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Fungus Pigments

I. Cinnabarin, a Colouring Matter from *Trametes Cinnabarina* Jacq.

JARL GRIPENBERG

Chemistry Department, Finland Institute of Technology, Helsingfors, Finland

Trametes cinnabarina Jacq., a red coloured fungus growing on decaying wood, particularily mountain-ash, was investigated some sixty years ago by Zopf[1], who obtained therefrom a red crystalline substance for which he proposed the name xanthotrametin. Zopf extracted the fungus with absolute alcohol and evaporated the extract *in vacuo*. Treatment of the residue with ether removed a yellow substance, leaving xanthotrametin undissolved.

When the fungus is extracted with acetone, a compound is obtained, which can be purified by recrystallisation from quinoline or nitrobenzene, but which is almost insoluble in water and the common organic solvents. It thus differs markedly from xanthotrametin, which is said to be soluble in water and alcohol[1], and the name *cinnabarin* is proposed for it.

Due to the scarcity of the fungus, the amount of cinnabarin which has been available for investigation has been very limited, but the compound exhibits some interesting properties and it is the purpose of this communication to give a short account of the, admittedly incomplete, studies so far carried out.

Cinnabarin has no melting point but decomposes gradually on heating above 300 °. For this reason its purity is difficult to assess and analysis have given somewhat varying results, which, however, seem to indicate either $(C_{14}H_{10}O_5N_2)_n$ or $(C_{20}H_{17}O_7N_3)_n$. It contains neither methoxyl nor C-methyl groups. Due to the low solubility of cinnabarin it has not been possible to carry out any molecular weight determination, but analysis and molecular weight determinations on some of its derivatives, described below, show that the simple formula $C_{14}H_{10}O_5N_2$ is the most probable.

Although a number of nitrogen containing pigments have been isolated from bacteria [2], phomazarin, a derivative of aza-antraquinone [3,4], isolated

by Kögl and Sparenburg [5] from *Phoma terrestris* appears to be the only one previously obtained from a higher fungus.

Reference has already been made to the low solubility of cinnabarin in organic solvents. On the other hand it dissolves very readily in conc. sulphuric and nitric acids and less readily in conc. hydrochloric acid, forming in all cases deep red coloured solutions which give a red amorphous precipitate on dilution. This appears to be mostly unchanged cinnabarin, but there are indications that some decomposition also takes place. It has not been possible to prepare any stable salts of cinnabarin.

Cinnabarin is easily soluble in dilute sodium hydroxide and sodium carbonate solutions in the cold, and in sodium hydrogen carbonate solution on warming. The colour in sodium hydroxide is violet at first but soon changes to a dull red. It is not possible to regenerate the cinnabarin from the alkaline solution.

When cinnabarin is heated with dilute sodium hydroxide one molecule of ammonia is evolved, and the alkaline solution gives, after acidification, one equivalent of volatile acid. The nature of this acid has not been determined, and all attempts to obtain any identifiable products from the remaining solution have been fruitless.

Attempts to prepare derivatives of cinnabarin have usually met with little success. Acetylation with acetic anhydride under various conditions has invariably led to dark resinous material. With diazomethane the starting material was recovered unchanged. Although methylation with dimethyl sulphate in alkaline solution gave only resinous material, methylation with dimethyl sulphate and potassium carbonate in acetone has given, in low yield, a red, beautifully crystalline substance. However the analysis of this compound, which is easily soluble in ether, alcohol and acetone, does not correspond with any reasonable formula. The carbon, hydrogen and methoxyl values fit well for a formula $C_{16}H_{14}O_5N_2$, with one methoxyl group, but the nitrogen value is far too high. The relationship of this compound to cinnabarin is thus obscure.

Reductive acetylation yields two different products depending on the conditions. Using zinc and acetic anhydride in the presence of a small amount of pyridine, a yellow, neutral compound is obtained. This compound, m. p. 200—202 °, is only sparingly soluble in alcohol but readily soluble in chloroform giving a solution which shows a strong green fluorescence. Analyses correspond with the formula $C_{18}H_{16}O_6N_2$ and the compound is provisionally named cinnabarin leucoacetate B.

When, on the other hand, the reductive acetylation is carried out in the presence of acetic acid there is formed, in addition to leucoacetate B, another

substance. The latter is also yellow, but is strongly acidic, being easily soluble in sodium hydrogen carbonate. It melts at 213—214 °, is sparingly soluble in chloroform but readily soluble in alcohol, corresponds to the formula $C_{16}H_{16}O_6N_2$, and is termed cinnabarin leucoacetate A.

The reduction leading to the leucoaceate A has been difficult to duplicate. In one experiment a fair yield of both compounds was obtained, but other experiments gave only very small amounts of the leuco derivatives. Lack of material has prevented any thorough study of the conditions for optimum yields.

Both leucoacetates give ammonia on alkaline hydrolysis. Leucoacetate B gives nearly one molecule, but with leucoacetate A no quantitative determination has been made. Leucoacetate B gives, further, three equivalents of volatile acids.

On catalytic hydrogenation leucoacetate B takes up one molecule of hydrogen. The colour of the yellow solution does not change noticeably during hydrogenation, but the product, which has not been isolated, is extremely sensitive. The colour changes to an orange red, which is quite different from the original light yellow, as soon as air is admitted to the hydrogenation vessel.

The most important of all the reactions of the two leucoacetates is that when leucoacetate A is treated with acetic anhydride and a drop of pyridine it is converted into leucoacetate B. This is not however a simple acetylation as leucoacetate B differs from leucoacetate A only by two carbon atoms, *i. e.*, acetylation has been accompanied by the simultaneous removal of an extra molecule of water. The disappearance of the acidity when leucoacetate A is converted into leucoacetate B may be due to acetylation of a strongly acidic phenolic hydroxyl group, or to the formation of a lactone from a carboxyl group. This last mentioned possibility would also account for the loss of one molecule of water.

The facts presented above are clearly too meagre to allow any discussion on possible structures for cinnabarin. The following points may however be stressed: 1) The extremely low solubility and the high stability towards heat of a compound with such a comparatively low molecular weight, as corresponds to the formula $C_{14}H_{10}O_5N_2$ seem to indicate a zwitter ionic structure; 2) It contains two nitrogen atoms, one of which is removable as ammonia. As the molecule, however, suffers deep-seated decomposition during the alkaline hydrolysis is it difficult to draw any conclusions regarding the nature of this nitrogen atom. Likewise the origin of the equivalent of volatile acid which is formed in the alkaline hydrolysis is obscure; 3) The reductive acetylation suggests the presence of a quinonoid system, a hypothesis which is supported by the fact that cinnabarin is reduced by sodium hyposulphite.

It must, however, be borne in mind that neither of the two acetylation products are normal leucoacetates. Leucoacetate A can be regarded as a monoacetyltetrahydrocinnabarin and leucoacetate B as diacetylanhydrotetrahydrocinnabarin, whereas a normal leucoacetate would be a derivative of dihydrocinnabarin. There must, therefore, be some group which is responsible for the uptake of the second molecule of hydrogen. The formation of three equivalents of volatile acids in the hydrolysis of leucoacetate B, while cinnabarin itself gives only one equivalent, supports the formulation of leucoacetate B as a diacetylderivative; 4) Leucoacetate B contains one double bond which can be hydrogenated catalytically. The reaction which takes place when the hydrogenation product comes in contact with air is evidently not a simple dehydrogenation back to leucoacetate B.

This investigation will be continued as soon as more fungus material becomes available.

EXPERIMENTAL

Isolation of cinnabarin

The fungus material was collected on Omberg, Sweden. 360 G of the finely ground, air dried, material was extracted with acetone in a Soxhlet apparatus. The brown precipitate which formed (10 g) was filtered off and freed from waxy contaminants by extraction with ether. The crude cinnabarin thus obtained was dissolved in boiling quinoline, the solution filtered hot, allowed to cool to about 100°, and a large amount of alcohol added. The product, 2.3 g (0.65 %), separated in the form of small glistening leaflets. It was found advisable to carry out this purification with small portions at a time and the material thus obtained was used for the reactions described in this paper.

For analysis it was further purified by recrystallisation from a mixture of nitrobenzene and anisole. It decomposed, without melting, above 300°.

$C_{14}H_{10}O_5N_2$	Calc.	C	58.72	H	3.53	N	9.79
$C_{20}H_{17}O_7N_3$	»	»	58.66	»	3.70	»	10.27
	Found	»	57.69	»	3.93	»	9.66
	»	»	58.98	»	3.52	»	10.17
	»	»	59.42	»	3.58		

Methylation of cinnabarin

A mixture of cinnabarin (100 mg) suspended in dry acetone (50 ml) to which was added potassium carbonate (2 g) and dimethyl sulphate (1 ml) was boiled for twenty hours, the solvent removed *in vacuo*, and water added. After twelve hours the brown powder (30 mg) was filtered off and purified by recrystallisation from very dilute acetone, forming long, dark red needles of m. p. 183—185°.

$C_{15}H_{11}O_4N_2(OCH_3)$	Calc.	C	61.12	H	4.50	N	8.91	OCH_3	9.88	Mol. wt. 314
	Found	»	61.35	»	4.57	»	11.22	»	9.74	» (Rast) 338

Hydrolysis of cinnabarin

Cinnabarin was warmed with 2 *N* sodium hydroxide solution in a slow stream of nitrogen, the exit gases being passed through dilute hydrochloric acid. This was evaporated to dryness, leaving a white crystalline residue, which sublimed without melting, gave a strong smell of ammonia when treated with sodium hydroxide, and gave a negative test for alkylaminohydrochlorides [6].

In a quantitative experiment 62.8 mg (0.22 mmole) of cinnabarin was hydrolysed in the same way, but the exit gases were passed through 15 ml of 0.0515 *N* sulphuric acid. After 10 hours the sulphuric acid required 10.1 ml of 0.055 *N* sodium hydroxide for neutralisation, corresponding to an absorption of 0.216 mmole of ammonia.

The alkaline solution remaining after the hydrolysis was acidified with sulphuric acid and steam distilled. The distillate consumed on titration 3.86 ml of 0.055 *N* sodium hydroxide, corresponding to 0.212 m.equiv. of volatile acid.

The acidic solution containing the nonvolatile products was extracted with ether in a continuous extractor but only a small amount of brown resinous material was obtained.

Reductive acetylation of cinnabarin

1. Cinnabarin (200 mg) was suspended in acetic anhydride (5 ml) containing a few drops of pyridine. Zinc powder was added in small portions and the reaction mixture gently warmed. The cinnabarin gradually dissolved giving a yellow solution, which was finally boiled for some minutes. Unreacted zinc was filtered off and washed with hot acetic anhydride, and the filtrate was poured into water and set aside overnight. The yellow precipitate that was formed was taken up in chloroform and the aqueous solution extracted with additional portions of chloroform. The combined chloroform extracts were washed with sodium hydrogen carbonate and evaporated, leaving a residue (150 mg) which crystallised immediately on the addition of alcohol. Purification by chromatography on aluminium oxide, and recrystallisation from alcohol containing a small amount of chloroform, gave cinnabarin leucoacetate B as yellow needles, melting at 198—200°.

$C_{18}H_{16}O_6N_2$	Calc.	C 60.65	H 4.54	N 7.86	Mol.wt.		356
	Found	» 60.00	» 4.14	» 7.65	»	(Rast)	360

2. Cinnabarin (200 mg) was reduced and worked up in the same way as above, except that a few drops of acetic acid was added to the reaction mixture. In this case the sodium hydrogen carbonate washings of the chloroform solution acquired a bright red colour. Acidification of the sodium hydrogen carbonate solution gave a yellow precipitate of cinnabarin leucoacetate A which was recrystallised from alcohol and formed yellow micro-crystals of m. p. 213—214°.

$C_{16}H_{16}O_6N_2$	Calc.	C 57.80	H 4.87	N 8.43	Mol.wt.		332
	Found	» 57.63	» 4.93	» 8.42	»	(Rast)	330

From the chloroform solution, leucoacetate B was obtained as before.

Upon acetylation leucoacetate A gives a substance with m. p. 200—202°, which does not depress the m. p. of leucoacetate B.

Hydrolysis of leucoacetate B

Leucoacetate B (97.3 mg, 0.268 mmole) was hydrolysed as described for cinnabarin. The ammonia evolved was absorbed in 15 ml of 0.0515 *N* sulphuric acid, which, after the reaction, required 9.58 ml of 0.055 *N* sodium hydroxide for neutralisation, corresponding to 0.245 mmole of ammonia.

The alkaline hydrolysate was acidified with sulphuric acid and steam distilled. The distillate consumed 14.77 ml of 0.055 *N* sodium hydroxide corresponding to 0.81 m.equiv. of volatile acids.

Hydrogenation of leucoacetate B

Cinnabarin leucoacetate B (13.9 mg) was hydrogenated in acetic acid in the presence of hydrogenated PtO_2-catalyst. The uptake of hydrogen amounted to 0.86 ml (Calculated for one molecule of hydrogen, 0.875 ml). When the yellow solution was exposed to air, its colour immediately changed to an orange red.

SUMMARY

A nitrogen containing pigment, for which the name cinnabarin is proposed, has been isolated from the fungus *Trametes cinnabarina* Jacq. Some of its reactions are described.

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