The Distribution of Acetate-derived Oxygens in the Tetronic Acids of *Penicillium multicolor*

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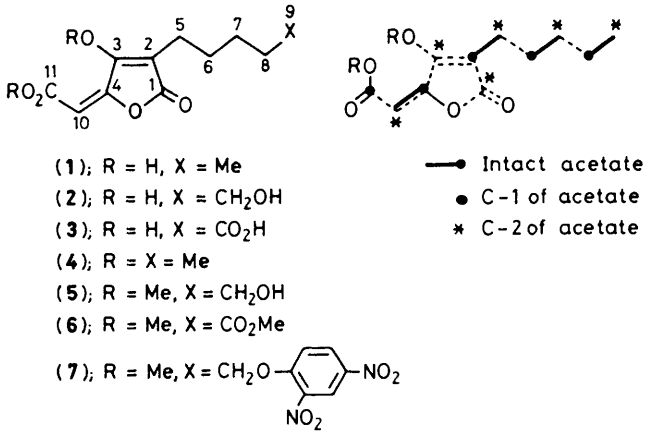
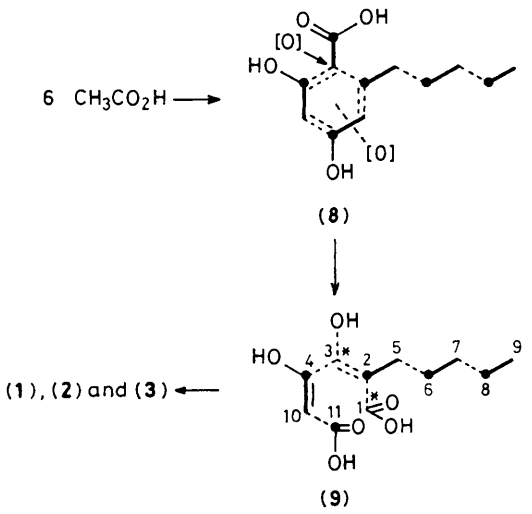
Incorporation of sodium [1-^{13}C, $^{18}O_2$]acetate by *Penicillium multicolor* into multicolanic, multicolic, and multicolosic acids, (**1**), (**2**), and (**3**), respectively, has given samples which when derivatised and examined by spin echo Fourier transform (S.E.F.T.)^{13}C n.m.r. spectroscopy show ^{18}O-induced ^{13}C-satellites, confirming the previously proposed biosynthetic pathway involving oxidative fission of the β-ketide derived intermediate 6-pentylresorcylate (**8**) and establishing that lactonisation of the tetronic acid ring occurs by displacement from the C-1 carboxy-group by the C-4 enolic oxygen, rather than by lactol formation.

^{18}O-Induced changes in the ^{13}C-chemical shifts of directly attached carbon atoms have recently been shown to provide an extremely valuable addition to the armoury of n.m.r. techniques available for the investigation of biosynthetic path-





Scheme 1

ways.[1-3] These induced shifts, although small (0.01—0.055 p.p.m.), can readily be resolved in ^{13}C n.m.r. spectra measured at 100 MHz, particularly with spin echo Fourier transform (S.E.F.T.) techniques.[4] Most of the biosynthetic studies carried out to date involve feeding experiments with sodium [1-^{13}C, $^{18}O_2$]acetate on poly-β-ketide derived metabolites.

It has been shown by feeding experiments with sodium [1-^{13}C]-, [2-^{13}C]-, [1,2-^{13}C]-acetate and ethyl [2-^{14}C]-6-pentylresorcylate that the *Penicillium multicolor* metabolites multicolanic, multicolic, and multicolosic acids, (**1**), (**2**), and (**3**) respectively, are derived from acetate by oxidative fission of a β-ketide-derived 6-pentylresorcylate intermediate (**8**), as outlined in Scheme 1.[5] On the basis of this pathway a key intermediate would be the diacid (**9**) in which the oxygen atom at C-4 and one of the two in the C-11 carboxy-group would be the only ones derived from the original acetate precursor. The question which interested us was whether the lactonisation of this involved in the generation of the metabolites (**1**), (**2**), and (**3**) would involve loss of oxygen from C-1, C-4, or both. In principle, a feeding experiment with [1-^{13}C, $^{18}O_2$]-acetate and determination of the ^{13}C-n.m.r. spectra of the resultant metabolites would not only permit this distinction, but would also provide excellent confirmatory evidence for the proposed pathway.

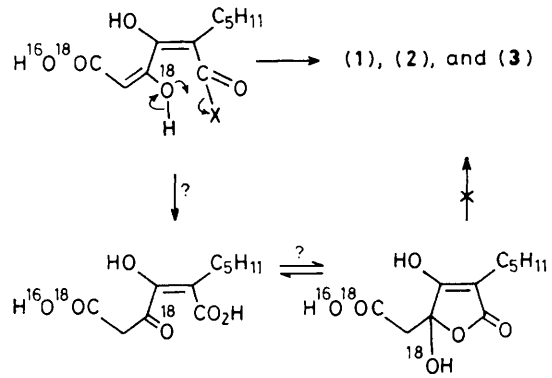
Accordingly, sodium [1-^{13}C, $^{18}O_2$]acetate was added to cultures of *P. multicolor*, as previously described for [1,2-^{13}C] acetate incorporations,[5] and the resultant mixture of metabolites was methylated with diazomethane and separated in the usual way[5] to give the derivatives (**4**), (**5**), and (**6**) respectively. Rigorous purification of the oily methylated derivatives by repeated preparative layer chromatography (p.l.c.) produced pure samples of methyl *O*-methylmulticolanate (**4**) and dimethyl *O*-methylmulticolosate (**6**). However, methyl *O*-methylmulticolate (**5**) still retained minor impurities which interfered with the required ^{13}C n.m.r. studies. It was, therefore, converted into a 2,4-dinitrophenyl ether (**7**) by treatment with 2,4-dinitrofluorobenzene in the presence of triethylamine in deuteriodichloromethane.[6] The resultant crystalline derivative was rigorously purified by p.l.c. and characterised by mass spectrometry (M^+ 436.1118; $C_{19}H_{20}N_2O_{10}$ requires M 436.1118) and ^1H and ^{13}C n.m.r. spectroscopy.

The ^{13}C n.m.r. spectra of compounds (**4**), (**6**), and (**7**) recorded in the normal Fourier transform (F.t.) mode at 100.6 MHz showed relatively complex signals due to long range couplings resulting from multiple incorporation of ^{13}C labels within the same molecule. By utilizing the S.E.F.T. technique, and spectral expansion (32 K data block/2000 Hz) of all the signals due to oxygen-bearing carbon atoms, it was

Table 1. ^{13}C-Chemical shifts of compounds (**4**), (**6**), and (**7**) derived from [1-^{13}C, $^{18}O_2$]acetate (90% ^{13}C, 81% $^{18}O_2$, 18% $^{18}O_1$).

Carbon	δ (p.p.m.)[a]	Δ (p.p.m. × 10^2)[b]	^{16}O:^{18}O[c]
Methyl *O*-methylmulticolanate (**4**)			
1	161.3	—	—
3	168.8	—	—
4	151.1	1.9	59:41
11	164.6	1.2; 3.6	60:20:20
Dimethyl *O*-methylmulticolosate (**6**)			
1	160.7	—	—
3	168.1	—	—
4	150.4	2.1	60:40
9	172.9	—	—
11	164.2	0.75; 3.25	67:17:17
Methyl *O*-methylmulticolate 2,4-dinitrophenyl ether (**7**)			
1	161.5	—	—
3	168.8	—	—
4	150.7	1.7	60:40
9	70.4	—	—
11	164.4	1.2, 3.5	60:20:20

[a] F.t. spectra were measured on a Bruker WH400 spectrometer at 100.6 MHz for solutions in $CDCl_3$ with Me_4Si as internal standard. [b] ^{18}O Upfield shift values are relative to the ^{13}C-^{16}O signal as internal reference and are ±0.1 (p.p.m. × 10^2). [c] Approximate values obtained from relative peak heights of the ^{13}C–^{16}O and ^{13}C-^{18}O signals in S.E.F.T. spectra.



Scheme 2

found that only the C-4 and C-11 signals showed upfield ^{18}O-isotope shift satellites. The C-4 had one satellite signal but C-11 had two due to contributions from $-C^{18}O\cdot OMe$ and $-CO\cdot{}^{18}OMe$ (see Table 1). Furthermore, the combined intensities of the two satellite signals due to C-11 were approximately equal to that due to C-4, indicating equal incorporation of intact [1-^{13}C, $^{18}O_2$]acetate at both sites.

The finding that [1-^{13}C, $^{18}O_2$]acetate precursor leads to equal labelling of C-4 and C-11 with all other oxygen atoms being otherwise derived is entirely compatible with the previous proposals summarised in Scheme 1. Since doubly labelled [^{13}C, ^{18}O]-precursors permit detection of carbon–oxygen bonds which remain intact during biosynthesis,[1] this equal labelling of C-4 and C-11 establishes that the lactonisation of the tetronic acid ring does not involve lactol formation and dehydration, but rather displacement from C-1, probably *via* an activated thioester (see Scheme 2).

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References

1 J. C. Vederas, *Can. J. Chem.*, 1982, **60**, 1637, and references cited therein.
2 A. E. DeJesus, W. E. Hull, P. S. Steyn, F. R. van Heerden, and R. Vleggaar, *J. Chem. Soc., Chem. Commun.*, 1982, 902.
3 G. K. Smith, W. T. Mueller, L. J. Slieker, C. W. DeBrosse, and S. J. Benkovic, *Biochemistry*, 1982, **21**, 2870.
4 M. P. Lane, T. T. Nakashima, and J. C. Vederas, *J. Am. Chem. Soc.*, 1982, **104**, 913.
5 J. A. Gudgeon, J. S. E. Holker, T. J. Simpson, and K. Young, *Bioorg. Chem.*, 1979, **8**, 311.
6 L. F. Fieser and M. Fieser, 'Reagents for Organic Synthesis,' Wiley, New York, 1967, vol. 1, p. 321, and references cited therein.