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Fungus Pigments

VIII *. The Structure of Cinnabarin and Cinnabarinic Acid

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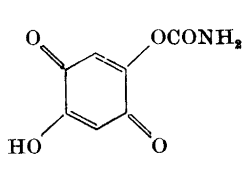
Hydrolysis of cinnabarin with alkali gives cinnaquinone, $C_7H_5NO_5$, which upon further hydrolysis gives 2,5-dihydroxybenzoquinone. The formation of these compounds and the results of reductive acetylation of cinnabarin and its derivatives has led to the conclusion that cinnabarin is 2-amino-9-hydroxymethylphenoxazin-3-one-1-carboxylic acid (IX). Cinnabarinic acid is formulated as 2-aminophenoxazin-3-one-1,9-dicarboxylic acid (XV), and it is identical with a compound obtained earlier by oxidation of 3-hydroxyanthranilic acid.

Since cinnabarin was first isolated, it has been known that alkaline hydrolysis gives one molecule of ammonia, but no other definite product could be isolated from this reaction [1-3]. It has now been found that on hydrolysis with a small volume of 2 N sodium hydroxide at room temperature for a short time a sparingly soluble sodium salt is precipitated. Acidification yields an orange-red crystalline compound, $C_7H_5NO_5$, which we have called cinnaquinone. Further hydrolysis of cinnaquinone gives 2,5-dihydroxybenzoquinone, ammonia and carbon dioxide [5].

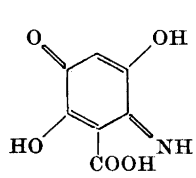
In view of the mild conditions for this further hydrolysis and the similarity of the U.V.-spectra of cinnaquinone and of 2,5-dihydroxybenzoquinone (Fig. 1) it is assumed that the reaction is not accompanied by any deepseated changes in structure. The structures I—VI therefore represent the theoretical possibilities for the structure of cinnaquinone.

Of these, I can be dismissed at once because cinnaquinone has peaks at 1 683 and 1 613 cm^{-1} in the carbonyl region, whereas I should have a peak at $\sim$ 1 725 cm^{-1} typical of carbamates [4]. It is more difficult to decide between the other possible structures. VI, incorporating the carboxamide group suspected in cinnabarin [3,6] is the structure most likely to have been formed under the conditions used, and was considered the most probable alternative [5].

* The paper in *Proc. Chem. Soc.* **1957** 233 is regarded as Part VII of this series. Part VI *Suomen Kemistilehti B* **30** (1957) 134.



I



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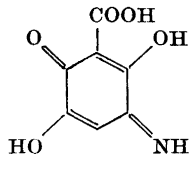

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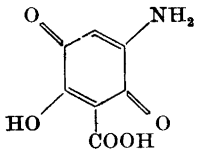

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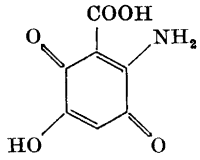

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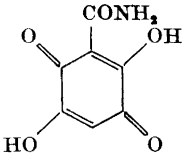
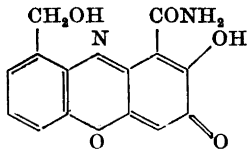
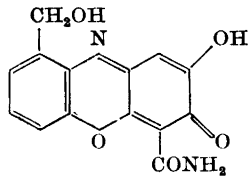


VI

The formation of a hydroxybenzoquinone derivative on alkaline hydrolysis of cinnabarin is in complete accordance with its formulation as a phenoxazin-3-one derivative [7,8] because phenoxazin-3-ones are known to give hydroxybenzoquinones, although more drastic conditions have usually been employed [9,10]. Cinnabarin was accordingly formulated [5] as VII or VIII



VII



VIII

Both these formulae, however, suffer from the disadvantage that they do not permit any rational formulation of the products of reductive acetylation of cinnabarin and its derivatives.

The first paper in this series [1] described the formation of two substances by reductive acetylation of cinnabarin. They were designated as cinnabarin leucoacetate A and cinnabarin leucoacetate B, respectively. The former was given the composition $C_{16}H_{16}N_2O_6$ and the latter $C_{18}H_{16}N_2O_6$ and it was shown that leucoacetate A upon further acetylation gives leucoacetate B. Cavill *et al.*[3] also prepared leucoacetate B, but assigned it the composition $C_{20}H_{16}N_2O_7$. More recent analytical evidence is also more consistent with this formula, and we therefore regard it as correct. Cinnabarin leucoacetate B is thus triacetylanhydrodihydrocinnabarin. Cavill *et al*[3] also prepared a triacetyl-O-methyldihydrocinnabarin. Since no anhydro compound was formed in this case they drew the natural conclusion that the hydroxylgroup, which is methylated is that involved in the formation of the anhydro compound.

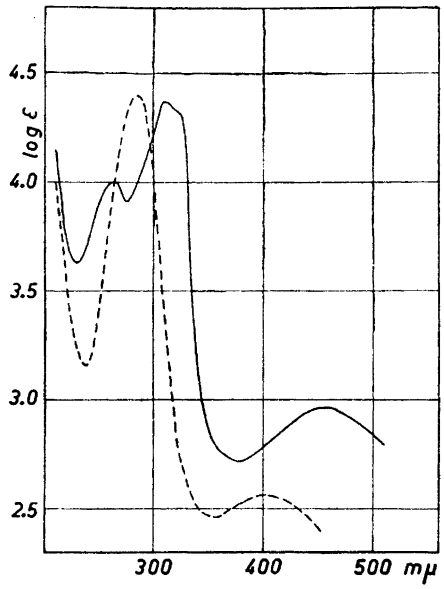


Fig. 1. Absorption curves for cinnaquinone (———). and 2,5-dihydroxybenzoquinone (– – – –). Both in alcohol.

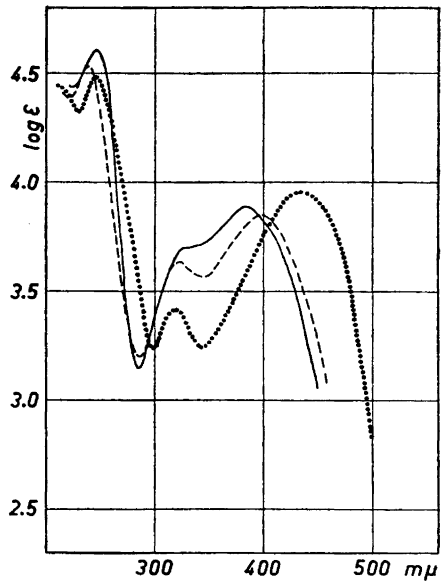


Fig. 2. Absorption curves for triacetyldihydrocinnabarin (XI) (————), triacetyldihydrocinnabarin methyl ester (XII) (– – – –) and triacetylanhydrodihydrocinnabarin (XIII) (·····). All in alcohol.

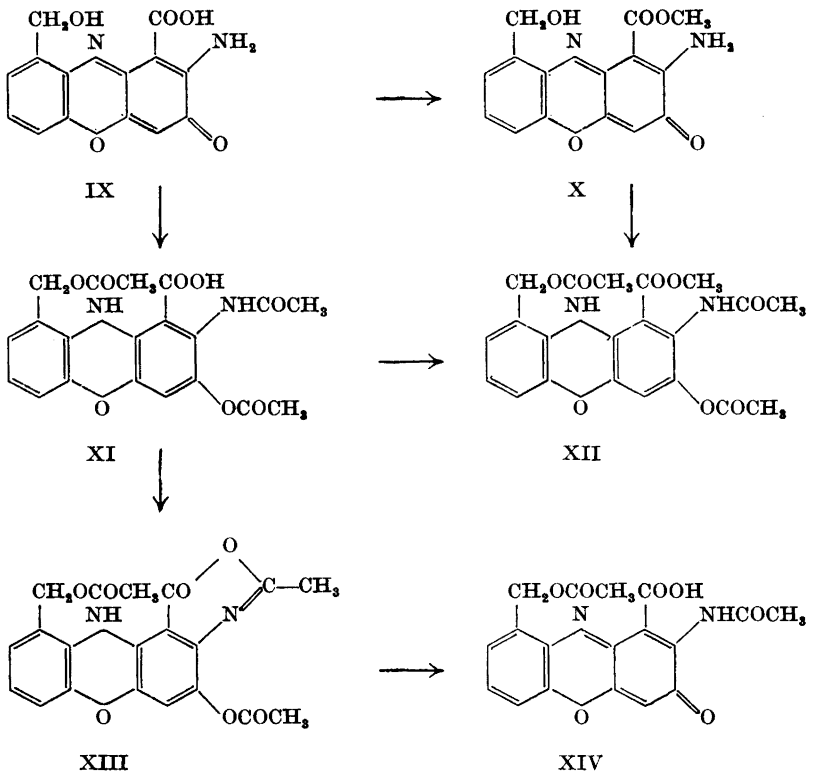
A comparison of the U.V.-spectra of cinnabarin leucoacetate A and triacetyl-O-methyldihydrocinnabarin indicated a close relationship between them (Fig. 2). This was also established by methylation of leucoacetate A with diazomethane, which gave triacetyl-O-methyldihydrocinnabarin. In our hands this substance had m.p. 173.5—174.5°, whether prepared by methylation of cinnabarin leucoacetate A or by reductive acetylation of O-methyl cinnabarin, whereas Cavill *et al.*[3] report 156°. This difference is probably due to dimorphism. Cinnabarin leucoacetate A must thus be triacetyldihydrocinnabarin with a composition $C_{20}H_{18}N_2O_8$, although the nitrogen value (8.42 %) is much higher than that required by the above formula (6.76 %). The carbon and hydrogen values (C 57.63; H 4.93) fit this formula reasonably well (Calc. C 57.97; H 4.38).

All attempts to repeat the preparation of leucoacetate A have given only very minute amounts of material insufficient for further analysis.

The results of reductive acetylation show that the hydroxyl group in triacetyldihydrocinnabarin, which is responsible for the acidity [1], is also present in cinnabarin, and is that which is methylated in the formation of O-methylcinnabarin. This hydroxyl group also takes part in the reaction leading to the formation of triacetylanhydrodihydrocinnabarin.

Formulae VII or VIII for cinnabarin do not give a satisfactory explanation of the behaviour of cinnabarin.

If, however, cinnabarin has the structure IX these reactions can be formulated as shown below,



O-Methylcinnabarin (X) is, on this basis, more properly named cinnabarin methyl ester. Triacetyldihydrocinnabarin (leucoacetate A) is XI, triacetyldihydrocinnabarin methyl ester (triacetyl-O-methyldihydrocinnabarin) is XII and triacetylanhydrodihydrocinnabarin (leucoacetate B) is XIII. The conversion of XI into XIII is a well known reaction [11]. That the NH-group of the phenoxazine ring cannot be acetylated can be ascribed to steric hindrance.

These structures are also supported by the I.R.-spectra (Our I.R.-spectra differ in some respects from these reported by Cavill *et al.*[3], but these differences are probably due to the difference in technique employed [12], and in the case of triacetyldihydrocinnabarin methyl ester also to different crystalline forms.) The bands at 1 672 and 1 653 cm^{-1} in the spectrum of cinnabarin, which were originally assigned to the carboxamide group [3], can equally well be due to strongly chelated carboxyl and the carbonyl of the phenoxazin-3-one [13]. Cinnabarin methyl ester has a single much stronger band at 1 657 cm^{-1} in this region [3]. The strong, partly resolved absorption just below 1 600 cm^{-1} is typical of phenoxazin-3-ones [13] and disappears on reductive acetylation. Instead there are a number of bands above 1 650 cm^{-1}. In the spectrum

of XI the bands at 1 770 and 1 740 cm^{-1} can be assigned to the aromatic acetate and the aliphatic acetate, and those at 1 690 and 1 677 cm^{-1} to the carboxyl and the N-acetyl-group, or *vice versa*. In XII the corresponding bands occurs at 1 760, 1 740, 1 682 and 1 657 cm^{-1}. XIII has, as expected, a third band above 1 700 cm^{-1}, due to the carbonyl group of the oxazinone ring [11]. The band at 1 775 cm^{-1} can be assigned to the aromatic acetate and the band at 1 750 cm^{-1} to the oxazinone carbonyl or vice versa and the one at 1 732 cm^{-1} to the aliphatic acetate. The band at 1 655 cm^{-1} is probably due to the C = N-bond in the oxazinone ring [11].

One objection that could be raised against these formulations for the leucoderivatives of cinnabarin is, that the transformation of XI into XIII causes a large bathochromic shift in the spectrum (see Fig. 2) while the change from N-acetylanthranilic acid into 2-methylbenzoxazin-4-one is accompanied by a slight hypsochromic shift [11]. It is, however, doubtful whether any significant conclusion can be put on a comparison of systems that are as different as these.

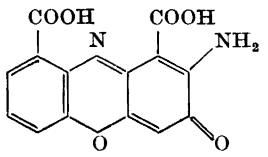
These leucoderivatives of cinnabarin are quite stable, unlike the leucoacetate of actinomycin, which according to Brockmann and Franck [14] can be readily partially hydrolysed and reoxidised to N-acetylactinomycin. Such a hydrolysis appears to take place with triacetylanhydrodihydrocinnabarin (XIII) only in the presence of an oxidant, *e.g.* nitrous acid. Cavill *et al.*[15] who discovered this reaction called their product diacetyl*iso*polystictin (in our nomenclature diacetyl*iso*cinnabarin) but it might just as well be diacetylcinnabarin (XIV). Their assumption that this product should be identical with our leucoacetate A is incorrect, as shown by the completely different U.V.- and I.R.-spectra for the two compounds and by the strong mixed m.p. depression.

The formulation of cinnabarin as IX and not as VII or VIII finds further support in a colour reaction. Brockmann and Franck [16] have observed that actinomycin, which has an amino-group in the 2-position of the phenoxazin-3-one ring gives a yellow colour with stannous chloride, while desaminoactinomycin, with a hydroxyl group in the 2-position gives a green colour. This difference between 2-amino- and 2-hydroxyphenoxazin-3-ones is even more pronounced in the parent compounds; 2-aminophenoxazin-3-one gives with stannous chloride a colourless solution and 2-hydroxyphenoxazin-3-one a blue colour [9]. Cinnabarin gives in accordance with its formulation as a 2-aminoderivative only a yellow colour.

If cinnabarin is IX it means that cinnaquinone must be V. Its properties agree equally well with this structure, although it is somewhat surprising that such a compound is stable under alkaline conditions. The formation of oxamic acid on oxidation of cinnabarin under slightly alkaline conditions [6] can of course also be explained on the basis of structure IX for cinnabarin.

In a previous paper [6] we described the isolation of cinnabarinic acid, which accompanies cinnabarin. The assumed close similarity of cinnabarinic acid and cinnabarin, which was inferred from their almost identical U.V.-absorption in pyridine solution has now found further support in that both show the same characteristic shift of absorption maximum in strongly acidic solution (see below) and both give the same yellow colour reaction with stannous chloride.

Cinnabarinic acid has the composition $C_{14}H_8N_2O_6$, having two hydrogen atoms less and one oxygen atom more than cinnabarin. It was therefore very tempting to speculate that cinnabarinic acid differs from cinnabarin in having a carboxyl group instead of the primary hydroxyl group. The presence of a second carboxyl group in cinnabarinic acid, which finds strong support in a band at 1 727 cm^{-1} also explains the greater acidity of cinnabarinic acid [6]. If cinnabarin has structure IX, cinnabarinic acid should be XV.



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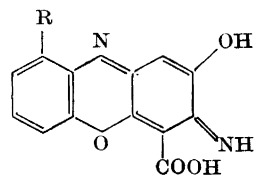

XVI R = COOH
XVII R = CH_2OH

XV has been proposed by Butenandt *et al.*[10] as an alternative structure for the product obtained by oxidation of 3-hydroxyanthranilic acid, the other being XVI. Through the kindness of Professor Butenandt we received a sample of his »Farbstoff IV». Although the I.R.-spectrum of »Farbstoff IV» is not quite as sharp as that of cinnabarinic acid, there can be no doubt that the two substances are identical (Fig. 3). The identity of the U.V.-spectra both in neutral and strongly acidic solution [10] confirms this identity further.

One remarkable difference between cinnabarin and cinnabarinic acid is in their behaviour towards alkali. Cinnabarin, as has been shown above, splits off cinnaquinone, while cinnabarinic acid is converted into 2-hydroxyphenoxazin-3-one-9-carboxylic acid [10]. Attempts to obtain cinnaquinone from cinnabarinic acid gave only a minute amount of a substance, which showed the characteristic U.V. spectrum of cinnaquinone but the amount available was not sufficient for further purification.

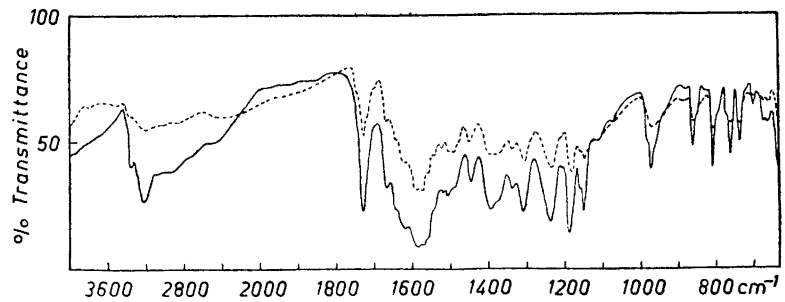

Fig. 3. Infrared absorption curves for natural cinnabarinic acid (————) and synthetic cinnabarinic acid (— — — — —). In KBr-discs.

In spite of this difference, which can be accounted for by the influence of the 9-carboxylic group on the rate of hydrolysis of the C = N-bond, it is considered that cinnabarin and cinnabarinic acid have analogous structures. This definitely excludes structures VII and VIII for cinnabarin. The possibility that cinnabarinic acid has structure XVI and cinnabarin XVII is highly improbable because XVII ought to form a dimethyl derivative. The formation of phenoxazin-3-one (or more probably 9-methylphenoxazin-3-one) on zinc dust distillation [6,7] and oxamic acid on oxidation of cinnabarin [6] is also difficult to reconcile with structure XVII.

IX and XV thus appear to be the most probable structures for cinnabarin and cinnabarinic acid, respectively.

After this paper had been written a paper by Cavill, Clezy and Tetaz [17] appeared, in which the structure IX is also suggested for cinnabarin.

EXPERIMENTAL

(The analyses are by Dr. A. Bernhardt, Mühlheim, and the I.R.-spectra by Mr. B. C. Fogelberg, Ab Centrallaboratorium, Helsingfors.)

Cinnaquinone. Cinnabarin (400 mg) was added to 2 N sodium hydroxide (2 ml) and the mixture was stirred with a glass rod. The cinnabarin dissolved in a few minutes and a brown crystalline precipitate soon formed. This was at once filtered off. A second crop of crystals forming in the filtrate was also filtered off and usually a third crop of crystals could also be obtained. The combined precipitates were dissolved in the minimum amount of water and the solution was acidified. Brown-yellow crystals of cinnaquinone, mixed with some dark-brown amorphous material were obtained. The precipitate was filtered off and treated with ethyl acetate which leaves the amorphous material undissolved. The filtrate which still contained a considerable amount of cinnaquinone was extracted with ethyl acetate. The combined ethyl acetate extracts were evaporated under reduced pressure and the crystalline residue recrystallised from dioxan giving orange-brown needles (70 mg), decomp. above 200° without melting. (Found: C 45.78; H 2.95; N 7.39; $C_7H_5NO_5$ requires C 45.91; H 2.75; N 7.65). I.R. maxima (KBr): 3 375 (m), 3 230 (m), 1 683 (m), 1 613 (m), 1 580 (s), 1 490 (m), 1 422 (m), 1 395 (s), 1 332 (s), 1 302 (m), 1 247 (m), 1 212 (w), 1 167 (m), 955 (w), 882 (w), 865 (w), 820 (w), 763 (w), 742 (w) cm^{-1}.

2,5-Dihydroxybenzoquinone. Cinnaquinone (10 mg) was added to 20 % sodium hydroxide (0.5 ml) and warmed slightly. In a few minutes a red precipitate was formed and was filtered off, treated with acid and extracted with ethyl acetate. The residue from the ethyl acetate extract was sublimed at 90°/0.005 mm. The red sublimate had an I.R.-spectrum indistinguishable from that of 2,5-dihydroxybenzoquinone prepared according to Jones and Shonle [18].

Triacetyldihydrocinnabarin methyl ester. a) Cinnabarin methyl ester [3] (30 mg) was suspended in acetic anhydride (2 ml). A few drops of pyridine and zinc dust were added and the mixture was warmed giving a yellow solution. This was filtered hot to remove unreacted zinc and then poured into water. The yellow precipitate was filtered off and recrystallised from benzene, small yellow needles m. p. 173.5—174.5° (Found: C 59.49; H 4.64; $C_{21}H_{20}N_2O_8$ requires C 58.87; H 4.71). I.R. maxima (KBr): 3 330 (w), 3 250 (w), 1 760 (s), 1 740 (s), 1 682 (s), 1 657 (s), 1 612 (w), 1 582 (w), 1 513 (s), 1 475 (s), 1 437 (m), 1 375 (m), 1 349 (w), 1 312 (m), 1 290 (s), 1 254 (s), 1 240 (s), 1 207 (s), 1 116 (m), 1 020 (m), 965 (w), 912 (w), 875 (w), 847 (w), 789 (w), 771 (m), 720 (w) cm^{-1}.

b) Triacetyldihydrocinnabarin [1] (10 mg) was suspended in ether and an ethereal solution of diazomethane was added. The clear solution which formed after some time was evaporated leaving fine yellow needles, m. p. 173—174°. No depression of m. p. when mixed with material prepared according to a).

Triacetylanhydrodihydrocinnabarin was prepared as described before [1]. (Found: C 60.24; H 4.39; N 7.03; O 28.29; $C_{20}H_{16}N_2O_7$ requires C 60.60; H 4.07; N 7.07; O 28.26). I.R. maxima (KBr): 3 340 (m), 1 775 (s), 1 750 (s), 1 732 (s), 1 655 (s), 1 619 (w), 1 589

(w), 1 519 (s), 1 481 (s), 1 465 (s), 1 432 (m), 1 405 (m), 1 375 (s), 1 351 (m), 1 317 (s), 1 292 (s), 1 260 (m), 1 230 (s), 1 198 (s), 1 163 (m), 1 131 (s), 1 063 (s), 1 027 (m), 1 009 (m), 999 (m), 970 (m), 914 (m), 883 (m), 790 (m), 780 (s), 716 (w), 685 (m) cm^{-1}.

Triacetyldihydrocinnabarin [1] has I.R. maxima (KBr): 3 380 (m), 1 770 (s), 1 740 (m), 1 690 (s), 1 677 (s), 1 630 (m), 1 587 (m), 1 508 (s), 1 480 (s), 1 435 (m), 1 381 (m), 1 295 (s), 1 260 (s), 1 237 (s), 1 198 (s), 1 120 (w), 1 022 (m), 993 (w), 955 (w), 938 (w), 920 (w), 878 (m), 847 (w), 796 (w), 779 (m), 768 (w), 745 (w), 730 (m), 715 (w), 695 (w) cm^{-1}.

Cinnabarinic acid [6] was purified further by extractive crystallisation, first from dioxan and then from acetone. I.R. maxima (KBr); 3 370 (m), 3 240 (s), 1 727 (s), 1 662 (m), 1 615 (s), 1 587 (s), 1 570 (s), 1 516 (m), 1 507 (m), 1 448 (m), 1 395 (s), 1 338 (m), 1 310 (s), 1 237 (s), 1 184 (s), 1 150 (s), 973 (m), 860 (m), 807 (m), 761 (m), 736 (m), 701 (w), 670 (w), 663 (w), 633 (m) cm^{-1}.

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