hydroxide in 8 cc. of alcohol, diluted and extracted with ether. The washed ethereal extract was evaporated, and the crystalline residue dissolved in 10 cc. of hot alcohol. To this solution was added a hot solution of 0.4 g. of digitonin in 20 cc. of alcohol. The next day the digitonide was filtered, dried and decomposed in the usual manner to give the carbinol. After recrystallization from slightly diluted alcohol, the pregnanol-3(β) was obtained as needles, m. p. 144°, which depressed with pregnanol-3(α) to 115–120°.

Anal. Calcd. for $C_{21}H_{36}O$: C, 82.8; H, 11.8. Found: C, 82.9; H, 11.8.

Twenty milligrams of pregnanol-3(β) was converted into its acetate by refluxing for a half hour with 2 cc. of acetic anhydride. After recrystallization from dilute alcohol, it melted at 87°.

Anal. Calcd. for $C_{23}H_{38}O_2$: C, 80.1; H, 11.3. Found: C, 80.4; H, 11.5.

3||4-Pregnane-3,4-diacid.—The 20-keto-3||4-pregnane-3,4-diacid used in these experiments was obtained by D. M. Jones of this Laboratory as a by-product in the preparation of pregnanedione, and it had a melting point of 270°. It formed a 2,4-dinitrophenylhydrazone which was crystallized from alcohol to a constant m. p. 210°.

Anal. Calcd. for $C_{27}H_{36}O_8N_4$: C, 59.6; H, 6.7. Found: C, 59.5; H, 7.0.

A mixture of 40 g. of amalgamated zinc, 1 g. of 20-keto-3||4-pregnane-3,4-diacid, 100 cc. of concentrated hydrochloric acid, and 100 cc. of acetic acid was refluxed for five hours, small portions of acetic acid and hydrochloric acid being added from time to time. The suspended acid was collected and washed with ether to give 0.53 g., m. p. 260–270°. This product was purified by leaching with boiling alcohol, in which, in contrast to 20-keto-3||4-pregnane-3,4-diacid, it is very insoluble. Extraction of the acetic acid–hydrochloric acid filtrate with ether, and concentration of the latter gave an additional quantity of the product, 3||4-pregnane-3,4-diacid, m. p. 297°. This acid is extremely insoluble in all ordinary solvents.

Anal. Calcd. for $C_{21}H_{34}O_4$: C, 71.4; H, 9.7. Found: C, 71.3; H, 9.5.

A solution of 30 mg. of 3||4-pregnane-3,4-diacid in 20 cc. of methanol and 10 drops of concentrated sulfuric acid was concentrated to one-third volume by heating for two hours on a steam-bath. The solution was diluted with water, extracted with ether, and the ethereal extract washed with sodium carbonate solution and water. After evaporation of the ether, the residue was crystallized from methanol to give the dimethyl ester of 3||4-pregnane-3,4-diacid, m. p. 147°.

Anal. Calcd. for $C_{23}H_{38}O_4$: C, 72.5; H, 10.1. Found: C, 72.2; H, 9.9.

We wish to thank Dr. Oliver Kamm and Parke, Davis and Company for their generous support of this work. We also wish to thank Dr. A. H. Popkin for the microanalyses reported in this paper.

Summary

The preparations of pregnanone-3 and the isomeric 3-pregnanols are described. The Clemmensen reduction, in the presence of acetic acid, of hydroxylated compounds may give rise to acetylated products.

STATE COLLEGE, PENNA. RECEIVED AUGUST 8, 1938

[CONTRIBUTION FROM THE SCHOOL OF CHEMISTRY AND PHYSICS OF THE PENNSYLVANIA STATE COLLEGE]

Sterols. XLV. The Neutral Reduction Products of Equilenin*

BY RUSSELL E. MARKER, EWALD ROHRMANN, EUGENE L. WITTLE AND FRANK H. TENDICK

While the phenolic reduction products of equilenin have been studied rather extensively[1-3] no such study has been reported on the neutral reduction products which often accompany the phenolic reduction products. Marker *et al.*[2] carried out the catalytic hydrogenation of equilenin in acidic solution and found that the main product of the reaction was $\Delta^{5,7,9}$-oestratrienol-17. All searches for completely saturated compounds in the reaction were unsuccessful, indicating the extreme stability of the $\Delta^{5,7,9}$-oestratrienol-17 to further reduction.

Marker[3] made an extensive study of the phenolic reduction products of equilenin, α-dihydroequilenin, and β-dihydroequilenin, using sodium and amyl alcohol. In every case it was found that the reaction gave approximately 20% of phenolic reduction products, the remainder consisting largely of neutral reduction products. The phenolic reduction products were shown to be the α- and β-oestradiols. We have now extended these studies to the neutral reduction products of these reactions. The neutral fraction from the reduction of equilenin gave a diol (I) $C_{18}H_{24}O_2$, melting at 172°. This same compound was produced by the analogous reduction of α-dihydroequilenin, thus establishing the configuration of the hydroxyl group at C_{17}. The

(*) Paper XLIV, THIS JOURNAL, **60**, 2438 (1938).

(1) Wintersteiner, Schwenk, Hirschmann and Whitman, THIS JOURNAL, **58**, 2652 (1936).

(2) Marker, Kamm, Oakwood and Tendick, *ibid.*, **59**, 768 (1937).

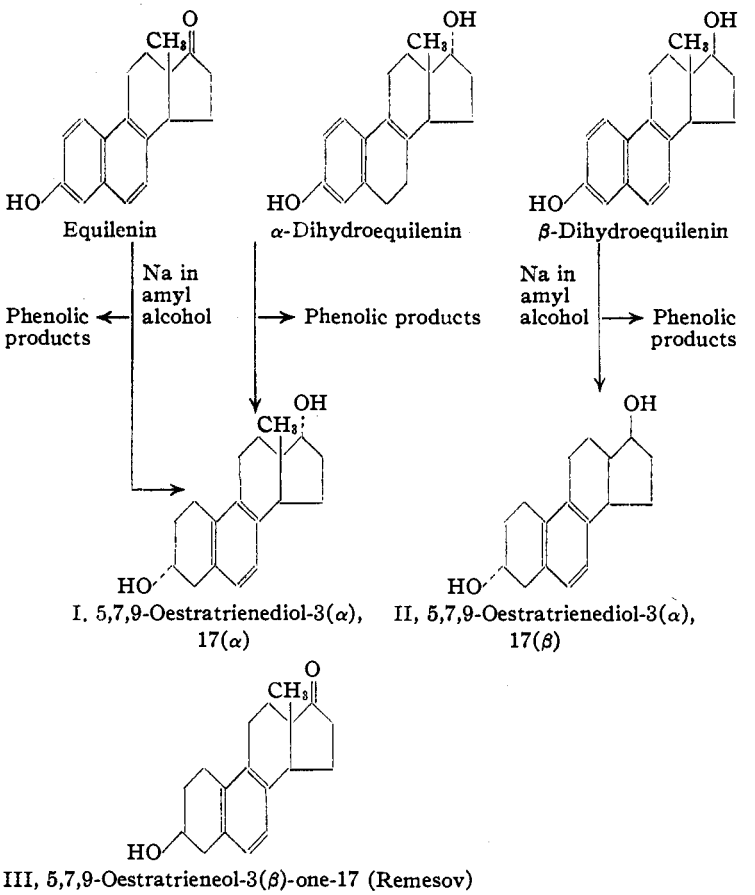
(3) Marker, *ibid.*, **60**, 1897 (1938).

reduction of β-dihydroequilenin gave a diol (II) melting at 179° and possessing the β-configuration at C_{17}. Attempts to oxidize these diols to diketones resulted in the formation of uncrystallizable oils. The fact that the only diol isolated from the equilenin reduction possessed the α-configuration at C_{17} indicates that the sodium and amyl alcohol reduction gives largely the α-form at C_{17} as in the case of oestrone reduction.

It is not possible to assign a definite configuration to the hydroxyl group at C_3, although it is most probable that the same configuration at C_3 is obtained in both of the diols.

A compound apparently related to the two reduction products which we have prepared is that obtained by Remesov[4] by the degradative oxidation of the side chain of neoergosterol. This compound, being derived from neoergosterol, must possess the β-configuration at C_3. Aside from the differences at C_{17}, this compound may differ from the present diols in the configuration of the hydroxyl group at C_3. Inasmuch as Remesov's compound possessed oestrogenic activity comparable to that of oestrone, one might expect the corresponding dihydro compounds to be high in oestrogenic activity. The fact that Windaus and Deppe[5] obtained *epi*-neoergosterol by the reduction of dehydroneoergosterol with sodium and amyl alcohol suggests that our reduction products of equilenin probably possess the α-configuration at C_3 in contrast to the β-configuration which Remesov's compound possesses.

We wish to thank Dr. Oliver Kamm and Parke, Davis and Company for their generous help and assistance in various phases of this work.



Experimental Part

5,7,9-Oestratrienediol-3(α),17(α) from Equilenin.—The neutral fraction obtained by the reduction of equilenin with sodium and amyl alcohol was used.[3] The crude fraction was sublimed in high vacuum at 160–200°. The sublimed material was crystallized from acetone to give white crystals melting at 172°.

Anal. Calcd. for $C_{18}H_{24}O_2$: C, 79.3; H, 8.9. Found: C, 79.2; H, 8.9.

A monobenzoate was prepared by allowing a solution of the diol in pyridine and a slight excess of benzoyl chloride to stand for two days at room temperature. The solution was diluted with water and after standing for several hours the product was extracted with ether. The ether solution was washed well with a saturated sodium carbonate solution and then with water, and evaporated to dryness. The benzoate thus obtained, after crystallization from ethyl acetate, melted at 195°.

Anal. Calcd. for $C_{25}H_{28}O_3$: C, 79.7; H, 7.5. Found: C, 79.7; H, 7.7.

5,7,9-Oestratrienediol-3(α),17(α).—The neutral fraction obtained by the reduction of α-dihydroequilenin with sodium and amyl alcohol was used.[3] The material was distilled in high vacuum, most of it distilling at 160–200°. The sublimed material was crystallized from acetone to give white crystals melting at 172°, uncorr. This gave no depression in melting point when mixed with a sample obtained by the reduction of equilenin with sodium and amyl alcohol.

5,7,9-Oestratrienediol-3(α),17(β) from β-Dihydroequilenin.—The neutral fraction from the reduction of β-dihydroequilenin with sodium and amyl alcohol[3] was purified by leaching with cold ether, followed by crystallization from dilute acetone. It gave a product crystallizing in thick needles, m. p. 179°. It gave a large depression in melting point when mixed with the 17(α)-diol.

(4) Remesov, *Rec. trav. chim.*, **56**, 1093 (1937).
(5) Windaus and Deppe, *Ber.*, **70**, 76 (1937).

Anal. Calcd. for $C_{18}H_{24}O_2$: C, 79.3; H, 8.9. Found: C, 79.4; H, 8.9.

Summary

5,7,9-Oestratrienediol-3(α),17(α) and 5,7,9-oestratrienediol-3(α),17(β) were obtained in the non-phenolic fraction from the reduction of α- and β-dihydroequilenin with sodium in amyl alcohol.

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[CONTRIBUTION FROM THE SCHOOL OF CHEMISTRY AND PHYSICS OF THE PENNSYLVANIA STATE COLLEGE]

Sterols. XLVI. The Steroid Content of Cows' Pregnancy Urine*

BY RUSSELL E. MARKER

Although numerous investigations of the steroid content of human and mare urines have been described,[1] relatively little work has been done with other urines. In order to supply this deficiency we have undertaken to investigate the steroids present in the urines of other *mammalia*. A preliminary study[2] has revealed the presence in stallions' urine of β-equistanol, an *allo*-triol, $C_{21}H_{36}O_3$, an *allo*-tetrol, $C_{21}H_{36}O_4$, and a uranetriol-3,11,20. A similar preliminary study of cows' pregnancy urine is presented in this paper.

The butanol extract of 200 gallons (760 liters) of cows' pregnancy urine was extracted with alkali to remove phenols, and then hydrolyzed by steam distillation in the presence of an excess of strong alkali. From a concentrated ethereal solution of the resulting non-volatile tar, a crude pregnanediol mixture was obtained. Fractional crystallization of the acetate mixture from this crude crop yielded the diacetate of pregnanediol-3(α),20(α), m. p. 179°, and from this the diol was obtained. The soluble acetate mother liquor after hydrolysis yielded *allo*-pregnanediol-3(α),20(α). The mother liquor from the crude pregnanediol mixture was freed of traces of ketones by the use of Girard's reagent, and treated with digitonin. The insoluble digitonide was decomposed, and after treatment with bromine this 3(β)-OH sterol mixture was again treated with digitonin. The insoluble digitonide gave, after decomposition, *allo*-pregnanediol-3(β),20(α), and the mother liquor from this digitonide gave cholesterol. The mother liquor from the original digitonin treatment yielded the soluble digitonide of β-equistanol, from which the latter was obtained on decomposition, and an ethereal solution free of 3(β)-OH sterols. After the latter was separated into hydroxylated and non-hydroxylated fractions by means of the acid succinates, the non-hydroxylated fraction was distilled in a high vacuum, and yielded a hydrocarbon, $C_{28}H_{58}$, m. p. 63°. The carbinol fraction was epimerized with sodium in boiling xylene, and then treated with digitonin. The insoluble digitonide yielded *allo*-pregnanediol-3(β),20(α), which had been formed from some *allo*-pregnanediol-3(α),20(α) not removed in the original separation of the crude pregnanediol mixture. The soluble digitonide yielded β-equistanol, which must have been produced by the epimerization of α-equistanol originally present. The mother liquor from the digitonides, containing no 3(β)-OH sterols, was distilled in a high vacuum, and sirupy fractions collected at 115–150° and 150–200°. Since these fractions could not be crystallized readily, they were oxidized with chromic acid. The higher boiling fraction gave no definite products, while the lower boiling fraction gave an aliphatic acid, $C_{18}H_{36}O_2$, m. p. 55°, suggesting the presence of an aliphatic primary alcohol in the original urine extract.

The isolation from cows' pregnancy urine of the three pregnanediols, in about half the amounts present in human pregnancy urine, lends further support to the theory of the interrelationships of the sex hormones advanced in a paper of this series.[1] The occurrence of both the β- and α-equistanols in cows' pregnancy as well as in stallions, urine[2] and in mares' pregnancy urine[3] but not in human pregnancy urine seems to indicate, as suggested earlier,[2] that their presence may be traced to the herbivorous diet of these animals. The hydrocarbon $C_{28}H_{58}$, m. p. 63°, has been found to occur in human pregnancy urine,[4] in stallions' urine,[4] and in mares' pregnancy urine[4] as well as

(*) Paper XLV, THIS JOURNAL, **60**, 2440 (1938).

(1) These investigations are reviewed by Marker, THIS JOURNAL, **60**, 1725 (1938).

(2) Marker, Lawson, Rohrmann and Wittle, *ibid.*, **60**, 1555 (1938).

(3) Marker, Rohrmann and Wittle, *ibid.*, **60**, 1561 (1938).

(4) Unpublished results from this Laboratory.