Kirk-Othmer Encyclopedia of Chemical Technology.

HORMONES, SEX HORMONES

Progestins are a class of steroids (qv), named for their progestational effects, which are essential for the initiation and continuation of pregnancy. The primary progestin in humans is progesterone [57-83-0] 1, a hormone and intermediate in the synthetic pathways of estrogens, androgens, and corticosteroids. Progesterone is synthesized from pregnenolone [145-13-1], $C_{21}H_{32}O_2$ 1, which is derived from the side-chain cleavage of cholesterol [57-88-5] 1 (Fig. 1). This cleavage is the rate-limiting step in the biosynthesis. Pregnenolone is converted to progesterone by a two-enzyme system, ie, it is dehydrogenated to an intermediate 5-ene-3-oxosteroid by the mitochondrial enzyme 5-ene-3β-ol-dehydrogenase (EC 1.1.1.51), and this intermediate is irreversibly converted to progesterone by a 5-4 isomerase (EC 5.3.3.3.1) (1). Numerous reviews on the biosynthesis of progesterone can be found (2–4).

The primary sources of progesterone in women are the corpora lutea of the ovary and the placenta. Ovarian production of progesterone varies over the menstrual cycle. Baseline levels are $0.75 - 4$ mg/d during the follicular phase, ie, days 1–14 of a typical 28-d cycle. After ovulation and formation of the corpus luteum, production rises reaching peak levels of $15 - 50$ mg/d midway through the luteal phase, ie, days 15–28 of the cycle (5–7). The plasma levels of progesterone reflect these differing rates of synthesis. Follicular levels are $<0.2 - 2.0$ ng/mL and luteal levels range from $9.5 - 22$ ng/mL (8, 9). Peak levels of plasma progesterone persist for only 4–6 days, then rapidly decline to initial baseline levels. This drop in plasma progesterone triggers the onset of menses in 1–2 days. During pregnancy, placental production of progesterone exceeds that of the ovary by nearly 10-fold and the resulting plasma progesterone concentrations reach $120 - 200$ ng/mL near term. In men and ovariectomized women, progesterone can be detected in the plasma at concentrations of $0.3 - 0.4$ ng/mL (5–7).

Other naturally occurring progestins such as pregnanediol (5α- [566-58-5] or 5β-pregnane-3α,20α-diol [80-92-2]) 4 and 20-dihydroprogesterone (20α- [145-14-2] or 20β-dihydroprogesterone [145-15-3]) 5 can result from progesterone metabolism in steroid-responsive tissues (Fig. 1) (10–12). These progestins have much weaker biological activity than progesterone and their physiological significance remains unclear. Progesterone has been used as a therapeutic agent, but is rapidly metabolized and has little or no oral activity and a short duration of action. In humans, it is metabolized primarily by the liver into the biologically inactive pregnanediol. Reviews of the physical characteristics, pharmacokinetics, and metabolism of progesterone can be found in several references (4, 13).

All of the progestational agents in clinical use (ca 1994) are synthetic steroidal progestins. Since the discovery of ethisterone [434-03-7] (**6**), the first progestin having reasonable oral activity, an enormous number of progestins has been synthesized. As a class, progestins have been identified by their biological activity, rather than their basic chemical structure.

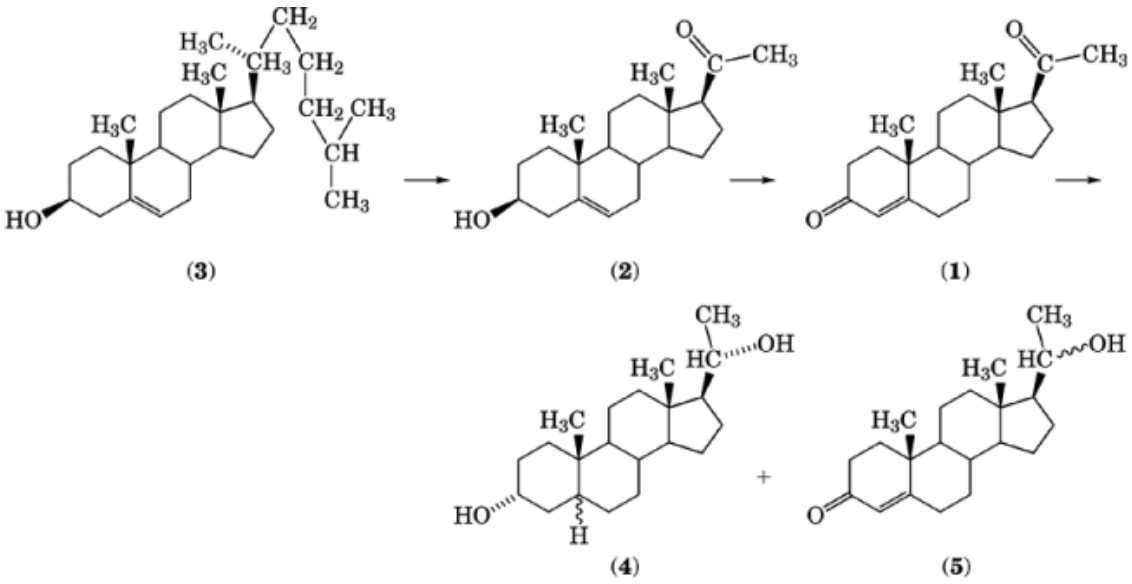
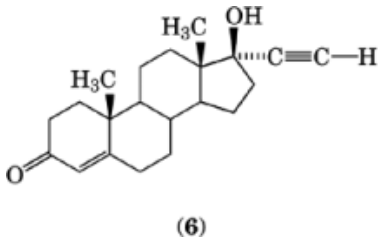


Fig. 1. Compounds involved in the biosynthesis and metabolism of progesterone (**1**).



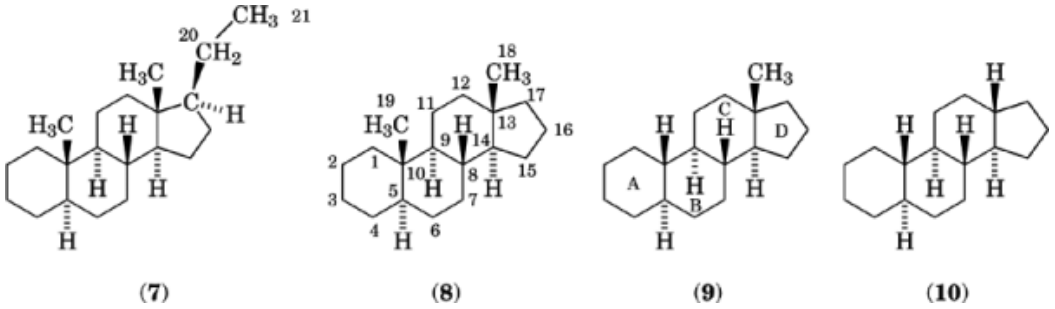
(6)

Most progestins prescribed in the early 1990s were developed a number of years prior. However, developments in the use of progestins have led to several newer progestins being brought to the marketplace (ca 1994). In addition to newer progestin agonists, potent progestin antagonists are available and may eventually replace progestin agonists for many therapeutic uses. Steroids, once given only orally or by injection, can be administered in sustained release formulations such as depot injection, vaginal silicone ring, or subcutaneous silicone rods (see Controlled release technology, pharmaceutical; Drug delivery systems).

The many advances in molecular biology have aided in understanding the actions of progestins at the receptor and genomic level. The interaction of hormone and receptor is an active area of research, the results of which are the progestin agonists and antagonists being developed. As the effects of progestins at the molecular level are better understood, precise assays for progestin action have been devised. Progestin agonists and antagonists are being evaluated for the ability to stimulate the transcription of specific genes in cell culture.

1. Synthesis of Steroidal Agonists

Progestins are derivatives of a planar tetracyclic structure and fall into one of four structural classes: 5α-pregnanes 2, 5α-androstanes 2, estranes 2, and gonanes 2 (Fig. 2). Substituents that lie above the plane of the ring system are assigned a β-stereochemical configuration, indicated by a bold wedge. Substituents lying



(7) (8) (9) (10)

Fig. 2. The four structural classes of progesterone. Carbon atoms are numbered as shown in (**7**) and (**8**) and the rings are referred to as detailed in (**9**).

below the ring system are termed α-substituents and are joined to the ring system by a dashed line. Angular methyl groups at C-10 and C-13 have the β-configuration and are usually indicated by solid bonds only. When there are no methyl groups in these positions the structures are referred to as 19-nor and 18-nor compounds, respectively. Tertiary hydrogen atoms at C-5, C-8, C-9, C-14, and C-17 are generally not indicated unless their stereochemistry differs from 2 (14).

Extracts of corpora lutea were known in the early twentieth century to inhibit ovulation in animals. Pure progesterone 1, the active component of the extracts, was isolated in 1934 and its structure reported (15). Several problems limited its use and drove efforts to develop progesterone analogues, ie, it was available only in small quantities from animal sources, was not orally active, and was discovered to cause androgenic side effects.

The isolation of progesterone from animal sources has never been commercially viable, although enough has been purified to allow early pharmacological studies. Progress in improving the biological profile of progestins, therefore, depended on finding a source of steroid precursors. Three methods of obtaining steroids have been developed for the commercial preparation of progestins, ie, chemical degradation of steroids isolated from plants, microbial degradation of steroids isolated from plants, and total chemical synthesis. Advantages of using naturally occurring steroids as raw materials include availability of large quantities and presence of the required tetracyclic ring structure possessing the correct stereochemical orientation. Chemical degradation methodology was developed first. The discovery of mutant strains of bacteria that incompletely degrade steroids complemented the chemical degradation routes. Microbial degradation allowed the use of plant materials not amenable to chemical modification and the synthesis of precursors not easily produced chemically. The total synthesis of steroids from materials that do not contain the steroid nucleus allow the synthesis of steroids containing structural features not found naturally. Industrial processes (ca 1994) for the synthesis of progestins make use of all three methods (16, 17). Most progestins are prepared from a few steroidal precursors.

1.1. Pregnanes

The first process to produce progesterone in quantity (15, 18) used diosgenin [512-04-9] 3 isolated from the Mexican yam, a species of *Dioscorea*, as the starting material (Fig. 3). Treatment of 3 with hot acetic anhydride in the presence of a catalyst, eg, *p*-toluenesulfonic acid, provides a dihydrofuran. Oxidation of the dihydrofuran with chromium trioxide cleaves the side chain and results in the desired C-20 ketone 3. Treatment of this ester with acetic anhydride gives 16-dehydropregnenolone acetate [979-02-2] 3 and selective reduction of the conjugated ketone by catalytic hydrogenation provides pregnenolone acetate [1778-02-5] 3. Hydrolysis of the acetate and oxidation with aluminum tri-*tert*-butoxide, ie, Oppenauer oxidation, accompanied by *in situ* migration of the double bond, gives progesterone 1. Several modifications in the extraction and degradation

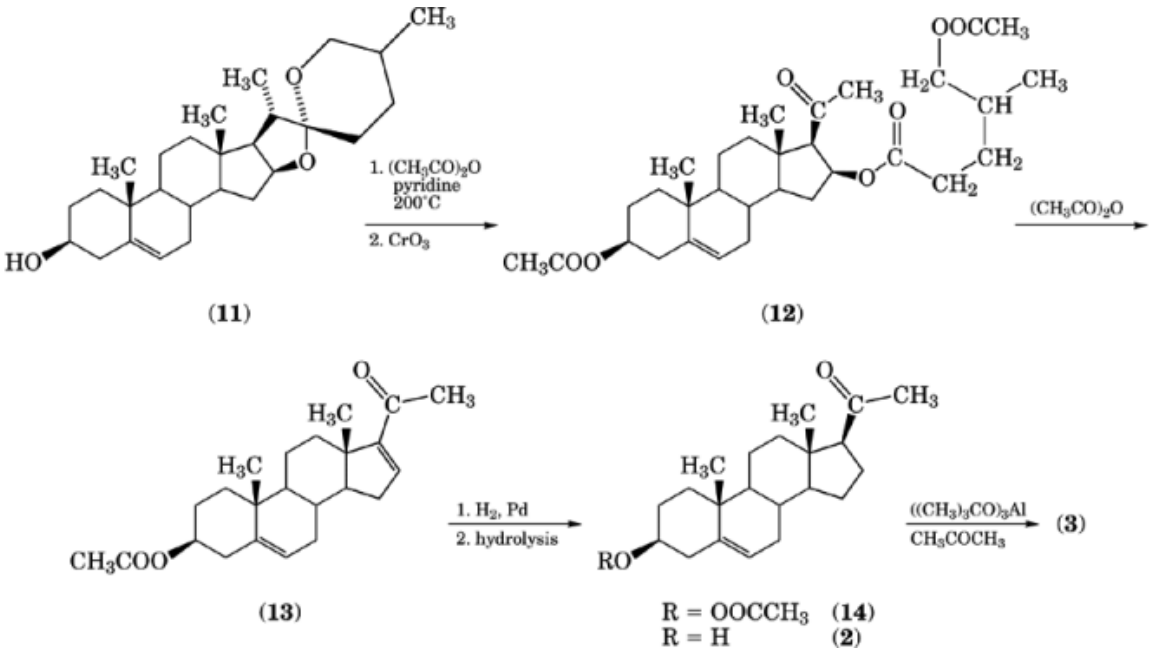


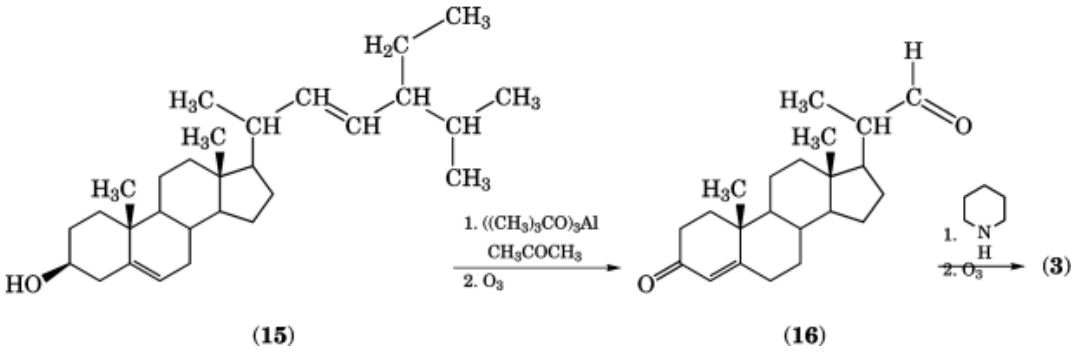
Fig. 3. First degradation process used to produce progesterone.

process have appeared in the literature (19, 20). Extensive surveys have been made of plant materials in an effort to find the optimal raw material for processing (18, 19); diosgenin isolated from *Dioscorea composita*, barbasco root, has been found to be the preferred source (21, 22).

It is possible to leave the sapogenin side chain intact while making modifications in the A- and B-rings of the steroid. Conversion of diosgenin to its tosylate, followed by solvolysis, induces the *i*-steroid rearrangement. Oxidation using chromium trioxide—pyridine provides a ketone which upon reaction with methyl magnesium iodide gives a mixture of alcohols that are thus dehydrated under acidic conditions. After degradation of the side chain, the mixture is carried on to progestins, such as elengestrol acetate [2919-66-6], $C_{25}H_{32}O_4$, and medrogestone [977-79-7], $C_{23}H_{32}O_2$ (23).

At one time, the processing of diosgenin 3 provided most of the supply of steroid raw materials (16). The development of microbial degradation processes and total synthesis, together with a rapid rise in the price of diosgenin in the 1970s, has led to the increased use of other processes (16, 24). Nevertheless, diosgenin remains an important source of steroid raw materials, particularly in countries with indigenous sources (19).

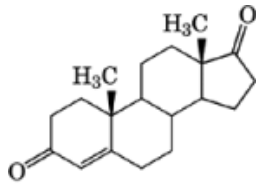
Soybeans provide another rich source of plant steroids. A process has been developed by Upjohn to isolate and purify one of the components, stigmasterol [83-48-7] (**15**) (25). Stigmasterol contains a double bond in its side chain making it amenable to chemical degradation. Treatment of stigmasterol under Oppenauer oxidation conditions results in the oxidation of the A-ring alcohol and migration of the double bond. Ozonolysis of the side chain leads to aldehyde (**16**). Formation of the enamine and further degradation through ozonolysis provides progesterone 1 (25).



(15) (16)

1.2. Androstanes

Androstanes, ie, pregnanes without the C-17 side chain, are important intermediates in the synthesis of progestins. One approach to the synthesis of androstanes is simply side-chain degradation of pregnanes. 16-Dehydropregnenolone acetate 3, an intermediate in the diosgenin to progesterone scheme, may be converted to androstenedione [63-05-8] (**17**) 16-Dehydropregnenolone acetate 3 is converted to its oxime. Treatment of the oxime with a Lewis acid induces a Beckmann rearrangement providing an enamide; hydrolysis and Oppenauer oxidation of the resulting alcohol forms androstenedione (**17**) (22).



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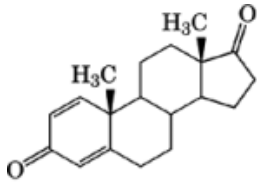


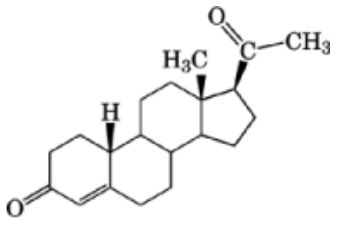
(18)

Microbial processes for transforming steroids such as cholesterol 1 or sitosterol, directly into androstanes are available (26). Neither steroid can be used easily as a substrate in the chemical degradation pathways owing to their relatively inert side chains. As a result sitosterol was an unused by-product of the stigmasterol (**15**) extraction from soybeans until it was discovered that it can be transformed into androstenedione (**17**) and androstadienedione [897-06-03] (**18**) by *Mycobacterium fortuitum* mutants (25, 27). Cholesterol 1 has been degraded with *Mycobacterium phlei*, with added nickel sulfate, to androstadienedione (28). The use of microorganisms to degrade steroids continues to be examined as a source of raw materials (29).

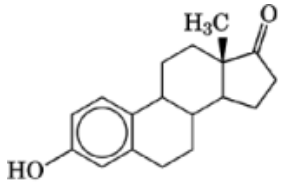
Methods for reconstructing the C-17 side chain from intermediates such as androstendione have been developed. These are used when commercial considerations favor the production of androstanes, but pregnanes are the ultimate goal (25, 30).

1.3. Estranes

Estranes lack the C-19 methyl group found in pregnanes and androstanes and are commonly referred to as 19-norsteroids. As a class, they represent many of the oral contraceptives on the market as of 1994 (see Contraceptives). Interest in 19-norsteroids resulted from the observation that 19-norprogesterone [472-54-8] (**19**) possesses high oral progestational activity (31). Small quantities of norprogesterone were produced from strophanthidin [66-28-4], $C_{23}H_{32}O_6$, through a complex multistep procedure. The final oily product probably does not possess the same stereochemistry as progesterone at C-14 and C-17; it is known that small changes in steroid structure often result in loss of activity (32). The Birch reduction, a method developed for the reduction of aromatic rings to dihydrobenzenes, provides an improved route to 19-norprogesterone (33). Application of the Birch reduction allows the synthesis of stereochemically pure, crystalline 19-norprogesterone in sufficient quantities to be fully evaluated (32). It has proven to be four to eight times as biologically active as progesterone 1 (34).



(**19**)



(**20**)

Interest in 19-norsteroids, and the expedient route provided to them by the Birch reduction, has led to the need for A-ring aromatic steroids. However, none of the plant steroids available in commercial quantities contained aromatic A-rings (16). The principal difficulty in transforming available plant steroids into aromatic A-rings is the presence of the angular methyl group at the A–B ring junction. The methyl group is unactivated, making its removal relatively difficult. *In vivo* removal is accomplished enzymatically and is a key step in the biosynthetic conversion of androgens to estrogens (35). Loss of the methyl group and subsequent aromatization of the A-ring through chemical methods has been accomplished (36). Passing a solution of androstadienedione (**18**) in mineral oil through a hot tube at approximately 500°C yields estrone [53-16-7] (**20**) in reasonable amounts (37).

Several alternative methods followed this early work. In one, aromatization is effected by treating the ketal of androstadienedione with the radical anion obtained from lithium and diphenyl in refluxing tetrahydrofuran. Diphenylmethane is added to quench the methyllithium produced from the departing methyl group. An acidic work-up assures hydrolysis of the ketal, providing estrone (**20**) in good yield (38). In another method, elimination of the unactivated 19-methyl group is accomplished via intramolecular functionalization of C-19 (39, 40). Figure 4 illustrates one route applicable to a wide variety of 5-ene-3-ols. The acetate 4 is converted to bromohydrin 4. Irradiation of 4 in the presence of lead tetraacetate, iodine, and calcium carbonate provides the C-19

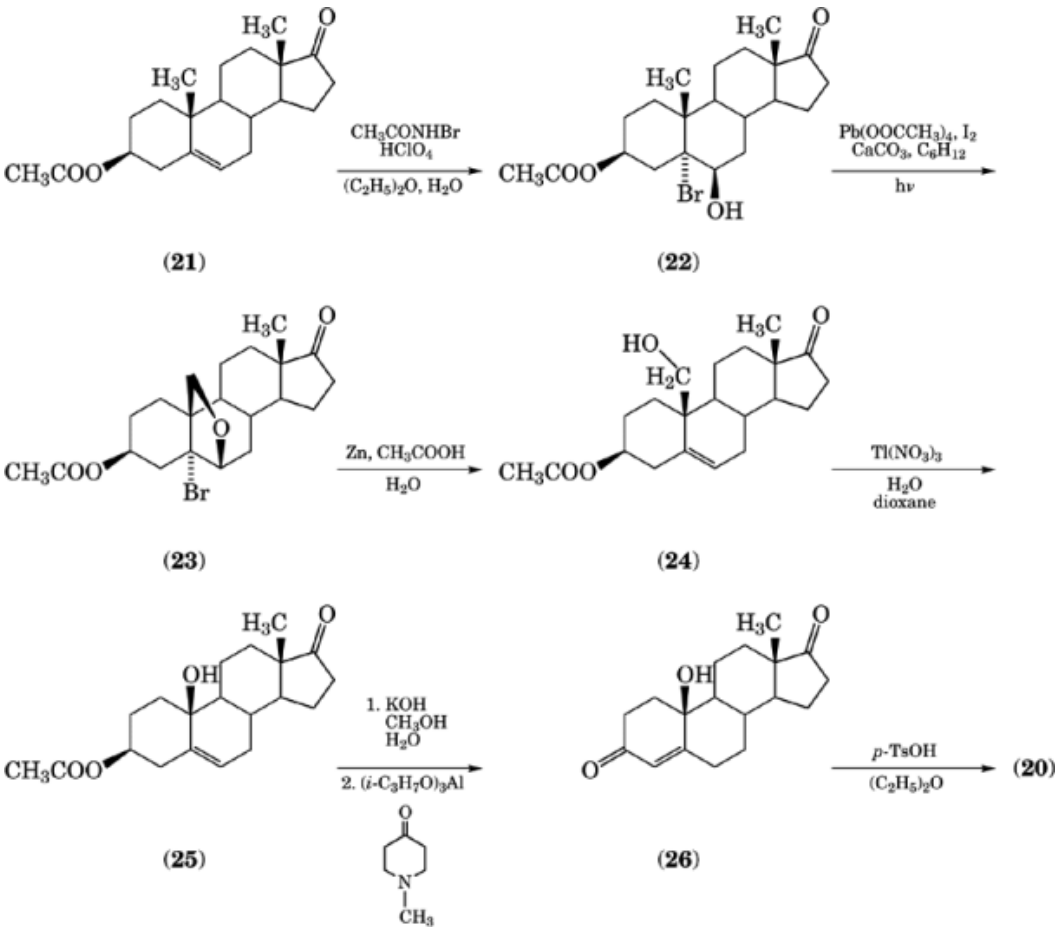


Fig. 4. Synthesis of estrone (**20**) through elimination of unactivated 19-methyl group via intramolecular functionalization of C-19. *p*-TsOH $_{=p-\text{toluenesulfonic acid}}$.

functionalized cyclic ether 4. Treatment of 4 with zinc in acetic acid/water provides the 19-hydroxy derivative 4. This intermediate can be converted to a 19-nor derivative in several ways (40–42). In one method, the conversion of 4 to estrone (**20**) is accomplished by treatment of 4 with thallium nitrate to provide the acetate 4, which is saponified and oxidized to 4 using modified Oppenauer conditions (43). Enone 4 is treated with *p*-toluenesulfonic acid to give estrone (**20**) (42).

The preparation of estranes via 19-hydroxy steroids has also been accomplished microbially. 19-Hydroxycholesterol, prepared chemically (39), is incubated with *Nicardia restrictus* to provide estrone (**20**) directly (44). Similarly, 19-hydroxyandrost-4-en-3,17-dione is converted to estrone (**20**) (45).

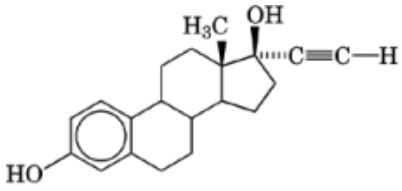
A direct, one-step aromatization of 19-substituted steroids has appeared in the literature, ie, cholesterol 1, dehydroisoandrosterone, androsterone, progesterone 1, and testosterone react with an electrophilic ruthenium complex, C^*Ru^+, where Cp* represents η^5-cyclopentadienyl, obtained through protonation of $[Cp^*Ru(OCH_3)]_2$ using triflic acid, to provide estrone (**20**) directly (46).

1.4. Gonanes

Progress at dissociating progestagenic from androgenic activity was made with the discovery of 13β-ethyl substituted gonane derivatives, originally prepared by total synthesis. Methodology has since developed that allows these compounds to be prepared from the natural product intermediates discussed in the preceding sections. Compounds of this type include levonorgestrel [797-63-7], norgestrel [6533-00-2], gestodene [60282-87-3], desogestrel [54024-22-5], and norgestimate [35189-28-7].

1.5. 17-Ethynyl Steroids

Although 17-ethynyl steroids do not represent a separate skeletal class, they are significant in the development of an orally active progestin. Progress toward orally active progestins has been spurred by the observation that ethynyl groups, introduced at the 17α-position of stradiol and testosterone, result in orally active compounds (47). Ethynylestradiol [57-63-6] (**27**) is the estrogenic component of most oral contraceptives (ca 1994).



(27)

Ethynyltestosterone (ethisterone) (**6**) was the first marketed orally active progestin and has been used to treat gynecological disorders. Introducing an ethynyl substitution into a 19-norsteroid has led to the synthesis of norethindrone [68-22-4] 5 and norethynodrel [68-23-5] 6, the first two progestins used as oral contraceptives (32, 48). Preparation of norethindrone is outlined in Figure 5 (32). Treatment of the monomethyl ether of estradiol 5 to Birch reduction conditions, followed by hydrolytic work-up, provides 19-nortestosterone 5. Chromium trioxide oxidation affords enone 5. Reaction of 5 with ethyl orthoformate, under controlled conditions, results in the selective protection of the A-ring ketone 5. Addition of the ethynyl group, followed by cleavage of the enol ether with aqueous mineral acid gives norethindrone 5. The route to norethynodrel 6 is shown in Figure 6. Estrone (**20**) is treated with dimethylsulfate to form the methyl ether, and with lithium aluminum hydride to reduce the C-17 ketone. The product 6 undergoes a Birch reduction to provide 6. The enol ether 6 is oxidized to 6. The enol ether formed in the Birch reduction (49) is used as the protecting group during the ethynylation. Subsequent mild hydrolysis maintains the double bond out of conjugation to provide norethynodrel 6 (48). Under more vigorous hydrolytic conditions, ie, mineral acids, the double bond migrates to provide norethindrone 5 (32).

2. Agonists and Antiprogestins

2.1. Steroidal Agonists

A comprehensive list of steroids possessing progestational activity has been compiled (50). Many drugs were originally prepared from optically active natural products and contain one enantiomer. In general they are stable, white to off-white crystalline solids. Because of possible instability of the A-ring, it has been recommended that many of them be stored protected from light (51). Table 1 lists data for a variety of steroidal progestin agonists. A list of progestins used in the United States and Europe is given in Table 2. General references for the analysis of steroids are also available (54).

Table 1. Physical Properties of Progestins[a]

Common name	CAS Registry Number	Structure number	Molecular formula	Mol wt	Melting point, °C	$[\alpha]_D$[b]
allylestrenol	[432-60-0]	(**37**)	$C_{21}H_{32}O$	300.483	79.5–80	+39[c]
chlormadinone acetate	[302-22-7]		$C_{23}H_{29}ClO_4$	404.932	211–212	+8
cyproterone acetate	[427-51-0]	(**41**)	$C_{24}H_{29}ClO_4$	416.943	200–201	
desogestrel	[54024-22-5]	7	$C_{22}H_{30}O$	310.478	109–110	+55
ethynodiol diacetate	[297-76-7]	(**53**)	$C_{24}H_{32}O_4$	384.514	129–132, 126–127	−72.5
gestodene	[60282-87-3]	8	$C_{21}H_{26}O_2$	310.435	198	−185.7
hydroxyprogesterone	[68-96-2]	(**39**)	$C_{21}H_{30}O_3$	330.466	221	+97
hydroxyprogesterone acetate	[17308-02-0]	(**42**)	$C_{23}H_{32}O_4$	372.503	246.5	+76.4
hydroxyprogesterone caproate	[630-56-8]	(**59**)	$C_{27}H_{40}O_4$	428.611	119–121	+61
levonorgestrel	[797-63-7]	(−)-9	$C_{21}H_{28}O_2$	312.451	238–242	−32.4
lynestrenol	[52-76-6]	(**73**)	$C_{20}H_{28}O$	284.441	162–164	−13
medroxyprogesterone acetate	[71-58-9]	10	$C_{24}H_{34}O_4$	386.530	205–209	+66
megestrol acetate	[595-33-5]	(**79**)	$C_{24}H_{32}O_4$	384.514	214–216	+5
norgestrel	[6533-00-2]	9	$C_{21}H_{28}O_2$	312.451	205–207	
norethindrone	[68-22-4]	5	$C_{20}H_{26}O_2$	298.424	203–204	−31.7
norethindrone acetate	[51-98-9]	(**80**)	$C_{22}H_{29}O_3$	340.461	161–163	−33
norgestimate	[35189-28-7]	(**82**)	$C_{23}H_{31}NO_3$	369.25	214–218	+41
progesterone	[57-83-0]	1	$C_{21}H_{30}O_2$	314.476	121–122, 127–131	+192
promegestone	[34184-77-5]	(**83**)	$C_{22}H_{30}O_2$	326.478	152	−262[d]

[a] Ref. 50, unless noted.
[b] $[\alpha]_D$ = specific optical rotation for the sodium D line.
[c] Ref. 52.
[d] Ref. 53.

Table 2. Progestins Marketed in the United States and Europe for Contraceptive and Noncontraceptive Uses

		Primary use[a]				Primary use[a]	
Progestin	CAS Registry Number	C	NC		CAS Registry Number	NC	Progestin
algestone acetophenide	[595-77-7] [24351-94-3]	+		lynestrenol	[52-76-6]	+	+
allylestrenol	[432-60-0]		+	medrogestone	[977-79-7]		+
chlormadinone	[1961-77-9]	+	+	medroxyprogesterone acetate	[71-58-9]	+	+
chlormadinone acetate	[302-22-7]	+	+	megestrol acetate	[595-33-5]		+
cyproterone acetate	[427-51-0]		+	milbolerone			+
demegestone	[10116-22-0]	+		nomegestrol acetate			+
desogestrel	[54024-22-5]	+		norethindrone	[68-22-4]	+	+
dydrogesterone	[152-62-5]		+	norethindrone acetate	[51-98-9]	+	+
ethisterone	[434-03-7]		+	norethindrone enanthate	[3836-23-5]	+	+
ethynodiol diacetate	[297-76-7]	+		norethynodrel	[68-23-5]		+
gestodene	[60282-87-3]	+		norgestimate	[35189-28-7]	+	
gestrinone	[16320-04-0]		+	norgestrel	[6533-00-2]	+	+
gestronol hexanoate	[1253-28-7]		+	norgestrienone	[848-21-5]	+	
hydroxyprogesterone hexanoate	[630-56-8]		+	progesterone	[57-83-0]	+	+
levonorgestrel	[797-63-7]	+	+	promegestrone	[34184-77-5]		+

[a] C = contraceptive; NC = noncontraceptive.

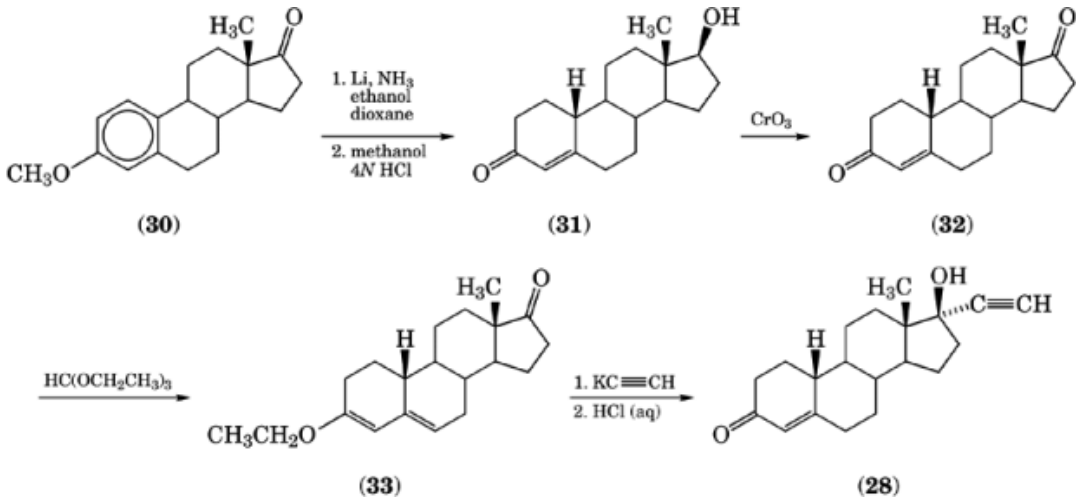


Fig. 5. Preparation of norethindrone (28).

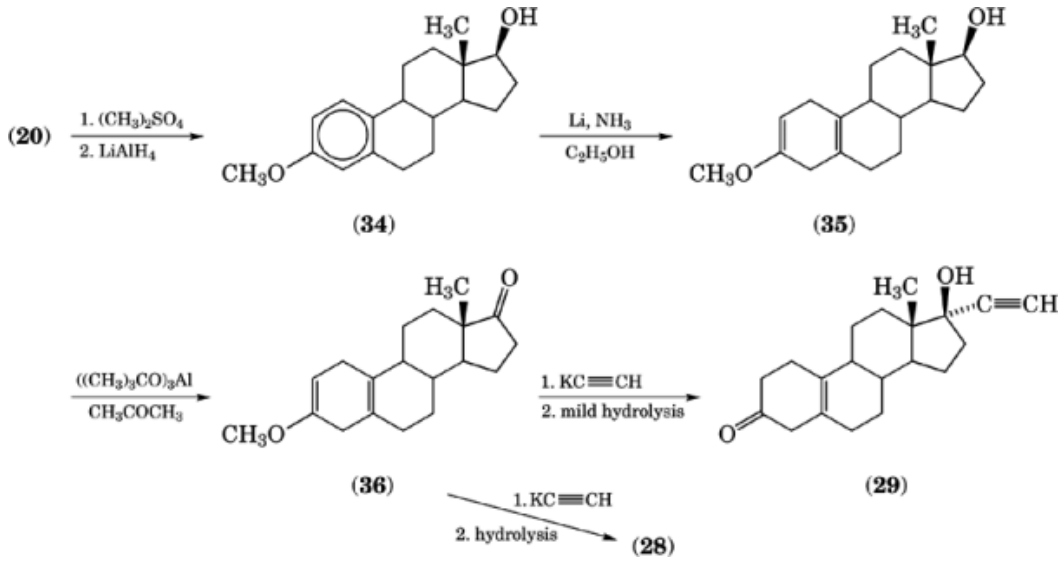
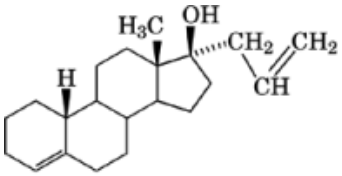


Fig. 6. Preparation of norethynodrel (29).

2.1.1. Allylestrenol

Allylestrenol (**37**), which has been used to treat cases of habitual abortion (55), can be recrystallized from ether/petroleum ether (56). It is soluble in acetone, ethanol, ether, and chloroform and practically insoluble in water (57). The uv and ir spectra have been reported (58). Allylestrenol is sensitive to oxidizing agents (57).

Allylestrenol (**37**) is prepared from 5, an intermediate in the synthesis of norethindrone. Treatment of 5 with ethanedithiol and catalytic boron trifluoride provides a thioketal. Reduction with sodium in liquid ammonia results in the desired reductive elimination of the thioketal along with reduction of the 17-keto group. Oxidation of this alcohol with chromic acid in acetone followed by addition of allyl magnesium bromide, completes the synthesis (52).

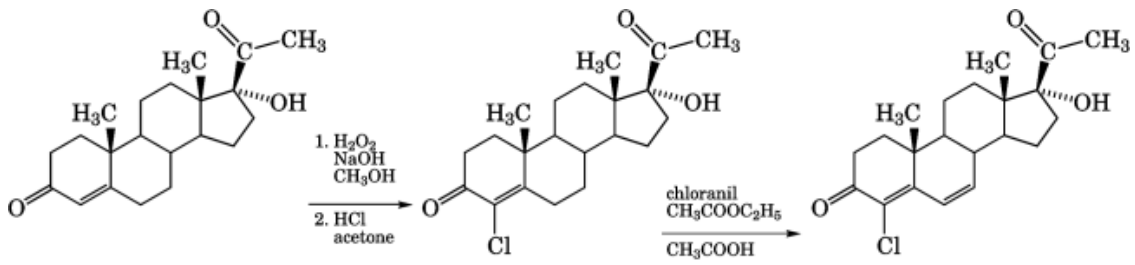


(37)

2.1.2. Chlormadinone Acetate

Chlormadinone acetate is used in combination with ethynylestradiol or mestranol [72-33-3], $C_{21}H_{26}O_2$, in the treatment of menstrual disorders and as an oral contraceptive. It has been reported to be slightly estrogenic (59). It may be recrystallized from acetone/ether (60), methanol, or ether (61) and is soluble in chloroform, benzene, acetone, ethyl acetate, and dimethylformamide, and insoluble in water and hexane (62). The uv and ir spectrum for chlormadinone acetate, along with other chromatographic data, have been summarized (62).

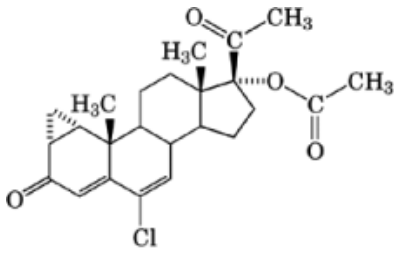
Chlormadinone (**38**) is prepared from hydroxyprogesterone (**39**) by epoxidation and treatment of the epoxide with hydrochloric acid to provide the chloroalkene (**40**). Oxidation, ie, dehydrogenation, with chloranil, provides chlormadinone (**38**), which may be acetylated to provide chlormadinone acetate (63, 64).



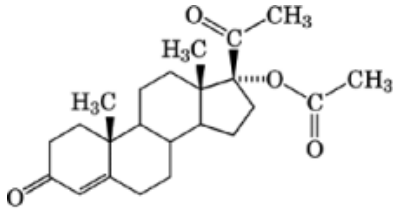
(39) (40) (38)

2.1.3. Cyproterone Acetate

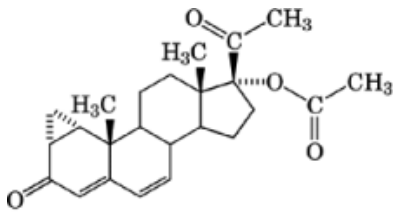
Cyproterone, the free alcohol of cyproterone acetate, is an antiandrogen. Cyproterone acetate (**41**) is both an antiandrogen and a progestin. It is used in prostatic carcinoma treatment and for the control of libido. In females, it is used in conjunction with ethynylestradiol for the control of acne and hirsutism (65). Cyproterone acetate may be recrystallized from diisopropyl ether (66). It may be synthesized from 17-acetoxyprogesterone (**42**). The triene (67) reacts with diazomethane to give a pyrazoline; pyrolysis, to effect loss of nitrogen, provides a cyclopropane (**43**); and oxidation with perbenzoic acid leads to an epoxide. Treatment with hydrochloric acid results in both the desired opening of the epoxide and the undesired cleavage of the cyclopropane. The cyclopropane is re-formed through treatment with collidine to afford cyproterone acetate (**41**) (68–70).



(41)



(42)



(43)

2.1.4. Desogestrel

Desogestrel is used in oral contraceptives. It is a white powder and can be recrystallized from pentane (71). It is practically insoluble in water and its crystal structure has been reported (72).

The structural features that distinguish desogestrel 7 are the absence of the 3-keto group, the presence of an 11-alkylidene, and the 13-ethyl substitution. Figure 7 outlines a route to desogestrel 7 that addresses the synthesis of these features. In this synthesis, the key step is the intramolecular oxidation of bis-ketal 7 to the lactone 7. This allows the transformation of a naturally occurring methyl group into the synthetically derived ethyl group. Treatment of 7 with lead tetraacetate, iodine, and azobisisobutyronitrile [78-67-1] (AIBN) as the radical initiator provides the lactone 7. Grignard reaction with methyl magnesium bromide gives a nearly quantitative yield of a solution phase equilibrium of 7 and 7. Wolff-Kishner reduction of the mixture gives 7. Oxidation with chromium trioxide/pyridine and Wittig reaction of the resulting 11-ketone provides 7. Removal of the oxo-ketals and selective protection of the 3-ketone of 7 as a thioketal yields 7. The synthesis of desogestrel 7 is completed by reaction with potassium acetylide and reductive removal, using Na/NH_3 of the thioketal (73). The introduction of 11-alkylidene groups in various steroid skeletons has been discussed (17, 74). An alternative to the thioketalization/reduction step has been described; ie, 17-β-hydroxy-4-androsten-3-

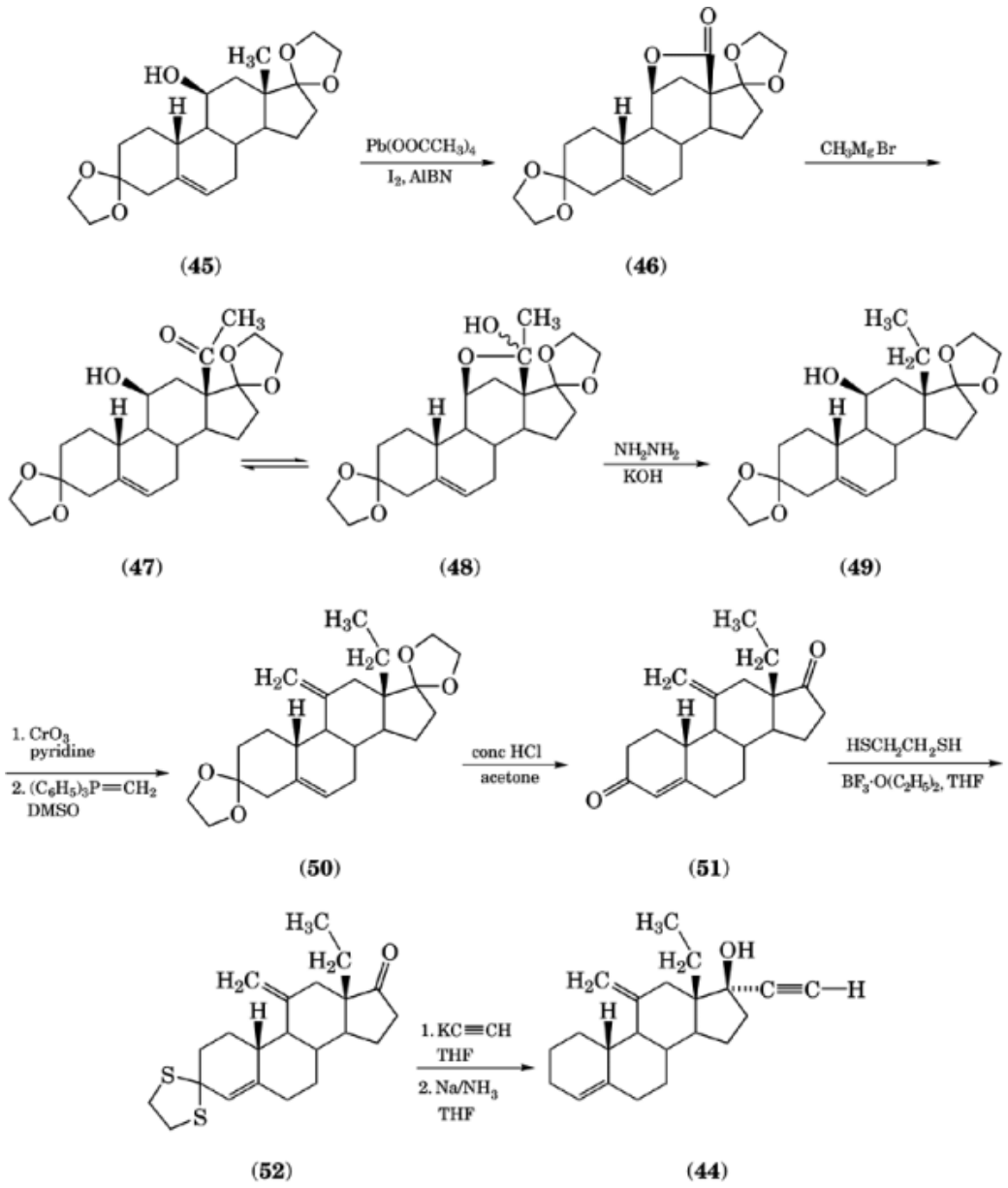


Fig. 7. Synthesis of desogestrel (44).

ones are reduced to the corresponding 17-β-hydroxy-4-androstenes by treatment with sodium borohydride and trifluoroacetic or richloroacetic acid (75).

2.1.5. Ethynodiol Diacetate

Ethynodiol diacetate has been used alone, and in combination with an strogen, as an oral contraceptive and to treat disorders associated with progesterone deficiency (76). It may be crystallized from aqueous methanol

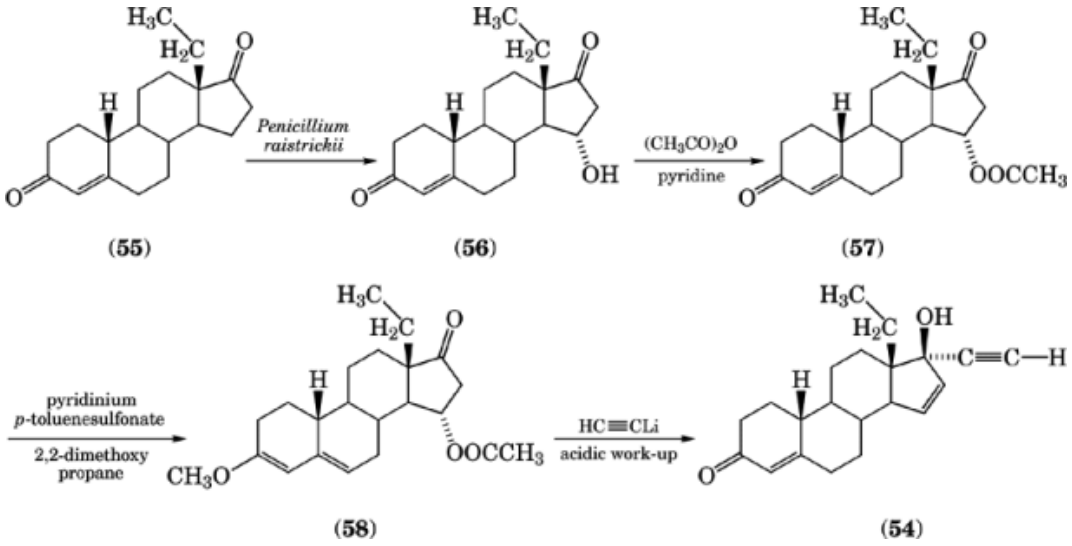
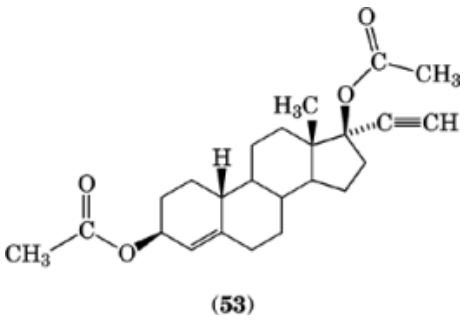


Fig. 8. Preparation of gestodene (54).

(77) and is soluble in chloroform, ether, and ethanol; sparingly soluble in fixed oils; and insoluble in water (76). Extensive spectral and chromatographic data have been compiled (78).

Ethynodiol diacetate (**53**) is prepared by reduction of the 3-oxo group of norethindrone 5 with lithium tributoxyaluminum hydride, followed by acylation with acetic anhydride-pyridine (78, 79). It has been reported that higher yields can be obtained in the reduction step by using triethylaminoaluminum hydride (80).



2.1.6. Gestodene

Gestodene 8, along with norgestimate and desogestrel, are the progestin components of the third-generation oral contraceptives (see Contraceptives). It may be crystallized from hexane/acetone (81) or ethyl acetate (82), and its crystal structure (83) and other spectral data have been reported (84).

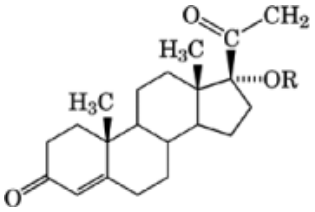
Gestodene has been prepared in several ways (85). The route that provides the highest yield is shown in Figure 8. Microbial oxidation of 8 with *Penicillium raistrickii* results in the 15-alcohol 8. Protection of the alcohol as the acetate 8 and protection of the ketone as a dienolether provides 8. In a one-pot procedure 8 is treated with lithium acetylide and subjected to a hydrolytic work-up to provide gestodene 8 (86).

2.1.7. Hydroxyprogesterone Acetate and Caproate

Hydroxyprogesterone (**39**) itself does not possess progestational activity, but its esters are active progestins. Hydroxyprogesterone caproate (**59**) is used mainly to treat threatened or habitual abortion (87). Although other esters are known (88), the caproate is the most widely used. The ester group of hydroxyprogesterone caproate is resistant to hydrolysis (89).

Hydroxyprogesterone is a colorless crystalline solid which may be recrystallized from acetone or ethanol (90) and is practically insoluble in ethanol and water (87). Hydroxyprogesterone acetate can be recrystallized from chloroform/methanol (90). Hydroxyprogesterone caproate, ie, hexanoate, can be recrystallized from isopropyl ether or methanol (90) and is soluble in chloroform, ethanol, ether, benzene, and fixed oils, and insoluble in water (91). An extensive profile of hydroxyprogesterone caproate, including spectral and analytical data, has been compiled (89). The crystal structures of hydroxyprogesterone and hydroxyprogesterone acetate have been discussed (92).

Hydroxyprogesterone (**39**) has been prepared from 3. Treatment of 3 in alkaline hydrogen peroxide provided an epoxide. Opening of the epoxide is accomplished by reaction with hydrogen bromide in acetic acid. Reductive removal of the bromide is performed in the presence of ammonium acetate, which prevents reduction of the double bond. The reaction mixture is heated with formic acid to provide the formate which is acylated. Subsequent Oppenauer oxidation (43) provides hydroxyprogesterone acetate (**42**). Saponification of the acetate with potassium hydroxide in methanol provides hydroxyprogesterone (**39**). This method is efficient in that direct oxidation of the formate avoids a deprotection step (93). Hydroxyprogesterone acetate (**42**) and hydroxyprogesterone caproate (**59**) also have been prepared from hydroxyprogesterone (**39**) (94).



R = H (**39**)
R = $COCH_3$ (**42**)
R = $CO(CH_2)_4CH_3$ (**59**)

2.1.8. Levonorgestrel and Norgestrel

Both of these compounds are used in oral contraceptives. Norgestrel 9 is a racemic mixture; the biologically active isomer is known as levonorgestrel or (−)-norgestrel 9. Norgestrel can be recrystallized from ethyl acetate or methanol (95), and levonorgestrel can be recrystallized from chloroform—methanol (95). Both compounds are soluble in chloroform, slightly soluble in ethanol, and practically insoluble in water (96). Extensive physical, spectral, and analytical properties have been compiled (97).

Compound 9 in Figure 9, obtained by the reaction of 6-methoxytetralone and vinyl magnesium bromide, is condensed with 2-ethyl-1,3-cyclopentanedione 9. Cyclization of 9 to 9 is effected with hydrochloric acid in ethanol. Reduction of the D-ring double bond, 17-keto group, and finally the B-ring double bond provides 9. Birch reduction and Oppenauer oxidation, followed by addition of acetylide, completes the synthesis of norgestrel 9 (98). Early syntheses of levonorgestrel relied on chiral separation to obtain optically active material (98). Later, chiral syntheses were developed (99, 100). One chiral synthesis is shown in Figure 9 (85). The optical activity is obtained through an asymmetric aldol and subsequent dehydration of the indanedione 9 (101). The chiral hydrindane 9 reacts with paraformaldehye and benzenesulfinic acid to provide 9, which is hydrogenated to form sulfone 9. Sulfone reacts with the anion formed from 9 to provide the crude product 9, which is cyclized, hydrolyzed, and decarboxylated, resulting in 9. Hydrogenation, followed by treatment with acid to effect ketal cleavage and cyclization, leads to 8, which can be further converted to levonorgestrel (−)-9 by ethynylation (86).

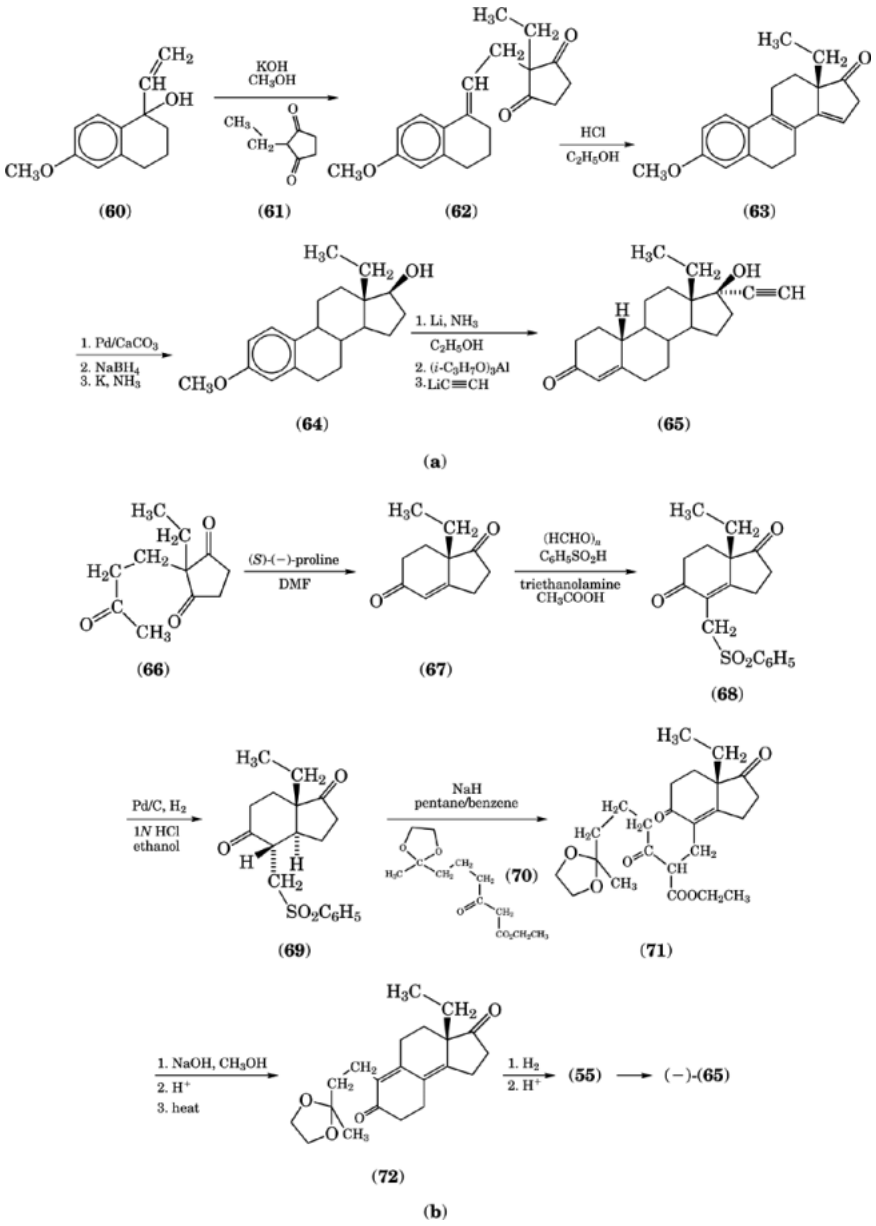
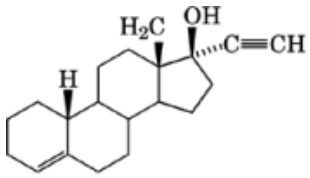


Fig. 9. Synthesis of (**a**) norgestrel and (**b**) levonorgestrel (−)-(**65**).

2.1.9. Lynestrenol

Lynestrenol (**73**) has been used in oral contraceptives and to treat menstrual disorders. It is converted *in vivo* to its active metabolite norethindrone (102, 103). It can be recrystallized from methanol, and is soluble in ethanol, ether, chloroform, and acetone, and insoluble in water (102). The crystal structure (104) and other spectral and analytical data have been reported for lynestrenol (62).

Lynestrenol is the des-3-oxo derivative of norethindrone 5. It has been prepared through a similar synthetic pathway as allylestrenol (**37**) (52), ie, addition of potassium acetylide, rather than allyl magnesium bromide, affords lynestrenol (**73**). Lynestrenol is also available from norethindrone 5. Reduction of the 3-keto group is accomplished by treating norethindrone 5 with sodium borohydride in the presence of trifluoro- or trichloroacetic acid (75).



(**73**)

2.1.10. Medroxyprogesterone Acetate

Medroxyprogesterone acetate (**74**), ie, Depo-Provera (Upjohn), is used as an injectable, long-acting contraceptive. It is also used in the treatment of menstrual disorders, endometriosis, and in the palliative treatment of hormone-responsive malignant neoplasms. Medroxyprogesterone acetate can be recrystallized from methanol (105). It is soluble in chloroform, acetone, and dioxane; sparingly soluble in ethanol and methanol; slightly soluble in ether; and insoluble in water (102). Its crystal structure (92) and other spectral and analytical data have been reported (62).

Medroxyprogesterone acetate (**74**) is structurally related to and has been prepared from hydroxyprogesterone (**39**) (Fig. 10). Formation of the bis-ketal accomplishes the protection of the ketones and the required migration of the double bond. Epoxidation with peracetic acid produces a mixture of epoxides 10, with α predominating. Treatment of the α-epoxide with methyl magnesium bromide results in diaxial opening of the epoxide. Deprotection of the ketones provides 10, which is dehydrated to 10 by treatment with dilute sodium hydroxide in pyridine. Upon treatment with gaseous hydrochloric acid in chloroform, the 6β-methyl in 10 is epimerized to the more stable 6α-methyl 10. Acylation of 10 completes the synthesis of medroxyprogesterone acetate (**74**) (106).

2.1.11. Megestrol Acetate

This compound is used outside the United States as an oral contraceptive. In the United States, it is used for the palliative treatment of breast cancer and endometrial cancer, or as an adjunct to other therapies. Its use has been associated with an increased appetite and food intake and has been evaluated in the treatment of anorexia and cachexia (107).

Megestrol acetate can be recrystallized from aqueous methanol (108). It is soluble in acetone, chloroform, and ethanol; slightly soluble in ether and fixed oils; and insoluble in water (107). Additional spectral and physical data have been published (62).

Megestrol acetate (**79**) is structurally related to progesterone 1. It has been prepared from medroxyprogesterone acetate (**74**) by chloranil-mediated dehydrogenation. It also has been prepared from hydroxyprogesterone acetate (**42**) via 6-methylenation and double-bond migration (109, 110).

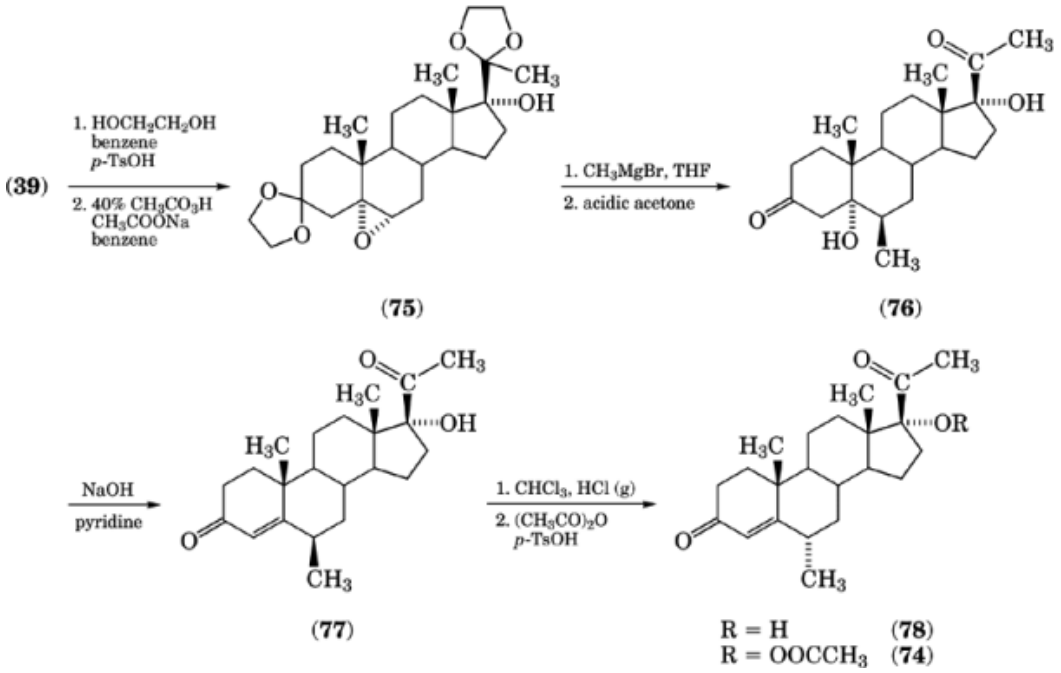
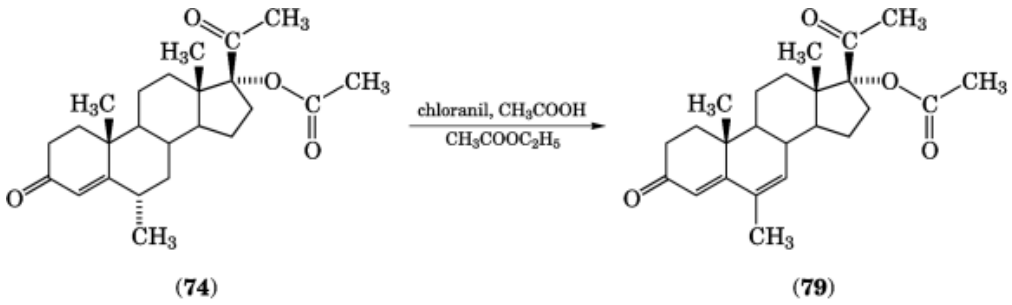
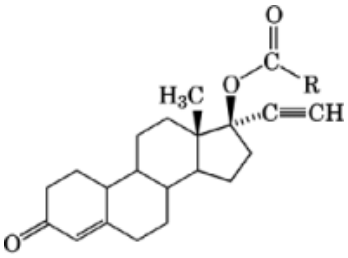


Fig. 10. Preparation of medroxyprogesterone acetate (74) and medroxyprogesterone (78) from hydroxyprogesterone (39). *p*−TsOH=*p*−toluenesulfonic acid.

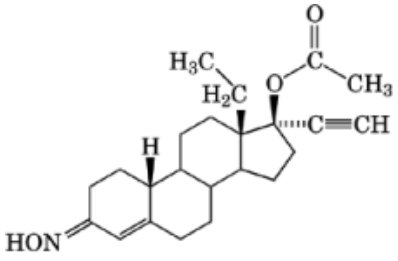


2.1.12. Norethindrone and Norethindrone Acetate

Norethindrone 5 and its acetate or enanthate are used in oral contraceptives. Norethindrone may be recrystallized from ethyl acetate (111). It is soluble in acetone, chloroform, dioxane, ethanol, and pyridine; slightly soluble in ether, and insoluble in water (112, 113). Its crystal structure has been reported (114), and extensive analytical and spectral data have been compiled (115). Norethindrone acetate can be recrystallized from methylene chloride/hexane (111). It is soluble in acetone, chloroform, dioxane, ethanol, and ether, and insoluble in water (112). Data for identification have been reported (113). The preparation of norethindrone 5 has been described (see Fig. 5). Norethindrone acetate (**80**) is prepared by the acylation of norethindrone. Norethindrone esters have been described; ie, norethindrone, an appropriate acid, and trifluoroacetic anhydride have been shown to provide a wide variety of norethindrone esters including the acetate (**80**) and enanthate (**81**) (116).



R = CH_3 (**80**)
R = $(CH_2)_5CH_3$ (**81**)



(**82**)

2.1.13. Norgestimate

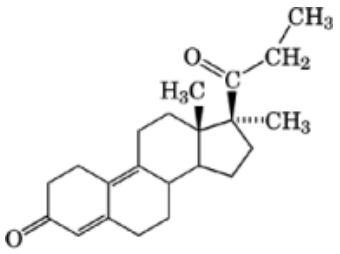
Norgestimate (**82**) is used in oral contraceptives. It can be recrystallized from methylene chloride (117) and is the 17-acetoxy-3-oxime derivative of levonorgestrel. It may be prepared from levonorgestrel by bisacylation, hydrolysis of the enol acetate, and oximation (118, 119). In an alternative route, the A-ring is formed through cyclization to provide an enamine. Hydrolysis and formation of the oxime affords norgestimate (**82**) (120).

2.1.14. Progesterone

Progesterone 1 is not orally active. Although seldom used clinically, it can be administered as an intramuscular injection, pessaries, or suppositories in the treatment of menstrual disorders and habitual abortion (121). Progesterone can be recrystallized from dilute alcohol and exists in two crystalline forms (122). It is soluble in chloroform and ethanol; sparingly soluble in acetone, dioxane, ether, and fixed oils; and practically insoluble in water (121). Two syntheses of progesterone 1 are described in Figure 3.

2.1.15. Promegestone

Promegestone (**83**) or R5020, is an antineoplastic agent and is used in the treatment of menstrual disorders. Tritiated R5020 is widely used as a radioligand in progestin-binding studies (see Radioactive tracers) (123). Promegestone can be recrystallized from isopropyl ether, and is soluble in acetone and benzene and insoluble in water (53). In the synthesis of promegestone (**83**) (124), an enone is reduced with lithium in ammonia and the intermediate enolate is quenched with methyl iodide (125). The resulting compound is then subjected to Birch reduction and the intermediate enol ether is hydrolyzed under mild conditions to maintain the double bond out of conjugation. The resulting alcohol is oxidized, dehydrohalogenated, selectively protected then alkylated and hydrolyzed, to afford promegestone (**83**) (124–127).

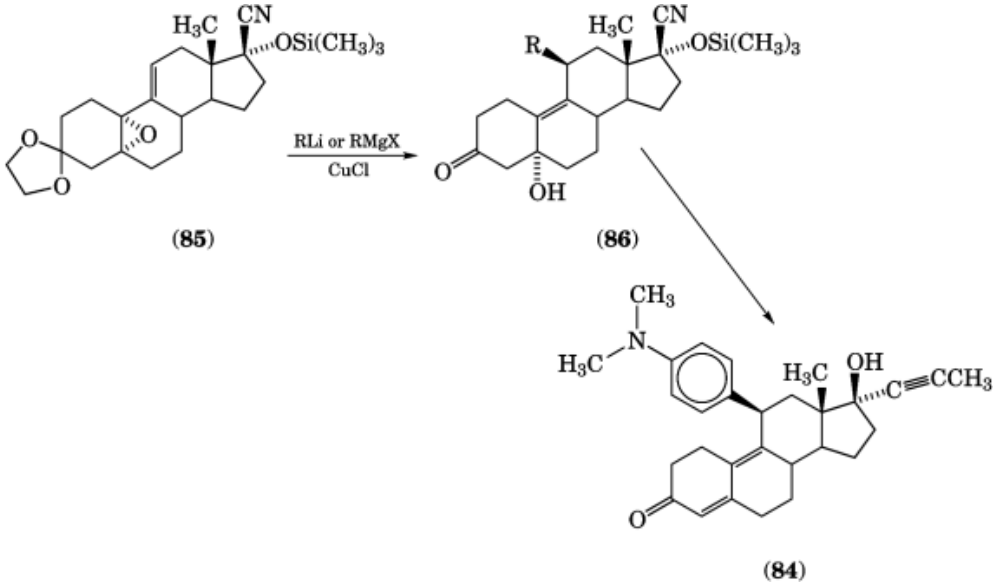


(**83**)

2.2. Steroidal Antiprogestins

A significant breakthrough in the progestin antagonist area was the discovery of the 11β-substituted progestin antagonists. The first of these compounds to be used clinically was RU 38486, ie, RU 486 or mifespristone [84371-65-3] (**84**). It has proven to be a potent antiprogestin, but has the liability of also displaying antiglucocorticoid activity. Although this activity has been exploited in the treatment of Cushing's syndrome, for antiprogestational applications, a compound with reduced antiglucocorticoid activity is desirable. Synthetic changes have been made in an effort to improve the antiprogestational activity of RU 38486 while minimizing antiglucocorticoid activity (128–130).

The methodology used in the preparation of RU 486 (**84**) and other 11β-steroids is shown. Conjugate addition of a cuprate reagent to the α,β-unsaturated epoxide (**85**) provides the 11β-substituted steroid (**86**) stereospecifically (131). Subsequent steps lead to the synthesis of RU 486 (**84**).



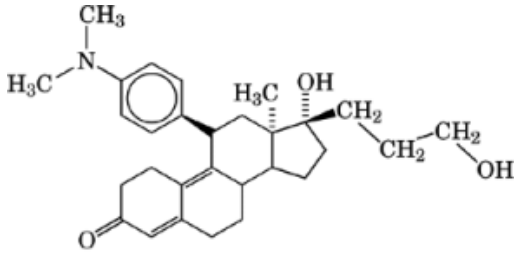
(**85**) (**86**) (**84**)

A wide variety of R groups have been added using this methodology. Provided that the corresponding Grignard or lithium reagent is available, all but the most bulky substituents can be added. The assignment of the β-orientation is based on both nuclear magnetic resonance (nmr) and x-ray crystallographic data (132).

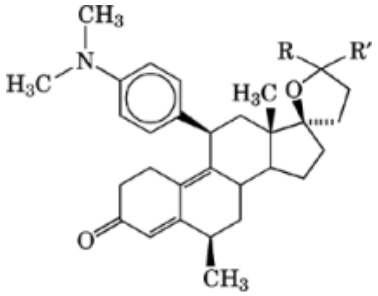
A series of compounds with an epimeric methyl at C-13 has been prepared. A modification of the Teutsch route starting from a deconjugated ketone is used in the synthesis (132, 133). Dehydrogenation (126) provides

the dienone, which is protected as a ketal. 2,2-Dimethyl-1,3-propanediol is used to protect the ketone because it provides a more crystalline product than is obtained with ethylene glycol (134). Selective epoxidation, addition of the 11β-phenyl substituent, and oxidation provide the substrate for epimerization. Irradiation of this substrate leads to the C-13 epimer. It is preferable to epimerize at this stage, because the stereo- and regioselective epoxidation step used in the synthesis of RU 486 fails in the C-13 epimeric series. In contrast to the natural series, alkynylation of the C-13 epimer occurs less selectively and predominantly from the β-face (135). The alkyne is reduced and treated with acid to effect hydrolysis and dehydration of the C-5 alcohol to afford onapristone [96346-61-1] (ZK 98299) (**87**). On a technical scale, a modification that involved epoxidation followed by epimerization was reported to be the preferred pathway (133). Onapristone has been evaluated clinically. Compounds of this type have been shown to retain antiprogestational effects and to have reduced antiglucocorticoid activity (136).

Introduction of a 6β-methyl substituent, but not a 6α-methyl, into an 11β-phenyl steroid causes a reduction in affinity for the glucocorticoid receptor while maintaining affinity for the progesterone receptor (137). The starting material used is the hydroxy-steroid 7 (see Fig. 7). This is epoxidized and treated with methyl magnesium chloride to provide the desired 6β-methyl product in high yield. This material is then carried through using the chemistry of Teutsch (131) to introduce the 11β-phenyl substituent. Other chemistry (138) is used to introduce the 17α-substitution that ultimately will be converted into the 17-spiro compounds. ORG 31710 (**88**) and (**89**) have an affinity for the progesterone receptor similar to RU 486, whereas (**90**) has less affinity. All compounds have a lower affinity for the glucocorticoid receptor than RU 486 (**84**) (137).



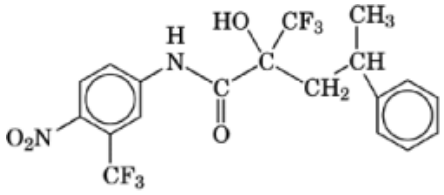
(**87**)



R, R′ = H (**88**)
R = H, R′═OH (**89**)
R, R′ = R═O (**90**)

2.3. Nonsteroidal Agonists and Antagonists

There are no nonsteroidal progestin agonists or antagonists in clinical use. This is in contrast to the wide variety of known nonsteroidal estrogens and androgens (139). Nonsteroidal compounds having affinity for the progesterone receptor or activity typical of such compounds have been reported. A series of acylanilides (**91**), which are structurally related to the antiandrogen flutamide [13311-84-7], has been reported to possess potent progestational activity and no antiandrogenic activity (140).



(**91**)

A closely related series has been reported to be antiandrogenic, and to possess some progestational and antiprogestational activity (141). Other compounds have been reported that either have affinity for the progesterone receptor or demonstrate progestational activity or antiprogestational activity (142–148). The antihormonal action of compounds with modified steroid ring structures has been reviewed (149). Potential mechanisms of action and a chemical classification of nonsteroidal hormone antagonists have been discussed (139). The relative binding affinities for the progesterone receptor of a large number of steroidal and nonsteroidal compounds have been compiled (143).

3. Structure–Activity Studies

An understanding of the ligand–receptor interaction is crucial in the effort to develop more potent and more selective ligands. Ideally, the structure of the receptor and the activity of a variety of ligands are known, and conclusions can be drawn about structure–activity relationships (SAR). Although the amino acid sequence of the progesterone receptor is known, the three-dimensional structure remains unsolved (150). In the absence of the purified progestin receptor, or even of the hormone-binding domain fragment, several techniques have been used to gain insight into ligand–receptor interactions (151). Techniques designed to identify which amino acids are important for ligand binding have been developed, including site-directed mutagenesis (151, 153), affinity labeling (154–158), and reactions of specific amino acids with chemical reagents (159).

Another approach has been to use binding data and structures of ligands to develop a three-dimensional map of the receptor. Extensive work has been done in this area. The picture of the binding pocket that has emerged consists of a hydrophobic core with polar regions able to form hydrogen bonds on each end. There is also a hydrophobic region above and below the plane of the steroid. The large but narrowly defined hydrophobic region above the plane of the steroid is associated with agonist versus antagonist activity. Except for specific polar interactions, steroid–ligand binding is generally considered to be hydrophobic in nature (160–163). Antibodies have been raised to steroids in an effort to examine how steroids interact with receptors (164).

The prediction of protein structure through computational techniques has been applied to steroid hormone receptors. Hydrophobic cluster analysis (HCA) has been used to develop a model of the hormone binding domain of steroid receptors. Using HCA as a first approximation, the hormone-binding domains are proposed to be similar to a cleaved portion of human α_1-antitrypsin, the crystal structure of which is known (151). Another

analysis has predicted that the hormone-binding domains of steroid receptors are similar to the substilisin-like serine proteases (165). Both models await further validation through comparison with biological data and, hopefully, the eventual solution of the three-dimensional structure of the progestin receptor.

3.1. Modes of Structural Studies

Determining the three-dimensional structure of relatively small steroids has been accomplished through x-ray crystallography and nuclear magnetic resonance (nmr) spectroscopy. If the structure is unknown or theoretical, or the compound is difficult to crystallize, molecular modeling techniques may be employed to predict the preferred conformation. This is a much easier task for the relatively small ligands, as compared to large receptors. From these techniques geometrical, conformational, and electronic information may be obtained. Many studies have focused on the conformation of the A-ring, as it is believed to be important in receptor binding (161); however, each method has its limitations. The structure of a steroid in its natural environment may not be the same as that obtained by any of these methods due to crystal packing forces, solvent effects, or an error in minimizing the structure.

3.1.1. Nuclear Magnetic Resonance Spectroscopy

The data from nmr studies complement information obtained by x-ray crystallography in that nmr is performed in the solution phase (see Magnetic spin resonance). Selected studies have used nmr to draw conclusions about steroid structure or SAR. In a study of three 19-nor-4-en-3-ones, the normal A-ring conformation has been shown to predominate (166). An nmr study of 10 4-ene-3-one steroids has found that the normal $1\alpha,2\beta$-half-chair conformation predominates in all but one 2β-acetoxy-substituted steroid. A-Ring conformations have been found to vary less in solution than in the solid phase (167). The A- and D-ring conformations of 17 5α-androstanes and one pregnane have been discussed based on observed nmr data and calculated minimized structures. The A-ring sofa and D-ring half-chair/envelope intermediate conformations have been found to be preferred in solution (168).

3.1.2. X-Ray Crystallography

Structural data exists on more than one thousand steroids. Comparisons of the conformations obtained by x-ray crystallography have been made to the conformations obtained by other methods (161). Several studies use x-ray crystal structures in the study of progestins.

Crystal structures of phenylseleno-substituted progesterone analogues support the theory that a hydrogen-bond donor is present in the receptor toward O-20 in the β-region of C-16; eg, 21-(phenylseleno)progesterone bound with moderate affinity to the progesterone receptor, but 17α-(phenylseleno)progesterone bound with very little affinity. From the crystal structures, it has been observed that in the 21-substituted progesterone, the C-16–C17–C20–O20 orientation is closer to that of other compounds known to bind to the progesterone receptor (169). An inverted $1\beta,2\alpha$-half-chair A-ring conformation has been proposed to be the preferred conformation for progestin binding and activity (161). Steroids with affinity for the progestin receptor are able to form hydrogen bonds in the A-ring region. Based on crystal structure data, this hydrogen-bonding region is predicted to be located closer to the C-2 side than to the C-4 side of the ketone and above the β-face of the steroid (163, 170).

3.1.3. Molecular Modeling

Molecular modeling (qv) techniques have been used to predict the conformation of progestins. Combined with computer graphics techniques, such tools allow visualization of a three-dimensional structure. Steric, hydrophobic, and electronic information can be incorporated into the model. The conformation of the progesterone side chain determined from x-ray crystallographic data has been found to be identical to that predicted from molecular mechanics calculations. Calculations using older force-field techniques have led to a discrepancy

between the x-ray and calculated structures (161). When the tendency of a carbonyl group to eclipse neighboring aliphatic bonds is considered, a better correlation is obtained (171). A semi-empirical, all-valance-electron molecular orbital method successfully predicts the x-ray crystal structure of progesterone and these results imply the structures of other steroids can be calculated (172). A study of 4-ene-3-ones in the androstane, estrane, and estradiene classes has been reported. To exclude the possibility of crystal packing forces, structures are determined by molecular mechanics calculations. 10-Methyl group steric hindrance and/or steroid backbone flexibility play an important role in ligand binding (173).

3.2. Quantitative Structure–Activity Relationships

Many quantitative structure–activity relationship (QSAR) studies of progestins have appeared in the literature and an extensive review of this work is available (174). QSAR studies attempt to correlate electronic, steric, and/or hydrophobic properties to progestational activity or receptor binding affinity. A review focusing on the problems associated with QSAR of steroids has been published (175).

Examination by ^{13}C nmr of eight analogues of norethindrone, where structures varied at the C-11, C-18, and $\Delta^{15,16}$ position, suggests a correlation between the ^{13}C-resonance of C-17 and the relative binding affinity (RBA) to the progesterone receptor and to the progestin/androgen RBA ratios (176). A nonlinear relationship has been found between the lipophilicity of esters of norethindrone and levonorgestrel (177). The lipophilicity is expressed in terms of high performance liquid chromatography (hplc) retention times. It has been proposed that the rate of release and dissolution of the esters, as well as partitioning through biological membranes, not *in vivo* ester hydrolysis, are the rate-determining steps in obtaining biologically active drug. A pilot study has attempted to find a relationship between the calculated lipophilicity and steric effects of a 13β-substituent and progestational potency. The activity of the 13β-ethyl and 13β-acetyl pregnanes have been measured by the subcutaneous Clauberg assay (178). Binding energies of progesterone analogues, calculated from their binding affinities for the rabbit uterine progesterone receptor, have been used to draw conclusions about receptor–ligand interactions. Binding is attributed to hydrogen bonds involving the C-3 and C-20 carbonyls, and van der Waals' interactions at C-2, C-4, C-7, C-9, C-12, C-18, and C-19. A greater distance between the ligand and the receptor occurs at C-6, C-11, C-14, C-15, C-16, and C-21. An extensive discussion of structure and binding affinity correlations has been presented (179). A successful QSAR has been obtained for affinity to the progesterone receptor for 55 progesterone derivatives using the minimal steric, ie, topological, method (180).

Two correspondence analysis methods have been described (151, 181). Using the minimum spanning tree method, many test compounds have been found to separate into four main branches corresponding to favorable androgen receptor (AR) binding, progesterone receptor (PR) binding, glucocorticoid receptor (GR) binding, and a nonspecific branch with AR/PR and GR binding. The favored PR branch is characterized by 19-norpregnanes or sultine structures. A related method, that of correspondence factoral analysis, also has been developed (181, 182). This method allows representation of compounds and biological variables on the same two-dimensional graph. The results agree with those found using the minimum spanning tree method, ie, the same compounds show the highest specificities for the tree receptors (151). A comparison of the binding of steroids to the progestin and androgen receptors using the comparative molecular field analysis (CoMFA) technique has been published. Using relative binding affinities from the literature, 48 steroids have been analyzed. Differences in the electrostatic requirements in the C-17 region have been noted. The progestin model is found to be more predictive than the androgen model (183).

4. Mechanism of Action of Progestins and Antiprogestins

Like all steroid hormones, progestins exert their biological effects by altering the rate at which certain genes are expressed in hormone-responsive cells. Steroids bind in a competitive and reversible fashion to hormone-specific

receptor proteins which act as transcription factors regulating ribonucleic acid (RNA) synthesis within the cell nucleus. For the progestin receptor, the binding of steroid is an absolute requirement for the receptor to interact with deoxyribonucleic acid (DNA) and initiate gene transcription in intact cells (184, 185). The progestin receptor has two isoforms, A and B, having molecular weights of approximately 94,000 and 120,000, respectively. The isoforms are produced from the same gene but differ structurally; the B form is 164 amino acids longer at the amino-terminus than the A form (186). The isoforms show similar DNA and hormone-binding affinities (187, 188). The entire amino acid sequence for the human progestin receptor is known and has been cloned (150). The progestin receptor is a member of the same steroid hormone receptor superfamily as the glucocorticoid, mineralcorticoid, estrogen, vitamin D_3, and thyroid receptors. There is considerable structural homology between the members of this receptor superfamily. The structure of the steroid receptors, including the progestin receptor, can be divided into several functional domains, ie, the progestin-binding domain at the carboxyl terminus, the hinge region, the DNA-binding domain, and the variable region at the amino-terminus (189). The structure of the progestin receptor has been under intense study and many reviews are available (190–192).

The binding of hormone to the receptor triggers a number of changes in the receptor that allow the hormone-receptor complex to bind to DNA and stimulate transcription. The occurrence of this ligand-induced transcriptional activity is termed receptor activation. The binding of progestin to the receptor induces the phosphorylation of serine residues in the receptor (193, 194), the release of heat shock proteins normally associated with the hormone-free receptor (195), and the dimerization of activated receptors (196). Because there are A and B isoforms of the progestin receptor, three species of dimers may exist: A:A, A:B, and B:B. All three dimers can bind to the same specific DNA sequences, known as progesterone response elements (196). However, there is evidence that the three dimeric forms may differentially regulate target genes (197, 198). To study the A and B isoforms in isolation, molecular biological techniques have been used to produce cells which express either the A isoform, the B isoform, or some known ratio of the two. In some cell types, the B isoform but not the A isoform activates a specific gene promoter (198). When the A isoform is coexpressed in the same cells as the B isoform, the transcriptional activity of the B isoform is repressed in direct proportion to the amount of A isoform present. Apparently, the A isoform has dual activities, acting both to promote and repress the expression of certain genes. The A and B isoforms show additional differences in their response to progestin antagonists. The A isoform is not transcriptionally activated by progestin antagonists, but only by progestin agonists. In contrast, the B isoform, in the absence of the A isoform, is activated to induce gene transcription by both progestin agonists and antagonists (199, 200). The binding of progestin antagonists induces changes in receptor structure and phosphorylation different from those due to progestin agonist binding (201–203). A monoclonal antibody directed to the carboxyl terminus of the human progestin receptor can differentiate between agonist- and antagonist-receptor complexes (204). It is these ligand-induced changes in receptor structure which are believed to be the basis for the biological effects of progestin agonists and antagonists.

4.0.1. RU 486

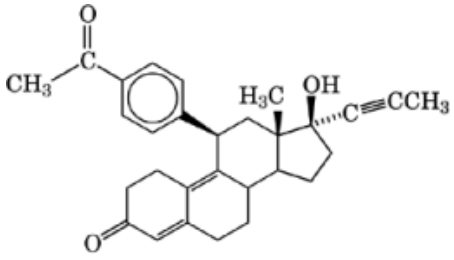
The most well-studied progestin antagonist is RU 486 (**84**). RU 486 is a potent antiprogestin and antiglucocorticoid which has been used in the research laboratory for exploring receptor structure and in the clinic for terminating first-trimester pregnancies. The progestin receptor binding characteristics of RU 486 have been described (205, 206). It binds to both A and B progestin receptor isoforms with high affinity and can induce receptor activation. RU 486 does not, however, bind to the progestin receptors of all species. Human but not chick progestin receptors recognize RU 486 (207). The species differences seen with RU 486 receptor binding result from as little as a single amino acid difference in the structures of the receptor. The substitution of a cysteine at position 575 with a glycine in the chick receptor allows RU 486 to bind to the modified receptor and antagonize agonist-induced gene transcription (208). Substitution with other amino acids containing side chains, ie, methionine or leucine, at position 575 produces receptors with the same properties as the native

chick receptor, suggesting that the presence of an amino acid side chain at this critical position sterically hinders RU 486 binding.

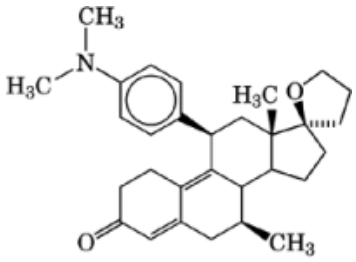
RU 486 blocks the actions of agonists in two ways. The antagonist binds to and competes for the same receptor as the agonists. In addition, after receptor binding has occurred, the antagonist-receptor complex binds to and competes for the same progesterone response elements on the DNA as the agonist-receptor complex (209). By allowing receptor-DNA binding, RU 486 may actually induce the transcription of some genes. This may explain some of the agonistic effects of RU 486 that have been reported. RU 486 alone does not alter insulin receptor concentrations in breast cancer cells, but can partially block progestin-induced increases in insulin receptors (see Insulin and other antidiabetic drugs). In contrast, progestin suppression of breast cell growth is not blocked by RU 486. Further, RU 486 alone suppresses cell growth as effectively as the progestin agonist, R5020 (205). RU 486 can induce gene transcription when bound to either the A or B isoform of the human progestin receptor (199, 200). In rats, RU 486 has some weak agonistic effects on sexual behavior (210). In women, RU 486 alone induces the same secretory changes in the uterine endometrium as progesterone; administered together, RU 486 blocks the effects of progesterone (211).

4.1. Classifications of Progestin Antagonists

Progestin antagonists can be classified based on their ability to form hormone-receptor complexes capable of binding to progesterone response elements (212). Type I antagonists bind to the receptor but do not induce the receptor activation necessary for DNA binding. Type II antagonists, such as RU 486, bind to the receptor and induce the binding of receptor to DNA. Type I antagonists may be viewed as pure antagonists because, owing to their blockade of receptor-DNA binding, no gene transcription is possible. However, type II antagonists may induce transcription of some genes. Presently there are several type I and type II antagonists being used for studying the progestin receptor. Type I antagonists include ZK 98299 [96349-61-1] (**87**) (209, 213); type II antagonists such as RU 486 include ZK 112993 [105114-63-4] (**92**) (214), ORG 31710 [118968-41-5] (**88**), and ORG 31806 [123916-70-1] (**93**) (215).



(**92**)



(**93**)

4.2. Regulation of Progestin Receptors

The biological response of a tissue to a steroid hormone depends on the number of steroid receptors in the tissue. This makes regulation of receptor concentration an issue in determining the effectiveness of a steroid (216, 217). Progestin receptors can be regulated by a number of hormones. Estrogens increase the levels of the progestin receptor in most target tissues and cells (218). Progestin receptor messenger RNA (mRNA) levels rise several hours prior to any increase in receptor concentration indicating that the effects of estrogen are due to an increase in progestin receptor synthesis (219, 220). In contrast, progestins decrease the number of progestin receptors (221), increasing the rate of receptor degradation and decreasing the rate of receptor synthesis (222). Whereas estrogens and progestins are the primary regulators of progestin receptor concentrations, other factors and hormones may also play a role. Insulin, insulin-like growth factor, and epidermal growth factor can increase progestin-receptor concentrations in cultured breast and uterine cells (223, 224). Luteinizing hormone and follicle stimulating hormone, the pituitary hormones responsible for ovulation, induce progestin receptors in cultured ovarian granulosa cells (225).

4.3. Assay Methods for Evaluating Progestin Agonists and Antagonists

4.3.1. Progestin-Binding Assays

The initial evaluation of a progestin often begins with the determination of its relative binding affinity for the progestin receptor. Compounds can be directly compared based on their ability to compete with a radiolabeled progestin for a receptor binding site. Classically, progestin receptor studies have been performed using a crude receptor preparation made from the uteri of estrogen-primed rabbits. The assay itself involves combining the receptor preparation with a ^{3}H- or ^{125}I-labeled progestin and varying concentrations of the test compound. After incubating under conditions allowing equilibrium to be reached, the receptor-bound radioligand is separated from the free radioligand and is quantitated. The relative binding affinity is calculated from the displacement curve and is often expressed as either an IC_{50} value or as the ratio of the IC_{50} of progesterone, or another standard, versus the test compound IC_{50}. There are many detailed descriptions of the assay methodology (226–228). Differences between species, and even tissues within the same species, have been noted in progestin binding (229, 230). However, the relative binding affinities of progestins in rabbit uterine preparations show a reasonable correlation with binding affinities measured in human tissues (143, 231). In addition, the relative binding affinities of progestins are related to and can predict their relative biological activity *in vitro* and *in vivo* (232–234).

The utility of receptor binding assays in identifying active compounds and in predicting biological activity is not as clear with progestin antagonists. The potent progestin antagonist RU 486 shows a high degree of species specificity with regard to its receptor-binding affinity. It binds with a high affinity to the progestin receptors in human, rat, rabbit, and calf tissues (205, 235–237) but does not bind to progestin receptors in chick oviduct (207) or hamster uterus (238). Receptor-binding assays cannot differentiate agonists from antagonists, nor can they predict the biological actions of mixed agonists/antagonists. An example of such a compound is RU 486 which antagonizes the effects of progestins and yet by itself can demonstrate agonistic properties *in vitro* and *in vivo* (205, 211).

4.3.2. Progestin Effects In Vitro

To quantitate hormone action in a defined system, several cell lines have been used to evaluate the biological activity of progestins. The proliferation of human breast cells has become one of the most commonly used *in vitro* assays of progestin action. Normal breast cells obtained from mammoplasties and breast tumor-derived cells respond to progestins with a concentration-dependent reduction in their rate of growth (239, 240). Under different experimental conditions, a progestin-induced increase in breast cancer cell proliferation also has been reported (241, 242). Possible explanations for these discrepancies include differences in the cell lines

used between laboratories, the confounding estrogenic and androgenic properties of the progestational steroids used, and the presence of phenol red in the media (243–245). Human breast cancer cells synthesize a 250,000 mol wt protein in response to progestin stimulation. In further studies, this protein has been found to be fatty acid synthetase and has been shown to be produced in a dose-response fashion following progestin treatment (246). Another progestin-induced protein found in human breast cells is a 48,000 mol wt protein secreted from T47D breast cells as a specific response to progestin treatment (247).

Rather than use the synthesis of an endogenous protein as an index of progestin activity, a newer assay methodology has become available which uses cells modified or transfected to produce a foreign protein in response to progestins. To perform this transactivation assay, cells containing progestin receptors are transiently transfected, ie, made to take up a DNA construct comprising the gene for an easily quantitated enzyme (the reporter gene) linked to a hormonally responsive promoter (see Genetic engineering). The reporter enzymes commonly used are chloramphenicol acetyltransferase [9014-00-0] or luciferase [9040-07-7], neither of which is found in mammalian cells. Addition of a progestin agonist to the transfected cells activates the endogenous progestin receptor which binds to the hormonally responsive promoter, triggers transcription of the reporter gene, and causes the synthesis of the reporter enzyme (248, 249). Progestin antagonists also can be identified with this assay based on ability to block transactivation by the agonist R5020. Another method for exploring the action of antagonists is to measure gene activation in a hormone-dependent, cell-free system (250). In this system, the effect of an agonist or antagonist can be explored by measuring gene expression under controlled, well-defined conditions.

4.3.3. Progestin Effects In Vivo

A variety of methods has been devised to evaluate and compare progestins *in vivo.* Perhaps the best known is the Clauberg assay in which the end point is the glandular proliferation of the uterine epithelium in rabbits (251). Intact, immature rabbits are given estradiol for several days to prime the uterus; the progestin is then given orally or by subcutaneous injection for several days. The uterus is removed and histological sections are prepared for analysis. In response to a progestin, the endometrium shows a characteristic pattern of proliferation which is related to the dose and potency of the progestin. The progestin-induced proliferation of the endometrium is quantitated using the McPhail index (252). This index grades the histological changes in the endometrium on a 1 to 4 scale with +4 as the maximum proliferative response. A variation on this method is the McGinty test in which spayed adult rabbits have the drug administered directly into the uterine horn (182). The direct application of the progestin to the target tissue makes this a more sensitive test for progestational activity. Using these methods there can be problems quantifying the results. The dose-response curves for many synthetic progestins are not parallel with progesterone. Also, those compounds that produce shallow, nonparallel dose-response curves do not produce the maximal McPhail ratings (253).

There are other whole animal models to evaluate progestins based on their reproductive effects. Using rats or mice, progestational activity can be determined by measuring uterine deciduoma formation (251). Deciduoma formation is a proliferative response of the uterine stroma to a physical trauma which, in the case of this assay, is usually a silk suture sewn through the uterine wall. The magnitude of decidual reaction, quantitated by uterine weight or histology, occurs in direct proportion to the dose or potency of the progestin used to pretreat the animal. Progestins can also be compared based on their ability to inhibit parturition in rats when given near the time of birth (254). The maintenance of pregnancy in rats following ovariectomy is also a model of progestational activity (251).

In addition to histological changes, the effects of progestins on the endometrium can be quantitated by measuring the production of several proteins. Carbonic anhydrase, measured in rabbit uterine homogenates, increases in a dose-response fashion after progestin treatment (251, 255). The synthesis of uteroglobin is also stimulated by progestins in the rabbit (256). After five days of progesterone treatment, the concentration of

uteroglobin in the uterine luminal fluid is increased nearly 3000-fold (257). Estrogens and androgens, however, can also stimulate uterine uteroglobin levels (256).

4.4. Effects of Progestins on Various Target Tissues

Progestins exert a variety of direct and indirect effects on all tissues and organs within the reproductive system. Organs that contain progestin receptors, and thus can respond directly to progestins, include the uterus, cervix, vagina, breast, ovary, brain, and pituitary gland (258, 259). Receptors have also been identified in rat thymus (260), human lymphocytes (261), and bone cells (262, 263). Several factors are responsible for progestin effects including the dose and potency of the drug itself, the duration of treatment, the route of administration, and the ability of the drug to affect progestin-receptor number and estrogen levels.

The uterus is exceptionally sensitive to the effects of estrogens and progestins. Estrogens stimulate the proliferation and vascularization of the uterine endometrium (see Hormones, estrogens and antiestrogens). Progestins serve to suppress the stimulatory effects of estrogen on uterine growth, an effect termed antiestrogenic. Progestins blunt the responsiveness of the uterus to estrogen by reducing estrogen receptor levels in the uterus (264, 265). In addition, progestins affect estrogen metabolism within the uterus by inducing 17β-hydroxysteroid dehydrogenase which converts estradiol to the inactive estrone (266). Morphologically, the uterine effects of progestins can be seen as a reduction in the number and size of endometrial glands and a decrease in the proliferation of both glandular and stromal cells (253, 267, 268). Progestins also act on the uterine myometrium reducing the frequency of contractions (269). In nonpregnant women, menstrual bleeding is the most noticeable effect of progestins on the lower reproductive tract. After being transformed from a proliferative to a secretory state by the action of progesterone or a synthetic progestin, the uterine endometrium requires constant steroidal stimulation or it regresses and is lost in menses. Intermenstrual bleeding, or breakthrough bleeding, is a common side effect with the use of synthetic progestins, as in oral contraceptives. Elsewhere within the lower reproductive tract, progestins have other effects. The quantity of cervical mucus is lessened and the mucus is more viscous under the influence of progestins (253). The motility of the oviduct is affected by progestins which can act to delay the transport of ova from the ovary to the uterus (270).

The breast is another tissue sensitive to estrogens and progestins. In the presence of estrogens, which presumably increase progestin receptors in the breast, progestins stimulate ductal branching and lobuloalveolar development (271, 272). While acting to promote mammary gland growth in concert with estrogens, progestins suppress milk production (268). The actions of progestins on breast cells in culture have been examined in a number of studies with conflicting results as to whether cell proliferation is stimulated or inhibited (201).

The effects of progestins are not limited to the reproductive system. Progestins exert direct actions on bone osteoblast cells, acting as a bone-forming hormone (273). Within the brain, progestins modulate neuroendocrine function, mood, and behavior via intracellular nuclear receptors (274, 275). On the membrane of some neurons however, naturally occurring A-ring-reduced metabolites of progesterone bind to the chloride ion-$GABA_A$ receptor complex. The principal neuroactive metabolite of progesterone is 3α-hydroxy-5α-pregnan-30-one [516-54-1] (allopregnanolone). Allopregnanolone and similar A-ring-reduced pregnanes potentiate GABA effects at these receptors. These steroids mimic the effects of the benzodiazepines, changing chloride ion conductance and producing sedative and hypnotic behavioral effects (276, 277). Neuroactive steroids can be therapeutically useful as anticonvulsants, anxiolytics, or anesthetics (qv) (see also Hypnotics, sedatives, anticonvulsants, and anxiolytics).

Progestins have indirect effects on other nonreproductive systems and organs, effects not necessarily involving progestin receptors. Some synthetic progestins, especially medroxyprogesterone acetate (MPA) and other acetoxyprogesterone derivatives, have sufficient glucocorticoid-like activity to lower adrenocorticotrophin hormone (ACTH) and cortisol levels via a negative feedback effect (278). Progesterone has antimineralcorticoid activity, and some other progestins, eg, gestodene, show surprisingly high affinity for the mineralcorticoid receptor (279, 280). The hepatic production of sex hormone-binding globulin [111566-18-8] (SHBG) is lowered

by some progestins, eg, MPA or norgestrel, but not others (280). It is not uncommon for progestins, especially 19-nortestosterone derivatives, to bind to SHBG, as do androgens (279, 280). Many progestins show varying degrees of affinity for the androgen receptor and produce androgenic effects *in vivo.* Gonane-based progestins tend to be more androgenic than estrane-based progestins (281). High blood glucose levels owing to insulin insensitivity occur during pregnancy, a time of high endogenous progesterone secretion. Depending on dose and potency, synthetic progestins can produce similar effects, impairing glucose tolerance and increasing insulin resistance (282).

There has been great interest in understanding the impact of synthetic progestins on the cardiovascular system and the risk of coronary heart disease in women (283). Coronary heart disease has been linked with high plasma levels of total cholesterol and low density lipoprotein cholesterol (LDL) and low plasma levels of high density lipoprotein cholesterol (HDL). Estrogens tend to increase plasma levels of cardiac-protective HDL and decrease LDL levels, whereas progestins have opposite effects (284). At clinically used doses, norgestrel and levonorgestrel have a greater effect on HDL and LDL levels than estrane derivatives such as norethindrone (285, 286). The newer gonane-based progestins, eg, desogestrel, gestodene, and norgestimate, have minimal or even favorable effects on the LDL/HDL ratio (287).

5. Therapeutic Uses of Progestins

5.1. Progestins Used for Contraception

The primary therapeutic use of progestins is in combination with estrogens for contraception. Estrogen-containing contraceptives work primarily by inhibiting pituitary gonadotropin secretion and thus ovulation (288). Progestins are given with estrogens not so much as to increase their contraceptive effectiveness, but to prevent uncontrolled, estrogen-driven proliferation of the uterine endometrium. Synthetic progestins have a number of effects on the reproductive system, many of which by themselves provide some degree of contraception. Progestins counter the effects of estrogens on the uterine endometrium, making it less suitable for implantation (253, 264–268). Progestins block sperm passage into the uterus by thickening the cervical mucus (253). The movement of the egg through the fallopian tubes is also slowed by progestins (270). The combined effects of progestins on the uterus, cervix, and fallopian tubes are sufficient to achieve contraception as evidenced by the use of progestin-alone contraceptives.

As of 1994, there were approximately 47 progestin-containing contraceptive drug formulations sold in the United States for use as oral contraceptives (Table 3). In addition, there are three nonoral contraceptive formulations containing progestins; ie, one injectable (Depo-Provera), one as an intrauterine device (IUD) (Progestasert), and one implantable (Norplant). Of the oral formulations, all but two also contain an estrogen component, ethynylestradiol.

Progestins are formulated in a variety of doses. Oral contraceptives are available in a monophasic formulation, where the doses of progestin and estrogen are constant through the 21–28 day dosing regimen, and in a triphasic formulation, where the doses of progestin and estrogen are varied in three stages to more accurately mimic the normal changes in steroid levels during the menstrual cycle. The triphasic regimen was designed to reduce the total monthly dose of progestin and thus reduce side effects but still provide effective contraception (289). A number of studies have shown that this approach to lowering the total progestin dose does lessen the negative changes in plasma lipids, androgenic effects, and glucose tolerance that occur owing to the progestin component in oral contraceptives (290, 291).

Contraception can also be achieved using progestins administered in a fashion to provide sustained release. Depo-Provera (Upjohn) is a microcrystalline solution of MPA designed to provide contraception for three months after a single administration. Each intramuscular injection contains 150 mg MPA, a dose appropriate for all body weights. Plasma MPA concentrations peak at $1 - 7$ ng/mL within three weeks and fall to

Table 3. Progestin Formulations Marketed in the United States[a]

Progestin	Doses, mg	Ethynylestradiol component, μg	Trade name	Manufacturer
		Contraceptive uses		
desogestrel	0.15	30	Desogen, Ortho-Cept	Organon Ortho
ethynodiol diacetate	1.0	35	Demulen 1/35	Searle
	1.0	50	Demulen 1/50	Searle
levonorgestrel	0.15	30	Nordette	Wyeth-Ayerst
	0.05/0.075/0.125	30/40/30	Triphasil	Wyeth-Ayerst
	6×36[b]		Norplant	Wyeth-Ayerst
medroxyproges-terone acetate (MPA)	150[c]		Depo-Provera	Upjohn
norethindrone	0.35		Micronor, Nor-Q D	OrthoSyntex
	0.4	35	Ovcon 35	Mead Johnson
	1.0	50	Ovcon 50	Mead Johnson
	1.0	50	Ortho Novum 1/50 Norinyl $1 + 50$,	Ortho Syntex
	0.5	35	Ortho Novum 10/11, Brevicon	Ortho Syntex
	1.0	35	Ortho Novum 1/35, Norinyl $1 + 35$	Ortho Syntex
	0.5/0.75/1.0	35	Ortho Novum 7/7/7	Ortho
	0.5/1.0/0.5	35	Tri-Norinyl	Syntex
norethindrone acetate	1.0	20	Loestrin 1/20	Parke-Davis
	1.5	30	Loestrin 1.5/30	Parke-Davis
norgestimate	0.25	35	Ortho-Cyclen	Ortho
	0.18/0.215/0.25	35	Ortho Tri-Cyclen	Ortho
norgestrel	0.075		Ovrette	Wyeth-Ayerst
	0.5	50	Ovral	Wyeth-Ayerst
	0.3	30	Lo/Ovral	Wyeth-Ayerst
progesterone	38[c]		Progestasert	Alza
		Noncontraceptive uses		
medroxyproges-terone acetate	2.5, 5, 10[e]		Amen	Carnrick
			Provera	Upjohn
			Cycrin	ESI-Pharma
norethindrone acetate	5[e]		AygestinNorluate	Wyeth-AyerstParke-Davis

[a] Oral formulations unless noted.
[b] Implant.
[c] Injection.
[d] Intrauterine device.
[e] Doses available.

undetectable levels 120–200 days after injection (292). The Norplant system consists of six capsules made of Silastic (dimethylsiloxane–methylvinylsiloxane copolymer), each containing 36 mg levonorgestrel. When inserted subcutaneously, the Norplant capsules provide contraception for five years. During Norplant development, levonorgestrel was chosen as the most suitable progestin owing to its slow release rate, approximately 10% of original load per year (293). Initially 85 μg of levonorgestrel is released per day producing blood levels of $0.25 - 0.3$ ng/mL. The rate of release slowly falls over time, reaching 50 μg/d by nine months, 35 μg/d by 18 months, and 30 μg/d thereafter (294). Unlike oral contraceptives which show dramatic hepatic metabolism following absorption from the gastrointestinal tract ie, the first-pass effect, the release of progestins from Depo-Provera and Norplant avoids this initial hepatic metabolism. Other methods of progestin administration

now being investigated include progestin-containing IUDs (Progestasert), progestin-filled Silastic vaginal rings (295), and injectable microcapsules of poly(lactide-*co*-glycolide) containing norethindrone (296).

5.2. Noncontraceptive Uses of Progestins

Progestins have other therapeutic uses aside from contraception. Hormone-dependent tumors and cysts involving reproductive tissues respond to progestins. Megestrol acetate and MPA are the two most commonly used progestins to treat breast cancer (297). The progestin doses necessary for effective cancer therapy are very high, reaching $1000 - 2000\ \mathrm{mg/d}$. These high concentrations of progestins may work through several modes of action, including inhibition of estrogen-induced growth by both lowering estrogen receptors and increasing the oxidative activity of 17 β-hydroxy-steroid dehydrogenase which converts estradiol to less active estrone (297), and directly inhibiting the proliferation of breast cancer cells, as has been shown *in vitro* (258). Benign breast masses, ie, fibrocystic breast cysts, can be treated with progestins at doses found in oral contraceptives (298). The uterine endometrium is very sensitive to progestins and therefore abnormal growth of the endometrium, whether benign or malignant, often responds to progestin treatment. Endometrial cancer, endometriosis, and endometrial hyperplasia all show reduced growth and regression during progestin treatment (299, 300). For the treatment of endometrial adenocarcinomas, MPA is given at a dose of 1 g/w orally (301). This dose produces therapeutically effective serum levels of approximately $90\ \mathrm{ng/mL}$ within one week. Part of the benefit of MPA treatment is believed to be its ability to make the tumor more sensitive to radiation therapy (302). In disease states involving excess androgen production, ie, polycystic ovary syndrome or excess androgen of adrenal origin, progestins can be used therapeutically to suppress the synthesis of androgenic steroids at the ovary and adrenal gland (303, 304). For this purpose progestins having low androgenicity are the logical choices, ie, not levonorgestrel.

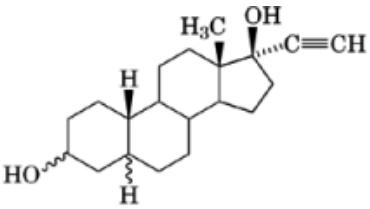
Progestins are commonly given in combination with estrogen in hormone replacement therapy following menopause or ovariectomy. The primary function of the progestin component is to reduce the likelihood of endometrial hyperplasia and endometrial carcinoma (305). The usual course of treatment calls for 12–13 days of progestin per month with continuous daily estrogen administration. To protect the endometrium, doses of 10–20 mg dydrogesterone, 10 mg MPA, 1 mg norethindrone, or 150 μg levonorgestrel are effective (306, 307). By themselves, however, progestins have beneficial effects on the events associated with menopause. The vasomotor symptoms of menopause, ie, hot flushes, are decreased with MPA administered as either a 150-mg depot injection or $10\ \mathrm{mg/d}$ orally (308, 309). Osteoporosis occurs in post-menopausal women primarily because of the loss of estrogen. In the absence of estrogens the rate of bone resorption exceeds the rate of bone formation and a steady loss of bone mass occurs. Estrogen replacement slows bone resorption but does not cause the replacement of lost bone (310, 311). There is mounting evidence that progestins can prevent bone resorption and may also induce bone formation (273). *In vitro,* progestins stimulate the growth and activity of osteoblasts, the cells which lay down new bone (312). MPA, norethindrone, and progesterone have been reported to exert positive effects on bone mass in animal models and women, either stimulating bone formation and/or suppressing bone resorption (273, 313–315). The drug most commonly used off-label is MPA (Provera, see Table 3), prescribed with estrogen for the prevention of post-menopausal osteoporosis (316).

5.3. Pharmacokinetics and Metabolism of Therapeutic Progestins

5.3.1. Norethindrone

Norethindrone 5 is rapidly and completely absorbed after oral administration (317). There is a significant loss of norethindrone owing to a first-pass effect. Approximately 36% of an oral dose is lost as a result of metabolism within the intestinal wall and liver (318). In women, 60.8% of norethindrone in the blood is bound to albumin, 35.5% to sex hormone binding globulin (SHBG), and 3.7% is free (319). The half-life for elimination has been measured in a number of studies in which the values ranged from 3.4 to 13.4 h, thereby giving an average of

approximately 8 h (320). Using single daily dosing, steady-state levels are reached in 5–10 days. Norethindrone is quickly distributed to all tissues, accumulating in, and being metabolized by, the liver. The primary metabolic pathway is reduction of the α, β-unsaturated oxo group in the A-ring producing four stereoisomeric tetrahydro-reduced metabolites (3α,5β; 3β,5β; 3α,5α; 3β,5α) (**94**). Metabolites are conjugated with glucuronic acid and excreted in the urine. Sulfate conjugation and hydroxylation also occur to a lesser degree (321–323). The most widely used method to measure norethindrone and some of its metabolites is radioimmunoassay (320, 324) but a gc/ms method has been described (325).



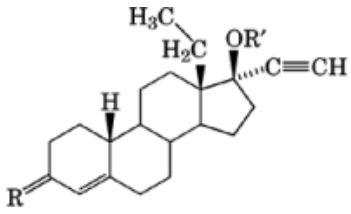
(94)

5.3.2. Levonorgestrel

Levonorgestrel (−)-9 is 100% orally absorbed with no first-pass effect. Peak plasma levels are reached between 0.5 and 2 h (326, 327). Plasma levels of levonorgestrel decline in a biphasic fashion; the initial phase has a half-life of 50 to 180 min followed by a second phase with a half-life of 10 to 26 h. In plasma, approximately 98% of levonorgestrel circulates bind; $\sim$50% to albumin, 48% to SHBG, and 2% free (328). The metabolic pathways of levonorgestrel are A-ring reduction and or hydroxylation in the liver. Secretion is in the form of sulfate and glucuronide conjugates in both urine and feces. All four tetrahydro-metabolites of levonorgestrel can be detected in the urine with the 3α,5β-isomer as the principal compound (329). The analytical method used for measuring levonorgestrel is radioimmunoassay (326).

5.3.3. Norgestimate

Norgestimate (**82**) is completely and rapidly absorbed after oral administration with peak plasma levels reached within 2 h (330). Over the course of two weeks following administration of ^{14}C-norgestimate, 35–49% is excreted in the urine and 16–49% in the feces (330, 331). The urine does not contain any intact norgestimate or any metabolite retaining the oxime group. The principal metabolites of norgestimate are levonorgestrel (−)-9, 3-ketonorgestimate (**95**), and 3α,5β-levonorgestrel and trihydroxy compounds; minor metabolites include 16β-hydroxylevonorgestrel, 2α-hydroxylevonorgestrel, and 3,16-dihydroxy-5α-tetrahydrolevonorgestrel (331). Based on these active metabolites, the argument has been raised that norgestimate is a prodrug for levonorgestrel. Several studies have refuted this possibility. Norgestimate itself binds to the progestin receptor and is biologically active when administered directly into the uterus in a McGinty test (332). Norgestimate is rapidly deacetylated to 17-deacetylnorgestimate (**96**) by the human gastric mucosa and liver *in vitro*. 17-Deacetylnorgestimate shows a pharmacological profile similar to that of norgestimate but different from that of the levonorgestrel-based metabolites (333). Norgestimate and 17-deacetylnorgestimate can be measured in plasma by either radioimmunoassay or hplc (330).



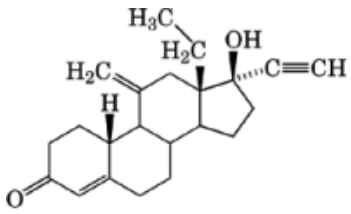
R = O; R′ = $COCH_3$ (**95**)
R = NOH; R′ = H (**96**)

5.3.4. Gestodene

Gestodene 8 is completely absorbed after oral administration with no first-pass metabolism (334). The half-life of elimination is approximately 20 h. Repeated daily dosing results in steady-state serum levels after about 10 days (335). These levels are approximately four to five times those following a single dose, due to an increase in serum SHBG. The vast majority of gestodene in the serum is protein-bound. At steady-state conditions, about 75% of gestodene is bound to SHBG and 24% to albumin. In contrast, gestodene is 50% SHBG-bound and 48% albumin-bound after a single dose (336, 337). Regardless of the frequency of administration, less than 1–2% of circulating gestodene is free and biologically active. The plasma clearance of gestodene is biphasic. The first half-life is 1.5 h; the second, 18 h (338). The metabolites of gestodene are highly water soluble and are excreted equally in the urine and feces following conjugation by the liver. Gestodene undergoes extensive reduction and oxidation in the liver. The metabolites measured in urine are mainly hydroxylated derivatives of A-ring-reduced gestodene. Hydroxylation occurs at several positions including C-1, C-6, and C-11 (338). Gestodene is measured in plasma by radioimmunoassay (334).

5.3.5. Desogestrel

Desogestrel 7 is rapidly and nearly completely converted to the biologically active metabolite 3-ketodesogestrel [54048-10-1] (**97**) (339, 340) The oral administration of equal doses of desogestrel or 3-ketodesogestrel produces nearly identical serum concentrations of 3-ketodesogestrel (341). The affinity of 3-ketodesogestrel for the progestin receptor in human uterine myometrium is approximately six times greater than that of progesterone and 57 times greater than that of desogestrel (342). 3-Ketodesogestrel binds extensively to albumin and SHBG; only approximately 2.5% of 3-ketodesogestrel is free in circulation (343). Four metabolites of desogestrel 7 have been identified in *in vitro* studies; 3α- and 3β-hydroxydesogestrel, 3α-hydroxy-5α-desogestrel, and 3-ketodesogestrel (**97**). The half-life of elimination from the plasma for 3α-hydroxydesogestrel is 16 hours (340). The 3α- and 3β-hydroxydesogestrel metabolites are believed to be intermediates in the conversion of desogestrel to 3-ketodesogestrel (344). Human intestinal mucosa as well as liver can perform this conversion (344, 345). Desogestrel and 3-ketodesogestrel can be measured by radioimmunoassay or hplc (339, 345).



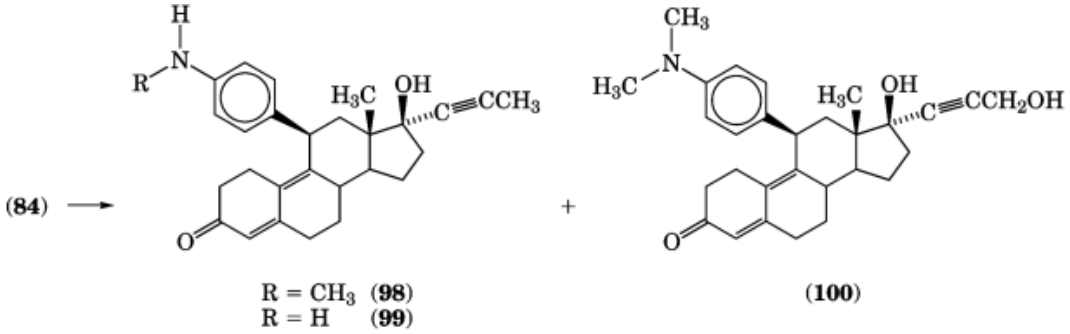
(**97**)

5.3.6. Medroxyprogesterone Acetate

Accurate pharmacokinetic and metabolism studies on MPA have been difficult because the radioimmunoassays employed cannot differentiate between MPA and its metabolites (346). Comparison of MPA plasma levels assayed by hplc and radioimmunoassay show that radioimmunoassay may overestimate intact MPA concentrations by about fivefold (347). However, values of the mean elimination half-life of MPA were similar, being 33.8 and 39.7 h when measured by hplc and radioimmunoassay, respectively (347). Approximately 94% of MPA in the blood is bound to albumin (348). When taken orally, MPA is rapidly absorbed with little or no first-pass metabolism (13). Peak serum levels are reached after 3 h. Steady state occurs after three days of daily administration (349). The pharmacokinetics of MPA when administered in a depot formulation have been described (350).

5.3.7. Mifepristone

After oral administration, peak plasma levels of mifepristone (**84**) (RU 486) are reached in 1 h and over 95% was bound to plasma proteins (351, 352). The plasma half-life of RU 486 is approximately 24 h (352, 353). In humans, monodemethylated (**98**), didemethylated (**99**) and alcoholic nondemethylated (**100**) metabolites of RU 486 have been identified (351). These metabolites show some progestin-binding affinity, approximately five to ten times lower than that of RU 486 itself. RU 486 and its metabolites can be measured by radioimmunoassay and hplc (353, 354).



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