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COMPLETE SPECIFICATION

Pregnadienes

We, SCHERING CORPORATION, a Corporation organised and existing under the laws of the State of New Jersey, United States of America, of 60 Orange Street, Bloomfield, New Jersey, United States of America, 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 steroids and in particular to 1,4-pregnadienes. It covers certain new compounds of this class which have been found to be therapeutically useful. These can all be regarded as derivatives of the hydro-carbon pregnane.

According to the invention we provide compounds of the pregnane series having therapeutic activity and possessing a double bond in the 4-position, which compounds are substituted by ketonic oxygen in the 3- and 20-positions, by a hydroxyl group or ketonic oxygen atom in the 11-position, by a hydroxyl or an esterified hydroxyl group in the 21-position and by a hydroxyl group in the 17α-position and which may be substituted by halogen at the 9α-position, which compounds are also characterised by the presence of a double bond in the 1-position.

The invention also provides a therapeutic composition containing a compound as defined above, mixed with a pharmaceutical carrier.

To aid in an understanding of the invention, reference will be made to the accompanying drawings in which Fig. 1 shows the conventional numbering of the pregnane skeleton. It is conventional to indicate the CH_3 groups attached to the 10 and 13 carbon atoms by vertical lines only. The letters A, B, C, D represent the conventional mode of identifying the four rings.

The principal compounds of the invention are represented by the formulae of Fig. 2 (1-dehydrocortisone and its 21-esters). Fig. 3 (1-dehydrocortisol and its 21-esters), Fig. 4 (9α - halogeno - 1 - dehydrocortisones and their 21-esters), and Fig. 5 (9α-halogeno-1-dehydrocortisols and their 21-esters), all these formulae being combined in the composite formula of Fig. 6. The formula of Fig. 7 represents 11 - epi - 1 - dehydrocortisol and its 21-esters, i.e., 1,4-pregnadiene - 11α,17α,21 - triol - 3,20 - dione and its 21-esters, these compounds being valuable intermediates for preparing 1-dehydrocortisone (Fig. 2).

A large variety of starting compounds can be employed for preparing the compounds of the invention and in the course of treatment of the different starting compounds, described in detail hereinbelow, various new intermediates are obtained, a number of which are themselves of therapeutic value.

In carrying out the synthesis of the com-

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pounds of the present invention, a saturated pregnane or an unsaturated pregnane having an oxygen function (that is to say, ketonic oxygen or a group convertible thereinto) at the 3- and 20-positions, but lacking one or more of the following characteristics, namely (a) the 1,2-double bond; (b) the 4,5-double bond; (c) the X group; (d) the 17α-OH group; (e) the 21-OR group; and (f) the 9α-halogen group, is subjected to one or more treatments whereby the absent characteristic or characteristics are introduced into the molecule.

The compounds of the invention can be produced by introducing a second double bond at a carbon of the A-ring of a compound the A-ring of which already has a double bond attached to a carbon thereof. Thus, a double bond may be introduced at the 1,2 position of a suitable starting compound, with or without one or more of the following modifications of such compound as may be necessary for producing any of the compounds of the invention, carried out in any suitable order:

(1) introduction of a 4,5- or 5,6- double bond (such as 5,6-double bond ultimately shifting to the 4,5-position);
(2) introduction of an 11-oxygen function;
(3) introduction of a 17α-oxygen function;
(4) introduction of a 21-oxygen function;
(5) introduction of a 9α-halogen.

The keto group at the 3-position is not listed as a separate characteristic to be introduced into the starting compound because in a novel procedure for introducing the 1,2-double bond, a hydroxyl or ester group at the 3-position is simultaneously replaced by ketonic oxygen.

An unsaturated starting compound may have a double bond at one or more of the 4, 6, 9(11), and 16-positions. In one modification of our process, the starting compounds may, in fact, already possess the two double bonds in the A-ring, characteristic of the final products represented by the formulae of Figs. 2 to 5.

Various routes may be employed for the synthesis of the compounds of Figs. 2 to 5, depending upon the nature of the starting compound, all such routes having in common the feature that they utilize procedures for introducing one or more of the characteristics (a) to (f) listed hereinabove, and yielding final products in the form of 1,4-pregnadienes having the substituents at the 3, 11, 17, 20 and 21-positions, indicated by the structural formulae of Figs. 2 to 5 and with or without the 9α-halogeno group.

The operations by which the necessary changes in the starting compound are effected may be all microbiological, or all chemical, or a combination of microbiological and chemical procedures, depending on the character of the starting compound, or as may be preferred in particular cases where certain conversions can be effected conveniently either with the aid of certain microorganisms or with purely chemical reagents.

While a number of reactions, both chemical and biochemical, herein described are broadly known in connection with the production of other compounds, one of the novel transformations effected by the present process involves the use of cultures of dehydrogenating micro-organisms having the specific property of introducing a double bond between 1,2-carbons without simultaneous degradation of the pregnane carbon skeleton, such as the splitting off of the side chain at the 17-carbon or opening up of the D-ring. The dehydrogenating microorganisms are capable also of hydrolyzing ester groups, usually at the 3- and 21-positions, and with different speeds or completeness effect also oxidation of 3- and 20-hydroxyl groups. These microorganisms are highly effective in introducing the Δ^1-unsaturation, particularly in compounds having a double bond attached to the 4,5- or 5,6-carbons.

The dehydrogenating microorganisms operate best on ~~sobstrates~~ (i.e., the steroid compound to be modified) which have a double bond attached to the 5-carbon and contain either a hydroxyl group which is relatively stable to the organism, i.e., is not quickly oxidized to ketonic oxygen, or an ester of such an hydroxyl group which is hydrolyzed by the culture of the microorganism. For this reason, compounds like progesterone are not satisfactory starting compounds, and the same is true of pregnenolone (5-pregnene-3-ol-20-one), since its 3-hydroxyl group is quickly oxidized to ketonic oxygen, yielding progesterone.

The 11-hydroxyl group can be introduced into compounds devoid of a substituent on the 11-carbon by the use of a culture of 11α - hydroxylating or 11β - hydroxylating microorganisms. Such 11-hydroxylation can be effected not only on 4-pregnene or 5-pregnene compounds, but also on 1,4-pregnadienes, 1,4,16-pregnatrienes, and on 4,6-pregnadienes. As the 11α-hydroxyl compounds usually have little or no physiological activity, the 11α-hydroxyl is preferably oxidized by a mild oxidizing agent, such as chromic acid in pyridine at room or lower temperatures, and preferably after acylating the 21-hydroxyl when the latter group is present.

Our synthesis of the compounds of Figs. 2 to 5 includes also the introduction of a 17α-hydroxyl group when such group is absent in the starting compound. Such 17α-hydroxylation can be accomplished either chemically, by starting with a 16-dehydro pregnane compound, as by forming the corresponding 16,17-epoxide, followed by hydrolysis, or by starting with compounds which

ar...saturated in the D-ring and introducing the 17α-hydroxyl group with the aid of cultures of known 17α-hydroxylating microorganisms.

With regard to the 21-hydroxyl group, while such hydroxyl can be introduced into the 21-methyl group in known manner by purely chemical means, we prefer to accomplish such result by means of a culture of a 21-hydroxylating microorganism.

As already indicated, the unsaturation in the A-ring can be introduced also by purely chemical means, as by bromination followed by dehydrobromination. In this procedure, the starting compound is a saturated pregnane and two double bonds are introduced into ring A by dehydrohalogenation of the 2,4-dibromo compound.

It is also possible, however, to form only a single bond in the A-ring of an already singly unsaturated pregnene compound by purely chemical means. This can be accomplished by substituting an oxygenated function at the 2-position of cortisone and hydrocortisone and their esters. The oxygenated function is preferably an ester group which is ultimately replaced by a 2-hydroxyl. The 2-hydroxy-3-keto-4-pregnane compound so attained is then dehydrated, as with an acid, to remove the 2-hydroxyl together with a hydrogen atom at the 1-position to produce the corresponding 3 - keto - 1,4 - pregnadiene compound.

Still another route for forming the 3-keto-1,4-pregnadiene structure involves starting with the singly unsaturated 1-pregnene analogue of cortisone, hydrocortisone and their esters. There is formed first the 1,2-epoxide of the starting compound, after which the oxido ring is opened up to form the saturated halohydrin (1 - hydroxy - 2-halogeno - 3 - keto pregnane). After removal of the halogen and acylation of the 1-hydroxyl, the saturated compound is halogenated in the 4-position and then dehydrogenated to form the 1 - acyloxy - 3 - keto-4 - pregnene. Upon splitting off of the 1-acyloxy group, the double bond at the 1,2-position is introduced, forming the 1,4-pregnadiene compound.

The invention contemplates also the preparation of certain esters of the compounds of the formulae of Figs. 2 to 5 which have been found to increase the duration of activity of the compounds, whereby less frequent administration, and especially injection, is required.

The 9α-halogeno group can be present in the 11-keto or 11β-hydroxy starting compound, or can be introduced by various chemical procedures at any suitable stage in the manufacture of the compounds of Figs. 2 to 5 at which an 11α or 11β-hydroxyl group is present, resulting in both instances in a 9α - halogeno - 11β - hydroxy compound which can be oxidized to the 9α - halogeno-11 - keto form.

The compounds 1-dehydrocortisone and 1-dehydrocortisol and their 21-esters have been found by clinical testing to have an extraordinarily improved physiological action over the corresponding singly unsaturated compounds (cortisone, hydrocortisone, and their 21-esters, respectively). Thus, they exhibit several times the potency of the corresponding known hormones, such as cortisone and hydrocortisone, in the treatment of rheumatoid arthritis. In addition, the undesirable side reactions of the known hormones appear in only a diminished degree on use of the new compounds; while the intensity of these side reactions is still further reduced by the fact that much lower dosages of the new compounds can be employed as compared with the corresponding singly unsaturated, known compounds. Both the initial doses and the maintenance doses in the treatment of rheumatoid arthritis can be greatly reduced by the use of the new compounds as compared with the dosages required with cortisone and hydrocortisone. Even with the reduced dosages, the new compounds do not require the concomitant use of codeine or other analgesic for the relief of pain, whereas such analgesics have frequently been used together with cortisone and hydrocortisone.

The above therapeutically active dienes are preferably administered by mouth in the form of tablets containing a full daily dosage, for example 50 mg., or a sub-multiple of such dosage, say 25 or 20 mg., or even 10 mg., mixed with a solid pharmaceutical carrier containing one or more of the usual ingredients, such as starch, sugar, gums, or clays. They may, however, also be administered in suitable cases by intravenous and intramuscular injection, dissolved or suspended in a suitable non-toxic liquid vehicle; or they can be administered in the solid form by subcutaneous implantation, or in the form of suppositories dissolved or suspended in a fatty or waxy vehicle which melts at approximately body temperature. They, as well as their 9α-halogeno derivatives, can also be administered topically in the form of an ointment or cream dissolved in an unguent or cream base of known composition.

The introduction of the second double bond into various singly unsaturated intermediates known to have therapeutic activity has been found to improve the activity also of such compounds. Examples of the latter are corticosterone and 11-dehydrocorticosterone.

The various conversions briefly referred to above will now be explained in greater detail.

A. *Introduction of a Δ^1-double bond with a culture of a dehydrogenating microorganism*

We have found that the chemical modification of pregnene compounds, and especially the A-ring dehydrogenation of pregnenes already possessing a double ~~bend~~ bond attached to a carbon of the A-ring, with or without one or more of the operations of oxidation, reduction, and ester-hydrolysis, can be acomplished in an efficient and inexpensive manner by incubating or fermenting the starting steroid with a culture medium containing a dehydrogenating microorganism which does not at the same time degrade or split the pregnane molecule. Suitable microorganisms of this type belong to the family *Corynebacteriaceae*. Microorganisms of the genus *Corynebacterium* and more particularly of the species *Corynebacterium simplex* (American Type Culture Collection 6946) and *Corynebacterium hoagii* (A.T.C.C. 7005) have given very satisfactory results. The first of these is a soil bacterium, while the second is found in the human throat (where it apparently produces no pathologic condition) and sometimes as a contaminant of cultures exposed to the atmosphere, although its real or original ~~habitate~~ habitat is not known.

The starting compounds can have hydroxyl, keto, halogen, and ester groups in various positions of the nucleus or side chain; thus, hydroxyl groups may be present in one or more of the 3, 11, 17, 20 and 21 - positions; keto groups may occupy one or more of the 3, 11, and 20 - positions; while halogen, such as fluorine or bromine may be attached to the 9-carbon, or at other points of the nucleus or side chain. The presence of a free hydroxyl group appears to promote the chemical transformation. Ester groups of great variety, and preferably the esters of acids usually employed in steroid synthesis and in preparing steroid hormones for therapeutic use, and particularly of the lower alkanoic acids, such as acetate, may be located at one or more of the 3,11,17,20 and 21-positions. The specific character of the ester is not controlling in our process and other esters, both of organic and inorganic acids may be employed, such ~~a~~ cyclopentyl and cyclohexyl acetates, propionates and butyrates; and also the phosphates, polyphosphates and sulfates; it being necessary only that the esters be non-toxic ~~towards~~ the microorganism. The hydroxylated products of our process can, if desired, be converted into their corresponding esters by known procedures, for example, into their lower alkanoic and particularly their acetic acid esters. The hydroxyl groups at the 3- and 11-positions can be either the α- or β-epimers. As disclosed more fully hereinafter, the ester, ~~specially~~ especially at the 21-position, may be of an acid which acts to prolong the duration of activity of the therapeutic compounds.

With the use of a dehydrogenating microorganism one can convert, for example, 4-pregnene - 17α,21 - diol - 3,11,20 - trione (cortisone, Kendall's Compound E) into 1,4-pregnadiene - 17α,21 - diol - 3,11,20 - trione (1 - dehydrocortisone); 4 - pregnene - 11β,17α,21 - triol - 3,20 - dione (hydrocortisone or cortisol, Kendall's Compound F) into 1,4-pregnadiene - 11β,17α,21 - triol - 3,20 - dione (1 - dehydrocortisol); 4 - pregnene - 17α,21 - diol - 3,20 - dione (Reichstein's Compound S) into 1,4 - pregnadiene - 17α,21 - diol - 3,20 - dione and 1,4 - pregnadiene - 17α,20β,21 - triol - 3 - one; 5 - pregnene - 3β,20 - diol into 1,4 - pregnadiene - 3,20 - dione; 4 - pregnene - 11β,21 - diol - 3,20 - dione (corticosterone) into 1,4 - pregnadiene - 11β,21 - diol - 3,20 - dione; 4 - pregnene - 21 - ol - 3,20 - dione (desoxycorticosterone) into 1,4 - pregnadiene - 21 - ol - 3,20 - dione; 4 - pregnene - 21 - ol - 3,11,20 - trione (Kendall's Compound A) into 1,4 - pregnadiene - 21 - ol - 3,11,20 - trione; 9α - fluoro - 4-pregnene - 11β,17α,21 - triol - 3,20 - dione (fluoro—Compound F) into 9α - fluoro - 1,4 - pregnadiene - 11β,17α,21 - triol - 3,20 - dione; 5 - pregnene - 3β,17α,21 - triol - 20 - one 3,21 - diacetate into 1,4 - pregnadiene - 17α,21 - diol - 3,20 - dione and 4 - pregnene - 17α,21 - diol - 3,20 - dione 21-acetate; 4 - pregnene - 20 - ol - 3 - one into 1,4 - pregnadiene - 3,20 - dione; 5 - pregnene - 3,17α,20,21 - tetrol - 11 - one and its 3- and/or 21 - esters into 1,4 - pregnadiene - 17α,21 - diol - 3,11,20 - trione and its 21-ester; and 5 - pregnene - 3,,11α,17α,20,21-pentol and its 3- and/or 21 - esters into 1,4 - pregnadiene - 11α,17α,21 - triol - 3,20-dione and its 21 - ester.

Instead of the 3-keto starting compound the corresponding 3-hydroxy compounds and their 3-esters can be employed, like 5-pregnene - 3,11β,17α,21 - tetrol - 20 - one and 5 - pregnene - 3,17α,21 - triol - 11,20 - dione and their 3-acetates or 3,21 diacetates, produce the same 3-keto diene end product the 3-ester group being hydrolyzed and the 3-hydroxyl being then oxidized to ketone oxygen. Ester groups in the 11- and 17-positions are generally not hydrolyzed, at least not to any significant extent; while an ester group in the 21-position may or may not be hydrolyzed, depending on the reaction conditions. Thus, where the starting compound is a 3,21-diester, the reaction product may be a 3 - keto - 21 - ester compound or a 3 - keto - 21 - hydroxy compound. Along with 3-hydroxy, a 20-hydroxyl may frequently be oxidized to a keto group. It will thus be seen that the dehydrogenating organisms employed are selective with respect to the oxidation step, this being limited practically completely to the 3- and 20-po-

tions; while the hydrolysis may be restricted to 3-ester groups.

The process is applicable also to the treatment of the 11α-hydroxy epimers of the above-mentioned 11β-hydroxy starting compounds, such as 4 - pregnen - 11α,17α,21-triol - 3,20 - dione, 4 - pregnen - 11α,17α, 20,21 - tetrol - 3 - one, 5 - pregnen - 3,11α, 17α,21 - tetrol - 20 - one, 5 - pregnen - 3, 11α,17α,20,21 - pentol, and their mono- and poly-esters, such as the 3-acetates, 3,21-diacetates, and 3,17α,21-triacetates, these starting compounds yielding 1,4 - pregnadien-11α,17α,21 - triol - 3,20 - dione or an ester thereof. The 11-epimers of the 1,4-diene of Compound F (1-dehydrocortisol) and its esters can be converted into the 1,4-diene of cortisone and its esters by oxidation of the 11α-hydroxyl group in known manner, as with the theoretical amount of chromic acid, with or without pyridine or acetic acid, at room temperature or below (5 to 15° C.) preferably after esterifying the 21-hydroxyl if it is free. These 11α-hydroxy starting compounds are relatively easily prepared in high yield, as is known in the art, and therefore represent desirable starting compounds for the preparation of the 1-dehydrocortisone and 1-dehydrocortisol.

Additional transformations that can be accomplished with the dehydrogenating microorganisms include the conversion of 5-pregnene - 3,17α,21 - triol - 20 - one 3,21-diacete into a mixture of 1,4 - pregnadiene-17α,21 - diol - 3,20 - dione and 4 - pregnene-17α,21 - diol - 3,20 - dione 21 - acetate. By alteration of the fermenting medium, Compound S can be made to yield not only 1,4-pregnadiene - 17α,21 - diol - 3,20 - dione but also 1,4 - pregnadiene - 17α,20β,21 - triol-3 - one, i.e., reduction is effected on the 20-keto group. As already indicated, the microorganisms can also effect oxidation of a 20-secondary hydroxyl group, as in the conversion of 5 - pregnene - 3,20 - diol into 1,4 - pregnadiene - 3,20 - dione.

The process is applicable also to 9α-fluoro, chloro and bromo steroids and will yield the corresponding substituted reaction products. The various transformations may be represented by equations of Figs. 16 to 18, which illustrate the formation of the desired end products and also intermediates convertible thereinto.

In the equation of Fig. 16 X is H_2, $=O$, $<\overset{H}{\underset{OH}{}}$ (α or β); Y is —$CO.CH_2OH$; and Z^1 is OH or H. In the equation of Fig. 17, W is H, F, Cl or Br.; X is H_2, $=O$ or $<\overset{H}{\underset{OH}{}}$; Y is —$CO.CH_2OH$, —$CO.CH_2OOCR^1$, or —$CHOH.CH_2OH$; Z^1 is OH or H; and R^1 is a lower alkyl radical. In the equation of Fig. 18, R is a lower alkanoyl group; Y is —$CO.CH_2OH$, —$CHOH.CH_3$, —$CO.CH_2OOCR^1$, or $CHOH.CH_2OH$; Z^1 is H or OH, and R^1 is a lower alkyl radical.

The terms "lower alkanoyl" and "lower alkyl" are to be understood as referring to radicals having not more than eight carbon atoms.

In order to obtain a desirable growth of the dehydrogenating microorganism, such as *Corynebacterium simplex* and *C. hoagii* for the process described, a suitable nutrient medium may be prepared containing carbohydrate, organic nitrogen, cofactors, and inorganic salts. It is possible to omit the use of carbohydrate without completely impairing the growth of the organism. After cultivation of the microorganism in the nutrient medium, the cell mass may be harvested by centrifuging the nutrient broth, decanting the supernatant liquid and suspending the cell mass in saline. A suitable volume of the cell suspension is then seeded into a desirable nutrient medium for supporting growth of the microorganism. The nutrient medium employed may be a yeast extract such as that sold under the Registered Trade Mark "DIFCO," casein hydrolysate (N-Z-Amine) (Type B Sheffield), corn steep liquor, water extract of soybean oil meal, lactalbumin hydrolysate (Edamine-Sheffield Enzymatic) or fish solubles.

The steroid compound as a solid or dissolved or suspended in ethanol, acetone or any other water-miscible solvent which is non-toxic toward the organism, is added to the cultivated microorganism in the broth medium under sterile conditions. This culture is then shaken, aerated, or simultaneously aerated and agitated in order to enhance the growth of the microorganism and the biochemical conversion of the steroid substrate. The steroid may be added to the broth medium and then inoculated with the bacterium, or the cultivated microorganism in broth medium may be added to the steroid. in certain cases, depending on the conditions of the reaction medium, it may be more desirable to obtain optimum growth of the microorganism before the addition of the steroid. Alternatively, enzyme preparations obtained in known manner from cultures of the microorganisms may be used for carrying out the process.

Inorganic salts are desirable to maintain a pH level in the reaction medium of between 6.8 and 7.2. However, the use of inorganic salts for buffering the reaction mixture may be omitted. The omission of inorganic salts cause the pH to rise from an initial value of 6.8 to about 7.7—8. This, however, will still permit the formation of the desired steroidal end products. The optimum temperature for growth of the microorganism is 37° C., but the temperature may vary be-

tween 25° and 37°, and even between 20° and 40° C. The time of reaction may vary from as little as 3 hours to as much as 48 hours. The length of time which is employed will depend on the steroid which is being transformed. Any water-miscible solvent which is non-toxic to the organism may be employed to dissolve or suspend the steroid. The use of ethanol or acetone is preferred in such amounts that the final concentration of these solvents in the reaction mixture is no higher than about 7% and may amount to only traces; owing to evaporation, the final concentration of the organic solvent may even be practically zero.

Following the completion of the oxidation or dehydrogenation process, which may be accompanied by partial or complete hydrolysis when mono- or poly-esters are used, the products of reaction may be recovered from the mixture by extraction with a suitable water-immiscible solvent, by filtration, by adsorption on a suitable adsorbent, or by any other procedures commonly used in the art. For extraction, chlorinated lower hydrocarbons, ketones, and alcohols are useful. These include chloroform, methylene chloride, trichloroethane, ethylene dichloride, butanol, diethylketone, and others. We prefer to use extraction as the method for isolating the steroidal products. Following extraction, the products may be isolated by concentration of the extracts to a small volume or to dryness. Purification of the residues may be then accomplished by simple recrystallizations from a suitable solvent or solvent mixture, such as acetone, methylene chloride, ethanol, acetone-hexane, methylene chloride-hexane, etc., yielding the desired diene in excellent yield and high state of purity.

The chemical transformations which can be accomplished by subjecting the various pregnenes to the action of a culture of the dehydrogenating microorganisms are thus of widely different kinds, and can take place singly, or two or more of such transformations can occur simultaneously or in sequence. The various reactions appear to be unaffected by other substituents in the steroid nucleus or in the side chain.

It will be evident from the foregoing that it is not essential that the starting compound have its double bond in the 4-position of the A-ring; thus, the double bond can be in 5-position. The starting compound may also have more than one double bond. Thus 4,6-pregnadienes can be dehydrogenated and otherwise transformed in the manner above described to yield 1,4,6-pregnatrienes, the latter being readily reduced in known manner to 1,4-pregnadienes. By way of example, 4,6-pregnadiene - 17α,21 - diol - 3,11,20 - trione-21-acetate can be converted into 1,4,6-pregnatriene - 17α,21 - diol - 3,11,20 - trione or its 21-ester. The corresponding 11α- or 11β-hydroxy compounds will be similarly converted to the 1,4,6-trienes. The trienes can be reduced by known procedures into 1-dehydrocortisone or 1-dehydrocortisol or analogous compounds. The starting compound may also have a double bond in the 16-position, or in the 9(11)-position, as explained below.

The fish solubles referred to hereinabove are presently available commercially as an extract of herring, menhaden, and various mixtures thereof, which has been subjected to an enzymatic hydrolysis. This material can be added directly to the culture broth for supplying the nutrient material. Where fish solubles (50% solid content) are available which have not been subjected to enzymatic hydrolysis, such extracts should be diluted with water and steamed for about 10 minutes at 90° C, followed by filtration, preferably with the aid of the substance known under the Registered Trade Mark "FILTER-CEL".

A further example of a dehydrogenating microorganism which has been found to accomplish the above-described reactions is *Bacillus sphaericus var. fusiformis* (American Type Culture Collection 7055) which likewise is capable of introducing a double bond into the A-ring without simultaneously causing degradation of the side chain or splitting of the D-ring. The resulting A-ring unsaturated steroids are highly stable in a culture of this microorganism and no special precautions are necessary to prevent destruction of the desired end product. The time of incubation can be as high as 96 hours and the yields are quite high, being frequently almost quantitative.

The use of microorganisms of the family *Corynebacteriaceae*, particularly the genus *Corynebacterium*, and of the species *B. sphaericus* is described and claimed in our Specifications Nos. 807,227 and 843,217 (5076/57). Specification No. 807,227 also claims 3,20-diketo-1,4-pregnadienes, which may be additionally substituted at the 11-position by ketonic oxygen or a hydroxy group and/or at the 21-position by a hydroxy or an acyloxy group.

As with the other dehydrogenating microorganisms named above, there can be employed in place of a growing culture of *B. sphaericus var, fusiformis* the separated enzymes or enzymatic extract of such culture. The starting pregnenes can similarly be of great variety, but it is preferred to employ starting material having a double bond either at C-4 or C-5, as exemplified by a 3-keto-4- or a 3-hydroxy-5-pregnene.

In general, *B. sphaericus var. fusiformis* will effect the same chemical transformation in the pregnene compounds named hereinabove as the *Corynebacterium* organisms, except that it does not as readily, and in some cases not at all, hydrolyze a 3-ester group, and we therefore recommend that with this

organism there be employed 3-keto and 3-hydroxy pregnenes, and not the corresponding 3-esters. The steroid substrates can contain nuclear double bonds in addition to that at C-4 or C-5, and such additional double bonds can be saturated with halogen or hydrogen halide, or by formation of adducts of dienophiles like maleic acid and maleic anhydride. With the exception that 3-esters are not employed, the various starting compounds will be converted to the same end products by *B. sphaericus var. fusiformis* as by *Corynebacterium simplex* and *hoagii*.

The manner of cultivation of *B. sphaericus var. fusiformis* and method of inoculating the nutrient medium, the nature of the nutrient medium, the manner of bringing the steroid substrate into contact with the growing culture of the microorganism can be the same as with the *Corynebacterium* species, except that, as already indicated, the period of incubation can be longer, and is generally about 24 to 96 hours. In all cases, the steroid-containing culture is subjected to agitation and is aerated.

In addition to the nutrient media hereinabove disclosed, there may also be employed protopeptones. Growth of the microorganism is preferably initiated at a pH of about 6.6 to 7.2; the optimum temperatures for growth of *B. sphaericus var fusiformis* is about 28° C, but the temperature may vary between 19° and 32° C.

The products of the biochemical reaction are preferably recovered by extraction with the solvents and in the manner hereinabove described, but any other suitable method known to chemists may be employed.

Obviously, in place of the dehydrogenating microorganisms hereinabove described, their mutants can be used which have similar dehydrogenating activity without acting destructively on the pregnane carbon skeleton.

More elaborate syntheses, starting from simpler compounds, for manufacturing the compounds of Figs. 2 to 5, and also intermediates which are converted or are convertible thereinto, but employing a dehydrogenating microorganism for introducing a double bond into ring A, will now be described.

B. Manufacture of 1-Dehydrocortisone and 1-Dehydrocortisol from 17α-Hydroxy Progesterone and 5-Pregnene-3,17α-diol-20-one.

According to a modification of our process, the dehydrogenation of the A-ring by means of the dehydrogenating microorganisms described above can be combined, as we have found, with other microbiological conversions which are individually broadly known, and with a chemical oxidation step (when 1-dehydrocortisone is to be produced), to convert 17α-hydroxy progesterone (4-pregnene-17α-ol-3,20-dione) and 5-pregnene-3,17α-diol-20-one into the more highly valuable Δ^1-dehydro derivatives of cortisone and hydrocortisone, such operations being capable of being employed in different sequences, except that the chemical oxidation step is subsequent to the microbiological process which introduces an hydroxyl group in the 11-position. The microbiological processes are basically of three types and are as follows:—

(1) introduction of a 1α-unsaturation (i.e., double bond in the 1,2-position) with simultaneous oxidation of a 3-hydroxyl, when present, by the action of a dehydrogenating microorganism, such as those above disclosed, to produce a 1,4-diene-3-keto system;

(2) introduction of an 11α- or an 11β-hydroxyl group by the action of an 11-hydroxylating microorganism of the type of *Rhizopus nigricans* and *Curvularia lunata;* and

(3) introduction of a 21-hydroxyl group by the action of a member of the genus *Ophiobolus.*

The sequences of these three microbiological transformations may be varied and may, for example, be (1), (2), (3); (2), (1), (3); (1), (3), (2); or (3), (1), (2).

If 1-dehydrocortisone is to be produced, then at any point after the introduction of the 11-hydroxyl group, the intermediate is oxidized, as with chromic acid in pyridine, preferably at room temperatures or below, to convert the 11-hydroxyl into ketonic oxygen. Where, however, the 11-hydroxyl has the β-configuration, and it is desired to obtain the 1-dehydro-derivative of hydrocortisone, the oxidation step is omitted.

The introduction of an 11α-hydroxyl, as above indicated, is effected preferably by the action of a culture (or of the separated enzymatic material) of *Rhizopus nigricans,* in the manner described in the United States patent to Murray et al, No. 2,602,769 dated July 8, 1952; while the introduction of an 11β-hydroxyl group is effected preferably with a culture (or the separated enzymatic material of a culture) of *Curvularia lunata,* as described in United States patent to Shull et al, No. 2,658,023 dated November 3, 1953. However, other 11α-hydroxylating organisms, such as *Aspergillus niger, Rhizopus arrhizus,* and the like may also be employed; while other β-hydroxylating organisms, such as *Cunninghamella blakesleeana,* and the like, may also be used.

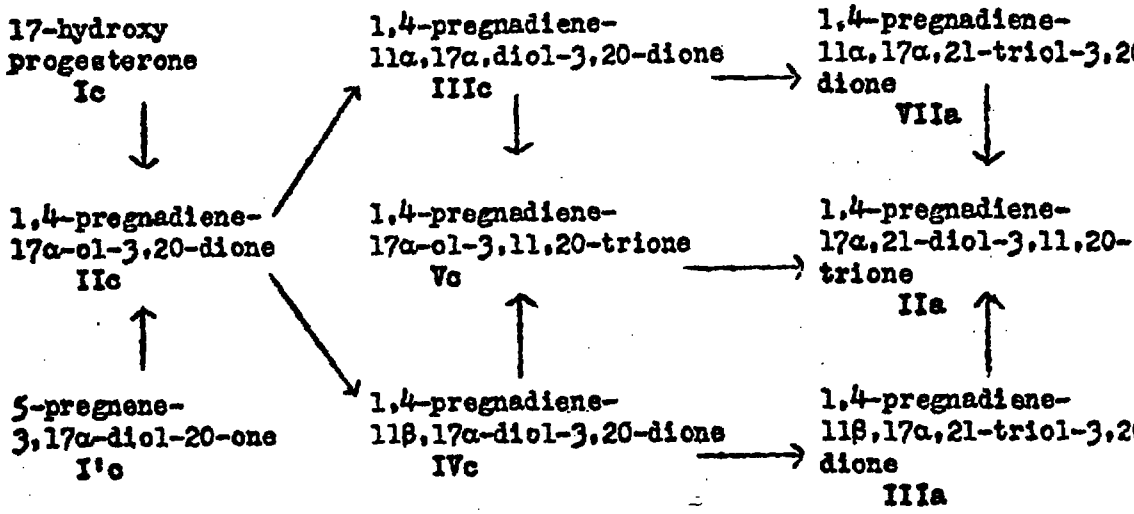
The 21-hydroxyl group is introduced into the steroid molecule by an hydroxylating organism of the genus *Ophiobolus,* preferably *O. herbotrichus* in the manner described by Meystre et al, Helvetica Chim. Acta *37,* 1548 (1954).

The dehydrogenation of the A-ring of the steroid compound to introduce a Δ^1-double bond is accomplished by a dehydro-

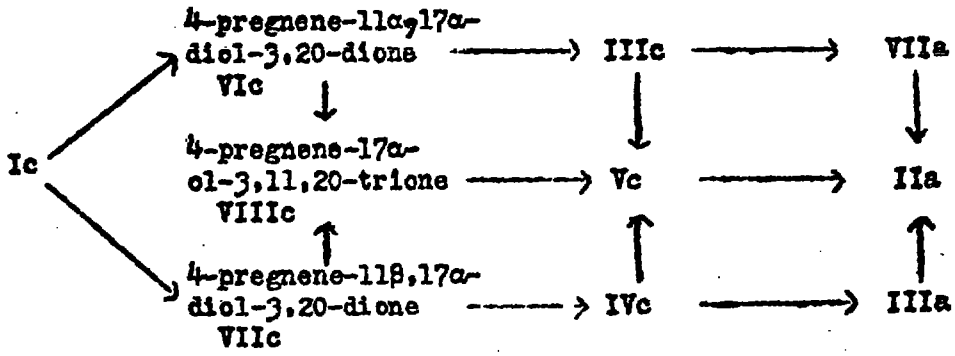
genating organism, such as one of those hereinbefore described. This dehydrogenating step can be applied to the starting compound or at any intermediate point in the complete process.

The various sequences above indicated may be represented by the following equations:

ROUTE A:



ROUTE B:



In the first sequence (Route A), 17α-hydroxyprogesterone (Ic), or 5 - pregnene - 3,17α-diol - 20 - one (I'c) is readily transformed by the action of *C. simplex* or *C. hoagii* into 1,4 - pregnadiene - 17α - ol - 3,20 - dione (IIc). Hydroxylation of IIc *Rhizopus nigricans* (in the manner described in United States patent No. 2,602,769) yields compound IIIc. On the other hand, hydroxylation of IIc with *Curvularia lunata* (following the method described in United States patent No. 2,658,023), gives compound IVc.

Other 11α-hydroxylating organisms such as *Aspergillus niger, Rhizopus arrhizus*, etc. are equally efficacious in transforming IIc to IIIv; while other 11β-hydroxylating organisms, such as *Cunninghamella blakesleeana*, may be used to convert IIc to IVc. Either IIIc or IVc may be oxidized to Vc by the action of a mild oxidizing agent, such as pyridine-chromic acid reagent. Hydroxylation of IIIc, IVc or Vc at the 21-position is effected with, for example, *Ophiobolus herbotrichus*, affording respectively VIIa, IIIa, or IIa (see Figs. 7, 3 and 2). Both VIIa and IIIa (preferably in the form of their 21-acetates or other 21-lower alkanoyl esters) are convertible to IIa by mild oxydation with pyridine-chromic acid reagent. The ester group can be subsequently saponified, as by refluxing with alcoholic potassium bicarbonate.

In the second sequence (Route B), the order of steps (1) and (2) (listed above) is reversed, resulting in the production of the three intermediates, Vc, VIIc and VIIIc. The individual steps are, however, the same and the ultimate products are likewise the same.

As will be readily seen from the above equations, compounds IIIc, IVc and Vc are produced by both sequences; these compounds represent important intermediates for

manufacture of the 1-dehydro derivatives of cortisone and hydrocortisone. They are embraced by the formula of Fig. 10 wherein X is =O, β-OH,H or α-OH,H.

C. Conversion of Desoxycorticosterone and 5 - Pregnene - 3,21 - diol - 20 - one and and Their Esters into 1-Dehydrocortisone and 1-Dehydrocortisol.

The preferred end products, namely 1-dehydrocortisone and 1-dehydrocortisol, and their esters, can be obtained also from desoxycorticosterone (Id, below) (4-pregnene-21 - ol - 3,20 - dione) and from 5 - pregnene - 3,21 - diol - 20 - one, and their esters, by a combination of certain of the steps described hereinabove, with the step of introducing the 17α-hydroxyl by means of a 17α-hydroxylating microorganism, such as one chosen from the genus *Trichothecium*, the process including also the step of oxidizing an 11α- or an 11β-hydroxyl, introduced during the process, when 1-dehydrocortisone is the desired end product.

The various steps can be employed in any desired sequence, except that the chemical oxidation step is subsequent to the microbiological process which introduces the 11-hydroxyl.

The microbiological transformations are of three types, and are as follows:

(1) introduction of a Δ^1-unsaturation (i.e., a double bond in the 1,2-position) with simultaneous oxidation of a 3-hydroxyl, when present, or hydrolysis of a 3-ester followed by oxidation of the resulting 3-hydroxyl, by the action of a dehydrogenating microorganism;

(2) introduction of an 11α- or 11β-hydroxyl group by the action of an organism of the type of *Rhizopus nigricans* or of *Curvularia lunata;* and

(3) introduction of a 17α-hydroxyl group by the action of a culture of an hydroxylating microorganism chosen from the genus *Trichothecium.*

The sequences of these three microbiological transformations may be varied at will and may be (1), (2), (3); or (2), (1), (3); or (1), (3), (2); or (3), (1), (2). If 1-dehydrocortisone or its esters are to be produced, then at any point after the introduction of the 11-hydroxyl group, the intermediate is oxidized, preferably in the form of its 21-ester, as with chromium trioxide at low temperatures, to ~~conver~~ the 11-hydroxyl into ketonic oxygen. Where, however, the 11-hydroxyl has the β-configuration, and it is desired to obtain the 1-dehydro-derivative of hydro-cortisone, the oxidation step is omitted.

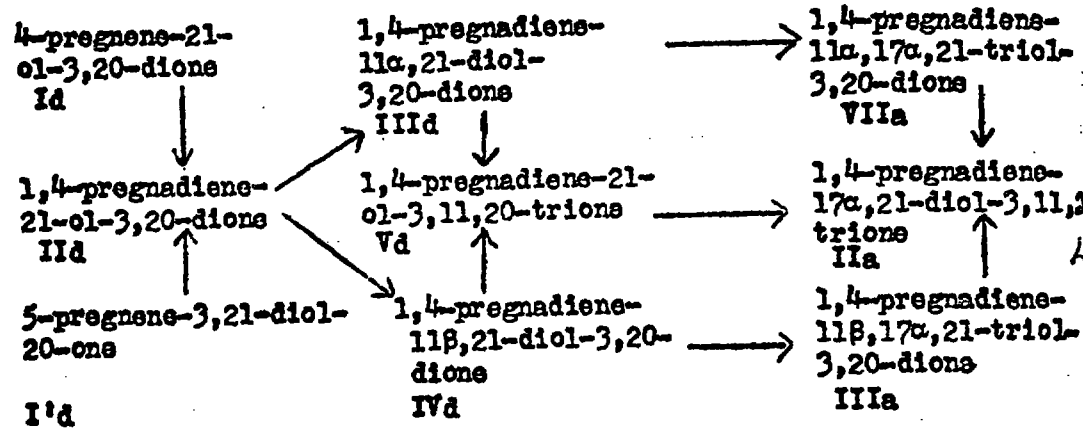
The 17α-hydroxyl group is attached by an hydroxylating organism chosen from the genus *Trichothecium,* and preferably *T. roseum,* in the manner and with the apparatus described by Meystre et al, Helvetica Chim. Acta, *37* 1548 (1954).

Following the completion of the dehydrogenation of ring-A, which may be accompanied by partial or complete hydrolysis when mono- or poly-esters are used, the products of reaction may be recovered from the mixture by extraction with a suitable water-immiscible solvent, by filtration, by adsorption on a suitable absorbent or by any other procedures commonly used in the art, suitable solvents being disclosed hereinabove.

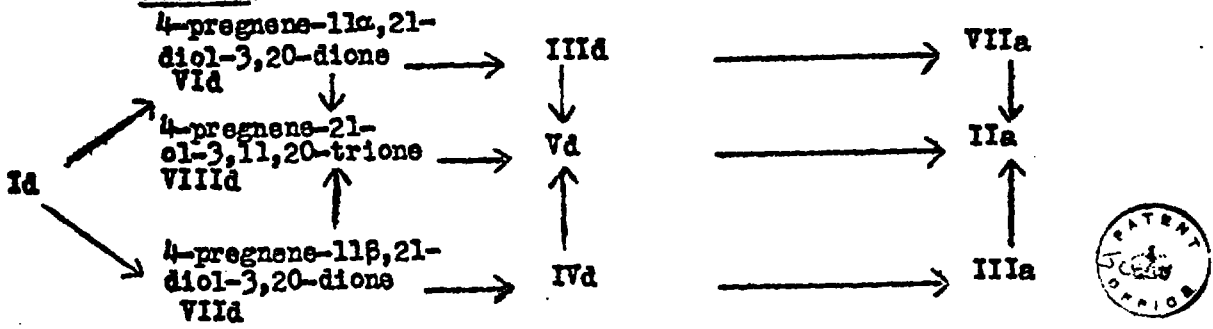
The ester groups can be the same as above disclosed. The final products can, if they contain a free 21-hydroxyl, be converted into their corresponding esters by known procedures, for example, into their lower alkanoic and particularly their acetic acid esters.

The different sequences in which this modification of our process can be carried out are illustrated by the following scheme:

ROUTE C:



ROUTE D:



In the above scheme, compounds IIId, IVd, VId, VIId, IIIa and VIIa have been shown, for the sake of simplicity, as free 21-alcohols, but in actual practice we prefer to esterify the 21-OH group, as with a lower alkanoylating agent, prior to oxidation to compounds Vd, VIIId and IIa, the ester group being hydrolyzed at any time thereafter. In the case of compounds ~~VId~~ and ~~VIId~~, the hydrolysis may be effected simultaneously with the introduction of the Δ^1-double bond by the *Corynebacterium* or other dehydrogenating culture.

In the first sequence (Route C), desoxycorticosterone (Id) is transformed readily by the action of *C. simplex* into 1,4-pregnadiene-21 - ol - 3,20 - dione (IId), which is formed also by the similar treatment of 5-pregnene-3,21 - diol - 20 - one (I^1d). Hydroxylation of IId by *Rhizopus nigricans* affords IIId. On the other hand, hydroxylation of IId with *Curvularia lunata* yields IVd. Either of these hydroxylating processes may be carried out with facility.

As above indicated, other 11α-hydroxylating organisms, such as *Aspergillus niger, Rhizopus arrhizus*, etc. are equally efficacious in transforming IId to IIId; while other 11β-hydroxylating organisms, such as *Cunninghamella blakesleeana* may be used to convert IId to IVd. Either IIId or IVd or preferably their 21-acetates or other lower alkanoyl esters may be oxidized to Vd or its corresponding ester by the action of a mild oxidizing agent such as pyridine-chromic acid reagent. Hydroxylation of IIId, IVd or Vd at 17- is effected with *T. roseum*, affording respectively VIIa, IIIa, or IIa. Both VIIa and IIIa (or preferably their 21 - acetates) are convertible to IIa by mild oxidation by pyridine-chromic acid reagent.

In the second sequence (Route D), the starting compound (Id) is first 11-hydroxylated and then dehydrogenated in ring A, resulting in the production of the three ~~intermediate~~, VId, VIId and VIIId. The ultimate products remain the same, however, and likewise the procedures employed.

The use of 11-hydroxylating microorganisms in the manufacture of 11-hydroxylated derivatives of 1,4 - pregnadiene - 17α, 21 - diol - 3,20 - dione and 21 - acyloxy esters thereof is described and claimed in our Specification No. 5078/57 (Serial No. 843,218).

D. Conversion of 16 - Dehydropregnenolone and its Esters and 16 - Dehydroprogesterone into 1-Dehydrocortisone and 1-Dehydrocortisol.

It is possible also to convert the relatively cheap and readily available 16-dehydropreg-

nolone (Ie) and its esters, and likewise 16-dehydroprogesterone (IIIe) into the highly active cortical hormone substances 1,4-pregnadiene - 11β,17α,21 - triol - 3,20 - dione (1-dehydrocortisol) and 1,4-pregnadiene-17α, 21-diol-3,11,20-trione (1 - dehydrocortisone) and their esters, such as their lower alkanoyl esters, by a relatively small number of chemical and biochemical operations, in the course of which new intermediate compounds are obtained. These operations include:

(a) conversion of the 3-hydroxy-Δ^5-system to the 3-keto-Δ^4-system (in the case of 16-dehydro-pregnenolone);
(b) introduction of a 17α-hydroxyl via the 16,17-epoxide;
(c) introduction of a 21-hydroxyl group by the action of a member of the genus *Ophiobolus,* for example *O. herbotrichus;*
(d) introduction of an 11α- or 11β-hydroxyl group by the action of an appropriate microorganism, as above described; and
(e) introduction of the Δ^1-double bond by means of *Corynebacterium simplex* or similarly functioning dehydrogenating microorganisms, as above described.

The oxidation of the 3-hydroxyl group of 16-dehydropregnenolone (Ie) can be accomplished by the Oppenauer reaction or by careful oxidation with chromic acid at room or below room temperature, but we prefer to effect such oxidation simultaneously with the introduction of the Δ^1-double bond by treatment with a culture of a dehydrogenating microorganism or with an enzymatic extract of such culture, as described more fully hereinabove, but such treatment should follow the addition of a hydroxyl group at one or more of the 11,17 and 21-positions, as otherwise the yields are poor. If desired, esters, such as lower alkanoyl esters of 16-dehydropregnenolone can be used as starting material for this microbiological conversion. The introduction of the 17α-hydroxyl group can be effected by atment with a per-acid, like perphthalic and perbenzoic acids, yielding the intermediate 16,17-epoxide. The latter is then preferably subjected to the action of hydrogen iodide in admixture with acetic acid and acetic anhydride, yielding the 16-iodo-17α-hydroxy compound. The iodine atom is then removed by refluxing the compound in solution in a lower aliphatic alcohol, like ethanol, and containing a small quantity of an organic acid, like acetic acid, in the presence of Raney nickel catalyst. In this reaction, the hydrogen adsorbed on the catalyst operates to replace the iodo group with hydrogen.

The 21-hydroxyl group is introduced in the manner already described by subjecting the 21-methyl compound to the action of a culture of a member of the genus *Ophiobolus;* while the mode of introduction of the 11α- or 11β-hydroxyl is likewise accomplished biochemically, as described in connection with other compounds hereinabove. We have found that the 11β-hydroxyl group can be introduced at the desired positions even in the case of 1,16- and 1,4-pregnadienes and of 1,4,16-pregnatrienes and in the case of 1,4-pregnadienes, also the 11α-hydroxyl, without saturating any of the double bonds.

The dehydrogenating step whereby a double bond is introduced at the 1-position of the pregnene compound is accomplished by subjecting the starting compound, preferably after hydroxylation as above stated, to the action of a dehydrogenating organism such as above described. Cultures of the dehydrogenating microorganisms are capable of introducing a Δ^1-double bond even though the steroid molecule already contains a double bond at C_{16} and also a double bond attached to C_5. As with the other starting compounds above described, the culture is capable not only of introducing a Δ^1-double bond, but also of oxidizing a 3-hydroxyl group to a keto group with simultaneous shifting of a 5,6-double bond to the 4,5-position; and under certain conditions it can also effect oxidation of a 20-hydroxyl to a 20-keto group. Where the compound operated on has an ester group in the 3- or 21-positions, such ester group will be hydrolyzed, the hydrolysis of the C_{21}-ester group being generally favored by a pH of 6.8 to 7.1, and a temperature of about 26 to 29° C.

Where the ultimate product is to be 1-dehydrocortisone, we prefer to introduce an 11α- rather than an 11β-hydroxyl group, as the 11α-hydroxyl can generally be more easily introduced and can then subsequently be oxidized to an 11-keto group. Where an 11β-hydroxyl is introduced into the steroid molecule, such group may be retained unchanged in the final product to yield the 1-dehydro-derivative of hydrocortisone, which is likewise highly active.

Intermediates for the preparation of the 1-dehydro-derivatives of cortisone, hydrocortisone and their esters, and prepared by the modification of our process now being described, have the structures indicated in the formula of Figs. 8 and 9. In these formulae,

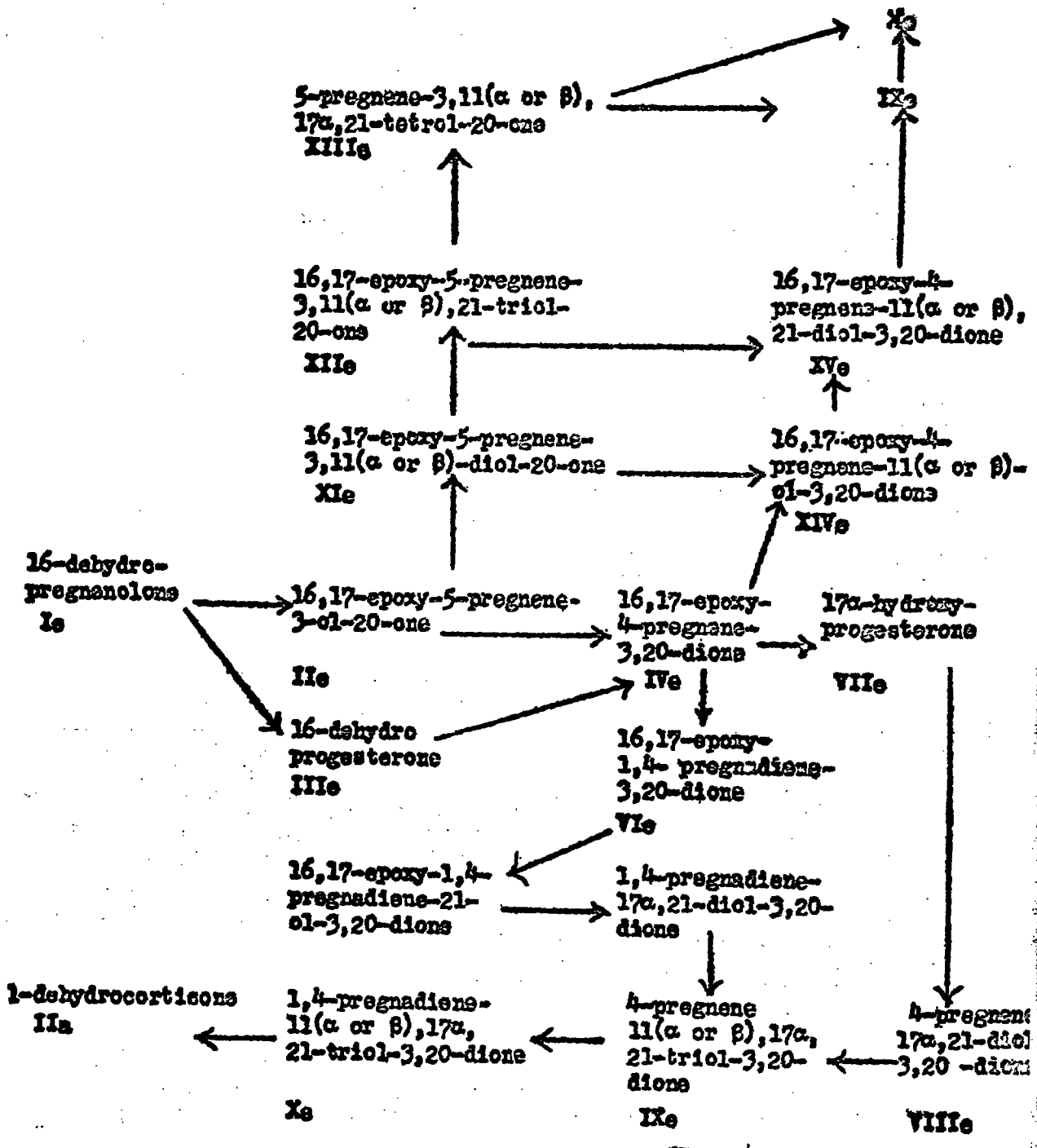
X is H_2, O, or $<^{OH\ (\alpha \text{ or } \beta)}_{H}$, while R stands

for H, OH, or OAcyl, the acyl group in each case being that of an organic carboxylic acid, but preferably of a lower alkanoic acid, that is to say, having not more than eight carbon atoms, such as formic, acetic, propionic, butyric, valeric or caproic acids) although other acids, like substituted alkanoic and

aromatic acids, such as cyclohexyl acetic, cyclopentyl propionic, and benzoic acids, are not excluded. These compounds and the preparation thereof are claimed in our Application No. 5074/57 (Serial No. 843,216).

Compounds in which X represents hydroxyl and R an acyl group are prepared by esterifying 16,17 - oxido-1,4-pregnadiene-11, 21-diol-3,20-dione to obtain the 21-ester preliminarily to the oxidation of the 11-hydroxyl, after which the oxido ring is opened up with HI or HBr to form the 16,17-halohydrin; the halogen is then removed with Raney nickel or with hydrogen in the presence of palladium or charcoal catalyst. The hydrolysis of the 21-ester group can be effected at any time after oxidation.

As already indicated, the several steps involved in this form of our process can be conducted in various sequences, a number of which are indicated in the following scheme:



4-pregnene-17α,21-diol-3,20-dione

Most of these steps are completely independent of each other, so that the sequence of operations may be successfully changed in a variety of ways. For example, compound Ie may first be converted to 16,17-epoxy-pregnenolone (IIe), and then to 16,17 - epoxy-progesterone (IVe), or it can be converted to 16-dehydroprogesterone (IIIe), and thence to IVe. Any of these known intermediates (IIe, IIIe, IVe) may be hydroxylated at position 11 (either α or β) and at position 21, and dehydrogenated to the 3-keto-$\Delta^{1,4}$-diene system, and these three sequences can be done in any order except that hydroxylation or epoxylation preferably precedes treatment with the dehydrogenating microorganism. Compound IIIe containing a Δ^{16}-double bond, is converted to the 16,17 - epoxide (IVe) by the usual procedures (either with per-acids or alkaline hydrogen peroxide). Compound IVe is then treated with an acid to split open the oxido ring to form 17α-hydroxy-progesterone (VIIe). The latter is then treated with a culture of a 21-hydroxylating microorganism to yield VIIIe; the latter can then be 11-hydroxylated to form compound IXe. Compound IXe is then treated with a culture of a dehydrogenating microorganism to give the 1,4-pregnadiene compound (Xe) which is then oxidized to 1-dehydrocortisone (IIa).

Compound Xe can also be produced by first 11-hydroxylating the 16,17-epoxy compound IIe, yielding XIe which is then 21-hydroxylated to yield compound XIIe. The epoxide ring of the latter compound is opened up to introduce the 17α-hydroxyl group, as indicated in compound XIIIe, which can be chemically oxidized to convert the 3-hydroxyl into ketonic oxygen, yielding IXe, which is then dehydrogenated to Xe, or compound XIIIe can be treated immediately with the dehydrogenating microorganism to yield Xe directly.

As further indicated by the diagram, compound IVe can be 11-hydroxylated (XIVe) and then 21-hydroxylated to give compound XVe. The latter is then treated to open up the 16,17-epoxide ring to give compound [illegible] which is then dehydrogenated to Xe. The diagram shows several other transformations which will be readily understood from the foregoing description.

It does not matter at what point in the process the 16,17-epoxide ring is opened up, for example, by hydrogen iodide (HI), to give the 17α-hydroxyl group, or at what point the 11-hydroxyl is oxidized to the 11-ketone, provided that during these reactions any hydroxyls present at 3 and 21 positions are protected by ester groups which can subsequently be removed.

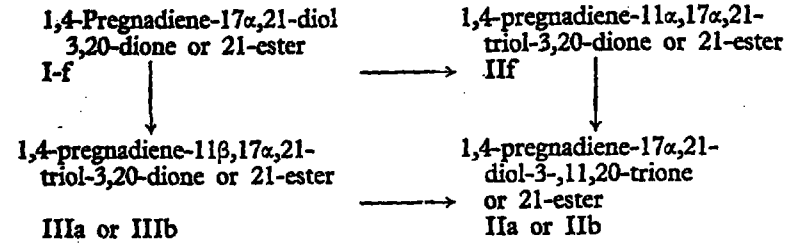
E. The Conversion of 1,4-Pregnadiene-17α-21-Diol-3,20-Dione and its Esters into 1 - Dehydrocortisone and 1 - Dehydrocortisol and their Esters.

An example of the use of a starting compound which already has the 3-keto-1,4-pregnadiene structure for the preparation of 1, dehydrocortisone and 1-dehydrocortisol and their esters is 1,4-pregnadiene-17α,21-diol-3, 20-dione (the 1-dehydro derivative of Reichstein's Compound S) which can be manufactured from Compound S either chemically (see United States patent to Djerassi et al, No. 2,579,479, dated December 25, 1951), or by treatment with a dehydrogenating microorganism as hereinabove described.

We have found that cultures of microorganisms, or their separated enzymes, having the known property of introducing a hydroxyl group into the 11-position of saturated or singly unsaturated pregnanes, and either in the 11α or 11β-position, are capable of introducing such 11-hydroxyl group also into 1,4-pregnadienes, which are unsubstituted in such position; and that the conjugated double bond system in the A-ring is not modified by such microorganisms. Thereby 1-dehydrocortisone and 1-dehydrocortisol can be prepared in simple manner when the Δ^1-dehydro derivative of Compound S is available.

According to the present modification of our process, and as we have found, 1,4-pregnadiene-17α,21-diol-3,20-dione and its 21-esters (If, below) can be converted, despite the presence of the double unsaturation in ring-A, into the corresponding 11 - hydroxyl compound, by treatment with various known 11-hydroxylating microorganisms. Thus, compound If can be either 11α-hydroxylated by the action of one of the various known 11α-hydroxylating organisms, such as *Rhizopus arrhizus, Rhizopus delmar, Rhizopus nigricans, Rhizopus reflexus, Aspergillus niger, Phycomyces blakesleeanus*, to yield IIf; or it may be 11β-hydroxylated by the action of *Cunninghamella blakesleeana, Curvularia lunata* or one of the other known 11β-hydroxylating organisms to yield IIIa or IIIb. The product of the 11α-hydroxylation, IIf, in the form of its 21-ester, preferably the acetate, is then oxidized by the action of chromic acid in pyridine solution or by other mild oxidizing agents of equivalent potential, to 1,4-pregnadiene - 17α,21 - diol - 3,11,20 - trione 21 - acetate (IIb), which can be readily hydrolyzed to the 21-OH compound (1-dehydrocortisone) (IIa). The product of the 11β-hydrolysis is the 21-ester of 1-dehydrocortisone (IIIb) which can be oxidized to the 21-acetate of 1-dehydrocortisone, followed by hydrolysis of the 21-ester.

The reactions involved in this procedure are indicated by the following scheme:



If I-f is in the form of the free 21-alcohol, then after 11-hydroxylation, it is intermediately converted to the 21-ester prior to the oxidation. The acyl radical, as before, is preferably that of acetic acid, but it may also be that of other lower fatty acids, like formic, propionic, butyric, valeric, or succinic, or of aromatic acids, like benzoic, salicylic, phthalic and veratric. In the case of the dibasic acids, the ester is preferably in the form of a half ester. If desired, the acyl group may be hydrolyzed, as by heating with alcoholic potassium bicarbonate.

F. Introduction of A-Ring Unsaturation by Chemical Means.

It will be evident from the foregoing description that the dehydrogenating microorganisms can effect either single unsaturation of the A-ring or double unsaturation, as in the case of 5-pregnene-3-ol compounds (or their esters, which are hydrolyzed to the free 3-OH compounds) wherein upon oxidation of the 3-OH to a keto group, the 5,6-double bond is caused to shift to the 4,5-position. However, one or both of the A-ring double bonds can be introduced also by purely chemical means.

We have found that, for example, the 21-esters of pregnane-17α,21-diol-3,11,20-trione (Ig, below) and likewise the 21-ester and the 11,21-diesters of pregnane-11(α or β), 17α,21-triol-3,20-dione (IIg, 11β-OH compound), can be halogenated at the 2- and 4-positions by treatment with a halogen having an atomic weight higher than that of fluorine, to produce the corresponding 2,4-dihalogenides in satisfactory yield. The esterification, especially of the 11β-hydroxyl, improves the yield. The halogenation at the two positions can be carried out in one or two steps, and in the latter case, different halogens may be employed, like chlorine and bromine. The dihalogenide is then treated with a dehydrohalogenating agent to introduce two double bonds into ring A at the 1,2- and 4,5-positions, yielding IIb, IIIb (21-ester or 11β,21-diester) or VIIb. The resulting diene ester can then, if desired, be hydrolyzed in known manner with an acid or base to produce the primary alcohol of the dienes (IIa, ~~IIa~~ or VIIa).

We prefer to employ bromine as the halogenating agent and this modification of the process (which is claimed in our Application No. 5071/57 (Serial No. 843,213)) will accordingly be further described in connection with the use of such halogen. Upon reaction with bromine, the 2,4-dibromides of the 21-esters of pregnan-17α,21-diol-3,11,20-trione and of pregnan-11,17α,21-triol-3,20-dione are obtained. We prefer to effect the dehydrobromination by refluxing with collidine, followed by chromatographic or other separation of the 1,4-diene-21-ester. In place of collidine, other high-boiling organic bases can be employed, like quinaldine and dimethyl aniline.

Our process is applicable to the allo as well as to the normal pregnane series. Thus, dibromination of allo-pregnan-17α,21-diol-3,11,20-trione 21-acetate and of allopregnan-11β,17α,21-triol-3,20-dione 21-acetate yields the corresponding dibromides (IIIg and III¹g) which upon dehydrobromination give the 21-acetates of the dienes II and III, respectively.

The dienes of 11α-hydroxylated steroids can also be prepared in the same manner. Dibromination of pregnan-11α,17α,21-triol-3,20-dione 21-acetate gives the corresponding 2,4-dibromide (IVg) which, upon dehydrobromination, gives 1,4-pregnadien-11α,17α,21-triol-3,20-dione 21-acetate (VIIb). Oxidation of VIIb gives IIb (R=acetyl) in good yield. Hydrolysis of IIb yields IIa.

The reactions involved in this chemical procedure are typified by the following equations:

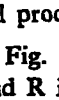
(1) Pregnane-17α, 21-diol-3,11,20-trione 21-ester Ig —Br_2→ 2,4-dibromo-compound —→ 1,4-pregnadiene-17α,21-diol-3,11,20-trione 21-ester IIb —→ IIa

(2) Pregnane-11β,17α, 21-triol-3,20-dione 21-ester IIg —Br_2→ 2,4-dibromo compound —→ 1,4-pregnadiene-11β,17α,21-triol-3,20-dione 21-ester IIIb —→ IIIa

(3) 2,4-dibromo-allo-pregnane-17α, 21-diol-3,11,20-trione 21-ester IIIg —→ IIb

(4) 2,4-dibromo-allo-pregnane-11β,17α, 21-triol-3,20-dione 21-ester III¹g —→ IIIb

(5) 2,4-dibromo pregnane-11α,17α, 21-triol-3,20-dione 21-ester IVg —→ 1,4-pregnadiene 11α,17α,21-triol-3,20-dione 21-ester VIIb —CrO_3→ IIb —→ IIa

The intermediates obtained by this chemical procedure are represented by the formula of Fig. 11, wherein X is O, or  and R is H or a lower alkanoyl radical. The pregnane nucleus may have either the normal or allo-configuration.

The bromination of the starting compound, which is preferably a 21-ester, is conducted in acetic acid or other substantially anhydrous organic solvent which is inert toward the bromine. It is not necessary to purify the so-formed dibromide prior to reaction with the dehydrobrominating agent which, as already stated, is preferably a high-boiling organic base, collidine being generally most suitable. The resulting doubly unsaturated ester can then, if desired, be hydrolyzed with an alkali metal base, such as alkali metal carbonates, bicarbonates, and hydroxides in known manner in a suitable neutral solvent like methyl and ethyl alcohols.

Both the 11α- and 11β-hydroxyls can be oxidized to ketonic oxygen either before or after the hydrolysis. While various oxidizing agents may be employed, we have found chromic acid to be an efficient and inexpensive agent for this purpose.

We have shown hereinabove that the 1,2-double bond can be introduced into 4-pregnene-3-ones having a hydroxyl group elsewhere in the molecule, by treatment with a dehydrogenating microorganism. Such 1,2-double bond can be introduced into the unsaturated pregnenes by chemical means; moreover, the 4,5-double bond can also be introduced by purely chemical reactions into 3-keto pregnenes in which the double bond is located in the 1,2-position.

To effect introduction of the 1,2-double bond into 4-pregnene-3-ones, we may start with the available 6-bromo-4-pregnene-17α, 21-diol-3,11,20-trione (Kendall, et al., J. Biol. Chem., *197*, 261 (1952)) and 6-bromo-4-pregnene-11β,17α,21-triol-3,20-dione 11-formate 21-acetate (from the bromination according to Kendall of 4-pregnene-11β,17α, 21-triol-3,20-dione 11-formate 21-acetate) which are treated with an alkali metal salt of a lower alkanoic acid, preferably sodium acetate or potassium acetate. In this way, the respective 2-alkanoates are prepared. Mild hydrolysis with aqueous or alcoholic acids or bases may be employed to convert the 2-alkanoates to the 2-hydroxysteroids. Dehydration by elimination of the 2-hydroxyl together with hydrogen at the 1-carbon effects introduction of the 1,2-double bond. The reactions involved are indicated in the following series of equations:

(1) 6-bromo-4-pregnene-17α,21-diol-3,11,20-trione 21-ester Ih → 4-pregnene-2,17α,21-triol-3,11,20-trione → 2,21-diester IIh

4-pregnene-2,17α,21-triol-3,11,20-trione IIIh → IIa

(2) 6-bromo-4-pregnene-11β,17α,21-triol-3,20-dione 11-formate 21-alkanoate IVh → 4-pregnene-2,11β,17α,21-tetraol-3,20-dione 11-formate 21-alkanoate Vh

4-pregnene-2,11β,17α,21-tetrol,3,20-dione VIh → IIIa

The structural formulae of IIIh and VIh are in Figs. 12 and 13. In these formulae, R is hydrogen or a lower alkanoic acid radical. In the formula of Fig. 13, the 11β-OH group can be replaced by OR.

Instead of dehydrating compound IIIh or VIh, the 2,21-diesters can be subjected to the action of the dehydrating agent. Thus, the 2,21-diacetate of compound IIIh can be treated with hydrogen bromide in acetic acid to give the 21-acetate of 1-dehydrocortisone.

The 1,2-double bond can also be introduced by way of an hydroxy or acyloxy substituent at the 1-carbon of, for example, the 21-esters of cortisone and hydrocortisone. The structure of these compounds is shown in Fig. 14, and may be prepared from 1-pregnenes by the following sequence of reactions, wherein X stands for =O or H, OH; and Y is OH or OAcyl:

1-pregnene-11X-17α,21-diol-3,20-dione 21-ester Ii → 1,2-epoxy-11X-pregnane-17α,21-diol-3,20-dione 21-ester IIi

2-bromo-pregnane-11X-1,17α,21-triol-3,20-dione 21-ester IIIi → pregnane-11X-1,17α,21-triol-3,20-dione 21-ester IVi

pregnane-11X-1,17α,21-triol-3,20-dione 1,21-diester Vi → 4-bromo-pregnane-11X-1,17α,21-triol-3,20-dione 1,21-diester VIi

4-pregnene-11X-1,17α,21-triol-3,20-dione 1,21-diester VIIi → II or III

The ester groups in the above equations are preferably those of lower alkanoic radicals. For example, in converting 1-pregnene-17α,21-diol - 3,11,20 - trione 21 - acetate (Mattox and Kendall, J.B.C., *188*, 287 (1951)) to 4 - pregnene - 1,17α,21 - triol - 3,11,20 - trione and the corresponding 1,21-diacetate, the starting material (Ii) is epoxidized by means of one of the well known epoxidizing agents, such as perbenzoic acid, monoperphthalic acid, peracetic acid, etc. Preferably perbenzoic acid or monoperphthalic acid in an inert medium such as chloroform or methylene chloride is used in this step. The product of epoxidation (IIi) is then treated with an anhydrous hydrohalic acid such as hydrogen bromide in chloroform, hydrogen iodide in glacial acetic acid, etc. The resultant halohydrin (IIIi), preferably the iodohydrin, but also the bromohydrin, is then dehalogenated with Raney nickel in acetic acid, or with hydrogen and palladium-on-calcium carbonate catalyst to pregnane-1,17α,21 - triol - 3,11,20 - trione (IVi). Compound IVi is acylated with a lower alkanoic acid anhydride or acid chloride, such as acetic anhydride, in pyridine solution to yield Vi. Alternatively, compound Vi may be prepared by reversing the order of the last two steps. Bromination and dehydrobromination of Vi affords the desired 4-pregnene-1,17α,21-triol-3,11,20 - trione 1,21 - diacetate (VIIi). The bromination is preferably conducted with bromine, in the presence of

soc n acetate to control the acidity. The dehydrobromination is most conveniently accomplished with the aid of semicarbazide. The resulting semicarbazone is converted to VIIi by the action of pyruvic acid

In similar fashion, IVi is convertible to 4 - pregnene - 1,17α,21 - triol - 3,11,20 - trione 21-acetate (VIIIi) by omitting the acylation step. Bromination in the presence of sodium acetate followed by the same dehydrobromination procedure effects the desired transformation.

The corresponding 11β-hydroxy-compounds are prepared in the same way starting from 1 - pregnene-11β,17α,21 - triol - 3,20 - dione 21 - acetate.

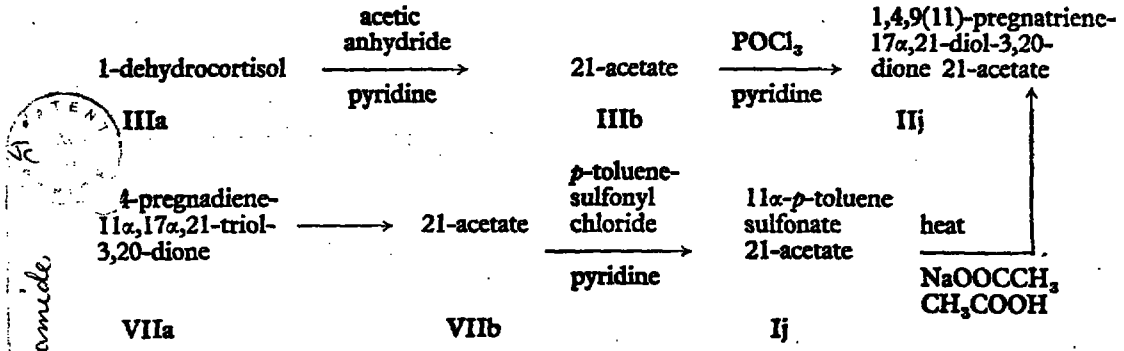
Compounds VIIi, VIIIi and the related 11β-hydroxy compounds can be treated with an acid or base, such as alumina or the form of tetramethyl ammonium hydroxide known by the Registered Trade Mark "Triton B", to split off the 1-substituent, yielding the powerfully anti-arthritic substances whose formulae are shown in Figs. 2 and 3.

These 1-hydroxy and 2-hydroxy 11-substituted 4-pregnene compounds, and their esters, and the preparation thereof, are described and claimed in our Applications Nos. 5072/57 (Serial No. 843,214) and 36612/58 (Serial No. 843,219).

G. Substitution of Halogen in the 9α-Position.

It is not necessary for the manufacture of compounds of the formulae of Figs. 4 and 5 that the starting compounds contain the halogen atom (fluorine, chlorine, or bromine) at the 9α-position. The substituent can be introduced into the steroid molecule after the dehydrogenation of the A-ring. The substitution is accomplished by starting with the 11(α or β)-hydroxy-1,4-pregnadiene compound and converting it into the 1,4,9(11)-pregnatriene which can then be treated in various ways to introduce the desired halogen at the 9α-position and at the same time an 11β-hydroxyl. The 11-hydroxyl can then be oxidized under mild conditions to replace it with ketonic oxygen to form 9α - halogeno-1-dehydrocortisone.

We prefer to employ as the starting material either 1,4-pregnadiene-11β,17α,21-triol-3,20-dione (Fig. 3) or the corresponding 11α-hydroxy compound (Fig. 7) or their 21-esters. The starting compound is first esterified (IIIb, Fig. 3) at the 21-position (if it is not already a 21-ester) by treatment with an acid anhydride in pyridine or utilizing the acid chloride so as to produce, for example, a lower alkanoyl ester, such as the 21-acetate or propionate or any other suitable ester, like the 21-cyclopentylpropionate, 21-enanthate, and the like. Dehydration to effect introduction of the 9,11-double bond is conveniently accomplished in the case of compound IIIb by the action of phosphorus oxychloride in pyridine. We prefer, however, to form the 1,4,9(11) - triene by treating the 21-ester of the 11α - hydroxy compound (VIIb, Fig. 7) with *p*-toluenesulfonylchloride in pyridine to produce the corresponding 11α-tosylate (11α-*p*-toluenesulfonate) (Ij). By treating the latter compound with a basic compound, such as sodium acetate in acetic acid (or with potassium acetate, lithium propionate and the like), the elimination of the *p*-toluenesulfonic acid is effected with introduction of the 9(11)-double bond. In place of the *p*-toluenesulfonate there may be produced, as an intermediate, the corresponding methanesulfonate, benzenesulfonate, or other similar sulfonic acid esters. These reactions are indicated in the following scheme:



The structure of the 1,4,9(11)-pregnatrienes of which compound IIj is an example, is shown in (Fig. 15) in which R is H or acyl, preferably lower alkanoyl.

The triene (IIj) is now treated with a compound which provides a bromium ion, such as N-bromoacetamide or N-bromsuccinimide in perchloric acid or other strong acid like sulfonic. Thereby the 9α-bromo derivative (IIIj) is obtained. If it is desired to substitute fluorine or chlorine at the 9α-position, the 9α-bromo compound (the bromohydrin IIIj) is dehalogenated by the action of a weak base such as potassium or sodium acetate in a relatively neutral solvent, like acetone or alcohol, thereby yielding the epoxide IVj. The latter is subjected to the action of anhydrous hydrogen fluoride, whereupon the epoxide ring is

opened up and a 9α-fluorine atom is introduced together with an 11β-hydroxyl group yielding compound V (Z=F). By employing anhydrous hydrogen chloride, the 9α-chloro analogue is formed. The latter can then be hydrolyzed to split off the 21-acyl group and produce the 21-alcohol as by means of aqueous acid.

The 9α-fluoro compound can be subjected to a mild oxidation as with chromic acid in pyridine at room temperature to replace the 11β-hydroxyl with ketonic oxygen, to yield compound IVb, which can then be hydrolyzed with acid to produce 9α-fluoro-1,4-pregnadiene-17α,21-diol-3,11,20-trione.

It is preferred to employ 1,4-pregnadiene-11α,17α,21-triol-3,20-dione to the corresponding 11β-hydroxy compound as a starting material, this new 11α-hydroxy compound being produced in the manner hereinabove described. It is important that, prior to the treatment with the *p*-toluenesulfonyl chloride, the 21-hydroxyl be selectively protected before the 11α-hydroxyl group is esterified with a sulfonic acid derivative. We have found that failure to protect the 21-hydroxyl group against the action of a sulfonyl halide, such as *p*-toluene sulfonyl chloride, results in non-rectifiable mixtures. Furthermore, we prefer, in the esterification of the 21-position, to use a relatively mild acylating agent such as the anhydrides heretofore described whereby true selectivity is obtained and the 11-hydroxyl remains unesterified.

These processes and the 1,4,9(11)-pregnatriene compounds referred to are described and claimed in our Application No. 5073/57 (Serial No. 843,215).

Specification

H. Manufacture of Esters of Increased Duration of Activity.

The invention contemplates also the compounds of the formulae of Figs. 2 to 5 in the form of 21-esters which are not as rapidly metabolized in the body as the free 21-alcohol compounds, so that the physiological activity of the compounds is maintained for a longer period of time and the frequency of injection thereby greatly reduced.

The acids whose esters have been found to possess a longer period of activity are cyclohexanecarboxylic acid, 4-methylcyclohexanecarboxylic acid, 3-ethyl-cyclohexylacetic acid, cyclohexylpropionic acid, cyclopentylpropionic acid, phenylacetic acid, trimethylacetic acid, *t*.-butylacetic acid, butoxybutyric acid, ethoxycaproic acid, methylthiovaleric acid, isopropylthioacetic acid, phenylthioacetic acid, caproic acid, isobutyric acid, enanthic acid, isocaprylic acid, cyclohexylcaproic acid, undecylenic acid, 2-ethylbutyric acid, toluic acid and ethoxybenzoic acid.

Particularly we have found that acids of the aryloxyalkanoic and furoic acid groups give outstandingly superior duration of hormonal activity, thus providing a more efficient, convenient and useful therapy than can be obtained with the parent hormones. Typically useful acids of these groups are: phenoxyacetic acid, *p*-chlorophenoxyacetic acid, 2,4-dichlorophenoxyacetic acid, 2,4,5-trichlorophenoxyacetic acid, 5-chlorofuroic acid, 5-methylfuroic acid, 5-bromofuroic acid, 4-bromophenoxyacetic acid, 4-methylphenoxyacetic acid, 4-methoxyphenoxyacetic, 4-*t*.-butyl-phenoxyacetic acid, 5-*t*.-butylfuroic-2[1] acid, furoic-2[1] acid and phenoxypropionic acid.

The esters of the above-named acids have been found to increase the duration of activity not only of 1-dehydrocortisone, 1-dehydrocortisol, dehydro-9α-halogeno-cortisone, and 1-dehydro-9α-halogeno-cortisol (in which the halogen atom is chlorine or bromine), but also of the physiologically active intermediates like 1-dehydrocorticosterone, 1-dehydro-11-desoxycorticosterone, 1-dehydro-11-desoxycortisone, 1-dehydro-17-desoxycortisone, and 1-dehydro-aldosterone.

As examples of the increased duration of activity, it has been found that in adrenalectomized mice, injection of 0.25 mg. of 1-dehydrocortisone or 1-dehydrocortisol or their acetates causes eosinopenia for from two or four days. A similar injection of 1-dehydrocortisone 21-(2[1],4[1]-dichlorophenoxyacetate) lasts 12—20 days; its 21-(5[1]-bromofuroate-2[1]) lasts 10—18 days; the 21-furoate-2[1] of 1-dehydrocortisol lasts 10—16 days; and its 21-(4[1]-*t*.-butylphenoxyacetate) lasts 14—20 days.

In another test, an injection of 1-dehydrocortisone 21-(5[1]-chlorofuroate-2[1]) in rats caused the weight of the thymus gland to be decreased for a period from five to eight times longer than the free hormone; while 1-dehydrocortisol 21-(4[1]-tertiary-butylphenoxyacetate) caused thymus involution from four to six times longer than the free hormone. These tests are standard indications of glucocorticoid (i.e., anti-arthritic) activity. Likewise, the mineralo-corticoid activity of the hormones is enhanced by esterification with these acids. Thus 1-dehydro-11-desoxycorticosterone 21-(4[1]-chlorophenoxyacetate) maintains the life of adrenalectomized mice five to seven times longer than the free hormone, and 1-dehydroaldosterone 21-furoate maintains life six to ten times longer than the corresponding hormone.

In human therapy, the same protection of the hormone from destruction is observed, with concomitant extension of duration of the therapeutic level of activity. Thus, one or two injections per week of 1-dehydrocortisone 21-furoate or 1-dehydrocortisol 21-(4[1]-*t*.-butylphenoxyacetate) replace 7 daily medications with the free hormone. Similarly, 50—100 mg. injections of 1-dehydrocortisol 21-(5[1]-*t*.-butylfuroate) or 1-dehydrocortisone 21-(4[1]-

chlorophenoxy-acetate) have activity lasting to 10 days compared to the one-day effectiveness of the free hormone.

Specific examples of 21-esters of 9α-halogeno-derivatives of 1-dehydrocortisone and 1-dehydrocortisol are the 21 - (2^1 - furoate),21-[(5^1 - *t* - butyl)2^1 - furoate],21 - phenoxy-acetate, and 21 - (2^1,4^1,5^1 - trichlorophenoxy-acetate).

The esters may be administered in the usual manner of cortical hormones. Thus they may be compounded into tablets with inert material for oral or sublingual use. Another form commonly used is an ointment or lotion for topical use. Generally the most effective mode of administration is by injection intramuscularly, subcutaneously or intra-articularly, of solutions or suspensions in oil or aqueous media.

The esters may be prepared in several ways, the simplest being esterification of the corresponding free hormones, for example, by treatment of the hydroxysteroid with the corresponding acid anhydride or chloride in the presence or absence of a base; or by treatment of the hydroxysteroid with the free acid in the presence of an acid catalyst under dehydrating conditions.

The esters may also be prepared by esterification of intermediates in the preparation of the hormones, using these esters rather than the acetate as protecting groups. Thus, in the preparation of 1-dehydrocortisone and 1-dehydrocortisol by the chemical methods above described, there can be intermediately formed the 21-ester of one of the above-named depot or reservoir-producing acids prior to the introduction of halogen (usually bromine) at the 2- and 4-position, this procedure being conveniently carried out by reacting the 21-bromo compound of the steroid with a metal salt of the acid. This is illustrated in the following equation in which it is to be understood that the starting pregnane compound can be of the normal or allo configuration:

21-bromo-pregnane-11X-17α-ol-3,20-dione ⟶ pregnane-11X-17,21-diol-3,20-dione 21-ester ⟶ 2,4-dibromo-derivative ⟶ 1,4-pregnadiene-11X-17α,21-diol-3,20-dione 21-ester

In this equation, X stands for O or H,OH; while the ester is that of any of the above-described acids which increases the duration of the activity of the diene.

Detailed procedures for producing a number of compounds of the invention or intermediates for use in the production of such compounds are presented for purposes of illustration in the following examples, the dehydrogenation of ring A with the aid of microorganisms being described first.

Example 1

Conversion of Compound E (Cortisone) to 1,4 - Pregnadiene - 17α,21 - diol - 3,11, 20 - trione (1 - Dehydrocortisone)

From a solution of 30 g. of yeast extract ("Difco") in 3.0 l. of tap water containing 13.2 g. of potassium dihydrogen phosphate and 26.4 g. of disodium hydrogen phosphate (pH of the solution 6.9) 27 portions of 100 each are withdrawn, placed in 300 ml. Erlenmeyer flasks and sterilized by autoclaving for 15 minutes at 15 lb. per sq. in. steam pressure (120° C.). After autoclaving and cooling of the broth one ml. of a suspension of *Corynebacterium simplex* (A.T.C.C. 6946) is placed in each flask. The flasks are then shaken on a shake table at 220 rpm and 28° C. for 24 hours.

Into each of 27 Erlenmeyer flasks are placed 150 mg. of Kendall's Compound E. The flasks and contents are then sterilized for 15 minutes at 15 lb. per sq. in. steam pressure (120° C.). To each flask are then added 5.0 ml. of ethanol. The 24-hour bacterial culture is then transferred aseptically and the resulting suspensions are shaken on a shake table at 220 rpm, and 28° C. for 48 hours. The final pH is 7.2.

The contents of all the flasks are combined and extracted with a total of 9.0 l. of chloroform in three equal portions. The combined extracts are then concentrated to a residue which is crystallized from acetone-hexane. There results 1.1 g. of 1,4 - pregnadiene-17α,21 - diol - 3,11,20 - trione, m.p. 210—215° (dec.). Several additional recrystallizations raised the m.p. to 230—232° (dec.); $[\alpha]_D^{25}$ + 175.3 (dioxane); ϵ_{238} 15,400 (methanol).

Anal.: Calcd for $C_{21}H_{28}O_5$; C, 70.37; H, 7.31. Found: C, 70.38; H, 7.67.

21-Acetylation of 1,4 - Pregnadiene - 17α,21-diol - 3,11,20 - trione

To a solution of 0.5 g. of 1,4 - pregnadiene - 17α,21 - diol - 3,11,20 - trione in 5 ml. of anhydrous pyridine are added 3 ml. of acetic anhydride. The reaction mixture is permitted to stand overnight at room temperature, and is then diluted with ice and water. The resulting precipitate is filtered and recrystallized from acetone-hexane. There is obtained 0.35 g. of 1,4 - pregnadiene-17α, 21 - diol - 3,11,20 - trione 21 - acetate, m.p. 227—228° d. After several recrystallizations from acetone-hexane it melted at 233—236° (dec.).

Example 2

Conversion of Compound F (Hydrocortisone) to 1,4 - Pregnadiene - 11β,17α,21 - triol-3,20 - dione (1 - Dehydrocortisol).

150 mg. of Kendall's Compound F in each

of 27 Erlenmeyer flasks are treated as described in Example 1. The pH at the end of the shake period is 7.0.

The contents of all the flasks are combined and extracted with a total of 9.0 l. of chloroform in three equal portions. The combined extracts are then concentrated to a residue which weighs 3.75 g. The m.p. of the residue is 227—232°. From 2.75 g. of this crude material on sludging with 50 ml. of acetone and cooling, there is recovered on filtration 1.35 g. of 1,4 - pregnadiene - 11β,17α,21-triol - 3,20 - dione. m.p. 237—239° (dec.). Additional product can be recovered from the mother liquor. Recrystallization from acetone raised the m.p. to 239—241° (dec.), $[\alpha]_D^{25}+107°$ (dioxane); ϵ_{243} 14,600 (methanol).

Anal.: Calcd for $C_{21}H_{28}O_5$; C, 69.97; H, 7.83. Found: C, 70.24; H, 8.13.

21 - Acetylation of 1,4 - Pregnadiene - 11β, 17α,21 - triol - 3,20 - dione

To a solution of 0.85 g. of 1,4-pregnadiene-11β,17α,21 - triol - 3,20 - dione in 5 ml. of pyridine are added 3 ml. of acetic anhydride. The reaction mixture is allowed to stand at room temperature overnight and is then diluted with ice water. The resulting precipitate is filtered from the mixture and recrystallized from acetone-hexane. There is recovered 0.45 g. of 1,4 - pregnadiene - 11β, 17α,21 - triol - 3,20 - dione 21 - acetate, m.p. 235—239°. On recrystallization, the m.p. rose to 237—239°; $[\alpha]_D^{25}+116°$ (dioxane); ϵ_{243} 15,000 (methanol).

Anal.: Calcd. for $C_{23}H_{30}O_6$; C, 68.63; H, 7.51. Found: C, 68.62; H, 7.78.

Example 3

Conversion of Compound S to 1,4 - Pregnadiene - 17α,21 - diol - 3,20 - dione

One hundred ml. of 1.0% yeast extract concentrate including 9.0 ml. of 0.2 M KH_2PO_4 and 9.0 ml. of 0.2 M Na_2HPO_4 is sterilized as before and inoculated with a 1.0% suspension of *Corynebacterium simplex* (A.T.C.C. 6946) from a 24-hour broth culture. The newly seeded culture is incubated and shaken (shake table) for 20 hours at 28° C. After incubation, the broth culture is transferred aseptically to a second sterile 300 ml. Erlenmeyer flask containing 150.0 mg. of sterile Reichstein's Compound S (4-pregnene - 17α,21 - diol - 3,20 - dione) in 5.0 ml. ethanol or acetone. The pH of the reaction mixture is 7.0. The bacterial culture containing steroid and solvent is incubated and shaken for a period of 48 hours at 28° C. The final pH of the reaction mixture is 7.2—7.4. The culture is then extracted thoroughly with chloroform. The extracts are pooled and concentrated on a steam bath to dryness. The crude extract weighs 196.0 mg.

The total crude extract is sludged with methanol and there is obtained 80 mg. of crystalline solid, m.p. 246—250°. After two crystallizations from acetone, the m.p. is 246—249° dec. $(\alpha)_D^{25}+76°$ ($CHCl_3$), ϵ_{243} 15,500 (C_2H_5OH).

Calcd for $C_{21}H_{28}O_4$: C, 73.22; H, 8.19. Found: C, 73.56; H, 8.40.

Infrared spectrum and chemical analysis established that the product is 1,4 - pregnadiene - 17α,21 - diol - 3,20 - dione.

21 - Acetylation of 1,4 - Pregnadiene - 17α, 21 - diol - 3,20 - dione

To a solution of 0.25 g. of 1,4 - pregnadiene - 17α,21 - diol - 3,20 - dione in 2 ml. of pyridine was added 1 ml. of acetic anhydride. The reaction mixture was allowed to stand at room temperature overnight and was then diluted with ice and water. The resulting precipitate was filtered and recrystallized from methylene chloride-hexane, affording 0.20 g. of 1,4 - pregnadiene - 17α-21 - diol - 3,20 - dione 21 - acetate, m.p. 226.5—228°.

Example 4

Conversion of Compound S to 1,4 - Pregnadiene - 17α,21 - diol - 3,20 - dione and 1,4 - Pregnadiene - 17α,20,21 - triol-3 - one

One hundred ml. of a medium consisting of 1% fish solubles, 0.1% yeast extract, 9.0 ml. of 0.2M KH_2PO_4 and 9.0 ml. of 0.2M Na_2HPO_4 are sterilized as described hereinabove and inoculated with a suspension of *Corynebacterium simplex* from a 24-hour broth culture. The newly seeded culture is incubated and shaken (shake table) for 20 hours at 28° C. After incubation, the broth culture is transferred aseptically to a second sterile 300 ml. Erlenmeyer flask containing 150 mg. of sterile Compound S in 5.0 ml. of ethanol. The pH of the reaction mixture is 6.9. The bacterial culture containing steroid and solvent is incubated and shaken for 48 hours at 28° C. The final pH is 7.3. The culture is extracted with 2 l. of chloroform in 5 equal portions, the extracts are pooled, and the pool is concentrated on the steam bath.

The crude residue is taken up in methylene chloride and chromatographed over the adsorbent sold under the name "Florisil." There is isolated from the chromatogram, starting material (15 mg.), 1,4-pregnadiene-17α,21 - diol - 3,20 - dione (30 mg) and 1,4 - pregnadiene - 17α,20,21 - triol - 3 - one (90 mg.). The triol is recrystallized from acetone-hexane and melts at 195—196°, $(\alpha)_D^{25}+33°$ (methanol).

Anal.: Calcd. for $C_{21}H_{30}O_4$: C, 72,80; H, 8.73. Found: C, 72.79; H, 9.08.

In place of ethanol there can be employed other water-soluble organic solvents which

are non-toxic to the microorganism, such as acetone, mixture of ethanol and acetone, and the like. mixtures

EXAMPLE 5

Reaction of 5-Pregnen-3β,20-diol

One hundred ml. of 0.1% yeast extract concentrate including 9.0 ml. of 0.2M KH_2PO_4 and 9.0 ml of 0.2M Na_2HPO_4 is autoclaved in a 300 ml. Erlenmeyer flask. After autoclaving for 15 minutes at 15 lbs. per sq. in. pressure (120° C.), the flask is allowed to cool to room temperature. The flask is then seeded with a suspension of *Corynebacterium simplex*. The seeded flask is incubated and shaken (shake table) for 24 hours at 28° C.

A second 300 ml. Erlenmeyer flask containing 150 mg. of 5 - pregnene - 3α,20 - diol is sterilized in an autoclave for 15 minutes at 15 lbs. (120° C.). To this flask is then added 5.0 ml. of acetone or ethanol to dissolve the steroid. The 24-hour growth culture of *Corynebacterium simplex* is transferred aseptically to the flask containing the steroid and the reaction mixture is shaken (shake table) for 36 hours at 28° C. At the end of the transformation period, the pH is 7.1—7.2.

The reaction mixture is then extracted thoroughly with chloroform, the chloroform extracts are pooled and the resulting solution is concentrated to a residue (0.20 g.). The crude extract is crystallized from ether as long prisms, m.p. 135—138°. Two crystallizations from methylene chloride-hexane afford 0.06 g. of 1,4 - pregnadiene - 3,20-dione, m.p. 152—153° $(\alpha)_D^{25}$ + 122° ($CHCl_3$), ϵ_{245} 15,000 (C_2H_5OH). The infrared spectrum indicated the presence of a $\Delta^{1,4}$-diene - 3 - one system, another carbonyl (6-membered ring or side-chain), and the complete absence of hydroxyl.

EXAMPLE 6

1,4-Pregnadiene-17α,21-diol-3,20-dione

A one hundred ml. broth culture containing a 0.1% yeast extract concentration, 9.0 ml. of 0.2M KH_2PO_4 and 9.0 ml. of 0.2M Na_2HPO_4 is seeded with 1 ml. of a 24-hour broth culture of *Corynebacterium simplex*. The flask is incubated at 28° C. for 24 hours. 150 ml. of sterile 5 - pregnene - 3β,17α,21-triol - 20 - one 3,21 - diacetate in 50 ml. acetone in a 300 ml. Erlenmeyer flask are inoculated with the 24- hour culture and incubated for 48 hours at 28° to 30° C.

The products are extracted with chloroform, the chloroform extracts are concentrated to a small volume and chromatographed on "Florisil." The order of elution is unreacted starting material (75 mg.) first, then Compound S 21-acetate (15 mg.) and finally 1,4-pregnadiene-17α,21 - diol - 3,20 - dione (30 mg). The products were all identified by comparison of their infrared spectra with those of authentic samples.

By use of the same reaction medium and organism as in Example 6, and with the same proportion of steroid substrate, 4-pregnene-11β,21 - diol - 3,20 - dione (corticosterone) is converted into crystalline 1,4-pregnadiene - 11β,21 - diol - 3,20 - dione. Similarly, 4 - pregnene - 21 - ol - 3,20-dione (desoxycorticosterone) yields 1,4-pregnadiene - 21 - ol - 3,20 - dione as a crystalline solid, while 4 - pregnene - 21 - ol - 3,11, 20 - trione (11 - dehydrocorticosterone) is transformed into crystalline 1,4 - pregnadiene - 21 - ol - 3,11,20 - trione.

With reference to the acetylation of the dienes described in Examples 1, 2 and 3, it will be evident that other esters of the diene compounds can be similarly prepared, as by reaction with the anhydride of the acid or with its chloride in known manner, such as the formates, propionates, butyrates, and valerates, and likewise the esters of other non-toxic acids, like the benzoates, and also the neutral and acid esters of polybasic acids, like succinic, maleic, malic, citric, tartaric, phthalic and hexahydrophthalic. In the case of the acid esters, the metal salts can be formed in the usual manner by reaction with the hydroxide, carbonate or bicarbonate of the metal, as of the alkali and alkaline earth metals.

Instead of forming the 1,4-dienes of cortisone and hydrocortisone and subsequently esterifying the products, the corresponding esters of cortisone and hydrocortisone and of their intermediates can be subjected to the process described to yield the dienes of the esters. Thus, in Example 1, Compound E can be replaced by its 21-ester or by its 17α,21-diester, or by 5-pregnene-3,17α,21-triol - 11,20 - dione 21 - acetate or 17α,21-diacetate, or 3,17α,21 - triacetate or other esters; while in Example 2, Compound F can be replaced by its 21-acetate, or 17α,21-diacetate, or 11β,17α,21-triacetate; or by 5-pregnene - 3,11β,17α,21 - tetrol - 20 - one 3,21 - diacetate, 3,17α,21 - triacetate or 3,11β, 17α,21 - tetraacetate. The latter group of compounds can be replaced by the corresponding 11α-hydroxy epimers, to yield the 11-epimers of the diene of Compound F and its 21-esters and 17α,21-diesters. The polyesters can in all cases be mixed esters, like 3-propionate-21-acetate.

For example, by a treatment as described for the transformation of Compound F to the corresponding diene (Example 2), Compound F acetate can be converted to the 1,4-diene or its 21-acetate, and the product is isolated by chloroform extraction and crystallization from acetone. From 1.0 g. of Compound F 21-acetate there is obtained 0.22 g. of 1-dehydrocortisol (IIIa), m.p. 239—241° (dec.).

When it is desired to suppress the deacetylation reaction, the same conditions as above are used with the exception that the tem-

perature of the environment for the growth and reaction phases of the process is raised to 36° C. The product is isolated in the usual way. From 1.0 g. of Compound F 21-acetate there results 0.13 g. of 1-dehydrocortisol 21-acetate (IIIb), m.p. 237—239° (dec.).

Similarly, following the procedure of Example 1, 1.0 g. of cortisone 21-acetate is converted into 1-dehydrocortisone, of which 0.17 g. is isolated, m.p. 230—232° C. If the temperature of the environment for the growth and reaction phases of the process is raised to 36° C., then from 1.0 g. of cortisone acetate there results 0.11 g. of 1-dehydrocortisone 21-acetate, m.p. 230—233° (dec.).

Example 7

Conversion of Compound F (Hydrocortisone) to 1-Dehydrocortisol.

From a solution of 1 gram yeast extract ("Difco") in 1.0 liter of tap water containing 4.4 gm. of potassium dihydrogen phosphate and 8.8 g. of disodium hydrogen phosphate (pH of the solution 6.9), 10 portions of 100 ml. each are withdrawn, placed in 300 ml. Erlenmeyer flasks and sterilized by autoclaving for 15 minutes at 15 lbs. per sq. in. steam pressure (120° C.). After autoclaving and cooling of the broth, one ml. of a suspension of *Corynebacterium ~~hoagii~~* (American Type Culture ~~collection~~ 7005) is placed in each flask. The flasks are then shaken on a shake table at 220 rpm and 28° C. for 16½ hours.

Into each of the 10 Erlenmeyer flasks, 50 mg. of Kendall's Compound F, dissolved in 1 ml. of 90% methanol is added aseptically. The flasks are replaced on the shaker and incubated for 7 hours. The pH at the end of the shake period is 6.82.

The contents of all flasks are combined and extracted with a total of 3 liters of chloroform in 3 equal portions. The combined extracts are concentrated to a residue of 425 mg. Crystallization of the residue from acetone affords 248 mg. of 1,4-pregnadiene-11β,17α,21-triol-3,20-dione.

Example 8

Conversion of Compound F (Hydrocortisone) to 1-Dehydrocortisol.

From a solution of 0.5 gram ~~Besamin~~ Busch (Anheuser-Busch) in 1 liter of tap water, 10 portions of 100 ml. each are withdrawn, placed in 300 ml. Erlenmeyer flasks and sterilized by autoclaving for 15 minutes at 15 lb. steam pressure (120° C.). After autoclaving and cooling of the broth, one ml. of a suspension of *Corynebacterium simplex* is placed in each flask. The flasks are then shaken on a shake table at 220 rpm at 28° C. for 24 hours.

Into each of the 10 Erlenmeyer flasks 50 mg. of Kendall's Compound F, dissolved in 0.8 ml. of absolute methanol, are added aseptically. The flasks are replaced on the shaker and incubated for an additional 4—7 hours. The pH at the end of the shake period is 7.2—7.6.

The contents of all flasks are combined and extracted with a total of 3 liters of chloroform in 3 equal portions. The combined extracts are concentrated to a residue of 490 mg. Crystallization of the residue from acetone affords 403 mg. of 1,4-pregnadiene - 11β,17α,21 - triol - 3,20 - dione, m.p. 238—240° C. (dec.); $(\alpha)_D^{25}$ + 105° (dioxone); ϵ_{243} 14,500 (methanol).

By using Compound E in place of Compound F in this example, and increasing the conversion time to 6—12 hrs., crude 1-dehydrocortisone is obtained in a yield of 85%.

Example 8 shows that the yield is increased with reduction in the concentration of the starting compound.

The other 4-pregnene compounds hereinabove disclosed can likewise be treated according to the procedures of Example 8. As with the other examples hereinabove set forth, the 21-esters can be employed as starting compounds and likewise the 5-pregnene-3-hydroxy analogues.

Example 9

Conversion of Compound E (Cortisone) to 1-Dehydrocortisone.

Bacillus sphaericus var. fusiformis (A.T.C.C. 7055) is incubated on a nutrient agar composed of Bacto-beef extract, 3 g.; Bacto-peptone, 5 g.; sodium chloride, 8 g.; agar, 15 g.; and tap water, 1 liter, for 24 hours at 28° C.

To 100 ml. of a sterile nutrient broth (composed of Bacto-beef extract, 3 g.; Bacto-peptone, 5 g.; and made up to 1 liter with tap water) in a 300 ml. flask is added one loopful of the incubated culture and the broth mixture is further incubated for 24 hours at 28° C. on a shaking machine under aerobic conditions. The broth culture so obtained is employed as a one per cent inoculum.

Into each of 10 flasks containing 100 ml. of sterile nutrient broth is added one ml. of the inoculum. The flasks are agitated on a rotary shaker aerobically for 8 hours at 28° at 240 strokes per minute. After this growth period, a solution of 25 mg. of cortisone in 0.5 ml. of methanol is aseptically added to each flask which in turn is reshaken and incubated for an additional 24 hours. The final pH is about 7.8. The contents of the flasks are combined, extracted with chloroform and processed in the usual manner, yielding 1-dehydrocortisone.

In place of Compound E, there can be employed as starting material 5-pregnen-3,17α,21 - ~~tetrol~~ - 11,20 - dione, 5 - pregnen-3,17α,20,21 - tetrol - 11 - one or their 3-acetates or other esters which are non-toxic toward the microorganism or inhibiting to-

ward its enzyme; while similarly, in place of Compound F, there can be used 5-pregnen-3,11β,17α,21 - tetro - 20 - one, 5 - pregnen-3,11β,17α,20,21 - pentol, or their 3 - acetates or other esters. As indicated above, the 20-hydroxy group will be oxidized to a keto group.

EXAMPLE 10

Conversion of Compound F (Hydrocortisone) to 1-Dehydrocortisol.

A growth medium is prepared from 30 g. of condensed fish solubles (enzyme-treated), 4.49 g. of KH_2PO_4 and 8.83 g. of $Na_2HPO_4.7H_2O$, all diluted to 1 liter, with tap water. The pH of this medium is about 6.8. Into each of ten 300 ml. flasks are placed 100 ml. of the growth medium and the flasks and contents are autoclaved for 15 minutes at 15 pounds steam pressure. To each cooled flask is then added 1 ml. of the inoculum described in Example 9, and the growth is permitted to occur for 12 hours. To each flask is then aseptically added 25 mg. of hydrocortisen and aerobic incubation is resumed for an additional 24 hours. The fermentation mixture is then worked up as above described for the recovery of 1-dehydrocortisol.

The procedures of Examples 9 and 10 can be varied by employing as the nutrient medium a 1% yeast extract ("Difco") in tap water.

EXAMPLE 11

Conversion of 4,6 - Pregnadiene - 17α,21-diol - 3,11,20 - trione 21 - acetate into 1 - Dehydrocortisone.

0.85 g. of 4,6-pregnadiene-17α,21 - diol-3,11,20 - trione 21 - acetate (also known as 6-hydrocortisone acetate) obtained in the manner described in J. Biol. Chem. *197*, 261 (1952) is converted into 1,4,6 - pregnatriene-17α,21 - diol - 3,11,20 - trione in the manner above described by the action of the enzymes of a culture of *Corynebacterium simplex*, in a yield of about 18%. This new triene exhibits adrenocorticol hormone properties, but may be partially hydrogenated into the corresponding 1,4-diene by reduction under relatively mild conditions, as by means of zinc, acetic and ascorbic acids, in the manner described in the cited publication.

In the manner described, other 1,4,6-pregnatrienes may be prepared from starting compounds having a system of conjugated double bonds distributed in the A- and B-rings, and such pregnatrienes may be similarly partially hydrogenated to produce the corresponding 1,4-pregnadienes. Also the substituent in the 3-position may be a hydroxyl group, in which case the conjugated double bonds will generally be attached to the 5,6- and 7,8-carbons. The substituent in the 11-position may be an α- or β-hydroxyl or may be absent altogether, and the group in the 21-position may be methyl in place of hydroxy-methyl or acyloxy-methyl. Thus, 4,6 - pregnadiene - 11β,17α,21 - triol - 3,20-dione and 5,7 - pregnadiene - 3,11β,17α,21-tetrol - 20 - one can be converted by the action of the above disclosed cultures into 1,4,6 - pregnatriene - 11β,17α,21 - triol - 3,20 - dione; while 4,6 - pregnadiene - 17α,21-diol - 3,20 - dione can be converted into 1,4,6 - pregnatriene - 17α,21 - diol - 3,20-dione. In each case, the 21-ester can be employed, which may or may not be hydrolyzed in the process. By partial hydrogenation, as above described, the corresponding 1,4-pregnadienes will be obtained.

EXAMPLE 12

Conversion of 4 - Pregnene - 11α,17α,21-triol - 3,20 - dione to 1,4 - Pregnadiene-11α,17α,21 - triol - 3,20 - dione (Epi-1-dehydrocortisol).

The reaction is run exactly as described in Example 2 for the transformation of Compound F to the corresponding diene, and the product is isolated by chloroform extraction and crystallized from acetone-hexane. From 1.0 g. of 11-epi Compound F there is isolated 0.25 g. of 1,4 - pregnadiene - 11α,17α,21-triol - 3,20 - dione as a crystalline solid. M.p. 245—246° C (dec.).

Acetylation of epi-1-dehydrocortisol (1.0 g.) is accomplished by solution in 15 ml. of anhydrous pyridine followed by the addition of 0.3 g. of acetic anhydride. The reaction mixture is allowed to stand at room temperature overnight and is then poured into ice-water. The resulting precipitate is separated by filtration and recrystallized as a crystalline solid (21-acetate) from acetone-hexane.

EXAMPLE 13.

1,4 - Pregnadiene - 9α - fluoro - 21 - ol - 3,11,20 - trione.

To a 300 ml. Erlenmeyer flask are added 100 ml. of 0.1% yeast extract ("Difco") containing 9.0 ml. of 0.2M potassium dihydrogen phosphate and 9.0 ml. of 0.2M disodium hydrogen phosphate. The flask and its contents are sterilized by autoclaving for 15 minutes at 120° C. and to the sterile medium is added 1 ml. of a 1% suspension of *Corynebacterium simplex*. The flask and its contents are incubated at 28° C. for 24 hours.

To a second 300 ml. Erlenmeyer flask are added 2 ml. of ethanol and 25 mg. of 9α-fluoro-11-dehydrocorticosterone. The 24-hour growth culture is transferred aseptically to the flask containing the steroid and the mixture is incubated at 28° C. and shaken for 10 hours. At the end of that time the reaction mixture is extracted thoroughly with chloroform and the chloroform extracts are concentrated to a residue. The residue is crystallized from acetone-hexane, affording 4 mg. of 9α-fluoro-1,4-pregnadiene-21-ol-3,11, 20 - trione.

The 21-acetate of the compound of this

example is prepared by reacting 0.3 g. of acetic anhydride with a solution of 1 g. of the 21-ol in 20 ml. of anhydrous pyridine at room temperature overnight, followed by dilution with ice water.

Similarly, by the procedure of Example 13, 9α-fluoro-1,4-pregnadiene-11β,21-diol-3,20-dione is obtained in a yield of 5 mg. from 25 mg. of singly unsaturated starting compound. The 21-acetate is obtained by the usual acetylation.

By employing a 1% suspension of *Corynebacterium hoagii*, in place of the *C. simplex* of Example 13, the conditions being otherwise the same, 25 mg. of 9α-chlorocorticosterone are converted to 9α-chloro-1,4-pregnadiene-11β,21-diol-3,20-dione in a yield of 7 mg. The product can be converted to the 21-propionate by reaction with propionic anhydride. With the same culture (*C. hoagii*) 9α-bromocorticosterone 21-acetate is converted into 9α-bromo-1,4-pregnadiene-11β,21-diol-3,20-dione.

Following the procedure of Example 13, but employing an incubation period of 48 hours at 28 to 30° C., the 9α-fluoro derivative of Compound F is converted into the corresponding 1,4-diene. With the same procedure, the 9α-fluoro, 9α-chloro and 9α-bromo derivatives of Compound E (cortisone) are converted into the 9α-fluoro, 9α-chloro and 9α-bromo derivatives of 1-dehydrocortisone, respectively. The 9α-chloro- and 9α-bromo-derivatives of Compound F are in the same fashion converted into the 9α-chloro- and 9α-bromo derivatives of 1-dehydrocortisol, respectively.

The 17-desoxy compounds can be converted to the corresponding 17α-hydroxy compounds (9α-halogeno derivatives of 1-dehydrocortisol and 1-dehydrocortisone) by the actions of a culture of *Trichothecium roseum*, as described below.

Also other 1,4-pregnadienes produced with the aid of a dehydrogenating microorganism can, by the procedures described more fully hereinbelow, be converted to the desired highly potent end products.

EXAMPLE 14.

1,4-Pregnadiene-17α,21-diol-3,20-dione

One ml. of a broth culture prepared as an inoculum (1%), according to Example 9, is added to each of ten flasks containing 100 ml. of the sterile nutrient broth as described in such example. The flasks are agitated on a rotary shaker for 8 hours at 28° at 240 strokes per minute. After this growth period, a solution of 25 mg. of Reichstein's Compound S in 0.5 ml. of methanol is aseptically added to each flask which in turn is reshaken and incubated for an additional 24 hours. The final pH is 7.8.

The contents of the flasks are then combined and extracted 3 times with 2 liters of chloroform per extraction. The combined chloroform extracts are evaporated to dryness yielding 310 mg. of crude product. The crude steroid is purified by chromatography on the chromatographic system described by G. M. Shull, Abstracts of Papers of the 126th Meeting of the American Chemical Society, December 12—17, 1954, page 9a, paper No. 24. Chromatographic evaluation shows a quantitative conversion of the starting material to the diene when an authentic sample of the S-diene is used as a control.

Alternatively, the crude product is recrystallized from acetone affording 225 mg. of 1,4-pregnadiene-17α-21-diol-3,20-dione, m.p. 246—248°.

EXAMPLE 15

1-Dehydrocortisol

From a solution of 1% yeast extract ("Difco") in tap water, 10 portions of 100 ml. each are withdrawn, placed in 300 ml. flasks and sterilized by autoclaving for 15 minutes at 15 pounds per sq. in. steam pressure. After autoclaving and cooling of the broth, 1 ml. of a shake culture of *Bacillus sphaericus var, fusiformis* grown in nutrient broth as described in Example 14, is placed in each flask. The flasks are then shaken on a shake table for 12 hours at 28° C. at 240 strokes per minute.

Into each flask is aseptically added a solution of 25 mg. of Compound F in 0.5 ml. of methanol and the flasks and contents are shaken and incubated for an additional 8 hours.

Upon combining the contents of all the flasks and extracting with chloroform as described in Example 14, there is obtained 304 mg. of crude diene. Purification is effected by recrystallization from acetone using decolorizing charcoal whereupon there is obtained 230 mg. of 1,4-pregnadiene-11β,17α,21-triol-3,20-dione, m.p. 240—241°.

By employing a total of 250 mg. of the 21-acetate of Compound F in place of Compound F itself, there are obtained 220 mg. of 1,4-pregnadiene-11β,17α,21-triol-3,20-dione 21-acetate, m.p. 237—239° C. after recrystallization from acetone-hexane.

EXAMPLE 16

1-Dehydrocortisone

A growth medium is prepared from 30 g. of condensed fish solubles (enzyme-treated), 4.49 g. of KH_2PO_4 and 8.83 g. of $Na_2HPO_4 \cdot 7H_2O$, diluted to 1 liter with tap water. (The pH of this medium is about 6.8). Into each of ten 300 ml. flasks are placed 100 ml. of the growth medium and the flasks and contents are autoclaved for 15 minutes at 15 pounds steam pressure. Each flask is inoculated with 1 ml. of *Bacillus sphaericus var. fusiformis* as in Example 15, and growth is permitted to occur for 12 hours. To each flask is then aseptically added a solution of 50 mg. of Compound E in 1 ml. of methanol and shaking and incuba-

tion resumed for an additional 24 hours.

The contents of the flasks are combined and extracted with chloroform as described in Example 14, yielding 630 mg. of crude Compound E diene. Purification is effected by recrystallization from acetone in the presence of decolorizing charcoal, whereupon there is obtained 420 mg. of 1-dehydrocortisone, m.p. 233—235°.

Similarly, in accordance with the procedure in Example 16, corticosterone is converted into 1,4 - pregnadiene - 11β,21 - diol - 3,20-dione. By utilizing the same reaction medium and organism as described in Example 14, 9α - fluoro - 4 - pregnene - 11β,17α,21-triol - 3,20 - dione is transformed into the corresponding 1,4 - pregnadiene which is obtained as a crystalline solid.

With the culture described in Example 16, 5 - pregnene - 3β,20 - diol is converted into 1,4 - pregnadiene - 3,20 - dione, m.p. 152—153° C. after extraction with chloroform, evaporation, and recrystallization of the residue from ether.

Desoxycorticosterone can be similarly converted into 1,4 - pregnadiene - 21 - ol - 3,20-dione with the culture described in Example 14.

As will be apparent from the foregoing, the dehydrogenating microorganisms effect introduction of a double bond in the 4,5-position through oxidation of the 3-hydroxyl of 3 - hydroxy - 5 - pregnenes with simultaneous dehydrogenation at the 1,2-carbons; and while cultures of dehydrogenating microorganisms may be caused to act on 3-hydroxy or 3 - keto - 1 - pregnenes, we prefer to employ starting compounds having a double bond attached to the 5-carbon.

The following examples 17 to 19 illustrate various combinations of the step for introducing a 1,2-double bond into the steroid molecule with the aid of the dehydrogenating microorganisms above described, in combination with other microbiological procedures whereby various substituents are introduced into the molecule for converting various known starting compounds into the therapeutically active pregnadienes with the production of intermediates which are themselves therapeutically active or can be converted into 1-dehydrocortisone or 1-dehydrocortisol, and their esters.

EXAMPLE 17

A. 1,4 - Pregnadiene - 17α - ol - 3,20 - dione (IIc).

Employing the culture described in Example 3, 150.0 mg. of sterile 4 - pregnene-17α - ol - 3,20 - dione (17α - progesterone) (Ic) in 5.0 ml. ethanol are dehydrogenated. The crude extract weighs 178 mg. The crude product is crystallized from acetone-hexane, yielding pure crystals of 1,4 - pregnadiene-17α - ol - 3,20 - dione (IIc).

B. 1,4 - pregnadiene - 11α,17α - diol - 3,20-dione (IIIc).

This conversion is effected with the aid of a culture of *Rhizopus nigricans* in the manner described in detail in J. Am. Chem. Soc., *74*, 5933 (1952). To 6 liters of a 24-hour growth culture of *Rhizopus nigricans* is added 1.0 g. of IIc in 200 ml of ethanol. After a 48-hour transformation period in the equipment and medium described in the just-mentioned publication, the culture is extracted with methylene chloride, the solvent evaporated, and the crude crystalline residue is recrystallized from acetone-hexane, affording crystalline 1,4 - pregnadiene - 11α,17α - diol-3,20 - dione (IIIc).

C. 1,4 - Pregnadiene - 17α - ol - 3,11,20-trione (Vc).

A solution of 3.0 g. of 11c in 30 ml. of pyridine is added slowly to a slurry of 1.5 g. of chromic acid in 15 ml. of pyridine (Poos, et al, J. am. Chem. Soc., *75*, 442 (1953)) and the resulting mixture is stirred overnight at room temperature. To the reaction mixture are then added 4.5 g. of sodium sulfite in 45 ml. of water and stirring is continued for two hours. The reaction mixture is poured into 600 ml. of water and the resulting solution is extracted with methylene chloride. The extracts are washed neutral with dilute sulfuric acid, aqueous sodium carbonate and water, and dried over magnesium sulfate. Concentration of the dried solution and crystallization of the residue from acetone-hexane affords crystalline 1,4 - pregnadiene - 17α - ol - 3,11,20-trione (Vc).

D. 1 - Dehydrocortisone (IIa).

Four liters of 1% yeast extract ("Difco") is sterilized in a shake flask and inoculated with a strain of *Ophiobolus herbotrichus*. The culture is incubated for three days with shaking at 27° C. Then 1.0 g. of Vc in 25 ml. of acetone (sterile) is added aseptically to the shake flask and shaking is continued for another three days at 27° C. The mycelium is separated from the reaction mixture and both the mycelium and aqueous filtrate are extracted thoroughly with three portions of chloroform. The extracts are combined, washed with water, dried and concentrated. The concentrated solution is chromatographed over Florisil (30 g.) and the column is washed with methylene chloride. Elution with 0.5% and 1.0% methanol-in-methylene chloride removes 1-dehydrocortisone which is recrystallized from acetone-hexane.

EXAMPLE 18

A. Preparation of 1,4 - Pregnadiene - 11β, 17α - diol - 3,20 - dione (IVc) from IIc.

A culture of *Curvularia lunata* (QM 120h) is grown in flasks containing the same medium as is described in Example 17 (see also U.S. Patent No. 2,658,023). One hundred milliliters

of this inoculum is added under sterile conditions to two liters of an aqueous medium containing the following:

Sucrose	1
"Difco" tryptone	1
Sodium nitrate	0.2
Dipotassium hydrogen phosphate	0.1
Magnesium sulfate heptahydrate	0.05
Potassium chloride	0.05
Ferrous sulfate heptahydrate	0.001

This mixture is adjusted to pH 7 with sulfuric acid and 0.25% of calcium carbonate is added before the mixture is sterilized. The inoculated medium is aerated at the rate of about one-half to one volume of air per volume of solution per minute at 27° to 28° C. for 24 hours. During this time, the mixture is agitated with a stirrer rotating at about 1700 revolutions per minute. One-half gram of 1,4 - pregnadiene - 17α - ol-3,20 - dione (IIc), is dissolved in 20 milliliters of 95% ethanol. The solution is added to the fermentation mixture unuder sterile conditions. The reaction is then continued for a further 24 hours under exactly the same conditions as described above.

The fermentation mixture is extracted thoroughly with three portions of methylene chloride and the combined extracts are dried over magnesium sulfate and concentrated to a small volume. The concentrated solution is added to a "Florisil" column (30 g.) prepared with hexane and the column is stripped with hexane containing ether in amounts varying upward from 1% to 99%. The desired product is collected from the most polar eluates (25% ether→99% ether) and is crystallized from acetone-hexane, affording crystalline 1,4 - pregnadiene - 11β, 17α - diol - 3,20 - dione (IVc).

B. Preparation of Vc from IVc

This reaction is run exactly like the transformation of IIIc to Vc, except that IVc is employed in place of IIIc. The product is then converted into IIa in the manner described above.

EXAMPLE 19

Preparation of 1,4 - Pregnadiene - 11α,17α,21-triol - 3,20 - dione (VIIa) from IIIc.

The same procedure is employed as is used to convert Vc to IIa with the exception that the steroid substrate is IIIc and the product, VIIa, is isolated by chromatography over "Florisil" in the 1% and 2% methanol-in-methylene chloride eluates. Recrystallization of VIIa from acetone gives a crystalline product, m.p. 243—245°.

By the same procedure as is employed to convert Vc to IIa, IVc is converted to IIIa. Recrystallization of IIIa from acetone gives crystals of m.p. 238—240°.

Oxidation of IIIa to VIIa, preferably as the 21-acetates or other lower alkanoyl esters (IIIb and VIIb), is accomplished according to the procedure described for converting IIIc to Vc. The 21-acetate group is hydrolyzed with potassium bicarbonate in aqueous methanol to recover IIa.

The conversion of Ic to 4 - pregnene - 11α, 17α - diol - 3,20 - dione (VIc) (Route B), is accomplished by the same procedure as that employed in the conversion of compound IIc to compound IIIc (Example 17 B).

Similarly, 4 - pregnene - 11β,17α - diol-3,20 - dione (VIIc) is prepared from Ic in the same manner as IVc from IIc. Compounds VIc and VIIc are both oxidized to 4 - pregnene - 17α - ol - 3,11,20 - trione (VIIIc) by the procedure employed for the transformation of compounds IIc and IVc to compound Vc.

The remainder of the transformations according to Route B similarly follow the procedures of Route A. Thus, compound VIc is converted to IIIc, and compound VIIIc is converted to Vc, while compound VIIc is converted to IVc, in the same way as Ic is converted to IIc, i.e., by treatment with a culture or an enzymatic extract of a culture of *Corynebacterium simplex* or *hoagii*. From this point, the transformations are the same as in Route A.

As applied specifically to the conversion of desoxycorticosterone (4 - pregnene - 21-ol - 3,20 - dione), 5 - pregnene - 3,21 - diol-20 - one and their esters into 1 - dehydrocortisone and 1 - dehydrocortisol (1 - dehydro Compound F) and their esters, the process of the invention can be applied as indicated in the following Examples 20 and 21.

EXAMPLE 20

A. 1,4 - pregnadiene - 21 - ol - 3,20 - dione (IId).

150.0 mg. of sterile 4 - pregnene - 21 - ol-3,20 - dione (Id) in 5.0 ml. ethanol are subjected to fermentation in the same medium, with the same microorganism and in the same manner ~~described in Example 17 B. The~~ crude extract weighing 137 mg. The crude product is crystallized from acetone-hexane, affording crystalline 1,4 - pregnadiene - 21-ol - 3,20 - dione (IId).

B. 1,4 - Pregnadiene - 11α,21 - diol - 3,20-dione (IIId).

1.0 g. of IId in 200 ml. of ethanol is treated with a culture of *Rhizopus nigricans* in the manner described in Example 17. The crude crystalline residue is recrystallized from acetone-hexane, yielding crystalline 1,4-pregnadiene - 11α,21 - diol - 3,20 - dione (IIId).

C. 1,4 - Pregnadiene - 21 - ol - 3,11,20-trione (Vd).

A solution of 3.0 g. of IIId in 30 ml. of pyridine is oxidized in the manner described in Example 17A, yielding a

scribed in Example 17 C. Evaporation of the dried methylene chloride extract and crystallization of the residue from acetone-hexane affords crystalline 1,4 - pregnadiene - 21-ol - 3,11,20 - trione (Vd).

D. 1-Dehydrocortisone (IIa).

Two liters of Czapek-Dox medium are sterilized in a shake flask and inoculated with a strain of *Trichothecium roseum*. After three days' shaking at 27° C., there are added aseptically to the culture 500 mg. of 1,4-pregnadiene - 21 - ol - 3,11,20 - trione (Vd) in 15 ml. of acetone. Shaking is continued for 60 hours at 27° C. The mycelium is separated and both the mycelium and the aqueous filtrate are extracted thoroughly with methylene chloride. The combined extracts are washed well with water, dried and concentrated. The concentrated solution is chromatographed over "Florisil" and 1-dehydrocortisone (IIa) is eluted in the 0.5% and 1.0% methanol-in-methylene chloride fractions, following washing of the column with methylene chloride. Recrystallization of the indicated fractions from acetone affords crystals of IIa, m.p. 226—229° (dec.).

Example 21

A. 1,4 - Pregnadiene - 11β,21 - diol - 3,20-dione (IVd).

A culture of *Curvularia lunata* (QM 120h) is grown in flasks containing the same medium as described in Example 1 of U.S. patent to Gilbert M. Shull et al, No. 2,658,023, and is employed exactly as described in Example 18A for the transformation of 1,4 - pregnadiene - 21 - ol - 3,20 - dione (IId) likewise dissolved in 20 milliliters of 95% ethanol. From the most polar eluates (25% ether→99% ether) there is obtained 1,4-pregnadiene-11β,21 - diol - 3,20 - dione (IVd) which is recrystallized from acetone-hexane.

B. Preparation of 1,4 - Pregnadiene - 21-ol - 3,11,20 - trione (Vd).

This reaction is run exactly as the transformation of IIId to Vd, except that IVd is employed in place of IIId.

Compound IIId is converted to 1,4-pregnadiene - 11α,17α,21 - triol - 3,20 - dione (VIIa) by the same procedure as is used to convert Vd to IIa. The product is isolated by chromatography over Florisil in the 1% and 2% methanol-in-methylene chloride eluates. Recrystallization of VIIa from acetone gives the product in crystalline form, m.p. 243—245° C.

Similarly, the procedure employed to convert IVd to hydrocortisone (IIIa) is that used to convert Vd to IIa, and the product IIIa is isolated by chromatography over "Florisil" in the 1% and 2% methanol-in-methylene chloride eluates. Recrystallization of IIIa from acetone gives a crystalline product m.p. 232—240° C.

Oxidation of IIIa or VIIa, preferably as the 21-acetate, obtained by reacting the 21-alcohols at room temperature with one equivalent of acetic anhydride, is accomplished according to the procedure described for converting IIId. to Vd. The 21-acetate group is hydrolyzed by heating with potassium bicarbonate in aqueous methanol to recover IIa.

The transformation of 4 - pregnene - 11α, 21 - diol - 3,20 - dione (VId), 4 - pregnene-11β,21-diol-3,20-dione (VIId), and 4-pregnene - 21 - ol - 3,11,20 - trione (VIIId), to IIId, IVd, and Vd, respectively, is effected in the same manner as the conversion of Id to IId.

Analogously, the conversion of Id. to the intermediates VId and VIId follows the procedures described above for the conversion of IId to IIId and of IId to IVd, respectively. Compounds VId and VIId are oxidized to compound VIIId under mild oxidizing conditions such as are described above.

As indicated hereinabove, in place of desoxycorticosterone and its esters, we may use 5 - pregnene - 3,21 - diol - 20 - one and its 21-esters, such as the acetate and other lower alkanoyl esters, as starting compounds, as in the course of the treatment with a culture or separated enzyme of the dehydrogenating microorganism, such as *C. simplex* or *C. hoagii*, in the manner already described, the 3-hydroxyl is oxidized to ketonic oxygen, while the 5,6-double bond shifts to the 4,5-position. Thereby 5 - pregnene - 3,21 - diol-20 - one and its 21 - esters are converted into IIa and its 21 - esters.

Better yields in the oxidation of the 11α-OH and 11β-OH compounds to the corresponding 11-keto compounds will generally be obtained and under less critical conditions if the 21-hydroxyl is first esterified in the procedures described in Examples 20C and 21B, followed, if desired, by hydrolysis. Methods which are suitable for the partial esterification of the 11,21-diols and hydrolysis of the 11 - keto - 21 - esters obtained on oxidation have already been described.

The 21-acetate of Vd can be obtained by the oxidation of the 11-hydroxyl of the 21-acetates of IIId and IVd with chromic acid in pyridine as already described in connection with related compounds. The 21-ester of Vd is readily hydrolyzed by refluxing with potassium carbonate in aqueous methanol.

Compounds VId and VIId are convertible into their 21-acetates in the same way as IIId and IVd. Oxidation of VId 21-acetate and VIId 21-acetate to VIIId 21-acetate follows the procedure of the oxidation of IIId 21-acetate to Vd 21-acetate. Hydrolysis of VIIId 21-acetate to VIIId, where such hydrolysis has not been accomplished or has not been completed by the microorganism employed to introduce the 1,2-double bond, as

when higher fermenting temperatures are used, follows the procedure for the hydrolysis of Vd 21-acetate.

In one of the diagrams hereinabove there is shown the conversion of 16-dehydropregneneolone (Ie) and 16 - dehydroprogesterone (IIIe) into 1-dehydrocortisone (IIa) by way of the 16,17-epoxides and by various routes. These routes involve also the addition of an 11(α or β)-hydroxyl, the addition of a 21-hydroxyl, the introduction of a 17α-hydroxyl by opening up of the 16,17-epoxide ring, the introduction of the 1,2-double bond, and the oxidation of the 11-hydroxyl to ketonic oxygen. While the order of these reactions can be varied within limits (for example, the oxidation step must follow the introduction of the 11-hydroxyl), we prefer to conduct the treatment with the dehydrogenating microorganism after the introduction of a hydroxyl group at least one of the 11, 17, and 21-positions, or after the formation of the 16,17-epoxide. Illustrative procedures for conducting different orders of conversions on 4,16- and 5,16-pregnadienes are indicated in Examples 22 and 23.

Example 22
1-Dehydrocortisone

One g. of 16-dehydroprogesterone (IIIe) dissolved in 25 ml. of chloroform is reacted at room temperature for about 15 hours with the equivalent amount of perbenzoic acid dissolved in chloroform. Water is then added to the reaction mixture, the organic layer is washed with sodium sulfite solution, then with sodium bicarbonate solution, water, and then dried over sodium sulfite. The chloroform is evaporated and the residue is crystallized from a mixture of acetone and hexane, yielding 16,17 - epoxy - 4 - pregnene - 3,20-dione (IVe).

A suspension of 1 g. of the 16,17-epoxide in 10 ml. of glacial acetic acid is mixed with a solution of 1.1 g. of 47% aqueous hydrogen iodide, 10 ml. of acetic acid and 4.1 g. of acetic anhydride. The temperature is maintained at about 15 to 20° C. throughout the addition and for a half hour afterwards. The mixture is then poured into five volumes of water, the precipitate filtered, washed with water and dried. The crude product is 16 - iodo - 4 - pregnene - 17α - ol - 3,20-dione and is dissolved in 100 ml. of ethanol and 1 ml. of acetic acid and refluxed for about 6 hours with 2.5 g. of Raney nickel catalyst. The catalyst is then removed by filtration, the filtrate concentrated, and upon the addition of water, there is obtained a precipitate of 4 - pregnene - 17α - ol - 3,20-dione (17α - hydroxy - progesterone, VIIe).

Four liters of a 1% yeast extract (Difco) is sterilized in a shake flask and inoculated with a strain of *Ophiobolus herbotrichus*. The culture is incubated for three days with shaking at 27° C.; then 1.0 g. of 17α-hydroxyprogesterone in 25 ml. of acetone (sterile) is added aseptically to the shake flask, and shaking continued for another three days at 27° C. The mycelium is separated from the reaction mixture and both the mycelium and aqueous filtrate are extracted thoroughly with three portions of chloroform. The extracts are combined, washed with water, dried, concentrated, and chromatographed on "Florisil." The material obtained on elution, as with 0.5% to 1.5% methanol in methylene chloride, is crystallized from acetone-hexane to give 4 - pregnene - 17α,21-diol - 3,20 - dione (VIIIe).

A solution of 1.0 g. of VIIIe in 150 ml. of ethanol is added to one liter of a 24-hour growth culture of *Rhizopus nigricans*. After a 48-hour transformation period, the mixture is extracted with methylene chloride and the organic extract evaporated to dryness. The crude product is crystallized from acetone-hexane to give 4 - pregnene - 11α,17α,21-triol - 3,20 - dione (IXe).

The compound IXe is now subjected to the action of a culture of a dehydrogenating microorganism, such as *Corynebacterium simplex*. After incubation for about 48 hours at 28° C., there is obtained 1,4 - pregnadiene - 11α,17α,21 - triol - 3,20 - dione (Xe, VIIa). This compound is then oxidized as hereinabove described to 1-dehydrocortisone (IIa).

By employing a culture of *Curvularia lunata*, (QM 120h), as described in Example 18A, in place of a culture of *Rhizopus nigricans* there is obtained from IXe the compound 1,4-pregnadiene - 11β,17α,21 - triol-3,20-dione (1-dehydrocortisol, IIIa), which can likewise be oxidized to 1-dehydrocortisone.

Example 23
1-Dehydrocortisone

16-Dehydropregnenolone is converted to the 16,17-epoxide by treatment with perphthalic acid. The 16,17 - epoxy - 5 - pregnene - 3 - ol - 20 - one (IIe) is then converted to the 11α-hydroxy derivative (XIe) by treatment with a culture of *Rhizopus nigricans*. Compound XIe is then subjected to the action of a culture of *Ophiobolus herbotrichus* to produce 16,17 - epoxy - 5-pregnene - 3,11α,21 - triol - 20 - one (XIIe). The epixode ring is then opened up by treatment with hydriodic acid, as above described, yielding 5 - pregnene - 3,11α,17α,21 - tetrol-20 - one (XIIIe). On subjecting the latter compound to the action of a culture of a dehydrogenating microorganism there is obtained 1,4 - pregnadiene - 11α,17α,21 - triol-3,20 - dione (Xe), which is then oxidized to 1-dehydrocortisone.

If desired, 16-dehydropregnenolone can first be treated with a culture of *Ophiobolus herbotrichus* to yield 5,16 - pregnadiene - 3, 21 - diol - 20 - one which is then acetylated

in known manner to produce the diacetate. On treatment with a dehydrogenating microorganism, the diacetate is converted into 1,4,16 - pregnatriene - 21 - ol - 3,20 - dione. This is then converted to the 16,17-epoxide by treatment with perphthalic acid, after which the epoxide ring is opened up with hydriodic acid followed by refluxing with Raney nickel catalyst, yielding 1,4 - pregnadiene - 17α,21 - diol - 3,20 - dione. The latter can then be converted to 1-dehydrocortisol by the action of a culture of *Curvularia lunata.*

In place of the per-acids, like peracetic, perbenzoic, and perphthalic acids for the formation of the oxido ring, there may be employed hydrogen peroxide, together with an alkali metal base, preferably sodium or potassium hydroxide. This mixture may contain a quantity of an alcohol, like methanol and t-butanol, to aid in dissolving the steroid compound, but such alcohol is generally not absolutely necessary.

Additional starting compounds are 5,16-pregnadiene - 3,11 - diol - 20 - one and its 3 - esters, 5,16 - pregnadiene - 3,21 - diol-20 - one and its 3- and 21 - esters, 5,16-pregnadiene - 3,11,21 - triol - 20 - one and its 3- and 21-esters, and 4,16 - pregnadiene - 21 - ol - 3,11,20 - trione and its 21-esters, which can be subjected to the culture of a dehydrogenating microorganism, to form the conjugated 3 - keto - 1,4 - diene system, and to the action of a per-acid, or of hydrogen peroxide, to form the 16,17-oxido ring, in either order.

An example of a compound already having the 1,4 - diene - 3 - keto structure which can be converted into 1-dehydrocortisone and 1-dehydrocortisol is the 1-dehydro derivative of Compound S. The necessary transformations can be conducted according to Examples 24 and 25.

EXAMPLE 24

(A) 1,4 - Pregnadiene - 11α,17α,21 - triol-3,20 - dione (VIIa).

Following the procedure described in J. Am. Chem. Soc., *74*, 5933 (1952), there is added to 6 liters of a 24 hr. growth culture of *Rhizopus nigricans*, 1.0 g. of 1,4 - pregnadiene - 17α,21 - diol - 3,20 - dione (which may be prepared as described in Examples 3 and 6 hereinabove or by known chemical means) in 200 ml. of ethanol. After a 48 hr. transformation period, in the equipment and medium described in the aforementioned article, the methylene chloride extraction is performed and the crude crystalline solid residue is washed three times with 5 ml. portions of ice-cold chloroform. The yield of compound VIIa is 0.9 g. The product is recrystallized from acetone-hexane.

(B) 1-Dehydrocortisone (IIa)

To a solution of 0.4 g. of VIIa in 10 ml. of anhydrous pyridine is added 0.11 g. of acetic anhydride. The reaction mixture is allowed to stand at room temperature overnight and is then added to a mixture of 0.15 g. of chromium trioxide in 15 ml. of pyridine (the chromium trioxide-pyridine reagent is prepared according to Poos et al., J. Am. Chem. Soc., *75*, 422 (1953)). The resulting mixture is allowed to stand at room temperature overnight and is then poured into 5 volumes of water. The product is extracted with methylene chloride and the extracts are washed with water, dilute sulfuric acid and again with water. The methylene chloride solution is dried over magnesium sulfate and concentrated in vacuo. Crystallization of the 21-acetate of IIa is effected from acetone-hexane, resulting in 0.21 g. of crystalline solid (IIb).

To a solution of 0.12 g. of the 21-acetate of IIa in 3.3 ml. of chemically pure methanol is added 0.0315 g. of potassium bicarbonate in 0.5 ml. of water. The mixture is refluxed for 3 minutes and cooled rapidly. It is then diluted with water, neutralized with dilute hydrochloric acid, and extracted with methylene chloride. The extracts are dried and concentrated. The residue is crystallized from acetone-hexane, affording 0.051 g. of 1-dehydrocortisone (IIa).

EXAMPLE 25

(A) 1,4 - Pregnadiene - 11β,17α,21 - triol-3,20 - dione (IIIa).

1,4 - Pregnadiene - 17α,21 - diol - 3,20-dione (0.5 g). is treated with a culture of *Curvularia lunata* (QM 120H) which is prepared in the following way. *Curvularia lunata* is grown for two days in shake flasks at 28° on a malt-sucrose-salts medium. One hundred ml. of the resulting growth is used to inoculate 2000 ml. of the same medium contained in a fermenter equipped with a submerged aerator. The inoculated medium is agitated at 1700 r.p.m. and aerated at the rate of 0.5 volume of air per volume of medium per minute for 22 hours in a 28° water-bath. Fifty grams of damp mycelium, obtained by filtration of the resulting broth, is added to 2000 ml. of water and the aforementioned steroid in a similar fermenter. After the mycelium-steroid suspension is agitated in the previously described fashion for 22 hours, it is extracted with chloroform. The chloroform extracts are dried, concentrated to a residue, and chromatographed on "Florisil." The Florisil column is prepared with hexane, the steroid mixture is added in methylene chloride solution and elution is carried out with methylene chloride and methylene chloride-methanol mixtures. Unreacted starting material is eluted in 0.5% methanol in methylene chloride and the desired product IIIa is eluted in 1—2% methanol in methylene chloride. From 0.5 g. of the 1-dehydro compound S there is obtained 0.17

g. of IIIa as a crystalline solid, m.p. 237—239° C.

(B) 1-Dehydrocortisone (IIa).

Compound IIIa is converted into compound IIa by the same procedure as is employed for converting compound VIIa to compound IIa. From 0.400 g. of IIIa there is obtained 0.205 g. of IIa.

A chemical procedure for the introduction of the 1,2-double bond is described in the following Examples 26 and 27.

EXAMPLE 26

1-Dehydrocortisone (IIa)

A solution of 1 g. of allopregnane-17α,21-diol - 3,11,20 - trione 21 - acetetate in 15 ml. of acetic acid is brominated by the addition of 0.80 g. of bromine in 10 ml. of acetic acid. Addition of water precipitates the 2,4-dibromoallopregnane - 17α,21 - diol - 3,11,20-trione 21 - acetate.

Without purification, the dibromide (1.5 g.) is refluxed in 50 ml. of S-collidine for 1 hour. Chloroform and water are added, the organic extract washed with dilute sulfuric acid, then with water, and dried and evaporated. The crude product is chromatographed on Florisil to give 1,4 - pregnadiene - 17α,21-diol - 3,11,20 - trione - 21 - acetate (Ib, R= acetyl) m.p. 232—235° (dec.).

In similar fashion, by brominating pregnane - 17α,21 - diol - 3,11,20 - trione 21-acetate with 2 equivalents of bromine and dehydrobrominating by means of collidine, there is obtained the 21 - acetate of 1 - dehydrocortisone.

By bromination of allo-pregnane-11β,17α, 21 - triol - 3,20 - dione 21 - acetate at positions 2 and 4 by treatment with bromine followed by dehydrobromination with collidine, there is obtained the 21-acetate of 1-dehydrocortisol.

Similarly, dibromination of pregnane - 11β,17α,21 - triol - 3,20 - dione 21 - acetate, with 2 moles of bromine followed by dehydrobromination with collidine, yields the 21-acetate of 1-dehydrocortisol.

The allo-pregnane - 17α,21 - diol - 3,11,20-trione 21 - acetate employed in this modification of our process may be prepared by hydrogenating 2.00 g. of cortisone acetate in 200 ml. of ethyl-acetate with hydrogen at atmospheric pressure and room temperature, using 0.20 g. of 5% palladium on charcoal as catalyst. The reaction is allowed to proceed over night, the catalyst is then removed by filtration and the filtrate evaporated under reduced pressure. The allo-pregnane compound is recrystallized from acetone.

Similarly, the allo - pregnane - 11β,17α, 21 - triol - 3,20 - dione 21 - acetate can be obtained by hydrogenating 5.0 g. of Compound F 21-acetate in 750 ml. of ethyl acetate with hydrogen in the presence of 0.5 g. of 5% palladium on charcoal as catalyst.

The obtained 21-acetate of IIa and likewise that of IIIa can be hydrolysed by refluxing with potassium bicarbonate in aqueous methanol to give the free alcohol.

EXAMPLE 27

1,4 - Pregnadiene - 11α,17α,21 - triol - 3,20-dione 21 - acetate

A solution of 2.0 g. of pregnane - 11α,17α, 21 - triol - 3,20 - dione 21 - acetate in 25 ml. of acetic acid is brominated in positions 2 and 4 by the addition of 1.60 g. of bromine in 15 ml. of acetic acid. Crude 2,4 - dibromopregnane - 11α,17α,21 - triol - 3,20 - dione 21 - acetate is precipitated by the addition of water.

Dehydrobromination with 100 ml. of collidine for 1 hr. gives, after chromatography, 1,4 - pregnadiene - 11α,17α,21 - triol - 3,20-dione 21 - acetate (VIIb).

Hydrolysis with potassium bicarbonate in aqueous methanol for 10 minutes at reflux gives 1,4 - pregnadiene - 11α,17α,21 - triol-3,20 - dione (VIIa).

Oxidation of the acetate with CrO_3, N-bromoacetamide, or N - bromosuccinimide yields IIb.

Examples 28 to 32 illustrate methods for introducing the 1,2-double bond by chemical means.

EXAMPLE 28

A. 4 - Pregnene - 2,17α,21 - triol - 3,11,20-trione 2,21 - diacetate (IIh).

A solution of 2.0 g. of 6 - bromo - 4 - pregnene - 17α,21 - diol - 3,11,20 - trione 21-acetate in 100 ml. of glacial acetic acid is heated under reflux in a nitrogen atmosphere with 10 g. of anhydrous potassium acetate for four hours. The reaction mixture is then diluted with water and the products are extracted with ethyl acetate. The extracts are dried, concentrated in vacuo and the residue is taken up in a small volume of methylene chloride. The resulting solution is chromatographed on "Florosil" (magnesium silicate) and the solid material which is eluted with 50% ether/hexane is recrystallized several times from acetone-hexane, affording 4 - pregnene-2,17α,21 - triol - 3,11,20 - trione 2,21-diacetate, m.p. 210—215° C. $\lambda_{max}^{alcohol}$ 238 (ε=14,700).

B. 4 - Pregnene - 2,17α,21 - triol - 3,11,20-trione (IIIh).

To a refluxing solution of 1.0 g. of IIh in 100 ml. of chemically pure methanol, under a nitrogen atmosphere, is added 0.45 g. of potassium bicarbonate dissolved in 10 ml. of water. The mixture is refluxed 3—5 minutes and the pH is then adjusted to 6.8—7.0 by the addition of acetic acid. The resulting mixture is then concentrated in vacuo to a solid residue, the residue is leached

thoroughly with ethyl acetate and the extracts are concentrated. The residues are then crystallized from acetone-hexane, affording crystalline 4 - pregnene - 2,17α,21 - triol - 3,11, 20 - trione.

EXAMPLE 29

A. 6 - Bromo - 4 - pregnene - 11β, 17α,21-triol - 3,20 - dione 11 - formate 21-acetate (IVh).

To 160 ml. of chlorobenzene is added 1.6 g. of 4 - pregnene - 11β,17α,21 - triol - 3,20-dione 11 - formate 21 - acetate and the mixture is warmed until solution of the steroid is complete. Thereupon 180 ml. of dry carbon tetrachloride are added and the mixture is boiled to remove water. Then 4.2 ml. of a 10% solution of pyridine in carbon tetrachloride and 0.79 g. of N-bromsuccinimide are added, carbon dioxide is bubbled through the mixture, the mixture is illuminated by a 50-watt, frosted bulb placed in contact with the flask and the reaction is heated rapidly to boiling. After 10—20 minutes of heating the N-bromsuccinimide has reacted completely; the solution is cooled, washed with water and concentrated in vacuo. The residue may be crystallized from methylene chloride-hexane, yielding crystalline compound IVh.

B. 4 - Pregnene - 2,11β,17α,21 - tetrol - 3, 20 - dione 11-formate 2,21 - diacetate.

The reaction is conducted exactly as described in Example 28, except that IVh is the starting steroid. The above-named product is chromatographed over Florisil and the crystalline fractions (50% ether-hexane and 100% ether) are pooled and recrystallized from acetone-hexane.

C. 4 - Pregnene - 2,11β,17α,21 - tetra - 3, 20 - dione (VIh).

A solution of 0.5 g. of the tri-ester in 50 ml. of chemically pure methanol is treated with a solution of 0.165 g. of sodium hydroxide in 5 ml. of water under an argon atmosphere. The reaction mixture is allowed to stand at room temperature overnight and is then neutralized with acetic acid. The solution is concentrated in vacuo and the residue is extracted thoroughly with ethyl acetate. The extracts are concentrated and the residue is crystallized from acetone-hexane, giving crystalline 4 - pregnene - 2,11β,17α,21 - tetrol-3,20 - dione (VIh).

EXAMPLE 30

A. 1,2 - Oxidopregnane - 17α,21 - diol - 3, 11,20 - trione 21 - Acetate (IIi).

To a solution of 0.4 g. of 1 - pregnene-17α,21 - diol - 3,11,20 - trione 21 - acetate (Ii) in 100 ml. of chloroform at 5° C. is added 0.14 g. of perbenzoic acid in 25 ml. of chloroform. The resulting mixture is allowed to stand at 5° C. overnight and is then washed with dilute aqueous sodium bicarbonate. The chloroform solution is then dried and concentrated in vacuo. The residue is crystallized from acetone-hexane, affording crystalline compound IIi.

B. 2 - Bromopregnane - 1,17α,21 - triol - 3, 11,20 - trione 21 - Acetate (IIIi).

To a solution of 0.42 g. of IIi in 100 ml. of chloroform at 5° C. is added 0.08 g. of anhydrous hydrogen bromide dissolved in 100 ml. of chloroform. The mixture is allowed to stand at 5° C. for one hour and is then concentrated in vacuo. The residue is crystallized from methylene chloride-hexane, giving crystalline compound IIIi.

C. Pregnane - 1,17α,21 - triol - 3,11,20-trione 21 - Acetate (IVi).

A solution of 1 g. of IIIi in 100 ml. of methanol is shaken with 10 g. of 10% palladium-on-calcium carbonate catalyst in an atmosphere of hydrogen at atmospheric pressure. After one equivalent of hydrogen has been taken up (several hours' shaking), the catalyst is filtered from the solution and the filtrate is concentrated in vacuo to a solid residue (IVi).

D. Pregnane - 1,17α,21 - triol - 3,11,20-trione 1,21 - Diacetate (Vi).

The crude product IVi is dissolved in a mixture of 0.5 ml. of acetic anhydride and 5 ml. of pyridine. The reaction is allowed to proceed overnight and then the mixture is diluted with ice-water. The resulting precipitate is filtered and recrystallized from methylene chloride-hexane, yielding crystalline pregnane-1,17α,21 - triol - 3,11,20 - trione 1,21 - diacetate (Vi).

E. 4 - Bromopregnane - 1,17α,21 - triol - 3, 11,20 - trione 1,21 - Diacetate (VIi).

To a solution of 0.46 g. of Vi in 50 ml. of glacial acetic acid is added 0.5 ml. of 0.28 N hydrogen bromide in acetic acid. Then there is added dropwise, with good agitation, a solution containing 0.16 g. of bromine, 0.08 g. of sodium acetate and 15 ml. of glacial acetic acid at such a rate that each drop has the opportunity to react before another is added. When addition is complete the reaction mixture is diluted with five volumes of water and the precipitated compound VIi is collected by filtration.

F. 4 - Pregnene - 1,17α,21 - triol - 3,11,20 trione 1,21 - Diacetate (VIIi).

To a solution of 0.54 g. of VIi in 50 ml. of glacial acetic acid is added, under an atmosphere of carbon dioxide, a solution containing 0.25 g. of semi-carbazide hydrochloride, 180 mg. of anhydrous sodium acetate, 10 ml. of water and 10 ml. of glacial acetic acid. The mixture is agitated for ten minutes and there are then added 20 ml. of 1N sodium acetate in glacial acetic acid.

17α,21 - triol - 3,20 - dione 21 - acetate.

A solution of 1.0 g of the 9α-bromo compound in 100 ml. of methanol containing 2 g. of anhydrous potassium acetate is refluxed for one hour and then concentrated to a residue. The solid is leached thoroughly with methylene chloride and the extracts are washed well with water. After drying, the extracts are concentrated and crystallized from acetone-hexane, affording crystalline 9,11 - oxido - 1,4 - pregnadiene - 17α,21-diol - 3,20 - dione 21 - acetate.

A solution of 1.0 g. of the oxido compound in 50 ml. of alcohol-free chloroform is saturated with anhydrous hydrogen fluoride at 0°. After 5 hours at this temperature, the excess hydrogen fluoride and the solvent are removed *in vacuo* at 0° and the residue is crystallized from acetone-hexane to give crystalline 9α - fluoro - 1 - dehydrocortisol 21-acetate. The latter is then hydrolyzed in methanol-chloroform solution with aqueous hydrochloric acid at 20—25° C. over a period of about 48 hours to yield the free 21-ol (9α - fluoro - 1 - dehydrocortisol) which is crystallized from acetone-hexane.

The 21-acetate can be oxidized to the 21-acetate of 9α - fluoro - 1 - dehydrocortisone by adding to a solution of 162 mg. of the former in 10 ml. of glacial acetic acid, dropwise, a solution of 30 mg. of chromium trioxide in 0.5 ml. of water and 2 ml. of glacial acetic acid. After 6 hours, the green solution is diluted with 2 ml. of methanol and concentrated *in vacuo*. The residue is leached thoroughly with methylene chloride, and the extracts are dried over anhydrous magnesium sulfate. Concentration of the dry solution affords crystalline 9α-fluoro-1-dehydrocortisone 21 - acetate which can be readily hydrolyzed to the free 21-ol (Fig. 4 Z = F).

EXAMPLE 35

9α - Chloro - 1,4 - pregnadiene - 11β,17α,21-triol - 3,20 - dione (9α - Chloro - 1 - dehydrocortisol).

A solution of 1.0 g. of 9(11) - oxido - 1,4-pregnadiene - 17α,21 - diol - 3,20 - dione 21-acetate in 50 ml. of alcohol-free chloroform is saturated with anhydrous hydrogen chloride at 0°. After 5 hours at this temperature the excess hydrogen chloride and the solvent are removed *in vacuo* at 0° and the residue is crystallized from acetone-hexane to give crystalline 9α - chloro - 1 - dehydrocortisol 21-acetate. The latter is then hydrolyzed to give the free 21-ol compound.

By oxidizing the acetate in the manner described in the preceding example, there is obtained 21 - acetate of 9α - chloro - 1 - dehydrocortisone which can then be hydrolyzed to the free 21-ol.

The ester of Example 33 can be hydrolyzed by refluxing a mixture of 1.0 g. of the ester in 10 ml. of methanol under nitrogen and adding thereto 0.22 g. of sodium bicarbonate in 1 ml. of water. Refluxing is continued for ten minutes and then the reaction mixture is neutralized with acetic acid. The solvents are removed *in vacuo*, the residue is leached with methylene chloride and crystallization of the free 21-alcohol is induced by the addition of hexane to the concentrated methylene chloride solution. The following examples involve the preparation of long-acting esters of the 1,4-pregnadienes and indicate how these ester groups may be utilized to protect the 21-hydroxyl during the course of the synthesis of the pregnadienes.

EXAMPLE 36

1,4 - Pregnadiene - 11β,17α,21 - triol - 3,20-dione 21 - (5¹ - *t* - Butylfuroate - 2¹).

One gram of 1,4 - pregnadiene - 11β,17α,21 - triol - 3,20 dione is dissolved in 10 ml. of pyridine, chilled with stirring in an ice bath and treated with 0.6 g. of 5 - *t*-butylfuroylchloride - 2¹. After stirring overnight, the mixture is poured into dilute sulfuric acid and the solid extracted with ether. The crude product is crystallized from a benzenemethanol mixture to give the pure product which melts with decomposition at about 237—239° C.

EXAMPLE 37

A. Pregnane - 3α,17α,21 - triol - 11,20-dione 21 - (2¹,4¹ - Dichlorophenoxyacetate).

One hundred grams of pregnane - 3α,17α-diol - 11,20 - dione is dissolved in 1 liter of chloroform containing 1% ethanol, and the mixture is chilled to 10° C. and treated with 12 g. of gaseous hydrogen bromide. After being chilled to −20° C. and while holding below −10° C., the mixture is treated with a solution of 48 g. of bromine in 650 ml. of chloroform over a period of three hours. The reaction mixture is then concentrated to 250 ml. *in vacuo* below 25° C. To the solution are added 800 ml. of acetone followed by 200 g. of potassium 2,4 - dichlorophenoxy - acetate (obtained by vacuum evaporation of a methanol solution of 2,4-dichlorophenoxyacetic acid neutralized by potassium carbonate). The mixture is treated with 200 ml. of water, mixed well and refluxed 16 hours. The solvent is then removed by steam distillation after adding 500 ml. of water, and the mixture is heated 30 min. at 100° C. The product is collected by filtration and washed three times with hot water. This is crystallized from aqueous methanol to give pure pregnane - 3α,17α,21-triol - 11,20 - dione 21 - (2¹,4¹ - dichlorophenoxyacetate).

B. Pregnane - 17α,21 - diol - 3,11,20 - trione 21 - (2¹,4¹ - Dichlorophenoxyacetate).

The crude damp residue from Example 37A is dissolved in 1170 ml. of acetone and 100 ml. of water. The solution is chilled

Agitation is continued for 10 minutes longer, 2 ml. of pyruvic acid are added and the mixture is refluxed for ten minutes. The cooled solution is diluted with water and extracted with methylene chloride. The extracts are washed free of acid with water and the solution is dried over magnesium sulfate. Concentration of the dry solution and addition of hexane induces crystallization of VIIi.

Pregnane - 1,17α,21 - triol - 3,11,20 - trione 21 - acetate may be brominated and dehydrobrominated by the procedure described in Example 30 E and F, yielding crystalline 4-pregnene - 1,17α, 21 - triol - 3,11,20-trione 21 - acetate.

EXAMPLE 31

Conversion of 1 - pregnene - 11β,17α,21-triol - 3,20 - dione 21 - acetate into 4 - pregnene - 1,11β,17α,21 - tetrol - 3, 20 - dione 1,21 - diacetate.

By the same series of reactions disclosed in Example 30 A to 30 F, and under substantially the same conditions, 1 - pregnene-11β,17α,21 - triol - 3,20 - dione 21 - acetate is transformed by the same steps and by way of 1,2 - oxidopregnane - 11β,17α,21-triol - 3,20 - dione 21 - acetate, 2 - bromopregnane - 1,11β,17α,21 - tetrol - 3,20 - dione 21 - acetate, pregnane - 1,11β,17α,21 - tetrol-3,20 - dione 21 - acetate, pregnane - 1,11β, 17α,21 - tetrol - 3,20 - dione 1,21 - diacetate, and 4 - bromopregnane - 1,11β,17α,21 - tetrol-3,20 - dione 1,21 - diacetate into 4 - pregnene - 1,11β,17α,21 - tetrol - 3,20 - dione 1,21 - diacetate.

Pregnane - 11β,17α,21 - tetrol - 3,20 - dione 21 - acetate may be similarly brominated and dehydrobrominated by the procedures above described to give 4 - pregnene - 1,11β, 17α,21 - tetrol - 3,20 - dione 21 - acetate.

EXAMPLE 32

Elimination of the 1-Hydroxyl Group with Alumina

A. A solution of 0.1 g. of 4 - pregnene-1,11β,17α,21 - tetrol - 3,20 - dione 21 - acetate in 100 ml. of chloroform is passed over an activated alumina (30 g. of 100—200 mesh) column and the column is then washed with methanol. The combined eluates are concentrated and the residue, which crystallizes from acetone, is identical in all respects with 1,4 - pregnadiene - 11β,17α,21 - triol - 3,20-dione 21 - acetate (1 - dehydrocortisol 21-acetate).

B. A solution of 0.1 g. of 4 - pregnene-1,17α,21 - triol - 3,11,20 - trione 21 - acetate in 100 ml. of chloroform is similarly passed over an activated alumina (30 g. of 100—200 mesh) column and the column is then washed with methanol. The combined eluates are concentrated and the residue is crystallized from acetone. The product is 1,4 - pregnadiene - 17α,21 - diol - 3,11,20 - trione 21-acetate (1 - dehydrocortisone 21 - acetate).

Methods for the introduction of the 9α-halogeno group into 1,4 - pregnadiene - 2 - ol compounds are described in Examples 33 to 35.

EXAMPLE 33

1,4,9(11) - Pregnatriene - 17α,21 - diol - 3,20-dione 21 - acetate (IIj).

1,4 - Pregnadiene - 11β,17α,21 - triol - 3,20-dione (IIIa) is acetylated with acetic acid in pyridine, and to 1.22 g. of such ester dissolved in pyridine and cooled in ice is added 0.30 ml. of phosphorus oxychloride and the reaction mixture is allowed to stand at room temperature overnight. At the end of this time the solution is concentrated *in vacuo* at 25° C. to a volume of 5 ml., and 50 ml. of water are added. The oil which separates is taken up in ethyl acetate and the extracts are washed once with water, with 1N hydrochloric acid until free of pyridine, again with water, and then with 5% sodium bicarbonate solution. The ethyl acetate solution is then dried and concentrated *in vacuo*. The residue is triturated with ether, resulting in the crystallization of 1,4,9(11) - pregnatriene - 17α, 21 - diol - 3,20 - dione 21 - acetate.

EXAMPLE 34

9α - Fluoro - 1,4 - pregnadiene - 11β,17α,21-triol - 3,20 - dione (9α - Fluoro - 1 - dehydrocortisol).

1,4 - Pregnadiene - 11α,17α,21 - triol - 3,20-dione (VIIa) is 21-acetylated; to a solution of 1.0 g. of the acetate in 15 ml. of anhydrous pyridine at 0° is added 1.0 g. of *p*-toluene sulfonyl chloride. The reaction mixture is allowed to warm to room temperature and stand overnight. It is then diluted with ice water and the resulting precipitate of the 11α - *p*- toluene - sulfonate - 21 - acetate (Ij) is filtered from the solution.

A solution of 1.0 g. of Ij in 25 ml. of acetic acid containing 2.0 g. of sodium acetate is refluxed for forty-five minutes. The resulting solution is concentrated *in vacuo* and the residue is leached with methylene chloride. The methylene chloride extract is washed free of acetic acid with water and dried over anhydrous magnesium sulfate. The dry solution is concentrated and the residue is triturated with ether. The product (IIj) is crystallized from acetone-hexane.

To a suspension of 2.0 g. of finely-divided IIj in 200 ml. of purified dioxane and 20 ml. of water is added 1.0 g. of N-bromacetamide and 10 ml of 1 N aqueous perchloric acid. The mixture is agitated occasionally until the suspended solid is all in solution. After one hour, aqueous sodium sulfite is added to destroy the excess N-bromacetamide. The resulting solution is then extracted thoroughly with methylene chloride and the extracts are washed free of acid with water. After drying over magnesium sulfate, the solution is concentrated *in vacuo* and the residue is crystallized from acetone, affording crystalline 9α - bromo - 1,4 - pregnadiene-

to −5° C. and the pH is adjusted to 2.4 with 6N hydrochloric acid (0.2—1.0 ml.). In the dark 89.8 g. of N-bromosuccinimide are added and the mixture is held at −5° C. for one hour. To the red solution is added enough sodium sulfite solution (123 g. of sodium sulfite in 670 ml. of water) to bring the pH to 4.5—5.0 and the mixture is steam distilled until the temperature reaches 100° C. for one hour. The product is filtered off and washed three times with hot water and dried. The crude pregnane - 17α,21 - diol - 3,11,20-trione 21 - (2¹,4¹ - dichlorophenoxyacetate) is purified by crystallization from an acetone-methanol mixture.

C. 2,4 - Dibromopregnane - 17α,21 - diol-3,11,20 - trione 21 - (2¹,4¹ - Dichlorophenoxy acetate).

Ten grams of the product of Example 37B are dissolved in 250 ml. of glacial acetic acid, stirred, and treated dropwise with solution of 5.85 g. of bromine in 50 ml. of acetic acid as rapidly as the color is discharged (about thirty minutes). Addition of water precipitates the crude dibromide which is filtered off. Crystallization from acetone-methanol gives the pure 2,4 - dibromopregnane - 17α,21 - diol - 3,11,20 - trione 21-(2¹,4¹ - dichlorophenoxyacetate).

D. 1,4 - Pregnadiene - 17α,21 - diol - 3,11, 20 - trione 21 - (2¹,4¹ -Dichlorophenoxyacetate).

Five grams of the product of Example 37C are dissolved in 25 ml. of quinaldine and held at 95—100° C. for 16 hours. The mixture is then steam distilled to remove quinaldine and the ester is extracted with ether. The solution is washed with dilute sulfuric acid and water, dried, and evaporated to a residue. Chromatography from benzene on activated magnesium silicate gives the pure 1,4 - pregnadiene - 17α,21 - diol-3,11,20 - trione 21 - (2¹,4¹ - dichlorophenoxyacetate), which is eluted with benzene and has a rotation of about $(\alpha)_D + 116$ (dioxane).

EXAMPLE 38

A. Pregnane - 11β,17α,21 - triol - 3,20-dione 21 - (4¹ - Chlorophenoxyacetate).

In the same manner as in Example 36, two grams of pregnane - 11β,17α,21 - triol-3,20 - dione are treated with 1.40 g. of 4-chlorophenoxyacetyl chloride. The crude product is dissolved in methanol and concentrated to give crystalline pregnane-11β, 17α,21 - triol - 3,20 - dione 21 - (4¹ - chlorophenoxyacetate).

B. 1,4 - Pregnadiene - 11β,17α,21 - triol-3,20 - dione 21 - (4¹ - Chlorophenoxyacetate).

One gram of the product of Example 38A is brominated as in Example 37A with 0.62 g. of bromine in acetic acid. The crude water-precipitated dibromide is dried and dissolved in 10 ml of toluene and 5 ml. of 2,4,6-collodine. The mixture is refluxed for 16 hours and steam distilled to remove solvents. The crude product is worked up and chromatographed as in Example 37D, and the benzene eluate is crystallized from methanol to give 1,4 - pregnadiene - 11β,17α,21 - triol - 3,20-dione 21 - (4¹ - chlorophenoxyacetate), m.p. 184—186° C.

EXAMPLE 39

A. Allopregnane - 11β,17α,21 - triol - 3,20-dione 21 - (4¹ - Chlorophenoxyacetate).

Ten grams of 4 - pregnene - 11β,17α,21-triol - 3,20 - dione is esterified as in Example 36 with 7.0 g. of 4-chlorophenoxyacetyl chloride. The crude product is crystallized from 95% ethanol to give pure 4-pregnene - 11β,17α,21 - triol - 3,20 - dione 21 - (4¹ - chlorophenoxyacetate).

Five grams of this ester are suspended in 150 ml. of ethylacetate and 1 g. of 5% palladium on charcoal is added. The mixture is shaken with hydrogen until one equivalent (231 cc. at 25° C.) is absorbed and the uptake stops. The mixture is filtered and the solution is concentrated to 25 ml. The product which crystallizes out is filtered off and dried. It may be further crystallized from acetone to give allopregnane - 11β,17α, 21 - triol - 3,20 - dione 21 - (4¹ - chlorophenoxyacetate). This ester can also be prepared by hydrolysis of allopregnane - 11β, 17α,21 - triol - 3,20 - dione 21 - acetate and re-esterification. Ten grams of the 21-acetate are dissolved in 200 ml. of methanol and treated with a solution of 2.95 g. of potassium bicarbonate in 25 ml. of water. The mixture is stirred for 24 hours with nitrogen slowly bubbling through. Acetic acid is then added to neutrality, 50 ml. of water are added and the mixture concentrated *in vacuo* to 125 ml. The precipitate is collected, washed with water and crystallized from dilute methanol to give allopregnane - 11β,17α,21-triol - 3,20 - dione. Five grams of the latter are esterified as in Example 36 with 3.5 g. of 4-chlorophenoxyacetyl chloride. The product is crystallized from benzene-methanol to give allopregnane - 11β,17α,21 - triol - 3, 20 - dione 21 - (4¹ - chlorophenoxyacetate).

B. 1,4 - Pregnadiene - 11β,17α,21 - triol - 3, 20 - dione 21 - (4¹ - Chlorophenoxyacetate).

Two grams of the product of Example 39A are covered with 25 ml. of glacial acetic acid, stirred, and treated dropwise with 1.25 g. of bromine in 5 ml. of acetic acid. After the color disappears, water is added and the crude dibromide is filtered off and dried.

1 g. of the dibromide is suspended in 5 ml. of diethylaniline and warmed at 95—100° C. on the steam bath with stirring for six

hours. The mixture is then poured into dilute sulfuric acid and extracted with methylene chloride. The solution is dried and evaporated and the residue is crystallized three times from methanol-acetone to give 1,4-pregnadiene - 11β,17α,21 - triol - 3,20 - dione 21 - (4¹ - chlorophenoxyacetate), m.p. 184—186° C., the same product as in Example 38B.

EXAMPLE 40

9α - Fluoro - 1,4 - pregnadiene - 17α,21 - diol-3,11,20 - trione 21 - (2¹ - Furoate).

1.0 g. of 9α - fluoro - 1,4 - pregnadiene-17α,21 - diol - 3,11,20 - trione is dissolved in 20 ml. of dry pyridine and the resulting solution is cooled to 0° C. There is added dropwise to the stirred solution 0.45 g. of 2 - furoyl - chloride. The reaction mixture is allowed to warm slowly to room temperature and is stirred overnight. The mixture is then poured into 200 ml. of cold 10% aqueous sulfuric acid. The resulting precipitate is filtered and washed successively with water, aqueous sodium bircarbonate, and water. Recrystallization from methanol-water yields the 21 - (2¹ - furoate) ester as a crystalline substance.

In similar fashion, the 21 - (2¹ - furoate) ester of 9α - fluoro - 1,4 - pregnadiene - 11β, 17α,21 - triol - 3,20 - dione, and of the 9α-chloro analogues of both compounds can be obtained. By employing 5 - *t* - butyl - 2 - furoyl - chloride, phenoxyacetylchloride and 2,4,5 - trichlorophenoxyacetylchloride, 21-[(5¹ - *t*- butyl) - 2¹ - furoate], 21 - phenoxyacetate, and 21 - (2¹,4,¹5¹ - trichlorophenoxyacetate) of the 9α - fluoro and 9α - chloro derivatives of 1-dehydrocortisol and of 1-dehydrocortisone are produced.

By reacting the corresponding free 21-alcohol with the chloride of the acid, the following additional esters have been obtained:

1,4-pregnadiene - 21 - ol - 3,20 - dione 21-(5¹ - bromofuroate);

1,4-pregnadiene - 11β,17α,21 - triol - 3,20-dione 21 - furoate - 2¹, melting point 240—2° C. (with decomposition);

1,4-pregnadiene-11β,21 - diol - 3,20 - dione (4¹ - methylphenoxyacetate);

1,4-pregnadiene - 11β,17α,21 - triol - 3,20-dione 21 - (5¹ - chlorofuroate - 2¹);

1,4-pregnadiene - 11β,17α - 21 - triol - 3,20-dione 21 - (4¹- *t* - butylphenoxyacetate) melting point 203—6° C. (with decomposition);

1,4-pregnadiene - 21 - ol - 3,20 - dione 21-phenoxyacetate;

1,4-pregnadiene - 17α,21 - diol - 3,11,20-trione 21 - (4¹ - chlorophenoxyacetate), melting point 180—182° C.;

1,4-pregnadiene - 17α,21 - diol - 3,11,20-trione 21 - (4¹ - methoxyphenoxyacetate), melting point 130—135° C. (with loss of solvate);

1,4-pregnadiene - 17α,21 - diol - 3,11,20-trione 21 - (5¹ - bromofuroate - 2¹);

1,4-pregnadiene - 11β,17α,21 - triol - 3,20-dione 21-phenoxyacetate, melting point 196—199° C.;

1,4-pregnadiene - 11β,17α,21 - triol--3,20 - dione 21 - (4¹ - bromophenoxyacetate);

1,4-pregnadiene - 17α,21 - diol - 3,11,20-trione 21 - furoate - 2¹, in two forms, 233—235° C. and 251—254° C.;

1,4-pregnadiene - 17α,21 - diol - 3,11,20-trione 21 - (4¹ - *tert.* - butylphenoxyacetate), melting point 130—135° C. (with loss of solvate);

1,4-pregnadiene - 17α,21 - diol - 3,11,20-trione 21 - phenoxyacetate, melting point 205—207° C.; and

1,4-pregnadiene - 17α,21 - diol - 3,11,20-trione 21 - (5¹ - tert. - butylfuroate-2¹), melting point 241—243° C.

These esters are administered in the different ways described hereinabove.

As will be understood from the foregoing, the sequence of operations to produce a desired product from a given starting compound will be selected with due regard to the possible effect of interfering groups or configurations on subsequent steps of the process, and to the necessary presence or prior introduction of a group on which a particular step of the process operates.

WHAT WE CLAIM IS:—

1. Compounds of the pregnane series having therapeutic activity and possessing a double bond in the 4-position, which compounds are substituted by ketonic oxygen in the 3- and 20-positions, by a hydroxyl group or ketonic oxygen atom in the 11-position, by a hydroxyl or an esterified hydroxyl group in the 21-position and by a hydroxyl group in the 17α-position and which may be substituted by halogen at the 9α-position, which compounds are also characterised by the presence of a double bond in the 1-position.

2. 1-dehydrocortisone.

3. 1-dehydrocortisol.

4. 9α-fluoro-1-dehydrocortisone.

5. 9α-fluoro-1-dehydrocortisol.

6. A 21-ester of a compound as claimed in any one of claims 2 to 5.

7. A compound as claimed in claim 1, having an esterified hydroxyl group in the 21-position, or as claimed in claim 6, wherein the ester radical is organic.

8. A compound as claimed in claim 7, wherein the ester radical is that of a polybasic aliphatic or cyclo-aliphatic acid.

9. An alkali or alkaline earth metal salt of a partial ester as claimed in claim 8.

10. A compound as claimed in claim 7, wherein the ester radical is that of an unsubstituted or cycloalkyl-substituted lower alkanoyl acid, having not more than eight carbon atoms in the alkanoyl group.

11. A 21-ester as claimed in claim 7,

hours. The mixture is then poured into dilute sulfuric acid and extracted with methylene chloride. The solution is dried and evaporated and the residue is crystallized three times from methanol-acetone to give 1,4-pregnadiene - 11β,17α,21 - triol - 3,20 - dione 21 - (4¹ - chlorophenoxyacetate), m.p. 184—186° C., the same product as in Example 38B.

EXAMPLE 40

9α - Fluoro - 1,4 - pregnadiene - 17α,21 - diol-3,11,20 - trione 21 - (2¹ - Furoate).

1.0 g. of 9α - fluoro - 1,4 - pregnadiene-17α,21 - diol - 3,11,20 - trione is dissolved in 20 ml. of dry pyridine and the resulting solution is cooled to 0° C. There is added dropwise to the stirred solution 0.45 g. of 2 - furoyl - chloride. The reaction mixture is allowed to warm slowly to room temperature and is stirred overnight. The mixture is then poured into 200 ml. of cold 10% aqueous sulfuric acid. The resulting precipitate is filtered and washed successively with water, aqueous sodium bircarbonate, and water. Recrystallization from methanol-water yields the 21 - (2¹ - furoate) ester as a crystalline substance.

In similar fashion, the 21 - (2¹ - furoate) ester of 9α - fluoro - 1,4 - pregnadiene - 11β, 17α,21 - triol - 3,20 - dione, and of the 9α-chloro analogues of both compounds can be obtained. By employing 5 - *t* - butyl - 2 - furoyl - chloride, phenoxyacetylchloride and 2,4,5 - trichlorophenoxyacetylchloride, 21-[(5¹ - *t*- butyl) - 2¹ - furoate], 21 - phenoxyacetate, and 21 - (2¹,4,¹,5¹ - trichlorophenoxyacetate) of the 9α - fluoro and 9α - chloro derivatives of 1-dehydrocortisol and of 1-dehydrocortisone are produced.

By reacting the corresponding free 21-alcohol with the chloride of the acid, the following additional esters have been obtained:

1,4-pregnadiene - 21 - ol - 3,20 - dione 21-(5¹ - bromofuroate);

1,4-pregnadiene - 11β,17α,21 - triol - 3,20-dione 21 - furoate - 2¹, melting point 240—2° C. (with decomposition);

1,4-pregnadiene-11β,21 - diol - 3,20 - dione (4¹ - methylphenoxyacetate);

1,4-pregnadiene - 11β,17α,21 - triol - 3,20-dione 21 - (5¹ - chlorofuroate - 2¹);

1,4-pregnadiene - 11β,17α - 21 - triol - 3,20-dione 21 - (4¹- *t* - butylphenoxyacetate) melting point 203—6° C. (with decomposition);

1,4-pregnadiene - 21 - ol - 3,20 - dione 21-phenoxyacetate;

1,4-pregnadiene - 17α,21 - diol - 3,11,20-trione 21 - (4¹ - chlorophenoxyacetate), melting point 180—182° C.;

1,4-pregnadiene - 17α,21 - diol - 3,11,20-trione 21 - (4¹ - methoxyphenoxyacetate), melting point 130—135° C. (with loss of solvate);

1,4-pregnadiene - 17α,21 - diol - 3,11,20-trione 21 - (5¹ - bromofuroate - 2¹);

1,4-pregnadiene - 11β,17α,21 - triol - 3,20-dione 21-phenoxyacetate, melting point 196—199° C.;

1,4-pregnadiene - 11β,17α,21 - triol--3,20 - dione 21 - (4¹ - bromophenoxyacetate);

1,4-pregnadiene - 17α,21 - diol - 3,11,20-trione 21 - furoate - 2¹, in two forms, 233—235° C. and 251—254° C.;

1,4-pregnadiene - 17α,21 - diol - 3,11,20-trione 21 - (4¹ - *tert.* - butylphenoxyacetate), melting point 130—135° C. (with loss of solvate);

1,4-pregnadiene - 17α,21 - diol - 3,11,20-trione 21 - phenoxyacetate, melting point 205—207° C.; and

1,4-pregnadiene - 17α,21 - diol - 3,11,20-trione 21 - (5¹ - tert. - butylfuroate-2¹), melting point 241—243° C.

These esters are administered in the different ways described hereinabove.

As will be understood from the foregoing, the sequence of operations to produce a desired product from a given starting compound will be selected with due regard to the possible effect of interfering groups or configurations on subsequent steps of the process, and to the necessary presence or prior introduction of a group on which a particular step of the process operates.

WHAT WE CLAIM IS:—

1. Compounds of the pregnane series having therapeutic activity and possessing a double bond in the 4-position, which compounds are substituted by ketonic oxygen in the 3- and 20-positions, by a hydroxyl group or ketonic oxygen atom in the 11-position, by a hydroxyl or an esterified hydroxyl group in the 21-position and by a hydroxyl group in the 17α-position and which may be substituted by halogen at the 9α-position, which compounds are also characterised by the presence of a double bond in the 1-position.

2. 1-dehydrocortisone.

3. 1-dehydrocortisol.

4. 9α-fluoro-1-dehydrocortisone.

5. 9α-fluoro-1-dehydrocortisol.

6. A 21-ester of a compound as claimed in any one of claims 2 to 5.

7. A compound as claimed in claim 1, having an esterified hydroxyl group in the 21-position, or as claimed in claim 6, wherein the ester radical is organic.

8. A compound as claimed in claim 7, wherein the ester radical is that of a polybasic aliphatic or cyclo-aliphatic acid.

9. An alkali or alkaline earth metal salt of a partial ester as claimed in claim 8.

10. A compound as claimed in claim 7, wherein the ester radical is that of an unsubstituted or cycloalkyl-substituted lower alkanoyl acid, having not more than eight carbon atoms in the alkanoyl group.

11. A 21-ester as claimed in claim 7,

wherein the ester radical is acetic.

12. A 21-ester as claimed in claim 7, wherein the ester radical is trimethylacetic.

13. A 21-ester as claimed in claim 7, wherein the ester radical is *t*-butylacetic.

14. A 21-ester as claimed in claim 7, wherein the ester radical is a cyclopentyl- or cyclohexyl-substituted lower alkanoyl radical, the alkanoyl group having not more than eight carbon atoms.

15. A 21-ester as claimed in claim 7, wherein the ester radical is cyclopentylacetic.

16. A 21-ester as claimed in claim 7, wherein the ester radical is cyclopentylpropionic.

17. A 21-ester as claimed in claim 7, wherein the ester radical is cyclohexylpropionic.

18. A 21-ester as claimed in claim 7, wherein the ester radical is hemi-succinic.

19. A 21-ester as claimed in claim 7, wherein the ester radical is hemi-hexahydrophthalic.

20. A 21-ester as claimed in claim 7, wherein the ester radical is 2^1-furoic.

21. A 21-ester as claimed in claim 7, wherein the ester radical is 5^1-*t*-butyl-2^1-furoic.

22. A 21-ester as claimed in claim 7, wherein the ester radical is 5^1-chloro-2^1-furoic.

23. A 21-ester as claimed in claim 7, wherein the ester radical is 5^1-bromo-2^1-furoic.

24. A 21-ester as claimed in claim 7, wherein the ester radical is phenoxyacetic.

25. A 21-ester as claimed in claim 7, wherein the ester radical is 4^1-chlorophenoxyacetic.

26. A 21-ester as claimed in claim 7, wherein the ester radical is $2^1,4^1$-dichlorophenoxyacetic.

27. A 21-ester as claimed in claim 7, wherein the ester radical is $2^1,4^1,5^1$-trichlorophenoxyacetic.

28. A 21-ester as claimed in claim 7, wherein the ester radical is 4^1-bromophenoxyacetic.

29. A 21-ester as claimed in claim 7, wherein the ester radical is 4^1-*t*-butylphenoxyacetic.

30. A 21-ester as claimed in claim 7, wherein the ester radical is 4^1-methoxyphenoxyacetic.

31. 1-Dehydrocortisone 21-phosphate.

32. 1-Dehydrocortisol 21-phosphate.

33. 1-Dehydrocortisone 21-sulphate.

34. 1-Dehydrocortisol 21-sulphate.

35. A therapeutic composition comprising a compound as claimed in any one of the preceding claims, mixed with a pharmaceutical carrier.

36. A therapeutic composition as claimed in claim 35, in oral dosage unit form.

37. A therapeutic composition as claimed in claim 35, wherein the carrier is a cream base.

38. A therapeutic composition as claimed in claim 35, wherein the carrier is an aqueous suspending medium.

39. A therapeutic composition as claimed in claim 35, wherein the carrier is an ointment base.

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