Use of Singly and Doubly Labelled ^{13}C-Acetate in the Elucidation of the Structures and Biosynthesis of Multicolic and Multicolosic Acids, New Tetronic Acids from *Penicillium multicolor*

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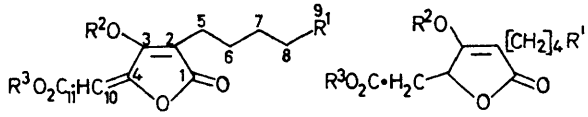
Summary Multicolic and multicolosic acid, isolated from cultures of *Penicillium multicolor*, are shown to have structures (I) and (II) respectively by ^{1}H- and ^{13}C-n.m.r., and u.v. spectroscopic studies, ^{13}C-couplings observed in the spectrum of methyl *O*-methylmulticolate (III), prepared from multicolic acid enriched with [1,2-^{13}C]-acetate establishing the substitution pattern in the tetronic acid chromophore; the biosynthesis of these metabolites from acetate, *via* oxidative fission of a pre-formed aromatic precursor, *e.g.*, 6-pentylresorcylic acid, is suggested by the ^{13}C-n.m.r. spectra of derivatives, enriched with [1-^{13}C]-, [2-^{13}C]-, and [1,2-^{13}C]-acetate.

Two new optically inactive metabolites, multicolic and multicolosic acids, $C_{11}H_{14}O_6$, m.p. 129—131°, and $C_{11}H_{12}O_7$, m.p. 150—153°, respectively† have been isolated from the fermentation liquors of *P. multicolor* (IMI 104602), which had previously been reported to produce pencolide.[1] The structures (I) and (II) respectively have been established for these compounds on the basis of the following evidence. (a) Multicolic acid (I) gave methyl *O*-methylmulticolate (III) with diazomethane. Oxidation of this derivative with chromic oxide gave the acid (IV) which was converted into the methyl ester (V) with diazomethane, identical with the product obtained by similar methylation of multicolosic acid (II).



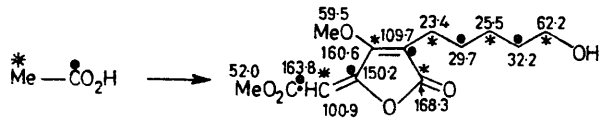
	R^1	R^2	R^3
(I)	CH_2OH	H	H
(II)	CO_2H	H	H
(III)	CH_2OH	Me	Me
(IV)	CO_2H	Me	Me
(V)	CO_2Me	Me	Me

	R^1	R^2	R^3
(VI)	CH_2OH	H	H
(VII)	CH_2OH	Me	Me
(VIII)	CO_2Me	Me	Me

(b) Hydrogenation of multicolic acid with Pd–C in ethyl acetate gave the dihydro-derivative (VI) which showed spectral properties characteristic of a tetronic acid chromophore: λ_{max} (EtOH) 234 nm (ϵ 7000); λ_{max} (EtOH–KOH)

† All compounds described gave satisfactory elemental analyses.

262 nm (ε 11,000), *cf.* α-ethyl-tetronic acid,[2] λ_{max} (EtOH) 233 nm (ε 12,000), λ_{max} (EtOH–KOH) 258 nm (ε 18,000).



(III)

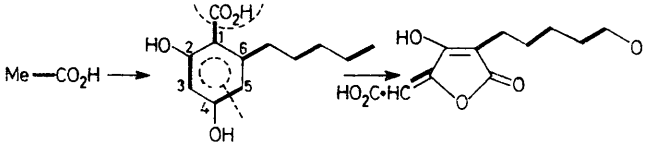
FIGURE 1. ^{13}C-n.m.r. chemical shifts (δ) for methyl *O*-methyl multicolate (in $CDCl_3$) and labelling pattern established for [1-^{13}C]- and [2-^{13}C]-acetate incorporations.

(c) The presence of the residue $-[CH_2]_4\cdot CH_2OH$ in multicolic acid was clearly demonstrated by the ^{1}H n.m.r. spectrum of methyl *O*-methylmulticolate (III); τ ($CDCl_3$), 6·41 (2H, t, *J* 7 Hz), 7·52 (2H, t *J* 7 Hz) 7·75 (1H, exchangeable in D_2O), and 8·50 (6H, m). Lanthanide induced shift (L.I.S.) studies with Eu(fod)$_3$ showed that the principal co-ordination site was the primary alcoholic hydroxy-group and separate resolution of the individual methylene groups of the pentyl side-chain was achieved.

(d) The residue $>C{=}CH\cdot CO_2H$ in both metabolites was demonstrated by the presence of a singlet proton, τ 4·10 in the ^{1}H n.m.r. spectra of the methylated compounds (III) and (V), which was replaced in the dihydro-derivatives (VII) and (VIII) by typical ABX patterns (τ_A 7·15, τ_B 7·50, τ_X 5·01; J_{AB} 16 Hz, J_{AX} 4 Hz, and J_{BX} 8 Hz). The conjugation of this residue with the tetronic acid chromophore was shown by the u.v. spectra of the parent metabolites; λ_{max} (EtOH) 262 and 295 nm (ε 15,000 and 8000).

(e) The ^{13}C-assignments shown in Figure 1 for methyl *O*-methylmulticolate (III) are based on measurements of proton noise- and off-resonance-decoupled ^{13}C-n.m.r. spectra of compounds (III), (V), (VII), and (VIII) and L.I.S. studies on the spectrum of compound (III). The only ambiguity is a possible reversal of the C(1) and C(3) assignments. The ^{13}C-n.m.r. spectrum (Figure 2) of compound (III), derived from multicolic acid enriched by feeding the organism with [1,2-^{13}C]acetate, shows ^{13}C-couplings of 48 and 90 Hz between C(2)–C(5) and C(4)–C(10) respectively. These values, which are typical of those for sp^2–sp^3 and sp^2–sp^2 hybridised coupled carbon atoms respectively,[3] establish the positions of the substituent groups in the tetronic acid chromophore and complete the structure elucidation, apart from the stereochemistry of the 4,10 double bond, which has not been determined.

Two separate biosynthetic pathways have been established for fungal tetronic acids. (a) Oxidative cleavage of an aromatic or quinonoid precursor, as in the formation of penicillic acid from orsellinic acid,[4] and (b) condensation of a polyketide derived β-ketoacid with a Kreb's cycle acid, *e.g.* succinic acid, as in the formation of carolic and carlosic acids.[5] In the present case, the ^{13}C-n.m.r. spectra (Figure 2) of the derivative (III), prepared from multicolic acid enriched with [1-^{13}C]- and [2-^{13}C]-acetate respectively, establish the labelling patterns indicated in Figure 1. These are compatible only with a biosynthetic origin by oxidative cleavage of a polyketide derived aromatic precursor, *e.g.* fission between C(4) and C(5) in 6-pentylresorcylic acid (IX). The ^{13}C-n.m.r. spectrum of derivative (III), derived from [1,2-^{13}C]acetate enriched multicolic acid, (Figure 2), confirms this postulate since the observed couplings C(8)–C(9), C(6)–C(7), C(2)–C(5), and C(4)–C(10), are those between pairs of atoms derived from the same acetate residues in the aromatic precursor. The absence of couplings at C(1), C(3), and C(11) rules out the possibility of 1,2-cleavage in (IX) and precludes the biosynthetic intermediacy of any symmetrical aromatic intermediate, *e.g.* 5-pentylresorcinol. This route which is summarised in the Scheme, appears to be the first example of the use of ^{13}C-doubly labelled acetate to establish the intermediacy of an aromatic precursor in the biosynthesis of a fungal metabolite. This technique, due to Seto and his co-workers[6] is finding increasing application in fungal biosynthesis, where the necessary high incorporations can be obtained relatively easily. Similar findings have



(IX) (I)

SCHEME: Expected couplings in multicolic acid derived from [1,2-^{13}C] acetate, *via* 4,5-fission of 6-pentylresorcylic acid.

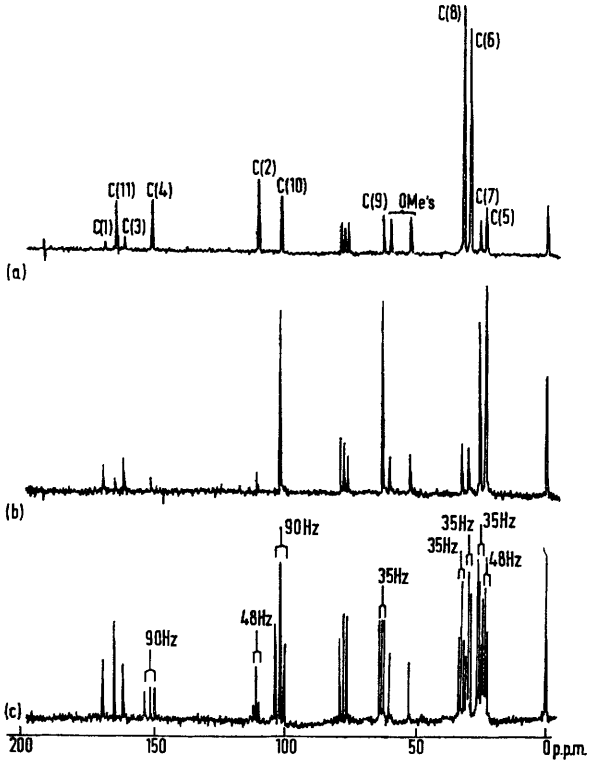


FIGURE 2. The proton-noise decoupled F.T. ^{13}C-n.m.r. spectra of methyl *O*-methylmulticolate (III) labelled with: (a) [1-^{13}C] acetate, (b) [2-^{13}C] acetate, and (c) [1,2-^{13}C] acetate (in $CDCl_3$).

recently been reported for the biosynthesis of penicillic acid, where it has been shown by tritium labelling studies that orsellinic acid undergoes specific 4,5-cleavage.[7]

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