Wooton, and probably from *Artemisia Wrightii*, all of which grow in the region of New Mexico and Mexico.[11]

Only very small quantities of material were available for experimental work. Attempts to obtain fresh and larger supplies of *Artemisia mexicana* and *Artemisia neo-mexicana* have thus far been unsuccessful. Sufficient plants could not be collected in time, and the seeds obtained failed to grow.

The closed flower heads, which in the case of *Artemisia cina*, contain the largest quantities of santonin, were used in the survey whenever possible. In many cases the open flower heads of old plants of various species had to be examined. The negative results, therefore, are not absolute proof of the absence of santonin in the species mentioned.

The results indicate a distinct possibility of utilizing domestic plants, growing as weeds in barren fields, for the manufacture of santonin.

Summary

Of 56 species of domestic *Artemisia* pronounced tests for santonin were obtained from *A. mexicana* Willd., from *A. neo-mexicana* Wooton and probably from *A. Wrightii*, all of which grew in the region of New Mexico and Mexico.

The results indicate a distinct possibility of utilizing domestic plants, growing as weeds in barren fields, for the manufacture of santonin.

WASHINGTON, D. C.

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

A STUDY OF THE PHYTOSTEROLS OF CORN OIL, COTTONSEED OIL AND LINSEED OIL[1]

BY R. J. ANDERSON WITH M. G. MOORE

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Introduction

The present investigation was undertaken in connection with the work on the phytosterols in the fat from corn pollen.[2] The properties of the phytosterols from corn pollen differed so markedly from other plant phytosterols that we desired to extend the investigation in the hope of finding similar substances in other plant products. But the materials which we examined, corn oil, cotton seed oil and linseed oil, did not yield any phytosterols similar to those found in corn pollen.

The first substance to be investigated was corn oil. The unsaponifiable matter in corn oil was called cholesterol by Hoppe-Seyler,[3] but no data were

[11] Copies of the illustrations of the plants may be obtained from the author.

[1] Read at the meeting of the American Chemical Society, New Haven, Conn., April, 1923.

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

[3] Hoppe-Seyler, "Med. Chem. Unters.," **1866**, p. 162.

given regarding the physical or chemical properties of this substance. Hopkins[4] reported that the "cholesterol" of maize oil melted at 137° to 137.5°, and gave the color reactions of cholesterol. Gill and Tufts[5] questioned the correctness of the term "cholesterol" as applied to the unsaponifiable matter in maize oil. These authors found that the purified substance melted at 137.5° to 138°, and the acetyl derivative melted at 127.1°, and they concluded that "the above results seem sufficient to prove that the alcohol of maize oil is not cholesterol.....the compound studied is undoubtedly identical with the compound found in wheat and rye and described by Burian[6] under the name of 'Sitosterol'." König and Schluckebier[7] report the isolation of a phytosterol from corn oil which melted after the seventh recrystallization at 140.4°, and the acetate melted at 137°. The high melting point of this substance must have been due to imperfect or partial acetylation.

Our results agree with those reported by Gill and Tufts. The phytosterol which we isolated from corn oil appeared to be homogeneous. We believe that it is identical with sitosterol and we could not find any evidence of the presence of stigmasterol. Upon bromination of the acetyl derivative by the method of Windaus and Hauth[8] only a dibromo compound was obtained. Ritter[9] reported for sitosterol in chloroform solution a specific rotation of —33.91°, while our preparation dissolved in chloroform gave a specific rotation of —34.38°.

Several investigators have reported upon the unsaponifiable matter in cottonseed oil.[10] Bömer and Winter[11] isolated a preparation which melted at 136° to 137°. Siegfeld[12] obtained 3 different crystalline substances from the unsaponifiable matter of cottonseed oil. A phytosterol preparation was finally obtained from the mother liquors which melted at 138.8° to 139.8°, and its acetate melted at 131.5° to 132.5°. König and Schluckebier[7] found a phytosterol which melted at 137° to 138° and the acetate melted at 125.8°, after the fifth recrystallization. Heiduschka and Gloth[13] were unable to find any stigmasterol in the phytosterol from cottonseed oil by the method of Windaus and Hauth.[8] They obtained a dibromo-acetate that melted at 127°, and that upon reduction with zinc

[4] Hopkins, THIS JOURNAL, **20**, 948 (1898).

[5] Gill and Tufts, *ibid.*, **25**, 251 (1903).

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

[7] König and Schluckebier, *Z. Nahr. Genussm.*, **15**, 641 (1908).

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

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

[10] For a more complete list of references see Lewkowitsch, "Chemical Technology and Analysis of Oils, Fats and Waxes," MacMillan and Co., Ltd., London, 6th Ed., **1922**, vol. I, p. 280 and vol. II, pp. 208–209.

[11] Bömer and Winter, *Z. Nahr. Genussm.*, **4**, 865 (1901).

[12] Siegfeld, *ibid.*, **7**, 577 (1904).

[13] Heiduschka and Gloth, *Pharm. Zentr.*, **49**, 863 (1908).

dust and saponification gave the original phytosterol which melted at 136°. In an investigation of the "Soapstock" residues from cottonseed oil, Wagner and Clement[14] isolated a phytosterol which after 3 or 4 recrystallizations melted at 137.5° to 138°. The above-mentioned authors were unable to separate any stigmasterol from the preparation by the method of Windaus and Hauth,[8] thus confirming the results of Heiduschka and Gloth.[13] They were able, however, by repeated recrystallization to separate this phytosterol into 2 fractions. One melted at 139°, and gave an acetate melting at 125°; the other melted at 130–131°, and its acetate melted at 120°. Although both of these fractions gave the usual phytosterol reactions, these authors believed that the phytosterol of cottonseed oil was not homogeneous, but consisted of two different substances.

Matthes and Heintz[15] separated the unsaponifiable matter obtained from cottonseed oil into a solid and a liquid portion. The liquid portion was further separated by distillation under reduced pressure into several fractions from which, however, no definite compounds could be obtained.

The solid portion consisted of phytosterol which melted at 139°. Its optical rotation was —23.14° which is much lower than has usually been reported for phytosterol. The acetyl derivative gave only a dibromo derivative melting at 125°, which upon reduction with zinc dust gave the original phytosterol melting at 139°.

Our results confirm the findings of Wagner and Clement. By fractional crystallization we were able to separate the phytosterol of cottonseed oil into 2 fractions that differed slightly in their melting points and also in optical rotation.

The phytosterol of linseed oil was investigated by Bömer and Winter[11] who isolated a preparation which melted at 137° to 138°, and the acetate melted at 128° to 129°.

We were able to separate the phytosterol of linseed oil by fractional crystallization into 2 fractions that were very similar to those we obtained from cottonseed oil. We believe, therefore, that cottonseed oil and linseed oil contain at least 2 phytosterols that differ in their melting points and also in their optical rotation. These substances, however, so far as one can determine by chemical analysis, are identical in composition and they give identical color reactions. The differences in physical properties which we have noted must depend upon slight variations in constitution.

It is very evident that the phytosterol occurring in plants is not a single homogeneous substance but may consist of 2 or more fractions that differ slightly in physical properties and sometimes in chemical composition, particularly stigmasterol. Occasionally these fractions may be separated with ease and in great purity as shown by Windaus and Hauth.[8] However,

[14] Wagner and Clement, *Z. Nahr. Genussm.*, **17**, 266 (1909).

[15] Matthes and Heintz, *Arch. Pharm.*, **247**, 161 (1909).

in some mixtures of phytosterols this method is not applicable as shown by Wagner and Clement[14] in the case of the phytosterols from cottonseed oil.

Phytosterols occur in all parts of the plants[16] but the function of these substances in the metabolism of the plant is not known. The fact that phytosterols are largely deposited with the fats in seeds and pollen grains would seem to justify the assumption that these substances play an important role in the life of the plant cells.

An important relation must undoubtedly exist between the phytosterols and the corresponding alcohol which occurs in animal cells and tissues, namely cholesterol, the phytosterols contained in vegetable foods being the source of the cholesterol found in animal tissues.

Experimental Part

Crude corn oil[17] was saponified in lots of 300 g. by boiling it with 600 cc. of alcoholic potassium hydroxide. Two kg. of the oil was saponified in this way. The soap solution was largely diluted with water and extracted with 3 portions of ether. The ethereal solution was washed with dilute potassium hydroxide and with water, filtered and evaporated. The residue was yellowish-brown in color and it contained some oily substance. It was again boiled with 300 cc. of alcoholic potassium hydroxide for $^1/_2$ hour. As the solution cooled, the greater part of the substance crystallized. This was filtered off, washed in dil. alcohol until free from alkali, and dried in a vacuum over sulfuric acid.

A smaller amount of material was recovered from the filtrate after diluting it with water and extracting with ether. After the ether had evaporated an oily residue remained that was dissolved in hot alcohol. A mixture of crystals and a yellow oil separated as the solution cooled. The oil was eliminated by repeated crystallization from alcohol, and we obtained finally a pure snow-white product that crystallized in plates. The dry substance melted[18] at 137.5°.

The first crystalline product mentioned above weighed 16.8 g. It was recrystallized thrice from 300 cc. of 95% alcohol and was then snow-white in color. It crystallized in 2 forms, either as compact elongated plates when the solution was cooled slowly, or in very large thin plates when the solution was cooled rapidly. The purified substance weighed 12.6 g. Heated in a capillary tube it melted at 137.5°. Drying in a vacuum at 105° did not change its melting point. It gave the Liebermann-Burchard reaction and it readily absorbed bromine in chloroform solution. For analysis it was dried at 105°, in a high vacuum over phosphorus pentoxide.

Analyses. Subs., 0.1434: H_2O, 0.1578; CO_2, 0.4437. Calc. for $C_{27}H_{45}OH$ (386): C, 83.93; H, 11.91. Found: C, 84.38; H, 12.31.

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[16] Czapek, "Biochemie der Pflanzen," Gustav Fischer, Jena, 2nd ed., **1905**, vol. I, p. 163.

[17] The crude and refined corn oils used in this investigation were kindly furnished by Mr. Lloyd Bosworth of the Patent Cereals Company, Geneva, N. Y.

[18] Unless otherwise specified, the melting points given in this paper are uncorrected.

The substance was again recrystallized four times from absolute alcohol and analyzed after it was dried as described above.

Analysis. Found: C, 83.72; H, 12.41.

The substance again melted at 137.5°. It was then recrystallized from 80% alcohol. The melting point did not change. After it had been dried as described above it was analyzed.

Analysis. Found: C, 83.78; H, 12.12.

In chloroform solution its specific rotation was $[\alpha]_D^{20} = -34.38°$.

The analyses agree closely with the calculated composition of phytosterol, $C_{27}H_{45}OH$, and the melting point is identical with that of sitosterol.

Water of Crystallization.—The various preparations that we analyzed contained some water of crystallization.

Analyses. Subs., 0.1532, 0.1384, 0.1093: loss on drying at 105°, 0.0063, 0.0054, 0.0036. Calc. for $C_{27}H_{45}OH + H_2O$: H_2O, 4.45. Found: 4.11, 3.90, 3.29.

The first sample had been crystallized from 95% alcohol and dried in a vacuum over sulfuric acid. The second had been crystallized from absolute alcohol and dried in the air. The third had been crystallized from 80% alcohol and dried in the air.

It is evident from the above data that all of our preparations contained less water of crystallization than is required for 1 H_2O.

Acetyl Derivative.—The acetyl derivative was prepared by boiling 1 g. of phytosterol with 35 cc. of acetic anhydride. The acetic anhydride was distilled in a vacuum and the residue purified by recrystallizations from absolute alcohol. The substance was obtained as snow-white crystals that melted at 127°. It did not lose in weight on drying in a vacuum over phosphorus pentoxide.

Analysis. Subs., 0.1410: H_2O, 0.1435; CO_2, 0.4207. Calc. for $C_{27}H_{45}O.OC.CH_3$ (428): C, 81.31; H, 11.21. Found: C, 81.37; H, 11.38.

Bromination of Phytosterol.—The direct bromination of phytosterol did not lead to a satisfactory product. To a solution of 1 g. of dry phytosterol in 10 cc. of ether was added 0.85 g. of bromine dissolved in 10 cc. of glacial acetic acid. Nothing crystallized from this solution even after it had stood in a freezing mixture for some time. The substance was therefore extracted with ether, the ethereal solution was washed with dil. alkali and water, filtered and the ether evaporated. The yellowish crusts that remained weighed 1.4 g. after they had dried in a vacuum over sulfuric acid. This indicates that 2 atoms of bromine had been absorbed. The substance could not be obtained in crystalline form from any of the usual solvents. It separated more readily from methyl alcohol than from any other solvent but the product was amorphous.

The bromination of the acetyl derivative was more successful. A solution of 5.1 g. of the acetyl derivative in 30 cc. of ether was brominated by the method of Windaus and Hauth.[8] After it had stood for about 1/2 hour at room temperature a small amount of substance without any distinct crystal form separated from the bromination mixture. When the flask was placed in a freezing mixture the amount of this substance was greatly increased. It was filtered, washed thoroughly in cold glacial acetic acid and dried in a vacuum over sulfuric acid and potassium hydroxide. The dry substance showed only a faint yellowish color, and it weighed 3.3 g.

The remainder of the bromine derivative was obtained when the filtrate from the precipitate described above was diluted with water after the ether had been distilled under reduced pressure. It separated as a voluminous, amorphous precipitate. This was filtered off, washed with water and dried in a vacuum over sulfuric acid. The dry substance was brownish and weighed 3.7 g.

The total weight of the bromine derivatives was 7 g., which represents a quantitative yield provided 2 atoms of bromine had been absorbed.

These fractions were purified separately. The first fraction separated from absolute alcohol in the form of microscopic granules that showed no definite crystalline structure. The product was snow-white. It was impossible to obtain the substance in definite crystals from any of the usual solvents. When heated in a capillary tube the substance melted at 120° to 120.5°. It did not give the Liebermann-Burchard reaction. Evidently the development of the color in this reaction depends upon the double bond.

It did not lose in weight on drying at 105° in a high vacuum over phosphorus pentoxide.

Analyses. Subs., 0.2075: H_2O, 0.1569; CO_2, 0.4500. Subs., 0.1780: AgBr, 0.1182. Calc. for $C_{27}H_{45}O.CO.CH_3Br_2$ (587.84): C, 59.20; H, 8.16; Br, 27.19. Found: C, 59.14; H, 8.46; Br, 28.25.

The bromine found was about 1% too high but it is evident that the substance was essentially a dibromo-phytosterol acetate.

The second preparation was purified in the same way and gave an identical product that melted at 120° to 120.5°.

It is evident from the above results that the phytosterol of corn oil does not contain any stigmasterol.

Regeneration of Phytosterol from the Bromo-acetyl Derivative.—To 3 g. of the bromo-acetyl derivative dissolved in 200 cc. of alcohol was added 10 cc. of glacial acetic acid and the solution was boiled for 3 hours with 15 g. of zinc dust under a reflux condenser. More zinc dust was added in small portions from time to time. The excess of zinc was then filtered off and the filtrate boiled with 40 g. of potassium hydroxide for 1 hour. The phytosterol was extracted with ether and purified by recrystallization several times from absolute alcohol. So far as one could judge by crystal form, solubilities and reactions, the substance was identical with the original phytosterol and melted at 137.5°.

Quantitative Determination of the Unsaponifiable Matter in Corn Oil.—We followed the method outlined by Bömer[19] in determining the unsaponifiable matter in corn oil. It was found necessary, however, to extract the soap solution 7 times with ether before all of the crystallizable phytosterol had been removed. On evaporation of the ether after the sixth extraction there was a slight but distinct crystalline residue. Even the seventh extraction left a slight residue on evaporation of the ether but this was a yellow oily substance that did not show any crystals of phytosterol.

The total residue after the evaporation of the ether was again boiled with alcoholic potassium hydroxide, diluted with water, and extracted thrice with ether. After the ethereal solution had been washed and filtered, it was evaporated and the residue dried to constant weight at 100° and weighed.

The crude phytosterol thus obtained was mixed with some yellow oil and the residues from the crude corn oil contained a larger amount of this oily substance than those from the refined oil. We did not determine the nature of this unsaponifiable oily substance but it would seem worthy of further investigation.

[19] Bömer, *Z. Nahr. Genussm.*, **1**, 21 (1898).

In each determination 50 g. of oil was saponified and the following results were obtained.

Crude corn oil		Refined edible corn oil	
Unsaponifiable		Unsaponifiable	
G.	%	G.	%
1.0740	2.15	0.8346	1.67
1.0018	2.00	.8260	1.65
0.9711	1.94	.8654	1.73
0.9813	1.96	....	..
Av. 1.0070	2.01	Av. 0.8420	1.68

It is evident from the above figures that corn oil contains a high percentage of unsaponifiable matter and that in the process of refining less than 0.5% of the unsaponifiable material is removed.

The Phytosterol of Cottonseed Oil

About 2 kg. of cottonseed oil[20] was saponified and the phytosterol isolated as described under corn oil.

The unsaponifiable residue left on evaporation of the ether was dark brown, largely due to the admixture of a dark oil. The substance was dissolved in 500 cc. of methyl alcohol and the solution boiled with norite, filtered and allowed to cool. It was still dark in color and the product that separated in large thin plates was yellowish-brown. It was recrystallized thrice from methyl alcohol and 4 times from ethyl alcohol. The dry substance weighed 2 g. and was slightly yellowish; m. p., 135°. The mother liquors were saved and examined, as will be described later.

Acetyl Derivative.—For further purification the phytosterol was acetylated and the acetyl derivative recrystallized twice from methyl alcohol, from which it crystallized in rosets of fine needles, and twice from ethyl alcohol from which it crystallized in thin plates. After the final filtration, washing the substance in cold alcohol and drying it in a vacuum over sulfuric acid, it weighed 1.7 g. It was snow-white; m. p., 119°. It did not lose in weight on drying at 105°, in a high vacuum over phosphorus pentoxide.

Analyses. Subs., 0.1103: H_2O, 0.1151; CO_2, 0.3288. Calc. for $C_{27}H_{45}O.CO.CH_3$ (428): C, 81.31; H, 11.21. Found: C, 81.30; H, 11.67.

Saponification of Acetyl Derivative.—The remainder of the purified acetyl derivative was saponified with alcoholic potassium hydroxide and the phytosterol extracted with ether in the usual way. It was crystallized from methyl alcohol and recrystallized from ethyl alcohol from which it separated in thin colorless plates. The dry product was snow-white and it weighed 1.1 g. Heated in a capillary tube it melted at 134–135°. Drying it at 105° in a vacuum over phosphorus pentoxide did not change the melting point. The substance was again recrystallized from 95% ethyl alcohol, but the melting point was still 134° to 135°.

Water of Crystallization.—The purified phytosterol suffered some loss in weight when dried at 105° in a vacuum over phosphorus pentoxide, corresponding to about 0.5 H_2O.

Analyses. Subs., 0.1201, 0.8760: loss, 0.0033, 0.0185. Calc. for $C_{27}H_{45}OH.H_2O$: H_2O, 4.45. Calc. for $C_{27}H_{45}OH.{}^1/_2H_2O$; H_2O, 2.27. Found: 2.74, 2.11; av., 2.42.

[20] The oil was bought in the open market in sealed tin cans which were marked "Wesson Oil. The Southern Cotton Oil Company."

The purified and dried phytosterol in chloroform solution gave $[\alpha]_D^{20}$, —33.61°. After drying in a vacuum at 105°, over phosphorus pentoxide the substance was analyzed.

Analysis. Subs., 0.1168: H_2O, 0.1277; CO_2, 0.3588. Calc. for $C_{27}H_{45}OH$: C, 83.93; H, 11.91. Found: C, 83.78; H, 12.23.

Phytosterol from the Mother Liquors.—The total mother liquors from which the phytosterol preparation described above had been crystallized, were evaporated in a vacuum. The residue was crystallized several times from methyl alcohol until it was snow-white. It was then acetylated and the acetyl derivative purified by recrystallization from ethyl alcohol. It was saponified by alcoholic potassium hydroxide, extracted with ether, and the product was recrystallized many times from methyl alcohol. It was snow-white and crystallized in large thin plates. After drying in the air it weighed about 1 g.; m. p., 138–139°. After the substance was again recrystallized from methyl alcohol the melting point was still the same; in chloroform $[\alpha]_D^{20}$, —34.19°.

When dried at 105° in a vacuum over phosphorus pentoxide the substance was found to contain about the same amount of water of crystallization as the first preparation.

Analyses. Subs., 0.1192, 0.7807: loss, 0.0036, 0.0212. Found: H_2O, 3.02, 2.71; av., 2.86.

The dried preparation was analyzed.

Analysis. Subs., 0.1156: H_2O, 0.1257; CO_2, 0.3546. Calc. for $C_{27}H_{45}OH$ (386): C, 83.93; H, 11.91. Found: C, 83.65; H, 12.16.

Acetyl Derivative.—The acetyl derivative prepared from this fraction and recrystallized from methyl alcohol melted at 124°. It was recrystallized several times without change in the melting point.

The 2 phytosterol preparations described above gave identical colors in the Liebermann-Burchard reaction and both preparations absorbed bromine in chloroform solution.

It is evident from the data obtained that the phytosterol contained in cottonseed oil is not homogeneous but consists of at least 2 fractions which differ in melting point and probably in optical rotation since the rotation was slightly greater in the fraction having the higher melting point. If there are 2 phytosterols in cottonseed oil they have apparently the same chemical composition. The differences in physical properties must depend upon slight differences in constitution. It seems doubtful whether these products can ever be separated into homogeneous substances by simple recrystallization.

The Phytosterol of Linseed Oil

The crude phytosterol was isolated from 2 kg. of saponified raw linseed oil in the manner described under corn oil.

The residue obtained on the final evaporation of the ether contained a considerable amount of dark brown oil. This oil was eliminated by recrystallizing the substance several times from ethyl alcohol and then from methyl alcohol but the crystals retained a brownish color. The alcoholic solution was finally decolorized with norite and the phytosterol was obtained pure white in color. The substance was now recrystallized 5 times from methyl alcohol from which it separated in colorless plates. The dry product was snow-white and it weighed 2.8 g. Heated in a capillary tube it began to soften at 132° and to melt at 134°, but it was not completely fluid until heated to 136°. The mother liquors were examined, as will be described later.

Acetyl Derivative.—The whole substance was acetylated and the product crystallized from acetic anhydride. It was then twice recrystallized from 95% alcohol from which it separated in small plate-shaped crystals. It was snow-white and weighed 2.4 g.

It softened at 122° and melted at 124°. It did not lose in weight when dried at 105° over phosphorus pentoxide.

Analysis. Subs., 0.1246: H_2O, 0.1299; CO_2, 0.3709. Calc. for $C_{27}H_{45}O.CO.CH_3$ (428): C, 81.31; H, 11.21. Found: C, 81.18; H, 11.66.

Saponification of Acetyl Derivative.—The balance of the acetyl derivative was saponified with alcoholic potassium hydroxide and the phytosterol was isolated in the usual way. After it had been recrystallized several times from alcohol it was obtained as snow-white, plate-shaped crystals which weighed 1.9 g. It began to soften at 130° but it was not melted to a clear fluid until heated to 134°. When dried at 105° in a high vacuum over phosphorus pentoxide it lost 2.37% in weight, corresponding to $^1/_2$ mol. of water; in chloroform solution $[\alpha]_D^{20}$, —31.16°. The dry substance was analyzed.

Analysis. Calc. for $C_{27}H_{45}OH$(386): C, 83.93; H, 11.91. Found: C, 83.88; H, 12.34.

Phytosterol from the Mother Liquors.—The mother liquors left on crystallizing the above preparations were saved, concentrated in vacuum and the material that crystallized as the solution cooled was purified by recrystallization from methyl alcohol until it was snow-white. This fraction weighed 1.4 g.; m. p., 138°; in chloroform solution, $[\alpha]_D^{20}$, —34.22°.

Water of Crystallization.—The loss in weight when the substance was dried at 105° in a high vacuum over phosphorus pentoxide corresponded to $^1/_2$ H_2O.

Analyses. Subs., 0.1045, 1.0131: loss, 0.0028, 0.0186. Calc. for $C_{27}H_{45}OH^1/_2H_2O$: H_2O, 2.27. Found: 2.67, 1.83; av., 2.25.

The dried preparation was analyzed.

Analysis. Subs., 0.1017: H_2O, 0.1125; CO_2, 0.3130. Calc. for $C_{27}H_{45}OH$ (386): C, 83.93; H, 11.91. Found: C, 83.93; H, 12.37.

Acetyl Derivative.—The acetyl derivative was prepared in the usual way and allowed to crystallize from acetic anhydride. It was recrystallized from methyl alcohol from which it separated in tufts of needles. The product was snow-white; m. p., 129–130°. Both fractions gave identical colors with the Liebermann-Burchard reaction.

The data obtained indicate that the phytosterol contained in raw linseed oil is very similar to the phytosterol of cottonseed oil. From both oils 2 fractions of phytosterol were isolated one of which melted at 134°, and the other at 138°. The higher-melting fraction showed a slightly higher specific rotation. However, the acetyl derivatives of the phytosterol from linseed oil melted 5° higher than the corresponding products derived from cottonseed oil.

It is an interesting point that the higher-melting fractions of phytosterol were always obtained from the mother liquors.

Summary

1. Corn oil contains a relatively high percentage of unsaponifiable matter, amounting in the crude oil to 2.01% and in the refined edible oil to 1.68%. This unsaponifiable matter consists largely of phytosterol which is identical with sitosterol. Its melting point was 137.5°, and the optical rotation of the dry phytosterol was —34.38°. The acetate melted at 127°. The phytosterol of corn oil does not contain any stigmasterol.

2. Cottonseed oil contains at least two phytosterols which differ in melting points and probably in optical rotation. Two fractions of phytosterol were separated by fractional crystallization: (a) m. p., 138–139°;

$[\alpha]_D^{20}$, —34.19°; the acetate melted at 124°; (b) m. p., 134–135°; $[\alpha]_D^{20}$, —33.61°; the acetate melted at 119°.

The complete separation of these fractions by crystallization is very difficult, if not impossible, and we do not believe that either of the above preparations was homogeneous.

3. Linseed oil contains at least two phytosterols which differ in melting point and optical rotation. As in the case of cotton seed oil 2 fractions of phytosterol were separated by fractional crystallization: (a) m. p., 138°; $[\alpha]_D^{20}$, —34.22°; the acetate melted at 129–130°; (b) m. p., not sharp, 134°; $[\alpha]_D^{20}$, —31.16°; the acetate melted at 124°.

None of the phytosterol preparations that we isolated contained 1 molecule of water of crystallization. The loss in weight on drying was somewhat irregular but corresponded more nearly to $^1/_2$ molecule of water of crystallization.

Mixtures of phytosterols such as are obtained from cottonseed oil and linseed oil are so nearly alike in solubility in the usual solvents that it is practically impossible to effect a complete separation by simple fractional crystallization.

GENEVA, NEW YORK

[CONTRIBUTION FROM THE HULL LABORATORIES OF PHYSIOLOGICAL CHEMISTRY AND PHARMACOLOGY, THE UNIVERSITY OF CHICAGO]

A QUANTITATIVE METHOD FOR THE DETERMINATION OF TOTAL SULFUR IN BIOLOGICAL MATERIAL[1]

BY MABEL STOCKHOLM AND FRED C. KOCH

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The estimation of total sulfur in biological materials has been the subject of many investigations. The most generally recognized source of error, the difficulty of forming a pure barium sulfate, has been largely eliminated by the now well-known modifications introduced as a result of the careful studies by Folin,[2] Allen and Johnston,[3] Johnston and Adams[4] and Krieble and Mangum.[5] There are, however, several other factors which have not been sufficiently emphasized. These are complete retention of the sulfur, complete oxidation to sulfate and complete precipitation of sulfate in blanks and in materials low in sulfur. The first two factors appear to be well controlled in the special methods for total sulfur in urine as developed by

[1] The greater part of the experimental work of this paper was carried out by Miss Mabel Stockholm in 1918 as part of the requirement for the degree of Master of Science.

[2] Folin, *J. Biol. Chem.*, **1**, 130 (1905).

[3] Allen and Johnston, THIS JOURNAL, **32**, 588 (1910).

[4] Johnston and Adams, *ibid.*, **33**, 829 (1911).

[5] Krieble and Mangum, *ibid.*, **41**, 1317 (1919).