CHEMICAL EXAMINATION OF MORINDA LONGIFLORA. 1907

CLXXXV.—Chemical Examination of the Root and Leaves of Morinda longiflora.

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SEVERAL plants belonging to the genus Morinda (Nat. Ord. Rubiacea) have from time to time attracted the attention of chemists, chiefly on account of the tinctorial properties possessed by them. The Indian dye-stuff "Suranji," which consists of the roots of M. citrifolia and M. tinctoria, was investigated by Anderson (Annalen, 1849, **71**, 216), who obtained from it a substance designated "morindin," which, when heated, gave a sublimate of a red compound named "morindon." Subsequent workers have shown that morindin is a glucoside, $C_{26}H_{28}O_{14}$, and that morindone, yielded by its hydrolysis, is a trihydroxymethylanthraquinone. An exhaustive examination of "Mang-Koudu," the root-bark of *M. umbellata*, was conducted by Perkin and Hummel (Trans., 1894, 65, 857), who obtained from it morindin, morindone, and five other anthraquinone derivatives. More recently, Oesterle (*Arch. Pharm.*, 1907, 245, 287) has examined the wood of *M. citrifolia*, and obtained from it a dihydroxymethoxyanthraquinone.

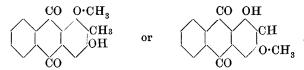
The West African plant, Morinda longiflora, G. Don, was brought to our notice by an article in the Journal of the Society of Arts (1905, 53, 1069), where it is referred to, under the name of "Ojuologbo"— Woody Vine, as one of the most valuable plants of that region, and is stated to be used as a medicine by nearly all West African tribes.

Through the kindness of Dr. W. Renner, Medical Officer, Colonial Hospital, Freetown, Sierra Leone, we were supplied with a quantity of the roots and leaves of "Ojuologbo," and the identity of the material thus obtained with the products of *Morinda longiflora* was kindly confirmed by Mr. E. M. Holmes, F.L.S. A complete botanical description of this plant may be found in the *Flora of Tropical Africa*, by Daniel Oliver, F.R.S., Vol. III, p. 192, London, 1877, where it is stated to be known under the native name of "Mbogga."

As a preliminary test, the leaves and root were each examined for the presence of an alkaloid, but with a negative result. The ground materials were subsequently extracted with alcohol, and the resulting extracts separately investigated.

On examining the extract yielded by the root, no morindin or morindone was obtained, but a quantity of a hydroxymethoxymethylanthraquinone and a small amount of a monomethyl ether of alizarin were isolated. The root also yielded, in addition to resins and other amorphous products, small quantities of formic, acetic, butyric, palmitic, and citric acids, a little of a phytosterol, $C_{27}H_{46}O$ (m. p. 130°), and a considerable amount of a sugar which yielded d-phenylglucosazone (m. p. 216°.)

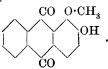
The hydroxymethoxymethylanthraquinone, $C_{16}H_{12}O_4$, forms yellow needles which melt at 290°, and gives an *acetyl* derivative melting at 173°. When heated with 70 per cent. sulphuric acid, it yields a dihydroxy-compound identical with the 1:3-dihydroxy-2-methylanthraquinone prepared by Schunck and Marchlewski (Trans., 1894, 65, 182). It must, therefore, be represented by one of the following formulæ:



On heating with hydriodic acid it yields a dihydroxymethylanthranol, $C_{15}H_{12}O_3$ (m. p. 235°), and on methylation gives 1:3-dimethoxy-2-methylanthraquinone (m. p. 181°)—a compound which is also readily obtained from the 1:3-dihydroxy-2-methylanthraquinone prepared by Schunck and Marchlewski.

The monomethyl ether of alizarin present in "Ojuologbo" root was obtained in needles (m. p. 175°), and yielded an acetyl derivative melting at 209°. It was shown to be identical with the compound of this nature isolated by Perkin and Hummel (Trans., 1893, 63, 1174) from the root of *Oldenlandia umbellata* ("Chay root").

Alizarin was methylated by Schunck (Mem. Manchester Phil. Soc., 1873), also by Schunck and Marchlewski (loc. cit.), and more recently by Gräbe and Aders (Annalen, 1901, **318**, 369), but the product was, in all cases, a monomethyl ether melting at $228-229^{\circ}$. Since, as was shown by Kostanecki and Dreher (Ber., 1893, **26**, 76), the hydroxyl group in the monohydroxyxanthones will not undergo methylation when it is situated in the 1-position with respect to the carbonyl group, whereas it readily does so when in the 2-, 3-, or 4-position, it was suggested by Schunck and Marchlewski that the hydroxyanthraquinones might show a similar behaviour. They therefore concluded that the monomethylalizarin prepared by them was probably the 2-methoxy-compound. This opinion, which was also shared by Gräbe and Aders (loc. cit.), would lead to the conclusion that the monomethylalizarin occurring in Morinda longiflora and Oldenlandia umbellata possesses the following formula:



It would appear, however, that the hydroxyanthraquinones, on methylation, may not always exhibit a behaviour analogous to that of the hydroxyxanthones, as it has been shown in this investigation that 1:3-dihydroxy-2-methylanthraquinone readily undergoes complete methylation. It is impossible, therefore, from the above considerations to arrive at a safe conclusion regarding the position occupied by the methoxyl group in the above-described methyl ether of alizarin.

The extract obtained from the "Ojuologbo" leaves differed from that yielded by the root, inasmuch as it contained a considerable proportion VOL. XCI. 6 L

of material which was soluble in petroleum. This petroleum extract yielded palmitic acid, together with traces of acetic and formic acids, and a small amount of the hydrocarbon hentriacontane. The leaves were also found to contain some of the previously-mentioned hydroxy-methoxymethylanthraquinone (m. p. 290°), but their most interesting constituent is a new, crystalline *alcohol*, which it is proposed to designate *morindanol*. This substance possesses the formula $C_{38}H_{62}O_4$, melts at 278°, and has $[a]_D + 65 \cdot 9^\circ$. It yields *methylmorindanol*, $C_{38}H_{61}O_3 \cdot O \cdot CH_3$ (m. p. 116°), on treatment with sodium ethoxide and methyl iodide.

Morindanol is represented by the same general formula, $C_nH_{2n-14}O_4$, as three other crystalline alcohols which have recently been isolated in these laboratories, namely, lippianol, $C_{25}H_{36}O_4$ (m. p. $300-308^\circ$; $[a]_D + 64.9^\circ)$, obtained from *Lippia scaberrima*, Sonder (Power and Tutin, Arch. Pharm., 1907, 245, 344), and micromerol, $C_{33}H_{52}O_4$ (m. p. 277° ; $[a]_D + 57^\circ)$, and micromeritol, $C_{30}H_{46}O_4$ (m. p. $294-296^\circ$; $[a]_D + 61.4^\circ)$, both recently isolated by Drs. F. B. Power and A. H. Salway from *Micromeria Chamissonis*. These four substances, moreover, exhibit a marked similarity in properties, and it would therefore appear probable that they are chemically related. No similar compounds appear previously to have been described.

In order to ascertain whether "Ojuologbo" possesses any pronounced physiological action, the following experiments were conducted for us by Mr. H. H. Dale, Director of the Wellcome Physiological Research Laboratories, to whom our thanks are due. Amounts of the extracts corresponding respectively to 17 grams of the dried leaves and to 7.5 grams of the dried root were administered to small dogs, but no definite effects of any kind could be observed.

Experimental.

I. Examination of the Root.

For the purpose of this investigation, a quantity (10.9 kilograms) of the finely-ground root was completely extracted by continuous percolation with hot alcohol. The liquid thus obtained was freed from the greater part of the solvent, the resulting dark brown extract then mixed with water, and distilled with steam until the liquid flowing from the condenser was no longer acid. The distillate contained drops of a heavy, yellow oil possessing a peculiar odour. It was extracted with ether, and the ethereal liquid shaken with a solution of sodium carbonate. The deep red, alkaline liquid thus obtained yielded, on acidification and extraction with ether, a small quantity of a red oil which deposited crystals on standing. These, after being separated and recrystallised from alcohol, melted at $59-60^{\circ}$, and apparently consisted of palmitic acid.

The ethereal liquid which had been freed from acids by means of sodium carbonate yielded a very small amount of a yellow essential oil, whilst the aqueous portion of the distillate was found to contain formic, acetic, and butyric acids.

The contents of the distillation flask consisted of a quantity of resinous matter, and a dark-coloured, aqueous liquid. The latter was decanted from the resin, which was then washed several times with hot water.

Examination of the Resins.

The resin was boiled with several successive portions of alcohol, and the liquids filtered. In this way there were obtained a dark coloured solution, which deposited a small amount of a solid on standing, and a quantity of an amorphous, brown powder. The latter product was very sparingly soluble in alcohol or glacial acetic acid, and insoluble in the other usual solvents. Nothing crystalline could be separated from it, and, in view of the possibility of its being a glucoside, it was submitted to the action of dilute alcoholic hydrochloric acid, but with a negative result.

The solution of that portion of the resins which had been dissolved by treatment with alcohol was evaporated on purified sawdust, and extracted successively in a Soxhlet apparatus with light petroleum (b. p. $33-50^{\circ}$), ether, chloroform, ethyl acetate, and alcohol.

Petroleum Extract of the Resins.

This was small in amount. The greater portion of it was soluble in a solution of sodium carbonate, and a further quantity was dissolved by cold aqueous potassium hydroxide. Nothing crystalline was obtained from it.

Ethereal Extract of the Resins.

This weighed 35 grams. On attempting to redissolve it in ether, a portion did not readily pass into solution, and this was collected on a filter and independently examined. The ethereal filtrate, containing the more readily soluble constituents of the extract, was shaken with several successive portions of a solution of sodium carbonate, and the alkaline liquids thus obtained, which were all dark red, were examined separately.

Isolation of a Hydroxymethoxymethylanthraquinone, $C_{16}H_{12}O_4$.

On acidifying the first two sodium carbonate extracts, a viscid, oily substance separated, but the liquids from the subsequent shakings with this alkali yielded a precipitate of a dark yellow solid. This was dissolved in hot alcohol, from which, on cooling, it separated in brown crystals melting at about 280° As these crystals could not readily be purified, the entire amount of the substance was dissolved in acetic anhydride and the mixture boiled. The resulting *acetyl* derivative was crystallised from ethyl acetate, when it was obtained in deep yellow needles melting at 173° :

The portion of the ethereal extract of the resins which was only sparingly soluble in ether, and which was separated as described above, was found to consist of the substance which yielded this acetyl derivative, as on heating it with acetic anhydride the same product (m. p. 173°) was obtained.

The pure acetyl derivative was hydrolysed, and the product crystallised from absolute alcohol. It separated in golden-yellow needles melting at 290° :

No substance of the formula $C_{16}H_{12}O_4$ and possessing properties agreeing with those of this compound has hitherto been described. A methoxyl determination by means of Perkin's modification of Zeisel's method gave the following result:

The flask which had been employed in this estimation contained, together with the hydriodic acid, a quantity of a solid *substance*. This was collected, and crystallised from ethyl acetate, when it was obtained in dark yellow needles melting at 235° :

0.1030 gave 0.2802 CO₂ and 0.0446
$$H_2O$$
. C = 74.2; H = 4.8.
C₁₅ $H_{12}O_3$ requires C = 75.0; H = 5.0 per cent.

A further quantity of this substance (m. p. 235°) was prepared, and oxidised with chromic acid, when a compound was obtained which crystallised from ethyl acetate in deep golden leaflets melting at 290°. The latter substance was also produced, and in much better yield, on heating the original methoxy-derivative with 70 per cent. sulphuric acid. It was analysed with the following result:

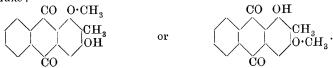
0.0885 gave 0.2290 CO_2 and 0.0326 H_2O . C = 70.6; H = 4.1.

 $C_{15}H_{10}O_4$ requires C = 70.9; H = 3.9 per cent.

This compound agrees in composition and properties with the 1:3-dihydroxy-2-methylanthraquinone prepared by Schunck and Marchlewski (Trans., 1894, **65**, 182), and is evidently identical with it. In order to confirm this conclusion the acetyl derivative was prepared, and was obtained in slender, pale yellow needles melting at 225° ,—the temperature recorded by Schunck and Marchlewski for the melting point of the acetyl derivative of the substance prepared by them :

0.1208 gave 0.2992 CO₂ and 0.0462 H₂O. C = 67.5; H = 4.2. C₁₅H₈O₄(CO·CH₃)₂ requires C = 67.5; H = 4.1 per cent.

The yellow substance melting at 290° , isolated from the root of *Morinda longiflora*, must, therefore, be a *hydroxymethoxymethylanthraquinone* possessing a constitution represented by one of the following formulæ:



The compound, $C_{15}H_{12}O_3$ (m. p. 235°), which was formed from this hydroxymethoxymethylanthraquinone by the action of hydriodic acid, and which yielded 1:3-dihydroxy-2-methylanthraquinone on oxidation, must, therefore, be a *dihydroxymethylanthranol*.

1:3-Dimethoxy-2-methylanthraquinone.—On solution in alcohol and treatment with sodium ethoxide and methyl iodide, the hydroxymethoxymethylanthraquinone yielded 1:3-dimethoxy-2-methylanthraquinone. This new compound is also readily produced in a similar manner from 1:3-dihydroxy-2-methylanthraquinone. It crystallises in golden-coloured needles melting at 181° :

0.1142 gave 0.3020 CO_2 and 0.0537 H_2O . C = 72.1; H = 5.2. $C_{17}H_{14}O_4$ requires C = 72.3; H = 5.0 per cent.

Isolation of a Monomethyl Ether of Alizarin.

The oily product which separated on acidifying the first two alkaline liquids obtained by shaking the ethereal solution of the readily soluble portion of the ether extract of the resins with sodium carbonate, as already described, deposited no solid on standing. It was, however, thought probable that it might contain some of the above-described hydroxymethoxymethylanthraquinone, or other phenolic substance, together with carboxylic acids. It was therefore heated with acetic anhydride, the product dissolved in ether, and the ethereal liquid shaken with a solution of sodium carbonate. Acidification of the dark-coloured alkaline liquid thus obtained yielded a viscid substance from which nothing crystalline could be separated, but on evaporating the ethereal solution containing the non-acidic, acetylated product an oily liquid was obtained which deposited a small quantity of a solid on standing. This was collected, and, after several crystallisations from ethyl acetate, was obtained in pale yellow needles melting sharply at 209°. It was hydrolysed with alcoholic potash, and the product crystallised from ethyl acetate, when it formed yellow needles melting at 175°. Unlike its acetyl derivative, this substance is freely soluble in alcohol.

On dissolving a trace of this compound in concentrated sulphuric acid a brownish-red colour was produced, which, on heating the liquid to 150° , changed to an intense red. It dissolved in a solution of barium hydroxide with a red colour, and on boiling the liquid for some time a violet-coloured precipitate was deposited. The substance melting at 175° was analysed with the following result:

0.0629 gave 0.1638 CO₂ and 0.0228 H₂O. C=71.0; H=4.0. C₁₅H₁₀O₄ requires C=70.9; H=3.9 per cent.

The composition and properties of this substance agree with those of the monomethyl ether of alizarin which was isolated by Perkin and Hummel (Trans., 1893, **63**, 1174) from the root of Oldenlandia umbellata, Linn. (" Chay root"),—a plant belonging to the same natural order (*Rubiaceæ*) as Morinda longiflora. Unfortunately, sufficient material was not available to permit of our making a methoxyl determination. The melting point found by Perkin and Hummel for the compound isolated by them is 3° higher than that found for our substance, but the 'acetyl derivative of each preparation melts at 209°. No doubt can therefore be entertained regarding the identity of the above-described substance, $C_{15}H_{10}O_4$, with the monomethyl ether of alizarin obtained from "Chay root."

The oily liquid, from which the acetyl derivative of the monomethyl ether of alizarin had been separated, was hydrolysed. The product yielded a further quantity of the hydroxymethoxymethylanthraquinone, but no other solid substance could be obtained from it.

After the ethereal solution of the readily soluble portion of the ether extract of the resins had been treated with a solution of sodium carbonate, as already described, it was shaken with a dilute solution of sodium hydroxide. The product which separated on acidifying the alkaline liquid thus obtained was only small in amount, and nothing crystalline could be obtained from it. The ethereal liquid, which was now free from substances of an acidic or phenolic nature, was evaporated, when a product was obtained which deposited a solid on standing. This was several times crystallised from alcohol, and obtained in colourless leaflets melting at 130° . On treatment with acetic anhydride and sulphuric acid, it gave the colour reaction characteristic of the phytosterols. After drying at 110° it was analysed:

0.0828 gave 0.2533 $\rm CO_2$ and 0.0889 $\rm H_2O.~~C=83.4$; H=11.9,

 $C_{27}H_{46}O$ requires C = 83.9; H = 11.9 per cent.

Chloroform, Ethyl Acetate, and Alcohol Extracts of the Resins.

The chloroform extract of the resins was small in amount, and yielded only a little of the above-described hydroxymethoxymethylanthraquinone.

The portion of the resins which was extracted by ethyl acetate amounted to 22 grams. Various attempts were made to obtain a crystalline substance from it, but without success.

The remainder of the resins, which was soluble only in alcohol, was the largest extract obtained, and amounted to 90 grams. Although submitted to a prolonged investigation, it yielded nothing crystalline.

Examination of the Aqueous Liquid.

The combined aqueous liquid and washings, which had been separated from the resins, as previously described, were filtered, and extracted five times with ether. The ethereal liquid was then shaken with six successive small portions of a solution of sodium carbonate. On acidifying the first alkaline liquid thus obtained, a dark red, oily product was precipitated, which did not solidify, but the subsequent extracts gave a yellow solid, which was found to consist of the hydroxymethoxymethylanthraquinone previously isolated from the resins.

The aqueous liquid which had been extracted with ether was treated with a solution of barium acetate until no further precipitate was formed, then filtered, and the precipitate washed. This precipitate was examined, but nothing crystalline was obtained from it. A slight excess of a solution of basic lead acetate was added to the filtrate from the barium precipitate, the resulting insoluble lead compounds collected, washed, suspended in water, decomposed by means of hydrogen sulphide, and the liquid filtered. The filtrate was concentrated, mixed with purified sawdust, and dried, after which it was extracted with various solvents. From the portion removed by means of ether, a solid was obtained which, after crystallisation from ethyl acetate, melted at 151--- 152° and gave the reactions of anhydrous citric acid.

The filtrate from the lead acetate precipitate was freed from lead, and concentrated. It was found to contain a large amount of a hexose, as it readily yielded *d*-phenylglucosazone melting at 216° .

II. Examination of the Leaves.

The ground leaves (5.8 kilograms) were completely extracted by continuous percolation with hot alcohol, the liquid concentrated, and the residual dark green extract mixed with water and distilled with steam. The distillate, on extraction with ether, gave a small amount of an essential oil possessing an unpleasant odour, and the aqueous liquid from which this had been removed was found to contain formic, acetic, and butyric acids.

The distillation flask then contained a quantity (135 grams) of dark green resin, which was separated from the aqueous liquid and well washed with hot water.

Examination of the Resins.

The resinous mass was dissolved in alcohol, the solution mixed with purified sawdust, and the mixture dried, after which it was extracted successively in a Soxhlet apparatus with light petroleum (b. p. $33-50^{\circ}$), ether, chloroform, ethyl acetate, and alcohol.

Petroleum Extract of the Resins.

The petroleum extract amounted to 59 grams. It was dissolved in ether and the resulting liquid extracted successively with solutions of sodium carbonate and potassium hydroxide. The products obtained by this treatment were small, and yielded nothing definite, although the liquid obtained on shaking with the latter alkali had a violet colour.

The ethereal solution was then evaporated, the residue dissolved in an alcoholic solution of potassium hydroxide, and the mixture heated for two hours. The greater part of the alcohol was then removed, water added, and the alkaline liquid extracted with ether. The ethereal liquid thus obtained yielded, on evaporation, an oily product, which was distilled under diminished pressure. The distillate deposited a solid on standing which, when crystallised from ethyl acetate, formed leaflets melting at 68° :

0.0816 gave 0.2551 CO_2 and 0.1085 H_2O . C = 85.3; H = 14.8.

 $C_{31}H_{64}$ requires C = 85.3; H = 14.7 per cent.

This substance was therefore the hydrocarbon hentriacontane.

The oily liquid from which this hydrocarbon had been separated was found to contain oxygenated substances, probably alcohols, but it gave no reaction for phytosterol.

The alkaline liquid from which the hydrocarbon had been removed by means of ether was acidified, and distilled with steam. This removed a little acetic and formic acids. The contents of the distillation flask were then shaken with ether, the ethereal liquid separated, concentrated to a small bulk, and a considerable volume of light petroleum added. This caused the precipitation of a quantity of green resin, from which the petroleum liquid was decanted. The latter was then evaporated and the residue distilled under diminished pressure. The distillate, which solidified on cooling, was crystallised from methyl alcohol, when it yielded a product melting at 60° :

0.1053 gave 0.2906 CO_2 and 0.1152 H_2O . C = 75.3; H = 12.2.

 $C_{16}H_{32}O_2$ requires C = 75.0; H = 12.5 per cent.

This, therefore, consisted largely of palmitic acid.

Ethereal Extract of the Resins.

This was a soft, green mass weighing 57 grams. On treating with a limited amount of ether, a quantity of a green solid remained undissolved, and was collected on a filter. The ethereal filtrate was then shaken with a solution of sodium carbonate. The alkaline liquid thus obtained yielded, on acidification, a product which appeared to consist, to a considerable extent, of the hydroxymethoxymethylanthraquinone previously isolated from the root, but it could not be purified. After extracting the ethereal liquid with sodium carbonate solution, it was washed with water, when a large quantity of chlorophyll was removed, and subsequent extraction with potassium hydroxide yielded a further quantity of a similar, uncrystallisable product. The ethereal liquid finally left on evaporation only a soft, green resin.

Isolation of a New, Crystalline Alcohol, Morindanol, C₃₈H₆₁O₃·OH,H₂O.

The above-mentioned sparingly soluble solid was dissolved in a considerable quantity of hot alcohol and the solution digested with animal charcoal, by which means the greater part of the green colour was removed. On concentrating the solution, an amorphous, slightly green product was obtained, which melted from 240° to 270° . This was again dissolved and treated with animal charcoal, which removed the last traces of colour, when, after repeated fractionations from alcohol, the greater part of it was obtained in colourless, glistening needles melting at 278° . The substance, after drying at 100° , was analysed: 0.1077 gave 0.3088 CO₂ and 0.1041 H₂O. C = 78.2; H = 10.7. C₈₈H₆₂O₄ requires C = 78.3; H = 10.7 per cent.

The air-dried substance gave the following result :

0.1025 gave 0.2852 CO_2 and 0.0978 H_2O . C = 75.9; H = 10.6.

 $C_{38}H_{62}O_4, H_2O$ requires C = 76.0; H = 10.7 per cent.

0.4808, dissolved in 100 c.c. of absolute alcohol, gave $a_{\rm D} + 0^{\circ}38'$ in a 2-dcm. tube, whence $\lceil a \rceil_{\rm D} + 65.9^{\circ}$.

The composition and properties of this substance do not agree with those of any compound previously described, and, being of an alcoholic nature, it is proposed to designate it *morindanol*.

If a small quantity of morindanol is dissolved in alcohol and ammonia added, no precipitate is produced on diluting the solution with water, even if the greater part of the alcohol has previously been evaporated. The clear liquid thus obtained seems to have the character of a colloidal solution, as no morindanol will separate from it until an aqueous solution of an electrolyte is added, and, on allowing it to stand, it forms a perfectly transparent jelly.

Methylmorindanol, $C_{38}H_{61}O_3 \cdot O \cdot CH_3$.—A quantity of morindanol was dissolved in alcohol, sodium ethoxide and methyl iodide added, and the mixture heated for three hours. The product was then dissolved in ether and freed from unchanged material by means of potassium hydroxide. The methylmorindanol was crystallised from alcohol, when it formed rosettes of needles which melted at 116°. This substance contained no solvent of crystallisation. A methoxyl determination gave the following result:

0.1034 gave 0.0405 AgI. CH₃·O = 5.2.

 $C_{38}H_{61}O_3 \cdot O \cdot CH_3$ requires $CH_3 \cdot O = 5 \cdot 2$ per cent.

Chlcroform, Ethyl Acetate, and Alcohol Extracts of the Resins.— These were all small in amount, and nothing crystalline could be obtained from any of them.

Examination of the Aqueous Liquid.

The aqueous liquid obtained from the extract of the leaves, as previously described, was examined in a manner similar to that recorded in connexion with the corresponding product from the root, with the exception that the treatment with barium acetate was omitted. A further quantity of the hydroxymethoxymethylanthraquinone (m. p. 290°) was obtained, and the aqueous liquid, after purification by means of basic lead acetate, yielded *d*-phenylglucosazone melting at 215°. No citric acid could be obtained from the leaves.

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