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MANUFACTURE OF HYDROXY-KETO COMPOUNDS HAVING A CYCLOPENTANOPOLYHYDROPHENANTHRENE NUCLEUS

Rupert Oppenauer, Amsterdam, The Netherlands; vested in the Alien Property Custodian

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This invention relates to manufacture of hydroxy-keto compounds having a cyclopentanopolyhydrophenanthrene nucleus; and it comprises a process in which an organic compound, containing a cyclopentanopolyhydrophenanthrene nucleus and having at least one keto and one non-tertiary hydroxy group attached thereto, is subjected to the action of a metal alcoholate in the presence of an inert solvent, whereby isomerization and/or dismutation takes place; all as more fully hereinafter set forth and as claimed.

This application is a continuation-in-part of my copending application, Serial No. 150.758, filed June 28, 1937. In this prior application I have described and claimed the same generic invention as that which is disclosed in the present application but in the present application the disclosure has been amplified by the inclusion of an additional specific example to which the claims are particularly directed.

The raw materials employed in the production of the 3-keto-17-hydroxy compounds having a cyclopentanopolyhydrophenanthrene nucleus, are usually the isomeric 3-hydroxy-17-keto compounds. Thus testosterone, which is a 3-keto-17-hydroxy compound, is usually prepared from dehydroandrosterone, which is a 3-hydroxo-17-keto compound. The 3-keto-17-hydroxy compounds, such as testosterone and dihydrotestosterone for example, not only have a greater activity than the isomeric 3-hydroxy-17-keto compounds but in addition they often show another physiological action, for example on the seminal vesicle and prostata.

The prior art methods which have been developed for the production of the 3-keto-17-hydroxy compounds from the isomeric 3-hydroxy-17-keto compounds or from natural starting materials containing such compounds have had the disadvantages that they are complicated and that the reactions employed do not produce the desired compounds directly but only via intermediate products, such as the hydroxy-esters, as described by Ruzicka, Helv. Chim. Acta 18, 1478 (1935), for example.

I have found that the desired compounds can be produced in a very simple and direct way by means of an isomerization reaction in which the raw materials are treated with lower molecular metal alcoholates in the presence of inert solvents. By this treatment there are formed not only the lower molecular alcohols derived from the alcoholate employed but also the alcoholates of the 3-hydroxy-17-keto compounds, the latter having the property, in the presence of an inert solvent, of undergoing isomerization to form the desired 3-keto-17-hydroxy compounds with substantial yields which can then be recovered in the form of pure crystals. I have found that this isomerization reaction is applicable to all hydroxy-keto compounds having a cyclopentanopolyhydrophenanthrene nucleus in which it is theoretically possible for at least one of the hydroxyl groups to be replaced by or to exchange places with a keto group. This means that at least one of the hydroxyl groups must be non-tertiary. The hydroxyl and keto groups may be attached to one of the rings in the cyclopentanopolyhydrophenanthrene nucleus or to a side chain attached to said nucleus, for example in the 17 position. The more important compounds which are operative in my process are those containing at least one secondary hydroxyl group and in which the keto and hydroxyl groups are attached to different rings. By this method I have succeeded in converting the less active 3-hydroxy-17-keto compounds of the androsterone and dehydroandrosterone type into the more strongly active 3-keto-17-hydroxy compounds of the dihydrotestosterone and testosterone type, for example.

My process can be generally applied to the isomerization and dismutation of hydroxy-keto compounds of the types described and in which it is desired to convert these compounds into their isomeric hydroxy-keto compounds or into their polyalcoholic or polyketonic derivatives. My process is not limited to the use of the hydroxy-keto compounds in their pure state but crude or natural products, such as urine extracts, which contain hydroxy-keto compounds of the types defined are useful in my process. The reaction products and compounds obtained in this invention are useful as pharmaceuticals.

Various metal alcoholates can be used in the described process among which there can be mentioned sodium ethylate, aluminum isopropylate, chloro-magnesium-triphenylcarbinolate and tertiary aluminium butylate. The inert solvent employed should be a substance having no reactive hydroxyl or keto groups. Gasoline, hexane, benzene and cyclohexane can be mentioned as examples.

The reaction of this invention may be effected with such a small quantity of metal alcoholate that the reducing action produced by the alcoholate is negligible. Primary and secondary alcoholates can be employed but the tertiary alcoholates are more advantageous. In some cases, particularly when primary or secondary alco-

holates are used, I advantageously remove the low molecular alcohol by evaporation, for example in vacuo, at the beginning of the reaction. During the reaction a certain quantity of diol and dione derivatives is formed and it is possible, if desired, to adapt my reaction to the production of these particular compounds. The hydroxy-ketone fraction can be easily separated from the diol-dione fraction by known methods. Either of these fractions can be treated again with a metal alcoholate, preferably a tertiary alcoholate, in accordance with my invention in order to produce an increased yield of the other fraction. Before effecting this re-treatment, one of the components of the fraction to be re-treated may be separated. For example, it is possible to separate testosterone from the hydroxy-ketone fraction by known methods, before re-treatment of the residue. When re-treating the diol-dione fraction, it is advantageous to have present substantially equal numbers of hydroxyl and keto groups.

Which of the two fractions should be re-treated depends, of course, upon whether it is desired to recover one of the hydroxy-ketone compounds, which are formed in the process, or crystallized diols and diones. In the treatment of dehydroandrosterone by my process, for example, it is possible to recover either testosterone or androstendiol and androstendione, the latter compounds being recovered from the diol-dione fraction and the former from the hydroxy-ketone fraction. If it is desired to obtain a hydroxy-ketone compound, the diol-dione fraction may be subjected to a treatment, this fraction usually being mixed with the unconverted hydroxy-ketone which is first separated from the hydroxy-ketone fraction. On the other hand if it is desired to recover diol and dione compounds, the hydroxy-ketone fraction should be subjected to re-treatment. Since substantially no by-products are formed in my reaction, it is possible by successive re-treatments to increase the yield of the desired compound or compounds to approximately the theoretical yield. It is also possible, of course, to obtain by my method various mixtures of compounds having a certain physiological action, for example a greater or smaller action on the growth of the capon's comb with a correspondingly smaller or greater action on the seminal vesicle, this result being possible by controlling the conditions under which the reaction is effected, such as temperature, concentration of solvent and time of reaction, or by choice of the fractions to be re-treated or by mixing the fractions to be re-treated in various proportions.

My invention can be described in somewhat greater detail by reference to the following specific examples which represent illustrative embodiments thereof.

Example 1

I refluxed 20 g. of dehydroandrosterone with 25 g. of tertiary aluminum butylate in 500 cc. of anhydrous benzene for a period of 14 hours. The resulting yellowish solution was shaken with dilute sulfuric acid for a short time to remove the aluminum. The benzene solution was then washed with water, dried with sodium sulfate and subjected to evaporation. A nearly colorless syrup, fraction I, was obtained which was found to have an activity in the capon's comb test which was 2 to 3 times that of the starting material. This syrup was then rubbed with 250 cc. of ether and the resulting mixture was allowed to stand for three days during which it was found that crystals (II) separated, which were found to consist of substantially pure delta-5,6-androstendiol. These crystals were filtered off and, after the etheric solution was evaporated, a syrup (III) remained. This syrup was then treated with a ketone reagent in order to separate the diols (IV). As ketone reagents it is possible to use semicarbazide, hydroxylamine, beta-ine-hydrazine-hydrochloride etc. The ketone condensation products were then split up and the syrup (V) thus formed eontained the hydroxy-ketones and the androstendione. This syrup was separated by methods known per se into a hydroxy-ketone fraction (VI) and into androstendione by treatment with phthalic anhydride. Chloro-sulfonic acid in pyridine can also be used in this separation.

The hydroxy-ketone fraction (VI) was then treated with a 1 per cent alcoholic digitonine solution, forming a digitonide precipitate which was filtered off and split up by boiling in xylene to obtain a syrupy fraction (VII), which was found to consist mainly of dehydroandrosterone. The filtrate from the digitonine precipitation (VIII) was allowed to stand until crystals separated, these consisting mainly of testosterone. These crystals can be recovered and purified by recrystallization with a yield of 1.5 to 2.2 grams.

If desired, the process described above can be interrupted at any of the intermediate stages indicated. For example, the fractions (I), (III), (V), (VI) and (VIII) are useful for pharmaceutical purposes without further purification. If it should be desired to obtain fraction (VI) in a large yield, the fractions (II) and (IV) and the androstendione from (V) may be united and again subjected to the reaction or added to a subsequent charge of dehydroandrosterone used in a repetition of the process. If it is desired to obtain crystallized testosterone in high yield, this can be accomplished by uniting all fractions obtained prior to the separation of fraction (VIII), as well as the mother liquor from (VIII), this mixture being subjected to a new isomerization treatment or being added to a subsequent charge in a repetition of the process.

Example 2

In this example 10 grams of an extract from urine was used, which was prepared aceording to the method described by Funk and Harrow (Proc. Amer. Soc. Biol, Chem., 7, LXX, 1931) and which had an activity of 1 international capon's comb unit per mg. This extract was refluxed with 250 g. of tertiary aluminum amylate in 1.5 liters of anhydrous toluene. After the reaction was completed, the aluminum was removed by shaking with dilute sulfuric acid and the toluene solution was washed with water. The resulting solution may be used as such for pharmaceutical purposes. It was found to show an activity about 3 to 5 times that of the starting material in the capon's comb test. Moreover, in contrast to the starting material, it was found to have a strong action on the primary sexual organs. Thus, in the seminal vesicle test on rats, it was found that 0.3 mg. corresponded to 1 unit. The yield in this reaction was 100 per cent, since the material had not changed in weight during the treatment. Urine extracts obtained by the method described by Butenandt and Tscherning (Z. Physiol. Chem. 167, 229, 1934) can be substituted for that used in the above described reaction.

Example 3

I refluxed a mixture of 24 g. of delta 5-pregnenol-3-one-20 with 600 cc. of toluene and 60 g. of tertiary aluminum butylate for a period of 24 hours. The resulting yellow reaction products were thoroughly shaken with dilute sulfuric acid and then washed with water, the toluene being then removed by evaporation in vacuum. The residue was heated with a mixture of 180 cc. of pyridine and 50 g. of phthalic anhydride on the steam bath for a period of 3 hours, then poured into 2 liters of N/2 NaOH. The reaction mixture was then extracted with ether. The ether solution was thoroughly washed with water, dried with sodium sulfate and the ether was removed by evaporation. Crude progesterone remained behind, which was crystallized from methanol. A yield of 4.3 g. was obtained, having a melting point of 129–130° C.

The aqueous layer obtained as described above was refluxed in the absence of oxygen for a period of 4 hours. After the liquid was cooled, it was extracted with ether. The ether solution was washed with water and the ether removed by evaporation. During this evaporation crystals separated out consisting of delta 5-pregnanediol-3,20. The remaining solution, containing approximately 150 cc. of ether was allowed to stand for 24 hours. The crystals formed in the solution were filtered off and the mother liquor was concentrated by evaporation. The residue obtained was heated in 150 cc. of glacial acetic acid with 25 g. of Girard-reagent-T on the steam bath for 20 minutes. The solution was then separated by adding ether and an aqueous alkaline solution into an etheric ketone-free fraction and an aqueous ketonic fraction (see Girard, Sandulesco. Helv. Chim. Acta 19, 1095, 1936). The ketone-free fraction consisted for the main part of delta 5-pregnenediol-3,20. The total yield of this substance together with the crystals already recovered amounted to 4.8 g. The aqueous fraction was found to contain the hydroxy-ketones in which unchanged delta 5-pregnenol-3-one-20 was present. The latter was separated by precipitation with digitonine in 90 per cent alcohol. The clear solution was diluted with water, extracted with ether and the etheric layer was subjected to evaporation. The residue was subjected to sublimation in high vacuo (140° C. and 0.01 mm. mercury pressure). Upon the addition of acetone it was found that crystals separated after a short time, which crystals were found to melt at 161–162° C. These crystals were found to be identical to the delta 4-pregnenol-20-one-3 obtained by Butenandt and Schmidt (Ber. 67, 1901, 1934) by means of a different process.

If it is desired to prepare progesterone, the whole hydroxy-ketone fraction can be added to the following charge of starting material, preferably after a preliminary distillation in a high vacuum.

Example 4

I refluxed 500 mg of delta 5-pregnene-diol-3,21-one-20 with 500 mg. of tertiary aluminum butylate and 20 cc. of benzene for several hours in an oil bath. Ether was then added and dilute sulfuric acid was added to remove the aluminum. The etheric solution was washed with water, dried and then evaporated.

By treatment with digitonine in alcohol of about 80 per cent concentration, approximately half the material was found to be precipitated as the digitonide. The compound precipitated by the digitonine was regenerated but was found to be inactive. The unprecipitated fraction was freed from the excess digitonine and then acetylated at room temperature by treatment with acetic acid anhydride in pyridine. The reaction products were poured into water and dissolved in ether, the pyridine being removed by repeated washing of the etheric solution. The solution was then dried and subjected to evaporation. It was found that 1–2 mg. of the residue, when dissolved in oil, gave a strong cortine activity in the Everse-de-Fremery test while the starting material was substantially inactive in this test. The active component in this product is believed to be the 21-acetate of delta 4-pregnene-dione-3,20-ol-21.

In the above reaction it is evident that theoretically no less than 8 different compounds are formed.

While I have described what I consider to be the most advantageous embodiments of my process, it is evident that the specific procedures disclosed can be varied widely without departing from the purview of this invention.

RUPERT OPPENAUER.