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PHYTOCHEMICAL STUDY. SEED OF THE MAGNOLIA GRANDIFLORA.*

BY ST. ELMO BRADY.[1]

The magnolia tree is one of the most interesting and beautiful of the trees which are embraced in the flora of North America. In the southern part of this country it grows to a magnificent height and is prized highly as an ornamental tree primarily for its beautiful, well-scented white flowers. No complete chemical examination has hitherto been made of the seed of this tree, and the constituents of the fatty oil have been only imperfectly known. Most of the published works have been confined chiefly to the examination of the volatile oil of the leaves and bark. Proctor (1) made an examination of the fruit of the *Magnolia tripetala* and reported the isolation of crystals during the course of several experiments without indicating anything as to their composition. Rawlins (2) examined the leaves of the *Magnolia glauca*, L., and recorded the presence of crystals, calcium oxalate, volatile oil, and extracts which possess a bitter taste, impart fluorescence to chloroform, and after boiling with sulfuric acid reduce Fehling's solution. Randolph (3) made a study of the bark of *Magnolia grandiflora* and recorded the following constituents: tannin, starch, saccharine and coloring matter. Greshoff (4) examined the bark of several species of the magnolia and reported the presence of a bebeerine-like alkaloid.

The present study is the first of a series which is being carried out in this laboratory on the *Magnolia grandiflora*. Interests in the tree grew out of the reported statements that the bark has been used, domestically, in infusion or decoction for the treatment of rheumatism and malaria; and tinctures of the bark in brandy or whisky are said to produce cures in chronic cases of chills and fevers

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when quinine failed. The future study will be made on the bark with the hope of isolating the supposed principle or principles responsible for the reported efficacy of the bark in malaria.

The seeds used in the present study were collected on the campus of the University during the latter part of October, after they had fully matured. A large quantity of the seed was collected and immediately subjected to examination.

EXPERIMENTAL PART.

Preliminary Examination.—Twenty grams of the seed were separated into the skin with the fleshy portion and the inner seed which is covered with a bony-like shell. No effort was made to separate the bony covering from the embryo.

Weight of the whole fresh seed	20.00 Gm.
Weight of skin and fleshy portion	9.87 Gm.
Weight of embryo and covering	10.13 Gm.

The parts of the seed were extracted separately with ether. The color of the ethereal extract of the skin and fleshy portion was a decided orange but on the concentration of the solution the color changed to a red, and the residue was of a beautiful dark red color. After drying to constant weight the residue amounted to 4.5 Gm. or 22.5 per cent of the whole seed. The residue from the ether extract of the embryo and covering was of a yellow-orange color and amounted to 4.2 Gm. or 21 per cent of the whole seed.

The total ether extract, therefore, amounted to 43.5 per cent of the total weight of the seed.

Alcohol was added to each of the residues and allowed to remain over night in the refrigerator. The alcoholic solution of the residue from the skin and fleshy portion of the seed showed the presence of a small amount of crystals which were separated and gave the Liebermann reaction for phytosterol; the solution of the residue of the embryo, however, did not deposit such crystals.

A portion of the ground seed was tested for alkaloids with Prollius' fluid but with negative results.

Another portion of the ground seed was extracted in a Soxhlet apparatus with light petroleum (b. p. 30–50°) which yielded 42.5 per cent of fatty oil.

Ash Content.—The ash content was determined from the whole fresh seed and the following results were obtained.

TABLE I.—ASH DETERMINATION.

	Per Cent.*
Total ash	2.16
Water-soluble ash	0.40
Water-insoluble ash	1.77
Acid-soluble ash	1.66
Acid-insoluble ash	0.113

* Each result represents the average of several determinations.

The whole fresh seed was first boiled with alcohol to remove a wax-like coating. This material introduces difficulties in the study of the ground seed as examination progresses unless removed. After the removal of the alcoholic extract the seed was ground in a mill and extracted with petroleum ether. The extraction was continued until the seed was exhausted. The solvent was removed and the remaining oil heated under reduced pressure to remove the last traces of the solvent. The clear red oil possessed a distinctly pleasant odor recalling that of the magnolia blossom. It colored cotton cloth a deep yellow which was removed only with difficulty. The oil solidified to an orange-colored solid between 5–7°.

Another portion of the ground seed was extracted by continuous percolation with hot alcohol. After the removal of the exhausted seed pulp there separated a heavy oil which was collected. On cooling more of the oil separated. The oil was permitted to stand undisturbed for a week when a small amount of a red solid separated, which was removed by filtration. The solid

was practically insoluble in cold alcohol but dissolved more readily in hot alcohol to an orange solution. This solid will be discussed later.

The residual seed cake from the above extraction with alcohol was further extracted with petroleum ether to remove any residual oil.

EXAMINATION OF ALCOHOL FILTRATE.

The alcohol extract from which the oil separated was concentrated and more oil separated, and was removed. The solvent was finally removed as far as possible and there remained a dark semi-solid residue which was mixed with a little water and subjected to steam distillation. Several liters of the distillate were collected and extracted with ether. The ethereal extract was dried over anhydrous sodium sulfate and the solvent removed. There remained a small amount of oil (1.456 Gm.) with a very pleasant aromatic odor. This was fractionally distilled, giving two fractions. Fraction (1), amounting to 0.8693 Gm., was of a light yellow color with the following physical constants: b. p. 115–120° at 6 mm.; specific gravity, 0.9474 at 25/25°; refractive index, 1.4995/25°. The oil formed no additional product with sodium bisulfite nor did it give a coloration with ferric chloride solution. Fraction (2), amounting to 0.4256 Gm., boiled at 120–135° at 6-mm. pressure with slight decomposition. The refractive index was 1.519 at 25°. It gave a slightly greenish yellow coloration with ferric chloride solution but no additional product with the bisulfite.

There remained a very small amount of a dark viscous residue.

After the steam distillation as described there was left in the distillation flask a dark-colored aqueous liquid which had the odor of burnt sugar and a heavy oily layer. These layers were separated.

THE AQUEOUS LIQUID.

On standing for several weeks there was deposited a quantity of a dark reddish brown solid in the aqueous liquid which was removed by filtration. The aqueous solution was repeatedly extracted with ether, the ethereal liquid washed, dried and the solvent removed. A small amount of a brown, viscous mass remained which was soluble in sodium carbonate but could not be purified sufficiently to obtain a product which could be analyzed.

The aqueous liquid, after extraction with ether, was treated with basic lead acetate which produced a light yellow precipitate. This was filtered, thoroughly washed, suspended in water and decomposed by hydrogen sulfide. The filtrate from the lead-sulfide precipitation was concentrated under diminished pressure but deposited nothing crystalline on standing.

The filtrate from the basic lead-acetate precipitate deposited a light yellow solid on standing which was not further investigated. This solid was removed and the liquid freed of the excess lead with hydrogen sulfide, filtered and concentrated to a heavy reddish brown syrupy liquid which yielded a *d*-phenylglucosazone, melting at 214°. Nothing crystalline could be extracted from this syrupy liquid.

WAX-LIKE SEED COVERING.

The solvent was removed from the alcoholic extract of the whole fresh seed and there remained a semi-solid, yellow material. This was taken up in alcohol and the solution permitted to stand for several hours at room temperature. An oily substance settled out and was removed. The alcoholic solution was placed in the refrigerator over night and only a small amount of a yellow solid separated, which was removed. Water was added to the alcoholic solution just to cause permanent turbidity; and on standing, a large quantity of a white solid separated. Recrystallization of this from alcohol gave white needles, melting at 59°. Further study is being made of this coating material.

EXAMINATION OF THE OIL.

The red oil obtained by extraction with petroleum ether and by percolation with alcohol was filtered and the following physical and chemical constants determined.

The oil was first subjected to steam distillation. The distillate contained no volatile oil and a very slight acid reaction was indicated.

The extracted oil was separated from the water, the latter extracted with ether to remove the suspended oil, and the solvent removed. The remaining oil was added to the main portion

TABLE II.—CONSTANTS OF OIL.

	Extracted Oil.	Neutral Oil.
Specific gravity, 25/25°	0.9652	0.9537
Refractive index at 25°	1.480	1.4702
Iodine number (Hanus)	89.5	95.3
Saponification value	182.5	181.7
Unsaponifiable matter %	2.83	2.17
Saturated acids (corrected) %	20.20	19.18
Unsaturated acids (correction) %	72.63	73.77
Iodine number of unsaturated acids	114.4	.....

and the whole was hydrolyzed in the usual manner by heating with alcoholic potassium-hydroxide solution. The greater part of the alcohol was removed, water added and the solution of the potassium salts was extracted repeatedly with ether. The combined ethereal solutions were washed with water, dried over anhydrous sodium sulfate, and the solvent removed when a small amount of a yellow crystalline solid was obtained. This was fractionally crystallized from ethyl acetate and alcohol and was obtained in colorless needles, melting at 132°.

This substance, when dissolved in chloroform to which a little acetic anhydride had been added and treated with a drop of sulfuric acid, developed a blue coloration, rapidly changing to green, and finally to brown. This substance is evidently a phytosterol.

THE FATTY ACIDS.

The alkaline aqueous solution of the potassium salts, after extraction with ether, was acidified with dilute sulfuric acid and the liberated fatty acids taken up with ether. The ethereal solution was washed with water, dried over anhydrous sodium sulfate and the solvent removed. A determination of the acetyl value of the mixed fatty acids showed a negative result. No volatile acids could be detected either in mixed acids or in the aqueous filtrate from which the acids had been extracted.

The percentages of the saturated and unsaturated acids were determined by the lead-salt ether method (5) and by the method of Twitchell (6) with the following results.

Lead-Salt Ether Method.	Lead-Salt Alcohol Method.
Saturated acids 19.18 per cent	20.20 per cent*
Unsaturated acids 72.25 per cent	72.63 per cent

* The iodine number was small enough to be negligible.

SATURATED ACIDS.

The saturated acids which were separated by the above methods were esterified with absolute alcohol, dry hydrogen chloride being used, and the resulting esters fractionally distilled under diminished pressure. The data for the distillation are given in Table III. The preliminary distillation was made from a 500-cc. Claissen flask, giving two fractions and a residue.

Fraction.	Tempt.	Pressure.	Refractive Index.	Color.	Amounts in Gm.
A	To 170°	4 mm.	1.4380/27°	Sl. yellow	76
B	170–185°	4 mm.	1.4405/27°	Sl. yellow	43
	Residue, dark red solid				1
					120

The final distillation was made from a 125-cc. Claissen flask. The auxiliary neck of the flask was extended by sealing on a modified Vigreux distilling column nine inches in length and this proved very efficient in the distillation. Fractions A and B were distilled under a pressure of less than 1 mm., giving four fractions and a residue and three fractions and a residue, respectively.

The further purification of the various fractions involved fractional crystallization of the esters. Inasmuch as the same procedure was used in each case, the process will be described in some detail. Fraction (1) (b. p. 145°) was dissolved in absolute alcohol in which it is very soluble and the solution placed in the refrigerator at a temperature of 0° to 5° over night. Beautiful white

TABLE III.—FRACTIONAL DISTILLATION OF ESTERS.

Fraction A (b. p. −170°/4 mm.).

Fraction.	Tempt.	Pressure.	Refractive Index.	Color.	Amount in Gm.
1	To 145°	>1 mm.	1.4380/25°	Colorless	28.0
2	145–155°	>1 mm.	1.4385/25°	Colorless	14.0
3	155–160°	>1 mm.	1.4395/25°	Colorless	7.5
4	160–165°	>1 mm.	1.4400/25°	Colorless	23.0
	Residue, solid				1.5
					74.0

Fraction B (b. p. 170–185° /4 mm.).

1	To 160°	>1 mm.	1.4385/25°	Colorless	20
2	160–165°	>1 mm.	1.4412/25°	Colorless	11
3	165–170°	>1 mm.	1.4420/25°	Colorless	10
	Residue, solid				1
					42

needles separated which were filtered without removing the flask from the refrigerator. Care was taken to remove the alcohol as far as possible by suction. The crystals were removed to a glass evaporating dish and allowed to melt. The melted crystals were placed in a vacuum desiccator and the last traces of the alcohol removed. These crystals melted at 25–25.5°, and the melted crystals had a refractive index of 1.4385 at 25°.

The melted crystals were again dissolved in absolute alcohol and submitted to the above procedure. The crystals obtained were long slender needles which melted at 25.5°, the whole thermometer in the bath, and the melted crystals showed a refractive index of 1.4385 at 25°.

Seventeen grams of the crystallized ester were dissolved in a little alcohol and saponified in the usual manner. The potassium salt was obtained in long, flat glistening needles arranged in mats. The free acid crystallized in white, fan-shaped crystals, melting at 62°. Recrystallization from alcohol gave glistening white plates, while from acetone glistening, almost transparent plates were obtained. In each case the melting point was 63°.

The silver salt of the acid was prepared and analyzed.

0.0732 Gm. of salt gave on ignition 0.022 Gm. Ag.
Ag = 30.05 per cent.
0.0733 Gm. of salt gave on ignition 0.0216 Gm. Ag.
Ag = 29.46 per cent.
$C_{16}H_{31}O_2AG$ requires Ag = 29.7 per cent.

The crystals were thus identified as palmitic acid.

Recrystallization of products obtained from the above alcoholic filtrates did not show any substance with a different melting point.

Fraction 2 (b. p. 145–155°) gave white crystals by the above procedure, melting at 25°. The ester on saponification yielded an acid which, after recrystallization from alcohol and acetone had a melting point of 63°. This acid was analyzed and identified as palmitic acid.

Fraction 3 (b. p. 155–160°) showed the presence of palmitic acid.

Fraction 4 (b. p. 160–165°). The colorless fraction was dissolved in absolute alcohol and crystallized by the above procedure. The crystals showed a melting point 18–21°. These were converted into the acids and fractionally crystallized when the following fractions were obtained: Fractions, (*a*) m. p. 62°; (*b*) m. p. 62°; (*c*) m. p. 60°; (*d*) m. p. 59.5°; (*e*) m. p. 59–59.5°; (*f*) m. p. 59°.

It is obvious that the first three fractions are palmitic acid while the fractions, *d*, *e*, *f*, represent a mixture of palmitic acid and a small amount of a lower melting acid.

The filtrate from the final crystallization was diluted with water and the precipitated acid collected and dried. The melting point was 56°. Recrystallization from acetone, methyl and ethyl alcohols did not materially alter the melting point.

The silver salt of this acid was prepared and analyzed.

0.0985 Gm. of salt gave on ignition 0.0315 Gm. Ag.
Ag = 31.99 per cent.
0.0705 Gm. of salt gave on ignition 0.0225 Gm. Ag.
Ag = 31.90 per cent.
$C_{14}H_{28}Ag$ requires Ag = 32.2 per cent.

The acid was thus identified as myristic acid.

Fraction B (B. P. 170–185).—The various fractions obtained by a more careful separation of this fraction were subjected to the same procedure as indicated. Fraction 1 (b. p. to 160°) was primarily palmitic acid; fraction 2 (b. p. 160 – 165°), a mixture of palmitic and myristic acids.

Fraction 3 (B. P. 165–170°).—The pure ester melted at 22.5°. The acid obtained by saponification of the ester had a melting point of 64.66°. On dissolving the acid and in cold alcohol a portion did not go into solution very readily and was filtered off and called the "insoluble portion." Recrystallization of this "insoluble portion" gave a product melting at 68°; while the acid obtained from the soluble ester melted at 62°.

The silver salt of the acid melted at 68°, was prepared and analyzed.

0.125 Gm. of salt gave on ignition 0.0343 Gm. Ag.
Ag = 27.44 per cent.
0.096 Gm. of salt gave on ignition 0.0263 Gm. Ag.
Ag = 27.39 per cent.
$C_{18}H_{35}O_2Ag$ requires Ag = 27.55 per cent.

The acid was thus identified as stearic acid.

The solid residues were mixed and treated with cold alcohol and a portion of it was more soluble than another. The sparingly soluble portion was removed and recrystallized. The ester had a melting point of 49.5°. The acid obtained by the hydrolysis of the ester, after recrystallization from dilute alcohol, melted at 76–77°.

The silver salt of this acid was prepared and analyzed.

0.0932 Gm. of salt gave on ignition 0.0238 Gm. Ag.
Ag = 25.53 per cent.
0.0754 Gm. of salt gave on ignition 0.0192 Gm. Ag.
Ag = 25.46 per cent.
$C_{20}H_{39}O_2Ag$ requires Ag = 25.67 per cent.

The acid was thus identified as arachidic acid.

SEPARATION AND IDENTIFICATION OF UNSATURATED ACIDS.

Oxidation of the Unsaturated Acids.—Thirty grams of the mixed acids were oxidized with dilute alkaline permanganate solution according to the method of Hazura and Grüssner as given by Lewkowitsch (7), and the oxidation products separated into two fractions: (*a*) 5.06 Gm. representing sativic acid having a melting point of 169.5° which, after recrystallization, melted at 172.5°. The acid value was found to be 162.9 as compared with the theoretical value of 161.2. (*b*) 7.56 Gm. from the ether after evaporation was fractionally crystallized and two acids were obtained in a pure form, (1) melting at 121°, and (2) melting at 130°. Both were dihydroxystearic acids with acid values of 178.9 and 179.0, respectively, compared with the theoretical value of 180.5. The presence of sativic acid indicates linoleic acid. The two dihydroxystearic acids indicate the presence of oleic acid. The isolation of the two oxidation products of oleic acid with different melting points leads one to assume the presence of isomeric oleic acids in the mixture.

Preparation of the Bromine Addition Products.—Twenty-five grams of the unsaturated acids were dissolved in dry ether, 200 cc., and 20 cc. of glacial acetic acid added. The ethereal solution was cooled to −5° and bromine slowly added, maintaining the temperature between −5° and 0° throughout the addition. When a permanent bromine color had been attained the whole was set aside in the refrigerator over night. No separation of solid material was noticed indicating the absence of linolenic acid. The bromination products soluble in ether were freed from the excess bromine by treatment with sodium thiosulfate, the ethereal solution washed, dried over anhy-

drous sodium sulfate, the ether removed and the residue refluxed with petroleum ether and the whole permitted to stand over night in the refrigerator. The tetrabromide of linoleic acid separated in wart-like crystals which, after washing with petroleum ether and drying, melted at 114°. Analysis of the bromide gave the following results.

0.4253 Gm. bromide gave 0.5320 Gm. AgBr.
Br = 53.20 per cent.
0.4020 Gm. bromide gave 0.5026 Gm. AgBr.
Br = 53.18 per cent.
$C_{18}H_{32}O_2Br_4$ requires Br = 53.33 per cent.

Thus indicating the presence of linoleic acid.

The filtrate was concentrated and permitted to stand for several hours but no further precipitation of the bromide took place. The solvent was completely removed, the last traces in the vacuum. This fraction should contain the oleic dibromides and the soluble tetrabromides of linoleic acid. After standing for a week some more of the solid separated, which was filtered, washed well with petroleum ether, and dried in the desiccator. These crystals melted at 114°.

The residual liquid bromide was analyzed for bromine.

1.755 Gm. bromide gave 1.553 Gm. AgBr.
Br = 37.16 per cent.
2.264 Gm. bromide gave 1.971 Gm. AgBr.
Br = 37.04 per cent.
$C_{18}H_{34}O_2Br_2$ requires Br = 36.18 per cent.

Thus oleic acid is identified.

The analyses of the bromine addition products of the unsaturated acids together with the oxidation products of these acids confirm the presence of oleic and linoleic acids. The analytical results of the residual liquid bromide show the product to be almost pure oleic dibromide.

In another experiment, after the concentration of the liquid bromides under reduced pressure, a bromide separated which melted at 56°. Recrystallization from petroleum ether resulted in white needles, melting at 57–58.5°. This amounted to one Gm. Unfortunately after several attempts with other portions of the liquid bromides no more of the low melting isomer could be isolated.

TABLE IV.—ANALYSIS OF UNSATURATED ACIDS.

Weight of unsaturated acids	25.00 Gm.	
Weight of linoleic tetrabromide (m. p. 114°)	7.54 Gm.	
Weight of linoleic tetrabromide (m. p. 57–58.5°)	1.00 Gm.	
Weight of liquid bromide	30.10 Gm.	
Bromine in tetrabromide %	53.20	53.18
Bromine in liquid bromide %	37.16	37.04

REGENERATION OF THE UNSATURATED ACIDS FROM THEIR RESPECTIVE BROMIDES.

The procedure of Rollett (8) was used in the preparation of the unsaturated acids from their bromides. Fifty grams of the liquid bromide were dissolved in methyl alcohol, 50 Gm. of zinc added and methyl alcoholic hydrogen chloride (150 cc. of 11%) was dropped into the refluxing solution. After all of the alcoholic hydrogen chloride had been added the whole was refluxed for several hours. The solvent was removed and the ester was saponified in the cold by dissolving in 5 per cent potassium hydroxide and allowing to stand over night. The acid obtained was purified and had an iodine value of 91, theoretical value for oleic acid is given as 90.07.

The same procedure was used in the case of the tetrabromide of linoleic acid. The free acid was distilled and the pure fraction boiled at 225° at 12-mm. pressure. Two samples showed iodine values of 178.6 and 179.1, respectively. The theoretical value for linoleic acid is 181.42.

Thus the presence of the oleic and linoleic acids were established by the analysis of the oxidation products of the mixed unsaturated acids, the preparation and analysis of the respective bromides, and finally, from the iodine values of the regenerated acids.

OXIDATION OF THE NEUTRAL FAT.

A study of the oxidation products of the neutral fat was made. For this purpose 310 Gm. of the fat were boiled under a reflux with aqueous sodium carbonate. The mixture was allowed to separate and cool. The oil was removed and dried. No examination was made of the brownish colored aqueous layer at this time.

The neutral oil was filtered to remove any sediment and dissolved in ether so that the solution represented approximately a 25 per cent one. Two volumes of warm acetone were added and the beautiful cherry-red solution was placed in the cold room for twenty-four hours. During this time a light yellow, soft material was deposited. This was removed by the aid of the pump. The solvent was removed from the oil and the last traces under reduced pressure. The oil remaining was of a dark red color. This process was repeated until no more of the solid separated. This solid, after purification, crystallized in needles melting at 62°. Further study is being made on this product.

The physical and chemical constants of this purified oil are given in Table II.

Two hundred grams of the neutral purified oil were dissolved in 2000 cc. of dry acetone and boiled gently on the water-bath. Two hundred grams of powdered potassium permanganate were added in small quantities and the mixture refluxed for four hours. The addition of the permanganate must be done cautiously as the reaction can become violently reactive. After this time the acetone was removed as far as possible and the residue thoroughly mixed with about 200 Gm. of sodium bisulfite and poured into 1000 cc. of water. This solution was boiled and dilute sulfuric acid added until the solution was free of manganese dioxide. On cooling the solution was extracted several times with ether. The ethereal extracts were combined, washed with water, dried and the solvent removed. The residue was taken up in hot water and dilute sodium carbonate solution added until it was alkaline to litmus. The aqueous solution was extracted with ether to remove any unchanged oil and neutral substances.

THE AQUEOUS PORTION.

The aqueous layer was made acid with dilute sulfuric acid and extracted with ether. After the removal of the solvent a residue remained, consisting of a solid and heavy liquid. The mixture was separated and the solid washed with petroleum ether. This ether was added to the liquid part of the extract.

The white solid was recrystallized from alcohol several times and finally crystals were obtained which had the constant melting point of 106–107°. The acid was identified as azelaic acid which melts at 106.5°.

A second acid was obtained from this crystallization which had the melting point 56–57°.

The liquid acid fraction was soluble in petroleum ether and the solution was filtered to remove some sediment. After the removal of the solvent the remaining oily liquid was fractionated at atmospheric pressure with the following results:

Fraction.	Temperature.
1	To 90°
2	90–120°
3	120–205°
4	205–230°
5	230–240°
6	240–255°
7	Solid residue

Fractions (1) and (2) represent in the main the solvent and acetic acid; fraction (3), lower acid with a mean equivalent of 123.2. Fractions (4), (5) and (6) were mixed and refractionated and finally a product was obtained which boiled at 250 – 255° and represented the greater portion of the material used.

Further purification of the fraction consisted in its conversion into the barium salt which is sparingly soluble in hot alcohol and practically insoluble in cold alcohol. On dissolving the salt in a large volume of boiling alcohol and allowing to cool the characteristic silky needles of the barium salt separated. These were removed and after drying, analyzed.

0.2323 Gm. salt gave 0.120 Gm. $BaSO_4$.
Ba = 30.37 per cent
$Ba(C_9H_{17}O_2)_2$ requires Ba = 30.45 per cent.

The acid is thus identified as nonanoic acid.
The residue was identified as azelaic acid.

UNSAPONIFIABLE MATTER.

The unsaponifiable matter was obtained by extracting the aqueous solution of the potassium salts from the hydrolysis of the oil with ether. After drying the ethereal solution over anhydrous sodium sulfate and removing the ether, a red solid material remained. A chemical study of this is in progress. The work done so far would indicate that there are at least two phytosterols, an oil and coloring matter.

COLORING MATTER.

Mention has already been made of the separation of a dark red solid material from the oil which had been standing for a week. This colored solid was placed in a desiccator and after some time it was bleached out. This solid was practically insoluble in the usual organic solvents after bleaching. A small portion was burned on a platinum foil and a residue remained which was insoluble in water but readily soluble in hydrochloric acid. This solution gave a decided test for magnesium. It is not known at this time whether the magnesium is a definite part of the color molecule or simply an admixture. At various times during the preparation and analysis of the oil a gray solid would separate at the point in the procedure where the unsaponifiable matter was extracted from the aqueous solution of the potassium salts. This gray solid collected between the aqueous and ether layer. Several tests have been made with this material. Refluxing with alcoholic potassium hydroxide and with concentrated hydrochloric acid apparently caused no change. It gave the test for magnesium.

In the examination of the unsaponifiable matter a small amount of red coloring matter was isolated which was very intimately associated with another pigment which was of an orange color. It is unusually difficult to separate the two pigments from the oil. All are exceedingly soluble in carbon disulfide and the resulting solution is blood red. A large volume of absolute alcohol was added to the carbon disulfide solution and the whole allowed to stand over night in the refrigerator. A dark red solid separated which was removed by the aid of suction. The process of purification was repeated and the final product melted at 158–164° which indicated the presence of other substances, possibly oil which is removed with difficulty. The red solid showed all the solubility reactions of lycopin. Solutions in hot ether, alcohol, chloroform and benzene were yellow while in carbon disulfide a blood-red solution was obtained which persisted even in dilute solutions. It bleached to a white solid in the desiccator over phosphorus pentoxide.

Attempts were made to determine the absorption spectra but seemingly there was just sufficient of the orange pigments present to show an almost continuous absorption spectrum. This is to be expected in the case of a mixture because of the relative positions of the absorption bands of carotin and lycopin.

The refractive index of the isolated red pigment was determined in carbon disulfide solution and was found to be 1.603 at 25°, Kohl (9) gives 1.628 for the pure substance. The combustion analysis of this substance was far from satisfactory.

The alcohol-carbon disulfide filtrates were combined and concentrated. After standing over night in the refrigerator a mixture of red oil and an orange-yellow solid separated. This solid was removed and dissolved in absolute alcohol to a yellow solution. Very little oil separated with the orange-yellow solid after standing over night. It was impossible, under the conditions, to remove the last traces of oil from the pigment because of the small quantity of the material at our command. This orange-yellow pigment, like the red, bleached when exposed to the air.

The blood-red oil remaining was of a more limpid nature than the original oil and showed some differences in properties. For comparison the specific gravity and refractive index of the original oil and this oil are indicated.

	Original Oil.	Final Oil.
Specific gravity	0.9652 25/25°	0.9456 25/25°
Refractive index	1.480/25°	1.4950/25°

There is little doubt that the red color of this oil is due to the presence of the pigment.

A critical study of the pigments of the magnolia seed is now in progress together with that of the whole unsaponifiable matter.

SUMMARY.

The material employed for this investigation consisted of the whole fresh seed of the *Magnolia grandiflora* which was collected on the campus of the University during the month of October.

The seed was subjected to preliminary tests and gave no evidence of alkaloid or glucoside.

The ash content of the fresh seed was determined and gave the following results: Total ash, 2.16 per cent; water-soluble ash, 0.40 per cent; water-insoluble ash, 1.77 per cent; acid-soluble ash, 1.66 per cent; and acid-insoluble ash, 0.113 per cent. In view of the high acid solubility it is desirable to make a complete analysis of the ash which is in progress.

An alcoholic extract of the ground seed, when distilled with a current of steam, yielded a small amount of a light yellow volatile oil with a very pleasant aromatic odor. The oil was fractionated, giving two fractions: (*a*) light yellow oil, very pleasant odor, and boiling at 115–120° at 6-mm. pressure. It had a specific gravity of 0.9474 at 25/25°; index of refraction 1.4995/25°. It formed no addition product with sodium bisulfite nor did it give any coloration with ferric chloride solution. (*b*) This fraction was darker in color but possessed an odor similar to the other fraction. It boiled at 120–135° at 6-mm. pressure with slight decomposition. The refractive index was 1.519/25°. It gave a slightly greenish yellow coloration with ferric chloride solution but no addition product with the bisulfite.

The portion of the extract which was soluble in water contained a sugar which yielded a *d*-phenylglucosazone, melting at 214°, some tannin but nothing crystalline. A quantity of reddish brown solid separated from the aqueous solution on standing, which needs further investigation.

The fatty oil of the entire seed when ground and extracted with petroleum ether amounted to 42.5 per cent. This oil showed the following constants: Specific gravity, 0.9652 at 25/25°; refractive index, 1.428 at 25°; iodine number (Hanus), 89.5; saponification value, 182.5. The composition of the oil was as follows: Saturated acids, 20.20 per cent; unsaturated acids, 72.63 per cent; unsaponifiable matter, 2.83 per cent. The saturated acids resolved themselves into myristic, palmitic, stearic and arachidic acids; while the unsaturated acids were, isomeric, oleic and linoleic acids. Of the saturated acids palmitic acid predominated, the others in small quantities totaling probably less than 5 per cent. Oleic acid predominated in the mixture of unsaturated acids.

Oxidation of the oil in alkaline permanganate solution gave sativic and two dihydroxystearic acids; oxidation in boiling acetone with permanganate yielded acetic and some other lower acids, nonanoic and azelaic acids.

Evidence was obtained of the presence of at least two different pigments belonging, no doubt, to the carotinoid group from their color reactions and bleaching properties. The presence of phytosterol was indicated.

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ESTIMATION OF IODIDES IN COMPLEX MIXTURES.*

BY J. B. FULLERTON, W. J. WATKINS AND C. L. GRAHAM.

The estimation of inorganic iodine in complex mixtures has frequently been a source of difficulty. There have been numerous methods in the literature for the determination of iodine in ferrous iodide pills; but there will be no attempt made to list the papers describing the various methods. None of the methods recommended were successful in this laboratory, and it is assumed they were not generally satisfactory, else we would not find references to so many attempts on the problem.

Scott (1) gives a method for the decomposition of an iodide with nitrous acid and the subsequent extraction of the free iodine by means of carbon disulfide. The solution of iodine is then titrated with standard thiosulfate. In the same connection there is also given a method for liberation of iodine by the addition of hydrogen peroxide to a solution acidified with phosphoric acid. The liberated iodine is then distilled into a solution of potassium iodide and titrated with thiosulfate.

Working on a modification of Scott's method we have arrived at the following procedure:

Weigh enough of the finely powdered material to represent approximately 5 grains of potassium iodide (or an equivalent amount of iodine), and transfer to a separatory funnel. Add 50 cc. of water and, if alkaline, neutralize with phosphoric acid, finally adding 5 cc. in excess. Add 25 cc. of hydrogen peroxide and agitate thoroughly. Allow to stand a few minutes to be sure the reaction is complete and extract the liberated iodine with several portions of chloroform until the iodine has been removed, as can be told from the color. Collect the chloroform extractions in an iodine flask containing about 4 Gm. of potassium iodide in 25 cc. of water. Titrate with $N/10$ thiosulfate solution using starch as indicator.

The reaction between hydrogen peroxide and iodide in an acid solution is expressed by the following equation:

$$H_2O_2 + 2I^- + 2H \rightleftharpoons 2H_2O + I_2$$

We have found this method to be rapid, accurate and capable of a wide variety of uses. As previously stated, it was originally devised for the determination of iodine in ferrous iodide pills and has since been used for the estimation of iodine in various complex pill and tablet mixtures. It is satisfactory for mixtures of iodides with drug extracts, ferrous salts, reduced iron or arsenic. Obviously the same method could be used for the separation of iodine from the other halogens; but for simple mixtures of such, it is not as convenient as the well-known method of titration with KIO_3.

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