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

THE PHYTOSTEROLS OF RICE BRAN FAT[1]

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Introduction

The ether extract of rice bran has been examined by Weinhagen[2] who reported about 10% of fat consisting mostly of free fatty acids and containing about 5% of unsaponifiable matter. The above-mentioned author described two crystalline substances obtained from the unsaponifiable matter, a phytosterol (m. p., 131–133°; $[\alpha]_D$, —24.62°) which was called sitosterol and a saturated hydrocarbon melting at 79.5–80.5° to which the formula $C_{27}H_{48}$ was assigned. He believed that this hydrocarbon was closely related to phytosterol and that it was similar to or identical with β-cholestan[3a] which may be prepared from cholesterol.

Rice bran oil had been examined earlier by Smetham[3b] and by Browne[3c] who reported that the oil consisted largely of free fatty acids. More recently the material has been investigated by Jamieson[3d] who reported that the unsaponifiable material contained some myricyl alcohol in addition to phytosterol.

The unsaponifiable matter derived from rice-bran fat was examined in this Laboratory for the presence of the saturated sterol, dihydrositosterol, previously found in the fat extracted from the endosperm and bran of corn[4] and wheat.[5] Incidentally some other components of this material were identified and are reported in this paper.

The rice bran gave about 10% of fat which contained about 5% of unsaponifiable matter. The latter consisted largely of a brown, viscous liquid and crystalline sterols formed but a small fraction of this material. From the crystalline portion it was possible to isolate some myricyl alcohol and small amounts of dihydrositosterol and stigmasterol. The sitosterol fraction which remained, after the above-mentioned constituents had been separated, was probably not homogeneous because it could be resolved into several fractions that showed variation in properties. The purest sample obtained melted at 140–141°; $[\alpha]_D$, —37.7°. The substance may have contained a trace of stigmasterol as well as other impurities since the method of separating the bromides of the acetyl derivatives does not ex-

[1] Presented before a meeting of the Rochester Section of the American Chemical Society, March 16, 1925.

[2] Weinhagen, *Z. physiol. Chem.*, **100**, 159 (1917).

[3] (a) Diels and Linn. *Ber.*, **41**, 548 (1908). (b) Smetham, *Analyst*, **18**, 191 (1893); *J. Soc. Chem. Ind.*, **12**, 848 (1893). (c) Browne, THIS JOURNAL, **25**, 948 (1903). (d) Jamieson, *J. Oil Fat Ind.*, **3**, 256 (1926).

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

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

clude the possibility of some stigmasteryl acetate tetrabromide dissolving in the ether.

The yield of dihydrositosterol was not so great as had been anticipated. The amount of crystalline sterols that was available was too small to permit of separating the saturated sterol by fractional crystallization. The method of removing the unsaturated sterols with sulfuric acid and acetic anhydride[6] was therefore used. The amount of dihydrositosterol obtained from the top fraction by this method was only sufficient for its identification.

Stigmasterol which was first found by Windaus and Hauth[7] in the phytosterol isolated from calabar beans has also been found by Matthes and Dahle[8] in soy bean oil and by Beschke[9] in the fat extracted from carrots. The sample prepared from rice-bran fat had a somewhat higher rotation than that given by Windaus and Hauth but the amount of pure substance at our disposal was so small that it was difficult to determine the optical rotation very accurately. The melting points of the sterol and of the acetyl derivative, however, corresponded very closely to the values given by the authors mentioned above.

We were unable to separate from the unsaponifiable matter any substance that resembled the hydrocarbon described by Weinhagen.

The non-crystalline portion of the unsaponifiable matter was subjected to distillation. The products that were obtained were similar to those found by Gardner[10] in the unsaponifiable matter from feces. The lower fractions were mobile oils with strong aromatic odor. As the boiling point rose, the odor diminished and the viscosity increased, the higher fractions being extremely viscous. Fractions distilling above 200° at 1 mm. pressure gave the Liebermann-Burchard reaction, evidently due to the presence of phytosterol which had volatilized at this high temperature.

Experimental Part

Extraction of the Oil from Rice Bran

Eighteen kg. of finely ground rice bran, on extraction with petroleum ether, yielded 1800 g. of a dark green oil. The oil, which partly solidified on cooling, was almost completely soluble in dilute alkali and hence was composed almost entirely of free fatty acids.

Separation of the Unsaponifiable Matter

The oil was boiled with alcoholic potassium hydroxide for two hours, diluted with water and extracted thrice with ether. The ether was distilled and the residue, after it had been dried, weighed 95 g. The product was a viscous brown oil with a strong odor and gave a marked Liebermann-Burchard reaction. It was separated with much difficulty by means of acetone and alcohol into a crystalline fraction and a non-crystalline oil.

[6] Anderson and Nabenhauer, THIS JOURNAL, **46**, 1957 (1924).

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

[8] Matthes and Dahle, *Arch. Pharm.*, **249**, 436 (1911).

[9] Beschke, *Ber.*, **47**, 1853 (1914).

[10] Gardner, *Biochem. J.*, **15**, 244 (1915).

The Crystalline Fraction

Separation of Myricyl Alcohol.—The crystalline material was obviously impure and contained in addition to sterols a considerable amount of some less soluble substance; which was found to be myricyl alcohol. A separation was effected by dissolving the crude crystalline material in a large volume of hot alcohol. The sterols are much more soluble in cold alcohol than is myricyl alcohol; hence, they remained in the mother liquor. By repeating this treatment a fairly good separation was effected. The crude myricyl alcohol weighed about 4 g.; m. p., about 80°.[11] After several recrystallizations the melting point was 85°. The acetyl derivative melted at 73°.

Anal. Subs., 0.1399: H_2O, 0.1762; CO_2, 0.4231. Calcd. for $C_{30}H_{61}OH$ (438): C, 82.19; H, 14.15. Found: C, 82.48; H, 14.09.

Dihydrositosterol.—The sterols, after the myricyl alcohol had been removed, were recrystallized several times. The substance separated in colorless plates and after drying weighed 9 g.; m. p., 137–138°; $[\alpha]_D$, —27.67.°[12] The material was recrystallized five times when the top fraction weighed 3 g. and the rotation was —22.94°. As will be described elsewhere the top fraction yielded 0.1 g. of dihydrositosterol. The substance melted at 144–145°; $[\alpha]_D$, +24°.

The Sitosterol Fractions

The sterols found in the mother liquors of the above mentioned recrystallizations were combined and acetylated. The acetyl derivative, 11.5 g., was dissolved in 250 cc. of ether, and an excess of bromine in glacial acetic acid was added. When the volume was made up to 500 cc. with glacial acetic acid a slight precipitate separated which was filtered off. Since no further precipitate was produced by adding more acetic acid, water was added to bring about fractional precipitation. By this treatment the bromides were separated into two nearly equal fractions which were filtered off and dried. Each fraction was digested in ether, about 10 cc. of ether per gram of bromide, when a slight, insoluble residue remained which was filtered off and identified as stigmasteryl acetate tetrabromide as shown below. The two fractions were again fractionally precipitated, the first by adding acetic acid and the second by methyl alcohol.

The four fractions were debrominated separately by boiling with zinc dust and acetic acid in alcoholic solution. The free sterols were obtained by saponifying the acetyl derivatives. The properties of the sterol fractions and their acetates are given in Table I. An analysis was made of Fraction B since this was regarded as the purest sample.

Anal. Subs. (dried), 0.0916, 0.1080: H_2O, 0.1003, 0.1189; CO_2, 0.2825, 0.3324. Calcd. for $C_{27}H_{45}OH$(386): C, 83.93; H, 11.91. Found: C, 84.11, 83.94; H, 12.25, 12.32.

Stigmasterol.—The ether-insoluble portion of the bromides was crystallized from a mixture of benzene and ether. Colorless, four-sided platelets were obtained which melted at 205° with decomposition. The substance crystallized in a similar form from a mixture of chloroform and alcohol. The crystals were debrominated by the method described by Windaus and Hauth[7] and the acetyl derivative was recrystallized from alcohol. Colorless crystals were obtained which melted at 143°; $[\alpha]_D$, —56.°

The free stigmasterol was obtained by saponifying the acetyl derivative with alcoholic potassium hydroxide. It crystallized in colorless plates that contained one

[11] The melting points given in this paper are corrected.

[12] All determinations of optical rotations were made in chloroform solution and with sodium light.

molecule of water of crystallization. The substance weighed about 0.12 g.; m. p., 169–170°; $[\alpha]_D$, —50°.

Anal. Subs., 0.1168 g., lost 0.0056 g. on drying at 105° in a vacuum over phosphorus pentoxide. Calcd. for $C_{30}H_{49}OH + H_2O$: H_2O, 4.05. Found: 4.79.

TABLE I

PROPERTIES OF THE STEROLS FROM RICE-BRAN FAT

Substance	M. p., °C.	$[\alpha]_D$	Acetyl derivative M. p., °C.	Acetyl derivative $[\alpha]_D$
Dihydrositosterol	144–145	+24°		
Stigmasterol	169–170	−50°	143	−56°
Phytosterol Frac. A	139–140	−38°	135.5–136.5	−41.6°
B	140–141	−37.7°	134–135	−40.9°
C	140	−36.7°	131–133	−40°
D				−29.7°
Myricyl alcohol	85–86		73	

The Uncrystallizable Oil

The only material which remained after the crystalline fraction had been separated was subjected to fractional distillation at about 1mm. pressure. The first three fractions were distilled from an oil-bath but an air-bath was used for the remainder. The results are summarized in Table II.

TABLE II

FRACTIONAL DISTILLATION OF THE OIL

Fraction	Wt., g.	B. p., °C.	Temp. of bath, °C.	Remarks
1	0.4	110	130	Yellow mobile oil of strong odor.
2	2.5	110–140	130–165	Yellow oil.
3	3.4	140–180	165–210	Yellow oil depositing few crystals on standing.
4	3.9	180–230	.....	Light brown, viscous oil. Liebermann-Burchard reaction positive. Deposited a few crystals with negative L.-B. reaction.
5	4.0	230–245	.....	Light brown, viscous liquid giving strong Liebermann-Burchard reaction. Crystals deposited giving negative L.-B. reaction.
6	1.0	245–260	.....	Light brown, very viscous liquid. Crystals of phytosterol separated from acetone solution on cooling.
7	3.0	.....	.....	Residue from second distillation. Brown, semi-solid, varnish-like mass giving strong Liebermann-Burchard reaction.
8	...	.....	.....	Residue from first distillation. Similar to Fraction 7.

Fraction 5 was analyzed. The results of the analysis indicate that the substance contains about 1% more oxygen than does phytosterol.

Anal. Subs., 0.2072: H_2O, 0.2133; CO_2, 0.6333. Found: C, 83.35; H, 11.51.

A small amount of crystals separated from Fractions 4, 5 and 6 but only those from Fraction 6 gave the Liebermann-Burchard reaction. The phytosterol was determined

in Fraction 6 by precipitation with digitonin. The substance, 0.3860 g., was dissolved in 50 cc. of hot alcohol and to this was added a solution containing 1.25 g. of digitonin in 100 cc. of alcohol. After standing at room temperature for two hours and in ice water for the same length of time, the precipitate was filtered off and washed with cold alcohol and ether. The precipitate, after drying at 90°, weighed 0.3800 g., corresponding to 0.0924 g. or 24% of phytosterol.

The digitonide was extracted with boiling xylene and the sterol, obtained on evaporation of the solvent, was recrystallized from alcohol. It had the characteristic crystal form of sitosterol, melted at 138–139°, and gave the Liebermann-Burchard reaction.

The filtrate from the crystalline digitonide was concentrated, diluted with water and extracted with chloroform. On evaporation of the chloroform, an amber-colored, very viscous liquid was obtained. In the Liebermann-Burchard reaction a brown coloration developed at first which changed rapidly to green and then to yellowish-green. The solution had a strong green fluorescence which, when the solution was diluted, resembled that of fluorescein. The reaction is markedly different from that obtained with the sterols and would interfere greatly with the accurate colorimetric determination of sterols.

Summary

1. Extraction of rice bran with petroleum ether yields about 10% of an oil consisting largely of free fatty acids.

2. The unsaponifiable matter from this oil amounts to about 5% and consists chiefly of a viscous oil.

3. The crystalline portion of the unsaponifiable matter contains myricyl alcohol, dihydrositosterol, stigmasterol and phytosterol which is probably not homogeneous sitosterol.

4. The oily part of the unsaponifiable matter yields, on distillation, yellowish to light brown oils. The higher fractions give sterol color reactions which are partly due to the presence of phytosterol.

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THE PHYTOSTEROLS OF CORN OIL[1]

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Introduction

In an earlier paper,[2] in which will be found a brief review of the literature, a phytosterol preparation isolated from corn oil was described which melted at 137.5° ($[\alpha]_D$ —34.38°) and the acetyl derivative melted at 127°. Since these values were identical with those given for sitosterol[3] it was assumed that corn oil contained this sterol. A similar assumption had been made by Gill and Tufts.[4]

[1] An abstract of this paper was presented in the Symposium, "Chemistry and Plant Life," at the meeting of the American Chemical Society, Los Angeles, California, 1925.

[2] Anderson and Moore, THIS JOURNAL, **45**, 1944 (1923).

[3] Burian, *Monatsh.*, **18**, 551 (1897). Ritter, *Z. physiol. Chem.*, **34**, 461 (1901).

[4] Gill and Tufts, THIS JOURNAL, **25**, 251 (1903).