

of Jamieson and Baughman are here recalculated from percentages in the oil to percentages in the mixture of acids.

PERCENTAGE COMPOSITION OF FATTY ACIDS

Acid	J. and B. %	W. and B. %
Myristic.....	..	0
Palmitic.....	6.8	10
Stearic.....	4.4	2
Arachidic.....	0.7	1
Lignoceric.....	0.1	..
Linolenic.....	2.3	87.7
Linolic.....	51.8	
Oleic.....	33.6	

The chief discrepancy between the two sets of results lies in the distribution between palmitic acid and stearic acid of the saturated acids. No explanation of this discrepancy is offered.

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[CONTRIBUTION FROM THE BIOCHEMICAL LABORATORY, NEW YORK AGRICULTURAL EXPERIMENT STATION]

REDUCTION OF SITOSTEROL
PREPARATION OF DIHYDROSITOSTEROL OR SITOSTANOL

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Introduction

The properties of the saturated sterols that we have isolated from the endosperm of corn¹ and wheat² as previously described resembled to a considerable degree those of dihydrocholesterol or β -cholestanol, which substance was first prepared by Willstätter and Mayer³ by reducing cholesterol with hydrogen in the presence of platinum black. In order that the properties of these substances might be compared we decided to make some attempts to reduce sitosterol with hydrogen using platinum black as the catalyst. The dihydrositosterol that should be formed if the reduction were successful might prove to be identical with the natural dextro-rotatory saturated sterol occurring in the endosperm of different grains.

So far as we are aware, dihydrositosterol has never been prepared by reduction with hydrogen. The substance, prepared by Windaus and Hauth⁴ by boiling a solution of sitosterol in amyl alcohol with metallic sodium and described by these authors as dihydrophytosterol, melted at 175°, and it was not saturated since it absorbed bromine. It was probably

¹ Anderson, *THIS JOURNAL*, **46**, 1450 (1924).

² Anderson and Nabenhauer, *ibid.*, **46**, 1717 (1924).

³ Willstätter and Mayer, *Ber.*, **41**, 2199 (1908).

⁴ Windaus and Hauth, *Ber.*, **40**, 3681 (1907).

analogous to α -cholestanol that is produced when cholesterol is boiled with metallic sodium in amyl alcohol.⁵ Windaus and Uibrig⁶ have shown, however, that α -cholestanol is a condensation product of cholesterol and amyl alcohol.

Preliminary experiments indicated that the procedure outlined by Voorhees and Adams⁷ was quite suitable for the reduction of the unsaturated sitosterol. The crude apparatus that we employed did not permit the determination of the amount of hydrogen that was absorbed, but the analysis of the reduction product showed clearly that a saturated sterol of the formula $C_{27}H_{47}OH$ had been formed.

The reduction did not proceed absolutely to completion. A small quantity of sitosterol escaped reduction and it had to be eliminated by recrystallization.

The dihydrositosterol or sitostanol was found to be dextrorotatory. The specific rotation in chloroform solution of the purest product obtained was $+24.16^\circ$. The dried substance melted⁸ between 141° and 142° . The Liebermann-Burchard reaction was negative. The color reactions for sterols recently elaborated by Whitby⁹ when applied to dihydrositosterol gave either negative or atypical results indicating that unchanged sitosterol was absent. In properties and composition and in the failure to give the usual phytosterol color reactions, dihydrositosterol is very similar to, if not identical with, the saturated sterol that occurs in the endosperm of corn and wheat. That the alcoholic hydroxyl has not been changed was shown by the fact that an acetyl derivative, m. p. $138-139^\circ$, was readily formed on heating the substance with acetic anhydride.

Dihydrositosterol has the same melting point as β -cholestanol and the specific rotations are nearly alike but the acetyl derivatives differ greatly in melting point.

Experimental Part

The platinum oxide was prepared according to the method of Voorhees and Adams.⁷ The reduction was carried out in a liter distilling flask. The side tube was connected with a mercury manometer and the hydrogen was admitted through a glass stopcock passing through a tightly fitting rubber stopper at the top of the flask.

The sitosterol used in the first experiment had been isolated from wheat bran and its specific rotation was -30.90° .

A solution of 10 g. of this product in 300 cc. of ether, contained in the flask mentioned above, was mixed with 0.65 g. of platinum oxide. Practically all of the air was

⁵ Diels and Abderhalden, *Ber.*, **39**, 1155 (1906).

⁶ Windaus and Uibrig, *Ber.*, **46**, 2487 (1913).

⁷ Voorhees and Adams, *THIS JOURNAL*, **44**, 1397 (1922).

⁸ The melting points given in this paper are uncorrected.

⁹ Whitby, *Biochem. J.*, **17**, 5 (1923).

removed from the flask by means of a vacuum pump and hydrogen was introduced to a pressure of $1/2$ atmosphere. After the contents of the flask had been agitated for a few minutes the brown color of the platinum oxide changed to black and hydrogen was absorbed very rapidly. The pressure of hydrogen was maintained at 310–362 mm. and the flask was shaken constantly. The reaction was practically completed in about 0.5 hour and the platinum black which had been uniformly distributed throughout the liquid settled to the bottom. The solution was allowed to stand under the above-mentioned pressure of hydrogen overnight. In the morning the solution was found to contain a considerable amount of long prismatic crystals which dissolved on the addition of more ether. The liquid was filtered and the ether distilled. The snow-white crystalline residue was dissolved in 500 cc. of boiling alcohol. The substance was only slightly soluble in the alcohol, and continued boiling was necessary to dissolve it. After the solution had been filtered and allowed to cool, the substance separated in beautiful, elongated, hexagonal plates that appeared to be identical with the usual crystals of dihydrositosterol. The substance was again crystallized in the same manner, filtered off, washed with cold alcohol and dried in the air. The crystals weighed 9.1 g.

In chloroform solution the substance did not absorb bromine. In the Liebermann-Burchard reaction it gave no immediate coloration, but after the reaction mixture had stood for some time a faint green color developed. The typical reaction was, however, entirely absent. The air-dried substance melted at 141° , and after drying at 105° it melted between 142° and 143° .

Rotation. Dried subs., 0.8293: dissolved in chloroform and made up to 18 cc., it gave in a 2dm. tube a reading of $+2.17$; $[\alpha]_D^{20}$, $+23.55^\circ$.

The substance was dried for analysis at 105° in a vacuum over phosphorus pentoxide. The loss in weight on drying was 4.75% and 4.72% corresponding to one molecule of water of crystallization.

Anal. Subs., 0.1864: H_2O , 0.2062; CO_2 , 0.5705. Calc. for $C_{27}H_{47}OH(388)$: C, 83.50; H, 12.37. Found: C, 83.47; H, 12.37.

The reactions and composition of this substance indicated that it was dihydrositosterol. The specific optical rotation was a little lower, however, than that of the saturated sterols previously isolated from the endosperm of corn and wheat. This lower rotation might have been due to a slight contamination with unchanged sitosterol.

In order to make sure that the reduction was complete the substance was dissolved in 500 cc. of ether and again shaken with platinum oxide and hydrogen in the manner described above. After isolating and recrystallizing the substance from alcohol 7.37 g. of colorless crystals was recovered. The melting point was the same as before and on analysis the following results were obtained.

Found: H_2O , 4.73, 4.72; C, 83.72; H, 12.49; $[\alpha]_D^{20}$, $+23.52^\circ$.

These results are practically identical with those obtained in the first analysis.

For further purification the substance was twice recrystallized from 300 cc. of light petroleum ether from which it separated in tufts of fine needles, and it was twice recrystallized from alcohol. It crystallized from alcohol in large, hexagonal plates. The air-dried substance weighed 2.2 g., and melted between 140° and 141° . After it had been dried in a vacuum at 105° over phosphorus pentoxide it melted between 141° and 142° . The melted substance began to solidify at 138° and the solid remelted at 143° .

The Liebermann-Burchard reaction was completely negative. In the Whitby reaction A, the chloroform gradually assumed a yellow color that changed slowly to pale orange and the sulfuric acid slowly assumed a faint yellow color. The addition of acetic anhydride to the orange colored chloroformic solution gave a cherry-red that changed

rapidly into purple and more slowly to brown with strong green fluorescence. The Whitby reaction B gave no coloration.

On drying as described above the substance lost 4.48% and 4.42% in weight, corresponding to one molecule of water of crystallization. The dried substance was analyzed.

Anal. Subs., 0.1874: H₂O, 0.2084; CO₂, 0.5730. Calc. for C₂₇H₄₇OH(388): C, 83.50; H, 12.37. Found: C, 83.39; H, 12.44.

Rotation. Dried subs., 1.0430: dissolved in chloroform and made up to 18 cc., it gave in a 2dm. tube a reading of +2.80°; $[\alpha]_D^{20}$, +24.16°.

ACETYL DERIVATIVE.—The substance, 0.9 g., was boiled with 15 cc. of acetic anhydride for two hours. After the solution had cooled the crystals were filtered off, washed with acetic anhydride and recrystallized from alcohol. The large, colorless, hexagonal plates were dried in the air, and then weighed 0.75 g. The substance melted between 138° and 139°. There was no loss in weight on drying at 105°.

Anal. Subs., 0.1950: H₂O, 0.2039; CO₂, 0.5770. Calc. for C₂₇H₄₇O.CO.CH₃(430): C, 80.93; H, 11.62. Found: C, 80.70; H, 11.70.

Summary

The unsaturated levorotatory sitosterol has been reduced with hydrogen, in ethereal solution, in the presence of platinum black. In this reduction dextrorotatory dihydrositosterol or sitostanol, C₂₇H₄₇OH, is formed.

The dihydrositosterol obtained by reducing sitosterol appears to be identical with the dihydrositosterol that has been isolated from corn and wheat endosperm. The dried substance melts between 141° and 142°. In chloroform solution the specific dextrorotation was +24.16°.

The substance crystallized in large colorless hexagonal plates containing one molecule of water of crystallization. The Liebermann-Burchard reaction is negative and the Whitby reactions are atypical. The substance does not absorb bromine.

The acetyl derivative crystallizes in large, hexagonal plates free from water of crystallization. It melts between 138° and 139°.

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