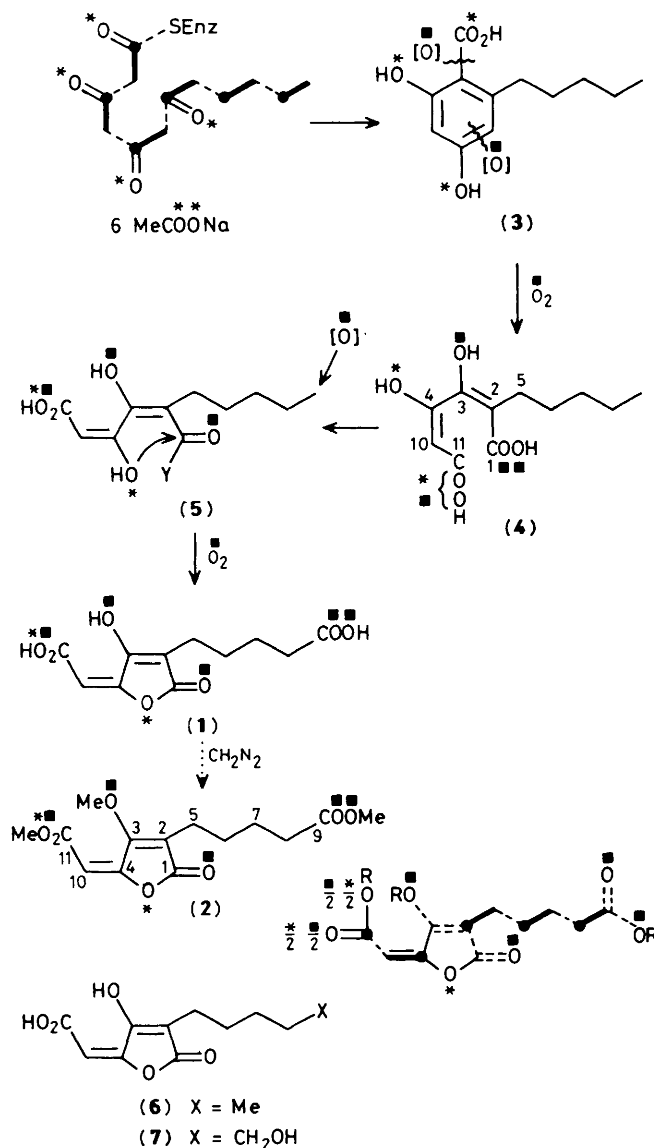
Biosynthesis of Multicolosic Acid, a Polyketide Metabolite from *Penicillium multicolor*: Occurrence of Large ^{18}O-Induced β-Isotope Shifts in ^{13}C N.M.R. Spectra

John S. E. Holker,*[a] Miyuki Kaneda,[b] Shawn E. Ramer,[b] and John C. Vederas*[b]

[a] *Robert Robinson Laboratories, University of Liverpool, P.O. Box 147, Liverpool L69 3BX, U.K.*
[b] *Department of Chemistry, University of Alberta, Edmonton, Alberta, Canada T6G 2G2*

Fermentation of *Penicillium multicolor* under an atmosphere containing $^{18}O_2$ produces multicolosic acid (**1**) whose methylated derivative (**2**) displays both α-isotope shifts and unusually large β-isotope shifts in its ^{13}C n.m.r. spectra; the results define the origins of all oxygens of (**1**) and provide information about its formation from an aromatic polyketide precursor, 6-pentylresorcylate (**3**).

The varied natural distribution and interesting biological activities of ylidene tetronic acids have prompted numerous synthetic and structural studies on such compounds.[1,2] Multicolosic acid (**1**) is one of several related metabolites [*e.g.*, multicolanic acid (**6**) and multicolic acid (**7**)] isolated by Holker and coworkers from *Penicillium multicolor*.[3,4] Incorporation experiments with sodium [1-^{13}C]-, [2-^{13}C]-, [1,2-$^{13}C_2$]-, [1-^{13}C,$^{18}O_2$]-acetate and ethyl [2-^{14}C]-6-pentylresorcylate (**3**) have indicated that (**1**) is a polyketide metabolite derived from oxidative cleavage of an aromatic precursor (Scheme 1).[4,5] In particular, labelling studies with [1-^{13}C, $^{18}O_2$]acetate have shown that the bond between C-4 and the lactone oxygen remains intact during biosynthesis of (**1**) and that such an intact bond can also be found in the C-11 carboxy group. We now report incorporation experiments with $^{18}O_2$ gas which define the origin of all remaining oxygens in multicolosic acid (**1**). During these investigations we also observed unusually large ^{13}C n.m.r. chemical shift changes for a carbon two bonds away from the oxygen-18 (β-isotope shifts).



Scheme 1

Cultures of *P. multicolor* were grown as previously described[4] except that the fermentation was done in a closed system[6] containing an atmosphere in which normal oxygen was gradually replaced with $^{18}O_2$ (50% isotopic purity). The resulting mixture of metabolites was methylated with diazomethane and separated in the usual way to afford pure dimethyl *O*-methylmulticolosate (**2**).[4]

Incorporation of ^{18}O was detected by observation of upfield ^{18}O-induced shifts in the ^{13}C n.m.r. spectra[7] of (**2**) (Table 1 and Figure 1). All carbons bearing oxygen, except C-4, show α-isotope shifts. The lactone carbonyl (C-1) displays a single ^{18}O-shifted resonance ($\Delta\delta$ = 18.0 p.p.b.) which, together with the absence of an isotope shift at C-4, indicates that only the doubly-bonded oxygen is labelled. The same ^{16}O : ^{18}O ratio (70:30) is seen at C-3. As expected on the basis of [1-^{13}C,$^{18}O_2$]acetate labelling studies,[5] the C-11 carbon exhibits two upfield isotope-shifted resonances ($\Delta\delta$ = 12.1, 35.3 p.p.b.). The ratio of unlabelled peaks is 69:16:15 since $^{18}O_2$ and acetate each contribute one oxygen to this position, and these are randomized in the free carboxy group of (**1**). Although little if any exchange with the medium occurs at C-1, C-3, or C-11, the 90:5:5 ratio at C-9 suggests that a

Table 1. ^{13}C N.m.r. chemical shifts of (2) derived from (1) obtained by fermentation of *P. multicolor* under an $^{18}O_2$ atmosphere.[a]

Carbon	δ	Δδ/p.p.b.[b]	^{16}O : ^{18}O
1	161.4	18.0	72:28
2	110.1	7.2, 22.3, 29.9	42:24:19:15
3	168.5	34.5	70:30
4	150.7	—	—
9	173.5	13.7, 37.8	90:5:5
11	164.3	12.1, 35.3	69:16:15
(3-OMe)	59.7	31.1	70:30
(9-OMe)	51.4	25.3	94:6
(11-OMe)	52.0	26.5	84:16

[a] Spectra were measured on a Bruker WH400 spectrometer at 100.6 MHz in $CDCl_3$ solutions (*ca.* 0.05 M) of (2) with Me_4Si as internal standard. [b] All isotope shifts are upfield relative to the ^{13}C–^{16}O signal and are ±0.5 p.p.b.

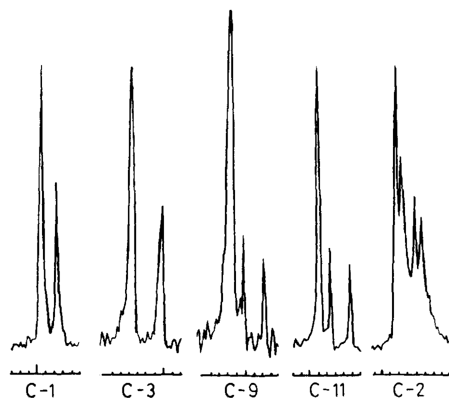


Figure 1. 100.6 MHz ^{13}C n.m.r. spectra of carbons of (**2**) which exhibit ^{18}O isotope shifts (methoxy carbons not shown).

considerable amount of the oxygen at the side chain carboxy terminus originates from water. Possibly this is due to the intermediacy of an aldehyde functionality (which can exchange oxygen readily with the medium) *en route* from multicolic acid (**7**) to multicolosic acid (**1**). Unfortunately this could not be tested because insufficient quantities of (**7**) were produced in controlled atmosphere fermentations.

Unexpectedly large upfield β-isotope shifts (7.2, 22.3, and 29.9 p.p.b.) were observed for C-2 which bears no oxygen. Since such isotope shifts are directly additive,[8] the 29.9 p.p.b. shift is caused by molecules of (**2**) bearing ^{18}O at both C-1 and C-3. Generally ^{18}O-induced β-isotope shifts are less than 10 p.p.b.,[7,8] but recently two other examples have been found in which an olefinic carbon two bonds away from an enolic oxygen displays a very large β-isotope shift.[6] On this basis it appears that the 22.3 p.p.b. shift at C-2 results from molecules of (**2**) bearing ^{18}O at C-3 and only ^{16}O at C-1. Hence the 7.2 p.p.b. shift at C-2 is due to molecules bearing ^{16}O at C-3 and ^{18}O at the C-1 carbonyl oxygen. Neither C-10 nor C-8 displays visible β-isotope shifts. Examination of a large number of [^{18}O]carbonyl compounds has demonstrated that their β-isotope shifts are usually too small to be readily observed despite their large α-isotope shifts.[9] The situation at C-2 is obviously an exception.

These results support the biosynthetic pathway proposed in Scheme 1. The appearance of equal amounts of ^{18}O label from $^{18}O_2$ at both C-1 and C-11 demonstrates that the cleavage of the aromatic ring proceeds by a different mechanism than that observed in patulin formation.[10] It is interesting to note that a variety of pathways for oxidative aromatic ring cleavage are available in *Penicillium* species. The occurrence of unusually large ^{18}O β-isotope shifts in ^{13}C n.m.r. spectra, whose magnitudes are similar to those of α-shifts, emphasizes the need for caution in using this technique for detection of ^{18}O labelling.

Financial support from the Natural Sciences and Engineering Research Council of Canada is gratefully acknowledged.

Received, 17th February 1987; Com. 212

References

1 For recent leading references see: B. C. Vanwagenen and J. H. Cardellina, II, *Tetrahedron*, 1986, **42**, 1117; J. Buck, N. G. Clemo, and G. Pattenden, *J. Chem. Soc., Perkin Trans. 1*, 1985, 2399; A. C. Campbell, M. S. Maidment, J. H. Pick, and D. F. M. Stevenson, *ibid.*, 1985, 1567.

2 For syntheses of multicolanic acid and its esters see: R. Ramage and P. P. McCleery, *J. Chem. Soc., Perkin Trans. 1*, 1984, 1555; D. R. Gedge and G. Pattenden, *ibid.*, 1979, 89; S. C. M. Fell, J. Heaps, and J. S. E. Holker, *J. Chem. Soc., Chem. Commun.*, 1979, 81.

3 J. A. Gudgeon, J. S. E. Holker, and T. J. Simpson, *J. Chem. Soc., Chem. Commun.*, 1974, 636.

4 J. A. Gudgeon, J. S. E. Holker, T. J. Simpson, and K. Young, *Bioorg. Chem.*, 1979, **8**, 311.

5 J. S. E. Holker, E. O'Brien, R. N. Moore, and J. C. Vederas, *J. Chem. Soc., Chem. Commun.*, 1983, 192.

6 J. C. Vederas, 'Mycotoxins and Phycotoxins,' eds. P. S. Steyn and R. Vleggaar, Elsevier Science Publ., Amsterdam, 1986, pp. 97—108.

7 J. C. Vederas, *Can. J. Chem.*, 1982, **60**, 1637; *Nat. Prod. Rep.*, 1987, in the press.

8 R. N. Moore, J. Diakur, T. T. Nakashima, S. L. McLaren, and J. C. Vederas, *J. Chem. Soc., Chem. Commun.*, 1981, 501.

9 P. E. Hansen, *Annu. Rep. NMR Spectrosc.*, 1983, **15**, 105.

10 H. Iijima, H. Noguchi, Y. Ebizuka, U. Sankawa, and H. Seto, *Chem. Pharm. Bull.*, 1983, **31**, 362; J. Sekiguchi, T. Shimamoto, Y. Yamada, and G. M. Gaucher, *Appl. Environ. Microbiol.*, 1983, **45**, 1939.