then give the cis-hydration product (81b). This explanation accounts for the fact that as the ability of the aryl group to stabilize positive charge at the benzyl position increases, cis-hydration increases.

\[
\begin{align*}
(83) & \quad \rightarrow \\
(84) & \quad \downarrow \\
(85)
\end{align*}
\]

a, X=H; b, X=OMe.

The predominant trans-hydration of the trans-diol epoxide series (78a-c) has been rationalised by assuming that
the epoxide opens to give the cation (86) already in the more stable conformation, with the hydroxyl groups equatorial. Subsequent attack by water would then lead to the newly formed hydroxyl group in the axial position (fig. 7).

Fig. 7.

It has already been mentioned that mestranol (14) is demethylated in the liver to ethynylestradiol (15). The major metabolites of ethynylestradiol (EE) (15), found both from in vitro incubation experiments with liver tissue and in the urine, are 2-methoxy-EE (87), 16β-OH-EE (88), as well as estrone (8), estradiol (9) and estriol (7) derived from de-ethynylation. Also present in urine
are 2-methoxyestradiol (12) and 2-hydroxy-EE(20). The urinary metabolites are almost entirely present in conjugated form and about 15% are de-ethynylated (identified by radio labelling). De-ethynylation has also been reported by Helton, Williams and Goldzieher\textsuperscript{45} on incubation of EE (15) with baboon liver microsomes. Helton and Goldzieher have proposed a mechanism for this de-ethynylation which involves the reactive oxirene (89, fig. 9).

\begin{align*}
(14) & \ R^1=\text{Me}; \ R^2=\text{R}^3=\text{H}. \\
(15) & \ R^1=R^2=\text{R}^3=\text{H}. \\
(20) & \ R^1=\text{R}^3=\text{H}; \ R^2=\text{OH}. \\
(87) & \ R^1=\text{R}^3=\text{H}; \ R^2=\text{OMe}. \\
(88) & \ R^1=R^2=\text{H}; \ R^3=\text{OH}.
\end{align*}

The major metabolic pathway for 3-oxo-Δ\textsuperscript{4}-steroids (90) is reduction of the double bond (to give 91) followed by reduction of the keto-group to afford the 3-hydroxy-steroids (92)\textsuperscript{34}.

\begin{align*}
(90) & \\
(91) & \\
(92)
\end{align*}

Fig. 8. \hspace{1cm} R=\text{H or Me}
Fig. 9.
Incubation with beagle liver of the 3-oxo-Δ4-steroid, norethisterone (28), led to the isolation of two additional metabolites - the 4β,5β-epoxide (27) and the 6-oxo-compound (93). It is suggested that the epoxide (27) is formed by direct epoxidation of norethisterone (28) and that the 6-oxo-compound (93) is formed via 6-hydroxylation.

Incubation of testosterone (16), another 3-oxo-Δ4-steroid, with liver microsomes from dexamethasone-treated rats has recently been found to produce the Δ4-steroid - 17β-hydroxy-4,6-androstadiene-3-one (94).
4. Proposed investigations.

The aim of this project was to synthesise potential steroid metabolites with alkylating properties so that their reactions with important cellular constituents and their mutagenicity can be investigated. Examples of these potential metabolites are the epoxides (95) and (96).

\[
\begin{align*}
(95) & \quad \text{R} = \text{H; b, R=OH or OMe.} \\
(96) & \quad \text{6,7-Epoxyestradiol (95a) is a potential metabolite of estradiol (9) and other estrogens. As described previously, 6-hydroxyestrogens (e.g. 40) are established metabolites. and it has also been reported}\text{ that 6-hydroxyestrogens will readily dehydrate to give } \Delta^6\text{-estrogens under acidic conditions. Epoxidation of 6,7-dehydroestradiol (97) would yield compound (95a).}
\end{align*}
\]
As reported above, benzylic epoxides have been implicated in the carcinogenic behaviour of certain hydrocarbons, and addition of a 2-hydroxy or a 2-methoxy group (to give 95b) would be expected to increase the alkylating activity of the epoxide still further.

The epoxy-alcohol (96) can be considered as a potential metabolite of ethynylestradiol (15) and is related to the oxirene (89). Its formation could involve reduction to the 17α-vinyl compound (98) and oxidation to the epoxide (96).

\[ \text{HO} \quad \text{(98)} \]

It was also envisaged that the potential target estrogen compounds may have alkylating properties and still retain their hormonal properties. They may therefore be of importance as selectively toxic compounds to estrogen dependent tumours.
Chapter 1: Production of 17β-hydroxy-17α-vinyl- and 20,21-epoxy-steroids from 17α-ethynyl-steroids.

As indicated in the introduction 17β-hydroxy-17α-vinyl- and 20,21-epoxy-steroids are potential metabolites of 17α-ethynyl-steroids.

1.1 Preparation of 17β-hydroxy-17α-vinyl-steroids.

The 17β-hydroxy-17α-vinyl compounds (99) and (98) were synthesised from their 17α-ethynyl counterparts, (28) and (15).

Initially partial hydrogenation of the 17α-ethynyl derivative (28) in pyridine solution over a 5% Pd on CaCO₃ catalyst was attempted. However, the reaction end-point was indeterminate and 5% Pd on BaSO₄ was found to be a more suitable catalyst for the preparation of both (99) and (98). The catalyst was pre-reduced before addition of the steroid and the reaction end-point was judged by a slowing in the uptake of hydrogen. The
reduction to afford 17α-vinylestradiol (98) always produced some over-reduced compound, 17α-ethylestradiol (100), which could not be removed completely either by recrystallisation or preparative tlc. The solvent was changed from pyridine to ethyl acetate containing a small percentage of quinoline in the hope that the reaction would be more selective, but no improvement was found. The best results were obtained using pyridine, stirring the reaction vigorously in a large flask to increase the surface area and hence the rate of hydrogen uptake, and by stopping the reaction as soon as the rate of hydrogen uptake slowed.

Samples of (99) and (98) were sent to the Chester Beatty group (see appendix).

1.2 Attempted 20,21-epoxidation of 20,21-dihydro-norethisterone (99).

A series of experiments were tried to epoxidise 20,21-dihydronorethisterone (99) selectively at C-20,21 with m-chloroperoxybenzoic acid (MCPBA). When (99) was
treated with 1.1 molar equivalents of MCPBA in dichloromethane, the reaction was only two thirds complete after two days. Addition of a further two molar equivalents of MCPBA and allowing the reaction to continue for five days removed all the starting material but, from tlc and the 'Hnmr spectrum it appeared that at least two other compounds had been produced as well as the required compound (101). It was thought that this may be due to acid-catalysed rearrangement and as a consequence, an experiment was tried in diethylether, adding sodium bicarbonate to neutralise any m-chlorobenzoic acid produced. No 20,21-epoxide was produced. Instead an alternative reaction, thought to be the Baeyer-Villiger oxidation, took place to produce compound (102). The acid-catalysed Baeyer-Villiger oxidation of this A-ring system with perbenzoic acid has been reported. The structural assignment of the product (102) was based on the 'Hnmr spectrum (the signal for the C-4 proton shifted down-field from 65.87 [for (99)] to 66.16) and the carbonyl absorption in the ir spectrum at 1750 cm⁻¹ [cf carbonyl absorption at 1740 cm⁻¹ for the related compound (103)].
In the light of this reaction and from the 'Hnmr spectroscopic data (table 1), the products produced in the initial dichloromethane reaction were thought to be (104) [12.5%], (105) [37.5%] and (101) [50%]. Examination of 'Hnmr spectra taken before the reaction was complete showed that the by-products (104) and (105) were being produced before all the starting material had reacted. It would appear that with no base present the mild acid conditions catalysed an attack at the A-ring alongside the C-20 epoxidation, but when base is added, A-ring attack takes place preferentially.

Table 1. 'Hnmr spectroscopic data of the crude product mixture from the MCPBA/CH₂Cl₂ oxidation of (99).

<table>
<thead>
<tr>
<th>Steroid</th>
<th>I</th>
<th>δ</th>
<th>%</th>
<th>I</th>
</tr>
</thead>
<tbody>
<tr>
<td>101</td>
<td>I</td>
<td>5.8</td>
<td></td>
<td>50%</td>
</tr>
<tr>
<td>104</td>
<td>I</td>
<td>6.1</td>
<td></td>
<td>12.5%</td>
</tr>
<tr>
<td>105</td>
<td>I</td>
<td>4.7 and 4.8</td>
<td></td>
<td>37.5%</td>
</tr>
<tr>
<td></td>
<td>I</td>
<td>(α- and β-H)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

δ - δ value for the C-4 proton singlet.
I - integration of the C-4 proton signal expressed as a percentage of the integration of the C-20 proton signal at δ3.0-3.3.
Other solvents were tried, namely diethylether, benzene, and chloroform, to see whether the attack at the A-ring could be reduced. In all cases, some Baeyer-Villiger oxidation took place. Chloroform proved to be the best solvent, with only a small amount of (104) being produced, although some starting material also remained. Preparative tlc of the chloroform reaction product on alumina produced a mixture of (101) (70%) and starting material (99) (30%) in about 34% yield. This is obviously not a very satisfactory preparation. It was anticipated that better results may be achieved by use of $t$-butylhydroperoxide and $VO(acac)_2$, as this reaction is specific to 2,3-epoxy alcohols. 
1.3 Preparation of 3,17β-dihydroxy-20,21-epoxy-19-nor-
pragna-1,3,5(10)-triene (96).

An attempt to epoxidise 17α-vinylestradiol (98) with MCPBA in chloroform caused reaction at the A-ring. To avoid this, the 3-O-acetyl derivative was prepared and allowed to react with MCPBA in chloroform. Purification by preparative tlc on silica gave a 56:44 mixture of the (20R)- and (20S)-20,21-epoxides (106b) and (107b) in 37% yield (the assignments of these configurations are explained below). The silica, in fact, caused some breakdown of the epoxides, and alumina chromatography would probably have been preferable. Later work showed that oxidation with t-butylhydroperoxide and VO(acac)₂ was a better procedure for this reaction (see below).

In one attempt to repeat the above acetylation and epoxidation, the acetylation reaction with acetic anhydride in pyridine produced a compound which was at first assumed to be 17α-vinylestradiyl diacetate (108). Epoxidation with MCPBA gave two compounds which, when
separated by preparative tlc on alumina, and were found to be the (20S)-20,21-epoxide (107b) and a compound thought to be the 13,21-oxy-compound (109). The structure of this compound was assigned from the 'Hnmr spectrum (δ3.4 (1H, t, J=10Hz, 20-CH) and 3.88-4.17 (2H, m, 21-CH₂)) and mass spectral data (M⁺, 356.1996), and also from the 'Hnmr spectrum of its O-acetyl derivative which compares with that of the O-acetyl derivative of an acid rearrangement product of the 17,20-epoxide (118a, see §4.2).

As mentioned above, the starting material used in the above epoxidation was assumed at first to be 17α-vinylestradiyl diacetate (108). However, this material could not be synthesised again using acetic anhydride in pyridine, leading to the conclusion that the original reaction contained an unknown reagent. For the purposes of this thesis, this unknown diacetate will be referred to as diacetate-I.

17α-Vinylestradiyl diacetate (108) was synthesised both by acetylating 17α-vinylestradiol (98) with acetic anhydride
and 4-dimethylaminopyridine in triethylamine\(^{31}\), and by acetylation of 17\(\alpha\)-ethynylestradiol (15) with acetic anhydride and \(p\)-toluenesulphonic acid\(^{32}\) followed by partial hydrogenation. The latter method was found to be preferable because the former acetylation did not go to completion. The product from both these reactions was 17\(\alpha\)-vinylestradiyl diacetate (108) [henceforth known as diacetate-II] and this was found to be different from diacetate-I. The principle differences in the \(^1\)Hnmr spectra are the signals for the 17-AcO \([\delta 1.19\) (diacetate-I) and \(\delta 2.25\) (diacetate-II)], the C-21 protons \([\delta 5.11-5.38\) (diacetate-I) and \(\delta 4.96-5.37\) (diacetate-II)] and the C-20 protons \([\delta 6.00-6.36\) (diacetate-I) and \(\delta 5.80-6.16\) (diacetate-II)]. This suggests that the diacetate-I is a rearranged product. Acid-catalysed rearrangements of 17\(\alpha\)-vinyl steroids of the type shown in fig.10 have been reported\(^{33}\). It may be that diacetate-I is the rearranged product 3,17\(\alpha\)-diacetoxy-17\(\beta\)-vinylestra-1,3,5(10)-triene (110, fig. 11). However, sufficient data was not obtained to confirm this proposal or explain the oxidation of diacetate-I.

As indicated above, the epoxidation of 17\(\alpha\)-vinylestradiol (98) with \(t\)-butylhydroperoxide (\(t\)-BuOOH) and VO(acac)\(_2\) proved preferable to using MCPBA. The specificity of the reaction meant that prior acetylation was not necessary. Treatment of 17\(\alpha\)-vinylestradiol (98) with \(t\)-BuOOH/VO(acac)\(_2\)\(^{34}\) gave a 65:35 mixture (determined by
Fig. 10.

Fig. 11.
integration of the 20-H triplets at 63.22 and 63.34 in the ¹Hnmr spectrum of the (20R)- and (20S)-20,21-epoxides (63a) and (64a) respectively.

The diastereoisomers were separated by fractional recrystallisation to give (106a) in 39% yield and (107a) in 11% yield. The assignment of the 20R-configuration to the major product follows arguments similar to those presented by Sharpless et al. The proposed mechanism for the epoxidation is shown in fig.12. The slow step in the catalytic cycle is thought to be the oxygen transfer step (111) → (112). This is also the step which will determine the stereoselectivity. The predicted transition states leading to the two epoxides (106a) and (107a) are shown in fig.13. The O-C-C=C dihedral angle of ~50° was determined by Sharpless from a study of simpler acyclic allylic alcohols. It is thought that transition state (113) will be preferred over (114) because, in the latter, there is significant steric interaction between the 20-H and the 12α-H (this can be seen most clearly from a model). Consequently, the major product is most likely to be (106a).

Samples of the epoxides (106a) and (107a) were sent to the Chester Beatty group. They were also tested at Loughborough University as part of the on-going project to find target-specific anti-tumour agents (see appendix).
Fig. 12. Proposed mechanism for Vanadium catalysed epoxidations.

Fig. 13.
Chapter 2: Reactions of the 20,21-epoxides (106a) and (107a)

2.1 The Payne Rearrangement.

The Payne rearrangement of 2,3-epoxy alcohols (fig. 14.) has been well documented\textsuperscript{56,57} The rearrangement of the 20,21-epoxides (106a) and (107a) was of interest because it is possible that this rearrangement is induced metabolically, and it was anticipated that compound (106a) would undergo the rearrangement far more readily than compound (107a) (see the discussion below). The reaction could therefore serve to confirm the configurational assignments proposed for (106a) and (107a).

![Diagram of Payne rearrangement](image)

Fig. 14.

It would be expected that conformation (115), where the OH group is antiperiplanar to the C(20)-O bond, would be preferred for the Payne rearrangement. It was anticipated that this conformation (116) could be easily attained by the (20R)-20,21-epoxide (106a), whereas (117) for the
(20S)-20,21-epoxide (107a) would experience considerable steric hindrance between the 21-methylene group and the 12α-H. Thus, it would be expected that (106a) would undergo the Payne rearrangement more readily than (107a) if the correct structure is assigned to each compound.

The (20R)-20,21-epoxide (106a) did in fact readily rearrange in MeOH/K₂CO₃ to the (20S)-17β,20-epoxide (118a, fig.15), whilst the (20S)-20,21-epoxide (107a) did not rearrange under these conditions. The reaction with the (20R)-20,21-epoxide (106a) went to completion, showing the rearranged product (118a) to be the preferred structure. This agrees well with the literature data on 2,3-epoxy alcohols, which reports that, generally, 2,3-epoxy alcohols with a primary hydroxyl are more stable than those with a tertiary hydroxyl. The 400MHz ¹Hnmr spectrum of (118a) showed a triplet at δ3.17 (1H, J=5Hz, 20-CH) and a multiplet at δ3.66 (2H, 21-CH₂). On adding D₂O, this multiplet simplified to an octet, the AB component of an ABX system (JAB=12Hz), thereby showing that some coupling between the C21-OH and the C21 protons is exhibited in the original spectrum. In the 60MHz ¹Hnmr spectrum of the diacetate (118b), the triplet
became a quartet at δ3.2 (1H, J_Ax=4Hz, J_Bx=6Hz, 20-CH) and the multiplet (δ3.66) became an octet, shifted downfield to δ4.0 (2H, J_AB=12Hz, 21-CH$_2$).

\[
\begin{align*}
\text{Fig. 15.}
\end{align*}
\]

The use of a stronger base (t-BuOK/t-BuOH) was required to induce the Payne rearrangement of the (20S)-20,21-epoxide (107a) to the (20R)-17β,20-epoxide (119a, fig.16). Again, the reaction went to completion. Interestingly, when the reaction was worked up by first diluting the reaction mixture with water, the expected epoxide (119a) was produced. However, if the reaction mixture was first diluted with EtOAc the 21-acetate (119b) was produced, presumably via attack of the 21-oxygen anion on the EtOAc added. The 'Hnmr spectrum of (119a) showed two multiplets which upon D$_2$O exchange became a quartet at δ3.09 (1H, J_Ax=4Hz, and J_Bx=7Hz, 20-CH) and an octet at δ3.90 (2H, J_AB=12Hz, 21-CH$_2$). The 'Hnmr spectrum of the acetate (119b) had a quartet at δ3.15 (1H, J_Ax=2Hz, J_Bx=8Hz, 20-CH). The signal for the C21 protons were shifted downfield and split into two

\[
a, R=H ; b, R=Ac.
\]
quartets at $\delta 4.13$ (1H, $J_{AB}=12$Hz, $J_{AX}=8$Hz, 21-CH) and $\delta 4.64$ (1H, $J_{AB}=12$Hz, $J_{AX}=2$Hz, 21-CH). The larger ABX splitting patterns of the (20R)-17β,20-epoxides (119a) and (119b) as compared to those of the (20S)-17β,20-epoxides (118a) and (118b) may be explained by steric interaction. With the (20R)-17β,20-epoxides (119a) and (119b) there is considerable steric interaction between the 12α-H and the C21 protons and oxy group which will greatly hinder the rotation about C21. Consequently, the difference in the environments of the two C21 protons is increased.

Fig. 16. a, R=H; b, R=Ac

Samples of (118a) and (119a) were sent to the Chester Beatty group, and were tested at Loughborough University as part of the project to find target-specific anti-tumour agents (see appendix).
2.2 Acid hydrolysis of the 17β,20-epoxide (118a).

Acid hydrolysis was attempted on the (20S)-17β,20-epoxide (118a) because in addition to providing information on the electrophilic behaviour, it was hoped that the epoxide would open at C17 to provide a novel route to steroids containing the dihydroxyacetone side chain of cortical hormones (fig.17.).

Hydrolysis of the (20$S$)-17β,20-epoxide (118a) in aqueous THF with HClO$_4$ produced three products in roughly equal yields, which proved to be compounds (120a), (121a), and (122a), fig.18.
Fig. 18. \( a, R=H \); \( b, R=Ac \).
Preparative tlc of the mixture afforded (122a) and a an inseparable mixture of (120a) and (121a). Acetylation of this mixture and further preparative tlc gave the acetates (120b) and (121b). The 13α,21-epoxide (122a) was acetylated separately to give (122b) which was more readily characterised.

The structural assignment of product (120b) was based on important bands in the 'Hnmr spectrum [δ4.14 (1H, q, J=9 and 12Hz, 21-CH), 4.65 (1H, q, J=2 and 12Hz, 21-CH), 5.44 (1H, q, J=2 and 9Hz, 20-CH)]. However, the stereochemistry was uncertain, as this structure could arise from H₂O attack at C-17 or C-20 of the (20S)-17β,20-epoxide (118a), to give the isomers (120a) or (123), fig. 19. (Isomer (124), arising from a non-concerted addition, was discounted as only one isomer was produced and a stepwise reaction would not be expected to be this selective.) The stereochemistry was tentatively assigned as being that of (120a), because the product (120a) proved stable to the same acid hydrolysis conditions as employed on the (20S)-17β,20-epoxide (118a). Isomer (123) would be expected to undergo acid rearrangement of the type shown in fig. 20.

The structural assignment of product (121b) was based on important bands in the 'Hnmr spectrum [δ1.12 (3H, s, 17β-CH₃), 4.05 (1H, q, J=9 and 12Hz, 21-CH), 4.41 (1H, q, J=3 and 12Hz, 21-CH), 5.32 (1H, q, J=3 and 9Hz, 20-CH)]
Fig. 19.

Fig. 20.
and the ',Cnmr spectrum (139.2 and 138.2 (C-13 and C-14)). The stereochemistry was assigned assuming the Δ¹⁵₇-17β-methyl-20,21-dihydroxide (121a) arose via rearrangement of the (20S)-17β,20-epoxide (118a) as shown in fig. 21.

The structural assignment of product (122b) was based on important bands in the ',Hnmr spectrum (1.20 (3H, s, 17β-CH₃), 3.49 (1H, q, J=7 and 9Hz, 21-CH), 4.08 (1H, q, J=7 and 9Hz, 21-CH), 4.89 (1H, t, J=7Hz, 20-CH)) and on the oxidised product (125). Several methods were employed in order to oxidise the diol (122a) directly, namely CrO₃/pyridine, CrO₃-dipyridine/dichloromethane, DMSO/tri-
fluoroacetic acid/dichloromethane and DMSO/\(\text{SO}_3\)-pyridine/triethylamine, but none were successful. The main problem in finding a suitable oxidising agent was the insolubility of the diol (122a) in solvents such as dichloromethane and diethylether. The 3-O-methyl ether was therefore synthesised by treating the diol (122a) with diazomethane/diethylether/methanol. This 3-O-methyl ether of (122a) was then successfully oxidised to the ketone (125) with pyridinium chlorochromate(pcc)/dichloromethane. The resulting ketone (125) had an ir band at 1756 cm\(^{-1}\), which is characteristic of 13\(\alpha\),21-epoxy-20-keto-17\(\beta\)-methyl steroids. The ir absorption ruled out any possibility of the product being the isomeric ketone (126), as systems of this type have a characteristic absorption of 1810 cm\(^{-1}\).

The stereochemistry of the hydrolysis product (122a) was assigned assuming it arose from the rearrangement shown in fig.22.
The (20R)-20,21-epoxide (106a) was also hydrolysed with HClO₄ in aqueous THF to give the same products as those obtained from the identical hydrolysis of the (20S)-17β,20-epoxide (118a) (namely 120a, 121a and 122a), along with another 17,20,21-trihydroxide (124). The common products (120a, 121a and 122a) probably arose via the Payne rearrangement (fig. 23) to give the (20S)-17β,20-epoxide (118a), followed by the hydrolysis and rearrangements described previously. The 17,20,21-trihydroxide (120a) could also have arisen via H₂O attack at C-21 of the (20R)-20,21-epoxide (106a, fig. 24). The stereochemistry of the
17,20,21-trihydroxide (124) was assigned assuming it arose via H₂O attack at C-20 of the (20R)-20,21-epoxide (106a, fig. 24).

Fig. 23.

Fig. 24.
Precedence for the rearrangements which led to compounds (121a) and (122a) was found in work performed by Brown. Brown reported that treatment of 17β,21-dihydroxy-3-methoxy-19-norpregna-1,3,5(10)-triene (127) with HCl in refluxing ethanol yielded three products (128, 129, and 130, fig. 25). The Δ13⁰-compound (128) was a minor product, the extreme reaction conditions presumably causing it to react further to produce the 14α,21-epoxy compound (130).

Fig. 25.
Hydrolysis of the \((20S)-17\beta,20\)-epoxide (118a) in MEK with 
HClO₄ also gave the 13α,21-epoxide (122a) in about 28% 
yield along with less polar material which was separated 
by preparative tlc on alumina. The less polar band 
appeared, from the 'Hnmr spectroscopic data, to contain 
several products and was not analysed further.

2.3 Reaction of the 20,21-epoxides, \((106a)\) and \((107a)\), 
and the 17β,20-epoxides, \((118a)\) and \((119a)\), with 
nucleophiles other than water.

The epoxides \((106a)\), \((107a)\), \((118a)\), and \((119a)\) were 
allowed to react with thiophenol and various amines as a 
model for their reactions with biological nucleophiles, 
such as proteins and DNA.

The reactions of these epoxides with thiophenol are 
outlined in fig 26. All the epoxides opened by attack at 
the least hindered position. The reactivity of the 
epoxides towards thiophenol decreased qualitatively in 
the following order: \((106a)\) > \((107a)\) >> \((118a)\) > \((119a)\).

Both the 20,21-epoxides, \((106a)\) and \((107a)\), were treated 
with 3 molar equivalents of thiophenol and 4 molar 
equivalents of triethylamine in DMF. Following the 
reaction by tlc, the majority of the \((20R)-20,21\)-epoxide 
\((106a)\) had reacted to form the thiol adduct (131) after 
half an hour. The reaction with the \((20S)-20,21\)-epoxide
Fig. 26.
(107a) was only about 50% complete at this time. After 3 hours, however, the majority of (107a) had also reacted to form the thiol adduct (132). The difference in the reactivity of the two epoxides is probably related to minor differences in the steric constraints imposed on the thiophenoxide anion approach. In the preferred conformation (135), the (20R)-20,21-epoxide (106a) would impose little hindrance towards the thiophenoxide approach. However, in the preferred conformation (136), the 16α-proton of the (20S)-20,21-epoxide (107a) would hinder the thiophenoxide approach.

\[
\text{(135)} \quad \text{(136)}
\]

The reactions of the 17β,20-epoxides, (118a) and (119a), with thiophenol did not go to completion even when they were treated with 12 molar equivalents of thiophenol and 16 molar equivalents of triethylamine in DMF for 13 days. After purification by preparative tlc, the thiol adduct (133) was obtained in 44% yield from (118a), and the thiol adduct (134) was obtained in 81% yield from (119a). The differences in the reactivity of (118a) and (119a) may be explained if (119a) experiences greater steric relief on forming the thiol adduct than (118a). From
models it would appear that the hindrance to approach of the nucleophile is not significantly different for (118a) and (119a), but relief of the steric congestion between the 12α-H and the C-21 protons and hydroxyl in (119a) could cause steric acceleration. The reduced activity of the 17β,20-epoxides, (118a) and (119a), towards the thiophenoxide anion, as compared to that of the 20,21-epoxides, (106a) and (107a), can be explained by the fact that the thiophenoxide approach at C-20 will be considerably more hindered than the approach at C-21.

It is interesting that the 17β,20-epoxides (118a, and 119a) did not produce any of the C-21 adducts (131 and 132 respectively) via their Payne rearrangements to (106a) and (107a). Under the basic conditions employed, Payne rearrangement and attack at the less hindered C-21 might have been expected.

Of the four thiophenol adducts, only compound (131) was a stable crystalline product. The other three adducts, (132), (133), and (134), which were obtained by preparative tlc as oily foams, could not be persuaded to crystallise. Their 3,20- (in the case of compound 132) and 3,21- (in the case of compounds 133 and 134) O-acetyl and O-3,5-dinitrobenzoyl derivatives were synthesised in an attempt to produce a stable crystalline product, but these derivatives also refused to crystallise. Tlc showed the compounds to deteriorate on attempting
recrystallisation and they also deteriorated on standing at room temperature overnight. The 'Hnmr spectrum of the freshly purified adducts (131) and (132), showed three distinct quartets for the C-20 and C-21 protons (for (131) 63.00 (J=10 and 14Hz, 21-CH), 3.73 (J=2 and 14Hz, 21-CH), 3.94 (J=2 and 10Hz, 20-CH) and for (132) 63.12 (J=10 and 14Hz, 21-CH), 3.50 (J=3 and 14Hz, 21-CH), 3.84 (J=3 and 10Hz, 20-CH)). The 3,20-O-acetyl derivatives, however, showed one multiplet for the C-21 protons. The results may indicate hydrogen bonding between the C-17 and C-20 hydroxyls, and possibly the C-21 thiol (fig. 27).

![Chemical structures](image)

Fig. 27.

The 20,21-epoxides (106 and (107a) were reacted with imidazole in methanol/DMF as a model for their reactions with DNA bases. No reaction took place either at room temperature or when the reaction was heated to 50°-55°C. Added water did not catalyse the reaction, although Hewett and Savage report that water catalyses epoxide cleavage with amines. Sharpless reports that
Ti(O-i-Pr)$_4$ can greatly facilitate the opening of epoxy alcohols with nucleophiles by coordination with the epoxy alcohol (fig. 28). The (20R)-20,21-epoxide (106a) was therefore allowed to react with 3 molar equivalents of imidazole and 3 molar equivalents of Ti(O-i-Pr)$_4$ in THF, but no reaction occurred. The basic work-up, consisting of adding ethyl acetate and 10% NaOH in brine and stirring overnight, caused some Payne rearrangement to (118a).

![Diagram](image)

**Fig. 28**

The epoxide (106a) was also allowed to react in diethylamine with 3 molar equivalents of Ti(O-i-Pr)$_4$. After preparative tlc a band was removed, the 'Hnmr spectrum of which indicated that it was a mixture of the diethylamine adduct (137) [60.08 (s, 18-CH$_2$), 0.18 (t, Et$_2$-CH$_3$), 3.7-4.2 (m, 20-CH)] plus another compound with an 18-CH$_3$ signal at 61.29. The down-field shift of the 18-CH$_3$ indicates a carbon-carbon transfer to C-17; the additional compound could therefore be the 17S-methyl...
compound (138). Compounds (137) and (138), if correctly assigned, were produced in the ratio of ca. 4:3 and the total yield was ca. 20%. It would appear that the Ti(O-i-Pr)$_4$ also catalyses 18-CH$_3$ transfer. No further purification and analysis of these compounds was attempted.

The (20R)-20,21-epoxide (106a) was also allowed to react with benzylamine. Two reactions were attempted - one was catalysed with water, the other with phenol$^6$. Both reactions, were heated to 50°C until all the starting material had disappeared as shown by tlc. The results from both reactions were similar. Preparative tlc yielded two bands which, from their $^1$Hnmr spectrum, appeared to contained several products. The majority of the products appeared to have $^1$Hnmr singlets at ca. 61.3, indicating an 18-CH$_3$ shift to C-17. No further attempts were made to identify these products.

It would appear that conditions strong enough to encourage the addition of amines, also cause the 20,21-epoxides to rearrange.
Chapter 3: Attempted preparation of 15,16-dehydro- and 15,16-epoxy-estrone, (144) and (145).

The enone (144) and its derived epoxide (145) can be considered as potential metabolites of the 16α-hydroxylated estrogens (e.g. 7). Attempts were made to produce these estrogens via 16α-bromoestrone (141).

16α-Bromoestrone (141) was produced using the method of Numazawa et al., by treating estrone (8) with copper II bromide in methanol. Attempts to dehydrobrominate 16α-bromoestrone (141) directly, either by heating with lithium bromide and lithium carbonate in DMF or via the semicarbazone, were unsuccessful. The required enone (144) was not detected among the many reaction products. Johnson et al. attempted to dehydrobrominate 16-bromopiandrosterone acetate (139) by heating with Y-collidine. Considerable tarry material was produced, probably due in part to polymerisation of the sensitive cyclopentenone system, and the only alkene isolated was the 14,15-dehydro compound (140) in 5% yield.
It may be that the failure to produce the required cyclopentenone system was due to the highly strained nature of this trans-fused cyclopentenone ring.

The required enone (144), however, has been synthesised by both Cantrall and Linder via the ketals (142) and (143). Forming the ketal may relieve some of the strain on the trans-fused five membered ring, increasing the flexibility of the ring to allow introduction of the double bond (fig. 29). Cantrall induced the dehydrobromination by refluxing with potassium t-butoxide in xylene, whilst Linder heated the bromoketal (141) with 1,5-diazo-bicyclo(5,4,0)-5-undecene in a sealed tube.

Initial attempts to repeat Cantrall's work were unsuccessful, and attempts at this synthesis were discontinued in favour of other work.
Chapter 4: Epoxidation of 3-oxo-Δ4-steroids

A series of 3-oxo-Δ4-steroids were epoxidised with alkaline hydrogen peroxide to form their potential metabolites, 3-oxo-4β,5β-epoxysteroids (27) and (146)-(149). As reported in the introduction the 3-oxo-4β,5β-epoxide (27) is a known metabolite of norethisterone (28). All of these compounds, with the exception of (149), have been previously reported, but have not been evaluated for their potential alkylating properties.

\[(146)\ R_1=\text{Me},\ R_2=\text{C}_3\text{H}_7,\ R_3=\text{H}\]
\[(147)\ R_1=\text{Me},\ R_2=\text{COCH}_3,\ R_3=\text{H}\]
\[(148)\ R_1=\text{Me},\ R_2=\text{OH},\ R_3=\text{H}\]
\[(27)\ R_1=\text{H},\ R_2=\text{OH},\ R_3=\text{C}=\text{CH}\]
\[(149)\ R_1=\text{Me},\ R_2=\text{OH},\ R_3=\text{C}=\text{CH}\]

Samples of these epoxides were sent to the Chester Beatty group (see appendix). The 4β,5β-epoxide of progesterone (147) contained ca. 28% α-epoxide and the 4β,5β-epoxide of testosterone (148) contained 10-15% α-epoxide. Some α-epoxide was produced with each of the β-epoxides (Table 2), but compounds (146), (27), and (149) were purified by recrystallisation. It has been suggested that the rate of α-epoxide production is dependent on the overall polarity of the steroid. However no attempt was made in our work to evaluate this proposal.
Table 2. Ratio of $\alpha$ and $\beta$ epoxides produced with alkaline Hydrogen peroxide on 3-oxo-$\Delta^4$-steroids.

<table>
<thead>
<tr>
<th>3-oxo-$\Delta^4$-steroids</th>
<th>Product</th>
<th>% $\alpha$-epoxide$^a$</th>
<th>% $\beta$-epoxide$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholestenone</td>
<td>146</td>
<td>28</td>
<td>72</td>
</tr>
<tr>
<td>Progesterone</td>
<td>147</td>
<td>30</td>
<td>70</td>
</tr>
<tr>
<td>Testosterone</td>
<td>146</td>
<td>21</td>
<td>79</td>
</tr>
<tr>
<td>Norethisterone</td>
<td>27</td>
<td>&lt;5</td>
<td>&gt;95</td>
</tr>
<tr>
<td>Ethisterone</td>
<td>149</td>
<td>13</td>
<td>87</td>
</tr>
</tbody>
</table>

$^a$ Determined from $^1$Hnmr spectra by integration of the C-4 proton signals.

Holland et al.$^4$ found that 3-oxo-4$\beta$,5$\beta$-epoxysteroids could be synthesised stereospecifically and in good yield, by the treatment of 3-oxo-$\Delta^4$-steroids with $t$-butylhydroperoxide in the presence of lithium hydroxide. It is believed that the bulky peroxide group prevents the formation of the intermediate species leading to the $\alpha$-epoxide (fig. 30), the 1,3-diaxial interactions between the peroxide group at C-5 and the hydrogens at C-7 and C-9 being highly unfavourable. It is probably this interaction which causes the $\beta$-epoxidation to be also favoured with the less bulky hydrogen peroxide epoxidation.
Our attempts to repeat Holland's work with testosterone (16), under slightly modified conditions (using 70% t-butylhydroperoxide solution rather than the reported 90% solution) were unsuccessful. No epoxide was detected and it appeared that over oxidation had taken place. When a 3.8M solution of t-butylhydroperoxide in toluene was used, it appeared that over oxidation and a reaction involving the addition of the t-butyl group was taking place. Reducing the temperature to 0°C reduced the amount of over oxidation, but still no epoxide was detected. The reaction was not investigated further.
Chapter 5: Preparation of 6α,7α-epoxyestradiyl diacetate (150b)

As indicated in the introduction 6α,7α-epoxyestradiol (150a) is a possible metabolite of estradiol (9), which may arise from 6-hydroxyestradiol (40) via dehydration and epoxidation.

6α,7α-Epoxyestradiyl diacetate (150b) was prepared by epoxidation of 6,7-dehydroestradiyl diacetate (97b). 6,7-Dehydroestradiyl diacetate (97b) has been prepared both from androstane-3,17-dione (151) via Δ1.4.6-androstatriene-3,17-dione (152), (see fig. 31)?, and from estradiol (9) via 6-ketoestradiyl diacetate (153) and 6α-hydroxyestradiyl-3,17β-diacetate (154), (see fig. 32)??.

The literature reports good yields for the preparation 6,7-dehydroestradiyl diacetate (97b) from the 6α-hydroxy compound (154)??. However, the yield of (154) was poor, due to the reduction of 6-ketoestradiyl diacetate (153) being accompanied by the loss of the 3-O-acetyl group. As a consequence dehydration of the 3-hydroxy derivative of
(154) with HCl/H$_2$O/DMF at 37°C was attempted, but the 17β-O-acetyl derivative of 6,7-dehydroestradiol (97) was only produced in only ca. 10% yield. Alternative reactions appeared to be taking place at the A-ring, reducing the yield of the required compound.

Fig. 31.
Because (154) was obtained in only poor yield, it was decided to attempt the introduction of the 6,7-alkene via the Shapiro reaction\(^7\) (fig. 33). The synthesis is outlined in fig. 34. Estradiyl diacetate (155) was oxidised with CrO\(_3\)-3,5-dimethylpyrazole\(^7\) to give 6-ketoestradiyl diacetate (153) in 42% yield. Another more polar compound was also obtained, and \(^1\)Hnmr and ir spectra indicate that it was 9α-hydroxy-6-ketoestradiyl diacetate (156) (obtained in 38% yield).

TPMI - Triphenylphosphite Methiodide.
When 6-ketoestradiyl diacetate (153) was treated with tosylhydrazine in glacial acetic acid, no reaction took place, but when the solvent was changed to ethanol and the reaction refluxed for four hours, the reaction went to completion. The tosylhydrazone (157b) crystallised from the reaction mixture in 79% yield, and the remaining 21% was obtained by column chromatography on the filtrate. Butyl lithium in THF would not induce the Shapiro reaction with the tosylhydrazone (157b), but as might be expected, the acetate groups were removed. It was decided to remove the acetate groups prior to the alkyl lithium reaction, in order to simplify the reaction. When the hydrolysed tosylhydrazone (157a) was treated with MeLi (6 molar equivalents) in THF overnight, 6,7-dehydroestradiol (97a) was obtained in 70% yield after preparative tlc. Acetylation with acetic anhydride/pyridine gave the required compound (97b).
Fig. 34. Synthesis of 6,7-dehydroestradiol (97a) via the Shapiro reaction.
The high yields in this preparation suggest it to be an improvement over previous synthetic routes to 6,7-dehydroestradiol (97a).

6.7-Dehydroxyestradiyl diacetate (97b) was epoxidised with MCPBA in chloroform according to the method described by Neeman et al., except that the reaction mixture was kept at 0-10°C overnight rather than 28°C for 1.5 hours. The crude product proved to be relatively pure 6α,7α-epoxyestradiyl diacetate (150b) (96% yield). Attempts to synthesise 6α,7α-epoxyestradiol (150a) by hydrolysis with K₂CO₃ in MeOH caused the epoxide to open to form two polar products. The 'Hnmr spectrum indicated the presence of a methoxy group; possibly the products arose via addition of methanol and water to give compounds such as (158) and (159); see fig. 35. Tlc of the reaction mixture during the hydrolysis of (150b) indicated that the 3-O-acetyl group was initially removed and the epoxide opened before the 17β-O-acetyl could be removed.

Attempts to epoxidise 6,7-dehydroestradiol (97a) directly with MCPBA in dichloromethane and a suspension of sodium hydrogen carbonate to scavenge any acid, produced 6α,7α-epoxyestradiol (97a) contaminated with other by-products. The products would not crystallise to purity and attempts to purify by chromatography caused decomposition of the epoxide. Using monoperphosphoric acid in
a mixture of diethylether and THF also produced several products and isolation of the 6α,7α-epoxide (150a) was impossible.

\[ \text{Fig. 35.} \]

![Chemical structures](image)

The problem with epoxidising 6,7-dehydroestradiol (97a) directly, is probably due to the 3-hydroxy group enhancing the opening of the epoxide produced. It therefore seems possible that a pure sample of 6α,7α-epoxyestradiol (150a) might be obtained by epoxidising 6,7-dehydroestradiol (150a) which has been selectively acetylated at C-3, followed by removal of the 3-O-acetyl group by very mild basic hydrolysis.

The above route was not tried due to lack of time, and consequently 6α,7α-epoxyestradiyl diacetate (150b) was
submitted for biological testing to the Chester Beatty group as a model for a possible esterified metabolite (see appendix).

Nucleophilic additions to the 6α,7α-epoxide (150b) were not investigated, and relatively few are reported in the literature (fig. 37). However, in each case reported, as might be expected, the epoxide opened via axial attack at the benzylic position. Some nucleophilic additions to the 6β,7β-epoxyestradiol derivative (160b) are also shown in fig. 36. The β-epoxide also opened via attack at the benzylic position as opposed to axial attack at C-7. Epoxide opening was even observed with acetic anhydride/pyridine, showing the increased reactivity of these benzylic epoxides.

Fig. 36.
Fig. 37.
As mentioned in the introduction the electrophilic properties of the 6,7-epoxides of the 2,3-dihydroxy-estra-1,3,5(10)-triene were of interest, as such epoxides would be expected to be more reactive than the 6,7-epoxy-3-hydroxyestra-1,3,5(10)-triene (see chapter 5).

C-2 hydroxylation is a major metabolic pathway for estrogens\textsuperscript{94}, and consequently much work has been done to produce 2-hydroxyestrogens synthetically. The most successful syntheses have been reported by Numazawa \textit{et al}\textsuperscript{94} and Zhao \textit{et al}\textsuperscript{95}.

Zhao\textsuperscript{95} synthesised the 17\(\beta\)-O-acetyl derivative of 2-hydroxyestradiol (163) and 2-hydroxyestrone (19) via the Dakin oxidation of their 2-\(\alpha\)-acetyl derivatives (161) and (162); see fig.38.

Numazawa\textsuperscript{94} synthesised 2-methoxyestradiol (12) and 2-methoxyestrone (48) via their 2-iodo derivatives (164) and (165); see fig.39.

Our attempt to repeat Numazawa's preparation of 2-iodoestradiol (164) only produced impure (164) in 29\% yield. The Zhao preparation is not without its problems.
Fig. 38. (a), yield from (9); (b), yield from (8).
Fig. 39. (a), yield from (164); (b), yield from (165).
either, the Dakin oxidation requiring rigid control on the pH of the reaction medium. Consequently it was decided to attempt a novel synthesis via an (arene)tricarbonylchromium complex.

6.1 o-Hydroxylation of a (methoxyarene)tricarbonyl-
chromium complex.

Co-ordination of arene rings to the electron withdrawing Cr(CO)$_3$ group increases the acidity of the ring and benzylic protons. The acidity of the ring protons is increased more than that of the benzylic protons, and the removal of the these protons is dependent upon the nature of the base and the reaction conditions employed. A strong base such BuLi will, as a rule, preferentially remove an aromatic proton, whereas NaH or t-BuOK will preferentially remove a benzylic proton. The introduction of aromatic substituents which are either electron withdrawing (such as F or Cl) or have a co-ordinating property towards the lithium atom (such as MeO) greatly increases the metallation of the aromatic ring with BuLi. What is more, these substituents direct the metallation to the ortho position.

There have been reports in the literature of Cr(CO)$_3$ complexes of estradiol derivatives being synthesised in order to induce alkylation at C-6 (e.g. fig. 40). The benzylic proton was removed with (Me$_3$Si)$_2$NNa in THF.
or DMSO, and the electrophilic addition (of either an alkyl halogen or paraformaldehyde) was to the least hindered face of the steroid; so that a $\beta$-Cr(CO)$_3$ complex produced an $\alpha$-alkyl addition, and vice versa.

![Diagram]

Fig. 40. $R = t$-BuMe$_2$Si

To date, there have been no reports of electrophilic additions to the A-ring of estradiol, or its derivatives, via the Cr(CO)$_3$ complex. However, electrophilic addition to the arene ring of the 7-methoxytetralol complex (166, fig. 41) has been reported$^{99}$. (166) was treated with $n$-BuLi and TMEDA at $-78^\circ$C for two hours, and then quenched with 2-formyl-3-methoxy-N,N-diethylbenzamide. Subsequent decomplexation by exposure to sunlight gave a diastereoisomeric mixture of the hydroxy-phthalide (167) in 40-50% yield.

Electrophilic addition of a hydroxy group to a benzylic anion (produced by the treatment of an (arene)Cr(CO)$_3$ complex with $n$-BuLi) has been reported by Davies$^{90}$, using oxodiperoxymolybdenum(pyridine)hexamethylphosphoramide.
as the electrophile. (Generally MoOPH has been used to \( \alpha \)-hydroxylate ketones via the production of the enol\textsuperscript{\textcircled{\textdagger}}.) However, to date, MoOPH has not been used to introduce a hydroxyl group into an arene ring.

\[
\begin{align*}
\text{MeO} & \quad \text{Me} \\
\text{Cr(CO)}_3 & \quad \text{OH} \\
(166) & \quad \text{n-BuLi} \\
& \quad \text{TMEDA}
\end{align*}
\]

\[
\begin{align*}
\text{MeO} & \quad \text{Me} \\
\text{O} & \quad \text{Me} \\
\end{align*}
\]

Fig. 41.

We proposed to introduce a 2-hydroxy group into estradiol, via lithiation at C-2 (produced by the action of BuLi on the Cr(CO)\textsubscript{5} complex) followed by treatment with MoOPH. Initially, the reaction was attempted on 6-methoxytetralin (168), in the hopes that a new general procedure for synthesis of catechols could be found. The
methyl protecting group was chosen as this is known to promote o-lithiation.

The successful hydroxylation at C-7 of 6-methoxytetralin (168) is shown in fig. 42. Refluxing (168) with Cr(CO)₆ in n-Bu₄O for 24 hours, followed by purification with flash chromatography and recrystallisation from diethyl ether/petrol ether(40/60) gave (6-methoxytetralin)tricarbonylchromium (169) in 68% yield. The 'Hnmr spectrum showed an up-field shift from 66.8-7.2 (for (168)) to 64.8-5.6 for the aromatic proton signals on forming the complex. When the yellow crystalline (6-methoxytetralin)tricarbonylchromium (169) was treated with n-BuLi (2 molar equivalents) and TMEDA (2 molar equivalents) in THF at -78°C for two hours, followed by treatment with MoOPH (5 molar equivalents) at -40°C for one hour and decomplexation, a crude product was obtained in ca. 85% yield. The 'Hnmr spectrum and tlc indicated that this crude product was a mixture of ca. 30% starting material (168) and ca. 70% 7-hydroxy-6-methoxytetralin (170) (the product ratio was calculated by comparing the height of the 6-methoxy proton signals at 63.71 and 63.77 in the 'Hnmr spectrum). The products from this reaction were not isolated, but products from a previous less successful reaction (the 'Hnmr spectrum of the crude product mixture indicated that the reaction was only ca. 40% complete) in which only 2 molar equivalents of MoOPH had been used, were separated by preparative tlc to give
(170) in 33% yield and starting material (168) in 49% yield. The identity of (170) was determined from its melting point and from the two singlets at δ6.45 and δ6.55 in its 'Hnmr spectrum (the C-5 and C-8 aromatic protons). No other products were observed and no hydroxylation appears to have taken place at C-4 or C-5. Indeed, when (169) was quenched with D₂O after treating with n-BuLi and TMEDA, the 7-deuterio product was obtained in 100% yield (the 'Hnmr spectrum showed two singlets for the aromatic protons at δ6.55 and δ6.92).

When the chromium complex (169) was allowed to react with lithium diisopropylamide (LDA) in THF for two hours at -75°C, followed by addition of MeI, allowing the temperature to rise to room temperature, 6-methoxy-7-methyltetralin (171) was obtained in quantitative yield after decomplexation (fig. 42). LDA is therefore an alternative reagent to n-BuLi for the preferential abstraction of the C-7 proton.

Decomplexation of the chromium complexes was achieved by standing an ether solution of the complex in sunlight until the yellow colour disappeared. Filtration and evaporation then yielded the decomplexed compound. The ease with which solutions of the chromium complexes decomplex, whilst the solids are very stable, is one of the properties which make (arene)tricarbonylchromium complexes favourable synthetic intermediates. However,
solutions of the complexes, where decomplexation is not required, must be kept in the dark and/or under nitrogen.

The success with hydroxylating 6-methoxytetralin at C-7 via the Cr(CO)$_3$ complex, encouraged attempts to use this synthesis for the preparation of 2-hydroxyestrogens.
6.2 Preparation of the 3-O-methyl derivative of 2-hydroxyestradiol (178) via a tricarbonylchromium complex.

It was again decided to protect the 3-hydroxy group of estradiol (9) as the 3-O-methyl ether. K$_2$CO$_3$/MeI in acetone was found to be a more efficient reagent for this methylation than the usual diazomethane in diethylether/methanol. Estradiol (9) was refluxed in acetone with K$_2$CO$_3$/MeI for three days. Only enough heat was applied to keep the reaction mixture just at reflux, and two condensers were used to limit the loss of MeI via evaporation. Additional MeI was introduced when tlc showed the reaction to have slowed. After the reaction work-up and recrystallisation from methanol, the 3-O-methyl derivative of estradiol (172) was obtained in 91% yield.

Although there are reports in the literature of preparing Cr(CO)$_3$ complexes of estradiol derivatives without protecting the 17β-hydroxyl group, attempts at preparing (17β-hydroxy-3-methoxyestra-1,3,5(10)-triene)-tricarbonylchromium (173) by refluxing the 3-O-methyl derivative of estradiol (172) with Cr(CO)$_3$ in n-Bu$_2$O caused oxidation at C-17 to produce (3-methoxyestra-1,3,5(10)-triene-17-one)tricarbonylchromium (174) as a by-product. Although (3-methoxyestra-1,3,5(10)-triene-17-one)tricarbonylchromium (174) can be readily reduced
back to \((17\beta\text{-hydroxy}-3\text{-methoxyestra-1,3,5(10)-triene})\text{tri-carbonylchromium (173)}\) with \(\text{LiAlH}_4\), an attempt to hydroxylate at C-2 with (173), directly, failed. Consequently, it was decided to protect the 17-hydroxyl group prior to preparing the \(\text{Cr(CO)}_3\) complex.

The successful preparation of the 3-O-methyl derivative of 2-hydroxyestradiol (178) is shown in fig. 43. \(17\beta\)-(dimethyl-t-butyldimethylsilox)-3-methoxyestra-1,3,5(10)-triene (175)\) was prepared in quantitative yield by treating the 3-O-methyl derivative of estradiol (172) with dimethyl-t-butyldimethylsilyl chloride and imidazole in DMF at 35-40°C for 21 hours\(^\text{a2}\). Refluxing the silyl ether (175) with \(\text{Cr(CO)}_3\) in \(\text{n-Bu}_3\text{O}\) for 24 hours produced a mixture of the \(\alpha\)- and \(\beta\)-\(\text{Cr(CO)}_3\) complexes (176a) and (176b). Separation by flash chromatography gave the \(\alpha\)-\(\text{Cr(CO)}_3\) complex (176a) in 24% yield and the \(\beta\)-\(\text{Cr(CO)}_3\) complex (176b) in 28% yield (an overall ratio of \(\alpha:\beta = 46:54\)). The \(\alpha\) and \(\beta\) isomers were assigned by comparing their 'Hnmr spectra with those reported for the \(\alpha\) and \(\beta\) \(\text{Cr(CO)}_3\) complexes of 3-benzyloxy-17\(\beta\)-(dimethyl-t-butyldimethylsilox)estra-1,3,5(10)-triene, (179a) and (179b)\(^\text{a3}\).
Fig. 43. Preparation of the 3-O-methyl derivative of 2-hydroxyestradiol (178).
No difference was found between the reactivities of the α- and β-isomers, (176a) and (176b), towards the 2-hydroxylation reaction, and consequently there was no advantage in separating them. Chromatography of the Cr(CO)$_3$ complexes (176) caused some decomplexation, and an improved yield was obtained when the crude product was purified by recrystallisation (recrystallising from diethylether/petrol ether(40/60) produced a 50:50 mixture of the α- and β-Cr(CO)$_3$ complexes, (176a) and (176b), in 85% yield).

Treatment of the (17ß-(dimethyl-t-butyldimethyl)siloxy)-3-methoxy-estra-1,3,5(10)-triene)tricarbonylchromium complexes (176) with BuLi/TMEDA (5 molar equivalents) and MoOPH (10 molar equivalents) gave, after decomplexation in sunlight and separation by preparative tlc, 17ß-(dimethyl-t-butyldimethyl)siloxy)-2-hydroxy-3-methoxyestra-1,3,5(10)-triene (177) in 55% yield, and starting material (175) in 17% yield (making the overall yield of (177), allowing for the recovery of starting material, 66%).

Attempts to desilylate the 17ß-O-silyl ether (177) with $n$-Bu$_4$NF did not go to completion, but treatment of (177) with BF$_3$.Et$_2$O in chloroform$^a$ produced the 3-O-methyl derivative of 2-hydroxyestradiol (178) in quantitative yield.
The high yields in this reaction make it a reasonable alternative synthesis for the preparation of 2-hydroxyestrogens, and an improvement on previous syntheses for the preparation of the 3-0-methyl derivative of 2-hydroxyestradiol (178) in particular.

Kirk has reported the preparation of the 3-0-methyl derivative of 2-hydroxyestrone (51) via the treatment of 17-ethylenedioxy-3-methoxyestra-1,3,5(10)-triene (180) with n-BuLi/TMEDA, diborane/trimethyl borate and subsequent oxidation with H₂O₂/NaOH. It was thought that this procedure might be an improvement over the oxidation with MnO₂. However, treatment of the Cr(CO)₆ complex (176) with n-BuLi/TMEDA, diborane/trimethyl borate, H₂O₂/NaOH produced no 2-hydroxy compound.

To test the generality for producing 2-hydroxyestrogens via Cr(CO)₆ complexes, the preparation of the 3-0-methyl derivative of 2-hydroxyestrone (51) was attempted.

6.3 Preparation of the 3-0-methyl derivative of 2-hydroxyestrone (51) via a tricarbonylchromium complex.

The successful synthesis of the 3-0-methyl derivative of 2-hydroxyestrone (51) via the preparation of (17-ethylenedioxy-3-methoxyestra-1,3,5(10)-triene)tricarbonylchromium (182) is shown in fig. 44.
Fig. 44. Preparation of the 3-O-methyl derivative of 2-hydroxyestrone (51)
The 17-ethylene acetal (180) was prepared in 76% yield from estrone (8) via the usual reflux with ethyleneglycol and PTSA in toluene. The 3-methyl ether (181) was prepared in quantitative yield by refluxing (180) with K₂CO₃/MeI in acetone (see §6.2).

Refluxing 17-ethylenedioxy-3-methoxyestra-1,3,5(10)-triene (181) with Cr(CO)₆ in n-Bu₂O for 24 hours afforded (17-ethylenedioxy-3-methoxyestra-1,3,5(10)-triene)tricarbonyl chromium (182) in quantitative yield. The 'Hnmr spectrum showed the ratio of the α:β isomers (182a and 182b) to be 36:64. Recrystallisation from diethylether/petrol ether (40/60) gave a very pure mixture of the α and β isomers (182a and 182b) in 88% yield. No attempt was made to separate the diastereoisomers by chromatography, but the β-isomer (182b) was isolated by careful, successive recrystallisations from diethylether/petrol ether (40/60). Again, the assignment of the α and β stereochemistry was based on the 'Hnmr spectra.

The Cr(CO)₆ complex (182) was treated with n-BuLi/TMEDA (5 molar equivalents), followed by treatment with MoOPH (10 molar equivalents) and decomplexation, to give a white solid. The 'Hnmr spectra indicated it to be ca. 35% starting material (181) and 65% 17-ethylenedioxy-2-hydroxy-3-methoxyestra-1,3,5(10)-triene (183) (estimated from the 3-methoxy signals at 63.85 and 63.78
respectively). Attempts to separate the products by flash chromatography, caused loss of the 17-acetal group. Consequently, the 17-acetal group was removed from the crude reaction mixture prior to the use of chromatography. The crude acetals were hydrolysed by refluxing in MeOH/HCl, and separated by preparative tlc to give the 3-0-methyl derivative of 2-hydroxyestrone (51) in 47% yield and the 3-O-methyl derivative of estrone (8a) in 19% yield (making the overall yield of (51), allowing for the recovery of the 3-O-methyl derivative of the starting estrone, 58%).

This synthesis is an improvement over previous reports for the preparation of the 3-O-methyl derivative of 2-hydroxyestrone (51) and shows Cr(CO)₃ complexes to be good intermediates for the selective 2-hydroxylation of estrogens.

6.4. **Rearrangement of (3,17β-bis(dimethyl-t-butyldimethylsiloxy)-estra-1,3,5(10)-trienetricarbonylchromium (185).**

(3,17β-Bis(dimethyl-t-butyldimethylsiloxy)estra-1,3,5(10)-trienetricarbonylchromium (185) was prepared, as it was thought that hydroxylation at C-2 and subsequent desilylation might provide a new and useful synthesis for the preparation of 2-hydroxyestradiol (11).
3,17β-Bis(dimethyl-t-butylsiloxy)estra-1,3,5(10)-triene (184) was prepared in quantitative yield by the treatment of estradiol (9) with t-BuMe₂SiCl and imidazole. The di-silyl compound (184) was refluxed with Cr(CO)₆ in n-Bu₂O to afford (3,17β-bis(dimethyl-t-butylsiloxy)estra-1,3,5(10)-triene)tricarbonylchromium (185) in quantitative yield. The α- and β-diastereoisomers (185a and 185b), were produced in approximately equal quantities (estimated from the 18-CH3 signals at δ0.71 and 0.77 in the 'Hnmr spectrum) and could not be separated by chromatography. [Jaouen separated these isomers, (185a and 185b), by preparing the Cr(CO)₆ complex of the mono-silylated estradiol (188), separating the α- and β-isomers on a silica gel column and then O-silylating at C-17. However, Jaouen's overall yield was only 45%.] The crude product mixture was recrystallised from diethylether/petrol ether (40/60) to give a very pure mixture of (185a and 185b) in 65% yield.

Treatment of the Cr(CO)₆ complex (185) with BuLi/TMEDA (5 molar equivalents) for two hours at -78°C, followed by quenching with D₂O, afforded the rearranged product (186a) in ca. 65% yield, and the 3-O desilylated product (187) in ca. 35% yield. When the BuLi/TMEDA treatment was prolonged to six hours, the yield of the rearranged product (186a) was reduced to about 40%. Treating the Cr(CO)₆ complex (185) with LDA (5molar equivalents), followed by quenching with D₂O, afforded, after
\[ (9) \rightarrow \text{OSiMe}_2\text{Bu}^+ \]

\[ \text{Bu}^+\text{Me}_2\text{SiO} \]

\[ (184) \]

\[ (186a), R^1 = H, R^2 = \text{SiMe}_2\text{Bu}^+ \]
\[ (186b), R^1 = \text{Ac}, R^2 = \text{SiMe}_2\text{Bu}^+ \]
\[ (187), R^1 = R^2 = H \]

\[ \text{Cr(CO)}_3 \]

\[ (185a), \text{Cr(CO)}_3 \]
\[ (185b), \text{Cr(CO)}_3 \]

\[ (11) \]

\[ \text{Bu}^+\text{Me}_2\text{SiO} \]

\[ \text{Cr(CO)}_3 \]

\[ (188) \]
preparative tlc, the rearranged product (186a) in 82% yield. Reducing the addition of LDA to 1.5 molar equivalents did not reduce the yield of the C-2 adduct.

The rearranged product (186a) was also produced, along with the 3-O desilylated product (187), when (185) was treated with LDA followed by treatment with MoOPH or Mel. Attempts to prepare (186a) from the Cr(CO)₃ complex (185) with LDA and H₂O, showed the quantity of H₂O added to be crucial. An optimum yield of 83% (186a) was obtained when 0.1g (185) was treated with LDA (2 molar equivalents) in THF (10ml), and then quenched with H₂O (2ml).

The structural assignment of (186a) was based on the ¹Hnmr spectrum [δ6.29 (1H, s, 4-CH) and δ7.14 (1H, s, 1-CH)], the mass spectroscopy data which showed (186a) to have the same mass ion as the starting disilyl compound (184), and the desilylation of (186a) with BF₃·Et₂O, which produced estradiol (9). Precedence for the silyl rearrangement is found in work reported by Oishi.⁹⁷ (Dimethyl-t-butylsilyloxybenzene)tricarbonylchromium (189) was treated with n-BuLi/TMEDA and the resulting species quenched with benzaldehyde, after the subsequent decomplexation, 2-dimethyl-t-butylsilyl phenol (190) and 3-(α-hydroxy-benzyl)phenol (191) were obtained in 35% and 40% yields respectively.
The 2-silyl compound (186a) was found to be unstable to recrystallisation, and consequently the 3-O-acetyl derivative (186b) was prepared for the full characterisation of this compound. It was thought that this acetyl derivative (186b) might be oxidised with Pb(OAc)$_4$ in CF$_3$CO$_2$H to produce, after removal of the protecting groups, 2-hydroxyestradiol (11). Unfortunately, there was not time available to allow investigation into this oxidation, but, in view of the high yields obtained for the preparation of (186), it may be that this oxidation will lead to a new high yielding synthetic route for 2-hydroxyestradiol (11).

6.5 Attempted introduction of a benzylic hydroxy group.

In Chapter 5, the preparation of 6,7-dehydroestradiol (97a) was discussed. 6,7-Dehydroestradiol diacetate (97b) is reported to have been prepared in good yield from
6α-hydroxyestradiol diacetate (154), but the 6α-hydroxy compound (154) could only be produced in poor yield. As mentioned above (§6.1), metallation at C-6 of Cr(CO)_3 complexes of estradiol derivatives have been reported. It was thought that if a hydroxy group could be introduced at C-6 with MoOPH, this might provide an improved synthesis for the preparation of 6-hydroxyestrogens.

It was decided to attempt benzylic hydroxylation of (6-methoxytetralin)tricarbonylchromium (169), the preparation of which is described in §6.1. The proposed oxidation is shown in fig. 45.

![Figure 45](image)

No reaction took place when (169) was treated with NaH in DMF, followed by treatment with MoOPH or MeI. The reaction with MeI showed that no benzylic anion had been produced. Treatment of (169) with t-BuOK/DMSO followed by addition of MeI, afforded, after decomplexation, 7-methoxy-1-methyltetralin (192) in quantitative yield. However, when MoOPH was added to the anion produced by
the action of \( \text{t-BuOK/DMSO} \) on (169), only starting material was recovered. It appeared that the \( \text{MoOPH} \) was preferentially reacting with the DMSO.

![Diagram](Diagram.png)

Treatment of (169) with \( (\text{Me}_3\text{Si})_2\text{NNa} \) in THF, followed by addition of \( \text{MeI} \), afforded only starting material. However, when the solvent was changed to DME, some methyl adduct (192) was produced. Interestingly, a better yield was obtained when the reaction was performed at room temperature than when the reaction was heated to 40°C or 50°C. Treatment of (169) in DME with \( (\text{Me}_3\text{Si})_2\text{NNa} \) (5 molar equivalents) at room temperature for 1.5 hours, followed by treatment with \( \text{MeI} \) (10 molar equivalents), gave, after decomplexation, both optical isomers of 7-methoxy-1-methyltetralin (192) in about 60% yield (estimated from the 'Hnmr spectrum of the crude product mixture, by comparing the integration of the C-1 methyl signal at \( \delta1.27 \) with that of the methoxy signal at \( \delta3.73 \)). Treatment of (169) with \( (\text{Me}_3\text{Si})_2\text{NNa} \) in DME, followed by addition of MoOPH at -20°C, showed very little reaction after two hours. However, when the reaction mixture was left overnight at room temperature, several products were formed. Separation of these
products by preparative tlc afforded the over-oxidised product (193) in 24% yield.

![Compound 193](image)

An attempt was made to metallate the benzylic position of (169) with (Me₃Si)₂NLi/TMEDA in THF, THF being the usual solvent for MoOPH oxidations, but subsequent treatment with MeI afforded no methyl adduct, and only starting material recovered.

It was not considered to be within the scope of this project to proceed any further with this investigation. The production of the benzylic ketone (193), however, does indicate that a more detailed study may be fruitful.
Chapter 7: Synthesis with the view to preparing 2,17β-diacetoxy-6α,7α-epoxy-3-methoxyestra-1,3,5(10)-triene (201).

As indicated in the introduction and chapter 6 the electrophilic properties of the 6,7-epoxides of the 2-hydroxyestrogens were of interest as potential alkylating metabolites of estradiol (9). Consequently, the synthesis of the 6α,7α-epoxide (201, fig. 46) was attempted.

It was proposed to prepare the 6,7-dehydro derivative of the 3-0-methyl derivative of 2-hydroxyestradiol (178) (the preparation of which is described in §6.2) via the Shapiro reaction described in chapter 5. The diacetate (194) was prepared in quantitative yield by the treatment of (178) with acetic anhydride/pyridine. Oxidation of (194) with CrO3·3,5-dimethylpyrazole afforded the 6-keto compound (195) in 29% yield, along with another more polar compound, assumed to be the 9α-hydroxy compound (195), in 26% yield. The yield for (195) is an improvement over that reported in the literature\(^\text{100}\) for the preparation of this compound (namely 5%), but less than the yield obtained for the preparation of 6-ketoestradiyl diacetate (153) (namely 42%, see chapter 5). The reaction rate was greater for the oxidation of (194) than for (155); the oxidation of (194) being
Fig. 46. Proposed synthetic route to epoxide (201)
complete in 45 minutes, whereas the oxidation of estradiol diacetate (155) took five hours.

The 6-keto compound (195) was refluxed for one hour with tosylhydrazine in ethanol. Crystallisation from the reaction mixture gave the 6-tosylhydrazone (197) in 51% yield [chromatography on the filtrate would probably yield more 6-tosylhydrazone (197)]. Hydrolysis of (197) with 2% KOH in methanol produced the dihydroxy compound (198) in 90% yield.

To date, no further progress has been made on the synthesis. No difficulty is envisaged with the preparation of the dehydro compound (199) from (198) with MeLi.

We would expect the epoxide (201) to be unstable. Attempts by other workers\(^{101}\) to epoxidise 2,3,17β-tribenzyloxyestra-1,3,5(10),6-tetraene (202) with MCPBA only yielded a diastereo mixture of 6α- and 6β-m-chlorobenzoates (203). Apparently, the epoxide formed opens immediately to form a cation at C-6. It is hoped that the electron withdrawing effect of the 2-O-acetyl will be enough to stabilize (201).
Chapter 8: Experimental.

8.0 Instrumentation.

The practical work presented in this thesis was carried out in the Department of Chemistry, The University of Loughborough between September 1983 and March 1987.

(i) Infra-red Spectra.

These were recorded either as KBr discs, nujol mulls, or as neat films, using a Perkin-Elmer 177 spectrometer (calibrating the spectra with a polystyrene reference film) or a Pye Unicam 9516 spectrometer linked to an IBM personal computer XT.

(ii) 'Hnmr Spectra.

The 60MHz spectra were recorded using a Varian EM360A spectrometer. The 90MHz spectra were recorded using a Perkin-Elmer R32 spectrometer. The 400MHz spectra were recorded with a Bruker WH400 spectrometer. In all cases tetramethylelsilane (TMS) was used as an internal standard. All spectral chemical shifts are quoted in $\delta$ units (ppm). The following abbreviations are utilised in the spectral interpretations:

- $s$ - singlet, $d$ - doublet, $dd$ - doublet of doublets,
- $t$ - triplet, $q$ - quartet, $m$ - multiplet.
(iii) $^{13}$Cnmr Spectra.

These were recorded using a Bruker WP-80 spectrometer in the pulsed F.T. mode. TMS was used as the internal standard.

(iv) Accurate Mass Spectrometry.

Measurements were carried out using a Kratos MS80 spectrometer linked to a DS-55 data system. Both the measured and required masses are quoted together with the intensity of the measured peak.

(v) Elemental Analyses.

These were performed by the microanalytical department of Manchester University.

(vi) Melting Points.

Melting points were recorded using a Ko fler hot stage apparatus and are uncorrected.

(vii) Chromatography.

Preparative and analytical thin layer chromatography (tlc) was performed using either Merck silica gel 60 PF$_{254}$-366 (70-230 mesh) or alumina gel 60 PF$_{254}$. 

-115-
(Typ E). Flash chromatography was carried out using Merck silica gel (230-400 mesh).

(viii) **Optical Rotation.**

Rotations were measured at ambient temperature using a AA-10 polarimeter.

8.1(i) **Preparation of 20,21-dihydronethisterone (99).**

A suspension of 5%Pd on BaSO₄ (0.10g) in pyridine (20ml) was pre-reduced by stirring overnight in an atmosphere of hydrogen, at atmospheric pressure and ambient temperature. A solution of norethisterone (28) (1.0g, 3.36 mmol) in pyridine (20ml) was introduced to the pre-reduced catalyst via a pressure equalising dropping funnel. After stirring vigorously for 15-20 minutes, the hydrogen uptake began to slow. The reaction mixture was filtered and the pyridine removed *in vacuo*. The residue was dissolved in ethyl acetate, washed with 2M HCl, 8% NaHCO₃ solution and water, dried (MgSO₄) and the solvent removed *in vacuo* to afford a white solid (0.99g). Recrystallisation from ethyl acetate gave pure 20,21-dihydronethisterone (99) (0.76g, 75%): mp 171-172°C (lit 169-170°C); 'Hnmr (90MHz; CDCl₃) δ 0.98 (3H, s, 18-CH₃), 5.07-5.35 (2H, m, 21-CH₂), 5.87 (1H, s, 4-CH), 5.93-6.30 (1H, dd, 20-CH); **Accurate Mass** [Found:
8.1(ii) **Preparation of 17α-vinylestradiol (98).**

17α-Ethynylestradiol (15) (2.8g, 9.46mmol) was treated as described for 8.1(i) to afford a white solid (3.0g). Recrystallisation from methanol gave 17α-vinylestradiol (98) (2.22g, 78%), ca. 4% of which was contaminated with 17α-ethylestradiol (100) (calculated from the 18-CH₃ singlets in the 'Hnmr spectra). [Recrystallisation of the mother liquors from methanol gave a second crop of 17α-vinylestradiol (98) (0.15g, 5%), ca. 7% of which was 17α-ethylestradiol (100).]

- mp 149.5-150°C (lit 155-156°C);
- 'Hnmr (90MHz; CDCl₃/DMSO d₆) δ [0.85 (ca. 4%, 3H, s, 18-CH₃ of 17α-ethylestradiol)], 0.90 (3H, s, 18-CH₃), 2.75-3.00 (2H, m, 6-CH₂), 5.15-5.45 (2H, m, 21-CH₂) 6.05-6.44 (1H, dd, 20-CH), 6.62-7.32 (3H, m, A-ring CH's).

8.1(iii) **Epoxidation of 20,21-dihydronorethisterone (99) with MCPBA in dichloromethane.**

20,21-Dihydronorethisterone (99) (0.15g, 0.5mmol) in dichloromethane (30ml) was treated dropwise with 80% MCPBA (0.12g, 1.1 molar equivalents) in dichloromethane (15ml). The resulting solution was stirred for 2 days and then washed with NaSO₃ solution, 8% NaHCO₃ solution and...
water, dried (MgSO₄) and the solvent removed in vacuo to afford a clear oil (0.17g). The 'Hnmr spectra showed the crude product to be about one-third starting material. Consequently the crude product was redissolved in dichloromethane (30ml) and treated with 80% MCPBA (0.22g, 2 molar equivalents) in dichloromethane (30ml). The resulting solution was stirred for 5 days, after which tlc showed no further reaction to be taking place. The reaction mixture was worked up as described above to afford an oily white solid (0.16g). Analytical tlc showed the crude product to be a mixture of 3 products. Examination by 'Hnmr spectroscopy (see §1.2 for details) indicated that the crude product consisted of ca. 50\% 20,21-epoxy-17β-hydroxy-19-norpregn-4-ene-3-one (101), ca. 12.5\% A-homo-3a-oxa-20,21-epoxy-17β-hydroxy-19-norpregn-4-ene-3-one (104) and ca. 37.5\% A-homo-3a-oxa-4,5-epoxy-20,21-epoxy-17β-hydroxy-19-norpregnan-3-one (105): ir (neat) 3450 (OH), 1740 (lactone C=O), 1655 (enone C=O) cm⁻¹.

8.1(iv) Reaction of 20,21-dihydronorethisterone (99) with MCPBA and NaHCO₃ in diethyl ether.

20,21-Dihydronorethisterone (99) (0.21g, 0.7mmol) in diethyl ether (40ml) with a suspension of NaHCO₃ was treated with 80% MCPBA (0.30g, 2 molar equivalents) in diethyl ether (20ml). After stirring for 22 hours the reaction mixture was worked up as described for 8.1(iii)
to yield a white solid (0.22g). Examination by 'Hnmr spectroscopy showed the crude product to consist of approximately 50% starting material (99) and 50% another compound, tentatively assigned as A-homo-3a-oxa-
-17β-hydroxy-19-norpregna-4,20-diene-3-one (102): ir (nujol mull) 3420 (OH), 1745 (lactone C=O), 1657 (enone C=O) cm⁻¹; 'Hnmr (60MHz; CDCl₃) 0.94 and 0.98 (3H, s×2 (ca. 50:50), 18-CH₃'s), 4.9-5.3 (2H, m, 21-CH₂), 5.77 (ca. 50%, 1H, s, (99)-4-CH), 6.0 (1H, dd, 20-CH), 6.05 (ca. 50%, 1H, s, (102)-4-CH).

8.1(v) Epoxidation of 20,21-dihydronorethisterone (99) with MCPBA in chloroform.

20,21-Dihydronorethisterone (99) (0.10g, 0.33mmol) in chloroform (20ml) was treated with 80% MCPBA (0.15g, 2 molar equivalents) in chloroform (10ml). The resulting solution was stirred at ambient temperature, excluding all light. After 45 hours no MCPBA could be detected with starch iodide paper, and the reaction mixture was worked up as described for 8.1(iii) to afford a clear oil (0.13g). Separation by preparative tlc on alumina ran with chloroform gave 20,21-epoxy-17β-hydroxy-19-norpregn-
-4-ene-3-one (101) (0.036g, 34%), ca. 30% of which was contaminated with starting material (99) (estimated from the 'Hnmr spectra): 'Hnmr (90MHz; CDCl₃) 6 0.95 and 0.99 (3H, s×3 (2 at 0.99), 18-CH₃ of the two epoxide diastereoisomers (101) and of (99)), 2.72-3.05 (ca. 70%,
2H, m, (101)-21-CH₂), 3.05-3.35 (ca. 70%, 1H, m, (101)-20-CH), 5.07-5.35 (ca. 30%, 2H, m, (99)-21-CH₂), 5.80 (1H, s, 4-CH), 5.95-6.30 (ca. 30%, 1H, dd, (99)-20-CH).

8.1(vi) Preparation of the 3-O-acetyl derivative of 17α-vinylestradiol (98).

17α-Vinylestradiol (98) [contaminated with ca. 10% 17α-ethylestradiol (100)] (0.29g, 0.99mmol) was dissolved in the minimum of pyridine and treated with acetic anhydride (0.5g, 5 molar equivalents). After standing over night, the reaction mixture was poured into ice and extracted with diethyl ether. The ether extracts were washed with 2M HCl, 8% NaHCO₃ solution and water, dried (MgSO₄), and the solvent removed in vacuo to afford a white solid (0.31g). Recrystallisation from aqueous methanol gave 17α-vinylestra-17β-oyl-3-acetate (0.20g, 61%), ca. 9% of which was contaminated with 17α-ethylestra-17β-oyl-3-acetate (estimated from the 18-CH₃ singlets in the 'Hnmr spectrum): 'Hnmr (90MHz; CDCl₃) δ (0.93 (ca. 9%, 3H, s, 18-CH₃ of 17α-ethylestra-17β-oyl-3-acetate), 0.95 (3H, s, 18-CH₃), 2.29 (3H, s, 3AcO-CH₃), 5.11-5.38 (2H, m, 21-CH₂), 6.00-6.36 (1H, dd, 20-CH), 6.28-7.42 (3H, m, A-ring CH's)
8.1(vii) Epoxidation of the 3-O-acetyl derivative of 17α-vinylestradiol (98) with MCPBA.

The 3-O-acetyl derivative of 17α-vinylestradiol (98) [contaminated with ca. 9% of the 3-O-acetyl derivative of 17α-ethylestradiol (100)] (0.196g, 0.58mmol) was treated as described for 8.1(v). After 21 hours the reaction appeared complete by tlc and was worked up as described for 8.1(iii) to afford a white foam (0.18g). Purification by preparative tlc on silica gel ran with diethyl ether gave a mixture (ca. 56:44) of (20R)-3-acetoxy-20,21-epoxy-17β-hydroxy-19norpregna-1,3,5(10)-triene (106b) and (20S)-3-acetoxy-20,21-epoxy-17β-hydroxy-19norpregna-1,3,5(10)-triene (107b) (0.07g, 37% allowing for the 17α-ethyl impurity in the starting material): 'Hnmr (60MHz; CDCl₃) δ 0.89 and 0.92 (3H, sX2, 18-CH₃ of (20S)- and (20R)-20,21-epoxides respectively), 2.25 (3H, s, 3AcO-CH₃), 2.5-3.0 (4H, m, 6-CH₂ and 21-CH₂), 3.0-3.4 (1H, m, 20-CH), 6.5-7.4 (3H, m, A-ring CH's); Accurate Mass [Found: m/z 356.1978 (M⁺, 26.46%) C₂₂H₂₈O₄ requires M⁺ 356.1987].

8.1(viii) Reaction of 17-vinylestradiyl diacetate-I with MCPBA.

17-Vinylestradiyl diacetate-I (anomalous product from 17α-vinylestradiol (98) acetylation with acetic anhydride/pyridine - 'Hnmr (60MHz; CDCl₃) as for the
3-O-acetyl derivative of 17α-vinylestradiol [8.1(vi)] with δ 1.91 (3H, s, 17OAc-CH$_3$) (0.23g, 0.60mmol) was treated as described for 8.1(vi) to afford a cream solid (0.27g). Separation by preparative tlc on alumina gel ran with ethyl acetate/toluene (8:1) gave two major products: (20S)-3-acetoxy-20,21-epoxy-17β-hydroxy-19-norpregna-1,3,5(10)-triene (107b) (0.034g, 16%): 'Hnmr (90MHz; CDCl$_3$) δ 0.92 (3H, s, 18-CH$_3$), 2.26 (3H, s, 3AcO-CH$_3$), 2.67-3.07 (4H, m, 6-CH$_2$ and 21-CH$_2$), 3.34 (1H, t, 20-CH), 6.80-7.45 (3H, m, A-ring CH's); Accurate Mass [Found: m/z 356.1996 (M$^+$. 15.35%)] C$_{22}$H$_{28}$O requires M$^+$ 356.1987; and 3-acetoxy-13α,21-epoxy-20-hydroxy-17β-methyl-18,19-dinorpregna-1,3,5(10)-triene (109) (0.049g, 23%): 'Hnmr (90MHz; CDCl$_3$) δ 1.17 (3H, s, 18-CH$_3$), 2.29 (3H, s, 3AcO-CH$_3$), 2.70-3.05 (2H, m, 6-CH$_2$), 3.47 (1H, t, J=10Hz, 20-CH), 3.88-4.17 (2H, m, 21-CH$_2$), 6.77-7.43 (3H, m, A-ring CH's); Accurate Mass [Found: m/z 356.1996 (M$^+$. 34.86%)] C$_{22}$H$_{24}$O$_4$ requires M$^+$ 356.1987]. Acetylation of (109) with acetic anhydride/pyridine gave the 3,21-diacetate (122b): ir (neat) 1755 (20AcO C=O), 1740 (3AcO C=O) cm$^{-1}$; 'Hnmr (90MHz; CDCl$_3$) δ 1.17 (3H, s, 18-CH$_3$), 2.11 (3H, s, 20AcO-CH$_3$), 2.19 (3H, s, 3AcO-CH$_3$), 2.70-3.00 (2H, m, 6-CH$_2$), 3.56 (1H, dd, J$_{v=1}=7$Hz, J$_{g=m}=9$Hz, 21-CH), 4.16 (1H, dd, J$_{v=1}=7$Hz, J$_{g=m}=9$Hz, 21-CH), 4.95 (1H, t, J$_{v=1}=7$Hz, 20-CH), 6.78-7.46 (3H, m, A-ring CH's).
8.1(ix) Preparation of 17α-ethynylestradiyl diacetate.

17α-Ethynylestradiol (15) (1.0g, 3.38mmol) in acetic anhydride (10ml) was treated with PTSA (0.3g) and stirred at ambient temperature for 18 hours. The reaction mixture was then poured into water and after 1 hour extracted into ethyl acetate. The ethyl acetate extracts were washed with 8% NaHCO₃ solution and water, dried (MgSO₄), and the solvent removed in vacuo to afford a white solid (1.25g). Recrystallisation from aqueous methanol gave 17α-ethynylestradiyl diacetate (0.78g, 60%): ¹Hnmr (60MHz; CDCl₃) δ 0.88 (3H, s, 18-CH₃), 2.00 (3H, s, 17AcO-CH₃), 2.23 (3H, s, 3AcO-CH₃), 2.57 (1H, s, 21-CH), 6.6-7.5 (3H, m, A-ring CH's).

8.1(x) Preparation of 17α-vinylestradiyl diacetate-II (108).

17α-Vinylestradiyl diacetate (0.96g, 2.5mmol) was partially hydrogenated as described for 8.1(i), except that the reaction was continued until the hydrogen uptake ceased. The crude product appeared, from its tlc and ¹Hnmr spectra, to be relatively pure 17α-vinylestradiyl diacetate (108) (0.92, 95%); no further purification was attempted: ¹Hnmr (90MHz; CDCl₃) δ 0.94 (3H, s, 18-CH₃), 2.25 (3H, s, 3AcO-CH₃), 4.96-5.37 (2H, m, 21-CH₂), 5.80-6.16 (1H, dd, 20-CH), 6.76-7.47 (3H, m, A-ring CH's).
8.1(xi) Epoxidation of 17α-vinylestradiol (98) with t-BuOOH/VO(acac)$_2$.

VO(acac)$_2$ (44 mg, 0.17 mmol) was added to a stirred solution of 17α-vinylestradiol (98) (2.5 g, 8.39 mmol) in CH$_2$Cl$_2$ (500 ml) at 0°C. Anhydrous t-BuOOH in toluene solution (4.4 ml, 3.8 M) was added dropwise, and the reaction mixture was stirred overnight while the temperature was maintained below 10°C. The reaction mixture was washed with sodium sulphite solution and water, dried (MgSO$_4$) and the solvent removed in vacuo to afford a brown solid. Purification by flash chromatography, eluting with dichloromethane/ethyl acetate (6:1), gave a mixture (ca. 65:35) of the (20R)- and (20S)-20,21-epoxides (106a) and (107a) (1.8 g, 68%). Fractional crystallisation of the mixture from chloroform gave (20R)-3,17β-dihydroxy-20,21-epoxy-19-norpregna-1,3,5(10)-triene (106a) (39%): mp 154-158°C; [α]$_D$ +41.8° (c 1.0, dioxan); IR (KBr) 3380 (OH) cm$^{-1}$; $^1$H NMR (90 MHz; CDCl$_3$/(CD$_3$)$_2$CO/D$_2$O) δ 0.96 (3H, s, 18-CH$_3$), 2.7-3.0 (4H, m, 6-CH$_2$ and 21-CH$_2$), 3.22 (1H, t, J=3.5 Hz, 20-CH), 6.6-7.3 (3H, m, A-ring CH's); $^{13}$C NMR see Table 3;

**Accurate Mass** [Found: m/z 314.1880 (M$^{+}\cdot$, 65.10%) C$_{20}$H$_{26}$O$_{3}$ requires M$^{+}\cdot$, 314.1882]. **Elemental Analysis** [Found: C, 76.5%; H, 8.4%; C$_{20}$H$_{26}$O$_{3}$ requires C, 76.4%; H, 8.3%]. Fractional crystallisation from methanol gave the (20S)-3,17β-dihydroxy-20,21-epoxy-19-norpregna-1,3,5(10)-triene (107a) (11%): mp 184-200°C [Both (106a) and
(107a) melted over a relatively wide range, but (107a) melted over a particularly wide range with decomposition; \([\alpha]_D +46.6^\circ\) (c 0.9, dioxan); ir (KBr) 3600,3510 and 3420,3240 (OH) cm\(^{-1}\); \(^1\)Hnmr (90MHz; CDCl\(_3\)/(CD\(_3\)\(_2\)CO/D\(_2\)O) \(\delta\) 0.94 (3H, s, 18-CH\(_3\)), 2.7-3.0 (4H, m, 6-CH\(_2\) and 21-CH\(_2\)), 3.34 (1H, t, J=3.5Hz, 20-CH), 6.6-7.3 (3H, m, A-ring CH's); \(^{13}\)Cnmr see Table 3; Accurate Mass (Found: \(m/\text{z} 314.1876\) (M\(^+\), 61.33%) \(\text{C}_{20}\)\(\text{H}_{26}\)\(\text{O}_{3}\) requires M\(^+\), 314.1882]; Elemental Analysis (Found: C,76.5; H,8.4%; \(\text{C}_{20}\)\(\text{H}_{26}\)\(\text{O}_{3}\) requires C,76.4; H,8.3%].

8.2(i) Preparation of \((20S,3,21\text{-dihydroxy-178,20-epoxy-19-norpregna-1,3,5(10)-triene (118a).}

\(\text{K}_2\text{CO}_3\) (1.31g, 7.9mmol) was added to a solution of \((20R,3,17\beta\text{-dihydroxy-20,21-epoxy-19-norpregna-1,3,5(10)-triene (106a) (1.01g, 3.2mmol) in methanol (69ml). The resulting suspension was stirred overnight at ambient temperature during which time the suspended solid dissolved. Ethyl acetate was added to the methanol solution, and the resulting solution was washed with water, dried (MgSO\(_4\)) and the solvent removed in vacuo to afford a white solid (0.81g, 80%), which on recrystallisation from ethyl acetate gave \((20S,3,21\text{-dihydroxy-178,20-epoxy-19-norpregna-1,3,5(10)-triene (118a) (0.63g, 62%): mp 173-260°C (The compound partially resolidified before melting and finally decomposed); \([\alpha]_D +37.6^\circ\) (c 0.9, dioxan); ir (KBr) 3420,
3320 (OH) cm⁻¹; 'Hnmr (90MHz; (CD₂)₂CO/D₂O) 0.87 (3H, s, 18-CH₃), 2.6-3.0 (2H, m, 6-CH₂), 3.17 (1H, t, J=5.5Hz, 20-CH), 3.66 (2H, m, 21-CH₂), 6.5-7.3 (3H, m, A-ring CH's). At 400MHz the multiplet at δ 3.66 is fully resolved into the expected 8 lines of the AB component of an ABX system (J_AB=12Hz); 'Cnmr see Table 3; Accurate Mass (Found: m/z 314.1885 (M⁺, 62.96%) C₂₀H₂₈O₅ requires M⁺ 314.1882; Elemental Analysis (Found: C,76.1; H,8.3%; C₂₀H₂₈O₅ requires C,76.4; H,8.3%). Acetylation with acetic anhydride/pyridine yielded the diacetate (118b) 'Hnmr (60MHz; CDCl₃) 0.9 (3H, s, 18-CH₃), 2.1 (3H, s, 21AcO-CH₃), 2.2 (3H, s, 3AcO-CH₃), 2.5-3.0 (2H, m, 6-CH₂), 3.2 (1H, q, J_Ak=4Hz, J_Bk=6Hz, 20-CH), 4.0 (2H, m(8 lines), J_AB=12Hz, 21-CH₂), 6.6-7.2 (3H, m, A-ring CH's).


KOT-Bu (1.0g, 8.20mmol) was added to a solution of (20S)-3,17β-dihydroxy-20,21-epoxy-19-norpregna-1,3,5(10)-triene (107a) (0.93g, 2.96mmol) in t-BuOH (30ml) at 30°C. The reaction mixture was stirred overnight while the temperature was maintained at 30-40°C, after which it was diluted with water and extracted (2x) with ethyl acetate. The combined extracts were washed with water, dried (MgSO₄) and the solvent removed in vacuo to afford a white solid (0.83g, 89%). Recrystallisation from ethyl
acetate (2x) gave (20R)-3,21-dihydroxy-17β,20-epoxy-19-norpregna-1,3,5(10)-triene (119a) (0.37g, 40%): mp 185-189°C; [α]D +29.9° (c 1.0, dioxan); ir (KBr) 3160,3400 (OH) cm⁻¹; ¹Hnmr (90MHz; (CD₃)₂CO/D₂O) δ 0.99 (3H, s, 18-CH₃), 2.6-2.9 (2H, m, 6-CH₂), 3.09 (1H, q, Jₓ=4Hz, Jₓₓ=7Hz, 20-CH), 3.90 (2H, m(8 lines), Jₓₓₓ=12Hz, 21-CH₂), 6.5-7.2 (3H, m, A-ring CH's); ¹³Cnmr see Table 3; Accurate Mass [Found: m/z 314.1876 (M⁺, 1.2%)] C₂₀H₂₆O₉ requires M⁺ 314.1882]; Elemental Analysis [Found: C,76.2; H,8.5%; C₂₀H₂₆O₉ requires C,76.4; H,8.3%].


(20S)-3,17β-Dihydroxy-20,21-epoxy-19-norpregna-1,3,5(10)-triene (107a) (0.20g, 0.54mmol) was treated as described for 8.2(ii) except that the reaction mixture was worked up by first diluting with ethyl acetate. The diluted mixture was then washed with water, dried (MgSO₄), and the solvent removed in vacuo to afford a white solid (0.22g, 97%). Recrystallisation from aqueous methanol gave (20R)-21-acetoxy-17β,20-epoxy-3-hydroxy-19-norpregna-1,3,5(10)-triene (119b) (0.11g, 49%): mp 171-178°C; [α]D +46.7° (c 1.24, dioxan); ir (neat) 3370 (OH), 1725 (C=O) cm⁻¹; ¹Hnmr (90MHz; CDCl₃/(CD₃)₂CO) δ 0.98 (3H, s, 18-CH₃), 2.12 (3H, s, 21AcO-CH₃), 2.7-3.0 (2H, m, 6-CH₂), 3.15 (1H, q, Jₓ=2Hz, Jₓₓ=8Hz, 20-CH),
4.13 (1H, q, \( J_{AB} = 12 \text{Hz}, J_{AX} = 8 \text{Hz}, 21-\text{CH} \)), 4.64 (1H, q, \( J_{AB} = 12 \text{Hz}, J_{AX} = 2 \text{Hz}, 21-\text{CH} \)), 6.6-7.3 (3H, m, A-ring CH's);

\(^{13}\text{C}n\text{mr} \text{ see Table 3; Accurate Mass [Found: m/z 356.1981 (M\(^+\), 8.24%) C}_{22}\text{H}_{22}\text{O}_{4} \text{ requires M}^+ \text{: 356.1987].}


(20S)-3,21-Dihydroxy-17β,20-epoxy-19-norpregna-1,3,5(10)-triene (118a) (1.4g, 4.5mmol) in THF (90ml) and water (30ml) was treated with 60% HClO\(_4\) (90 drops). After stirring for 20 hours, ethyl acetate was added and the reaction mixture was washed with 8% NaHCO\(_3\) solution (2x) and water (2x), dried (MgSO\(_4\)) and the solvent removed in vacuo to afford a white solid (1.37g). Separation by preparative tlc on silica gel ran with ethyl acetate/chloroform (1:1) gave a mixture of (20R)-3,17β,20,21-tetrahydroxy-19-norpregna-1,3,5(10)-triene (120a) and (20S)-3,20-dihydroxy-13α,21-epoxy-17β-methyl-18,19-dinorpregna-1,3,5(10)-triene (122a) (0.33g, 24%): ir (KBr) 3280 (OH) cm\(^{-1}\); \(^{1}H\text{nmr (90MHz; CDCl}_{3}/\text{DMSO d}_{6}/\text{D}_{2}O\) \(\delta \) 1.12 (3H, s, 17β-CH\(_3\)), 2.5-2.9 (2H, m, 6-CH\(_2\)), 3.2-3.6 (2H, m, 21-CH\(_2\)), 3.7-4.2 (1H, m, 20-CH), 4.87 (1H, s(broad),
20-OH, exchanges on adding D$_2$O), 6.4-7.2 (3H, m, A-ring CH's), 8.80 (1H, s, 3-OH, exchanges on adding D$_2$O);

**Accurate Mass** (Found: m/z 314.1871 (M$^+$, 50%) C$_{20}$H$_{26}$O$_3$ requires M$^+$ 314.1882).

The mixture of (120a) and (121a) was acetylated with acetic anhydride/pyridine and then separated by preparative tlc on silica gel ran with diethyl ether/petrol ether(40/60) - (2:1) to give (20R)-17B-hydroxy-3,20,21-triacetoxy-19-norpregna-1,3,5-(10)-triene (120b) (0.20g, 10%) [recrystallisation from ethyl acetate/petrol ether(40/60) afforded pure (120b) (0.15, 7%): mp 190-192°C; $\alpha_0$ +7.9° (c 0.9, CHCl$_3$); ir (KBr) 3500 (OH), 1740 (AcO C=O's) cm$^{-1}$; Hnmr (90MHz; CDC$_3$) δ 0.91 (3H, s, 18-CH$_3$), 2.06 (3H, s, 21AcO-CH$_3$), 2.13 (3H, s, 20AcO-CH$_3$), 2.30 (3H, s, 3AcO-CH$_3$), 2.7-3.0 (2H, m, 6-CH$_2$), 4.14 (1H, q, J=9 and 12Hz, 21-CH), 4.65 (1H, q, J=2 and 12Hz, 21-CH), 5.44 (1H, q, J=2 and 9Hz, 20-CH), 6.8-7.4 (3H, m, A-ring CH's); $\delta$Cnmr see Table 3; **Accurate Mass** (Found: m/z 458.2295 (M$^+$, 5.67%) C$_{26}$H$_{34}$O$_7$ requires M$^+$. 458.2304); **Elemental Analysis** (Found: C, 67.6; H, 7.5%; C$_{26}$H$_{34}$O$_7$ requires C, 68.1; H, 7.5%.)] and (20S)-17B-methyl-3,20,21-triacetoxy-18,19-dinorpregna-1,-3,5(10)-13-tetraene (121b) (0.27g, 14%) [recrystallisation from ethyl acetate/petrol ether(40/60) afforded pure (121b) (0.09g, 5%): mp 168-171°C; $\alpha_0$ -57.0° (c 1.0%, CHCl$_3$); ir (KBr) 1732 (AcO C=O's) cm$^{-1}$; Hnmr (90MHz; CDC$_3$) δ 1.12 (3H, s, 17β-CH$_3$), 2.03 (3H,
s, 21AcO-CH₃), 2.12 (3H, s, 20AcO-CH₃), 2.30 (3H, s, 3AcO-CH₃), 4.05 (1H, q, J=9 and 12Hz, 21-CH), 4.41 (1H, q, J=3 and 12Hz, 21-CH), 5.32 (1H, q, J=3 and 9Hz, 20-CH), 6.8-7.5 (3H, m, A-ring CH's); ¹³Cnmr see Table 3; Accurate Mass [Found: m/z 440.2203 (M⁺, 0.91%) C₂₆H₃₂O₆ requires 440.2199]; Elemental Analysis [Found: C, 70.9; H, 7.4%; C₂₆H₃₂O₆ requires C, 70.9; H, 7.3%].

(122a) (0.10g) was acetylated with acetic anhydride/pyridine to afford a white solid (0.12g) which crystallised from aqueous methanol to give pure (20S)-3,20-diacetoxy-13α,21-epoxy-17β-methyl-18,19-dinorpregna-1,3,5(10)-triene (122b) (0.045g, 36%): mp 101-104°C; [α]D +59.3° (c 0.9%, dioxan); ir (KBr) 1752 (AcOC=O's) cm⁻¹; ¹Hnmr (90MHz; acetone d₆) δ 1.20 (3H, s, 17β-CH₃), 2.08 (3H, s, 20AcO-CH₃), 2.23 (3H, s, 3AcO-CH₃), 2.6-3.0 (2H, m, 6-CH₂), 3.49 (1H, q, J=7 and 9Hz, 21-CH), 4.08 (1H, q, J=7 and 9Hz, 21-CH), 4.89 (1H, t, J=7Hz, 20-CH), 6.7-7.4 (3H, m, A-ring CH's); Accurate Mass [Found: m/z 398.2089 (M⁺, 2.24%) C₂₄H₃₀O₆ requires M⁺ 398.2093]; Elemental Analysis [Found: C, 72.2; H, 7.95%; C₂₄H₃₀O₆ requires C, 72.3; H, 7.6%].
8.2(v) **Oxidation of (20S)-3,20-dihydroxy-13α,21-epoxy-17β-methyl-18,19-dinorpregna-1,3,5(10)-triene (122a).**

Diazomethane solution was prepared by gradually adding N-nitroso-N-methylurea (3.0g, 29mmol) to a mixture of 40% aqueous KOH (10ml) and diethyl ether (30ml) at 0°C, whilst stirring vigorously. When all the N-nitroso-N-methylurea had reacted the ether layer was decanted and dried over KOH pellets for 2 hours. The diazomethane in ether solution was then added to a stirred suspension of (20S)-3,20-dihydroxy-13α,21-epoxy-17β-methyl-18,19-dinorpregna-1,3,5(10)-triene (122a) (0.21g, 66mmol) in dry methanol (10ml). After three days the solvents were removed in vacuo to leave a cream solid (0.25g), which was taken up into dichloromethane and filtered to remove any insoluble starting material. Subsequent removal of the dichloromethane in vacuo afforded (20S)-13α,21-epoxy-20-hydroxy-3-methoxy-17β-methyl-18,19-dinorpregna-1,3,5(10)-triene (the 3-O-methyl ether of (122a)) (0.19g, 87%): "Hnmr (90MHz; acetone d₆) δ 1.16 (3H, s, 17β-CH₃), 2.6-3.0 (2H, m, 6-CH₂), 3.2-3.6 (2H, m, 21-CH₂), 3.74 (3H, s, 3MeO-CH₃), 3.8-4.2 (1H, m, 20-CH), 6.5-7.3 (3H, m, A-ring CH's).

The crude product from the above methylation reaction (0.19g, 0.82mmol) was dissolved in dichloromethane (50ml), cooled to 0°C and treated with pcc (0.26g, 2
molar equivalents). The resultant mixture was stirred over night while the temperature was maintained below 10°C. After 18 hours the reaction mixture was washed with 2M HCl (2x), 8% NaHCO₃ solution (2x) and brine (2x), dried (MgSO₄), and the solvent removed in vacuo to afford a brown oil (0.15g). Purification by preparative tlc on silica gel ran with diethyl ether/petrol ether(40/60) (2:1) gave a light brown oil (0.095g, 50%) which crystallised from ethyl acetate/petrol ether(40/60) to give pure 13α,21-epoxy-3-methoxy-17β-methyl-18,19-dinorpregn-1,3,5(10)-triene-20-one (125) (0.018g, 10%): mp 97-100°C; [α]D +45° (c 0.29%, CHCl₃); ir 1756 (C=O) cm⁻¹; ¹Hnmr (90MHz; CDCl₃) δ 1.23 (3H, s, 17β-CH₃), 2.7-3.0 (2H, m, 6-CH₂), 3.37 (3H, s, 3MeO-CH₃), 4.17 (2H, q, Jₘₗ=22Hz, 21-CH₂), 6.7-7.4 (3H, m, A-ring CH's); Accurate Mass [Found: m/z 326.1882 (M⁺, 39.75%) C₂₅H₃₂O₂ requires M⁺: 326.1882]. Compound (83) readily decomposed, and an accurate elemental analysis could not be obtained.


(20R)-3,17β-Dihydroxy-20,21-epoxy-19-norpregn-1,3,5(10)-triene (106a) (0.10g, 0.32mmol) in THF (6ml) and water (2ml) was treated with 60% HClO₄ (6 drops) and stirred for 24 hours at ambient temperature. The reaction mixture was worked up as described for 8.2(iv) to afford a white solid (0.09g). Separation by preparative tlc on silica
gel ran with ethyl acetate/chloroform (1:1) afforded 4 fractions: starting material (106a) (14mg, 14%), (20S)-3,20-dihydroxy-13α,21-epoxy-17β-methyl-18,19-dinorpregna-1,3,5(10)-triene (122a) (19mg, 19%) [acetylated with acetic anhydride/pyridine to give (20S)-3,20-diacetoxy-13α,21-epoxy-17β-methyl-18,19-dinorpregna-1,3,5(10)-triene (122b) (22mg): 'Hnmr see §8.2(iv)], (20S)-17β-methyl-3,20,21-trihydroxy-18,19-dinorpregna-1,3,5(10),13-tetraene (121a) (31mg, 31%) [acetylated with acetic anhydride/pyridine to give (20S)-17β-methyl-3,20,21-triacetoxy-18,19-dinorpregna-1,3,5(10),13-tetraene (121b) (31mg): 'Hnmr see §8.2(iv)], and a fraction tentatively assigned as a =50:50 mixture of (20R)-3,17β,20,21-tetrahydroxy-19-norpregna-1,3,5(10)-triene (120a) and (20S)-3,17β,20,21-tetrahydroxy-19-norpregna-1,3,5(10)-triene (124) (26mg, 25%) [acetylated with acetic anhydride/pyridine to give a ca. 50:50 mixture of (20R)- and (20S)-17β-hydroxy-3,20,21-triacetoxy-19-norpregna-1,3,5(10)-triene, (120b) and (124b), (31%): 'Hnmr (90MHz; CDCl₃) δ 0.91 (ca.50%, 3H, s, (120b)-18-CH₃), 0.94 (ca.50%, 3H, s, (124b)-18-CH₃), 2.7-3.1 (2H, m, 6-CH₂), 4.0-4.7 (2H, m, 21-CH₂), 5.2-5.6 (1H, m, 20-CH), 6.8-7.5 (3H, m, A-ring CH's)].
8.2(vii) **Acid hydrolysis of (20$)-3,21-dihydroxy-17B,20-epoxy-19-norpregna-1,3,5(10)-triene (118a) with HClO$_4$ in MEK.**

(20$)-3,21-Dihydroxy-17B,20-epoxy-19-norpregna-1,3,5(10)-triene (118a) (0.10g, 0.32mmol) in MEK (10ml) was treated with 60% HClO$_4$ (2 drops) and stirred rapidly for 10 minutes. After diluting with ethyl acetate, the reaction mixture was washed with 8% NaHCO$_3$ solution (2x) and water (2x), dried (MgSO$_4$) and the solvents removed in vacuo to afford a cream solid (0.10g). Separation by preparative tlc on alumina gel ran with ethyl acetate / chloroform / ethanol (5:5:1) gave 2 major fractions: (20S)-3,20-dihydroxy-13a,21-epoxy-17B-methyl-1a,19-dinorpregna-1,3,5(10)-triene (122a) (0.028, 28%) [see 8.2(iv) for analytical data], and a mixture of unidentified less polar products with the same RF value (0.015g) [ir (KBr) 3340 (OH) cm$^{-1}$; 'Hnmr (90MHz; CDCl$_3$/acetone d$_6$) δ 0.90, 0.97, 0.99, 1.05, 1.11, 1.13, 1.28, 1.36 (singlets, major signals are underlined) 2.7-3.1 (2H, m, 6-CH$_2$), 3.5-4.3 (ca.3H. m), 6.6-7.3 (3H, m, A-ring CH's)].

8.2(viii) **Addition of thiophenol to (20$)-3,17B-dihydroxy-20,21-epoxy-19-norpregna-1,3,5-(10)-triene (106a).**

(20$)-3,17B-Dihydroxy-20,21-epoxy-19-norpregna-1,3,5(10)-triene (106a) (0.50g, 1.59mmol) in DMF (2.5ml) was added
to a mixture of thiophenol (0.50ml, 3 molar equivalents) and triethylamine (0.88ml, 4 molar equivalents) in DMF (1ml) under nitrogen. After 22 hours ethyl acetate was added and the reaction mixture was washed with 8% NaHCO₃ solution (3x), 2M HCl (3x) and water (2x), dried (MgSO₄) and the solvent removed in vacuo to afford a oily white crystals (0.73g). Purification by preparative tlc on silica gel ran with diethyl ether / petrol ether(40/60) (4:1) gave a white solid (0.47g, 70%), which recrystallised from dichloromethane to give pure

(20S)-21-phenylthio-3,17β,20-trihydroxy-19-norpregna-1,3,5(10)-
3,5(10)-triene (131) (0.12g, 18%): mp 173-176°C; [α]D
+82.5° (c 0.9%, CHCl₃); ir (CHCl₃ solution) 3585, 3350
(OH) cm⁻¹; ¹Hnmr (90MHz; CDCl₃/acetone d₆) 0.93 (3H, m, 18-CH₃), 2.7-3.0 (2H, m, 6-CH₂), 3.00 (1H, q, J=14 and 10Hz, 21-CH), 3.71 (1H, q, J=14 and 2Hz, 21-CH), 3.94 (1H, q, J=10 and 2Hz, 20-CH), 6.5-7.2 (3H, m, A-ring CH's), 7.2-7.6 (5H, m, S-Ph CH's); Accurate
Mass [Found: m/z 424.2079 (M⁺, 8.14%) C₂₀H₃₂O₉S requires
M⁺ 424.2072].

Acetylation with acetic anhydride/pyridine gave the
3,20-diacetoxy derivative of (131) - (20S)-3,20-di-
acetoxy-17β-hydroxy-21-phenylthio-19-norpregna-1,3,5(10)-
-triene: ¹Hnmr (90MHz; CDCl₃) δ 0.90 (3H, s, 18-CH₃),
2.05 (3H, s, 20AcO-CH₂), 2.31 (3H, s, 3AcO-CH₂), 2.7-3.0
(2H, m, 6-CH₂), 3.2-3.5 (2H, m, 21-CH₂), 5.27 (1H, q, J=8
and 3Hz, 20-CH), 6.7-7.7 (8H, m, A-ring and S-Ph CH's).

(20S)-3,17β-dihydroxy-20,21-epoxy-19-norpregna-1,3,5(10)-triene (107a) (0.5g, 1.59mmol) was treated as described for 8.2(viii) to afford, after the work up, a clear oil (0.78g). Purification by preparative tlc on silica gel ran with diethyl ether / petrol ether (40/60) (4:1) gave (20R)-21-phenylthio-3,17β,20-trihydroxy-19-norpregna-1,3,5(10)-triene (132) (0.56g, 83%) as a white foam which would not crystallise to further purity: [α]D 0.0° (c 1.1%, CHCl₃); ir (CHCl₃ solution) 3680, 3585, 3540, 3340 (OH) cm⁻¹; ¹Hnmr (90MHz; CDCl₃/D₂O) δ 0.92 (3H, s, 18-CH₃), 2.6-3.0 (2H, m, 6-CH₂), 3.12 (1H, q, J=14 and 10Hz, 21-CH), 3.50 (1H, q, J=14 and 3Hz, 21-CH), 3.85 (1H, q, J=10 and 3Hz, 20-CH), 6.5-7.2 (3H, m, A-ring CH's), 7.2-7.6 (5H, m, S-Ph CH's); Accurate Mass [Found m/z 424.2076 (M⁺, 7.99%) C₂₆H₃₂O₆S requires M⁺ 424.2072].

Acetylation with acetic anhydride/pyridine gave the 3,20-diacetoxy derivative of (132) - (20R)-3,20-diacetoxy-17β-hydroxy-21-phenylthio-19-norpregna-1,3,5(10)-triene, which could not be persuaded to crystallise: [α]D +3.6° (c 1.2%, CHCl₃); ir (neat) 3500 (OH) 1730 (C=O's) cm⁻¹; ¹Hnmr (90MHz; CDCl₃) δ 0.91 (3H, s, 18-CH₃), 2.00 (3H, s, 20AcO-CH₃), 2.27 (3H, s, 3AcO-CH₃),
2.7-3.0 (2H, m, 6-CH₂), 3.2-3.7 (2H, m, 21-CH₂), 5.25 (1H, q, J=7 and 4Hz, 20-CH₂), 6.7-7.6 (8H, m, A-ring and S-Ph CH's); Accurate Mass [Found m/z 508.2281 (M⁺, 1.26%) C₉₀H₃₆O₆S requires M⁺·508.2283].


(20S)-3,21-Dihydroxy-17β,20-epoxy-19-norpregna-1,3,5(10)-triene (118a) (0.40g, 1.27mmol) in DMF (2ml) was added to a mixture of thiophenol (0.40ml, 3 molar equivalents) and triethylamine (0.70ml, 4 molar equivalents) in DMF (1ml) under nitrogen. After 6 days additional thiophenol (0.40ml) and triethylamine (0.70ml) was introduced, and again after a further 2 days thiophenol (0.8ml) and triethylamine (1.4ml) was added. The reaction mixture was stirred for a further 5 days (total reaction time 13 days) and worked up as described for 8.2(viii) to afford a white foam (1.6g). Purification by preparative tlc on silica gel ran with diethyl ether / petrol ether (40/60) (8:1) gave (20R)-20-phenylthio-3,17β,21-trihydroxy-19-norpregna-1,3,5(10)-triene (133) (0.24g, 44%) as a clear oil which would not crystallise: [α]₀ < -25° (c 1.1%, dioxan); ir (CHCl₃ solution) 3590, 3360 (OH) cm⁻¹; 'Hnmr (90MHz; CDCl₃/D₂O) δ 0.92 (3H, s, 18-CH₃), 2.6-3.0 (2H, m, 6-CH₂), 3.4-3.7 (1H, m, 20-CH), 3.7-4.4 (2H, m, 21-CH₂), 6.5-7.2 (3H, m, A-ring CH's), 7.2-7.7 (5H, m,
Acetylation with acetic anhydride/pyridine gave the 3,21-diacetoxy derivative of (133) - (20R)-3,21-diacetoxy-17β-hydroxy-20-phenylthio-19-norpregna-1,3,5(10)-triene, as a clear oil which would not crystallise:

'Hnmr (90MHz; CDCl₃) δ 0.94 (3H, s, 18-CH₃), 1.78 (3H, s, 21AcO-CH₃), 2.27 (3H, s, 3AcO-CH₃), 2.7-3.0 (2H, m, 6-CH₂), 3.57 (1H, q, J=8 and 4Hz, 20-CH), 4.3-4.8 (2H, m, 21-CH₂), 6.8-7.7 (8H, m, A-ring and S-Ph CH's).

(20R)-3,21-Dihydroxy-17β,20-epoxy-19-norpregna-1,3,5(10)-triene (119a) (0.40g, 1.27mmol) was treated as described for 8.2(x) to afford, after the work up, a white foam (1.9g). Purification by preparative tlc on silica gel ran with diethyl ether/petrol ether (40/60) (8:1) gave (20S)-20-phenylthio-3,17β,21-trihydroxy-19-norpregna-1,3,5(10)-triene (134) (0.44g, 81%) as a clear oil which would not crystallise to further purity: [α]D +26° (c 1.3%, dioxan); ir (CHCl₃ solution) 3580, 3520, 3360 (OH) cm⁻¹; 'Hnmr (90MHz; CDCl₃/D₂O) δ 0.99 (3H, s, 18-CH₃), 2.6-3.0 (2H, m, 6-CH₂), 3.4-3.6 (1H, m, 20-CH), 4.0-4.2 (2H, m, 21-CH₂), 6.5-7.2 (3H, m, A-ring CH's).
7.2-7.7 (5H, m, S-Ph CH's); Accurate Mass [Found m/z 406.1963 (M+-18, 4.42%) C_{26}H_{30}O_{2}S requires 406.1966].

Acetylation with acetic anhydride/pyridine gave the 3,21-diacetoxy derivative of (134) - (20R)-3,21-di-acetoxy-17β-hydroxy-20-phenylthio-19-norpregna-1,3,5(10)-triene, as a clear oil which would not crystallise:

'Hnmr (90MHz; CDCl₃) 6 1.00 (3H, s, 18-CH₃), 1.97 (3H, s, 21AcO-CH₃), 2.28 (3H, s, 3AcO-CH₃), 3.60 (1H, t, J=6Hz, 20-CH), 4.54 (2H, m(8 lines), J_{AB}=12Hz, 21-CH₂), 6.7-7.7 (8H, m, A-ring and S-Ph CH's).

8.2(xii) Addition of diethylamine to (20R)-3,17β-dihydroxy-20,21-epoxy-19-norpregna-1,3,5(10)-triene (106a) with Ti(i-PrO)₄.

(20R)-3,17β-Dihydroxy-20,21-epoxy-19-norpregna-1,3,5(10)-triene (106a) (0.10g, 0.32g) in diethylamine (5ml) was stirred under nitrogen and treated with Ti(i-PrO)₄ (0.15ml, 1.5 molar equivalents). After 24 hours additional diethylamine (5ml) and Ti(i-PrO)₄ (0.15ml, 1.5 molar equivalents) was added. After a further 22 hours ethyl acetate (15ml) and 10% NaOH in brine (10ml) was introduced and the resulting mixture stirred for 6.5 hours, filtered, washed with water (2x), dried (MgSO₄) and the solvent removed in vacuo to afford a yellow oil (0.097g). Separation by preparative tlc on silica gel ran with ethyl acetate / chloroform / methanol (5:5:1) gave
starting material (106a) (36mg, 36%), (20S)-3,21-dihydroxy-17β,20-epoxy-19-norpregna-1,3,5(10)-triene (118a) (11mg, 11%): see §8.2(1) for analysis, and a fraction tentatively assigned as a ≈4:3 mixture of (20R)-21-(diethylamino)-3,17β,20-trihydroxy-19-norpregna-1,3,5(10)-triene (137) and 21-(diethylamino)-3,20-dihydroxy-17β-methyl-18,19-dinorpregna-1,3,5(10)-triene (138) (25mg, ca. 20%): 'Hnmr (90MHz; CDCl₃) δ 0.88 (s, (137)-18-CH₃), 0.18 (t, Et₂N-CH₃), 0.29 (s, (138)-18-CH₃), 2.6-3.1 (m, 6-CH₂, 21-CH₂ and Et₂N-CH₂), 3.7-4.2 (m, 20-CH), 6.6-7.2 (m, A-ring CH's).

8.3 Preparation of 16α-bromoestrone (141).

Estrone (8), (2.0g, 7.40mmol) and dry CuBr₂ (5.0g, 22.4mmol) in dry methanol (500ml) were heated under reflux for 24 hours. The methanol was removed in vacuo and the residue dissolved in ethyl acetate. The organic extract was washed with water, dried (MgSO₄) and the solvent removed in vacuo to afford an oily light brown solid (3.18g). Purification by flash chromatography, eluting with dichloromethane/ethyl acetate (40:1), gave 16α-bromoestrone (141) (1.1g, 43%): ir (nujol mull) 3400 (OH), 1740 (C=O) cm⁻¹; 'Hnmr (60MHz; CDCl₃/DMSO d₆) δ 0.94 (3H, s, 18-CH₃), 4.67 (1H, m, 16β-H), 6.5-7.2 (3H, m, A-ring CH's). The ir and 'Hnmr spectra compare well with the literature data on this compound."
8.4 (i) Preparation of 4β,5β-epoxycholestan-3-one (146).

Cholestenone (1.0g, 3.79mmol) in methanol (100ml) was cooled to 0°C and treated with 30% aqueous H₂O₂ (4ml) and 4M NaOH (4ml). The resulting solution was stirred for 2 hours at 0°C and for a further 5 hours at ambient temperature. The reaction mixture was diluted with H₂O and extracted into diethyl ether (100ml x 2). The combined organic extracts were washed with sat. FeSO₄ solution (until no colour was produced) and water, dried (MgSO₄) and evaporated in vacuo to afford a cream solid (0.88g). Examination by 'Hnmr spectroscopy showed the crude product to consist of predominantly 4α,5α-epoxy- and 4β,5β-epoxy-cholestan-3-one (ca. 28:72, calculated by integration of the 4-CH at δ 3.05 and 2.99). Recrystallising twice from methanol afforded pure 4β,5β-epoxycholestan-3-one (146) (0.45g, 42%): mp 118-120°C (lit7 mp 116-117°C); ir (nujol mull) 1710 (C=O) cm⁻¹; 'Hnmr (90MHz; CDCl₃) δ 0.92 (3H, s, 18-CH₃), 1.17 (3H, s, 19-CH₃), 2.99 (1H, s, 4α-H).


Progesterone (25) (1.0, 3.10mmol) in methanol (30ml) was cooled to 0°C and treated with 30% H₂O₂ (6ml) and 10% NaOH (2ml). After 20 hours at 0°C, the reaction was worked up as described for 8.4(i) to afford an oily white...
solid (0.92g). Examination by 'Hnmr spectroscopy showed the crude product to consist of predominantly 4α,5α-epoxy- and 4β,5β-epoxy-pregnan-3,20-dione (ca. 30:70, calculated by integration of the 4-CH at δ 2.97 and 2.92). Recrystallising twice from methanol gave a pure mixture (ca. 28:72) of 4α,5α-epoxy- and 4α,5α-epoxy-pregnan-3,20-dione (147) (0.51g, 49%): 'Hnmr (60MHz; CDCl₃) δ 0.62 (3H, s, 18-CH₃), 1.12 (3H, s, 19-CH₃), 2.92 (ca. 72%, 1H, s, 4α-H), 2.97 (ca. 28%, 1H, s, 4β-H).

8.4(iii) Preparation of 4α,5α-epoxyandrostan-17β-ol-3-one (148).

Testosterone (16) (1.0g, 3.47mmol) was treated as described for 8.4(ii), to afford a white crystalline solid (0.89g). Examination by 'Hnmr spectroscopy showed the crude product to consist of predominantly 4α,5α-epoxy- and 4β,5β-epoxy-androstan-17β-ol-3-one (ca 21:79, calculated by integration of the 4-CH at δ 3.07 and 3.01). Recrystallising twice from acetone/hexane afforded a pure mixture of 10-15% 4α,5α-epoxy- and 85-90% 4β,5β-epoxy-androstan-17β-ol-3-one (148) (0.18g, 17%): mp 137-156°C (lit² 138-150°C for a mixture of α- and β-epoxides; 157-158°C for pure β-epoxide); 'Hnmr (90MHz; CDCl₃) δ 0.79 (3H, s, 18-CH₃), 1.19 (3H, s, 19CH₃), 3.01 (85-90%, 1H, s, 4α-H), 3.07 (10-15%, 1H, s, 4β-H), 3.7 (1H, m, 17α-H).

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8.4(iv) **Preparation of 48,58-epoxy-17\(\beta\)-hydroxy-19-norpregn-20-yn-3-one (27).**

Norethisterone (28) (0.5g, 1.68mmol) in methanol (40ml) was treated with 18% \(\text{H}_2\text{O}_2\) (2ml) and 4M NaOH (2ml) at 0°C, and the resulting solution was stirred at ambient temperature for 1 hour. The reaction was worked up as described for 8.4(i) to afford a white solid (0.42g), whose 'Hnmr spectra showed it to consist of predominantly the required \(\beta\)-epoxide. The crude product would not crystallise to purity, but separation by preparative tlc on silica gel ran with diethyl ether/petrol ether (40/60) (3:1) afforded pure 48,58-epoxy-17\(\beta\)-hydroxy-19-norpregn-20-yn-3-one (27) (0.19g, 36%). Further purification by recrystallisation from propan-2-ol gave a very pure sample for biological testing: mp 146-148°C (lit. 141.5-142°C); 'Hnmr (60MHz; CDCl\_3) \(\delta\) 0.87 (3H, s, 18-CH\(_3\)), 2.56 (1H, s, 21-CH), 3.01 (1H, s, 4\(\alpha\)-H).

8.4(v) **Preparation of 48,58-epoxy-17\(\beta\)-hydroxypregn-20-yn-3-one (149).**

Ethisterone (1.0g, 3.2mmol) in methanol (150ml) was treated with 30% \(\text{H}_2\text{O}_2\) (6ml) and 10% NaOH (2ml) at 0°C. After 3 days at 0°C, the reaction was worked up as described for 8.4(i) to afford a white solid (0.41g). Examination by 'Hnmr spectroscopy showed the crude product to consist of predominantly 4\(\alpha\),5\(\alpha\)-epoxy- and
4,5-epoxy-17-hydroxypregn-20-yn-3-one (ca. 13:87, calculated by integration of 4-CH at δ 3.00 and 2.93). Separation by preparative tlc on silica gel ran with diethyl ether/petrol ether (40/60) (4:1) afforded pure 4,5-epoxy-17-hydroxypregn-20-yn-3-one (149) (0.17g, 16%). Further purification from propan-2-ol gave a very pure sample for biological testing: mp 166-168°C; [α]D +75.5° (c 1.2, CHCl₃); ir (KBr) 3456 (OH), 3304 (C=O), 1684 (C=O) cm⁻¹; ¹Hnmr (60MHz, CDCl₃) δ 0.85 (3H, s, 18-CH₃), 1.14 (3H, s, 19-CH₃), 2.55 (1H, s, 21-CH), 2.93 (1H, s, 4α-H); Accurate Mass [Found: m/z 328.2037 (M⁻, 34.70%) C₂₁H₂₈O₃ requires M⁻, 328.2038].

8.5(i) Preparation of 3,17β-diacetoxyestra-1,3,5(10)-tri-en-6-one (153).

3,5-Dimethylpyrazole (24g) was added in one portion to a stirred mixture of dry CrO₃ (25.2g) in dichloromethane (160ml) at -25°C. After 15 minutes a solution of estradiyl diacetate (155) [prepared in quantitative yield by the treatment of estradiol (9) with acetic anhydride/pyridine] (3.6g, 0.01mol) in dichloromethane (20ml) was added to the stirred complex. The stirred reaction mixture was maintained at -15 to -20°C for 5 hours and then treated with NaOH solution (4M, 125ml). The resultant mixture was stirred for a further 45 minutes at -5 to -10°C. The organic layer was separated, and the aqueous layer was extracted with
dichloromethane (2×). The combined organic layers were washed with 2M HCl (2×) and brine (2×), dried (MgSO₄) and the solvent removed in vacuo to afford a brown solid (5.5g). Separation by flash chromatography ran with petrol ether (40/60) / ethyl acetate (4:1) and petrol ether (40/60) / ethyl acetate (1:1) gave 2 compounds:

3,17β-diacetoxyestra-1,3,5(10)-trien-6-one (153) (1.59g, 42%), recrystallisation from ethanol gave pure (153) (0.96g, 26%): mp 173-175°C (lit. 173-174°C); ir (neat) 1765 (3AcO C=O), 1730 (17AcO C=O), 1685 (6 C=O) cm⁻¹; 

'Hnmr (60MHz; CDCl₃) δ 0.83 (3H, s, 18-CH₃), 2.03 (3H, s, 17α-H), 7.0-7.5 (3H, s, A-ring CH's), 4.5-4.9 (1H, m, J=2.5Hz, 4-CH), and a compound tentatively assigned as 3,17β-diacetoxy-9α-hydroxyestra-1,3,5(10)-trien-6-one (156) (1.50g, 38%): ir (neat) 3450 (OH), 1765 (3AcO C=O), 1730 (17AcO C=O), 1685 (6 C=O) cm⁻¹; 

'Hnmr (60MHz; CDCl₃/acetone d₆) δ 0.82 (3H, s, 18-CH₃), 1.96 (3H, s, 17AcO-CH₂), 2.20 (3H, s, 3AcO-CH₂), 3.86 (1H, s[broad], exchanges on adding D₂O, 9α-OH), 4.4-4.9 (1H, m, 17α-H), 6.9-7.6 (3H, m, A-ring CH's).

8.5(ii) Preparation of 3,17β-diacetoxyestra-1,3,5(10)-trien-6-one tosylhydrazone (157b).

3,17β-diacetoxyestra-1,3,5(10)-trien-6-one (153) (1.57g) and tosylhydrazone (1.57g, ca 2 molar equivalents) in ethanol (100ml) were refluxed for 4 hours and then left
standing at ambient temperature overnight. The resultant precipitate was filtered to give 3,17β-diace	
toxyestra-1,3,5(10)-trien-6-one tosylhydrazone (157b) (1.8g, 79%). The filtrate, when evaporated in vacuo and purified by flash chromatography ran with petrol ether(40/60) / ethyl acetate (2:1), gave (157b) (0.48g, 21%). Recrystallisation from ethanol gave pure (157b); mp 232-233°C; [α]D +73.9° (c 1.1, CHCl₃); ir (nujol mull) 3184 (N-H) 1758,1734 (C=O's) cm⁻¹; ¹Hnmr (60MHz; CDCl₃/DMSO d₆) δ 0.77 (3H, s, 18-CH₃), 2.00 (3H, s, 17AcO-CH₃), 2.27 (3H, s, 3AcO-CH₃), 2.37 (3H, s, tosyl-CH₃), 3.20 (1H, s[broad] exchanges on adding D₂O, tosylhydrazone-NH), 4.4-4.9 (1H, m, 17α-H), 6.8-7.9 (7H, m, A-ring and tosyl CH's); Accurate Mass [Found: m/z 354.1837 (M+-NNHSO₂C₆H₄CH₃, 27.34%) C₂₂H₂₆O₄ requires m/z 354.1831].

8.5(iii) Preparation of 6,7-dehydroestradiol (97a).

The diacetate (157b) (1.6g 3.0mmol) was hydrolysed by treating with 2% KOH in ethanol to give 6-ketoestradiol tosylhydrazone (157a) (1.4g, 100%): ir (KBr) 3480, 3210 (OH) cm⁻¹; ¹Hnmr (60MHz; CDCl₃/DMSO d₆/D₂O) δ 0.67 (3H, s, 18-CH₃), 2.36 (3H, s, tosyl-CH₃), 3.2-3.7 (1H, m, 17α-H), 6.4-7.8 (7H, m, A-ring and tosyl CH's).

MeLi (1.7M, 2.4ml, 5 molar equivalents) was added dropwise, under nitrogen, to the crude hydrolysed
material (157a) (0.36g, 0.79mmol) in dry DMF (65ml) at 0°C. After stirring the reaction mixture at ambient temperature, under nitrogen, for 3.5 hours additional MeLi (1.7M, 0.5ml, 1 molar equivalent) was introduced and the resultant mixture was stirred for a further 15 hours. The reaction mixture was then poured into ice and acidified with 2M HCl. The resultant acidic mixture was extracted with ethyl acetate (2x), and the combined organic extracts were washed with 8% NaHCO₃ solution (3x) and water (2x), dried (MgSO₄) and the solvent removed in vacuo to give an orange solid (0.22g). Purification by preparative tlc on silica gel ran with ethyl acetate / petrol ether(40/60) (4:3) gave 6,7-dehydroestradiol (97a) (0.15g, 70%). Recrystallisation from ethyl acetate gave very pure (97a): mp 226-229°C (lit mp 225-227°C); $^1$Hnmr (60MHz; acetone d₆) δ 0.75 (3H, s, 18-CH₃), 3.04 (1H, s[ broad]), exchanges on adding D₂O, 17α-OH), 3.3-3.9 (1H, m, 17α-H), 5.6-6.4 (2H, m, 6-CH and 7-CH), 6.4-7.1 (3H, m, A-ring CH's), 7.90 (1H, s[broad], exchanges on adding D₂O, 3-OH).

Acetylation of (97a) (0.11g, 0.41mmol) with acetic anhydride/pyridine gave 6,7-dehydroestradiyl diacetate (97b) (0.14g, 97%). Recrystallisation from methanol gave pure (97b) (0.073g, 51%): mp 154-157°C (lit mp 154-156°C); $^1$Hnmr (60MHz; CDCl₃) δ 0.80 (3H, s, 18-CH₃), 2.03 (3H, s, 17AcO-CH₃), 2.24 (3H, s, 3AcO-CH₃), 4.5-4.9 (1H, m,
17α-H), 5.7-6.6 (2H, m, 6-CH and 7-CH), 6.7-7.4 (3H, m, A-ring CH's).

8.5(iv) **Preparation of 6α,7α-epoxyestradiyl diacetate**

(150b),

6,7-Dehydroestradiyl diacetate (97b) (0.15g, 0.42mmol) in dry dichloromethane (5ml) at 0°C was treated with 80% MCPBA (0.14g, 1.5 molar equivalents) in dry dichloromethane over 10 minutes. The reaction mixture was left overnight, maintaining the temperature below 10°C, and then washed with saturated Na₂SO₄ solution, 8% NaHCO₃ solution and water, dried (MgSO₄) and the solvent removed *in vacuo* to afford a cream solid (0.15g, 96%). Recrystallisation from methanol gave 6α,7α-epoxyestradiyl diacetate (150b) (82mg, 52%): mp 167-170°C (lit 167-169°C); 'Hnmr (60MHz; CDCl₃) 0.81 (3H, s, 18-CH₃), 2.02 (3H, 17AcO-CH₂), 2.24 (3H, s, 3AcO-CH₂), 3.46 (1H, d, J=4Hz, 7-CH), 3.78 (1H, d, J=4Hz, 6-CH), 4.5-4.9 (1H, m, 17α-H), 6.8-7.3 (3H, m, A-ring CH's).
8.6(i) Preparation of (6-methoxytetralin)tricarbonylchromium (169).

6-Methoxytetralin (168) (2.00g, 12.3mmol) and Cr(CO)₆ (4.00g, 18.2mmol) in di-n-butylether (100ml) were refluxed under nitrogen for 24 hours. After cooling to ambient temperature the reaction mixture was filtered and the solvent removed in vacuo to afford a yellow solid (3.56g). Purification by flash chromatography eluted with ethyl acetate / petrol ether (40/60) gave (6-methoxytetralin)tricarbonylchromium (169) (3.17g). Recrystallisation from diethyl ether/petrol ether (40/60) gave pure (169) (2.5g, 68%): mp 90-91°C; ir (KBr) 1944, 1870, 1840 (CO) cm⁻¹; ¹Hnmr (60MHz; CDCl₃) δ 1.5-2.0 (4H, m, 2-CH₂ and 3-CH₂), 2.2-2.9 (4H, m, 1-CH₂ and 4-CH₂), 3.65 (3H, s, 6MeO-CH₃), 4.8-5.6 (3H, m, aromatic CH's); Elemental Analysis [Found: C, 56.5; H, 4.8%; C₁₄H₁₀O₄Cr requires C, 56.4; H, 4.7%].

8.6(ii) Preparation of 7-hydroxy-6-methoxytetralin (170).

(6-Methoxytetralin)tricarbonylchromium (170) (0.5g, 1.68mmol) in THF (25ml) was added dropwise to a stirred solution of n-BuLi (15% in hexane solution, 1.5ml, 2 molar equivalents) and TMEDA (0.5ml, 2 molar equivalents) in THF (25ml) at -78°C under nitrogen. After 2 hours at -78°C, 10ml of the reaction mixture was removed and quenched with D₂O, acidified with 2M HCl and
extracted with diethyl ether (2×). The organic extracts were combined and washed with water (2×), dried (MgSO₄) and decomplexed by standing in sunlight until the yellow solution turned colourless. After filtering the solvent was removed in vacuo to afford 7-deutero-6-methoxy-tetralin (0.071g) only, showing 100% C-7 proton extraction to have taken place: 'Hnmr (60MHz; CDCl₃) δ 1.4-2.0 (4H, m, 2-CH₂ and 3-CH₂), 2.5-2.9 (4H, m, 1-CH₂ and 4-CH₂), 3.72 (3H, s, 6MeO-CH₃), 6.55 (1H, s, "5-CH"), 6.92 (1H, s, 8-CH).

The remaining reaction mixture was treated with MoOPH₂O₄ (3.0g, ca. 5 molar equivalents) and stirred at -40°C for 1 hour. Saturated Na₂SO₄ solution (50ml) was added and the resultant mixture stirred for 15 minutes at ambient temperature and extracted into diethyl ether (2×). The combined ether extracts were washed with 2M HCl (2×), 8% NaHCO₃ solution (2×) and water, dried (MgSO₄) and decomplexed by standing in sunlight until the yellow solution turned colourless. After filtering the solvent was removed in vacuo to afford a white solid (0.19g, ca. 86%, assuming 100% recovery of the deuterated material).

The 'Hnmr spectra indicates that the product is ca. 30% 6-methoxytetralin (168) and ca. 70% 7-hydroxy-6-methoxy-tetralin (170) (assessed from the methoxy signals at δ 3.71 and 3.77 respectively). A previous experiment which used only 2 molar equivalents of MoOPH gave a crude product whose 'Hnmr spectra indicated it to be
ca. 60% 6-methoxytetralin (168) and ca. 40% 7-hydroxy-6-methoxytetralin (170). Separation by preparative tlc with silica gel ran with ethyl acetate / petrol ether (40/60) (3:4) gave 6-methoxytetralin (168) (49%) and 7-hydroxy-6-methoxytetralin (170) (33%), recrystallisation from methanol gave pure (170): mp 82-83°C (lit. '05 mp 81-83°C); 'Hnmr (60MHz; CDCl₃) δ 1.4-2.1 (4H, m, 2-CH₂ and 3-CH₂), 2.3-2.9 (4H, m, 1-CH₂ and 4-CH₂), 3.77 (3H, s, 6MeO-CH₃), 5.40 (1H, s [broad], 7-OH), 6.45 (1H, s, 8-CH), and 6.53 (1H, s, 5-CH).

8.6(iii) Preparation of 6-methoxy-7-methyltetralin (171) with LDA and MeI.

BuLi (17% in hexane solution, 0.25ml, 2 molar equivalents) and i-Pr₂NH (0.10ml, 2 molar equivalents) in THF (5ml) were stirred, under nitrogen, for 10 minutes at -10°C. The resultant mixture was then cooled to -78°C and (6-methoxytetralin)tricarbonylchromium (169) (0.1g, 0.34mmol) in THF (5ml) was added. After 2 hours MeI (0.1ml, 5 molar equivalents) was added and the reaction mixture stirred for a further 1 hour at ambient temperature. After diluting with water, acidifying with 2M HCl and extracting into diethyl ether (2×), the combined ether extracts were washed with 8% NaHCO₃ solution and water, dried (MgSO₄) and decomplexed by standing in sunlight. After filtering the solvent was removed in vacuo to afford 6-methoxy-7-methyltetralin.'05
(171) (0.056g, 95%): 'Hnmr (60MHz; CDCl₃) δ 1.5-2.0 (4H, m, 2-CH₂ and 3-CH₂), 2.15 (3H, s, 7Me-CH₃), 2.4-2.9 (4H, m, 1-CH₂ and 4-CH₂), 3.77 (3H, s, 6MeO-CH₃), 6.48 (1H, s, 5-CH), 6.79 (1H, s, 8-CH); Accurate Mass [Found: m/z 176.1193 (M⁺, 100%) C₁₂H₁₆O requires M⁺, 176.1201]. No further purification or analysis was attempted.

8.6(iv) Preparation of 17β-hydroxy-3-methoxyestratriene (172).

Estradiol (9) (10.0g, 36.8mmol) in dry acetone (400ml) was refluxed with K₂CO₃ (16.0g, 3 molar equivalents) and MeI (23ml, 10 molar equivalents). Additional portions of MeI (10ml) were introduced if tlc showed the reaction to have ceased. After 3 days the reaction mixture was filtered and the solvent removed in vacuo. The residue was dissolved in ethyl acetate and washed with water (2×), dried (MgSO₄) and the solvent removed in vacuo to afford a white solid (11.9g). Recrystallisation from methanol gave 17β-hydroxy-3-methoxyestratriene-1,3,5(10)-triene (172) (9.6g, 91%): mp 98-99°C (lit' 97-99°C); 'Hnmr (60MHz; acetone d₆) δ 0.76 (3H, s, 18-CH₃), 2.5-3.0 (2H, m, 6-CH₂), 3.70 (3H, s, 3MeO-CH₃), 6.5-7.3 (3H, m, A-ring CH's).
8.6(v) **Preparation of 17β-(dimethyl-t-butyl-siloxy)-3-methoxyestra-1,3,5(10)-triene (175).**

17β-Hydroxy-3-methoxyestra-1,3,5(10)-triene (172) (11.5g), Me₂Bu₅SiCl (12.1g, 2 molar equivalents) and imidazole (8.2g, 3 molar equivalents) in DMF (115ml) were stirred at 35-40°C for 24 hours. After dilution with water and extraction into ethyl acetate (2x), the organic extracts were washed with 2M HCl (2x), 8% NaHCO₃ solution (2x) and water (2x), dried (MgSO₄) and the solvent removed in vacuo to afford 17β-(dimethyl-t-butyl-siloxy)-3-methoxyestra-1,3,5(10)-triene (175) (15.7g, 98%). Recrystallisation from ethanol gave very pure (175) (13.5g, 85%): mp 95-97°C; [α]₀ +57.7° (c 1.1%, CHCl₃); ir (nujol) 1615, 1505, 1260, 1250 cm⁻¹; 'Hnmr (90MHz; CDCl₃) δ 0.03 (6H, s, SiMe₂-CH₃'s), 0.76 (3H, s, 18-CH₃), 0.90 (9H, s, SiBu₅-CH₃'s), 2.6-3.0 (2H, m, 6-CH₂), 3.68 (3H, s, 3MeO-CH₃), 6.5-7.2 (3H, m, A-ring CH's); **Accurate Mass** [Found: m/z 400.2799 (M⁺, 36.74%) C₂₅H₄₈O₂Si requires M⁺ 400.2797]; **Elemental Analysis** [Found: C, 74.7; H, 10.3%; C₂₅H₄₈O₂Si requires C, 74.9; H, 10.1%].

8.6(vi) **Preparation of (17β-(dimethyl-t-butyl-siloxy)-3-methoxyestra-1,3,5(10)-triene)tricarbonyl chromium (176).**

17β-(Dimethyl-t-butyl-siloxy)-3-methoxyestra-1,3,5(10)-triene (175) (14.5g, 0.036mol) and Cr(CO)₆ (24g, 3 molar
equivalents) in di-\textit{n}-butylether were refluxed under nitrogen for 24 hours. After cooling to ambient temperature the reaction mixture was filtered and the solvent removed \textit{in vacuo} to afford a yellow solid (19.2g, 99%). Recrystallisation from diethyl ether / petrol ether(40/60) gave a 1:1 mixture of the $\alpha$ and $\beta$ isomers of \((17\beta-(\text{dimethyl-\textit{t}-butylsiloxy})-3\text{-methoxyestra-1,3,5(10)-triene})\text{tricarboxylicromium, (176a) and (176b), (16.6g, 85%). Separation of the crude complex by flash chromatography eluted with petrol ether(40/60) / diethyl ether (20:1) gave the $\alpha$-isomer (176a) (24%): mp 138-141°C; $[\alpha]_D +59.7^\circ$ (c 0.9%, CHCl$_3$); ir (nujol) 1940, 1875, 1865 (CO) cm$^{-1}$; $^1$Hnmr (90MHz; CDCl$_3$) $\delta$ 0.03 (6H, s, SiMe$_2$-CH$_3$'s), 0.73 (3H, s, 18-CH$_3$), 0.88 (9H, s, SiBu$^t$-CH$_3$'s), 2.6-3.0 (2H, m, 6-CH$_2$), 3.5-3.8 (1H, m, 17$\alpha$-H), 3.68 (3H, s, 3MeO-CH$_3$), 4.95-5.25 (2H, m, 2-CH and 4-CH), 5.78 (1H, d, J=7Hz, 1-CH); Elemental Analysis [Found: C, 62.5; H, 7.6%; C$_{26}$H$_{46}$O$_5$SiCr requires C, 62.7; H, 7.5%], and the $\beta$-isomer (176b) (28%): mp 150-152°C; $[\alpha]_D +58.9^\circ$ (c 0.9%, CHCl$_3$); ir (nujol) 1943, 1897, 1865, 1845 (CO) cm$^{-1}$; $^1$Hnmr (90MHz; CDCl$_3$) $\delta$ 0.03 (6H, s, SiMe$_2$-CH$_3$'s), 0.78 (3H, s, 18-CH$_3$), 0.88 (9H, s, SiBu$^t$-CH$_3$'s), 2.7-3.2 (2H, m, CH$_2$), 3.5-3.8 (1H, m, 17$\alpha$-H), 3.73 (3H, s, 3MeO-CH$_3$), 4.85-5.05 (2H, m, 2-CH and 4-CH), 5.65 (1H, d, J=7Hz, 1-CH); Elemental analysis [Found: C, 62.9; H, 7.9%; C$_{26}$H$_{46}$O$_5$SiCr requires C, 62.7; H, 7.5%].
8.6(vii) **Preparation of 2,17\(\beta\)-dihydroxy-3-methoxyestra-1,3,5(10)-triene (178).**

(17\(\beta\)-(Dimethyl-\(t\)-butylsiloxy)-3-methoxyestra-1,3,5(10)-triene) tricarbonylchromium (176) (0.20g, 0.37mmol) in THF (10ml) was added dropwise to a solution of n-BuLi (15% in hexane, 0.8ml, 5 molar equivalents) and TMEDA (0.3ml, 5 molar equivalents) in THF (10ml) at -78°C under nitrogen. After 2 hours at -78°C, MoOPH (1.6g, 10 molar equivalents) was added and the resultant mixture stirred at -40 to -30°C for 3.5 hours. Saturated \(\text{NaSO}_3\) solution (25ml) was then introduced, and after 15 minutes at ambient temperature the reaction mixture was extracted with diethyl ether (2\(\times\)). The combined organic extracts were washed with HCl (2\(\times\)), 8% \(\text{NaHCO}_3\) solution (2\(\times\)) and water (2\(\times\)), dried (\(\text{MgSO}_4\)) and decomplexed by standing in sunlight until the yellow solution became colourless. After filtering, the solvent was removed \textit{in vacuo} to afford a cream solid (0.13g). Separation by preparative tlc on silica gel ran with diethyl ether / petrol ether(40/60) \((1:6)\) gave the starting material 17\(\beta\)-(dimethyl-\(t\)-butylsiloxy)-3-methoxyestra-1,3,5(10)-triene (175) (26mg, 17%) and 17\(\beta\)-(dimethyl-\(t\)-butylsiloxy)-2-hydroxy-3-methoxyestra-1,3,5(10)-triene (177) (65mg, 55%): mp 160-162°C (methanol); \(^1\text{H}nmr\) (60MHz; CDCl\(_3\)) \(\delta\) 0.03 (6H, s, SiMe\(_2\)-CH\(_3\)'s), 0.74 (3H, s, 18-CH\(_3\)), 0.88 (9H, s, SiBu\(^t\)-CH\(_3\)'s), 2.6-3.0 (2H, m, 6-CH\(_2\)), 3.4-3.9 (1H, m, 17\(\alpha\)-H), 3.84 (3H, s, 3MeO-CH\(_3\)), 5.40 (1H,
s, exchanges on adding D₂O, 2-OH), 6.54 (1H, s, 1-CH),
6.87 (1H, s, 4-CH).

BF₃·Et₂O (0.5ml, ca. 4 molar equivalents) was added,
under nitrogen, to a stirred solution of the 2-hydroxy
derivative (177) (53mg, 0.13mmol) in dry chloroform
(5ml). After 10 minutes the reaction mixture was basified
with 8% NaHCO₃ solution and extracted into chloroform
(2×). The combined organic extracts were washed with
water (2×), dried (MgSO₄) and the solvent removed in
vacuo to afford a white solid (38mg, 100%).
Recrystallisation from acetone / petrol ether (40/60) gave
pure 2,17B-dihydroxy-3-methoxyestra-1,3,5(10)-triene
(178) (71%): mp 178-181°C (lit= 179-181°C); ¹Hnmr
(90MHz; CDCl₃) δ 0.76 (3H, s, 18-CH₃), 2.6-3.0 (2H, m,
6-CH₂), 3.55-3.85 (1H, m, 17α-H), 3.80 (3H, s, 3MeO-CH₃),
5.52 (1H, s, exchanges on adding D₂O), 6.47 (1H, s,
1-CH), 6.77 (1H, s, 4-CH).

8.6(viii) Preparation of 17-ethylenedioxy-3-methoxy-
estra-1,3,5(10)-triene (181).

17-Ethylenedioxy-3-hydroxyestra-1,3,5(10)-triene
(180) [prepared in 76% yield by refluxing estrone (8) with
ethyleneglycol and PTSA⁷⁻] (2.5g, 8.0mmol) was treated as
described for 8.6(iv) to afford a white solid (2.6g, 100%).
Recrystallisation from methanol gave pure
17-ethylenedioxy-3-methoxyestra-1,3,5(10)-triene
(181)
Preparation of (17-ethylenedioxy-3-methoxyestra-1,3,5(10)-triene)tricarbonylchromium (182).

17-Ethylenedioxy-3-methoxyestra-1,3,5(10)-triene (181) (0.63 g, 1.92 mmol) and Cr(CO)$_5$ (1.5 g, 3 molar equivalents) in di-n-butylether (40 ml) were refluxed under nitrogen for 24 hours. After cooling to ambient temperature the reaction mixture was filtered and the solvent removed in vacuo to afford a yellow solid (0.88 g, 99%). Examination of the $^1$Hnmr spectra indicated the product to be a mixture of the $\alpha$- and $\beta$- isomers (182a and 182b) in the ratio 36:64 (estimated from the 3-MeO signals at $\delta$ 3.64 and 3.70). Recrystallisation from diethyl ether / petrol ether (40/60) gave a pure mixture of the (17-ethylenedioxy-3-methoxyestra-1,3,5(10)-triene)tricarbonylchromium $\alpha$- and $\beta$- isomers (182a and 182b) (0.78 g, 88%): ir (nujol) 1950, 1880, 1840 (CO) cm$^{-1}$; $^1$Hnmr (90MHz; CDCl$_3$) $\delta$ 0.88 (s, 18-CH$_3$ of $\alpha$-isomer), 0.95 (s, 18-CH$_3$ of $\beta$-isomer) 2.5-3.2 (2H, m, 6-CH$_2$), 3.64 (s, 3MeO-CH$_3$ of $\alpha$-isomer), 3.70 (s, 3MeO-CH$_3$ of $\beta$-isomer), 3.88 (4H, s, 17-ethylenedioxy CH$_2$'s), 4.8-5.3 (2H, m, 2-CH and 4-CH), 5.11 (d, $J$=7Hz, 1-CH of $\beta$-isomer), 5.22 (d, $J$=7Hz, 1-CH of $\alpha$-isomer); **Elemental Analysis** [Found: C, 62.1; H,
6.2%; C$_{24}$H$_{28}$O$_6$Cr requires C, 62.1; H, 6.1%. Careful recrystallisation from diethyl ether / petrol ether (40/60) allowed the isolation of pure β-isomer (182b): mp 190-191°C; [α]$_D$ +39.9 (c 0.6%, CHCl$_3$); 'Hnmr (90MHz; CDC$_3$) δ 0.95 (3H, s, 18-CH$_3$), 2.5-3.2 (2H, m, 6-CH$_2$), 3.70 (3H, s, 3MeO-CH$_3$), 3.88 (2H, s, 17-ethylenedioxy CH$_2$'s), 4.83-5.05 (2H, m, 2-CH and 4-CH), 5.11 (1H, d, J=7Hz, 1-CH); Elemental Analysis (Found C, 61.8; H, 6.2%; C$_{24}$H$_{28}$O$_6$Cr requires C, 62.1; H, 6.1%).

8.6(x) Preparation of 2-hydroxy-3-methoxyestra-1,3,5(10)-trien-17-one (51).

(17-Ethylenedioxy-3-methoxyestra-1,3,5(10)-triene)tricarbonylchromium (182) (0.5g, 1.1mmol) in THF (30ml) was added dropwise to a solution of n-BuLi (15% in hexane, 2.3ml, 5 molar equivalents) and TMEDA (0.8ml, 5 molar equivalents) in THF (30ml) at -78°C under nitrogen. After 2 hours at -78°C, MoOPH (5g, 10 molar equivalents) was added and the resultant mixture stirred for 2.5 hours at -40 to -30°C. Subsequent reductive work-up and decomplexation as described for 8.6(vii) afforded a white solid (0.37g).

The crude product was refluxed in methanol (50ml) with conc. HCl (0.2ml) for 15 minutes. After basifying with 8% NaHCO$_3$ solution and extraction with ethyl acetate (3x),
the combined organic extracts were washed with water, dried \((\text{MgSO}_4)\) and the solvent removed \textit{in vacuo} to afford a cream solid \((0.31\text{g})\). Separation by preparative tlc on silica gel ran with diethyl ether / petrol ether \((40/60)\) \((3:1)\) gave 3-methoxyestrone \((8a)\) \((58\text{mg}, 19\%)\) and 2-hydroxy-3-methoxyestra-1,3,5(10)-trien-17-one \((51)\) \((0.152\text{g}, 47\%)\) : mp \((183.5-185^\circ\text{C})\) (ethanol) \((\text{lit}^\circ 182.5-185^\circ\text{C})\); \(^1\text{Hnmr} (60\text{MHz}; \text{CDCl}_3)\) \(\delta 0.90\) \((3\text{H}, \text{s}, 18-\text{CH}_3), 2.6-3.1\) \((2\text{H}, \text{m}, 6-\text{CH}_2), 3.86\) \((3\text{H}, \text{s}, 3\text{MeO-CH}_3), 5.80\) \((1\text{H}, \text{s}{\text{ broaden}}, \text{exchanges on adding D}_2\text{O}, 2-\text{OH}), 6.60\) \((1\text{H}, \text{s}, 1-\text{CH}), 6.88\) \((1\text{H}, \text{s}, 4-\text{CH})\).

8.6(xi) \textbf{Preparation of 3,17\beta-bis(dimethyl-t-butylsiloxy)estra-1,3,5(10)-triene (184).}

Estradiol \((9)\) \((4.0\text{g}, 0.015\text{mol})\) was stirred in dry DMF with \(\text{Me}_2\text{Bu}^+\text{SiCl}\) \((6.6\text{g}, 3 \text{ molar equivalents})\) and imidazole \((4.0\text{g}, 4 \text{ molar equivalents})\) at 35 to 40\(^\circ\text{C}\) for 21 hours. The reaction mixture was then diluted with water and extracted with ethyl acetate \((2\times)\). The combined organic extracts were washed with \(2\text{M HCl} (2\times)\) and water \((2\times)\), dried \((\text{MgSO}_4)\) and the solvent and residual \(\text{Me}_2\text{Bu}^+\text{SiCl}\) removed \textit{in vacuo} to afford a white solid \((7.1\text{g}, 97\%)\). Recrystallisation from methanol gave pure \textbf{3,17\beta-bis-(dimethyl-t-butylsiloxy)estra-1,3,5(10)-triene (184)} \((74\%)\): mp \(128-130^\circ\text{C}\) \((\text{lit}^\circ \geq 120^\circ\text{C} \text{ (ether/petrol ether)})\); \(^1\text{Hnmr} (60\text{MHz}; \text{CDCl}_3)\) \(\delta 0.03\) \((6\text{H}, \text{s}, 17\beta-\text{OSiMe}_2\text{ CH}_3's), 0.18\) \((6\text{H}, \text{s}, 3-\text{OSiMe}_2\text{ CH}_3's), 0.74\) \((3\text{H}, \text{s}, 18-\text{CH}_3), 0.90\).
Preparation of (3,17β-bis(dimethyl-t-butylsiloxy)estra-1,3,5(10)-triene)tricarbonylchromium (185).

3,17β-Bis(dimethyl-t-butylsiloxy)estra-1,3,5(10)-triene (184) (4.0 g, 8.0 mmol) and Cr(CO)₆ (5.3 g, 3 molar equivalents) in di-n-butylether (100 ml) were refluxed for 24 hours under nitrogen. After cooling to ambient temperature the reaction mixture was filtered and the solvent removed in vacuo to afford a yellow solid (4.8 g, 94%). The 'Hnmr spectra indicated the product to be a 1:1 mixture of the α- and β-isomers, (185a) and (185b). Recrystallisation from diethyl ether / petrol ether (40/60) gave a pure mixture of α- and β-(3,17β-bis(dimethyl-t-butylsilox)estra-1,3,5(10)-triene)tricarbonylchromium, (185a) and (185b), (3.3 g, 65%): 'Hnmr (90 MHz; CDCl₃) δ 0.0 (6H, s, 17β-OSiMe₂), 0.25 (6H, s, 30SiMe₂), 0.71 (s, 18-CH₃ of α-isomer), 0.77 (s, 18-CH₃ of β-isomer), 0.87 (9H, s, 17β-OSiBu⁺ CH₃), 0.93 (9H, s, 3-OSiBu⁺ CH₃), 2.5-3.2 (2H, m, 6-CH₂), 3.4-3.7 (1H, m, 17α-H), 4.7-5.1 (2H, m, 2-CH and 4-CH), 5.4-5.8 (1H, m, 1-CH).
8.6(xiii) Preparation of 17β-(dimethyl-t-butyldimethylsiloxyl) -
-2-(dimethyl-t-butyldimethylsilyl)-3-hydroxyestra-
-1,3,5(10)-triene (186a).

\( n\text{-BuLi} \) (15% in hexane, 0.3ml, 2 molar equivalents) and
di-\( t\)-propylamine (0.11ml, 2.5 molar equivalents) in THF
(10ml) were stirred at \(-10^\circ\text{C}\) for 10 minutes. The
resultant mixture was then cooled to \(-78^\circ\text{C}\) and
\((3,17β-(dimethyl-t-butyldimethylsiloxyl)estra-1,3,5(10)-triene) -
tricarbonylchromium (185) (0.2g, 0.31mmol) in THF (10ml)
added. After 1 hour at \(-78^\circ\text{C}\), water (4ml) was added and
the reaction mixture stirred for a further 15 minutes at
ambient temperature. After further dilution with water
and extraction into diethyl ether (2×), the combined
ether extracts were washed with 2M HCl (2×), 8% NaHCO\(_3\)
solution (2×) and water (2×), dried (MgSO\(_4\)) and
decomplexed by standing in sunlight until the yellow
solution turned colourless. After filtering the solvent
was removed in vacuo to afford a cream solid (0.15g).
Separation by preparative tlc on silica gel run with
diethyl ether / petrol ether (40/60) (1:6) gave
17β-(dimethyl-t-butyldimethylsiloxyl)-2-(dimethyl-t-butyldimethylsilyl)-3-
-hydroxyestra-1,3,5(10)-triene (186a) (0.13g, 83%): 'Hnmr
(90MHz; CDCl\(_3\)) \( \delta \) 0.03 (6H, s, 17β-OSiMe\(_2\) CH\(_2\)'s), 0.30
(6H, s, 2-SiMe\(_2\) CH\(_2\)'s), 0.71 (3H, s, 18-CH\(_3\)), 0.87 (18H,
s, SiBu\(_3\)'s) 2.5-2.9 (2H, m, 6-CH\(_2\)), 3.45-3.75 (1H, m,
17α-H), 6.29 (1H, s, 4-CH), 7.14 (1H, s, 1-CH); Accurate
Mass (Found: m/z 500.3504 (M\(^+\), 23.62%)
\( \text{C}_{30}\text{H}_{52}\text{O}_{2}\text{Si}_{2} \)
\(-161\)
requires M$^+$. 500.35061, and 17β-(dimethyl-t-butyldimethylsilyloxy)-3-hydroxyestra-1,3,5(10)-triene (187) (0.02g, 16%);

$^1$Hnmr (90MHz, CDCl$_3$) δ 0.03 (6H, s, SiMe$_2$CH$_3$'s), 0.73 (3H, s, 18-CH$_3$), 0.90 (9H, s, SiBu$_t$CH$_3$'s), 2.6-2.9 (2H, m, 6-CH$_2$), 3.5-3.8 (1H, m, 17α-H), 6.4-7.2 (3H, m, A-ring CH's).

Acetylation of (186a) with acetic anhydride/pyridine gave 3-acetoxy-17β-(dimethyl-t-butyldimethylsilyl)estra-1,3,5(10)-triene (186b) mp 66-68°C (MeOH); [α]$_D$ +51.5° (c 1.2, CHCl$_3$), ir (neat) 1764 (C=O) cm$^{-1}$;

$^1$Hnmr (60MHz; CDCl$_3$) δ 0.03 (6H, s, 17β-OSiMe$_2$CH$_3$’s), 0.23 (6H, s, 2-SiMe$_2$CH$_3$’s), 0.72 (3H, s, 18-CH$_3$), 0.89 (18H, s, SiBu$_t$CH’s) 2.24 (3H, s, 3AcO-CH$_3$), 2.5-3.1 (2H, m, 6-CH$_2$), 3.3-3.8 (1H, m, 17α-H), 6.77 (1H, s, 4-CH), 7.38 (1H, s, 1-CH); Elemental Analysis [Found: C, 70.7; H, 10.0%; C$_{32}$H$_{54}$O$_3$Si$_2$ requires C, 70.8; H, 10.0%].

Preparation of 7-methoxy-1-methyltetralin (192) with t-BuOK and MeI.

(6-Methoxytetralin)tricarbonylchromium (169) (0.1g, 0.34mmol) in DMSO (20ml) under nitrogen was treated with t-BuOK (0.38g, 10 molar equivalents). The resultant mixture was stirred for 1 hour at ambient temperature and then treated with MeI (0.2ml, 10 molar equivalents). After stirring for a further 2 hours the reaction mixture was acidified and extracted with diethyl ether (2×). The
combined ether extracts were washed with 8% NaHCO₃ solution (2x) and water (2x), dried (MgSO₄) and decomplexed by standing in sunlight. After filtering the solvent was removed in vacuo to afford 7-methoxy-1-methyltetralin<sup>o</sup> (192) (0.059g, 100%): 'Hnmr (60MHz; CDCl₃) δ 1.27 (3H, d, J=7.5Hz, 1Me-CH₃), 1.5-2.2 (4H, m, 2-CH₂ and 3-CH₂), 2.4-3.0 (3H, m, 1-CH and 4-CH₂), 3.73 (3H, s, 7MeO-CH₃), 6.5-7.2 (3H, m, aromatic CH's). No attempt was made to purify the product further.

8.6(xv) **Preparation of 7-methoxy-1-methyltetralin (192)**

(6-Methoxytetralin)tricarboxylchromium (169) (0.1g, 0.34mmol) in dry DME (5ml) under nitrogen was treated with (Me₃Si)₂NNa (1.0M, 1.7ml, 5 molar equivalents) and stirred at ambient temperature for 1.5 hours. MeI (0.2ml, 10 molar equivalents) was added and the resultant mixture stirred for a further 1 hour. The reaction mixture was then acidified and extracted with diethyl ether (2x). The combined ether extracts were washed with 8% NaHCO₃ solution and water, dried (MgSO₄) and decomplexed by standing in sunlight. After filtering the solvent was removed in vacuo to afford a clear oil (0.058g). Examination of the 'Hnmr spectra indicated that the product was ca. 40% 6-methoxytetralin (168) and ca. 60% 7-methoxy-1-methyltetralin<sup>o</sup> (192): 'Hnmr (60MHz; CDCl₃) δ 1.27 (ca. 60%, 3H, d, J=7.5Hz, 1Me-CH₃), 1.5-2.2 (4H,
m, 2-CH$_2$ and 3-CH$_2$), 2.4-3.0 (m, benzylic protons), 3.73 (3H, s, MeO-CH$_3$), 6.5-7.2 (3H, m, aromatic CH's).

8.6(xvi) **Preparation of 7-methoxy-1-tetralone (193).**

(6-Methoxytetralin)tricarbonylchromium (169) (0.1g, 0.34mmol) in dry DME (5ml) under nitrogen was treated with (Me$_3$Si)$_2$NNa (1.0M, 1.7ml, 5 molar equivalents) and stirred for 2 hours at ambient temperature. The resultant mixture was then cooled to -20°C and treated with MoOPH (1.5g, 10 molar equivalents). After stirring for 2 hours at -20°C the acetone/dry ice bath was removed and the reaction mixture stirred overnight at ambient temperature. Subsequent reductive work-up and decomplexation as described for 8.6(vii) afforded a yellow oil (0.069g). Separation by preparative tlc on silica gel ran with ethyl acetate / petrol ether(40/60) (1:2) gave 7-methoxytetralone$^{193}$ (14mg, 24%): ir (neat) 1678 (C=O) cm$^{-1}$; $^1$Hnmr (90MHz; CDCl$_3$) $\delta$ 2.13 (2H, m, 3-CH$_2$), 2.62 (2H, t, J=6Hz, 2-CH$_2$), 2.88 (2H, t, J=5.5Hz, 4-CH$_2$), 3.71 (3H, s, 7MeO-CH$_3$), 7.02 (2H, m, 5-CH and 6-CH), 7.43 (1H, d, J=2.5Hz, 8-CH).
8.7(i) **Preparation of 2,17β-diacetoxy-3-methoxyestra-1,3,5(10)-triene (194).**

2,17β-dihydroxy-3-methoxyestra-1,3,5(10)-triene (178) (1.2g, 4.0mmol) was treated with acetic anhydride/pyridine to give 2,17β-diacetoxy-3-methoxyestra-1,3,5(10)-triene (194) (1.5g, 98%). Recrystallisation from methanol gave pure (194) (0.79g, 52%): mp 126-127°C (lit 135-136.5°C); 'Hnmr (90MHz; CDCl₃) δ 0.80 (3H, s, 18-CH₃), 2.02 (3H, s, 17βAcO-CH₃), 2.25 (3H, s, 2AcO-CH₃), 2.6-3.0 (2H, m, 6-CH₂), 3.72 (3H, s, 3MeO-CH₃), 4.5-4.8 (1H, m, 17α-H), 6.56 (1H, s, 1-CH), 6.80 (1H, s, 4-CH).

8.7(ii) **Preparation of 2,17β-diacetoxy-3-methoxyestra-1,3,5(10)-tri-en-6-one (195).**

3,5-Dimethylpyrazole (3.2g) was added in one portion to a stirred mixture of dry CrO₃ (3.37g) in dichloromethane (20ml) at -25°C. After 15 minutes, a solution of 2,17-diacetoxy-3-methoxyestra-1,3,5(10)-triene (194) (0.43g, 1.1mmol) in dichloromethane (3ml) was added to the stirred complex. The stirred reaction mixture was maintained at -15 to -20°C for 45 minutes, after which NaOH solution (4M, 20ml) was added and the resultant mixture stirred for a further 45 minutes at -5 to -10°C. The reaction was worked-up as described for 8.5(i) to afford a brown solid (0.56g). Separation by flash
chromatography eluted with diethyl ether / petrol ether (40/60) (1:1) gave 2,17β-diacetoxy-3-methoxyestra-1,3,5(10)-triene-6-one (195) (0.13g, 29%): ir (neat) 1767, 1731 (AcO), 1682 (6-C=O) cm⁻¹; 'Hnmr (90MHz; CDCl₃) δ 0.82 (3H, s, 18-CH₃), 2.05 (3H, s, 17βAcO-CH₃), 2.29 (3H, s, 2AcO-CH₃), 3.81 (3H, s, 3MeO-CH₃), 4.5-4.8 (1H, m, 6-CH₂), 6.97 (1H, s, 1-CH), 7.54 (1H, s, 4-CH), and a product tentatively assigned as 2,17β-diacetoxy-9α-hydroxy-3-methoxyestra-1,3,5(10)-triien-6-one (196) (0.12g, 26%): ir (neat) 3460 (OH), 1768, 1734 (AcO), 1684 (6-C=O) cm⁻¹; 'Hnmr (60MHz; CDCl₃) δ 0.85 (3H, s, 18-CH₃), 2.07 (3H, s, 17βAcO-CH₃), 2.35 (3H, s, 3AcO-CH₃), 3.90 (3H, s, 3MeO-CH₃), 7.23 (1H, s, 1-CH), 7.62 (1H, s, 4-CH).

8.7(111) Preparation of 2,17β-diacetoxy-3-methoxyestra-1,3,5(10)-triien-6-one tosylhydrazone (197).

2,17β-diacetoxy-3-methoxyestra-1,3,5(10)-triien-6-one (195) (0.22g, 0.55mmol) and tosylhydrazine (0.22g) in ethanol (10ml) were refluxed for 1 hour and then cooled to 0°C. The resulting precipitate was filtered to afford 2,17β-diacetoxy-3-methoxyestra-1,3,5(10)-triien-6-one tosylhydrazone (197) (0.61g, 51%): mp 216-217°C (EtOH); ir (KBr) 3212 (N-H), 1766,1732 (C=O's) cm⁻¹; 'Hnmr (60MHz; CDCl₃) δ 0.75 (3H, s, 18-CH₃), 2.05 (3H, 17βAcO-CH₃), 2.31 (3H, s, 3AcO-CH₃), 2.42 (3H, s, tosyl-CH₃), 3.85 (3H, s, 3MeO-CH₃), 4.4-5.0 (1H, m,
17α-CH), 6.91 (1H, s, 1-CH), 7.40 and 8.03 (4H, dx2, J=8Hz, tosyl-CH's), 7.55 (1H, s, 4-CH), Accurate Mass [Found: m/z 384.1936 (M--NNHSO₂C₆H₄CH₃, 10.41%) C₂₃H₂₈O₅ requires m/z 384.1937].

Hydrolysis of (197) with 2% KOH in methanol gave 2,17β-dihydroxy-3-methoxyestra-1,3,5(10)-trien-6-one tosylhydrazone (198) (0.12g, 90%): 'Hnmr (90MHz; CDCl₃) δ 0.73 (3H, s, 18-CH₃), 2.58 (3H, s, tosyl-CH₃), 3.65 (1H, m, 17α-CH), 3.83 (3H, s, 3MeO-CH₃), 6.65 (1H, s, 1-CH), 7.29 and 7.81 (4H, dx2, J=8Hz, tosyl-CH's), 7.41 (1H, s, 4-CH).
Table 3. Selected $^13$Cnmr spectral data in ppm.

<table>
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<tr>
<th>Compound</th>
<th>No.</th>
<th>(106a)$^a$</th>
<th>(107a)$^a$</th>
<th>(118a)$^a$</th>
<th>(119a)$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-3</td>
<td></td>
<td>155.1</td>
<td>154.9</td>
<td>155.2</td>
<td>155.1</td>
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<tr>
<td>C-13</td>
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<td>46.3</td>
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<td>80.3</td>
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<td>18.6</td>
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<td>79.0</td>
<td>60.3</td>
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<td>43.4</td>
<td>42.7</td>
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<table>
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<tr>
<th>Carbon No.</th>
<th>(119b)$^a$</th>
<th>(120b)$^c$</th>
<th>(121b)$^c$</th>
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</thead>
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<td>148.3</td>
<td>148.6</td>
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<tr>
<td>C-13</td>
<td>41.6</td>
<td>46.4</td>
<td>138.2, 139.2</td>
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<td></td>
<td></td>
<td></td>
<td>(C-13 and C-14)</td>
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<tr>
<td>C-17</td>
<td>73.9</td>
<td>84.2</td>
<td>52.1</td>
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<td>C-18</td>
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<td>14.3</td>
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<tr>
<td>C-21</td>
<td>64.2</td>
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<td>63.9</td>
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</table>

Solvent: a, DMSO $d_6$; b, CDCl$_3$/DMSO $d_6$; c, CDCl$_3$. 

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APPENDIX: Results of the Biological Testing

Summarised below are the results, available to date, of the tests performed by Dr. P.L.Grover and Dr. D.H.Phillips of the Chester Beatty Research Institute on the compounds submitted.

The alkylating activity of the submitted steroids was tested by applying the steroid to a silica tlc plate and spraying it with 0.2M Tris HCl buffer, pH 7.4, and 4-(p-nitrobenzyl)pyridine in ethylene glycol. The plate was then heated to 120°C for 5 minutes, cooled and sprayed with triethylamine in acetone. Alkylating agents yield a blue colouration. The 3-oxo-4β,5β-epoxides (27) and (146)-(149) all proved negative to this test. The 20,21-epoxides (106a) and (107a), however, did exhibit alkylating activity. No results are currently available for the 17β,20-epoxides (118a) and (119a) or the 6α,7α-epoxyestradiyl diacetate (150b)

The 20,21-epoxides, (106a) and (107a), were further tested for any DNA binding ability, by incubation with calf thymus DNA. However, no DNA binding was detected. The method used to detect any modified nucleotides - enzymic degradation of the incubated DNA to deoxyribonucleoside 3-monophosphates, 32P-postlabelling and tlc - would detect an adduct level of about 1 in 10^7 nucleotides.

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The mutagenicity of the 20,21-epoxides, (106a) and (107a), was also tested. However, they both proved to be negative to the Ames test and a SOS-chromotest.

The 20,21-epoxides, (106a) and (107a), and 17β,20-epoxides, (118a) and (109a), were tested by Dr. J. Traynor and Dr. P. M. Lockey of Loughborough University as part of an ongoing project to find target-selective anti-tumour agents for hormone responsive tissues. No results are currently available for the 17β,20-epoxides. The 20,21-epoxides, (106a) and (107a) were found to be toxic to both HeLaS3 cells (human cervical carcinoma) and GH3 cells (rat pituitary adenoma), and both epoxides bound to estrogen receptor sites. (106a) bound ten times more effectively than (107a). The difference in the estrogen receptor ability of the two epoxides can probably be ascribed to the epoxide group in (107a) disrupting the H-bonding of the 17β-hydroxyl to the estrogen receptor, either by steric inhibition or by H-bonding to the 17β-hydroxyl itself (see §2.3 for the preferred conformations, (135) and (136), of (106a) and (107a)). Cytotoxicity and a good affinity for estrogen binding sites, are both properties required for a potentially selective antitumor drug for estrogen dependent tumours.
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111. Work performed by Dr. S. Venitt, Institute of Cancer Research, Royal Cancer Hospital.

