

ALIEN PROPERTY CUSTODIAN

PRODUCTION OF MALE SEXUAL HORMONE

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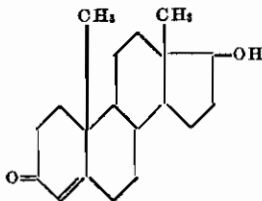
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This invention relates to a male sex hormone in a crystalline state, called testosterone, capable of affecting growth of the comb of capons and of certain reproductive organs of mammals.

It has particular reference to the pure crystalline product which can be obtained by a process in which organs containing testosterone are extracted and which extracts are purified, this being a division of application No. 122,324.

A process for the preparation of the crystalline substance shall now be described.

Organs containing testosterone, for instance testicles, are extracted with organic solvents being miscible with water. Preferably acetone is used in such a quantity that during the extraction a concentration of the acetone of 60% is maintained. Thereupon the organic solvent of the extract is removed, for instance by distillation and the remaining liquid is repeatedly extracted with a solvent immiscible with water, preferably benzene is used. A distribution of the extract between the two layers of a solvent mixture forming two phases may be inserted, for instance between 70% alcohol and petroleum ether. In case the solvent wherein the crude testosterone is dissolved is not indifferent to sulphuric acid it has to be replaced by such a solvent, for instance benzene. This solution is repeatedly extracted with 55%-75% sulphuric acid. The sulphuric acid layers are separated, diluted with water and extracted with an organic solvent. After evaporation of the solvent the residue is distilled in a high vacuum, whereby the fraction distilling between 110°-130° C is separated. This fraction is recrystallized from organic solvents, preferably hexane and acetone until a constant melting point of 154.5°C (corr) is reached. This material consists of needles having the formula $C_{19}H_{28}O_2$. It has a specific rotation $(\alpha)_D^{20} = +109^\circ$ (C=1 in abs. alcohol). Its maximum of absorption of ultraviolet light lies between 2.400 A. Its structure is represented by the following formula



It forms an acetate having a melting point of 140-141°C (corr) and an oxime having a melting point of 222 - 223°C (corr). About 15% of this material contain 1 capon unit, i. e. the effect of 100% androsterone.

A method for the preparation of the new crystalline hormone, called testosterone, will now be described:

Example 1.—235 kgs testicles of bulls are extracted according to Gallagher and Koch (J. Biol. Chem. 84, 495, 1929) with alcohol, the alcohol is evaporated, the residing solution is extracted with benzene, the benzene is evaporated and the residue extracted with acetone, the acetone solution is frozen, the precipitate is removed and after evaporation of the acetone the residue is distributed between a mixture of 70% alcohol and petroleum ether. From the alcohol layer which contains the main part of the testosterone, the solvent is evaporated, whereby 283 gs residue remain. The residue is dissolved in 3,6 Lts benzene and twice shaken with 600 ccm 60% sulphuric acid and once with 600 ccm 70% sulphuric acid. The separated sulphuric acid layers are united and poured into 3,6 Lts icewater and four times extracted with 800 ccm ether. The ether solution is washed with 100 ccm 10% KOH and thereupon with distilled water until it has become neutral. It is dried with some sodium sulfate. After evaporation of the ether 345 mgs (=1,47 mg per kg testicles) residue remain, forming a viscous yellow-brown oil. The estimation of the physiological activity of this fraction shows that it contains 1 capon unit per 50 γ , that means that 30 capon units per kg testicles are obtained. This oil is submitted to a fractionating high vacuum distillation at a pressure less than 0,001 mm whereupon the fraction distilling between 110-130°C is three times boiled with 20 ccm hexane. The united hexane solutions are concentrated by evaporation to a third of their volumes and kept at about 0°C. After several hours a yellow-white granular material separates, which is filtered off, washed with cold hexane and recrystallized several times from diluted acetone until white prismatic needles [M.P. 154-154,5°C [corr]] are obtained. Per kilogramme testicles 10 capon units are gained as crystals (See page 3, 1. 13/14).

Example 2.—250 kgs testicles of bulls are extracted with 250 Lts of 98% acetone. The liquid is separated and the residue is extracted again, this time with 250 Lts of 60% acetone. From the united extracts the acetone is evaporated and the remaining solution is extracted with 100 Lts benzene. The benzene solution, which contains 280 gs of solid matter, is submitted to the process described in Example 1 beginning with the treatment with sulphuric acid.

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