Chemistry of Fungi. Part VIII.[1] Constituents of *Valsaria rubricosa* and the Identification of Papulosin with Valsarin

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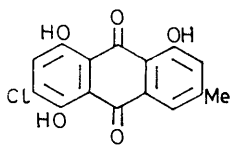
Valsarin (3), 7-chloroemodin (5), emodin, and mannitol have been isolated from *Valsaria rubricosa*. There is mass spectral evidence also for the presence of three further compounds, probably 5,7-dichloroemodin (6) parietin (7), and 1,2,4,5-tetrahydroxy-7-methylanthraquinone (8). ' Papulosin ' has been shown to be identical with valsarin.

A PRELIMINARY publication [2] described the isolation from *Valsaria rubricosa* of a chlorinated anthraquinone, valsarin, with properties consistent with one of the formula (1)—(3). Subsequent work,[3] involving biosynthetic, n.m.r., and chemical considerations, clearly showed that it had the formula (3).



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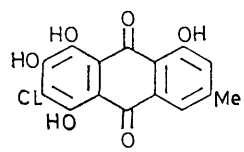

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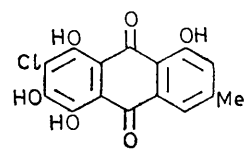

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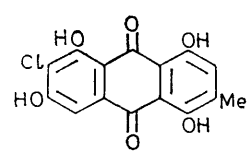
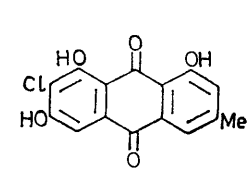


(4)

Unaware of our publication, Fox, Maass, and Forrest [4] described the isolation of ' papulosin ' from the lichen *Lasallia papulosa* (Ach.) Llano, and adduced clear-cut evidence for its formulation as (3). Bohman [5] later isolated valsarins I and II from *V. rubricosa* from different sources and showed by co-chromatography that they were identical with the same two compounds from *Lasallia papulosa* var. *rubiginosa*. She suggested structures (4) and (3) for valsarins I and II, respectively.

Direct comparison has now shown that ' papulosin ' is identical with valsarin (3).

On chromatography of the extracted mycelia of *V. rubricosa* another chlorinated anthraquinone was isolated and shown [3] to be 7-chloroemodin (5). This was later isolated as compound *AO-1* by Japanese workers [6] from the lichen, *Anaptychia obsurata* (Nyl.) vain (syn. A. *heterochroa*) and clearly shown to have formula (5). Direct comparison showed that the products from the different sources were identical. 7-Chloroemodin has also been isolated from *Lasallia papulosa* both by Fox *et al.*[4] and by Bohman,[5] as well as from *V. rubricosa* [5]



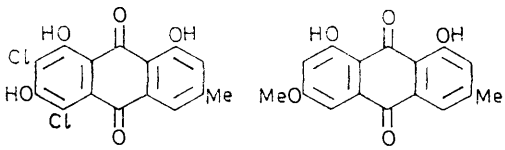
(5)

[1] Part VII, R. C. Cambie and J. C. Parnell, *New Zealand J. Sci.*, 1971, **14**, 292.
[2] L. H. Briggs and D. R. Castaing, *Bull. Nat. Inst. Sci. India*, 1965, 71.
[3] E. F. Orgias, Thesis, University of Auckland, 1965.
[4] C. H. Fox, W. S. G. Maass, and T. P. Forrest, *Tetrahedron Letters*, 1969, 919.
[5] G. Bohman, *Acta Chem. Scand.*, 1969, **23**, 2241.
[6] I. Tosioka, H. Yamaguchi, K. Morimato, and I. Kitagawa, *Tetrahedron Letters*, 1968, 1149.

and other sources.[7-10] A synthesis has been described by Sargent *et. al.*[11]

In agreement with Bohman,[5] emodin has also been isolated from *V. rubricosa*. The acetone extract of the mycelia yielded mannitol.

In the mass spectrum of a sample of 7-chloroemodin (5) purified by crystallisation and not by preparative t.l.c. there was evidence for the presence of two further compounds. One showed a triplet of peaks at *m/e* 338, 340, 342 corresponding to 5,7-dichloroemodin (6) (compound *AO-2* from *Anaptychia obscurata*[6]). The other, *m/e* 284, corresponded to parietin (7), also isolated from *V. rubricosa* by Bohman.[5] Similarly, in a sample of valsarin there was a further molecular ion peak at *m/e* 286 which on biosynthetic grounds is probably due to 1,2,4,5-tetrahydroxy-7-methylanthraquinone (8), occurring in *Laurera purpurina* (Nyl.) Zahlbr.[12] and as the methyl ether, xanthorin, in the same fungus and in *Xanthoria elegans* (Link) Th. Fr.[13,14]



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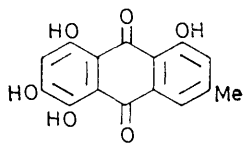

(8)

A synthesis of valsarin is described in the following paper.

EXPERIMENTAL

M.p.s were determined with an electrically heated copper block or a Kofler hot-stage apparatus. I.r. spectra were measured with Perkin-Elmer Infracord or 237 instruments and u.v. spectra with a Perkin-Elmer 137 UV spectrophotometer. N.m.r. spectra were determined for solutions in deuteriochloroform with a Varian A60 spectrometer (tetramethylsilane as internal reference). Microanalyses were performed by Dr A. D. Campbell and his associates, University of Otago. Mass spectra were measured with an A.E.I. MS9 instrument.

Growth and Harvesting of Valsaria rubricosa.—Raw blended potato in water (40% w/w suspension; 120 ml) was introduced into 1 l Erlenmeyer flasks and sterilised for 30 min at 103 kPa. Small pieces of mycelia of *V. rubricosa* (New Zealand Reference Culture Collection, No. 10020), grown on agar slopes, were added to a sterilised solution of glucose (45%), the suspension was shaken, and and a sample (5 ml) was added by a sterile tip measure to each of the flasks. These were incubated at 20—25° under artificial light for 4—8 weeks. The mycelial mats were separated by centrifugation and dried (yield *ca.* 1·8—3 g per flask). The mycelia was extracted (Soxhlet) separately with various solvents.

Crystalline material separating from an acetone extract was identified as mannitol (needles from methanol), m.p. and mixed m.p. 166° (acetate, m.p. and mixed m.p. 123—124°).

Prolonged extraction with light petroleum afforded a red crystalline solid from which valsarin was readily obtained by crystallisation successively from ethyl acetate, ethanol–light petroleum, and aqueous acetic acid. The extract was also amenable to column chromatography (as later).

The concentrated ethyl acetate extract was treated with dilute sodium hydroxide solution, and the purple alkaline solution was separated and acidified with hydrochloric acid. The precipitate was taken up in ether. After evaporation of the solvent the material was chromatographed on magnesium carbonate–Celite (1 : 1) packed in acetone, and developed with acetone. Four bands, yellow, grey, red, and purple, appeared on the chromatogram, which was then eluted with acetone, the elution being followed by t.l.c. with benzene–acetone (1 : 1). The purple band was unaffected but t.l.c. of the eluates indicated a poor separation on the column of two coloured compounds, later shown to be emodin and 7-chloroemodin (5). These two compounds, however, were separated by preparative t.l.c. on silica gel with benzene–acetone (1 : 1) and crystallised from glacial acetic acid and aqueous acetone, respectively.

The purple band was extruded, acidified with hydrochloric acid, and extracted with ether. Valsarin (3) was obtained from the extract and was recrystallised repeatedly from glacial acetic acid. It formed red needles, m.p. 285—286° (with sublimation from 235°), and gave a single spot, R_F 0·81, with butanol–acetic acid–water (5 : 2 : 4).

A sample of ' papulosin ' (provided by Dr. Fox) after crystallisation from glacial acetic acid had m.p. 284—285° and a u.v. spectrum in good agreement with that recorded.[1] Valsarin had $\nu_{max.}$ (KBr) 3280br, 2920w, 2850w, 1610s, 1590m, 1465s, 1435m, 1410w, 1385w, 1370w, 1345w, 1290m, 1270m, 1245m, 1210w, 1185w, 1165w, 1140w, 1115w, 1030s, 1005w, 960w, 935w, 870m, 785s, 750m, and 710m cm^{-1}. Our own i.r. data for ' papulosin ', however, were not in full agreement with these figures. Examination of ' papulosin ' by t.l.c. and mass spectroscopy, however, indicated that it was not homogenous.

Valsarin dissolves in sodium hydrogen carbonate solution to give a purple solution, and in concentrated sulphuric acid to give a blue solution. When an alcoholic solution of valsarin is spotted on filter paper and sprayed with alcoholic magnesium acetate solution a pink-violet colour is observed different from the purple and red colours produced respectively by morindone[15] (with a 1,2,5-trihydroxyanthraquinone structure) and 1,4-dihydroxyanthraquinone, but similar to that produced by 1,2,4-trihydroxyanthraquinone.

The *acetate*, prepared by acetylation in pyridine or with

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[9] G. Bohman, *Arkiv Kemi*, 1969, **30**, 217.
[10] J. Santesson, *Acta Chem. Scand.*, 1970, **24**, 371.
[11] M. V. Sargent, D. O. Smith, and J. A. Elix, *J. Chem. Soc.* (*C*), 1970, 307.
[12] K. E. Stensiö and C. A. Wachtmeister, *Acta Chem. Scand.*, 1969, **23**, 144.
[13] W. Steglich, W. Losel, and W. Reininger, *Tetrahedron Letters*, 1967, 4719.
[14] B. Franck and I. Zimmer, *Chem. Ber.*, 1965, **98**, 1514.
[15] R. Bhattacharya and J. L. Simonsen, *J. Indian Inst. Sci.*, 1927, **10***A*, 6.

perchloric acid, crystallised from aqueous ethanol, methanol, or ethyl acetate–light petroleum in yellow needles, m.p. 221·5—222°, $\lambda_{max.}$ (EtOH) 215 (ε 31,700), 265 (ε 39,000), and 345 nm (ε 13,200), $\nu_{max.}$ (KBr) 2940w (Me), 1790s and 1780s (acetate (C=O), 1680s (C=O), 1610s, 1568m, 1475sh, 1430m, 1375s, 1332s, 1267m, 1185br, 1142w, 1121m, 1095m, 1017s, 960m, 933s, 891m, 855m, 822w, 810w, 782m, 755w, 717w, 690w, and 677w cm^{-1}, δ 2·44 (3H, s, 7-Me), 2·39 and 2·46 (12H, 2 × s, 1-, 2-, 4-, and 5-OAc), 7·17br (1H, $W_{\frac{1}{2}}$ 3 Hz, 6-H), and 7·88br p.p.m. (1H, $W_{\frac{1}{2}}$ 3 Hz, 8-H) (Found: C, 56·8; H, 3·6; Cl, 7·35; O, 31·7; Ac, 38·2. $C_{23}H_{17}ClO_{10}$ requires C, 56·5; H, 3·5; Cl, 7·25; O, 32·7; Ac, 35·2%). The acetate of 'papulosin', prepared similarly, was identical (m.p., mixed m.p., and i.r. spectrum).

Valsarin tetramethyl ether [4] crystallised from ethanol–light petroleum in yellow needles, m.p. 180—180·5° (lit.,[4] 173—174°), $\nu_{max.}$ ($CHCl_3$) 2980m (OMe), 2910m (OMe) 2836w (OMe), 1667s (C=O), 1603s (C=O), and 1587s cm^{-1} (aromatic), δ 2·35 (3H, s, 7-Me), 3·94 and 3·98 (12H, 2 × s, 1-, 2-, 4-, and 5-OMe), 7·04br (1H, $W_{\frac{1}{2}}$ 2 Hz, 6-H), and 7·54br p.p.m. (1H, $W_{\frac{1}{2}}$ 2·5 Hz, 8-H).

Oxidation of Valsarin with Manganese Dioxide.—Valsarin (25 mg) was heated at 60° in concentrated sulphuric acid (9 ml) while manganese dioxide (50 mg) was added during 25 min. The mixture was poured into water and extracted with ether. Crystallisation of the product from aqueous acetone yielded dark brown plates (15 mg), m.p. 278—279°, $\nu_{max.}$ (KBr) 3400 (OH), 1580 (C=O), and 785 cm^{-1} (CCl), $\lambda_{max.}$ (cyclohexane) 240 (ε 15,000), 512 (9000), 523 (10,500), 550 (12,000), and 563 nm (13,000) (u.v. spectrum almost identical with that of 1,4,5,8-tetrahydroxyanthraquinone [16] in the same solvent).

7-Chloroemodin (yellow needles) had m.p. and mixed m.p. (with *AO-1* from Professor Kitagawa and a sample from Dr. Fox from *Lasallia papulosa*) 287—288° (with sublimation from 233°). The spectral data (i.r. and mass) were also identical. The *acetate*, prepared by acetylation with acetic anhydride and perchloric acid, crystallised from ethanol in pale yellow needles, which sublimed at *ca.* 241° (Found: C, 59·2; H, 3·8. $C_{21}H_{15}ClO_8$ requires C, 58·6; H, 3·5%).

The emodin obtained had m.p. and mixed m.p. 259—261° (i.r. spectrum identical with that of authentic material).

We thank Dr. L. D. Colebrook for measuring an n.m.r. spectrum, Drs. R. T. Aplin, J. S. Shannon, and D. Rosenthal for mass spectral measurements, the Nuffield Foundation for a grant, and Miss J. M. Dingley for the fungal cultures.

[1/2312 *Received*, 6*th December*, 1971]

[16] H. Brockmann and W. Müller, *Chem. Ber.*, 1959, **92**, 1164.