The two enzyme preparations studied behaved alike and the data of many experiments with both preparations showed that in each case the change in the direction of migration occurs between P_H 4.3 and 4.5. On the acid side of this range the enzyme migrates toward the cathode, and on the alkaline side it migrates toward the anode.

Thus the iso-electric point or zone of malt amylase is definitely established at P_H 4.3 to 4.5 which coincides with that of its optimum enzymic action upon starch.

NEW YORK, N. Y.

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

THE PHYTOSTEROLS OF WHEAT ENDOSPERM

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Introduction

It has been shown in a previous paper from this Laboratory that corn endosperm[1] contains two different phytosterols. One is identical with sitosterol and the other was found to be a saturated sterol corresponding to dihydrositosterol, $C_{27}H_{47}OH$. We have extended the investigation to include other plant material and in this paper we report upon the results obtained in an examination of the phytosterols occurring in the wheat endosperm.

We have separated the unsaponifiable matter from the fat extracted from wheat endosperm into three parts as follows: (1) an unsaturated sterol identical with sitosterol; (2) a saturated sterol corresponding to dihydrositosterol, $C_{27}H_{47}OH$; (3) a non-crystallizable oil that we have not examined.

The purest dihydrositosterol obtained from wheat endosperm melted[2] between 144° and 145°, and $[\alpha]_D^{20}$ in chloroform was +25.82°. These values are slightly higher than those given by the saturated sterol isolated from corn endosperm but the analyses agree closely with the calculated composition of dihydrositosterol. Whether these sterols are chemically identical or if they are isomers cannot be determined from the present data. The two preparations possess the same crystal form and solubility and give the same reactions and in general are so nearly alike that we are inclined to believe that they are identical.

Experimental Part

The material for this investigation was obtained from a near-by flour mill and consisted of the crushed wheat endosperm after the bran and germ

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

[2] Melting points given in this paper are uncorrected.

had been sifted out. The flour, 3 kg., was macerated in petroleum ether for three days. It was then filtered out and washed with three portions of petroleum ether. The solvent was recovered by distillation and the residue, after drying, formed a pale yellow, semi-solid fat that weighed 20.5 g. The flour was further digested with ether on the water-bath under the reflux condenser for five hours. It was then filtered off and washed and the ether was distilled. The residue, after drying, weighed 4 g. The total fat obtained is therefore 0.81%.

The two fractions of fat were united, saponified by boiling with alcoholic potassium hydroxide, and the unsaponifiable matter was extracted with ether. After the ether was distilled and the residue dried, 2.05 g. of a yellow crystalline mass was obtained. This crude phytosterol was purified by crystallization from alcohol until it was snow white; m. p., 138.5°; $[\alpha]_D^{20}$ in chloroform, —15.6°.

Some pure wheat bran was also examined in the same manner as described above. Extraction with petroleum ether gave 3% of fat and this fat was found to contain about 6% of unsaponifiable matter. The crude phytosterol was purified by crystallization from alcohol until colorless crystals were obtained. The substance melted between 129–130°. In chloroform solution it showed hardly any optical activity. It was decided, therefore, to prepare from wheat bran a sufficient quantity of these sterols to use in this investigation.

From 65 kg. of wheat bran we obtained by extraction with petroleum ether about 2 kg. of fat. The fat was saponified and the crude phytosterol was purified by crystallizing it from petroleum ether which eliminated adhering oil and most of the coloring matter. The nearly colorless crystals weighed 50 g. The substance was dissolved in alcohol, decolorized with Norite, and allowed to crystallize. We have found that phytosterol preparations retain with great tenacity certain impurities that are insoluble in chloroform. For the purpose of removing these impurities the substance was dissolved in chloroform and the slight amount of insoluble matter was removed by filtration. After distilling the chloroform, the residue was recrystallized thrice from large volumes of alcohol. After drying in the air, the snow-white crystals weighed 17 g.; m. p., 128–129°; $[\alpha]_D^{20}$ in chloroform, —4.80°.

The mother liquors were always saved and the phytosterol retained therein was recovered and examined as will be described later.

The recrystallization of the top fraction was continued and there was observed a slow but steady rise in melting point. The levo rotation sank to 0°, and then a slow rise in dextrorotation began. These data are indicated briefly in Table I.

Lack of material prevented us from continuing the recrystallization any further but we are inclined to believe that the substance was very

nearly pure after the 38th crystallization. It gave only a very slight coloration in the Liebermann-Burchard reaction and it showed no visible absorption of bromine in chloroform solution.

TABLE I

INCREASE IN MELTING POINT AND DEXTROROTATION OF WHEAT BRAN PHYTOSTEROL ON RECRYSTALLIZATION

Recrystallization	Weight of substance G.	M. p. °C.	$[\alpha]_D^{20}$ in chloroform solution
5th	17	128–129	− 4.80°
9th	11	138–139	+ 1.32°
13th	7.8	139–141	+11.83°
18th	5.1	140–141	+17.58°
23rd	3.1	143–144	+21.40°
33rd	1.3	144.5	+24.94°
38th	0.9	144–145	+25.82°

On drying at 105° in a vacuum over phosphorus pentoxide the substance lost 3.78% in weight, being somewhat less than the calculated quantity for 1 molecule of water of crystallization.

Analysis. Dried subs., 0.1143: H_2O, 0.1292; CO_2, 0.3493. Calc. for $C_{27}H_{47}OH$ (388): C, 83.50; H, 12.37. Found: C, 83.34; H, 12.64.

ACETYL DERIVATIVE.—The remainder of the substance was boiled with acetic anhydride for 1.5 hours. The acetyl derivative crystallized, as the solution cooled, in large thin plates. It was filtered and washed with acetic anhydride and recrystallized from 125 cc. of methyl alcohol. It separated when the solution cooled in elongated hexagonal plates, the crystal form being identical with that of the free sterol. After the substance had been dried in the air it weighed 0.55 g. It did not lose in weight on drying at 105°. Heated in a capillary tube, it melted at 140°.

Analysis. Subs., 0.0997: H_2O, 0.1076; CO_2, 0.2964. Calc. for $C_{27}H_{47}O.CO.CH_3$ (430): C, 80.93; H, 11.62. Found: C, 81.08; H, 12.07.

A small quantity of the acetyl derivative was dissolved in 4 cc. of chloroform and a few drops of a dilute solution of bromine in chloroform were added. The bromine color persisted. A considerable excess of the bromine solution was then added and the solution was allowed to stand for several hours. The solvent was evaporated and the residue was recrystallized from methyl alcohol. The substance separated in large, colorless, hexagonal plates and, after it had been dried in the air, it melted between 139° and 140°. The crystal form and the melting point indicate that the substance was recovered unchanged and it is evident that the saturated sterol does not absorb any bromine.

Attempt to Separate the Wheat Bran Sterols after Brominating a Mixture of the Two Substances

It has been shown by Bondzynski and Humnicki[3] that the saturated coprosterol can be separated from cholesterol after brominating a mixture of the two substances by extracting the resulting product with petroleum ether which dissolves only the coprosterol. The wheat bran sterols, however, cannot be separated so easily because the dibromositosterol is per-

[3] Bondzynski and Humnicki, *Z. physiol. Chem.*, **22**, 396 (1896–1897).

ceptibly soluble in petroleum ether. But dibromositosterol is very much more soluble in alcohol than is the saturated sterol and it was hoped that the two substances could be separated by crystallizing the bromination product from alcohol.

An intermediate fraction of the wheat bran sterols, obtained from the mother liquors, that had a specific rotation of +10.41° was acetylated. The acetyl derivative, 1 g., was dissolved in 10 cc. of chloroform. A solution of 5 g. of bromine in 50 cc. of chloroform was added gradually. The bromine was absorbed very rapidly at first, but when 1.2 cc. of the solution described above had been added the absorption ceased and the color remained brown, indicating an excess of bromine. The solvent was evaporated and the brownish residue was dissolved in alcohol, decolorized with Norite, and recrystallized six times from alcohol. It separated in colorless, hexagonal plates that appeared to be pure, but qualitative tests indicated the presence of bromine. The substance was saponified with alcoholic potassium hydroxide, extracted with ether and recrystallized five times from alcohol. The snow-white crystals still contained traces of bromine. In chloroform solution the specific optical rotation was +22.16°. It is evident from the increase in the rotation that a large part of the sitosterol had been eliminated, but since it was impossible to obtain the substance free from bromine the method is not suitable for the separation of these sterols.

Separation of Sitosterol from the Saturated Sterol by means of Acetic Anhydride and Sulfuric Acid

A solution of 3 g. of the acetyl derivative in 50 cc. of carbon tetrachloride was treated with acetic anhydride and sulfuric acid as described in an earlier paper.[1] The substance was isolated, recrystallized thrice from alcohol and dried in the air and then weighed 1.6 g. It lost 4.99% in weight on drying at 105°, corresponding to 1 molecule of water of crystallization; m. p., 143° and 144°; $[\alpha]_D^{20}$ in chloroform solution, +23.79°. In the Liebermann-Burchard reaction no immediate coloration was produced, but after the solution had stood for some time a faint green color developed.

Analysis. Dried subs., 0.1709: H_2O, 0.1866; CO_2, 0.5222. Calc. for $C_{27}H_{47}OH$ (388): C, 83.50; H, 12.37. Found: C, 83.34; H, 12.22.

The acetyl derivative was prepared and twice recrystallized from alcohol. It melted at 139° and was free from water of crystallization.

Analysis. Subs., 0.1573: H_2O, 0.1632; CO_2, 0.4651. Calc. for $C_{27}H_{47}O.CO.CH_3$ (430): C, 80.93; H, 11.62. Found: C, 80.64; H, 11.61.

This method of removing sitosterol appears to be satisfactory and is very rapid. Judging by the analysis, the product was quite pure dihydrositosterol although the rotation was somewhat lower than that of the preparation purified by crystallization.

Examination of the Mother Liquors

The mother liquors obtained in the recrystallizations of the above-mentioned mixture of sterols from wheat bran were saved and examined separately. The middle fractions yielded 10 g. of material that showed a rotation in chloroform of +10.41°. From the mother liquors of the first fractions we isolated 16.7 g. of substance having a levorotation of —30.9°. This latter material was fractionated as follows. It was dissolved in 900 cc. of boiling alcohol and as the solution cooled 13.5 g. of crystals separated. The substance was again recrystallized from 900 cc. of alcohol, yielding 10 g. of crystals. The mother liquors were concentrated and the material that separated as the solution cooled was recrystallized several times from alcohol, yielding 4.7 g. of colorless, plate-shaped crystals; $[\alpha]_D^{20}$ in chloroform —33.46°. This bottom fraction represents therefore nearly pure sitosterol.

Summary

The phytosterols occurring in wheat endosperm have been examined. Wheat endosperm contains at least two different sterols, namely, ordinary sitosterol, $C_{27}H_{45}OH$, and dihydrositosterol, $C_{27}H_{47}OH$, m. p. 144–145°; $[\alpha]_D^{20}$, +25.82°. The dihydrositosterol from wheat bran appears to be identical with the saturated sterol that occurs in corn endosperm. The substance exists throughout the wheat endosperm but the bran is particularly rich in this sterol.

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[CONTRIBUTION FROM THE LABORATORY OF ORGANIC CHEMISTRY OF THE UNIVERSITY OF WISCONSIN]

PIPERIDINE DERIVATIVES. A CYCLIC AND AN OPEN-CHAIN COMPOUND RELATED IN STRUCTURE TO COCAINE

BY S. M. MCELVAIN

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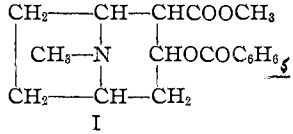
The piperidine nucleus occurs in many of the alkaloids, either as the single nucleus or in a bicyclic system. Such a bicyclic system is the basic structure upon which the molecule of cocaine is built. Since the functional groups of the cocaine molecule (I) are attached to the piperidine nucleus, piperidine derivatives of that general structure should be of interest. This communication, in part, describes the synthesis of a compound, 1-methyl-3-carbo-ethoxy-4-piperidyl benzoate (II), which has the same structure as the piperidine portion of the cocaine molecule.



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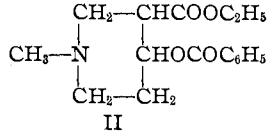

II