was oxidized first with alkaline potassium permanganate in pyridine solution. The 14,15-diol thus obtained was then dissolved in acetic acid and oxidized with Kiliani's reagent yielding the diketo acid VIII, m.p. 195–199°. Sodium azide (40 mg.) was added to a cooled solution of this diketo acid (28 mg.) in a mixture of chloroform (0.4 ml.) and concentrated sulfuric acid (0.5 ml.). The mixture was warmed to 45–50° and the evolved carbon dioxide was collected as barium carbonate (8.1 mg.).

The Structures of Mitorubrin and Mitorubrinol

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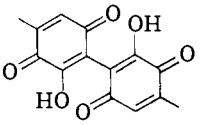
Contribution from the Department of Chemistry and the Department of Nutrition and Food Science, Massachusetts Institute of Technology, Cambridge, Massachusetts. *Received April 12, 1965*

Two new metabolites, for which the names mitorubrin and mitorubrinol are suggested, have been isolated from Penicillium rubrum cultures. Spectral evidence was used to derive their structures.

The isolation and characterization of mold and fungal metabolites has generally been impelled by some conspicuous feature of the microorganism, such as its pigmentation or its ability to elaborate a toxic or therapeutic agent. *Penicillium rubrum* is notable both for the coloration which its mycelium develops during growth and for its ability to synthesize a substance of high toxicity. The latter feature has attracted attention in association with the poisoning of livestock through feedstuff which was shown to be infected with *P. rubrum*.[2,3] During the course of the present work it was found that the substances responsible for pigmentation of the fungus are distinct from the agent(s) causing toxicity and in the present paper we shall discuss the isolation and structure determination of two pigments.

When *P. rubrum* is grown on a natural medium such as wheat or corn its mycelium develops a brilliant orange coloration interspersed with flecks of darker red. Extraction of the culture with ethyl acetate after 2 to 3 weeks of growth affords a dark red extract which contains, in addition to pigments, a large amount of lipid and fat-soluble material. Since the pigments are relatively insoluble in nonpolar solvents, they can be concentrated by extracting with solvents of increasing polarity. The lipid material is removed in the initial extraction with petroleum ether and the final residue after extraction with ethyl ether contains, according to thin layer chromatography, three principal pigments together with some colorless, polar material. Chromatography of this residue on silicic acid yields two pure pigments which we have named mitorubrin and mitorubrinol, respectively.

A search among the known mold products disclosed only one metabolite of *P. rubrum* which has been previously isolated and identified.[4] This substance, phoenicin, has been assigned the bisquinonoid structure **1**, and its synthesis has been reported.[5] The obvious dissimilarity between the physical and spectral properties of phoenicin and those of mitorubrin (and mitorubrinol) precluded the possibility of close structural resemblance.



1

Mitorubrin was obtained as orange-yellow prisms, m.p. 218°, and is optically active. Bioassays with mice showed that it possesses none of the physiological properties associated with the toxic metabolite which is elaborated by the same fungus. The infrared spectrum of the pigment shows broad absorption attributable to hydroxyl groups, a carbonyl band at 1715, and broad, complex absorption in the region of 1600–1660 cm.$^{-1}$. The ultraviolet spectrum of mitorubrin shows maxima, in ethanol, at 216 mμ (ε 18,200), 266 (18,200), 292 (10,100), and 346 (16,100). Solutions of this substance change from yellow to orange on addition of sodium hydroxide and this is accompanied by a large shift in the absorption spectrum, with maxima occurring, in base, at 246 mμ (ε 20,200), 320 (23,600), 346 (28,600), and 485 (5600). The original spectrum is regenerated upon immediate acidification, but prolonged contact with base results in degradation of the molecule. Attempts to match the rather complex ultraviolet spectrum of mitorubrin with known chromophores were not immediately successful and led to the suggestion that the spectrum might be the result of a superposition of two separate chromophores present in the molecule.

The appearance of a clearly defined molecular ion peak in the mass spectrum indicated that the molecular weight of mitorubrin is 382 and a high-resolution mass spectrum established the composition as $C_{21}H_{18}O_7$.[6]

We now wish to consider the spectroscopic and chemical properties of mitorubrin in terms of structure **2** which we feel is uniquely compatible with the evidence available.

(1) National Institutes of Health Predoctoral Fellow, 1963–1965.

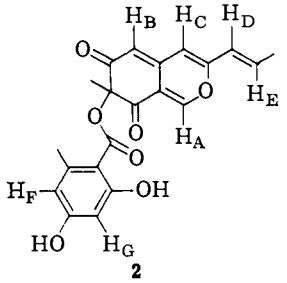
(2) J. E. Burnside, *et al.*, *Am. J. Vet. Res.*, **18**, 817 (1957); J. Forgacs, H. Koch, W. T. Carll, and R. H. White-Stevens, *ibid.*, **19**, 744 (1958).

(3) B. J. Wilson and C. H. Wilson, *J. Bacteriol.*, **83**, 693 (1962); **84**, 293 (1962).

(4) E. A. Friedheim, *Helv. Chim. Acta*, **21**, 1464 (1938); T. Curtin, G. Fitzgerald, and J. Reilly, *Biochem. J.*, **34**, 1605 (1940).

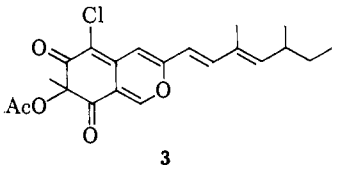
(5) T. Posternak, H. W. Ruelius, and J. Tcherniak, *Helv. Chim. Acta*, **26**, 2031 (1943).

(6) The high resolution mass spectrum was obtained through the courtesy of Professor K. Biemann, Massachusetts Institute of Technology.



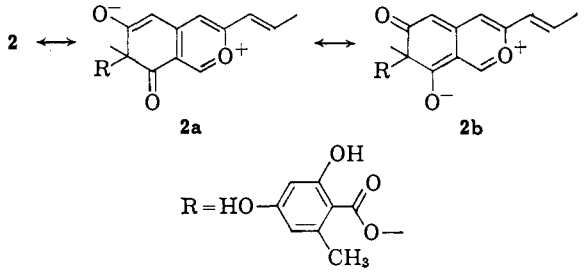
2

Structure **2** contains one asymmetric carbon atom to account for the optical activity of mitorubrin. The extended conjugation of the carbonyl groupings, in conjunction with the chelated orsellinate ester, would be expected to give rise to the broad absorption at 1600–1660 cm.$^{-1}$ and, in this respect, the infrared spectrum of mitorubrin bears a close resemblance to that of sclerotiorin (**3**),[7,8] in which there is a band at 1715 and intense, complex absorption centered at 1640 cm.$^{-1}$.



3

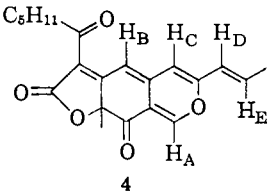
The n.m.r. spectrum of mitorubrin (see Figure 1) is seen to be in good agreement with the proposed structure **2**. Two low-field singlets at 10.4 and 10.3 p.p.m. are assigned to the two phenolic protons. A singlet at 2.48 is attributed to the benzenoid methyl substituent, the doublet at 1.89 to the methyl group of the propenyl side chain, and the singlet at 1.67 p.p.m. to the methyl group attached to quaternary carbon. The signal at 8.25 is assigned to H_A and that at 5.61 p.p.m. to H_B. The large difference in chemical shift of these two protons clearly reflects their different molecular environments; H_A is deshielded both by the adjacent oxygen atom and the neighboring carbonyl group in the peri position, while H_B can be shielded by delocalization of the nonbonding electrons of the pyrone oxygen atom. This difference will be maximal in the charge-separated resonance form **2a**, in which H_A lies in the plane of the pyrylium ring. The form **2a** may be expected to make a larger contribution to the structure than **2b**, which does not possess a resonance stabilized pyrylium structure.



2a **2b**

Dissection of the signal pattern in the region of 6.0–6.8 p.p.m. (see Figure 2) shows that it consists of a one proton singlet at 6.57 p.p.m., which must arise from H_C, and two overlapping AB systems. The two protons H_F and H_G of the orsellinate residue appear as a four-line pattern with chemical shifts of 6.19 and 6.28 p.p.m.; the coupling constant of 2 c.p.s. is as expected for coupling between *meta* protons of a benzene nucleus. The vinylic proton H_D gives rise to an unsymmetrical doublet (J = 16 c.p.s.) centered at 6.21 p.p.m., and the predicted pattern for H_E is therefore an unsymmetrical pair of quartets with coupling constants of 16 and 6 c.p.s. In fact, only the center two peaks of the quartet at higher field can be clearly distinguished and these have the anticipated line separation of 6 c.p.s. The outer lines of this quartet are obscured by other more intense signals while the expected low-field quartet, which should have considerably diminished intensity, is not differentiated from the background. The chemical shift of H_E, estimated from the centers of gravity of the AB system, is 6.55 p.p.m. The coupling between H_D and H_E of 16 c.p.s. indicates that the side-chain double bond has the *trans* configuration.

A detailed comparison with the n.m.r. spectrum of rubropunctatin (**4**)[9] provides firm support for the above interpretation of the spectrum of mitorubrin. The splitting patterns of the protons compared are virtually identical and their chemical shifts, with the exception of



4

H_B, correspond closely. The low-field position of H_B in rubropunctatin may be attributed to the deshielding effect of the side-chain carbonyl grouping in this molecule which, for steric and electronic reasons, should be aligned in the plane of the ring system and oriented towards H_B (see Table I).

Table I[a]

	Chemical shifts, p.p.m.	
	Mitorubrin (**2**)	Rubropunctatin (**4**)
H_A	8.25 (1)	7.88 (1)
H_B	5.61 (1)	6.89 (1)
H_C	6.57	6.17
H_D	6.21 (16)	6.06 (17)
H_E	6.55 (16, 6)	6.58 (17, 7)
CH_3—C=C	1.89 (6)	1.97 (7)
CH_3—C—O	1.67	1.72

[a] Coupling constants (J) are in cycles per second.

A deuterium-exchange study of mitorubrin using D_2O in d_7-dimethylformamide showed that, in addition

(7) F. M. Dean, J. Staunton, and W. B. Whalley, *J. Chem. Soc.*, 3004 (1959), and references cited.

(8) J. S. E. Holker, W. J. Ross, J. Staunton, and W. B. Whalley, *ibid.*, 4150 (1962).

(9) "Varian Catalog of NMR Spectra," Vol. 2, Varian Associates, Palo Alto, Calif., Spectrum No. 687. (The molecular formula and structure given are those of rubropunctatin (**4**) although the compound is designated as monascorubrin. Since the two substances differ only in the number of methylene groups in the side chain, this discrepancy does not affect the present argument.)

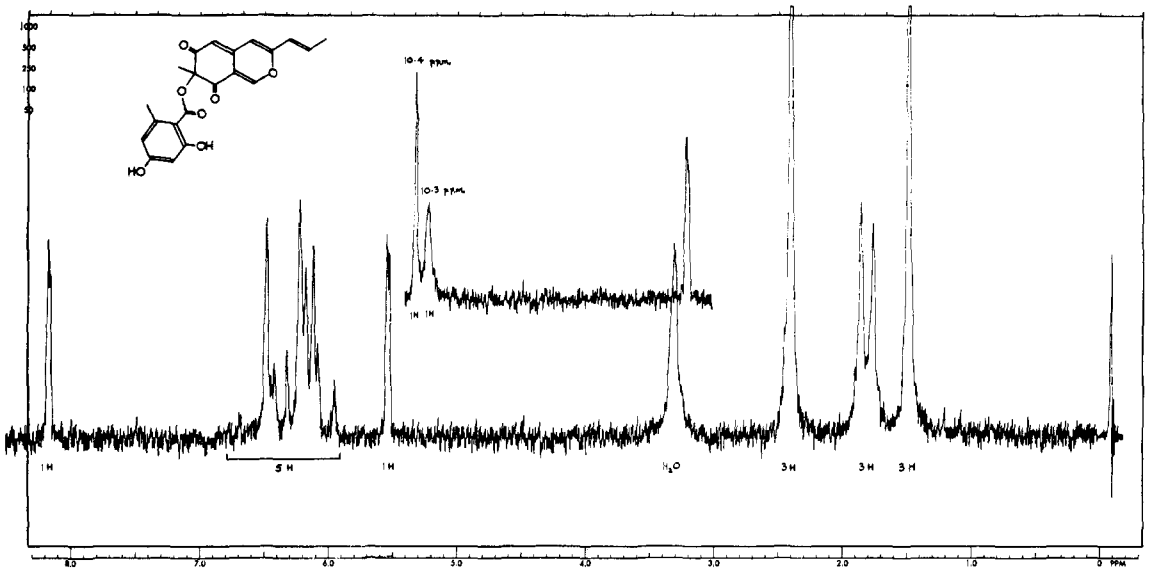


Figure 1. N.m.r. spectrum of mitorubrin.

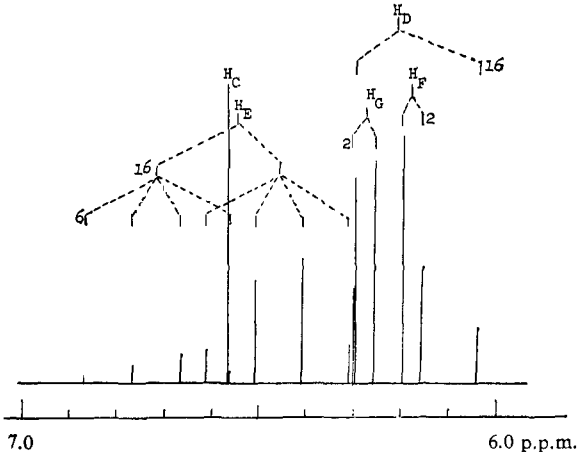
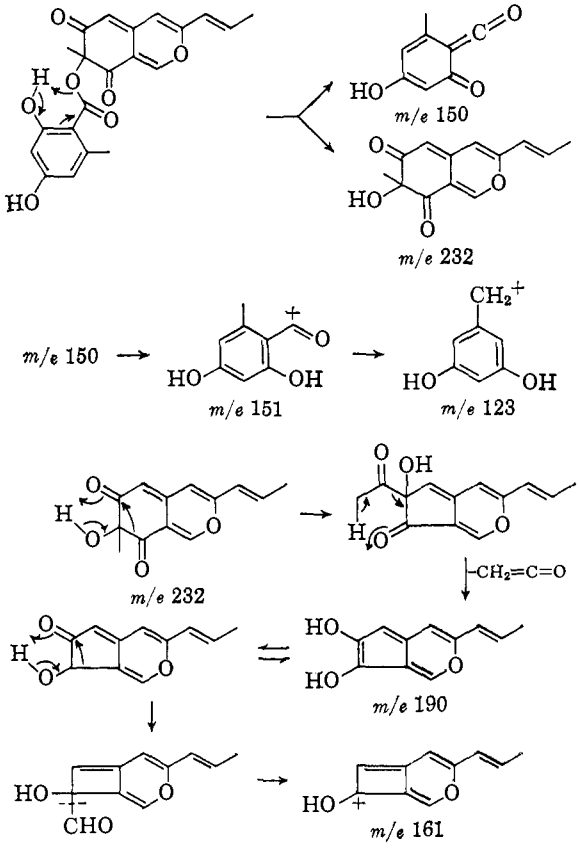


Figure 2. Analysis of the 6–7-p.p.m. region of the n.m.r. spectrum of mitorubrin (coupling constants are shown in c.p.s.).

to replacement of the two hydroxyl protons by deuterium, H_B was fully exchanged and H_C was partly exchanged after 72 hr. at room temperature. Other signals remained unaltered except that due to H_A, which became a singlet in the absence of proton coupling with H_B. Exchange of H_B and H_C is reasonable in terms of resonance of the type **2** ↔ **2a**, in which the carbon atoms to which these protons are attached become centers of high electron density. The more rapid exchange of H_B is also to be expected on this basis.

All of the major peaks in the mass spectrum (see Figure 3) of mitorubrin (**2**) can be rationalized in terms of energetically favorable fragmentation processes and thus constitute additional support for the structural assignment. Initial cleavage of the orsellinate residue affords fragments of 150 and 232 mass units, which then decay by independent pathways. The fragment of *m/e* 232 can undergo a rearrangement, with concomitant ring contraction, to give a hydroxyacetyl ketone, which can subsequently eliminate ketene in a cyclic process to yield a fragment of *m/e* 190. The tautomeric hydroxy ketone form of this diol may undergo a second rearrangement, with a further ring contraction, to give a hydroxyaldehyde. Loss of the aldehyde grouping produces the fragment of *m/e* 161. This fragmentation pathway is supported by the high-resolution mass spectrum which confirmed the composition of the fragments.



The ultraviolet spectrum of mitorubrin must represent the sum of absorption due to the orsellinate chromophore and that due to the vinylogous pyrone system in the remaining portion of the molecule. In order to compare the spectral properties with those of a model, methyl orsellinate was synthesized. Orcinol (**5**) was

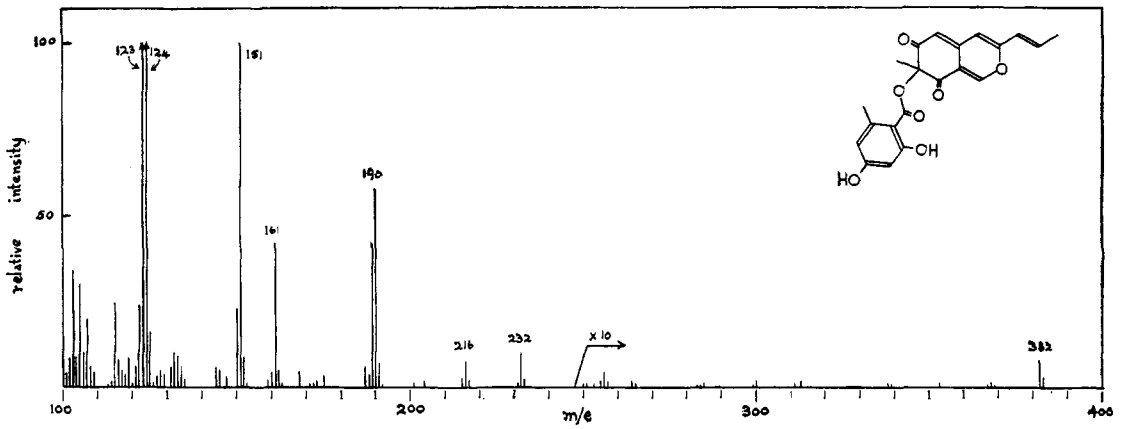
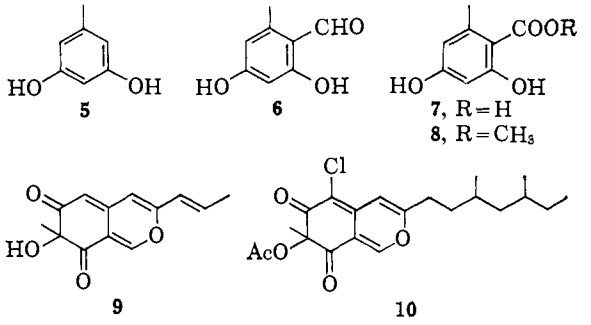
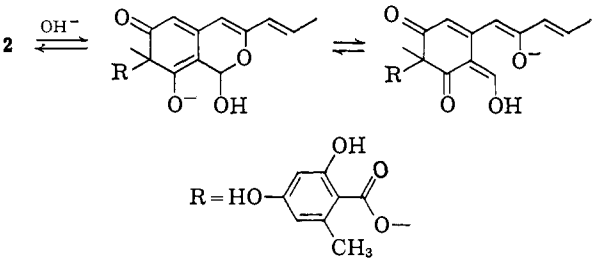


Figure 3. Mass spectrum of mitorubrin.

converted to orcylaldehyde (**6**) by treatment with zinc cyanide and anhydrous hydrogen chloride, followed by hydrolysis of the intermediate iminium salt, according to the method of Adams.[10] Oxidation of the aldehyde with potassium permanganate (with protection of the phenolic hydroxy groups) gave orsellinic acid (**7**),[11] which on treatment with 1 equiv. of diazomethane afforded methyl orsellinate (**8**).[12]



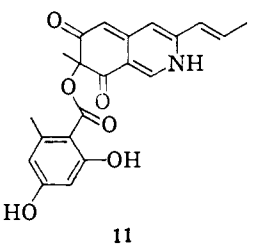
The ultraviolet spectrum of methyl orsellinate, in ethanol, showed maxima at 216 mμ (ε 18,100), 264 (12,400), and 297 (5100); on addition of base, maxima occurred at 242 mμ (ε 8300) and 306 mμ (ε 17,900). The spectrum in both neutral and basic solution is thus in excellent agreement with that of mitorubrin. The hypothetical ultraviolet spectrum of **9** calculated by subtraction, is predicted to have maxima, in ethanol, at 241 mμ (ε 8200), 278 (9200), and 346 (16,000) and, in base, at 249 (12,000), 346 (27,000), and 485 (5600). Comparison of predicted and observed spectra in this case is made difficult by the absence of a good model of either natural or synthetic origin. Tetrahydrosclerotiorin (**10**) provides an approximate analogy and has maxima, in ethanol, at 225 mμ (ε 13,500) and 343 mμ (ε 20,200).[13] Although no spectrum is reported for basic solution, both sclerotiorin and its tetrahydro derivative undergo a color change from yellow to orange on addition of sodium hydroxide solution. As in the case of mitorubrin, this change is reversible upon immediate acidification. A possible explanation of the appearance of absorption in the visible region of the spectrum, and the accompanying transformation, when mitorubrin (and, presumably, also sclerotiorin) is treated with base involves attack by hydroxide ion at the unsubstituted α-position of the pyrone nucleus. Opening of the pyrone ring forms an extensively conjugated enolate system and is thus the feature upon which reversibility of the reaction depends.



It had been hoped that methyl orsellinate (**8**), in addition to providing a model for the ultraviolet spectrum of mitorubrin, would also be a suitable analog for a comparison of n.m.r. spectra. It was of particular interest to see whether the chemical shift and splitting pattern of the two aromatic protons in **8** accounted satisfactorily for the AB system assigned to this grouping in the spectrum of the pigment. Somewhat unexpectedly, the aromatic protons in **8** proved to be equivalent, appearing as a two-proton singlet at 6.36 p.p.m. In contrast, the aromatic protons in the spectrum of orcylaldehyde (**6**) gave rise to the anticipated AB pattern, with chemical shifts of 6.28 and 6.37 p.p.m. and J = 2 c.p.s. The proton at lower field is split further by long range coupling (J = 0.8 c.p.s.) with the aldehyde proton. The chemical shift of the aromatic methyl group (2.46 p.p.m.) in **8** is in good agreement with the corresponding signal in mitorubrin (2.48 p.p.m.) and the two hydroxyl protons appear as a broad absorption at 11 p.p.m.

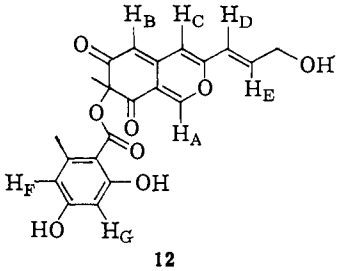
The assignment of structure **2** to mitorubrin places it in the group of mold metabolites known as azaphilones,[14] a name derived from the facile reaction of these pigments with ammonia to yield vinylogous γ-pyridones. To provide chemical support for its structure, mitorubrin (**2**) was treated with ammonia. The anticipated product, mitorubramine, was formed smoothly and has spectral properties fully in accord with structure **11**.

(10) R. Adams and I. Levine, *J. Am. Chem. Soc.*, **45**, 2373 (1923).
(11) K. Hoesch, *Ber.*, **46**, 886 (1913).
(12) J. Herzig and F. Wenzel, *Monatsh.*, **24**, 898 (1903).
(13) R. A. Eade, H. Page, A. Robertson, K. Turner, and W. B. Whalley, *J. Chem. Soc.*, 4913 (1957).
(14) A. D. G. Powell, A. Robertson, and W. B. Whalley, Special Publication No. 5, The Chemical Society, London, 1956, p. 27.



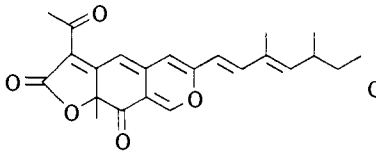
11

The second pigment, mitorubrinol, which was isolated from the extract of *P. rubrum* was found to possess properties similar to those of mitorubrin. The ultraviolet spectrum, in both neutral and basic solution, is identical with that of mitorubrin and the infrared spectrum indicates that the two substances differ structurally in only minor detail. Mitorubrinol is most clearly distinguished from its copigment by its lower R_f value on thin layer chromatography, and hence is the more polar of the pair. Although the mass spectrum of mitorubrinol fails to show a peak for the molecular ion, fragments of *m/e* 151, 150, 124, and 123 suggest that the orsellinate part structure is retained. Higher mass fragments occur at *m/e* 248, 206, and 177. The difference of 16 mass units between these fragments and those in the spectrum of mitorubrin implies that mitorubrinol contains one extra oxygen atom and must therefore have the molecular formula $C_{21}H_{18}O_8$. The n.m.r. spectrum of mitorubrinol strongly suggests structure **12** for this pigment. In d_6-dimethyl sulfoxide as solvent, the two protons of the phenolic hydroxyl groups appear as singlets at 10.3 and 10.2 p.p.m. As in mitorubrin, H_A and H_B appear as doublets (J = 1 c.p.s.) at 8.27 and 5.62 p.p.m., respectively, and the two aromatic protons of the orsellinate residue give rise to the previously observed AB pattern with chemical shifts of 6.18 and 6.27 p.p.m. The methyl groups attached to the benzenoid nucleus and quaternary carbon are present as singlets at 2.47 and 1.58 p.p.m., respectively, but the methyl doublet at 1.89 p.p.m. in the spectrum of mitorubrin is absent and a new unresolved multiplet, accounting for two protons, appears at 4.20 p.p.m. This change, together with the appearance of a broad signal at 5.1 p.p.m. which can be attributed to a hydroxylic proton, confirms that the vinylic methyl group in mitorubrin has been replaced by $-CH_2OH$ in mitorubrinol. In association with this change, H_D now occurs as an unsymmetrical doublet (J = 16 c.p.s.) at 6.35 p.p.m. and H_E as a multiplet with a calculated shift of 6.70 p.p.m. Of the unsymmetrical pair of triplets predicted for H_E, only the center component of the higher field and more intense triplet can be clearly distinguished.



12

The structure of mitorubrin is in good agreement with current biogenetic theory, and the relationship between this substance and the other members of the "azaphilone" group is more readily understood when their probable biosynthetic origin is considered. In mitorubrin (**2**) both the orsellinic acid moiety and the alcohol **9** (or their equivalents), from which the ester is presumably derived, can originate from acetate. It has been shown by labeling that sclerotiorin (**3**),[7,8] rotiorin (**13**),[15] monascin (**14**),[16,17] and rubropunctatin (**4**)[17,18] are all derived from acetate in the manner expected for cyclization of the appropriate polyketide precursor.[19,20,21]



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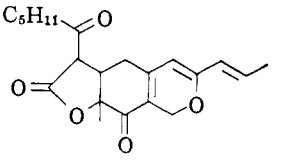

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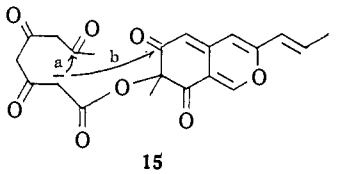
The alcohol part (**9**) of the mitorubrin skeleton can be formed schematically from a ketide chain derived by condensation of six acetate units, with subsequent transformations involving reduction of the initial and terminal carbonyl groups and introduction of a methyl group.

Mitorubrin itself appears to be most closely related biogenetically to rubropunctatin (**4**) since both can originate, at least in principle, by alternative modes of condensation of the same precursor **15**. The possibility that preformed orsellinate is incorporated into mitorubrin cannot be excluded, however. Orsellinic acid (**7**) is a well-known metabolite of fungi (it has been isolated, for example, from *Penicillium griseofulvum*, *Penicillium barnense*, and *Chaetomium cochlioides*). Mitorubrinol (**12**) is probably derived from mitorubrin (**2**) since the oxidation of a terminal methyl group to a primary alcohol in an acetate-derived chain has substantial analogy in microorganism chemistry.



15

Experimental

Isolation of Mitorubrin and Mitorubrinol. *P. rubrum* (P-13) was grown on a wheat–sucrose medium and harvested after 2 weeks of growth at 30°. The culture medium was extracted with ethyl acetate and the ex-

(15) J. S. E. Holker, J. Staunton, and W. B. Whalley, *J. Chem. Soc.*, 3641 (1963).
(16) Y. Inouye, K. Nakanishi, H. Nishikawa, M. Ohashi, A. Terahara, and S. Yamamura, *Tetrahedron*, **18**, 1195 (1962).
(17) B. C. Fielding, J. S. E. Holker, D. F. Jones, A. D. G. Powell, K. W. Richmond, A. Robertson, and W. B. Whalley, *J. Chem. Soc.*, 4579 (1961).
(18) E. J. Haws, J. S. E. Holker, A. Kelly, A. D. G. Powell, and A. Robertson, *ibid.*, 3598 (1959).
(19) A. J. Birch, P. Fitton, E. Pride, A. J. Ryan, H. Smith, and W. B. Whalley, *ibid.*, 4576 (1958).
(20) A. J. Birch, A. Cassera, P. Fitton, J. S. E. Holker, H. Smith, G. A. Thompson, and W. B. Whalley, *ibid.*, 3583 (1962).
(21) J. S. E. Holker, J. Staunton, and W. B. Whalley, *ibid.*, 16 (1964).

tract was filtered, washed with water, and dried (magnesium sulfate). Solvent was removed under vacuum at 40–45° leaving a dark red residue which was shaken vigorously with 250 ml. of petroleum ether followed by 250 ml. of benzene. The residue, after decanting the benzene solution, was triturated with 100 ml. of ether and divided into ether-soluble and -insoluble portions. The ether-insoluble portion was chromatographed on a column of 100 mesh silicic acid packed in chloroform. Continuous change of solvent from chloroform to ethyl acetate eluted first mitorubrin and then mitorubrinol. Fractional crystallization of the ether-soluble portion from ethyl acetate–ether afforded further quantities of mitorubrinol.

Mitorubin was recrystallized from ethyl acetate to give orange-yellow prisms: m.p. 218°; $[\alpha]^{25}D$ −405° (*c* 1.02, dioxane); mol. wt. 382 (mass spectrum); λ_{max}^{EtOH} 216 mμ (ε 18,200), 266 (18,200), 292 (10,100), and 346 (16,100); λ_{max}^{NaOH} 246 mμ (ε 20,200), 320 (23,600), 346 (28,600), and 485 (5600); ν_{max}^{KBr} 3400 (broad), 1715, 1660–1600 (many bands), 1545, 1505, 1450, 1380, 1345, 1315, 1270, 1240, 1220, 1160, 1130, 1115, 1075, 979, 960, 921, 916, 884, 872, 860, and 801 cm.$^{-1}$. Recrystallization of mitorubrinol from ethyl acetate–ether gave yellow microcrystalline needles: m.p. 219–221°; $[\alpha]^{25}D$ −375° (*c* 1.70, dioxane); ultraviolet spectrum identical with that of mitorubrin: ν_{max}^{KBr} 3400 (broad), 1715, 1650–1580 (many bands), 1540, 1445, 1365, 1320, 1260, 1235, 1200, 1165, 1105, 1075, 997, 972, 937, 881, 858, and 799 cm.$^{-1}$.

Deuterium Exchange with Mitorubrin. To a sample of 40 mg. of mitorubrin dissolved in 0.2 ml. of d_7-dimethylformamide was added 0.04 ml. of D_2O. The solution was allowed to stand at room temperature for intervals of 5 min., 12 hr., and 72 hr. After each period, the solvent was removed under vacuum and replaced with 0.2 ml. of fresh d_7-dimethylformamide, and the n.m.r. spectrum was determined.

Mitorubramine. Mitorubrin (9 mg.) was added to 1 ml. of a 1:1 mixture of 0.880 ammonium hydroxide and water. The pigment slowly dissolved to form a dark red solution which, on acidification with 10% hydrochloric acid, precipitated an orange colored solid. The precipitate was collected, washed with water, and dried *in vacuo* to yield 8 mg. of mitorubramine: m.p. 196–199° dec.; λ_{max}^{EtOH} 212 mμ (ε 25,800), 275 (32,600), 293 (shoulder), and 365 (18,500); $\lambda_{max}^{10\% NaOH}$ 240 mμ (ε 13,500), 297 (43,800), and 335 (18,500); mass spectrum *m/e* (381), 337, 229, 215, 214, 200, 189, 150, 124, and 123.

Orcylaldehyde (**6**). This substance was prepared according to the method of Adams[10] and after purification by sublimation had m.p. 183.5° (lit.[10] 178–180°); ν_{max}^{Nujol} 3150, 1630, 1600, 1390, 1295, 1270, 1230, 1205, 1165, 999, 989, 877, 836, and 821 cm.$^{-1}$. The n.m.r. spectrum of **6**, in d_7-dimethylformamide, showed two very broad signals at 11.0 and 12.5 p.p.m. for the hydroxyl protons, a singlet at 10.2 p.p.m. for the aldehyde proton, and a three proton singlet at 2.55 p.p.m. corresponding to the methyl group. The two aromatic protons gave rise to an AB quartet, with chemical shifts of 6.28 and 6.37 p.p.m. and a coupling constant of 2 c.p.s.

Methyl Orsellinate (**8**). Addition of 1 equiv. of diazomethane to an ice-cold solution of orsellinic acid (**7**) in anhydrous ether gave a quantitative yield of methyl orsellinate (**8**). The ester was obtained, after recrystallization from aqueous ethanol, as a microcrystalline solid: m.p. 135–138° dec. (lit.[12] m.p. 138–139°); λ_{max}^{EtOH} 216 mμ (ε 18,100), 264 (12,400), and 297 (5100); λ_{max}^{NaOH} 242 mμ (ε 8300), and 306 mμ (ε 17,900); ν_{max}^{Nujol} 3400, 3100–2500 (broad), 1650–1605 (many bands), 1580, 1500, 1315, 1260, 1215, 1200, 1170, 1160, 1115, 1065, 1005, 998, 957, 858, 840, 802, and 704 cm.$^{-1}$. The n.m.r. spectrum, in d_7-dimethylformamide, showed signals at 11 (2 H, broad), 6.36 (2 H, singlet), 3.93 (3 H, singlet), and 2.46 p.p.m. (3 H, singlet).

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Transformation of L-Serine Peptides to L-Cysteine Peptides[1]

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The serine moieties of the peptides N-carbobenzoxy-L-serylglycine ethyl ester and N-carbobenzoxyglycyl-L-serylglycine ethyl ester were O-tosylated to give the corresponding O-tosyl derivatives IV and VI, respectively. Displacement of the O-tosyl groups of IV and VI by thiobenzoate anion afforded in excellent yields N-carbobenzoxy-S-benzoyl-L-cysteinylglycine ethyl ester (Va) and N-carbobenzoxyglycyl-S-benzoyl-L-cysteinylglycine ethyl ester (VIIa), respectively. A similar displacement by thioacetate anion afforded N-carbobenzoxy-S-acetyl-L-cysteinylglycine ethyl ester (Vb) and N-carbobenzoxyglycyl-S-acetyl-L-cysteinylglycine ethyl ester (VIIb).

Introduction

Previous communications from this laboratory dealt with the possibility of the transformation of L-serines incorporated into a peptide chain to L-cysteine residues.[2] This should be brought about by conversion of the O-tosyl group of serine to an easily removable S-protected

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(2) L. Zervas and I. Photaki, *Chimia* (Aarau), **14**, 375 (1960).