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

Sterols. CXX. Anterior Pituitary Gland Extracts

BY RUSSELL E. MARKER AND EMERSON L. WITTBECKER

Because of the importance of the pituitary glands in the regulation of the function of the other endocrines, we have attempted to separate their chemical components and establish their composition. Of primary interest to us was the sterol fraction. This consisted entirely of cholesterol. We were unable to find even traces of other sterols.

After removal of the greater part of the cholesterol, a product crystallized from the filtrate upon standing for several months. The ether insoluble portion of this was found to be sodium stearate.

A water-soluble product of the composition $C_8H_{10}N_4O_4$ or $C_{10}H_{13}N_5O_5$ was obtained from this filtrate. This product is being studied further. The fraction which was not soluble in water was saponified and the carbinols separated by means of their succinic esters. The carbinol fraction was treated with digitonin. The fraction which precipitated yielded only cholesterol upon decomposition. The non-digitonin precipitable fraction of the carbinols gave a product melting at 79–81°.

The non-carbinol fraction yielded two products, one of which analyzed for $C_{20}H_{40}O_2$, and the other was a hydrocarbon which was identical with the hydrocarbon obtained previously by us from pregnancy urine.[1]

We wish to thank Parke, Davis and Company for their assistance.

Experimental Part

The acetone extract of 1000 pounds of beef anterior pituitary glands was concentrated *in vacuo*. It weighed 3.5 kg. This was refluxed with 4 liters of methanol, cooled in a refrigerator overnight and filtered. The solid was recrystallized from methanol and melted at 147–148°; yield 640 g. When mixed with cholesterol there was no depression in melting point. With acetic anhydride it gave an acetate which melted at 115° and gave no depression when mixed with an authentic sample of cholesteryl acetate.

The greater part of the solvent was removed from the filtrate and the residue was allowed to stand at room temperature for three months. A precipitate which settled out was obtained by dilution with 6 liters of ether and filtering. This gave a white salt, 7 g., which was crystallized from methanol. A methanolic solution of 100 mg. of this salt was shaken with 2 cc. of concentrated hydrochloric acid and 50 cc. of ether. The ether was separated and the aqueous layer was vacuum distilled. The residue was crystallized from ethanol–water and was sodium chloride. The solvent was removed from the organic material and crystallized from methanol; m. p. 66–68°. It gave no depression in melting point when mixed with an authentic sample of stearic acid.

Anal. Calcd. for $C_{18}H_{36}O_2$: C, 75.97; H, 12.77; neut. equiv., 284. Found: C, 75.73; H, 12.63; neut. equiv., 276.

The filtrate from the sodium stearate was extracted several times with water and the aqueous layer was then extracted once with ether, then evaporated *in vacuo* to about one liter. It was then treated with Norit, filtered and the water removed *in vacuo*. The residue was dissolved in methanol. It crystallized upon standing in a refrigerator for three weeks, and was purified by sublimation at 190° in a high vacuum, and by crystallization from methanol. It is soluble in water, methanol and organic acids, but sparingly soluble in ether and pentane; m. p. 281–284°; yield 1.6 g. Sodium fusion showed the absence of sulfur.

Anal. Calcd. for $C_8H_{10}N_4O_4$: C, 42.5; H, 4.4; N, 24.4; mol. wt., 226. Calcd. for $C_{10}H_{13}N_5O_5$: C, 42.4; H, 4.6; N, 24.7; mol. wt., 283. Found: C, 42.7; H, 4.3; N, 24.7; mol. wt., 218.

It has no free carboxyl groups.

The ether layer from the water extraction was evaporated and the residue was hydrolyzed with methanolic potassium hydroxide. It was diluted with water and extracted with ether. The ether layer was washed with water, evaporated and the residue was taken up in acetone and methanol. Upon cooling in a refrigerator overnight, an additional quantity of cholesterol precipitated out. This was filtered and the filtrate was concentrated. The carbinol fraction was separated by means of its half succinic esters. After hydrolysis this fraction was allowed to stand in methanol to obtain an additional crop of cholesterol. The methanol was removed from the filtrate. The residue was treated with a hot solution of 50 g. of digitonin in 2 liters of ethanol. The digitonide was filtered and dried. It was decomposed in the usual way with pyridine. This gave pure cholesterol.

The excess digitonin was removed from the filtrate and the product was dissolved in a small amount of methanol. Upon standing overnight the crystals which were formed were filtered. It was recrystallized three times from acetone; m. p. 79–81°; yield 2 g.

Anal. Found: C, 76.47; H, 12.53; mol. wt., 551.7 (Rast).

The non-carbinol fraction was dissolved in ether, washed with water, and the solvent removed. The residue was dissolved in acetone, treated with Norit and filtered. Upon standing for two weeks at room temperature, crystals separated from a concentrated solution. These were fil-

(1) Marker, THIS JOURNAL, 60, 2442 (1938).

tered, washed with pentane and recrystallized from acetone, m. p. 96-98°.

Anal. Calcd. for $C_{20}H_{40}O_2$: C, 76.8; H, 12.6; mol. wt., 312. Found: C, 76.5; H, 12.4; mol. wt., 324.

The non-carbinol fraction after removal of the above product was sublimed in a high vacuum; a fraction was collected at 100° and crystallized from acetone, m. p. 63°. When mixed with a hydrocarbon isolated from pregnancy urines, it gave no depression in melting point.

Anal. Calcd. for $C_{28}H_{58}$: C, 85.3; H, 14.8; mol. wt., 394. Found: C, 85.3; H, 14.9; mol. wt., 384 (Rast).

Summary

Anterior pituitary extract has been partially studied. 1. The only sterol isolated was cholesterol. 2. Sodium stearate was found. 3. A water-soluble nitrogenous product, $C_8H_{10}N_4O_4$ or $C_{10}H_{13}N_5O_5$ has been isolated. 4. The hydrocarbon found in pregnancy urines also was obtained.

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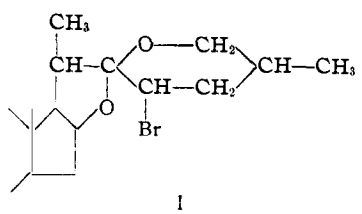
Sterols. CXXI. Sapogenins. XLVIII. Bromosarsasapogenin and Bromodiosgenin[1]

BY RUSSELL E. MARKER, D. L. TURNER, ANTHONY C. SHABICA AND PAUL R. ULSHAFER

Marker and Rohrmann[1a] obtained tetrahydrosarsasapogenin by the Clemmensen reduction of bromosarsasapogenin, and sarsasapogenin by reduction with sodium in alcohol. This suggests that bromosarsasapogenin contains the ketal structure like sarsasapogenin. It seems probable that the bromine atom is adjacent to the potential carbonyl group at C-22 (I).



I

In order to determine the position of the bromine atom, we have oxidized the acetate of bromosarsasapogenin at 60° with chromic acid under the conditions employed for the oxidation of sarsasapogenin by Fieser and Jacobsen.[2] The only material in the neutral fraction from this oxidation was unchanged bromosarsasapogenin. The C-22 keto acid (3-hydroxy-16-keto-*bis-nor*-cholanic acid) of Marker and Rohrmann[3] was obtained in excellent yield from the acidic fraction. No intermediate oxidation products analogous to the C-22 lactone of Farmer and Kon[4] or to sarsasapogenoic acid and the C-27 neutral product of Fieser and Jacobsen,[2] were found. The isolation of the C-22 keto acid indicates that the bromine is at C-23 rather than at C-20, and the structure of the side-chain in the bromosapogenins may be represented by I.

Diosgenin acetate can be brominated at the double bond without affecting the side-chain.[5] We have found that it is also possible to brominate the side-chain; this gives 5,6,23-tribromodiosgenin acetate. When this is debrominated with potassium iodide in ethanol[6] the bromine in the side-chain is not affected and 23-bromodiosgenin acetate results.

Because of the relative stability of the brominated side-chain it is possible to carry out reactions depending on the presence of the nuclear double bond which fail with diosgenin acetate. Thus selenium dioxide, which attacks the sapogenin side-chain[1a] but does not react with bromosarsasapogenin,[1a] gives the reaction discovered by Rosenheim and Starling[7,8] in the case of bromodiosgenin acetate.

The oxidation of bromodiosgenin acetate under the conditions employed for the preparation of 7-keto compounds,[9] however, gives mostly acid products in addition to the expected 7-keto-bromodiosgenin acetate. The acid appears to be Δ^5-3-acetoxy-7,16-diketo-*bis-nor*-cholenic acid, although it gives only a monosemicarbazone instead of the expected disemicarbazone.

We wish to thank Parke, Davis and Company for their generous help.

(1) Original manuscript received August 12, 1940.
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(2) Fieser and Jacobsen, *ibid.*, 60, 28, 2753 (1938).
(3) Marker and Rohrmann, *ibid.*, 61, 1285 (1939).
(4) Farmer and Kon, *J. Chem. Soc.*, 414 (1937).
(5) Tsukamoto, Ueno and Ohta, *J. Pharm. Soc., Japan*, 57, 9 (1937).
(6) Linnemann and von Zotta, *Ann.*, 192, 102 (1878).
(7) Rosenheim and Starling, *J. Chem. Soc.*, 377 (1937).
(8) Butenandt and Hausmann, *Ber.*, 70, 1154 (1937).
(9) Windaus, Lettré and Schenck, *Ann.*, 520, 98 (1935).