hydrogen and no active group other than the system C=NO which is characteristic of isoxazoline oxides.

2. Triphenyl-isoxazoline oxide while less readily "stripped" than the oxides, which contain an acyl group, can be dehydrated with basic or acidic dehydrating agents and to some extent by heat alone. There is no evidence that the process involves an intermediate opening of the ring.

3. Phosphorus pentachloride removes the oxido oxygen; in this respect alone the oxide behaves like diphenyl-furoxan.

4. The oxide, when reduced with mild reducing agents, combines with two atoms of hydrogen and forms a substance which is sufficiently acidic to give a copper compound when it is shaken with copper acetate.

CAMBRIDGE 38, MASSACHUSETTS

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

SITOSTEROL

BY R. J. ANDERSON AND FRED P. NABENHAUER

RECEIVED JUNE 9, 1924 PUBLISHED SEPTEMBER 5, 1924

Introduction

There is much confusion in the literature dealing with plant sterols regarding such physical properties as melting points and optical rotation, but the substance known as sitosterol is probably more fully characterized than any other plant sterol. The original description of sitosterol by Burian[1] was amplified by Ritter,[2] and the properties described by these authors have in general been confirmed by other investigators.

Sitosterol is stated to melt at 137.5° and to have a specific rotation in chloroform solution of about —34°. The acetyl derivative melts at 127°. A phytosterol possessing the above-mentioned properties is rather easily obtained from fats extracted from the germ of wheat, rye or corn. It would seem, therefore, that the sterol deposited in the germ is practically homogeneous.

It has been found in this Laboratory that the endosperm of corn[3] and of wheat[4] contains a mixture of sterols consisting of sitosterol and dihydrositosterol. It would not seem impossible that a mixture of these two sterols might also be deposited in the germ although they would probably exist there in different proportions from that in the endosperm.

In the hope of removing any dihydro-sitosterol that might be present we have attempted to purify some sitosterol preparations by fractional

---

[1] Burian, *Monatsh.*, **18**, 551 (1897).

[2] Ritter, *Z. physiol. Chem.*, **34**, 461 (1901).

[3] Anderson, THIS JOURNAL, **46**, 1450 (1924).

[4] Anderson and Nabenhauer, *ibid.*, **46**, 1717 (1924).

precipitation of the dibromo compound from its solution in ether and glacial acetic acid. It was expected that any dihydro-sitosterol that might be present in the material would remain in the ether-glacial acetic acid solution.

It is well known that sitosterol is easily regenerated from the dibromo compound on boiling it with zinc dust in glacial acetic acid or acidified alcohol. A number of such regenerated sitosterols have been described by Windaus and Hauth,[5] Heiduschka and Gloth,[6] Wagner and Clement,[7] Matthes and Heintz,[8] and by Anderson.[9] It has always been found, however, that the regenerated sitosterol preparations possessed the same melting point as the starting material.

Windaus and Hauth[10] were able to separate stigmasterol from sitosterol by brominating the mixed acetates in ether-glacial acetic acid solution. The tetrabromo-stigmasterol is much less soluble than the dibromo-sitosterol and it separates from the bromination mixture in a crystalline form. We have observed on brominating sitosterol acetate[9] that a part of the dibromo compound can be precipitated on cooling the brominating solution in a freezing mixture and the remainder may be obtained on diluting the solution with water.

Samples of rather pure sitosterol were fractionated in this manner and the fractions separately examined. The results show clearly that the first fraction possesses a higher melting point and a higher levorotation than the last fraction which is obtained by precipitation with water.

It is probable, therefore, that even the purest preparations of sitosterol that have been obtained by crystallization in the usual way have been contaminated by small amounts of dihydro-sitosterol. As a result of such an admixture the melting point as well as the levorotation has been depressed.

Different preparations of sitosterol, after being purified as will be described in the experimental part, melted between 138° and 139°, and one preparation as high as 140–141°, while the specific optical rotation varied from —34.9° to —36.64°. The acetyl derivative melted between 130° and 131°. The melting point of the purified sitosterol is nearly identical with that of dihydro-sitosterol but mixtures of these substances, as shown in another paper,[4] may melt as low as 129°.

Experimental Part

Purification of Sitosterol from Corn Gluten.—This preparation was obtained from the bottom fraction of the corn gluten sterols as described in another paper.[3]

[5] Windaus and Hauth, *Ber.*, **40,** 3681 (1907).

[6] Heiduschka and Gloth, *Pharm. Zentr.*, **49,** 863 (1908).

[7] Wagner and Clement, *Z. Nahr. Genussm.*, **17,** 266 (1909).

[8] Matthes and Heintz, *Arch. Pharm.*, **247,** 161 (1909).

[9] Anderson, THIS JOURNAL, **45,** 1944 (1923).

[10] Windaus and Hauth, *Ber.*, **39,** 4378 (1906).

The substance weighed 21 g. and in chloroform solution it had a specific rotation of —27.96°. It was dissolved in 1000 cc. of alcohol and on cooling 16.1 g. of crystals separated. The mother liquor was concentrated and allowed to cool whereupon 4.8 g. of crystals was obtained. Another fraction obtained in a similar manner was added to this material, thus making a total of 7.7 g. of the bottom fraction. This substance, after decolorizing with Norite, and twice recrystallizing from alcohol, yielded 6.5 g. of colorless crystals. It melted between 136° and 137°; in chloroform solution, $[\alpha]_D^{20}$ —32.34°. This preparation, which consisted of nearly pure sitosterol, was acetylated and the acetyl derivative brominated by the method of Windaus and Hauth[9] as follows. It was dissolved in 60 cc. of ether and to this solution was added 3.5 g. of bromine dissolved in 100 cc. of glacial acetic acid. The solution was brown, indicating an excess of bromine. No precipitate formed at room temperature, but on cooling in a freezing mixture a white amorphous precipitate separated. It was collected on a filter, washed with 75% acetic acid and dried in a vacuum over sulfuric acid and potassium hydroxide. The dibromo-sitosterol acetate formed a faintly yellowish, amorphous powder which softened at 120° and melted at 124°. It was debrominated by boiling with 5 g. of zinc dust, 10 cc. of glacial acetic acid and 100 cc. of alcohol for three hours. The colorless solution was filtered to remove the excess of zinc, diluted with water and extracted with ether. The ether was evaporated and the residue saponified by boiling it with alcoholic potassium hydroxide. The solution was then diluted with water, extracted with ether and the solvent evaporated, when it yielded a product which was dissolved in alcohol, decolorized with Norite and recrystallized thrice from alcohol. Snow-white crystals were then obtained which, after drying in the air, weighed 2.5 g.; m. p., 138–139°.

Rotation. Dry subs., 0.9074: dissolved in chloroform, made up to 20 cc. in a 2-dm. tube, —3.33°; whence $[\alpha]_D^{20}$, —36.69°.

For analysis the substance was dried at 105° in a vacuum over phosphorus pentoxide.

Anal. Subs., 0.1661: H_2O, 0.1745; CO_2, 0.5096. Calc. for $C_{27}H_{45}OH$: C, 83.93; H, 11.91. Found: C, 83.67; H, 11.75.

The substance was again boiled with alcoholic potassium hydroxide, isolated as before and twice recrystallized from alcohol; m. p., 138–139°.

Rotation. $[\alpha]_D^{20}$, —36.64°.

Anal. Found: C, 83.94; H, 11.75.

The acetyl derivative was prepared and twice recrystallized from alcohol. It separated in fine, colorless crystals containing no water of crystallization. It melted at 130–131°; in chloroform solution, $[\alpha]_D^{20}$, —40.20°.

Anal. Subs., 0.1295: H_2O, 0.1307; CO_2, 0.3873. Calc. for $C_{27}H_{45}O.CO.CH_3$: C, 81.31; H, 11.21. Found: C, 81.56; H, 11.29.

For the purpose of comparison, two other sitosterol preparations were purified in the manner described above. One of these had been isolated

TABLE I

MELTING POINT, ROTATION AND COMPOSITION OF SITOSTEROL AND OF THE ACETYL DERIVATIVE AFTER PURIFICATION THROUGH THE DIBROMO COMPOUND

	Sitosterol, $C_{27}H_{45}OH$				Acetyl derivative			
Source	M. p. °C.	$[\alpha]_D^{20}$	C %	H %	M. p. °C.	$[\alpha]_D^{20}$	C %	H %
Wheat bran..	140–141	−34.90°	84.01	11.82	130–131	.....	81.45	11.26
Crude corn oil	138–139	−34.87°	83.80	11.93	128–130	−38.81°	81.29	11.24
Corn gluten..	138–139	−36.69°	83.94	11.75	130–131	−40.20°	81.56	11.29

from wheat bran, m. p. 137°, $[\alpha]_D^{20}$ —33.46°; and the other had been obtained from crude corn oil, m. p. 137.5°, $[\alpha]_D^{20}$ —34.38°. The melting point, optical rotation and composition of the purified preparations are shown in Table I.

The data show that the top fractions of dibromo-sitosterol acetate yield, after debromination, saponification and recrystallization, sitosterol preparations that have higher melting points and greater levorotations than the original material.

The bottom fractions of the dibromo compound that were precipitated by the addition of water were debrominated and purified as described above. These preparations all had lower levorotations than the original substances. The bottom fraction from wheat bran, for instance, had $[\alpha]_D^{20}$, —28.21°.

Water of Crystallization in Purified Sitosterol

The purified sitosterol lost its water of crystallization very easily when dried in the air at a temperature of about 60°. Some loss of water occurred even when the preparations were dried in the air at room temperature. Different preparations that we analyzed, after having been dried in the air at room temperature, lost on further drying at 105° in a vacuum over phosphorus pentoxide from 3.21 to 3.87% in weight. Every preparation that we examined contained less than one molecule of water of crystallization.

Relation between Rotation and Percentage Composition of Mixtures of Sitosterol and Dihydro-sitosterol

The results of our investigations indicate that the phytosterol occurring in corn and wheat consists of a mixture of sitosterol and dihydro-sitosterol. The two compounds occur throughout the grain but the germ contains mostly sitosterol while the endosperm is richer in dihydro-sitosterol and the latter is present in even larger amounts in the bran.

The percentage of the two components that may be present in any mixture may be calculated by means of the formulas, $S = (25.82 - R)/0.6246$, and $P = (36.64 + R)/0.6246$, where S and P are the percentages of sitosterol and dihydro-sitosterol, respectively, and R is the specific rotation in chloroform.

When the composition is plotted against the rotation the curve obtained is a straight line as shown in Fig. 1. The approximate composition of any mixture may therefore be determined directly from the specific rotation by means of this curve.

The values of —36.64° for sitosterol and +25.82° for dihydro-sitosterol were chosen, having been the highest values obtained and representing the purest preparations of these two compounds which were isolated.

In order to substantiate the assumption that a straight line would represent the relation between the composition and optical rotation, several artificial mixtures were prepared and the observed rotations compared with those calculated. As no pure compounds were available in large enough quantities, two mixtures with rotations of + 23.2° and —29.03° designated as A and E, respectively, were used. Three mixtures were made of these two and the observed rotations are given in Table II.

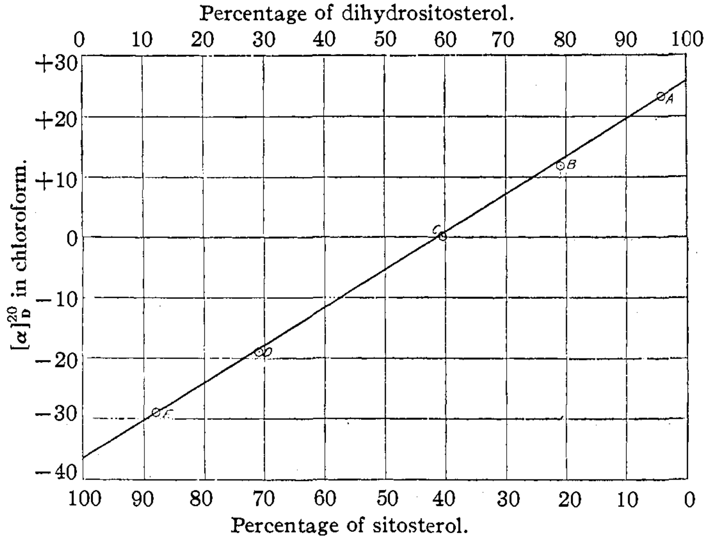

Fig. 1.

Table II

Observed and Calculated Rotations of Mixtures of Sitoserol and Dihydrositosterol

Mixture	Composition % A	 % E	 Sitosterol calcd. %	 Dihydrositosterol calcd. %	Rotation in chloroform Calcd.	 Found
A	100	0	4.1	95.9	.....	+23.20°
B	79.8	20.2	20.9	79.1	+12.6°	+11.93°
C	56.6	43.4	40.5	59.5	+ 0.5°	+ 0.19°
D	20.1	79.9	70.8	29.2	−18.5°	−19.20°
E	0	100	87.8	12.2	.....	−29.03°

The value found corresponds quite closely with the calculated value in each case.

The percentages of sitosterol in the two mixtures A and E were calculated from the rotation, and in B, C and D from the proportions of A and E used in making these mixtures. These five points are on the graph, although they were not used directly in drawing the curve.

It should be noted that the above formulas and curve hold only for mixtures containing the two sterols having the rotations of +25.82° and

—36.64° as is the case in the oils from various parts of the two grains studied, namely, wheat and corn.

Summary

An effort has been made to prepare sitosterol in as pure a condition as possible and to study the physical properties of the purified preparations.

The results indicate that sitosterol as ordinarily prepared is contaminated with small amounts of dihydro-sitosterol. The greater part of this impurity can be removed by fractional precipitation of the dibromo compound.

The sitosterol regenerated from the dibromo compound possesses a higher melting point and a higher levorotation than preparations purified only by ordinary recrystallization.

GENEVA, NEW YORK

[CONTRIBUTION FROM THE COLOR LABORATORY, BUREAU OF CHEMISTRY, UNITED STATES DEPARTMENT OF AGRICULTURE, NO. 91]

THE INFLUENCE OF CONSTITUTIONAL VARIATION IN DYES UPON THEIR RELATIVE ABSORPTION IN AQUEOUS AND ALCOHOLIC SOLUTIONS[1]

BY WALTER C. HOLMES

RECEIVED JUNE 11, 1924 PUBLISHED SEPTEMBER 5, 1924

It was recognized by the earliest investigators in spectroscopy that colorless solvents affect the absorption spectra of colored solutes. The rule was formulated by Kundt[2] that the position of the absorption band of the solute depends on the refractive index of the solvent, being displaced toward the red end of the spectrum as the refractive index increases.

Subsequent investigations bearing on the question[3] have not, for the greater part, confirmed the validity of Kundt's rule. It has been established that if any fundamental relation exists between the refractive index of the solvent and the absorption of the solute its influence is greatly obscured by the predominant effect of other factors. In numerous instances, as in the recent investigation of the absorption of alizarin-cyanine in organic solvents by Meek,[4] it has been made apparent that the chemical nature of the solvent has a decisive influence on the phenomenon.

The method of procedure employed in these investigations was a study of the absorption of a comparatively small number of dyes in as compre-

---

[1] Presented before the Dye Section of the American Chemical Society at its meeting, April 21–26, 1924.

[2] Kundt, *Pogg. Ann. Jubelband,* **1874,** 615–624. *Sitzb. Bayer. Akad.,* **7,** 234 (1877). *Wied. Ann.,* **4,** 34 (1878).

[3] Most of these have been reviewed and discussed by Kayser, "Handbuch der Spectroscopie," S. Hirzel, Leipzig, **1905,** III, pp. 80–89.

[4] Meek, *J. Chem. Soc.,* **111,** 969 (1917).