manner. It is also hoped to extend the work to lower hydrocarbons, particularly ethane.

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RECEIVED MARCH 11, 1937

CHEMICAL STUDIES ON TOAD POISONS. FURTHER CONTRIBUTIONS TO THE CHEMICAL CONSTITUTION OF MARINOBUFAGIN, CINOBUFAGIN AND GAMABUFAGIN

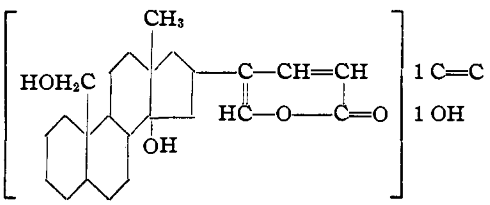
Sir:

Marinobufagin, $C_{24}H_{32}O_5$ [Jensen and Evans, Jr., *J. Biol. Chem.*, **104,** 307 (1934)], and cinobufagin, $C_{26}H_{34}O_6$ [Crowfoot and Jensen, THIS JOURNAL, **58,** 2018 (1936)], are unsaturated hydroxy lactones, cinobufagin being the acetyl derivative of the compound $C_{24}H_{32}O_5$, an isomer of marinobufagin. Tschesche and Offe [*Ber.*, **68,** 1998 (1935)] and independently Jensen [THIS JOURNAL, **57,** 2733 (1935)] showed that cinobufagin on dehydrogenation with selenium yields the Diels hydrocarbon $C_{18}H_{16}$. It has been found that marinobufagin (5 g. used) under the conditions described for cinobufagin [Jensen, *ibid.*, **57,** 2733 (1935)] also yields this hydrocarbon, found m. p. 122–123°, no depression when mixed with methylcyclopentenophenanthrene (m. p. 123–124°). *Anal.* Calcd. for $C_{18}H_{16}$: C, 93.05; H, 6.95. Found: C, 92.92; H, 7.17.

Repetition of catalytic hydrogenation of marinobufagin and cinobufagin has revealed the presence of three double bonds instead of two as previously reported by Jensen and Evans [*J. Biol. Chem.*, **104,** 307 (1934)]; α-hexahydromarinobufagin m. p. 212–213°, β-derivative m. p. 225–227°; α-hexahydrocinobufagin m. p. 230–232°; β-derivative m. p. 210–212°. The found analytical data agree with the calculated. Simultaneously small amounts of acidic products are produced, probably by the opening of the lactone ring. Tschesche and Offe [*Ber.*, **69,** 2361 (1936)] have since reported similar findings.

Ozonization of marinobufagin and cinobufagin (ozone was passed under cooling for three hours through a solution of 1 g. of substance in chloroform, the working up of the reaction product was carried out in the usual manner) was found to yield formic acid, identified by qualitative tests, and glyoxylic acid, identified by color reactions and as the dinitrophenylhydrazone, yellow needles m. p. 190° with decomposition, no depression with the derivative prepared from glyoxylic acid. A small amount of oxalic acid, formed by oxidation of glyoxylic acid, was also found and identified as the calcium salt. These findings indicate the presence of two double bonds in the lactone group, which is a six-membered ring and apparently identical with that in scillaren [Stoll, Hofmann and Peyer, *Helv. Chim. Acta*, **18,** 1247 (1935)]. The third double bond is present in the sterol ring; its exact position is still uncertain.

It has been found that both marinobufagin and gamabufagin contain a —CH_2OH group attached either at C_{10} or at C_{13}, corresponding to one of the angular methyl groups of the sterols. Under the influence of strong acid or alkali this primary alcoholic group is eliminated as formaldehyde. On oxidation with chromic acid marinobufagin gives an aldehyde, the oxidation product giving the typical reactions for an aldehyde group. Evidence has been obtained that a tertiary hydroxyl group is attached at C_{14} in all three principles. Whether or not the third hydroxyl group in marinobufagin is also tertiary is somewhat uncertain, as is its position. Under the influence of acid two hydroxyl groups in marinobufagin are eliminated as water. On the basis of the experimental findings the following structure for marinobufagin is suggested



Analytical data obtained for gamabufagin, acetylgamabufagin and anhydrogamabufagin indicate the empirical constitution of $C_{24}H_{34}O_5$ for gamabufagin, as suggested by Wieland and Vocke [*Ann.*, **481,** 215 (1930)]. Gamabufagin contains only two double bonds, both in the lactone ring. Under the influence of acid gamabufagin loses one molecule of water. Both cinobufagin and gamabufagin have a structure similar to that of marinobufagin.

The author is greatly indebted to Dr. K. K. Chen, Lilly Research Laboratories, Indianapolis, for a supply of the secretion of the Japanese toad.

Owing to the temporary interruption of this 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-cholanic 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-cholanic 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 $C_{36}H_{49}OCl$: C, 81.1; H, 9.2. Found: C, 81.1; H, 9.3. It was oxidized by chromic acid to 3-chloro-allo-*nor*-cholanic 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-cholanic 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