

investigation, it is necessary to defer publication of the details of the work until a later date.

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**STEROLS. XII. SYNTHETIC PREPARATION OF
EPI-ALLO-PREGNANOLONE, THE ANDROGENIC
PRINCIPLE OF HUMAN PREGNANCY URINE**

Sir:

That human pregnancy urine contains a substance which has male hormone activity has been reported by several workers. Because of the similarity in physiological activities in the male hormone tests between human pregnancy and male urine extracts, it has been assumed that the male principle in pregnancy urine was androsterone. Heretofore this androgenic principle has not been isolated.

Recently, Marker, Kamm and McGrew [THIS JOURNAL, **59**, 616 (1937)] reported the isolation and identification of *epi*-allo-pregnanolone from the sterol fraction of human pregnancy urine after the removal of theelin and theelol. At the time of this report we did not have the physiological assays on this compound. Since then it has been found that the substance isolated from human pregnancy urine promotes the growth of the seminal vesicle in the rat tests. Preliminary tests have shown this to have about the same activity as androsterone.

We have now prepared synthetically *epi*-allo-pregnanolone from 3-chloro-*allo*-cholanolic acid, a by-product from the preparation of chloroandrosterone [Marker, Whitmore and Kamm, THIS JOURNAL **57**, 2358 (1935)]. This was prepared by the stepwise degradation of the side-chain according to the method of Wieland [*Z. physiol. Chem.*, **161**, 80 (1926)].

3-Chloro-*allo*-cholanolic acid, m. p. 180°, was converted into its methyl ester, m. p. 133°. *Anal.* Calcd. for $C_{25}H_{41}O_2Cl$: C, 73.2; H, 10.1; Found: C, 72.8; H, 10.3. This was treated with a phenyl Grignard reagent and the resulting carbinol melted at 171°. *Anal.* Calcd. for $C_{30}H_{49}OCl$: C, 81.1; H, 9.2. Found: C, 81.1; H, 9.3. It was oxidized by chromic acid to 3-chloro-*allo*-*nor*-cholanolic acid. *Anal.* Calcd. for $C_{23}H_{37}O_2Cl$: C, 72.5; H, 9.8. Found: C, 72.8; H, 9.6. This acid was converted to its methyl ester, m. p. 178°.

Anal. Calcd. for $C_{24}H_{39}O_2Cl$: C, 73.0; H, 10.0. Found: C, 73.3; H, 10.2. By treating with a phenyl Grignard reagent, 3-chloro-*allo*-bis-*nor*-cholanyldiphenylcarbinol was obtained, m. p. 183°. *Anal.* Calcd. for $C_{35}H_{47}OCl$: C, 80.9; H, 9.1. Found: C, 80.8; H, 9.3. This carbinol was oxidized by chromic acid to 3-chloro-bis-*nor*-*allo*-cholanolic acid, m. p. 231°. *Anal.* Calcd. for $C_{22}H_{35}O_2Cl$: C, 71.9; H, 9.6. Found: C, 71.8; H, 9.3. This acid was converted to its methyl ester, m. p. 150°. *Anal.* Calcd. for $C_{23}H_{37}O_2Cl$: C, 72.5; H, 9.8. Found: C, 72.5; H, 9.8. By treating with a phenyl Grignard reagent the carbinol was obtained, m. p. 146°. *Anal.* Calcd. for $C_{34}H_{45}OCl$: C, 80.8; H, 9.0. Found: C, 80.9; H, 9.2. This carbinol was dehydrated by refluxing with acetic acid and acetic anhydride. The resulting product was ozonized and the ozonolysis product treated with potassium acetate to convert the chloro derivative into an *epi*-OH compound [Marker, Whitmore and Kamm, THIS JOURNAL, **57**, 2358 (1935)]. The hydroxy ketone was purified by means of its half succinic ester and then as the semicarbazone. It gave *epi*-allo-pregnanolone, m. p. 170°. *Anal.* Calcd. for $C_{21}H_{34}O_2$: C, 79.2; H, 10.7. Found: C, 78.9; H, 10.9. This was identical with the natural product which we isolated from human pregnancy urine.

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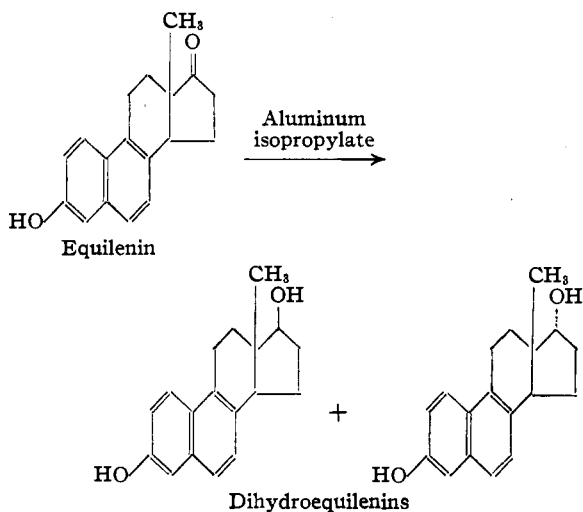
STEROLS. XIII. THE DIHYDROEQUILENINS

Sir:

Recently Wintersteiner, Schwenk, Hirschmann and Whitman [THIS JOURNAL, **58**, 2652 (1936)] in their studies on the isolation of the δ -follicular hormone from the phenolic fraction of mare's urine obtained as the δ -follicular fraction a molecular compound of two components, one of which formed a picrate. This was isolated and identified as dihydroequilenin. They suggested that possibly the high estrogenic activity of the originally reported δ -follicular hormone [Wintersteiner, Schwenk and Whitman, *Proc. Soc. Exptl. Biol. Med.*, **32**, 1087 (1935)] is due to the other

isomer of dihydroequilenin, differing from their product only in the configuration of the —OH group in the 17-position.

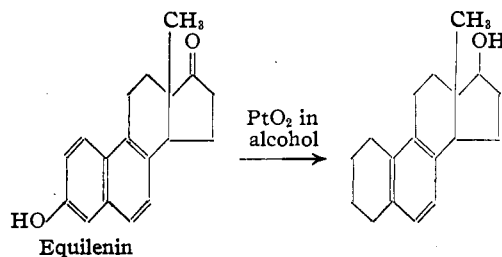
We have now prepared both isomers of dihydroequilenin by the reduction of equilenin with aluminum isopropylate. These isomers were separated by crystallization from alcohol, the more soluble dihydroequilenin melting at 215°. *Anal.* Calcd. for $C_{18}H_{20}O_2$: C, 80.5; H, 7.5. Found: C, 80.4; H, 7.7. This gave a monobenzoate of m. p. 204°. *Anal.* Calcd. for $C_{25}H_{24}O_3$: C, 80.4; H, 6.7. Found: C, 80.4; H, 6.7. These products are identical in melting points with the products isolated by Wintersteiner and co-workers from mare's urine.



From the less soluble reduction product we obtained the epimers of Wintersteiner's dihydroequilenin. This product melts at 248°. *Anal.* Calcd. for $C_{18}H_{20}O_2$: C, 80.5; H, 7.5. Found: C, 80.3; H, 7.6. This gives a diacetate of m. p. 124° [*Anal.* Calcd. for $C_{22}H_{24}O_4$: C, 74.9; H,

6.9. Found: C, 74.8; H, 7.0] and a monobenzoate m. p. 215° [*Anal.* Calcd. for $C_{25}H_{24}O_3$: C, 80.4; H, 6.7. Found: C, 80.5; H, 6.7]. This is the product that Wintersteiner suggests may be the other component of the δ -follicular hormone mixture which causes the high estrogenic activity. Each isomer gives a large melting point depression when mixed with the other, or with the original equilenin.

We attempted to prepare dihydroequilenin by the reduction of equilenin with sodium as tried by David [*Acta brevia Neerland.*, 4, 63 (1934)]. We duplicated his results, obtaining only an oil. The sodium apparently effects a partial reduction of the naphthol ring. Catalytic hydrogenation of equilenin with Adams platinum oxide catalyst in alcohol reduced the first ring with the loss of an —OH group to give a 70% yield of a product containing only one oxygen atom.



The product melted at 148°. *Anal.* Calcd. for $C_{18}H_{24}O$: C, 84.3; H, 9.4. Found: C, 84.3; H, 9.6. Acetate, m. p. 104°. *Anal.* Calcd. for $C_{20}H_{26}O_2$: C, 80.4; H, 8.8. Found: C, 80.0; H, 9.0.

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