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.

GENEVA, NEW YORK

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

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]

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[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).

The object of the present investigation was to secure a larger quantity of pure sitosterol, and corn oil was selected as the source of this substance because it is rich in unsaponifiable matter. From 10 kg. of refined corn oil about 100 g. of crystalline sterols was obtained. In purifying this preparation it was found that it could be separated into a number of fractions which showed marked differences in properties, especially in optical rotation. In studying this matter more closely it was found that the preparation was a complex mixture containing several sterols.

In the first place an appreciable quantity of the saturated sterol, dihydrositosterol, $C_{27}H_{47}OH$, which is a constituent of the fat extracted from the endosperm of corn[5] and wheat[6] was isolated.

The sterol which remained after dihydrositosterol had been removed was supposed to represent pure sitosterol. Numerous recrystallizations from alcohol and acetone gave fractions that had practically identical properties. The substance behaved, therefore, like a homogeneous compound. Exhaustive fractionation of the acetyl derivative led, however, to a partial separation but it was impossible to obtain even the least soluble fraction in an approximate state of purity by this method. The top fraction after some 40 recrystallizations was further purified by bromination by the method of Windaus and Hauth,[7] when a sparingly soluble compound was precipitated. This product on recrystallization from a mixture of benzene and methyl alcohol yielded a small quantity of stigmasteryl acetate tetrabromide. The amount of stigmasterol present in corn oil is so small that it cannot be detected by the usual method of Windaus and Hauth[7] and it was stated in the publication referred to above[2] that corn oil did not contain any of this sterol.

After the stigmasterol had been removed from the top fraction of the acetyl derivative the remainder of the substance was further fractionated and finally brominated, when a crystalline precipitate separated. This bromo compound crystallized in colorless needles from a mixture of benzene and methyl alcohol. Analyses and molecular-weight determinations proved that this substance was a dibromide having the composition $C_{27}H_{45}Br_2O.CO.CH_3$. Debromination of this compound yielded the acetyl derivative of a new sterol which crystallized in colorless plates; m. p., 143–144°; $[\alpha]_D$, —46.09°.

The free sterol, prepared by saponifying the acetyl derivative, crystallized in colorless plates containing one molecule of water of crystallization. The crystals were very similar in appearance to sitosterol. The substance melted at 145–146°, $[\alpha]_D$ —42.43°, and agreed in composition with the formula $C_{27}H_{45}OH$. The new sterol is, therefore, isomeric with sitosterol

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[5] Anderson, *ibid.*, **46**, 1450 (1924).

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

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

but differs from the latter in melting point and in optical rotation. It is proposed to designate this substance by the name γ-sitosterol.

When γ-sitosterol is reduced with hydrogen in the presence of platinum black a saturated sterol, dihydro-γ-sitosterol, $C_{27}H_{47}OH$, is obtained. It crystallizes in large, colorless plates; m. p., 144–145°; $[\alpha]_D$, +17.82°.

Some years ago a substance called paraphytosterol was isolated from the seed coats of *Phaseolus vulgaris* by Likiernik.[8] The properties of this compound, m. p. 149–150°, $[\alpha]_D$ —44.10°, are similar to those of γ-sitosterol and it is possible that these substances may be identical.

The remainder of the acetyl derivative, after dihydrositosterol, stigmasterol and γ-sitosterol had been removed, was especially examined for sitosterol. A number of fractions had been collected that possessed the properties ascribed to sitosteryl acetate but on further fractionation the properties always changed. The top fraction had a higher melting point and rotation than the intermediate fractions. An attempt to purify some of this material by means of the dibromo compound led to the observation that a portion of the substance was eliminated. In brominating these mixtures bromine was not only absorbed on the double bond but there was also some substitution with liberation of hydrobromic acid. The bromine substitution product could not be debrominated completely but as it was easily soluble in alcohol it was eliminated in recrystallizing the regenerated acetyl derivative.

The acetyl derivative after being purified in this manner possessed a slightly higher levorotation than before, indicating that some substance of a lower rotation had been removed. On fractionation the substance behaved exactly as stated above, that is, an increase in melting point and rotation of the top fraction was observed. It is believed, therefore, that this fraction contains two sterols, a small amount of γ-sitosterol and an isomeric compound which may be designated as β-sitosterol.

Analyses and molecular-weight determinations of the dibromide prepared from the purified acetyl derivative were in agreement with the formula $C_{27}H_{45}Br_2O.CO.CH_3$. The acetyl derivative, m. p. 127–128°, $[\alpha]_D$ about —39°, and the free sterol, m. p. 139–140°, $[\alpha]_D$ about —36°, also gave results on analyses that were in close agreement with the usual sitosterol formula, $C_{27}H_{45}OH$. The properties of the sterol fraction designated β-sitosterol correspond closely with those described in the literature for sitosterol.

When β-sitosterol is reduced with hydrogen and platinum black, dihydro-β-sitosterol is formed. The properties of this compound differ from those of dihydro-γ-sitosterol. It melts at 140–141°; $[\alpha]_D$, +24.91°.

The bottom fractions of the original sterol, corresponding to the parasitosterol mentioned by Burian,[3] differed from the intermediate fractions

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[8] Likiernik, *Z. physiol. Chem.*, **15**, 426 (1891).

by possessing a low levorotation and a lower melting point. The low rotation might be due to the presence of a sterol isomeric with sitosterol which was either optically inactive or else had a very low levorotation and which may be called α-sitosterol. This sterol was eliminated from the intermediate fractions of the acetyl derivative by bromination, as stated above, but since the resulting bromine substitution product could not be debrominated by any of the usual methods, the properties of the pure substance could not be determined.

Sterol mixtures apparently rich in α-sitosterol are reduced with great difficulty by hydrogen and platinum black but the reduction product which is finally obtained appears to be identical with that prepared from β-sitosterol. The substance melted at 139–140°; $[\alpha]_D$, +23.53°.

α-Sitosterol differs, therefore, from β-sitosterol in forming a bromine substitution product which cannot be debrominated. The two compounds must be closely related, nevertheless, since both give saturated sterols on reduction which are identical in composition and have very similar properties. It is interesting to note that the melting points and rotations of these reduction products are very similar to those of the natural dihydrositosterol.

Experimental Part

Preparation of the Unsaponifiable Material.[9]—Refined corn oil,[10] 10 kg., was saponified by heating on the water-bath with alcoholic potassium hydroxide. The soap solution was diluted with water and extracted with four portions of ether. The ether was distilled and the residue was again boiled with alcoholic potassium hydroxide for two hours. The solution was diluted with water, extracted with three portions of ether and the ethereal solution was washed thoroughly with water, filtered and the ether was distilled. The dark brown, crystalline residue was dissolved in hot alcohol and on cooling 87.5 g. of light yellow crystals separated. The filtrate was concentrated and cooled when 32.7 g. of dark brown crystals was obtained. The last filtrate was evaporated to dryness when a dark brown, semisolid oil remained which weighed 26 g. The total yield of unsaponifiable material was therefore about 1.5%.

The last two fractions were dissolved in a little hot acetone and the solutions were cooled in a freezing mixture when light yellow crystals, practically free from oil, separated. They were filtered off, washed with a little cold acetone and allowed to dry. The crystals which weighed 16.7 g. were united with the main crystalline portion mentioned above.

The acetone mother liquors were united and evaporated to dryness. The residue was a dark brown oil which weighed 42 g.

Purification of the Sterols.—The crude crystalline sterols which weighed 104.2 g. were dissolved in hot alcohol, treated with Norite, and recrystallized thrice from large volumes of alcohol.[11] The beautiful, snow-white preparation consisted of colorless

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[9] We wish to thank Mr. W. F. Morton, formerly Associate Chemist at this Station, for assistance in preparing the unsaponifiable material.

[10] Supplied by the Patent Cereals Co., Geneva, N. Y.

[11] Unless otherwise specified, ethyl alcohol purified by distillation with potassium hydroxide was used in all the crystallizations mentioned in this paper.

plates and weighed 64 g.; m. p.,[12] 139–140°; $[\alpha]_D$, —31.43°.[13] The substance was then recrystallized 15 times and separated into Fractions 1, 2 and 3. The first three mother liquors yielded on concentration, successively, Fractions 4, 5, 6 and 7. The properties of these various fractions are recorded in Table I.

TABLE I

FRACTIONATION OF THE UNSAPONIFIABLE MATTER FROM CORN OIL

Fraction	Wt., g.	M. p., °C.	$[\alpha]_D$	Description
1	19.7	139–140	−17.45°	Colorless plates
2	25.1	139–140	−35.63°	Colorless plates
3	18.4	139–140	−36.85°	Colorless plates
4	13.1	139–140	−36.79°	Colorless plates
5	11.2	139–140	−32.45°	Colorless plates
6	3.8	135–136	−13.85°	Colorless plates
7	12.9	impure crystals from the last mother liquors		
8	42.0	brown, uncrystallizable oil		

Although the melting points of the first five fractions are identical it is evident from the variations in optical rotation that the sterol could not be homogeneous. It was believed that the low rotation of Fraction 1 was due to the presence of the dextrorotatory dihydrositosterol but the low rotations of Fractions 5 and 6 must be caused by the presence of some other substance, namely, the parasitosterol mentioned by Burian[3] and Ritter[3] and which is called α-sitosterol in this paper.

Isolation of Dihydrositosterol from Fraction 1.—By methods that will be described in another paper it was possible to isolate 1.65 g. of pure dihydrositosterol from Fraction 1. The substance possessed the properties that are characteristic of dihydrositosterol, m. p. 143–144°, $[\alpha]_D$ +23.81°, and the composition agreed with the formula $C_{27}H_{47}OH$.

Purification of the Corn Oil Sitosterol.—Judging by the properties recorded in Table I, it was believed that Fractions 3 and 4 represented nearly pure sitosterol. Attempts were made to fractionate these portions by five and ten recrystallizations from alcohol and by five recrystallizations from acetone, but no perceptible difference could be observed, the properties of both top and bottom fractions being practically identical. Attempts to separate the substance by means of fractional precipitation of the dibromoacetyl derivative, as described in an earlier paper,[14] were hardly more successful. After

TABLE II

FRACTIONATION OF STEROL FRACTION 3 FROM ALCOHOL AND ACETONE

	5 Recrystallizations from alcohol		5 Recrystallizations from acetone		10 Recrystallizations from alcohol	
Fraction	M. p., °C.	$[\alpha]_D$	M. p., °C.	$[\alpha]_D$	M. p., °C.	$[\alpha]_D$
Top	140–141	−36.16°	140–141	−37.28°	140–141	−37.14°
Bottom	140–141	−37.09°	140–141	−36.93°	139–140	−37.09°

TABLE III

STEROL FRACTION 4 AFTER FRACTIONAL PRECIPITATION OF THE DIBROMO-ACETYL DERIVATIVE

All Fractions Melted at 139–140°

$[\alpha]_D$	−36.84°	−36.56°	—37.93°	−38.15°

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[12] All melting points are corrected.

[13] All rotations were observed in chloroform solution and with sodium light.

[14] Anderson and Nabenhauer, THIS JOURNAL, **46**, 2113 (1924).

debromination and saponification, the sterol was recovered and separated into four fractions by crystallization. The results obtained in the operations mentioned above are given in Tables II and III.

The slightly higher levorotations of the two bottom fractions in Table III indicated a possibility that the sterol was not homogeneous. In order to test this matter further, the acetyl derivative was prepared from the sterol listed in the top fraction, Table II, m. p. 140–141°, $[\alpha]_D$ —36.16°, and it was recrystallized ten times. The first five mother liquors on concentration gave Fraction 3. The last five mother liquors gave Fraction 2. The final mother liquors were combined, yielding Fraction 4. The results are summarized in Table IV.

TABLE IV

FRACTIONATION OF THE ACETYL DERIVATIVE; M. P., 132–133°; $[\alpha]_D$, —39.50°

Fraction	1	2	3	4
M. p., °C.	136–137	129–130	127–128	121–122
$[\alpha]_D$	−42.58°	−39.89°	−38.43°	−34.06°

The data show that the acetyl derivative may be separated by crystallization into fractions that vary greatly in properties and it is evident that the sterol is not a homogeneous compound.

The other sterols included in Fractions 2, 3 and 4, Table I, were acetylated and recrystallized separately. In every case the top fractions melted at about 140° and the rotation was about —45° after the substance had been recrystallized from 20 to 25 times, as is shown in Table V.

TABLE V

PROPERTIES OF THE TOP FRACTIONS OF THE ACETYL DERIVATIVE

No. of recrystallizations	Wt., g.	M. p., °C.	$[\alpha]_D$
19	1.7	140	−45.13°
20	2.5	140–141	−45.27°
25	2.8	140	−45.13°
25	3.5	140–141	−44.79°

The various top fractions were united and again recrystallized 15 times, when a slow but steady rise in melting point and rotation was observed. The final top fraction weighed 3.5 g.; m. p., 143–144°; $[\alpha]_D$, —46.96°. Since this material had been recrystallized about 40 times and as it was apparently not yet homogeneous it seemed impossible to obtain a pure compound by crystallization. It was decided therefore to brominate the substance in the hope of effecting a more complete separation by this procedure.

Bromination of the Top Fraction. Isolation of Stigmasterol.—The following preparations were brominated by the method of Windaus and Hauth:[7] (a) Top Fraction, 3.5 g.; $[\alpha]_D$ —46.96°; (b) 1.5 g.; $[\alpha]_D$, −45.41°; (c) 4.0 g.; $[\alpha]_D$, −42.33°. Fractions b and c had been obtained from the mother liquors in recrystallizing the top fraction. The solutions, on standing at room temperature, deposited small amounts of crystalline precipitates. After the mixtures had stood in the ice box for four hours the crystals were filtered off, washed with glacial acetic acid and dried in a vacuum over sulfuric acid and potassium hydroxide. The slightly yellow substance weighed 1.6 g.

The filtrates from the products described above were united and precipitated by adding water. The substance was filtered off, washed with water and dried in a vacuum over sulfuric acid. The white, amorphous dibromide, which weighed 10.8 g., was examined as will be described later.

The crystalline product, obtained as described above, was recrystallized 14 times from benzene and methyl alcohol before the melting point was constant. The substance was identified as stigmasteryl acetate tetrabromide. The colorless quadratic plates weighed 0.4 g.; m. p., 210–211° with decomposition.

The substance was dried at 78° in a vacuum over phosphorus pentoxide but there was no loss in weight.

Anal. Subs., 0.1070: AgBr, 0.1034. Calcd. for $C_{32}H_{52}O_2Br_4$ (787.68): Br, 40.58. Found: 41.12.

After the substance had been debrominated and recrystallized, 0.15 g. of the acetyl derivative was obtained as colorless plates: m. p., 143°; $[\alpha]_D$, —50.28°. The acetyl derivative was saponified and recrystallized, yielding 0.12 g. of stigmasterol as colorless plates; m. p., 170–171°; $[\alpha]_D$, —45.28°.

The amount of stigmasterol present in corn oil is so small that it cannot be detected on brominating the original acetyl derivative. It evidently accumulates in the top fraction since it was possible to isolate the small quantity mentioned above only after the mixed acetates had been recrystallized forty times.

Examination of the Amorphous Dibromide.—The amorphous dibromide, obtained as mentioned above, was easily soluble in benzene, ether and chloroform. It dissolved in hot alcohol but separated as colorless globules as the solution cooled. Crystals of the tetrabromide could not be obtained. The substance was dissolved in benzene and separated into three fractions by the gradual addition of methyl alcohol. These fractions were debrominated separately and the acetyl derivatives were recrystallized. The properties of the three preparations were identical; m. p., 141°; $[\alpha]_D$, —43°. The fractions were united and recrystallized ten times. The top fraction weighed 3.1 g.; m. p., 142–143°; $[\alpha]_D$, —45.14°.

The results indicated that the substance was a mixture that was slowly separated on recrystallization. The observed increase in melting point and levorotation was probably due to the presence of a new sterol that had not previously been recognized in corn oil. In order to secure a larger amount of this substance it became necessary to work up the numerous intermediate fractions of the acetyl derivative that had been accumulated during the previous fractionations.

Examination of the Intermediate Fractions of the Acetyl Derivative. Removal of α-Sitosterol.—It had been noticed in earlier experiments in purifying the acetyl derivative by means of the dibromo compound that the regenerated substance had a levorotation about 1° higher than the original acetate and that the alcoholic mother liquors contained a small amount of a yellow, non-crystalline substance which contained bromine. It was believed that this material represented a bromine substitution product of α-sitosterol because larger amounts of the non-crystalline substance were obtained from acetyl derivatives that had a low levorotation.

In order to eliminate the α-sitosterol from the intermediate fractions before subjecting them to further fractionation, the united material was brominated by the method of Windaus and Hauth.[7] Eight fractions, ranging in melting point from 121° to 132° and in rotation from —35° to —39.4°, were united giving a total weight of 34.8 g. The bromination mixture remained clear at room temperature but a small amount of hydrogen bromide was liberated, indicating some substitution of bromine. After the bromo compound had been isolated, it was dissolved in alcohol and the solution was boiled with zinc dust and acetic acid. The substance was then precipitated with water, filtered, washed with water, dried and thrice recrystallized.

The alcoholic mother liquors were freed as much as possible from the crystalline acetyl derivative and evaporated to dryness when 5.7 g. of a yellow, resinous residue remained. It was readily soluble in ethyl and methyl alcohol but could not be obtained in crystalline form. It gave a strong reaction for bromine but it could not be debrominated by any of the usual methods.

The regenerated acetyl derivative was obtained as snow-white crystals that weighed 24.2 g.; m. p., 129–130°; $[\alpha]_D$, —40.81°. It gave no reaction for bromine. The substance was united with 13.3 g. of another preparation, m. p. 129–130°, $[\alpha]_D$ —40.20° that had been purified previously by means of the bromo compound. The material was recrystallized forty times and fractions were collected from every three and later from every five mother liquors. The properties of these fractions are shown in Table VI.

TABLE VI

FRACTIONATION OF THE ACETYL DERIVATIVE

Fraction	Wt., g.	M. p., °C.	$[\alpha]_D$
1	2.9	124–125	−38.01°
2	4.6	126–127	−39.15°
3	4.0	127–128	−39.13°
4	3.9	127–128	−39.58°
5	4.2	128–129	−39.93°
6	3.4	129–130	−40.42°
7	5.4	131–132	−40.96°
8	3.3	134–135	−42.04°
9	4.2	140–141	−44.62°

Preparation of γ-Sitosterol

The substance described on p. 2982, 3.1 g., m. p. 142–143°, $[\alpha]_D$ —45.14°, was united with fraction 9 (Table VI). The material was brominated by the method already described. The solution, on standing at room temperature, deposited 1.2 g. of colorless crystals. The mother liquor was partly freed from ether under reduced pressure and then cooled, when 6.3 g. of colorless, needle-shaped crystals was obtained. The substance contained in the filtrate was discarded.

The crystals were treated with ether, 10 cc. per g. of substance, and a small amount of insoluble material was filtered off. Glacial acetic acid was added to the filtrate and the solution was cooled. The substance again crystallized in needles. The crystals were digested in ether and the insoluble material was filtered off. These operations were repeated until the bromo compound was completely soluble in ether.

The ether-insoluble material weighed 0.2 g. When it was crystallized from benzene and methyl alcohol, colorless quadratic plates were obtained which melted at 210–211°, with decomposition. The properties indicate that the substance was stigmasteryl acetate tetrabromide.

The ethereal solution of the bromo compound, after removal of the stigmasterol as described above, was brought to crystallization by the addition of glacial acetic acid and cooling. The colorless needles, 2.8 g., were dissolved in 25 cc. of ether, and warm methyl alcohol was added until the solution turned cloudy. Colorless, silky needles separated as the solution cooled. The crystals were filtered off, washed with methyl alcohol and dried.

The substance contained in the filtrates was recovered as will be described later.

The colorless crystals weighed 1.5 g.; m. p., 136–137°. When dried at 105° the substance turned dark but drying at 78° in a vacuum over phosphorus pentoxide caused no change in color and there was no loss in weight.

Anal. Subs., 0.2105: AgBr, 0.1346. Calcd. for $C_{29}H_{48}O_2Br_2$ (587.84): Br, 27.19. Found: 27.21.

Mol. wt. (Cryoscopic method; solvent, benzene.) Subs., 0.5300: 18.92 g. of benzene; Δt, 0.239°. Calcd. for $C_{29}H_{48}O_2Br_2$: mol. wt., 587.84. Found: 586.

The analysis and molecular-weight determination indicate that the substance is a dibromide and that the sterol possesses the formula ascribed to sitosterol, $C_{27}H_{45}OH$.

The remainder of the crystalline dibromide was debrominated by boiling with zinc dust and acetic acid. The acetyl derivative was isolated and twice recrystallized, when colorless plates were obtained which weighed 0.75 g. The substance melted at 143–144°, solidified at 136° and remelted at 143–144°; $[\alpha]_D$, —46.09°.

The mother liquors from which the crystalline dibromide had been obtained were mixed with zinc dust and boiled for two hours. The resulting acetyl derivative was recrystallized six times, yielding colorless plates that weighed 2 g. It melted at 143–144° and the rotation was —46.13°. It is evident, therefore, that the two acetyl derivatives had identical properties.

The free sterol was prepared by saponifying 1 g. of the acetyl derivative with alcoholic potassium hydroxide. It was precipitated by water, filtered off, washed with water, dried and recrystallized four times. It separated in colorless plates similar to those of sitosterol. The air-dried sterol melted at 145–146°, solidified at 138° and remelted at 146°; $[\alpha]_D$, —42.43°. It gave the Liebermann-Burchard reaction. When dried at 105° in a vacuum over phosphorus pentoxide the loss in weight corresponded to one molecule of water of crystallization.

Anal. Subs., 0.5014: loss on drying, 0.0216. Calcd. for $C_{27}H_{45}OH + H_2O$: H_2O, 4.45. Found: 4.30.

Dried subs., 0.1136: CO_2, 0.3483; H_2O, 0.1236. Calcd. for $C_{27}H_{45}OH$ (386): C, 83.93; H, 11.91. Found: C, 83.62; H, 12.17.

It is proposed to designate this new sterol, which is isomeric with sitosterol, differing from the latter only in melting point and in optical rotation, by the name γ-sitosterol.

Dihydro-γ-sitosteryl Acetate.—A portion of the acetyl derivative described above was reduced in alcoholic solution with hydrogen in the presence of platinum black. The reduction product was recrystallized and separated from alcohol in large, colorless plates. In the Liebermann-Burchard reaction it gave no immediate coloration and when the reaction mixture was allowed to stand only a faint green color developed. The substance melted at 143°, solidified at 135° and remelted at 143–144°; $[\alpha]_D$, +8.98°. The melting point was the same as before reduction but the rotation had changed from —46° to +9°.

Anal. Subs., 0.0884: CO_2, 0.2614; H_2O, 0.0939. Calcd. for $C_{27}H_{47}O.CO.CH_3$ (430): C, 80.93; H, 11.62. Found: C, 80.65; H, 11.88.

Dihydro-γ-sitosterol.—The reduced acetyl derivative was saponified with 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. It separated in large, colorless plates, m. p. 144–145°, solidified at 140° and remelted at 144–145°; $[\alpha]_D$, +17.82°.

Anal. Dried subs., 0.1048: CO_2, 0.3213; H_2O, 0.1179. Calcd. for $C_{27}H_{47}OH$ (388): C, 83.50; H, 12.37. Found: C, 83.61; H, 12.58.

Attempt to Prepare Pure Sitosteryl Acetate.—A study of the data presented in Table VI shows that a gradual change occurs in the properties of the different fractions of the acetyl derivative on fractionation. Fractions 2, 3 and 4 possessed the melting point usually given for pure sitosteryl acetate.

A number of fractions of the acetyl derivative were united, total weight 14.5 g., m. p. 126° to 128°, $[\alpha]_D$ —38.83° to —39.41°, and the material was recrystallized ten times. The mother liquors were concentrated and the substance that separated was twice recrystallized, giving Fraction 2. The last mother liquors on concentration gave Fraction 3. The properties of these preparations are shown below.

Fraction	Wt., g.	M. p., °C.	$[\alpha]_D$
1	7.1	127–129	−39.90°
2	5.6	124	−38.63°
3	1.2	122–123	−37.41°

The variation in melting point and in optical rotation indicates that the preparation was a mixture that was slowly separated by recrystallization.

Bromination of Fraction 1.—A portion of the acetyl derivative, Fraction 1, was brominated by the method already described. The dibromo compound was precipitated by water, filtered off, washed with water and dried. It was dissolved in a little benzene, and hot methyl alcohol was added until the solution turned cloudy. The substance separated on the form of colorless globules as the solution cooled. The precipitate was collected on a filter, washed with methyl alcohol and dried. The substance could not be obtained crystalline. It separated from hot alcohol in colorless globules and its solution in ether was precipitated in the same form by the addition of methyl alcohol; m. p., 124–125°; $[\alpha]_D$, —30.68°.

The substance turned dark when dried at 105° in a vacuum over phosphorus pentoxide but there was no change in color when dried at 78° and there was no loss in weight.

Anal. Subs., 0.2248: AgBr, 0.1418. Calcd. for $C_{29}H_{48}O_2Br_2$ (587.84): Br, 27.19. Found: 26.85

Mol. wt. (Cryoscopic method; solvent, benzene.) Subs., 0.3725: 17.22 g. of benzene Δt, 0.184°. Calcd. for $C_{29}H_{48}O_2Br_2$: mol. wt., 587.84. Found: 587.8.

The results of the analyses indicate that the substance is a dibromide identical in composition and molecular weight with the crystalline dibromide which was prepared from γ-sitosteryl acetate.

Preparation and Properties of the Free Sterol.—Another portion of Fraction 1 was saponified with alcoholic potassium hydroxide. The sterol was precipitated with water, filtered, washed with water, dried and twice recrystallized. It separated in colorless plates; m. p., 139–140°; $[\alpha]_D$, —36.11°.

Anal. Dried subs. 0.1036: CO_2, 0.3192; H_2O, 0.1120. Calcd. for $C_{27}H_{45}OH$ (386): C, 83.93; H, 11.91. Found: C, 84.03; H, 12.09.

No difference in composition could be detected by analyses or molecular-weight determinations between the mixture described above and the substance called γ-sitosterol; both correspond to the formula $C_{27}H_{45}OH$. The differences observed in the physical properties, melting point and optical rotation, must be due to a difference in arrangement of the asymmetric carbon atoms in the configuration of the molecule.

It appears probable that at least three isomeric sterols constitute what has been called sitosterol. γ-Sitosterol, the least soluble isomer, has been isolated in a fairly pure state as described above and the presence of α-sitosterol has been indicated earlier. It is evident, however, that an intermediate third isomer which may be called β-sitosterol must be present in the mixture just described. In fact, it is believed that these fractions consist largely of β-sitosterol with a small admixture of γ-sitosterol.

Reduction of β-Sitosterol.—A portion of the β-sitosterol mentioned above was reduced with hydrogen and platinum black. The dihydro-β-sitosterol separated in large, colorless plates when it was recrystallized from alcohol. It melted at 140–141°; $[\alpha]_D$, +24.91°.

Anal. Dried subs., 0.0738, 0.0878: CO_2, 0.2257, 0.2687; H_2O, 0.0852, 0.0993. Calcd. for $C_{27}H_{47}OH$ (388): C, 83.50; H, 12.37. Found: C, 83.41, 83.46; H, 12.91, 12.65.

Examination of Fraction 6 of the Original Sterol.—This fraction had been obtained from the first mother liquors on recrystallizing the crude sterol. It was much more soluble in alcohol than the other fractions and differed especially in that it possessed a very low rotation. It was apparently rich in α-sitosterol and corresponds to the parasitosterol mentioned by Burian.[3]

The substance was twice recrystallized and it separated in irregular plates; m. p., 135–136°; $[\alpha]_D$, —13.45°. The acetyl derivative was prepared and it crystallized in colorless plates; m. p., 127–128°; $[\alpha]_D$, —17.18°.

The small amount of substance available precluded any extensive fractionations but a portion was reduced with hydrogen and platinum black in order to compare the properties of the reduction product with other reduced sterols. The reduction was very slow and repeated treatments with hydrogen and fresh catalyst were necessary before the Liebermann-Burchard reaction became negative. The reduced sterol which was finally obtained crystallized in large, colorless plates. The air-dried substance melted at 139–140°. solidified at 135° and remelted at 141–142°; $[\alpha]_D$, +23.53°.

Anal. Dried subs., 0.1109: CO_2, 0.3392; H_2O, 0.1251. Calcd. for $C_{27}H_{47}OH$ (388): C, 83.50; H, 12.37. Found: C, 83.41; H, 12.62.

The acetyl derivative was prepared and recrystallized. It separated in large, colorless plates; m. p., 137–138°; $[\alpha]_D$, +13.64°.

The properties of this substance are very similar to those of dihydro-β-sitosterol but they differ from dihydro-γ-sitosterol; α- and β-sitosterol evidently yield identical reduction products and it is interesting to notice that their properties are very similar to those of the natural dihydrositosterol.

Summary

1. The phytosterols occurring in corn oil have been examined.

2. The crystalline portion of the unsaponifiable material which has been called sitosterol is a complex mixture of several sterols.

3. About 1.6% of the crude crystals consisted of dihydrositosterol, $C_{27}H_{47}OH$.

4. A very small quantity of stigmasterol, $C_{30}H_{50}O$, was also isolated.

5. The substance corresponding in composition to sitosterol, $C_{27}H_{45}OH$, is not homogeneous. It is a mixture containing at least three isomeric sterols which differ in their physical properties.

6. It is proposed to name these isomers α-, β- and γ-sitosterol.

It has been possible to prepare only γ-sitosterol in a pure condition. It crystallizes in colorless plates; m. p., 145–146°; $[\alpha]_D$, —42.43°.

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