On the Biosynthesis of the Mycoticins, Metabolites of *Streptomyces ruber*

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Summary The biosynthesis of mycoticins A and B has been studied using labelled acetate, propionate, and methionine.

WE have previously shown that mycoticin,[1]† a neutral metabolite of *Streptomyces ruber* possessing activity against pathogenic fungi, is a mixture of two polyolefinic, polyhydroxy-macrocyclic lactones, A, $C_{36}H_{58}O_{10}$ (Ia), and B, $C_{37}H_{60}O_{10}$ (Ib).[2]

In the light of earlier investigations on macrolide antibiotics,[3] certain biogenetic pathways for the formation of (Ia) and (Ib) seem reasonable, *a priori*. In path A, the basic carbon skeletons (C-1 to C-33) in both pigments could be derived from three propionate units (at carbons C-13 to C-14′, C-29 to C-30′, and C-31 to C-33) plus 13 acetate units. Mycoticin B would then be formed by acylation at C-32, and mycoticin A by methylation at C-32. Alternatively, (path A′), mycoticin B, derived as above, could lead to mycoticin A *via* degradation of the C-34 + C-35 ethyl side-chain or its equivalent during the biogenesis.

In another biogenetic sequence for the formation of mycoticin B, (path B), carbons C-1 to C-32 and C-34 to C-35 might be derived from 17 acetate units with methylation taking place at C-14, C-30, and C-32. Similarly, mycoticin A biogenesis could involve a pathway utilizing propionate at C-31 to C-33 as a starter unit followed by 15 acetate units to complete the lactone skeleton (C-1 to C-33) with methylation at C-14, C-30 and C-32. Our studies were undertaken in order to differentiate among the above possibilities, and, as outlined below, are consistent with the scheme designated as path A′.

TABLE 1

Labelled precursor	Mycoticin isolated (d.p.m./mg)	Specific activity[a] of mycoticin (d.p.m./mmol)	Specific incorporation (%)
Sodium [2-^{14}C]propionate	13,429	$8{\cdot}82 \times 10^6$	0·10
Sodium [2-^{14}C]acetate	30,935	$20{\cdot}32 \times 10^6$	0·45
[*Methyl*-^{14}C]-L-methionine	0	—	—
[*Methyl*-^{3}H]-L-methionine	1,747	$11{\cdot}50 \times 10^5$	0·00013

[a] Since it was not possible to separate mycoticins A and B, an average molecular weight of the two was used for calculation of the specific activity. This value has an error of 1%.

Sodium [2-^{14}C]propionate (specific activity, 3·77 mCi/mmol) sodium [2-^{14}C]acetate (specific activity, 2·00 mCi/mmol), [*methyl*-^{14}C]-L-methionine (specific activity, 11·10 mCi/mmol), and [*methyl*-^{3}H]-L-methionine (specific activity, 134 mCi/mmol) were fed, respectively, to 2·5 day old cultures of *S. ruber* in 2% dextrose–Difco Bacto Peptone solutions. Mycoticin was isolated in each case and crystallized to constant activity (Table 1).

In order to determine the origin of the C-29 to C-34 and C-29 to C-35 portions of mycoticin A (Ia) and mycoticin B (Ib), respectively, the following degradation scheme was employed. The isolated mycoticin‡ was ozonized in acetic

† In this discussion "mycoticin" refers to the inseparable mixture of mycoticin A and mycoticin B isolated from the mycelium of *S. ruber* (ATCC 3348).

‡ All products obtained in this study were compared to authentic samples with respect to m.p. and mixed m.p.; t.l.c. analysis of the phenylhydrazones showed them to have identical R_F values with authentic samples.

acid,[1] reduced with Zn–HOAc–H_2O and the volatile products were steam-distilled into a saturated 2% solution of 2,4-dinitrophenylhydrazine hydrochloride. The 2,4-dinitrophenylhydrazones of 2,4-dimethylpent-2-enal (IIa) (C-29 to C-34 fragment) and of 2,4-dimethylhex-2-enal (IIb) (C-29 to C-35 fragment) were separated[4] on a column of Voclay-Betonite : Celite mixtures (22·5 g : 10·5 g) with ether–hexane elution. The radioactive hydrazones were diluted with known quantities of the corresponding pure unlabelled hydrazones and crystallized to constant activity.

TABLE 2

Labelled precursor	2,4-DNP isolated (d.p.m./mg)	Specific activity of 2,4-DNP (d.p.m./mmol) 2,4-dimethylpent-2-enal (IIa)	2,4-dimethylhex-2-enal (IIb)
[2-^{14}C]Propionate	20,356	$5{\cdot}94 \times 10^6$	—
"	19,153	—	$5{\cdot}86 \times 10^6$
[2-^{14}C]Acetate	11,878	$3{\cdot}47 \times 10^6$	—
"	14,756	—	$4{\cdot}52 \times 10^6$

As shown in Table 2, the 2,4-dinitrophenylhydrazones of 2,4-dimethylpent-2-enal and 2,4-dimethylhex-2-enal obtained from the propionate feeding showed specific activities of $5{\cdot}94 \times 10^6$ and $5{\cdot}86 \times 10^6$ d.p.m./mmol, respectively. Since these values are approximately equal and 2/3 the specific activity of the propionate-derived mycoticin ($8{\cdot}82 \times 10^6$ d.p.m./mmol), it appears most likely that the two propionate units comprise six carbons in the C-29 to C-33 fragments of both (Ia) and (Ib). Furthermore, these results suggest that the remaining 1/3 of the propionate activity not accounted for in the aldehydes (IIa) and (IIb) is located at C-13 to C-14′ in both mycoticins.

Support for the conclusion that the units C-29 to C-33 and also C-13 to C-14′ are propionate-derived is obtained from feeding experiments with labelled methionine (Table 1). The negligible incorporation of both [*methyl*-^{3}H]- and [*methyl*-^{14}C]-L-methionine shows that the C-14′, C-30′, and C-34 methyl groups (Ia) are not derived from this one-carbon source.

One of the most interesting aspects of the biogenesis of the mycoticins concerns the origin of the C-34 carbon in mycoticin A and the C-34 to C-35 unit in mycoticin B. As shown in Table 2, the specific activity of the 2,4-dinitrophenylhydrazone of 2,4-dimethylhex-2-enal (IIb) obtained from the sodium [2-^{14}C]acetate feeding was found to be significantly greater ($4{\cdot}52 \times 10^6$ d.p.m./mmol) than the activity of the similarly derived DNP of 2,4-dimethylpent-2-enal (IIa) ($3{\cdot}47 \times 10^6$ d.p.m./mmol). These findings along with the observed specific activity ($20{\cdot}36 \times 10^6$ d.p.m./mmol) of the mycoticin mixture in the [2-^{14}C]-acetate feeding are consistent with the conclusion that mycoticin B contains 14 acetate units and mycoticin A 13 acetate units.§ According to this view, the C-34 + C-35 fragment in mycoticin B is acetate-derived, while in mycoticin A, the methyl group at C-34 constitutes a one-carbon residue of this unit. It thus appears that the C-35 carbon atom (active in the [2-^{14}C]acetate) is lost in the degradation of mycoticin B to mycoticin A (or their precursors) during biosynthesis, and mycoticin A thus shows no activity in the C-34 fragment.

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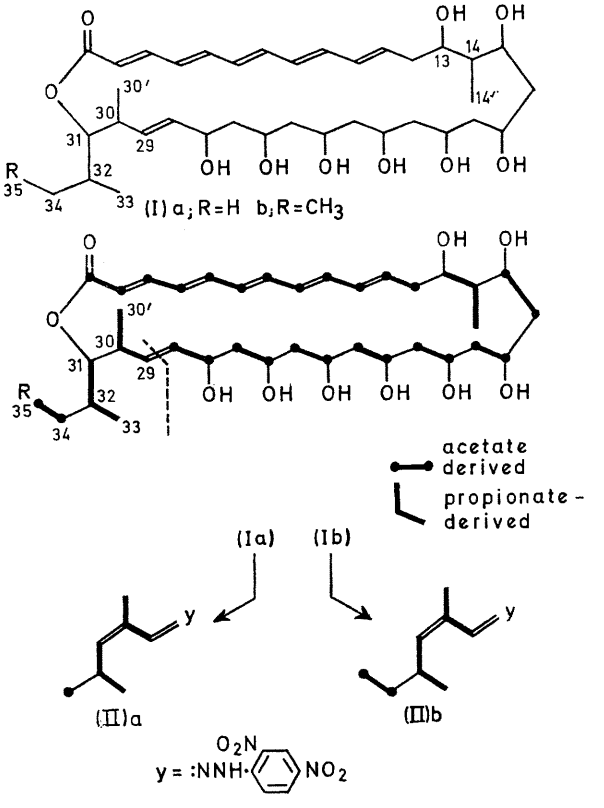

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§ We have assumed that the activity in the pentenal fragment from the [2-^{14}C]acetate feeding results from acetate to propionate conversion[5] which should be equal for mycoticins A and B. Each acetate unit then accounts for $4{\cdot}52 \times 10^6 - 3{\cdot}47 \times 10^6 = 1{\cdot}05 \times 10^6$ d.p.m./mmol. Each propionate unit contributes $1/2 \times 3{\cdot}47 \times 10^6 = 1{\cdot}73 \times 10^6$ d.p.m./mmol.

[1] H. H. Wasserman, J. E. Van Verth, D. J. McCaustland, I. J. Borowitz, and B. Kamber, *J. Amer. Chem. Soc.*, 1967, **89**, 1535.

[2] Recently, R. Bognar, B. O. Brown, W. J. S. Lockley, S. Makleit, T. P. Toube, B. C. L. Weedon, and K. Zsupan (*Tetrahedron Letters*, 1970, 471) have isolated flavofungin from *Streptomyces flavofungin* and have shown it to be a mixture of mycoticins A and B. Their results are in complete agreement with our previous structural assignments.[1]

[3] (a) A. J. Birch, *Fortschr. Chem. org. Naturstoffe*, 1957, **14**, 186; (b) A. J. Birch, R. J. English, R. A. Massy-Westropp, M. Slaytor, and Herchel Smith, *J. Chem. Soc.*, 1958, 365; (c) R. B. Woodward, *Angew. Chem.*, 1956, **68**, 13; (d) K. Gerzon, E. H. Flynn, M. V. Sigal, jun., P. F. Wiley, R. Monahan, and U. C. Quarck, *J. Amer. Chem. Soc.*, 1956, **78**, 6396.

[4] For analogous separations see J. W. White, *Analyt. Chem.*, 1948, **20**, 726.

[5] For analogous examples of acetate–propionate conversion see Z. Vanek, *et al.*, *Folia Microbiol.*, 1961, **6**, 408; R. Bentley, *Ann. Rev. Biochem.*, 1962, 605, and references therein.