during the reduction. In some cases reduction was complete in from two to ten hours but frequently reduction stopped before it was complete.

A convenient method for reducing dehydroascorbic acid is with bacteria of the *B. coli* group.[11] Using a few drops of cell suspension per 10 cc. of dehydroascorbic acid solution, reduction is complete in fifteen minutes at 40°. The details of the procedure have been described in another publication.[12]

Experiments made on the reversibility of the oxidation of vitamin C using *B. coli* and glucose as the reducing agent showed that reversibility also varied with the nature of the catalyst The results are shown in Table II.

(11) W. B. Esselen, Jr., and J. E. Fuller, *J. Bact.*, **37**, 501 (1939).
(12) I. C. Gunsalus and D. B. Hand, *J. Biol. Chem.*, **141**, 853 1941).

Summary

The amount of oxygen used in the oxidation of vitamin C depends on the nature of the catalyst: with cucumber oxidase 1.0, with copper 1.19 to 1.67, and with riboflavin in the light 1.57 to 2 atoms of oxygen are involved. The oxidation is completely reversible with 1.0 but only partially reversible with more than 1.0 atom of oxygen. The partial irreversibility is due to the production of hydrogen peroxide. Two molecules of hydrogen peroxide oxidize one molecule of ascorbic acid.

ITHACA, N. Y. RECEIVED SEPTEMBER 22, 1941

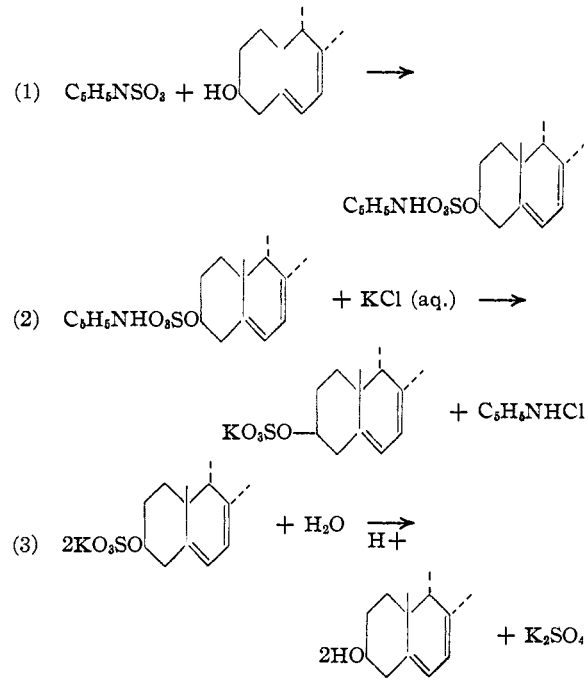
[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF THE POLYTECHNIC INSTITUTE OF BROOKLYN, BROOKLYN, NEW YORK, AND THE PEDIATRIC RESEARCH LABORATORY OF THE JEWISH HOSPITAL OF BROOKLYN]

Steryl Sulfates. II. Isolation and Separation of Sterols

BY ALBERT E. SOBEL[1] AND PAUL E. SPOERRI

As pointed out in the first paper of this series[2] the preparation of steryl sulfates may be of aid in the isolation and separation of sterols. The properties that recommend the sulfate derivatives for this purpose are: (1) ease of formation in quantitative yields, (2) insolubility in lipid solvents, (3) inexpensiveness when compared to digitonides, which are commonly employed because of their insolubility in lipid solvents.

An illustration of the usefulness of the sulfate derivative for the separation of sterols is the complete removal of cholesterol from the reaction products of the thermal decomposition of calcium and potassium cholesteryl sulfates. Previously, the costly digitonin would have been employed in such a process. A second application of the sulfate derivative is the quantitative removal of cholesterol from cholesteryl acetate. Here, again, digitonin would have been employed in the past. A third application is the successful isolation of ergosterol from a natural product, *i. e.*, brewer's yeast. The lipid fraction of the yeast was isolated by refluxing with hot alcohol in order to break up the combined form of ergosterol (probably in combination with protein[3]) and by extracting with ether. The combined extracts were then evaporated to dryness. Attempts made to use this product directly were unsuccessful. Therefore, the oil was purified by extracting it with petroleum ether and discarding the insoluble portion. The lipid extracts were then saponified and the ergosterol isolated by the following reactions



(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) C. E. Bills, *Physiol. Rev.*, **15**, 1 (1935).

Since the purpose of this last experiment was to illustrate the isolation of a sterol from a natural product by means of the sulfate derivative, no attempt was made to make the process quantitative.

There are many other applications where chemists working with sterols may employ the sulfate derivatives. The above are only typical examples of problems commonly encountered in which the use of the sulfate derivatives offers a simple solution.

Experimental

(1) **The Removal of Cholesterol from Reaction Mixtures.**—One of the products of the thermal decomposition of calcium and potassium cholesteryl sulfates (to be described in a subsequent article), in the presence of traces of water, is cholesterol, as is shown by the digitonin test.[4] Three to 4 g. of the ether soluble portion of the reaction mixture was dissolved in 40 to 50 ml. of benzene to which was added 2 g. of pyridine sulfur trioxide. The mixture was heated to 56–60° for thirty minutes and then cooled; 250 ml. of petroleum ether (35–60°) was then added to the reaction mixture. The precipitate which formed was filtered off and washed with petroleum ether. The combined mother liquors were evaporated to dryness either under vacuum or with an electric fan; 5.0 mg. of the dry residue in 4 ml. of 95% alcohol was examined for the presence of cholesterol by the digitonin test. No digitonide was formed. Since this test is sensitive to less than 0.05 mg. of cholesterol, the complete or practically complete removal of cholesterol was indicated in these experiments.

(2) **Separation of Cholesterol from Cholesteryl Acetate.**—To 1.191 g. of cholesterol and 1.027 g. of cholesteryl acetate dissolved in 23 ml. of benzene, was added 1.2 g. of pyridine sulfur trioxide. The mixture was heated to 56–60° for forty minutes, with frequent shaking, under anhydrous conditions. The reaction mixture was then cooled and 110 ml. of petroleum ether (30–60°) added. The precipitate was filtered off and washed twice with petroleum ether. This precipitate is pyridonium cholesteryl sulfate. The cholesterol may be regenerated by refluxing for thirty minutes in 100 cc. of 70% methyl alcohol which is 2 *N* with respect to sulfuric acid. On cooling, the crystals of pure cholesterol may be filtered off.

The mother liquors were carefully evaporated on a steam-bath at 60°. The weight of the desiccator-dried residue (cholesteryl acetate) was 1.011 g. This was tested for the presence of pyridine sulfur trioxide. The tests were negative. Another portion of the solid was quantitatively examined for the presence of free cholesterol by the digitonin method[4]; 20 mg. contained between 0.01 to 0.02 mg. of free cholesterol. Thus, a practically quantitative separation of free cholesterol was accomplished in one step.

(3) **Isolation of Ergosterol from Yeast.**—The yeast for these experiments (which was donated by the Mead Johnson Company) was of the drug store type which is supplied in 6-oz. bottles. Before use, a small specimen was tested for antirachitic activity both before and after irradiation. The irradiated specimen produced healing in rachitic rats while the non-irradiated specimens were biologically inactive. Thus, the presence of the provitamin D (ergosterol) was shown. 170 grams of yeast was refluxed with 300 cc. of 95% alcohol for thirty minutes. The solution was filtered while hot on a Büchner funnel and the residue again extracted with 200 cc. of hot alcohol and filtered. The residue thus obtained was extracted with 300 cc. of ether. All the extracts were combined and the solvents removed under reduced pressure. The oily residue was extracted with two portions of hot petroleum ether (35–60°), a total volume of 200 cc. being used. The petroleum ether extracts were filtered and evaporated to dryness under reduced pressure. The residue was then taken up in 200 cc. of hot 95% alcohol and transferred to a three-necked flask equipped with a mercury sealed stirrer and a reflux condenser. Twenty grams of finely ground $Ba(OH)_2 \cdot 8H_2O$ was added and the mixture refluxed for sixty minutes with vigorous stirring. At the end of this period, the mixture was cooled and carbon dioxide gas passed into the solution to precipitate the excess barium hydroxide. The mixture was then heated, filtered while hot, and the precipitate extracted once with 200 cc. of hot 95% alcohol. The alcoholic extracts were taken to dryness and the dry residue dissolved in 50 cc. of benzene. The benzene extract after drying over calcium chloride was filtered and treated with 2 g. of pyridine sulfur trioxide, 5 cc. of pyridine and 5 cc. of acetic anhydride under conditions similar to those described for the preparation of pyridonium cholesterol sulfate by method B.[2] The weight of the crude precipitate was 2.4 g. This was suspended in 50 cc. of water and 50 cc. of 10% potassium chloride added. It was then shaken for ten minutes, allowed to stand for three hours and filtered. The precipitate, which was yellow and gummy in appearance, was then washed three times with water, once with methyl alcohol and finally once with petroleum ether. The resultant product was white (the methyl alcohol removing most of the color). It decomposed sharply at 211°, which is identical with the decomposition point of potassium ergosteryl sulfate.[2] The yield was 0.3 g. It gave a positive reaction with Rosenheim and Callow's mercuric acetate reagent,[5] indicating the presence of a conjugated double bond, which is characteristic of ergosterol.

This potassium salt was decomposed in a sealed tube at 105° with 5 cc. of water and one drop of sulfuric acid (one hour treatment). The contents of the sealed tube were extracted with ether and the extract dried over sodium sulfate. The dried ether extract was filtered and evaporated to dryness with an electric fan. The residue was a white solid. The yield was 0.16 g., m. p. 160–163°. It gave no depression with a sample of ergosterol melting at 160–162°. The compound on irradiation produced healing in rachitic rats. It gave a positive test with Rosenheim and Callow's mercuric acetate reagent.[5] Furthermore, it exhibited a high negative rotation characteristic of ergosterol $[\alpha]^{25}D$ −103.5° ($CHCl_3$, C = 2.165). These criteria indicate the presence of a good grade of ergosterol.

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

(5) O. Rosenheim and R. K. Callow, *Biochem. J.*, **25**, 74 (1931).

Summary

Sterols may be isolated and separated as steryl sulfates because of their (1) ease of formation in quantitative yields, (2) insolubility in lipid solvents, (3) inexpensiveness when compared to digitonides, which are commonly employed due to their insolubility in lipid solvents.

Three examples are given. (1) Cholesterol was removed from a reaction mixture, *i. e.*, the thermal decomposition products of calcium and potassium cholesteryl sulfates. (2) Cholesterol was quantitatively separated from its ester, *i. e.*, cholesteryl acetate. (3) Ergosterol was isolated from a natural product, *i. e.*, brewer's yeast.

BROOKLYN, N. Y. RECEIVED OCTOBER 10, 1941

[CONTRIBUTION NO. 256 FROM THE RESEARCH LABORATORY OF ORGANIC CHEMISTRY OF THE MASSACHUSETTS INSTITUTE OF TECHNOLOGY]

The Partial Reduction of Acetylenes to Olefins Using an Iron Catalyst. II. Enyne and Dienyne Reduction[1]

BY A. F. THOMPSON, JR., AND E. N. SHAW[2]

Not much has been published describing partial hydrogenation of the triple bond in substances containing the conjugated enyne and dienyne systems. Zal'kind and Khudekova[3] reported the formation of 5-methyl-heptadiene-1,3-ol-5 by the addition of one molecule of hydrogen to the corresponding vinylethynylcarbinol, in the presence of a palladium-starch catalyst. Golovchanskaya,[4] by electrolytic reduction of the same vinylethynyl derivative, obtained a very complex mixture of products, with evidence of 1,4 addition, 1,2 addition and isomerization. The production of butadiene by partial reduction of vinylacetylene has been patented.[5] Blomquist and Marvel,[6] have described experiments designed to introduce one molecule of hydrogen into divinylacetylene, using platinum oxide as a hydrogenation catalyst, but they obtained a mixture of products.

Partial hydrogenation of enynes and dienynes is most unpromising unless a catalyst is used which acts selectively on the acetylenic link. Otherwise a complex mixture of hydrocarbons is very likely to result, difficult to separate due to small differences in properties of the component hydrocarbons. Thus, electrolytic reduction as practiced by Golovchanskaya[4] and hydrogenation with most catalysts are likely to give discouraging results. However, palladium and Raney nickel[7] have been shown to exhibit a selective action which makes hydrogen attack on the triple bond proceed virtually to completion before the olefin linkage begins to be reduced. In addition, these catalysts have a further important characteristic; when all acetylenic linkages have been partially reduced, further hydrogenation of the double bonds proceeds at a measurably slower rate. Recently, Paul and Hilly[8] reported the preparation of an iron catalyst which was not only selective but had the valuable property of catalyzing acetylene hydrogenation without being measurably effective in catalyzing reduction of olefins. In the first paper of this series,[1] the properties of this iron catalyst were studied, and Paul and Hilly's results to some extent confirmed. Most interesting was the isolation of isoprene, characterized as its maleic anhydride addition product, from persistent reduction of the corresponding methylvinylacetylene derivative. This result suggested possible application of the iron catalyst for partial reduction of enynes and dienynes now being prepared in connection with synthetic work in progress in this Laboratory.

The hydrogenation of a number of acetylenes, most of them enynes and dienynes, has been studied in the presence of the iron catalyst. It can now definitely be stated that the catalyst is not completely specific for the carbon–carbon triple bond. On the other hand, all the evidence available shows that the substance has the same selective action as palladium and Raney nickel, and that there is a marked decrease in the rate of reduction of the olefin as compared with the acetylene. Moreover, in a number of cases the

(1) First paper of this series: THIS JOURNAL, **62**, 2555 (1940).

(2) From the thesis submitted by E. N. Shaw to the Massachusetts Institute of Technology in partial fulfillment of the requirements for the degree of Bachelor of Science.

(3) Zal'kind and Khudekova, *J. Gen. Chem.* (U. S. S. R.), **10**, 435 (1940).

(4) Golovchanskaya, *ibid.*, **10**, 521 (1940).

(5) U. S. Patents 1,920,242 and 2,207,070.

(6) Blomquist and Marvel, THIS JOURNAL, **55**, 1655 (1933).

(7) Campbell and O'Connor, *ibid.*, **61**, 2897 (1939). This paper includes numerous references to earlier investigations of partial hydrogenation.

(8) Paul and Hilly, *Bull. soc. chim.*, [5] **6**, 218 (1939).