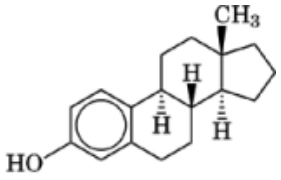
Kirk-Othmer Encyclopedia of Chemical Technology.

HORMONES, ESTROGENS AND ANTIESTROGENS

Estrogens are a group of naturally occurring steroid sex hormones which are characterized by their ability to induce estrus in the female mammal. They are derivatives of the planar tetracyclic structure estra-1,3,5(10)-trien-3-ol [53-63-4]**(1)**, and the three principal estrogens in humans are estrone [56-16-7] (E_1) **(2)**, estradiol [50-28-2] (E_2) **(3)**, and estriol [50-27-1] (E_3) **(4)**.



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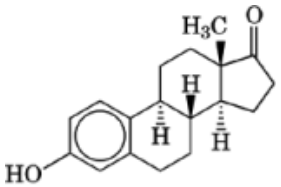

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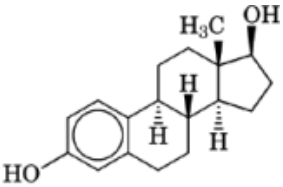

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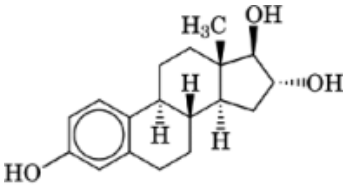

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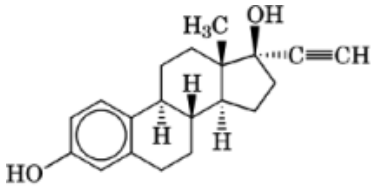
The two synthetic steroidal estrogens which have attained the greatest degree of therapeutic use are ethinyl estradiol [57-63-6] (EE) **(5)** and its 3-methyl ether, mestranol [72-33-3]**(6)**. In contrast to the naturally occurring estrone derivatives, these acetylenic analogues are orally active and are the main estrogenic components of combination oral contraceptives (see Contraceptives) and certain estrogen replacement products.



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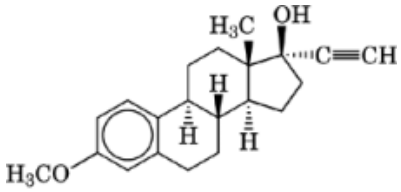

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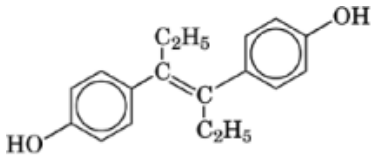
Diethylstilbestrol [56-53-1] (DES)**(7)**, which was first synthesized in the 1930s, is the most widely studied nonsteroidal estrogen and has been extensively reviewed. It is an extremely potent estrogen, possessing four times the oral potency of estradiol **(3)**, but carcinogenicity problems have limited its use.



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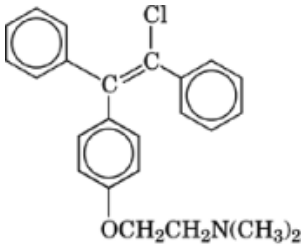
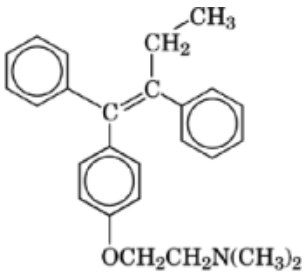
In the 1980s little progress occurred in the introduction of new estrogens, either steroidal or nonsteroidal, into human medicine. However, there has been a resurgence of interest in the development of novel steroidal estrogens for specific medical uses in the 1990s and a significant advancement in the understanding of the mechanism of estrogen action at the molecular level. For instance, the estrogen receptor has been characterized as consisting of three major domains, one of them being a DNA-binding domain which specifically binds to estrogen response elements in the target genes. The estrogen receptor will be discussed in more detail in the pharmacology section of this article. Further elucidation of the nature of this steroid-receptor complex will supply the groundwork for the development of new estrogens with unique and tissue-specific therapeutic effects via a structure-based approach to their design and discovery.

For the purposes of this article, antiestrogens are compounds that counteract the biological activity of estrogens at the receptor level. In the late 1970s, there were no steroidal antiestrogens in widespread clinical use. Clomiphene [911-45-5]**(8)** and tamoxifen [10540-29-1]**(9)** were nonsteroidal antiestrogens that had been employed for the treatment of female infertility and breast cancer, respectively.



(**8**)



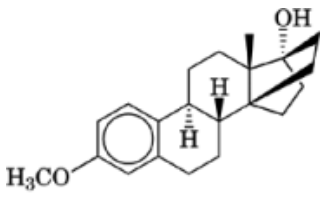
(**9**)

This article highlights the progress in the development of steroidal antiestrogens such as ICI 182780, compounds with greatly reduced partial estrogenic activity, and selected nonsteroidal antiestrogens classified as triarylethylenes (TAEs), chromenes, benzofurans, benzothiophenes, carbocyclic TAEs, diphenylmethanes, diphenylethanes, and indoles. Representative studies which have attempted to delineate the mechanism of action of estrogens and antiestrogens based on binding studies, molecular modeling strategies, or the like have also been highlighted. An overview of the pharmacology of estrogens is presented with a particular emphasis on receptor-mediated molecular events. Therapeutically, estrogens are used for contraception, hormone replacement therapy, and chemotherapy.

1. Chemistry

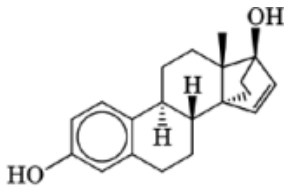
1.0.1. Steroidal and Nonsteroidal Estrogens.

Modification of the basic steroid skeleton and the nature of the functional groups in the B, C, and D rings while maintaining the phenolic A-ring has continued to be a primary approach in the development of new estrogens with unique biological profiles.



(**10**)

A series of patents from Schering AG has described the synthesis of various 14–17 α- and β-ethano-bridged estratriene compounds represented by compounds [116229-12-0] **(10)**, [130693-81-1] **(11)**, and [135768-83-1] **(12)**. All are reported to be potent estrogens (1–3). A series of 17-halomethylene estratrienes represented by compound [123651-64-9] **(13)** have also been reported by Schering AG to be potent estrogens (4).



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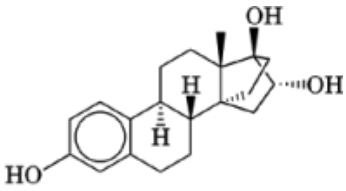

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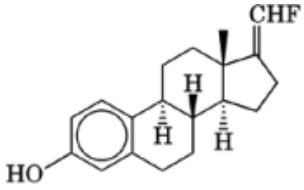

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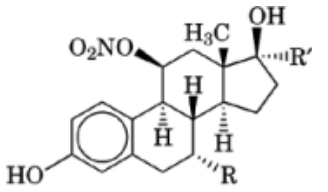
1.0.2. 11β-Nitrate Esters

It was discovered in the late 1930s that introduction of a 17α-alkynyl group in estradiol gave orally active estrogens such as EE (ethinyl estradiol), which have been widely employed with synthetic progestins (pharmaceutical agents which have effects similar to progesterone) in oral contraceptives. The 17-alkynyl group is more resistant to metabolism in the liver than are the naturally occurring estrogens, and attention has refocused on the search for new orally active super estrogens which could be used at lower doses and theoretically reduce the metabolic burden. As was the case with the hormone antagonists, introduction of functionality at the 11-position of the estrane skeleton has resulted in the generation of orally active potent estrogen agonists. Agonists are drugs that stimulate activity at cell receptors normally stimulated by naturally occurring substances. Various estrane derivatives have been converted with ceric ammonium nitrate selectively and efficiently to the corresponding 9α,11β-hydroxy nitrate esters which were then deoxygenated at C-9 with triethylsilane—boron trifluoride etherate to yield the desired 11β-nitrate esters (5); standard transformations then gave the 7α-methyl target compounds such as **(14)**. The results of uterotropic and post-coital antifertility activities in rats and estrogen withdrawal bleeding studies in monkeys for a similar series of estradiol analogues and ethinyl estradiol analogues were determined (6). The estrogenic potency in rats of the estradiol derivatives CDB-3280 and CDB-1357 [108887-25-8] when taken orally were 0.63 and 14.41 times the potency of EE **(5)**, respectively, whereas the corresponding ethinyl estradiol derivatives,

CDB-3294 and CDB-3322, were surprisingly only 5.09 and 2.0 times the potency of EE, respectively. Post-coital activity in the rat was on the same order as estrogenic potency, with the order being CDB-1357 (ED_{100} = (5 μg/kg)/d for each of5days) > CDB − 3294(ED_{100} = (< 10 μg/kg)/d for each of 5 days) > CDB − 3322(ED_{100} = (< 20 μg/kg)/d for each of 5 days) > CDB − 3280(ED_{100} = (100 μg/kg)/d for each of 5 days).



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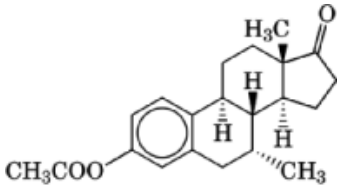
C-11 functionalized estrone derivatives are synthetically available by the following process (5): treatment of 7α-methylestrone acetate [36014-09-2]**(15)** with four equivalents of ceric ammonium nitrate in 90% acetic acid provides the 9α-hydroxy-11β-nitrate ester in good yield. Subsequent deoxygenation of the C-9 benzylic position of the nitrate ester with retention of configuration is effected utilizing triethylsilane and boron trifluoride etherate to produce compound **(16)**. Reduction with $NaBH_4$ affords the key target **(14)**. A nearly identical approach has been used to prepare **(16)** which was converted to **(14)** and also ethinylated at C-17 with acetylene and potassium *t*-butoxide to give CDB-3322 (6).



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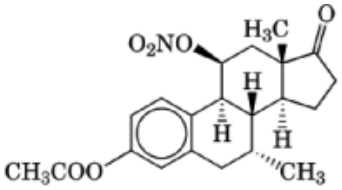
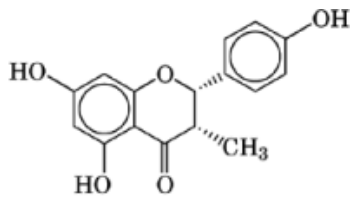


(16)

WS-7528 [132147-69-4]**(17)**, a nonsteroidal estrogen, is an isoflavone which has been isolated from *Streptomyces sp. No. 7528* and is an estrogen agonist. It inhibits [3*H*]-estradiol binding to its receptor in rat uterine cytosol at an inhibitor for 50% of the rats tested (IC_{50}) concentration of 5.7 n*M*. It also induces the growth of estrogen-dependent human breast cancer cell line MCF-7 (7).



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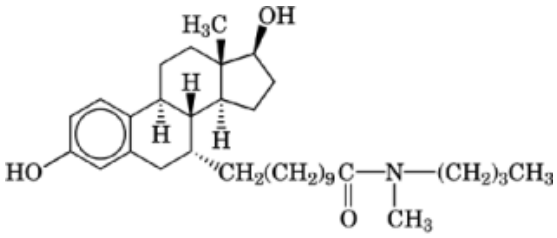
2. Steroidal Antiestrogens

The balance of estrogenic and antiestrogenic activity expressed by the nonsteroidal antiestrogens varies widely across species, target organs, cells, and genes, depending on which indicator of response is measured (8). Tamoxifen**(9)**, Nolvadex (ICI 46474), is a synthetic nonsteroidal anti-estrogen which has been used for the control of hormone-sensitive breast cancer (see Chemotherapeutics, anticancer). However, tamoxifen is a partial estrogen agonist and as such as estrogen-like stimulatory activity on the uterus, vagina, mammary glands, and the pituitary-ovarian axis in animals. Attempts to synthesize nonsteroidal antiestrogens devoid of partial estrogen agonist activity have met with limited success. The emphasis has returned to the synthesis of steroidal antiestrogens. The ICI compounds ICI 164384 [98007-99-9]**(18)** and ICI 182780 [129453-61-8]**(19)** are examples of "pure" antagonists.



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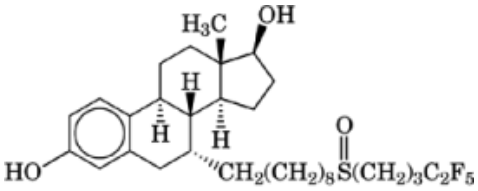
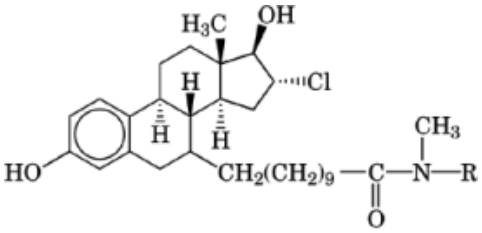


(19)

ICI 164384 is an antiestrogenic agent with a greater affinity for the rat uterus estrogen receptor than tamoxifen (0.19 and 0.025, respectively, relative to estradiol = 1), which dose-dependently inhibits estradiol-induced growth of ZR-71-1 human breast cancer cells *in vitro;* it is devoid of estrogenic activity in the rat and mouse uterus (9). Further exploration of the structure-activity relationships of the 7α-alkylamide analogues of 17β-estradiol led to the synthesis of ICI 182780, a compound with a fivefold increase of intrinsic potency compared to ICI 164384, measured by receptor binding and cell growth inhibition in human breast cancer cells.

More importantly, ICI 182780 is 10-fold more potent *in vivo* and represents a candidate for further clinical evaluation (10). Biological activity in this series is confined to the 7α-isomers (11, 12).

Based on the potent antagonist activity of the above ICI compounds and the observations that halogenation of the 16α-position of the D-ring in the steroid nucleus often leads to compounds with increased affinity for the estrogen receptor, a series of 7α-undecanamide-substituted 17β-estradiols with 16-halogen substituents were synthesized (13, 14). These 16-halo-7-alkylamide antiestrogens, characterized by EM-139 [131811-54-6]**(20)** (13) and EM-170 [131811-55-7] (**21**, R = $n-$ butyl) (14), demonstrated potent and pure antagonistic activity *in vivo* in screens where tamoxifen exhibited estrogenic activity and was only a weak partial antiestrogen.



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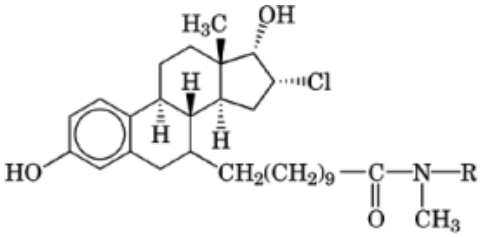


(21)

The synthesis of EM-139 and related 16α-halosteroids has been carried out utilizing an enol acetate (22) as a key intermediate; synthesis of (22) is shown in Figure 1. Stereospecific chlorination of (22) is effected with *tert*-butyl hypochlorite in acetone buffered with sodium acetate, acetic acid, and water to yield the 16α-chloro ketone amide. Reduction of the 17-keto group with lithium aluminum hydride gives a mixture of epimers from which EM-139 **(20)** is isolated by chromatography. In addition, the enol diacetate amide (22) is stereospecifically brominated with bromine in acetic acid to give the corresponding 16α-bromo ketone amide. The 16α-iodo analogue is synthesized from the above bromo derivative by treatment with sodium iodide in refluxing butanone under equilibrium conditions.

Related 7-substituted 19-norsteroids have appeared in the patent literature as potential antiestrogens including the 14α,17α-ethanoestratriene [134514-24-2] **(25)** (Schering AG) and the 7-dimethylaminoethoxyphenyl analogue [119286-92-9] **(26)** (Roussel-Uclaf). Compound **(25)** is reported to be antiestrogenic without the partial agonistic effects when given orally. It inhibited an estradiol-induced increase in uterine weight by 53 and 92%, respectively, and vaginal weight by 13 and 92% when administered at 3 and 30 (mg/kg)/d to adult ovariectomized rats (15). Compound **(26)** is a potent inhibitor of the growth of MCF-7 mammary tumor cells (IC_{50} = 0.1 nM) and thus has potential utility on hormone-dependent carcinomas (16).

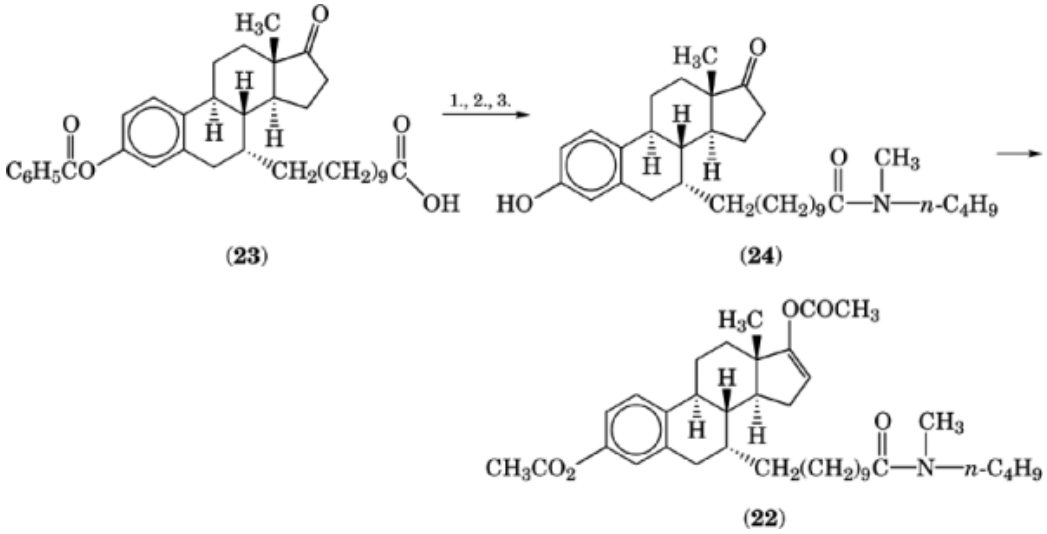
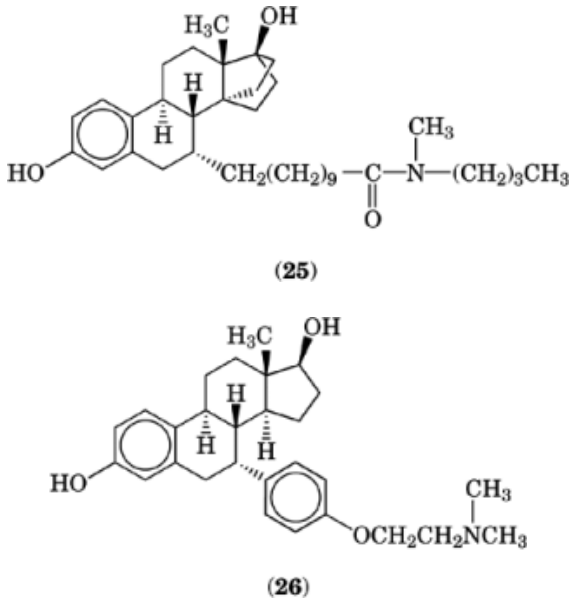
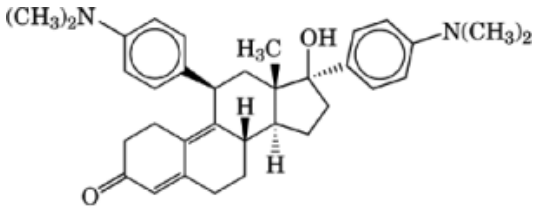


Fig. 1. Treatment of 11-[3′-(benzoyloxy)-17′-oxoestra-1′,3′,5′(10′)-trien-7′ α-yl]undecanoic acid [55592-11-5] (**23**) in $CH_2Cl_2/(C_4H_9)_3N$ with 1. isobutyl chloroformate followed by 2. methylbutylamine gave the protected *N*-butyl-*N*-methyl undecanamide which was 3. deprotected with aqueous NaOH to give the estrone derivative [98013-89-9] (**24**). The enol diacetate amide [131811-72-8] (**22**) was then prepared under standard conditions with isopropenyl acetate and *p*-toluenesulfonic acid.



The 11-position of estradiol analogues has been a fruitful site of exploration in the development of hormone antagonists, eg, the antiprogestin RU 486 (see Contraceptives). The Roussel group has also uncovered novel anti-estrogens by investigating various substituents at the 11β-position. The bisdimethylaminophenyl compound [123955-65-7] **(27)** and the phenoxyoctanamide [134411-59-9] **(28)** are examples of such an approach. The dimethylaminophenyl analogue **(27)** is also, like **(26)**, effective against MCF-7 mammary tumor cells, but

is much less potent (IC_{50} = 500 nM) (17). The importance of the long alkyl side chain for potency is evident as compound **(28)** (IC_{50} = 0.006 nM) is extremely potent and has potential use in a variety of indications (18).



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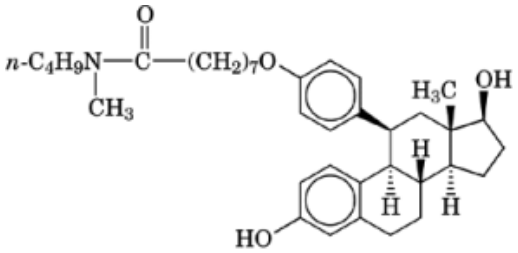

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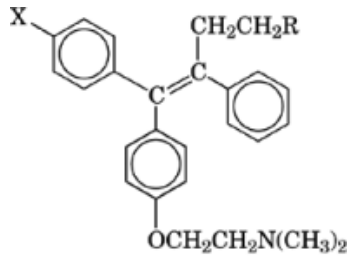
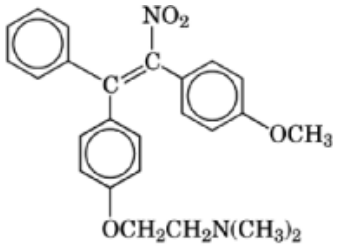
2.0.3. Nonsteroidal Antiestrogens

Clomiphene **(8)** and tamoxifen **(9)** which have been reviewed previously, are prime examples of nonsteroidal antiestrogens which have gained some degree of medical use in the treatment of female infertility and breast cancer, respectively (see Chemotherapeutics, anticancer). However, the first generation of nonsteroidal antiestrogens all demonstrated some degree of agonist activity. Synthetic nonsteroidal antiestrogens are bound intracellularly by two high affinity saturable binding sites, the estrogen receptor and the antiestrogen-binding site (AEBS) (19, 20). The physiological significance of the AEBS(s) is still an open question. The search for compounds with improved specificity for each of these receptors has continued and representative examples of selected structural types are listed below. The first structural type is triarylethylenes (TAEs). Toremifene [89778-26-7]**(29)** is a chlorinated analogue of tamoxifen which is indicated for the treatment of post-menopausal breast cancer (21).



(9), R = X = H
(29), R = Cl; X = H
(30), R = H; X = I
(31), R = H; X = Br



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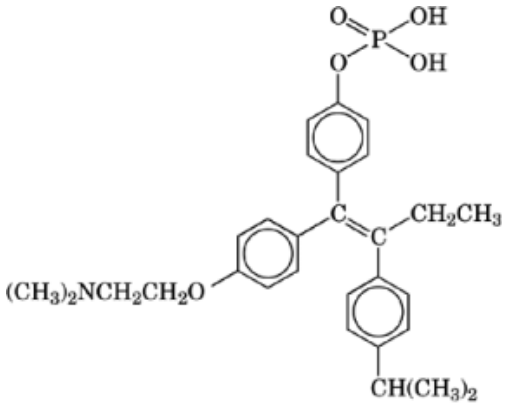
4-Iodotamoxifen [116057-66-0]**(30)** and 4-bromotamoxifen [116057-69-3]**(31)** have been reported in the patent literature (22) as more potent anti-estrogens than tamoxifen. When the iodine is radioisotopic, compound **(30)** has potential utility for radiotherapy of breast cancer or as an imaging agent for diagnosis. However, its relatively low affinity for the estrogen receptor limits its practical usefulness. Nitromifene [76313-96-7] (CI 628) **(32)** (23) is the prototype of a series of nitrostilbenes with antiestrogen activity. Structure–activity relationship studies of a series of 32 nitromifene analogues indicated that replacement of the 4-OCH_3 with a 3-CF_3 moiety gave the analogue of highest affinity for the AEBS in both rat liver and MCF-7 human breast cancer cell preparations (24). Metabolites resulting from transformation of the pyrrolidine ring and of the nitro group were synthesized and the estrogen receptor affinities and estrogen agonist and antagonist properties evaluated vs nitromifene (25). Additional TAEs which have been reported are **(33)–(36)**. Compound **(33)** is TAT 59 [115767-74-3] (26). Compounds **(34)** [109517-76-2], **(35)** [115767-77-6], and (36) [129612-87-9] are from Taiho (27–29). Compound (36) is structure **(33)** wherein the phosphate has been hydrolyzed to the phenolic OH.



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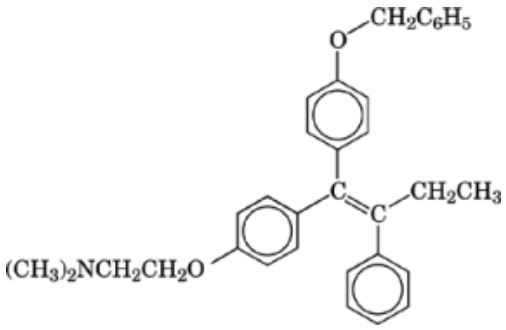

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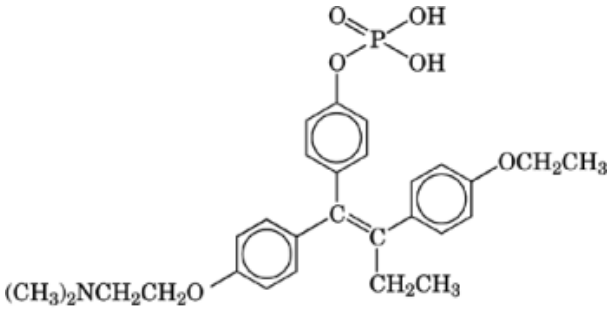
A structurally related series of phenylhydrazones resulted in the selection of compound A-007 [2675-35-6] (DEKK-TEC)**(37)** for the treatment of hormone-dependent tumors. A-007 is an antiestrogen that, in contrast to tamoxifen, demonstrated inhibitory activity both in the presence and absence of estradiol in ZR-75-1 estrogen-dependent human breast cancer cells, and afforded more protection than tamoxifen in the 7,12-dimethylbenz[*a*]anthracene [57-97-6] (DMBA) rat breast cancer model (30).



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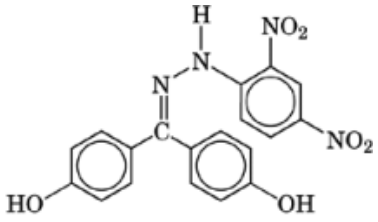
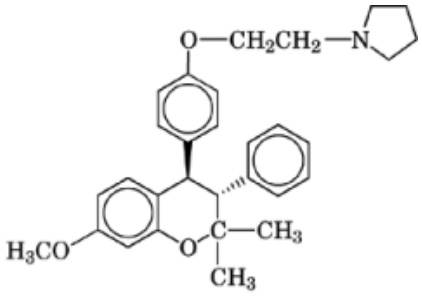


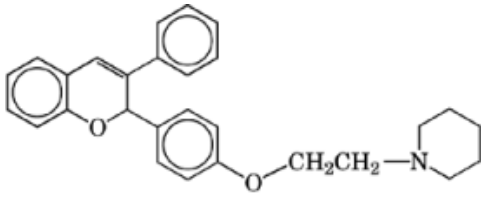
(37)

Another structural type is chromenes. Centchroman [31477-60-8]**(38)** is a pyrrolidinoethoxyphenyl chromane which is a potent antiestrogen with weak estrogenic activity. In India, it is used as a weekly contraceptive pill based on its reported ability to inhibit the uterine preparation for the attachment of the fertilized ova to the wall of the uterus (see Contraceptives) (31).



(38)

Related benzopyran derivatives include the compound CDRI-85/287 [130064-18-5]**(39)** from the Central Drug Research Institute (32). Analogues such as the pyrrolidinoethoxyphenyl have also been evaluated (33). An alternative series of basic ethers of 3-(*p*-halophenyl)-4-arylchrom-3-enes (**40**, X = F [128040-44-8], Cl, Br), has been synthesized and all found to be selective ligands for AEBS *in vitro* (34).



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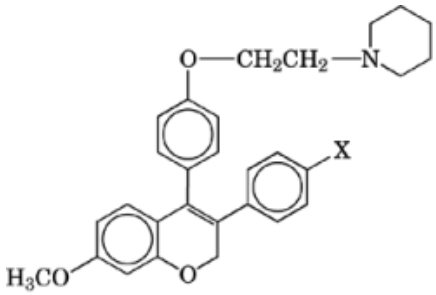
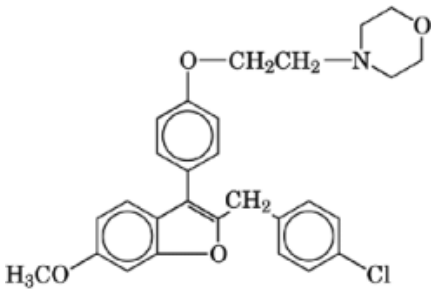
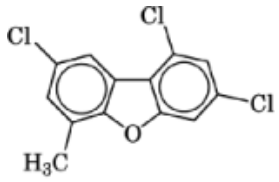


(40)

In an extension of the initial studies with chromenes related to **(40)**, the synthesis of the five-membered benzofuran ring analogues such as the morpholino derivative **(41)** [139276-13-4] was carried out (34). In general, this series of benzofurans are ligands for AEBS and display no significant interaction with the estrogen receptor. Based on a comparison with a number of other reported antiestrogens, the high binding affinity for AEBS is postulated to be attributable to the following structural features: (*1*) the presence of a triarylethylene moiety in a fixed heterocyclic oxygen ring system, (*2*) the presence of an (alkylamino)-ethoxy side chain at the 4′-position of the 3-phenyl ring, and (*3*) the presence of an additional methylene group at the C-2 position of the benzofuran ring.



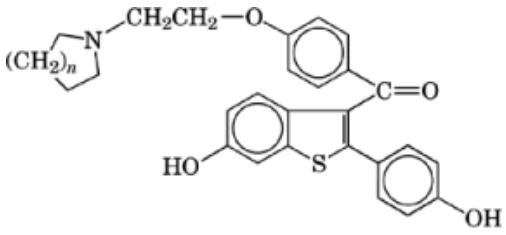
(**41**)



(**42**)

1,3,8-Trichloro-6-methyldibenzofuran [118174-38-2] (MCDF)**(42)** has been described as an antiestrogen which induces a broad spectrum of antiestrogenic responses in the female rat and in human breast cancer cells which are mediated through the aryl hydrocarbon (Ah) receptor. In contrast to tamoxifen, MCDF does not bind directly to the estrogen receptor. Neither tamoxifen or 17β-estradiol bind to the Ah receptor. 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin [1746-01-6] (TCDD) was the initial lead in this structural series. However, although MCDF is 500 times less potent than TCDD as an antiestrogen, it is 10,000 to 100,000 times less toxic than TCDD for the traditional Ah receptor-mediated toxic responses (35).

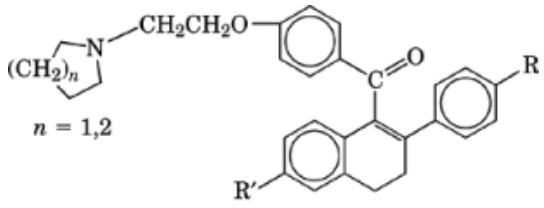
A series of benzothiophenes characterized by LY 117018 [63676-25-5] (**43**, $n = 1$) and LY 156758 [82640-04-8] (**43**, $n = 2$) was synthesized with the aim of producing estrogen antagonists with minimal intrinsic estrogenicity using trioxifene [63619-84-1] (**44**, $n = 1$, R = OCH_3, R′ = H) as the structural prototype (20, 36). Each of these benzothiophenes has an affinity for the estrogen receptor approximately equal to estradiol and approximately 100 times that of tamoxifen. In addition, LY 117018 was 100–1000 times as potent as tamoxifen in suppressing MCF-7 human breast cancer cell growth (see Chemotherapeutics, anticancer) and neither compound induced characteristics in these cells believed to be linked to residual estrogenicity.



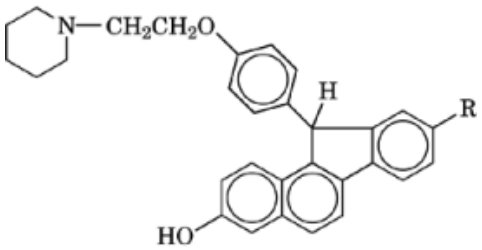
(**43**)

The results on the chromenes and benzothiopenes led to a further extension of the structure—activity relationship of the carbocyclic prototype trioxifene. As a class, these are called carbocyclic triarylethylenes. Dihydronaphthalene derivatives with a hydroxy moiety at R′ and the corresponding benzo[*a*]fluorenes ([138666-

17-8], R = H; [138630-67-8], R = OH) **(45)** were synthesized and evaluated for their antiestrogenic properties (37). Both the dihydronaphthalenes and the benzo[*a*]fluorenes had potent antiproliferative effects on MCF-7 cells in culture, but their binding to estrogen receptors was similar to that of other TAEs. The inhibitory effects of (**44**, R = H , OH, or OCH_3; R′ = H or OH) and (**45**, R = H or OH) could not be reversed completely by estradiol, indicating interaction with receptors other than the estrogen receptor or a nonspecific toxic effect. Studies of these analogues with calmodulin, a putative target for TAEs, indicated only a weak interaction.

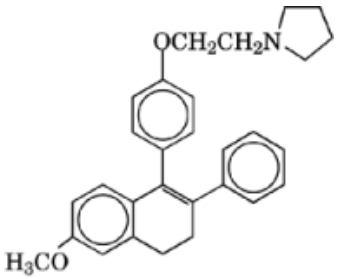


(44)



(45)

The synthesis of compounds of general structure **(44)** and **(45)** is carried out as shown in Figures 2 and 3. Nafoxidine [1845-11-0]**(52)** (38), the prototype dihydronaphthalene lacking an acyl group at C-1, was one of the first compounds found to have higher affinity at the AEBS than at the estrogen receptor (20).



(52)

Another group of antiestrogens are diphenylmethanes and diphenyl-ethanes. CGS-20267 [112809-51-5] (**53**, Ciba-Geigy) is a potent aromatase inhibitor (IC_{50} = 11 n*M*) with potential utility in the treatment of estrogen-dependent disease; it was derived from CGS-16949 A [102676-96-0] (fadrozole HCl)**(54)** which is in clinical trials for breast cancer (39). Compounds **(53)** and **(54)** most likely exert their antagonist effects by inhibition of estrogen biosynthesis. D-18954 [96826-17-4]**(55)** is reported to be an antineoplastic agent which inhibits DMBA-induced mammary carcinoma in rats (74% remission at 5 mg/kg) and is active on

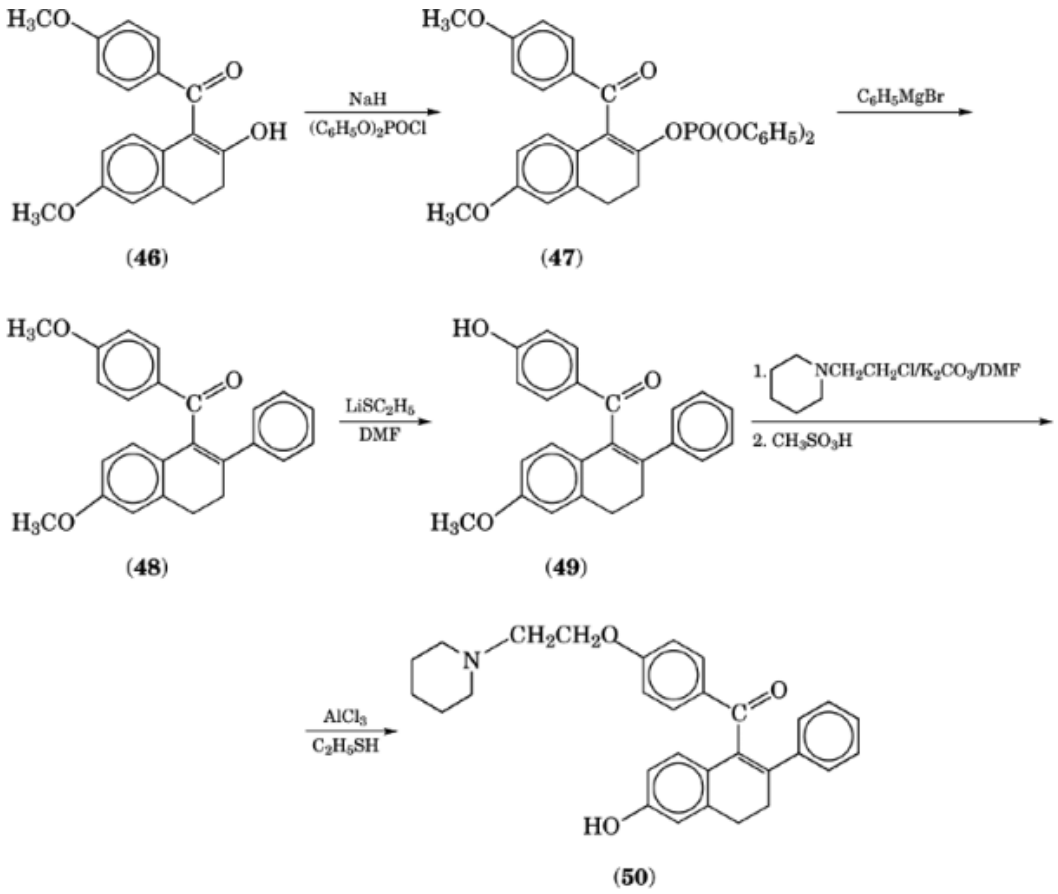


Fig. 2. The highly enolized diketone [138630-68-9] (**46**) is synthesized by acylation of the sodium salt of 6-methoxy-2-tetralone. The enol is then converted to the enol phosphate, tentatively assigned structure [138630-69-0] (**47**), with sodium hydride followed by diphenyl chlorophosphate. Addition of Grignard reagents, eg, phenylmagnesium bromide, to (**47**) gives dihydronaphthalene [138630-70-3] (**48**). Compound (**48**) is selectively demethylated with lithium thioethylate/DMF to remove the methoxy group para to the carbonyl group in good yield, producing monophenol [138630-73-6] (**49**). The basic piperidinylethoxy amine side chain is introduced using conventional conditions, and the remaining methoxy group is cleaved by the use of aluminum trichloride—ethyl mercaptan to give dihydronaphthalenes of general structure [138630-65-6] (**50**).

transplantable Dunning R 3327 prostate carcinoma of rats owing to its antiestrogenic activity (40). The *gem*-dichlorocyclopropane [126987-63-1] **(56)** was selected from a series of cyclopropane analogues which again demonstrated greater antitumor activity when compared to tamoxifen in MCF-7 human breast cell proliferation assays *in vitro* (see Chemotherapeutics, anticancer) (41).

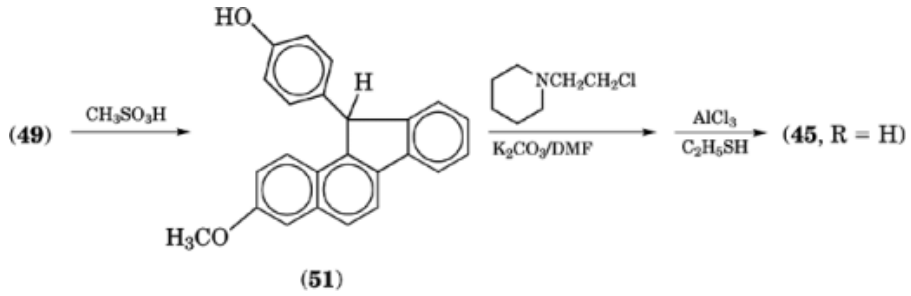
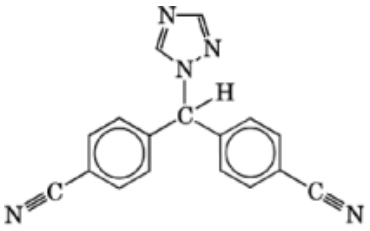
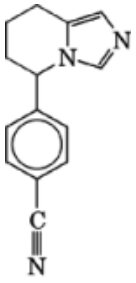
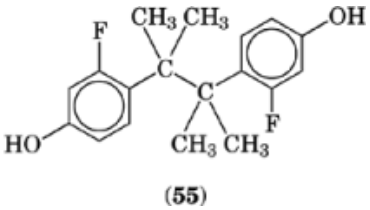
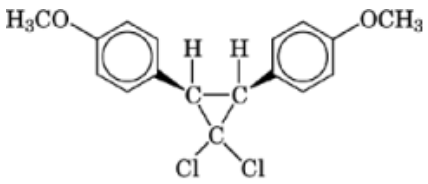


Fig. 3. After exposure of 1-acyl-2-aryl-3,4-dihydronaphthalene derivatives of structure (**49**) to strong acidic conditions, cyclization of the 2-aryl moiety onto the carbonyl group occurs rapidly and is accompanied by rapid dehydration of the carbinol intermediate to provide the 11-aryl-11*H*-benzo[*a*]fluorene compounds of structure [138630-78-1] (**51**). The corresponding analogues containing the aminoethoxy side chain [138630-80-5], $C_{31}H_{31}NO_2$, are then prepared by treatment with *N*-(2-chloroethyl)piperidine $_{HCl/K_2CO_3}$ in refluxing DMF. Cleavage of the remaining methoxy group is again accomplished with aluminum trichloride–ethyl mercaptan, as described in Figure 2.



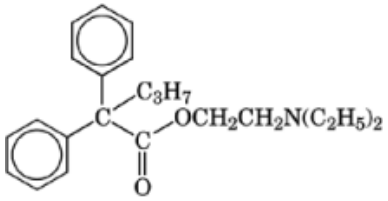




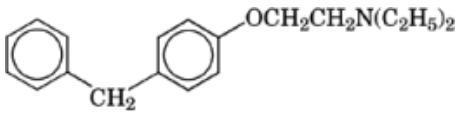


(**56**)

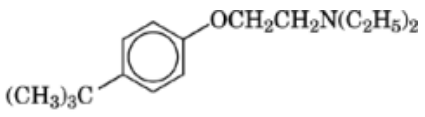
Other compounds of this general class which have been found to have antiestrogenic properties include the cytochrome P-450 inhibitor, SKF 525A [302-33-0]**(57)** (24); *N, N*-diethyl-2-[(4-phenylmethyl)phenoxy]ethanamine [98774-23-3] (DPPE)**(58)** (42); *t*-Butylphenoxyethyl diethylamine [57586-10-4] (BPEA)**(59)** (43); and cyclofenil [110042-18-7] (**60**, R = C_2H_5) (24) analogues.



(**57**)

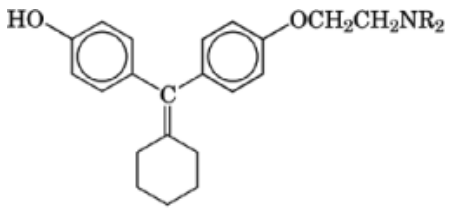


(**58**)

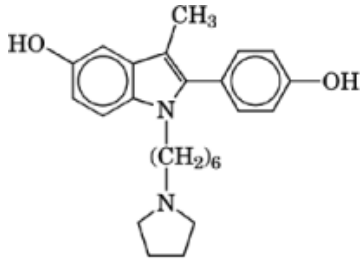


(**59**)

ZK-119010 [127457-69-8] (Schering AG) **(61)**, a pyrrolidinohexyl indole derivative, is the prototype from this series of antiestrogens, with high affinity for and competitive inhibition of calf uterus [3*H*]-17β-estradiol receptors. ZK-119010 **(61)** inhibits hormone-related tumor growth, especially of human mammary carcinoma cells (MCF-7) and was reported to be superior to ICI-164384 **(18)** and tamoxifen **(9)** in antiuterine potency when evaluated in rats and mice (44). The indolo–isoquinoline [123312-72-5] **(62)** is reported to be a DNA-intercalating agent with high affinity for steroid hormone receptors and has anticancer properties (45).



(60)



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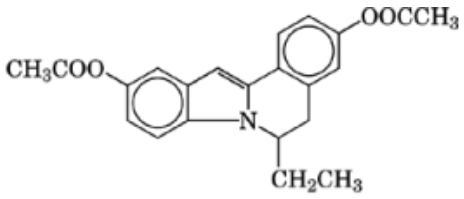

(62)

The area of nonsteroidal antiestrogens along with other classes of nonsteroidal antagonists of sex-steroid hormone action has been reviewed to 1986, and these compounds have been grouped by chemical structure as a basis of classification rather than any biochemical or biological test system utilized to assess antagonist activity (46).

3. Modeling and Crystallographic Studies of Estrogen Agonists and Antagonists

There have been a number of studies that have attempted to describe the mechanism of action of estrogen agonists and antagonists at the molecular level based on binding studies, molecular modeling strategies, conformational analyses, and similar strategies. This is an area where limited progress has been achieved owing primarily to the lack of potent specific antagonists which totally block the action of endogenous and exogenous steroids.

The nature of inducible hormone effects and ligand structure remains of intense interest as the relationship between ligand–receptor binding and subsequent post-binding events such as induction of gene expression continues to be explored. One of the models proposed for the mechanism of estrogen and antiestrogen action is based on the structure of the human estrogen receptor (ER) cloned from complementary DNA (cDNA) libraries prepared from the MCF-7 breast cancer cell line which encodes a 595 amino acid protein of 66 kiloDaltons (47, 48). In this model, estrogen promotes dimerization of the ER which binds tightly to the estrogen response

element (ERE), resulting in transcription via TAF-2, a ligand-inducible transcriptional activating factor and TAF-1, a constitutive transcriptional activating factor. Tamoxifen and other related antiestrogens promote dimerization and DNA binding and appear to act only through TAF-1, whereas "pure" steroidal antiestrogens such as ICI 164384 **(18)** activate neither TAF-1 nor TAF-2. The interaction of tamoxifen **(9)** with the calcium-binding protein calmodulin was investigated by using computerized molecular modeling methods (49). In addition, six analogues of tamoxifen were also examined, exploring various portions of the molecule. These analogues included side-chain variants (diethylamino, piperazino, and pyrrolidino in place of dimethylamino), introduction of aromatic substitution in the 4-position in the phenyl ring cis to the ethyl substituent (iodo and hydroxyl), and replacement of the ethyl with a methyl group. The interaction models for tamoxifen with calmodulin support the observations that were obtained experimentally, which indicated that the ethyl group is essential for potency. The model also predicts the poor binding properties of the piperazino and C-methyl analogues; however, it does not account for the superior binding affinity of the iodo analogue which is observed experimentally. X-ray crystallographic data on estradiol, tamoxifen, and other estrogens and antiestrogens, combined with energy minimization of the observed crystal conformations, suggested that estrogen receptor binding is primarily the result of a tight fit only at the A-ring of the steroid. The most potent antagonists have phenolic rings capable of promoting binding but do not elicit a response due to a lack of the appropriate functional group elsewhere in the molecule (ie, hydrogen bond donor) or are sterically hindered in some way to prevent events subsequent to binding from taking place (50, 51). Conformational analyses on a recently reported estrogen, bis(4-hydroxphenyl) [2-phenoxysulfonyl)-phenyl] methane, and related analogues showed that the spatial orientation of the phenyl sulfonate ring was a critically important determinant of receptor binding (52).

4. Pharmacology

4.0.4. Mechanism of Estrogen Action

4.0.4.1. Estrogen Receptor and Receptor-Mediated Molecular Events. Estrogen molecules are lipophilic and diffuse through the plasma membrane of all cells. The steroid ligands encounter their specific receptors only within target cells. In cells lacking estrogen receptors, estrogens are not readily retained and exit the cell. Estrogen receptors appear in target tissues before ovary maturation (53, 54), and the concentration of estrogen receptor in the uterus correlates with the level of estrogen in the blood (55). The number of estrogen-binding sites in the uterus changes during the menstrual cycle from a minimum of 1000 sites per cell during estrus (ovulation), increasing to 3500 sites per cell and reaching a maximum at proestrus, which is immediately prior to the next cycle, of 5000 sites per cell. It has been reported that aging is associated with a decrease of estrogen receptor concentration (56). However, there is no decrease in binding affinity for estrogens. The affinity, specificity, and large concentration of estrogen receptors in cells allow a low concentration of estrogen to produce biological responses (57).

The estrogen receptors are large protein molecules that are mainly localized in the cell nucleus (58). The receptor contains three principal domains: an estrogen-binding site at the C-terminal region, a DNA-binding domain in the middle of the protein molecule which is capable of binding to specific regions within the target genes called estrogen response elements, and a modulating domain at the N-terminus (59). Recent evidence showed that the estrogen receptor is bound to specific estrogen response elements of a variety of genes with or without estrogen (60).

Estrogen exerts hormonal effects by first binding with high affinity to the receptor to form an estrogen receptor complex. The hormone binding then induces conformational changes in the steroid-binding domain and other domains of the receptor, a process which is referred to as receptor activation or transformation. The transformed or activated estrogen receptor complex alters the interaction with target genes, leading to an

increase of the affinity to DNA and other nuclear components, such as the nuclear matrix, nuclear proteins, as well as alteration of structure of a complex of nucleic acids and proteins which surround the genes. As a result, estrogen induces changes in the activity of transcription machinery associated with the target genes, which in turn modulates the expression of target genes and further regulates cell function, growth, or differentiation. Reviews with more detailed references on this topic have been published (61–64).

4.0.4.2. Cell- or Tissue-Specific Effects of Estrogen.. Out of the trillions of cells in the human body, only special cell types or tissues elicit biochemical or physiologic responses to estrogen. These target cells possess estrogen receptors, although the receptor concentration varies between cell types. The responses to estrogen of target cells are also divergent. The mechanism for the cell- or tissue-specificity of estrogen response is still under active investigation. However, several elements are considered to modulate the nature of cell- or tissue-specific effects of estrogen following estrogen receptor binding, eg, specific target genes and the associated gene network in each type of cell; cell-specific regulating factors for transcription of the genes, such as regulatory sequences, transcriptional factors, and local chromatin conformation of the genes and nuclear matrix; and the relative response of the cell to other synergistic molecules of estrogen action (64, 65).

4.0.4.3. Current Concept of Estrogen Agonist, Antagonist, and Cell- or Tissue-Specific Response of Estrogen. The presence of estrogen-receptor and estrogen-target genes containing estrogen response elements as well as appropriate transcription machinery determine the cell or tissue's competence to respond to estrogen. Factors such as the cell- or tissue-specific array of target genes, the associated transcription regulatory factors, the chromatin structure, and nuclear matrix surrounding the genes allow different target cells to elicit diverse cell-specific responses to a certain ligand. The molecular features of different ligands affect the stability of ligand-receptor binding and the interaction of ligand-receptor complex with DNA and transcription machinery which in turn governs the nature of induced estrogen responses, ie, active or latent, strong or weak, being agonist in one tissue and being antagonist in another tissue (10, 66–68).

4.0.5. Biosynthesis

Natural estrogens are produced by steroidogenesis in various tissues. The ovary is the primary source of the hormone in nonpregnant women (69). Estradiol**(3)** is the most potent and primary product of the ovary, although the organ also produces estrone**(2)**. The estrogens are ultimately formed from either androstenedione or testosterone as immediate precursors. The key reaction is the aromatization of the A-ring to yield a phenolic hydroxyl at C-3 (70). Pathological conditions, such as hirsutism and virilism, are thought to be caused by a defect of the aromatization reaction. During pregnancy the placenta produces large amounts of estrogens, especially estriol **(4)**. Other tissues such as liver, adipose tissue, skeleton muscle, and hypothalamus are also sources of estrogens where androgens are converted to estrone (71). In post-menopausal women, peripheral aromatization of adrenal androgens to estrone is the principal source of estrogen (72). Because significant extraglandular estrogen production occurs in adipose tissue, estrogen production is greater in obese than in thin post-menopausal women, and total estrogen production in the massively obese may be as great as, or greater than, in premenopausal women (73).

4.0.6. Metabolism and Distribution

Estrogens are readily absorbed through the gastrointestinal (GI) tract. During this process the unconjugated estrogens are converted primarily to estrone. Therefore, estrogens, if taken orally, cause increased serum estrone levels. Both endogenous and exogenous estrogens are metabolized similarly (74). Maximal serum estrogen levels after oral ingestion are reached in 4–6 h (75).

Inactivation of estrogen in the body is carried out mainly in the liver. A certain proportion of the estrogen reaching that organ is secreted into the bile, which is then reabsorbed from the intestine. Degradation of estradiol and estrone in the liver is mainly through conversion to less active products such as estriol and numerous other estrogens, through oxidation to nonestrogenic substances, and through conjugation with sulfuric and

glucuronic acids. The end products are secreted by the kidney. A portion of estriol is metabolized and excreted in the gallbladder, and the majority is cleared in the urine. Many of the synthetic derivatives of steroidal estrogens, conjugated estrogens, and the nonsteroidal estrogens are orally active and inactivated slowly. The course of metabolism of ethinyl estradiol is different. The insertion of an ethinyl group at the 17α-position allows estrogen to be absorbed efficiently and inhibits intestinal or hepatic metabolism, which accounts for its high intrinsic potency (76).

The absorption of estrogen is efficient through other modes of delivery (transdermal, vaginal, nasal, or intramuscular) (77). Various vehicles by which estrogen is administered give different rates of absorption. Injected estrogens are rapidly absorbed and lead to a fast increase in blood concentration. However, esterification or polymerization slows the process. Vaginal, transdermal, or intramuscular administration of estradiol results in higher levels in plasma of estradiol than estrone, because the intestinal metabolism of oral estrogens is bypassed (78–80).

Circulating estrogens are tightly conjugated with sex hormone-binding globulin and weakly bound to albumin. The concentration of sex hormone-binding globulin in blood is regulated by hormones. Plasma sex hormone-binding globulin levels are increased by estrogens and pregnancy and decreased by testosterone administration (81). The physiological role of plasma protein binding of estrogen is still not fully understood. Binding is not necessary for estrogen transport, because estrogens are sufficiently water-soluble at physiological concentrations. Besides, only free estrogens are available to the tissues. It is suggested that the principal function of sex hormone-binding proteins may be to ensure uniform hormone distribution among all the cells of target tissue (82).

4.0.7. Pharmacological Effects

Three principal natural estrogens (E_1, E_2, and E_3) are produced by the ovary and play important roles in the development and support of female reproduction (65). The normal menstrual cycle is modulated by a variety of ovarian steroids and peptides, among which estrogen is a primary regulator. Cells in the anterior hypothalamus secrete gonadotropin-releasing hormone (GnRH, also known as luteinizing hormone releasing hormone, LHRH) in a pulsating manner. LHRH stimulates the gonadotrope cells of the anterior pituitary to release both luteinizing hormone (LH) and follicle-stimulating hormone (FSH). These pituitary gonadotropic hormones induce maturation of the oocyte (an ovarian cell which produces an ovum) and stimulate the ovarian follicles to synthesize estrogen and progestins as well as the peptide hormone inhibin. Estrogen and progestins directly inhibit LHRH and pituitary gonadotropin secretion and inhibit further stimulation of the ovary (83). The effect of estrogen on pituitary gonadotropin is biphasic. Early in the cycle when the concentration of estrogen is relatively low ($20 - 60$ pg/mL), estrogen inhibits LH secretion. The increasing concentration of estrogen in midcycle (200 pg/mL) leads to an increase of the frequency and amplitude of the LH pulse. The LH surge leads to ovulation of the dominant follicle and coincides with a transient decrease in estrogen levels. Ovulation typically occurs on day 14 of the typical 28-d cycle. The period after ovulation is the luteal phase, which is characterized by a decreasing LH pulse and a rise in progesterone concentration (83, 84).

Estrogens coordinate the systemic response during the ovulatory cycle, including the growth and maintenance of the reproductive tract, pituitary, breasts, and other tissues. Estrogens are also responsible for maturation of the skeleton and development of female secondary sex characteristics when females enter puberty. The other important functions of estrogens include modulation of many metabolic processes (76).

Estrogens stimulate cellular proliferation, induce RNA and protein synthesis of uterine endometrium and the fibrous connective tissue framework for ovaries, and increase the size of the cells. This effect leads to the growth and regeneration of the endometrial layer and spiral arterioles, and increase in the number and size of endometrial glands. Under the influence of estrogen, vaginal mucosa becomes thicker, as cervical mucus becomes thinner (85, 86).

Breast development is initiated by estrogens with both ductal and stromal growth, resulting in breast enlargement. Estrogens also promote body hair and female distribution of fat in the breasts, buttocks, and thighs. Estrogens modulate bone growth in a biphasic manner. At low dosages, estrogens help to maintain bone growth primarily by inhibiting bone resorption. At high dosages, the hormones stimulate closure of the shafts of the long bone. The positive effects of estrogens on salt and water retention usually cause edema and decrease of bowel motility. Estrogens also stimulate the synthesis and secretion of prolactin in pituitary lactotrophic cells (87).

Estrogens have an influence on hepatic metabolism. The production of sex hormone-binding globulin, thyroxine-binding globulin, blood-clotting factors (VII to X) and plasminogen in the liver is stimulated by estrogens. Estrogens promote the production of high density lipoprotein (HDL), especially HDL2 and its apolipoproteins A1 and A2, in liver. Contrarily, estrogens inhibit the hepatic formation of low density lipoprotein (LDL). These effects vary with different types and doses of estrogens and the route of administration (88, 89).

5. Therapeutic Uses

Estrogens, along with progestins, are used to suppress ovulation for fertility control in the form of contraceptives (see Contraceptives). Estrogens are applied to treat gonadal failure, to induce and maintain secondary sexual characteristics due to inadequate production of ovarian steroids in hypogonadal individuals. Estrogens are also indicated for hormone replacement therapy in postmenopausal women, for the preparation of the endometrium of hypogonadal women before donor egg and embryo transfer, and for treatment of breast cancer (see Chemotherapeutics, anticancer).

5.0.8. Contraceptive Agents

Oral contraceptives consist of estrogens and progestins in combination or progestin alone. Both hormones act primarily to inhibit the production of gonadotropins, follicle-stimulating hormone (FSH), and luteinizing hormone (LH) in the pituitary. As a result, the midcycle surge of LH is suppressed and ovulation is prevented. Measurements of circulating FSH and LH show that estrogen–progestin combinations suppress both gonadotropins. Experience with the most common types of oral contraceptives shows them to be 99–100% effective (90).

The estrogen component consists of either ethinyl estradiol**(5)**, a potent synthetic estrogen, or mestranol**(6)**, the 3-methyl ether of ethinyl estradiol, a less potent compound. Mestranol is demethylated to ethinyl estradiol in the liver, which accounts for the majority of its estrogenic activity. Ethinyl estradiol is metabolized and conjugated in the liver very slowly, which enhances the oral activity (91). Peak levels of ethinyl estradiol in plasma are reached 1 h after oral administration, followed by an initial rapid decline and a second, slower phase of decline. Up to 60% of an oral dose is excreted in urine after 24 h (92). Early oral contraceptive formulations contained up to 100–150 μg of estrogen. Numerous studies were carried out to find the lowest doses of estrogen and progestogen, a hormone whose effects mimic those of progesterone, consistent with efficacy and having the least amount of side effects. By the early 1990s the majority of oral contraceptives contained only 30–35 μg of estrogen. These low dose products are commonly referred to as second-generation oral contraceptives, or are termed “low dose” (see Contraceptives).

There were reports that the appearance of male characteristics, androgenicity, associated with the progestational component of some of the original progestogens was related to changes in lipid metabolism (93, 94). New, pharmacologically more selective progestogens with no or less undesirable adverse effects have been investigated. Using medicinal chemistry approaches, companies like Ortho Pharmaceutical Corp., Organon, and Schering AG have dissociated the androgenicity and progestational activity of steroidal progestogens. More selective progestogens such as norgestimate, desogestrel, and gestodene emerged from this research

(95–98). In combination with ethinyl estradiol, these new progestogens compose the third generation of oral contraceptives. The usage of these contraceptives is expected to continue to grow in the 1990s and beyond.

Estrogens alone have only been used as short-term "emergency" contraimplantative agents. Under high dose estrogen treatment, the endometrium becomes nonreceptive to the blastocyst, thus preventing implantation. Therefore, large doses of estrogens can prevent pregnancy when given within 48 h following unprotected coitus. The "morning-after pill" is effective; however, its use is associated with side effects such as nausea, vomiting, and menstrual disturbances. It is recommended only in case of rape, incest, failure of a barrier method, or unprotected intercourse (99). The use of 50 μg of ethinyl estradiol and 0.5 mg of norgestrel is equally effective and results in fewer side effects (100). Detailed reviews on contraceptive agents have been published (see Contraceptives) (92, 101, 102).

5.0.9. Hormone Replacement Therapy

5.0.9.1. Estrogen Deficiency. Treatment with cyclic estrogen has been proven to benefit young women with estrogen deficiency, caused by primary ovarian failure or by hypogonadotropic hypogonodolism resulting from luteinizing hormone-releasing hormone (LH-RH) deficiency or hypopituitarism (103, 104). The therapy should be initiated at the time of expected puberty for the promotion and maintenance of female sexual characteristics. The breasts and endometrium respond to only a high dosage of ethinyl estradiol. Low dosage estrogen therapy is used to promote the growth of the long bones. Estrogen is usually prescribed in a cyclic fashion with an initial dose of 0.3 mg/d of conjugated estrogens until growth ceases, at which time the daily estrogen dose is increased to 0.625–1.25 mg to augment breast development. Adding a progestogen at the time of the first breakthrough bleeding episode could induce cyclical withdrawal bleeding (105, 106).

5.0.9.2. Menopause. The depletion of functional ovarian follicles leads to the natural cessation of menses called menopause (107). After menopause, ovarian production of estradiol falls to less than 20 μg/d from a mean value of 220 μg/d during the normal menstrual cycle (108, 109). This causes a deficiency of estrogen and progesterone in post-menopausal women. However, the menopause does not result in total cessation of estrogen production. Androstenedione is continuously aromatized to estrone in peripheral tissues such as fat, liver, the skeletal muscles, hypothalamus, and hair follicles. Because estrone is a weak estrogen, most menopausal women are hypoestrogenic.

Estrogen replacement is successful in treating the symptoms in menopause (vasomotor symptoms and vaginal atrophy) and has been reported to reduce the risk of osteoporosis and atherosclerotic heart disease (110). A recent study suggests that estrogen replacement treatment prevents increases in abdominal fat that occur after menopause (111). For long-term use estrogens should be given in the minimally effective dose (0.625 mg conjugated estrogen, 0.625 mg estrone sulfate, or 1 mg of micronized estradiol) (112, 113). The hormones are given orally or in estrogen-containing vaginal cream or transdermal estradiol patches. Progestogens given cyclically $(10 - 14\ \mathrm{d/mo})$ are recommended to avoid withdrawal bleeding (114) and to minimize the risk of endometrial cancer (115, 116). Estrogens are most effective for the relief of vasomotor symptoms. The symptoms of genitourinary atrophy improve with estrogen replacement therapy of all forms, and it has been suggested that estrogens could be given before atrophy occurs.

5.0.9.3. Osteoporosis. Bone is constantly renewed to meet the stresses imposed upon it and to ensure proper calcium homeostasis. The process involves the removal of bone (resorption) by the osteoclasts and formation of bone by the osteoblasts. It is believed that estrogens may induce production of cytokines (nonantibody proteins) in osteoblasts or other progenitors of bone cells. In turn, the hormone inhibits the osteoclastic resorption and as a result, reduces the net bone loss (117, 118). The administration of estrogens daily (and of progestogens intermittently if the woman has a uterus) is an effective method of preventing bone loss, slowing the progress of osteoporosis, and reducing the risk of fractures of the hip, radius, and vertebrae in postmenopausal women (119, 120). Case-control and other retrospective studies suggest that the long-term use of estrogen in post-menopausal women, starting soon after the menopause, reduces the risk of subsequent

vertebral fracture by up to 90% and of Colles' (wrist) and hip fractures by up to 50%, especially if therapy is started within 5 yr of the last menses (120–122). The use of hormone replacement therapy to treat established osteoporosis by preventing further bone resorption has been reported (123, 124). The minimal effective doses of oral estrogen that reduce the loss of bone and the incidence of fracture are $0.625\ \mathrm{mg/d}$ of conjugated equine estrogens, $0.625\ \mathrm{mg/d}$ of estrone sulfate, or $0.02\ \mathrm{mg/d}$ of ethinyl estradiol. Tamoxifen**(9)**, a partial-antiestrogen in certain tissues, acts as an estrogen agonist on trabecular bone, which supports connective tissue, causing decreases in the usual high levels of bone resorption and a net preservation of bone mineral mass (125–127). Detailed reviews on this subject have been published (128–130).

5.0.9.4. Cardiovascular System. Concerns about negative cardiovascular effects as associated with the use of estrogen originated from the use of oral contraceptives when the steroid dose was much higher than that utilized in current low dose preparations. Recent data show that estrogens have positive effects on the cardiovascular system in post-menopausal women receiving estrogen replacement therapy (89, 131, 132). Post-menopausal estrogen treatment reduced the risk for angiographically significant coronary artery disease and prolonged survival when coronary artery disease was present, but had less effect in the absence of coronary artery disease. Epidemiological studies have shown that estrogen treatment after menopause significantly reduces the mortality rate from cardiovascular disease (89).

Table 1. Preparations of Estrogens[a]

Preparation	Form[b]	Trade name	Manufacturer
estrone aqueous suspension	I, 2 – 5 mg/mL	Estrone Aqueous	Wyeth-Ayerst
	Bestrone	Bluco	
		Estrone-A	Kay Pharmaceuticals
		Estronol	Central
		Theelin aqueous	Parke-Davis
		Estrone 5	Keene Pharmaceuticals
		Kestrone-5	Hyrex
		Theogen	Jones-Western
		Estragyn 5	Clint Pharmaceuticals
estrogenic substance aqueous suspension	I, 2 – 5 mg/mL	Estrogenic Substance Aqueous	Wyeth-Ayerst
		Estrofol	Reid-Provident
		Estroject-2	Mayrand
		Estromone	Endo
		Gynogen	Forest Pharmaceuticals
		Hormogen-A	Mallard
		Kestrin Aqueous	Hyrex
		Unigen	Vortech
		Wehgen	Hauck
micronized estradiol	T, 1–2 mg	Estrace	Mead Johnson Laboratories
	C, 0.1 mg/g	Estrace	Mead Johnson Laboratories
	Tr, 0.05–0.1 mg/d	Estraderm	Ciba-Geigy
estradiol cypionate in oil	I, 1–5 mg	Depo-Estradiol	Upjohn
	Depostra	Tennessee Pharmaceuticals	
		Depgyhogen	Forest Pharmaceuticals
		Depogen	Hyrex
		Dura-Estrin	Hauck
		E-lonate	Tunex
		Estra-D	Seatrace
		Estro-Cyp	Keene Pharmaceuticals

Table 1. ***Continued***

Preparation	Form[b]	Trade name	Manufacturer
		Estrofem	Pasadena Research
		Estroject-LA	Mayrand
		Estronol-LA	Central
		Estragyn LA 5	Clint Pharmaceuticals
		Estro-L.A.	Hauser
		E-Cypionate	Legere
		Estro-Span C	Primedics
		Esdinate	Shoals Pharmaceuticals
estradiol valerate in oil	I, 10–40 mg	Delestrogen	Mead Johnson Laboratories
		Dioval, XX, 40	Keene Pharmaceuticals
		Duragen-10, 20, 40	Hauck
		Estradiol L.A., 20, 40	Vortech
		Estroval-10	Reid-Provident
		Feminate-10, 20, 40	Jones-Western
		Gynogen L.A. 10, 20	Forest Pharmaceuticals
		Valergen-10, 20, 40	Hyrex
		L.A.E. 20	Seatrace
		Clinagen LA 40	Clint Pharmaceuticals
		Deladiol-40	Dunhall
		Menaval-20	Legere
		Medidiol 10	Med-Tek Pharmaceuticals
		Estra-L	Pasadena Research
		Estro-Span	Primedics
		Repository Hormone	Rugby
		Esdival-10	Shoals Pharmaceuticals
polyestradiol phosphate	I, 40 mg	Estradurin	Wyeth-Ayerst
conjugated estrogens[c]	T, 0.3–2.5 mg	Premarin	Wyeth-Ayerst
		Progens	Major
		Estrocon	Savage Laboratories
	P, 25 mg	Premarin Intravenous	Wyeth-Ayerst
	VC, 0.625 mg/g	Premarin Cream	Wyeth-Ayerst
esterified estrogens[d]	T, 0.3–2.5 mg	Estratab	Solvay Pharmaceuticals
		Menest	Smith Kline Beecham
estropipate piperazine estrone sulfate	T, 0.625–5 mg	Ogen	Abbot
	VC, 1.5 mg/g	Ogen	Abbott
ethinyl estradiol	T, 0.02–0.5 mg	Estinyl	Schering
		Feminone	Upjohn
quinestrol	T, 100 μg	Estrovis	Parke-Davis
diethylstilbestrol (DES)	T, 1–5 mg, entric coated; 0.1–5 mg	Diethylstilbestrol	Lilly
	VS, 1–0.5 mg		Lilly
chlorotrianisene	Ca, 12–72 mg	Tace	Marion Merrell Dow
dienestrol	VC, 0.1 mg/g	Ortho Dienestrol	Ortho-McNill
		DV	Marion Merrell Dow
		Estraguard	Reid-Provident

[a]Ref. 134.
[b]I = injection; T = tablet; C = cream; Tr = transdermal, continuous delivery, P = parenteral; VC = vaginal cream; VS = vaginal suppositories; Ca = capsule.
[c]Contain 50–65% sodium estrone sulfate and 20–35% sodium equillin sulfate.
[d]Contain 75–80% sodium estrone sulfate and 6–15% sodium equillin sulfate.

One theory is that the protective effect by estrogens against atherosclerosis is partially a result of hormonal effects on circulating lipid levels. Estrogen replacement therapy changes the overall lipid profiles of older women

(133). The rise in high density lipoprotein (HDL) cholesterol and the lowering of low density lipoprotein (LDL) cholesterol may provide a protective effect from atherosclerotic heart disease (88) (see Cardiovascular agents). Table 1 lists preparations of estrogens for hormone replacement therapy (134).

5.0.10. Chemotherapy

5.0.10.1. Prostate Cancer. Estrogen has an inhibitory effect on the prostate in addition to its suppression of gonadotropin secretion by the pituitary. The three- and five-year survival rates in prostate cancer patients with metastatic disease improved when treated with DES **(7)** alone or along with castration. However, DES does not improve the survival rates in patients whose carcinoma is confined to the prostate. Small doses of DES (1 mg/d) appear to retard prostate cancer growth and could reduce the cardiovascular complications associated with larger doses (5 mg/d) (135) (see Chemotherapeutics, anticancer).

5.0.10.2. Breast Cancer. Although the mechanism of action is unknown, estrogens (DES, conjugated estrogens, and ethinyl estradiol) have been used in the treatment of advanced metastatic breast cancer in post-menopausal women (see Chemotherapeutics, anticancer). However the estrogen responsiveness is not predictable. Some patients with breast cancer show tumor regression when treated with pharmacological doses of estrogens (136, 137). Remission rates of 30–37% have been reported when estrogen is used as the initial therapy. In a randomized trial, estrogen gave a 29% remission rate. The duration of response to estrogen is relatively long-lived in most series. Remission rates of less than 10% have been reported when estrogens are used in patients who have relapsed from other therapy. A more recent clinical report concluded that DES remains a useful, active agent in the management of advanced breast cancer in post-menopausal women, even in patients with tumors unresponsive to other endocrine therapy (138).

Triphenylethylene derivatives, such as tamoxifen **(9)** and clomiphene **(8)**, are long-acting antiestrogens. They act by binding to estrogen receptors and interfering with estrogen function. Both are mixed agonists–antagonists of estrogen action, which partially mimic the response of estrogen in some cell types, yet block estrogen action in others (139).

Tamoxifen is useful in the treatment of estrogen-receptor-positive breast cancer (140, 141). The antiestrogen induces a therapeutic response in about a third of men and women with breast cancer. Tamoxifen acts as a cell cycle phase-specific which controls a biochemical process critical in cycle regulation of breast cancer cells. The mechanism of the antiestrogen action is not clear yet. *In vitro* studies show that tamoxifen stimulates hormone-dependent breast cancer cells to secrete the peptide transforming growth factor β (TGF-β), which can inhibit the growth of estrogen receptor-negative breast cancer cells which are negative for estrogen receptors (142–144).

Tamoxifen is converted to a number of well-characterized metabolites in patients. The majority of these metabolites exhibit antiestrogenic effects. The primary metabolite of tamoxifen is *N*-desmethyltamoxifen which has low affinity for the estrogen receptor. 4-Hydroxytamoxifen is a minor metabolite, but binds to the estrogen receptor with an affinity 25–50 times that of tamoxifen and equal to that of estradiol (145, 146). Therefore, although 4-hydroxytamoxifen is found at lower levels in serum than *N*-desmethyltamoxifen, its potency is 1250 times that of *N*-desmethyltamoxifen. It suggests that 4-hydroxytamoxifen is primarily responsible for the observed antiestrogenic effect. The metabolites are secreted largely in the bile as conjugates. The initial blood half-life of tamoxifen is less than 8 h. The secondary half-life is about 7 d. It takes 16 weeks to reach steady state (147–149). At a dose of 20 mg twice a day, tamoxifen levels range from 285 to 310 mg/L, *N*-desmethyltamoxifen levels from 462 to 481 mg/L, and 4-hydroxytamoxifen levels from 6 to 7 mg/L. The relationship between the response and the dose of tamoxifen is minimal. However, increasing dose has been reported to result in a second remission after relapse (150–152). Animal carcinogenicity studies and human studies suggest that tamoxifen treatment of preclinical or early breast cancer (asymptomatic) has a major beneficial effect. With long-term therapy, a majority of preclinical breast-cancer clones should be suppressed.

5.0.11. Adverse Effects

The side effects of estrogens vary according to the type and dosage of the estrogen and if progestins are coadministrated. The most common side effect is nausea. However, it does not usually interfere with eating and cause weight loss. Increasing the dosage may further induce anorexia and vomiting. Breast enlargement, enlargement of endometrial tissue, and intermenstrual bleeding are other common side effects. Low dose estrogens, such as multiphasic oral contraceptives which contain 30–35 μg of estrogen and new versions of progestogen, significantly reduce intermenstrual bleeding (153).

5.0.11.1. The DES Story. Diethylstilbestrol (DES), a potent estrogen, was in widespread use in midcentury for maintenance of pregnancies (154, 155). It has been estimated that up to 4.5×10^6 children born during this period were exposed to DES*in utero* (156). It was not until the 1970s that an increase in cases of a rare form of vaginal adenocarcinoma was found in girls who had been exposed to DES *in utero,* a link between prenatal exposure to DES and this rare cancer was suggested (157, 158). The annual incidence rate of genital cancer (per 10×10^6) for 16-yr-old females born in 1955 was 17.8 as compared to 0 for 16-yr-old females born in 1949 (158). In addition to the malignant growth (neoplasia), benign anomalies were also reported in women exposed to DES *in utero* (159–161). Approximately 0.14–1.4 per thousand of the exposed offspring developed clear cell adenocarcinoma of the vagina and cervix, peaking at age 19 and decreasing to lower levels by age 30. Functional abnormalities include an increased incidence of spontaneous abortion, premature labor, and ectopic pregnancies. In male adults, the reproductive tracts were adversely affected by prenatal exposure to DES. Decreased fertility, abnormalities in quantity and quality of sperm, and epididymal cysts were present more often in men who were exposed to DES *in utero* compared with the unexposed controls (160, 161). Since there have been no reports correlating DES treatment during pregnancy with genital carcinogenesis in the mother, it is believed that the teratogenic effects of DES are exerted only during the first 18 weeks of pregnancy, when the fetal urogenital tract is differentiating and appears to be sensitive to the harmful effects of DES (54).

5.0.11.2. Breast Cancer. Epidemiological studies of breast cancer have suggested that in the presence of other cocarcinogens such as virus, chemicals, and radiation, estrogen could induce breast cancer (162–164). The causation of breast cancer also includes genetic factors. A history of breast cancer in a first-degree relative elevates a woman's risk of contracting breast cancer more than twofold (165, 166). Other predictors of risk include age, age at menarche and menopause, age at first pregnancy, geographic area of residence, dietary factors, and smoking habit. However, knowledge of the specific hormones involved in the carcinogenesis and their relative roles remains elusive. The results of many retrospective analyses and designed studies on the risk of breast cancer in various groups of estrogen users are conflicting (167–172).

5.0.11.3. Endometrial Cancer. Clinical, biological, and epidemiological data indicate that exogenous estrogens are linked to endometrial cancer (173, 174). Increased risk (two- to eightfold) has been found in women under estrogen replacement therapy (175–179). The incidence depends on duration of the hormone treatment. Every type of estrogen that has been investigated has shown this relationship, including conjugated equine estrogens, ethinyl estradiol, and DES. The endometrial cancer associated with estrogen users is a less aggressive form, to which the relatively lower death rate from this disease can be attributed. The opposed estrogen treatment (estrogen–progesterone in sequence) reduces the frequency of hyperplasia (tissue enlargement) and atypical hyperplasia which is associated with unopposed estrogen treatment. Studies on combination oral contraceptives clearly show a 50–60% protection against endometrial cancer. The protective effect may be absent among the obese, long-term estrogen users, and women who have borne more than one child (180, 181).

5.0.11.4. Cervical Cancer. Studies of cervical cancer found two principal risk factors for this malignancy: a large number of different sexual partners and an early age at first intercourse. However, the cervix is an endocrine target organ, and therefore its neoplastic potential may depend on hormonal influence. A series of case-control and follow-up studies have linked "high dose" oral contraceptive use to cervical intraepithelial neoplasia and frankly invasive cervical carcinoma (182–184). These studies report a risk that rises with the duration of use to approximately twofold among long-term users of "high dose" contraceptives. A systematic

follow-up of women who were exposed to diethylstilbestrol has revealed an increased incidence of cervical intraepithelial neoplasia among these women compared with women unexposed to the drug (185).

5.0.11.5. Liver and Gallbladder. High dosages of oral estrogens have been reported to increase the risk for jaundice, cholestatic hepatitis, gallstones, and hepatic vein blood clots. Estrogens promote the development of hepatic neoplasms associated with increased hepatic cell regenerative activity (186, 187).

Estrogens change the hepatic production of many proteins and metabolites, which can alter the rate of metabolism and excretion of other hormones and drugs, thus further influencing the interaction between these compounds and the hepatocytes (188). For example, estrogen enhances the synthesis of carrier proteins corticosteroid-binding globulin (CBG), thyroxine-binding globulin (TBG), sex hormone-binding globulin (SHBG), transferrin, and ceruloplasmin, which influences the results of laboratory tests used to determine the levels of substances bound to these proteins.

BIBLIOGRAPHY

"Synthetic Estrogens" under "Hormones" in *ECT* 1st ed., Vol. 7, pp. 536–547, by P. F. Dreisbach, Calco Chemical Division, American Cyanamid Company; "Nonsteroidal Estrogens" under "Hormones" in *ECT* 2nd ed., Vol. 11, pp. 127–141, by R. I. Dorfman, Syntex Research Division of Syntex Corporation; "Nonsteroidal Estrogens" under "Hormones" in *ECT* 3rd ed., Vol. 12, pp. 658–691, by G. C. Crawley, Imperial Chemical Industries Limited.

Cited Publications

1. U.S. Pat. 4,789,671 (Dec. 6, 1988), J. R. Bull, R. I. Thomson, H. Laurent, H. Schroder, and R. Wiechert (to Schering AG).
2. Ger. Offen. 3,838,779 (May 17, 1990), G. Kirsch, G. Neef, H. Laurent, R. Wiechert, S. Beier, W. Elger, and J. R. Bull (to Schering AG).
3. Ger. Offen. 3,939,893; 3,939,894 (June 6, 1991), S. Beier, J. R. Bull, W. Elger, P. Esperling, G. Kirsch, H. Laurent, G. Neef, and R. Wiechert (to Schering AG).
4. Ger. Offen. 3,741,800 (June 15, 1989), P. Jungblut, R. Wiechert, and R. Bohlmann (to Schering AG).
5. R. H. Peters and co-workers, *J. Med. Chem.* **32**, 2306 (1989).
6. H. K. Kim, in B. K. Kim, E. B. Lee, C. K. Kim, and Y. N. Han, eds., *Advances in New Drug Development* (Invited Lectures of the International Congress of New Drug Development, Seoul, South Korea, August 18, 1991) The Pharmaceutical Society of Korea, Seoul, 1991, 547–555.
7. O. Nakayama and co-workers, *J. Antibiot.* **43**, 1394 (1990); H. Kondo and co-workers, *J. Antibiot.* **43**, 1533 (1990).
8. A. E. Wakeling and J. Bowler, *Biochem. Soc. Trans.* **19**, 899 (1991).
9. P. J. Weatherill and co-workers, *J. Steroid Biochem.* **30**, 263 (1988).
10. A. E. Wakeling, M. Dukes, and J. Bowler, *Cancer Res.* **51**, 3867 (1991).
11. A. E. Wakeling and J. Bowler, *J. Steroid Biochem.* **30**, 141 (1988).
12. A. E. Wakeling and J. Bowler, *J. Endocrinol.* **112**, R7 (1988).
13. C. Levesque and co-workers, *J. Med. Chem.* **34**, 1624 (1991).
14. Eur. Pat. 367576 (May 9, 1990), F. Labrie (to Endorecherche).
15. Ger. Offen. 3,925,507 (Jan. 31, 1991), R. Bohlmann, D. Henderson, H. Kunzer, Y. Nishino, M. Schneider, R. Wiechert, and H. Kuenzer (to Schering AG).
16. U.S. Pat. 4,874,754 (Oct. 17, 1989), M. Bouton, L. Nedelec, F. Nique, and D. Philibert (to Roussel-Uclaf).
17. U.S. Pat. 4,943,566 (July 24, 1990), F. Nique, D. Philibert, M. Moquilewsk, M. Bouton, and L. Nedelec (to Roussel-Uclaf).
18. Eur. Pat. 384842 (Aug. 29, 1990), A. Claussner, L. Nedelec, D. Philibert, and P. Vandevelde (to Roussel-Uclaf).
19. R. L. Sutherland and co-workers, *Nature* **288**, 273 (1980).
20. C. B. Lazier and B. V. Bapat, *J. Steroid Biochem.* **31**(4B), 665 (1988).
21. R. Valavaara and co-workers, *Eur. J. Cancer Clin. Oncol.* **24**, 785 (1988).
22. Eur. Pat. 260066 (Mar. 16, 1988), R. McCague (to National Research and Development Corp.).

23. *Drugs Fut.* **8**, 673 (1983).
24. C. K. W. Watts and R. L. Sutherland, *Mol. Pharmacol.* **31**, 541 (1987).
25. P. C. Ruenitz, J. R. Bagley, and C. M. Mokler, *J. Med. Chem.* **26**, 1701 (1983).
26. Jpn. Pat. 88503618 (April 7, 1988) T. Asao and co-workers (to Taiho).
27. Jpn. Pat. 87099352 (May 8, 1987) T. Asao and co-workers (to Taiho).
28. U.S. Pat. 4,897,503 (Jan. 30, 1990) T. Asao, S. Takeda, and Y. Sugimoto (to Taiho).
29. T. Asao, *16th Int. Cong. Chemother.* (June 11–16, Jerusalem), 248 (1989).
30. L. R. Morgan and co-workers, *Proc. Am. Assoc. Cancer Res.* **31**, Abst. 2629 (1990); *Drugs Fut.* **17**, 369 (1992).
31. N. C. Misra and co-workers, *Int. J. Cancer* **43**, 781 (1989).
32. A. P. Sharma and co-workers, *J. Med. Chem.*, **33**, 3216 (1990).
33. A. Saeed and co-workers, *J. Med. Chem.* **33**, 3210 (1990).
34. C. C. Teo and co-workers, *J. Med. Chem.* **35**, 1330 (1992).
35. B. Astroff and co-workers, *Mol. Pharmacol.* **33**, 231 (1988).
36. C. D. Jones and co-workers, *J. Med. Chem.* **27**, 1057 (1984).
37. C. D. Jones and co-workers, *J. Med. Chem.* **35**, 931 (1992).
38. *Drugs Fut.* **3**, 211 (1978).
39. M. Lang and co-workers, *Proc. Amer. Assoc. Cancer Res.* **31**, Abst. 1305 (1990).
40. R. W. Hartmann and co-workers, *J. Med. Chem.* **28**, 1295 (1985).
41. M. B. Hossain and co-workers, *Acta Crystallogr. Sect. B* **B47**, 511 (1991).
42. L. J. Brandes and M. W. Hermonat, *Biochem. Biophys. Res. Commun.* **123**, 724 (1984).
43. Y. Y. Sheen, D. M. Simpson, and B. S. Katzenellenbogen, *Endocrinology* **117**, 561 (1985).
44. M. R. Schneider, *Eur. J. Cancer* **115**, Abst. TH 11 (1989).
45. S. von Angerer and co-workers, *6th NCI-EORTC Symp. New Drugs Cancer Ther.* (March 7–10, Amsterdam) Abst. 060 (1989).
46. J. F. Miquel and J. Gilbert, *J. Steroid Biochem.* **31**(4B), 525 (1988).
47. S. Green and co-workers, *Nature* **320**, 134 (1986).
48. K. J. Edwards, C. A. Laughton, and S. Neidle, *J. Med. Chem.* **35**, 2753 (1992).
49. S. Green, *J. Steroid Biochem. Molec. Biol.* **37**, 747 (1990).
50. W. L. Duax and co-workers, *J. Steroid Biochem.* **31**(4B), 481 (1988).
51. W. L. Duax and J. F. Griffin, *J. Steroid Biochem.* **27**, 271 (1987).
52. R. D. Bindal, J. T. Golab, and J. A. Katzenellenbogen, *J. Amer. Chem. Soc.* **112**, 7861 (1990).
53. C. Holdregger and D. Keefer, *Am. J. Anat.* **177**, 285 (1986).
54. T. L. Greco, T. M. Duello, and J. Gorski, *Endocr. Rev.* **14**, 59 (1993).
55. J. H. Clark, J. Anderson, and E. J. Peck, Jr., *Science* **176**, 528 (1972).
56. G. S. Roth and G. D. Hess, *Mech. Ageing Dev.* **20**, 175 (1982).
57. A. Barbea and J. Gorsic, *Biochemistry* **9**, 1899 (1970).
58. W. V. Welshons, M. S. Lieberman, and J. Gorski, *Nature* **122**, 1165 (1984).
59. V. Kumar, S. Green, and G. Stack, *Cell* **51**, 941 (1987).
60. F. E. Murdoch and co-workers, *Biochemistry* **29**, 8377 (1990).
61. M. Beato, *Cell* **56**, 335 (1989).
62. R. M. Evans, *Science* **240**, 889 (1988).
63. J. Gorski and co-workers, *Biol. Reprod.* **48**, 8 (1993).
64. J. H. Clark, W. T. Schrader, and B. W. O'Malley, in J. D. Wilson and D. W. Foster, eds., *Textbook of Endocrinology*, 8th ed., W. B. Saunders, Philadelphia, Pa., 1991, p. 9.
65. J. E. Griffin and S. R. Ojeda, eds., *Textbook of Endocrine Physiology*, 2nd ed. Oxford University Press, New York, 1992.
66. L. Martin, in J. A. McLachlan, ed., *Estrogen in the Environment*, Elsevier North Holland Inc., New York, 1980, p. 103.
67. K. L. Kelner, H. J. Kirchick, and E. J. Peck, Jr., *Endocrinology* **111**, 1986 (1982).
68. S. W. Curtis and K. S. Korach, *Mol. Endocrinol.* **5**, 959 (1991).
69. A. J. W. Hsueh, E. Y. Adashi, and P. B. C. Jones, *Endocr. Rev.* **5**, 76 (1984).
70. E. R. Simpson, *Mol. Cell Endocrinol.* **13**, 213 (1979).
71. P. K. Siiteri and P. C. MacDonald, in R. O. Greep, and E. B. Astwood, eds., *Handbook of Physiology, Section 7: Endocrinology*, Vol. **II**, American Physiology Society, Williams & Wilkins, Baltimore, Md., 1973, p. 615.

72. D. L. Hemsell, J. M. Grodin, and P. F. Brenner, *J. Clin. Endocrinol. Metab.* **38**, 476 (1974).
73. C. D. Edman and P. C. MacDonald, *Am. J. Obstet. Gynecol.* **130**, 456 (1978).
74. K. Fotherby and F. James, *Adv. Steroid Biochem. Pharmacol.* **3**, 67 (1972).
75. D. E. Englund and E. D. B. Johansson, *Brit. J. Obstet. Gynecol.* **85**, 957 (1978).
76. G. I. Zatuchni, in K. L. Becker, ed., *Principles and Practice of Endocrinology and Metabolism*, Lippincott, Philadelphia, Pa., 1990, p. 868.
77. L. A. Rigg, B. Milanes, and B. Villanueva, *J. Clin. Endocrinol. Metab.* **45**, 1261 (1977).
78. M. S. Power, L. Schenkel, and P. E. Darley, *Am. J. Obstet. Gynecol.* **152**, 1099 (1985).
79. I. Schiff, D. Tulchinsky, and K. J. Ryan, *Fertil. Steril.* **28**, 1063 (1977).
80. S. Hass and co-workers, *Obstet. Gynecol.* **71**, 671 (1988).
81. W. Rosner, *Endocr. Rev.* **11**, 80 (1990).
82. W. M. Pardridge, *Amer. J. Physiol.* **252**, E157 (1987).
83. R. T. Scott, Jr. and G. D. Hodhen, *Clin. Obstet. Gynecol.* **33**, 551 (1990).
84. D. L. Healy and co-workers, *Fertil. Steril.* **41**, 114 (1984).
85. R. W. Rebar, D. Kenigsberg, and G. D. Hodgen, in K. L. Becker, ed., *Principles and Practice of Endocrinology and Metabolism*, Lippincott, Philadelphia, Pa., 1990, p. 788.
86. A. R. LaBarbera and R. W. Rebar, *Clin. Obstet. Gynecol.* **33**, 576 (1990).
87. P. A. Lee, *Semin. Reprod. Endocrinol.* **6**, 13 (1988).
88. J. Ginsburg, in H. Burger and M. Boulet, eds., *A Portrait of the Menopause*, Parthenon Publishing Group, Carnforth, Lancs, U.K., 1991, p. 45.
89. J. M. Sullivan and co-workers, *Arch. Intern. Med.* **150**, 2557 (1990).
90. *Oral Contraceptives*, Population Reports, Series A, No. 6, Population Information Program, The Johns Hopkins University, Baltimore, Md. 1982, p. A190.
91. U. J. Gospard, *Amer. J. Obstet. Gynecol.* **157**, 1029S (1987).
92. L. E. DeLia and M. G. Emery, *Clin. Obstet. Gynecol.* **24**, 879 (1981).
93. K. Fotherby, *Brit. J. Family Planning* **11**, 86 (1985).
94. G. Silfverstolpe and co-workers, *Acta Obstet. Gynecol. Scand.* **88** (Suppl), 89 (1979).
95. A. Phillips and co-workers, *Contraception* **36**, (1987).
96. A. Phillips and co-workers, *Contraception* **41**, 399 (1990).
97. H. J. Kloosterboer, C. A. Von Knoordegraaf, and E. W. Turpijn, *Contraception* **38**, 325 (1988).
98. D. W. Hahn, A. Phillips, and J. L. McQuire, in D. W. Hahn, A. Phillips, and J. L. McQuire, *Aktuelle Aspekte der Hormonalen Kontrazeption*, P. J. Keller, ed., Karger, New York, 1991, p. 46.
99. T. A. Grillo and R. Hatcher, *Bull. Woodruff Med. Cent.* **11**, 183 (1980).
100. A. Yuzpe, R. P. Smith, and A. W. Rademaker, *Feretil. Steril.* **37**, 508 (1982).
101. C.-R. Garcia and D. L. Rosenfeld, *Human Fertility: The Regulation of Reproduction*, F. A. Davis Company, Philadelphia, Pa., 1977, p. 59.
102. L. Mastroiani, Jr., P. J. Donaldson, and T. T. Kane, *Developing New Contraceptives*, National Academic Press, Washington, D.C., 1990, p. 11.
103. M. R. Soules, *Pediatr. Clin. North Amer.* **43**, 1083 (1987).
104. D. T. Baird, in L. J. LeGroot and co-workers, eds., *Endocrinology*, 2nd ed., W. B. Saunders, Philadelphia, Pa., 1989, p. 1950.
105. J. L. Ross and co-workers, *N. Engl. J. Med.* **309**, 1104 (1983).
106. J. L. Ross and co-workers, *J. Pediatr.* **109**, 950 (1986).
107. M. J. Odom, B. R. Carr, and P. C. MacDonald, in R. Andres, E. Bierman, and J. P. Blass, eds., *Principles of Geriatric Medicine and Gerontology*, 2nd ed., McGraw-Hill, Book Co., Inc., New York, 1990, p. 777.
108. D. T. Baird and I. S. Fraser, *J. Clin. Endocrinol. Metab.* **38**, 1009 (1974).
109. C. Longcope, *Amer. J. Obstet. Gynecol.* **111**, 778 (1971).
110. G. A. Colditz and co-workers, *JAMA* **264**, 2648 (1990).
111. J. Haarbo and co-workers, *Metabolism* **40**, 1323 (1991).
112. B. Ettinger and co-workers, *Amer. J. Obstet. Gynecol.* **166**, 479 (1992).
113. Consensus Development Conference, *Brit. Med. J.* **295**, 914 (1987).
114. A. T. Leather, M. Savvas, and J. W. W. Studd, *Obstet. Gynecol.* **78**, 1008 (1991).

115. I. Persson and co-workers, *Brit. Med. J.* **298**, 147 (1989).
116. L. F. Voigt and co-workers, *Lancet* **338**, 274 (1991).
117. M. C. Horowitz, *Science* **260**, 626 (1993).
118. R. L. Jilka and co-workers, *Science* **257**, 88 (1992).
119. K. Hunt, *Brit. J. Hosp. Med.* **38**, 450 (1987).
120. D. M. Hart, in J. O. Drife and J. W. W. Studd, eds., *HRT and Osteoporosis*, Springer-Verlag, London, 1990, p. 263.
121. N. S. Weiss, C. L. Ure, and J. H. Ballard, *N. Engl. J. Med.* **303**, 1195 (1980).
122. B. Ettinger, H. K. Genant, and C. E. Cann, *Ann. Intern. Med.* **102**, 319 (1985).
123. E. G. Lufkin and co-workers, *Ann. Intern. Med.* **117**, 1 (1992).
124. R. Lindsay and J. F. Tohme, *Obstet. Gynecol.* **76**, 290 (1990).
125. R. R. Love and co-workers, *Breast Cancer Res. Treat.* **12**, 297 (1988).
126. T. Fornander, L. E. Rutqvist, and H. E. Sjorberg, *Proc. Am. Soc. Clin. Oncol.* **8**, 21 (A-77) (1989).
127. S. Turken and co-workers, *J. Nat. Cancer Inst.* **81**, 1086 (1989).
128. R. Lindsay, *Lancet* **341**, 801 (1993).
129. J. D. Drife and J. W. W. Studd, *HRT and Osteoporosis*, Springer-Verlag, London, 1990, p. 251.
130. G. A. Greendale, K. J. Carlson, and I. Schiff, *J. Gener. Intern. Med.* **5**, 464 (1990).
131. J. M. Sullivan and R. A. Lobo, *Amer. J. Obstet. Gynecol.* **168**, 2006 (1993).
132. G. A. Colditz and co-workers, *N. Engl. J. Med.* **316**, 1105 (1987).
133. T. L. Bush and co-workers, *Circulation* **75**, 1102 (1987).
134. *Red Book*, Medical Economics Data, Montvale, N.J., 1993.
135. D. P. Byer, *Cancer* **32**, 1126 (1973).
136. B. J. Kennedy, *Surg. Gynecol. Obstet.* **113**, 635 (1965).
137. B. J. Kennedy, *Cancer* **24**, 1345 (1969).
138. M. J. Boyer and M. H. Tattersall, *Med. Pediatr. Oncol.* **18**, 317 (1990).
139. R. R. Love, *Prevent. Med.* **20**, 64 (1991).
140. V. C. Jordan and C. S. Murphy, *Endocr. Rev.* **11**, 578 (1990).
141. J. S. Patterson, L. A. Buttersby, and D. G. Edward, *Rev. Endocr. Related Cancer* **9**, 563 (1982).
142. C. Knabbe and co-workers, *Cell* **48**, 417 (1987).
143. B. Dickson and co-workers, *J. Steroid Biochem. Mol. Biol.* **43**, 69 (1992).
144. R. Clarke, R. B. Dickson, and M. E. Lippman, *Critical Rev. Oncol. Hematol.* **12**, 1 (1992).
145. C. Fabian, L. Tilzer, and L. Sternson, *Biopharmaceut. Drug Dispos.* **2**, 281 (1981).
146. R. I. Nicholson and co-workers, *Eur. J. Cancer* **15**, 317 (1979).
147. J. M. Fromson, S. Pearson, and S. Bramah, *Xenobiotica* **3**, 711 (1973).
148. C. Fabian and co-workers, *Cancer* **48**, 876 (1981).
149. B. M. Sherman and co-workers, *J. Clin. Invest.* **64**, 398 (1979).
150. J. V. Kemp and co-workers, *Biochem. Pharmacol.* **32**, 2045 (1983).
151. P. Daniel and co-workers, *Eur. J. Cancer Clin. Oncol.* **17**, 1183 (1981).
152. J. S. Patterson and co-workers, in H. T. Mourison and T. Palshoff, eds., *Breast Cancer—Experimental and Clinical Aspects*, Pergamon, Oxford, UK, 1980, pp. 89.
153. B. R. Carr and J. E. Griffin, in Ref. 64, p. 1007.
154. O. W. Smith, *Amer. J. Obstet. Gynecol.* **56**, 821 (1948).
155. O. W. Smith and G. V. S. Smith, *N. Engl. J. Med.* **241**, 562 (1949).
156. J. M. Reinisch and W. G. Karow, *Arch. Sex. Behav.* **6**, 257 (1977).
157. A. L. Herbst, H. Ulfelder, and D. C. Poskanzer, *N. Engl. J. Med.* **284**, 878 (1971).
158. A. L. Herbst and co-workers, *Amer. J. Obstet. Gynecol.* **128**, 43 (1977).
159. A. L. Herbst and co-workers, *N. Engl. J. Med.* **292**, 334 (1975).
160. W. B. Gill, G. F. B. Schumacher, and M. Bibbo, *J. Reprod. Med.* **16**, 147 (1976).
161. M. Bibbo and co-workers, *J. Obstet. Gynecol.* **49**, 1 (1977).
162. W. R. Miller and T. J. Anderson, in J. J. Studd and M. I. Whitehead, eds., *The Menopause*, Blackwell Scientific Publishers, Oxford, UK, 1988, p. 241.
163. J. L. Kelsey and co-workers, *J. Natl. Cancer Inst.* **67**, 327 (1981).
164. A. B. Miller, *Cancer Res.* **38**, 3985 (1978).

165. B. L. Weber and J. E. Garber, *JAMA* **270**, 1602 (1993).
166. M. L. Slattery and R. A. Kerber, *JAMA* **270**, 1563 (1993).
167. R. K. Ross and co-workers, *JAMA* **243**, 1635 (1980).
168. R. Hoover and co-workers, *J. Natl. Cancer Inst.* **67**, 815 (1981).
169. L. A. Brinton, R. Hoover, and J. F. Fraumeni, Jr., *Brit. J. Cancer* **54**, 825 (1986).
170. B. S. Hulka and co-workers, *Amer. J. Obstet. Gynecol.* **143**, 638 (1982).
171. P. A. Wingo and co-workers, *JAMA* **257**, 209 (1987).
172. K. Hunt and co-workers, *Brit. J. Obstet. Gynecol.* **94**, 620 (1987).
173. L. A. Brinton, *Cancer Detect. Prev.* **7**, 159 (1984).
174. R. D. Gambrel, *Clin. Obstet. Gynecol.* **130**, 695 (1986).
175. A. Paganini-Hill, R. K. Ross, and B. E. Henderson, *Br. J. Cancer* **59**, 445 (1989).
176. J. L. Kelsey and co-workers, *Amer. J. Epidemiol.* **116**, 333 (1982).
177. S. Shapiro and co-workers, *N. Engl. J. Med.* **303**, 485 (1980).
178. B. S. Hulka and co-workers, *Amer. J. Obstet. Gynecol.* **137**, 92 (1980).
179. C. M. F. Antunes and co-workers, *N. Engl. J. Med.* **300**, 9 (1979).
180. D. W. Sturdee and co-workers, *Brit. Med. J.* **1**, 1575 (1978).
181. M. I. Whitehead and co-workers, *J. Reprod. Med.* **27** (Suppl), 539 (1982).
182. L. A. Brinton and co-workers, *Int. J. Cancer* **38**, 339 (1986).
183. M. P. Vessey and co-workers, *Lancet* **2**, 930 (1983).
184. S. H. Swan and W. L. Brown, *Amer. J. Obstet. Gynecol.* **139**, 52 (1980).
185. S. J. Robboy and co-workers, *JAMA* **252**, 2979 (1984).
186. I. R. Wanless and A. Medline, *Lab. Invest.* **46**, 313 (1982).
187. L. E. Porter, D. H. Thiel, and P. K. Eagon, *Semin. Liver Dis.* **7**, 24 (1987).
188. J. Dickerson, R. Bressler, and C. D. Christian, *Contraception* **22**, 597 (1980).

J. Z. GUO
D. W. HAHN
M. P. WACHTER
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