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

Sterols. XLIII. The 3(β)-Hydroxysteroids in Human Pregnancy Urine

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On the basis of a theory of the origin and interrelationships of the sex and cortical steroids[1] we have predicted the absence in glandular extracts and urines of 3(β)-hydroxy saturated steroids of the coprostanol type. The absence of this type of steroid from mare pregnancy urine already has been reported.[2] As further verification of this prediction, we are now able to report the absence of this type of steroid in human pregnancy urine. Our examination of the 3(β)-hydroxy steroid fraction from 1000 gallons (3780 liters) of human pregnancy urine has revealed the presence, in more than mere traces, of only cholesterol (4 mg. per gallon) and *allo*-pregnanediol-3(β),20(α) (1–1.5 mg. per gallon).

The carbinol fraction of 1000 gallons (3780 liters) of human pregnancy urine from which pregnanediol-3(α),20(α), and *allo*-pregnanediol-3(α),20(α) had been removed was treated with digitonin solution to isolate the 3(β)-hydroxysteroids. The 3(β)-hydroxysteroid fraction thus obtained was distilled and the fraction subliming up to a bath temperature of 170° was investigated. (The residue which did not distil, consisting of triols, tetrols and so forth, was too small to warrant investigation.) The distillate, consisting of mono- and dihydroxy steroids was separated into a saturated and unsaturated steroid fraction, making use of the fact that the unsaturated sterol dibromides do not precipitate with digitonin. Crystallization of the unsaturated sterol fraction yielded cholesterol, and an examination of the mother liquors from this showed the presence of only small amounts of other substances. Similarly, the saturated sterol fraction proved to be almost entirely *allo*-pregnanediol-3(β),20(α). To prove the absence of pregnanediol-3(β),20(α) and similar coprostanol derivatives, the sterols in the mother liquors from the isolation of *allo*-pregnanediol-3(β),20(α) were epimerized with sodium in boiling xylene, the cholestanol derivatives precipitated with digitonin, and the filtrate examined for the presence of *epi*-coprostanol type derivatives, but none were found present.

(1) Marker, THIS JOURNAL, **60**, 1725 (1938).
(2) Marker, Rohrman and Wittle, *ibid.*, **60**, 1561 (1938).

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

Experimental Part

The carbinol fraction from 1000 gallons (3780 liters) of human pregnancy urine after removal of the preganediol, *allo*-pregnanediol and ketones was dissolved in a small amount of ethyl alcohol. To this was added a solution of 40 g. of digitonin in 2 liters of 90% ethyl alcohol. It was allowed to stand overnight; then the insoluble digitonide was filtered and washed with ethyl alcohol until colorless. After drying it weighed 38 g.

The digitonide was decomposed by heating for thirty minutes on a steam-bath with 150 cc. of pyridine and pouring into 2 liters of ether. The digitonin was filtered and the pyridine was removed by washing with hydrochloric acid. The ether was evaporated, giving a residue of 9.8 g. of sterols. This residue was sublimed *in vacuo* up to 170° using a mercury vapor pump for forty hours. Only mono- and dihydroxysterols distil up to this temperature. The distillate, weighing 7.0 g., was dissolved in 500 cc. of ethyl alcohol, cooled to 0° and enough bromine added to give a permanent yellow color. Then an excess of a 1% solution of digitonin in 90% alcohol was added. After standing overnight, the digitonide was filtered. Upon drying this weighed 6.4 g. From the filtrate was obtained, after debromination with zinc dust, 3.2 g. of cholesterol. The filtrate contained a considerable amount of cholesterol in addition to some other unsaturated steroids in smaller amounts. The insoluble digitonide was decomposed by pyridine as previously described. Upon crystallizing the liberated sterol from acetone, 0.8 g. of a product was obtained melting at 216°, which gave no depression in melting point when mixed with *allo*-pregnanediol-3(β),20(α) (m. p. 216°).

Anal. Calcd. for $C_{21}H_{36}O_2$: C, 78.8; H, 11.3. Found: C, 79.9; H, 11.4.

Oxidation of this gave *allo*-pregnanedione of m. p. 200° which showed no depression in melting point when mixed with a known sample.

To see whether the filtrate from the *allo*-pregnanediol-3-(β),20(α) contained any carbinols of the pregnane series with β-OH groups in the 3-position, the residue after evaporation of the solvent was epimerized by refluxing for nine hours with 5 g. of sodium in 100 cc. of xylene. The sodium was destroyed with aqueous alcohol and the product was extracted with ether. The solvent was removed and the residue dissolved in a small amount of alcohol. An excess of digitonin in 90% alcohol was added, and after standing overnight the digitonide was filtered. The alcohol was removed from the filtrate and the residue was extracted for twenty-four hours with ether. No residue remained after evaporating the ethereal solution, showing that all of the sterols in human pregnancy urine precipi-

table by digitonin and containing one or two hydroxyl groups are either unsaturated sterols or belong to the *allo*-saturated series, with none of the regular pregnane series present.

Summary

Cholesterol and *allo*-pregnanediol-3(β),20(α) were isolated from the digitonin precipitable fraction of human pregnancy urine sterols. No mono- or dihydroxy-steroids of the pregnane configuration at C-5 with 3(β)-OH groups were found present.

STATE COLLEGE, PENNA. RECEIVED JUNE 15, 1938

[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF NORTHWESTERN UNIVERSITY]

The Rearrangement of Vinyl Allyl Ethers

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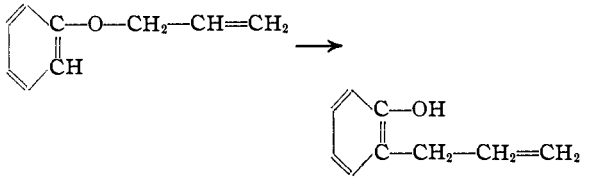
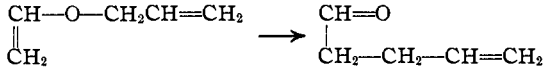
That phenyl allyl ether undergoes thermal rearrangement into *o*-allylphenol has been known for several years. Recent investigations have demonstrated the intramolecular nature of the process,[1] the inversion of the allyl group,[2] the failure of α,α-disubstituted allyl ethers to rearrange,[3] and the unusual rearrangement of γ-ethylallyl phenyl ether.[4]

That part of the aryl allyl ethers which is concerned in the rearrangement possesses the skeleton C=C—O—C—C=C



There is no previous mention in the literature of vinyl allyl ether, the simplest compound to possess this structure. It seemed important, therefore, to prepare it so that its pyrolytic behavior might be studied. If a comparable rearrangement occurred, allylacetaldehyde would be anticipated.



The enol modification of this aldehyde, CH_2=CH—CH_2—CH=CHOH, would be analogous to the *o*-allylphenol.

Since neither vinyl allyl ether nor any of its simple analogs are known, several synthetical approaches were tested. One of these methods consisted in the condensation between allyl bromoacetal and sodium. A similar reaction had been employed successfully by Wislicenus[5] in the preparation of vinyl ethyl ether: $BrCH_2CH(OC_2H_5)_2 + Na \longrightarrow CH_2{=}CHOC_2H_5 + NaBr + C_2H_5ONa$, but in the present adaptation, the yield was trivial. When only one-fifth of the theoretical amount of sodium was added to the acetal, the reaction mixture became a thick brown mass, to which further addition of alkali metal proved ineffectual. No improvement was realized with xylene as solvent. Somewhat similar results were obtained by Hibbert and Hill[6] from the reaction between sodium and 3-bromo-1,2-propanediol bromoacetal, $BrCH_2CH(O{-}CH{-}CH_2Br)(O{-}CH_2)$.

Another procedure tried was related to the last step in Boord's[7] synthesis of α-olefins from β-bromo ethers: $BrCH_2CHROC_2H_5 + Zn \longrightarrow CH_2{=}CHR + BrZnOC_2H_5$. Here again, however, the analogy was misleading for only slight yields of vinyl allyl ether were obtained following the action of zinc on allyl bromoacetal, $BrCH_2CH(OC_3H_5)_2$. With no solvent, very little reaction occurred below 148°, and above that temperature the violence of reaction led to the formation of a black tar. When boiling alcohol was used as a medium, it was found that the rate of reaction was very slow, only a small amount of ether being obtained in six hours.

Another approach to the synthesis of this material was based on the fact that enol ethers may be prepared by heating certain ketals in the presence of an acid catalyst.[8] When allyl acetal was heated with a trace of *p*-toluenesulfonic acid, a small amount of vinyl allyl ether was obtained, but most of the material remained unreacted.

(1) Hurd and Schmerling, THIS JOURNAL, **59**, 107 (1937).

(2) Claisen and Tietze, *Ber.*, **58**, 275 (1925).

(3) Claisen and co-workers, *J. prakt. chem.*, **105**, 67 (1922); Hurd and Cohen, THIS JOURNAL, **53**, 1919 (1931).

(4) Lauer and Filbert, *ibid.*, **58**, 1388 (1936).

(5) Wislicenus, *Ann.*, **192**, 106–112 (1879).

(6) Hibbert and Hill, THIS JOURNAL, **45**, 746 (1923).

(7) Dykstra, Lewis and Boord, *ibid.*, **52**, 3401 (1930).

(8) Johannissian and Akunian, *Bull. univ. etat R. S. S.-Armenie* No. 5, 245–249 (1930); *C. A.* **25**, 921 (1931); Killian, Hennion and Nieuwland, THIS JOURNAL, **57**, 544 (1935).