Anal. C, 63.4; H, 8.3; N, 12.8.

Similar to acid II, it gave an oxime which crystallized in fiber clusters from methyl alcohol and water, m. p. and mixed m. p. 181–182°.

Anal. N, 9.17.

Treatment of Pregnanetriol-B with Periodic Acid.—To a solution of 0.67 g. of pregnanetriol-B in 250 cc. of alcohol was added a solution of 100 cc. of 0.01 *M* periodic acid and 3 cc. of 5 *N* sulfuric acid. After standing for fifteen hours the mixture was distilled into 0.7 g. of Dimedon (dimethyldihydroxyresorcinol) in 50 cc. of alcohol. When most of the water and alcohol had been removed, 300 cc. of water was added to the residue and the distillation was repeated. The distillate was concentrated to 150 cc. to remove the alcohol, but no precipitate of the formaldehyde derivative was obtained. Pregnanetriol-B was recovered substantially unchanged from the residual solution.

Isomerization of Pregnanetriol-B.—A solution of 0.5 g. of triol-B in 200 cc. of dry xylene and 3 g. of sodium was refluxed for six hours and then 50 cc. of amyl alcohol was added to dissolve the sodium. The solution was washed thoroughly with water, steam distilled, and the remaining water solution filtered. The solid was dissolved in 250 cc. of ethyl alcohol and treated with 2 g. of digitonin in 200 cc. of ethyl alcohol to yield 400 mg. of digitonide.

Treatment of Pregnanetriol-B with Lead Tetraacetate.—To a solution of 2 g. of lead tetraacetate in 200 cc. of acetic acid was added 0.5 g. of triol-B. It dissolved readily with shaking and the solution was allowed to stand at 25° for three days. It was then diluted with one liter of water and washed with 300-cc. portions of ether. The ether solution was evaporated and the residue was dissolved in 25 cc. of hot acetic acid and treated with 5 cc. of 30% hydrogen peroxide over a period of four hours on the steam-bath. The solution was diluted with ether and after washing with water was extracted with sodium carbonate. No acid was obtained on acidifying the carbonate solution and the triol was recovered in the ether solution. This procedure gave a good yield of Diels saturated acid from 4-hydroxycholestanol.

Summary

Pregnanetriol-B, a trihydroxy steroid occurring in mares' pregnancy urine, has been identified as 3(α),4(β),20(α)-trihydroxypregnane.

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Sterols. XXXI. Oxidation of Sitosterol by Selenium Oxide

BY RUSSELL E. MARKER, OLIVER KAMM AND EUGENE L. WITTLE

In the preceding article in this series[1] we reported that the structure of one of the triols (pregnanetriol-B) obtained from mares' pregnancy urine is quite probably that of a glycol having hydroxyl groups at positions C_3 and C_4 of the pregnane nucleus. Since it represents the first compound of this type which has been isolated from urine and is probably closely related to the sex hormones, we have decided to prepare similar compounds from readily available materials to learn more of the chemistry of these glycols and also as a possible means for the synthesis of pregnanetriol-B.[1] This paper reports such work on sitosterol.

Oxidation of sitosteryl acetate by selenium dioxide has been found to take place very readily following a course similar to that with cholesterol,[2] giving 4-hydroxysitosteryl acetate and 6-hydroxysitosteryl acetate. The two oxidation products were separated readily through their diacetates, the 4-hydroxy derivative being obtained by direct crystallization whereas 6-hydroxysitosterol was isolated from the acetic anhydride mother liquors after hydrolysis.

Catalytic reduction of the diacetate of 4-hydroxysitosterol gave the corresponding derivative of 4-hydroxysitostanol which was hydrolyzed to the saturated diol. Oxidation of this 4-hydroxysitostanol with chromium trioxide in acetic acid took place very readily, yielding a dibasic acid which was characterized by its dimethyl ester. We have found also that the oxidation of 4-hydroxycholestanol with chromium trioxide in acetic acid gives Diels' saturated acid which is identical with the compound prepared from the diol by oxidation with lead tetraacetate and hydrogen peroxide. It is apparent that C_3—C_4 diols of the *allo* series (β-configuration at C_5) on oxidation with chromium trioxide undergo splitting to yield C_3—C_4 dibasic acids. This is in marked contrast to the oxidation of C_3—C_4 glycols of the pregnane series,[1] which yield a monobasic acid of unknown structure.

When 4-hydroxysitosterol is heated with alcoholic hydrochloric acid it undergoes dehydration and rearrangement to form sitostenone.

(1) Marker, Kamm, Wittle, Oakwood and Lawson, THIS JOURNAL, **60**, 000 (1938).

(2) Rosenheim and Starling, *J. Chem. Soc.*, 377 (1937); Butenandt and Hausmann, *Ber.*, [5] **70**, 1154 (1937).

The reactions involved in the present work are strictly analogous to those illustrated by the formulas in Chart I of our Sterol Paper No. XXXII, which deals with the selenium oxidation of stigmasterol and therefore are not duplicated here.

We wish to thank the Gaylord Bag and Paper Company of Bogalusa, Louisiana, for contributing the tallol from which we obtained the β-sitosterol used in these experiments.

The microanalyses herein reported have been performed by Dr. George H. Fleming.

Experimental Part

Diacetate of 4-Hydroxysitosterol.—A solution of 40 g. of sitosteryl acetate in 200 cc. of warm benzene was added to a solution of 20 g. of selenium dioxide in 400 cc. of hot 98% acetic acid. The mixture, from which red selenium precipitated almost immediately, was refluxed on the steam-bath for an hour, after which 40 g. of sodium acetate was added and refluxing continued for an additional ten minutes. Water was added and the product was extracted with ether. The solvent was evaporated and the residue was refluxed with 120 cc. of acetic anhydride for thirty minutes. The solution was cooled and the crystalline mass was filtered off and dissolved in 200 cc. of ether. Upon addition of 400 cc. of methanol the diacetate crystallized in needles upon cooling. It was recrystallized from acetone and ethyl acetate to a constant melting point of 167°.

Anal. Calcd. for $C_{33}H_{54}O_4$: C, 77.0; H, 10.6. Found: C, 77.1; H, 10.7.

4-Hydroxysitosterol.—A solution of 2 g. of the diacetate of 4-hydroxysitosterol in 50 cc. of ethanol was refluxed with potassium hydroxide solution for thirty minutes. The product after crystallization from acetone and ethyl acetate melted at 184°.

Anal. Calcd. for $C_{29}H_{50}O_2$: C, 80.8; H, 11.7. Found: C, 80.4; H, 11.8.

Diacetate of 4-Hydroxysitostanol.—To a solution of 2 g. of the diacetate of 4-hydroxysitosterol in 100 cc. of acetic acid was added 100 mg. of platinum oxide and the product was shaken with hydrogen at 45 pounds (3 atm.) pressure for one hour. The catalyst was filtered off and after evaporation of the acetic acid the residue was crystallized from ether–ethanol, and acetone. Its melting point was 153°.

Anal. Calcd. for $C_{33}H_{56}O_4$: C, 76.7; H, 10.9. Found: C, 76.7; H, 10.6.

4-Hydroxysitostanol.—An alcoholic solution of 500 mg. of the diacetate of 4-hydroxysitostanol was hydrolyzed by potassium hydroxide. The product after crystallization from ether, ether–ethanol, and acetone melted at 203°. It is sparingly soluble in ether.

Anal. Calcd. for $C_{29}H_{52}O_2$: C, 80.5; H, 12.1. Found: C, 80.1; H, 12.2.

Sitostenone from 4-Hydroxysitosterol.—A solution of 1 g. of 4-hydroxysitosterol diacetate in 100 cc. of ethanol was refluxed for ten minutes with 5 cc. of concd. hydrochloric acid. The product was recrystallized from ethyl acetate and acetone. It melted at 83° and gave no depression in melting point when mixed with sitostenone prepared by distilling sitosterol from copper.

3-Acetoxy-4-hydroxysitosterol.—This product was prepared by the same method as described above for the diacetate, except that the acetic anhydride treatment was omitted. The benzene–ether solution was concentrated to crystallization, cooled and filtered. The dark colored crystals were dissolved in a mixture of ether and acetone (2:1) and treated with Norite. The mixture was filtered and about half of the solvent removed by distillation. Upon cooling the solution the product separated in the form of white plates which were recrystallized from methanol and ethyl acetate. The melting point was found to be 192°.

Anal. Calcd. for $C_{31}H_{52}O_3$: C, 78.6; H, 11.2. Found: C, 78.7; H, 11.1.

When heated with acetic anhydride this 3-acetoxy derivative was converted into the diacetate melting at 164–165°.

6-Hydroxysitosterol.—The acetic anhydride filtrate from the preparation of the diacetate of 4-hydroxysitosterol was evaporated to dryness *in vacuo* and the residue was dissolved in 1 liter of alcohol and hydrolyzed by refluxing for one hour with 30 g. of potassium hydroxide in 500 cc. of ethyl alcohol. The solution was diluted with water and the solids were filtered off, washed with ether, dissolved in ethyl alcohol, treated with Norite, and filtered. The solution on cooling gave fine needles of 6-hydroxysitosterol. When recrystallized from ethyl acetate, in which solvent it is only sparingly soluble, it melted at 250°.

Anal. Calcd. for $C_{29}H_{50}O_2$: C, 80.85; H, 11.7. Found: C, 81.04; H, 11.75.

The diacetate was prepared by refluxing a portion of the diol with excess acetic anhydride, evaporating to dryness and crystallizing the product from ethyl acetate and methyl alcohol to give plates, m. p. 107°. These on hydrolysis gave the original diol, m. p. 250°.

Anal. Calcd. for $C_{33}H_{54}O_4$: C, 77.0; H, 10.6. Found: C, 77.1; H, 10.6.

Oxidation of 4-Hydroxystigmastanediol.—To a solution of 1 g. of 4-hydroxystigmastanol in 100 cc. of acetic acid was added at 25° a solution of 1.2 g. of chromium trioxide in 25 cc. of 90% acetic acid. The solution was allowed to stand overnight at 25°, diluted with methyl alcohol and evaporated *in vacuo* to dryness. The residue was dissolved in ether and water and the ether solution after being washed with water was extracted with sodium carbonate. The sodium carbonate solution was extracted with ether, acidified, and the acid so liberated was filtered off and crystallized from benzene–pentane, m. p. 200–205°. As the acid did not have a sharp melting point it was converted to the dimethyl ester by treatment with diazomethane and this was readily crystallized from methyl alcohol to yield 200 mg., m. p. 123–124°.

Anal. Calcd. for $C_{31}H_{54}O_2$: C, 75.9; H, 11.1. Found: C, 75.7; H, 11.0.

Oxidation of 4-Hydroxycholestanol.—To a solution of 1.0 g. of 4-hydroxycholestanol, m. p. 198–200°,[2] in 200 cc. of acetic acid at 15° was added dropwise over a period of

one hour a solution of 1.2 g. of chromium trioxide in 25 cc. of 90% acetic acid. The solution was stirred for several hours at 20° and allowed to stand overnight at 25°. Methyl alcohol (20 cc.) was added, the solution was evaporated *in vacuo* to dryness and the residue was dissolved in 250 cc. of water and 250 cc. of ether. The ether solution was washed thoroughly with water and then extracted with sodium carbonate solution and the sodium carbonate solution, after extraction with ether, was acidified to liberate an acid which was filtered off and crystallized from benzene–pentane and also from dilute acetone: m. p. 250°; yield 0.3 g. It gave no depression with the acid prepared by lead tetraacetate–hydrogen peroxide oxidation of this 4-hydroxycholestanol.[2]

Conversion to the dimethyl ester with diazomethane and crystallization from methyl alcohol gave needles, m. p. 123–124°.

Anal. Calcd. for $C_{27}H_{46}O_4$: C, 74.6; H, 10.7. Found: C, 74.5; H, 10.7.

Summary

The oxidation of sitosterol by selenium oxide gave 4-hydroxy- and 6-hydroxysitosterol. Hydrogenation of the 4-hydroxy compound gave 4-hydroxysitostanol. 4-Hydroxycholestanol upon oxidation with chromic acid gave the same dicarboxylic acid as upon treatment with lead tetraacetate. An analogous dicarboxylic acid was obtained upon the oxidation of 4-hydroxysitostanol by chromic acid.

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Sterols. XXXII. Oxidation of Stigmasterol by Selenium Oxide

BY RUSSELL E. MARKER AND EWALD ROHRMANN

Rosenheim and Starling[1] studied the oxidation of cholesterol by selenium dioxide in acetic acid solution and reported the formation of two stereoisomers, namely, *cis*-4-hydroxycholesterol and *trans*-4-hydroxycholesterol. Butenandt and Hausmann,[2] however, showed that the assumed *trans*-4-hydroxycholesterol actually is 6-hydroxycholesterol.

We have now extended this oxidation to stigmasterol and stigmasteryl acetate and find that the reaction is similar to that with cholesterol. In analogy to the reactions in the cholesterol series, the diacetate of 4-hydroxystigmasterol is less soluble than the diacetate of the 6-hydroxy compound while with the free diols the solubility relationship is reversed. These factors make possible an easy separation of the two diols. The oxidations were carried out in acetic acid in the presence of benzene at about 90°. Contrary to the results reported by Rosenheim and Starling[1] for cholesterol, we find that the oxidation of the acetate of stigmasterol readily yields the monoacetate of 4-hydroxystigmasterol. The main reactions involved in this study are illustrated in the chart.

Hydrogenation of the diacetate of 4-hydroxystigmasterol with platinum oxide catalyst yielded the diacetate of 4-hydroxystigmastanol which proved to be identical with the diacetate of 4-hydroxysitostanol.[3] Reduction of the diacetate of 4-hydroxystigmastanol by the method of Clemmensen gave a hydrocarbon which was identical with sitostane prepared by the action of sodium on sitostyl chloride. Hydrolysis of the diacetate of 4-hydroxystigmastanol yielded 4-hydroxystigmastanol which was identical with 4-hydroxysitostanol. These facts give further evidence that stigmasterol differs from sitosterol only in the presence of a double bond in the side chain.

The microanalyses herein reported have been performed by Dr. George H. Fleming.

Experimental Part

Diacetate of 4-Hydroxystigmasterol.—To a solution of 10 g. of stigmasteryl acetate in 50 cc. of benzene was added a hot solution of 4 g. of selenium dioxide in 100 cc. of 98% acetic acid. The solution was refluxed for one hour, then 10 g. of sodium acetate was added and refluxing continued for ten minutes to coagulate the selenium. The mixture then was poured into 200 cc. of water and the benzene layer was separated. After removal of the solvent the residue was dissolved in 30 cc. of acetic anhydride and heated under a reflux condenser during one-half hour. The acetyl derivative which separated on cooling was filtered off, dissolved in ether (100 cc.) and treated with decolorizing charcoal (Norite). After filtration and partial evaporation of the ether the crystalline product was obtained by the addition of 200 cc. of cold

(1) Rosenheim and Starling, *J. Chem. Soc.*, 377 (1937).
(2) Butenandt and Hausmann, *Ber.*, **70**, 1154 (1937).
(3) Marker, Kamm and Wittle, THIS JOURNAL, **60**, 1071 (1938).