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

THE PHYTOSTEROLS OF THE ENDOSPERM OF CORN[1]

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Introduction

We have shown previously that corn pollen[2] contains several fractions of phytosterols that differ from ordinary phytosterol in melting points and also in that they crystallize without water of crystallization and are optically inactive. Similar phytosterols could not be isolated from corn oil, cottonseed oil or linseed oil.[3]

So far as we are aware, the unsaponifiable matter derived from the endosperm of corn has never been examined. The present investigation was begun at the suggestion of Dr. T. B. Osborne of New Haven, Connecticut, who kindly furnished us with the raw material which consisted of the alcohol-ether soluble by-product obtained from corn gluten in the purification of zein.

This material contained some free phytosterol that crystallized when the concentrated solution was allowed to stand. The phytosterol was evidently not homogeneous since on fractional crystallization we obtained two fractions that varied slightly in melting point and in optical rotation.

The unsaponifiable matter extracted by ether after complete saponification was separated by fractional crystallization. The interesting observation was made on the first or principal fraction that the optical rotation decreased on continued recrystallization and finally the product was obtained entirely inactive. The results of the chemical analyses seemed to indicate that the preparation was a saturated phytosterol, having the formula $C_{27}H_{47}OH$. On treating this phytosterol in chloroform solution with bromine there was a slight absorption of bromine and at the same time hydrobromic acid was evolved but no definite bromine containing compound could be isolated.

It was found later that this optically inactive phytosterol could be separated by fractional crystallization from alcohol into two parts, one of which had an optical rotation of $+ 10.9°$ and the other showed an equal amount of levorotation. It was thus shown very clearly that the inactive product was a mixture of dextro- and levorotatory phytosterols.

Several fractions of levorotatory phytosterol were separated from the mother liquors from which the above inactive product had been crystallized. The fraction that appeared to be the purest corresponded in melt-

[1] Read at the meeting of the Rochester Section of the American Chemical Society, January 21, 1924.

[2] Anderson, *J. Biol. Chem.*, **55**, 611 (1923).

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

ing point with sitosterol but the optical rotation was about 2° lower, which may have been due to the presence of a small quantity of the dextrorotatory substance.

These preliminary observations indicated that the endosperm of corn contained at least two different phytosterols. In the hope of securing a sufficient quantity of these sterols to permit a complete examination we extracted 23 kilograms of corn gluten and saponified the fat thus obtained. The yield was 55 g. of crude crystalline phytosterol. After twice recrystallizing the substance from petroleum ether and once from alcohol it was obtained in colorless crystals and showed a specific rotation of —5.93°. The material was then recrystallized 20 times from alcohol, as a result of which the specific optical rotation was raised to + 20.54°. It was again recrystallized 10 times and the rotation rose to + 22.89°. Six further crystallizations from alcohol brought the rotation to + 24.23°. This seems to be the upper limit of the dextrorotation attainable by crystallization.

The substance still contained some of the unsaturated sitosterol because it gave a slight but distinct coloration in the Liebermann-Burchard reaction.

A portion of the substance that had a rotation of + 20.54° was treated in chloroform solution with acetic anhydride and concd. sulfuric acid as will be described elsewhere. The product finally obtained showed a specific rotation of + 25.04°. It was evidently free from sitosterol because the Liebermann-Burchard reaction was entirely negative.

The sterols obtained from some very pure corn bran were also examined. After this material was purified by crystallization from petroleum ether and alcohol as just described, snow-white crystals were obtained that showed a specific rotation of —9.54°. Recrystallization of this substance five times from alcohol gave 8.9 g. of material that had a specific rotation of + 3.17°. Some of this material was dissolved in chloroform and treated with acetic anhydride and concd. sulfuric acid. The purified product finally obtained had a specific rotation of + 24.05°.

The analysis of the dextrorotatory substance indicates that it is a saturated sterol corresponding to dihydrositosterol, $C_{27}H_{47}OH$. That it is a saturated sterol is shown further by the facts that it does not absorb bromine and it does not give the Liebermann-Burchard reaction.

The dihydrositosterol crystallizes from alcohol with one molecule of water of crystallization. The water of crystallization is not lost on drying in the air or under reduced pressure over sulfuric acid but it is lost very rapidly on drying in a vacuum at 105° over phosphorus pentoxide. The air-dried substance when heated in a capillary tube melts between 138° and 139°, and after it has been dried at 105° it melts between 140° and 141°.

Dihydrositosterol is decidedly less soluble in alcohol than is sitosterol When a hot alcoholic solution of the substance is allowed to cool slowly, large, usually elongated, hexagonal plates separate. The crystals are larger and denser than those of sitosterol but the angles of the crystals of the two substances are apparently identical. There is no difference in the appearance of the crystals obtained from a mixture of the two substances. Sitosterol and dihydrositosterol are evidently capable of forming mixed crystals in any proportion.

The acetyl derivative is formed easily on boiling dihydrositosterol with acetic anhydride. It crystallizes from alcohol in large hexagonal plates that are free from water of crystallization. The melting point of the acetyl derivative is nearly identical with that of the free sterol. Different preparations varied slightly in the melting point from 136° to 139°. The specific optical rotation in chloroform solution was + 14.41°.

The discovery of dihydrositosterol as a constituent of the unsaponifiable matter of plant fats will help to explain some of the conflicting statements that are found in the literature regarding such physical constants of phytosterol as the melting point and the optical rotation. The levorotatory sitosterol and the dextrorotatory dihydrositosterol exist, as will be shown later, in various plant fats. The separation of these substances is extremely difficult and a mixture of the two may be recrystallized a great number of times without showing any appreciable change in the melting point. In fact, we have found that the magnitude of the optical rotation is a safer and more reliable criterion of purity in these compounds than is the melting point.

It should be noted that in recrystallizing a mixture of these sterols the dihydrositosterol accumulates in the top fraction. The levorotation of this fraction, therefore, gradually sinks toward 0°, and after this point has been reached there is a slow rise in dextrorotation. It will be shown later that the melting points of pure sitosterol and dihydrositosterol are very nearly identical, but a mixture of the two may show any melting point from 129° to 141°. The sitosterol accumulates in the mother liquors and the bottom fractions, therefore, show the higher levorotation and a higher melting point. In an earlier paper[3] we called attention to the fact that the higher-melting fractions of phytosterols showed the highest levorotation and that these fractions were always obtained from the mother liquors. At that time we were unable to give any explanation of this phenomenon.

It is interesting that plants may elaborate several different phytosterols and that these may be deposited in special parts of the plant. Schulze and Barbieri[4] found in the cotyledons of lupin seedlings a phytosterol, m. p. 136–137°, with a specific rotation of —36.4°, while from the roots

[4] Schulze and Barbieri, *J. prakt. Chem.* **25**, 159 (1882).

of the same seedlings the above authors isolated a preparation called caulosterol; m. p., 158–159°; optical rotation, —49.6°. Windaus and Hauth[5] showed that stigmasterol occurred in the phytosterol obtained from calabar beans and from rape oil, and it has since been isolated from various other oils. Apparently it has not been determined whether stigmasterol is specially deposited in certain parts of the plants.

We have found that corn contains several different phytosterols. From corn pollen we obtained various fractions of optically inactive phytosterols. Corn oil, derived from the germ, contains a substance that is identical with sitosterol. The endosperm, as shown in this paper, contains at least 2 different phytosterols, one of which is the dextrorotatory dihydrositosterol; the other is levorotatory and is identical with sitosterol.

Experimental Part

The raw material was a dark brown, alcoholic, oily solution contained in a 300cc. bottle. It had been obtained in the following way as described by Dr. Osborne.

"Fourteen hundred g. of commercial corn gluten was ground very fine and added slowly to 30 liters of boiling 80% alcohol. This mass was allowed to simmer for a few minutes, after which dry filter paper clippings were added to facilitate pressing in the hydraulic press. The strong zein solution thus obtained was filtered clear, through a dense pulp filter. For convenience the solution was divided into 4-liter portions and ordinary ether added with constant stirring until the zein was precipitated in a coherent mass in the bottom of the vessel. The top portion containing the ether-soluble substances, coloring matter, etc., was decanted and the ether distilled and used over again on another portion. The higher-boiling portion remaining in the still was concentrated to a small volume and shaken out with fresh ether to precipitate any zein which might have remained in solution. The ether-alcohol soluble substances other than zein thus obtained are contained in this bottle."

The solution, contained in the bottle referred to above, deposited, after a few days, a considerable amount of crystalline substance. The amount of the crystals increased on standing in the ice box for a few days. The crystals were filtered and washed with several portions of cold alcohol to remove as much as possible of the adhering oil.

The filtrate and washings were saponified as will be described later.

The yellowish, crystalline substance after drying in a vacuum desiccator over sulfuric acid weighed 3.8 g. It gave the Liebermann-Burchard reaction, indicating that it was phytosterol.

Purification of the Crude Phytosterol

The substance retained some yellow color after recrystallization thrice from ethyl alcohol and twice from methyl alcohol. It weighed 1.7 g For further purification it was converted into the acetyl derivative by boiling with 25 cc. of acetic anhydride under a reflux condenser for one

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

hour. A large part of the acetyl derivative crystallized on cooling. The crystals were filtered and washed with a little cold acetic anhydride.

The alcoholic mother liquors from the first crystallizations as well as the acetic anhydride solution mentioned above were treated as will be described later.

The acetyl derivative was decolorized by boiling its alcoholic solution with Norite. It was then recrystallized twice from ethyl alcohol and once from methyl alcohol. The substance was then obtained as snow-white, elongated, hexagonal plates; m. p.,[6] 123°.

The purified acetyl derivative was saponified by boiling with alcoholic potassium hydroxide. The solution was diluted with a little water and allowed to cool. The phytosterol that crystallized was filtered, washed free from alkali with water and recrystallized several times from ethyl alcohol. The product was snow-white and weighed 1 g. It gave the Liebermann-Burchard reaction. Heated in a capillary tube, it began to soften at 132° and melted at 134°. It was then twice recrystallized from alcohol but there was no change in the melting point.

The dried preparation in chloroform solution gave a specific rotation of —30.80° at 23°.

On drying in a vacuum over phosphorus pentoxide it lost 4.33% and 4.24% in weight. The dried preparation was analyzed.

Analyses. Subs., 0.1125: H_2O, 0.1241; CO_2, 0.3449. Calc. for $C_{27}H_{45}OH$(386): C, 83.93; H, 11.91. Found: C, 83.61; H, 12.34. Calc. for $C_{27}H_{45}OH + H_2O$: H_2O, 4.45. Found: 4.33, 4.24.

Examination of the Mother Liquors

The alcoholic mother liquors obtained in all of the above-mentioned crystallizations, including those from the acetyl derivative, were concentrated to about 100 cc.

The acetic anhydride mother liquors were evaporated to dryness and the residue was added to the alcoholic solution. After the addition of 15 cc. of 50% potassium hydroxide solution, the solution was boiled under a reflux condenser for 2 hours. It was then diluted with water and extracted with ether. The ethereal solution was washed with water, filtered, and the ether distilled. The slightly yellow residue was dissolved in alcohol, decolorized with Norite, concentrated and allowed to crystallize. The substance was further recrystallized 5 times from alcohol. It separated as large snow-white, plate-shaped crystals. The dried preparation weighed 1 g.; m. p., 137°. After two more recrystallizations from alcohol, the melting point was still 137°.

On drying at 105° in a vacuum over phosphorus pentoxide it lost 4.82% and 4.71% in weight, corresponding to 1 molecule of water of crystallization. The dried preparation, dissolved in chloroform, had a specific rotation of —32.23° at 26°. The dried substance was analyzed.

Analyses. Subs., 0.1460: H_2O, 0.1589; CO_2, 0.4500. Calc. for $C_{27}H_{45}OH$ (386): C, 83.93; H, 11.91. Found: C, 84.06; H, 12.17.

[6] Unless otherwise specified, the melting points mentioned in this paper are uncorrected.

The melting point and composition of this substance agreed with those of sitosterol but the specific rotation was about 2° lower.

The acetyl derivative, prepared from this fraction and recrystallized several times from methyl alcohol, melted at 127°.

Examination of the Unsaponifiable Matter in the Fat from the Endosperm of Corn

The original filtrate and washings containing the fat, after the free phytosterol had been removed as described above, were boiled for two hours with alcoholic potassium hydroxide.

The very dark brown soap solution was largely diluted with water and extracted with three portions of ether. The ether was distilled and the residue, a dark brown, semi-crystalline mass, was again boiled with alcoholic potassium hydroxide. While the mass cooled, a considerable amount of phytosterol separated in crystalline form. This was filtered off, washed free from alkali with dil. alcohol and dried in a vacuum over sulfuric acid. The dark brown substance weighed 9.4 g.

The alcoholic solution, after the above-mentioned crystals had been filtered off, was largely diluted with water and extracted with three portions of ether. The ethereal solution was washed with water, filtered, and the ether removed by distillation. The residue, after it had dried in a vacuum over sulfuric acid, formed a dark brown, thick oil that weighed 7.2 g. This non-crystallizable oil was not further examined.

The total amount of unsaponifiable matter contained in 1400 g. of corn gluten was 20.4 g. or 1.45%, made up as follows: free phytosterol, 3.8 g.; crude phytosterol obtained after saponification, 9.4 g.; unsaponifiable oily substance, 7.2 g.; total unsaponifiable matter, 20.4 g.

Purification of the Crude Crystalline Phytosterol

The crude crystalline substance was dissolved in 400 cc. of ethyl alcohol and the solution boiled with several portions of Norite before all of the coloring matter was removed.

The solution was then concentrated to about 100 cc. On cooling, the phytosterol separated in large, plate-shaped, colorless crystals. It was further recrystallized 5 times from alcohol and after drying in the air it weighed 4.4 g. Heated in a capillary tube, it melted at 132–133° and began to solidify at 129°; 0.4610 g. of the dry substance dissolved in 15 cc. of chloroform gave in a 1-dm. tube a barely perceptible levorotation.

The substance was further recrystallized thrice from ethyl alcohol. After drying in the air the large, colorless, plate-shaped crystals weighed 3.2 g. It melted at 133° and began to solidify at 129°. It was entirely inactive; 0.8486 g. of the dried substance dissolved in 15 cc. of chloroform gave no rotation in a 1-dm. tube. It gave the usual Liebermann-Burchard color reaction. Dissolved in chloroform, it appeared to absorb a trace of bromine, but the color immediately changed to greenish, which made it impossible to determine when an excess of bromine had been added.

Water of Crystallization.—On drying the substance in a high vacuum over phosphorus pentoxide at 105° the loss in weight corresponded to 1 molecule of water of crystallization.

Analyses. Subs., 0.8866, 0.1175: loss on drying, 0.0380, 0.0057. Calc. for $C_{27}H_{45}OH + H_2O$: H_2O, 4.45. Found: 4.06, 4.86; av. 4.46.

The dried substance was analyzed.

Analyses. Subs., 0.1118: H_2O, 0.1264; CO_2, 0.3429. Calc. for $C_{27}H_{45}OH$ (386): C, 83.93; H, 11.91. Calc. for $C_{27}H_{47}OH$ (388): C, 83.50; H, 12.37. Found: C, 83.65; H, 12.65.

The inactive phytosterol was then recrystallized five times from rather large volumes of alcohol. The mother liquors were saved, concentrated, and the substance that crystallized was also examined. The top fraction had a specific rotation of +10.91° while the bottom fraction showed a specific rotation of —10.79°.

It is evident, therefore, that the inactive phytosterol is a mixture of dextro- and levorotatory sterols. While there was a great difference in the magnitude of the optical rotation of the fractions described above, there was no appreciable difference in their melting points.

Examination of the Mother Liquors from which the Inactive Phytosterol Had Been Crystallized

The mother liquors obtained on recrystallizing the inactive phytosterol were concentrated to about 100 cc. As they cooled, a considerable amount of colorless crystals separated as elongated plates.

These were filtered, washed with cold methyl alcohol and recrystallized from ethyl alcohol. After drying in the air this preparation weighed 1.3 g.

When the mother liquors described above were concentrated, a further fraction was obtained. After recrystallizing from alcohol and drying in the air the substance weighed 1 g.; m. p., 136–137°.

The first fraction which weighed 1.3 g. melted between 137° and 138°. This substance was again fractionated by dissolving it in 125 cc. of boiling methyl alcohol. A small amount of thin, colorless plates separated on cooling. After it had been filtered, washed with cold methyl alcohol and dried in the air it weighed 0.3 g. This fraction melted between 137° and 138°.

The mother liquor was concentrated to 30 cc. and on cooling, the remainder of the phytosterol crystallized. It was filtered and recrystallized from alcohol. After drying in the air it weighed 0.65 g. This fraction melted at 138°. In chloroform solution it had a specific rotation of —32.36° at 32°.

On drying at 105° in a high vacuum over phosphorus pentoxide it lost 4.27% in weight, which corresponds to 1 molecule of water of crystallization. The dried preparation was analyzed.

Analyses. Subs., 0.1364: H_2O, 0.1477; CO_2, 0.4182. Calc. for $C_{27}H_{45}OH$ (386): C, 83.93; H, 11.91. Found: C, 83.62; H, 12.11.

The acetyl derivative was prepared and recrystallized from methyl alcohol. It melted at 129°.

The composition, melting point and water of crystallization of this substance agree with those of sitosterol. The specific optical rotation, however, was about 2° lower. This low optical rotation may have been due to a slight contamination with the dextrorotatory phytosterol.

Examination of the Sterols Occurring in Corn Gluten

Preliminary experiments showed that a mixture of equal parts of alcohol and ether was the most satisfactory solvent for the fats and sterols in

corn gluten. Both chloroform and petroleum ether were tried as solvents, but the amount of phytosterol obtained from such extracts was comparatively small.

Twenty-three kilograms of corn gluten[7] was macerated in a mixture of equal parts of alcohol and ether during several days with occasional stirring. It was then filtered through a percolator with suction and the solvent was distilled. The fat was saponified with an excess of alcoholic potassium hydroxide and the resulting soap solution was diluted with water, and extracted with several portions of ether. The ethereal solution was washed with water, filtered and distilled. The dark brown, crystalline residue was again boiled with alcoholic potassium hydroxide. On cooling, the greater part of the phytosterol crystallized. It was filtered out, washed with diluted alcohol and dried. The crude, dark brown, crystalline substance weighed 55 g.

The filtrate, after dilution with water and extraction with ether, yielded 30 g. of a dark brown oil that was not further examined.

The crude phytosterol was purified by crystallizing it twice from light petroleum ether, the solution being cooled in a freezing mixture. A slight yellow color that remained was removed by boiling the substance with Norite in 1 liter of alcohol. After the solution had been filtered and cooled, the substance separated in colorless crystals that were dried in air and then weighed 28 g. This material in chloroform solution had the specific rotation of —5.93°.

The substance was recrystallized 20 times from rather large volumes of alcohol. The snow-white crystals were finally washed with cold alcohol and dried in the air, and then weighed 5.7 g. On drying at 105° in a vacuum over phosphorus pentoxide, they lost 4.32% in weight, corresponding to one molecule of water of crystallization. The dried substance melted at 141°. When dissolved in chloroform it had the specific rotation of +20.54°.

Four g. of this material was further recrystallized 10 times from alcohol, using from 200 cc. to 150 cc. of solvent for each crystallization. The air-dried substance weighed 2.3 g. and melted at 139°. After it had dried at 105° it melted between 140° and 141°. It gave a faint coloration in the Liebermann-Burchard reaction.

Analyses. Subs., 1.1754, 0.1645: loss on drying at 105° in a vacuum over phosphorus pentoxide, 0.0483, 0.0068. Calc. for $C_{27}H_{47}OH.H_2O$ (406): H_2O, 4.43. Found: 4.10, 4.13.

The dried preparation was analyzed.

Analyses. Subs., 0.1577: H_2O, 0.1757; CO_2, 0.4824. Calc. for $C_{27}H_{47}OH$ (388): C, 83.50; H, 12.37. Found: C, 83.42; H, 12.46.

Rotation. Dry subs., 1.0811: dissolved in chloroform and made up to 18 cc. gave in a 2-dm. tube a reading of +2.75°. $[\alpha]_D^{20}$ + 22.89°.

For further purification the substance was converted into the acetyl derivative and the latter, after recrystallization 4 times from alcohol, was saponified by boiling with alcoholic potassium hydroxide. The substance was extracted with ether and recrystallized twice from alcohol. The air-dried preparation melted at 138°; after it had dried at 105° it melted at 141°. It still gave a very faint coloration in the Liebermann-Burchard reaction. It lost 4.33% in weight when dried at 105° as mentioned above, corresponding to one molecule of water of crystallization.

Rotation. Dry subs., 0.5497: dissolved in chloroform and made up to 18 cc. gave in a 2-dm. tube a reading of +1.48°. $[\alpha]_D^{20}$, + 24.23.

This substance had then been recrystallized 39 times and we believe that the specific

[7] Supplied by the Corn Products Co., New York.

rotation $[\alpha]_D^{20}$ +24.23 is very close to the upper limit of dextrorotation attainable by crystallization. The substance was, however, not completely free from sitosterol, because it gave a faintly positive Liebermann-Burchard reaction. The amount of sitosterol that was present was too small to affect the composition which as shown by the analysis given above, agrees closely with the calculated composition of dihydrositosterol.

Acetyl Derivative.—The acetyl derivative, prepared as mentioned above, crystallized from alcohol in large, hexagonal plates without water of crystallization; m. p., 136°.

Rotation. Subs., 0.7619: dissolved in chloroform and made up to 18 cc. gave in a 2-dm. tube a reading of +1.22°. $[\alpha]_D^{20}$ +14.41°.

Analyses. Subs., 0.1355: H_2O, 0.1432; CO_2, 0.4031. Calc. for $C_{27}H_{47}O.CO.CH_3$ (430): C, 80.93; H, 11.62. Found: C, 81.13; H, 11.82.

Removal of Sitosterol from Dihydrositosterol by Means of Acetic Anhydride and Sulfuric Acid

To a solution of 1.6 g. of the preparation mentioned above (rotation, +20.54°) in 60 cc. of chloroform was added 10 cc. of acetic anhydride, and to this, contained in a small separatory funnel, was slowly added 10 cc. of concd. sulfuric acid. When this mixture was cooled and shaken it became intensely blue but after 1 cc. of water was added the color changed to deep green and the sulfuric acid, containing most of the coloring matter, settled to the bottom and was drawn off. The chloroformic solution was shaken thrice with 10cc. portions of acetic anhydride and sulfuric acid until it showed only a faint green color. After the chloroform was washed thrice with water, it was distilled and the residue was boiled for one hour with alcoholic potassium hydroxide. The substance was extracted with ether, and twice recrystallized from alcohol. It crystallized in the usual form, as elongated hexagonal plates. The air-dried substance weighed 0.64 g. and melted at 139°. It gave no immediate coloration in the Liebermann-Burchard reaction, but after some time a very faint green color developed. When dissolved in chloroform, it did not absorb any bromine.

Rotation. Dry subs., 0.5790: dissolved in chloroform and made up to 20 cc. gave in a 2-dm. tube a reading of +1.45°. $[\alpha]_D^{20}$ + 25.04°.

When the substance was dried at 105° in a vacuum over phosphorus pentoxide it lost 4.49% in weight, corresponding to one molecule of water of crystallization.

Analyses. Dry subs., 0.1912: H_2O, 0.2120; CO_2, 0.5835. Calc. for $C_{27}H_{47}OH$ (388): C, 83.50; H, 12.37. Found: C, 83.23; H, 12.40.

The acetyl derivative was prepared as mentioned above and was recrystallized from alcohol. It was free from water of crystallization and melted between 136° and 137°.

Analyses. Calc. for $C_{27}H_{47}O.CO.CH_3$ (430): C, 80.93; H, 11.62. Found: C, 80.75; H, 11.61.

It is evident from the experiment described above that sitosterol may be removed from dihydrositosterol by means of sulfuric acid and acetic anhydride and that the pure dihydrositosterol is easily recovered after this treatment. This method of purification of sterols will be described in more detail in another paper.

Examination of the Sterols Occurring in Corn Bran

It occurred to us that the dihydrositosterol that we had found in corn gluten might be derived from corn bran, since the commercial corn gluten

contains a considerable proportion of bran. Some very pure corn bran[8] free from germ and practically free from starch was therefore examined.

From 15 kg. of bran we obtained by alcohol-ether extraction 540 g. of fat which, after saponification, yielded 56.4 g. of crude unsaponifiable matter. The phytosterol was purified, as already described, by crystallization from petroleum ether, decolorization with Norite and recrystallization from alcohol. The colorless crystals that were obtained weighed 15.6 g. When heated in a capillary tube the substance began to soften at 129° and melted at 133°. In chloroform solution $[\alpha]_D^{20}$ was —9.54°.

This substance was recrystallized five times from rather large volumes of alcohol. The air-dried, snow-white substance weighed 8.9 g. and melted at 133–134°. In chloroform solution $[\alpha]_D^{20}$ was +3.17°. It is evident from the rotation of this substance that corn bran is relatively rich in dihydrositosterol.

Of this preparation, 3 g. was treated, in chloroform solution, with acetic anhydride and concd. sulfuric acid and the dihydrositosterol was isolated in the manner already described. After twice recrystallizing the substance from alcohol 0.8 g. of snow-white crystals was obtained. The air-dried preparation melted at 139° and that dried at 105° melted between 141° and 142°. It gave no immediate coloration in the Liebermann-Burchard reaction, but after the reaction mixture had stood for half an hour or longer a faint green color developed. Dissolved in chloroform, the substance did not absorb bromine. It is evident, therefore, that the preparation was practically free from sitosterol.

Rotation. Dry subs., 0.7731: dissolved in chloroform and made up to 20 cc. gave in a 2-dm. tube a reading of +1.86°. $[\alpha]_D^{20}$ + 24.05°.

When dried at 105° in a vacuum over phosphorus pentoxide the substance lost 4.70% in weight, corresponding to one molecule of water of crystallization. The dried substance was analyzed.

Analyses. Subs., 0.1494: H_2O, 0.1634; CO_2, 0.4563. Calc. for $C_{27}H_{47}OH$ (388): C, 83.50; H, 12.37. Found: C, 83.30; H, 12.23.

The acetyl derivative was prepared by boiling 0.4 g. of the substance described above with 7 cc. of acetic anhydride. On recrystallization from alcohol it separated in large, colorless, hexagonal plates. It was free from water of crystallization and melted between 138° and 139°.

Analyses. Subs., 0.1669: H_2O, 0.1768; CO_2, 0.4963. Calc. for $C_{27}H_{47}O.CO.CH_3$ (430): C, 80.93; H, 11.62. Found: C, 81.09; H, 11.85.

The color reactions for sterols recently developed by Whitby[9] were applied to dihydrositosterol. Reaction A did not give the colorations typical of unsaturated sterols and Reaction B was negative.

In conclusion we desire to express our indebtedness to Dr. T. B. Osborne of New Haven, Connecticut, to the Patent Cereals Company of Geneva, New York and to the Corn Products Company, New York, for kindly supplying the raw material.

Summary

1. The unsaponifiable matter derived from the endosperm of corn has been examined. This portion of the corn kernel contains at least two fractions of phytosterol that differ in properties and composition.

[8] Kindly supplied by Mr. Lloyd Bosworth of the Patent Cereals Co., Geneva.

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

2. The endosperm of corn contains some free phytosterol; m. p., 137–137.5°; specific rotation —32.23°; acetyl derivative, m. p. 127°.

3. After saponification, the unsaponifiable matter was separated into the following 3 parts. (*1*). The optically active dihydrositosterol, $C_{27}H_{47}OH.H_2O$; m. p., 138–139°. The dried preparation melts between 140° and 141°; $[\alpha]_D^{20}$, + 25°; acetyl derivative, m. p. about 138°; $[\alpha]_D^{20}$, + 14.41°. (*2*). Rather large quantities of the ordinary sitosterol associated with the dihydrositosterol in the endosperm and bran of corn. (*3*). A brownish-yellow oily substance that has not been examined.

4. Dihydrositosterol crystallizes in the same form as sitosterol but the crystals are larger and denser. It does not give the Liebermann-Burchard reaction, and the Whitby reactions are atypical. It does not absorb bromine.

GENEVA, NEW YORK

[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF THE JOHNS HOPKINS UNIVERSITY]

ATTEMPTS TO PREPARE 1-METHYL-2-METHOXYPIPERIDINE. THE HYDROGENATION OF CERTAIN PYRIDINE DERIVATIVES[1]

BY T. B. GRAVE

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Introduction

This research was started with a view to synthesizing 1-methyl-2-methoxypiperidine and some of its homologs, in order to study their physiological action, but these have not yet been obtained. However, certain 2-substituted pyridines have been prepared and their transformations into the corresponding piperidines attempted; this paper deals with the results of these trials.

Four pyridine derivatives were used, successively, as starting points for the synthesis: 2-methoxypyridine, 2-chloropyridine, 2-aminopyridine, and 1-methyl-2-pyridone.

1. The most likely method appeared to consist in the hydrogenation and methylation of 2-methoxypyridine. Von Pechmann and Baltzer[2] first prepared this compound by the action of methyl iodide on the silver salt of 2-pyridone, and later von Pechmann[3] made it from 2-pyridone and

[1] From a dissertation submitted in partial fulfilment of the requirements for the degree of Doctor of Philosophy in the Johns Hopkins University. The author is indebted to Dr. D. I. Macht for suggesting this problem, and to Dr. E. E. Reid for his helpful criticism during the progress of work on it.

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[2] Von Pechmann and Baltzer, *Ber.*, **24**, 3144 (1891).

[3] Von Pechmann, *Ber.*, **28**, 1624 (1895).