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THE PHYTOSTEROLS OF WHEAT GERM OIL[1]

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Introduction

The crystalline portion of the unsaponifiable material prepared from the oil extracted from wheat and rye germ was investigated by Burian[3] in 1897. The substance was apparently homogeneous and it received the name sitosterol. The mother liquors from which the sitosterol had been crystallized gave on concentration a very small amount of crystalline substance called parasitosterol which differed from sitosterol in having a lower melting point and rotation. These results were confirmed by the work of Ritter.[4] All subsequent investigators[5] who have worked with sitosterol have regarded it as a homogeneous compound and the substance is usually referred to as the best known and most thoroughly studied plant sterol.[6]

Sitosterol is an unsaturated secondary alcohol containing one double bond. Burian[3] recorded the following properties: m. p., 137.5°; $[\alpha]_D$, —26.87° in ethereal solution; acetyl derivative, m. p. 127°. Ritter found[4] the melting point to be 136.5° and $[\alpha]_D$ —33.91° in chloroform. Sitosterol is isomeric with cholesterol, corresponding in composition to the formula $C_{27}H_{45}OH$, and the work of Windaus and Brunken[5d] has shown that the molecules of the two compounds are probably structurally identical.

The object of the present investigation was to secure pure sitosterol in order that its properties might be compared with those of other plant sterols. Since it had been impossible to obtain any definitely homogeneous sitosterol from corn oil[7] an attempt was made to prepare the pure substance from wheat germ.

Two different samples of sterols prepared from wheat germ oil were

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

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

[5] (a) Windaus and Hauth, *Ber.*, **40**, 3681 (1907). (b) Pickard and Yates, *J. Chem. Soc.*, **93**, 1928 (1908). (c) Windaus and Rahlen, *Z. physiol. Chem.*, **101**, 223 (1918). (d) Windaus and Brunken, *ibid.*, **140**, 52, (e) 109 (1924).

[6] Meyer and Jacobson, "Lehrbuch der Organischen Chemie," Walter De Gruyter and Co., Berlin and Leipsic, **1924**, vol. 2, pt. 4, p. 226. Lewkowitsch, "Chemical Technology and Analysis of Oils, Fats and Waxes," Macmillan and Co., Ltd., London, **1921**, vol. 1, p. 280. Trier, "Chemie der Pflanzenstoffe," Gebrüder Borntraeger, Berlin, **1924**, p. 164.

[7] Anderson and Shriner, THIS JOURNAL, **48**, 2976 (1926).

examined very carefully but no homogeneous sitosterol could be obtained from either lot. The crystalline sterols were very similar in properties but investigation proved that they were complex mixtures. An appreciable quantity of dihydrositosterol[8] was isolated but no evidence was found of the presence of stigmasterol, thus confirming the results reported by Windaus and Hauth.[9] Whether dihydrositosterol is present in absolutely pure wheat germ cannot be determined from the data which are given in this paper because the commercial wheat germ that was used in this investigation contained a slight admixture of fine particles of bran and starch.

The remainder of the sterol, after the dihydrositosterol had been removed, was acetylated and the acetyl derivative was fractionated by crystallization from alcohol. A series of fractions were obtained that ranged in melting point[10] from 143–144° to 113–114° and in rotation[11] from —45° to —23°. These fractions were identical with those obtained from the corn oil sterols[7] and consisted of α-, β- and γ-sitosterol.

The γ-sitosterol was obtained in a fairly pure condition by recrystallizing the acetyl derivative from alcohol from 30 to 35 times. The top fraction then melted at 143–144° and the rotation was about —44°. Further purification was effected by bromination according to the method of Windaus and Hauth.[9] The γ-sitosteryl acetate dibromide is not very soluble and a portion of it separates directly from the bromination mixture, on standing at room temperature, either as needle-shaped crystals or as colorless globules. γ-Sitosteryl acetate may be obtained by boiling the purified dibromide with zinc dust and acetic acid. It crystallizes in colorless plates, m. p. 143–144°, and the rotation is about —45°. The free sterol, γ-sitosterol, is obtained when the acetyl derivative is saponified. It crystallizes in colorless plates that resemble sitosterol. It melts at 147–148° and the rotation is about —42°.

When γ-sitosterol is reduced with hydrogen and platinum black, dihydro-γ-sitosterol or γ-sitostanol is formed. The substance crystallizes in large, colorless plates; m. p., 143–144°; $[\alpha]_D$, about +18°.

β-Sitosterol could not be obtained in pure state because it was impossible to remove γ-sitosterol completely. It is contained in the bottom fractions of the acetyl derivative that has been purified by bromination and subsequent fractionation for the removal of γ-sitosterol. The fractions designated β-sitosterol possess nearly the same properties that are ascribed in the literature to sitosterol.

β-Sitosterol gives on reduction with hydrogen and platinum black a

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

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

[10] All melting points given in this paper are corrected.

[11] Rotations were observed in chloroform solution by sodium light.

saturated sterol, dihydro-β-sitosterol or β-sitostanol, which crystallizes in colorless plates; m. p., 139–140°; $[\alpha]_D$, about +24°. The properties of this substance are very similar to those of the natural dihydrositosterol.

The bottom fractions of the original sterol, as already stated, had a low melting point and a low levorotation. These fractions which undoubtedly correspond to the parasitosterol mentioned by Burian[3] are now designated α-sitosterol. The substance is decidedly more soluble in ethyl and methyl alcohol than β- and γ-sitosterol. It has been impossible, however, to remove the latter compounds completely by crystallization from the material that has been available. When these fractions are brominated the color turns greenish-brown and some hydrogen bromide is liberated, indicating some substitution of bromine. The resulting bromo-acetyl derivatives that are formed cannot be debrominated completely and after boiling with zinc dust and acetic acid the reaction product can be separated by crystallization from alcohol into a bromine-free acetyl derivative corresponding to β-sitosterol containing a small amount of γ-sitosterol and a non-crystalline substance containing bromine which is retained in the mother liquors. This latter substance evidently represents a bromine substitution production of α-sitosterol. If some method could be found by which this material could be debrominated it would offer a means of preparing pure α-sitosterol.

Burian[3] called attention to the fact that in brominating sitosterol an excess of bromine caused the formation of dark-colored compounds that were very difficult to purify.

The results obtained on analyses of α-sitosterol fractions and of the corresponding acetyl derivatives are in agreement with the usual formula for sitosterol, $C_{27}H_{45}OH$. These compounds are reduced very slowly when treated with hydrogen and platinum black but the saturated sterol which is formed appears to be identical with dihydro-β-sitosterol.

The fractions designated α-, β- and γ-sitosterol are undoubtedly isomeric and differ mainly in their physical properties of melting point and optical rotation, but α-sitosterol differs from the other two in that it forms a bromine substitution product that cannot be debrominated. Analyses of all these fractions give values that are in agreement with the formula assigned to sitosterol, $C_{27}H_{45}OH$, and molecular-weight determinations of the dibromo-acetyl derivatives of the β- and γ-sitosterols also agree closely with the formula $C_{29}H_{48}Br_2O_2$.

The results of this investigation indicate that the crystalline sterols obtained from the germ of American grown wheat compose a mixture containing dihydrositosterol and at least three other sterols which are isomeric with sitosterol. No satisfactory explanation can be given as to why the results herein reported differ from those of European investigators. European wheat may possibly contain different sterols from wheat grown in

America. It is also possible that the sterols in the same species of plant may vary from season to season according to climatic conditions. Further investigation of these matters will be necessary before any safe conclusions can be drawn.

Experimental Part

Preparation of the Unsaponifiable Material.—The crude sterol used in these experiments had been prepared according to the following process by Evans and Burr. A good grade of wheat germ, containing very little material besides the yellow flakes was obtained from the Sperry Mills at Vallejo, California. The wheat had been grown on the Pacific Coast. The germ was extracted with ether in a large tin extractor and the oil was recovered by evaporating the ether on a steam-bath. The oil is golden-yellow and has a strong odor of wheat germ when heated.

The oil was saponified and the unsaponifiable matter was recovered by the following standardized procedure. An approximately 20% solution of alcoholic potassium hydroxide is made by mixing 1000 cc. of 95% alcohol with 335 g. of 60% aqueous potassium hydroxide. After settling, the clear solution is decanted into 500 g. of the wheat germ oil. The clear amber soap solution is kept at 35° for eight hours. It is then diluted with water and extracted five times with ether in large separatory funnels. The ethereal solution is washed with water until free from soaps and the ether is distilled. The yellow residue, after drying at 100° in a vacuum, is washed three or more times with ice-cold pentane, in which solvent the non-crystalline part of the unsaponifiable matter is easily soluble while the crystalline sterols are very slightly soluble. The residual crude sterol mixture is a nearly white product with a melting point close to that of pure sitosterol.

The yields are relatively high. About 10% of oil is obtained from the germ. The total unsaponifiable matter amounts to about 4% and the crude crystalline sterols represent about 65% of the latter.

The alcohol that is used is purified by distillation over sodium ethoxide. The alcoholic potassium hydroxide, prepared as described, above shows no coloration after one hour of refluxing.

Purification of the Sterols.—The faintly yellow crystals which weighed 100 g. were boiled for two hours with alcoholic potassium hydroxide. The solution was diluted with water and extracted with ether. The ethereal solution was washed thoroughly with water and the ether was distilled. The residue was dissolved in hot alcohol, treated with Norite and separated into four fractions by crystallization from alcohol.[12] The properties of these fractions are shown in Table I.

[12] Unless some other solvent is stated, all crystallizations mentioned in this paper were made from ethyl alcohol purified by distillation over potassium hydroxide.

TABLE I

PROPERTIES OF DIFFERENT FRACTIONS OF THE WHEAT GERM STEROL

All Formed Colorless Plates

Fraction	Wt., g.	M. p., °C.	$[\alpha]_D$
1	66	140–141	−28.67°
2	16.8	138–139	−25 07°
3	4.2	138–139	−31.91°
4	10.2	139–140	−32.23°

Fractions 1 and 2, Table I, were united and recrystallized 25 times. The observed changes in properties are recorded in Table II.

TABLE II

CHANGES IN PROPERTIES OF THE TOP FRACTION

No. of recrystallizations	Wt., g.	M. p., °C.	$[\alpha]_D$
10	35.3	139–140	−22.23°
15	23.3	138–139	−18.45°
25	3.6	138	0°

While 25 recrystallizations had changed the melting point but slightly the effect on the optical rotation was very great and it was believed that the change in optical activity was due to the presence of the dextrorotatory dihydrositosterol.

Isolation of Dihydrositosterol.—The last fraction, Table II, which was optically inactive, was acetylated and the acetyl derivative was treated in carbon tetrachloride solution with acetic anhydride and sulfuric acid until the unsaturated sterol was removed. As will be described elsewhere 1.6 g. of dihydrositosterol, m. p. 143–144°, $[\alpha]_D$ +23.61°, was obtained after this treatment.

Fractionation of the Acetyl Derivative.—During the crystallizations mentioned above a number of sterol fractions had been collected that had similar properties; m. p., about 140°; $[\alpha]_D$, —32° to —33°. Two different fractions were acetylated and the acetyl derivatives were recrystallized separately. The properties of these products were very similar; m. p., about 129–130°; $[\alpha]_D$, about —36°. Each preparation was recrystallized ten times and separated into three fractions. The results are shown in Table III.

TABLE III

PROPERTIES OF DIFFERENT FRACTIONS OF THE ACETYL DERIVATIVE

Fraction	Wt., g.	M. p., °C.	$[\alpha]_D$
1	9.9	135–136	−41.01°
2	3.9	129	−38.51°
3	8.6	118–119	−27.51°
1	17.5	135–136	−41.01°
2	7.0	126–127	−36.07°
3	12.0	118–119	−25.64°

The data in Table III indicate very plainly that the wheat germ sterol, even after the dihydrositosterol had been removed, was a mixture which could be separated by fractionation of the acetyl derivative. The results are very similar to those reported for the corn oil sterols.[7]

Isolation of γ-Sitosterol.—The top fractions of the acetyl derivatives, Table III, were united and purified by means of the bromo compound in order to remove any α-sitosterol that might be present. The material was brominated by the method of Windaus and Hauth,[9] precipitated by water, filtered, washed with water and dried. The substance was debrominated by boiling its solution in alcohol with zinc dust and acetic acid. The regenerated acetyl derivative was recrystallized 20 times. The top fraction consisted of colorless plates and then weighed 5.4 g.; m. p., 143–144°; $[\alpha]_D$, —43.74°. This substance was again brominated in the manner described above. The bromination mixture, which was clear at first, gradually turned cloudy and deposited on standing at room temperature overnight 1.6 g. of colorless crystals. The mother liquor was cooled in a freezing mixture when an amorphous, white precipitate separated which was filtered off, washed with acetic acid and water and dried in a vacuum over sul furic acid. These two fractions were examined separately. The substance retained in the last mother liquor was discarded.

The crystalline substance mentioned above appeared to consist of colorless prisms but when examined closely under the microscope it was seen to be made up of rods of fine, colorless globules joined together. It melted at 129–130°. The substance was readily soluble in ether and no characteristic crystals of stigmasteryl acetate tetrabromide could be obtained. For analysis the substance was dried at 78° in a vacuum over phosphorus pentoxide but there was no loss in weight.

Anal. Subs., 0.1882: AgBr, 0.1177. Calcd. for $C_{29}H_{48}Br_2O_2$ (587.84): Br, 27.19. Found: 26.61.

The results of the analysis indicate that the substance is a dibromide and it evidently cannot contain any stigmasteryl acetate tetrabromide.

The remainder of the substance was debrominated and the regenerated acetyl derivative was twice recrystallized. The colorless plates weighed 0.9 g.; m. p., 143–144°; $[\alpha]_D$, —45.37°.

The amorphous bromo compound was debrominated and the acetyl derivative was twice recrystallized. The colorless plates weighed 3.5 g.; m. p., 143–144°; $[\alpha]_D$, —45.28°. The properties are identical with those of the top fraction. There was no loss in weight on drying at 105°.

Anal. Subs., 0.1090, 0.1133: CO_2, 0.3249, 0.3384; H_2O, 0.1121, 0.1167. Calcd. for $C_{29}H_{48}O_2$ (428): C, 81.31; H, 11.21. Found: C, 81.29, 81.45; H, 11.50, 11.52.

The two acetyl fractions described above were united and saponified with boiling alcoholic potassium hydroxide. The sterol was precipitated with water, filtered off, washed with water and dried. It was dissolved in alcohol, treated with Norite and twice recrystallized. The γ-sitosterol separated in colorless, hexagonal plates which were similar in appearance to sitosterol crystals, and like the latter it gave the Liebermann-Burchard reaction. The crystals weighed 2.9 g. and melted at 147–148°; $[\alpha]_D$, —42.47°.

The substance was dried at 105° in a vacuum over phosphorus pentoxide. The loss in weight was somewhat less than the calculated amount for one molecule of water of crystallization.

Water. Calcd. for $C_{27}H_{45}OH + H_2O$: H_2O, 4.45. Found: 3.90, 4.10.

Anal. Subs., 0.1171: CO_2, 0.3598; H_2O, 0.1265. Calcd. for $C_{27}H_{45}OH$ (386): C, 83.93; H, 11.91. Found: C, 83.80; H, 12.08.

The molecular weight of the free γ-sitosterol could not be determined very accurately. The method of Rast[13] gave results that varied from 401 to 408. The values obtained by the cryoscopic method using benzene as solvent were also too high, varying from 434 to 446, evidently due to the fact that some of the sterol crystallized with the solvent.

The dibromo-acetyl derivative gave better values by the cryoscopic method using benzene as solvent. The dibromide was prepared from the pure acetyl derivative. The substance was reprecipitated from benzene with methyl alcohol when it separated in the form of fine, colorless globules: m. p., 129–130°.

Mol. wt. Subs., 0.5925, 0.7801: 17.1557 g. of benzene; Δ*t* 0.296°, 0.387°. Calcd. for $C_{29}H_{48}Br_2O_2$: mol. wt., 587.84. Found: 583, 587.

A further quantity of γ-sitosterol was obtained by the following method. During the recrystallizations of the acetyl derivative, mentioned above, a number of intermediate fractions had been collected. The fractions that melted between 132° and 134°, $[\alpha]_D$ about —41°, were united (total weight, 20.3 g.) and recrystallized 35 times. The top fraction consisted of colorless plates which weighed 3.3 g.; m. p., 142–143°; $[\alpha]_D$, —44.26°. The acetyl derivative was saponified and the sterol was isolated and recrystallized as described above. The substance crystallized in colorless plates and weighed 2.75 g.; m. p., 146–147°; $[\alpha]_D$, —41.32°. These values are nearly the same as those recorded for the γ sitosterol that had been purified by means of the dibromo-acetyl derivative and the results indicate that approximate purity may be attained by crystallization.

Dihydro-γ-sitosterol.—Both of the γ-sitosterol preparations mentioned above were reduced with hydrogen in the presence of platinum black. The reduction products had similar properties: (1) m. p. 143–144°, $[\alpha]_D$ +18.89°; (2) m. p. 143–144°, $[\alpha]_D$ +18.01°. The acetyl derivative melted at 142–143°; $[\alpha]_D$, +9.98°.

The dihydro-γ-sitosterol as well as other reduction experiments mentioned in this paper will be more fully described in a separate publication.

β-Sitosterol.—A number of fractions of the acetyl derivative that melted at 127–128° had been collected during the preceding recrystallizations in the purification of γ-sitosteryl acetate. The melting point corresponds to that of sitosteryl acetate but the preparations were obviously not homogeneous since both melting point and optical rotation changed on further fractionation.

The following data are recorded as a typical example of the behavior of these preparations. A fraction consisting of snow-white prisms weighed 6.3 g.; m. p., 127–128°; $[\alpha]_D$, —39.56°. In composition it corresponded to sitosteryl acetate as shown by the following analysis.

Anal. Subs., 0.1371: CO_2, 0.4078; H_2O, 0.1402. Calcd. for $C_{29}H_{48}O_2$ (428): C, 81.31; H, 11.21. Found: C, 81.12; H, 11.44.

The substance was recrystallized ten times and separated into three fractions which had the properties shown in Table IV.

TABLE IV

CHANGES IN PROPERTIES OF SITOSTERYL ACETATE ON RECRYSTALLIZATION

Fraction	Wt., g.	M. p., °C.	$[\alpha]_D$
1	2.7	131–132	−40.39°
2	1.2	126–127	−39.94°
3	1.7	124–125	−38.91°

Several other fractions of the acetyl derivative similar to that described above were

[13] Rast, *Ber.*, **55**, 1051 (1922).

united; m. p., 127°; $[\alpha]_D$, —39.10°. The material was saponified and the sterol was twice recrystallized from alcohol, from which it separated in colorless plates. It melted at 140°, solidified at 127° and remelted at 140°; $[\alpha]_D$, —35.06°. This substance represents the purest "sitosterol" that could be obtained from the wheat germ sterols but, as stated above, it is not a homogeneous compound. The substance is a mixture of β-sitosterol with a small amount of γ-sitosterol.

Dihydro-β-sitosterol.—When β-sitosterol preparations such as those described above, are reduced with hydrogen in the presence of platinum black, saturated sterols are obtained that are different from dihydro-γ-sitosterol. The reduction products are, however, either identical with or very similar to the natural dihydrositosterol which occurs in certain plant fats. γ-Sitosterol with a rotation of —42° gives a saturated sterol that has a rotation of +18°. Mixtures of β- and γ-sitosterol with rotations of —35° or —36° yield saturated sterols that have a rotation of about +24°. In both cases the total change of rotation is the same, amounting to about 60°.

The β-sitosterol mentioned above, m. p. 140°, $[\alpha]_D$ —35.06°, was reduced with hydrogen and platinum black. The reduction product crystallized in large, colorless hexagonal plates; m. p., 139–139.5°; $[\alpha]_D$, +24.22°. The acetyl derivative melted at 137–138°; $[\alpha]_D$, +13.57°.

α-Sitosterol.—When the acetyl derivative of the original sterol was fractionated the bottom fractions possessed a low melting point and a low levorotation as shown in Table III. The two bottom fractions, Table III, were united and separated by ten recrystallizations into four fractions. The results are shown in Table V.

TABLE V

PROPERTIES OF THE BOTTOM FRACTIONS OF THE ACETYL DERIVATIVE

Fraction	Wt., g.	M. p., °C.	$[\alpha]_D$
1	6.0	122–123	−29.84°
2	3.5	118–119	−28.03°
3	3.8	117–118	−26.81°
4	6.1	117–118	−25.69°

Fractions 3 and 4, Table V, were united and again fractionated in the same manner, when a bottom fraction was obtained that melted at 113–114°; $[\alpha]_D$, —23.16°.

The acetyl derivative of the wheat germ sterol corresponding to sitosterol may therefore be separated into fractions that range in melting point from 143–144° to 113–114° and in rotation from —45° to —23°. The upper fraction consists of γ-sitosterol and it is assumed that the portion having the lower melting point contains an isomeric compound which is designated α-sitosterol. The latter substance as obtained by crystallization is far from pure, since it evidently contains a large proportion of β-sitosterol and a smaller quantity of γ-sitosterol.

When the fractions described in Table V were brominated some hydrogen bromide was liberated, indicating substitution of bromine. After the bromo-acetyl derivatives had been boiled with zinc dust and acetic acid, the reaction product could be separated by crystallization into two fractions: (a) a crystalline acetyl derivative which was free from bromine, m. p. 127–128°, $[\alpha]_D$ —about —39°, that represents a mixture, as suggested in this paper, of β- and γ-sitosterol; (b) a non-crystalline resinous mass, containing bromine which represents a bromine substitution product of α-sitosterol, was obtained from the mother liquor. The non-crystalline substance is readily soluble in alcohol and is, therefore, easily removed from the crystalline acetyl derivative. The substance may be boiled for 24 hours or longer with zinc dust or zinc amalgam and acetic or hydrochloric acid without becoming debrominated.

The bottom fractions are identical in composition with β- and γ-sitosterol. An acetyl derivative, m. p. 114–116°, $[\alpha]_D$ —23.91°, was analyzed.

Anal. Subs., 0.1264: CO_2, 0.3766; H_2O, 0.1291. Calcd. for $C_{29}H_{48}O_2$ (428): C, 81.31; H, 11.21. Found: C, 81.26; H, 11.42.

A portion of the same preparation was saponified and the free sterol was twice recrystallized. It separated in dense, colorless plates. The melting point was not sharp, 138–141°; $[\alpha]_D$, —23.41°. The substance was analyzed after it had been dried at 105°.

Anal. Subs., 0.1160: CO_2, 0.3575; H_2O, 0.1252. Calcd. for $C_{27}H_{45}OH$ (386): C, 83.93; H, 11.91. Found: C, 84.05; H, 12.07.

Another portion of the same preparation was reduced with hydrogen and platinum black. The substance was reduced very slowly and repeated, prolonged treatments with fresh portions of catalyst were necessary before the reduction product gave a negative Liebermann-Burchard reaction. The reduced acetyl derivative crystallized in large, colorless plates; m. p., 136–137°; $[\alpha]_D$, +13.69°.

After saponification and recrystallization, the saturated sterol separated in colorless plates; m. p., 137–138°; $[\alpha]_D$, +23.20°. These properties are very similar to those of β-sitostanol.

Further Investigation of the Sterols from Wheat Germ

In order to secure more evidence regarding the composition of the wheat germ sterols a fresh preparation was made. Wheat germ,[14] 44 kg., was extracted with a mixture of ether and alcohol and nearly 4 kg. of oil was obtained. The oil was saponified with alcoholic potassium hydroxide, diluted with water, extracted with ether and the ether was distilled. The residue was again boiled with alcoholic potassium hydroxide and extracted as before. The crude unsaponifiable matter which weighed 158 g. was purified by crystallization from alcohol, yielding 73 g. of crystalline sterols; m. p., 135–136°; $[\alpha]_D$, —27.47°.

The substance was fractionated by crystallization from alcohol and the results that were obtained coincided completely with those described before. After 35 recrystallizations the top fraction melted at 130–133°; $[\alpha]_D$, —3.18°.

Isolation of Dihydrositosterol.—The top fraction mentioned above was acetylated and treated in carbon tetrachloride solution with acetic anhydride and sulfuric acid until no further coloration was produced. From the solvent it was possible to isolate by the method already described 0.44 g. of pure dihydrositosterol; m. p., 143–144°; $[\alpha]_D$, +22.20°.

Preparation of γ-Sitosterol.—The intermediate sterol fractions were acetylated. The acetyl derivative, after it had been recrystallized, weighed 42.7 g.; m. p., 128–130°; $[\alpha]_D$, —32.93°. The substance was recrystallized 35 times and separated into a series of fractions whose melting points ranged from 143–144° to 114–116° and the rotations from —43.33° to —23°. The top fraction, m. p. 143–144°, $[\alpha]_D$ —43.33°, was brominated, but no stigmasterol could be found. The dibromo-acetyl derivative was purified by crystallization from benzene and methyl alcohol. It separated as colorless needles; m. p., 136–137°.

The crystalline dibromide was analyzed and its molecular weight was determined by the freezing-point method, using benzene as solvent.

[14] The wheat germ, supplied by Pillsbury Flour Mills Co., Buffalo, N. Y., appeared to be as pure as this material can be obtained commercially but a microscopic examination revealed a slight contamination with particles of bran and starch.

Anal. Subs., 0.1960, 0.1967: AgBr, 0.1242, 0.1253. Calcd. for $C_{29}H_{48}Br_2O_2$ (587.84): Br, 27.19. Found: 26.97, 27.11.

Mol. wt. Subs., 0.5250, 0.7336: 16.09, 19.12 g. of benzene; Δt 0.279°, 0.327°. Calcd. for $C_{29}H_{48}Br_2O_2$: mol. wt., 587.84. Found: 584.7, 586.6.

γ-Sitosteryl acetate was obtained by debrominating the crystalline dibromide. The substance crystallized in colorless plates and melted at 143–144°; $[\alpha]_D$, —45.05°.

Dihydro-γ-sitosterol.—A portion of the γ-sitosterol acetate was reduced with hydrogen and platinum black. The reduction product melted at 144–145°; $[\alpha]_D$, +10.11°. The dihydro-γ-sitosterol was obtained by saponifying the acetyl derivative described above; m. p., 144–145°; $[\alpha]_D$, +17.88°.

β-Sitosterol.—A portion of the intermediate fractions of the acetyl derivative, corresponding in melting point to sitosteryl acetate, was purified by means of the dibromo-compound for the purpose of removing α-sitosterol. The bromo compound was boiled with zinc dust and acetic acid and the reaction product was recrystallized from alcohol. From the mother liquors was isolated a small amount of a yellow, non-crystalline mass that contained bromine. The purified acetyl derivative corresponded to the mixture of the β- and γ-sitosteryl acetates previously described. The analytical results were in agreement with the formula $C_{29}H_{48}O_2$. Analyses and molecular-weight determinations of the dibromide prepared from the purified acetyl derivative were in agreement with the formula $C_{29}H_{48}Br_2O_2$. The saturated sterol obtained by reduction with hydrogen and platinum black had very similar properties to those of the dihydro-β-sitosterol previously obtained.

α-Sitosterol.—The bottom fractions of the original acetyl derivative differed from the intermediate fractions in that they were decidedly more soluble in alcohol and the melting point and rotation were much lower. Analyses gave results that were in agreement with the usual formula for sitosterol. When reduced with hydrogen and platinum black a saturated sterol was obtained that appeared to be identical with dihydro-β-sitosterol.

The results of the investigations of the two sterol preparations obtained from wheat germ were very similar in all respects.

Summary

1. The crystalline sterols obtained from the germ of American-grown wheat have been examined.

2. No pure, homogeneous sitosterol could be found.

3. The wheat germ sterols constitute a mixture containing dihydrositosterol and at least three sterols isomeric with sitosterol which are designated α-, β- and γ-sitosterol.

4. α- and β-Sitosterol could not be obtained in pure form.

5. γ-Sitosterol which is the least soluble of the three isomers was obtained in a fairly pure state. It crystallizes in colorless plates containing one molecule of water of crystallization. In appearance the crystals are similar to sitosterol. The substance melts at 147–148°; $[\alpha]_D$, about —42°. The acetyl derivative melts at 143–144°; $[\alpha]_D$, about —45°.

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