10 DEC. 1963

PATENT SPECIFICATION

NO DRAWINGS

825,210

Date of Application and filing Complete Specification Feb. 6, 1956.
No. 3693/56.
Application made in Switzerland on Feb. 7, 1955.
Application made in Switzerland on Feb. 25, 1955.
Application made in Switzerland on April 1, 1955.
Application made in Switzerland on May 18, 1955.
Application made in Switzerland on June 28, 1955.
Application made in Switzerland on July 21, 1955.
Complete Specification Published Dec. 9, 1959.

Index at acceptance:—**Class 2(3)**, U(2:3), U4(A1:A2:B1:B2:C1:C2:C3:C4:C5:D:X), U(5:7).

International Classification:—**C07c.**

COMPLETE SPECIFICATION

Dehydro-Compounds of the Steroid Series and process for their Manufacture

We, CIBA LIMITED, a Body Corporate organised according to the laws of Switzerland, of Basle, Switzerland, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

This invention relates to dehydro compounds of the steroid series, containing double bonds in the 1:2- and the 4:5-positions, and to a process for their manufacture.

Specification No. 792,803 (24354/54) describes a process for the manufacture of products of the steroids series by the biochemical degradation of the side chain of pregnane compounds to form androstane compounds and the splitting up, in certain cases, of the ring D, with the simultaneous introduction of a double bond into the 1,2-, or, if desired, into the 1,4-position. In this manner, for example, progesterone, as also Δ^5-3β-hydroxy-20-oxo-pregnene, 11-desoxycorticosterone of 3,20-dioxo-allopregnane can be converted in one step into $\Delta^{1,4}$-3,17-dioxo-androstadiene or 1,2-dehydro-testolactone (lactone of $\Delta^{1:4}$-13:17-seco-13-hydroxy-androstadiene-17-acid).

The present invention is based on the observation that in steroid compounds, especially of the pregnane series, the 1:2-double bond and/or 4:5 double bond can be introduced by a biochemical method without degradation of the side chain or splitting up of a ring, when steroid compounds which are saturated in 1:2-position and/or in 4:5-position are subjected to the aerobic action of enzymes of *Didymella lycopersici, Calonectria decora, Alternaria passiflorae, Ophiobolus heterostrophus* or *Ophiobolus Miyabeanus*.

[*Price 3s. 6d.*]

The steroid compounds used as starting materials are, for example, derivatives of spirostane, allospirostane, furostane, allofurostane, cholane, allocholane, pregnane, allopregnane, androstane and testane and contain, preferably in 3-position, a free or functionally converted hydroxyl or oxo group. They may be saturated or may contain double bonds, for example in 1- or 4-position, and also in 5-, 6-, 7-, 8-, 9:11-, 14- or 16-position, or additional substituents such as free or converted hydroxyl, or oxo or carboxyl groups or epoxy groups or halogen atoms, for example in 6-, 7-, 8-, 9-, 11-, 12-, 14-, 15-, 16-, 17-, 18-, 20-, or 21-position, or methyl groups, for example, in 17α- or 17β-position. The above mentioned starting materials are of any steric configuration and may also exist as racemates. Especially important starting materials are compounds of the pregnane series which contain in 3- and 20-position free or functionally converted hydroxyl or oxo groups, for example progesterone, 11-dehydro-progesterone, 11-, 12-, 14-, 15-, 16-, 17- or 18-hydroxy-progesterones, Δ^5- or Δ^4-pregnen-3β-ol-20-ones, Δ^5-pregnene-3:20-diols, 11-desoxy-corticosterone, cortisone, hydrocortisone, 11-epi-hydrocortisone, aldosterone, 18-hydroxy-corticosterone, 11-epi-18-hydroxy-corticosterone, 17α-hydroxy-aldosterone, 18-hydroxy-hydrocortisone, 18-hydroxy- and 18-oxo-cortisone, 18-hydroxy- and 18-oxo-cortexone, Substance S (Reichstein), 18-hydroxy- and 18-oxo-Substance S (Reichstein), corresponding compounds, which are unsaturated, not in the 4-, but in the 1-position, pregnane-3:20-dione, pregnan-3-ol-20-one, allopregnane-3:20-dione, pregnane- and allopregnane-3:11-20-trione-17α:21-diol, pregnane- and allopregnane-3:

Price 4s 6d

20-dione-11β:17α:21-triol, pregnane- and allopregnane-3:18:20-trione-11β:21-diol, pregnane and allopregnane-3:20-dione-11β:18:21-triol, pregnane- and allopregnane-3:20-dione-18:21-diol, the corresponding 21-oxo-compounds and/or 9α-fluoro- and chloro-derivatives for example 9α-fluoro-cortisone, 9α-fluoro-hydrocortisone, 9α-fluoro-aldosterone and 9α-fluoro-18-hydroxy-corticosterone, also androstane compounds saturated in 1:2-position, and/or in 4:5-position, for example Δ^4- or Δ^5-androstene-3:17-dione, testosterone, Δ^5-androsten-3-ol-17-one, andrenosterone, androstane-3:17-dione, etianic acids, for example Δ^4- or Δ^5-etianic acids, alloetianic acids which contain hydroxyl or oxo-groups, especially in 3-, 11-, and/or 18-position, or functional derivatives of the same compounds, that is to say corresponding compounds with functionally converted hydroxyl or oxo-groups. In the starting materials the functionally converted hydroxyl group is, for example, a hydroxyl group esterified with an aliphatic, aromatic or heterocyclic carboxylic acid, for example, acetic acid, propionic acid, benzoic acid or furane carboxylic acid or an etherified hydroxyl group, for example the tetrahydropyranyloxy-, benzyloxy- or triphenylmethoxy-group. The functionally converted oxo-group is advantageously a ketalised oxo-group, derived especially from a dihydric alcohol, such as the ethylenedioxy group.

The specified starting materials are, in accordance with the invention, brought into reaction with enzymes of *Didymella lycopersici, Calonectria decora, Alternaria passiflorae, Ophiobolus heterostophus* or *Ophiobolus Miyabeanus*.

For their preparation cultures of these fungi in the known media for this purpose are suitable, for example such as contain sugars, such as glucose or lactose, or peptones, corn steep liquor, soya products and the like, or with mineral salts or also synthetic nutrient solutions. The operation is conducted especially under aerobic conditions for example in a surface culture or preferably submerged with stirring or shaking and passage of air through the culture liquid. The specified fungi are distinguished from other micro-organisms, for example the bacteria, by good growth under relatively simple culture conditions. The reaction according to the present invention takes place in the described fungi cultures or with the aid of the enzymes contained therein, if desired after enrichment or separation, that is to say in the simplest case in a suspension of the separated fungus mycelium, or of the homogenised fungus mycelium or in filtrates or aqueous extracts thereof.

The isolation of the products of the process can be carried out by known methods. Their separation can take place, for example, by extraction of the reaction mixture with an organic solvent, for example methylene chloride or ethyl acetate. For the further purification of the resulting extract there can suitably be applied, especially chromatography, for example on alumina or silica gel, or distribution methods, for example the counter-current process, or separation by means of Girard reagents such as trimethyl ammonium or pyridinium acetic acid hydrazide. Subsequent to or instead of purification as above, preferably recrystallization is used from organic or aqueous-organic solvents.

By the introduction of the 1:2-double bond and/or 4:5-double bond valuable medicaments of the steroid series, especially of the pregnane series are obtained, which are distinguished on comparison with therapeutically active compounds which are saturated in 1:2-position, by an enhanced activity.

The new dehydro-compounds of the steroid series provided by the present invention include 1:4-bis-dehydro-pregnadiene compounds having in 18-position a free or functionally converted hydroxyl or oxo group and 1:4-bis-dehydro-9α-halogen-pregnadiene compounds as well as 17α-methyl-3:20-dioxo-1:4-bis dehydro pregnanes. Specific new compounds include: 1-dehydro-aldosterone and its functional derivatives, $\Delta^{1:4}$-3:20-dioxo-18:21-dihydroxypregnadiene and its functional derivatives, $\Delta^{1:4}$-3:18:20-trioxo-11β:17α:21-trihydroxypregnadiene and its functional derivatives, $\Delta^{1:4}$-3:18:20-trioxo-9α-fluoro-11β:21-dihydroxypregnadiene and its functional derivatives, $\Delta^{1:4}$-3:18:20-trioxo-9α-fluoro-11β:17α:21-trihydroxy-pregnadiene and its functional derivatives, $\Delta^{1:4}$-3:11:20-trioxo-9α-fluoro-17α:21-dihydroxy-pregnadiene and its functional derivatives, 1-dehydro-9α-fluoro-hydrocortisone and its functional derivatives, 1:6-bis-dehydro-cortexone and its functional derivatives, 1:11-bis-dehydro-progesterone and its functional derivatives, 1-dehydro-11-oxo-17α-methyl-cortexone and its functional derivatives, 1-dehydro-17α-methyl-testosterone and its functional derivatives, 1-dehydro-17α-ethinyl-testosterone and its functional derivatives. Other 18-oxygenated compounds are: $\Delta^{1:4}$-3:11:18:20-tetraoxo-21-hydroxy-pregnadiene, $\Delta^{1:4}$-3:20-dioxo-11β:18:21-trihydroxy-pregnadiene $\Delta^{1:4}$-3:11:18:20-tetraoxo-17α:21-dihydroxy-pregnadiene, $\Delta^{1:4}$-3:20-dioxo-11β:17α:18:21-tetrahydroxy-pregnadiene, $\Delta^{1:4}$-3:18:20-trioxo-21-hydroxy-pregnadiene, $\Delta^{1:4}$-3:20-dioxo-17α:18:21-trihydroxy-pregnadiene, $\Delta^{1:4}$-3:18:20-trioxo-17α:21-dihydroxy-pregnadiene, the corresponding 21-oxo- and 21-desoxy-compounds and also corresponding functional derivatives, such as esters, ethers, halogen derivatives, for example 9α-halogen, especially the fluorine or chlorine compounds. Among the many other 1-dehydro-steroids that can be obtained according to the new dehydration process there may be further mentioned: 1-dehydro-cortisone,

1-dehydro-hydrocortisone and the 1-dehydro derivative of Substance S of Reichstein. If the products of the process do not have the configuration and do not contain the substituents of therapeutically useful steroids, they can serve as intermediate products for the manufacture of such compounds, for example the above specified compounds.

The reaction products obtainable according to the process of the invention can be converted in the customary manner into their functional derivatives, such as oxygen, sulphur or nitrogen derivatives, for example esters, ethers, enol esters, enol ethers, ketals, thio-ethers and thioketals, also hydrazones, oximes and enamines. In these compounds the hydroxyl and/or oxo groups can be completely or partially functionally converted.

In the esters and enol esters the acid residues are derived from any organic or inorganic acids, such as aliphatic, alicyclic, araliphatic, aromatic or heterocyclic carboxylic, thione carboxylic, thiol carboxylic or sulphonic acids, preferably from formic acid, acetic acid, chloracetic acids, trifluoracetic acid, propionic acid, butyric acids, valeric acids, diethylacetic acid, caproic acids, œnanthic acids, caprylic acids, palmitic acids, crotonic acid, undecanic acid, undecylenic acid, oxalic acid, succinic acid, pimelic acid, maleic acid, lactic acid, carbamic acids, alkoxy-carboxylic acids, β-cyclopentyl-propionic acid, hexahydrobenzoic acid, benzoic acid, phenylacetic acid, cyclohexylacetic acid, phenylpropionic acids, trimethylgallic acid, phthalic acid, furane-2-carboxylic acid, isonicotinic acid, methane sulphonic acid, toluene sulphonic acid, sulphuric acids, hydrohalic acids or phosphoric acids.

If desired, in resulting compounds functionally converted hydroxyl or oxo groups can be converted into free groups. In a similar manner in polysubstituted derivatives the functionally converted groups can also be partially liberated. This takes place, for example, by chemical or enzymatic hydrolysis, especially with the use of acid or basic agents, by re-esterification or re-acetalisation. From derivatives obtained in this manner or also directly and which are only partially converted, for example esterified or etherified, by subsequent functional conversion, for example esterification or etherification, polysubstituted derivatives, especially mixed ethers or mixed ethers or ester-ethers, can be produced.

The products of the present process can be used as medicaments or as intermediate products for the manufacture thereof.

The following examples illustrate the invention:—

EXAMPLE 1.

4 litres of 70% beerwort are sterilized in a shaking vessel (pH 5.1) and inoculated with 150 cc of a 2-day old agitation culture of *Calonectria decora* in 70% beerwort. The vessel is shaken for 24 hours at 27° C., whereby the culture becomes well-developed. A solution of 1 gram of cortexone in 25 cc of acetone is then added under sterile conditions and the whole further shaken at 27° C. After 2 days, the mycelium is separated and well washed with water and ethyl acetate. After exhaustive extraction of the combined filtrates with a total quantity of 4 litres of ethyl acetate, the extracts are washed with water, dried and evaporated under vacuum. The resulting crude extract (1.1 grams) is chromatographed over a column of 30 grams of silica gel by fractional elution with chloroform and with chloroform-acetone mixtures of increasing acetone content. The individual fractions (100 cc in each case) are investigated by paper chromatography. The fractions eluted with chloroform contain only impurities, whereas in the chloroform-acetone (9:1) fractions a new substance is found which in a paper chromatogram (system propylene glycol-toluene) runs for a shorter distance than cortexone. The fractions of higher acetone content contain only a small quantity of highly polar material. The fractions containing the said new substances are combined and evaporated under vacuum. By recrystallization of the residue from acetone-petroleum ether mixtures, the pure 1-dehydro-cortexone is obtained of m.p. 185 to 192° C. in practically quantitative yield. This compound reduces alkaline silver diamine solution rapidly and strongly. The analysis agrees with the empirical formula $C_{21}H_{28}O_3$ (calculated C 76.79 H 8.59%, found C 76.67 H 8.84%). The ultraviolet spectrum of the free compound exhibits a strong band having a maximum at 244 mμ (ε=14.200), that of the dinitrophenyl hydrazone exhibits a maximum at 388 mμ (ε=39.000). The corresponding bands in the case of cortexone are at 240 mμ (ε=16.200) and 378 mμ (ε=39.000).

EXAMPLE 2.

By using for the dehydrogenation of cortexone instead of the culture of *Calonectria decora* described in Example 1, in an analogous manner a culture of *Alternaria passifloræ*, *Ophiobolus heterostrophus* or *Ophiobolus Miyabeanus*, using the method of working up described in Example 1, 1-dehydrocortexone is obtained in high yield.

EXAMPLE 3.

By the method described in Example 1, in a shaking vessel, 4 litres of a culture of *Calonectria decora* are produced and, under sterile conditions, a solution of 1 gram of cortisone in 25 cc of methanol is added thereto. The vessel is then shaken at 27° C. After 4 days, the mycelium is separated off and the culture filtrate is exhaustively extracted with a total quantity of 3 litres of ethyl acetate. The combined extracts are washed with water, dried and evaporated under vacuum. The

resulting crude residue is chromatographed as described in Example 1 on 30 grams of silica gel by the fractional elution method. The fractions eluted with chloroform-acetone (6:4) mixtures consist for the most part of a substance which is somewhat more polar than the starting material. These fractions are combined and evaporated under vacuum. From acetone-ether mixtures the 1-dehydro-cortisone is crystallized; m.p. 231 to 234° C. The substance reduces alkaline silver diamine solution rapidly and strongly and exhibits in the ultraviolet spectrum a strong absorption at 240 mμ (ϵ=15.800). By acetylation with pyridine-acetic anhydride, the corresponding 21-acetate is obtained of m.p. 224—230° C. In a similar manner there can be obtained, for example, the œnanthate or undecylenate.

EXAMPLE 4.

To 4 litres of a well developed culture of *Calonectria decora* there is added under sterile conditions a solution of 1 gram of hydrocortisone in 25 cc of methanol. After shaking for 4 days at 27° C., extraction is carried out as described in Example 1 followed by chromatography in also similar manner. The fractions eluted with chloroform-acetone (1:1) mixtures contain a substance which is somewhat more polar than hydrocortisone. These fractions are combined and evaporated. The 1-dehydro-hydrocortisone is ~~recrystallized~~ from methanol or mixtures of acetone-ether; m.p. 238—241° C. It reduces alkaline silver diamine solution rapidly and strongly and exhibits in the ultraviolet spectrum a strong absorption at 244 mμ (ϵ=15.100).

EXAMPLE 5.

In an Erlenmeyer flask of 500 cc capacity 150 cc of 70% beerwort are sterilized and inoculated with *Calonectria decora*. The whole is shaken for 2 days at 27° C. and there is added under sterile conditions to the now well developed culture a solution of 30 mg of aldosterone in 1.5 cc of acetone. Shaking is then continued at 27° C. and after 38 hours the mycelium is filtered off. The culture filtrate is exhaustively extracted with ethyl acetate and the extracts washed with water, dried and evaporated. The residue is chromatographed, as described in Example 1, on 1 gram of silica gel and the individual fractions (20 cc in each case) are examined by paper chromatography. In the fractions eluted with chloroform-acetone (1:1) mixtures there is a substance which is somewhat more polar than aldosterone and reduces alkaline silver diamine solution. It is crystallized from acetone-ether mixtures and constitutes 1-dehydro-aldosterone. In the ultraviolet spectrum it exhibits strong absorption at 244 mμ.

EXAMPLE 6.

In 14 Erlenmeyer flasks each of 200 cc capacity 50 cc of 70% beerwort per flask are sterilized and inoculated with *Calonectria decora*. After 2 days shaking at 27° C. the cultures are well developed. Under sterile conditions there is added to each of the 14 cultures one of the following 14 compounds (in each case 10 mg dissolved in 0.5 cc of methanol); cortexone, cortisone, hydrocortisone, 17α-hydroxy-cortexone, corticosterone, 11-epi-corticosterone, 11-epi-hydrocortisone, aldosterone, 17α-hydroxy-aldosterone, progesterone, pregnenolone, 21-hydroxy-pregnenolone, Δ^5-androsten-3β-ol-17-one and Δ^4-androsten-3:17-dione. Shaking is continued in each case for 2 days further at 27° C. and the cultures are then extracted with ethyl acetate. Paper chromatographic examination of the individual extracts shows that all of the starting substances have been converted into the corresponding 1-dehydrocompounds which compared with the starting materials possess a slightly increased polarity and are characteristically distinguished therefrom in their spectra.

EXAMPLE 7.

If there are used for the dehydrogenation of the substances specified in Example 6 instead of the cultures of *Calonectria decora* therein described, cultures of *Alternaria passiflorae*, *Ophiobolus heterostrophus* or *Ophiobolus Miyabeanus*, paper chromatographic examination of the extracts proves the presence of the same reaction products as are described in Example 6.

EXAMPLE 8.

A solution of 1 g of 3:11:20-triketo-17α:21-dihydroxy-allopregnane in 25 cc of methanol is added under sterile conditions to 4 litres of a well developed culture of *Calonectria decora*. The mixture is agitated at 27° C. for 3 days and the mycelium then separated. The extraction of the culture filtrate and the chromatography of the crude extract are carried out in the manner described in Example 1. The portions extracted with mixtures of chloroform and acetone (1:1) contain the 1-dehydro-cortisone which is crystallized from mixtures of acetone and ether. Its melting point is at 230—233° C. and it has the properties described in Example 3.

When instead of the solution of 3:11:20-triketo-17α:21-dihydroxy-allopregnane a solution of 3:11:20-triketo-17α:21-dihydroxy-pregnane in 25 cc of methanol is added to an analogous culture of *Calonectria decora* and the product worked up in the manner described, the 1-dehydro-cortisone is also obtained in good yield.

EXAMPLE 9.

Under sterile conditions, a solution of 1 g of 3:20-diketo-11β:17α:21-trihydroxy-allopregnane in 25 cc of methanol is added to 4 litres of a culture of *Calonectria decora* and the mixture agitated at 27° C. for 4 days. The

mycelium is then separated and the extraction of the culture filtrate and the chromatography of the extract carried out in the manner described in Example 1. From the portions eluted with mixtures of chloroform and acetone (1:1) the 1-dehydro-hydrocortisone crystallizes in good yield. Its melting point is at 239—242° C. and it has the properties described in Example 4.

EXAMPLE 10.

A solution of 1 g of androstane-3:17-dione in 25 cc of acetone is added under sterile conditions to 4 litres of a culture of *Calonectria decora*. After 36 hours' agitation at 27° C. the mycelium is separated and the culture filtrate exhaustively extracted by agitation with methylene chloride. The extract is washed twice with 0.1N-hydrochloric acid and twice with sodium bicarbonate solution of 1 per cent strength, washed three times with water, dried, and evaporated under reduced pressure. The residue (1.2 g) is chromatographed over 30 g. of alumina by the fractional elution method, the elution being carried out with mixtures of petroleum ether and benzene, benzene, mixtures of benzene and ether, and ether. The fractions eluted with mixtures of petroleum ether and benzene and those from benzene contain the known $\Delta^{1:4}$-androstadiene-3:17-dione, which crystallizes from a mixture of acetone and petroleum ether in the form of rhombic platelets. Its melting point is at 145—146° C.; $[\alpha]_D = +110°$ ($CHCl_3$).

EXAMPLE 11.

A solution of 40 mg of 1-dehydro-cortexone ($[\alpha]_D^{22} = +120°$ in $CHCl_3$) in 2 cc of pyridine is mixed with 2 cc of acetic anhydride and allowed to stand at room temperature. After 16 hours the solution is evaporated *in vacuo* at 30° C. and the residue taken up in a mixture of chloroform and ether (1:4). The solution is washed three times with 0.1N-hydrochloric acid, three times with sodium bicarbonate solution of 2 per cent strength, and three times with water, then dried and evaporated under reduced pressure. By recrystallizing the residue from a mixture of acetone and petroleum ether there is obtained the pure 1-dehydro-cortexone-21-acetate of melting point 203—205° C. $[\alpha]_D = +134°$ ($CHCl_3$).

EXAMPLE 12.

A solution of 1 gram of 11-dehydrocorticosterone in 25 cc of acetone is added under sterile conditions to 4 litres of a culture of *Calonectria decora*, grown as described in Example 1. The whole is agitated at 27° C. for 3 days and the mycelium then filtered off. The extraction of the culture filtrate and the chromatography of the crude extract on silica gel is performed as described in Example 1. The chloroform fractions and the chloroform-acetone (95:5) fractions contain impurities and some starting material, whereas the chloroform-acetone (90:10) fractions consist mainly of a substance which on the paper chromatogram flows somewhat more slowly than the starting material. This substance is the $\Delta^{1,4}$-3,11,20-triketo-21-hydroxy-pregnadiene, which is crystallized from a mixture of acetone and ether. The crystals decompose between 200 and 220° C., depending on the speed of heating. In the ultraviolet light the substance absorbs at 240 mμ, and in the infrared spectrum it shows the bands at 5.99 μ, 6.14 μ and 6.22 μ characteristic of the $\Delta^{1,4}$-3-keto grouping. On acetylation with pyridine-acetic anhydride the corresponding 21-acetate of melting point 188—191° C. is obtained. In a similar manner there can be prepared e.g. the enanthate or undecylenate.

EXAMPLE 13.

A solution of 1 gram of corticosterone in 25 cc of acetone is added under sterile conditions to 4 litres of a culture of *Calonectria decora*, grown as described in Example 1. The whole is agitated at 27° C. for 4 days and the mycelium then filtered off. The extraction of the culture filtrate and the chromatography of the crude extract on silica gel is performed as described in Example 1. The chloroform fractions and the chloroform-acetone (95:5) fractions contain impurities and some starting material, whereas the chloroform-acetone (90:10) and (18:20) fractions consist mainly of a substance which on the paper chromatogram flows somewhat slower than the starting material. The substance is 1-dehydro-corticosterone, which is crystallized from acetone. It melts at 216—220° C. (with decomposition). $[\alpha]_D = +158°$ (alcohol). In ultraviolet light the substance absorbs at 244 mμ (ε=15300) and in the infrared spectrum it shows bands at 2.76 μ, 2.86 μ, 5.84 μ, 5.99 μ, 6.14 μ, 6.22μ, 7.44 μ, 8.73 μ, 9.22 μ, 9.38 μ and 9.63 μ.

The 21-acetate prepared by means of a mixture of pyridine and acetic anhydride at 20° C. melts at 159—161° C.; $[\alpha]_D = +151°$ (dioxane); UV absorption spectrum λ_{max} 243 mμ (ε=14800).

EXAMPLE 14.

A solution of 1 gram of 17α-hydroxy-cortexone (Reichstein's substance S) in 25 cc of methanol is added under sterile conditions to 4 litres of a culture of *Calonectria decora*, grown as described in Example 1. The whole is agitated for 3 days at 27° C. and the mycelium then filtered off. The extraction of the culture filtrate and the chromatography of the crude extract on silica gel is performed as described in Example 1. The chloroform fractions and the chloroform-acetone (97.5:2.5) fractions contain impurities, while with a mixture of chloroform and acetone (95:5) some starting material is eluated. The chloroform-acetone (90:10) fractions consist of a mixture

containing, in addition to a small amount of starting material and more polar products, mainly 1-dehydro-17α-hydroxy-cortexone. This substance is obtained in pure form on crystallization from acetone; melting point, 227—233° C. (decomposition); $[\alpha]_D = +76°$ (ethanol); UV absorption spectrum λ_{max} 244 mμ ($\varepsilon = 16200$). The infrared spectrum shows bands at 2.76 μ, 2.85 μ, 5.84 μ, 6.00 μ, 6.15 μ, 6.23 μ, 9.01μ, 9.20 μ, 9.55μ, and 10.04 μ.

The 21-acetate prepared by means of pyridine-acetic anhydride melts at 216—222° C., $[\alpha]_D$ +86° (dioxane); UV absorption spectrum λ_{max} 244 mμ ($\varepsilon = 15400$).

EXAMPLE 15.

A solution of 1 gram of progesterone in 25 cc of acetone is added under sterile conditions to 4 litres of a culture of *Calonectria decora* grown as described in Example 1. The culture is shaken for 24 hours at 27° C., and the mycelium is then filtered off. The culture filtrate is extracted as described in Example 1. The crude extract is chromatographed over a column of 30 grams of aluminium oxide free from alkali, the separate fractions (of 100 cc each) being examined by paper-chromatography. The petroleum ether-benzene fractions contain starting material, whilst with benzene chiefly 1-dehydro-progesterone is eluated. The latter is crystallized from a mixture of ether and petroleum ether. Melting point=151—152° C.; $[\alpha]_D^{23} = +120°$ (ethanol); ultraviolet absorption spectrum λ maximum 245 mμ ($\varepsilon = 16150$). In the infrared there are bands *inter alia* at 5.86 μ, 5.99 μ, 6.14 μ, 6.23 μ, 7.23 μ, 7.38 μ, 8.33 μ, 8.62 μ, 10.55 μ and 12.24 μ.

EXAMPLE 16.

In a shaking vessel 1 litre of a nutrient solution containing 15 grams of peptone, 10 cc of corn steep liquor, 0.1 gram of crude glucose and tap water, is adjusted to a pH value of 6.8 and sterilized. It is then inoculated with a culture of *Calonectria decora*. The culture is shaken for 48 hours at 27° C. A solution of 250 mg of cortisone in 8 cc of methanol is then added to the well developed culture under sterile conditions. The culture is further shaken at 27° C., after three days the mycelium is filtered off and the culture filtrate is extracted with ethyl acetate as described in Example 1. The examination by paper-chromatography of the crude extract shows that in addition to few more polar components only 1-dehydro-cortisone but no starting material is present any longer. By means of crystallization form a mixture of acetone and ether and from methanol the 1-dehydro-cortisone is obtained in a pure form.

EXAMPLE 17.

2 grams of sodium nitrate, 1 gram of primary potassium ortho-phosphate, 0.5 gram of magnesium sulphate heptahydrate, 0.5 gram of potassium chloride, 50 grams of glucose and 1 gram of "Difco" (Registered Trade Mark) yeast extract are dissolved in 1 litre of tap water, brought to pH 5 by addition of a sodium hydroxide solution and sterilized. The resulting nutrient solution is inoculated with 50 cc of a four day-old shaking culture of *Didymella lycopersici* and shaken for 48 hours at 27° C., whereby the culture becomes well developed. Then under sterile conditions a solution of 250 mg of cortexone in 10 cc of acetone is added. The whole is shaken for a further two days at 27° C., the mycelium then filtered off with suction, washed with water and ethyl acetate and the combined filtrates extracted with ethyl acetate. The ethyl acetate solutions are washed with water, dried and evaporated under vacuum and the resulting crystalline residue, on paper-chromatographic examination in the system Bush B_3 (ligroin: benzene: methanol: water (6.67:3.33:8.0:2.0)) is found to be practically pure 1-dehydro-cortexone. For purification, a solution thereof in chloroform is run through a layer of silica gel. The chloroform filtrate contains a little oil. The further filtrates, obtained with chloroform with the addition of 1—3% of tertiary butanol are combined and evaporated. The residue, on recrystallization from a mixture of acetone-isopropyl ether yields 220 mg of 1-dehydrocortexone of mp 189—195° C. (with decomposition).

EXAMPLE 18.

To 1 litre of a well developed culture of *Didymella lycopersici*, prepared as described in Example 17, there is added under sterile conditions a solution of 250 mg of cortisone in 10 cc of methanol and the whole is shaken for 72 hours at 27° C., after which the culture liquid is worked up as described in Example 17. On the basis of paper-chromatographic examination in the system propylene glycol-toluene, the residue of the ethyl acetate extracts contains as steroid fraction 1-dehydro-cortisone practically exclusively. The residue, in chloroform solution, is filtered through silica gel. The chloroform filtrates give a little of an oil. With chloroform-acetone (6.4) a crystalline residue is obtained which on recrystallization from a mixture of acetone-and ether, gives 225 mg of pure 1-dehydrocortisone of m.p. 231—234° C.

EXAMPLE 19.

To 1 litre of a well developed culture of *Didymella lycopersici*, prepared as described in Example 17, there is added under sterile conditions a solution of 250 mg of hydrocortisone in 10 cc of methanol. After shaking for ten days at 27° C., the culture is worked up as described in Example 17. The residue of the ethyl acetate solutions, in a paper-chromatogram (system propylene-glycol-

toluene) proves to be practically pure 1-dehydrohydrocortisone. For separation of the accompanying substances it is taken up in chloroform and the solution filtered through silica gel. The chloroform eluates, which contain a small quantity of an oil, are rejected. The chloroform-acetone (1:1) eluates are combined and evaporated. The residue is recrystallized from methanol or an acetone-ether mixture, whereby 210 mg of pure 1-dehydrohydrocortisone of mp 238—241° C. are obtained.

EXAMPLE 20.

To 120 cc of a well developed culture of *Didymella lycopersici* obtained as described in Example 17, is added under sterile conditions a solution of 30 mg of 3:11:20-triketo-17α:21-dihydroxy-allopregnane in 2 cc of methanol. The whole is shaken for ten days at 27° C. and the culture worked up as described in Example 17. On the basis of paper-chromatographic examination in the system propylene glycol-toluene, the residue of the ethyl acetate extracts contains, together with unchanged starting material, 1-dehydrocortisone, which is obtained in pure form by chromatography on silica gel and subsequent crystallization from a mixture of acetone and ether.

EXAMPLE 21.

In an analogous manner to Example 20 the corresponding $\Delta^{1:4}$-dienes are obtained from progesterone, Δ^{1}-allopregnene-3:20-dione, pregnane-3:20-dione, corticosterone, 11-dehydrocorticosterone, 17α-hydroxy-cortexone, testosterone and Δ^{1}- or Δ^{4}-androstene-3:17-dione.

EXAMPLE 22.

To 2 litres of a well developed culture of *Didymella lycopersici* obtained as described in Example 17, there is added under sterile conditions a solution of 500 mg of Δ^{4}-3:11:20-trioxo-17α-methyl-21-acetoxy-pregnene in 15 cc of acetone. Shaking is carried out for 3 days at 27° C. and working up then effected as described in Example 17. The extraction residue (585 mg), on the basis of paper-chromatographic examination, consists practically exclusively of $\Delta^{1:4}$-3:11:20-trioxo-17α-methyl-21-hydroxy-pregnadiene. By crystallization from an acetone-petroleum ether mixture it is obtained in needles which melt at 183—186° C. with slight decomposition. $[\alpha]_D = +120°$ (alcohol), ultra violet absorption λ_{max} 240 mμ (ε=14,600). The infrared spectrum shows the characteristic bands for $\Delta^{1:4}$-3-ketones at 5.98 μ; 6.13 μ and 6.21 μ. The 21-acetate prepared with pyridine-acetic anhydride melts at 198—201° C.; $[\alpha]_D^{24} = +135°$ (chloroform), UV absorption spectrum λ_{max} 240 mμ (ε=14,800).

EXAMPLE 23.

To four litres of a well developed culture of *Didymella lycopersici* obtained as described in Example 17, there is added under sterile conditions a solution of 1 gram of 17α-ethinyl-testosterone in 25 cc of acetone. Shaking is carried out for 8 days at 27° C. and the culture then worked up as described in Example 17. Paper-chromatographic examination of the extraction residue shows that it consists practically exclusively of 1-dehydro-17α-ethinyl-testosterone. By crystallization from a mixture of acetone and ether the pure substance is obtained. M.p. 228—233° C. $[\alpha]_D = -17°$ (chloroform).

EXAMPLE 24.

To 2 litres of a well developed culture of *Didymella lycopersici* prepared as described in Example 17, there is added under sterile conditions a solution of 180 mg of 6-dehydro-cortexone-21-acetate in 10 cc of acetone and the whole is then shaken for three days at 27° C. The culture is then worked up as described in Example 17. According to paper-chromatographic examination the extraction residue (190 mg) consists practically exclusively of free 1:6-bis-dehydroxy-cortexone, which is crystallized from acetone-ether mixtures. In the infrared spectrum are the characteristic bands for the $\Delta^{1:4}$-3-ketones at 5.98 μ, 6.13 μ and 6.22 μ. It melts at 166—168° C.; $[\alpha]_D = +112°$ (acetone). The 21-acetate prepared with pyridine- acetic anhydride melts at 158—162° C.; $[\alpha]_D^{25} = +123°$ (ethanol). UV-absorption spectrum λ_{max} 223 mμ (ε=11,600), 256 mμ (ε=9,900) and 300 mμ (ε=12,800).

EXAMPLE 25.

To 4 litres of a well developed culture of *Didymella lycopersici* obtained as described in Example 17, is added under sterile conditions a solution of 1 gram of 11-dehydro-progesterone in 20 cc of acetone. Shaking is carried out for eight days at 27° C. and working up then effected as described in Example 17. In the extraction residue, by paper-chromatographic examination, only 1:11-bis-dehydro-progesterone can be detected. It is obtained in crystalline form from an acetone-petroleum ether mixture and in the infrared spectrum exhibits, among others, bands at 5.98 μ, 6.17 μ, 6.22 μ. It melts at 166—168° C. $[\alpha]_D^{24} = +112°$ (acetone); UV absorption spectrum λ_{max} 245 mμ (ε=17,750).

EXAMPLE 26.

To 2 litres of a well developed culture of *Didymella lycopersici* obtained as described in Example 17, under sterile conditions a solution of 500 mg of 17α-methyl-testosterone in 15 cc of acetone is added. Shaking is carried out for three days at 27° C. and the culture then worked up as described in Example 17. The extraction residue is dissolved in a little acetone. On addition of ether, the 1-dehydro-17α-methyl-testosterone is obtained in compact crystals. M.p. 163—164° C. $[\alpha]_D = \pm 0°$ (chloroform). UV absorption spectrum λ_{max} 245 mμ (ε=15,600).

EXAMPLE 27.

2 grams of sodium nitrate, 1 gram of primary potassium orthophosphate, 0.5 gram of magnesium sulphate heptahydrate, 0.5 gram of potassium chloride, 50 grams of glucose and 1 gram of "Difco" yeast extract are dissolved in 1 litre of tap water, brought to pH 5 by addition of a sodium hydroxide solution and sterilized. The resulting nutrient solution is inoculated with 50 cc of a four day-old shaking culture of *Didymella lycopersici* and the whole shaken for 48 hours at 27° C. as a result of which the culture becomes well developed. To 120 cc of this culture of *Didymella lycopersici* is added under sterile conditions a solution of 30 mg of 9α-fluoro-hydrocortisone in 2 cc of methanol. Shaking is carried out for five days at 27° C. and then the mycelium is filtered off with suction and washed with water and ethyl acetate and the combined filtrates extracted with ethyl acetate. The ethyl acetate solutions are washed with water, dried and evaporated under vacuum. The extraction residue contains, on the basis of paper-chromatographic examination, 1-dehydro-9α-fluoro-hydrocortisone, which can be separated from unchanged starting material by means of a preparative paper-chromatogram (propylene glycol-toluene system). From acetone it forms crystals of melting point 263—266° C. with decomposition), $[\alpha]_D^{23} = +108°$ (ethanol). UV absorption spectrum λ_{max} 240 mμ (ε=15,800). The product obtained in this way is dried under vacuum and acetylated in the usual way at room temperature with 4 cc of a pyridine-acetic anhydride-(1:1)-mixture. The resulting 21-acetate of 1-dehydro-9α-fluoro-hydrocortisone is recrystallized from an acetone-petroleum ether mixture and melts at 244—246° C. (with decomposition), $[\alpha]_D^{23} = +108°$ (dioxane), UV absorption spectrum λ_{max} 240 mμ (ε=16,250).

EXAMPLE 28.

2 grams of sodium nitrate, 1 gram of primary potassium orthophosphate, 0.5 gram of magnesium sulphate heptahydrate, 0.5 gram of potassium chloride, 50 grams of glucose and 1 gram of "Difco" yeast extract are dissolved in 1 litre of tap water, brought to pH 5 by addition of a sodium hydroxide solution and sterilized. The resulting nutrient solution is inoculated with 50 cc of a four day old shaking culture of *Didymella lycopersici* and the whole shaken for 48 hours at 27° C. as a result of which the culture becomes well developed. To 120 cc of this culture of *Didymella lycopersici* is added under sterile conditions a solution of 30 mg of 9:11β-oxido-17α-hydroxy-cortexone-21- acetate in 2 cc of methanol. Shaking is carried out for five days at 27° C. and then the mycelium is filtered off with suction and washed with water and ethyl acetate and the combined filtrates extracted with ethyl acetate. The ethyl acetate solutions are washed with water, dried and evaporated under vacuum. The extraction residue contains, on the basis of paper-chromatographic examination, 1-dehydro-9:11β-oxido-17α-hydroxy cortexone, which is separated by means of a preparative paper-chromatogram (system propylene glycol-toluene) from unchanged starting material. The crude product is dried under high vacuum and acetylated in the usual way at room temperature with 4 cc of a pyridine-acetic anhydride-(1:1)-mixture.

The resulting 1-dehydro-9:11β-oxido-17α-hydroxy-cortexone-21- acetate (30 mg) is dissolved in 5 cc of dioxane and the solution mixed with 1.25 cc of 2.5 N hydrofluoric acid in chloroform and the whole allowed to stand for 1 hour at room temperature. Then water is added and extraction carried out with chloroform-ether (1:3). After washing with water, drying and evaporation of the solvent under vacuum there is obtained the 1-dehydro-9α-fluoro-hydrocortisone-21-acetate of m.p. 235—237° C. After recrystallization the pure product melts at 244—246° C.

WHAT WE CLAIM IS:—

1. A process for the manufacture of 1:4-bis-dehydro compounds of the steroid series, wherein a steroid compound saturated in the 1:2- and/or 4:5-position is subjected to the aerobic action of an enzyme of *Didymella lycopersici, Calonectria decora, Alternaria passiflorae, Ophiobolus heterostrophus,* or *Ophiobolus Miyabeanus,* and if desired, the resulting product is converted into a functional derivative thereof.

2. A process for the manufacture of 1:4-bis-dehydro compounds of the steroid series, wherein a steroid compound saturated in the 1:2-position is subjected to the aerobic action of an enzyme of *Calonectria decora, Alternaria passiflorae, Ophiobolus heterostrophus,* or *Ophiobolus Miyabeanus,* and if desired, the resulting product is converted into a functional derivative thereof.

3. A process as claimed in Claim 1 or 2, wherein the steroid compound starting material contains in the 3-position a free or functionally converted hydroxyl or oxo group.

4. A process as claimed in any one of Claims 1—3, wherein the steroid compound starting material is a pregnane compound.

5. A process as claimed in any one of Claims 1—4, wherein cortisone is used as starting material.

6. A process as claimed in any one of Claims 1—4, wherein hydrocortisone is used as starting material.

7. A process as claimed in any one of Claims 1—4, wherein 11-epi-hydrocortisone is used as starting material.

8. A process as claimed in any one of Claims 1—4, wherein corticosterone is used as starting material.

9. A process as claimed in any one of Claims

EXAMPLE 27.

2 grams of sodium nitrate, 1 gram of primary potassium orthophosphate, 0.5 gram of magnesium sulphate heptahydrate, 0.5 gram of potassium chloride, 50 grams of glucose and 1 gram of "Difco" yeast extract are dissolved in 1 litre of tap water, brought to pH 5 by addition of a sodium hydroxide solution and sterilized. The resulting nutrient solution is inoculated with 50 cc of a four day-old shaking culture of *Didymella lycopersici* and the whole shaken for 48 hours at 27° C. as a result of which the culture becomes well developed. To 120 cc of this culture of *Didymella lycopersici* is added under sterile conditions a solution of 30 mg of 9α-fluoro-hydrocortisone in 2 cc of methanol. Shaking is carried out for five days at 27° C. and then the mycelium is filtered off with suction and washed with water and ethyl acetate and the combined filtrates extracted with ethyl acetate. The ethyl acetate solutions are washed with water, dried and evaporated under vacuum. The extraction residue contains, on the basis of paper-chromatographic examination, 1-dehydro-9α-fluoro-hydrocortisone, which can be separated from unchanged starting material by means of a preparative paper-chromatogram (propylene glycol-toluene system). From acetone it forms crystals of melting point 263—266° C. with decomposition), $[\alpha]_D^{23} = +108°$ (ethanol). UV absorption spectrum λ_{max} 240 mμ (ε=15,800). The product obtained in this way is dried under vacuum and acetylated in the usual way at room temperature with 4 cc of a pyridine-acetic anhydride-(1:1)-mixture. The resulting 21-acetate of 1-dehydro-9α-fluoro-hydrocortisone is recrystallized from an acetone-petroleum ether mixture and melts at 244—246° C. (with decomposition), $[\alpha]_D^{23} = +108°$ (dioxane), UV absorption spectrum λ_{max} 240 mμ (ε=16,250).

EXAMPLE 28.

2 grams of sodium nitrate, 1 gram of primary potassium orthophosphate, 0.5 gram of magnesium sulphate heptahydrate, 0.5 gram of potassium chloride, 50 grams of glucose and 1 gram of "Difco" yeast extract are dissolved in 1 litre of tap water, brought to pH 5 by addition of a sodium hydroxide solution and sterilized. The resulting nutrient solution is inoculated with 50 cc of a four day old shaking culture of *Didymella lycopersici* and the whole shaken for 48 hours at 27° C. as a result of which the culture becomes well developed. To 120 cc of this culture of *Didymella lycopersici* is added under sterile conditions a solution of 30 mg of 9:11β-oxido-17α-hydroxy-cortexone-21- acetate in 2 cc of methanol. Shaking is carried out for five days at 27° C. and then the mycelium is filtered off with suction and washed with water and ethyl acetate and the combined filtrates extracted with ethyl acetate. The ethyl acetate solutions are washed with water, dried and evaporated under vacuum. The extraction residue contains, on the basis of paper-chromatographic examination, 1-dehydro-9:11β-oxido-17α-hydroxy cortexone, which is separated by means of a preparative paper-chromatogram (system propylene glycol-toluene) from unchanged starting material. The crude product is dried under high vacuum and acetylated in the usual way at room temperature with 4 cc of a pyridine-acetic anhydride-(1:1)-mixture.

The resulting 1-dehydro-9:11β-oxido-17α-hydroxy-cortexone-21- acetate (30 mg) is dissolved in 5 cc of dioxane and the solution mixed with 1.25 cc of 2.5 N hydrofluoric acid in chloroform and the whole allowed to stand for 1 hour at room temperature. Then water is added and extraction carried out with chloroform-ether (1:3). After washing with water, drying and evaporation of the solvent under vacuum there is obtained the 1-dehydro-9α-fluoro-hydrocortisone-21-acetate of m.p. 235—237° C. After recrystallization the pure product melts at 244—246° C.

WHAT WE CLAIM IS:—

1. A process for the manufacture of 1:4-bis-dehydro compounds of the steroid series, wherein a steroid compound saturated in the 1:2- and/or 4:5-position is subjected to the aerobic action of an enzyme of *Didymella lycopersici, Calonectria decora, Alternaria passiflorae, Ophiobolus heterostrophus,* or *Ophiobolus Miyabeanus,* and if desired, the resulting product is converted into a functional derivative thereof.

2. A process for the manufacture of 1:4-bis-dehydro compounds of the steroid series, wherein a steroid compound saturated in the 1:2-position is subjected to the aerobic action of an enzyme of *Calonectria decora, Alternaria passiflorae, Ophiobolus heterostrophus,* or *Ophiobolus Miyabeanus,* and if desired, the resulting product is converted into a functional derivative thereof.

3. A process as claimed in Claim 1 or 2, wherein the steroid compound starting material contains in the 3-position a free or functionally converted hydroxyl or oxo group.

4. A process as claimed in any one of Claims 1—3, wherein the steroid compound starting material is a pregnane compound.

5. A process as claimed in any one of Claims 1—4, wherein cortisone is used as starting material.

6. A process as claimed in any one of Claims 1—4, wherein hydrocortisone is used as starting material.

7. A process as claimed in any one of Claims 1—4, wherein 11-epi-hydrocortisone is used as starting material.

8. A process as claimed in any one of Claims 1—4, wherein corticosterone is used as starting material.

9. A process as claimed in any one of Claims

1—4, wherein 11-desoxy-corticosterone is used as starting material.

10. A process as claimed in any one of Claims 1—4, wherein Δ^5-3β-hydroxy-20-oxo-pregnene is used as starting material.

11. A process as claimed in any one of Claims 1—4, wherein aldosterone is used as starting material.

12. A process as claimed in any one of Claims 1—4, wherein 18-hydroxy-corticosterone is used as starting material.

13. A process as claimed in any one of Claims 1—4, wherein 18-hydroxy-cortexone is used as starting material.

14. A process as claimed in any one of Claims 1—4, wherein 18-oxo-cortexone is used as starting material.

15. A process as claimed in any one of Claims 1—4, wherein progesterone is used as starting material.

16. A process as claimed in Claim 1, wherein there is used as starting material a compound which corresponds to the compound mentioned in any one of Claims 5—9 and 11—15 but which contains the double bond in 1:2-position instead of in 4:5-position.

17. A process as claimed in any one of Claims 1—3, wherein an allopregnane-3:20-dione is used as starting material.

18. A process as claimed in any one of Claims 1—4 and 17, wherein pregnane- or allopregnane-3:11:20-trione-17α:21-diol is used as starting material.

19. A process as claimed in any one of Claims 1—4 and 17, wherein pregnane- or allopregnane-3:20-dione-11β-17α:21-triol is used as starting material.

20. A process as claimed in any one of Claims 1—3, wherein there is used as starting material an androstane compound saturated in 1:2- and/or in 4:5-position and having in the 17-position a free hydroxyl, oxo or carboxyl group.

21. A process as claimed in Claim 1 or 2, wherein a functional derivative of the compound mentioned in any one of Claims 5—20 is used as starting material.

22. A process as claimed in Claim 1 or 2, wherein there is used as starting material a 9α-fluoro- or -chloro derivative of the compound mentioned in any one of Claims 5—8, 11—12, 18 or 19.

23. A process as claimed in Claim 1 or 2, wherein 9:11β-oxido-17α-hydroxy-cortisone acetate is used as starting material.

24. A process as claimed in Claim 23, wherein the resulting 1-dehydro-9:11β-oxido-17α-hydroxy-cortexone acetate is split up by means of a hydrohalic acid to form the corresponding 9:11-halogen hydrin.

25. A process for the manufacture of $\Delta^{1:4}$-unsaturated compounds of the steroid series conducted substantially as described in any one of Examples 1—7 herein.

26. A process for the manufacture of $\Delta^{1:4}$-unsaturated compounds of the steroid series conducted substantially as described in any one of Examples 8—11 herein.

27. A process for the manufacture of $\Delta^{1:4}$-unsaturated compounds of the steroid series conducted substantially as described in any one of Examples 12—15 herein.

28. A process for the manufacture of $\Delta^{1:4}$-unsaturated compounds of the steroid series conducted substantially as described in Example 16 herein.

29. A process for the manufacture of $\Delta^{1:4}$-unsaturated compounds of the steroid series conducted substantially as described in any one of Examples 17—21 herein.

30. A process for the manufacture of $\Delta^{1:4}$-unsaturated compounds of the steroid series conducted substantially as described in any one of Examples 22—26 herein.

31. A process for the manufacture of $\Delta^{1:4}$-unsaturated compounds of the steroid series conducted substantially as described in either of Examples 27 and 28 herein.

32. 1:4-bis-dehydro-pregnadiene compounds having in 18-position a free or functionally converted hydroxyl or oxo group.

33. 1-dehydro-aldosterone and its functional derivatives.

34. $\Delta^{1:4}$-3:20-dioxo-18:21-dihydroxy-pregnadiene and its functional derivatives.

35. $\Delta^{1:4}$-3:18:20-trioxo-11β:17α:21-trihydroxy-pregnadiene and its functional derivatives.

36. 1:4-bis-dehydro-9α-halogen-pregnadiene compounds.

37. $\Delta^{1:4}$-3:18:20-trioxo-9α-fluoro-11β:21-dihydroxy-pregnadiene, and its functional derivatives.

38. $\Delta^{1:4}$-3:18:20-trioxo-9α-fluoro-11β:17α:21-trihydroxy-pregnadiene, and its functional derivatives.

39. $\Delta^{1:4}$-3:11:20-trioxo-9α-fluoro-17α:21-dihydroxy-pregnadiene, and its functional derivatives.

40. 1-dehydro-9α-fluoro-hydrocortisone and its functional derivatives.

41. 1:6-bis-dehydro-cortexone and its functional derivatives.

42. 1:11-bis-dehydro-progesterone and its functional derivatives.

43. 17α-methyl-3:20-dioxo-1:4-bis-dihydro-pregnanes.

44. 1-dehydro-11-oxo-17α-methyl-cortexone and its functional derivatives

45. 1-dehydro-17α-methyl-testosterone and its functional derivatives.

46. 1-dehydro-17α-ethinyl-testosterone and its functional derivatives.

ABEL & IMRAY,
Agents for the Applicants,
Quality House, Quality Court,
Chancery Lane, London, W.C.2.

Leamington Spa: Printed for Her Majesty's Stationery Office, by the Courier Press.—1959.
Published at The Patent Office, 25, Southampton Buildings, London, W.C.2, from which copies may be obtained.