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The Structures and Biosynthesis of Multicolanic, Multicolic, and Multicolosic Acids, Novel Tetronic Acid Metabolites of Penicillium Multicolor[1]

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Multicolanic, multicolic, and multicolosic acids, metabolites of *Penicillium multicolor*, are shown by chemical transformations and spectroscopic methods to be 4-ylidenetetronic acids with structures (**I**), (**II**), and (**III**), respectively. The biosynthesis of these metabolites from acetate, via oxidative fission of preformed 6-pentylresorcylic acid is established by incorporation studies with [1-^{13}C]-, [2-^{13}C]-, [1,2-^{13}C]acetate and ethyl [2-^{14}C]-6-pentylresorcylate.

Two new optically inactive crystalline metabolites multicolic acid, $C_{11}H_{14}O_6$ (**II**), and multicolosic acid, $C_{11}H_{12}O_7$ (**III**), have been isolated from the fermentation liquors of a strain of *Penicillium multicolor* (CMI 104602), which has previously been reported to produce pencolide (1). Evidence for the presence of a third compound, multicolanic acid, $C_{11}H_{14}O_5$ (**I**), has also been obtained. Spectral and chemical evidence leading to the structures of these compounds and the elucidation of their biosynthesis is now reported in full.[3]

Three acidic groups in multicolosic acid (**III**) were demonstrated by potentiometric titration against sodium hydroxide when ionizations with p*K*s of 2.9, 9.8, and 11.4, respectively, were observed. Furthermore, methylation with diazomethane gave dimethyl *O*-methylmulticolosate (**VI**). Under similar conditions multicolic acid (**II**) gave methyl *O*-methylmulticolate (**V**) showing two acidic groups in the parent. When the crude fungal extract was similarly treated with diazomethane, prior to chromotography, a small amount of methyl *O*-methylmulticolanate (**IV**) was isolated, in addition to larger amounts of the two previous derivatives, (**V**) and (**VI**), respectively. Clearly, methyl *O*-methylmulticolanate (**IV**) is derived from the parent multicolanic acid (**I**), but attempts to isolate the latter have been unsuccessful.

Hydrogenation of multicolic acid (**II**) gave a dihydro-derivative (**VIII**), which showed spectral properties characteristic of a tetronic acid chromophore, viz λ_{max} (EtOH) 234 nm (ε 7000); λ_{max} (EtOH–KOH) 262 nm (ε 12 000), cf α-ethyltetronic acid, λ_{max} (EtOH) 233 nm (ε 12 000); λ_{max} (EtOH–KOH) 258 nm (ε 18 000) (*3*). Hydrogenation of methyl *O*-methylmulticolate (**V**) and dimethyl *O*-methylmulticolosate (**VI**) similarly

[1] This work is part of a series, X, "The Biosynthesis of Fungal Metabolites." This series of papers and many other related contributions are based on work carried out in Liverpool during the occupancy of the Heath Harrison Chair by the late Professor G. W. Kenner. Throughout this period of 21 years the senior author (J.S.E.H.) received enormous academic stimulation, great encouragement, and warm friendship from George Kenner, to whom this paper is gratefully dedicated.

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[3] A preliminary communication on part of this study has already been published (*2*).

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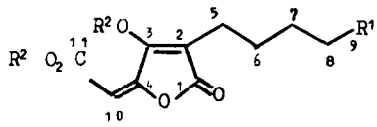
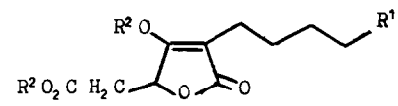
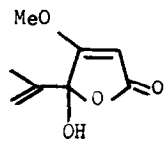


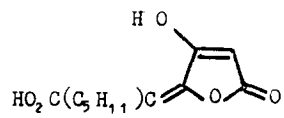


	R_1	R_2
(I)	Me	H
(II)	CH_2OH	H
(III)	CO_2H	H
(IV)	Me	Me
(V)	CH_2OH	Me
(VI)	CO_2Me	Me
(VII)	CH_2OAc	Me

	R_1	R_2
(VIII)	CH_2OH	H
(IX)	CH_2OH	Me
(X)	CO_2Me	Me



(XI)



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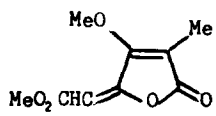

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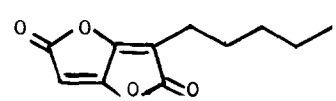


(XIV)

gave dihydro-derivatives, (**IX**) and (**X**), respectively. The residue >C=CH · CO_2Me in compounds (**IV**), (**V**), and (**VI**) was shown by a vinylic proton, τ ca. 4.15, in their ^1H nmr spectra (Table 1) and resonances at ca. 164, 150, 101, and 52 ppm in their ^{13}C nmr spectra (Table 2). In the dihydro-derivatives (**IX**) and (**X**), these were replaced by the diagnostic ABX pattern (τ_A 7.15, τ_B 7.50, τ_X 5.01; J_{AX} 4 Hz, J_{BX} 8 Hz, and J_{AB} 16 Hz) in the ^1H nmr spectra and resonances at 169, 73.5, 38, and 52 ppm in the ^{13}C nmr spectra. Conjugation of this residue with the tetronic acid chromophore was apparent from the change in uv spectra on hydrogenation. Thus methyl *O*-methylmulticolate (**V**) had λ_{max} (EtOH) 266 nm (ε 11 600), whereas the corresponding dihydro-derivative (**IX**) had λ_{max} (EtOH) 227 nm (ε 7100). No further reduction takes place, consistent with the known resistance of the tetronic acid chromophore to hydrogenation (*3*).

TABLE 1

CHEMICAL SHIFTS (τ ppm) AND MULTIPLICITIES (J Hz) OF PROTONS IN 100 MHz nmr SPECTRA OF *P. multicolor* METABOLITES AND DERIVATIVES (TMS AS INTERNAL STANDARD)

Compound	10-H^a	10-$CH_2{}^b$	5-$CH_2{}^c$	6-$CH_2{}^d$	7-$CH_2{}^d$	8-CH_2	4-H^b	*Me*-O[a]	Others
(II)[e]	4.10		7.66	8.5	8.5	8.5[d]			6.38 (6 Hz) 9-CH_2
(III)[e]	4.10		7.70	8.4	8.4	7.70[c]			
(IV)[f]	4.17		7.52	8.5	8.5	8.5[d]		5.93, 6.26	9.08 (7 Hz) 9-*Me*
(V)[f]	4.16		7.52	8.5	8.5	8.5[d]		5.93, 6.28	6.41 (7 Hz) 9-CH_2 7.75 CH_2OH
(VI)[f]	4.12		7.50	8.4	8.4	7.66[c]		5.93, 6.26 6.36	
(VII)[f]	4.13		7.51	8.5	8.5	8.5[d]		5.94, 6.26	5.97 (7 Hz) 9-CH_2 7.98 CH_3-CO
(VIII)[g]		6.65	7.40	8.7	8.7	8.7[d]	5.25		2.5–4.0 (3*H*, exchangeable)
(IX)[f]		7.31	7.61	8.5	8.5	8.59[d]	5.01	5.94, 6.32	6.39 (7 Hz) 9-CH_2
(X)[f]		7.30	7.60	8.4	8.4	7.60[c]	4.98	5.96, 6.30 6.36	

[a] Singlet.
[b] ABX system J_{AB} 16 Hz, J_{AX} 4 Hz, J_{BX} 8 Hz.
[c] Triplet (7 Hz).
[d] Broad multiplet.
[e] $CDCl_3/d_6$-DMSO.
[f] $CDCl_3$.
[g] d_6-DMSO.

The remainder of the multicolic acid structure is accounted for by the residue $-(CH_2)_4 \cdot CH_2OH$. This was clearly demonstrated by the ^1H nmr spectrum of methyl *O*-methylmulticolate (**V**): τ 6.41 (2H, t, J 7 Hz), 7.52 (2H, t, J 7 Hz), 7.75 (1H,

TABLE 2

^{13}C-CHEMICAL SHIFTS (ppm DOWNFIELD FROM INTERNAL TMS FOR $CDCl_3$ SOLUTIONS); MULTIPLICITIES IN OFF-RESONANCE SPECTRA

Carbon	(IV)	(V)	(VI)	(IX)	(X)
3	168.6s	168.3s	168.1s	173.2s	173.2s
2	110.4s	109.7s	109.3s	102.6s	102.3s
1	160.9s	160.6s	160.7s	171.4s	171.5s
4	150.7s	150.2s	150.9s	73.5d	73.5d
10	101.0d	100.9d	101.0d	38.4t	37.1t
11	164.3s	163.8s	163.7s	168.9s	168.8s
5	23.5t	23.4t	23.1t	22.9t	22.7t
6	29.7t	29.7t	29.2t	29.8t	29.4t
7	22.4t	25.5t	24.5t	25.4t	24.5t
8	31.6t	32.1t	33.4t	32.3t	33.5t
9	13.9q	62.2t	172.9s	62.3t	173.2s
3-MeO	59.6q	59.5q	59.5q	58.7q	58.7q
11-MeO	52.1q	52.0q	52.0q	52.0q	52.0q
9-MeO			51.4q		51.4q

exchangeable in D_2O), and 8.50 (6H, m). The presence of the primary alcoholic hydroxyl group was established by acetylation of methyl *O*-methylmulticolate to give the acetate (**VII**) and by oxidation with chromic oxide to give the corresponding carboxylic acid, which was not characterized but converted directly to dimethyl *O*-methylmulticolosate (**VI**) with diazomethane. The latter experiment confirms the structural relationship between multicolic and multicolosic acids. Comparison of the ^1H and ^{13}C nmr spectra of methyl *O*-methylmulticolanate (**IV**) and methyl *O*-methylmulticolate (**V**) (Tables 1 and 2, respectively) clearly demonstrates the presence of the pentyl side-chain in the former compound, compared with the pentanol side-chain in the latter.

TABLE 3

^1H- AND ^{13}C-RESONANCE SHIFTS INDUCED BY ADDITION OF $Eu(fod)_3$ TO METHYL *O*-METHYLMULTICOLATE (**V**)[a]

Position	^1H	^{13}C
1		0.40
2		0.28
3		0.31
4		0.32
5	0.70	0.40
6	1.09	0.60
7	1.80	0.89
8	2.59	1.11
9	4.21	6.04
10	0.32	0.32
11		0.08
3-OMe	0.31	0.20
11-OMe	0.18	0.12

[a] Values quoted are for ratios of $Eu(fod)_3$:compound of 2:5 and 1:10 for ^1H- and ^{13}C-spectra, respectively.

The ^{13}C assignments in Table 2 are based on comparisons of the proton noise decoupled (pnd) and off-resonance decoupled spectra, standard chemical shift data (*4*), and lanthanide induced shift (LIS) studies on methyl *O*-methylmulticolate (**V**) (Table 3). The latter showed that the principal site of coordination was the primary alcoholic hydroxyl group. The study permitted unambiguous assignments of the ^{13}C-chemical shifts of the individual side-chain carbons, and separate resolution of the individual pairs of methylene protons in the ^1H nmr spectrum. The ^{13}C-assignments of C-1 and C-3 are made by analogy with the corresponding spectrum of penicillic acid (**XI**), which has been assigned unambiguously (*5*). These represent a reversal of our original assignments for compound (**V**), which were made (*2*) prior to publication of the work on penicillic acid.

The ^{13}C nmr spectrum of compound (**V**), derived from multicolic acid enriched by feeding of [1,2-^{13}C]acetate to cultures of *P. multicolor* (see below), showed ^{13}C–^{13}C couplings of 48 and 90 Hz between C-2 and C-5 and between C-4 and C-10,

respectively. These values, which are typical for those of sp^2–sp^3 and sp^2–sp^2 hybridized coupled carbons, respectively, confirmed the positions of the substituent groups in the tetronic acid chromophore. It is worthy of note that the alternative type of structure (**XII**), which would be consistent with most of the structural data, and also biogenetically reasonable (see below), is excluded by the observation of the ABX system (see above) in the ^1H nmr spectra of the dihydro-derivatives (**IX**) and (**X**) and by the multiplicities observed for C-4 and C-10 in their off-resonance decoupled ^{13}C nmr spectra.

The only remaining ambiguity in the structures of this group of tetronic acids is the stereochemistry around the 4,10-double bond. It was observed that on prolonged standing, samples of the methylated derivative (**IV**), (**V**), and (**VI**) which had been recovered from $CDCl_3$ solutions, but not further purified, were partially isomerized to compounds in which the vinyl proton resonated at ca. τ 4.4, compared with 4.10 in the original compounds. This equilibration is presumably due to traces of acid remaining after removal of the solvent. This observation suggested the *E*-stereochemistry for the natural products, as the vinyl proton in the corresponding *Z*-isomers would be expected to resonate at higher field due to the shielding effect of the tetronic acid enolic ether oxygen atom. This conclusion has been confirmed by recent synthetic studies. Methyl *O*-methylmulticolanate (**IV**), the corresponding *Z*-isomer, together with the *E*- and *Z*-isomers of the 2-methyl analog (**XIII**), have been synthesized; X-ray crystallographic studies were carried out on the latter (*6*). It is found that the isomer in which the vinyl proton resonates at lower field does indeed have the *E*-stereochemistry. Furthermore, multicolanic acid (**I**) has been synthesized and shown to give the bislactone (**XIV**) on dehydration with bicyclohexylcarbodiimide (*7*), which could only arise from the *E*-isomer.

Two separate biosynthetic pathways to tetronic acids have been established: (a) oxidative ring cleavage of an aromatic or quinonoid intermediate, which may itself be polyketide or shikimate derived, and (b) condensation of a poly-β-ketide derived β-ketoacid with a C_4-dicarboxylic acid from the Kreb's Cycle (*8*). In the case of the new tetronic acids from *P. multicolor*, the biosynthesis was demonstrated by ^{13}C-incorporation experiments. Extracts from this organism, to which [1-^{13}C]-, [2-^{13}C]-, and [1,2-^{13}C]acetate had been fed were methylated and separated in the usual way to give suitably enriched samples of methyl *O*-methylmulticolate (**V**) and dimethyl *O*-methylmulticolosate (**VI**). The pnd ^{13}C nmr spectra of these compounds are summarized in Tables 4 and 5, respectively. The spectra from [1-^{13}C]- and [2-^{13}C]acetate-derived samples show that all the carbon atoms of the multicolic and multicolosic acid skeleta are acetate derived. However, the spectra from the [1,2-^{13}C]acetate-derived samples show ^{13}C–^{13}C couplings only in the carbon atoms 8–9, 6–7, 2–5, and 4–10, indicating that these are the only four intact acetate residues in the molecules. The complete absence of couplings in carbons 1, 3, and 11 indicate their origin from acetate units which have been cleaved during biosynthesis.

These results are best accommodated by the assumption that the poly-β-ketide derived 6-pentylresorcylic acid (**XV**) is a biosynthetic intermediate, as shown in Scheme 1. However, it is necessary to postulate that this intermediate is not converted at any stage to a symmetrical aromatic compound, e.g., 5-pentylresorcinol (**XVI**), since this would give rise to scrambling of ^{13}C–^{13}C couplings in the compounds derived from [1,2-

TABLE 4

^{13}C-ENRICHMENT DATA FOR METHYL *O*-METHYLMULTICOLATE (V)

Carbon	Enrichment from [1-^{13}C]Acetate[a]	Enrichment from [2-^{13}C]Acetate[b]	^{13}C–^{13}C-coupling constants from [1,2-^{13}C]acetate (Hz)
1		+	
2	+		48
3		+	
4	+		90
5		+	48
6	+		35
7		+	35
8	+		35
9		+	35
10		+	90
11	+		

[a] Average enrichment: 3.6 atom% ^{13}C over natural abundance.
[b] Average enrichment: 3.1 atom% ^{13}C over natural abundance.

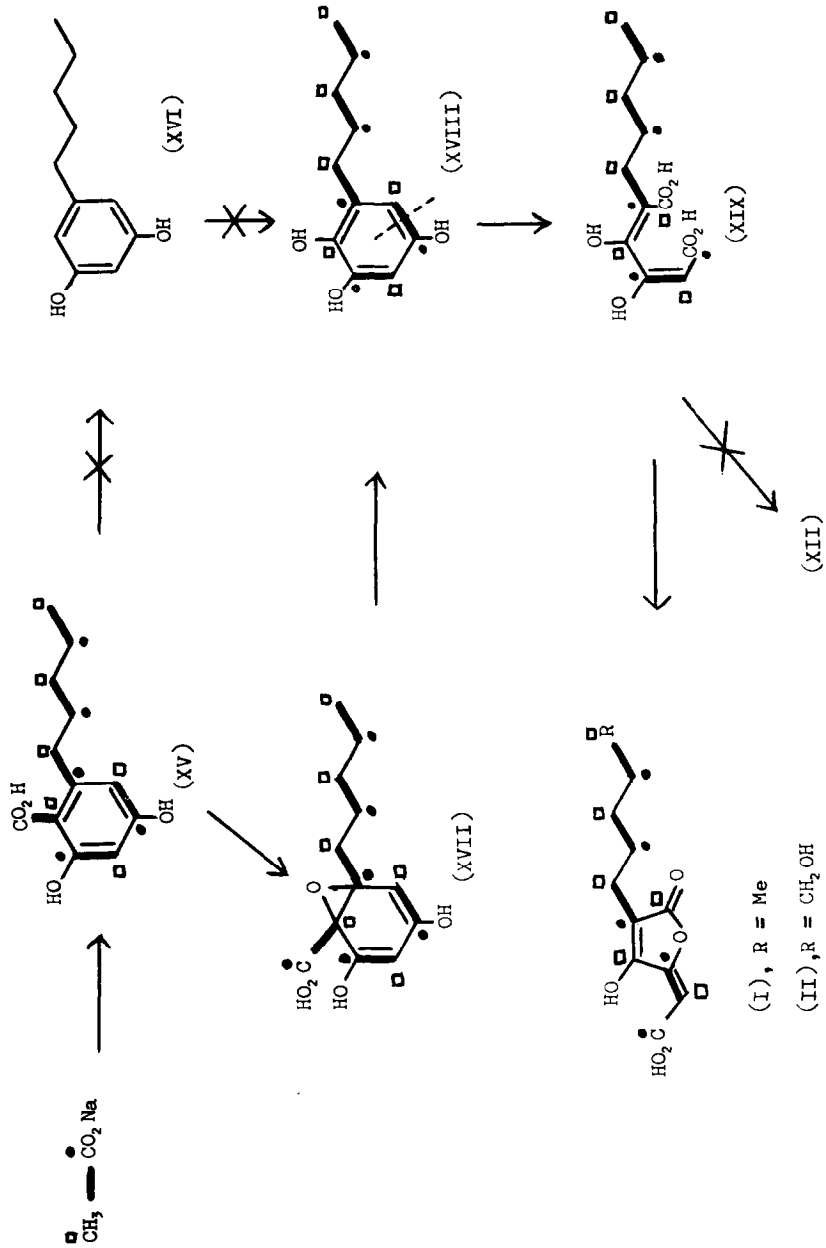
^{13}C]acetate. A possible intermediate is the trihydric phenol (**XVIII**), which could arise from (**XV**) via an arene oxide of type (**XVII**). Ring scission would have to occur at the position shown in (**XVIII**) to give the diacid (**XIX**). Enol lactone formation could then lead either to the observed metabolites (**I**), (**II**), and (**III**) or to a compound of type (**XII**). It is interesting that the latter has not been detected as a natural product, either because of enzyme mediation in the formation of tetronic acids (**I**), (**II**), and (**III**) or possibly because of a compound of type (**XII**) is much less thermodynamically stable than the observed compounds. This point is being investigated.

TABLE 5

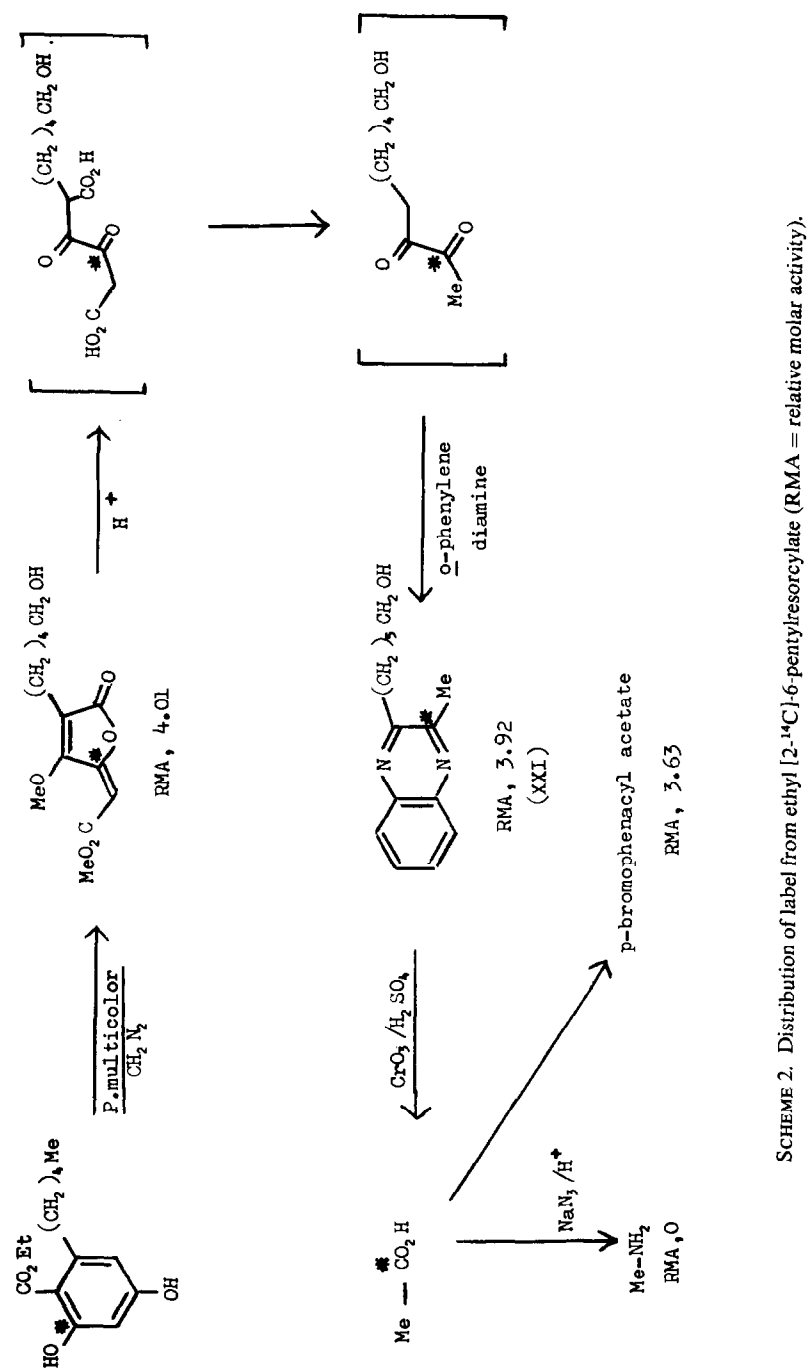
^{13}C-ENRICHMENT DATA FOR DIMETHYL *O*-METHYLMULTICOLOSATE (VI)

Carbon	Enrichment from [1-^{13}C]Acetate[a]	Enrichment from [2-^{13}C]Acetate[b]	^{13}C–^{13}C-coupling constants from [1,2-^{13}C]acetate
1		+	
2	+		45
3		+	
4	+		90
5		+	45
6	+		35
7		+	35
8	+		55
9		+	55
10		+	90
11	+		

[a] Average enrichment: 1.2 atom% ^{13}C over natural abundance.
[b] Average enrichment: 1.0 atom% ^{13}C over natural abundance.



SCHEME 1. Incorporations of [1-^{13}C]-, [2-^{13}C]-, and [1,2-^{13}C]acetate into metabolites of *P. multicolor* (heavy bonds denote inact acetate residues).



SCHEME 2. Distribution of label from ethyl [2-^{14}C]-6-pentylresorcylate (RMA = relative molar activity).

To confirm the intermediacy of 6-pentylresorcylic acid on the biosynthetic pathway, ethyl [2-^{14}C]-6-pentylresorcylate was synthesized from ethyl [3-^{14}C]acetoacetate and methyl oct-2-enoate (*9*) and fed to cultures of *P. multicolor*. Methyl *O*-methylmulticolate and dimethyl *O*-methylmulticolosate were isolated in the usual way, after methylation, and found to contain 0.25% of the fed radiolabel. To establish the specificity of labeling the former compound (4.01×10^{-3} μC mmol^{-1}) was degraded by dilute sulfuric acid to the α-diketone (**XX**), which was isolated as its quinoxaline derivative (**XXI**) (3.92×10^{-3} μC mmol^{-1}) (see Scheme 2). Kuhn–Roth oxidation of this compound gave acetic acid, which was characterized as its *p*-bromophenacyl ester (3.63×10^{-3} μC mmol^{-1}). Schmidt degradation of the acetate gave methylamine, which was isolated as the hydrochloride and found to contain negligible radioactivity. Hence essentially all of the radioactivity of the acetate is located in the carboxyl group. Since this accounts for 90% of the total activity of the methyl *O*-methylmulticolate, and corresponds to C-4 of this compound, it is clear that 6-pentylresorcylic acid is a specific precursor, which is incorporated with minimal randomization of label. It seems likely that ω-oxidation of the pentyl side-chain to give multicolic and multicolosic acids (**II**) and (**III**), respectively, occurs at a late stage in the biosynthetic sequence, although this has not yet been established.

In many respects the biosynthesis of multicolic acid is analogous to that of the extensively investigated compound, penicillic acid (**XI**) (*8*). In this case the precursor has been shown to be orsellinic acid, which undergoes *O*-methylation at the 2-position, followed by exactly analogous oxidative decarboxylation and ring scission reactions to those observed above. In this case 2-methyl-6-methoxybenzoquinone has been shown to be an intermediate (*10*). The absence of symmetrical intermediates in penicillic and multicolic acid biosynthetic pathways is in direct contrast to ravenelin (*11*) and griseofulvin (*12*) pathways, where studies with [1,2-^{13}C]acetate have demonstrated scrambling of couplings, presumably arising from the participation of intermediates containing symmetrically substituted aromatic rings.

EXPERIMENTAL SECTION

Unless otherwise stated, ir spectra were measured for solutions in carbon tetrachloride with a Perkin–Elmer 125 instrument, uv spectra for solutions in ethanol with a Unicam SP800 instrument, ^{1}H nmr spectra with a Varian HA-100 or XL-100 instrument, the latter also being used for ^{13}C nmr spectra. Mass spectra were determined with an A.E.I. MS-12 instrument at 70 eV, and accurate masses with an A.E.I. MS-9 instrument. tlc was performed on silica gel GF.254 (Merck). mp's were determined with a Kofler hot-stage instrument. For general details of ^{13}C-incorporation studies, see Part IV (*13*).

Isolation of metabolites. Penicillium multicolor (CMI 104602) was grown from spore suspension in static culture for 8 days at 25°C in 16 flat vessels (ca. 1 liter capacity), each containing Raulin-Thom medium (500 ml). After filtration, the culture broth was basified with a saturated solution of potassium carbonate, extracted with ethyl acetate, until the organic phase was colorless, acidified with concentrated hydrochloric acid, and reextracted with ethyl acetate. After removal of the solvent, a solution of the residual

brown gum (10 g) in ether containing 5% methanol (200 ml) was filtered and silica gel (300 g, Grace 200–300 mesh) was stirred in. After evaporation of the solvent, benzene (500 ml) was added, and the resultant slurry added to the top of a column (90 × 12 cm) of silica gel (2 kg) in benzene. Gradient elution was then carried out using benzene, containing increasing proportions of ether:dichloromethane (1:1). Five hundred ml fractions were collected and after every fourth fraction the concentration of ether:dichloromethane was increased by 1%, so that after 200 fractions had been taken, a mixture of benzene:ether:dichloromethane (2:1:1) was being used. Slowing increasing proportions of methanol were then added until most of the color had been removed from the column.

Multicolosic acid (**III**) was obtained in fractions 100–116 (ether:dichloromethane:benzene, 1:1:20) and separated from dichloromethane in needles (500 mg), mp 150–153°C, ν_{max} (CH_2Cl_2) 3460, 3200, 2800–2400, 1785, 1640 cm^{-1}, λ_{max} 262, 295 nm (ε 15 000 and 8000), *m/e* 256 (0.08), 239 (0.23), 238 (1.00), 221 (0.15), 220 (0.88), 210 (0.40), 207 (0.20), 192 (0.45), 182 (0.82), 178 (0.45), 164 (0.55), *m*/e* 221.2 (256→238), 205.4 (238→221), 203.4 (238→220), 193.4 (221→207), 185.5 (238→210), 147.6 (182→164) (Found: C, 51.6; H, 4.7. $C_{11}H_{12}O_7$ requires C, 51.5; H, 4.7%).

Multicolic acid (**II**) was obtained in fractions 190–215 (while the first trace of methanol was being added) and separated from dichloromethane in needles (1.2 g), mp 129–131°C, ν_{max} (CH_2Cl_2) 3420, 2920, 2850, 1785, 1700, 1640 cm^{-1}, λ_{max} 263, 295 nm (ε 15 000 and 8000), *m/e* 242 (0.30), 224 (0.24), 207 (0.06), 206 (0.50), 196 (0.15), 182 (0.80), 178 (0.40), 164 (1.00), 157 (0.18), *m*/e* 207.3 (242→224), 189.4 (224→206), 161.7 (196→178), 147.8 (182→164) (Found: C, 54.0; H, 5.8. $C_{11}H_{14}O_6$ requires C, 54.5; H, 5.8%).

Methyl O-methylmulticolanate (**IV**), *methyl O-methylmulticolate* (**V**), *and dimethyl O-methylmulticolosate* (**VI**). (a) Compounds (**V**) and (**VI**) were obtained as colorless gums from multicolic and multicolosic acids, respectively, by treatment with excess ethereal diazomethane in the usual way. *Methyl O-methylmulticolate* (**V**) had ν_{max} 3605, 3500 (br), 1742, 1665, and 1185 cm^{-1}, λ_{max} 266 nm (ε 11 600) (Found: *m/e*, 270.110. $C_{13}H_{18}O_6$ requires *m/e*, 270.110). *Dimethyl O-methylmulticolosate* (**VI**) had ν_{max} 1793, 1739, 1718, 1672, and 1643 cm^{-1}, λ_{max} 266 nm (ε 11 500) (Found: *m/e*, 298.105. $C_{14}H_{18}O_7$ requires *m/e*, 298.106). The *acetate* (**VII**) was prepared from methyl *O*-methylmulticolate (50 mg) with acetic anhydride/pyridine at room temperature for 4 hr, in the usual way. Purified by preparative tlc, with ether as developing solvent, it was obtained as an oil (48 mg), ν_{max} 1786, 1738, 1635, and 1230 cm^{-1}, λ_{max} 266 nm (ε 10 500) (Found: *m/e*, 312.123. $C_{15}H_{20}O_7$ requires *m/e*, 312.123). (b) The crude extract from the organism was methylated with diazomethane and the total product partitioned by preparative tlc with ether as the developing solvent. Methyl *O*-methylmulticolate (60 mg $liter^{-1}$ of culture broth) was eluted with ethyl acetate from a band with R_f 0.3, and dimethyl *O*-methylmulticolosate (55 mg $liter^{-1}$) from a band with R_f 0.45. A third band, R_f 0.8, was isolated and further purified by multiple elution tlc, using light petroleum (bp 60–80°C):ether (1:1) as developing solvent. A band with R_f 0.2 was extracted in ethyl acetate to give *methyl O-methylmulticolanate* (**IV**) as an oil (3 mg $liter^{-1}$), ν_{max} 1775, 1727, 1630, and 1138 cm^{-1}, λ_{max} 256 nm (ε 12 600) (Found: *m/e*, 254.116. $C_{13}H_{18}O_5$ requires *m/e*, 254.116).

Oxidation of methyl-O-methylmulticolate. To a solution of this compound (60 mg) in acetone (5 ml) at 0°C, Jones reagent (three drops) was added, and after 5 min the reaction was quenched with ethanol (1 ml). The product was isolated in ethyl acetate (2 × 10 ml), after dilution of the reaction mixture with water (20 ml), and methylated with ethereal diazomethane. Purified by tlc dimethyl *O*-methylmulticolosate, with identical spectral properties to those above, was isolated as a gum (40 mg).

Dihydromulticolic acid (**VIII**), *methyl O-methyldihydromulticolate* (**IX**), *and dimethyl O-methyldihydromulticolosate* (**X**). Multicolic acid, methyl *O*-methylmulticolate and dimethyl *O*-methylmulticolosate were each hydrogenated in ethyl acetate containing 10% palladium on carbon, with hydrogen gas at atmospheric pressure and room temperature, to give quantitative yields of the respective dihydro-derivatives. Thus obtained, *dihydromulticolic acid* (**VIII**) was an amorphous solid, λ_{max} 234 nm (ε 6900), λ_{max} (EtOH–NaOH) 262 nm (ε 12 400), *methyl O-methyldihydromulticolate* (**IX**), an oil, λ_{max} 227 (ε 7100), ν_{max} ($CHCl_3$) 1740 and 1665 cm^{-1} (Found: *m/e*, 272.126. $C_{13}H_{20}O_6$ requires *m/e*, 272.126), and *dimethyl O-methyldihydromulticolosate* (**X**), an oil, λ_{max} 228 (ε 7200), ν_{max} ($CHCl_3$) 1735 and 1665 cm^{-1} (Found: *m/e* 300.119. $C_{14}H_{20}O_7$ requires *m/e*, 300.118).

Incorporations of [1-^{13}C]-, [2-^{13}C]-, and [1,2-^{13}C]acetate into the metabolites. Sodium [1-^{13}C]- and [2-^{13}C]acetate (200 mg, respectively) were each added to a 7-day-old growth culture pan of *P. multicolor*. After a further 3 days the culture liquors were extracted and methylated in the usual way to give [^{13}C]methyl *O*-methylmulticolate (92 and 84 mg, respectively, from [1-^{13}C]- and [2-^{13}C]acetate) and [^{13}C]dimethyl *O*-methylmulticolosate (63 and 67 mg, respectively). Similarly [1,2-^{13}C]acetate (250 mg) was added to a 6-day-old culture pan of *P. multicolor* to give, after work-up and isolation, [^{13}C]methyl *O*-methylmulticolate (70 mg) and [^{13}C]dimethyl *O*-methylmulticolosate (30 mg).

Ethyl [2-^{14}C]-6-pentylresorcylate. Prepared from ethyl [3-^{14}C]acetoacetate (483 mg, 134.9 mC $mmol^{-1}$) and methyl oct-2-enoate (478 mg) by the literature method (*9*), ethyl [2-^{14}C]-6-pentyldihydroresorcylate was obtained as prisms (535 mg, 134.1 mC $mmol^{-1}$) from light petroleum (bp 40–60°C), mp 64°C [lit. mp (*14*) 64–65°C]. Bromination of this compound (530 mg) with bromine (1 g) in acetic acid (1.3 ml), by the literature method (*9*) gave a mixture which was separated by chromatography on silica. Elution with light petroleum (bp 60–80°C) : acetone (1 : 1) gave successively ethyl [2-^{14}C]dibromo-6-pentylresorcylate, prisms (320 mg, 134.0 mC $mmol^{-1}$), mp 66–67°C [lit. mp (*14*) 67°C] from light petroleum (bp 40–60°C) and *[1,3-^{14}C]-2,4,6-tribromo-5-pentylresorcinol*, needles (100 mg) from light petroleum (bp 40–60°C), mp 64–65°C, ν_{max} 2475, 1520, 1400, 1358, 1320 and 1110 cm^{-1}, λ_{max} 213 nm (ε 13 000), τ 3.91 (2H, s, exchangeable), 7.03 (2H, t, *J* 7.5 Hz), ca. 8.5 (6H, m) and 9.09 (3H, t, *J* 5.5 Hz) (Found: C, 31.7: H, 3.1; Br, 57.5. $C_{11}H_{13}O_2Br_3$ requires C, 31.7; H, 3.1; Br, 57.5%). Hydrogenolysis of ethyl [2-^{14}C]dibromo-6-pentylresorcylate (300 mg) in 1 *M* sodium hydroxide (3.5 ml), containing 5% palladium on calcium carbonate (150 mg) with hydrogen at atmospheric pressure and room temperature gave, after isolation in ether (4 × 25 ml), ethyl [2-^{14}C]-6-pentylresorcylate, which formed prisms (160 mg, 134.6 mC $mmol^{-1}$) from light petroleum (bp 40–60°C), mp 69°C [lit. mp (*14*) 69°C [Found: C, 66.7; H, 8.0. Calcd for $C_{14}H_{10}O_4$: C, 66.7; H, 8.0%].

[^{14}C]-2-methyl-3-(6-hydroxylhexyl)-quinoxaline (**XXI**). Radiolabeled methyl *O*-

methylmulticolate was diluted with inactive material to give a sample (90 mg, 4.01×10^{-3} μC mmol^{-1}) which was heated under reflux with 1.5 *M* sulfuric acid (10 ml), under nitrogen, for 48 hr. After neutralization of the mixture with sodium hydrogen carbonate, *o*-phenylenediamine (50 mg) in ethanol (3 ml) was added and the whole warmed to 100°C for 15 min. The product was isolated in chloroform (4 × 10 ml) and purified by preparative tlc, using acetone : light petroleum (bp 60–80°C) (1 : 3) as the developing solvent. The band with R_f 0.25 was eluted with ethyl acetate and the material crystallized from aqueous ethanol to constant radioactivity giving [^{14}C]-*2-methyl-3-(6-hydroxyhexyl)-quinoxaline* (**XXI**) as needles (55 mg, 3.92×10^{-3} μC mmol^{-1}), mp 139°C, ν_{max} 3480 (br), 1563, 1450, 1392, 1365, 1340, 1328, 1315, 1155, 1140, and 1124 cm^{-1}, λ_{max} 204, 237, and 315 nm (ε 13 200, 12 300, and 9700), τ 1.89–2.42 (4H), 6.40 (2H, t, *J* 7.5 Hz), 7.0 (2H, t, *J* 7.0 Hz), 7.33 (3H, s) and 8.2–8.8 (8H, m) [Found: C, 73.5; H, 8.3; N, 11.7; *m/e*, 244.156. $C_{15}H_{20}N_2O$ requires C, 73.7; H, 8.3; N, 11.5; *m/e*, 244.158].

Degradation of quinoxaline (**XXI**). Kuhn–Roth oxidation of compound (**XXI**) was carried out by the standard procedure (*15*) and a portion of the acetic acid isolated was converted to its *p*-bromophenacyl ester. This was purified by tlc and crystallized by constant activity from light petroleum (bp 60–80°C), giving plates (3.63×10^{-3} μC mmol^{-1}), mp 86°C. The remainder of the acetic acid was subjected to Schmidt degradation, as previously described (*16*). Methylamine was isolated as its hydrochloride and found to be essentially radiochemically inactive.

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