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THE CHEMISTRY OF MOULD METABOLITES—III*

STRUCTURE OF CINNABARIN (POLYSTICTIN)†

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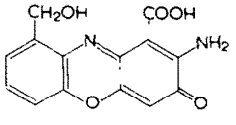
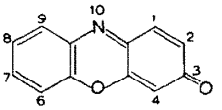
Abstract—Cinnabarin (polystictin) is shown to be 1-carboxy-2-amino-9-hydroxymethylphenoxazin-3-one. Previous degradative and spectroscopic studies on cinnabarin and its derivatives are interpreted on the basis of this structure, which is confirmed by the transformation of methyl cinnabarin into the known 1:9-dimethoxycarbonyl-2-aminophenoxazin-3-one.

FOLLOWING upon the isolation of the red pigment, cinnabarin (polystictin), from the wood-rotting fungi, *Coriolus sanguineus*[1,2] and *Trametes cinnabarina*,[3] various degradative studies have been reported.[4,5,6,7] In a preliminary communication,[8] structure (I) was proposed for this mould metabolite and it is now intended to detail the evidence that has led to this formulation.

The acidic character of cinnabarin, $C_{14}H_{10}O_5N_2$, which is converted into methyl cinnabarin by the action of diazomethane, or by methyl iodide and potassium carbonate in acetone,[2] is attributed to a carboxyl group. On mild treatment with methanolic potassium hydroxide solution cinnabarin yields the non-acidic decarboxy-cinnabarin,[1] and as the elimination of the elements of carbon dioxide is achieved without significant change in the characteristic absorption spectrum,[5] the reaction is represented as a simple decarboxylation. In addition, cinnabarin contains a hydroxyl group which is acetylated by the action of acetic anhydride–sulphuric acid.[2] Similarly methyl cinnabarin yields methyl O-acetylcinnabarin, by the action of acetic anhydride–pryidine.‡[5]



(I)

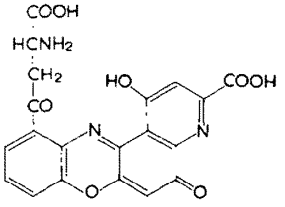


(II)

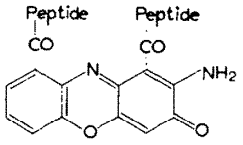
Now, the nature of the nucleus has been established by the isolation of phenoxazin-3-one (II), on fusion of cinnabarin with zinc dust,[4,5] and this mould metabolite is

* Part II, G. W. K. Cavill, P. S. Clezy and J. R. Tetaz, *J. Chem. Soc.* 2646 (1957).
† For preliminary communication, see *Proc. Chem. Soc.* 346 (1957).
‡ Methyl O-acetylcinnabarin was originally described[5] as an N-acetyl derivative, cf. Gripenberg.[6]
[1] Report *Nat. Health Med. Res. Council Australia* p. 12 (1946); R. Lemberg, *Aust. J. Exp. Biol. Med. Sci.* **30**, 271 (1952).
[2] G. W. K. Cavill, B. J. Ralph, J. R. Tetaz and R. L. Werner, *J. Chem. Soc.* 525 (1953).
[3] J. Gripenberg, *Acta Chem. Scand.* **5**, 590 (1951).
[4] G. W. K. Cavill and J. R. Tetaz, *Chem. & Ind.* 986 (1956).
[5] G. W. K. Cavill, P. S. Clezy and J. R. Tetaz, *J. Chem. Soc.* 2646 (1957).
[6] J. Gripenberg, E. Honkanen and O. Patoharju, *Acta Chem. Scand.* **11**, 1485 (1957).
[7] J. Gripenberg, *Proc. Chem. Soc.* 233 (1957).
[8] G. W. K. Cavill, P. S. Clezy and J. R. Tetaz, *Proc. Chem. Soc.* 346 (1957).

thus grouped with the ommochromes, for example xanthommatin[9] (III), and with the actinomycins[10,11] (IV), as a new class of organic pigments in Nature. The latter compounds contain a nitrogen atom in the 2-position, and considerable support for a 2-aminophenoxazin-3-one chromophore in cinnabarin is provided by a comparison of the light absorption data of cinnabarin and its derivatives with that of various natural and synthetic 2-aminophenoxazin-3-ones.[5] For example, the spectrum of



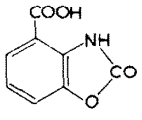
(III)



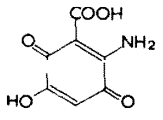
(IV)

decarboxycinnabarin (max at 237 and 421, 437 mμ; log ε 4·15 and 4·10, 4·12) is almost identical with that of 2-aminophenoxazin-3-one (max at 237 and 421–436 mμ; log ε 4·17 and 4·10).

The isolation of benzoxazolone-4-carboxylic acid (V), on oxidation of cinnabarin with potassium permanganate,[6] may be compared with the known conversion of 2-aminophenoxazin-3-one into benzoxazolone, and of actinomycin (IV) into 7-methylbenzoxazolone-4-carboxylic acid,[12] thus confirming the phenoxazone nucleus. In addition, the isolation of this C_7 moiety, a degradation product of the benzenoid ring, establishes the presence of a carbon substituent at position 9 of the phenoxazone nucleus.[6] Now (V) has been obtained by the oxidation of cinnabarin with potassium permanganate, but not by the action of alkaline hydrogen peroxide, hence the carboxyl group results from further oxidation of the original substituent in position 9.[6] As cinnabarin contains a 2-aminophenoxazin-3-one chromophore, and possesses a carboxyl group, the above substituent must be a hydroxymethyl group (cf. I).



(V)



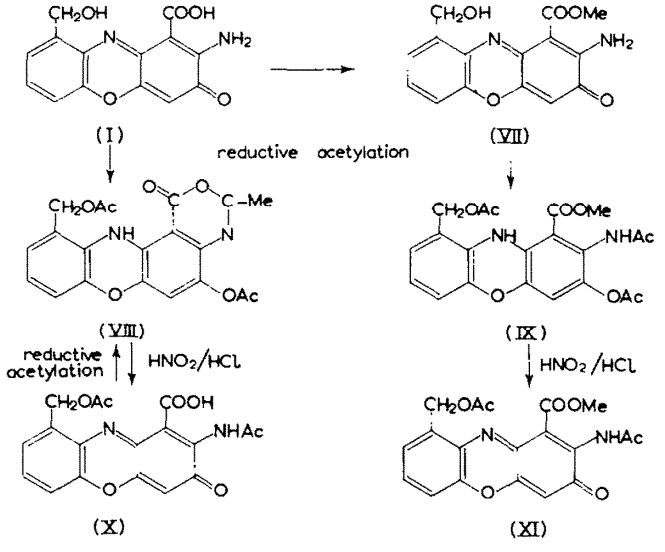
(VI)

The action of aqueous alkali on cinnabarin has resulted in the ready liberation of one molar proportion of ammonia[1,2,3] and corresponds to a hydrolysis of the 2-amino group. More recently a milder hydrolysis of cinnabarin, with aqueous sodium hydroxide solution, has given a quinone, $C_7H_7O_5N$, which is further hydrolysed to 2:5-dihydroxy-*p*-benzoquinone, carbon dioxide and ammonia.[7] Such a C_7 moiety must correspond to the quinonoid ring of the aminophenoxazone nucleus, and thus, is represented as a 2-amino-5-hydroxy-*p*-benzoquinone carboxylic acid, presumably (VI). The carboxyl group is that originally present in cinnabarin. The alternative

[9] A. Butenandt, U. Schiedt and E. Bickert *Liebigs Ann.* **588**, 106 (1954); A. Butenandt, U. Schiedt, E. Bickert and R. J. T. Cromartie, *Ibid.* **590**, 75 (1955).
[10] H. Brockmann and H. Muxfeldt, *Angew. Chem.* **68**, 69 (1956).
[11] S. J. Angyal, E. Bullock, W. G. Hanger, W. C. Howell and A. W. Johnson, *J. Chem. Soc.* 1592 (1957).
[12] E. Bullock and A. W. Johnson, *J. Chem. Soc.* 1602 (1957).

formulation[7] of this compound as a 2:5-dihydroxy-*p*-benzoquinone carboxamide is untenable for cinnabarin does not contain an amide group.[5] Rather the oxamic acid, acid, obtained on treatment of cinnabarin with alkaline hydrogen peroxide solution,[6] results from the same hydrolytic fission of the quinonoid ring as yields pyruvic acid, oxalic acid, carbon dioxide and ammonia.*,[5]

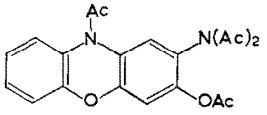
Evidence for the *ortho*- relationship of the 1-carboxy and 2-amino substituents on the quinonoid ring is provided by a re-interpretation of the experimental data[2,5] on the reductive acetylation of cinnabarin and its derivatives (cf. Part II).[5] In the conversion of cinnabarin into triacetylanhydrodihydrocinnabarin (VIII), and of methyl cinnabarin (VII) into methyl triacetyldihydrocinnabarin (IX), three acetyl groups have been introduced into these products. As O-acetylcinnabarin and methyl O-acetylcinnabarin are also converted into the triacetyldihydro- derivatives (VIII) and (IX) respectively, one acetyl group is accommodated on the primary alcoholic group at C_9.[7] Methyl cinnabarin and methyl O-acetylcinnabarin do not form the anhydro compound, hence the hydroxyl group involved in this cyclisation must be that of the carboxylic acid, and the anhydro compound is then the oxazine (VIII) formed by a an *ortho*-hydroxyl (or potential hydroxyl) group with an adjacent acetylamino ring closure of the 1-carboxyl with the 2-acetylamino group. Previously cyclisation of substituent was considered,[5] but it is now shown that no oxazole formation results from the reductive acetylation of 2-aminophenoxazin-3-one. The third acetyl group, placed on the hydroxyl group now located in position 3, stabilises the reduced products (VIII) and (IX).



2-Aminophenoxazin-3-one, on similar treatment with zinc dust, acetic anhydride and a trace of pyridine, is converted into a tetra-acetyldihydro- compound formulated as (XII). Thus the triacetyldihydro- derivative (IX), from methyl cinnabarin, is

* The fact that cinnabarin shows a small acetyl value (approximately one third to one quarter of an acetyl group) and that the results of acetyl determinations on many of the derivatives of cinnabarin are high, is attributed to this hydrolytic fission.

comparable with a diacetyldihydro- derivative of the unsubstituted aminophenoxazone. Presumably additional acetylation of methyl cinnabarin* is prevented by the steric effects of the substituents in position 1 ($—COOCH_3$) and position 9 ($—CH_2OCOCH_3$). The structures of the reduced products (VIII) and (IX) are confirmed



(XII)

by their oxidation, with nitrous acid in dilute hydrochloric acid, to give the acidic O:N-diacetylcinnabarin (X, with the re-addition of the elements of water), and its methyl ester (XI), respectively. Only one acetyl group, that in position 3, is removed in each case and the infra-red data shows the loss of the appropriate carbonyl absorption $\sim$1770 cm^{-1}. In addition the ultra-violet spectrum of methyl O:N-diacetylcinnabarin (XI) (max at 237 and 404 mμ; log ε 4·48 and 4·40) closely resembles that of 2-acetylaminophenoxazin-3-one (max at 241 and 404 mμ; log ε 4·56 and 4·45).

The infra-red spectrum of cinnabarin† and of O-acetylcinnabarin ("Nujol" mull) show no features in the 3 mμ region characteristic of a carboxylic acid, an observation that previously led us to attribute the acidity of the parent compound to a phenolic group.[2] However, a carboxyl group in position 1 may hydrogen bond with the ring nitrogen atom and with the 2-amino group, whence cinnabarin is represented as (Ia). (Flett[14] attributes poor absorption of the hydroxyl group in 3-amino-2-naphthoic acid to internal hydrogen bonding). The band at 3505 cm^{-1} in cinnabarin is then assigned to the alcoholic hydroxyl group and, in agreement, is absent from O-acetylcinnabarin Further the band at 1672 cm^{-1} in cinnabarin, and at 1691 cm^{-1} in O-acetylcinnabarin, is assigned to the carbonyl group of the carboxylic acid, whilst that at 1727 cm^{-1} in the latter compound is characteristic of the acetate ester. Methyl cinnabarin (VIIa), which showed absorption at 1656 cm^{-1} ("Nujol" mull),[2] exhibits bands at 1676 cm^{-1} and 1654 cm^{-1} in solution (carbon tetrachloride), and the higher frequency is assigned to the ester carbonyl group which is hydrogen bonded wih the 2-amino group, (cf. absorption of methyl N-methylanthranilate at 1685 cm^{-1} in carbon tetrachloride).[15] The lower frequency (1654 cm^{-1}) is that of the phenoxazin-3-one carbonyl group.[5,16] Methyl O-acetylcinnabarin exhibits comparable bands at 1675 cm^{-1} and 1655 cm^{-1}, and in agreement with Gripenberg,[6] the additional absorption at 1745 cm^{-1} is assigned to the acetate carbonyl group. The consistently low values recorded for the NH stretching modes of the amino group in cinnabarin[2] and in O-acetylcinnabarin,[2] and in methyl cinnabarin (3441 cm^{-1}, 3319 cm^{-1} in carbon tetrachloride) and in methyl O-acetylcinnabarin (3446 cm^{-1}, 3319 cm^{-1} in carbon tetrachloride), parallel the low values already noted for the carbonyl absorption of the acid or ester in these

* The compound, m.p. 176°, which has been isolated as a by-product of the reductive acetylation of methyl cinnabarin,[13] is probably a tetra-acetyldihdro- derivative.

† Infra-red absorption spectra ("Nujol" mull) for cinnabarin and its derivatives are recorded in Part I.[2] (An absorption at 1799 cm^{-1} originally reported[2] for triacetylanhydrodihydrocinnabarin (VIII) has not been duplicated and is attributed to an artifact).[13] Many of these compounds are insufficiently soluble for complete examination in solution (chloroform or carbon tetrachloride).

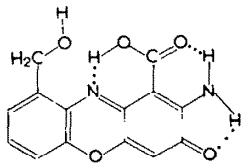
[13] J. R. Tetaz, Thesis, N.S.W. University of Technology (1955).

[14] M. St. C. Flett, *J. Chem. Soc.* 962 (1951).

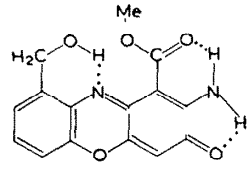
[15] R. S. Ramussen and R. R. Brattain, *J. Amer. Chem. Soc.* **71**, 1073 (1949).

[16] H. Musso and H. G. Matthies, *Chem. Ber.* **90**, 1814 (1957).

compounds, and further support the hydrogen bonded formulations (Ia and VIIa). In contrast, but in agreement with structure (XIII), decarboxycinnabarin which lacks the carboxyl group in position 1, exhibits higher NH stretching values (3514 cm^{-1}, 3392 cm^{-1} in chloroform). Suprisingly methyl cinnabarin and decarboxycinnabarin



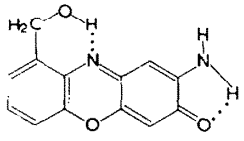
(Ia)



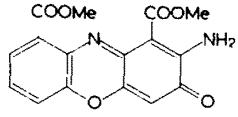
(VIIa)

show no absorption attributable to the alcoholic hydroxyl group; hence on esterification, or on elimination of the carboxyl group in position 1, the alcoholic group may hydrogen bond with the ring nitrogen atom (see VIIa and XIII). The orientation of the substituents—1-carboxy, 2-amino, and 9-hydroxymethyl—is thus strongly supported by the infrared spectroscopic data.

Confirmation of structure (I) for cinnabarin is provided by the transformation of methyl cinnabarin into the known 1:9-dimethoxycarbonyl-2-aminophenoxazin-3-one (XIV). As the oxidation has been achieved under mild conditions, employing successively manganese dioxide in chloroform, and chromium trioxide-sulphuric acid in acetone, direct evidence is provided for the presence of the hydroxymethyl substituent in position 9. The yield of the 9-carboxy-1-methoxycarbonyl-intermediate is small, much of the methyl cinnabarin being recovered, and the unexpected resistance to oxidation shown by this aromatic carbinol may be attributed to the "locked" position of the hydrogen atom of the hydroxyl group (see structure VIIa). Such strong intramolecular hydrogen bonding is an especial feature of cinnabarin and its derivatives.



(XIII)



(XIV)

EXPERIMENTAL

Melting points are uncorrected. Light petroleum has b.p. 60–80°. Neutralised alumina is prepared by washing Peter Spence (grade H) alumina with methanol–acetic acid (9 : 1), triturating it with hot methanol until all washings are neutral, and drying it at 150–200°. Carbon, hydrogen and nitrogen microanalyses are by Dr. E. Challen of this University, additional microanalyses by C.S.I.R.O. Microanalytical Laboratory (Melbourne), and infra-red spectra by Mr. I. Reece.

Decarboxycinnabarin.[1] Cinnabarin (100 mg) in methanolic potassium hydroxide solution (20 ml, 20%) was heated at 100° on the steam bath. After 3 min the dark red solution was cooled rapidly to room temp, acidified (2 N H_2SO_4), and extracted with chloroform (10 × 50 ml). The chloroform layer was washed (2% sodium

carbonate solution, then water), dried (anhydrous sodium sulphate), and on evaporation gave a red-brown product (40 mg). After repeated recrystallisation from chloroform, *decarboxycinnabarin* was isolated as dark red needles, m.p. >250° with decomposition. (Found: C, 64·1; H, 4·2; N, 11·4%. $C_{13}H_{10}O_3N_2$ requires: C, 64·5; H, 4·2; N, 11·6%).

Reductive acetylation of 2-aminophenoxazin-3-one. 2-Aminophenoxazin-3-one (0·70 g) in acetic anhydride (30 ml) and pyridine (1 ml) was heated under reflux with excess zinc dust for 10 min. The zinc was filtered off, and the pale yellow solution poured onto ice when a colourless solid slowly precipitated. This reductive acetylation *product* (0·70 g) was recrystallised from benzene-light petroleum, and finally obtained as colourless needles, m.p. 184–185°, from ethanol. (Found: C, 62·9; H, 4·7; N, 7·4; Ac, 44·7; $C_{20}H_{18}O_6N_2$ requires: C, 62·8; H, 4·8; N, 7·3; 4Ac, 45·0%). No evidence of oxazole formation was noted.

*Oxidation of methyl cinnabarin.** Methyl cinnabarin (30 mg) in chloroform (40 ml) was shaken for 72 hr with manganese dioxide (300 mg), prepared according to the method of Attenburrow *et al.*[17] The manganese dioxide was then filtered off and well washed with chloroform. The combined filtrates, on evaporation, yielded an orange product which was dissolved in acetone (120 ml), then the solution was treated (under nitrogen) with one drop of chromium thioxide-sulphuric acid reagent.[18] Excess of the oxidant was still present after 1 hr at room temp, when the mixture was poured into water. The product was extracted into chloroform and the acidic material separated by treatment with 1% sodium carbonate solution. The chloroform layer yielded methyl cinnabarin† (25 mg), m.p. 236–238° with decomposition, undepressed on admixture with the starting material. The acidic product, after re-extraction into chloroform, was treated with an excess of diazomethane in ether, and the crude orange ester so obtained was purified by chromatography on neutralised alumina, being eluted with benzene–chloroform (3 : 1). The product, 1:9-dimethoxycarbonyl-2-aminophenoxazin-3-one, was finally isolated as bright orange needles (2·0 mg), m.p. 224–226° with decomposition, undepressed on admixture with an authentic specimen.[11] The identity of the oxidation product with the synthetic specimen was confirmed by comparison of the spectra in the ultra-violet (acid, neutral and alkaline)[11] and in the infra-red region and by paper chromatography.‡

Acknowledgments—We thank Dr. R. Lemberg, F.R.S., for his interest and for facilities afforded one of us (P. S. C.), and Professor A. W. Johnson for a specimen of 1:9-dimethoxycarbonyl-2-aminophenoxazin-3-one. A National Health and Medical Research Council grant (to P. S. C.) is gratefully acknowledged.

* We are indebted to Mr. F. W. Whitfield for the paper chromatography of methyl cinnabarin, 1:9-dimethoxycarbonyl-2-aminophenoxazin-3-one and the oxidation product.

† R_f 0·59, in water–*n*-butanol–*iso*proponal–propionic acid (75 : 5 : 5 : 15), and R_f 0·56, in water–*n*-butanol–*iso*propanol–piperidine (75 : 5 : 5 : 15), in each case identical with that of the starting material.

‡ R_f 0·73, in water–*n*-butanol–*iso*propanol–propionic acid (75 : 5 : 5 : 15), and R_f 0·83, in water–*n*-butanol–*iso*propanol–piperidine (75 : 5 : 5 : 15).

[17] J. Attenburrow, A. F. B. Cameron, J. H. Chapman, R. M. Evans, B. A. Hems, A. B. A. Jansen and T. Walker, *J. Chem. Soc.* 1094 (1952).

[18] C. Djerassi, R. R. Engle and A. Bowers, *J. Org. Chem.* **21**, 1547 (1956).