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REPORT
of
COMMITTEE ON EXAMINATION

This is to certify that we the undersigned, as a Committee of the Graduate School, have given Arthur Rosson Cade final oral examination for the degree of Master of Science. We recommend that the degree of Master of Science be conferred upon the candidate.

Minneapolis, Minnesota

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Geo B Frankforter
Chairman
H. A. Hunt

Earl Brown for G. H. Hirschfelder

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REPORT
of
Committee on Thesis

The undersigned, acting as a Committee of the Graduate School, have read the accompanying thesis submitted by Arthur Rosson Cade for the degree of Master of Science.

They approve it as a thesis meeting the requirements of the Graduate School of the University of Minnesota, and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of ~~ARTS~~ Science

Geo B Frankforter
Chairman

H. A. Hunt

Ed. Brown for G. S. H. H. H. H. H.

Contributions to Our Knowledge of
Carminic Acid

A thesis submitted to the
Faculty of the Graduate School of the
University of Minnesota

by

Arthur E. Cade

In partial fulfilment of the requirements
for the degree of
Master of Science

JUNE

1917

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Acknowledgement

Acknowledgement is due to Dr. Wulf Kritchevsky under whose guidance this work was begun, and to Dean George B: Frankforter under whom it was later carried to completion, for the kindly interest shown and the valuable assistance rendered in the course of this work.

Introduction

For centuries the natural dyestuff, Cochineal, has been known and used as a source of red coloring matter for medicines, food-stuffs and the like, but with over one hundred years of experimental work upon this substance scientists are yet at a loss to know what is the composition of the actual coloring matter present-which they have named Carminic Acid.

Much extensive reasearch has been done upon this subject and many varied theories have been expounded. It is with the idea of checking up some of the previous work and to work out some new ideas along this line that this thesis subject was taken up.

Cochineal

Cochineal, of which carminic acid constitutes about ten percent, is one of the few coloring matters of importance which are of animal origin. Cochineal is the female of the *Coccus Cacti*, an insect which feeds upon various species of cacti. It is collected largely in Mexico, Guatemala, the Canary Islands and Java. The insects, which have no wings, are merely brushed off the plants and killed by boiling. They are then dried and ground, being put on the market in this condition, having gone thru no other method of preparation. The bugs are dark red brown in color and in size resemble a lady bug.

It is thot by some that most of the carminic acid occurs in the eggs of the insect as a glucoside, but this fact is yet to be more satisfactorily proven. This glucoside then, upon hydrolysis, is supposed to produce a sugar and carminic acid.

Since the introduction of coal tar colors the consumption of

cochineal has greatly diminished, it being used only in a minor degree in the arts at the present day.

Carminic Acid

Carminic acid is a bright crimson red (turning to dark brown upon standing) powder, appearing both in the amorphous and in the crystalline state. It is readily soluble in water and in alcohol, less soluble in chloroform and benzene, insoluble in carbon tetrachloride and ether. In alkalies it is readily soluble to a dark blue color, which can be brought back to red by the addition of acid (except in the case of ammonia). It is soluble in mineral acids, being decomposed by them if boiled in concentrated solutions.

Carminic acid has no definite melting point. It darkens at 130°-135° and blackens with decomposition at about 190°-200°.

Historical

Carminic acid was first isolated by Pelletier and Caventou¹ about the year 1800. They named it carmin and further investigated the composition of cochineal.

In 1832 Pelletier analysed carminic acid and found it to be of the composition $C_{16}H_{13}NO_{10}$, which however is incorrect as there is no nitrogen in the molecule. The author apparently used impure material, as the cochineal itself contains a considerable amount of nitrogenous matter.

The acid and cochineal were subsequently examined by Preisser² in 1844 and by Arpee³ in 1845, but no definite work of importance was accomplished by either of them.

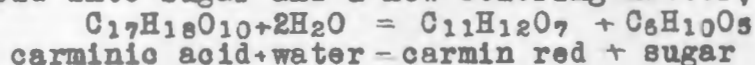
Warren de la Rue⁴ in 1847 was the first man to isolate the acid in the pure form, and he described it as a purple brown mass, easily soluble in water and alcohol, but not in ether. By analysis he found the acid to contain C 54.13, H 4.62, O 41.25. Thus from these figures he deduced the formula $(C_{14}H_7O_8)_2$.

Carminic acid was not obtained in the crystalline form by the previous authors. Schutzenberger⁵, in 1858, being the first man to accomplish this. He claims to have found two products in his purified substance, namely, - Carminic acid, $C_9H_8O_8$ and oxy carminic acid, $C_9H_8O_6$. The latter being soluble in ether, thus a means of separating the two.

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1. Ann. Chim. Phys. (2) 8, 250
 2. Ann. 52, 375 JI. Pharm. Chim. (3) 5, 191
 3. Ann 55, 101
 4. Ann. 64, 1 Jahresber. 1847, 788 JI. Pharm. Chim. (3) 13, 386
 5. Jahresber. 1858, 462 Ann. Chim. Phys. (3) 54, 52
Comp. Rend. 46, 47 JI. Prak. Chem: 74, 444
 6. Bull. Soc. Chim. (2) 2, 414 Jahresber. 1864, 410

Schaller¹ in 1864 prepared the acid and gave the substance the formula $C_9H_{10}O_6$, which differs from the previous formula by one molecule of water. He believed the acid to be a dibasic one, forming salts of the type $C_9H_8O_5M_2$ or $C_9H_8O_5ME$.

In 1867 Hlaziwetz and Grabowski² indicated that carminic acid was a glucoside, which could be decomposed by hydrolysis with dilute acid into sugar and a new coloring matter, carmin red.



Lieberman and Van Dorp³ in 1871 worked upon the action of carminic acid and nitric acid, obtaining the same substance that De la Rue obtained, namely, nitrococcus acid. They found that carminic acid when treated with concentrated sulphuric acid formed a compound which they called ruficoccin, and which had properties and actions similar to rufigallic acid. They believed carminic acid to be a derivative of dimethyl anthracene.

The next man to work upon carminic acid was Furth⁴ in 1883. He obtained ruficoccin by carminic acid and sulphuric, and by the action of zinc dust upon this compound he obtained a hydrocarbon similar to the one obtained from coccinin (to be described later). This hydrocarbon upon analysis was found to be $C_{16}H_{12}$, so Furth concluded that the previous formulae of C_{14} etc must be changed to C_{16} . He gave as his formula, $C_{16}H_{14}O_6$.

In 1886 Will and Leyman⁵ did extensive researching upon the acid and from their results concluded that the coloring matter was not an anthracene derivative, and that such substances as ruficoccin, coccinin, coccinone (anthracene derivatives) have been

1. See reference 6 on previous page.

2. Ann. 141, 329

3. J.C.S. Trans. 1871, 910 Ber. 4, 655

4. Ber. 16, 2159

5. Ber. 18, 3180

obtained by energetic reactions which may have led to a modification in the molecule. These authors did most of their work upon bromine derivatives of carminic acid.

Miller and Rhode¹ were the next authors to take up this subject. In 1893 they proved carminic acid not to be a glucoside and claimed that the acid was not altered by boiling with dilute sulphuric. They too worked upon the bromine derivatives of the acid and concluded from their investigations that carminic acid was a naphthaquinone derivative, being related to one of the two hydroxy derivatives of methyl alpha naphthaquinone. Their analysis of the acid checked quite well with those of De la Rue and they gave as their formula for the acid $C_{22}H_{20}O_{13}$.

Further work by these same authors in 1897² showed the compound not to be a hexa hydroxy alpha methyl naphthaquinone, owing to the recognition of a carboxyl group.

Schunk and Marchlewski³ who worked in 1894 investigated Will and Leyman's bromine compounds and concluded that carminic acid was a di oxy methyl alpha naphthaquinone, by they were uncertain as to the amount of water of crystallization present in the molecule. They gave as their formula $C_{11}H_{12}O_6$ or $C_{11}H_8O_4 \cdot 2H_2O$.

The next authors to make carminic acid the source of their study were Lieberman and Voswinckle⁴ in 1897. They studied chiefly the oxidation products.

Carl Lieberman⁵ in 1898 beleived that carminic acid was an indene derivative. He found the acid to be soluble in water, but

1. Ber. 26, 2647

2. Ber. 1897, 1759

3. Ber. 27, 2979

4. Ber. 1897, 688

5. Ber. 31, 2079

Ber 30, 1731

J.C.S. Trans. 1911, 1712

upon heating that it became insoluble with the elimination of water, this change being characteristic of keto indene derivatives.

Some small amount of work upon cochenillic acid, a decomposition product of the acid by oxidation, was done by Landau¹ in 1900.

Lieberman² again in 1900, working in conjunction with Horing and Wiederman found the formula of the acid to be $C_{22}H_{22}O_{13}$, which formula seems to be the correct one. These men prepared many salts and put the acid thru an elaborate process of purification before analysing it.

Perkin and Wilson³ in 1903 made an exhaustive study of the reactions of phenol coloring matters and studied carminic acid in this connection, assuming it to be an anthracene derivative containing hydroxyl groups. They found the action of the acid to be quite similiar to that of alizarin and purpurin.

In 1904 Lieberman and Voswinckle⁴ did a little more work on this subject, as did Lieberman and Lieberman⁵ in 1909.

Otto Dimroth⁶ published a paper in 1909 on some oxidation work on carminic acid with the results obtained. This work has thrown quite a bit of light upon this subject. From his investigations he concluded the acid to be a naphthaquinone derivative. By oxidizing the acid with $KMnO_4$ and sulphuric acid in an aqueous solution, then heating for an hour at 90° CO_2 came off and a substance called carminazarin was formed. Upon further oxidation in acid solution at 70° (5) -- see formulae on next page-- was formed. This when heated with H_2SO_4 eliminated CO_2 and formed cochenillic acid.

1. Ber. 33, 2442

2. Ber. 33, 149

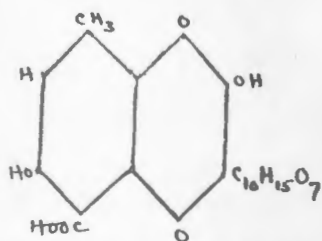
3. J.C.S. Trans. 1903, 139

4. Ber. 37, 3344

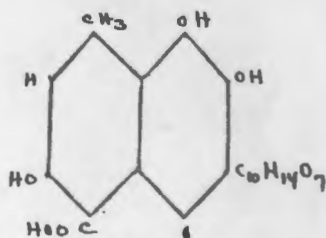
5. Ber. 42, 1922

6. Ber. 42, 1611 J.C.S. 1909, 485

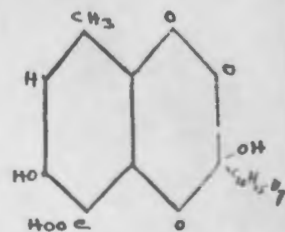
(1)



(1a)

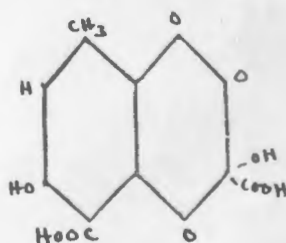


(2)



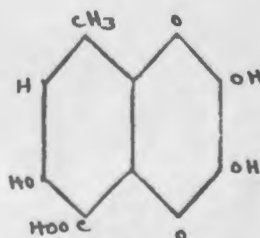
Keto form--Carminic Acid--Enol form.

(3)



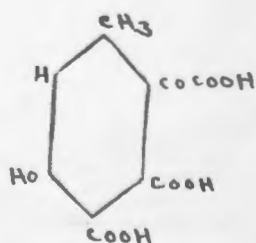
Intermediate oxidation product
Carminoquinone
(not isolated)

(4)



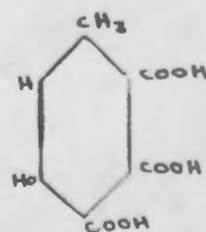
Carminazarin

(5)



Intermediate step to

(6)



Cochenillic Acid

As an intermediate step between the carminic acid and the carminazarin Dimroth found a substance, carminoquinone to which he gave the formula (3) as shown on previous page. Lieberman however thought that that compound was an indene derivative which readily passed into carminazarin as shown on previous page also.

Furthermore Dimroth found that carminic acid with MnO_2 in acetic gave carminoquinone, which when oxidized with $KMnO_4$ gave carminazarin.

The most valuable and complete work upon cochineal coloring matter was done by Dimroth¹ in 1913, when he continued his work upon oxidations with the hopes of getting closer to the molecule. In this work he obtained, by oxidation and thru caustic fusions, two substances, namely, - coccinin and coccinone, which are anthraquinone derivatives. Thus his idea of the acid molecule was changed from the naphthaquinone one to an anthraquinone derivative.

Lieberman and Lieberman² published a second paper in 1914 disputing Dimroth's anthraquinone theory, claiming the anthracene ring if found present was probably formed by energetic reactions in the process, probably condensations being brought about.

The most recent work has been done by Dimroth in 1916 but the original article was published in the Berichte and is not available.

1. Ber. 1913, 1
2. Ber. 47, 1213

Experimental Part

To add onto carminic acid various radicals or groups and try to form new compounds which might be identified with some known products seems quite impractical, for the acid molecule evidently is a large somewhat heavily laden one.

It seems therefore that better results are obtainable by breaking down the acid into its various constituents-identifying these decomposition products and then trying to build up these known substances again into the carminic acid.

To split the compound gradually seems to be very difficult. In nearly every case the action seems to be to the extent that all is broken down to the initial benzene ring.

By studying the oxidation, nitration, reduction, halogenation, addition products, salts etc of carminic acid and their decomposition products; then comparing the resulting products with corresponding products of compounds of known constitution, it was hoped that much insight into the constitution of the carminic acid molecule might be gained.

Before working upon the constitution of the acid it was first prepared and purified, as will be explained later, starting with crude cochineal as the initial product.

Nitrations

To begin with attempts were made to nitrate the acid with the hope of after nitrating that the acid could be reduced to an amine. It could then be diazotized and coupled with other compounds perhaps and a new series of dyes obtained. At the same time this would give an opportunity to load down the end ring and allow the other two rings to split off together, possibly.

Attempts to form the desired nitro compound failed. All the nitrations producing the same effect, ie,- instead of forming the desired nitro compound the ring was split and broken down forming the known nitro coccussic acid,- a white crystalline substance melting at 106°.

As nitrating agents concentrated nitric in glacial acetic, concentrated nitric in concentrated sulphuric, and benzoyl nitrate (made by the action of benzoyl chloride on silver nitrate under anhydrous conditions) were used.

Oxidations

The most important work, that from which the greatest knowledge concerning the composition of the acid is obtained, has been done upon oxidations. Many previous authors, as well, have devoted much time to oxidations, both strong and mild.

Such oxidation agents as $K_2S_2O_8$, MnO_2 , $KMnO_4$, Air, PbO_2 , H_2O_2 , HNO_3 , caustic fusions, and $Ba(MnO_4)_2$ -- in acetic acid, in water, and in sulphuric acid-- have been used with very varying results,

All strong oxidations break the original substance to a single ring as in the case of nitrations. Therefore attempts have been made with weak oxidizers, in order to oxidize the acid, if possible, without breaking the ring; thereby discovering the composition of the side chain which seems to be present in the last ring of the molecule and which is lost in almost all of the reactions upon the acid.

Carminic acid when treated with potassium persulphate gave sharp needles of a sparingly soluble acid, while the filtrate from this contained another acid which was precipitated with lead acetate as the lead salt. When treated with nitric acid both of these

formed the nitro coccussic acid previous explained. These results are quite in accord to those found by Lieberman and Voswinckle.

An attempt was made to oxidize the acid by allowing air (previously dried by passing thru concentrated sulphuric acid and then thru caustic soda) to pass thru an alkaline solution of it for several days. However upon acidifying the solution at the end of a couple of weeks, the red color of the carminic acid was noticeable and upon evaporation the acid was reobtained.

Another attempt to subject carminic acid to mild oxidation was carried out as follows,- 3 gms. of carminic acid were suspended in 500 cc. of benzene. This was shaken up with 30 gms. of PbO_2 for an hour. Then 25 gms. more of PbO_2 added and the entire mixture refluxed for an hour. After cooling the mass was extracted with ether (ie. after being filtered from the benzene) and then refluxed for two hours with more PbO_2 and the benzene. This mass was again extracted as above,. The two ether extractions were then mixed and evaporated, with the hope of obtaining some quinone compound therein. Upon evaporation however too small amount of anything was obtained to warrant further investigation.

Acids and Bases

Upon treatment with acids and alkalies carminic acid goes thru various changes depending upon the amount of reagents added and the strength of the reagents, as well as the temperature at which the reaction is carried out.

If carminic acid is treated with concentrated sulphuric in H_2SO_4 the cold apparently no reaction takes place. If, however, the mixture is heated to 140° , a new compound is formed. This compound, obtained by taking 5 gms of the acid and 30 cc. of water together with 30 cc. of concentrated sulphuric and boiling for 15 minutes,

was dark red in color. It was recrystallized from alcohol and melted at 107°. It corresponded very well to Dimroth's ruficoccin, to which he gave the formula $C_{16}H_{10}O_6$. It would not dissolve in hot water, but went into solution quite readily in benzene, ether, chloroform and the alkalies.

The actions of carminic acid with dilute alkalies will be found later under the heading of "Salts of Carminic Acid".
NaOH
Caustic fusions on the acid have quite a different effect than aqueous solutions of the same reagents have. Work upon this was not carried out, but works by Hlasiwetz and Grabowski and by Dimroth show two substances, - coccinin and its oxidation product coccinone to be formed thereby.

Salts of Carminic Acid.

The fact that carminic acid contains hydroxyl and carboxyl groups makes it possible for it to form many salts.

The alkalies and the alkaline earths as well as the heavy metals form salts with the acid; while several amines have been found to form additive products with it.

Altho not completely carried out yet, it appears also that condensation products with compounds such as hydroxyl amine, phenyl hydrazine and hydrazine are formed. This is due probably to the presence of two ketone (carbonyl) groups.

Sodium and potassium hydroxides appear to form two salts, depending upon the amount of the reagent added, the first being lighter in color than the latter.

Repeating some work done by Perkin and Wilson it was found that if an insufficient amount of potassium acetate was added to carminic acid (i.e. when one molecule was added drop by drop to one molecule of the acid in a boiling solution) a red precipitate

However, when two molecules were added a violet precipitate was formed. As these salts agreed in appearance and actions with those found by the previous mentioned authors they were not further analysed, but were assumed to be the mono and di K salts of carminic acid-- $C_{22}H_{23}O_{12}K$ and $C_{22}H_{20}O_{12}K_2$

$BaCl_2$ added to an aqueous solution of the acid formed a violet black precipitate as did $CaCl_2$.

Lead, silver and copper salts when added to carminic acid threw down heavy precipitates of hard dark insoluble salts. An analysis of the lead salt was made, which will appear later on.

Ammonium hydroxide seems to have a somewhat different reaction from that of the alkalies. A small amount of the acid was dissolved in concentrated ammonia and the same kept in a closed bottle-in the dark -- for four months. The color changed from red to violet immediately and remained the same. However upon the addition of acid the red color of the carminic acid did not return, as it does in the case of the other alkalies. The properties of the acid are apparently changed somewhat and it is probable that a new compound--perhaps an amine-- is formed. This new compound was soluble in water and in alcohol, but was not obtained in large enough quantity to purify and analyse.

The following additive products of the acid were also prepared, but as there appeared to be nothing in particular to gain by analysing them, nothing more than preparing them was done.

Several amines, of one, two and double benzene rings were coupled with the acid and precipitates were formed. Early authors who prepared the anilin and quinolin salts of carminic acid thought that they were condensation products, i.e. that a molecule of water was split out where the amine joined the acid ring. However recent

work upon the action of amines forming additive salts with acids makes the former idea quite improbable and that the additive salt formation the most plausible explanation.

When 3 gms. of the acid were dissolved in a small amount of water and a few cc. of anilin added, the solution thoroly mixed and allowed to stand several hours, crimson brown crystals separated out.

When 3 gms. of acid were dissolved in water and a solution of benzidine was added an immediate precipitate appeared.

When 3 gms. of the acid were dissolved in water and a slightly acid solution of alpha naphthol amine was added, solution thoroly stirred and allowed to stand, red brown crystal appeared. More came out upon evaporation.

Nitrous acid formed a brown precipitate with carminic acid, when 3 gms. of the acid dissolved in water was treated with NaNO_2 and a few drops of HCl . An evolution of gas (NO_2 , probably from the excess of nitrite present) appeared at the same time.

An attempt to dehydrate carminic acid was carried out as follows. Some of the dry acid was placed on a watch glass in an air bath at 160° for about one hour. At the end of that time the color had changed to red brown and upon boiling this with water, the red color of the acid could not be obtained. In fact the substance was practically insoluble in water.

Carboxyl Determination

That at least one carboxyl group is present in the carminic acid molecule is evident by the fact that CO_2 can be eliminated from the molecule by heating. In order to find out the percent of COOH present, thus the number of carboxyls in the molecule, a special apparatus devised by Dr. W.H.Hunter and J.D.Edwards¹ was made use of.

The method for determining the carboxyls present depends upon the fact that organic acids disengage hydrogen sulphide from potassium sulphhydrate solutions of 10th. normal strength, stable solutions of which may be made by saturating the KOH solution with H_2S (generated by dropping H_2SO_4 upon a solution of NaOH which had been saturated with H_2S directly from Kipp generator) and keeping an excess of this gas in contact with the liquid. When the gas was evolved by the dropping in of the sample an equal quantity of air was expelled from the apparatus and led into a eudiometer, where it was measured.

From the volume of the gas expelled the weight of COOH present was calculated by the use of the following formula

$$\text{Wt. COOH} = \frac{V(b-w).000002632/}{1 \quad 0.00367 \quad t}$$

where V is the volume of gas evolved
 b is the barometric pressure
 w is the vapor pressure of water at temperature t
 t is the temperature of the apparatus

In order to be more certain of the results of the COOH determination in carminic acid, a test was first run upon maleic acid, which is known to contain 2 COOH radicals. Then the run was made upon the carminic acid, followed by a test upon alizarin, which is known to contain no COOH groups and which contains OH groups.

1. J.A.C.S. 35, 452

As long as carminic acid contains several OH groups and as it is quite similiar in many ways to alizarin, a test upon alizarin was deemed necessary; for a blank test with the latter substance would show a positive result upon carminic acid to be correct, and that the hydroxyl groups were not interfering.

The analyses gave the following results,-

Temp. 22.4°
Bar. 744 mm.

Maleic acid

----- A sample of .1105 gms. gave 48 cc of gas, which by the use of the previous formula shows 75.9 % COOH. Maleic acid is known to contain 77.28 % COOH.

Carminic Acid

----- A sample of .0528 gms. gave 2.9 cc of gas, which shows the percent of COOH to be 9.5%.

A sample of .0355 gms. gave 2 cc. of gas, which shows the percent of be 9.8 % of COOH.

If carminic acid had one ^{carb}hydroxyl present the percent would be 9.1%. The above results are a little bit high, probably due to inexperience with the apparatus, which was new to the author. However the results are close enough to make certain that one and only one COOH is present in the carminic acid molecule.

Alizarin

----- Two samples of alizarin failed to give off any volume of gas, which shows that the OH groups did not effect the results in the above analyses.

Lead Salt Determination

An analysis of the lead salt of carminic acid was determined in order to find the amount of lead present, from which to draw conclusions as to the hydroxyl and carboxyl groups present.

The lead salt was prepared by adding a solution of lead acetate to a water solution of the acid, filtering off the dark purple precipitate and drying it at 100°.

The sample was then placed in a large porcelain crucible, treated with an excess of concentrated H₂SO₄ and very cautiously heated in the covered crucible over a free flame, until all of the acid was expelled. The crucible was then gently ignited until the residue was white, allowed to cool and weighed with the following results:

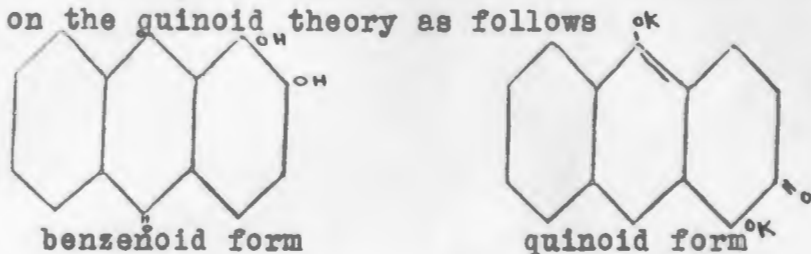
	No. 1	No. 2
Wt. sample and crucible	16.4998gm	16.9462gm
Wt. crucible	15.8196	16.2269
-----	-----	-----
Wt. sample	0.6802gm	0.7193gm
Wt. crucible and PbSO ₄	16.2470gm	16.6909gm
Wt. "	15.8196	16.2269
-----	-----	-----
Wt. PbSO ₄	0.4274gm	0.4640gm.
Wt. PbSO ₄ 303	0.4274	0.4640
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Wt. Pb 207	X	Y

X/0.6802 42.90 % Pb. Y/0.7193 44.08 % Pb.

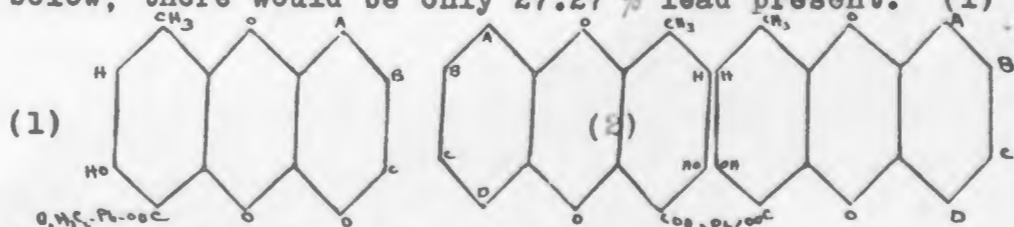
Average 43.49 % Pb.

The fact that the alkalie salts of carminic acid are very similar to those of alizarin, forming a mono and a di salt with one and two molecules of the base respectively; as well as the fact

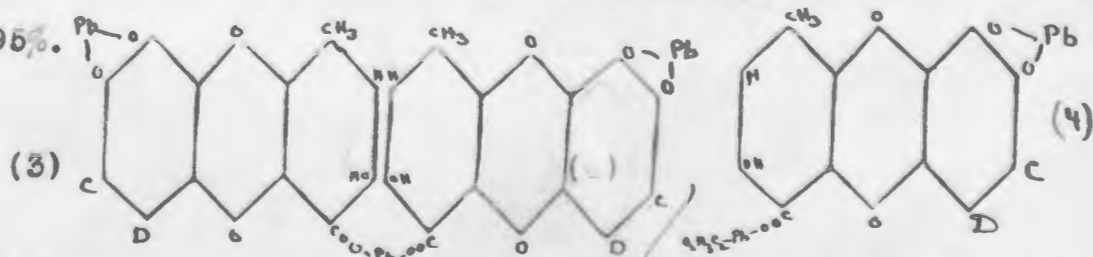
that carminic acid has dyeing properties similar to alizarin leads to the belief that the acid has two hydroxyl groups in the adjacent position in the molecule; thus explaining the dyeing property on the quinoid theory as follows



From the lead salt analysis, where it was found that the salt contained 43.49 % of lead, this adjacent hydroxyl idea is again verified; for if the lead combined with one molecule of the acid as below, there would be only 27.27 % lead present. (1)



If it combined with two molecules as in (2) above, then the percent of lead would be 17.35%. If the lead combined with two molecules, both in the carboxyls and in the adjacent hydroxyls as in figure (2) the lead would be present as 38.74 %. However if the combination of the acid and the lead was as (4), which seems to be the most likely combination, the percentage of lead would be 42.95%.



This checks very closely to one of my results (42.90%) and close enough to the average of the two results to satisfy the theory that there are two hydroxyls present adjacent to each other.

The lead salt is no longer a dye, and this is evident if we keep

to this reasoning for the quinoid possibility has disappeared by the lead going into the two hydroxyl groups. If it did not go in there, but only in the COOH group, then the quinoid possibility would still remain; and the lead salt should have dyeing properties, as long as the acid has dyeing properties before the lead is introduced into the molecule.

Preparation and Purification

To prepare the carminic acid from the crude cochineal several processes were tried, among which were,-

A. Cochineal extracted with ether and the residue exhausted with successive portions of boiling alcohol. On cooling, small crystals of the acid separated out, more being obtained by the evaporation of the solution and allowing it to cool. The acid was then freed of adhering fatty matter by dissolving it in alcohol and adding an equal quantity of ether. Pure carminic acid deposited after a couple days standing.

B. Cochineal extracted with hot water and precipitated the filtrate by the addition of lead acetate. The lead salt was suspended in water and decomposed by H_2S . The liberated acid was obtained by filtering and evaporating the filtrate at a low temperature in a vacuum. It was further purified by dissolving in the least possible amount of alcohol and adding an equal amount of ether.

C. The third process, which will be given in detail, proved more satisfactory than either of the two above so was used in the manufacturing of the carminic acid which was used in this work.

Much trouble was encountered with it at first, a great deal of which probably would have been eliminated by the use of a filter-press. Various methods of filtering, using different kinds of filters and at different temperatures, were tried in order to prevent, as much^h as possible, the fine gritty matter from coming thru the filter; as well as to prevent the gelatinous colloidal matter from clogging the filters.

Mexican cochineal was used as the initial product, from which the carminic acid was extracted by means of hot water as a solvent.

Three preliminary methods of extraction were used in order to find which gave the best results; namely,-

1. Extraction in a Soxhlet apparatus.
2. Extraction in a vacuum, in order to prevent oxidation by the air.
3. Extraction in an open vessel exposed to the air.

The latter method gave the most satisfactory results so this process was carried out as follows;

100 grams of cochineal in 8 liters of water were placed in a large evaporating dish and heated up to 70°-80°, being kept there for about 20 minutes with occasional stirring. The solution was then allowed to settle for about 1/2 hour and filtered by decantation, warm. The remaining residue was extracted twice more as above with the quantity of water diminished by one half each time. The residue was then discarded as a fourth extraction showed by the faintness of the color that practically all of the soluble carminic acid had been removed by the three previous extractions.

When filtered by suction or thru glass wool clogging occurred almost at the start, which made such means very difficult and quite impractical. However by filtering the solution while quite warm thru linen much more satisfactory results were obtained. By filtering twice in this manner- the first filtering removing most of the colloidal gritty matter- a filtrate practically free from impurities was obtained.

From this solution the carminic acid was precipitated by means of the addition of lead acetate. After being allowed to stand over night the solution was decanted and filtered by suction and the precipitate was dried at 80°. A very hard purple amorphous substance was thus obtained.

To obtain the pure acid from the lead salt, 300 grams of the latter were taken and to that was added 2 liters of methyl alcohol and 42 grams of concentrated H_2SO_4 (just sufficient to break up the lead salt and form lead sulphate).

The mixture was then stirred continuously for four hours by means of a mechanical stirred and the solution then filtered. More alcohol was then added to the residue and a small amount (few cc) of acid and the mixture stirred again for two hours. This was repeated a third time and then all filtrates were added together and distilled in a vacuum to about 1/10 th. of the volume. On cooling crystals of carminic acid appeared, which were filtered, washed with ether and dried; being purified later. On further evaporation the mother liquors yielded another quantity of the acid.

Purification

The carminic acid as obtained by the breaking up of the lead salt was sufficiently pure for most purposes; but for certain experiments it was necessary to subject the acid to further purification. The acid was dissolved in alcohol and reprecipitated by ether. This process however did not give very good results, as the yield was small and a comparatively large quantity of ether was necessary.

Another method of purification by dissolving the acid in hot glacial acetic and allowing to cool slowly gave quite satisfactory results.

The best method however was found to be as follows,-

25 grams of the acid were dissolved in 75 cc. of hot water and 325 cc. of dry methyl alcohol. The solution was filtered hot immediately thru a hot water funnel. On cooling a yield of about 18 grams of the pure acid was obtained. There was a slight differ-

ence in color of the acid as purified by the two different methods just explained, the former being a bit lighter shade of red. Upon standing exposed to the air, both darkened. However when kept in the dark in closed bottles no color change was visible.

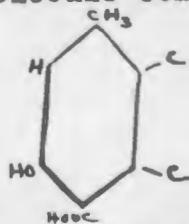
Conclusions

Thus far all that is definitely known is that the carminic acid contains a phthalic acid ring; this fact having been proven by the splitting down of the acid to cochenillic acid¹ and to nitrococussic acid², by the isolation of a brom phthalic derivative from brom carmin³, and by the formation of carminazarin⁴.

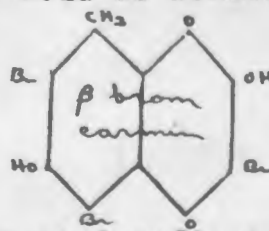
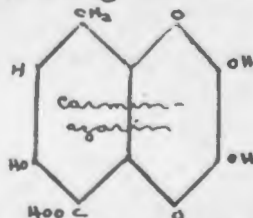
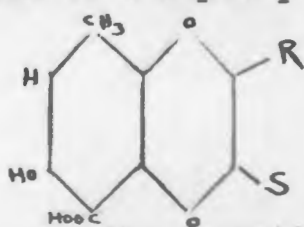
That the initial benzene ring contains a methyl, a hydroxyl and a carboxyl group has been proven, as well as the orientation of the same, by summing up the works of various authors in the formation of tri brom cresotic acid, cochenillic acid and nitrococussic acid. (See references 1 and 2).

Thus it is definitely known that the molecule contains the following ring formation.

The fact that upon oxidation with H₂O₂ carminazarin is formed⁵, and that



by bromination⁶ beta brom carmin is formed- both of which are in all probability naphthaquinone compounds- lead to the belief that there is a naphthaquinone ring in the carminic acid as follows,-

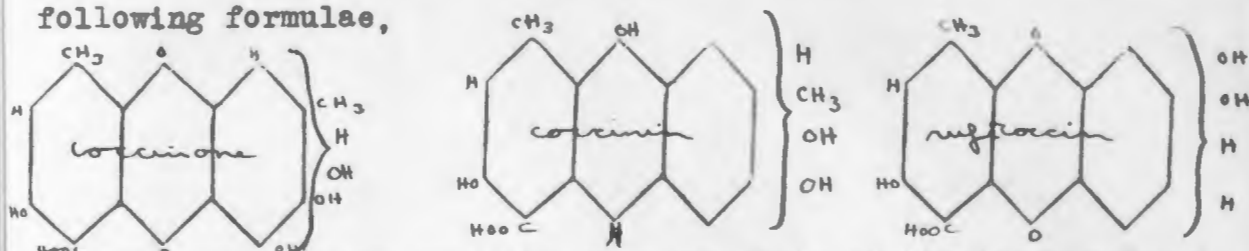


By the formation of coccinin, coccinonon, and ruficoccin⁷-

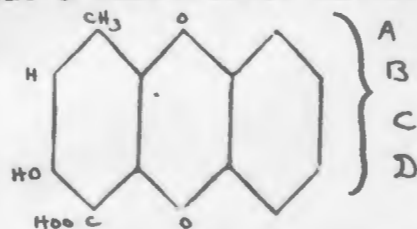
all of which have properties like and act very similar to

-
1. Lieberman and VanDorp (Ber.4, 655) ; Nolting and Salis (Ber 15, 1858); and St. Kostanecki (Ber. 18,250)
 2. Lieberman and Voswinckle (Ber 30, 688); Muldrum (J.C.S. Trans 1911, 1713)
 3. Will and Leyman- See Historical part
 4. Dimroth (Ber. 1909) - See Historical part
 5. " " "
 6. Miller and Rhode " " "

anthraquinone compounds, and which Dimroth believes to have the following formulae,



it would seem quite probable that the basis of the large molecule of carminic acid was a substituted anthraquinone compound of the formula



What one of the groups (D) is, remains uncertain; altho there is but little doubt now that A and B are hydroxyls and that C is an H atom.

From the molecular weight determinations it is evident that D is a much larger molecule than either A, B or C and it probably is a saturated side chain. Whatever it is, it is not heavy enough to hold down the last ring to which it is attached and thus to allow the other two rings to split off. Neither does it seem possible to break it off as a whole from the rest of the anthraquinone ring, at its point of union, and thus give a chance to identify it.

Owing to the fact that benzoyl and acetyl chlorides form hexa derivatives with carminic acid (see Lieberman and Wiederman's works) and that these derivatives eliminate CO_2 when heated, and that there are but three hydroxyls known to be present in the acid molecule, all lead to the further assumption that there are still three more hydroxyls in the acid. These must be present in the unknown $\text{C}_8\text{H}_{13}\text{O}_6$ group.

Attempts to hydrolyse the acid by means of boiling with dilute sulphuric or hydrochloric failed to give any indication as to what the side chain might be. It was thought that it might be a sugar radical and that perhaps the early authors' ideas on carminic acid being a glucoside might be correct, but such facts could not be definitely proven.

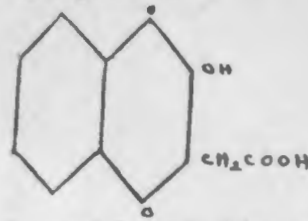
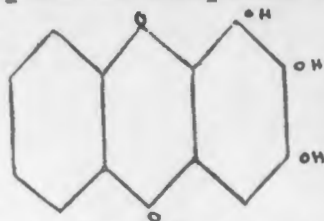
Lieberman in his most recent works opposes the anthraquinone theory upon the following basis,- first, that the solubility of carminic acid in water is not shared by the other hydroxy anthraquinones; second, that anthraquinone compounds do not behave like carminic acid, particularly in being dehydrated to an insoluble compound at 145°; thirdly, that the amounts of anthraquinone derivatives obtained by zinc dust distillation of carminic acid; as well as the amounts of anthraquinone compounds, such as rufiococcin, coccinin and coccinone; are present in too small a quantity. He believes that they were produced by condensation reactions. He still keeps to his indené or bisindene theory.

The fact that anthraquinone ring compounds can be made from carminic acid by oxidations or alkaline fusions seems to be more favorable than unfavorable to that theory. There is no precedent for such a ring forming by alkalie fusion, as it is a well known fact that anthraquinone and oxy anthraquinones are split to benzene and oxy benzenes by that means.

There might come the question, if the acid is an anthraquinone compound why do you get a naphthaquinone compound when the former is oxidized to carminazarin? As a rule you do not get such a result. However Bamberger and Practorius¹ have shown that anthragallol in an alkaline solution will react as follows and form

1. Monat. 23, 688

an anthraquinone compound a naphthaquinone compound.



The action of zinc dust upon carminic acid and its compounds to form a C_{16} hydrocarbon is very favorable to the anthraquinone idea.

In some ways the dyeing properties of carminic acid are quite different from those of the anthraquinone dyes, yet there are many likenesses. The fact that the acid is readily soluble in water can be explained by the fact that there are a large number of hydroxyls present. Also the fact that the acid does not sublime, as do most of the anthraquinone compounds, can be explained by the fact that the large number of hydroxyls present lower the volatility of the compound. Furthermore the fact that the acid has dyeing properties cannot be explained by the naphthaquinone theory or the indene theory.

Therefore in conclusion it would seem from these results that the carminic acid molecule, which has the formula of $C_{22}H_{22}O_{13}$ is an anthraquinone compound, in all probability a derivative of purpurin as shown below, -

