and the Board of Research of the University of California.

Summary

A modified procedure for obtaining molecular extinction coefficients has been described.

Working with the vapor phase, the ultraviolet absorption spectra of crotonaldehyde and acrolein have been determined quantitatively.

Experiments designed to test for the presence of *cis*-crotonaldehyde in the commercial product gave results which indicate that this form of the aldehyde was not present.

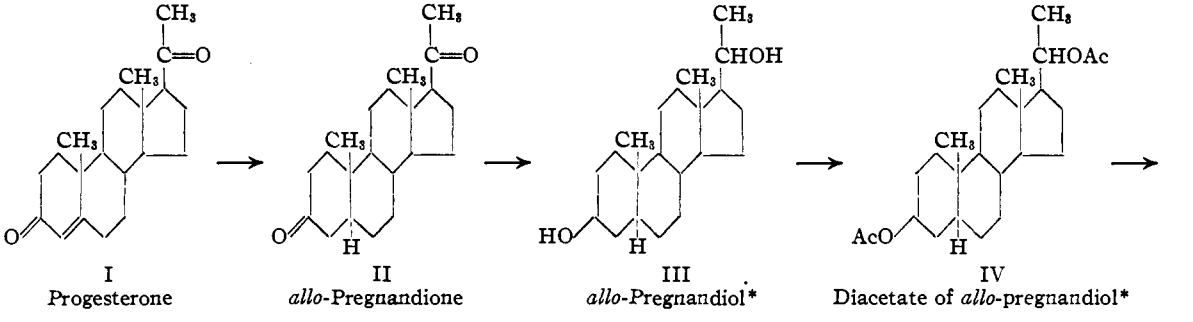
LOS ANGELES, CALIF. RECEIVED SEPTEMBER 30, 1936

[CONTRIBUTION FROM THE SCHOOL OF CHEMISTRY AND PHYSICS OF THE PENNSYLVANIA STATE COLLEGE AND THE PARKE, DAVIS & CO. RESEARCH LABORATORIES]

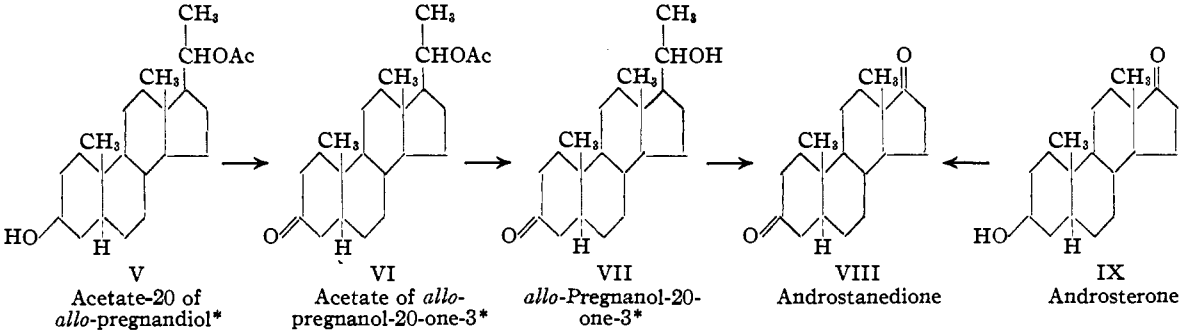
Sterols. VIII. Preparation of Androstanedione from *allo*-Pregnandiol

BY RUSSELL E. MARKER, OLIVER KAMM, DAVID M. JONES AND THOMAS S. OAKWOOD

The present paper serves to establish a complete structural relationship between the female sex hormone, progesterone (I) and the male sex hormone, androsterone (IX). This has been accomplished by supplementing the known preparation[1] of androstanedione (VIII) by oxidation of androsterone, with a new method of preparation of the same compound starting with *allo*-pregnandione (II). The last mentioned compound has been described previously by Butenandt,[2] who obtained it by reducing progesterone to a mixture of pregnandiol and *allo*-pregnandiol, oxidizing the mixture, and separating from it pregnandione and *allo*-pregnandione. The reactions involved in our work are illustrated by formulas II–VIII.



I Progesterone — II *allo*-Pregnandione — III *allo*-Pregnandiol* — IV Diacetate of *allo*-pregnandiol*



V Acetate-20 of *allo*-pregnandiol* — VI Acetate of *allo*-pregnanol-20-one-3* — VII *allo*-Pregnanol-20-one-3* — VIII Androstanedione — IX Androsterone

* The designation *trans* has been omitted at the suggestion of the referee. It seems to us, however, that such a designation is needed to differentiate our new compounds from previously known isomers.

allo-Pregnandione (II) was obtained by oxidation with chromic anhydride of the mixture of pregnandiol and *allo*-pregnandiol obtained from concentrates of human pregnancy urine. Crystallization from acetone gave pure *allo*-pregnandione. The latter was reduced in acetic acid at room temperature with platinum oxide and hydrogen to *allo*-pregnandiol (III). This gave a digitonide readily. The diol was converted to the diacetate (IV). The acetyl group in the 3-

(1) Butenandt and Tscherning, *Z. physiol. Chem.*, **229**, 189 (1934).
(2) Butenandt and Fleischer, *Ber.*, **68**, 2094 (1935).

position was then hydrolyzed with potassium hydroxide in methyl alcohol solution at 15–20°, giving the 3-hydroxy-20-acetoxy compound (V). The 3-hydroxy group was then oxidized to a keto group to give Compound VI. Hydrolysis of the acetyl group at position 20 resulted in *allo*-pregnanol-20-one-3 (VII).

The conversion of the ol-one (VII) to androstanedione was carried out without isolation of the intermediate compounds. The ol-one was dehydrated by heating in acetic acid with anhydrous zinc chloride. The product was freed of acetic acid and the chloride was dissolved in chloroform and ozonized. The ozonide was decomposed by heating with acetic acid. The neutral fraction yielded two products, one a high-melting material of undetermined constitution, and the other androstanedione melting at 126-128°. This was found to be identical with androstanedione obtained by the oxidation of androsterone.

Experimental

***allo*-Pregnandione.**—A solution of 50 g. of pregnandiol-*allo*-pregnandiol mixture in 3500 cc. of glacial acetic acid was cooled to 10° and treated with a solution of 50 g. of chromic anhydride in 600 cc. of 90% acetic acid, added over a period of four hours. The resulting mixture was allowed to stand overnight at room temperature, when 100 cc. of methyl alcohol was added and the acetic acid removed under reduced pressure. The residue was extracted with ether and the ether solution washed successively with dilute hydrochloric acid, water, 10% sodium bicarbonate solution, and finally again with water. The ether was then evaporated, giving 45 g. of oily white solid. This was dissolved in 190 cc. of boiling acetone, the solution cooled to 0°, and allowed to stand for twelve hours. The precipitate of *allo*-pregnandione was filtered off, washed with a small amount of acetone, and recrystallized from acetone. Its melting point was found to be 199–200°. The mother liquors contained the isomeric pregnandione.

Anal. Calcd. for $C_{21}H_{32}O_2$: C, 79.7; H, 10.2. Found: C, 79.4; H, 10.3.

***allo*-Pregnandiol.**—To a solution of 3 g. of *allo*-pregnandione in 100 cc. of glacial acetic acid was added 2 g. of platinum oxide. The mixture was shaken with hydrogen at 45 pounds (3 atm.) pressure during seventy-five minutes, the catalyst filtered off, and the acetic acid concentrated under reduced pressure. The residue was crystallized from ethyl alcohol. Its melting point was 195–196°.

Anal. Calcd. for $C_{21}H_{36}O_2$: C, 78.7; H, 11.3. Found: C, 78.8; H, 11.4.

***allo*-Pregnandiol Diacetate.**—A solution of 300 mg. of *allo*-pregnandiol in 2 cc. of acetic anhydride was refluxed during two hours. The reaction product which solidified on cooling was taken up with water and filtered. After crystallization first from ethyl alcohol and then from ethyl acetate it melted at 142–143°.

Anal. Calcd. for $C_{25}H_{40}O_4$: C, 74.2; H, 10.0. Found: C, 74.2; H, 10.1.

***allo*-Pregnandiol Monoacetate.**—To a solution of 1.55 g. of the diacetate in 375 cc. of methyl alcohol was added 28.3 cc. of a methyl alcohol–potassium hydroxide solution containing 0.0062 g. of potassium hydroxide per cc. The mixture was kept at 15–20° for forty-two hours. It was then neutralized with dilute hydrochloric acid and evaporated to a small volume. Water was added and the product filtered. After crystallization from petroleum ether it melted at 170–171°.

Anal. Calcd. for $C_{23}H_{38}O_3$: C, 76.2; H, 10.6. Found: C, 76.3; H, 10.7.

Acetate of *allo*-Pregnanol-20-one-3.—To a solution of 360 mg. of the monoacetate of *allo*-pregnandiol in 25 cc. of acetic acid was added 100 mg. of chromic anhydride in acetic acid. The solution was kept at room temperature for sixteen hours after which methyl alcohol was added and the acetic acid removed under reduced pressure. Water was added and the product filtered off. After crystallization from ethyl alcohol and finally from petroleum ether it melted at 156°.

Anal. Calcd. for $C_{23}H_{36}O_3$: C, 76.6; H, 10.1. Found: C, 76.5; H, 10.1.

***allo*-Pregnanol-20-one-3.**—A solution of 180 mg. of the acetate of *allo*-pregnanol-20-one-3 in ethyl alcohol was treated with an excess of potassium hydroxide solution and refluxed during two hours. The solution was neutralized with hydrochloric acid and concentrated under reduced pressure. Water was added and the solid filtered off and crystallized from dilute ethyl alcohol when it showed a melting point of 195°.

Anal. Calcd. for $C_{21}H_{34}O_2$: C, 79.2; H, 10.7. Found: C, 79.4; H, 10.8.

Androstanedione.—To a solution of 500 mg. of *allo*-pregnanol-20-one-3 in 8 cc. of glacial acetic acid was added 500 mg. of freshly fused zinc chloride. The solution was refluxed for three hours, cooled, and diluted with water. A semi-solid precipitate separated which after standing overnight was filtered off. It was dissolved in 100 cc. of chloroform and ozone was passed into the solution during thirty minutes at 0° at the rate of 7 liters of ozone per hour. The chloroform was evaporated at 25° and the residue taken up in 100 cc. of glacial acetic acid and heated on a steam-bath during one hour. The solution was then cooled to room temperature and 100 mg. of chromic anhydride added. After thirty minutes, methyl alcohol was added, the solvents evaporated at 40° and the residue dissolved in 50 cc. of water and 50 cc. of ether. The ether solution was separated, washed with 5% sodium hydroxide to free it of organic acids, and evaporated to dryness. The residue upon sublimation in a high vacuum yielded two fractions. The higher fraction gave a material melting at 185°. The lower fraction was resublimed at 110°, and then crystallized from acetone. The yield was 40 mg. of a product melting at 128°.

Anal. Calcd. for $C_{19}H_{28}O_2$: C, 79.1; H, 9.8. Found: C, 78.4; H, 9.9.

Oxidation of Androsterone.—To a solution of 335 mg. of androsterone in 120 cc. of acetic acid was added 116 mg. of

chromic oxide. This was kept at 15–20° for thirteen hours and then at 25–30° for eight hours. Methyl alcohol was added and the solvents evaporated under reduced pressure. Water was added and the precipitate crystallized from acetone. It melted at 132°.

Anal. Calcd. for $C_{19}H_{28}O_2$: C, 79.1; H, 9.8. Found: C, 79.3; H, 9.7.

A mixture of the two samples of androstandione gave a melting point of 128–129°.

Summary

allo-Pregnandiol, a reduction product of progesterone, was converted into androstanedione, an oxidation product of androsterone, thus establishing a complete structural relationship between the female sex hormone, progesterone, and the male sex hormone, androsterone.

STATE COLLEGE, PA. DETROIT, MICH. RECEIVED OCTOBER 10, 1936

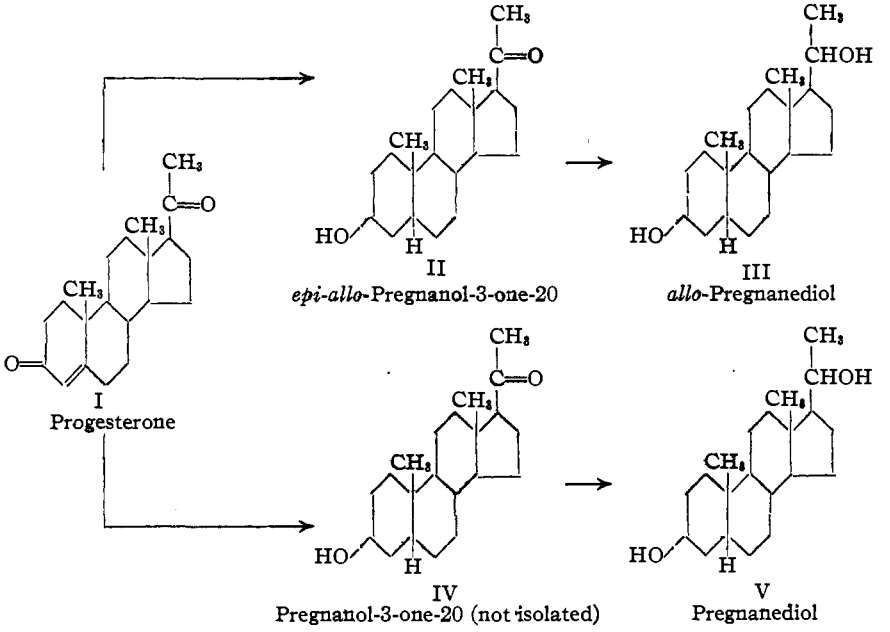
[CONTRIBUTION FROM THE SCHOOL OF CHEMISTRY AND PHYSICS OF THE PENNSYLVANIA STATE COLLEGE, AND THE PARKE, DAVIS & COMPANY RESEARCH LABORATORIES]

Sterols. IX. Isolation of *epi*-Pregnanol-3-one-20 from Human Pregnancy Urine

BY RUSSELL E. MARKER, OLIVER KAMM AND RALPH V. MCGREW

A reinvestigation of the sterol derivatives contained in human pregnancy urine fails to show the presence of progesterone. Since our experiments were conducted on a large scale using the sterol fraction from 10,000 gallons of human pregnancy urine, it seems reasonable to conclude that the corpus luteum hormone is not excreted as such by the human subject during pregnancy. Whether or not the *normal* female excretes the hormone is the subject of an investigation now in progress.

It is known that the neutral carbinol fraction from human pregnancy urine consists mainly of a mixture of pregnanediol and *allo*-pregnanediol, which differ only in the configuration of rings A and B of the sterol nucleus. We have now found, however, that sterols which may be considered as reduction products intermediate between progesterone and the pregnanediols *are* eliminated. In this article we describe the isolation of a compound of that type, namely, a pregnanolone.



Butenandt[1] in the isolation of progesterone from hog corpora lutea obtained *allo*-pregnanol-3-one-20 melting at *195°* which he prepared later by the degradation of stigmasterol. Since stigmasterol possesses the normal arrangement (of cholesterol) in respect to the hydroxyl group and since his method of synthesis did not produce an inversion of this group, the naturally occurring allopregnanolone also has the hydroxyl group in the normal arrangement.

The epimeric form of *allo*-pregnanol-3-one-20 has been obtained from the residues of human pregnancy urine after removal of the theelin, theelol and other known sterol derivatives. It is present in quantities of 1-2 mg. per gallon of urine. This product does not absorb bromine

(1) Butenandt and co-workers, *Ber.*, **67**, 1441, 1897 (1934).