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9β-ISOMERS OF ESTRADIOL, PROCESSES OF MAKING THE SAME AND THEIR UTILIZATION

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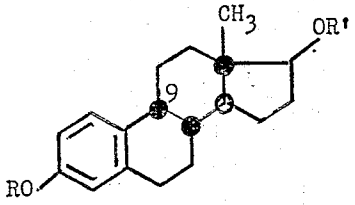
11 Claims. (Cl. 167—74)

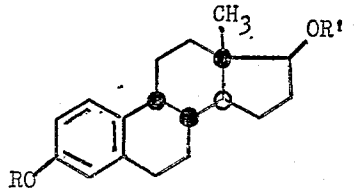
The present invention relates to new 9β-isomers of estradiol and in particular to 9β-estradiol, stereoisomeric in the 9-position with the natural product, and its functional derivatives, which are compounds which correspond to the formula:



wherein R and R' can be the same or different and represent hydrogen, an acyl radical of an organic carboxylic acid having from 1 to 18 carbon atoms or a lower alkyl. In addition the invention relates to processes of preparing said 9β-estradiol and its derivatives and to the process of utilizing the same.

The present application is a continuation-in-part of United States patent application Serial No. 191,263, filed April 30, 1962, and now abandoned.

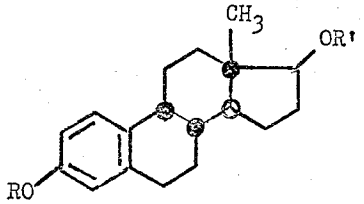
It is an object of the present invention to obtain estradiol derivatives of the formula:



wherein R and R' can be the same or different and represent radicals selected from the group consisting of hydrogen, lower alkyl and the acyl radical of an organic carboxylic acid having from 1 to 18 carbon atoms.

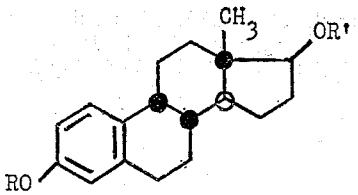
Another object of the invention is the development of processes for the preparation of the new estradiol derivatives.

A further object of the invention is the development of a process for the improvement of estrogenic deficiency at the situs of the vaginal epithelium which comprises the administration of a safe but effective amount of an estradiol derivative of the formula:



wherein R and R' can be the same or different and represent radicals selected from the group consisting of hydrogen, lower alkyl and the acyl radical of an organic carboxylic acid having from 1 to 18 carbon atoms.

A still further object of the invention is to prepare a composition having an estrogenic action on the vaginal epithelium consisting of an estradiol derivative of the formula:

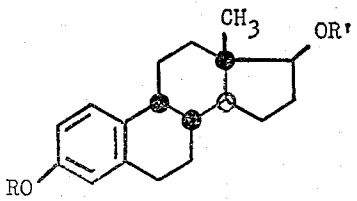


wherein R and R' can be the same or different and represent radicals selected from the group consisting of hydrogen, lower alkyl and the acyl radical of an organic carboxylic acid having from 1 to 18 carbon atoms, and a non-toxic vehicle.

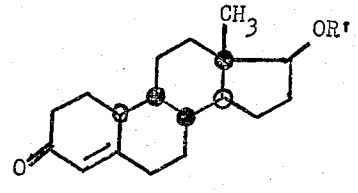
These and other objects of the invention will become more apparent as the description thereof proceeds.

It has been found that 9β-estradiol and its derivatives possess an interesting hormonal activity. In particular they exercise and estrogenic action. Contrary to other known estrogens, 9β-estradiol and its derivatives are considerably more active in promoting an estrogenic response on the vaginal epithelium than on other organs such as the uterus. This represents an unexpected dissociation of the generally multiple hormonal effects of estrogenic steroids, due to the critical steroidal configuration.

The estradiol derivatives of the formula:



wherein R and R' can be the same or different and represent radicals selected from the group consisting of hydrogen, lower alkyl and the acyl radical of an organic carboxylic acid having from 1 to 18 carbon atoms, can be produced by heating a compound of the formula:



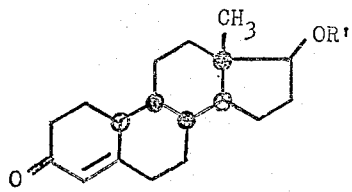
wherein R' has the above assigned meanings in an inert organic solvent in the presence of a catalyst having a palladium base and recovering said estradiol derivatives.

The process results in the recovery of the corresponding 3-hydroxy derivatives where R=H. This compound can be transformed into all other desired functional derivatives.

The desired 9β-estradiol is obtained from the above re-

action in admixture with some of the naturally occurring estradiol. 9β-estradiol may be recovered from the mixed isomers by taking advantage of the property of the 17β-benzoate of estradiol to form with isopropyl ether a solvate which is sparsely soluble in isopropyl ether. By this recovery process the natural isomer is removed and the 17β-benzoate of 9β-estradiol is recovered from the solvent by customary procedures.

The starting 19-nor-steroids of the formula:



where R' has the above assigned meanings are described in copending, commonly-assigned United States patent application Serial No. 111,499, filed May 22, 1961, now United States Patent No. 3,138,617.

The process of the invention is advantageously executed by using as the starting compound 19-nor-/9β/,/10α/-testosterone which can be named 19-nor-Δ^4-/9β/,10α/androsterene-17β-ol-3-one. However, its esters with organic carboxylic acids having from 1 to 18 carbon atoms, such as the alkanoates and alkenoates, for example, the acetate, the trimethylacetate, the propionate, the 4,4-dimethyl-pentanoate, the 10-undecenoate; the cycloalkylalkanoates, for example, the β-cyclopentyl-propionate; the arylalkanoates, for example, the phenyl-propionate; the cycloalkanoates, for example, the hexahydrobenzoate, the hexahydroterephthalate and other phenyl-carboxylic acids as well as its lower alkyl ethers may also be used without departing from the scope of the invention.

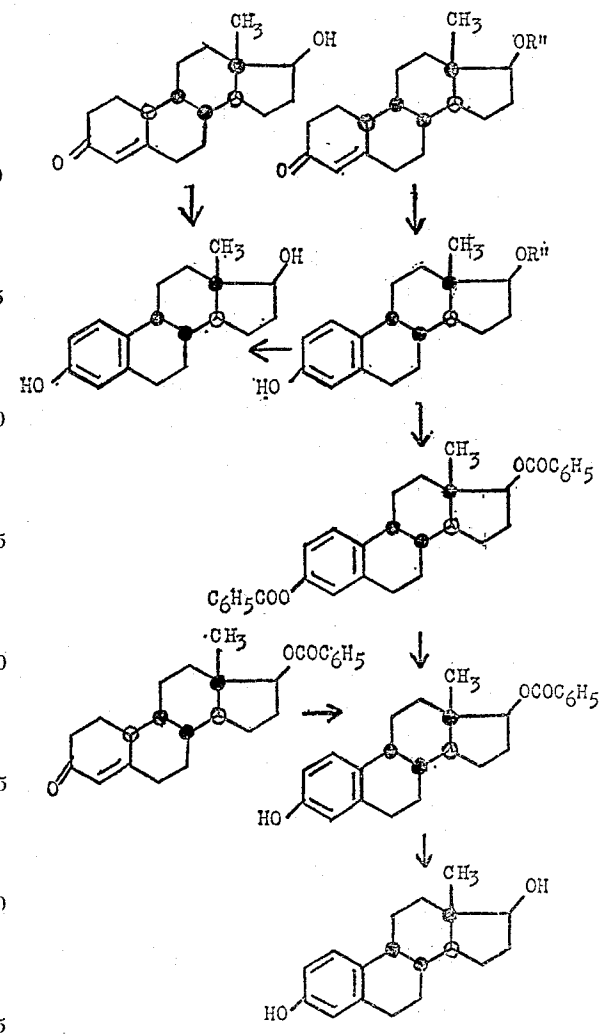
In the execution of the process according to the invention, the dehydrogenation step is preferentially conducted in the presence of an anhydrous lower alkanol such as ethanol as the inert organic solvent at the reflux temperature in an inert atmosphere utilizing a dehydrogenation catalyst based on palladium. It is particularly advantageous to use the palladium catalyst in the form of palladium hydroxide deposited and supported on an alkaline reacting compound such as, for example, magnesia.

In order to recover 9β-estradiol, it is advantageous to prepare the 17β-benzoate ester of the raw reaction product from the above reaction. This can be done by the process outlined by Miescher et al., Helv. Chim. Acta 20, 263 and 1237 (1937) by reacting the raw reaction product with an excess of benzoyl chloride in the presence of pyridine and saponifying the ester linkage in the 3-position by action of alcoholic potassium hydroxide. The mixture of esters of benzoic acid in the 17β-position, an oily residue is refluxed in isopropyl ether and cooled. The precipitate of the estradiol benzoate solvate with isopropyl ether is separated. The isopropyl ether solution of 9β-estradiol benzoate is dried and the residue is saponified by refluxing with methanolic potassium hydroxide to give the desired 9β-estradiol.

Even more preferable is to utilize as starting material an ester of 19-nor-/9β/,/10α/-testosterone, preferably the benzoic acid ester described in United States patent application Serial No. 111,499, now United States Patent No. 3,138,617. Dehydrogenation of this product leads directly to the 17β-benzoate ester of 9β-estradiol in admixture with small amounts of the benzoate of estradiol. The desired product can be recovered as outlined above.

Obviously, in the case of an ester other than the benzoate, or an ether of 19-nor-/9β/,/10α/-testosterone as the starting material, it is necessary, after the dehydrogenation step to liberate the 17-hydroxy group.

The reaction scheme is illustrated in the following table:



R'' is selected from the group consisting of lower alkyl and the acyl radical of an organic carboxylic acid having from 1 to 18 carbon atoms, other than the benzoyl radical.

It is to be understood that either the 3 or 17- position or both positions of the 9β-estradiol obtained by the above process can be etherified or esterified, after a saponification step, if desired.

As esters of 9β-estradiol, the invention comprises particularly the esters in the 17-position, such as the acetate, the pivalate, the cyclopentylpropionate, the benzoate, the hexahydrobenzoate, etc.

The following examples, which are given purely for illustration, are non-limitative in character and will make the invention better understood to one skilled in the art.

EXAMPLE I

Preparation of 9β-estradiol

(A) *Preparation of the catalyst.*—4.5 grams of calcined magnesia were introduced into 35 cc. of distilled water. The solution was agitated and 4.5 cc. of a hydrochloric acid solution containing 20% of palladium chloride were introduced. The solution was agitated for a period of 15 minutes at room temperature. The precipitate formed was vacuum filtered and dried at 100° C. for a period of 15 hours.

(B) *Dehydrogenation.*—0.450 gram of 19-nor-/9β/,/10α/-testosterone (obtained according to United States patent application Serial No. 111,499) were dissolved in

50 cc. of ethanol at 60° C. under an atmosphere of nitrogen. 0.450 gram of palladium hydroxide on magnesia, prepared as indicated above, were added and the mixture was heated at reflux while agitating for a period of 20 hours. 0.200 gram of catalyst were readded and the heating was continued for another period of 20 hours. After cooling, the solution was filtered. The filtrate was evaporated to dryness under vacuum and the residue was dissolved in the following mixture:

	Cc.
Methanol	5
Methylene chloride	25
N sodium hydroxide solution	25

This mixture was agitated. The aqueous layer was decanted. The organic phase was extracted with N sodium hydroxide solution containing 20% of methanol. The aqueous phases were combined and reduced to a small volume. Several cc. of concentrated hydrochloric acid were added. The raw product containing 9β-estradiol precipitated. The solution was vacuum filtered and the precipitate was purified by subjecting it to chromatography over magnesium silicate. Elution with methylene chloride containing 2.5% of ether supplied a product melting at 223° C. and having a specific rotation $[\alpha]_D^{20}=-4°$ (c.=0.39% in methanol). This product comprises 9β-estradiol containing small amounts of isometric estradiol and occurred in the form of white prismatic crystals. It was soluble in alcohol, acetone, benzene and chloroform, slightly soluble in ether and insoluble in water.

(C) *Esterification.*—In order to separate the small amount of estradiol contained in the reaction product, the 17β-benzoate ester was prepared according to Miescher et al., Helv. Chim. Acta 20, 263 and 1237 (1937) by reacting the raw product with an excess of benzoyl chloride in the presence of pyridine. The 3,17β-dibenzoate ester was separated.

(D) *Partial saponification and isolation of the 17β-benzoate of 9β-estradiol.*—The crude 3,17β-dibenzoate ester was placed in suspension in ethanol. The suspension was heated to reflux and, while refluxing, an aqueous solution of potassium hydroxide (1.5 mol KOH for 1 mol of dibenzoate) was added. Refluxing was maintained until entire dissolution under an atmosphere of inert gas. Next the solution was neutralized by adding acetic acid and the mixture was poured while agitating into a concentrated aqueous solution of sodium chloride.

The crude 17β-benzoate ester of the isomeric diols precipitated. This precipitate was extracted repeatedly with methylene chloride. The methylene phases were separated, combined, washed with water, dried with magnesium sulfate, filtered, then washed with water and finally brought to dryness under vacuum.

The amorphous residue thus obtained was treated at reflux with isopropyl ether. The solution was iced, then triturated until crystallization.

Thereafter the suspension was placed in the refrigerator for one hour. The precipitate of the estrodiol benzoate solvate was separated by vacuum filtration, washed with iced isopropyl ether and dried.

The ethereal phases were combined and brought to dryness. The dry residue was essentially composed of the 17β-benzoate of 9β-estradiol.

(E) *Saponification of the 17β-benzoate of 9β-estradiol.*—8.29 gram of 17β-benzoate of 9β-estradiol obtained above and 83 cc. of a 10% methanolic solution of potassium hydroxide were combined under an inert atmosphere. The mixture was heated to reflux for two hours. The solution was neutralized with 12 cc. of acetic acid, then 150 cc. of water were added. The precipitate was vacuum filtered, washed with water and dried in an oven. 5.82 g. of crude 9β-estradiol were thus obtained.

The crude product was triturated with ether at room temperature, then placed in a refrigerator. The precipitate was vacuum filtered, washed with iced ether and the ethereal phases were separated. The residue, weighing 1.02 g. was dried. A product slightly colored a light yellow-beige was obtained, melting at 220° C., and insoluble in ether. This was dissolved in 15 cc. of refluxing methanol, treated with carbon black and filtered. The spent carbon black was washed in boiling methanol. The methanol phases were combined, concentrated to 10 cc., 1.5 cc. of water was added, iced for one hour at 0° C., and vacuum filtered. The precipitate was dried.

In this manner 0.700 g. of a product melting at 226° C. were recovered. An additional recrystallization raised the melting point to 227° C.

The ethereal and methanolic phases previously recovered were evaporated to dryness. The residue was dissolved in 75 cc. of methylene chloride containing 2% acetone. The mixture was subject to chromatography through a column of silica gel and eluated with methylene chloride containing 2% acetone, then with methylene chloride containing 3% acetone. A second fraction of 9β-estradiol was thus recovered on evaporation of the solvent. By trituration of the residue in ether and recrystallization, first from 90% methanol, then from ethyl acetate, 0.35 g. of pure 9β-estradiol was further recovered.

Pure 9β-estradiol occurred in the form of colorless prisms, slightly soluble in alcohols, acetone and benzene, very slightly soluble in chloroform, and insoluble in ether and water. The pure product had a melting point of 227° C. and a specific rotation

$[\alpha]_D^{20}=-55°\pm2°$ (c.=1% in dioxane) U.V. spectra (in alcohol):

$\lambda_{max.}$ 281 mμ $E^{1\%}_{1cm.}=75$

$\lambda_{max.}$ 287 mμ $E^{1\%}_{1cm.}=67$

The I.R. spectra is different from that of estradiol in the part corresponding to the carbon skeleton. It shows also the absence of carbonyl function and the presence of a hydroxyl function.

This compound is not described in the literature.

By reacting this compound with acid chlorides such as those of acetic acid, pivalic acid, cyclopentylpropionic acid, benzoic acid, hexahydrobenzoic acid, etc. in the presence of pyridine, the corresponding esters can be obtained.

EXAMPLE II

17β - benzoyloxy - 19 - nor - Δ^4 - /9β/,/10α/ - androsten-3-one, having a melting point of 160° C. and a specific rotation $[\alpha]_D^{20}=+2°$ (c.=0.5% in methanol) was dehydrogenated under the same conditions as Example I(B). The crude mixture of 17β-benzoate esters of estradiol and 9β-estradiol, in which the latter predominated, was obtained and purified according to the process of Example I(E) and 9β-estradiol was recovered having the same characteristics as above.

The 9β-estradiol, its esters and its ethers can be used for its effect on the vaginal epithelium where a simultaneous effect on other organs ordinarily effected by estrogenic therapy is not desired.

The 9β-estradiol, its esters and its ethers are administered orally, perlingually, transcutaneously or locally by a topical application on the skin and mucous membanes, or by rectal or vaginal methods.

The product can be utilized in the form of extracts, of injectable solutions prepared with the usual adjunctives, in ampules, in multiple-dose flacons, and in prepared injection syringes, in the form of implants, of tablets, of glossettes, of suppositories, of ovules and of pomades.

The dosology is controlled between 25 γ/kg. and 2 mg./kg. per day in the warm-blooded animal as a function of the method of administration. The pharmaceutical forms such as injectable solutions and suspensions, tab-

lets, glossettes, suppositories, ovules and pomades are prepared according to the usual procedures.

EXAMPLE III

Pharmacological study of 9β-estradiol

The comparative study of the estrogenic effects of natural estradiol and of 9β-estradiol was carried out with the aid of the following tests:

(a) The Allen-Doisy test, such as that described by Feyel-Cabanes, C. R. Soc. Biol. 1956, 150, 1881.

This test of estrogenic activity was made on lots of female rats, weighing about 200 grams. The female rats were castrated and tested three weeks thereafter by a single subcutaneous injection of 1 μg. of estradiol benzoate. After a rest of three weeks, the female rats, whose sensitivity to the estrogen was found to be normal, were divided into lots and treated with the experimental products. Vaginal smears were made every day at the same hour. Only those smears formed exclusively of keratinized cells were reported as positive.

(b) Test of the weight of the uterus, as described by Lauson et al., Endrocrinology, 24, 35 (1939). Immature female rats, 22 to 23 days old, were treated with the experimental products for three consecutive days and sacrificed on the fourth day. The uterus of each of the test animals was then separated, vacuum dried and weighed.

(c) Test of Velardo, as described by Velardo, Ann. N. Y. Acad. Sc., 75, 441 (1959). Female rats, about 90 days old, were castrated on zero day. Thereafter they were treated with the experimental products on the 7th, 8th and 9th days and sacrificed on the 10th day. The uterus and vagina of each of the test animals were then separated, vacuum dried, and the uterus weighed. The uterus and the vagina were mounted and stained in accordance with customary histological techniques.

For all these tests the products to be studied were placed in solution in neutral olive oil containing 5% benzyl alcohol and administered by subcutaneous injections.

RESULTS

(a) Allen-Doisy test. Estradiol in a dose of 5 μg., administered at one time gave a positive response in 50–75% of the cases. 9β-estradiol must be injected at a total dose of 750 μg. at one time to give the same result. According to this test, the ratio of the active doses of estradiol to 9β-estradiol is 1 to 150.

(b) Test of the weight of the uterus. The comparative dosage was administered to six groups of rats. Estradiol was administered in total doses of 0.05; 0.10 and 0.20 μg., for which the average weights of the uterus were 34.18; 51.52 and 75.82 mg., respectively. 9β-estradiol was injected in total doses of 37.5; 75.0 and 150.0 μg., for which the average weights of the uterus were 37.86; 60.34 and 79.60 mg., respectively. The statistical study of the results with the aid of the variant analysis showed FΔI for the difference between estradiol and 9β-estradiol, demonstrating a parallelism. Under these conditions the ratio of the active doses on the test of the weight of the uterus of estradiol to 9β-estradiol was, therefore, 1 to 750. A comparison of this ratio with the one obtained in the Allen-Doisy test shows that there exists a clear dissociation between the amount of 9β-estradiol necessary to obtain the vaginal keratinization based on the Allen-Doisy test, and that which was required to obtain a true reversion superimposable on that the estradiol based on the test of the weight of the uterus.

(c) Velardo test. It seemed important to verify this first evaluation of the dissociation of properties by using the same animal for purpose of observing the response of the two affected organs with regard to the same stimulus. This was undertaken according to the Velardo test. The female rats, castrated on zero day, received on the 7th, 8th and 9th days, either the solvent, or 0.3 μg. (total dose) of estradiol, or 450 μg. (total dose) of 9β-estradiol. On autopsy, performed on the 10th day, the average weights of the uterus were the following: control=127.1 mg.; estradiol=346.7 mg. (liquid excluded); 9β-estradiol=170.1 mg. For the rat in physiological estrus, the morphological aspect of the uterus and of the vagina is characteristic of this state. The uterus is more developed than in diestrus or in metaestrus; the motility of the musculature is increased; the chorion is oedematosed and the cylindrical endometral epithelium shows basal nucleation; the multistratified vaginal epithelium casts off from its orifice its kerationized superficial layers by exfoliation. These two aspects of the uterus and of the vagina are concomitant on the day of estrus.

Castration places at arrest these target organs which quickly assume the characteristic aspect for diestrus; the whitish and filiform uterus has a musculature of little mobility; its chorion is thick and the lower endometral epithelium is covered with nuclei over all its length; such uterus corresponds with a vagina whose flattened bistratified epithelium indicates the hormonal arrest.

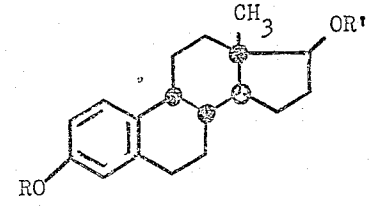
Inspection of the mounted and stained specimens according to the Velardo test showed that when, for 3 days, 0.3 μg. of estradiol was subcutaneously administered to the female rats placed at arrest by castration, on the day after the last injection identical resemblances in every respect to those described for the physiological estrus phase were observed. The action of the injected hormone was simultaneous and of the same intensity on the uterus and the vagina as that of the endogenous hormone. However, when the estradiol was replaced by its isomer in 9-position, 9β-estradiol, the picture obtained was different. With a total dose of 450 μg., injected subcutaneously over 3 days, the uterus was in diestrus and the vagina was in estrus. In this case as shown by the previous tests, a dissociation was observed between the steroid action on the uterus and on the vagina. These results confirmed exactly those obtained in the two preceding tests.

The divergences of action here observed between estradiol and its artificial isomer, 9β-estradiol, were solely due to the spatial configuration. The inversed position of the 9-carbon atom reflected in a striking manner on the biological response. It is seen from the above tests that, while estradiol reacts in a simultaneous manner on both normally conditioned target organs, 9β-estradiol does not.

It will be understood that the invention is not limited to the specific modes of execution described above. Particularly, it is evident to one skilled in the art that equivalent techniques may be employed, such as varying the temperatures, the nature of the solvents, or the ester of the organic carboxylic acid and the lower alkanol solvent, without departing from the spirit of the invention or the scope of the appended claims.

We claim:

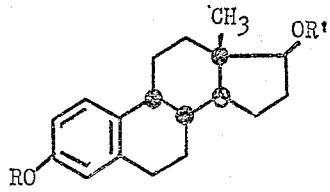
1. A member selected from the group consisting of:



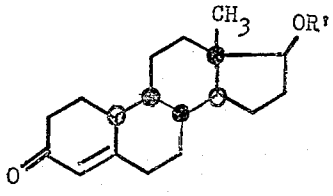
wherein R and R' can be different and represent radicals selected from the group consisting of hydrogen, lower alkyl and the acyl radical of an organic carboxylic acid having from 1 to 18 carbon atoms selected from the group consisting of alkanoic acids, alkenoic acids, cycloalkyl-alkanoic acids, phenyl-alkanoic acids, cycloalkanoic acids and phenyl carboxylic acids.

2. 9β-estradiol.

3. The process of producing estradiol derivatives selected from the group consisting of



wherein R and R′ can be different and represent radicals selected from the group consisting of hydrogen, lower alkyl and the acyl radical of an organic carboxylic acid having from 1 to 18 carbon atoms selected from the group consisting of alkanoic acids, alkenoic acids, cycloalkyl-alkanoic acids, phenyl-alkanoic acids, cycloalkanoic acids and phenyl carboxylic acids which comprises the steps of heating a compound of the formula:



wherein R′ has the above assigned meanings, in an inert organic solvent in the presence of a catalyst having a palladium base, and recovering said estradiol derivatives.

4. The process of claim 3 wherein the starting compound is 19-nor-/9β/,/10α/-testosterone.

5. The process of claim 3 wherein the starting compound is the benzoate ester of 19-nor-/9β/,/10α/-testosterone.

6. The process of claim 3 wherein said inert organic solvent is ethanol.

7. The process of claim 3 wherein said catalyst is palladium hydroxide.

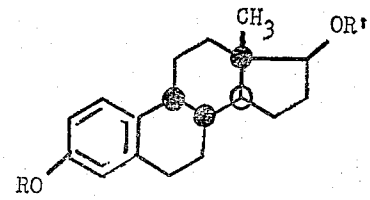
8. The process of claim 6 wherein said catalyst is palladium hydroxide deposited and supported on magnesia.

9. The process of preparing 9β-estradiol which comprises the steps of heating 19-nor-/9β/,/10α/-testosterone in an anhydrous lower alkanol at the reflux temperature

in an inert atmosphere in the presence of palladium hydroxide deposited and supported on an alkaline reacting compound, recovering a mixture of estradiol and 9β-estradiol, preparing the 17β-benzoate ester of said mixture, suspending said 17β-benzoate esters in isopropyl ether, separating the insoluble isopropyl solvate of the 17β-benzoate of estradiol, and recovering said 9β-estradiol.

10. The process of preparing 9β-estradiol which comprises the steps of heating the benzoate ester of 19-nor-/9β/,/10α/-testosterone in an anhydrous lower alkanol at the reflux temperature in an inert atmosphere in the presence of palladium hydroxide deposited and supported on an alkaline reacting compound, recovering a mixture of 17β-benzoate esters of estradiol and 9β-estradiol, suspending said 17β-benzoate esters in isopropyl ether, separating the insoluble isopropyl solvate of the 17β-benzoate of estradiol, and recovering said 9β-estradiol.

11. A process for the promotion of an estrogenic response on the vaginal epithelium substantially free of an estrogenic response on other sites which comprises the administration to warm-blooded animals of between 25 γ/kg. and 2 mg./kg. per day of an estradiol derivative of the formula:



wherein R and R′ can be different and represent radicals selected from the group consisting of hydrogen, lower alkyl and the acyl radical of an organic carboxylic acid having from 1 to 18 carbon atoms selected from the group consisting of alkanoic acids, alkenoic acids, cycloalkyl-alkanoic acids, phenyl-alkanoic acids, cycloalkanoic acids and phenyl carboxylic acids.

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Steroids (Fieser et al.), published by Reinhold Publishing Corporation (1959), page 445 relied on.

LEWIS GOTTS, *Primary Examiner.*