

at 3 atm. for eight hours. The solution was filtered and concentrated. The product crystallized from the methanol and was recrystallized from the same solvent. It melted at 262–264°. Admixture with the 20-methyl-*allo*-pregnanetriol described above gave no depression of the melting point. The product, unlike the unsaturated triol, did not absorb bromine in acetic acid.

*Anal.* Calcd. for  $C_{22}H_{34}O_3$ : C, 75.4; H, 10.9. Found: C, 75.4; H, 11.1

**Oppenauer Oxidation of 20-Methyl-pregnane-3( $\beta$ ),16-,20-triol.**—A mixture of 4.0 g. of triol, 20 g. of aluminum *t*-butylate, 400 cc. of dry acetone and 2 liters of dry benzene was refluxed for thirty hours. The mixture was distilled to dryness *in vacuo* and the product decomposed with dilute hydrochloric acid and extracted with ether.

The product remaining after the evaporation of the ether was treated with acetone in which a little starting material remained undissolved. The acetone soluble material was recrystallized from ether-pentane. It melted at 193–195°.

*Anal.* Calcd. for  $C_{22}H_{32}O_2$ : C, 80.4; H, 9.8. Found: C, 80.2; H, 10.2.

### Summary

The oxidation products obtained from the pseudosapogenin diacetates have been treated with methylmagnesium iodide to give the related triols.

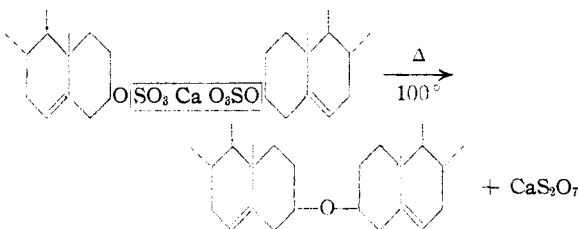
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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF THE POLYTECHNIC INSTITUTE OF BROOKLYN AND THE PEDI-  
ATRIC RESEARCH LABORATORY OF THE JEWISH HOSPITAL OF BROOKLYN]

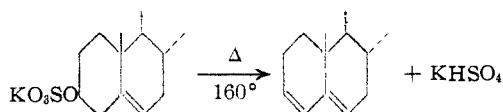
## Steryl Sulfates. IV. Thermal Decomposition of Calcium Cholesteryl Sulfate

BY ALBERT E. SOBEL<sup>1</sup> AND PAUL E. SPOERRI

Since a great variety of cholesteryl sulfates are available at present,<sup>2</sup> an interesting question arises: Does the cation in the cholesteryl sulfates influence the thermal decomposition of these compounds? This may be answered in the affirmative as a result of present investigations in which the thermal decomposition of calcium cholesteryl sulfate was studied as a typical representative of the divalent alkali earth cations. This decomposition takes place readily at 100° (in the dry state) with the formation of dicholesteryl ether.



In contrast to this, the monovalent potassium salt decomposes (in the dry state) to form 3,5-cholestadiene<sup>3</sup>



The presence of water inhibits the thermal de-

composition of calcium cholesteryl sulfate in some manner as shown by the fact that on heating calcium cholesteryl sulfate for one hour in refluxing water, only 6 to 7% decomposition takes place with the formation of cholesterol. This may be compared to the complete decomposition of the dry solid in forty minutes. It was also observed on further investigation that complete decomposition takes place in refluxing benzene (b. p. 80.1°) in thirty minutes with the formation of calcium sulfate, sulfuric acid and some unidentified reaction product in which cholesterol and sulfur are absent. This decomposition in benzene takes place also in the presence of barium oxide, used to remove the sulfuric acid formed in the decomposition. Here again the main reaction products were not identified, but free cholesterol and sulfur were absent. Thus the presence of inert solvents which boil even lower than water does not inhibit thermal decomposition. Water, therefore, must have some specific inhibitory effect which may be due to the dissociation of the calcium cholesteryl sulfate as shown



Or possibly the hydrated form of the compound is more resistant to thermal decomposition.

### Experimental

**Thermal Decomposition of Calcium Cholesteryl Sulfate in a Sealed Tube.**—Four grams of calcium cholesteryl sulfate was sealed in an evacuated Pyrex tube which was

(1) From the dissertation submitted to the Polytechnic Institute of Brooklyn in partial fulfillment of the requirements for the degree of Doctor of Philosophy, June, 1940.

(2) A. E. Sobel and P. E. Spoerri, *THIS JOURNAL*, **63**, 1259 (1941).

(3) S. Natelson and S. P. Gottfried, *ibid.*, **61**, 971 (1939); A. E. Sobel and M. J. Rosen, *ibid.*, **63**, 3536 (1941).

immersed in a boiling water-bath, and lead weights attached to prevent it from floating. After thirty-five minutes of heating, a sudden shrinking was observed with a change from white to grayish-black or brown color. The heating was continued for five more minutes. The tube was then removed from the water-bath, allowed to cool, broken open and the material extracted with ether. The ether-insoluble portion was dried and found to be inorganic on ashing.

The ether extract was filtered and taken down to 50 cc. on a steam-bath. About 100 cc. of methyl alcohol was added, and the mixture evaporated on a steam-bath until crystals appeared. At this stage, sufficient ether was added to the hot solution to just barely cause the crystals to go back into solution. On standing in the refrigerator, white crystals appeared (1.0 g., m. p., 174–176°).

The crystals were recrystallized twice from methyl alcohol and ether; m. p. 192–194°; Lieberman–Burchard test positive, Carr-Price and Rosenheim tests negative. Calcd. for  $(C_{27}H_{46})_2O$ ; C, 85.87; H, 12.01; mol. wt., 755.26; iodine no., 67.3. Found C, 85.46; H, 11.95; mol. wt. (Rast), 732; iodine no., 64.5.

**Treatment of Calcium Cholesteryl Sulfate in Boiling Water.**—Two and one-half grams of calcium cholesteryl sulfate was placed for one hour in 80 cc. of refluxing water. The cooled reaction mixture was extracted with several portions of ether. The ether extracts were dried over anhydrous sodium sulfate and evaporated to dryness. The white residue weighed 132 mg.; 111 mg. of this was free cholesterol as determined by the digitonin method.<sup>4</sup> In a repeat experiment, the residue from 1.47 g. of starting material weighed 74 mg.

The ether-extracted water layer contained unchanged calcium cholesteryl sulfate which was insoluble in cold water and in ether.

**Thermal Decomposition of Calcium Cholesteryl Sulfate in Benzene.**—Two grams of anhydrous calcium cholesteryl sulfate<sup>3</sup> was refluxed in 100 cc. of benzene for thirty minutes with anhydrous precautions. The reaction mixture was extracted with water. The water extracts contained calcium sulfate and sulfuric acid. The washed benzene solution was evaporated to dryness and the residue ex-

tracted with ether. The ether-soluble material was evaporated to dryness. A resin-like residue was found (1.5 g., 94% yield). Various attempts to obtain crystals were unsuccessful. Three portions of 100 mg. each were fed to three rats but there was no antirachitic activity in 10 days.

A similar experiment carried out in the presence of 1.0 g. of barium oxide has also resulted in decomposition. The yield from 4 g. of calcium cholesteryl sulfate was 2.8 g. of brown, gummy-looking substances which appeared to recrystallize from a mixture of ethyl methyl ketone and alcohol. The precipitate, however, became gummy-looking. It was antirachitically inactive, and was not investigated further.

Cholesterol was absent in the above two products as shown by negative digitonin tests.

**Treatment of Calcium Cholesteryl Sulfate with Ether and Isopropyl Ether.**—Two 4-g. portions of calcium cholesteryl sulfate were refluxed for two hours with anhydrous precautions, in 100 cc. of anhydrous ethyl and isopropyl ether, respectively. On evaporating the filtered off solvents there was no residue. The precipitate decomposed at the same temperature as the original salt (135°) and gave a similar analysis for calcium: 4.10, 4.09, 4.11; present originally, 4.10. Thus there was no decomposition in these solvents.

### Summary

The thermal decomposition of calcium cholesteryl sulfate was investigated under various conditions.

This salt decomposes in the dry state, on heating at 100°, with the formation of dicholesteryl ether. Under similar conditions in water only a slight degree of decomposition takes place with the formation of cholesterol as the main product. Thus the presence of water inhibits thermal decomposition.

Thermal decomposition takes place in refluxing benzene, but not in ethyl or isopropyl ether.

(4) I. J. Drekter, A. E. Sobel and S. Natelson, *J. Biol. Chem.*, **115**, 391 (1936).