## 106. *The Chemistry of Mould Metabolites. Part I. Isolation and Characterisation of a Red Pigment from* Coriolus sanguineus *(Fr.).*

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Naturally occurring and the artificially grown *Coriolus sanguineus* contain a compound, $C_{14}H_{10}O_5N_2$, believed identical with Lemberg's polystictin and Gripenberg's cinnabarin, which is shown by chemical and spectroscopic studies to contain an acidic hydroxyl group, an amide group, and possibly an ether link. The basic skeleton is of a polycyclic unsaturated type containing a quinonoid system and heterocyclic nitrogen.

A bright-red wood-rotting fungus, *Coriolus sanguineus* (Fr.), is a conspicuous feature on decaying pines and eucalypts in the natural bushlands close to Sydney. This fungus would seem to be cosmopolitan in distribution and, as there is considerable variation in its general appearance and colour, it is not surprising that it is known by many synonyms: *Polyporus cinnabarinus* Fr., *P. sanguineus*, Fr., *P. coccineus*, Fr., *P. puniceus*, Kalch; *Polystictus cinnabarinus* (Jacq.), *P. sanguineus*, L., *P. semi-sanguineus*, Lloyd, *Trametes cinnabarina* (Jacq.), Fr. In the herbarium of the Royal Botanical Gardens, Kew, Australian specimens are placed under the names *Polystictus sanguineus* and *P. cinnabarinus*, but it is the opinion of Cunningham (*Proc. Linn. Soc. N.S.W.*, 1950, **75**, 240), who has recently re-examined the type specimens, that the collections should be merged under the name of *Coriolus sanguineus* (Fr.).

Recently, as the result of one afternoon's collecting in the vicinity of Sydney, over 5 kg. of fresh fungus were obtained. The same red pigment is isolated from cultures grown on synthetic medium (described below) as from the natural fungus. Lemberg [Report of Nat. Health and Med. Res. Council, C'wealth of Australia (Canberra), 1946, p. 12; *Aust. J. Exp. Biol. Med. Sci.*, 1952, in the press] reported the isolation of a red nitrogenous pigment, polystictin, from the Australian fungus, *Polystictus cinnabarinus*, and, in Finland, Gripenberg (*Acta Chem. Scand.*, 1951, **5**, 590) has described the isolation of the red pigment cinnabarin from *T. cinnabarina*. We therefore record our studies on the chemistry of the red mould metabolite from *C. sanguineus* (Fr.). It appears that these compounds are the same, and we retain the name polystictin.

Polystictin is a red crystalline compound, $C_{14}H_{10}O_5N_2$, which decomposes above 320°. No satisfactory determination of the molecular weight of polystictin or its simple derivatives has yet proved possible, but on present chemical evidence, and in agreement with Gripenberg (*loc. cit.*), we believe the above formula a likely one. The pigment is extremely insoluble in most organic solvents, readily soluble in cold concentrated sulphuric acid to a deep violet solution, whilst in cold 2N-sodium hydroxide the colour is violet, changing to a dull red. Polystictin is slowly soluble in cold 3N-sodium carbonate and in saturated sodium hydrogen carbonate solution on slight warming. It can be regenerated from alkaline solution.

With diazomethane, or with methyl iodide or methyl sulphate and potassium carbonate in acetone, polystictin gives a monomethyl ether (a red acidic crystalline compound, possibly $C_{15}H_{11}O_6N$, is obtained as a by-product with methyl sulphate and potassium carbonate) which, in contrast to polystictin, is very readily soluble in dilute mineral acid and insoluble in cold 2N-sodium hydroxide. With acetic anhydride–sulphuric acid it gave an acetate,

insoluble in dilute mineral acid but slowly soluble in cold 3N-sodium carbonate. Thus, polystictin contains a hydroxyl group, to which we attribute its acidity. *O*-Methylpolystictin gives no colour with triphenyl borate in dioxan, whereas polystictin shows an orange-red solution after several minutes, suggesting that the free hydroxyl group is *peri* to a carbonyl system (Anderson, O'Brien, and Reuter, *Anal. Chem. Acta*, 1952, in the press).

A pale yellow " diacetylanhydrotetrahydrocinnabarin, $C_{18}H_{16}O_6N_2$," m. p. 200—202°, was obtained by Gripenberg on reductive acetylation of cinnabarin. We isolated an apparently identical compound, m. p. 200—204°, by this method but an acetyl estimation shows the presence of three such groups, and we believe this compound is triacetylanhydrodihydropolystictin, $C_{20}H_{16}O_7N_2$. Reductive acetylation of *O*-methylpolystictin gave a pale yellow, neutral, triacetyldihydro-*O*-methylpolystictin, without loss of water. We assume that a quinonoid system was reductively acetylated, and that the molecule of water lost from polystictin involved the hydroxyl group which was methylated. We have still to account for an additional acetyl group present in the reduction products.

Polystictin is reductively benzoylated by benzoyl chloride and sodium dithionite in cold sodium hydroxide solution, to a tetrabenzoyldihydropolystictin insoluble in cold mineral acid and alkali. The quinonoid structure is also supported by the ease with which polystictin is reduced with zinc and dilute acid and re-oxidised on exposure to the atmosphere. There is no apparent reaction between polystictin and *o*-phenylenediamine.

Ammonia and carbon dioxide are evolved on hydrolysis of polystictin with warm aqueous barium hydroxide, followed by acidification. These products are presumably derived from an amide (or imide) group, which is converted into a carboxylic acid, capable of ready decarboxylation.

The following infra-red spectroscopic evidence confirms and supplements the chemical examination. The spectrum of solid polystictin in the high-frequency region (Fig. A), which is the same irrespective of the source of the compound, consists of three sharp bands. That at 3505 cm.$^{-1}$ is normally associated with the hydroxyl group; the other two, of the longer wave-length, are assigned to the two hydrogen atoms attached to a nitrogen of a primary amine or of an unsubstituted amide (Williams, Hofstadter, and Herman, *J. Chem. Phys.*, 1939, **7**, 802). Around 2900 cm.$^{-1}$, where one normally expects to find the –C–H bands associated with saturated carbon, polystictin is transparent, indicating an almost, if not complete, lack of such groups. However, the presence of unsaturated =C–H groups is shown by the band at 3165 cm.$^{-1}$, suggesting an unsaturated polycyclic system.

There are two bands, at 1672 and 1653 cm.$^{-1}$, of which the former is the more intense, and such bands (of similar intensity) have been assigned to the >C=O and $-NH_2$ groups, respectively, of an amide (Randall, Fowler, Fuson, and Dangl, " Infra-red Determination of Organic Structures," New York, 1949, p. 10). A simple carbonyl group conjugated to an aromatic nucleus, as in acetophenone, would explain the 1672-cm.$^{-1}$ band but there is no chemical evidence for such, nor are there spectroscopic features in the 3- and 4-$\mu$ regions characteristic of a carboxylic acid. It is more difficult to rule out the possibility of an unconjugated –C=N– system; were it conjugated with –C=C– groupings, as is probable, it would move to longer wave-lengths and become indistinguishable as part of the ring vibrations between 1600 and 1480 cm.$^{-1}$ (Angyal and Werner, *J.*, 1952, 2911; Blout, Fields, and Karplus, *J. Amer. Chem. Soc.*, 1948, **70**, 194). Both the 3- and the 6-$\mu$ bands therefore support the suggestion that an amide group is present.

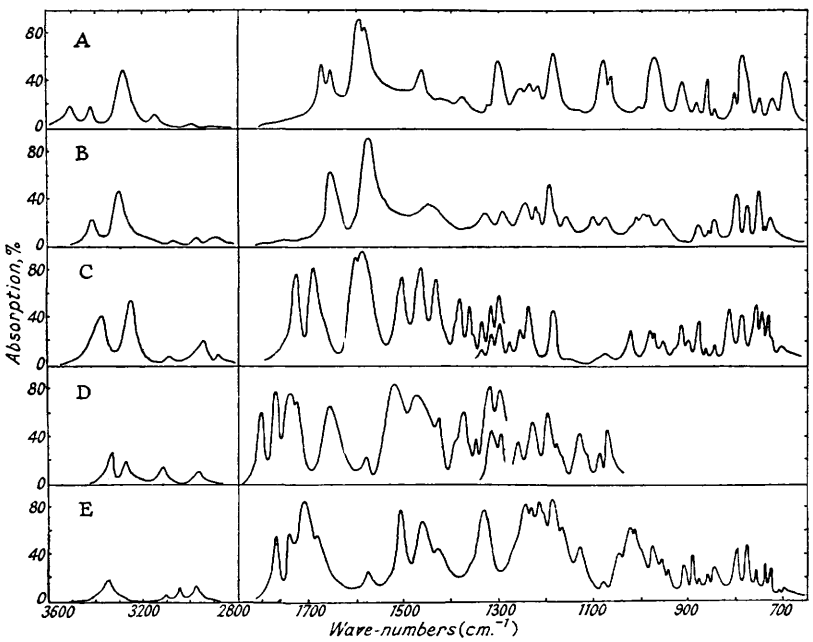
Polystictin gives intense bands at 1600 and 1586 cm.$^{-1}$, which are normally associated with the ring vibrations of unsaturated cyclic systems, including quinonoid structures. Further, the absence of bands at 1380 and 1450 cm.$^{-1}$ confirms the lack of $-CH_2$ and $-CH_3$ groups while the 1466-cm.$^{-1}$ band is suggestive of the bending vibration of hydrogen attached to unsaturated carbon (Thompson and Torkington, *Trans. Faraday Soc.*, 1945, **61**, 250).

The spectrum of *O*-methylpolystictin (Fig. B) shows the expected conversion of a hydroxyl into a methoxyl group and that of *O*-acetylpolystictin (Fig. C) indicates the conversion into an acetoxy-group (cf. Barnes, Gore, Liddel, and Williams, " Infra-Red Spectroscopy," New York, 1944, pp. **67**, **68**). In all three compounds there are strong

bands in the 700—800-cm.$^{-1}$ region, usually associated with nuclear substitution, but it remains for the basic system to be identified before information can be obtained from these bands.

The most striking feature of the spectra of the two compounds produced by reductive acetylation (Figs. D and E) is the change in the ring vibrations. The strong bands around 1600 cm.$^{-1}$ have been replaced by bands of lower frequency, between 1400 and 1600 cm.$^{-1}$, which indicate a new common structure. The new system is unusual in that strong bands are now present around 1500 cm.$^{-1}$ rather than higher, and it may be significant that a shift to longer wave-lengths has been observed in heterocyclic nitrogenous compounds or where several rings are fused together (Canon and Sutherland, *Spectrochim. Acta*, 1951, **4**, 373). Reductive acetylation also introduced a number of bands above 1700 cm.$^{-1}$, three of which appear to be due to common structures, namely, at 1722, 1736, and 1769

*Infra-red spectra of polystictin and some derivatives.*




A, *Polystictin*. B, O-*Methylpolystictin*. C, O-*Acetylpolystictin*. D, *Reduced acetylated polystictin*. E, *Reduced acetylated O-methylpolystictin*.

cm.$^{-1}$ in the reduced polystictin and at 1709, 1730, and 1768 cm.$^{-1}$ in the reduced *O*-methylpolystictin, and these are assigned to acetate groups. There is a further band at 1799 cm.$^{-1}$ in the reduced polystictin indicative of the carbonyl group in a β-lactam or other strained ring system. In all the spectra a sharp band of medium intensity near 1190 cm.$^{-1}$ may be assigned to an ether linkage, unaffected by any of the reactions described.

It is concluded that polystictin contains an amide group, and an acidic hydroxyl group most likely *peri* to a carbonyl group, itself being part of a quinonoid structure. In the absence of a positive test for a nitrogenous system we believe the second nitrogen atom to be heterocyclic and that this is acetylated during the reductive acetylation. The fifth oxygen atom is accommodated as an inert ether link. Finally, in the absence of methylene and methyl groups, we assume the remaining carbon and hydrogen to be part of the fundamental unsaturated polycyclic system. Degradative studies on polystictin and its derivatives are in progress and will be reported later.

## Experimental

*The Fungus*.—The sporophores varied in colour from a bright vermilion to a very dark blood-red. The fruiting bodies are commonly laterally attached throughout, but many speci-

mens are thin and coriaceous and attached by a short stem, such characteristics being generally considered to be typical of the tropical forms, usually described as *Polystictus sanguineus* L. Our experience indicates much variation in the characteristics of the pure cultures derived from different forms of the organism, and some current investigations are directed towards the detection of corresponding variations in metabolism.

*Isolation of Polystictin.*—(a) *From naturally occurring* Coriolus sanguineus. The following is typical of numerous isolations. The dried, milled fungus (8 × 50 g.) was extracted (Soxhlet) with acetone. To the vermilion solid (3·45 g.) which separated from the extracts was added the waxy material (2·5 g.) obtained on evaporation of the combined filtrates. The total solid (5·95 g.) was washed with hot alcohol (2 × 200 ml.), giving crude polystictin (3·5 g.). Repeated treatment of this (1·0 g.) with boiling dioxan gave *polystictin* (0·3 g.) as red needles, decomp. >300° (Found : C, 58·9; 58·3, 58·3; H, 3·9, 3·6, 3·4; N, 9·0, 9·4, 9·9. $C_{14}H_{10}O_5N_2$ requires C, 58·7; H, 3·4; N, 9·8%), difficultly soluble in organic solvents (acetone, 0·04% at 20°; dioxan, 0·1% at 100°), fairly soluble in cold concentrated hydrochloric acid.

(b) *From fungi cultivated on artificial medium.* The following pure fungal cultures were grown as surface mats on a modified William–Saunders medium (*Biochem. J.*, 1934, **28**, 1887) containing " Marmite " (0·1 g. per l.) with glucose (50 g. per l.) in place of sucrose, and glycine (2 g. per l.) in place of asparagine. All inoculations were made with slips of mycelium-covered agar from stock slope cultures on Sabaraud's medium.

(i) Culture 105A (Forest Products Research Laboratories Collection, Princes Risborough, England), described as *Polystictin sanguineus* (L.) Mey, was grown in 66 flasks each containing 500-ml. of medium. Approx. 10 g. of cotton wool were placed in each flask to form an " island " to support initial growth since the mycelium of this organism tends to sink. The organism was grown for 257 days at 25°, followed by 543 days at room temperature; the mycelium was a light vermilion colour with some dark-red patches and a few fluffy white areas; there were numerous abortive fructifications. At the time of harvesting, the mycelium could be readily separated from the cottom wool which was stained a light brown, and did not appear too much degraded since it retained its mechanical strength. The yield of vacuum-dried mycelium was 4·5 g. per flask. This mycelium (175 g.), on extraction, gave a brown solid (1·6 g.) and a further tarry residue (0·9 g.) from the evaporation of filtrates. Polystictin (0·16 g.) was finally isolated as orange-red prisms, m. p. 300—320° (decomp.), from dioxan (600 ml.) (Found : C, 58·8, 58·9; H, 3·9, 3·8; N, 10·2, 10·0%).

[With Anne Edwards and J. G. Wilson] (ii) Culture 6D (Division of Forest Products Collection, Melbourne), described as *T. cinnabarina*, and originally isolated in 1947 from a sporophore on *Eucalyptus regnans*, was grown at 30° for 49 days in 102 flat-sided bottles, each containing 150 ml. of medium. The yield of the light vermilion mycelium, washed and air-dried, was 0·54 g. per flask. This mycelium (55 g.) on extraction gave a red waxy solid (1·65 g.), yielding polystictin (0·1 g.) as light red prisms, decomp. >300° (Found : C, 58·0; H, 3·8; N, 9·5%).

[With Anne Edwards and J. G. Wilson] (iii) Culture 6F (D.F.P.), from the Department of Agriculture, Ottawa (Culture Collection No. 17765), described as *Polyporus cinnabarinus*, was originally isolated in 1946 from a sporophore on *Acer rubrum*. It was grown as in (ii) (103 bottles) for 41 days, the yield being 0·58 g. per flask. This mycelium (60 g.) was treated as above, yielding polystictin (0·01 g.) which crystallised in light red-brown prisms (from dioxan), decomp. >300° (Found : C, 57·8; H, 3·5%).

*Acetylation of Polystictin.*—Polystictin (0·5 g.) was added to sulphuric acid (36N; 1 ml.) and acetic anhydride (10 ml.) and after 10 min. the black solution was poured on ice. The resulting red solid was extracted with chloroform and the extract treated with sodium hydrogen carbonate solution, removing a red fluorescent compound. O-*Acetylpolystictin* (0·13 g.), isolated on evaporation of the washed and dried chloroform solution, crystallised as red needles, m. p. 250—252° (decomp.), from acetone–light petroleum (Found : C, 58·1; H, 3·5; N, 8·4. $C_{16}H_{12}O_6N_2$ requires C, 58·5; H, 3·7; N, 8·5%). Acetylation in aqueous alkali or pyridine failed.

O-*Methylpolystictin.*—(*a*) Polystictin (1·5 g.) was suspended in dry acetone (1500 ml.) with potassium carbonate (30 g.) and methyl sulphate (9 ml.) and refluxed for 7 hours. The crude product, after filtration and evaporation of acetone, was taken up in chloroform. Extraction of the chloroform solution, with sodium hydrogen carbonate solution, followed by acidification, gave a *substance* (0·02 g.) as red prisms, m. p. >200° (decomp.), from ethyl acetate–light petroleum (Found : C, 57·7, 59·2; H, 3·5, 3·7; N, 4·7, 4·3. $C_{15}H_{11}O_6N$ requires C, 59·8; H, 3·7; N, 4·6%). O-*Methylpolystictin* (0·4 g.) was obtained on evaporation of the

washed and dried chloroform extract and crystallised as orange-red prisms, m. p. 200—202° (decomp.), from ethyl acetate–light petroleum (Found : C, 60·2; H, 3·8; N, 9·1; OMe, 8·3. $C_{14}H_9O_4N_2$·OMe requires C, 60·0; H, 4·0; N, 9·3; OMe, 10·3%).

(*b*) Methylation as above, with methyl iodide instead of methyl sulphate, gave *O*-methylpolystictin (0·3 g.), orange-red prisms, m. p. 222—223° (decomp.), from acetone–light petroleum or from ethyl acetate–light petroleum (Found : C, 60·2; N, 4·0; N, 9·5%).

(*c*) Methylation of polystictin (2 g.) with ethereal diazomethane in methanol (30 ml.) and water (10 ml.) gave unchanged polystictin (1 g.) and *O*-methylpolystictin (0·04 g.), which crystallised as orange-red prisms, m. p. 236—240° (decomp.), from ethyl acetate (Found : C, 60·3; H, 4·2; H, 9·3%). It is extremely difficult to obtain reproducible m. p.s for *O*-methylpolystictin, the decomposition temperature depending on the rate of heating.

*Reductive Acetylation of Polystictin.*—Polystictin (0·7 g.) with zinc dust and acetic anhydride (20 ml.) in the presence of pyridine yielded a *substance* (isolated by Gripenberg's method) as yellow plates, m. p. 200—204°, from ethyl acetate–acetone (Found : C, 59·7; H, 3·7; N, 7·5%). Sublimation at 200—220°/7 × $10^{-3}$ mm. gave a yellow solid, m. p. 195°, mixed m. p. with compound above 196° (Found : C, 60·5, 60·1; H, 4·0, 4·0; N, 7·3, 7·4; 3Ac, 33·7. $C_{20}H_{16}O_7N_2$ requires C, 60·6; H, 4·0; N, 7·1; 3Ac, 32·6%).

*Reductive Acetylation of* O-*Methylpolystictin.*—Treatment of *O*-methylpolystictin (0·6 g.) as described for polystictin gave a yellow *substance* (0·4 g.), crystallising in yellow needles, m. p. 156°, from acetone–light petroleum (Found : C, 59·0, 58·2; H, 5·2, 4·6; N, 6·2, 6·3; OMe, 6·5; 3Ac, 34·4. $C_{21}H_{20}O_8N_2$ requires C, 58·8; H, 4·7; N, 6·5; OMe, 7·2; 3Ac, 31·5%).

*Regeneration of Polystictin from Alkaline Solution.*—Polystictin (0·14 g.) was dissolved in cold dilute sodium hydroxide solution, and the purple-red filtered solution acidified at 10—20° with concentrated hydrochloric acid. The red solid which separated recrystallised from dioxan (300 ml.) as red needles (0·02 g.) (Found : C, 58·1; H, 3·6; N, 9·4%).

*Reductive Benzoylation of Polystictin.*—Addition of benzoyl chloride (10 ml.) to polystictin (1 g.) dissolved in dilute sodium hydroxide solution containing excess of sodium dithionite gave a yellow solid on shaking. The crude product was extracted with chloroform, and the solution washed with sodium hydrogen carbonate solution. Evaporation of the chloroform layer gave the *substance* (0·15 g.) as an oil, which on repeated crystallisation, gave an ill-defined solid, m. p. 98 104°, from ethanol [Found : C, 72·4, 71·3; H, 4·1, 3·9; N, 3·3, 4·0. $C_{14}H_8O_5N_2(C_6H_5{\cdot}CO)_4$ requires C, 71·6; H, 4·0; N, 4·0%].

*Alkaline Degradation of Polystictin.*—Polystictin (1·5 g.) was refluxed with 2N-barium hydroxide (100 ml.) for 1 hour, and the alkaline distillate was collected in N-hydrochloric acid. After evaporation of the distillate and addition of chloroplatinic acid, yellow prisms of ammonium chloroplatinate separated [Found : N, 6·3. Calc. for $(NH_4)_2PtCl_6$ : N, 6·3%]. Acidification of the residual barium hydroxide solution and distillation liberated carbon dioxide, identified as barium carbonate.

*Identifications.*—Analyses of polystictin and its derivatives vary considerably within replicates and much difficulty is also experienced with m. p.s of other than the reduced acetylated compounds. However, infra-red spectroscopic examination of all samples of polystictin, *O*-methylpolystictin, and other derivatives from their respective sources confirmed the identities in each case.

*Infra-red Spectra.*—Infra-red spectra of the different samples of polystictin and its derivatives were recorded with a Perkin-Elmer Model 12-C spectrometer with sodium chloride optics. This instrument was calibrated against ammonia, water vapour, and carbon dioxide from the data of Oetjen, Kao, and Randall (*Rev. Sci. Instr.*, 1942, **13**, 515) and comparison with the data used indicated a resolving power of about 2 cm.$^{-1}$ at 1000 cm.$^{-1}$, 5 cm.$^{-1}$ at 1700 cm.$^{-1}$, and 20 cm.$^{-1}$ at 3000 cm.$^{-1}$. Each sample was run as a paste with paraffin oil according to the method of Randall *et al.* (*op. cit.*), and also as a mull with tetrachloroethylene which has been found suitable to cover the C–H regions obscured by the paraffin oil. The percentage transmission recorded may not correctly indicate the percentage absorption to any better than 5% because of differential scattering from the solid particles.

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