Biosynthesis of the Antimitotic Antitumour Antibiotic Rhizoxin by *Rhizopus chinensis*; Origins of the Carbon Atoms†

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^{13}C N.m.r. spectroscopy has been used to determine the labelling patterns of the antibiotic rhizoxin derived from ^{13}C labelled acetate, methionine, serine, and glycine which are all incorporated during biosynthesis by the fungus, *Rhizopus chinensis*.

We have recently reported the structures of rhizoxin,[1] a novel 16-membered macrolide, and of its homologues[2] obtained from *Rhizopus chinensis* Saito Rh-2 strain. They show activity against fungi, plants, and tumour cells, appearing to interfere with cell division.[3] We now report results of a study of the biosynthesis of rhizoxin.

Preliminary studies with ^{14}C-labelled precursors indicated that acetate, methionine, and serine were incorporated during the biosynthesis of the antibiotic while propionate was not. Thus we fed ^{13}C enriched acetate (both [1-^{13}C]acetate and [1,2-$^{13}C_2$]acetate), [*methyl*-^{13}C]methionine, [1-^{13}C]serine, and [1,2-$^{13}C_2$]glycine to *R. chinensis* grown in a medium as previously described;[1] the antibiotic was purified by silica gel column chromatography and h.p.l.c. as reported previously.[1] Subsequent analysis of ^{13}C n.m.r. spectra allowed the position of incorporation of the various precursors to be assigned as shown in Figure 1.

The proton noise decoupled ^{13}C n.m.r. spectrum of rhizoxin (see Table 1), obtained after feeding with [1,2-$^{13}C_2$]acetate, indicated the incorporation of thirteen intact acetate units (0.8% enrichment). Twelve of these formed a polyketide chain from C-1 to C-22 with branching of C-5a to C-5b, and the remainder an intact unit forming the C-26, C-26a portion on the methyl substituted oxazole ring. Addition of [*methyl*-

Table 1. ^{13}C N.m.r. data[a] for the incorporation of [1,2-$^{13}C_2$]acetate, [*methyl*-^{13}C]methionine, [1-^{13}C]serine, and [1,2-$^{13}C_2$]glycine into rhizoxin.

Carbon	δ	J_{CC}/Hz[b]	Carbon	δ	J_{CC}/Hz
1	169.3	77.0	15	77.6	37.4
2	55.3		16	39.2	
3	57.4	44.0	16a	10.0	
4	36.6		17	90.5	44.8
5	30.4	32.3	18	137.0	
6	35.2		18a	12.1	
5a	37.2	50.6	19	130.6	57.2
5b	172.5		20	124.9	
7	84.3	38.2	21	138.5	[c]
8	46.5		22	138.3	
8a	17.4		22a	14.8	
9	140.2	71.2	23	121.0	70.4[d]
10	127.8		24	139.1	
11	63.6	30.1	25	137.4	
12	65.7		26	162.4	60.9
12a	11.9		26a	13.5	
13	78.4	38.9	17-OCH_3	56.5	
14	34.2				

[a] Data were recorded on a Jeol JNM FX-400 n.m.r. spectrometer at 100.7 MHz. Chemical shifts in $CDCl_3$ are given in p.p.m. relative to Me_4Si. [b] Unless otherwise stated, coupling constants for [1,2-$^{13}C_2$]acetate incorporation are given. [c] Coupling constant could not be determined because of overlapping of the signals due to C-19 and -20. [d] Coupling constant for [1,2-$^{13}C_2$]glycine incorporation.

† For Part IX of the series Studies on Macrocyclic Lactone Antibiotics see reference 2.

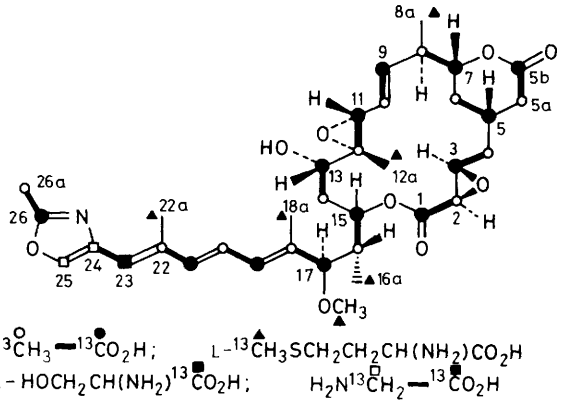

Figure 1. Schematic representation of biosynthetic incorporation into rhizoxin.

^{13}C]methionine to the medium caused enhancement of the intensity of six signals in the ^{13}C n.m.r. spectrum attributed to C-8a, C-12a, C-16a, C-18a, C-22a, and the methoxy methyl carbon (by *ca.* 36%). Thus all methyl groups apart from C-26a are derived from methionine.

Feeding with [1-^{13}C]serine caused intensity enhancement of the C-23 signal in the ^{13}C n.m.r. spectrum of rhizoxin (*ca.* 1% enrichment). Similar experiment with [1,2-$^{13}C_2$]glycine caused intensity enhancement of three signals due to C-23, C-24, and C-25 (*ca.* 2% enrichment). In this spectrum, C–C coupling between C-23 and C-24 was observed (J_{CC} 70.4 Hz) while coupling was not observed between C-24 and C-25. This indicates that a C_1 unit from a glycine molecule coupled with another unit of glycine to form a serine unit, one of which was incorporated in each rhizoxin molecule. In the same [1,2-$^{13}C_2$]glycine feeding experiment, all the methyl carbon atoms except for C-26a were also enriched by about 2%, suggesting that the C_1 unit of glycine was incorporated also into methionine.

In conclusion, we have been able to demonstrate by ^{13}C n.m.r. spectroscopy the origin of all the carbon atoms in rhizoxin. Presumably, *O*-acetyl-L-serine acts as the starter unit of the polyketide chain C-22 to C-1, prior to or after formation of a 2-methyloxazole-4-carboxylic acid unit, the polyketide chain then being alkylated with six moles of methionine at C-8, C-12, C-16, C-18, C-22, and a hydroxy group (at C-17), and with an acetate unit at C-5.

Since compounds without methoxymethyl and epoxy groups were also isolated,[2] *O*-methylation and epoxidation presumably occur after formation of the macrocyclic ring.

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