# Chemistry of Fungi. Part III.[1] Constituents of *Coriolus sanguineus* Fr.

By R. C. Cambie and P. W. Le Quesne

In addition to mixed crystals of ergosterol and ergosta-7,22-dien-3β-ol, ergosta-7,22-dien-3-one, polyporenic acid C, n-heptanol, n-octanol, mannitol, trehalose, cinnabarin, and the n-alkanes from $C_{24}$ to $C_{31}$, have been identified as constituents of the fungus, *Coriolus sanguineus* Fr.



THE fungus *Coriolus sanguineus* Fr. which is also known under a number of synonyms [2] [*e.g.*, *Trametes cinnabarina* (Jacq.) Fr., *Polyporus cinnabarinus* Fr.] contains the red pigment cinnabarin [3–5] and the related cinnabarinic acid.[6] Extensive investigation by two groups of workers [3–8] has shown that cinnabarin is 2-amino-9-(hydroxymethyl)-3-oxo-3*H*-phenoxazine-1-carboxylic acid and that cinnabarinic acid is 2-amino-3-oxophenoxazine-1,9-dicarboxylic acid. The presence of a third pigment, polystictinin, has been reported by Lemberg.[3]

We now report the colourless compounds obtained from the fungus. The principal constituent (0·32% yield) of an ether-soluble neutral fraction had m. p. 171—172° and possessed typical ergostadienoid ultraviolet absorption.[9] Quantitative estimation from optical rotation and extinction coefficients indicated that the "ergosterol-like" compound in the most highly purified material was present to the extent of only 3%. This material was obtained by direct crystallisation, by chromatography of non-saponifiable residues, or directly from the fungus by extraction with light petroleum. In each case the melting points, optical rotations, and extinction coefficients were identical and showed no change after repeated crystallisation and chromatography. Reduction of the material with sodium in ethanol [10] gave ergosta-7,22-dien-3β-ol only, while preferential decomposition of ergosteryl benzoate with bromine in the mixed benzoates derived from the material,[11] gave ergosta-7,22-dien-3β-yl benzoate. Thus, it appeared to be a mixture of ergosterol and its 5,6-dihydro-derivative. This was supported by the mass spectrum which showed all the peaks associated with [12] or expected for [13] the fragmentation of the side-chain of ergosterol and in each case a corresponding and stronger peak two mass units higher.

The presence of ergosterol and "ergosterol-like" compounds in extracts of fungi has frequently been recorded and in most cases such mixtures were difficult to separate.[14] In particular, Barton and Cox [15] showed that "neosterol" [16] was a mixture of ergosterol (75·5%) and 5,6-dihydroergosterol (24·5%) whose composition was unaltered by fractional crystallisation of the sterol or its derivatives. Wieland and his co-workers,[17] however, isolated ergosterol and 5,6-dihydroergosterol as well as the "neosterol" mixture by fractional crystallisation of the benzoates from yeast fat sterols. Although our attempts to separate ergosterol and 5,6-dihydroergosterol by gas–liquid chromatography gave no resolution and the natural mixture formed mixed crystals of an acetate containing 3% of ergosteryl acetate, fractional crystallisation of the benzoate afforded pure 5,6-dihydroergosteryl benzoate. The identical properties (m. p., ultraviolet and infrared spectra, optical rotation, and integrated n.m.r. spectra) of a synthetic mixture of 5,6-dihydroergosterol containing 3% of ergosterol confirmed the composition of the mixed crystals from the fungus.

A minor constituent of the neutral fraction was a ketone, m. p. 183—185°, identified as erogsta-7,22-dien-3-one from its n.m.r. spectrum [18] and comparison with a sample preparared from ergosta-7,22-dien-3β-ol by Bladon, Henbest, and Wood's method.[19] The ketone has been isolated previously from the fungi *Fomes fomentarius* [20] and *F. applanatus*.[21] An acidic fraction from *C. sanguineus* contained mainly pigments but methylation followed by alumina chromatography gave a trace of a hydroxyketo-ester, $C_{32}H_{48}O_4$, m. p. 196—198°, which possessed a typical 7,9(11)-diene chromophore.[22] Although insufficient was obtained for a detailed investigation its properties corresponded with those [23] of methyl polyporenate C and a mixed melting point showed no depression.

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Accompanying the neutral sterols was a mixture of three low-molecular-weight alcohols in which n-heptanol and n-octanol were identified as their acetates by comparative gas–liquid chromatography. These alcohols do not appear to have been previously recognised as fungal metabolites but n-octanol has been found to accompany sterols in the saponified neutrals of the catus *Lophocereus schottii*.[24] Initial fractions from the chromatography of neutral non-saponifiable material gave a waxy fraction, the principal component of which appeared to be octacosane. Gas–liquid chromatography of the wax, however, showed the presence of all the n-alkanes from $C_{24}$ to $C_{31}$. n-Octacosane is the only alkane previously reported from a fungus.[25]

An aqueous extract of the fungus gave a carbohydrate fraction which was purified by Lindberg and Wickberg's method.[26] Thin-layer chromatography showed the presence of mannitol and trehalose and these were isolated by column chromatography on charcoal–Celite. Finally, cinnabarin was isolated from a mixture of three pigments by chromatography on magnesium oxide columns.

EXPERIMENTAL

Infrared spectra were determined on a Perkin-Elmer Infracord and ultraviolet spectra for ethanol solutions on a Perkin-Elmer spectrophotometer, model 137. Optical rotations were determined for chloroform solutions at 22°. Alumina for chromatography was grade H, supplied by P. Spence and Co., and light petroleum refers to the fraction with b. p. 50—60°. Gas–liquid chromatography of sterol fractions was carried out on a Pye Argon instrument using a 1% E 301 column at 214°, and that of alkanes using a 1% SE-30 column at 200 and 225°. Analyses are by Dr. A. D. Campbell and associates, University of Otago, New Zealand.

*Extraction of* Coriolus sanguineus.—The finely ground fungus (1·5 kg.) was extracted twice with cold methanol for periods of 1 week. The combined extracts were concentrated to small volume, ether (2 l.) was added, and the filtered solution was separated into acidic and neutral fractions in the usual way. Removal of solvent from the neutral fraction and trituration of the residue with methanol containing a little light petroleum gave a crystalline mass (1·4 g.). Gum (6·4 g.), obtained from the filtrate, was steam distilled for 6 hr. and the distillate was saturated with sodium chloride and extracted with ether. The non-steam distillable fraction was then hydrolysed with 1% methanolic potassium hydroxide following Halsall and Sayer's procedure.[14]

*Ergosterol and 5,6-Dihydroergosterol.*—The crystalline mass was chromatographed from benzene–light petroleum (1 : 3) on alumina (500 g.). Fractions eluted with benzene–ether (1 : 2) gave mixed crystals of ergosterol and 5,6-dihydroergosterol, which crystallised from methanol–acetone as plates or needles, m. p. 171—172°, unchanged by further chromatography or repeated crystallisation. Similar chromatography of the neutral non-saponifiable fraction gave the same material (4·92 g.) and it was also isolated (0·1% yield) by extraction of a further sample of the fungus with light petroluem [Found (for sample crystallised from ethanol): C, 81·2; H, 11·4. Calc. for $C_{28}H_{46}O$,EtOH: C, 81·0; H, 11·8%]. Analyses at intervals of sample dried for long periods indicated the gradual loss of solvent of crystallisation. The material had $[\alpha]_D$ −21·65° (*c* 1·0) and $\lambda_{max.}$ 262 (log ε 2·48), 271 (log ε 2·60), 281 (log ε 2·70), and 293 mμ (log ε 2·60).

Acetylation with acetic anhydride and pyridine at 20° for 24 hr. gave mixed crystals of ergosteryl acetate (3%) and 5,6-dihydroergosteryl acetate (97%), which crystallised from acetone as plates, m. p. 179—180°, $[\alpha]_D$ −21·5° (*c* 1·0) (lit.,[27] m. p. for 5,6-dihydroergosteryl acetate, 180—182°, $[\alpha]_D$ −20·5°) (Found: C, 81·25; H, 11·1. Calc. for $C_{30}H_{48}O_2$: C, 81·8; H, 11·0%).

Benzoylation of the sterol mixture with benzoyl chloride at 100° for 4 hr., and fractional crystallisation of the product from acetone, gave 5,6-dihydroergosteryl benzoate as plates, m. p. 198—200°, $[\alpha]_D$ −8·6° (*c* 1·0) (lit.,[28] m. p. 200°, $[\alpha]_D$ −10°) (Found: C, 83·4, 83·7; H, 10·2, 10·5. Calc. for $C_{35}H_{50}O_2$: C, 83·6; H, 10·0%). Treatment of the crude benzoate, m. p. 187—189°, with bromine in carbon tetrachloride followed by fractional precipitation with ethanol [11] also gave 5,6-dihydroergosteryl benzoate (60%), m. p. 195—197°, $[\alpha]_D$ −10° (Found: C, 83·7; H, 10·3%).

Reduction of the mixed sterols (400 mg.) with sodium and absolute ethanol by Windaus and Brunken's method [10] gave 5,6-dihydroergosterol (330 mg.), recrystallised from ethanol as plates, m. p. 175—176°, $[\alpha]_D$ −19° (lit.,[28] m. p. 176°, $[\alpha]_D$ −19°) [Found (after drying at 140° for 24 hr.): C, 84·0; H, 11·7. Calc. for $C_{28}H_{46}O$: C, 84·35; H, 11·6%]. This product furnished an acetate crystallising from acetone–methanol as plates, m. p. 182—184°, $[\alpha]$ −19·5° (lit.,[27] m. p. 180—182°, $[\alpha]_D$ −20·5°) (Found: C, 78·4; H, 10·7. Calc. for $C_{30}H_{48}O_2$,$CH_3OH$: C, 78·8; H, 11·1%), and a benzoate, m. p. 196·5—197·5° (lit.,[28] m. p. 200°) (Found: C, 83·3; H, 10·3. Calc. for $C_{35}H_{50}O_2$: C, 83·6; H, 10·0%).

A synthetic mixture of ergosterol (3%) and 5,6-dihydroergosterol (97%) gave a single peak on g.l.c. with retention time identical to that given by the mixed fungal sterols. Similar results were also obtained on g.l.c. of the mixed acetates.

*Ergosta-7,22-dien-3-one.*—Fractions eluted with benzene–ether (1 : 1) from chromatography above, crystallised from methanol–ether to yield ergosta-7,22-diene-3-one (8 mg.), $[\alpha]_D$ +3° (*c* 0·1), m. p. 183—185° (lit.,[20] m. p. 184—187°, $[\alpha]_D$ +6°), undepressed by a sample prepared from ergosta-7,22-dien-3β-ol by Bladon, Henbest, and Wood's method [19] (Found: C, 81·75; H, 12·0. Calc. for $C_{28}H_{44}O$,$CH_3OH$: C, 81·25; H, 11·3%), $\nu_{max.}$ ($CCl_4$) 1709 (cyclohexanone), 1427, 1383, and 1374 cm.$^{-1}$ (2 adj. $CH_2$). N.m.r. 0·57 ($C_{(18)}$Me), 0·82 ($C_{(26)}$,$C_{(27)}$Me, doublet), 0·92 ($C_{(28)}$Me, doublet), 1·02 ($C_{(19)}$Me), 1·02 ($C_{(21)}$Me, doublet), and 5·24 δ ($C_{(7)}$,$C_{(22)}$,$C_{(23)}$, multiplet).

*Polyporenic Acid C.*—The acidic fraction (1·1 g.), comprised mainly of pigments, was suspended in ether and treated twice at 0° with an excess of an ethereal solution of diazomethane. Removal of solvent gave a brown gum, the benzene-soluble portion of which was chromatographed on alumina. Fractions eluted with ether gave methyl polyporenate C which after repeated crystallisation from methanol as needles (12 mg.) had m. p. 196—198°, undepressed

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by an authentic sample, and $[\alpha]_D$ +8° (*c* 0·1) (lit.,[23] $[\alpha]_D$ +10°) (Found: C, 77·2; H, 9·8. Calc. for $C_{32}H_{48}O_4$: C, 77·35; H, 9·75%), $\lambda_{max.}$ 236 (log ε 4·16), 243 (log ε 4·22), 251 (log ε 4·06), and 276—280 mμ (infl., log ε 1·76); $\nu_{max.}$ ($CHCl_3$) 3559 (OH), 1739 (ester CO), 1709 (cyclohexanone), 1640, and 891 $cm.^{-1}$ (C:$CH_2$).

The ester (5 mg.) formed a 2,4-dinitrophenylhydrazone, m. p. 190—196° (lit.,[20] m. p. 195—198°), with $\lambda_{max.}$ 366 (cf. ref. 20) but which was not obtained pure.

*n-Alkanes*.—Initial fractions from alumina chromatography of the non-saponifiable material, which were eluted with light petroleum, crystallised from ethanol as plates (30 mg.), m. p. 61—62° (Found: C, 85·4; H, 14·5%; *M* (Rast), 390. Calc. for $C_{28}H_{58}$: C, 85·2; H, 14·8%; *M*, 392). The material corresponded to n-octacosane but was probably a mixture. G.l.c. of the waxy residues (50 mg.) after removal of the above material showed the presence of n-alkanes with the following chain lengths (approximate relative areas of the peaks in parentheses): $C_{24}$(5), $C_{25}$(5), $C_{26}$(5), $C_{27}$(5), $C_{28}$(5), $C_{29}$(7), $C_{30}$(1·5), and $C_{31}$(1). A number of minor peaks between those of the above were indicative of branched alkanes.

*Heptan-1-ol and Octan-1-ol*.—The oil (1·1 g.) obtained from the steam distillation was treated with acetic anhydride–pyridine at 20° for 6 hr. Comparative g.l.c. of the product on a 10% silicone oil column at 125° against authentic samples of n-alkyl acetates of chain length $C_4$—$C_8$ showed three peaks. Two corresponded to heptanyl and octanyl acetates and an intermediate peak was ascribable to a branched-chain octyl or higher acetate.

*Mannitol and Trehalose*.—The fungus (500 g.) was heated under reflux with water for 6 hr. and the extract was concentrated *in vacuo* to give a dark viscous resin (6 g.) from which pigments were partially removed by chromatography on a short column of alumina (cf. ref. 26). Further chromatography on charcoal–Celite (1 : 1) and elution with 3% aqueous ethanol (500 ml.) gave mannitol (633 mg.), m. p. and mixed m. p. 165—166° (identical infrared spectra); hexa-acetate, m. p. and mixed m. p. 123—125°.

Elution with 6·8% aqueous ethanol (500 ml.) gave trehalose hydrate (50 mg.), m. p. and mixed m. p. 97—98° (identical infrared spectra); octa-acetate, m. p. and mixed m. p. 100—102°.

*Cinnabarin* (with D. E. Cooper).—An acetone extract (Soxhlet) of the fungus, previously extracted with light petroleum, was chromatographed on magnesium oxide. The presence of three pigments was shown by development of the chromatogram with acetone. A pale yellow band, eluted rapidly from the column with acetone containing a few drops of acetic acid, gave cinnabarin (0·4% yield). Five recrystallisations from pyridine or nitrobenzene gave red needles, decomp. >320°, which were chromatographically pure and which showed no increase in ultraviolet extinction coefficients on further recrystallisation (Found: C, 59·1, 59·1; H, 3·6, 3·6; N, 9·4. Calc. for $C_{14}H_{10}N_2O_5$: C, 58·7; H, 3·4; N, 9·8%); $\lambda_{max.}$ 230 (log ε 4·20), 270 (infl., log ε 4·04), and 430—438 mμ (log ε 3·89).

A cooled suspension of cinnabarin (50 mg.) in nitrobenzene (16 ml.) and acetic anhydride (4 ml.) was treated with one drop of 60% perchloric acid. The temperature was allowed to rise to 20° and excess of water was added. The nitrobenzene solution was separated, dried, and the solvent was removed. Chromatography of the crystalline residue from acetone solution on magnesium carbonate–Celite gave *O*-acetylcinnabarin (22 mg.) which crystallised from ethanol as red needles, m. p. 241° (decomp.) (lit.,[5] m. p. 250—251° decomp.) (Found: C, 58·35; H, 3·85; N, 8·8. Calc. for $C_{16}H_{12}N_2O_6$: C, 58·5; H, 3·7; N, 8·5%).

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Department of Chemistry, University of Auckland, Auckland, New Zealand.

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