A REINVESTIGATION OF THE PROXIMATE COMPOSITION OF RHAMNUS FRANGULA.*

BY JOHN ABERDEEN GUNTON WITH GEORGE D. BEAL.

I. Introduction.—While engaged in a comparative series of investigations on the various drugs which owe their therapeutic value to their anthraquinone content a redetermination of the proximate composition of *Rhamnus frangula*, or alder buckthorn, was undertaken. This bark, because of its agreeable cathartic properties, has been in use in Continental Europe since the Middle Ages. It was early investigated by Gerber[1] who stated in 1828 that the active principles were resinous and hard to separate, and by Binswanger[2] who isolated the substance which he called *rhamnoxanthin* and which he assumed to be simply a coloring agent, and also a bitter principle of resinous nature to which its physiological action was ascribed.

Buchner[3] again isolated rhamnoxanthin, which he called *frangulin*, and made a note of its properties as being similar to those of hydroxy-anthraquinones. These same properties he found to be duplicated by the yellow coloring substances obtained from rhubarb. Casselman[4] compared this frangulin with the substance which he had obtained from rhubarb and found that they were not identical. Kubly[5] isolated a glucoside-like substance which he named *avornin* and from which he obtained on hydrolysis *avornic acid* and a sugar. Faust[6] found that the substances isolated from the bark by Kubly and Casselman were identical and that the avornic acid of Kubly, which he himself called frangulic acid, had an anthracene nucleus.

Liebermann and Waldstein[7] in 1876 determined that the principal constituent of the bark was emodin, which they obtained with a melting point of 259° and an empirical formula of $C_{15}H_{10}O_5$. Liebermann[8] later stated that emodin was identical with frangulic acid and that it was a trihydroxy-methyl-anthraquinone. *Chrysophanic acid*, hereinafter referred to as *chrysophanol*, he found to be a dihydroxy-methyl-anthraquinone.

Schwabe[9] illustrated the hydrolysis of the previously described frangulin by the equation

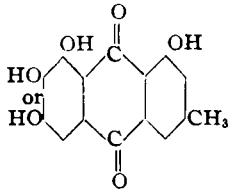
$$C_{21}H_{20}O_8 + H_2O = C_{15}H_{10}O_5 + C_6H_{12}O_6.$$

He furthermore confirmed Liebermann's formula for emodin and stated that the sugar accompanying the same was in all probability *rhamnodulcite*. Thorpe and Robinson[10] agreed on the formula $C_{21}H_{20}O_8$ assigned to frangulin by Schwabe, and Thorpe and Miller[11] found that the sugar obtained on the hydrolysis of frangulin was identical with the rhamnose obtained by the hydrolysis of quercitrin.

A compound with the same characteristics as frangulin, with a melting point of 237°, was obtained by Dohne and Engelhardt[12] in small quantities from cascara. This substance was thought to be a glucoside, since on hydrolysis there were obtained emodin and a sugar other than rhamnose, and was accordingly given the name *purshianin*. This has been since questioned by other chemists who have investigated cascara. Free emodin and chrysophanol have been obtained from frangula by Tschirch and Pool.[13]

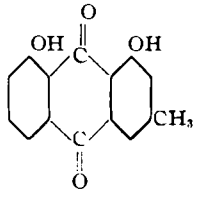
* Abstracted from a thesis presented by J. A. Gunton in partial fulfillment of the requirements for the Degree of Doctor of Philosophy in Chemistry in the Graduate School of the University of Illinois. Read in abstract at the New Orleans meeting of the American Pharmaceutical Association.

It may be said that the drugs which owe their cathartic properties to anthraquinone derivatives all contain emodin or its isomer, *aloe-emodin*, which is a dihydroxy-anthraquinone with a primary alcohol group. Emodin is characteristic of frangula, cascara, rhubarb and the species of Rumex which have been examined, while aloe-emodin is characteristic of aloes and senna. The structure of emodin is probably represented by the formula



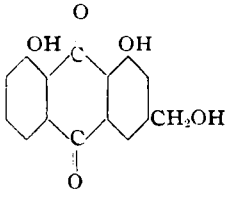
Its monomethyl ether, having less acidic properties than emodin because of the neutralization of one hydroxyl group, is associated with emodin in smaller amounts.

Chrysophanic acid, or chrysophanol, is found in practically all of these cathartic drugs, and has been assigned the formula

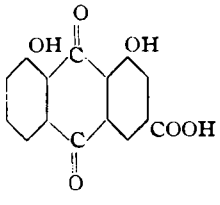


From inspection of this formula it will be seen that the properties of chrysophanol and emodin-monomethyl ether must be similar.

Two other important derivatives of β-methyl anthracene are the aloe-emodin, previously mentioned, the formula for which is given as

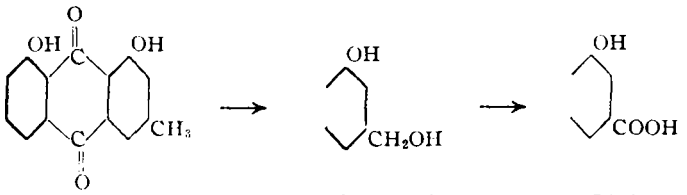


and *rhein*, which is a carboxylic acid with the probable formula



There is no mention anywhere in the literature of the finding of either of these substances in any of the varieties of Rhamnus.

Oesterle[14] has shown that chrysophanol, aloe-emodin and rhein are three stages in the oxidation of the same hydroxy-methyl-anthraquinone, as follows:



Chrysophanol Aloe-emodin Rhein

Our purpose in taking up this investigation was primarily to determine whether the anthraquinone derivatives which have been regarded as the important active constituents of the drug were present in the free form or principally in some sort of combination, possibly as a glucoside. Questions of lesser importance developed from time to time during the study.

The material used in this investigation was a fifty-pound lot of the air-dried drug obtained in France by Fuller, Morrison and Company of Chicago. This was in the form of small quills having a grayish black color on the outside and a reddish tint on the inner surface. Even when viewed superficially the drug could be distinguished readily from cascara. The taste, when macerated in water, was pleasant in its bitterness.

A small quantity of fresh bark was obtained from the garden of Doctor James H. Beal in Urbana. This was taken in January, and was dried at room temperature. Our object in taking this small sample was to determine, if possible, the emodin content at a time of year when the plant was inactive.

Apparatus.—The percolations with alcohol were carried out in the continuous extraction apparatus invented and patented by John Uri Lloyd, and the thanks of the authors are extended to Professor Lloyd for permission to use a replica of his apparatus manufactured by Deckebach and Sons of Cincinnati, Ohio. In this apparatus, which in our laboratory could be used for the percolation of a 5.5 kilo sample of drug, the percolate flows directly into a concentrator where the menstruum is removed by surface evaporation with the aid of a steam jacket surrounding a portion of the concentrator. The concentrated extract falls by circulatory displacement into a cooled zone. The time during which heat is applied to the extractives is thus reduced to a minimum and the decomposing and hydrolytic action of the heat accordingly diminished.

The large-scale extractions with ether and chloroform upon the dry resins were made with the aid of the Gramercy Extraction Apparatus of Eimer and Amend and also in part with a modified Soxhlet apparatus composed of an inverted aspirator bottle as extractor, with a condenser and a round-bottomed flask as a receiver for the extract. This improvization has been frequently described by chemists and needs no further mention.

The extractions with immiscible solvents, which necessitated the handling of hundreds of liters of liquids, were performed in twelve liter bottles, securely stoppered with rubber stoppers, and rotated about their longitudinal axes in an Abbé Vertical Ball Mill. There was practically no shaking effect obtained with this arrangement, the liquids coming into intimate contact with each other with a

rolling motion. In only one instance was an emulsion formed, and it was believed that this was due to an improper placing of the bottle in the holder, imparting a slight jerk to the bottle during each revolution.

II. Preliminary Examination. Moisture.—All investigations were made using the air-dried drug. For the determination of moisture a sample was dried to constant weight in a vacuum oven at 98°.

Nitrogen.—The nitrogen content was determined by the Kjeldahl method, using copper sulphate as the catalyst and potassium sulphate to raise the boiling point of the digestion mixture.

Ash.—Portions of the drug were ashed in an electric muffle at 950°, using shallow platinum dishes. The ash was light gray in color, with a green cast.

Hydrocyanic Acid.—The presence of this acid has been claimed by a number of investigators, and one author has ascribed its popularity as a cathartic to its "peach-kernel" taste. The HCN content was determined by the method outlined by Whirthle and Rheinberger[15] for the examination of Rangoon beans. The ground bark was digested for twenty-four hours with water containing one percent of tartaric acid and the liquid then distilled with steam. The distillate was titrated with $N/100$ silver nitrate according to Liebig's method.

The results of the above determinations were as follows:

Moisture	6.01%
Ash	5.75%
Nitrogen	0.74%
Protein, N × 6.25	4.62%
Hydrocyanic acid	0.005%

Manganese.—The green tint of the ash suggested the presence of manganese, a frequent but small constituent of drugs. The ashes of cascara and rhubarb have a distinctly green color, and Westman[16] has found the "manganese number" of the ash of cascara to be of fairly constant value in the identification and determination of this drug in medicines. Accordingly the manganese content of the drug was determined by the method of the A. O. A. C.,[17] that of cascara and rhubarb being determined for comparison. The values obtained were:

Frangula	0.0084%
Cascara	0.0402%
Rhubarb	0.0223%

Since the purgative properties of these drugs are admittedly due to their content of anthraquinone derivatives and this content with regard to frangula and cascara is about equal there seems to be no relationship between the manganese content and the medicinal value of the bark.

Sublimation.—In charring the bark during the ashing process copious yellow fumes were given off before the combustion temperature was reached. A portion of drug was heated in a small test-tube having a side arm through which it could be evacuated and containing an inner test-tube to serve as a condenser. A moist yellow-brown sublimate was obtained in which a few microscopic crystals could be seen. When the sublimate was moistened with dilute alkali it gave a reddish brown color similar to that obtained at times with derivatives of hydroxy-anthraquinones.

Alkaloids.—Eccles[18] having found traces of an alkaloid in fluidextract of cascara, there seemed to be a possibility that members of this group might exist in frangula. A portion was thoroughly extracted with Prollius solution and the extract in turn shaken with five percent hydrochloric acid. The light yellow acid solution was clarified with lead subacetate and the filtrate deleaded. Mayer's reagent, picric acid and iodine gave precipitates of doubtful significance.

A second portion of drug was barely moistened with water and mixed with an equal volume of magnesium oxide, then dried and extracted with ether. The drug mixture became brick-red in a short time and almost black on standing. The ether extract was deep yellow in color and deposited a crust of anthraquinones on the inner surface of the flask. This ether extract was shaken with acid as above, obtaining the same yellow solution, which was rendered faintly alkaline with sodium hydroxide and again extracted with ether. The resulting extract was shaken with one percent hydrochloric acid, and this solution tested with the same series of precipitants. The negative results obtained led us to believe that the first reactions had been given by proteins not completely separated by the clarifier or by ammonia passing over from the Prollius solution.

Total Extraction.—In order to determine the nature and amount of extractives obtained by various solvents a twenty-five gram portion of bark was successively extracted with petroleum ether, ethyl ether, chloroform, ethyl acetate and ninety-five percent alcohol. The petroleum ether extract consisted chiefly of fatty material, the ether extract of anthraquinones with a small amount of fat, while the other extracts were resinous in nature and contained the bitter principles of the bark as well as the anthraquinones. The yields to the various solvents were as follows:

Petroleum ether	3.8%
Ethyl ether	2.3%
Chloroform	4.8%
Ethyl acetate	6.8%
95% alcohol	36.0%

Hydroxy-anthraquinones.—Various methods have been devised for the quantitative determination of the members of this group together, all since 1900. These depend on colorimetric or precipitation reactions of the derivatives. They have been quantitatively studied by Tschirch and Hebeisen[19] and found to yield comparable results. Four methods have been suggested by Tschirch[20] and his students as follows:

1. A spectroscopic method based on the absorption spectra of hydroxy-anthraquinones dissolved in normal alkali.

2. A colorimetric method based on the characteristic red color of alkaline solutions of hydroxy-anthraquinones.

3. A modification of the above for use without a colorimeter.

4. A gravimetric method for the precipitation of their diazonitranilin derivatives.

Another colorimetric method by Warin[21] is based upon the color given by nickel salts and alkali. This is the reaction which was used by Beal and Okey[22] to distinguish the various emodin-containing drugs. The most recent and probably the most rapid method is that of Daels,[23] who extracts the anthraquinones present

in the drug with chloroform, both before and after acid hydrolysis, obtaining values for "free" and "combined" anthraquinones.

Determination of Free Hydroxy-anthraquinones.—Five grams of the finely ground bark, dried at 70°, were placed in a 400-cc Erlenmeyer flask and weighed after the addition of two hundred cc of chloroform. The flask was connected with a reflux condenser and the solvent boiled for fifteen minutes, then cooled decanted through a filter and the residue washed with chloroform. This solution was shaken with five percent sodium hydroxide solution until the color was removed from the chloroform and the alkali extract no longer became pink. These combined alkaline extracts were shaken with small amounts of chloroform to remove emulsified fats and then acidified with hydrochloric acid and the liberated anthraquinones extracted with chloroform. The chloroform solution was filtered and evaporated to dryness in a tared beaker, then dried at 100° and weighed. Assuming that the "primary" glucosides of the plant are insoluble in chloroform, the residue represents the free acidic constituents.

Determination of Combined Hydroxy-anthraquinones.—All of the residual drug from the first extraction was returned to the flask and chloroform added to the previous weight. After adding fifty cc of 25% sulphuric acid the flask was again weighed and the mixture refluxed at the boiling point of the chloroform for two and one-half hours. After cooling and making up to the original weight with chloroform, one hundred and fifty cc of the chloroform were filtered and shaken with fifty cc of 10% sodium bisulphite solution. The chloroform was then shaken with one hundred cc of 1% hydrochloric acid and the chloroform finally evaporated and the residue weighed as before. A number of other drugs were examined at the same time for comparative purposes.

Drug.	Free.	Anthraquinones combined.	Total.
Frangula	1.14%	2.63%	3.77%
Frangula (recent)	0.87	3.39	4.26
Cascara	1.11	2.70	3.81
Rhubarb	0.29	2.32	2.61
Rumex crispus	0.72	1.18	1.90
Rumex ecklonianus	0.26	1.36	1.62

For the specimen of *Rumex ecklonianus* we are indebted to Doctor Rudolph Marloth of Capetown, South Africa.

Cold Water Extract.—Extraction of a small portion of the bark with cold water yielded an extract which was quite bitter in taste and gave the color reactions of hydroxy-anthraquinones. The soluble matter amounted to 18% and the ash thereof to 0.80% of the bark. Sugars were indicated by the Molisch and Fehling reactions. Shaking out the water extract with chloroform removed material equal to 0.56% of the bark.

A portion of the aqueous extract was allowed to react with emulsin according to the procedure of Bourquelot[24] and the quantity of chloroform extract compared with that obtained before action of the enzyme. An increased amount of chloroform extract was obtained, which was taken as evidence of the occurrence of some of the extractives in combined form.

Hot Water Extract.—Another small portion of bark was digested repeatedly with water on the steam-bath and the percentage of extractives found to be 36.80%.

The ash amounted to 1.95% and the chloroform extract to 2.40%. More strongly positive tests for sugars and hydroxy-anthraquinones were obtained, while the extract also gave a test for pentoses with phloroglucin.

A larger portion of the bark, four hundred grams in all, was digested on a steam-bath at 70° with distilled water until exhausted. This solution contained all of the bitter principles and had cathartic properties. After spontaneous evaporation to a thick syrup the extract was taken up with alcohol and mixed with sawdust which had been purified by exhaustion with alcohol. This mixture after drying was extracted in a Soxhlet with petroleum ether, ethyl ether, chloroform, ethyl acetate and ethyl alcohol.

The petroleum ether extract was oily with a slight yellow stain giving a feeble reaction with alkalies. The ether extract showed a few orange crystals in an oily residue. These had the appearance and reactions of impure emodin. The chloroform extract was very small and the slight ethyl acetate residue was resinous with an odor suggestive of tanbark. The alcoholic extract was resinous, nothing crystalline being obtained therefrom, but contained all the bitter material of the aqueous extract.

During the latter part of the water extraction there remained in suspension over the bark some fine golden yellow crystals, while the epidermal layers of the bark showed a yellow incrustation. This was crystalline and homogenous, soluble in dilute alkali with a red color, and was supposed to be anthraquinone derivatives liberated by hydrolysis and crystallized from the water.

Extraction with Hot Ammonium Hydroxide.—The bark which had been extracted with water was exhausted with warm 2% ammonia, obtaining fifteen liters of red solution. This was neutralized with hydrochloric acid and filtered, and the residue, after drying, extracted with petroleum ether and ethyl ether. The acid filtrate yielded only a trace of matter to ether.

The petroleum ether removed a small amount of brown oil, a portion of which was undoubtedly due to the solvent. A few crystals of emodin were seen therein. The ether extract yielded small brown nodules, m. p., 232°, which on recrystallization were found to be emodin. The material was not split by boiling dilute acid.

Extraction with Cold Ammonium Hydroxide.—Another four hundred gram portion of the bark was completely extracted with cold 2% ammonia and after filtration the solution was acidified and again filtered. The precipitate showed some evidence of hydrolysis with acid, but no definite compound was isolated.

Extraction with 95% Alcohol.—Four and one-half kilos of the bark were extracted with 95% alcohol in the Lloyd apparatus and the extract concentrated to a thick syrup. This was allowed to stand for four months in a cool place and the separated material removed. This was digested on the steam-bath with 95% alcohol to dissolve as much as possible and the solution freed from fatty matter with petroleum ether. Water was added gradually while the flask was on the steam-bath and the digestion continued while a light brown granular material gradually deposited. This melted at 212° with some decomposition, subliming slightly above 100°. Purification by solution in alcohol and precipitation with ether raised the melting point to 229°.

Digestion of some of the alcoholic extract with water in the same fashion yielded material melting at 228°. This corresponds to the melting point of the

rhamnoside frangulin described by Thorpe and Miller (*loc. cit.*). A small portion of the brown material was suspended in 10% sulphuric acid and heated at 100° for two hours in an attempt to hydrolyze the possible glucosides present. The orange-colored insoluble product was filtered off and after crystallization from alcohol was identified as emodin.

The filtrate, which was brown in color and had an odor of caramel, was tested for sugars. It gave the Molisch reaction, reduced Fehling's solution and gave the phloroglucin-hydrochloric reaction for pentoses and a positive reaction for methyl pentose with alcohol and sulphuric acid. The sugar was evidently rhamnose and its original state of combination was probably as the glucoside frangulin.

Study of the Sugars.—Hudson and Harding's[25] method of obtaining xylose was applied to the bark. The extract obtained with 2% ammonia was hydrolyzed by boiling with sulphuric acid of 7% concentration. The acid was neutralized with barium carbonate and the filtrate concentrated.

An osazone prepared from this solution melted at 180°, corresponding with that for rhamnose. The precipitate forming during the acid hydrolysis consisted of anthraquinones. Water and ammonia had failed to remove enough sugar from the bark to form an osazone, therefore it must have been removed by the ammonia in glucosidic combination and liberated by acid hydrolysis.

Following the ammonia digestion the bark was suspended in 7% sulphuric acid and refluxed for several hours. After filtration, neutralization with barium carbonate and refiltration, a syrup was obtained by evaporation *in vacuo*. No crystals were obtained on standing but the addition of five volumes of absolute alcohol brought down a few white needle-like crystals. The osazone of the sugar melted at 152° after crystallization from alcohol as fine yellow needles. The crystalline form of the sugar and its osazone and the melting point of the osazone identified the sugar as xylose.

III. Systematic Examination.—This was carried out following in general the scheme of analysis which has been developed by Power and his associates at the Wellcome Chemical Research Laboratories. The bark was percolated with alcohol in the Lloyd apparatus and the concentrated extract, a heavy syrup, mixed with purified sawdust and dried. The mixture was extracted successively with petroleum ether, ether, chloroform and ethyl acetate. Each of these extracts was shaken in turn with 8% ammonium carbonate, dissolving strongly acidic substances; 5% sodium carbonate, dissolving less acidic substances; and 2% sodium hydroxide dissolving the most weakly acidic substances, including phenols. The further treatment of the extracts was as suggested by their character.

Following the use of these solvents the resin was dissolved in alcohol and hydrochloric acid added to 1% concentration and the mixture then boiled on the steam-bath under a reflux condenser for twenty-four hours or more. The hydrolyzed resin was precipitated by pouring the solution into six volumes of water, the trace of acid facilitating precipitation of the resin. The precipitate was filtered, washed and dried and treated in the same fashion as the original resin. The water solution was extracted with the same series of solvents.

Legend of Operations.—In order to facilitate identification of the different fractions a code was devised in which the common reagents and operations were represented by letters, as follows:

A. Original bark.
B. 95% alcoholic extract.
C. Petroleum ether extract.
D. Ethyl ether extract.
E. 8% ammonium carbonate extract.
F. 5% sodium carbonate extract.
G. 2% sodium hydroxide extract.
H. 50% alcoholic extract.
I. Neutralized with hydrochloric acid.
J. Precipitate.
K. Residue after ABCD.
L. Hydrolysis with 1% hydrochloric acid.
M. Aqueous portion.
N. Resin.
P. Amyl alcohol extract.
R. Residue after ABCEFG.
S. Residue after ABCDEFG.
T. Chloroform extract.
U. Solution in glacial acetic acid.
V. Extraction with 2% ammonia.
W. Extraction with water.
X. Extraction with ethyl acetate.

(Example: ABCDEFGIMDEFGIJU. A 95% alcoholic extract of the bark was extracted successively with petroleum ether and ethyl ether. The ether extract was washed with ammonium carbonate, sodium carbonate and sodium hydroxide and the latter solution acidified with hydrochloric acid and filtered. The filtrate was shaken with ether and the ether solution extracted with the three alkalies. The sodium hydroxide solution neutralized with hydrochloric acid gave a precipitate which was dissolved in glacial acetic acid for crystallization.)

The 95% Alcoholic Extract. AB.—Seventeen and one-half kilos of the bark in No. 30 powder were extracted in the Lloyd apparatus, the concentrated extract amounting to about four and one-half liters. This was dark brown in color with a greenish shade suggestive of chlorophyll. This was mixed with purified sawdust and dried, leaving a friable mass which was extracted with the series of solvents described above.

A. Petroleum Ether Extract. ABC.—This was greenish yellow in color, containing chlorophyll and fat. The fat furthered emulsification during the alkaline extractions.

1. 8% Ammonium Carbonate Extract. ABCE.—This was deep red in color, becoming yellow when neutralized with acid and depositing a fine brownish yellow precipitate, *ABCEIJ*. The filtrate, *ABCEIM*, was yellow and was extracted with ether and amyl alcohol. These solutions were concentrated, shaken with the series of alkalies and the alkaline solutions neutralized and shaken with ether.

The Ether Extract, ABCEIMD, yielded crystallizable material only to sodium carbonate, *ABCEIMDEFIMD*. This was obtained from its ether solution as a slight yellow-orange residue, melting at 249° when freed from oily matter. This was undoubtedly emodin. The other fractions gave yellow oily residues with mere traces of anthraquinones.

The Amyl Alcohol Solution, ABCEIMDP, while deeply colored with extractives, yielded little material to alkalies and that only fatty or resinous. Whenever amyl alcohol was used for extraction we noted that even a very dilute solution

of anthraquinone derivatives was deeply colored, while the color of an ether solution did not become deep until the solution was quite concentrated.

The Precipitate, ABCEIJ, was extracted with chloroform, the extract, *ABCEIJT*, being dark colored. This left on evaporation a fatty resin with no crystals, and was probably a mixture of anthraquinone, as emodin, and fat.

2. The 5% Sodium Carbonate Fraction, ABCEF.—The deep red solution was siphoned into concentrated hydrochloric acid, stirring the mixture with air. The precipitate, *ABCEFIJ*, was dried and extracted with ether and chloroform. The material thus obtained was too small for identification.

The filtrate, *ABCEFIM*, was shaken with ether and amyl alcohol and the ether extract shaken with the three alkalies. Ammonium carbonate removed a small amount of semi-liquid material with an odor of ethyl acetate and butyrate and traces of anthraquinones. Sodium carbonate extracted impure emodin, while sodium hydroxide extracted chrysophanol with a small amount of emodin-monomethyl ether.

3. The Sodium Hydroxide Fraction, ABCEFG.—Difficulty was encountered in this extraction because of emulsification. The main portion of the emulsion was acidified, when the semi-solid material could be separated from the aqueous portion. The filtrate, *ABCEFGIM*, was yellow and deposited on standing a fine yellow powder found to be chrysophanol.

The semi-solid material, *ABCEFGIJ*, was saponified and the aqueous soap solution extracted with ether. When the solvent was evaporated a waxy brown mass with an acrid soapy taste resulted, which on recrystallizing eight times from alcohol yielded white crystals, m. p. 134°. This melting point has been previously given for the phytosterol *rhamnol*, and the product agreed with previous preparations of this laboratory. The acetyl derivative melted at 120° and the compound gave the Liebermann-Burchard[26] reaction for phytosterols with acetic anhydride and sulphuric acid. This phytosterol was first discovered by Jowett[27] in cascara and has been reported by Beal and Okey[28] in *Rumex crispus* and by Tutin and Clewer[29] in *Rumex ecklonianus*. A neutral hydrocarbon accompanied the rhamnol but could not be purified for identification.

The soap solution was neutralized and extracted with ether and the ether extract in turn shaken with sodium carbonate, removing emodin, and with sodium hydroxide, removing chrysophanol. The latter when recrystallized from absolute alcohol formed golden yellow plates melting at 191°. When mixed with the corresponding material obtained by Beal and Okey from *Rumex crispus* the melting point remained unchanged. The diacetyl derivative prepared after long acetylation was greenish yellow in color and melted at 240°.

B. The Ethyl Ether Extract, ABCD.—The combined ether extracts from the resin were concentrated to five liters and shaken with the three alkalies. The ammonium and sodium carbonate fractions were deeply colored, the sodium hydroxide fraction much less so. Each of the extracts was acidified and filtrate and precipitate examined. The neutral material left in the ether was found to be the same mixture of rhamnol and hydrocarbon found in the petroleum ether extract.

1. Ammonium Carbonate Fraction, ABCDE.—This formed a yellow-brown precipitate on acidifying and the filtrate continued to deposit yellow flocks until practically colorless, yielding finally no extractives to ether. The precipitated

matter, *ABCDEIJ*, was extracted with petroleum ether, ether and chloroform. The petroleum ether removed a very small quantity of yellow resin which was found to be a mixture of emodin and fat.

Ether dissolved practically all of the precipitated material, the dark brown solution, *ABCDEIJD*, depositing dark red crystals in the Soxhlet flask during the extraction. In all about two grams of red crystals were obtained as a result of the extraction. The crystals as first deposited melted at 225°, but were shown by failure of acid hydrolysis to be non-glucosidic and on crystallization from glacial acetic acid very pure emodin was obtained. This emodin agreed in all respects with samples which had been prepared from *Rumex crispus* and cascara. The material extracted by chloroform was of the same character as the ether extract but very small in amount.

2. The Sodium Carbonate Fraction, ABCDEF.—Practically all of the extractives were precipitated on acidifying. The precipitate, *ABCDEFIJ*, yielded a trace of emodin to petroleum ether and a mixture of emodin with some chrysophanol to ether. Since it was evident that the sodium carbonate had some solvent action on the less acidic constituents, the precipitate was dissolved in ether and the sodium carbonate-sodium hydroxide separation repeated. Pure emodin and chrysophanol were thus obtained from the proper fractions.

3. The Sodium Hydroxide Fraction, ABCDEFG.—Additional quantities of emodin and chrysophanol were obtained from this fraction.

C. Chloroform and Ethyl Acetate Fractions.—These solvents dissolved very little anthraquinone, the ones present being emodin and chrysophanol.

The Hydrolyzed Resin, KBL.—The sawdust marc was percolated with alcohol and the extract concentrated to a thin syrup, having a volume of eight liters, to which was added one percent of concentrated hydrochloric acid. The mixture was refluxed on the steam-bath for four days and the resin precipitated by pouring the solution into six volumes of water. The precipitate, *KBLMN*, which was dark brown in color, was filtered and dried and the filtrate concentrated *in vacuo* to twelve liters, keeping the reaction of the solution neutral. Some humus-like material, *KBLMJ*, separated during the concentration and was saved for examination.

D. The Water-Insoluble Resin, KBLMN.—The resin was dissolved in a small amount of alcohol, mixed with purified sawdust, dried and extracted with the usual solvents.

1. Petroleum Ether Extract, KBLMNC.—This was light yellow in color and consisted of a fatty residue, resulting in part from the solvent, with only enough nanthraquinone to give a color.

2. Ethyl Ether Extract, KBLMNCD.—So much of the resin dissolved in ether and was deposited in the flasks that frequent changing of the flasks and solvent was necessary. On standing the solution deposited orange-red rosettes, altogether forty grams of ether-soluble material being obtained. The remaining ether solution, concentrated to about five liters, was extracted successively with the three alkalies, leaving but a trace of fatty matter in the ether.

a. Ammonium Carbonate Fraction, KBLMNCDE.—A dark red, almost crystalline, precipitate formed between the solvent layers. This material was filtered but even on careful drying the ammonium salt which had been formed was broken

down with loss of ammonia, the residue yielding pure emodin on crystallization from glacial acetic acid. Acidifying the ammoniacal solution and extracting with ether gave a further crop of emodin. The extraction of emodin at this point was due to its high concentration in the ether solution, favoring the formation of the unstable ammonium salt.

b. Sodium Carbonate Fraction, KBLMNCDEF.—Several extractions with 5% sodium carbonate solution were required to remove all of the soluble material, which was identified as emodin.

c. Sodium Hydroxide Fraction, KBLMNCDEFG.—On acidifying the brilliant red solution a light yellow precipitate formed, which on crystallization from alcohol melted slowly at 160°. This, evidently a mixture, was thought to consist of chrysophanol and emodin-monomethyl ether. These were separated, as described by Jowett (*loc. cit.*), by dissolving 0.3 gram of the mixture in concentrated sulfuric acid and heating slowly to 160° with an oil-bath, then cooling and slowly pouring the mixture into water. There was thus formed a green amorphous precipitate which was partially soluble in chloroform. The mixture was shaken with chloroform until no more yellow coloration was obtained and the chloroform extract shaken with sodium carbonate and sodium hydroxide. Emodin, from the demethylation of the monomethyl ether, was obtained from the sodium carbonate fraction, and chrysophanol from the sodium hydroxide fraction.

The Precipitated Material, KBLMNCDJ, which deposited from the ether solution during extraction in the Soxhlet apparatus, crystallized from glacial acetic acid in orange-red needles. This was the purest emodin obtained. It melted at 253°, its acetyl derivative at 195° and its dibenzoyl derivative at 224°.

3. The Chloroform and Ethyl Acetate extracts contained resinous material and traces of emodin, its methyl ether and chrysophanol. It was noticed that prolonged heating of the chloroform solution caused decomposition of the extractives.

E. Water-Soluble Hydrolyzed Resin, KBLM.—During concentration of this solution under diminished pressure the first portion of distillate was colored brown by a volatile substance which could be extracted with ether. Its odor was that of tanning extracts and its taste extremely bitter. It gradually resinified on standing so that nothing therein could be identified.

The concentrated filtrate yielded to ether a small amount of emodin and a few crystals of cinnamic acid. Rhamnose was identified in the clarified solution.

Discussion of Constituents.—The glucoside frangulin was found to agree in properties with that which has been previously described. It melted sharply at 229° and on hydrolysis with acid or emulsin yielded emodin and rhamnose. It is quite insoluble in cold ether and the other immiscible solvents, which accounts for its being found in the original alcoholic extract and not in subsequent fractions.

One molecule of emodin is apparently so linked with one of rhamnose as to allow two hydroxyls of the emodin to retain acidic properties. These groups are probably neutralized by other molecules in the plant in such a way as to form much larger complex primary glucosides to which the real purgative action is due. Neither pure frangulin nor the pure hydroxy-methy-anthraquinones has cathartic properties which are comparable with those of the drug.

Emodin, the principal hydroxy-anthraquinone present, is found in free and combined forms and agrees with the properties of that from cascara and *Rumex*

crispus. Its melting point, even when mixed with preparations from the other drugs, remained at 253°, with slight sublimation above 210°. The acetyl derivative melted at 197°, the dibenzoyl at 224° and the monobrom compound at 250°. As a trihydroxy-methyl-anthraquinone it is isomeric with morindone from Morinda citrifolia.

Chrysophanol, or chrysophanic acid, is found free and associated with emodin-monomethyl ether. It melts at 190° and forms an acetyl derivative melting at 204°. This agrees with other preparations obtained in this laboratory. Chrysophanol is probably present in both free and combined form in the bark.

Emodin-monomethyl ether while associated with the chrysophanol was not present in sufficient quantity to isolate. Its presence was easily demonstrated by the demethylating action of concentrated sulfuric acid.

Rhamnol, the phytosterol $C_{20}H_{34}O$ isolated from the unsaponified portion of the petroleum ether extract, is an alcohol of the quebrachol type. It is identical with that from cascara and Rumex.

The sugars isolated were rhamnose and xylose, the latter from hydrolysis of the woody tissue. The rhamnose was apparently present in glucosidic combination. It is identical with that obtained by hydrolysis of quercitrin.

IV. An Attempted Synthesis of Frangulin.—Rhamnose was prepared by the acid hydrolysis of quercitrin as described by Walton.[30] The quercitrin was contained in Lemon Flavin which was courteously furnished us by J. S. Young and Company of Hanover, Pa. The crystallized rhamnose was dissolved in acetic anhydride and saturated with gaseous hydrobromic acid in the presence of a few small globules of mercury. The product, which was a viscous liquid, was purified by solution in ether and precipitation with petroleum ether. It could not be crystallized.

Five grams of acetyl-brom-rhamnose were added to a mixture of four grams of pure emodin and 2.48 grams of potassium hydroxide dissolved in absolute alcohol. The solution had an odor of ethyl acetate after standing a short time, became deep brown in color and deposited a dark precipitate after refluxing on the steam-bath for one hour. The mixture after spontaneous evaporation was dissolved in a small amount of water and carefully neutralized with hydrochloric acid. Just enough alcohol was added to dissolve the brown precipitate and the mixture was digested on the steam-bath with the occasional addition of a little water. The brown precipitate which formed was filtered, washed and dried and extracted thoroughly with ether until the ether washings gave no test for anthraquinones. When this brown substance was shaken with water no test for carbohydrate could be obtained in the aqueous portion.

The compound could not be crystallized from alcohol and was insoluble in ether. It apparently decomposed at 220° with some sublimation and when mixed with frangulin from the bark the mixture melted at 224°. The substance had a bitter taste. When boiled with dilute hydrochloric acid and the solution shaken with ether the ether washings gave reactions for emodin and the aqueous liquid reactions for carbohydrates. This leads us to believe that a combination of glucosidic character had taken place with the formation of a substance akin to frangulin. This phase of the work is being continued at the present time.

Acknowledgment.—The authors wish to take this opportunity of expressing their thanks to the American Pharmaceutical Association for the aid which they have received from a grant made from the Research Fund of the Association and which has been used in part in the purchase of materials for the investigation.

URBANA, ILLINOIS, JUNE 1921.

BIBLIOGRAPHY.

1. *Brandes Archiv.*, 26, 1828.
2. *Annalen*, 76, 356, 1850.
3. *Ibid.*, 87, 218, 1853.
4. *Ibid.*, 104, 77, 1857.
5. *Pharm. Zeit. f. Russ.*, 5, 160, 1866.
6. *Zeit. f. Chem.*, 5, 17, 1869; 7, 340, 1871. *Annalen*, 165, 229, 1872. *Arch. Pharm.*, 187, 8, 1869.
7. *Ber.*, 9, 1775, 1876.
8. *Jour. Chem. Soc.*, 31, 609, 1877.
9. *Arch. Pharm.*, (3) 26, 569, 1882.
10. *Jour. Chem. Soc.*, 57, 38, 1890.
11. *Chem. News*, 64, 305, 1891. *Jour. Chem. Soc.*, 61, 1, 1892.
12. PROC. A. PH. A., 45, 193, 1897.
13. *Arch. Pharm.*, 246, 317, 1908.
14. *Ibid.*, 249. 445, 1911; 250, 301, 1912.
15. *Zts. Nahr.-Genussm.*, 39, 346, 1920.
16. *Jour. Amer. Chem. Soc.*, 40, 558, 1918. JOUR. A. PH. A., 7, 759, 1918.
17. "Methods of Analysis," A. O. A. C., p. 17, 1920.
18. *Druggists' Circular*, 32, 59, 1888.
19. "Thesis," Berne, 1915.
20. *Pharm. Post*, Nos. 17, 19, 1904. *Arch. Pharm.*, 245, 151, 1907.
21. *Jour. d. pharm.*, 1905, 253.
22. *Jour. Amer. Chem. Soc.*, 39, 716, 1917.
23. *Jour. d. pharm. d'Anvers*, 1913, 401.
24. *Arch. Pharm.*, 245, 172, 1907.
25. *Jour. Amer. Chem. Soc.*, 40, 1601, 1918.
26. *Ber.*, 18, 1804, 1885.
27. PROC. A. PH. A., 52, 288, 1904.
28. *Jour. Amer. Chem. Soc.*, 41, 693, 1919.
29. *Jour. Chem. Soc.*, 97, 1, 1910
30. *Jour. Amer. Chem. Soc.*, 43, 127, 1921.

A NEW COLORIMETRIC DETERMINATION OF METHYL ALCOHOL.

BY A. B. LYONS.

In a comprehensive paper published in the *Journal of Industrial and Engineering Chemistry* last June, Robert M. Chapin describes a modification of the Denigès test for methyl in presence of ethyl alcohol, by which it is possible to determine colorimetrically with a fair degree of precision the proportion of the former. The author points out the fact that when alcoholic beverages are under consideration small quantities of formaldehyde may occur normally in the oxidized solution to which the test is applied so that the test is inconclusive unless it shows approximately the quantity of methyl alcohol it indicates. No limit is set by the author to the quantity which may be regarded as possibly "normal," but it seems unlikely that it can ever amount to as much as 1 percent of the original sample, if this be